Full Paper

Elaborating on Efficient Anti-Proliferation Agents of Cancer Cells and Anti-Inflammatory-Based *N*-Bisphosphonic Acids

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Methylenebisphosphonic acid tetraethyl ester (**1**) was added to 2-azido-**7a–e** and 2-chloroquinoline-3-chalcones **10a–e** in boiling sodium ethanolate solution to give, *via* Michael addition, tetrazolo[1,5-*a*]quinoline-**8a–d**, **13a** and 2-chloroquinoline-based bisphosphonates **11a–d**, **14a** in *E*-configuration. Further acid hydrolysis afforded the respective BP-acid analogues *E*-**9a–d**, **12a–d**, **13b**, and **14b** in excellent yields. Anti-tumor activity screening for the new BP-acids at a dose of 10 μM utilizing 44 different human tumor cell lines representing breast, ovary, prostate, lung, and CNS cancer as well as leukemia and melanoma was carried out. Eight of ten tested compounds exhibited remarkable anti-tumor activity against breast and prostate cancer, and a promising anti-tumor sensitivity toward ovarian cancer and melanoma. Conversely, there was only scattered activity against leukemia and no noticeable action of these BP-acids on CNS or lung cancer. Based on the prediction results (PASS program), anti-inflammatory activity of the new acids was also determined *in vivo*, by the acute carrageenin induced paw edema in rats. Many of these compounds showed anti-inflammatory properties at a dose of 50 mg/kg body weight.

Keywords: Anti-inflammatory activity / Anti-tumor screening / Bisphosphonates / Chalcones / Tetrazoloquinolines

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Introduction

In addition to inhibiting bone resorption, bisphosphonates (BPs) have also been shown to exhibit anti-tumor effects. *In vitro*, BPs inhibit proliferation and induce apoptosis in cultured human breast cancer cells [1–4]. There is now a much greater appreciation of the benefits of introduction of BPs in oncology, which has dramatically changed the management of patients with metastatic bone disease. Recently, BPs have been, however, proven to be the drugs of choice to: i) decrease in bone resorption in tumor bone disease; ii) decrease in hypercalcemia as a result of diminution of bone resorption that leads to a big decrease of new osteolytic lesions; and iii) decrease of fractures, which results in amelioration of pain and an improvement of the quality of life [5]. The nitrogen-containing BPs (*N*-BPs) are the latest and most potent addition to this family of compounds and have the widest use. They have high potency, are specifically targeted to the osteoclasts on bone and are used at very low doses (~10 mg clinically). Over the last decade [6], there was significant progress in elucidating the mechanism of action of *N*-BPs. For all *N*-BPs, the molecular target is the isoprenoid biosynthetic enzyme, farnesyl diphosphate synthase, in the cholesterol biosynthesis pathway. Although inhibition of this enzyme by *N*-BPs results in the suppression of sterol biosynthesis, it is actually disruption of a branch pathway, isoprenylation, that is responsible for *N*-BP pharmacological activity. Isoprenylation involves covalent linkage of the 15 or 20 carbon isoprene moiety farnesyl diphosphate or geranyl-geranyl diphosphate, respectively, to the carboxy-terminus of regulatory proteins, including the small GTPases Ras, Rac, Rho and Cdc42. The latter three, as well as numerous others, are geranyl-geranylated and play a rate-limiting role in the activity of the bone-resorbing osteoclast. This targeted osteoclast inhibition accounts for the potency of the *N*-BPs and for their ability to elicit the desired therapeutic response of suppressing bone turnover.

Our contribution to this field of BPs that started in 2002 included synthesis of a series of BPs for the treatment of bone resorption disorder [7–14]. Indeed, all BPs have significant potency on bone resorption. We have also utilized the high bone/joint specificity of this class of compounds together

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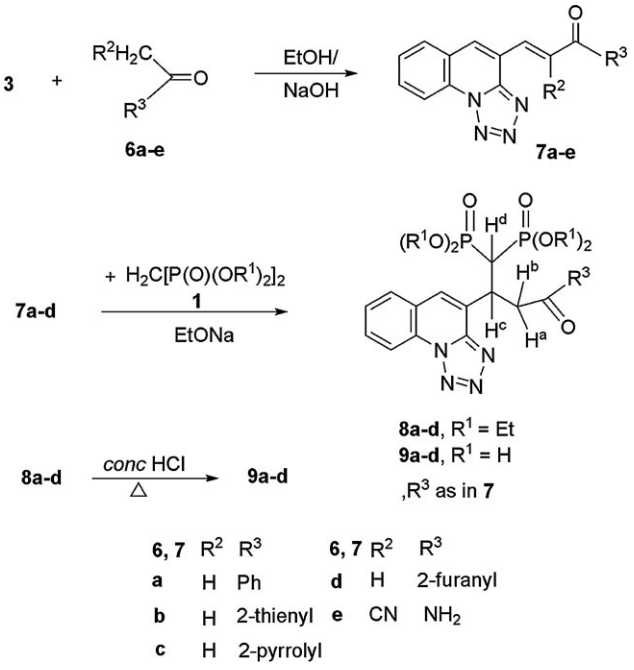
with other chemical moieties of potential anti-catabolic pharmacology in the design of *N*/*S*-bisphosphonic acids as anti-inflammatory and anti-arthritic agents [7–9, 12]. Several of the newly synthesized BP-acids have shown *in vivo* to be potent agents in rat adjuvant model of inflammation.

As a sequel, the present work aimed at investigating the synthesis of novel quinoline-based bisphosphonate derivatives utilizing accessible starting materials and facile synthetic approaches. Furthermore, the prospective potency of the products for treating cancer and inflammatory diseases was investigated. The computer-assisted molecular modeling (CAMM), PASS program, was adopted for designing, *in silico*, the structures of potentially active molecules for future synthesis. Later on, *in vitro* the anti-tumor activity screening; and the *in-vivo* anti-inflammatory properties of the synthesized bisphosphonic acids in the rat adjuvant model were also studied in terms of structure-activity relationships (SARs). The interest in the synthesis of these analogues is mainly driven by the growing use of quinolines in anti-tumor drugs [15, 16]. Several quinoline derivatives exhibit potency as inhibitors of the enzyme farnesyl protein transferase and are therefore believed to be useful as anti-cancer agents [16].

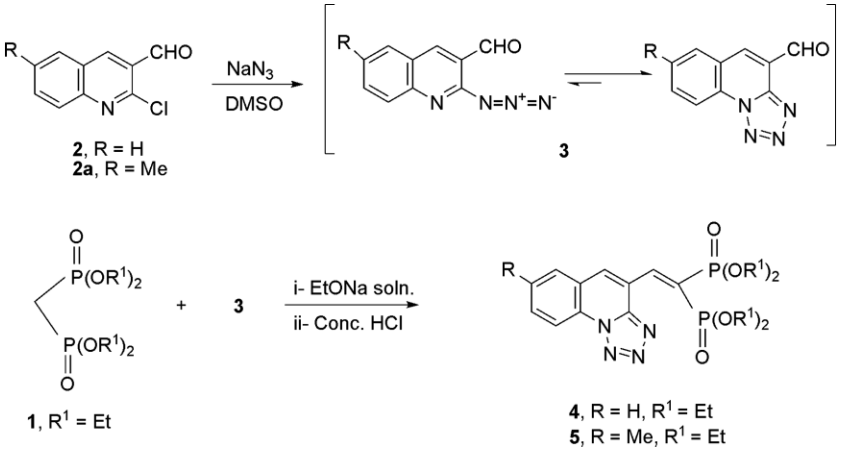


Scheme 2. Preparation of BPs **8a–d** and BP-acids **9a–d**.

Results and discussion

In a previous investigation [8] Perkin-type condensation between tetraethyl methylenebisphosphonate (**1**) and 2-azidoquinolines-3-carbaldehyde (**3**) led to tetrazoloquinoline-based ethylene-bisphosphonates **4** and **5** with anti-inflammatory properties (Scheme 1). The investigation reported notwithstanding the azide **3** reacts with phosphorus reagents mainly in the tautomeric-tetrazole form, the involvement of N_3 group in some reactions was also reported [8, 17]. The results reflected the versatility of the azido group and the phosphorus reagents as well.

In parallel, the bisphosphonate reagent **1** was applied to 3-substituted chalcones of 2-chloro- and 2-azidoquinolines, to produce the corresponding bisphosphonate derivatives (Scheme 2). Chalcones **7a–e** were smoothly obtained in more than 85% yield through the condensation of 3-formyl tetrazolo[1,5-*a*]-quinoline (**3**) with the ketone derivatives **6a–e** in ethyl alcohol containing 10% NaOH. Accoding to the ^{1}H-NMR data, we assigned the configuration (*E*) for the C–C double bond based on the vicinal proton–proton coupling $J \sim 15$ Hz. Similarly, (*E*)-isomers were also assigned for other



Scheme 1. Preparation of BPs **4** and **5**.

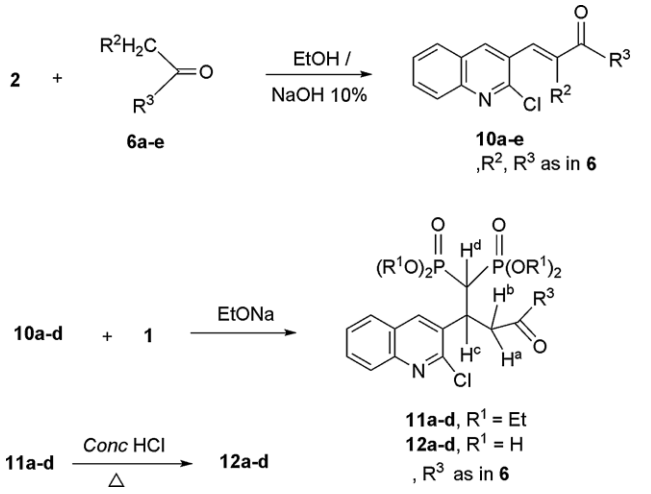
chalcones [18]. However, the selectivity in configuration may derive from the repulsion between the two moieties bonding on C–C double bond, which makes the geometric isomer (*Z*) highly unfavorable. Compounds **7a–e** were then treated with slight excess of tetraethyl methylenebisphosphonate (**1**) in ethanolate solution, and the reaction mixture was refluxed for 4–6 h (TLC) to give the corresponding tetraethyl 4-oxo-4-aryl-2-(tetrazolo[1,5-*a*]quinolin-4-yl)butane-1,1-diyl-diphosphonates **8a–d** in about 70% yield. Obviously, compounds **8a–d** were formed *via* Michael addition at the exocyclic α,β-unsaturated system. The formation of the tetrazole ring in **8** is however consistent with the data reported for the azide **3** [19].

