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Potent and Nontoxic Chemosensitizer of P-Glycoprotein-Mediated Multidrug Resistance in Cancer: Synthesis and Evaluation of Methylated Epigallocatechin, Gallocatechin, and Dihydromyricetin Derivatives

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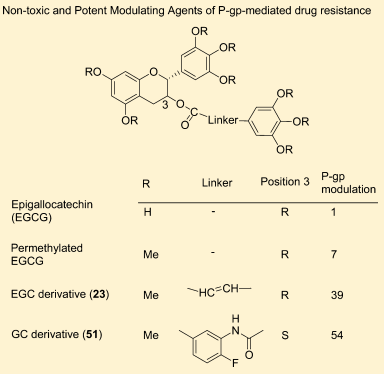
Supporting Information

ABSTRACT: We are interested in developing novel natural product-derived P-gp inhibitors to reverse cancer drug resistance. Here, we have synthesized 55 novel derivatives of methylated epigallocatechin (EGC), gallocatechin (GC), and dihydromyricetin (DHM). Three EGC derivatives (**23**, **35**, and **36**) and three GC derivatives (**50**, **51**, and **53**) are significantly better than epigallocatechin gallate (EGCG) with a relative fold (RF) ranging from 31.4 to 53.6. The effective concentration (EC_{50}) of **23** and **51** ranges from 102 to 195 nM. Compounds **23** and **51** are noncytotoxic to fibroblasts with IC_{50} > 100 μM. Compound **23** is specific for P-gp without modulating activity toward MRP1 or BCRP. Compounds **23** and **51** are non-P-gp substrates. Important pharmacophores for P-gp modulation were identified. In summary, methylated EGC and GC derivatives represent a new class of potent, specific, noncytotoxic, and nonsubstrate P-gp modulators.



INTRODUCTION

Multidrug resistance (MDR) is a major clinical problem in cancer chemotherapy. One important cause of MDR is the overexpression of the ATP-binding cassette (ABC) transporters which can efflux different kinds of drugs across the cell membrane.[1−3] Fifteen ABC transporters have been reported to extrude anticancer drugs *in vitro*.[4,5] Among them, P-glycoprotein (P-gp; MDR1; ABCB1) has been widely studied and is known to be the major contributor to clinical MDR.[2−4] Modulation of P-gp can potentially reverse clinical MDR.[2,4−8]

P-gp was the first identified and best characterized mammalian ABC protein.[9−11] In the past two decades, considerable effort has been spent in the discovery of P-gp modulators. The first-generation P-gp inhibitors, such as the calcium channel blocker verapamil[12,13] and immunosuppressive agent cyclosporin A,[14,15] were found to be competitive inhibitors.[2−5,16] Their low potency, however, precluded them from further development for clinical use. Some of the first generation modulators, e.g., verapamil, have side effects and were undesirable for clinical use. Second generation P-gp modulators include dexverapamil,[17] dexniguldipine,[18] and valspodar (an analogue of cyclosporin A; also known as PSC833).[19,20] Although they were less toxic and more potent, their uses were limited by their unpredictable pharmacokinetic interactions with the anticancer drugs.[2−5,21] Third generation P-gp modulators including tariquidar,[22−24] elacridar,[25,26] zosuquidar,[27,28] and laniquidar[29] were potent and safe in *in vitro* studies. Some of them have been used in clinical trials but without much success.[21,30] There is an urgent need to discover new noncytotoxic and potent P-gp modulators.

Noncytotoxic natural phenolic compounds like flavonoids,[31−38] catechins,[39−41] curcumin,[42] lamallarines,[43] and ningalins[44−47] are potential candidates for good MDR modulators.[35,48] We have previously demonstrated that methylation can improve the P-gp modulating activity of flavonoid. Examples include methylated quercetin with side chain modifications (compounds **1** and **2** shown in Chart 1)[36] and permethyl ningalin B and its synthetic analogues (compounds **3** and **4** shown in Chart 1).[49,50] They displayed significantly better P-gp modulating activity than the parental compounds. These results suggest that the methylation of polyphenolic compounds is a reasonable and important modification to improve P-gp modulating activity. We also found that methylated quercetin derivatives containing O-3 substituents (compounds **5**, **6**, and **7** shown in Chart 1) exhibited promising P-gp- and/or BCRP-modulating activity in cancer cell lines and did not possess any inherent cytotoxicity toward cancer or normal mouse fibroblast cells.[51] We also found that permethyl epigallocatechin gallate (permethyl EGCG, shown in Chart 1) is a good P-gp inhibitor.[52] Herein, we report the design, synthesis, and evaluation of a novel series methylated epigallocatechin (EGC),

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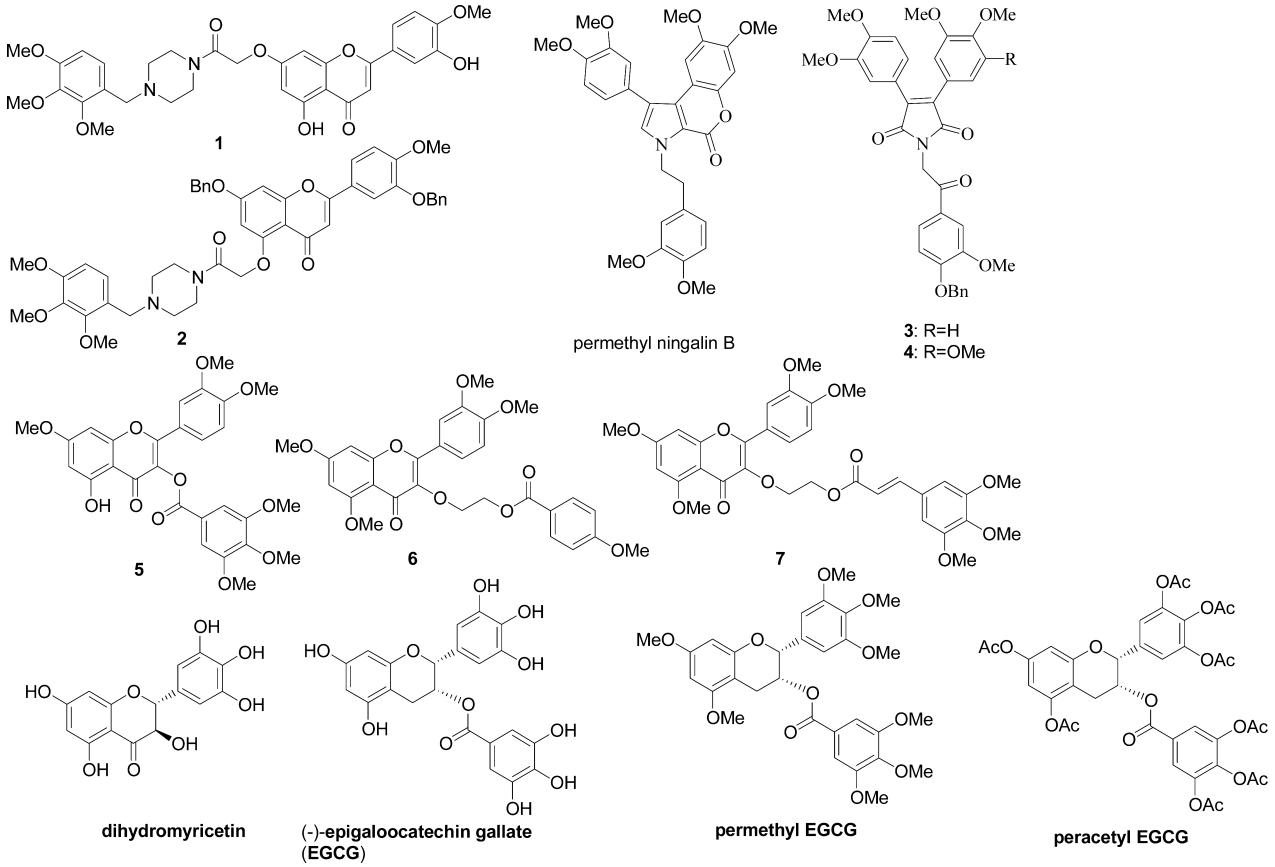
Chart 1. P-gp Inhibitors of Methylated Phenolic Compounds



methylated gallocatechin (GC), and methylated dihydromyricetin (DHM) derivatives in reversing P-gp-mediated drug resistance.[35,36,48−50]

RESULTS AND DISCUSSION

Chemistry. A series of methylated EGC derivatives with various substituents at the C3 position were synthesized using the synthetic route shown in Scheme 1. Pentamethyl epigallocatechin **8** was reacted with methoxy-, ethoxy-, allyoxy-fluoro-, or acetamido-substituted benzoic acid catalyzed by EDCI to give the target compounds **9**−**17**, respectively, with 80−88% yield. Similarly, compounds **18**−**23** were also prepared from compound **8** with 81−90% yield. Hydrogenation of compounds **21**, **22**, and **23** afforded compounds **24**, **25**, and **26**, respectively.

Seven methylated EGC derivatives with various substituents at C3, containing a 3-amino-4-fluorobenzoyloxy linker, were synthesized as shown in Scheme 2. Coupling of methyl 3-amino-4-fluorobenzoate **27** with (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid or (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, followed by hydrolysis, provided intermediates **28** and **29**, respectively. Using a similar method, intermediates **32**−**34** and **37**−**39** were prepared from methyl 3-amino-4-fluorobenzoate and 3,4,5-trimethoxybenzoic acid, 3,4-dimethoxybenzoic acid, or 4-methoxybenzoic acid, respectively. Catalyzed by EDCI, pentamethyl (−)-EGC **8** was reacted either with compounds **28** and **29** to generate the target compounds **30** and **31** or with compounds **33** and **34** to give **35** and **36** or with compounds **37**, **38**, and **39** to produce **40**, **41**, and **42**, respectively.

To investigate the importance of the chiral center C3, a series of methylated GC derivatives were made which have 2R,3S configuration instead of the 2R,3R in EGC derivatives. Synthesis of this series of methylated GC derivatives with the 2R,3S configuration is shown in Scheme 3. The Mitsunobu method was adopted in the configuration inversion at C3, which is more efficient than that reported.[53] (−)-(2*R*,3*R*)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol **8** (pentamethyl EGC)[54] was reacted with 3,4,5-trimethoxybenzoic acid in the presence of PPh_3 and DIAD to produce (+)-(2*R*,3*S*)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4,5-trimethoxybenzoate **43** (permethyl GCG). Hydrolysis of permethyl GCG **43** gave (−)-(2*R*, 3*S*)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol **44** (pentamethyl GC). Pentamethyl GC **44** was reacted with 4-methoxybenzoic acid or 3,4-dimethoxybenzoic acid catalyzed by EDCI to give the target compounds **45** or **46**, respectively. Similarly, target compounds **47**−**53** were obtained using pentamethyl GC **44** as the starting material.

To investigate analogues of permethyl EGCG with a similar scaffold, a series of methylated dihydromyricetin[55] derivatives with a chromanone scaffold was synthesized as shown in Scheme 4. Pentamethyl dihydromyricetin **54** was acetylated to provide **55**. Pentamethyl dihydromyricetin **54** was reacted with 4-methoxybenzoic acid, 3,4-dimethoxybenzoic acid, 3,4,5-trimethoxybezoic acid, or 3,4,5-triallyloxybezoic acid catalyzed by EDCI to give target compounds **56**−**59**, respectively. Using a similar method, target compounds **60**−**66** were obtained.

BIOLOGICAL EVALUATION

Structure−Activity Relationship Study of the P-gp Modulating Activity of Methylated EGC, Methylated GC, and Methylated DHM Derivatives. In this study, the P-gp-transfected breast cancer cell line (MDA435/LCC6MDR) and

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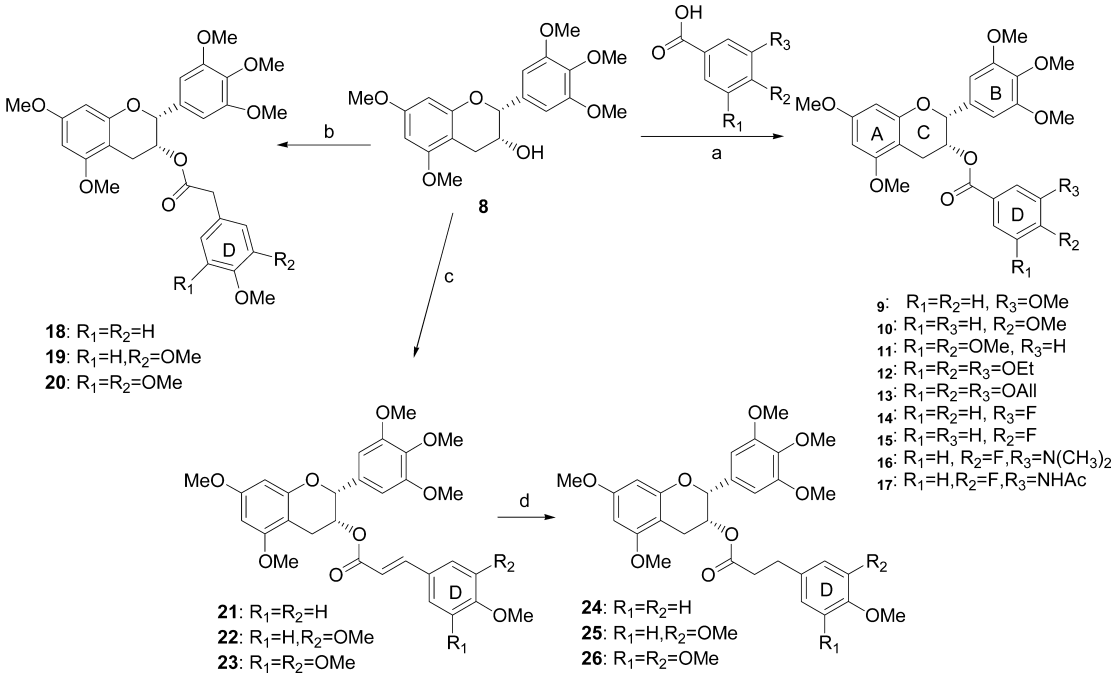
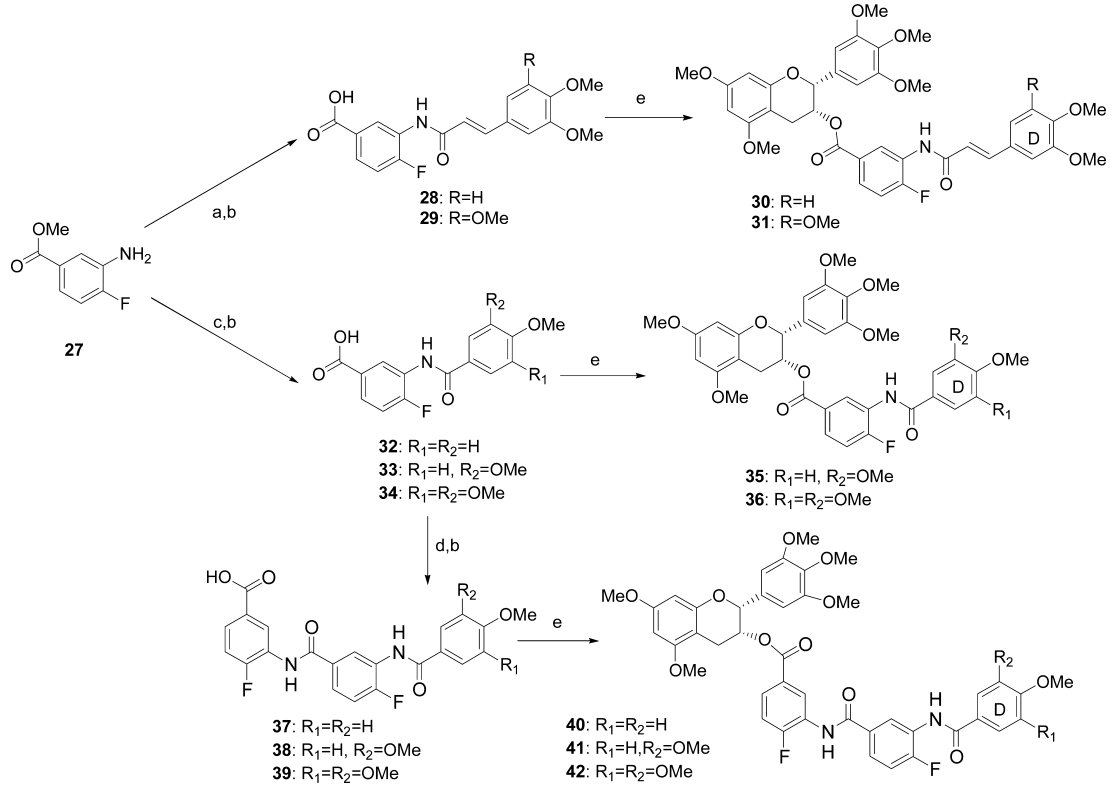
Scheme 1. Synthetic Route of Compounds 9−26[a]



[a]Reagents and conditions: (a) EDCI/DMAP/DCM, rt; (b) EDCI/DMAP/DCM, 4-methoxyphenylacetic acid, 3,4-dimethoxyphenylacetic acid, 3,4,5-trimethoxyphenylacetic acid, rt; (c) EDCI/DMAP/DCM, (*E*)-3-(4-methoxyphenyl) acrylic acid, (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid, or (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, rt; (d) Pd/C, H_2, CH_3OH, rt.

