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Synergism and Antagonism of Lenalidomide–(β-D-Ribofuranose 2',3'-phosphodiester)–Paclitaxel as a Selective Anticancer Codrug

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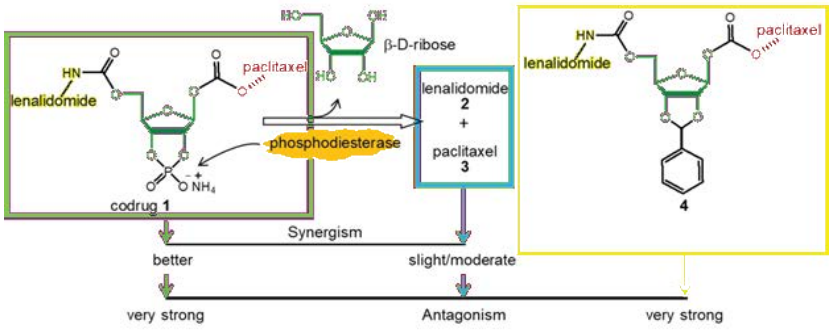
Abstract

A novel codrug **1** is designed through the covalent conjugation of lenalidomide (**2**, an immunomodulatory agent) and paclitaxel (**3**, an anticancer drug) via a D-ribofuranose scaffold as the central linker. The ribofuranose core is further equipped with a phosphodiester moiety as an enzymatically recognizable trigger and as a controlled drug-release site. Codrug **1** exhibits potent cytotoxicity against OECM-1 oral cancer cells (IC_{50} = 8.82–9.93 nM) while showing markedly reduced toxicity toward HUVEC normal endothelial cells (IC_{50} = 686 nM). Compared with paclitaxel, codrug **1** displays a 3.04-fold increase in the anticancer activity and a 113-fold decrease in normal-cell toxicity. Combination-index analysis shows that codrug **1** improves from "slight synergism" (CI <0.724) to "synergism" (CI < 0.338) against OECM-1 cells after 24–48 h and surpasses the efficacy of the corresponding 1:1 physical combination (i.e., **2** + **3**). Remarkably, antagonism toward HUVEC cells is enhanced by more than a two-order-magnitude — from "moderate antagonism" to "nearly additive" for the physical mixture (CI = 1.276–1.040) to "very strong antagonism" for codrug **1** (CI > 41.98; ≈131.8). Codrug **1** represents the first codrug capable of **simultaneously enhancing anticancer synergism while increasing antagonism toward healthy cells**. Such a design offers a promising new paradigm for the development of selective chemotherapy.

Graphical Abstract



Keywords: Antagonism; Codrug; Phosphodiester; D-ribofuranose; Synergism

Introduction

The formulation of combination therapies incorporating two or more pharmacologically active constituents with synergistic interactions can yield

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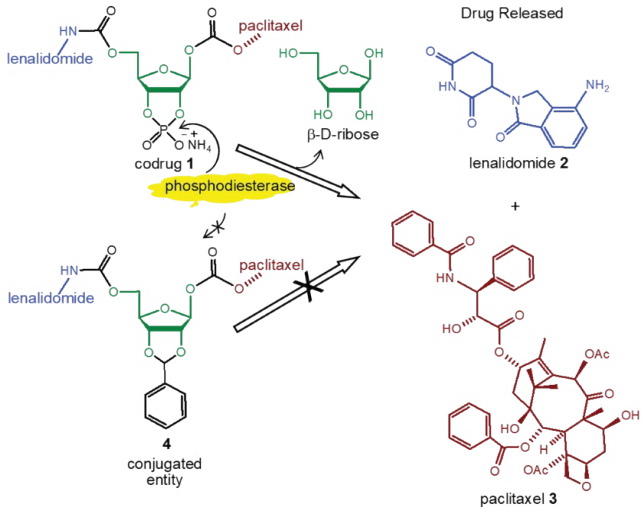
therapeutic outcomes exceeding the additive effects of each drug's individual potency [1]. In contrast, when drugs exhibit antagonistic interactions, the efficacy of at least one drug is diminished when administered together [2, 3]. The ideal therapeutic pairing would demonstrate potent synergism against diseased cells while simultaneously display antagonism toward healthy cells. Cokol et al. [4] outlined the most favourable examples among numerous reported drug combinations; however, none has been shown to possess concurrently both pronounced synergistic and antagonistic properties [5].

One innovative strategy to enhance the pharmacological performance of synergistic drug pairs is to link them covalently into a single molecular entity, referred to as a "codrug" [6]. The active components of a codrug are typically indicated for the same pathological condition yet operate through distinct and complementary mechanisms of action [7]. Notable examples of clinically relevant codrugs demonstrated either significant synergism or antagonism include ampicillin, benorylate, sulfasalazine, and sultamicillin (Unasyn® Oral) [8]. Despite this, relatively few codrugs have been developed with anticancer activity. Examples in this domain include conjugates such as butyric acid, retinoic acid, 5-fluorouracil–cytarabine, paclitaxel–captopril, and 5-fluorouracil–diazeniumdiolate [7]. Recently, a novel codrug, designated as the compound **1**, is synthesized, through incorporation of two established therapeutic agents—lenalidomide (**2**, LENA, Revlimid®) and paclitaxel (**3**, PTX, Taxol®) as shown in



Scheme 1: The possibility of codrug **1** and conjugated entity **4** to release the drugs **2** and **3** from their skeletons in the presence of phosphodiesterase.

