

1-(3,4,5-Trimethoxyphenyl)ethane-1,2-diyl esters, a novel compound class with potent chemoreversal activity

Hsin-Yi Hung^{a,c}, Emika Ohkoshi^a, Masuo Goto^a, Kyoko Nakagawa-Goto^{a,b}, Kuo-Hsiung Lee^{a,c,*}

^a Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7568, USA

^b Division of Pharmaceutical Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

^c Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung, Taiwan 401, Republic of China

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ABSTRACT

1-(3,4,5-Trimethoxyphenyl)ethane-1,2-diyl esters, which share a fragment from (±)-3'-O-4'-O-bis(3,4-dimethoxycinnamoyl)-*cis*-khellactone (DMDCK) and 3'*R*,4'*R*-disubstituted-2',2'-dimethyldihydropyrano[2,3-*f*]chromone (DSP), exhibited remarkable chemoreversal activity on multidrug resistant human nasopharyngeal carcinoma (KB) when combined with three anticancer drugs, paclitaxel, vincristine and doxorubicin. Among 15 novel synthesized analogs, bis-trimethoxybenzoyl derivative **15** was the most active (340-fold more active than verapamil when used with vincristine) followed by two di-cinnamoyl derivatives, **10** and **11**, and then di-cyclohexanecarbonyl derivative **9**. All aliphatic chain derivatives, **3–5**, showed no activity. Structure-activity relationship study indicated that a di-ester structure was critical to enhance the activity resulting from the maintenance of the spatial arrangement proposed by the pharmacophore based on the verapamil-binding site. Further mechanism of action study showed **15** inhibited mainly P-glycoprotein efflux pump function, while **13** exhibited an additional multidrug resistance-associated protein efflux pump function.

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Chemotherapy is an important strategy for cancer treatment. However, it is difficult to eliminate all tumor cells, resulting in relapse with resistant cancer cells. Emerging evidence suggests that epithelial-mesenchymal transition (EMT)-type cells and cancer stem cells (CSCs) or initiating cells are more resistant to conventional chemotherapy and are linked to anticancer drug resistance leading to failure of chemotherapy.¹ Another issue of chemotherapeutic resistance is the tumor microenvironment, in which cancer cells are surrounded by various sets of non-transformed cells and a heterogeneous stromal compartment.² The interaction between tumor cells and normal cells in this tumor microenvironment occurs via secreted and surface-bound proteins, and is critical for tumor progression.²

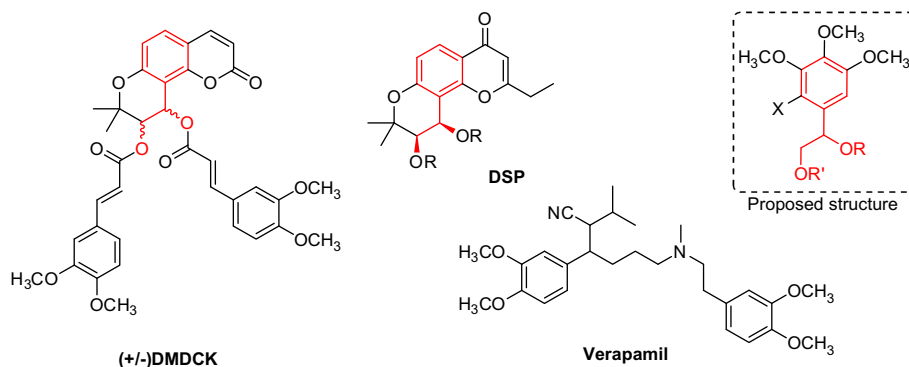
In addition to the tumor microenvironment, cancer cells also adapt other mechanisms to deal with chemotherapeutic agents.³ Over-expression of drug efflux transporters such as P-glycoprotein (P-gp) and multidrug resistance-associated proteins (MRPs) is the primary cause leading to multidrug resistance. Numerous studies have explored chemosensitizer development. However, clinical drugs are still unavailable.

