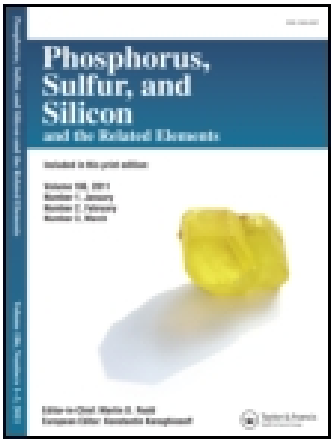

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Synthesis, Antimicrobial, and Antioxidant Activity of New α-Aminophosphonates

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SYNTHESIS, ANTIMICROBIAL, AND ANTIOXIDANT ACTIVITY OF NEW α-AMINOPHOSPHONATES

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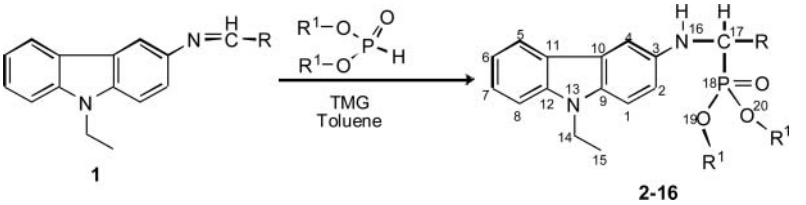
GRAPHICAL ABSTRACT



Abstract *A new class of α-aminophosphonates* ***2–16*** *has been synthesized by the reaction of equimolar quantities of Schiff's bases with diethyl/dimethyl/diphenyl phosphite in dry toluene under reflux conditions using tetramethylguanidine (TMG) as a catalyst via Pudovik reaction in high yields (78–89%). The structures of the title compounds have been established by elemental; infrared (IR);* ^{1}*H-,* ^{13}*C-, and* ^{31}*P-NMR; and mass spectral data analyses. They were found to possess significant antimicrobial and antioxidant activity.*

Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.*

Keywords α-Aminophosphonates; antimicrobial activity; antioxidant activity; 9-ethyl-3-aminocarbazole; tertramethyl guanidine

INTRODUCTION

The Pudovik reaction is one of the most versatile pathways for the formation of carbon–phosphorus bonds. It involves addition of organophosphorus compounds containing a labile P-H bond to an unsaturated system[1] and affords corresponding α-aminophosphonate addition products. α-Aminophosphonates are considered to be structural analogues of α-amino acids and peptidomimetics.[2] Low mammalian toxicity of these compounds makes them attractive for use in agriculture as herbicides[3] and in medicine as antitumor agents.[4]

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α-Aminophosphonates find application in many areas such as inhibitors of protease,[5] catalytic antibodies,[6] and antibacterial agents.[7] In addition, α-aminophosphonate derivatives have effects on parasites such as *Toxoplasma* and *Cryptosporidium* that cause opportunistic infections in AIDS patients.[8] α-Aminophosphonic acids have been found to act as inhibitors of specific enzymes such as HIV protease, thrombin, and human collagenase and to suppress the growth of various tumors and viruses.[9] Indeed, it has been shown that some aminophosphonic acids can also act as antioxidants.[10] Mechanistic studies have been performed by adopting various synthetic methodologies[11] using triethylamine, potassium or cesium fluoride, potassium fluoride on alumina, quinine, lithium diisopropylamide (LDA), MgO, 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), and tetramethylguanidine (TMG).[12] Lewis acids that have been used are $AlCl_3$, $InCl_3$, BF_3, $SnCl_4$, $ZrCl_4$, Mg $(ClO_4)_2$,[13] and $ZrOCl_2.8H_2O$.[14] A closer look at the literature showed that all of these catalysts are redundant. TMG, as a strong base (p*K*a = 10.78), has the ability to react with substrates by virtue of its structure and not only catalyzes this Pudovik reaction but also guides its course to avoid the formation of side products.[15] Therefore, the use of TMG to perform the addition of dialkyl phosphites to unsaturated systems could offer significant advantages, especially in terms of experimental simplicity, easy workup, high yield, reduced time, and low cost, and is a convenient catalyst for the synthesis of a variety of phosphonates. Carbazole derivatives were found to be useful as antibacterial, antifungal,[16] anticancer, and HIV agents.[17] Their pharmacological applications prompted us to use 9-ethyl-3-amino carbazole as a starting compound for the synthesis of α-aminophosphonates. Herein we report the synthesis, spectral characterization, and antimicrobial and antioxidant activities of novel α-aminophosphonates by the Pudovik reaction.

RESULTS AND DISCUSSION

Synthesis

A new class of α-aminophosphonates **2–16** was synthesized by equimolar quantities of N-arylidene-9-ethyl-9*H*-carbazol-3-imines **1** and dialkyl/diaryl phosphite in dry toluene at reflux conditions (4–5 h) using TMG as a catalyst via the Pudovik reaction. The reaction's progress was monitored by thin-layer chromatography (TLC) analysis (ethyl acetate : hexane, 2:3) at different time intervals and the products were purified by column chromatography using ethyl acetate : hexane (2:3) as step gradient mixtures as eluents. TMG was found to be more effective than other catalysts in terms of yields, reaction times, and cost of the catalyst.

