Full Paper

Carbodiimides in the Synthesis of Enamino- and α-Aminophosphonates as Peptidomimetics of Analgesic/Antiinflammatory and Anticancer Agents

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Carbodiimide that was generated from the condensation reaction of iminophosphorane with phenylisocyanate was allowed to react with different phosphorus nucleophiles. Thus, the *in situ* resulted heterocumulene reacted with dialkylhydrogenphosphonates in tetrahydrofuran (THF)/$FeCl_3$/H_2O system to give fused pyrrole- (≈14%) and pyrimidinephosphonates (≈57%). On the other hand, with *tris*-(dialkyl)aminophosphines, the reaction afforded the corresponding hexaalkylphosphinic diamides as a water-sensitive fine powder, quite stable for a few days in a desiccator. When a protonating agent was present in the reaction medium, the reaction was markedly accelerated leading to the formation of the phosphamides. Next, some saturated and unsaturated Horner–Emmons reagents were applied *in situ* to the same carbodiimide to obtain more phosphorylated *N*-heterocycles. The analgesic and antiinflammatory activities of the newly synthesized compounds were investigated and showed significant activities. Finally, we further estimated the antitumor activity of five new phosphonates against four carcinoma cell lines.

Keywords: α-Aminophosphonates / Analgesic/antiinflammatory agents / Antitumor bioassay / Enaminophosphonates / Horner–Emmons reagents / Pyrazoles

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Introduction

Due to the structural similarity of α-aminophosphonates with α-amino acids the former have received an increasing attention. The potential of α-aminophosphonates as enzyme inhibitors and antibiotics as well as pharmacological agents has been established [1–3]. Their inhibitory activity on protein tyrosine phosphatases (PTPs) is proposed and a recent review article in regard to heterocyclic glycopeptide-mimetics as PTPs inhibitors has recently been reported [4].

Although a variety of synthetic methods of α-aminophosphonates are available, approaches to enaminophosphonates are somewhat even less known. In connection with our interest in preparation of β-enamino- and α-aminophosphonates [5–11], we have recently reported a strategy for the synthesis of a series of these compounds. The latter arose from one-pot three-component synthesis, *via* Kabachnik–Fields reaction, starting from substituted amines, aldehydes, and tri-/dialkyl phosphites, in the presence of tetrahydrofuran (THF)–$FeCl_3$ (10%) solution [12]. In this article, we report a convenient approach to the synthesis of substituted *N*-heterocylces enamino- and α-aminoposphonates from the reaction of the *in situ* generated heterocumulene with dialkylhydrogenphosphonates **5a–c**, *tris*-(dialkyl)aminophosphines **10a**,**b**, as well as some saturated **14a–d**, and unsaturated Horner–Emmons reagents **18**, **20**. The effects of selected compounds on carrageenin-induced hind paw edema and *p*-benzoquinone-induced writhing tests as well as their anticancer activity against several different cancer cell lines were investigated.

Results and discussion

Synthesis of the required α-amino-/enaminophosphonates is outlined in Schemes 1–6. First, the iminophosphorane **2** was obtained from the reaction of ethyl 4-amino-5-cyano-1-phenyl-1*H*-pyrazole-3-carboxylate (**1**) [13] with *in situ* generated dichlorotriphenylphosphorane using a hexachloroethane-triphenylphosphine-triethylamine reagent system.

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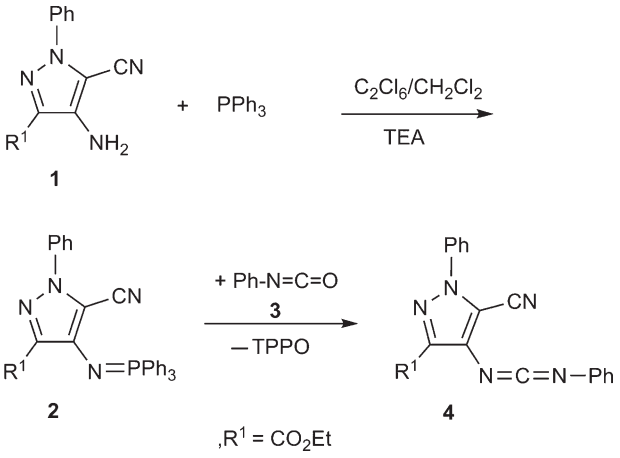
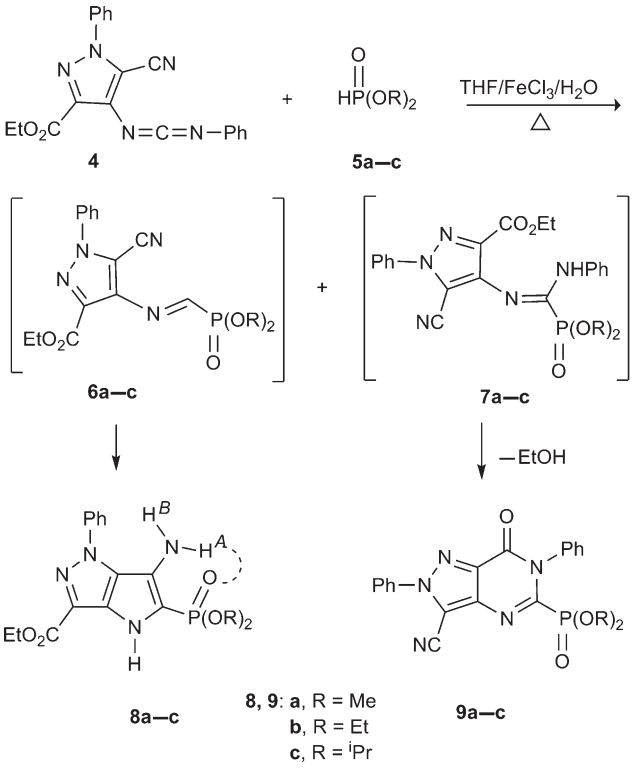


Scheme 1. Preparation of the carbodiimide **4**.



Scheme 2. Preparation of the phosphonates **8a**–**c** and **9a**–**c**.

Subsequent treatment of **2** with phenylisocyanate (**3**) in THF solution resulted in the formation of the carbodiimide **4** and triphenylphosphine oxide (TPPO) *via* aza-Wittig reaction (Scheme 1) [14].

The heterocumulene **4** is highly unstable, and should be quickly freed from TPPO, followed by adding dialkylhydrogenphosphonates **5a**–**c** in THF–$FeCl_3$ (aq.) solution. The reaction mixture was refluxed for ≈6 h to give after the usual workup the two phosphonates **8** and **9** (Scheme 2).

The minor product **8a** (14%) was analyzed correctly for $C_{16}H_{19}N_4O_5P$: *m*/*z* (%): 378 (100) [M^+], this is equivalent to an additional product (**4** + **5a**) minus 91 [M^+−NPh]. The structure of **8** was based on the spectroscopic data. The IR spectra ν_{max}, cm^{-1} of **8a**–**c** revealed, in each case, no bands at ≈2220 cm^{-1} due to the nitrile group (CN), and a showed strong absorption band at ≈1710 assigned to the carboxylate carbonyl (C=O) stretching (these excluding any cycloaddition reaction involving the carboxylate group). Moreover, the ^{1}H NMR spectrum of **8a**, for example, revealed two signals at δ ppm 1.31 (t) and 3.64 (q) due to the ethoxy (ester) protons. The phosphonate moiety [$P(OCH_3)_2$] was located as a doublet ($^3J_{P-H}$ = 11.6 Hz) at 4.38, whereas the amino group exhibited two types of (H) protons, [(δ (H^A) = 6.98 (br, 1H) and δ (H^B) = 9.01 (br, 1H)]. The different chemical shifts of the NH_2-protons are the spectroscopic evidence for the presence of an intramolecular hydrogen bond between one of the NH_2-protons and the oxygen atom of the P=O binding in the phosphonate group. The ^{13}C NMR spectra of **8** displayed, among others, three signals at ≈159, 128, 125 (d, J_{P-C} = ≈196.3 Hz) ppm assignable to *C*=O (ester), *C*–NH_2, and *C*–P, respectively. Their ^{31}P NMR spectra showed a sharp singlet at δ_p (DMSO-d^6) ≈24 ppm. The formation of the products **8** is assumed to take place *via* the nucleophilic addition of the phosphite-phosphorus to the carbodiimide **4** in tandem of the fission of the imino-double bond (C=NPh) [15, 16], giving rise to the intermediate **6**, following intramolecular cyclization, and hydrolysis of the nitrile moiety afforded **8** (due to the competitive interaction with the carboxylate moiety [17, 18]). The presence of the $FeCl_3$ solution may have provoked the reduction of the nitrile group [14]. Furthermore, intramolecular cyclization of the intermediate **6** through the interaction of CN in **4** has previously been observed on similar occasions [19].

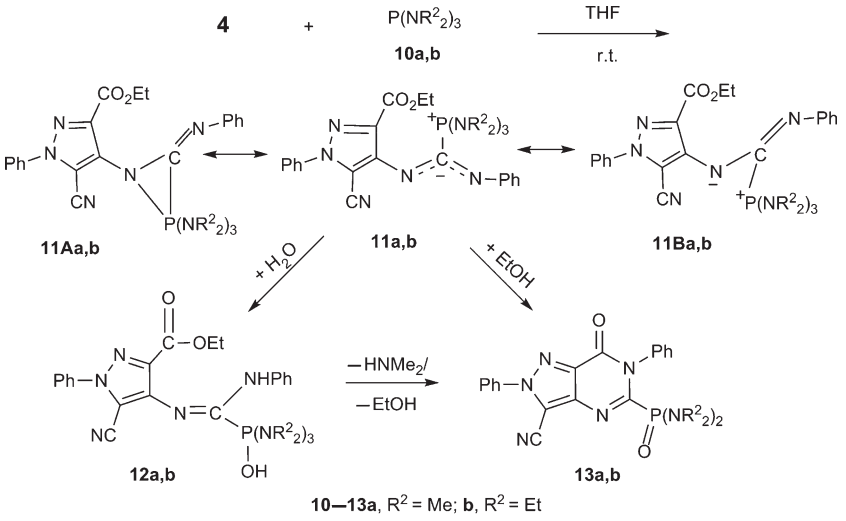
The IR spectra of **9a**–**c** showed strong absorption bands at ν_{max}, cm^{-1}: ≈2230, 1685, and 1590 assigned to nitrile (CN), C=O vibration of cyclic imide, and the imino (C=N) stretching. The major product **9a** (58%) gave microanalysis for the formula $C_{20}H_{16}N_5O_4P$ (*m*/*z* (%): 421(22), which is equivalent to a 1:1 addition product minus 46 (C_2H_5OH). In the ^{1}H NMR spectrum (DMSO-d^6) of **9a**, the characteristic signal at δ_H 4.18 ppm was assigned to the methoxy-phosphorus ester group while the aromatic protons were displayed in the region 7.29–8.18 ppm. The ^{31}P NMR spectra of **9** showed a sharp singlet at δ_p (DMSO-d^6) ≈27 ppm. The structure **9** was assumed to be formed *via* the parallel intermediate **7**, which underwent intramolecular cyclization and subsequent aromatization accompanied with concomitant loss of an ethyl alcohol molecule under the reaction conditions to afford the

final enaminone-phosphonate products **9a–c** as depicted in Scheme 2.

