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

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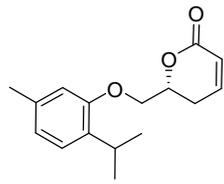
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Compound 11

11 (5 μ M)
MG132(0.05 μ M)
MG132(0.1 μ M)
Bortezomib (2.5nM)

MGC803

-	+	-	+	-	+	-	+
-	-	+	+	-	-	-	-
-	-	-	-	+	+	-	-
-	-	-	-	-	-	+	+

CRM1



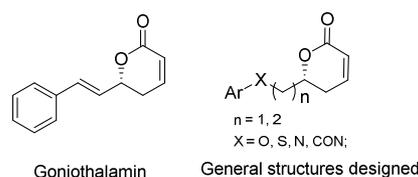
Actin



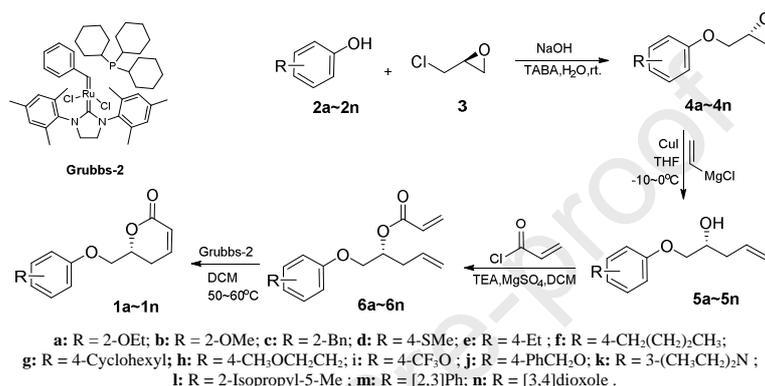
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2. Results and Discussion

2.1. Chemistry

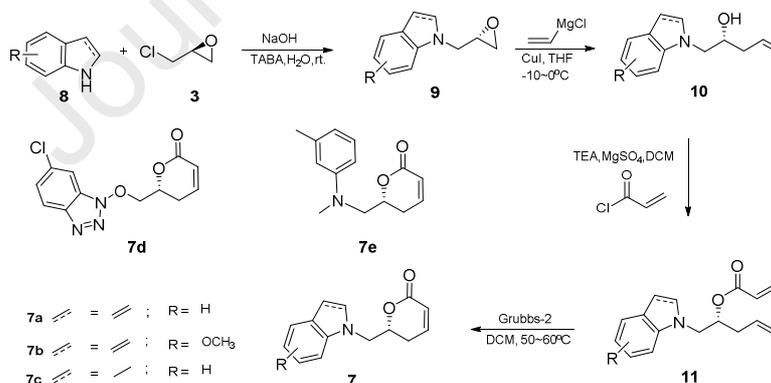


Natural products LMB, Goniotalamin and their analogues showed CRM1 inhibition and anti-proliferation activity on cancer cell lines. They were reported to bind to CRM1 through the α , β -unsaturated lactone moiety [18, 19]. Based on the pharmacophore of α , β -unsaturated lactone, a series of compounds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



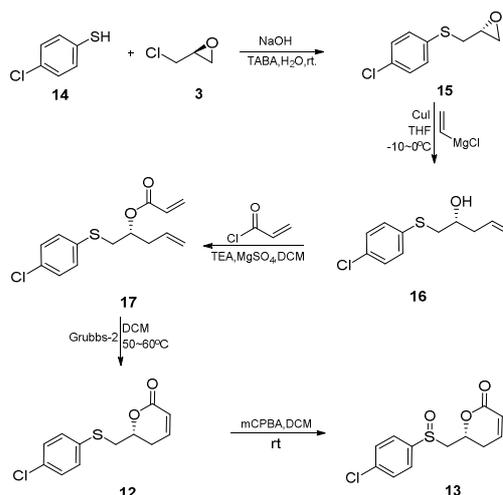
Scheme 1. Synthesis of (*R*)-6-(phenyloxidemethyl)-5,6-dihydro-2*H*-pyran-2-ones (**1a-1n**)

Compounds **1a-1n** with phenoxy methyl 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].



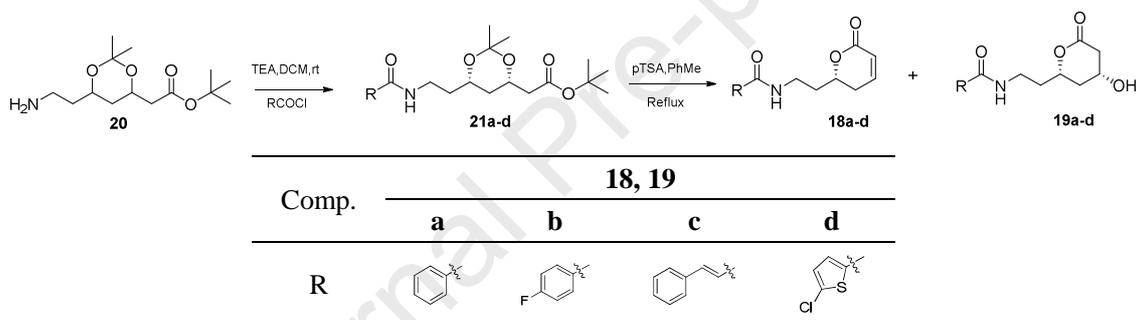
Scheme 2. Synthesis of (*R*)-6-((1*H*-indol-1-yl)methyl)-5,6-dihydro-2*H*-pyran-2-ones (**7a-7d**)

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**).



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

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 transferred to compounds **21a-d** by the acylation. Compounds **18a-18d** and **19a-19d** were prepared by an intramolecular esterification in toluene under reflux in the presence of *p*TSA.



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

2.2. Biology

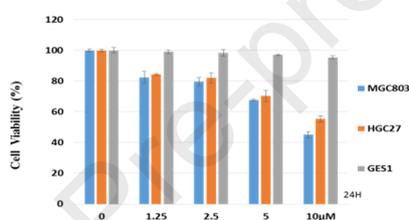
The anti-proliferation of all synthesized compounds were evaluated on MGC803 by MTT assay (Table 1). Compounds (**1a-1n**) containing benzyl groups and a two atoms length linker showed moderate anti-proliferation activity on MGC-803 with IC₅₀ of 2.3 - 47.7 μM. The most potent compound **1l** had an IC₅₀ 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 IC₅₀>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.

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	>50μM
1j	35.439±1.549	18d	>50μM
1k	>50μM	19a	>50μM
1l	2.314±0.364	19b	>50μM
1m	47.687±1.678	19c	>50μM
1n	11.976±1.078	19d	>50μM
5-Fu	24.803±1.395		

The anti-proliferation activity of compound **11** (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 **11** on reducing cell viabilities were assayed by MTT. As shown in Fig.1B, following treatment with compound **11**, the viability of the gastric cancer cell lines decreased in a dose dependent manner. MGC803 and HGC27 cells lines showed the sensitivity to compound **11** 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 **11** has selective anti-proliferation on gastric cancer cell lines versus normal human gastric epithelial cell lines.



