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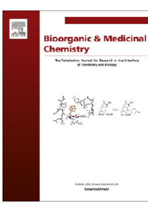

Design and discovery of flavonoid-based HIV-1 integrase inhibitors targeting both the active site and the interaction with LEDGF/p75

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A B S T R A C T

HIV integrase (IN) is an essential enzyme for the viral replication. Currently, three IN inhibitors have been approved for treating HIV-1 infection. All three drugs selectively inhibit the strand transfer reaction by chelating a divalent metal ion in the enzyme active site. Flavonoids are a well-known class of natural products endowed with versatile biological activities. Their β-ketoenol or catechol structures can serve as a metal chelation motif and be exploited for the design of novel IN inhibitors. Using the metal chelation as a common pharmacophore, we introduced appropriate hydrophobic moieties into the flavonol core to design natural product-based novel IN inhibitors. We developed selective and efficient syntheses to generate a series of mono 3/5/7/3′/4′-substituted flavonoid derivatives. Most of these new compounds showed excellent HIV-1 IN inhibitory activity in enzyme-based assays and protected against HIV-1 infection in cell-based assays. The 7-morpholino substituted **7c** showed effective antiviral activity (EC_{50} = 0.826 μg/mL) and high therapeutic index (TI > 242). More significantly, these hydroxyflavones block the IN–LEDGF/p75 interaction with low- to sub-micromolar IC_{50} values and represent a novel scaffold to design new generation of drugs simultaneously targeting the catalytic site as well as protein–protein interaction domains.

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1. Introduction

The virally encoded HIV integrase (IN) is an attractive target for the development of anti-AIDS drugs because it is an essential enzyme for the viral replication and has no mammalian counterparts.[1,2] IN catalyzes the integration of viral DNA into the host genome in a two-step process, 3′-processing (3′-P) and strand transfer (ST).[3] The active site of the enzyme comprises a DDE motif (D116, D64, E152), a catalytic triad of acidic amino acid residues that coordinates two divalent metal ions. The divalent metals are required for not only the 3′-P and ST steps, but also for the assembly of IN into specific viral donor DNA to form a complex that is competent to carry out either function.[3–5] Thus the chelation of the critical metal cofactors can cause functional impairment of IN, offering an excellent opportunity for the design and development of highly efficient IN inhibitors.[6–8] In fact, most of the current IN inhibitors that exhibit potent antiviral activity by the inhibition of viral DNA integration, commonly contain a structural motif that coordinates the metal cofactors in the active site.[6,7,9–11] Typically, the aryl diketoacid and its bioisosteres are the most developed class that selectively inhibit the ST step by chelating the two divalent metal ions.[12,13] Accordingly, the ketoenol carboxyl structure as the metal chelation motif and the coplanar aromatic group as the hydrophobic binding motif have been recognized as the pharmacophores for the HIV-1 IN ST inhibitors, that were exemplified by the FDA-approved HIV-1 IN inhibitors (Raltegravir, Elvitegravir, and Dolutegravir) (Fig. 1) and those under advanced clinical trials.[14]

Although great progresses have been made in the design and discovery of IN inhibitors as antiviral agents,[7,8] the emergence of viral strains resistant to clinically studied IN inhibitors and the dynamic nature of the HIV-1 genome demand a continued effort toward the discovery of novel inhibitors to keep a therapeutic advantage over the virus. Besides searching for new active site-directed HIV-1 inhibitors, allosteric inhibition of IN catalytic

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Figure 1. Structures of the three FDA-approved HIV-1 integrase inhibitors and a pharmacophore model for the metal binding in the active site.[1,2]

activity has emerged as an interesting trend in current R&D of the antiretroviral drugs.[15] Targeting a non-active-site region on IN that is still essential for its catalysis, such as a cofactor-binding site or dimerization hotspots,[16] could yield the same inhibitory potency as active site inhibitors, yet evoke a different resistance profile. So far, the most studied cellular cofactor of HIV-1 integration is the lens epithelium-derived growth factor/p75 (LEDGF/p75). This human protein was originally found to co-localize with IN and stimulate its activity[17] and later found to exert this stimulation by tethering IN to host cell chromatin.[18] In a proof-of-concept

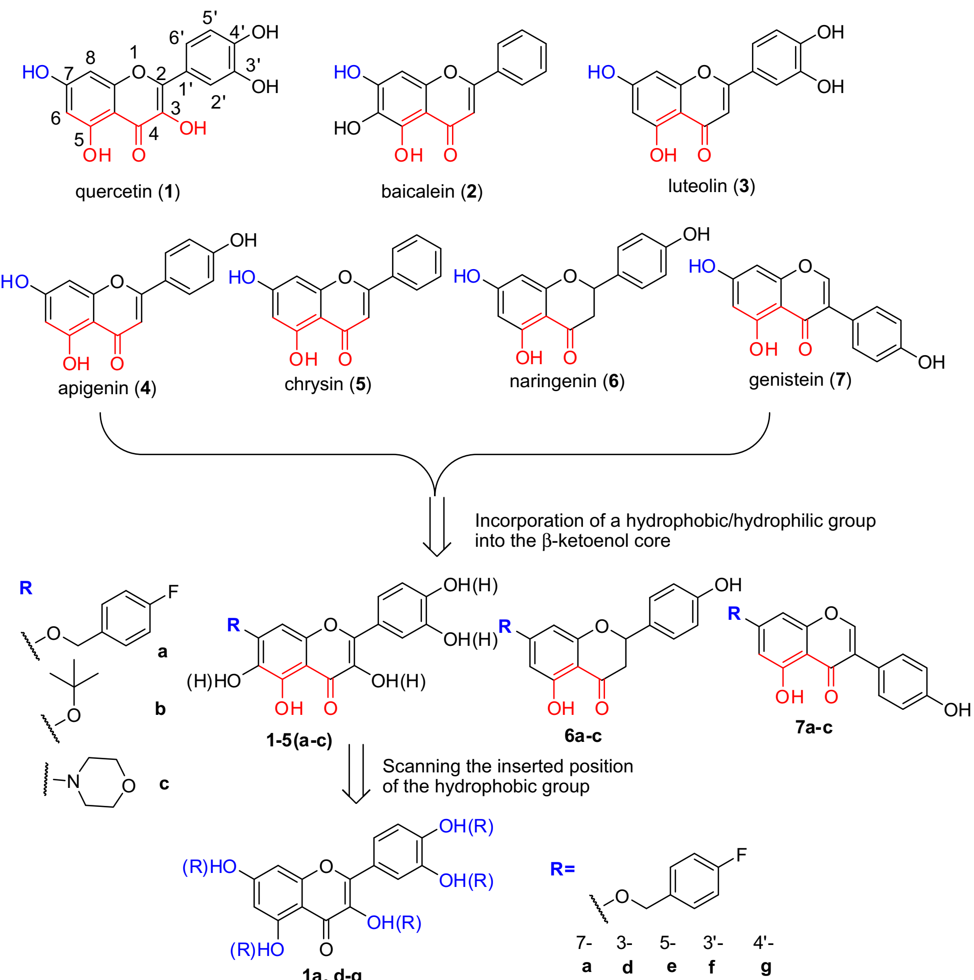

Figure 2. The pharmacophore-based design and SAR study on select β-ketoenol-containing flavonoids as novel HIV-1 IN inhibitors.

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study that validated the IN-LEDGF/p75 interaction as an antiviral target, the IN-binding LEDGF/p75 domain (IBD) was overexpressed in human cells and was proven to compete for IN binding with the endogenous full-length cofactor, thereby inhibiting HIV replication.[19]

Natural products are an important source for the discovery of new molecular entity drugs. Flavonoids are a well-known class of natural products demonstrating versatile biological activities, such as anti-inflammatory, anti-oxidative and anti-viral activity.[20–25] Inspired by the β-ketoenol structure inherently present in some flavonoids, we were interested in developing novel IN inhibitors by the pharmacophore-based design and SAR study on select natural flavonoids containing β-ketoenol module (Fig. 2 and **1**–**7**). First, a hydrophobic module (aromatic or alkyl group) was designed to incorporate into the β-ketoenol skeleton of these selected flavonoids to deliver novel metal chelators. For comparison, a hydrophilic morpholine group was introduced into the 7-position. Then, an optimal position of the hydrophobic moiety on

Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 4-fluorobenzyl bromide, rt → 70 °C, 67–95%; or BOC_2O, $MgClO_4$, DCM, 40 °C, 12–17%; (b) Ac_2O, pyridine, 70 °C, 94–96%; (c) PhSH, NMP, imidazole, 86–88%; (d) NH_3/CH_3OH, 0 °C → rt, 90–95%; (e) Ph_2CCl_2, diphenyl ether, 180 °C, 40%; (f) HAc/H_2O (4:1), reflux, 65%; (g) H_2, 20% $Pd(OH)_2/C$, rt.

Scheme 2. Reagents and conditions: (a) Ac_2O, pyridine, 70 °C, 92%; (b) PhSH, NMP, imidazole, 85%; (c) TsCl, TEA, THF, 0 °C, 90%; (d) NH_3/CH_3OH, 0 °C → rt, 92%; (e) BnBr, K_2CO_3, DMF, 80 °C, 86%; (f) $Pd(OAc)_2$, Xphos, *t*-BuOK, morpholine, Mw, 80 °C; (g) 10% $Pd(OH)_2/C$, rt, 81%.

Scheme 3. Reagents and conditions: (a) benzyl bromide, K_2CO_3, DMF, rt, 81–92%; (b) 4-flurobenzyl bromide, K_2CO_3, DMF, 80 °C, 43–91%; (c) Ph_2CCl_2, diphenyl ether, 180 °C, 40%; (d) HAc/H_2O (4:1), reflux, 44–65%; (e) 10% Pd–C, H_2, rt, 18–25%.

the β-ketoenol core was further surveyed by using quercetin as a model substrate. All derivatives were evaluated with respect to the inhibitory activities against the enzyme, the IN–LEDGF/p75 interaction and the viral infection in the C8166 cells. The optimal combination of the β-ketoenol and hydrophobic pharmacophores indeed resulted in an effective inhibition of IN catalytic activities and viral infection. Significantly, some hydroxyflavonoids displayed excellent inhibition of the IN–LEDGF/p75 interaction. This is the first report on the rational design and systematic SAR study of flavonoid-based HIV-1 IN inhibitors that also block IN–LEDGF/p75 interaction.