Scheme 1 [9].

As an immunomodulatory compound, lenalidomide (**2**) is endowed with antiangiogenic, antitumor, and pro-apoptotic activities. In combination with dexamethasone, it exhibits substantial efficacy as a first-line treatment for multiple myeloma [10]. Furthermore, its synergistic potential has been demonstrated in combination with bortezomib [11]. While lenalidomide possesses broad activities against various haematological malignancies and solid tumours, its dose-limiting toxicity remains a significant concern [12]. A modular Phase I clinical trial of lenalidomide (**2**) in combination of paclitaxel (**3**) in patients with metastatic castration-resistant prostate cancer examined prostate-specific antigen (PSA) kinetics following lenalidomide lead-in therapy. It also assessed the feasibility of co-administration of the two drugs [13]. Additionally, lenalidomide continues to be investigated in clinical trials for pancreatic carcinoma, chronic lymphocytic leukaemia, and lymphoma.

On the other hand, paclitaxel (**3**), the naturally occurring (–)-enantiomer, is widely used in the treatment of diverse malignancies, including bladder, breast, oesophageal, lung, ovarian, and prostate cancers, as well as Kaposi's sarcoma and melanoma [14, 15]. Its remarkable clinical impact is attributed to its unique mechanism of action on microtubule stabilization [16]. However, its therapeutic utility is limited by poor aqueous solubility and inadequate tumour selectivity.

The design of codrug **1** is on the basis of a β-D-ribofuranose scaffold linked to a phosphodiester moiety. It enables the controlled and selective release of lenalidomide (**2**) and paclitaxel (**3**). The D-ribofuranose core is inherently non-toxic, while the phosphodiester group serves both as an enzymatic recognition site and as a targeting element, potentially enhancing selectivity toward cancer cells while minimizing effects on normal tissues. In contrast, although conjugated entity **4** contains both drugs **2** and **3**, it lacks a phosphodiester moiety and is therefore unable to undergo phosphodiesterase-mediated activation or drug release.

We evaluate the synergistic and antagonistic effects of compounds **1**–**4** using oral squamous cell carcinoma cells (OECM-1) and human umbilical vein endothelial cells (HUVECs), respectively. OECM-1 cells are derived from malignant tumours originating in the squamous epithelial lining of the oral cavity. Oral squamous carcinoma is one of the most common forms of oral cancer and may arise in regions, such as the lips, tongue, floor of the mouth, and gums [17]. In contrast, HUVECs are primary endothelial cells isolated from the human umbilical vein. They serve as a well-established model for investigation of endothelial cell biology, with applications in studies of hypoxia, inflammation, oxidative stress, infection response, and physiological/tumour-associated angiogenesis [18].

Herein, we describe the dual synergistic–antagonistic profile of the synthesized anticancer codrug **1**. Its biological activities are evaluated alongside those of a physically mixed lenalidomide (**2**) and paclitaxel (**3**) as well as the structurally related conjugated entity **4**. To the best of our knowledge, this is the first reported codrug to achieve simultaneously

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substantial gains in both anticancer synergism and healthy-cell antagonism.

Materials and Methods

Cell viability assay: For evaluation and comparison of the cytotoxicity of codrug **1**, conjugate entity **4**, and the constituent drugs **2** and **3**, OECM-1 and HUVEC cells were seeded in 96-well plates at a density of 5,000 cells/well and allowed to attach overnight. On the following day, cells were treated with serial dilutions of the test compounds and incubated for an additional 24 or 48 hours. Cell viability was then assessed by use of the MTT assay (Catalog No. M5655, Sigma-Aldrich, St. Louis, MO, USA) [19]. Absorbance at 570 nm was measured with a microplate reader (Sunrise Absorbance Reader; Tecan, Männedorf, Switzerland). Cell viability was expressed as a percentage relative to untreated control cells. The half-maximal inhibitory concentration (IC_{50}) values were calculated from the corresponding dose–response curves and defined as the concentrations required to reduce cell viability by 50% after 24 and 48 h of treatment.

Results

Biological Activities of Codrug 1, a Physical Mixture of Lenalidomide (2) and Paclitaxel (3), and Conjugated Entity 4 Against OECM-1 and HUVEC Cells

We evaluated the cytotoxic activities of codrug **1**, lenalidomide (**2**), paclitaxel (**3**), a 1:1 physical mixture of **2** and **3**, and conjugated entity **4** against OECM-1 and HUVECs. Cell viability was determined by the MTT assay [19], and IC_{50} values were calculated from the dose–response curves shown in Figures 1–5.

