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A low toxic CRM1 degrader: Synthesis and anti-proliferation on MGC803 and HGC27

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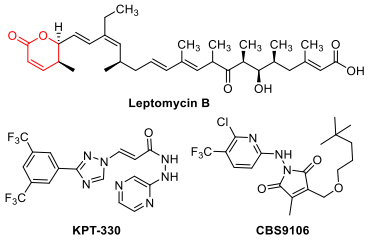
ABSTRACT

Chromosome region maintenance 1 (CRM1) is the sole nuclear exporter of several tumor suppressor, a growth regulatory protein as an attractive cancer drug target. In the present work, a novel CRM1 degrader was discovered from newly synthesized α, β-unsaturated-δ-lactone based on a natural product Goniothalamin. It induces apoptosis of both MGC803 and HGC27 cell lines *via* degrading CRM1. Selective inhibition was observed for the proliferation of gastric cancer cell lines MGC803, HGC27 comparing to Human Gastric Mucosal Epithelial Cell Line (GES1). For the first time, CRM1 inhibitor or degrader inducing apoptosis in gastric carcinoma was investigated.

1. Introduction

Chromosome region maintenance 1 (CRM1), also known as exportin 1 (XPO1), is the protein transporter. It is responsible for the nucleocytoplasmic shuttling of most of the tumor suppressor proteins (TSP) and growth regulatory factors(GRP), which belongs to the karyopherin β super family of transport receptors [1,2]. CRM1, the sole nuclear exporter of TSP, GRP including p53, Rb1 and so on, is an attractive cancer drug target [3]. Currently, an increasing number of drug-like compounds that target CRM1 have been isolated or synthesized such as Leptomycin B (LMB) [4,5], KPT-330 (selinexor) [6,7], KPT-335(verdinexor) [8,9], KPT-185 [9,10], KPT-276 [11,12], KPT-251 [13], S109 [14] and CBS9106 [15]. Some of them have been investigated in clinical trials as a single agent or in combination with bortezomib, selinexor, or dexamethasone [16], while CRM1 inhibitors have not demonstrated adequate potency in the clinical settings. Among them, CBS9106 is a novel reversible CRM1 inhibitor with unique CRM1 degrading activity [15] and it is the only reported CRM1 degrader. Therefore it is needed to discover and research new CRM1 inhibitor or degrader.



Gastric cancer (GC) is a highly aggressive malignant tumor, especially in East Asia such as China, South Korea and Japan. Its high mortality rate prompts the urgent need for novel therapeutic agents [17]. In our screening of therapeutic drugs against GC, some

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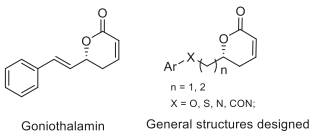
synthesized compounds showed apoptosis activity in MGC803. Among them compound **ll** led to the downregulation of CRM1 in a dose dependent manner in MGC803. The downregulation of CRM1 led by compound **1l** was for the degradation of CRM1 through proteasome pathway. It was approved to be a novel CRM1 degrader with a different skeleton of the first degrader CBS9106 [15], had selective anti-proliferation and induced apoptosis activity on MGC803, HGC27 cell lines comparing with Human Gastric Mucosal Epithelial Cell Line (GES1).

2. Results and discussion

2.1. Chemistry



Natural products LMB, Goniothalamin and their analogues showed CRM1 inhibition and anti-proliferation activity on cancer cell lines. They were reported to bind to CRM1 through the α, β-unsaturated lactone moiety [18,19]. Based on the pharmacophore of α, β-unsaturated lactone, a series of compounds with various linkers and aryl groups were designed and synthesized to investigate the SAR for their anti-proliferation on MGC803.

Compounds **1a~1n** with phenoxymethyl groups were synthesized shown in Scheme 1. They were prepared from key intermediates (**6a~6n**) *via* olefin metathesis reaction catalyzed by grubbs-2. Compounds **6a~6n** were obtained by the acylation of compounds **5a~5n** with Vinylmagnesium chloride**,** which were synthesized by grignard reaction of compounds **4a-4n.** Compounds **4a-4n** was prepared by the reaction of substituted phenols (**2a-2n**) and *S*-epoxy chloropropane (**3**) [20].

Compounds **7a~7d** were synthesized in a similar way with compounds **1a~1n**, and the only difference was in the first step where indol analogues instead of the phenyl derivatives were used as the start materials to react with *S*-epoxy chloropropane (**3**) (Scheme 2). Compound **12** with phenyl sulfane was synthesized by the same strategy, which was oxidized using *m*CPBA to yield compound **13** (Scheme 3).

Compounds **18a-18d** and **19a~19d** with a linker of 3 atoms length were synthesized from compound **20** which can be obtained commercially (Scheme 4). Compound **20** was transfer to compounds **21a-d** by the acylation. Compounds **18a-18d** and **19a~19d** were prepared by an intramolecular esterification in toluene under refluxing in the present of *p*TSA.

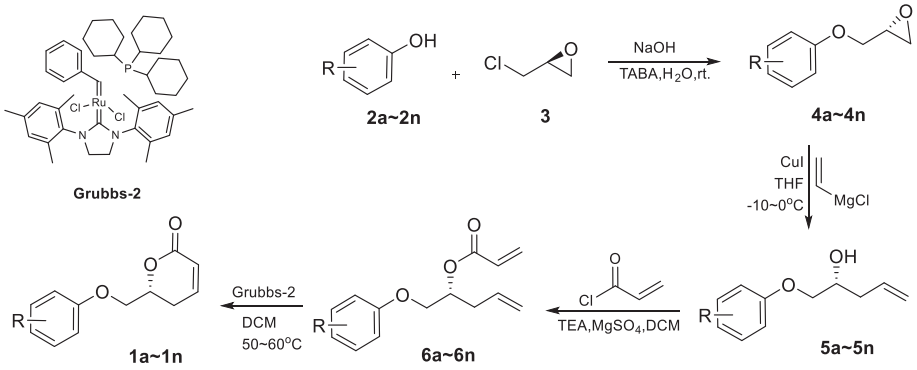
2.2. Biology

The anti-proliferation of all synthesized compounds were evaluated on MGC803 by MTT assay (Table 1). Compounds (**1a~1n)** containing benzyl groups and a two atoms length linker showed moderate anti-proliferation activity on MGC-803 with IC_{50} of 2.3−47.7 μM. The most potent compound **1l** had an IC_{50} of 2.3 μM. Compounds **7a~7e** with aniline or indole groups and compounds **18a~18d** with a linker of 3 atoms length linker led to significantly decreasing of efficacy with IC_{50} > 50 μM. Compounds **19a~19d** did not inhibit the proliferation of MGC803. The SAR data clearly suggested that the benzyl groups and the two atoms length linker were very important for their anti-proliferation on MGC803. The steric hindrance at the terminal of the linker and the more atoms length linkers (compounds **7a-7d** and **18a-18d**) were not beneficial for efficacy. Thus compound **1l** was chosen for further study based on all the above data.

The anti-proliferation activity of compound **1l** (Fig. 1A) was then evaluated on human gastric cancer cell lines. Two gastric cancer cell lines (MGC803 and HGC27) and human gastric epithelial cells lines(GES1) were incubated with it (24 h) at different concentrations, and then the effects of compound **1l** on reducing cell viabilities were assayed by MTT. As shown in Fig. 1B, following treatment with compound **1l**, the viability of the gastric cancer cell lines decreased in a dose dependent manner. MGC803 and HGC27 cells lines showed the sensitivity to compound **1l** in related to control treatment, causing 40−60% viability reduction at 10 μM for 24 h. However, it is almost no toxicity to human gastric epithelial cells (GES1) (Fig. 1B). Taken together, these results suggested that compound **1l** has selective anti-proliferation on gastric cancer cell lines versus normal human gastric epithelial cell lines.

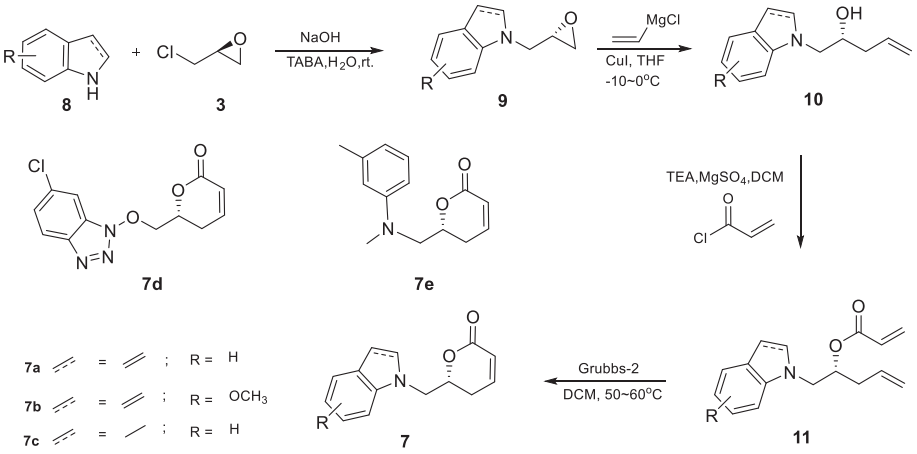
2.2.1. Compound *1l* induced apoptosis by the activation of Bcl-2 family proteins in gastric cancer cell lines

Further experiments were conducted to determine whether the anti-proliferation of compound **1l** on the gastric cancer cell lines were the result of apoptotic cell death. The inducing apoptosis of compound **1l** on MGC803 and HGC27 were evaluated by Annexin V and PI (Propidium Iodide) staining. As shown in Fig. 2A, the numbers of Annexin V positive cells gradually increased in a dose

