Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Amine Linked Flavonoid Dimers as Modulators for P-Glycoprotein-Based Multidrug Resistance: Structure−Activity Relationship and Mechanism of Modulation

Kin-Fai Chan,[†,‡,∥] Iris L. K. Wong,[†,‡,∥] Jason W. Y. Kan,[†,‡] Clare S. W. Yan,[†,‡] Larry M. C. Chow,*[,†,‡] and Tak Hang Chan*[,†,‡,§]

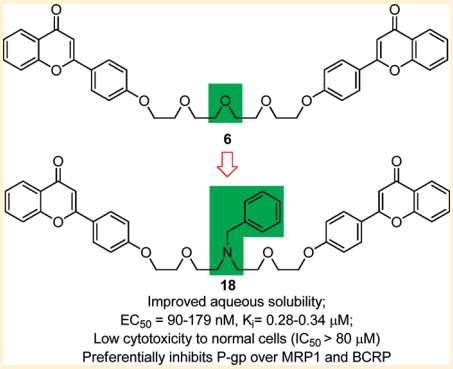
[†]Department of Applied Biology and Chemical Technology, State Key Laboratory of Chirosciences, The Hong Kong Polytechnic University, Hong Kong SAR, China

[‡]State Key Laboratory for Chinese Medicine and Molecular Pharmacology, The Hong Kong Polytechnic University, Shenzhen, China

[§]Department of Chemistry, McGill University, Montreal, Quebec, H3A 2K6, Canada

Supporting Information

ABSTRACT: Here we report a great improvement in reversal potency of cancer drug resistance when flavonoid dimers possess a functionally substituted aminopolyethylene glycol linker. The most potent compound, **18**, contains a *N*-benzyl group at the linker. It has many advantages including (1) high potencies in reversing P-glycoprotein (P-gp) mediated resistance in LCC6MDR cells to various anticancer drugs with EC_{50} in the nanomolar range, (2) low toxicity and high therapeutic index, and (3) preferential inhibition of P-gp over multidrug resistance protein 1 and breast cancer resistance protein. Compound **18** stimulates P-gp-ATPase activity by 2.7-fold and mediates a dose-dependent inhibition of doxorubicin (DOX) transport activity. Lineweaver−Burk and Dixon plots suggest that **18** is a competitive inhibitor to DOX in binding to P-gp with a K_i of 0.28−0.34 μM and a Hill coefficient of 1.17. Moreover, the LCC6MDR cell displays about 2.1-fold lower intracellular accumulation of **18** compared to the wild type, suggesting that **18** is a P-gp substrate as well.



INTRODUCTION

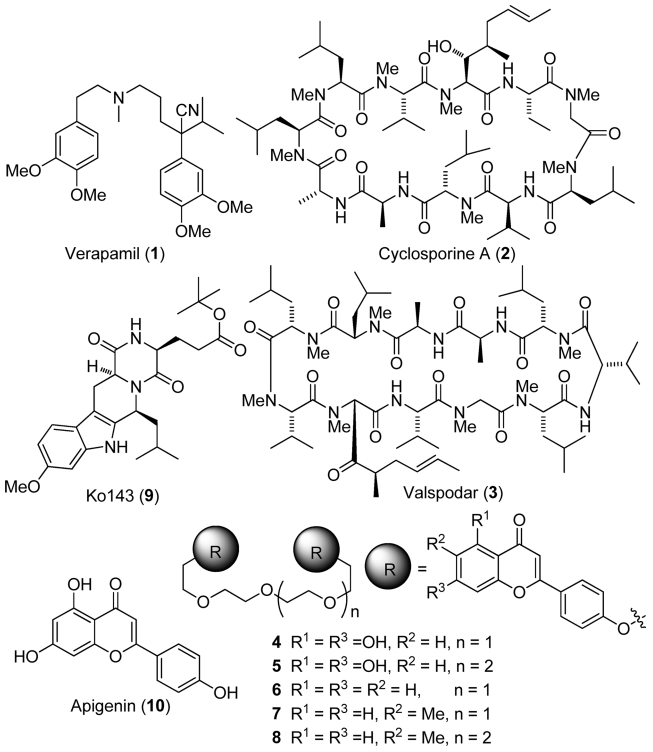
Treatment of cancer patients with chemotherapeutic drugs often leads to emergence of tumors with a multidrug resistance (MDR) phenotype, which constitutes a major obstacle for cancer chemotherapy. Many mechanisms of MDR have been described. One of the well-characterized resistance mechanisms is overexpression of a membrane transporter, permeability glycoprotein (P-gp), which recognizes and pumps a variety of anticancer drugs out of the cancer cells, resulting in a lowered intracellular drug accumulation. P-gp belongs to the ATP binding cassette (ABC) transporter superfamily, which has a broad substrate specificity.[1,2] Three-dimensional structure of the mouse homologue of human P-gp revealed that it has a pseudodimeric structure.[3] In its nucleotide-free state, the nucleotide-binding domains (NBDs) were separated by about 30 Å and the two bundles of six transmembrane helices formed a large internal cavity for substrate binding open to both the membrane and the inside of the cell.[3]

There has been intensive search for potent P-gp modulators. The first P-gp inhibitor was verapamil (**1**) developed by Tsuruo et al.[4] Subsequently, a range of compounds including calcium channel blockers,[5,6] calmodulin inhibitors,[7,8] indole alkaloids,[9,10] cyclosporine A (**2**),[11−14] quinolines,[15] and steroids[16−18] have been demonstrated to have P-gp inhibitory activity to different extents. The second generation MDR modulators resulted in the less toxic and more potent agents such as dexverapamil,[19] dexniguldipine,[20] valspodar (**3**),[17,21] and biricodar.[22,23] The third generation MDR modulators developed by structure−activity relationships and combinatorial chemistry approaches included zosuquidar, tariquidar, laniquidar, acridonecarboxamide, and the substituted diarylimidazole derivatives.[24,25] Among them, only very few were selected for clinical trial and none of them have been approved for therapeutic use.

Flavonoids are polyphenolic compounds commonly found in fruits, vegetables, and plant-derived products of the human diet.[26] Humans consume large amounts of flavonoids daily, and it is therefore generally accepted that flavonoids are not toxic. Some flavonoids have been shown to have a low modulating activity on cancer MDR.[27−33] We have previously reported that synthetic apigenin homodimers, linked by polyethylene glycol (PEG), can modulate P-gp and multidrug resistance protein 1 (MRP1) in human cancer[34−36] and parasitic protozoan *Leishmania*.[37,38] Such bivalency approach resulted in a significantly higher reversal activity in apigenin homodimers than monomeric apigenin. The most potent apigenin homodimers for reversing P-gp- and MRP1-mediated drug resistance were **4** and **5** (Chart 1), which have four and five ethylene glycol (EG)

Received: August 22, 2011
Published: February 9, 2012

Chart 1. Chemical Structures of Compounds 1−10



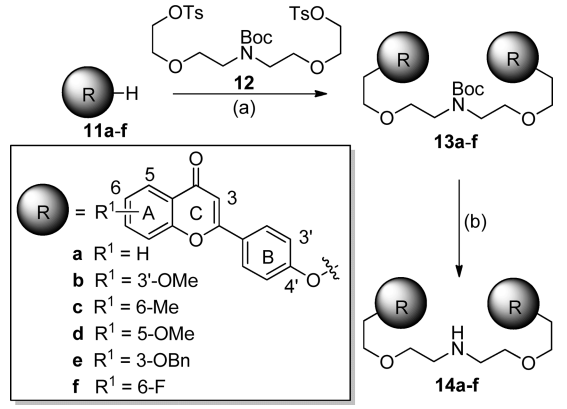
units, respectively.[34,36] Modulating activity of flavonoid dimers has been further optimized by structural modification of the A ring.[35,36] In general, replacement of the OH group in the A ring with H (e.g., **6**) led to more potent P-gp modulators. Furthermore, nonpolar and hydrophobic substituents (e.g., methyl, ethyl, or fluoro groups) were better than polar and hydrophilic substituents (e.g., hydroxyl groups) at the C3, C6, and C7 positions.[35,36] The EC_{50} of compound **7** (Chart 1) for vincristine was determined to be 110 nM. This efficacy was close to other highly efficient modulators such as tariquidar (EC_{50} = 22−38 nM for vincristine).

