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Synthesis and Bioevaluation of Substituted Chalcones, Coumaranones and other Flavonoids as anti-HIV agents

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Abstract. A series of chalcone, flavone, coumaranone and other flavonoid compounds were screened for their anti HIV-1 activity in two cell culture models using TZM-bl and PM1 cells. Within the systems evaluated, the most promising compounds contained either an α- or β-hydroxy-carbonyl motif within their structure (e.g., **8** and **9**)**.** Efficacious substituents were identified and used to design new HIV inhibitors with increased potency and lower cytotoxicity. Of the scaffolds evaluated, specific chalcones were found to provide the best balance between anti-HIV potency and low host cell toxicity. Chalcone **8l** was shown to inhibit different clinical isolates of HIV in a dose-dependent manner (e.g., IC_{50} typically $\leq$ 5 μM). Inhibition of HIV infection experiments using TZM-bl cells demonstrated that chalcone **8l** and flavonol **9c** had IC_{50} values of 4.7 μM and 10.4 μM, respectively. These insights were used to design new chalcones **8o** and **8p.** Rewardingly, chalcones **8o** and **8p** (at 10 μM) each gave >92% inhibition of viral propagation without impacting PM1 host cell viability. Inhibition of viral propagation significantly increased (60% to 90%) when PM1 cells were pre-incubated with chalcone **8o**, but not with the related flavonol **9c**. These results suggested that chalcone **8o** may be of value as both a HIV prophylactic and therapy. In summary, *O*-benzyl-substituted chalcones were identified as promising anti-HIV agents for future investigation.

1.1 Introduction

Human immunodeficiency virus-1 (HIV-1) leads to acquired immunodeficiency syndrome (AIDS) via destruction of T-cells after the virus successfully enters and replicates within the human host. [1] The current treatment for HIV/AIDS includes the use of antiretroviral therapy (ART) to control viral load and indirectly minimize the spread of the virus.[1] This treatment consists of a multi-drug cocktail that inhibits various processes required for HIV propagation. Although relatively successful in slowing disease progression and enabling HIV patients to live longer, long-term ART often leaves patients susceptible to unwanted side effects and drug-resistant viral quasi-species.[2] Furthermore, HIV-infected individuals have higher rates of chronic disease, cancer, and immune defects compared to the general population[3] and HIV/AIDS remains an important epidemic of global concern. According to the 2014 UNAIDS report, there are nearly 35 million infected people worldwide and roughly 2.1 million new cases each year.[3a] Alarmingly, the HIV-1 acquisition rate continues to outpace morbidity from HIV/AIDS, causing a steady global increase in the number of infected people each year. Without a vaccine, attention must be given to identifying candidate drugs that are inexpensive to produce in large scale and show promise for preventing transmission of HIV-1 between serodiscordant sexual partners or for suppressing viral load with minimal daily pill burden and side effects.

Flavonoids are a class of compounds that are typically found in nature in plants, and have demonstrated a number of medicinal properties. They exhibit antitumor, anti-inflammatory, and antiviral activity, as well as the ability to act as an antioxidant.[4] Chalcones are common synthetic precursors to flavones and other flavonoid structures. The chalcones themselves have also demonstrated inhibitory activity against a variety of enzymes and have been shown to be useful

in treating inflammatory or infectious diseases.[5] Their broad range of medicinal properties, along with the simplicity of synthesizing these compounds make them a desirable compound class to be further investigated.

Flavonoids have also been evaluated as antiviral agents both in our laboratory[6] and others.[7] For example, baicalein **1** and quercetin **2** (Figure 1) are examples of naturally-occurring flavonoids with activity against human cytomegalovirus (HCMV),[8] as well as human immunodeficiency virus (HIV).[9] The chalcone butein **3** has been shown to have moderate activity against HIV-1 protease[10] and xanthohumol **4** has been shown to have antiviral activity against several herpes viruses, including HCMV.[11] Previous literature also indicates that 5-bromo-2-hydroxy-3-[3-(2,3,6-trichlorophenyl)acryloyl benzoic acid **5** is effective against HIV-1 integrase and inhibits the replication of HIV-1 with an IC_{50} of 8.7 μM.[2]

Prior work in our group investigated the ability of both chalcone and flavonoid structures to inhibit the US28 signaling of HCMV.[6] Since there was literature precedent for the anti-HIV activity of these types of compounds (Figure 1),[4b, 12] we elected to test the efficacy of a small library of chalcone and flavonoid compounds against HIV.[6] Specifically, we examined the anti-HIV-1 activities of the flavonoid library in two different cell culture assays. In addition, we assessed each compound's toxicity profile in two cell lines. These studies demonstrated that the chalcone series (**8l-8p**) represents a drug scaffold capable of HIV-1 suppression in the low μM range along with low toxicity to human cells.

In this report, we describe our findings with this flavonoid series (Figure 1) in the inhibition of HIV as well as our efforts to improve their efficacy and to reduce their toxicity to host cells.

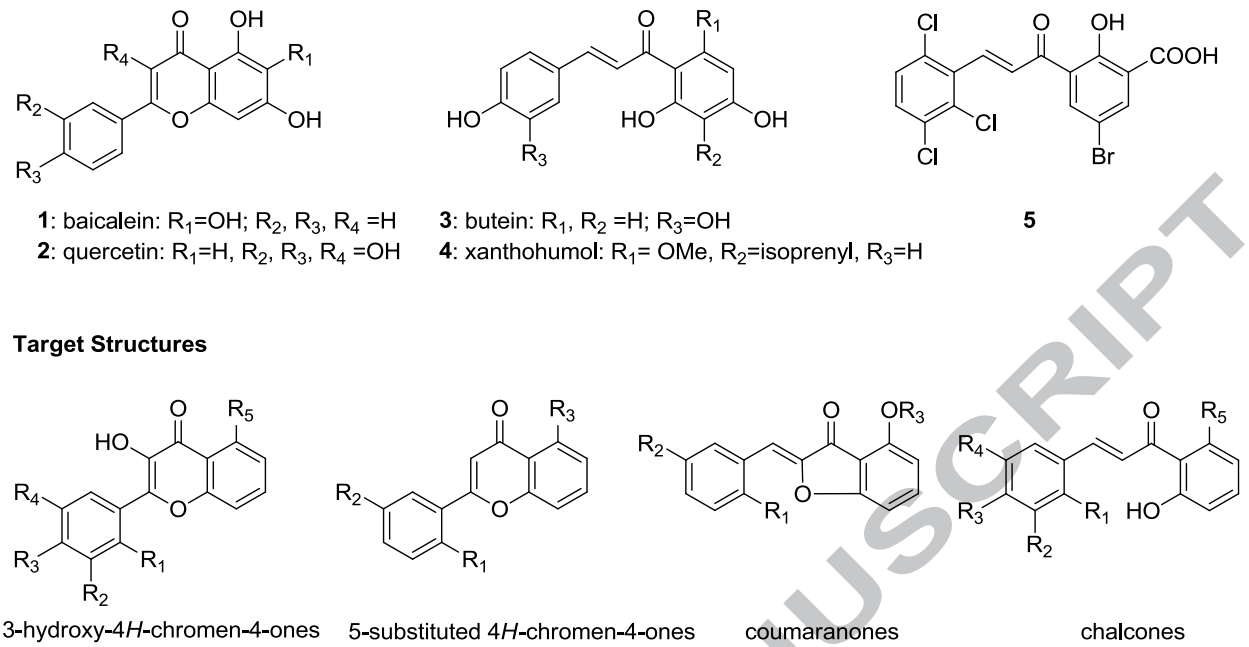


Figure 1. Structures of bioactive flavones (**1, 2**) and chalcone motifs **3-5** and synthetic target structures

2.1 Materials and Methods.

General. The compounds **7a-d**, **8a-m, 9a-j,** and **11-13** were prepared previously and the new chalcones **8n-8p** were prepared using published methods. [6] The coumaranones **14a** and **14b** were made using a modified hydrogen peroxide method for generating aurones.[13] Coumaranone **14c** was made previously and was incorrectly assigned as flavonol **9f** in a prior reference.[6] All tested compounds were ≥ 95% pure as evidenced by their elemental analyses. Elemental analyses for **8n, 8o, 8p, 14a** and **14b** are provided in the Supporting Information.

5-Bromo-2-(octyloxy)benzaldehyde (7e).[14] 5-Bromosalicylaldehyde (1.01 g, 5 mmol) and 1-bromooctane (1.46 g, 7.5 mmol), K_2CO_3 (1.38 g: 10 mmol) were combined with DMF (5 mL) and stirred at rt for 2 days. The DMF was removed under reduced pressure and CH_2Cl_2 added and the solution washed with 1M HCl. The organic layer was separated, dried over anhydrous

Na_2SO_4, filtered and concentrated to give 2.63 g of crude. Column chromatography (10% CH_2Cl_2 in hexane to elute the 1-bromooctane impurity followed by 40% CH_2Cl_2 in hexane to elute the product) gave **7e** as a white solid, 1.26 g (80% yield). ^{1}H NMR ($CDCl_3$): δ 10.34 (s, 1H, CHO), 7.84 (d, 1H), 7.53 (dd, 1H), 6.80 (d, 1H), 3.98 (t, 2H), 1.77 (m, 2H), 1.41 (m, 2H), 1.24 (m, 8H), 0.82 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 188.5, 160.4, 138.2, 130.8, 126.2, 114.6, 113.2, 69.0, 31.8, 29.3, 29.2, 29.0, 26.0, 22.6, 14.1.