2. Chemistry

In order to investigate the optimal combination of the β-ketoenol and the hydrophobic moieties on the flavone core, we needed to synthesize various flavonoid derivatives with substitutions at C3, C3′, C4′, C5, and C7 hydroxyl functionalities with various alkoxy, and heterocycle moieties. Since the flavonoids selected in this study bear polyphenolic groups that could display non-selective reactivities, we introduced various substituents into the core ring at the C7-position. This site is susceptible to nucleophilic substitution (Scheme 1). The C7-hydroxy group is located *para* to the electron-withdrawing pyrone carbonyl functionality and thus is the most acidic site. Furthermore, the C5-hydroxy could form an intramolecular hydrogen bonding with the C4-carbonyl group. Therefore, the nucleophilic substitution of naringenin **5** and chrysin **6** predominantly occurred at the C7-position, generating the benzyloxy or *tert*-butyloxy-substituted products **5a**–**b**, **6a**–**b** by etherification of **5**, **6** with 4-fluorobenzyl bromide and K_2CO_3 in DMF or $Mg(ClO_4)_2$ and Boc_2O in DCM, respectively.

For other polyhydroxy substituted flavonoids (**1**–**4** and **7**), the regioselective nucleophilic substitution required orthogonally protected precursors, as depicted in Scheme 1.[26–32] In general, the polyphenolic flavonoids (**1**, **2**, **4** and **7**) were globally acetylated by the treatment with excess amount of acetic anhydride in pyridine (**8**–**11**), then the selective deacylation at 7-OH using imidazole-catalyzed acyl transfer to an aromatic thiol under basic conditions afforded the key intermediates **12**–**14**. Subsequently, the nucleophilic attack on the C7-hydroxy by 4-fluorobenzyle bromide or Boc_2O followed by the basic hydrolysis with methanolic ammonia furnished the desired 7-benzyloxy/*t*-butyloxy flavonoids [(**1**,**4**,**7**)**a**–**b**]. It is worth noting that we modified the traditional *tert*-butyl etherification reaction by using Boc_2O and $Mg(ClO_4)_2$ as the *tert*-butylation reagents to produce 7-*O*-*tert*-butylflavonoids under mild conditions. This protocol avoids the harsh conditions required by the conventional isobutylene as the nucleophile.[33] However, the very poor solubility of compounds **1** and **3** in the reaction solvent of dichloromethane abolished the production of the *tert*-butyl substituted derivatives.

For baicalein **2**, the selective 7-deacetylation reaction caused the removal of the C6- and C7-acetate groups by PhSH, NMP and imidazole. Alternatively, direct treatment of the globally acetylated intermediate **18a** with benzyl halide and potassium carbonate in dry acetone at 70 °C resulted in a selective C7-alkylation, affording the desired product **2a**.

For the luteolin **3**, bearing a catechol moiety, the benzophenone ketal protection of the 3′,4′-dihydroxy groups by treatment with dichlorodiphenylmethane at 180 °C (the intermediate **19**) ensured the selective benzylation of the 7-hydroxy (the precursor **20**). Cleavage of the dibenzyl group of **20** with a mixture of acetic acid and water (4:1) yielded the target compound **3a**.

Table 1
Inhibition of HIV-1 IN catalytic activities, IN–LEDGF/p75 interaction and the antiviral effect of the flavonoid derivatives with various substitution at 7-position

Compd	R	HIV-1 IN IC_{50}[a] (μM)		Antiviral activity (μg/mL)		TI[d]	LEDGF/p75 IC_{50} (μM)
		3′-P	ST	EC_{50}[b]	CC_{50}[c]		
1	OH	>20	8.0	2.05	104.9	44	0.6
1a	OBnF[f]	20	4	>5.68	5.68		0.94
1c	MOR[f]	>20	>20	5.05	24.7	5	0.97
2	OH	7.5	0.78	1.67	30.3	18	2.7
2a	OBnF	>20	7	3.04	5.80	2	>20
2b	O^{t}Bu[f]	>100	>100	8.45	40.5	5	>20
3	OH	>20	8.9	2.48	17.9	7	0.45
3a	OBnF	>20	>20	7.37	16.2	2	ND[e]
3c	MOR	>20	>20	2.21	63.6	29	1.6
4	OH	>20	>20	4.46	17.2	4	1.5
4a	OBnF	80	35	10.7	16.8	1.6	>20
4b	O^{t}Bu	>20	>20	3.20	25.0	8	>20
4c	MOR	>20	>20	68.4	105.4	1.5	1.7
5	OH	>20	>20	13.4	110.0	8	1.4
5a	OBnF	>20	>20	>8.35	8.35		>20
5b	O^{t}Bu	>100	50	12.1	61.2	5	>20
5c	MOR	>20	>20	>200	>200		>20
6	OH	>20	>20	9.47	141.6	15	1.7
6a	OBnF	>100	>100	2.06	11.5	6	ND[e]
6b	O^{t}Bu	>100	>100	3.05	25.4	8	>20
7	OH	>20	>20	13.9	36.2	3	1.1
7a	OBnF	>100	80	11.0	>200	>18	>20
7b	O^{t}Bu	>100	70	>47.7	47.7		>20
7c	MOR	>20	>20	0.826	>200	>242	1.7
30	OH	>20	>20	ND	ND		>20

[a] IC_{50}: the drug concentration that produced 50% inhibition of the enzyme function.
[b] EC_{50}: effective concentration required to protect C8166 cells against the cytopathogenicity of HIV-1 by 50%.
[c] CC_{50}: cytotoxic concentration required to reduce C8166 cell proliferation by 50% tested by MTT method.
[d] TI: therapeutic index is a ratio of the CC_{50} value/EC_{50} value.
[e] ND: not determined.
[f] OBnF: 4-fluorobenzyloxy, O^{t}Bu: *tert*-butyloxy, MOR: morpholino.

The synthesis of C7-morpholino substituted analogs of the flavonoids **1c**–**7c** adopted a modified procedure using 7-tosylated flavone as the key intermediate. Taking compound **5** as an example (Scheme 2), upon global acetylation and selective removal of the 7-*O*-acyl group, the 5-acetylated-7-hydroxy flavone **21**-**5** was converted to the 7-tosyl derivative **22**-**5**. After the protecting group exchange at 5-hydroxy from acetyl to benzyl, the resulting 5-benzyloxy-7-tosylate flavonoid was subjected to the nucleophilic replacement by morpholine using $Pd(OAc)_2$/XPhos as a catalyst. The following hydrogenolysis catalyzed by palladium hydroxide on carbon delivered the desired product **5c**.

The installation of proper hydrophobic fluorobenzyl group around the core of quercetin **1** demanded a regioselective synthesis. Quercetin bears five hydroxyl groups and each of them displays unique nucleophilicity under different conditions. According to the published acidity order of the polyphenol hydroxyls (3,7 > 4′ > 3′ >> 5),[34] an individualized orthogonal protection strategy was developed to achieve a selective alkylation of each

Table 2
Inhibition of HIV-1 integrase catalytic activities, integrase–LEDGF/p75 interaction and the antiviral effect of 4-fluorobenzyl substituted quercetins

Compd	R_7	R_5	R_3	$R_{3'}$	$R_{4'}$	HIV-1 IN IC_{50}[a] (μM)		Antiviral activity (μg/mL)		TI[d]	LEDGF/p75 (μM)
						3′-P	ST	EC_{50}[b]	CC_{50}[c]		
1a	BnF	H	H	H	H	20	4	>5.68	5.68		0.94
1d	H	H	BnF	H	H	>20	>20	1.95	22.5	12	0.38
1e	H	BnF	H	H	H	>20	7.6	4.54	24.0	5	1.8
1f	H	H	H	BnF	H	>20	>20	0.47	23.8	51	1.8
1g	H	H	H	H	BnF	>20	>20	4.10	23.4	6	1.4

[a] IC_{50}: the drug concentration that produced 50% inhibition of the enzyme function.
[b] EC_{50}: effective concentration required to protect C8166 cells against the cytopathogenicity of HIV-1 by 50%.
[c] CC_{50}: cytotoxic concentration required to reduce C8166 cell proliferation by 50% tested by MTT assay.
[d] TI: therapeutic index is a ratio of the CC_{50}/EC_{50} values.

hydroxyl group on quercetin (Scheme 3). Treatment of quercetin with benzyl bromide and potassium carbonate in DMF at room temperature produced *O*-benzyl protected derivatives with the free OH on 5-C (**26**) or both 5-C and 3′-C (**27**). Then the selective alkylation of 5- or 3′-OH was readily achieved by reaction with 4-fluorobenzyl bromide and potassium carbonate at 80 °C or room temperature, respectively. By careful control of the reaction time, it was possible to stop the Pd-C-catalyzed hydrogenolysis at the debenzylation stage with the 4-fluorobenzyl group remaining.

For the regioselective substitution of 3- and 4′-OH, the benzophenone ketal of the 3′,4′-dihydroxy was employed as an efficient orthogonal protection group. Starting from the resulting 3′,4′-benzophenone ketal protected quercetin **30**, a direct etherification by halide afforded the 3-*O*-alkyl substituted derivatives (**31**–>**1d**), while the selective 4′-*O*-alkylation was realized by an additional 3,7-O-benzylation followed by an acidic removal of the 3′,4′-benzophenone ketal protecting group (**32**–>**1g**).

3. Results and discussion

All new flavonoids were tested in biochemical assays for inhibition of IN catalytic function, IN–LEDGF/p75 interaction, cytotoxicity, and cell-based viral infectivity assays (Tables 1 and 2).