A

B

Figure 1. the structure of compound **11** and its anti-proliferation activity on MGC803 and HGC27

2.2.1 Compound **11** 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 **11** on the gastric cancer cell lines were the result of apoptotic cell death. The inducing apoptosis of compound **11** 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 dependent manner in these two cell lines. The results exhibited that exposure to compound **11** 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 **11**, JC-1 was used to measure MMP ($\Delta\Psi$). After treatment with compound **11** in a dose dependent manner, MMP ($\Delta\Psi$) 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 **11**, 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 **11** 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 **11** mediated apoptosis in gastric cancer cells.

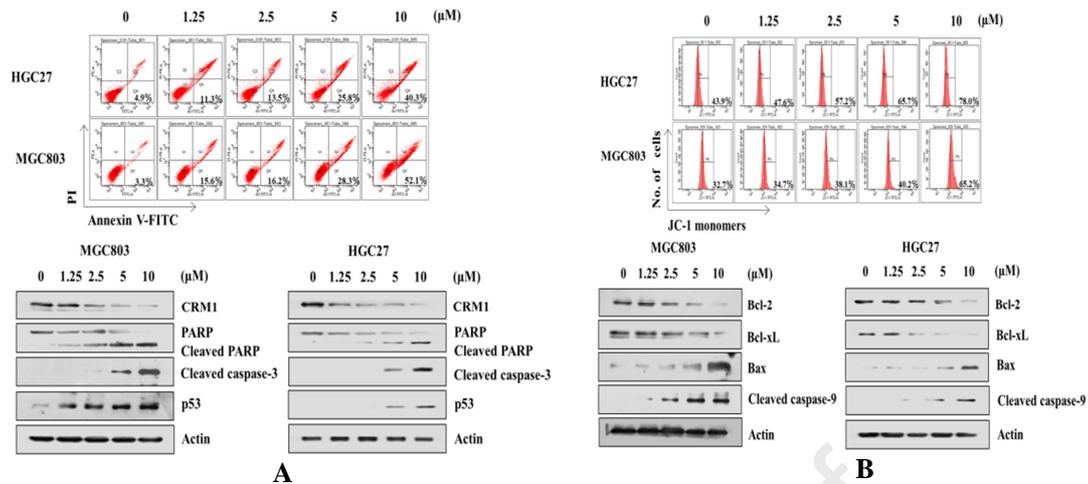


Figure 2. Apoptosis induced by compound 11 involves Mitochondrial pathway in gastric cancer cell lines. (A) Cell apoptosis of MGC803 and HGC27 was detected by flow cytometry after treatment by compound 11 for 24 h. The protein levels of CRM1, PARP, Cleaved PARP, Cleaved caspase-3 and p53 were detected at different concentrations by Western blot in MGC803 cells. (B) MMP ($\Delta\Psi$) was decreased by compound 11 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 11 treatment.

2.2.2 Compound 11-mediated CRM1 depletion depends on ubiquitin-proteasome pathway

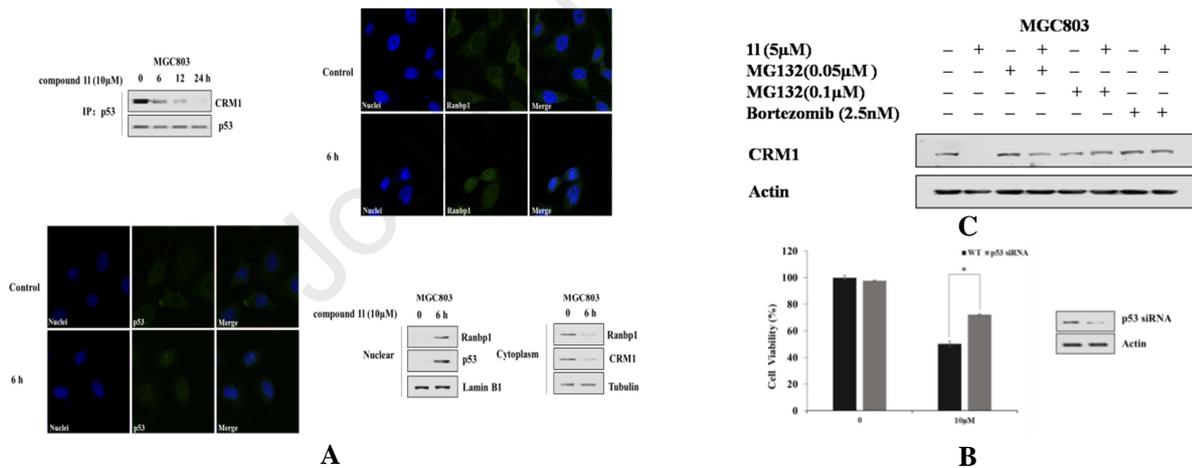


Figure 3. Mitochondrial p53 pathway is involved in the inducing apoptosis of compound 11 in MGC803 cells and compound 11 induces CRM1 protein degradation via the ubiquitin-proteasome pathway.

(A) CRM1 bound with p53 detected by immunoprecipitation assay in MGC803 cell lines after treatment with compound 11 at 10 μM at various time points. Accumulation of Ranbp1 or p53 in nucleus was observed in MGC803 cells with treatment of compound 11 at 10 μM for 6 h. The treated and untreated MGC803 cells with compound 11 at 10 μM for 0 and 6 h are stained with Ranbp1 antibody (Green) and DAPI (Blue) and analyzed by confocal microscopy. Western blot 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 11 (10 μM) in the present or absent of MG132 (0.05 μM), MG132 (0.1 μM) and bortezomib (2.5nM) for 24 h. Western blot was used to detect the expression levels of CRM1.

The levels of CRM1 protein in MGC803 and HGC27 cell lines with treatment of compound **11** 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 and HGC27 cell lines comparing with controls (Fig. 2A), which suggested that compound **11** was possible a CRM1 degrader. Therefore, we detected whether compound **11** 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 **11** treatment. After exposure to compound **11** 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 **11** were decreased evidently, as shown in Fig. 3B. The results showed that p53 is required for compound **11** 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 **11**. MGC803 and HGC27 cells were treated with compound **11** (5 μ M) for 24h in the presence or absence of MG132(0.05 μ M). Reduced CRM1 protein levels were observed in compound **11**-treated cell lines. In contrast, in the presence of MG132, depletion of CRM1 protein by compound **11** was almost completely abolished (Fig. 3C). Similar results were obtained with the use of bortezomib, another proteasome inhibitor. These data demonstrated that compound **11** mediated CRM1 depletion depends on ubiquitin-proteasome pathway.

2.2.3. Compound **11** 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 **11** 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 **11** 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 **11** 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 **11** was completely blocked.