Scheme 2. Synthetic Route of Compounds 28−42[a]



[a]Reagents and conditions: (a) DMAP/DCM, (*E*)-3-(3,4-dimethoxyphenyl)acryloyl chloride or (*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl chloride, rt; (b) LiOH, $MeOH/H_2O$, rt; (c) DMAP/EDCI/DCM, 4-methoxybenzoic acid, 3,4-dimethoxybenzoic acid, or 3,4,5-trimethoxybenzoic acid, rt; (d) DMAP/EDCI/DCM, methyl 3-amino-4-fluorobenzoate, rt; (e) DMAP/EDCI/DCM, compound **8**.

its parent (MDA435/LCC6) were used. LCC6MDR cells were about 90.4-fold more resistant to paclitaxel (PTX) than the parental LCC6 cells (Table 1). P-gp modulating activity of compounds was evaluated by a parameter known as relative

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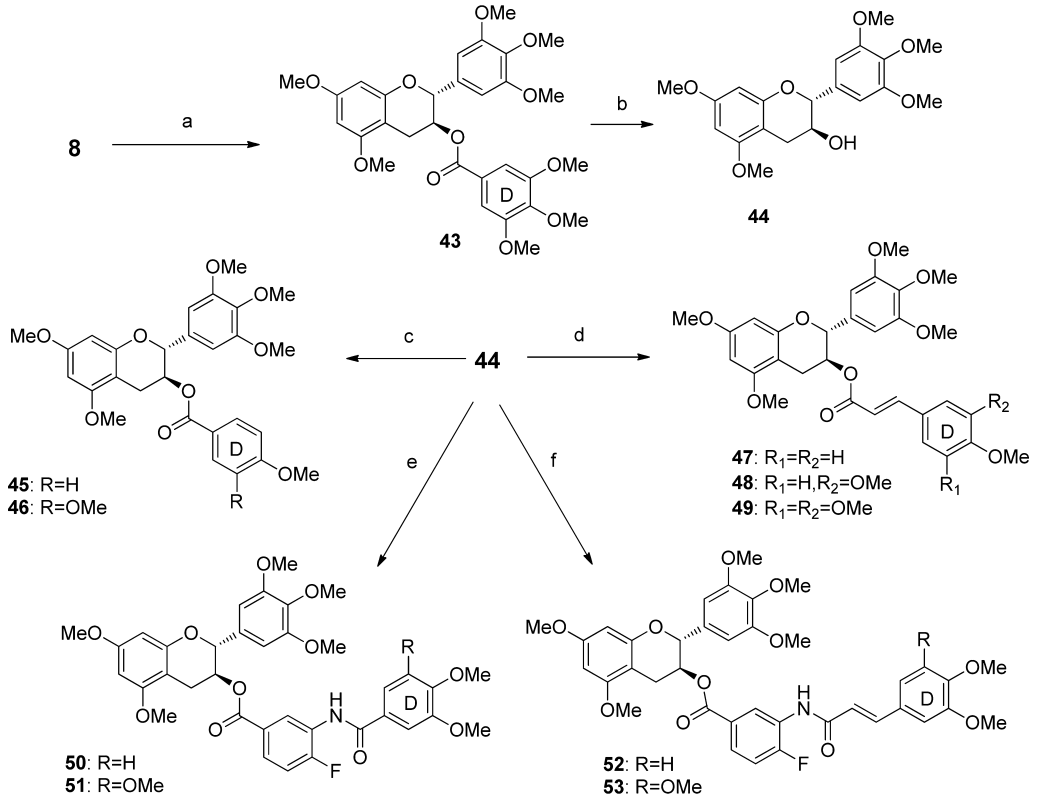
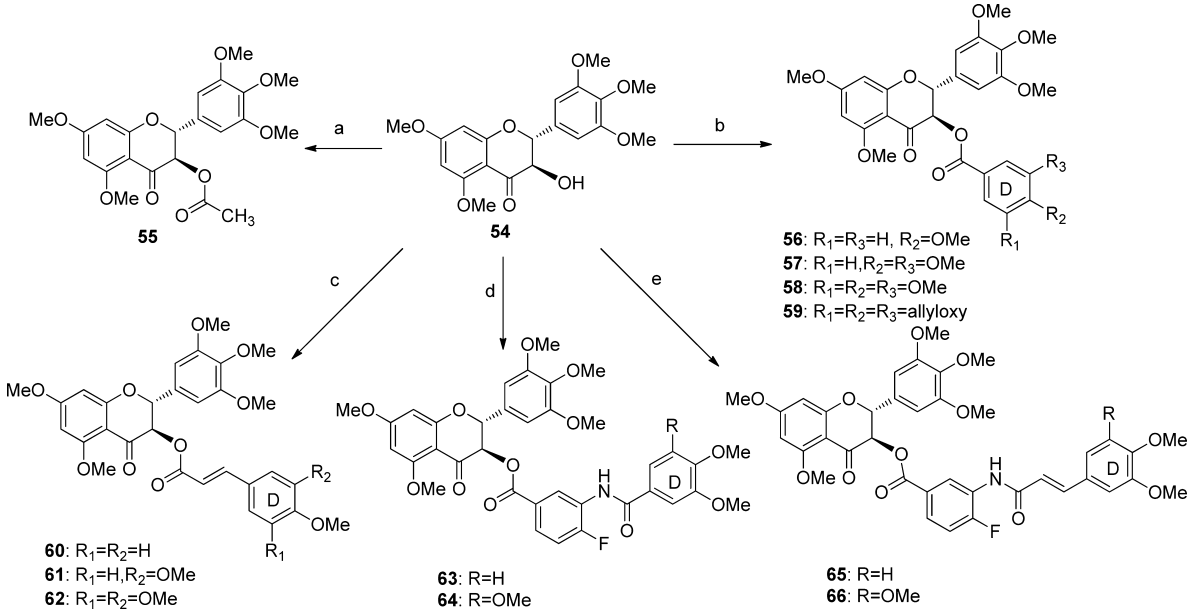
Scheme 3. Synthetic Route of Compounds 43−53[a]



[a]Reagents and conditions: (a) Ph_3P/DIAD, 0 °C−rt; (b) K_2CO_3/MeOH/DME, rt; (c) DMAP/EDCI/DCM, 3,4-dimethoxybenzoic acid, or 3,4,5-trimethoxybenzoic acid, rt; (d) EDCI/DMAP/DCM, (*E*)-3-(4-methoxyphenyl)acrylic acid, (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid, or (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, rt; (e) EDCI/DMAP/DCM, compounds **33** or **34**, rt; (f) EDCI/DMAP/DCM, compounds **28** or **29**, rt.

Scheme 4. Synthetic Route of Compounds 55−66[a]



[a]Reagents and conditions: (a) Ac_2O/Py; (b) DMAP/EDCI/DCM, 4-methoxybenzoic acid, 3,4-dimethoxybenzoic acid, 3,4,5-trimethoxybenzoic acid, or 3,4,5-triallyloxybenzoic acid, rt; (d) EDCI/DMAP/DCM, (*E*)-3-(4-methoxyphenyl)acrylic acid, (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid, or (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, rt; (e) EDCI/DMAP/DCM, compounds **33** or **34**, rt; (f) EDCI/DMAP/DCM, compounds **28** or **29**, rt.

fold (RF), which is defined as the ratio of IC_{50} toward PTX in LCC6MDR cells in the absence of 1 μM of modulators relative to that with modulator. Higher RF means better P-gp modulating activity (Table 1). Verapamil, a first-generation P-gp modulator, displayed a moderate activity with a RF of 3.8 (Table 1).

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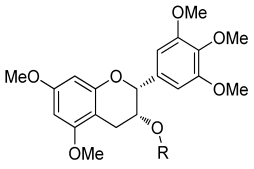
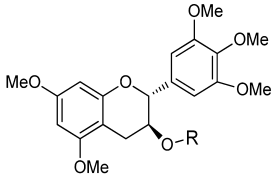
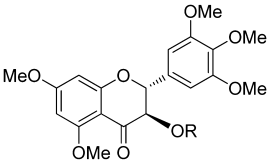
Table 1. Cytotoxicity, P-gp-Modulating Activity, Calculated LogP, and Calculated PSA of Methylated EGC, Methylated GC, and Methylated Dihydromyricetin Derivatives*



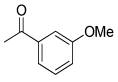
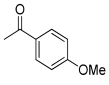
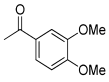
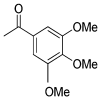
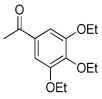
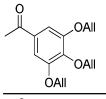
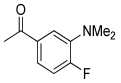
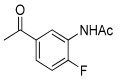
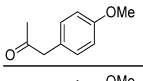
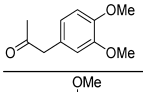
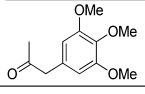
EGC derivatives (EGC)



GC derivatives (GC)



Dihydromyricetin derivative (DHM)

Series	Cpds	R group	Cytotoxicity IC_{50} (μM)			IC_{50} PTX in LCC6MDR [nM]	RF	cLogP	PSA (A^2)
			LCC6	LCC6MDR	L929				
I (EGC)	EGCG (1 μM)	/	69.1±5.0	56.0±13.9	82.3±7.7	124.1 ± 13.7	1.2	1.49	380.1
	EGCG (10 μM)	/				122.6 ± 29.0	1.2		
	Peracetyl EGCG	/	71.5±16.8	31.7±11.5	41.4±10.6	176.1 ± 31.7	0.8	0.02	361.2
	8	H	>100	>75	>100	155.2 ± 28.1	0.9	2.28	100.5
II (EGC)	**9**		>100	>100	>100	45.2 ± 5.7	3.2	5.26	95.5
	10		>100	>84	>100	147.7 ± 23.2	1.0	5.26	90.4
	11		>100	>100	>100	19.5 ± 3.7	7.4	4.96	117.3
	Permethyl EGCG		>100	>100	>100	21.0 ± 2.8	6.9	4.58	82.2
	12		>100	>100	>100	112.9 ± 14.2	1.3	6.17	100.1
	13		>100	>100	>100	12.0 ± 3.2	12.1	7.02	98.5
	14		>100	>100	>100	97.1 ± 28.9	1.5	5.23	90.3
	15		>100	>100	>100	53.3 ± 3.0	2.7	5.23	65.4
	16		>100	>100	>100	36.0 ± 6.1	4.0	5.76	81.3
	17		>100	>100	>100	14.8 ± 2.5	9.8	4.20	135.2
III (EGC)	**18**		/	/	/	34.2 ± 1.4	4.2	4.72	75.7
	19		/	/	/	32.2 ± 2.7	4.5	4.46	69.0
	20		/	/	/	25.0 ± 0.7	5.8	4.10	73.8

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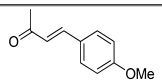
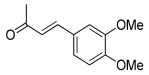
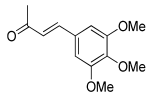
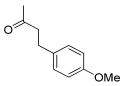
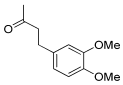
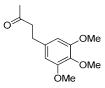
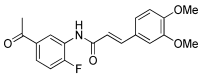
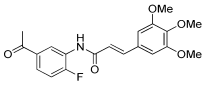
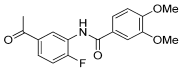
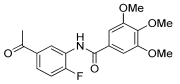
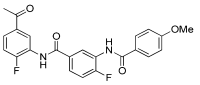
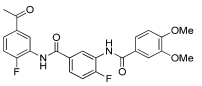
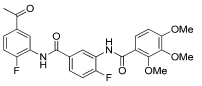
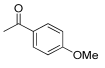
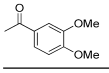
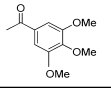
A total of 55 methylated EGC, methylated GC, and methylated DHM derivatives were synthesized. They were divided into 12 series and studied for their P-gp modulating activity. These new derivatives were designed by (1) making substitutions at ring D, (2) changing linker length and rigidity between rings C and D at the C3 position, and (3) using various scaffolds with stereoselectivity.

Substituents at Phenyl Ring D of Methylated EGC Derivatives. In series I, EGCG (RF = 1.2) was a weak P-gp

Table 1. continued

Series	Cpds	R group	Cytotoxicity IC_{50} (μM)			IC_{50} PTX in LCC6MDR [nM]	RF	cLogP	PSA (A^2)
			LCC6	LCC6MDR	L929				
IV (EGC)	21		>100	>100	>100	21.9 ± 5.0	6.6	5.36	66.0
	22		>100	>100	>100	8.7 ± 0.9	16.6	5.09	71.2
	23		>100	>100	>100	3.7 ± 0.9	39.1	4.74	108.9
V (EGC)	24		/	/	/	40.1 ± 1.7	3.6	5.20	81.8
	25		/	/	/	19.0 ± 2.1	7.6	4.94	80.1
	26		/	/	/	18.0 ± 1.7	8.0	4.58	82.1
VI (EGC)	30		>100	>100	>100	8.1 ± 0.4	17.9	6.36	108.6
	31		>100	>100	>100	6.2 ± 0.7	23.3	6.01	141.4
VII (EGC)	35		>100	>100	>100	3.0 ± 0.6	48.2	5.43	152.6
	36		>100	>100	>100	3.3 ± 0.6	43.8	5.02	169.0
VIII (EGC)	40		/	/	/	15.7 ± 2.1	9.2	6.23	131.9
	41		/	/	/	13.6 ± 1.0	10.6	5.87	162.9
	42		/	/	/	13.4 ± 0.6	10.8	5.46	186.2
IX (GC)	44	H	/	/	/	135.9 ± 17.9	1.1	2.28	92.2
	45		/	/	/	67.1 ± 6.9	2.2	5.26	112.7
	46		/	/	/	12.0 ± 1.4	12.1	4.96	102.4
	43		/	/	/	49.0 ± 30.5	3.0	4.58	109.4

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modulator. Even at the highest concentration tested (10 μM), the RF of EGCG remained 1.2. Peracetylation of EGCG at all OH groups of A, B, and D rings (peracetyl EGCG) showed no improvement (RF = 0.8). In contrast, permethylation of EGCG (permethyl EGCG; grouped in series II) resulted in significant improvement to RF = 6.9, suggesting that methoxy rather than acetyl groups on rings A, B, and D are preferred. Removal of ring D from permethyl EGCG abolished all activity in **8** (RF = 0.9), suggesting that ring D is critical.

In view of the critical importance of ring D, we investigated the effect of substituents in ring D. In series II, all EGC derivatives have ring D attached to C3 via an oxycarbonyl linker. Monosubstituted compounds with either methoxy at the meta-position (compound **9** with RF = 3.2) or para-position (compound **10** with RF = 1.0), or fluoro at the meta-position (compound **14** with RF = 1.5) or para-position (compound **15** with RF = 2.7) yielded relatively weak P-gp modulators. Disubstitution at meta- and para-positions in general yielded better P-gp modulators. These include compound **11** with two methoxys at the meta- and para-positions (RF = 7.4), compound **16** with a para-fluoro and meso-dimethylamino (RF = 4.0), or compound **17** with a para-fluoro and meso-acetylamino at

Table 1. continued

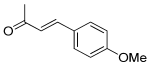
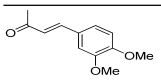
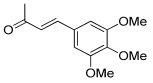
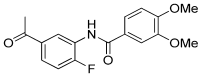
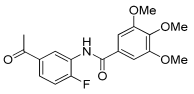
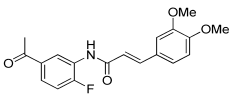
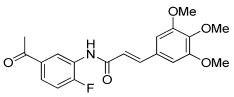
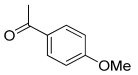
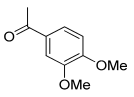
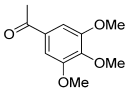
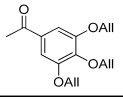
Series	Cpds	R group	Cytotoxicity IC_{50} (μM)			IC_{50} PTX in LCC6MDR [nM]	RF	cLogP	PSA (A^2)
			LCC6	LCC6MDR	L929				
X (GC)	47		/	/	/	18.5 ± 1.5	7.8	5.36	83.9
	48		>100	>100	>100	11.8 ± 2.3	12.3	5.09	94.6
	49		>100	>100	>100	11.6 ± 0.7	12.5	4.74	83.7
	50		>100	>100	>100	4.6 ± 0.5	31.4	5.43	162.4
	51		>100	>100	>100	2.7 ± 0.6	53.6	5.02	132.7
	52		>100	>100	>100	6.6 ± 1.2	21.9	6.36	142.6
	53		>100	>100	>100	4.2 ± 0.7	34.4	6.01	124.9
Quercetin	5						2.1[a]		
	6						6.0[a]		
	7						11.2[a]		
XI (DHM)	55		/	/	/	107.3 ± 26.1	1.4	2.43	90.3
	56		/	/	/	47.9 ± 0.1	3.0	4.32	107.3
	57		/	/	/	36.6 ± 6.1	4.0	4.02	97.8
	58		/	/	/	44.4 ± 4.5	3.3	3.64	123.9
	59		/	/	/	31.6 ± 1.9	4.6	6.09	115.8

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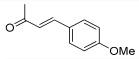
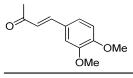
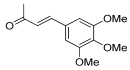
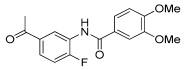
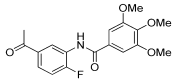
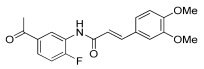
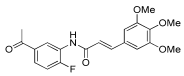
Table 1. continued

Series	Cpds	R group	Cytotoxicity IC_{50} (μM)			IC_{50} PTX in LCC6MDR [nM]	RF	cLogP	PSA (A^2)
			LCC6	LCC6MDR	L929				
XII (DHM)	60		/	/	/	40.7 ± 2.0	3.6	4.47	114.8
	61		/	/	/	26.2 ± 0.3	5.5	4.21	76.4
	62		/	/	/	15.9 ± 0.1	9.1	3.86	101.9
	63		/	/	/	9.4 ± 1.6	15.4	4.49	132.5
	64		/	/	/	36.5 ± 3.7	4.0	4.08	191.7
	65		/	/	/	14.4 ± 0.3	10.0	5.43	132.9
	66		/	/	/	27.8 ± 4.4	5.2	5.07	174.8
	Verapamil		63.8±0.1	63.9±1.7	89.2±8.2	38.0 ± 7.0	3.8	/	/
	LCC6MDR[b]					144.6 ± 9.4	1.0	/	/
	LCC6[b]					1.6 ± 0.3	90.4	/	/

*Methylated EGC, GC, and DHM derivatives are divided into 12 series with their R group indicated in the Table. P-gp modulating activity was measured by determining IC_{50} toward PTX in LCC6MDR cells with or without 1.0 μM of the modulator. RF reflects P-gp modulating activity and is calculated as [IC_{50} of PTX without modulator/IC_{50} with 1.0 μM modulator]. All modulators were dissolved in DMSO and used at 1 μM concentration unless indicated otherwise. Each experiment was repeated at least three times independently, and the IC_{50} value is presented as the mean ± standard error of the mean. In each experiment, each concentration of PTX with or without modulators was done in triplicate. The cytotoxicity of these modulators toward LCC6, LCC6MDR, and normal fibroblast L929 cells was determined. [a]RF values of compounds **5**, **6**, and **7** have been reported. [b]Cytotoxicity of PTX alone toward LCC6 and LCC6MDR in the absence of modulators was determined. Verapamil, a first generation P-gp inhibitor, was included for comparison. L929: mouse fibroblasts. cLogP and PSA were calculated as described in Experimental Section. /: Not determined.

ring D (RF = 9.8). Addition of the third methoxy (permethyl EGCG with RF = 6.9) cannot further improve the activity of compound **11** (RF = 7.4), which already has disubstitution. Trisubstitution with other functional groups yields the following compounds with the rank order of P-gp modulating activity: allyloxy (compound **13**; RF = 12.1) > methoxy (permethyl EGCG; RF = 6.9) ≫ ethoxy (compound **12**; RF = 1.3). We have previously observed a similar structural requirement in the study of ningalin B analogues.[49] The above results suggest that tri- and disubstitutions are in general better than monosubstitution in ring D, and allyloxy or acetyl amino at ring D may improve the P-gp modulating activity of methylated EGC derivatives.

Linker between C3 and Ring D of Methylated EGC Derivatives. As ring D was critical to the P-gp modulating activity of methylated derivatives, we investigated the structural requirements for the linker between rings D and C3. All linkers used were attached to C3 via an oxycarbonyl group. Each series has either one, two, or three methoxy groups at the meta- or para-position of ring D. They were oxycarbonyl (series II: **9**, **11**, and permethyl EGCG), oxycarbonylmethylene (series III: **18**, **19**, **and 20**), oxycarbonylvinyl (series IV: **21**, **22**, and **23**), oxycarbonylethylene (series V: **24**, **25**, and **26**), oxycarbonylphenylcarbamoylvinyl (series VI: **30** and **31**), and oxycarbonylphenylcarbamoyl (series VII: **35** and **36**).

