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ORIGINAL PAPER

Synthesis and in vivo anti- or pro-inflammatory activity of new bisphosphonates and vinylphosphonates

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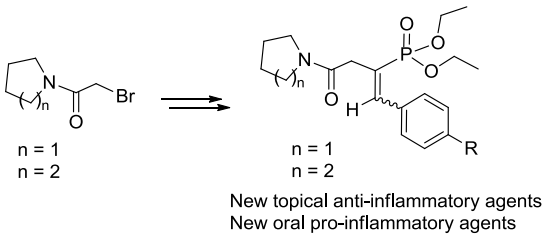
Abstract

We herein report the synthesis and in vivo anti-inflammatory activity of a series of new bisphosphonate and vinylphosphonate derivatives of pyrrolidine and piperidine through a short route of synthesis. The *C*-alkylation of tetraethylmethylene diphosphonate with *N*-(bromoacetyl)pyrrolidine or *N*-(bromoacetyl)piperidine, respectively, yielded the corresponding α-substituted bisphosphonates in excellent yields (82–89%). Next, the Horner–Wadsworth–Emmons reaction of these bisphosphonates with aromatic aldehydes afforded final vinylphosphonates in moderate yields (26–36%). Synthesized bisphosphonates and vinylphosphonates were tested by two models of acute inflammation in male BalB/c mice, founding excellent edema inhibition by topical TPA (12-*O*-tetradecanoylphorbol-13-acetate) model (67.53–72.10% in comparison with indomethacin = 64.89%). However, remarkably pro-inflammatory effect by systematic carrageenan model (− 9.78 to − 36.18) was observed, probably due to biotransformation. In conclusion, the new vinylphosphonates emerged as attractive topical anti-inflammatory compounds that "twist" its pharmacological activity to route of administration. Further research is needed to understand the dual effect.

Graphical abstract



Keywords Phosphorus compounds · Alkenes · Wittig reaction · Inflammation

Introduction

In recent years, vinylphosphonates have been gaining attention in need of their interesting and underexplored pharmacological activities. For example, many vinylphosphonates have applications as antimicrobials [1–3], enzymatic inhibitors [4, 5], anticancer agents [6, 7] and have also been used in siRNA gene silencing technology [8–11] and drug delivery systems [12]. In this context, it is worth mentioning dehydrophos [1], a natural broad spectrum antibiotic, tamiphosphor [2], a synthetic bioisostere of the antiviral

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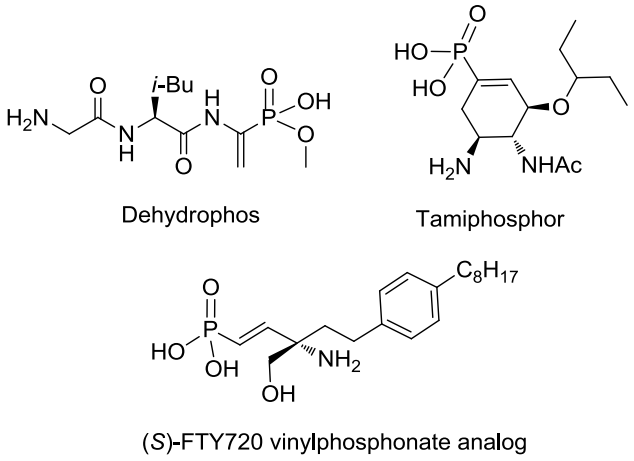


Fig. 1 Structure of some biologically active vinylphosphonates

oseltamivir and (*S*)-FTY720, a potential anticancer agent [6] (Fig. 1). On the other hand, bisphosphonates have been used as the primary therapeutic agents in the treatment of bone diseases such as osteoporosis, metastatic and osteolytic bone disease, Paget's disease of bone and osteopenia. For instance, zoledronate, alendronate, pamidronate, and etidronate (all commercial drugs) inhibit bone resorption interfering with the action of the bone-resorbing osteoclasts by different molecular modes of action [13–15].

In other instances, the synthesis of vinylphosphonates has been carried out through Horner–Wadsworth–Emmons (HWE) reaction [16, 17], hydrophosphorylation of alkynes [18–21], cross coupling of dialkylphosphites and dibromoalkenes [22, 23], and Heck reaction starting from simple vinylphosphonates and aryl halides [24, 25], mainly. Despite the importance and potential of these compounds to medicinal chemistry, anti-inflammatory activity studies on vinylphosphonates have just received modest attention [26–28]. An interesting example of in vitro anti-inflammatory activity of vinylphosphonates was reported by Al Quntar et al. [28]. Compounds **1–3** (Fig. 2a) modulated the production of reactive oxygen species, nitric oxide, and tumoral necrosis factor (TNF-α) (all of them involved in the development of inflammation) in cultured murine macrophages.