The infrared (IR) spectra of the title compounds **2–16** showed absorption bands at 3340–3408 cm^{-1} (N-H), 1232–1254 cm^{-1} (P=O), 1015–1034 cm^{-1} (P-O-$C_{aliphatic}$), and 735–760 cm^{-1} (P-$C_{aliphatic}$) stretching frequencies.[18]

Aromatic protons of the title compounds **2–16** showed complex multiplets in the region δ 6.45–8.03. P-C-H protons resonated as multiplet in the region δ 4.77–5.36 due to its coupling with phosphorus and neighboring N-H proton.[19] The N-H proton gave a triplet in the range of δ 5.44–5.52 (J = 9.0–9.6 Hz) due to its coupling with the neighboring proton and phosphorus. The methoxy protons of the dimethyl phosphite moiety resonated as two distinct doublets in the range of δ 3.60–3.69 ($^3J_{C\text{-}H}$ = 10.8 Hz), 3.68–3.78 ($^3J_{C\text{-}H}$ = 10.8–11.4 Hz), showing their nonequivalence. The methylene protons of P-O-$\underline{CH_2}$-CH_3 showed a multiplet, and methyl protons of P-O-CH_2-$\underline{CH_3}$ gave a triplet in the region of δ 3.75–3.96 and 1.18–1.78 (J = 8.0–8.4 Hz), respectively.[20]

The ^{13}C-NMR chemical shifts for P-C-H appeared in the region δ 49.1–51.8 ppm as a doublet (d, $^{1}J_{\text{P-C}} = 150$ Hz, P-C).[20] The diethyl moiety carbon gave two doublets, one at 63.1–64.9 ppm (d, $^{2}J_{\text{P-O-C-}} = 12.6$ Hz, P-O-CH_2) and the other at δ 15.6–15.9 ppm (d, $^{3}J_{\text{P-O-C-C}} = 6.9$ Hz, P-O-CH_2-$\underline{CH_3}$).[21] Methoxyl carbon of P-O-CH_3 resonated as a doublet at 54.2–55.3 ppm (d, $^{2}J_{\text{P-O-C}} = 7.4$–$8.2$ Hz)[21] due to coupling with phosphorus.

The ^{31}P-NMR signals appeared as singlets in the region 22.1–23.8 ppm in all of the title compounds.[15]

The α-aminophosphonate derivatives **6** showed significant zones of inhibition against all represented microorganisms; similarly, derivative **8** exhibited significant activity against *Staphylococcus aureus* and *Escherichia coli*. Compounds **5** and **10** showed high activity against the fungal cultures *Aspergillus niger* and *Helminthosporium oryzae*. These results clearly indicate that the phosphorus atom directly attached to sulfur and nitrogen with their aliphatic chains ending with OH and Cl was found to possess promising antimicrobial activity compared with other moieties at their ends. The derivatives **6**, **7**, and **10** also showed considerable activity against *E. coli* and *S. aureus*, which indicates that their activity was enhanced by the presence of a terminal –OH group.

Most of the compounds showed high activities for catalase (CAT), superoxide dismutase (SOD), lipid peroxidation (LPO), and glutathione (GSH) inhibition compared to vitamins C and E. The fragments 2-thiophene carboxy aldehyde and carbazole linked to phosphorus led to a great change in CAT, SOD, LPO, and GSH inhibitory potency (i.e., **8**). Attachment of different moieties to the phosphorus atom could affect the ability of the molecule to interact with the peripheral and active sites of the enzymes simultaneously and thereby influence the CAT, SOD, LPO, and GSH inhibitory potency. The bis-(2-chloroethyl) amino fragment series (i.e., **6–11**), compounds **4**, **7**, and **8** showed high inhibitory potency for CAT, SOD, LPO, and GSH when compared to vitamins C and E (Scheme 1).

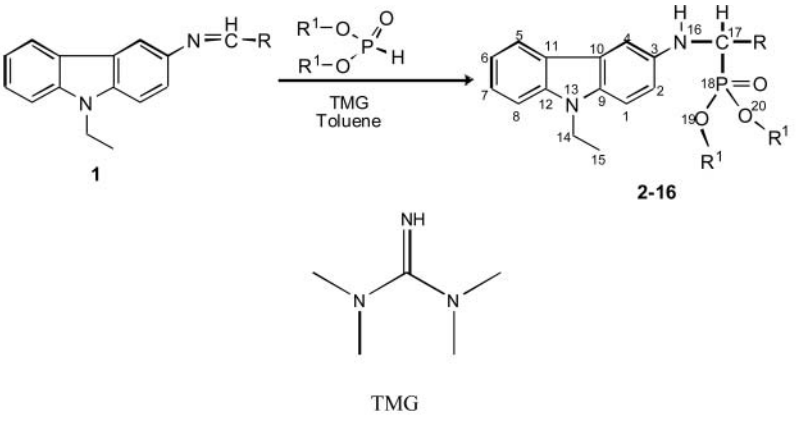
Antimicrobial Studies

In the present study, the antimicrobial activity was evaluated for the title compounds **2–16**. Specifically, the antibacterial activity[22] was evaluated against *S. aureus* ATCC-25923 (Gram-positive) and *E. coli* ATCC-25922 (Gram-negative) bacteria along with a standard reference penicillin G at three different concentrations (100, 50, and 25 ppm) in dimethyl formamide (DMF). All of the compounds exhibited moderate to high antibacterial activity when compared with the reference compound, and the data are given in Table S1. The title compounds **6** and **7** exhibited higher activity than that of the standard.

Similarly, the antifungal activity was evaluated[23] against *A. niger* and *H. oryzae* species along with standard reference griseofulvin at three different concentrations (100, 50, and 25 ppm) in DMF. All of the compounds exhibited moderate to high antifungal activity when compared with the reference compound, and the data are given in Table S2. The title compounds **5** and **10** exhibited higher activity than that of the standard.