1-(2-Benzyloxy-6-hydroxy-phenyl)-3-(5-bromo-2-octyloxy-phenyl)-propenone (8n). Aldehyde **7e** (0.155g: 0.5mmol) and 6-*O*-benzyl-2-hydroxy-acetophenone[6] (0.12 g: 0.5 mmol) were combined and dissolved in methanol (3 mL) and combined with 40% KOH in MeOH (2 mL) at rt. The orange solution was stirred for 2h at 80^{o}C. Workup included evaporating the solvent and adding water (20 mL) followed by addition of CH_2Cl_2. 1M HCl (20 mL) was added until the pH of the water layer was pH 1. The organic layer was then separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to provide a crude orange oil. Column chromatography was performed (100% CH_2Cl_2, then 50:50 Hexane/CH_2Cl_2) to obtain the pure product (179 mg, 67%). **8n**: m.p. 138-140^{o}C, ^{1}H NMR ($CDCl_3$): δ 13.09 (s, 1H, OH), 8.04 (d, 1H, J= 15.9Hz), 7.80 (d, 1H, J= 15.9 Hz), 7.40 (m, 2H), 7.37 (m, 2H), 7.33 (d, 1H, J = 2.4 Hz), 7.28 (m, 3H), 6.73 (d, 1H, J=8.8 Hz), 6.65 (dd, 1H, J=8.3 Hz and 1 Hz), 6.53 (dd, 1H, J= 8.3 Hz and 1 Hz), 5.14 (s, 2H), 3.91 (t, 2H, J=6.7 Hz), 1.78 (m, 2H), 1.43 (m, 2H), 1.29 (m, 8H), 0.87 (t, 3H, J=6.85 Hz). ^{13}C NMR ($CDCl_3$)**:** δ 194.58, 164.76, 160.01, 156.99, 136.18, 135.81, 135.46, 133.75, 130.28, 128.84, 128.68, 128.58, 128.45, 127.46, 126.11, 113.69, 112.64, 112.22, 111.11, 102.58, 71.26, 68.83, 31.72, 29.26, 29.14, 28.96, 25.94, 22.60, 14.05. Anal. $C_{30}H_{33}BrO_4$: C, H. HRMS $C_{30}H_{33}BrO_4$ (M + H): theory 537.1635; found 537.1636.

1-(2-Benzyloxy-6-hydroxy-phenyl)-3-(5-bromo-2-ethoxy-phenyl)-propenone (8o). 5-Bromo-2-ethoxy-benzaldehyde **7a** (473 mg, 2.06 mmol), 1-(2-Benzyloxy-6-hydroxy-phenyl)-ethanone[6] (500 mg, 2.06 mmol), and MeOH (9 mL) were combined and KOH in methanol (40% weight/volume, 8.24 mL) added at room temperature. A water condenser was attached to the flask, and the orange solution quickly turned dark red upon heating and was stirred at reflux in an oil bath at 85 °C for 3h. Workup involved evaporation of the solvent and addition of 1N HCl until the solution was at pH 1, followed by extraction with ethyl acetate (twice). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give an orange solid. Column chromatography was performed (10% EtOAc:hexane) to purify the product. A crystallization step was then performed (ethyl acetate/hexane) to obtain the pure product (75% yield). **8o**: m.p. 107-109^{o}C, ^{1}H NMR ($CDCl_3$): δ 13.08 (s, 0.76H), 8.07 (d, 1H), 8.03 (d, 0.53H), 7.81 (d, 0.58H), 7.78 (d, 0.47H), 7.41 (m, 2H), 7.37 (m, 2H), 7.33 (d, 1H), 7.29 (m, 3H), 6.73 (d, 2H, J = 8.8 Hz), 6.66 (d, 1H, J = 1.2 Hz), 6.64 (d, 1H, J = 1 Hz), 6.55 (d, 1H, J = 1 Hz), 6.53 (d, 1H, J = 0.7 Hz), 5.14 (s, 2H), 4.00 (1, 2H), 1.54 (s, 0.93H), 1.41 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 165.05, 160.17, 157.1, 136.20, 133.87, 130.38, 128.76, 127.59, 126.20, 113.68, 112.76, 102.71, 71.36, 64.31, 14.68; Anal. $C_{24}H_{21}BrO_4$: C, H; HRMS For $C_{24}H_{22}BrO_4$ (M + H): theory 453.0701; found 453.0691.

1-(2-Benzyloxy-6-hydroxy-phenyl)-3-(5-chloro-2-ethoxy-phenyl)-propenone (8p). 5-Chloro-2-ethoxy-benzaldehyde **7c** (500 mg, 2.7mmol), 1-(2-Benzyloxy-6-hydroxy-phenyl)-ethanone[6] (655 mg, 2.7mmol), and MeOH (10 mL) were combined and KOH in methanol (40% weight/volume, 10 mL) added at room temperature. A water condenser was attached to the flask, and the yellow solution quickly turned red upon heating and was stirred at reflux in an oil bath at 85 °C for 4 hours. Workup involved evaporation of the solvent and addition of 1N HCl until the

solution was at pH 1, followed by extraction with ethyl acetate (twice). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give an orange/yellow solid (1.3 g). Column chromatography was performed (15% ethyl acetate:hexane) to obtain the pure product (56% yield). **8p**: m.p. 103-105^oC, ^1H NMR ($CDCl_3$): δ 13.08 (s, 0.76H), 8.09 (d, 2H, J = 15.9 Hz), 7.82 (d, 2H, J = 15.7 Hz), 7.78 (d, 0.47H), 7.43-7.25 (m, 8H), 7.15 (d, 2 H, J = 2.7 Hz), 6.79 (d, 2H, J = 8.8), 6.66 (dd, 2H, J = 8.6), 6.55 (dd, 2H, J = 8.2 Hz), 5.14 (s, 2H), 4.00 (q, 2H), 1.54 (s, 0.93H), 1.41 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 194.79, 164.98, 160.01, 156.37, 136.49, 135.92, 135.53, 130.93, 128.77, 128.60, 127.64, 127.35, 125.62, 125.46, 113.26, 112.25, 111.24, 102.61, 71.40, 64.38, 14.69. Anal. $C_{24}H_{21}ClO_4$: C, H.; HRMS $C_{24}H_{22}ClO_4$ (M + H): theory 409.1207; found 409.1211.

4-Benzyloxy-2-(5-bromo-2-ethoxy-benzylidene)-benzofuran-3-one (14a). (*E*)-1-(2-(benzyloxy)-6-hydroxyphenyl)-3-(5-bromo-2-ethoxyphenyl)prop-2-en-1-one, **8o** (174 mg, 0.38 mmol) was dissolved in a 3 M KOH solution in 96% ethanol (2.7 mL). Then 35% H_2O_2 (0.8 mL) was added dropwise to the mixture cooled in an ice bath (the mixture turned viscous) and stirred for 2 h. TLC (10% ethyl acetate:hexane) showed the completion of the reaction via the consumption of **8o**. The mixture was the acidified to pH 1 with 1M HCl at 0°C. A precipitate formed as the acid was added. A crude solid was collected by vacuum filtration and purified by column chromatography (60% CH_2Cl_2:hexane) to obtain the bromo-coumaranone derivative **14a** (74 mg, 43%). **14a**: m.p. 153-155^oC, ^1H NMR: δ 8.36 (d, 1H, J = 2.4 Hz), 7.53 (m), 7.41 (m), 7.34 (m), 7.33 (s), 7.31 (m), 6.92 (d, 1H, J = 0.5 Hz), 6.91 (d, 1H, J = 0.5 Hz), 6.79 (d, 2H, J = 8.8 Hz), 6.64 (d, 2H, J = 8.3Hz), 5.35 (s), 4.10 (q), 2.18 (s), 1.57 (s), 1.49 (t). ^{13}C NMR ($CDCl_3$): δ 182.00, 166.90, 157.49, 157.01, 147.28, 138.16, 136.15, 133.95, 133.37, 128.63, 127.91,

126.69, 123.57, 113.26, 112.92, 111.40, 107.22, 105.14, 104.44, 70.65, 64.51, 14.71. Anal. $C_{24}H_{19}BrO_4$: C, H.; HRMS For $C_{24}H_{19}BrO_4$ (M + H): theory: 451.0539 ; found 451.0530.