First, we found that di- or trimethoxy groups at ring D generally displayed stronger P-gp modulating activity than those with the monomethoxy group irrespective of the linkers used. Second, we found that linker length did not have a significant effect on P-gp modulating activity. Activity was equally mild when the length of the flexible linkers was increased from oxycarbonyl (series II: **9**, **11**, and permethyl EGCG with RF = 3.2, 7.4, and 6.9) to oxycarbonylmethylene (series III: **18**, **19**, and **20** with RF = 4.2. 4.5, and 5.8) and to oxycarbonylethylene (series V: **24**, **25**, and **26** with RF = 3.6, 7.6, and 8.0). Third, when the flexible oxycarbonylethylene linker (series V: **24**, **25**, and **26** with RF = 3.6, 7.6, and 8.0) was replaced with a rigid oxycarbonylvinyl linker of the same length (series IV: **21**, **22**, and **23** with RF = 6.6, 16.6, and 39.1), P-gp modulating activity was significantly improved. The requirement of a rigid linker was further exemplified when the rigid oxycarbonylphenylcarbamoyl linker was used and found to give the most potent activity (series VII: **35** and **36** with RF = 48.2 and 43.8). However, the advantage of a rigid linker was compromised when the length of the rigid linker was further increased from oxycarbonylphenylcarbamoyl (series VII: **35** and **36** with RF = 48.2 and 43.8) to oxycarbonylphenylcarbamoylvinyl (series VI: **30** and **31** with RF = 17.9 and 23.3) to the longest oxycarbonylphenylcarbamoylphenylcarbamoyl linker (series VIII: **40**, **41**, and **42** with RF = 9.2, 10.6, and 10.8). This

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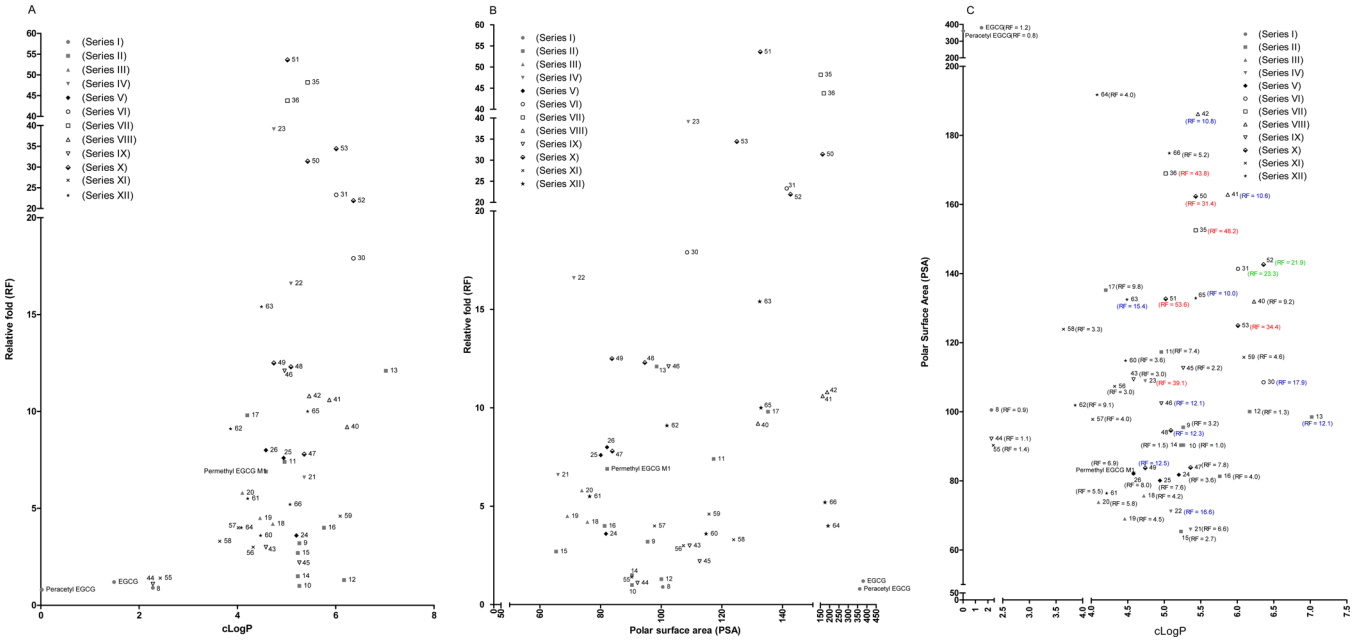

Figure 1. Comparison of cLogP or PSA with the RF values of methylated EGC, methylated GC, and methylated dihydromyricetin derivatives. (A) RF versus cLogP, (B) RF versus PSA, and (C) cLogP versus PSA. RF > 30 is in red, RF = 20−30 is in green, RF = 10−20 is in blue, and RF <10 is in black in (C).

result suggests that having a rigid linker between rings D and C3 is important for the P-gp modulating activity of these methylated EGC analogues. The optimal linker length was between 7.75 and 13.37 Å, which corresponds to the length of oxycarbonylvinyl and oxycarbonylphenylcarbamoyl.

Chiral Configuration at C3 of Methylated GC Derivatives. EGC and GC differ from each other at how ring D attaches to the chiral center C3. In EGC, ring D is in cis-configuration with ring B (2R,3R) whereas in GC, ring D is in trans-configuration (2R,3S). After testing the EGC series above, we studied the GC series here (series IX and X). Each series has one, two, or three methoxy groups at the meta- or para-position in ring D. All linkers used were attached to C3 via oxycarbonyl groups. Ring D was important in GC derivatives as compound **44** only has OH at C3, and it displayed no P-gp modulating activity (RF = 1.1), whereas all other GC derivatives with different forms of ring D (compounds **43** and **45**−**53**) all have higher levels of P-gp modulating activity (RF = 2.2 to 53.6).

We then investigated the effect of linker length and rigidity on P-gp modulating activity. Similar to EGC derivatives, we found that rigid linkers were more desirable than flexible linkers. In general, when the short and flexible oxycarbonyl linker was used (**45**, **46**, and **43** with RF = 2.2, 12.1, and 3.0), their P-gp modulating activity was weaker than the longer and more rigid linkers (**47**−**53** with RF = 7.8 to 53.6). Among the rigid linkers (**47** to **53**), P-gp modulating activity improved when the rigid linker length was increased from oxycarbonyvinyl (**47**, **48**, and **49** with RF = 7.8, 12.3, and 12.5) to oxycarbonylphenylcarbamoyl (**50** and **51** with RF = 31.4 and 53.6) but further increase in length resulted in a drop of activity in oxycarbonylphenylcarbamoylvinyl (**52** and **53** with RF = 21.9 and 34.4). This requirement for linker rigidity with optimal linker length was identical to that observed in EGC derivatives.

When GC derivatives were compared with EGC derivatives with the same linker and substituents in ring D, we found that the best two methylated GC derivatives (**50** and **51** with RF = 31.4 and 53.6) have the same linker (oxycarbonylphenylcarbamoyl) as methylated EGC derivatives (**35** and **36** with RF = 48.2 and 43.8), but the methylated GC derivatives prefer trimethoxy ring D, whereas the methylated EGC derivative slightly prefer dimethoxy ring D. Overall, the activity of GC derivatives was in general slightly better than that of EGC derivatives. Another interesting observation is that dimethoxy or trimethoxy substitutions in ring D were always preferred over monomethoxy substitution in EGC derivatives; however, it was not the case in GC derivatives. Compound **43** with trimethoxy substitution in ring D (RF = 3.0) was equally inactive as monomethoxyl **45** (RF = 2.2) and much less active than dimethoxyl substituted **46** (RF = 12.1). Overall, the pharmacophore for the GC derivative is similar to that of EGC derivatives with GC being slightly more potent.

Chromanone Scaffold of Methylated Dihydromyricetin (DHM) Derivatives. Here, we studied the structure−activity relationship of the P-gp modulating activity in methylated dihydromyricetin (DHM). DHM belongs to flavonolol (3-hydroxy-2,3-dihydro-2-phenylchromen-4-one). We have previously reported that methylated quercetin derivatives with a chromene scaffold have potent P-gp and BCRP modulating activities.[51] As shown in Chart 1 and Table 1, compounds **5** (RF = 2.1) and **7** (RF = 11.2) (quercetin derivatives with a chromene scaffold) possess weaker P-gp inhibitory activity than cis-methylated EGC derivatives containing a chromanol scaffold, permethyl EGCG (RF = 6.9), and **23** (RF = 39.1). However, trans-methylated GC derivatives, **43** (RF = 3.0) and **49** (RF = 12.5), showed P-gp modulating activity similar to that of compounds **5** and **7** even though they also have the chromanol scaffold. There is no particular rule governing the structural requirement for quercetin derivatives and methylated DHM derivatives in terms of their P-gp modulating activity.

A group of methylated DHM derivatives with a chromanone scaffold similar to that of permethyl EGCG (series XI and XII of Table 1) were studied. Linkers with different lengths were introduced into DMH derivatives and compared for their P-gp

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Table 2. Effective Concentration EC_{50} (nM) of Methylated EGC and Methylated GC Derivatives for Reversing the Multidrug Resistance of LCC6MDR[a]

	cytotoxicity toward	EC_{50} (nM) for reversing drug resistance of LCC6MDR				
Cpds	L929 (IC_{50}, μM)	PTX	vinblastine	vincristine	DOX	selective index
22	>100	157 ± 13	123 ± 19	167 ± 47	160 ± 30	>638
23	>100	127 ± 30	195 ± 50	123 ± 2	130 ± 24	>786
35	>100	159 ± 23	193 ± 29	181 ± 7	139 ± 48	>698
36	>100	214 ± 25	/	/	/	>467
50	>100	171 ± 11	173 ± 12	147 ± 19	208 ± 81	>585
51	>100	140 ± 0	108 ± 4	102 ± 6	107 ± 4	>714
52	>100	280 ± 30	/	/	/	>357
53	>100	225 ± 5	/	/	/	>444
verapamil	89 ± 8	446 ± 41	503 ± 92	385 ± 35	245 ± 23	200
cyclosporin A	30 ± 6	32 ± 1	/	/	43 ± 4	934

[a]EC_{50} values were presented as the mean ± standard error of the mean. N = 3−4 independent experiments. Each experiment was done in triplicate. Verapamil and cyclosporin A were used as positive controls. Selective index value = (IC_{50} of modulators toward L929 fibroblasts)/(EC_{50} of modulators for reversing PTX resistance in LCC6MDR cells). /: not determined.

modulating activity. These linkers include oxycarbonyl (**56**−**59**), oxycarbonylvinyl (**60**−**62**), oxycarbonylphenylcarbamoyl (**63**−**64**), and oxycarbonylphenylcarbamoylvinyl (**65**−**66**). In contrast to methylated EGC and GC derivatives, no obvious linker length effect was observed among these dihydromyricetin derivatives. Overall, they showed moderate P-gp modulating activity with RF values ranging from 3.0 to 15.4. This result suggests that the chromanol scaffold of permethyl EGCG derivatives, rather than the chromanone scaffold of methylated dihydromyricetin derivatives, is important in conferring P-gp modulating activity.

It was reported that P-gp substrates are usually amphipathic and lipid soluble. Fifty-two percent of P-gp substrates have a topological polar surface area (tPSA) over 90 Å^2.[56] LogP and the polar surface area (PSA) may reflect the P-gp modulating activity.[49,57] Here, we have calculated the PSA and cLogP value of the 55 compounds (Figure 1 and Table 1). Most compounds have cLogP values of 3.8−7.0 and PSA values of 65.3−191.7 Å^2. For the potent P-gp modulators (RF > 20), the PSA values fall between 108.9 and 169.0 Å^2, while cLogP values fall between 4.7−6.4.

EC_{50} and Selective Index Values of Methylated EGC and Methylated GC Derivatives for Reversing Multidrug Resistance in LCC6MDR. We have chosen four potent methylated EGC derivatives (compounds **22**, **23**, **35**, and **36**) and four potent methylated GC derivatives (compounds **50**−**53**) for further characterization in terms of their effective concentration (EC_{50}) in reversing P-gp and their selective index (Table 2). All eight compounds were noncytotoxic toward L929 normal fibroblasts with IC_{50} greater than 100 μM (Table 2). Their EC_{50} for reversing P-gp mediated resistance toward PTX, vinblastine, vincristine, and DOX resistance in LCC6MDR cells ranged from 102 to 280 nM (Table 2). Their selective indices ranged from >357 to >786. Verapamil was less potent with EC_{50} ranging from 245 to 503 nM and a selective index of 200 (Table 2). Cyclosporin A was the most potent modulator with EC_{50} ranging from 32 to 43 nM. It is, however, slightly toxic toward L929 with an IC_{50} of 30 μM, giving it a selective index of 934.

MRP1- and BCRP-Modulating Activity of Methylated EGC and Methylated GC Derivatives. We have also determined the specificity of methylated EGC and methylated GC toward P-gp, MRP1, and BCRP transporters. All of them are ABC transporters with two transmembrane domains and two nucleotide binding domains. They can mediate drug efflux in an ATP-dependent manner. MRP1 activity was measured as DOX resistance in MRP1-transfected ovarian cancer cell line 2008/MRP1 when compared to its parent 2008 cell line. BCRP activity was measured as topotecan resistance in BCRP-transfected human kidney embryonic cell line HEK293/R2 when compared to the empty vector-transfected HEK293/pcDNA3.1 cell line. 2008/MRP1 was about 5.8-fold more resistant to DOX than 2008/P cells, whereas HEK293/R2 was about 29.6-fold more resistant to topotecan than HEK293/pcDNA3.1 cells. Compound **4e** is a flavonoid homodimer previously reported to have potent MRP1-modulating activity with a RF of 8.9.[58] As shown in Table 3, compounds **23**, **35**, and **36** displayed no MRP1-modulating activity, whereas **50** and **51** showed moderate MRP1 inhibition (RF = 2.6). Ko143 is a known specific BCRP modulator (RF = 19.5). Compounds **23**, **35**, and **36** displayed no or very low BCRP-modulating activity (RF = 1.3 to 2.7), whereas compounds **50** and **51**

Table 3. Specificity of Methylated EGC and Methylated GC Derivatives toward MRP1 and BCRP Transporters[a]

	MRP1-modulating activity		BCRP-modulating activity	
Cpds	IC_{50} of DOX (nM)	RF	IC_{50} of topotecan (nM)	RF
23	322 ± 80	1.1	357 ± 141	1.3
35	434 ± 139	0.8	176 ± 43	2.7
36	418 ± 127	0.9	207 ± 73	2.3
50	142 ± 4	2.6	45 ± 1	10.4
51	141 ± 6	2.6	38 ± 4	12.3
4e	41 ± 13	8.9	/	
Ko143	/		24 ± 2	19.5
2008/MRP1	365 ± 80	1.0	/	
2008/P	63 ± 5	5.8	/	
HEK293/R2	/		468 ± 33	1.0
HEK293/pcDNA3.1	/		16 ± 2	29.6

[a]MRP1- and BCRP-modulating activity of **23**, **35**, **36**, **50**, **51**, **4e**, and Ko143 (all at 1.0 μM) were investigated using 2008/MRP1 and HEK293/R2 cells, respectively (N = 2−4 independent experiments, and the values are presented as the mean ± standard error of the mean. Each experiment was done in triplicate). IC_{50} toward DOX and topotecan in these two cell lines were determined with or without modulators to determine RF. IC_{50} values were also determined for their parental cell lines (2008/P and HEK293/pcDNA3.1) for reference.

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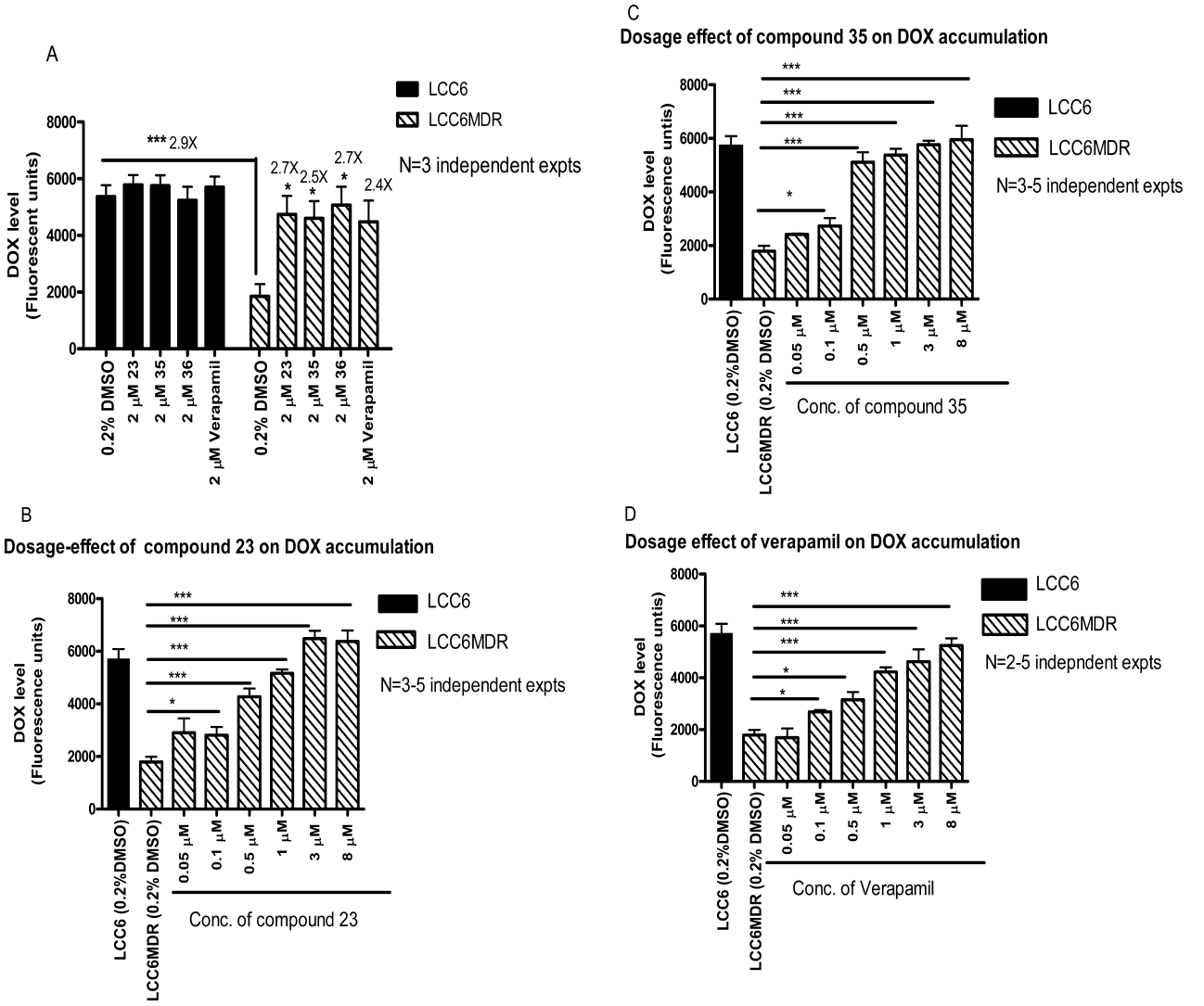

Figure 2. Effect of methylated EGC derivatives on DOX accumulation in LCC6MDR cells. Effect of compounds **23** and **35** or verapamil on intracellular DOX accumulation was studied. (A) LCC6 or LCC6MDR cells were incubated with 20 μM DOX with or without 2 μM of modulators (**23** and **35** or verapamil) for 150 min at 37 °C. DMSO (0.2%) was used as the negative control. After the incubation period, cells were lysed, and the supernatant was saved for measuring the DOX level by spectrofluorometry. (B, C, and D) LCC6MDR cells were incubated with 20 μM DOX and with increasing doses (0, 0.05, 0.1, 0.5, 1, 3, and 8 μM) of **23** (B), **35** (C), or verapamil (D). After incubation, cells were lysed, and their intracellular DOX level was measured by spectrofluorometry. $N = 2-5$ independent experiments. The results are presented as the mean ± standard error of the mean. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$ relative to the LCC6MDR negative control.

surprisingly exhibited very promising BCRP-modulating activity (RF = 10.4 and 12.3). All in all, cis-methylated EGC derivatives are likely monospecific toward the P-gp transporter, especially compound **23**. Trans-methylated GC derivatives, **50** and **51**, are multispecific toward P-gp, MRP1, and BCRP transporters. Modulators with different transporter specificity (mono-, dual-, and multi-) are helpful for treating multidrug resistant cancers caused by the overexpression of ABC transporters.

