European Journal of Medicinal Chemistry 51 (2012) 239–249

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Inhibitory effect of novel *S*,*N*-bisphosphonates on some carcinoma cell lines, osteoarthritis, and chronic inflammation

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ARTICLE INFO

Article history:
Received 3 November 2011
Received in revised form
17 February 2012
Accepted 23 February 2012
Available online 6 March 2012

Keywords:
S,*N*-bisphosphonates
Thiazinethiones
Antitumor activity
Antiarthritic- and anti- inflammatory activity

ABSTRACT

A new series of *S*,*N*-bisphosphonate derivatives was synthesized and evaluated as antitumor agents against breast-, cervix-, liver, and colon cancer diseases. Antiarthritic and antichronic inflammatory properties of the new bisphosphonates (BPs) were also investigated. The studies demonstrated an efficient site selective method for making condensation products of BP-derivatives in high yields from thiazinethiones and tetraethyl methylenebisphosphonate reagent. The bioscreening evaluation showed that one of the tested BPs exhibited remarkable antitumor activity against the four tested carcinoma cell lines; nevertheless, all tested *S*,*N*-BP-derivatives (11 compounds) showed significant to moderate anti-inflammatory activity and capable of inhibiting polyarthritis.

1. Introduction

Recently, bisphosphonates (BPs) have been proven to be an important asset in the treatment and prevention of bone metastatic breast cancer [1–5]. In addition, a number of in vitro studies indicated growth inhibition and induction of apoptosis of multiple myeloma cells by BP-drugs [1]. This result of antitumor activity-based (*N*-BPs) and their relevant BP-acids has been confirmed and reported by our group [6–8]. Our findings also showed that the order of BP-potency on inflammatory is not, however, equivalent to that of inhibiting cell viability in carcinoma cells [6].

Over the last two decades, there was a significant progress in elucidating the mechanism of action of BPs, which in general can shorten the life span of osteoclasts by induction of programmed cell death (apoptosis) [9–11]. For all *N*-BPs, in particular, apoptosis seems to be caused by the inhibition of important biosynthetic enzymes, e.g. farnesyl diphosphate synthase, in the mevalonate pathway, on which depends the synthesis of cholesterol and isoprenoid lipids. Isoprenylation involves covalent linkage of the 15 or 20 carbon of isoprene moiety of farnesyl diphosphate or geranyl–geranyl diphosphate, respectively, to the carbon-terminus of regulatory proteins, including the small GT-Pases Ras, Rac, Rho and Cdc42. The latter three, as well as numerous others, are signaling proteins that regulate a variety of cellular processes. In this way, *N*-BPs can deprive osteoclasts of important regulators of intramolecular dynamics, leading to poor cell functioning and eventually programmed cell death. 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 [1,5].

In the present article, it is intended to utilize the chemistry of the easily available thiobenzothiazine scaffold for formation of a new series of *N*-heterocyclic methylenethiobis-phosphonates in order to compare their chemistry with their oxygen-counterparts previously studied [7], and to evaluate their antitumor- as well as chronic inflammation properties. The work is a pursuance of our research activity directed toward construction of bioactive heterocycle gem-diphosphor esters, especially those associated with antitumor [6–8], anti-inflammatory [6,12–15], and antiosteoporosis potencies [12,15–17].

In an earlier stage, we had adopted the computer-assisted approach, PASS program [18,19] for designing in silico the structures of potentially active molecules for the future synthesis. An innovative approach to the simultaneous computer-aided prediction and structure-activity relationship analysis of many biological activities has been developed and widely used in finding and optimization of new leads [20,21].

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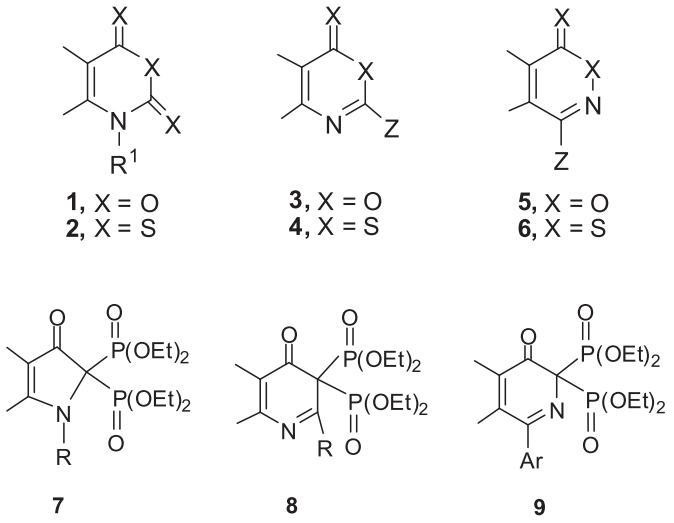
doi:10.1016/j.ejmech.2012.02.047

2. Results and discussion

In a recent study [7], we have prepared a number of methylenediphosphonate compounds **7**, **8** and **9** by conducting the condensation reaction of oxazines **1**, **3** and **5** with Horner reagent, tetraethyl methylenebisphosphonate. Antitumor properties of the bisphosphonate products of types **7**–**9** were discussed.

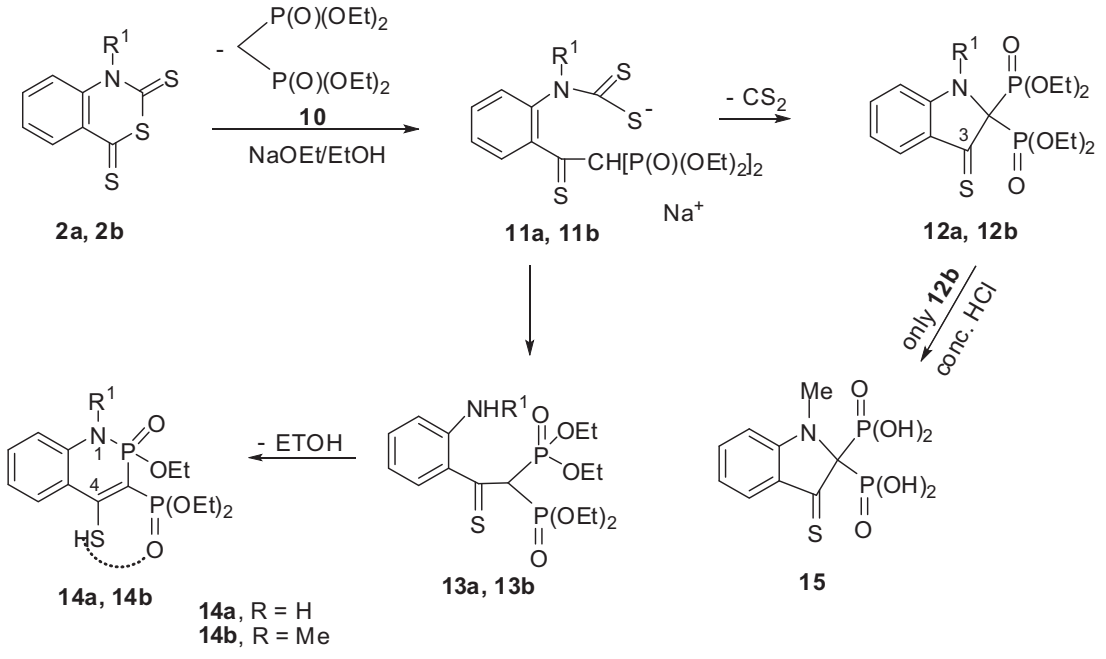


In the context of this work, the synthesis of the required sulfur and nitrogen containing-BP derivatives was accomplished by applying the same methodology on the analogously thiothiazines **2**, **4** and **6**. When 2*H*-3,1-benzothiazine-2,4(1*H*)-dithiones **2a** and **2b** were allowed to react with tetraethyl methylenebisphosphonate reagent **10** in boiling ethanol solution containing sodium ethoxide, BPs of types **12** and **14** in almost equal yields (≈39%) were formed. The spectroscopic data showed **14a** and **14b** to be present in the thiol form. Formation of the reaction products **12** and **14** was attained according to the transformations outlined in Scheme 1. The initial nucleophilic attack of the carbanion center in **10** on the C(1)=S group with subsequent ring cleavage leads to the formation of the sodium salt **11**, which is the key intermediate for subsequent transformations. **12a** and **12b** were formed from **11**, as previously reported [22–24], via carbon disulfide elimination whereas intramolecular cyclization of the protonated intermediates **13** yielded **14a** and **14b** with concomitant loss of ethanol moiety (Scheme 1). Considering the previous report [7], the results of the reaction of the oxygen analog **1** with **10** showed that we were able to isolate the products **12** parallel to those oxygen-analogs, but with **14a** and **14b** we have isolated different condensed products. The structures **12** and **14** were deduced on the basis of IR, ^{1}H, and ^{13}C spectroscopy and elemental analyses.

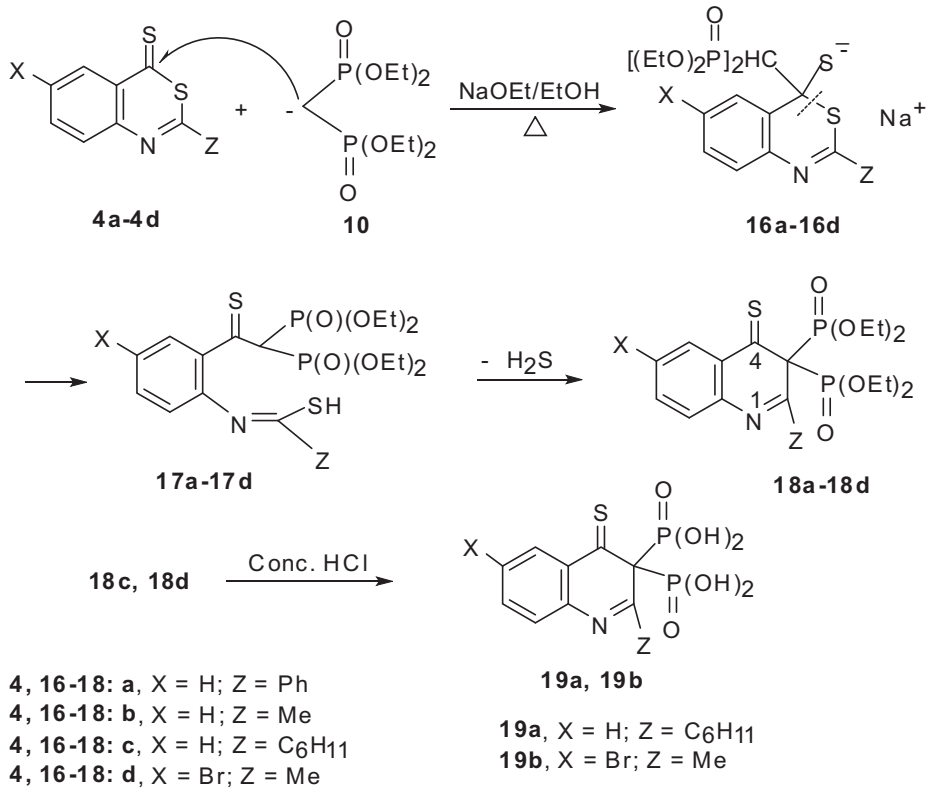
Tetraethyl 1-methyl-3-thioxoindoline-2,2-diyldiphosphonate, **12b** was obtained in 41.4% yield. The IR spectrum of **12b** showed absorptions bands at 1329 (C=S), 1258, 1242 (2P=O), and at 1135, 1052 (2P–O–C groups). Its ^{1}H NMR ($CDCl_3$, δ ppm) spectrum exhibited two doublet-triplets and two doublet-quartets recognized as arising from the two P-ester moieties (1.11, 1.41 and 3.84, 3.97 Hz, respectively). One doublet signal at 3.14 ppm and two doublet signals at 7.42, 7.85 ppm are due to the H_3CN and the *H*–Ar, respectively. The ^{1}H decoupled ^{13}C NMR ($CDCl_3$, δ ppm) spectrum of **12b** showed signals at 256.4 (d, *C*=S), 82.9 (t, $^1J_{P-C}$ = 136.4 Hz, *C*–P_2), and 38.3 (d, CH_3N). The ^{31}P NMR spectrum of **12b** displayed two separate doublets with equal $^2J_{P-P}$ coupling constants = 28 Hz.