Antioxidant Studies

Antioxidant activity was determined by the thiocyanate method. The amount of peroxide was determined by reading absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation.[24] The amount of peroxides formed in emulsion during incubation was determined spectrophotometrically by measuring absorbance at



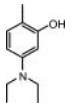
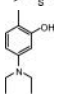
Scheme 1

500 nm. High absorbance is an indication of high concentrations of formed peroxides. Oxidative stress can be derived from a variety of sources and includes events such as the production of reactive oxygen species by mitochondrial oxidative phosphorylation, ionizing radiation exposure, and metabolism of exogenous compounds.[25] In addition to these sources of oxidative stress, a decrease in the activity of antioxidant enzymes, such as lipid peroxidation, glutathione peroxidase, superoxide dismutase, and catalase, may contribute to oxidative stress in some disease states. All of the compounds exhibited moderate to high antioxidant activity when compared with the reference compound, and the data are given in Table S3.

EXPERIMENTAL

Chemicals were obtained from Sigma-Aldrich (Lancaster, Hyderabad, India), and used as such without further purification. All solvents (analytical reagent [AR] or extrapure grade) used for spectroscopic and other physical studies were further purified by literature methods.[26] The melting points were determined in open capillary tubes on a Mel-Temp apparatus (Tempo Instruments and Equip (Pvt.) Ltd., Mumbai, India) and are uncorrected. IR spectra (ν_{max} in cm^{-1}) were recorded in KBr pellets on a Nicolet (San Diego, CA, USA) 380 Fourier transform infrared (FT-IR) spectrophotometer at the Environmental Engineering Laboratory, Sri Venkateswara University, Tirupati, India. The ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra were recorded on a Bruker (Ettlingen, Germany) AMX 400 MHz nuclear

Table 1 Physical data of the synthesized compounds 2–16

Compound	R	R^1	Color	Melting Point (°C)	Yield (%)
2		Me	Brown-red solid	181–183	85
3		Me	Brick-red solid	178–180	84
4		Me	Brown-red solid	180–182	89
5		Me	Brown solid	187–189	88
6		Me	Brownish yellow solid	169–171	87
7		Et	Brick-red solid	185–187	86
8		Et	Brown-red solid	167–169	85
9		Et	Brown-red solid	175–177	82
10		Et	Yellowish brown solid	179–181	86
11		Et	Brown solid	187–189	79
12		Ph	Brown-red solid	165–167	78
13		Ph	Brown solid	184–186	84
14		Ph	Yellowish brown solid	166–168	81
15		Ph	Brown solid	163–165	83
16		Ph	Brownish yellow solid	174—176	85

magnetic resonance (NMR) spectrometer operating at 400 MHz for ^{1}H-NMR, 100.57 MHz for ^{13}C-NMR, and 161.9 MHz for ^{31}P-NMR. All compounds were dissolved in $CDCl_3$ and chemical shifts were referenced to tetramethylsilane (TMS; ^{1}H-NMR and ^{13}C-NMR) and 85% H_3PO_4 (^{31}P NMR). Mass spectra were recorded on a Jeol SX 102DA/600 (Tokyo, Japan) mass spectrometer using argon/xenon (6 keV, 10 mA) as the FAB gas." Microanalysis was performed with a Thermo Finnigan (Courtaboeuf, France) Flash EA 1112 I instrument at University of Hyderabad, Hyderabad, India. Table 1 summarizes the physical data of the compounds.

Synthesis

Schiff's bases (**1**) were prepared by reacting 9-ethyl-3-aminocarbazole with various aldehydes in refluxing ethanol.

Dimethyl (9-Ethyl-9H-carbazole-3-ylamino)(5-methyl thiophen-2-yl) methyl phosphonate (2)

To a stirred solution of 9-ethyl-N-[((5-methyl thiophen-2-yl)methylene)]-9H-carbazole-3-imine(**1**) (1.59 g, 5 mmol) and TMG catalyst (1.0 mL) in dry toluene (25 mL) was added dimethyl phosphite (0.45 mL, 5 mmol) in dry toluene (20 mL) at room temperature. After the addition, the temperature of the reaction was raised to 60–65 °C and maintained for 5 h. Progress of the reaction was monitored by TLC analysis (silica gel) at different time intervals using ethyl acetate and hexane (2:3 by volume) as a mobile phase. After completion of the reaction, the solvent was removed under reduced pressure in a rota-evaporator and the crude product obtained was washed with petroleum ether and water and purified by column chromatography on 60- to 120-mesh silica gel using ethyl acetate : hexane (2:3) as eluent to afford the pure brown-red solid (**2**), yield 1.82 g (85%), m.p. 181–183 °C. Other compounds (**3–16**) were prepared by adopting the same procedure and were characterized by IR; ^{1}H-, ^{13}C-, and ^{31}P-NMR; and mass spectral studies; the physical data are tabulated for all of the compounds **2**–**16** in Table 1.

