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Synthesis and Cytotoxicity Evaluation of Certain Coumarin–α-Aminophosphonates

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Abstract: A series of coumarin α-aminophosphonates were synthesised *via* multi- component reaction using 2-3 drops of acetic acid in ethanol under heating. The compounds **3a-j** were evaluated in *vitro* against the four cancer cell lines (HepG2, HeLa, PANC-1 and SKNSH) using an SRB assay. Most of the coumarin derivatives **3a-j** showed strong inhibitory activities against the four tested cell lines.

Keywords: Coumarin, α-aminophosphonates, *in vitro,* Cytotoxicity

Introduction

Coumarin and its derivatives are a common structural subunit in both natural and synthetic compounds possessing important biological activities [1-2]. Coumarins are also used as additives to food and cosmetics *etc* [3]. 3-aminocoumarin analogues constitute an important class of therapeutic agents in medicinal chemistry including antibacterial [4], antifungal [5], antiviral [6], anticoagulant [7], anti-inflammatory [8] and antitumor [9] activity. Burlison *et al.* have studied such as antibiotic Novobiocin containing 3-aminocoumarin derivative as an ATP competitive inhibitor of gyrase B subunit [10]. Zhao *et al.* demonstrated that coumarin derivatives are important lead compounds for the development of new drugs against HIV [11]. Weber *et al.* showed coumarin and its metabolite 7-hydroxycoumarin exhibits antitumor activity against several human tumor cell lines [12]. Coumarin and its derivatives have extensively used as potential inhibitors of cellular proliferation in various carcinoma cell

lines [13-14]. Budzisz *et.al.* have studied series of coumarin derivatives and their phosphonic analogue **I(a-c)**, **II(a-c)**and **III(a-c)** (Figure 1). They showed these compounds exhibits inhibition of cell proliferation on the two leukemia cell lines HL-60 and NALM-6[15]. Grotz *et al.* demonstrated that coumarin used not only to treat cancer but also to treat the side effects caused by radiotherapy [16]. Recently, Krešimir Benci *et al.* reported anti-tumor effect of coumarin derivatives bearing triazole, dicyanoimidazole and purine against human tumor cell line [17].

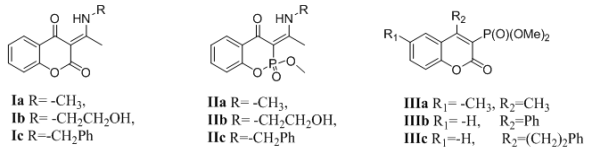


Figure 1. Coumarin derivatives **Ia-c,IIa-c** and **IIIa-c**.

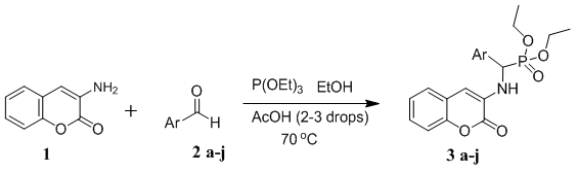
On the other hand, synthesis and biological activity study of α-aminophosphonate has attracted much attention from chemists [18-22]. Pratt *et al.* have studied phosphonates and showed these compounds to be an important class of biologically active agents such as antibacterial [23]. Beers *et al.* showed phosphonate compounds acting as inhibitor of phosphatase activity [24]. Such phosphonate and their derivatives were prepared to study diverse pharmacological activities such as antiviral, antifungal [25], and antitumor [26-29].

In continuation of our interest in α-aminophosphonate chemistry, the present report describes the synthesis and cytotoxic activity study of certain coumarin-α-aminophosphonates with a hope to find more potent leads for anticancer activity.

Results and Discussion

Chemistry

The synthesis of coumarin-α-aminophopshonate **3a-j** is illustrated in the scheme 1. One of the starting compound 3-aminocoumarin **(1**) was prepared from the straightforward condensation of salicylaldehyde and *N*-acetylglycine [30]. The synthesis of designed α-aminophosphonates (**3a-j**) was achieved from 3-aminocoumarin (**1**), aldehydes (**2a-j**) and triethylphosphate in the presence of ethanol as a solvent at elevated temperature. We found that the use of 2-3 drops of acetic acid is an efficient catalytic system (Scheme1) [31]. As expected the corresponding phosphonate products (**3a-j)** were obtained in good yields (Table1) using the above protocol. The structure of all the synthesised compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass and HRMS spectroscopic techniques.



