Research Paper

Synthesis and Antiretroviral Activity of 6-Acetyl-coumarin Derivatives against HIV-1 Infection

V. K. SRIVASTAV, M. TIWARI*, X. ZHANG[1] AND X-J. YAO[1]

Department of Pharmacy, Laboratory of Medicinal and Pharmaceutical Chemistry, Shri G. S. Institute of Technology and Science, 23 Park Road, Indore-452 003, India, [1]Department of Medical Microbiology, Laboratory of Molecular Human Retrovirology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, R3E 0W3, Canada

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A series of 6-acetyl-coumarin derivatives (2a-n) were synthesized and evaluated for antiretroviral activity in C8166 T-cell line infected with HxBru-Gluc strain of human immunodeficiency virus-1. Michael Addition followed by re-aromatization and subsequent acid-induced elimination of water leads to the formation of coumarin intermediate, which undergoes Claisen-Schmidt condensation with substituted bezaldehydes using silica sulphuric acid as a catalyst to form 6-acetyl-coumarin derivatives, 2a-n. *In silico* absorption, distribution, metabolism, excretion and toxicity parameters of compounds 2a-n were found within their reference limits. All synthesized compounds were devoid of cytotoxicity as they have shown cell viability count more than 80 % in cytotoxicity assay. Compounds 2a, 2g and 2h showed potent inhibitory activity against human immunodeficiency virus infection with IC_{50} value of 4.7, 4.5 and 0.35 µM, respectively. It was found that electron-withdrawing group at phenyl ring, attached to the coumarin nucleus was crucial for activity against human immunodeficiency virus. The present study may be helpful in the development of some potent antiretroviral agents.

Key words: 6-acetyl-coumarin, synthesis, *in silico* ADMET, *in vitro* toxicity, antiretroviral activity

Acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus type-1 (HIV-1) is final stage of infection that occurs when CD4 cell (also called T-cells) count of a HIV-1 infected person falls below 200[1]. Now, it became the fifth leading cause of death among all adults (between 25 to 44 y age-group) in the United States. Globally, about 36.7 million people were living with HIV in year 2015[2]. Rapid development of viral resistance to currently available antiretroviral drugs and drug combinations invoked an imperative need to develop novel antiretroviral agents. One of the active natural products, coumarin, has attracted attention in recent years due to its diverse pharmacological properties[3-8]. Coumarins are highly variable in structure due to possibility of various types of substitutions in its ring skeleton, which influence a variety of pharmacological actions[9-15]. Various reports showed the antiretroviral potency of coumarins via inhibition of retroviral enzymes i.e. reverse transcriptase, protease and integrase[16-20]. Due to wide range of biological activity, synthesis of coumarins in laboratory has now become the prime interest of medicinal chemists. Previously, several coumarin derivatives were designed as antiHIV agents by quantitative structure-activity relationship (QSAR) and docking studies[21,22]. The present study explores the synthesis and antiretroviral activity of 6-acetyl-coumarin derivatives against HIV infection.

The classical synthesis routes of coumarins include condensation reactions such as Knoevenagel, Pechmann, Perkin, Reformatsky and Wittig[23-25]. Phenols, o-hydroxy-benzaldehydes and acetophenones are the commonly used precursors for synthesis of coumarin ring[26-27]. To increase the yield of classical reactions, several variations have been made in terms of catalysts and reaction conditions[28]. It has now became advantageous to use solid catalysts in organic synthesis due to their non-toxicity, operational simplicity, low cost, environmental compatibility, reusability, and ease of isolation[29]. Silica sulphuric acid (SSA) is

*Address for correspondence
E-mail: drmeenatiwari@gmail.com

such a versatile catalyst that makes chemical reactions economic, convenient and environment friendly[30-32]. In the present study, 6-acetyl-coumarin derivatives were synthesized in good yield using SSA as a catalyst.

About 40 % of drug candidates fail in clinical trials because of poor pharmacokinetics, and toxicity. The late-stage detection of such failures significantly increases the cost of new drug development process. In order to determine absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of developed compounds at an early stage, *in silico* approaches are preferred nowadays. Therefore, a preliminary drug-likeness, *in silico* ADME profiling, and toxicity risk assessment (i.e. mutagenicity, tumorigenicity, irritant and reproductive effects) studies were performed to determine the pharmacokinetic properties and toxicity of synthesized compounds.

The *in vitro* toxicity study of synthesized compounds was performed using Trypan Blue exclusion method[33] while the antiretroviral activity of synthesized compounds against HIV-1 infection was determined by measuring the Gaussia luciferase (Gluc) activity[34]. The present study indicated that synthesized compounds have potential of inhibiting the HIV-1 infection in low micromolar concentration.

MATERIALS AND METHODS

All reactions were carried out under anhydrous conditions and in solvents dried over molecular sieves type 4 Å. Melting points (MP) of all the synthesized compounds were determined by open capillary method and are uncorrected. Reactions were monitored by thin layer chromatography (TLC) using hexane:ethyl acetate (3:7) as mobile phase. Spots on TLC plate were detected in UV cabinet. All synthesized compounds were purified by column chromatography using optimized proportions of hexane and ethyl acetate. The wavelength of maximum absorption (λ_{max}) and LogP (octanol-water partition coefficient) of synthesized compounds was determined by Shimadzu 1700 UV/Vis double beam spectrophotometer. IR spectra were recorded on Bruker Alpha spectrophotometer. Proton nuclear magnetic resonance (¹H-NMR) spectra were recorded on Bruker Avance II NMR spectrometer using tetramethylsilane as an internal reference standard. Chemical shift values are reported in parts per million (δ in ppm), where s, d, dd and m designate singlet, doublet, doublet of doublet and multiplet, respectively. Mass spectra were recorded on Varian 500 Mass

spectrometer and elemental analysis was done using Vario EL III CHN elemental analyser.

