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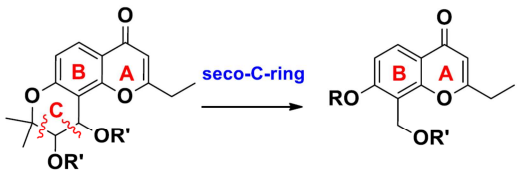

DSP **seco-DSPs: 7c-e and 8b**

compound	R	R'	reversal ratio
7c	ethyl	2-(3,4,5-trimethoxyphenyl)acetyl	677
7d	ethyl	cinnamoyl	503
7e	ethyl	(*E*)-3-(4-methoxyphenyl)acryloyl	>1471
8b	isopropyl	2-(3,4,5-trimethoxyphenyl)acetyl	576

New Seco-DSP Derivatives as Potent Chemosensitizers

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Abstract

Thirty-four seco-3'R,4'R-disubstituted-2',2'-dimethyldihydropyrano[2,3-*f*]chromone (seco-DSP) derivatives were designed, synthesized and evaluated for chemo-reversal activity when combined with paclitaxel or vincristine in P-gp overexpressing A2780/T and KB-VIN drug-resistant cancer cell lines. Most of the compounds displayed moderate to significant MDR reversal activities. Compound **7e** showed the most potent chemo-sensitization activity with more than 1471 reversal ratio at a concentration of 10 μM, which was higher than verapamil (VRP) (212-fold). Unexpectedly the newly synthesized compounds did not show chemosensitization activities in a non-P-gp overexpressing cisplatin resistant human ovarian cancer cell line (A2780/CDDP), implying that the MDR reversal effects might be associated with P-gp overexpression. Moreover, the compounds did not exhibit significant anti-proliferative activities against non-tumorigenic cell lines (HUVEC, HOSEC and T29) compared to VRP at the tested concentration and might be safer than VRP. In

preliminary pharmacological mechanism studies, the compounds increased accumulation of DOX and promoted P-gp ATPase activity in A2780/T cell lines. Western blot analysis indicated they did not affect the expression level of P-gp in the tested MDR cell lines. Thus, further studies on these seco-DSP derivatives are merited with the goal of developing a desirable chemosensitizer drug candidate.

Keywords: Seco-DSP • MDR reversal activity • Chemosensitizer • P-gp

1. Introduction

Tumor cell resistance, especially multidrug resistance (MDR), is a major obstacle to cancer treatment [1]. Statistically, 90% of failures in metastatic cancer chemotherapy are caused by multidrug resistance [2]. Reasons for the production of MDR in tumor cells include i) MDR gene expression and P-glycoprotein (P-gp)-mediated drug efflux [3]; ii) reduced tumor sensitivity resulting from decreased expression levels of intracellular DNA topoisomerase (Topo II) [4]; iii) apoptosis-related gene expression, such as bc1-2, mutant p53, etc. [5]; iv) decreased intracellular drug concentration resulting from multidrug resistance protein (MRP) recognition and coupling of chemotherapeutic drugs [6]; v) enhanced drug pump function caused by intracellular protein kinase C (PKC) promoting phosphorylation of P-gp [7]; and vi) up-regulation of caveolin and some of its components in tumor multidrug-resistant cells [8]. Since overexpression of P-gp leading to MDR is very common, the development of P-gp inhibitors is one of the most effective strategies to overcome MDR mediated by P-gp overexpression.

Presently, three generations of P-gp inhibitors have been developed. Verapamil (VRP), a representative first-generation P-gp inhibitor, enhances the intracellular accumulation of many anticancer drugs, e.g., doxorubicin (DOX) [9]. However, due to strong toxic side effects at its effective concentration, it has not been developed for use in cancer chemotherapy [10-11]. The second-generation P-gp inhibitors displayed improved affinity for P-gp and enhanced activity compared to the first-generation inhibitors. However, strong CYP3A4 inhibitory activity remained [12]. Although the third-generation P-gp inhibitors generally have stronger P-gp affinity and fewer toxic

side effects compared with the first- and second-generation inhibitors [13], currently, no safe and effective P-gp inhibitor has been approved and marketed.

Wu, J. Y. *et al.* reported that (±)-praeruptorin A (PA, **Figure 1**), a pyranocoumarin natural product isolated from *Peucedanum Praeruptorum Dunn*, exhibited multidrug resistance reversal activity [14]. Through structural modification, a series of (±)-3'-*O*-4'-*O*-dicinnamoyl-*cis*-khellactone (DCK) compounds were synthesized as non-competitive P-gp inhibitors. Notably, (±)-3'-*O*-4'-*O*-bis (3,4-dimethoxy-cinnamoyl)-*cis*-khellactone (DMDCK) displayed remarkable activity with reversal ratios ranging from 110- to 167-fold at a concentration of 4 μM when combined with different anticancer drugs in MDR HepG2/Dox cell lines [15]. Moreover, 3'R,4'R-disubstituted-2',2'-dimethyldihydropyrano[2,3-*f*]chromone (DSP) derivatives, obtained by scaffold hopping from DMDCK, also showed good MDR reversal activity. Among them, the resistance reversal ratio of DSP-**1** in vincristine (VCR)-resistant KB-VIN cells was more than 300-fold [16].

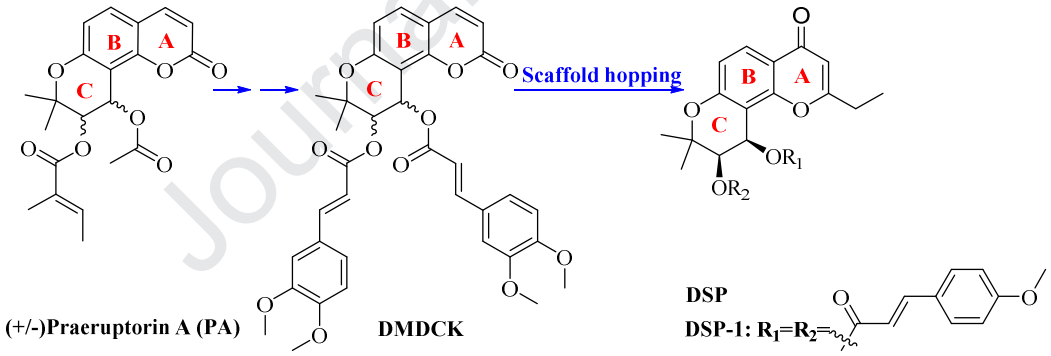


Figure 1. Structures of PA and DMDCK.

In our previous research aimed at the development of a desirable P-gp inhibitor, we opened the C-ring of 4-methyl-DMDCK to prepare seco-DMDCK derivatives (**Figure 2**) [17]. The synthesized compounds showed better multidrug resistance reversal activity than 4-methyl-DMDCK. Especially, the drug resistance reversal ratios of seco-DMDCK-1 in paclitaxel (PTX)-resistant A2780/T cells and VCR-resistant KB-VIN cells were more than 700-folds, much better than those of VRP.

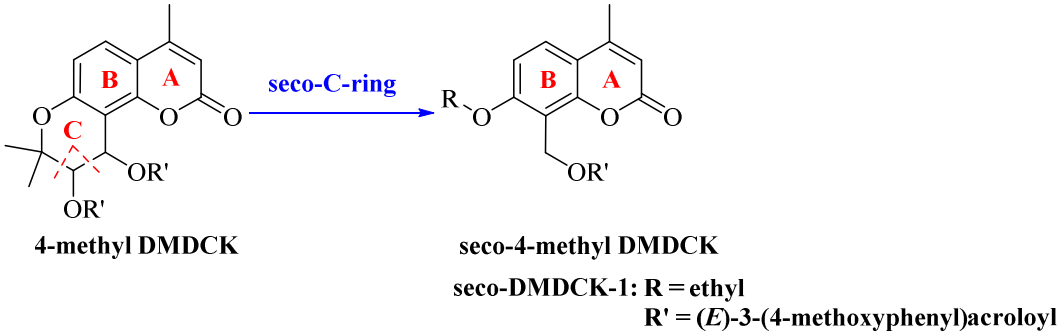


Figure 2. Seco-C-ring strategy for 4-methyl-DMDCK.

Motivated by the above results with DSP-1 and seco-4-methyl-DMDCK compounds, we further designed and synthesized 34 seco-DSP derivatives (**Figure 3**) based on both modification strategies. The newly synthesized compounds with lower molecular weights and fewer chiral centers might show maintained or even enhanced MDR reversal activity but improved drug-like properties. Herein, the synthesis and bioactivity evaluation of seco-DSPs containing 7-methoxy, -ethoxy or -isopropoxy moieties and various acyl substituted 8-hydroxymethyl groups are reported.

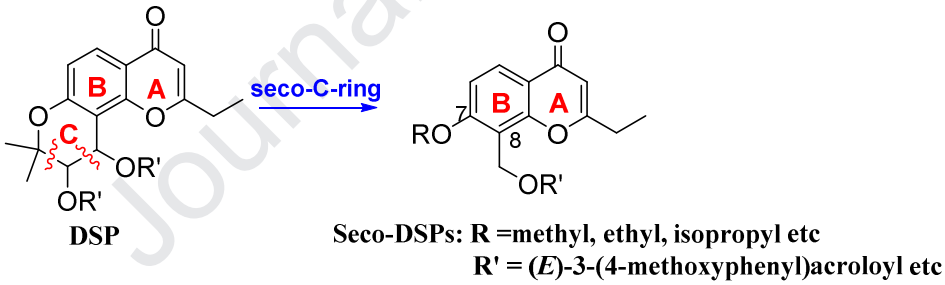


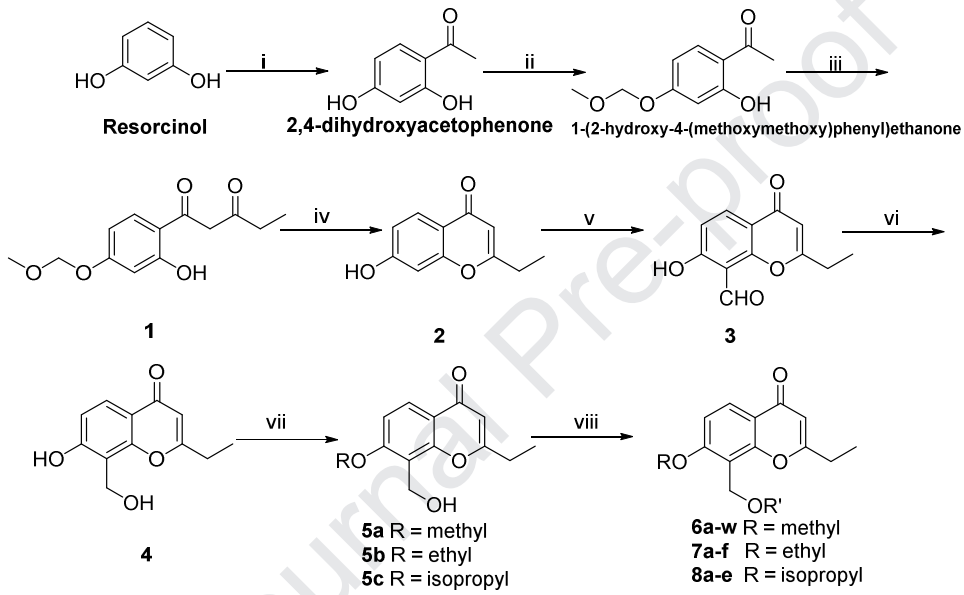
Figure 3. Seco-C-ring strategy for new derivatives.

2. Results and Discussion

2.1 Chemistry

As shown in **Scheme 1**, Friedel-Crafts acylation of resorcinol with acetic acid using $ZnCl_2$ as a catalyst gave 2,4-dihydroxyacetophenone. The 4-hydroxy group was then selectively protected to provide 1-(2-hydroxy-4-(methoxymethoxy)phenyl)ethanone, which was condensed with ethyl propionate using NaH in THF to give intermediate **1**.

Compound **4** was synthesized in three steps: cyclization of **1** with concentrated HCl in EtOH to give **2**, a Duff reaction to introduce an aldehyde group at C-8 to give **3**, and reduction of the aldehyde group using $NaBH_4$ in MeOH. Then, the 7-hydroxyl of **4** was alkylated successively with iodomethane, bromoethane and 2-bromopropane in the presence of K_2CO_3 and KI to provide the three key intermediates **5a-c**. Finally, 34 seco-DSP derivatives (**6a-w**, **7a-f** and **8a-e**) were prepared by esterification of **5a-c** with various aromatic acids catalyzed by 4-dimethylaminopyridine (DMAP) and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDCI) in yields from 40.8% to 57.5%.