Dimethyl(9-ethyl-9H-carbazole-3-ylamino)(5-methylthiophen-2-yl)methyl Phosphonate (2)

IR (KBr) (ν_{max} cm^{-1}): 3394 (N-H), 1247 (P=O), 1022 (P-O-$C_{aliphatic}$), 759 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.71–7.01 (m, 9H, ArH), 4.72–4.89 (m, 1H, P-CH), 5.41 (t, J = 9.0 Hz, 1H, NH), 4.45 (q, 2H, NCH_2-), 1.15 (t, 3H, J = 8.2 Hz, NCH_2-CH_3), 3.68 (d, J = 11.8 Hz, 3H, P-OCH_3), 3.57 (d, J = 11.0 Hz, 3H, P-OCH_3), 2.50 (s, 3H, Ar-CH_3); ^{13}C-NMR (δ ppm): 112.1 (C-1), 105.1 (C-2), 136.4 (C-3), 111.1 (C-4), 119.7 (C-5), 122.1 (C-6), 120.2 (C-7), 111.5 (C-8), 130.7 (C-10), 106.1 (C-11), 125.2 (C-12), 124.9 (C-13), 48.7 (C-14), 15.1 (C-15), 70.1 (C-17), 139.6 (C-1^{1}), 136.1 (C-3^{1}), 126.2 (C-4^{1}), 125.4 (C-5^{1}), 16.8 (C-6^{1}), 54.3 (d, J = 7.4 Hz-P-OCH_3); ^{31}P-NMR (δ ppm): 22.1; liquid chromatography–mass spectrometry (LC-MS): (m/z) 428 (M^+), 369, 317, 289, 251, 204, 112. Anal. Calcd. for $C_{22}H_{25}N_2O_3PS$: C, 61.67; H, 5.88; N, 6.54. Found: C, 61.65; H, 5.85; N, 6.52.

Dimethyl(9-ethyl-9H-carbazole-3-ylamino)(3-methylthiophen-2-yl)methyl Phosphonate (3)

IR (KBr) (ν_{max} cm^{-1}): 3399 (N-H), 1232 (P = O), 1029 (P-O-$C_{aliphatic}$), 744 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.60–7.97 (m, 9H, ArH), 4.80–5.11 (m, 1H, P-CH), 4.52 (q, 2H, NCH_2-), 1.46 (t, 3H, J = 8.0 Hz, NCH_2-CH_3), 5.43 (t, J = 9.6 Hz, 1H, NH), 3.72 (d, J = 12.1 Hz, 3H, P-OCH_3), 3.63 (d, J = 11.8 Hz, 3H, P-OCH_3), 2.23 (s, 3H, Ar-CH_3); ^{13}C-NMR (δ ppm): 112.3 (C-1), 105.0 (C-2), 136.7 (C-3), 112.3 (C-4), 119.1 (C-5), 121.9 (C-6), 121.0 (C-7), 111.9 (C-8), 130.5 (C-10), 107.3 (C-11), 124.6 (C-12), 125.3 (C-13), 47.9 (C-14), 16.0 (C-15), 69.1 (C-17), 135.3 (C-1^{1}), 124.1 (C-3^{1}), 124.6 (C-4^{1}), 134.2 (C-5^{1}), 17.7 (C-6^{1}), 56.7 (d, J = 7.2 Hz-P-OCH_3); ^{31}P-NMR (δ ppm): 23.3; LC-MS: (m/z) 428 (M^+), 369, 317, 251, 204, 112. Anal. Calcd. for $C_{22}H_{25}N_2O_3PS$: C, 61.67; H, 5.88; N, 6.54. Found: C, 61.63; H, 5.83; N, 6.53. (Selected spectra are shown in the Supplemental Materials, Figures S1–S5, available online.)

Dimethyl(4-(diethylamino)-2-hydroxyphenyl)(9-ethyl-9H-carbazole-3-ylamino) Methylphosphonate (4)

IR (KBr) (ν_{max} cm^{-1}): 3391 (N-H), 1236 (P=O), 1032 (P-O-$C_{aliphatic}$), 760 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 10.2 (s, 1H, Ar-OH), 6.56–7.99 (m, 10H, ArH), 4.54 (q, 2H, NCH_2-), 1.45 (t, 3H, $J = 8.1$ Hz, NCH_2-$\underline{CH_3}$), 4.63 (q, 4H, Ar-N-CH_2), 1.54 (t, $J = 8.2$ Hz, 6H, Ar-N-CH_2-$\underline{CH_3}$), 4.01–5.13 (m, 1H, P-CH), 5.46 (t, $J = 9.2$ Hz, 1H, NH), 3.69 (d, $J = 11.6$ Hz, 3H, P-$\overline{OCH_3}$), 3.60 (d, $J = 12.0$ Hz, 3H, P-OCH_3); ^{13}C-NMR (δ ppm): 113.0 (C-1), 105.2 (C-2), 137.1 (C-3), 112.5 (C-4), 119.9 (C-5), 122.2 (C-6), 121.3 (C-7), 110.9 (C-8), 131.2 (C-10), 106.2 (C-11), 126.7 (C-12), 124.1 (C-13), 47.8 (C-14), 15.5 (C-15), 69.2 (C-17), 111.5 (C-1^1), 155.2 (C-2^1), 99.6 (C-3^1), 150.5 (C-4^1), 106.7 (C-5^1), 130.3 (C-6^1), 44.8 (C-8^1), 14.1 (C-9^1), 53.9 (d, $J = 7.4$ Hz -P-OCH_3); ^{31}P-NMR (δ ppm): 23.3. LC-MS: (m/z) 495 (M^+), 423, 382, 317, 273, 218, 110. Anal. Calcd. for $C_{27}H_{34}N_3O_4P$: C, 65.44; H, 6.92; N, 4.48. Found: C, 65.39; H, 6.88; N, 4.43.