Only one isomer of the respective bisphosphonates **8a–d** was identified and was assigned as (*E*)-isomer. The configuration of the exo heterocyclic substituent was examined by NMR analyses. The ^{1}H-NMR spectrum of **8a**, taken as an example, revealed four types of methine protons with different chemical shifts. The diastereotopic protons H^a and H^b resonated at δ 3.47 (dd, $J_{a,b} = 2.8$, $J_{a,c} = 10.5$ Hz), 3.51 (dd, $J_{b,a} = 2.8$, $J_{b,c} = 15.3$ Hz) ppm, respectively. The multiplet at δ 4.98 ppm assigned to the H^c-proton, while the P-CH^d-proton resonated at δ 4.17 (dd, $J_{d,c} = 16.5$, $^2J_{P\text{-}H} = 22.4$ Hz) ppm. This large coupling constant ($J_{H\text{-}H}$) of H^d with H^c as well as its coupling with phosphorus clearly indicates that H^d is anti-configuration to H^c. The structure of **8a–d** was also verified by careful inspection of a model in terms of the Newman projection that confirmed the staggered anti-conformation of H^c and H^d.

As structure-activity studies in several pharmaceutical laboratories have identified distinct therapeutic characteristics of bisphosphonic acid compared to bisphosphonate analogs [20, 21], acid hydrolysis of compounds **8a–d** was therefore undertaken to give the corresponding BP-acids (*E*) **9a–d**.

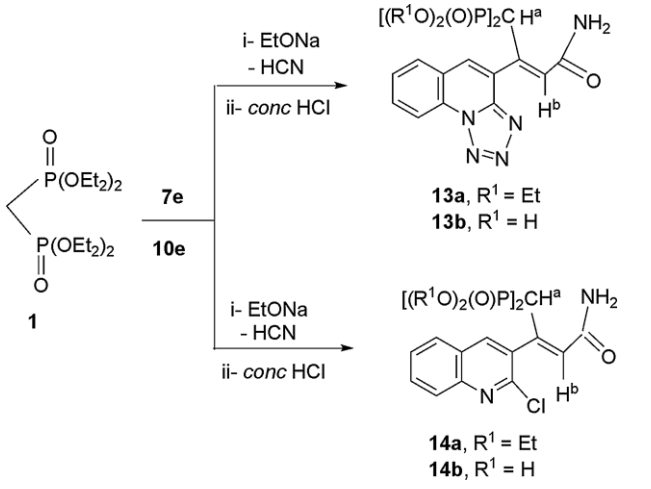
The reactions of the bisphosponate reagent **1** with the chalcones **10a–e** were then investigated, in an effort to study the possible influence on the potency of the chlorine atom compared to the tetrazole ring. The required chalcones **10a–e** were obtained in high yields (≥85%) *via* the condensation of the parent 2-chloro-3-formylquinoline (**2**) with the ketones **6a–e** in ethanol containing NaOH (10%) solution. Treatment of **10a–d** with an equivalent amount of **1** under the same previous experimental conditions afforded the bisphosponate analogues **11a–d** in reasonable yields (>70%). Acid hydrolysis (conc. HCl) of **11a–d** afforded the respective acids **12a–d** (Scheme 3). Elemental analyses and spectral data substantiated the chemical structure **11**. On the same bases and by spectral analogy with structure **8**, the configuration of the exocyclic substituents in **11** was confirmed as staggered anti-conformation.

Conversely, coupling reaction of **1** with **7e** and **10e** with concomitant loss of HCN molecule was observed to give the



Scheme 3. Preparation of BPs **11a–d** and BP-acids **12a–d**.

vinylbisphosphonates **13a** and **14a** in *E*-configuration, respectively (Scheme 4). The ^{1}H-NMR spectrum of **13a** showed among others, two doublet of doublets (δ 4.66, $^2J_{P\text{-}H} = 23.8$, $^4J_{Ha\text{-}Hb} = 2.8$ Hz, and δ 7.08, $^4J_{P\text{-}H} = 4.2$, $^4J_{Ha\text{-}Hb} = 2.8$ Hz) readily recognized as arising from the diastereotopic methine protons of the allylic moiety H^a and H^b. In the ^{1}H-NMR spectrum of **14a**, the vinyl proton of C-3 appeared as an allylic defined doublet of doublet ($^4J_{H\text{-}H} = 2.6$, $^4J_{P\text{-}H} = 3.8$ Hz, H^b) at 7.12 ppm while the CH^a-P_2 proton (dd, $^4J_{H\text{-}H} = 2.2$, $^2J_{P\text{-}H} = 23.7$ Hz) was deshielded at 4.62 ppm. The observation of the allylic coupling constants H-C-C = C-H ($^4J_{H\text{-}H} = 2.8$ & 2.6 Hz) and the large P-H coupling constants of H^a ($^2J_{P\text{-}H} = 23.8$ & 23.7 Hz) in **13a** and **14a** indicates the dominance of anti-configuration. The inspection of a model in terms of the Newman projection also



Scheme 4. Preparation of BPs **13a**, **14**, and BP-acids **13b** and **14b**.

confirmed this result. In the case of **13a** the configuration of the exocyclic double bond was examined by selective NOE experiments, which were also useful for the assignment of the ^{13}C-NMR signals. The irradiation of CH^b-proton (7.08 ppm) resulted only in a little enhancement of 1-C-triplet (56.8 ppm) and the C-2-doublet (133.3 ppm). Irradiation of the CH^a proton (4.66 ppm) produced a small NOE effect at the 3-C-doublet (122.8 ppm) and at 2-C-doublet (133.2 ppm), indicating the anti-configuration of the H^a proton to H^b proton in compounds **13** and **14**.

Biological assays

The prospective potency of our products for treating cancer and inflammatory diseases was based on the results of the prediction that had been carried out in the earlier stage. The computer-assisted molecular modeling (CAMM), PASS program, was adopted for designing, *in silico*, the structures of potentially active molecules for synthesis.

Anti-tumor activity screening

Antitumor activity for the synthesized bisphosphonic-acids **9a–d**, **12a–d**, **13b**, and **14b** at a dose of 10 μM utilizing 44 different human tumor cell lines, representing breast, ovarian, prostate, lung, and brain cancer as well as leukemia and melanoma was carried out according to the previously reported standard method [22, 23]. Substrates **7a** and **10a** were also biologically tested in a trial to reflect the effect of introducing BP moiety. The obtained results represent percentage growth of the tumor cell lines treated with compounds under investigation relative to control cell.

The pharmacological results in Table 1 showed that other than the substrates **7a** and **10a**, all synthesized compounds reflect remarkable anti-tumor activity against breast (especially MDA-MB-23/ATCC), and prostate cancer whereas a moderate effect was observed on ovarian cancer and melanoma. On the other hand, only BP-acids bearing the tetrazole moiety **9a–d** and **13b** showed sensitivity against leukemia, brain and lung cancer cells. However, we considered that cell line growth inhibition with >50% at a concentration of 10 μM usually seems to be a noticeable activity. SARs correlation for these reported observations reveal that the presence of the fused tetrazole moiety to the quinoline-ring usually associates with the enhancement in anti-tumor properties as indicated in compounds **9a–d** and **13b**.

Anti-inflammatory screening

The anti-inflammatory activity of the synthesized bisphosphonic acids (BP-acids) **9a–d**, **12a–d**, **13b**, and **14b** was determined *in vivo* by the acute carrageenin-induced paw edema standard method in rats; the substrates **7a** and **10a** were also tested to reflect the effect of introducing the BPs moiety [24, 25]. Carrageenin which is a sulfated polysaccharide, extracted from sea weed has been extensively used to induce inflammation in a number of animal species. Furthermore, the carrageenin induced rat hind paw edema is now routinely used for the assay of anti-inflammatory agents. The reproducibility and the fact that the edema depends entirely on a local inflammatory reaction devoid of antigenic properties, has made carrageenin a most widely used phlogistic agent in experimental pharmacology and a good correlation has been shown to exist between the antiphlogistic and anti-inflammatory effects of several drugs. In all experiments, carrageenin was administered into the left hind paw. Anti-inflammatory activity of the tested compounds (at a dose of 50 mg/kg body weight) was measured at successive time intervals (1, 2, and 4 h after carrageenin injection) and compared with that of the bisphosphonate drug, clodronic acid (**A**) at the same dose. The reference **A** was used as a reference standard on similar occasions [26, 27]. However, when the rats were reused, carrageenin injection was given into the right hind paw (Table 2, Figs. 1 and 2).

The recorded data in Table 2 show that 8 of 10 new synthesized BP-acids have moderate to good anti-inflammatory properties when compared with available indomethacin-drug, without toxic side-effects. The bisphosphonates **9c** and **9b** possess maximum inhibitory effect at all detected time intervals when compared to the standard group. Nevertheless, the substrates **7a** and **10a** showed poor considerable effect as anti-inflammatory agents. Other compounds, i.e., **9a**, **9d**, **12a–d**, **13b**, and **14b** have displayed good to a moderate effect on inhibitory properties, at least after 1 h.

Structure-activity correlation based on the obtained results indicates that the presence of the bisphosphonate moiety is an essential factor in developing the total pharmacological properties for these compounds. The type of the substituent attached on the 2-position or the 3-substituents on quinoline nucleus is a determining element for the potency; e.g., the presence of fused tetrazole ring highly enhanced the efficacy. Also it could be concluded that the activity of the hetero-ring of the chalcone on the biological potency for **9** or **12** increases in the succession: pyrrolyl > thienyl > furanyl > phenyl. In sequel, the BP-acids **9c** and **9b**, which are the most active products as anti-inflammatory compounds are along the line of our observations.

Toxicity of the most promising products

Toxicological studies of the most promising synthesized anti-inflammatory active compounds **9a** and **12a** were performed using LD_{50} standard method in mice in 500, 750 and 1000 mg/kg (body weight), i.e. 10–20-fold of the used anti-inflammatory effective dose. However, no toxic symptoms or mortality rates were observed after 24 h post-administrations explaining the safe behavior of the used doses.

Table 1. Concentrations resulting in growth inhibition of 50% (GI_{50}, mg/L) of *in vitro* human tumor cell lines.

