Monatshefte für Chemie - Chemical Monthly
https://doi.org/10.1007/s00706-018-2196-9

ORIGINAL PAPER

Computer-aided design, synthesis, and biological studies of anticological nitrogen-containing tetraphosphonic acids against melanoma

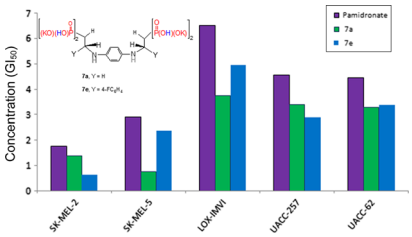
Mohamed S. Bekheit[1] · Reham F. Barghash[1] · Wafaa M. Abdou[1]

Received: 16 January 2018 / Accepted: 3 April 2018

Abstract

As a part of our quest to develop new bioactive bisphosphonic acids, we synthesized a series of bis(α-aminobisphosphonic acids) in good yields (66–78%) as new entities for treating malignant melanoma. The reaction of the Schiff bases, 1,4-pheneylenediimines with the Horner–Emmons–Wadsworth reagent, tetraethyl methylene-1,1-bisphosphonate in DMF/LiOH (aq) solution led exclusively to a *meso* form of nitrogen-containing tetraphosphonates (NTPs) (bis(α-aminobisphosphonates)). Next, hydrolysis of the ester moieties of the tetraphosphonate products yielded the corresponding tetraphosphonic acids, which treated with MeOH/KOH (aq, 20%) to give the respective NTP-tetrapotassium salts. Prior to synthesis, the suggested structures (and others) were applied to the computer-assisted molecular modeling, PASS program to investigate their prospective biological properties. Cytotoxic properties were later evaluated against five malignant melanoma cell lines that originated from different categories of malignant melanoma primary stage (I/II), histologically advanced stage (III/IV), and metastasized malignancy. Almost all tested compounds showed antitumor activity though on different levels. Three NTP salts were found to have activity in the range of GI_{50} 0.650–5.73 μM vs. control reference GI_{50}: 1.745–6.50 μM. Structure activity relationship is also discussed.

Graphical abstract



Keywords Bis(α-aminobisphosphonic acids) · Pudovik reaction · Malignant melanoma · Structure activity relationship

Introduction

The alarming rates of the growing resistance to chemotherapeutic drugs, which are used for treating different cancers, have become a major concern for members of the scientific community as well as public health officials worldwide [1, 2]. Research also shows that mortality rates among patients are increasing due to not only the numerous side effects but also the resistance to prescribed drugs [3, 4]. Therefore, research targeting the development of more effective therapeutic agents has been recently intensified. Melanoma, also known as malignant melanoma, is a type of cancer that originates from pigments containing skin cells known as melanocytes [5]. Despite melanoma is less common than other kinds of skin cancer

✉ Wafaa M. Abdou
wabdou@link.net

[1] Chemical Industries Research Division, National Research Centre, Elbuhouth St., Dokki, Cairo 12622, Egypt

Published online: 27 June 2018

(basal and squamous cell carcinoma), it is the most serious type of skin malignancy. The melanoma cancerous growth develops from unprepared mutagenized DNA of skin cells, which is mostly caused by either intense occasional exposure to sun-UV irradiation, over-using tanning bed device leading to skin burn, or could be due to heredity genetic defects. These factors trigger the skin cells to split rapidly and multiply increase causing the DNA mutation followed by skin cancer [6, 7]. In 2016, the WHO announced that an estimated 76,330 melanoma cases were recorded with about 46,810 cases reported in males and 29,510 cases in women. It is also reported that melanoma kills an estimated 10,130 people in the USA annually. Fortunately, melanoma malignancy is almost always curable if it is diagnosed in the early stage (I/II) phase. Otherwise the proliferation can advance rapidly generating early metastasis. In addition, the melanoma (III/IV) phase is characterized by its relatively high therapeutically resistance [8] (Fig. 1 [9]) and, therefore, new treatment strategies need to be developed.

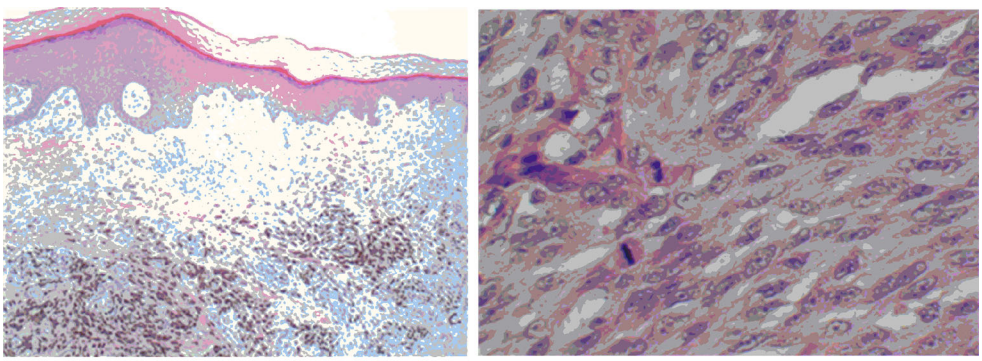
Nitrogen-containing bisphosphonates (NBPs) are well known as potent inhibitors of bone resorption and are frequently used for the treatment of Paget's disease, osteoporosis, and cancer-induced osteolysis [10]. The main mechanism of the inhibitory effect of NBPs on bone metastasis relies on the inhibition of osteoclast's growth [11, 12]. Furthermore, clinic findings showed that NBPs revealed a direct apoptotic effect on several types of carcinoma cells. This is attributed to the capability of NBPs to inhibit farnesyl diphosphate synthase, which induces blocking of the synthesis of higher isoprenoids [13, 14]. As a result of this mechanism, NBPs inactivate monomeric G-proteins of Ras and Rho families for which proliferation is a functional requirement. Encouraged by these findings, the beneficial use of NBPs in the treatment of different kinds of cancer, especially breast, colon, prostate, cervix, and multiple myeloma has been intensively reported by us [15–20] and others [21–24]. Nevertheless, only a limited number of investigations studied the effect of NBPs on melanoma [25, 26]. In a detailed study, Geilen et al. showed that the NBPs drug, pamidronate, could induce apoptosis and inhibits the proliferation of all types of human malignant melanoma cells [27, 28].

Building on this information, we demonstrate that treating malignant melanoma cell lines with bis(α-aminobisphosphonate) tetrapotassium salts induced medium to excellent apoptosis and inhibited the proliferation in a concentration-dependent manner. Remarkably, the concept of our present investigation was developed using the predicted spectrum of the biological activity of the suggested compounds (and others). The prediction process was run in the early stage of the work, using the computer-assisted molecular modeling, PASS 2014 program (prediction activity spectra of substances) [29, 30]. It is based on a robust analysis of structure–activity relationships [30] in a heterogeneous training set that currently includes 7000 types of biological activity. There are many examples of successful use of the PASS approach for finding new pharmaceutical agents [31–34].

Results and discussion

Prediction spectra of biological activity

The analysis of the biological activity spectra prediction for NTP acids **6a–6j** is a good example of in silico study of chemical compounds before running their experimental processes. It is available on the free website with the internet version of PharmaExpert: http://www.pharmaexpert.ru [29–34]. The biological activity spectrum of a compound is a list of biological activity types for which the probability to be revealed is represented by *Pa* and the probability not to be revealed is represented by *Pi*. The probabilities *Pa* and *Pi* are independent and their values vary from 0 to 1. In this work, the biological activity



A B

Fig. 1 **a** Regression in I/II stage of melanoma; **b** subcapsular lymph node metastasis (malignant melanoma III/IV stage)

spectra were predicated for the new acid structures **6a–6j** using the PASS.2014.1 version. By default, in PASS $Pa = Pi$ is chosen as a threshold and that compounds with $Pa > Pi$ are suggested to be active. Table 1 summarizes the prediction spectra of most activity types for compounds **6a–6j**. Accordingly, other than bone resorption and relative diseases, the cytotoxic activity for these specified compounds was the promising probability and, therefore, was considered.