Verapamil (VRP), the first generation chemosensitizer, was precluded from clinical use due to its high toxicity, but it is used as a

positive control in various chemosensitizing research efforts. Before the structure of P-glycoprotein (P-gp) was resolved, a pharmacophore model based on the verapamil-binding site was proposed.⁴ In this model, the pharmacophore for P-gp substrates or inhibitors consists of two hydrophobic planes, three optional hydrogen bond (HB) acceptor points, and one optional HB donor point spatially arranged like a butterfly shape.⁴ Based on this model, (±)-3'-O-4'-O-bis-(3,4-dimethoxycinnamoyl)-*cis*-khellactone (DMDCK) was found to be a hit and was also confirmed to exhibit chemosensitizing ability.⁵ Moreover, 3'*R*,4'*R*-disubstituted-2',2'-dimethyldihydropyrano[2,3-*f*]chromone (DSP) analogs sharing similar structures to DMDCK have also been reported to overcome multidrug resistance (MDR) in an acquired MDR human nasopharyngeal carcinoma (KB) cell line.⁶ With inspiration from the above results, a simplified scaffold, 1-(3,4,5-trimethoxyphenyl)ethane-1,2-diyl ester, was designed (Fig. 1) to provide an ethane-1,2-diyl ester partial structure mimicking a fragment in both DSP and DMDCK. By esterifying different substituents, the importance of the spatial arrangement mentioned in the pharmacophore model can be evaluated. According to results based on another model, P-gp inhibitors should show high hydrophobicity, with two or more aromatic rings, a methoxy group (HB acceptor) on the aromatic ring, and one or two protonatable nitrogens.⁷ Verapamil also contains two methoxy group on an aromatic ring. In addition, previous study of DDB substitutions also showed

* Corresponding author. Tel.: +1 919 962 0066; fax: +1 919 966 3893.

E-mail address: khlee@unc.edu (K.-H. Lee).

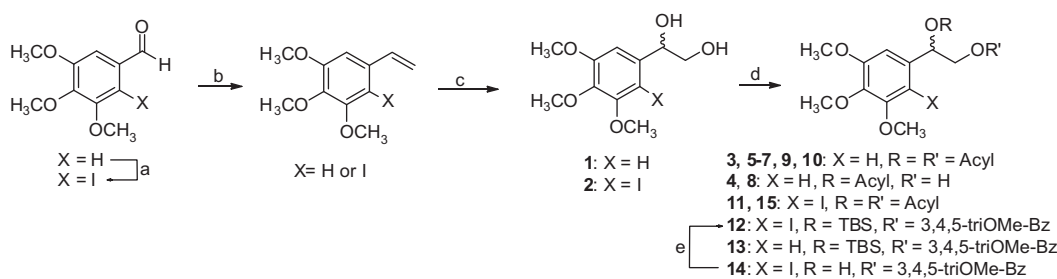


increasing activity along with an increased number of methoxy substituents.⁸ Therefore, a trimethoxy-substituted aromatic ring was designed as the basic scaffold in this study.

1-(3,4,5-Trimethoxyphenyl)ethane-1,2-diol (**1**), a common intermediate for all synthesized compounds, was obtained through Wittig reaction of 3,4,5-trimethoxybenzaldehyde following dihydroxylation with osmium tetroxide and *N*-methylmorpholine *N*-oxide (Scheme 1). Various acyl groups, including linear alkyl (**3–5**), unsaturated fatty acid (**8–9**), cyclic alkyl (**6–7**), and aromatic (**10–15**) side chains, were selected for the structure-activity relationship (SAR) study. Both mono- and di-esters were also synthesized by controlling the equivalents of the acyl chloride to investigate the importance of the spatial arrangement and hydrophobicity. Diesterification of diol was achieved with excess acyl chloride under basic conditions to generate the related diesters, **3**, **5–7**, **9**, and **10**. The bulky acyl chlorides, such as butyryl chloride and cyclohexanecarbonyl chloride, were selectively introduced on only the primary alcohol to produce mono-ester derivatives, **4** and **8**. Monobenzoyl ester **13** was prepared through the esterification of the primary alcohol using 1.1 equiv of benzoyl chloride, following silylation of the secondary alcohol. Halogenated compounds are abundant among drug candidates. Therefore, iodinated analogs, **2**, **11**, **12**, **14**, and **15**, were also designed and synthesized through iodination of 3,4,5-trimethoxybenzaldehyde following the same reaction sequence as mentioned above (Scheme 1). Newly synthesized derivatives, **1–15** (Table 1), were screened for cytotoxicity and chemoreversal effects on KB and MDR KB cells in the absence (–) or presence (+) of paclitaxel.

