Synthesis and α-Glucosidase Inhibitory, DPPH Scavenging Activity of Substituted 2-Oxo-2*H*-chromen-7-yl-dihydrogen Phosphate Derivatives

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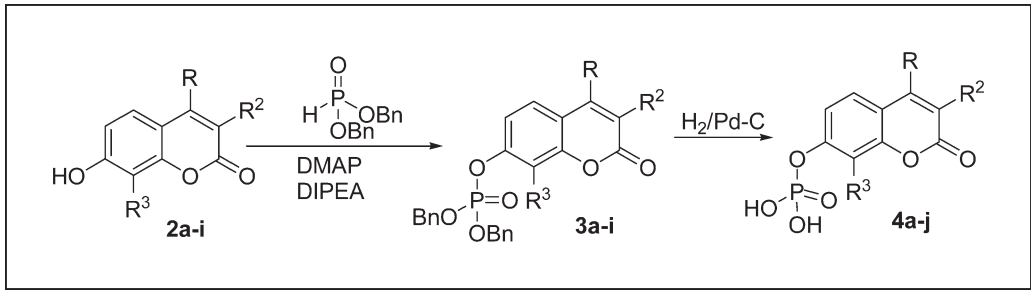

Series of phosphorylated coumarin derivatives (**4a**–**j**) were synthesized by Pechmann condensation, phosphorylation, and debenzylation reactions in very good yields. Thus, synthesized compounds (**4a**–**j**) were evaluated for their α-glucosidase and 1,1-diphenyl-2-picrylhydrazyl scavenging activities; few compounds showed moderate to good activity.

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INTRODUCTION

Among heterocyclic compounds, coumarin (2*H*-1-benzopyran-2-ones) and its derivatives are important compounds present in many biological systems [1]. Among various applications of these coumarins, the pharmaceutical applications are more important. Extensive studies have been done on synthesis of coumarin compounds owing to their wide range of biological activities [2].

Postprandial hyperglycemia (PPHG) remains a serious public health problem of diabetes and cardiovascular disease [3]. PPHG is an exaggerated rise in blood sugar due to excessive intake of carbohydrate rich diet. It also induces overt free radical generation that leads to oxidative damage of biomolecules, which is better defined as postprandial oxidative stress (PPOS). PPOS is associated with higher risk for atherosclerosis, diabetic complications, and obesity [4].

Therefore, combination of agents that reduce PPHG and PPOS may become therapeutics of interest in combating these multiple disorders. This could be achieved either by dietary manipulations or by intestinal α-glucosidase inhibitors that reduce the digestion and absorption of glucose from dietary sources. α-Glucosidase inhibitory drugs such as acarbose, voglibose, and miglitol have shown promise in reducing PPHG, hyperinsulinemea, and burden of PPOS.

We have observed α-glucosidase inhibitory and free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity with 3,4- and 3,6-disubstituted 2*H*-chromenone derivatives [**5b**]. In this communication, we report synthesis of substituted dibenzyl-2-oxo-2*H*-chromen-7-yl-phosphates and substituted 2-oxo-2*H*-chromen-7-yl-dihydrogen phosphate derivatives. The α-glucosidase inhibitory, DPPH scavenging potentials and the structure activity relationship of such moieties were also discussed.

RESULTS AND DISCUSSION

Chemistry. Phosphorylation reaction is an important reaction in the area of pharmaceutical, natural product, pesticide, and synthetic organic chemistry to form oxygen-phosphorous bonds that generate better biologically active molecules. ''For that purpose, we applied Pechmann condensation of resorcinol with ethylacetoacetate in presence of *p*-TsOH using toluene under reflux conditions to afford 7-hydroxy-8-methylcoumarin (**2b**).'' The compound **2b** on phosphorylation using dibenzyl phosphite in presence of *N*,*N*-diisopropylethylamine (DIPEA) with catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) in acetonitrile and dry CCl_4 solvents at −10°C resulted **3b** in 80% yield. The compound **3b** was characterized by spectral data ^{1}H NMR, ^{13}C NMR, IR, Mass,