Chemistry

The roadmap for the synthesis of new series of *N*-tetraphosphonates (NTPs) and relevant acid salts is depicted in Schemes 1 and 2. The key Schiff-base substrates, N^1,N^4-[(diarylidene/dihetarylidene)phenylene]-1,4-diamines **3a–3j** were prepared by treating 1,4-phenylenediamine (**1**) with the appropriate aldehydes **2a–2j** according to the reported methods [35–37]. Treating N^1,N^4-1,4-phenylenediimines **3a–3j** with the Horner–Emmons–Wadsworth (HEW) reagent tetraethyl methylene-1,1-bisphophonate (**4**) in dimethylformamide solution containing aqueous lithium hydroxide (DMF/LiOH aq), our targets octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-(aryl/hetryl)ethane-2-1,1-triyl]tetraphosphonates **5a–5j** were obtained in good yields (66–78%), via Pudovik reaction (66–78%) [38] (Scheme 1).

The structures and the purity of **5a–5j** were substantiated by the spectrometric (MS, IR, ^{1}H, ^{13}C, and ^{31}P NMR) data and the elemental analysis. Due to the symmetry of the molecules [39, 40], some shifts overlapped in the NMR spectra. We determined by the NMR experiments that [except **5a** (Y = H)] the occurring products **5b–5j** present exclusively in the *meso* diastereoisomeric form. Compounds **5b–5j** revealed the presence of one sharp signal of at $\delta_p \sim 25.3$ ppm in the ^{31}P NMR spectra. In their ^{1}H NMR spectra, they displayed two signal sets for methine protons. The first was ascribed to the H^a and $H^{a'}$ protons, appeared at $\delta_H \sim 2.5$ ppm as two doublet of triplets as a result of the coupling with the (2)^{31}P nucleus and the CH proton whereas the second set appeared at $\delta_H \sim 4.3$ ppm as a multiplet obscured by the 8 CH_2OP protons and assigned to H^b and $H^{b'}$ protons. To exclude the formation of the second diastereoisomeric configuration, the postreaction crude mixture was subjected to NMR spectroscopy in each case. The NMR analysis disclosed no presence of the other diastereoisomeric isomer and, therefore, we can safely state that the stereoselectivity was not formed from the preferential crystallization of one of the isomers. Since these molecules possess a mirror plane and in analogy to the previous reports in similar cases [41–44], one would obtain *meso* compounds. Consequently, we proposed that bis(α-aminobisphosphonate) are *meso* form. Indeed, this result is expected since these compounds are achiral compounds that have two chiral centers (C2 and C2′) and are superimposed on their mirror image. According to previous reports [44–46], the mechanism of the formation of the *meso* isomer, exclusively, is considered a two-step reaction. The stereogenic center that created in the first mono-aminobisphosphonates would probably control the attack of the second bisphosphonate molecule strictly at one face.

Next, ester cleavage of NTPs **5a–5j** was carried out by boiling the ester in chloroform solution containing trimethylsilyl bromide (TMS-Br) to give the respective NTP-acid counterparts **6a–6j**. The resulting acids were dramatically hygroscopic and could be kept in a vacuumed desiccator only for few days, which were enough for identification purposes. Subsequently, **6a–6j** were stirred in MeOH/KOH (10% aq) solution (20 cm^3, 1:3 v/v) for 1 h, followed by concentration to give the respective tetrapotassium salts **7a–7j** [40, 47]. Furthermore, in analogy to **5b–5j**, the acids **6b–6j** and **7b–7j** were also assigned *meso* form (Scheme 2).

Pharmacology

Reports by pharmaclinic laboratories confirmed the remarkable therapeutic potency of bisphosphonic acids for many pathogens as compared to the parent bisphosphonate esters [47, 48]. Therefore, in this work only *N*-tetraphosphonic acids **6a–6j** were applied to the prediction process while their salts were applied for the biological screening assessment.

Antitumor evaluation

Screening of the antineoplastic properties of the synthesized new salts **7a–7j** was in vitro evaluated against a panel of human malignant melanoma cell lines, Sk-MEL-2, SK-MEL-5 (obtained from primary tumor), LOX-IMMVI (originated from metastasized malignancy), and UACC-257, UACC-62 (derived from patients with histologically confirmed advanced stage by surgical intervention). The biological process was carried out utilizing the sulforhodamine-B (SRB) protein assay [49–51]. Each compound of **7a–7j** and the reference standard pamidronate (3-amino-1-hydroxypropylidene-1,1-bisphosphonic acid, Fig. 2) was tested at a minimum of five concentrations at tenfold dilution against every cell line in the panel with 48 h continuous drug exposure protocol. The results are displayed in Table 2 while the flow of GI50 of the most active NBP salts vs. the malignant melanoma carcinoma cell lines is displayed in Fig. 3.

The findings in Table 2 show that all tested NTP salts display significant inhibition of SK-MEL-2 and SK-MEL-5

Table 1 *Pa* values of biological activities for the most expected potent types of activities for *N*-tetraphosphonic acids (NTP acids) **6a–6j**

Cmpd.	Predictive activity	*Pa*	Cmpd.	Predictive activity	*Pa*
6a	Antiosteoporotic	0.850	**6f**	Bone formation stimulant	0.543
	Hypolipemic	0.750		Acute neurologic disorders treatment	0.358
	Bone diseases treatment	0.613		Leukopoiesis stimulant	0.390
	Acute neurologic disorders treatment	0.482		Antineoplastic (bone cancer)	0.527
	Leukopoiesis stimulant	0.571		Antineoplastic (lung cancer)	0.432
	Antineoplastic (bone cancer)	0.693		Antineoplastic (ovarian cancer)	0.504
	Antineoplastic (melanoma)	0.750		Antineoplastic (melanoma)	0.761
	Antineoplastic (lymphoma)	0.580		Antileukemic	0.418
	Antitumor (ovarian cancer)	0.513		Antineoplastic (brain cancer)	0.273
	Immunosuppressant	0.582		Immunosuppressant	0.414
	Antiinflammatory	0.511		Antiinflammatory	0.442
	Toxicity	0.139		Toxicity	0.331
6b	Antiosteoporotic	0.750	**6g**	Bone formation stimulant	0.596
	Bone formation stimulant	0.724		Antineoplastic (bone cancer)	0.529
	Contraceptive female	0.421		Antineoplastic (lung cancer)	0.294
	Antineoplastic (bone cancer)	0.512		Antineoplastic (bone cancer)	0.683
	Antineoplastic (lung cancer)	0.703		Antitumor (ovarian cancer)	0.555
	Antitumor (ovarian cancer)	0.571		Antineoplastic (brain cancer)	0.270
	Antineoplastic (melanoma)	0.641		Antineoplastic	0.694
	Antineoplastic	0.533		Antiosteoporotic	0.535
	Antineoplastic (bone cancer)	0.550		Antineoplastic (melanoma)	0.875
	Immunosuppressant	0.537		Immunosuppressant	0.270
	Antiinflammatory	0.550		Antiinflammatory	0.375
	Toxicity	0.537		Toxicity	0.470
6c	Antiosteoporotic	0.693	**6h**	Antiosteoporotic	0.842
	Bone diseases treatment	0.537		Bone diseases treatment	0.780
	Antiarthritic	0.484		Antiarthritic	0.451
	Antineoplastic (lung cancer)	0.368		Antineoplastic (lung cancer)	0.294
	Antitumor (ovarian cancer)	0.278		Antineoplastic (ovarian cancer)	0.510
	Antitumor (ovarian cancer)	0.750		Antineoplastic (ovarian cancer)	0.503
	Antineoplastic (brain cancer)	0.124		Antineoplastic (brain cancer	0.684
	Antineoplastic (melanoma)	0.651		Antineoplastic (melanoma)	0.773
	Immunosuppressant	0.512		Immunosuppressant	0.426
	Antiinflammatory	0.326		Antiinflammatory	0.571
	Toxicity	0.312		Toxicity	0.212
6d	Antiosteoporotic	0.504	**6i**	Bone formation stimulant	0.842
	Bone formation stimulant	0.519		Bone diseases treatment	0.780
	Hypolipemic	0.442		Hypolipemic	0.551
	Bone diseases treatment	0.780		Antiarthritic	0.394
	Acute neurologic disorders treatment	0.415		Antineoplastic (bone cancer)	0.510
	Antineoplastic (bone cancer)	0.614		Antineoplastic (ovarian cancer)	0.503
	Leukopoiesis stimulant	0.410		Antineoplastic (lung)	0.484
	Antineoplastic (lung cancer)	0.342		Antineoplastic (gastric cancer)	0.473
	Antitumor (ovarian cancer)	0.480		Antineoplastic (ovarian cancer)	0.424
	Antineoplastic (melanoma)	0.751		Antineoplastic (melanoma)	0.610
	Antileukemic	0.194		Antileukemic	0.503
	Antiinflammatory	0.451		Antiinflammatory	0.424
	Toxicity	0.251		Toxicity	0.118