Diethyl (2-ethoxy-4-mercapto-1-methyl-2-oxido-1,2-dihydro-1,2-benzazaphosphinin-3-yl)-phosphonate, **14b** was obtained in 37.2% that showed stretching bands at ν 3429, 1424 cm^{-1} that were attributed to the bonded SH group. In its ^{1}H NMR ($CDCl_3$, δ ppm) the thiol and *N*-methyl protons were given as two doublet at 1.91 and 3.23, respectively. The two phosphor ester moieties were displayed at 0.99, 1.12 (2dt) and at 3.88–4.01 [m (2dq)], respectively. Furthermore, ^{13}C NMR spectrum of **14b** revealed, among other signals, a doublet at 162.7 (C–SH), one triplet at 102.6 (*C*–P_2), and a doublet at 31.1 (CH_3N) ppm. Its ^{31}P NMR spectrum showed two doublets at δ_P 19.8 and 25.6 ppm ($^2J_{P-P}$ = 22 Hz, C–P_2).

Application of the phosphorus reagent **10** to 3,1-benzothiazine-4-thiones **4a**–**4d** was next investigated. Treatment of **4a**–**4d** with a slight excess of **10** under the same reaction conditions, afforded the corresponding bisphosphonate products **18a**–**18d** in high yields (≈70%) as a sole reaction product (Scheme 2).



Scheme 1. Synthesis of BPs **12a**, **14a**, **12b**, **14b**, and BP-acid **15**.



Scheme 2. Synthesis of BPs **18a**–**18d**, and BP-acids **19a**, **19b**.

A reasonable mechanism of the condensation of **4a**–**4d** with **10** involved an initial attack [22,25–28] of the carbanion carbon in **10** on the C(1)=S group with subsequent ring opening to afford the intermediated **17** via **16**. Under thermal conditions and the basic medium, **17** was formed. Further intramolecular cyclization led to the final BPs **18a**–**18d** accompanied with the loss of hydrogen sulfide molecule (Scheme 2).

Finally, the inserted products **21a**–**21d** were obtained in high yields when 4-substituted-1*H*-2,3-benzothiazine-1-thiones **6a**–**6d** were treated with Horner reagent **10** under the alkaline condition. The substituted isoquinoline-bisphosphonate esters were produced via the intermediates **20** according to Scheme 3.

As recent advances in pharma laboratories have identified impressively remarkable therapeutics from 1,1-bisphosphonic acid to 1,1-bisphosphonate ester counterparts [29], hydrolysis of the representatives **12b**, **18c**, **18d** and **21d** was undertaken to give the corresponding BP-acids **15**, **19a**, **19b** and **22** (Schemes 1–3).

The structures suggested for all new compounds are in good agreement with their analytical and spectral data (see Experimental section).

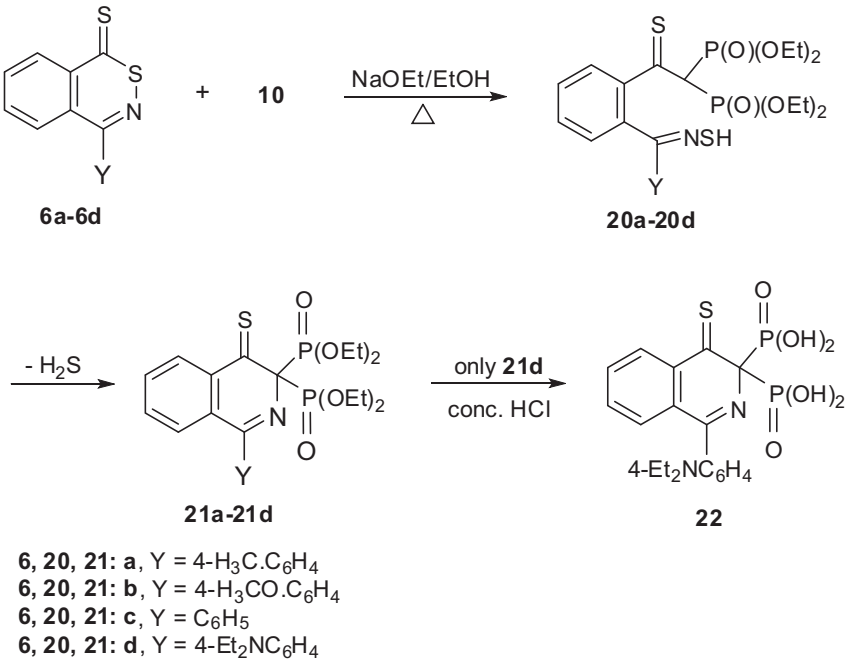
3. Pharmacological evaluation

3.1. Antitumor activity

Biological activity spectra were predicted for all synthesized BPs and the known drugs: zoledronic acid and clodronate with the free available internet version of PASS 2009.1: http://www.ibmc.msk.ru/PASS. The potency spectrum for a substance is a list of the biological activity types for which the probability to be revealed (*Pa*) and the probability not to be revealed (*Pi*) are calculated. *Pa* and *Pi* values are independent and their values vary from 0 to 1. As it is observed from the PASS results (Supplementary), **12b**, **18a**, and **21a** are the most three out of eight predicted compounds showed a promised cytotoxicity. Some common types of activities for new class of BPs and the known BP-drugs (zoledronic acid and clodronate) were observed. Thus, such activities as cytotoxicity and breast cancer were predicted almost for all tested new compounds as well as for zoledronic acid and clodronate. By default, in PASS $Pa = Pi$ value is chosen as a threshold, therefore all compounds with $Pa > Pi$ are suggested to be active.

In sequel, antitumor activity screening of **12b**, **18a**, and **21a** in assays applying a human breast carcinoma (MCF7)-, a human cervix (HeLa)-, a human liver (HEPG2)-, and a human colon (HCT116) cell lines was investigated. The evaluation was considered *vc* the known anticancer drugs: doxorubicin (**DOX**) or cisplatin (**CIS**) by sulfo-rhodamine-B-Stain (SRB) using the method of Skehan et al. [30]. The obtained results (Table 1) represent concentrations (four different concentrations: 5, 12.5, 25, and 50 μg/mL) of the used investigated compounds resulting in growth inhibition of 50% (IC_{50}) for the tested human cell lines compared to **DOX** or **CIS**; the highest concentration of each compound used was 50 μg/mL. Each concentration is evaluated three times (each dose is incubated with the cells in three different wells) thereby the data represent the average of the total inhibition observed. The deviation in the obtained data was ranged between: $p < 0.001$ and $p < 0.05$ (Table 1).

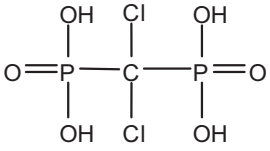
The antitumor activity results displayed in Table 1, indicated that the tested BP-derivatives **12b**, **18a**, and **21a** reflect good to moderate activity against the used human tumor cells. The order of activity is **12b** > **18a** > **21a**. It is also been noticed that **12b** exhibited remarkable antitumor activity against the four tested carcinoma cell lines. The relation between surviving fraction and drug concentration of the tested compounds **12b**, **18a**, and **21a** *vc* **DOX** or **CIS** is plotted in Fig. 1, to get the survival curve of each tumor cell for each compound. Further studies in experimental tumors in vivo for evaluating the possible antineoplastic potential by these and the other synthesized compounds are warranted.



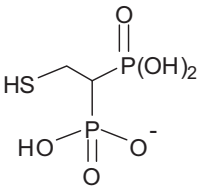
Scheme 3. Synthesis of BPs **21a**–**21d** and BP-acid **22**.

3.2. Antiarthritis bioassay

All bisphosphonates (BPs) are similar in terms to their inhibitory effects on bone resorption, but seem to have different effects on hypocalcemia resulted from diseases such as tumor-induced-osteolytic bone diseases, and human arthritis. However, it is recently reported that sulfur-containing BPs [31–33], e,g., mercaptomethyl-1,1-bisphosphonic acid (HSEDP®) [31], have demonstrated remarkable activity in the rat adjuvant arthritis model. In sequel, it is encouraging to evaluate the new *S*,*N*-BPs in animal models of arthritis: rat adjuvant-induced polyarthritis (AIP) and delayed type hypersensitivity granuloma assay as a model of chronic inflammation.



23



HSEDP

Table 1
Anti-tumor properties of the tested compounds **DOX**, **CIS**, **12b**, **18a**, and **21a**.

Compd.	IC_{50}, μg/mL (μM)			
	MCF7 (breast cancer)	HeLa (cervical cancer)	HEPG2 (liver cancer)	HCT116 (colon cancer)
DOX	4.4 (8.09)*	–	3.10 (5.70)***	3.73 (6.86)*
CIS	–	2.8 (9.30)**	–	–
12b	7.20 (16.54)***	6.60 (15.16)*	6.40 (14.70)**	3.60 (8.27)*
18a	7.95 (15.60)*	13.80 (27.08) ***	10.40 (20.40)*	11.60 (22.77)***
21a	15.90 (30.37)**	14.00 (26.74)*	13.20 (25.20)**	9.60 (18.34)*

DOX = doxorubicin; **CIS** = cisplatin.
Deviation error: (***) $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$; p is the percentage of inhibition.

According to the histopathology of the human arthritic joint, the adjuvant-induced polyarthritis (AIP) model expresses synovitis, infiltration of the subsynovial tissue with numerous inflammatory cell types, and pannus formation leading to erosion of articular cartilage and bone. Perhaps the most distinctive symptom of the joint pathology in AIP is the marked resorption of bone that is caused by a granulomatous reaction and periosteal bone formation. The rapid onset (*the first appearance of the signs or symptoms of an illness*) (24 h) of arthritis in the injected paw is considered to be largely a nonimmune inflammatory response to complete Freund**'s** adjuvant. In contrast, the subsequent arthritic reaction in the noninjected hindpaw and forepaws is delayed in onset and is mediated by immunological components. The suppression of bone destruction and periarticular inflammation in the noninjected paw in AIP, therefore, is considered to be an indication of potential antiarthritic activity in human rheumatoid arthritis.