Methylated EGC Derivatives Increase DOX Accumulation by Inhibiting P-gp-Mediated Efflux in LCC6MDR Cells. DOX is a known fluorescent P-gp substrate, and its fluorescence can be used for monitoring the intracellular DOX level. We found that LCC6 cells accumulated about 2.9-fold more DOX than LCC6MDR cells (Figure 2A). Treatment of LCC6MDR cells with 2 μM of **23**, **35**, **36**, or verapamil can inhibit P-gp and increase DOX accumulation by 2.4- to 2.7-fold, to a level similar to that of LCC6 cells (Figure 2A). Restoration of DOX accumulation in LCC6MDR cells by **23**, **36**, and verapamil was dosage-dependent (Figure 2B, C, and D). Three micromolar **23** and **36** was sufficient to restore DOX accumulation of LCC6MDR to the parental LCC6's level (Figure 2B and C). For verapamil, the highest concentration of 8 μM was not enough to restore DOX accumulation of LCC6MDR to the parental LCC6's level (Figure 2D). Compounds **23** and **36** are therefore roughly at least 2.7-fold more potent than verapamil in inhibiting P-gp's DOX transport activity.

Is reduced DOX accumulation in LCC6MDR due to decreased DOX influx or increased DOX efflux? Here, we studied the effect of **23** and **35** on P-gp-mediated DOX influx and efflux. In the DOX efflux studies, cells were first loaded with DOX, followed by incubation in DOX-free medium. In LCC6 cells, there is no difference in the DOX efflux rate among **23**, **35**, and the DMSO control (Figure 3A). On the contrary, **23** and **35** can reduce DOX efflux significantly compared to that of the DMSO control (Figure 3B). In the DOX influx studies, there is no significant difference in influx rate for both LCC6

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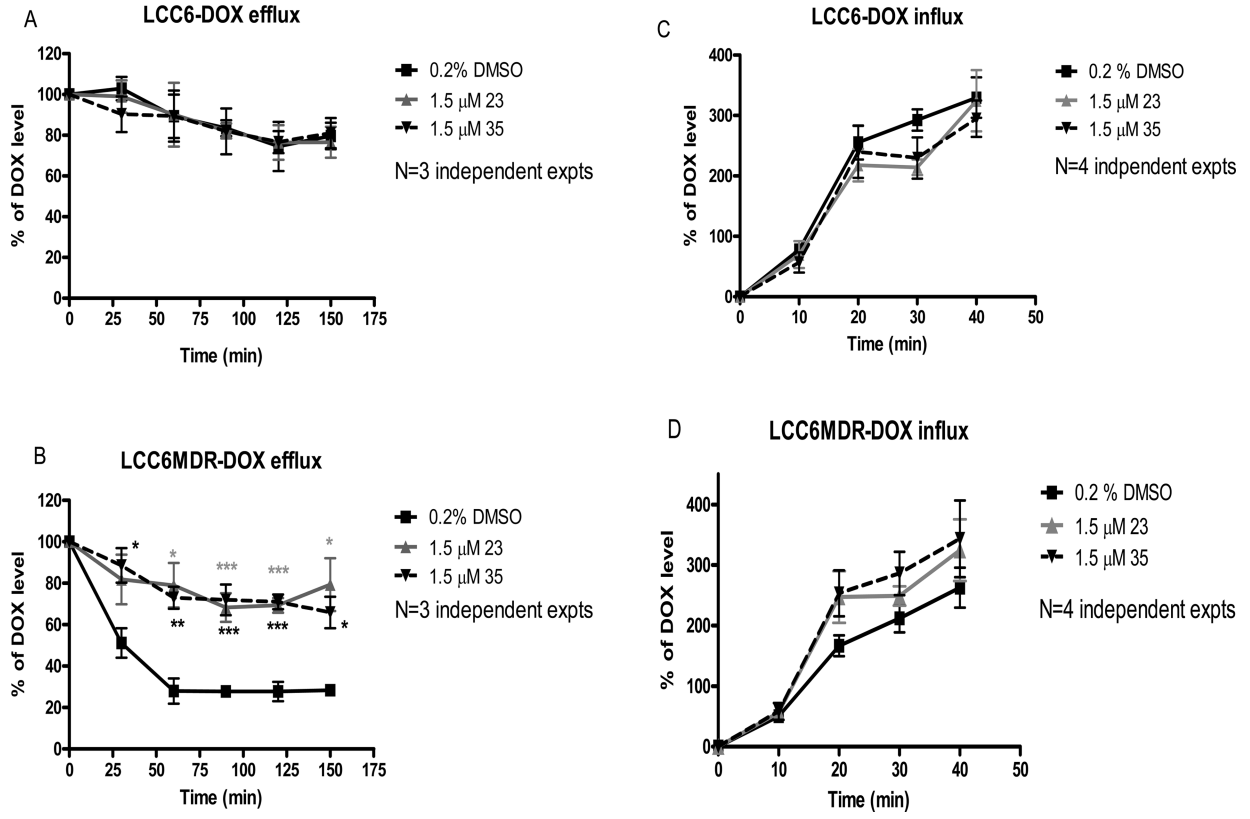

Figure 3. Effect of methylated EGC derivatives on DOX influx and DOX efflux. Effect of methylated EGC derivatives on DOX influx and DOX efflux was studied. For the efflux study, LCC6 (A) or LCC6MDR cells (B) were incubated with 20 μM DOX for 60 min. Cells were then washed with ice cold PBS and resuspended with DMEM media with or without 1.5 μM **23** or **35** to inhibit efflux if there is any. After incubation for 30, 60, 90, 120, and 150 min, cells were collected by centrifugation, and the intracellular DOX level was determined by spectrofluorometry. For the influx study, LCC6 (C) or LCC6MDR cells (D) were incubated with 5 μM of DOX. After incubation for 10, 20, 30, and 40 min, cells were collected, and the intracellular DOX level was determined by spectrofluorometry. N = 3−4 independent experiments. The values are presented as the mean ± standard error of the mean. *P < 0.05, **P < 0.01, and ***P < 0.005 relative to the LCC6MDR negative control at each time point.

(Figure 3C) and LCC6MDR (Figure 3D) cells treated with compounds **23**, **35**, or the DMSO control (Figure 3C and D). The above data suggests that the methylated EGC derivatives can inhibit P-gp-mediated DOX efflux.

Intracellular Accumulation of Methylated EGC and Methylated GC Derivatives in LCC6 and LCC6MDR Cells. Some P-gp modulators are competitive inhibitors of anticancer drugs to bind P-gp, but they are also substrates of P-gp and are effluxed out of the cells. Here, we study whether methylated EGC or methylated GC derivatives are P-gp substrates. We found that both LCC6 and LCC6MDR cells accumulated a similar level of methylated EGC **23** and methylated GC **51** at 20 or 200 μM (Figure 4A and B), indicating that compounds **23** and **51** are not substrates of P-gp. Here, we used DOX as a positive control because it is a known P-gp substrate. At 20 μM, parental cells LCC6 accumulated 2.9-fold more DOX than P-gp overexpressed cells LCC6MDR (Figure 4C), indicating that DOX can bind the P-gp transporter and was exported out by P-gp in LCC6MDR cells.

CONCLUSIONS

In the present study, a total of 55 novel methylated EGC, methylated GC, and methylated dihydromyricetin derivatives were synthesized and evaluated for their P-gp modulating activity in a P-gp overexpressing breast cancer cell line LCC6MDR. The SAR study illustrates several important pharmacophores for modulating P-gp, including (1) methoxy, allyloxy, or acetylamino substitution at ring D, (2) rigid linkers of oxycarbonylvinyl and oxycarbonylphenylcarbamoyl with the optimal linker length ranging from 7.75 to13.37 Å between rings D and C3, (3) chiral configuration at C3, and (4) the chromanol scaffold of permethyl EGCG derivatives. Among the four potent cis-methylated EGC derivatives (**22**, **23**, **35**, and **36**), compound **23** with trimethoxy substituents at ring D and an oxycarbonylvinyl linker between ring D and the C3 position was the most potent with EC_{50} ranging from 123 nM to 195 nM. Among the four potent trans-methylated GC derivatives (**50**, **51**, **52**, and **53**), compound **51** with a linker containing an oxycarbonylphenylcarbamoyl and trimethoxy groups at ring D was the most potent with EC_{50} values ranging from 102 to 140 nM. The mechanism of methylated EGC derivatives in reversing P-gp mediated drug resistance is by virtue of inhibiting the active drug efflux of P-gp transporter (Figure 3B). Importantly, compounds **23** and **51** are not substrates of the P-gp transporter (Figure 4). They will be predicted to stay inside P-gp overexpressing cells for a longer time and result in a longer-lasting P-gp modulating effect. In summary, our study demonstrates that methylated EGC or methylated GC derivatives are noncytotoxic, effective, and specific P-gp modulators that can be used in the future for reversing P-gp mediated clinical cancer drug resistance.

EXPERIMENTAL SECTION

General. All moisture sensitive reactions were conducted under a nitrogen atmosphere in anhydrous, freshly distilled solvents. Starting

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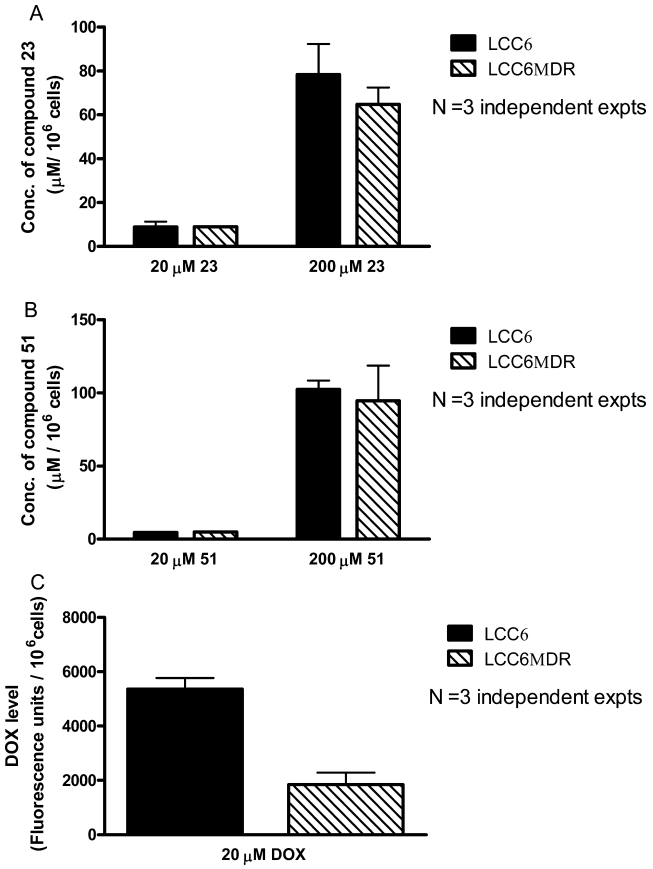

Figure 4. Intracellular accumulation of methylated EGC and methylated GC derivatives in LCC6 and LCC6DR cells. LCC6 or LCC6MDR cells (3.0×10^6) were incubated with 20 or 200 μM of compound **23** (A), **51** (B), or DOX (C) at 37 °C for 150 min. After incubating with compounds **23** and **51**, the cell pellet was resuspended with 100 μL of 100% methanol and vortexed for 5 min. The lysate was spinned down, and the supernatant was used to measure the level of **23** or **51** by HPLC. After incubating with DOX, the cell pellet was lysed with 100 μL of lysis buffer, and the intracellular DOX fluorescence level was measured using a spectrofluorometer. N = 2−3 independent experiments. The data are represented as the mean ± standard error of the mean.

materials and reagents, unless otherwise stated, were of commercial grades and were used without further purification. Solvents were dried according to standard procedures. TLC chromatography was performed on aluminum sheets (Silica gel 60-F254, E. Merck). ^{1}H (600 or 500 MHz) and ^{13}C (150 or 125 MHz) NMR experiments were determined on an instrument when $CDCl_3$ was used as a solvent. Chemical shifts are given in ppm (δ) values with TMS as an internal standard and coupling constants (J) in Hz. High-resolution (ESI) MS spectra were performed with a QTOF-2 micromass spectrometer. In addition to NMR and high-resolution (ESI) MS, HPLC analysis was used to determine the purity (>95%) of the compounds. Compounds were dissolved in methanol (1.5 mL). A reversed phase Diamonsil C18 (2) (4.6 × 150 mm) column attached to a Gilson 322 pump coupled to a Gilson UV−vis-152 detector was used. Each sample was injected at a volume of 20 μL and eluted with methanol, and the flow rate was 1 mL/min. Melting points were determined with a micromelting point apparatus MP-500D and were uncorrected.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol(**8**).* Permethyl EGCG (500 mg, 1.09 mmol) was dissolved in methanol (10 mL) and dimethoxyethane (10 mL), then K_2CO_3 (243 mg, 1.76 mmol) was added. The mixture was stirred for 2 h until TLC showed the reaction had been completed. The solvent was removed under reduced pressure. The residue was dissolved with EtOAc (20 mL). Insoluble substance in the reaction mixture was removed by filtration. The filtrate was evaporated under reduced pressure and purified by flash chromatography on silica gel (EtOAc/PE = 1/3, v/v) to afford the desired compound **8** (300 mg, 90.0% yield): mp 157−159 °C; $[\alpha]^{20}_D$ = −57.6 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.74 (s, 2 H), 6.20 (d, J = 2.2 Hz, 1 H), 6.12 (d, J = 2.2 Hz, 1 H), 4.92 (bs, 1 H), 4.28 (bs, 1 H), 3.88 (s, 6 H), 3.85 (s, 3 H), 3.79 (s, 3 H), 3.77 (s, 3 H), 2.97 (A of ABX, J = 17.1, 1.2 Hz, 1 H), 2.90 (B of ABX, J = 17.1,4.3 Hz,1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 159.8, 159.3, 155.1, 153.5, 137.7, 134.1, 103.3, 103.4, 93.4, 92.3, 78.8, 66.5, 60.9, 56.3, 55.6, 55.5, 28.2; HRMS calcd for $(C_{20}H_{24}O_7 + H)^+$ 377.1600; found, 377.1588.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-methoxybenzoate (**9**).* A mixture of compound **8** (200 mg, 0.53 mmol), 3-methoxybenzoic acid (100 mg, 0.66 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 200 mg, 1.04 mmol), and 4-dimethylaminopyridine (DMAP, 130 mg, 1.06 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature overnight under a nitrogen atmosphere. Then the solution was washed by diluted hydrochloric acid two times, and brine in sequence. The organic layer was dried by anhydrous $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography to afford the title compound **9** (242 mg, 89.4% yield); mp 131−134 °C; $[\alpha]^{20}_D$ = −182.1 (c = 0.06, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.53 (d, J = 7.7 Hz, 1 H), 7.45 (d, J = 1.1 Hz, 1 H), 7.24 (d, J = 7.7 Hz, 1 H), 7.03 (m, 1 H), 6.69 (s, 2 H), 6.23 (d, J = 2.2 Hz, 1 H), 6.10 (d, J = 2.2 Hz, 1 H), 5.64 (bs, 1 H), 5.05 (bs, 1 H), 3.78 (m, 12 H), 3.69 (m, 6 H), 3.04 (d, J = 3.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.4, 159.7, 159.5, 158.9, 155.5, 153.2, 137.9, 133.4, 129.3, 122.0, 119.1, 114.7, 104.0, 100.2, 93.5, 92.0, 77.9, 68.7, 65.8, 60.7, 56.0, 55.4, 26.0; HRMS calcd for $(C_{28}H_{30}O_9 + H^+$ 511.1968; found, 511.1957.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-methoxybenzoate (**10**).* Following the procedure for the preparation of compound **9**, but with 4-methoxybenzoic acid as starting material, compound **10** was obtained. Yield 89.8%; mp 67−69 °C; $[\alpha]^{20}_D$ = −179.3 (c = 0.10, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.91 (d, J = 8.8 Hz, 2 H), 6.84 (d, J = 8.8 Hz, 2H), 6.70 (s, 2 H), 6.25 (d, J = 2.2 Hz, 1 H), 6.11 (d, J = 2.2 Hz, 1 H), 5.65 (bs, 1 H), 5.06 (bs, 1 H), 3.80 (m, 12 H), 3.71 (m, 6 H), 3.04 (d, J = 2.8 Hz, 2 H); ^{13}C NMR($CDCl_3$, 150 MHz) δ 165.3, 163.6, 159.7, 159.0, 155.6, 153.2, 137.8, 133.5, 131.9, 131.8, 122.5, 113.6, 104.0, 93.5, 92.0, 78.1, 68.2, 56.0, 56.0,55.5, 26.2; HRMS calcd for $(C_{28}H_{30}O_9 + H)^+$ 511.1968; found, 511.1954.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4-dimethoxybenzoate (**11**).* Following the procedure for the preparation of compound **9**, but with 3,4-dimethoxybenzoic acid as starting material, compound **11** was obtained. Yield 88.9%; mp 146−148 °C; $[\alpha]^{20}_D$ = −170.4 (c = 0.03, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.60 (dd, J = 1.9, 8.5 Hz, 1 H), 7.42 (d, J = 1.9 Hz, 1 H), 6.80 (d, J = 8.5 Hz, 1 H), 6.70 (s, 2 H), 6.24 (d, J = 2.2 Hz, 1 H), 6.11 (d, J = 2.2 Hz, 1 H), 5.66 (bs, 1 H), 5.06 (bs, 1 H), 3.88 (m, 15 H), 3.71 (s, 6 H), 3.04 (d, J = 3.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.3, 159.7, 158.9, 155.6, 153.2, 153.1, 148.7, 137.9, 133.4, 123.7, 122.6, 112.3, 110.2, 104.1, 100.3, 93.4, 91.9, 78.0, 68.3, 65.8, 60.8, 56.0, 55.4, 26.0; HRMS calcd for $(C_{29}H_{32}O_{10} + H)^+$ 541.2073; found, 541.2085.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4,5-triethoxybenzoate (**12**).* Following the procedure for the preparation of compound **9**, but with 3,4,5-triethoxybenzoic acid as starting material, compound **12** was obtained. Yield 88.5%; mp 47−51 °C; $[\alpha]^{20}_D$ = −129.8 (c = 0.05, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.13 (s, 2 H), 6.68 (s, 2 H), 6.23 (d, J = 1.1 Hz, 1 H), 6.10 (d, J = 1.1 Hz, 1 H), 5.62 (bs, 1 H), 5.06 (bs, 1 H), 4.06 (q, J = 7.1 Hz, 2 H), 4.00 (q, J = 7.1 Hz, 4 H), 3.78 (m, 9 H), 3.69 (s, 6 H), 3.02 (d, J = 2.8 Hz, 2 H), 1.38 (t, J = 7.1 Hz, 6 H), 1.31 (t, J = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.3, 159.8, 159.0, 155.6, 153.2, 152.7, 142.5, 137.9, 133.5, 124.9, 108.7, 108.7,104.0, 100.3, 93.3, 91.9, 77.9, 69.0, 68.6, 64.9, 56.0, 55.5, 26.0, 15.6, 14.9; HRMS calcd for $(C_{33}H_{40}O_{11} + H)^+$ 613.2649; found, 613.2661.