As shown in Table 1, for select β-ketoenol-containing flavonoids, those bearing an additional catechol ketoenol structure displayed distinct ST inhibitory activity (e.g., compounds **1**–**3**). Compounds containing one ketoenol moiety only were inactive in inhibiting the IN catalytic function (e.g., compounds **4**–**7**). For the 22 new derivatives, the introduction of 4-fluorobenzyl group into the 7-position of the flavone core tended to display favorable inhibition of ST reaction. However, the hydrophobic aliphatic *tert*-butyl group exerted little impact on potency. In comparison, the hydrophilic group such as morpholine was disfavored for activity. It is worth noting that the destruction of the catechol or the ketoenol structure by the substitution on the hydroxyls led to the drop of the IN inhibitory activity (Table 1, **2a** vs **2**, **30** vs **1**; Table 2, **1d**, **1f**, **1g** vs **1**). This suggests that the flavonoids' chelation motif played an important role in enzyme inhibition. Accordingly, the position of the inserted hydrophobic aromatic moiety was equally critical for the IN inhibitory activity of the β-ketoenol-containing flavonoids.

Therefore, the most potent flavonoid **1** (quercetin) was chosen for the investigation of the optimal substitution position, and the privileged hydrophobic 4-fluorobenzyl functionality was employed to screen the 5 hydroxyl positions on the quercetin core. As indicated in Table 2, the best position of the hydrophobic moiety on the quercetin core was C-7 (**1a**: IC_{50} = 4 μM vs **1**: IC_{50} = 8 μM for ST), which resulted in an improvement in the IN inhibition. The substitutions at other positions (the phenolic hydroxyls at the 3, 6, 3′, 4′-site) with 4-F-benzyl caused a loss of activity for 3′-P and ST reactions. This could be perhaps due to the impairment of the divalent metal ion chelation structure embodied by catechol or β-ketoenol moiety.

More significantly, these β-ketoenol containing flavonoids and their derivatives inhibited IN–LEDGF/p75 interaction (Tables 1 and 2). As an essential cofactor of HIV-1 IN, LEDGF/p75 promotes the viral integration by tethering the pre-integration complex to the chromatin. Thus small molecules that could block the binding of IN catalytic core domain with the LEDGF/p75 IBD (integrase binding domain) have been recognized as promising new anti-AIDS chemotherapeutics. So far only few small molecule inhibitors of the IN–LEDGF/p75 interaction have been reported.[35] Our group previously reported the salicylic acid and catechol-merged molecules as novel IN–LEDGF/p75 inhibitors.[36] Interestingly, some of the hydroxyflavones presented here display sub-micromolar IC_{50} values for the inhibition of IN–LEDGF/p75 interaction. The introduction of the hydrophilic morpholine into the 7-position was beneficial for the IN–LEDGF/p75 inhibition as exemplified by compounds **1c**, **3c**, **4c** and **7c** (IC_{50} = 0.97, 1.6, 1.7 and 1.7 μM, respectively). Even with benzyl substitution at the phenolic hydroxyl functionality, all the quercetin derivatives (**1a**–**1g**) showed potent LEDGF/p75 inhibitory activities, with IC_{50} values ranging from 0.38 to 1.8 μM. Similar to the inhibition of catalytic activities, the ketoenol compounds with a catechol group efficiently blocked IN–LEDGF/p75 interaction (**1**, **1a**–**1d**, **3**, IC_{50} = 0.38–0.94 μM). Consistently, the IN–LEDGF/p75 inhibitory activity was reduced for **2** (IC_{50} = 2.7 μM) and **3** (IC_{50} = 0.45 μM) when the catechol structure was protected by hydrophobic substitutions (**2a**–**b**, IC_{50} >20 μM; **3c**, IC_{50} = 1.6 μM). However, contrary to the ST inhibition, the presence of the ketoenol functionality conferred the IN–LEDGF/p75 inhibitory activity to most of the select flavonoids regardless of the catechol structure co-occurrence, and the 7-hydroxy or polar group such as morpholine at C7-position was beneficial for the protein–protein interaction disruption.

Encouragingly, most of the synthesized flavonoid derivatives displayed effective antiviral activity in C8166 cells. Some hydoxyflavones such as **1**–**2**, **1f**, **3c**, **4b**, **7c** (EC_{50} = 0.5–3.2 μg/mL) showed promising therapeutic index (TI = 18–242). More significantly,

the antiviral potency was generally consistent with the inhibitory activities against the ST reaction and the IN–LEDGF/p75 interaction.

4. Conclusions

Starting from the natural product flavonoids, we successfully designed and synthesized novel inhibitors targeting both the catalytic domain of IN and the interaction with the cellular cofactor LEDGF/p75. Using a previously established pharmacophore model important for the selective inhibition of strand transfer, we incorporated a hydrophobic aromatic group into the proper position of the flavonoid core to design novel analogs. The presence of a catechol structure was beneficial for these β-ketoenol containing flavonoids, confirming the metal-chelation mechanism of IN inhibition. Significantly, this study revealed for the first time that the β-ketoenol containing flavonoids are potent novel HIV-1 IN inhibitors targeting the IN-LEDGF/p75 interaction. Furthermore, the substitution of morpholino group at the phenolic hydroxyl position conferred sub- to low-micromolar IN-LEDGF/p75 inhibitory activity. Most of the active IN inhibitors displayed effective antiviral effects in cellular settings. Further optimization of the active compounds will lead to second generation derivatives targeting both the active site of IN and the IN–LEDGF/p75 interaction.

5. Experimental section

5.1. General synthetic methods

All Reagents and solvents were obtained from commercial of analytical grade and used without further purification. ^{1}H NMR spectra were recorded on a Varian Mercury-400 or 300 MHz and ^{13}C NMR spectra on a Bruker AVANCEIII 400 or 500 MHz. Mass spectra were recorded using a Finnigan MAT-95 instrument for EI and Finnigan LCQ Deca for ESI.

5.1.1. 2-(3,4-Diacetoxyphenyl)-4-*oxo*-*4H*-chromene-3,5,7-triyl triacetate (8)

Acetic anhydride (1 mL, 10.7 mmol) was added to a solution of **1** (604 mg, 2 mmol) in anhydrous pyridine (8 mL) at room temperature. The reaction mixture was stirred for 6 h at 70 °C. After the completion of reaction, the resultant mixture was cooled to room temperature (rt). After concentration, the crude product was recrystallized with acetone to afford **8** as a white solid (973 mg, 95% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75–7.67 (m, 2H), 7.36 (s, 1H), 7.33 (d, J = 1.8 Hz, 1H), 6.87 (d, J = 2.1 Hz, 1H), 2.42 (s, 3H), 2.37–2.31 (m, 12H).

5.1.2. 2-(4-Acetoxyphenyl)-4-*oxo*-*4H*-chromene-5,7-diyl diacetate (9)

Compound **9** was prepared from **4** according to the same procedure described for **8** (640 mg, 94% yield), as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (d, J = 8.8 Hz, 2H), 7.35 (d, J = 2.2 Hz, 1H), 7.26 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 2.3 Hz, 1H), 6.63 (s, 1H), 2.43 (s, 3H), 2.38 (s, 3H), 2.34 (s, 3H).

5.1.3. 4-(5,7-Diacetoxy-4-*oxo*-*4H*-chromen-2-yl)-1,2-phenylene diacetate (10)

Compound **10** was prepared from **7** according to the same procedure described for **8** (670 mg, 96% yield), as a white solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.09–7.99 (m, 3H), 7.60 (d, J = 2.1 Hz, 1H), 7.51 (d, J = 9.0 Hz, 1H), 7.10 (d, J = 2.1 Hz, 1H), 6.96 (d, J = 1.0 Hz, 1H), 2.35–2.31 (m, 9H).

5.1.4. 4-*oxo*-2-Phenyl-*4H*-chromene-5,6,7-triyl triacetate (11)

Compound **11** was prepared from **2** according to the same procedure described for **8** (620 mg, 94% yield), as a white solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.11–8.02 (m, 3H), 7.65 (d, J = 2.1 Hz, 1H), 7.44 (d, J = 9.0 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 6.90 (d, J = 1.0 Hz, 1H), 2.28–2.35 (m, 9H).

5.1.5. 4-(3,5-Diacetoxy-7-hydroxy-4-*oxo*-*4H*-chromen-2-yl)-1,2-phenylene diacetate (12)

PhSH (0.15 mL, 1.51 mmol) was slowly added to a stirred mixture of **8** (972 mg, 1.9 mol) and imidazole (52 mg, 0.77 mol) in NMP (20 mL) at 0 °C and then stirred for another 2 h at rt. The mixture was diluted with EtOAc and washed with 2 N HCl. The organic layer was concentrated under reduced pressure and dried over $MgSO_4$. The crude product was purified by column chromatography on silica gel (hexanes/EtOAc = 1:1) to give **12** as a white solid (804 mg, 86% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 7.83–7.73 (m, 2H), 7.42 (d, J = 8.3 Hz, 1H), 6.88 (d, J = 2.3 Hz, 1H), 6.59 (d, J = 2.3 Hz, 1H), 2.34 (s, 3H), 2.33–2.28 (m, 9H).

5.1.6. 4-(5-Acetoxy-7-hydroxy-4-*oxo*-*4H*-chromen-2-yl)phenyl acetate (13)

Compound **13** was prepared from **9** according to the same procedure described for **12** (697 mg, 88% yield), as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.04 (d, J = 8.7 Hz, 2H), 7.35–7.26 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 2.3 Hz, 1H), 6.66 (s, 1H), 6.56 (d, J = 2.3 Hz, 1H), 2.34 (s, 3H), 2.31 (s, 3H).

5.1.7. 4-(5-Acetoxy-7-hydroxy-4-*oxo*-*4H*-chromen-3-yl)phenyl acetate (14)

Compound **14** was prepared from **10** according to the same procedure described for **12** (680 mg, 87% yield), a white solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.37 (s, 1H), 7.51 (d, J = 6.9 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 2.1 Hz, 1H), 6.60 (d, J = 2.1 Hz, 1H), 2.30 (s, 3H), 2.26 (s, 3H).