4-Benzyloxy-2-(5-chloro-2-ethoxy-benzylidene)-benzofuran-3-one (14b). 1-(2-Benzyloxy-6-hydroxy-phenyl)-3-(5-chloro-2-ethoxy-phenyl)-propenone **8p** (300 mg, 0.73 mmol) was dissolved in a 3 M KOH solution in 96% ethanol (5.3 mL). Then 35% H_2O_2 (1.49 mL) was added dropwise to the mixture cooled in an ice bath (the mixture turned viscous) and stirred for 30 min. The ice bath was removed and the reaction stirred at room temperature for 3 h. TLC (15% EtOAc:hexane) showed the completion of the reaction via the consumption of **8p**. The mixture was the acidified to pH 1 with 1M HCl at 0°C. A precipitate formed as the acid was added. A crude solid was collected by vacuum filtration and purified by column chromatography (85% $CHCl_3$: hexane) to give the chloro-coumaranone derivative **14b** (60% yield). **14b**: m.p. 148-149^{o}C; ^{1}H NMR ($CDCl_3$): δ 8.22 (d, 2H, J = 2.7H), 7.52 (m), 7.39 (t), 7.31 (m), 7.27 (d, 1 H, J = 2.7 Hz), 7.26 (s), 6.91 (d, 2H, J = 8.1 Hz), 6.83 (d, 2H, J = 9 Hz), 6.64 (d, 2H, J = 8.3 Hz), 5.33 (s), 4.09 (q), 1.48 (t). ^{13}C NMR ($CDCl_3$): δ 181.87, 166.97, 156.70, 147.42, 138.15, 131.10, 130.45, 128.63, 127.94, 126.70, 125.62, 123.05, 112.83, 111.0, 107.25, 105.13, 104.59, 70.67, 64.59, 14.76, Anal. $C_{24}H_{19}ClO_4$: C, H; HRMS For $C_{24}H_{20}ClO_4$ (M + H): theory: 407.1050 ; found : 407.1050.

Cell lines and viruses. TZM-bl cells (Dr. John C. Kappes, Dr. Xiaoyun Wu, and Tranzyme, Research Triangle Park, NC), and PM1 cells (Dr. Marvin Reitz) were acquired from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Both cell lines express high levels of HIV-1 co-recepters CD4, CCR5, and CXCR4, and TZM-bl cells are also endowed with an HIV-1 *tat* luciferase reporter construct that is useful for quantifying HIV infection in microtiter assays. TZM-bl cells were maintained in Dulbecco's modified Eagles's

medium (DMEM) (4.5 g/L glucose) containing penicillin, streptomycin, and 10% (v/v) fetal bovine serum (FBS) (termed "D10" hereafter). PM1 cells are a pro-myelocytic cell line capable of propagating HIV-1 virions following exposure to infectious virus. These were maintained in RPMI 1640 supplemented with penicillin, streptomycin, 10mM HEPES, and 20% (v/v) FBS ("R20"). The following HIV-1 isolates were acquired from the NIH AIDS Research and Reference Reagent Program: BaL (R5), 92UG029 (X4, subtype A), 93UG070 (X4, subtype D), 93UG067 (R5X4). Stocks were prepared via propagation in PM1 cells for 5-11 days (isolate-dependent), and cell supernatants containing fresh virions were clarified of cellular debris by centrifugation followed by filtration through a 0.45 uM syringe filter, and stored at -80C in aliquots to be used as viral stocks. Quantification of viral stocks were performed by HIV-1 p24 ELISA (Perkin Elmer, Waltham, MA), and viral infectious titer was determined by infecting PM1 and TZM-bl cells with serial dilutions of viral stock and identification of the dilution that resulted in infection of 50% of the culture wells ($TCID_{50}$/mL).

CHO cells were grown as previously described.[6] Briefly, cell growth was assayed in sterile 96-well microtiter plates (Costar 3599, Corning, NY, USA) in the presence of each compound. Experiments were conducted in triplicate. CHO cells were plated at 10,000 cells/mL. Drug solutions were prepared in 100% DMSO and dosed so that the final DMSO concentration was <1%. Drug additions occured after an initial overnight incubation of CHO cells in each well. For example, 1 μL of flavonoid solution was added to the CHO cells plated in each well in 100 μL of media. After flavonoid compound was added, the cells were incubated in 5% CO_2 for 48 h at 37^{o}C. The MTS reagent (Promega Cell Titer 96 AQueous non-radiactive cell proliferation reagent) was added (20 μL) and the CHO cells were incubated for an additional 4h and then absorbance at 490 nm was measured on a BioTek Synergy MX plate reader. Controls run using

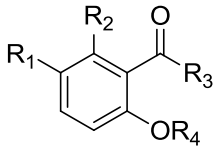
1% DMSO in the media and no compound showed no toxicity over the 48 h period compared to CHO cells grown in media only. IC_{50} values were determined from the corresponding plot of relative absorbance at 490 nm vs flavonoid concentration. The data is tabulated in Table 1.

Antiviral and cytotoxicity assays. TZM-bl cells were trypsinized and seeded to 6×10^4 cells/ml (6000 cells/well) in 96-well dishes, then incubated (37°C/5% CO_2) overnight to allow for cell attachment. The following day, when cells were 50-60% confluent, maintenance media was aspirated and cells were treated in triplicate with 50 µL of D10 containing diluted compound or the volume equivalent of DMSO vehicle. After 5 min (or for select experiments, 3 hr) HIV was diluted in D10 and applied to pre-treated wells (50 uL/well) at a final concentration of 60 $TCID_{50}$/0.1mL (MOI=0.01) and incubated at 37°C/5% CO_2. After 24 h, cells were lysed and processed for luciferase assay (Bright Glo luciferase system, Promega, Madison, WI), and luciferase was quantified with a Spectramax luminometer (Molecular Devices Corp., Sunnyvale, CA, USA), with an integration time of 5 s/well. Ability to prevent HIV-1 infection was measured as a percentage reduction in luciferase (relative light units) compared to the positive viral control (medium, DMSO vehicle, and virus), while the negative control (medium, DMSO, no virus) represented the baseline. To test whether reduced luciferase activity could be due to cytotoxic effects of certain compounds rather than reduced HIV infection, replicate dishes of treated TZM-bl cells were assayed for dead cell and total cell numbers using CytoTox Glo reagent (Promega). Percent differences in live cell luminescence were calculated for each compound versus the matched DMSO vehicle control. Select compounds were assayed additionally for cytotoxic effects using confluent TZM-bl cells and MTT reduction assay (R&D Systems, Minneapolis, MN) to monitor cell metabolic activity. CHO IC_{50} values were determined using established methods.[6]

PM1 cells (1.5×10^5/0.1 mL) were treated with compound or matched DMSO volume-equivalent diluted in R20 maintenance media, incubated for 10 min, then infected with HIV-1 BaL (150 $TCID_{50}$/0.1 mL, MOI=0.001) for 2 h. Cells were then washed and resuspended in fresh R20 containing compounds or DMSO vehicle. Culture supernatants were collected on day 3, and cells were re-suspended and incubated in treatment media until day 5, when supernatant was collected and assayed for HIV p24 by ELISA. Cell viability was monitored by trypan blue dye exclusion.

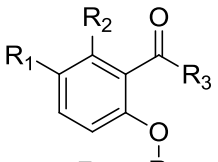
3.1 Results and Discussion

The new target structures were inspired by the bioactive structures **1-5** and the previously designed compounds **6-13** (Figure 2).



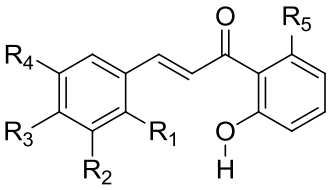
6

6a: R_1=Br, R_2=R_3=R_4=H
6b: R_1=Cl, R_2=R_3=R_4=H
6c: R_1=R_4=H, R_2=OH, R_3=Me
6d: R_1=R_2=R_3=H, R_4=Me
6e: R_1=Br, R_2=R_3=H, R_4=Me



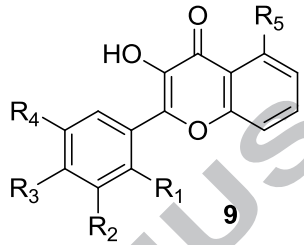
7

7a: R_1=Br, R_2=R_3=H, R_4=Et (95%)
7b: R_1=Cl, R_2=R_3=H, R_4=Me (98%)
7c: R_1=Cl, R_2=R_3=H, R_4=Et (96%)
7d: R_1=H, R_2=OH, R_3=R_4=Me (60%)



8

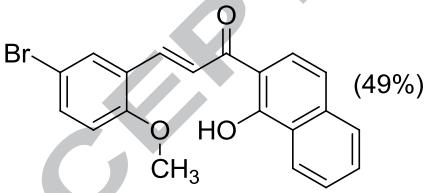
8a: R_1=OMe, R_2=R_3=R_4=R_5=H (54%)
8b: R_1=OMe, R_2=R_3=R_5=H, R_4=Br (71%)
8c: R_1=OEt, R_2=R_3=R_5=H, R_4=Br (42%)
8d: R_1=R_2=OMe, R_3=R_5=H, R_4=Br (48%)
8e: R_1=R_3=OMe, R_2=R_5=H, R_4=Br (94%)
8f: R_1=OMe, R_2=R_3=H, R_4=Br, R_5=OMe (51%)
8g: R_1=OMe, R_2=R_3=R_5=H, R_4=I (65%)
8h: R_1=OMe, R_2=R_3=R_5=H, R_4=Cl (32%)
8i: R_1=OEt, R_2=R_3=R_5=H, R_4=Cl (30%)
8j: R_1=OMe, R_2=R_3=R_5=H, R_4=Me (46%)
8k: R_1=OMe, R_2=R_3=R_4=H, R_5=OBn (60%)
8l: R_1=OMe, R_2=R_3=H, R_4=Br, R_5=OBn (82%)