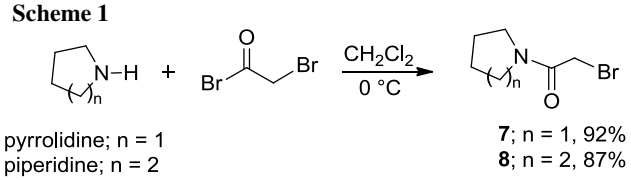
In spite of all the above mentioned and rheumatoid arthritis as an emerging problem of public health [29], we herein report the synthesis of new potential anti-inflammatory agents. These vinylphosphonates are structurally related to parent compounds **1–3**. The new vinylphosphonates **4–6** conserve the α,β-unsaturated phosphonate in **1–3**, an aromatic ring and also a nitrogen atom at four-bond distance from phosphonate (Fig. 2b). Hence, the synthesis of target compounds **4–6** was achieved through HWE reaction of bisphosphonates **9–10** with aromatic aldehydes as the key step. Furthermore, an in vivo evaluation of **4–6** anti-inflammatory agents was carried out to develop new promising antiarthritic compounds.

Results and discussion

Synthesis

In addition to the structural relationship between target compounds **4–6** and anti-inflammatory vinylphosphonates **1–3**,

Scheme 1



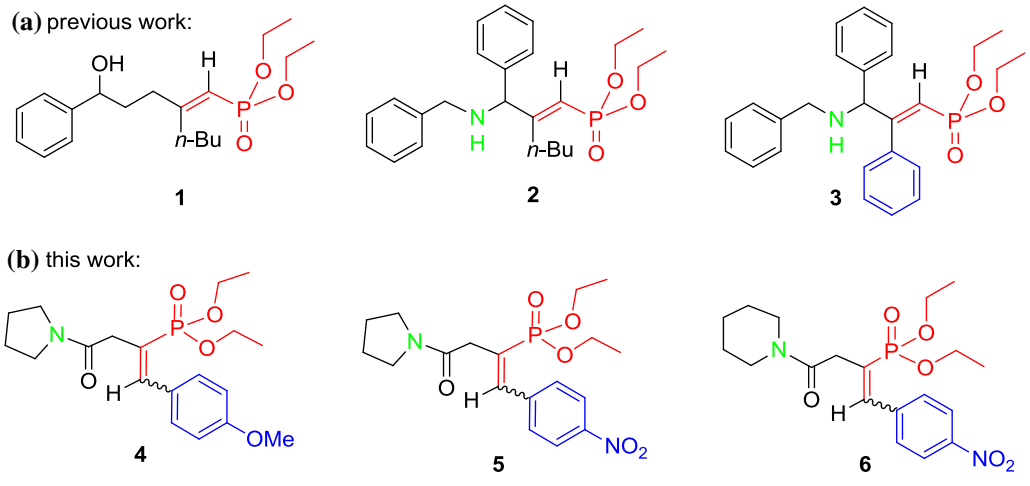


Fig. 2 **a** Previously reported in vitro anti-inflammatory vinylphosphonates [28]; **b** newly proposed vinylphosphonates as anti-inflammatory agents

we used the Prediction of Activity Spectra for Substances (PASSOnline) database, getting probability of activity as antiarthritic agents > 0.9 (in scale from 0 to 1) in **4–6**. PASSonline predicts more than 7000 types of biological activity with an average accuracy > 95% [30] and has been used successfully to predict in vitro and in vivo diverse pharmacological activities of new compounds (and possible adverse effects) [31–34]. Moreover, proposed vinylphosphonates **4–6** agreed with Lipinski's rules [35] as a clear indicator of its drug-likeness.

For the synthesis of target compounds **4–6** our strategy started with amidation of pyrrolidine or piperidine with bromoacetyl bromide, respectively, under previously established conditions [36], obtaining excellent yields without purification (Scheme 1).

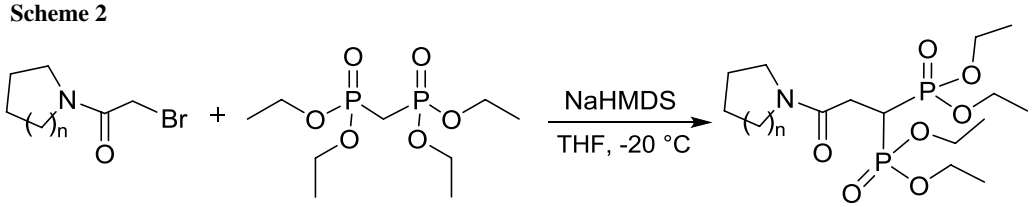
Then we tried to prepare the key bisphosphonate intermediates **9** and **10** through *C*-alkylation reaction of tetraethylmethylene diphosphonate with alkyl halides **7** and **8** under anhydrous conditions. At the first attempt, using NaHMDS as base and alkyl halide **7**, product **9** was detected by NMR analysis. Due to the instability of **9** in silica gel column chromatography, the product could not be obtained under these conditions. Therefore, the use of neutral alumina as stationary phase during purification of **9** or **10** under identical reaction conditions allowed obtention of desired products in excellent yields (Scheme 2).

