https://doi.org/10.1016/j.ejmech.2016.09.070

Extending the Structure−Activity Relationship study of Marine Natural Ningalin B Analogues as P-glycoprotein Inhibitors

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Keywords

Ningalin B, Multidrug resistance (MDR); P-glycoprotein (P-gp); P-gp chemosensitizer, ATP-binding cassette (ABC) transporter.

Abstract

In the present study, a total of 25 novel ningalin B analogues were synthesized and evaluated for their P-gp modulating activity in a P-gp overexpressed breast cancer cell line LCC6MDR. Preliminary structure-activity study shows that A ring and its two methoxy groups are important pharmacophores for P-gp modulating activity. Among all derivatives, **23** is the most potent P-gp modulator with EC_{50} of 165 nM in reversing paclitaxel resistance. It is relatively safe to use with selective index greater than 606 as compared to verapamil. Mechanism study demonstrates that compound **23** reverses P-gp mediated drug resistance by inhibiting transport activity of P-gp, thereby restoring intracellular drug accumulation. In summary, our study demonstrates that ningalin B analogue **23** is a non-cytotoxic and effective P-gp chemosensitizer that can be used in the future for reversing P-gp mediated clinical cancer drug resistance.

1. Introduction

P-glycoprotein (P-gp or ABCB1) belongs to the ATP-Binding Cassette (ABC) super-family of proteins[1]. Overexpression of P-gp in cancer patients has been correlated with chemoresistance and poor prognosis. It can pump various anticancer drugs out of cancer cells and reducing intracellular drug concentration below their therapeutic levels[2-5]. P-gp has also been found in highly drug resistant cancer stem cells[6]. Considerable effort has been spent on developing P-gp inhibitors[7-14]. However, only a few non-toxic and specific P-gp inhibitors have been found[15-20] and none of these inhibitors can be used clinically. Therefore, searching for novel P-gp inhibitors with low toxicity and high potency remains an important approach to reverse clinical multidrug resistance (MDR).

Although P-gp was the most characterized ABC family member in terms of its structure and mechanism of action [3, 21, 22], its exact binding sites of inhibitors remains elusive. Recently, non-toxic natural products including curcumin[23], kaempferol[24], quercetin[25], epigallocatechin gallate[26], lamallarine K, lamallarine I, lamallarine O[27]and their derivatives or analogues such as quercetin pentamethyl ether[28], permethyl lamallarine D, permethyl ningalin D and permethyl ningalin B (shown in **Figure 1**)[29-31] have been discovered as a novel source for providing new P-gp modulators[10]. Recently, we have synthesized and characterized analogues of natural marine product ningalin B for their P-gp inhibitory activity. We found that 4 ningalin B analogues have potent P-gp inhibitory activity, were safe to use and specific to P-gp (compounds **1-4** in **Figure 1**)[32-34]. Structure-activity relationship study revealed that substituent at C-ring and the linkers between N atom of pyrroledione and C-ring were important. Compounds **3** and **4** were the most potent and non-cytotoxic lead compounds. In this report, we will study the effects of substituent in rings A and C on P-gp modulating activity.

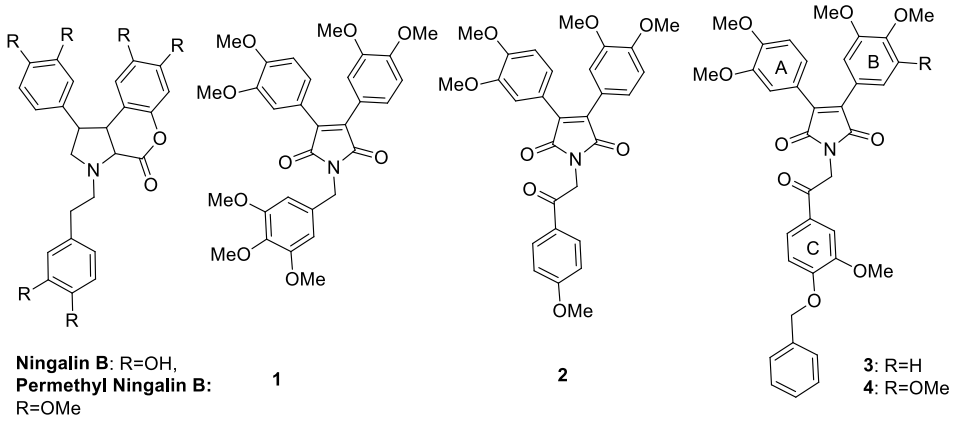


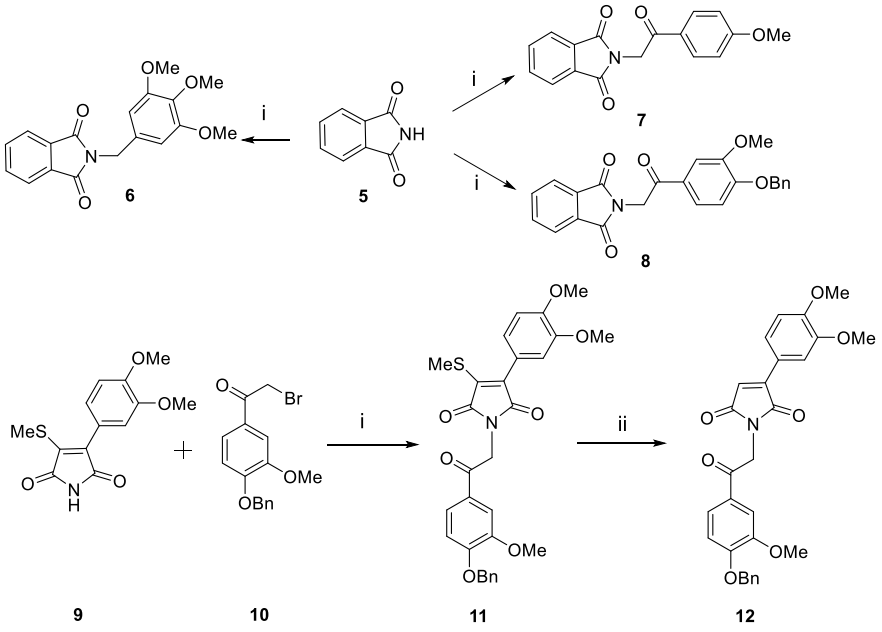
Figure 1. Ningalin B analogues as P-gp inhibitors.

2. Results and Discussion

2.1. Chemistry

As shown in **Scheme 1**, three N-substituted phthalimide derivatives and two N-substituted 3-arylpyrroledione derivatives were synthesized. Phthalimide was reacted with 3,4,5-trimethoxybenzylmethanesulfonate,2-bromo-1-(4-methoxyphenyl)ethanone, and 2-bromo-1-(4-benzoloxy-3-methoxyphenyl)ethanone in the presence of K_2CO_3 in DMF to afford compounds **6**, **7**, and **8** respectively.Synthetic compound **9**[35] was coupled with 2-bromo-1-(4-benzoloxy-3-methoxyphenyl)ethanone in the presence of K_2CO_3 in DMF to produce compound **11** which was then reduced to compound **12.**

Scheme 1. Synthetic route of compounds **6**, **7**, **8**, **11**, and **12.**

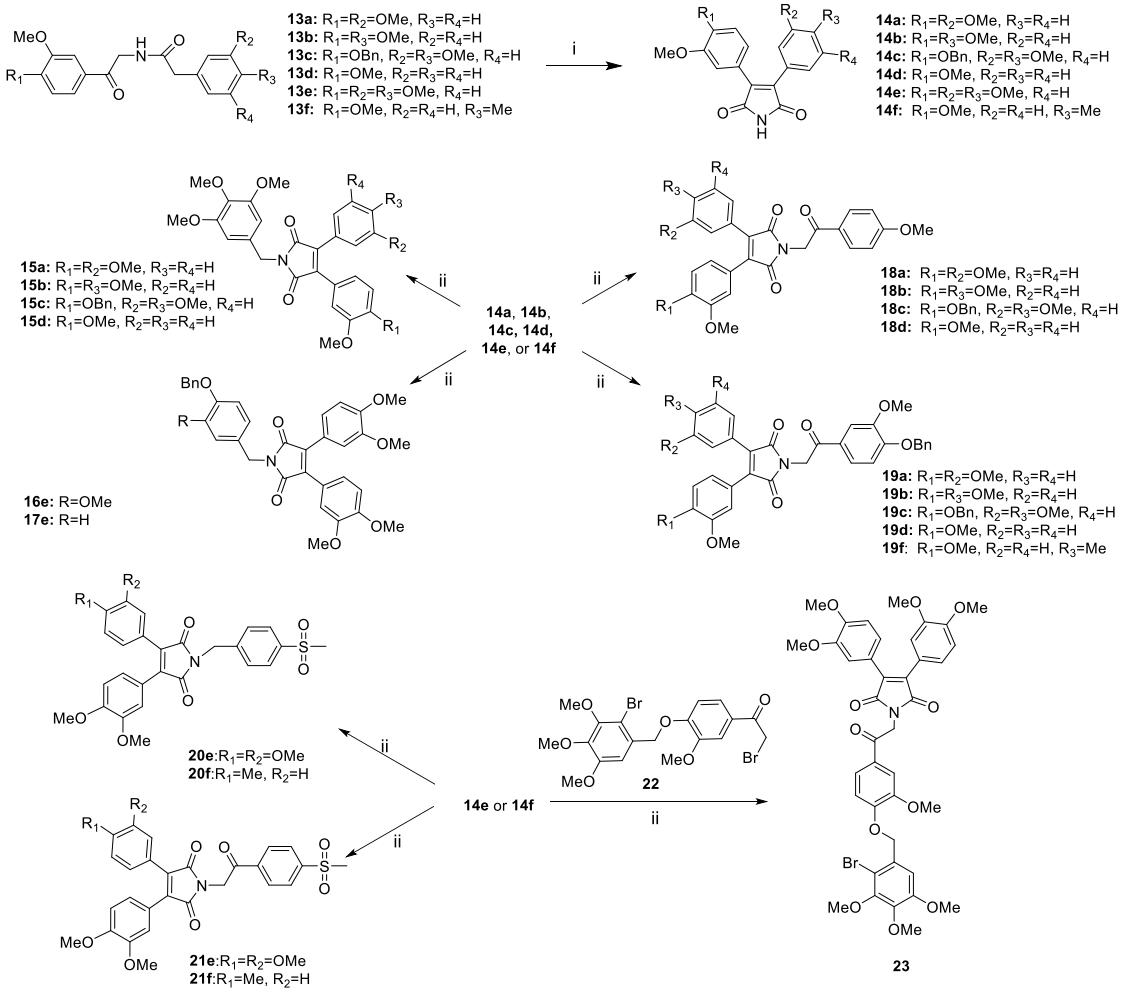


Reagents and conditions: (i) K_2CO_3, DMF,40 °C, 5-6 h; (ii) Zn, MeOH, acetic acid, chloroform, rt, 8 h, then, Et_3N, EtOH, rt.