Dimethyl 1-(9-ethyl-9H-carbazole-3-ylamino)-3-(2-nitrophenyl) allylphosphonate (5)

IR (KBr) (ν_{max} cm^{-1}): 3408 (N-H), 1254 (P=O), 1034 (P-O-$C_{aliphatic}$), 753 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.71–8.01 (m, 11H, ArH), 6.45 (d, $J = 16.3$ Hz, 1H, Ar-$\underline{CH}$), 6.02–6.08 (m, 1H, Ar-CH-$\underline{CH}$), 4.75–4.85 (m, 1H, P-CH), 5.52 (t, $J = 9.3$ Hz, 1H, $\overline{NH}$), 4.52 (q, 2H, NCH_2-), 1.44 (t, 3H, $J = 8.0$ Hz, NCH_2-CH_3), 3.65 (d, $J = 11.6$ Hz, 3H, P-OCH_3), 3.52 (d, $J = 11.8$ Hz, 3H, P-OCH_3); ^{13}C-NMR (δ ppm): 112.3 (C-1), 105.4 (C-2), 136.4 (C-3), 111.7 (C-4), 119.5 (C-5), 122.1 (C-6), 120.3 (C-7), 111.9 (C-8), 130.8 (C-10), 106.7 (C-11), 125.3 (C-12), 124.7 (C-13), 48.5 (C-14), 15.9 (C-15), 69.1 (C-17), 128.5 (C-1^1), 108.4 (C-2^1), 123.4 (C-3^1), 128.9 (C-4^1), 113.7 (C-5^1), 149.8 (C-6^1), 110.8 (C-7^1), 159.8 (C-8^1), 54.3 (d, $J = 7.2$ Hz -P-OCH_3); ^{31}P-NMR (δ ppm): 23.7; LC-MS: (m/z) 479 (M^+), 392, 310, 284, 253, 182, 128. Anal. Calcd. for $C_{25}H_{26}N_3O_5P$: C, 62.63; H, 5.47; N, 8.76. Found: C, 62.57; H, 5.42; N, 8.73.

Dimethyl(5-chloro-2-hydroxyphenyl)(9-ethyl-9H-carbazole-3-ylamino) methylphoshonate (6)

IR (KBr) (ν_{max} cm^{-1}): 3394 (N-H), 1251 (P=O), 1015 (P-O-$C_{aliphatic}$), 744 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 10.4 (s, 1H, Ar OH), 6.52–7.79 (m, 10H, ArH), 4.75–4.85 (m, 1H, P-CH), 5.49 (t, $J = 9.4$ Hz, 1H, NH), 4.53 (q, 2H, NCH_2-), 1.45 (t, 3H, $J = 8.0$ Hz, NCH_2-CH_3), 3.74 (d, $J = 12.0$ Hz, 3H, P-OCH_3), 3.66 (d, $J = 10.8$ Hz, 3H, P-OCH_3); ^{13}C-NMR (δ ppm): 113.2 (C-1), 105.1 (C-2), 136.8 (C-3), 113.1 (C-4), 118.9 (C-5), 123.5 (C-6), 121.8 (C-7), 110.4 (C-8), 131.1 (C-10), 106.5 (C-11), 126.9 (C-12), 124.3 (C-13), 48.5 (C-14), 15.8 (C-15), 69.4 (C-17), 121.5 (C-1^1), 155.7 (C-2^1), 116.3 (C-3^1), 134.3 (C-4^1), 121.4 (C-5^1), 130.1 (C-6^1), 56.0 (d, $J = 7.4$ Hz -P-OCH_3); ^{31}P NMR (δ ppm): 22.9; LC-MS: (m/z) 458 (M^+), 459, 460, 369, 317, 289, 251, 204, 112. Anal. Calcd. for $C_{23}H_{24}ClN_2O_4P$: C, 60.20; H, 5.27; N, 5.27. Found: C, 60.16; H, 5.25; N, 5.24.

Diethyl(9-ethyl-9H-carbazole-3-ylamino)(5-methylthiophen-2-yl)methylphosphonate (7)

IR (KBr) (ν_{max} cm^{-1}): 3377 (N-H), 1244 (P=O), 1018 (P-O-$C_{aliphatic}$), 735 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.55–7.90 (m, 9H ArH), 4.88–5.17 (m, 1H, P-CH), 5.44 (t, $J = 9.6$ Hz, 1H, NH), 4.52 (q, 2H, NCH_2-), 1.46 (t, 3H, $J = 8.0$Hz, NCH_2-CH_3), 3.68

(m, 4H, P-OCH_2), 2.23 (s, 3H, Ar-CH_3), 1.45 (t, $J = 8.2$ Hz, 6H, $OCH_2\underline{CH_3}$); ^{13}C-NMR (δ ppm): 113.2 (C-1), 105.2 (C-2), 137.2 (C-3), 111.3(C-4), 120.3 (C-5), 122.2 (C-6), 120.7 (C-7), 111.6 (C-8), 130.5 (C-10), 106.3 (C-11), 124.9 (C-12), 125.2 (C-13), 48.9 (C-14), 15.0 (C-15), 69.5 (C-17), 139.7 (C-1^1), 136.2 (C-3^1), 127.1 (C-4^1), 125.5 (C-5^1), 16.9 (C-6^1), 63.2 (d, $J = 12.6$ Hz-P-OCH_2), 15.6 (d, $J = 6.9$ Hz-P-OCH_2-$\underline{C}H_3$). ^{31}P-NMR (δ ppm): 23.3. LC-MS: (m/z) 456 (M^+), 341, 317, 296, 240, 198, 107. Anal. Calcd for $C_{24}H_{29}N_2O_3PS$: C, 63.14; H, 6.40; N, 6.14. Found: C, 63.00; H, 6.37; N, 6.06.