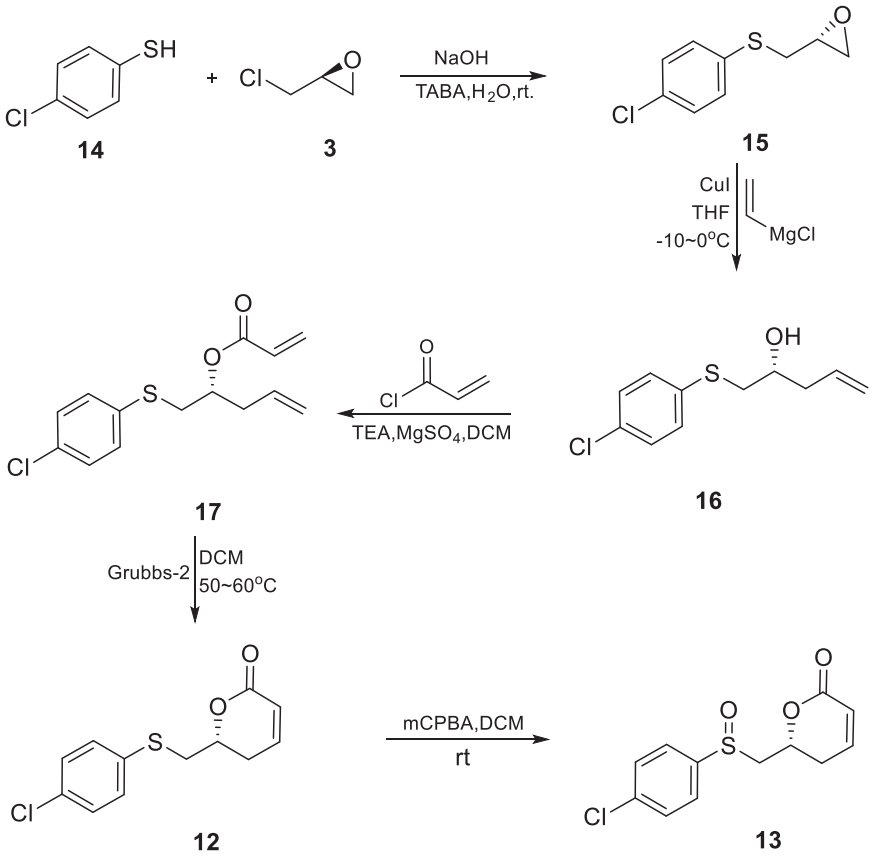


a: R = 2-OEt; **b:** R = 2-OMe; **c:** R = 2-Bn; **d:** R = 4-SMe; **e:** R = 4-Et ; **f:** R = 4-$CH_2(CH_2)_2CH_3$;
g: R = 4-Cyclohexyl**; h:** R = 4-$CH_3OCH_2CH_2$; **i:** R = 4-CF_3O ; **j:** R = 4-$PhCH_2O$; **k:** R = 3-$(CH_3CH_2)_2N$;
l: R = 2-Isopropyl-5-Me ; **m:** R = [2,3]Ph; **n:** R = [3,4]dioxole .

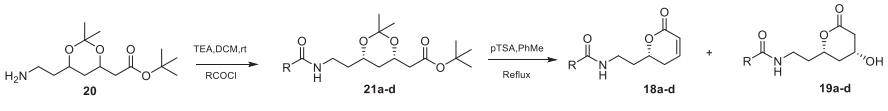
Scheme 1. Synthesis of (*R*)-6-(phenyloxidemethyl)-5, 6-dihydro-*2H*-pyran-2-ones (**1a-1n**).



Scheme 2. Synthesis of (*R*)-6-((1H-indol-1-yl)methyl)-5,6-dihydro-2H-pyran-2-ones (**7a~7d**).



Scheme 3. Synthesis of (*R*)-6-(((4-chlorophenyl)sulfinyl) methyl)-5,6-dihydro-*2H*- pyran-2- ones (**12, 13**).



Comp.	18, 19			
	a	**b**	**c**	**d**
R				

Scheme 4. Synthesis of compounds **18a-d** and **19a-d**.

Table 1
The anti-proliferation of synthesized compounds on MGC803.

Comp.	MGC803/IC_{50}(μM)	Comp.	MGC803/IC_{50}(μM)
1a	10.353 ± 1.015	7a	>50 μM
1b	24.159 ± 1.383	7b	25.620 ± 1.409
1c	22.978 ± 1.36	**7c**	>50 μM
1d	>50	**7d**	>50 μM
1e	12.425 ± 1.094	**7e**	>50 μM
1f	16.950 ± 1.229	**13**	>50 μM
1g	10.593 ± 1.025	**18a**	>50 μM
1h	>50 μM	**18b**	>50 μM
1i	8.489 ± 0.929	**18c**	>50 μM
1j	35.439 ± 1.549	**18d**	>50 μM
1k	>50 μM	**19a**	>50 μM
1l	2.314 ± 0.364	**19b**	>50 μM
1m	47.687 ± 1.678	**19c**	>50 μM
1n	11.976 ± 1.078	**19d**	>50 μM
5-Fu	24.803 ± 1.395		

dependent manner in these two cell lines. The results exhibited that exposure to compound **1l** for 24 h significantly upregulated the levels of p53 and apoptosis-related proteins including Cleaved PARP and Cleaved caspase-3, whereas downregulated CRM1 in MGC803 and HGC27 cells were observed.

In order to confirm the function of mitochondrial in the apoptosis caused by compound **1l**, JC-1 was used to measure MMP (ΔΨ). After treatment with compound **1l** in a dose dependent manner, MMP (ΔΨ) was decreased in MGC803 and HGC27 cell lines comparing with controls, as shown in Fig. 2B. The pro-apoptotic and anti-apoptotic members of the Bcl-2 super family play primary roles in regulating mitochondrial associated apoptosis. After treatment with compound **1l**, the levels of the pro-apoptotic protein Bax was increased and the levels of the anti-apoptosis proteins such as Bcl-2 and Bcl-xL were decreased in MGC803 and HGC27 cell lines comparing with controls (Fig. 2B). In addition, it was observed that compound **1l** increased the expression of cleaved caspase-3 and cleaved caspase-9 in MGC803 and HGC27 cells in comparison with controls. These results indicated that the mitochondrial pathway is an important factor in compound **1l** mediated apoptosis in gastric cancer cells.

*2.2.2. Compound **1l**-mediated CRM1 depletion depends on ubiquitin-proteasome pathway*

The levels of CRM1 protein in MGC803 and HGC27 cell lines with treatment of compound **1l** were analyzed. The results showed that compound **11** decreased CRM1 protein level in a dose dependent manner and the levels of the p53 were increased in MGC803

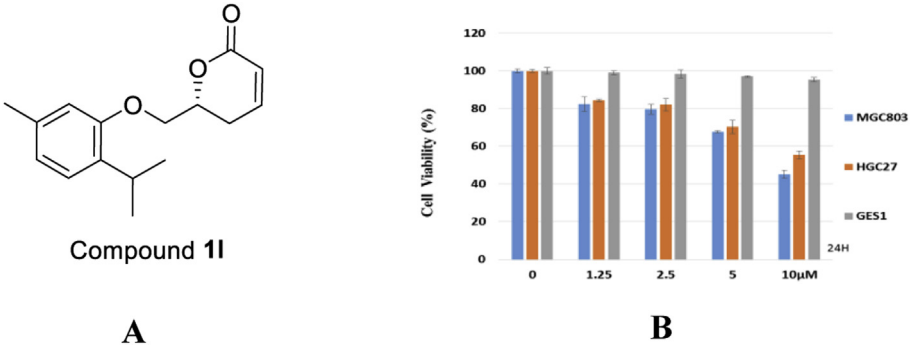


Fig. 1. The structure of compound **1l** and its anti-proliferation activity on MGC803 and HGC27.

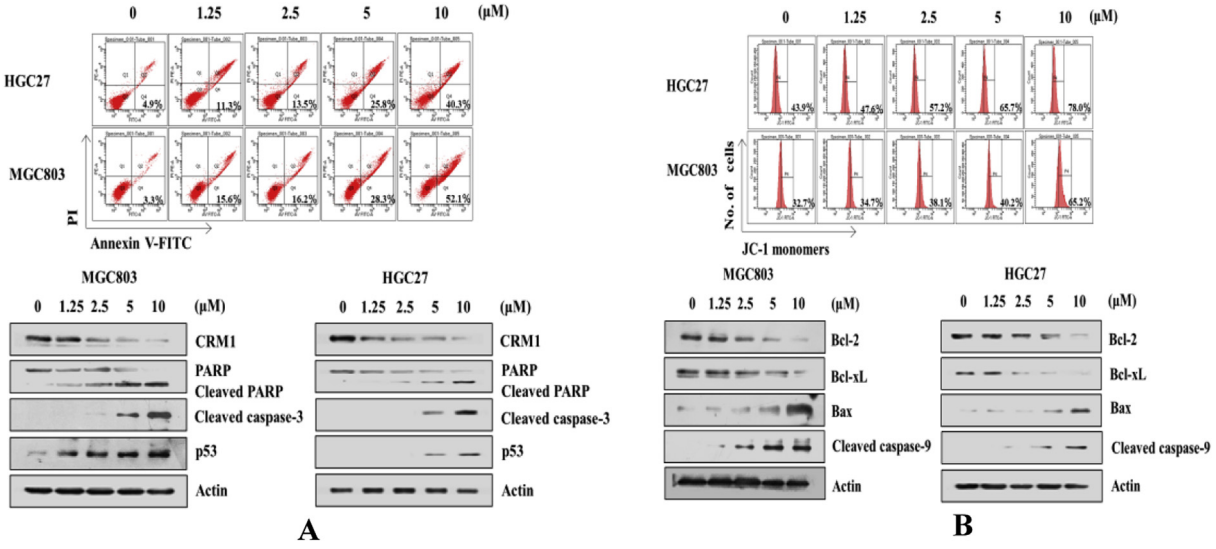


Fig. 2. Apoptosis induced by compound 1l involves Mitochondrial pathway in gastric cancer cell lines.
(A) Cell apoptosis of MGC803 and HGC27 was detected by flow cytometry after treatment by compound **1l** for 24 h. The protein levels of CRM1, PARP, Cleaved PARP, Cleaved caspase-3 and p53 were detected at different concentrations by Western blot in MGC803 cells. (B) MMP (ΔΨ) was decreased by compound **1l** treatment. Membrane potential was measured by JC-1 dye retention using Flow Cytometry. The protein levels of Bcl-2, Bcl-xL, Bax and Cleaved caspase-9 were determined by Western blot after compound **1l** treatment.

