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An efficient one pot synthesis of 2-amino-3-cyano-4*H*-chromenyl phosphonates and their anti-oxidant activity

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Abstract: 2-Amino-3-cyano-4*H*-chromen-4-ylphosphonates are synthesized *via* one-pot, multi-component reaction of structurally diverse salicylaldehydes, malononitrile and dialkylphosphites using a catalytic amount of potassium carbonate in ethanol at ambient temperature. Use of potassium carbonate as an inexpensive catalyst makes the protocol more economical. Mild reaction conditions and simple work-up procedure are the added advantages of the present method. The bio-assay revealed that the title compounds exhibited moderate to good antioxidant activities, particularly **4b, 4e, 4h, 4k, and 4l** showed high activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide (H_2O_2) methods.

Keywords: Chromenyl phosphonates, Multicomponent reaction, Potassium carbonate, room temperature, Antioxidant activity.

Introduction

Multi-Component Reactions (MCRs) are surprisingly appealing in their capacity to manufacture to develop C-C, C-P, C-N or C-S bonds in a single step [1]. The achievement of numerous alterations in a single operation is enormously well matched with the goals of green chemistry [2]. Hence, industrial and business research agencies have increasingly targeted on the use of MCRs to synthesize a broad variety of products. Nowadays development of MCRs can lead to a new efficient synthetic methodology to many small organic compounds within the discipline of current organic, bioorganic, and medicinal chemistry [3]. The chromene moiety frequently appears as pivotal auxiliary segment in both organically and natural

compounds [4]. It is widely accomplished in natural alkaloids, flavonoids, tocopherols and anthocyanins. Furthermore, in recent years functionalized chromenes have played ever growing role inside the synthetic strategies to auspicious compounds in the area of medicinal chemistry. Among different types of chromene systems, 2-amino-4*H*-chromenes are significant heterocyclic compounds with a number of biotic and pharmacological possessions like antimicrobial [5], antiviral [6], anticonvulsant [7], antiproliferative [8], antitumor [9], anti-cancer [10] and central nervous system [11], accomplishments and are comprehensively working as cosmetics [12], pigments and intoxicating eco-friendly agrochemicals [13].

Phosphonates are an important class of molecules in organophosphorus compounds. The synthesis of phosphonates and their products have fascinated much attention over the last few years due to their extensive range of applications in material chemistry, catalysis and medicinal chemistry as enzyme inhibitors [14], peptide mimics [15], antibiotics [16] and pharmacological agents. Enlargement of proficient etiquettes for the synthesis of phosphonates, *via* C-P bond formation enjoy the growing interest [17]. The synthesis of 2-amino-4*H*-chromen-4-yl phosphonate has fascinated much attention recently due to their important biological activities.

A sum of processes has been reported for the synthesis of 2-amino-3-cyano-4*H*-chromen-4-yl phosphonates. Some of which include phospha-Michael addition catalysed by diethylamine [18], ethylene diamine diacetate [19], K_3PO_4 [20], I_2 [21], PEG [22], *β*-cyclodextrin [23], $InCl_3$ [24], silica-bonded 2-hydroxyethylammoniumacetate(HEAA)[25], dibutyl amine [26] and tetramethylguanidine [27]. These approaches appearance changeable degrees of success as well as precincts, such as extended reaction times, low yields, requirement of surplus reagent or catalyst, use of noxious solvent, and laborious work-up methods based on this still they need substitute milder and ecologically workable procedures for the synthesis of 2-amino-3-cyano-4*H*-chromen-4-yl phosphonates. Keeping in view of the above boundaries, efforts were made to improve a new and better synthetic protocol for one pot three component condensation of salicylaldehydes, malononitrile and dialkyl phosphites using a catalytic amount of potassium carbonate in ethanol at ambient temperature under room temperature. The synthesized 2-amino-3-cyano-4*H*-chromen-4-yl phosphonates were screened for antioxidant activity by using DPPH, NO and H_2O_2 methods [28-32].

EXPERIMENTAL

Chemistry

Materials and methods

Reagents were purchased from common commercial sources. All solvents were purified and dried by standard procedures. All the reactions were monitored by thin layer chromatography (TLC) on silica gel GF254 plates from Qingdao Haiyang Chemical Co. Ltd (China) visualized in an iodine chamber or with an UV lamp (254 nm). Column chromatography was performed using silica gel (100–200 mesh). The melting points of the products were determined on a Guna Digital melting point apparatus (China) and are uncorrected. The IR spectra were recorded on a Bruker Alpha ECO-ATR FTIR (Attenuated total reflection–Fourier transform infrared) interferometer with a single reflection sampling module equipped with Zn-Se crystal. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. NMR spectra were recorded on a Jeol instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C, 125 MHz for ^{31}P, using $CDCl_3$ and DMSO-d_6 as solvents. TMS ($\delta = 0$) served as an internal standard for

^{1}H-NMR & ^{13}C-NMR and H_3PO_4 (δ = 0) was used as an external standard for ^{31}PNMR, Mass spectra were recorded on a LC–MS/MS-TOF API QSTARPULSAR spectrometer, samples were introduced by the infusion method using the Electrospray Ionization Technique (ESI). All other chemicals were of analytical grade.