5.1.8. 4-(3,5-Diacetoxy-7-((4-fluorobenzyl)oxy)-4-*oxo*-*4H*-chromen-2-yl)-1,2-phenylene diacetate (15a)

To a solution of **12** (470 mg, 1 mmol) in DMF (10 mL) were added potassium carbonate (152 mg, 1.1 mmol) and benzyl bromide (150 μL, 1.2 mmol) under argon. The reaction mixture was agitated vigorously at rt for 12 h. The resulting mixture was diluted with 200 mL of EtOAc and was washed with water (100 mL). The residue obtained after evaporation of the solvent was purified by flash column chromatography (hexanes/EtOAc = 2:1) to afford **15a** (531 mg, 92%) as a yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.83–7.73 (m, 2H), 7.37–7.26 (m, 4H), 7.42 (d, J = 8.3 Hz, 1H), 6.88 (d, J = 2.3 Hz, 1H), 6.59 (d, J = 2.3 Hz, 1H), 5.19 (s, 2H), 2.34 (s, 3H), 2.33–2.28 (m, 9H).

5.1.9. 4-(5-Acetoxy-7-((4-fluorobenzyl)oxy)-4-*oxo*-*4H*-chromen-2-yl)phenyl acetate (16a)

Compound **16a** was prepared from **13** according to the same procedure described for **15a** (295 mg, 94%), as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.10 (d, J = 7.5 Hz, 2H), 7.53 (d, J = 7.5 Hz, 2H), 7.40–7.29 (m, 4H), 7.29–7.16 (m, 2H), 6.83 (s, 1H), 5.24 (s, 2H), 2.30 (s, 3H) 2.28 (s, 3H).

5.1.10. 4-(5-Acetoxy-7-((4-fluorobenzyl)oxy)-4-*oxo*-*4H*-chromen-3-yl)phenyl acetate (17a)

Compound **17a** was prepared from **14** according to the same procedure described for **15a** (210 mg, 91% yield), as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.42 (s, 1H), 7.59–7.50 (m, 2H), 7.41 (d, J = 8.7 Hz, 2H), 7.30–7.21 (m, 2H), 6.84 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 2.3 Hz, 1H), 6.50 (d, J = 2.2 Hz, 1H), 5.23 (s, 2H), 2.30

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(s, 3H), 2.28 (s, 3H).

5.1.11. 2-(3,4-Dihydroxyphenyl)-7-((4-fluorobenzyl)oxy)-3,5-dihydroxy-*4H*-chromen-4-one (1a)

A cold mixture of **15a** (289 mg, 0.50 mmol) in NH_3/MeOH (12 mL, 0 °C) was stirred for 2 h at rt. After concentration under reduced pressure, the residue was recrystallized with acetone to give **1a** (182 mg, 89% yield), as a yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.75 (d, J = 2.1 Hz, 1H), 7.64 (dd, J = 8.6, 2.1 Hz, 1H), 7.48 (dd, J = 8.5, 5.4 Hz, 2H), 7.12 (t, J = 8.8 Hz, 2H), 6.88 (d, J = 8.5 Hz, 1H), 6.62 (d, J = 2.1 Hz, 1H), 6.35 (d, J = 2.1 Hz, 1H), 5.12 (s, 2H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 176.39, 164.22, 162.40 (d, J = 244.1 Hz), 160.88, 156.44, 148.30, 147.80, 145.52, 136.52, 132.89, 130.67 (d, J = 8.4 Hz), 122.30, 120.47, 115.86 (d, J = 21.4 Hz), 104.62, 98.47, 93.24, 69.69. EI-MS m/z: 410 $(M)^+$. HR-EI MS calcd for $C_{22}H_{15}FO_7$ $(M)^+$: 410.0802, found: 410.0795.

5.1.12. 7-((4-Fluorobenzyl)oxy)-5-hydroxy-2-(4-hydroxyphenyl)-*4H*-chromen-4-one (4a)

Compound **4a** was prepared from **16a** according to the same procedure described for **1a** (61 mg, 76% yield), as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 13.03 (s, 1H), 7.98 (d, J = 8.7 Hz, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.31–7.21 (m, 2H), 6.99–6.91 (m, 2H), 6.89–6.85 (m, 2H), 6.47 (d, J = 2.2 Hz, 1H), 5.23 (s, 2H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 182.32, 164.51, 164.40, 163.33, 162.35 (d, J = 244.2 Hz), 161.74, 161.65, 161.38, 157.56, 132.79, 130.60 (d, J = 8.2 Hz), 128.95, 121.46, 116.40, 115.79 (d, J = 21.4 Hz), 105.23, 103.46, 98.98, 93.91, 69.66. EI-MS m/z: 378 $(M)^+$. HR-EI MS calcd for $C_{22}H_{15}FO_5$ $(M)^+$: 378.0904, found: 378.0903.

5.1.13. 7-((4-Fluorobenzyl)oxy)-5-hydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (7a)

Compound **7a** was prepared from **17a** according to the same procedure described for **1a** (176 mg, 91% yield), as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 12.73 (s, 1H), 9.68 (s, 1H), 8.42 (s, 1H), 7.59–7.50 (m, 2H), 7.41 (d, J = 8.7 Hz, 2H), 7.30–7.21 (m, 2H), 6.83 (d, J = 8.7 Hz, 2H), 6.76 (d, J = 2.3 Hz, 1H), 6.51 (d, J = 2.2 Hz, 1H), 5.20 (s, 2H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 207.01, 180.88, 164.54, 162.41 (d, J = 244.1 Hz), 162.23, 157.92 (d, J = 7.7 Hz), 154.93, 132.75, 130.78 (d, J = 8.4 Hz), 122.97, 121.48, 115.85 (d, J = 21.4 Hz), 106.00, 99.12, 93.74, 67.05, 31.16. EI-MS m/z: 378 $(M)^+$. HR-EI MS calcd for $C_{22}H_{15}FO_5$ $(M)^+$: 378.0904, found: 378.0903.

5.1.14. 4-(5-Acetoxy-7-(*tert*-Butoxy)-4-*oxo*-*4H*-chromen-2-yl)phenyl acetate (16b)

To a suspension of **13** (130 mg, 0.4 mmol), $Mg(ClO_4)_2$ (12 mg, 0.06 mmol) in DCM was added Boc_2O (283 mg, 1.3 mmol). The mixture was stirred for 24 h at 70 °C. The resulting mixture was diluted with 200 mL of DCM and washed with water (100 mL). The residue obtained after evaporation of the solvent was used in the next step without further purification. ESI-MS m/z: 411 $(M+H)^+$.

5.1.15. 7-(*tert*-Butoxy)-5-hydroxy-2-(4-hydroxyphenyl)-*4H*-chromen-4-one (4b)

A cold mixture of **16b** (230 mg, 0.56 mmol) in NH_3/MeOH (12 mL, 0 °C) was stirred for 2 h at rt. The residue obtained after evaporation of the solvent was purified by flash column chromatography using hexanes/EtOAc (4:1) as eluent to afford **4b** (26 mg, 16% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 7.91 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 6.65 (s, 1H), 6.46 (d, J = 2.1 Hz, 1H), 6.23 (d, J = 2.0 Hz, 1H), 1.43 (s, 9H). EI-MS m/z: 326 $(M)^+$. HR-EI MS calcd for $C_{19}H_{18}O_5$ $(M)^+$: 326.1154, found: 326.1152.

5.1.16. 4-(5-Acetoxy-7-(*tert*-Butoxy)-4-*oxo*-*4H*-chromen-3-yl)phenyl acetate (17b)

Compound 1**7b** was prepared from **14** according to the same procedure described for **16b**, as a yellow solid. ESI-MS m/z: 411 $(M+H)^+$.

5.1.17. 7-(*tert*-Butoxy)-5-hydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (7b)

Compound **7b** was prepared from **17b** according to the same procedure described for **4b** (28 mg, 15% yield), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.69 (s, 1H), 7.87 (s, 1H), 7.40 (d, J = 7.8 Hz, 2H), 6.90 (d, J = 7.8 Hz, 2H), 6.48 (d, J = 12 Hz, 1H), 5.09 (s, 1H), 1.48 (d, J = 1.4 Hz, 9H). EI-MS m/z: 326 $(M)^+$. HR-EI MS calcd for $C_{19}H_{18}O_5$ $(M)^+$: 326.1154, found: 326.1155.

5.1.18. 7-((4-Fluorobenzyl)oxy)-4-oxo-2-phenyl-*4H*-chromene-5,6-diyl diacetate (18a)

A mixture of **11** (396 mg, 1 mmol), 4-flurobenzyl bromide (372 µL, 3 mmol) and anhydrous K_2CO_3 (552 mg, 4 mmol) in acetone (250 mL) was refluxed for 24 h with stirring. The reaction mixture was cooled down to room temperature, filtered and the solvent was evaporated under reduced pressure to give the crude product as a pale white solid. The solid washed with EtOAc to give compound **18a** (416 mg, 90%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89–7.80 (m, 2H), 7.57–7.47 (m, 2H), 7.46–7.31 (m, 4H), 7.28–7.25 (m, 1H), 7.00 (s, 1H), 6.62–6.58 (m, 1H), 5.20 (s, 2H), 2.45 (s, 3H), 2.30 (s, 3H).

5.1.19. 7-(*tert*-Butoxy)-4-oxo-2-phenyl-4*H*-chromene-5,6-diyl diacetate (18b)

A suspension of **18a** (93 mg, 0.2 mmol) in MeOH (10 mL) was treated with 10% $Pd(OH)_2$/C (8 mg) under a flow of hydrogen until the starting material disappeared. The reaction mixture was then filtered on Celite and eluted with MeOH. After concentration of the filtrate under vacuum, the residue was purified by column chromatography on silica gel (hexanes/EtOAc = 1:1) to afford a yellow solid. Then, from the yellow solid, the compound **18b** was prepared as a white solid according to the same procedure described for **16b**. ESI-MS m/z: 411 $(M+H)^+$.

5.1.20. 7-((4-Fluorobenzyl)oxy)-5,6-dihydroxy-2-phenyl-*4H*-chromen-4-one (2a)

A cold mixture of **18a** (520 mg, 1.12 mmol) in NH_3/MeOH (12 mL) was stirred at room temperature for 2 h. After concentration under reduced pressure, the residue was recrystallized with acetone to afford **2a** (385 mg, 91% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91–7.85 (m, 2H), 7.57–7.41 (m, 5H), 7.15–7.06 (m, 2H), 6.69 (m, 1H), 6.64 (s, 1H), 5.21 (s, 2H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 182.81, 163.69, 162.37 (d, J = 243.8 Hz), 153.91, 150.10, 146.93, 132.98, 132.46, 131.31, 130.88, 130.54 (d, J = 8.3 Hz), 129.62, 126.80, 115.77 (d, J = 21.4 Hz), 105.90, 105.21, 93.00, 69.97. EI-MS m/z: 378 $(M)^+$. HR-EI MS calcd for $C_{22}H_{15}FO_5$ $(M)^+$: 378.0904, found: 378.0899.