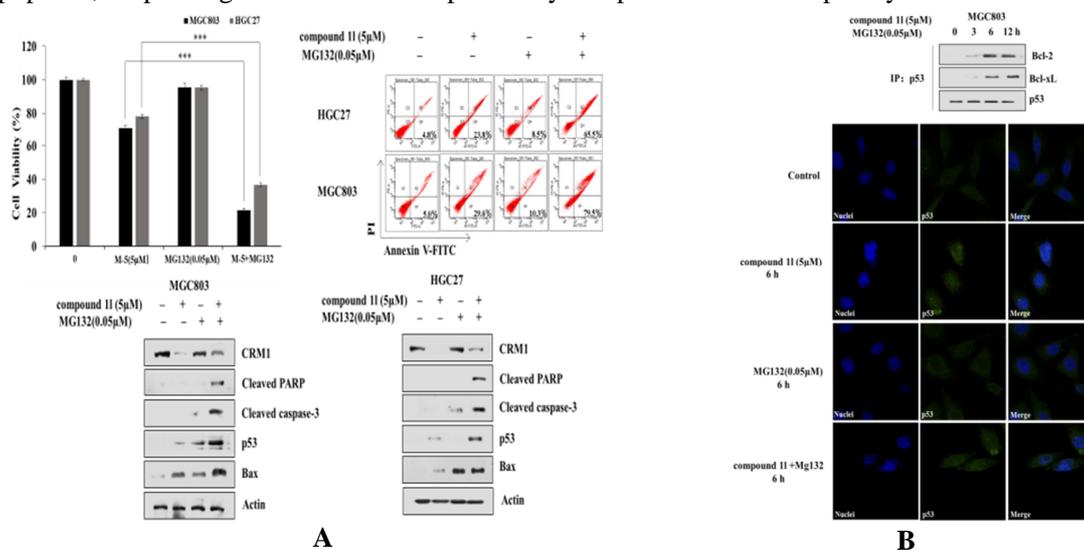


Figure 4. Compound 11 work synergistically with MG132 increased apoptosis

(A) MGC803 and HGC27 cells were pretreated with/without 0.05 μ M of MG132 for 1 h before exposure to 10 μ M

of compound **11** 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 **11** 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 hour, followed by 24 h of compound **11** (10 μM) incubation. (B) MGC803 cells were pretreated with 0.05 μM of MG132 for 1 h before exposure to 10 μM of compound **11** 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 **11** for 6 h. p53 in the nucleus or cytoplasm was detected by Immunofluorescence analysis. The treated and untreated samples are stained with p53 antibody (Green) and DAPI (Blue) and analyzed by confocal microscopy.

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 **11** in gastric cancer cells. Compound **11** 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 **11** 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 **11** 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 **11** 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 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 **11** is associate with tumor suppressor proteins p53. We observed that the expression levels of CRM1 was decreased and p53 accumulated within nuclear after compound **11** treatment. In addition, knockdown of p53 by specific siRNA in MGC803 cells, compound **11** 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, a 26S 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 **11** was almost completely blocked. In addition, we observed that compound **11** 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 **11** 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 **11** 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 **11** 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 **11** has a good permeability possibly.

In summary, the results in this work show that compound **11** 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%. ^1H 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 200~300 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, 2.21mmol) was dissolved in 2 mL DCM and added drop wise under an nitrogen atmosphere. The reaction was stirred for 15 hours at 50 °C ~60 °C. The crude product was purified by chromatography on silica gel with petroleum ether/ ethylacetate (10:1) to afford **1a** as a white solid (173 mg, 31.46%). ^1H 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, 1.98mmol) 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; ^1H 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 $\text{C}_{13}\text{H}_{14}\text{NaO}_4$ $[\text{M}+\text{Na}]^+$ 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, 1.71mmol) was converted to **1c**(220mg, 43.81%) as a white solid by the same procedure as described for compound **1a**. m.p. 86.5~87.2°C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.27 – 7.21m, 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, $\text{DMSO}-d_6$) 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 $\text{C}_{19}\text{H}_{18}\text{NaO}_3$ $[\text{M}+\text{Na}]^+$ 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, 1.80mmol) was converted to **1d**(200mg, 44.32%) as a gray solid by the same procedure as described for compound **1a**. m.p. 100.5~101.7°C; ^1H 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 $\text{C}_{13}\text{H}_{14}\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$ 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, 2.15mmol) 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; ^1H 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 $\text{C}_{14}\text{H}_{16}\text{NaO}_3$ $[\text{M}+\text{Na}]^+$ 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, 1.82mmol) 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; ^1H 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 $\text{C}_{17}\text{H}_{22}\text{NaO}_3$ $[\text{M}+\text{Na}]^+$ 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; ^1H NMR (400 MHz, CDCl_3) δ 7.13 (d, 7.5 Hz, 2H), 6.98 – 6.89 (m, 1H), 6.84 (d, $J = 8.0$ Hz, 2H), 6.07 (d, 9.7 Hz, 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 $\text{C}_{18}\text{H}_{22}\text{NaO}_3$ $[\text{M}+\text{Na}]^+$ 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; ^1H 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 $\text{C}_{15}\text{H}_{18}\text{NaO}_4$ $[\text{M}+\text{Na}]^+$ 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; ^1H 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 $\text{C}_{13}\text{H}_{11}\text{F}_3\text{NaO}_4$ $[\text{M}+\text{Na}]^+$ 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; ^1H 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 $\text{C}_{19}\text{H}_{18}\text{NaO}_4$ $[\text{M}+\text{Na}]^+$ 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; ^1H 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**. ^1H 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 (1m)* 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; 1H 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; 1H 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 30 min. The reaction system was added drop wise 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 \times 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 and ethylacetate to afford **4a~4n**.

4.4. *General Synthesis Procedure for analogues of (R)-1-phenoxy-pent-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 drop wise 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 \times 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 and ethylacetate to afford **5a~5n**.

4.5. *General Synthesis Procedure for analogues of (R)-1-phenoxy-pent-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 drop wise. The reaction mixture was stirred at room temperature for 2 h. After the reaction is completed, The resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (20 mL \times 3), dried ($MgSO_4$), and concentrated to afford a crude product. The crude product was purified by chromatography on silica 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 drop wise under a nitrogen atmosphere. The reaction was stirred for 15 hours at 50 °C~60 °C. The crude product was purified by chromatography on silica gel with petroleum ether/ethylacetate (10:1) to afford **7a** as a colorless oily liquid (143 mg, 42.28%). 1H 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 pale yellow solid by the same procedure as described for compound **7a**. ^1H 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 $\text{C}_{15}\text{H}_{15}\text{NNaO}_3$ $[\text{M}+\text{Na}]^+$ 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 liquid by the same procedure as described for compound **7a**. ^1H 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 $\text{C}_{14}\text{H}_{15}\text{NNaO}_2$ $[\text{M}+\text{Na}]^+$ m/z : 252.1000, found: 252.1003.