Scheme 1. Synthesis of coumarin-α-aminophosphonate (**3a-j**).

In the IR spectra, the stretching vibration of NH of compound **3a** appeared at 3400 cm^{-1} and for P=O at 1246 cm^{-1}. In the^{1}H NMR spectra the POCH proton peak appeared as multiplates at 5.86 ppm. Furthermore, in the ^{13}C NMR spectra a signal for the α-carbon appeared at δ 63.5 ppm and peak appered at δ 21.4 in ^{31}P NMR spectrum. For all compounds **3a-j**, a detailed description of the spectral data is given in the experimental section.

Biological activity

In vitro cytotoxic activity of these coumarin-α-aminophosphonate compounds (**3a-j**) were evaluated on four human tumour cell lines including human liver carcinoma (HepG2), human cervical cancer (HeLa), human Pancreatic cancer cells (PANC-1) and human neuroblastomacellline (SKNSH). The growth

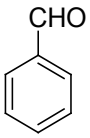
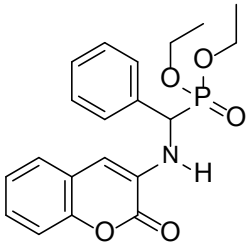
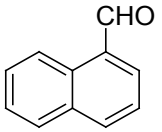
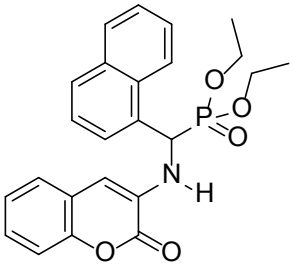
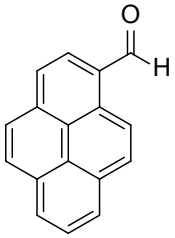
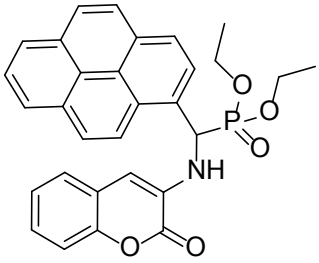
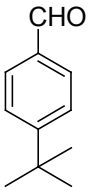
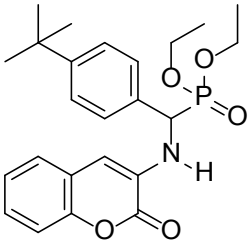
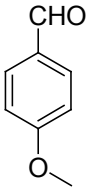
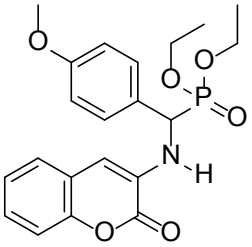
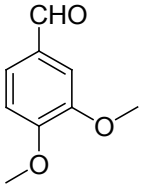
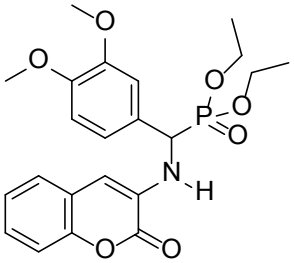
inhibition was evaluated using standard SRB assay following reported protocol in literature [32]. The obtained activity results were expressed as 50% cell growth inhibition (GI_{50}) in μM and summarised in Table 2. The reported GI_{50} values were the average of three independent experiments performed. The cytotoxic activities of synthesised compounds were compared with the activity exhibited by Doxorubicin in *vitro* investigation.

