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Synthesis and evaluation of stereoisomers of methylated catechin and epigallocatechin derivatives on modulating P-glycoprotein-mediated multidrug resistance in cancers

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ABSTRACT

P-glycoprotein (P-gp; ABCB1)-mediated drug efflux causes multidrug resistance in cancer. Previous synthetic methylated epigallocatechin (EGC) possessed promising P-gp modulating activity. In order to further improve the potency, we have synthesized some novel stereoisomers of methylated epigallocatechin (EGC) and gallocatechin (GC) as well as epicatechin (EC) and catechin (C). The (2R, 3S)-*trans*-methylated C derivative **25** and the (2R, 3R)-*cis*-methylated EC derivative **31**, both containing dimethyoxylation at ring B, tri-methoxylation at ring D and oxycarbonylphenylcarbamoyl linker between ring D and C3, are the most potent in reversing P-gp mediated drug resistance with EC_{50} ranged from 32 nM to 93 nM. They are non-toxic to fibroblast with $IC_{50} > 100$ μM. They can inhibit the P-gp mediated drug efflux and restore the intracellular drug concentration to a cytotoxic level. They do not downregulate surface P-gp protein level to enhance drug retention. They are specific for P-gp with no or low modulating activity towards MRP1- or BCRP-mediated drug resistance. In summary, methylated C **25** and EC **31** derivatives represent a new class of potent, specific and non-toxic P-gp modulator.

Keywords: P-glycoprotein (P-gp); Epigallocatechin (EGC); Gallocatechin (GC); Catechin (C); Epicatechin (EC)

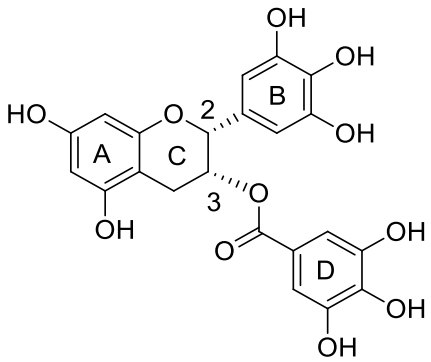
1. INTRODUCTION

The multidrug resistance (MDR) in cancer cells has been a major obstacle to successful cancer chemotherapy in clinic. An important mechanism for MDR is the enhanced cellular efflux of anticancer drugs by over-expression of ATP-binding cassette (ABC) transporter proteins in tumor cells.[1] So far, P-glycoprotein (P-gp; ABCB1; MDR1) is the most well-characterized ABC transporter and can transport a broad range of structurally diverse anticancer drugs. Therefore, P-gp is a good drug target for treating multidrug resistant cancers.

Numerous P-gp inhibitors have been studied, including calcium channel blocker verapamil[2-4] or its derivative dexverapamil,[5] antimalarial drug quinidine,[6] calmodulin antagonists,[7, 8] the immunosuppressant cyclosporine A[9-12] or its derivatives PSC833 (valspodar),[13] some steroids,[14-16] dexniguldipine,[17] VX-710 (biricodar),[18, 19] zosuquidar LY335979, tariquidar XR9576, laniquidar R101933, elacridar GF120918 and the substituted diarylimidazole ONT-090.[20, 21] Among them, only a very few were selected for clinical trial and none of them has been approved yet for clinical application.[22-25] These failures may be because the previous clinical trials did not include patient selection to evaluate the expression of drug transporters in the tumors. P-gp inhibitors might fail to overcome MDR due to the overexpression of other ABC transporters like MRP1 or BCRP. It is better to monitor the expression of ABC transporters in patient tumors before using any P-gp modulators. Other factors may enhance the toxicity including drug-drug interaction between the anticancer drugs and inhibitors and low specificity of the inhibitors itself. Further improvement of inhibitors of ABC transporters should focus on potency, specificity and safety.

P-gp can be modulated by natural compounds including flavonoid, curcumin, ginsenosides, piperine, catechins and silymarin for the purpose of reversing MDR in tumor cells.[26-32] We have previously found that methylation of polyphenolic compounds such as ningalin B and quercetin was effective in improving their P-gp modulating activities.[33-35] The presence of ring D, O-methylation and linker modification of epigallocatechin (EGC, with 2R, 3R configuration) have been demonstrated to significantly improve their P-gp inhibitory activities (**Figure 1**).[32] The EC_{50} value of EGC **4** was at least 5-fold lower than EGCG and EGC **1** (**Figure 1**).[32]

Up till now, there was still no report concerning the P-gp modulating activities of methylated epicatechin (EC) and catechin (C) derivatives. Currently, EC and C are not promising P-gp modulators because they are rare components in green tea and their P-gp modulating activity was low with effective concentration at 10 μM.[36] Despite this, our previous study suggested that structural modifications of EC and C including methylation of all hydroxyl groups on the rings and varying the linker rigidity between ring D and C3 position can significantly improve their P-gp modulating activities.[32] To further understand the effect of stereochemistry on the P-gp modulating activity of catechins, we have designed, synthesized and evaluated more novel catechin stereoisomers including methylated EGC, methylated GC, methylated epicatechin (methylated EC) and methylated catechin (methylated C) for their P-gp modulating activities in breast cancer cells.

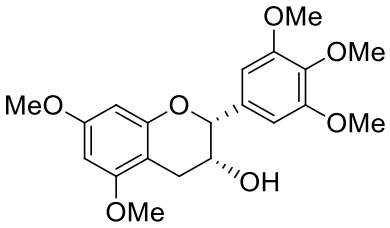


(-)-epigaloocatechin gallate [EGCG]

EC_{50} > 1000 nM

75

76

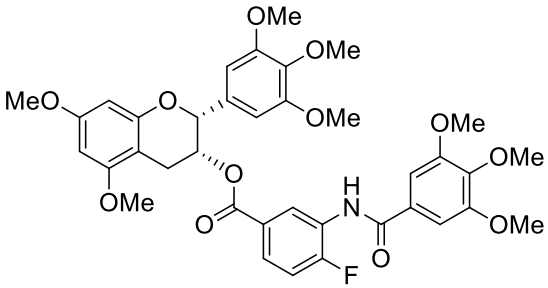


(2*R*,3*R*)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol [EGC **1**]

EC_{50} > 1000 nM

77

78



(2*R*,3*R*)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoate [EGC **4**]

EC_{50} = 214 nM

79

80 **Figure 1.** Improvement of P-gp modulating activity of EGC by presence of D ring, O-

81 methylation and linker modification. EGC **1** and EGC **4** was named as **8** and **36** in previous

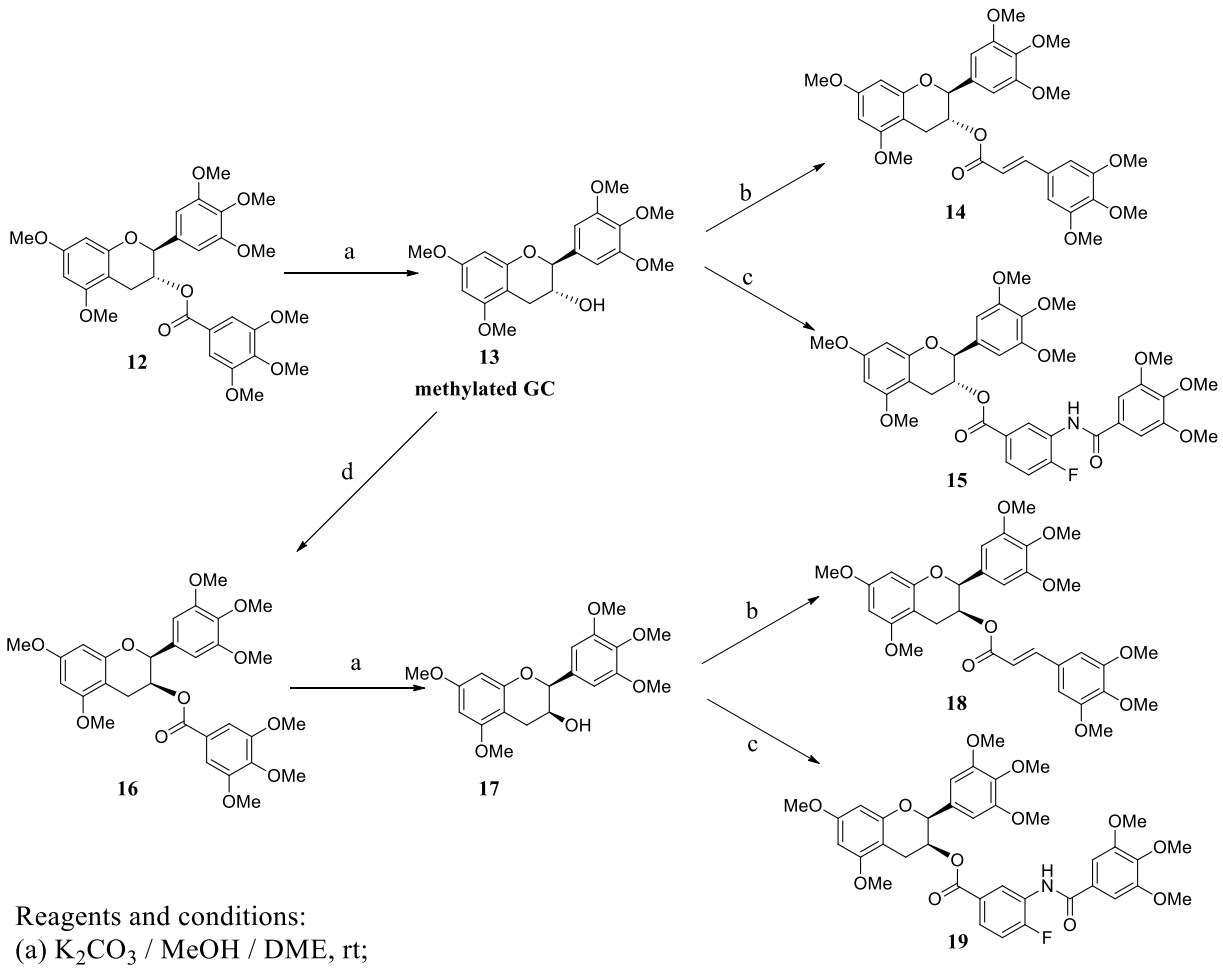
82 study.[32]

83

2. RESULTS

2.1 Chemistry

Scheme 1 Synthetic route of stereoisomers of methylated GC derivatives

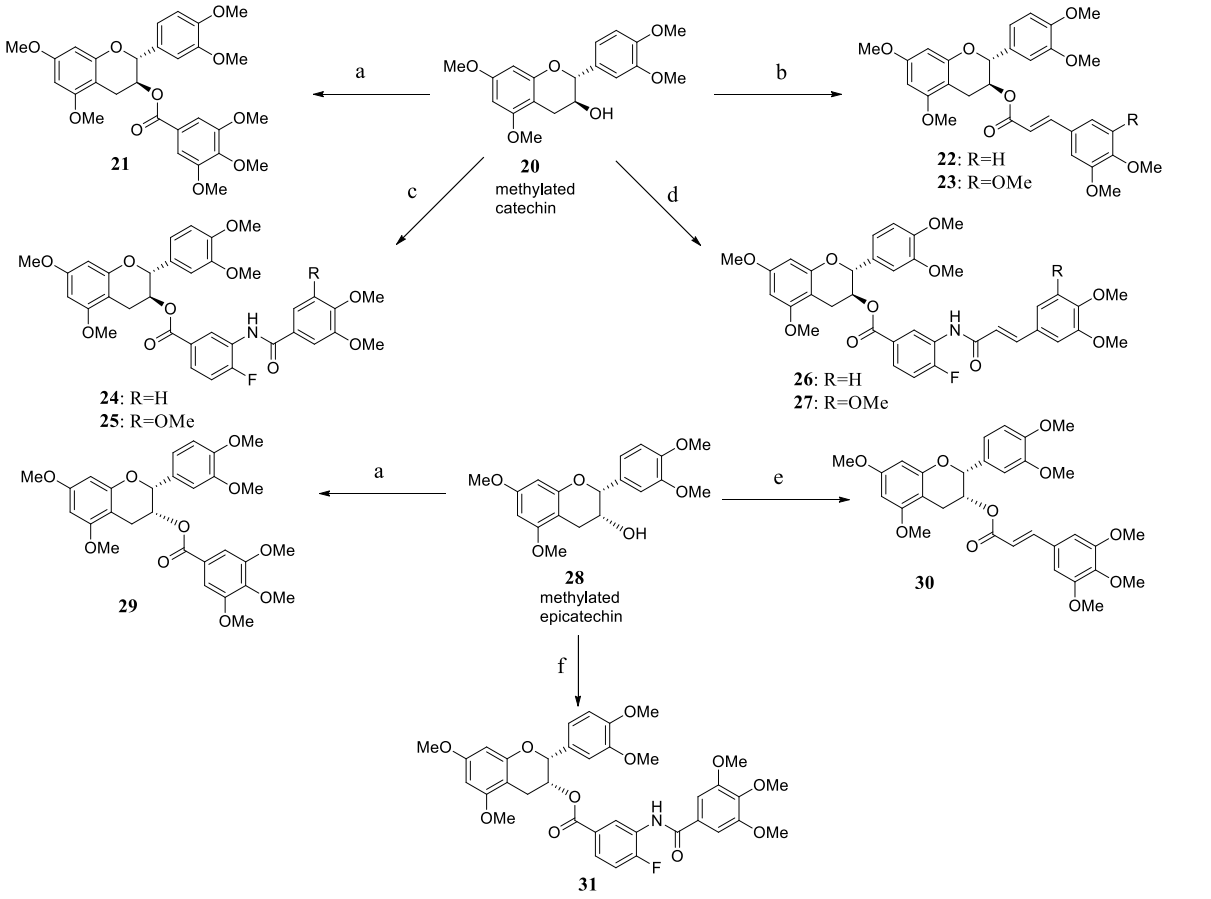


Reagents and conditions:
(a) K_2CO_3 / MeOH / DME, rt;
(b) DMAP / EDCI / DCM, (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid;
(c) 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid;
(d) 3,4,5-trimethoxybenzoic acid, Ph_3P / DIAD, 0°C-rt.

Synthesis of methylated gallocatechin derivatives is shown in scheme 1. (2S, 3R)-pentamethylated gallocatechin **12**, which was obtained from methylation of commercial available (2S, 3R)-gallocatechin, was hydrolyzed by K_2CO_3 to afford intermediate **13** (with 2S, 3R configuration)**.** Catalyzed by EDCI and DMAP, esterification of **13** with (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid or 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid

produced target compounds **14** or **15**, respectively. Compound **13** was reacted with 3,4,5-trimethoxybenzoic acid, catalyzed by PPh_3 and DIAD, gave a configuration-inversion product of (2S, 3S)-pentamethylated epigallocatechin gallate **16**. Hydrolysis of **16** provided **17** (2S, 3S), the diastereomer of intermediate **13** (2S, 3R). Esterification of **17** with (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid or 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid, catalyzed by EDCI and DMAP, produced target compounds **18** or **19**, respectively.

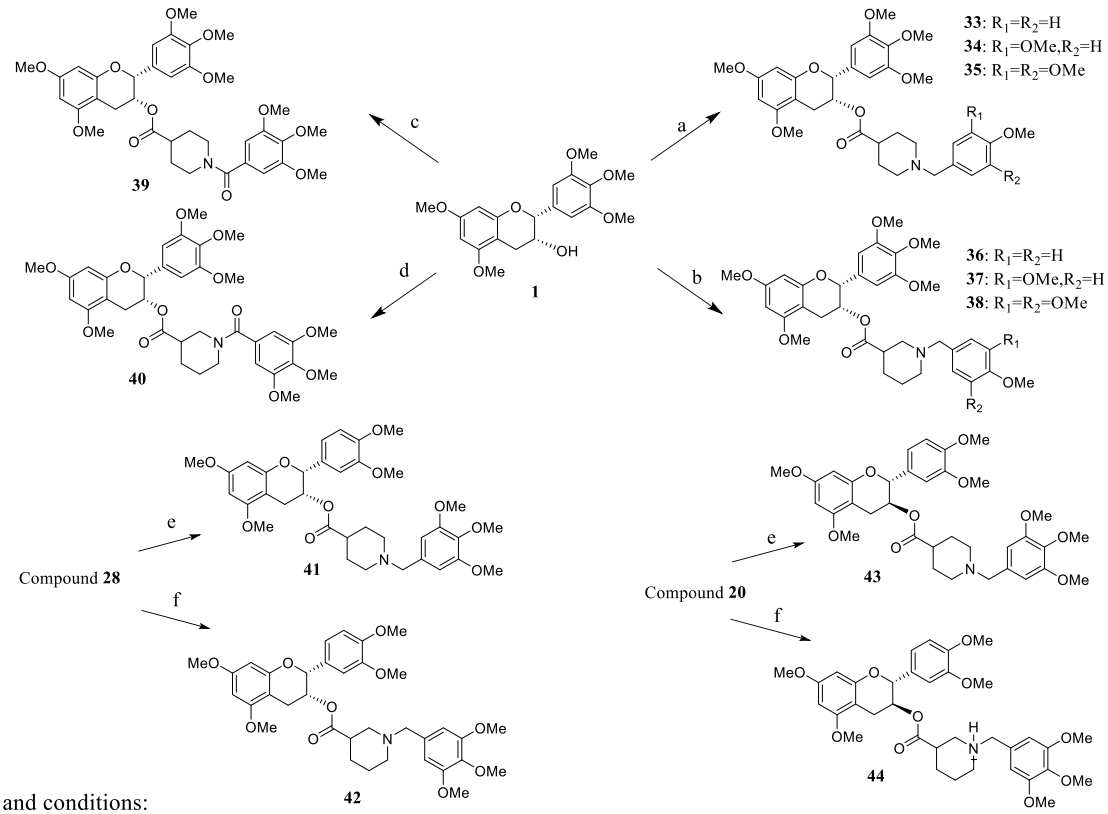
Scheme 2 Synthetic route of methylated catechin and epicatechin derivatives



Reagents and conditions:
(a) EDCI/DMAP/DCM, 3,4,5-trimethoxybenzoic acid;
(b) EDCI/DMAP/DCM (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid or (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid;
(c) EDCI/DMAP/DCM, 3-(3,4-dimethoxybenzamido)-4-fluorobenzoic acid, or 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid;
(d) EDCI/DMAP/DCM, (*E*)-3-(3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoic acid, or (*E*)-4-fluoro-3-(3-(3,4,5-trimethoxyphenyl)acrylamido)benzoic acid;
(e) EDCI/DMAP/DCM, (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid;
(f) EDCI/DMAP/DCM, (*E*)-4-fluoro-3-(3-(3,4,5-trimethoxyphenyl)acrylamido)benzoic acid.

Synthetic route of methylated catechin and epicatechin derivatives was shown in scheme 2. (2R, 3S)-tetramethylated catechin **20** was produced by methylation of commercial available (2R, 3S)-catechin. Catalyzed by EDCI and DMAP, compound **20** was coupled with 3,4,5-trimethoxybenzoic acid, (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid, (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, 4-fluoro-3-(3,4-dimethoxybenzamido)benzoic acid, 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid, (*E*)-3-(3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoic acid, or (*E*)-3-(3-(3,4,5-trimethoxyphenyl)acrylamido)-4-fluorobenzoic acid provided target compounds **21**, **22**, **23**, **24**, **25**, **26** or **27**, respectively. (2R, 3R)-tetramethylated epicatechin **28** was obtained from methylation of commercial available (2R, 3R)-epicatechin, a diastereomer of (2R, 3S)-epicatechin. Compound **28** was reacted with 3,4,5-trimethoxybenzoic acid, (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid, or 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid catalyzed by EDCI and DMAP produced target compounds **29**, **30** or **31**, respectively.