The antiarthritic effect of seven new BPs **12a**,**b**, **14a**,**b**, **18a**,**b** and **21d** as well as four BP-acids **15**, **19a**,**b** and **22**, in the rat adjuvant-induced polyarthritis (AIP) over 28 days, was examined. Clodronate **23**, the BP drug was used as the relative control in our experiments as it demonstrated activity in this model [33]. The changes in paw volume over time (APV), which occurred in the injected and noninjected hindpaws of treated and control rats were quantitated by mercury displacement plethysmography. Clodronate, when administered at 10–50 mg/kg, exerted significant inhibitory effects ($p < 0.001$) on the noninjected hindpaw arthritis, whereas it was less effective (23–38% inhibition) against the swelling in the injected hindpaw. None of the tested BPs significantly altered the arthritis in the 28-day injected paw; however, **12b** and **14b** (10–50 mg/kg) caused a marginal suppression (18–37%) of this component of AIP (*injected hindpaw*) and matched that observed with the standard control **23**. In contrast, all tested BPs significantly inhibited ($p < 0.05$) noninjected paw arthritis when given at the same doses. The suppressive effects of tested BPs as well as the standard control **23** on AIP were not dose-related. BPs **14a** and **14b**, which contain free thiol group, exerted the highest inhibitory effect (56–76%) on the noninjected hindpaw arthritis. Also, the results displayed in Table 2 indicated that *N*-alkylated

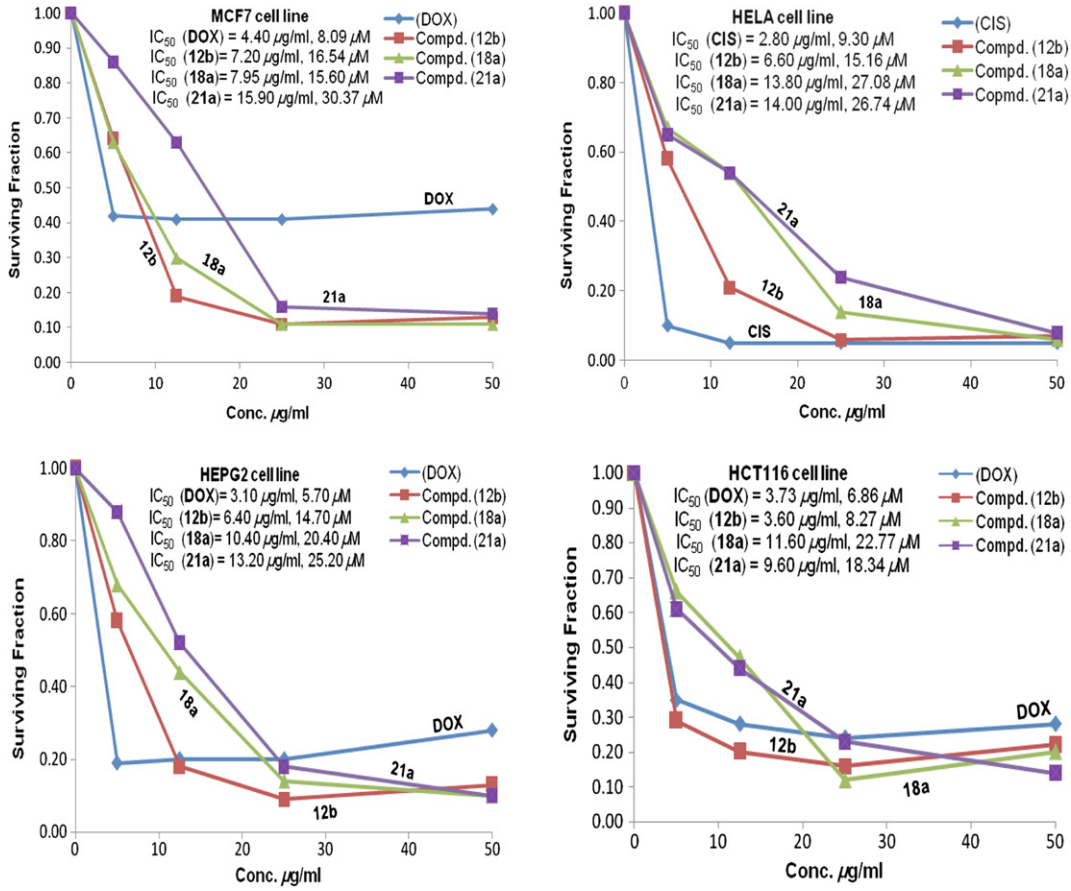


Fig. 1. Dose response curves of compounds **12b**, **18a**, **21a** *vc* **DOX** or **CIS** against HCT116, HEPG2, HeLa, and MCF7 cell lines.

derivatives **12b** and **14b** caused better inhibition than their counterparts **12a** and **14a** at the same doses whereas conversion of BPs **12b**, **18c**, **18d**, and **21d** to the corresponding BP-acids, **15**, **19a**, **19b**, and **22**, resulted in loss of activity even at higher dose (100 mg/kg) in noninjected hindpaw-associated arthritis.

Next, the same standard **23**, BPs **12a**,**b**, **14a**,**b**, **18a**,**b** and **21d** as well as BP-acids **15**, **19a**,**b** and **22** were profiled in a delayed-type hypersensitivity granuloma model using the reported procedure by Nugent et al. [33]. The compounds were administered subcutaneously in mice, which were previously sensitized to methylated bovine serum albumin (mBSA) and surgically implanted with hydroxyapatite disks (two per mouse), soaked in mBSA, in order to generate granulomas. This model is unaffected by traditional nonsteroidal anti-inflammatory drugs, such as aspirin, indomethacin, or ibuprofen [34]. Clodronate reproducibly inhibited granuloma wet and dry weights and served as a positive control in our experiments; the results are shown in Table 2.

Both **14a** and **14b** significantly inhibited the granuloma in a dose-dependent manner at the doses examined (25–100 mg/kg). **12a**, **12b**, **18a**, **18b**, and **21d** all displayed inhibitory activities, which were equivalent matched to that of clodronate at 100 mg/kg. BP-acids **15**, **19a**, **19b**, and **22**, on the other hand, showed only marginal activity against the dry and the wet weight granuloma. This result is not surprising as it has been reported that, in some cases, conversion of BPs to the corresponding acid, resulted in the loss of activity [33]. Also data in Table 2 showed that replacement of the acidic N-1 hydrogen in **12a**, **14a** with a methyl group, resulted in enhancing the activity.

Prediction of anti-inflammatory was made by using the computer-assisted approach, PASS program [18,19] at the earlier stage in order to decide if it is worthy to be in vivo evaluated, and to compare the results of the prediction with the experimental one. The prediction result is presented as a list of activities (Table 2) with appropriate *Pa* and *P*/*E*, which reflects the accuracy of the prediction with the experiment results thereby it can be deduces the average accuracy of prediction (AAP) for anti-inflammatory activity to 54.5%. This observation suggests that these compounds differ significantly from the classic ant-inflammatory compounds and that they may be new chemical entities (NCEs).

3.3. Toxicity of the most promised products

Toxicological studies of the most promising synthesized anti-inflammatory active compounds **12b**, **14b** and **21d** were performed using LD_{50} standard method in mice in 500, 750 and 1000 mg/kg (body weight), i.e. 10–20 folds 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.

4. Conclusion

The studied reactions in the previous [7] and the present investigations are offered as an easy route for the transformations of easily available starting materials to the title BPs and the related BP-acids in satisfactory yields. In addition, our protocol demonstrates an efficient site selective method for making condensation products in high yields from thiazinethiones and methylene-bisphosphonate reagent under mild conditions. We have also attempted in this research work to utilize the high bone (joint) specificity of sulfur

Table 2
Antiarthritic activity and delayed-type hypersensitivity granuloma results[a] of BPs **23**, **12a**,**b**, **14a**,**b**, **18a**,**b**, **21d**, and BP-acids **15**, **19a**,**b**, **22**.

No	AIP (% inhibn) (APV, 28 days)			Dose (mg/kg) sc	% inhibn of granuloma		Prediction (AIA) *Pa*	Coincidence *P*/*E*
	Dose (mg/kg)	Injected paw	Noninjected paw		Dry wt	Wet wt		
23	50	35	68***	100	48***	44***	0.786	+/+
	30	34	62***	60	44	40		
	20	28	70**	25	33	30		
	10	23	56***	–	–	–		
12a	50	15	64***	100	46***	44***	–	–/+
	30	11	58***	60	42**	31*		
	20	7	60**	25	30	24		
	10	5	48***	–	–	–		
12b	50	30	68*	100	48*	38*	–	–/+
	30	32	70***	60	45	45		
	20	23	55**	25	36	33		
	10	15	45***	–	–	–		
14a	50	25	75**	100	50***	51***	0.741	+/+
	30	14	68**	60	42	44		
	20	10	56***	25	38	33		
	10	<5	66***	–	–	–		
14b	50	37	76***	100	55***	50**	0.641	+/+
	30	35	73***	60	53	48		
	20	27	60***	25	50	33		
	10	18	66***	–	–	–		
15	100	13	27	100	32***	25**	0.213	+/+
	50	<5	18	50	16	15		
18a	50	28	70***	100	48***	43***	0.442	+/+
	30	30	68***	60	32	35		
	20	12	60***	25	22	13		
	10	<5	40**	–	–	–		
18b	50	20	66***	100	44**	40**	0.311	+/+
	30	18	72***	60	37	30		
	20	23	61***	25	24	20		
	10	<5	38***	–	–	–		
19a	100	16	14	100	36	24*	0.466	+/+
	50	<5	8	50	28	19*		
19b	100	21	18	100	25	13	0.284	–/+
	50	<5	12	50	18	<10		
21d	50	24	65**	100	44***	34***	0.533	+/–
	30	16	52***	60	40**	31*		
	20	20	56***	25	32	22		
	10	<5	44***	–	–	–		
22	100	18	24	100	30	23**	–	–/–
	50	<5	10	50	26	16**		

sc: subcutaneously.
(*P*/*E*): *P* – prediction; *E* – experiment; *P*/*E* – accuracy of prediction.
+/+ means that both prediction and experiment give positive results; –/– means that both prediction and experiment give negative results; +/– means that prediction gives positive results but experiment gives negative results; –/+ means that prediction gives negative results but experiment give positive results.
[a] (***) $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$; *p* is the percentage of inhibition.

containing bisphosphonic acids [31–33] with other chemical moieties of potential anticatabolic pharmacology for testing as a new series of compounds for the treatment of human rheumatoid arthritis. Screening results indicated that **14b** and **14b** that have free thiol group are the highest potent BP-product for inhibition chronic arthritis, which may bind to a metal atom in the active site of the matrix metalloproteinases (MMPs) [35]. In parallel, the bioassays are in agreement with the previously reported [29] that small changes in the structure of the *N*-heterocyclic moiety in the BP-derivatives can lead to extensive alterations in their physicochemical, biological and therapeutic characteristics.

5. Experimental section

Melting points (uncorrected) were determined with open capillary tube on an electrothermal (variable heater) melting point apparatus. IR spectra were recorded on a JASCO FT-IR 6100 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 appropriate precautions in handling moisture-sensitive compounds were observed. The purity of all new samples was verified by microchemical analysis (H/C/N) and spectroscopy. All international principles and local regulations concerning the care and use of laboratory animals were considered during the pharmacological screening. The known starting substrates **2a**, **2b** [36], **4a** [37], **4b** [38], **4c** [39], and **4d** [40] were prepared according to the reported methods.