Most of the analogs exhibited no significant cytotoxicity at 10 μ M against four tumor cell lines, A549, DU145, KB and KBvin (Supplementary data, Table S1). Reversal activity of 1-(3,4,5-trimethoxyphenyl)ethane-1,2-diyl esters using paclitaxel is shown in Table 2. In the absence of paclitaxel, most compounds had no cytotoxic effect, while chemoreversal effects were seen on KBvin cells

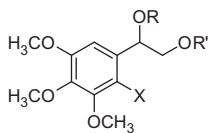
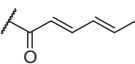
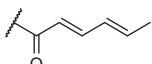
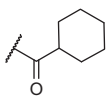
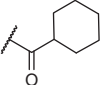
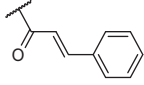
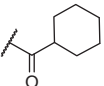
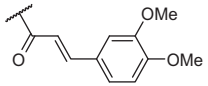
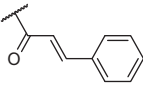
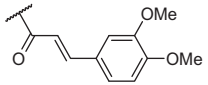
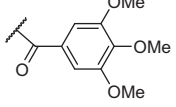
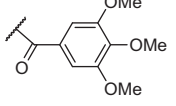
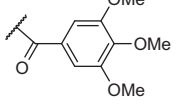
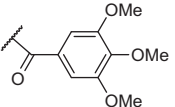
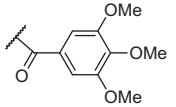
but not on KB cells in the presence of paclitaxel. This result indicates that these compounds are specifically toxic for MDR KB cells when combined with the current anticancer drug. Brief SAR correlations were observed as follows. Among the alkyl esters, acetyl (**3**), butyryl (**4**) and dodecanoyl (**5**) groups showed no chemoreversal activity. In the case of short fatty acid esters (**6** and **7**), moderate reversal effects were observed, indicating a conjugated system may contribute to chemoreversal activity. Interestingly, compound **9** with bulky di-cyclohexanecarbonyl esters displayed remarkable reversal activity, while mono-cyclohexanecarbonyl ester (**8**) totally lost activity. This fact suggested that di-esters would be better than mono-esters, probably due to higher hydrophobicity, a critical feature for drug efflux pump inhibitors or the importance of the butterfly shape proposed in the pharmacophore model.⁴ The ester derivatives containing an aromatic ring showed relatively better reversal ability than the aliphatic ester derivatives. Especially, cinnamoyl ester **10** and 2-iodo-3,4-dimethoxycinnamoyl ester **11**, with the same side chain as DMDCK, totally reversed the cytotoxicity of paclitaxel in the KBvin cell line. Similarly, 2-iodo-mono-3,4,5-trimethoxybenzoyl (**14**) as well as 2-iodo-di-3,4,5-trimethoxybenzoyl (**15**) esters displayed high chemoreversal effects. These four compounds resulted in 0% of KBvin cell survival at 10 μ M. The comparison of **10** and **11**, as well as **12** and **13**, suggested that iodination of the C-2 position is not very important for the activity. In the case of benzoyl ester, similar effects were shown regardless of mono-ester (**14**) or di-ester (**15**). In a comparison of **12** and **14**, the silyl group led to reduced chemoreversal when an iodo group was present at the C-2 position and also increased cytotoxicity in the presence of paclitaxel. Among the potent derivatives **9–15**, compound **12** displayed cytotoxicity. Therefore, only **9–11** and **13–15**, mainly cinnamoyl and trimethoxybenzoyl derivatives, were selected for further evaluation with three anticancer drugs, paclitaxel, vincristine and doxorubicin (Table 3).



Reagents and conditions: (a) trifluoroacetate, iodine; (b) $\text{Ph}_3\text{PCH}_2\text{Br}$, tBuOK , toluene; (c) OsO_4 , NMO, THF, 55°C ; (d) RCOCl , Et_3N , CH_2Cl_2 ; (e) 2,6-lutidine, TBSOTf, CH_2Cl_2 , -78°C

Scheme 1. Synthetic route to 1-(3,4,5-trimethoxyphenyl)ethane-1,2-diyl esters

Table 1
Structures of 1-(3,4,5-trimethoxyphenyl)ethane-1,2-diyl esters

				
No	x	R	R'	
1	H	H	H	
2	I	H	H	
3	H			
4	H	H		
5	H			
6	H			
7	H			
8	H	H		
9	H			
10	H			
11	I			
12	I	TBS		
13	H	TBS		
14	I	H		
15	I			

Among the six compounds, trimethoxybenzoyl analogs, **13–15**, generally had better chemoreversal ability than cinnamoyl derivatives (**10** and **11**) when combined with the three anticancer drugs. The reversal activity was similar between **10** and **11**. Among compounds **13–15** with mono- or di-3,4,5-trimethoxybenzoyl modification, di-ester **15** was the most active showing 340-fold more potent than verapamil when used with vincristine. Compound **13** with a bulky and hydrophobic *tert*-butyldimethylsilyl group also showed significant activity. Cyclohexanoyl ester **9** displayed less activity than the other five compounds. These results indicated