4.6.3. Synthesis of (R)-6-(((6-chloro-1H-benzo[d][1,2,3]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 grey-green solid by the same procedure as described for compound **7a**. m.p. 109.5~110.1 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.94 (d, 8.8 Hz, 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 $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{NaO}_3$ $[\text{M}+\text{Na}]^+$ 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 liquid by the same procedure as described for compound **7a**. ^1H 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 $\text{C}_{14}\text{H}_{17}\text{NNaO}_2$ $[\text{M}+\text{Na}]^+$ 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 equi) and NaH (3 equi) was dissolved in 20 mL MeCN at ice-bath in a flask. Subsequently, The reaction mixture was stirred at room temperature for 30 min. (S)-epichlorohydrin (3 equi) was added drop wise. 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 \times 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 equi) and CuI (0.2 equi) 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 equi) was added drop wise 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 \times 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 and ethylacetate 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 equiv), anhydrous MgSO_4 (2 equiv), and TEA (1.5 equiv) in Anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature. Acryloylchloride (1.5 equiv) was added drop wise. The reaction mixture 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 \times 3), dried (MgSO_4), and concentrated to afford crude product. The crude product was purified by chromatography on silica 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 drop wise under an nitrogen atmosphere. The reaction was stirred for 15 hours at 50 °C ~60 °C. The crude product was purified by chromatography on silica gel with petroleum ether/ 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 (812mg, 4.71mmol) is added to the system under ice bath conditions. After the reaction is complete, a mixture of saturated solution of $\text{Na}_2\text{S}_2\text{O}_3$ (10 mL) and NaHCO_3 (10 mL) was added. The organic phase was washed with water (3 \times 10mL) 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; ^1H 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 $\text{C}_{12}\text{H}_{11}\text{ClNaO}_3\text{S}$ [$\text{M}+\text{Na}$] $^+$ 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, 13.83mmol) and NaOH (1.66 g, 41.49mmol) 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 drop wise to a 50 mL round bottom flask containing (s)-epichlorohydrin (3.84 g, 41.49mmol) and tetrabutylammonium bromide (223 mg, 0.69mmol). 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 \times 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 and ethylacetate 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 drop wise 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 \times 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 and ethylacetate 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** (950mg, 4.15mmol), anhydrous MgSO_4 (999.79mg, 8.31mmol), and TEA (630.43mg, 6.23mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature. Acryloylchloride (563.85mg, 6.23mmol) was added drop wise. The reaction mixture 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 \times 3), dried (MgSO_4), and concentrated to afford crude product. The crude product was purified by chromatography on silica gel with petroleum ether/ethylacetate to afford **17** (970mg, 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, 6.6mmol) was dissolved in 25 mL toluene in a flask. Subsequently, the p-TSA (56.79mg, 0.33mmol) was added. The reaction was stirred at 110°C for 6 hours, reflux. The reaction mixture was filtered and the toluene was removed by rotary evaporation. The resulting mixture was diluted with water (20 mL) and extracted with ethylacetate (20 mL × 3). The combined organic phases were dried with Na₂SO₄, 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 crystal by the same procedure as described for compound **18a**.m.p. 128.2~129.3°C. ¹H 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). ¹³C NMR (100 MHz, CDCl₃) 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* = 21.8 Hz), 76.78, 36.62, 34.29, 29.37. HRMS: calcd for C₁₄H₁₅FNO₃ [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) cinnamide (18c) Compound **21c** (1.79 g, 4.44 mmol) was converted to **18c** (265 mg, 22.02%) as a white acicular crystal by the same procedure as described for compound **18a**.m.p. 113.1~114.2°C. ¹H 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). ¹³C 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 C₁₆H₁₇NNaO₃ [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 crystal by the same procedure as described for compound **18a**.m.p. 145.0~145.7°C. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₂H₁₂ClNNaO₃S [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, 6.6mmol) was dissolved in 25 mL toluene in a flask. Subsequently, the p-TSA (56.79mg, 0.33mmol) was added. The reaction was stirred at 110°C for 6 hours, 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. ¹H 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). ¹³C 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 C₁₄H₁₇NNaO₄ [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 crystal by the same procedure as described for compound **19a**.m.p. 182.7~183.6°C. ¹H 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 $\text{C}_{14}\text{H}_{16}\text{FNNaO}_4$ $[\text{M}+\text{Na}]^+$ 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 crystal by the same procedure as described for compound **19a**. m.p. 171.6–172.3 °C. ^1H 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 $\text{C}_{16}\text{H}_{19}\text{NNaO}_4$ $[\text{M}+\text{Na}]^+$ 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 crystal by the same procedure as described for compound **19a**. m.p. 172.4–173.1 °C. ^1H 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 $\text{C}_{12}\text{H}_{14}\text{ClNNaO}_4\text{S}$ $[\text{M}+\text{Na}]^+$ m/z : 326.0230, found: 326.0231.

4.17. *General Synthesis Procedure for analogues of tert-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 drop wise. The reaction mixture was stirred for 2h. After the reaction is completed, the resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (20 mL \times 3), dried (MgSO_4), and concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether/ethyl acetate to afford **21**.

5. Material and Methods

5.1. Reagent and antibodies

Fetal bovine serum (FBS), Roswell Park Memorial Institute 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 bortezomib were 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 from Cell Signaling Technology (Danvers, MA). Antibodies against Bcl-xL, PARP, Lamin B1, Tubulin, Actin, Cleaved caspase-3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against Bax, Bcl-2, p53 siRNA, Cleaved caspase-9 were purchased from 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 **11**. Then, 20 μL of MTT solution (5 mg/mL) was added to the cells in each well, and maintained at 37 °C

for 4h. Finally, formazan crystals were solubilized with 150 μ l of DMSO, and the absorbance intensity was analyzed with a 96-well plate reader at 490 nm. Every experiment was duplicated three times.

5.4 Immunofluorescence analysis

MGC803 cells were treated with compound **11** and fixed for 30 minutes with 4% paraformaldehyde. Next, cell membranes were permeabilized by 0.1% Triton X-100 for 10 minutes. 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 at a 100-mm tissue culture plate and cultured for 24 h. After culturing, the cells treated with different concentrations of compound **11** 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 \times loading buffer and resolved by 10% or 12% SDS-PAGE and blotted onto PVDF membranes. The membranes were blocked with 5% skimmed milk in PBST for 2 h at 37 $^{\circ}$ C, they were washed and treated overnight at 4 $^{\circ}$ C with appropriate antibodies, followed by HRP conjugated anti-mouse, anti-goat or anti-rabbit secondary antibodies. followed by HRP conjugated anti-mouse, anti-goat or anti-rabbit secondary antibodies for 2 h at 37 $^{\circ}$ 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 **11** for 24 h. Cells then washed with PBS twice, and collected by centrifugation. After that, cells incubated with fluorescein isothiocyanate (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 assayed using 2.5 μ M 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 **11** for 1, 3, 6, 12 and 24 h. After that, cells were collected and stained with 2.5 μ M JC-1 at 37 $^{\circ}$ 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 \pm SD. Statistical evaluation of significant differences was performed using the unpaired Student's t-test. *, ** and *** respectively represent $p < 0.05$, $p < 0.02$ and $p < 0.001$.

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Appendix A. Supplementary data

The Supporting Information, NMR for compounds, is available free of charge on the ACS Publications website.

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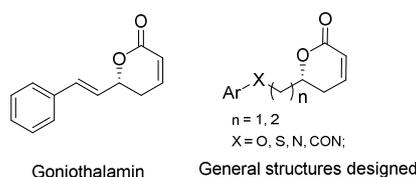
Highlight:

- Novel small molecular CRM1 degrader with different skeleton from CBS9106.
- CRM1 inhibitor or degrader induce the apoptosis in gastric carcinoma
- Selective inhibition for the proliferation of gastric cancer cell line MGC803, HGC27 comparing to Human Gastric Mucosal Epithelial Cell Line (GES1).
- Combination treatment of compound **11** and MG132 resulted in synergistic effects.