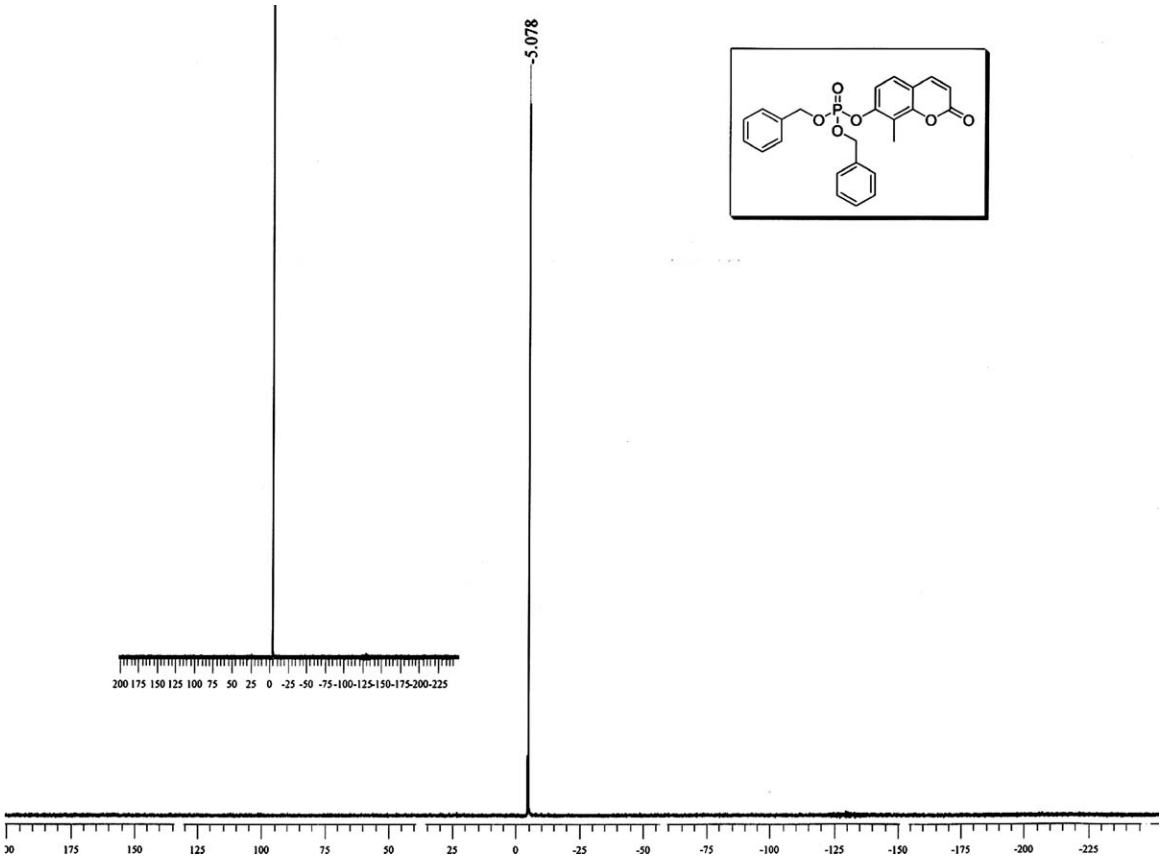
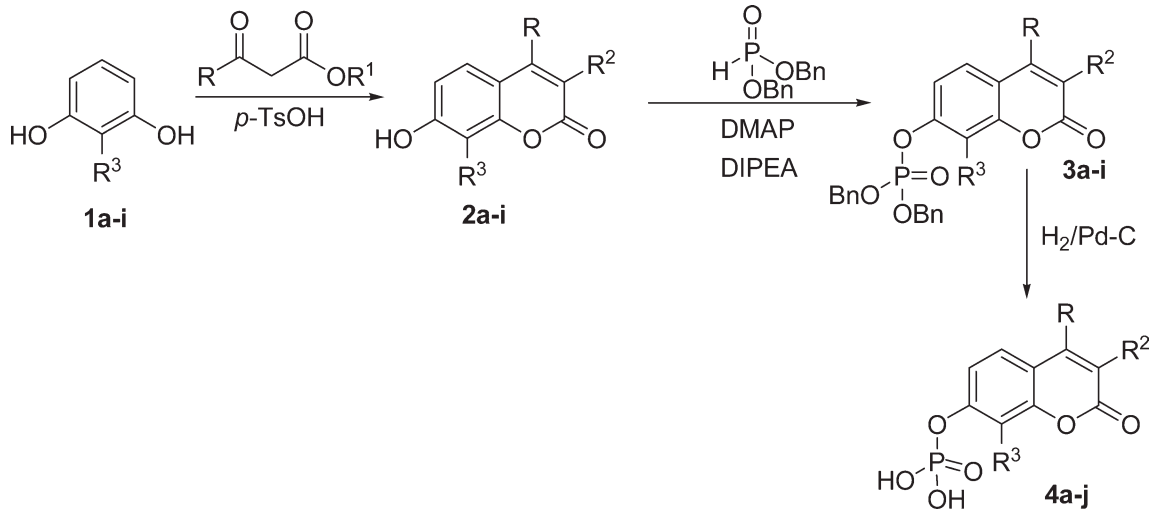


Figure 1. ^{31}P NMR of compound **3b.**

and further confirmed by ^{31}P NMR, where the phosphorous appeared at δ −5.08 ppm (Fig. 1). Thus, obtained compound **3b** was subjected for debenzylation using 10% Pd-C in methanol solvent resulted **4b** in 98% yield (Scheme 1). Compound **4b** was characterized by ^{1}H NMR, ^{13}C NMR, IR, Mass, and ^{31}P (δ −0.02 ppm, Fig. 2) spectral data.