General procedure for the preparation of 2-Amino-3-cyano-4*H*-chromen-4-yl phosphonates (4a-l)

K_2CO_3 was added to a mixture of salicylaldehydes (1 mmol), malononitrile (1 mmol) and dialkyl phosphites (1 mmol) in ethanol as solvent. The resulting mixture was stirred at room temperature. After completion of the reaction (monitored by TLC), distilled water (15 mL) was added to the reaction mixture and the stirring continued till a free flowing solid was obtained. It was filtered and then washed successively with water, *n*-hexane. The combined organic extracts were washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from ethanol. The similar synthetic procedures were adopted for the synthesis of all the 2-amino-3-cyano-4*H*-chromen-4yl phosphonates. Structures of all the synthesized compounds were confirmed by analytical and spectral data.

Dibutyl (2-amino-3-cyano-4H-chromen-4-yl) phosphonate **(4a)**

White solid, mp: 146-148°C, IR (ZnSe, cm^{-1}) υ_{max}; 3293 (N-H), 2190 (CN), 1233 (P=O), 799(P-C-H $_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.33-6.95 (m, 4H, Ar-H), 4.97 (s, 2H, -NH_2), 4.05-4.02 (m, 4H, -OC$\underline{H}_2$), 3.85 (d, 1H, *J* = 8 Hz, C$\underline{H}$P), 1.86-1.28 (m, 8H, -$(CH_2)_4$), 0.93 (t, 3H, *J*= 8.0 Hz, -C$\underline{H}_3$), 0.84 (t, 3H, *J* = 7.20 Hz, -C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm) 161.69, 150.06, 130.06 , 129.03, 125.45, 110.01,119.81, 116.66, 67.11 , 52.67, 36.22, 35.11, 19.36, 14.50 ; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm 22.36. LC-MS m/z: 365.30 $(M+1)^+$.

Dibutyl (2-amino-6-chloro -3-cyano-4H-chromen-4-yl) phosphonate **(4b)**

Yellow solid, mp : 138-140°C, IR (ZnSe, cm^{-1}) υ_{max}; 3327 (N-H), 2186 (CN), 1228 (P=O), 815 (P-C-H$_{aliphatic}$); ^{1}H ,NMR (400 MHz, $CDCl_3$, δppm): 7.31-6.92 (m, 3H, Ar-H), 4.93 (s, 2H, -NH_2) , 4.08-4.05 (m, 4H,- OC$\underline{H}_2$), 3.86 (d, 1H, *J* = 8 Hz, C$\underline{H}$P), 1.69-1.30 (m, 8H, -$(CH_2)_4$), 0.93 (t, 3H, *J* = 8.0 Hz, -C$\underline{H}_3$), 0.87 (t, 3H, *J* = 8.00 Hz, -C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 162.03, 149.03 ,130.11, 129.07,119.21, 118.48, 115.05, 105.50 ,67.48, 51.97, 36.55, 34.80, 18.98, 14.08 ; ^{31}PNMR (162.0 MHz, $CDCl_3$, δ ppm): 21.32; LC-MS m/z: 399.6 $(M+1)^+$.

Dibutyl (2-amino-6-bromo-3-cyano-4H-chromen-4-yl) phosphonate **(4c)**

Brown solid, mp: 148-150°C, IR (ZnSe, cm^{-1}) υ_{max}; 3294 (N-H), 2199 (CN), 1226 (P=O), 809 (P-C-H$_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.47-6.85 (m, 4H, Ar-H), 5.02 (s, 2H, -NH_2), 4.03-4.01 (m, 4H, -OC$\underline{H}_2$-CH_3), 3.84 (d, 1H, *J* = 8 Hz, C$\underline{H}$P), 1.65-1.30 (m, 8H, -$(CH_2)_4$), 0.96 (t, 3H, *J*= 8.0 Hz, -C$\underline{H}_3$), 0.92 (t, 3H, *J* = 8.00 Hz, -C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 162.00, 149.34, 132.51 , 119.19, 118.49, 117.37, 115.20, 105.50 , 67.50, 51.68 , 36.95, 36.21 , 18.04 , 14.08; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 21.62; LC-MS m/z: 444.3 $(M+1)^+$.

Dibutyl (2-amino -3-cyano-5, 7 -dichloro-4H-chromen-4-yl) phosphonate **(4d)**

Yellow solid, mp: 156-158°C, IR (ZnSe, cm^{-1}) υ_{max}; 3320 (N-H), 2192 (CN), 1235 (P=O), 841 (P-C-H $_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$,

δppm): 7.31-6.92 (m, 2H, Ar-H), 4.92 (s, 2H, -NH_2), 4.06 - 4.03 (m, 4H, OC$\underline{H}_2$), 3.82 (d, 1H, *J* = 8 Hz, C$\underline{H}$P), 1.64-1.32 (m, 8H, -$(CH_2)_4$), 0.93 (t, 3H, *J*= 8.0 Hz, -C$\underline{H}_3$), 0.87 (t, 3H, *J* = 8.00 Hz, -C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 163.05, 150.05, 134.11, 132.07, 124.21, 120.48, 115.50, 105.30 69.35, 52.85, 38.60, 35.50, 18.48, 14.08; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 22.56; LC-MS m/z; 434.2 $(M+1)^+$.