and HGC27 cell lines comparing with controls (Fig. 2A), which suggested that compound **1l** was possible a CRM1 degrader. Therefore, we detected whether compound **1l** could regulate the expression of p53 by acting on the CRM1 protein. Immuno precipitation indicated that CRM1 bound with p53 dramatically decreased at 6 h (Fig. 3A). As revealed by immunofluorescence staining, notable nuclear accumulation of Ranbp1 and p53 occurred at 6 h after compound **1l** treatment. After exposure to compound **1l** at 6 h, the accumulation of p53 in the nucleus increased, whereas the cytoplasmic level of Ranbp1 and CRM1 decreased, as shown in Fig. 3A. In addition, the utilization of p53 siRNA with MGC803 cell lines, the cell death led by compound **1l** were decreased evidently, as shown in Fig. 3B. The results showed that p53 is required for compound **1l** mediated apoptosis.

The degradation of CRM1 proceeds by the ubiquitin-proteasome pathway. To investigate this, MG132, a proteasome inhibitor, was used to block the CRM1 degradation led by compound **1l**. MGC803 and HGC27 cells were treated with compound **1l** (5 μM) for 24h in the presence or absence of MG132(0.05 μM). Reduced CRM1 protein levels were observed in compound **1l**-treated cell lines. In contrast, in the presence of MG132, depletion of CRM1 protein by compound **1l** was almost completely abolished (Fig. 3C). Similar results were obtained with the use of bortezomib, another proteasome inhibitor. These data demonstrated that compound **1l** mediated CRM1 depletion depends on ubiquitin-proteasome pathway.

*2.2.3. Compound **1l** synergize with MG132 to increase the apoptosis of MGC803 and HGC27 cell lines*

Some of CRM1 inhibitors have been investigated in clinical trials, while they have not demonstrated adequate potency in the clinical settings as a single agent and they are more potency in combination with proteasome inhibitor such as bortezomib [16]. In Fig. 4A, pretreatment with MG132 significantly increased apoptosis in MGC803 and HGC27 cells, whereas treatment with compound **1l** or MG132 alone decreased the activity of gastric cancer cells only slightly or not at all. In addition, the data showed that pretreatment with MG132 increased expression of Cleaved PARP, Cleaved caspase-3, p53 and Bax in two gastric cancer cells (Fig. 4A). To investigate the role of p53 in MG132 induced enhancement of compound **1l** mediated apoptosis. Fig. 4B revealed that p53 translocated in cytoplasm and bound with anti-apoptotic Bcl family proteins including Bcl-2, Bcl-xl. These data indicated that, combination of compound **1l** with MG132 increased apoptosis via accumulate p53 in the cytoplasmic, p53 translocated in cytoplasm and bound with Bcl-2 and Bcl-xl, which increased Bax expression and induced apoptosis, despite degradation of CRM1 protein by compound **1l** was completely blocked.

3. Discussion

Gastric cancer is one of highly aggressive malignant tumor, while impactful chemotherapies for it are limited [21]. Therefore, developing novel therapeutic drugs is an attractive area of gastric cancer research. In this study, we examined the cytotoxic effects of a novel compound **1l** in gastric cancer cells. Compound **1l** exhibited strong cytotoxic effect against gastric cancer cell lines with low toxic in Human Gastric Mucosal Epithelial Cell Line. Furthermore, we discovered that p53 is required for compound **1l** mediated apoptosis.

In recent years, CRM1 over expression has been detected in gastric cancer and caused dysfunction of cell fate controls [22]. A number of studies have shown that targeting CRM1 as promising therapeutic targets for cancer research. Our results support this suppose because we found that compound **1l** showed antitumor effects in gastric cancer cell lines *in vitro*. It was reported that CRM1 inhibitor KPT-185 induced apoptosis in ovarian and breast cancer cell lines [23]. However, the mechanism of anti-gastric cancer activity of CRM1 inhibitor remain unknown. Our study showed that compound **1l** suppresses proliferation and induces apoptosis in MGC803 and HGC27 cell lines in a dose dependent manner through mitochondrial pathway.

Up to present, CRM1 mediates the transport of more than 230 proteins, many of which are tumor suppressors [24]. Therefore, the inhibition of CRM1 function induced the accumulation of tumor suppressor proteins in the nucleus and reduced cytoplasmic

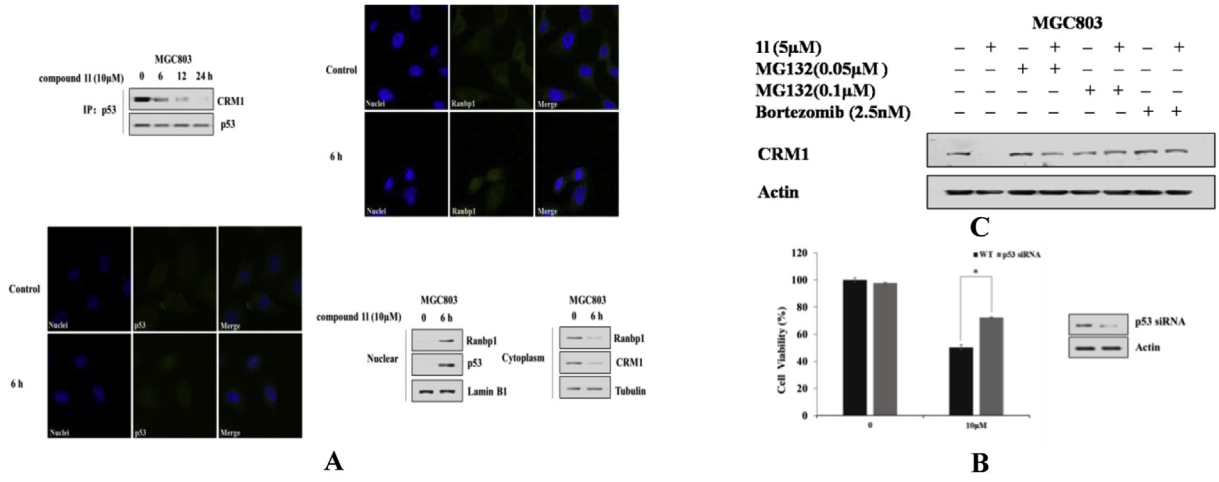


Fig. 3. Mitochondrial p53 pathway is involved in the inducing apoptosis of compound 1l in MGC803 cells and compound 1l induces CRM1 protein degradation via the ubiquitin-proteasome pathway.
(A)CRM1 bound with p53 detected by immunoprecipitation assay in MGC803 cell lines after treatment with compound **1l** at 10 μM at various time points. Accumulation of Ranbp1 or p53 in nucleus was observed in MGC803 cells with treatment of compound **1l** at 10 μM for 6 h. The treated and untreated MGC803 cells with compound **1l** at 10 μM for 0 and 6 h are stained with Ranbp1 antibody (Green) and DAPI (Blue) and analyzed by confocal microscopy. Westernblot analysis for expression of Ranbp1, p53 and CRM1 in the nucleus and cytoplasm. (B) MTT assay detected the effect of siRNA targeting to p53 on the survival rate of MGC803 cells, *P < 0.05 vs control group. (C) MGC803 cells were treated with compound **1l** (10 μM) in the present or absent of MG132 (0.05 μM), MG132 (0.1 μM) and bortezomib (2.5 nM) for 24 h. Westernblot was used to detect the expression levels of CRM1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