These flavonoid dimers are, however, highly hydrophobic and barely soluble in water. Attempts to use these compounds for in vivo animal experiments proved to be impractical (data not shown). In order to improve their physicochemical properties as potential drug candidates, we must introduce some hydrophilic groups into the structure. Since we already knew that introduction of hydrophilic groups into the flavonoid moiety reduced potency, it leaves the linker as a possible site for structural modification. Here we synthesize a series of new flavonoid dimers with nitrogen-containing PEG linker and evaluate their P-gp-modulating activity, cytotoxicity against cancer cells and normal cells, and their specificity toward MRP1 and breast cancer resistance protein (BCRP). The most effective amine linked flavonoid dimer has been further investigated regarding its mechanism of reversing P-gp-mediated drug resistance.

RESULTS AND DISCUSSION

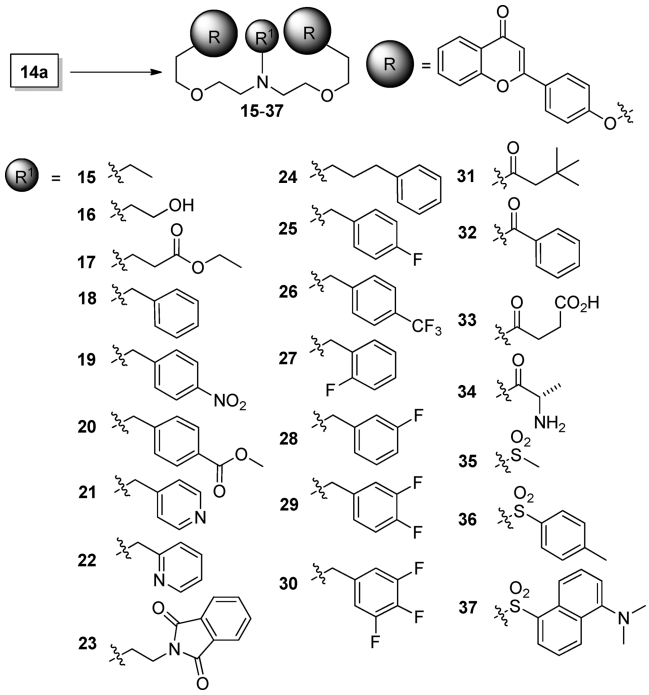
I. Chemistry. By use of **6** as the starting template, the synthesis of amine-containing PEG linker is considerably simplified by replacing the central oxygen with nitrogen. The designed flavonoid dimers were synthesized as summarized in Schemes 1 and 2. As depicted in Scheme 1, by reaction of the *tert*-butyloxycarbonyl (*N*-Boc) protected ditosylate **12** with

Scheme 1. Synthesis of Flavonoid Dimers 13 and 14[a]



[a]Reagents and conditions: (a) K_2CO_3, DMF, reflux, 2 h; (b) trifluoroacetic acid, CH_2Cl_2, 0 °C to room temp, 2 h.

Scheme 2. Synthesis of Compounds 15−37[a]



[a]Reagents and conditions: (a) for compounds **15**−**30**, K_2CO_3, RX, ACN, or DMF, reflux, 3 h; (b) for compounds **31** and **32**, RCOCl, pyridine, 0 °C to room temp; (c) for compound **33**, succinic anhydrite, pyridine, room temp, 4 h; (d) for compound **34**, coupling with *t*-Boc-alanine followed by acid hydrolysis; (e) for compounds **35**−**37**, RSO_2Cl, pyridine, 0 °C to room temp.

corresponding 4′-hydroxyflavones **11a**−**f**, prepared as previously reported[35] in dimethylformamide (DMF) under basic condition at refluxing temperature, the desired *N*-Boc protected flavonoid dimers **13a**−**f** with various substituents on the flavone rings were obtained in reasonable yield. Their dimeric nature was evident from the high-resolution mass spectrum. Treatment of these compounds with trifluoroacetic acid (TFA) in CH_2Cl_2 followed by extraction at basic pH led to the corresponding flavonoid dimers **14a**−**f** with the N−H group at the linker region in high yield. This synthetic pathway offers advantages and flexibility to access desired flavonoid dimers for structure−activity relationship (SAR) study at the linker region.

dx.doi.org/10.1021/jm201121b | J. Med. Chem. 2012, 55, 1999−2014

It should be mentioned that the hydrochloride salt of compound **14a** was obtained simply by treatment with concentrated hydrochloric acid followed by evaporation to dryness.

After preliminary biological screening of these flavonoid dimers, **14a**−**f** and their hydrochloride salts **14a** and **14f** showed not only good P-gp modulating activity but also greatly improved aqueous solubility compared with the PEG linked flavonoid dimer **6** or **7**. These encouraging results prompted us to carry out further structural optimization at the amino linker region. Compound **14a** was selected to be the focus of attention because of inexpensive starting material 2-hydroxyacetophone, which is commercially available in bulk. Treatment of **14a** with various alkyl halides in acetonitrile (ACN) or DMF at refluxing temperature afforded tertiary amine derivatives (**15**−**30**) in good yield (Scheme 2). Amide derivatives (**31**, **32**) were also obtained under similar conditions in high yield by mixing **14a** with the corresponding acid chlorides. Amide derivative **33** was furnished by treatment of **14a** with succinic anhydride in pyridine at room temperature. Amide derivative **34** was prepared in two steps from commercially available Boc-Ala-OH followed by acid hydrolysis using TFA. Treatment of **14a** with various sulfonyl chlorides in pyridine at 0 °C afforded sulfonamide derivatives (**35**−**37**) in reasonably good yields.

II.A. Structure−Activity Relationship Study. *Effect of Amine Linked Flavonoid Dimers on Reversing Paclitaxel Resistance in MDA435/LCC6MDR Cells.* We employed two cell lines in this study, namely, MDA435/LCC6 (an estrogen-independent human breast cancer cell line) and its P-gp-transfected derivative (MDA435/LCC6MDR).[39] LCC6MDR (IC_{50} for paclitaxel of 153.1 ± 2.9 nM) displayed about 95.7-fold greater resistance than LCC6 cells (IC_{50} of 1.6 ± 0.3 nM) (Table 1). Cytotoxicity of flavonoid dimers alone, measured by the IC_{50} toward LCC6, LCC6MDR, and a normal mouse fibroblast cell line L929, ranged from 2.4 to above 100 μM. At the concentration we used for assaying P-gp reversing activity (0.5 or 1.0 μM), there was at least 80% survival (data not shown). Flavonoid dimers synthesized can be structurally divided into six series (A−F) according to the chemical substitutions on the amine linker. Table 1 summarizes the results of cytotoxicity and P-gp-modulating activity of all flavonoid dimers.

II.A.1. Boc-Group Protected Amine Linked Flavonoid Homodimers (Series A, Table 1). All flavonoid dimers in this series have a Boc group protecting the amine group on the PEG linker. The unsubstituted parent compound **13a** displayed a promising P-gp-modulating activity with a RF of 69.6 at 1.0 μM. The *N*-Boc group in the linker offers a significant improvement over **6**[35] (RF = 17.2), which has a central oxygen in the PEG linker. Compound **13a** was about 20-fold more potent than **1** (RF = 3.5). Addition of a methoxy group at the C3′ position of the B ring (**13b**), however, caused a reduction in reversal potency with a RF of 16.8 at 1.0 μM, suggesting that hydrophobicity at position 3′ in the B ring is unfavorable for P-gp-modulating activity. On the other hand, addition of a methyl group at the C6 position (**13c**) or a methoxy group at the C5 position (**13d**) of the A ring displayed an improved efficacy with a RF of 139.2 and 109.4 at 1.0 μM, respectively. This result suggests that the increased hydrophobicity in ring A is important to produce efficient P-gp inhibitor. However, a bulky benzyloxy group at the C3 position of the C ring (**13e**) showed no modulating activity with a RF of 1.2 at 1.0 μM. This loss of P-gp inhibitory activity may be due to the steric hindrance of benzyloxy group. Addition of a fluorine atom at the C6 position (**13f**) of the A ring resulted in a moderate efficacy with a RF of 31.9 at 1.0 μM. In terms of cytotoxicity, the unsubstituted compound (**13a**) and C5-methoxy substituted compound (**13d**) displayed killing activity to the cancer lines (IC_{50} < 8.0 μM) and normal cell L929 (IC_{50} < 11.1 μM). In contrast, C6-methyl substituted compound (**13c**) showed no cytotoxicity to all of the different cell lines (IC_{50} > 100 μM).