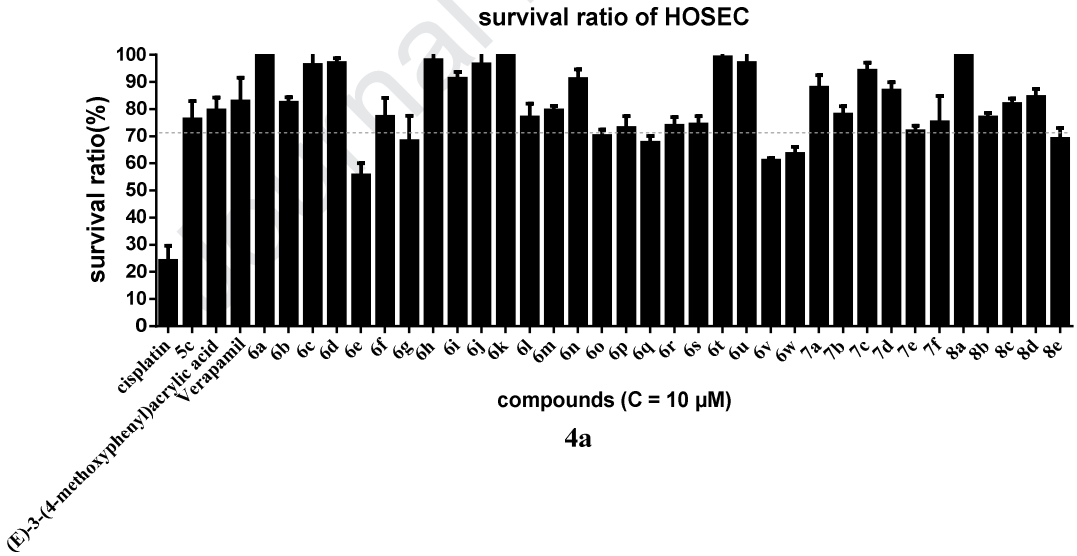
6a R' = 4-fluorobenzoyl
6b R' = 2-bromobenzoyl
6c R' = 2-nitrobenzoyl
6d R' = 2,6-difluorobenzoyl
6e R' = 4-chloro-3-nitrobenzoyl
6f R' = 2-chloro-5-nitrobenzoyl
6g R' = 2,4-dichlorobenzoyl
6h R' = 4-amino-3-methylbenzoyl
6i R' = 3-(dimethylamino)benzoyl
6j R' = 2-(4-chlorophenyl)acetyl
6k R' = 2-(o-tolyl)acetyl
6l R' = 4-propylbenzoyl
6m R' = 4-pentylbenzoyl
6n R' = 4-(tert-butyl)benzoyl
6o R' = (E)-3-(4-chlorophenyl)acryloyl
6p R' = 3-methoxybenzoyl
6q R' = 3,4-dimethoxybenzoyl
6r R' = 2,4,5-trimethoxybenzoyl
6s R' = 3,4,5-trimethoxybenzoyl
6t R' = 2-(3,4,5-trimethoxyphenyl)acetyl
6u R' = cinnamoyl
6v R' = (E)-3-(4-methoxyphenyl)acryloyl
6w R' = 4-cyanobenzoyl
7a R' = 2,4,5-trimethoxybenzoyl
7b R' = 3,4,5-trimethoxybenzoyl
7c R' = 2-(3,4,5-trimethoxyphenyl)acetyl
7d R' = cinnamoyl
7e R' = (E)-3-(4-methoxyphenyl)acryloyl
7f R' = 4-cyanobenzoyl
8a R' = 3,4,5-trimethoxybenzoyl
8b R' = 2-(3,4,5-trimethoxyphenyl)acetyl
8c R' = cinnamoyl
8d R' = (E)-3-(4-methoxyphenyl)acryloyl
8e R' = 4-cyanobenzoyl

Scheme 1. Syntheses of seco-DSP derivatives (**6a-w**, **7a-f** and **8a-e**). Reagents and conditions: (i) $ZnCl_2$, HOAc, reflux; (ii) MOMCl, K_2CO_3, acetone, rt; (iii) ethyl propionate, NaH, THF, reflux; (iv) EtOH, conc. HCl, reflux; (v) hexamethylenetetramine, HOAc, 95 °C, then HCl (conc.HCl:H_2O = 84:100 v/v), 70 °C; (vi) $NaBH_4$, MeOH, rt; (vii) iodomethane, bromoethane or 2-bromopropane, K_2CO_3, KI, acetone, reflux; (viii) aromatic acids, EDCI, DMAP, CH_2Cl_2, 0 °C-r.t.

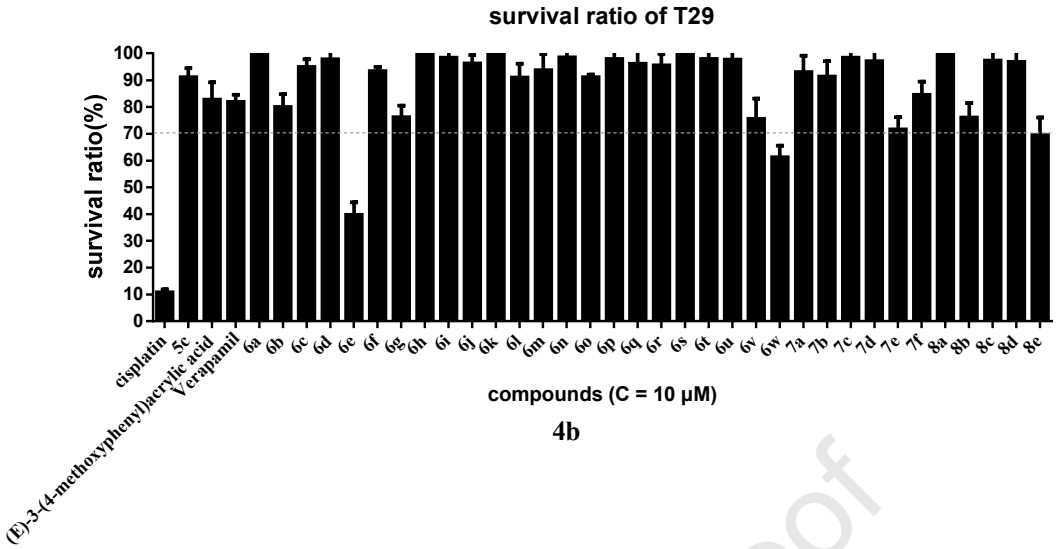
2.2 Biological evaluation

2.2.1. In vitro cytotoxicity

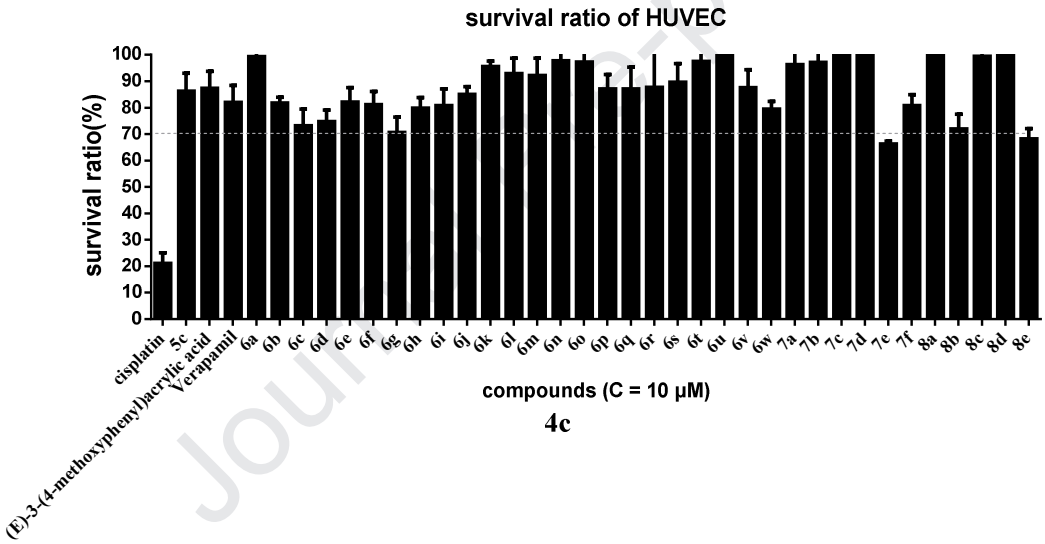
The 34 target compounds **6a-w**, **7a-f** and **8a-e** were screened for cytotoxicity at a concentration of 10 μM in an MTT assay against three non-tumorigenic cell lines (HOSEC, HUVEC, and T29) and three drug-resistant cancer cell lines including PTX- or cisplatin (CDDP)-resistant human ovarian (A2780/T and A2780/CDDP) and VCR-resistant human oral epidermoid cancer cell lines (KB-VIN). VRP was used as a positive control. As shown in **Figure 4a-c**, at 10 μM, only three compounds, **6e** (R' = 4-chloro-3-nitrobenzoyl), **6v** (R' = (*E*)-3-(4-methoxyphenyl)acryloyl) and **6w** (R' = 4-cyanobenzoyl), were slightly toxic to two (HOSEC and T29) of the normal human ovarian epithelial cell lines, the remaining 31 seco-DSP derivatives did not significantly affect the proliferation of normal cells. The inhibition rates were lower than that of VRP, suggesting that seco-DSP derivatives might be less toxic and safer than VRP.



4a

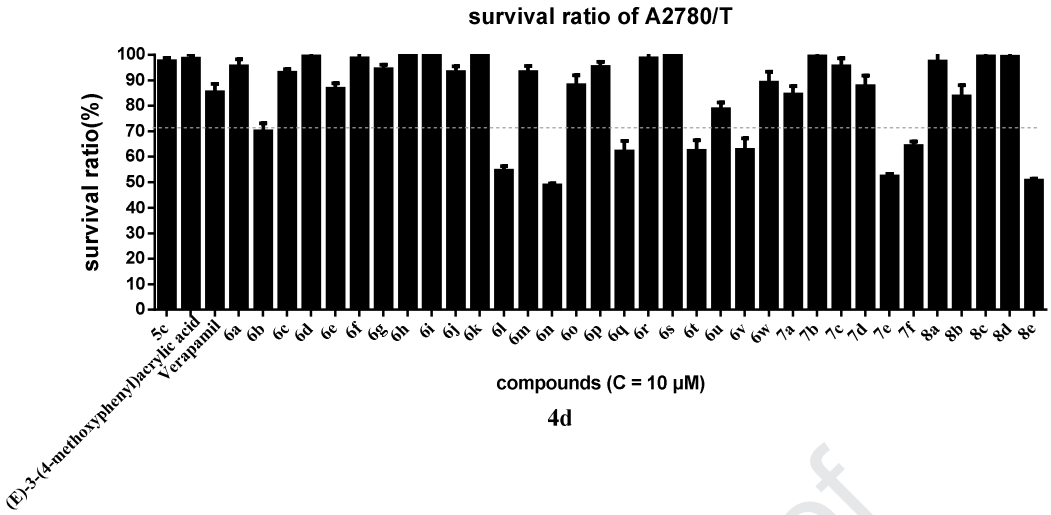


4b



4c

As shown in **Figure 4d**, more than two thirds of seco-DSP derivatives had no significant effect compared with VRP on the proliferation of A2780/T, while less than one third of the compounds, including ones with 2-bromobenzoyl (**6b**), 4-propylbenzoyl (**6l**), 4-*tert*-butylbenzoyl (**6n**), 3,4-dimethoxybenzoyl (**6q**), 2-(3,4,5-trimethoxyphenyl)acetyl (**6t**), 4-methoxycinnamoyl (**6v**, **7e**) and 4-cyanobenzoyl (**7f**, **8e**) esters, were slightly more cytotoxic than VRP against A2780/T.