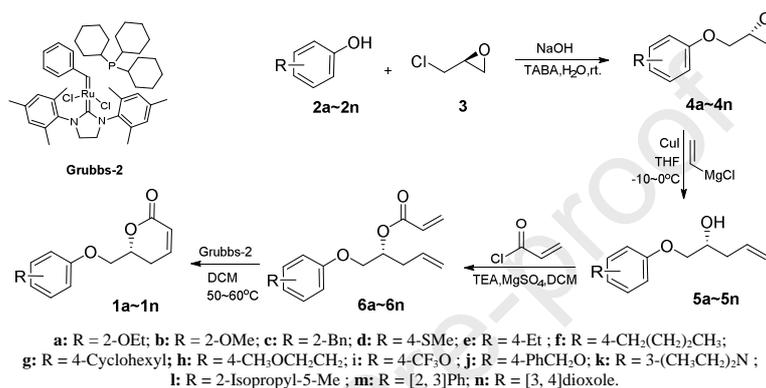
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2. Results and Discussion

2.1. Chemistry

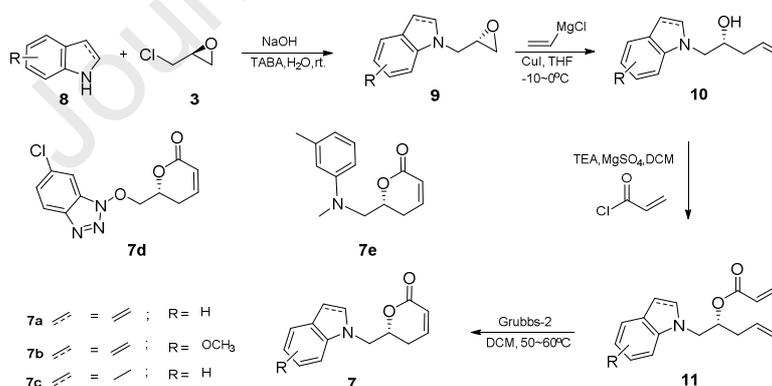


Natural products LMB, Goniotalamin and their analogues showed CRM1 inhibition and anti-proliferation activity on cancer cell lines. They were reported to bind to CRM1 through the α , β -unsaturated lactone moiety^[18, 19]. Based on the pharmacophore of α , β -unsaturated lactone, a series of compounds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



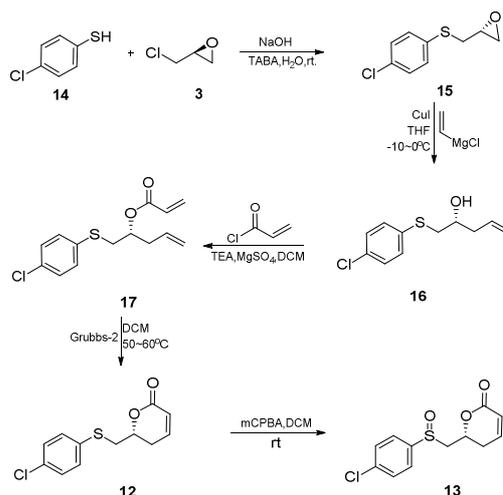
Scheme 1 Synthesis of (*R*)-6-(phenyloxidemethyl)-5,6-dihydro-2*H*-pyran-2-ones (**1a-1n**)

Compounds **1a ~ 1n** with phenoxy methyl 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 acryloyl chloride, which were synthesized by the reaction of compounds **4a-4n** and vinylmagnesium chloride. Compounds **4a-4n** was prepared by the reaction of substituted phenols (**2a-2n**) and *S*-epoxy chloropropane (**3**)^[20].



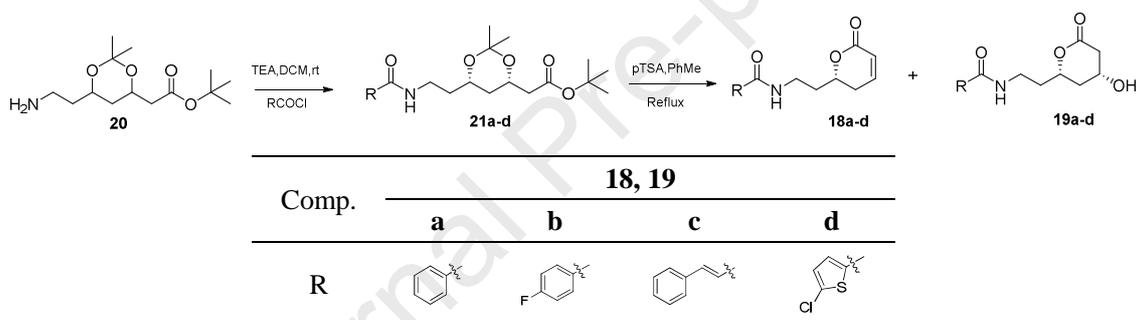
Scheme 2 Synthesis of (*R*)-6-((1*H*-indol-1-yl)methyl)-5,6-dihydro-2*H*-pyran-2-ones (**7a ~ 7d**)

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).



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

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 transferred to compounds **21a-d** by the acylation. Compounds **18a-18d** and **19a ~ 19d** were prepared by an intramolecular esterification in toluene under reflux in the presence of *p*-TSA.



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

2.2. Biology

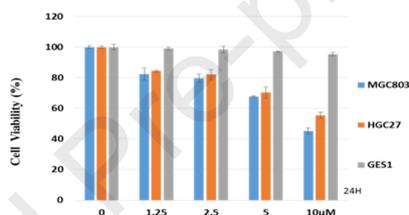
The anti-proliferation of all synthesized compounds were evaluated on MGC803 by MTT assay (Table 1). Compounds (**1a ~ 1n**) containing benzyl groups and a two atoms length linker showed moderate anti-proliferation activity on MGC-803 with IC₅₀ of 2.3 - 47.7 μM. The most potent compound **1l** had an IC₅₀ 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 IC₅₀>50μM. 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.

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	>50μM
1b	24.159±1.383	7b	25.620±1.409
1c	22.978±1.36	7c	>50μM
1d	>50	7d	>50μM
1e	12.425±1.094	7e	>50μM
1f	16.950±1.229	13	>50μM
1g	10.593±1.025	18a	>50μM
1h	>50μM	18b	>50μM

1i	8.489±0.929	18c	>50μM
1j	35.439±1.549	18d	>50μM
1k	>50μM	19a	>50μM
1l	2.314±0.364	19b	>50μM
1m	47.687±1.678	19c	>50μM
1n	11.976±1.078	19d	>50μM
5-Fu	24.803±1.395		

The anti-proliferation activity of compound **11** (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 **11** on reducing cell viabilities were assayed by MTT. As shown in Fig.1B, the viability of the gastric cancer cell lines decreased in a dose dependent manner after treatment with compound **11**. MGC803 and HGC27 cells lines showed the sensitivity to compound **11** 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 **11** has selective anti-proliferation on gastric cancer cell lines versus normal human gastric epithelial cell lines.



A

B

Figure 1. the structure of compound **11** and its anti-proliferation activity on MGC803 and HGC27

2.2.1 Compound **11** 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 **11** on the gastric cancer cell lines were the result of apoptotic cell death. The inducing apoptosis of compound **11** 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 dependent manner in these two cell lines. The results exhibited that exposure to compound **11** 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.

The MMP ($\Delta\Psi$) was measured by using JC-1 to confirm the function of mitochondrial in the induction of apoptosis caused by compound **11** on MGC 803 and HGC27 cell lines. After treatment with compound **11** in a dose dependent manner, MMP ($\Delta\Psi$) 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 **11**, 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 **11** 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 **11** mediated apoptosis in gastric cancer cells.