As a final step in the synthesis, using bisphosphonates **9–10**, respectively, we carried out the HWE reaction with aromatic aldehydes, under inert conditions. The reaction of **9** with *p*-anisaldehyde using NaHMDS as base, afforded product **4** as trace. A ^{31}P NMR analysis of crude reaction mixture showed an *E*/*Z* ratio of 65:35. Next, the use of LDA as a hard base improved yield to 12%; the crude reaction's NMR analysis showed an *E*/*Z* ratio of 13:87. Subsequent chromatographic purification afforded 8% of (*Z*)-**4** and 4% of (*E*)-**4**. Finally, the use of the hardest base *n*-BuLi produced compound **4** in 36% yield, with an *E*/*Z* ratio of 17:83. This time again it was possible to separate diastereomers (*Z*)-**4** in 16% and (*E*)-**4** in 20% yields. Employing *n*-BuLi in subsequent experiments afforded vinylphosphonates **5** and **6** in moderate yields (26–32%). It is worth to mentioning that for compounds **5** and **6**, just *E* isomer was detected and isolated (Scheme 3).

The *E* and *Z* configuration in **4–6** was unambiguously assigned through ^{1}H NMR spectra, specifically to vinylic $^{3}J_{H\text{-}P}$ coupling, observing a typical $J = 23.9$ Hz for *E* isomer and $J = 47.1$ Hz for *Z* isomer, as has been well documented elsewhere [37] and it is depicted in Fig. 3.

To the best of our knowledge, both bisphosphonates **9** and **10** and vinylphosphonates **4–6** have not been reported in the literature, hence constitute new and unexplored bioactive compounds.

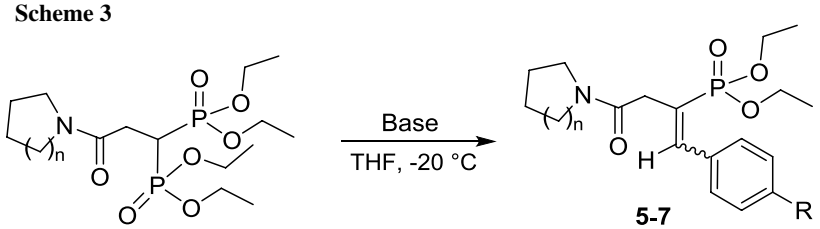
Scheme 2



7; n = 1
8; n = 2

9; n = 1, 89%
10; n = 2, 82%

Scheme 3



9; n = 1
10; n = 2

5-7

[a] NaHMDS
[b] LDA
[c] *n*-BuLi

	(E):*(Z)* ratio
4; n = 1, R = 4-$MeOC_6H_4$, trace[a]	65:35
4; n = 1, R = 4-$MeOC_6H_4$, 12%[b]	13:87
4; n = 1, R = 4-$MeOC_6H_4$, 36%[c]	17:83
5; n = 1, R = 4-$O_2NC_6H_4$, 32%[c]	-
6; n = 2, R = 4-$O_2NC_6H_4$, 26%[c]	-