II.A.2. N−H Linked Flavonoid Homodimers (Series B, Table 1). All N−H linked flavonoid dimers in this series have their Boc group removed from the linker by acid hydrolysis. Similar to their Boc-protected parent compounds of **13a** (RF = 69.6) and **13c** (RF =139.2), the unsubstituted **14a** and C6-methyl substituted **14c**, both displayed potent reversal activity with RF of 139.2 at 1.0 μM. Interestingly, C3′-methoxy substituted compound **14b** (RF = 1.1) and C5-methoxy substituted compound **14d** (RF = 1.2) completely lost their P-gp-modulating potency compared to corresponding compounds **13b** (RF = 16.8) and **13d** (RF = 109.4). In contrast, C3-benzyloxy substituted compound **14e** (RF = 127.6) regained the promising reversal potency compared to the corresponding compound **13e** (RF = 1.2). These data suggest that there may be some positive or negative interacting activity between the Boc group on the linker and the substitution on the flavone moiety and their combined effect would influence the P-gp-mediated resistance reversal potency. Nevertheless, all N−H linked dimers in this series were found to exhibit toxicity toward all cell lines tested (IC_{50} < 23.8 μM), suggesting that a free N−H group on the linker would potentiate its cytotoxicity.

II.A.3. Functional-Group-Substituted Amine Linked Flavonoid Homodimers (Series C−F, Table 1). The amine group on the linker in series C−F was substituted with various functional groups such as alkyl group (C), amide group (D), sulfonamide group (E), and benzyl group or pyridylmethyl group (F). We found that the P-gp inhibitory potency and toxicity toward L929 varied greatly among these amine linked flavonoid dimers. In general, all compounds exhibited moderate to potent paclitaxel reversing activity at 1.0 μM (RF = 18.9 to 139.2), except those that showed no reversal potency included amides **33** and **34** and sulfonamide **37**.

To further differentiate reversal potency of those active compounds, we tested them at lower concentration (0.5 μM). The presence of a benzyl group on **18** (RF = 58.9) conferred the highest activity. In addition, reversal potency has been diminished slightly by unfavorable electron-withdrawing substituents on the phenyl ring, such as fluoro (**25**, **27**, and **28**), difluoro (**29**), trifluoro (**30**), trifluoromethyl (**26**), nitro (**19**), and methyl ester (**20**). The distance between the nitrogen atom and the phenyl ring appeared to be a crucial element, as the lengthened compound **23** or **24** was less potent than **18**. One methylene group seems to be optimal. Besides, a phenyl ring without any nitrogen substitution seems to be favorable, as a pyridyl ring (**21**, **22**) also displayed much lower modulating activity than **18**. Moreover, compounds **31**, **32**, **35**, and **36** were prepared to explore the effects of replacing the benzylic carbon with other functional groups, such as carbonyl and sulfonyl. It was found that their modulating activities were much lower than that of **18**. This may be attributed to the fact that both amide and sulfonamide bonds are less basic than amines. Finally, we synthesized compounds **15**, **16**, and **17** to investigate the role of the phenyl ring of **18**. These derivatives generally displayed much lower modulating activity than **18**. It therefore seems reasonable to hypothesize that there may be an

Table 1. Cytotoxicity and Paclitaxel-Resistance Reversal Activity of Amine Linked Flavonoid Dimers[a]

		cytotoxicity IC_{50} (μM)[f]			IC_{50} of paclitaxel of LCC6MDR [nM] (RF)[f]			
compd[b]	series	LCC6MDR	LCC6	L929	compd at 1.0 μM		compd at 0.5 μM	
13a	A	6.2 ± 0.8	8.0 ± 2.5	11.1 ± 0.0	2.2 ± 0.5	(69.6)	13.8 ± 3.7	(11.1)
13b	A	6.9 ± 2.9	8.3 ± 1.4	33.3	9.1 ± 3.6	(16.8)	17.6 ± 1.8	(8.7)
13c	A	>100	>100	>100	1.1 ± 0.3	(139.2)	16.0 ± 3.1	(9.6)
13d	A	5.4 ± 0.5	4.6 ± 0.2	5.5 ± 0.4	1.4 ± 0.3	(109.4)	10.3 ± 3.2	(14.9)
13e	A	>100	>100	nd	131.4 ± 4.9	(1.2)	137.6 ± 20.6	(1.1)
13f	A	>100	>100	>100	4.8 ± 1.4	(31.9)	13.5 ± 0.5	(11.3)
14a	B	2.4 ± 0.7	3.3 ± 1.0	5.9 ± 0.6	1.1 ± 0.1	(139.2)	2.1 ± 0.3	(72.9)
14b	B	23.8 ± 12.8	12.8 ± 1.1	nd	134.9	(1.1)	194.0 ± 5.1	(0.8)
14c	B	7.0 ± 1.4	7.5 ± 0.9	6.0 ± 0.6	1.1 ± 0.1	(139.2)	11.0 ± 2.0	(13.9)
14d	B	10.2 ± 3.8	8.8 ± 2.7	nd	126.6 ± 11.4	(1.2)	124.9 ± 10.4	(1.2)
14e	B	4.4 ± 0.1	4.3 ± 0.5	4.2	1.2 ± 0.1	(127.6)	2.8 ± 0.6	(54.7)
14f	B	3.3 ± 0.5	5.3 ± 1.0	5.7 ± 0.4	1.4 ± 0.1	(109.4)	2.1 ± 0.4	(72.9)
15	C	17.3 ± 3.8	10.3 ± 2.2	6.9 ± 2.0	1.6 ± 0.3	(95.7)	5.7 ± 0.2	(26.9)
16	C	14.8 ± 5.9	10.7 ± 3.3	6.9 ± 1.4	4.7 ± 0.6	(32.6)	30.6 ± 3.8	(5.0)
17	C	17.7 ± 3.7	13.5 ± 0.5	18.1 ± 3.4	3.5 ± 0.5	(43.7)	28.9 ± 9.5	(5.3)
23	C	4.9 ± 0.1	8.6 ± 3.4	17.0 ± 4.3	1.3 ± 0.0	(117.8)	16.0 ± 0.8	(9.6)
24	C	>100	>100	9.5 ± 0.6	2.7 ± 1.7	(56.7)	12.2 ± 8.5	(12.5)
31	D	>100	>100	>100	2.1 ± 0.5	(72.9)	8.1 ± 1.4	(18.9)
32	D	>100	>100	95.0	3.6 ± 0.7	(42.5)	20.4 ± 2.0	(7.5)
33	D	>100	78.4 ± 8.1	nd	164.5	(0.9)	144.6	(1.1)
34	D	4.6 ± 0.2	4.4 ± 0.0	nd	173.1 ± 40.0	(0.9)	145.9 ± 13.8	(1.0)
35	E	42.6 ± 5.4	27.5 ± 8.4	13.4 ± 3.9	7.6	(20.1)	12.0	(12.8)
36	E	>100	>100	>100	7.1 ± 0.9	(21.6)	26.3 ± 5.6	(5.8)
37	E	>100	>100	nd	116.8 ± 9.5	(1.3)	111.2 ± 10.2	(1.4)
18	F	>63	>75	85.0 ± 5.0	1.1 ± 0.1	(139.2)	2.6 ± 0.6	(58.9)
19	F	>90	86.8 ± 1.8	88.0 ± 6.1	1.2 ± 0.1	(127.6)	3.5 ± 1.2	(43.7)
20	F	64.8 ± 14.1	33.3 ± 10.8	11.4 ± 4.4	8.1 ± 3.5	(18.9)	72.3 ± 15.4	(2.1)
21	F	>100	>100	26.4 ± 2.7	5.5 ± 1.4	(27.8)	22.3 ± 0.2	(6.9)
22	F	29.7 ± 16.4	10.0 ± 1.2	10.0 ± 2.8	1.6 ± 0.1	(95.7)	9.7 ± 0.1	(15.8)
25	F	>83	>100	19.7 ± 0.1	1.4 ± 0.1	(109.4)	4.9 ± 1.8	(31.2)
26	F	>100	>100	32.7 ± 8.7	1.7 ± 0.4	(90.1)	4.1 ± 1.1	(37.3)
27	F	>100	>100	>100	1.5 ± 0.2	(102.1)	3.5 ± 0.5	(43.7)
28	F	>100	>100	>87	1.3 ± 0.0	(117.8)	4.2 ± 1.0	(36.5)
29	F	33.6 ± 4.3	32.5 ± 11.0	42.2 ± 2.1	1.4 ± 0.0	(109.4)	3.1 ± 0.6	(49.3)
30	F	>100	>75	>100	1.9 ± 0.3	(80.6)	46.8 ± 23.2	(3.3)
6		nd	nd	nd	8.9 ± 2.6	(17.2)	24.7 ± 4.9	(6.2)
1		63.9 ± 1.7	63.8 ± 0.1	89.2 ± 8.2	43.9 ± 5.2	(3.5)	76.4 ± 10.4	(2.0)
3		14.6 ± 2.2	25.3 ± 4.3	>100	1.8 ± 0.3	(85.1)	nd	nd
2		2.8 ± 0.6	8.3 ± 1.5	33.9 ± 5.2	2.0 ± 0.2	(76.6)	nd	nd
DMSO		nd	nd	nd	148.0 ± 13.9[c]	(1.0)	138.4 ± 8.6[d]	(1.1)
LCC6MDR		nd	nd	nd	153.1 ± 2.9[e]	(1.0)	nd	
LCC6		nd	nd	nd	1.6 ± 0.3[e]	(95.7)	nd	

