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Synthesis, *in vitro* evaluation, and docking studies of novel chromone derivatives as HIV-1 protease inhibitor

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ABSTRACT

Novel chromone derivatives with a benzopyran-4-one scaffold have been prepared by the one-pot cyclization reaction. The *in vitro* inhibitory activity of these new compounds towards HIV-1 protease have been evaluated using stop time HPLC method as the preliminary screening. The most potent compound, 7,8-dihydroxy-2-(3′-trifluoromethyl phenyl)-3-(3″-trifluoromethylbenzoyl)chromone (**32**), showed IC_{50} = 0.34 μM. The molecular docking study supported results from experimental activity testing and also provided structure–activity relationship of this series.

1. Introduction

HIV-1 protease (HIV-1 PR), a member of aspartic protease superfamily, is an important target for the design of specific antiviral agents dedicated to the treatment of HIV-1 infection and acquired immunodeficiency syndrome (AIDS) [1–3]. It plays an important role in the replication and maturation of infectious viral particles since it is responsible for the processing of *gag* and *gag-pol* polyprotein precursors into active viral structural proteins and replicative enzymes such as reverse transcriptase, protease, and integrase [4]. Inhibitors of the HIV-1 PR (amprenavir, atazanavir, darunavir, indinavir, fosamprenavir, lopinavir, nelfinavir, ritonavir, saquinavir and tipranavir) have been approved by the United States Food and Drug Administration (US FDA) and are currently in use in combination with reverse transcriptase inhibitors [5,6]. Despite combination chemotherapy, resistant variants have developed with reduced sensitivity to these inhibitors [7]. Therefore, there is still need for new and improved drugs against HIV-1 PR due to increasing viral resistance. Most of the HIV-1 PR inhibitors reported are peptidomimetic, *e.g.*, saquinavir, indinavir, ritonavir, etc [8,9]. The major disadvantages of peptidomimetic compounds are low bioavailability due to their high molecular weight, poor solubility, and synthetic difficulties. Therefore, efforts have been concentrated on developing nonpeptidic compounds.

One of the most intriguing small molecule, nonpeptidic HIV PR inhibitors was warfarin (**I**) with IC_{50} = 30 mM (Fig. 1) [10]. A low molecular weight template is very compelling. Focused screening based on this result revealed the 4-hydroxy coumarin derivative, phenprocoumon (**II**), exhibited improved enzymatic inhibitory activity with Ki value of 1 mM [10]. Compounds such as 4-hydroxy-5,6-dihydropyrone series (**III**) have been reported as potent HIV-1 PR inhibitor with IC_{50} in the nanomolar range [11,12]. In our search for new series of potent HIV-1 PR inhibitors, we focused on chromone derivatives whose general structure as shown in Fig. 2.

2. Experimental

2.1. Physical measurements

All melting points were determined on an Electrothermal model 9100 capillary melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on an ADVANCE 300 MHz Digital NMR Spectrophotometer (Bruke Switzerland DPX-3000). Chemical shifts were reported in ppm relative to the internal standard, tetramethylsilane (TMS). The NMR solvent used was deuterated dimethylsulfoxide (DMSO, δ = 2.54 ppm). FAB mass spectra were determined on a MAT 90 (Finnigan) mass spectrometer and EI mass spectra using INCOS 50. Elemental analyses were performed by CHNS/O analyzer (Perkin Elmer PE2400 series II). Silica gel, E. Merck (70–230 mesh), was used for column chromatography.

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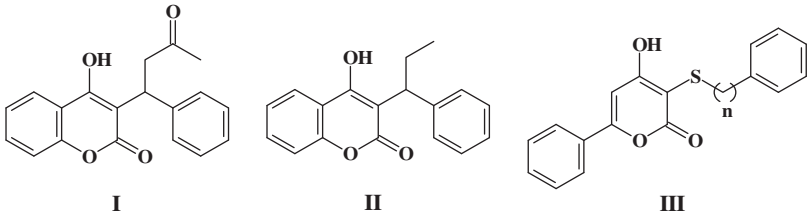


Fig. 1. Nonpeptidic HIV PR inhibitors.

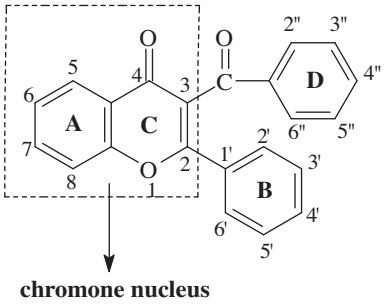


Fig. 2. General structure of synthesized chromone derivatives.

2.2. General procedure

To a stirred solution of phenolic ketone (1 mmol) in pyridine (20 mL) was slowly added the appropriate acid chloride (1.5–2 equivalents) and then DBU (1,8-diazabicyclo[5,4,0] undec-7-ene) (2 equivalents) in small portions. The reaction mixture was refluxed at 120–140 °C for 24 h and pyridine was evaporated *in vacuo*. The mixture was poured into 5 mL of concentrated HCl in 100 mL water and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (2 × 50 ml), dried over anhydrous sodium sulfate, and filtered. After evaporation, the crude product was hydrolyzed by the mixture of dioxane (2 mL), methanol (4 mL), water (1 mL) and 10% NaOH (2 mL), the mixture was stirred at room temperature for 3–4 h. The mixture was acidified with 4 N HCl and poured into 50 mL water, and then extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (2 × 50 ml), dried over anhydrous sodium sulfate, and filtered. After evaporation, the crude product was purified by column chromatography using mixture of EtOAc and hexane as eluent.

2.2.1. 8-Hydroxy-2-phenylchromone (**1**)

Yield 22.4%; white solid; mp 218–219 °C. ^{1}H NMR: 6.21 (s, 1H, H3), 6.85–6.88 (m, 2H, H6, H7), 7.57 (t, *J* = 7.51 Hz, 2H, H3′, H5′), 7.71 (t, *J* = 7.51 Hz, 1H, H4″), 7.96 (d, *J* = 9.32 Hz, 1H, H5), 8.07 (d, *J* = 7.44 Hz, 2H, H2′, H6′). MS *m*/*z* 238 $(M)^+$. Anal. Calcd. for $C_{15}H_{10}O_3$ (238.25 g/mol): C, 75.61; H, 4.24; O, 20.15. Found: C, 75.39; H, 4.34; O, 20.27.

2.2.2. 7,8-Dihydroxy-2-methylchromone (**2**)

Yield 19.2%; yellow crystal; mp 239–242 °C. ^{1}H NMR: 2.43 (s, 3H, CH_3), 6.49 (s, 1H, H3), 6.91 (d, *J* = 8.76 Hz, 1H, H6), 7.36 (d, *J* = 8.76 Hz, 1H, H5). MS *m*/*z* 194 $(M+2H)^+$. Anal. Calcd. for $C_{10}H_8O_4{\cdot}0.25H_2O$ (196.68 g/mol): C, 61.06; H, 4.36; O, 34.58. Found: C, 61.00; H, 4.27; O, 34.73.

2.2.3. 7,8-Dihydroxy-2-benzylchromone (**3**)

Yield 24.3%; yellow crystal; mp 259–260 °C. ^{1}H NMR: 2.14 (s, 2H, CH_2Ph), 6.15 (s, 1H, H3), 6.81 (d, *J* = 8.74 Hz, 1H, H6), 7.14 (d, *J* = 8.74 Hz, 1H, H5), 7.20 (d, *J* = 8.24 Hz, 2H, H2′, H6′), 7.32–7.43 (m, 3H, H3′, H4′, H5′). MS *m*/*z* 268 $(M)^+$. Anal. Calcd. for $C_{16}H_{12}O_4$ (268.28 g/mol): C, 71.63; H, 4.52; O, 23.85. Found: C, 71.39; H, 4.27; O, 23.82.

2.2.4. 7,8-Dihydroxy-2-pheny chromone (**4**)

Yield 44.9%; yellow crystal; mp 191–192 °C. ^{1}H NMR: 6.79 (d, *J* = 2.56 Hz, 1H, H3), 6.94 (d, *J* = 8.72 Hz, 1H, H6), 7.39 (d, *J* = 8.72 Hz, 1H, H5), 7.52–7.56 (m, 3H, H3′, H4′, H5′), 8.01–8.08 (m, 2H, H2′, H6′). MS *m*/*z* 255 $(M+H)^+$. Anal. Calcd. for $C_{15}H_{10}O_4$ (254.25 g/mol): C, 70.86; H, 3.97; O, 25.17. Found: C, 70.59; H, 3.93; O, 25.18.

2.2.5. 7-Hydroxy-2-methylchromone (**5**)

Yield 21.7%; yellow crystal; mp 205–206 °C. ^{1}H NMR: 2.47 (s, 3H, CH_3), 6.22 (s, 1H, H3), 6.49 (d, *J* = 2.37 Hz, 1H, H8), 6.72 (dd, *J* = 8.62, 2.37 Hz, 1H, H6), 7.79 (d, *J* = 8.62 Hz, 1H, H5). MS *m*/*z* 176 $(M)^+$. Anal. Calcd. for $C_{10}H_8O_3{\cdot}H_2O$ (194.20 g/mol): C, 61.84; H, 5.20; O, 32.96. Found: C, 61.98; H, 5.03; O, 32.99.