Dibutyl (2-amino- 3-cyano- 7-(diethylamino)-4H-chromen-4-yl) phosphonate **(4e)**

Brown solid, mp: 152-154°C, IR (ZnSe, cm^{-1}) υ_{max}; 3327(N-H), 2193 (CN), 1198 (P=O), 793(P-C-$H_{aliphatic}$) ; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.28-7.11 (m, 3H, Ar-H), 4.77 (s, 2H, -NH_2), 4.10-4.01 (m, 4H,- OC$\underline{H}_2$) 3.78 (d, 1H, *J* = 8 Hz, C$\underline{H}$P), 3.32-327 (m, 4H, N-C$\underline{H}_2$-CH_3) 1.73-1.26 (m, 8H, -$(CH_2)_4$), 1.14-1.11 (m, 6H, N-CH_2- C$\underline{H}_3$) 0.93 (t, 3H, *J*= 8 Hz, -C$\underline{H}_3$), 0.82 (t, 3H, *J* = 8.00 Hz, -C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 161.99, 151.18, 148.32, 130.04, 119.83, 108.98, 101.96 , 98.47 , 66.77, 53.10, 44.31 , 35.14, 32.64, 18.97, 14.08, 12.35; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 23.13 LC-MS m/z; 436.3 $(M+1)^+$.

Dibutyl (2-amino- -3-cyano- 5-Ethoxy -4H-chromen-4-yl) phosphonate **(4f)**

White solid, mp: 146-148°C, IR (ZnSe, cm^{-1}) υ_{max}; 3230 (N-H), 2193 (CN), 1241 (P=O), 730 (P-C-$H_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$,δ ppm): 7.28-7.25 (m, 3H,Ar-H), 4.93 (s, 2H, -NH_2), 4.08-4.05 (m, 4H, OC$\underline{H}_2$, 2H Ar-O-C$\underline{H}_2$-CH_3) 3.86 (d, 1H, *J* = 8 Hz, C$\underline{H}$P) 1.69-1.65 (m, 8H, -$(CH_2)_4$), 1.4 - 1.2 (m, 6H, -OCH_2-C$\underline{H}_3$) 0.92 (t, 3H, *J* = 8.0 Hz, -C$\underline{H}_3$), 0.82 (t, 3H, *J* = 8.00 Hz, -C$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$ δ ppm): 162.04, 146.59, 139.54, 124.76, 121.01, 119.48, 112.88, 108.50, 66.60, 65.05, 52.10, 36.23, 32.66 ,18.90, 14.97, 13,67; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 22.32; LC-MS m/z; 409.1 $(M+1)^+$.

Diethyl (2-amino-3-cyano-4H-chromen-4-yl) phosphonate **(4g)**

White solid, mp: 142-144°C, (ZnSe, cm^{-1}) υ_{max}; 3198 (N-H), 2214 (CN), 1148 (P=O), 751 (P-C-$H_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.33-6.92 (m, 4H, Ar-H), 4.96 (s, 2H, -NH_2), 4.22-3.90 (m, 4H, OC$\underline{H}_2$-CH_3), 3.8 (d, 1H, *J* = 8 Hz, C$\underline{H}$P), 1.38 (t, 3H, *J* = 8.0 Hz, -OCH_2-C$\underline{H}_3$), 1.28 (t, 3H, *J* =7.20 Hz, O-CH_2-C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$ δ ppm): 161.50 , 149.02, 138.14, 132.18 , 131.36 , 120.24 , 120.20 ,116.82 , 77.20 , 50.20 ,35.96 , 16.44 ; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 21.43; LC-MS m/z; 309.10 $(M+1)^+$.

Diethyl (2-amino-3-cyano-(6-chloro)-4H-chromen-4-yl) phosphonate **(4h)**

Yellow solid, mp: 156-158°C, IR (ZnSe, cm^{-1}) υ_{max}; 3301 (N-H), 2221 (CN), 1174 (P=O), 752 (P-C-$H_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.47- 7.26 (m, 3H, Ar-H), 5.0 (s, 2H, -NH_2), 4.20-4.00 (m, 4H, -O-C$\underline{H}_2$-CH_3), 3.8 (d, 1H, *J* = 18Hz, C$\underline{H}$P), 1.36-1.33 (t, 3H, *J* = 7.20Hz, O-CH_2-C$\underline{H}_3$), 1.28 (t, 3H, *J* = 7.20 Hz, O-CH_2-C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$ δ ppm): 161.87, 150.50, 148.49, 129.98, 129.25, 119.20, 118.44, 117.83, 63.51, 50.93, 36.07 , 16.46; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 21.52; LC-MS m/z; 343.40 $(M+1)^+$.