The high yield of compounds encouraged us to synthesize various substituted coumarins **2a–i** followed by phosphorylation reaction that resulted phosphorylated coumarins **3a–i** and subsequent hydrogenation afforded **4a–i** in very good yields. Compound 3,4-dihydro-2-oxo-2*H*-chromen-7-yl-dihydrogen phosphate (**4j**) was obtained when **3a** was

Scheme 1. Synthesis of 2-oxo-2*H*-chromen-7-yl-dihydrogen phosphate derivatives.



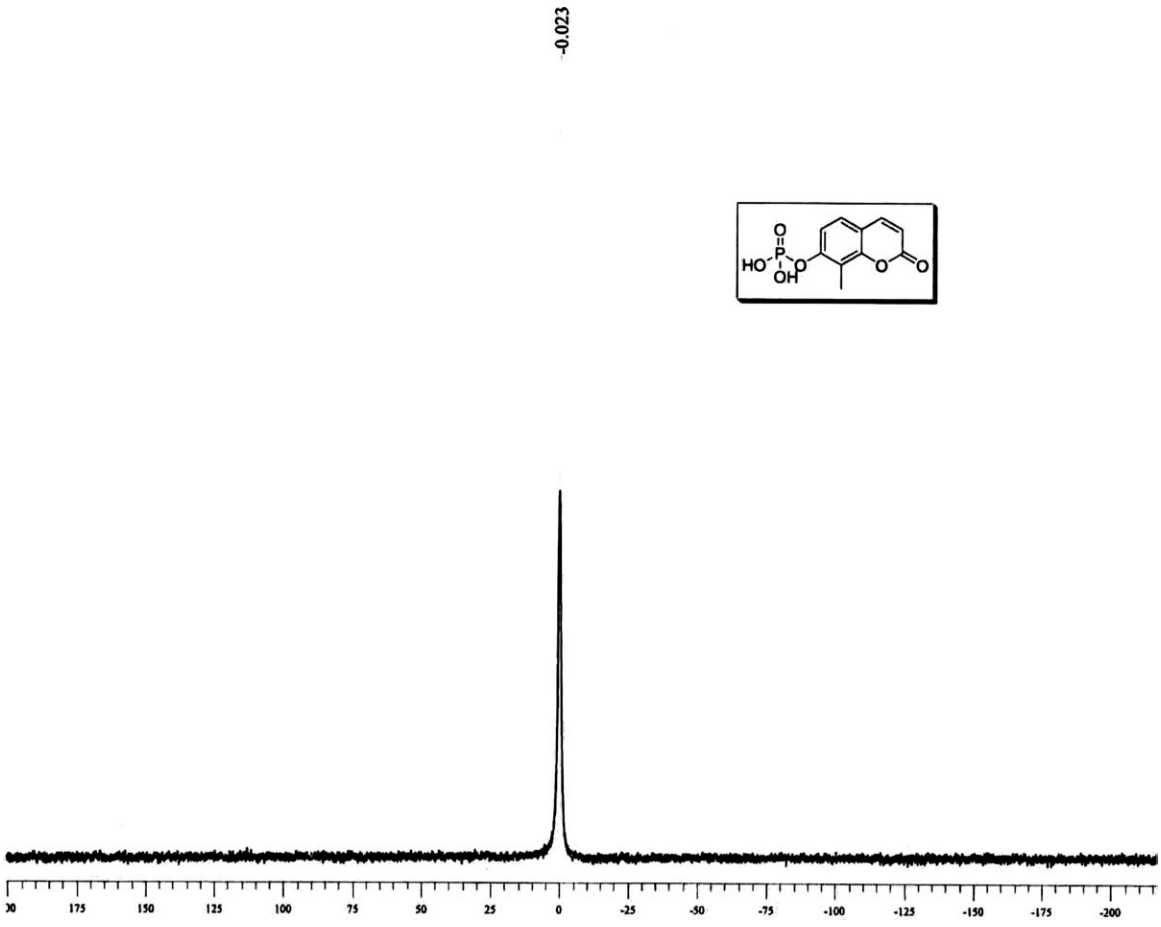


Figure 2. ^{31}P NMR of compound **4b.**

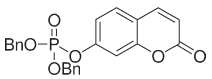
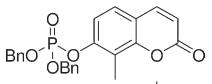
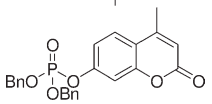
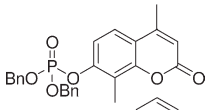
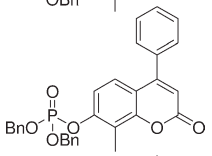
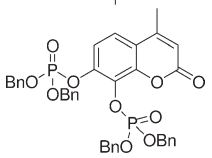
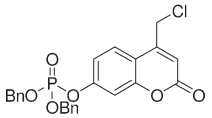
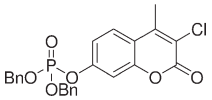
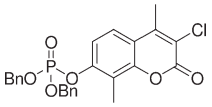
subjected to hydrogenation for 2 h, and the double bond of coumarin **4a** [6] was found reduced. Among the synthesized compounds **3a–i** and **4a–j**, compounds **3a**, **4a** [6], and **4c** [7] are known compounds. Compounds **3a–i** and **4a–j** were screened *in vitro* for α-glucosidase inhibitory and DPPH free radical scavenging activities (Tables 1 and 2).

Biology. DPPH free radical scavenging and rat intestinal α-glucosidase inhibitory activity potentials are presented in Tables 1 and 2. Table 1 describes the substituted dibenzyl-2-oxo-2*H*-chromen-7-yl-phosphate activities of **3a–i**. It was observed that all the compounds displayed similar DPPH free radical scavenging potential at primary concentration of 25 μg/mL. 4-Methyl (**3c**) and 3-chloro-4-methyl (**3h**) substitutions improve the α-glucosidase inhibition activity of **3a**, rather than 8 methyl (**3b**), 4,8-dimethyl (**3d**), 3-chloro-4,8-dimethyl (**3i**), and 4-phenyl-8-methyl (**3e**) derivatives. On the other hand, further phosphorylation on eighth position (**3f**) or 4-chloromethyl (**3g**) could not improve α-glucosidase inhibitory potential. Substitution of chloro at third position (**3h**) significantly improved α-glucosidase inhibition activity.