2.2.2. MDR reversal activity of compounds against A2780/T, KB-VIN, and A2780/CDDP

In our previous research [17], the result of expression of P-gp in these MDR cell lines (A2780/CDDP, A2780/T and KB-VIN) has been detected by Western blot analysis (see in supporting information). The result indicated P-gp overexpression in the A2780/T and KB-VIN drug-resistant cell lines, but not in the cisplatin-resistant A2780/CDDP. Furthermore, the newly synthesized compounds **6a-w**, **7a-f** and **8a-e** at 10 μM were co-administered with PTX at different concentrations to evaluate the MDR reversal effects in PTX-resistant A2780/T cell lines. The reversal ratio (r.r.) was defined as IC_{50} (PTX only) / IC_{50} (PTX with 10 μM synthesized compound), as a standard of the evaluation. As shown in **Table 1**, 27 compounds exhibited moderate to significant MDR reversal activity (r.r. > 50), and 15 compounds (**6i**, **6q-s**, **6u-v**, **7a-e** and **8a-d**) showed greater reversal activity (r.r. 260-1471) than VRP (r.r. 211). The reversal ratios of compounds bearing 3,4,5-trimethoxybenzoyl, 2-(3,4,5-trimethoxyphenyl) acetyl, cinnamoyl, (*E*)-3-(4-methoxyphenyl)acryloyl esters at position-8 of the seco-DSP core (**6s**, **6u-v**, **7b-e** and **8a-d**) were over 400. Notably, compound **7e** with an ethoxy group at position-7 and an (*E*)-3-(4-methoxyphenyl)acryloyl ester at position-8 showed the most potent reversal effect (r.r. 1471), much higher than that of VRP. Meanwhile compounds **6b**, **6d-e**, **6w**, **7f** and **8e** with an electron-withdrawing group, such as fluoro, chloro, bromo, nitro and cyano, on the benzene ring in the aromatic ester at position-8 showed lower reversal activity (r.r. < 50). These results indicated that an electron-donating group, such as alkoxy, on the aromatic ester was more favorable for improved reversal activity. Also, the C-ring and chiral centers of the

DSP scaffold were not needed to produce chemosensitization. In addition, we further tested the reversal activity of the best potency four compounds (**7c**, **7d**, **7e** and **8b**) in two lower doses of 1 μM and 200 nM (See the detailed data in the supporting information). The results showed that in the concentration of 1 μM, the compounds **7c**, **7d**, **7e** and **8b** had reversal ratios of 4.13, 4.24, 128 and 3.97 folds, respectively. And in the concentration of 200 nM, their reversal activities hardly were observed with reversal ratios from 1.05 to 1.11-fold in A2780/T. This result indicated that our newly synthesized compounds still can keep a good reversal activity at lower a concentration as 1 μM, especially the compound **7e** had a reversal ratio of 128-fold. While the much lower concentration as 200 nM was not favorable for the reversal activity of **7c**, **7d**, **7e** and **8b**.The result suggested that the concentration of 10 μM might be a relatively suitable dose for these compounds reversal MDR action.

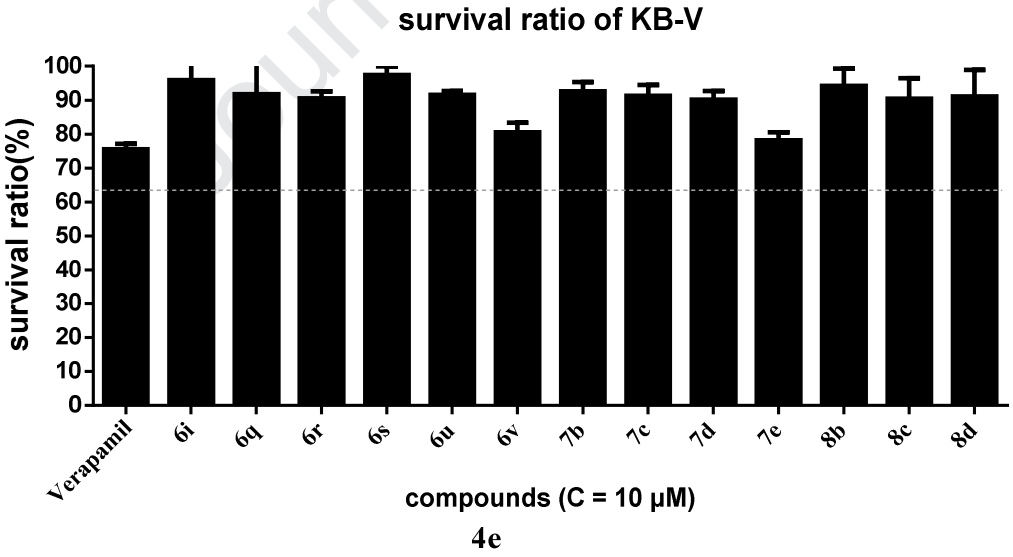
Table 1 MDR reversal activity of **6a-w**, **7a-f** and **8a-e** (10 μM) in A2780/T.

Compd	PTX IC_{50} (μM)	r.r.[a]	Compd	PTX IC_{50} (μM)	r.r.[a]
VRP	0.0188	211	**6r**	0.0140	284
6a	0.0745	53	**6s**	0.0074	537
6b	0.1695	23	**6t**	0.0735	54
6c	0.0231	172	**6u**	0.0093	427
6d	0.0934	43	**6v**	0.0096	414
6e	0.2770	14	**6w**	0.4988	8
6f	0.0701	57	**7a**	0.0087	457
6g	0.0315	126	**7b**	0.0087	457
6h	0.0225	177	**7c**	0.0059	677
6i	0.0082	484	**7d**	0.0079	503
6j	0.0448	89	**7e**	<0.0027	>1471
6k	0.0403	99	**7f**	0.2004	20
6l	0.1091	36	**8a**	0.0079	503
6m	0.0230	173	**8b**	0.0069	576
6n	0.0252	158	**8c**	0.0058	685

6o	0.0747	53	**8d**	0.0081	490
6p	0.0270	147	**8e**	0.3993	10
6q	0.0153	260	**PTX**	3.972	1

[a] reversal ratio (r.r.) defined as IC_{50} (PTX only) / IC_{50} (PTX with 10 μM synthesized compound)

Next, compounds **6i**, **6q-s**, **6u-v**, **7b-e** and **8b-d**, which displayed high MDR reversal activity in A2780/T cell lines, were evaluated for antiproliferative and reversal activities when administered alone and together with VCR in drug-resistant KB-VIN cell lines. As observed in **Figure 4e**, all 13 compounds exhibited lower antiproliferative activity than VRP against KB-VIN cells. However, most of the tested compounds displayed significant MDR reversal activity (r.r 105–633) in the KB-VIN cell line (**Table 2**). Among them, **6r**, **7c**, **7d** and **8b** with 2,4,5-trimethoxybenzoyl, 2-(3,4,5-trimethoxyphenyl)acetyl (**7c** and **8b**), and cinnamoyl esters, respectively, at position-8 of the seco-DSP scaffold showed more potent reversal activity than VRP (r.r. 579, 628, 633, 604, and 418, respectively).



4e

Table 2 MDR reversal activity of **6i**, **6q-v**, **7b-e** and **8b-d** (10 μM) in KB-VIN.

Compd	VCR IC_{50} (nM)	r.r.[a]	Compd	VCR IC_{50} (nM)	r.r.[a]

VRP	1.593	418	**7b**	2.692	248
6i	2.842	234	**7c**	1.061	628
6q	6.331	105	**7d**	1.053	633
6r	1.151	579	**7e**	2.228	299
6s	1.570	424	**8b**	1.103	604
6u	1.942	343	**8c**	1.429	466
6v	1.588	420	**8d**	1.947	342
VCR	666.3	1			

[a] reversal ratio (r.r.) was defined as IC_{50} (VCR only) / IC_{50} (VCR with 10 μM synthesized compound)

Interestingly, when co-administered with CDDP in the drug-resistant A2780/CDDP cell line, neither VRP (r.r. 1.07) nor compounds **6i**, **6s**, **7a-e**, **8a** and **8c-d** showed reversal activity (r.r. 0.8-1.05) (**Table 3**). And in view of P-gp inhibitors being developed for three generations, we chose a third generation P-gp inhibitor tariquidar, which was at phase III clinic trial, as a positive control for the reversal activity assay in A2780/CDDP. And the result indicated that at 10 μM, tariquidar only showed a weak reversal ratio of 1.9 folds in A2780/CDDP. (See the detailed data in supporting information). These results were consistent with our previously reported research; both seco-DSPs and seco-4-methyl-DCK analogs were strong chemosensitizers in A2780/T and KB-VIN cell lines but not in the A2780/CDDP cell line. The results of western blot analysis suggested that overexpression of P-gp partially contributed to MDR in the A2780/T and KB-VIN drug-resistant cell lines, but not in the CDDP-resistant A2780/CDDP cell lines [17]. Thus, we speculated that the MDR reversal activity of our newly synthesized seco-DSPs might also depend on the overexpression of P-gp in the relevant drug-resistant cancer cell lines.

Table 3 MDR reversal activity of **6i, 6s**, **7a-e, 8a** and **8c-d** (10 μM) in A2780/CDDP.

Compd	CDDP IC_{50} (μM)	r.r.[a]
VRP	40.11	1.07
6i	53.24	0.81

6s	53.17	0.81
7a	48.86	0.88
7b	41.13	1.05
7c	44.25	0.97
7d	47.06	0.92
7e	48.95	0.88
8a	43.59	0.99
8c	53.02	0.81
8d	44.22	0.97
CDDP	43.11	1

[a] reversal ratio (r.r.) was defined as IC_{50} (CDDP only) / IC_{50} (CDDP with 10 μM synthesized compound)

2.2.3. Structure-activity relationships

Some preliminary structure-activity relationship correlations are summarized as follows. Electron withdrawing substituents, such as F, Cl, Br, NO_2, and CN, on a benzoyl ester at position-8 were disadvantageous for the drug resistance reversal activity. Notably, the introduction of a methoxy group on a benzoyl ester generally led to improved reversal activity. The rank order of potency was 3-methoxy (**6p**, r.r. 147) < 3,4-dimethoxy (**6q**, r.r. 260) < 2,4,5-trimethoxy (**6r**, r.r. 284) < 3,4,5-trimethoxy (**6s**, r.r. 537). Electron-donating groups other than methoxy affected the reversal activity to different extents; compound **6i** with a 3-*N*,*N*-dimethylaminobenzoate on position-8 of the seco-DSP had a good r.r of 484. Furthermore, except for **6t**, compounds with phenylacetyl, cinnamoyl, or phenylacryloyl esters rather than benzoyl esters (**6u-v**, **7c-e**, and **8b-d**) exhibited good to remarkably improved potency (r.r. 414–1471). Among them, compound **7e** exhibited outstanding MDR reversal activity with a r.r. greater than 1471 and completely reversed PTX resistance in A2780/T cells at a concentration of 10 μM. Additionally, the potencies of 7-methoxy seco-DSPs were only slightly lower than those of corresponding 7-ethoxy or isopropoxy substituted derivatives; thus, the substituent at position-7 on the DSP A-ring affected the reversal activity to a smaller degree compared with the diverse aroyl substituents at the adjacent position-8. The type of ester groups might be key to maintain or improve the drug resistance reversal activity.

2.2.4. Effects on the expression of P-gp in A2780/T cells

The reversal of P-gp-mediated MDR can be achieved by inhibiting the function of P-gp or suppressing its expression. Wu, J. Y. et al. reported that PA mainly affected cellular P-gp activity by suppressing the expression of P-gp [14]. To determine whether the reversal activity of the seco-DSP compounds was caused by inhibition of P-gp expression, we incubated A2780/T cells with **7c**, **7d**, **7e** and **8b** at 10 μM and used Western blot analysis to determine the protein expression level of P-gp. As shown in **Figure 5**, the expression level neither increased nor decreased compared to the control after exposure to the four compounds. Wang-Fun Fong reported that DCK did not affect the P-gp expression [18], which is consistent with our experimental results. Because the results indicated that the compounds did not interfere with P-gp genetic expression and seco-DSP derivatives are derived from DCK/DSP compounds, they likely inhibited the efflux function of P-gp.