Table 1 (continued)

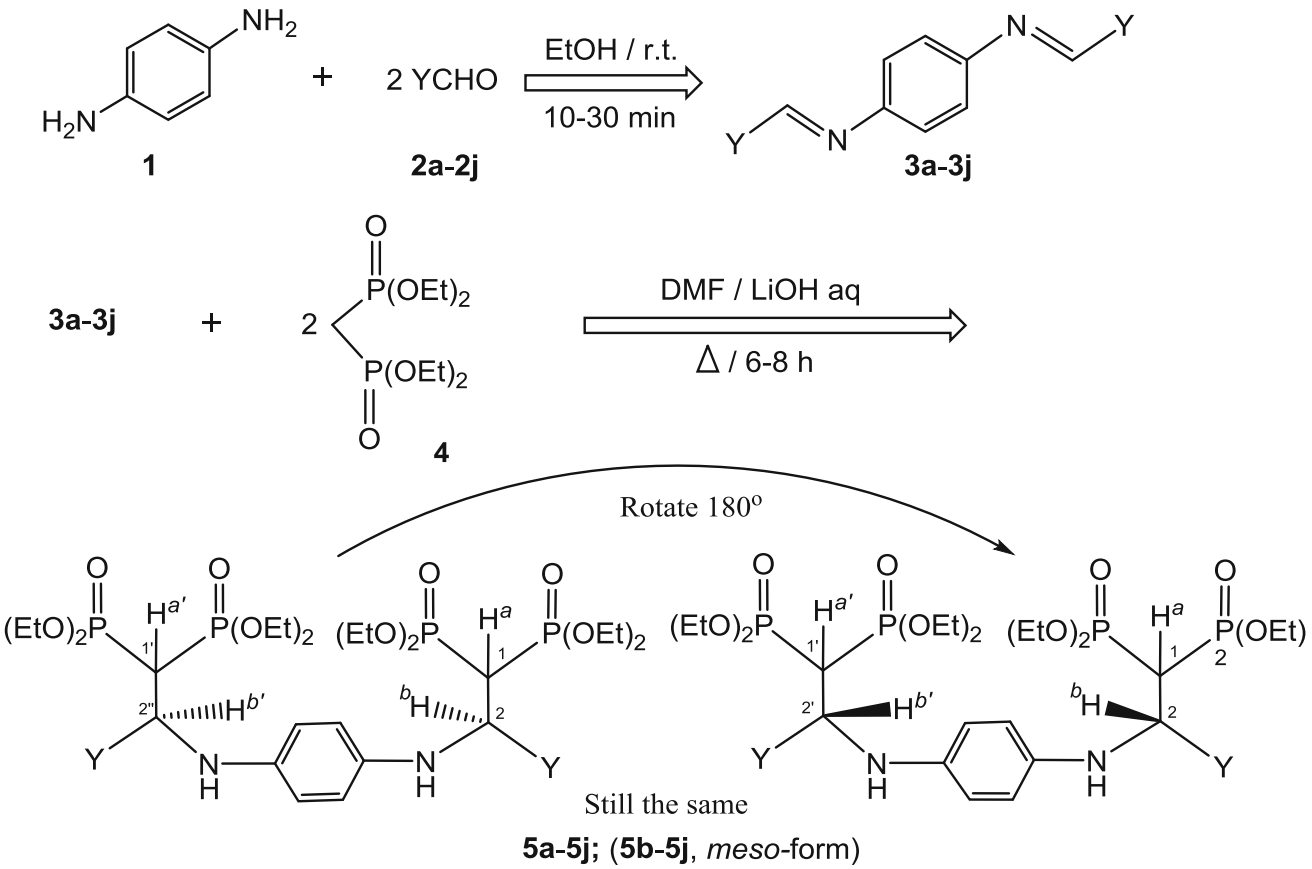
Cmpd.	Predictive activity	*Pa*	Cmpd.	Predictive activity	*Pa*
6e	Bone diseases treatment	0.843	**6j**	Bone diseases treatment	0.643
	Acute neurologic disorders treatment	0.443		Acute neurologic disorders treatment	0.243
	Leukopoiesis stimulant	0.558		Leukopoiesis stimulant	0.528
	Contraceptive female	0.690		Contraceptive female	0.490
	Antineoplastic (lung cancer)	0.327		Antineoplastic (lung cancer)	0.362
	Antineoplastic (non-Hodgkin's lymphoma)	0.414		Antineoplastic (melanoma)	0.648
				Antineoplastic (bone cancer)	0.532
	Antineoplastic (melanoma)	0.691		Antineoplastic (ovarian cancer)	0.502
	Antitumor (ovarian cancer)	0.468		Immunosuppressant	0.581
	Immunosuppressant	0.253		Antiinflammatory	0.468
	Antiinflammatory	0.514		Immunosuppressant	0.253
	Toxicity	0.274		Toxicity	0.414

(I/II-stages) cell lines in the range of GI_{50} 0.634–3.846 μM vs. the control reference GI_{50} 1.745 and 2.89 μM. The remarkable effect of the low concentrations on I/II-phases confirmed the importance of diagnosing the skin malignant melanoma in an early stage. However, higher concentrations of the tested salts and the control drug were needed to decrease the cell viability of UACC-257 and UACC-62 cell lines that originated from patients with histologically confirmed advanced stage by surgical invention with GI_{50} ranging from 2.885 to 10.15 μM vs. reference drug with GI_{50} 4.550 and 4.454 μM. Contrary to these results, the tumor cell line LOX-IMVI that derived from metastasized malignancy showed highest resistance toward all tested compounds thereof only relatively high concentrations could affect the inhibition with GI_{50} (**7a**–**7j**: from 3.74 to 22.7 μM vs. the control GI_{50} 6.5 μM). Furthermore, the biological results of **7a**–**7j** showed that the substituents at C2 as well as at the aryl ring played a vital role in the activity of these compounds, suggesting specific interactions of these groups with the biological targets. Among the ten NTP salts, **7a** (Y = H) and **7e** (Y = F) were the most active, and more potent than the positive control toward all the tested cell lines. However, the efficacy dramatically dropped on replacing the H at C2 by phenyl (**7b**) or thiophene (**7j**), taking into account that the hetero ring (**7j**) imposed slightly higher activity than the phenyl moiety (**7b**, Y = Ph). Considering the substituents at the aryl ring, the structure activity relationships revealed that the cytotoxic activity depends not only on the nature of the substituent but also on its position and number. Thus, introduction of the fluorine atom **7e** and the dimethylamino group **7g** to the parent phenyl group in **7b** imposed a significant activity on the molecule. These two substituents were found to be the most favorable, followed by a chlorine atom at 2- or 4-positions in **7c** and **7d**, whereby the introduction of the chlorine in the *para* position caused better inhibition than that at 2-position. Replacing the fluorine atom with the CF_3 group (**7f**) resulted in an obvious decrease in activity when compared with the salt **7e** (Y = 4-FC_6H_4) whereas insertion of methoxy groups **7h**, **7i** in the molecule exhibited less inhibitory action (**7h** > **7i**) among other substituents at the phenyl nucleus against all tested carcinoma cells. The order of activity is: **7a** (H) > **7e** (4-FC_6H_4) > **7g** (4-$Me_2NC_6H_4$ > **7d** (4-ClC_6H_4) > **7c** (2-ClC_6H_4) > **7f** (4-$F_3CC_6H_4$) > **7h** (4-$MeOC_6H_4$) > **7i** (2,5-$(MeO)_2C_6H_3$) > **7j** (thiophene) > **7b** (Ph).

