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Anti-AIDS agents 79. Design, synthesis, molecular modeling and structure–activity relationships of novel dicamphanoyl-2′,2′-dimethyldihydropyranochromone (DCP) analogs as potent anti-HIV agents

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ABSTRACT

In a continued study, 23 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyldihydropyrano[2,3-*f*]chromone (DCP) derivatives (**5–27**) were synthesized, and screened for anti-HIV activity against both a non-drug-resistant NL4-3 strain and multiple reverse transcriptase (RT) inhibitor-resistant (RTMDR-1) strain, using 2-EDCP (**4**) and 2-MDCP (**35**) as controls. New DCP analogs **5**, **9**, **14**, and **22** exhibited potent anti-HIV activity against HIV_{NL4-3} with EC_{50} and therapeutic index (TI) values ranging from 0.036 μM to 0.14 μM and from 110 to 420, respectively. Compounds **5** and **9** also exhibited good activity against RTMDR-1 (EC_{50} 0.049 and 0.054 μM; TI 310 and 200, respectively), and were twofold more potent than the leads **4** and **35** (EC_{50} 0.11 and 0.19 μM; TI 60 and 58, respectively). Evaluation of water solubility showed that **5** and **22** were 5–10 times more water soluble than **4**. Quantitative structure–activity relationship (QSAR) modeling results were first performed on this compound type, and the models should aid in design of future anti-HIV DCP analogs and potential clinical drug candidates.

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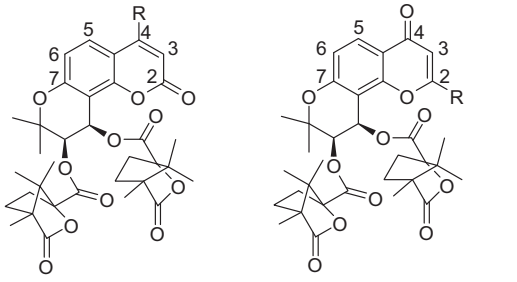
1. Introduction

Although over 30 formulations are now approved by the US FDA to treat AIDS, drug resistance problems have dramatically reduced the efficacy of these current anti-HIV agents.[1] Therefore, research to find new anti-HIV agents with either higher potency or novel mechanisms has attracted great attention to overcome this problem.[2]

In our prior studies, 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK, **1**) and 4-methyl DCK (4-MDCK, **2**) showed high potency against HIV-1_{IIIB} replication in H9 lymphocytes. The EC_{50} and therapeutic index (TI) values were reported as 0.049 μM and 328 for DCK, and, 0.0059 μM and 6660 for 4-MDCK, respectively (Fig. 1).[3,4] More specifically, preliminary mechanism of action-related studies indicated that 4-MDCK inhibited the activity of HIV-RT through inhibition of DNA-dependent DNA polymerase activity, in contrast to currently available NNRTIs that block HIV-RT by inhibiting RNA-dependent DNA polymerization.[5] However, DCK had reduced activity against the multi-RT inhibitor resistant (RTMDR-1) strain. In the course of our continuing exploration of DCK analogs as potent anti-HIV agents,



1 DCK, R = H
2 4-Me-DCK, R = CH_3

3 DCP, R = H
4 2-Ethyl-DCP, R = CH_2CH_3

Figure 1. DCK and DCP analogs.

4*H*-chrom-4-one derivatives (DCPs) were designed and synthesized as DCK positional isomers (Fig. 1).[6,7] Compared with DCKs, DCP analogs not only retained high activity against wild-type HIV, but also showed potency against RTMDR-1 HIV.[7] Among the previously reported DCP derivatives, 2-ethyl DCP (2-EDCP, **4**) exhibited the best anti-HIV activity against both wild-type and drug-resistant strains with EC_{50} values of 0.070 and 0.11 μM and TI values of 94 and 60, respectively. The uniqueness of DCP analogs opens a new avenue for us to discover a distinct class of potent, effective anti-HIV drugs for AIDS therapy.

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The structure–activity relationship (SAR) information provided from our previous study on the DCP series led to the following conclusions. Steric effects of substitutions on position-2, -3, and -6 of the chromone system could influence the anti-HIV activity. Bulky substituents at position-3 or -6 dramatically reduced anti-HIV activity. In addition, appropriate alkyl substitution at position-2 was crucial to maintain high activity against both wild-type and multi-RT inhibitor-resistant strains. 2-EDCP (**4**), with an ethyl group at position-2 of the chromone ring, exhibited the most potent activity against both virus strains.

However, the preliminary SAR information on DCPs was not extensive enough to establish a feasible pharmacological profile. Except for the steric effect, prior data could not illustrate how other factors such as electronic and hydrogen-bond effects might influence activity. In addition, all active DCP analogs synthesized had poor water-solubility. Therefore, additional DCP analogs with varying substituents, particularly different from the prior analogs, are needed in the search for an optimal anti-HIV-1 drug candidate from this compound class.

In our present study, DCP analogs with different structural functionalities on the pyranochromone have been synthesized towards this aim. We first designed and synthesized several 5-alkyl-substituted DCPs to explore the steric effect at position-5, which was not a major focus in prior studies. Then, we introduced combinations of diverse functional groups at position-2, -3, -5 and -6, including halogen, cyano, and amino groups, to explore electronic and hydrogen-bonding effects. Furthermore, we introduced hydrophilic heterocyclic amine moieties at position-2 to generate compounds with better water solubility. All newly synthesized DCPs were evaluated for their activity against both wild-type and RTMDR-1 strains. Two of the active and more polar compounds, **5** and **22**, were selected for water solubility analysis in comparison with the active lead compound **4**.

A QSAR molecular modeling study was also performed in this research using Partial Least Square (PLS) method with QuaSAR-Model module of MOE 2009 to systematically study the structure–anti-HIV–activity relationships of DCP-class compounds. With this study, we aimed not only to establish a pharmacological profile of DCPs, but also to study the DCP pharmacophores that play an important role in anti-HIV activity.

In this paper, we report and discuss the chemistry and synthesis of the newly synthesized DCP analogs, the results of anti-HIV activity evaluation, water solubility analysis, QuaSAR-model and pharmacophore studies, as well as structure–anti-HIV–activity relationship conclusions resulting from the studies.

2. Results and discussion

2.1. Chemistry

Scheme 1 illustrates the synthesis of 6-methyl- and 6-ethyl-2,4-dihydroxyphenyl ethanones (**30b**–**c**, respectively). Compounds **29b** (commercially available) and **29c** [synthesized by reduction of 3′,5′-dihydroxyacetophenone (**28**)] were acylated through a Friedel–Crafts reaction in the presence of the Lewis acid $ZnCl_2$ to afford **30b** and **30c**, respectively (Scheme 1).[8–10]

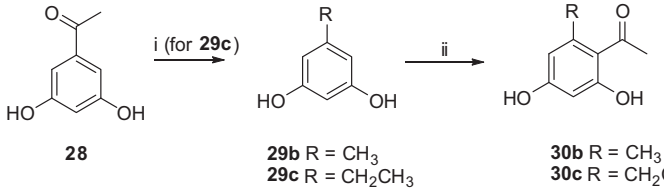
The synthesis of 2,3,5-alkyl substituted DCP analogs is shown in Scheme 2. Commercially available 1-(2,4-dihydroxyphenyl)ethanone (**30a**) and the synthesized **30b–c** were converted to **31a–c** by alkylation with 4,4-dimethoxy-2-methyl-2-butanol in pyridine. This reaction was conducted using a modified method by reaction of ethanone and butanol in a microwave initiator at 220 °C for 4 h.[11] Compounds **4**–**5**, **9**–**10**, and **35**–**36** were synthesized following literature procedures.[7] Briefly, reaction of **31a–c** with diverse ethyl alkanoates in the presence of NaH followed by hydrolysis with Amberlyst 15 resin in isopropanol afforded the chromone ring closure products (**32a**–**e**). 2′,2′-Dimethyl-3-methylpyaranochrmone (**32f**) was synthesized from propiophenone (**30d**) in two steps. Commercially available **30d** was treated with methanesulfonyl chloride in dry DMF to afford 7-hydroxy-3-methyl-chromone (**33**).[12] Compound **33** was converted to the corresponding pyranochromone (**32f**) by alkylation with 4,4-dimethoxy-2-methyl-2-butanol in pyridine under microwave conditions. The asymmetric dihydroxylation of **32a**–**f** was accomplished using a catalytic Sharpless asymmetric dihydroxylation,[13,14] in which $K_2OsO_2(OH)_4$ served as catalyst and $(DHQ)_2PYR$ as chiral auxiliary.[14,15] After drying in vacuo overnight, the diols (**34a–f**) were reacted with excess (*S*)-camphanic chloride in anhydrous dichloromethane in the presence of excess DMAP at rt for 2 h to afford the target compounds **4–5, 9–10**, and **35–36**.

Scheme 3 illustrates the synthesis of novel DCP analogs with various functional groups at position-2. The synthesis of **11** and **12** was accomplished by benzylic bromination with NBS in anhydrous carbon tetrachloride in the presence of 3-chloroperbenzoic acid as a radical initiator. Dibromo-substituted DCP analog (**13**) was also obtained during the reaction as previously reported.[16] Compound **11** was treated with KCN under mild condition in DMF to give **15**.[17] Reaction of **11** with appropriate amine groups in THF at rt afforded compounds **16** and **25–27**.[18]

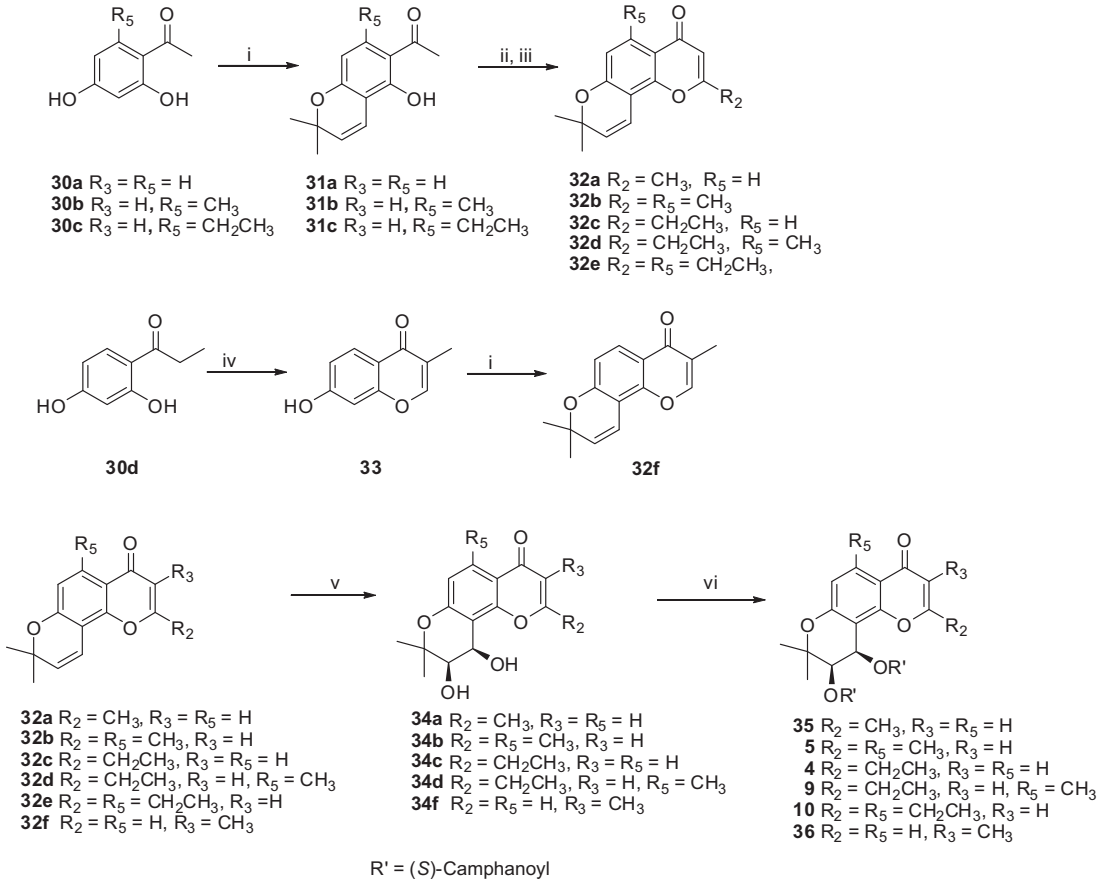
The synthesis of novel DCP analogs **17**–**24** with 3-substitutions is shown in Scheme 4. Selective bromination of **35** or **4** in acetonitrile gave **17** or **18**, respectively.[19] Compound **18** was subsequently converted to **20** in the presence of KCN in a mixture of DMF and 95% aq EtOH.[17] Reaction of **17** or **18** with 33% aqueous ammonium solution or methylamine at rt gave **21**–**24**.[18] Compound **35** was treated with I_2 in the presence of CF_3CO_2Ag as catalyst to obtain **19** in almost quantitative yield.[20]