Panel/cell line	Compounds										
	7a/10a	**9a**	**9b**	**9c**	**9d**	**13b**	**12a**	**12b**	**12c**	**12d**	**14b**
Breast cancer											
McF7	>46	17.58	9.80	5.21	8.40	30.64	27.6	26.3	24.23	32.4	38.55
NCl/ADR-RES	>46	6.50	9.37	6.46	20.1	33.52	25.5	20.8	22.41	22.5	38.05
MDA-MB-231/ATCC	>46	8.13	11.12	8.35	9.3	30.9	25.8	18.9	17.05	20.7	33.36
HS578T	>46	14.80	32.92	26.10	24.2	38.2	19.6	20.2	8.36	18.6	34.2
MDA-MB-435	>46	16.60	9.85	5.32	13.4	38.2	30.4	17.6	36.20	14.5	21.0
BT-549	>46	13.3	18.13	5.05	11.97	32.1	17.6	NT	NT	NT	NT
T-47D	>46	11.65	14.12	14.06	11.08	30.4	17.8	13.57	13.42	14.7	13.55
Ovarian cancer											
IGROVI	>46	28.8	11.6	7.9	26.1	32.3	23.6	20.6	16.8	30.8	>50
OVCAR-3	>46	27.41	4.4	7.4	27.2	30.3	24.2	14.5	10.7	18.9	NT
OVCAR-4	>46	10.18	10.6	3.6	10.5	14.6	24.8	24.6	4.3	9.8	>52
OVCAR-5	>46	40.04	25.6	17.8	40.6	30.6	16.3	10.3	16.5	36.6	>52
OVCAR-8	>46	40.6	28.4	14.6	36.5	21.4	13.5	11.4	13.7	28.6	>52
SK-OV-3	>46	16.5	32.3	11.5	13.6	16.7	13.6	12.6	8.4	16.5	NT
Prostate cancer											
PC-3	>46	16.3	5.6	3.82	14.2	>52	19.4	10.82	19.6	18.3	>52
DU-145	>46	11.4	16.42	2.38	11.6	>52	11.52	8.75	15.3	14.5	>52
Melanoma											
LOXIMVI	>50	36.52	34.51	8.83	13.16	33.42	29.37	24.52	24.23	32.42	>50
MALME-3M	>50	18.42	14.73	8.36	11.5	28.61	37.3	31.31	22.82	21.53	>50
MI4	>50	33.42	15.72	5.64	9.50	40.23	47.82	27.52	19.36	27.42	>50
SK-MEL-2	>50	29.5	27.33	11.43	14.23	30.35	30.73	26.47	23.71	12.62	15.34
SK-MEL-28	>50	11.22	14.35	14.69	26.21	18.36	38.92	40.35	24.64	34.06	>50
SK-MEL-5	>50	16.40	11.44	17.36	12.06	40.22	46.42	37.4	28.56	27.6	36.15
UACC-257	>50	9.62	8.64	10.46	10.54	19.23	14.33	23.2	28.76	>42	NT
UACC-62	>50	24.3	7.21	11.62	9.52	22.6	12.53	26.67	18.63	16.23	NT
Non small cell lung cancer											
A549/ATCC	>50	>50	36.6	>52	>50	>50	>52	>52	>52	>52	>52
EKVX	>50	44.8	36.4	38.2	35.9	>52	>52	>52	>52	>52	>52
HOP-62	>50	>50	>50	>52	>46	>52	>52	>52	>52	>52	>52
HOP-92	>50	>52	>52	>52	>52	>52	>52	>52	>52	>52	>52
NCI-H226	>50	>48	>52	>52	>52	>52	>52	>52	>52	>52	>52
NCI-H23	>50	39.7	25.3	15.6	40.6	>52	>52	>52	>52	>52	>52
NCI-H322M	>50	>50	>52	>52	36.9	>52	>52	>52	>52	>52	>52
NCI-H460	>50	>50	>52	>52	>52	>52	>52	>52	>52	>52	>52
NCI-H522	>50	>50	28.6	30.7	41.4	>52	>52	>52	>52	>52	>52
Leukemia											
CCRF-CEM	>50	>46	33.25	>42	30.52	38.9	40.0	42.5	>46	>46	>50.5
HL-60 (TB)	>50	36.55	30.35	33.53	17.54	40.0	>46	37.25	30.36	34.27	>50.3
K-562	>50	34.23	31.24	30.55	38.82	36.9	35.36	23.37	>50	17.39	>50.4
MOLT-4	>50	NT	32.14	40.23	NT	26.52	28.21	27.36	NT	NT	NT
RPMI-8226	>50	>46	28.25	41.00	24.50	14.25	NT	NT	31.62	23.59	>50
SR	>50	NT	24.29	28.32	NT	NT	20.35	18.20	22.36	NT	>50
CNS Cancer											
SF-268	>50	>50	>50	>52	26.72	>50	>50	>50	>47	>50	>50
SF-295	>50	>50	>50	>52	26.72	>50	>50	>50	>47	>50	>50
SF-539	>50	>50	>50	>50	26.72	>50	>50	>50	>47	>50	>50
SNB-19	>50	>50	>50	>50	26.72	>50	>50	>50	>47	23.11	>50
SNB-75	>50	>50	>50	21.36	26.72	>50	>50	>50	20.32	>50	>50
U251	>50	>50	>50	28.25	26.72	>50	>50	>50	>47	20.36	>50

Nt: not tested

Conclusion

The present investigation offered an easy approach for the transformation of available starting materials to the title BPs and the related BP-acids from chalcones and methylene-bisphosphonate. The convenience and novelty of this work is reflected in its several advantages such as mild reaction conditions, short reaction time, and easy workup

Table 2. Anti-inflammatory activity of **9a-d**, **12a-d, 13b, 14b, 7a,** and **10a** in acute carrageenin induced paw edema in rats.

Cmpd	Mean swelling[a] volume (mL) (percentage inhibition of edema)			Potency[d] (%)/4 h
	1 h	2 h	4 h	
Control	0.585 ± 0.192[c] (00.0)	0.649 ± 0.067[c] (00.0)	0.869 ± 0.058[c] (00.0)	–
A[b]	0.287 ± 0.031[c] (50.9)	0.290 ± 0.011[c] (55.3)	0.415 ± 0.022[c] (52.2)	100.0
9a	0.256 ± 0.026[c] (54.9)	0.326 ± 0.039[c] (49.7)	0.436 ± 0.048[c] (49.8)	101.2
9b	0.218 ± 0.023[c] (59.8)	0.308 ± 0.024[c] (52.5)	0.403 ± 0.030[c] (53.6)	108.9
9c	0.211 ± 0.025[c] (61.0)	0.297 ± 0.032[c] (54.2)	0.401 ± 0.033[c] (53.8)	109.3
9d	0.247 ± 0.032[c] (56.5)	0.317 ± 0.037[c] (51.17)	0.425 ± 0.045[c] (51.0)	103.6
12a	0.364 ± 0.039[c] (35.9)	0.378 ± 0.102[c] (41.7)	0.632 ± 0.094[c] (27.2)	55.3
12b	0.338 ± 0.055[c] (40.4)	0.551 ± 0.092[c] (15.1)	0.538 ± 0.048[c] (38.0)	77.2
12c	0.310 ± 0.028[c] (46.9)	0.378 ± 0.102[c] (41.7)	0.502 ± 0.012[c] (42.2)	85.7
12d	0.348 ± 0.034[c] (40.4)	0.498 ± 0.062[c] (23.2)	0.625 ± 0.052[c] (28.0)	56.9
13b	0.495 ± 0.045[c] (12.9)	0.442 ± 0.062[c] (31.8)	0.725 ± 0.052[c] (16.5)	33.5
14b	0.510 ± 0.034[c] (12.3)	0.542 ± 0.077[c] (16.5)	0.721 ± 0.102[c](17.0)	34.5
7a	0.558 ± 0.055[c] (8.8)	0.594 ± 0.077[c] (8.5)	0.822 ± 0.066[c] (5.9)	12.0
10a	0.580 ± 0.055[c] (6.8)	0.600 ± 0.077[c] (7.5)	0.827 ± 0.052[c] (4.8)	9.7

(a) Data are means of two independent determinations at least, and the deviation in absorbance values was less than 10%. (b) **A** = Clodronic acid (used as a reference standard), each value represents the mean ± of two independent experiments with 6 animals in each group. (c) (SEM: standard error of the mean) Statistical significance of results was established using the Student's test from the standard at $P < 0.05$. (d) Potency was expressed as percentage edema inhibition of the tested compounds relative to percentage edema inhibition of **A** at 4 h effect.

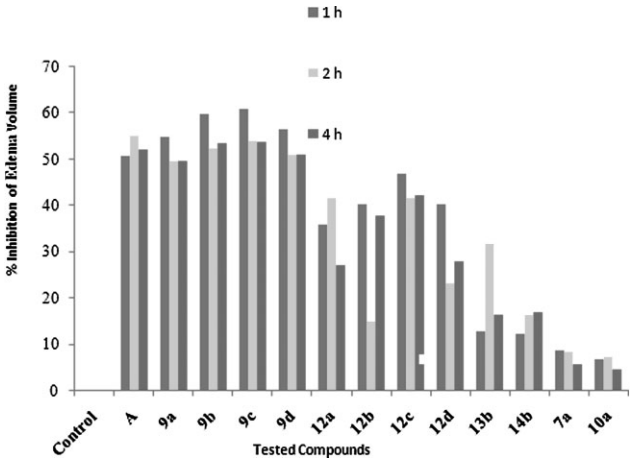


Figure 1. Percentage inhibition of edema for the tested compounds at successive intervals.

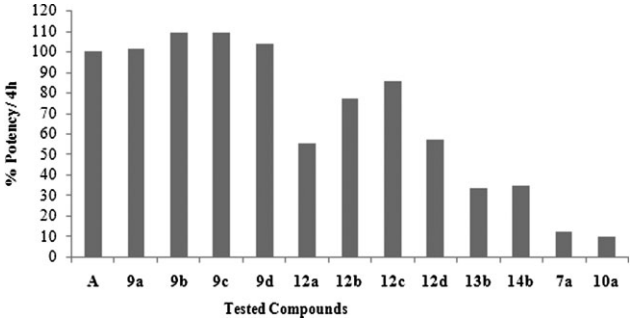


Figure 2. Anti-inflammation activity of the tested compounds relative to indomethacin.

procedure without the need for chromatographic purification.

In parallel, screening results showed that the BP moiety is an essential factor in developing the total pharmacological properties for the synthesized compounds whereas the type of the substituents attached to the quinoline nucleus determines and controls the potency. Finally, the presence of the fused tetrazole ring markedly enhanced the anti-tumor and the anti-inflammatory efficacy of the synthesized bisphosphonates.

Experimental section

General

Melting points were determined with open capillary tube on an Electrothermal (variable heater) melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer spectrophotometer model 297 using KBr disc. NMR spectra were measured with a JEOL E.C.A-500 MHz (^{13}C: 125.8 MHz, ^{1}H: 500.6 MHz, ^{31}P: 200.7 MHz) spectrometer. ^{31}P-NMR spectra were recorded with H_3PO_4 (85%) as external reference. ^{1}H and ^{13}C NMR spectra were recorded with trimethylsilane as internal standard in $CDCl_3$ or DMSO-d_6. Chemical shifts (δ) are given in ppm. The mass spectra were performed at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer provided with a data system. The purity of all new samples was verified by microchemical analysis (H/C/N) and in some cases via ^{13}C-, ^{31}P-, and ^{1}H-NMR spectroscopy. Materials and reagents were purchased from Aldrich Company. All international principles and local regulations concerning

the care and use of laboratory animals were considered during the pharmacological screening.