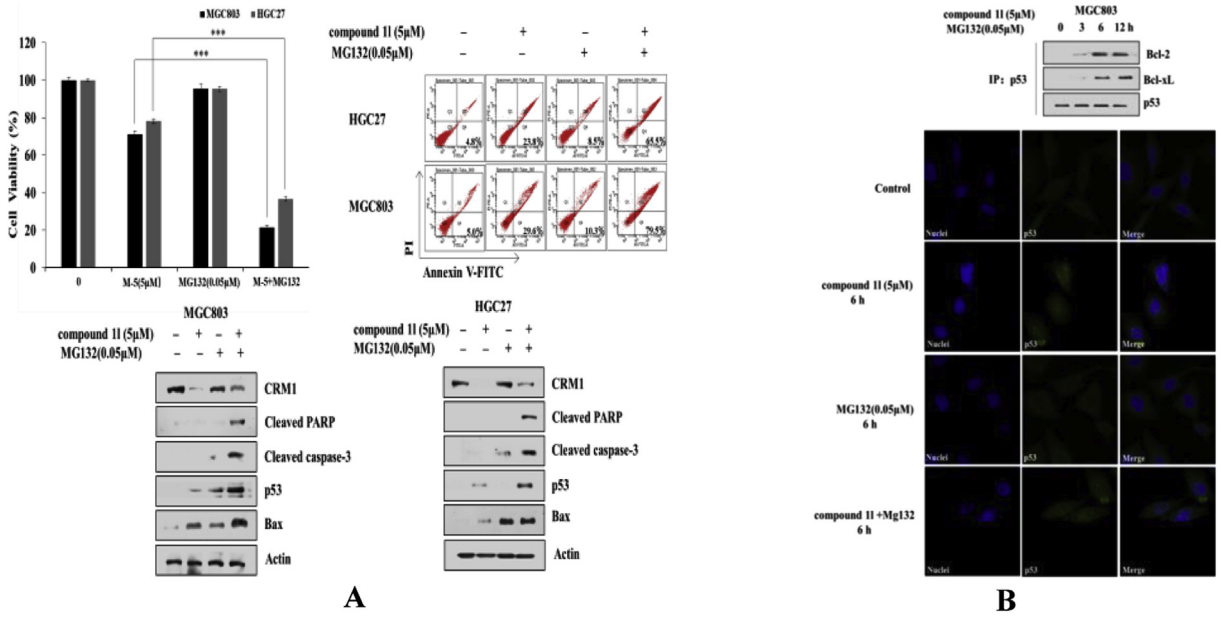


Fig. 4. Compound 1l work synergistically with MG132increased apoptosis
(A) MGC803 and HGC27 cells were pretreated with/without 0.05 μM of MG132 for 1 h before exposure to 10 μM of compound **1l** for 24 h. Cell viability was determined by MTT assays. ***P < 0.01 vs. untreated group.MGC803 and HGC27 cells were pretreated with/without 0.05 μM of MG132 for 1 h before exposure to 10 μM of compound **1l** for 24 h. Cells apoptosis of MGC803 and HGC27 was detected by flow cytometry. Western blot assay was used to detect the expression levels of CRM1. Cleaved PARP, Cleaved caspase-3, p53 and Bax after pretreatment with the MG132 (0.05 μM) in MGC803 and HGC27 cells for 1 h, followed by 24 h of compound **1l**(10 μM) incubation. (B) MGC803 cells were pretreated with 0.05 μM of MG132 for 1 h before exposure to 10 μM of compound **1l** for 24 h. The indicated proteins were detected by immunoprecipitation with an antibody for p53.MGC803 cells were pretreated with/without 0.05 μM of MG132 for 1 h before exposure to 10 μM of compound **1l** for 6 h p53 in the nucleus or cytoplasm was detected by Immunofluorescence analysis. The treated and untreated samples are stained with p53antibody (Green) and DAPI (Blue) and analyzed by confocal microscopy. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

degradation of these proteins, has been thought to be as significant mechanism of the anti-gastric cancer activity of CRM1 inhibitor. In our study, the data suggest that the antitumor effects of compound **1l** is associate with tumor suppressor proteins p53. We observed that the expression levels of CRM1 was decreased and p53 accumulated within nuclear after compound **1l** treatment. In addition, knockdown of p53 by specific siRNA in MGC803 cells, compound **1l** induced mitochondrial apoptotic pathway was decreased evidently.

The ubiquitin-proteasome system (UPS) and autophagy are major intracellular protein degradation systems [25]. The UPS pathway is essential to the cell cycle, transcription and cell survival. Numerous studies have revealed that blockage of proteasome mediated protein degradative pathway stabilized the tumor suppressor p53, and the proapoptotic proteins Bax [16,26]. MG132, a26S proteasome inhibitor, whereas inhibition of the 26S proteasome lead to accumulation of non-degraded ubiquitinated proteins in the cytoplasmic. In our study, we found that co-incubated with the proteasome inhibitor MG132 or bortezomib, degradation of CRM1 protein by compound **1l** was almost completely blocked. In addition, we observed that compound **1l** treatment in combination with MG132 exhibited enhanced expression of p53, Cleaved PARP, Cleaved caspase-3 and Bax and induced higher levels of apoptosis in two gastric cancer cells when compared to cells treated with compound **1l** or inhibitors alone. p53 transcription-independent apoptosis is initiated by interacts with anti-apoptotic Bcl family proteins including Bcl-2, Bcl-xl, induced cell apoptosis [27]. In this study, we found that p53 translocated in cytoplasm and bound with Bcl-2 and Bcl-xl. Pretreatment with MG132, these results indicated that combined treatment significantly increased apoptosis via accumulate p53 in the cytoplasmic, despite degradation of CRM1 protein by compound **1l** was completely blocked.

A critical component of any degrader/drug is permeability. Passive diffusion permeability is the most important permeability for small molecules. Cell permeability of a drug depends on its molecular weight, polar surface area and clogP. The permeability of the lipophilic molecules is better than that of polar molecules. Neutral molecules are much more permeable than their charged forms. Compound 1l is a neutral and lipophilic molecule with clogP of 3.32 and polar surface area of 35.5. Its molecular weight is 260.4. The small molecule structure and the reasonable clogP suggest that compound **1l** has a good permeability possibly.

In summary, the results in this work show that compound **1l** is a novel CRM1 degrader that has major cytotoxicity in gastric cancer cells and have little toxicity in human gastric epithelial cells. **It** is a potential pro-apoptotic agent for prevention and treatment of gastric cancer.

4. Experimental section

4.1. General information

Reagents and solvents were purchased from Bide Pharmatech Ltd, Aladdin, Sinopharm Chemical Reagent Co, Ltd. with purities of at least 97%. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectrawere recorded with a Bruker spectrometer. All reactions were monitored by thin-layer chromatography (TLC) on 25.4 mm × 76.2 mm silica gel plates (GF-254) and UPLC-Mass on Waters ACQUITY UPLC H-Class or Q-Tof Micro HRMS on waters. Melting points were determined on a Beijing Keyi XT4A apparatus. The silica gel used for column chromatography was 200300 mesh or recrystallization with solvents specified in the corresponding experiments.

4.2. General synthetic procedure for the compounds

4.2.1. General synthesis of (R)-6-((2-ethoxyphenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1a)

As a sample Grubbs-2 (188 mg, 0.221 mmol) was dissolved in 20 mL anhydrous DCM in an oven dried flask. Subsequently, compound **6a**(612 mg, 2.21 mmol) was dissolved in 2 mL DCM and added dropwise under an nitrogen atmosphere. The reaction was stirred for 15 h at 50 °C–60 °C. The crude product was purified by chromatography on silica gel with petroleumether/ethylacetate (10:1) to afford **1a** as a white solid (173 mg, 31.46%).^{1}H NMR (400 MHz, $CDCl_3$)δ 7.06–6.84 (m, 5H), 6.14–6.04(m, 1H), 4.95–4.75(m, 1H), 4.30 (dd, J = 10.2, 4.5 Hz, 1H), 4.21 (dd, J = 10.2, 6.2 Hz, 1H), 4.19–4.00 (m, 2H), 2.79–2.58 (m, 2H), 1.45(t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$)δ 163.48, 148.11, 144.86, 122.71, 121.34, 121.09, 116.01, 113.97, 75.69, 70.53, 64.49, 26.41, 14.96.

4.2.2. Synthesis of (R)-6-((2-methoxyphenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1b)

Compound **6b** (520 mg, 1.98 mmol) was converted to **1b**(145 mg, 31.22%) as a white solid by the same procedure as described for compound **1a**. m.p. 90.2–91.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06–6.84 (m, 5H), 6.10–6.04 (m, 1H), 4.88–4.80 (m, 1H), 4.28 (dd, J = 10.2, 4.6 Hz, 1H), 4.19 (dd, J = 10.2, 6.2 Hz, 1H), 3.85 (s, 3H), 2.75–2.53 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.42, 149.95, 147.78, 144.81, 122.48, 121.36, 121.00, 115.03, 112.31, 75.62, 70.19, 55.93, 26.45.HRMS: calcd for $C_{13}H_{14}NaO_4$ $[M+Na]^+$ m/z: 257.0790, found: 257.0792.

4.2.3. Synthesis of (R)-6-((2-benzylphenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1c)

Compound **6c** (550 mg, 1.71 mmol) was converted to **1c**(220 mg, 43.81%) as a white solid by the same procedure as described for compound **1a**. m.p. 86.5–87.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27–7.21 m, 4H), 7.21–7.13 (m, 3H), 7.12–7.04 (m, 1H), 7.00 (d, J = 7.9 Hz, 1H), 6.91 (t, J = 7.4 Hz, 1H), 6.02–5.97 (m, 1H), 4.85–4.77 (m, 1H), 4.25–4.14 (m, 2H), 3.91 (s, 2H), 2.50–2.44 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 163.26, 155.68, 146.80, 140.85, 130.28, 129.45, 128.65, 128.16, 127.58, 125.71, 120.82, 119.95, 111.85, 75.63, 68.57, 35.45, 25.10. HRMS: calcd for $C_{19}H_{18}NaO_3$ $[M+Na]^+$ m/z: 317.1154, found: 317.1153.

4.2.4. Synthesis of (R)-6-((4-(methylthio)phenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1d)

Compound **6d** (500 mg, 1.80 mmol) was converted to **1d**(200 mg, 44.32%) as a gray solid by the same procedure as described for compound **1a**. m.p. 100.5–101.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 2H), 7.02–6.91 (m, 1H), 6.86 (d, J = 7.6 Hz, 2H), 6.08 (d, J = 9.6 Hz, 1H), 4.84–4.74 (m, 1H), 4.22–4.08 (m, 2H), 2.77–2.50 (m, 2H), 2.45 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.27, 156.63, 144.61, 129.99,129.89, 121.38, 115.30, 75.47, 68.72, 26.24, 17.77.HRMS: calcd for $C_{13}H_{14}NaO_3S$ $[M+Na]^+$$m/z$: 273.0561, found: 273.0561.

4.2.5. Synthesis of (R)-6-((4-ethylphenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1e)

Compound ***6e*** (560 mg, 2.15 mmol) was converted to **1e**(213 mg, 42.63%) as a white solid by the same procedure as described for compound **1a**. m.p. 65.1–66.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, J = 8.5 Hz, 2H), 6.98–6.90 (m, 1H), 6.84 (d, J = 8.5 Hz, 2H), 6.07 (dd, J = 9.8, 1.7 Hz, 1H), 4.83–4.73 (m, 1H), 4.19 (dd, J = 10.0, 4.5 Hz, 1H), 4.13 (dd, J = 10.0, 5.6 Hz, 1H), 2.71–2.59 (m, 2H), 2.59–2.49 (m, 2H), 1.21 (t, J = 7.6 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.43, 156.21, 144.81, 137.30, 128.85, 121.30, 114.47, 75.60, 68.62, 27.99, 26.29, 15.87.HRMS: calcd for $C_{14}H_{16}NaO_3$ $[M+Na]^+$$m/z$: 255.0997, found:255.0998.