The synthesis of 2-cyano-3-methyl-DCP (**14**) is given in Scheme 5. Stirring **36** with NBS in dichloromethane and heating to reflux gave **37**, which was further reacted with NaCN to give **14**, with a cyano substituent at position-2,[17] rather than displacement of bromide to give **14a**. The postulated Michael addition-elimination mechanism is illustrated in Scheme 6. Tautomerization of intermediate **38** regains resonance stabilization and produces compound **14**.

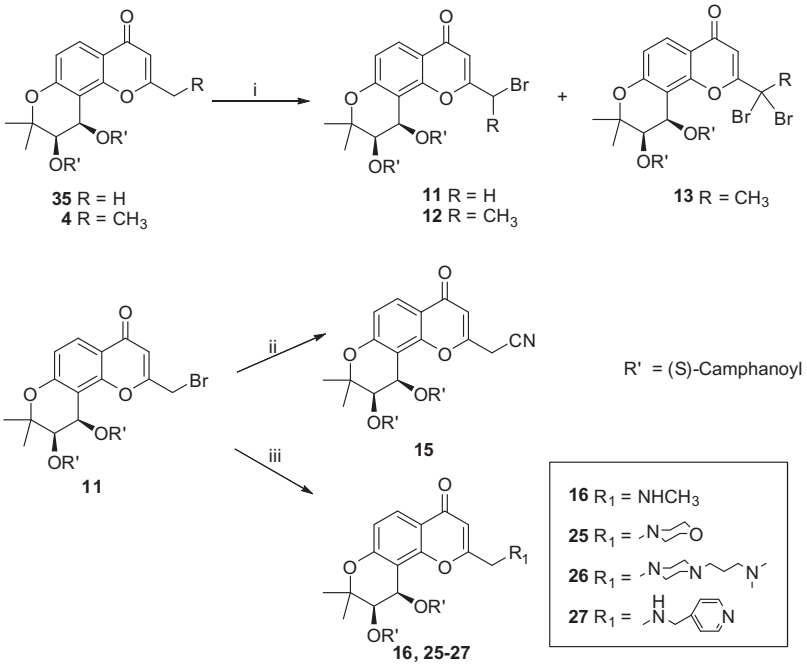
The synthesis of **6**–**8** is shown in Scheme 7. Different solvents were used as mentioned above to selectively generate **6** and **8**.[16,19] Compound **7** was obtained by using excess NBS.



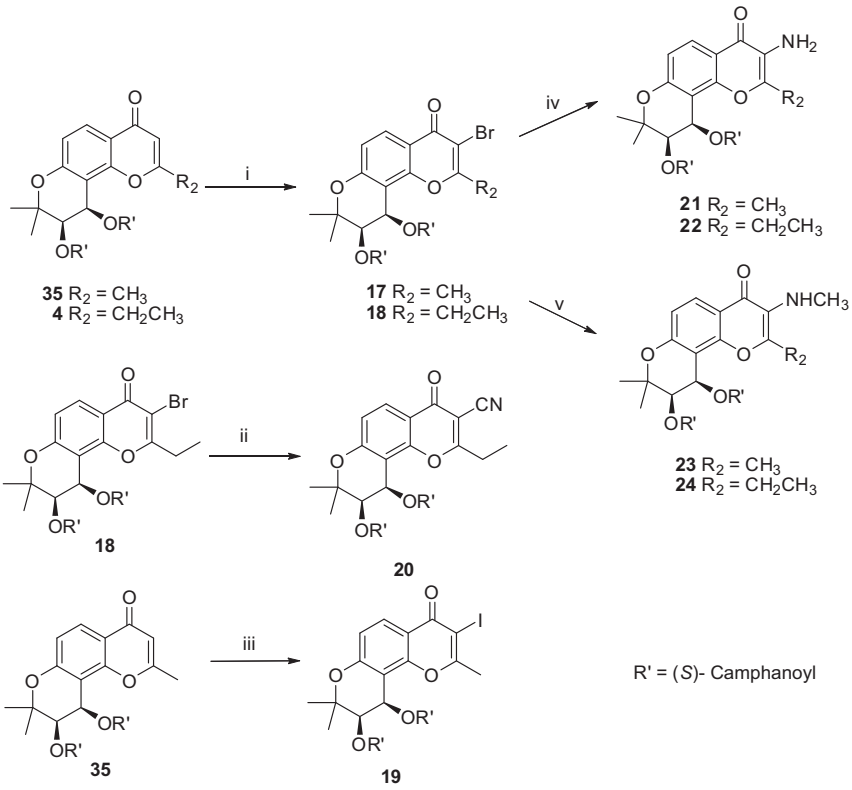
Scheme 1. Synthesis of **30b** and **30c**. Regents and conditions: (i) Pd/C, H_2, 4% HCl, rt; (ii) CH_3CN, HCl, $ZnCl_2$, diethyl ether, 0 °C.



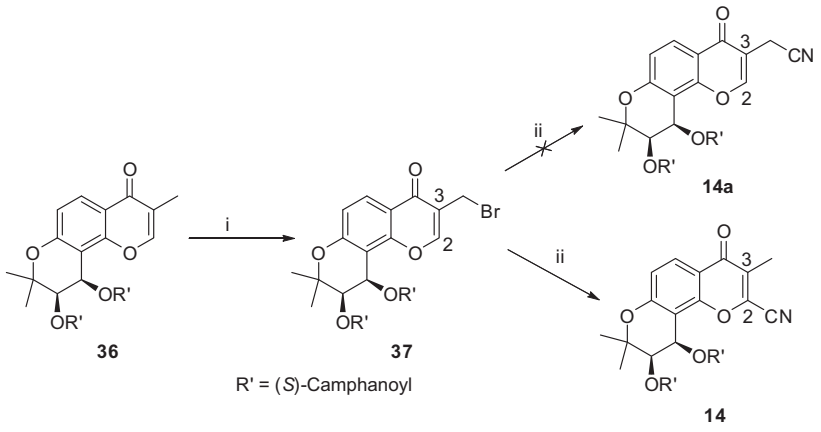
Scheme 2. Synthesis of alkyl substituted DCP analogs. Reagents and conditions: (i) 4,4-dimethoxt-2-methyl-2-butanol, pyridine, microwave; (ii) ethyl alkanoates, NaH, THF, reflux; (iii) Amberlyst 15 resin, isopropanol, reflux; (iv) methanesulfonyl chloride, DMF, (v) $K_3Fe(CN)_6$, $(DHQ)_2PYR$; $K_2OsO_2(OH)_4$, K2CO3, *t*-butanol/H_2O, 0 °C; (vi) (*S*)-camphanoyl chloride, DMAP, CH_2Cl_2.



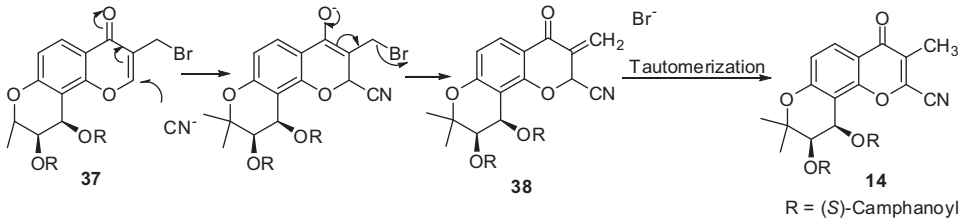
Scheme 3. Synthesis of novel 2-subsituted DCP analogs (**11**–**13**, **15**, **16**, **25**–**27**). Reagents and conditions: (i) NBS, 3-chloroperbenzoic acid, CCl_4, reflux; (ii) KCN, DMF, 95% aq EtOH; (iii) THF, diverse amine.



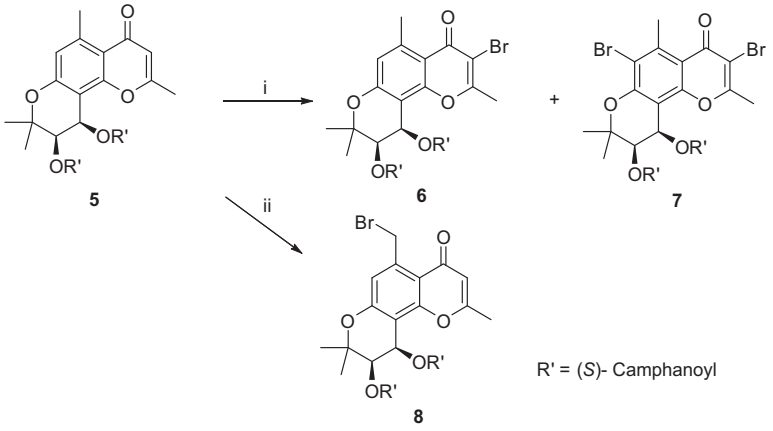
Scheme 4. Synthesis of novel 3-subsituted DCP analogs (**17**–**24**). Reagents and conditions: (i) NBS, CH_2Cl_3, reflux; (ii) KCN, DMF, 95% aq EtOH; (iii) I_2, CF_3COOAg, CH_3Cl_2, 0 °C; (iv) NH_4OH, THF; (v) $NHCH_3$ H_2O, THF.



Scheme 5. Synthesis of **14**. Reagents and conditions: (i) NBS, CH_3CN, reflux; (ii) NACN, DMF, 95% aq EtOH.



Scheme 6. Speculated mechanism for production of **14**.