Synthesis

*Preparation of 2-azidoquinoline-3-carbaldehyde (**3**)*

To a solution of (26 mmol) of 2-chloroquinoline-3-carbaldehyde (4.98 g) in 15 mL of DMSO, 2 g of sodium azide (30 mmol) was added and the resulting mixture was heated to 90°C for 4 h. The precipitated product was collected, washed with acetone, dried, and crystallized to give the azide **3** as straw yellow crystals 4.12 g (80%); mp 260–262°C (EtOH) ([28]: mp 260°C).

*Preparation of quinoline-3-chalcones **7a–e** and **10a–e***

A mixture of 5 mmol of the ketone derivatives **6a–e** and 3-formyl tetrazolo[1,5-*a*]-quinoline (**3**) or 2-chloroquinoline-3-carbaldehde (**2**) in 20 mL ethanol containing 5 mL of NaOH (10% aq.) was stirred at r.t. for about 6 h. The resulting precipitate was filtered, washed with 10 mL dil. EtOH, and dried. Crystallization of the collected residue from the proper solvent afforded the corresponding known chalcones **10a–e** [29–31] in >85% yield, and the new products **7a–e**.

*(E) 1-Phenyl-3-(tetrazolo[1,5-a]quinolin-4-yl)-prop-2-en-1-one (**7a**)*

Yellow substance, mp 238–240°C (MeOH), yield 93%. IR: ν_{max}, cm^{-1}: 1657 (C=O), 1594 (C=C), 1180 (tetrazole). EI-MS: *m/z* (%) 300 (9) [M^+], 274 (100). Anal. calcd. for $C_{18}H_{12}N_4O$ (300.3): C, 71.99; H, 4.03; N, 18.66. Found: C, 72.05; H, 4.08; N, 18.61. For NMR-spectral data see Tables 3 and 4.

*(E) 3-(Tetrazolo[1,5-a]quinolin-4-yl)-1-(thiophen-2-yl)-prop-2-en-1-one (**7b**)*

Yellow crystals, mp 272–274°C (EtOH), yield 92%. IR: ν_{max}, cm^{-1}: 1660 (C=O), 1592 (olefin), 1184 (tetrazole). EI-MS: 306 (7) [M^+], 280 (100). Anal. calcd. for $C_{16}H_{10}N_4OS$ (306.3): C, 62.73; H, 3.29; N, 18.29; S, 10.47. Found: C, 62.81; H, 3.33; N, 18.35; S, 10.58. For NMR-spectral data see Tables 3 and 4.

*(E) 1-(1H-Pyrrol-2-yl)-3-(tetrazolo[1,5-a]quinolin-4-yl)-prop-2-en-1-one (**7c**)*

Orange crystals, mp 278°C (EtOH), yield 93%. IR: ν_{max}, cm^{-1}: 3434 (NH), 1659 (C=O), 1590 (C=C), 1182 (tetrazole). EI-MS: 289 (10) [M^+], 263 (100). Anal. calcd. for $C_{16}H_{11}N_5O$ (289.3): C, 66.43; H, 3.83; N, 24.21. Found: C, 66.51; H, 3.89; N, 24.18. For NMR-spectral data see Tables 3 and 4.

*(E) 1-(Furan-2-yl)-3-(tetrazolo[1,5-a]quinolin-4-yl)-prop-2-en-1-one (**7d**)*

Yellow powder, mp 243°C (EtOH), yield 95%. IR: ν_{max}, cm^{-1}: 1663 (C=O), 1595 (C=C), 1182 (tetrazole). EI-MS: *m/z* (%) 290 (13) [M^+], 264 (100). Anal. calcd. for $C_{16}H_{10}N_4O_2$ (290.3): C, 66.20; H, 3.47; N, 19.30. Found: C, 66.27; H, 3.51; N, 19.37. For NMR-spectral data see Tables 3 and 4.

*(E)-2-Cyano-3-(tetrazolo[1,5-a]quinolin-4-ylacrylamide (**7e**)*

Yellow powder, mp 277°C (MeCN), yield 92%. IR: ν_{max}, cm^{-1}: 1663 (C=O), 1595 (C=C, olefin), 1182 (tetrazole). EI-MS: *m/z* (%) = 264 (100) [M^+]. Anal. calcd. for $C_{13}H_8N_6O$ (264.2): C, 59.09; H, 3.05; N, 31.80. Found: C, 59.14; H, 3.12; N, 31.74. For NMR-spectral data see Tables 3 and 4.

Reaction of chalcones 7a–d and 10a–d with tetraethyl methylenebisphosphonate (1)

*Preparation of the bisphosphonate derivatives **8a–d**, **11a–d***

General Method

A solution 1.25 mmol of **1** in 20 mL of absolute ethanol containing 2.4 mmol sodium ethoxide was stirred at 0°C for about 0.5 h. A solution of 1.0 mmol **7a–d** (or **10a–d**) in 10 mL of absolute ethanol was then added in one portion, and the reaction was completed by heating under reflux for ≈6 h (TLC). The resulting mixture was allowed to warm to r.t. The product mixture was cooled, poured onto iced-water, and acidified with conc HCl to pH ~6, followed by extraction with AcOEt (3 × 50 mL), and the combined organic phase was dried over anhydr. Na_2SO_4. After removal of the solvent under vacuum, the resulting residue was washed several times with light petroleum (40–60°C), and crystallized from the proper solvent to give the corresponding quinolinyl-biphosphonates **8a–d**, **11a–d**, respectively.

*(E) Tetraethyl 4-oxo-4-phenyl-2-(tetrazolo[1,5-a]-quinolin-4-yl)butane-1,1-diyl-diphosphonat (**8a**)*

Dark orange crystals, mp = 210–212°C (MeCN), yield 75%. IR: ν_{max}, cm^{-1}: 1677 (C=O), 1254, 1236 (2P=O), 1184 (tetrazole), 1164, 1030 (P–O–C). EI-MS: *m/z* (%) 588 (11) [M^+], 276 (100). Anal. calcd. for $C_{27}H_{34}N_4O_7P_2$ (588.5): C, 55.10; H, 5.82; N, 9.52; P 10.53. Found: C, 55.13, H, 5.93; N, 9.56; P, 10.64. For NMR-spectral data see Tables 3 and 4.

*(E)Tetraethyl 4-oxo-2-(tetrazolo[1,5-a]quinolin-4-yl)-4-(thiophen-2-yl)butane-1,1-diyl-diphosphonate (**8b**)*

Dark orange crystals, mp 234–236°C (MeCN), yield 72%. IR: ν_{max}, cm^{-1}: 1685 (C=O), 1487 (tetrazole), 1268, 1234 (2 P=O), 1161, 1074 (2 P–O–C). EI-MS: *m/z* (%) = 594 (13) [M^+], 282 (100). Anal. calcd. for $C_{25}H_{32}N_4O_7P_2S$ (594.2): C, 50.50; H, 5.42; N, 5.42; P, 10.42; S, 5.39. Found: C, 50.56; H, 5.47; N, 5.49; P, 10.46; S, 5.51. For NMR-spectral data see Tables 3 and 4.

Table 3. Spectral data ^{31}P- and ^{1}H-NMR of BPs **7a–7e**, **8a–8d**, **11a–11d**, **13a**, **14a**, and BP-acids **9a-9d**, **12a-12d**, **13b**, and **14b**.