Conclusion

A novel class of nitrogen-tetraphosphonic acid derivatives as new entities for malignant melanoma treatment was developed. The cytotoxic activity was in vitro evaluated using the sulforhodamine-B (SRB) protein assay on a panel of five melanoma carcinoma cell lines. Almost all tested compounds showed antitumor activity but on different levels and this activity was even better than that of the positive control reference Pamidronate, especially toward SK-MEL-2 and SK-MEL-5 (I/II-stage) cell lines. Nevertheless, only five compounds (out of ten) **7a** (H), **7e** (4-FC_6H_4), **7g** (4-$Me_2NC_6H_4$), **7d** (4-ClC_6H_4), and **7c** (2-ClC_6H_4) displayed good activity on UACC-257, UACC-62 cell lines obtained from histologically advanced stage (III/IV-phases). On the other hand, the cell line, LOX-IMVI, that originated from metastasized melanoma showed high resistance, and only compounds **7a**, **7e**, and **7g** exhibited significant activity on utilizing relatively higher concentration. It could be concluded that these three compounds were the most potent and even better than the positive

Scheme 1



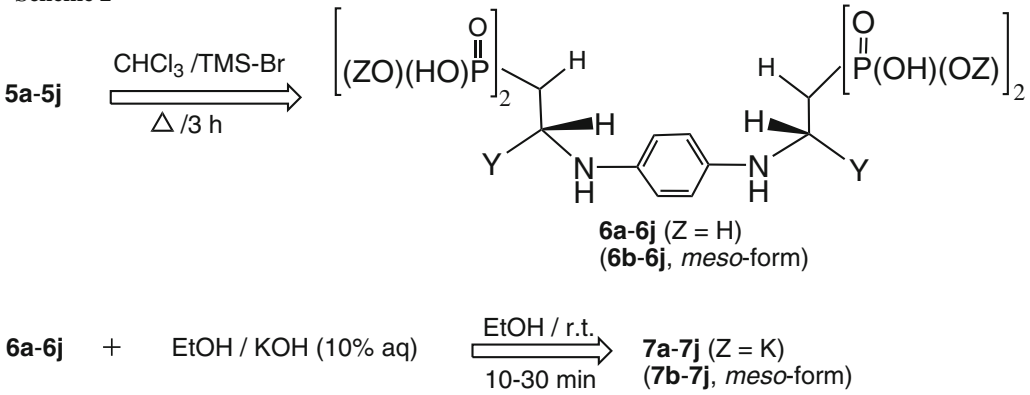
2, 5	Y	2, 5	Y	2, 5	Y
a	H	**e**	4-FC_6H_4	**h**	4-$MeOC_6H_4$
b	C_6H_5	**f**	4-$F_3CC_6H_4$	**i**	2,5-$(MeO)_2C_6H_3$
c	2-ClC_6H_4	**g**	4-$Me_2NC_6H_4$	**j**	2-Thienyl
d	4-ClC_6H_4				

control, Pamidronate toward all tested malignant melanoma cell lines. The antitumor potential among the new NTP salts **7a**–**7j** could be presented as **7a** > **7e** > **7g** > **7d** > **7c** > **7f** > **7h** > **7i** > **7j** > **7b**.

Experimental

Melting points were determined with an open capillary tube on an Electro-thermal (variable heater) melting point apparatus and were corrected. IR spectra were recorded on a JASCO FT-IR 6100 using KBr disc (JASCO, Japan). NMR spectra were measured with a JEOL E.C.A-500 MHz (^{13}C: 125.7 MHz, ^{1}H: 500 MHz, ^{31}P: 202.4 MHz) spectrometer (JEOL, Japan). ^{1}H and ^{13}C NMR spectra were recorded with trimethylsilane as internal standard in DMSO solution. Chemical shifts (δ) are given in ppm; ^{31}P NMR spectra were recorded with H_3PO_4 (85%) as external reference. The mass spectra were performed at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer provided with a data system (spectrometer (Kratos, UK). Elemental analyses were carried out at the Microanalysis Laboratory, Cairo University, Cairo, Egypt, using elementary Analysen-systeme GmbH-vario EL III Element Analyzer, Germany;

Scheme 2



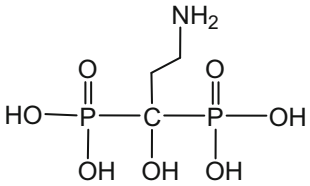
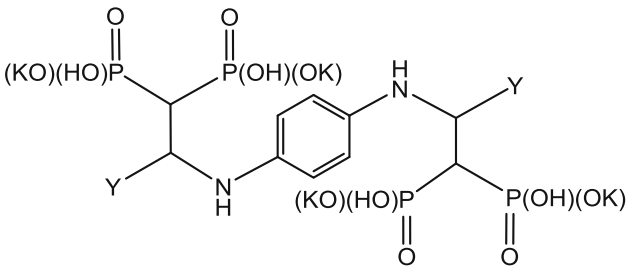


Fig. 2 Structure of pamidronate

their results were in good agreement with the calculated data. The appropriate precautions in handling moisture-sensitive compounds were observed. The purity of all new samples was verified by microchemical analysis (C/H/N/S) and spectroscopy. Thin-layer chromatography (TLC): Merck 0.2 mm silica gel 60 F254 analytic aluminum plates. The substrates **3a**–**3j** were prepared according to Refs. [35–37].

Table 2 Antitumor properties of bis(α-aminobisphosphonate) salts (NTP salts) **7a**–**7j** vs. melanoma cell lines using pamidronate drug as standard reference



NTP salts **7a-7j**

Cmpd.	Y	GI_{50}*/μM				
		SK-MEL-2	SK-MEL-5	LOX-IMVI	UACC-257	UACC-62
7a	H	1.393 ± 0.11	0.77 ± 0.26	3.74 ± 1.99	3.381 ± 3.76	3.274 ± 0.57
7b	Ph	3.650 ± 0.47	3.74 ± 0.35	22.70 ± 3.59	10.15 ± 0.78	9.679 ± 0.49
7c	2-ClC_6H_4	1.618 ± 0.92	0.650 ± 0.26	8.13 ± 2.86	4.833 ± 3.78	5.830 ± 2.17
7d	4-ClC_6H_4	1.729 ± 1.67	2.57 ± 0.78	8.03 ± 0.37	4.736 ± 0.08	5.111 ± 3.47
7e	4-FC_6H_4	0.634 ± 1.51	2.36 ± 0.68	4.93 ± 0.72	2.885 ± 0.87	3.371 ± 2.05
7f	4-$CF_3C_6H_4$	3.846 ± 0.24	1.76 ± 2.56	9.16 ± 1.88	5.714 ± 0.45	6.677 ± 1.76
7g	4-Me_2N-C_6H_4	1.590 ± 1.83	2.53 ± 3.06	5.73 ± 1.69	3.715 ± 0.92	3.712 ± 3.87
7h	4-MeO-C_6H_4	3.536 ± 2.66	2.41 ± 0.88	12.31 ± 0.48	8.255 ± 0.77	7.226 ± 1.55
7i	$(2,5\text{-MeO})_2$-C_6H_3	3.970 ± 2.52	3.30 ± 0.07	10.53 ± 4.57	8.816 ± 1.06	7.684 ± 2.35
7j	Thiophene	3.294 ± 1.82	3.23 ± 3.27	20.11 ± 4.08	9.433 ± 1.10	8.083 ± 1.18
Pamidronate [27]		1.745 ± 0.18	2.89 ± 0. 37	6.50 ± 0.33	4.550 ± 1.48	4.454 ± 0.34