Synthetic route of compounds **15a-23** is shown in **Scheme 2.** The key intermediates

13a-13f and **14a-14f** were prepared as previously described[33, 34]. 3,4,5-Trimethoxybenzyl methanesulfonate was reacted with **14a**, **14b**, **14c**, or **14d** in the presence of K_2CO_3 in DMF to afford compounds **15a**, **15d**, **15c**, or **15d**, respectively. Similarly, reaction of 4-benzoloxybenzyl methanesulfonate or 3-methoxy-4-benzoloxybenzyl methanesulfonate with **14e** gave compounds **16e** or **17e.** Catalyzed by K_2CO_3, coupling of 2-bromo-1-(4-methoxyphenyl)ethanone with **14a**, **14b**, **14c**,or **14d** produced target compounds **18a**, **18b**, **18c**, or **18d**, respectively. Using the same procedure，compounds **19a**, **19b**, **19c**, **19d**, or **19f** were obtained**. 14e** or **14f** was coupled with 4-(methylsulfonyl)benzyl methanesulfonate, 2-bromo-1-(4-(methylsulfonyl)phenyl)ethanone, or 2-bromo-1-(2-bromo-5-methoxy-4-((3,4,5-trimethoxybenzyl)oxy)phenyl)ethanone (**22**) in the presence of K_2CO_3 in DMF to provide compounds **20e**, **20f**, **21e**, **21f**, or **23** , respectively.

Scheme 2. Synthetic route of compounds **15a-23 .**



Reagents and conditions: (i) t-BuOK, t-BuOH, N_2, 8-10 h, then to O_2, 2 h; (ii) K_2CO_3, DMF, 40 ℃, 6 h.

2.2. P-gp-modulating activity of ningalin B analogues

P-gp-transfected breast cancer cell line MDA435/LCC6MDR was 87.1-fold more resistant to paclitaxel (PTX) than its parental LCC6 cells (Table 1). Based on our lead compounds **1**-**3**[32-34], twenty five new ningalin B analogues were synthesized and divided into 3 series according to their (1) type and number of substituent at rings A and C and (2) type of linker between C1 of ring C and N atom of pyrrole-2,5-dione (**Table 1**).

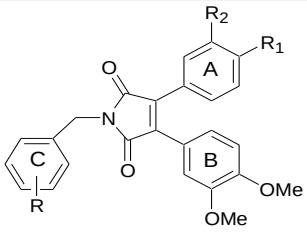
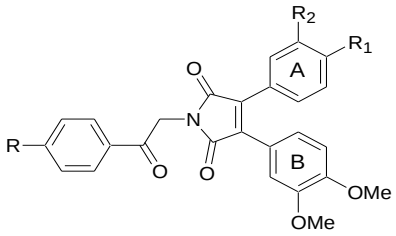
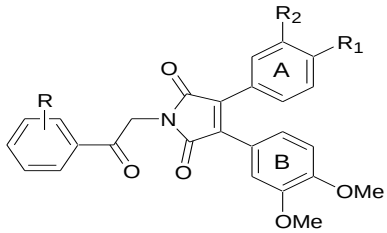
The importance of substitution at rings A and C of ningalin B analogues on P-gp modulating activity was studied. In series I, compound **1** (RF = 18.2) [34] with methylene linker and di-methoxy groups on rings A and B and tri-methoxy groups on ring C was the lead compound. Removal of phenyl rings A and B in compound **6** (RF = 1.2) completely abolished P-gp modulating activity, indicating that methylated polyphenol structure is an important pharmacophore. Reducing the number of methoxy group at phenyl ring A gradually diminished the potency to reverse P-gp-mediated PTX resistance: di-methoxylated compound **1** (RF = 18.2) > mono-methoxylated **15a** (RF = 11.4) and **15b** (RF = 5.1) and non-substituted **15d** (RF = 7.1). Substitution position at ring A is also important because mono-methoxylation at meta-position (**15a** with RF = 11.4) displayed 2-fold higher RF value than that at para-position (**15b** with RF = 5.1). Furthermore, the size of substituent is also important. Smaller para-methoxy group at ring A in compound **1** (RF = 18.2) results in a higher activity than the bulkier group of para-benzyloxy group in compound **15c** (RF = 11.9). This result suggests that smaller substituent is preferred. This effect of substituent size was also observed in ring C among this series. At ring C when trimethoxy groups (compound **1** with RF = 18.2) were replaced by methoxybenzyloxy, benzyloxy and methylsulfonyl substituent, there was a reduction in P-gp modulating activity (compounds **16e**, **17e**, **20e** and **20f** with RF = 9.3, 4.2, 3.4 and 3.0).

Compounds of series II are analogues of lead compound **2**. They have longer carbonylmethylene linker than that of series I (methylene linker). They only have mono-

substitution at ring C instead of tri-substitution as in series I. Compound **2** (RF = 9.9) displayed the weakest P-gp modulating activity compared to the other 2 lead compounds (**1** with RF = 18.2 and **3** with RF = 42.7) [34]. Similar to series I, when rings A and B are removed in compound **7** (RF = 1.7) in series II, the P-gp modulating activity is completely lost. The influence of number and size of substituent on P-gp-modulating activity is investigated in series II. First, di-methoxylation at ring A (**2** with RF = 9.9) displayed better activity than mono-methoxylation (**18a** and **18b** with RF = 3.5 and 2.7) or non-methoxylation (**18d** with RF = 2.6). Second, when the substituent at ring A gradually increased in size from di-methoxy in **2** (RF = 9.9) to benzyloxy in **18c** (RF = 4.5), P-gp modulating activity was reduced. Similar to series I, methylsulfonyl group in ring C was not preferred (**21e** with RF = 1.4 or **21f** with RF = 2.0).

In series III, either removal of both A and B rings or A ring alone consistently resulted in a complete loss of P-gp modulating activity (**8**, **11** and **12** with RF = 1.1, 1.4 and 1.4 respectively), indicating that both rings A and B were important pharmacophores. Same as series I and II, di-substitution at ring A (**3** with RF = 42.7) displayed stronger activity than mono- (**19a**, **19b** and **19f** with RF = 8.8, 22.1 and 8.6) and non-substituted analogues (**19d** with RF = 5.9). Contrary to series I, mono-methoxylation at para-position (**19b** with RF = 22.1) yielded higher RF values than that at meta-position (**19a** with RF = 8.8). Replacing the para-methoxy group of compound **3** (RF = 42.7) at A ring by para-benzyloxy (**19c** with RF = 1.4) or replacing para-methoxy group of **19b** (RF = 22.1) at A ring by para-methyl group (**19f** with RF = 8.6) both caused a marked reduction in the P-gp modulating activity. Surprisingly, additional modification at C ring of compound **3** (RF = 42.7)with a 2-bromo, 3-methoxy and 4-(3,4,5-trimethoxybenzloxy) groups resulted in a highly potent P-gp modulator **23** (RF = 48.0). Further modification at C ring should be considered in the future.