Scheme 3 Synthetic route of compounds **33**-**44**



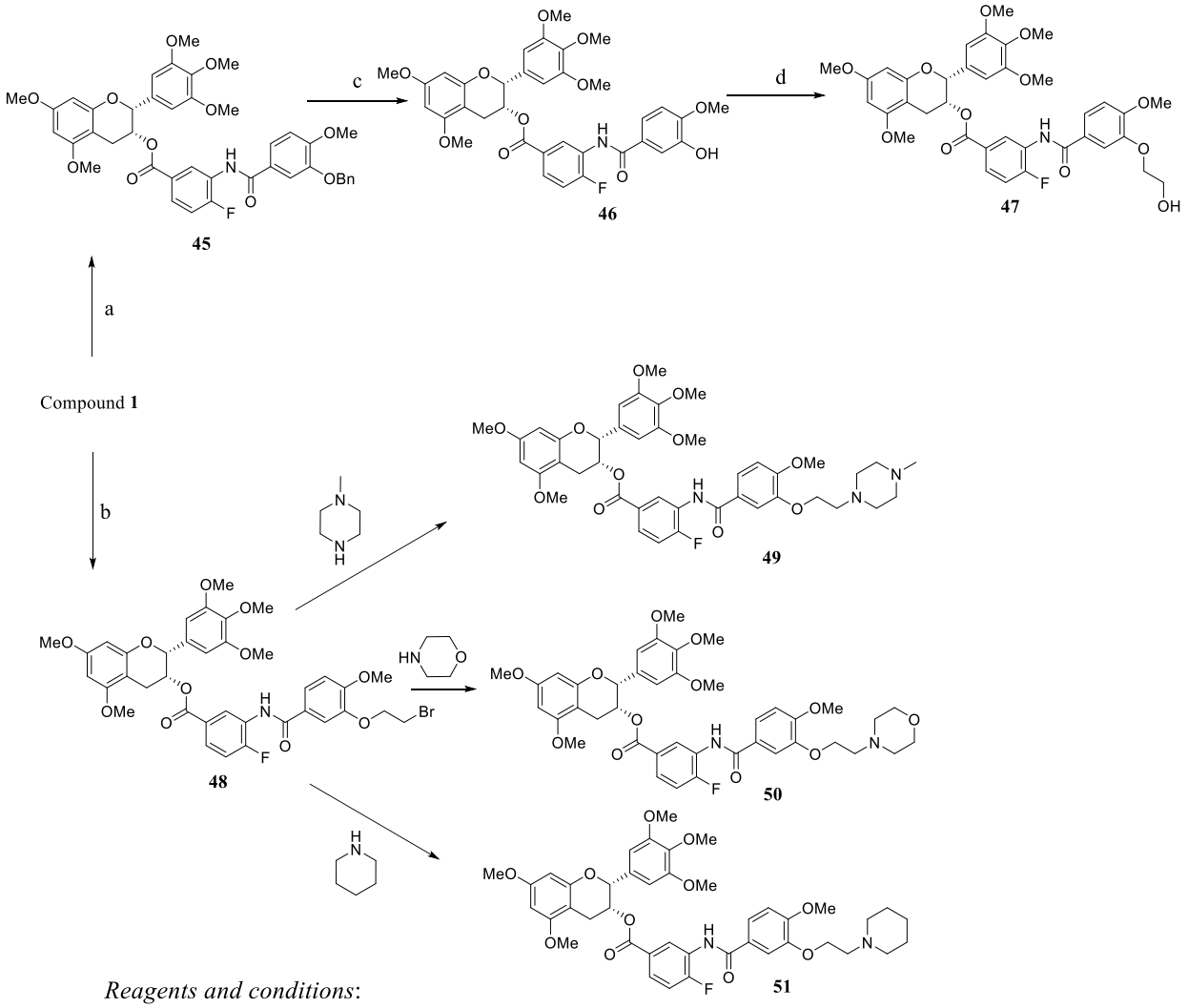
Reagents and conditions:
(a) EDCI/DMAP/DCM, 1-(4-methoxybenzyl)piperidine-4-carboxylic acid, 1-(3,4-dimethoxybenzyl)piperidine-4-carboxylic acid, or 1-(3,4,5-trimethoxybenzyl)piperidine-4-carboxylic acid, rt;
(b) EDCI/DMAP/DCM, 1-(4-methoxybenzyl)piperidine-3-carboxylic acid, 1-(3,4-dimethoxybenzyl)piperidine-3-carboxylic acid, or 1-(3,4,5-trimethoxybenzyl)piperidine-3-carboxylic acid, rt;
(c) EDCI/DMAP/DCM, 1-(3,4,5-trimethoxybenzoyl)piperidine-4-carboxylic acid, rt;
(d) EDCI/DMAP/DCM, 1-(3,4,5-trimethoxybenzoyl)piperidine-3-carboxylic acid, rt;
(e) EDCI/DMAP/DCM, 1-(3,4,5-trimethoxybenzyl)piperidine-4-carboxylic acid, rt;
(f) EDCI/DMAP/DCM, 1-(3,4,5-trimethoxybenzyl)piperidine-3-carboxylic acid, rt.

Eight basic groups containing epigallocatechin derivatives and four basic groups containing catechin derivatives were prepared and their synthetic routes were shown in Scheme 3. Catalyzed by EDCI and DMAP, important intermediates **1**, **20** and **28** were reacted with substituted piperidine-4-carboxylic acid or substituted piperidine-3-carboxylic acid to produce target compounds **33**-**44**, respectively.

In Scheme 4, 3-(3-(benzyloxy)-4-methoxybenzamido)-4-fluorobenzoic acid was treated with pentamethylated epigallocatechin **1** to produce compound **45**. Hydrogenation of compound **45** with Pd/C and H_2 afforded compound **46,** which subsequently reacted with 2-

iodoethan-1-ol in DMF to provide compound **47**. 3-(3-(2-bromoethoxy)-4-methoxybenzamido)-4-fluorobenzoic acid was coupled with pentamethylated epigallocatechin **1** to provide the key intermediate **48**. Compound **48** was then dissolved in 1-methylpiperazine, morpholine, or piperidine and stirred at room temperature to afford basic ring-containing compounds **49**, **50**, and **51**, respectively.

Scheme 4 Synthetic route of compounds **45-51**



Reagents and conditions:
(a) EDCI/DMAP/DCM, 3-(3-(2-bromoethoxy)-4-methoxybenzamido)-4-fluorobenzoic acid, rt;
(b) EDCI/DMAP/DCM, 3-(3-benzyloxy-4-methoxybenzamido)-4-fluorobenzoic acid, rt;
(c) H_2, 10%Pd/c, MeOH, rt;
(d) 2-iodoethan-1-ol, 85^{o}C, DMF.

2.2 Biological evaluation

2.2.1 Structure-activity relationship study of the P-gp modulating activity of methylated EGC, methylated GC, methylated EC and methylated C derivatives

In this study, we have used a P-gp-transfected breast cancer cell line (MDA435/LCC6MDR) to study the structure activity relationship of how catechins modulate the P-gp. MDA435/LCC6MDR cells were about 95.3-, 38.6-, 43.8- and 107.3-fold more resistant to paclitaxel (PTX), DOX, vinblastine and vincristine than the non-transfected parental cells (MDA435/LCC6) (**Table 1 and Table S1**). P-gp modulating activity was measured using a parameter known as relative fold (RF) which is defined as the ratio of IC_{50} towards PTX in MDA435/LCC6MDR cells without 1 μM of modulator relative to that with modulator. Higher RF means higher P-gp modulating activity. A known P-gp modulator verapamil showed weak P-gp modulating activity with RF = 4.0.

A total of 39 methylated EGC, methylated GC, methylated EC and methylated C derivatives were synthesized for studying their P-gp modulating activities (**Table 1**). These new derivatives differ from each other at (1) stereoselectivity at C2 and C3 position of ring C; (2) linker length and rigidity between C3 and ring D or (3) substitutions at ring D.

Natural (-)-EGCG (at either 1 or 10 μM) did not show any significant P-gp-modulating activity with RF = 1.2 (**Table 1**). Peracetylation of EGCG at all OH groups in rings A, B and D did not improve either (RF = 0.9) (**Table 1**). In contrast, permethylation of EGCG, resulted in a significant improvement with RF =7.3 (**Table 1**). These data suggest that O-methylation in rings A, B and D is crucial.

We first investigated if ring D is needed for the P-gp modulating activity. We have compared a pair of catechins with or without ring D in all 6 series (I to VI) and found that addition of ring D can significantly increase the P-gp modulating activity in all 6 series by 2.8-fold (in series II) to 17.8-fold (series IV) (**Table 2**). This result is consistent with our previous observation that ring D was important for the P-gp modulating activity in EGC and GC.[32]

2.2.1.1 Effect of linker length between C3 and ring D and stereochemistry at C2 and C3 on P-gp modulating activity

Catechin is composed of rings A and B and a dihydropyran heterocycle C ring in between. C2 and C3 in ring C contain chiral centers. Ring D is attached to C3 of ring C. The importance of these 2 chiral centers in P-gp modulating activity has not been studied before. Here we have attached a trimethoxylated ring D to C3 of ring C with all 4 possible stereoisomers of EGC (2R, 3R in series I and 2S, 3S in series III) and GC (2R, 3S in series II and 2S, 3R in series IV). The length of the linker between rings D and C was varied from 1 atom to 8 atoms generating 21 compounds (**Table 3**). The linker lengths are as follows: oxycarbonyl (with 1 atom) < oxycarbonylvinyl (with 3 atoms) < oxycarbonylphenylcarbamoyl (with 6 atoms) < oxycarbonylphenylcarbamoylvinyl (with 8 atoms). Their P-gp modulating activities are summarized in **Table 3.** In general, the P-gp modulating activity increased with linker length, up till the linker has reached 6 atoms. Afterwards, the activity would drop when the linker reached 8 atoms in length. This can be observed in all 6 series. In series I: permethyl EGCG, **2**, **4** and **5** with RF = 7.3, 41.2, 46.2 and 24.6. In series II: **7**, **8**, **10** and **11** with RF = 3.1, 13.1, 56.5 and 23.1. In series III: **16**, **18** and **19** with RF = 14.4, 4.1 and 38.1. In series IV: **12**, **14** and

15 with RF = 23.1, 29.9, 56.5. In series V: **29**, **30** and **31** with RF = 12.8, 12.6, 69.3. In series VI: **21**, **23**, **25** and **27** with RF = 10.3, 12.8, 84.7 and 58.7. Overall, the oxycarbonylphenylcarbamyol linker with 6 atoms was the optimal length, yielding the most potent methylated catechin derivatives (EGC, GC, EC and C) as P-gp modulators, irrespective of their stereochemistry at C2 and C3 position.

To study the importance of stereochemistry at C2 and C3 position, we compared the 4 stereoisomers namely series I (2R, 3R-EGC), series II (2R, 3S-GC), series III (2S, 3S-EGC) and series IV (2S, 3R-GC) and the 2 stereoisomers of series V (2R, 3R-EC) and series VI (2R, 3S-C) (**Table 3**). In general, we observed that stereochemistry at C2 and C3 position was important in those weaker modulators with short oxycarbonyl or medium length oxycarbonylvinyl linkers, but not in those with long linkers of oxycarbonylphenylcarbamoyl. When short linker (oxycarbonyl, 1 atom) was used, series IV GC (2S, 3R) displayed the highest P-gp modulating activity (RF=23.1). But when medium linker length (oxycarbonylvinyl, 3 atoms) was used, series I EGC (2R, 3R) exhibited the highest activity (RF=41.2) (**Table 3**). When longest linker (oxycarbonylphenylcarbamoyl, 6 atoms) was used, all stereoisomers have similar activity (RF = 38.1 to 56.5) (**Table 3**). These results suggested that the stereochemistry at C2 and C3 position only matters when shorter linker was used. When longer linker was used, the P-gp modulating activity of all stereoisomers were all highly potent and stereochemistry at C2 and C3 position plays a lesser role. In series V and VI, *trans*-(2R, 3R)-EC and *cis*-(2R, 3S)-C derivatives exhibited similar activity no matter what linker length was conjugated at C3 and ring D (**Table 3**).

2.2.1.2 Effect of linker rigidity on P-gp modulating activity

To study the effect of linker rigidity on P-gp modulation, we designed stereoisomers with various linker flexibility. All of them have the same optimal linker length of 6 atoms. Three levels of linker rigidity were studied: oxycarbonylphenylcarbamyol > *N*-acyl-piperidine-4-carboxylate > *N*-alkyl-piperidine-4-carboxylate (**Table 4**). The CO-NH- (amide bond) in oxycarbonylphenylcarbamyol or *N*-acyl-piperidine-4-carboxylate linker is conformationally rigid whereas *N*-alkyl can freely rotate. In addition, the planar phenyl ring is more constrained than the saturated piperidine ring in the linker. It was found that the strongest linker rigidity (oxycarbonylphenylcarbamoyl) yielded the highest P-gp modulating activity (RF =46.2 to 84.7) in series I, V and VI (**Table 4**). The most flexible linker (*N*-alkyl-piperidine-carboxylate) caused the lowest activity with (RF=9.1 to 15.7) in series I, V and VI (**Table 4**).

Moreover, we found that *N*-atom at either *para* or *meta* positions in piperidine ring of flexible linker had no effect on P-gp modulation, giving similar RF values such as **39** and **40**; **41** and **42**; and **43** and **44** (**Table 1**).

2.2.1.3 Effect of substitutions at phenyl ring D on P-gp modulating activity

Next, we determined if mono-, di- or trimethoxylation on ring D is preferred. Seven groups of compounds with different number of methoxy group on ring D were compared (**Table 5**). They had the same chiral configuration at C2 and C3 positions, linker length/rigidity and number of methoxy groups on ring B (**Table 5**). The number of methoxy substituent at ring D had no essential influence on P-gp modulating activity in those weaker modulators such as **36**, **37**, **38** (RF = 6.7 to 10.3 in series I); **33**, **34**, **35** (RF = 9.1 to 13.3 in series I) or **22**, **23** (RF =

12.2 to 12.8 in series VI) (**Table 5**). For potent modulators with *cis*-configuration, 2R, 3R-EGC **3** (RF = 50.8) and **4** (RF = 46.2) in series I either with di or tri-methoxylation on ring D yielded similar activity. In contrast, other potent modulators with *trans*-configuration in series II and VI, trimethoxylated-substituted phenyl ring D displayed higher P-gp inhibitory activity than dimethoxylated-substituted ring D when comparing 2R, 3S-GC **9** (RF = 33.2) and **10** (RF = 56.5); 2R, 3S-C **24** (RF = 32.4) and **25** (RF = 84.7); or 2R, 3S-C **26** (RF = 29.3) and **27** (RF = 58.7)**,** respectively (**Table 5**).

Other than methoxy substitution at ring D, we also studied the effect of functional group size and polarity in series I (**Table 1**). The 3-methoxy group in compound **3** (RF = 50.8) was replaced by bulky benzyloxy group (**45**, RF = 8.9), there was about 5.7-fold reduction, indicating that smaller substitution is preferred. When replacing by 3-OH group (**46**, RF =6.2), 8.2 folds of diminishment was noted, suggesting that polar functional group was not preferred. These data suggests that methoxy group at ring D with smaller size and non-polarity is a good pharmacophore. When comparing other substituents at C3 position of ring D, 2-bromoethoxy (**48,** RF = 1.6) and 2-hydroxyethoxy (**47,** RF = 15.0), polar hydroxyl group is better than bromide to improve the P-gp modulating activity (**Table 1**).

Not only the size, we also study the hydrophobicity effect on P-gp modulation. Different heterocyclic rings were substituted at *meta* position of ring D and the order of hydrophobicity is as follows: 2-(piperidin-1-yl)ethoxy (**51**) > 2-morpholinoethoxy (**50**) > 2-(4-methylpiperazin-1-yl)ethoxy) (**49**) (**Table 1**). The potency of derivatives was positively correlated with the hydrophobicity of heterocyclic ring. The piperidine ring (**51** with RF = 56.5)

exhibited the highest hydrophobicity and casued the highest RF values, then morpholine (**50** with RF = 37.2) and finally hydrophilic piperazine resulted in the lowest RF value (**49** with RF = 1.4). It is likely believed that more hydrophobic side chain would bind more easily to the transmembrane domain of P-gp than the hydrophilic side chain and finally result in higher potency. Nevertheless, active compound **51** with hydrophobic piperidine ring at the ring D also displayed severe toxicity towards L929 cells (IC_{50} = 5.0 ± 1.7 μM, **Table S2**). We did not select it for further characterization. So far, trimethoxylation at ring D is highly preferred because it retains the high P-gp inhibitory potency of catechin derivatives and causes no toxic effect.

Table 1. P-gp-modulating activity of methylated epigallocatechin, methylated gallocatechin, methylated epicatechin and methylated catechin derivatives.

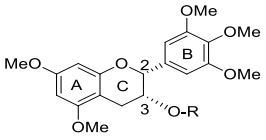
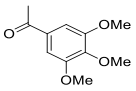
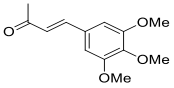
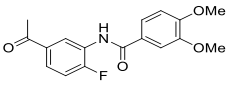
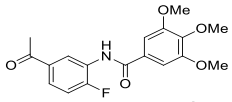
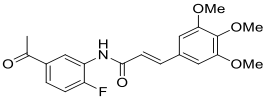
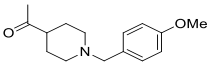
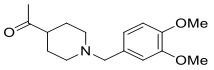
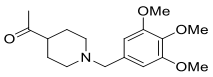
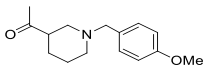
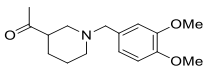
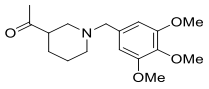
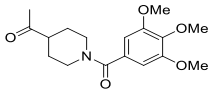
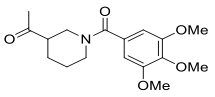
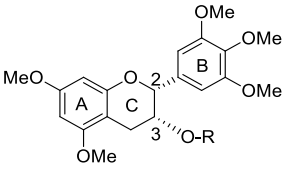
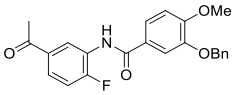
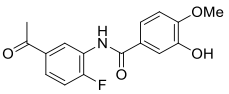
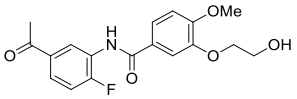
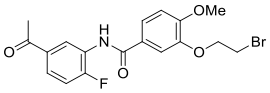
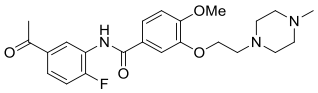
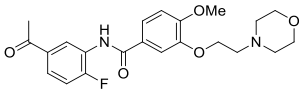
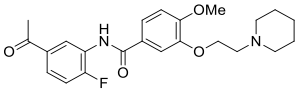
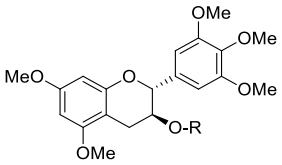
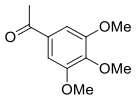
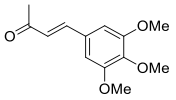
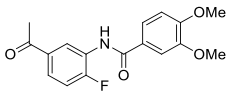
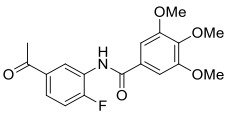
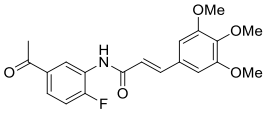
Series	Cpds	R group	No. of atom between C3 and ring D	P-gp modulating activity mean IC_{50} of PTX (nM)	RF
LCC6MDR	0.1% DMSO	/	/	152.5 ± 9.7	1.0
LCC6	0.1% DMSO	/	/	1.6 ± 0.3	95.3
	Verapmail	/	/	38.0 ± 7.0	4.0
	EGCG (1 μM)[a]	/	1	124.1 ± 13.7[a]	1.2
	EGCG (10 μM)[a]	/	1	122.6 ± 29.0[a]	1.2
	peracetyl EGCG[a]	/	1	176.1 ± 31.7[a]	0.9
I (2R, 3R) *cis*-methylated EGC derivatives 	**1**[a]	H	0	155.2 ± 28.1[a]	1.0
	permethyl EGCG[a]		1	21.0 ± 2.8[a]	7.3
	2[a]		3	3.7 ± 0.9[a]	41.2
	3[a]		6	3.0 ± 0.6[a]	50.8
	4[a]		6	3.3 ± 0.6[a]	46.2
	5[a]		8	6.2 ± 0.7[a]	24.6
	33		6	11.5 ± 1.2	13.3
	34		6	16.8 ± 3.7	9.1
	35		6	15.2 ± 1.2	10.0
	36		5	14 ± 2.1	10.9
	37		5	14.8 ± 1.4	10.3
	38		5	22.9 ± 5.0	6.7
	39		6	44.7 ± 4.2	3.4
	40		5	49.1 ± 6.2	3.1

Table 1……. to be continued.