Diethyl (2-amino-3-cyano-(6-bromo)-4H-chromen-4-yl) phosphonate **(4i)**

Yellow solid, mp:160-162°C, IR (ZnSe, cm^{-1}) υ_{max}; 3320 (N-H), 2189 (CN), 1172 (P=O), 738 (P-C-$H_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.24-7.21(m 3H, Ar-H), 5.01 (s, 2H, -NH_2), 4.16-4.02 (m, 4H, O-C$\underline{H}_2$-CH_3), 3.8 (d, 1H, *J =18* Hz, C$\underline{H}$P), 1.36-1.33 (t, 3H, *J*= 7.20 Hz, O-CH_2-C$\underline{H}_3$), 1.27-1.23 (t, 3H, *J*= 16 Hz, O-CH_2-C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$, δ

ppm): 161.80, 150.50, 149.02, 138.14, 132.18, 119.15, 118.20, 117.38, 63.50, 51.04, 35.96 , 16.45; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 22.42; LC-MS m/z; 388.10 $(M+1)^+$.

Diethyl (2-amino-5, 7-dichloro-3-cyano-4H-chromen-4-yl) phosphonate **(4j)**

White solid mp: 150-152°C, IR (ZnSe, cm^{-1}) υ_{max}; 3293 (N-H), 2190 (CN), 1233 (P=O), 799 (P-C-$H_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.40-7.36 (d, 2H,Ar-H), 5.02 (s, 2H, -NH_2), 4.22-3.92 (m, 4H, O$C\underline{H}_2$-CH_3), 3.96 (d, 1H, *J* = 8 Hz, $C\underline{H}$P), 1.34 (t, 3H, *J* = 8.0 Hz, -OCH_2-$C\underline{H}_3$), 1.28 (t, 3H, *J* = 7.20 Hz, O-CH_2-$C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 160.50, 150.50, 134.50, 130.48, 126.40, 124.16, 128.40, 120.50, 63.38, 50.05, 36.07, 16.20 ; ^{31}P NMR(162.0 MHz, $CDCl_3$, δ ppm): 21.42; LC-MS m/z; 378.1 $(M+1)^+$.

Diethyl (2-amino-3-cyano-7-(diethylamino)-4H-chromen-4-yl) phosphonate **(4k)**

Yellow solid, mp : 160-162°C, IR (ZnSe, cm^{-1}) υ_{max}; 3322 (N-H), 2195 (CN), 1216 (P=O), 794 (P-C-$H_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.26-6.22 (m, 3H, Ar-H), 4.36 (s, 2H, -NH_2), 4.16-4.05 (m, 4H, O-$C\underline{H}_2$-CH_3), 1.16-1.14 (m, 6H, N-CH_2- $C\underline{H}_3$), 4.00 (d, 1H, *J* = 10.64 Hz, $C\underline{H}$P), 3.95-3.93 (m, 4H, N-$C\underline{H}_2$-CH_3), 1.33 (t, 3H, *J*= 7.20 Hz, O-CH_2-$C\underline{H}_3$), 1.21 (t, 3H, *J*= 7.15 Hz, O-CH_2-$C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 162.15, 151.08 , 148.65, 130.0 , 120.09, 115.50, 108.0 , 101.43, 62.73 , 52.31, 44.56, 33.84 , 16.58 , 12.51 ; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 23.52; LC-MS m/z; 380.1 $(M+1)^+$.

Diethyl (2-amino-3-cyano-5-ethoxy-4H-chromen-4-yl) phosphonate **(4l)**

Yellow solid, mp: 154-156°C, IR (ZnSe, cm^{-1}) υ_{max}; 3304 (N-H), 2191 (CN), 1219 (P=O), 745 (P-C-$H_{aliphatic}$); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.47-7.26 (m, 3H, Ar-H), 5.0 (s, 2H, -NH_2), 4.25-4.05 (Ar-O-$C\underline{H}_2$-CH_3), 4.20-4.00 (m, 4H, O-$C\underline{H}_2$-CH_3), 3.8 (d, 1H, *J* = 18Hz, $C\underline{H}$P), 1.36-1.33 (t, 3H, J = 7.20 Hz, O-CH_2-$C\underline{H}_3$), 1.28 (t, 3H, J = 7.20 Hz, O-CH_2-$C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$ δ ppm): 161.87, 148.49, 129.98, 129.25, 119.20, 118.44, 117.83, 104.20, 65.31, 50.93, 36.07, 34.59, 16.46, 15.45; ^{31}P NMR (162.0 MHz, $CDCl_3$, δ ppm): 22.36; LC-MS m/z; 353.24 $(M+1)^+$.

PHARMACOLOGY

Antioxidant activity

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the compound was measured from the bleaching of the purple coloured methanol solution of 2, 2- diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent [28]. 1mL of different concentrations of the compound (50, 100, 150 and 200µg/mL) in methanol were added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After 30 minutes of incubation period at room temperature, the absorbance was read against blank at 517 nm. Along with, a standard ascorbic acid was used as analyser in the test compounds in same concentration. The percent of inhibition (I %) of free radical production from DPPH was calculated by using the following equation.

$$I\% = [(A_{control} - A_{sample} / A_{control})] \times 100.$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate. IC_{50} values for both the compounds and standard ascorbic acid were calculated by plotting a graph concentration *vs* percent of scavenging activity. IC_{50} value denotes

the concentration of the compound, which is required to scavenge 50% of DPPH free radicals.