Diethyl (9-ethyl-9H-carbazole-3-ylamino)(3-methyl thiophen-2-yl) methylphosphonate (8)

IR (KBr) (ν_{max} cm^{-1}): 3404 (N-H), 1239 (P=O), 1033 (P-O-$C_{aliphatic}$), 739 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.59–7.88 (m, 9H, ArH), 4.81–5.11 (m, 1H, P-CH), 5.49 (t, $J = 9.4$ Hz, 1H, NH), 4.55 (q, 2H, NCH_2-), 2.23 (s, 3H, Ar-CH_3), 1.46 (t, 3H, $J = 8.2$ Hz, NCH_2-$\underline{CH_3}$), 3.71 (m, 4H, P-OCH_2), 1.47 (t, $J = 8.4$ Hz, 6H, $OCH_2\underline{CH_3}$); ^{13}C-NMR (δ ppm): 113.1 (C-1), 105.7 (C-2), 137.1 (C-3), 112.6 (C-4), 119.2 (C-5), 122.1 (C-6), 121.2 (C-7), 111.7 (C-8), 130.6 (C-10), 107.2 (C-11), 124.9 (C-12), 125.5 (C-13), 47.8 (C-14), 16.1 (C-15), 69.1 (C-17), 135.5 (C-1^1), 124.2 (C-3^1), 124.7 (C-4^1), 134.1 (C-5^1), 17.9 (C-6^1), 63.1(d, $J = 12.6$ Hz, P-OCH_2), 15.7 (d, $J = 6.8$ Hz, P-OCH_2-$\underline{C}H_3$); ^{31}P-NMR (δ ppm): 23.3; LC-MS: (m/z) 456 (M^+), 341, 317, 296, 240, 198, 107. Anal. Calcd for $C_{24}H_{29}N_2O_3PS$: C, 63.14; H, 6.40; N, 6.14. Found: C, 63.02; H, 6.35; N, 6.07.

Diethyl(4-(diethylamino)-2-hydroxyphenyl)(9-ethyl-9H-carbazole-3-ylamino)methylphosphonate (9)

IR (KBr) (ν_{max} cm^{-1}): 3350 (N-H), 1248 (P=O), 1025 (P-O-$C_{aliphatic}$), 753 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 10.2 (s, 1H, Ar $\underline{OH}$), 6.53–7.90 (m, 10H ArH), 4.88–5.15 (m, 1H, P-CH), 5.48 (t, $J = 9.4$ Hz, 1H NH), 4.52 (q, 2H, NCH_2-), 1.47 (t, 3H, $J = 8.1$ Hz, NCH_2-$\underline{CH_3}$), 4.62 (q, 4H, Ar-N-CH_2), 1.55 (t, $J = 8.1$ Hz, 6H, Ar-N-CH_2-$\underline{CH_3}$), 3.75 (m, 4H, P-OCH_2), 1.51 (t, $J = 8.0$ Hz, 6H, $OCH_2\underline{CH_3}$); ^{13}C-NMR (δ ppm): 113.0 (C-1), 105.3 (C-2), 137.3 (C-3), 112.5 (C-4), 118.9 (C-5), 122.7 (C-6), 121.4 (C-7), 110.9 (C-8), 131.3 (C-10), 106.3 (C-11), 126.7 (C-12), 124.2 (C-13), 47.7 (C-14), 15.7 (C-15), 69.0 (C-17), 111.4 (C-1^1), 155.3 (C-2^1), 99.7 (C-3^1), 150.6 (C-4^1), 106.6 (C-5^1), 130.5 (C-6^1), 44.9 (C-8^1), 14.9 (C-9^1), 63.2 (d, $J = 12.7$ Hz, P-OCH_2), 15.9 (d, $J = 6.9$ Hz, P-OCH_2-$\underline{C}H_3$); ^{31}P-NMR (δ ppm): 23.6; LC-MS: (m/z) 523 ($M^{+\cdot}$), 432, 343, 289, 239, 149, 91. Anal. Calcd. for $C_{29}H_{38}N_3O_4P$: C, 66.52; H, 7.32; N, 8.02. Found: C, 66.47; H, 7.29; N, 7.97.

Diethyl 1-(9-Ethyl-9H-carbazole-3-ylamino)-3-(2-nitrophenyl) allylphosphonate (10)

IR (KBr) (ν_{max} cm^{-1}): 3398 (N-H), 1239 (P=O), 1023 (P-O-$C_{aliphatic}$), 743 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.55–8.01 (m, 11H, ArH), 6.43 (d, $J = 16.3$ Hz, 1H, Ar-$\underline{CH}$), 6.04–6.11 (m, 1H, Ar-CH-$\underline{CH}$), 4.85–5.12 (m, 1H, P-CH), 5.52 (t, $J = 9.4$ Hz, 1H, $\underline{NH}$), 4.53 (q, 2H, NCH_2-), 1.46 (t, 3H, $J = 8.2$ Hz, NCH_2-$\underline{CH_3}$), 3.96 (m, 4H, P-OCH_2), 1.48 (t, $J = 8.0$ Hz, 6H, $OCH_2\underline{CH_3}$); ^{13}C-NMR (δ ppm): 112.7 (C-1), 105.6 (C-2), 136.1 (C-3), 111.9 (C-4), 119.6 (C-5), 122.2 (C-6), 120.4 (C-7), 111.5 (C-8), 130.8 (C-10), 106.6 (C-11), 125.4 (C-12), 124.6 (C-13), 48.6 (C-14), 15.8 (C-15), 69.1 (C-17), 128.6 (C-1^1), 108.5 (C-2^1), 123.5 (C-3^1), 128.8 (C-4^1), 113.9 (C-5^1), 149.6 (C-6^1), 110.6 (C-7^1), 159.9

(C-8[1]), 64.9 (d, $J = 12.6$ Hz, P-OCH_2), 15.8 (d, $J = 6.7$ Hz, P-OCH_2-CH_3); ^{31}P-NMR (δ ppm): 24.3; LC-MS: (m/z) 523 (M^+), 564, 448, 324, 280, 224, 141, 103. Anal. Calcd. for $C_{27}H_{30}N_3O_5P$: C, 63.90; H, 5.96; N, 8.28. Found: C, 63.85; H, 5.91; N, 8.24.