2.2.6. 7-Hydroxy-2-benzylchromone (**6**)

Yield 42.2%; white crystal; mp 228–229 °C. ^{1}H NMR: 2.15 (s, 2H, CH_2Ph), 6.17 (s, 1H, H3), 6.71 (d, *J* = 2.44 Hz, 1H, H8), 6.80 (dd, *J* = 8.80, 2.44 Hz, 1H, H6), 7.20 (d, *J* = 8.12 Hz, 2H, H2′, H6′), 7.32–7.44 (m, 3H, H3′, H4′, H5′), 7.62 (d, *J* = 8.80 1H, H5). MS *m*/*z* 252 $(M)^+$. Anal. Calcd. for $C_{16}H_{12}O_3$ (252.28 g/mol): C, 76.17; H, 4.80; O, 19.03. Found: C, 76.19; H, 4.71; O, 19.10.

2.2.7. 7-Hydroxy-2-benzyl-3-methylchromone (**7**)

Yield 27.3%; yellow crystal; mp 231–232 °C. ^{1}H NMR: 1.05 (s, 3H, CH_3), 2.26 (s, 2H, CH_2Ph), 6.74 (s, 1H, H8), 6.81 (d, *J* = 8.83 Hz, 1H, H6), 7.18 (d, *J* = 7.14 Hz, 2H, H2′, H6′), 7.36–7.42 (m, 3H, H3′, H4′, H5′), 7.65 (d, *J* = 8.83 Hz, 1H, H5). MS *m*/*z* 266 $(M)^+$. Anal. Calcd. for $C_{17}H_{14}O_3$ (266.31 g/mol): C, 76.67; H, 5.31; O, 18.02. Found: C, 76.31; H, 4.97; O, 18.72.

2.2.8. 7-Hydroxy-2-phenylchromone (**8**)

Yield 48.6%; yellow crystal; mp 246–247 °C. ^{1}H NMR: 6.04 (s, 1H, H3), 6.33 (d, *J* = 2.25 Hz, 1H, H8), 6.48 (dd, *J* = 8.76, 2.25 Hz, 1H, H6), 7.34–7.46 (m, 5H, H2′, H3′, H4′, H5′, H6′), 7.60 (d, *J* = 8.76 Hz, 1H, H5). MS *m*/*z* 239 $(M+H)^+$. Anal. Calcd. for $C_{15}H_{10}O_3$ (238.25 g/mol): C, 75.61; H, 4.24; O, 20.15. Found: C, 75.60; H, 4.22; O, 20.18.

2.2.9. 7-Hydroxy-3-methyl-2-phenylchromone (**9**)

Yield 29.5%; yellow crystal; mp 268–269 °C. ^{1}H NMR: 1.94 (s, 3H, CH_3), 6.81 (d, *J* = 2.18 Hz, 1H, H8), 6.89 (dd, *J* = 8.78, 2.18 Hz, 1H, H6), 7.52–7.54 (m, 3H, H3′, H4′, H5′), 7.62–7.64 (m, 2H, H2′, H6′), 7.88 (d, *J* = 8.78 Hz, 1H, H5). MS *m*/*z* 252 $(M+H)^+$. Anal. Calcd. for $C_{16}H_{12}O_3$ (252.28 g/mol): C, 76.17; H, 4.80; O, 19.03. Found: C, 76.19; H, 4.76; O, 19.05.

2.2.10. 7-Hydroxy-2-(4′-nitrophenyl)chromone (**10**)

Yield 12.1%; yellow crystal; mp 298–299 °C. ^{1}H NMR: 6.95 (dd, *J* = 8.76, 2.17 Hz, 1H, H6), 7.02 (d, *J* = 2.17 Hz, 1H, H8), 7.10 (s, 1H,

H3), 7.90 (d, J = 8.76 Hz, 1H, H5), 8.31–8.39 (m, 4H, H2′, H3′, H5′, H6′). MS m/z 283 $(M)^+$. Anal. Calcd. for $C_{15}H_9NO_5{\cdot}0.25H_2O$ (287.49 g/mol): C, 62.67; H, 3.24; N, 4.87. Found: C, 62.87; H, 3.32; N, 4.75.

*2.2.11. 7-Hydroxy-2-(3′-trifluoromethylphenyl)chromone (**11**)*

Yield 42.9%; yellow crystal; mp 258–260 °C. ^{1}H NMR: 6.94 (dd, J = 8.85, 2.43 Hz, 1H, H6), 7.06 (d, J = 2.43 Hz, 1H, H8), 7.09 (s, 1H, H3), 7.80 (t, J = 7.96 Hz, 1H, H5′), 7.88 (d, J = 8.85 Hz, 1H, H5), 7.95 (d, J = 7.96 Hz, 1H, H6′), 8.33–8.40 (m, 2H, H2′, H4′). MS m/z 306 $(M)^+$. Anal. Calcd. for $C_{16}H_9F_3O_3$ (306.24 g/mol): C, 62.75; H, 2.96. Found: C, 62.63; H, 3.09.

*2.2.12. 7-Hydroxy-2-(4′-fluorophenyl)chromone (**12**)*

Yield 41.6%; yellow crystal; mp 247–248 °C. ^{1}H NMR: 6.87 (s, 1H, H3), 6.92 (dd, J = 8.76, 2.19 Hz, 1H, H6), 7.00 (d, J = 2.19 Hz, 1H, H8), 7.40 (t, J = 8.83 Hz, 2H, H3′, H5′), 7.88 (d, J = 8.76 Hz, 1H, H5), 8.12 (dd, J = 8.83, 5.23 Hz, 2H, H2′, H6′). MS m/z 256 $(M)^+$. Anal. Calcd. for $C_{15}H_9FO_3{\cdot}0.25H_2O$ (260.73 g/mol): C, 69.10; H, 3.67. Found: C, 69.10; H, 3.54.

*2.2.13. 7-Hydroxy-2-(3′,5′-dinitrophenyl)chromone (**13**)*

Yield 22.1%; yellow crystal; mp 347–348 °C. ^{1}H NMR: 6.95 (dd, J = 8.75, 2.16 Hz, 1H, H6), 7.10 (d, J = 2.16 Hz, 1H, H8), 7.32 (s, 1H, H3), 7.90 (d, J = 8.75 Hz, 1H, H5), 8.95 (t, J = 1.95 Hz, 1H, H4′), 9.14 (d, J = 1.95 Hz, 2H, H2′, H6′). MS m/z 328 $(M)^+$. Anal. Calcd. for $C_{15}H_8N_2O_7$ (328.23 g/mol): C, 54.89; H, 2.49; N, 8.53. Found: C, 54.91; H, 2.27; N, 8.46.

*2.2.14. 7-Hydroxy-2-(3′-chlorophenyl)chromone (**14**)*

Yield 16.3%; yellow crystal; mp 268–269 °C. ^{1}H NMR: 6.92 (dd, J = 8.64, 2.08 Hz, 1H, H6), 6.99 (s, 1H, H3), 7.04 (d, J = 2.08 Hz, 1H, H8), 7.58 (t, J = 7.33 Hz, 1H, H5′), 7.65 (d, J = 7.33 Hz, 1H, H4′), 7.89 (d, J = 8.64 Hz, 1H, H5), 8.03 (d, J = 7.33 Hz, 1H, H6′), 8.14 (s, 1H, H2′). MS m/z 272 $(M)^+$. Anal. Calcd. for $C_{15}H_9ClO_3$ (272.68 g/mol): C, 66.07; H, 3.33. Found: C, 66.08; H, 3.22.

*2.2.15. 7-Hydroxy-2-(3′,4′-dichlorophenyl)chromone (**15**)*

Yield 20.3%; yellow crystal; mp 285–286 °C. ^{1}H NMR: 6.91 (dd, J = 8.48, 2.50 Hz, 1H, H6), 7.01 (s, 1H, H3), 7.03 (d, J = 2.50 Hz, 1H, H8), 7.82 (d, J = 8.72 Hz, 1H, H5′), 7.88 (d, J = 8.48 Hz, 1H, H5), 8.05 (dd, J = 8.72, 2.01 Hz, 1H, H6′), 8.34 (d, J = 2.01 Hz, 1H, H2′). MS m/z 308 $(M+2H)^+$. Anal. Calcd. for $C_{15}H_8Cl_2O_3$ (307.13 g/mol): C, 58.66; H, 2.63. Found: C, 58.50; H, 2.55.

*2.2.16. 7-Hydroxy-2-(4′-tert-butylphenyl)chromone (**16**)*

Yield 44.7%; yellow crystal; mp 271–272 °C. ^{1}H NMR: 1.35 (s, 9H, $C(CH_3)_3$), 6.83 (s, 1H, H3), 6.92 (dd, J = 8.64, 2.18 Hz, 1H, H6), 6.98 (d, J = 2.18 Hz, 1H, H8), 7.57 (d, J = 8.38 Hz, 2H, H3′, H5′), 7.88 (d, J = 8.64 Hz, 1H, H5), 7.97 (d, J = 8.38 Hz, 2H, H2′, H6′). MS m/z 294 $(M)^+$. Anal. Calcd. for $C_{19}H_{18}O_3{\cdot}0.25H_2O$ (298.85 g/mol): C, 76.43; H, 6.19. Found: C, 76.77; H, 5.68.