[a] IC_{50} value toward paclitaxel was determined in the presence of 1.0 or 0.5 μM modulator. Relative fold (RF) represents fold-change in drug sensitivity. N = 1−4 independent experiments, and the values were presented as the mean ± standard error of mean. RF = (IC_{50} without modulator)/(IC_{50} with 1.0 or 0.5 μM modulator). Known P-gp inhibitors **1**, **2**, **3**, and **6** were included for comparison. [b] All compounds were dissolved in DMSO for testing. [c] 0.1% of DMSO was used as solvent control for testing the P-gp modulating activity. [d] 0.05% of DMSO was used as solvent control for testing the P-gp modulating activity. [e] The cytotoxicity of LCC6 and LCC6MDR to paclitaxel was tested in the absence of modulators. For cytotoxicity assay, IC_{50} values of different flavonoid dimers for LCC6, LCC6MDR, and L929 cell lines were determined. N = 1−3 independent experiments, and the values were presented as the mean ± standard error of mean. L929: mouse fibroblasts. [f] nd: not determined.

interaction between the phenyl ring of **18** and the amino acid with aromatic substituents of P-gp such as tryptophan, tyrosine, and phenylalanine through $\pi-\pi$ interaction.

Other than modulating activity, the amine linker also plays an important role in determining its cytotoxic effect. For a proper comparison, three levels of toxicity were employed: high (cytotoxicity, IC_{50} < 20 μM), moderate (20−60 μM), and low (cytotoxicity, >60 μM). From series B to F, all N−H linked homodimers (**14a−f**) (series B) displayed a high level of toxicity toward cancer cells and normal fibroblast. In series C, all compounds (**15−17**, **23**) were at high levels of toxicity to cancer cells and L929 cells except **24** which was nontoxic to both cancer cells. In general, flavonoid homodimer possessing unsubstituted N−H or small *N*-alkyl linkers would result in high killing activity toward cancer cells or mouse fibroblast. When the bulkiness of the amine linker is increased, the cytotoxicity level would generally be reduced. From the series D, E, and F, 3 out of 18 dimers (**20**, **22**, and **34**) displayed a

Table 2. EC_{50} and Therapeutic Indexes of Amine Linked Flavonoid Dimers for Lowering Anticancer Drug Resistance[a]

	EC_{50} for reversing anticancer drug resistance (nM)						
compd	paclitaxel	vinblasinte	vincristine	DOX	daunorubicin	mitoxantrone	therapeutic index
13a	320 ± 40	nd	nd	nd	nd	nd	34.7
14a	305 ± 35	nd	nd	nd	nd	nd	19.3
15	200	nd	nd	nd	nd	nd	34.5
18	148 ± 18	173 ± 27	179 ± 32	131 ± 13	95 ± 25	90 ± 20	574.3
19	189 ± 1	nd	nd	nd	nd	nd	465.6
24	250	nd	nd	nd	nd	nd	38.0
25	255 ± 5	nd	nd	nd	nd	nd	77.3
26	150	nd	nd	nd	nd	nd	218.0
27	179 ± 44	nd	nd	nd	nd	nd	>558.7
28	258 ± 103	nd	nd	nd	nd	nd	>337.2
1	428 ± 40	503 ± 92	385 ± 35	245 ± 23	nd	nd	208.4

[a]EC_{50} values were presented as the mean ± standard error of mean. N = 1−7 independent experiments. Therapeutic index = (IC_{50} of modulators towards L929 fibroblasts)/(EC_{50} of modulators for reversing paclitaxel resistance in LCC6MDR cells). nd: not determined.

Table 3. Effect of 18 on P-gp, MRP1, and BCRP Modulating Activity[a]

			IC_{50} [nM] (RF)		
cell line	overexpress	resistant to	no modulator	**18**	+ve control[b]
LCC6MDR	P-gp	paclitaxel	153.1 ± 2.9 (1.0)	2.6 ± 0.6 (58.9)	1.8 ± 0.5 (85.1)
LCC6	nd	nd	1.6 ± 0.3 (95.7)		
2008/MRP1	MRP1	DOX	404.2 ± 41.9 (1.0)	192.0 ± 58.0 (2.1)	44.8 ± 10.4 (9.0)
2008/P	nd	nd	50.3 ± 5.6 (8.0)		
HEK292/R2	BCRP	topotecan	563.4 ± 41.1 (1.0)	147.0 ± 3.9 (3.8)	40.5 ± 8.3 (13.9)
HEK293/pcDNA3.1	nd	nd	22.4 ± 2.4 (25.2)		

[a]Modulating activity of **18** and +ve control modulators (all at 0.5 μM) on P-gp, MRP1, and BCRP were investigated using LCC6MDR, 2008/MRP1, and HEK293/R2 cells, respectively (N = 2−4 independent experiments, and the values are presented as the mean ± standard error of mean). IC_{50} values toward paclitaxel, DOX, and topotecan in these three cell lines were determined with or without modulators to determine RF. IC_{50} values were also determined for their parental cell lines (LCC6, 2008/P, and HEK293/pcDNA3.1) for reference. [b]+ve controls used for modulating P-gp, MRP1, and BCRP (all at 0.5 μM) are compounds **2**, **8**, and **9**, respectively. nd: not determined.

high level of toxicity toward normal cells L929, as their IC_{50} values were below 11 μM. A total of 10 compounds (**18**, **19**, **27**, **28**, and **30** in series F; **31**, **32**, and **33** in series D; and **36** and **37** in series E) were found to be nontoxic to the cancer cells and L929 cells. Overall, a benzyl group appeared to be a desirable substitution for making a potent P-gp inhibitor while maintaining low toxicity to the normal cells.

*II.B. Effect of Compound **18** on Reversing P-gp toward Other Anticancer Drugs.* Among the most potent N-linked flavonoid dimers listed in Table 1, we have determined the EC_{50} values for modulating P-gp toward various anticancer drugs from a selected group of compounds (**13a**, **14a**, **15**, **18**, **19**, and **24**−**28**). EC_{50} refers to the concentration of modulator required to reduce the IC_{50} by half compared to control without modulators. Compounds **18** and **26** were the most active N-linked flavonoid dimers in modulating P-gp toward paclitaxel with EC_{50} equal to 148 ± 18 nM and 150 nM, respectively (Table 2). Compound **18** was also very potent in modulating P-gp-mediated resistance toward vinblastine, vincristine, DOX, daunorubicin, and mitoxantrone with EC_{50} in nanomolar range between 90 and 179 nM (Table 2). These EC_{50} values are lower than that of **1** and is within 2- to 3-fold difference from a highly effective modulator like tariquidar (EC_{50} = 60 nM for paclitaxel resistance).[40]

We have also measured the therapeutic index (ratio of IC_{50} toward L929 to EC_{50} for reversing paclitaxel resistance) for compounds listed in Table 2. The most nontoxic compounds (highest therapeutic index) are **18** (therapeutic index of 574.3), **19** (therapeutic index of 465.6), **27** (therapeutic index of >558.7), and **28** (therapeutic index of >337.2). These compounds are considerably less toxic than **1** (therapeutic index of 208.4).

On the basis o fthe P-gp-modulating activity and cytotoxicity of the amine linked flavonoid dimers, we can draw the following conclusion related to their structure−activity relationships: (1) introduction of Boc group on the amine linker would greatly improve the P-gp-modulating activity compared to the previously reported PEG linked flavonoid dimers (e.g., **6**); (2) introduction of a hydrophobic group at the C6 or C5 position of ring A has a dramatic effect on the P-gp-mediated MDR reversal potency, and its effect is dependent on the size of the group; (3) removal of a Boc group from the amine linker would markedly potentiate its toxicity in a number of compounds, suggesting that flavonoid dimers with free N−H linker would be a toxic P-gp chemosensitizer; (4) the nature of the N-substituent on the amine linker has a strong influence on both P-gp-modulating activity and cytotoxicity. Balancing all the above factors, we have chosen **18** for further investigation.