We could not get the appreciable level of α-glucosidase inhibition with benzylated phosphonocoumarins. Therefore, we made an effort to debenzylate these coumarins (**3a–i**) under hydrogenation conditions to make hydroxyphosphorylated coumarins (**4a–j**, Table 2). To our surprise, the DPPH scavenging activity increased more than 1.5 times, and all the chromenyldihydrogen phosphates displayed better α-glucosidase inhibition than benzylated phosphorylated coumarins. In this case, we also observe that 4-methyl-2-oxo-2*H*-chromen-7-yl-dihydrogen phosphate (**4c**, IC_{50}; 61.76) displayed better α-glucosidase inhibition than methyl at eighth position (**4b**). Substitution of methyl at fourth and eighth position (**4d**, IC_{50}; 94.70) could not influence α-glucosidase activity. However, the compound (**4e**) having phenyl substitution at fourth position and methyl substitution at eighth position appreciably enhanced α-glucosidase inhibitory potential (IC_{50}; 80.05). Absence of eighth methyl (**4g**, **4h**) drastically reduced α-glucosidase inhibition when compared with (**4i**). In contrast to lower α-glucosidase inhibition activity of the bis(dibenzyl ester) **3f**, debenzylated bis(dihydrogenphosphate) **4f** shows

Table 1

Synthesis of dibenzyl 2-oxo-2*H*-chromen-7yl phosphate derivatives and their activity profiles.

Entry	Compound (3)	% DPPH Scavenging	% AGH inhibition (IC_{50}, μ*M*)
a		26.23	0.00
b		24.50	0.00
c		20.32	11.65
d		21.41	0.00
e		26.20	0.00
f		56.59	12.03
g		31.52	9.02
h		21.50	55.59
i		25.84	0.00

% DPPH scavenging activity is based on values obtained with primary screening concentration of 25 μg/mL and that for α-glucosidase inhibition concentration was 100 μg/mL. Values in parentheses represent μ*M* IC_{50} value for the respective compound. AGH; α-glucosidase.

improved enzyme inhibition activity. On the other hand, it is important to note that reduction of chromenone to chromanone (**4j**; 3,4-dihydro derivative of **4a**) displayed lower α-glucosidase inhibition potential.

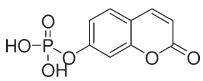
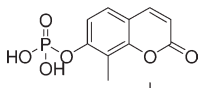
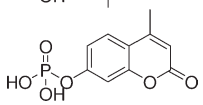
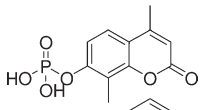
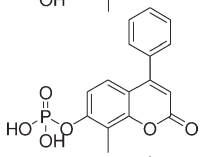
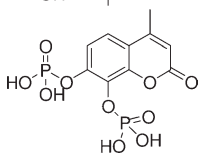
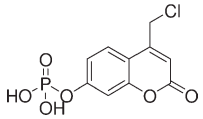
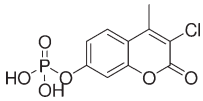
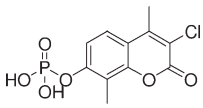
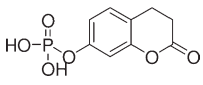
In conclusion, phosphorylated coumarin derivatives **3a–i** were synthesized by the Pechmann condensation and phosphorylation reaction, subsequent debenzylation resulted in **4a–j**. Compound **4e**, which has phenyl at fourth position and methyl at eighth position, displayed better α-glucosidase inhibition with DPPH scavenging activity.