Series	Cpds	R group	No. of atom between C3 and ring D	P-gp modulating activity mean IC_{50} of PTX (nM)	RF
I (2R, 3R) *cis*-methylated EGC derivatives 	**45**		6	17.1 ± 5.0	8.9
	46		6	24.7 ± 3.0	6.2
	47		6	10.2 ± 2.5	15.0
	48		6	93.7 ± 8.6	1.6
	49		6	107.7 ± 15.3	1.4
	50		6	4.1 ± 0.9	37.2
	51		6	2.7 ± 0.7	56.5
II (2R,3S) *trans*-methylated GC derivatives 	**6**[a]	H	0	135.9 ± 17.9[a]	1.1
	7[a]		1	49.0 ± 30.5[a]	3.1
	8[a]		3	11.6 ± 0.7[a]	13.1
	9[a]		6	4.6 ± 0.5[a]	33.2
	10[a]		6	2.7 ± 0.6[a]	56.5
	11[a]		8	4.2 ± 0.7[a]	36.3

252 Table 1……. to be continued.

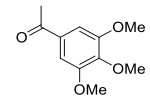
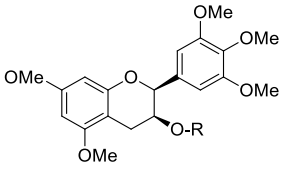
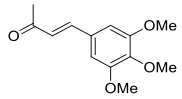
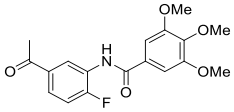
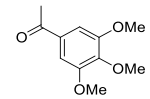
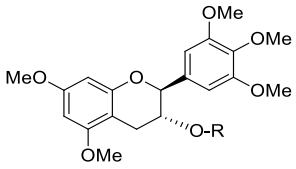
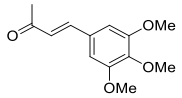
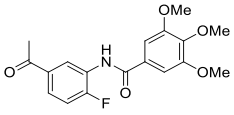
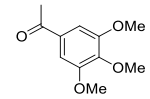
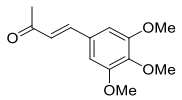
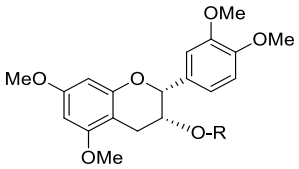
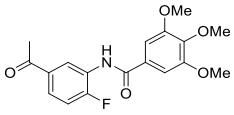
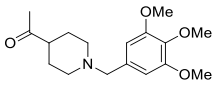
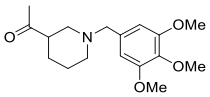
Series	Cpds	R group	No. of atom between C3 and ring D	P-gp modulating activity mean IC_{50} of PTX (nM)	RF
	17	H	0	154.2 ± 15.7	1.0
III					
	16		1	10.6 ± 1.4	14.4
(2S, 3S) *cis*-methylated EGC derivatives 	**18**		3	36.9 ± 5.8	4.1
	19		6	4.0 ± 0.3	38.1
	13	H	0	116 ± 9.1	1.3
IV					
	12		1	6.6 ± 1.1	23.1
(2S, 3R) *trans*-methylated GC derivatives 	**14**		3	5.1 ± 0.7	29.9
	15		6	2.7 ± 0.4	56.5
	28	H	0	120 ± 4.5	1.3
	29		1	11.9 ± 1.5	12.8
V					
	30		3	12.1 ± 0.9	12.6
(2R,3R) *cis*-methylated EC derivatives 	**31**		6	2.2 ± 0.1	69.3
	41		6	16.6 ± 4	9.2
	42		5	18.6 ± 2.5	8.2

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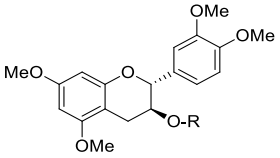
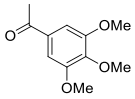
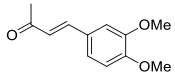
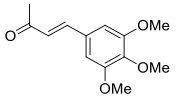
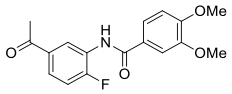
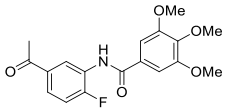
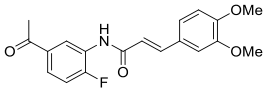
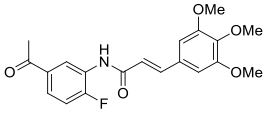
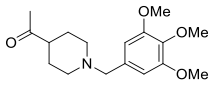
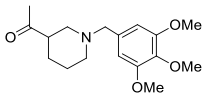
Table 1……. to be continued.

Series	Cpds	R group	No. of atom between C3 and ring D	P-gp modulating activity mean IC_{50} of PTX (nM)	RF
VI (2R,3S) *trans* -methylated C derivatives 	**20**	H	0	117.5 ± 9.4	1.3
	21		1	14.8 ± 1.7	10.3
	22		3	12.5 ± 0.4	12.2
	23		3	11.9 ± 1.8	12.8
	24		6	4.7 ± 1.1	32.4
	25		6	1.8 ± 0.2	84.7
	26		8	5.2 ± 0.6	29.3
	27		8	2.6 ± 0.1	58.7
	43		6	9.7 ± 2.3	15.7
	44		5	8.4 ± 2.2	18.2

Methylated EGC, GC, EC and C derivatives are divided into six series with their R group indicated in the Table. P-gp modulating activity was measured by determining IC_{50} towards PTX in P-gp overexpressing LCC6MDR cells in the absence or presence of 1.0 μM of modulator. Relative Fold (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. Each experiment was repeated three times independently and average RF is presented. The IC_{50} presented as mean ± standard error of mean. [a] IC_{50} values of these compounds had been published [32] and included

here for comparison. Compound **1** was named as [(**8)** in J Med Chem, 2015, 58, 4529−4549], **2**(**23**), **3**(**35**), **4**(**36**), **5**(**31**), **6**(**44**), **7**(**43**), **8**(**49**), **9**(**50**), **10**(**51**) and **11**(**53**).[32] LCC6 and LCC6MDR cells incubated with 0.1% DMSO were solvent control. The chemical structures of compounds are shown in Supporting Information.

Table 2. Effect of ring D on P-gp modulating activity of methylated EGC, GC, EC and C derivatives.

Series	*Trans* /*Cis* Configuration	Derivatives	No. of methoxy group in ring B	No. of methoxy group in ring D	Linker used	Cpds	RF
Series I	2R, 3R	EGC	3	No ring D	oxycarbonyl	**1**	1.0
Series I	2R, 3R	EGC	3	3	oxycarbonyl	permethyl EGCG	7.3
Series II	2R, 3S	GC	3	No ring D	oxycarbonyl	**6**	1.1
Series II	2R, 3S	GC	3	3	oxycarbonyl	**7**	3.1
Series III	2S, 3S	EGC	3	No ring D	oxycarbonyl	**17**	1.0
Series III	2S, 3S	EGC	3	3	oxycarbonyl	**16**	14.4
Series IV	2S, 3R	GC	3	No ring D	oxycarbonyl	**13**	1.3
Series IV	2S, 3R	GC	3	3	oxycarbonyl	**12**	23.1
Series V	2R, 3R	EC	2	No ring D	oxycarbonyl	**28**	1.3
Series V	2R, 3R	EC	2	3	oxycarbonyl	**29**	12.8
Series VI	2R, 3S	C	2	No ring D	oxycarbonyl	**20**	1.3
Series VI	2R, 3S	C	2	3	oxycarbonyl	**21**	10.3

For easy analysis of effect of ring D on P-gp modulating activity of derivatives, the RF values of respective compounds were extracted from Table 1.

Table 3. Effect of linker length and stereochemistry on P-gp modulating activity of methylated EGC, GC, EC and C derivatives.

Series	*Trans* /*Cis* Configuration	Derivatives	No. of methoxy group in ring B	No. of methoxy group in ring D	**Different linker length between C3 and ring D**							
					Oxycarbonyl (1 atom)	RF	**Oxycarbonylvinyl (3 atoms)**	RF	**Oxycarbonyl-phenylcarbamoyl (6 atoms)**	RF	**Oxycarbonyl-phenylcarbamoylvinyl (8 atoms)**	RF
Series I	2R, 3R	EGC	3	3	**Permethyl EGCG**	7.3	**2**	41.2	**4**	46.2	**5**	24.6
Series II	2R, 3S	GC	3	3	**7**	3.1	**8**	13.1	**10**	56.5	**11**	23.1
Series III	2S, 3S	EGC	3	3	**16**	14.4	**18**	4.1	**19**	38.1	/	/
Series IV	2S, 3R	GC	3	3	**12**	23.1	**14**	29.9	**15**	56.5	/	/
Series V	2R, 3R	EC	2	3	**29**	12.8	**30**	12.6	**31**	69.3	/	/
Series VI	2R, 3S	C	2	3	**21**	10.3	**23**	12.8	**25**	84.7	**27**	58.7

For easy analysis of effect of linker length and stereochemistry on P-gp modulating activity of derivatives, the RF values of respective compounds were extracted from Table 1. /: not determined.

Table 4. Effect of linker rigidity on P-gp modulating activity of methylated EGC, GC, EC and C derivatives.

Series	*Trans* /*Cis* Configuration	Derivatives	No. of methoxy in ring B	No. of methoxy in ring D	Different linker rigidity					
					***		**		*	
					Oxycarbonylphenyl carbamoyl (6 stoms)	RF	N-acyl-piperidine-4-carboxylate (6 atoms)	RF	N-alkyl-piperidine-4-carboxylate (6 atoms)	RF
Series I	2R, 3R	EGC	3	2	**3**	50.8	/	/	**34**	9.1
Series I	2R, 3R	EGC	3	3	**4**	46.2	**39**	3.4	**35**	10.0
Series V	2R, 3R	EC	2	3	**31**	69.3	/	/	**41**	9.2
Series VI	2S, 3R	C	2	3	**25**	84.7	/	/	**43**	15.7

For easy analysis of effect of linker rigidity on P-gp modulating activity of derivatives, the RF values of respective compounds were extracted from Table 1. ***: strong linker rigidity, ** medium level of linker rigidity and * weak linker rigidity. /: not determined.

Table 5. Effect of methoxy substitution at ring D on P-gp modulating activity of methylated EGC, GC, EC and C derivatives.

Series	*Tans/Cis* Configuration	Derivatives	Linker used	No. of methoxy in ring B	No. of methoxy in ring D	Cpds	RF
Series I	2R, 3R	EGC	*N* -alkyl-piperidine-4-carboxylate (5 atoms)	3	1	**36**	10.3
Series I	2R, 3R	EGC	*N* -alkyl-piperidine-4-carboxylate (5 atoms)	3	2	**37**	10.3
Series I	2R, 3R	EGC	*N* -alkyl-piperidine-4-carboxylate (5 atoms)	3	3	**38**	6.7
Series I	2R, 3R	EGC	*N* -alkyl-piperidine-4-carboxylate (6 atoms)	3	1	**33**	13.3
Series I	2R, 3R	EGC	*N* -alkyl-piperidine-4-carboxylate (6 atoms)	3	2	**34**	9.1
Series I	2R, 3R	EGC	*N* -alkyl-piperidine-4-carboxylate (6 atoms)	3	3	**35**	10.0
Series I	2R, 3R	EGC	Oxycarbonylphenylcarbamoyl (6 atoms)	3	2	**3**	50.8
Series I	2R, 3R	EGC	Oxycarbonylphenylcarbamoyl (6 atoms)	3	3	**4**	46.2
Series II	2R, 3S	GC	Oxycarbonylphenylcarbamoyl (6 atoms)	3	2	**9**	33.2
Series II	2R, 3S	GC	Oxycarbonylphenylcarbamoyl (6 atoms)	3	3	**10**	56.5
Series VI	2R, 3S	C	Oxycarbonylvinyl (3 atoms)	2	2	**22**	12.2
Series VI	2R, 3S	C	Oxycarbonylvinyl (3 atoms)	2	3	**23**	12.8
Series VI	2R, 3S	C	Oxycarbonylphenylcarbamoyl (6 atoms)	2	2	**24**	32.4
Series VI	2R, 3S	C	Oxycarbonylphenylcarbamoyl (6 atoms)	2	3	**25**	84.7
Series VI	2R, 3S	C	Oxycarbonylphenylcarbamoylvinyl (with 8 atoms)	2	2	**26**	29.3
Series VI	2R, 3S	C	Oxycarbonylphenylcarbamoylvinyl (with 8 atoms)	2	3	**27**	58.7

For easy analysis of effect of methoxylation at ring D on P-gp modulating activity of derivatives, the RF values of respective compounds were extracted from Table 1.

2.2.2 EC_{50} and selective index values of methylated GC, C and EC derivatives for reversing multidrug resistance in LCC6MDR

Four potent compounds with RF > 50 were chosen for further characterization in terms of their effective concentration (EC_{50}) in reversing P-gp mediated drug resistance and their selective index (**Table 5**) including (2S, 3R)-*trans*-methylated GC **15,** (2R, 3R)-*cis*-methylated EC **31**, and (2R, 3S)-*trans*-methylated C **25, 27**. It is desirable for modulators to affect only LCC6MDR, but not the normal cells. Selective index may be used as a safety indicator of a new compound. We therefore determined the selective index of modulators by dividing IC_{50} of modulators in L929 by the EC_{50} of modulator for reversing drug resistance in LCC6MDR cells.

EC_{50} values for reversing P-gp mediated resistance towards PTX, vinblastine, vincristine and DOX resistance in LCC6MDR cells ranged from 32 to 178 nM (**Table 5**). Their selective indices ranged from > 563 to > 1112 which was higher than verapamil (selective index = 200). (2R, 3S)-*trans*-methylated C **25** and (2R, 3R)-*cis*-methylated EC **31** with tri-methoxy substituents at ring D and oxycarbonylphenylcarbamoyl linker between ring D and C3 position were the most potent with EC_{50} ranging from 32 nM to 93 nM. Cyclosporin A showed moderate cytotoxicity towards L929 cell, but our compounds did not. After considering the toxicity itself, the selective indices of **25** (> 1112) and **31** (>1078) are highly comparable to cyclosporine A with the selective index of 934. Overall, our modulators are non-toxic and effective P-gp modulators.

Table 6. EC_{50} of potent methylated GC, methylated EC and methylated C derivatives for reversing multidrug resistance in LCC6MDR cells.

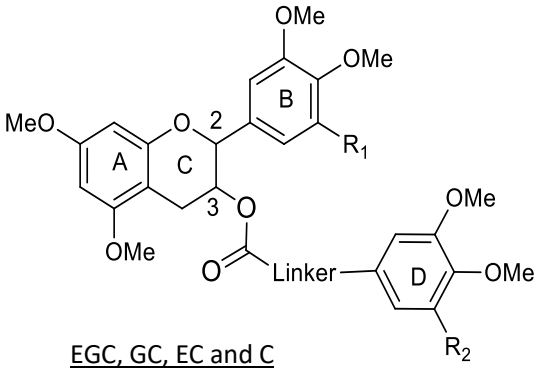
Cpds	L929 (IC_{50}, μM)	Selective index (relative to EC_{50} of PTX)	Mean EC_{50} (nM) for reversing drug resistance using LCC6MDR cells			
			PTX	DOX	Vinblastine	Vincristine
15	>100	>741	135.0 ± 5.0	ND	ND	ND
25	>100	>1112	89.9 ± 3.5	31.8 ± 10.9	60.0 ± 15.1	66.0 ± 4.0
27	>100	>563	177.5 ± 2.5	ND	ND	ND
31	>100	>1078	92.8 ± 5.4	37.3 ± 4.3	60.7 ± 5.5	77.7 ± 6.7
Verapamil	89.2±8.2[a]	200[a]	445.7 ± 40.7[a]	254.4 ± 22.9	502.5 ± 91.7	385.0 ± 35.1
Cyclosporin A	29.9±5.7[a]	934[a]	32.0 ± 1.0[a]	ND	ND	ND

EC_{50} values were presented as mean ± standard error of mean. N = 3 - 8 independent experiments. Selective index value = (IC_{50} of modulators towards L929 fibroblasts) / (EC_{50} of modulators for reversing PTX resistance in LCC6MDR cells). ND = not determined. [a] the IC_{50} values, EC_{50} values and selective index values of verapamil and cyclosporin A had been published.[32]

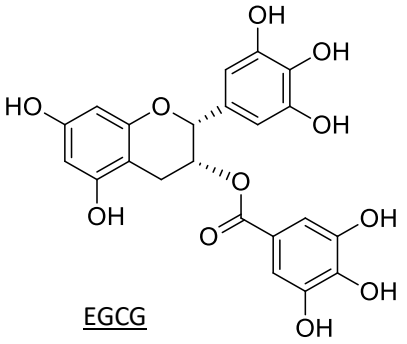
2.2.3 Effect of number of methoxy group at rings B and D on P-gp modulating activity of EGC and EC as well as GC and C derivatives

EGC **3**, **4** and EC **31** (2R, 3R-configuration) as well as GC **9**, **10** and C **25** (2R, 3S-configuration) derivatives were structurally similar (**Table 7**). All of them possessed the optimal oxycarbonylphenylcarbamyol linker and O-methylated A, B and D rings. Surprisingly, there was a correlation between the EC_{50} for reversing PTX resistance and number of methoxy group at B and D rings (**Table 7**): EC **31** (EC_{50} = 93 nM with 2 methoxy groups at B ring + 3 methoxy groups at D ring) < EGC **3** (EC_{50} = 159 nM with 3 methoxy groups at B ring + 2 methoxy groups at D ring) < EGC **4** (EC_{50} = 214 nM with 3 methoxy groups at B ring + 3 methoxy at D ring). EC **31** was about 1.7- to 2.3-fold more potent than EGC **3** and **4**. Similarly, C **25** (EC_{50} = 90 nM with 2 methoxy groups at B ring + 3 methoxy groups at D ring) < GC **10** (EC_{50} = 140 nM with 3 methoxy groups at B ring + 3 methoxy group at D ring) < GC **9** (EC_{50} = 171 nM with 3 methoxy groups at B ring + 2 methoxy groups at D ring). C **25** displayed about 1.6- to 1.9-fold higher potency than GC **9** and **10**, respectively. It suggests that the number of methoxy group at B and D rings can affect the P-gp modulating activity. Derivatives EC **31** and C **25** containing dimethoxylated B ring and trimethoxylated D ring gave the highest P-gp inhibitory activity as compared to other EGC and GC **3, 4**, **9** and **10** which had trimethoxylation at ring B and either di- or tri-methoxylation at ring D. It suggests that dimethoxylation at ring B is an important pharmacophore of catechins for strong P-gp modulation.

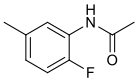
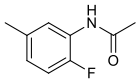
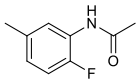
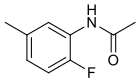
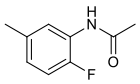
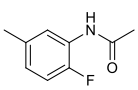
Table 7. Effect of number of methoxy group at rings B and D on P-gp modulating activity of EGC and EC as well as GC and C derivatives.



EGC, GC, EC and C



EGCG

Cpds	R_1	R_2	Linker	Position C2	Position C3	Mean EC_{50} (nM) for reversing PTX resistance in LCC6MDR cells
EGCG	/	/	/	/	/	>1000
EGC **4**	OMe	OMe		R	R	214 ± 25[a]
EGC **3**	OMe	H		R	R	159 ± 23[a]
EC **31**	H	OMe		R	R	93 ± 5
GC **10**	OMe	OMe		R	S	140 ± 0[a]
GC **9**	OMe	H		R	S	171 ± 11[a]
C **25**	H	OMe		R	S	90 ± 4

EC_{50} values for reversing PTX resistance were presented as mean ± standard error of mean. N = 3-8 independent experiments. [a] EC_{50} values of compounds **3**, **4**, **9** and **10** had been published.[32]

2.2.4 MRP1- and BCRP-modulating activity of methylated C 25 and methylated EC 31 derivatives

We have also determined the selectivity of methylated C **25** and methylated EC **31** towards P-gp, MRP1 and BCRP transporters. They can transport a broad range of drugs out of cell with the aid of ATP hydrolysis. MRP1 transfected ovarian cancer cell line 2008/MRP1 and its wild type 2008/P, and BCRP transfected human kidney embryonic cell line HEK293/R2 and empty vector-transfected

337 HEK293/pcDNA3.1 were employed. 2008/MRP1 was about 7.1-fold more resistant to DOX than
338 2008/P cells (**Table 8**), whereas HEK293/R2 displayed about 18.7-fold higher level of topotecan
339 resistance than HEK293/pcDNA3.1 cells (**Table 8**). **4e** is a flavonoid homodimer and reported to have
340 potent MRP1-modulating activity with a RF of 17.7.[37] As shown in **Table 8**, compounds **25** and **31**
341 displayed no MRP1-modulating activity. Ko143 is a known specific BCRP modulator and it resulted in
342 a high RF value of 17.5. Compounds **25** and **31** displayed low BCRP-modulating activity (RF = 2.9 and
343 6.5) (**Table 8**). On the contrary, they specifically exhibited high P-gp modulating activity (RF = 69.3
344 and 84.7) (**Table 8**). Therefore, (2R, 3S) *trans*-methylated C **25** and (2R, 3R) *cis*-methylated EC **31**
345 derivative are likely strong P-gp inhibitor but weak BCRP inhibitor.