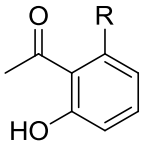
9

9a: R_1=OMe, R_2=R_3=R_4=R_5=H (51%)
9b: R_1=OMe, R_2=R_3=R_5=H, R_4=Br (80%)
9c: R_1=OEt, R_2=R_3=R_5=H, R_4=Br (47%)
9d: R_1=R_2=OMe, R_3=R_5=H, R_4=Br (39%)
9e: R_1=R_3=OMe, R_2=R_5=H, R_4=Br (81%)
9g: R_1=OMe, R_2=R_3=R_5=H, R_4=I (44%)
9h: R_1=OMe, R_2=R_3=R_5=H, R_4=Cl (49%)
9i: R_1=OEt, R_2=R_3=R_5=H, R_4=Cl (60%)
9j: R_1=OMe, R_2=R_3=R_5=H, R_4=Me (71%)

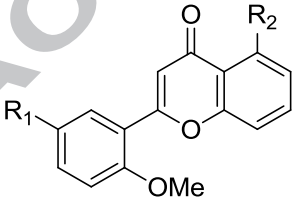
8m:

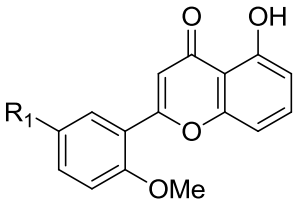


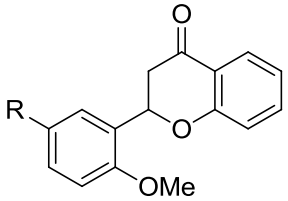
(49%)



10a: R=OH
10b: R=OBn







11a: R_1=H, R_2= OBn (80%)
11b: R_1=Br, R_2 =OBn (55%)
11c: R_1= R_2=H (70%)
11d: R_1= Br, R_2=H (95%)

12a: R_1=H (67%)
12b: R_1=Br (70%)

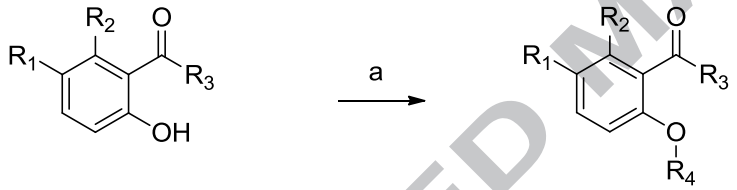
13b: R=Br (11%)
Note: **13h**: R=Cl

Figure 2. Aryl Precursors and final Chalcone and Flavonoid compounds

3.2 Synthesis. The synthesis of compounds **8a-m**, **9a-j** and **10-13** from their precursors **6** and 7 has been previously described.[6] During the course of our biological investigations of **8** and **9** as antivirals, we discovered that certain ring substituents provided enhanced efficacy against HIV and significantly reduced toxicity to host cells. This insight led to the design of new molecules which combined these favorable substituents onto the same scaffold.

While many of the required benzaldehyde and acetophenone starting materials were commercially available, several were made via *O*-alkylation of 2-(hydroxy)phenyl derivatives, **6**. As shown in Scheme 1, the desired alkyl groups were readily introduced in good yields.[6]

Scheme 1[a]



6a: R_1=Br, R_2=R_3=H
6b: R_1=Cl, R_2=R_3=H
6c: R_1=H, R_2=OH, R_3=Me

7a: R_1=Br, R_2=R_3=H, R_4=Et (95%)
7b: R_1=Cl, R_2=R_3=H, R_4=Me (98%)
7c: R_1=Cl, R_2=R_3=H, R_4=Et (96%)
7d: R_1=H, R_2=OH, R_3=R_4=Me (60%)
7e: R_1=Br, R_2=R_3=H, R_4=octyl (80%)

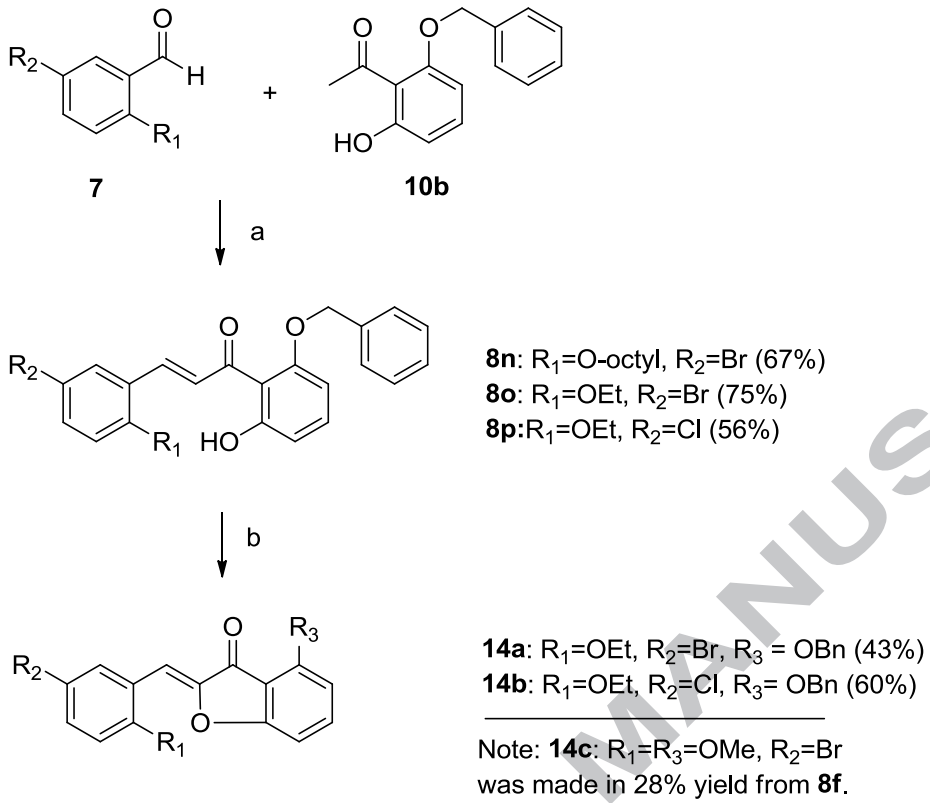
[a]*Reagents:* a) MeI or EtBr or n-octylbromide, K_2CO_3, DMF, 2 days, rt

The newly-designed target chalcones were synthesized following Scheme 2 and the chalcones **8n**, **8o**, and **8p** were generated in good yields. The chalcones were accessed using strong base at 85°C via the crossed aldol condensation of the respective carbonyl compound **7** and acetophenone **10b** to obtain the chalcone products, **8n-8p**. We found that a reaction time of 3 h produced higher yields, and the products were isolated using column chromatography.

These compounds incorporated the most effective substituents, such as bromine or chlorine at the R_4 position, as well lengthening the alkyl chain at the R_1 position. Compounds **8o** and **8p** incorporated an *O*-ethyl substituent, whereas **8n** incorporated an *O*-(n-octyl) substituent to evaluate the effects of increasing lipophilicity at this position. This strategy was predicated upon indications in our initial HIV screens that the *O*-ethyl substituent was desirable. The new generation of compounds also contained an *O*-benzyl substituent at the R_3 position (See Scheme 2) found to be so effective in prior antiviral studies. [6]

The conversion of the chalcones **8o** and **8p** to the substituted benzyl-coumaranones **14a** and **14b** was accomplished with 35% H_2O_2 in the presence of KOH (Scheme 2).[15] Upon mixing the reagents at 0^oC, the solution was allowed to slowly warm to room temperature to facilitate conversion. The final yields of these compounds, however, were still low due to the formation of significant by-products. The presence of the R_3 alkoxy group (Scheme 2) is known to direct ring closure to form the five membered ring in lieu of the six membered ring.[13] Indeed systems lacking oxygen at this position generated six membered rings under these conditions.[6] The coumaranones **14a** and **14b** were isolated and purified via column chromatography.

Scheme 2[a]



[a]*Reagents* : a) 40%KOH/MeOH, 85°C ; b) 35% H_2O_2, KOH/EtOH, then 1M HCl

In summary, the expanded library of compounds was tested for their activity in inhibiting HIV infection and cytotoxicity using infectious HIV-1 and the human target cell lines TZM-bl and PM1.