Nitric oxide scavenging activity

Sodium nitro prusside generates nitric oxide in aqueous solutions, at physiological pH, which is converted into nitric and nitrous acids on contact withdissolved oxygen and water. This method is based onthe inhibition of nitric oxide radical generation from sodium nitro prusside. The liberated nitrous acid is estimated using a modified Griess-Illosvoy method, where nitrous acid reacts with Griess reagent, to forma purple azo dye. In the presence of antioxidants the amount of nitrous acid will decrease and the degree of this reduction in formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed is measured at 540 nm. [29]

$$I\% = [(A_{control} - A_{sample} / A_{control})] \times 100.$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate. IC_{50} values for both the compounds and standard ascorbic acid were calculated by plotting a graph concentration *vs* per cent of scavenging activity. IC_{50} value denotes the concentration of the compound, which is required to scavenge 50% of NO free radicals.

Hydrogen peroxide scavenging activity

According to the method reported by Ruch *et. al.* [30] the ability of test compounds to scavenge hydrogen peroxide was estimated with slight modifications. A solution of hydrogen peroxide (43 mM) is prepared in phosphate buffer (1 M pH 7.4). A different concentration of 12 test compounds (50-200 µg/ml) was added to a series of test tubes containing hydrogen peroxide solution (2 ml, 43 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating % inhibition as described below.

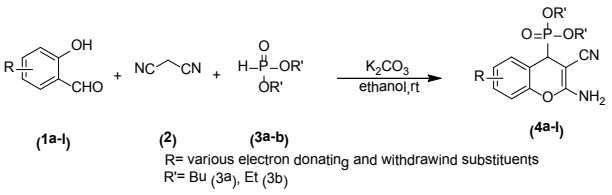
$$I\% = [(A_{control} - A_{sample} / A_{control})] \times 100.$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate. IC_{50} values for both the compounds and standard ascorbic acid were calculated by plotting a graph concentration *vs* per cent of scavenging activity. IC_{50} value denotes the concentration of the compound, which is required to scavenge 50% of H_2O_2 free radicals.

RESULTS AND DISCUSSIONS

Chemistry

A Strategic approach has been made for the C-P bond formation in the one-pot synthesis of 2-amino-3-cyano-4*H*-chromen-4-yl phosphonates (**4a-l**) by the reaction of salicylaldehydes (**1a-l**), malononitrile (**2**), and dialkylphosphites (**3a-b**) using K_2CO_3 as a catalyst under room temperature (rt) conditions in ethanol, which offered good to excellent yields after simple work-up procedure.



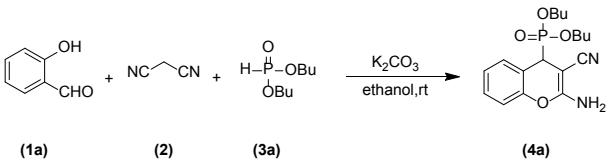
Scheme-1: Synthesis of 2-amino-3-cyano-4*H*-chromen-4yl phosphonates (**4a-l**)

In order to define the best experimental conditions for the synthesis of dibutyl (2-amino-3-cyanochromen-4-yl) phosphonate

(**4a**), a model reaction has been carried out by taking 2-Hydroxy benzaldehyde (1; 1mmol), malononitrile (2; 1mmmol) and dibutylphosphite (3; 1mmol) using ethanol as solvent.

Initially the reaction was run under room temperature without a catalyst which results no yields of the product (**Table 1, entry 1**). Then we moved to different catalysts such as TiO_2, ZnO, $LaCl_3$, Cs_2O_3, CAN, CeO_2, $(NH_4)_2CO_3$ and K_2CO_3 (**Table 1, entries 2-9**). Among all of them, K_2CO_3 in 10mol% showed better catalytic activity; the reaction proceeded very smoothly and gave the **4a** in 96% yields at room temperature. Increasing the amount of catalyst beyond 10% had no effect on the product yields.

Table 1: Effect of the various catalysts on the synthesis of 2-amino-3-cyano-4*H*-Chromen-4-yl phosphonate (**4a**)[a]



(1a) (2) (3a) (4a)

Entry	Catalyst (mol (%))	Time(min)	Yield (%)[b]
1	Catalyst free	1h	nr[c]
2	TiO_2(10)	15 min	59
3	ZnO(10)	15 min	46
4	$LaCl_3$(10)	15 min	65
5	Cs_2CO_3(10)	15 min	48
6	CAN(10)	15 min	67
7	CeO_2(10)	15 min	63
8	$(NH_4)_2CO_3$(10)	15 min	70
9	K_2CO_3(10)	15 min	96
10	K_2CO_3(15)	15 min	94

[a] Reaction of 2-hydroxy benzaldehyde (1 mmol), malononitrile (1 mmol) and dibutylphosphite (1 mmol) using various catalysts along with K_2CO_3 in different concentrations under r.t and solvent of ethanol.