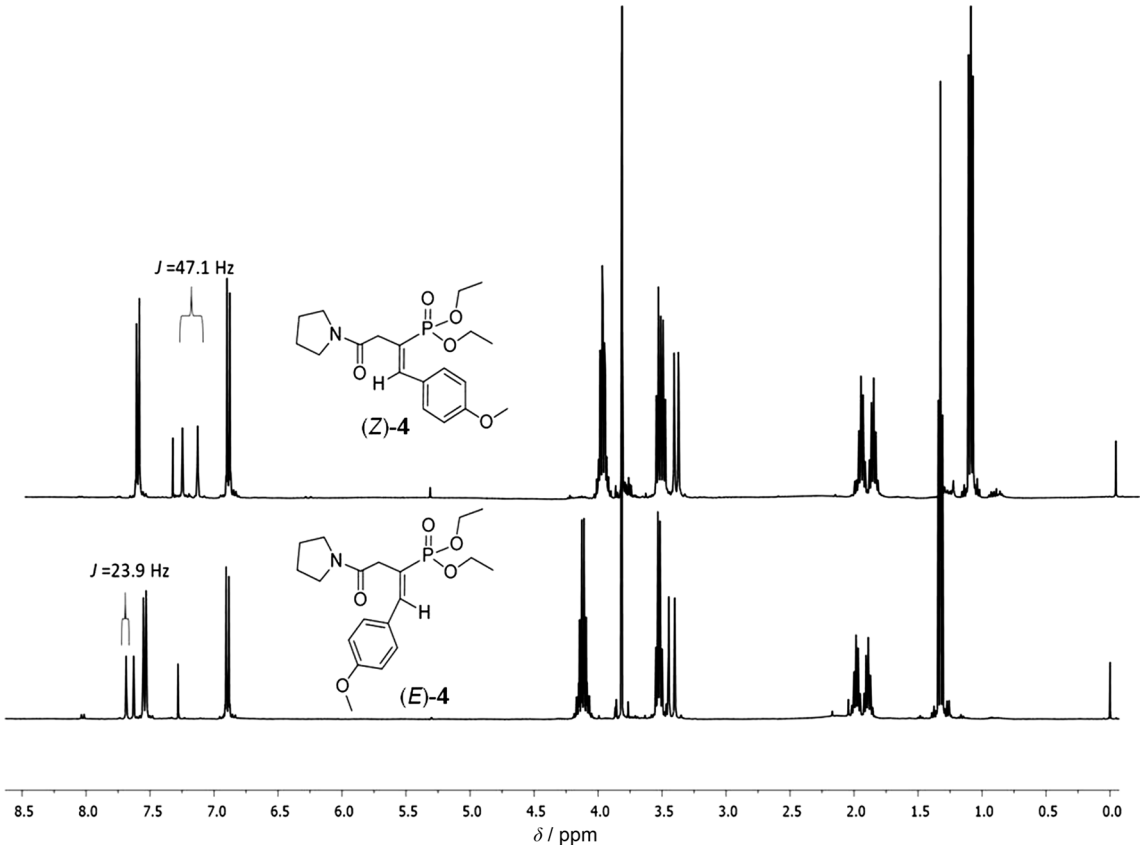


Fig. 3 NMR ^{1}H spectra of diastereomers (*Z*)-**4** and (*E*)-**4**

Table 1 Anti-inflammatory activity of **9**, **10**, and **4–6**, 5 h after administration, carrageenan model

Treatment	Paw edema/mm	% inhibition
Carrageenan	0.48 ± 0.07	–
Indomethacin 20 mg/kg[a]	0.38 ± 0.02	21.86
Test compounds 50 mg/kg		
9[b]	0.45 ± 0.06	7.01
10[b]	0.38 ± 0.02	22.15
(*E*)-**4**[b]	0.42 ± 0.09	14.35
(*Z*)-**4**[b]	0.57 ± 0.01	− 35.78
5[b]	0.53 ± 0.06	− 9.78
6[b]	0.66 ± 0.08	− 36.18

Each group represents the mean ± standard error of the mean (SEM). The percentage of inhibition of edema is with respect to the carrageenan group. Two-way analysis of variance (ANOVA) and post hoc Student–Newman–Keuls ($p \leq 0.05$)

[a]Versus carrageenan control

[b]Versus indomethacin

Table 2 Anti-inflammatory activity of **9**, **10**, and **4–6**, 6 h after administration, TPA model

	Auricular edema/mg	% inhibition
TPA control	8.31 ± 0.52	–
Indomethacin 2 mg/ear[a]	2.92 ± 0.41a	64.89
Test compounds 2 mg/ear		
9[b]	4.70 ± 0.62b	43.48
10[b]	3.66 ± 0.18b	55.99
(*E*)-**4**[b]	2.70 ± 0.16a	67.53
(*Z*)-**4**[b]	2.36 ± 0.30a	71.62
5[b]	2.45 ± 0.56a	70.54
6[b]	2.32 ± 0.33a	72.10

Each group represents the mean ± standard error of the mean (SEM). The percentage of inhibition of the edema is with respect to the TPA group. One-way analysis of variance (ANOVA) and post hoc Student–Newman–Keuls ($p \leq 0.05$)

[a]Versus carrageenan control

[b]Versus indomethacin

Anti-inflammatory activity

Anti-inflammatory activity evaluation [38] of compounds **9** and **10**, and **4–6** was carried out by oral gavage at 50 mg/kg in male BalB/c mice using carrageenan inducing paw edema as phlogistic agent and indomethacin as positive control. Astonishingly, just bisphosphonates **9** and **10** exhibited activity with 7.01 and 22.15% edema inhibition,