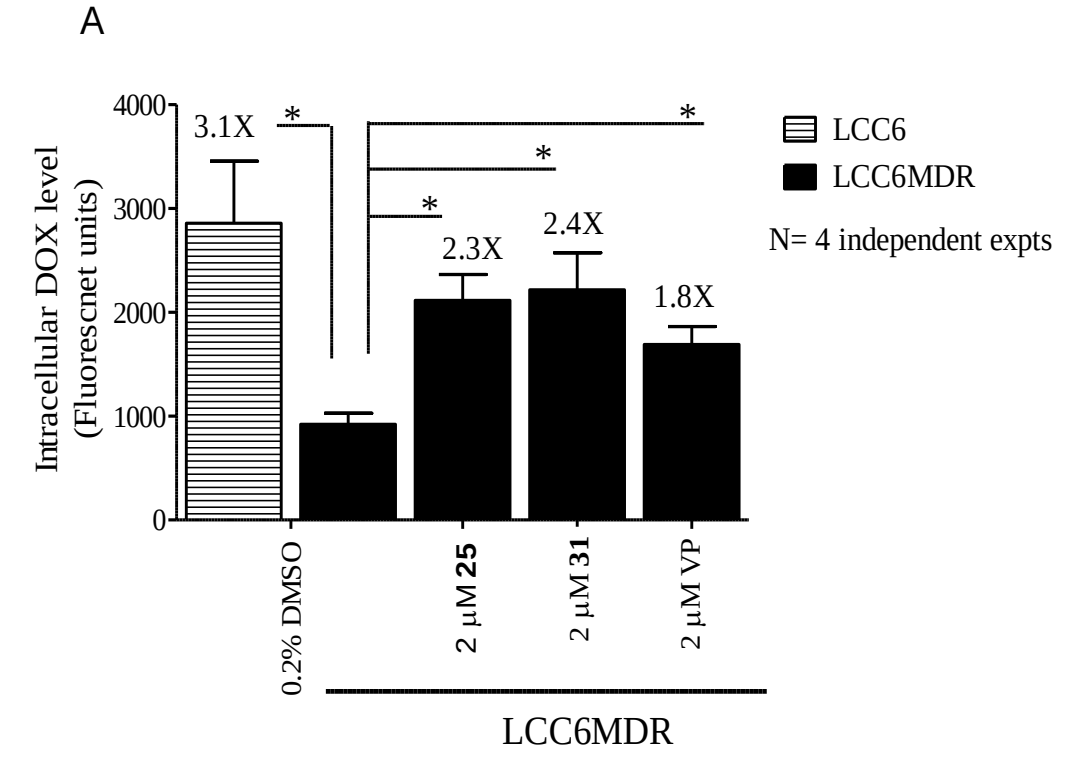
346 **Table 8.** MDR modulating activity of compounds **25** and **31**.

Cpds	MRP1-modulating activity in 2008/MRP1		BCRP-modulating activity in HEK293/R2		P-gp-modulating activity in LCC6MDR	
	IC_{50} of DOX (nM)	RF	IC_{50} of Topotecan (nM)	RF	IC_{50} of PTX (nM)	RF
Control	426.5 ± 134.8	1.0	295.6 ± 54.2	1.0	152.5 ± 9.7	1.0
1 μM **25**	353.7 ± 148.0	1.2	45.5 ± 14.6	6.5	1.8 ± 0.2	84.7
1 μM **31**	341.1 ± 128.2	1.3	100.8 ± 28.6	2.9	2.2 ± 0.1	69.3
1 μM **4e**	24.1 ± 10.6	17.7	/	/	/	/
1 μM Ko143	/	/	16.9 ± 3.1	17.5	/	/
1 μM verapamil	/	/	/	/	38.0 ± 7.0	4.0
2008/P	60.3 ± 5.0	7.1	/	/	/	
HEK293/pcDNA3.1	/	/	15.8 ± 1.5	18.7	/	/
LCC6	/	/	/	/	1.6 ± 0.3	95.3

348 MDR modulating activity of **25** and **31** (all at 1.0 μM) were investigated using 2008/MRP1, HEK293/R2 and LCC6MDR, respectively (N = 2-4 independent
349 experiments and the values are presented as mean ± standard error of mean). **4e,** Ko143 and verapamil (tested at 1 μM) are specific MRP1, BCRP and P-
350 gp modulator, respectively. IC_{50} towards DOX in 2008/MRP1 cell lines, IC_{50} towards topotecan in HEK293/R2 and IC_{50} towards PTX in LCC6MDR were
351 determined with or without modulators to determine RF. IC_{50} were also determined for their parental cell lines (2008/P, HEK293/pcDNA3.1 and LCC6)
352 for reference. /: not determined.

2.2.5 Methylated C 25 and methylated EC 31 derivatives increases DOX and rhodamine 123 accumulation by inhibiting transport activity of P-gp

DOX and rhodamine 123 are known fluorescent P-gp substrates and their fluorescence levels can be used for monitoring intracellular drug concentration. We found that LCC6 cells accumulated about 3.1-fold ($P < 0.05$) more DOX and 5.2-fold ($P < 0.05$) more rhodamine 123 than LCC6MDR cells (**Figure 2A** and **2B**). Treatment of LCC6MDR cells with 2 μM of **25**, **31** or verapamil can significantly increase DOX accumulation by 2.3-, 2.4 and 1.8-fold (**Figure 2A**) or rhodamine 123 accumulation by 3.5-, 3.5- and 1.3-fold (**Figure 2B**). It is suggesting that methylated C **25** and methylated EC **31** can inhibit the functionality of P-gp, restore the drug concentration and finally re-sensitize the LCC6MDR cells to the anticancer drug again.



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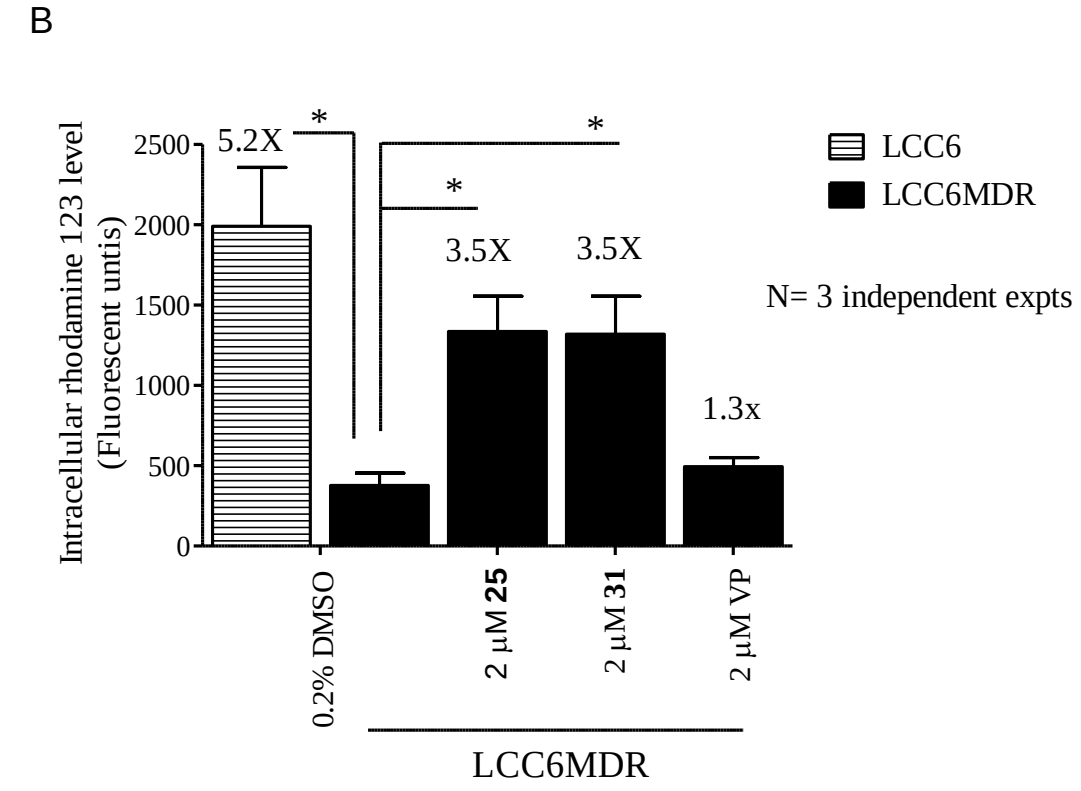


364

365 **Figure 2.** Effect of compounds **25** and **31** on DOX and rhodamine 123 accumulation in LCC6

366 and LCC6MDR cells.

367 LCC6 or LCC6MDR cells were incubated with 20 μM DOX (A) and 10 μg/mL rhodamine 123
368 (B) with or without 2 μM of modulators (**25, 31**, or verapamil) for 150 minutes at 37ºC. 0.2%
369 of DMSO was used as negative control. After the incubation period, cells were lysed and the
370 supernatant was saved for measuring the DOX and rhodamine 123 level by spectrofluorometry.
371 N = 3-4 independent experiments. The values are presented as mean ± standard error of mean.
372 * $P < 0.05$ relative to the LCC6MDR negative control.

373

374 ***2.2.6 Methylated C 25 and methylated EC 31 have no effect on plasma membrane P-gp level***

375 Without modulator, LCC6MDR displayed about 8.0-fold higher plasma membrane P-gp
376 level than its parental cell line LCC6. After treating LCC6MDR cells with compounds **25** or
377 **31** at 1 or 2 μM for 48 hrs, the level of P-gp had slightly increased (**Figure 3**), suggesting that
378 these potent methylated C and EC derivatives do not decrease the plasma membrane level of
379 P-gp. They rather than inhibit the functionality of P-gp transporter to increase the intracellular
380 drug accumulation (**Figure 2**) and finally re-sensitize the cells to anticancer drugs again
381 (**Tables 1**).

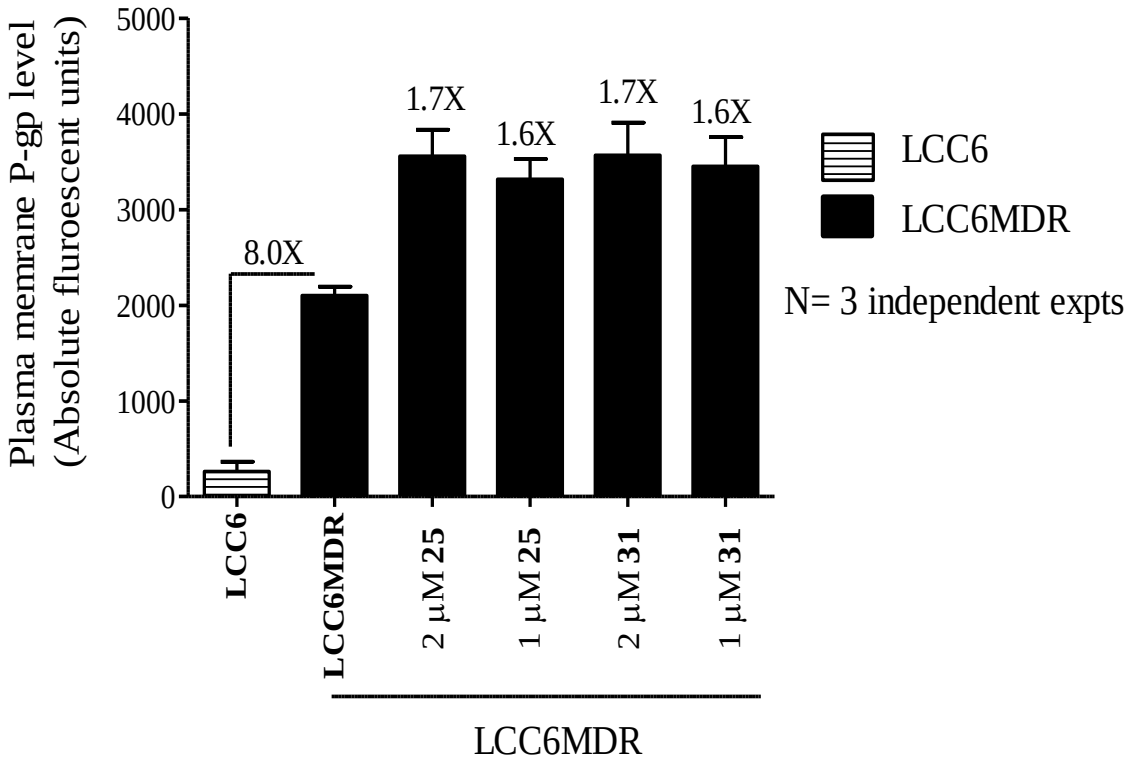


Figure 3. Effect of compounds **25** and **31** on plasma membrane P-gp protein levels in LCC6 and LCC6MDR cells.

$1x10^6$ cells of LCC6 and LCC6MDR were incubated with 2 μM and 1 μM of **25** or **31** for 48 hrs at 37ºC with 5% CO_2. After 48 hrs, the cells were incubated with vinblastine and PE-labelled human P-gp antibody for 1 hr at 37ºC. The level of P-gp was determined by flow cytometery. N = 3 independent experiment and each treatment was duplicated in every experiment. 0.2% DMSO was the negative control.

2.2.7 Methylated C 25 inhibited DOX efflux in LCC6MDR cells.

We then performed experiment to determine whether the increased DOX retention in LCC6MDR cells caused by **25** was due to inhibition of DOX efflux (**Figure 2A**). In the efflux experiment, the DOX pre-loaded cells were incubated with or without 2 μM of compound **25**.

After 0, 15, 30, 60, 90 and 120 min, the amount of DOX remained inside the cells was measured by flow cytometry. In the absence of **25**, the intracellular DOX level of wild type LCC6 cells kept 100% from 0 min to 120 min, indicating that LCC6 cells had no DOX efflux (**Figure 4)**. In contrast, the intracellular DOX level of LCC6MDR cells was gradually reduced from 100% at 0 min to 35% at 120 min (**Figure 4**), indicating that the efflux rate of LCC6MDR cells was higher than the wild type. This difference in efflux rate may explain why LCC6MDR cells had less accumulation and were resistant to DOX as compared to the wild type. In the presence of 2 μM of **25**, DOX efflux rate kept the same in the wild type, whereas in LCC6MDR cells, the DOX efflux rate was almost inhibited. After 60, 90 and 120 min, the intracellular DOX levels still retained 87% ($P < 0.01$), 89% ($P < 0.01$) 90% ($P < 0.01$) in LCC6MDR cells, respectively (**Figure 4)**. The above results demonstrate that reversal of DOX resistance by **25** is due to an inhibition of P-gp mediated drug efflux, leading to an increased drug accumulation and thus restoring the drug sensitivity.

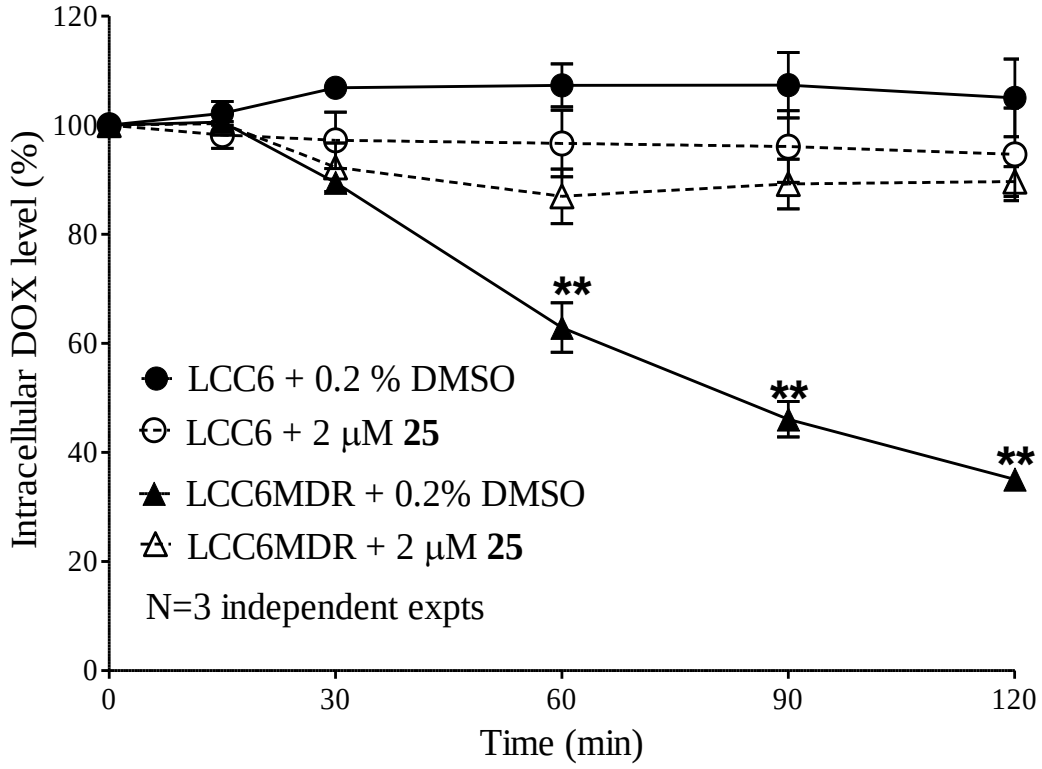


Figure 4. Effect of compound **25** on DOX efflux in LCC6 and LCC6MDR cells. DOX pre-loaded cells were incubated with or without compound **25** (2 μM) at 37ºC. At 0, 15, 30, 60, 90 and 120 min, cells were harvested and intracellular DOX concentration was measured by flow cytometer at FL-2 channel. The values were presented as mean ± standard error of mean. N = 3 independent experiments. Student paired t test was conducted at each time point in LCC6MDR cells after incubating with or without **25**. ** $P < 0.01$.

3. DISCUSSION AND CONCLUSION

In the present study, a total of 39 novel methylated EGC, methylated GC, methylated EC and methylated C derivatives were synthesized and evaluated for their P-gp modulating activities in a P-gp overexpressing breast cancer cell line LCC6MDR. EGCG is a natural compound and abundantly found in green tea. It has a lot of beneficial properties such as antibacterial, anticancer, antioxidant and antiatherogenic.[38-40] Its effect on P-gp modulation has been firstly reported in 2002.[40] In such study, EGCG at 50 μM potentiated the cytotoxicity of vinblastine in P-gp overexpressing cell line CH^RC5 cells and resulted in low IC_{50} value as its wild type.[40] EGCG is a potential agent to reverse MDR in cancer, however, its high effective concentration preclude it from further development. In order to improve its P-gp inhibitory potency, we firstly replaced all –OH groups in A, B and D rings with -OAc and -OMe groups (**Figure 5A**).[32] Only permethylation but not peracteylation yielded 7.3-fold improvement and therefore, permethyl EGCG became our parent compound for further structural modification (**Figure 5A**).[32] Importantly, removal of ring D from permethyl EGCG completely resulted in no activity (**1** with RF =1.0), indicating that ring D is an essential pharmacophore (**Figure 5A**).

Secondly, the oxycarbonyl (1 atom) linker located between C3 and ring D in the parent compound permethyl EGCG was substituted by different length linkers including oxycarbonylvinyl (3 atoms in compound **2**), oxycarbonylphenylcarbamoyl (6 atoms in compound **4**) and oxycarbonylphenylcarbamoyllvinyl (8 atoms in compound **5**). It was demonstrated that linker length played an important role in controlling P-gp modulating

activity and oxycarbonylphenylcarbamoyl (6 atoms) linker was the optimal linker to give the highest RF values (compound **4** with RF = 46.2) (**Figure 5A**). After the above two structural modifications, nonactive EGCG (RF= 1.0) has been significantly improved by 46 folds. Further replacing the linker of the potent EGC **4** by flexible linkers such as *N*-acyl-piperidine-4-carboxylate (**39**) and *N*-alkyl-piperidine-4-carboxylate (**35**) caused poor activity (**Figure 5A**). Once again, oxycarbonylphenylcarbamoyl linker with optimal length and rigidity is the most preferable for making P-gp modulator. In future, EGC derivatives with more rigid linkers than oxycarbonylphenylcarbamyol should be made in order to get more hints on the effect of linker rigidity on P-gp modulation.