3.3 Bioevaluation. To determine the efficacy of these flavonoid derivatives against HIV-1, compounds were initially screened at 10 µM using the target cell line TZM-bl, which expresses an HIV-1 *tat* luciferase reporter construct for quantifying HIV infection.[16] To provide a strict test of antiviral activity, cells were treated and infected while at 50-60% confluence, which provided more surface area for viral fusion and entry than if the cells formed a confluent monolayer. Luciferase production in response to HIV-1 *tat* expression was measured after 24 hr.

Compounds were tested for cytotoxicity on replicate dishes of TZM-bl cells, as well as on CHO cells for comparison. CHO cells were chosen in order to compare the relative toxicities of the new flavonoid derivatives to previously investigated compounds.[6] Table 1, column 2, displays the mean % inhibition of HIV-1 infection for each compound. The third column in Table 1 shows the relative % decrease each compound had on uninfected TZM-bl cell viability. In order to select potent non-toxic compounds for further investigation, we applied a screening criteria to the datasets obtained. Compounds, which gave >50% HIV-1 inhibition at 10 μM and showed limited toxicity to unifected TZM-bl cells (arbitrarily set as a ≤25% decrease in cell viability for this early screen, see column 3), are highlighted in bold in Table 1, column 2. The fourth column in Table 1 shows the cell proliferation IC_{50} value for each compound in CHO cells after 48 h.

Taken together, this efficacy and safety information allowed for an assessment of the selectivity of each compound to target the virus and not the host cell. A relative ranking of the compounds via their toxicity profiles was also performed. In each case, the chalcone system **8** was more toxic than the corresponding ring-closed 4H-chromen-4-one **9**. With the exception of **11a** (CHO IC_{50} = 17.5 μM), compounds **11-13** were found to be relatively non-toxic to CHO cells (CHO IC_{50} values > 64 μM). In sum, these studies provided general toxicity profiles of each compound as well as insights into the molecular design needed to lower cell toxicity and still maintain potency against HIV-1.

Table 1. Effect of selected compounds **8-9** and **11-14** on HIV-1 infection, viability of TZM-bl cells treated with each compound at 10 µM, and the cell proliferation IC_{50} values (µM) in CHO cells. [a,b,c]

Compound	% Inhibition of HIV-1 infection by 10 µM compound (mean±SEM)	% decrease in TZM-bl cell viability by 10 µM compound (mean±SEM)	CHO 48 h cell proliferation IC_{50} value (µM)[a]
8a	none	16.5±9.1	25.5 ± 2.7
8b	78.6±1.0	62.6±7.0	8.7 ± 0.3
8c	88.1±0.2	51.9±6.6	7.2 ± 0.5
8d	85.8±1.7	72.3±4.4	7.2 ± 0.4
8e	28.6±5.3	1.9±9.6	16.9 ± 1.3
8f	95.1±0.8	90.4±4.5	6.1 ± 0.8
8g	99.7±0.2	99.6±1.2	6.7 ± 0.7
8h	96.4±0.5	99.5±3.6	8.4 ± 0.6
8i	86.1±1.4	86.4±3.9	6.8 ± 0.9
8j	63.1±1.0	46.9±3.5	14.2 ± 2.7
8k	ND	ND	ND
8l	**86.5**±5.4	23.4±4.9	8.1 ± 1.0
8m	**53.3**±3.5	19.9±5.0	15.6 ± 1.0
9a	21.3±3.6	none	>100
9b	22.4±1.6	none	88.5 ± 10.1
9c	**51.7**±1.5	none	13.5 ± 1.3
9d	25.9±0.8	none	>100
9e	43.6±0.7	none	18.3 ± 2.5
9g	**61.7**±1.2	15.3±3.5	17.9 ± 1.6
9h	**60.7**±1.2	23.5±5.3	25.0 ± 2.2
9i	**61.4**±1.5	21.9±5.9	>100
9j	13.0±0.9	4.8±5.8	54.6 ± 4.2

11a	ND	ND	17.5 ± 1.9
11b	16.4±1.6	6.5±5.5	96.6 ± 5.6
11c	74.8±0.9	39.5±4.9	64.8 ± 8.1
11d	38.4±1.9	23.6±8.4	>100
12a	83.8±0.9	44.5±5.6	>100
12b	52.4±1.5	34.8±3.7	75.2 ± 8.7
13b	2.8±2.8	none	67.9 ± 9.3
14a	12.6±9.3	8.4±7.9	ND
14b	15.7±7.5	none	ND
14c	9.2±1.1	0.2±8.6	17.3 ± 1.9

[a] CHO cells were incubated for 48 h at 37°C with the respective compound and relative cell viability assessed. We noted that compounds **9a, 9j, 11b** and **13b** demonstrated low toxicity across multiple cell types but were relatively ineffective against HIV-1. [b]Compounds, which gave >50% HIV-1 inhibition at 10 μM and showed limited toxicity to unifected TZM-bl cells (arbitrarily set as a ≤25% decrease in TZM-bl cell viability, see column 3), are highlighted in bold in column 2 and were selected for further study. [c]Compound **8k** was not evaluated in bioassays due to its previously observed high toxicity in HEK 293T cells (see ref 6).

As shown in Table 1, interpreting the efficacy of each compound against HIV-1 required an understanding of the compound's toxicity because dead or damaged cells would not be able to synthesize luciferase protein following HIV-1 infection. For example, a highly toxic compound like **8g** which inhibits 99% of HIV-1 but also damaged 99% of the host cells at the same concentration (10 μM) was undesirable. In contrast, a compound like **8l** (10 μM) provided a better balance between efficacy (86.5% inhibition of HIV-1 infection) and toxicity (23.4% decrease in host cell viability). The flavonols **9c, 9g, 9h**, and **9i** each had >50% inhibition of

HIV-1 and had modest toxicity (<23.5% decrease in host cell toxicity). Interestingly, the nature of the 2-alkoxy substituent in the 5-bromo derivatives **9b** (22.4% inhibition of HIV-1) and **9c** (51.7%) suggested that the O-ethyl substituent was more advantageous than OMe. However, this was not the case with the related 5-chloro derivatives **9h** (OMe) and **9i** (OEt) which had similar activities (~61% inhibition of HIV-1). The nature of the 5-halo group was interesting in the OMe series (**9b, 9g,** and **9h**) and suggested that the 5-chloro and 5-iodo substituents (both gave ~61% HIV inhibition) may be more efficacious than the 5-bromo (**9b**, 22.4%). In the presence of the 2-*O*-ethyl group, however, this difference is less pronounced where **9c** (Br/OEt, 51.7% HIV inhibition) and **9i** (Cl/OEt, 61.7% HIV inhibition).

Next, we screened selected compounds from Table 1 for their dose-dependent effectiveness in protecting TZM-bl cells from HIV-1 infection. These studies allowed for the determination of IC_{50} values for this subset of compounds and are listed in Table 2.

Table 2. Anti-HIV potency of each compound (anti-HIV IC_{50} value), the toxicity of each compound when tested in uninfected TZM-bl cells at the anti-HIV IC_{50} concentration. Each compound's substituents are shown for comparisons.

Compound	Anti-HIV HIV-1 $_{BAL}$ IC_{50} (μM)	% Toxicity at its respective HIV-1 $_{BAL}$ IC_{50} dose	Substituents
8l	4.7	not toxic	OMe/Br/OBn/benzene core
8m	20	90%	OMe/Br/H/naphthyl core
9a	30.4	not toxic	H/OMe
9b	43.7	not toxic	Br/OMe
9c	10.4	not toxic	Br/OEt
9d	30.9	not toxic	Br/ diOMe
9i	12.6	20%	Cl/OEt
11b	42.3	not toxic	OMe/Br/OBn
12a	4.4	42%	OMe

As shown in Table 2, compound **12a** was the most potent (anti-HIV IC_{50}: 4.4 μM) but also resulted in significant toxicity at 4.4 μM (42% toxicity). In this regard, compound **8l** was selected as the better scaffold to pursue as it also displayed good potency (4.7 μM) along with derivatives **9c** (10.4 μM) and **9i** (12.6 μM). The other systems **11b** and **12a** were not pursued due to either low efficacy or high toxicity properties. As shown in Figure 3, compound **8l** was also shown to inhibit diverse isolates of HIV-1 in a dose-dependent manner (Panel A, Fig 3). The laboratory strain BaL is an R5 (M-tropic) strain representing the type of HIV-1 typcially spread by sexual contact. Clinical isolates 92UG029, 93UG070, and 93UG067 were tested because they represent both dual tropic (R5X4) and T-tropic (X4) strains isolated from patients with advanced disease. Compound **8l** was not toxic to the host cells (Panel B, Fig 3) and no changes in relative cell metabolic activity were noted when **8l** was dosed up to 10 μM (Panel C, Fig 3). Note: Compound **8l** gave a CHO 48h IC_{50} value of 8.1 μM in Table 1, but this toxicity was not

observed in the TZM-bl cells at 4.7 µM (Table 2) nor in Figure 3 at 10 µM and is likely cell line dependent.