Diethyl(5-chloro-2-hydroxyphenyl)(9-ethyl-9H-carbazole-3-ylamino)methylphosphonate (11)

IR (KBr) (ν_{max} cm^{-1}): 3401 (N-H), 235 (P=O), 1017 (P-O-$C_{aliphatic}$), 747 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 10.1 (s, 1H, Ar-OH), 6.51–7.98 (m, 10H, ArH), 4.78–4.95 (m, 1H, P-CH), 5.45 (t, $J = 9.6$ Hz, 1H, NH), 4.52 (q, 2H, NCH_2-), 1.45 (t, 3H, $J = 8.0$ Hz, NCH_2-CH_3), 3.95 (m, 4H, P-OCH_2), 1.47 (t, $J = 8.4$ Hz, 6H, OCH_2CH_3); ^{13}C-NMR (δ ppm): 113.3 (C-1), 105.2 (C-2), 136.5 (C-3), 113.3 (C-4), 119.0 (C-5), 123.6 (C-6), 121.9 (C-7), 110.6 (C-8), 131.0 (C-10), 106.7 (C-11), 126.8 (C-12), 124.5 (C-13), 48.6 (C-14), 16.1 (C-15), 69.4 (C-17), 121.7 (C-1[1]), 155.9 (C-2[1]), 116.7 (C-3[1]), 134.1 (C-4[1]), 120.9 (C-5[1]), 130.1 (C-6[1]), 63.2 (d, $J = 6.9$ Hz, P-OCH_2), 15.7 (d, $J = 12.6$ Hz, P-OCH_2-CH_3); ^{31}P-NMR (δ ppm): 23.3; LC-MS: (m/z) 486 (M^+), 487, 488, 368, 243, 208, 128. Anal. Calcd. for $C_{25}H_{28}ClN_2O_4P$: C, 61.67; H, 5.80; N, 5.75. Found: C, 61.62; H, 5.78; N, 5.76.

Diphenyl(9-ethyl-9H-carbazole-3-ylamino)(5-methylthiophen-2-yl)methylphosphonate (12)

IR (KBr) (ν_{max} cm^{-1}): 3408 (N-H), 1244 (P=O), 749 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.45–7.97 (m, 19H, ArH), 4.75–4.85 (m, 1H, P-CH), 5.46 (t, $J = 9.6$ Hz, 1H, NH), 4.53 (q, 2H, NCH_2-), 1.46 (t, 3H, $J = 8.0$ Hz, NCH_2-CH_3), 2.45 (s, 3H, Ar-CH_3); ^{13}C-NMR (δ ppm): 112.4 (C-1), 105.1 (C-2), 136.7 (C-3), 111.1(C-4), 119.8 (C-5), 122.0 (C-6), 120.1 (C-7), 111.7 (C-8), 130.9 (C-10), 106.2 (C-11), 125.3 (C-12), 124.8 (C-13), 48.9 (C-14), 15.3 (C-15), 69.2 (C-17), 139.8 (C-1[1]), 136.0 (C-3[1]), 126.1 (C-4[1]), 125.5 (C-5[1]), 16.9 (C-6[1]), 150.8, 121.1, 131.6, 124.2 (P-O-Ar); ^{31}P-NMR (δ ppm): 23.5; LC-MS: (m/z) 552 (M^+), 341, 277, 231, 185, 104. Anal. Calcd. for $C_{32}H_{29}N_2O_3PS$: C, 69.55; H, 5.29; N, 5.07. Found: C, 69.50; H, 5.24; N, 5.03.

Diphenyl(9-ethyl-9H-carbazole-3-ylamino)(3-methylthiophen-2-yl)methylphoshonate (13)

IR (KBr) (ν_{max} cm^{-1}): 3403 (N-H), 1251 (P=O), 758 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.51–7.98 (m, 19H, ArH), 4.97–5.36 (m, 1H, P-CH), 5.44 (t, $J = 9.2$ Hz, 1H NH), 4.52 (q, 2H, NCH_2-), 1.47 (t, 3H, $J = 8.0$ Hz, NCH_2-CH_3), 2.22 (s, 3H, Ar-CH_3); ^{13}C-NMR (δ ppm): 112.3 (C-1), 105.0 (C-2), 136.7 (C-3), 112.3 (C-4), 119.1 (C-5), 121.9 (C-6), 121.0 (C-7), 111.9 (C-8), 130.4 (C-10), 107.4 (C-11), 124.7 (C-12), 125.3 (C-13), 47.9 (C-14), 16.2 (C-15), 69.1 (C-17), 135.4 (C-1[1]), 124.3 (C-3[1]), 124.5 (C-4[1]), 134.3 (C-5[1]), 17.9 (C-6[1]), 56.9, 150.1, 121.0, 131.9, 124.1 (P-O-Ar); ^{31}P-NMR (δ ppm): 23.3; LC-MS: (m/z) 552 (M^+), 387, 312, 293, 294, 207, 171, 91. Anal. Calcd. for $C_{32}H_{29}N_2O_3PS$: C, 69.55; H, 5.29; N, 5.07. Found: C, 69.48; H, 5.25; N, 5.03.