EXPERIMENTAL

^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded on a Varian, Gemini 200, and Avance 300 MHz spectrometer in $CDCl_3$ and CH_3OH-d_4 with TMS as internal standard. IR spectra were recorded on Nicollet 740 FT spectrometer. EI-MS obtained on 7070H spectrometer operating at 70 eV using a

Table 2

Synthesis of 2-oxo-2*H*-chromen-7yl-dihydrogen phosphate derivatives and their activity profiles.

Entry	Compound (4)	% DPPH Scavenging	% AGH inhibition (IC_{50}, μM)
a		45.32	65.54
b		36.82	27.43
c		41.00	61.76 (97.41)
d		27.46	69.15 (94.70)
e		42.24	70.73 (80.05)
f		63.86	66.13 (523.48)
g		31.02	21.27
h		37.63	18.99
i		44.43	66.65 (523.49)
j		46.32	15.09

% DPPH scavenging activity is based on values obtained with primary screening concentration of 25 μg/mL and that for α-glucosidase inhibition concentration was 100 μg/mL. Values in parentheses represent μ*M* IC_{50} value for the respective compound. AGH; α-glucosidase.

direct inlet system. Melting points were determined in open glass capillary tubes on a Metler FP 51 melting point apparatus and are uncorrected. The CHN analyses were recorded on Vario EL analyzer. HRMS were measured on Agilent Technologies 6510, Q-TOFLC/MS ESI-Technique. All reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh); spots were visualized under UV light. Merck silica gel (60–120; 100–200 mesh) was used for chromatography.

General procedure for the synthesis of 4-substituted chromenones (2a–i). Resorcinol (1 mmol), ethylacetoacetate (1.1 mmol), and *p*-TsOH (20 mol %) in dry toluene are refluxed for 20 min. The crude compound on flash chromatography using silica gel gave 4-methyl-7-hydroxy-2*H*-2-chromenone (**2a**) in 90% yield. Similarly, the compounds (**2b** and **2i**) were prepared by using the corresponding β-ketoesters.

General procedure for the synthesis of phosphorylated chromenones (3a–i). The 7-hydroxy-8-methylcoumarin (**2b**, 1 mmol) and the anhydrous acetonitrile (5 mL) are charged to an oven-dried three-necked round-bottom flask fitted with septa containing a stir bar under nitrogen atmosphere. The mixture was cooled to −10°C, $CC1_4$ (10 equiv), DIPEA (2.1 equiv) followed by DMAP (0.1 equiv) and dibenzyl phosphite (1.5 equiv) was added and the contents were stirred at the same temperature for 10 h. After completion of the reaction (TLC), 0.5*M* aqueous KH_2PO_4 (32 mL/100 mL H_2O) was added at 0°C, and the mixture was extracted with ethyl acetate (3 × 30 mL), the organic phase was washed with water and saturated aqueous NaC1, dried over Na_2SO_4, and the crude product was purified by column chromatography using silica gel to give dibenzyl-8-methyl-2-oxo-2*H*-chromen-7-yl-phosphate (**3b**) in 86% yield.

Dibenzyl-8-methyl-2-oxo-2H-chromen-7-yl-phosphate (3b). Semisolid, Yield: 82%. IR (KBr): 3405, 2924, 2855, 1731, 1605, 1492, 1556, 1380, 1246, 1017, 958, 755 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.66 (d, 1H, aromatic), 7.22–7.38 (m, 12H, aromatic), 6.36 (d, 1H, aromatic), 5.12 (d, 4H, OCH_2), 2.26 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 160.59, 153.01, 143.29, 134.95, 128.73, 128.56, 128.48, 128.00, 127.70, 125.57, 116.33, 115.18, 115.02, 70.37, 70.29, 8.76. ^{31}P NMR ($CDCl_3$): −5.07. Mass (LC-MS): *m*/*z* 437 [M+H]. Anal. Cacld. for $C_{24}H_{21}O_6P$: C, 66.05; H, 4.81. Found: C, 66.02; H, 4.85.