*2.2.17. 5,7-Dihydroxy-2-(3′-trifluoromethylphenyl)chromone (**17**)*

Yield 70.1%; yellow crystal; mp 280–281 °C. ^{1}H NMR: 6.21 (d, J = 2.18 Hz, 1H, H6), 6.58 (d, J = 2.18 Hz, 1H, H8), 7.17 (s, 1H, H3), 7.81 (t, J = 7.95 Hz, 1H, H5′), 7.98 (d, J = 7.95 Hz, 1H, H6′), 8.33–8.39 (m, 2H, H2′, H4′). MS m/z 322 $(M)^+$. Anal. Calcd. for $C_{16}H_9F_3O_4$ (322.24 g/mol): C, 59.64; H, 2.82. Found: C, 59.65; H, 2.70.

*2.2.18. 5,7-Dihydroxy-2-(4′-fluorophenyl)chromone (**18**)*

Yield 71.0%; yellow crystal; mp 270–271 °C. ^{1}H NMR: 6.21 (d, J = 2.02 Hz, 1H, H6), 6.52 (d, J = 2.02 Hz, 1H, H8), 6.98 (s, 1H, H3), 7.41 (t, J = 8.88 Hz, 2H, H3′, H5′), 8.14 (dd, J = 8.88, 5.25 Hz, 2H, H2′, H6′). MS m/z 272 $(M)^+$. Anal. Calcd. for $C_{15}H_9FO_4{\cdot}0.50H_2O$ (280.73 g/mol): C, 64.18; H 3.41. Found: C, 64.31; H, 3.12.

*2.2.19. 5,7-Dihydroxy-2-(3′,4′-difluorophenyl)chromone (**19**)*

Yield 26.1%; yellow crystal; mp 359–360 °C. ^{1}H NMR: 6.21 (d, J = 2.03 Hz, 1H, H6), 6.55 (d, J = 2.03 Hz, 1H, H8), 7.04 (s, 1H, H3), 7.58–7.70 (m, 1H, H5′), 7.93–8.00 (m, 1H, H2′), 8.17–8.29 (m, 1H, H6′). MS m/z 290 $(M)^+$. Anal. Calcd. for $C_{15}H_8F_2O_4{\cdot}0.25H_2O$ (294.72 g/mol): C, 61.13; H, 2.91. Found: C, 61.09; H, 2.88.

*2.2.20. 5,7-Dihydroxy-2-(4′-tert-butylphenyl)chromone (**20**)*

Yield 35.4%; yellow crystal; mp 263–264 °C. ^{1}H NMR: 1.35 (s, 9H, $C(CH_3)_3$), 6.21 (d, J = 2.04 Hz, 1H, H6), 6.50 (d, J = 2.04 Hz, 1H, H8), 6.91 (s, 1H, H3), 7.59 (d, J = 8.50 Hz, 2H, H3′, H5′), 7.98 (d, J = 8.50 Hz, 2H, H2′, H6′). MS m/z 310 $(M)^+$. Anal. Calcd. for $C_{19}H_{18}O_4{\cdot}0.25H_2O$ (314.74 g/mol): C, 72.54; H, 5.84. Found: C, 72.49; H, 5.44.

*2.2.21. 5,7-Dihydroxy-2-(4′-nitrophenyl)chromone (**21**)*

Yield 26.5%; yellow crystal; mp 285–286 °C. ^{1}H NMR: 6.22 (d, J = 1.66 Hz, 1H, H6), 6.55 (d, J = 1.66 Hz, 1H, H8), 7.18 (s, 1H, H3), 8.30–8.43 (m, 4H, H2′, H3′, H5′, H6′). MS m/z 299 $(M)^+$. Anal. Calcd. for $C_{15}H_9NO_6{\cdot}0.75H_2O$ (312.74 g/mol): C, 57.61; H, 3.28; O, 34.63; N, 4.48. Found: C, 57.38; H, 2.92; O, 35.0; N, 4.70.

*2.2.22. 5,7-Dihydroxy-2-(3′,5′-dinitrophenyl)chromone (**22**)*

Yield 11.0%; yellow crystal; mp 373–374 °C. ^{1}H NMR: 6.26 (d, J = 2.02 Hz, 1H, H6), 6.63 (d, J = 2.02 Hz, 1H, H8), 7.44 (s, 1H, H3), 8.97 (t, J = 2.06 Hz, 1H, H4′), 9.16 (d, J = 2.06 Hz, 2H, H2′, H6′). MS m/z 299 $(M)^+$. Anal. Calcd. for $C_{15}H_8N_2O_8$ (344.23 g/mol): C, 52.34; H, 2.34; N, 8.14. Found: C, 52.20; H, 2.11; N, 7.83.

*2.2.23. 5,7-Dihydroxy-2-(3′-chlorophenyl)chromone (**23**)*

Yield 58.1%; yellow crystal; mp 240–242 °C. ^{1}H NMR: 6.21 (d, J = 2.13 Hz, 1H, H6), 6.57 (d, J = 2.13 Hz, 1H, H8), 7.07 (s, 1H, H3), 7.59 (t, J = 8.02 Hz, 1H, H5′), 7.68 (dt, J = 8.02, 1.72 Hz, 1H, H4′), 8.05 (dt, J = 8.02, 1.72 Hz, 1H, H6′), 8.13 (t, J = 1.72 Hz, 1H, H2′). MS m/z 299 $(M)^+$. Anal. Calcd. for $C_{15}H_9ClO_4{\cdot}0.50H_2O$ (297.19 g/mol): C, 60.62; H, 3.22. Found: C, 60.34; H, 2.89.

*2.2.24. 5,7-Dihydroxy-2-(3′,4′-dichlorophenyl)chromone (**24**)*

Yield 65.0%; yellow crystal; mp 334–335 °C. ^{1}H NMR: 6.21 (d, J = 1.98 Hz, 1H, H6), 6.58 (d, J = 1.98 Hz, 1H, H8), 7.10 (s, 1H, H3), 7.83 (d, J = 8.60 Hz, 1H, H5′), 8.08 (dd, J = 8.60, 2.10 Hz, 1H, H6′), 8.37 (d, J = 2.10 Hz, 1H, H2′). MS m/z 299 $(M)^+$. Anal. Calcd. for $C_{15}H_8Cl_2O_4{\cdot}0.50H_2O$ (331.63 g/mol): C, 54.33; H, 2.50. Found: C, 54.42; H, 2.42.

*2.2.25. 5,7-Dihydroxy-2-(4′-methoxyphenyl)chromone (**25**)*

Yield 43.2%; yellow crystal; mp 243–244 °C. ^{1}H NMR: 3.90 (s, 3H, OCH_3), 6.19 (d, J = 2.10 Hz, 1H, H6), 6.49 (d, J = 2.10 Hz, 1H, H8), 6.86 (s, 1H, H3), 7.10 (d, J = 9.00 Hz, 2H, H3′, H5′), 8.02 (d, J = 9.00 Hz, 2H, H2′, H6′). MS m/z 284 $(M)^+$. Anal. Calcd. for $C_{16}H_{12}O_5$ (284.26 g/mol): C, 67.60; H, 4.25. Found: C, 67.61; H, 4.05.

*2.2.26. 5,7-Dihydroxy-2-(3′-methoxyphenyl)chromone (**26**)*

Yield 33.4%; yellow crystal; mp 242–243 °C. ^{1}H NMR: 3.90 (s, 3H, OCH_3), 6.21 (d, J = 2.10 Hz, 1H, H6), 6.53 (d, J = 2.10 Hz, 1H, H8), 7.01 (s, 1H, H3), 7.18 (dd, J = 8.05, 2.20 Hz, 1H, H4′), 7.48 (t, J = 8.05 Hz, 1H, H5′), 7.58 (dd, J = 2.20, 1.72 Hz, 1H, H2′), 7.64 (d, J = 8.05 Hz, 1H, H6′). MS m/z 284 $(M)^+$. Anal. Calcd. for $C_{16}H_{12}O_5$ (284.26 g/mol): C, 67.60; H, 4.25. Found: C, 67.57; H, 3.86.

*2.2.27. 6-Hydroxy-2-(3′-methoxyphenyl)chromone (**27**)*

Yield 12.0%; pink solid; mp 226–228 °C. ^{1}H NMR: 3.84 (s, 3H, OCH_3), 6.98 (s, 1H, H3), 7.15 (d, J = 7.53, 1H, H4′), 7.25 (dd, J = 8.91, 2.75 Hz, 1H, H7), 7.31 (d, J = 2.75 Hz, 1H, H5), 7.47 (t, J = 7.53 Hz, 1H, H5′), 7.57 (s, 1H, H2′), 7.64 (d, J = 7.53 Hz, 1H, H6′), 7.65 (d, J = 8.91 Hz, 1H, H8), 10.07 (s, 1H, C_6–OH). MS m/z 268 $(M)^+$. Anal. Calcd. for $C_{16}H_{12}O_4$ (268.27 g/mol): C, 71.63; H, 4.51. Found: C, 71.45; H, 4.57.