Table 1. P-gp modulating activity, cLogP and PSA of ningalin B analogues

Cpds	Group at A ring R_1	Group at A ring R_2	Groups at C ring (R)	Type of linker	Mean IC_{50} of PTX (nM)	RF
						
	Series I					
1	OMe	OMe	3,4,5-triOMe	methylene	10.1 ± 0.6	18.2[a]
6	No A ring	No B ring	3,4,5-triOMe	methylene	111.5 ± 11	1.2
15a	H	OMe	3,4,5-triOMe	methylene	12.2 ± 0.5	11.4
15b	OMe	H	3,4,5-triOMe	methylene	27.2 ± 2.3	5.1
15c	OBn	OMe	3,4,5-triOMe	methylene	11.7 ± 1.2	11.9
15d	H	H	3,4,5-triOMe	methylene	19.6 ± 0.9	7.1
16e	OMe	OMe	3-OMe, 4-OBn	methylene	14.9 ± 6.7	9.3
17e	OMe	OMe	4-OBn	methylene	33.0 ± 2.2	4.2
20e	OMe	OMe	4-CH_3SO_2	methylene	40.8 ± 3.9	3.4
20f	Me	H	4-CH_3SO_2	methylene	46.3 ± 2.8	3.0
						
	Series II					
2	OMe	OMe	OMe	carbonylmethylene	18.4 ± 1.1	9.9[a]
7	No A ring	No B ring	OMe	carbonylmethylene	110.2 ± 14	1.3
18a	H	OMe	OMe	carbonylmethylene	39.6 ± 7.5	3.5
18b	OMe	H	OMe	carbonylmethylene	52.0 ± 2.3	2.7
18c	OBn	OMe	OMe	carbonylmethylene	31.3 ± 1.7	4.5
18d	H	H	OMe	carbonylmethylene	53.2 ± 7.1	2.6
21e	OMe	OMe	CH_3SO_2	carbonylmethylene	98.0 ± 16	1.4
21f	Me	H	CH_3SO_2	carbonylmethylene	68.9 ± 1.7	2.0
						
	Series III					
3	OMe	OMe	3-OMe, 4-OBn	carbonylmethylene	3.5 ± 0.3	42.7[a]
8	No A ring	No B ring	3-OMe, 4-OBn	carbonylmethylene	129.0 ± 11	1.1
11	SMe (No A ring)		3-OMe, 4-OBn	carbonylmethylene	102.0 ± 7.5	1.4
12	No A ring		3-OMe, 4-OBn	carbonylmethylene	98.6 ± 12	1.4
19a	H	OMe	3-OMe, 4-OBn	carbonylmethylene	15.9 ± 5.7	8.8
19b	OMe	H	3-OMe, 4-OBn	carbonylmethylene	6.3 ± 0.5	22.1
19c	OBn	OMe	3-OMe, 4-OBn	carbonylmethylene	101.7 ± 4.8	1.4
19d	H	H	3-OMe, 4-OBn	carbonylmethylene	23.5 ± 6.2	5.9
19f	Me	H	3-OMe, 4-OBn	carbonylmethylene	16.2 ± 2.6	8.6
23	OMe	OMe	2-Bromo, 3-OMe, 4-(3,4,5-triOMe)-OBn	carbonylmethylene	2.9 ± 0.2	48.0
LCC6					1.6 ± 0.3	87.1
LCC6MDR					139.3 ± 7.5	1.0

Ningalin B analogues are divided into 3 series according to their R_1 and R_2 groups at ring A, R

group at ring C and types of linker used. Lead compounds **1**, **2** and **3** of series I, II and III, respectively have been reported previously and they were used as starting points for modification[34]. P-gp-modulating activity was measured by determining IC_{50} towards PTX in LCC6MDR cells in the absence or presence of 1.0 μM of modulator. At 1.0 μM, none of the modulators displayed any cytotoxicity towards LCC6MDR cells was found. Relative Fold (RF) reflects P-gp-modulating activity and is calculated as [IC_{50} of PTX without modulator / IC_{50} with 1.0 μM modulator]. All modulators were dissolved in DMSO. Each experiment was done in triplicates and repeated at least three times. IC_{50} values are presented as mean ± standard error of mean. [a]These data have been reported previously[34].

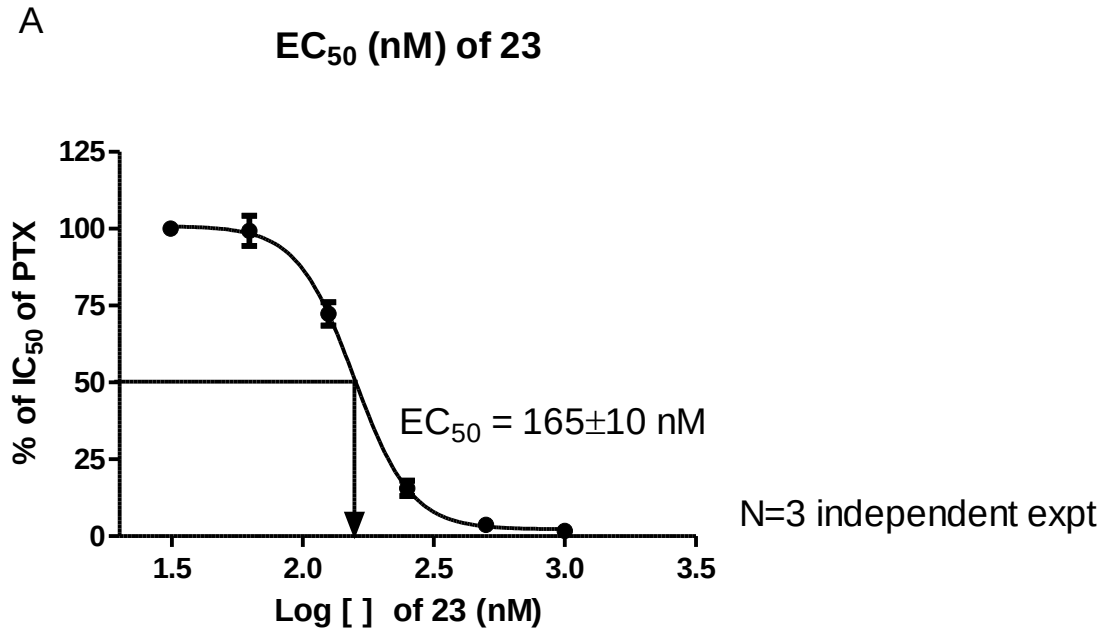
*2.3. EC_{50} (nM) and selective index values of ningalin B analog **23***

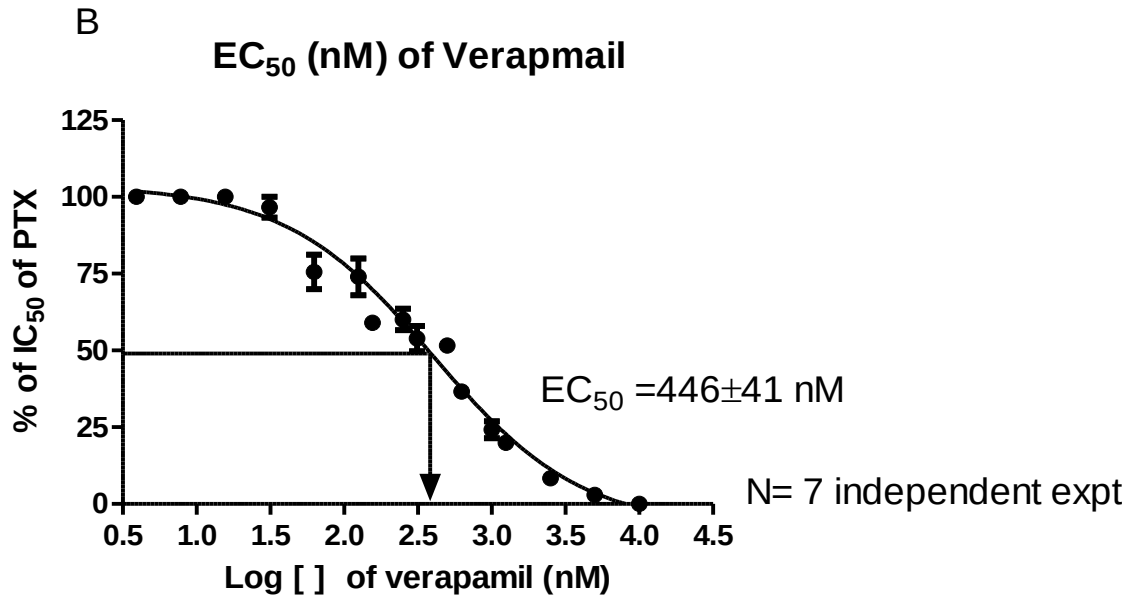
We have chosen one potent ningalin analogue **23** for further characterization in terms of its effective concentration (EC_{50}) in reversing P-gp-mediated PTX resistance and its selective index (**Table 2**). Compound **23** was non-cytotoxic towards L929 normal fibroblasts with IC_{50} greater than 100 μM (**Table 2**). Its EC_{50} for reversing P-gp mediated resistance towards PTX was165 nM, which is about 2.7-fold more potent than verapamil (EC_{50} = 446 nM) (**Table 2**). After considering toxicity, selective index of **23** was greater than 606, which is about 3-fold higher than verapamil (selective index = 200). Overall, **23** is a non-cytotoxic and effective P-gp chemosensitizer.

Table 2. Effective concentration EC_{50} (nM) and selective index value of **23** in reversing PTX resistance of LCC6MDR.

Cpds	**Cytotoxicity towards L929 (IC_{50}, μM)**	**Reversing PTX resistance in LCC6MDR (EC_{50}, nM)**	**Selective index**
23	>100	165 ±10	> 606
Verapamil	89±8	446 ± 41	200

EC_{50} values were presented as mean ± standard error of mean. N= 3-4 independent experiments. Each experiment was done in triplicate. Verapamil was used as positive control. Selective index value = (IC_{50} of modulators towards L929 fibroblasts) / (EC_{50} of modulators for reversing PTX resistance in LCC6MDR cells).





*2.4. Ningalin B analogue **23** increases DOX and rhodamine 123 accumulation in LCC6MDR cells*

Doxorubicin (DOX) and rhodamine 123 are known fluorescent P-gp substrates. We found that LCC6 cells accumulated about 2.6- and 8.0-fold more DOX and rhodamine 123 than

LCC6MDR cells (**Figure 3A** and **3B**). Treatment of LCC6MDR cells with 2 μM of **23** or verapamil can inhibit P-gp and increase DOX accumulation by 2.4- or 2.3-fold to a level similar to that of LCC6 cells (**Figure 3A**). Treating LCC6MDR cells with 2 μM of **23** or verapamil partially restored rhodamine 123 accumulation by 4.8- and 2.7-fold, respectively (**Figure 3B**). Compound **23** was 1.7-fold more potent than verapamil in inhibiting P-gp-mediated transport of rhodamine 123.