5.1.21. 7-(*tert*-Butoxy)-5-hydroxy-2-phenyl-*4H*-chromen-4-one (5a)

To a solution of **5** (510 mg, 2 mmol) in DMF (10 mL) were added K_2CO_3 (276 mg, 2 mmol) and 4-flurobenzyl bromide (372 µL, 3 mmol) under argon. The reaction mixture was agitated vigorously at room temperature for 12 h. The mixture was diluted with EtOAc and washed with water. The organic phase was evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (hexanes/EtOAc = 4:1) to afford **5a** (707 mg, 95% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.76 (s, 1H), 7.89 (m, 2H), 7.59–7.49 (m, 3H), 7.46–7.36 (m, 2H), 7.10 (m, 2H), 6.68 (d, J = 2.5 Hz, 1H), 6.57 (d, J = 2.5 Hz, 1H), 6.45 (s, 1H), 5.10 (s, 2H). ^{13}C NMR (125 MHz,

d_6-DMSO): δ 182.46, 164.64, 163.90, 162.36 (d, J = 244.1 Hz), 161.64, 157.70, 132.73, 132.54, 130.99, 130.60 (d, J = 8.4 Hz), 129.54, 126.83, 115.79 (d, J = 21.5 Hz), 105.79, 105.49, 99.15, 94.04, 69.71. EI-MS m/z: 362 $(M)^+$. HR-EI MS calcd for $C_{22}H_{15}FO_5$ $(M)^+$: 362.0954, found: 362.0954.

5.1.22. 7-((4-Fluorobenzyl)oxy)-5-hydroxy-2-phenylchroman-4-one (6a)

Compound **6a** was prepared from **6** according to the same procedure described for **5a** (600 mg, 67% yield), as a yellow solid. ^1H NMR (300 MHz, d_6-DMSO): δ 12.23 (s, 1H), 9.77 (s, 1H), 7.54–7.43 (m, 2H), 7.37–7.30 (m, 2H), 7.28–7.18 (m, 2H), 6.84–6.77 (m, 2H), 6.22–6.14 (m, 2H), 5.50 (dd, J = 12.9, 2.9 Hz, 1H), 5.15 (s, 2H), 3.07 (dd, J =3.9, 30.3 Hz, 1H), 2.73 (dd, J = 20.1, 14.4 Hz, 1H). EI-MS m/z: 380 $(M)^+$. HR-EI MS calcd for $C_{22}H_{17}FO_5$ $(M)^+$: 380.1060, found: 380.1056.

5.1.23. 7-(*tert*-Butoxy)-5-hydroxy-2-phenyl-*4H*-chromen-4-one (5b)

To a suspension of **5** (255 mg, 1 mmol), $Mg(ClO_4)_2$ (24 mg, 0.12 mmol) in DCM was added Boc_2O (849 mg, 4 mmol). The mixture was stirred for 24 h at 70 °C. The resulting mixture was diluted with 200 mL of DCM and was washed with water (100 mL). The organic phase was evaporated under reduced pressure to give the crude product that was purified by column chromatography on silica gel (hexanes/EtOAc = 10:1) to afford **5b** (40 mg, 13% yield) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 12.58 (s, 1H), 7.92–7.86 (m, 2H), 7.58–7.46 (m, 3H), 6.67 (s, 1H), 6.60 (d, J = 2.1 Hz, 1H), 6.46 (d, J = 2.1 Hz, 1H), 1.48 (s, 9H). EI-MS m/z: 310 $(M)^+$. HR-EI MS calcd for $C_{18}H_{15}O_4$ $(M)^+$: 310.1205, found: 310.1209.

5.1.24. 7-(*tert*-Butoxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (6b)

Compound **6b** was prepared from **6** according to the same procedure described for **5b** (21 mg, 12% yield), as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 12.15 (s, 1H), 7.91–7.84 (m, 3H), 7.57–7.48 (m, 2H), 6.02 (m, 2H), 5.58 (dd, J = 11.7, 2.7 Hz, 1H), 3.07 (dd, J = 3.6, 27.3 Hz, 1H) 2.73 (dd, J = 19.1, 12.4 Hz, 1H), 1.49 (m, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.02, 164.94, 164.26, 163.22, 133.00, 127.00, 124.29, 103.03, 96.73, 95.52, 78.97, 43.20, 28.91, 28.79. EI-MS m/z: 328 $(M)^+$. HR-EI MS calcd for $C_{19}H_{20}O_5$ $(M)^+$: 328.1311, found: 328.1313.

5.1.25. 7-(*tert*-Butoxy)-5,6-dihydroxy-2-phenyl-*4H*-chromen-4-one (2b)

Compound **2b** was prepared from **18b** according to the same procedure described for **4b** (18 mg, 10% yield) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.92–7.84 (m, 2H), 7.57–7.51 (m, 3H), 6.66 (s, 1H), 6.62 (s, 1H), 1.37 (s, 9H). EI-MS m/z: 326 $(M)^+$. HR-EI MS calcd for $C_{19}H_{18}O_5$ $(M)^+$: 326.1154, found: 326.1153.

5.1.26. 2-(2,2-Diphenylbenzo[*d*][1,3]dioxol-5-yl)-5,7-dihydroxy-*4H*-chromen-4-one (19)

A mixture of **3** (2 g, 6.96 mmol) and dichlorodiphenylmethane (2 mL, 10.42 mmol) in 5 mL diphenyl ether was heated at 180 °C for 20 min. The resulting products was washed by ether and diluted with hot acetone. The filtrate was concentrated and purified by flash column chromatography using petroleum ether/EtOAc (6:1 to 1:1) as eluent to give **19** (1.26 g, 40% yield) as a yellow solid. ^1H NMR (300 MHz, d_6-DMSO): δ 7.80–7.72 (m, 2H), 7.62–7.51 (m, 4H), 7.46–7.33 (m, 7H), 7.20 (d, J = 8.1 Hz, 1H), 6.44 (d, J = 1.8 Hz, 1H), 6.12 (s, 1H).

5.1.27. 2-(2,2-Diphenylbenzo[*d*][1,3]dioxol-5-yl)-7-((4-fluorobenzyl)oxy)-5-hydroxy-*4H*-chromen-4-one (20)

Compound **20** was prepared from **19** according to the same procedure described for **15a** (210 mg, 91% yield), as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.64–7.50 (m, 4H), 7.48–7.32 (m, 9H), 7.10 (d, J = 8.4 Hz, 2H), 7.02–6.87 (m, 2H), 6.84 (d, J = 2.1 Hz, 1H), 6.50 (s, 1H), 6.42 (d, J = 2.1 Hz, 1H), 5.09 (s, 2H).

5.1.28. 2-(3,4-Dihydroxyphenyl)-7-((4-fluorobenzyl)oxy)-5-hydroxy-*4H*-chromen-4-one (3a)

A mixture of compound **20** (117 mg, 0.21 mmol) in acetic acid/water (4:1, 25 mL) was refluxed for 9 h. Then, EtOAc (100 mL) and water (100 mL) were added. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and dried over anhydrous Mg_2SO_4. After concentration, the residue was recrystallized with ether/EtOAc to afford **3a** (54 mg, 65% yield) as a yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.56–7.43 (m, 2H), 7.41–7.27 (m, 2H), 7.18–7.06 (m, 2H), 6.79 (d, J = 8.1 Hz, 1H), 6.66 (d, J = 2.1 Hz, 1H), 6.50 (s, 1H), 6.37 (d, J = 2.1 Hz, 1H), 5.12 (s, 2H). EI-MS m/z: 394 $(M)^+$. HR-EI MS calcd for $C_{22}H_{15}FO_5$ $(M)^+$: 394.0853, found: 394.0858.

5.1.29. 7-Hydroxy-4-*oxo*-2-phenyl-*4H*-chromen-5-yl acetate (21-5)

Compound **21-5** was prepared from **5** according to the same procedure described for **12** (700 mg, 77% yield), as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.99–8.06 (m, 2H), 7.58–7.54 (m, 3H), 6.83 (d, J = 2.1 Hz, 1H), 6.69 (s, 1H), 6.47 (d, J = 2.1 Hz, 1H), 2.27 (s, 3H).

5.1.30. 4-(5,7-Diacetoxy-4-*oxo*-*4H*-chromen-2-yl)-1,2-phenylene diacetate (21-3)

Compound **21-3** was prepared from **3** according to the same procedure described for **12** (670 mg, 94% yield), as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 8.05–7.96 (m, 2H), 7.49 (d, J = 9.0 Hz, 1H), 6.96 (s, 1H), 6.75 (s, 1H), 6.58 (d, J = 2.1 Hz, 1H), 2.32 (m, 9H).

5.1.31. 4-*oxo*-2-Phenyl-7-(tosyloxy)-*4H*-chromen-5-yl acetate (22-5)

The mixture of compound **21**–**5** (521 mg, 1.76 mmol), TsCl (665 mg, 3.5 mmol) and TEA (300 μL, 2 mmol) in 20 mL of THF was agitated at room temperature for 8 h. The reaction mixture was diluted with 150 mL of EtOAc and washed with water. The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography on silica gel (DCM) to afford **22-5** (712 mg, 90% yield) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.82–7.88 (m, 2H), 7.77 (d, J = 8.1 Hz, 2H), 7.49–7.57 (m, 3H), 7.30–7.39 (m, 3H), 6.63–6.66 (m, 2H), 2.46 (s, 3H), 2.40 (s, 3H).

5.1.32. 4-(3,5-Diacetoxy-4-*oxo*-7-(tosyloxy)-*4H*-chromen-2-yl)-1,2-phenylenediacetate (22-1)

Compound **22-1** was prepared from **12** according to the same procedure described for **22-5** (1.2 g, 73% yield), as a yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.81–7.70 (m, 4H), 7.48–7.30 (m, 4H), 6.82–6.78 (d, J = 2.3 Hz, 1H), 2.43 (s, 3H), 2.35–2.24 (m, 12H).