*II.C. Preferential Inhibition P-gp over MRP1 and BCRP by **18**.* Other than P-gp, MRP1 and BCRP have also been implicated in clinical MDR. Here, we investigated whether **18** might have any MRP1- and/or BCRP-modulating activity. At 0.5 μM, **18** restored chemosensitivity of LCC6MDR to paclitaxel, with RF = 58.9 (Table 3). At the same concentration (0.5 μM), **18** mediated a weak modulating activity for both MRP1 (RF = 2.1 in 2008/MRP1 cells) and BCRP (RF = 3.8 in HEK293/R2 cells). Positive controls **2**, **8**, and **9** can effectively modulate P-gp, MRP1, and BCRP with RF equal to 85.1, 9.0, and 13.9,

dx.doi.org/10.1021/jm201121b | J. Med. Chem. 2012, 55, 1999−2014

respectively. These data suggest that compound **18** is more likely a preferential inhibitor toward P-gp than MRP1 or BCRP.

III. Mechanism of P-gp Modulation by 18. How does compound **18** modulate P-gp? It has been suggested that **10** binds at the NBD of P-gp, with C4 carbonyl and C7 phenolic functionalities of apigenin mimicking N1 and N7 of ATP.[27,28] Interestingly, we previously observed that apigenin dimer **4** can stimulate, instead of inhibit, ATPase activity of P-gp. This is more in line with the general observation that P-gp substrates can stimulate its ATPase activity. On the contrary, compounds that bind to the NBD of P-gp would inhibit ATP hydrolysis.[34] This suggested that **4** is not binding to the NBD region of P-gp.[34] Others have suggested that alkyl-substituted flavonoids may bind to the hydrophobic steroidal interacting region or the drug binding sites of the transmembrane domain (TMD) of P-gp.[30] Our previous observation that hydrophobic substitution in the flavonoid moiety can enhance modulating activity of flavonoid dimers was consistent with the latter model.[35] Here, we investigated if **18**, now carrying a hydrophilic amine linker, will still be able to bind to the drug binding site of P-gp.

*III.A. Effect of **18** on P-gp-ATPase Activity.* We first examined the effect of **18** on P-gp-ATPase activity. Compound **1**, being an inhibitor as well as a substrate of P-gp, is one of the best simulators of P-gp-ATPase. At 200 μM, **1** strongly increased the P-gp-ATPase activity over the basal level by 5.3-fold (Figure 1). Amine linked flavonoid dimer **18**, at 200 μM, can also stimulate P-gp-ATPase over the basal level by 2.7-fold (Figure 1). This result suggests that **18**, even though with the more hydrophilic amine group, is similar to **4** in not binding to the NBD region of P-gp.[34] Compound **18**, like **1**, may bind to the substrate recognition site of P-gp and stimulate its P-gp-ATPase activity.

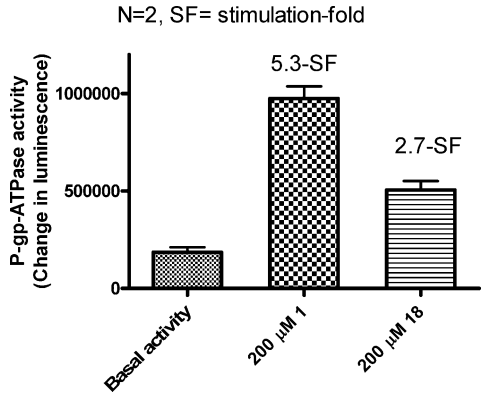


Figure 1. Effect of **1** and **18** on P-gp-ATPase activity. Sodium vanadate inhibitable ATPase (P-gp ATPase) was studied as described in Experimental Section. P-gp ATPase was measured in the absence (basal activity) or presence of P-gp modulators (**1** or **18**, both at 200 μM). The latter is normalized to the basal activity and presented as stimulation fold (SF). Results are presented as the mean ± standard error of mean. N = 2 independent experiments.

*III.B. **18** Can Increase Intracellular DOX Accumulation in MDA435/LCC6MDR Cells.* We investigated whether the modulation of P-gp-mediated drug resistance by **18** is associated with a concomitant increase in DOX accumulation. In the absence of modulator, DOX accumulation in parental LCC6 cells was about 2.9-fold (P < 0.001) higher than that of LCC6MDR cells (Figure 2). This is probably due to P-gp-mediated DOX efflux. Both **18** and **1** can increase DOX accumulation in LCC6MDR

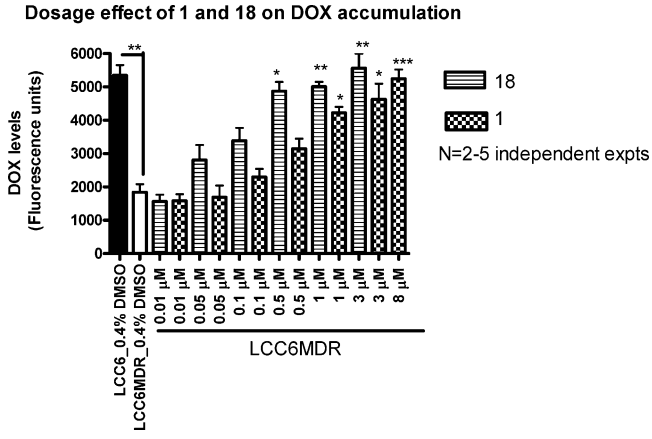


Figure 2. Effect of **1** and **18** on intracellular DOX accumulation in LCC6MDR cells. LCC6MDR cells were incubated with 20 μM DOX for 150 min at 37 °C with different concentrations of **18** (0, 0.01, 0.05, 0.1, 0.5, 1, and 3 μM) and **1** (0, 0.01, 0.05, 0.1, 0.5, 1, 3, and 8 μM). 0.4% of DMSO was used as negative control. After the incubation period, cells were lysed and the supernatant was measured for the DOX level by spectrofluorometry. N = 2–5 independent experiments. The results are presented as the mean ± standard error of mean: (*) P < 0.005, (**) P < 0.001, and (***) P < 0.0005 relative to the negative control (LCC6MDR).

cells in a dose-dependent manner (Figure 2). Minimum concentration required for **18** and **1** to restore DOX accumulation of LCC6MDR to that of LCC6's level is about 0.5 and 8 μM, respectively (Figure 2). Compound **18** is therefore roughly 16-fold more potent than **1** in inhibiting the DOX transport activity of P-gp.

*III.C. Compound **18** Is a Competitive Inhibitor of DOX in Binding to P-gp.* Lineweaver–Burk and Dixon plots were employed to further characterize the inhibition mechanism of **18** on P-gp's DOX transport activity. Lineweaver–Burk analysis found that V_{max} was unaffected, whereas K_m was decreased in the presence of either **1** or **18**, suggesting that **1** and **18** were both competitive inhibitors of DOX in binding to P-gp (Figure 3A and Figure 3B). From a plotting of the slopes of the Lineweaver–Burk plot versus the corresponding modulator concentrations used, K_i can be determined for **1** (K_i = 1.75 μM, inset of Figure 3A) and **18** (K_i = 0.34 μM, inset of Figure 3B).

The Dixon plot was also used to determine K_i for **1** (1.75 μM, Figure 3C) and **18** (0.28 μM, Figure 3D). These values are very similar to the K_i values determined using the Lineweaver–Burk plot. The linear regression line obtained from plotting the slopes of the Dixon plot versus reciprocal of substrate concentration can be used to distinguish a competitive, noncompetitive, or partially competitive relationship between inhibitor and substrate.[41,42] A competitive inhibitor will fit a line through the origin, whereas a noncompetitive inhibitor would not.[41,42] It was found that the regression lines almost coincide with the origin for **1** (inset of Figure 3C) and **18** (inset of Figure 3D), suggesting that both **1** and **18** are competitive inhibitors of DOX binding to P-gp, which is consistent with the Lineweaver–Burk analysis.