Synthesis of 5-hydroxy-6-acetyl-4-methylcoumarin (1):

Aluminum chloride (7.5 mmol) and dry nitrobenzene (10 ml) were added to a mixture of resacetophenone (20 mmol) and ethyl acetoacetate (20 mmol) in a three naked round bottom flask. The mixture was protected from moisture and heated around 130-140° for about an hour until the evolution of hydrogen chloride gas ceased. It was then cooled in ice and nitrobenzene was removed by steam distillation. Solid left behind was coumarin intermediate 1 (5-hydroxy-6-acetyl-4-methylcoumarin), which was re-crystallized from boiling alcohol to give colourless needle shaped crystals (MP=189-195°, % yield=62).

Synthesis of 6-acetyl-coumarin derivatives (2a-n):

Substituted bezaldehydes (10 mmol), intermediate 1 (7.5 mmol) and SSA (0.7 g) were mixed in a glass tube. The mixture was heated at 70-80° for 2 h with intermittent mixing every 15 min with a glass rod. After completion of the reaction, the product was extracted with 50 ml of dichloromethane. The dichloromethane extract was filtered and concentrated under vacuum to give solid mass. Recrystallization from ethanol finally gave 6-acetyl-coumarin derivatives, 2a-n, which was further purified by column chromatography. The physicochemical properties and spectral data of synthesized compounds were as follows:

6-(3-(4-chlorophenyl)-acryloyl)-5-hydroxy-4-methyl-2H-chromen-2-one (2a):

Compound 2a was synthesized by reaction of 1 with 4-chloro-benzaldehyde using SSA as a catalyst; yellow crystals; % yield: 79; MP: 215-218°; Rf: 0.5; UV (λ_{max}): 267; LogP: 3.42; IR (KBr): 3245 (-OH stretch), 2926 (Ar, C-H stretch), 2840 (aliphatic, C-H stretch), 1696 (O-C=O stretch), 1624 (C=O stretch), 1489, 1427 (C=C ring stretch), 1287, 1181, 1142 (Ar, C-H bend, in plane), 1091 (C-Cl stretch), 840, 805, 762 (Ar, C-H bend, out of plane) and 441 cm⁻¹ (C=C ring bend, out of plane); ¹H NMR (300 MHz, CDCl₃) δ 7.95 (d, *J*=15.0 Hz, 1H, 3' –CH), 7.86 (d, *J*=7.5 Hz, 1H, 7 –CH), 7.48 (dd, *J*=11.3, 3H, 2', 2", 6" –CH), 7.41 (d, *J*=7.5 Hz, 2H, 3", 5" –CH), 6.95 (d, *J*=7.4 Hz, 1H, 8 –CH), 6.05 (d, *J*=0.5 Hz, 1H, 3 –CH), 4.98 (s, 1H, 5 –OH), 2.54 (s, *J*=0.6 Hz, 3H, 4 –CH₃); calculated mol. wt.: 340; found: EIMS m/z: 340 (M+ peak), 341 (M+1 peak); Molecular formula: C₁₉H₁₃ClO₄; Elemental analysis, calculated, C, 66.97; H, 3.85; found, C, 66.85; H, 4.01.

6-(3-(4-fluorophenyl)acryloyl)-5-hydroxy-4-methyl-2H-chromen-2-one (2b):

Compound 2b was synthesized by reaction of 1 with 4-fluoro-benzaldehyde using SSA as a catalyst; light brown crystals; % yield: 78; MP: 212-217°; Rf: 0.7; UV (λ_{max}): 278; LogP: 3.22; IR (KBr): 3385 (O-H), 3056 (Ar, C-H stretch), 2927 (aliphatic, C-H stretch), 1692 (O-C=O stretch), 1624 (C=O stretch), 1599, 1468, 1429 (C=C ring stretch), 1293, 1158 (Ar, C-H bend, in plane), 1229 (C-F stretch), 834 (Ar, C-H bend, out of plane) and 436 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.18 (d, J=15.1 Hz, 1H, 3' –CH), 7.79 (d, J=7.6 Hz, 1H, 7 –CH), 7.62–7.49 (m, 3H, 2', 2", 6" –CH), 7.15 (t, J=7.7 Hz, 2H, 3", 5" –CH), 6.96 (d, J=7.4 Hz, 1H, 8 –CH), 6.05 (d, J=0.9 Hz, 1H, 3 –CH), 5.71 (s, 1H, 5 –OH), 2.54 (s, J=0.5 Hz, 3H, 4 –CH$_3$); calculated mol. wt.: 324; found: EIMS m/z: 324 (M+ peak), 325 (M+1 peak); Molecular formula:C$_{19}$H$_{13}$FO$_4$; Elemental analysis: calculated, C, 70.37; H, 4.04; found, C, 69.98; H, 4.43.

5-hydroxy-6-(3-(4-hydroxyphenyl)acryloyl)-4-methyl-2H-chromen-2-one (2c):

Compound 2c was synthesized by reaction of 1 with 4-hydroxy-benzaldehyde using SSA as a catalyst; off white crystals; % yield: 85; MP: 239-243°; Rf: 0.45; UV (λ_{max}): 264; LogP: 2.56; IR (KBr): 3176 (-OH stretch), 3033 (Ar, C-H stretch), 2848 (aliphatic, C-H stretch), 1672 (O-C=O stretch), 1631 (C=O stretch), 1579, 1454 (C=C ring stretch), 1289, 1221, 1159 (Ar, C-H bend, in plane), 855, 789, 706 (Ar, C-H bend, out of plane) and 454 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.93 (d, J=15.1 Hz, 1H, 3' –CH), 7.79 (d, J=7.6 Hz, 1H, 7 –CH), 7.39 (d, J=15.0 Hz, 1H, 2' –CH), 7.29 (d, J=7.4 Hz, 2H, 2", 6" –CH), 6.97 (d, J=7.4 Hz, 1H, 8 –CH), 6.87 (d, J=7.4 Hz, 2H, 3", 5" –CH), 6.48 (s, 1H, 5 –OH), 6.05 (d, J=0.6 Hz, 1H, 3 –CH), 3.94 (s, 1H, 4" –OH), 2.53 (s, J=0.5 Hz, 3H, 4 –CH$_3$); calculated mol. wt.: 322; found: EIMS m/z: 322 (M+ peak), 323 (M+1 peak); Molecular formula: C$_{19}$H$_{14}$O$_5$; Elemental analysis; calculated, C, 70.80; H, 4.38; found, C, 70.89; H, 4.54.