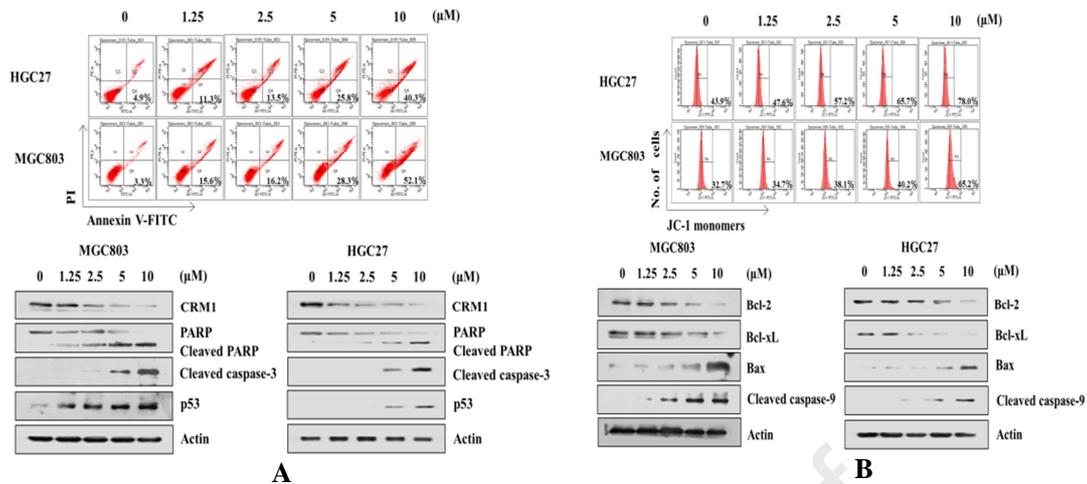


Figure 2. Apoptosis induced by compound 11 involves Mitochondrial pathway in gastric cancer cell lines.

(A) Cell apoptosis of MGC803 and HGC27 was detected by flow cytometry after treatment by compound 11 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 ($\Delta\Psi$) was decreased by compound 11 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 11 treatment.

2.2.2 Compound 11-mediated CRM1 depletion depends on ubiquitin-proteasome pathway

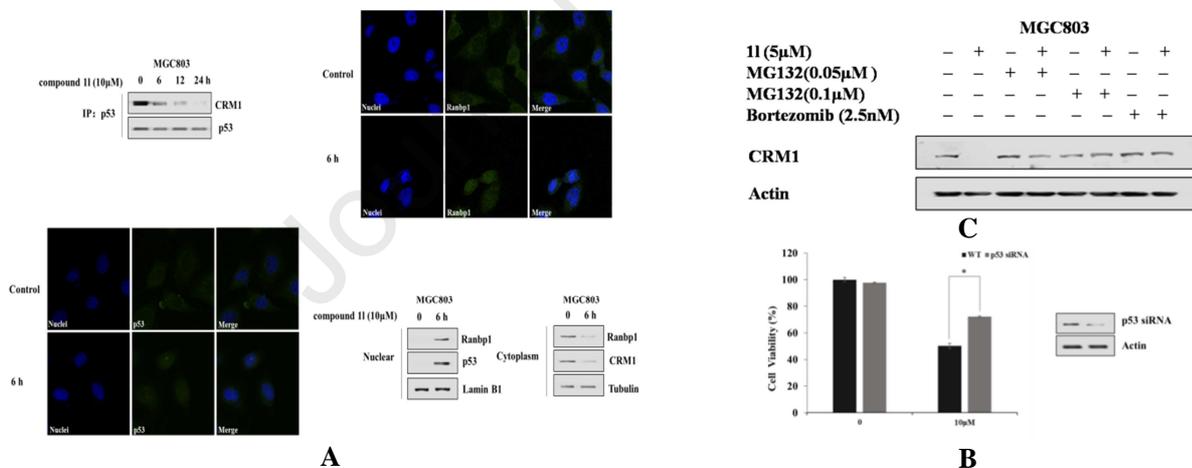


Figure 3. Mitochondrial p53 pathway is involved in the inducing apoptosis of compound 11 in MGC803 cells and compound 11 induces CRM1 protein degradation via the ubiquitin-proteasome pathway.

(A) CRM1 bound with p53 detected by immunoprecipitation assay in MGC803 cell lines after treatment with compound 11 at 10 μM at various time points. Accumulation of Ranbp1 or p53 in nucleus was observed in MGC803 cells with treatment of compound 11 at 10 μM for 6 h. The treated and untreated MGC803 cells with compound 11 at 10 μM for 0 and 6 h are stained with Ranbp1 antibody (Green) and DAPI (Blue) and analyzed by confocal microscopy. Western blot 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 11 (10 μM) in the presence or absence of MG132 (0.05 μM), MG132 (0.1 μM) and bortezomib (2.5nM) for 24 h. Western blot was used to detect the expression levels of CRM1.

The levels of CRM1 protein in MGC803 and HGC27 cell lines with treatment of compound **11** 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 and HGC27 cell lines comparing with controls (Fig. 2A), which suggested that compound **11** was possible a CRM1 degrader. Therefore, we detected whether compound **11** 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 **11** treatment. 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 **11** were decreased evidently, as shown in Fig. 3B. The results showed that p53 is required for compound **11** 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 **11**. MGC803 and HGC27 cells were treated with compound **11** (5 μ M) for 24h in the presence or absence of MG132 (0.05 μ M). Reduced CRM1 protein levels were observed in compound **11**-treated cell lines. In contrast, in the presence of MG132, depletion of CRM1 protein by compound **11** was almost completely abolished (Fig. 3C). Similar results were obtained with the using of bortezomib, another proteasome inhibitor. These data demonstrated that compound **11** mediated CRM1 depletion depends on ubiquitin-proteasome pathway.

2.2.3. Compound **11** 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 **11** 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 **11** 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 **11** 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 **11** was completely blocked.

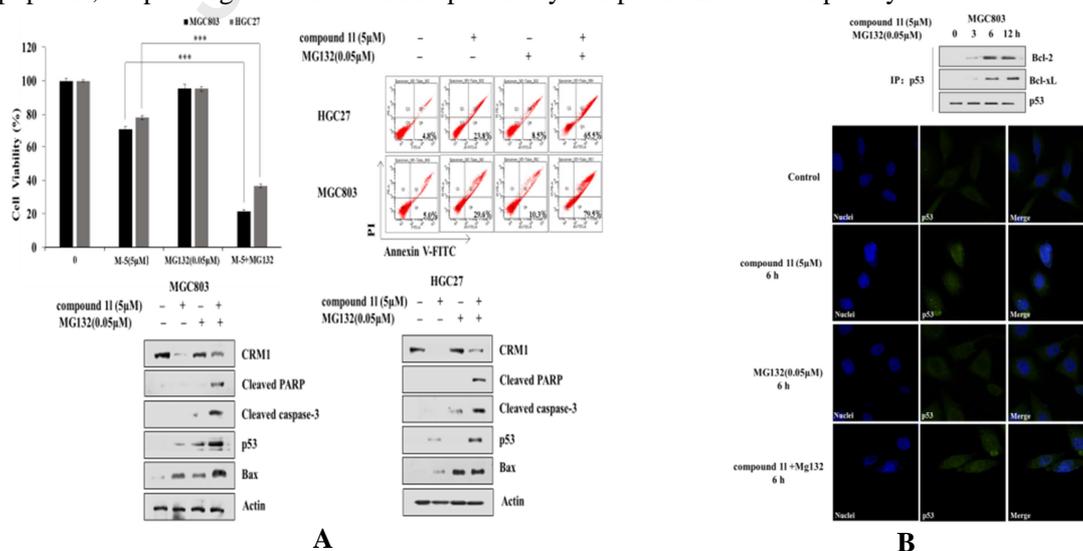


Figure 4. Compound 11 work synergistically with MG132 increased apoptosis

(A) MGC803 and HGC27 cells were pretreated with/without 0.05 μ M of MG132 for 1 h before exposure to 10 μ M of compound **11** 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 **11** 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 hour, followed by 24 h of compound **11** (10 μM) incubation. (B) MGC803 cells were pretreated with 0.05 μM of MG132 for 1 h before exposure to 10 μM of compound **11** 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 **11** for 6 h. p53 in the nucleus or cytoplasm was detected by Immunofluorescence analysis. The treated and untreated samples are stained with p53 antibody (Green) and DAPI (Blue) and analyzed by confocal microscopy.