Figure 1 illustrates the dose-dependent cytotoxic effects of paclitaxel (**3**) on OECM-1 and HUVEC cells. After 24 hours of exposure (left graph), cell viability decreased progressively with increased paclitaxel concentration in both cell lines. The IC_{50} values were 25.7 nM for OECM-1 cells and 24.4 nM for HUVEC cells. These comparable IC_{50} values indicate that the two cell lines exhibited similar sensitivity to paclitaxel. Thus, selectivity was limited between cancerous and normal cells at this time point.

After 48 hours of exposure (right graph), HUVEC cells exhibited markedly increased sensitivity to paclitaxel, with the IC_{50} value decreasing to 5.23 nM, whereas the IC_{50} value for OECM-1 cells remained essentially unchanged at 26.8 nM. These results indicate that prolonged exposure to paclitaxel substantially increased cytotoxicity toward normal endothelial cells while no additional cytotoxic advantage was provided against OECM-1 cells. Consequently, the selectivity of paclitaxel between cancerous and normal cells was further diminished after extended treatment. The data also demonstrate a pronounced time-dependent increase in paclitaxel-induced cytotoxicity in HUVEC cells from 24 to 48 hours of exposure. The potential toxicity was highlighted associated with prolonged paclitaxel treatment toward normal tissues.

Similar experiments were applied to lenalidomide (**2**). The results, shown in Figure 2, illustrate the cytotoxic effects of lenalidomide on OECM-1 and HUVEC cells after 24 and 48 hours of treatment. At both exposure times, cell viability remained consistently high across the entire concentration range tested, which indicate that lenalidomide exhibited minimal cytotoxicity toward either cell line. After 24 hours of treatment (left graph), neither OECM-1 nor HUVEC cells showed a significant reduction in viability, even at the highest concentration tested (1000 nM). Following 48 hours of exposure (right graph), both cell lines also maintained high viability with no apparent dose-dependent decrease in cell survival was observed.

Consistent with these findings, the IC_{50} values of lenalidomide (**2**) in both OECM-1 and HUVEC cells were close to 1000 nM at both 24 and 48 hours. These results demonstrate that lenalidomide did not induce substantial cytotoxic effects in either cancerous or normal cells within the concentration range and exposure periods examined.

The dose–response curves shown in Figure 3 illustrate the cytotoxic effects of conjugate entity **4** on OECM-1 and HUVEC cells. This compound, which contains paclitaxel

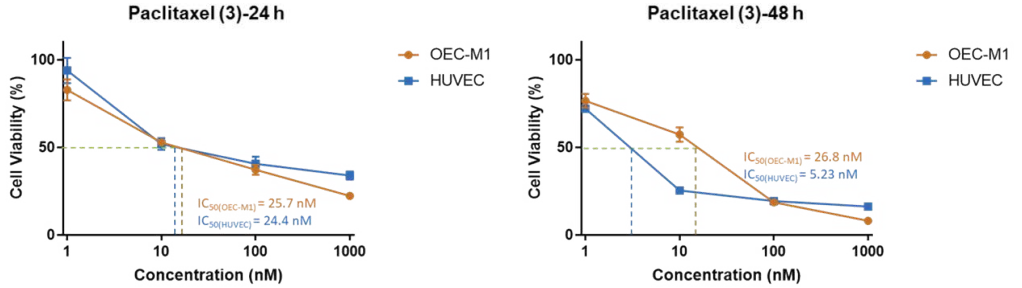

Figure 1: Cell viability curves and corresponding IC_{50} values of paclitaxel (**3**) in OECM-1 and HUVEC cells, as determined by the MTT assay after 24 and 48 hours of treatment.

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(**3**) and lenalidomide (**2**) through a D-ribofuranose scaffold, exhibited a cytotoxic profile distinct from those of its parent drugs. After 24 hours of treatment, OECM-1 cells were more sensitive to conjugate entity **4**, with an IC_{50} value of 90.1 nM, whereas HUVEC cells were markedly less sensitive with an IC_{50} value close to1000 nM. This result suggests a favorable degree of selectivity toward cancer cells during the early stage of exposure. Following 48 hours of treatment, the cytotoxicity toward OECM-1 cells increased modestly, as reflected by a decrease in the IC_{50} value to 54.0 nM. In contrast, HUVEC cells exhibited a substantially greater increase in sensitivity, with the IC_{50} value decreased to 351.8 nM. These findings indicate a time-dependent enhancement of cytotoxicity in both cell lines particularly in HUVEC cells between 24 and 48 hours of exposure.

The delayed cytotoxic response observed in HUVEC cells suggests that prolonged exposure to conjugate entity **4** may increase cellular susceptibility over time. By comparison, OECM-1 cells displayed only a moderate increase in sensitivity; a substantial portion of the cytotoxic effect has already been established during the initial exposure period.