5-hydroxy-4-methyl-6-(3-p-tolylacryloyl)-2H-chromen-2-one (2d):

Compound 2d was synthesized by reaction of 1 with 4-methyl-benzaldehyde using SSA as a catalyst; light red crystals; % yield: 89; MP: 209-214°; Rf: 0.65; UV (λ_{max}): 298; LogP: 3.29; IR (KBr): 3432 (-OH stretch), 3020 (Ar, C-H stretch), 2922 (aliphatic, C-H stretch), 1682 (O-C=O stretch), 1622 (C=O stretch), 1465, 1423

(C=C ring stretch), 1288, 1237, 1183 (Ar, C-H bend, in plane), 847, 809, 756 (Ar, C-H bend, out of plane) and 435 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.96 (d, J=15.1 Hz, 1H, 3' –CH), 7.79 (d, J=7.4 Hz, 1H, 7 –CH), 7.47 – 7.35 (m, 3H, 2', 2", 6" –CH), 7.24 (d, J=7.5 Hz, 2H, 3", 5" –CH), 6.97 (d, J=7.4 Hz, 1H, 8 –CH), 6.36 (s, 1H, 5 –OH), 6.05 (d, J=0.6 Hz, 1H, 3 –CH), 2.53 (s, 3H, 4 –CH$_3$), 2.34 (s, 3H, 4" –CH$_3$); calculated mol. wt.: 320; found: EIMS m/z: 320 (M+ peak), 321 (M+1 peak); Molecular formula: C$_{20}$H$_{16}$O$_4$; Elemental analysis: calculated, C, 74.99; H, 5.03; found, C, 75.22, H, 4.89.

5-hydroxy-6-(3-(4-methoxyphenyl)acryloyl)-4-methyl-2H-chromen-2-one (2e):

Compound 2e was synthesized by reaction of 1 with 4-methoxy-benzaldehyde using SSA as a catalyst; reddish brown crystals; % yield: 87; MP: 203-207°; Rf: 0.76; UV (λ_{max}): 287; LogP: 2.89; IR (KBr): 3406 (-OH stretch), 2968 (Ar, C-H stretch), 2932 (aliphatic C-H stretch), 1632 (O-C=O stretch), 1606 (C=O stretch), 1427 (C=C ring stretch), 1248, 1175 (Ar, C-H bend, in plane), 1030 (C-O-C asymmetric stretch), 831 (Ar, C-H bend, out of plane) and 432 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.90 (dd, J=28.5, 11.3 Hz, 2H, 7, 3' –CH), 7.52 (d, J=7.5 Hz, 2H, 2", 6" –CH), 7.43 (d, J=15.0 Hz, 1H, 2'–CH), 6.99 (dd, J=23.7, 7.4 Hz, 3H, 8, 3", 5" –CH), 6.05 (d, J=0.9 Hz, 1H, 3 –CH), 4.97 (s, 1H, 5 –OH), 3.81 (s, 3H, 4" –OCH$_3$), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 336; found: EIMS m/z: 336 (M+ peak), 337 (M+1 peak); Molecular formula: C$_{20}$H$_{16}$O$_5$; Elemental analysis; calculated, C, 71.42; H, 4.79; found, C, 71.59, H, 5.00.

6-(3-(4-(dimethylamino)phenyl)acryloyl)-5-hydroxy-4-methyl-2H-chromen-2-one (2f):

Compound 2f was synthesized by reaction of 1 with p-dimethyl-amino-benzaldehyde using SSA as a catalyst; off white crystals; % yield: 80; MP: 196-200°; Rf: 0.58; UV (λ_{max}): 236; LogP: 3.26; IR (KBr): 3255 (-OH stretch), 2998 (Ar, C-H stretch), 2956 (aliphatic, C-H stretch), 1739 (O-C=O stretch), 1631 (C=O stretch), 1508, 1444 (C=C ring stretch), 1323 (C-N stretch), 1275, 1223, 1175 (Ar, C-H bend, in plane), 1143 (C-N stretch, aliphatic), 879, 839, 730 (Ar, C-H bend, out of plane) and 432 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.89 (dd, J=29.1, 11.2 Hz, 2H, 7, 3' –CH), 7.38 (dd, J=11.3, 6.8 Hz, 3H, 2, 2", 6" –CH), 6.96 (d, J=7.4 Hz, 1H, 8 –CH), 6.75 (d, J=7.5 Hz, 2H, 3", 5" –CH), 6.05 (d, J=0.9 Hz, 1H, 3

–CH), 4.77 (s, 1H, 5 –OH), 2.92 (s, 6H, 4" –N(CH$_3$)$_2$), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 349; found: EIMS m/z: 349 (M+ peak), 350 (M+1 peak); Molecular formula: C$_{21}$H$_{19}$NO$_4$; Elemental analysis: calculated, C, 72.19; H, 5.48; N, 4.01; found, C, 71.98; H, 5.59; N, 3.95.