[b] Isolated yield, [c] No reaction

To study the effect of solvent on the reaction, we tried with various solvents such as Toluene, THF, DMF, DCM, CH_3CN, $CHCl_3$, MeOH and EtOH (**Table 2, Entries 1-8**) at different temperatures. Finally the reaction proceeds well in ethanol at room temperature and hence these conditions are selected as the optimized experimental conditions. Different 2-amino-3-cyano-4*H*-chromen-4-ylphosphonates were synthesized from the corresponding salicylaldehydes in a one-step reaction procedure and used as substrates to accomplish the title compounds (**4a-l**) (**Table-3**)

Table 2: Optimization of reaction, conditions on solvent and catalyst loading for the synthesis of **4a**

Entry	catalyst	Solvent	Temperature (°C)	Time (min)	Yield (%)[b]
1	K_2CO_3	Toluene	100	120	75
2	K_2CO_3	THF	60	60	60
3	K_2CO_3	DMF	100	90	70
4	K_2CO_3	DCM	20	120	65
5	K_2CO_3	CH_3CN	50	60	80
6	K_2CO_3	$CHCl_3$	50	90	72
7	K_2CO_3	MeOH	50	60	81
8	K_2CO_3	EtOH	RT	15	96

[a]Reaction conditions: 2-hydroxy benzaldehyde (1.0 mmol), malononitrile (1.0 mmol), and dibutylphosphite (1.0 mmol) in the presence of K_2CO_3 Catalyst.Ethanol solvent and r.t conditions

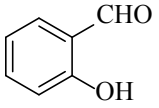
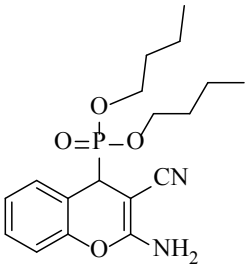
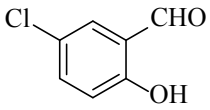
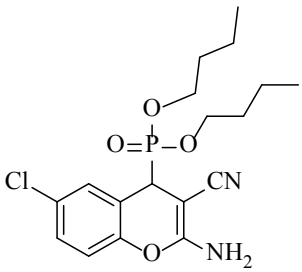
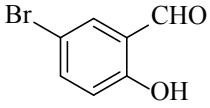
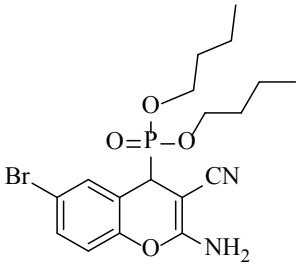
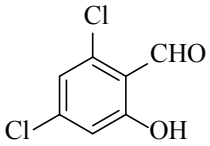
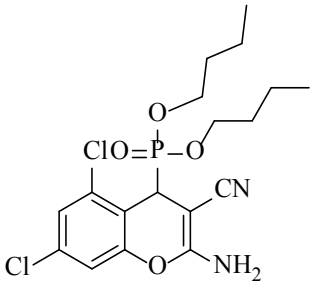
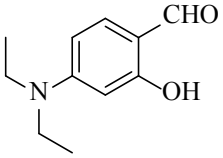
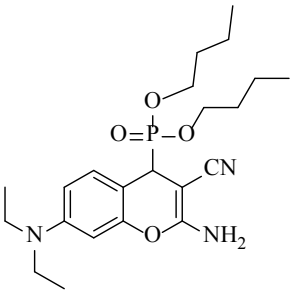
[b]yield of the isolated product.

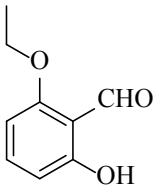
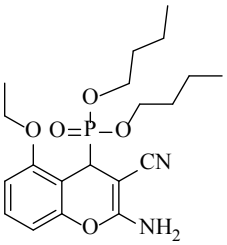
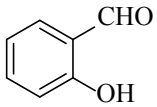
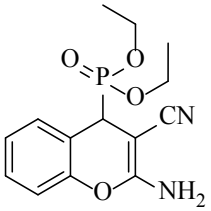
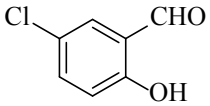
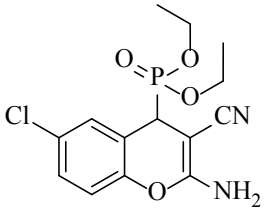
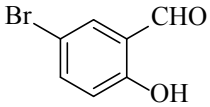
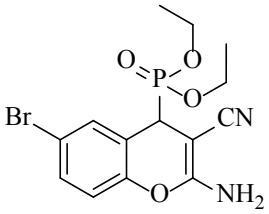
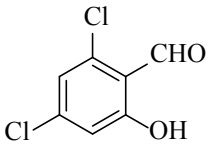
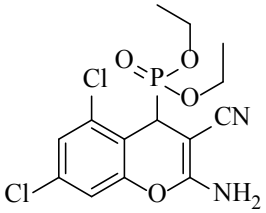
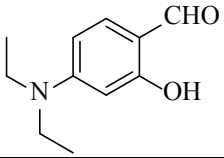
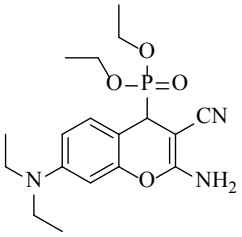
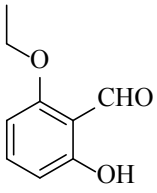
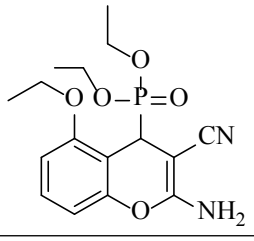
The 2-amino-3-cyano-4*H*-chromen-4-ylphosphonates were obtained in a shorttime in high yields. Next, we investigated the replacement of DBP with DEP. The reaction was observed to proceed smoothly for all DAPs in good yield; while the reaction with DEP also proceeded smoothly but in a comparatively higher yield than that of DBPs.