4.2.6. Synthesis of (R)-6-((4-pentylphenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1f)

Compound ***6f*** (550 mg, 1.82 mmol) was converted to **1f**(246 mg, 49.30%) as a white solid by the same procedure as described for compound **1a**. m.p. 68.0–69.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, J = 8.5 Hz, 2H), 6.98–6.92 (m, 1H), 6.83 (d, J = 8.6 Hz, 2H), 6.14–6.02 (m, 1H), 4.85–4.75 (m, 1H), 4.19 (dd, J = 10.0, 4.5 Hz, 1H), 4.13 (dd, J = 10.0, 5.7 Hz, 1H), 2.72–2.50 (m, 4H), 1.62–1.56 (m, 2H), 1.39–1.24 (m, 4H), 0.89 (t, J = 6.9 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 156.15, 144.75, 135.97, 129.37, 121.32, 114.33, 77.33, 75.56, 68.55, 35.00, 31.43, 31.38, 26.31, 22.54, 14.05.HRMS: calcd for $C_{17}H_{22}NaO_3$ $[M+Na]^+$$m/z$: 297.1467, found: 297.1467.

4.2.7. Synthesis of (R)-6-((4-cyclohexylphenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1g)

Compound ***6g***(470 mg, 1.49 mmol) was converted to **1g**(196 mg, 45.79%) as a white solid by the same procedure as described for compound **1a**. m.p. 85.5–86.3 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13(d, 7.5Hz, 2H), 6.98–6.89(m, 1H), 6.84 (d, J = 8.0 Hz, 2H), 6.07 (d, 9.7Hz, 1H), 4.85–4.75 (m, 1H), 4.25–4.07 (m, 2H), 4.13 (dd, J = 10.0, 5.7 Hz, 1H), 2.72–2.50 (m, 1H), 2.50–2.36 (m, 1H), 1.93–1.79 (m, 4H), 1.73 (d, J = 13.0 Hz, 1H), 1.46–1.30 (m, 4H), 1.30–1.15 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.37, 156.22, 144.71, 141.28, 127.76, 121.34, 114.36, 75.55, 68.56, 43.70, 34.68, 26.91, 26.33, 26.14. HRMS: calcd for $C_{18}H_{22}NaO_3$ $[M+Na]^+$ m/z: 309.1467, found: 309.1466.

4.2.8. Synthesis of (R)-6-((4-(2-methoxyethyl)phenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1h)

Compound **6h**(556 mg, 1.91 mmol) was converted to**1h**(223 mg, 44.40%) as a white solid by the same procedure as described for compound **1a**. m.p. 47.3–48.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 8.5 Hz, 2H), 7.03–6.90 (m, 1H), 6.84 (d, J = 8.6 Hz, 2H), 6.06 (dd, J = 9.8, 1.7 Hz, 1H), 4.84–4.74(m, 1H), 4.18 (dd, J = 10.0, 4.6 Hz, 1H), 4.13 (dd, J = 10.0, 5.6 Hz, 1H), 3.56 (t, J = 7.0 Hz, 2H), 3.35 (s, 3H), 2.82 (t, J = 7.0 Hz, 2H), 2.70–2.59 (m, 1H), 2.58–2.48 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.40, 156.62, 144.83, 131.91, 129.86, 121.22, 114.48, 75.56, 73.72, 68.53, 58.64, 35.26, 26.21.HRMS: calcd for$C_{15}H_{18}NaO_4$ $[M+Na]^+$$m/z$: 285.1103, found: 285.1104.

4.2.9. Synthesis of (R)-6-((4-(trifluoromethoxy)phenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1i)

Compound **6i**(570 mg, 1.80 mmol) was converted to **1i**(250 mg, 48.13%) as a white solid by the same procedure as described for compound **1a**. m.p. 71.1–72.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, J = 8.7 Hz, 2H), 7.01–6.95 (m, 1H), 6.95–6.90 (m, 2H), 6.11 (dd, J = 9.4, 2.1 Hz, 1H), 4.88–4.78 (m, 1H), 4.26–4.13 (m, 2H), 2.75–2.64 (m, 1H), 2.63–2.50 (m, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 163.22, 156.63, 144.59, 143.26, 122.57, 121.37, 119.24, 115.41, 75.39, 68.96, 26.15.HRMS: calcd for$C_{13}H_{11}F_3NaO_4$ $[M+Na]^+$$m/z$: 311.0507, found: 311.0506.

4.2.10. Synthesis of (R)-6-((4-(benzyloxy)phenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1j)

Compound **6j** (530 mg, 1.57 mmol) was converted to**1j**(210 mg, 43.20%) as a white solid by the same procedure as described for compound **1a**. m.p.146.0–147.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.28 (m, 5H), 6.98–6.93 (m, 1H), 6.92–6.88 (m, 2H), 6.88–6.82 (m, 2H), 6.11–6.04 (m, 1H), 5.02 (s, 2H), 4.83–4.73 (m, 1H), 4.16 (dd, J = 10.0, 4.5 Hz, 1H), 4.11 (dd, J = 10.0, 5.6 Hz, 1H), 2.71–2.59 (m, 1H), 2.58–2.48(m, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.42, 153.53, 152.53, 144.74, 137.14, 128.57, 127.94, 127.47, 121.34, 115.91, 115.64, 75.62, 70.66, 69.27, 29.70.HRMS: calcd for-$C_{19}H_{18}NaO_4$ $[M+Na]^+$ m/z: 333.1103, found: 333.1102.

4.2.11. Synthesis of **(R)-6-((3-(diethylamino)phenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1k)**

Compound **6k** (360 mg, 1.19 mmol) was converted to **1k**(150 mg, 45.91%) as a white solid by the same procedure as described for compound **1a**. m.p. 50.2–51.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (t, J = 8.2 Hz, 1H), 6.98–6.91 (m, 1H), 6.34 (dd, J = 8.4, 1.8 Hz, 1H), 6.26–6.15 (m, 2H), 6.11–6.04 (m, 1H), 4.85–4.75 (m, 1H), 4.21 (dd, J = 10.0, 4.5 Hz, 1H), 4.13 (dd, J = 10.0, 6.0 Hz, 1H), 3.33 (q, J = 7.1 Hz, 4H), 2.71–2.50 (m, 2H), 1.15 (t, J = 7.1 Hz, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.46, 159.56, 144.79, 130.01, 121.35, 105.72, 100.50, 98.87, 75.63, 68.30, 44.36, 29.70, 26.41, 12.58.

4.2.12. Synthesis of **(R)-6-((2-isopropyl-5-methylphenoxy)methyl)-5,6-dihydro-2H-pyran-2-one (1l)**

Compound **6l** (460 mg, 1.60 mmol) was converted to **1l**(186 mg, 44.79%) as a colorless oily liquid by the same procedure as described for compound **1a**.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, J = 7.7 Hz, 1H), 7.05–6.91 (m, 1H), 6.79 (d, J = 7.7 Hz, 1H), 6.65 (s, 1H), 6.09 (d, J = 11.4 Hz, 1H), 4.90–4.80 (m, 1H), 4.21 (dd, J = 9.9, 4.3 Hz, 1H), 4.16 (dd, J = 9.9, 5.7 Hz, 1H), 3.30–3.20 (m, 1H), 2.76–2.50 (m, 2H), 2.32 (s, 3H), 1.19 (d, J = 6.9 Hz, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.47, 155.02, 144.76, 136.49, 134.06, 126.06, 121.93, 121.33, 112.14, 75.63, 68.51, 26.54, 26.43, 22.76, 22.73, 21.31.HRMS: calcd for$C_{16}H_{20}NaO_3$ $[M+Na]^+$ m/z: 283.1310, found: 283.1311.

4.2.13. Synthesis of **(R)-6-((naphthalen-1-yloxy)methyl)-5,6-dihydro-2H-pyran-2-one (1 m)**

Compound **6m** (480 mg, 1.70 mmol) was converted to **1m**(153 mg, 35.39%) as a white solid by the same procedure as described for compound **1a**. m.p. 87.0–88.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33–8.10 (m, 1H), 7.93–7.87 (m, 1H), 7.61–7.48 (m, 3H), 7.44 (t, J = 7.9 Hz, 1H), 7.20–7.13 (m, 1H), 7.03 (d, J = 7.5 Hz, 1H), 6.08–6.02 (m, 1H), 5.05–4.95 (m, 1H), 4.46–4.34 (m, 2H), 2.78–2.60 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 163.36, 153.53, 146.95, 133.99, 127.47, 126.53, 126.14, 125.43, 124.80, 121.48, 120.39, 120.03, 105.45, 75.68, 68.90, 25.30.HRMS: calcd for $C_{16}H_{14}NaO_3$ $[M+Na]^+$ m/z: 277.0841, found: 277.0842.

4.2.14. Synthesis of **(R)-6-((benzo[d] [1,3]dioxol-5-yloxy)methyl)-5,6-dihydro-2H-pyran-2-one (1n)**

Compound **6n** (530 mg, 1.92 mmol) was converted to **1n**(198 mg, 41.58%) as a white solid by the same procedure as described for compound **1a**. m.p. 96.5–97.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00–6.90 (m, 1H), 6.71 (d, J = 8.4 Hz, 1H), 6.50 (d, J = 2.5 Hz, 1H), 6.33 (dd, J = 8.5, 2.5 Hz, 1H), 6.10–6.06 (m, 1H), 5.93 (s, 2H), 4.83–4.72 (m, 1H),4.13 (dd, J = 10.0, 4.6 Hz, 1H), 4.08 (dd, J = 10.0, 5.4 Hz, 1H), 2.70–2.46 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.36, 153.66, 148.35, 144.68, 142.25, 121.33, 107.95, 105.79, 101.28, 98.34, 75.53, 69.59, 26.22.HRMS: calcd for$C_{13}H_{12}NaO_5$ $[M+Na]^+$$m/z$: 271.0582, found: 271.0582.