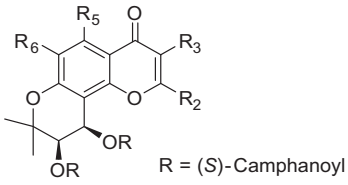
Scheme 7. Synthesis of compounds **6**–**8**. Reagents and conditions: (i) NBS, CH_3CN, reflux; (ii) NBS, 3-chloroperbenzoic acid, CCl_4, reflux.

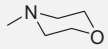
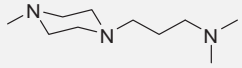
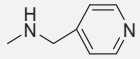
2.2. Biological evaluation

All newly synthesized DCP analogs (**5**–**27**) were evaluated for anti-HIV activity against both HIV-1_{NL4-3} and HIV-1 RTMDR1, a multi-RT inhibitor-resistant viral strain, in a single cycle infection assay using TZM-bl cells. The data are given in Table 1.

Compounds **5–10** are novel DCP analogs with short alkyl groups at both position-2 and -5. Compounds **5**, **9**, and **10** with methyl and ethyl substituents at these positions exhibited promising anti-HIV activity against the non-drug-resistant strain HIV-1_{NL4-3}. They also showed comparable or greater TI values compared with both positive DCP reference standards **4** and **35**. Compound **5** (2-CH_3, 5-CH_3)

Table 1
Anti-HIV activity of DCP analogs **5**–**27**[a]



Compd	R_2	R_3	R_5	R_6	IC_{50} [b] (μM)	NL4-3		HIV-1 RTMDR1	
						EC_{50} (μM)	TI	EC_{50} (μM)	TI
5	CH_3	H	CH_3	H	15	0.036	420	0.049	310
6	CH_3	Br	CH_3	H	>14	0.59	>24	0.91	>15
7	CH_3	Br	CH_3	Br	>12	10	>1.2	>12	1
8	CH_3	H	CH_2Br	H	8.9	0.81	11	1.2	7.4
9	CH_2CH_3	H	CH_3	H	11	0.10	110	0.054	200
10	CH_2CH_3	H	CH_2CH_3	H	>30	0.25	>120	0.34	>88
11	CH_2Br	H	H	H	1.7	0.55	3.0	0.56	3.0
12	$CHBrCH_3$	H	H	H	3.5	0.46	7.6	0.24	15
13	$C(Br)_2CH_3$	H	H	H	1.8	1.0	1.8	0.45	4.0
14	CN	CH_3	H	H	40	0.14	290	1.9	21
15	CH_2CN	H	H	H	17	1.8	9.4	1.4	12
16	CH_2NHCH_3	H	H	H	>30	0.29	>100	0.69	>43
17	CH_3	Br	H	H	7.7	0.55	14	0.57	14
18	CH_2CH_3	Br	H	H	>27	1.1	>24	0.99	>28
19	CH_3	I	H	H	13	0.73	18	1.6	8.0
20	CH_2CH_3	CN	H	H	>30	0.55	>54	0.79	>37
21	CH_3	NH_2	H	H	32	0.25	130	0.47	68
22	CH_2CH_3	NH_2	H	H	23	0.12	190	0.31	74
23	CH_3	$NHCH_3$	H	H	>60	0.30	>200	0.45	>130
24	CH_2CH_3	$NHCH_3$	H	H	32	0.44	73	1.1	29
25		H	H	H	>28	2.0	>14	4.4	>6.3
26		H	H	H	17	3.7	4.5	7.5	2.2
27		H	H	H	>27	21	>1.3	21	>1.3
4	CH_2CH_3	H	H	H	6.6	0.070	94	0.11	60
35	CH_3	H	H	H	11	0.10	110	0.19	58

[a] All data presented in this table were averaged from at least three independent experiments.
[b] Cytotoxicity was determined using a Promega CytoTox-Glo™ assay kit.

had the highest potency (EC_{50} 0.036 μM, TI 420) among these six compounds, and was two times more potent than **4** (EC_{50} 0.07 μM, TI 94). However, changing the 5-CH_3 in **5** to 5-CH_2Br in **8** was unfavorable to anti-HIV activity and also decreased the TI value (**8**: 0.81 μM, TI 11). Likewise, adding bromine at position-3 (**6**) or position-3 and -6 (**7**) led to decreased potency and TI.

Compounds **11**–**16** and **25**–**27** are novel 2-substituted DCPs. Similarly to **8**, bromination of the alkyl group at position-2 (**11**–**13**) was unfavorable to anti-HIV activity. The potency of **12** with bromoethyl substitution (EC_{50} 0.46 μM, TI 7.6) was six times lower than that of **4**; while **13** with dibromoethyl substitution, exhibited only mild potency (EC_{50} 1.0 μM, TI 1.8). Compound **14** with a cyano group at position-2 (EC_{50} 0.14 μM, TI 290) exhibited comparable anti-HIV activity and lower cytotoxicity compared with **4** and **35**. However, the anti-HIV activity of **15** with a cyanomethyl group at position 2 decreased significantly. With EC_{50} of 1.8 μM, **15** was ten times less potent than **14**, suggesting that a slight variation in the substitution at position-2 may result in a significant change in anti-HIV activity. It is postulated that expanding the conjugation of the coumarin core structure by adding a cyano group at position-2 might contribute to high activity. Compound **16**, with 2-CH_2NHCH_3 substitution, also showed considerable anti-HIV activity (EC_{50} 0.29 μM, TI >100). These results suggest that analogs with polar groups, such as cyano and amino, introduced appropriately at position-2, can maintain anti-HIV activity. In addition, these groups should increase the compounds' polarity, which may improve water solubility. However, **25**–**27** contain large hydrophilic moieties at position-2, and showed either very weak (**25** and **26**) or no (**27**) activity.

Compounds **17**–**24** are novel 3-substitued DCPs. Introduction of halogens such as bromine (**17** and **18**) and iodine (**19**) at position-3 reduced anti-HIV activity and TI. Compounds **17** (3-Br. EC_{50} 0.55 μM, TI 14) and **19** (3-I. EC_{50} 0.73 μM, TI 18) were five and seven times less potent, respectively, than the corresponding non-brominated **35** (EC_{50} 0.10 μM, TI 110). 3-Cyano-2-ethyl-DCP (**20**) showed moderate activity (EC_{50} 0.55 μM, TI >54), and analogs with NH_2 and $NHCH_3$ substituents at position-3 (**21**–**24**) maintained good anti-HIV activity. With a low EC_{50} value of 0.12 μM, **22** (3-NH_2) was equipotent with **4** (3-H) and **t**hree times more potent than **24** (3-$NHCH_3$). The 3-NH_2 analogs (**21** and **22**) showed comparable or greater potency and TI values than corresponding 3-$NHCH_3$ analogs (**23** and **24**).

In summary, the influence of position-2 and -3 substituents on anti-HIV activity was generally equivalent. Electronic and hydrogen-bonding effects from halogen, cyano, and amino groups at these positions could influence both anti-HIV activity and therapeutic index. Halogens were not favorable and led to decreased anti-HIV potency and lower TI, while amino moieties resulted in both potent anti-HIV activity and high TI values. Analogs with a cyano substituent, particularly at position-2, maintained good anti-HIV-1 activity. In addition, the extended conjugation of the chromone ring system might be important to the high-potency, and led to the potency difference of **14** and **15**. Anti-HIV-1 activity was quite sensitive to the substituent size at position-2, and large moieties were not tolerable. Functional groups at position-5 of the chromone ring are also important for potent anti-HIV activity. Addition of a methyl group at this position led to increased anti-HIV activity, as exemplified by **5** and **9**. 2,5-Dimethyl DCP (**5**) had the highest TI values against both wild-type and drug-resistant HIV.

Most of the new DCP analogs were active against HIV RTMDR-1 strain, but were approximately two to three times less potent than against wild-type virus. Compounds **5** and **9** showed the most promising activity against HIV-$1_{RTMDR-1}$ with EC_{50} values of 0.049 and 0.054 μM and TI values of 310 and 200, respectively. These two compounds were approximately twofold more potent than **4** against drug-resistant virus. Thus, the functional group at position-5 of the chromone ring is critical for potent activity against the drug-resistant strain. Halogen-substituted DCPs (**6–8**, **11–13**, **17–19**) showed reduced anti-HIV activity against HIV RTMDR-1 when compared with **4** and **35**. Among the halogen-substituted DCPs, **12** showed the best activity with EC_{50} of 0.24 μM. Amino-substituted DCP analogs (**16**, **21**–**24**) showed considerable activity against wild-type HIV-1_{NI4-3}, but reduced activity against the drug-resistant strain. The EC_{50} values of **16** and **22** against HIV-$1_{RTMDR-1}$ were 0.69 and 0.31 μM, respectively, which are approximately three times higher than EC_{50} against wild-type virus (0.29 μM and 0.12 μM). The SAR analysis of the synthesized DCP derivatives against the drug-resistant strain is similar to that for the wild-type virus.

2.3. Water solubility (WS) analysis

Because prior active DCP analogs showed poor water solubility, we were interested in improving this molecular parameter. We selected two active compounds from the preliminary SAR work for further analysis: **5**, which had the best anti-HIV activity against both virus strains, and **22**, which contains a hydrophilic amine group and maintains high anti-HIV activity against wild-type virus (Table 1). Both compounds showed lower predicted log *P* values than 2-EDCP (**4**) (Table 2), indicative of increased polarity that may improve the water solubility. We then performed a WS analysis with **5** and **22**, in comparison to **4**. We first established a standard curve of each tested compound by dissolution of the compound in acetonitrile at rt at various concentrations. The solubility in water could be determined by HPLC through the correlation between the saturated concentration of each compound in water and the correlating area detected by HPLC. With a solubility value less than 0.9 mg/L, 2-EDCP (**4**) showed the lowest WS among the three compounds. Compound **5** had an improved WS value (5.2 mg/L), and compound **22** presented the best WS value of 10.3 mg/L. (Table 2) This latter result confirmed that increasing the polarity of DCP analogs by introducing polar functional groups could result in improved water solubility. While both compounds **5** and **22** showed better WS than 2-EDCP (**4**), **5** also showed more potent anti-HIV activity than **4**, and thus, could merit further development study as a drug candidate.

2.4. Molecular modeling

2.4.1. Partial least square (PLS) QSAR

The PLS QSAR method was employed in the study using the QuaSAR-Model module of MOE 2009.[21] This method is relatively less sophisticated among those traditional available QSAR approaches. It was explored here to test if reliable models could be built for underlying data sets. A set of 2489 theoretical molecular descriptors used in this calculation was computed using the software Dragon v.5.5.[22] The number of components was set to no limit on the degree of the fit. The maximum condition number of the principal component transform of the correlation matrix S, the condition limit, was set to be a very large number of 1.0∗106.

We used the structures of the 25 DCP analogs listed in Table 1 and their anti-HIV activities (EC_{50} in μM) against both NL_{4-3} and

Table 2
Log *P* values and water solubility results of **4**, **5** and **2**

Compound	Predicted log *P* value[a]	Water solubility (mg/L)
5	3.85	5.2
22	2.88	10.3
4 (2-EDCP)	4.01	<0.9

[a] Calculated using ACD program.