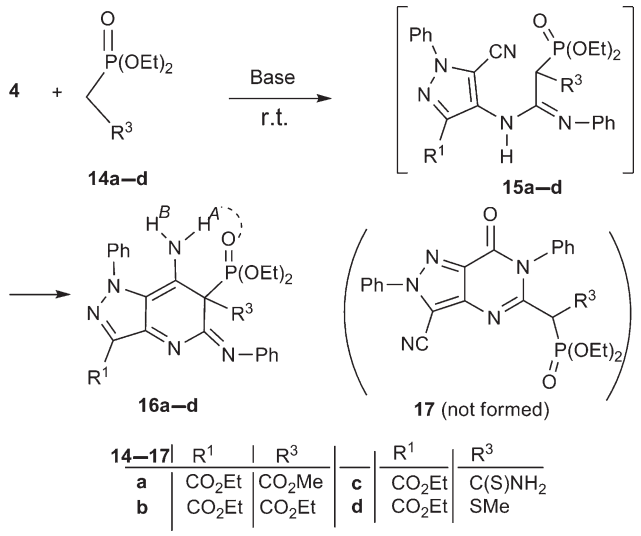
The carbodiimide **4** reacted also with *tris*-(dialkylamino)-phosphines **10a**,**b** in THF at room temperature (r.t.). The reaction was rapid and exothermic at 5–10°C. The resulting crystalline 1:1 adduct (≈84% yield) is formulated as *tris*-(dialkylamino)methylenephosphoranes **11a**,**b**, **11Aa**,**b**, **11Ba**,**b** from the spectral data presented below. Evidently, the unsaturated carbodiimide (–N=C=) the carbon, but the resulting *C*-phosphonium-betaines **11** and/or **11B** may rearrange also to the cyclic structure **11A**. In the ^{1}H NMR spectrum of **11a**, the 18 ^{1}H of the six methyl groups attached to nitrogen gave a doublet (J_{P-H} = 9.8 Hz) at 2.83 ppm; and the ^{31}P NMR shift of the amino-ylide **11a** was displayed at δ_p 58.3 ppm, which favor the open structure [20]. Compounds **11** are water sensitive, quite stable for a few days in a desiccator (Scheme 3). When a protonating agent (*e.g.*, 1 mL H_2O) was present in the reaction medium, the reaction was markedly accelerated leading to the formation of the phosphonic diamides **13a**,**b** (≈80%). Furthermore, when compounds **11** were boiled in alcohol, **13a**,**b** were again obtained. Obviously, compounds **13a**,**b** were formed through the initial formation of **11**, followed by quenching a molecule of H_2O to give **13** *via* the intermediate **12** with concomitant loss of a dimethyl amine and an ethanol molecule (Scheme 3). The spectroscopic data of **13** confirmed the suggested structure, the ^{31}P NMR signal was found at $\delta_p \approx 34$ ppm [21].

The behavior of heterocumulene **4** toward some phosphonyl carbanions was next investigated, and the reactions are displayed in Schemes 4–6. Thus, when the *in situ* generated carbodiimide **4** was treated with ≈1.3 equivalents of diethylmethylphosphonoacetate (**14a**) in alcoholic sodium ethoxide solution (or LiH/DMF) at r.t., the fused pyridine-phosphonate derivative **16a** (68%) was the only isolated product. A similar treatment of **4** with triethyl methylphosphonate (**14b**), diethyl (2-amino-2-thioxoethyl)-phosphonate (**14c**), or diethyl (methylthiomethyl)-phosphonate (**14d**), the reaction yielded the respective analogs **16b–d** in 66, 72, and 74% yield, respectively. The other possible structure **17** (based on a competition between the nitrile group and the carboxylate moiety) was ruled out on the basis of the spectroscopic data of the isolated products. For example, the IR spectra ν_{max}, cm^{-1} of **16a–d** showed the characteristic bands due to the amino (≈3480, 3370), the exocyclic imino (≈1633), the two carbonyl esters (1708, 1694), and the P=O groups (1227, bonded) as well as the disappearance of the band at ≈2233 cm^{-1} corresponding to CN. The ^{1}H NMR spectrum of **16a** (δ_p = 27.9 ppm) showed, as previously described, two signals of NH_2 protons [δ (H^A) = 6.02 (br, 1H) and δ (H^B) = 9.67 ppm (br, 1H)]. The fused pyridines **16** were formed *via* cyclization and transformations of the cyano group of the initially 1:1 addition intermediate **15** (Scheme 4).

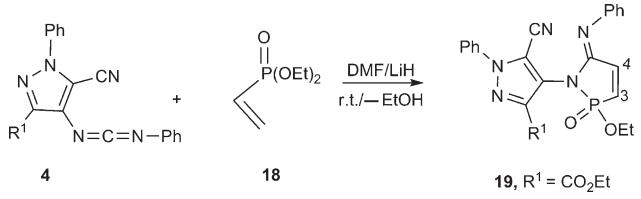
Conversely, the direct reaction of the *in situ* generated carbodiimide **4** with the unsaturated Horner–Emmon reagent, diethyl vinylphosphonoate **18** in dimethylformamaide (DMF) solution containing an excess of lithium hydride (LiH) at r.t., produced the azaphosphone **19** (72%). The formation of **19** can have resulted through Diels–Alder addition accompanied with an extrusion of an ethanol molecule in a one-step reaction (Scheme 5) [16]. The azaphosphone **19** was proved by IR, NMR, and mass spectroscopic analyses. Its IR spectrum exhibited absorption bands due to the stretching vibrations of CN, C=O (ester), P=O (free), and P–O–C groups at ν_{max}, cm^{-1}: 2222, 1712, 1260, and 1083, respectively. Its ^{1}H NMR spectrum (δ_p = 15.3 ppm) showed the *H*(3)- and *H*(4) of the azaphosphone ring as two doublets at 7.55 ($^2J_{P-H}$ = 17.2 Hz) and 6.95 ($^3J_{P-H}$ = 6.7 Hz) ppm. The latter's were attested in its ^{13}C NMR spectrum at 123.8



Scheme 3. Preparation of phosphoniomethylamides, **11a**,**b**, and phosphonic diamides, **13a**,**b**.



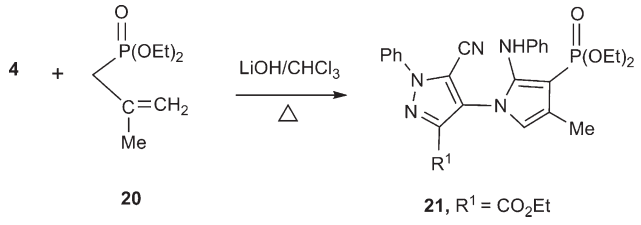
Scheme 4. Preparation of the phosphonates **16a–d**.



Scheme 5. Preparation of the azaphosphone **19**.

(d, $^{1}J_{P-C} = 144$ Hz, *C*(3)) and 138.63 (d, $^{2}J_{P-C} = 8.5$ Hz, *C*(4)) ppm, respectively.

Finally, coupling reaction of **4** with diethyl (2-methylallyl)-phosphonate (**20**) in a mixture of $LiOH/H_2O/CHCl_3$ at r.t. yielded ethyl 5-cyano-4-(3-(diethoxyphosphoryl)-4-methyl-2-(phenylamino)-1*H*-pyrrol-1-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**21**, 70%; Scheme 6). The ^{1}H NMR spectrum of **21** displayed the methine proton of the pyrrole ring as a doublet ($^{4}J_{P-H} = 4.2$ Hz) at 6.24 ppm. Its ^{13}C NMR spectrum exhibited, among others, signals at 158.9 (*C*=O, ester), 129.9 (d, $^{1}J_{P-C} = 211.8$ Hz, *C*–P), 116.2 [C(2)-pyrrole] ppm. α-Aminophosphonate **21** may be regarded as a result of (3 + 2) cycloaddition as depicted in Scheme 6.



Scheme 6. Preparation of the phosphonate **21**.

In summary, we have developed a facile and efficient regioselective method for the preparation of a new series of α-amino-/enaminophosphonates in moderate to high yields. Carbodiimide that was generated *via* the aza-Wittig reaction of iminophosphorane with phenylisocyanate is the key intermediate and could react with different types of phosphorus reagents. This attractive method is effective for the reaction of dialkylhydrogenphosphonates, trisaminophosphines as well as saturated and unsaturated phosphonyl carbanions with carbodiimide, and provides excellent yields of the products in a very short time, which makes it a novel and economically viable process.