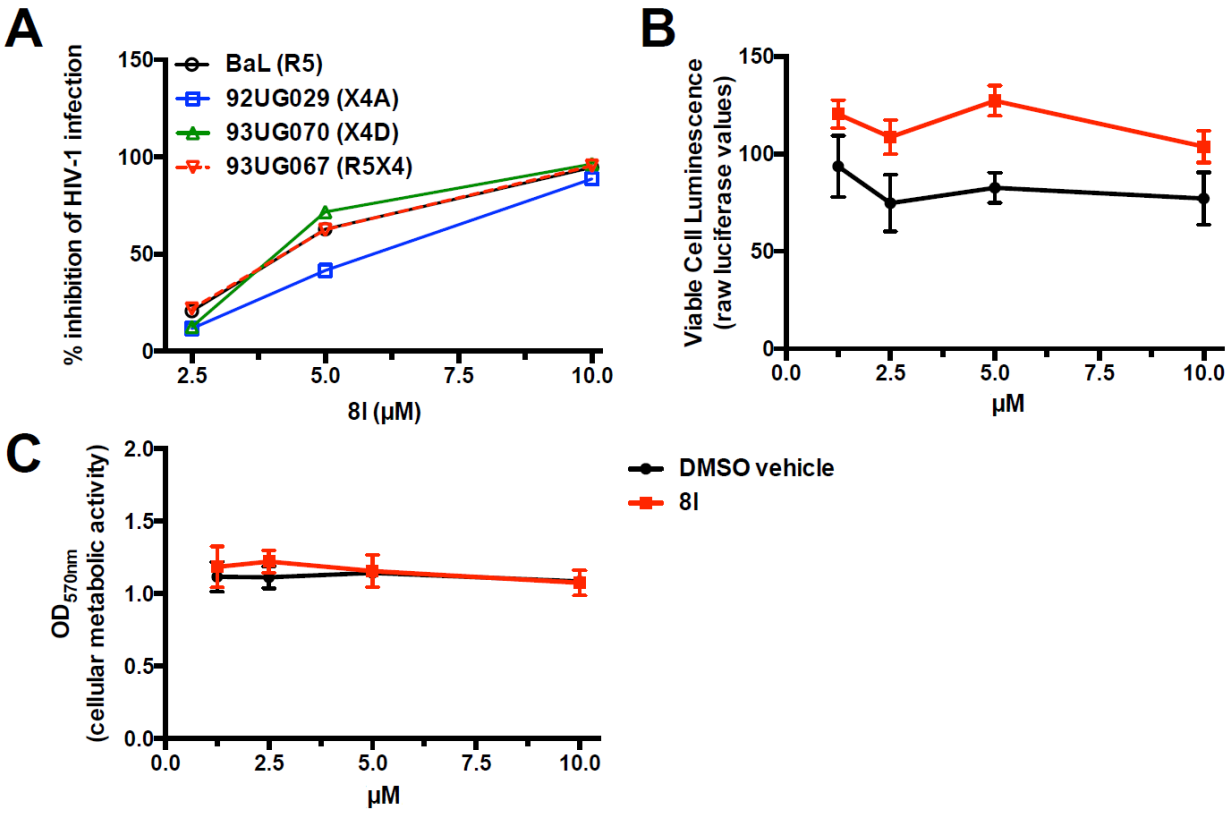


Figure 3. Chalcone **8l** dose-dependently inhibits diverse isolates of HIV-1. Panel A) Compound **8l** was tested in TZM-bl HIV reporter cells at 2.5-10 µM and compared with volume-matched DMSO vehicle controls. Presented is the mean % inhibition of infection for assays performed in triplicate. Panels B-C) Compound **8l** (red lines) or matched vehicle (black lines) was applied to confluent TZM-bl cells and assessed for cytotoxicity using a live/dead cell detection assay (CytoTox Glo, Promega, panel B) or MTT reduction assay (C). Mean ± SEM, n=3.

We noted that the most active compounds of all those tested had similar O-alkyl and halogen substituents. The most potent chalcone **8l** also contained the unique 5'-O-benzyl substituent suggesting that this modification was key to its performance as **8b,** which does not contain this group, was less effective and significantly more toxic to TZM-bl cells (Table 1). We noted that **8f,** which contains a smaller OMe group at the 5'-position, was also more toxic than **8l,** which contained the 5'-O-benzyl group. Moreover, **8c** (2-OEt) was less toxic and more potent than **8b** (2-OMe) suggesting that the longer *O*-alkyl group improved performance. This trend was also seen in the **9** series in Table 2, where the 2-*O*-ethyl derivative **9c** was significantly more potent (anti HIV IC_{50}: 10.4 µM) than its related 2-*O*-methyl analogue **9b** (43.7 µM), again suggesting that increased length of the alkoxy substituent may be beneficial. This relationship, however, was not evident in **9h** (OMe) and **9i** (OEt) which had similar toxicities in TZM-bl cells in Table 1 (~23 µM). Therefore, we designed a new series of chalcones, **8n-8p**, which pursued these insights in order to increase potency and lower toxicity. Compound **8n** contained a long n-octyl substituent to further improve lipophilicity at the 2-position, whereas **8o** and **8p** investigated the role of the 5-halo substituent in the presence of the 5'-*O*-benzyl substituent.

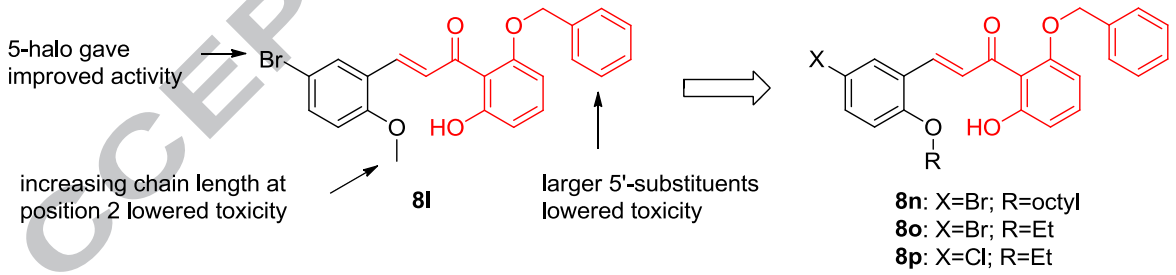


Figure 4. New inhibitor designs, **8n-8p**

We then synthesized the new compounds that contained the desirable 5'-*O*-benzyl group and longer 2-*O*-alkyl substituents (**8n-8p**, described above in the Synthesis section). Interestingly, the n-octyl derivative **8n** was devoid of anti-HIV activity and was not pursued,

while **8o** and **8p** exhibited anti-HIV-1 activity and were essentially non-toxic under the experimental conditions (Table 3). To test whether exposure time impacted efficacy, TZM-bl cells were pre-treated with each compound for either 5 min (standard assay) or 3 hours prior to inoculation with HIV-1. The anti-HIV activity of compounds **8o** and **8p** was higher when tested using longer pre-incubation times (Table 3). Both **8l** (2-OMe/5'-OBn) and **8o** (2-OEt/5'-OBn) inhibited HIV infection by ~90% after a three hour pre-incubation period. In contrast, the potency of the **9** series compounds (**9c** and **9i**) did not improve with the extended pre-incubation period.

Table 3: Bioevaluation of selected compounds (10 μM) in TZM-bl cells challenged with HIV-1 [a]

Compound	HIV % Inhibition after 5 min pre-treatment (at 10 μM)	HIV % Inhibition after 3 hr pre-treatment (at 10 μM)	% Inhibition of Cell Viability (compd at 10 μM)
8l	86.2±5.4	90.5±3.5	23.4±4.9
8n	<10	27.9±10.6	ND
8o	60.2±6.7	89.9±2.2	none
8p	64.8±5.3	79.0±12.9	8.7±5.1
9c	57.2±4.5	54.9±6.2	none
9i	67.2±5.6	54.1±6.8	none

[a] ND = not determined

Having shown that these compounds can inhibit HIV-1 infection of TZM-bl cells, we assessed their ability to inhibit viral propagation by PM1 cells, a pro-myelocytic cell line that is readily infected by HIV. As shown in Table 4, compounds **9c** and **9i** had no effect on HIV-1 infection of cultured PM1 cells compared to the DMSO control at 10 μM. In contrast, the chalcones **8l-8p**

showed >92% inhibition of HIV propagation at 10 µM and >65% inhibition at 5 µM. Regarding host-cell viability, compound **8l** was toxic to PM1cells at 10 µM (38% viability), whereas the chalcones **8o** and **8p** were relatively non-toxic to PM1 cells at 10 µM (96.8 and 87.7 % viability, respectively, Table 4).