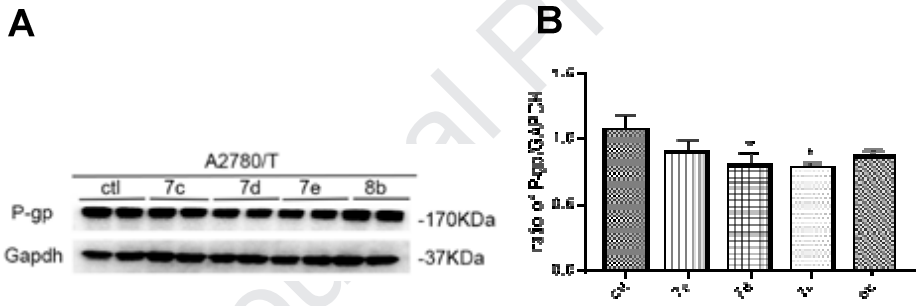


Figure 5. (A) Western blot analysis shows the expression of P-gp after exposure to 10 μM **7c**, **7d**, **7e** and **8b**. Band intensity was analyzed by Quantity One software, and protein expression was presented as the ratio of target protein's band intensity to that of β-tubulin. (B) Ratio of P-gp/GAPDH.

2.2.4. Inhibition of DOX efflux mediated by P-gp in A2780/T

Chemotherapy of MDR phenotypes is often associated with decreased intracellular drug concentration. An accumulation assay is commonly used to explore drug-efflux mediated by P-gp. Hence, we used this method to investigate whether the reversal effect of our seco-DSP compounds was also associated with a concomitant increase in DOX accumulation. Due to the autofluorescence of DOX, its accumulation can be determined by spectrofluorometry. The results are depicted in **Figure 6**. At 10 μM,

modulators **7c**, **7d**, **7e** and **8b** significantly enhanced DOX accumulation in A2780/T cells. Moreover, these results indicate that **7c**, **7d**, **7e** and **8b** are potent P-gp inhibitors, which are more potent than VRP in increasing accumulation of DOX in A2780/T cells.

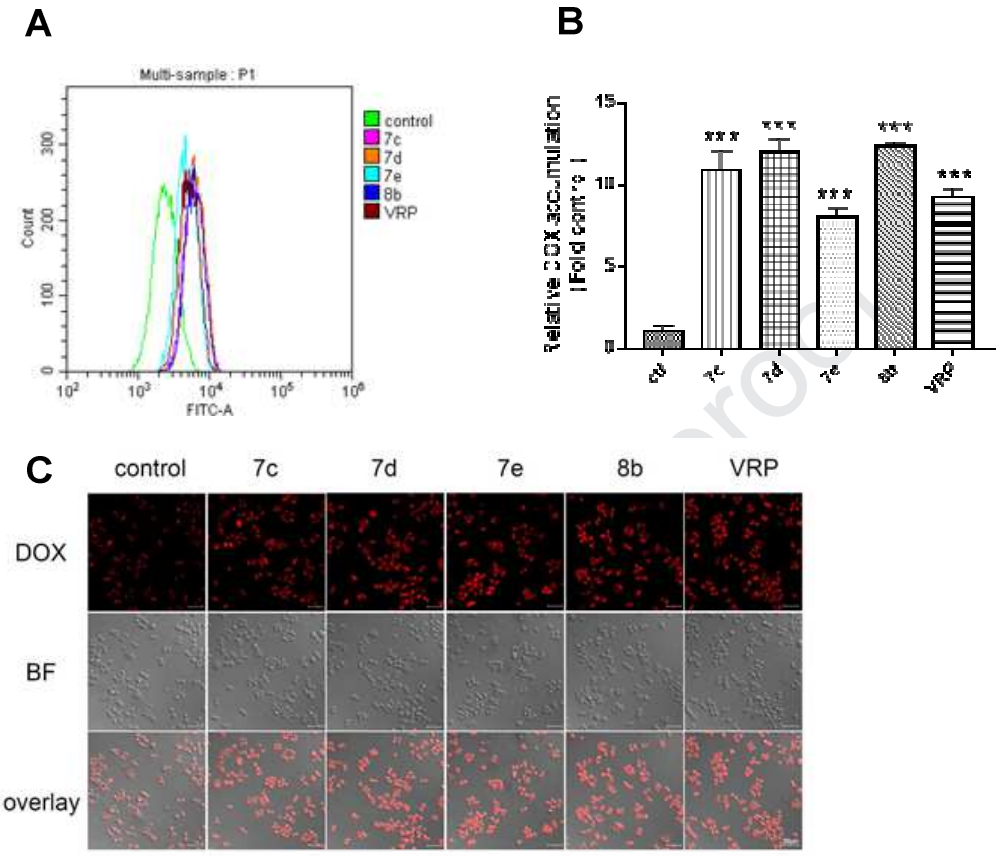


Figure 6. Intracellular accumulation of DOX in A2780/T cells. (A) Flow cytometry analysis of the accumulation of DOX (10 μM) in A2780/T cells with various test substances (10 μM) or VRP (10 μM), 0.1% DMSO as a negative control; (B) Quantification of DOX in A2780/T cells by flow cytometry; (C) Fluorescence images of DOX (red) under fluorescence microscopy. The results are presented as the mean ± SD: *P < 0.005; **P < 0.001; ***P < 0.0005, compared to negative control.

2.2.5. Effects on P-gp-ATPase activity

VRP is not only an inhibitor as well as a substrate of P-gp but also one of the best stimulators of P-gp-ATPase [19]; thus, we determined the effects of **7c**, **7d**, **7e** and **8b** on P-gp-ATPase activity. As shown in **Figure 7**, VRP visibly increased the P-gp-ATPase activity over the basal level by 1.8-fold at 0.5 mM, while **7c**, **7d**, **7e** and **8b** stimulated the activity by 1.2- to 2.3-fold at same tested concentration. These

findings suggest that the new compounds might function similarly to VRP toward P-gp-ATPase.

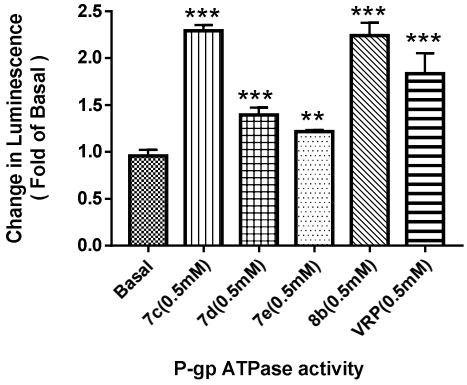


Figure 7. Effects of **7c**, **7d**, **7e** and **8b** on P-gp-ATPase activity. P-gp ATPase was measured in the absence (basal activity) or presence of P-gp modulators (VRP, **7c**, **7d**, **7e** and **8b**). Results are presented as the mean ± SD of three independent experiments: (∗∗∗) $P < 0.001$, (∗∗) $P < 0.01$, (∗) $P < 0.05$ relative to the basal activity.

3. Conclusions

In summary, we synthesized 34 seco-DSPs with three alkoxy groups at position-7 and various aryl-containing esters at position-8 and evaluated their ability to chemosensitize PTX and VCR in A2780/T and KB-VIN cell lines. Most of the compounds displayed lower cytotoxicity against HOSEC, HUVEC, T29, A2780/T, A2780/CDDP and KB-VIN cell lines and greater MDR reversal activity compared with VRP. Among them, compound **7e** [(7-ethoxy-2-ethyl-4-oxo-4*H*-chromen-8-yl) methyl (*E*)-3-(4-methoxyphenyl)acrylate] showed the most potent reversal activity (r.r. >1471). A preliminary structure-activity relationships said that electron-withdrawing group substitution on the benzoylbenzene ring was against the MDR reversal activity, while methoxy as an electron-donating group was a favourable groupto significantly improve the reversal activity. In addition, the activity also was greatly increased by using cinnamoyl or p-methoxycinnamoyl insteady of benzoyl. And the MDR reversal activity of the compounds with methoxyl group at position-7 was slight poor than that of ethoxyl and isopropoxyl groups substituted compounds.

Moreover, we explored the pharmacologic mechanism of four seco-DSP derivatives, **7c**, **7d**, **7e** and **8b**. The four compounds significantly inhibited the transport activity of

P-gp, as shown by intracellular accumulation of DOX in resistant A2780/T cells. However, Western blot analysis showed no significant difference between compound-treated and untreated cells, suggesting that the MDR reversal of the four compounds was likely due to the inhibition of P-gp efflux function rather than P-gp protein expression. In addition, the new compounds clearly promoted P-gp ATPase activity similarly to VRP. Overall, these results implied that C-ring of DSP is not required for P-gp modulations and MDR reversal. Further studies on seco-DSP derivatives are and merited to find and develop a promising P-gp-mediated MDR reversal modulator candidate.

4. Experimental section

4.1 Chemistry

All starting materials, reagents, and solvents were obtained from commercial sources and used without further purification unless otherwise indicated. Column chromatography was performed on silica gel (100-200 mesh), monitored by thin layer chromatography (TLC) on GF/UV 254 plates, and visualized using UV light at 254 and 365 nm. Melting points were measured on an SGW X-4 microscopy melting point apparatus without correction. ^{1}H and ^{13}C NMR data were recorded with a Varian 400 MHz spectrometer at 303 K using TMS as an internal standard. Mass spectra were recorded on Agilent Technologies 1260 infinity LC/MS instrument, and HRMS spectra were recorded on an Agilent Technologies LC/MSD TOF instrument.

4.2 Materials for biological studies

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin (DOX), verapamil (VRP) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, USA. The antibodies including anti-GAPDH (ab181602) and anti-P Glycoprotein (ab170904) were purchased from Abcam plc, USA (1:1000 dilutions). Horseradish peroxidase (HRP)-conjugated secondary antibodies used for Western blot were obtained from MR Biotech (Shanghai, China). Pgp-Glo assay systems were purchased from Promega Corporation.

4.3 Cell lines and cell culture

The A2780/CDDP, A2780/T and KB-VIN cell lines were kindly provided by Shanghai Institute of Materia Medica Chinese Academy of Science. HUVEC, HOSEC and T29 cells were kindly provided by Fudan University Shanghai Cancer Center. HUVEC, HOSEC and T29 cell lines were cultured in RPMI-1640 medium supplemented with 10% heat inactivated FBS in a humidified incubator at 37 °C with 5% CO_2. A2780/CDDP, A2780/T and KB-VIN cell lines were cultured in DMEM/F12 medium supplemented with 10% heat inactivated FBS in a humidified incubator at 37 °C with 5% CO_2.

4.4 Growth inhibitory and MDR reversal assays

For MTT assay, the *in vitro* anti-proliferation of the chemical compounds was measured by the MTT reagent, as described in the literature [20]. Briefly, 3~5×10^3 cells in 100 μL of medium per well were plated in 96-well plates. In cytotoxicity assays, after being incubated for 24 h, the cells were treated with different concentrations of test compound or DMSO (as negative control) for 48 h. In the MDR reversal assays, after being incubated for 24 h, the compounds and paclitaxel/cisplatin were added together to the 96-well plates or DMSO (as negative control) for 48 h. Then, the medium with compound or DMSO was replaced with 200 μL of fresh medium containing 10% MTT (5 mg/mL in PBS) in each well and incubation continued at 37 ºC for 4 h. Lastly, the MTT-containing medium was removed and 150 μL of DMSO per well was added to dissolve the newly formed formazan crystals. Absorbance of each well was determined by a microplate reader (Synergy H4, Bio-Tek) at a 490 nm wavelength. The inhibition rates of proliferation were calculated with the following equation:

$$\text{Inhibition ratio} = (OD_{DMSO}-OD_{compd.})/(OD_{DMSO}-OD_{blank})\times 100\%.$$

The concentrations of the compounds that inhibited cell growth by 50% (IC_{50}) were calculated using Graph Pad Prism version 6.0.

4.5 Cellular doxorubicin accumulation

Experiments for the cellular doxorubicin accumulation were carried out with the following modified method [21]. Approximately 5×10^5 cells of A2780/T were grown in six-well plates. After 24 h incubation, the cells were treated with 10 μM DOX and various test substances (10 μM) or VRP (10 μM) at 37 °C for 4 h with 0.1% DMSO as a negative control. Then, the cells were washed with ice-cold phosphate-buffered saline (PBS) three times and resuspended in ice-cold PBS. Cellular doxorubicin fluorescence level was monitored by CytoFLEX flow cytometer (Beckman Coulter, CA) and confocal microscopy.