Notably, covalent conjugation of paclitaxel and lenalidomide into a single molecular entity altered the biological activity profile relative to either parent drug alone. Conjugate entity **4** exhibited greater cytotoxicity than lenalidomide (**2**) while maintaining a degree of selectivity toward OECM-1 cells. Compared with paclitaxel (**3**), conjugate entity **4** showed reduced toxicity toward HUVEC cells, particularly after 24 h of exposure. These results indicate that chemical conjugation can effectively modulate drug activity and cellular response.

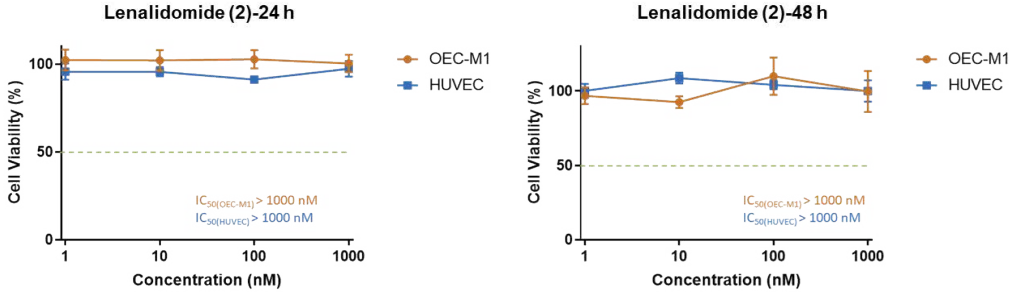

Figure 2: Cell viability curves and corresponding IC_{50} values of lenalidomide (**2**) in OECM-1 and HUVEC cells, as determined by the MTT assay after 24 and 48 hours of treatment

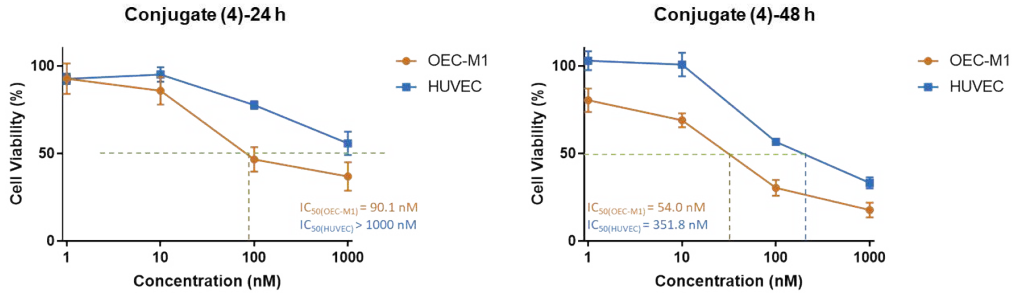

Figure 3: Cell viability curves and IC_{50} values of conjugated entity **4** in OECM-1 and HUVEC cells, as determined by use of the MTT assay after 24 and 48 hours of treatment.

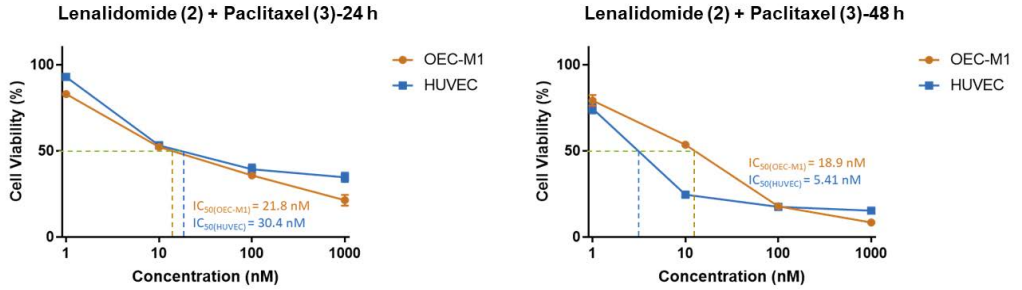

Figure 4: Cell viability curves and corresponding IC_{50} values of a physical mixture of lenalidomide (**2**) and paclitaxel (**3**) (1:1 molar ratio) in OECM-1 and HUVEC cells, as determined by use of the MTT assay after 24 and 48 hours of treatment.

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Furthermore, Figure 4 illustrates the cytotoxic effects of a physical mixture of lenalidomide (**2**) and paclitaxel (**3**) at a 1:1 molar ratio on OECM-1 and HUVEC cells treatment. The results indicate that simple physical mixing of the two drugs did not provide a significant advantage over paclitaxel alone. After 24 hours of exposure, the IC_{50} values of the drug mixture were 21.8 nM for OECM-1 cells and 30.4 nM for HUVEC cells. These data indicate moderate cytotoxicity toward both cell lines and were comparable with those observed for paclitaxel alone. The outcomes suggest that the addition of lenalidomide did not alter the initial cytotoxic response substantially.

Following 48 hours of treatment, OECM-1 cells exhibited a slight increase in sensitivity, with the IC_{50} value decreased to 18.9 nM. In contrast, HUVEC cells showed a marked increase in susceptibility, with the IC_{50} value decreasing dramatically to 5.41 nM. This response closely mirrored the enhanced toxicity observed for paclitaxel alone after prolonged exposure.