RTMDR1 HIV strains to establish PLS models in the present study. The activity of each compound was transformed to the commonly used logarithm format and the log ($1/EC_{50}$) ranged from −1.31 to 1.44 for the activity against NL_{4-3} HIV and from -1.31 to 1.31 for the activity against RTMDR1 HIV. The leave-one-out cross validation scheme was used to test the reliability and robustness of the resulting models. One of the 25 compounds was excluded, and a PLS model was developed for the remaining 24 compounds. Then the model was used to predict the anti-HIV activity of the excluded compound. This procedure was repeated 25 times for each type of activity until each compound was used as the external test compound. From the leave-one-out cross validation procedure for the PLS model, the correlation coefficients (R^2)/mean absolute errors (MAE) for the wild type and drug resistant HIV strains were 0.67/0.30 (Fig. 2a) and 0.60/0.35, respectively (Fig. 2b). Compounds **25**, **26**, and **27** had relatively larger MAE than the remaining compounds, and compound **26** was a common outlier in both models. A probable reason is that these compounds have dissimilar R_2 substituents compared with the rest of the dataset. The R^2 and MAE values obtained from both models indicated that the newly established models can reliably be used to screen external chemical libraries in future studies.

2.4.2. Pharmacophore analysis

To explore DCP pharmacophores, the chemical structures of the three most potent compounds (**4**, **5**, and **9**) and the three weakest compounds (**7**, **26** and **27**) were energy minimized and superimposed using the Flexible Alignment of MOE 2009. Then the pharmacophore analysis was performed using the Pharmacophore Query. The results are shown in Figure 3a and b. The yellow balls shown in Figure 3 represent the identified pharmacophore. In both sets of compounds, the planar chromone ring, carbonyl group at position-4, and the oxygen at position-1′ were identified as part of the corresponding pharmacophore. However, in the most potent compounds (Fig. 3a), the carbonyl group of the 4′-camphanoyl ester, which represents a hydrogen bond acceptor, was identified as a unique pharmacophore. In the three weakest compounds (Fig. 3b), the orientation of both camphanoyl groups varied dramatically due to the introduction of bulky substitutions at position-2, which suggested that the orientations of the 3′- and 4′-camphanoyl groups might be critical for maintaining high anti-HIV activity.

3. Conclusions

Our study identified a series of new DCP analogs with high anti-HIV potency against both wild-type and drug-resistant HIV-1 strains. The following SAR conclusions were drawn from these results. (1) Position-5 of the DCP chromone ring system is critical for anti-HIV activity against both wild-type and drug-resistant HIV-1 strains, and appropriate alkyl groups on this position can improve anti-HIV activity against both virus strains. (2) Electronic and hydrogen-bonding effects at position-2 and -3 can influence the anti-HIV activity as well as therapeutic index. 3. The orientations of the 3′- and 4′-camphanoyl groups are critical to maintain high anti-HIV activity against both virus strain, and the carbonyl group in the 4′ position camphanoyl ester was identified as a potential hydrogen-bond acceptor by pharmacophore analysis. We also analyzed the water solubility of selected newly synthesized DCP analogs and confirmed that increasing polarity can dramatically improve the water-solubility of DCP analogs. In addition,

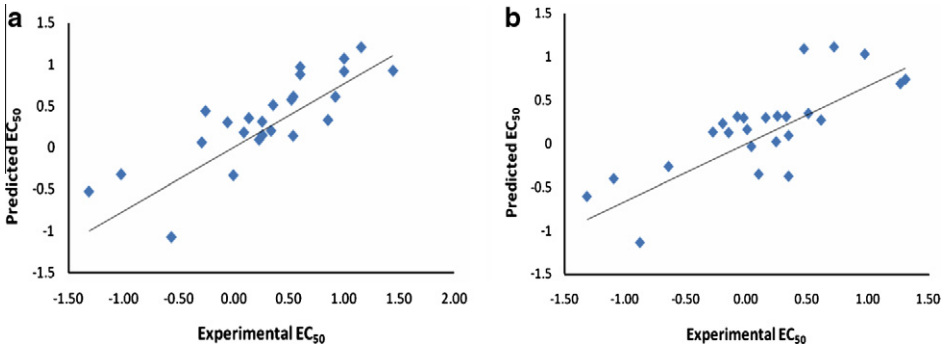


Figure 2. The correlation between experimental and predicted EC_{50} values obtained from leave one out cross validation for (a) NL4-3 HIV strain and (b) RTMDR1 HIV strain.

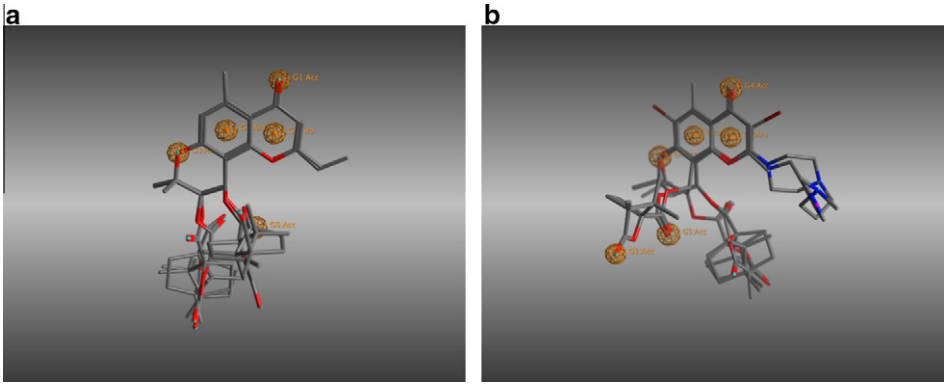


Figure 3. The pharamacophore analysis of the three most active compounds (a) and three most inactive compounds (b) using MOE 2009. The default setting was used except the tolerance (neighbor distance) and consensus score threshold (percentage of the compounds containing the pharmacophore) were changed to 0.5% and 100%, respectively. Atom coloring: gray, carbon; blue, nitrogen; red, oxygen. Pharmacophore schemes: purple, hydrogen bond donor; light blue, hydrogen bond acceptor; yellow, aromatic center.

we successfully established reliable PLS QSAR models. These models should help to predict the EC_{50} values of newly designed DCP analogs, which may be a useful tool for design of future new DCP analogs.

4. Experimental section

4.1. Chemistry

Melting points were measured with a Fisher Johns melting apparatus without correction. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Microwave reactions were performed with a Biotage initiator EXP US. Mass spectra were measured on Shimadzu LCMS-2010 (ESI-MS). Optical rotation was measured with a Jasco Dip-2000 digital polarimeter at 20 °C at the sodium D line. Thin-layer chromatography (TLC) was performed on PLC Silica Gel 60 F_{254} plates (0.5 mm, Merck). Biotage Flash and Isco Companion systems were used as medium-pressure column chromatography. Shimadzu LC-20AT prominence liquid chromatography was used as HPLC system. Alltima 2.1 mm × 100 mm C18 3u was used as HPLC column. Silica gel (200–400 mesh) from Aldrich, Inc. was used for column chromatography. All other chemicals were obtained from Aldrich, Inc. All final compounds are >95% pure on the basis of the two HPLC conditions.

4.1.1. Preparation of 5-ethylbenzene-1,3-diol (29c)

A reaction mixture of 2 g (13.1 mmol) of 3′,5′-dihydroxyacetophenone (**28**), 1 g of Pd/C (10%) and 150 mL aqueous HCl (4%) was hydrogenated overnight (900 mL of H_2). The mixture was filtered and extracted with three portions of Et_2O. The dried solution was evaporated at reduced pressure. The residue was purified by column chromatography with hexanes/EtOAc = 10:1 to afford **29c** as a white solid. 80% yield; MS (ESI+) *m*/*z* (%) 137 (M^++1, 100); ^{1}H NMR δ 6.26 (2H, s, H-4, 6), 6.18 (1H, s, H-2), 4.90 (2H, br, OH-1, 3), 2.53 (2H, q, *J* = 7.5 Hz, CH_2CH_3-5), 1.20 (3H. t, *J* = 7.5 Hz, CH_2CH_3-5).

4.1.2. Preparation of 1-(2,4-dihydroxy-6-methylphenyl)ethanone (30b)

MeCN (0.7 mL, 20 mmol) and dry $ZnCl_2$ (1.36 g, 10 mmol) were added to a solution of 3,5-dihydroxytoluene **29b** (1.24 g, 10 mmol) in Et_2O (5 mL). Hydrogen chloride gas was then bubbled through the mixture, and the resulting precipitate was filtered off and dissolved in water. This solution was neutralized by adding aqueous ammonia solution (33%) and was subsequently stirred for 30 min at 100 °C. The crude product was purified by column chromatography with hexanes/EtOAc = 7:3 to afford **30b** (680 mg). 41% yield; MS (ESI-) *m*/*z* (%) 165 (M^-−1, 100); ^{1}NMR δ 6.24 (1H, s, H-5), 6.23 (1H, s, H-3), 5.44 (2H, br, OH-2, 4), 2.62 (3H, s, $COCH_3$-1), 2.55 (3H, s, CH_3-6).

4.1.3. Preparation of 1-(2,4-dihydroxy-6-ethylphenyl)ethanone (30c)

The procedure was identical to that used for the preparation of **30b**. 40% yield (starting with 2.36 g of **29c**); MS (ESI+) *m*/*z* (%) 181 (M^++1, 100); ^{1}NMR δ 6.28 (1H, s, H-5), 6.19 (1H, s, H-3), 4.85 (2H, br, OH-2, 4), 2.91 (2H, q, *J* = 7.2 Hz, CH_2CH_3-6), 2.67 (1H, s, $COCH_3$-1), 1.30 (3H, t, *J* = 7.2 Hz, CH_2CH_3-6).

4.1.4. General procedure for the preparation of 31a–c and 32f

A mixture of starting compound **30a–c** (1 equiv) or **33**, 4,4-dimethoxy-2-methyl-2-butanol (1.5–2 equiv) and pyridine (2–3 mL) was heated at 220 °C for 4 h under high absorption microwave conditions. The reaction mixture was cooled to rt, diluted with EtOAc and washed with aqueous HCl (10%) and brine. The organic layer was separated, and solvent was removed in vacuo. The residue was purified by column chromatography with hexanes/EtOAc = 97:3 to afford **31a–c** and **32f**.

4.1.4.1. 6-Acetyl-2,2-dimethyl-5-hydroxy-2*H*-chromone (31a). 57.3% yield (starting with 1 g of **30a**); MS (ESI+) *m*/*z* (%) 219 (M^++1, 100); ^{1}NMR δ 7.52 (1H, d, *J* = 8.7 Hz, H-7), 6.72 (1H, d, *J* = 7.5 Hz, H-4), 6.33 (1H, d, *J* = 8.7 Hz, H-8), 6.58 (1H, d, *J* = 7.5 Hz, H-3), 2.54 (3H, s, $COCH_3$-1), 1.45 (6H, s, CH_3-2,2).

4.1.4.2. 6-Acetyl-2,2,7-trimethyl-5-hydroxy-2*H*-chromone (31b). 66.4% yield (starting with 77.2 mg of **30b**); mp 56–67 °C; MS (ESI+) *m*/*z* (%) 233 (M^++1, 100); ^{1}NMR δ 6.69 (1H, d, *J* = 10.2 Hz, H-4), 6.19 (1H, s, H-8), 5.52 (1H, d, *J* = 10.2 Hz, H-3), 3.31 (3H, s, $COCH_3$-1), 2.53 (3H, s, CH_3-7), 1.43 (6H, s, CH_3-2,2).