Pharmacological evaluation

Inflammation is a natural and beneficial reaction in response to infections and trauma. Inflammation process begins when an unknown antigen gains access to patient's tissue and combines with antibody in the joint. This activates an antigen complement–antibody immune complex, which precipitates in the synovial and joint fluid. In turn, it leads to the release of chemical mediators that cause migration of polymorphonuclear leukocytes phagocytizing the immune complex, the lysosomal membrane discharges protease and collagenate, causing continued inflammation, tissue destruction, and loss of physical properties of the connective tissue and joints. Management of inflammatory disorder involves the use of therapeutic agents for relieving pain and reducing inflammation. Physical therapy, non-steroidal antiinflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), and finally, immunosuppressive agents are the generally accepted stepwise approach to achieve these goals.

Among different types of NSAIDs, pyrazoles and fused pyrazole with six-membered rings [22, 23] occupy focus position to be used as analgesic and antiinflammatory agents. Furthermore, our previous findings with certain pyrazole-based α-aminophosphonates resulted in derivatives with potent *in vivo* antiinflammatory effects, with no toxic symptoms [12]. Also, based on the previous findings that indicated pyrazole-nucleus as suitable for anticancer activity [24, 25], bioscreening of selected synthesized substituted pyrazole phosphorus derivatives was determined. In sequel, by keeping the pyrazole core structure intact, we studied the effect of different phosphorus containing moieties side chains: **9a**,**b**, **13a**,**b**, **16a–d**, **19**, and **21** on their analgesic, antiinflammatory, and anticancer activities. Substrate **1** was also tested to reflect the effect of its transformations to our products.

Analgesic evaluation

Evaluation of analgesic activity of the synthesized compounds was assessed *in vivo* in mice using the *p*-benzoquinone-induced writhing test [26]. Aspirin was used as the positive control in

Table 1. Analgesic and antiinflammatory effects of **9a**,**b**, **13a**,**b**, **16a–d**, **19**, and **21**.

Test compds	Number of writhings ± SEM (Analgesic activity, %)[a)]	Swelling in thickness ($\times 10^{-2}$ mm) ± SEM (inhibtion of edema, (%))[b)]			
		90 min	180 min	270 min	360 min
Control	28.2 ± 2.8	38.0 ± 2.0	50.0 ± 3.0	74.0 ± 3.0	77.0 ± 3.0
9a*	11.5 ± 3.6 (59.2%)*	34.0 ± 4.0 (10.5%)*	28.0 ± 8.0 (44%)*	31.0 ± 5.0 (58.1%)***	44.0 ± 5.0 (42.8%)***
9b*	12.2 ± 4.2 (56.9%)*	33.5 ± 5.8 (11.8%)**	30.6 ± 5.0 (38.8%)**	58.0 ± 4.0 (21.6%)***	45.0 ± 4.0 (41.5%)**
13a*	24.0 ± 3.4 (14.9%)**	37.2 ± 5.0 (2.1%)*	46.0 ± 4.0 (8%)**	69.6 ± 5.0 (5.9%)**	62.0 ± 7.0 (19.4%)*
13b*	25.1 ± 3.6 (10.9%)*	36.0 ± 6.1 (5.2%)**	47.0 ± 4.0 (6%)***	70.0 ± 4.0 (5.4%)***	60.8 ± 3.0 (21%)***
16a*	8.8 ± 2.9 (68.9%)***	29.8 ± 6.0 (21.5%)**	37.4 ± 8.0 (25.2%)*	42.0 ±7.0 (43.2%)***	38.0 ±6.0 (50.6%)***
16b*	7.5 ± 3.5 (73.4%)***	28.8 ± 4.0 (24.2%)***	34.0 ± ± 6.0 (32%)**	36.0 ± 6.0 (51.3%)***	32.0 ± 7.0 (58.4%)***
16c*	4.8 ± 1.7 (83.2%)***	33.0 ± 4.3 (13.1%)***	25.0 ± 4.0 (50%)**	34.0 ± 3.0 (54%)***	28.0 ± 4.0 (63.6%)***
16d*	6.5 ± 2.1 (77.0%)***	28.0 ± 4.0 (26.3%)*	27.0 ± 5.0 (46%)**	35.0 ± 4.0 (52.7%)***	30.0 ± 5.0 (61%)***
19	15.3 ± 1.8 (45.7%)*	27.0 ± 5.0 (28.9%)**	36.8 ± 8.0 (26.4%)*	37.0 ± 5.0 (50%)***	40.0 ± 3.0 (48%)***
21	14.4 ± 3.5 (48.9%)**	29.0 ± 6.0 (23.6%)**	35.8 ± 8.0 (28.4%)***	42.0 ± 7.0 (43.2%)***	44.0 ± 6.0 (42.8%)***
1	26.4 ± 3.6 (6.4%)	40.0 ± 6.1	47.0 ± 4.0 (6%)**	68.0 ± 6.0 (8.1%)***	76.2 ± 3.0 (1%)***
Aspirin	8.8 ± 2.9 (68.9%)***				
Indomethacin		32.0 ± 4.0 (15.5%)**	35.0 ± 4.0 (31.7%)*	41.0 ± 3.0 (45.6%)***	42.0 ± 4.0 (45.7%)***

Data obtained from animal experiments were expressed as means ± SEM.
ASA, aspirin, 100 mg/kg (body weight); INDO (A), indomethacin, 100 mg/kg (body weight); and all test drugs, 100 mg/kg (body weight) were s.c. administered to mice ($n = 6$–12) for [a)]*p*BQ induced writhing and [b)]CG-induced paw edema tests, respectively.
*Statistical significance was evaluated from the control by one-way ANOVA *post hoc* Dunnett's test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

our experiments. The results shown in Table 1 indicate that the substituted 4-aminopyridine phosphonate derivatives **16a–d** are the compounds with the most potent analgesic activity. Thus, **16c** (83%) and **16d** (77%) demonstrated higher potency than aspirin at the same dose of 100 mg/kg. In addition, compounds **16a** and **16b** resulted in comparable activity at 68 and 73%, respectively, with respect to reference aspirin with a small decrease in the inhibitory potential of writhing movements. In the case of pyrimidinone-phosphonates **9a** and **9b** there was a discrepancy in favor of the substituted pyridine core that caused a decrease in analgesic potency to 59 and 56%, respectively. However, phosphonic diamide derivatives **13a** and **13b** failed to induce analgesic activity and lacked the similar potency (≈12%). Nevertheless, azaphospholyl-2-oxide **19** and substituted pyrrole phosphonate **21** restored the analgesic efficiency to 45 and 48%, respectively.

Antiinflammatory bioassay

Antiinflammatory activities of the previous compounds **9a**,**b**, **13a**,**b**, **16a–d**, **19**, and **21** were also in good correlation with the analgesic activity results, tested by using the carrageenin-induced hind paw edema model [27] (Table 1). As shown, the fused *p*-aminopyridine phosphonate derivatives (**16a–d**) as well as fused pyrimdinone phosphonate derivatives (**9a** and **9b**) resulted in potent antiinflammatory activity, which was comparable to indomethacin. It is claimed that edema produced by carrageen is a biphasic event for the inhibitory effects of compounds: The action on the first stage is attributable to the inhibition of the release of chemical mediators such as histamine, serotonin, and bradykinin [26]. However, the second phase of edema might be related to the release of arachidonic acid (AA) metabolites since it is potently suppressed by aspirin, indomethacin, and other cyclooxygenases (COX-1 and COX-2) inhibitor drugs [28]. As shown in Table 1, the most active derivatives showed a similar pattern as indomethacin with an absolute gradual increase in the second phase (after 270 min), therefore suggesting that these compounds might exert their antiinflammatory activities through the mechanisms involving the inhibition of the formation of AA metabolites. Taken together, along with our previous findings, we can conclude that α-aminophosphonate derivatives incorporating a pyrazole core can be modified to potent analgesic/antiinflammatory drugs for novel treatment of inflammation-related disorders. %Inhibition of edema for the tested compounds at successive intervals is displayed in Fig. 1.

Acute toxicity

At the end of these experiments after 48 h of observation of all animals, no morbidity or mortality was recorded.

Antitumor activity

Since the pro-inflammatory eicosanoids (*i.e.*, leukotrienes (LTs) and prostaglandins (PGs)), are evidenced to be visible key regulators of cell proliferation and neo-angiogenesis, the

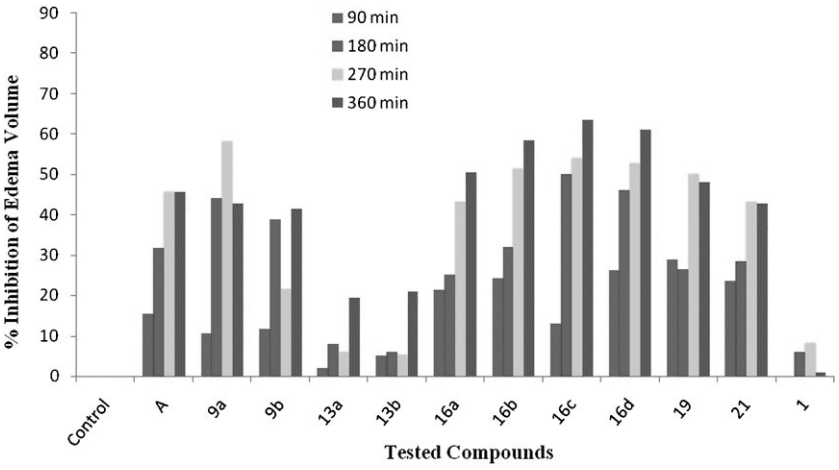


Figure 1. %Inhibition of edema for the tested compounds at successive intervals.

inhibitors of the pathways for their formation are being investigated as potential anticancer drugs [29, 30]. Considering that our compounds displayed promising *in vivo* antiinflammatory activities presumably by inhibiting the formation of AA metabolites, we selected the above cell lines which provide a suitable cellular model system to investigate the anticancer potential of these derivatives. The results for each tested compound at 50 μM concentration are reported.

Respectively, antitumor activity screening of **9a**, **9b**, **13a**, **16a**, and **16c** in assays applying a human liver (HEPG2) and a human colon (HCT116), a human breast carcinoma (MCF7) and a human cervix (HeLa) cell line 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 and Storeng [31]. The obtained results (Table 2) represent concentrations (four different concs.: 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 was evaluated three times (each dose was 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 2).