4.6 Western blot analysis

Approximately 5×10^5 cells of A2780/T were grown in six-well plates. After 24 h incubation, the cells were treated with various seco-DSP derivatives (10 μM) at 37 °C for 48 h. Cells were incubated in ice-cold RIPA lysis buffer containing 1 mM PMSF for 30 min and collected by centrifugation (12500 rpm) at 4 °C for 15 min. The protein concentrations of supernatants were then subjected to BCA protein analysis. Then, equal amounts of proteins (20 μg) were separated on 12% SDS-PAGE gel and electro-transferred to PVDF membranes. Membranes were blocked with 3% bovine serum albumin (BSA). The membranes were then incubated with the specific primary antibodies (anti- P Glycoprotein antibody or GAPDH antibody, 1:1000 dilution) for night at 4 °C, followed by horseradish–peroxidase-conjugated secondary antibodies (1:10000 dilutions) for 1h at room temperature. Protein bands were visualized via enhanced chemiluminescence. Band intensity was calculated with Image J software.

4.7 P-gp ATPase assay

The effects of test substances on P-gp ATPase were detected by a P-gp-Glo assay system. Initially, about 25 mg of human P-gp membrane fraction were added into different wells: (1) "NT" well (20 μL of P-gp-Glo assay buffer), (2) "Na_3VO_4" well (20 μL of 0.25 mM Na_3VO_4), (3) "Ver" well (20 μL of 0.5 mM VRP), (4) "test-substances" well (20 μL of 0.5 mM test compounds), respectively. The cells were incubated at 37 °C for about 5 min. Secondly, reactions were initiated by adding and mixing 10 μL of 25 mM MgATP to all wells, and then the cells were incubated for 120 min at 37 °C. Thirdly, ATP detection reagent (50 μL) was added to all wells to stop the reactions and initiate luminescence. Incubation was then continued at room temperature for about 20 min. Finally, the luminescence signal was detected on a Biotek multi-functional microplate reader (Molecular Devices, USA).

4.8 Procedure for the synthesis of intermediate compound

2,4-Dihydroxyacetophenone. After zinc chloride (92.80 g, 681.2 mmol) was dissolved in 75 mL of acetic acid with heating, resorcinol (50.0 g, 454.1 mmol) was added. The reaction mixture was heated to 130-140 °C. After reaction completion, the mixture was cooled to room temperature, and the resulting solid was filtered. The reaction mixture was poured into ice-water (500 mL) and extracted with EtOAc (3×100 mL). The product was further purified by column chromatography to obtain 2,4-dihydroxyacetophenone (35.71 g). Yield 51.1%, m.p. 143.1-145.0 °C, MS(ESI) (*m/z*) 155.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 12.83 (s, 1H), 7.3-7.7 (m, 3 H),

6.46 (s, 1 H), 2.72 (s, 3 H).

1-(2-Hydroxy-4-(methoxymethoxy)phenyl)ethanone. To a stirred solution of 2,4-dihydroxyacetophenone (20.0 g, 131.4 mmol) in acetone (500 mL) was added anhydrous potassium carbonate (45.41 g, 328.5 mmol) at 0 °C. After stirring for 20 min at 0 °C, chloromethyl methyl ether (19.96 mL, 262.8 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 4 h and monitored by TLC. After completion of the reaction, the reaction was quenched with water (300 mL). EtOAc (300 mL) was added, the layers were separated, and the aqueous layer was extracted with EtOAc (2×100 mL). The combined organic layer was dried over anhydrous sodium sulfate. The residue was purified by silica gel column chromatography to provide 1-(2-hydroxy-4-(methoxymethoxy)phenyl)ethanone (18.1 g) as a colorless liquid. Yield 84.4%, MS(ESI) (*m/z*) 197.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.77 (s, 1H), 7.54 (d, J = 8.8 Hz, 1H), 6.58 (d, J = 2.0 Hz, 1H), 6.55 (dd, J = 8.8, 2.0 Hz, 1H), 5.24 (s, 2H), 3.46 (s, 3H), 2.54 (s, 3H).

2-Ethyl-7-hydroxy-4H-chromen-4-one (**2**). Sodium hydride (8.86 g, 222 mmol) was added to a well-stirred suspension of 1-(2-hydroxy-4-(methoxymethoxy) phenyl) ethanone (13.28 g, 67.69 mmol) in dry THF (100 mL) at 0 °C under N_2. After 30 min, ethyl propionate (104.5 mL, 135.4 mmol) was added dropwise, and the reaction mixture was heated to reflux for 2 h. The mixture was then cooled to room temperature, and the excess sodium hydride was quenched with distilled water. The reaction mixture was then poured on ice, followed by addition of 2M HCl, and the products were extracted with EtOAc (4×50 mL). The organic layers were combined and were washed with a saturated solution of sodium chloride (2×100 mL). The EtOAc layer was dried over anhydrous sodium sulfate and then evaporated to obtain the crude product **1**. Compound **1** (16.22 g, 64.31 mmol) was dissolved in 500 mL of EtOH, then conc. HCl (10 mL) was added. The reaction mixture was heated to reflux for 30 min. After reaction completion, EtOH was evaporated to obtain the crude product. The crude product was purified by silica gel column chromatography to provide **2** (10.91 g). Yield 85.0% (two steps), m.p. 194.2-196.1 °C, MS(ESI) (*m/z*) 191.1 $[M+H]^+$. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 10.78 (s, 1H), 7.83 (d, J = 8.7 Hz, 1H), 6.89 (d, J = 8.7 Hz, 1H), 6.84 (s, 1H), 6.07 (s, 1H), 2.62 (d, J = 7.4 Hz, 2H), 1.21 (t, J = 7.4 Hz, 3H).

2-Ethyl-7-hydroxy-4-oxo-4H-chromene-8-carbaldehyde (**3**). A reaction mixture of compound **2** (30.0 g, 157 mmol) and hexamethylenetetramine (154.8 g, 1104 mmol) in HOAc (500 mL) was stirred for 5.5 h at 80-90 °C. Aqueous HCl (800 mL, conc.

HCl/H_2O 84:100, v/v) was then added, and the reaction mixture was stirred for 0.5 h at 70 °C. After cooling, the reaction mixture was poured into ice-water (1.0 L) and extracted with EtOAc (3×500 mL). The combined organic fraction was dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. The residue was recrystallized from EtOAc to give **3** (8.65 g) as a light yellow solid. Yield 25.0%, m.p. 187.8-188.9 °C, MS(ESI) (*m/z*) 219.1 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.43 (s, 1H), 10.56 (s, 1H), 8.30 (d, *J* = 9.0 Hz, 1H), 6.98 (d, *J* = 9.0 Hz, 1H), 6.22 (s, 1H), 2.72 (q, *J* = 7.5 Hz, 2H), 1.35 (t, *J* = 7.5 Hz, 3H).

2-Ethyl-7-hydroxy-8-(hydroxymethyl)-4H-chromen-4-one (**4**). Sodium borohydride (3.48 g, 92.1 mmol) was added to a well-stirred suspension of **3** (13.41 g, 61.4 mmol) in MeOH (150 mL) at 0 °C. Then reaction mixture was stirred at room temperature. After reaction completion, reaction mixture was poured on ice, followed by addition of 2M HCl. The resulted solid was filtered to provide the crude product **4**. The crude product was purified by silica gel column chromatography to give **4** (12.85 g). Yield 95.1%, m.p. 164.0-166.1 °C, MS(ESI) (*m/z*) 221.1 $[M+H]^+$. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 10.70 (s, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 1H), 6.09 (s, 1H), 4.83 (s, 1H), 4.67 (s, 2H), 2.66 (d, *J* = 7.3 Hz, 2H), 1.25 (d, *J* = 7.3 Hz, 3H).

2-Ethyl-8-(hydroxymethyl)-7-methoxy-4H-chromen-4-one (**5a**), ***7-Ethoxy-2-ethyl-8-(hydroxymethyl)-4H-chromen-4-one*** (**5b**), ***2-Ethyl-8-(hydroxymethyl)-7-isopropoxy-4H-chromen-4-one*** (**5c**). Compound **4** (0.50 g, 1.8 mmol) and potassium carbonate (0.63 g, 4.5 mmol) were added to stirred DMF (5 mL). The temperature was raised to 80 °C, and iodomethane, bromoethane or 2-bromopropane was added. The reaction mixture was stirred at 80 °C for 1 h and then the insoluble solid was removed by filtration. The 30 mL of water were added to the mixture solution, which was then extracted with EtOAc (3×10 mL). The combined organic layers were washed with saturated sodium chloride solution (2×40 mL). The organic layer was dried over anhydrous sodium sulfate. Then, the solvent was removed under reduced pressure, and the residue was purified by column chromatography to yield **5a-c**. **5a**: yield 85.0%; m.p. 138.0-140.3 °C; MS(ESI) (*m/z*) 235.1 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 8.9 Hz, 1H), 7.01 (d, *J* = 8.9 Hz, 1H), 6.13 (s, 1H), 4.97 (s, 2H), 4.00 (s, 3H), 2.68 (q, *J* = 7.5 Hz, 2H), 1.32 (t, *J* = 7.5 Hz, 3H). **5b**: yield 80.2%; m.p. 107.1-109.2 °C; MS(ESI) (*m/z*) 249.1 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.9 Hz, 1H), 6.98 (d, *J* = 8.9 Hz, 1H), 6.11 (s, 1H), 4.97 (s, 2H), 4.23 (q, *J* = 7.0 Hz, 2H), 2.68 (q, *J* = 7.5 Hz, 2H), 1.51 (t, *J* = 7.0 Hz, 3H), 1.32 (t, *J* = 7.5 Hz, 3H). **5c**: yield 87.0%, m.p. 107.9-109.8 °C, MS(ESI) (*m/z*) 263.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 9.0 Hz, 1H), 6.99 (d, *J* = 9.0 Hz, 1H), 6.11 (s, 1H), 4.95 (s, 2H), 4.79 (m, *J* = 6.0 Hz, 1H), 2.67 (q, *J* = 7.5

Hz, 2H), 1.43 (d, *J* = 6.0 Hz, 6H), 1.31 (t, *J* = 7.5 Hz, 3H).

*4.9 General procedure for the synthesis of compounds **6a-w**, **7a-f** and **8a-e***

The corresponding aromatic acids (1.2 eq) and EDCI (1.2 eq) were dissolved and stirred in CH_2Cl_2 (10 mL) in an ice bath for 30 min, and then the corresponding alcohol **5a-c** (1.0 eq) was added to the reaction solution. The reaction mixture was removed to room temperature and monitored by TLC. After reaction completion, 15 mL of water were added to the mixture solution. The organic layers were washed with saturated sodium chloride solution (2×15 mL) and dried over anhydrous sodium sulfate. Then, the solvent was removed under reduced pressure, and the residue was purified by column chromatography to yield **6a-w**, **7a-f** and **8a-e**.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 4-fluorobenzoate (**6a**). Yield: 45.1%. m.p. 147.8-149.4 °C. MS(ESI) (*m/z*) 357.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 357.1133, Found: 357.1136. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 9.0 Hz, 1H, 5-H), 8.03 (ddd, *J* = 8.3, 5.2, 2.4 Hz, 2H, (2',6'-Ph-H)), 7.12-7.03 (m, 2H, (3',5'-Ph-H)), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.64 (s, 2H, 8-CH_2), 3.99 (s, 3H, OCH_3), 2.62 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 1.25 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 165.8, 165.7, 162.3, 156.3, 132.3, 132.2, 128.2, 126.4, 117.7, 115.6, 115.4, 111.2, 108.8, 108.6, 56.4, 55.5, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2-bromobenzoate (**6b**). Yield: 50.2%. m.p. 145.3-147.8 °C. MS(ESI) (*m/z*) 417.0 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 417.0332, Found: 417.0334. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 1H, 5-H), 7.75-7.62 (m, 2H, (3',6'-Ph-H)), 7.35-7.28 (m, 2H, (4',5'-Ph-H)), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.15 (s, 1H, 3-H), 5.68 (s, 2H, 8-CH_2), 4.00 (s, 3H, OCH_3), 2.66 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 1.29 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 166.2, 162.3, 156.2, 134.3, 132.5, 132.3, 131.3, 128.3, 127.1, 121.6, 117.6, 110.9, 108.7, 108.6, 56.4, 55.9, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2-nitrobenzoate (**6c**). Yield: 56.0%. m.p. 144.0-145.3 °C. MS(ESI) (*m/z*) 384.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 384.1078, Found: 384.1081. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.91 (d, *J* = 7.7 Hz, 1H, 3'-Ph-H), 7.75-7.70 (m, 1H, 5'-Ph-H), 7.69-7.58 (m, 2H, (4',6'-Ph-H)), 7.02 (d, *J* = 9.0 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.68 (s, 2H, 8-CH_2), 4.00 (s, 3H, OCH_3), 2.72 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 1.32 (t, *J* = 7.5 Hz, 3H,