3. Discussion

Gastric cancer is one of highly aggressive malignant tumor, while impactful chemotherapies for that are limited^[21]. Therefore, development of the novel therapeutic drugs is an attractive topic. The over expression of CRM1 has been detected in gastric cancer and caused the dysfunction of cell fate controls^[22], which became a promising therapeutic target for cancer^[23]. In this research, compound **11** showed anti-proliferative activity via the degradation of CRM1 in gastric cancer cell lines *in vitro*. For the first time, a new degradation of CRM1, compound **11** suppresses proliferation and induces apoptosis on 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 or degradation of CRM1 induced the accumulation of tumor suppressor proteins in the nucleus and reduced cytoplasmic degradation of these proteins. This has been thought to be as significant mechanism of the anti-gastric cancer activity of CRM1 inhibitor or degrader. In our study, the expression levels of CRM1 was decreased and p53 accumulated in the nuclear of MGC803 and HGC27 cell lines treated by compound **11**. In addition, the mitochondrial apoptotic pathway induced by compound **11** was decreased evidently in MGC803 cell lines with knockdown of p53 by specific siRNA. The result suggest that the anti-proliferation activity of compound **11** is associate with the accumulation of the tumor suppressor proteins p53 in the nuclear. 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, a 26S proteasome inhibitor, whereas inhibition of the 26S proteasome lead to accumulation of non-degraded ubiquitinated proteins in the cytoplasmic. In our study, it was observed that the degradation of CRM1 protein induced by compound **11** was almost completely blocked by co-incubation with the proteasome inhibitor MG132 or bortezomib. The combination treatment with MG132 and compound **11** enhanced expression of p53, cleaved PARP, cleaved caspase-3 and Bax, and induced more apoptosis in two gastric cancer cells compared to cell lines treated with compound **11** or proteasome inhibitors alone. p53 transcription-independent apoptosis is initiated by interacts with anti-apoptotic Bcl family proteins including Bcl-2, Bcl-xl^[27]. In this study, p53 translocated in cytoplasm and bound with Bcl-2 and Bcl-xl by pretreatment with MG132, which indicated that the treatment by combination with compound **11** and proteasome inhibitors significantly increased apoptosis via accumulate p53 in the cytoplasmic, despite degradation of CRM1 protein by compound **11** 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 **11** 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 **11** has a good permeability possibly.

In summary, the results in this work show that compound **11** 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%. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded with a Bruker spectrometer. All reactions were monitored by thin-layer chromatography (TLC) on 25.4 mm \times 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 200~300 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**)

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 drop wise under an nitrogen atmosphere. The reaction was stirred for 15 hours at 50 °C ~ 60 °C. The crude product was purified by chromatography on silica gel with petroleum ether/ethylacetate (10:1) to afford **1a** as a white solid (173 mg, 31.46%). ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₃H₁₄NaO₄ [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; ¹H 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). ¹³C 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 C₁₉H₁₈NaO₃ [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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₃H₁₄NaO₃S [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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₄H₁₆NaO₃ [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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₇H₂₂NaO₃ [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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₈H₂₂NaO₃ [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** (556mg, 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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₅H₁₈NaO₄ [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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (101 MHz, CDCl₃) 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 C₁₃H₁₁F₃NaO₄ [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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₉H₁₈NaO₄ [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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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**. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₆H₂₀NaO₃ [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 (**1m**)

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; ¹H 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). ¹³C 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 C₁₆H₁₄NaO₃ [M+Na]⁺ m/z: 277.0841, found: 277.0842.

4.2.14. Synthesis of (R)-6-((benzo[d][1,3]dioxol-5-yl)oxy)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; ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₃H₁₂NaO₅ [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 H₂O in a flask. Subsequently, the reaction mixture was stirred at room temperature for 30 min. The reaction system was added drop wise to a 50 mL round bottom flask containing (S)-epoxy chloropropane (3 equvi) and tetrabutyl ammonium bromide (0.05 equvi). The reaction mixture was stirred at room temperature. After the reaction completed, the resulting mixture was diluted with water (10 mL) and extracted with ethylacetate (20 mL × 3). The combined organic phases were dried with Na₂SO₄, concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether and ethylacetate 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 drop wise under an argon atmosphere. After the reaction completed, the resulting mixture was diluted with saturated ammonium chloride (50 mL) and the system was filtered with celite under reduced pressure, and then evaporated and extracted with ethylacetate (15 mL × 3). The combined organic phases were dried with Na₂SO₄, concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether and ethylacetate 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 MgSO₄ (2 equvi), and TEA (1.5 equvi) in Anhydrous CH₂Cl₂ (20 mL) was stirred at room temperature. Acryloylchloride (1.5 equvi) was added drop wise. The reaction mixture was stirred at room temperature for 2h. After the reaction completed, the resulting mixture was diluted with water (20 mL) and extracted with CH₂Cl₂ (20 mL × 3), dried (MgSO₄), and concentrated to afford a crude product. The crude product was purified by chromatography on silica 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**)

Grubbs-2 (126 mg, 0.219 mmol) was dissolved in 20 mL anhydrous DCM in adried flask. Subsequently, compound **11a** (380 mg, 1.49 mmol) was dissolved in 2 mL DCM and added drop wise under a nitrogen atmosphere. The reaction was stirred for 15 hours at 50 °C ~ 60 °C. The crude product was purified by chromatography on silica gel with petroleum ether/ethylacetate (10:1) to afford **7a** as a colorless oily liquid (143 mg, 42.28%). ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 pale yellow solid by the same procedure as described for compound **7a**. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₅H₁₅NNaO₃ [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 liquid by the same procedure as described for compound **7a**. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₄H₁₅NNaO₂ [M+Na]⁺ *m/z*: 252.1000, found: 252.1003.

4.6.3. Synthesis of (R)-6-(((6-chloro-1H-benzo[d][1,2,3]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 grey-green solid by the same procedure as described for compound **7a**. m.p. 109.5 ~ 110.1 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.94 (d, 8.8 Hz, 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). ¹³C NMR (101 MHz, CDCl₃) 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 C₁₂H₁₀ClN₃NaO₃ [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 liquid by the same procedure as described for compound **7a**. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₄H₁₇NNaO₂ [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 in a flask at ice-bath. Subsequently, the reaction mixture was stirred at room temperature for 30 min. (S)-epoxy chloropropane (3 equvi) was added drop wise. The reaction mixture was stirred at room temperature. After the reaction 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 Na₂SO₄, 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 drop wise under an argon atmosphere. After the reaction completed, the resulting mixture was diluted with saturated ammonium chloride (50 mL) and the system was filtered with celite in induced pressure, and then evaporated and extracted with ethylacetate (15 mL × 3). The combined organic phases were dried with Na₂SO₄, concentrated to

afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether and ethylacetate to afford **10**.