Table 2
Screening of reversal activity of 1-(3,4,5-trimethoxyphenyl)ethane-1,2-diyl esters using paclitaxel

Compd	Conc. (μM)	Cell viability (%) ± SD ^a			
		KB		KBvin	
		(–)	(+)	(–)	(+)
1	10	101.4 ± 4.2	96.8 ± 4.4	92.4 ± 17.8	99.0 ± 10.5
2	10	95.1 ± 0.8	97.0 ± 0.9	90.0 ± 22.0	91.8 ± 11.6
3	10	98.0 ± 3.6	96.0 ± 2.0	91.5 ± 19.4	92.0 ± 9.1
4	10	99.3 ± 4.3	95.1 ± 3.2	90.0 ± 14.0	90.4 ± 10.1
5	10	92.9 ± 3.8	85.5 ± 6.7	85.7 ± 8.5	81.4 ± 6.6
6	10	91.1 ± 0.9	83.9 ± 6.3	79.9 ± 10.2	44.0 ± 15.1
7	10	91.2 ± 3.9	76.8 ± 9.1	81.5 ± 9.9	31.3 ± 11.8
8	10	98.9 ± 7.8	96.7 ± 5.0	109.9 ± 8.9	102.8 ± 8.6
9	10	91.1 ± 7.4	88.1 ± 5.4	89.3 ± 6.5	6.2 ± 14.4
10	10	82.2 ± 2.2	73.8 ± 4.9	74.3 ± 7.0	0 ± 5.7
11	10	85.9 ± 2.7	76.5 ± 4.0	95.3 ± 2.0	0 ± 4.4
12	10	64.2 ± 5.5	58.0 ± 9.3	41.0 ± 5.5	19.6 ± 5.1
13	10	83.9 ± 1.0	70.3 ± 6.8	74.3 ± 3.2	12.3 ± 11.9
14	10	77.0 ± 4.1	75.7 ± 3.2	83.3 ± 12.9	0 ± 6.0
15	10	84.2 ± 4.8	73.7 ± 8.7	87.1 ± 6.9	0 ± 6.9

^a Cell viability was measured by SRB method in the absence (–) or presence (+) of non-toxic concentration of paclitaxel. (1 nM for KB cell and 100 nM for KBvin cell)

Table 3
Reversal activity of selected 1-(3,4,5-trimethoxyphenyl)ethane-1,2-diyl esters

Compd	Conc. (μM)	Paclitaxel (nM)		Vincristine (nM)		Doxorubicin (nM)	
		IC ₅₀ ± SD	Fold ^b	IC ₅₀ ± SD	Fold	IC ₅₀ ± SD	Fold
9	10	43.1 ± 7.33	30	9.49 ± 1.22	215	106.5 ± 13.8	14
10	10	11.0 ± 5.65	116	3.89 ± 0.83	524	39.0 ± 1.58	37
11	10	7.66 ± 1.54	166	3.29 ± 0.28	620	61.9 ± 11.1	23
13	10	5.91 ± 0.69	216	1.21 ± 0.03	1684	18.4 ± 7.99	79
14	10	7.79 ± 0.55	164	2.56 ± 0.42	798	28.4 ± 9.42	51
15	10	9.04 ± 4.55	141	0.89 ± 0.25	2285	9.29 ± 2.71	156
VRP ^a	10	28.47 ± 8.64	72	21.0 ± 4.63	97	249.4 ± 78.5	6

^a VRP: verapamil.

^b The reversal fold values were calculated as: reversal fold = IC₅₀ (anticancer drug alone)/IC₅₀ (anticancer drug + test compound). The IC₅₀ values of paclitaxel, vincristine, and doxorubicin against KBvin cells were determined in the presence of 10 μM compound, and expressed with mean ± SD of three independent experiments.