5.1.33. 4-(5-Acetoxy-4-*oxo*-7-(tosyloxy)-*4H*-chromen-2-yl)-1,2-phenylene diacetate (22-3)

Compound **22-3** was prepared from **21-3** according to the same procedure described for **22-5** (700 mg, 90% yield), as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.82–7.66 (m, 4H), 7.41–7.32 (m, 3H), 7.30 (d, J = 2.3 Hz, 1H), 6.66 (d, J = 2.4 Hz, 1H), 6.60 (s, 1H), 2.46 (s, 3H), 2.40 (s, 3H), 2.36 (s, 3H), 2.33 (s, 3H).

5.1.34. 4-(5-Acetoxy-4-*oxo*-7-(tosyloxy)-*4H*-chromen-2-yl)phenyl acetate (22-4)

Compound **22-4** was prepared from **13** according to the same procedure described for **22-5** (1.1 g, 83% yield), as a yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, *J* = 8.8 Hz, 2H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.62–7.52 (m, 2H), 7.35 (d, *J* = 2.2 Hz, 1H), 7.28 (s, 1H), 7.24 (s, 1H), 6.83 (d, *J* = 2.3 Hz, 1H), 6.63 (s, 1H), 2.51 (s, 3H), 2.37 (d, *J* = 3.0 Hz, 6H).

5.1.35. 4-(5-Acetoxy-4-*oxo*-7-(tosyloxy)-*4H*-chromen-3-yl)phenyl acetate (22-7)

Compound **22**-**7** was prepared from **17** according to the same procedure described for **22**-**5** (1.1 g, 75% yield), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89–7.85 (s, 1H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.18–7.12 (m, 3H), 6.71 (d, *J* = 2.4 Hz, 1H), 2.47 (s, 3H), 2.47 (s, 3H), 2.32 (s, 3H).

5.1.36. 5-Hydroxy-4-*oxo*-2-phenyl-*4H*-chromen-7-yl 4-methylbenzenesulfonate (23-5)

Compound **23**-**5** was prepared from **22**-**5** according to the same procedure described for **1a** (580 mg, 92% yield), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92–7.86 (m, 2H), 7.82–7.75 (m, 2H), 7.62–7.52 (m, 3H), 7.40–7.33 (m, 2H), 6.94 (d, *J* = 2.1 Hz, 1H), 6.74 (s, 1H), 6.34 (d, *J* = 2.1 Hz, 1H), 2.46 (s, 3H). EI-MS *m*/*z*: 408 (M) $^+$.

5.1.37. 2-(3,4-Dihydroxyphenyl)-3,5-dihydroxy-4-*oxo*-*4H*-chromen-7-yl4-methylbenzenesulfonate (23-1)

Compound **23**-**1** was prepared from **22**-**1** according to the same procedure described for **1a** (585 mg, 77% yield), as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.82–7.75 (m, 2H), 7.72 (d, *J* = 2.2 Hz, 1H), 7.62–7.57 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.50–7.42 (m, 2H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.71 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 2.45 (s, 3H). ESI-MS *m*/*z*: 457 (M+H)$^+$.

5.1.38. 2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-*oxo*-*4H*-chromen-7-yl4-methylbenzenesulfonate (23-3)

Compound **23**-**3** was prepared from **22**-**3** according to the same procedure described for **1a** (580 mg, 80% yield), as a yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.79 (d, *J* = 8.5 Hz, 2H), 7.50–7.34 (m, 4H), 6.90 (d, *J* = 8.2 Hz, 1H), 6.81 (d, *J* = 2.1 Hz, 1H), 6.66 (s, 1H), 6.37 (d, *J* = 2.0 Hz, 1H), 2.46 (s, 3H). ESI-MS *m*/*z*: 441 (M+H)$^+$.

5.1.39. 5-Hydroxy-2-(4-hydroxyphenyl)-4-*oxo*-*4H*-chromen-7-yl4-methylbenzenesulfonate (23-4)

Compound **23**-**4** was prepared from **22**-**4** according to the same procedure described for **1a** (585 mg, 90% yield), as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 7.98 (d, *J* = 8.8 Hz, 2H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.56–7.48 (m, 2H), 7.05 (d, *J* = 2.2 Hz, 1H), 7.00–6.89 (m, 3H), 6.42 (s, 1H), 2.43 (s, 3H). ESI-MS *m*/*z*: 425 (M+H) $^+$.

5.1.40. 5-Hydroxy-3-(4-hydroxyphenyl)-4-*oxo*-*4H*-chromen-7-yl4-methylbenzenesulfonate (23-7)

Compound **23**-**7** was prepared from **22**-**7** as according to the same procedure described for **1a** (600 mg, 92% yield), as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.70 (s, 1H), 8.50 (s, 1H), 7.85 (d, *J* = 8.1 Hz, 2H), 7.52 (d, *J* = 7.9 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 2H), 6.91–6.79 (m, 3H), 6.50 (d, *J* = 2.1 Hz, 1H), 2.44 (s, 3H). ESI-MS *m*/*z*: 425 (M+H)$^+$.

5.1.41. 5-(Benzyloxy)-4-*oxo*-2-phenyl-*4H*-chromen-7-yl 4-methylbenzenesulfonate (24-5)

Compound **24**-**5** was prepared from **23**-**5** according to the same procedure described for **15a** (400 mg, 86% yield), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73–7.63 (m, 2H), 7.50–7.43 (m, 2H), 7.39–7.24 (m, 5H), 7.22–7.05 (m, 5H), 6.71 (d, *J* = 2.1 Hz, 1H), 6.51 (s, 1H), 6.35 (d, *J* = 2.1 Hz, 1H), 4.93 (s, 2H), 2.24 (s, 3H). EI-MS *m*/*z*: 498 (M)$^+$.

5.1.42. 3,5-Bis(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-4-*oxo*-*4H*-chromen-7-yl-4-methylbenzenesulfonate (24-1)

Compound **24**-**1** was prepared from **23**-**1** as a yellow solid according to the same procedure described for **15a** (520 mg, 84% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73–7.64 (m, 3H), 7.57–7.27 (m, 20H), 7.25–7.20 (m, 3H), 6.98–6.92 (m, 1H), 6.83–6.77 (m, 1H), 6.48–6.43 (m, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 5.07 (s, 2H), 4.97 (s, 2H), 2.44 (s, 3H). ESI-MS *m*/*z*: 817 (M+H)$^+$.

5.1.43. 5-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-4-*oxo*-*4H*-chromen-7-yl4-methylbenzenesulfonate (24-3)

Compound **24**-**3** was prepared from **23**-**3** according to the same procedure described for **15a** (430 mg, 80% yield), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79–7.75 (m, 1H), 7.74–7.68 (m, 2H), 7.53 (d, *J* = 7.5 Hz, 2H), 7.58–7.25 (m, 16H), 7.06–6.98 (d, *J* = 7.2 Hz, 2H), 6.73 (s, 1H), 6.53 (s, 1H), 5.16 (s, 2H), 5.11 (s, 4H), 2.46 (s, 3H). ESI-MS *m*/*z*: 711 (M+H)$^+$.

5.1.44. 5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-4-*oxo*-*4H*-chromen-7-yl4-methylbenzenesulfonate (24-4)

Compound **24**-**4** was prepared from **23**-**4** according to the same procedure described for **15a** (400 mg, 75% yield), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.6 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.55 (d, *J* = 7.5 Hz, 2H), 7.49–7.28 (m, 10H), 7.08 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 2.1 Hz, 1H), 6.62 (s, 1H), 6.48 (d, *J* = 2.2 Hz, 1H), 5.15 (s, 2H), 5.10 (s, 2H), 2.45 (s, 3H). ESI-MS *m*/*z*: 605 (M+H) $^+$.

5.1.45. 5-(Benzyloxy)-3-(4-(benzyloxy)phenyl)-4-*oxo*-*4H*-chromen-7-yl4-methylbenzenesulfonate (24-7)

Compound **24**-**7** was prepared from **23**-**7** according to the same procedure described for **15a** (410 mg, 74% yield), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.74–7.68 (m, 2H), 7.53 (d, *J* = 7.5 Hz, 2H), 7.48–7.29 (m, 12H), 7.06–6.98 (m, 2H), 6.73 (d, *J* = 1.8 Hz, 1H), 6.53 (d, *J* = 1.8 Hz, 1H), 5.11 (s, 4H), 2.45 (s, 3H). ESI-MS *m*/*z*: 605 (M+H)$^+$.

5.1.46. 5-(Benzyloxy)-7-morpholino-2-phenyl-*4H*-chromen-4-one (25-5)

The mixture of compound **25**-**5** (50 mg, 0.1 mmol), the morpholine (20 μL, 0.2 mmol), $Pd(OAc)_2$ (5 mg, 0.02 mmol), XPhos (20 mg, 0.04 mmol) and Cs_2CO_3 (41 mg, 0.125 mmol) in 1 mL of 1,4-dioxane was stirred at 110 °C for 3 h under microwave irradiation. Then the reaction mixture was filtered on Celite and eluted with MeOH. After concentration of the filtrate under vacuum, the residue was washed by ether/EtOAc to give the yellow solid, which was used in the next step without further purification. ESI-MS *m*/*z*: 414 (M+H)$^+$.

5.1.47. 3,5-Bis(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-7-morpholino-*4H*-chromen-4-one (25-1)

Compound **25**-**1** was prepared from **24**-**1** according to the same procedure described for **25**-**5**, as a yellow solid. ESI-MS *m*/*z*: 732 (M+H)$^+$.

5.1.48. 5-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-7-morpholino-*4H*-chromen-4-one (25-3)

Compound **25**-**3** was prepared from **24**-**3** according to the same procedure described for **25**-**5**, as a yellow solid. ESI-MS *m*/*z*: 626 (M+H)$^+$.

5.1.49. 5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-7-morpholino-*4H*-chromen-4-one (25-4)

Compound **25**-**4** was prepared from **24**-**4** according to the same procedure described for **25**-**5**, as a yellow solid. ESI-MS *m*/*z*: 520 (M+H)$^+$.