6-(3-(3,4-dichlorophenyl)acryloyl)-5-hydroxy-4-methyl-2H-chromen-2-one (2g):

Compound 2g was synthesized by reaction of 1 with 3, 4-dichloro-benzaldehyde using SSA as a catalyst; grayish white solid; % yield: 86; MP: 225-230°; Rf: 0.62; UV (λ$_{max}$): 289; LogP: 3.88; IR (KBr): 3386 (-OH stretch), 3054 (Ar, C-H stretch), 2925 (aliphatic C-H stretch), 1702 (O-C=O stretch), 1623 (C=O stretch), 1469, 1429 (C=C ring stretch), 1287, 1242, 1194 (Ar, C-H bend, in plane), 1054 (C-Cl stretch), 881, 815 (Ar, C-H bend, out of plane) and 436 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.95 (d, J=15.1 Hz, 1H, 3' –CH), 7.85 (d, J=7.6 Hz, 1H, 7 –CH), 7.60 (d, J=1.1 Hz, 1H, 2" –CH), 7.49 (d, J=15.0 Hz, 1H, 2' –CH), 7.41 – 7.30 (m, 2H, 5", 6" –CH), 6.95 (d, J=7.4 Hz, 1H, 8 –CH), 6.05 (d, J=0.9 Hz, 1H, 3 –CH), 5.07 (s, 1H, 5 –OH), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 375; found: EIMS m/z: 374 (M+ peak), 375 (M+1 peak); Molecular formula: C$_{19}$H$_{12}$Cl$_2$O$_4$; Elemental analysis: calculated, C, 60.82; H, 3.22; found, C, 60.69; H, 3.44.

6-(3-(3,4-dimethoxyphenyl)acryloyl)-5-hydroxy-4-methyl-2H-chromen-2-one (2h):

Compound 2h was synthesized by reaction of 1 with 3, 4-dimethoxy-benzaldehyde using SSA as a catalyst; brown crystals; % yield: 87; MP: 203-207°; Rf: 0.65; UV (λ$_{max}$): 293; LogP: 2.78; IR (KBr): 3405 (-OH stretch), 3045 (Ar, C-H stretch), 2934, 2837 (aliphatic C-H stretch), 1631 (O-C=O stretch), 1512 (C=O stretch), 1463, 1423 (C=C ring stretch), 1268 (Ar, C-H bend, in plane), 1137 (C-O-C asymmetric stretch), 849, 731 (Ar, C-H bend, out of plane) and 433 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.15 (d, J=15.1 Hz, 1H, 3' –CH), 7.79 (d, J=7.4 Hz, 1H, 7 –CH), 7.57 (d, J=15.1 Hz, 1H, 2' –CH), 7.23 – 7.14 (m, 2H, 2", 6" –CH), 6.98 (dd, J=9.8, 7.4 Hz, 2H, 8, 5" –CH), 6.08 – 5.99 (m, 2H, 3 –CH, 5 –OH), 3.82 (d, J=7.8 Hz, 6H, 3", 4" –OCH$_3$), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 366; found: EIMS m/z: 366 (M+ peak), 367 (M+1 peak); Molecular formula: C$_{21}$H$_{18}$O$_6$; Elemental analysis: calculated, C, 68.85; H, 4.95; found, C, 68.88; H, 4.99.

5-hydroxy-6-(3-(4-hydroxy-3-methoxyphenyl) acryloyl)-4-methyl-2H-chromen-2-one (2i):

Compound 2i was synthesized by reaction of 1 with 4-hydroxy-3-methoxy-benzaldehyde using SSA as a catalyst; brownish white crystals; % yield: 75; MP: 232-238°; Rf: 0.73; UV (λ$_{max}$): 276; LogP: 2.56; IR (KBr): 3390 (-OH stretch), 2922 (Ar, C-H stretch), 2910 (aliphatic C-H stretch), 1650 (O-C=O stretch), 1633 (C=O stretch), 1590, 1453, 1433 (C=C ring stretch), 1273, 1175, 1150 (Ar, C-H bend, in plane), 1025 (C-O-C asymmetric stretch), 806, 730 (Ar, C-H bend, out of plane) and 437 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.95 (d, J=15.0 Hz, 1H, 3' –CH), 7.86 (d, J=7.4 Hz, 1H, 7 –CH), 7.43 (d, J=15.0 Hz, 1H, 2' –CH), 7.08 – 6.99 (m, 2H, 2", 6" –CH), 6.96 (d, J=7.6 Hz, 1H, 8 –CH), 6.86 (d, J=7.4 Hz, 1H, 5" –CH), 6.05 (d, J=0.9 Hz, 1H, 3 –CH), 4.79 (s, 1H, 5 –OH), 4.33 (s, 1H, 4" –OH), 3.82 (s, 3H, 3" –OCH$_3$), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 352; found EIMS m/z: 352 (M+ peak), 353 (M+1 peak); Molecular formula: C$_{20}$H$_{16}$O$_6$; Elemental analysis: calculated, C, 68.18; H, 4.58; found, C, 68.11; H, 4.70.

5-hydroxy-6-(3-(3-hydroxy-4-methoxyphenyl) acryloyl)-4-methyl-2H-chromen-2-one (2j):

Compound 2j was synthesized by reaction of 1 with 3-hydroxy-4-methoxy-benzaldehyde using SSA as a catalyst; off white solid; % yield: 76; MP: 232-235°; Rf: 0.62; UV (λ$_{max}$): 294; LogP: 2.44; IR (KBr): 3299 (-OH stretch), 2935 (Ar, C-H stretch), 2847 (aliphatic C-H stretch), 1673 (O-C=O stretch), 1607 (C=O stretch), 1579, 1443 (C=C ring stretch), 1277, 1246, 1214 (Ar, C-H bend, in plane), 1120 (C-O-C asymmetric stretch), 865, 791, 758 (Ar, C-H, bend, out of plane) and 438 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.90 (dd, J=23.1, 11.2 Hz, 2H, 7, 3' –CH), 7.43 (d, J=15.1 Hz, 1H, 2' –CH), 7.12 – 7.02 (m, 2H, 2", 6" –CH), 6.95 (d, J=7.6 Hz, 1H, 8 –CH), 6.88 – 6.81 (m, 1H, 5" –CH), 6.05 (d, J=0.6 Hz, 1H, 3 –CH), 5.16 (s, 1H, 5 –OH), 3.88 (s, 1H, 3" –OH), 3.81 (s, 3H, 4" –OCH$_3$), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 352; found EIMS m/z: 352 (M+ peak), 353 (M+1 peak); Molecular formula: C$_{20}$H$_{16}$O$_6$; Elemental analysis: calculated, C, 68.18; H, 4.58; found, C, 68.20; H, 4.68.