After establishing the optimum reaction conditions, we investigated the scope of the reaction by condensing various commercially

available substituted aryl salicylaldehydes **(1a-l)**, malononitrile **(2)** and dialkylphosphites **(3a-b)** to form corresponding 2-amino-3-cyano-4*H*-chromenyl phosphonates. The details of the physical data, such as yield and melting points, are illustrated in **Table 3.**

Table 3: Physical data of the title compounds (**4a-l**)

S.NO	Structures of salicylaldehydes (1a-l)	Structures of products (4a-l)	Time(Min)	Yield[a](%)	Melting point(°C)
1			15	96	146-148
2			15	90	138-140
3			15	90	148-150
4			15	91	156-158
5			15	89	152-154

6			15	92	146-148
7			15	95	142-144
8			15	97	156-158
9			15	93	160-162
10			15	95	150-152
11			15	96	160-162
12			15	94	154-156

[a] Reaction conditions: Various substituted salicylaldehydes (1 mmol), malononitrile (1 mmol), and dialkylphosphites (1 mmol) in the presence of 10 mol% of K_2CO_3 as catalyst at solvent in ethanol and room temperature conditions.
[b] Isolated yields

The products were obtained as semi solids and purified by column chromatography using silica gel as adsorbent and ethyl acetate-hexane (2:3) as eluent. The chemical structures of **4a–l** were confirmed by elemental analysis, IR, ^{1}H-, ^{13}C-, ^{31}P-NMR and mass spectral data. Compounds **4a–l** exhibited characteristic IR stretching frequencies in the regions 3209–3330,1150–1240 and 730–841 cm^{-1} for N–H, P=O (phosphonates), and P–C respectively. The P–C–H proton, signal appeared at δ 3.75–4.10 (d, *J*= 8.0 Hz) due to its coupling with phosphorus. The methylene protons of P (O) $CH_2CH_2CH_2CH_3$ group showed a multiplet at δ1.73-1.20. The methyl protons signal of P (O) $CH_2CH_2CH_2CH_3$ group appeared as triplet signal at δ 0.96-0.82. The ^{13}CNMR chemical shifts for P (O) $\underline{CH_2}CH_3$ and P (O) $CH_2\underline{CH_3}$ group occurred at δ 62.73–70.00 and *δ* 14.4–16.9, respectively. The ^{31}P-NMR chemical shifts were present in the range of δ 21.32–23.52 (P=O phosphonates) in all the compounds.

BIOLOGY

Antioxidant activity

DPPH radical scavenging activity

One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index. In the DPPH free radical scavenging activity, 12 compounds were evaluated for their free radical scavenging activity along with ascorbic acid as standard compound. The IC_{50} was calculated for each compound along with standard ascorbic acid and summarized in **Table 4** and graphically represented in **Fig. 1.** The scavenging effect increased with the increasing concentrations of test compounds. Among the title compounds (**4a-l**) tested for antioxidant activity by DPPH method, the compounds **4b, 4e, 4h, 4k** and **4l** showed remarkably high antioxidant activity while other compounds showed moderate activity when compared with that of standard ascorbic acid.

Table 4: In vitro antioxidant activity of title compounds (**4a-l**) by DPPH method

S. No	T1 (50µg/mL)	T2 (100µg/mL)	T3 (150µg/mL)	T4 (200µg/mL)	IC_{50}
1	9.80	4.65	-0.98	-2.45	255.10
2	16.42	24.50	40.68	45.83	218.19
3	27.20	22.79	12.5	2.94	91.91
4	17.64	7.10	0.00	-1.22	141.72
5	15.93	22.54	49.50	54.41	151.51
6	-177.9	-207.35	-215.44	-219.85	-----
7	-0.73	-8.33	-17.15	-20.83	-----
8	7.84	21.81	39.70	57.84	172.89
9	-161.76	-175.73	-215.44	-219.85	----
10	11.76	7.35	0.49	-3.92	212.58
11	14.46	25.73	45.09	52.94	188.89
12	25.98	45.09	60.78	68.62	110.88
Ascorbic acid	20.09	46.56	61.27	75.00	107.38

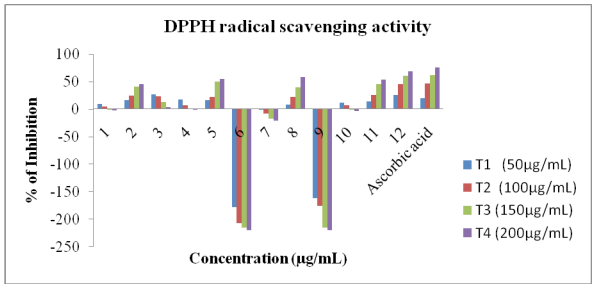


Figure 1: *In vitro* antioxidant activity of title compounds (**4a-l**) by DPPH method