*2.2.28. 6-Hydroxy-2-(3′-chlorophenyl)chromone (**28**)*

Yield 28.1%; yellow crystal; mp 247–248 °C. ^{1}H NMR: 7.03 (s, 1H, H3), 7.25 (dd, J = 8.92, 2.85 Hz, 1H, H7), 7.30 (d, J = 2.85 Hz, 1H, H5), 7.55–7.66 (m, 2H, H5′, H6′), 7.69 (d, J = 8.92 Hz, 1H, H8), 8.04 (dd, J = 7.74, 1.15 Hz, 1H, H4′), 8.13 (s, 1H, H2′), 10.10 (s, 1H, C_6–OH). MS m/z 272 $(M)^+$. Anal. Calcd. for $C_{15}H_9O_3Cl$ (272.69 g/mol): C, 66.07; H, 3.33. Found: C, 66.19; H, 3.19.

*2.2.29. 6-Hydroxy-2-(4′-fluorophenyl)chromone (**29**)*

Yield 6.8%; pale yellow crystal; mp 261–262.5 °C. ^{1}H NMR: 6.95 (s, 1H, H3), 7.25 (dd, J = 8.93, 2.67, 1H, H7), 7.30 (d, J = 2.67 Hz, 1H, H5), 7.41 (t, J = 8.78 Hz, 2H, H3′, H5′), 7.64 (d, J = 8.93 Hz, 1H, H8), 8.13–8.17 (m, 2H, H2′, H6′), 10.04 (s, 1H, C_6–OH). MS m/z 256 $(M)^+$. Anal. Calcd. for $C_{15}H_9O_3F$ (256 g/mol): C, 70.31; H, 3.51. Found: C, 70.30; H, 3.26.

*2.2.30. 6-Hydroxy-2-(3′-trifluoromethylphenyl)chromone (**30**)*

Yield 3.0%; yellow crystal; mp 259.1–260.3 °C. ^{1}H NMR: δ 7.15 (s, 1H, H3), 7.26 (dd, J = 8.96, 2.82, 1H, H7), 7.31 (d, J = 2.82 Hz, 1H, H5), 7.72 (d, J = 8.96 Hz, 1H, H8), 7.80 (t, J = 7.86 Hz, 1H, H5′), 7.95 (d, J = 7.86 Hz, 1H, H6′), 8.40 (m, 2H, H2′, H4′), 10.06 (s, 1H, C_6–OH). MS m/z 306 $(M)^+$. Anal. Calcd. for $C_{16}H_9O_3F_3$ (306 g/mol): C, 62.75; H, 2.94. Found: C, 63.26; H, 2.72.

*2.2.31. 6-Hydroxy-2-(4′-tert-butylphenyl)chromone (**31**)*

Yield 7.4%; white crystal; mp 233.5–235 °C. ^{1}H NMR: δ 1.31 (s, 9H, $C(CH_3)_3$), 6.89 (s, 1H, H3), 7.24 (dd, J = 8.97, 2.95 Hz, 1H, H7), 7.30 (d, J = 2.95 Hz, 1H, H5), 7.57 (d, J = 8.49 Hz, 2H, H3′, H5′), 7.63 (d, J = 8.97 Hz, 1H, H8), 7.98 (d, J = 8.49 Hz, 2H, H2′, H6′), 10.60 (s, 1H, C_6–OH). MS m/z 294 $(M)^+$. Anal. Calcd. for $C_{19}H_{18}O_3$ (294.35 g/mol): C, 77.53; H, 6.16. Found: C, 78.13; H, 6.16.

*2.2.32. 7,8-Dihydroxy-2-(3′-trifluoromethylphenyl)-3-(3″-trifluoromethylbenzoyl)chromone (**32**)*

Yield 8.3%; yellow crystal; mp 208–209 °C. ^{1}H NMR: 7.10 (d, J = 8.68 Hz, 1H, H6), 7.41 (d, J = 8.68 Hz, 1H, H5), 7.62–7.75 (m, 2H, H5′, H5″), 7.82–7.87 (m, 2H, H4′, H4″), 7.97–8.00 (m, 2H, H2″, H6″), 8.18 (s, 1H, H2′), 8.22 (d, J = 8.04, 1H, H6′). MS m/z 517 $(M+Na)^+$. Anal. Calcd. for $C_{24}H_{12}F_6O_5$ (494.34 g/mol): C, 58.31; H, 2.45. Found: C, 58.28; H, 2.77.

*2.2.33. 7,8-Dihydroxy-2-(3′-chlorohenyl)-3-(3″-chlorobenzoyl) chromone (**33**)*

Yield 11.5%; yellow crystal; mp 256–257 °C. ^{1}H NMR: 7.01 (d, J = 8.71, 1H, H6), 7.39 (d, J = 8.71 Hz, 1H, H5), 7.45–7.58 (m, 4H, H5′, H4′, H5″, H4″), 7.69 (d, J = 6.87 Hz, 1H, H6′), 7.76 (s, 1H, H2′), 7.88 (d, J = 7.69 Hz, 1H, H6′), 7.96 (s, 1H, H2′). MS m/z 426 $(M)^+$. Anal. Calcd. for $C_{22}H_{12}O_5Cl_2{\cdot}0.25H_2O$ (431.74 g/mol): C, 61.20; H, 2.92. Found: C, 61.25; H, 2.82.

*2.2.34. 7,8-Didroxy-2-(3′-methoxyphenyl)-3-(3″-methoxybenzoyl)chromone (**34**)*

Yield 15.0%; yellow crystal; mp 255–257 °C. ^{1}H NMR: 3.66 (s, 3H, 3′-OCH_3), 3.85 (s, 3H, 3″-OCH_3), 7.01 (d, J = 8.62 Hz, 1H, H6), 7.05 (d, J = 9.17, 1H, H4′), 7.18 (s, 1H, H2′), 7.18–7.20 (m, 2H, H6′, H5), 7.34 (d, J = 7.79 Hz, 1H, H4″), 7.38 (s, 1H, H2″) 7.38–7.41 (m, 2H, H5′, H5″), 7.47 (d, J = 7.79 Hz, 1H, H6″). MS m/z 418 $(M)^+$. Anal. Calcd. for $C_{24}H_{18}O_7$ (418.40 g/mol): C, 68.89; H, 4.34. Found: C, 68.38; H, 4.28.

*2.2.35. 7,8-Dihydroxy-2-(4′-fluorophenyl)-3-(4″-fluorobenzoyl)chromone (**35**)*

Yield 10.0%; yellow crystal; mp 292–293 °C. ^{1}H NMR: 7.00 (d, J = 8.72 Hz, 1H H6), 7.22–7.34 (m, 4H, H3′, H3″, H5′, H5″), 7.39 (d, J = 8.72 Hz, 1H, H5), 7.65 (dd, J = 8.86, 5.50 Hz, 2H, H2″, H6″) 7.99 (dd, J = 8.78, 5.40 Hz, 2H, H2′, H6′). MS m/z 417 $(M+Na)^+$. Anal. Calcd. for $C_{22}H_{12}F_2O_5$ (394.32 g/mol): C, 67.01; H, 3.07. Found: C, 67.06; H, 3.48.

*2.2.36. 7,8-Dihydroxy-2-(4′-nitrophenyl)-3-(4″-nitrobenzoyl)chromone (**36**)*

Yield 5%; yellow crystal; mp 327–328 °C. ^{1}H NMR: 7.03 (d, J = 8.57 Hz, 1H, H6), 7.41 (d, J = 8.57 Hz, 1H, H5), 7.88 (d, J = 8.57 Hz, 2H, H2″, H6″), 8.20 (d, J = 8.57 Hz, 2H, H3″, H5″), 8.22–8.35 (m, 4H, H2′, H3′, H5′, H6′). MS m/z 448 $(M)^+$. Anal. Calcd. for $C_{22}H_{12}N_2O_9$ (448.34 g/mol): C, 58.94; H, 2.70; N, 6.25. Found: C, 58.95; H, 2.74; N, 5.98.

*2.2.37. 7,8-Dihydroxy-2-(4′-methoxyphenyl)-3-(4″-methoxybenzoyl)chromone (**37**)*

Yield 5.8%; pale yellow crystal; mp 253–254 °C. ^{1}H NMR: 3.74 (s, 3H, 4′-OCH_3), 3.79 (s, 3H, 4″-OCH_3), 6.91–6.99 (m, 5H, H6, H5′, H3′, H5″, H3″), 7.37 (d, J = 8.71 Hz, 1H, H5), 7.61 (d, J = 8.79, 2H, H2′, H6′), 7.84 (d, J = 8.76 Hz, 2H, H2″, H6″). MS m/z 418 $(M)^+$. Anal. Calcd. for $C_{24}H_{18}O_7{\cdot}0.75H_2O$ (431.91 g/mol): C, 66.74; H, 4.20. Found: C, 66.63; H, 4.36.