In contrast to conjugate entity **4**, which displayed an altered cytotoxic profile and improved selectivity at early exposure times, the physical mixture largely retained the cytotoxic characteristics of paclitaxel (**3**). These findings suggest that simple co-administration of lenalidomide (**2**) and paclitaxel (**3**) did not significantly modify the biological activity of paclitaxel. Therefore, covalent conjugation of the two drugs **2** and **3** appears to be essential for alteration of their biological activity and cellular response.

The anticancer activity was markedly enhanced when lenalidomide (**2**) and paclitaxel (**3**) were covalently linked to form codrug **1**. Unlike the simple physical mixture, codrug **1** incorporated a phosphodiester linkage designed to undergo enzymatic cleavage. Thereby, it enables the intracellular release of both therapeutic agents.

As shown in Figure 5, codrug **1** exhibited potent cytotoxicity toward OECM-1 cells, with an IC_{50} value of 9.93 nM after 24 hours of treatment. Cytotoxicity increased slightly upon prolonged exposure, with the IC_{50} value decreased to 8.82 nM after 48 hours. There values were lower than those observed for paclitaxel alone and for the 1:1 physical mixture of lenalidomide (**2**) and paclitaxel (**3**). Thus, codrug **1** enhanced antiproliferative activity against OECM-1 cells.

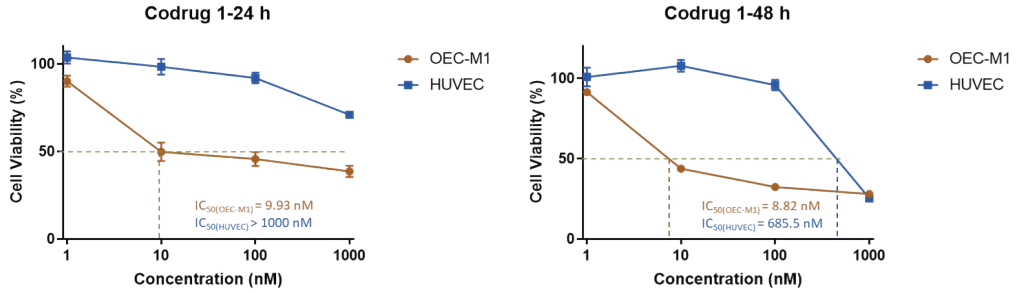

Figure 5: Cell viability curves and corresponding IC_{50} values of codrug **1** in OECM-1 and HUVEC cells, as determined by use of the MTT assay after 24 and 48 hours of treatment.

In contrast, HUVEC cells remained substantially less sensitive to codrug **1**, and exhibited an IC_{50} value close to 1000 nM after 24 hours of exposure and 685.5 nM after 48 hours. Compared with paclitaxel (**3**) alone, which displayed pronounced cytotoxicity toward HUVEC cells after prolonged treatment, codrug **1** maintained a markedly improved selectivity profile.

The distinct cytotoxic responses observed in OECM-1 and HUVEC cells suggest that covalent incorporation of the phosphodiester linker significantly altered the biological behaviour of the parent drugs. The enhanced activity toward OECM-1 cells, together with the reduced toxicity toward HUVEC cells, supports the potential utility of the codrug strategy for improvement of the therapeutic index of paclitaxel-based treatment. These findings demonstrate that *codrug **1** achieved both enhanced anticancer activity and reduced toxicity toward normal cells relative to the parent compounds and their physical mixture*.

Discussion

The IC_{50} values determined for OECM-1 and HUVEC cells are summarized in Table 1. Selectivity indices (SI) were calculated as the ratio of IC_{50}(HUVEC) to IC_{50}(OECM-1). We found that codrug **1**, conjugate entity **4**, and paclitaxel (**3**) exhibited significant antiproliferative activity against OECM-1 cells. After 24 hours of incubation, their IC_{50} values were 9.93, 90.1, and 25.7 nM, respectively (column 2 of Table 1). Thus, codrug **1** was nearly one order of magnitude (90.1/9.93 = 9.07) more potent than conjugate entity **4** and approximately 2.60–3.04 times more potent than paclitaxel (**3**). These findings suggest that codrug **1** effectively delivered the active anticancer component (i.e., paclitaxel) under the in vitro assay conditions. This interpretation is consistent with our previous work [9], which demonstrates that codrug **1** undergoes phosphodiesterase-catalyzed cleavage in sodium pyrophosphate buffer to release paclitaxel (**3**) in vitro. The enhanced potency of codrug **1** may also be attributed, at