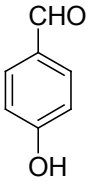
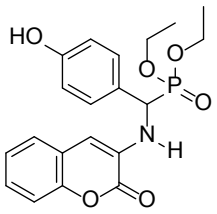
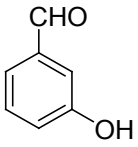
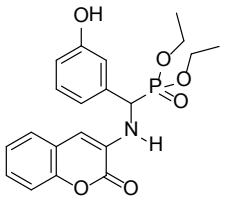
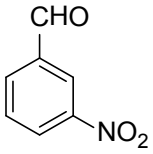
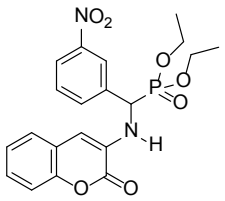
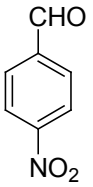
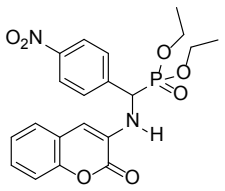
The coumarin α-aminophosphonate **3a-j** derivatives were tested against cancer cell line HepG2. The cytotoxic activity study revealed that the compound **3a** (GI_{50} = 96 ± 8.7 μM) having phenyl ring at α-carbonfailed to show effective inhibition as compare to **3b** (GI_{50} = 7.7 ± 0.5 μM) and **3c** (GI_{50} = 8.22 ± 1.1 μM) contacting naphthalene and pyrene moiety at α-carbon respectively. The comparison of these three derivatives activity indicates that **3b** and **3c** exhibits excellent activity and are superior as compare to **3a.** Here we assume that increase in aromatic characteristic increases potency of the compounds inhibition ability. It was also found that compound **3d** (GI_{50} = 22.3 ± 4.6 μM) having tertiry butyl substituent on phenyl ring showed moderate cytotoxic activity. The derivative **3e** (GI_{50} = 16.8 ± 1.59 μM) containing methoxy group at para position of phenyl ring is more potent than **3d**. Furthermore, the derivative **3f** (GI_{50} = 11.07 ± 1.5 μM) containing two methoxy groups on phenyl ring showed excellent inhibitory activity as compare to **3d** and **3e**. Thus it was found that compounds **3d-f** having electron donating (tertiry butyl, methoxy and two methoxy) groups on phenyl ring exhibits excellent inhibition activity as compare to unsubstituted phenyl containing compound **3a**. The cytotoxic activity of compounds **3g** and **3h** containing hydroxy substituents on the phenyl ring was also investigated. When the hydroxy group is at *para*-position in **3g**, it exhibits very little inhibition. While, derivative containing hydroxy functionality at *meta*-position of phenyl ring in **3h** showed good activity. It is interesting to note that presence of nitro functional group (electron withdrawing) as substituent on phenyl ring at *para* position of coumarin α-aminophosphonate **3j** exhibits excellent activity where as **3i** having nitro functional group at *meta*-position showed overall potency is decreased as compare to **3j**.

The inhibitory effects of the test compounds **3a-j** were determined against the HeLa cell line in vitro. Compound **3a** (GI_{50} = 20.7 ± 0.8 μM) showed moderate inhibition and **3b** (GI_{50} = > 100 μM) showed no inhibition whereas **3c** (GI_{50} = 6.4 ± 0.7 μM) exhibits excellent inhibition. The structure activity relationship result of these three derivatives implies that compound **3c** containing pyrene is most efficient among them. Introduction of an electron-donating substituents at the α-carbon substituent showed inhibition activity of **3f** (GI_{50} = 10.1 ± 1.6 μM) is superior to **3e** (GI_{50} = 18.7 ± 0.5 μM) and **3d** (GI_{50} = 39.0 ± 0.8 μM). The derivative **3g** (GI_{50} = > 100 μM) failed to show any inhibition against HeLa cell line. The derivative **3h** (GI_{50} = 1.9 ± 0.04 μM) showed excellent inhibitory activity. The coumarin derivatives containing electron withdrawing substituent nitro group at *meta*- and *para*- positions in **3i** (GI_{50} = 2.4 ± 1.4 μM) and **3j** (GI_{50} = 3.7 ± 1.2 μM) respectively possess a strong inhibitory activity.

In the growth inhibition of PANC-1 cell line using SRB assay was also investigated by using coumarin α-aminophosphonate derivatives **3a-j**. Compound **3a** (GI_{50} = 1.4 ± 0.1 μM), **3b** (GI_{50} = 1.39 ± 0.6 μM) and **3c** (GI_{50} = 1.3 ± 0.2 μM) having aromatic moiety at α-carbon atom was found more promising agents for inhibition of growth of PANC-1 cells. The derivative **3d** also showed excellent activity. The compound **3e** (GI_{50} = 0.64 ± 0.03μM) is one of the most active compounds among the series may be due to the presence of methoxy substituent at *para*- position on phenyl ring. Whereas, compound **3f** (GI_{50} = 3.0 ± 1.2 μM) containing two methoxy substituents at *meta*- and *para*-

Table 1. Coumarin-α-aminophosphonates (**3 a-j**).

Entry	Aldehyde (**2a-j**)	Product (**3a-j**)	Time (h)	Yield[a] (%)
3a			3.0	81
3b			4.2	85
3c			5.0	78
3d			3.4	83
3e			4.1	84
3f			3.8	75

3g			3.9	69
3h			4.2	70
3i			5.0	63
3j			5.0	62

[a] Pure isolated product.