Dibenzyl-4-methyl-2-oxo-2H-chromen-7-yl-phosphate (3c). White solid; mp 61–63°C. Yield: 84%. IR (KBr): 3430, 3064, 2924, 1733, 1616, 1389, 1277, 1039, 895 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.50 (d, 1H, aromatic), 7.30−7.42 (m, 10H, aromatic), 7.12 (dd, 1H, aromatic), 7.02 (s, 1H, aromatic), 6.22 (s, 1H, aromatic), 5.14 (d, 4H, OCH_2), 2.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 160.38, 154.12, 152.75, 152.66, 151.80, 134.97, 134.89, 128.76, 128.57, 128.09, 125.64, 117.11, 116.37, 116.31, 114.08, 108.68, 108.61, 70.37, 70.29, 18.62. ^{31}P NMR ($CDCl_3$): −5.38. Mass (LC-MS): *m*/*z* 459 [M+H]. Anal. Cacld. for $C_{24}H_{21}O_6P$: C, 66.05; H, 4.81. Found: C, 65.97; H, 4.82.

Dibenzyl-4,8-dimethyl-2-oxo-2H-chromen-7-yl-phosphate (3d). White solid; mp 94–96°C. Yield: 78%. IR (KBr): 3427, 2925, 1724, 1602, 1378, 1279, 1089, 1037, 890 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.26–7.39 (m, 12H, aromatic), 6.22 (s, 1H, aromatic), 5.14 (d, 4H, $2OCH_2$), 2.42 (s, 3H, CH_3), 2.28 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): 163.22, 155.53, 153.78, 124.74, 123.91, 119.07, 117.66, 113.67, 70.33, 70.24, 18.85, 8.90. Mass (LC-MS): *m*/*z* 451 [M+H]. HRMS (ESI): *m*/*z* calcd for $C_{25}H_{23}O_6P$ $[M+H]^+$ 450.12322, found 450.12252.

Dibenzyl-8-methyl-2-oxo-4-phenyl-2H-chromen-7-yl-phosphate (3e). White solid; mp 86–88°C. Yield: 72%. IR (KBr): 3425, 3057, 2924, 1724, 1600, 1371, 1283, 1017, 925 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.36–7.42 (m, 3H, aromatic), 7.26–7.33 (m, 2H, aromatic), 7.16–7.24 (m, 10H, aromatic), 7.04–7.15 (m, 2H, aromatic), 6.16 (s, 1H, aromatic), 5.04 (d, 4H, OCH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 160.39, 155.52, 135.38, 135.19, 135.10, 129.69, 128.88, 128.76, 128.62, 128.53, 128.39, 128.07, 127.94, 124.84, 116.05, 113.92, 70.33, 70.26, 9.16. ^{31}P NMR ($CDCl_3$): δ −5.075. Mass (LC-MS): *m*/*z* 513 [M+1]. HRMS (ESI): *m*/*z* calcd for $C_{30}H_{25}O_6P$ $[M+H]^+$ 512.13888, found 512.13972.

Tetrabenzyl-4-methyl-2-oxo-2H-chromene-7,8-diyl-diphosphate (3f). Semisolid, Yield: 68%. IR (KBr): 3064, 2956, 2924, 2855, 2337, 1736, 1611, 1574, 1450, 1382, 1280, 1013, 917, 871, 738, 694 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.21–7.44 (m, 22H, aromatic), 6.26 (s, 1H, aromatic), 5.34 (d, 4H, OCH_2), 5.08 (d, 4H, OCH_2), 2.42 (s, 3H, CH_3). Mass (LC-MS): *m*/*z* 713 [M+H]. Anal. Cacld. for $C_{38}H_{34}O_{10}P_2$: C, 64.05; H, 4.81. Found: C, 64.10; H, 4.78.

Dibenzyl-4-(chloromethyl-2-oxo-2H-chromen-7-yl-phosphate (3g). Semisolid, Yield: 70%. IR (KBr): 3413, 2924, 1713, 1655, 1607, 1449, 1239, 926 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.50 (d, 1H, aromatic), 7.21–7.32 (m, 10H, aromatic), 7.06 (dd, 1H, aromatic), 6.96 (d, 1H, aromatic), 6.42 (s, 1H, aromatic), 5.09 (d, 4H, OCH_2), 4.55 (s, 2H, CH_2Cl). Mass (LC-MS): *m*/*z* 471 [M+H]. Anal. Cacld. for $C_{24}H_{20}ClO_6P$: C, 61.27; H, 4.25. Found: C, 61.18; H, 4.27.

Dibenzyl-3-chloro-4-methyl-2-oxo-2H-chromen-7-yl-phosphate (3h). Semisolid, Yield: 70%. IR (KBr): 3421, 2924, 2855, 1729, 1612, 1552, 1275, 1146, 1016, 880, 730 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.52 (d, 1H, aromatic), 7.28–7.38 (m, 10H, aromatic), 7.12 (dd, 1H, aromatic), 7.02 (d, 1H, aromatic), 5.14 (d, 4H, OCH_2), 2.59 (s, 3H, CH_3). Mass (LC-MS): *m*/*z* 471 [M+H]. Anal. Cacld. for $C_{24}H_{20}ClO_6P$: C, 61.27; H, 4.25. Found: C, 61.25; H, 4.32.

