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Seco-4-methyl-DCK derivatives as potent chemosensitizers

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ABSTRACT

Twenty-five seco-4-methyl-DCK derivatives were designed, synthesized and evaluated for chemoreversal activity when combined with paclitaxel or vincristine in two drug-resistant cancer cell lines (A2780/T and KB-V) respectively. Most of the new compounds displayed moderate to significant MDR reversal activities in the P-gp overexpressing A2780/T and KB-V cells. Especially, compounds **7o** and **7y** showed the most potent chemosensitization activities with more than 496 and 735 reversal ratios at a concentration of 10 μM. Unexpectedly the newly synthesized compounds did not show chemosensitization activities observed 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, these compounds did not exhibit significant antiproliferative activities against nontumorigenic cell lines (HUVEC, HOSEC and T29) compared to the positive control verapamil at the tested concentration, which suggested better safety than verapamil. The pharmacological actions of the compounds will be studied further to explore their merit for development as novel candidates to overcome P-gp mediated MDR cancer.

Multidrug resistance (MDR), described first by Biedler and Riehm in 1970, leads to over 90% failure in the treatment of metastatic cancer, because the cancer cells resist several structurally unrelated drugs simultaneously.[1–3] While several factors can lead to MDR, the overexpression of ABC (ATP Binding Cassette) transporters is one of the most probable. Among these transporters, the most prevalent is P-glycoprotein (P-gp, also known as MDR1).[4] Using the energy of ATP hydrolysis, P-gp transports hundreds of substrates, including various anticancer drugs, out of cells. The resultant downregulation in the intracellular concentration of anticancer drugs can lead to MDR.[5–8] Therefore, the development of P-gp inhibitors is one of the most effective strategies to overcome MDR mediated by P-gp overexpression. Presently, although three generations of small molecule P-gp inhibitors have been identified,[9] none have been approved as a drug for clinical use. Unfortunately, a phase III clinical trial with tariquidar, a third-generation P-gp inhibitor, was terminated due to an undesirable benefit/risk ratio.[10,11] Although another third-generation P-gp inhibitor HM30181 is currently in Phase I-II clinical trials,[11,12] efforts continue to identify and develop highly effective P-gp inhibitors with low toxicity as chemotherapy sensitizers.

Fong *et al.* reported that (±)-praeruptorin A (PA), a naturally occurring pyranocoumarin, reversed P-gp mediated MDR, while its 3′,4′-modified khellactone analog, (±)-3′-*O*,4′-*O*-bis(3,4-dimethoxycinnamoyl)-*cis*-khellactone (DMDCK) (Fig. 1), was even more potent with reversal ratios ranging from 110 − 167 fold when combined at a concentration of 4 μM with different anticancer drugs in MDR HepG2/Dox cell lines.[13,14] To potentially improve the druggability, we designed a series of seco-4-methyl-DCK derivatives (Fig. 2), which lacked the C-ring and two chiral centers of PA and its khellactone analogs. Additionally, because the prior study indicated that 3′,4′-acyl groups were important to maintain reversal activity, we also introduced variously substituted benzoyl and cinnamoyl acyl groups at the 8-position of the coumarin, as well as including an ethyl or isopropyl ether at the 7-position. The 25 new synthetic coumarin derivatives (**7a–y**) were screened as chemosensitizers in comparison with the first-generation P-gp inhibitor verapamil used as a positive control.

As shown in Scheme 1, a Duff reaction of **1** gave **2**, and the generated aldehyde was protected to produce **3**. The 7-hydroxyl of **3** was

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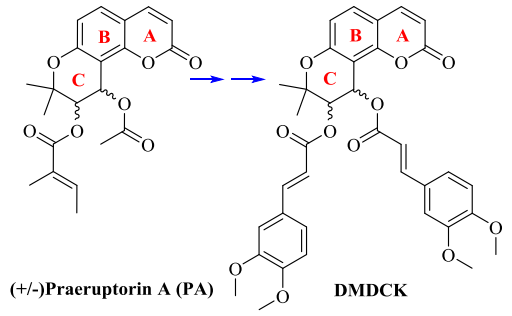


Fig. 1. Structures of PA and DMDCK.

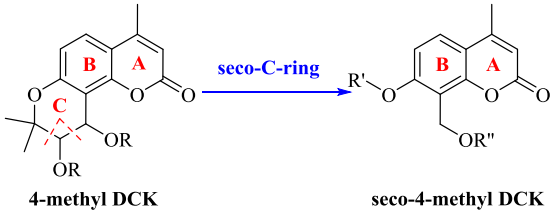


Fig. 2. Seco-C-ring strategy for new derivatives.

reacted with bromoethane or 2-bromopropane to give **4a** and **4b**, respectively. Deprotection regenerated the 8-aldehydes of **5a–b**. Key intermediates **6a–b** were synthesized by reduction of the aldehyde to a hydroxymethyl group. Finally, 25 seco-4-methyl-DCK derivatives (**7a–y**) were prepared via a esterification reaction between **6a–b** and various aromatic acids catalyzed by 4-dimethylaminopyridine (DMAP) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI).