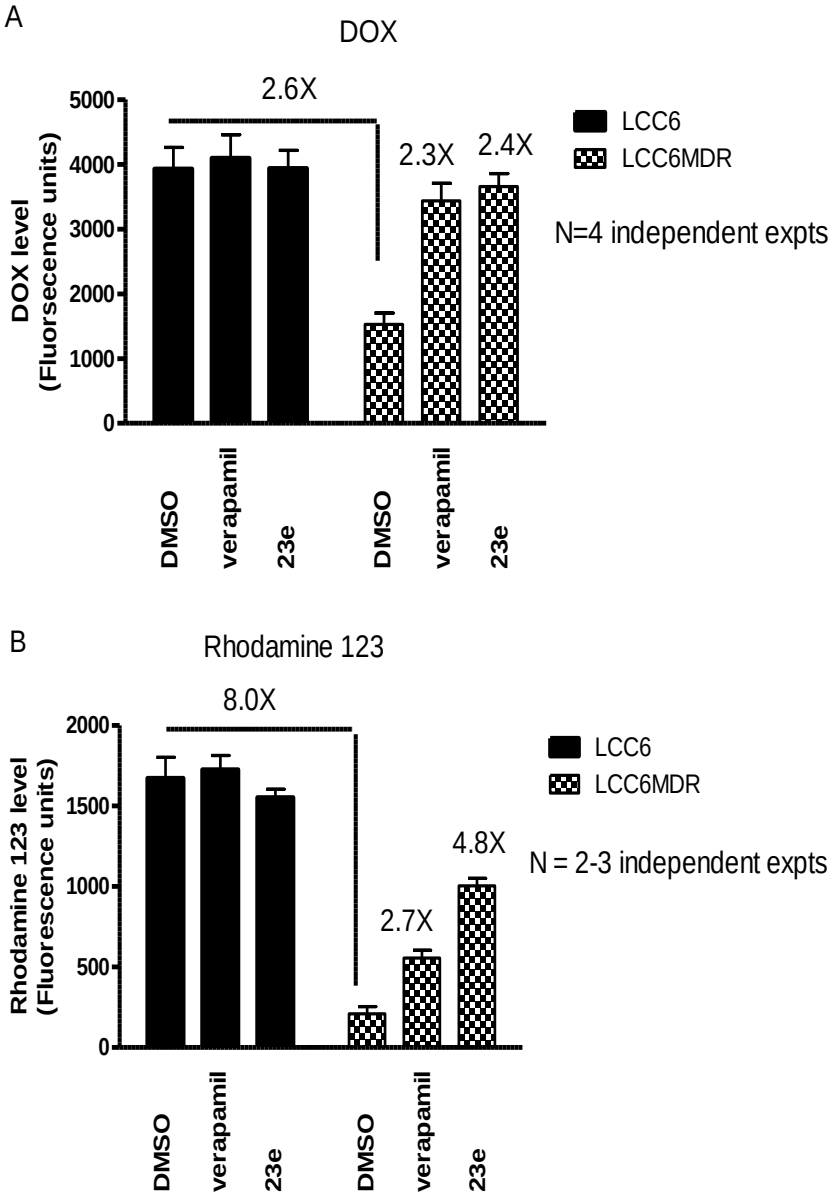


Figure 3. Effect of **23** on DOX and rhodamine 123 accumulation in LCC6MDR cells.

Effect of compound **23** or verapamil on intracellular DOX and rhodamine 123 was studied. LCC6 or LCC6MDR cells were incubated with20 μM DOX (**A**) with or without 2 μM of

modulators or 10 μg/mL rhodamine 123 (**B**) with or without 2 μM of modulator for 150 minutes at 37ºC. 0.2% of DMSO was used as negative control. After the incubation period, cells were lysed and the DOX level in supernatant was measured by spectrofluorometry. N = 2-4 independent experiments and values were presented as mean ± standard error of mean.

3. Conclusion

In the present study, a total of 25 novel ningalin B analogs were synthesized and characterized for their P-gp modulating activity in a P-gp overexpressed breast cancer cell line LCC6MDR. Several important pharmacophores for modulating P-gp were found including (1) phenyl rings A and B, (2) di-methoxylation at rings A and (3) tri-substitution at ring C with ortho-bromo, meta-methoxy and para-trimethoxybenzyloxy groups. Among all ningalin B derivatives, **23** with dimethoxy groups at rings A and B and tri-substitution at ring C with ortho-bromo, meta-methoxy, and para-trimethoxybenzyloxy groups is the most potent P-gp inhibitor with EC_{50} of 165 nM in reversing PTX resistance (**Table 2**). It is safe with selective index greater than 606 compared to verapamil (**Table 2**). The mechanism of **23** in reversing P-gp mediated drug resistance is by virtue of inhibiting transport activity of P-gp and restoring intracellular drug accumulation (**Figure 3**). In summary, our study demonstrates that ningalin B analogue **23** is non-cytotoxic and effective P-gp chemosensitizer that can be used in future for reversing P-gp mediated clinical cancer drug resistance.

4. Experimental Section

General. All non-aqueous reactions were carried out under nitrogen atmosphere in freshly distilled anhydrous solvents. Starting materials and reagents were reagent-grade and were used without further purification unless otherwise stated. Solvents were dried according to standard procedures. Analytical thin-layer chromatography (TLC) was performed on pre-coated plates (silica gel 60 F_{254}) purchased from Merck KGaA and they were visualized under short (254 nm) and long (365 nm) UV light. Column chromatography was carried out using silica gel (200–300 mesh). Melting points were recorded on a micro melting point apparatus MP-500D and were uncorrected. All NMR measurements were carried out at room temperature and the chemical shifts are reported as parts per million (ppm) in δ units relative to the resonance of

$CDCl_3$ (7.26 ppm in the ^1H, 77.0 ppm for the central line of the triplet in the ^{13}C modes). Melting points were recorded on a micro melting point apparatus MP-500D and were uncorrected. High-resolution (ESI) MS spectra were performed with a QTOF-2 Micromass spectrometer.

2-(3,4,5-trimethoxybenzyl)isoindoline-1,3-dione (6)

A mixture of compound **5** (100 mg, 0.68 mmol), 3,4,5-trimethoxybenzyl methanesulfonate (227 mg, 0.82 mmol) and K_2CO_3 (282 mg, 2.04 mmol) in anhydrous DMF (20 mL) was stirred at room temperature for 6 h. The resulting solution was poured into water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/PE = 1/2, v/v) to afford the desired compounds **6** (161 mg, 73%) as white solid; mp114-116°C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.84 (m, 2 H), 7.70 (m, 2 H), 6.70 (s, 2 H), 4.75 (s, 2 H), 3.86 (s, 6 H), 3.79 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.0, 153.3, 134.0, 132.0, 128.4, 106.0, 60.8, 56.1, 41.9; HRMS calcd for $(C_{18}H_{17}O_5N + H)^+$ 328.1179, found 328.1185.

2-(2-(4-methoxyphenyl)-2-oxoethyl)isoindoline-1,3-dione (7)

7 was prepared as described for the synthesis of **6** using **5** (100 mg, 0.68 mmol), 2-bromo-1-(4-methoxyphenyl)ethanone (188 mg, 0.82 mmol) and K_2CO_3 (282 mg, 2.04 mmol). Yield 78%; mp140-142°C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.98 (d, J = 8.6 Hz, 2 H), 7.86 (m, 2 H), 7.74 (m, 2 H), 6.97 (d, J = 8.6 Hz, 2 H), 5.08 (s, 2 H), 3.88 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.3, 167.9, 164.2, 134.3, 134.1, 132.6, 132.3, 130.4, 127.4, 123.5, 114.1, 55.5, 43.9, 29.7; HRMS calcd for $(C_{17}H_{13}O_4N + H)^+$ 296.0917, found 296.0925.

2-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)isoindoline-1,3-dione (8)

8 was prepared as described for the synthesis of **6** using **5** (100 mg, 0.68 mmol), 1-(4-(benzyloxy)-3-methoxyphenyl)-2-bromoethanone (275 mg, 0.82 mmol) and K_2CO_3 (282 mg, 2.04 mmol); Yield 71%; mp146-148°C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.90 (dd, J = 3.0, 5.0 Hz, 2 H), 7.75 (dd, J = 3.0, 5.0 Hz, 2 H), 7.58 (d, J = 8.4 Hz, 1 H), 7.54 (s, 1 H), 7.44 (d, J = 7.4 Hz, 2 H), 7.39 (t, J = 7.4 Hz, 2 H), 7.33 (t, J = 7.2 Hz, 1 H), 6.94 (d, J = 8.4 Hz, 1 H), 5.26 (s, 2 H), 5.08 (s, 2 H), 3.93 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.5, 168.0, 153.1, 149.8, 136.0, 134.1, 132.3, 128.7, 128.2, 127.8, 127.2, 123.5, 122.5, 112.3, 110.7, 70.9, 56.1, 43.9, 29.7; HRMS calcd for $(C_{24}H_{19}O_5N + H)^+$ 402.1336, found 402.1342.