Cmpd. #	^{31}P-NMR δ (ppm)	^{1}H-NMR δ (ppm)
7a[a]	–	6.31, 7.20 (2d, $J_{H\text{-}H}$ = 15.7 Hz, 2 × 1H, *HC*=C*H*, exocycl.), 7.81–8.69 (m, 10 H, *H*-Ar & Ph)
7b[a]	–	6.31, 7.45 (2d, $J_{H\text{-}H}$ = 16.12 Hz, 2 × 1H, *HC*=C*H*, exocycl.), 7.76 - 8.53 (m, 8 H, *H*-het, & Ar)
7c[a]	–	6.25, 7.34 (2d, $J_{H\text{-}H}$ = 15.6 Hz, 2 × 1H, *HC*=C*H*, exocycl.), 7.63 - 8.29 (m, 8 H, *H*-het & -Ar), 11.84 (s br, 1H, N*H*)
7d[a]	–	6.29, 7.17 (2d, $J_{H\text{-}H}$ = 15.8 Hz, 2 × 1H, *HC*=C*H*, exocycl.), 7.57–8.36 (m, 8H, *H*-Ar)
7e[a]	–	7.78 (s, 1H, =C*H*, exoxcycl.), 7.82 (d, $J_{H\text{-}H}$ = 2.4 Hz, 2H, NH_2), 7. 84, 8.21, 8.36 (3m, 5H, *H*-Ar)
8a[a]	21.8, 29.2 (2d, $^{2}J_{P\text{-}P}$ = 34.6 Hz, P-C-P)	1.28, 1.33 (2dt, $J_{H\text{-}H}$ = 7.6, $^{4}J_{P\text{-}H}$ = 4.3 Hz, 2 × 6H, 4 H_3CCO), 3.47 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.5 Hz, 1H, H^aC), 3.51 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.3 Hz, 1H, H^bC), 3.94, 4.35 (2 quint, $^{3}J_{P\text{-}H}$ = 12.8 Hz, 2 × 4H, 4 H_2CO), 4.17 (dd, $J_{Hd\text{-}Hc}$ = 16.5, $^{2}J_{P\text{-}H}$ = 22.4 Hz, 1H, H^d-C-P), 4.98 (m, 1H, H^cC), 7.24–7.83, 8.13, 8.32 (m, 10 H, *H*-Ph & Ar)
8b[a]	24.6, 29.8 (2d, $^{2}J_{P\text{-}P}$ = 33.4 Hz, P-C-P)	1.23, 1.69 (2dt, $J_{H\text{-}H}$ = 7.7, $^{4}J_{P\text{-}H}$ = 5.2 Hz, 2 × 6H, 4 H_3CC.O), 3.47 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.2 Hz, 1H, H^aC), 3.72 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.6 Hz, 1H, H^bC), 3.94, 4.13 (2 quint, $^{3}J_{P\text{-}H}$ = 12.8 Hz, 2 × 4H, 4 H_2CO), 4.20 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.18 (m, 1H, H^cC), 7.24–7.88, 8.15, 8.64 (m, 8H, *H*-Het & Ar)
8c[a]	22.6, 28.8 (2d, $^{2}J_{P\text{-}P}$ = 40.2 Hz, P-C-P)	1.22, 1.52 (2dt, $J_{H\text{-}H}$ = 7.4, $^{4}J_{P\text{-}H}$ = 4.8 Hz, 2 × 6H, 4 H_3CCO), 3.38 (dd, $J_{Ha\text{-}Hb}$ = 2.6, $J_{Ha\text{-}Hc}$ = 10.4 Hz, 1H, H^aC), 3.65 (dd, $J_{Hb\text{-}Ha}$ = 2.6, $J_{Hb\text{-}Hc}$ = 14.8 Hz, 1H, H^bC), 3.96, 4.08 (2 quint, $^{3}J_{P\text{-}H}$ = 11.7 Hz, 2 × 4H, 4 H_2CO), 4.42 (dd, $J_{Hd\text{-}Hc}$ = 16.2, $^{2}J_{P\text{-}H}$ = 22.3 Hz, 1H, H^d-C-P), 5.48 (m, 1H, H^cC), 7.24–7.77, 8.23, 8.54 (m, 8H, *H*-Het & -Ar), 11.74 (s br, 1H, *H*N)
8d[a]	25.3, 28.2 (2d, $^{2}J_{P\text{-}P}$ = 36.7 Hz, P-C-P)	1.23, 1.63 (2dt, $J_{H\text{-}H}$ = 6.8, $^{4}J_{P\text{-}H}$ = 5.6 Hz, 2 × 6H, 4 H_3CCO), 3.38 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.4 Hz, 1H, H^aC), 3.46 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.6 Hz, 1H, H^bC), 3.99, 4.18 (2quint, $^{3}J_{P\text{-}H}$ = 12.6 Hz, 2 × 4H, 4 H_2COP), 4.46 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^{2}J_{P\text{-}H}$ = 22.4 Hz, 1H, H^d-C-P), 5.42 (m, 1H, H^cC), 7.24–7.78, 8.18, 8.35 (m, 8H, *H*-Het & Ar)
11a[a]	24.6, 27.8 (2d, $^{2}J_{P\text{-}P}$ = 34.6 Hz, P-C-P)	1.28, 1.33 (2dt, $J_{H\text{-}H}$ = 7.6, $^{4}J_{P\text{-}H}$ = 4.3 Hz, 2 × 6H, 4 H_3CCO), 3.43 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}H}$ = 10.4 Hz, 1H, H^aC), 3.62 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.6 Hz, 1H, H^bC), 3.94, 4.35 (2quint, $^{3}J_{P\text{-}H}$ = 12.8 Hz, 2 × 4H, 4 H_2CO), 4.38 (dd, $J_{Hd\text{-}Hc}$ = 16.0, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.22 (m, 1H, H^cC), 7.24–7.78, 8.18, 8.35 (m, 8H, *H*-Het & Ar)
11b[a]	23.5, 29.4 (2d, $^{2}J_{P\text{-}P}$ = 36.8 Hz, P-C-P)	1.22, 1.47 (2dt, $J_{H\text{-}H}$ = 6.6, $^{4}J_{P\text{-}H}$ = 6.4 Hz, 2 × 6H, 4 H_3CCO), 3.38 (dd, $J_{Ha\text{-}Hb}$ = 2.6, $J_{Ha\text{-}Hc}$ = 10.4 Hz, 1H, H^aC), 3.46 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$= 15.6 Hz, 1H, H^bC), 3.94, 4.35 (2 quint, $^{3}J_{P\text{-}H}$ = 12.8 Hz, 2 × 4H, 4 H_2CO), 4.38 (dd, $J_{Hd\text{-}Hc}$ = 16.0, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.22 (m, 1H, H^cC), 7.24–7.78, 8.18, 8.35 (m, 8H, *H*-Het & Ar)
11c[a]	20.6, 27.9 (2d, $^{2}J_{P\text{-}P}$ = 34 Hz, P-C-P)	1.21, 1.58 (2dt, $J_{H\text{-}H}$ = 7.2, $^{4}J_{P\text{-}H}$ = 5.6 Hz, 2 × 6H, 4 H_3CCO), 3.38 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.2 Hz, 1H, H^aC), 3.72 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.6 Hz, 1H, H^bC), 3.94, 4.13 (2 quint, $^{3}J_{P\text{-}H}$ = 12.8 Hz, 2 × 4H, 4 H_2CO), 4.26 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.38 (m, 1H, H^cC), 7.24–7.88, 8.15, 8.23 (m, 8H, *H*-Het & Ar), 11.76 (s, 1H, *H*N, D_2O exch.)
11d[a]	23.6, 27.5 (2d, $^{2}J_{P\text{-}P}$ = 34 Hz, P-C-P)	1.19, 1.43 (2dt, $J_{H\text{-}H}$ = 6.7, $^{4}J_{P\text{-}H}$ = 6.6 Hz, 2 × 6H, 4 H_3CCO), 3.42 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 9.8 Hz, 1H, H^aC), 3.62 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 16.6 Hz, 1H, H^bC), 3.88, 4.06 (2 quint, $^{3}J_{P\text{-}H}$ = 12.8 Hz, 2 × 4H, 4 H_2CO), 4.36 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^{2}J_{P\text{-}H}$ = 20.6 Hz, 1H, H^d-C-P), 5.28 (m, 1H, H^cC), 7.24–7.88, 8.13, 8.2 (m, 8H, *H*-Het & Ar)
13a[a]	22.7, 28.3 (2d, $^{2}J_{P\text{-}P}$ = 29 Hz, P-C-P)	1.14, 1.47 (2dt, $J_{H\text{-}H}$ = 7.3, $^{4}J_{P\text{-}H}$ = 4.4 Hz, 2 × 6H, 4 H_3CCO), 3.89–4.12 (m, 8H, 4 H_2CO), 4.66 (dd, $J_{Ha\text{-}Hb}$ = 1.6, $^{2}J_{P\text{-}Ha}$ = 23.8 Hz, 1H, H^aC), 5.08 (d, $J_{H\text{-}H}$ = 2.4 Hz, 2H, H_2N), 7.08 (dd, $^{4}J_{Hb\text{-}Ha}$ = 1.8, $^{4}J_{H\text{-}P}$ = 4.2 Hz, 1H, H^bC), 7.37, 7.77, 8.23, 8.46 (3m, 5H, *H*-Ar)
14a[a]	24.7, 28.7 (2d, $^{2}J_{P\text{-}P}$ = 32 Hz, P-C-P)	1.16, 1.43 (2dt, $J_{H\text{-}H}$ = 6.5, $^{4}J_{P\text{-}H}$ = 4.8 Hz, 2 × 6H, 4 H_3CCO), 3.88–4.02 (m, 8H, 4 H_2CO), 4.62 (dd, $J_{Ha\text{-}Hb}$ = 1.6, $^{2}J_{P\text{-}Ha}$ = 23.7 Hz, 1H, H^aC), 4.86 (d, $J_{H\text{-}H}$ = 2.8 Hz, 2H, H_2N), 7.12 (dd, $^{4}J_{Hb\text{-}Ha}$ = 1.6, $^{4}J_{H\text{-}P}$ = 3.8 Hz, 1H, H^bC), 7.43, 7.76, 8.25, 8.5 (3m, 5H, *H*-Ar)
9a[b]	21.2, 22.7	3.35 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.5 Hz, 1H, H^aC), 3.65 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.3 Hz, 1H, H^bC), 3.98 (dd, $J_{Hd\text{-}Hc}$ = 16.5, $^{2}J_{P\text{-}H}$ = 22.4 Hz, 1H, H^d-C-P), 5.12 (m, 1H, H^cC), 7.62–7.86, 8.23, 8.52 (m, 10 H, *H*-Ar & Ph)
9b[b]	20.3, 22.5	3.67 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.2 Hz, 1H, H^aC), 3.81 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$= 15.6 Hz, 1H, H^bC), 4.35 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.26 (m, 1H, H^cC), 7.24–7.88, 8.15, 8.64 (m, 8H, *H*-Het & Ar)
9c[b]	20.5, 22.7	3.58 (dd, $J_{Ha\text{-}Hb}$ = 2.6, $J_{Ha\text{-}Hc}$ = 10.4 Hz, 1H, H^aC), 3.73 (dd, $J_{Hb\text{-}Ha}$ = 2.6, $J_{Hb\text{-}Hc}$ = 14.8 Hz, 1H, H^bC), 4.62 (dd, $J_{Hd\text{-}Hc}$ = 16.2, $^{2}J_{P\text{-}H}$ = 22.3 Hz, 1H, H^d-C-P), 5.48 (m, 1H, H^cC), 7.62–7.79, 8.23, 8.56 (3m, 8H, *H*-Het & Ar)
9d[b]	20.7, 21.8	3.62 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.4 Hz, 1H, H^aC), 3.76 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.6 Hz, 1H, H^bC), 4.86 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^{2}J_{P\text{-}H}$ = 22.4 Hz, 1H, H^d-C-P), 5.42 (m, 1H, H^cC), 7.24–7.78, 8.18, 8.35 (m, 8H, *H*-Het & Ar)
12a[b]	20.2, 22.6	3.53 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.4 Hz, 1H, H^aC), 3.75 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.6 Hz, 1H, H^bC), 4.48 (dd, $J_{Hd\text{-}Hc}$ = 16.0, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.32 (m, 1H, H^cC), 7.26–7.82, 8.20, 8.35 (m, 8H, *H*-Ar & Ph)
12b[b]	20.45, 22.35	3.38 (dd, $J_{Ha\text{-}Hb}$ = 2.6, $J_{Ha\text{-}Hc}$ = 10.4 Hz, 1H, H^aC), 3.46 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 15.6 Hz, 1H, H^bC), 4.38 (dd, $J_{Hd\text{-}Hc}$ = 16.0, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.22 (m, 1H, H^cC), 7.74–7.88, 8.18, 8.35 (3m, 8H, *H*-Het & Ar)

Table 3. (*continued*)

Cmpd. #	^{31}P-NMR δ (ppm)	^{1}H-NMR δ (ppm)
12c[b]	20.5, 22.6	3.42 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 10.2 Hz, 1H, H^aC), 3.84 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$= 15.6 Hz, 1H, H^bC), 4.46 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^2J_{P\text{-}H}$ = 21.6 Hz, 1H, H^d-C-P), 5.45 (m, 1H, H^cC), 7.64–7.88, 8.15, 8.23 (m, 8H, *H*-Het & Ar)
12d[b]	21.3, 23.1	3.52 (dd, $J_{Ha\text{-}Hb}$ = 2.8, $J_{Ha\text{-}Hc}$ = 9.8 Hz, 1H, H^aC), 3.73 (dd, $J_{Hb\text{-}Ha}$ = 2.8, $J_{Hb\text{-}Hc}$ = 16.6 Hz, 1H, H^bC), 4.42 (dd, $J_{Hd\text{-}Hc}$ = 16.6, $^2J_{P\text{-}H}$ = 20.6 Hz, 1H, H^d-C-P), 5.36 (m, 1H, H^cC), 7.24–7.89, 8.14, 8.21 (m, 8H, *H*-Het & Ar)
13b[b]	20.9, 22.7	4.69 (dd, $J_{Ha\text{-}Hb}$ = 1.6, $^2J_{P\text{-}Ha}$ = 23.8 Hz, 1H, H^aC), 7.18 (dd, $^4J_{Hb\text{-}Ha}$ = 1.8, $^4J_{H\text{-}P}$ = 4.2 Hz, 1H, H^bC), 7.42–8.28, 8.48 (ms, 5H, *H*-Ar)
14b[b]	21.5, 22.8	4.72 (dd, $J_{Ha\text{-}Hb}$ = 1.6, $^2J_{P\text{-}Ha}$ = 23.7 Hz, 1H, H^aC), 7.12 (dd, $^4J_{Hb\text{-}Ha}$ = 1.6, $^4J_{H\text{-}P}$ = 3.8 Hz, 1H, H^bC), 7.43, 7.76, 8.25, 8.5 (3m, 5H, *H*-Ar)

*Solvents of NMR: [a] DMSO-d_6; [b] D_2O.