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*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxy phenyl)chroman-3-yl 3,4,5-tris(allyloxy)benzoate (**13**).* Following the procedure for the preparation of compound **9**, but with 3,4,5-tris(allyloxy)benzoic acid as starting material, compound **13** was obtained. Yield 87.9%; $[\alpha]^{20}_{D}$ = −138.2 (*c* = 0.10, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.17 (s, 2 H), 6.67 (s, 2 H), 6.24 (d, *J* = 2.8 Hz, 1 H), 6.12 (d, *J* = 2.8 Hz, 1 H), 6.03 (m, 1 H), 5.97 (m, 2 H), 5.65 (bs, 1H), 5.35 (A of ABX, *J* = 17.3, 1.3 Hz, 2 H), 5.28 (A of ABX, *J* = 17.3, 1.4 Hz, 1H), 5.24 (B of ABX, *J* = 10.5, 1.3 Hz, 2 H), 5.15 (B of ABX, *J* = 10.5, 1.4 Hz, 1H)., 5.06 (bs, 1 H), 4.57 (d, *J* = 6.0 Hz, 2 H), 4.52 (d, *J* = 5.3 Hz, 4 H), 3.79 (m, 9 H), 3.68 (s, 6 H), 3.03 (d, *J* = 2.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.9, 159.8, 158.9, 155.5, 153.2, 152.2, 142.1, 138.1, 134.1, 133.4, 133.0, 133.0, 125.0, 118.0, 117.9, 117.8, 109.2, 109.1, 104.1, 100.2, 93.4, 91.9, 77.8, 74.0, 70.0, 68.5, 60.7, 56.0, 55.4, 55.3, 26.0; HRMS calcd for $(C_{36}H_{40}O_{11} + H)^+$ 649.2649; found, 649.2637.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-fluorobenzoate(**14**).* Following the procedure for the preparation of **9**, but with 3-fluorobenzoic acid as starting material, compound **14** was obtained. Yield 89.5%; mp 59−62 °C; $[\alpha]^{20}_{D}$ = −22.7 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.74 (d, *J* = 7.7 Hz, 1 H), 7.63 (d, *J* = 9.3 Hz, 1 H), 7.35 (m, 1 H), 7.21 (m, 1 H), 6.70 (s, 2 H), 6.26 (d, *J* = 2.2 Hz, 1 H), 6.13 (d, *J* = 2.2 Hz, 1 H), 5.66 (bs, 1 H), 5.08 (bs, 1 H), 3.80 (m, 9 H), 3.74 (s, 6 H), 3.07 (d, *J* = 3.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ164.4, 163.3, 161.6, 159.8, 158.9, 155.5, 153.2, 138.0, 133.3, 130.0, 129.9, 125.5, 120.2, 120.0,116.6, 116.5, 103.8, 100.0, 93.6, 92.1, 77.8, 69.1, 60.8, 56.0, 55.4, 26.0; HRMS calcd for $(C_{27}H_{27}FO_8 + H)^+$ 499.1768; found, 499.1776.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-fluorobenzoate (**15**).* Following the procedure for the preparation of **9**, but with 4-fluorobenzoic acid as starting material, compound **15** was obtained. Yield 88.6%; mp 55−57 °C; $[\alpha]^{20}_{D}$ = −10.8 (*c* = 0.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.96 (m, 2 H), 7.03 (m, 2 H), 6.69 (s, 2 H), 6.25 (d, *J* = 1.9 Hz, 1H), 6.12 (d, *J* = 1.9 Hz, 1 H), 5.67 (bs, 1 H), 5.06 (bs, 1 H), 3.79 (m, 9 H), 3.71 (s, 6 H), 3.04 (d, *J* = 2.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.6, 164.9, 164.5, 159.7, 158.9, 155.5, 153.2, 137.9, 133.3, 132.2, 126.3, 115.5, 115.4, 100.1, 93.5, 92.0, 77.8, 68.7, 60.7, 55.9, 55.4, 26.1; HRMS calcd for $(C_{27}H_{27}FO_8 + H)^+$ 499.1768; found, 499.1779.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(dimethylamino)-4-fluorobenzoate (**16**).* Following the procedure for the preparation of **9**, but with 3-(dimethylamino)-4-fluorobenzoic acid as starting material, compound **16** was obtained. Yield 87.8%; mp 56−58 °C; $[\alpha]^{20}_{D}$ = −17.6 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.47 (m, 2 H), 6.96 (m, 1 H), 6.69 (s, 2 H), 6.25 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.65 (m, 1 H), 5.06 (bs, 1 H), 3.79 (m, 9 H), 3.71 (s, 6 H), 3.04 (d, *J* = 3.3 Hz, 2 H), 2.81 (s, 6 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.1, 159.8, 159.0, 158.8, 155.6, 153.2, 153.2, 140.9, 137.9, 133.5, 126.4, 122.9, 119.9, 115.9,103.9, 103.9, 100.2, 93.4, 92.0, 78.0, 68.6, 60.9, 56.0, 55.5, 42.6, 26.1; HRMS calcd for $(C_{29}H_{32}FNO_8 + H)^+$ 542.2190; found, 542.2178.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-acetamido-4-fluorobenzoate (**17**).* Following the procedure for the preparation of **9**, but with 3-acetamido-4-fluorobenzoic acid as starting material, compound **17** was obtained. Yield 86.9%; mp 93−96 °C; $[\alpha]^{20}_{D}$ = −106.8 (*c* = 0.04, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.84 (bs, 1 H), 7.67 (bs, 1 H), 7.33 (bs, 1 H), 7.06 (m, 1 H), 6.72 (s, 2 H), 6.27 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.64 (bs, 1 H), 5.07 (bs, 1 H), 3.80 (m, 9 H), 3.75 (s, 6 H), 3.05 (d, *J* = 3.3 Hz, 2 H), 2.19 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 168.6, 164.5, 159.7, 158.9, 156.5, 155.5, 153.1, 137.7, 133.4, 126.6, 126.4, 124.4, 114.9, 114.8, 103.9, 100.1, 93.6, 92.0, 77.7, 69.0, 60.7, 56.0, 55.3, 25.9, 24.0; HRMS calcd for $(C_{29}H_{30}FNO_9 + H)^+$ 556.1983; found, 556.1998.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 2-(4-methoxyphenyl)acetate (**18**).* Following the procedure for the preparation of **9**, but with 4-methoxyphenylacetic acid as starting material, compound **18** was obtained. Yield 88.5%; mp 46−48 °C; $[\alpha]^{20}_{D}$ = −63.7 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.89 (d, *J* = 8.4 Hz, 2 H), 6.70 (d, *J* = 8.4 Hz, 2 H),6.68 (s, 2 H), 6.24 (d, *J* = 2.0 Hz, 1H), 6.12 (d, *J* = 2.0 Hz, 1H), 5.49 (bs, 1H), 4.98 (bs, 1H), 3.85 (s, 3 H), 3.81 (s, 6 H), 3.78 (s, 3 H), 3.75 (s, 3 H), 3.72 (s, 3 H), 3.43 (A of AB, *J* = 15.0 Hz, 1H), 3.39 (B of AB, *J* = 15.0 Hz, 1H), 2.91 (d, *J* = 2.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 159.5, 158.7, 158.3, 155.1, 152.9, 137.5, 133.2, 129.7, 125.4, 113.6, 103.3, 99.8, 93.2, 91.7, 67.9, 60.5, 55.8, 55.1, 54.8, 40.0, 25.6; HRMS calcd for $(C_{29}H_{32}O_9 + Na)^+$ 517.1939; found, 517.1934.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 2-(3,4-dimethoxyphenyl)acetate (**19**).* Following the procedure for the preparation of **9**, but with 3,4-dimethoxyphenylacetic acid as starting material, compound **19** was obtained. Yield 88.4%; mp 51−53 °C; $[\alpha]^{20}_{D}$ = −56.2 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.66 (s, 2 H),6.65 (d, *J* = 8,2 Hz, 1H), 6.59 (d, *J* = 1.9 Hz, 1H), 6.51 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.21 (d, *J* = 2.3 Hz, 1H), 6.10 (d, *J* = 2.3 Hz, 1H), 5.50 (bs, 1H), 4.99 (bs, 1H), 3.83 (s, 3H), 3.81 (s, 6 H), 3.81 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 3.70 (s, 3H), 3.43 (A of AB, *J* = 15.2 Hz, 1 H), 3.39 (B of AB, *J* = 15.2 Hz, 1H), 2.93 (A of ABX, *J* = 17.9, 4.3 Hz, 1H), 2.88 (B of ABX, *J* = 17.9, 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 159.6, 158.8, 155.2, 153.0, 148.7, 147.9, 137.6, 133.2, 125.9, 121.1, 112.1, 110.9, 103.5, 99.9, 93.3, 91.9, 68.0, 60.7, 56.0, 55.7, 55.5, 55.3, 40.7, 25.8; HRMS calcd for $(C_{30}H_{34}O_{10} + Na)^+$ 577.2024; found, 577.2030.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 2-(3,4,5-trimethoxyphenyl)acetate (**20**).* Following the procedure for the preparation of **9**, but with 3,4,5-trimethoxyphenylacetic acid as starting material, compound **20** was obtained. Yield 89.7%; mp 56−58 °C; $[\alpha]^{20}_{D}$ = −54.3 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.68 (s, 2H), 6.29 (s, 2H), 6.21 (d, *J* = 2.2 Hz, 1H), 6.10 (d, *J* = 2.2 Hz, 1H), 5.52 (bs, 1H), 5.00 (bs, 1H), 3.83 (s, 9 H), 3.75 (m, 9 H), 3.70 (s, 6H), 3.43 (A of AB, *J* = 15.2 Hz, 1 H), 3.39 (B of AB, *J* = 15.2 Hz, 1H), 2.93 (A of ABX, *J* = 17.9, 4.5 Hz, 1H), 2.89 (B of ABX, *J* = 17.9, 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.7, 159.8, 158.9, 155.3, 153.2, 137.8, 137.0, 133.3, 129.1, 106.1, 103.7, 100.0, 93.4, 92.1, 68.2, 60.8, 56.2, 55.9, 55.4, 41.4, 26.0; HRMS calcd for $(C_{31}H_{36}O_{11} + Na)^+$ 607.2150; found, 607.2144.

*(E)-(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(4-methoxyphenyl)acrylate (**21**).* Following the procedure for the preparation of compound **9**, but with (*E*)-3-(4-methoxyphenyl)-acrylic acid as starting material, compound **21** was obtained. Yield 85.4%; mp77−81 °C; $[\alpha]^{20}_{D}$ = −186.2 (*c* = 0.05, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.55 (d, *J* = 16.0 Hz, 1 H), 7.39 (d, *J* = 8.5 Hz, 2 H), 6.86 (d, *J* = 8.5 Hz, 2 H), 6.73 (s, 2 H), 6.25 (d, *J* = 16.0 Hz, 1 H), 6.24 (bs, 1 H), 6.12 (bs, 1 H), 5.61 (bs, 1 H), 5.03 (bs, 1 H), 3.82 (m, 18 H), 2.99 (bs, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.5, 161.6, 159.7, 159.0, 155.5, 153.2, 145.1, 137.9, 133.4, 129.8, 127.0, 115.3, 114.4, 104.0, 100.4, 93.5, 92.1, 77.9, 67.6, 60.9, 56.2, 55.5, 55.4, 26.2; HRMS calcd for $(C_{30}H_{32}O_9 + H)^+$ 537.2124; found, 537.2113.

*(E)-(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman −3-yl 3-(3,4-dimethoxyphenyl)acrylate (**22**).* Following the procedure for the preparation of compound **9**, but with (*E*)-3-(3,4-dimethoxyphenyl)-acrylic acid as starting material, compound **22** was obtained.Yield 87.5%: mp 76−79 °C; $[\alpha]^{20}_{D}$ = −175.0 (*c* = 0.03, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.53 (d, *J* = 15.9 Hz, 1 H), 7.01 (d, *J* = 8.2 Hz, 1 H), 6.95 (s, 1 H), 6.81 (d, *J* = 8.2 Hz, 1 H), 6.72 (s, 2 H), 6.25 (d, *J* = 15.9 Hz, 1 H), 6.24 (d, *J* = 1.7 Hz, 1 H), 6.12 (d, *J* = 1.7 Hz, 1 H), 5.63 (bs, 1 H), 5.03 (bs, 1 H), 3.82 (m, 21 H), 2.99 (bs, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.3, 159.6, 158.9, 155.3, 153.1, 151.2, 149.1, 145.2, 137.8, 133.2, 127.1, 122.7, 115.4, 110.9, 109.3, 103.8, 100.3, 93.3, 92.0, 77.8, 67.4, 60.8, 56.1, 55.9, 55.8, 55.4, 55.3, 26.1; HRMS calcd for $(C_{31}H_{34}O_{10} + H)^+$ 567.2230; found, 567.2241.

*(E)-(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4,5-trimethoxyphenyl)acrylate (**23**).* Following the procedure for the preparation of compound **9**, but with (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid as starting material, compound **23** was obtained. Yield 86.5%; mp111−113 °C; $[\alpha]^{20}_{D}$ = −177.8 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.50 (d, *J* = 16.0 Hz, 1 H), 6.72 (s, 2 H), 6.66 (s, 2 H), 6.28 (d, *J* = 16.0 Hz, 1 H), 6.25 (d, *J* = 2.2 Hz, 1H), 6.14 (d, *J* = 2.2 Hz, 1H), 5.66 (bs, 1 H), 5.05 (bs, 1 H), 3.84 (m, 18 H), 3.83 (s, 6 H), 3.00 (bs, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.2, 159.8, 159.1, 155.4, 153.5, 153.3, 145.4, 140.3, 137.8, 133.3, 129.8, 117.1, 105.3, 104.0, 100.4, 93.5, 92.1, 77.8, 67.6, 61.0, 60.9, 56.2, 55.5, 26.2; HRMS calcd for $(C_{32}H_{36}O_{11} + H)^+$ 597.2336; found, 597.2339.

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*(E)-(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl3-(4-methoxyphenyl)propionate (**24**).* Compound **21** (300 mg, 0.56 mmol) was dissolved in CH_3OH (20 mL) and carefully added to 10% palladium on carbon. Hydrogen was introduced to the flask, and the mixture was allowed to stir under a positive hydrogen atmosphere for 12 h. The catalyst was filtered from the reaction mixture, and the filtrate was evaporated to dryness to give the title compound **24** (236 mg, 78.5% yield): mp 52−54 °C; $[\alpha]^{20}_D$ = −72.3 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz)*δ* 6.90 (d, *J* = 8.4 Hz, 2 H), 6.72 (d, *J* = 8.4 Hz, 2 H), 6.71 (s, 2 H), 6.23 (d, *J* = 2.0 Hz, 1 H), 6.12 (d, *J* = 2.0 Hz, 1H), 5.46 (bs, 1H), 4.98 (bs, 1H), 3.85 (s, 6 H), 3.84 (s, 3 H), 3.77 (s, 6 H), 3.73 (s, 3 H), 2.96 (A of ABX, *J* = 17.8, 4.5 Hz, 1H), 2.88 (B of ABX, *J* = 17.8, 1.8 Hz, 1H), 2.67 (m, 2 H), 2.42 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz)*δ* 172.0, 159.6, 158.8, 157.9, 155.2, 153.1, 137.6, 133.4, 132.1, 128.9, 113.7, 103.5, 100.0, 93.3, 91.9, 67.8, 60.7, 56.1, 55.2, 55.0, 36.0, 29.8, 25.8; HRMS calcd for $(C_{30}H_{34}O_9 + Na)^+$ 561.2095; found, 561.2089.

*(E)-(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl3-(3,4-dimethoxyphenyl)propionate (**25**).* Following the procedure for the preparation of compound **24**, but with compound **22** as starting material, compound **25** was obtained. Yield 79.3%; mp 85−87 °C; $[\alpha]^{20}_D$ = −71.2 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* 6.70 (s, 2 H), 6.68 (d, *J* = 8.2 Hz, 1 H), 6.58 (d, *J* = 1.6 Hz, 1 H), 6.52 (dd, *J* = 8.2, 1.6 Hz, 1 H), 6.21 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.47 (bs, 1 H), 4.99 (bs, 1 H), 3.85 (s, 6 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 6 H), 2.95 (A of ABX, *J* = 17.9, 4.7 Hz, 1H), 2.87 (B of ABX, *J* = 17.9, 1.7 Hz, 1H), 2.72 (m, 2 H), 2.52 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* 172.2, 159.7, 158.9, 155.3, 153.2, 148.8, 147.4, 137.7, 133.4, 132.8, 119.9, 111.4, 111.2, 103.6, 100.1, 93.4, 92.1, 67.9, 60.9, 56.2, 55.9, 55.5, 55.4, 36.0, 30.3, 26.0; HRMS calcd for $(C_{31}H_{36}O_{10} + H)^+$ 569.2381; found, 569.2388; $(C_{31}H_{36}O_{10}+Na)^+$ 591.2201; found, 591.2206.

*(E)-(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl3-(3,4,5-trimethoxyphenyl)propionate (**26**).* Following the procedure for the preparation of compound **24**, but with compound **23**as starting material, compound **26** was obtained. Yield 78.2%; mp 52−54 °C; $[\alpha]^{20}_D$ = −64.6 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) *δ*: 6.69 (s, 2 H), 6.26 (s, 2 H), 6.19 (d, *J* = 2.2 Hz, 1 H), 6.09 (d, *J* = 2.2 Hz, 1 H), 5.48 (bs, 1 H), 4.98 (bs, 1 H), 3.82 (m, 24 H), 2.96 (A of ABX, *J* = 17.9, 4.7 Hz, 1H), 2.87 (B of ABX, *J* = 17.9, 1.7 Hz, 1H), 2.69 (m, 2 H), 2.51 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* 172.1, 159.7, 158.9, 155.3, 153.2, 137.7, 136.3, 136.0, 133.4, 104.9, 103.6, 100.0, 93.4, 92.0, 68.0, 60.9, 56.2, 56.0, 55.5, 35.8, 31.1, 26.0; HRMS calcd for $(C_{32}H_{38}O_{11} + Na)^+$ 621.2306; found, 621.2300.