4.1.4.3. 6-Acetyl-2,2-dimethyl-5-hydroxy-7-ethyl-2*H*-chromone (31c). 72.4% yield (starting with 500 mg of **30c**); MS (ESI+) *m*/*z* (%) 247 (M^++1, 100%); ^{1}H NMR δ 6.70 (1H, d, *J* = 10.2 Hz, H-4), 6.25 (1H, s, H-8), 6.52 (1H, d, *J* = 10.2 Hz, H-3), 2.86 (2H, q, *J* = 7.5 Hz, CH_2CH_3-7), 2.64 (3H, s, CH_3CO-6), 1.42, 1.41 (each 3H, s, CH_3-2,2), 1.26 (3H, t, *J* = 7.5 Hz, CH_2CH_3-7).

4.1.4.4. 2′,2′,3-Trimethyl-pyrano[2,3,*f*]-chromone (32f). 60% yield (starting with 120 mg of **33**); mp 66–67 °C; MS (ESI+) *m*/*z* (%) 243 (M^++1, 100); ^{1}H NMR δ 7.98 (1H, d, *J* = 8.7 Hz, H-5), 7.73 (1H, s, H-2), 6.81 (1H, d, *J* = 8.7 Hz, H-6), 6.76 (1H, d, *J* = 9.9 Hz, H-4′), 5.67 (1H, d, *J* = 9.9 Hz, H-3′), 2.00 (3H, s, CH_3-3), 1.48 (6H, s, CH_3-2′,2′).

4.1.5. General procedure for the preparation of 32a–e

A mixture of **31a–c** and ethyl alkanoate in absolute THF was added slowly to a sodium hydride/THF suspension under nitrogen. The mixture was warmed to reflux temperature for 2–6 h monitored by TLC, followed by neutralization with 10% aq HCl, and extraction three times with CH_2Cl_2. The organic layer was collected and the solvent evaporated under reduced pressure. The residue and Amberlyst 15 resin were stirred in isopropanol at reflux temperature to give 2-substituted dimethylpranochromone **32a–e**.

4.1.5.1. 2,2′,2′-Trimethyl-pyrano[2,3,*f*]-chromone (32a). 56% yield (starting with 558.2 mg of **31a**); mp 123–125 °C; MS (ESI+) *m*/*z* (%) 243 (M^++1, 100); ^{1}H NMR δ 7.92 (1H, d, *J* = 8.7 Hz, H-5), 6.80 (1H, d, *J* = 8.7 Hz, H-6), 6.78 (1H, d, *J* = 9.9 Hz, H-4′), 6.09 (1H, s, H-3), 5.68 (1H, d, *J* = 9.9 Hz, H-3′), 2.36 (3H, s, CH_3-2), 1.48 (6H, s, CH_3-2′,2′).

4.1.5.2. 2,2′,2′-Trimethyl-5-methylpyrano[2,3,*f*]-chromone (32b). 38% yield (starting with 770 mg of **31b**); mp 128 130 °C; MS (ESI+) *m*/*z* (%) 257 (M^++1, 100); ^{1}H NMR δ 6.74 (1H, d, *J* = 9.9 Hz, H-4′), 6.56 (1H, s, H-6), 6.00 (1H, s H-3), 5.64 (1H, d, *J* = 9.9 Hz, H-3′), 2.76 (3H, s, CH_3-5), 2.31 (3H, s, CH_3-2), 1.46 (6H, s, CH_3-2′,2′).

4.1.5.3. 2′,2′-Dimethyl-2-ethylpyrano[2,3,*f*]-chromone (32c). 66% yield (starting with 1.1 g of **31a**); mp 97–98 °C; MS (ESI+) *m*/*z* (%) 279 (M^++Na, 100); ^{1}H NMR δ 7.92 (1H, d, *J* = 8.7 Hz, H-5), 6.80 (1H, d, *J* = 8.7 Hz, H-6), 6.77 (1H, d, *J* = 10.2 Hz, H-4′), 6.10 (1H, s, H-3), 5.69 (1H, d, *J* = 10.2 Hz, H-3′), 2.65 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 1.44, 1.48 (each 3H, s, CH_3-2′,2′), 1.30 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2).

4.1.5.4. 2′,2′-Dimethyl-2-ethyl-5-methylpyrano[2,3-*f*]-chromone (32d). 45% yield (starting with 64.8 mg of **31b**); mp 123–124 °C; MS (ESI+) *m*/*z* (%) 271 (M^++1, 100); ^{1}H NMR δ 7.75 (1H,

d, J = 10.5 Hz, H-4′), 6.57 (1H, s, H-6), 6.01 (1H, s, H-3), 5.64 (1H, d, J = 10.5 Hz, H-3′), 2.77 (3H, s, CH_3-5), 2.60 (2H, q, J = 7.5 Hz, CH_2CH_3-2), 1.59, 1.47 (each 3H, s, CH_3-2′,2′), 1.29 (3H, t, J = 7.5 Hz, CH_2CH_3-2).

4.1.5.5. 2′,2′-Dimethyl-2,5-diethylpyrano[2,3-*f*]chromone (32e). 36% yield (starting with 120 mg of **31c**); MS (ESI+) m/z (%) 285 (M^++1, 100); ^{1}H NMR δ 6.76 (1H, d, J = 10.2 Hz, H-4′), 6.61 (1H, s, H-6), 6.01 (1H, s H-3), 5.64 (1H, d, J = 10.2 Hz, H-3′), 3.24 (2H, q, J = 7.5 Hz, CH_2CH_3-5), 2.58 (2H, q, J = 7.5 Hz, CH_2CH_3-2), 1.48 (6H, s, CH_3-2′,2′), 1.29 (3H, t, J = 7.5 Hz, CH_2CH_3-5), 1.22 (3H, t, J = 7.5 Hz, CH_2CH_3-2).

4.1.6. Preparation of 7-hydroxy-3-methylchromone (33)

The commercially available phenol **30d** (400 mg, 2.41 mmol) in dry DMF (6 mL) was heated to 50 °C, and a solution of methanesulfonyl chloride (0.5 mL) in dry DMF (1 mL) was added slowly. The mixture was then reacted at 60 °C for 6 h. After cooling, the reaction mixture was poured into a large volume of ice-cold aqueous sodium acetate (12 g/100 mL). The crude product was filtered off and purified by column chromatography with hexanes/EtOAc = 7:3 to afford **33** (120 mg). 28% yield; mp 155–157 °C; MS (ESI+) m/z (%) 199 (M^++Na, 100); ^{1}H NMR δ 10.72 (1H, s, OH-7), 8.11 (1H, s, H-2), 7.88 (1H, d, J = 9.0 Hz, H-5), 6.89 (1H, dd, J = 9.0, 2.4 Hz, H-6), 6.80 (1H, d, J = 2.4 Hz, H-8), 1.87 (3H, s, CH_3-3).

4.1.7. General procedure for the preparation of 34a–d and 34f

A mixture of $K_3Fe(CN)_6$ (3 equiv), K_2CO_3 (3 equiv), $(DHQ)_2$-PYR (2% equiv), and $K_2OsO_2(OH)_4$ (2% equiv) was dissolved in *t*-BuOH/H_2O (v/v, 1:1) at rt. The solution was cooled to 0 °C and methanesulfonamide (1 equiv) was added with stirring. After 20 min, substituted pyranochromone (**32a–f**) was added. The mixture was stirred at 0 °C for 1–2 days, monitored by TLC. At completion, $Na_2S_2O_5$ (excess), water and CH_2Cl_2 were added, and stirring was continued for 1 h at rt. The mixture was extracted with CH_2Cl_2 three times, and the combined organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography with hexanes/EtOAc = 3:7 to afford the pure substituted (+)-*cis*-3′,4′-dihydroxypyranochromones (**34a–f**).

4.1.7.1. 3′*R*,4′*R*-Dihydroxy-2,2′,2′-trimethylpyrano[2,3-*f*]chromone (34a). 66% yield (starting with 1.1 g of **32a**); mp 176–178 °C; MS (ESI+) m/z (%) 276 (M^++1, 100); ^{1}H NMR (DMSO) δ 7.95 (1H, d, J = 9.0 Hz, H-5), 6.84 (1H, d, J = 9.0 Hz, H-6), 6.10 (1H, s, H-3), 5.20 (1H, t, J = 4.2, 4.2 Hz, H-4′), 3.87 (1H, t, J = 7.2, 4.2 Hz, H-3′), 3.44 (1H, d, J = 4.2 Hz, OH-4′), 3.18 (1H, d, J = 7.2 Hz, OH-3′), 2.40 (3H, s, CH_3-2), 1.50, 1.44 (each 3H, s, CH_3-2′,2′).

4.1.7.2. 3′*R*,4′*R*-Dihydroxy-2, 5, 2′,2′-tetramethylpyrano[2,3-*f*]chromone (34b). 35% yield (starting with 325 mg of **32b**); mp 114–116 °C; MS (ESI+) m/z (%) 291 (M^++1, 100); ^{1}H NMR δ 6.61 (1H, s, H-6), 6.04 (1H, s, H-3), 5.15 (1H, t, J = 3.9, 4.5 Hz, H-4′), 3.85 (1H, dd, J = 4.5, 6.6 Hz, H-3′), 3.08 (1H, d, J = 3.9 Hz, OH-4′), 3.05 (1H, d, J = 6.6 Hz, OH-3′), 2.74 (3H, s, CH_3-5), 2.35 (3H, s, CH_3-2), 1.46, 1.43 (each 3H, s, CH_3-2′,2′).

4.1.7.3. 3′*R*,4′*R*-Dihydroxy-2′,2′-dimethyl-2-ethylpyrano[2,3-*f*]-chromone (34c). 28% yield (starting with 120 mg of **32c**); mp 153–155 °C; MS (ESI+) m/z (%) 291 (M^++1, 100); ^{1}H NMR (DMSO) δ 7.80 (1H, d, J = 9.0 Hz, H-5), 6.83 (1H, d, J = 9.0 Hz, H-6), 6.13 (1H, s, H-3), 4.97 (1H, t, J = 4.8, 4.2 Hz, H-4′), 3.64 (1H, t, J = 6.6, 4.8 Hz, H-3′), 3.08 (1H, d, J = 4.2 Hz, OH-4′), 2.99 (1H, d, J = 6.6 Hz, OH-3′), 2.58 (2H, q, J = 7.5 Hz, CH_2CH_3-2), 1.38, 1.37 (each 3H, s, CH_3-2′,2′), 1.26 (3H, t, J = 7.5 Hz, CH_2CH_3-2).

4.1.7.4. 3′*R*, 4′*R*-Dihydroxy-5,2′,2′-trimethyl-2-ethylpyrano[2,3-*f*]chromone (34d). 40% yield (starting with 272 mg of **32d**); mp 114–116 °C; MS (ESI+) m/z (%) 305 (M^++1, 100); ^{1}H NMR δ 6.62 (1H, s, H-6), 6.06 (1H, s, H-3), 5.15 (1H, dd, J = 3.6, 5.1 Hz, H-4′), 3.86 (1H, dd, J = 5.1, 6.9 Hz, H-3′), 3.01 (1H, d, J = 6.9 Hz, OH-3′), 2.98 (1H, d, J = 3.9 Hz, OH-4′), 2.76 (3H, s, CH_3-5), 2.64 (2H, q, J = 7.5 Hz, CH_2CH_3-2), 1.46, 1.42 (each 3H, s, CH_3-2′,2′), 1.31 (3H, t, J = 7.5 Hz, CH_2CH_3-2).