Target compounds **7a–y** were screened for cytotoxicity at a concentration of 10 μM in an MTT assay against three non-tumorigenic cell lines (HOSEC, HUVEC, and T29), paclitaxel- or cisplatin-resistant human ovarian cancer cell lines (A2780/T and A2780/CDDP) and a vincristine-resistant human oral epidermoid cancer cell line (KB-V). Verapamil was used as a positive control. As shown in Table 1, survival ratios of the above six cell lines ranged from 50% to 131% after treatment with 10 μM of **7a–y**. Most test compounds produced higher survival ratios than those with verapamil (76–114%). The data imply that some seco-4-methyl-DCK derivatives might have lower toxicity and higher safety than verapamil.

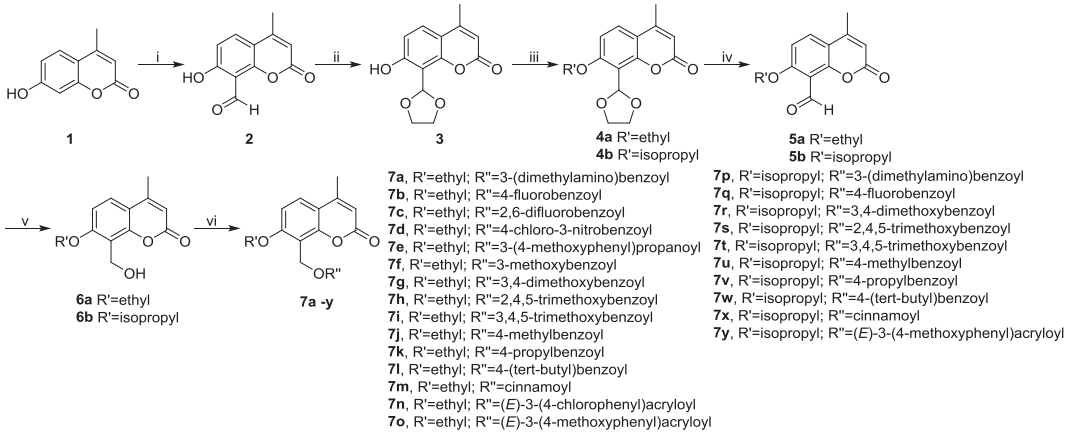
Next, the newly synthesized compounds **7a** − **y** at 10 μM were co-administered with different concentrations of paclitaxel to evaluate

Table 1
Cytotoxicity in non-tumorigenic and cancer cell lines.[a]

Compound	Survival Ratio (%)					
	HOSEC	HUVEC	T29	A2780/T	KB-V	A2780/CDDP
7a	97.24	96.31	106.35	97.05	103.72	117.41
7b	78.26	87.23	88.58	93.41	ND	ND
7c	78.25	86.33	94.62	100.64	ND	ND
7d	50.10	81.02	72.61	78.72	ND	ND
7e	73.45	85.01	91.34	101.38	ND	ND
7f	68.42	79.76	83.38	76.50	ND	ND
7g	72.01	83.36	86.14	83.07	ND	ND
7h	78.15	88.78	89.51	84.07	ND	ND
7i	86.71	99.71	103.19	97.63	ND	ND
7j	64.65	77.12	86.09	95.81	ND	ND
7k	76.03	78.44	97.25	84.02	ND	ND
7l	79.79	76.00	87.07	95.14	ND	ND
7m	92.23	84.72	98.67	98.94	ND	ND
7n	90.70	101.03	105.99	102.49	ND	ND
7o	84.88	93.77	98.91	83.57	104.60	122.78
7p	75.96	84.78	90.75	102.17	ND	ND
7q	70.48	79.66	85.32	102.01	ND	ND
7r	106.85	84.17	101.12	101.86	ND	ND
7s	86.31	72.67	90.94	105.70	ND	ND
7t	61.73	73.08	81.53	94.58	96.14	65.55
7u	79.80	75.63	91.21	95.16	ND	ND
7v	87.51	82.25	92.68	100.55	ND	ND
7w	90.64	83.70	96.04	104.30	ND	ND
7x	105.03	95.86	104.38	102.74	ND	ND
7y	93.59	98.90	90.40	98.09	108.46	131.10
6b	80.18	84.66	88.57	94.64	ND	ND
Verapamil	82.82	82.07	81.85	85.34	75.58	113.84
Cisplatin	24.04	20.98	10.80	ND	ND	ND