*2.2.38. 7-Hydroxy-2-(3′,4′-difluorophenyl)-3-(3″,4″-difluorobenzoyl)-chromone (**38**)*

Yield 7.0%; yellow crystal; mp 242–243 °C. ^{1}H NMR: 6.98 (dd, J = 8.30, 2.14 Hz, 1H, H6), 7.01 (d, J = 2.14 Hz, 1H, H8), 7.32–7.38 (m, 1H, H2″), 7.48–7.60 (m, 2H, H5′, H5″), 7.72–7.79 (m, 1H, H6″), 7.80–7.87 (m, 1H, H2′), 7.89 (d, J = 8.30 Hz, 1H, H5), 8.00–8.08 (m, 1H, H6′). MS m/z 437 $(M+Na)^+$. Anal. Calcd. for $C_{22}H_{10}F_4O_4$ (414.40 g/mol): C, 63.78; H, 2.43. Found: C, 63.46; H, 2.70.

*2.2.39. 7-Hydroxy-2-(3′-trifluoromethylphenyl)-3-(3″-trifluoromethylbenzoyl)chromone (**39**)*

Yield 10.9%; yellow crystal; mp 234–235 °C. ^{1}H NMR: 7.01 (dd, J = 8.43, 1.64 Hz, 1H, H6), 7.03 (d, J = 1.64 Hz, 1H, H8), 7.62–7.73 (m, 2H, H5′, H5″), 7.81–8.00 (m, 5H, H2″, H4″, H5, H4′, H6″), 8.18 (s, 1H, H2′), 8.20 (d, J = 8.00 Hz, 1H, H6′). MS m/z 501 $(M+Na)^+$. Anal. Calcd. for $C_{24}H_{12}F_6O_4$ (478.34 g/mol): C, 60.26; H, 2.53. Found: C, 60.29; H, 2.33.

*2.2.40. 7-Hydroxy-2-(3′-chlorophenyl)-3-(3″-chlorobenzoyl)-chromone (**40**)*

Yield 23.1%; yellow crystal; mp 303–305 °C. ^{1}H NMR: 6.98 (dd, J = 8.13, 1.81 Hz, 1H, H6), 7.00 (d, J = 1.81, 1H, H8), 7.44–7.58 (m, 4H, H5′, H5″, H4′, H6′), 7.68 (s, 1H, H2′), 7.70 (d, J = 1.67 Hz, 1H, H4″), 7.86–7.91 (m, 2H, H5, H6″), 7.95 (d, J = 1.36 Hz, 1H, H2″). MS m/z 410 $(M)^+$. Anal. Calcd. for $C_{22}H_{12}O_4Cl_2$ (411.24 g/mol): C, 64.25; H, 2.94. Found: C, 63.94; H, 2.81.

*2.2.41. 7-Hydroxy-2-(3′-methoxyphenyl)-3-(3″-methoxybenzoyl)chromone (**41**)*

Yield 14.1%; yellow crystal; mp 253–254 °C. ^{1}H NMR: 3.65 (s, 3H, 3″-OCH_3), 3.75 (s, 3H, 3′-OCH_3), 6.97 (dd, J = 8.04, 2.29 Hz, 1H, H6), 6.99 (d, J = 2.29 Hz, H8), 7.04 (dd, J = 8.91, 1.98 Hz, 1H, H4″), 7.12–7.22 (m, 3H, H2″, H4′, H5″), 7.32–7.41(m, 3H, H2′, H5′, H6″), 7.45–7.50 (m, 1H, H6′), 7.89 (d, J = 8.04 Hz, 1H, H5). MS m/z

Table 1
Composition of HPLC assay mixture.

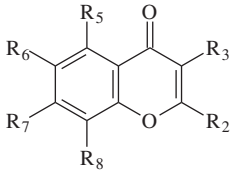
Reagents	Negative control (μL)	Positive control (μL)	Sample (μL)
Assay buffer	20.4	20.4	20.4
Substrate	1.6	1.6	1.6
50% DMSO	1.6	–	–
Inhibitor	–	–	1.6
Pepstatin A	–	1.6	–
Enzyme	1.4	1.4	1.4
Total volume	25.0	25.0	25.0

402 $(M)^+$. Anal. Calcd. for $C_{24}H_{18}O_6$ (402.40 g/mol): C, 71.64; H, 4.51. Found: C, 72.46; H, 4.71.

*2.2.42. 7-Hydroxy-2-(4′,-fluorophenyl)-3-(4″-fluorobenzoyl)chromone (**42**)*

Yield 8.5%; yellow crystal; mp 239-240 °C. ^{1}H NMR: 6.95–7.02 (m, 2H, H6, H8), 7.25–7.37 (m, 4H, H3′, H3″, H5′, H5″), 7.63 (dd, J = 8.84, 5.38 Hz, 2H, H2″, H6″), 7.89 (d, J = 9.39 Hz, 1H, H5), 8.00 (dd, J = 8.70, 5.48 Hz, 2H, H2′, H6′). MS m/z 401 $(M+Na)^+$. Anal. Calcd. for $C_{22}H_{12}F_2O_4$ (378.41 g/mol): C, 69.85; H, 3.20. Found: C, 69.92; H, 3.02.

Table 2
Structures of chromone derivatives and % inhibition at 12.5 μg/mL concentration.