Diphenyl(4-(diethylamino)-2-hydroxyphenyl)(9-ethyl-9H-carbazole-3-ylamino)methyl phosphonate (14)

IR (KBr) (ν_{max} cm^{-1}): 3394 (N-H), 1248 (P=O), 760 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 10.3 (s, 1H, Ar OH), 6.51–7.98 (m, 20H, ArH), 4.75–4.85 (m, 1H, P-CH), 5.46 (t, $J = 9.4$ Hz, 1H NH), 4.54 (q, 2H, NCH_2-), 1.47 (t, 3H, $J = 8.2$ Hz, NCH_2-CH_3), 4.61 (q,

4H, Ar-N-CH_2), 1.56 (t, $J = 8.0$ Hz, 6H, Ar-N-CH_2-CH_3); ^{13}C-NMR (δ ppm): 113.1 (C-1), 105.2 (C-2), 137.2 (C-3), 112.7 (C-4), 120.1 (C-5), 122.3 (C-6), 121.1 (C-7), 110.7 (C-8), 131.1 (C-10), 106.3 (C-11), 126.8 (C-12), 124.2 (C-13), 47.9 (C-14), 15.3 (C-15), 69.5 (C-17), 111.6 (C-1^1), 155.5 (C-2^1), 99.6 (C-3^1), 150.4 (C-4^1), 106.8 (C-5^1), 130.2 (C-6^1), 44.9 (C-8^1), 14.9 (C-9^1), 150.3, 121.7, 131.6, 124.5 (P-O-Ar); ^{31}P-NMR (δ ppm): 24.3; LC-MS: (m/z) 619 ($M^{+\cdot}$), 511, 429, 357, 321, 229, 207, 116. Anal. Calcd. for $C_{37}H_{38}N_3O_4P$: C, 71.71; H, 6.18; N, 6.78. Found: C, 71.65; H, 6.15; N, 6.73.

Diphenyl-1-(9-ethyl-9H-carbazole-3-ylamino)-3-(2-nitrophenyl) allylphosphonate (15)

IR (KBr) (ν_{max} cm^{-1}): 3390 (N-H), 1253 (P=O), 759 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 6.56–8.03 (m, 21H, ArH), 6.45 (d, $J = 16.3$ Hz, 1H, Ar-CH), 6.02–6.06 (m, 1H, Ar-CH-CH), 4.77–5.05 (m, 1H, P-CH), 5.50 (t, $J = 9.5$ Hz, 1H, NH), 4.53 (q, 2H, NCH_2-), 1.46 (t, 3H, $J = 8.1$ Hz, NCH_2-CH_3); ^{13}C-NMR (δ ppm): 112.5 (C-1), 105.7 (C-2), 136.5 (C-3), 112.2 (C-4), 119.5 (C-5), 122.2 (C-6), 120.4 (C-7), 111.7 (C-8), 131.2 (C-10), 106.8 (C-11), 125.5 (C-12), 124.5 (C-13), 48.7 (C-14), 16.3 (C-15), 69.1 (C-17), 128.7 (C-1^1), 108.5 (C-2^1), 123.3 (C-3^1), 128.8 (C-4^1), 113.6 (C-5^1), 149.9 (C-6^1), 110.7 (C-7^1), 159.6 (C-8^1), 150.7, 121.7, 131.7, 124.6 (P-O-Ar); ^{31}P-NMR (δ ppm): 23.3; LC-MS: (m/z) 603 (M^+), 531, 398, 345, 234, 220, 154, 123. Anal. Calcd. for $C_{35}H_{30}N_3O_5P$: C, 69.64; H, 5.01; N, 6.96. Found: C, 69.58; H, 4.97; N, 6.92.

Diphenyl-(5-chloro-2-hydroxyphenyl)(9-ethyl-9H-carbazole-3-ylamino)methylphosphonate (16)

IR (KBr) (ν_{max} cm^{-1}): 3340 (N-H), 1247 (P=O), 739 (P-$C_{aliphatic}$); ^{1}H-NMR (δ ppm): 10.3 (s, 1H, Ar OH), 6.51–7.98 (m, 20H, ArH), 6.42 (s, 1H, Ar-CH), 4.77–4.95 (m, 1H, P-CH), 5.45 (t, $J = 9.2$ Hz, 1H, NH), 4.50 (q, 2H, NCH_2-), 1.46 (t, 3H, $J = 8.0$ Hz, NCH_2-CH_3); ^{13}C-NMR (δ ppm): 113.3 (C-1), 105.2 (C-2), 136.7 (C-3), 113.3 (C-4), 118.8 (C-5), 123.1 (C-6), 121.9 (C-7), 110.7 (C-8), 131.2 (C-10), 106.6 (C-11), 126.7 (C-12), 124.3 (C-13), 48.7 (C-14), 15.9 (C-15), 69.4 (C-17), 121.1 (C-1^1), 155.8 (C-2^1), 116.5 (C-3^1), 134.4 (C-4^1), 121.3 (C-5^1), 130.2 (C-6^1), 150.5, 121.1, 131.6, 124.5 (P-O-Ar); ^{31}P-NMR (δ ppm): 22.7; LC-MS: (m/z) 582 (M^+), 583, 584, 434, 310, 286, 243, 198, 109. Anal. Calcd. for $C_{33}H_{28}ClN_2O_4P$: C, 67.98; H, 4.84; N, 4.80. Found: C, 67.92; H, 4.78; N, 4.75.

Biological Testing

Please see the Supplemental Materials (available online).

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