The antitumor activity results displayed in Table 2 indicate that the tested phosphonate derivatives **9a**, **9b**, **13a**, **16a**, and **16c** reflect good to moderate activity against the used human tumor cells. The order of activity is **16c** > **16a** > **9a** > **9b** > **13a**. It can also be noticed that **16c** exhibited remarkable antitumor activity against the four tested carcinoma cell lines. Further studies in experimental tumors *in vivo* for evaluating the possible antineoplastic potential by these and the other synthesized compounds are warranted.

Finally, from the structure–activity relationship (SAR) viewpoint, the data displayed in Tables 1 and 2 show that the analgesic/antiinflammatory and antineoplastic activities of *p*-aminopyridine-phosphorus derivatives were found to be

Table 2. Antitumor properties of the tested compounds DOX, CIS, **9a**, **9b**, **13a**, **16a**, and **16c**.

Compd.	IC_{50}, μg/mL (μM)			
	HEPG2 (liver cancer)	**HCT116 (colon cancer)**	**MCF7 (breast cancer)**	**HeLa (cervical cancer)**
Doxorubicin (DOX)	3.10 (5.7)*	3.73 (6.86)**	4.4 (8.09)***	—
Cisplatin (CIS)	—	—	—	2.8 (9.30)**
9a	6.40 (15.20)***	7.30 (17.33)*	7.20 (17.10)**	6.60 (15.67)*
9b	10.40 (23.16)*	11.60 (25.83)***	7.95 (17.70)*	7.80 (17.37)***
13a	20.20 (45.19)**	13.60 (30.42)*	15.90 (35.57)**	14.00 (31.31)*
16a	5.62 (9.91)*	5.23 (9.22)***	3.88 (6.84)*	4.16 (7.33)**
16c	3.44 (6.19)**	3.33 (6.00) ***	3.20 (5.76)**	2.66 (4.79)*

Deviation error: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$; p is the percentage of inhibition.
(IC_{50}, μM) = IC_{50}, μg/mL × 1000 M.Wt.

higher than those of the fused-pyrimidinone phosphonates-based pyrazole as a core. In addition, we observed that there is a close analogy among the potencies of the tested compounds toward the analgesic/antiinflammatory or antineoplastic effects. Thus, while **16a** and **16c** showed significant properties toward the three activities, compounds **13a** and **13b** failed to induce any effect. It can be deduced that there is a correlation between the modes of action of this class of compounds toward these types of diseases.

Experimental

General

Melting points were determined with open capillary tubes on an electrothermal (variable heater) melting point apparatus and were uncorrected. IR spectra were recorded on a PerkinElmer 297 grating IR spectrophotometer using KBr pellets. NMR spectra were measured with a JEOL E.C.A-500 MHz (^{13}C: ≈125 MHz, ^{1}H: ≈500 MHz, and ^{31}P: ≈200 MHz) spectrometer. ^{31}P NMR spectra were recorded with H_3PO_4 (85%) as external reference. ^{1}H and ^{13}C NMR spectra were measured using $SiMe_4$ as an internal reference in $CDCl_3$ or DMSO-d^6. Chemical shifts (δ) are given in ppm. Mass spectrometry was performed on a JEOL JMS-AX 500 spectrometer. The appropriate precautions in handling moisture-sensitive compounds were considered. Solvents were dried by standard techniques. Thin-layer chromatography (TLC) used Merck 0.2 mm silica gel 60 F254 analytic aluminum plates. Elemental analyses were carried out at the Micro Analytical Centre, Cairo University, Cairo, Egypt, using Elemental C, H, N Analyzer Vario EL III Germany. All international principles and local regulations concerning the care and use of laboratory animals were considered during the pharmacological screening.

Synthesis

*Preparation of the iminophosphorane **2***

The procedure reported by Liu et al. [13] for the preparation of **4** was modified as follows: To a mixture of 2.04 g ethyl 4-amino-5-cyano-1-phenyl-1*H*-pyrazole-3-carboxylate (**1**, 8 mmol), 3.14 g PPh_3 (12 mmol), and 2.84 g C_2Cl_6 (12 mmol) in 40 mL dry THF, was added dropwise 2.42 g NEt_3 (24 mmol) at r.t. The color of the reaction mixture quickly turned yellow. After stirring for 4–6 h, solvents were removed under reduced pressure to give the iminophosphorane **2**.

*Ethyl 5-cyano-4-[(triphenylphosphoranylidene)amino]-1,4-dihydropyrazol-3-carboxylate (**2**)*

Colorless crystals, mp: 126–128°C (cyclohexane), yield: (3.6 g, 87%). IR: ν_{max}, cm^{-1}: 2232 (CN), 1709 (C=O). ^{1}H NMR [DMSO-d^6]: δ 1.36 (t, J_{H-H} = 7.2 Hz, 3H, H_3CC), 3.69 (q, J_{H-H} = 7.2 Hz, 2H, H_2CO), 7.43–8.01 (m, 20H, *H*–Ph) ppm. ^{13}C NMR [DMSO-d^6]: δ 160.6 (*C*=O), 136.5 (*C*=N), 143.6, 137.1, 133.4, 132.3, 131.7, 129.8, 129.6, 129.4, 128.8, 124.4 (*C*–Ph), 121.0 (*C*N), 107.4 (*C*–CN), 60.9 (CH_2O), 14.1 (CH_3CO) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 21.4 ppm. EI–MS: *m*/*z* (%): 516 (100) [M$^+$], 487 (88), 499 (82), 262 (66), 254 (48), 237 (65), 77 (44). Anal. calcd. for $C_{31}H_{25}N_4O_2P$ (516.2): C, 72.08; H, 4.88; N, 10.85; P, 6.00. Found: C, 72.06; H, 4.85; N, 10.83; P, 6.03.

*Preparation of carbodiimide **4***

To a solution of 1.2 g iminophosphorane **2** (2.3 mmol) in 15 mL dry THF was added 0.28 g phenylisocyanate (**3**, 2.3 mmol) under nitrogen at r.t. After the reaction mixture was stirred for 12 h at 0–5°C, the solvent was removed under reduced pressure and 20 mL ether/petroleum ether (1:2) was added to precipitate TPPO. After filtration the solvent was removed to give carbodiimide **4**, which was generally used directly without further purification. Compound **4** was also isolated, for analytical purposes, from the reaction mixture by column chromatography on silica gel.

*Ethyl 5-cyano-1-phenyl-4-{[(phenylimino)methylene]-amino}-1,4-dihydropyrazorol-3-carboxylate (**4**)*

White material, mp: 110–111°C (ligroin), yield: (0.65 g, 77%). IR: ν_{max}, cm^{-1}: 2233 (CN), 1709 (C=O), 1665, 1595, 1490 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.36 (t, J_{H-H} = 7.2 Hz, 3H, H_3CC), 4.33 (q, J_{H-H} = 7.2 Hz, 2H, H_2CO), 7.23–7.37 (m, 10H, *H*–Ph), ^{13}C NMR ($CDCl_3$): δ 164.2, 161.6, 147.6, 144.7, 136.0, 135.4, 132.4, 129.8, 129.4, 126.1, 124.8, 120.4, 61.2, 60.2, 14.5, 14.3. EI–MS *m*/*z* (%): 357 (100) [M$^+$], 328 (84), 302 (60), 77 (37). Anal. calcd. for $C_{20}H_{15}N_5O_2$ (357.1): C, 67.22; H, 4.23; N, 19.60. Found: C, 67.27; H, 4.18, N, 19.67.

Reaction of carbodiimide 4 with dialkylhydrogenphosphonates 5a–c

*Preparation of the phosphonate derivatives **8a–c** and **9a–c***

General Method: To the solution of 0.8 g **4** (2.2 mmol) prepared above, a solution of 2.7 mmol dimethyl- (**5a**), diethyl- (**5b**), or diisopropylhydrogenphosphite (**5c**) in 15 mL THF containing 10% $FeCl_3$, and 1 mL H_2O was added. The reaction mixture was refluxed for 3–6 h. After completion of the reaction (TLC), 10 mL AcOEt was added to the mixture. The organic phase was separated, washed with 20 mL distilled water, and dried over anhydrous sodium sulfate. Solvents were evaporated under vacuum, and the residue was crystallized from the proper solvent to give the corresponding phosphonates **8a–c**, **9a–c**, which had been separated by fractional crystallization.

*Ethyl 6-amino-5-(dimethoxyphosphoryl)-1-phenyl-1,4-dihydropyrrolo[3,2-c]pyrazole-3-carboxylate (**8a**)*

Colorless crystals, mp 240–242°C (EtOH), yield: (120 mg, 14%). IR: ν_{max}, cm^{-1}: 3415–3323 (NH & NH_2), 1717 (C=O, ester), 1233 (P=O, bonded), 1090 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.31 (t, J_{H-H} = 7.2 Hz, 3H, H_3CC), 3.64 (q, J_{H-H} = 7.2 Hz, 2H, H_2CO), 4.38 (d, $^{3}J_{P-H}$ = 11.6 Hz, 6H, $(H_3CO)_2$P), 6.98 (br, 1H, H^AN), 7.29–7.79 (m, 5H, *H*–Ph), 9.01 (br, 1H, H^BN), 9.88 (br, 1H, *H*N, pyrrole) ppm. ^{13}C NMR [DMSO-d^6]: δ 159.6 (*C*=O), 136.5 (*C*=N), 143.1, 128.7, 121.2, 120.3, 119.9, 111.8 (*C*–Ph, *C*-pyrazole), 128.5 (d, $^{2}J_{p-c}$ = 17.6 Hz, *C*–NH_2), 125.4 (d, $^{1}J_{p-c}$ = 196.3 Hz, *C*–P), 60.9 (OCH_2), 52.3 (d, $^{2}J_{p-c}$ = 14.8 Hz, CH_3OP), 14.1 (CH_3, ester) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 23.4ppm. EI–MS: *m*/*z* (%): 378 (100) [M$^+$], 360 (68) [M$^+$–18, H_2O], 349 (11) [M$^+$–29, C_2H_5], 222 (24) [M$^+$–156, [P(O)(OMe)$_2$)]+H_2O+C_2H_5], 109 (18) (P(O)(OMe)$_2$), 77 (66). Anal. calcd. for $C_{16}H_{19}N_4O_5P$ (378.1): C, 50.80; H, 5.06; N, 14.81; P, 8.19. Found: C, 50.76; H, 5.01; N, 14.78; P, 8.25.