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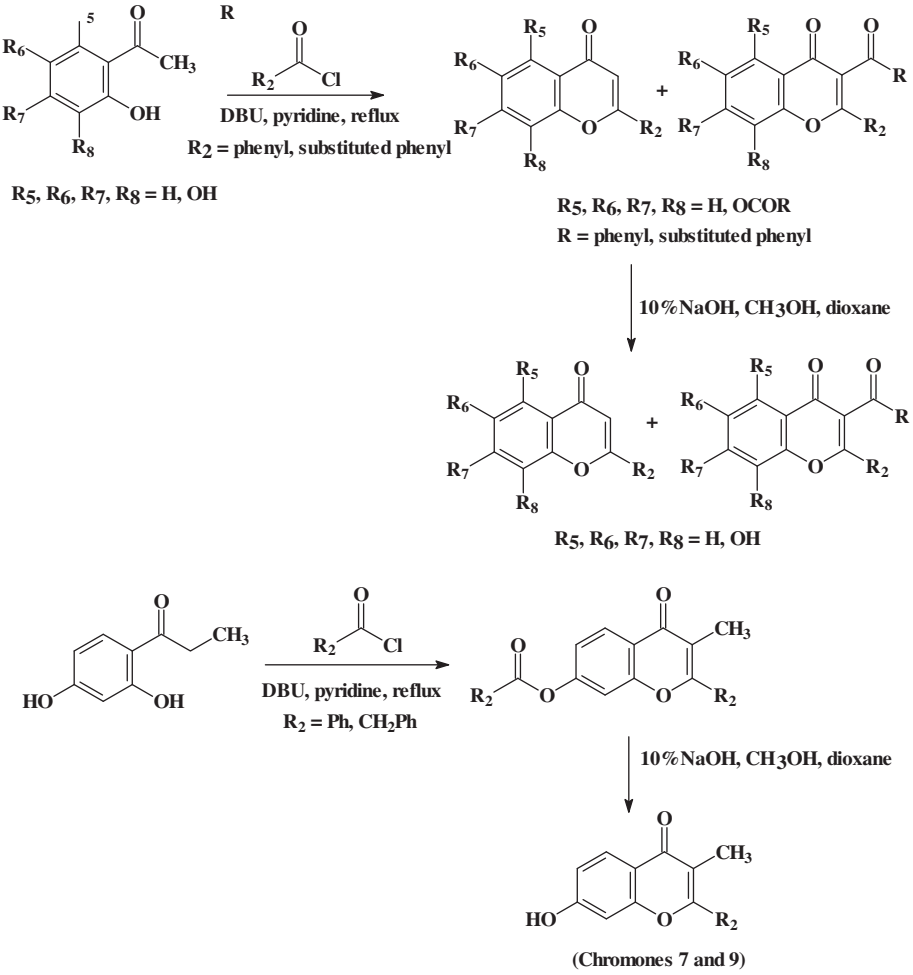
Compd	R_2	R_3	R_5	R_6	R_7	R_8	% Inhibition
1	Phenyl	H	H	H	H	OH	64.34 ± 1.01
2	CH_3	H	H	H	OH	OH	92.02 ± 0.24
3	Benzyl	H	H	H	OH	OH	93.30 ± 0.07
4	Phenyl	H	H	H	OH	OH	88.17 ± 1.09
5	CH_3	H	H	H	OH	H	56.00 ± 1.74
6	Benzyl	H	H	H	OH	H	50.26 ± 4.73
7	Benzyl	CH_3	H	H	OH	H	18.97 ± 5.79
8	Phenyl	H	H	H	OH	H	75.04 ± 1.72
9	Phenyl	CH_3	H	H	OH	H	57.29 ± 5.06
10	4′-(NO_2)-Phenyl	H	H	H	OH	H	63.52 ± 1.63
11	3′-(CF_3)-Phenyl	H	H	H	OH	H	29.27 ± 1.10
12	4′-(F)-Phenyl	H	H	H	OH	H	37.52 ± 7.03
13	3′,5′-(diNO_2)-Phenyl	H	H	H	OH	H	26.32 ± 4.49
14	3′-(Cl)-Phenyl	H	H	H	OH	H	27.36 ± 3.84
15	3′,4′-(diCl)-Phenyl	H	H	H	OH	H	47.63 ± 2.11
16	4′-(*t*-butyl)-Phenyl	H	H	H	OH	H	78.89 ± 5.71
17	3′-(CF_3)-Phenyl	H	OH	H	OH	H	74.80 ± 0.26
18	4′-(F)-Phenyl	H	OH	H	OH	H	88.13 ± 2.33
19	3′,4′-(diF)-Phenyl	H	OH	H	OH	H	80.25 ± 3.25
20	4′-(*t*-butyl)-Phenyl	H	OH	H	OH	H	89.29 ± 3.47
21	4′-(NO_2)-Phenyl	H	OH	H	OH	H	27.20 ± 0.82
22	3′,5′-(diNO_2)-Phenyl	H	OH	H	OH	H	10.48 ± 2.52
23	3′-(Cl)-Phenyl	H	OH	H	OH	H	73.62 ± 0.58
24	3′,4′-(diCl)-Phenyl	H	OH	H	OH	H	85.26 ± 1.20
25	4′-(OCH_3)-Phenyl	H	OH	H	OH	H	88.68 ± 2.27
26	3′-(OCH_3)-Phenyl	H	OH	H	OH	H	97.48 ± 1.72
27	3′-(OCH_3)-Phenyl	H	H	OH	H	H	75.29 ± 2.13
28	3′-(Cl)-Phenyl	H	H	OH	H	H	47.48 ± 0.94
29	4′-(F)-Phenyl	H	H	OH	H	H	54.56 ± 4.68
30	3′-(CF_3)-Phenyl	H	H	OH	H	H	66.68 ± 0.32
31	4′-(*t*-butyl)-Phenyl	H	H	OH	H	H	31.98 ± 2.46
32	3′-(CF_3)-Phenyl	3″-(CF_3)-benzoyl	H	H	OH	OH	93.16 ± 1.74
33	3′-(Cl)-Phenyl	3″-(Cl)-benzoyl	H	H	OH	OH	50.59 ± 1.39
34	3′-(OCH_3)-Phenyl	3″-(OCH_3)-benzoyl	H	H	OH	OH	53.13 ± 2.95
35	4′-(F)-Phenyl	4″-(F)-benzoyl	H	H	OH	OH	84.94 ± 1.54
36	4′-(NO_2)-Phenyl	4″-(NO_2)-benzoyl	H	H	OH	OH	92.24 ± 1.70
37	4′-(OCH_3)-Phenyl	4″-(OCH_3)-benzoyl	H	H	OH	OH	35.23 ± 4.07
38	3′,4′-(diF)-Phenyl	3″,4″-(diF)-benzoyl	H	H	OH	H	88.98 ± 1.44
39	3′-(CF_3)-Phenyl	3″-(CF_3)-benzoyl	H	H	OH	H	74.98 ± 3.86
40	3′-(Cl)-Phenyl	3″-(Cl)-benzoyl	H	H	OH	H	54.17 ± 0.81
41	3′-(OCH_3)-Phenyl	3″-(OCH_3)-benzoyl	H	H	OH	H	24.67 ± 4.31
42	4′-(F)-Phenyl	4″-(F)-benzoyl	H	H	OH	H	50.79 ± 0.97
43	4′-(NO_2)-Phenyl	4″-(NO_2)-benzoyl	H	H	OH	H	74.47 ± 2.76
44	4′-(OCH_3)-Phenyl	4″-(OCH_3)-benzoyl	H	H	OH	H	34.06 ± 9.89
45	4′-(*t*-butyl)-Phenyl	4″-(*t*-butyl)-benzoyl	H	H	OH	H	88.53 ± 1.75
46	3′-(OCH_3)-Phenyl	3″-(OCH_3)-benzoyl	OH	H	OH	H	53.39 ± 3.22
47	4′-(NO_2)-Phenyl	4″-(NO_2)-benzoyl	OH	H	OH	H	88.39 ± 0.11
48	4′-(*t*-butyl)-Phenyl	4″-(*t*-butyl)-benzoyl	H	OH	H	H	43.19 ± 4.07
Pepstatin A (100 μM)							91.07 ± 1.53



Scheme 1. Synthesis of chromone derivatives by one-pot cyclization reaction.

*2.2.43. 7-Hydroxy-2-(4′-nitrophenyl)-3-(4″-nitrobenzoyl)chromone (**43**)*

Yield 5.1%; yellow crystal; mp 290–291 °C. ^{1}H NMR: 6.98–7.07 (m, 2H, H6, H8), 7.83 (d, *J* = 8.78 Hz, 2H, H2″, H6″), 7.91 (d, *J* = 9.05 Hz, 1H, H5), 8.21 (d, *J* = 8.78 Hz, 2H, H3″, H5″), 8.28 (d, *J* = 8.72 Hz, 4H, H2′, H3′, H5′, H6′). MS *m*/*z* 455 $(M+Na)^+$. Anal. Calcd. for $C_{22}H_{12}N_2O_8$ (432.34 g/mol): C, 61.12; H, 2.80; N, 6.48. Found: C, 61.11; H, 2.87; N, 6.87.

*2.2.44. 7-Hydroxy-2-(4′-methoxyphenyl)-3-(4″-methoxybenzoyl)chromone (**44**)*

Yield 41.2%; yellow crystal; mp 300–301 °C. ^{1}H NMR: 3.75 (s, 3H, 3″-OCH_3), 3.79 (s, 3H, 3′-OCH_3), 6.90–7.02 (m, 6H, H3′, H3″, H5′, H5″, H6, H8), 7.57 (d, *J* = 8.98, 2H, H2″, H6″), 7.83 (d, *J* = 8.86, 2H, H2′, H6′), 7.87 (d, *J* = 8.50 Hz, 1H, H5). MS *m*/*z* 402 $(M)^+$. Anal. Calcd. for $C_{24}H_{18}O_6$: (402.40 g/mol): C, 71.64; H, 4.51. Found: C, 71.62; H, 4.23.

*2.2.45. 7-Hydroxy-2-(4′-tert-butylphenyl)-3-(4″-tert-butylbenzoyl)chromone (**45**)*

Yield 25.4%; white crystal; mp 271–273 °C. ^{1}H NMR: 1.23 (s, 9H, 4′-*t*-butyl), 1.27 (s, 9H, 4″-*t*-butyl), 6.95 (dd, *J* = 7.95, 1.13, 1H, H6), 6.98 (d, *J* = 1.13 Hz, 1H, H8), 7.45 (d, *J* = 8.32 Hz, 2H, H3′, H5′), 7.49 (d, *J* = 8.48 Hz, 2H, H3″, H5″), 7.55 (d, *J* = 8.32 2H, H2′, H6′), 7.82 (d, *J* = 8.48 2H, H2″, H6″), 7.86 (d, *J* = 7.95 Hz, 1H, H5). MS *m*/*z* 454 $(M)^+$. Anal. Calcd. for $C_{30}H_{30}O_4$ (454.56 g/mol): C, 79.27; H, 6.65. Found: C, 79.09; H, 6.73.

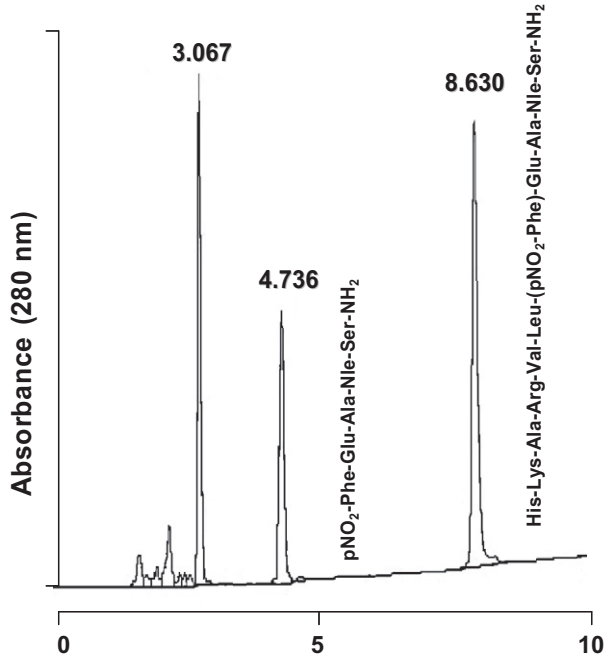


Fig. 3. HPLC profile of a reaction mixture of HIV-1 PR and substrate after incubated for 2 h at 37 °C.