5-hydroxy-4-methyl-6-(3-(3,4,5-trimethoxyphenyl) acryloyl)-2H-chromen-2-one (2k):

Compound 2k was synthesized by reaction of 1 with 3,4,5-trimethoxy-benzaldehyde using SSA as a catalyst;

brownish white crystals; % yield: 84; MP: 202-206°; Rf: 0.54; UV (λ_{max}): 280; LogP: 2.65; IR (KBr): 3372 (-OH stretch), 3008 (Ar, C-H stretch), 2939, 2839 (aliphatic C-H stretch), 1691 (O-C=O stretch), 1623 (C=O stretch), 1590, 1462, 1420 (C=C ring stretch), 1279, 1233, 1184 (Ar, C-H bend, in plane), 1125 (C-O-C asymmetric stretch), 835, 754, 722 (Ar, C-H bend, out of plane) and 438 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.96 (d, J=15.1 Hz, 1H, 3' –CH), 7.79 (d, J=7.6 Hz, 1H, 7 –CH), 7.40 (d, J=15.1 Hz, 1H, 2' –CH), 7.11 (s, 1H, 5 –OH), 6.96 (d, J=7.6 Hz, 1H, 8 –CH), 6.60 (s, 2H, 2", 6" –CH), 6.05 (d, J=0.6 Hz, 1H, 3 –CH), 3.80 (s, 9H, 3", 4", 5" –OCH$_3$), 2.56 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 396; found EIMS m/z: 396 (M+ peak), 397 (M+1 peak); Molecular formula: C$_{22}$H$_{20}$O$_7$; Elemental analysis: calculated, C, 66.66; H, 5.09; found, C, 66.59; H, 5.20.

5-hydroxy-6-(3-(4-hydroxy-3,5-dimethoxyphenyl) acryloyl)-4-methyl-2H-chromen-2-one (2l):

Compound 2l was synthesized by reaction of 1 with 4-hydroxy-3,5-dimethoxy-benzaldehyde using SSA as a catalyst; grayish white solid; % yield: 80; MP: 229-234°; Rf: 0.51; UV (λ_{max}): 270; LogP: 2.34; IR (KBr): 3267 (-OH stretch), 2968 (Ar, C-H stretch), 2940, 2839 (aliphatic C-H stretch), 1671 (O-C=O stretch), 1631 (C=O stretch), 1586, 1464, 1425 (C=C ring stretch), 1276, 1208, 1142 (Ar, C-H bend, in plane), 1108 (C-O-C asymmetric stretch), 841, 830, 728 (Ar, C-H bend, out of plane) and 433 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.92 (d, J=15.1 Hz, 1H, 3' –CH), 7.80 (d, J=7.4 Hz, 1H, 7 –CH), 7.39 (d, J=15.1 Hz, 1H, 2' –CH), 6.97 (d, J=7.4 Hz, 1H, 8 –CH), 6.72 (s, 1H, 5 –OH), 6.60 (s, 2H, 2", 6" –CH), 6.05 (d, J=0.9 Hz, 1H, 3 –CH), 3.87 (s, 1H, 4" –OH), 3.79 (s, 6H, 3", 5"–OCH$_3$), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 382; found EIMS m/z: 382 (M+ peak), 383 (M+1 peak); Molecular formula: C$_{21}$H$_{18}$O$_7$; Elemental analysis: calculated, C, 65.96; H, 4.74; O, 29.29; found, C, 67.00; H, 5.00.

6-(3-(1H-indol-3-yl)acryloyl)-5-hydroxy-4-methyl-2H-chromen-2-one (2m):

Compound 2m was synthesized by reaction of 1 with 1H-indole-3-carbaldehyde using SSA as a catalyst; white solid; % yield: 76; MP: 250-254°; Rf: 0.55; UV (λ_{max}): 298; LogP: 2.56; IR (KBr): 3298 (-OH stretch), 3180 (Ar, C-H stretch), 2931 (aliphatic C-H stretch), 1631 (O-C=O stretch), 1607 (C=O stretch), 1509, 1442 (C=C ring stretch), 1276, 1208, 1143 (Ar, C-H bend, in plane), 879, 839, 729 (Ar, C-H bend, out of plane) and

442 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.90 (d, J=15.1 Hz, 1H, 3' –CH), 7.76 (d, J=7.6 Hz, 1H, 7 –CH), 7.57 (s, 1H, 2" –CH), 7.40 (s, 1H, 3" –NH), 7.32 (d, J=15.1 Hz, 1H, 2' –CH), 7.21 – 6.99 (m, 4H, 5", 6", 7", 8" –CH), 6.92 (d, J=7.6 Hz, 1H, 8 –CH), 6.00 (d, 1H, 3 –CH), 5.10 (s, 1H, 5 –OH), 2.43 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 345; found EIMS m/z: 345 (M+ peak), 346 (M+1 peak); Molecular formula: C$_{21}$H$_{15}$NO$_4$; Elemental analysis: calculated, C, 73.03; H, 4.38; N, 4.06; O, 18.53; found, C, 73.00; H, 4.58; N, 3.99.