[a] The survival ratio was determined in the presence of 10 μM compound and expressed as the mean of three independent experiments. ND: Not determined.

their MDR reversal effects in the paclitaxel-resistant A2780/T cell line. The reversal ratio (r.r.) was defined as $IC_{50(\text{drug only})}/IC_{50(\text{drug with synthesized compound})}$, as a standard of the evaluation. As shown in Table 2, **18** compounds exhibited moderate to significant MDR reversal activity (r.r. > 50), and four compounds (**7a**, **7o**, **7t**, and **7y**) exhibited greater reversal activity (r.r. 224–735) than verapamil (r.r. = 211). Notably, compounds **7o** and **7y** with an (*E*)-3-(4-methoxyphenyl)acryloyl group at the 8-position showed the most potent reversal effects with 735- and 496-fold reversal ratios. In other words, the IC_{50} of paclitaxel against A2780/T was 3.97 μM, while those of paclitaxel when combined with **7o** or **7y** were 5.4 and 8.0 nM, respectively. Thus, the C-ring and chiral centers of the 4-methyl DCK scaffold were not necessary to produce



Scheme 1. Syntheses of seco-4-methyl-DCK derivatives (**7a–y**). Reagents and conditions: (i) hexamethylenetetramine (2.5 equiv), HOAc, 95 °C, 4.5 h; then HCl (conc. HCl:H_2O = 84:100 v/v), 70 °C, 1 h, 22%; (ii) ethylene glycol (2 equiv), *p*-toluenesulfonic acid (catalytic amount), benzene, circumfluence to repel water, 2 h, 48%; (iii) bromoethane or 2-bromopropane (1.2 equiv), K_2CO_3 (3 equiv), KI (0.1 equiv), acetone, reflux, 5 h, 81–93%; (iv) 2 N HCl (30 mL/mmol compound **4a** or **4b**), rt, 24 h, 85–90%; (v) $NaBH_4$ (1.4 equiv), MeOH, rt, 2 h, 95–99%; (vi) aromatic acids (1.1 equiv), EDCI (1.2 equiv), DMAP (0.5 equiv), DCM, 2 h, 0 °C ~ rt, 39–82%.

Table 2
MDR reversal activity of **7a–y** (10 μM) against MDR cell lines.

Compd	A2780/T Paclitaxel (nM)		A2780/CDDP Cisplatin (μM)		KB-V VCR (nM)	
	IC_{50}	r.r.	IC_{50}	r.r.	IC_{50}	r.r.
7a	17.7	224	51.97	0.83	2.12	314
7b	64.7	61	ND[a]	ND	ND	ND
7c	54.9	72	ND	ND	ND	ND
7d	129.2	31	ND	ND	ND	ND
7e	668.6	6	ND	ND	ND	ND
7f	71.2	56	ND	ND	ND	ND
7g	39.2	101	ND	ND	ND	ND
7h	83.8	47	ND	ND	ND	ND
7i	77.4	51	ND	ND	ND	ND
7j	155	26	ND	ND	ND	ND
7k	74.2	54	ND	ND	ND	ND
7l	25.1	158	ND	ND	ND	ND
7m	40.8	97	ND	ND	ND	ND
7n	288.4	14	ND	ND	ND	ND
7o	5.4	735	56.59	0.76	0.84	793
7p	46.4	86	ND	ND	ND	ND
7q	42.9	93	ND	ND	ND	ND
7r	20.2	197	ND	ND	ND	ND
7s	24.6	161	ND	ND	ND	ND
7t	11.2	354	50.13	0.86	4.99	134
7u	41.8	95	ND	ND	ND	ND
7v	88.6	45	ND	ND	ND	ND
7w	55.5	72	ND	ND	ND	ND
7x	124.7	32	ND	ND	ND	ND
7y	8	496	54.98	0.78	0.53	1257
Verapamil	18.8	211	40.11	1.07	1.31	509
Paclitaxel	3970	1418[b]	ND	ND	ND	ND
Cisplatin	ND	ND	43.11	17[c]	ND	ND
VCR	ND	ND	ND	ND	666.3	3173[d]

[a] ND: Not determined.
[b] Drug resistance ratio of A2780/T, defined as $IC_{50\ (A2780/T)}/IC_{50\ (A2780)}$ of paclitaxel = 3970 nM/2.8 nM.
[c] Drug resistance ratio of A2780/CDDP, defined as $IC_{50\ (A2780/CDDP)}/IC_{50\ (A2780)}$ of cisplatin = 43.11 μM/2.55 μM.
[d] Drug resistance ratio of KB-V, defined as $IC_{50\ (KB-V)}/IC_{50\ (KB)}$ of VCR = 666.3 nM/0.21 nM.