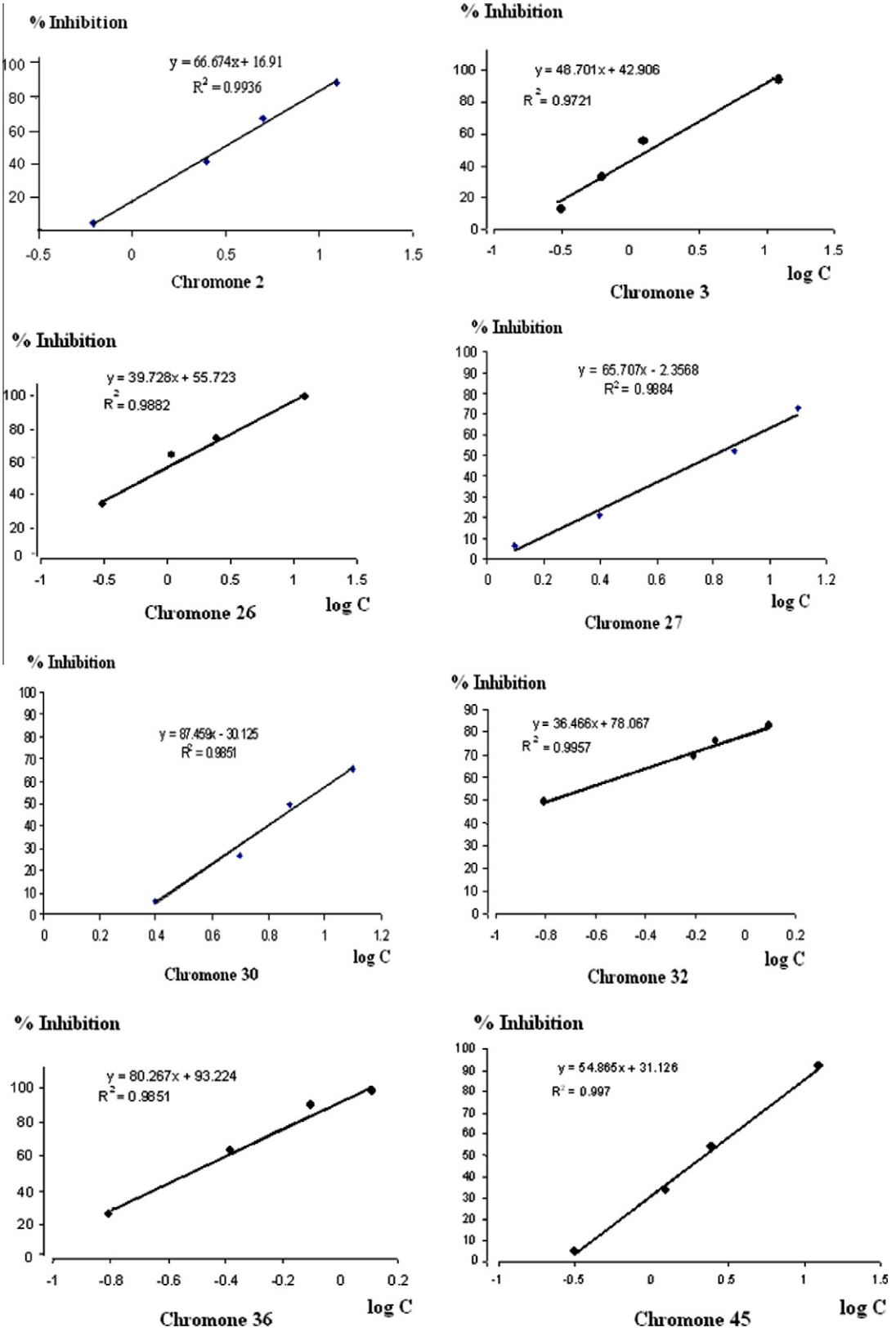


Fig. 4. Plots of the logarithm of concentrations (μg/mL) and % inhibition profiles and IC_{50} of chromones **2**, **3**, **26**, **27**, **30**, **32**, **36** and **45**.

*2.2.46. 5,7-Dihydroxy-2-(3′-methoxyphenyl) 3-(3″-methoxybenzoyl)chromone (**46**)*

Yield 6.7%; yellow crystal; mp 167–168 °C. ^{1}H NMR: 3.64 (s, 3H, 3′-OCH_3), 3.76 (s, 3H, 3″-OCH_3), 6.26 (d, *J* = 1.68 Hz, 1H, H6), 6.51 (d, *J* = 1.68, 1H, H8), 7.05 (dd, *J* = 8.24, 2.49 Hz, 1H, H4′), 7.10 (s, 1 H, H2′), 7.13 (dd, *J* = 8.24, 2.49 Hz, 1 H, H6′), 7.21 (dd, *J* = 7.81, 2.25 Hz, 1H, H4″), 7.34 (t, *J* = 8.24 Hz, 1 H, H5′), 7.38 (t, *J* = 7.81 Hz, 1 H, H5″), 7.41 (s, 1H, H2″), 7.54 (dd, *J* = 7.81, 2.25 Hz, 1H, H6″). MS *m*/*z* 418 $(M)^+$. Anal. Calcd. for $C_{16}H_{12}O_4 \cdot 0.25H_2O$ (422.90 g/mol): C, 68.16; H, 4.41. Found: C, 68.16; H, 4.32.

*2.2.47. 5,7-Dihydroxy-2-(4′-nitrophenyl)-3-(4″-nitrobenzoyl)chromone (**47**)*

Yield 4.1%; yellow crystal; mp 288–289 °C. ^{1}H NMR: 6.27 (d, J = 1.75 Hz, 1H, H6), 6.54 (d, J = 1.75 Hz, 1H, H8), 7.82 (d, J = 8.78 Hz, 2H, H2″, H6″), 8.22–8.38 (m, 6H, H2′, H3′, H3″, H5′, H5″, H6′). MS m/z 448 $(M)^+$. Anal. Calcd. for $C_{22}H_{12}N_2O_9$ (448.34 g/mol): C, 58.94; H, 2.70; N, 6.25. Found: C, 58.95; H, 2.74; N, 6.41.

*2.2.48. 6-Hydroxy-2-(4′-tert-butylphenyl)-3-(4″-tert-butylbenzoyl)chromone (**48**)*

Yield 2.8%; pale orange solid; mp 232–234.0 °C. ^{1}H NMR: 1.22 (s, 9H, 4′-*t*-butyl), 1.68 (s, 9H, 4″-*t*-butyl), 7.30 (broad d, J = 2.95 Hz, 1H, H5), 7.31 (dd, J = 8.82, 2.95 Hz, 1H, H7), 7.45–7.54 (m, 6H, H3′, H5′, H3″, H5″, H2′, H6′), 7.66 (d, J = 8.82 Hz, 1H, H8), 7.83 (d, J = 8.32 Hz, 2H, H2″, H6″), 10.25 (s, 1H, C_6–OH). MS m/z 454 $(M)^+$. Anal. Calcd. for $C_{30}H_{30}O_4{\cdot}0.5H_2O$ (463.58 g/mol): C, 77.73; H, 6.74. Found: C, 77.74; H, 6.76.

2.3. HIV-1 PR assay

A stopped time HPLC assay [13] was performed by incubating peptide substrate with the enzyme in small volumes (<0.1 mL). A modified peptide, anthraniryl His-Lys-Ala-Arg-Val-Leu-(*p*-NO_2-Phe)-Glu-Ala-Nle-Ser-Amide, whose amino acid sequence corresponds to the p24-p15 cleavage site of viral polyprotein was used as a substrate. The following reagents were prepared for the assay: anthranirylHis-Lys-Ala-Arg-Val-Leu-(*p*-NO_2-Phe)-Glu-Ala-Nle-Ser-Amide (1 μg/L), substrate buffer (50 mM NaOAc, pH 4.9), inhibitors (chromone derivatives, 12.5 μg/mL), positive control (pepstatin A, 0.1 mM) dissolved in DMSO, assay buffer (50 mM NaOAc, pH 4.9, 200 mM NaCl, 5 mM dithiothreitol (DTT) and 10% (v/v) glycerol in water and purified HIV-1 PR biosynthetic enzyme from *E. coli* (0.005 μg/μL).

The composition of negative, positive control and sample reaction mixtures for HPLC assay is shown in Table 1.