Stereochemistry could influence biological activity of catechins. It has been reported that *cis*-EGCG has higher potency than *trans*-GCG in inhibiting glucose-stimulated insulin secretion from pancreas β-cell [41] and killing colorectal cancer cells.[39] For P-gp modulation, we have synthesized four stereoisomers of (2R, 3R and 2S, 3S)-EGC and (2R, 3S and 2S, 3R)-GC. Stereochemistry only influence weaker modulators such as oxycarbonyl and oxycarbonylvinyl linked EGC and GC, but not the potent oxycarbonylphenylcarbamoyl linked stereoisomers (**Table 3** and **Figure 5B**).

It has been reported minor component of green tea ECG and CG derivatives were better than major component EGCG in suppressing pancreatic tumor growth.[42] In order to further improve the activity of 2R ,3R-EGC **4** (RF = 46.2), a *cis*-(2R, 3R)-EC **31** and a *trans*-(2R, 3S)-C **25** with identical structure as EGC **4** except for dimethoxylation at ring B were synthesized. A 1.5- and 1.8- fold increase in RF was noted, respectively (**Figure 5B**). The effective

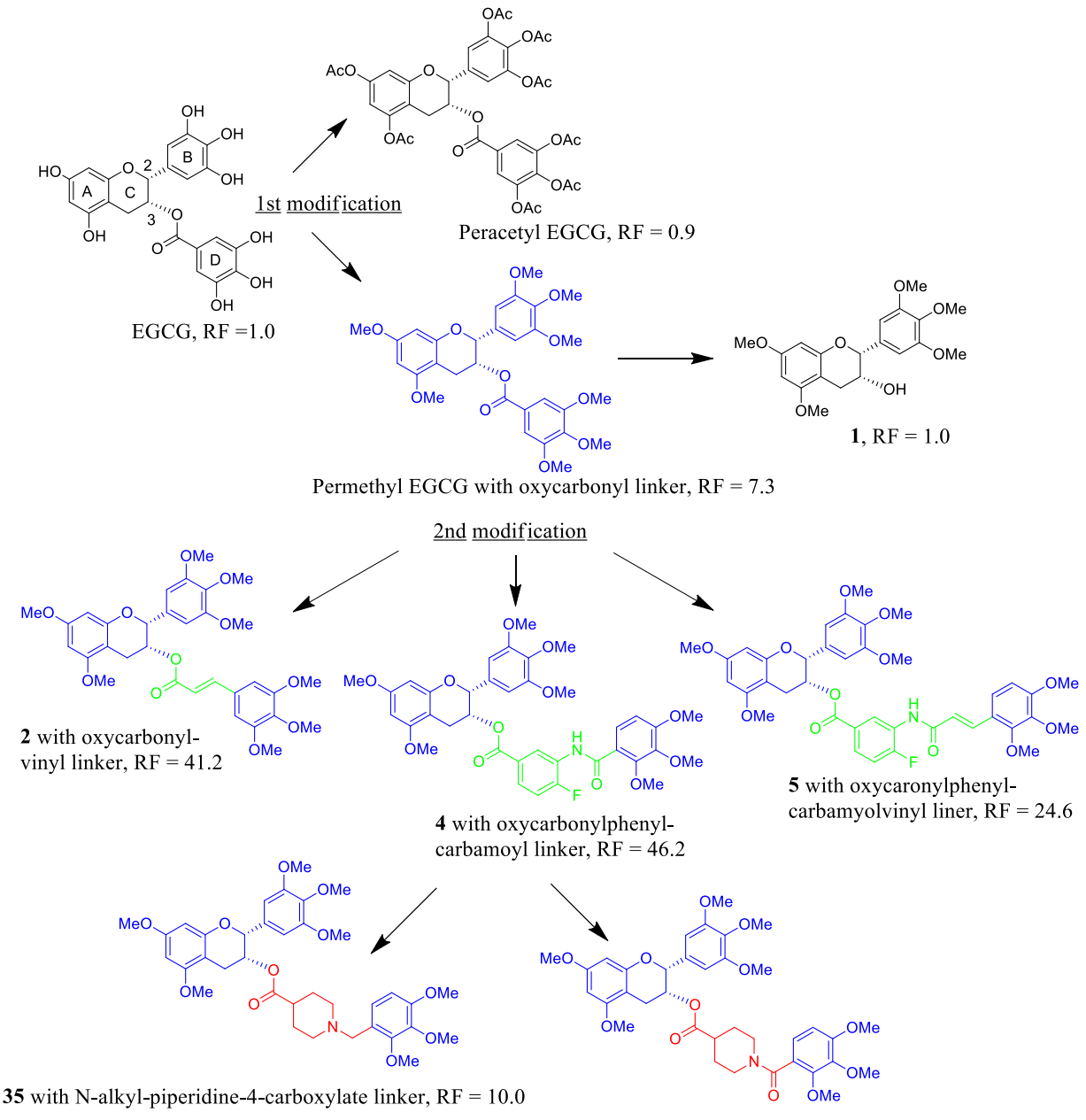
concentration (EC_{50}) of EC **31** and C **25** were about 2.3-fold lower than EGC **4** for reversing PTX-mediated resistance (**Figure 5B** and **Table 7**). It is believed that the number of methoxy group in ring B might be a crucial factor to control P-gp modulating activity of catechins. Therefore, it is suggesting that 2S, 3S-EC and 2S, 3R-C derivatives should be synthesized to study their activity. More modifications at ring B of catechin is also a potential strategy to further potentiate the P-gp inhibitory potency of catechins.

By virtue of detailed SAR, the order of factors for controlling P-gp modulating activity of catechins is as follows: phenyl ring D >> linker length/rigidity between C3 and ring D > methoxy substitution at A, B and D rings > stereochemistry. Four important pharmacophores of catechins for modulating P-gp transporter include (1) phenyl ring D, (2) oxycarbonylphenylcarbamoyl linker with the optimal length and rigidity between ring D and C3, (3) dimethoxylation at ring B and (4) trimethoxylation at ring D. Among the 39 derivatives, two potent compounds, C **25** and EC **31** were found. Compound **25** is a (2R, 3S)-*trans*-methylated C derivative, whereas compound **31** is a (2R, 3R)-*cis*-methylated EC derivative. They were a pair of epimer and possessed di-methoxylation at ring B, tri-methoxylation at ring D and oxycarbonylphenylcarbamoyl linker between ring D and C3 position**.**

The mechanism of methylated C **25** and methylated EC **31** derivatives in reversing P-gp mediated drug resistance is by virtue of inhibiting efflux activity of P-gp transporter (**Figure 4**) and restoring the drug accumulation to a cytotoxic level (**Figure 2**). They did not downregulate the plasma membrane P-gp protein level to enhance the drug retention (**Figure 3**) Compounds **25** and **31** were specific for P-gp with no or weak modulating activity towards

MRP1- and BCRP-mediated drug resistance (**Table 8**). In summary, our study demonstrates that methylated C **25** or methylated EC **31** derivatives are non-toxic, effective and specific P-gp modulators that can be used in future for reversing P-gp mediated clinical cancer drug resistance.

A



B

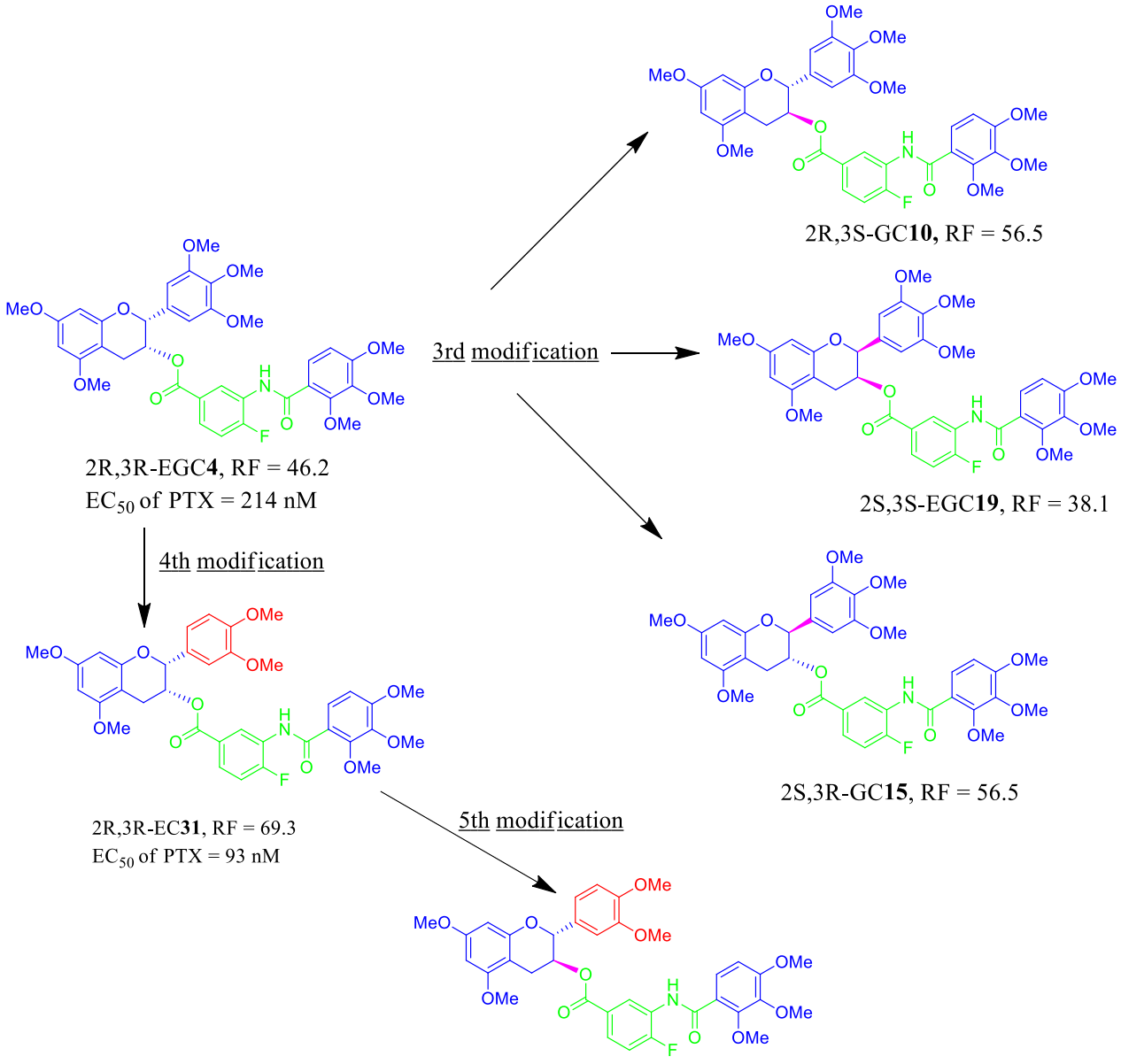


Figure 5. SAR analysis of catechins. (A) Effect of substitution on rings and linker length/rigidity between C3 and ring D on P-gp modulating activity and (B) Effect of stereochemistry on P-gp modulating activity. The RF values at 1 μM of compounds were extracted from Table 1.

EXPERIMENTAL SECTION

4.1. General

Experiments with air and moisture sensitive materials were carried under a nitrogen atmosphere. All solvents were dried and freshly distilled prior to use. Tetrahydrofuran was distilled from benzophenone and sodium immediately prior to use. Anhydrous methylene chloride was distilled under nitrogen from CaH_2. Unless otherwise mentioned all the solvents and reagents used are of commercial grade. Reactions were magnetically stirred and monitored by thin layer chromatography using aluminium sheets (Silica gel 60-F254, E.Merck). The TLC plates were visualized by exposure to ultraviolet light (UV, 254 nm) and exposure to an aqueous solution of potassium permanganate ($KMnO_4$) followed by heating with a heat gun. ^{1}H NMR and ^{13}C NMR spectra were measured at 500 and 126 MHz respectively, with TMS as internal standard when $CDCl_3$ was used as solvent. 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.

4.1.1. Synthesis of compounds peracetyl EGCG, permethyl EGCG, 1-11

These compounds were obtained according to the procedure as described previously.[32]

4.1.2. Synthesis of (2S,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4,5-trimethoxybenzoate (12)

To a solution of green tea crude extractings (15 g) in acetone (150 mL), potassium carbonate (13.56 g, 98 mmol) was added. After stirring the suspension at room temperature for 1 h, dimethyl sulfate (27.97 mL) was added dropwise and then the reaction mixture was heated

to reflux for 72 h. The TLC showed that the reaction was completed, then the solvent was removed under reduced pressure and the resultant mixture was added 100 mL EtOAc and 100 mL water. The organic layer was dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel to afford the title compound **12** (3.86 g, 20.7% yield)，$[a]^{20}_D$ = -45.6 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.11 (s, 2 H), 6.66 (s, 2 H), 6.20 (s, 1 H), 6.12 (s, 1 H), 5.50 (dd, *J* = 13.2, 7.1 Hz, 1 H), 5.10 (d, *J* = 7.4 Hz, 1 H), 3.88 – 3.75 (m, 24 H), 3.15 (dd, *J* = 16.5, 5.4 Hz, 1 H), 2.81 (dd, *J* = 16.5, 7.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.4, 160.1, 158.8, 155.1, 153.5, 153.0, 142.6, 138.2, 133.5, 125.1, 107.1, 104.1, 101.0, 93.2, 92.1, 79.2, 70.4, 61.1, 60.9, 56.4, 56.3, 55.6, 55.6, 25.0. HRMS calcd for $(C_{30}H_{34}O_{11} + H)^+$ 571.2174, found 571.2174.

4.1.3. Synthesis of (2S,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol (13)

To a solution of compound **12** (1 g, 1.75 mmol) in methyl alcohol (50 mL) and DME (50 mL) was added potassium carbonate (0.73 g, 5.3 mmol). The reaction mixture was stirred at room temperature for 10 h. Then the solvent was removed under reduced pressure and the resultant mixture was added 50 mL EtOAc and 50 mL water. The organic layer was dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel to afford the title compound **13** (606 mg, 92.0 % yield). $[a]^{20}_D$ = 17.1 (c = 1.0, CH_2Cl_2) mp 131-133 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.68 (s, 2 H), 6.13 (dd, *J* = 15.5, 2.3 Hz, 2 H), 4.63 (d, *J* = 8.5 Hz, 1 H), 4.09 – 4.03 (m, 1 H), 3.87 (s, 6 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.76 (s, 3 H), 3.10 (dd, *J* = 16.3, 5.8 Hz, 1 H), 2.59 (dd, *J* = 16.3, 9.3 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.7, 158.7, 155.1, 153.5, 138.1, 133.4, 104.1, 101.7, 92.9, 92.0, 82.1, 68.3, 60.8, 56.1, 55.5, 27.8. HRMS calcd for $(C_{20}H_{24}O_7 + H)^+$ 377.1595,

found 377.1593.

4.1.4. Synthesis of (E)-(2S,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4,5-trimethoxyphenyl)acrylate (14)

Following the procedure for the preparation of compound **22**, but with compound **13** and (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid as starting material, the titled compound **14** (399mg**,** 84.0% yield) was prepared. $[a]^{20}_{D}$ = -13.6 (c = 1.0, CH_2Cl_2); mp 65-67 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.52 (d, *J* = 15.9 Hz, 1 H), 6.70 (s, 2 H), 6.62 (s, 2 H), 6.25 (dd, *J* = 33.0, 8.9 Hz, 2 H), 6.11 (d, *J* = 2.1 Hz, 1 H), 5.51 (m, *J* = 5.9 Hz, 1 H), 5.16 (d, *J* = 5.9 Hz, 1 H), 3.86 (s, 9 H), 3.84 (s, 1 H), 3.81 (s, 8 H), 3.77 (m, *J* = 2.7 Hz, 6 H), 2.91 (dd, *J* = 16.9, 5.3 Hz, 1 H), 2.77 (dd, *J* = 16.9, 6.1 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.1, 159.9, 158.6, 154.6, 153.3, 145.3, 140.2, 137.8, 133.5, 129.7, 117.0, 105.2, 103.5, 100.6, 92.9, 91.8, 78.4, 69.0, 60.9, 56.1, 55.4, 23.6. HRMS calcd for $(C_{32}H_{36}O_{11}+ H)^+$ 597.2330, found 597.2334.

4.1.5. Synthesis (2S,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoate (15)

Following the procedure for the preparation of compound **22**, but with compound **13** and 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid as starting material, the titled compound **15** (497mg**,** 85.0% yield) was prepared. $[a]^{20}_{D}$ = -74.2 (c = 1.0, CH_2Cl_2); mp 67-69 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.97 (d, *J* = 7.5 Hz, 1 H), 7.93 (s, 1 H), 7.70 (m *J* = 8.6, 5.0, 2.0 Hz, 1 H), 7.15 (dd, *J* = 10.2, 8.6 Hz, 1 H), 7.09 (s, 2 H), 6.70 (s, 2 H), 6.20 (d, *J* = 2.2 Hz, 1 H), 6.13 (d, *J* = 2.2 Hz, 1 H), 5.51 (m, *J* = 8.1, 5.9 Hz, 1 H), 5.09 (d, *J* = 8.1 Hz, 1 H), 3.95 (s, 6 H), 3.92 (s, 3 H), 3.79 (s, 15 H), 3.21 (dd, *J* = 16.5, 5.8 Hz, 1 H), 2.81 (dd, *J* = 16.5, 8.2 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.5, 164.3, 159.9, 158.6, 156.5, 154.9, 154.5, 153.4, 153.2, 141.7,

137.8, 133.2, 129.4, 126.9, 126.8, 126.7, 126.6, 126.5,123.4, 115.1, 114.9, 104.5, 103.9, 100.9, 93.0, 92.0, 79.1, 70.5, 61.0, 60.8, 56.4, 56.0, 55.4, 25.3 HRMS calcd for $(C_{37}H_{38}O_{12}NF + H)^+$ 708.2451, found 708.2461.

4.1.6. Synthesis of (2S,3S)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4,5-trimethoxybenzoate (16)

A mixture of compound **13** (300 mg, 0.8 mmol), triphenylphosphine (1.31 g, 4.82 mmol) and 3, 4, 5-Trimethoxy benzoic acid were dissolved in anhydrous THF (25 mL). Diisopropyl azodicarboxylate (1.5 mL) was added dropwise under a nitrogen atmosphere at -25 °C. After 1 h, the reaction was left at room temperature overnight. The TLC showed that the reaction was completed, then the solvent was removed under reduced pressure and the resultant mixture was added 20 mL EtOAc and 20 mL water. The organic layer was dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel to afford the title compound **16** (136 mg**,** 30.0% yield). $[a]^{20}_D$ = 111.8 (c = 1.0, CH_2Cl_2) mp 51-53 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.16 (s, 2 H), 6.69 (s, 2 H), 6.23 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.65 (m, *J* = 3.0 Hz, 1 H), 5.07 (s, 1 H), 3.84 (s, 3 H), 3.80 (s, 6 H), 3.78 (s, 3 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.70 (s, 6 H), 3.04 (d, *J* = 3.4 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.1, 159.7, 158.9, 155.5, 152.8, 142.5, 137.9, 133.4, 125.1, 107.2, 103.9, 100.1, 93.2, 91.9, 77.8, 68.7, 60.8, 56.2, 56.0, 55.4, 25.9. HRMS calcd for $(C_{30}H_{34}O_{11} + H)^+$ 571.2174, found 571.2173.

4.1.7. Synthesis of (2S,3S)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol (17)

Following the procedure for the preparation of compound **13**, but with compound **16** as starting material, the titled compound **17** (594 mg, 90.0% yield) was prepared. $[a]^{20}_D$ = 54.9 (c

= 1.0, CH_2Cl_2) mp 67-69 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.73 (s, 2 H), 6.19 (d, *J* = 2.3 Hz, 1 H), 6.11 (d, *J* = 2.3 Hz, 1 H), 4.92 (s, 1 H), 4.27 (s, 1 H), 3.88 (s, 6 H), 3.84 (s, 3 H), 3.79 (s, 3 H), 3.76 (s, 3 H), 2.95 (dd, *J* = 17.1, 1.2 Hz, 1 H), 2.88 (dd, *J* = 17.1, 4.3 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 159.8, 159.4, 155.1, 153.5, 137.7, 134.1, 103.4, 100.4, 93.4, 92.3, 78.8, 66.6, 60.9, 56.3, 55.5, 28.2. HRMS calcd for $(C_{20}H_{24}O_7 + H)^+$ 377.1595, found 377.1594.