4.3. General synthesis procedure for **analogues of (R)-2-(phenoxymethyl)oxirane** (**4a~4n**)

Phenol derivatives **2a~2n** (1 equvi) and NaOH (3 equvi) was dissolved in 5 mL H_2O in a flask.Subsequently, The reaction mixture was stirred at room temperature for 30min. The reaction system was added dropwise to a 50 mL round bottom flask containing (s)-epichlorohydrin (3 equvi) and tetrabutylammonium bromide (0.05 equvi). The reaction mixture was stirred at room temperature. After the reaction is completed, The resulting mixture was diluted with water (10 mL) and extracted with **ethylacetate** (20 mL × 3).The combined organic phases were dried with Na_2SO_4, concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether andethylacetate to afford**4a~4n.**

4.4. General synthesis procedure for analogues of (R)-1-phenoxypent-4-en-2-ol (5a~5n)

(R)-2-(phenoxymethyl)oxirane derivatives 4a~4n (1 equvi) and CuI (0.2 equvi) was dissolved in 20 mL anhydrous THF in a flask. The reaction mixture was stirred at-10–0 °C for 10 min and ethylene magnesium chloride (1.5 equvi) was added dropwise under an argon atmosphere. After the reaction is completed, The resulting mixture was diluted with saturated ammonium chloride (50 mL) and the system was suction filtered with Celite, and then evaporated and extracted with ethylacetate (15 mL × 3).The combined organic phases were dried with Na_2SO_4, concentrated to afford a crude product.The crude product was purified by chromatography on silica gel with petroleum ether andethylacetate to afford 5a~5n.

4.5. General synthesis procedure for analogues of (R)-1-phenoxypent-4-en-2-yl acrylate (6a~6n)

A solution of 5a~5n(1 equvi), anhydrous$MgSO_4$ (2 equvi), and TEA (1.5 equvi) in Anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature. Acryloylchloride (1.5 equvi) was added dropwise. The reactionmixture was stirred atrt for 2h. After the reaction is completed, The resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3), dried ($MgSO_4$), and concentrated to afford acrude product. The crude product was purified by chromatography onsilica gel with petroleum ether/ethylacetate to afford 6a~6n.

4.6. General synthesis of **(R)-6-((1H-indol-1-yl)methyl)-5,6-dihydro-2H-pyran-2-one (7a)** *as a sample*

Grubbs-2 (126 mg, 0.219 mmol) was dissolved in 20 mL anhydrous DCM in an oven dried flask. Subsequently, compound 11a(380 mg, 1.49 mmol) was dissolved in 2 mL DCM and added dropwise under an nitrogen atmosphere. The reaction was stirred for 15 h at 50 °C–60 °C. The crude product was purified by chromatography on silica gel with petroleumether/ethylacetate (10:1) to afford **7a** as a colorless oily liquid(143 mg, 42.28%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.26–7.19 (m, 1H), 7.19–7.04 (m, 2H), 6.89–6.66 (m, 1H), 6.54 (d, J = 2.5 Hz, 1H), 5.99 (d, J = 9.7 Hz, 1H), 4.90–4.68 (m, 1H), 4.46 (dd, J = 15.1, 4.8 Hz, 1H), 4.39 (dd, J = 15.0, 5.5 Hz, 1H), 2.36–2.17 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.17 (s), 144.32, 136.28 128.66, 128.57, 122.02, 121.30, 121.22, 119.83, 109.06, 102.46, 76.35, 48.99, 26.88.

4.6.1. Synthesis of **(R)-6-((5-methoxy-1H-indol-1-yl)methyl)-5,6-dihydro-2H-pyran-2-one (7b)**

Compound **11b**(430 mg, 1.51 mmol) was converted to **7b**(176 mg, 45.39%) as a paleyellow solid by the same procedure as described for compound **7a**.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, J = 8.9 Hz, 1H), 7.14 (d, J = 3.1 Hz, 1H), 7.09 (d, J = 2.4 Hz, 1H), 6.89 (dd, J = 8.9, 2.4 Hz, 1H), 6.84–6.73 (m, 1H), 6.46 (d, J = 3.1 Hz, 1H), 6.05–5.95 (m, 1H), 4.85–4.70 (m, 1H), 4.43 (dd, J = 15.1, 4.9 Hz, 1H), 4.37 (dd, J = 15.1, 5.6 Hz, 1H), 3.85 (s, 3H), 2.30–2.19 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.16, 154.27, 144.28, 131.56, 129.09, 121.32, 112.33, 109.82, 102.83, 102.03, 76.37, 55.86, 49.20, 26.86.HRMS: calcd for$C_{15}H_{15}NNaO_3$ $[M+Na]^+$$m/z$: 280.0950, found: 280.0951.

4.6.2. Synthesis of ***(R)-6-(indolin-1-ylmethyl)-5,6-dihydro-2H-pyran-2-one (7c)***

Compound **11c** (500 mg, 1.94 mmol) was converted to **7c**(210 mg, 47.14%) as a colorless oily liquidby the same procedure as described for compound **7a**.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (dd, J = 13.3, 7.1 Hz, 2H), 6.98–6.85 (m, 1H), 6.68 (t, J = 7.3 Hz, 1H), 6.44 (d, J = 7.8 Hz, 1H), 6.05 (dd, J = 10.1, 2.0 Hz, 1H), 4.78–4.60 (m, 1H), 3.59–3.46 (m, 2H), 3.38 (d, J = 5.2 Hz, 2H), 3.00 (t, J = 8.4 Hz, 2H), 2.64–2.39 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.84, 152.14, 144.95, 129.48, 127.35, 124.65, 121.29, 117.98, 106.27, 55.13, 53.38, 29.69, 28.77, 27.25.HRMS: calcd for$C_{14}H_{15}NNaO_2$ $[M+Na]^+m/z$: 252.1000, found: 252.1003.

4.6.3. Synthesis of ***(R)-6-(((6-chloro-1H-benzo[d] [1–3]triazol-1-yl)oxy)methyl) -5,6-dihydro-2H-pyran-2- one (7d)***

Compound **11d**(450 mg, 1.46 mmol) was converted to **7d**(55 mg, 13.45%) as a gray-green solid by the same procedure as described for compound **7a**.m.p. 109.5–110.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, 8.8Hz, 1H), 7.72 (d, J = 1.3 Hz, 1H), 7.43–7.35 (m, 1H), 7.07–6.92 (m, 1H), 6.13 (dd, J = 9.8, 1.9 Hz, 1H), 4.95–4.84 (m, 1H), 4.81–4.68 (m, 2H), 2.80–2.65 (m, 1H), 2.65–2.50 (m, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 162.47, 144.04, 141.94, 135.22, 127.76, 126.40, 121.54, 121.26, 108.64, 80.29, 73.96, 25.49.HRMS: calcd for-$C_{12}H_{10}ClN_3NaO_3$ $[M+Na]^+m/z$: 302.0308, found: 302.0308.

4.6.4. Synthesis of ***(R)-6-((methyl(m-tolyl)amino)methyl)-5,6-dihydro-2H-pyran-2-one (7e)***

Compound **11e** (500 mg, 1.93 mmol) was converted to **7e**(210 mg, 47.09%) as a yellow oily liquidby the same procedure as described for compound **7a**.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19–7.06 (m, 1H), 6.97–6.80 (m, 1H), 6.57 (d, J = 7.4 Hz, 1H), 6.52 (d, J = 6.2 Hz, 2H), 6.11–5.90 (m, 1H), 4.76–4.60 (m, 1H), 3.68 (dd, J = 15.4, 5.8 Hz, 1H), 3.60 (dd, J = 15.4, 5.8 Hz, 1H), 3.04 (s, 3H), 2.46–2.37 (m, 2H), 2.32 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.79, 148.86, 144.68, 139.12, 129.21, 121.47, 117.96, 112.89, 109.36, 76.15, 55.96, 39.96, 27.45, 21.95.HRMS: calcd for$C_{14}H_{17}NNaO_2$ $[M+Na]^+m/z$:254.1157, found: 254.1156.

4.7. General synthesis procedure for analogues of (S)-1-(oxiran-2-ylmethyl)-1H-indole (9)

Indole analogues 8 (1 equvi) and NaH (3equvi) was dissolved in 20 mL MeCN at ice-bathin a flask.Subsequently, The reaction mixture was stirred at room temperature for 30min. (S)-epichlorohydrin (3 equvi) was added dropwise. The reaction mixture was stirred at room temperature. After the reaction is completed, The resulting mixture was diluted with water (10 mL) and evaporated, and then extracted with ethylacetate (20 mL × 3).The combined organic phases were dried with Na_2SO_4, concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether/acetone to afford 9.

4.8. General synthesis procedure for analogues of (R)-1-(1H-indol-1-yl)pent-4-en-2-ol (10)

Compounds 9 (1 equvi) and CuI (0.2 equvi) was dissolved in 20 mL anhydrous THF in a flask. The reaction mixture was stirred at-10–0 °C for 10 min and ethylene magnesium chloride (1.5 equvi) was added dropwise under an argon atmosphere. After the reaction is completed, The resulting mixture was diluted with saturated ammonium chloride (50 mL) and the system was suction filtered with Celite, and then evaporated and extracted with ethylacetate (15 mL × 3).The combined organic phases were dried with Na_2SO_4, concentrated to afford a crude product.The crude product was purified by chromatography on silica gel with petroleum ether andethylacetate to afford 10.