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5.1.50. 5-(Benzyloxy)-3-(4-(benzyloxy)phenyl)-7-morpholino-*4H*-chromen-4-one (25-7)

Compound **25**-**7** was prepared from **24**-**7** according to the same procedure described for **25**-**5**, as a yellow solid. ESI-MS *m*/*z*: 542 $(M+Na)^+$

5.1.51. 5-Hydroxy-7-morpholino-2-phenyl-*4H*-chromen-4-one (5c)

A suspension of **25-5** (44 mg, 0.11 mmol) in MeOH (15 mL) was treated with 10% $Pd(OH)_2/C$ (8 mg) under a flow of hydrogen until the starting material disappeared. The reaction mixture was then filtered on Celite and eluted with MeOH. After concentration of the filtrate under vacuum, the residue was purified by column chromatography on silica gel (hexanes/EtOAc = 2:1) to afford **5c** (26 mg, 81% yield), as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.92–7.84 (m, 2H), 7.56–7.47 (m, 3H), 6.63 (d, *J* = 1.1 Hz, 1H), 6.41 (s, 1H), 6.30 (d, *J* = 2.4 Hz, 1H), 3.86 (t, *J* = 5.0 Hz 4H), 3.36 (t, *J* = 5.0 Hz, 4H). EI-MS *m*/*z*: 323 $(M)^+$. HR-EI MS calcd for $C_{19}H_{17}NO_4$ $(M)^+$: 323.1158, found: 323.1163.

5.1.52. 2-(3,4-Dihydroxyphenyl)-3,5-dihydroxy-7-morpholino-*4H*-chromen-4-one (1c)

Compound **1c** was prepared from **25-1** according to the same procedure described for **5c** (34 mg, 70% yield), as a yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.74 (d, *J* = 2.0 Hz, 1H), 7.63 (d, *J* = 2.2 Hz, 1H), 6.87 (d, *J* = 2.2 Hz, 1H), 6.48 (d, *J* = 2.2 Hz, 1H), 6.33 (d, *J* = 2.1 Hz, 1H), 3.80 (t, *J* = 4.7 Hz, 4H), 3.36 (d, *J* = 4.7 Hz, 4H). ESI-MS *m*/*z*: 370 $(M-H)^-$. HR-ESI MS calcd for $C_{19}H_{17}NO_7$ $(M-H)^-$: 370.0932, found: 370.0928.

5.1.53. 2-(3,4-Dihydroxyphenyl)-5-hydroxy-7-morpholino-*4H*-chromen-4-one (3c)

Compound **3c** was prepared from **25**-**3** according to the same procedure described for **5c** (25 mg, 60% yield), as a yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.16–7.04 (m, 2H), 6.60 (d, *J* = 7.7 Hz, 1H), 6.24 (d, *J* = 12.3 Hz, 2H), 6.05 (s, 1H), 3.53 (t, *J* = 3.3 Hz, 4H) 3.06 (t, *J* = 3.3 Hz, 4H). EI-MS *m*/*z*: 355 $(M)^+$. HR-EI MS calcd for $C_{19}H_{17}NO_6$ $(M)^+$: 355.1056, found: 355.1102.

5.1.54. 5-Hydroxy-2-(4-hydroxyphenyl)-7-morpholino-*4H*-chromen-4-one (4c)

Compound **4c** was prepared from **25-4** according to the same procedure described for **5c** (31 mg, 80% yield), as a yellow solid. ^1H NMR (300 MHz, d_6-DMSO): δ 12.76 (s, 1H), 7.98–7.89 (m, 2H), 6.98–6.88 (m, 2H), 6.75 (s, 1H), 6.65 (d, *J* = 2.2 Hz, 1H), 6.35 (d, *J* = 2.2 Hz, 1H), 3.60 (t, *J* = 4.9 Hz, 4H), 3.39 (t, *J* = 4.9 Hz, 4H). ESI-MS *m*/*z*: 338 $(M)^-$. HR-ESI MS calcd for $C_{19}H_{16}NO_5$ $(M-H)^-$: 338.1134, found: 338.1030.

5.1.55. 5-Hydroxy-3-(4-hydroxyphenyl)-7-morpholino-*4H*-chromen-4-one (7c)

Compound **7c** was prepared from **25-7** according to the same procedure described for **5c** (22 mg, 66% yield), as a yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 8.00 (s, 1H), 7.42 (d, *J* = 8.1 Hz, 2H), 6.85 (d, *J* = 8.1 Hz, 2H), 6.42 (d, *J* = 2.4 Hz, 1H), 6.35 (d, *J* = 2.4 Hz, 1H), 3.81 (t, *J* = 4.8 Hz, 4H), 3.35 (t, *J* = 4.8 Hz, 4H). ESI-MS *m*/*z*: 338 $(M-H)^-$.

5.1.56. 3,7-Bis(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-*4H*-chromen-4-one (26)

and

5.1.57. 3,7-Bis(benzyloxy)-2-(4-(benzyloxy)-3-hydroxyphenyl)-5-hydroxy-4H-chromen-4-one (27)

To a solution of quercetin (5.00 g, 14.79 mmol) in DMF (40 mL), were added potassium carbonate (7.14 g, 51.77 mmol) and benzyl bromide (6.19 mL, 51.77 mmol) under argon. The reaction mixture was stirred vigorously at room temperature for 16 h. The resulting mixture was diluted with 400 mL of AcOEt and washed with water. The residue obtained after evaporation of the solvent was purified by column chromatography on silica gel (hexanes/DCM = 1:1) to afford **26** (5.51 g, 56% yield) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.72 (d, *J* = 2.1 Hz, 1H), 7.55 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.51–7.20 (m, 20H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 6.44 d, *J* = 2.2 Hz, 1H), 5.26 (s, 2H), 5.13 (s, 2H), 5.04 (s, 2H), 5.00 (s, 2H); **27** (1.68 g, 20%) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.66–7.60 (m, 2H), 7.48–7.27 (m, 15H), 6.95 (d, *J* = 9.1 Hz, 1H), 6.53–6.41 (m, 2H), 5.24–5.17 (m, 2H), 5.17–5.10 (s, 2H), 5.08 (s, 2H).

5.1.58. 3,7-Bis(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-((4-fluorobenzyl)oxy)-*4H*-chromen-4-one (28)

Compound **26** (1.0 g, 1.51 mmol), 4-flurobenzyl bromide (1.24 g, 3.02 mmol) and potassium carbonate (552 mg, 4 mmol) were dissolved in 20 mL DMF under argon. The mixture was agitated at 60 °C for 6 h. The reaction mixture was diluted with 150 mL of EtOAc and washed with water. The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc = 85:15) to afford **28** (1.1 g, 88%) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.76–7.72 (d, *J* = 2.3 Hz, 1H), 7.66–7.29 (m, 21H), 7.25–7.21 (m, 2H), 7.15–7.05 (m, 2H), 6.96 (d, *J* = 8.6 Hz, 1H), 6.55 (d, *J* = 2.2 Hz, 1H), 6.45 (d, *J* = 2.3 Hz, 1H), 5.22 (s, 4H), 5.10 (s, 4H), 4.97 (s, 2H).

5.1.59. 3,7-Bis(benzyloxy)-2-(4-(benzyloxy)-3-((4-fluorobenzyl)oxy)phenyl)-5-hydroxy-*4H*-chromen-4-one (29)

Compound **27** (340 mg, 0.5 mmol), K_2CO_3 (83 mg, 0.6 mmol) and 4-flurobenzyl bromide (100 μL, 80 mmol) were dissolved in 20 mL DMF under argon. The mixture was agitated at room temperature for 16 h. Then, the reaction mixture was diluted with 150 mL of EtOAc and washed with water. The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 8:1) to afford **29** (243 mg, 85%) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, *J* = 2.1 Hz, 1H), 7.57 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.50 –7.23 (m, 17H), 7.01 (m, 3H), 6.48–6.42 (dd, *J* = 8.8, 2.2 Hz, 2H), 5.23 (s, 2H), 5.14 (s, 2H), 5.05 (s, 2H), 4.90 (s, 2H). ESI-MS *m*/*z*: 681 $(M+H)^+$.

5.1.60. 2-(3,4-Dihydroxyphenyl)-5-((4-fluorobenzyl)oxy)-3,7-dihydroxy-*4H*-chromen-4-one (1e)

A suspension of **28** (92 mg, 0.12 mmol) in MeOH (15 mL) was treated with 10% Pd/C (8 mg) under a flow of hydrogen until the starting material disappeared. The reaction mixture was then filtered on Celite and eluted with MeOH. After concentration of the filtrate under vacuum, the residue was purified by column chromatography on silica gel (hexanes/EtOAc = 2:1) to afford **1e** (9 mg, 18% yield), as a yellow solid: ^1H NMR (400 MHz, CD_3OD): δ 7.75 (d, *J* = 2.3 Hz, 1H), 7.69–7.59 (m, 3H), 7.13 (t, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H), 6.42 (d, *J* = 2.1 Hz, 1H), 5.26 (s, 2H). ESI-MS *m*/*z*: 433 $(M+Na)^+$.

5.1.61. 3,7-Bis(benzyloxy)-2-(4-(benzyloxy)-3-hydroxyphenyl)-5-hydroxy-*4H*-chromen-4-one (1f)

Compound **1f** was prepared from **29** according to the same procedure described for **1e** (20 mg, 25% yield), as a yellow solid. ^1H NMR (400 MHz, d_6-DMSO) δ 12.56 (s, 1H), 7.80 (s, 1H), 7.62 (d, *J* = 8.6 Hz, 1H), 7.61–7.53 (m, 2H), 7.22 (t, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.47 (s, 1H), 6.20 (s, 1H), 5.20 (s, 2H). ESI-MS *m*/*z*: 409 $(M-H)^-$. HR-ESI MS calcd for $C_{22}H_{15}FO_7$ $(M-H)^-$: 409.0729, found: 409.0724.