4.1.8. Synthesis of (E)-(2S,3S)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3,4,5-trimethoxyphenyl)acrylate (18)

Following the procedure for the preparation of compound **22**, but with compound **17** and (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid as starting material, the titled compound **18** (408 mg, 85.9% yield) was prepared. $[a]^{20}_D$ =68.22 (c = 1.0, CH_2Cl_2); mp 69-71 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.49 (d, *J* = 15.9 Hz, 1 H), 6.73 (s, 2 H), 6.66 (s, 2 H), 6.28 (d, *J* = 15.9 Hz, 1 H), 6.25 (d, *J* = 2.2 Hz, 1 H), 6.14 (d, *J* = 2.2 Hz, 1 H), 5.66 (s, 1 H), 5.05 (s, 1 H), 3.86 (s, 3 H), 3.85 (s, 6 H), 3.83 (s, 6 H), 3.82 (s, 3 H), 3.80 (s, 6 H), 3.01 (d, *J* = 4.1 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.2, 159.8, 159.1, 155.5, 153.5, 153.3, 145.4, 140.3, 137.9, 133.3, 129.8, 117.1, 105.3, 104.0, 100.4, 100.0, 93.5, 92.2, 77.8, 77.2, 77.0, 67.6, 61.0, 56.2, 55.5, 29.8, 26.2 HRMS calcd for $(C_{32}H_{36}O_{11} + H)^+$ 597.2330, found 597.2335.

4.1.9. Synthesis of (2S,3S)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoate (19)

Following the procedure for the preparation of compound **22**, but with compound **17** and 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid as starting material, the titled compound **19** (468 mg, 83.0% yield) was prepared. $[a]^{20}_D$ = 128.6 (c = 1.0, CH_2Cl_2); mp 98-100 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.90 (dd, *J* = 7.5, 1.9 Hz, 1 H), 7.91 (s, 1 H), 7.74 – 7.69 (m, 1 H),

7.11 (d, J = 10.0 Hz, 1 H), 7.07 (d, J = 3.7 Hz, 2 H), 6.74 (s, 2 H), 6.27 (d, J = 2.2 Hz, 1 H), 6.11 (d, J = 2.3 Hz, 1 H), 5.66 (s, 1 H), 5.08 (s, 1 H), 3.92 (s, 6 H), 3.90 (s, 3 H), 3.79 (s, 6 H), 3.76 (d, J = 8.3 Hz, 9 H), 3.06 (d, J = 3.3 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.2, 164.5, 159.7, 159.0, 155.6, 155.0, 153.5, 153.2, 141.7, 137.8, 133.4, 129.6, 127.0, 126.6, 124.2, 115.1, 115.0, 104.6, 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 $(C_{37}H_{38}O_{12}NF + H)^+$ 708.2451, found 708.2460.

4.1.10. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-ol (20)

To a solution of (+) – Catechin (348 mg, 1.2 mmol) in acetone (30 mL), potassium carbonate (994 mg, 7.2 mmol) was added. After stirring the suspension at room temperature for 1.h, dimethyl sulfate (1 mL) was added dropwise and then the reaction mixture was heated to reflux for 8 h. The TLC showed that the reaction was completed, then the solvent was removed under reduced pressure and the resultant mixture was added 50 mL EtOAc and 50 mL water. The organic layer was dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel to afford the title compound **20** (315 mg, 75.9% yield). $[a]^{20}_D$ = -11.0 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.00 (d, J = 8.2 Hz, 1 H), 6.98 (s, 1 H), 6.90 (d, J = 8.2 Hz, 1 H), 6.13 (dd, J = 15.7, 2.0 Hz, 2 H), 4.66 (d, J = 8.3 Hz, 1 H), 4.10 – 4.02 (m, 1 H), 3.89 (s, 6 H), 3.80 (s, 3 H), 3.75 (s, 3 H), 3.07 (dd, J = 16.3, 5.7 Hz, 1 H), 2.59 (dd, J = 16.3, 9.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.9, 158.9, 155.4, 149.5, 130.4, 120.1, 111.4, 110.1, 101.8, 93.2, 92.1, 82.0, 68.4, 56.1, 56.1, 55.6, 55.5, 27.8; HRMS calcd for $(C_{19}H_{22}O_6 + H)^+$ 347.1489, found 347.1494.

4.1.11. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 3,4,5-trimethoxybenzoate (21)

Following the procedure for the preparation of compound **22**, but with 3,4,5-trimethoxybenzoic acid as starting material, the titled compound **21** (421 mg 90.0 % yield) was prepared.$[a]^{20}_D$ = 85.7 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.09 (s, 2 H), 7.00 – 6.96 (m, 1 H), 6.95 (s, 1 H), 6.80 (d, *J* = 8.2 Hz, 1 H), 6.17 (d, *J* = 2.0 Hz, 1H), 6.09 (d, *J* = 2.0 Hz, 1H), 5.47 (dd, *J* = 13.4, 7.6 Hz, 1 H), 5.09 (d, *J* = 7.7 Hz, 1 H) 3.85 (s, 3 H), 3.81 (d, *J* = 4.3 Hz, 12 H), 3.75 (d, *J* = 6.0 Hz, 6 H), 3.15 (dd, *J* = 16.5, 5.5 Hz, 1 H), 2.78 (dd, *J* = 16.5, 7.8 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.2, 159.8, 158.6, 155.1, 152.8, 149.0, 142.2, 130.3, 125.0, 119.5, 110.9, 109.7, 106.8, 100.9, 93.0, 91.8, 78.8, 70.3, 60.8, 56.1, 55.8, 55.4, 25.0. HRMS calcd for $(C_{29}H_{32}O_{10} + H)^+$ 541.2068, found 541.2066.

4.1.12. Synthesis of (E)-(2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl-3-(3,4-dimethoxyphenyl)acrylate (22)

A mixture of compound **20** (300 mg, 0.9 mmol), (E)-3-(3,4-dimethoxyphenyl)acrylic acid (208 mg, 1.0 mmol), EDC·HCl (306 mg, 1.6 mmol) and DMAP (195 mg, 1.6 mmol) were dissolved in anhydrous CH_2Cl_2 (20 mL) under a nitrogen atmosphere and the solution was stirred at room temperature for 12 h. The reaction was diluted with water and extracted with CH_2Cl_2, The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by flash chromatography on silica gel to afford the title compound **22** (408 mg, 87.9% yield). $[a]^{20}_D$ = 62.2 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.55 (d, *J* = 16.0 Hz, 1 H), 7.05 (d, *J* = 8.1 Hz, 1 H), 7.00 (s, 1 H), 6.95 – 6.92 (m, 2 H), 6.83 (t, *J* = 7.3 Hz, 2 H), 6.23 (d, *J* = 16.0 Hz, 1 H), 6.20 (s, 1 H), 6.10 (s, 1 H), 5.50 (q, *J* = 5.8 Hz, 1 H), 5.16 (d, *J* = 6.1 Hz, 1 H), 3.89 – 3.76 (m, 18 H), 2.92 (dd, *J* = 16.9, 5.1 Hz, 1 H), 2.76 (dd, *J* = 16.8, 6.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.5, 160.0, 158.8, 154.9, 151.3, 149.3, 149.1, 149.0,

145.3, 130.6, 127.4, 123.0, 119.2, 115.6, 111.2, 111.1, 109.7, 109.6, 100.9, 93.1, 91.9, 78.4, 69.0, 56.1, 56.0, 56.0, 55.5, 55.5, 23.8; HRMS calcd for ($C_{30}H_{32}O_9$ + H)$^+$ 537.2119, found 537.2123.

4.1.13. Synthesis of (E)-(2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl.3-(3,4,5-trimethoxyphenyl)acrylate (23)

Following the procedure for the preparation of compound **20**, but with (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid as starting material, the titled compound **23** (425 mg, 86.7% yield) was prepared.[a]$^{20}_D$ = 53.1 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (d, *J* = 15.9 Hz, 1 H), 6.94 (dd, *J* = 8.2, 2.0 Hz, 1 H), 6.92 (d, *J* = 2.0 Hz, 1 H), 6.82 (d, *J* = 8.3 Hz, 1 H), 6.70 (s, 2 H), 6.27 (d, *J* = 15.9 Hz, 1 H), 6.21 (d, *J* = 2.3 Hz, 1 H), 6.11 (d, *J* = 2.3 Hz, 1 H), 5.52 (q, *J* = 6.1 Hz, 1 H), 5.17 (d, *J* = 6.1 Hz, 1 H), 3.88 – 3.77 (m, 21 H), 2.90 (dd, *J* = 16.9, 5.3 Hz, 1 H), 2.77 (dd, *J* = 16.7, 6.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.2, 159.9, 158.7, 154.8, 153.5, 149.1, 149.0, 145.3, 140.2, 130.5, 129.8, 119.1, 117.1, 111.1, 109.6, 105.3, 100.8, 93.1, 91.9, 78.3, 69.1, 61.1, 56.2, 56.0, 55.5, 23.7; HRMS calcd for ($C_{31}H_{34}O_{10}$ + H)$^+$ 567.2225, found 567.2230.

4.1.14. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 3-(3,4-dimethoxybenzamido)-4-fluorobenzoate (24)

Following the procedure for the preparation of compound **20**, but with 3-(3,4-dimethoxybenzamido)-4-fluorobenzoic acid as starting material, the titled compound **24** (481 mg, 85.9% yield) was prepared. [a]$^{20}_D$ = 85.6 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.98 (d, *J* = 7.5 Hz, 1 H), 8.00 (s, 1 H), 7.69 – 7.62 (m, 1 H), 7.50 (d, *J* = 1.5 Hz, 1 H), 7.41 (dd, *J* = 8.4, 1.6 Hz, 1 H), 7.11 (t, *J* = 9.5 Hz, 1 H), 7.04 (d, *J* = 8.2 Hz, 1 H), 6.99 (s, 1 H), 6.92

(d, J = 8.4 Hz, 1 H), 6.82 (d, J = 8.3 Hz, 1 H), 6.15 (dd, J = 38.3, 1.9 Hz, 2 H), 5.50 (dd, J = 13.8, 7.8 Hz, 1 H), 5.10 (d, J = 7.9 Hz, 1 H), 3.96 – 3.76 (m, 18 H), 3.17 (dd, J = 16.5, 5.7 Hz, 1 H), 2.80 (dd, J = 16.5, 8.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.0, 164.5, 160.0, 158.7, 155.2, 152.7, 149.5, 149.1, 149.0, 130.3, 127.0, 126.8, 126.6, 123.6, 119.8, 119.6, 115.1, 114.9, 111.2, 110.8, 110.6, 110.0, 101.1, 93.2, 92.1, 78.9, 77.4, 77.2, 76.9, 70.6, 56.2, 55.9, 55.6, 55.5, 27.0, 25.3. HRMS calcd for $(C_{35}H_{34}O_{10}NF + H)^+$ 648.2240, found 648.2248.

4.1.15. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoate (25)

Following the procedure for the preparation of compound **20**, but with 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid as starting material, the titled compound **25** (507 mg, 86.4% yield) was prepared. $[a]^{20}_D$ = 74.1 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.95 (dd, J = 7.6, 2.0 Hz, 1 H), 7.93 (d, J = 3.0 Hz, 1 H), 7.68 (m, J = 8.4, 5.0, 2.1 Hz, 1 H), 7.13 (dd, J = 10.3, 8.7 Hz, 1 H), 7.10 (s, 2 H), 7.04 (dd, J = 8.3, 2.0 Hz, 1 H), 6.98 (d, J = 2.0 Hz, 1 H), 6.83 (d, J = 8.3 Hz, 1 H), 6.19 (d, J = 2.3 Hz, 1 H), 6.12 (d, J = 2.3 Hz, 1 H), 5.51 (td, J = 7.9, 5.8 Hz, 1 H), 5.11 (d, J = 7.9 Hz, 1 H), 3.95 – 3.77 (m, 21 H), 3.17 (dd, J = 16.5, 5.7 Hz, 1 H), 2.81 (dd, J = 16.5, 8.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.3, 164.5, 160.0, 158.8, 155.2, 154.9, 153.6, 149.1, 149.0, 141.8, 130.3, 129.6, 126.9, 123.7, 119.6, 115.2, 115.1, 111.2, 110.0, 104.7, 101.0, 93.2, 92.1, 78.9, 70.7, 61.1, 56.5, 55.9, 55.6, 55.5, 25.3. HRMS calcd for $(C_{36}H_{36}O_{11}NF + H)^+$ 678.2345, found 678.2359.

4.1.16. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 3-((E)-3-(3,4-dimethoxyphenyl)acrylamido)-4-fluorobenzoate (26)

690 Following the procedure for the preparation of compound **20**, but with (E)-3-(3-(3,4-
691 dimethoxyphenyl)acrylamido)-4-fluorobenzoic acid as starting material, the titled compound
692 **26** (500 mg, 85.8% yield) was prepared.$[a]^{20}_{D}$ = 90.1 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500
693 MHz) δ 9.03 (d, *J* = 7.1 Hz, 1 H), 7.72 (d, *J* = 15.4 Hz, 1 H), 7.66 – 7.60 (m, 1 H), 7.50 (s, 1
694 H), 7.14 (dd, *J* = 8.3, 1.3 Hz, 1 H), 7.07 (dd, *J* = 18.7, 11.9 Hz, 3 H), 6.99 (s, 1 H), 6.88 (d, *J* =
695 8.3 Hz, 1 H), 6.83 (d, *J* = 8.2 Hz, 1 H), 6.45 (d, *J* = 15.4 Hz, 1 H), 6.19 (d, *J* = 1.6 Hz, 1 H),
696 6.11 (d, *J* = 1.7 Hz, 1 H), 5.49 (dd, *J* = 13.9, 7.9 Hz, 1 H), 5.10 (d, *J* = 8.0 Hz, 1 H), 3.93 – 3.77
697 (m, 18 H), 3.18 (dd, *J* = 16.5, 5.7 Hz, 1 H), 2.80 (dd, *J* = 16.5, 8.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$,
698 125 MHz) δ 164.5, 164.2, 160.0, 158.8, 155.2, 151.3, 149.4, 149.1, 149.0, 130.4, 127.4, 126.9,
699 123.4, 122.7, 119.7, 117.9, 115.0, 101.1, 93.2, 92.1, 78.9, 70.7, 56.1, 56.0, 56.0, 55.6, 55.5,
700 25.4. HRMS calcd for $(C_{37}H_{36}O_{10}NF + H)^+$ 674.2396, found 674.2408.

701 ***4.1.17. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 4-fluoro-***
702 ***3-((E)-3-(3,4,5-trimethoxyphenyl)acrylamido)benzoate (27)***

703 Following the procedure for the preparation of compound **20**, but with (E)-4-fluoro-3-(3-
704 (3,4,5-trimethoxyphenyl)acrylamido)benzoic acid as starting material, the titled compound **27**
705 (526 mg, 86.4% yield) was prepared. $[a]^{20}_{D}$ = 82.8 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500
706 MHz) δ 9.03 (d, *J* = 6.5 Hz, 1 H), 7.70 (d, *J* = 15.4 Hz, 1 H), 7.66 – 7.63 (m, 1 H), 7.51 (s, 1
707 H), 7.12 – 7.07 (m, 1 H), 7.05 (dd, *J* = 8.3, 1.7 Hz, 1 H), 6.99 (d, *J* = 1.7 Hz, 1 H), 6.83 (d, *J* =
708 8.3 Hz, 1 H), 6.79 (s, 2 H), 6.49 (d, *J* = 15.4 Hz, 1 H), 6.19 (d, *J* = 2.2 Hz, 1 H), 6.12 (d, *J* =
709 2.2 Hz, 1 H), 5.52 – 5.46 (m, 1 H), 5.11 (d, *J* = 8.0 Hz, 1 H), 3.91 – 3.77 (m, 21 H), 3.18 (dd,
710 *J* = 16.5, 5.7 Hz, 1 H), 2.81 (dd, *J* = 16.5, 8.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.5,
711 163.9, 160.0, 158.8, 155.2, 153.6, 149.1, 149.0, 143.5, 140.3, 130.4, 130.0, 123.5, 119.7, 119.4,

115.1, 111.3, 110.1, 105.4, 101.1, 93.3, 92.1, 78.9, 70.7, 61.1, 56.3, 56.0, 55.6, 55.5, 25.4. HRMS calcd for $(C_{38}H_{38}O_{11}NF + H)^+$ 704.2502, found 704.2509.

4.1.18. Synthesis of (2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-ol (28)

Following the procedure for the preparation of compound **20**, but with L-Epicatechin as starting material, the titled compound **28** (358 mg, 86.4% yield) was prepared. $[a]^{20}_D$ = -51.9 (c = 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.08 (d, *J* = 1.7 Hz, 1 H), 7.05 (dd, *J* = 8.3, 1.6 Hz, 1 H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.20 (d, *J* = 2.3 Hz, 1 H), 6.12 (d, *J* = 2.3 Hz, 1 H), 4.96 (s, 1 H), 4.28 (s, 1 H), 3.92 (s, 3 H), 3.90 (s, 3 H), 3.79 (s, 3 H), 3.77 (s, 3 H), 2.95 (dd, *J* = 17.2, 1.6 Hz, 1 H), 2.88 (dd, *J* = 17.2, 4.3 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.7, 159.3, 155.2, 149.1, 148.8, 130.8, 118.6, 111.2, 109.68, 100.3, 93.3, 92.2, 78.4, 66.4, 56.0, 55.4, 28.1. HRMS calcd for $(C_{19}H_{22}O_6 + H)^+$ 347.1489, found 347.1488.

4.1.19. Synthesis of (2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 3,4,5-trimethoxybenzoate (29)

Following the procedure for the preparation of compound **22**, but with compound **28** and 3,4,5-trimethoxybenzoic acid as starting material, the titled compound **29** (327 mg, 70.0 % yield) was prepared. $[a]^{20}_D$ = -166.2 (c = 1.0, CH_2Cl_2); mp 63-65 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.16 (s, 2 H), 7.02 (d, *J* = 8.2 Hz, 2 H), 6.82 (d, *J* = 8.2 Hz, 1 H), 6.24 (d, *J* = 2.0 Hz, 1 H), 6.11 (d, *J* = 2.0 Hz, 1 H), 5.63 (s, 1 H), 5.12 (s, 1 H), 3.85 (s, 3 H), 3.84 (s, 3 H), 3.82 (s, 6 H), 3.79 (s, 3 H), 3.77 (s, 3 H), 3.69 (s, 3 H), 3.04 (d, *J* = 2.5 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.2, 159.7, 158.9, 155.6, 152.8, 148.8, 142.3, 130.4, 125.1, 119.0, 110.8, 109.8, 107.0, 100.1, 93.2, 91.8, 77.5, 68.9, 60.9, 56.2, 25.8. HRMS calcd for $(C_{29}H_{32}O_{10} + H)^+$ 541.2063, found 541.2068.

4.1.20. Synthesis of (E)-(2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 3-(3,4,5-trimethoxyphenyl)acrylate (30)

Following the procedure for the preparation of compound **22**, but with compound **28** and (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid as starting material, the titled compound **30** (420 mg, 86.0% yield) was prepared. $[a]^{20}_{D}$ = -163.5 (c = 1.0, CH_2Cl_2); mp 77-79 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 (d, *J* = 15.9 Hz, 1 H), 7.05 (s, 1 H), 7.01 (d, *J* = 8.3 Hz, 1 H), 6.85 (d, *J* = 8.3 Hz, 1 H), 6.67 (s, 2 H), 6.28 (d, *J* = 15.9 Hz, 1 H), 6.24 (s, 1 H), 6.13 (s, 1 H), 5.64 (s, 1 H), 5.08 (s, 1 H), 3.85 (d, *J* = 4.8 Hz, 15 H), 3.79 (s, 6 H), 3.00 (d, *J* = 5.3 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.1, 159.75, 159.0, 155.5, 153.0, 148.8, 145.2, 140.1, 130.2, 129.7, 119.0, 117.0, 110.8, 109.8, 105.1, 100.1, 93.3, 92.0, 77.5, 67.6, 61.0, 56.1, 55.8, 55.4, 26.1. HRMS calcd for $(C_{31}H_{34}O_{10}+ H)^+$ 567.2225, found 567.2225.