*(E) Tetraethyl 4-oxo-4-(1H-pyrrol-2-yl)-2-(tetrazolo-[1,5-a]quinolin-4-yl)butane-1,1-diyl-diphosphonate (**8c**)*

Dark orange crystals, mp 260–262°C (MeCN), yield 72%. IR: ν_{max}, cm^{-1}: 3423 (NH), 1676 (C=O), 1561 (C=N), 1263, 1237 (2 P=O), 1164, 1065 (2 P–O–C). EI-MS: *m/z* (%) = 577 (13) [M$^+$], 265 (100). Anal. calcd. for $C_{25}H_{33}N_5O_7P_2$ (577.5): C, 51.99; H, 5.76; N, 12.13; P, 10.73. Found: C, 52.07; H, 5.82; N, 12.19; P, 10.81. For NMR-spectral data see Tables 3 and 4.

*(E) Tetraethyl 4-(furan-2-yl)-4-oxo-2-(tetrazolo-[1,5-a]quinolin-4-yl)butane-1,1-diyl-diphosphonate (**8d**)*

Dark orange crystals, mp 250–252°C (MeCN), yield 70%. IR: ν_{max}, cm^{-1}: 1679 (C=O), 1478 (tetrazole), 1257, 1231 (2 P=O), 1154, 1043 (2 P–O–C). EI-MS: *m/z* (%) = 578 (13) [M$^+$], 266 (100). Anal. calcd. for $C_{25}H_{32}N_4O_8P_2$ (578.5): C, 51.91; H, 5.58; N, 9.68; P, 10.71. Found: C, 51.95; H, 5.64; N, 9.73; P, 10.79. For NMR-spectral data see Tables 3 and 4.

*(E) Tetraethyl 2-(2-chloroquinolin-3-yl)-4-oxo-4-phenylbutane-1,1-diyl-diphosphonate (**11a**)*

Yellow crystals, mp 150–152°C (EtOH), yield 74%. IR: ν_{max}, cm^{-1}: 1681 (C=O), 1567 (C=N), 1254, 1231 (2 P=O), 1164, 1030 (P–O–C). EI-MS: *m/z* (%) = 581 (13) [M$^+$], 582 (2.5) [M$^+$+1], 583 (4.4) [M$^+$+2], 584 (0.8) [M$^+$+3], 295 (100), 297 (32). Anal. calcd. for $C_{27}H_{34}ClNO_7P_2$ (581.9): C, 55.72; H, 5.89; Cl, 6.09; N, 2.41; P, 10.64. Found: C, 55.78; H, 5.96;

Table 4. ^{13}C-NMR Spectral Data for BPs **7a-7e**, **8a-8d**, **11a-11d**, **13a**, and **14a**.

Cmpd. #	^{13}C-NMR [DMSO-d_6] δ (ppm)
7a	192.2 (C=O), 140.1, 138.2, 137.2, 134.6, 131, 130.3, 129.0, 128.2, 119.3, 114.6 (*C*-Ar & Ph), 128.4, 120.6 (*C*= *C*, exocycl.)
7b	168.7 (C=O), 140.4, 138.6, 137.2, 134.8, 130.7, 129.6, 128.2, 119.3, 114.2 (*C*-Het, Ar), 127.4, 120.3 (*C*=*C*, exocycl.)
7c	168.9 (C=O), 140.2, 138.4, 137.2, 134.6, 131.7, 129.06, 128.7, 119.3, 114.3 (*C*-Het, Ar), 128.1, 120.8 (*C*=*C*, exocycl.)
7d	168.9 (C=O), 141.4, 138.5, 137.4, 134.8, 130.9, 129.1, 128.3, 119.6, 114.3 (*C*-Het & Ar), 127.8, 121.2 (*C*=*C*, exocycl.)
7e	163.2 (C=O), 131.8 (H*C*=C, exocycl.), 140.2, 139.7, 134.8, 130.9, 129.1, 124.8 (*C*-Het, Ar), 117.6 (*C*N), 101.6 (C-*C*N)
8a	196.6 (C=O), 141.2, 138.5, 135.1, 134.8, 134.6, 133.3, 129.6, 127.8, 123.8, 113.8 (*C*-Ar, Ph), 60.7 (t, $^2J_{C\text{-}P}$ = 9.7 Hz, 4 CH_2O), 46.8 (t, $^2J_{P\text{-}C}$ = 14.2 Hz, *C*H), 43.2 (t, $^1J_{P\text{-}C}$ = 144.3 Hz, *C*-P_2), 42.8 (t, $^3J_{P\text{-}C}$ = 8.2 Hz, *C*H_2), 15.4 (t, $^3J_{P\text{-}C}$ = 8.8 Hz, 4 *C*H_3)
8b	191.6 (C=O), 145.3, 143.5, 136.4, 134.6, 132.3, 129.6, 126.7, 123.5, 114.3 (*C*-Het & Ar), 60.8 (t, $^2J_{C\text{-}P}$ = 10.3 Hz, 4 CH_2O), 48.4 (t, $^2J_{P\text{-}C}$ = 15.4 Hz, *C*H^c), 42.2 (t, $^1J_{P\text{-}C}$ = 148.4 Hz, *C*-P_2), 41.6 (t, $^3J_{P\text{-}C}$ = 8.2 Hz, *C*H_2), 15.6 (t, $^3J_{P\text{-}C}$ = 7.2 Hz, 4 *C*H_3)
8c	188.2 (C=O), 145.4, 137.5, 134.6, 132.6, 131.3, 127.8, 125.4, 121.5, 114.2 (*C*-Ar, Ph), 61.4 (t, $^2J_{C\text{-}P}$ = 9.7 Hz, 4 CH_2O), 49.6 (t, $^2J_{P\text{-}C}$ = 18.4 Hz, *C*H^c), 43.4 (t, $^1J_{P\text{-}C}$ = 142.4 Hz, *C*-P_2), 41.8 (t, $^3J_{P\text{-}C}$ = 8.2 Hz, *C*H_2), 15.3 (t, $^3J_{P\text{-}C}$ = 7.2 Hz, 4 *C*H_3)
8d	190.6 (C=O), 152.6, 148.2, 136.1, 134.8, 132.6, 126.8, 125.3, 123.8, 114.6 (*C*-Ar, Ph), 61.8 (t, $^2J_{C\text{-}P}$ = 8.8 Hz, 4 CH_2O), 45.6 (t, $^2J_{P\text{-}C}$ = 24.2 Hz, *C*H^c), 42.6 (t, $^1J_{P\text{-}C}$ = 140.4 Hz, *C*-P_2), 41.2 (t, $^3J_{P\text{-}C}$ = 6.2 Hz, *C*H_2), 15.4 (t, $^3J_{P\text{-}C}$ = 7.2 Hz, 4 *C*H_3)
11a	199.1 (C=O), 145.5, 142.4, 135.4, 133.1, 130.3, 128.3, 127.7, 124.4, 114.4 (*C*-Ar, Ph), 62.1 (t, $^2J_{C\text{-}P}$ = 10.7 Hz, 4 CH_2O), 45.2 (t, $^2J_{P\text{-}C}$ = 16.5 Hz, *C*H), 43.7 (t, $^1J_{P\text{-}C}$ = 168.3 Hz, *C*-P_2), 41.8 (t, $^3J_{P\text{-}C}$ = 8.8 Hz, *C*H_2), 15.4 (t, $^3J_{P\text{-}C}$ = 7.5 Hz, 4 *C*H_3).
11b	193.6 (C=O), 145.5, 143.1, 133.4, 132.3, 129.2, 128.3, 127.4, 127.2, 123.8, 119.8 (*C*-Ar, Ph), 61.2 (t, $^2J_{C\text{-}P}$ = 8.7 Hz, 4 CH_2O), 47.2 (t, $^2J_{P\text{-}C}$ = 14.6 Hz, *C*H), 44.2 (t, $^1J_{P\text{-}C}$ =158.4 Hz, *C*-P_2), 42.8 (t, $^3J_{P\text{-}C}$ = 8.2 Hz, *C*H_2^{c}), 16.2 (d, $^3J_{P\text{-}C}$ = 7.2 Hz, 4 *C*H_3)
11c	190.6 (C=O), 146.8, 146.2, 135.4, 133.4, 127.6, 126.4, 125.7, 124,7, 123.8, 119.8 (*C*-Het & Ar), 61.8 (t, $^2J_{C\text{-}P}$ = 9.2 Hz, 4 *C*H_2O), 48.2 (t, $^2J_{P\text{-}C}$ = 15.6 Hz, *C*H), 45.6 (t, $^1J_{P\text{-}C}$ =154.5 Hz, *C*-P_2), 44.6 (t, $^3J_{P\text{-}C}$ = 10.7 Hz, *C*H_2), 15.8 (t, $^3J_{P\text{-}C}$ = 7.8 Hz, 4 *C*H_3)
11d	192.8 (C=O), 152.5, 146.1, 135.0, 130.4, 127.4, 126.7, 125.9, 124,7, 123.8, 114.8 (*C*-Ar, Ph), 60.7 (t, $^2J_{C\text{-}P}$ = 9.7 Hz, 4 *C*H_2O), 46.8 (t, $^2J_{P\text{-}C}$ = 18.4 Hz, *C*H), 43.2 (t, $^1J_{P\text{-}C}$ =156.6 Hz, *C*-P_2), 42.8 (t, $^3J_{P\text{-}C}$ = 10.3 Hz, *C*H_2^{c}), 15.8 (t, $^3J_{P\text{-}C}$ = 7.8 Hz, 4 *C*H_3)
13a	169.6 (C=O), 133.3 (t, $^2J_{P\text{-}C}$ = 33.7 Hz, *C*=CH^b), 122.8 (t, $^3J_{P\text{-}C}$ = 16.4 Hz, =*C*H^b), 146.4, 144.7, 136.7, 130.2, 129.4, 127.4, 119.6 (*C*-Ar), 60.8 (t, $^2J_{C\text{-}P}$ = 9.6 Hz, 4 *C*H_2O), 56.8 (t, $^1J_{P\text{-}C}$ = 196.6 Hz, *C*-P_2), 16.2 (t, $^3J_{P\text{-}C}$ = 7.8 Hz, 4 *C*H_3)
14a	169.4 (C=O), 133.7 (t, $^2J_{P\text{-}C}$ = 34.7 Hz, *C*=CH^b), 122.4 (t, $^3J_{P\text{-}C}$ = 14.8 Hz, =*C*H^b), 148.4, 146.1, 134.6, 130.4, 129.4, 127.4, (*C*-Ar), 61.6 (t, $^2J_{C\text{-}P}$ = 10.4 Hz, 4 *C*H_2O), 56.5 (t, $^1J_{P\text{-}C}$ = 196.6 Hz, *C*-P_2), 16.8 (t, $^3J_{P\text{-}C}$ = 7.8 Hz, 4 *C*H_3)

Cl, 6.19; N, 2.37; P, 10.77. For NMR-spectral data see Tables 3 and 4.