*Ethyl 6-amino-5-(diethoxyphosphoryl)-1-phenyl-1,4-dihydropyrrolo[3,2-c]pyrazole-3-carboxylate (**8b**)*

Colorless crystals, mp 222–224°C (EtOH), yield: (110 mg, 12%). IR: ν_{max}, cm^{-1}: 3374–3327 (NH & NH_2), 1710 (C=O, ester), 1233 (P=O,

bonded), 1090 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.36–1.45 (m(2t), 9H, H_3C(ester) & 2H_3C-phosphonate), 3.59 (q, J_{H-H} = 7.2 Hz, 2H, H_2C, ester), 4.37 (dq, J_{H-H} = 7.4, $^3J_{P-H}$ = 4.8 Hz, 4H, (CH_2O)$_2$P), 6.94 (br, 1H, H^AN), 7.27–7.87 (m, 5H, *H*–Ph), 9.11 (br, 1H, H^BN), 9.83 (br, 1H, *H*N, pyrrole) ppm. ^{13}C NMR [DMSO-d^6]: δ 159.8 (*C*=O), 136.4 (*C*=N), 143.3, 129.2, 122.1, 120.3, 119.1, 110.9 (*C*–Ph, *C*-pyrazole), 128.2 (d, $^2J_{p-c}$ = 17.8 Hz, *C*–NH$_2$), 126.7 (d, $^1J_{p-c}$ = 198.6 Hz, *C*–P), 63.2 (d, $^2J_{p-c}$ = 8.7 Hz, (CH_2O)$_2$P), 60.8 (O*C*H$_2$, ester), 15.9 (d, $^3J_{p-c}$ = 7.6 Hz, *C*H$_3$COP), 14.3 (*C*H$_3$, ester) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 23.8 ppm. EI–MS: *m*/*z* (%): 406 (88) [M$^+$], 388 (100) [M$^+$−18, H$_2$O], 377 (16) [M$^+$−29, C$_2$H$_5$], 222 (33) [M$^+$−184, [P(O)(OEt)$_2$)]+H$_2$O+C$_2$H$_5$], 137 (27) (P(O)(OEt)$_2$), 77 (60). Anal. calcd. for $C_{18}H_{23}N_4O_5P$ (406.1): C, 53.20; H, 5.70; N, 13.79; P, 7.62. Found: C, 53.17; H, 5.64; N, 13.71; P, 7.69.

*Ethyl 6-amino-5-(diisopropoxyphosphoryl)-1-phenyl-1,4-dihydropyrrolo[3,2-c]pyrazole-3-carboxylate (**8c**)*

Colorless crystals, mp 278–280°C (EtOH), yield: (124 mg, 13%). IR: ν_{max}, cm^{-1}: 3388–3330 (NH & NH$_2$), 1723 (C=O, ester), 1223 (P=O, bonded), 1110 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.14 (t, J_{H-H} = 7.2 Hz, 3H, H_3C, ester), 1.31 (dd, J_{H-H} = 6.6 Hz, $^4J_{P-H}$ = 5.8 Hz, 2 × 6H, (*Me*$_2$CHO)$_2$P), 3.66 (q, J_{H-H} = 7.2 Hz, 2H, H_2CO, ester), 4.77 (dsept, $^3J_{P-H}$ = 12.4 Hz, 2H, (*H*CO)$_2$P), 6.64 (br, 1H, H^AN), 7.54–7.81 (m, 5H, *H*–Ph), 9.06 (br, 1H, H^BN), 10.07 (br, 1H, *H*N, pyrrole) ppm. ^{13}C NMR [DMSO-d^6]: δ 159.2 (*C*=O), 136.7 (*C*=N), 143.0, 127.6, 122.8, 121.5, 119.2, 111.6 (*C*–Ph, *C*-pyrazole), 128.2 (d, $^2J_{p-c}$ = 17.8 Hz, *C*–NH$_2$), 126.7 (d, $^1J_{p-c}$ = 201.5 Hz, *C*–P), 75.0 (d, $^2J_{p-c}$ = 9.5 Hz, (*C*HO)$_2$P), 60.9 (*C*H$_2$O, ester), 23.7 (d, $^3J_{p-c}$ = 7.9 Hz, (*Me*$_2$CO), 14.3 (*C*H$_3$, ester) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 25.2 ppm. EI–MS: *m*/*z* (%): 434 (72) [M$^+$], 416 (100) [M$^+$−18, H$_2$O], 405 (18) [M$^+$−29, C$_2$H$_5$], 375 (45) [M$^+$−59, Et+2Me], 222 (33), 165 (27) [P(O)(OCHMe$_2$)$_2$], 77 (60). Anal. calcd. for $C_{20}H_{27}N_4O_5P$ (434.2): C, 55.29; H, 6.26; N, 12.90; P, 7.13. Found: C, 55.23; H, 6.30; N, 12.82; P, 7.19.

*Dimethyl 3-cyano-7-oxo-2,6-diphenyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-5-ylphosphonate (**9a**)*

Colorless crystals, mp 226–228°C (acetone), yield: (540 mg, 58%). IR: ν_{max}, cm^{-1}: 2234 (CN), 1690 (C=O), 1599 (C=N), 1262 (P=O, free), 1054 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 4.18 (d, $^3J_{P-H}$ = 11.4 Hz, 6H, (*H*$_3$CO)$_2$P), 7.29–8.18 (m, 10H, *H*–Ph) ppm. ^{13}C NMR [DMSO-d^6]: δ 174.2 (d, $^1J_{p-c}$ = 178.6 Hz, *C*–P), 150.8 (*C*=O), 150.5, 141.8, 138.8, 137.3, 132.5, 129.9, 129.8, 128.1, 127.5, 123.3 (*C*-pyrazole, *C*–Ph), 122.2 (*C*N), 103.2 (*C*–CN), 54.5 (d, $^2J_{p-c}$ = 7.6 Hz, (*C*H$_3$O)$_2$P) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 28.3 ppm. EI–MS: *m*/*z* (%): 421 (22) [M$^+$], 395 (100) [M$^+$−26, CN], 367 (14) [M$^+$−(26 + 28), (CN+C=O)], 286 (56) [M$^+$−(26 + 109), (CN+P(O)(OMe)$_2$)], 109 (28) (P(O)(OMe)$_2$), 77 (65). Anal. calcd. for $C_{20}H_{16}N_5O_4P$ (421.1): C, 57.01; H, 3.83; N, 16.62; P, 7.35. Found: C, 56.9; H, 3.92; N, 16.53; P, 7.29.

*Diethyl 3-cyano-7-oxo-2,6-diphenyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-5-ylphosphonate (**9b**)*

Colorless crystals, mp 214–216°C (EtOH), yield: (583 mg, 59%). IR: ν_{max}, cm^{-1}: 2225 (CN), 1687 (C=O), 1586 (C=N), 1256 (P=O, free), 1080 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.31 (dt, J_{H-H} = 6.6 Hz, $^4J_{P-H}$ = 5.3 Hz, 2 × 3H, (*H*$_3$CCO)$_2$P), 4.09 (dq, $^3J_{P-H}$ = 10.7 Hz, 4H, (*CH*$_2$O)$_2$P), 7.43–7.98 (m, 10H, *H*–Ph) ppm. ^{13}C NMR [DMSO-d^6]: δ 175.2 (d, $^1J_{p-c}$ = 179.4 Hz, *C*–P), 154.8 (*C*=O), 151.0, 141.6, 139.5, 138.8, 132.5, 129.8, 129.7, 128.1, 127.0, 123.3 (*C*-pyrazole, *C*–Ph), 122.6 (*C*N), 105.2 (*C*–CN), 60.2 (d, $^2J_{p-c}$ = 8.7 Hz, (*C*H$_2$O)$_2$P), 16.5 (d, $^3J_{p-c}$ = 7.6 Hz, (*Me*CO)$_2$P) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 26.9 ppm. EI–MS: *m*/*z* (%): 449 (13) [M$^+$], 423 (100) [M$^+$−26, CN], 395 (9) [M$^+$−(26+28), (CN+C=O)], 286 (22) [M$^+$−(26+137) (CN+P(O)(OEt)$_2$)], 137 (30) (P(O)(OEt)$_2$), 77 (72). Anal. calcd. for $C_{22}H_{20}N_5O_4P$ (449.1): C, 58.80; H, 4.49; N, 15.58; P, 6.89. Found: C, 58.75; H, 4.47; N, 15.53; P, 6.82.

*Diisopropyl 3-cyano-7-oxo-2,6-diphenyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-5-ylphosphonate (**9c**)*

Colorless crystals, mp 250–252°C (MeCN), yield: (0.6 g, 57%). IR: ν_{max}, cm^{-1}: 2233 (CN), 1685 (C=O), 1560 (C=N), 1258 (P=O), 1077 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.56 (dd, J_{H-H} = 6.6 Hz, $^4J_{P-H}$ = 5.8 Hz, 12H, (*Me*$_2$CHO)$_2$P), 4.77 (dsept, $^3J_{P-H}$ = 12.4 Hz, 2H, (*H*CO)$_2$P), 7.43–7.98 (m, 10H, *H*–Ph) ppm. ^{13}C NMR [DMSO-d^6]: δ 176.9 (d, $^1J_{p-c}$ = 165.1 Hz, *C*–P), 153.9 (*C*=O), 151.5, 141.7, 138.8, 132.5, 129.8, 129.9, 128.1, 127.4, 123.3 (*C*-pyrazole, *C*–Ph), 121.8 (*C*N), 108.3 (*C*–CN), 72.4 (d, $^2J_{p-c}$ = 9.5 Hz, (*C*HO)$_2$P), 24.6 (d, $^3J_{p-c}$ = 7.9 Hz, (*Me*$_2$CO)$_2$P) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 27.2 ppm. EI–MS: *m*/*z* (%): 477 (18) [M$^+$], 451 (100) [M$^+$−26, CN], 423 (11) [M$^+$−(26+28), (CN+C=O)], 286 (66) [M$^+$−(26+165) (CN+P(O)(OC$_3$H$_7$)$_2$)], 166 (17) [P(O)(OCHMe$_2$)$_2$], 77 (65). Anal. calcd. for $C_{24}H_{24}N_5O_4P$ (477.2): C, 60.37; H, 5.07; N, 14.67; P, 6.49. Found: C, 60.33; H, 5.02; N, 14.59; P, 6.45.