Table 4. Select compounds effectively inhibit HIV-1 propagation by host PM1 cells without impacting cell viability

	% inhibition of HIV propagation		% inhibition of Cell Viability	
Compound	**10 µM**	**5 µM**	**10 µM**	**5 µM**
8l	99.9±0.1	80.0±13.9	61.7±5.8	0.6±7.2
8o	92.3±2.7	72.2±9.2	none	none
8p	95.8±1.7	65.8±12.4	12.3±7.2	none
9c	1.3	none	23.1	16.5
9i	none	ND	25.8±14.2	ND

We noted that the 5-bromo-substituent in **8b** (64.2% decrease in cell viability) provided a compound which was less toxic to TZM-bl cells than **8h**, which contained the 5-chloro-substituent (98.9% decrease in cell viability). In the HIV propagation assay, however, the bromo-containing **8o** and chloro-containing **8p** had similar toxicity profiles and potencies in PM1 cells (Table 4).

In summary, chalcone **8o** was found to provide a good balance between potency and toxicity and was effective in inhibiting both HIV-1 infection and the spread of infection while at the same time remaining relatively non-toxic to the cell lines tested (TZM-bl and PM1). In contrast, the other systems investigated (e.g., substituted flavones **11** and coumaranones **14**) were found to be ineffective by comparison. These new chalcones **8o** and **8p** not only demonstrated

good anti-viral activity, but were significantly less toxic than the previously screened derivatives, showing an improvement in the overall drug design.

A comparison of the related pairs **11d** and **13b** indicated that the presence of the α,β-unsaturated system of **11d** increased toxicity to TZM-bl host cells. Michael acceptors (α,β-unsaturated carbonyls) like the chalcones explored here are often considered PAINS in medicinal chemistry due to their ability to react with a variety of biological nucleophiles and generate significant off-target effects.[17] However, some PAINS constructs can act as privileged scaffolds, which allow for the "quick identification of hits with sufficient target affinity whose selectivity can be tuned in subsequent development steps." [18] In the library of flavonoids evaluated here, we noted that not all Michael acceptors within the same series performed the same. For example, **9b** and **9c** were both not toxic to TZM-bl host cells at 10 μM (Table 1), but **9b** and **9c** gave significantly different potencies in terms of HIV inhibition (22.4% and 51.7% HIV inhibition, respectively). A comparison across related Michael scaffolds showed that **81** and **11d** had nearly identical toxicity to TZM-bl cells (23**%** decrease in TZM-bl cell viability at 10 μM, Table 1) but **8l** outperformed **11d** in terms of HIV inhibition in Table 1 (86.5% vs 38.4%, respectively). Thus, our results suggest that one can maintain HIV inhibition properties and reduce host cell toxicity via substituent changes on a chalcone platform.

Lastly we also noted that one of the best HIV inhibitors **8l** was also the immediate synthetic precursor to the HCMV inhibitor **11b**.[6] We speculated that this arrangement of 5-bromo, 2-alkoxy and 5'-benzyloxy substituents on a core platform may provide unique anti-virals. This hypothesis was tested via the related coumaranone **14a** and flavone **11b**. Both were shown to be ineffective even though they represent these same substituents annealed to a related core platform. This suggested that the activity we observed in HIV may be due, in part, to the

additional presence of the β-hydroxyketone of **8** and α-hydroxyketone of **9**; an observation which may also partially explain why β-hydroxyflavone **12b** has anti-HIV-1 activity, whereas flavanone **13b** did not (Table 1). In addition, flavone **11d** retains α,β-unsaturation, but lacks the adjacent hydroxyl group and underperforms in terms of HIV inhibition in Table 1 (**%** Inhibition of HIV-1 infection by 10 μM compound; **8l**: 86.5%; **11d**: 38.4%). Indeed, these hydroxyl-carbonyl motifs and related bidentate ligands are well known to interact with HIV integrase[12b, 12d, 12e] and is the central motif of the HIV integrase inhibitor, Raltegravir (Isentress).[19] In addition, Courter et al have described inhibitors of HIV-1 viral entry predicated upon related oxalamide structures.[20] Future work will explore these possible mechanisms of action.

4.1 Conclusion. In conclusion, 33 flavonoids including chalcones, flavones, flavanones, and coumaranones were screened for their anti HIV activity. In general, compounds which contained an α- or β-hydroxy carbonyl motif were typically more potent. Of the series tested, the chalcones **8o** and **8p** provided the best balance between high anti-viral potency and low cytotoxicity. These chalcones significantly inhibited HIV propagation at low micromolar doses (≤ 10 μM) and were relatively non-toxic. We note that the low micromolar potencies observed with chalcones **8l-o** are in the range of other known small molecule HIV inhibitors. For example, small molecule allosteric HIV-1 integrase inhibitors in pre-clinical development have demonstrated antiviral activity in the 0.1-5 μM range.[21] In addition, small ubiquitin-like modifier (SUMO)-specific protease inhibitors represent a novel class of anti-HIV-1 agents, and are active in the 1-100 μM range [22] and human antimicrobial peptides (1-5 kD) capable of inhibiting HIV-1 fusion exhibit EC_{50} values of 0.5-5 μM.[23] In this regard, chalcone **8o** provides a good starting point for future drug development.

5.1 Supporting Information. ^{1}H NMR and ^{13}C NMR spectra and elemental analyses for the new compounds **8n-8p**, **14a** and **14b**. This material is available free of charge via the Internet.

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Notes. The authors declare no financial interest.

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Abbreviations Used. HIV-1, Human immunodeficiency virus-1; AIDS, acquired immunodeficiency syndrome; ART, antiretroviral therapy; HCMV, human cytomegalovirus; CHO, Chinese hamster ovary; $TCID_{50}$, tissue culture infectious dose, i.e. the dose that resulted in infection of 50% of the cells in culture; MOI, multiplicity of infection; DMSO, dimethylsulfoxide

Keywords. Flavonoids, HIV, chalcones

6.1 References

1. Zhao, Y.; Jiang, F.; Liu, P.; Chen, W.; Yi, K., Catechins containing a galloyl moiety as potential anti-HIV-1 compounds. *Drug Discov Today* **2012,** *17* (11-12), 630-635.

2. Singh, P.; Anand, A.; Kumar, V., Recent developments in biological activities of chalcones: a mini review. *Eur J Med Chem* **2014,** *85*, 758-777.

3. (a) Nations, U., UNAIDS report on the global AIDS epidemic 2014. *http://www.unaids.org/sites/default/files/media_asset/20140716_FactSheet_en.pdf* **2014**, unaids.org; (b) van den Heuvel, D.; Driessen, G. J.; Berkowska, M. A.; van der Burg, M.; Langerak, A. W.; Zhao, D.; Charif, H.; Hartwig, N. G.; van Rossum, A. M.; Fraaij, P. L.; van Dongen, J. J.; van Zelm, M. C., Persistent subclinical immune defects in HIV-1-infected children treated with antiretroviral therapy. *AIDS* **2015,** *29* (14), 1745-1756; (c) Buckhold, F. R., 3rd, Primary Care of the Human Immunodeficiency Virus Patient. *Med Clin North Am* **2015,** *99* (5), 1105-1122.

4. (a) Olivero-Verbel, J.; Pacheco-Londono, L., Structure-activity relationships for the anti-HIV activity of flavonoids. *J Chem Inf Comput Sci* **2002,** *42* (5), 1241-1246; (b) Wu, J. H.; Wang, X. H.; Yi, Y. H.; Lee, K. H., Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus Desmos. *Bioorg Med Chem Lett* **2003,** *13* (10), 1813-1815.

5. Mahapatra, D. K.; Bharti, S. K.; Asati, V., Chalcone scaffolds as anti-infective agents: Structural and molecular target perspectives. *Eur J Med Chem* **2015,** *101*, 496-524.

6. Kralj, A.; Nguyen, M. T.; Tschammer, N.; Ocampo, N.; Gesiotto, Q.; Heinrich, M. R.; Phanstiel, O., IV, Development of flavonoid-based inverse agonists of the key signaling receptor US28 of human cytomegalovirus. *J Med Chem* **2013,** *56* (12), 5019-5032.

7. (a) Khadem, S.; Marles, R. J., Chromone and flavonoid alkaloids: occurrence and bioactivity. *Molecules* **2011,** *17* (1), 191-206; (b) Hu, Q. F.; Zhou, B.; Gao, X. M.; Yang, L. Y.; Shu, L. D.; Shen, Y.; Li, G. P.; Che, C. T.; Yang, G. Y., Antiviral chromones from the stem of Cassia siamea. *J Nat Prod* **2012,** *75* (11), 1909-1914; (c) Sharma, H.; Patil, S.; Sanchez, T. W.; Neamati, N.; Schinazi, R. F.; Buolamwini, J. K., Synthesis, biological evaluation and 3D-QSAR studies of 3-keto salicylic acid chalcones and related amides as novel HIV-1 integrase inhibitors. *Bioorg Med Chem* **2011,** *19* (6), 2030-2045; (d) Mehla, R.; Bivalkar-Mehla, S.; Chauhan, A., A flavonoid, luteolin, cripples HIV-1 by abrogation of tat function. *PLoS One* **2011,** *6* (11), e27915; (e) Singh, S.; Malik, B. K.; Sharma, D. K., Targeting HIV-1 through molecular modeling and docking studies of CXCR4: leads for therapeutic development. *Chem Biol Drug Des* **2007,** *69* (3), 191-203.