4.1.21. Synthesis of (2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoate (31)

Following the procedure for the preparation of compound **22**, but with compound **28** and 4-fluoro-3-(3,4,5-trimethoxybenzamido)benzoic acid as starting material, the titled compound **31** (440 mg, 75.0 % yield) was prepared. $[a]^{20}_{D}$ = -126.6 (c = 1.0, CH_2Cl_2); mp 93-95 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.88 (dd, *J* = 7.5, 1.9 Hz, 1 H), 7.92 (d, *J* = 2.0 Hz, 1 H), 7.71 (ddd, *J* = 8.4, 4.9, 2.0 Hz, 1 H), 7.10 (dd, *J* = 10.2, 8.9 Hz, 1 H), 7.07 (s, 3 H), 7.03 (dd, *J* = 8.3, 1.6 Hz, 1 H), 6.83 (d, *J* = 8.3 Hz, 1 H), 6.26 (d, *J* = 2.2 Hz, 1 H), 6.10 (d, *J* = 2.2 Hz, 1 H), 5.64 (d, *J* = 2.7 Hz, 1 H), 5.12 (s, 1 H), 3.91 (s, 6 H), 3.90 (s, 3 H), 3.83 (s, 3 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.73 (s, 3 H), 3.05 (s, 2 H) ; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.1, 164.5, 159.6, 158.8, 155.6, 154.8, 153.4, 148.7, 141.5, 130.3, 129.5, 126.8, 124.2, 119.1, 115.0, 114.9, 110.9, 109.7,

104.6, 100.1, 93.5, 91.9, 77.5, 69.2, 61.0, 56.4, 55.8, 55.4, 25.9. HRMS calcd for ($C_{36}H_{36}FNO_{11}$ + H)$^+$ 678.2345, found 678.2351.

4.1.22. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl1-(4-methoxybenzyl)piperidine-4-carboxylate (33)

Under a nitrogen atmosphere, permethyl EGC (600 mg, 1.6 mmol), 1-(4-methoxybenzyl) piperidine-4-carboxylic acid (500 mg, 2.0 mmol), EDC·HCl (1150 mg, 6 mmol), and DMAP (488 mg, 4 mmol) were dissolved in anhydrous CH_2Cl_2 (20 mL). Then DMF (5 mL) was added and the reaction mixture was stirred at room temperature until TLC showed that the reaction was completed. Then the reaction mixture was washed by water and brine for two times. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by flash chromatography on silica gel to afford the title compound **33** (36% yield). $[a]^{20}_D$ = -66.7 (c = 1.0, CH_2Cl_2); mp 56-58 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.15 (d, *J* = 8.6 Hz, 2 H), 6.82 (d, *J* = 8.6 Hz, 2 H), 6.69 (s, 2 H), 6.21 (d, *J* = 2.3 Hz, 1 H), 6.11 (d, *J* = 2.3 Hz, 1 H), 5.48 – 5.45 (m, 1 H), 5.01 (s, 1 H), 3.86 (d, *J* = 4.2 Hz, 6 H), 3.83 (s, 3 H), 3.79 (s, 9 H), 3.32 (s, 2 H), 2.95 (dd, *J* = 17.9, 4.6 Hz, 1 H), 2.88 (dd, *J* = 17.9, 1.5 Hz, 1 H), 2.71 – 2.58 (m, 2 H), 2.19 – 2.12 (m, 1 H), 1.87 (t, *J* = 10.9 Hz, 2 H), 1.66 (s, 1 H), 1.63 – 1.56 (m, 2 H), 1.56 – 1.49 (m, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.4, 159.6, 158.8, 158.6, 155.2, 153.1, 133.3, 130.2, 113.5, 103.5, 100.1, 93.4, 92.0, 77.3, 67.6 62.5, 60.9, 56.2, 55.5, 55.3, 55.2 52.6, 52.40, 41.1, 28.2, 27.9, 25.8.

4.1.23. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl1-(3,4-dimethoxybenzyl)piperidine-4-carboxylate (34)

The title compound was made by same synthetic method as that used for compound **33**,

but with 1-(3,4-dimethoxybenzyl)piperidine-4-carboxylic acid as starting material, compound **34** was obtained. Yield 35%; $[a]^{20}_{D}$ = -60.3 (c = 1.0, CH_2Cl_2); mp 61-63 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.82 (s, 1 H), 6.76 (d, *J* = 3.6 Hz, 2 H), 6.69 (s, 2 H), 6.21 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.48 (s, 1H), 5.02 (s, 1 H), 3.89 – 3.84 (s, 12 H), 3.82 (s, 3 H), 3.79 (s, *J* = 3.3 Hz, 6 H), 3.32 (d, *J* = 3.4 Hz, 2 H), 2.97 – 2.87 (m, 2 H), 2.68 (d, *J* = 10.9 Hz, 2 H), 2.21 – 2.12 (m, 1 H), 1.87 (s, 2 H), 1.67 (d, *J* = 10.3 Hz, 1 H), 1.53 (dd, *J* = 17.7, 7.1 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.6, 159.7, 159.0, 155.3, 153.2, 148.9, 148.1, 137.7, 133.5, 131.1, 121.2, 112.1, 110.8, 103.6, 100.2, 93.4, 92.1, 77.4, 67.7, 63.0, 61.0, 56.2, 56.0, 55.5 , 52.8, 52.6, 41.2, 28.4, 28.0, 25.9.

4.1.24. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl1-(3,4,5-trimethoxybenzyl)piperidine-4-carboxylate (35)

Using the same procedure for the preparation of compound **33**, but with 1-(3, 4, 5-trimethoxybenzyl) piperidine-4-carboxylic acid as the starting material, the titled compound **35** was prepared. Yield 37%; $[a]^{20}_{D}$ = -56.2 (c = 1.0, CH_2Cl_2); mp 58-60 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.68 (s, 2 H), 6.48 (s, 2 H), 6.20 (d, *J* = 1.9 Hz, 1 H), 6.09 (d, *J* = 1.9 Hz, 1 H), 5.48 (s, 1 H), 5.00 (s, 1 H), 4.01 – 3.60 (s, 24 H), 3.34 – 3.25 (s, 2 H), 2.92 (m, 2 H), 2.72 – 2.56 (m, 2 H), 2.17 (m, 1 H), 1.89 (t, *J* = 9.6 Hz, 2 H), 1.59 (m, 4 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.4, 159.6, 158.8, 155.2, 153.0, 137.6, 133.4, 105.6, 103.5, 100.0, 93.4, 92.0, 77.2, 67.6, 63.3, 60.8, 56.1, 55.4, 52.7, 52.5, 40.9, 28.2, 27.8, 25.8.

4.1.25. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 1-(4-methoxybenzyl)piperidine-3-carboxylate (36)

A mixture of permethyl EGC (400 mg, 1.0 mmol), 1-(4-methoxybenzyl) piperidine-3-

carboxylic acid(331 mg, 1.3 mmol), EDC·HCl (764 mg, 4.0 mmol), and DMAP (489 mg, 4.0 mmol) were dissolved in anhydrous CH_2Cl_2 (25 mL). DMF (5 mL) was added and the suspension was stirred at room temperature for overnight .The solution was washed with water (25 mL) and then extracted with EtOAc for three times. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by flash chromatography on silica gel to afford the title compound **36** (38% yield); $[a]^{20}_D$ = -59.7 (c = 1.0, CH_2Cl_2); mp 53-55 °C ; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.12 (d, *J* = 6.9 Hz, 2 H), 6.81 (dd, *J* = 8.5, 4.0 Hz, 2 H), 6.68 (s, 2 H), 6.22 (d, *J* = 2.2 Hz, 1 H), 6.11 (dd, *J* = 5.4, 2.2 Hz, 1 H), 5.45 (s, 1 H), 5.01 (s, 1 H), 3.86 (s, 6 H), 3.82 (s, 3 H), 3.81 – 3.75 (s, 9 H), 3.42 (d, *J* = 13.1 Hz, 1 H), 3.33 (s, 1 H), 3.25 (d, *J* = 13.0 Hz, 1 H), 2.93 (ddd, *J* = 37.4, 21.1, 11.3 Hz, 2 H), 2.77 (t, *J* = 12.9 Hz, 1 H), 2.65 – 2.57 (m, 1 H), 2.50 – 2.38 (m, 1 H), 2.05 – 1.73 (m, 3 H), 1.69 – 1.63 (m, 1 H), 1.58 – 1.49 (m, 1 H), 1.46 – 1.35 (m, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 159.6, 158.8, 158.6, 155.2, 153.1, 133.4, 130.2, 113.5, 103.5, 100.1, 93.3, 92.0, 77.3, 67.6, 62.5, 60.8, 56.1, 55.4, 55.2, 55.0, 53.1, 52.7, 42.2, 41.8, 27.0, 25.8, 24.4.

4.1.26. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 1-(3,4-dimethoxybenzyl)piperidine-3-carboxylate (37)

Compound **12** was made using the procedure described for compound **36**, but with 1-(3,4-dimethoxybenzyl)piperidine-3-carboxylic acid as the starting material, the compound **37** was obtained. Yield 36%; $[a]^{20}_D$ = -55.7 (c = 1.0, CH_2Cl_2); mp 54-56 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.81 (s, 1 H), 6.75 (m, 2 H), 6.69 (s, 2 H), 6.22 (s, 1 H), 6.11 (dd, *J* = 7.1, 2.2 Hz, 1 H), 5.46 (s, 1 H), 5.01 (s, 1 H), 3.90 – 3.84 (m, 12 H), 3.83 (d, *J* = 4.9 Hz, 3 H), 3.78 (dd, *J* = 12.4, 9.1 Hz, 6 H), 3.45 (d, *J* = 13.1 Hz, 1 H), 3.33 (d, *J* = 2.4 Hz, 1 H), 3.22 (d, *J* = 13.1 Hz, 1 H),

2.96 (m, 1 H), 2.88 (d, *J* = 18.2 Hz, 1 H), 2.78 (t, *J* = 12.6 Hz, 1 H), 2.62 (s, 1 H), 2.46 (m, 1 H), 2.05 – 1.90 (m, 2 H), 1.86 (m, 1 H), 1.76 (t, *J* = 10.1 Hz, 1 H), 1.66 (d, *J* = 12.3 Hz, 1 H), 1.46 – 1.36 (m, 1 H).^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.7, 159.7, 159.0, 155.3, 153.2, 148.9, 148.1, 137.7, 133.5, 121.2, 112.2, 110.8, 103.6, 100.2, 93.4, 92.1, 77.4, 67.7, 62.9, 61.0, 56.2, 55.9, 55.5, 55.4, 53.2, , 52.8, 27.3, 27.0, 25.9.

4.1.27. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 1-(3,4,5-trimethoxybenzyl)piperidine-3-carboxylate (38)

Following the procedure for the preparation of compound **36**, but with 1-(3, 4, 5-trimethoxybenzyl) piperidine-3-carboxylic acid as starting material, the titled compound **38** was prepared. Yield 38%; $[a]^{20}_D$ = -61.0 (c = 1.0, CH_2Cl_2); mp 55-57 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.69 (d, *J* = 3.7 Hz, 2 H), 6.48 (s, 2 H), 6.21 (d, *J* = 1.8 Hz, 1 H), 6.12 (d, *J* = 1.8 Hz, 1 H), 5.47 (s, 1 H), 5.01 (s, 1 H), 3.86 (s, 6 H), 3.82 (s, 12 H), 3.77 (s, 6 H), 3.45 (d, *J* = 13.3 Hz, 1 H), 3.20 (d, *J* = 13.3 Hz, 1 H), 2.99 – 2.93 (m, 1 H), 2.88 (d, *J* = 17.4 Hz, 1 H), 2.76 (d, *J* = 11.0 Hz, 1 H), 2.64 (d, *J* = 11 Hz, 1 H), 2.49 (td, *J* = 10.8, 5.5 Hz, 1 H), 1.96 (t, *J* = 10.8 Hz, 1 H), 1.77 (dd, *J* = 20.0, 11.2 Hz, 3 H), 1.58 – 1.52 (m, 1 H), 1.46 – 1.37 (m, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz)δ 173.4, 159.6, 158.9, 155.2, 153.1, 134.2, 133.4, 105.5, 103.5, 100.1, 93.3, 92.0, 77.3, 67.7, 63.2, 60.8, 56.1, 55.8, 55.4, 52.9, 42.2, 26.8, 25.8, 24.4.

4.1.28. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl1-(3,4,5-trimethoxybenzoyl)piperidine-4-carboxylate (39)

Following the procedure for the preparation of compound **33**, but with permethyl catechin (GC) and 1-(3,4,5-trimethoxybenzoyl)piperidine-4-carboxylic acid as starting material, the titled compound **39** was prepared. Yield 32.0%; $[a]^{20}_D$ =-60.1 (c = 1.0, CH_2Cl_2); mp 83-85 °C;

844 ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.68 (s, 2 H), 6.53 (s, 2 H), 6.20 (d, *J* = 2.2 Hz, 1 H), 6.10 (d, *J*
845 = 2.2 Hz, 1 H), 5.52 (dd, *J* = 2.8, 1.3 Hz, 1 H), 5.02 (s, 1 H), 3.85 (s, 6 H), 3.83 (s, 9 H), 3.80
846 (s, 3 H), 3.78 (d, *J* = 2.4 Hz, 6 H), 2.95 (d, *J* = 4.5 Hz, 3 H), 2.90 (s, 1 H), 2.44 (ddd, *J* = 14.2,
847 10.1, 3.9 Hz, 1 H), 1.90 – 1.34 (m, 6 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 170.0, 159.7,
848 158.8, 155.1, 153.2, 139.1, 137.7, 133.2, 131.2, 104.0, 103.3, 99.8, 93.4, 92.0, 77.0, 68.0, 60.8,
849 56.18, 55.4, 40.7, 25.8.

850 ***4.1.29. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 1-***
851 ***(3,4,5-trimethoxybenzoyl)piperidine-3-carboxylate (40)***

852 Following the procedure for the preparation of compound **36**, but with permethyl catechin
853 (GC) and 1-(3,4,5-trimethoxybenzoyl)piperidine-3-carboxylic acid as starting material, the
854 titled compound **40** was prepared. Yield 30.0%; $[a]^{20}_D$ = -99.3 (c = 1.0, CH_2Cl_2); mp 84-86 °C;
855 ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.67 (s, 2 H), 6.53 (s, 2 H), 6.19 (s, 1 H), 6.09 (d, *J* = 1.8 Hz, 1
856 H), 5.50 (s, 1 H), 4.99 (s, 1 H), 3.86 (s, 5 H), 3.81 (s, 7 H), 3.78 (s, 6 H), 3.76 (s, 6 H), 2.89
857 (ddd, *J* = 32.7, 21.6, 8.7 Hz, 4 H), 2.40 (s, 1 H), 1.97 – 1.75 (m, 2 H), 1.65 – 1.24 (m, 4 H). ^{13}C
858 NMR ($CDCl_3$, 125 MHz) δ 170.1, 159.7, 158.8, 155.1, 153.2, 139.1, 137.6, 133.2, 104.1, 103.3,
859 99.7, 93.4, 92.1, 68.1, 60.8, 56.2, 55.4, 27.30, 25.9. HRMS calcd for $(C_{36}H_{43}O_{12}N + H)^+$
860 682.2858, found 682.2861.

861 ***4.1.30. Synthesis of (2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl1-(3,4,5-***
862 ***trimethoxybenzyl)piperidine-4-carboxylate (41)***

863 Following the procedure for the preparation of compound **33**, but with permethyl
864 epicatechin (EC) and 1-(3, 4, 5-trimethoxybenzyl) piperidine-4-carboxylic acid as starting
865 material, the titled compound **41** was prepared. Yield 37%; $[a]^{20}_D$ = -52.3 (c = 1.0, CH_2Cl_2);

mp 60-62 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.02 (d, *J* = 1.7 Hz, 1 H), 6.97 (dd, *J* = 8.3, 1.7 Hz, 1 H), 6.85 (d, *J* = 8.3 Hz, 1 H), 6.50 (s, 2 H), 6.20 (d, *J* = 2.2 Hz, 1 H), 6.11 (d, *J* = 2.2 Hz, 1 H), 5.49 – 5.45 (m, 1 H), 5.04 (s, 1 H), 3.90 (s, 3 H), 3.87 (s, 3 H), 3.84 (s, 6 H), 3.82 (s, 3 H), 3.78 (s, 6 H), 3.32 (d, *J* = 2.2 Hz, 2 H), 2.96 (dd, *J* = 17.8, 4.7 Hz, 1 H), 2.87 (d, *J* = 16.5 Hz, 1 H), 2.69 (dd, *J* = 21.4, 11.0 Hz, 2 H), 2.17 (m, 1 H), 1.89 (m, 2 H), 1.65 (m, 3 H), 1.55 – 1.49 (m, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 158.6 157.9, 154.4, 152.0, 147.7, 133.3, 129.3, 117.7, 109.8, 108.7, 104.6, 99.1, 92.3, 90.9, 76.1, 66.7, 64.5, 63.0, 54.4, 51.8, 51.6, 40.0, 27.3, 26.9 24.8. HRMS calcd for $(C_{35}H_{43}O_{10}N + H)^+$ 638.2960, found 638.2965.

4.1.31. Synthesis of (2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 1-(3,4,5-trimethoxybenzyl)piperidine-3-carboxylate (42)

Following the procedure for the preparation of compound **36**, but with permethyl epicatechin (EC) and 1-(3, 4, 5-trimethoxybenzyl) piperidine-3-carboxylic acid as starting material, the titled compound **42** was prepared. Yield 33%; $[a]^{20}_D$ = -49.0 (c = 1.0, CH_2Cl_2); mp 59-61 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.02 – 6.90 (m, 2 H), 6.73 (d, *J* = 8.3 Hz, 1 H), 6.49 (s, 2 H), 6.19 (d, *J* = 2.2 Hz, 1 H), 6.09 (d, *J* = 2.2 Hz, 1 H), 5.44 – 5.39 (m, 1 H), 5.00 (s, 1 H), 3.91 – 3.79 (m, 15 H), 3.79 – 3.69 (m, 6 H), 3.44 – 3.24 (m, 2 H), 2.99 – 2.80 (m, 3 H), 2.77 (d, *J* = 10.3 Hz, 1 H), 2.64 (s, 1 H), 2.48 (d, *J* = 7.3 Hz, 1 H), 1.98 – 1.71 (m, 3 H), 1.62 – 1.52 (m, 1 H), 1.43 (d, *J* = 11.0 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.4, 159.6, 158.8, 155.4, 153.0, 148.7, 130.3, 118.8, 110.8, 109.8, 105.4, 100.1, 77.1, 67.8, 63.0, 60.8, 56.1, 55.9, 55.8, 55.4, 53.0, 41.9, 26.6, 25.8, 24.3. HRMS calcd for $(C_{35}H_{43}O_{10}N + H)^+$ 638.2960, found 638.2967.

4.1.32. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl1-(3,4,5-

trimethoxybenzyl)piperidine-4-carboxylate (43)

Following the procedure for the preparation of compound **36**, but with permethyl catechin (C) and 1-(3, 4, 5-trimethoxybenzyl) piperidine-4-carboxylic acid as starting material, the titled compound **43** was prepared. Yield:35.0%; $[a]^{20}_{D}$ = +15.5 (c = 1.0, CH_2Cl_2); mp 80-82 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.91 (dd, *J* = 8.2, 1.8 Hz, 1 H), 6.89 (d, *J* = 1.8 Hz, 1 H), 6.82 (d, *J* = 8.2 Hz, 1 H), 6.52 (s, 2 H), 6.14 (d, *J* = 2.2 Hz, 1 H), 6.09 (d, *J* = 2.2 Hz, 1 H), 5.37 (m, 1 H), 4.94 (m, 1 H), 3.87 – 3.83 (s, 12 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 3.75 (s, 3 H), 3.34 (s, 2 H), 2.97 (dd, *J* = 16.5, 5.6 Hz, 1 H), 2.73 (d, *J* = 10.9 Hz, 1 H), 2.63 (dd, *J* = 16.5, 7.6 Hz, 2 H), 2.22 – 2.14 (m, 1 H), 1.93 (d, *J* = 10.2 Hz, 2 H), 1.71 (d, *J* = 10.0 Hz, 1 H), 1.67 – 1.59 (m, 2 H), 1.54 (m, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.2, 159.8, 158.6, 155.0, 153.0, 149.0, 136.8, 134.3, 130.1, 119.7, 111.0, 109.9, 105.6, 100.8, 93.0, 91.9, 78.7, 68.8, 63.3, 60.8, 56.1, 55.9, 55.4, 52.8, 52.6, 41.0, 28.1, 27.9, 24.7. HRMS calcd for $(C_{35}H_{43}O_{10}N + H)^+$ 638.2960, found 638.2965.