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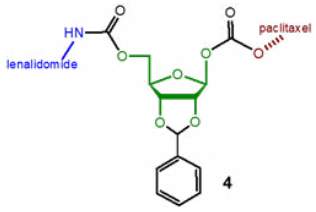
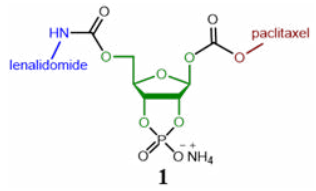
Table 1: IC_{50} Values and Selectivity Indices (SI) of Codrug **1**, Constituent Drugs **2** and **3**, Their Physical Mixture (1:1), and Conjugate Entity **4** in OECM-1 and HUVEC Cells after 24 and 48 Hours of Treatment.

compounds	24 h			48 h		
	IC_{50} (nM)			IC_{50} (nM)		
	OECM-1	HUVEC	SI [a]	OECM-1	HUVEC	SI [a]
lenalidomide (**2**)	>1000	>1000	~	>1000	>1000	~
paclitaxel (**3**)	25.7±3.24	24.4±3.54	0.95	26.8±2.99	5.23±0.11	0.19
lenalidomide + paclitaxel (1:1)	21.8±1.91	30.4±3.34	1.38	18.9±1.66	5.41±0.34	0.29
	90.1±7.19	>1000	>11.1	54.0±5.04	351.8±19.42	6.51
	9.93±0.07	>1000	>101	8.82±0.18	685.5±25.23	77.7
[a] SI = IC_{50} (HUVEC) / IC_{50} (OECM-1)						

least in part, to its ***substantially improved aqueous*** solubility (5.12×10^{-2} mg/mL), which is ~200 times greater than that of paclitaxel (2.50×10^{-4} mg/mL).

More importantly, codrug **1** exhibited substantially lower toxicity toward HUVEC cells than either paclitaxel (**3**) alone or the physical mixture of drugs **2** + **3**. Relative to paclitaxel (**3**), codrug **1** was >101 times (>1000/24.4) and 131 times (685.5/5.23) less toxic toward HUVEC cells after 24 and 48 hours of treatment, respectively (columns 3 and 6 of Table 1). Compared with the physical combination, the corresponding reductions in toxicity were >32.9 (>1000/30.4) and 127 (685.5/5.41) after 24 and 48 hours, respectively. These findings demonstrate a marked improvement in the therapeutic window of codrug **1**.

The foregoing results underscore the incorporative importance of the phosphodiester moiety into the molecular architecture of codrug **1**. *Located at the C2" and C3" positions of the ribose scaffold, the phosphodiester unit is expected to function as an enzyme-responsive trigger that modulates drug release and contribute to the observed selectivity profile.* This interpretation is supported by the exceptionally high selectivity indices of codrug **1** (SI = 77.7~101 shown in the last row of Table 1). Collectively, these results suggest that incorporation of the phosphodiester-containing scaffold plays a critical role in improving therapeutic discrimination between cancerous and normal cells.

Synergism and Antagonism

"Synergism" refers to an interaction between two or more agents in which the combined effect exceeds the sum of their individual effects [20]. In contrast, "antagonism" describes a situation in which the effect of a drug combination is less than the expected additive effect of the individual agents [21]. The synergistic and the antagonistic interactions of codrug **1**, the physical mixture of drugs **2** + **3** (1:1 molar ratio), and conjugate entity **4** were evaluated by use of the Chou–Talalay method [3]. The combination index (CI) was calculated according to the following equation:

$$CI = (IC_{50\ x+y}/IC_{50\ x}) + (IC_{50\ x+y}/IC_{50\ y})$$

where CI < 1 indicates synergism, CI = 1 indicates additivity, and CI > 1 indicates antagonism [22].

The calculated CI values are summarized in Table 2. The physical mixture of lenalidomide (**2**) and paclitaxel (**3**) exhibited slight synergism toward OECM-1 cells, with CI values of <0.870 and <0.724 after 24 and 48 hours of incubation, respectively (row 3 of Table 2). In contrast, codrug **1** displayed substantially stronger synergistic effects, with CI values of <0.396 and <0.338 at 24 and 48 hours, respectively (row 1 of Table 2). These results indicate that covalent linkage of the two drugs significantly enhanced their cooperative anticancer activity in OECM-1 cells.

In HUVEC cells, the physical mixture exhibited

moderate antagonism after 24 hours (CI <1.276) and approached additivity after 48 hours (CI <1.040, row 3 of Table 2). *By comparison, codrug **1** showed exceptionally strong antagonism, with CI values of >41.98 and ≈131.8 for 24 and 48 hours, respectively* (rows 1 and 2 of Table 2). These findings indicate that codrug **1** markedly reduced the combined cytotoxic effects of lenalidomide (**2**) and paclitaxel (**3**) toward normal endothelial cells.

Table 2: Combination Index (CI) Related to Synergistic and Antagonistic Effects of Codrug **1**, the Physical Mixture of Drugs **2** + **3** (1 : 1), and Conjugate **4**.