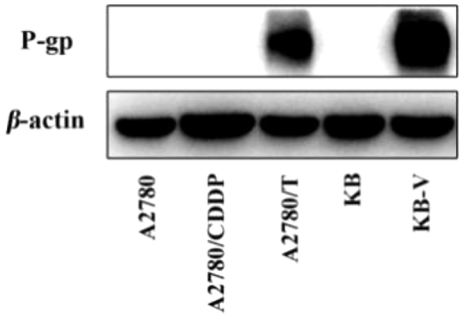


Fig. 3. P-gp expression in drug-sensitive and coupled drug-resistant cancer cell lines.

chemosensitization. The MDR reversal activity was not affected significantly or consistently by the choice of ethoxy or isopropoxy at the 7-position of the coumarin. As mentioned above, the most potent compounds contained methoxy-substituted cinnamoyl esters at the coumarin 8-position (**7o** and **7y**), while the next most potent compounds contained 3,4,5-trimethoxybenzoyl and 3-(dimethylamino)benzoyl esters (**7t** and **7a**, respectively). Introduction of electron-donating moieties on the aromatic ring of the ester group generally improved the reversal activity, while electron-withdrawing groups decreased the activity [e.g., **7g** (3,4-dimethoxybenzoyl) > **7d** (4-chloro-3-nitrobenzyl); **7y** (*p*-methoxycinnamoyl) > **7w** (cinnamoyl) > **7x** (*p*-chlorocinnamoyl)]. In addition, the potency decreased dramatically when the double bond of the acryloyl moiety was saturated; analog **7e** with a 4-methoxyphenylpropanoyl ester at the 8-position was the least potent compound.

Next, compounds **7a**, **7o**, **7t**, and **7y**, which displayed high MDR reversal activity in the A2780/T cell line, were further evaluated in two additional drug-resistant cell lines KB-V and A2780/CDDP, when co-administered with vincristine (VCR) and cisplatin, respectively. To our surprise, the four compounds showed strong drug-resistant reversal activity with high reversal ratios in KB-V cells, but no significant effects in A2780/CDDP cells. The r.r. values of **7o** and **7y** were 793 and 1257, higher than that of verapamil (5 0 9) in KB-V, but only 0.76 and 0.78, slightly lower than that of verapamil (1.07) in A2780/CDDP cells. The IC_{50} values of VCR against the KB-V cell line decreased dramatically from 666.30 nM alone to 0.84 nM and 0.53 nM when co-administered with **7o** or **7y**, respectively. The results were consistent with those for paclitaxel in A2780/T cells. In contrast, no reversal effects were seen in A2780/CDDP cells.

P-gp over-expression readily causes MDR in many cancer cell lines. Therefore, we measured the P-gp expression in the five tested cancer cell lines, two drug-sensitive (A2780 and KB) and three drug-resistant (A2780/T, A2780/CDDP and KB-V), using Western blot analysis. As shown in Fig. 3, we observed P-gp overexpression in the A2780/T and KB-V drug-resistant cell lines, but not in the cisplatin-resistant A2780/CDDP or A2780 and KB drug-sensitive cell lines. This outcome supported our above findings that the seco-C khellactone analogs were active as chemosensitizers in A2780/T and KB-V but not A2780/CDDP cell lines. A close relationship exists between the chemoreversal activity and P-gp over-expression in the drug-resistant cancer cell lines. We are performing studies on the detailed pharmacological mechanism and will report our findings in the future.

In brief, we designed and synthesized 25 seco-4-methyl-DCK derivatives and evaluated their ability to chemosensitize A2780/T and KB-V cell lines toward paclitaxel and vincristine. None of the compounds displayed significant cytotoxicity in HOSEC, HUVEC, T29, A2780/T, A2780/CDDP and KB-V cell lines, and some of them had stronger MDR reversal activity compared with verapamil. Among them, compounds **7o** and **7y** showed the most potent reversal activity. When paclitaxel and vincristine were administrated together with 10 μM of **7o** or **7y**, the reversal ratios (735 and 496 in A2780/T; 793 and 1257 in KB-V) were higher than those of verapamil (211 and 509). A preliminary structure-activity relationship (SAR) analysis indicated that MDR reversal activity was maintained with an appropriately substituted bicyclic coumarin core. Additionally, various benzoyl and cinnamoyl aromatic esters, particularly (*E*)-3-(4-methoxyphenyl)acryloyl, at the 8-position were favorable to the reversal ability. Interestingly, these compounds did not reverse resistance to cisplatin in the non-P-gp overexpressing A2780/CDDP cell line, indicating a close relationship between the MDR reversal activity of these newly synthesized compounds and the P-gp expression level of drug-resistant cancer cell lines. Further studies will be carried out to investigate the detailed mechanistic and pharmacokinetic properties of these seco-4-methy-DCK derivatives as potential chemosensitizer.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.11.023.

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