4.1.33. Synthesis of (2R,3S)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 1-(3,4,5-trimethoxybenzyl)piperidine-3-carboxylate (44)

Following the procedure for the preparation of compound **36**, but with permethyl catechin (C) and 1-(3, 4, 5-trimethoxybenzyl) piperidine-3-carboxylic acid as starting material, the titled compound **44** was prepared. Yield:32.0%; $[a]^{20}_{D}$ = +3.8 (c = 1.0, CH_2Cl_2); mp 49-51 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.89 (s, 2 H), 6.80 (dd, *J* = 18.7, 8.4 Hz, 1 H), 6.52 (d, *J* = 2.7 Hz, 2 H), 6.14 (d, *J* = 2.3 Hz, 1 H), 6.09 (d, *J* = 2.3 Hz, 1 H), 5.32 (m, 1 H), 4.94 (m, 1 H), 3.88 – 3.81 (s, 15 H), 3.78 – 3.74 (s, 6 H), 3.39 – 3.33 (m, 2 H), 3.01 – 2.96 (m, 1 H), 2.81 (d, *J* = 9.7 Hz, 1 H), 2.75 – 2.55 (m, 3 H), 2.50 – 2.42 (m, 1 H), 2.01 (ddd, *J* = 51.8, 20.4, 10.5 Hz, 3 H),

910 1.58 (dd, *J* = 8.4, 4.7 Hz, 1 H), 1.51 – 1.44 (m, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2,
911 159.8, 158.6, 155.0, 153.0, 149.0, 134.3, 130.1, 119.8, 110.9, 109.8, 105.5, 100.9, 93.0, 91.9,
912 78.8, , 68.9, 63.4, 60.8, 55.9, 55.8, 55.6, 55.3, 53.4, 53.1, 41.8, 26.9, 24.9, 24.4. HRMS calcd
913 for $(C_{35}H_{43}O_{10}N + H)^+$ 638.2960, found 638.2966.
914
915 ***4.1.34. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3-(3-***
916 ***(benzyloxy)-4-methoxybenzamido)-4-fluorobenzoate (45)***
917 Following the procedure for the preparation of compound **33**, but with (2R,3R)-5,7-
918 dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-(3-(benzyloxy)-4-methoxybenzamido)
919 -3-fluorobenzoateas starting material, the titled compound **45** was prepared. Yield 61.0%;
920 $[a]^{20}_D$ = -51.0 (c = 1.0, CH_2Cl_2); mp 56-59 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (dd, *J* = 7.5,
921 1.6 Hz, 1H), 7.83 (d, *J* = 2.8 Hz, 1H), 7.71 – 7.68 (m, 1H), 7.50 – 7.45 (m, 3H), 7.43 – 7.35
922 (m, 3H), 7.31 (t, *J* = 7.3 Hz, 1H), 7.08 (dd, *J* = 18.4, 9.5 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.74
923 (s, 2H), 6.28 (d, *J* = 2.1 Hz, 1H), 6.11 (d, *J* = 2.1 Hz, 1H), 5.66 (s, 1H), 5.21 (s, 2H), 5.08 (d, *J*
924 = 11.1 Hz, 1H), 3.94 (s, 3H), 3.81 – 3.75 (m, 16H), 3.07 (d, *J* = 3.2 Hz, 2H). ^{13}C NMR
925 ($CDCl_3$,125 MHz) δ 164.6, 164.4, 159.6, 158.5, 155.4, 153.1, 145.2, 137.7, 136.4, 133.3, 128.6,
926 128.1, 127.5, 126.8, 126.7, 126.6, 126.4, 126.3, 126.2, 123.7, 120.3, 114.8, 114.6, 113.0, 110.9,
927 103.7, 100.1, 93.5, 91.9, 77.8, 71.1, 69.0, 60.7, 56.1, 56.0, 55.4, 55.3, 25.9. HRMS calcd for
928 $(C_{42}H_{40}FNO_{11} + H)^+$ 754.2658, found 754.2651.
929 ***4.1.35. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-***
930 ***fluoro-3-(3-hydroxy-4-methoxybenzamido)benzoate (46)***

To a solution of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-(3-(benzyloxy)-4-methoxybenzamido)-3-fluorobenzoate（**45,** 500mg）in methanol,10% Pd/c was added. The material was reacted under hydrogen at room temperature and it accomplished in 4h. The catalyst is filtered off. Methanol is removed in vacuum and the residue was purified by column chromatography on silica gel to afford **46** (300 mg, 60%); $[a]^{20}_{D}$ = -48.0 (c = 1.0, CH_2Cl_2); mp 65-68 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.98 (d, *J* = 7.0 Hz, 1H), 7.87 (s, 1H), 7.72 – 7.67 (m, 1H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.12 – 7.06 (m, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.75 (s, 2H), 6.28 (d, *J* = 1.8 Hz, 1H), 6.11 (d, *J* = 1.7 Hz, 1H), 5.77 (s, 1H), 5.65 (s, 1H), 5.09 (s, 1H), 3.95 (s, 3H), 3.82 – 3.73 (m, 15H), 3.06 (d, *J* = 2.9 Hz, 2H). ^{13}C NMR ($CDCl_3$,125 MHz) δ 164.6, 1645, 159.6, 158.8, 156.5, 155.4, 154.5, 153.1, 149.8, 145.7, 137.7, 133.3, 127.2, 126.8, 126.7, 126.6, 126.3, 126.2, 123.7, 120.0, 114.8, 114.6, 113.2, 110.3, 103.7, 100.1, 93.5, 92.0, 77.8, 69.0, 60.7, 56.0, 55.3, 25.0. HRMS calcd for $(C_{35}H_{38}FNO_{11} + H)^+$ 664.2189, found 664.2202.

4.1.36. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 4-fluoro-3-(3-(2-hydroxyethoxy)-4-methoxybenzamido)benzoate (47)

Product **46** (300 mg) was dissolved in DMF, and then 2-iodoethan-1-ol (0.3ml) was added. The suspension is stirred under N_2 at 85℃ until the reaction completed (monitored by TLC). The mixture was washed with 20 ml of CH_2Cl_2. The aqueous phase was further extracted with CH_2Cl_2 (2×10 mL). The combined organic extract was dried over $MgSO_4$, filtered, and concentrated in vacuum. The crude product was purified by chromatography on silica gel, affording **47** (150 mg, 50%) as yellow oil. $[a]^{20}_{D}$ = -52.0 (c = 1.0, CH_2Cl_2); mp 68-70 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (dd, *J* = 10.1, 5.1 Hz, 1H), 7.91 (s, 1H), 7.71 (dd, *J* = 7.0, 4.2

953 Hz, 1H), 7.50 (d, *J* = 1.7 Hz, 1H), 7.44 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.13 – 7.06 (m, 1H), 6.94 (d,
954 *J* = 8.4 Hz, 1H), 6.74 (s, 2H), 6.28 (d, *J* = 1.8 Hz, 1H), 6.11 (d, *J* = 1.9 Hz, 1H), 5.66 (s, 1H),
955 5.09 (s, 1H), 4.59 – 4.55 (m, 2H), 4.33 (dd, *J* = 9.6, 5.2 Hz, 2H), 3.97 – 3.92 (m, 3H), 3.82 –
956 3.72 (m, 15H), 3.08 (t, *J* = 7.9 Hz, 2H). ^{13}C NMR ($CDCl_3$,125 MHz) δ 164.5, 160.7, 159.6,
957 158.8, 156.6, 155.4, 154.6, 163.1, 147.9, 137.7, 133.3, 126.9, 126.6, 126.5, 126.4, 126.3, 123.9,
958 120.8, 114.8, 114.7, 113.3, 111.1, 103.7, 100.1, 93.5, 91.9, 77.8, 69.0, 67.0, 62.0, 60.7, 56.0,
959 55.3, 25.9. HRMS calcd for $(C_{37}H_{39}FNO_{12} + H)^+$ 708.2451, found 708.2464.

960 ***4.1.37. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl3-(3-***
961 ***(2-bromoethoxy)-4-methoxybenzamido)-4-fluorobenzoate (48)***

962 Compound **48** was made using the procedure described for compound **36**, but with 3-
963 (3-(2-bromoethoxy)-4-methoxybenzamido)-4-fluorobenzoic acid, as the starting material, the
964 compound **48** was obtained. Yield 45.0%; $[a]^{20}_D$ =-53.0 (c = 1.0, CH_2Cl_2); mp 54-58 °C; ^{1}H
965 NMR ($CDCl_3$, 500 MHz) δ 8.96 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.96 (d, *J* = 2.9 Hz, 1H), 7.86 (dd, *J*
966 = 6.2, 3.3 Hz, 1H), 7.76 – 7.71 (m, 1H), 7.51 (d, *J* = 2.0 Hz, 1H), 7.49 (dd, *J* = 6.3, 3.2 Hz, 1H),
967 7.46 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.11 (dd, *J* = 10.2, 8.7 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.77
968 (s, 2H), 6.30 (t, *J* = 4.4 Hz, 1H), 6.14 (d, *J* = 2.3 Hz, 1H), 5.69 (t, *J* = 3.0 Hz, 1H), 5.11 (s, 1H),
969 4.41 (t, *J* = 6.4 Hz, 2H), 3.95 (d, *J* = 9.7 Hz, 3H), 3.84 – 3.75 (m, 13H), 3.70 (t, *J* = 6.4 Hz,
970 2H), 3.09(d,*J* = 3.3Hz, 2H). ^{13}CNMR($CDCl_3$,125 MHz) δ 164.6, 164.5, 159.7, 158.9, 156.6,
971 155.5, 154.9, 153.3, 153.2, 147.8, 137.7, 133.4, 126.9, 126.9, 126.8, 126.7, 126.6, 124.1, 124.0,
972 121.0, 113.7, 111.3, 103.8, 100.2, 92.0, 93.6. HRMS calcd for $(C_{37}H_{37}BrFNO_{11} + H)^+$ 770.1607, found
973 770.1611.

974 ***4.1.38. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl4-***

fluoro-3-(4-methoxy-3-(2-(4-methylpiperazin-1-yl)ethoxy)benzamido) benzoate (49)

Under a nitrogen atmosphere, compound **48** (78 mg, 0.1 mmol) were dissolved in 1-methylpiperazine (10 mL). The reaction mixture was stirred at room temperature until TLC showed that the reaction was completed. Then the reaction mixture was washed by water and dichloromethane for two times. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by flash chromatography on silica gel to afford the title compound **49.** Yield 78.0%; $[a]^{20}_D$ = -47 (c = 1.0, CH_2Cl_2); mp 50-51°C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.23 (d, *J* = 7.2 Hz, 1H), 7.78 – 7.74 (m, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.55 (s, 1H), 7.22 (t, *J* = 9.3 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.82 (s, 2H), 6.22 (s, 1H), 6.14 (s, 1H), 5.65 (s, 1H), 5.15 (s, 1H), 4.19 (t, *J* = 5.2 Hz, 2H), 3.89 (s, 3H), 3.75 (d, *J* = 10.1 Hz, 3H), 3.72 (d, *J* = 11.6 Hz, 3H), 3.71 – 3.61 (m, 8H), 3.07 (dd, *J* = 17.7, 4.6 Hz, 1H), 2.95 (d, *J* = 17.8 Hz, 1H), 2.84 (t, *J* = 5.2 Hz, 2H), 2.79 – 2.45 (m, 8H), 2.30 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 166.7, 164.1, 159.8, 158.8, 157.9, 155.3, 153.1, 152.8, 147.9, 137.1, 134.2, 128.2, 128.1, 127.7, 126.3, 126.0, 125.9, 125.7, 121.7, 115.8, 115.7, 112.8, 110.9, 103.6, 99.7, 93.3, 91.3, 77.3, 69.3, 66.6, 59.7, 56.5, 55.1, 55.1, 54.5, 54.4, 54.1, 52.5, 44.4 , 25.3. HRMS calcd for $(C_{42}H_{49}FN_3O_{11} + H)^+$ 790.3346, found 790.3338.

4.1.39. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl4-fluoro-3-(4-methoxy-3-(2-morpholinoethoxy)benzamido)benzoate (50)

Following the procedure for the preparation of compound **49**, but with morpholine as starting material, the titled compound **50** was prepared. Yield 70.0%; $[a]^{20}_D$ = -55 (c = 1.0, CH_2Cl_2); mp 51-54 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.24 (dd, *J* = 7.3, 2.1 Hz, 1H), 7.77 (ddd, *J* = 8.6, 4.7, 2.2 Hz, 1H), 7.58 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.24 –

7.19 (m, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.81 (s, 2H), 6.22 (d, *J* = 2.3 Hz, 1H), 6.14 (dd, *J* = 5.1, 2.4 Hz, 1H), 5.65 (dd, *J* = 2.7, 1.2 Hz, 1H), 5.14 (s, 1H), 4.19 (t, *J* = 5.5 Hz, 2H), 3.89 (d, *J* = 3.2 Hz, 3H), 3.76 (d, *J* = 5.1 Hz, 3H), 3.74 (s, 3H), 3.72 – 3.66 (m, 13H), 3.10 – 3.04 (m, 1H), 2.96 (d, *J* = 16.8 Hz, 1H), 2.82 (t, *J* = 5.5 Hz, 2H), 2.66 – 2.59 (m, 4H). ^{13}C NMR (125 MHz, CD_3OD) δ 166.71, 164.1, 159.8, 158.8, 157.8, 155.3, 153.1, 152.9, 147.9, 137.2, 134.2, 128.2, 128.1, 127.6, 127.6, 126.3, 126.3, 126.0, 125.9, 125.7, 121.7, 115.7, 115.6, 112.8, 110.9, 103.6, 99.7, 93.2, 91.3, 77.3, 69.2, 66.6, 66.2, 59.6, 57.1, 55.1, 55.0, 54.5, 54.3, 53.7, 25.3. HRMS calcd for $(C_{41}H_{46}FN_2O_{12} + H)^+$ 777.3029, found 777.3047.

4.1.40. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl4-fluoro-3-(4-methoxy-3-(2-(piperidin-1-yl)ethoxy)benzamido)benzoate (51)

Following the procedure for the preparation of compound **49**, but with piperidine as starting material, the titled compound **51** was prepared. Yield 80.0%; $[a]^{20}_D$ = -49 (c = 1.0, CH_2Cl_2); mp 58-63 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.24 (dd, *J* = 7.3, 2.1 Hz, 1H), 7.79 – 7.74 (m, 1H), 7.25 – 7.19 (m, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 6.82 (d, *J* = 5.4 Hz, 2H), 6.22 (d, *J* = 2.3 Hz, 1H), 6.18 – 6.13 (m, 1H), 5.67 – 5.63 (m, 1H), 5.14 (s, 1H), 4.24 (t, *J* = 5.6 Hz, 2H), 3.90 (d, *J* = 5.8 Hz, 3H), 3.79 – 3.72 (m, 6H), 3.72 – 3.64 (m, 9H), 3.10 – 3.04 (m, 1H), 2.97 (dd, *J* = 17.4, 11.8 Hz, 3H), 2.77 (s, 4H), 1.68 (dt, *J* = 11.3, 5.7 Hz, 4H), 1.60 – 1.46 (m, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 166.6, 164.1, 159.8, 158.8, 157.8, 155.3, 153.1, 152.9, 147.7, 137.2, 134.2, 128.2, 128.1, 127.6, 127.6, 126.3, 126.3, 126.7, 125.9, 125.7, 121.9, 115.8, 115.6, 113.1, 110.9, 103.6, 99.7, 93.2, 91.3, 77.3, 69.2, 65.8, 59.6, 56.9, 55.1, 55.1, 54.5, 54.3, 54.3, 25.3, 24.5, 23.0**.** HRMS calcd for $(C_{42}H_{48}FN_2O_{11} + H)^+$ 775.3237, found 775.3258.

4.2. Materials for biological studies

DMSO, verapamil, doxorubicin (DOX), rhodamine 123 (R123), 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)-2H-tetra zolium (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 Prof. P. Borst (The Netherlands Cancer Institute, Amsterdam, Netherlands). The HEK293/pcDNA3.1 and HEK293/R2 were kindly provided by Dr. Kenneth To (The Chinese University of Hong Kong, Hong Kong). The L929 cell line was purchased from ATCC.

4.3. 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 37ºC in a humidified atmosphere with 5% CO_2. The cells were split constantly after a confluent monolayer has been formed. To split cells, the plate was washed briefly with phosphate-buffered saline (PBS), treated with 0.05% trypsin-EDTA and harvested by centrifugation.

4.4 Cell proliferation assay

6,000 cells of LCC6 or LCC6MDR and PTX were mixed with or without modulators to a final volume of 200 µL in each well of 96-well plates. 4,000 cells of 2008/P or 2008/MRP1 and DOX were co-incubated with or without modulators to a final volume of 200 µL. 4,500 cells of HEK293/pcDAN3.1 or HEK293/R2 and topotecan were co-incubated with or without modulators to a final volume of 200 µL. The plates were then incubated for 5 days at 37 ºC. The cell viability was determined using the CellTiter 96 AQueous Assay (Promega) as reported previously.[37]

4.5. Cytotoxicity assay

10,000 cells of L929 were mixed with different concentrations (0, 0.4, 1.2, 3.7, 11.1, 33.3 and 100 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 ºC. 50 % inhibitory concentration (IC_{50}) of modulators was determined using MTS proliferation assay as described previously.

4.6. Intracellular DOX accumulation

1 x 10^6 cells of LCC6 or LCC6MDR cells were mixed with 20 µM DOX and 2 µM of modulator at 37ºC for 150 min. 0.2 % DMSO was used as a negative control. After incubation, the cells were spinned down and washed with cold PBS, pH7.4 and lysed with lysis buffer (0.75 M HCl, 0.2% Triton-X100 in isopropanol). The lysate was spinned down and the supernatant was saved. The fluorescence level of DOX was determined as reported previously.[37]

4.7. Intracellular rhodamine 123 accumulation

1 x 10^6 cells of LCC6 or LCC6MDR cells were mixed with 10 µg/mL DOX and 2 µM of modulator at 37ºC for 150 min. 0.2 % DMSO was used as a negative control. After incubation, the cells were spinned down and washed with cold PBS, pH7.4 and lysed with 2% Triton. The lysate was spinned down and the supernatant was saved. The fluorescence level of rhodamine 123 was determined as reported previously.[43]

4.8. Determination of plasma membrane P-gp protein levels

1x 10^6 cells of LCC6 or LCC6MDR was incubated with 2 or 1 µM of **25** or **31** for 48 hrs. After incubation, the cells were detached by incubating with 2.5 mM EDTA at 37ºC for 10 min. The cells were resuspended in 43 µL of FACS buffer (1% BSA and 1 mM ETDA in 1XPBS, pH7.4). Two µL of 1 µM vinblastine and 5 µL of PE labelled antihuman P-gp antibody (BD# 557003) were added to the cell suspension and then incubated at 37ºC for 1 hr.[44] After incubation, the cells were washed once with ice cold FACS buffer and finally resuspended in 300 µL FACS buffer. The mean signal of PE was measured by BD Accuri C6 flow cytometer using channel 2. A totol of 50,000 evens was recorded and the data was analyzed using BD Accuri software. Unstain control was included for each treatment with vinblastine and respective concentration of modulator only. An absolute fluroescence in each treatment was calculated by substracting the background fluorescence determined in the respective unstain control.

4.9. DOX efflux studies

To measure the DOX efflux, LCC6 or LCC6MDR cells were pre-incubated with 10 μM DOX for 1 hr at 37°C. After 1 hr, the cells were spun down and washed once with cold PBS. Then the cells were further incubated with or without compound **25** (2 μM). At 0, 15, 30, 60, 90 and 120 min, $5x10^5$ cells in 1 mL volume were harvested for measuring the intracellular DOX concentration. The % of DOX reduction was calculated = [(DOX level at final time point / DOX level at 0 min) * 100%]. The DOX level was determined by C6 Accuri flow cytometer at FL2 channel as described previously.

Supporting Information

Proton and Carbon NMR spectra of all the compounds tested can be found online.

Author contributions

Sheng-biao Wan and Larry M. C. Chow designed the project and revised the manuscript. Iris L. K. Wong and Xing-kai Wang conducted the experiments and wrote the manuscript. All the authors have read and approved the final version of the manuscript.

Conflict of interest

All authors in this article declare no conflict of interest.

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Abbreviation used:

P-gp, P-glycoprotein; MDR, multidrug resistance; ABC, ATP-binding cassette; DOX, doxorubicin; PTX, paclitaxel; EC_{50}, effective concentration; RPMI1640, Roswell Park Memorial Institute 1640; MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt.

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