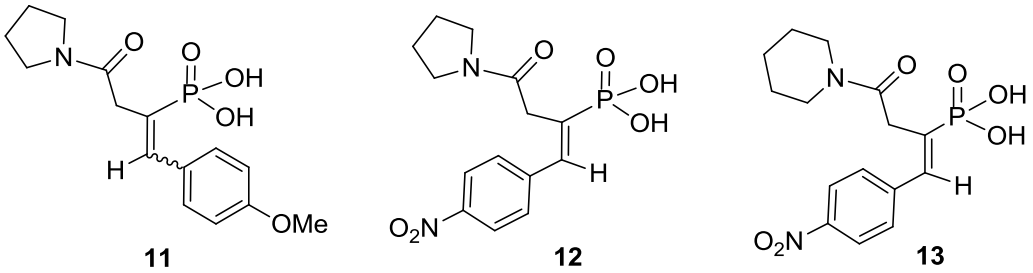


Fig. 4 Proposed pro-inflammatory metabolites presumably formed by oral administration

respectively (indomethacin showed 21.89% inhibition). In contrast, vinylphosphonates (*Z*)-**4**, **5**, and **6** were more pro-inflammatory than carrageenan itself (Table 1). Despite these previous results, we carried out topical administration of compounds **9** and **10**, and **4–6** at 2 mg per ear in BalB/c mice using TPA (12-*O*-tetradecanoylphorbol-13-acetate) as irritant. In this assay, moderate edema inhibition for bisphosphonates **9** and **10** was observed, but significant inhibition for vinylphosphonates **4–6** was achieved (67.53–72.10%), comparable or superior than indomethacin (64.89%) (Table 2). In view of the dual behavior of vinylphosphonates **4–6** in function of administration route, we propose a preliminary explanation: the hydrolysis of phosphonate functional group in **4–6** affords phosphonic acid derivatives **11–13** (Fig. 4) which could have pro-inflammatory activity. In this respect, it is important to mention that compounds **11–13** got probability of inflammation as adverse effect of 0.27–0.35 through PASSonline prediction; but this prediction was not obtained in bisphosphonates **9** and **10** or vinylphosphonates **4–6** at all.

In addition to the above-mentioned hypothesis, the successful use of phosphonates as prodrugs, which are hydrolyzed to bioactive phosphonic acids by enzymatic processes, has been well documented [39–41].

In spite of all, of the tested compounds (**9**, **10**, and **4–6**) only **9** and **10** were active when administered intragastric; however, compounds (*Z*)-**4**, **5**, and **6** induced inflammation. This effect may be a result of biotransformation of phosphonates **4–6** to its respective phosphonic acids **11–13**, predicted as potential pro-inflammatory metabolites. On the other hand, all compounds tested (**9**, **10**, and **4–6**) by the topical route were active, so they are candidates for further research to develop new clinically effective anti-inflammatory agents.

Finally, it should be noted that topical and systemic inflammation models carried out in this work are widely used to explore the anti-inflammatory effect of synthetic and natural products [42–44].

Conclusion

The synthesis of new bisphosphonates **9** and **10** and vinylphosphonates **4–6** provided in vivo anti- or pro-inflammatory agents, tested by two models of acute inflammation. Interestingly, in function of route of administration, we found pro-inflammatory activity in vinylphosphonates (*Z*)-**4**, **5**, and **6** with oral dosage (carrageenan model), but promising anti-inflammatory activity with topical administration (TPA model). A preliminary hypothesis was disclosed for this dual behavior, but further research is needed to clarify the dual effect. Lastly, synthesized compounds **9**, **10**, and **4–6** emerged as promising new topical anti-inflammatory agents which should be the object of future studies.

Experimental

All commercial materials were used as received from Sigma-Aldrich® without further purification. Flash chromatography was performed using 230–400-mesh Silica Flash 60® silica gel. Thin-layer chromatography was performed with pre-coated TLC sheets of silica gel (60F254, Merck). NMR spectra were recorded in a Varian System instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C, and 161.9 MHz for ^{31}P), and calibrated with $CDCl_3$ as the solvent and TMS as the internal standard signal. Chemical shifts (δ) are reported in parts per million. Multiplicities are recorded as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, td = triplet of doublets, bs = broad singlet, q = quartet, and m = multiplet. Coupling constants (J) are given in Hz. High-resolution mass spectra (HRMS) were obtained in a SYNAPT G2-Si (Waters) spectrometer equipped with single quadrupole mass filter and time of flight mass analyzer (Q-TOF).