4.9. General synthesis procedure for analogues of (R)-1-(1H-indol-1-yl)pent-4-en-2-yl acrylate (11)

A solution of 10(1 equvi), anhydrous$MgSO_4$ (2 equvi), and TEA (1.5 equvi) in Anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature. Acryloylchloride (1.5 equvi) was added dropwise. The reactionmixture was stirred atrt for 2h. After the reaction is completed, The resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3), dried ($MgSO_4$), and concentrated to afford acrude product. The crude product was purified by chromatography onsilica gel with petroleum ether/ethylacetate to afford 11.

4.10. General synthesis procedure for ***(R)-6-(((4-chlorophenyl) thio)methyl)-5,6-dihydro-2H-pyran-2-one****(**12**)*

Grubbs-2 (270 mg, 0.318 mmol) was dissolved in 20 mL anhydrous DCM in an oven dried flask. Subsequently, compound **17**(900 mg, 3.18 mmol) was dissolved in 2 mL DCM and added dropwise under an nitrogen atmosphere. The reaction was stirred for 15 h at 50 °C–60 °C. The crude product was purified by chromatography on silica gel with petroleumether/ethylacetate to afford **12** as a white solid (305 mg, 37.62%).

4.11. Synthesis of ***(6R)-6-(((4-chlorophenyl)sulfinyl)methyl)-5,6-dihydro-2H-pyran-2-one (13)***

A solution of **12** (1.00g, 3.93 mmol) in CH_2Cl_2 (20 mL),subsequently, m-chloroperoxybenzoic acid (812 mg, 4.71 mmol) is added to the system under ice bath conditions. After the reaction is complete, a mixture of saturated solution of $Na_2S_2O_3$ (10 mL) and $NaHCO_3$ (10 mL) was added. The organic phase was washed with water (3 × 10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether/acetone (8:1) to afford **13** as a gray solid (145 mg, 13.64%). m.p. 80.5–81.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H), 6.99–6.84 (m, 1H), 6.11 (d, J = 10.0 Hz, 1H), 5.17–5.01 (m, 1H), 3.11 (dd, J = 13.4, 9.4 Hz, 1H), 2.95 (dd, J = 13.4, 3.2 Hz, 1H), 2.62–2.28 (m, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 162.74, 144.29, 142.20, 137.74, 129.89, 125.18, 121.62, 71.67, 63.20, 29.24.HRMS: calcd for$C_{12}H_{11}ClNaO_3S$ $[M+Na]^+$ m/z: 293.0015, found: 293.0016.

4.12. General synthesis procedure for ***(R)-2-(((4-chlorophenyl) thio)methyl)oxirane*** *(**15**)*

4-chlorobenzenethiol**14** (2.00 g, 13.83 mmol) and NaOH (1.66 g, 41.49 mmol) was dissolved in 5 mL H_2O in a flask.Subsequently, The reaction mixture was stirred at room temperature for 30min. The reaction system was added dropwise to a 50 mL round bottom flask containing (s)-epichlorohydrin (3.84 g, 41.49 mmol) and tetrabutylammonium bromide (223 mg, 0.69 mmol). The reaction mixture was stirred at room temperature. After the reaction is completed, The resulting mixture was diluted with water (10 mL) and extracted with ethylacetate (20 mL × 3).The combined organic phases were dried with Na_2SO_4, concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether andethylacetate to afford colorless oil liquid**15** (1.05 g, 37.83%).

4.13. General synthesis procedure for ***(R)-1-((4-chlorophenyl) thio)pent-4-en-2-ol*** *(**16**)*

Compound **15** (1.00g, 4.98 mmol) and CuI (190 mg, 0.99 mmol) was dissolved in 20 mL anhydrous THF in a flask. The reaction

mixture was stirred at-10−0 °C for 10 min and ethylene magnesium chloride(648 mg, 7.47 mmol) was added dropwise under an argon atmosphere. After the reaction is completed, The resulting mixture was diluted with saturated ammonium chloride (50 mL) and the system was suction filtered with Celite, and then evaporated and extracted with ethylacetate (15 mL × 3).The combined organic phases were dried with Na_2SO_4, concentrated to afford a crude product.The crude product was purified by chromatography on silica gel with petroleum ether andethylacetate to afford colorless oil liquid**16** (970 mg, 85.10%).

*4.14. General synthesis procedure for **(R)-1-((4-chlorophenyl) thio)pent-4-en-2-yl acrylate** (**17**)*

A solution of **16**(950 mg, 4.15 mmol), anhydrous$MgSO_4$ (999.79 mg, 8.31 mmol), and TEA (630.43 mg, 6.23 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature. Acryloylchloride (563.85 mg, 6.23 mmol) was added dropwise. The reactionmixture was stirred at r.t. for 2h. After the reaction is completed, The resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3), dried ($MgSO_4$), and concentrated to afford acrude product. The crude product was purified by chromatography onsilica gel with petroleum ether/ ethylacetate to afford **17** (970 mg, 83.44%).

*4.15. General synthesis of **(R)-N-(2-(6-oxo-3,6-dihydro-2H-pyran-2-yl)ethyl) benzamide(18a)***

Compound **21a** (2.49g, 6.6 mmol) was dissolved in 25 mL toluene in a flask. Subsequently, the p-TSA (56.79 mg, 0.33 mmol) was added. The reaction was stirred at 110 °C for 6 h, reflux. The reaction mixture was filtered and the toluene was removed by rotaryevaporation. The resulting mixture was diluted with water (20 mL) and extracted with ethylacetate (20 mL × 3). The combined organic phases were dried with Na_2SO_4, concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether/acetone (2:1) to afford **18a** as a white acicular crystal (650 mg, 40.17%).m.p. 79.1−80.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86−7.76 (m, 2H),7.57−7.38 (m, 3H), 6.98−6.87 (m, 1H), 6.80 (s, 1H), 6.11−5.95 (m, 1H), 4.69−4.52 (m, 1H), 3.78−3.58 (m, 2H), 2.46−2.37 (m, 2H), 2.19−2.09 (m, 1H), 2.06−1.95 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 145.28, 131.54, 128.60, 126.91, 121.22, 76.67, 36.55, 34.35, 29.38.

*4.15.1. Synthesis of **(R)-4-fluoro-N-(2-(6-oxo-3,6-dihydro-2H-pyran-2-yl)ethyl)benzamide (18b)***

Compound **21b** (1.53 g, 3.87 mmol) was converted to**18b**(185 mg, 18.16%) as a white acicular crystalby the same procedure as described for compound **18a**.m.p. 128.2−129.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (t, J = 5.6 Hz, 1H), 8.01−7.83 (m, 2H), 7.29 (t, J = 8.9 Hz, 2H), 7.12−6.97 (m, 1H), 5.95 (dd, J = 9.8, 1.8 Hz, 1H), 4.58−4.48(m, 1H), 3.54−3.35 (m, 2H), 2.48−2.26 (m, 2H), 1.99−1.81 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 166.60, 164.72(d, J = 250 Hz), 164.01, 145.45, 130.41 (d, J = 2.8 Hz), 129.33 (d, J = 8.9 Hz), 121.13, 115.55 (d, J = 21.8Hz), 76.78, 36.62, 34.29, 29.37.HRMS: calcd for$C_{14}H_{15}FNO_3$ $[M+Na]^+$ m/z: 264.1036, found: 264.1036.

*4.15.2. Synthesis of **(R)-N-(2-(6-oxo-3,6-dihydro-2H-pyran-2-yl) ethyl) cinnamamide(18c)***

Compound **21c** (1.79 g, 4.44 mmol) was converted to **18c**(265 mg, 22.02%) as a white acicular crystalby the same procedure as described for compound **18a**.m.p. 113.1−114.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (t, J = 5.6 Hz, 1H), 7.56 (d, J = 6.8 Hz, 2H), 7.50−7.29 (m, 4H), 7.15−6.97 (m, 1H), 6.60 (d, J = 15.8 Hz, 1H), 5.95 (dd, J = 9.8, 1.8 Hz, 1H), 4.61−4.40 (m, 1H), 3.46−3.34 (m, 1H), 3.30−3.17 (m, 1H), 2.48−2.42 (m, 1H), 2.40−2.24 (m, 1H), 1.97−1.75 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 164.96, 163.71, 147.09, 138.59, 134.83, 129.39, 128.88, 127.45, 122.06, 120.15, 75.45, 34.63, 34.18, 28.69.HRMS: calcd for$C_{16}H_{17}NNaO_3$ $[M+Na]^+$ m/z: 294.1106, found: 294.1105.

*4.15.3. Synthesis of **(R)-5-chloro-N-(2-(6-oxo-3,6-dihydro-2H-pyran-2-yl)ethyl)thiophene-2-carboxamide (18d)***

Compound **21d** (1.60 g, 3.83 mmol) was converted to **18d**(305 mg, 27.88%) as a white acicular crystalby the same procedure as described for compound **18a**.m.p. 145.0−145.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 4.0 Hz, 1H), 6.95−6.91 (m, 1H), 6.89 (d, J = 4.0 Hz, 1H), 6.70 (br, 1H), 6.08−5.99 (m, 1H), 4.66−4.52 (m, 1H), 3.75−3.64 (m, 1H), 3.63−3.50 (m, 1H), 2.47−2.36 (m, 2H), 2.15−1.94 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.80, 161.17, 145.31, 137.53, 135.35, 127.11, 127.04, 121.18, 76.86, 36.65, 34.19, 29.37.HRMS: calcd for $C_{12}H_{12}ClNNaO_3S$ $[M+Na]^+$ m/z: 308.0124, found: 308.0123.