Reaction of carbodiimide 4 with *tris*-(dialkylamino)-phosphines 10a,b

*Preparation of **11a,b** and **13a,b***

General Method: The aminophosphine **10a** (R^2 = Me) or **10b** (R^2 = Et; 2.3 mmol) in 4 mL dry THF was added dropwise to 0.8 g **4** (2.2 mmol) in 15 mL of the same solvent, and the reaction mixture was stirred at r.t., for ≈10 h (TLC). The solvent was evaporated under vacuum; the residue was collected, washed with light petroleum, and crystallized from the proper solvent to give compounds **11a**,**b**.

*5-Cyano-3-(ethoxycarbonyl)-1-phenyl-1-H-pyrazol-4-yl)-(phenylimino)(tris-(dimethylamino)-phosphonio)-methylamide (**11a**)*

Straw yellow, mp 173–175°C (MeCN), yield: (0.98 g, 86%). IR: ν_{max}, cm^{-1}: 2221 (CN), 1715 (C=O), 1320, 838 (P[N(Me$_2$)]$_3$). ^{1}H NMR [DMSO-d^6]: δ 1.34 (t, J_{H-H} = 7.2 Hz, 3H, H_3CC, ester), 2.83 (d, $^3J_{P-H}$ = 9.8 Hz, 18H, (*Me*$_2$N)$_3$–P), 3.64 (q, J_{H-H} = 7.2 Hz, 2H, H_2CO, ester), 7.28–8.15 (m, 10H, *H*–Ph) ppm. ^{13}C NMR [DMSO-d^6]: δ 159.7 (*C*=O), 143.2, 137.6, 136.2, 129.0, 127.4, 127.1, 124.4, 122.7, 118.8 (*C*-pyrazole, *C*–Ph), 123.9 (*C*N), 98.5 (*C*–CN), 60.9 (*C*H$_2$O),), 57.6 (d, $^1J_{p-c}$ = 166.3 Hz, *C*–P), 34.2 (d, $^2J_{p-c}$ = 25.2 Hz, (*Me*$_2$N–P), 14.0 (*C*H$_3$CO) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 58.3 ppm. EI–MS: *m*/*z* (%): 520 (21) [M$^+$]. Anal. calcd. for $C_{26}H_{33}N_8O_2P$ (520.3): C, 59.99; H, 6.39; N, 21.53; P, 5.95. Found: C, 59.95; H, 6.38; N, 21.50; P, 5.92.

*5-Cyano-3-(ethoxycarbonyl)-1-phenyl-1H-pyrazol-4-yl)-((phenylimino)(tris-(diethylamino)phosphonio)-methylamide (**11b**)*

Straw yellow, mp 150–152°C (CH$_2$Cl$_2$), yield: (1.12 g, 84%). IR: ν_{max}, cm^{-1}: 2227 (CN), 1709 (C=O), 1317, 863 (P[N(Et$_2$)]$_3$). ^{1}H NMR [DMSO-d^6]: δ 1.34 (t, J_{H-H} = 7.2 Hz, 3H, H_3CC, ester), 1.28 (dt, $^4J_{P-H}$ = 5.8 Hz, 18H, [*Me*C]$_2$N)$_3$–P), 3.45–3.61 (m, 14H, [H_2C]$_2$N)$_3$, H_2CO, ester), 7.34–8.15 (m, 10H, *H*–Ph) ppm.

^{13}C NMR [DMSO-d^6]: δ 160.5 (*C*=O), 143.1, 137.4, 136.7, 129.6, 128.1, 127.4, 124.1, 123.9, 122.6 (*C*-pyrazole, *C*–Ph), 117.8 (*C*N), 102.5 (*C*–CN), 59.7 (O*C*H$_2$),), 56.5 (d, $^1J_{p-c}$ = 169.6 Hz, *C*–P), 37.8 (d, $^2J_{p-c}$ = 25.7 Hz, *C*H$_2$–N–P), 17.0 (d, $^3J_{p-c}$ = 8.8 Hz, *Me*CN–P), 14.0 (*C*H$_3$CO) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 58.5 ppm. EI–MS: *m*/*z* (%): 604 (17) [M$^+$]. Anal. calcd. for $C_{32}H_{45}N_8O_2P$ (604.3): C, 63.56; H, 7.50; N, 18.53; P, 5.12. Found: C, 63.53; H, 7.45; N, 18.51; P, 5.08.

When the above reaction (**4** + **10a**,**b**) was carried out in ethanol (or THF + catalytic amount of water), phosphamides **13a** and **13b** were obtained after the usual working up.

*[3-Cyano-7-oxo-2,6-diphenyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-N,N,N,N-tetramethyl-phosphonic diamide (**13a**)*

Colorless crystals, mp 150–152°C (CH_2Cl_2), yield: (0.8 g, 82%). IR: ν_{max}, cm^{-1}: 2221 (CN), 1685 (C=O), 1533 (C=N), 1256 (P=O), 1314, 859 (P[N(Me$_2$)]$_2$). ^{1}H NMR [DMSO-d^6]: δ 2.83 (d, $^3J_{P-H}$ = 10.4 Hz, 12H, (Me$_2$N)$_2$–P), 7.34–8.14 (m, 10H, *H*–Ph) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 33.6 ppm. EI–MS: *m*/*z* (%): 448 (62) [M$^+$+1], 421 (100) [M$^+$−26, CN], 286 (87) [M$^+$−(26+135), (CN+OP[NMe$_2$]$_2$)], 270 (15), 135 (48) OP[N–(Me$_2$)$_2$], 77 (74). Anal. calcd. for $C_{22}H_{22}N_7O_2P$ (447.2): C, 59.06; H, 4.96; N, 21.91; P, 6.92. Found: C, 58.98; H, 4.9; N, 21.85; P, 6.88.

*[3-Cyano-7-oxo-2,6-diphenyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-N,N,N,N-tetraethyl-phosphonic diamide (**13b**)*

Colorless crystals, mp 118–120°C (cyclohexane), yield: (0.86 g, 78%). IR: ν_{max}, cm^{-1}: 2221 (CN), 1682 (C=O), 1604 (C=N), 1254 (P=O), 1317, 863 (P[N(Et$_2$)]$_2$). ^{1}H NMR [DMSO-d^6]: δ 1.05 (dt, J_{H-H} = 6.4 Hz, $^4J_{P-H}$ = 4.2 Hz, 12H, (Me$_2$CN)$_2$–P), 3.21 (q, $^3J_{P-H}$ = 10.8 Hz, 8H, C*H*$_2$–N–P), 7.43–7.98 (m, 10H, *H*–Ph) ppm. ^{31}P NMR [DMSO-d^6]: δ_p 34.2 ppm; EI–MS: *m*/*z* (%): 504 (55) [M$^+$+1], 503 (50) [M$^+$], 477 (100) [M$^+$−26, CN], 286 (88) [M$^+$−(26+191), (CN+OP[NEt$_2$]$_2$)], 270 (15), 191 (48) OP[N(Et$_2$)$_2$], 77 (70). Anal. calcd. for $C_{26}H_{30}N_7O_2P$ (503.2): C, 62.02; H, 6.01; N, 19.47; P, 6.15. Found: C, 61.96; H, 5.94; N, 19.42; P, 6.12.

Reactions of carbodiimide 4 with phosphonates 14a–d, 18, and 20

*Preparation of phosphonate derivatives **16a–d**, **19**, and **21***

General Procedure: To the *in situ* 0.8 g carbodiimide **4** (2.2 mmol) a solution of the appropriate phosphonate **14a–d**, **18**, or **20** (2.5 mmol) containing 4.0 mmol of the assigned base was added, as indicated below. The reaction mixture was stirred at r.t. for ≈6–10 h (TLC). After the completion of the reaction, 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 *anh* 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 product **16a–d**, **19**, or **21**, respectively.

Preparation of **16a–c**

Reagents: Operation as above, 0.8 g compound **4** (2.2 mmol), diethyl methylphosphonoacetate (**14a**) (0.52 g, 2.5 mmol), triethyl phosphonoacetate (**14b**) (0.56 g, 2.5 mmol), or diethyl (2-amino-2-thioxoethyl)-phosphonate (**14c**) (0.53 g, 2.5 mmol) in 20 mL of absolute ethanol containing 103 mg sodium (4.5 mmol), at r.t. The product mixture was worked up as described in the general procedure to yield the phosphonate derivatives **16a–c**.