1-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)-3-(3,4-dimethoxyphenyl)-4-(methylthio)-1*H*-pyrrole-2,5-dione (11)

A mixture of compound 3-(3,4-dimethoxyphenyl)-4-(methylthio)-1H-pyrrole-2,5-dione **9** (300 mg, 1.07 mmol), 1-(4-(benzyloxy)-3-methoxyphenyl)-2-bromoethanone **10** (432 mg, 1.28 mmol) and K_2CO_3 (443 mg, 3.21 mmol) in anhydrous DMF (40 mL) was stirred at room temperature overnight. The resulting solution was poured into water (200 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/2, v/v/v) to afford the desired compounds **11** (354 mg, 62% yield) as yellow solid; mp 125-127°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54 (m, 2H), 7.40 (m, 6H), 7.32 (t, J = 7.2 Hz, 1H), 6.94 (dd, J = 12.7, 8.2 Hz, 2H), 5.24 (s, 2H), 4.95 (s, 2H), 3.93 (s, 3 H),3.92 (s, 3 H), 3.91 (s, 3 H) 2.72 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ189.8, 168.8, 167.8, 153.1, 150.2, 149.7, 148.5, 135.9, 135.7, 133.6, 128.7, 128.2, 127.6, 127.1, 123.6, 122.5, 121.8, 112.5, 112.2, 110.6, 110.5, 77.0, 76.7, 70.8, 56.0, 55.9, 55.8, 44.1, 15.8; HRMS calcd for $(C_{29}H_{27}O_7NS + H)^+$ 534.1581, found 534.1580.

1-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)-3-(3,4-dimethoxyphenyl)-1*H*-pyrrole-2,5-dione (**12**)

To a solution of compounds **11** (300 mg, 0.56 mmol) in methanol (20 mL), acetic acid (20 mL) and chloroform (5 mL) was added Zn (109 mg, 1.68 mmol). The reaction mixture was stirred for 8 h at room temperature, and then concentrated. Then, the residue was dissolved in ethanol (30 mL) and triethylamine (2 mL) was added, after stirred at room temperature for 10 h, the reaction mixture was poured into water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/PE = 1/2, v/v) to afford the desired compounds **12.**Yield 38%; mp 148-150 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.54 (m, 2H),7.40 (m, 6H), 7.32 (t, J = 7.1 Hz, 1H), 6.94 (m, 2 H), 6.72 (s, 1 H), 5.25 (s, 2 H), 4.95 (s, 2 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ189.8, 168.8, 167.8, 153.1, 150.2, 149.7, 148.5, 135.9, 135.7, 133.6, 128.7, 128.2, 127.6, 127.1, 123.6, 122.5, 121.8, 112.5, 112.2, 110.6, 110.5, 77.0, 76.7, 70.8, 56.0, 55.9, 55.8, 44.1; HRMS calcd for $(C_{28}H_{25}O_7N + H)^+$ 488.1704, found 488.1700.

3-(3,4-dimethoxyphenyl)-4-(3-methoxyphenyl)-1*H*-pyrrole-2,5-dione (14a)

Under a N_2 atmosphere, t-BuOK (2452 mg, 21.84 mmol) was added to a stirring solution of compound **13a** (2500 mg, 7.28 mmol) in t-BuOH (50 mL) at 0°C. Then the reaction was

allowed to slowly warm to room temperature. After 8-12 h, the reaction solution was exposed to air. After 2 h, the resulting reaction solution was poured into ice-cold water (300 mL), and then the pH was adjusted to 7 by adding 2 N hydrochloric acid to give a thick suspension. The above suspension was filtered and purified by flash chromatography on silica gel to afford compound **14a** (963 mg, 2.84 mmol); mp 187-189 °C; ESI-MS m/z $[M + H]^+$ 340.1; ^{1}H NMR ($CDCl_3$, 600 MHz) δ7.81 (s, 1H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.28 (m, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 7.03(m, 1H), 6.98 (d, *J* = 1.9 Hz, 1H), 6.93 (dd, *J* = 8.3, 2.6 Hz, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.74 (s, 3H), 3.64 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ170.7, 170.5, 159.6, 150.8, 148.7, 136.8, 135.1, 130.1, 129.8, 124.0, 122.2, 120.8, 115.7, 115.0, 112.7, 111.0, 77.3, 77.1, 76.9, 56.0, 55.7, 55.3.

3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-1*H*-pyrrole-2,5-dione (14b)

Following the procedure for the preparation of compound **14a**, but using compound **13b** as the starting material, the desired compound **14b** was obtained: yield 41%; mp 182-184 °C; ESI-MS m/z $[M + H]^+$ 340.1; ^{1}H NMR ($CDCl_3$, 600 MHz) δ8.11 (s, 1H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.21 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 3H), 3.68 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ171.0, 160.7, 150.4, 148.7, 135.0, 134.8, 131.5, 123.5, 121.2, 121.0, 114.0, 112.4, 111.0, 55.7, 55.3.

3-(4-(benzyloxy)-3-methoxyphenyl)-4-(3,4-dimethoxyphenyl)-1*H*-pyrrole-2,5-dione (14c)

Following the procedure for the preparation of compound **14a**, but using compound **13c** as the starting material, the desired compound **14c** was obtained: yield 44%; mp 188-190 °C; ESI-MS m/z $[M + H]^+$ 446.1; ^{1}H NMR ($CDCl_3$, 500 MHz)δ7.86 (s, 1H), 7.41 (d, *J* = 7.5 Hz, 2H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.22 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.12 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.06 (s, 1H), 6.99 (s, 1H), 6.86 (dd, *J* = 8.4, 5.3 Hz, 2H), 5.18 (s, 2H), 3.90 (s, 3H), 3.73 (s, 3H), 3.65 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ170.8, 150.4, 149.5, 149.2, 148.6, 136.4, 135.0, 134.9, 128.6, 128.0, 127.1, 123.6, 123.3, 121.5, 121.1, 113.2, 113.0, 112.4, 110.9, 70.7, 55.9, 55.7.

3-(3,4-dimethoxyphenyl)-4-phenyl-1*H*-pyrrole-2,5-dione (14d)

Following the procedure for the preparation of compound **14a**, but using compound **13d** as the starting material, the desired compound **14d** was obtained: yield 42%; mp 207-209 °C; ESI-MS m/z $[M + H]^+$ 309.1; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.89 (s, 1H), 7.51 (m, 2H), 7.38 (d, *J*

= 3.1 Hz, 3H), 7.27 (s, 1H), 6.94 (s, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.62 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.7, 170.6, 150.7, 148.6, 136.6, 135.2, 129.8, 129.7, 128.8, 128.6, 123.8, 120.8, 112.5, 110.9, 55.8, 55.5.

3-(3,4-dimethoxyphenyl)-4-(p-tolyl)-1*H*-pyrrole-2,5-dione (14f)

Following the procedure for the preparation of compound **14a**, but using compound **13f** as the starting material, the desired compound **14f** was obtained: yield 40%; mp 205-207 °C; ESI-MS m/z $[M + H]^+$ 324.1; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.65 (s, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.23 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 1.9 Hz, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.66 (s, 3H), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.7, 170.6, 150.5, 148.6, 140.0, 135.8, 135.4, 129.7, 129.3, 125.8, 123.6, 121.0, 112.5, 110.9, 55.8, 55.6, 29.6, 21.4.

3-(3,4-dimethoxyphenyl)-4-(3-methoxyphenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-pyrrole-2,5-dione (15a)

To a solution of compound **14a** (100mg, 0.29 mmol) and 3,4,5-trimethoxybenzyl methanesulfonate (96 mg, 0.35 mmol) in dry DMF (20 mL) was added K_2CO_3 (120 mg, 0.87 mmol). The reaction mixture was heated to 40 °C for 5-6 h and then poured into water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/2, v/v/v) to afford the desired compounds **15a** (108 mg, 72%); mp 115-117°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.28 (m, 2 H), 7.04 (d, *J* = 7.8 Hz, 2 H), 6.99 (s, 1 H), 6.92 (d, *J* = 7.8 Hz, 2 H), 6.84 (d, *J* = 8.4 Hz, 1 H), 6.72 (s, 2 H), 4.71 (s, 2 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.83 (s, 3 H), 3.73 (s, 3 H), 3.64 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.6, 170.5, 159.6, 153.3, 150.7, 148.6, 137.7, 135.9, 134.2, 132.1, 130.3, 129.7, 123.8, 122.2, 121.2, 121.0, 115.6, 114.9, 112.7, 110.9, 106.2, 60.8, 56.2, 55.9, 55.6, 55.3, 42.3, 29.7; HRMS calcd for $(C_{29}H_{29}O_8N + H)^+$ 520.1966, found 520.1981.

3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-pyrrole-2,5-dione (15b)

Following the procedure for the preparation of compound **15a**, but using compound **14b** as the starting material, the desired compound **15b** was obtained: yield 69%; mp 59-61°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 (d, *J* = 8.5 Hz, 2 H), 7.20 (d, *J* = 8.4 Hz, 1 H), 6.99 (s, 1 H),

6.88 (d, J = 8.5 Hz, 2 H), 6.85 (d, J = 8.4 Hz, 1 H), 6.72 (s, 2 H), 4.69 (s, 2 H), 3.88 (s, 3 H), 3.86 (s, 6 H), 3.83 (s, 6 H), 3.67 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.9, 160.7, 153.3, 150.4, 148.7, 134.3, 134.1, 132.2, 131.5, 123.5, 121.4, 121.2, 114.0, 112.5, 111.0, 106.2, 60.8, 56.2, 55.8, 55.3, 42.2, 29.7; HRMS calcd for $(C_{29}H_{29}O_8N + H)^+$ 520.1966, found 520.1972.