General procedure for the synthesis of alkyl halides 7 and 8

To an ice bath-cooled solution of bromoacetyl bromide (13.78 mmol) in 20 cm^3 of CH_2Cl_2 was added dropwise via addition funnel a solution of pyrrolidine or piperidine, respectively (2 equivalents) in 30 cm^3 of CH_2Cl_2, and the reaction mixture was stirred overnight. Then the reaction mixture was quenched by the addition of 20 cm^3 of distilled water and extracted with CH_2Cl_2 (3×20 cm^3). Next, to the combined organic extracts 30 cm^3 of HCl 2.0 N was added. After partition, the final organic extract was dried over Na_2SO_4, filtered, and evaporated under reduced pressure, yielding desired bromoamides **7** and **8**.

***N*-(Bromoacetyl)pyrrolidine (7)** Yield: 2.42 g (92%) as a white solid that melts at room temperature. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.87–1.94$ (m, 2H, CH_2), 1.98–2.05 (m, 2H, CH_2), 3.50 (t, $J = 6.9$ Hz, 2H, CH_2N), 3.54 (t, $J = 6.8$ Hz, 2H, CH_2N), 3.82 (AB system, $J = 20.0$ Hz, 2H, CH_2Br) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 24.31$ (CH_2), 26.15 (CH_2), 27.39 (CH_2Br), 46.43 (CH_2N), 47.06 (CH_2N), 165.07 (C=O) ppm; HRMS [ESI]: *m/z* calculated for $C_6H_{11}BrNO$ ($[M+H]^+$) 192.0024, found 192.0095.

***N*-(Bromoacetyl)piperidine (8)** Yield: 2.47 g (87%) as a pale yellow oil. ^{1}H and ^{13}C NMR spectra were found to be identical with the ones described in Ref. [45].

General procedure for the synthesis of bisphosphonates 9 and 10

To a solution of tetraethylmethylene bisphosphonate in 8 cm^3 THF was added dropwise NaHMDS (1.5 equiv) at − 5 °C under nitrogen. The solution was stirred for 15 min and then 10 min at room temperature. After this time, a solution of the respective alkyl halide **7** or **8** (2.5 equiv) was added at − 5 °C. Ten minutes later, reaction mixture was stirred at − 20 °C for 18 h. Finally, the reaction mixture was concentrated, and crude product was analyzed by ^{1}H and ^{31}P NMR and then purified by column chromatography supported on neutral alumina, CH_2Cl_2:AcOEt:MeOH (50:49:01), obtaining corresponding bisphosphonates **9** and **10**.

Tetraethyl [3-oxo-3-(pyrrolidin-1-yl)propane-1,1-diyl]-bis(phosphonate) (9, $C_{15}H_{31}NO_7P_2$) Yield: 687 mg (89%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.31–1.34$ (m, 12H, CH_3), 1.83–2.00 (m, 4H, CH_2), 2.80 (td, $J = 16.2$, 5.8 Hz, 2H, CH_2C=O), 3.42–3.57 (m, 5H, CH_2N, CHP), 4.10–4.25 (m, 8H, OCH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.30$ (d, $J = 6.1$ Hz, CH_3), 24.37 (CH_2), 25.99 (CH_2), 30.31 (t, $J = 4.1$ Hz, CH_2C=O), 31.59 (t, $J = 135.4$ Hz, CHP), 46.08 (CH_2N), 46.42 (CH_2N), 62.5 (d, $J = 6.6$ Hz, OCH_2), 62.90 (d, $J = 6.4$ Hz, OCH_2), 167.69 (t, $J = 7.6$ Hz, C=O) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$): $\delta = 23.86$ ppm; HRMS [ESI^+]: *m/z* calculated 400.1654, found 400.1720.

Tetraethyl [3-oxo-3-(piperidin-1-yl)propane-1,1-diyl]-bis(phosphonate) (10, $C_{16}H_{33}NO_7P_2$) Yield: 780 mg (82%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.33$ (t, $J = 7.1$ Hz, 12H, CH_3), 1.51–1.69 (m, 6H, CH_2), 2.85 (td, $J = 16.3$, 5.5 Hz, 2H, CH_2C=O), 3.43–3.65 (m, 5H, CH_2N, CHP), 4.12–4.23 (m, 8H, OCH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.33$ (d, $J = 6.4$ Hz, CH_3), 24.48 (CH_2), 25.53 (CH_2), 26.34 (CH_2), 28.73 (t, $J = 3.9$ Hz, CH_2C=O), 31.62 (t, $J = 135.4$ Hz, CHP), 43.55 (CH_2N), 46.43 (CH_2N), 62.51 (d, $J = 6.7$ Hz, OCH_2), 62.91 (d, $J = 6.5$ Hz, OCH_2), 167.30 (t, $J = 7.4$ Hz, C=O) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$): $\delta = 23.99$ ppm; HRMS [ESI^+]: *m/z* calculated 414.1811, found 414.1809.