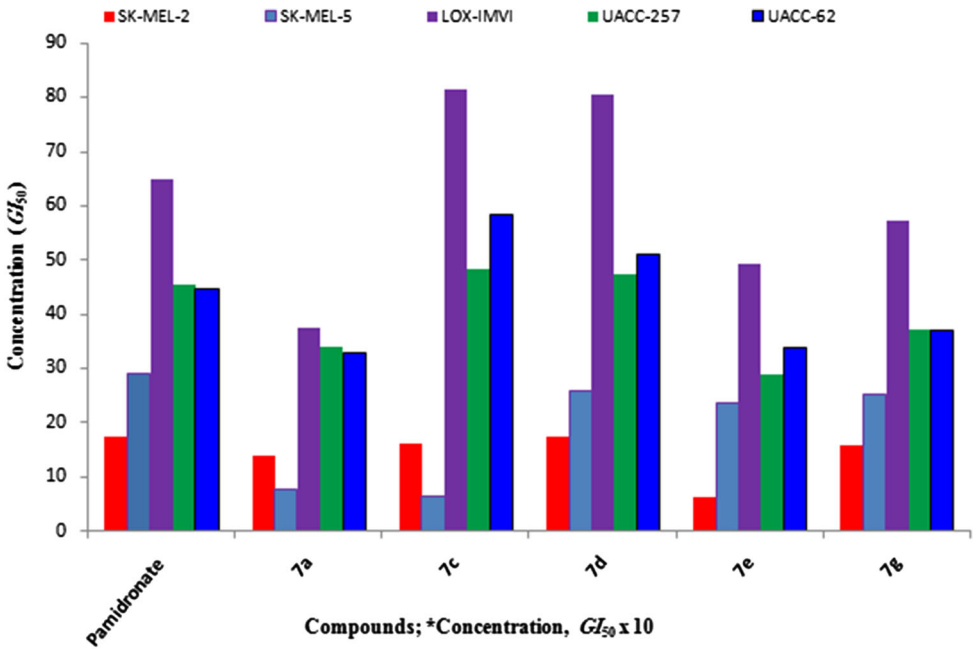


Fig. 3 Flow of GI_{50} of most active NTP salts compared to pamidronate vs. melanoma carcinoma cell lines

General procedure to prepare nitrogen-bisphosphonates (NBPs) 5a–5j

To a stirred solution of the starting materials **3a–3j** (2.2 mmol) dissolved in 20 cm^3 DMF, a solution of LiOH (6.6 mmol) dissolved into a least amount of distilled water was added, followed by the addition of tetraethyl methylenebiphosphonate (**4**, 5 mmol) in one portion. The reaction mixture was heated under reflux temperature for ≈ 6–8 h (TLC). After cooling, the product mixture was poured in ice water and acidified with HCl, followed by extraction with AcOEt (3 × 20 cm^3) and the combined organic phase was washed and dried over anhy Na_2SO_4. After removal of the volatile materials under vacuum, the resulting residue was collected and crystallized from the proper solvent to give the bisphosphonates **5a–5j** as colorless materials in good to high yields.