Table 2. Cytotoxic activities of coumarin-α-aminophosphonates

Entry	GI_{50} (in μM) values			
	HepG2	**HeLa**	**PANC-1**	**SKNSH**
3a	96±8.7	20.7±0.8	1.4±0.1	13.8±0.4
3b	7.7±0.5	>100	1.39±0.6	>100
3c	8.22±1.1	6.4±0.7	1.3±0.2	>100
3d	22.3±4.6	39.0±0.8	1.2±0.3	66.9±3.7
3e	16.8±1.59	18.7±0.5	0.64±0.03	2.9±0.1
3f	11.07±1.5	10.1±1.6	3.0±1.2	>100
3g	56.9±3.2	>100	0.74±0.02	0.4±0.02
3h	18±0.5	1.9±0.04	0.29±0.006	>100
3i	11.7±0.7	2.4±1.4	0.69±0.06	12.8±1.0
3j	6.2±1.6	3.7±1.2	0.26±0.01	>100
Doxorubicin	<0.01	<0.01	<0.01	<0.01

(a) Cell inhibition was measured employing SRB assay. (b) GI_{50} (in μM)values represent mean ± standard deviation for at least three independent experiments performed.

also showed excellent inhibition activity. The presence of hydroxyl group in **3g** (GI_{50} = 0.74 ± 0.02 μM) at *para*-position and **3h** (GI_{50} = 0.29 ± 0.006 μM) *meta*- position of phenyl ring in α-aminophosphonates enhances the cytotoxic activity on PANC-1 cell line. It was also observed that introduction of substituent nitro group also increases activity of the compounds **3i** (GI_{50} = 0.69 ± 0.06 μM) and **3h** (GI_{50} = 0.26 ± 0.01 μM). Thus the presence of nitro group at *para*- and *meta*- position on phenyl ring showed excellent inhibitory activity. These results allow us to establish the order of potency. Coumarin α-aminophosphonates **3a-j** exhibits excellent activity for compounds containing electron donating group, withdrawing group as well as more hydrophobic when compared with doxorubicin.

In the inhibition of SKNSH cells a variation was observed among the tested compounds **3a-j**. The derivatives **3a** (GI_{50} = 13.8 ± 0.4 μM) and **3i** (GI_{50} = 12.8 ± 1.0 μM) showed moderate activity. The compound **3e** (GI_{50} = 2.9 ± 0.1 μM) exhibits excellent activity. The derivatives **3g** (GI_{50} = 0.4 ± 0.02 μM) also showed excellent activity as compare to all other compounds. The compounds **3b** (GI_{50} = > 100 μM), **3c** (GI_{50} = > 100 μM), **3f** (GI_{50} = > 100μM), **3h** (GI_{50} = > 100μM) and **3j** (GI_{50} = > 100 μM) showed no inhibition and **3d** (GI_{50} = 66.9 ± 3.7 μM) showed little inhibition.

Conclusions

We have reported synthesis of α-aminophosphonates linked to coumarin in presence of acetic acid with the aim to enhance the cytotoxic activity. The cytotoxic activity of the synthesised compounds **3a-j** was evaluated and most of them showed moderate to excellent activity. The compounds **3b**, **3c**, and **3j** are excellent inhibitor of HepG2 cell line. The results of this stdy showed that α-aminophosphonates**3c**, **3i** and **3j** also showed excellent activity against HeLa cell line. Compound **3a-j** exhibits highest potency against PANC-1 cell line. Among them, compound **3g** was found to exhibits excellent activity against SKNSH cell line. We believe this study would be beneficial for the development of new lead compound as anticancer agents.

Experimental section

Chemistry

The chemicals were obtained from Sigma Aldrich and used without prior purification unless otherwise noted. All the solvents used were of laboratory grade and purified by standard methods. Thin-layer chromatography was performed using precoated silica gel glass plates (Merck). Visualisation of the spots on TLC plates is achieved either to iodine vapour or UV light. Infrared spectra were recorded on Perkin Elmer model FT-IR 400 Instruments and values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were obtained on AVANCE-300 MHz and 75 MHz respectively in $CDCl_3$. Chemical shifts were recorded in PPM using tetra Methyl Silane (Me_4Si) as an internal standard. Mass spectral data were recorded on an Agilent Technologies 1100 Series (Agilent Chemistation Software) mass spectrometer. High-resolution mass spectra (HRMS) were obtained by using ESI-Q-TOF mass spectrometry.