3-(4-(benzyloxy)-3-methoxyphenyl)-4-(3,4-dimethoxyphenyl)-1-(3,4,5-tri methoxybenzyl)-1*H*-pyrrole-2,5-dione(15c)

Following the procedure for the preparation of compound **15a**, but using compound **14c** as the starting material, the desired compound **15c** was obtained: yield 70%; mp 59-61°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (d, J = 7.3 Hz, 2 H), 7.35 (t, J = 7.4 Hz, 2 H), 7.29 (t, J = 7.2 Hz, 1 H), 7.22 (dd, J = 1.8, 8.4 Hz, 1 H), 7.12 (dd, J = 1.8, 8.4 Hz, 1 H), 7.06 (d, J = 1.8 Hz, 1 H), 7.00 (d, J = 1.8 Hz, 1 H), 6.85 (dd, J = 5.6, 8.4 Hz, 2 H), 6.71 (s, 2 H), 5.17 (s, 2 H), 4.69 (s, 2 H), 3.89 (s, 3 H), 3.85 (s, 6 H), 3.82 (s, 3 H), 3.72 (s, 3 H), 3.64 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.8, 153.3, 150.4, 149.5, 149.2, 148.6, 137.6, 136.5, 134.2, 132.2, 128.6, 128.1, 127.2, 123.6, 123.3, 121.7, 121.3, 113.3, 113.1, 112.5, 110.9, 106.1, 70.7, 60.8, 56.2, 55.8, 42.2; HRMS calcd for $(C_{36}H_{35}O_9N + Na)^+$ 648.2204, found 648.2215.

3-(3,4-dimethoxyphenyl)-4-phenyl-1-(3,4,5-trimethoxybenzyl)-1*H*-pyrrole-2,5-dione (15d)

Following the procedure for the preparation of compound **15a**, but using compound **14d** as the starting material, the desired compound **15d** was obtained: yield 69%; mp 65-66°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.46 (s, 2 H), 7.36 (s, 3 H), 7.24 (s, 1 H), 6.95 (s, 1 H), 6.83 (d, J = 8.5 Hz, 1 H), 6.72 (s, 2 H), 4.70 (s, 2 H), 3.88 (s, 3 H), 3.86 (s, 6 H), 3.82 (s, 3 H), 3.60 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.6, 153.3, 150.7, 148.6, 137.6, 135.8, 134.4, 132.1, 129.7, 129.1, 128.6, 123.8, 121.0, 112.6, 110.9, 106.2, 60.8, 56.2, 55.9, 55.6, 42.3; HRMS calcd for $(C_{28}H_{27}O_7N + H)^+$ 490.1860, found 490.1870.

1-(4-(benzyloxy)-3-methoxybenzyl)-3,4-bis(3,4-dimethoxyphenyl)-1*H*-pyrrole-2,5-dione (16e)

To a solution of compound **14e** (100mg, 0.27 mmol) and 4-(benzyloxy)-3-methoxybenzyl methanesulfonate (105 mg, 0.32 mmol) in dry DMF (20 mL) was added K_2CO_3 (112 mg, 0.81 mmol). The reaction mixture was heated to 40 °C for 5-6 h and then poured into water (100mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was

removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/3, v/v/v) to afford the desired compounds **16e** (125 mg, 78%); mp 116-118°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (d, *J* = 7.2 Hz, 2 H), 7.34 (t, *J* = 7.2 Hz, 2 H), 7.29 (d, *J* = 7.0 Hz, 1 H), 7.19 (d, *J* = 8.4 Hz, 2 H), 7.03 (s, 3 H), 6.95 (d, *J* = 8.1 Hz, 1 H),6.83 (t, *J* = 8.9 Hz, 3 H), 5.13 (s, 2 H), 4.70 (s, 2 H), 3.89 (s, 9 H), 3.70 (s, 6 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.8, 150.4, 149.6, 148.7, 137.1, 134.1, 129.7, 128.5, 127.8, 127.2, 123.6, 121.4, 113.8, 112.8, 112.6, 110.9, 70.9, 56.1, 55.8, 41.7, 29.7; HRMS calcd for $(C_{35}H_{33}O_8N + H)^+$ 596.2279, found 596.2296.

1-(4-(benzyloxy)benzyl)-3,4-bis(3,4-dimethoxyphenyl)-1*H*-pyrrole-2,5-dione (17e)

Following the procedure for the preparation of compound **16e**, but using compound 4-(benzyloxy)benzyl methanesulfonate as the starting material, the desired compound **17e** was obtained: yield 66%; mp 131-133°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.40 (m, 5H), 7.30 (m, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.04 (s, 2H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 5.04 (s, 2H), 4.72 (s, 2H), 3.89 (s, 6H), 3.70 (s, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ170.8, 158.4, 150.3, 148.6, 136.8, 134.1, 130.2, 129.1, 128.5, 127.9, 127.4, 123.5, 121.4, 114.8, 112.5, 110.9, 77.2, 77.0, 76.7, 69.9, 55.8, 55.7, 41.2; HRMS calcd for $(C_{34}H_{31}O_7N + H)^+$ 566.2173, found 566.2189.

3-(3,4-dimethoxyphenyl)-4-(3-methoxyphenyl)-1-(2-(4-methoxyphenyl)-2-oxoethyl)-1*H*-pyrrole-2,5-dione (18a)

To a solution of compound **14a** (100mg, 0.29 mmol) and 2-bromo-1-(4-methoxyphenyl)ethanone (80 mg, 0.35 mmol) in dry DMF (20 mL) was added K_2CO_3 (120 mg, 0.87 mmol). The reaction mixture was heated to 40 °C for 5-6 h and then poured into water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/3, v/v/v) to afford the desired compounds **18a** (102 mg, 72%); mp 130-132°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.98 (d, *J* = 8.6 Hz, 2 H), 7.29 (d, *J* = 6.8 Hz, 2 H), 7.08 (m, 3 H), 6.97 (d, *J* = 8.6 Hz, 2 H), 6.92 (d, *J* = 8.1 Hz, 1 H), 6.84 (d, *J* = 8.4 Hz, 1 H), 5.03 (m, 2 H), 3.90 (m, 3 H), 3.88 (m, 3 H), 3.74 (m, 3 H), 3.66 (m, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.8, 170.7, 170.4, 164.2, 159.5, 150.6, 148.6, 136.4, 134.7, 130.4, 129.6, 127.4, 123.9, 122.3, 121.1, 115.8, 114.8, 114.1, 112.8, 110.9, 55.9, 55.6, 55.3, 44.2, 29.7; HRMS calcd for $(C_{28}H_{25}O_7N + H)^+$ 488.1704, found 488.1716.

3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-1-(2-(4-methoxyphenyl)-2-oxoethyl)-1*H*-pyrrole-2,5-dione (18b)

Following the procedure for the preparation of compound **18a**, but using compound **14b** as the starting material, the desired compound **18b** was obtained: yield 77%; mp 99-101°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97 (d, *J* = 8.6 Hz, 2 H), 7.53 (d, *J* = 8.6 Hz, 2 H), 7.23 (d, *J* = 8.4 Hz, 1 H), 7.06 (s, 1 H), 6.96 (d, *J* = 8.6 Hz, 2 H), 6.88 (d, *J* = 8.6 Hz, 2 H), 6.85 (d, *J* = 8.4 Hz, 1 H), 5.02 (s, 2 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.82 (s, 3 H), 3.69 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.9, 170.9, 164.1, 160.7, 150.4, 148.7, 134.7, 134.5, 131.6, 130.4, 127.5, 123.5, 121.5, 114.0, 112.6, 111.0, 55.8, 55.5, 55.3, 44.1; HRMS calcd for $(C_{28}H_{25}O_7N + H)^+$ 488.1704, found 488.1717.

3-(4-(benzyloxy)-3-methoxyphenyl)-4-(3,4-dimethoxyphenyl)-1-(2-(4-methoxy- phenyl)-2-oxoethyl)-1*H*-pyrrole-2,5-dione (18c)

Following the procedure for the preparation of compound **18a**, but using compound **14c** as the starting material, the desired compound **18c** was obtained: yield 71%; mp 66-68°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.98 (d, *J* = 8.9 Hz, 2 H), 7.42 (d, *J* = 7.3 Hz, 2 H), 7.36 (t, *J* = 7.4 Hz, 2 H), 7.30 (t, *J* = 7.3 Hz, 1 H), 7.25 (m, 1 H), 7.15 (dd, *J* = 2.0, 8.4 Hz, 1 H), 7.12 (d, *J* = 1.9 Hz, 1 H), 7.06 (d, *J* = 1.9 Hz, 1 H), 6.97 (d, *J* = 8.9 Hz, 2 H), 6.86 (dd, *J* = 4.7, 8.4 Hz, 2 H), 5.18 (s, 2 H), 5.02 (s, 2 H), 3.89 (s, 3 H), 3.74 (s, 3 H), 3.66 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.8, 170.8, 164.1, 150.3, 149.4, 149.2, 148.6, 136.6, 134.6, 130.4, 128.6, 128.0, 127.4, 127.2, 123.7, 123.4, 121.8, 121.4, 114.1, 113.2, 112.6, 110.9, 70.7, 55.8, 55.6, 44.1; HRMS calcd for $(C_{35}H_{31}O_8N + H)^+$ 594.2122, found 594.2132.

3-(3,4-dimethoxyphenyl)-1-(2-(4-methoxyphenyl)-2-oxoethyl)-4-phenyl-1*H*-pyrrole-2,5-dione (18d)

Following the procedure for the preparation of compound **18a**, but using compound **14d** as the starting material, the desired compound **18d** was obtained: yield 80%; mp 167-169°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.99 (d, *J* = 8.6 Hz, 2 H), 7.53 (d, *J* = 3.4 Hz, 2 H), 7.38 (s, 3 H), 7.29 (d, *J* = 8.4 Hz, 1 H), 7.02 (s, 1 H), 6.98 (d, *J* = 8.6 Hz, 2 H), 6.85 (d, *J* = 8.4 Hz, 1 H), 5.04 (s, 2 H), 3.89 (s, 6 H), 3.63 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.8, 170.7, 170.5, 164.1, 150.6, 148.6, 136.2, 134.8, 130.4, 129.9, 129.6, 129.1, 128.5, 127.5, 123.8, 121.1, 114.1, 112.7, 110.9, 55.9, 55.6, 44.2; HRMS calcd for $(C_{27}H_{23}O_6N + H)^+$ 458.1598, found 458.1609.