General procedure for the synthesis of vinylphosphonates 4–6

A solution of *n*-BuLi (1.5 equiv) was added dropwise to a stirred solution of respective bisphosphonate **9** or **10** (1 equiv) in 7 cm^3 THF at − 5 °C under nitrogen. Resultant solution was stirred for 15 min at this temperature and then 10 min at room temperature, followed by the addition of respective aromatic aldehyde (1.1 equiv) at − 5 °C. Reaction mixture was stirred for 10 min at this temperature and then for 18 h at − 20 °C. Finally, the reaction mixture was concentrated, and crude product was analyzed by ^{1}H and ^{31}P NMR and then purified by column chromatography supported on silica gel, AcOEt:MeOH (90:10), yielding vinylphosphonates **4–6**.

(*Z*)-Diethyl [1-(4-methoxyphenyl)-4-oxo-4-(pyrrolidin-1-yl)-but-1-en-2-yl]phosphonate (4, $C_{19}H_{28}NO_5P$) Yield: 54 mg (16%) as yellow viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.12$ (t, $J = 7.1$ Hz, 6H, CH_3), 1.84–2.00 (m, 4H, CH_2), 3.40 (d, $J = 13.3$ Hz, 2H, CH_2C=O), 3.48–3.55 (m, 4H, CH_2N), 3.82 (s, 3H, OCH_3), 3.92–4.01 (m, 4H, OCH_2), 6.86 (AA′BB′, $J = 8.8$ Hz, 2H, H_{arom}), 7.16 (d, $J = 47.2$ Hz, 1H, CH), 7.56 (AA′BB′, $J = 8.9$ Hz, 2H, H_{arom}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 15.99$ (d, $J = 7.0$ Hz, CH_3), 24.43 (CH_2), 26.14 (CH_2), 41.81 (d, $J = 12.0$ Hz, CH_2C=O), 45.89 (CH_2N), 46.76 (CH_2N), 55.22 (OCH_3), 61.76 (d, $J = 5.7$ Hz, OCH_2), 113.12, 121.43 (d, $J = 178.7$ Hz, CP), 128.15 (d, $J = 7.5$ Hz), 131.14 (d, $J = 1.6$ Hz), 146.68 (d, $J = 8.8$ Hz), 159.85, 168.98 (d, $J = 4.7$ Hz, C=O) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$): $\delta = 18.12$ ppm; HRMS [ESI^+]: *m/z* calculated 382.1783, found 382.1952.

(*E*)-Diethyl [1-(4-methoxyphenyl)-4-oxo-4-(pyrrolidin-1-yl)-but-1-en-2-yl]phosphonate (4, $C_{19}H_{28}NO_5P$) Yield: 69 mg (20%) as a yellow viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.33$ (t, $J = 7.1$ Hz, 6H, CH_3), 1.86–2.02 (m, 4H, CH_2), 3.43 (d, $J = 18.7$ Hz, 2H, $CH_2C{=}O$), 3.50–3.55 (m, 4H, CH_2N), 3.82 (s, 3H, OCH_3), 4.07–4.17 (m, 4H, OCH_2), 6.90 (AA′BB′, $J = 8.8$ Hz, 2H, H_{arom}), 7.55 (AA′BB′, $J = 8.5$ Hz, 2H, H_{arom}), 7.66 (d, $J = 23.9$ Hz, 1H, CH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.48$ (d, $J = 6.9$ Hz, CH_3), 24.67 (CH_2), 26.35 (CH_2), 33.73 (d, $J = 10.5$ Hz, $CH_2C{=}O$), 46.20 (CH_2N), 47.05 (CH_2N), 55.43 (OCH_3), 62.08 (d, $J = 5.1$ Hz, OCH_2), 113.99, 121.99 (d, $J = 181.2$ Hz, CP), 127.88 (d, $J = 22.7$ Hz), 130.90, 146.61 (d, $J = 11.7$ Hz), 160.25, 168.35 (d, $J = 1.8$ Hz, C=O) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$): $\delta = 22.07$ ppm; HRMS [ESI$^+$]: *m/z* calculated 382.1783, found 382.1952.