*3-Ethyl-6-methyl-7-amino-6-(diethoxyphosphoryl)-1-phenyl-5-(phenylimino)-5,6-dihydro-1H-pyrazolo[4,3-b]-pyridine-3,6-dicarboxylate (**16a**)*

Yellow substance, mp 180–182°C (MeCN), yield: (0.85 g, 68%). IR: ν_{max}, cm^{-1}: 3482, 3379 (NH$_2$), 1708, 1694 (2C=O), 1633 (C=NPh, exocyclic), 1585 (C=N), 1227 (P=O, bonded), 1084 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.24–1.58 (m(2t), 9H, (*H*$_3$CC, ester & (*H*$_3$CCO)$_2$P), 3.48 (s, 3H, *H*$_3$CO, ester), 3.78 (q, J_{H-H} = 6.5 Hz, 2H, *H*$_2$CO, ester), 4.32 (dq, $^3J_{P-H}$ = 11.3 Hz, 4H, (*H*$_2$CO)$_2$P), 6.02 (br, 1H, *H*^{A}N), 7.32–7.89 (m, 10H, *H*–Ph), 9.67 (br, 1H, *H*^{B}N) ppm. ^{13}C NMR [DMSO-d^6]: δ 177.3 (d, $^2J_{P-C}$ = 11.1 Hz, *C*=NPh), 162.3, 159.5 (2*C*=O), 157.8, 154.4, 147.9, 143.7, 129.2, 128.5, 123.3, 111.4 (*C*–Ph, *C*-pyrazole), 119.5 (d, $^2J_{P-C}$ = 22 Hz, *C*–NH$_2$), 63.1 (d, $^2J_{P-C}$ = 8.7 Hz, (*C*H$_2$O)$_2$P), 61.8 (*C*H$_2$O), 52.1 (*C*H$_3$O), 47.8 (d, $^1J_{P-C}$ = 148.4 Hz, *C*–P), 16.3 (d, $^3J_{P-C}$ = 7.6 Hz, (*C*H$_3$CO)$_2$P), 14.2 (*C*H$_3$CO) ppm. ^{31}P NMR [DMSO-d^6]: δ_P 27.9 ppm. EI–MS: *m*/*z* (%) 568 (16) [M$^+$+1], 567 (16) [M$^+$], 523 (28) [M$^+$−44, (Me+Et)], 432 [M$^+$−135, (Me+Et+NPh)], 416 (55) [M$^+$−151, (Me+Et+NPh+NH$_2$)], 279 (100) [M$^+$−288, (Me+Et+NPh+NH$_2$+P(O)(OEt)$_2$], 212 (50), 137 (53) (P(O)(OEt)$_2$), 119 (17), 77 (66). Anal. calcd. for $C_{27}H_{30}N_5O_7P$ (567.2): C, 57.14; H, 5.33; N, 12.34; P, 5.46. Found: C, 57.12; H, 5.31; N, 12.32; P, 5.43.

*Diethyl 7-amino-6-(diethoxyphosphoryl)-1-phenyl-5-(phenylimino)-5,6-dihydro-1H-pyrazolo[4,3-b]pyridine-3,6-dicarboxylate (**16b**)*

Yellow substance, mp 152–154°C (CH_2Cl_2), yield: (0.84 g, 66%). IR: ν_{max}, cm^{-1}: 3477, 3350 (NH$_2$), 1715, 1700 (2C=O), 1633 (C=NPh, exocyclic), 1590 (C=N), 1225 (P=O, bonded), 1050 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.22–1.56 (m (3t), 12H, 4C*H*$_3$CO), 3.90–4.00 (m(2q), 4H, 2*H*$_2$CCO, 2ester), 4.27 (dq, $^3J_{P-H}$ = 10.4 Hz, 4H, (*H*$_2$CO)$_2$P), 6.02 (br, 1H, *H*^{A}N), 7.38–7.75 (m, 10H, *H*–Ph), 9.67 (br, 1H, *H*^{B}N) ppm. ^{13}C NMR [DMSO-d^6]: δ 177.1 (d, $^2J_{P-C}$ = Hz, *C*=NPh), 162.3, 159.8 (2C=O), 157.8, 154.5, 147.9, 143.7, 129.2, 128.5, 123.3, 111.4 (*C*–Ph, *C*-pyrazole), 119.3 (d, $^2J_{P-C}$ = 37 Hz, *C*–NH$_2$), 63.1 (d, $^2J_{P-C}$ = 8.7 Hz, (*C*H$_2$O)$_2$P), 61.8, 59.9 (2*C*H$_2$O), 47.4 (d, $^1J_{P-C}$ = 211 Hz, *C*–P), 16.3 (d, $^3J_{P-C}$ = 7.6 Hz, (*C*H$_3$CO)$_2$P), 14.2, 14.0 (2*C*H$_3$CO) ppm. ^{31}P NMR [DMSO-d^6]: δ_P 26.9 ppm. EI–MS: *m*/*z* (%): 582 (14) [M$^+$+1], 581 (11) [M$^+$], 523 (23) [M$^+$−58, 2C$_2$H$_5$], 432 [M$^+$−149, (2Et+NPh)], 416 (58) [M$^+$−165, (2Et+NPh+NH$_2$)], 279 (100) [M$^+$−302, (2Et+NPh+NH$_2$+P(O)(OEt)$_2$], 212 (48), 137 (53) (P(O)(OEt)$_2$), 119 (26), 77 (60). Anal. calcd. for $C_{28}H_{32}N_5O_7P$ (581.2): C, 57.83; H, 5.55; N, 12.04; P, 5.33. Found: C, 57.81; H, 5.53; N, 12.02; P, 5.31.

*Ethyl 7-amino-6-carbamothioyl-6-(diethoxyphosphoryl)-1-phenyl-5-(phenylimino)-5,6-dihydro-1H-pyrazolo[4,3-b]-pyridine-3-carboxylate (**16c**)*

Colorless needles, mp 186–188°C (MeCN), yield: (0.89 g, 72%). IR: ν_{max}, cm^{-1} 3461, 3349 (2NH$_2$), 1710 (C=O), 1628 (C=NPh, exocyclic), 1605 (C=N), 1232 (P=O, bonded), 1074 (P–O–C) cm^{-1}. ^{1}H NMR [DMSO-d^6]: δ 1.13 (t, J_{H-H} = 6.5 Hz, 3H, C*H*$_3$CO), 1.28 (dt, J_{H-H} = 6.5, $^4J_{P-H}$ = 3.8 Hz, 6H, (*H*$_3$CCO)$_2$P), 3.88 (q, J_{H-H} = 6.5 Hz, 2H, *H*$_2$CO, ester), 4.31 (dq, $^3J_{P-H}$ = 9.5 Hz, 4H, (*H*$_2$CO)$_2$P), 6.22 (br, 1H, *H*^{A}N), 7.14–7.76 (m, 10H, *H*–Ph), 8.00 [br, 2H, *H*$_2$N–C(*S*)], 9.67 (br, 1H, *H*^{B}N) ppm. ^{13}C NMR [DMSO-d^6]: δ 194.3 (*C*=S), 180.3 (d, $^2J_{P-C}$ = Hz, *C*=NPh), 162.3 (*C*=O), 158.0, 154.7,

147.9, 144.4, 129.2, 129.1, 128.5, 123.9, 123.3, 111.4 (*C*–Ph, *C*-pyrazole), 127.5 (d, $^2J_{P-C}$ = 37 Hz, *C*–NH_2), 62.2 (d, $^2J_{P-C}$ = 8.7 Hz, ($CH_2O)_2$P), 61.8 (CH_2O), 54.8 (d, $^1J_{P-C}$ = 211 Hz, *C*–P), 16.3 (d, $^3J_{P-C}$ = 7.6 Hz, ($CH_3CO)_2$P), 14.2 (CH_3CO, ester) ppm. ^{31}P NMR [DMSO-d^6]: δ_P 27.5 ppm. EI–MS: *m/z* (%): 569 (13) [M^++1], 568 (15) [M^+], 550 (13) [M^+−18, H_2O], 521 [M^+−47, (Et+H_2O)], 461 (76) [M^+−107, (H_2O+Et+C(S)NH_2)], 370 (68) [M^+−198, (H_2O+Et+C(S)NH_2+NPh)], 233 (100) [M^+−335, (H_2O+Et+NPh+C(S)NH_2+P(O)$(OEt)_2$], 137 (48) (P(O)$(OEt)_2$), 119 (24), 212 (38), 77 (65). Anal. calcd. for $C_{26}H_{29}N_6O_5PS$ (568.16): C, 54.92; H, 5.14; N, 14.78; P, 5.45; S, 5.64. Found: C, 54.90; H, 5.12; N, 14.76; P, 5.43; S, 5.62.

Preparation of **16d**

Reagents: Compound **4** (0.8 g, 2.2 mmol), diethyl (methylthiomethyl)-phosphonate (**14d**) (0.49 g, 2.5 mmol), LiH (44 mg, 5.5 mmol), and DMF (20 mL) were stirred at r.t, for 6 h. The product mixture was worked up as described in the general procedure to afford the phosphonate derivative **16d**.

*Ethyl 7-amino-6-(diethoxyphosphoryl)-6-(methylthio)-1-phenyl-5-(phenylimino)-5,6-dihydro-1H-pyrazolo[4,3-b]-pyridine-3-carboxylate (**16d**)*

Yellow substance, mp 143–145°C (cyclohexane), yield: (0.9 g, 74%). IR: ν_{max}, cm^{-1} 3497, 3381 (NH_2), 1715 (C=O), 1618 (C=NPh, exocyclic), 1598 (C=N), 1230 (P=O, bonded), 1097 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.23 (t, J_{H-H} = 6.5 Hz, 3H, H_3CCO, ester), 1.31 (dt, J_{H-H} = 6.5, $^4J_{P-H}$ = 3.8 Hz, 6H, ($H_3CCO)_2$P), 2.33 (s, 3H, H_3CS), 3.68 (q, J_{H-H} = 6.5 Hz, 2H, H_2CCO), 4.28 (dq, $^3J_{P-H}$ = 9.8 Hz, 4H, ($H_2CO)_2$P), 6.32 (br, 1H, H^AN), 7.14–7.65 (m, 10H, *H*–Ph), 9.67 (br, 1H, H^BN) ppm. ^{13}C NMR [DMSO-d^6]: δ 173.9 (d, $^2J_{P-C}$ = 14.2 Hz, *C*=NPh), 162.3 (*C*=O, ester), 155.5, 143.7, 129.2, 128.5, 128.2, 123.3, 123.2, 122.9 (*C*-pyrazole, *C*–Ph), 119.5 (d, $^2J_{P-C}$ = 17.4 Hz, *C*–NH_2), 63.3 (d, $^2J_{P-C}$ = 8.7 Hz, ($CH_2O)_2$P), 61.8 (CH_2O), 51.5 (d, $^1J_{P-C}$ = 144.6 Hz, *C*–P), 16.2 (d, $^3J_{P-C}$ = 8.6 Hz, ($CH_3CO)_2$P), 16.0 (CH_3–S), 14.29 (CH_3C–O) ppm. ^{31}P NMR [DMSO-d^6]: δ_P 26.9 ppm. EI–MS: *m/z* (%): 556 (17) [M^++1], 555 (12) [M^+], 526 (18) [M^+−29, Et], 508 (11) [M^+−47, SMe], 479 (70) [M^+−76, (Et+SMe)], 388 [M^+−167, (Et+SMe+NPh)], 372 [M^+−183, (Et+SMe+NPh+NH_2)], 235 (100) [M^+−320, (Et+SMe+NPh+NH_2+P(O)$(OEt)_2$], 212 (38), 137 (52) (P(O)$(OEt)_2$), 119 (30), 77 (68). Anal. calcd. for $C_{26}H_{30}N_5O_5PS$ (555.2): C, 56.21; H, 5.44; N, 12.61; P, 5.57; S, 5.77. Found: C, 56.20; H, 5.42; N, 12.63; P, 5.55; S, 5.75.