4.1.7.5. 3′*R*,4′*R*-Dihydroxy-3,2′,2′-trimethylpyrano[2,3-*f*]chromone (34f). 55% yield (starting with 1.3 g of **32f**); mp 180–182 °C; MS (ESI+) m/z (%) 277 (M^++1, 100); ^{1}H NMR δ 7.98 (1H, d, J = 8.7 Hz, H-5), 7.74 (1H, d, J = 1.0 Hz, H-2), 6.82 (1H, d, J = 8.7 Hz, H-6), 6.15 (1H, dd, J = 5.4, 3.6 Hz, H-4′), 3.85 (1H, dd, J = 5.7, 5.4 Hz, H-3′), 3.43 (1H, d, J = 3.6 Hz, OH-4′), 3.19 (1H, d, J = 5.7 Hz, OH-3′), 1.99 (3H, d, J = 1.0 Hz, CH_3-3), 1.41, 1.42 (each 3H, s, CH_3-2′,2′).

4.1.8. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-3-bromomethyl-dihydropyrano[2,3-*f*]chromone (37)

The mixture of **36** (40 mg, 0.06 mmol), NBS (18 mg, 0.1 mmol), and MeCN (2 ml) was heated to reflux for 4 h, monitored by TLC. At completion, the mixture was concentrated and purified by PTLC with an eluent of hexanes/EtOAc = 5:4 to afford pure **37** (30 mg): 70% yield; MS-ESI+ (*m/z,%*) 715 (M^++1, 100); ^{1}H NMR δ 8.21 (1H, d, J = 9.0 Hz, H-5), 7.96 (1H, s, H-2), 6.98 (1H, d, J = 9.0 Hz, H-6), 6.72 (1H, d, J = 4.8 Hz, H-4′), 5.38 (1H, d, J = 4.8 Hz, H-3′), 4.35 (2H, s, CH_2Br-3), 2.43, 2.20, 1.93, 1.85 (each 2H, m, camphanoyl CH_2), 1.53, 1.49 (each 3H, s, CH_3-2′,2′), 1.13, 1.10, 1.09, 1.02, 0.98, 0.91 (each 3H, s, camphanoyl CH_3).

4.1.9. General procedure for the preparation of 4–5, 9, 35–36

The substituted 3′*R*,4′*R*-dihydroxypyranochromones (**34a–f**), (*S*)-(−)-camphanic chloride (3 equiv), and DMAP (4 equiv) were stirred in CH_2Cl_2 for 1–2 h at rt, monitored by TLC. At completion, the mixture was diluted with CH_2Cl_2 and washed by water and brine. The solvent was then removed under reduced pressure and the residue was purified by PTLC with hexanes/EtOAc = 3:2 to afford the appropriately alkyl-substituted 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyldihydroprano[2,3-*f*]chromones (**4–5**, **9**, **35–36**).

4.1.9.1. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2,2′,2′-trimethyldihydropyrano[2,3-*f*]chromone (35). 70% yield (starting from 100 mg of **34a**); mp 146–148 °C; MS-ESI+ (*m/z,%*) 659 (M^++Na, 100); ^{1}H NMR δ 8.11 (1H, d, J = 8.8 Hz, H-5), 6.90 (1H, d, J = 8.8 Hz, H-6), 6.75 (1H, d, J = 4.6 Hz, H-4′), 6.12 (1H, s, H-3), 5.37 (1H, d, J = 4.6 Hz, H-3′), 2.46, 2.12, 1.92, 1.70 (each 2H, m, camphanoyl CH_2), 2.27 (3H, s, CH_3-2), 1.53, 1.46 (each 3H, s, CH_3-2′), 1.11, 1.10, 1.07, 1.00, 0.97, 0.94 (each 3H, s, camphanoyl CH_3); 60% de. $[R]_D$ −69.6 (*c* 0.25, $CHCl_3$).

4.1.9.2. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-3,2′,2′-trimethyldihydropyrano[2,3-*f*]chromone (36). 75% yield (starting from 200 mg of **34f**); mp 146–148 °C; MS-ESI+ (*m/z,%*) 659 (M^++Na, 100); ^{1}H NMR δ 8.16 (1H, d, J = 9.0 Hz, H-5), 7.61 (1H, s, H-2), 6.91 (1H, d, J = 9.0 Hz, H-6), 6.70 (1H, d, J = 4.8 Hz, H-4′), 5.36 (1H, d, J = 4.8 Hz, H-3′), 2.56, 2.32, 2.24, 1.85 (each 2H, m, camphanoyl CH_2), 2.12 (3H, s, CH3-3), 1.64, 1.59 (each 3H, s, CH3-2′), 1.32, 1.24, 1.22, 1.12, 1.10, 1.00 (each 3H, s, camphanoyl CH_3); 90% de. $[\alpha]_D$ −36.2 (*c* 0.23, $CHCl_3$).

4.1.9.3. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2′,2′-dimethyldihydropyrano[2,3-*f*]chromone (4). 71% yield (starting with 146 mg of **34c**); mp 90–92 °C; MS-ESI+ (*m/z,%*) 645 (M^++Na, 100); ^{1}H NMR δ 8.15 (1H, d, J = 9.0 Hz, H-5), 7.69 (1H, d, J = 6.3 Hz, H-2), 6.94 (1H, d, J = 9.0 Hz, H-6), 6.72 (1H, d, J = 4.8 Hz, H-4′), 6.32 (1H, d, J = 6.3 Hz, H-3), 5.37 (1H, d, J = 4.8 Hz, H-3′), 2.46, 2.20, 1.90, 1.74

(each 2H, m, camphanoyl CH_2), 1.52, 1.47 (each 3H, s, CH_3-2′), 1.11, 1.10, 1.08, 1.02, 0.99, 0.89 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −95.3 (*c* 0.17, $CHCl_3$).

4.1.9.4. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2,5,2′,2′-tetramethyldihydropyrano[2,3-*f*]chromone (5). 54% yield (starting with 290 mg of **34b**); mp 144–145 °C; MS-ESI+ (*m/z*,%) 645 (M^++1, 100); ^{1}H NMR δ 6.74 (1H, d, *J* = 4.8 Hz, H-4′), 6.67 (1H, s, H-6), 6.05 (1H, s, H-3), 5.37 (1H, d, *J* = 4.8 Hz, H-3′), 2.81 (3H, s, CH_3-5), 2.50, 2.20, 1.95, 1.85 (each 2H, m, camphanoyl CH_2), 2.24 (3H, s, CH_3-2), 1.54, 1.47 (each 3H, s, CH_3-2′,2′), 1.14, 1.13, 1.10, 1.01, 1.00, 0.96 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −71.2 (*c* 0.002, CH_2Cl_2).

4.1.9.5. 3′*R*,4′*R*-Di-*O* -(−)-camphanoyl-2′,2′-dimethyl-2-ethyl-5-methyldihydropyrano[2,3-f]chromone (9). 60% yield (starting with 100 mg of **34d**); mp 133–134 °C; MS-ESI+ (*m/z*,%) 665 (M^++1, 100); ^{1}H NMR δ 6.70 (1H, d, *J* = 4.5 Hz, H-4′), 6.64 (1H, s, H-6), 6.04 (1H, s H-3), 5.36 (1H, d, *J* = 4.5 Hz, H-3′), 2.78 (3H, s, CH_3-5), 2.50 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 2.50, 2.14, 1.91, 1.71 (each 2H, m, camphanoyl CH_2), 1.52, 1.44 (each 3H, s, CH_3-2′,2′), 1.21 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2), 1.11, 1.10, 1.07, 0.99, 0.97, 0.95, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −55.0 (*c* 0.003, CH_2Cl_2).

4.1.10. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2,5,2′,2′-tetramethyl-3-bromodihydropyrano[2,3-*f*]chromone (6)

A mixture of **5** (80 mg, 0.12 mmol), NBS (32.0 mg, 0.18 mmol) and MeCN (2 mL) was heated to 110 °C for 3 h under high-absorption microwave conditions. At completion, the mixture was concentrated and purified by PTLC with an eluent of hexanes/EtOAc = 1:1 to afford pure **6** (28 mg). 32% yield; mp 146–147 °C; MS-ESI+ (*m/z*,%) 729 (M^+, 100); ^{1}H NMR δ 6.72 (1H, d, *J* = 4.8 Hz, H-4′), 6.71 (1H, s, H-6), 5.36 (1H, d, *J* = 4.8 Hz, H-3′), 2.81 (3H, s, CH_3-5), 2.49 (3H, s, CH_3-2), 2.50, 2.15, 1.95, 1.72 (each 2H, m, camphanoyl CH_2), 1.53, 1.47 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.09, 1.00, 0.98, 0.95 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −65.8 (*c* 0.018, CH_3Cl).

4.1.11. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2,5,2′,2′-tetramethyl-3,6-dibromodihydropyrano[2,3-*f*]chromone (7)

The procedure was identical to that used for the preparation of **6**. 10% yield (starting with 80 mg of **5**); mp 148–150 °C; MS-ESI+ (*m/z*, %) 809 (M^+, 100); ^{1}H NMR δ 6.74 (1H, d, *J* = 4.5 Hz, H-4′), 5.39 (1H, d, *J* = 4.5 Hz, H-3′), 3.05 (3H, s, CH_3-5), 2.49 (3H, s, CH_3-2), 2.50, 2.16, 1.92, 1.73 (each 2H, m, camphanoyl CH_2), 1.56 (6H, s, CH_3-2′,2′), 1.13, 1.12, 1.09, 1.00, 0.99, 0.96 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −67.2 (*c* 0.018, CH_3Cl).

4.1.12. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2, 2′,2′-trimethyl-5-bromomethyldihydropyrano[2,3-*f*]chromone (8)

A mixture of **5** (100 mg, 0.15 mmol), NBS (29.4 mg, 0.17 mmol), and 3-chloroperbenzoic acid (2.6 mg, 0.015 mmol), dissolved in 2 mL of anhydrous CCl_4 was heated to 100 °C for 5 h under high-absorption microwave conditions. At completion, the mixture was concentrated and the residue was purified by PTLC with an eluent of hexanes/EtOAc = 1:1 to afford pure **8** (38 mg). 35% yield; mp 128–130 °C; MS-ESI+ (*m/z*,%) 729 (M^+, 100); ^{1}H NMR δ 6.97 (1H, s, H-6), 6.72 (1H, d, *J* = 4.5 Hz, H-4′), 6.15 (1H, s, H-3), 5.39 (1H, d, *J* = 4.5 Hz, H-3′), 5.29 (1H, d, *J* = 61.2 Hz, CH_2Br-5), 5.12 (1H, d, *J* = 61.2 Hz, CH_2Br-5), 2.50, 2.18, 1.94, 1.71 (each 2H, m, camphanoyl CH_2), 2.26 (3H, s, CH_3-2), 1.54, 1.48 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.09, 1.02, 0.99, 0.97 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −66.9 (*c* 0.018, CH_3Cl).