The K_i of **18** determined by both Lineweaver–Burk and Dixon plots was 0.28–0.34 μM. This is about 5.1- to 6.3-fold lower than the average K_i of **1** (1.75 μM) determined from Lineweaver–Burk and Dixon plots, indicating that **18** is more effective than **1** in displacing the DOX from P-gp. This is consistent with our observation that 16-fold more of **1**,

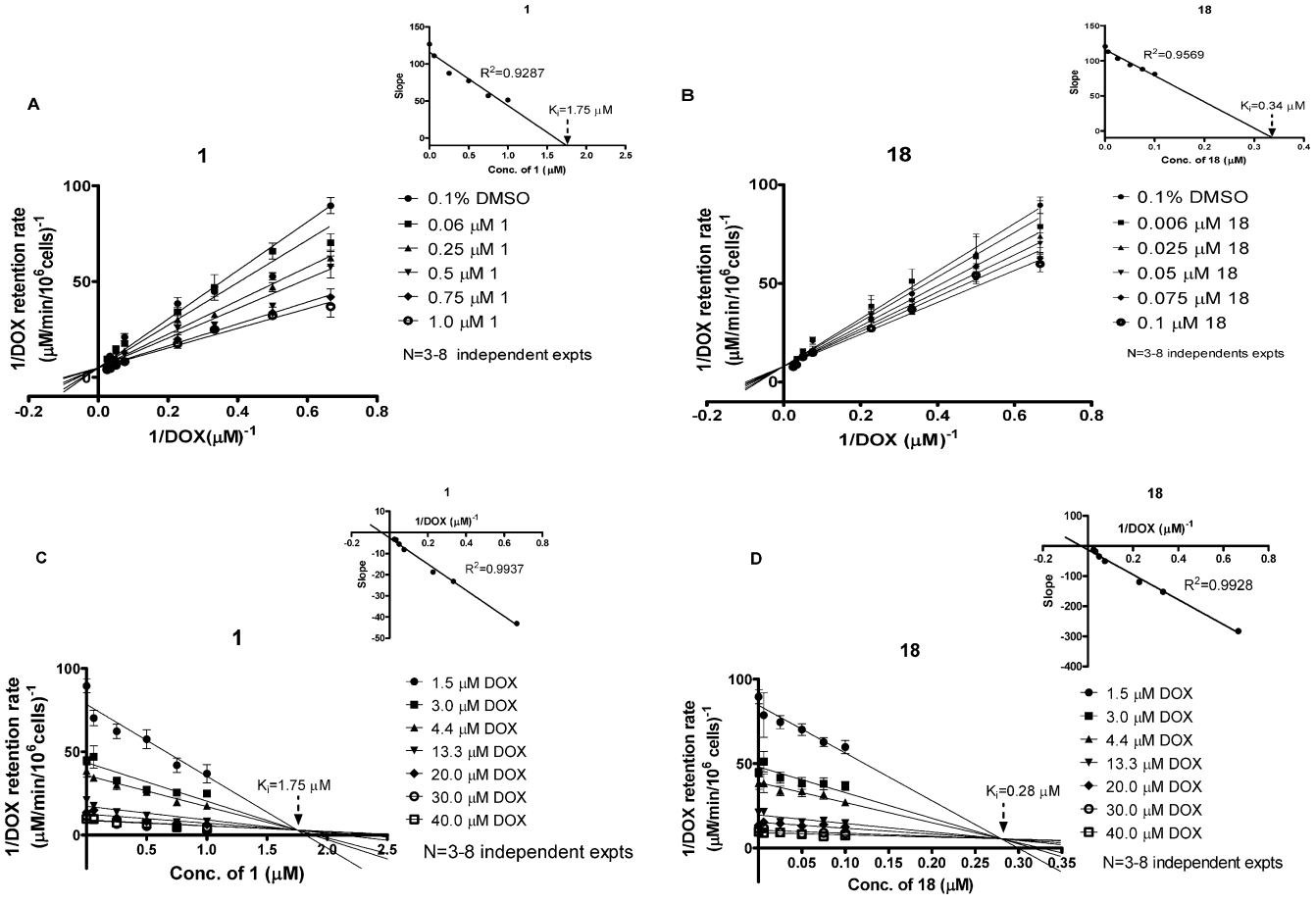


Figure 3. Lineweaver–Burk and Dixon analyses of the relationship between DOX and **1** or **18** in binding to P-gp. LCC6MDR cells were incubated with different concentrations of DOX (1.5, 3.0, 4.4, 13.3, 20.0, 30.0, and 40.0 μM) in the presence of **1** (0. 0.06, 0.25, 0.5, 0.75, or 1.0 μM) or **18** (0, 0.006, 0.025, 0.05, 0.075, or 0.1 μM). After incubation, the intracellular DOX level was measured by cell lysis followed by fluorescence spectrophotometry. Reciprocal of DOX retention rate is plotted against reciprocal of DOX concentration used (Lineweaver–Burk plot) in the presence of **1** (A) or **18** (B). Apparent K_i is determined by linear regression analysis of the slopes obtained from the double reciprocal plots versus the concentration of **1** or **18** used (insets of (A) and (B)). The relationship is also analyzed by Dixon plot: (C) **1** and (D) **18**. The reciprocal of DOX retention rate was plotted against concentration of **1** or **18**. The intersected point of the lines represented the K_i. The slope of the Dixon plot was plotted against reciprocal of DOX concentration to determine the relationship. N = 3–8 independent experiments, and the values were presented as the mean ± standard error of mean.

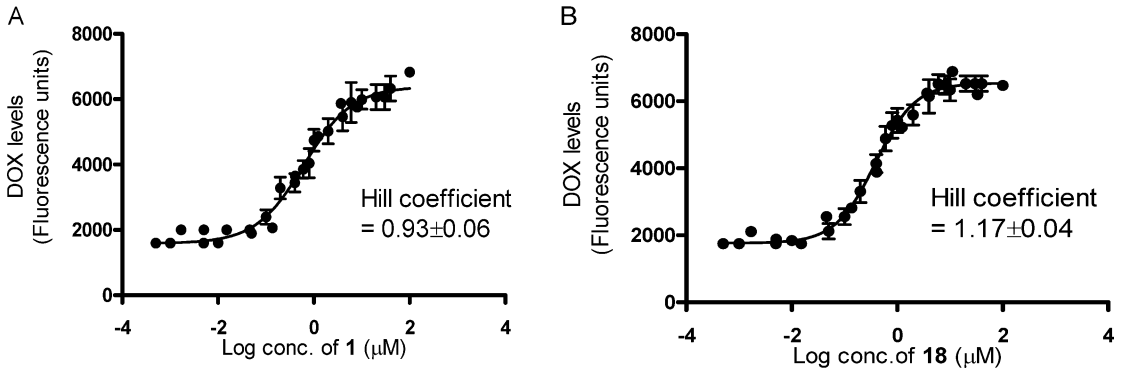


Figure 4. Hill coefficients of **1** and **18** for the DOX accumulation assay. LCC6MDR cells were incubated with 20 μM DOX in the presence of different concentrations of **1** (A) or **18** (B) (40, 30, 20, 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 μM). After incubation, the intracellular DOX level was measured by cell lysis followed by fluorescence spectrophotometry. The value of the Hill coefficient was determined by nonlinear regression of the following equation: Y = bottom + (top − bottom)/1 + $10^{(\log EC_{50}-X)\text{Hill coefficient}}$, where "bottom" is the baseline and "top" is the maximum effect, EC_{50} is the dose giving half the maximum effect, X is the modulator concentration (log). The Hill coefficient was presented as the mean ± standard error of mean from four to five independent experiments.

compared with **18**, is needed to fully restore DOX accumulation in LCC6MDR cells to the parental LCC6's level (Figure 2). In addition, the K_i of **18** (0.28–0.34 μM) for inhibiting P-gp-mediated DOX efflux is similar to the EC_{50} of **18**

dx.doi.org/10.1021/jm201121b | J. Med. Chem. 2012, 55, 1999–2014

(131 nM) for chemosensitizing LCC6MDR to DOX (Table 2). This result suggests that the chemosensitization mechanism of **18** is by virtue of its direct inhibition of P-gp-mediated DOX efflux (Figure 3), thereby increasing the intracellular DOX retention (Figure 2) and finally restoring DOX sensitivity (Tables 1 and 2).

In order to further understand the interaction between P-gp and **18**, the Hill slope (Hill coefficient) was determined. A Hill coefficient can reveal the biophysical nature of the receptor–ligand interaction.[43] A Hill coefficient of ~1 implies a single binding site with a simple mass action displacement. A Hill coefficient of >1 is indicative of cooperative binding, and a Hill value of <1 suggests negative cooperativity.[43] The Hill coefficient has been used to study the interaction between modulators and P-gp with respect to its drug efflux or ATPase activity.[44−46] Here, displacement of DOX from P-gp by **18** or **1** revealed a Hill coefficient of 1.17 ± 0.04 and 0.93 ± 0.06, respectively (Figure 4). This suggests that there is non-cooperative transport of DOX in the presence of **18** or **1**. In other words, there is independent binding of **18** or **1** with DOX.

In view of the stimulatory P-gp-ATPase activity and its competitive inhibition on DOX's transport, there is the possibility that compound **18** is itself a P-gp substrate as well. In order to further investigate whether compound **18** can be transported by P-gp, an intracellular accumulation assay of **18** was carried out. The LCC6MDR cell line was found to accumulate less **18** than the LCC6 cell line by 1.7-fold and 2.1-fold after incubating with 10 and 100 μM **18**, respectively (Figure 5). This indicates that compound **18** is a P-gp substrate.