5.1. General procedure of bisphosphonates *12a*, *12b*, *14a*, *14b*, and *18a–18d*

To a stirred solution of 9.12 mmol of sodium (Na) in 10 mL EtOH-was added drop wise 4.2 mmol of tetraethyl methylenebisphosphonate **10** followed by a solution of 3.8 mmol of 2*H*-3,1-

benzothiazine-2,4(1*H*)dithione **2a**, 1-methyl-2*H*-3,1-benzothiazine-2,4(1*H*)dithione **2b**, 2-phenyl-4*H*-3,1-benzothiazine-4-thione **4a**, 2-methyl-4*H*-3,1-benzothiazine-4-thione **4b**, 2-cyclohexyl-4*H*-3,1-benzothiazine-4-thione **4c**, or 6-bromo-2-methyl-4*H*-3,1-benzothiazine-4-thione **4d** in 20 mL EtOH at 0 °C. The resulting mixture was heated under reflux for 15–20 h (thin layer chromatography, TLC). The reaction mixture was poured into 100 mL of distilled water and HCl (1 N) was added at −5 °C until the pH of the reaction mixture became acidic, followed by extraction with AcOEt (3 × 50 mL). The combined organic phase was dried over *anhy* Na_2SO_4. After removal of the solvent, under reduced pressure, the resulting residue was washed several times with light petroleum (40–60 °C), and recrystallized from the solvent indicated after the m.p., to give the respective bisphosphonates (BPs) **12a**, **12b**, **14a**, **14b**, and **18a**–**18d**, respectively.

5.2. Reaction of *2a* with *10*

Reagents: NaOEt (0.2 g of Na, 9.1 mmol in 30 mL EtOH), BP-reagent **10** (1.2 mL, 4.2 mmol), and the substrate **2a** (0.8 g, 3.8 mmol). The product mixture was washed several times with light petroleum (40–60 °C), and recrystallized from the solvent indicated after the mp, to give BPs **12a** and **14a**.

5.2.1. Tetraethyl 3-thioxoindoline-2,2-diyldiphosphonate, *12a*

Yield 41.2%. Yellow crystals, mp 198–200 °C (from MeCN). IR (cm^{-1}, KBr): ν_{max} 3356$_w$ (NH), 1333 (C═S), 1256, 1246 (2P═O), 1153, 1061 (P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 0.99, 1.13 (2dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.4, 2 × 6H, 2($H_3CCO)_2$), 3.58, 3.78 (2dq, J_{H-H} = 6.6, $^3J_{P-H}$ = 4.7, 2 × 4H, 2($H_2CO)_2$), 7.38, 8.22 (2d, J_{H-H} = 8.1, 2 × 2H, *H*–Ar), 8.79 (d, $^3J_{P-H}$ = 4.6, 1H, *H*N). ^{13}C NMR [$CDCl_3$] ppm: δ 254.4 (d, $^2J_{P-C}$ = 12.4 Hz, *C*═S), 153.6, 134.3, 128.3, 126.5, 112.3 (*C*–Ar), 83.5 (t, $^1J_{P-C}$ = 137.4, *C*–P_2), 61.7 (d, $^2J_{P-C}$ = 13.7, CH_2OP), 16.4 (d, $^3J_{P-C}$ = 8.8, H_3CCOP). ^{31}P NMR [$CDCl_3$] ppm: δ 22.4, 25.7 (2d, $^2J_{P-P}$ = 28, CP_2). EI-MS: in *m*/*z* (%): 420 (11) [M^+ − 1], 388 (16) [M^+ − 32(S)], 282 (32) [M^+ − 137 ($C_4H_{10}O_3P$)], 146 (48) [M^+ − 274 ($C_4H_{10}O_3P)_2$], 114 (100) [M^+ − (32 + 274)S + ($C_4H_{10}O_3P)_2$}], 102 (38), 77 (96). Anal. Calcd for $C_{16}H_{25}NO_6P_2S$ (421.4): C, 45.60; H, 5.98; N, 3.32; P, 14.70; S, 7.61. Found: C, 45.67; H, 6.03; N, 3.27; P, 14.64; S, 7.52.

5.2.2. Diethyl (2-ethoxy-4-mercapto-2-oxido-1,2-dihydro-1,2-benzazaphosphinin-3-yl)phosphonate, *14a*

Yield 38.3%. Yellow needles, mp 219–221 °C (from $CHCl_3$). IR (cm^{-1}, KBr): ν_{max} 3429–3354 (br, NH, SH), 1421 (SH), 1248, 1226 (2P═O, bonded), 1156, 1086 (P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 1.02 (dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.5, 3H, H_3CCO), 1.26 (dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.6, 2 × 3H, ($H_3CCO)_2$P), 1.93 (br, 1H, *H*S), 3.85–4.03 [m, 6H, CH_2 OP & ($CH_2O)_2$ P], 7.41, 7.88 (2d, J_{HH} = 8.4, 2 × 2 H, *H*–Ar), 8.80 (br, 1H, *H*N). ^{13}C NMR [$CDCl_3$] ppm: δ 164.7 (d, $^2J_{P-C}$ = 13.5, *C*SH), 141.8, 131.3, 126.4, 123.5, 121.3 (*C*–Ar), 106.6 (t, $^1J_{P-C}$ = 148.4, *C*–P), 62.9, 60.1 (2d, $^2J_{P-C}$ = 11.7, 2 × CH_2O), 17.8, 17.6 (2d, $^3J_{P-C}$ = 4.8, 2 × CH_3C.O). ^{31}P NMR [$CDCl_3$] ppm: δ 20.4, 21.7 (2d, $^2J_{P-P}$ = 29, CP_2). EI-MS: in *m*/*z* (%): 376 (22) [M^+ − 1], 343 (17) [M^+ − 33 (SH)], 251 (41) [M^+ − (33 + 92) (SH + $C_2H_5O_2P$)], 114 (100) [M^+ − (33 + 229) (SH + $C_6H_{15}O_5P_2$)], 102 (46), 77 (92). Anal. Calcd for $C_{14}H_{21}NO_5P_2S$ (377.3): C, 44.56; H, 5.61; N, 3.71; P, 16.42; S, 8.50. Found: C, 44.61; H, 5.69; N, 3.67; P, 16.50; S, 8.57.

5.3. Reaction of *2b* with *10*

Reagents: NaOEt (0.2 g of Na, 9.1 mmol in 30 mL EtOH), BP-reagent **10** (1.2 mL, 4.2 mmol), and the substrate 2b (0.86 g, 3.8 mmol). The product mixture was washed several times with light petroleum (40–60 °C), and recrystallized from the solvent indicated after the mp, to give BPs **12b**, and **14b**.

5.3.1. Tetraethyl 1-methyl-3-thioxoindoline-2,2-diyldiphosphonate, *12b*

Yield 40.4%. Orange material, mp 119–121 °C (from cyclohexane). IR (cm^{-1}, KBr): ν_{max} 1329 (C═S), 1258, 1242 (2P═O), 1135, 1052 (P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 1.11, 1.41 (2dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.4, 2 × 6H, 2($H_3CCO)_2$), 3.14 (d, $^4J_{P-H}$ = 3.3, 3H, H_3CN), 3.84, 3.97 (2dq, J_{H-H} = 6.6, $^3J_{P-H}$ = 4.8, 2 × 4H, 2($H_2CO)_2$), 7.42, 7.85 (2d, J_{H-H} = 8.1, 2 × 2H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 256.4 (d, $^2J_{P-C}$ = 13.4, *C*═S), 155.2, 133.3, 131.2, 127.3, 124.5, 114.2 (*C*–Ar), 82.9 (t, $^1J_{P-C}$ = 136.4, *C*–P_2), 60.9 (d, $^2J_{P-C}$ = 13.6, CH_2O), 38.3 (d, $^3J_{P-C}$ = 8.2, CH_3N), 16.4 (d, $^3J_{P-C}$ = 3.9, CH_3CO). ^{31}P NMR [$CDCl_3$] ppm: δ 23.6, 27.5 (2d, $^2J_{P-P}$ = 28, CP_2). EI-MS: in *m*/*z* (%): 435 (17) [M^+], 420 (42) [M^+ − 15 (Me)], 388 (24) [M^+ − (15 + 32) (Me + S)], 283 (38) [M^+ − (15 + 137) (Me + $C_4H_{10}O_3P$)], 146 (55) [M^+ − (15 + 274) (Me + $C_4H_{10}O_3P)_2$], 114 (100) [M^+ − (15 + 32 + 274) {Me + S + ($C_4H_{10}O_3P)_2$}], 102 (36), 77 (98). Anal. Calcd for $C_{17}H_{27}NO_6P_2S$ (435.4): C, 46.89; H, 6.25; N, 3.22; P, 14.23; S, 7.36. Found: C, 46.94; H, 6.33; N, 3.15; P, 14.16; S, 7.28.

5.3.2. Diethyl (2-ethoxy-4-mercapto-1-methyl-2-oxido-1,2-dihydro-1,2-benzazaphosphinin-3-yl)phosphonate, *14b*

Yield 37.2%. Yellow crystals, mp 138–140 °C (from CH_2Cl_2). IR (cm^{-1}, KBr): ν_{max} 3429, 1424 (br, SH), 1245, 1222 (2P═O), 1155, 1096 (2P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 0.99 (dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.5, 3H, H_3CCOP), 1.12 (dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.6, 2 × 3H, (H_3CC.O)$_2$P), 1.91 (d, $^4J_{P-H}$ = 3.8, 1H, *H*S), 3.23 (d, $^3J_{P-H}$ = 4.7 Hz, 3H, H_3CN), 3.88–4.01 (m, 6H, CH_2OP & ($CH_2O)_2$ P), 7.46, 7.84 (2d, J_{H-H} = 8.4 Hz, 2 × 2H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 162.7 (d, $^2J_{P-C}$ = 13.7, *C*SH), 143.8, 131.3, 126.4, 125.5, 121.3 (*C*–Ar), 102.6 (t, $^1J_{P-C}$ = 137.4, *C*–P), 61.9, 59.7 (2d, $^2J_{P-C}$ = 11.7, CH_2O), 31.1 (d, $^2J_{P-C}$ = 15.2, CH_3N), 17.9, 17.3 (2d, $^3J_{P-C}$ = 8.8, CH_3CO). ^{31}P NMR [$CDCl_3$] ppm: δ 19.8, 25.6 (2d, $^2J_{P-P}$ = 22, CP_2). EI-MS: in *m*/*z* (%): 391 (15) [M^+], 376 (28) [M^+ − 15], 343 (14) [M^+ − (15 + 33)(Me + SH)], 251 (46) [M^+ − (15 + 33 + 92)(Me + SH + $C_2H_5O_2P$)], 114 (100) [M^+ − (15 + 33 + 229)(Me + SH + $C_6H_{15}O_5P_2$)], 102 (42), 77 (88). Anal. Calcd for $C_{15}H_{23}NO_5P_2S$ (391.4): C, 46.03; H, 5.92; N, 3.58; P, 15.83; S, 8.19. Found: C, 46.11; H, 5.98; N, 3.54; P, 15.76; S, 8.13.