$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 170.7, 165.5, 162.4, 156.2, 148.1, 132.9, 131.6, 129.8, 128.5, 127.8, 123.9, 117.6, 110.2, 108.6, 108.5, 56.7, 56.4, 27.4, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2,6-difluorobenzoate (**6d**). Yield: 43.4%. m.p. 139.0-141.9 °C. MS(ESI) (*m/z*) 375.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 375.1039, Found: 375.1042. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.38 (tt, *J* = 8.3, 6.2 Hz, 1H, 4'-Ph-H), 7.03 (d, *J* = 9.0 Hz, 1H, 6-H), 6.92 (t, *J* = 8.3 Hz, 2H, (3',5'-Ph-H)), 6.14 (s, 1H, 3-H), 5.70 (s, 2H, 8-CH_2), 3.99 (s, 3H, OCH_3), 2.68 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 1.30 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 170.6, 162.3, 161.6, 160.6, 160.5, 156.2, 132.6, 128.3, 117.6, 112.1, 111.8, 110.5, 108.7, 108.6, 56.4, 56.3, 27.4, 10.8.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 4-chloro-3-nitrobenzoate (**6e**). Yield: 48.9%. m.p. 185.4-187.0 °C. MS(ESI) (*m/z*) 418.0 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 418.0688, Found: 418.0691 ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, *J* = 2.0 Hz, 1H, 2'-Ph-H), 8.26 (d, *J* = 9.0 Hz, 1H, 5-H), 8.15 (dd, *J* = 8.5, 2.0 Hz, 1H, 6'-Ph-H), 7.62 (d, *J* = 8.5 Hz, 1H, 5'-Ph-H), 7.06 (d, *J* = 9.0 Hz, 1H, 6-H), 6.15 (s, 1H, 3-H), 5.69 (s, 2H, 8-CH_2), 4.00 (s, 3H, OCH_3), 2.64 (q, *J* = 7.3 Hz, 2H, $\underline{CH_2}CH_3$), 1.27 (t, *J* = 7.3 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.3, 163.7, 162.3, 156.2, 148.0, 133.6, 132.1, 131.7, 130.1, 128. 6, 126.6, 117.7, 110.4, 108.8, 108.7, 56.4, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2-chloro-5-nitrobenzoate (**6f**). Yield: 46.3%. m.p. 163.7-165.5 °C. MS(ESI) (*m/z*) 418.0 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 418.0688, Found: 418.0688. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 2.7 Hz, 1H, 6'-Ph-H), 8.25 (dt, *J* = 8.8, 1.2 Hz, 2H, (3',4'-Ph-H)), 7.64 (d, *J* = 9.0 Hz, 1H, 5-H), 7.06 (d, *J* = 9.0 Hz, 1H, 6-H), 6.16 (s, 1H, 3-H), 5.73 (s, 2H, 8-CH_2), 4.03 (s, 3H, OCH_3), 2.69 (q, *J* = 7.6 Hz, 2H, $\underline{CH_2}CH_3$), 1.31 (t, *J* = 7.6 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.4, 163.5, 162.4, 156.2, 146.1, 140.6, 132.3, 131.4, 128.6, 126.8, 126.5, 117.7, 110.3, 108.8, 108.7, 56.7, 56.5, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2,4-dichlorobenzoate (**6g**). Yield: 47.8%. m.p. 132.0-134.5 °C. MS(ESI) (*m/z*) 407.0 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 407.0448, Found: 407.0447. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 1H, 5-H), 7.73 (d, *J* = 8.4 Hz, 1H, 6'-Ph-H), 7.46 (s, 1H, 3'-Ph-H), 7.25 (s, 1H, 5'-Ph-H), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.66 (s, 2H, 8-CH_2),

3.99 (s, 3H, OCH_3), 2.65 (q, *J* = 7.4 Hz, 2H, $\underline{CH_2}CH_3$), 1.28 (t, *J* = 7.4 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 170.4, 164.7, 162.3, 156.2, 138.3, 134.9, 132. 5, 131.0, 128.4, 128.3, 127.0, 117.7, 110.8, 108.7, 108.6, 56.4, 56.0, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 4-amino-3-methylbenzoate (**6h**). Yield: 56.4%. m.p. 171.8-174.5 °C. MS(ESI) (*m/z*) 368.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 368.1492, Found: 368.1495. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.73 (s, 2H, (2',6'-Ph-H)), 7.70 (s, 2H, NH_2), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.63 (d, *J* = 9.0 Hz, 1H, 5'-Ph-H), 6.13 (s, 1H, 3-H), 5.59 (s, 2H, 8-CH_2), 3.98 (s, 3H, OCH_3), 2.61 (q, *J* = 7.6 Hz, 2H, $\underline{CH_2}CH_3$), 2.16 (s, 3H, 2'-$\underline{CH_3}$), 1.24 (t, *J* = 7.6 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 170.6, 166.9, 162.3, 156.3, 149.2, 132.3, 129.4, 127.8, 121.0, 119.6, 117.7, 113.6, 111.9, 108.8, 108.5, 56.4, 54.8, 27.5, 17.1, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 3-(dimethylamino)benzoate (**6i**). Yield: 54.2%. m.p. 165.2-167.0 °C. MS(ESI) (*m/z*) 382.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 382.1649, Found: 382.1650. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.41 (s, 1H, 6'-Ph-H), 7.34 (s, 1H, 2'-Ph-H), 7.23 (s, 1H, 5'-Ph-H), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.92 (s, 1H, 4'-Ph-H), 6.13 (s, 1H, 3-H), 5.64 (s, 2H, 8-CH_2), 3.98 (s, 3H, OCH_3), 2.97 (s, 6H, $N(CH_3)_2$), 2.62 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 1.26 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 167.2, 162.3, 156.3, 130.9, 129.0, 127.9, 117.7, 111.5, 108.8, 108.5, 56.4, 55.4, 40.8, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2-(4-chlorophenyl)acetate (**6j**). Yield: 48.3%. m.p. 94.4-96.5 °C. MS(ESI) (*m/z*) 387.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 387.0994, Found: 393.0997. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.8 Hz, 1H, 5-H), 7.27-7.20 (m, 4H, (2',3',5',6'-Ph-H)), 7.01 (d, *J* = 8.8 Hz, 1H, 6-H), 6.12 (s, 1H, 3-H), 5.42 (s, 2H, 8-CH_2), 3.93 (s, 3H, OCH_3), 3.59 (s, 2H, 1'-$\underline{CH_2}$COO), 2.52 (q, *J* = 7.4 Hz, 2H, $\underline{CH_2}CH_3$), 1.22 (d, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 171.0, 170.4, 162.2, 156.1, 133.1, 132.4, 130.6, 128.6, 128.2, 117.6, 111.0, 108.7, 108.5, 56.3, 55.4, 40.6, 27.3, 10.8.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2-(o-tolyl)acetate (**6k**). Yield: 50.3%. m.p. 84.0-85.6 °C. MS(ESI) (*m/z*) 367.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 367.1540, Found: 367.1544. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.9 Hz,

1H, 5-H), 7.21-7.09 (m, 4H, (3',4',5',6'-Ph-H)), 7.01 (d, *J* = 8.9 Hz, 1H, 6-H), 6.12 (s, 1H, 3-H), 5.43 (s, 2H, 8-CH_2), 3.93 (s, 3H, OCH_3), 3.64 (s, 2H, 1'-$\underline{CH_2}COO$), 2.54 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 2.27 (s, 3H, 2'-$\underline{CH_3}$), 1.24 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 171.4, 170.4, 162.2, 156.1, 136.8, 132.8, 130.3, 130.1, 128.0, 127.4, 126.1, 117.6, 111.2, 108.7, 108.5, 56.3, 55.2, 39.1, 27.3, 19.5, 10.8.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 4-propylbenzoate (**6l**). Yield: 42.5%. m.p. 93.2-97.4 °C. MS(ESI) (*m/z*) 381.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 381.1697, Found: 381.1700. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.91 (d, *J* = 8.3 Hz, 2H, (2',6'-Ph-H)), 7.20 (d, *J* = 8.3 Hz, 2H, (3',5'-Ph-H)), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.13 (s, 1H, 3-H), 5.63 (s, 2H, 8-CH_2), 3.98 (s, 3H, OCH_3), 2.65-2.58 (m, 4H, 2-$\underline{CH_2}CH_3$, 4'-$\underline{CH_2}CH_2CH_3$), 1.64 (m, 2H, 4'-$CH_2\underline{CH_2}CH_3$), 1.24 (t, *J* = 7.5 Hz, 3H, 2-$CH_2\underline{CH_3}$), 0.92 (t, *J* = 7.3 Hz, 3H, 4'-$CH_2CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 166.7, 162.3, 156.3, 148.3, 129.7, 128.5, 128.0, 127.7, 117.7, 111.5, 108.8, 108.5, 56.4, 55.2, 38.0, 27.5, 24.3, 13.7, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 4-pentylbenzoate (**6m**). Yield: 43.3%. m.p. 95.4-98.6 °C. MS(ESI) (*m/z*) 409.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 409.2010, Found: 409.2009. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.91 (d, *J* = 8.1 Hz, 2H, (2',6'-Ph-H)), 7.20 (d, *J* = 8.1 Hz, 2H, (3',5'-Ph-H)), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.13 (s, 1H, 3-H), 5.63 (s, 2H, 8-CH_2), 3.98 (s, 3H, OCH_3), 2.61 (q, *J* = 8.3, 7.6 Hz, 4H, 2-$\underline{CH_2}CH_3$, 4'-$\underline{CH_2}CH_2CH_2CH_2CH_3$), 1.61 – 1.58 (m, 2H, 4'-$CH_2\underline{CH_2}CH_2CH_2CH_3$), 1.31-1.22 (m, 7H, 2-$CH_2\underline{CH_3}$, 4' $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_3$), 0.87 (t, *J* = 6.8 Hz, 3H, 4'-$CH_2CH_2CH_2CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 166.7, 162.3, 156.3, 148.6, 129.7, 128.4, 128.0, 127.6, 117.7, 111.6, 108.8, 108.5, 56.4, 55.2, 36.0, 31.4, 30.8, 27.5, 22.5, 14.0, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 4-(tert-butyl)benzoate (**6n**). Yield: 57.5%. m.p. 131.6-133.6 °C. MS(ESI) (*m/z*) 395.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 395.1489, Found: 395.1491. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 1H, 5-H), 7.94 (d, *J* = 8.4 Hz, 2H, (2',6'-Ph-H)), 7.42 (d, *J* = 8.4 Hz, 2H, (3',5'-Ph-H)), 7.05 (d, *J* = 9.0 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.64 (s, 2H, 8-CH_2), 3.98 (s, 3H, OCH_3), 2.62 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 1.32 (s, 9H, $C(CH_3)_3$), 1.26 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 166.6, 162.3, 156.6, 156.3, 129.5, 128.0, 127.4, 125.3, 117.7, 111.5, 108.8, 108.5, 56.4, 55.2, 35.1, 31.1, 27.5, 10.9.