*(E) Tetraethyl 2-(2-chloroquinolin-3-yl)-4-oxo-4-(thiophen-2-yl)butane-1,1-diyl-diphosphonate (**11b**)*

Yellow crystals, mp 163–165°C (EtOH), yield 72%. IR: ν_{max}, cm^{-1}: 1675 (C=O), 1552 (C=N), 1259, 1236 (2 P=O), 1150, 1039 (2 P–O–C). EI-MS: *m/z* (%) = 587 (11) [M^+], 588 (2.1) [M^++1], 589 (3.7) [M^++2], 590 (1.0) [M^++3], 301(100), 303 (36). Anal. calcd. for $C_{25}H_{32}ClNO_7P_2S$ (587.9): C, 51.07; H, 5.49; Cl, 6.03; N, 2.38; P, 10.54; S, 5.45. Found: C, 51.15; H, 5.54; Cl, 6.11; N, 2.43; P, 10.59; S, 5.52. For NMR-spectral data see Tables 3 and 4.

*(E) Tetraethyl 2-(2-chloroquinolin-3-yl)-4-oxo-4-(1H-pyrrol-2-yl)butane-1,1-diyl-diphosphonate (**11c**)*

Yellow crystals, mp 145–147°C (MeOH), yield 72%. IR: ν_{max}, cm^{-1}: 3430 (NH), 1678 (C=O), 1562 (C=N), 1258, 1244 (2 P=O), 1154, 1035 (2 P–O–C). EI-MS: *m/z* (%) = 570 (19) [M^+], 571(3.6) [M^++1], 572 (6.5) [M^++2], 573 (6.5) [M^++3], 284 (100), 286 (32). Anal. calcd. for $C_{25}H_{33}ClN_2O_7P_2$ (570.9): C, 52.59; H, 5.83; Cl, 6.21; N, 4.91; P, 10.85. Found: C, 52.63; H 5.85; Cl, 6.25; N, 4.97; P, 10.92. For NMR-spectral data see Tables 3 and 4.

*(E) Tetraethyl 2-(2-chloroquinolin-3-yl)-4-(furan-2-yl)-4-oxobutane-1,1-diyl-diphosphonate(**11d**)*

Yellow crystals, mp 168–170°C (MeOH), yield 73%. IR: ν_{max}, cm^{-1}: 1679 (C=O), 1565 (C=N), 1256, 1232 (2 P=O), 1159, 1033 (2 P–O–C). EI-MS: *m/z* (%) 571 (15) [M^+], 572 (2.9) [M^++1], 573 (5.2) [M^++2], 574 (1.0) [M^++3], 285 (100), 287 (32). Anal. calcd. for $C_{25}H_{32}ClNO_8P_2$ (571.9): C, 52.50; H, 5.64; Cl, 6.20; N, 2.45; P, 10.83. Found: C, 52.54; H, 5.71; Cl, 6.24; N, 2.51; P, 10.87. For NMR-spectral data see Tables 3 and 4.

Reaction of chalcones 7e and 10e with tetraethyl methylenebisphosphonate (1)

*Preparation of the bisphosphonate derivatives **13a, 14a***

General Method

A solution 1.25 mmol of **1** in 20 mL of absolute ethanol containing 2.4 mmol sodium ethoxide was stirred at 0–(−5) °C for about 0.5 h. A solution of 1.0 mmol of (*E*-3-(tetrazolo[1,5-*a*]quinolin-3-yl)-2-cyanoacrylamide (**7e**) (or its 2-chloroquinolin analog **10e**) in 10 mL of absolute ethanol was then added in one portion, and the reaction was completed by heating under reflux for ≈6 h (TLC). After the usual work up and crystallization of the collected residue from the proper solvent, biphosphonates **13a** and **14a** were obtained, respectively.

*(E)-Tetraethyl 4-amino-4-oxo-2-(tetrazolo[1,5-a]quinolin-4-yl)-but-2-ene-1,1-diyl-diphosphonate (**13a**)*

Orange crystals, mp 234–260°C (MeCN), yield 75%. IR: ν_{max}, cm^{-1}: 1686 (C=O), 1616 (C=C), 1480 (tetrazole), 1258, 1244 (2 P=O), 1182, 1045 (2 P–O–C). EI-MS: *m/z* (%): 525 (18) [M^+], 509 (34) [M^+−16, NH_2], 479 (22), [M^+−44 (C(O)+NH_2], 205 (100) [M^+−(44+274) [P(O)$(OEt)_2]_2$]. Anal. calcd. for $C_{21}H_{29}C_5O_7P_2$ (525.4): C, 48.00; H, 5.56; N, 13.33; P, 11.79. Found: C, 48.12; H, 5.63; N, 13.41; P, 11.85. For NMR-spectral data see Tables 3 and 4.

*(E)-Tetraethyl 4-amino-2-(2-chloroquinolin-3-yl)-4-oxobut-2-ene-1,1-diyl-diphosphonate (**14a**)*

Yellow crystals, mp 258–260°C (MeCN), yield 71%. IR: ν_{max}, cm^{-1}: 1679 (C=O), 1618 (C=C), 1262, 1238 (2 P=O), 1159, 1033 (2 P–O–C). EI-MS: *m/z* (%): 518 (18) [M^+], 519 (3.5) [M^++1], 520 (6.3) [M^++2], 521 (1.4) [M^++3], 502 (34) [M^+−16, NH_2], 474 (22), [M^+−44 (C(O)+NH_2], 200 (100) [M^+−44+274 [P(O)$(OEt)_2]_2$]. Anal. calcd. for $C_{21}H_{29}ClN_2O_7P_2$ (518.8): C, 48.61; H, 5.63; Cl, 6.83; N, 5.40; P, 11.94. Found: C, 48.72; H, 5.74; Cl, 6.76; N, 5.49; P, 12.04. For NMR-spectral data see Tables 3 and 4.

Acid hydrolysis of synthesized BPs

*Preparation of the bisphosphonic acids **9a–d, 12a–d, 13b, 14b***

General procedure

0.3 g of bisphosphonate **8a–d**, **11a–d**, **13a** or **14a** was dissolved in 20 mL conc. HCl, and the mixture was heated under reflux for ∼12 h (TLC). After concentrating *in vacuo*, the crude material was diluted with AcOEt and water and then stirred for 30 min. The layers were separated, and the aqueous layer was evaporation to dryness. The precipitate was collected and dried to give the respective bisphosphonic acids **9a–d**, **12a–d**, **13b** or **14b**, respectively.

*(E) 4-Oxo-4-phenyl-2-(tetrazolo[1,5-a]quinolin-4-yl)butane-1,1-diyl-diphosphonic acid (**9a**)*

Dark brown substance, mp 300°C, yield 86%. IR: ν_{max}, cm^{-1}: 3450–3330 (P–OH), 1679 (C=O), 1256, 1235 (P=O), 1186 (tetrazole). EI-MS: *m/z* (%) = 472 (27) [M^+−4]. Anal. calcd. for $C_{19}H_{18}N_4O_7P_2$ (476.32): C, 47.91; H, 3.81; N, 11.76; P, 13.01. Found: C, 47.94; H, 3.87; N, 11.83; P, 13.08. For NMR-spectral data see Tables 3 and 4.

*(E) 4-Oxo-2-(tetrazolo[1,5-a]quinolin-4-yl)-4-(thiophen-2-yl)butane-1,1-diyl-diphosphonic acid (**9b**)*

Brown substance, mp > 300°C, yield 91%. IR: ν_{max}, cm^{-1}: 3325–3248 (P–OH), 1681 (C=O), 1254, 1238 (2 P=O), 1188 (tetrazole). EI-MS: *m/z* (%) = 478 (13) [M^+−4]. Anal. calcd.

for $C_{17}H_{16}N_4O_7P_2S$ (482.34): C, 42.33; H, 3.34, N, 11.62; P, 12.84; S, 6.57. Found: C, 42.37; H, 3.42; N, 11.59; P, 12.92; S 6.65. For NMR-spectral data see Tables 3 and 4.

*(E) 4-Oxo-4-(1H-pyrrol-2-yl)-2-(tetrazolo[1,5-a]quinolin-4-yl)-butane-1,1-diyl-diphosphonic acid (**9c**)*

Dark brown substance; mp > 300°C (H_2O/acetone); yield 72%. IR: ν_{max}, cm^{-1}: 3455–3328 (P–OH), 1679 (C=O), 1253, 1235 (P=O), 1186 (tetrazole). EI-MS: *m/z* (%) = 461 (9) [M^+-4]. Anal. calcd. for $C_{17}H_{17}N_5O_7P_2$ (465.29): C, 43.88; H, 3.68; N, 15.05; P 13.31; Found: C, 43.94; H, 3.75; N, 15.09; P, 13.9. For NMR-spectral data see Tables 3 and 4.

*(E) 4-(Furan-2-yl)-4-oxo-2-(tetrazolo[1,5-a]quinolin-4-yl)-butane-1,1-diyl-diphosphonic acid (**9d**)*

Dark brown crystals, mp > 300°C (H_2O/acetone), yield 86%. IR: ν_{max}, cm^{-1}: 3435–3353 (P–OH), 1683 (C=O), 1256, 1233 (P=O), 1186 (tetrazole). EI-MS: *m/z* (%) = 462 (13) [M^+-4]. Anal. calcd. for $C_{17}H_{16}N_4O_8P_2$ (466.28): C, 43.79; H, 3.46; N, 12.02; P, 13.29. Found: C, 43.86; H, 3.49; N, 12.06; P, 13.34. For NMR-spectral data see Tables 3 and 4.