4.1.13. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2,5-diethyldihydropyrano[2,3-f]chromone (10)

Compound **32e** (50 mg) was dihydroxylated using the identical procedure described above for 2 days. At completion, the mixture was extracted with CH_2Cl_2, and the combined organic layer was concentrated under reduced pressure to give crude 3′*R*,4′*R*-dihydroxyl-DCP. Without purification, the crude product was stirred with camphanic chloride (3 equiv) and DMAP (4 equiv) at rt for 2 h to give 30 mg of **10**. 30% yield; mp 108–110 °C; MS-ESI+ (*m/z*,%) 665 (M^++1, 100); ^{1}H NMR δ 6.70 (1H, d, *J* = 4.5 Hz, H-4′), 6.69 (1H, s, H-6), 6.04 (1H, s H-3), 5.37 (1H, d, *J* = 4.5 Hz, H-3′), 3.25 (2H, q, *J* = 7.5 Hz, CH_2CH_3-5), 2.50, (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 2.50, 2.15, 1.92, 1.70 (each 2H, m, camphanoyl CH_2), 1.53, 1.45 (each 3H, s, CH_3-2′,2′), 1.24 (3H, t, *J* = 7.5 Hz, CH_2CH_3-5), 1.21 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2), 1.12, 1.11, 1.07, 1.01, 0.98, 0.96, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −6.5 (*c* 0.003, CH_2Cl_2).

4.1.14. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-bromomethyldihydropyrano[2,3-*f*]chromone (11)

A mixture of **35** (200 mg, 0.31 mmol), NBS (60.6 mg, 0.34 mmol), and 3-chloroperbenzoic acid (5.4 mg, 0.031 mmol), dissolved in 2 mL of anhydrous CCl_4 was heated to 100 °C for 5 h under high-absorption microwave conditions. At completion, the mixture was concentrated and the residue was purified by PTLC with an eluent of hexanes/EtOAc = 1:1 to afford pure **11** (50 mg). 23% yield; mp 180–182 °C; MS-ESI+ (*m/z*,%) 717 (M^++1, 100); ^{1}H NMR δ 8.13 (1H, d, *J* = 9.0 Hz, H-5), 6.93 (1H, d, *J* = 9.0 Hz, H-5), 6.74 (1H, d, *J* = 4.5 Hz, H-4′), 6.40 (1H, s, H-3), 5.42 (1H, d, *J* = 4.5 Hz, H-3′), 4.12 (2H, s, CH_2Br-2), 2.50, 2.17, 1.90, 1.74 (each 2H, m, camphanoyl CH_2), 1.56, 1.47 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.09, 1.07, 1.02, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −13.9 (*c* 0.01, CH_2Cl_2).

4.1.15. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-(1-bromoethyl)dihydropyrano[2,3-f]chromone (12)

The procedure was identical to that used for the preparation of **11**: 25% yield (starting with 50 mg of **4**); mp 158–159 °C; MS-ESI+ (*m/z*,%) 731 (M^++1, 100); ^{1}H NMR δ 8.12 (1H, d, *J* = 9.0 Hz, H-5), 6.92 (1H, d, *J* = 9.0 Hz, H-5), 6.74 (1H, d, *J* = 4.5 Hz, H-4′), 6.34 (1H, s, H-3), 5.42 (1H, d, *J* = 4.5 Hz, H-3′), 4.76 (1H, t, *J* = 7.2, CHBrCH$_3$-2), 2.50, 2.19, 1.90, 1.74 (each 2H, m, camphanoyl CH_2), 1.57, 1.47 (each 3H, s, CH_3-2′,2′), 1.27 (3H, d, *J* = 7.2, CHBrCH_3-2), 1.12, 1.11, 1.08, 1.05, 1.03, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −31.9 (*c* 0.005, CH_2Cl_2).

4.1.16. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-(1-dibromoethyl)dihydropyrano[2,3-f]chromone (13)

A mixture of **4** (50 mg, 0.08 mmol), NBS (28.5 mg, 0.16 mmol), and 3-chloroperbenzoic acid (2 mg, 0.01 mmol), dissolved in 1 mL of anhydrous CCl_4 was heated to 100 °C for 5 h under high-absorption microwave conditions. At completion, the mixture was concentrated and the residue was purified by PTLC with an eluent of hexanes/EtOAc = 1:1 to afford pure **13** (8 mg). 12% yield; mp 166–168 °C; MS-ESI+ (*m/z*,%) 809 (M^++1, 100); ^{1}H NMR δ 8.13 (1H, d, *J* = 9.0 Hz, H-5), 6.94 (1H, d, *J* = 9.0 Hz, H-5), 6.80 (1H, d, *J* = 4.2 Hz, H-4′), 6.67 (1H, s, H-3), 5.42 (1H, d, *J* = 4.2 Hz, H-3′), 2.81 (3H, s, C(Br)$_2$CH$_3$-2), 2.50, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 1.57, 1.47 (each 3H, s, CH_3-2′,2′), 1.12, 1.11, 1.08, 1.05, 1.03, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −48.1 (*c* 0.004, CH_2Cl_2).

4.1.17. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-3,2′,2′-trimethyl-2-cyanodihydropyrano[2,3-f]chromone (14)

A solution of sodium cyanide in 95% EtOH (aqueous) was cooled in an ice-bath. Compound **37** (50 mg, 0.07 mmol) in 0.5 mL DMF was added slowly to the above solution over a 15–20 min period. The mixture was stirred at rt and monitored by TLC. At completion, the mixture was poured into ice-water and extracted with EtOAc three times. The combined organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated in vacuo and

the residue purified by PTLC to afford pure **14** (10 mg). 22% yield; mp 196–198 °C; MS-ESI+ (*m*/*z*,%) 684 (M^++Na, 100); ^{1}H NMR δ 8.13 (1H, d, *J* = 9.0 Hz, H-5), 6.99 (1H, d, *J* = 9.0 Hz, H-6), 6.61 (1H, d, *J* = 4.5 Hz, H-4′), 5.41 (1H, d, *J* = 4.5 Hz, H-3′), 2.55, 2.20, 1.96, 1.85 (each 2H, m, camphanoyl CH_2), 2.28 (3H, s, CH_3-3), 1.52, 1.48 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.10, 1.09, 1.05, 0.98 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −39.0 (*c* 0.002, CH_2Cl_2).

4.1.18. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-methylcyanodihydropyrano[2,3-f]chromone (15)

The procedure was identical to that used for the preparation of **14**: 27% yield (starting with 20 mg of **11**); mp 102–104 °C; MS-ESI+ (*m*/*z*,%) 684 (M^++Na, 100); ^{1}H NMR δ 8.14 (1H, d, *J* = 9.0 Hz, H-5), 6.97 (1H, d, *J* = 9.0 Hz, H-6), 6.72 (1H, d, *J* = 4.2 Hz, H-4′), 6.49 (1H, s, H-3), 5.40 (1H, d, *J* = 4.2 Hz, H-3′), 3.63 (2H, t, *J* = 25.5 Hz, CH_2CN-2), 2.50, 2.10, 1.95, 1.70 (each 2H, m, camphanoyl CH_2), 1.56, 1.49 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.09, 1.03, 1.00, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −27.9 (*c* 0.002, CH_2Cl_2).

4.1.19. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2,2′,2′-trimethyl-3-bromodihydropyrano[2,3-f]chromone (17)

The procedure was identical to that used for the preparation of **6**. 52% yield (starting from 21 mg of **35**); mp 160–161 °C; MS-ESI- (*m*/*z*,%) 713 (M^- - 1, 100); ^{1}H NMR δ 8.16 (1H, d, *J* = 9.0 Hz, H-5), 6.95 (1H, d, *J* = 9.0 Hz, H-6), 6.75 (1H, d, *J* = 4.5 Hz, H-4′), 5.38 (1H, d, *J* = 4.5 Hz, H-3′), 2.53 (3H, s, CH_3-2), 2.46, 2.16, 1.92, 1.73 (each 2H, m, camphanoyl CH_2), 1.54, 1.48 (each 3H, s, CH_3-2′,2′), 1.13, 1.12, 1.09, 1.01, 0.98, 0.96, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −47.7 (*c* 0.003, CH_2Cl_2).

4.1.20. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-ethyl-3-bromodihydropyrano[2,3-f]chromone (18)

The procedure was identical to that used for the preparation of **6**. 60% yield (starting from 20 mg of **4**); mp 166–168 °C; MS-ESI+ (*m*/*z*,%) 730 (M^++1, 100); ^{1}H NMR δ 8.18 (1H, d, *J* = 9.0 Hz, H-5), 6.95 (1H, d, *J* = 9.0 Hz, H-6), 6.74 (1H, d, *J* = 4.8 Hz, H-4′), 5.41 (1H, d, *J* = 4.8 Hz, H-3′), 3.02, 2.85 (each 1H, m, $CH_2$$CH_3$-2), 2.45, 2.10, 1.95, 1.85 (each 2H, m, camphanoyl CH_2), 1.55, 1.47 (each 3H, s, CH_3-2′,2′), 1.24 (3H, t, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 1.13, 1.11, 1.081.03, 0.98, 0.98 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −34.1 (*c* 0.006, CH_2Cl_2).

4.1.21. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2,2′,2′-trimethyl-3-iododihydropyrano[2,3-*f*]chromone (19)

An anhydrous CH_2Cl_2 solution of **35** (40 mg, 0.06 mmol) and CF_3CO_2Ag (13 mg, 0.06 mmol) was cooled to 0 °C in an ice-bath. I_2 (17.6 mg, 0.07 mmol) was added slowly under N_2 protection. The reaction mixture was stirred at 0 °C for 2 h, monitored by TLC. At completion, the mixture was concentrated and purified by PTLC to give pure **19** (43 mg): 90% yield; mp 179–180 °C; MS-ESI+ (*m*/*z*,%) 785 (M^++Na, 100); ^{1}H NMR δ 8.17 (1H, d, *J* = 9.0 Hz, H-5), 6.95 (1H, d, *J* = 9.0 Hz, H-6), 6.76 (1H, d, *J* = 4.5 Hz, H-4′), 5.39 (1H, d, *J* = 4.5 Hz, H-3′), 2.65 (3H, s, CH_3-2), 2.50. 2.15, 1.96, 1,85 (each 2H, m, camphanoyl CH_2), 1.55, 1.49 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.09, 1.02, 0.99, 0.96 (each 2H, s, camphanoyl CH_3); $[\alpha]_D$ −37.5 (*c* 0.002, CH_2Cl_2).

4.1.22. Preparation of 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-ethyl-3-cyanodihydropyrano[2,3-*f*]chromone (20)

The procedure was identical to that used for the preparation of **14**: 25% yield (starting with 50 mg of **18**); mp 193–194 °C; MS-ESI- (*m*/*z*,%) 674 (M^-−1, 100); ^{1}H NMR δ 7.75 (1H, d, *J* = 9.0 Hz, H-5), 6.78 (1H, d, *J* = 9.0 Hz, H-6), 6.65 (1H, d, *J* = 4.5 Hz, H-4′), 5.38 (1H, d, *J* = 4.5 Hz, H-3′), 2.51 (2H, q, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 2.40, 2.16, 1.92, 1.74 (each 2H, m, camphanoyl CH_2), 1.55, 1.49 (each 3H, s, CH_3-2′,2′), 1.24 (3H, t, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 1.21, 1.10, 1.07, 0.99, 0.98, 0.96, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −31.5 (*c* 0.002, CH_2Cl_2).