5.4. Reactions of *4a*–*4d* with *10*

Reagents: NaOEt (0.2 g of Na, 9.12 mmol in 30 mL EtOH), BP-reagent **10** (1.2 mL, 4.2 mmol), and the substrates **4a**–**4d** (3.8 mmol). The product mixture was washed several times with light petroleum (40–60 °C), and recrystallized from the solvent indicated after the mp, to give BPs **18a**–**d**.

5.4.1. Tetraethyl 2-phenyl-4-thioxo-3,4-dihydroquinoline-3,3-diyldiphosphonate, *18a*

Yield 72.4%. Yellow crystals, mp 191–193 °C (from MeCN). IR (cm^{-1}, KBr): ν_{max} 1556 (C═N), 1330 (C═S), 1262, 1250 (2P═O), 1168, 1110 (2P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 1.15, 1.43 (2dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.6, 2 × 6H, 2($H_3CCO)_2$), 4.00, 4.34 (2dq, J_{H-H} = 6.6, $^3J_{P-H}$ = 4.5, 2 × 4H, 2($H_2CO)_2$), 7.38, 7.56, 7.68 (3m, 7H, *H*–Ar), 8.39, 8.42 (2d, J_{H-H} = 7.8, 2H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 265.8 (d, $^2J_{P-C}$ = 18.6, *C*═S), 171.6 (d, $^2J_{P-C}$ = 22.7, *C*(2) = N), 151.1, 138.6, 135.7, 133.1, 130.7, 129.1, 128.2, 127.3 (*C*–Ar), 61.8 (d, $^2J_{P-C}$ = 9.7, CH_2O), 58.8 (t, $^1J_{P-C}$ = 148.6, *C*–P_2), 16.1 (d, $^3J_{P-C}$ = 8.2, CH_3CO). ^{31}P NMR [$CDCl_3$] ppm: δ 22.4, 25.3 (2d, $^2J_{P-P}$ = 28, CP_2). EI-MS: in *m*/*z* (%): 510 (17) [M^+ + 1], 509 (13) [M^+], 465 (20) [M^+ − 44 (CS)], 235 (100) [M^+ − 274($C_4H_{10}O_3P)_2$], 191 (88) [M^+ − (44 + 274){CS+ ($C_4H_{10}O_3P)_2$}],105 (67), 77 (78). Anal. Calcd for $C_{23}H_{29}NO_6P_2S$ (509.5): C, 54.22; H, 5.74; N, 2.75; P, 12.16; S, 6.29. Found: C, 54.28; H, 5.82; N, 2.69; P, 12.09; S, 6.23.

5.4.2. Tetraethyl 2-methyl-4-thioxo-3,4-dihydroquinoline-3,3-diyldiphosphonate, ***18b***

Yield 71.8%. Yellow crystals, mp 171–173 °C (from $CHCl_3$). IR (cm^{-1}, KBr): ν_{max} 1556 (C═N), 1329 (C═S), 1251, 1244 (2P═O), 1135, 1048 (2P–O–C). ^1H NMR [$CDCl_3$] ppm: δ 1.16, 1.33 (2dt, $J_{H-H} = 6.3$, $^4J_{P-H} = 3.5$, 2 × 6H, 2($H_3CCO)_2$), 2.41 (d, $^4J_{P-H} = 3.4$, 3H, H_3C), 4.04, 4.26 (2dq, $J_{H-H} = 6.6$, $^3J_{P-H} = 4.6$, 2 × 4H, 2($H_2CO)_2$), 7.25, 7.78 (2d, $J_{H-H} = 8.2$, 2 × 2 H, *H*–Ar). ^{13}C NMR [$CDCl_3$]: δ 264.5 (d, $^2J_{P-C} = 14.6$, *C*═S), 173.6 (d, $^2J_{P-C} = 16.8$, *C*═N), 151.7, 150.8, 137.3, 130.4, 128.3, 128.03 (*C*–Ar), 61.2 (d, $^2J_{P-C} = 9.7$, CH_2O), 57.8 (t, $^1J_{P-C} = 148.4$, *C*–P_2), 24.3 [d, $^3J_{P-C} = 7.8$, CH_3–C(2)], 16.9 (d, $^3J_{P-C} = 6.8$, CH_3CO). ^{31}P NMR [$CDCl_3$] ppm: δ 23.4, 28.7 (2d, $^2J_{P-P} = 34$, CP_2). EI-MS: in *m*/*z* (%): 448 (15) [M^+ + 1], 447 (12) [M^+], 432 (36) [M^+ − 15], 388 (18) [M^+ − (15 + 44)], 158 (100) [M^+ − (15 + 274){Me + ($C_4H_{10}O_3P)_2$}], 114 (80), [M^+ − (15 + 44 + 274){Me + CS + ($C_4H_{10}O_3P)_2$}],105 (64), 77 (84). Anal. Calcd for $C_{18}H_{27}NO_6P_2S$ (447.4): C, 48.32; H, 6.08; N, 3.13; P, 13.85; S, 7.17. Found: C, 48.38; H, 6.12; N, 3.07; P, 13.76; S, 7.19.

5.4.3. Tetraethyl 2-cyclohexyl-4-thioxo-3,4-dihydroquinoline-3,3-diyldiphosphonate, ***18c***

Yield 73.6%. Red crystals, mp 118–120 °C (from benzene); IR (cm^{-1}, KBr): ν_{max} 1548 (C═N), 1325 (C═S), 1259, 1242 (2P═O), 1135, 1066 (2P–O–C). ^1H NMR [$CDCl_3$] ppm: δ 1.24, 1.38 (2dt, $J_{H-H} = 7.6$, $^4J_{P-H} = 3.7$, 2 × 6H, 2($H_3CCO)_2$),1.98 (m, 10H, H_2C-hexyl), 3.43 (m, 1H, C*H*–hexyl), 4.11, 4.44 (2dq, $J_{H-H} = 7.6$, $^3J_{P-H} = 4.7$, 2 × 4H, 2($H_2CO)_2$), 7.55, 7.78 (2d, $J_{H-H} = 8.2$, 2 × 2H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 266.6 (d, $^2J_{P-C} = 13.6$, *C*═S), 178.6 (d, $^2J_{P-C} = 15.8$, *C*(2) = N), 151.8, 137.3, 136.3, 130.4, 128.3, 128.1(*C*–Ar), 61.2 (d, $^2J_{P-C} = 9.7$, CH_2O), 58.8 (t, $^1J_{P-C} = 148.4$, *C*–P_2), 34.3 (d, $^3J_{P-C} = 9.2$, *C*H–hexyl), 32.8, 26.6, 26.1 (*C*H_2–hexyl), 16.3 (d, $^3J_{P-C} = 9.2$, H_3CC.O). ^{31}P NMR [$CDCl_3$] ppm: δ 24.1, 28.9 (2d, $^2J_{P-P} = 18$, CP_2). EI-MS: in *m*/*z* (%): 516 (13) [M^+ + 1], 515 (9) [M^+], 513 (16) [M^+ − 2], 509 (18) [M^+ − 6], 465 (34) [M^+ − 6 + 44 (6H + CS)], 235 (100) [M^+ − (6 + 274) {6H + ($C_4H_{10}O_3P)_2$}], 191 (88) [M^+ − (6 + 44 + 274) {6H + CS + ($C_4H_{10}O_3P)_2$}], 105 (68), 77 (74). Anal. Calcd for $C_{23}H_{35}NO_6P_2S$ (515.5): C, 53.58; H, 6.84; N, 2.72; P, 12.02; S, 6.22. Found: C, 53.64; H, 6.91; N, 2.66; P, 12.12; S, 6.29.

5.4.4. Tetraethyl 6-bromo-2-methyl-4-thioxo-3,4-dihydroquinoline-3,3-diyldiphosphonate, ***18d***

Yield 70.6%. Yellowish brown substance, mp 177–179 °C (from CH_2Cl_2). IR (cm^{-1}, KBr): ν_{max} 1556 (C═N), 1331 (C═S), 1260, 1247 (P═O), 1154, 1078 (P–O–C). ^1H NMR [$CDCl_3$] ppm: δ 1.26, 1.30 (2dt, $J_{H-H} = 7.6$, $^4J_{P-H} = 4.3$, 2 × 6H, 2($H_3CCO)_2$), 2.49 (d, $^4J_{P-H} = 3.6$, 3H, H_3C), 4.04, 4.26 (2dq, $J_{H-H} = 6.6$, $^3J_{P-H} = 12.8$, 2 × 4H, 2($H_2CO)_2$), 7.20, 7.53 (2d, $J_{H-H} = 8.1$, 2 × 1H, *H*–Ar), 8.58 (s, 1H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 267.5 (d, $^2J_{P-C} = 15.6$, *C*═S), 173.6 (d, $^2J_{P-C} = 11.8$, *C*(2)═N), 152.7, 147.8, 131.3129.4, 128.4, 127.3 (*C*–Ar), 121.83 (*C*–Br), 61.2 (d, $^2J_{P-C} = 10.7$, CH_2O), 57.8 (t, $^1J_{P-C} = 148.4$, *C*–P_2), 24.3 (d, $^3J_{P-C} = 4.4$, CH_3), 16.4 (d, $^3J_{P-C} = 4.8$, CH_3CO). ^{31}P NMR [$CDCl_3$] ppm: δ 26.4, 29.2 (2d, $^2J_{P-P} = 28$, CP_2). EI-MS: in *m*/*z* (%): 526 (40) [M^+], 527 (3) [M^+ + 1], 528 (2) [M^+ + 2], 511 (28) [M^+ − 15 (Me)], 432 (46) [M^+ − (15 + 80)(Me + Br)], 387 (36) [M^+ − (15 + 80 + 44) (Me + Br + CS)], 193 (100) [M^+ − (15 + 44 + 274) {Me + CS + ($C_4H_{10}O_3P)_2$}], 114 (80), 105 (55), 77 (74). Anal. Calcd for $C_{18}H_{26}BrNO_6P_2S$ (526.3): C, 41.08; H, 4.98; Br, 15.18; N, 2.66; P, 11.77; S, 6.09. Found: C, 41.12; H, 5.06; Br, 15.12; N, 2.57; P, 11.71; S, 6.03.

5.5. General procedure of 4-aryl-1H-2,3-benzothiazine-1-thiones, ***6a–6d***

To a solution of 10 mmol of 4-(4-methylphenyl)-2,3-benzoxazin-1-one, **5a**, 4-(4- methoxy-phenyl)-2,3-benzoxazin-1-one, **5b**, 4-(4-methylphenyl)-2,3-benzoxazin-1-one, **5c** or 4-[4-(diethylamino)phenyl]-2,3-benzoxazin-1-one, **5d** in 10 mL xylene, was added 20 mmol of P_2S_5 in 100 mL of dry xylene in one portion. The mixture was boiled under reflux for 6–8 h (TLC), filtered upon hot and then concentrated to its half. The product that separated on cooling was recrystallized from the solvent indicated after the mp, to give the corresponding thiones **6a–6d**.