*(E)-3-(3-(3,4-Dimethoxyphenyl)acrylamido)-4-fluorobenzoic Acid (**28**).* (*E*)-3-(3,4-Dimethoxyphenyl)acrylic acid (300 mg, 1.4 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). The mixture was cooled to 0 °C under a nitrogen atmosphere. One drop of DMF and oxalyl chloride (330 mg, 2.6 mmol) was added dropwise to the reaction mixture. When the addition was completed, the ice bath was removed, and the reaction mixture was stirred at room temperature for 8 h. The solvent was evaporated to give a yellow solid, and the yellow solid was dissolved in dry CH_2Cl_2. A solution of methyl 3-amino-4-fluorobenzoate (compound **27**, 210 mg, 1.24 mmol) and DMAP (310 mg, 2.5 mmol)) in anhydrous CH_2Cl_2 (5 mL) was then added dropwise to the reaction mixture. The reaction mixture was stirred at room temperature for 12 h, and the solvent was evaporated to give a yellow solid. The yellow solid obtained above was dissolved in methanol (30 mL) and water (1 mL), then $LiOH{\cdot}H_2O$ (500 mg, 12.0 mmol) was added. The solution was stirred for 10 h at room temperature, and the solvent was evaporated. Water (30 mL) was added into the mixture and acidified with dilute hydrochloric acid until the pH test paper showed that it is neutral; a copious amount white solid was precipitated. The product was isolated by filtration, dried in vacuo to afford compound **28** (300 mg, 68.9% yield). mp 198−200 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) *δ* 9.97 (s, 1 H), 8.81 (d, *J* = 7.6 Hz, 1 H), 7.73 (m, 1 H), 7.57 (d, *J* = 15.4 Hz, 1 H), 7.40 (m, 1 H), 7.23 (d, *J* = 2.1 Hz, 1 H), 7.20 (d, *J* = 8.2 Hz, 1 H), 7.02 (d, *J* = 8.2 Hz, 1 H), 6.99 (d, *J* = 15.4 Hz, 1 H), 3.82 (s, 3 H), 3.80 (s, 3 H); ^{13}C NMR (DMSO-d_6, 150 MHz) *δ* 167.0, 164.9, 151.1, 149.4, 141.7, 127.9, 127.8, 127.3, 126.6, 124.8, 122.5, 119.7, 116.2, 116.1, 112.2, 110.5, 56.1, 55.9; HRMS calcd for $(C_{18}H_{16}O_5NF + H)^+$ 346.1085; found, 346.1082.

*(E)-4-Fluoro-3-(3-(3,4,5-trimethoxyphenyl)acrylamido)benzoic Acid (**29**).* Following the procedure for the preparation of compound **28**, but with 3-((*E*)-3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoic acid as starting material, compound **29** was obtained.Yield 73.6%. mp 186−188 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) *δ* 8.82 (d, *J* = 7.2 Hz, 1 H), 7.76 (d, *J* = 2.7 Hz, 1 H), 7.60 (d, *J* = 15.4 Hz, 1 H), 7.44 (m, 1 H), 7.14 (d, *J* = 15.4 Hz, 1 H), 7.01 (s, 3 H), 3.87 (s, 6 H), 3.73 (s, 3 H); ^{13}C NMR (DMSO-d_6, 150 MHz) *δ* 167.0, 164.7, 157.0, 155.4, 153.6, 141.7, 139.6, 130.8, 127.8, 127.3, 126.8, 124.9, 121.6, 116.3, 116.2, 105.8, 60.7, 56.4; HRMS calcd for $(C_{19}H_{18}O_6NF + H)^+$ 376.1191; found, 376.1185.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-((E)-3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoate (**30**).* Following the procedure for the preparation of compound **9**, but with 3-((*E*)-3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoic acid as starting material, compound **30** was obtained. Yield 85.8%; mp 93−95 °C; $[\alpha]^{20}_D$ = −159.6 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* 7.68 (d, *J* = 15.3 Hz, 1H), 7.66 (s, 1 H), 7.46 (d, *J* = 2.7 Hz, 1 H), 7.12 (dd, *J* = 8.2, 2.2 Hz, 1 H), 7.07 (m, 2 H), 6.87 (d, *J* = 8.2 Hz, 1 H), 6.74 (s, 2 H), 6.43 (d, *J* = 15.3 Hz, 1 H), 6.28 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.65 (t, *J* = 2.7 Hz, 1 H), 5.08 (s, 1 H), 3.91 (d, *J* = 2.2 Hz, 6 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.77 (s, 3 H), 3.76 (s, 6 H), 3.06 (d, *J* = 3.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz)*δ* 164.6, 163.9, 159.7, 158.9, 155.5, 153.2, 151.7, 149.2, 143.3, 143.2, 137.7, 133.4, 127.3, 126.9, 122.6, 122.5, 111.2, 111.1, 109.8, 109.7, 103.8, 103.7, 100.2, 69.1, 69.0, 60.9, 60.8, 56.1, 56.0, 55.9, 55.5, 55.4, 26.1; HRMS calcd for $(C_{38}H_{38}O_{11}NF + H)^+$ 704.2502; found, 704.2504.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-((E)-3-(3,4,5-trimethoxyphenyl)acrylamido)-4-fluorobenzoate (**31**).* Following the procedure for the preparation of compound **9**, but with 3-((*E*)-3-(3,4,5-trimethoxyphenyl) acrylamido)-4-fluorobenzoic acid as starting material, compound **31** was obtained. Yield 85.2%; mp 96−98 °C; $[\alpha]^{20}_D$= −141.0 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* 7.69 (m, 1 H), 7.66 (d, *J* = 15.4 Hz, 1 H), 7.54 (d, *J* = 2.7 Hz, 1 H), 7.07 (m, 1 H), 6.76 (s, 2 H), 6.74 (s, 2 H), 6.49 (d, *J* = 15.4 Hz, 1 H), 6.28 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.64 (bs, 1 H), 5.08 (bs, 1 H), 3.87 (s, 9 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 6 H), 3.06 (d, *J* = 3.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ*164.5, 163.7, 159.7, 158.9, 155.5, 153.5, 153.2, 143.3, 140.1, 137.7, 133.4, 129.9, 126.9, 126.8, 126.7, 126.3, 123.8, 119.3, 114.9, 114.7, 105.3, 103.8, 100.2, 93.6, 92.0, 69.1, 61.0, 60.8, 56.2, 56.1, 55.5, 42.0, 26.1; HRMS calcd for $(C_{39}H_{40}O_{12}NF + Na)^+$ 756.2427; found, 756.2427.

*3-(3,4-Dimethoxybenzamido)-4-fluorobenzoic Acid (**33**).* A mixture of methyl 3-amino-4-fluorobenzoate (150 mg, 0.89 mmol), 3,4-dimethoxybenzoic acid (200 mg, 1.07 mmol), 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 400 mg, 2.12 mmol), and 4-dimethylaminopyridine (DMAP, 260 mg, 2.12 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature overnight under a nitrogen atmosphere. Then, the solution was washed by diluted in hydrochloric acid two times and in brine in sequence. The organic layer was dried by anhydrous $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography to give a yellow solid. The yellow solid obtained above was dissolved in methanol (30 mL) and water (1 mL), then $LiOH{\cdot}H_2O$ (500 mg, 12.0 mmol) was added. The solution was stirred for 10 h at room temperature, then the solvent was evaporated. Water (20 mL) was added into the mixture and acidified with dilute hydrochloric acid until the pH test paper showed that it is neutral, and a copious amount of white solid was precipitated. The product was isolated by filtration and dried in vacuo to afford compound **33** (200 mg, 71.2% yield); mp 169−171 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) *δ* 10.16 (s, 1 H), 8.24 (d, *J* = 6.6 Hz, 1 H), 7.89 (m, 1 H), 7.69 (d, *J* = 6.6 Hz, 1 H), 7.62 (s, 1H), 7.48 (m, 1 H), 7.14 (d, *J* = 9.1 Hz, 1H), 3.88 (s, 6 H); ^{13}C NMR (DMSO-d_6, 150 MHz) *δ* 166.8, 165.4, 159.8, 1525, 148.9, 128.7,

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128.6, 127.7, 126.7, 126.2, 121.9, 116.8, 116.7, 111.5, 56.2, 56.1; HRMS calcd for $(C_{16}H_{14}O_5NF + H)^+$ 320.0929; found, 320.0924.

*3-(3,4,5-Trimethoxybenzamido)-4-fluorobenzoic acid (**34**).* Following the procedure for the preparation of compound **33**, but with 3,4,5-trimethoxybenzoic acids material, compound **34** was obtained. Yield 72.6%; mp 213−215 °C; 1H NMR (DMSO-d_6, 600 MHz) δ 10.26 (s, 1 H), 8.21 (dd, J = 2.2, 8.3 Hz, 1 H), 7.90 (m, 1H), 7.49 (m, 1 H), 7.37 (s, 2 H), 3.94 (s, 6 H), 3.78 (s, 3 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 166.8, 165.3, 159.8, 158.1, 153.2, 141.3, 129.2, 128.8, 127.9, 126.5, 116.9, 116.8, 105.9, 105.9, 60.7, 56.6; HRMS calcd for $(C_{17}H_{16}O_6NF + H)^+$ 350.1034; found, 350.1028.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4-dimethoxybenzamido)-4-fluorobenzoate (**35**).* Following the procedure for the preparation of compound **9**, but with3-(3,4-dimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **35** was obtained. Yield 85.8%; mp 103−105 °C; $[\alpha]^{20}_D$= −168.1 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.96 (d, J = 6.1 Hz, 1 H), 7.94 (d, J = 2.8 Hz, 1 H), 7.71 (m, 1 H), 7.47 (d, J = 1.7 Hz, 1 H), 7.37 (m, 1 H), 7.09 (m, 1 H), 6.91 (d, J = 8.3 Hz, 1 H), 6.74 (s, 2 H), 6.28 (d, J = 2.2 Hz, 1 H), 6.11 (d, J = 2.2 Hz, 1 H), 5.66 (bs, 1 H), 5.08 (bs, 1 H), 3.95 (m, 6 H), 3.79 (m, 15 H), 3.07 (d, J = 3.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.9, 164.6, 159.7, 158.9, 155.6, 154.9, 153.2, 152.5, 149.4, 137.7, 133.4, 127.0, 126.7, 126.5, 124.0, 119.7, 115.0, 114.8, 110.8, 110.5, 103.8, 100.2, 93.6, 92.1, 77.9, 69.1, 60.9, 56.2, 56.1, 26.1; HRMS calcd for $(C_{36}H_{36}FNO_{11} + H)^+$ 678.2350; found, 678.2364.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4,5- trimethoxybenzamido)-4-fluorobenzoate (**36**).* Following the procedure for the preparation of compound **9**, but with 3-(3,4,5-trimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **36** was obtained. Yield 83.8%; mp 98−100 °C; $[\alpha]^{20}_D$ = −172.1 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.89 (d, J = 7.2 Hz, 1 H), 7.93 (s, 1 H), 7.73 (m, 1 H), 7.10 (m, 1 H), 7.06 (s, 2 H),6.74 (s, 2 H), 6.27 (s, 1 H), 6.11 (s, 1 H), 5.66 (bs, 1 H), 5.08 (bs, 1 H), 3.91 (m, 9 H), 3.79 (m, 15 H), 3.06 (bs, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.1, 164.5, 159.7, 158.9, 155.6, 155.0, 153.5, 153.2, 141.7, 137.7, 133.4, 129.6, 127.0, 126.8, 124.2, 115.7, 114.9, 104.9, 104.6, 104.6, 103.8, 103.8, 100.2, 93.6, 92.1, 77.9, 69.1, 61.1, 60.9, 56.5, 56.1, 55.5, 26.1; HRMS calcd for $(C_{37}H_{38}FNO_{12} + H)^+$ 708.2456; found, 708.2468.

*4-Fluoro-3-(4-fluoro-3-(4-methoxybenzamido)benzamido)-benzoic Acid (**37**).* Following the procedure for the preparation of compound **33**, but with 3-(4-methoxybenzamido)-4-fluorobenzoic acid (compound **32**) as starting material, compound **37** was obtained. Yield 78.6%; mp 238−240 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.34 (s, 1H), 10.16 (s, 1H), 8.22 (m, 2H), 8.00 (d, J = 8.8 Hz, 1H), 7.92 (m, 1H), 7.85 (m, 1H), 7.48 (dd, J = 9.8, 8.8 Hz, 1H), 7.45 (dd, J = 9.8, 8.8 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 3.85 (s, 3H);^{13}C NMR (DMSO-d_6, 125 MHz) δ 166.4, 165.0, 164.4, 162.3, 159.3, 159.1, 157.3, 157.0, 130.0, 129.8, 127.2, 126.7, 126.2, 126.1, 125.9, 125.7, 116.2, 113.8, 55.5.HRMS calcd for $(C_{22}H_{16}O_5F_2N_2 - H)^+$ 425.0944; found, 425.0955.

*3-(3-(3,4-Dimethoxybenzamido)-4-fluorobenzamido)-4-fluoro-benzoic Acid (**38**).* Following the procedure for the preparation of compound **33**, but with 3-(3,4-dimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **38** was obtained. Yield 81.4%; mp 246−248 °C; ^{1}H NMR (, DMSO-d_6, 600 MHz) δ 10.33 (s, 1H), 10.19 (s, 1H), 8.22 (dd, J = 7.3, 2.0 Hz, 1H), 8.18 (d, J = 5.9 Hz, 1H), 7.91 (m, 1H), 7.84 (s, 1H), 7.66 (dd, J = 8.3, 2.1 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.46 (m, 1H), 7.38 (m, 1H), 7.11 (d, J = 8.6 Hz, 1H), 3.84 (s, 6H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 165.1, 164.4, 159.2, 157.2, 152.0, 148.5, 130.1, 128.2, 127.4, 126.2, 125.7, 121.5, 116.2, 111.1, 111.0, 55.8, 55.7. HRMS calcd for $(C_{23}H_{18}O_6F_2N_2 + Na)^+$ 479.1025; found, 479.1017.

*3-(3-(3,4,5-Trimethoxybenzamido)-4-fluorobenzamido)-4-fluoro-benzoic Acid (**39**).* Following the procedure for the preparation of compound **33**, but with 3-(3,4,5-trimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **39** was obtained. Yield 81.8%; mp 250−252 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.40 (s, 1H), 10.30 (s, 1H), 8.25 (m, 2H), 8.00−7.95 (m, 1H), 7.86 (m, 1H), 7.51 (m, 1H), 7.43 (m, 1H), 7.38 (s, 2H), 3.88 (s, 6H), 3.75 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 166.4, 165.0, 164.4, 159.4, 159.2, 157.4, 157.2, 152.8, 140.8, 130.1, 128.7, 128.2, 127.5, 127.4, 126.1, 126.0, 116.3, 105.5, 60.2, 56.2; HRMS calcd for $(C_{24}H_{20}O_7F_2N_2 + H)^+$ 487.1311; found, 487.1305.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3-(4-methoxybenzamido)-4-fluorobenzamido)-4-fluorobenzoate (**40**).* Following the procedure for the preparation of compound **9**, but with compound **37** as starting material, compound **40** was obtained. Yield 84.4%; mp 116−118 °C; $[\alpha]^{20}_D$ = −174.9 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.89 (d, J = 7.1 Hz, 1H), 8.84 (d, J = 7.4 Hz, 1H), 8.28 (s, 1H), 8.13 (s, 1H), 7.83 (d, J = 8.7 Hz, 2 H), 7.71 (m, 1H), 7.68−7.63 (m, 1H), 7.16 (m, 1H), 7.10−7.07 (m, 1H), 6.95 (d, J = 8.7 Hz, 2H), 6.74 (s, 2H), 6.27 (d, J = 2.1 Hz, 1H), 6.09 (d, J = 2.1 Hz, 1H), 5.65 (s, 1H), 5.07 (s, 1H), 3.84 (s, 3 H), 3.76 (m, 15H), 3.06 (s, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.3, 164.6, 164.4, 163.2, 159.8, 159.0, 155.6, 153.3, 137.9, 133.5, 131.0, 129.2, 127.1, 126.6, 126.2, 124.7, 120.0, 115.8, 115.6, 115.3, 115.1, 114.3, 103.9, 100.3, 93.7, 92.2, 78.0, 69.2, 60.9,58.6, 56.2, 55.7, 55.5,26.2, 18.6; HRMS (ESI) calcd for $(C_{42}H_{38}F_2N_2O_{11} + Na)^+$ 807.2336; found, 807.2339.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3-(3,4-dimethoxybenzamido)-4-fluorobenzamido)-4-fluoroben-zoate (**41**).* Following the procedure for the preparation of compound **9**, but with compound **38** as starting material, compound **41** was obtained. Yield 83.7%; mp 122−124 °C; $[\alpha]^{20}_D$ = −188.7 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.96 (d, J = 6.7 Hz, 1H), 8.88 (d, J = 7.2 Hz, 1H), 8.13 (s, 2H), 7.66 (m, 2H), 7.50 (s, 1H), 7.42 (d, J = 8.2 Hz, 1H), 7.22 (d, J = 9.1 Hz, 1H), 7.10 (m, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.74 (s, 2H), 6.28 (s, 1H), 6.10 (s, 1H), 5.66 (bs, 1H), 5.08 (bs, 1H), 3.96 (s, 3 H), 3.94 (s, 3H), 3.79 (s, 6 H), 3.77 (s, 9 H), 3.06 (bs, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.3, 164.6, 164.4, 159.8, 159.0, 155.6, 153.3, 152.8, 149.5, 137.9, 133.5, 131.0, 127.1, 126.5, 124.6, 120.1, 119.8, 115.8, 115.6, 115.2, 115.1, 110.9, 110.6, 103.9, 100.2, 93.7, 92.2, 78.0, 69.2, 60.9, 56.2, 55.5, 26.2; HRMS calcd for $(C_{43}H_{40}F_2N_2O_{12} + Na)^+$ 837.2442; found, 837.2436.