General experimental procedure for the synthesis of coumarin α-aminophosphonates (3a-j): In a 25 mL round bottom flask a mixture of 3-aminocoumarin (1 mmol) and substituted aldehyde (1 mmol) in anhydrous ethanol (8 mL) was stirred at room temperature for 30 min. Then equimolar amount of triethylphosphite and 2 to 3 drops of acetic acid were added. The reaction mixture was subjected to heating for 3-5 h. The completion of reaction was monitored by TLC. After being cooled solvent was evaporated under vaccume using rotary evaporator. The obtained crude product was loaded on silica gel and purified by column chromatography eluting

with an ethyl acetate /hexanes (2:8) gradients to afford desired coumarin α-Aminophosphonates.

Biological Screening: Inhibition bioassay [21,33,34]**:** The cell lines, HepG2, HeLa, PANC 1, and SKNSH (hepatic, cervical, pancreatic, and neuroblastoma) which were used in this study were procured from American Type Culture Collection (ATCC), United States. The synthesized test compounds were evaluated for their in*vitro* antiproliferative activity in these six different human cancer cell lines. A protocol of 48 h continuous drug exposure was used, and a SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100 µL aliquots at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, ,100 µM) of prepared derivatives. After 48 hours incubation at 37 °C, cell monolayers were fixed by the addition of 10% (w/v) cold trichloroacetic acid and incubated at 4 °C for 1h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein –bound dye was dissolved in 10 mM Tris base solution for OD determination at 510 nm using a microplate reader (Enspire, Perkin Elmer, USA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels.

Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti >/= Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti < Tz.

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from [(Ti-Tz)/Tz] x 100 = -50. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

Diethyl(2-oxo-2H-chromen-3-ylamino)(phenyl) methylphosphonate ***(3a).*** Brown semi solid, Yield: 81%, IR (KBr) cm^{-1}: 3400, 2981, 2825, 1713, 1628, 1501, 1364, 1248, 1212, 1164, 1021, 969. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.20-1.24 (m, 6H), 4.04-4.18 (m, 4H), 4.74-4.80 (m, 1H), 5.86 (bs, 1H), 6.26 (s, 1H), 7.15-7.29 (m, 4H), 7.48-7.49 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 16.0, 54.6, 55.8, 63.5, 107.9, 115.7,120.7, 125.2, 126.2, 128.0, 128.2, 128.6, 131.1, 133.6, 147.9, 159.0; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 21.4; Mass (ESI, 70 eV): m/z 388 $[M+H]^+$, 410 $[M+Na]^+$. HRMS (ESI) m/z: calculated for $C_{20}H_{23}NO_5P$: 388.1236 found: 388.1310; calculated for $C_{20}H_{22}NO_5PNa$: 410.1236 found: 410.1124.

Diethyl(2-oxo-2H-chromen-3-ylamino) (naphthalen-1-yl)methylphosphonate ***(3b).***

Light brown semisolid, Yield: 85%, IR (KBr) cm^{-1}: 3395, 3231, 3052, 2979, 2953, 2864, 1712, 1628, 1501, 1457, 1392, 1366, 1200, 11601, 1033, 966, 773. ^{1}H NMR(300 MHz,$CDCl_3$, TMS): δ 1.20-1.26 (m, 6H), 4.00-4.15 (m, 4H), 4.93-5.01 (bs, 1H), 5.51-5.65 (m, 1H), 6.03 (s, 1H), 6.98-7.05 (m, 2H) , 7.11-7.24 (m, 2H), 7.43-7.67 (m, 7H).^{13}C NMR (75 MHz,$CDCl_3$, TMS): δ 15.00, 49.0, 51.0, 64.9, 114.7, 119.9, 122.9, 127.48, 127.9, 128.0, 129.1, 129.8, 130.4, 132.5, 132.8, 158.0; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 21.5; Mass (ESI, 70 eV): m/z 460 $[M+Na]^+$. HRMS (ESI) m/z: calculated for $C_{24}H_{25}NO_5P$: 438.1392 found: 438.1466.