*4.16. General synthesis of **N-(2-((2S)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)benzamide (19a)***

Compound **21a** (2.49g, 6.6 mmol) was dissolved in 25 mL toluene in a flask. Subsequently, the p-TSA(56.79 mg, 0.33 mmol) was added. The reaction was stirred at 110 °C for 6 h, reflux. The reaction mixture was filtered to afford **19a** as a white acicular crystal (728 mg, 41.92%) directly.m.p. 178.8−179.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (t, J = 5.3 Hz, 1H), 7.85 (d, J = 7.1 Hz, 2H), 7.59−7.41 (m, 3H), 5.21 (br, 1H), 4.80−4.59 (m, 1H), 4.22−4.07 (m, 1H), 3.56−3.26 (m, 2H), 2.66 (dd, J = 17.3, 4.5 Hz, 1H), 2.41 (dd, J = 17.3, 1.7 Hz, 1H), 1.94−1.80 (m, 3H), 1.79−1.65 (m, 1H).^{13}C NMR (101 MHz, DMSO-d_6) δ 170.12, 166.17, 134.47, 131.04, 128.20, 127.09, 73.51, 61.23, 38.53, 35.44, 35.07, 34.98.HRMS: calcd for $C_{14}H_{17}NNaO_4$ $[M+Na]^+$ m/z: 286.1055, found m/z: 286.1058.

*4.16.1. Synthesis of **4-fluoro-N-(2-((2S)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)benzamide (19b)***

Compound **21b** (1.53 g, 3.87 mmol) was converted to **19b**(810 mg, 74.43%) as a white acicular crystalby the same procedure as described for compound **19a**.m.p. 182.7−183.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66−8.50 (m, 1H), 7.92 (dd, J = 8.7, 5.6 Hz, 2H), 7.29 (t, J = 8.8 Hz, 2H), 5.20 (br, 1H), 4.82−4.58 (m, 1H), 4.22−4.01 (m, 1H), 3.53−3.38 (m, 2H), 2.65 (dd, J = 17.3, 4.5 Hz, 1H), 2.40 (dd, J = 17.3, 1.7 Hz, 1H), 1.84 (dd, J = 13.5, 6.5 Hz, 3H), 1.77−1.64 (m, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.11, 165.10, 163.75 (d, J = 248.2 Hz), 130.92 (d, J = 2.8 Hz), 129.72 (d, J = 9.0 Hz), 115.11 (d, J = 21.7 Hz), 73.47, 61.22, 38.52, 35.48, 35.06, 34.95.HRMS: calcd for$C_{14}H_{16}FNNaO_4$ $[M+Na]^+$ m/z: 304.0961, found: 304.0961.

*4.16.2. Synthesis of **N-(2-((2S)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)cinnamamide (19c)***

Compound **21c**(1.79 g, 4.44 mmol) was converted to **19c**(810 mg, 63.11%) as a white acicular crystalby the same procedure as described for compound **19a**.m.p. 171.6−172.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (t, J = 5.3 Hz, 1H), 7.56 (d, J = 7.2 Hz, 2H), 7.49−7.30 (m, 4H), 6.62 (d, J = 15.8 Hz, 1H), 5.21 (br, 1H), 4.78−4.59 (m, 1H), 4.13 (s, 1H), 3.46−3.35 (m, 1H), 3.29−3.17 (m, 1H), 2.65 (dd, J = 17.3, 4.4 Hz, 1H), 2.40 (dd, J = 17.3, 1.2 Hz, 1H), 1.95−1.62 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.11, 164.93, 138.55, 134.84, 129.38, 128.89, 127.45, 122.10, 73.33, 61.23, 40.08, 38.52, 35.02, 34.84.HRMS: calcd for$C_{16}H_{19}NNaO_4$ $[M+Na]^+$ m/z: 312.1212, found: 312.1210.

4.16.3. Synthesis of ***5-chloro-N-(2-((2S)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)thiophene- 2-carboxamide(19d)***

Compound **21d** (1.60 g, 3.83 mmol) was converted to **19d**(650 mg, 55.90%) as a white acicular crystalby the same procedure as described for compound **19a**.m.p. 172.4–173.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (t, J = 5.5 Hz, 1H), 7.62 (d, J = 4.1 Hz, 1H), 7.18 (d, J = 4.0 Hz, 1H), 5.19 (br, 1H), 4.79–4.55 (m, 1H), 4.20–4.05 (m, 1H), 3.48–3.37 (m, 1H), 3.33–3.19 (m, 1H), 2.65 (dd, J = 17.3, 4.5 Hz, 1H), 2.39 (dd, J = 16.5, 3.9 Hz, 1H), 1.91–1.77 (m, 3H), 1.75–1.61 (m, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.03, 160.09, 139.15, 132.71, 127.95, 127.75, 73.33, 61.23, 38.52, 35.32, 35.05, 34.95.HRMS: calcd for $C_{12}H_{14}ClNNaO_4S$ $[M+Na]^+$ m/z: 326.0230, found: 326.0231.

4.17. General synthesis procedure for ***analogues oftert-butyl 2-((4S,6S)-6-(2-benzamidoethyl)-2, 2-dimethyl-1,3- dioxan-4-yl) acetate (21)***

A solution of compound **20**(1 equvi), anhydrous$MgSO_4$ (2 equvi), and TEA (1.5 equvi) in anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature. Acyl chloride analogue(1.5 equvi) was added dropwise. The reactionmixture was stirred atrt for 2h. After the reaction is completed, The resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3), dried ($MgSO_4$), and concentrated to afford acrude product. The crude product was purified by chromatography onsilica gel with petroleum ether/ethylacetate to afford **21**.

5. Material and methods

5.1. Reagent and antibodies

Fetal bovine serum (FBS), Roswell Park MemorialInstitute 1640 (RPMI-1640) medium, and penicillin-streptomycin were purchased from HyClone (Victoria, Australia).DAPI, MTT, JC-1 was purchased from Sigma-Aldrich (St.Louis, MO).MG132 and bortezomibwere purchased from MCE (MedChem Express, New Jersey, USA). The FITC/Annexin V Apoptosis Detection Kit was purchased from BestBio (Shanghai, China). The Nuclear and Cytoplasmic Protein Extraction Kit was purchased from Beyotime (Shanghai, China). The ECL Western blotting kit was purchased from ThermoFisher(Waltham, MA).Antibodies against CRM1, Ranbp1, p53 and Cleaved PARP, were purchased form Cell Signaling Technology (Danvers, MA). Antibodies against Bcl-xl, PARP, LaminB1, Tubulin, Actin, Cleaved caspase-3 were purchased formSanta Cruz Biotechnology (Santa Cruz, CA). Antibodies against Bax, Bcl-2, p53 siRNA, Cleaved caspase-9 were purchased form Abcam (Cambridge, MA).

5.2. Cell lines and culture

The human gastric cancer cell lines MGC803 and HGC27 and gastric epithelial cells (GES1) were purchased from the American Type Culture Collection (Manassas, VA). The cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, and were maintained at 37 °C in an incubator under an atmosphere containing 5% CO_2.

5.3. Cell viability assay

Cells were seeded into a 96-well plate at a density of 5 × 10^4/well, cultured overnight and then treated with compound **1l**. Then, 20 μL of MTT solution (5 mg/mL) was added tothe cells in each well, and maintained at 37 °C for 4h. Finally, formazan crystals were solubilized with 150 μl of DMSO, and the absorbance intensity was analyzed with a 96-well platereader at 490 nm. Every experiment was duplicated three times.

5.4. Immunofluorescence analysis

MGC803 cells were treated with compound **1l** and fixed for 30 min with 4% paraformaldehyde. Next, cell member were permeabilized by 0.1% Triton X-100 for 10 min. After blocked with 10% normal goat serum for 30 min, MGC803 cells incubation with relevant antibody.In order to locate the cell, DAPI was used for nuclei labeling, slides were analyzed using Nikon confocal microscopy.

5.5. Western blot

Cells were seeded ata 100-mm tissue culture plate and cultured for 24 h. After culturing, the cells treated with different concentrations of compound **1l** for 24 h. Cells then were collected and lysed with ice-cold RIPA buffer (Beyotime, P0013B), followed by centrifugation. Protein concentration was quantified and normalized using BCA assay kit.The total cellular protein extracts were boiled with 5 × loading buffer and resolved by 10% or 12% SDS-PAGE and blotted onto PVDF membranes. The membranes were blockedwith5% skimmed milk in PBST for 2 hat 37 °C, they were wash and treated with overnight at 4 °C with appropriate antibodies, followed by HRP conjugated anti-mouse, anti-goat or anti-rabbitsecondary antibodies. followed by HRP conjugated anti-mouse, anti-goat or anti-rabbitsecondary antibodies for 2 hat 37 °C. Finally, each protein were visualized by using an ECL Western blotting kit.

5.6. Apoptosis analysis

Cells were seeded at 50%confluency in a 6-well plate and overnight incubation, followed by treatment with different concentration of compound **1l** for 24 h. Cells then washed with PBS twice, and collected by centrifugation.After that, cells incubated with fluoresceinisothiocyanate (FITC) conjugated Annexin V and PI by use of FITC Annexin V/PI apoptosis kit according to the manufacturer's instructions.

5.7. Measurement of loss of mitochondrial membrane potential

Mitochondrial membrane potential was assed using 2.5 μM JC-1.In brief, cells were plated into a 6-well plate at a density of 2 × 10^5 cells/well and cultured overnight. Cells exposed to compound **1l** for 1, 3, 6, 12 and 24 h. After that, cells were collected and stained with 2.5 μM JC-1 at 37 °C for 10 min in darkness. The cells rinsed twice with PBS, suspended, and measured using flow cytometer.

5.8. Statistical analysis

All data are presented as mean ± SD. Statistical evaluation of signifcant differences was performed using the unpaired Student's t-test. *, ** and *** respectively represent $p < 0.05$, $p < 0.02$ and $p < 0.001$.

Declaration of competing interest

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.

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Appendix A. Supplementary data

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