6-(3-(1H-pyrrol-2-yl)acryloyl)-5-hydroxy-4-methyl-2H-chromen-2-one (2n):

Compound 2n was synthesized by reaction of 1 with 1H-pyrrole-2-carbaldehyde using SSA as a catalyst; grayish crystals; % yield: 80; MP: 219-224°; Rf: 0.57; UV (λ_{max}): 287; LogP: 1.52; IR (KBr): 3272 (-OH stretch), 3179 (Ar, C-H stretch), 2938 (aliphatic C-H stretch), 1739 (O-C=O stretch), 1630 (C=O stretch), 1508, 1444 (C=C ring stretch), 1275, 1175, 1143 (Ar, C-H bend, in plane), 879, 791, 730 (Ar, C-H bend, out of plane) and 439 cm^{-1} (C=C ring bend, out of plane); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.79 (dd, J=15.9, 11.6 Hz, 3H, 7, 3' –CH, 2"-NH), 7.35 (d, J=15.0 Hz, 1H, 2' –CH), 7.18 (d, J=7.4, 1.3 Hz, 1H, 3" –CH), 6.95 (d, J=7.4 Hz, 1H, 8 –CH), 6.37 (d, J=7.5 Hz,1H, 5" –CH), 6.17 (t, J=7.4 Hz, 1H, 4" –CH), 6.05 (d, J=0.6 Hz, 1H, 3 –CH), 5.85 (s, 1H, 5 –OH), 2.54 (s, 3H, 4 –CH$_3$); calculated mol. wt.: 295; found EIMS m/z: 295 (M+ peak), 296 (M+1 peak); Molecular formula: C$_{17}$H$_{13}$NO$_4$; Elemental analysis: calculated, C, 69.15; H, 4.44; N, 4.74; O, 21.67; found, C, 69.33; H, 4.59; N, 4.75.

Drug-likeness and *in silico* ADME/T study:

Drug-likeness and lead-likeness of synthesized compounds was assessed as per Lipinski's rule of five and Jorgensen's rule of three, respectively[35,36]. The rule of five states that a compound should have a molecular weight <500 Da, LogP ≤5, hydrogen bond donors (HBD) ≤5 and hydrogen bond acceptors (HBA) ≤10, while the rule of three states that a compound should have LogP ≤3, molecular weight <300 Da, HBD ≤3, HBA ≤3 and rotatable bonds ≤3. Polar surface area (PSA) is also a very good property for characterizing bioavailability, absorption (including intestinal absorption), blood-brain barrier penetration and Caco-2 permeability of a drug[37]. Molecules with PSA less than 140 Å^2 (angstroms squared) tends to be good at permeating cell membranes and also possesses

better drug-likeness properties[38]. In particular, compounds which meet only two criteria, 10 or fewer rotatable bonds and PSA equal to or less than 140 Å^2 are supposed to have good oral bioavailability[39]. *In silico* prediction of ADME properties and toxicity risks i.e. mutagenicity and tumorigenicity, irritant and reproductive effects, of compounds was done by Qikprop module of Schrödinger suite 2010[40] and OSIRIS property explorer[41], respectively.

Biological activity:

C8166 T-cells were cultured in Dulbecco modified Eagle medium and RPMI 1640 medium, each supplemented with 10 % fetal bovine serum. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy adult volunteers and cultured as described in previous study[42]. Antiretroviral drug, raltegravir (RAL) was obtained from the NIH AIDS Research and Reference Reagent Program. HIV-1 HxBru-Gluc virus was generated by transfecting 293T cells with corresponding HIV-1 proviral DNAs, as described previously[34,43].

Cytotoxicity assay:

The trypan blue exclusion method was used to analyze the cytotoxicity of synthesized compounds, which notably distinguishes non-viable cells from viable cells[33]. C8166 T-cells (0.1×10^6/well) were seeded in 96-well plate, and treated with corresponding concentrations of compounds (i. e. 0.25-2.0 μg/ml). Negative control was not seeded with C8166 T-cells. After 48 h treatment, viable (unstained) and nonviable (stained) cells were counted by TC 20 automated cell counter (Bio-RAD). The viable cell count was normalized as a percentage of the negative control and organized using following symbols –, +, ++, +++, which represent the mean cell viability of 80-100, 60-79, 40-59 and below 40 %, respectively.

Antiretroviral activity:

Synthesized compounds (2 mg/ml) were individually dissolved in dimethyl sulfoxide and stored at –20° until use. To screen the antiHIV-1 activity, each compound was added to a single well of 96-well plates at a final concentration of 0.25, 0.5, 1 and 2 μg/ml, separately. Meanwhile, 1×10^6 C8166 T cells were infected with HIV-1 HxBru-Gluc virus (HIVp24 10-50 pg/1×10^6 cells) for two hours, and this cell-virus mixture was immediately transferred to the 96-well plate containing the synthesized compounds such that each well contained a final concentration of 10^4 cells. Three days post-infection, supernatant were collected and the levels of virus infection were monitored by measuring the Gluc activity in the supernatants from each infected cell culture using a POLARstar OPTIMA microplate luminometer (BMG Labtech, Germany). Gluc activity measurement was used as biomarker to detect the HIV infection[34,44].

RESULTS AND DISCUSSION

The synthesis of target compounds 2a-n was carried out in two steps (fig. 1). Resacetophenone condensed with ethyl acetoacetate using aluminium chloride as condensing agent, to produce the coumarin intermediate 1 (5-hydroxy-6-acetyl-4-methylcoumarin) in high yield[25]. Claisen-Schmidt condensation of 1 with substituted bezaldehydes using SSA as a catalyst resulted in the formation of 6-acetyl-coumarin derivatives, 2a-n. IR bands at 3344 (-OH stretch), 2942 (Ar, C-H stretch), 2841 (aliphatic, C-H stretch) 1727 (C=O stretch, coumarin), 1662 (C=O stretch), 1585, 1456, 1421 (C=C, ring stretch), 1290, 1234, 1126 (Ar, C-H bend, in plane), 845, 794, 697 (Ar, C-H bend, out of plane), 423 (C=C ring bend, out of plane) showed the characteristic functional groups in 1. NMR spectrum of 1 showed two peaks at δ 2.0-3.0 which were attributed to three protons of -CH$_3$ at 4th position and -COCH$_3$ at 6th position of coumarin ring, respectively. The chemical shifts at δ 5.92, 7.80 and 6.87 were due to single protons at 3, 7 and 8 positions of coumarin ring. Mass spectrum of 1 showed characteristic molecular ion peak at m/z 218. Fragmentation of the molecule