Diethyl(2-oxo-2H-chromen-3-ylamino)(pyren-2-yl)methylphosphonate ***(3c).*** Light brown semi solid, Yield: 78%, IR (KBr) cm^{-1}: 3401, 2918, 2863, 1711, 1627, 1603, 1574, 1509, 1495, 1364, 1256, 1174, 1142, 1023, 996, 753. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.28-1.36 (m, 6H), 4.07-4.2 (m, 4H) ,5.13 (bs, 1H), 5.83-5.89 (m, 1H), 6.05 (s, 1H), 6.85-6.97 (m, 2H), 7.11-7.19 (m, 2H), 7.97-8.11 (m, 3H), 8.22-8.29 (m, 5H), 8.52-8.55 (m, 1H) ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 15.8, 50.9, 52.1, 63.3, 107.5, 115.5, 120.4, 121.6, 124.0, 124.3, 124.5, 125.0, 125.4, 125.9, 126.0, 127.1, 127.5, 128.1, 128.8, 130.2, 131.0, 131.2, 147.8, 159.0. Mass (ESI, 70 eV): m/z 512 $[M+H]^+$.

Diethyl(2-oxo-2H-chromen-3-ylamino)(4-tert-butylphenyl)methylphosphonate ***(3d).*** White semisolid, Yield: 83%, IR (KBr) cm^{-1}: 3408, 3061, 2964, 2933, 2870, 1704, 1639, 1572, 1507, 1520, 1366, 1246, 1056, 1016. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.23-1.26 (m, 6H), 1.29 (bs, 9H), 4.01-4.13 (m, 4H),4.67-4.71 (d, 1H), 5.79-5.81 (m, 1H), 6.23 (s, 1H) ,7.13-7.23 (m, 4H), 7.37 (bs, 4H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 16.1, 31.2, 29.6, 34.5, 54.1, 56.1, 63.5, 107.7, 116.0, 121.0, 124.5, 125.3, 125.7, 126.3, 127.21, 127.27, 131.6, 135.1, 148.0, 151.4, 159.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 21.8; Mass (ESI, 70 eV): m/z 444 $[M+H]^+$. HRMS (ESI) m/z: calculated for $C_{24}H_{30}NO_5P$: 444.1862 found: 444.1936.

Diethyl(2-oxo-2H-chromen-3-ylamino)(4-methoxyphenyl)methylphosphonate ***(3e).*** Reddish brown semisolid, Yield: 84%, IR (KBr) cm^{-1}: 3401, 3040, 2934, 2849, 1704, 1623, 1572, 1506, 1359, 1253, 1175, 1030, 963, 791, 751. ^{1}H NMR: (300 MHz, $CDCl_3$, TMS): δ 1.23-1.27 (m, 6H), 3.75 (s, 3H), 4.0-4.2 (m, 4H), 4.61-4.72 (m, 1H), 5.75 (m, 1H), 6.21 (s, 1H), 6.91 (d, 2H), 7.15-7.22 (m, 4H), 7.41 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 16.1, 55.1, 55.7, 63.3, 107.9, 114.1, 126.3, 128.7, 115.9, 120.8, 124.4, 125.3, 148.0, 159.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 21.7; Mass (ESI, 70 eV): m/z 418 $[M+H]^+$, 440 $[M+Na]^+$. HRMS (ESI) m/z: calculated for $C_{21}H_{25}NO_6P$: 418.1341 found: 418.1410.

Diethyl(2-oxo-2H-chromen-3-ylamino)(3,4-dimethoxyphenyl)methylphosphonate ***(3f).*** Yellow semisolid, Yield: 75%, IR (KBr) cm^{-1}: 3402, 2927, 2853, 1710, 1627, 1603, 1594, 1505, 1419, 1251, 1163, 1022, 963, 801, 753. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.28-1.36 (m, 6H), 3.8 (s, 6H), 4.07-4.2 (m, 4H), 4.75 (m, 1H) 5.82 (bs, 1H), 6.3 (s, 1H), 6.85-6.97 (m, 2H), 7.11 (m, 2H), 7.27-7.4 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 16.0, 54.2, 55.6, 63.2, 107.9, 110.2, 110.8, 115.6, 119.8, 120.6, 124.3, 125.1, 126.1, 131.1, 131.2, 147.8, 148.7, 148.9, 158.9; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.8; Mass (ESI, 70 eV): m/z 448 $[M+H]^+$. HRMS (ESI) m/z: calculated for $C_{22}H_{27}NO_7P$: 448.1447 found: 448.1518.