Dibenzyl-3-chloro-4,8-dimethyl-2-oxo-2H-chromen-7-yl-phosphate (3i). Semisolid, Yield: 76%. IR (KBr): 3391, 2927, 1687, 1603, 1386, 1278, 1110, 1037, 872 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.24–7.39 (m, 12H, aromatic), 5.15 (d, 4H, OCH_2), 2.58 (s, 3H, CH_3), 2.28 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 147.54, 128.78, 128.60, 128.49, 128.04, 127.63, 122.60, 116.65, 70.43, 70.34, 16.32, 9.02. Mass (LC-MS): *m*/*z* 485 [M+H]. Anal. Cacld. for $C_{25}H_{22}ClO_6P$: C, 61.98; H, 4.54. Found: C, 61.92; H, 4.59.

General procedure for the debenzylation of phosphorylated chromenones (4a–i). Dibenzyl-8-methyl-2-oxo-2*H*-chromen-7-yl-phosphate (**3b**, 1 mmol) in anhydrous methanol (5 mL) was charged to an oven-dried two-necked round-bottom flask fitted with septa containing a stir bar under nitrogen atmosphere. The Pd-C (10%) was charged, equipped with hydrogen balloon, and the contents were stirred at room temperature for 0.5–1 h. After completion of the reaction (TLC), the mixture was filtered through celite and washed with methanol (3 × 30 mL), the organic layer was dried over Na_2SO_4, and the crude product was purified by flash column chromatography using silica gel (60:120) to give 8-methyl-2-oxo-2*H*-chromen-7-yl-dihydrogen phosphate (**4b**) in 92% yield.

8-Methyl-2-oxo-2H-chromen-7-yl-dihydrogen phosphate (4b). Semisolid, Yield: 98%. IR (KBr): 3392, 2928, 1696, 1605, 1492, 1243, 1165, 1087, 971, 841 cm^{-1}. ^1H NMR

(MeOH-d_4): δ 7.76–7.82 (m, 1H, aromatic), 7.18–7.40 (m, 2H, aromatic), 6.28 (d, 1H, aromatic), 2.30 (s, 3H, CH_3). ^{13}C NMR (MeOH-d_4): δ 162.93, 154.36, 154.18, 145.80, 127.17, 119.13, 117.94, 116.88, 115.14, 8.90. ^{31}P NMR (MeOH-d_4): δ −0.023. Mass (LC-MS): *m/z* 255 [M−H]. HRMS (ESI): *m/z* calcd for $C_{10}H_9O_6P$ $[M+H]^+$ 256.0136, found 256.0141.

4,8-Dimethyl-2-oxo-2H-chromen-7-yl-dihydrogen phosphate (4d). White solid; mp 122–124°C. Yield: 98%. IR (KBr): 3422, 2928, 1686, 1602, 1384, 1276, 1036, 872 cm^{-1}. ^1H NMR (MeOH-d_4): δ 7.58 (s, 1H, aromatic), 7.30 (s, 1H, aromatic), 6.36 (s, 1H, aromatic), 2.48 (s, 3H, CH_3), 2.38 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 163.13, 155.61, 154.18, 153.74, 124.71, 123.90, 119.08, 117.67, 113.63, 18.85, 9.18. ^{31}P NMR (MeOH-d_4): δ −0.023. Mass (LC-MS): *m/z* 269 [M−H]. HRMS (ESI): *m/z* calcd for $C_{11}H_{11}O_6P$ $[M+H]^+$ 270.02932, found 270.03.

8-Methyl-2-oxo-4-phenyl-2H-chromene-7-yl-dihydrogen phosphate (4e). Semisolid, Yield: 98%. ^1H NMR (MeOH-d_4): δ 7.64 (d, 1H, aromatic), 6.22–6.40 (m, 6H, aromatic), 6.02 (s, 1H, aromatic), 2.52 (s, 3H, CH_3). Mass (LC-MS): *m/z* 333 [M+H]. Anal. Cacld. for $C_{16}H_{13}O_6P$: C, 57.84; H, 3.94. Found: C, 57.79; H, 4.04.

4-Methyl-2-oxo-2H-chromen-7,8-diyl-bis(dihydrogen phosphate) (4f). Semisolid, Yield: 98%. IR (KBr): 3449, 2924, 1728, 1604, 1379, 1267, 1217, 1019, 912, 760 cm^{-1}. ^1H NMR (MeOH-d_4): δ 7.18–7.41 (m, 1H, aromatic), 6.50–6.34 (m, 1H, aromatic), 6.10 (s, 1H, aromatic), 2.24 (s, 3H, CH_3). Mass (LC-MS): *m/z* 351 [M−H]. Anal. Cacld. for $C_{10}H_{10}O_{10}P_2$: C, 34.28; H, 2.85. Found: C, 34.19; H, 2.89.