1-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)-3-(3,4-dimethoxyphenyl)-4-(3-methoxyphenyl)-1*H*-pyrrole-2,5-dione (19a)

To a solution of compound **14a** (100mg, 0.29 mmol) and 1-(4-(benzyloxy)-3-methoxyphenyl)-2-bromoethanone (117 mg, 0.35 mmol) in dry DMF (20 mL) was added K_2CO_3 (120 mg, 0.87 mmol). The reaction mixture was heated to 40 °C for 5-6 h and then poured into water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/3, v/v/v) to afford the desired compounds **18a** (143 mg, 83%); mp 130-132°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.56 (m, 2 H), 7.44 (d, *J* = 7.5 Hz, 2 H), 7.39 (t, *J* = 7.4 Hz, 2 H), 7.34 (d, *J* = 7.2 Hz, 1 H), 7.29 (m, 2 H), 7.09 (m, 2 H), 7.05 (s, 1 H), 6.93 (m, 2 H), 6.85 (d, *J* = 8.5 Hz, 1 H), 5.25 (s, 2 H), 5.03 (s, 2 H), 3.94 (s, 3 H), 3.90 (s, 3 H), 3.74 (s, 3 H), 3.66 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.9, 170.7, 170.4, 159.5, 153.1, 150.7, 149.8, 148.6, 136.4, 136.0, 134.7, 130.3, 129.6, 128.7, 128.2, 127.8, 127.2, 123.9, 122.5, 122.3, 121.1, 115.8, 114.8, 112.8, 112.3, 110.9, 110.7, 70.8, 56.1, 55.9, 55.6, 55.3, 44.2; HRMS calcd for $(C_{35}H_{31}O_8N + H)^+$ 594.2122, found 594.2137.

1-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)-3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-1*H*-pyrrole-2,5-dione (19b)

Following the procedure for the preparation of compound **19a**, but using compound **14b** as the starting material, the desired compound **19b** was obtained: yield 80%; mp 67-69°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54 (m, 4 H), 7.43 (d, *J* = 7.3 Hz, 2 H), 7.38 (t, *J* = 7.4 Hz, 2 H), 7.32 (t, *J* = 7.2 Hz, 1 H), 7.23 (dd, *J* = 8.4, 1.8 Hz, 2 H), 7.05 (d, *J* = 1.6 Hz, 1 H), 6.93 (d, *J* = 8.4 Hz, 1 H), 6.88 (d, *J* = 8.8 Hz, 2 H), 6.84 (d, *J* = 8.4 Hz, 1 H), 5.24 (s, 2 H), 5.01 (s, 2 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.82 (s, 3 H), 3.68 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 190.1, 170.9, 160.7, 153.1, 150.4, 149.7, 148.7, 136.0, 134.7, 134.5, 131.6, 128.7, 128.2, 127.9, 123.5, 122.5, 121.5, 121.3, 114.0, 112.6, 112.3, 111.0, 110.7, 70.9, 56.1, 55.8, 55.3, 44.1; HRMS calcd for $(C_{35}H_{31}O_8N + H)^+$ 594.2122, found 594.2133.

3-(4-(benzyloxy)-3-methoxyphenyl)-1-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)-4-(3,4-dimethoxyphenyl)-1*H*-pyrrole-2,5-dione (19c)

Following the procedure for the preparation of compound **19a**, but using compound **14c** as the starting material, the desired compound **19c** was obtained: yield 77%; mp 140-142°C; ^{1}H

NMR ($CDCl_3$, 500 MHz) δ 7.55 (m, 2 H), 7.36 (m, 10 H), 7.24 (s, 1 H), 7.15 (d, *J* = 8.5 Hz, 1 H), 7.12 (s, 1 H), 7.06 (s, 1 H), 6.93 (d, *J* = 8.3 Hz, 1 H), 6.86 (m, 2 H), 5.24 (s, 2 H), 5.18 (s, 2 H), 5.01 (s, 2 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.73 (s, 3 H), 3.66 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 190.0, 170.8, 153.1, 150.4, 149.7, 149.5, 149.2, 148.6, 136.6, 136.0, 134.6, 128.7, 128.2, 128.0, 127.8, 127.2, 123.7, 123.4, 122.5, 121.8, 121.4, 113.2, 112.6, 112.3, 110.9, 110.6, 70.8, 56.1, 55.8, 44.1; HRMS calcd for $(C_{42}H_{37}O_9N + Na)^+$ 722.2361, found 722.2376.

1-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)-3-(3,4-dimethoxyphenyl)-4-phenyl-1*H*-pyrrole-2,5-dione (19d)

Following the procedure for the preparation of compound **19a**, but using compound **14d** as the starting material, the desired compound **19d** was obtained: yield 82%; mp 116-118°C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.54 (m, 4 H), 7.43 (d, *J* = 7.4 Hz, 2 H), 7.38 (m, 5 H), 7.32 (t, *J* = 7.2 Hz, 1 H), 7.28 (dd, *J* = 1.5, 8.4 Hz, 1 H), 7.01 (d, *J* = 1.3 Hz, 1 H), 6.93 (d, *J* = 8.4 Hz, 1 H), 6.84 (d, *J* = 8.4 Hz, 1 H), 5.25 (s, 2 H), 5.03 (s, 2 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.62 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 189.9, 170.7, 153.1, 150.6, 149.7, 148.6, 136.0, 134.8, 129.9, 129.6, 128.7, 128.3, 127.8, 127.2, 123.8, 122.5, 121.1, 112.7, 112.3,110.8, 70.8, 55.9, 55.8, 55.6, 44.2; HRMS calcd for $(C_{34}H_{29}O_7N + H)^+$ 564.2017, found 564.2023.

1-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-oxoethyl)-3-(3,4-dimethoxyphenyl)-4-(p-tolyl)-1*H*-pyrrole-2,5-dione (19f)

Following the procedure for the preparation of compound **19a**, but using compound **14f** as the starting material, the desired compound **19f** was obtained: yield 81%; mp 71-73°C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.54 (m, 2 H), 7.43 (d, *J* = 8.0 Hz, 4 H), 7.37 (t, *J* = 7.5 Hz, 2 H), 7.31 (t, *J* = 7.2 Hz, 1 H), 7.24 (d, *J* = 1.5 Hz, 1 H), 7.17 (d, *J* = 8.0 Hz, 2 H), 7.05 (s, 1 H), 6.92 (d, *J* = 8.3 Hz, 1 H), 6.83 (d, *J* = 8.5 Hz, 1 H), 5.23 (s, 2 H), 5.01 (s, 2 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.71 (s, 3 H), 2.35 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 190.1, 170.8, 153.1, 150.5, 149.7, 148.6, 139.9, 136.0, 135.5, 135.0, 129.8, 129.3, 128.7, 128.2, 127.8, 127.2, 126.1, 123.7, 122.5, 121.3, 112.7, 112.2, 110.9, 110.5, 70.8, 56.0, 55.9, 55.7, 44.1, 21.5; HRMS calcd for $(C_{35}H_{31}O_7N + H)^+$ 578.2173, found 578.2190.

3,4-bis(3,4-dimethoxyphenyl)-1-(4-(methylsulfonyl)benzyl)-1*H*-pyrrole-2,5-dione (20e)

To a solution of compound **14e** (100mg, 0.27 mmol) and 4-(methylsulfonyl)benzyl methanesulfonate (85 mg, 0.32 mmol) in dry DMF (20 mL) was added K_2CO_3 (112 mg, 0.81

mmol). The reaction mixture was heated to 40 °C for 5-6 h and then poured into water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/3, v/v/v) to afford the desired compounds **20e** (100 mg, 69%); mp 158-160°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (d, *J* = 8.2 Hz, 2 H), 7.63 (d, *J* = 8.2 Hz, 2 H), 7.21 (dd, *J* = 1.7, 8.4 Hz, 2 H), 7.04 (d, *J* = 1.6 Hz, 2 H), 6.85 (d, *J* = 8.4 Hz, 2 H), 4.86 (s, 2 H), 3.90 (s, 6 H), 3.70 (s, 6 H), 3.03 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.6, 150.6, 148.7, 142.5, 140.0, 134.1, 129.5, 127.9, 123.6, 121.1, 112.5, 110.9, 55.9, 44.5, 41.3; HRMS calcd for $(C_{28}H_{27}O_8NS + H)^+$ 538.1530, found 538.1530.

3-(3,4-dimethoxyphenyl)-1-(4-(methylsulfonyl)benzyl)-4-(p-tolyl)-1*H*-pyrrole-2,5-dione (20f)

Following the procedure for the preparation of compound **20e**, but using compound **14f** as the starting material, the desired compound **20f** was obtained: yield 73%; mp 166-168°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (d, *J* = 8.2 Hz, 2 H), 7.63 (d, *J* = 8.2 Hz, 2 H), 7.39 (d, *J* = 8.0 Hz, 2 H), 7.23 (dd, *J* = 1.5, 8.4 Hz, 1 H), 7.18 (d, *J* = 8.0 Hz, 2 H), 7.01 (d, *J* = 1.4 Hz, 1 H), 6.83 (d, *J* = 8.4 Hz, 1 H), 4.86 (s, 2 H), 3.89 (s, 3 H), 3.65 (s, 3 H), 3.03 (s, 3 H), 2.36 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.6, 150.7, 148.7, 142.5, 140.2, 140.0, 135.1, 134.5, 129.7, 129.5, 129.3, 127.9, 125.8, 123.6, 120.9, 112.5, 110.9, 55.9, 55.7, 44.5, 41.3, 21.5; HRMS calcd for $(C_{27}H_{25}O_6NS + Na)^+$ 514.1295, found 514.1301.