*(E) 2-(2-Chloroquinolin-3-yl)-4-oxo-4-phenylbutane-1,1-diyl-diphosphonic acid (**12a**)*

Brown crystals; mp 220°C (MeOH), yield 82%. IR: ν_{max}, cm^{-1}: 3410–3331 (P–OH), 1679 (C=O), 1607 (C=N), 1254, 1235 (2 P=O). EI-MS: *m/z* (%) = 465 (30) [M^+-4], 466 (5.8), 467 (10.2), 468 (<5). Anal. calcd. for $C_{19}H_{18}ClNO_7P_2$ (469.75): C, 48.58; H, 3.86; Cl, 7.55; N, 2.98; P, 13.19. Found: C, 48.62; H, 3.89; Cl, 7.65; N, 2.95; P, 13.27. For NMR-spectral data see Tables 3 and 4.

*(E) 2-(2-Chloroquinolin-3-yl)-4-oxo-4-(thiophen-2-yl)-butane-1,1-diyl-diphosphonic acid (**12b**)*

Brown powder; mp 231°C (MeCN); yield 78%. IR: ν_{max}, cm^{-1}: 3433–3325 (OH), 1688 (C=O), 1598 (C=N), 1258, 1233 (2 P=O). EI-MS: *m/z* (%) = 471 (27) [M^+-4], 472 (15), 473 (9.0), 574 (<5). Anal. calcd. for $C_{17}H_{16}ClNO_7P_2S$ (475.78): C, 42.92; H, 3.39; Cl, 7.45; N, 2.94; P, 13.02; S, 6.81. Found: C, 42.95; H, 3.43; Cl, 7.51; N, 3.11; P, 13.11; S, 6.74. For NMR-spectral data see Tables 3 and 4.

*(E) 2-(2-Chloroquinolin-3-yl)-4-oxo-4-(1H-pyrrol-2-yl)-butane-1,1-diyl-diphosphonic acid (**12c**)*

Brown powder; mp 225°C (EtOH); yield 84%. IR: ν_{max}, cm^{-1}: 3428–3648 (P–OH), 1679 (C=O), 1610 (C=N), 1254, 1236 (P=O). EI-MS: *m/z* (%) = 454 (17) [M^+-4], 455 (3.3), 456 (5.7), 457 (<5), Anal. calcd. for $C_{17}H_{17}ClN_2O_7P_2$ (458.73): C, 44.51; H, 3.74; Cl 7.73; N, 6.11; P, 13.50. Found: C, 44.56; H, 3.72; Cl, 7.77; N, 6.17; P, 13.57. For NMR-spectral data see Tables 3 and 4.

*(E) 2-(2-Chloroquinolin-3-yl)-4-(furan-2-yl)-4-oxobutane-1,1-diyl-diphosphonic acid (**12d**)*

Dark brown substance; mp > 300°C, yield 83%. IR: ν_{max}, cm^{-1}: 3425–3360 (P–OH), 1682 (C=O), 1608 (C=N), 1255, 1235 (2 P=O). EI-MS: *m/z* (%) = 455 (20) [M^+-4], 456 (4.0), 457 (6.5), 458 (<5). Anal. calcd. for $C_{17}H_{16}ClNO_8P_2$ (459.71): C, 44.42; H, 3.51; Cl, 7.71; N, 3.05; P, 13.48. Found: C, 44.52; H, 3.61; Cl, 7.67; N, 3.13; P, 13.53. For NMR-spectral data see Tables 3 and 4.

*(E)-4-Amino-4-oxo-2-(tetrazolo[1,5-a]quinolin-4-yl)but-2-ene-1,1-diyl-diphosphonic acid (**13b**)*

Yellow powder; mp > 300°C; yield 83%. IR: ν_{max}, cm^{-1}: 3445–3333 (OH, NH_2), 1679 (C=O), 1610 (C=N), 1254, 1235 (P=O), 1186 (tetrazole). EI-MS: *m/z* (%) = 409 (22) [M^+-4]. Anal. calcd. for $C_{13}H_{13}N_5O_7P_2$ (413.22): C, 37.79; H, 3.17; N, 16.95; P, 14.99. Found: C, 37.84; H, 3.23; N, 17.04; P, 15.07. For NMR-spectral data see Tables 3 and 4.

*(E)-4-Amino-2-(2-chloroquinolin-3-yl)-4-oxobut-2-ene-1,1-diyl-diphosphonic acid (**14b**)*

Brown powder; mp > 300°C; yield 83%. IR: ν_{max}, cm^{-1}: 3450–3328 (OH, NH_2), 1680 (C=O), 1606 (C=N), 1251, 1238 (2 P=O). EI-MS: *m/z* (%) = 402 (16) [M^+-4], 403 (3), 404 (5.5), 405 (<5). Anal. calcd. for $C_{13}H_{13}ClN_2O_7P_2$ (406.65): C, 38.40; H, 3.22; Cl, 8.72; N, 6.89; P, 15.23. Found: C, 38.53; H, 3.31; Cl, 8.79; N, 6.95; P, 15.29. For NMR-spectral data see Tables 3 and 4.

Anti-tumor activity screening

Anti-tumor potency of the new BP-acids **9a–d**, **12a–d**, **13b** and **14b** in addition to the substrates **7a** and **10a** was tested at a dose of 10 μM utilizing 44 different human tumor cell lines, representing breast, ovarian, prostate lung and brain cancer as well as leukemia and melanoma using adriamycin as a reference standard according to the reported methods [22, 23]. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 μL glutamine. For a typical screening experiment, cells are inoculated in 96-well-microtiter plates in 100 μL at plating densities ranging from 5000 to 40 000 cells/well depending on the doubling time individual cell lines. After cell inoculation, the microtiter plates are incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental tested compounds. After 24 h, two plates of each cell lines are fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of the tested compound addition (time zero, T_z). Experimental tested compounds are solubilized in dimethyl sulfoxide at 400-fold the desired

final maximum test concentration and stored frozen prior to use. At the time of the tested compound addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicine. Additional four, 10-fold or 1/2 log serial dilutions are made to provide a total of five tested compound concentrations (10^{-4} to 10^{-8} M concentrations) plus control. Aliquots of 100 μL of these different tested compound dilutions are added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final concentrations.

Following the tested compound addition, the plates are incubated for an additional 48 h at 37°C, 5% CO_2, 95% air and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant is discarded, and the plates are washed four times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing four times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 μM trizma base, and the absorbance is read on an automated plate at a wavelength of 515 nm. For cells suspension, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements (T_z), control growth (C) and test growth in the presence of the tested compound at the five concentration levels (T_i), the percentage growth is calculated at each of the tested compound concentration levels.

Percentage growth inhibition is calculated as:

$$[(T_i - T_z)/[(C - T_z) \times 100 \quad \text{with } T_i \geq T_z$$

$$[(T_i - T_z)/T_z)] \times 100 \quad \text{with } T_i < T_z$$

Growth inhibition of 50% (GI_{50}) is calculated from:

$$[(T_i - T_z)/[(C - Tz) \times 100 = 50$$

which is the tested compound concentration resulting in a 50% reduction in the net protein increase (as measured in SRB staining) in control cells during the compound incubation.

Table 1 represents the observed percentage growth of each cell line treated with a certain tested compound relative to control cell line experiments.

Anti-inflammatory activity experiments *in vivo*; carrageenin induced edema

Animals

All experiments have been conducted on adult Wistar strain albino rats of either sex, weighing between 150–200 g. Animals were kept in colony cages under identical housing conditions at an ambient temperature of 25 ± 2°C and 45–55% relative humidity with 12 h light-dark cycle in the departmental animal room and fed on standard diet. Animals were acclimatized for a week before use.

Experimental model of inflammation

Carrageenin induced paw edema was used throughout the investigation: Standard drug clodronic acid (**A**), substrates (**7a**, **10b**), synthesized BP-acids (**9a–d**, **12a–d**, **13b**, **14b**) and solutions:

(a) Powder of the pure carrageenin was used and fresh suspension was prepared in distilled water to make 1% carrageenin solution.
(b) Fresh tested compound solutions were made by adding 10 mg of the compound in 500 mg carboxymethyl cellulose (CMC) and 50 mL distilled water.
(c) Experimental inflammation: It was produced by the following method: carrageenin induced paw edema in rats: 1% carrageenin suspension was prepared as a homogeneous solution in distilled water. A volume of 0.1 mL of carrageenin solution was injected through a 26 gauge needle into the plantar surface of the left hind paw below the plantar aponeurosis. The volume of the paw was measured before and at different intervals for 4 h after injection of carrageenin. The difference in the paw volume before and after administration of the phlogistic agent was taken as the measure of pedal edema. The compounds whose effects have been studied on this particular model were administered as per schedule.
(d) Measurement of paw volume: The volume of hind paw of the rats up to the ankle joint was measured by plathysmographically by the mercury displacement method. The ankle joint of the rats was marked with a skin marking pencil and the paw was dipped in the mercury, so that the mark on the paw coincides with a prefixed line kept constant on the syringe. The level of the mercury was every time brought to the level of this line by adjusting the height of the displaced mercury. The difference in the paw volume before and after injection of the phlogistic agents was taken as a measure of pedal edema. The change in paw volume was expressed in ''mL'' of mercury displaced.

The anti-inflammatory activity was expressed as percentage inhibition of edema volume in the treated animals in

comparison with the control group.

$$\%\ \text{Inhibition of edema} = \frac{(V_c - V_t)}{V_c} \times 100$$

where V_c and V_t are the volumes of edema for the control and tested substance-treated animal groups, respectively, while potency of the tested compounds was calculated regarding clodronic acid, reference standard, treated group according to the following equation:

$$\%\ \text{Potency} = \frac{\%\text{Edema inhibition of tested compound treated groups}}{\%\text{Edema inhibition of clodronic acid treated groups}} \times 100$$

Toxicity evaluation

The LD_{50} determination of the most promising synthesized anti-inflammatory active agents (**9c** and **12c**) was determined by the standard known LD_{50} method in mice. Albino mice weighing 20–25 g were divided into 6 groups of 8 mice each. Administrations of the tested compounds (**9c** and **12c**) dissolved in the same vehicle solution in 500, 750, and 1000 mg/kg (body weight) were given intraperitoneally. The control groups were given in buffer solution only. The toxic symptoms, mortality rates and postmortem findings in each group were recorded 24 h post-administration.

LD_{50} of the tested compounds were calculated according to the following formula:

$$LD_{50} = D_m - \sum(z \times d)/n$$

where D_m = the largest dose which kills all animals, z = mean of dead animals between two successive groups, d = the constant factor between two successive doses, n = number of animals in each group, Σ = the sum of $(z \times d)$.

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