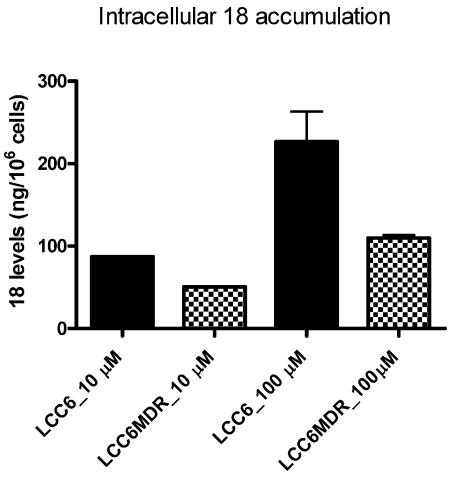


Figure 5. Intracellular accumulation of **18** in LCC6 and LCC6MDR cells. LCC6 and LCC6MDR cells were incubated with 10 or 100 μM **18** for 120 min at 37 °C. After the incubation period, cells were lysed and the supernatant was measured for the concentration of **18** by HPLC. N = 1−2 independent experiments. The results are presented as the mean ± standard error of mean.

The above results suggest that **18** directly interacts with the substrate recognition site of P-gp and competitively interferes with substrate binding. This inhibition restores intracellular accumulation of the substrate and effectively reverses P-gp-mediated MDR.

CONCLUSIONS

In summary, we observed a great improvement of the reversal potency of P-gp-mediated MDR when flavonoid dimers possess a substituted amine linker. The most potent amine linked flavonoid dimer is compound **18** containing a benzyl group at the amine functional group. Compound **18** significantly resensitizes LCC6MDR cells to various anticancer drugs including paclitaxel, vinblastine, vincristine, DOX, daunorubicin, and mitoxantrone with nanomolar EC_{50} values (EC_{50} ranged from 90 to 179 nM). Its mechanism of MDR modulation is associated with an increase in intracellular drug accumulation induced by directly inhibiting transport activity of P-gp. Kinetic characterization suggests that **18** likely acts as a competitive inhibitor of DOX. P-gp-ATPase activity indicates that **18**, like **1**, can stimulate P-gp-ATPase activity by binding to the substrate recognition site. The significant advantage of **18** includes its noncytotoxicity (IC_{50} for L929 of 85.0 μM) and its high therapeutic index (therapeutic index of 574.3). Because of the amine functional group, the hydrochloride salt of **18** can be readily prepared to improve water solubility. Pharmacokinetic studies and in vivo animal experiments with **18** will be reported in due course.

EXPERIMENTAL SECTION

General. All NMR spectra were recorded on a Bruker MHz DPX400 spectrometer at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C. All NMR measurements were carried out at room temperature, and the chemical shifts are reported as parts per million (ppm) relative to the resonance of $CDCl_3$ (7.26 ppm in the ^{1}H, 77.0 ppm for the central line of the triplet in the ^{13}C modes). Low-resolution and high-resolution mass spectra were obtained on a Micromass Q-TOF-2 by electron spray ionization (ESI) mode or on a Finnigan MAT95 ST by electron ionization (EI) mode. Melting points were measured using an Electrothermal IA9100 digital melting point apparatus and were uncorrected. All reagents and solvents were reagent grade and were used without further purification unless otherwise stated. The plates used for thin-layer chromatography (TLC) were E. Merck silica gel $60F_{254}$ (0.25 mm thickness), and they were visualized under short (254 nm) and long (365 nm) UV light. Chromatographic purifications were carried out using MN silica gel 60 (230−400 mesh). Substituted 4′-hydroxyflavones **11a−f** were prepared as we reported previously.[35] Ditosylate **12** was prepared according to the literature report.[47,48] The purity of tested compounds was determined by HPLC, which was performed by using an Agilent 1100 series installed with an analytic column of Agilent Prep-Sil Scalar column (4.6 mm × 250 mm, 5 μm) at UV detection of 320 nm (reference at 450 nm) with isocratic elution of hexane (50%)/ethyl acetate (25%)/methanol (25%) at a flow rate of 1 mL/min. All tested compounds were shown to >95% purity according to HPLC.

General Procedure I for the Preparation of Flavonoid Dimers 13a−f. A round-bottom flask was charged with corresponding 4′-hydroxyflavones **11a−f** (2 equiv), ditosylate **12** (1 equiv), K_2CO_3 (2.5 equiv), and DMF. The reaction mixture was stirred at refluxing temperature for 2 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. If the mixture could not be separated into two layers, small amount of 1 M HCl was added. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a brown crude reaction mixture. Purification of the flavonoid dimers was performed by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the desired product.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(*tert*-butyloxycarbonyl)-1,4,10,13-tetraoxa-7-azatridecane (13a). The titled compound **13a** was obtained from flavone **11a** (4.8 g, 20 mmol), ditosylate **12** (6.1 g, 10 mmol), K_2CO_3 (3.5 g), and DMF (30 mL) as a white foam (5.2 g, 70%) according to the general procedure described above: ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 3.45 (s, 4H), 3.62 (s, 4H), 3.69 (s, 4H), 4.08 (s, 4H), 6.60 (s, 2H), 6.94 (d, J = 8.4 Hz, 4H), 7.30 (d, J = 6.4 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 6.4 Hz, 2H), 7.75 (d, J = 8.4 Hz, 4H), 8.12 (d, J = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$)

δ 28.4, 47.6, 47.9, 67.5, 69.1, 69.2, 69.9, 70.1, 79.6, 106.0, 114.9, 117.9, 123.8, 123.9, 125.0, 125.4, 127.8, 133.5, 155.4, 156.0, 161.5, 163.1, 178.1; LRMS (ESI) *m/z* 734 (M^+ + H, 77), 756 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{43}H_{44}NO_{10}$ (M^+ + H) 734.2965, found 734.2979.

1,13-Bis[4′-((3-methoxy)-4*H*-chromen-4-on-2-yl)phenyl]-7-(*tert*-butyloxycarbonyl)-1,4,10,13-tetraoxa-7-azatridecane (13b). The titled compound **13b** was obtained from 4′-hydroxyflavone **11b** (0.18 g, 0.67 mmol), ditosylate **12** (0.20 g, 0.33 mmol), K_2CO_3 (0.12 g), and DMF (6 mL) as a white foam (0.14 g, 53%) according to the general procedure I described above: ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 3.45 (s, 4H), 3.61 (s, 4H), 3.79 (s, 4H), 3.87 (s, 6H), 4.13 (s, 4H), 6.63 (s, 2H), 6.89 (d, *J* = 6.4 Hz, 2H), 7.30 (d, *J* = 6.4 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 6.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 4H), 8.12 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 28.4, 47.6, 47.9, 56.0, 68.4, 69.0, 69.2, 69.9, 70.2, 79.5, 106.3, 109.1, 112.8, 117.9, 119.7, 123.7, 124.3, 125.0, 125.4, 133.5, 149.5, 151.4, 155.4, 156.0, 163.1, 178.1; LRMS (ESI) *m/z* 794 (M^+ + H, 27), 816 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{45}H_{48}NO_{12}$ (M^+ + H) 794.3177, found 794.3170.

1,13-Bis[4′-((6-methyl)-4*H*-chromen-4-on-2-yl)phenyl]-*N*-(*tert*-butyloxycarbonyl)-1,4,10,13-tetraoxa-7-azatridecane (13c). The titled compound **13c** was obtained from flavone **11c** (0.12 g, 0.48 mmol), ditosylate **12** (0.15 g, 0.25 mmol), K_2CO_3 (0.09 g), and DMF (6 mL) as a white foam (90 mg, 47%) according to the general procedure described above: ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 2.36 (s, 6H), 3.46 (t, *J* = 6.8 Hz, 4H), 3.64 (t, *J* = 3.6 Hz, 4H), 3.78 (s, 4H), 4.09 (s, 4H), 6.62 (s, 2H), 6.93 (d, *J* = 8.4 Hz, 4H), 7.31−7.39 (m, 4H), 7.75 (d, *J* = 8.4 Hz, 4H), 7.89 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 20.9, 28.5, 31.4, 47.8, 48.0, 67.6, 69.1, 70.0, 70.2, 79.7, 105.8, 115.0, 117.7, 123.3, 124.1, 124.9, 128.0, 134.9, 135.1, 154.4, 155.5, 161.7, 162.5, 163.3, 178.3; LRMS (ESI) *m/z* 762 (M^+ + H, 95), 784 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{45}H_{48}NO_{10}$ (M^+ + H) 762.3278, found 762.3289.