3,4-bis(3,4-dimethoxyphenyl)-1-(2-(4-(methylsulfonyl)phenyl)-2-oxoethyl)-1*H*-pyrrole-2,5-dione (21e)

To a solution of compound **14e** (100mg, 0.27 mmol) and 2-bromo-1-(4-(methylsulfonyl)phenyl)ethanone (89 mg, 0.32 mmol) in dry DMF (20 mL) was added K_2CO_3 (112 mg, 0.81 mmol). The reaction mixture was heated to 40 °C for 5-6 h and then poured into water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/3, v/v/v) to afford the desired compounds **21e** (119 mg, 78%); mp151-153°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.18 (d, *J* = 8.0 Hz, 2 H), 8.11 (d, *J* = 8.0 Hz, 2 H), 7.24 (s, 2 H), 7.08 (s, 2 H),6.87 (d, *J* = 8.4 Hz, 2 H), 5.08 (s, 2 H),

3.91 (s, 6 H), 3.71 (s, 6 H), 3.10 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 190.8, 170.5, 150.5, 148.7, 145.0, 138.3, 134.6, 129.0, 128.1, 123.7, 121.2, 112.6, 110.9, 55.9, 44.7, 44.3, 29.7; HRMS calcd for $(C_{29}H_{27}O_9NS + H)^+$ 566.1479, found 566.1485.

3-(3,4-dimethoxyphenyl)-1-(2-(4-(methylsulfonyl)phenyl)-2-oxoethyl)-4-(p-tolyl)-1*H*-pyrrole-2,5-dione (21f)

Following the procedure for the preparation of compound **21e**, but using compound **14f** as the starting material, the desired compound **21f** was obtained: yield 81%; mp 143-145°C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.19 (d, *J* = 8.2 Hz, 2 H), 8.11 (d, *J* = 8.2 Hz, 2 H), 7.43 (d, *J* = 7.9 Hz, 2 H), 7.27 (s, 1 H), 7.19 (d, *J* = 7.9 Hz, 2 H), 7.04 (s, 1 H), 6.85 (d, *J* = 8.4 Hz, 1 H), 5.09 (s, 2 H), 3.91 (s, 3 H), 3.67 (s, 3 H),3.10 (s, 3 H), 2.37 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 190.7, 170.5, 150.6, 148.6, 145.0, 140.1, 138.4, 135.6, 135.0, 129.8, 129.4, 129.0, 128.1, 125.9, 123.7, 121.1, 112.6, 110.9, 55.9, 55.7, 44.7, 44.3, 29.7, 21.5; HRMS calcd for $(C_{28}H_{25}O_7NS + H)^+$ 520.1424, found 520.1429.

2-bromo-1-(4-((2-bromo-3,4,5-trimethoxybenzyl)oxy)-3-methoxyphenyl) ethanone (22)

To a stirred solution of 1-(3-methoxy-4-((3,4,5-trimethoxybenzyl)oxy)phenyl)ethanone (200 mg, 0.57 mmol) in chloroform (30 mL) at 0 ℃, a solution of bromine (182 mg, 1.14 mmol) in chloroform (5 mL) was added slowly. After 5 h, the resulting reaction solution was poured into ice-cold water (100 mL), extracted with CH_2Cl_2, washed with brine, dried over anhydrous MgSO4, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/PE = 1/3, v/v) to afford the desired compounds 22 (92 mg, 32% yield) as white solid; mp 140-142 °C; ESI-MS m/z [M + H]+ 505.1; 1H NMR ($CDCl_3$, 500 MHz) δ 7.56 (s, 1 H), 7.52 (d, J = 8.4 Hz, 1 H), 6.94 (s, 1 H), 6.88 (d, J = 8.4 Hz, 1 H), 5.23 (s, 2 H), 5.03 (s, 2 H), 3.95 (s, 3 H), 3.91 (s, 3 H), 3.87 (s, 3 H), 3.80 (d, 3 H); 13C NMR ($CDCl_3$, 126 MHz) δ 196.7, 153.1, 152.0, 150.8, 149.4, 142.7, 131.0, 123.1, 112.4, 110.6, 108.1, 107.4, 70.3, 61.0, 61.0, 56.1.

1-(2-(2-bromo-5-methoxy-4-((3,4,5-trimethoxybenzyl)oxy)phenyl)-2-oxoethyl)-3,4-bis(3,4-dimethoxyphenyl)-1*H*-pyrrole-2,5-dione (23)

To a solution of compound **14e** (100mg, 0.27 mmol) and **22** (161 mg, 0.32 mmol) in dry DMF (20 mL) was added K_2CO_3 (112 mg, 0.81 mmol). The reaction mixture was heated to 40 °C for 6 h and then poured into water (100 mL), extracted with CH_2Cl_2, washed with brine,

dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/EtOAc/PE = 1/1/3, v/v/v) to afford the desired compounds **23** (141 mg, 66%); mp 158-160 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.61 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.57 (d, *J* = 1.9 Hz, 1H), 7.24 (dd, *J* = 8.4, 1.9 Hz, 2H), 7.09 (d, *J* = 1.9 Hz, 2H), 6.93 (t, *J* = 4.2 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.26 (s, 2H), 5.04 (s, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.90 (s, 6H), 3.88 (s, 3H), 3.82 (s, 3H), 3.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 190.0, 170.8, 153.1, 152.7, 150.8, 150.3, 149.7, 148.6, 142.7, 134.6, 130.8, 128.1, 123.6, 122.6, 121.4, 112.6, 112.5, 110.9, 110.7, 108.1, 107.3, 70.3, 61.1, 61.0, 56.1, 56.1, 55.8, 55.7, 44.1, 29.6; HRMS calcd for $(C_{39}H_{38}O_{12}NBr + H)^+$ 794.1630, found 794.1652.

4.2. Materials for biological studies.

DMSO, verapamil, doxorubicin (DOX), paclitaxel (PTX) and rhodamine 123 were purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM), trypsin-ethylenediaminetetracetic acid (EDTA), and penicillin/streptomycin were from Gibco BRL. Fetal bovine serum (FBS) was from Hyclone Laboratories. 2-(4,5-Dimethylthiazol-2-yl-)-5-[3-(carboxymethoxy) phenyl]- 2-(4-sulfophenyl)-2H-tetrazolium (MTS) and phenazine methosulfate (PMS) were purchased from Promega. Human breast cancer cell lines MDA435/LCC6 and MDA435/LCC6MDR were kindly provided by Dr. Robert Clarke (Georgetown University, Washington, DC).

4.3. Cell culture.

MDA435/LCC6, MDA435/LCC6MDR and L929 cell lines were cultured in supplemented DMEM media with 10% heat inactivated FBS and 100 U/mL penicillin and 100 μg/mL of streptomycin. They were maintained at 37ºC in a humidified atmosphere with 5% CO_2. The cells were split constantly after a confluent monolayer has been formed. To split cells, the plate was washed briefly with phosphate-buffered saline (PBS), treated with 0.05% trypsin-EDTA and harvested by centrifugation.

4.4. Cell proliferation assay.

6,000 cells of LCC6 or LCC6MDR were mixed with PTX (400, 133, 44, 15, 5, 1.6 or 0

nM) with or without modulators to a final volume of 200 μL in each well of a 96-well plate. The plates were then incubated for 5 days at 37 ºC. The cell viability was determined using the Cell Titer 96 A Queous Assay (Promega) as reported previously[36-38]. IC_{50}values of LCC6MDR was determined using non-linear regression dose-response curve analysis of Prism software.

4.5. Cytotoxicity assay.

10,000 cells of L929 were mixed with different concentrations (100, 33.3, 11.1, 3.7, 1.2, 0.4 and 0 μM) of modulators to a final volume of 100 μL in each well of a 96-well plate. The plates were then incubated for 3 days at 37 ºC. The percentage of survival was determined using MTS assay. IC_{50} of modulators was determined using non-linear regression dose-response curve analysis of Prism software.

4.6. Intracellular DOX and rhodamine 123 accumulation.

1 x 10^6 cells of LCC6 or LCC6MDR cells were mixed with 20 μM DOX or 10 μg/mL rhodamine 123 in the presence of 2 μM of modulator or0.2 % DMSO (as a negative control). Cells were incubated at 37ºC for 150 min. Cells were spinned down and washed with cold PBS, pH7.4 and lysed with lysis buffer. Supernatant was saved and kept in a 96-well black plate with flat bottom. Fluorescence level of DOX was determined by fluorescence spectrophotometer (BMG Technologies) using an excitation and an emission wavelength of 460 nm and 610 nm. Rhodamine 123 level was determined at wavelength of 480 nm for excitation and 520 nm for emission[33, 37, 38].

Acknowledgment

This project was funded by NSFC-Shandong Joint Fund for Marine Science Research Centers (U1406402), National Natural Science of China (NSFC 81172926), Project of Strategic Importance (1-ZE22) of the Hong Kong Polytechnic University and the General Research Fund (PolyU 5606/11M) of the Research Grant Council of Hong Kong.

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