Diethyl(2-oxo-2H-chromen-3-ylamino)(4-hydroxyphenyl)methylphosphonate ***(3g).*** Orange semisolid, Yield: 69%, IR (KBr) cm^{-1}: 3406, 3158, 2925, 2854, 1711, 1625, 1614, 1514, 1504, 1352, 1267, 1227, 1161, 1049, 1024. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.22-1.27 (m, 6H), 4.00-4.19 (m, 4H), 4.63-4.69 (d, 1H), 5.72 (m, 1H), 6.25 (s, 1H), 6.77 (d, 2H), 7.17-7.26 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ14.9, 54.4, 62.1, 112.0, 113.4, 113.9,

114.1, 114.5, 114.7, 120.3, 123.4, 123.7, 124.3, 125.1, 128.1, 130.8, 156.7; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 21.3; Mass (ESI, 70 eV): m/z 404 $[M+H]^+$. HRMS (ESI) m/z: calculated for $C_{20}H_{23}NO_6P$: 404.1185 found: 404.1255.

Diethyl(2-oxo-2H-chromen-3-ylamino) (3-hydroxyphenyl)methylphosphonate ***(3h).*** Light brown semisolid, Yield: 70%, IR (KBr) cm^{-1}: 3421, 2924,2853, 1716, 1603, 1460, 1371, 1231, 1096, 974, 753. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): *δ* 1.24-1.27 (m, 6H), 4.00 (m, 4H), 4.73 (m, 1H), 5.70 (m, 1H), 6.31 (s, 1H) ,6.70 (d, 2H), 6.80-6.87 (m, 4H), 7.14-7.23 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): *δ*16.6, 53.6, 54.5, 65.0, 114.1, 114.4, 116.2, 116.4, 119.4, 125.0, 125.9, 126.9, 129.8, 135.1, 137.8, 157.2, 157.9; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 21.5; Mass (ESI, 70 eV): m/z 404 $[M+H]^+$, 426 $[M+Na]^+$. HRMS (ESI) m/z: calculated for $C_{20}H_{23}NO_6P$: 404.1185 found: 404.1256.

Diethyl(2-oxo-2H-chromen-3-ylamino) (3-nitrophenyl)methylphosphonate ***(3i).*** Light brown semisolid, Yield: 63%, IR (KBr) cm^{-1}: 3352, 2924, 2863, 1709, 1531, 1412, 1385, 1351, 1231, 1216, 1079, 971, 812. ^{1}H NMR (300 MHz, $CDCl_3$, TMS) : *δ* 1.25-1.33 (m, 6H), 4.09-4.16 (m, 4H),5.15-5.18 (m, 1H), 5.79-5.81 (m, 1H), 6.23 (s, 1H),7.13-7.23 (m, 4H),7.53 (m, 1H), 7.67 (d, 1H), 8.10 (m, 1H), 8.21-8.23 (d, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): *δ* 16.2, 55.7, 63.4, 111.1, 115.8, 120.1, 120.8, 124.5, 125.3, 126.0, 126.4, 131.3, 131.4, 148.0, 149.0, 149.2, 159.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 21.0; Mass (ESI, 70 eV): m/z 433 $[M+H]^+$. HRMS (ESI) m/z: calculated for $C_{20}H_{22}N_2O_7P$: 433.1086 found: 433.1157.

Diethyl(2-oxo-2H-chromen-3-ylamino) (4-nitrophenyl)methylphosphonate ***(3j).*** Black semisolid, Yield: 62%, IR (KBr) cm^{-1}: 3421, 2925, 2853, 1716, 1630, 1607, 1523, 1458, 1347, 1248, 1166, 1035, 974. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): *δ* 1.27-1.30 (m, 6H), 4.06-4.11 (m, 4H),4.80-4.86 (m, 1H), 5.85-5.87 (m, 1H), 6.09 (s, 1H) ,7.13-7.14 (d, 2H), 7.23-7.24 (m, 2H), 7.65-7.67 (d, 2H), 8.21-8.23 (d, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, TMS): *δ* 16.02,54.4, 55.6, 108.1, 115.8, 120.1, 123.3, 123.6, 125.2, 126.7, 128.2, 130.8, 138.9, 141.6, 147.6, 147.9, 158.7; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 20.1; MASS (ESI, 70 eV): m/z 433 $[M+H]^+$. HRMS (ESI) m/z: calculated for $C_{20}H_{22}N_2O_7P$: 433.1086 found: 433.1156.

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