5.1.62. 2-(2,2-Diphenylbenzo[d][1,3]dioxol-5-yl)-3,5,7-trihydroxy-*4H*-chromen-4-one (30)

A mixture of **1** (2 g, 6.57 mmol) and dichlorodiphenylmethane (2 mL, 10.42 mmol) in 5 mL diphenyl ether was heated at 180 °C for 20 min. The reaction mixture was washed by ether and diluted with hot acetone. Then, the filtrate was purified by flash column chromatography using petroleum ether/EtOAc (6:1 to 1:1) as eluent to give **30** (1.25 g, 38% yield) as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 7.86–7.79 (m, 2H), 7.67–7.53 (m, 4H), 7.53–7.42 (m, 6H), 7.24 (d, *J* = 8.7 Hz, 1H), 6.59 (d, *J* = 2.1 Hz, 1H), 6.21 (s, 1H).

5.1.63. 2-(2,2-Diphenylbenzo[*d*][1,3]dioxol-5-yl)-3-((4-fluorobenzyl)oxy)-5,7-dihydroxy-*4H*-chromen-4-one (31)

Compound **31** was prepared from **30** according to the same procedure described for **15a** (210 mg, 91% yield), as a yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.61–7.53 (m, 4H), 7.48–7.38 (m, 8H), 7.19–7.10 (m, 2H), 6.95 (d, *J* = 8.8 Hz, 1H), 6.75–6.67 (m, 2H), 6.36 (d, *J* = 2.1 Hz, 1H), 6.19 (d, *J* = 2.1 Hz, 1H), 4.92 (s, 2H); EI-MS *m*/*z*: 574 $(M)^+$.

5.1.64. 2-(3,4-Dihydroxyphenyl)-3-((4-fluorobenzyl)oxy)-5,7-dihydroxy-*4H*-chromen-4-one (1d)

The solution of compound **31** (114 mg, 0.20 mmol) in acetic acid/water (4:1, 25 mL) was refluxed for 9 h. Then, EtOAc (100 mL) and water (100 mL) were added. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and dried over anhydrous Mg_2SO_4. After concentration, the residue was recrystallized with ether/EtOAc to afford **1d** (33 mg, 40% yield), as a yellow solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.52–7.37 (m, 4H), 7.15 (s, 2H), 6.85 (d, *J* = 8.6 Hz, 1H), 6.39 (s, 1H), 6.19 (s, 1H), 4.97 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 178.35, 164.71, 162.35 (d, *J* = 243.9 Hz), 161.75, 156.84, 149.14, 145.62, 136.53, 133.38, 131.14 (d, *J* = 8.4 Hz), 121.33, 116.00, 115.45 (d, *J* = 21.3 Hz), 104.57, 99.09, 94.09, 72.97. ESI-MS *m*/*z*: 433 $(M+Na)^+$. HR-ESI MS calcd for $C_{22}H_{15}FO_7$ $(M-H)^-$: 409.0729, found: 409.0727.

5.1.65. 3,7-Bis(benzyloxy)-2-(2,2-diphenylbenzo[*d*][1,3]dioxol-5-yl)-5-hydroxy-*4H*-chromen-4-one(32)

Compound **32** was prepared from **30** according to the same procedure described for **15a** (56 mg, 42%), as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65–7.35 (m, 16H), 7.34–7.27 (m, 4H), 7.14 (m, 2H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.55 (d, *J* = 2.3 Hz, 1H), 6.47 (d, *J* = 2.3 Hz, 1H), 5.29 (s, 2H), 5.07 (s, 2H).

5.1.66. 3,7-Bis(benzyloxy)-2-(3,4-dihydroxyphenyl)-5-hydroxy-*4H*-chromen-4-one (33)

Compound **33** was prepared from **32** as a yellow solid according to the same procedure described for **1d** (59 mg, 65% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 7.54–7.50 (d, *J* = 2.1 Hz, 1H), 7.49–7.29 (m, 8H), 7.29–7.22 (dt, *J* = 4.7, 1.8 Hz, 3H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.63 (d, *J* = 2.2 Hz, 1H), 6.41 (s, 1H), 5.20 (s, 2H), 5.16 (s, 2H). ESI-MS *m*/*z*: 483 $(M+H)^+$.

5.1.67. 3,7-Bis(benzyloxy)-2-(4-((4-fluorobenzyl)oxy)-3-hydroxyphenyl)-5-hydroxy-*4H*-chromen-4-one (34)

Compound **34** was prepared from **33** according to the same procedure described for **15a** (210 mg, 91% yield), as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.59 (m, 2H), 7.50 –7.08 (m, 14H), 6.98–6.90 (m, 1H), 6.54–6.43 (m, 2H), 5.16 (d, *J* = 7.2 Hz, 4H), 5.11 (s, 2H). EI-MS *m*/*z*: 590 $(M)^+$.

5.1.68. 2-(4-((4-Fluorobenzyl)oxy)-3-hydroxyphenyl)-3,5,7-trihydroxy-*4H*-chromen-4-one (1g)

Compound **1g** was prepared from **34** according to the same procedure described for **1e** (12 mg, 20% yield), as a yellow solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.46 (s, 1H), 7.81 (s, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.62–7.52 (m, 2H), 7.24 (t, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 8.6 Hz, 1H), 6.47 (s, 1H), 6.20 (s, 1H), 5.17 (s, 2H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 176.42, 164.48, δ 161.23 (d, *J* = 11.3 Hz), 161.19, 156.64, 148.54, 147.06, 146.60, 136.69, 133.64, 130.45 (d, *J* = 8.3 Hz), 124.28, 119.99, 115.67 (d, *J* = 21.4 Hz), 115.02, 114.13, 103.52, 98.69, 93.86, 69.45. EI-MS *m*/*z*: 409 $(M-H)^-$. HR-EI MS calcd for $C_{22}H_{15}FO_5$ $(M-H)^-$: 409.0729, found: 410.0793.

5.2. Biological materials, chemicals, and enzymes

All compounds were dissolved in DMSO and the 10 mM stock solutions were stored at −20 °C. Further dilutions were also performed in DMSO. The expression system used in purifying IN was a kind gift from Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD. The oligonucleotides used in the HIV-1 IN catalytic activity assay were synthesized at the USC Norris Cancer Centermicrosequencing core facility. The γ [^{32}P]-ATP was purchased from Perkin Elmer (Waltham, MA).

5.2.1. Preparation of oligonucleotide substrate

The HIV-1 IN catalytic activity assay uses a 21'mer top strand: (5′-GTGTGGAAAATCTCTAGCAGT-3′), and a 21'mer bottom strand: (5′-ACTGCTAGAGATTTTCCACAC-3′). The top strand was labelled at the 5′end with γ [^{32}P]-ATP by T4 polynucleotide kinase (Epicenter, Madison, WI). The mixture was then incubated at 95 °C for 15 min to inactivate the kinase and the bottom strand was added in 1.5 molar excess. The strands were allowed to anneal by cooling the mixture slowly to room temperature. Any unincorporated material was subsequently removed by centrifuging the mixture through a Spin-25 mini-column (USA Scientific, Ocala, FL).

5.2.2. Integrase catalytic activity assay

The extent of 3′-processing and strand transfer was analyzed by preincubating recombinant wild-type HIV-1 IN, at a final concentration of 200 nM, with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% DMSO, and 25 mM MOPS, pH 7.2) at 30 °C for 30 min. Then 20 nM of the ^{32}P 5′end-labeled linear 21'mer substrate was added, and incubation was continued for an additional 1 h. Reactions were then quenched by the addition of an equal volume (16 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue). An aliquot (7 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M Tris-borate pH 8.3, 2 mM EDTA, 20% acrylamide, 8 M urea).

Gels were dried, exposed in a PhosphorImagercassstte, analyzed using a Typhoon 8610 Variable Mode Imager (Amersham Biosciences), and quantified using ImageQuant 5.2. The percent inhibition (%I) was calculated using the following equation: %I = 100 * [1 − (D − C)/(N − C)] where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The IC50 values were determined by plotting the logarithm of drug concentration against percent inhibition of enzymatic activity to obtain the concentration that produced 50% inhibition.

5.2.3. LEDGF/p75–IN AlphaScreen assay

The AlphaScreen assay was performed according to the manufacturer's protocol (Perkin–Elmer, Benelux). Reactions were performed in 25 μL final volume in 384-well Optiwell microtiter plates (Perkin-Elmer). The reaction buffer contained 25 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$, 0.01% (v/v) Tween-20 and 0.1% (w/v) bovine serum albumin. His_6-tagged IN (300 nM

final concentration) was incubated with the inhibitory compound for 30 min at 4 °C. The compounds were added in varying concentrations spanning a wide range from 0.1 μM up to 20 μM. Afterwards, 100 nM Flag-LEDGF/p75 was added and incubation was prolonged for an additional hour at 4 °C. Subsequently, 5 μl of Ni-chelate-coated acceptor beads and 5 μl anti-Flag donor beads were added to a final concentration of 20 μg/mL of both beads. Proteins and beads were incubated for 1 h at 30 °C in order to allow association to occur. Exposure of the reaction to direct light was omitted as much as possible and the emission of light from the acceptor beads was measured in the EnVision plate reader (Perkin–Elmer) and analyzed using the EnVision manager software.

5.3. Antiviral assay

C8166 cells were maintained in RPMI-1640 supplemented with 10% heat-inactivated newborn calf serum (Gibco). The cells used in all experiments were in log-phase growth. *Syncytium reduction assay:* In the presence of 100 μL of various concentrations of compounds, C8166 cells (4×10^5 mL^{-1}) were infected with HIV-1_{IIIB} at a multiplicity of infection (MOI) of 0.06. The final volume per well was 200 μL. AZT was used as a positive control. After 3 days of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cell) in each well under an inverted microscope. Percentage inhibition of syncytial cell number in treated culture to that in infected control culture and 50% effective concentration (EC_{50}) was calculated. *Cytotoxicity assay:* The cellular toxicity of compounds on C8166 cells was assessed by MTT methods. Briefly, cells were seeded on a microplate in the absence or presence of various concentrations of compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2 for 72 h. The supernatants were discarded and MTT reagent (5 mg/mL in PBS) was added to each well then incubated for 4 h and 100 μL of 50% DMF-20% SDS was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek EL ×800 ELISA reader at 595/630 nm. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was calculated from dose–response curve.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.04.016.

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