8. Evers, D. L.; Chao, C. F.; Wang, X.; Zhang, Z.; Huong, S. M.; Huang, E. S., Human cytomegalovirus-inhibitory flavonoids: studies on antiviral activity and mechanism of action. *Antiviral Res* **2005,** *68* (3), 124-134.

9. (a) Cos, P.; Maes, L.; Vlietinck, A.; Pieters, L., Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection - an update (1998 - 2007). *Planta Med* **2008,** *74* (11), 1323-1337; (b) Kim, H. J.; Woo, E. R.; Shin, C. G.; Park, H., A new flavonol glycoside gallate ester from Acer okamotoanum and its inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase. *J Nat Prod* **1998,** *61* (1), 145-148.

10. Xu, H. X.; Wan, M.; Dong, H.; But, P. P.; Foo, L. Y., Inhibitory activity of flavonoids and tannins against HIV-1 protease. *Biol Pharm Bull* **2000,** *23* (9), 1072-1076.

11. Buckwold, V. E.; Wilson, R. J.; Nalca, A.; Beer, B. B.; Voss, T. G.; Turpin, J. A.; Buckheit, R. W., 3rd; Wei, J.; Wenzel-Mathers, M.; Walton, E. M.; Smith, R. J.; Pallansch, M.;

Ward, P.; Wells, J.; Chuvala, L.; Sloane, S.; Paulman, R.; Russell, J.; Hartman, T.; Ptak, R., Antiviral activity of hop constituents against a series of DNA and RNA viruses. *Antiviral Res* **2004,** *61* (1), 57-62.

12. (a) Critchfield, J. W.; Butera, S. T.; Folks, T. M., Inhibition of HIV activation in latently infected cells by flavonoid compounds. *AIDS Res Hum Retroviruses* **1996,** *12* (1), 39-46; (b) Hu, G.; Li, X.; Zhang, X.; Li, Y.; Ma, L.; Yang, L. M.; Liu, G.; Li, W.; Huang, J.; Shen, X.; Hu, L.; Zheng, Y. T.; Tang, Y., Discovery of inhibitors to block interactions of HIV-1 integrase with human LEDGF/p75 via structure-based virtual screening and bioassays. *J Med Chem* **2012,** *55* (22), 10108-10117; (c) Deng, J.; Dayam, R.; Al-Mawsawi, L. Q.; Neamati, N., Design of second generation HIV-1 integrase inhibitors. *Curr Pharm Des* **2007,** *13* (2), 129-141; (d) Deng, J.; Sanchez, T.; Al-Mawsawi, L. Q.; Dayam, R.; Yunes, R. A.; Garofalo, A.; Bolger, M. B.; Neamati, N., Discovery of structurally diverse HIV-1 integrase inhibitors based on a chalcone pharmacophore. *Bioorg Med Chem* **2007,** *15* (14), 4985-5002; (e) Ahn, H. C.; Lee, S. Y.; Kim, J. W.; Son, W. S.; Shin, C. G.; Lee, B. J., Binding aspects of baicalein to HIV-1 integrase. *Mol Cells* **2001,** *12* (1), 127-130; (f) Li, B. Q.; Fu, T.; Dongyan, Y.; Mikovits, J. A.; Ruscetti, F. W.; Wang, J. M., Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. *Biochem Biophys Res Commun* **2000,** *276* (2), 534-538; (g) Li, B. Q.; Fu, T.; Yan, Y. D.; Baylor, N. W.; Ruscetti, F. W.; Kung, H. F., Inhibition of HIV infection by baicalin--a flavonoid compound purified from Chinese herbal medicine. *Cell Mol Biol Res* **1993,** *39* (2), 119-124; (h) Liu, D. Y.; Ye, J. T.; Yang, W. H.; Yan, J.; Zeng, C. H.; Zeng, S., Ampelopsin, a small molecule inhibitor of HIV-1 infection targeting HIV entry. *Biomed Environ Sci* **2004,** *17* (2), 153-164.

13. Narasimhachari, N.; Rajagopalan, D.; Seshadri, T. R., A new effect of hydrogen bond formation (chelation) part VII. Hydrogen peroxide oxidation of some 2'-substituted chalkones and flavanones. *Proc. Indian Acad. Sci. - Section A* **1953,** *37* (6), 705-709.

14. Kiuchi, M.; Marukawa, K.; Kobayashi, N.; Sugahara, K., Preparation of 2-amino-4-phenylbutanol and 2-amino-4-phenyl-3-buten-1-ol derivatives and their phosphate esters as immunosuppressants. *PCT Int. Appl.* **2007,** *WO 2007069712 A1 20070621*.

15. Yamasaki, K.; Hishiki, R.; Kato, E.; Kawabata, J., Study of kaempferol glycoside as an insulin mimic reveals glycon to be the key active structure. *ACS Med Chem Lett* **2011,** *2* (1), 17-21.

16. (a) Platt, E. J.; Bilska, M.; Kozak, S. L.; Kabat, D.; Montefiori, D. C., Evidence that ecotropic murine leukemia virus contamination in TZM-bl cells does not affect the outcome of neutralizing antibody assays with human immunodeficiency virus type 1. *J Virol* **2009,** *83* (16), 8289-8292; (b) Platt, E. J.; Wehrly, K.; Kuhmann, S. E.; Chesebro, B.; Kabat, D., Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1. *J Virol* **1998,** *72* (4), 2855-2864.

17. Baell, J. B.; Holloway, G. A., New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J Med Chem* **2010,** *53* (7), 2719-2740.

18. Mendgen, T.; Steuer, C.; Klein, C. D., Privileged scaffolds or promiscuous binders: a comparative study on rhodanines and related heterocycles in medicinal chemistry. *J Med Chem* **2012,** *55* (2), 743-753.

19. (a) Zhang, X.; Pais, G. C.; Svarovskaia, E. S.; Marchand, C.; Johnson, A. A.; Karki, R. G.; Nicklaus, M. C.; Pathak, V. K.; Pommier, Y.; Burke, T. R., Azido-containing aryl beta-

diketo acid HIV-1 integrase inhibitors. *Bioorg Med Chem Lett* **2003,** *13* (6), 1215-1219; (b) Marchand, C.; Johnson, A. A.; Karki, R. G.; Pais, G. C.; Zhang, X.; Cowansage, K.; Patel, T. A.; Nicklaus, M. C.; Burke, T. R., Jr.; Pommier, Y., Metal-dependent inhibition of HIV-1 integrase by beta-diketo acids and resistance of the soluble double-mutant (F185K/C280S). *Mol Pharmacol* **2003,** *64* (3), 600-609; (c) Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S.; Vacca, J.; Hazuda, D. J., Diketo acid inhibitor mechanism and HIV-1 integrase: implications for metal binding in the active site of phosphotransferase enzymes. *Proc Natl Acad Sci U S A* **2002,** *99* (10), 6661-6666; (d) Wai, J. S.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; Tran, L. O.; Melamed, J. Y.; Langford, H. M.; Guare, J. P., Jr.; Zhuang, L.; Grey, V. E.; Vacca, J. P.; Holloway, M. K.; Naylor-Olsen, A. M.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S. D., 4-Aryl-2,4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells. *J Med Chem* **2000,** *43* (26), 4923-4926; (e) Evering, T. H.; Markowitz, M., HIV-1 Integrase Inhibitors. *The PRN Notebook* **2008,** *13*, 1-9.

20. Courter, J. R.; Madani, N.; Sodroski, J.; Schon, A.; Freire, E.; Kwong, P. D.; Hendrickson, W. A.; Chaiken, I. M.; LaLonde, J. M.; Smith, A. B., 3rd, Structure-based design, synthesis and validation of CD4-mimetic small molecule inhibitors of HIV-1 entry: conversion of a viral entry agonist to an antagonist. *Acc Chem Res* **2014,** *47* (4), 1228-1237.

21. Feng, L.; Dharmarajan, V.; Serrao, E.; Hoyte, A.; Larue, R. C.; Slaughter, A.; Sharma, A.; Plumb, M. R.; Kessl, J. J.; Fuchs, J. R.; Bushman, F. D.; Engelman, A. N.; Griffin, P. R.; Kvaratskhelia, M., The Competitive Interplay between Allosteric HIV-1 Integrase Inhibitor BI/D

and LEDGF/p75 during the Early Stage of HIV-1 Replication Adversely Affects Inhibitor Potency. *ACS Chem Biol* **2016**, in press.

22. Madu, I. G.; Li, S.; Li, B.; Li, H.; Chang, T.; Li, Y. J.; Vega, R.; Rossi, J.; Yee, J. K.; Zaia, J.; Chen, Y., A Novel Class of HIV-1 Antiviral Agents Targeting HIV via a SUMOylation-Dependent Mechanism. *Sci Rep* **2015,** *5*, 17808.

23. Cole, A. M., Antimicrobial peptide microbicides targeting HIV. *Protein Pept Lett* **2005,** *12* (1), 41-47.

7.1 Graphical Abstract.