	24 h		48 h	
drugs	OECM-1	HUVEC	OECM-1	HUVEC
codrug **1**	<0.396	>41.98	<0.338	≈131.8
	synergism	very strong	synergism	very strong
		antagonism		antagonism
combination drug	<0.870	<1.276	<0.724	<1.040
2 + **3** (1:1)	slight	moderate	moderate	nearly
	synergism	antagonism	synergism	additive
conjugate **4**	<3.596	>41.98	<2.069	≈67.62
	strong	very strong	antagonism	very strong
	antagonism	antagonism		antagonism

Conjugate entity **4**, which also contains lenalidomide (**2**) and paclitaxel (**3**) in a 1:1 ratio but lacks the phosphodiester moiety, exhibited very strong antagonism toward HUVEC, with CI values of >41.98 and ≈67.62 at 24 and 48 hours, respectively (rows 5 and 6 of Table 2). In OECM-1 cells, conjugate **4** also exhibited antagonistic interactions, with CI values of <3.596 and <2.069 at 24 and 48 hours, respectively (row 5 of Table 2). Thus, unlike codrug **1**, conjugate entity **4** failed to produce synergistic effects in OECM-1 cells despite displaying reduced toxicity toward HUVEC cells.

These findings suggest that the phosphodiester-containing framework of codrug **1** plays a critical role in modulating drug–drug interactions. Whereas conjugate entity **4** promoted antagonistic responses in both OECM-1 and HUVEC cells, codrug **1** uniquely combined synergistic activity toward OECM-1 cells with strong antagonistic effects toward HUVEC cells. Accordingly, codrug **1** contributed to its superior selectivity profile.

Conclusion

The present study demonstrates the dual phenomena of synergism and antagonism exhibited by a novel codrug **1**. It was constructed by covalently link of lenalidomide (**2**) and paclitaxel (**3**) through a D-ribofuranose scaffold bearing a phosphodiester moiety. Our design was intended to combine the therapeutic benefits of both agents while modulating their biological selectivity. Biological evaluation was conducted in human oral squamous carcinoma OECM-1 cells and normal HUVEC cells by use of the MTT assay.

In OECM-1 cells, codrug **1** exhibited potent antiproliferative activity, with IC_{50} values of 8.82–9.93 nM, while maintaining substantially lower toxicity toward HUVEC cells (IC_{50} = 685.5–>1000 nM). Compared with paclitaxel (**3**), codrug **1** displayed approximately 3-fold greater anticancer potency together with more than 100-fold lower toxicity toward normal endothelial cells. This profile demonstrates a markedly improved therapeutic selectivity.

Combination-index (CI) analysis revealed that codrug **1** produced substantially stronger synergistic effects in OECM-1 cells than the corresponding physical mixture of lenalidomide (**2**) and paclitaxel (**3**). In contrast, codrug **1** exhibited exceptionally strong antagonistic effects in HUVEC cells, whereas the physical mixture showed only weak antagonism or near-additive behavior. These findings indicate that covalent integration of the two drugs within a phosphodiester-containing framework profoundly alters their biological interaction profile.

To the best of our knowledge, *this is the first example in codrug design where both anticancer synergism and healthy-cell antagonism have been simultaneously and substantially optimized within a single molecular framework.* This achievement validates the design principles employed. It also highlights the broader potential of scaffold-based covalent conjugation as a strategy to fine-tune drug–drug interactions at the cellular level. We achieve a favourable therapeutic index through deliberate molecular architecture. Thus codrug **1** represents a highly selective and promising chemotherapeutic candidate. It aligns with the modern paradigm of precision oncology where efficacy is maximized while collateral toxicity is minimized.

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Additional Information

Supporting Information is available for Materials, Methods, References, Figures, and Spectra.

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Author Information

The authors declare no competing interests. Correspondence and requests for materials should be addressed to J.R.H. (jrhwu@mx.nthu.edu.tw).

Contributions

Syntheses of all compounds were done by A. P. and T. K, P.; analysis was performed by S.-C.T. and W.-C. H.; bioassay experimentation was done by C.-S. Y., W.-C. S. and L.-X. Y.; and manuscript was prepared by J. R. H. and D.-B. S.

Conflict of Interest

The authors of this paper report no conflicts of interest related to this publication.

Disclosure

The authors declare no conflicts of interest in this work.