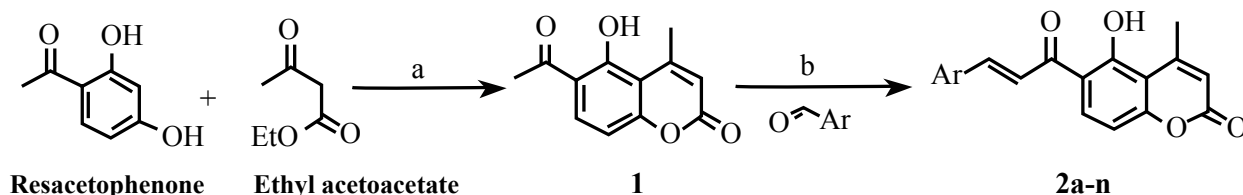**Fig. 1: Reaction scheme for synthesis of 2a-n**
Reagent and conditions: (a) AlCl$_3$, nitrobenzene, (b) SSA, substitutes bezaldehydes. 2a:Ar=4-Cl-Ph; 2b:Ar=4-F-Ph; 2c:Ar=4-OH-Ph; 2d:Ar=4-Me-Ph; 2e: Ar=4-MeO-Ph; 2f: Ar=4-(dimethylamino)-Ph; 2g: Ar=3,4-di-Cl-Ph; 2h:Ar=3,4-di-MeO-Ph; 2i:Ar=4-OH-3-MeO-Ph; 2j: Ar=3-OH-4-MeO-Ph; 2K:Ar=3,4,5-tri- MeO-Ph; 2l: Ar=4-OH-3,5-di-MeO-Ph; 2m:Ar=3-indole; 2n: Ar=2-pyrrol
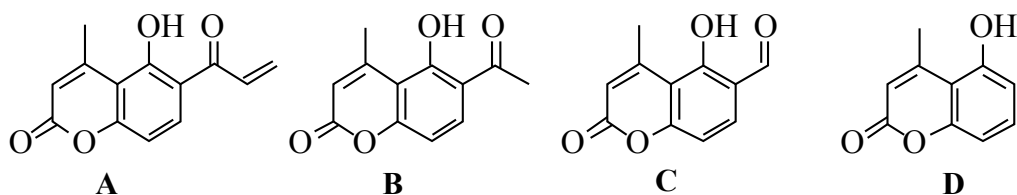
showed two peak at m/z 175 (due to removal of –COCH$_3$ fragment from parent ion) and m/z 43 (due to –COCH$_3$ fragment). Compounds 2a-n, which were derived from 1 showed only one peak in NMR spectrum at δ 2.0-3.0 due to three protons of -CH$_3$ group attached at 4 position of coumarin ring. This showed that –COCH$_3$ group of 1 was involved in formation of C=C bond. Mass spectrum of 2a showed M$^+$ peak at 340, M-15 at 325 and M-16 peak at 324, which confirm the molecular weight and CH$_3$ and OH fragments of 2a, respectively. Common fragments of mass 229, 216, 203 and 275 were found in all synthesized compounds (2a-n; fig. 2). The above spectral characterization was further confirmed by elemental analysis, which was in agreement with calculated percent values of C, H and N.

As per Lipinski's rule of five, an orally active compound has not more than one violation and as per Jorgensen's rule of three, if a compound complies with all parameters, then it is more likely to be orally available[45,46]. Compounds 2a-n complied with Lipinski's rule of five and Jorgensen's rule of three, as they have shown no rule violations in drug-likeness study (Table 1). All compounds showed values of ADME parameters i.e. LogS (aqueous solubility), #rot (number of rotatable bonds), #met (number of metabolic reactions), CNS activity, Caco2 permeability (permeability through intestinal epithelium), LogBB (ability to cross blood brain barrier), LogKp (permeability through skin), LogKhsa (serum protein binding) and % HOA (percent human oral absorption), within reference limits (Table 2). *In silico* toxicity study showed that all synthesized compounds have low toxicity risks for mutagenicity, tumorigenicity, irritant and reproductive effects (Table 3).

Cytotoxicity assay showed that all synthesized compounds were devoid of cytotoxicity, as their cell viability count was in the range of 80-100 %, except compound no. 2h, 2i and 2j, which showed cell viability count in the range of 60-79 %. The result of *in silico* toxicity study of synthesized compounds was in agreement with *in vitro* toxicity study results. The antiretroviral activity showed that most of synthesized compounds were effective in achieving protection against the cytopathic effect of HIV. Compounds with electron withdrawing groups i.e. 2a, 2g and 2h showed higher activity amongst all synthesized compounds as their IC$_{50}$ value was in the range of 0.35-4.7 μM. Fig. 3 showed that activity of compounds 2a, 2g and 2h was comparable with the activity of antiretroviral drug RAL. Structure activity relationship of synthesized compounds was explained by dividing the structure in two parts i.e. A and B. It was found that part B was crucial for activity as change in substitution resulted in variation of antiHIV activity while part A was kept unsubstituted (fig. 4). Compound with para chloro (Cl) substitution on phenyl ring of part B showed high antiHIV activity. As Cl was replaced with flouro (F) group, activity was reduced due to less electron affinity of F as compared to Cl. Introduction of hydroxyl (OH), methyl (CH$_3$), methoxy (OCH$_3$) and dimethylamino (N-(CH3)$_2$) groups further reduced activity as they are less electronegative as compared to Cl and F. The highest activity was found with 3,4-dimethoxy