4.9. General synthesis procedure for analogues of (*R*)-1-(1*H*-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 drop wise. The reaction mixture was stirred at room temperature for 2h. After the reaction completed, the resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (20 mL \times 3), dried (MgSO_4), and concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether/ethylacetate to afford **11**.

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

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

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

A solution of **12** (1.00g, 3.93 mmol) in CH_2Cl_2 (20 mL), subsequently, *m*-chloroperoxybenzoic acid (812mg, 4.71 mmol) is added to the system under ice bath conditions. After the reaction is complete, a mixture of saturated solution of $\text{Na}_2\text{S}_2\text{O}_3$ (10 mL) and NaHCO_3 (10 mL) was added. The organic phase was washed with water (3 \times 10mL) 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; ^1H 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 $\text{C}_{12}\text{H}_{11}\text{ClNaO}_3\text{S}$ [$\text{M}+\text{Na}$] $^+$ 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 drop wise to a 50 mL round bottom flask containing (S)-epoxy chloropropane (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 completed, the resulting mixture was diluted with water (10 mL) and extracted with ethylacetate (20 mL \times 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 and ethylacetate 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 drop wise under an argon atmosphere. After the reaction completed, the resulting mixture was diluted with saturated ammonium chloride (50 mL) and the system was filtered with celite, and then evaporated and extracted with ethylacetate (15 mL \times 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 and ethylacetate 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 drop wise. The reaction mixture was stirred at room temperature for 2h. After the reaction completed,

the resulting mixture was diluted with water (20 mL) and extracted with CH₂Cl₂ (20 mL × 3), dried (MgSO₄), and concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether/ethylacetate to afford **17** (970mg, 83.44%).

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

Compound **21a** (2.49 g, 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 hours. The reaction mixture was filtered and concentrated. The resulting mixture was diluted with water (20 mL) and extracted with ethylacetate (20 mL × 3). The combined organic phases were dried with Na₂SO₄, 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 crystal by the same procedure as described for compound **18a**. m.p. 128.2 ~ 129.3°C. ¹H 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). ¹³C NMR (100 MHz, CDCl₃) 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* = 21.8 Hz), 76.78, 36.62, 34.29, 29.37. HRMS: calcd for C₁₄H₁₅FNO₃ [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 crystal by the same procedure as described for compound **18a**. m.p. 113.1 ~ 114.2°C. ¹H 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). ¹³C 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 C₁₆H₁₇NNaO₃ [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 crystal by the same procedure as described for compound **18a**. m.p. 145.0 ~ 145.7°C. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₂H₁₂ClNNaO₃S [M+Na]⁺ m/z: 308.0124, found: 308.0123.

4.16. General synthesis of N-(2-((2*S*)-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 (56.79mg, 0.33 mmol) was added. The reaction was stirred and heated at 110°C for 6 hours. The reaction mixture was filtered to afford **19a** as a white acicular crystal (728 mg, 41.92%). m.p. 178.8 ~ 179.9°C. ¹H 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). ¹³C 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 C₁₄H₁₇NNaO₄ [M+Na]⁺ m/z: 286.1055, found m/z: 286.1058.

4.16.1. Synthesis of 4-fluoro-N-(2-((2*S*)-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 crystal by the same procedure as described for compound **19a**. m.p. 182.7 ~ 183.6°C. ¹H 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). ¹³C 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 C₁₄H₁₆FNNaO₄ [M+Na]⁺ *m/z*: 304.0961, found: 304.0961.

4.16.2. Synthesis of *N*-(2-((2*S*)-4-hydroxy-6-oxotetrahydro-2*H*-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 crystal by the same procedure as described for compound **19a**. m.p. 171.6 ~ 172.3°C. ¹H 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). ¹³C 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 C₁₆H₁₉NNaO₄ [M+Na]⁺ *m/z*: 312.1212, found: 312.1210.

4.16.3. Synthesis of 5-chloro-*N*-(2-((2*S*)-4-hydroxy-6-oxotetrahydro-2*H*-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 crystal by the same procedure as described for compound **19a**. m.p. 172.4 ~ 173.1°C. ¹H 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). ¹³C 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 C₁₂H₁₄ClNNaO₄S [M+Na]⁺ *m/z*: 326.0230, found: 326.0231.

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

A solution of compound **20** (1 equvi), anhydrous MgSO₄ (2 equvi), and TEA (1.5 equvi) in anhydrous CH₂Cl₂ (20 mL) was stirred at room temperature. Acyl chloride analogue (1.5 equvi) was added drop wise. The reaction mixture was stirred at room temperature for 2h. After the reaction completed, the resulting mixture was diluted with water (20 mL) and extracted with CH₂Cl₂ (20 mL × 3), dried (MgSO₄), and concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with petroleum ether/ethylacetate to afford **21**.

5. Material and Methods

5.1. Reagent and antibodies

Fetal bovine serum (FBS), Roswell Park Memorial Institute 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 bortezomib were purchased from MCE (Med Chem Express, New Jersey, USA). The FITC/Annexin V Apoptosis Detection Kit was purchased from Best Bio (Shanghai, China). The Nuclear and Cytoplasmic Protein Extraction Kit was purchased from Beyotime (Shanghai, China). The ECL Western blotting kit was purchased from Thermo Fisher (Waltham, MA). Antibodies against CRM1, Ranbp1, p53 and Cleaved PARP, were purchased from Cell Signaling Technology (Danvers, MA). Antibodies against Bcl-x1, PARP, LaminB1, Tubulin, Actin, Cleaved caspase-3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against Bax, Bcl-2, p53 siRNA, Cleaved caspase-9 were purchased from 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% CO₂.

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 **11**. Then, 20 μ L of MTT solution (5 mg/mL) was added to the cells in each well, and maintained at 37°C for 4h. Finally, formazan crystals were solubilized with 150 μ L of DMSO, and the absorbance intensity was analyzed with a 96-well plate reader at 490 nm. Every experiment was duplicated three times.

5.4. Immunofluorescence analysis

MGC803 cells were treated with compound **11** and fixed for 30 minutes with 4% paraformaldehyde. Next, cell membranes were permeabilized by 0.1% Triton X-100 for 10 minutes. 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 at a 100-mm tissue culture plate and cultured for 24 h. After culturing, the cells treated with different concentrations of compound **11** 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 \times loading buffer and resolved by 10% or 12% SDS-PAGE and blotted onto PVDF membranes. The membranes were blocked with 5% skimmed milk in PBST for 2 h at 37 °C, they were washed and treated overnight at 4°C with appropriate antibodies, followed by HRP conjugated anti-mouse, anti-goat or anti-rabbit secondary antibodies followed by HRP conjugated anti-mouse, anti-goat or anti-rabbit secondary antibodies for 2 h at 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 **11** for 24 h. Cells then washed with PBS twice, and collected by centrifugation. After that, cells incubated with fluorescein isothiocyanate (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 assayed using 2.5 μ M 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 **11** for 1, 3, 6, 12 and 24 h. After that, cells were collected and stained with 2.5 μ M 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 \pm SD. Statistical evaluation of significant differences was performed using the unpaired Student's t-test. *, ** and *** respectively represent $p < 0.05$, $p < 0.02$ and $p < 0.001$.

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Appendix A. Supplementary data

The Supporting Information, NMR for compounds, is available free of charge on the ACS Publications website.

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Declaration of interests

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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