Nitric oxide scavenging activity

NO scavenging activity was determined by the decrease in the absorbance at 540 nm, induced by antioxidants. In direction to calculate the antioxidant potency through NO scavenging by the test samples, the modification of optical density of NO was tested. Table 5 shows the comparative Nitric Oxide scavenging activity of the 12 test compounds along with a standard

ascorbic acid. The scavenging effect increased with the increasing concentrations of test compounds. The results are given in **Table-5** and **Fig. 2**. The NO free radical scavenging activity study of the title compounds (**4a-l)** reveals that the compounds **4b, 4e, 4h, 4k, and 4l** showed the highest NO scanning activity which is comparable to the reference standard. The remaining compounds exhibit good activity. The results were expressed as a percent of scavenged nitric oxide in **Table (5)**

Table 5: *In vitro* antioxidant activity of title compounds (**4a-l**) by NO method

S. No.	T1 (50µg/ mL)	T2 (100µg/ mL)	T3 (150µg/ mL)	T4 (200µg/ mL)	IC_{50}
1	18.59	15.45	11.74	5.47	134.48
2	20.74	27.39	29.15	22.11	257.28
3	17.61	14.87	9.00	5.67	141.96
4	20.54	11.74	9.39	2.93	121.71
5	27.78	40.70	42.85	58.51	175.02
6	-99.60	-118.19	-128.76	-133.65	-----
7	18.78	14.48	5.28	1.56	133.12
8	29.35	36.00	45.59	56.75	164.50
9	-116.43	-121.52	-129.54	-136.59	-----
10	14.09	10.95	9.39	-0.58	177.43
11	22.50	37.18	42.07	54.59	183.18
12	30.13	41.48	56.55	60.86	132.62
Ascorbic Acid	32.28	53.22	59.49	68.29	93.94

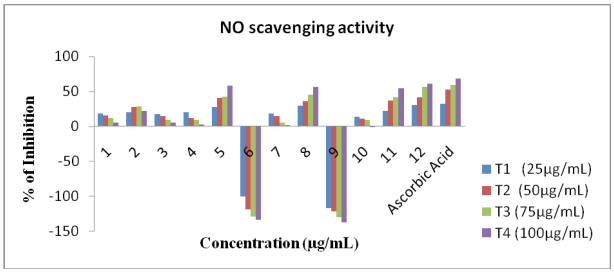


Figure 2: In vitro antioxidant activity of title compounds (**4a-l**) by NO method

Hydrogen peroxide scavenging activity

Hydrogen peroxide is generated *in vivo* by several oxidase enzymes. Hydrogen peroxide is scavenged via its reduction product hydroxyl radical (OH•). In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230nm.The H_2O_2 scavenging activity of 12 test compounds along with standard ascorbic acid was summarized in **Table 6** and **Fig 3.** The scavenging ability of hydrogen peroxide by compounds **4b, 4e, 4h, 4k, and 4l** was found to be comparable ascorbic acid **Table (6)**. This scavenging activity of hydrogen peroxide is found to be concentration dependent

Table 6: *In vitro* antioxidant activity of title compounds (**4a-l**) by H_2O_2 method

S. No.	T1 (50µg/ mL)	T2 (100µg/ mL)	T3 (150µg/ mL)	T4 (200µg/ mL)	IC_{50}
1	2.54	-2.86	-8.59	-12.42	984.25
2	5.09	10.50	16.56	28.34	352.85
3	14.96	6.05	3.82	0.63	167.11
4	21.65	13.69	6.68	2.22	115.47
5	14.33	31.21	41.40	45.22	221.14
6	-212.1	-235.0	-252.2	-255.0	------
7	19.10	13.69	10.50	7.64	130.89
8	31.21	40.76	47.77	53.82	157.00
9	-178.66	-183.75	-188.53	-197.45	----
10	5.09	0.63	-13.37	-9.87	491.15
11	22.61	33.12	43.94	53.50	186.91
12	28.02	35.98	46.49	57.96	140.18
Ascorbic Acid	35.66	43.31	54.77	65.60	136.93

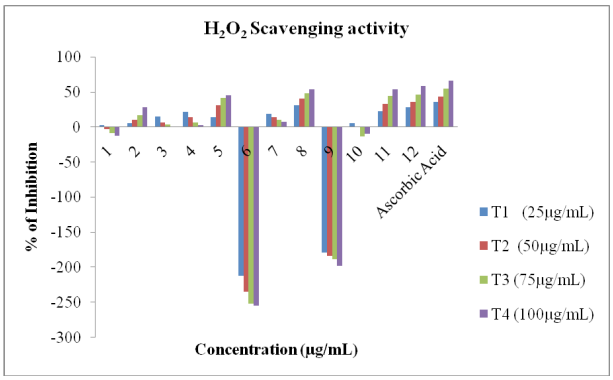


Figure 3: Hydrogen peroxide scavenging activity of chromenyl phosphonates (**4a-l**)

Conclusion

In conclusion, a simple and efficient green synthetic protocol has been developed for the synthesis of 2-amino-3-cyano-4*H*-chromen-4-ylphosphonates in presence of K_2CO_3 catalyst and ethanol. All the title compounds were screened for antioxidant activity by DPPH, NO and H_2O_2 methods. The bio-assay revealed that the title compounds exhibited moderate to good antioxidant activities, particularly **4b, 4e, 4h, 4k** and **4l** showed high activity in all the three methods.

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