(E)-(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 3-(4-chlorophenyl)acrylate (**6o**). Yield: 55.3%. m.p. 174.3-175.8 °C. MS(ESI) (*m/z*) 399.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 399.0994, Found: 399.0995. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 1H, 5-H), 7.65 (d, *J* = 16.0 Hz, 1H, CH=CHCOO), 7.43 (d, *J* = 8.5 Hz, 2H, (2',6'-Ph-H)), 7.34 (d, *J* = 8.5 Hz, 2H, (3',5'-Ph-H)), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.41 (d, *J* = 16.0 Hz, 1H, CH=CHCOO), 6.14 (s, 1H, 3-H), 5.54 (s, 2H, 8-CH_2), 3.99 (s, 3H, OCH_3), 2.65 (q, *J* = 7.6 Hz, 2H, CH_2CH_3), 1.29 (t, *J* = 7.6 Hz, 3H, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 170.4, 166.7, 162.3, 156.2, 143.6, 136.3, 132.9, 129.2, 128.1, 118.5, 117.7, 111.3, 108.7, 108.6, 56.4, 55.1, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 3-methoxybenzoate (**6p**). Yield: 42.6%. m.p. 112.4-115.4 °C. MS(ESI) (*m/z*) 369.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 369.1333, Found: 369.1336.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 1H, 5-H), 7.60 (d, *J* = 7.7 Hz, 1H, 6'-Ph-H), 7.56-7.52 (m, 1H, 5'-Ph-H), 7.31 (d, *J* = 8.0 Hz, 1H, 4'-Ph-H), 7.08 (dd, *J* = 8.0, 1.9 Hz, 1H, 3'-Ph-H), 7.05 (d, *J* = 9.0 Hz, 1H, 6-H), 6.13 (s, 1H, 3-H), 5.65 (s, 2H, 8-CH_2), 3.99 (s, 3H, 7-OCH_3), 3.83 (s, 3H, 3'-OCH_3), 2.62 (q, *J* = 7.5 Hz, 2H, CH_2CH_3), 1.25 (t, *J* = 7.5 Hz, 3H, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 166.5, 162.3, 159.5, 156.3, 131.5, 129.4, 128.1, 122.1, 119.2, 117.7, 114.3, 111.3, 108.8, 108.6, 56.4, 55.5, 55.5, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 3,4-dimethoxybenzoate (**6q**). Yield: 50.8%，m.p. 178.4-179.3 °C，MS(ESI) (*m/z*) 399.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 399.1438, Found: 399.1440. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 1H, 5-H), 7.63 (dd, *J* = 8.4, 1.6 Hz, 1H, 6'-Ph-H), 7.52(d, *J* =1.6 Hz, 1H, 2'-Ph-H), 7.05 (d, *J* = 9.0 Hz, 1H, 6-H), 6.84 (d, *J* = 8.4 Hz, 1H, 5'-Ph-H), 6.13 (s, 1H, 3-H), 5.62 (s, 2H, CH_2OOC), 3.98 (s, 3H, 7-OCH_3), 3.91 (s, 3H, 3'-OCH_3), 3.90 (s, 3H, 4'-OCH_3), 2.62 (q, *J* = 7.5 Hz, 2H, CH_2CH_3), 1.25 (t, *J* = 7.5 Hz, 3H, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 166.4, 162.3, 156.3, 153.0, 148.6, 128.0, 123.7, 122.7, 117.7, 112.1, 111.6, 110.2, 108.8, 108.6, 56.4, 56.1, 56.0, 55.4, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2,4,5-trimethoxybenzoate (**6r**). Yield: 46.3%. m.p. 165.1-167.1 °C. MS(ESI) (*m/z*) 429.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 429.1544, Found: 429.1547. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.31 (s, 1H, 6'-Ph-H), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.51 (s, 1H, 3'-Ph-H), 6.13 (s, 1H, 3-H), 5.61 (s, 2H, 8-CH_2), 3.99 (s, 3H, 7-OCH_3), 3.92 (s, 3H, 2'-OCH_3), 3.82 (s, 3H, 5'-OCH_3), 3.80 (s, 3H, 4'-OCH_3), 2.64 (q, *J* = 7.6 Hz, 2H, 2-CH_2CH_3), 1.27 (t, *J* = 7.6 Hz, 3H, 2-CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ

178.1, 170.5, 165.5, 162.3, 156.3, 155.8, 153.6, 142.5, 127.8, 117.6, 114.5, 111.8, 110.9, 108.7, 108.5, 97.9, 57.1, 56.5, 56.3, 56.0, 55.1, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 3,4,5-trimethoxybenzoate (**6s**). Yield: 42.6%. m.p. 147.9-150.4 °C. MS(ESI) (*m/z*) 429.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 429.1544, Found: 429.1547. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 9.0 Hz, 1H, 5-H), 7.26 (s, 2H, (2',6'-Ph-H)), 7.06 (d, *J* = 9.0 Hz, 1H, 6-H), 6.15 (s, 1H, 3-H), 5.64 (s, 2H, 8-CH_2), 4.00 (s, 3H, 7-OCH_3), 3.89 (s, 3H, 4'-OCH_3), 3.87 (s, 6H, 3'-OCH_3, 5'-OCH_3), 2.64 (q, *J* = 7.5 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.27 (t, *J* = 7.5 Hz, 3H, 3-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 170.4, 166.3, 162.3, 156.3, 152.9, 142.3, 128.1, 125.2, 117.7, 111.4, 108.8, 108.6, 107.0, 60.9, 56.4, 56.3, 55.7, 27.5, 11.0.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 2-(3,4,5-trimethoxyphenyl) acetate (**6t**). Yield: 46.5%. m.p. 156.4-158.0 °C. MS(ESI) (*m/z*) 443.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 443.1700, Found: 443.1702. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 443.1700, Found: 443.1697. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.9 Hz, 1H, 5-H), 7.01 (d, *J* = 8.9 Hz, 1H, 6-H), 6.48 (s, 2H, (2',6'-Ph-H)), 6.11 (s, 1H, 3-H), 5.44 (s, 2H, 8-CH_2), 3.92 (s, 3H, 7-OCH_3), 3.82 (s, 3H, 4'-OCH_3), 3.78 (s, 6H, 3'-OCH_3, 5'-OCH_3), 3.55 (s, 2H, 1'-$\underline{CH_2}$COO), 2.53 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 1.22 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 171.3, 170.4, 162.2, 156.1, 153.2, 137.2, 129.5, 128.1, 117.6, 111.2, 108.7, 108.5, 106.4, 60.8, 56.3, 56.1, 55.3, 41.6, 27.3, 10.8.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl cinnamate (**6u**). Yield: 40.8%. m.p. 140.2-140.9 °C. MS(ESI) (*m/z*) 365.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 365.1384, Found: 365.1386. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H, 5-H), 7.71 (d, *J* = 16.0 Hz, 1H, $\underline{CH}$=CHCOO), 7.53-7.47 (m, 2H, (2',6'-Ph-H)), 7.40-7.33 (m, 3H, (3',4',5'-Ph-H)), 7.04 (d, *J* = 9.0 Hz, 1H, 6-H), 6.45 (d, *J* = 16.0 Hz, 1H, CH=$\underline{CH}$COO), 6.14 (s, 1H, 3-H), 5.55 (s, 2H, 8-CH_2), 3.99 (s, 3H, OCH_3), 2.66 (q, *J* = 7.8 Hz, 2H, $\underline{CH_2}CH_3$), 1.29 (t, *J* = 7.8 Hz, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 166.9, 162.3, 156.3, 145.0, 134.4, 130.3, 128.9, 128.1, 117.9, 117.7, 111.4, 108.8, 108.6, 56.4, 55.0, 27.5, 10.9.

(E)-(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 3-(4-methoxyphenyl) acrylate (**6v**). Yield: 42.3%. m.p. 150.9-151.8 °C. MS(ESI) (*m/z*) 395.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 395.1489, Found: 395.1491.^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.22 (d, *J* = 8.9 Hz, 1H, 5-H), 7.66 (d, *J* = 16.0 Hz, 1H, CH=CHCOO), 7.45 (d, *J* = 8.7 Hz, 2H, (2',6'-Ph-H)), 7.04 (d, *J* = 8.9 Hz, 1H, 6-H), 6.89 (d, *J* = 8.7 Hz, 2H, (3',5'-Ph-H)), 6.31 (d, *J* = 16.0 Hz, 1H, CH=CHCOO), 6.14 (s, 1H, 3-H), 5.53 (s, 2H, 8-CH_2), 3.99 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 2.65 (q, *J* = 7.5 Hz, 2H, CH_2CH_3), 1.29 (t, *J* = 7.5 Hz, 3H, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.5, 167.3, 162.2, 161.4, 156.2, 144.7, 129.7, 128.0, 127.1, 117.7, 115.3, 114.3, 111.5, 108.7, 108.5, 56.4, 55.4, 54.8, 27.5, 10.9.

(2-Ethyl-7-methoxy-4-oxo-4H-chromen-8-yl)methyl 4-cyanobenzoate (**6w**). Yield: 45.6%. m.p. 197.1-198.2 °C. MS(ESI) (*m/z*) 364.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 364.1179, Found: 364.1182. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.9 Hz, 1H, 5-H), 8.12 (d, *J* = 8.2 Hz, 2H, (2',6'-Ph-H)), 7.72 (d, *J* = 8.2 Hz, 2H, (3',5'-Ph-H)), 7.06 (d, *J* =8.9 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.68 (s, 2H, 8-CH_2), 3.99 (s, 3H, OCH_3), 2.62 (q, *J* = 7.6 Hz, 2H, CH_2CH_3), 1.25 (t, *J* = 7.6 Hz, 3H, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.3, 164.9, 162.3, 156.2, 134.0, 132.2, 130.2, 128.4, 117.9, 117.7, 116.4, 110.7, 108.8, 108.7, 56.4, 56.2, 27.5, 10.9.

(7-Ethoxy-2-ethyl-4-oxo-4H-chromen-8-yl)methyl 2,4,5-trimethoxybenzoate **(7a)**. Yield: 50.4%. m.p. 159.8-161.7 °C. MS(ESI) (*m/z*) 443.0 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 443.1700, Found: 443.1702. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.9 Hz, 1H, 5-H), 7.32 (s, 1H, 6'-Ph-H), 7.01 (d, *J* = 8.9 Hz, 1H, 6-H), 6.51 (s, 1H, 3'-Ph-H), 6.13 (s, 1H, 3-H), 5.62 (s, 2H, 8-CH_2), 4.22 (q, *J* = 6.9 Hz, 2H, 7-OCH_2CH_3), 3.92 (s, 3H, 2'-OCH_3), 3.82 (s, 3H, 5'-OCH_3), 3.80 (s, 3H, 4'-OCH_3), 2.63 (q, *J* = 7.5 Hz, 2H, 2-CH_2CH_3), 1.46 (t, *J* = 6.9 Hz, 3H, 7-OCH_2CH_3), 1.26 (t, *J* = 7.5 Hz, 3H, 2-CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 170.5, 165.5, 161.8, 156.3, 155.8, 153.6, 142.6, 127.7, 117.5, 114.6, 111.9, 111.0, 109.6, 108.5, 98.0, 64.8, 57.1, 56.5, 56.0, 55.2, 27.5, 14.7, 10.9.

(7-Ethoxy-2-ethyl-4-oxo-4H-chromen-8-yl)methyl 3,4,5-trimethoxybenzoate **(7b)**. Yield: 52.6%. m.p. 162.0-164.1 °C. MS(ESI) (*m/z*) 443.0 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 443.1700, Found: 443.1704. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.9 Hz, 1H, 5-H), 7.26 (s, 2H, (2',6'-Ph-H)), 7.03 (d, *J* = 8.9 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.66 (s, 2H, 8-CH_2), 4.23 (q, *J* = 6.9 Hz, 2H, 7-OCH_2CH_3), 3.89 (s, 3H, 4'-OCH_3), 3.87 (s, 6H, 3'-OCH_3, 5'-OCH_3), 2.64 (q, *J* = 7.3 Hz, 2H, 2-CH_2CH_3), 1.46 (t, *J* = 6.9 Hz, 3H, 7-OCH_2CH_3), 1.27 (t, *J* = 7.3 Hz, 3H, 2-CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.4, 166.3, 161.8, 156.4, 153.0, 142.3, 128.0, 125.3, 117.6, 111.5, 109.7, 108.6, 107.0, 64.9, 60.9, 56.3, 55.8, 27.5, 14.8, 11.0.

***(7-Ethoxy-2-ethyl-4-oxo-4H-chromen-8-yl)methyl 2-(3,4,5-trimethoxyphenyl) acetate* (7c)**. Yield: 57.2%. m.p. 106.4-107.4 °C. MS(ESI) (*m/z*) 457.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 457.1857, Found: 457.1860. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.9 Hz, 1H, 5-H), 6.98 (d, *J* = 8.9 Hz, 1H, 6-H), 6.48 (s, 2H, (2',6'-Ph-H)), 6.12 (s, 1H, 3-H), 5.45 (s, 2H, 8-CH_2), 4.15 (q, *J* = 6.9 Hz, 2H, 7-O$\underline{CH_2}CH_3$), 3.82 (s, 3H, 4'-OCH_3), 3.78 (s, 6H, 3'-OCH_3, 5'-OCH_3), 3.56 (s, 2H, 1'-$\underline{CH_2}$COO), 2.53 (q, *J* = 7.6 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.39 (t, *J* = 6.9 Hz, 3H, 7-$OCH_2\underline{CH_3}$), 1.23 (t, *J* = 7.6 Hz, 3H, 2-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 171.3, 170.3, 161.6, 156.2, 153.2, 137.1, 129.6, 128.0, 117. 5, 111.3, 109.5, 108.5, 106.3, 64.9, 60.8, 56.0, 55.4, 41.7, 27.3, 14.6, 10.8.