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis(ethane-2,1,1-triyl)tetraphosphonate (5a, $C_{26}H_{52}N_2O_{12}P_4$) Yield: 1.02 g (66%); m.p.: 147 °C (from MeCN); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.02, 1.24 (2dt, 24H, $^3J_{HH}$ = 6.3, $^4J_{PH}$ = 4.5 Hz, 8 MeCO), 2.51, 2.63 (2dt, 2H, $^3J_{HH}$ = 13.4, $^2J_{PH}$ = 18.8 Hz, H^a, H$^{a''}$), 4.09–4.21 (2dq (m), 16H, 8 H_2CO), 4.31–4.39 (2tt (m), 4H, 2 H_2C), 6.69–6.75 (m, 4H, H/Ph), 8.86 (br, 2H, 2HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 144.6, 119.8 (4C/Ar), 61.3 (d, $^2J_{PC}$ = 11.3 Hz, 8 CH_2O), 43.5 (t, $^2J_{PC}$ = 8.4 Hz, CH_2), 42.6 (t, $^1J_{PC}$ = 155.3 Hz, C1, C1′), 14.2 (d, $^3J_{PC}$ = 8.8 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 23.6 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3398 (br, 2 NH), 1252 (P=O), 1121 (POC) cm^{-1}; MS (EI, 70 EV): *m/z* (%) = 708 (M^+, 18), 134 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis(2-phenyl-ethane-2,1,1-triyl)tetraphosphonate (5b, $C_{38}H_{60}N_2O_{12}P_4$) Yield: 1.3 g (69%); m.p.: 125 °C (from cyclohexane); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.18, 1.29 (2dt, 24H, $^3J_{HH}$ = 5.9, $^4J_{PH}$ = 4.1 Hz, 8 MeCO), 2.49, 2.59 (2dt, 2H, $^3J_{HH}$ = 12.9, $^2J_{PH}$ = 19.2 Hz, H^a, H$^{a'}$), 3.88–4.01 (2dq (m), 16H, 8 H_2CO), 4.41–4.44 (m, 2H, H^b, H$^{b'}$), 6.73 (m, 4H, H/Ph), 7.10–7.73 (m, 10H, H/Ar), 9.06 (br, 2H, 2HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 145.1, 141.3, 130.2, 128.3, 125.3, 121.8 (C/Ar), 61.9 (t, $^2J_{pc}$ = 12.8 Hz, C*2, C*2′), 58.2 (d, $^2J_{PC}$ = 12.5 Hz, 8 CH_2O), 41.5 (t, $^1J_{PC}$ = 156.3 Hz, C1, C1′), 14.4 (d, $^3J_{PC}$ = 8.7 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 24.7 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3420 (br, 2 NH), 1259 (br, P=O), 1097 (br, POC) cm^{-1}; MS (EI, 70 EV): *m/z* (%) = 860 (M^+, 22), 286 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-(2-chlor-ophenyl)ethane-2,1,1-triyl]tetraphosphonate (5c, $C_{38}H_{58}Cl_2N_2O_{12}P_4$) Yield: 1.6 g (78%); m.p.: 165 °C (from CH_3Cl); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.87, 1.29 (2dt, 24H, $^3J_{HH}$ = 6.9, $^4J_{PH}$ = 4.5 Hz, 8 MeCO), 2.65, 2.81 (2dt, 2H, $^3J_{HH}$ = 13.1, $^2J_{PH}$ = 18.9 Hz, H^a, H$^{a'}$), 4.09–4.18 (2dq (m), 16H, 8 H_2CO), 4.39–4.02 (m, 2H, H^b, H$^{b'}$), 6.31 (m, 4H, H/Ph), 7.21–7.51 (m, 8H, H/Ar), 9.32 (br, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 143.2, 137.3, 135.2, 134.6, 133.8, 129.1, 126.7, 121.5 (C/Ar), 62.5 (t, $^2J_{pc}$ = 12.8 Hz, C*2, C*2′), 59.2 (d, $^2J_{PC}$ = 11.8 Hz, 8 CH_2O), 42.7 (t, $^1J_{PC}$ = 157.1 Hz, C1, C1′), 15.6 (d, $^3J_{PC}$ = 6.7 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 22.8 (s, P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3415 (br, NH), 1256 (br, P=O), 1112 (br, POC) cm^{-1}; MS (EI, 70 EV): *m/z* (%) = 930 ($[M+2]^+$, 9), 928 (M^+, 15), 354 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-(4-chlorophenyl)ethane-2,1,1-triyl]tetraphosphonate (5d, $C_{38}H_{58}Cl_2N_2O_{12}P_4$) Yield: 1.46 g (72%); m.p.: 181 °C (from MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.08, 1.21 (2dt, 24H, $^3J_{HH}$ = 6.8, $^4J_{PH}$ = 4.8 Hz, 8 MeCO), 2.61, 2.82 (2dt, 2H, $^3J_{HH}$ = 12.4, $^2J_{PH}$ = 19.1 Hz, H^a, H$^{a''}$), 4.12–4.21 (2dq (m), 16H, 8 H_2CO), 4.31–4.38 (m, 2H, H^b, H$^{b'}$), 6.41 (m, 4H, H/Ph), 7.34–7.92 (m, 8H, H/Ar), 8.69 (br, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 141.7, 137.7, 136.1, 135.1, 133.9, 121.0 (C/Ar), 61.9 (t, $^2J_{pc}$ = 12.8 Hz, C*2, C*2′), 59.5 (d, $^2J_{PC}$ = 10.5 Hz, 8 CH_2O), 42.1 (t, $^1J_{PC}$ = 154.6 Hz, C1, C1′), 15.3 (d, $^3J_{PC}$ = 8.9 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 23.4 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3419 (br, 2 NH), 1234 (br, P=O), 1098 (br, POC) cm^{-1}; MS (EI, 70 EV): *m*/*z* (%) = 930 ([M + 2]$^+$, 5), 928 (M$^+$, 18), 354 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-(4-fluorophenyl)ethane-2,1,1-triyl]tetraphosphonate (5e, $C_{38}H_{58}F_2N_2O_{12}P_4$) Yield: 1.47 g (75%); m.p.: 151 °C (from EtOH); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.93, 1.29 (2dt, 24H, $^3J_{HH}$ = 6.5, $^4J_{PH}$ = 5.1 Hz, 8 MeCO), 2.61, 2.69 (2dt, 2H, $^3J_{HH}$ = 13.5, $^2J_{PH}$ = 18.9 Hz, H^a, H$^{a'}$), 3.92–4.39 (2dq (m), 16 H, 8 H_2CO), 4.37 (m, 2H, H^b, H$^{b'}$), 6.88 (m, 4H, H/Ph), 7.32–7.75 (m, 8H, H/Ar), 8.88 (br, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 160.5 (d, $^1J_{CF}$ = 271.3 Hz, CF), 144.7, 136.8, 131.8 (d, $^3J_{CF}$ = 10.8 Hz, *C*CCF), 122.8(*C*/Ar), 119.7 (d, $^2J_{CF}$ = 28 Hz, *C*CF), 62.1 (t, $^2J_{pc}$ = 12.8 Hz, C*2, C*2′), 58.2 (d, $^2J_{PC}$ = 12.5 Hz, 8 CH_2O), 41.7 (t, $^1J_{PC}$ = 154.6 Hz, C1, C1′), 15.4 (d, $^3J_{PC}$ = 8.1 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 26.3 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3422 (br, 2 NH), 1248 (br, P=O), 1091 (br, POC) cm^{-1}; MS (EI, 70 EV): *m*/*z* (%) = 896 (M$^+$, 21), 322 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-[4-(trifluoromethyl)phenyl]ethane-2,1,1-triyl]tetraphosphonate (5f, $C_{40}H_{58}F_6N_2O_{12}P_4$) Yield: 1.53 g (70%); m.p.: 175 °C (from MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.85, 1.19 (2dt, $^3J_{HH}$ = 7.2, 24H, $^4J_{PH}$ = 5.6 Hz, 8 MeCO), 2.54, 2.72 (2dt, 2H, $^3J_{HH}$ = 11.9, $^2J_{PH}$ = 17.6 Hz, H^a, H$^{a'}$), 4.12–4.29 (2dq (m), 16H, 8 H_2CO), 4.38 (m, 2H, H^b, H$^{b'}$), 6.84 (m, 4H, H/Ph), 7.31–7.61 (m, 8H, H/Ar), 8.78 (br, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 145.8, 144.1, 131.8, 128.3 (q, $^3J_{CF}$ = 8.8 Hz, *C*CCF$_3$), 125.2 (q, $^2J_{CF}$ = 28.6 Hz, *C*CF$_3$), 119.3 (*C*/Ar), 122.5 (q, $^1J_{CF}$ = 269.8 Hz, CF$_3$), 64.6 (t, $^2J_{pc}$ = 11.9 Hz, C*2, C*2′), 63.2 (d, $^2J_{PC}$ = 13.2 Hz, 8 CH_2O), 43.1 (t, $^1J_{PC}$ = 157.2 Hz, C1, C1′), 14.8 (d, $^3J_{PC}$ = 8.1 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 22.6 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3410 (br, 2 NH), 1247 (br, P=O), 1119 (br, POC) cm^{-1}; MS (EI, 70 EV): *m*/*z* (%) = 996 (M$^+$, 17), 422 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-[4-(dimethylamino)phenyl]ethane-2,1,1-triyl]tetraphosphonate (5g, $C_{42}H_{70}N_4O_{12}P_4$) Yield: 1.49 g (72%); m.p.: 234 °C (from MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.88, 1.26 (2dt, 24H, $^3J_{HH}$ = 6.3, $^4J_{PH}$ = 4.4 Hz, 8 MeCO), 2.57, 2.68 (2dt, 2H, $^3J_{HH}$ = 13.8, $^2J_{PH}$ = 20.6 Hz, H^a, H$^{a'}$), 3.07 (s, 12H, 2 Me_2N), 3.88–4.36 (2dq (m), 16H, 8 H_2CO), 4.37 (m, 2H, H^b, H$^{b'}$), 6.74 (m, 4H, H/Ph), 6.99–7.70 (m, 8H, H/Ar), 8.66 (br, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 145.8, 141.7, 132.4, 128.9, 121.1, 115.8 (C/Ar), 62.6 (t, $^2J_{pc}$ = 12.4 Hz, C*2, C*2′), 61.4 (d, $^2J_{PC}$ = 12.5 Hz, 8 CH_2O), 43.3 (t, $^1J_{PC}$ = 158.3 Hz, C1, C1′), 38.8 (2 Me_2N), 14.7 (d, $^3J_{PC}$ = 7.9 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 25.3 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3404 (br, NH), 1260 (P=O), 1089 (br, POC) cm^{-1}; MS (EI, 70 EV): *m*/*z* (%) = 946 (M$^+$, 18), 372 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-(4-methoxyphenyl)ethane-2,1,1-triyl]tetraphosphonate (5h, $C_{40}H_{64}N_2O_{14}P_4$) Yield: 1.37 g (68%); m.p.: 102–104 °C (from CH_2Cl_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.81, 1.30 (2dt, 24H, $^3J_{HH}$ = 7.7, $^4J_{PH}$ = 4.8 Hz, 8 MeCO), 2.62, 2.81 (2dt, 2H, $^3J_{HH}$ = 14.8, $^2J_{PH}$ = 21.3 Hz, H^a, H$^{a'}$), 3.65 (s, 6H, 2 MeO), 3.91–4.38 (2dq (m), 16 H, 8 H_2CO), 4.42 (m, 2H, H^b, H$^{b'}$), 6.45 (m, 4H, H/Ph), 7.31–7.65 (m, 8H, H/Ar), 9.01 (brs, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 154.7, 141.2, 131.8, 130.3, 121.9, 117.2 (C/Ar), 62.4 (t, $^2J_{pc}$ = 11.8 Hz, C*2, C*2′), 61.2 (d, $^2J_{PC}$ = 11.9 Hz, 8 CH_2O), 52.7 (2 MeO), 43.4 (t, $^1J_{PC}$ = 148.9 Hz, C1, C1′), 15.7 (d, $^3J_{PC}$ = 8.4 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 26.2 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3419 (br, 2 NH), 1250 (br, P=O), 1115 (br, POC) cm^{-1}; MS (EI, 70 EV): *m*/*z* (%) = 920 (M$^+$, 21), 346 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-(2,5-dimethoxyphenyl]ethane-2,1,1-triyl]tetraphosphonate (5i, $C_{42}H_{68}N_2O_{16}P_4$) Yield: 1.53 g (71%); m.p.: 147 °C (from MeCN); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.89, 1.08 (2dt, 24H, $^3J_{HH}$ = 6.7, $^4J_{PH}$ = 5.2 Hz, 8 MeCO), 2.46, 2.69 (2dt, 2H, $^3J_{HH}$ = 12.1, $^2J_{PH}$ = 19.61 Hz, H^a, H$^{a'}$), 3.65, 3.68 (2 s, 12H, 4 MeO), 3.82–4.26 (2dq (m), 16H, 8 (H_2CO)P), 4.42 (m, 2H, H^b, H$^{b'}$), 6.68 (m, 4H, H/Ph), 7.42–7.81 (2d, 6H, H/Ar), 8.82 (br, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 156.2, 146.1, 145.7, 129.9, 121.9, 117.8, 116.4, 112.6 (C/Ar), 64.4 (t, $^2J_{pc}$ = 14.2 Hz, C*2, C*2′), 61.8 (d, $^2J_{PC}$ = 12.4 Hz, 8 CH_2O), 55.7, 52.4 (2 MeO, 2′MeO), 42.7 (t, $^1J_{PC}$ = 161.3 Hz, C1, C1′), 14.2 (d, $^3J_{PC}$ = 7.3 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 25.7