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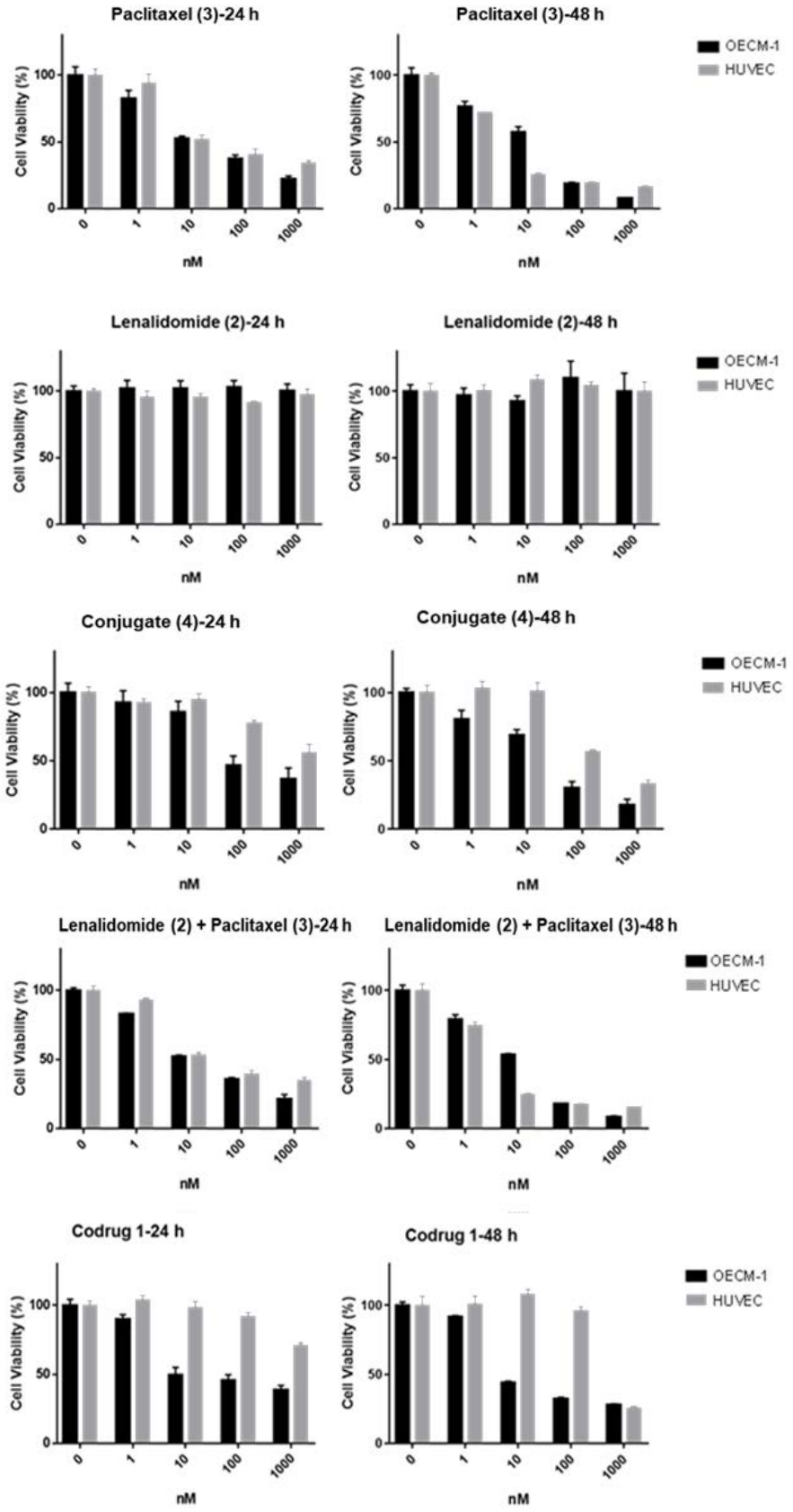

Figure S1: The Cytotoxicity Results of Various Compounds in OECM-1 and HUVEC by Use of the MTT Assay.

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Determination of Synergism and Antagonism by Calculation of the Combination Index (CI) by Use of the Chou-Talalay Equation[1]

Equation: $CI = (D_{x+y}/D_x + D_{x+y}/D_y)$, the CI equation leads to the quantitative definition for synergism (CI < 1), additive effect (CI = 1), and antagonism (CI > 1).

In OECM-1:

(a) for codrug **1**: $CI_{24\text{-h}}$ = (9.93/25.7 + 9.93/>1000) = <0.396

$CI_{48\text{-h}}$ = (8.82/26.8 +8.82/>1000) = <0.338

(b) for combination drug **2** + **3** (1 : 1): $CI_{24\text{-h}}$ = (21.8/25.7 + 21.8/>1000) = <0.870

$CI_{48\text{-h}}$ = (18.9/26.8 + 18.9/>1000) = <0.724

(c) for conjugate **4**: $CI_{24\text{-h}}$ = (90.1/25.7 + 90.1/>1000) = <3.596

$CI_{48\text{-h}}$ = (54.0/26.8 + 54.0/>1000) = <2.069

In HUVEC:

(a) for codrug **1**: $CI_{24\text{-h}}$ = (>1000/24.4 + >1000/>1000) = >41.98

$CI_{48\text{-h}}$ = (685.5/5.23 + 685.5/>1000) = ≈131.8

(b) for combination drug **2** + **3** (1 : 1): $CI_{24\text{-h}}$ = (30.4/24.4 + 30.4/>1000) = <1.276

$CI_{48\text{-h}}$ = (5.41/5.23 + 5.41/>1000) = <1.040

(c) for conjugate **4**: $CI_{24\text{-h}}$ = (>1000/24.4 + >1000/>1000) = >41.98

$CI_{48\text{-h}}$ = (351.8/5.23 + 351.8/>1000) = ≈67.62

Reference

1. Chou TC, Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. Cancer Res 70 (2010): 440−446.

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