***(7-Ethoxy-2-ethyl-4-oxo-4H-chromen-8-yl)methyl cinnamate* (7d)**. Yield: 46.4%. m.p. 128.7-130.7 °C. MS(ESI) (*m/z*) 379.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 379.1540, Found: 379.1542. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 9.0 Hz, 1H, 5-H), 7.71 (d, *J* = 16.0 Hz, 1H, $\underline{CH}$=CHCOO), 7.53-7.47 (m, 2H, (2',6'-Ph-H)), 7.41-7.34 (m, 3H, (3',4',5'-Ph-H)), 7.02 (d, *J* = 9.0 Hz, 1H, 6-H), 6.45 (d, *J* = 16.0 Hz, 1H, CH=$\underline{CH}$COO), 6.14 (s, 1H, 3-H), 5.56 (s, 2H, 8-CH_2), 4.22 (q, *J* = 7.0 Hz, 2H, 7-O$\underline{CH_2}CH_3$), 2.65 (q, *J* = 7.5 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.47 (t, *J* = 7.0 Hz, 3H, 7-$OCH_2\underline{CH_3}$), 1.29 (t, *J* = 7.5 Hz, 3H, 2-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.4, 166.9, 161.7, 156.3, 145.0, 134.4, 130.3, 128.9, 128.1, 127.9, 1179, 117.5, 111.4, 109.6, 108.6, 64.9, 55.1, 27.5, 14.7, 10.9.

***(E)-(7-Ethoxy-2-ethyl-4-oxo-4H-chromen-8-yl)methyl 3-(4-methoxyphenyl) acrylate* (7e)**. Yield: 43.2%. m.p. 128.4-126.9 °C. MS(ESI) (*m/z*) 409.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 409.1646, Found: 409.1649. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 9.0 Hz, 1H, 5-H), 7.67 (d, *J* = 15.9 Hz, 1H, $\underline{CH}$=CHCOO), 7.46 (d, *J* = 8.7 Hz, 2H, (2',6'-Ph-H)), 7.01 (d, *J* = 9.0 Hz, 1H, 6-H), 6.89 (d, *J* = 8.7 Hz, 2H, (3',5'-Ph-H)), 6.32 (d, *J* = 15.9 Hz, 1H, CH=$\underline{CH}$COO), 6.14 (s, 1H, 3-H), 5.54 (s, 2H, 8-CH_2), 4.22 (q, *J* = 6.9 Hz, 2H, 7-O$\underline{CH_2}CH_3$), 3.83 (s, 3H, 4'-OCH_3), 2.65 (q, *J* = 7.5 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.47 (t, *J* = 6.9 Hz, 3H, 7-$OCH_2\underline{CH_3}$), 1.29 (t, *J* = 7.5 Hz, 3H, 2-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.4, 167.3, 161.7, 161.4, 156.3, 144.6, 129.7, 127.8, 127.1, 117.5, 115.3, 114.3, 111.6, 109.6, 108.5 , 64.9, 55.4, 54.9, 27.5, 14.7, 10.9.

***(7-Ethoxy-2-ethyl-4-oxo-4H-chromen-8-yl)methyl 4-cyanobenzoate* (7f)**. Yield: 44.8%. m.p. 196.8-197.2 °C. MS(ESI) (*m/z*) 378.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 378.1336, Found: 378.1337. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.9 Hz, 1H, 5-H), 8.12 (d, *J* = 8.6 Hz, 2H, (2',6'-Ph-H)), 7.72 (d, *J* = 8.6 Hz, 2H, (3',5'-Ph-H)),

7.02 (d, *J* = 8.9 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.70 (s, 2H, 8-CH_2), 4.22 (q, *J* = 6.9 Hz, 2H, 7-O$\underline{CH_2}CH_3$), 2.62 (q, *J* = 7.5 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.44 (t, *J* = 6.9 Hz, 3H, 7-$OCH_2\underline{CH_3}$), 1.25 (t, *J* = 7.5 Hz, 3H, 2-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.3, 164.9, 161. 8, 156.3, 134.1, 132.2, 130.2, 128.3, 117.9, 117.5, 116.4, 110.8, 109.6, 108.7, 64.9, 56.3, 27.5, 14.7, 10.9.

(2-Ethyl-7-isopropoxy-4-oxo-4H-chromen-8-yl)methyl 3,4,5-trimethoxybenzoate **(8a)**. Yield: 48.6%. m.p. 67.4-69.3 °C. MS(ESI) (*m/z*) 457.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 457.1857, Found: 457.1860. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 9.0 Hz, 1H, 5-H), 7.26 (s, 2H, (2',6'-Ph-H)), 7.03 (d, *J* = 9.0 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.64 (s, 2H, 8-CH_2), 4.78 (hept, *J* = 6.0 Hz, 1H, 7-O$\underline{CH}(CH_3)_2$), 3.89 (s, 3H, 4'-OCH_3), 3.86 (s, 6H, 3'-OCH_3, 5'-OCH_3), 2.64 (q, *J* = 7.5 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.38 (d, *J* = 6.0 Hz, 6H, 7-OCH($\underline{CH_3}$)$_2$), 1.27 (t, *J* = 7.5 Hz, 3H, 2-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.3, 166.2, 161.1, 156.5, 152.9, 142.3, 127.7, 125.3, 117.3, 112.3, 110.8, 108.6, 106.9, 71.6, 60.9, 56.3, 55.8, 27.5, 22.1, 11.0.

(2-Ethyl-7-isopropoxy-4-oxo-4H-chromen-8-yl)methyl 2-(3,4,5-trimethoxypheny) acetate **(8b)**. Yield: 40.8%. m.p. 120.2-122.2 °C. MS(ESI) (*m/z*) 471.3 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 471.2013, Found: 417.2013. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 9.0 Hz, 1H, 5-H), 6.99 (d, *J* = 9.0 Hz, 1H, 6-H), 6.48 (s, 2H, (2',6'-Ph-H)), 6.10 (s, 1H, 3-H), 5.43 (s, 2H, 8-CH_2), 4.71 (hept, *J* = 6.0 Hz, 1H, 7-O$\underline{CH}(CH_3)_2$), 3.81 (s, 3H, 4'-OCH_3), 3.78 (s, 6H, 3'-OCH_3, 5'-OCH_3), 3.55 (s, 2H, 1'-$\underline{CH_2}$COO), 2.52 (q, *J* = 7.5 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.32 (d, *J* = 6.0 Hz, 6H, 7-OCH($\underline{CH_3}$)$_2$), 1.22 (t, *J* = 7.5 Hz, 3H, 2-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 171.3, 170.2, 160.9, 156.4, 153.2, 137.1, 129.5, 127.8, 117.3, 112.1, 110.8, 108.5, 106.3, 71.6, 60.8, 56.0, 55.5, 41.6, 27.3, 22.0, 10.8.

(2-Ethyl-7-isopropoxy-4-oxo-4H-chromen-8-yl)methyl cinnamate **(8c)**. Yield: 46.2%. m.p. 102.0-103.9 °C. MS(ESI) (*m/z*) 393.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 393.1697, Found: 393.1700. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.0 Hz, 1H, 5-H), 7.71 (d, *J* = 16.0 Hz, 1H, $\underline{CH}$=CHCOO), 7.54-7.47 (m, 2H, (2',6'-Ph-H)), 7.41-7.34 (m, 3H, (3',4',5'-Ph-H)), 7.02 (d, *J* = 9.0 Hz, 1H, 6-H), 6.45 (d, *J* = 16.0 Hz, 1H, CH=$\underline{CH}$COO), 6.14 (s, 1H, 3-H), 5.54 (s, 2H, 8-CH_2), 4.76 (hept, *J* = 6.0 Hz, 1H, 7-O$\underline{CH}(CH_3)_2$), 2.65 (q, *J* = 7.5 Hz, 2H, 2-$\underline{CH_2}CH_3$), 1.39 (d, *J* = 6.0 Hz, 6H, 7-OCH($\underline{CH_3}$)$_2$), 1.29 (t, *J* = 7.5 Hz, 3H, 2-$CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.3, 166.9, 161.0, 156.5, 144.9, 134.4, 130.3, 128.9, 128.1, 127.7, 117.9, 117.3, 112.3, 110.9, 108.6, 71.7, 55.2, 27.5, 22.1, 10.9.

***(E)-(2-Ethyl-7-isopropoxy-4-oxo-4H-chromen-8-yl)methyl 3-(4methoxy-phenyl) acrylate* (8d)**. Yield: 42.3%. m.p. 117.8-119.0 °C. MS(ESI) (*m/z*) 423.2 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 423.1802, Found: 423.1806. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.0 Hz, 1H, 5-H), 7.66 (d, *J* = 15.9 Hz, 1H, CH=CHCOO), 7.46 (d, *J* = 8.7 Hz, 2H, (2',6'-Ph-H)), 7.02 (d, *J* = 9.0 Hz, 1H, 6-H), 6.89 (d, *J* = 8.7 Hz, 2H, (3',5'-Ph-H)), 6.31 (d, *J* = 15.9 Hz, 1H, CH=CHCOO), 6.13 (s, 1H, 3-H), 5.53 (s, 2H, 8-CH_2), 4.76 (hept, *J* = 6.0 Hz, 1H, 7-OCH$(CH_3)_2$), 3.83 (s, 3H, 4'-OCH_3), 2.65 (q, *J* = 7.6 Hz, 2H, 2-CH_2CH_3), 1.39 (d, *J* = 6.0 Hz, 6H, 7-OCH$(CH_3)_2$), 1.28 (t, *J* = 7.6 Hz, 3H, 2-CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.3, 167.3, 161.4, 161.0, 156.5, 144.6, 129.7, 127.6, 127.1, 117.3, 115.4, 114.3, 112.5, 110.9, 108.6, 71.7, 55.4, 55.0, 27.5, 22.1, 11.0.

***(2-Ethyl-7-isopropoxy-4-oxo-4H-chromen-8-yl)methyl 4-cyanobenzoate* (8e)**. Yield: 48.9%. m.p. 175.3-175.9 °C. MS(ESI) (*m/z*) 392.1 $[M+H]^+$. ESI-HRMS (*m/z*) $[M+H]^+$ Calc.: 392.1492, Found: 392.1494. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.9 Hz, 1H, 5-H), 8.12 (d, *J* = 8.6 Hz, 2H, (2',6'-Ph-H)), 7.72 (d, *J* = 8.6 Hz, 2H, (3',5'-Ph-H)), 7.03 (d, *J* = 8.9 Hz, 1H, 6-H), 6.14 (s, 1H, 3-H), 5.68 (s, 2H, 8-CH_2), 4.76 (hept, *J* = 6.0 Hz, 1H, 7-OCH$(CH_3)_2$), 2.62 (q, *J* = 7.6 Hz, 2H, 2-CH_2CH_3), 1.36 (d, *J* = 6.0 Hz, 6H, 7-OCH$(CH_3)_2$), 1.25 (t, *J* = 7.6 Hz, 3H, 2-CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.2, 164.9, 161.1, 156.4, 134.1, 132.2, 130.1, 128.1, 117.9, 117.3, 116.4, 111.6, 110.7, 108.7, 71.6, 56.4, 27.5, 22.1, 10.9.

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Abbreviations: CDDP: cisplatin • DCK: (±)-3'-O,4'-O-dicinnamoyl-cis-khellactone • DMAP: dimethylaminopyridine • DOX: doxorubicin • DSP: 3'R,4'R-disubstituted-2',2'-dimethyldihydropyrano[2,3-f]chromone • EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride • MDR: multidrug resistance • MRP: multidrug resistance associate protein • MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide P-gp: P-glycoprotein • PKC: protein kinase C • PXL: paclitaxel • VCR: vincristine • VRP: verapamil

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HIGHLIGHTS:

- Thirty-four novel seco-DSP derivatives were synthesized.
- Most of compounds displayed moderate to significant MDR reversal activities in the P-gp overexpressing A2780/T and KB-VIN cells.
- These compounds were able to observably increase accumulation of doxorubicin in A2780/T cell line.
- These compounds hardly interfered protein expression, while likely inhibited the efflux function of P-gp.
- MDR reversal effects of the compounds might be associated with P-gp overexpression.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.