5.5.1. 4-(4-Methylphenyl)-1H-2,3-benzothiazine-1-thione, ***6a***

Yield 53.7%. Red crystals, mp 178–180 °C (from EtOH). IR (cm^{-1}, KBr): ν_{max} 1562 (C═N), 1369 (C═S). ^1H NMR [$CDCl_3$] ppm: δ 2.51 (s, 3H, H_3C–tolyl), 7.32–8.37 (m, 8H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 213.4 (*C*═S), 163.4 (C(4)═N), 139.1, 137.3, 130.9, 129.8, 129.2, 127.5, 125.9, 125.1 (*C*–Ar), 21.6 (CH_3–tolyl). EI-MS: in *m*/*z* (%): 269 (33) [M^+], 254 (100) [M^+ − 15 (Me)], 193 (68) [M^+ − 76 (CS_2)], 178 (21) [M^+ − (15 + 76) (Me + CS_2)], 102 (68), 77 (98). Anal. Calcd for $C_{15}H_{11}NS_2$ (269.4): C, 66.88; H, 4.12; N, 5.20; S, 23.81. Found: C, 66.91; H, 4.17; N, 5.13; S, 23.76.

5.5.2. 4-(4-Methoxyphenyl)-1H-2,3-benzothiazine-1-thione, ***6b***

Yield 57.4%. Yellow crystals, mp 190–193 °C (from $CHCl_3$). IR (cm^{-1}, KBr): ν_{max} 1552 (C═N), 1370 (C═S). ^1H NMR [$CDCl_3$] ppm: δ 3.53 (s, 3H, H_3CO), 7.12–8.37 (m, 8H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 213.4 (*C*═S), 163.4 (C(4)═N), 159.7, 137.2, 130.9, 129.2, 127.5, 125.8, 125.1 (*C*–Ar), 55.4 (CH_3O). EI-MS: in *m*/*z* (%): 285 (42) [M^+], 254 (100) [M^+ − 31(OMe)], 179 (27) [M^+ − (31 + 76) (OMe + CS_2)], 102 (58), 77 (98). Anal. Calcd for $C_{15}H_{11}NOS_2$ (285.4): C, 63.13; H, 3.89; N, 4.95; S, 22.47. Found: C, 63.17; H, 3.93; N, 4.89; S, 22.55.

5.5.3. 4-Phenyl-1H-2,3-benzothiazine-1-thione, ***6c***

Yield 62.4%. Yellow crystals, mp 158–160 °C (from MeCN). IR (cm^{-1}, KBr): ν_{max} 1554 (C═N), 1366 (C═S). ^1H NMR [$CDCl_3$]: δ 7.33–8.37 (m, 9H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 213.4 (*C*═S), 163.4 (C(4)═N), 137.2, 134.1, 133.9, 131.6, 129.6, 127.3, 125.9 (*C*–Ar). EI-MS: in *m*/*z* (%): 255 (100) [M^+]. 179 (78) [M^+ − 76 (CS_2)], 102 (76), 77 (90). Anal. Calcd for $C_{14}H_9NS_2$ (255.4): C, 65.85; H, 3.55; N, 5.49; S, 25.11. Found: C, 65.89; H, 3.61; N, 5.41; S, 25.15.

5.5.4. 4-[4-(Diethylamino)phenyl]-1H-2,3-benzothiazine-1-thione, ***6d***

Yield 66.3%. Yellow crystals, mp 111–113 °C (from MeCN). IR (cm^{-1}, KBr): ν_{max} 1558 (C═N), 1373 (C═S). ^1H NMR [$CDCl_3$] ppm: δ 0.94 (t, 6H, H_3C.CN), 3.54 (q, 4H, H_2CN), 6.48–8.37 (m, 8H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 213.4 (*C*═S), 163.4 (C(4)═N), 149.1, 146.8, 139.2, 130.9, 129.4, 127.2, 126.9, 115.1 (*C*–Ar), 44.8 (CH_2N), 12.7 (CH_3C. N). EI-MS: in *m*/*z* (%): 326 (32) [M^+], 254 (100) [M^+ − 72 (NEt_2)], 250 (38) [M^+ − 76 (CS_2)], 178 (78) [M^+ − (72 + 76) {NEt_2 + CS_2}], 102 (66), 77 (81). Anal. Calcd for $C_{18}H_{18}N_2S_2$ (326.5): C, 66.22; H, 5.56; N, 8.58; S, 19.64. Found: C, 66.26; H, 5.61; N, 8.51; S, 19.72.

5.6. General procedure of BPs ***21a–21d***

Following the general procedure, a mixture of 9.2 mmol of Na, 4.2 mmol of **10**, and 3.8 mmol of 4-(4-methylphenyl)-1*H*-2,3-benzothiazine-1-thione **6a**, 4-(4-methoxyphenyl)-1*H*-2,3-benzothiazine-1-thione **6b**, 4-phenyl-1*H*-2,3-benzothiazine-1-thione **6c** or 4-[4-(diethylamino)-phenyl]-1*H*-2,3-benzothiazine-1-thione **6d** in 30 mL EtOH was heated under reflux for 15–20 h (TLC). After the usual workup, the resulting residue was collected, washed several times with pentane and recrystallized from the solvent indicated after the mp, to give the respective BPs **21a–21d**.

5.6.1. Tetraethyl 4-thioxo-1-p-tolyl-3,4-dihydroisoquinoline-3,3-diyldiphosphonate, ***21a***

Yield 72.8%. Yellow crystals, mp 212–214 °C (from MeOH). IR (cm^{-1}, KBr): ν_{max} 1562 (C═N), 1369 (C═S), 1252, 1239 (2P═O),

1161, 1105 (2P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 1.25, 1.31 (2dt, J_{H-H} = 6.6, $^4J_{P-H}$ = 3.4, 2 × 6H, 2($H_3CCO)_2$), 2.38 (s, 3H, H_3C–tolyl), 4.02, 4.27 (2dq, J_{H-H} = 6.6, $^3J_{P-H}$ = 4.6, 2 × 4H, 2($H_2CO)_2$), 7.36, 7.58, 8.04, 8.74 (4m, 8H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 240.9 (d, $^2J_{P-C}$ = 14.4, *C*=S), 178.6 (d, $^3J_{P-C}$ = 7.4, *C*=N), 145.8, 137.7, 134.2, 133.3, 130.9, 127.2, 126.8 (*C*–Ar), 90.1 (t, $^1J_{P-C}$ = 168.7, *C*–P_2), 61.1 (d, $^2J_{P-C=}$ 8.3, CH_2O), 21.5 (CH_3–tolyl), 16.3 (d, $^3J_{P-C}$ = 5.3 Hz, H_3CCO). ^{31}P NMR [$CDCl_3$] ppm: δ 20.7, 24.7 (2d, $^2J_{P-P}$ = 28, CP_2). EI-MS: in *m/z* (%): 524 (14) [M^+], 508 (22) [M^+ – 15, Me], 464 (36) [M^+ – (15 + 44) (Me + CS)], 234 (100) [M^+ – (15 + 274) {Me + ($C_4H_{10}O_3P)_2$}], 190 (85) [M^+ – (15 + 44 + 274) {Me + CS + ($C_4H_{10}O_3P)_2$}], 102 (48), 77 (79). Anal. Calcd for $C_{24}H_{31}NO_6P_2S$ (523.5): C, 55.06; H, 5.97; N, 2.68; P, 11.83; S, 6.13. Found: C, 55.11; H, 6.02; N, 2.74; P, 11.76; S, 6.05.

5.6.2. Tetraethyl 1-(4-methoxyphenyl)-4-thioxo-3,4-dihydroisoquinoline-3,3-diyldiphosphonate, **21b**

Yield 76.4%. Red crystals, mp 252–254 °C (dec.) (from EtOH). IR (cm^{-1}, KBr): ν_{max} 1572 (C=N), 1370 (C=S), 1265, 1252 (2P=O), 1161, 1110 (2P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 1.29, 1.35 (2dt, J_{H-H} = 7.6, $^4J_{P-H}$ = 3.3, 2 × 6H, 2($H_3CCO)_2$), 3.48 (s, 3H, H_3COAr), 4.19, 4.18 (2dq, J_{H-H} = 7.6, $^3J_{P-H}$ = 4.8, 2 × 4H, 2($H_2CO)_2$), 6.68, 6.70, 7.25, 8.41 (4m, 8H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 239.8 (d, $^2J_{P-C}$ = 15.6, *C*=S), 178.6 (d, $^3J_{P-C}$ = 8.8, *C*=N), 159.5, 145.4, 133.9, 131.7, 130.1, 126.3, 113.5 (*C*–Ar), 88.3 (t, $^1J_{P-C}$ = 166.7, *C*–P_2), 61.5 (d, $^2J_{P-C}$ = 133, CH_2O), 55.5 (CH_3O), 16.3 (d, $^3J_{P-C}$ = 6.4 Hz, H_3CCO). ^{31}P NMR [$CDCl_3$] ppm: δ 24.7, 26.8 (2d, $^2J_{P-P}$ = 29, CP_2). EI-MS: in *m/z* (%): 539 (<8) [M^+], 508 (33) [M^+ – 31, OMe], 464 (24) [M^+ – (31 + 44) (OMe + CS)], 234 (100) [M^+ – (31 + 274) {OMe + ($C_4H_{10}O_3P)_2$}], 190 (73) [M^+ – (31 + 44 + 274) {Me + CS + ($C_4H_{10}O_3P)_2$}], 102 (62), 77 (88). Anal. Calcd for $C_{24}H_{31}NO_7P_2S$ (539.5): C, 53.43; H, 5.79; N, 2.60; P, 11.48; S, 5.94. Found: C, 53.51; H, 5.84; N, 2.53; P, 11.39; S, 5.86.

5.6.3. Tetraethyl 1-phenyl-4-thioxo-3,4-dihydroisoquinoline-3,3-diyldiphosphonate, **21c**

Yield 74.7%. Yellow crystals, mp 176–178 °C (from EtOH). IR (cm^{-1}, KBr): ν_{max} 1564 (C=N), 1374 (C=S), 1261, 1250 (2P=O), 1162, 1113 (2P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 1.25, 1.62 (2dt, J_{H-H} = 7.6, $^4J_{P-H}$ = 3.6, 12H, H_3CC.OP), 4.42, 4.67 (2dq, J_{H-H} = 7.6, $^3J_{P-H}$ = 4.6, 2 × 4H, 2($H_2CO)_2$), 7.08–8.23 (m, 9H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 239.2 (d, $^2J_{P-C}$ = 14.4, *C*=S), 178.6 (d, $^3J_{P-C}$ = 6.8 Hz, *C*=N), 145.8, 137.7, 133.7, 133.2, 128.9, 127.3, 126.1 (*C*–Ar), 90.4 (t, $^1J_{P-C}$ = 166.7, *C*–P_2), 61.1 (d, $^2J_{P-C=}$ 8.3 Hz, CH_2OP), 16.4 (d, $^3J_{P-C}$ = 5.3, H_3CCO). ^{31}P NMR [$CDCl_3$] ppm: δ 20.7, 24.7 (2d, $^2J_{P-P}$ = 32, CP_2). EI-MS: in *m/z* (%): 509 (14) [M^+], 465 (28) [M^+ – 44, CS], 235 (100) [M^+ – 274 ($C_4H_{10}O_3P)_2$], 190 (92) [M^+ – (44 + 274) {CS + ($C_4H_{10}O_3P)_2$}], 102 (65), 77 (96). Anal. Calcd for $C_{24}H_{31}NO_6P_2S$ (509.5): C, 54.22; H, 5.74; N, 2.75; P, 12.16; S, 6.29. Found: C, 54.30; H, 5.81; N, 2.68; P, 12.09; S, 6.13.