*(2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3-(3,4,5-trimethoxybenzamido)-4-fluorobenzamido)-4-fluoro-benzoate (**42**).* Following the procedure for the preparation of compound **9**, but with compound **39** as starting material, compound **42** was obtained. Yield 84.9%; mp 125−127 °C; $[\alpha]^{20}_D$ = −116.6 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.77 (dd, J = 7.4, 1.9 Hz, 1H), 8.71 (d, J = 7.0 Hz, 1H), 8.32 (s, 1H), 8.26 (s, 1H), 7.70 (m, 1H), 7.60−7.58 (m, 1H), 7.26 (s, 1H), 7.11 (d, J = 9.9 Hz, 1H), 7.09 (s, 2H), 7.02 (m, 1H), 6.72 (s, 2H), 6.24 (d, J = 2.2 Hz, 1H), 6.07 (d, J = 2.3 Hz, 1H), 5.62 (bs, 1H), 5.06 (d, J = 17.7 Hz, 1H), 3.87 (s, 3H), 3.86 (s, 6H), 3.76 (s, 3 H), 3.74 (s, 3 H), 3.73 (s, 3 H), 3.72 (s, 6 H), 3.03 (bs, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.5, 164.5, 164.4, 159.7, 158.9, 157.2, 156.1, 155.5, 154.4, 153.3, 153.1, 141.6, 137.7, 133.4, 130.7, 129.1, 127.1, 126.7, 126.6, 126.3, 124.8, 121.2, 115.7, 115.1, 104.7, 103.8, 100.1, 93.6, 92.0, 77.8, 69.1, 60.9, 60.8, 56.3, 56.0, 55.4, 26.0; HRMS calcd for $(C_{44}H_{42}F_2N_2O_{13} + Na)^+$ 867.2547; found, 867.2548.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4,5-trimethoxybenzoate (**43**).* Compound **8** (300.mg, 0.80 mmol), 3,4,5-trimethoxybenzoic acid (200 mg, 0.94 mmol), and Ph_3P (840 mg, 3.20 mmol) were dissolved in anhydrous THF (20 mL), then DIAD (650 mg, 3.20 mmol) was added at 0 °C. The mixture was stirred at room temperature for 6 h under a nitrogen atmosphere. The solvent was evaporated, and the residue was dissolved in EtOAc (50 mL), then the mixture was washed by diluted hydrochloric acid two times and in brine in sequence. The organic layer was dried with anhydrous $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography to afford the title compound **43** (240 mg, 52.2% yield); mp 61−63 °C; $[\alpha]^{20}_D$ = +49.5 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ7.10 (s, 1H), 6.65 (s, 1H), 6.19 (d, J = 2.3 Hz, 1H), 6.11 (d, J = 2.2 Hz, 1H), 5.49 (d, J = 7.4 Hz, 1H), 5.09 (d, J = 7.4 Hz, 1H), 3.86 (s, 3 H), 3.82 (s, 6 H), 3.81−3.74 (m, 15 H), 3.14 (A of ABX, J = 16.5, 5.4 Hz, 1H), 2.80 (B of ABX, J = 16.5, 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.3, 160.0, 158.7, 155.0, 153.4, 153.0, 142.5, 138.1, 133.4, 125.1, 107.0, 104.1, 101.0, 100.0, 93.1, 92.0, 79.1,

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70.3, 60.9, 56.3, 55.5, 25.0; HRMS calcd for $(C_{30}H_{34}O_{11} + H)^+$ 571.2174; found, 571.2161.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol (**44**).* Following the procedure for the preparation of compound **9**, but with compound **43**[58] as starting material, compound **44** was obtained. Yield 87.1%; mp 152−154 °C; $[\alpha]^{20}_D = -17.4$ (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.67 (s, 2 H), 6.14 (d, J = 2.1 Hz, 1H), 6.11 (d, J = 2.1 Hz, 1H), 4.62 (d, J = 8.5 Hz, 1H), 4.16−3.98 (m, 1H), 3.86 (s, 6 H), 3.84 (s, 3 H), 3.81 (s, 3 H), 3.75 (s, 3 H), 3.09 (A of ABX, J = 16.2, 5.8 Hz, 1H), 2.59 (B of ABX, J = 16.2, 9.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 159.8, 158.8, 155.3, 153.6, 138.2, 133.6, 104.2, 101.8, 93.1, 92.1, 82.3, 68.4, 60.9, 56.2, 55.6, 55.5, 27.9; HRMS calcd for $(C_{20}H_{24}O_7 + H)^+$ 377.1595; found, 377.1588.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-methoxybenzoate (**45**).* Compound **44** (400 mg, 1.06 mmol), 4-methoxybenzoic acid (0.20 g, 1.31 mmol), 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl,400 mg, 2.12 mmol), and 4-dimethylaminopyridine (DMAP, 260 mg, 2.12 mmol) were dissolved in anhydrous CH_2Cl_2 (30 mL), then the mixture was stirred at room temperature overnight under a nitrogen atmosphere. The solution was washed in diluted hydrochloric acid two times and in brine in sequence. The organic layer was dried in anhydrous $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography to afford the title compound **45** (480 mg, 89.5% yield); mp 69−71 °C; $[\alpha]^{20}_D = +25.4$ (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.88 (d, J = 8.8 Hz, 2 H), 6.86 (d, J = 8.8 Hz, 2 H), 6.65 (s, 2 H), 6.21 (d, J = 2.2 Hz, 1H), 6.12 (d, J = 2.2 Hz, 1H), 5.51 (dd, J = 12.6, 7.0 Hz, 1H), 5.13 (d, J = 7.0 Hz, 1H), 3.83 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 12 H), 3.07 (A of ABX, J = 16.7, 5.5 Hz, 1H), 2.79 (B of ABX, J = 16.7, 7.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.3, 163.5, 159.8, 158.6, 154.8, 153.2, 137.8, 133.5, 131.6 131.0, 122.3, 113.6, 103.7, 100.9, 93.0, 91.9, 78.8, 69.6, 60.8, 56.1, 55.3, 24.5, 21.4; HRMS calcd for $(C_{28}H_{30}O_9 + H)^+$ 511.1963; found, 511.1948.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4-dimethoxybenzoate (**46**).* Following the procedure for the preparation of compound **45**, but with 3,4-dimethoxybenzoic acid as starting material, compound **46** was obtained. Yield 87.4%; mp 70−72 °C; $[\alpha]^{20}_D$=+59.1 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz)δ 7.54 (dd, J = 8.4, 2.0 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.65 (s, 2H), 6.21 (d, J = 2.3 Hz, 1H), 6.12 (d, J = 2.3 Hz, 1H), 5.51 (m, 1H), 5.12 (d, J = 7.1 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.77 (m, 15H), 3.10 (A of ABX, J = 16.6, 5.5 Hz, 1H), 2.80 (B of ABX, J = 16.6, 7.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.5, 159.9, 158.7, 154.9, 153.3, 153.2, 148.7, 137.9, 133.6, 123.7, 122.5, 112.1, 110.2, 103.8, 101.0, 93.1, 92.0, 78.9, 69.9, 60.9, 56.2, 55.5, 24.7; HRMS calcd for $(C_{29}H_{32}O_{10} + H)^+$ 541.2068; found, 541.2055.

*(E)-(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl3-(4-methoxyphenyl)acrylate (**47**).* Following the procedure for the preparation of compound **45**, but with (*E*)-3-(4-methoxyphenyl)-acrylic acid as starting material, compound **47** was obtained. Yield 84.3%; mp 66−68 °C; $[\alpha]^{20}_D$= +36.1 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz)δ 7.57 (d, J = 16.0 Hz, 1H), 7.42 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 6.63 (s, 1H), 6.23 (d, J = 16.0 Hz, 1 H),6.21 (d, J = 2.3 Hz, 1H), 6.11 (d, J = 2.3 Hz, 1H), 5.48 (m, 1H), 5.13 (d, J = 6.5 Hz, 1H), 3.81 (s, 12 H), 3.78 (s, 6 H), 2.96 (A of ABX, J = 16.8, 5.3 Hz, 1H), 2.76 (B of ABX, J = 16.8, 6.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.5, 161.6, 159.9, 158.7, 154.8, 153.3, 145.1, 137.8, 133.6, 132.3, 129.9, 127.0, 115.2, 114.4, 113.5, 103.7, 100.8, 93.0, 91.9, 78.6, 69.0, 60.9, 56.2, 55.5, 24.0; HRMS calcd for $(C_{30}H_{32}O_9 + H)^+$ 537.2117; found, 537.2107.

*(E)-(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman −3-yl 3-(3,4-dimethoxyphenyl)acrylate (**48**).* Following the procedure for the preparation of compound **45**, but with (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid as starting material, compound **48** was obtained. Yield 85.1%; mp 73−76 °C; $[\alpha]^{20}_D$ = +37.1 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.55 (d, J = 15.9 Hz, 1H), 7.04 (dd, J = 8.4, 1.9 Hz, 1H), 6.99 (d, J = 1.9 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.62 (s, 2H), 6.24 (d, J = 15.9 Hz, 1H), 6.21 (d, J = 2.2 Hz, 1H), 6.10 (d, J = 2.2 Hz, 1H), 5.50 (m, 1H), 5.15 (d, J = 6.2 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.80 (m, 15 H), 2.92 (A of ABX, J = 16.8, 5.3 Hz, 1H), 2.76 (B of ABX, J = 16.8, 6.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.4, 159.9, 158.7, 154.7, 153.3, 151.3, 149.2, 145.3, 137.8, 133.6, 127.2, 122.9, 115.4, 111.0, 109.5, 103.6, 100.8, 93.0, 91.9, 78.5, 69.0, 60.9, 56.2, 56.0, 55.5, 23.8; HRMS calcd for $(C_{23}H_{34}O_{10} + H)^+$567.2225; found, 567.2211.

*(E)-(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4,5-trimethoxyphenyl)acrylate (**49**).* Following the procedure for the preparation of compound **45**, but with (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid as starting material, compound **49** was obtained. Yield 84.7%; mp 78−80 °C; $[\alpha]^{20}_D$ = +30.7 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.52 (d, J = 15.9 Hz, 1H), 6.70 (s, 2H), 6.62 (s, 2H), 6.28 (d, J = 15.9, 1H), 6.22 (s, 1H), 6.11 (s, 1H), 5.51 (m, 1H), 5.15 (d, J = 6.1 Hz, 1H), 3.87 (s, 9H), 3.81 (s, 9H), 3.78 (s, 6 H), 2.92 (A of ABX, J = 16.9, 5.1 Hz, 1H), 2.77 (B of ABX, J = 16.9, 6.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.2, 160.0, 158.8, 154.8, 153.5, 153.4, 145.4, 140.4, 138.0, 133.6, 129.8, 117.1, 105.4, 103.7, 100.8, 93.1, 92.0, 78.5, 69.2, 61.1, 60.9, 56.3, 55.6, 55.5, 23.7; HRMS calcd for $(C_{32}H_{36}O_{11} + H)^+$ 597.2330; found, 597.2321.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4 - dimethoxybenzamido)-4-fluorobenzoate (**50**).* Following the procedure for the preparation of compound **45**, but with 3-(3,4-dimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **50** was obtained. Yield 83.2%; mp 99−101 °C; $[\alpha]^{20}_D$ = +64.5 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.99 (d, J = 6.6 Hz, 1H), 8.01 (s, 1H), 7.69 (m, 1H), 7.48 (s, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.11 (m, 1H), 6.91 (d, J = 8.3 Hz, 1H), 6.70 (s, 2H), 6.19 (d, J = 1.7 Hz, 1H), 6.11 (d, J = 1.7 Hz, 1H), 5.49 (dd, J = 14.0, 7.9 Hz, 1H), 5.08 (d, J = 8.1 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.82−3.73 (m, 15H), 3.20 (A of ABX, J = 16.5, 5.7 Hz, 1H), 2.80 (B of ABX, J = 16.5, 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.0, 164.4, 160.0, 158.7, 156.6, 155.1, 154.6, 153.3, 152.6, 149.4, 138.0, 133.4, 127.8, 123.5, 119.8, 115.0, 110.7, 104.1, 101.0, 93.2, 92.1, 79.1, 70.6, 60.8, 56.2, 55.5, 25.4; HRMS calcd for $(C_{36}H_{36FN}O_{11} + H)^+$ 678.2345; found, 678.2337.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4,5- trimethoxybenzamido)-4-fluorobenzoate (**51**).* Following the procedure for the preparation of compound **45**, but with 3-(3,4,5-trimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **51** was obtained. Yield 84.6%; mp 99−101 °C; $[\alpha]^{20}_D$ = +64.5 (c = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.94 (d, J = 6.8 Hz, 1H), 7.97 (s, 1H), 7.72−7.64 (m, 1H), 7.13 (m, 1H), 7.08 (s, 2H), 6.69 (s, 2H), 6.19 (d, J = 1.2 Hz, 1H), 6.11 (d, J = 1.2 Hz, 1H), 5.50 (dd, J = 14.0, 8.0 Hz, 1H), 5.08 (d, J = 8.0 Hz, 1H), 3.92 (s, 6H), 3.91 (s, 3H), 3.81−3.74 (m, 15H), 3.19 (A of ABX, J = 16.5, 5.7 Hz, 1H), 2.80 (B of ABX, J = 16.5, 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.3, 164.4, 160.0, 158.7, 156.7, 155.0, 154.7, 153.5, 153.3, 141.7, 137.9, 133.4, 129.5,126.9, 126.5, 123.6, 115.1, 104.6, 104.0, 101.0, 93.1, 92.1, 79.1, 70.6, 61.0, 56.3, 55.5, 25.4; HRMS calcd for $(C_{37}H_{38}FNO_{12} + H)^+$ 708.2451; found, 708.2443.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-((E)-3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoate (**52**).* Following the procedure for the preparation of compound **45**, but with (*E*)-3-(3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoic acid as starting material, compound **52** was obtained. Yield 82.8%; mp 112−114 °C; $[\alpha]^{20}_D$ = +64.6 (c = 0.09, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.05 (d, J = 6.2 Hz, 1H), 7.70 (d, J = 15.4 Hz, 1H), 7.67−7.63 (m, 1H), 7.50 (bs, 1H), 7.14 (dd, J = 8.3, 1.7 Hz, 1H), 7.12−7.07 (m, 2 H), 6.88 (d, J = 8.3 Hz, 1H), 6.71 (s, 2H), 6.45 (d, J = 15.4 Hz, 1H), 6.20 (d, J = 2.0 Hz, 1H), 6.12 (d, J = 2.0 Hz, 1H), 5.49 (m, 1H), 5.08 (d, J = 8.1 Hz, 1H), 3.92 (m, 6H), 3.79 (m, 15 H), 3.21 (A of ABX, J = 16.5, 5.8 Hz, 1H), 2.81 (B of ABX, J = 16.5, 8.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.5, 164.1, 160.0, 158.8, 155.1, 153.4, 151.4, 149.4, 143.5, 133.4, 127.4, 123.3, 122.8, 117.8, 115.0, 111.3, 109.9, 104.2, 101.2, 93.3, 92.2, 79.2, 70.7, 60.9, 57.6, 55.6, 25.6; HRMS calcd for $(C_{38}H_{38}FNO_{11} + H)^+$ 704.2502; found, 704.2488.

*(2R,3S)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-((E)-3-(3,4,5-trimethoxyphenyl)acrylamido)-4-fluorobenzoate (**53**).* Following the procedure for the preparation of compound **45**, but with (*E*)-3-(3-(3,4,5-trimethoxyphenyl)acrylamido)-4-fluorobenzoic

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acid as starting material, compound **52** was obtained. Yield 83.0%; mp 111−113 °C; $[\alpha]^{20}_D$ = +80.2 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.03 (d, *J* = 7.2 Hz, 1H), 7.66 (m, 3 H), 7.08 (t, *J* = 9.3 Hz, 1H), 6.77 (s, 2 H), 6.70 (s, 2H), 6.56−6.48 (m, 1H), 6.19 (s, 1H), 6.12 (s, 1H), 5.57−5.43 (m, 1H), 5.08 (d, *J* = 8.1 Hz, 1H), 3.88 (s, 9 H), 3.81−3.74 (m, 15 H), 3.20 (A of ABX, *J* = 16.5, 5.6 Hz, 1H), 2.80 (B of ABX, *J* = 16.5, 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.5, 163.9, 160.0, 158.8, 155.1, 153.6, 153.4, 143.6, 140.4, 138.0, 133.4, 129.9, 123.4, 119.4, 115.1, 114.9, 105.5, 104.2, 101.1, 93.2, 92.2, 79.2, 70.7, 61.2, 60.9, 56.3, 56.2, 55.6, 55.5, 25.5; HRMS calcd for $(C_{39}H_{40}FNO_{12} + H)^+$ 734.2607; found, 734.2598.