(s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3422 (br, 2 NH), 1265 (br, P=O), 1179 (br, POC) cm^{-1}; MS (EI, 70 EV): *m/z* (%) = 980 (M^+, 19), 406 (100).

Octaethyl 2,2′-[1,4-phenylenebis(azanediyl)]bis[2-(thiophen-2-yl)ethane-2,1,1-triyl]tetraphosphonate (5j, $C_{34}H_{56}N_2O_{12}P_4S_2$) Yield: 1.46 g (76%); m.p.: 131–133 °C (from CH_2Cl_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.92, 1.21 (2dt, 24H, $^3J_{HH}$ = 7.8, $^4J_{PH}$ = 4.9 Hz, 8 MeCO), 2.55, 2.73 (2dt, 2H, $^3J_{HH}$ = 12.9, $^2J_{PH}$ = 21.5 Hz, H^a, $H^{a'}$), 3.73–4.23 (2dq (m), 16H, 8 H_2CO), 4.57 (m, 2H, H^b, $H^{b'}$), 6.82 (m, 4H, H/Ph), 7.13–7.34 (m, 6H, H/thiophene), 8.75 (br, 2H, 2 HN) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 146.3, 142.6, 140.1, 132.2, 126.2, 118.9 (C/Ar), 62.2 (t, $^2J_{pc}$ = 12.4 Hz, C*2, C*2′), 60.8 (d, $^2J_{PC}$ = 13.2 Hz, 8 CH_2O), 42.3 (t, $^1J_{PC}$ = 158.6 Hz, C1, C1′), 14.5 (d, $^3J_{PC}$ = 8.5 Hz, 8 MeCO) ppm; ^{31}P NMR (202.4 MHz, DMSO-d_6): δ = 24.8 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3421 (br, NH), 1255 (br, P=O), 1127 (br, POC) cm^{-1}; MS (EI, 70 EV): *m/z* (%) = 872 (M^+, 16), 298 (100).

2,2′-[1,4-Phenylenebis(azanediyl)]bis(ethane-2,1,1-triyl)tetraphosphonic acid (6a, $C_{10}H_{20}N_2O_{12}P_4$) A solution of 0.6 g bisphosphonate **5a** (0.84 mmol) in 10 cm^3 $CHCl_3$ was treated with trimethylsilyl bromide (TMS-Br, 5 mmol) and the reaction mixture was heated at 40°C for ~ 6 h. After concentrating the product mixture in vacuum, the crude residue was diluted with dist. water/AcOEt (2:1 v/v) and stirred for 30 min. The two layers were separated and the aqueous layer was evaporated to dryness. The precipitates were collected and dried to give the corresponding hygroscopic tetraacid **6a**, which could be kept under vacuum. Compound **6a** was directed without any further purification for the analytical purposes. White compound; yield: 0.27 g (66%); m.p.: > 300 °C; ^{1}H NMR (500 MHz, D_2O): δ = 2.45, 2.57 (2dt, 2H, $^3J_{HH}$ = 12.4, $^2J_{PH}$ = 20.7 Hz, H^a, $H^{a'}$), 4.28–4.29 (m, 4H, 2 H_2C), 6.74 (s, 4H, H/Ph) ppm; ^{13}C NMR (125.7 MHz, D_2O): δ = 143.1, 121.4 (C/Ar), 43.9 (t, $^2J_{PC}$ = 8.4 Hz, CH_2), 42.2 (t, $^1J_{PC}$ = 154.0 Hz, C1, C1′) ppm; ^{31}P NMR (202.4 MHz, D_2O): δ = 18.3 (s, 2 P_2C) ppm; IR (KBr): $\bar{\nu}$ = 3416–3387 (NH, OH), 124.8 (br, P=O) cm^{-1}; MS (EI, 70 EV): *m/z* (%) = 476 ($[M-8]^+$, 16), 136 (100).

The acid **6a** was stirred for 1 h in 20 cm^3 solution of aqueous KOH (10%)/methanol (1: 3 v/v), followed by concentration. The precipitate was collected and air dried to give the tetrapotassium salt **7a** (72% yield).

General procedure to prepare NBP-tetrapotassium salts 7a–7j

Bisphosphonates **5b–5j** (0.8 g) dissolved in 10 cm^3 $CHCl_3$ were treated with trimethylsilyl bromide (TMS-Br, 5 mmol) and then heated at 40°C for ~ 6 h. After the usual working up (as described for **6a**), the acids **6b–6j** were obtained. They were converted as above to their tetrapotassium salts **7b–7j** by adding in situ a solution (20 cm^3) of aq KOH (10%)/methanol (1: 3 v/v) and were stirred for 1 h, followed by concentration. The precipitates were collected/air dried to give the salts **7b–7j** in ~ 68% yields.

Bioassay screening

The effect of NTP-tetrapotassium salts **7a–7j** on melanoma cell proliferation was studied on: SK-MEL-2, SK-MEL-5, LOX-IMVI, UACC-257, and UACC-62 cell lines utilizing sulforodamine-B method [43, 45]. The cells were seeded at a density of 5000 cells per well in 96-multiwell micrometer plate and left for 36 h at 37 °C under atmosphere of 5% CO_2 to allow the attachment of the cell to the wall of the plate. The tested compounds **7a–7j** and the positive control pamidronate were dissolved in dimethylsulfoxide (DMSO) 99% and diluted 1000 fold in the assay. Concentrations of the tested compounds were increased as 0, 5, 10, and 12.5 μg/cm^3 and added to the cell monolayer. Triplicate wells were used for each individual dose. The monolayer cells were incubated with the tested compounds for 48 h at 36–38 °C. Then, the cells were fixed, washed and stained with sulforodamine-B (SRB). Excess stain was washed with acetic acid while the attached stain was recovered with Tris–EDTA buffer. Cell viability was determined by measuring the color intensity at 750 nm using ELISA micrometer reader (Meter-tech-Σ960, USA). Data were collected as mean values for experiments that proceeded by SRB assay. A control experiment did not exhibit significant change compared to the DMSO vehicle. The percentage of cell survival was calculated according to the equation:

$$\text{Surviving fraction} = \text{Optical density (OD) of treated cell/OD of control cells.}$$

The GI_{50} (concentration required to produce 50% inhibition of cell grown compared to control experiment) was determined using the Graph-Pad PRISM version-5 software statistical calculations for determination of the mean and standard error values by SPSS software [52, 53]. The results of antimelanoma properties are displayed in Table 2 and Fig. 3.