5.6.4. Tetraethyl 1-(4-(diethylamino)phenyl)-4-thioxo-3,4-dihydroisoquinoline-3,3-diyldi-phosphonate, **21d**

Yield 76.2%. Red crystals, mp 129–131 °C (from cyclohexane). IR (cm^{-1}, KBr): ν_{max} 1559 (C=N), 1367 (C=S), 1261, 1258 (2P=O), 1160, 1100 (2P–O–C). ^{1}H NMR [$CDCl_3$] ppm: δ 0.93 (t, 6H, H_3CCN), 1.23, 1.69 (2dt, J_{H-H} = 7.6, $^4J_{P-H}$ = 3.6, 2 × 6H, 2($H_3CCO)_2$), 3.55 (q, 4H, H_2CN), 3.69, 3.73 (2dq, J_{H-H} = 7.6, $^3J_{P-H}$ = 4.6, 2 × 4H, 2($H_2CO)_2$), 7.21, 7.57, 7.63, 8.96 (4m, 8H, *H*–Ar). ^{13}C NMR [$CDCl_3$] ppm: δ 239.2 (d, $^2J_{P-C}$ = 14.4, *C*=S), 179.1 (d, $^3J_{P-C}$ = 6.8, *C*=N), 149.8, 147.7, 133.7, 131.2, 128.9, 127.3, 126.7 (*C*–Ar), 92.1 (t, $^1J_{P-C}$ = 168.7, *C*–P_2), 61.2 (d, $^2J_{P-C}$ = 8.3, CH_2O), 44.8 (CH_2N), 16.4 (d, $^3J_{P-C}$ = 5.3, $H_3CC.O$), 15.8 ($H_3CC)_2N$. ^{31}P NMR [$CDCl_3$] ppm: δ 21.7, 25.7 (2d, $^2J_{P-P}$ = 24, CP_2). EI-MS: in *m/z* (%): 581 (14) [M^+ + 1], 580 (10) [M^+], 508 (26) [M^+ – 72 (NEt_2)], 464 (33) [M^+ – (72 + 44) (NEt_2 + CS)], 234 (100) [M^+ – (72 + 274) ($C_4H_{10}O_3P)_2$)], 191 (87) [M^+ – (72 + 44 + 274) {NEt_2 + CS + ($C_4H_{10}O_3P)_2$}], 102 (76), 77 (88). Anal. Calcd for $C_{27}H_{38}N_2O_6P_2S$ (580.6): C, 55.85; H, 6.60; N, 4.82; P, 10.67; S, 5.52. Found: C, 55.92; H, 6.54; N, 4.77; P, 10.59; S, 5.45.

5.7. General procedure of BP-acids **15**, **19a**, **19b**, and **22**

Bisphosphonate **12b**, **18c**, **18d**, and **21d** (0.5 g) was dissolved in 15 mL of *conc* HCl, and the mixture was heated under reflux for ≈ 10 h (TLC). After concentrating the product mixture to its half, under reduced pressure, the crude material was diluted with AcOEt and water and then stirred for 30 min. The layers were separated, and the aqueous layer was evaporated to dryness. The precipitate was collected and dried to give the corresponding BP-acid **15**, **19a**, **19b**, and **22**.

5.7.1. 1-Methyl-3-thioxo-2,3-dihydro-1H-indole-2,2-diyldiphosphonic acid, **15**

Yield 84.8%. White material, mp > 300 °C (from MeOH). IR (cm^{-1}, KBr): ν_{max} 3340–3325 (P–OH), 1329(C=S), 1232–1220 (P=O, bonded). ^{1}H NMR [DMSO/D_2O] ppm: δ 3.14 (d, $^4J_{P-H}$ = 3.3, 3H, H_3CN), 7.42, 7.85 (2d, J_{H-H} = 8.1, 2 × 2 H, *H*–Ar). ^{31}P NMR [DMSO/D_2O] ppm: δ 21.5, 23.7 (CP_2). EI-MS: in *m/z* (%): 319 (30) [M^+ – 4]. Anal. Calcd for $C_9H_{11}NO_6P_2S$ (323.2): C, 33.45; H, 3.43; N, 4.33; P, 19.17; S, 9.92. Found: C, 33.51; H, 3.51; N, 4.28; P, 19.25; S, 9.97.

5.7.2. 2-Cyclohexyl-4-thioxo-3,4-dihydroquinoline-3,3-diyldiphosphonic acid, **19a**

Yield 77.3%. White material, mp > 300 °C (from MeOH). IR (cm^{-1}, KBr): ν_{max} 3338–3332 (P–OH), 1556 (C=N), 1322 (C=S), 1233–1219 (P=O, bonded). ^{1}H NMR [DMSO/D_2O] ppm: δ 1.98 (m, 10H, CH_2–hexyl), 3.43 (m, 1H, C*H*–hexyl), 7.65, 7.88 (2d, J_{H-H} = 8.2, 2 × 2H, *H*–Ar). ^{31}P NMR [DMSO/D_2O] ppm: δ 20.6–21.8 (broad, CP_2). EI-MS: in *m/z* (%): 399 (21) [M^+ – 4]. Anal. Calcd for $C_{15}H_{19}NO_6P_2S$ (403.3): C, 44.67; H, 4.75; N, 3.47; P, 15.36; S, 7.95. Found: C, 44.73; H, 4.81; N, 3.41; P, 15.29; S, 7.88.

5.7.3. 6-Bromo-2-methyl-4-thioxo-3,4-dihydroquinoline-3,3-diyldiphosphonic acid, **19b**

Yield 80.7%. White material, mp > 300 °C (from MeOH). IR (cm^{-1}, KBr): ν_{max} 3342–3325 (P–OH), 1559 (C=N), 1322 (C=S), 1229–1217 (P=O, bonded). ^{1}H NMR [DMSO/D_2O] ppm: δ 2.49 (s, 3H, H_3C), 7.50, 7.75 (2d, J_{H-H} = 8.1, 2 × 1H, *H*–Ar), 8.58 (s, 1H, *H*–Ar). ^{31}P NMR [DMSO/D_2O] ppm: δ 20.4, 23.7 (CP_2). EI-MS: in *m/z* (%): 410 (52) [M^+ – 4]. Anal. Calcd for $C_{10}H_{10}BrNO_6P_2S$ (414.1): C, 29.00; H, 2.43; Br, 19.30; N, 3.38; P, 14.96; S, 7.74. Found: C, 29.08; H, 2.51; Br, 19.22; N, 3.31; P, 15.08; S, 7.81.

5.7.4. 1-(4-(Diethylamino)phenyl)-4-thioxo-3,4-dihydroisoquinoline-3,3-diyldiphosphonic acid, **22**

Yield 72.4%. White material, mp > 300 °C (from MeOH). IR (cm^{-1}, KBr): ν_{max} 3342–3325 (P–OH), 1550 (C=N), 1328 (C=S), 1228–1222 (P=O, bonded). ^{1}H NMR [DMSO/D_2O] ppm: δ 0.94 (t, 6H, $(H_3CC)_2N$), 3.54 (q, 4H, $(H_2C)_2N$), 7.21, 7.57, 7.63, 8.96 (4m, 8H, *H*–Ar). ^{31}P NMR [DMSO/D_2O] ppm: δ 19.5, 22.8 (broad, CP_2). EI-MS: in *m/z* (%): 464 (18) [M^+ – 4]. Anal. Calcd for $C_{19}H_{22}N_2O_6P_2S$ (468.4): C, 48.72; H, 4.73; N, 5.98; P, 13.23; S, 6.85. Found: C, 48.81; H, 4.79; N, 5.89; P, 13.16; S, 6.77.

5.8. Pharmacology

5.8.1. Antitumor activity screening

Following the technique, previously reported by Skehan et al. [30], antitumor activity screening of BPs **12b**, **18a**, and **21a** was evaluated at doses of 0, 5, 12.5, 25, and 50 μM/kg. Four different human carcinoma cell lines, representing breast, cervix, liver, and

colon were utilized based on comparison to the behavior of **DOX** or **CIS** (Table 1).

5.8.2. Methods and materials

(i) Cells were plated in 96-multivated plate (104 cells/well) for 24 h before treatment with compounds to allow the attachment of cells to the wall of the plate; (ii) different concentrations of each compound under test (5, 12.5, 25 and 50 μg/mL) were added to the cell monolayer triplicate wells that prepared for each individual dose. Each concentration is evaluated three times (each dose is incubated with the cells in three different wells) and the monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2; (iii) after 48 h, cells were fixed, washed and stained with sulfo-rhodamine-B-Stain; (iv) excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer (pH 7.4); (v) color intensity was measured in an ELISA plate reader; (vi) The relation between surviving fraction and drug concentration is displayed in Table 1, and it is plotted to get the survival curve of each tumor cell after the specified examined compound (Fig. 1).

5.9. Adjuvant-induced polyarthritis

Groups of 10 male rats (200 g) were challenged with an intradermal injection of complete Freund's adjuvant (*Mycobacterium tuberculosis* in mineral oil) in the left hindpaw on day 0. Tested compounds were dissolved or suspended in sterile saline and sonicated where appropriate to homogeneous doses. All compounds were then adjusted to pH 7.0 with 1 N NaOH and stored frozen in aliquots. Fresh aliquots were used for each day of dosing. Rats were dosed once daily (subcutaneous, sc) for 28 days on a mg/kg of body weight basis. The normal rat control group received vehicle po (prolecebo) and the arthritic rat control group received vehicle sc. Changes in paw volume over time (APV) which occurred in the arthritic control and treated rats (injected and noninjected hindpaw) were quantitated on day 28 by mercury displacement plethysmography. Results were analyzed by one way analysis of variants, then Student's unpaired *t* test. The results were displayed in Table 2.

5.10. Delayed type hypersensitivity granuloma

Groups of 10 female mice (25 g) were sensitized with an emulsion of methylated bovine serum albumin (mBSA) in saline with Freund's incomplete adjuvant and dextran by sc injection over the inguinal lymph node. Three weeks later, hydroxyapatite (HA) discs (6-mm diameter) soaked in mBSA solution (30 mg/mL saline) were implanted sc in the dorsum of the mice (two discs, bilaterally). All drugs were prepared as solutions, suspensions, or emulsions, and the pH was adjusted to 7.4 with 0.1 M NaOH. Each mouse received compound in a volume of 0.1 mL/10 g body weight sc in the scruff of the neck. Dosing commenced on the day of implantation of the mBSA soaked discs and was continued thereafter on a daily basis until day nine, when the mice were euthanized. The granulomatous lesions were then excised and both wet and dry tissue weights measured. Results were analyzed by Student's paired *t* test. The results were displayed in Table 2.