The reaction mixture was incubated at 37 °C for 2 h and then terminated by addition of 2.5 μL of 10% trifluoroacetic acid. During the incubation, the substrate was hydrolyzed at the site of Leu and (*p*-NO_2-Phe). After quenching with acid at various times (<60 min), the sample was subjected to reverse phase HPLC. The resulting peptidolytic products were thereby separated from the remaining substrate using linear gradient of acetonitrile in 0.1% trifluoroacetic acid (TFA) with spectrophotometric detection of the peptides at 280 nm. The percentage of inhibition was calculated from the ratio of the substrate peak area to the hydrolysate peak area.

$$\%\text{Inhibition} = \frac{A_{\text{negative control}} - A_{\text{sample}}}{A_{\text{negative control}}} \times 100$$

(A = relative peak of the hydrolysate)

HPLC condition:
Column: Hypersil® BDS C18, particle size: 5 μm, 250 × 4.6 mm
Injection volume: 20 μL
Solvent: A. 0.1% trifluoroacetic acid in water, B. acetonitrile/water (3:1)
Gradient: 22.5–40% of solvent B in 12 min
Flow rate: 1.5 mL/min
UV detector: λ 280 nm

2.4. Generation of the molecular structures and docking

The molecular structures of chromone derivatives were modeled with SYBYL 6.9 molecular modeling program (Tripos Associates, Saint Louis, MO) on an Indigo Elan workstation (Silicon Graphics Inc., Mountain View, CA) using the sketch approach. The fragment libraries in SYBYL database were used as building blocks for the construction of larger ones. Each structure was energy minimized using the standard Tripos force field (Powell method and 0.05 kcal/mol Å energy gradient convergence criteria) and electrostatic charge was assigned by the Gasteiger–Hückel method. These conformations were used as starting conformations to perform docking. The docking was performed using FlexiDock option in SYBYL/Biopolymer program. The crystal structure of the HIV-1 PR complexed with inhibitor (Aha001) was obtained from the protein data bank (pdb 1AJX). The Aha001 structure was first removed from the complex structure, then chromone inhibitor was placed into the binding site. Water molecules and ions were removed and hydrogen atoms were added at appropriate geometry. The charges were assigned by Kollman force field for protein and Gasteiger–Hückel for ligands.

3. Results and discussion

3.1. Chemical synthesis

Forty-eight chromone derivatives (Table 2) were prepared according to Scheme 1. The one pot cyclization reaction was developed from the method of Riva et al. [14]. The phenolic ester intermediate was initially formed from the reaction of phenolic ketone and acid chloride. Abstraction of the acidic methylene proton by DBU led to the formation of the carbanion. The carbanion nucleophile attacked the ester carbonyl carbon and provided the β-diketone intermediate. Cyclization of β-diketone followed by dehydration resulted in the 2-substituted chromone ester. During the cyclization process, the DBU could abstract more acidic hydrogen to generate another carbanion nucleophile which attacked the carbonyl carbon of excess acid chloride. After dehydration process, the 2,3-disubstitued chromone ester was obtained. Hydrolysis of the chromone esters by the mixture of NaOH, methanol, water, and dioxane finally yielded the chromone derivatives.

3.2. Evaluation of HIV-1 PR inhibitory activity

The ability of the chromone derivatives to inhibit the HIV-1 PR was determined using HPLC assay for preliminary testing. The principle of the assay is based on the catalytic cleavage of peptide substrates bearing the chromogenic *p*-nitrophenylalanine. His-Lys-Ala-Arg-Val-Leu-(*p*-NO_2-Phe)-Glu-Ala-Nle-Ser-Amide peptide usually used as substrate for HIV-1 PR. Only the peptide bond between the Leu and *p*-NO_2-Phe residues is hydrolyzed [13]. The substrate and Phe(NO_2)-bearing hydrolysate could be monitored at wavelength 280 nm and were eluted at 8.6 and 4.7 min, respectively (Fig. 3). The results were reported as % inhibition as summarized in Table 2. The inhibitory activity was in the range of 10–97%. Chromone **26** exhibited highest activity with 97% inhibition at concentration 12.5 μg/mL, while pepstatin A, a potent aspartyl prote-

Table 3
IC_{50} (μM) of the investigated chromone derivatives.

Compd	IC_{50} (μM)
2	6.89
3	5.22
26	2.53
27	23.35
30	26.92
32	0.34
36	0.65
45	11.50

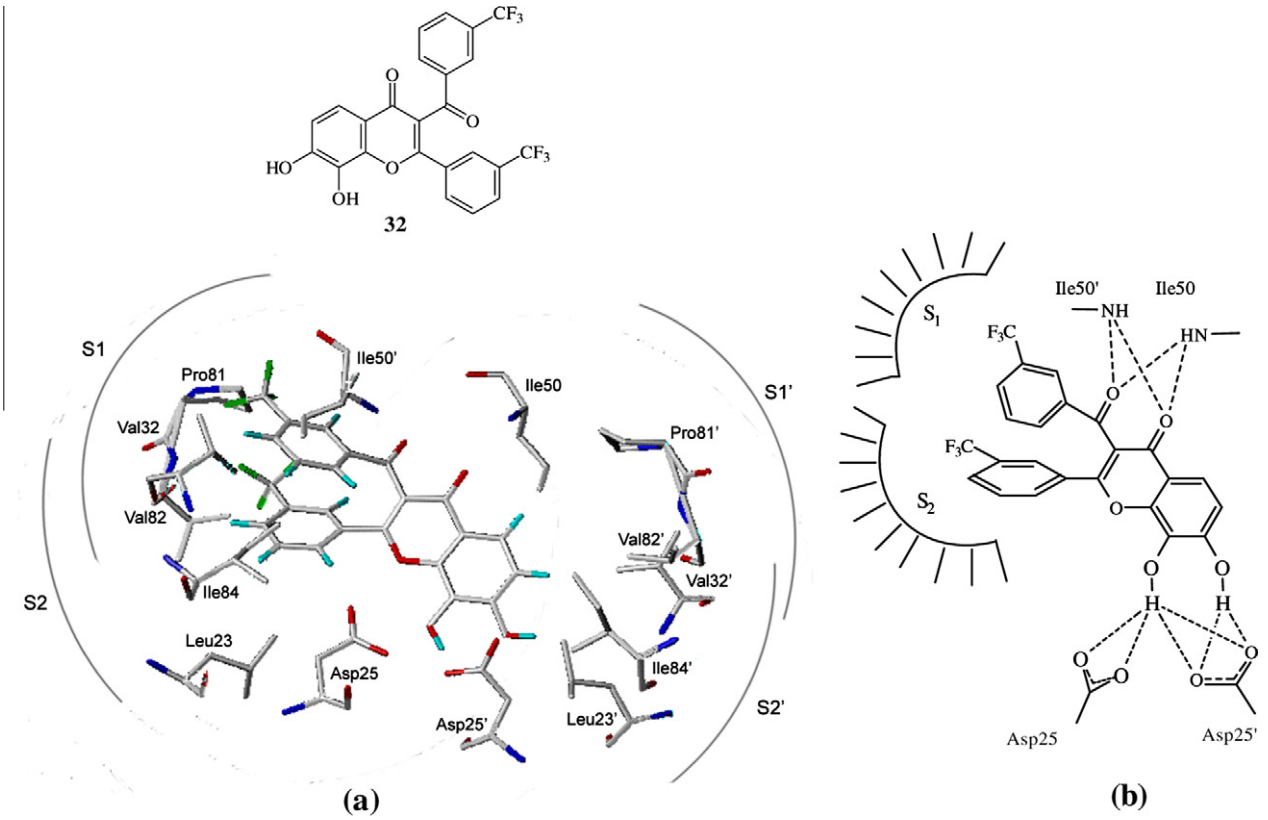


Fig. 5. (a) The hydrophobic interaction of chromone **32** in the active site of HIV-1 PR. (b) Schematic view of the binding conformation of chromone **32** in the enzyme active sites.

ase [15] showed 91% inhibition at 100 μM in the same experiment. Some derivatives that exhibited medium and high % inhibition were selected for further determination of IC_{50}. IC_{50} values were obtained from linear regression plots between log concentration (log *C*) versus % inhibition (Fig. 4). The three most potent inhibitors were chromones **32**, **36** and **26** with IC_{50} = 0.34, 0.65 and 2.53 μM, respectively (Table 3).

3.3. Binding interaction study

The most active compound in this series, chromone **32** was used as the representative molecule for discussion. As seen from Fig. 5, the 3-trifluoromethyl benzoyl ring binds to its binding site, S1 pocket (Val32, Ile50′, Pro81, and Val82) via hydrophobic interaction with the hydrophobic amino acid residues. The 3-trifluoro-methyl-phenyl ring also forms hydrophobic interaction with the hydrophobic residues in S2 pocket (Leu23, Val32, Val82, and Ile84). The 7-OH and 8-OH of the chromone form hydrogen bonds with the carbonyl oxygens of Asp25 and Asp25′. The docking results supported the experimental activity testing that most of the compounds which have both 7,8-dihydroxyl groups and hydrophobic substituents at positions 2 and 3 showed greater inhibition (the only exception was chromone **26**). Compounds with less than 20% inhibition such as chromones **7** and **22** are those whose structures are composed of only 7-hydroxy and hydrophobic substituent at position 2.

4. Conclusion

We have synthesized a series of chromone derivatives as nonpeptidic HIV-1 PR inhibitor. The *in vitro* HIV-1 PR inhibitory activity testing by HPLC assay was used as the preliminary screening. The most active compound, 7,8-dihydroxy-2-(3′-trifluoromethylphenyl)-3-(3″-trifluoromethylbenzoyl)chromone (**32**) showed IC_{50} = 0.34 μM which is more potent than the structurally related HIV-1 PR inhibitors in 4-hydroxycoumarins series. The docking study revealed that 7,8-dihydroxyl groups and hydrophobic substituents both at positions 2 and 3 are required for better enzyme inhibition.

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