Acknowledgements The authors would like to thank the National Research Centre, Dokki, Cairo, Egypt (project # 10010340) for the financial support of the present work. They also are grateful to Cancer Research Institute, NY, USA, for handling the antitumor properties.

References

1. Li J, Xu L-Z, He K-L, Guo W-J, Zheng Y-H, Xia P, Chen Y (2001) Breast Cancer Res 3:253

2. Engel J, Eckel R, Kerr J, Schmidt M, Furstenberger G, Richter R, Sauer H, Senn HJ (2001) Ital J Anat Embryol 106:59
3. Uchiyama-Kokubu N, Watanabe T (2001) Anticancer Drugs 12:769
4. Cree A, Knight L, Di Nicolantonio LF, Sharma S, Gulliford T (2002) Curr Opin Investig Drugs 3:634
5. Guo H, Miao Y (2013) Bioorg Med Chem Lett 23:2319
6. Ascierto PA, Kikwood JM, Marincola FM, Palmieri G (2011) J Skin Cancer 2011 **(article ID 710697)**
7. Divito SJ, Ferris LK (2010) Melanoma 20:450
8. Rondeau JM, Bitsch F, Bourgier E, Geiser M, Hemmig R, Kroemer M, Lehmann S, Ramage P, Rieffel S, Strauss A, Green JR, Jahnke W (2006) Chem Med Chem 1:267
9. Cancer Research UK. http://www.cancerresearchuk.org/about-melanoma/bowel-cancer/stages-grades/report2014-1
10. Balakrishna A, Reddy MVN, Rao PV, Kumar MA, Kumar BS, Nayak SK, Reddy CS (2011) Eur J Med Chem 46:1798
11. Hughes DE, Wright KR, Uy HL, Sasaki A, Yoneda T, Roodman GD, Mundy GR, Boyce BF (1995) J Bone Miner Res 10:1478
12. Reszka AA, Rodan GA (2003) Curr Osteoporos Rep 1:45
13. Aznar S, Lacal JC (2001) Cancer Lett 165:1
14. van Beek E, Pieterman E, Cohen L, Lowik C, Papapoulos S (1999) Biochem Biophys Res Commun 264:108
15. Abdou WM, Barghash RF, Bekheit MS, Geronikaki A (2016) Chem Select 1:3797
16. Abdou WM, Shaddy AA, Khidre RE, Awad GEA (2016) J Heterocycl Chem 53:524
17. Shaddy AA, Kamel AA, Abdou WM (2013) Synth Commun 43:236
18. Abdou WM, Barghash RF, Sediek AA (2012) Eur J Med Chem 57:362
19. Abdou WM, Kamel AA, Khidre RE, Geronikaki A, Ekonomopoulou MT (2012) Chem Biol Drug Des 79:719
20. Abdou WM, Khidre RE, Kamel AA (2012) Arch Pharm Chem Life Sci 345:123
21. Meta-analysis finds benefits of adjuvant bisphosphonates for postmenopausal breast cancer. Cancer Research Institute, NY, USA, Report September 9, 2015
22. Cheng F, Oldfield E (2004) J Med Chem 47:5149
23. Coleman RE (2001) Cancer Treat Rev 27:165
24. Fleisch H (2002) Breast Cancer Res 4:30
25. Shipman CM, Rogers MJ, Apperley JF, Russell RG, Croucher PI (1997) Br J Haematol 98:665
26. Shipman CM, Croucher PI, Russell RG, Helfrich MH, Rogers MJ (1998) Cancer Res 58:5294
27. Riebeling C, Forsea AM, Raisova M, Orfanos CE, Geilen CC (2002) Br J Cancer 87:366
28. Forsea A-M, Müller C, Riebeling C, Orfanos CE, Geilen CC (2004) Br J Cancer 91:803
29. Poroikov V, Filimonov D et al (1992–2014) PASS 14 Standard-prediction of activity spectra for substances. http://www.pharmaexpert.ru
30. Poroikov V, Filimonov D (2005) PASS: prediction of biological activity spectra for substances. In: Helma C (ed) Predictive toxicology. Taylor & Francis, London, p 459
31. Abdel-Fatah TM, McArdle SE, Agarwal D, Moseley PM, Green AR, Ball GR, Pockley AG, Ellis IO, Rees RC, Chan SY (2016) Clin Cancer Res 22:905
32. Goel RK, Singh A, Naidu PS, Mahajan MP, Kulkarni SK (2005) J Pharm Pharm Sci 8:182
33. Singh G, Bansal Y, Bansal G, Goel RK (2014) Med Chem 10:418
34. Poroikov VV, Filimonov DA (2002) J Comput Aided Mol Des 16:819
35. Das S, Das VK, Saikia L, Thakur AJ (2012) Green Chem Lett Rev 5:457
36. Kiviranta PH, Leppönen J, Kyrylenko S, Salo HS, Lahtela-Kakkonen M, Tervo AJ, Wittekindt C, Suuronen T, Kuusisto E, Jörvinen T, Salminen A, Poso A, Wallén EAA (2006) J Med Chem 49:7907
37. Sierra MA, Gómez-Gallego M, Alcázar R, Lucena JJ, Yuntab F, Garcia-Marcob S (2004) Dalton Trans (21):3741
38. Pudovik AN, Pudovik MA (1966) Zh Obshch Khim 36:1467
39. Manouni DE, Benech JM, Benramdane M, Lecouvey M, Leger G, Leroux Y (1999) Phosphorus. Sulfur Silicon Relat Elem 147:81
40. Kanaan M, Burgada R (1988) Phosphorus Sulfur Relat Elem 37:217
41. Vollhardt KPC, Shore N (2007) Organic Chemistry, 5th edn. Freeman WH, New York
42. Shore N (2007) Study guide and solutions manual for organic chemistry, 5th edn. Freeman WH, New York
43. Kraicheva I, Finocchiaro P, Failla S (2007) Phosphorus. Sulfur Silicon Relat Elem 182:57
44. Lewkowska J, Lewkowska ES (2006) Phosphorus. Sulfur Silicon Relat Elem 181:1323
45. Lewkowska J (2005) Phosphorus. Sulfur Silicon Relat Elem 180:179
46. Barycki J, Garncarz R, Milewska M, Tyka R (1995) Phosphorus. Sulfur Silicon Relat Elem 105:117
47. Nugent RA, Murphy M, Schlachter ST, Dunn CJ, Smith RJ, Staite ND, Galinet LA, Shields SK, Aspar DG, Richard KA, Rohloff NA (1993) J Med Chem 36:134
48. Ebetino FHM, Francis D, Rogers MJ, Russell RGG (1998) Rev Contemp Pharmacother 9:233
49. Grever MR, Schepartz SA, Chabner BA (1992) Semin Oncol 19:622
50. Boyd MR, Paull KD (1995) Drug Dev Res 34:91
51. Skehan P, Storeng R, Scudiero D, Monks A, Mahon JMC, Vistica D, Warren JT, Bokesh H, Kenney S, Boyd MR (1990) J Natl Cancer Inst 82:1107
52. Karber C (1931) Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol 162:480
53. Girgis AS, Mishriky N, Ellithey M, Hosni HM, Farag H (2007) Bioorg Med Chem 15:2403