5.11. Toxicity evaluation

The LD_{50} determination of the most promising synthesized anti-inflammatory active BPs (**12b**, **14b** and **21d**) 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 (**12b**, **14b** and **21d**) dissolved in the same *vehicle solution* in 500, 750, and 1000 mg/kg (body weight) were given intra-peritoneally. 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 kill 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 $(z \times d)$.

Acknowledgment

We thank National Cancer Institute, Cairo University, Egypt, for carrying out the anticancer screening.

Appendix A. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.02.047.

References

[1] T.V. den Wyngaert, M.T. Huizing, E. Fossion, J.B. Vermorken, Bisphosphonates in oncology: rising stars or fallen heroes, The Oncologist 14 (2009) 181–191.
[2] A. Lipton, R.L. Theriault, G.N. Hortobagyi, J. Simeone, R.D. Knight, K. Mellars, D.J. Reitsma, M. Heffernan, J. Seaman, Pamidronate prevents skeletal complications and is effective palliative treatment in women with breast carcinoma and osteolytic bone metastases, J. Cancer 88 (2000) 1082–1090.
[3] T. Yoneda, A. Sasaki, C. Dustan, P.J. William, F. Bauss, Y.A. De Clerck, G.R. Mundy, Inhibition of osteolytic bone metastasis of breast cancer by combined treatment with the bisphosphonate ibandronate and tissue inhibitor of the matrix metalloproteinase-2, J. Clin. Invest. 99 (1997) 2509–2517.
[4] M. Busch, M. Rave-Frank, A. Hille, E. Duhmke, Influence of clodronate on breast cancer cells in vitro, Eur. J. Med. Res. 3 (1998) 427–431.
[5] J.E. Brown, E.C. Robert, The role of bisphosphonates in breast cancer: the present and future role of bisphosphonates in the management of patients with breast cancer, Breast Cancer Res. 4 (2002) 24–29.
[6] W.M. Abdou, R.E. Khidre, A.A. Kamel, Elaborating on efficient anti-proliferation agents of cancer cells and anti-inflammatory-based on N-bisphosphonic acids, Arch. Pharm. Chem. Life Sci. 345 (2012) 123–136.
[7] W.M. Abdou, A.A. Kamel, R.E. Khidre, A. Geronikaki, M.T. Ekonomopoulou, Synthesis of 5- and 6- N- heterocyclic methylenebisphosphonate derivatives of cytogenetic activity in normal human lymphocyte cultures, Chem. Biol. Drug Des., in press.
[8] W.M. Abdou, M.D. Khidre, A.A. Sediek, The design and synthesis of sulfur and nitrogen containing bisphosphonic acids and their role in oncology, in: V.G. Kartsev (Ed.), The Chemistry and Biologically Activity of Synthetic and Natural Compounds, Modern Aspects of Chemistry of Heterocycles, Russian Academy of Natural Science, 2010, pp. 209–212.
[9] R.A. Baron, R. Tavaré, A.C. Figueiredo, K.M. Baewska, B.A. Kashemirov, C.E. McKenna, F.H. Ebetino, A. Taylor, M.J. Rogers, F.P. Coxon, M.C. Seabra, Phosphono-carboxylates inhibit the second geranylgeranyl addition by rab geranylgeranyl transferase, Biol. Chem. 284 (2009) 6861–6868.
[10] L.I. Plotkin, J.I. Aguirre, S. Kousteni, S.C. Manolagas, T. Bellido, Bisphosphonates and estrogens inhibit osteocyte apoptosis via distinct molecular mechanisms downstream of extracellular signal-regulated kinase activation, J. Biol. Chem. 280 (2005) 7317–7325.
[11] R.G. Russell, Bisphosphonates: from bench to bedside, Ann. NY Acad. Sci. 1068 (2006) 367–401.
[12] W.M. Abdou, A.A. Shaddy, An efficient practical synthesis of thiobisphosphonic acids for the treatment of arthritis, based on the chemistry of disulfides, J. Med. Chem. Res. 19 (2010) 39–40.
[13] W.M. Abdou, R.E. Khidre, A.A. Shaddy, Chemical synthesis of anti-inflammatory tetrazoloquinoline-based mono and bisphosphonate esters, J. Heterocyclic Chem. (2011) (accepted for publication).
[14] W.M. Abdou, A.A. Kamel, A.A. Shaddy, Use of phosphonyl carbanions in the synthesis of anti-inflammatory active phosphorus-containing fused heterocycles and relevance phosphonates, Eur. J. Med. Chem. 45 (2010) 5217–5224.
[15] W.M. Abdou, A.A. Shaddy, The development of bisphosphonates for therapeutic uses and bisphosphonate structure-activity consideration, Arkivoc 14 (2009) 143–182.

[16] W.M. Abdou, N.A. Ganoub, A.F.M. Fahmy, A.A. Shaddy, Symmetrical and asymmetrical bisphosphonate esters. Synthesis, selective hydrolysis, and isomerization, Monatsh. Chem. 136 (2006) 105–116.
[17] W.M. Abdou, N.A. Ganoub, Y.O. EL-Khoshnieh, Synthesis of new type of bisphosphonates bearing S- and N-Heterocycles, based on the reactions of methylene-bisphosphonate with alkenes, Synlett (2003) 785–790.
[18] A.V. Stepanchikova, A.A. Lagunin, D.A. Filimonov, V.V. Poroikov, Prediction of biological activity spectra for substances: evaluation on the diverse set of drug-like structures, Curr. Med. Chem. 10 (2003) 225–233.
[19] V.V. Poroikov, D.A. Filimonov, How to acquire new biological activities in old compounds by computer prediction, J. Comput. Aid. Mol. Des. 16 (2003) 819–824 Website: http://www.ibmc.msk.ru/PASS.
[20] C.H. Da Silva, V.B. Da Silva, J. Resende, P.F. Rodrigues, F.C. Bononi, C.G. Benevenuto, C.A. Taft, Computer-aided drug design and ADMET predictions for identification and evaluation of novel potential farnesyltransferase inhibitors in cancer therapy, J. Mol. Graph. Model. 28 (2010) 513–523.
[21] K. Mustafayeva, C. Di Giorgio, R. Elias, Y. Kerimov, O.E. Evelyne, D.M. Michel, DNA-Damaging, mutagenic, and clastogenic activities of gentiopicroside isolated from cephalaria kotschyi roots, J. Nat. Prod. 73 (2010) 99–103.
[22] D.T. Connor, M. von Strandtmann, Reaction of (carbethoxymethylene)triphenyl-phosphorane with omega-nitrostyrenes and isatoic anhydrides, J. Org. Chem. 38 (1973) 1047–1049.
[23] A.A. Kamel, W.M. Abdou, Alkylidenephosphoranes in heterocyclic synthesis. Reactivity of benzoxazinones with resonance-stabilized phosphorus ylides, Synlett (2007) 1269–1273.
[24] W.M. Abdou, A.A. Kamel, Remarkably efficient and direct route to quinolines and benzoazepines from the condensation of benzoxazinediones with phosphonium carbanion salts, Synth. Commun. 37 (2007) 3945–3960.
[25] F.G. Baddar, A.F.M. Fahmy, N.F. Aly, 2,3-Benzoxazin-1-ones. Part I. Reactions of aluminum chloride, sulphuric acid, and hydrazine hydrate with 4-aryl-2,3-benzoxazin-1-ones, J. Chem. Soc., Perkin Trans. 1 (1973) 2448–2450.
[26] W.M. Abdou, A.F.M. Fahmy, A.A. Kamel, Study of insertion reactions with phosphorus ylides. On reactions between 4-(4-methylphenyl)-2,3-benzoxazin-1-one and alkylidene phosphoranes, Eur. J. Org. Chem. 10 (2002) 1696–1701.
[27] W.M. Abdou, A.A. Kamel, M.D. Khidre, Phosphono substituted-isoindolines and indoles from 2,3- and 2,4-benzoxazin-1-ones, Heteroatom Chem. 15 (2004) 77–84.
[28] W.M. Abdou, A.A. Kamel, M.D. Khidre, Further insight into the reactivity of oxazinones toward phosphorus reagents, Synth. Commun. 34 (2004) 4119–4134.
[29] H. Fleisch, Bisphosphonates: a new class of drugs in diseases of bone and calcium metabolism, in: P.F. Baker (Ed.), In Handbook of Experimental Pharmacology, vol. 83, Springer, Berlin/Heidelberg, 1988, pp. 441–465.
[30] P. Skehan, R. Storeng, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Nat. Cancer Inst. 82 (1990) 1107–1112.
[31] W.K. Siestema, F.H. Ebetino, Bisphosphonates in development for metabolic bone disease, Expert Opin. Invest. Drugs 3 (1994) 1255–1276.
[32] N.G. Almstead, S.M. Dansereay, M.D. Francis, C.M. Snider, F.H. Ebetino, The design and synthesis of sulfur containing bisphosphonic acids for the treatment of arthritis, Phos. Sulf. Silicon 144–146 (1999) 325–328.
[33] R.A. Nugent, M. Murphy, S.T. Schlachter, C.J. Dunn, R.J. Smith, N.D. Staite, L.A. Galinet, S.K. Shields, D.G. Aspar, K.A. Richard, N.A. Rohloff, Pyrazoline bisphosphonate esters as novel antiinflammatory and antiarthritic agents, J. Med. Chem. 36 (1993) 134–139.
[34] C.J. Dunn, L.A. Galinet, A.J. Gibbons, S.K. Shields, Murine delayed type hypersensitivity granuloma: an improved model for the identification and evaluation of different classes of antiarthritic drugs, Int. J. Immunopharmacol. 12 (1990) 899–904.
[35] O. Teronen, Y.T. Konttinen, C. Lindqvist, T. Salo, A. Lauhio, Y. Ding, S. Santavirta, T. Sorsa, Human neutrophil collagenase MMP-8 in peri-implant sulcus fluid and its inhibition by clodronate, J. Dent. Res. 76 (1997) 1529–1537.
[36] W. Walter, T. Fleck, J. Vob, M. Gerwin, Über die struktur der thioamide und ihrer derivate, XXXII darstellung sowie IR- und 1H NMR-spektroskopische untersuchung von 2-aminobenzamiden und – thiobenzamiden, Justus Liebigs Ann. (1975) 275–294.
[37] M.F. Abdel-Megeed, A. Teniou, 1-Aryl-3-(3,4-dihydro-4-oxo-3-quinazolinyl) urea fungicidal agents, Rev. Roum. Chim. 33 (1988) 981–986.
[38] K. Clausen, S.-O. Lawesson, Studies on organophosphorus compounds XXIX–thiation of ethyl 2-acylaminobenzoates, Bull. Soc. Chim. Belg. 88 (1979) 305–311.
[39] M.M. Mahmoud, M.A. El-Hashash, Some reactions with 4H-3,1-benzoxazin-4-one and some studies on the growth of bacteria, Rev. Roum. Chim. 24 (1979) 849–858.
[40] M.A. El Hashash, M.A. Sayed, Some reactions with 2-benzyl-4H-3,1-benzoxazin-4-one, 6-bromo-2-methyl-4H-3,1-benzoxazin-4-one and 2-benzyl-3-phenyl-4(3H)-quinazolinone, Egypt. J. Chem. 21 (1978) 115–131.