Preparation of **19**

Reagents: A mixture of 0.8 g of compound **4** (2.2 mmol) in 10 mL DMF, and 0.41 g diethyl vinylphosphonate (**18**) (2.5 mmol) was treated, at r.t., with 44 mg LiH (5.5 mmol) in 20 mL DMF. The product mixture was worked up as described in the general procedure to afford the phosphonate derivative **19**.

*Ethyl-5-cyano-4-(2-ethoxy-5-(phenylimino)-2,5-dihydro-1H-1,2-azaphospholyl-2-oxide)-1-phenyl-1H-pyrazole-3-carboxylate (**19**)*

Colorless needles, mp 103–110°C (pentane), yield: (0.75 g, 72%. IR: ν_{max}, cm^{-1} = 2222 (CN), 1712 (C=O, ester), 1632 (C=NPh), 1608 (C=N), 1260 (P=O, free), 1083 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.24 (t, J_{H-H} = 6.7 Hz, 3H, CH_3CO), 1.29 (dt, J_{H-H} = 6.5 Hz, $^4J_{P-H}$ = 4.3 Hz, 3H, H_3COP), 4.22 (q, J_{H-H} = 6.7 Hz, 2H, H_2CO), 4.07 (dq, J_{H-H} = 6.5 Hz, $^3J_{P-H}$ = 5.0 Hz, 2H, H_2COP), 6.95 (d, $^3J_{P-H}$ = 6.7, 1H, H(4)), 7.55 (d, $^2J_{P-H}$ = 17.2, 1H, H(3)), 7.27–7.66 (m, 10H, H–Ph) ppm. ^{13}C NMR: δ 164.3 (d, $^3J_{P-C}$ = 6.8 Hz, C=NPh), 157.9 (*C*=O), 153.2, 142.5, 135.0, 128.2, 127.7, 127.4, 121.8, 120.9, 119.9 (C-pyrazole, *C*–Ph), 120.2 (*C*N), 138.63 [d, $^2J_{P-C}$ = 8.5 Hz, *C*(4)], 123.8 (d, $^1J_{P-C}$ = 144 Hz, *C*–P), 96.1 (*C*–CN), 60.9 (CH_2O), 60.6 (d, $^2J_{P-C}$ = 8.7 Hz, CH_2OP), 16.8 (d, $^3J_{P-C}$ = 7.6 Hz, CH_3COP), 14.05 (CH_3CO) ppm. ^{31}P NMR [DMSO-d^6]: δ_P 15.3 ppm. EI–MS: *m/z* (%) 475 (18) [M^+], 449 (26) [M^+−26, CN], 404 (28) [[M^+−71, (CN+OEt)], 331 (100) [M^+−144, (CN+OEt+CO_2Et)], 240 (91), 212 (23), 119 (16), 77 (44). Anal. calcd. for $C_{24}H_{22}N_5O_4P$ (475.1): C, 60.63; H, 4.66; N, 14.73; P, 6.51. Found: C, 60.61; H, 4.63; N, 14.71; P, 6.53.

Preparation of **21**

Reagents: Compound **4** (0.8 g, 2.2 mmol) and a solution of diethyl (2-methylallyl)phosphonate (**20**) (0.48 g, 2.5 mmol) in 30 mL CH_3Cl were treated with 5 mL of an aqueous LiOH solution (0.5 N), followed by heating under reflux for 6 h. The product mixture was then extracted with 2 × 20 mL of CH_3Cl. The combined extracts were washed with 40 mL of distilled H_2O, dried, and the solvent was removed under reduced pressure to give phosphonate **21**.

*Ethyl 5-cyano-4-(3-(diethoxyphosphoryl)-4-methyl-2-(phenylamino)-1H-pyrrol-1-yl)-1-phenyl-1H-pyrazole-3-carboxylate (**21**)*

White needles, mp 154–156°C (CH_2Cl_2), yield: (0.84 g, 70%). IR: ν_{max}, cm^{-1} = 3378 (NH), 2234 (CN), 1708 (C=O), 1227 (P=O), 1084 (P–O–C). ^{1}H NMR [DMSO-d^6]: δ 1.14 (t, J_{H-H} = 6.5 Hz, 3H, CH_3CO), 1.34 (dt, J_{H-H} = 6.5 Hz, $^4J_{P-H}$ = 4.3 Hz, 6H, ($H_3CO)_2$P), 2.10 (d, $^4J_{P-H}$ = 4.5 Hz, 3H, CH_3), 3.77 (q, J_{H-H} = 6.5 Hz, 2H, H_2CO), 4.23 (dq, $^3J_{P-H}$ = 9.7 Hz, 4H, ($H_2CO)_2$P), 6.24 (d, $^4J_{P-H}$ = 4.2 Hz, 1H, *H*C–pyrrole), 7.22–8.11 (m, 10H, *H*–Ph), 9.68 (br, 1H, *H*N) ppm. ^{13}C NMR [DMSO-d^6]: δ 158.9 (*C*=O), 143.2, 141.8, 129.4, 128.3, 127.4, 122.5, 120.0, 119.3 (*C*–Ph, *C*-pyrazole), 135.7 (d, $^2J_{P-C}$ = 14.0 Hz, *C*–NHPh), 129.9 (d, $^1J_{P-C}$ = 211.8 Hz, *C*–P), 120.3 (*C*N), 118.2 (d, $^2J_{P-C}$ = 14.8 Hz, *C*–Me), 116.2 [d, $^3J_{P-C}$ = 8.8 Hz, *C*(2)-pyrrole], 98.5 (*C*–CN), 62.7 (d, $^2J_{P-C}$ = 8.7 Hz, ($CH_2O)_2$P), 60.9 (CH_2O), 15.9 (d, $^3J_{P-C}$ = 7.6 Hz, ($CH_3CO)_2$P), 14.0 (CH_3CO), 13.0 (CH_3) ppm. ^{31}P NMR [DMSO-d^6]: δ_P 29.4 ppm. EI–MS: *m/z* (%) 547 (21) [M^+], 532 (31) [M^+−15, Me], 521 (18) [M^+−26, CN], 506 (58) [M^+−41, (Me+CN)], 414 (44) [M^+−133, (CN+Me+NHPh)], 277 (100) [M^+−270, (CN+Me+NHPh+P(O)$(OEt)_2$)], 212 (33), 137 (42) (P(O)$(OEt)_2$), 119 (18), 77 (39). Anal. calcd. for $C_{28}H_{30}N_5O_5P$ (547.2): C, 61.42; H, 5.52; N, 12.79; P, 5.66. Found: C, 61.40; H, 5.50; N, 12.77; P, 5.63.

Pharmacological screening

Animals

Male Swiss albino mice (25–30 g) were housed in a room with controlled temperature (22 ± 1°C), humidity (55 ± 10%) and photoperiod (12:12 h) light–dark cycle for at least a week before being used. They were maintained on standard pellet diet and water *ad libitum* throughout the experiment. A minimum of six animals were used in each group. Throughout the experiments, the animals were processed according to the suggested international ethical guidelines for the care of laboratory animals of animal ethics.

Analgesic activity

Analgesic activity was evaluated using the *p*-benzoquinone (*p*-BQ)-induced writhing test in mice [26]. Thirty minutes after

the subcutaneous administration of a test sample in dimethyl sulfoxide (DMSO; 100 mg/kg body weight), the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5% w/v (*p*-BQ) solution in distilled water. Control animals received an appropriate volume of dosing vehicle DMSO. The mice were then kept individually for the observation and the total number of the abdominal contractions (writhing movements) was counted for the following 15 min, starting 5 min after the pBQ injection. The data represent the average of the total number of writhes observed. Analgesic activity was then expressed as the percentage change from writhing controls.

$$\%\,\text{Potency} = \frac{(N_c - N_t)}{N_c} \times 100$$

where N_c and N_t are the total number of writhing movements at time 0 and after 15 min, respectively.

Antiinflammatory activity

Carrageenin-induced hind paw edema model was utilized for evaluation of antiinflammatory activity [27]. Each experimental group contained seven animals minimally. Thirty minutes after the subcutaneous administration of a test sample in DMSO (100 mg/kg body weight) or dosing vehicle, each mouse was injected with 0.1 mL/10 g body weight of freshly prepared suspension of carrageen (0.5 mg/25 mL) in physiological saline into sub-plantar tissue of the right hind paw. As a control group, 25 mL saline was injected into the left hind paw. Paw edema was measured in 90, 180, 270, and 360 min after carrageenin injections. Measurement of paw volume: The volume of the hind paw of the mouse up to the ankle joint was measured plathysmographically by the mercury displacement method. The ankle joint of the mouse was marked with a skin marking pencil and the paw was dipped into 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. Mean values of each treated group were compared with the control group and analyzed by using statistical methods. The antiinflammatory 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.

Antitumor activity screening

Following the technique previously reported by Skehan and Storeng [31], the antitumor activity of the phosphonates **9a**, **9b**, **13a**, **16a**, and **16c** 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 in comparison to the behavior of DOX or CIS (Table 2).

Materials and methods

(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 SRB stain; (iv) excess stain was washed away with acetic acid and the 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 2.

$$\text{Calcualtions}: \ (\text{IC}_{50}\ \mu\text{M}) = \frac{\text{IC}_{50}\ \mu\text{g/mL}}{\text{M.Wt.}} \times 1000$$

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