4.1.23. General procedure for the preparation of amino-substituted DCP derivatives (16, 21–27)

A THF solution of bromo-substituted DCP analogs (**11**, **17** or **18**) (1 equiv), various amines or aqueous amine solution (2.5 equiv) was stirred at rt for 3.5 h. The mixture was poured into water (excess) and extracted with EtOAc. After the usual workup, the crude product was purified by PTLC with an eluent of hexanes/EtOAc = 7:1 to afford corresponding amino-substituted DCP analogs (**16**, **21–27**).

4.1.23.1. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-(methylamino)methyldihydropyrano[2,3-*f*]chromone (16). 80% yield (starting from 50 mg of **11**); mp 137–138 °C; MS-ESI+ (*m*/*z*,%) 666 (M^++1, 100); ^{1}H NMR δ 8.14 (1H, d, *J* = 9.0 Hz, H-5), 6.92 (1H, d, *J* = 9.0 Hz, H-6), 6.75 (1H, d, *J* = 4.5 Hz, H-4′), 6.34 (1H, s, H-3), 5.40 (1H, d, *J* = 4.5 Hz, H-3′), 3.60, 3.55 (each 1H, d, *J* = 8.7 Hz, $CH_2$$NHCH_3$-2), 2.85 (1H, s, $CH_2NH$$CH_3$-2), 2.45, 2.14, 1.95, 1.71 (each 2H, m, camphanoyl CH_2), 2.45 (3H, s, CH_2NHCH_3-2), 1.55, 1.48 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.08, 1.02, 0.99, 0.97, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −35.8 (*c* 0.003, CH_2Cl_2).

4.1.23.2. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2,2′,2′-trimethyl-3-aminodihydropyrano[2,3-*f*]chromone (21). 50% yield (starting from 18 mg of **17**); mp 137–138 °C; MS-ESI+ (*m*/*z*,%) 652 (M^++1, 100); ^{1}H NMR δ 7.73 (1H, d, *J* = 8.7 Hz, H-5), 6.75 (1H, d, *J* = 4.8 Hz, H-4′), 6.70 (1H, d, *J* = 8.7 Hz, H-6), 5.39 (1H, d, *J* = 4.8 Hz, H-3′), 2.46 (2H, s, NH_2-3), 2.45, 2.20, 1.95, 1.85 (each 2H, m, camphanoyl CH_2), 2.17 (3H, s, CH_3-2), 1.48, 1.47 (each 3H, s, CH_3-2′,2′), 1.24, 1.10, 1.07, 0.99, 0.97, 0.86 (each 3H, s, camphanoyl CH_3; $[\alpha]_D$ −8.0 (*c* 0.004, CH_2Cl_2).

4.1.23.3. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-ethyl-3-aminodihydropyrano[2,3-*f*]chromone (22). 40% yield (starting from 100 mg of **18**); mp 145–146 °C; MS-ESI+ (*m*/*z*,%) 652 (M^++1, 100); ^{1}H NMR δ 7.70 (1H, d, *J* = 8.7 Hz, H-5), 6.73 (1H, d, *J* = 4.5 Hz, H-4′), 6.68 (1H, d, *J* = 8.7 Hz, H-6), 5.38 (1H, d, *J* = 4.5 Hz, H-3′), 5.0 (2H, br, NH_2-3), 2.50 (2H, q, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 2.40, 2.20, 1.91, 1.60 (each 2H, m, camphanoyl CH_2), 1.52, 1.46 (each 3H, s, CH_3-2′,2′), 1.21 (3H, t, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 1.11, 1.10, 1.07, 0.99, 0.97, 0.85, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −10.0 (*c* 0.003, CH_2Cl_2).

4.1.23.4. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-3,2′,2′-trimethyl-3-methylaminodihydropyrano[2,3-*f*]chromone (23). 80% yield (starting from 22.2 mg of **17**); mp 122–124 °C; MS-ESI+ (*m*/*z*,%) 666 (M^++1, 100); ^{1}H NMR δ 7.73 (1H, d, *J* = 8.4 Hz, H-5), 6.76 (1H, d, *J* = 4.8 Hz, H-4′), 6.70 (1H, d, *J* = 8.4 Hz, H-6), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 3.06 (3H, s, $NHCH_3$-3), 2.54 (1H, s, $NH$$CH_3$-3), 2.40, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 2.20 (3H, s, CH_3-2), 1.52, 1.47 (each 3H, s, CH_3-2′,2′), 1.12, 1.10, 1.06, 0.98, 0.95,0.83 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −21.4 (*c* 0.003, CH_2Cl_2).

4.1.23.5. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-ethyl-3-methylaminodihydropyrano[2,3-*f*]chromone (24). 75% yield (starting from 100 mg of **18**); mp 163–164 °C; MS-ESI+ (*m*/*z*,%) 680 (M^++1, 100); ^{1}H NMR δ 7.73 (1H, d, *J* = 8.4 Hz, H-5), 6.76 (1H, d, *J* = 4.5 Hz, H-4′), 6.69 (1H, d, *J* = 8.4 Hz, H-6), 5.39 (1H, d, *J* = 4.5 Hz, H-3′), 3.08 (3H, s, $NHCH_3$-3), 2.63 (2H, q, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 2.48, 2.20, 1.94, 1.77 (each 2H, m, camphanoyl CH_2), 1.55, 1.48 (each 3H, s, CH_3-2′,2′),1.13, 1.11, 1.07, 0.99, 0.96,0.87 (each 3H, s, camphanoyl CH_3), 1.08 (1H, br, $NH$$CH_3$-3); $[\alpha]_D$ −24.6 (*c* 0.013, CH_2Cl_2).

4.1.23.6. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-morpholinomethyl-dihydropyrano[2,3-*f*]chromone (25). 30% yield (starting from 30 mg of **11**); mp 140–142 °C; MS-ESI+ (*m*/*z*,%) 722 (M^++1, 100); ^{1}H NMR δ 8.13 (1H, d, J = 9.0 Hz, H-5), 6.92 (1H, d, J = 9.0 Hz, H-6), 6.74 (1H, d, J = 4.5 Hz, H-4′), 6.48 (1H, s, H-3), 5.38 (1H, d, J = 4.5 Hz, H-3′), 3.72 (4H, t, J = 4.5 Hz, 4.8 Hz, morpholine CH_2), 3.26, 3.42 (each 1H, d, J = 16.5 Hz, CH_2), 2.54 (4H, t, J = 4.5 Hz, 4.8 Hz, morpholine CH_2), 2.50, 2.15, 1.95, 1.70 (each 2H, m, camphanoyl CH_2), 1.54, 1.48 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.08, 1.00, 0.98, 0.95, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −28.5 (*c* 0.011, CH_2Cl_2).

4.1.23.7. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-(dimethylaminopropyl-piperazin1ylmethyl)–dihydropyrano[2,3-*f*]chromone (26). 15% yield (starting from 30 mg of **11**); mp 132–133 °C; MS-ESI+ (*m*/*z*,%) 806 (M^+, 100); ^{1}H NMR δ 8.13 (1H, d, J = 8.7 Hz, H-5), 6.92 (1H, d, J = 8.7 Hz, H-6), 6.73 (1H, d, J = 4.5 Hz, H-4′), 6.47 (1H, s, H-3), 5.38 (1H, d, J = 4.5 Hz, H-3′), 3.30, 3.44 (each 1H, d, J = 15.0 Hz, CH_2-2), 2.50 (8H, m, piperazine CH_2), 2.50, 2.15, 1.95, 1.70 (each 2H, m, camphanoyl CH_2), 2.41 (4H, m, amino-propylpiperazine CH_2), 2.28 (6H, s, dimethylamino-propylpiperazine CH_3), 1.70 (2H, m, amino-propylpiperazine CH_2), 1.54, 1.48 (each 3H, s, CH_3-2′,2′), 1.13, 1.11, 1.08, 1.01, 0.98, 0.95, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −20.3 (*c* 0.003, CH_2Cl_2).

4.1.23.8. 3′*R*,4′*R*-Di-*O*-(−)-camphanoyl-2′,2′-dimethyl-2-(pyridin-4-ylmethylamino)methyl-dihydropyrano[2,3-*f*]chromone (27). 25% yield (starting from 30 mg of **11**); mp 116–118 °C; MS-ESI+ (*m*/*z*,%) 806 (M^++1, 100); ^{1}H NMR δ 8.55 (2H, d, J = 6.0 Hz, pyridine CH), 8.13 (1H, d, J = 9.0 Hz, H-5), 7.29 (2H, d, J = 6.0 Hz, pyridine CH), 6.92 (1H, d, J = 9.0 Hz, H-6), 6.74 (1H, d, J = 4.5 Hz, H-4′), 6.36 (1H, s, H-3), 5.39 (1H, d, J = 4.5 Hz, H-3′), 3.85 (1H, s, CH_2NH-2), 3.59, 3.68 (each 1H, d, J = 15.9 Hz, CH_2-2), 2.50, 2.15, 1.95, 1.70 (each 2H, m, camphanoyl CH_2), 1.55, 1.48 (each 3H, s, CH_3-2′), 1.13, 1.11, 1.07, 0.99, 0.96, 0.94, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ −16.9 (*c* 0.003, CH_2Cl_2).

4.2. HIV-1 infectivity assay

Anti-HIV-1 activity was measured as reductions in Luc reporter gene expression after a single round of virus infection of TZM-bl cells. HIV-1 at 200 $TCID_{50}$ and various dilutions of test samples (eight dilutions, 4-fold stepwise) were mixed in a total volume of 100 μL growth medium in 96-well black solid plates (Corning-Costar). After 48-h incubation, culture medium was removed from each well and 100 μL of Bright Glo luciferase reagent was added to each culture well. The luciferase activity in the assay wells was measured using a Victor 2 luminometer. The 50% inhibitory dose (IC_{50}) was defined as the sample concentration that caused a 50% reduction in Relative Luminescence Units (RLU) compared to virus control wells after subtraction of background RLU.

4.3. Cytotoxicity assay

The general procedure was performed according to CytoTox-Glo™ cytotoxicity assay instructions for using product G9290, G9291 and G9292. (Promega)

4.4. Water solubility analysis assay

Each tested compound was added in excess to 1.5 mL Eppendorf tubes containing 1 mL of HPLC grade water. The tubes were placed into Branson 5510 ultrasonic tank at rt for 1 h. The excess solid was separated from the solution through a PTFE syringe filter (0.2 μM diameter). The supernatant was dispensed into glass HPLC vials. The concentration of the samples was determined with HPLC, on an Alltima C18 3u column (2.1 mm × 100 mm) and a flow rate of 200 μL/min. The samples (5 μL) were injected and run with a solution of 35% water and 65% MeCN. For each compound, a standard curve consisting of five concentrations (fivefold stepwise) in MeCN was established initially.

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Supplementary data

Supplementary data (HPLC conditions and summary of HPLC purity data for final compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.065.

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