*(2R,3R)-5,7-dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl Acetate (**55**).* Acetic anhydride (1 mL) and pyridine (1.5 mL) were stirred at 0 °C, and (2*R*,3*R*)-3-hydroxy-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-4-one**54** (200 mg, 0.51 mmol)[59] was added. The mixture was stirred at room temperature for 36 h and then poured into ice water (5 mL), and a copious amount of white solid was precipitated. The solid was dissolved in EtOAc (30 mL). Then, the solution was washed in diluted hydrochloric acid two times and in brine in sequence. The organic layer was dried with anhydrous $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography to afford the title compound **55** (190 mg, 85.4% yield); mp187−189 °C; $[\alpha]^{20}_D$= +6.6 (*c* = 0.1, CH_2Cl_2); ^{1}HNMR ($CDCl_3$, 600 MHz)δ6.69 (s, 2H), 6.19 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.2 Hz, 1H), 5.67 (d, *J* = 12.1 Hz, 1H), 5.27 (d, *J* = 12.1 Hz, 1H), 3.85 (m, 15 H), 2.21(s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ184.9, 169.6, 166.6, 164.1, 162.6, 153.5, 138.7, 131.1, 104.6, 104.5, 104.4, 93.6, 81.3, 73.5, 56.3, 56.2, 55.8; HRMS calcd for $(C_{22}H_{24}O_9 + H)^+$ 433.1493; found, 433.1498; $(C_{17}H_{16}O_6NF+Na)^+$ 455.1313; found, 455.1314.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl 4-methoxybenzoate (**56**).* (2*R*,3*R*)-3-hydroxy-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-4-one **54** (200 mg, 0.51 mmol), 4-methoxybenzoic acid (150 mg, 0.99 mmol), EDC·HCl (200 mg, 1.06 mmol), and DMAP (130 mg, 1.06 mmol) were dissolved in anhydrous CH_2Cl_2 (20 mL), then the mixture was stirred at room temperature overnight under a nitrogen atmosphere. Then, the solution was washed with diluted hydrochloric acid two times and in brine in sequence. The organic layer was dried with anhydrous $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography to afford the title compound **56** (230 mg, 85.4% yield); mp 124−126 °C; $[\alpha]^{20}_D$= +10.7 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.93 (s, 1 H), 7.91 (s, 1 H), 6.86 (s, 1 H), 6.84 (s, 1 H), 6.73 (s, 2H), 6.19 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.2 Hz, 1H), 5.86 (d, *J* = 12.1 Hz, 1 H), 5.44 (d, *J* = 12.1 Hz, 1 H), 3.82(s, 3H), 3.81 (s, 6 H), 3.80 (s, 9 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.1, 166.6, 164.9, 163.7, 162.6, 153.4, 138.6, 132.1, 131.3, 121.8, 113.7, 104.5, 93.7, 81.4, 73.8, 60.9, 56.2, 55.8, 55.5; HRMS calcd for $(C_{29}H_{33}O_9 + H)^+$ 525.1755; found, 525.1764.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl 3,4-dimethoxybenzoate (**57**).* Following the procedure for the preparation of compound **56**, but with 3,4-dimethoxybenzoic acid as starting material, compound **57** was obtained. Yield 83.3%; mp 125−127 °C; $[\alpha]^{20}_D$ = +5.2 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.61 (dd, *J* = 2.1, 8.3 Hz, 1H), 7.46 (d, *J* = 2.1 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 6.74 (s, 1H), 6.19 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.2 Hz, 1H), 5.87 (d, *J* = 12.1 Hz, 1H), 5.46 (d, *J* = 12.1 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.84 (s, 3 H), 3.81 (s, 9 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.1, 166.6, 164.2, 162.6, 153.4, 148.6, 138.7, 131.3, 124.1, 121.9, 112.4, 110.2, 104.5, 93.7, 81.4, 73.9, 60.9, 56.2, 56.1, 55.8; HRMS calcd for $(C_{29}H_{31}O_{11} + H)^+$ 555.1861; found, 555.1866.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4,5-trimethoxybenzoate (**58**).* Following the procedure for the preparation of compound **56**, but with 3,4,5-trimethoxybenzoic acid as starting material, compound **58** was obtained. Yield 84.3%; mp 126−128 °C; $[\alpha]^{20}_D$ = +4.1 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.21 (s, 2H), 6.74 (s, 2H), 6.19 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.2 Hz, 1H), 5.84 (d, *J* = 12.1 Hz, 1H), 5.46 (d, *J* = 12.1 Hz, 1H), 3.84 (m, 24 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 184.9, 166.7, 165.0, 164.3, 162.7, 153.5, 152.9, 142.7, 141.4, 138.8, 131.2, 124.5, 107.4, 104.6, 93.8, 81.4, 74.2, 61.1, 60.9, 56.4, 56.3, 55.8; HRMS calcd for $(C_{30}H_{33}O_{12} + H)^+$ 585.1967; found, 585.1972.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl 3,4,5-tris(allyloxy)benzoate (**59**).* Following the procedure for the preparation of compound **56**, but with 3,4,5-tris(allyloxy)benzoic acid as starting material, compound **59** was obtained. Yield 78.7%; mp 122−124 °C; $[\alpha]^{20}_D$ = +28.1 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.20 (s, 2H), 6.72 (s, 2H), 6.19 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.2 Hz, 1H), 6.03 (m, 3H), 5.85 (d, *J* = 12.1 Hz, 1H), 5.44 (A_1 of $A_1B_1X_1$, *J* = 12.0 Hz, 1.4 Hz, 1H), 5.39 (A_2 of $A_2B_2X_2$, *J* = 17.3, 1.2 Hz, 2H), 5.30 (B_1 of $A_1B_1X_1$, *J* = 17.2, 1.4 Hz, 1H), 5.26 (B_2 of $A_2B_2X_2$, *J* = 10.6, 1.2 Hz, 2H), 5.18 (d, J=12.1 Hz, 1H), 4.59 (d, *J* = 6.0 Hz, 2H), 4.55 (d, *J* = 5.1 Hz, 4H), 3.88 (s, 3H), 3.84 (s, 3 H), 3.81 (s, 6 H), 3.80 (s, 3 H); ^{13}CNMR ($CDCl_3$, 150 MHz) δ 184.1, 165.9, 164.1, 163.4, 161.9, 152.7, 151.5, 141.6, 138.0, 133.4, 132.2, 130.4, 123.5, 117.3, 117.1, 108.6, 103.8, 92.9, 80.6, 73.4, 69.4, 60.2, 55.5, 55.1. HRMS calcd for $(C_{36}H_{39}O_{12}H)^+$ 663.2436; found, 663.2446.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl (E)-3-(4-methoxyphenyl)acrylate (**60**).* Following the procedure for the preparation of compound **56**, but with (*E*)-3-(4-methoxyphenyl)acrylic acid as starting material, compound **60** was obtained. Yield 81.2%; mp 123−125 °C; $[\alpha]^{20}_D$ = +6.1 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.58 (d, *J* = 16.4 Hz, 1H),6.73 (s, 2H), 6.69 (s, 2H), 6.31 (d, *J* = 16.4 Hz, 1H), 6.16 (d, *J* = 2.2 Hz, 1H), 6.12 (d, *J* = 2.2 Hz, 1H), 5.85 (d, *J* = 12.1 Hz, 1H), 5.46 (d, *J* = 12.1 Hz, 1H), 3.85 (m, 18 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ185.0, 166.7, 165.4, 164.2, 162.6, 153.5, 146.1, 140.3, 138.8, 131.1, 129.8, 116.3, 105.4, 104.7, 104.3, 93.7, 81.4, 73.5, 61.0, 56.2, 55.8; HRMS calcd for $(C_{30}H_{31}O_{10} + H)^+$ 551.1912; found, 551.1919; $(C_{30}H_{31}O_{10} + Na)^+$ 573.1731; found, 573.1740.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl (E)-3-(3,4-Dimethoxyphenyl)acrylate (**61**).* Following the procedure for the preparation of compound **56**, but with (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid as starting material, compound **61** was obtained. Yield 80.6%; mp 121−123 °C; $[\alpha]^{20}_D$ = +12.7 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.61 (d, *J* = 15.9 Hz, 1H), 7.05 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.00 (d, *J* = 1.9 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.73 (s, 2H), 6.28 (d, *J* = 15.9 Hz, 1H), 6.16 (d, *J* = 1.7 Hz, 1H), 6.13 (d, *J* = 1.7 Hz, 1H), 5.83 (d, *J* = 11.9 Hz, 1H), 5.39 (d, *J* = 11.9 Hz, 1H), 3.90−3.83 (m, 21 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 185.1, 166.6, 165.7, 164.2, 162.6, 153.5, 151.3, 149.2, 146.1, 138.7, 131.1, 127.3, 122.9, 114.7, 111.0, 109.7, 104.7, 104.4, 93.6, 81.4, 73.4, 60.9, 56.3, 56.1, 55.8; HRMS calcd for $(C_{31}H_{33}O_{11} + H)^+$ 581.2017; found, 581.2021.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl (E)-3-(3,4,5-Trimethoxyphenyl)acrylate (**62**).* Following the procedure for the preparation of compound **56**, but with (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid as starting material, compound **62** was obtained. Yield 83.6%; mp 116−119 °C; $[\alpha]^{20}_D$ = +6.5 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.61 (d, *J* = 15.9 Hz, 1H), 7.41 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.73 (s, 2H), 6.26 (d, *J* = 15.9 Hz, 1H), 6.15 (d, *J* = 1.8 Hz, 1H), 6.12 (d, *J* = 1.8 Hz, 1H), 5.80 (d, *J* = 12.1 Hz, 1H), 5.38 (d, *J* = 12.1 Hz, 1H), 3.85 (m, 24 H); ^{13}CNMR ($CDCl_3$, 150 MHz) δ 185.0, 166.6, 165.8, 164.2, 162.6, 161.6, 153.4, 145.8, 138.7, 131.2, 129.9, 127.1, 114.4, 104.6, 104.4, 93.6, 81.4, 73.4, 60.9, 56.3, 55.8, 55.5. HRMS calcd for $(C_{32}H_{35}O_{12} + H)^+$ 611.2123; found, 611.2134; $(C_{30}H_{31}O_{10} + Na)^+$ 633.1942; found, 633.1953.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl 3-(3,4-dimethoxybenzamido)-4-fluorobenzoate (**63**).* Following the procedure for the preparation of compound **56**, but with 3-(3,4-dimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **63** was obtained. Yield 82.3%; mp 127−129 °C; $[\alpha]^{20}_D$ = +16.7 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.05 (s, 1H), 8.04 (s, 1H), 7.75 (s, 1H), 7.47 (s, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.12 (t, *J* = 8.8 Hz, 1H), 6.90 (d, *J* = 7.7 Hz, 1H), 6.79 (s, 2H),6.18 (d, *J* = 2.2 Hz, 1H), 6.12 (d, *J* = 2.2 Hz, 1H), 5.86 (d, *J* = 12.1 Hz, 1H), 5.47 (d, *J* = 12.1 Hz, 1H), 3.94 (s, 6H), 3.93 (s, 9 H), 3.83 (s, 3 H), 3.80 (s, 3 H); ^{13}CNMR ($CDCl_3$, 150 MHz) δ 184.6, 166.7, 164.2, 164.1,

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162.6, 156.6, 154.9, 153.4, 152.5, 149.3, 138.6, 131.3, 126.8, 123.8, 119.8, 115.1, 110.7, 110.5, 104.4, 104.3, 93.7, 81.3, 74.4, 60.9, 56.2, 55.8; HRMS calcd for $(C_{36}H_{35}O_{12}NF + H)^+$ 692.2138; found, 692.2141.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl 3-(3,4,5-trimethoxybenzamido)-4-fluorobenzoate (**64**).* Following the procedure for the preparation of compound **56**, but with 3-(3,4,5-trimethoxybenzamido)-4-fluorobenzoic acid as starting material, compound **64** was obtained. Yield 81.4%; mp117–119 °C; $[\alpha]^{20}_D$ = +10.2 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.97 (s, 1H), 8.04 (s, 1H), 7.77 (s, 1H), 7.14 (m, 1H), 7.08 (s, 2H), 6.78 (s, 2H), 6.18 (d, *J* = 2.2 Hz, 1H), 6.12 (d, *J* = 2.2 Hz, 1H), 5.86 (d, *J* = 12.1 Hz, 1H), 5.47 (d, *J* = 12.1 Hz, 1H), 3.85 (m, 24 H); ^{13}CNMR ($CDCl_3$, 150 MHz) δ 184.6, 166.7, 164.2, 164.2, 162.4, 156.9, 155.4, 153.7, 141.8, 138.9, 131.3, 129.8, 127.4, 127.3, 124.1, 115.3, 115.1, 104.9, 104.2, 104.3, 93.8, 81.5, 74.2, 61.1, 56.8, 56.2. HRMS calcd for $(C_{37}H_{37}O_{13}NF + H)^+$ 722.2243; found, 722.2260.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl 3-((E)-3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoate (**65**).* Following the procedure for the preparation of compound **56**, but with (*E*)-3-(3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoic acid as starting material, compound **65** was obtained. Yield 84.7%; mp 153–155 °C; $[\alpha]^{20}_D$ = +26.2 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.06 (d,*J* = 6.1 Hz, 1H), 7.71 (m, 1H), 7.68 (s, 1H), 7.64 (d, *J* = 15.4 Hz, 1H), 7.08 (m, 1H), 7.06 (s, 2H), 6.86 (d, *J* = 8.2 Hz, 1H), 6.79 (s, 2H), 6.47 (d, *J* = 15.4 Hz, 1H), 6.19 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.2 Hz, 1H), 5.88 (d, *J* = 12.1 Hz, 1H), 5.49 (d, *J* = 12.1 Hz, 1H), 3.85 (m, 21H); ^{13}CNMR ($CDCl_3$, 150 MHz) δ184.9, 166.8, 164.4, 164.3, 162.8, 153.8,151.2, 149.4, 143.4, 138.7, 131.2, 127.6, 126.8, 126.3, 123.8, 122.9, 118.4, 115.1, 114.8, 111.3, 109.9, 104.2, 93.8, 81.4, 74.2, 60.8, 56.4, 56.2. HRMS calcd for $(C_{38}H_{37}O_{12}NF + H)^+$ 718.2294; found, 718.2311.

*(2R,3R)-5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-chroman-3-yl 4-fluoro-3-((E)-3-(3,4,5-trimethoxyphenyl)-acrylamido)benzoate (**66**).* Following the procedure for the preparation of compound **56**, but with (*E*)-3-(3-(3,4,5-trimethoxyphenyl)-acrylamido)-4-fluorobenzoic acid as starting material, compound **66** was obtained. Yield 80.7%; mp 169–172 °C; $[\alpha]^{20}_D$ = +14.7 (*c* = 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.05 (d, *J* = 6.6 Hz, 1H), 7.71 (s, 2H), 7.62 (d, *J* = 15.4 Hz, 1H), 7.08 (t, *J* = 8.8 Hz, 1H), 6.79 (s, 2H), 6.77 (s, 2H), 6.52 (d, *J* = 15.4 Hz, 1H), 6.20 (d, *J* = 2.2 Hz, 1H), 6.14 (d, *J* = 2.2 Hz, 1H), 5.86 (d, *J* = 12.1 Hz, 1H), 5.49 (d, *J* = 12.1 Hz, 1H), 3.85 (m, 24 H); ^{13}CNMR ($CDCl_3$, 150 MHz) δ 184.7, 166.8, 164.3, 164.2, 162.7, 153.5, 143.1, 140.1, 138.6, 131.2, 130.1, 126.8, 126.1, 123.7, 119.7, 115.1, 114.9, 106.5, 105.3, 104.4, 93.8, 81.4, 74.4, 61.1, 60.9, 56.3, 55.9; HRMS calcd for $(C_{39}H_{39}O_{13}NF + H)^+$ 748.2400; found, 748.2421.

Materials for Biological Studies. DMSO, verapamil, doxorubicin (DOX), topotecan, and paclitaxel (PTX) were purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM), trypsin-ethylenediaminetetracetic acid (EDTA), and penicillin/streptomycin were from Gibco BRL. Fetal bovine serum (FBS) was from Hyclone Laboratories. 2-(4,5-Dimethylthiazol-2-yl-)-5-[3-(carboxymethoxy) phenyl]- 2-(4-sulfophenyl)-2*H*-tetrazolium (MTS), and phenazine methosulfate (PMS) were purchased from Promega. Human breast cancer cell lines MDA435/LCC6 and MDA435/LCC6MDR were kindly provided by Dr. Robert Clarke (Georgetown University, Washington, DC). The human ovarian carcinoma cell lines 2008/P and 2008/MRP1 were generous gifts from Professor P. Borst (The Netherlands Cancer Institute, Amsterdam, The Netherlands). The HEK293/pcDNA3.1 and HEK293/R2 cell lines were kindly provided by Dr. Kenneth To (The Chinese University of Hong Kong, Hong Kong). The L929 cell line was purchased from ATCC.

Cell Culture. MDA435/LCC6, MDA435/LCC6MDR, and L929 cell lines were cultured in supplemented DMEM media with 10% heat inactivated FBS and 100 U/mL penicillin and 100 μg/mL of streptomycin. 2008/P, 2008/MRP1, HEK293/pcDNA3.1, and HEK293/R2 cells were cultured in RPMI 1640 medium containing heat inactivated 10% FBS and 100 U/mL penicillin and 100 μg/mL of streptomycin. They were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells were split constantly after a confluent monolayer was formed. To split cells, the plate was washed briefly with phosphate-buffered saline (PBS), treated with 0.05% trypsin-EDTA, and harvested by centrifugation.

Cell Proliferation Assay. Six thousand cells of LCC6 or LCC6MDR and PTX (concentrations including 400, 133, 44, 15, 5, 1.6, and 0 nM) were mixed with or without modulators to a final volume of 200 μL in each well of 96-well plates. Four thousand cells of 2008/P or 2008/MRP1 and DOX (concentrations including 2000, 667, 222, 74, 25, 8, and 0 nM) were coincubated with or without modulators to a final volume of 200 μL. Four thousand five hundred cells of HEK293/pcDNA3.1 or HEK293/R2 and topotecan (concentrations including 2000, 667, 222, 74, 25, 8, and 0 nM) were coincubated with or without modulators to a final volume of 200 μL. The plates were then incubated for 5 days at 37 °C. The cell viability was determined using the CellTiter 96 AQueous Assay (Promega) as reported previously.[58,59] The IC_{50} of the anticancer drugs of the cell line was determined using nonlinear regression dose–response cuve analysis of the Prism software.

EC_{50} and Selective Index Value Determination. Six thousand LCC6MDR cells were incubated with different concentrations of PTX (400, 133, 44, 15, 5, 1.6, and 0 nM), vinblastine (20, 6.7, 2.2, 0.7, 0.2, 0.08, and 0 nM), vincristine (50, 16.7, 5.6, 1.9, 0.6, 0.2, and 0 nM) or DOX (10, 3.3, 1, 0.4, 0.1, 0.04, and 0 μM) with different concentrations of modulators (1000, 500, 250, 125, 62.5, 31.3, and 0 nM) for 5 days at 37 °C in a volume of 200 μL. The percentage of survival was determined using the MTS assay as mentioned previously.[58,59] $IC_{50\ (drug)}$ was determined using Prism 5.0 (GraphPad). EC_{50} was defined as the concentration of modulator needed to reduce the $IC_{50\ (drug)}$ by 50%. Selective index value is an indicator of compound safety. It is determined by dividing the IC_{50} of the modulator toward mouse fibroblast cells L929 by the EC_{50} of the modulator.

Cytotoxicity Assay. Ten thousand cells of LCC6 or LCC6MDR or L929 were mixed with different concentrations (100, 33.3, 11.1, 3.7, 1.2, 0.4, and 0 μM) of modulators to a final volume of 100 μL in each well of 96-well plates. The plates were then incubated for 3 days at 37 °C. The percentage of survival was determined using the MTS assay, and the IC_{50} of the modulators was determined using Prism software.

Intracellular DOX Accumulation. LCC6 or LCC6MDR cells (1×10^6 cells) were mixed with 20 μM DOX and 2 μM of the modulator at 37 °C for 150 min. DMSO (0.2%) was used as a negative control. After incubation, the cells were spun down and washed with cold PBS, pH 7.4, and lysed with lysis buffer (0.75 M HCl and 0.2% Triton-X100 in isopropanol). The lysate was spun down, and the supernatant was saved. The fluorescence level of DOX was determined as reported previously.[58,59]

DOX Influx and DOX Efflux. For the influx, 5 μM DOX with or without 1.5 μM of the modulator was added to LCC6 or LCC6MDR cells. After 0, 10, 20, 30, and 40 min, 1×10^6 cells were taken out and chilled on ice. For the efflux, LCC6 and LCC6MDR cells were preincubated with 20 μM oDOX for 1 h at 37 °C. After 1 h, the cells were washed once with 1× PBS. The washed cells were resuspended with supplemented DMEM media with or without 1.5 μM of the modulator. After 0, 30, 60, 90, 120, and 150 min, the cells were taken out and chilled on ice. The intracellular DOX level at each time point was measured as described previously.[58,59]

Intracellular Accumulation of Methylated EGC (23) and Methylated GC (51). LCC6 or LCC6MDR (3×10^6 cells) were incubated with 20 or 200 μM **23** or **51** at 37 °C for 150 min. The cell lysis and determination methods were according to a previous report.[49] A reversed phase Diamonsil C18 (4.6 × 150 mm) column attached to a Gilson 322 pump coupled to a Gilson UV–vis-152 detector was used. Each sample was injected at a volume of 20 μL and eluted with methanol, and the flow rate was 1 mL/min. The peak wavelength of **23** was detected at 308 nm, and the retention time was 3.26 min. The peak wavelength of **51** was detected at 270 nm, and the retention time was 3.23 min.

Calculated Molecular Descriptors. Calculated descriptors such as cLogP and PSA were determined by SYBYL-X 2.0. The structures of compounds **35–38** were built and energy minimized under the

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Tripos force field with 0.05 kcal/(mol Å). The Gasteiger−Huckel method was used to calculate the charges. Energy minimization was performed by the Powell method with 2000 iterations. Then, the distance of the linkers was calculated.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra and ^{13}C NMR spectra of all compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00085.

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Compounds **30**, **35**, **36**, **43**, **50**, and **51** were synthesized by J.Y.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MDR, multidrug resistance; ABC, ATP-binding cassette; P-gp, P-glycoprotein; RF, relative fold; DOX, doxorubicin; PTX, paclitaxel; PSA, polar surface area; cLogP, calculated log P values

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