TABLE 1: DRUG LIKENESS STUDY OF SYNTHESIZED COMPOUNDS

Comp.	MW	HBD	HBA	LogP	PSA	ROT	ROF
2a	340	0	4	3.611	81.025	0	0
2b	324	0	4	3.341	81.075	0	0
2c	322	1	5	2.597	103.380	0	0
2d	320	0	4	3.374	81.145	0	0
2e	336	0	5	3.166	89.007	0	0
2f	349	0	5	3.499	84.496	0	0
2g	375	0	4	4.066	81.027	0	0
2h	366	0	6	3.220	96.347	0	0
2i	352	1	6	2.746	107.210	0	0
2j	352	1	6	2.675	110.741	0	0
2k	396	0	7	3.311	103.841	0	0
2l	382	1	7	2.885	114.227	0	0
2m	345	1	4	3.513	94.404	0	0
2n	295	1	4	2.651	93.144	0	0
RAL	444	2	11	1.673	166.711	0	1
RR	<500	≤5	≤10	≤5	<140	0	1

ROT: Jorgensen's rule-of-three violations, ROF: Lipinski's rule-of-five violations, RR: Reference range for 95 % of known drugs

Fig. 2: Common molecular fragments of 6-acetyl-coumarin derivatives
A. m/z: 229; B. m/z: 216; C. m/z: 203; D. m/z: 175

TABLE 2: *IN SILICO* ADME PROFILE OF SYNTHESIZED COMPOUNDS

Comp.	#rot	#met	LogS	CNS	Caco-2	LogBB	LogKp	LogKhsa	% HOA
2a	5	2	-4.746	-1	573.585	-0.837	-2.399	0.236	100
2b	5	2	-4.347	-1	572.893	-0.881	-2.365	0.154	95.871
2c	6	3	-4.025	-2	181.524	-1.563	-3.268	0.119	82.585
2d	5	3	-4.564	-2	579.804	-1.014	-2.427	0.282	96.160
2e	6	3	-4.104	-2	590.902	-1.061	-2.306	0.079	95.089
2f	6	3	-4.728	-2	568.606	-1.123	-2.428	0.233	96.737
2g	5	2	-5.417	-1	573.912	-0.716	-2.529	0.358	100.000
2h	7	4	-4.125	-2	585.011	-1.138	-2.367	0.046	95.327
2i	7	4	-4.220	-2	264.441	-1.491	-2.972	0.108	86.378
2j	7	4	-4.335	-2	185.853	-1.669	-3.329	0.131	83.221
2k	8	5	-4.299	-2	577.417	-1.239	-2.449	0.032	95.756
2l	8	5	-4.432	-2	301.893	-1.528	-2.938	0.111	88.225
2m	5	2	-5.073	-2	327.267	-1.280	-2.601	0.466	92.528
2n	5	2	-3.917	-2	383.962	-1.141	-2.725	0.115	88.720
RAL	7	4	-4.629	-2	84.453	-2.04	-4.262	-0.278	58.268
RR	0 to 15	<7	-6.5to0.5	-2to2	<25 poor >500 good	-3 to1.2	-8to-1	±1.5	<25 poor

RR: Reference range for 95 % of known drugs

TABLE 3: *IN SILICO* TOXICITY RISK ASSESSMENT OF SYNTHESIZED COMPOUNDS

Comp.	Toxicity risks			
	MUT	TUM	IRR	RPRD
2a	Low	Low	Low	Low
2b	Low	Low	Low	Low
2c	Low	Low	Low	Low
2d	Low	Low	Low	Low
2e	Low	Low	High	Medium
2f	High	High	Low	Low
2g	Low	Low	Low	Low
2h	Low	Low	Low	Low
2i	Low	Low	Low	Low
2j	Low	Low	Low	Low
2k	Low	Low	Low	Low
2l	Low	Low	Low	Low
2m	Low	Low	Low	Low
2n	Low	Low	Low	Low
RAL	Low	Low	Low	Low

MUT: mutagenic, TUM: tumorigenic, IRR: irritant, RPRD: reproductive

Fig. 3: Antiretroviral activity of synthesized compounds against HIV-1 infection

■ PC; ■ NC; ■ RAL; ■ synthesized compounds (2a-n). HIV infection (HIVp24 10-50 pg/1×106 cells)

substituent but it showed cell viability count 60-79 % in cell culture, which was less than the cell viability count of compound 2a and 2g (Table 4). Compound with 3,4-dichloro substitution (i.e. 2 g) was equipotent as 2a. This shows that electronegative substituents were favourable for activity.

In conclusion, the present study indicated that all synthesized compounds (2a-n) have the potential of inhibiting HIV-1 infection in the low micromolar range. It was also found that early prediction of ADME/T properties reduce the risk of failure of compounds in

Fig. 4: Structure activity relationship of 6-acetyl coumarin derivatives

further studies. Still there is a scope of determination of exact mechanism of action, experimental pharmacokinetic profile and subsequent modification in synthesized compounds to improve activity, which is under progress in our laboratory.

TABLE 4: *IN VITRO* CYTOTOXICITY AND ANTIRETROVIRAL ACTIVITY OF SYNTHESIZED COMPOUNDS

Comp.	Cytotoxicity 0.25-2.0 µg/ml	Mean cell viability (%)	AntiHIV activity[a] IC_{50} (µM)
2a	-	91±1.5	4.7±0.12
2b	-	87±2.1	9.8±1.23
2c	-	89±3.6	36.33±2.09
2d	-	85±2.2	10.62±1.07
2e	-	84±1.9	10.71±1.23
2f	-	87±1.7	17.47±2.03
2g	-	89±1.3	4.5±0.23
2h	+	75±2.1	0.35±0.13
2i	+	72±3.9	21.02±1.92
2j	+	69±2.8	12.5±1.11
2k	-	85±2.1	17.82±2.42
2l	-	90±3.2	14.65±1.34
2m	-	87±2.4	20.57±2.09
2n	-	81±1.6	15.6±1.23
RAL	-	95±1.1	0.09±0.03

'-' and '+' represents the mean cell viability of 80-100 % and 60-79 %, respectively. [a]Data represent average results ±standard deviation from at least three separate experiments

Conflicts of interest:

There are no conflicts of interest.

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