(*E*)-Diethyl [1-(4-nitrophenyl)-4-oxo-4-(pyrrolidin-1-yl)-but-1-en-2-yl]phosphonate (5, $C_{18}H_{25}N_2O_6P$) Yield: 94 mg (32%) as viscous pale brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.34$ (t, $J = 7.1$ Hz, 6H, CH_3), 1.88–2.04 (m, 4H, CH_2), 3.36 (d, $J = 18.6$ Hz, 2H, $CH_2C{=}O$), 3.49–3.55 (m, 4H, CH_2N), 4.09–4.20 (m, 4H, OCH_2), 7.75 (d, $J = 23.6$ Hz, 1H, CH), 7.80 (AA′BB′, $J = 8.9$ Hz, 2H, H_{arom}), 8.22 (AA′BB′, $J = 8.8$ Hz, 2H, H_{arom}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.31$ (d, $J = 6.8$ Hz, CH_3), 24.49 (CH_2), 26.12 (CH_2), 33.44 (d, $J = 10.0$ Hz, $CH_2C{=}O$), 46.12 (CH_2N), 46.99 (CH_2N), 62.30 (d, $J = 5.2$ Hz, OCH_2), 123.63, 128.27 (d, $J = 179.3$ Hz, CP), 129.78 (d, $J = 1.4$ Hz), 141.56 (d, $J = 22.3$ Hz), 144.05 (d, $J = 11.8$ Hz), 147.71, 167.36 (d, $J = 1.6$ Hz, C=O) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$): $\delta = 19.21$ ppm; HRMS [ESI$^+$]: *m/z* calculated 397.1528, found 397.1576.

(*E*)-Diethyl [1-(4-nitrophenyl)-4-oxo-4-(piperidin-1-yl)-but-1-en-2-yl]phosphonate (6, $C_{19}H_{27}N_2O_6P$) Yield: 115 mg (26%) as viscous pale brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.35$ (t, $J = 7.1$ Hz, 6H, CH_3), 1.55–1.72 (m, 6H, CH_2), 3.40 (d, $J = 18.3$ Hz, 2H, $CH_2C{=}O$), 3.45–3.47 (m, 2H, CH_2N), 3.59–3.62 (m, 2H, CH_2N), 4.07–4.21 (m, 4H, OCH_2), 7.69 (AA′BB′, $J = 8.5$ Hz, 2H, H_{arom}), 7.74 (d, $J = 23.8$ Hz, 1H, CH), 8.22 (AA′BB′, $J = 8.8$ Hz, 2H, H_{arom}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.31$ (d, $J = 6.7$ Hz, CH_3), 24.46 (CH_2), 25.67 (CH_2), 26.49 (CH_2), 31.87 (d, $J = 9.5$ Hz, $CH_2C{=}O$), 43.35 (CH_2N), 47.25 (CH_2N), 62.33 (d, $J = 5.3$ Hz, OCH_2), 123.62, 128.87 (d, $J = 180.0$ Hz, CP), 129.71 (d, $J = 1.4$ Hz), 141.63 (d, $J = 22.6$ Hz), 143.69 (d, $J = 11.7$ Hz), 147.61, 167.15 (d, $J = 1.7$ Hz, C=O) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$): $\delta = 19.45$ ppm; HRMS [ESI$^+$]: *m/z* calculated 411.1685, found 411.1690.

Evaluation of anti-inflammatory activity

Carrageenan-induced edema procedure was performed as described by Pérez-González et al. [38]. Treated groups ($n = 5$) received indomethacin (20 mg/kg) by the intragastric route or tested compounds **9**, **10**, and **4–6** (50 mg/kg) 1 h prior to the injection of carrageenan (20 cm^3, 2%). The samples were solubilized in Tween 80:water (10:90) and the control received only vehicle. The percentage of inhibition was calculated by comparing the measurement of the paw edema at different times (1, 3, 5, 7, and 24 h) using a digital micrometer and the value of time zero (baseline) (E_0). The results were analyzed with the formula described by Pérez-González et al. [38]:

$$\%\ \text{Inhibition} = (E_t - E_0)\ carrageenan - (E_t - E_0)\ treated / (E_t - E_0\ \text{carrageenan}] \times 100. \quad (1)$$

TPA-induced ear edema was conducted as described by Pérez-González et al. [38]. Control was treated with TPA (2.5 µg) in acetone on the right ear (W's) and then left ear received only 25 mm^3 of acetone (Wo). The experimental groups ($n = 5$) received TPA and 30 min later were treated with test compounds **9**, **10**, and **4–6** (2 mg/ear) in the right ear (Ws). Anti-inflammatory activity was calculated according to the weight difference between the ear sections (6 mm) at 6 h, compared with the control group, using the following formula:

$$\%\ \text{Inhibition} = [(\text{W's} - \text{Wo})\ \text{control} - (\text{W's} - \text{Wo})\ \text{treated} / (\text{W's} - \text{Wo})\ \text{control}] \times 100. \quad (2)$$

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