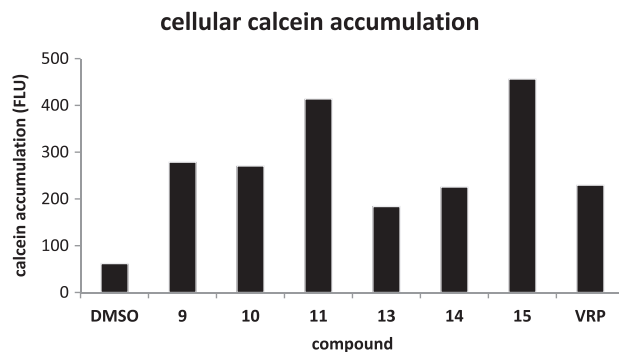


Figure 2. Inhibitory effects of compounds on P-gp function in KBvin Cells. The KBvin cells were pretreated with the compounds (10 μM) followed by addition of calcein-AM. The cellular accumulation of calcein is represented by fluorescent units (FLU). Cellular accumulation of calcein demonstrates inhibition of efflux activity of P-gp. Data with mean ± SD of three independent experiments are shown in Supplementary Table S2.

that hydrophobicity, higher electron density on aromatic group, as well as the maintenance of the spatial arrangement are critical for better chemoreversal activity.

All of the active compounds (**9–11** and **13–15**) were further evaluated for effects on P-gp efflux function in KBvin cells using

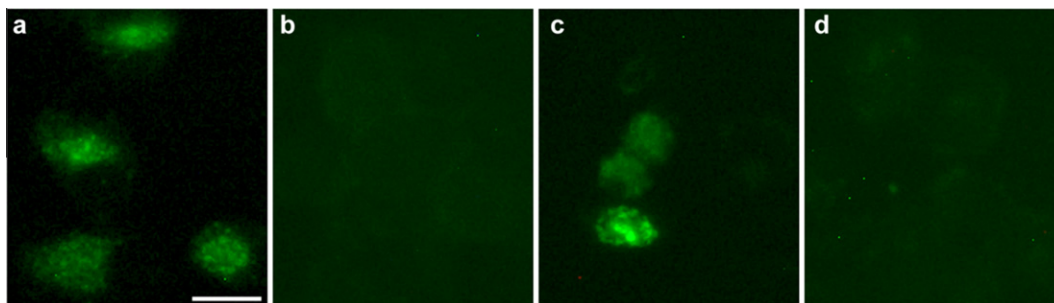


Figure 3. Effect of **13** on MRP function in KBvin Cells. KB (a) or KBvin (b, c, d) cells were pre-treated with DMSO (a, b), **13** (c), or **15** (d) for 1 h before addition of Fluo-3 AM for 2 h. Cellular accumulation of Fluo-3, a fluorescence MRPs substrate, was observed in KB cells (a), and effluxed from KBvin cells. (b). Cellular retention of Fluo-3 fluorescence was observed in KBvin cells treated with **13**. (c) yet undetectable in cells treated with **15**. (d). (Bar, 50 μ m.)

fluorescent P-gp substrate calcein-AM (Fig. 2).⁸ Cellular accumulation of calcein was observed in the presence of 10 μ M compounds, demonstrating that the compounds prevent calcein-AM efflux from KBvin cells by inhibiting P-gp. Compound **15** exhibited two-fold more calcein accumulation than verapamil at the same concentration, indicating **15** is more potent than verapamil at inhibiting P-gp function. Although **13** showed strong reversal activity compared with verapamil, its inhibitory effect on the P-gp efflux pump was relatively weaker than verapamil. Hence, **13** may inhibit other drug transporters, such as multidrug resistance-associated proteins (MRPs). The inhibitory effect of **13** on MRPs can be determined by using a fluorogenic MRP substrate Fluo-3 AM, which is accumulated in KB cells and pumped out from KBvin cells (Fig. 3). Although no significant Fluo-3 fluorescence was seen in KBvin cells pre-treated with **15**, cellular accumulation of Fluo-3 was observed in KBvin cells pre-treated with **13**. These results demonstrate that **13** inhibits efflux pump function of MRPs, whereas **15** does not affect MRPs. Taken together, **15** selectively inhibits P-gp and **13** can interfere with both P-gp and MRPs.

In this study, we demonstrated that a simplified scaffold containing the core of DMDC and DSP exhibited excellent chemoreversal activity. Fifteen ester analogs were designed, synthesized, and evaluated for chemoreversal activity. Based on the results, di-ester modification is critical for better activity, indicating the importance of maintaining the spatial arrangement of the compound. SAR study indicates that di-trimethoxylbenzoyl substitution resulted in the most activity, followed by di-3,4-dimethoxycinnamoyl and then di-cyclohexanecarbonyl substitutions. Aliphatic groups, such as acetyl or butyryl, led to no activity. Mechanism of action study showed that these compounds inhibited the drug efflux function of P-gp, which increases the cellular

accumulation of anticancer drugs, such as paclitaxel, vincristine, and doxorubicin. Moreover, compound **13** showed additional inhibitory activity against MRPs. Further modifications and more detailed SAR will be reported in the near future.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2012.09.096>.

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