4-(Chloromethyl)-2-oxo-2H-chromene-7-yl-dihydrogen phosphate (4g). Solid; mp 197–199°C. Yield: 98%. IR (KBr): 3426, 2925, 2855, 2309, 1679, 1614, 1393, 1247, 1168, 1043, 967 cm^{-1}. ^1H NMR (MeOH-d_4): δ 7.60 (d, 1H, $J = 6.61$ Hz, aromatic), 7.17–7.29 (m, 2H, aromatic), 6.26 (s, 1H, aromatic). Mass (LC-MS): *m/z* 289 [M−H]. Anal. Cacld. for $C_{10}H_8ClO_6P$: C, 41.37; H, 2.75. Found: C, 41.39; H, 2.69.

3-Chloro-4-methyl-2-oxo-2H-chromen-7-yl-dihydrogen phosphate (4h). Semisolid, Yield: 98%. IR (KBr): 3436, 2924, 2854, 1706, 1614, 1389, 1268, 1069, 758 cm^{-1}. ^1H NMR (MeOH-d_4): δ 7.74 (s, 1H, aromatic), 7.12-7.24 (m, 1H, aromatic), 6.26 (s, 1H, aromatic), 2.50 (s, 3H, CH_3). Mass (LC-MS): *m/z* 289 [M−H]. Anal. Cacld. for $C_{10}H_8ClO_6P$: C, 41.37; H, 2.75. Found: C, 41.29; H, 2.81.

3-Chloro-4,8-dimethyl-2-oxo-2H-chromen-7-yl-dihydrogen phosphate (4i). Semisolid, Yield: 98%. ^1H NMR (MeOH-d_4): δ 7.26 (s, 1H, aromatic), 6.38 (s, 1H, aromatic), 2.48 (s, 3H, CH_3), 2.38 (s, 3H, CH_3). Mass (LC-MS): *m/z* 327 [M+Na]. Anal. Cacld. for $C_{11}H_{10}ClO_6P$: C, 43.42; H, 3.28. Found: C, 43.46; H, 3.35.

3,4-Dihydro-2-oxo-2H-chromen-7-yl-dihydrogen phosphate (4j). Semisolid, Yield: 92%. IR (KBr): 3447, 2925, 2855, 1633, 1459, 1216, 760 cm^{-1} ^1H NMR (MeOH-d_4): δ 7.02 (d, 1H, aromatic), 6.54–6.72 (m, 2H, aromatic), 2.58 (t, 2H, CH_2), 2.86 (t, 2H, CH_2). ^{13}C NMR (MeOH-d_4): δ 175.70, 157.13, 131.30, 124.39, 112.05, 108.35, 115.13, 34.95, 26.56. Mass (LC-MS): *m/z* 245 [M+H]. Anal. Cacld. for $C_9H_9O_6P$: C, 44.26; H, 3.68. Found: C, 44.22; H, 3.71.

Biological activity: α-Glucosidase inhibitory assay. α-Glucosidase inhibitory activity was determined as reported earlier [5]. Rat intestinal acetone powder in normal saline (100:1; w/v) was sonicated properly, and the supernatant was used as a source of crude intestinal α-glucosidase after centrifugation.

In brief, 10 μL of test samples (5 mg/mL DMSO solution) were reconstituted in 100 μL of 100 m*M*-phosphate buffer (pH 6.8) in 96-well microplate and incubated with 50 μL of crude intestinal α-glucosidase for 5 min before 50-μL substrate (5 m*M*, *p*-nitrophenyl-α-D-glucopyranoside prepared in same buffer) was added. Release of *p*-nitrophenol was measured at 405-nm spectrophotometrically (SpectraMax plus 384, Molecular Devices, Sunnyvale, CA) 5 min after incubation with substrate. Individual blanks for test samples were prepared to correct background absorbance where substrate was replaced with 50 μL of buffer. Control sample contained 10-μL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as $(1 - B/A) \times 100$ where [*A*] represents absorbance of control without test samples, and [*B*] represents absorbance in presence of test samples.

DPPH free radical scavenging activity. Assay for the scavenging of stable free radical DPPH was done as reported earlier [5]. Briefly, in a 96-well microplate, 25 μL of test sample dissolved in DMSO (1 mg/mL), 100 μL of 0.1*M* tris-HCl buffer (pH 7.4), and 125 μL of 0.5 m*M* DPPH solution dissolved in absolute ethyl alcohol were added. The reaction mixture was shaken well and incubated in dark for 30 min and read at 517 nm spectrophotometrically (SpectraMax plus 384, Molecular Devices, Sunnyvale, CA). Percentage of DPPH scavenging was calculated as $(1 - B/A) \times 100$ where *A* represents absorbance of control without test samples, and *B* represents absorbance in presence of test samples.

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