1,13-Bis[4′-((5-methoxy)-4*H*-chromen-4-on-2-yl)phenyl]-7-(*tert*-butyloxycarbonyl)-1,4,10,13-tetraoxa-7-azatridecane (13d). The titled compound **13d** was obtained from 4′-hydroxyflavone **11d** (80 mg, 0.30 mmol), ditosylate **12** (90 mg, 0.15 mmol), K_2CO_3 (60 mg), and DMF (5 mL) as a white foam (69 mg, 58%) according to the general procedure I described above: ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 3.45 (t, *J* = 6.8 Hz, 4H), 3.63 (s, 4H), 3.71 (s, 4H), 3.92 (s, 6H), 4.09 (s, 4H), 6.55 (s, 2H), 7.73 (d, *J* = 7.6 Hz, 2H), 6.93 (d, *J* = 8.0 Hz, 4H), 7.02 (d, *J* = 8.0 Hz, 2H), 7.47 (dd, *J* = 7.6, 8.0 Hz, 2H), 7.74 (d, *J* = 8.0 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 28.4, 47.6, 47.9, 56.3, 68.4, 69.0, 69.2, 69.9, 70.1, 79.6, 106.3, 107.5, 110.0, 114.9, 123.7, 127.7, 133.6, 158.1, 159.7, 160.9, 161.3, 178.3; LRMS (ESI) *m/z* 794 (M^+ + H, 35), 816 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{45}H_{48}NO_{12}$ (M^+ + H) 794.3177, found 794.3175.

1,13-Bis[4′-((3-benzyloxy)-4*H*-chromen-4-on-2-yl)phenyl]-7-(*tert*-butyloxycarbonyl)-1,4,10,13-tetraoxa-7-azatridecane (13e). The titled compound **13e** was obtained from 4′-hydroxyflavone **11e** (0.19 g, 0.55 mmol), ditosylate **12** (0.17 g, 0.28 mmol), K_2CO_3 (0.10 g), and DMF (6 mL) as a white foam (0.13 g, 49%) according to the general procedure I described above: ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.50 (t, *J* = 6.8 Hz, 4H), 3.60 (s, 4H), 3.79 (s, 4H), 4.10 (s, 4H), 5.07 (s, 4H), 6.91 (d, *J* = 8.8 Hz, 4H), 7.22−7.41 (m, 14H), 7.55 (dd, *J* = 8.0, 8.4 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 4H), 8.20 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 28.4, 47.7, 47.9, 67.5, 69.1, 69.3, 69.9, 70.1, 73.8, 79.6, 114.3, 117.9, 123.3, 124.0, 124.5, 125.5, 128.0, 128.2, 128.7, 129.0, 130.4, 133.2, 136.7, 139.2, 155.0, 155.4, 156.0, 160.6, 174.8; LRMS (ESI) *m/z* 946 (M^+ + H, 25), 968 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{57}H_{56}NO_{12}$ (M^+ + H) 946.3803, found 946.3838.

1,13-Bis[4′-((6-fluoro)-4*H*-chromen-4-on-2-yl)phenyl]-7-(*tert*-butyloxycarbonyl)-1,4,10,13-tetraoxa-7-azatridecane (13f). The titled compound **13f** was obtained from 4′-hydroxyflavone **11f** (3.09 g, 12.1 mmol), ditosylate **12** (3.60 g, 5.98 mmol), K_2CO_3 (1.74 g), and DMF (20 mL) as a white foam (2.41 g, 52%) according to the general procedure I described above: ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 3.51 (s, 4H), 3.69 (s, 4H), 3.84 (s, 4H), 4.17 (s, 4H), 6.68 9s, 2H), 7.00 (d, *J* = 8.8 Hz, 4H), 7.39 (ddd, *J* = 2.0, 8.4, 8.4 Hz, 2H), 7.50 (dd, *J* = 4.4, 9.2 Hz, 2 H), 7.79 (d, *J* = 8.8 Hz, 4H), 7.81 (dd, *J* = 4.4, 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 28.4, 47.7, 47.9, 67.5, 69.1, 69.3, 69.9, 70.1, 73.8, 79.6, 105.4, 110.6 (d, *J* = 23.6 Hz, C5), 115.1, 119.9 (d, *J* = 7.8 Hz, C10), 121.7 (d, *J* = 25.2 Hz, C7), 123.8, 125.0 (d, *J* = 7.8 Hz, C8), 127.9, 152.2 (d, *J* = 1.5 Hz, C9), 159.5 (d, *J* = 245.3 Hz, C6), 161.7, 163.5, 177.4; LRMS (ESI) *m/z* 770 (M^+ + H, 100), 792 (M^+ + Na, 68); HRMS (ESI) calcd for $C_{43}H_{42}NO_{10}F_2$ (M^+ + H) 770.2777, found 770.2807.

General Procedure II for the Preparation of Flavonoid Dimers 14a−f. A round-bottom flask was charged with *N*-Boc protected flavonoid dimers **13** and CH_2Cl_2. The solution was cooled to 0 °C using an ice bath. An equal volume of TFA was then added dropwise, and the reaction mixture was stirred vigorously at 0 °C for 1 h and at room temperature for another 1 h. After the mixture was stirred, the reaction was quenched by pouring the mixture into a conical flask containing water. The resultant mixture was basified to pH 10 by using potassium hydroxide solution. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give the desired product.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-1,4,10,13-tetraoxa-7-azatridecane (14a). The titled compound **14a** was obtained from **13a** (5.10 g, 6.95 mmol), TFA (20 mL), and CH_2Cl_2 (20 mL) as a pale brown oil (4.30 g, 98%) according to the general procedure II described above: ^{1}H NMR ($CDCl_3$) δ 1.95 (br, 1H), 2.83−2.87 (m, 4H), 3.65−3.69 (m, 4H), 3.82 (t, *J* = 4.8 Hz, 4H), 4.14 (t, *J* = 4.8 Hz, 4H), 6.66 (s, 2H), 6.97 (d, *J* = 8.6 Hz, 4H), 7.34 (dd, *J* = 7.2, 8.0 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.61 (dd, *J* = 7.2, 8.0 Hz, 2H), 7.80 (d, *J* = 8.6 Hz, 4H), 8.15 (dd, *J* = 1.2, 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 49.2, 67.5, 69.3, 70.9, 106.1, 114.9, 117.9, 123.8, 124.0, 125.0, 125.5, 127.8, 133.5, 156.0, 161.5, 163.1, 178.2; LRMS (ESI) *m/z* 634 (M^+ + H, 100); HRMS (ESI) calcd for $C_{38}H_{36}NO_8$ (M^+ + H) 634.2441, found 634.2418.

1,13-Bis[4′-((3-methoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,10,13-tetraoxa-7-azatridecane (14b). The titled compound **14b** was obtained from **13b** (0.12 g, 0.15 mmol), TFA (4 mL), and CH_2Cl_2 (4 mL) as a pale brown oil (95 mg, 91%) according to the general procedure II described above: ^{1}H NMR ($CDCl_3$) δ 2.13 (br, 1H), 2.79−2.82 (m, 4H), 3.63−3.65 (m, 4H), 3.83−3.84 (m, 4H), 3.89 (s, 6H), 4.16−4.19 (m, 4H), 6.65 (s, 1H), 6.66 (s, 1H), 6.91−6.94 (m, 2H), 7.29−7.33 (m, 4H), 7.42−7.48 (m, 4H), 7.52−7.58 (m, 2H), 8.11−8.13 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 49.1, 56.0, 68.3, 69.2, 70.9, 79.6, 106.3, 109.2, 112.8, 117.9, 119.7, 123.8, 124.3, 125.0, 125.5, 133.5, 149.5, 151.3, 156.0, 163.1, 178.2; LRMS (ESI) *m/z* 694 (M^+ + H, 100), 716 (M^+ + Na, 19); HRMS (ESI) calcd for $C_{40}H_{40}NO_{10}$ (M^+ + H) 694.2652, found 694.2640.

1,13-Bis[4′-((6-methyl)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,10,13-tetraoxa-7-azatridecane (14c). The titled compound **14c** was obtained from **13c** (72 mg, 0.09 mmol), TFA (4 mL), and CH_2Cl_2 (4 mL) as a pale brown oil (56 mg, 90%) according to the general procedure II described above: ^{1}H NMR ($CDCl_3$) δ 2.03 (br, 1H), 2.41 (s, 6 h), 2.85 (t, *J* = 4.6 Hz, 4H), 3.68 (t, *J* = 4.2 Hz, 4H), 3.83 (t, *J* = 4.6 Hz, 4H), 4.14 (t, *J* = 4.6 Hz, 4H), 6.66 (s, 2H), 6.97 (d, *J* = 8.8 Hz, 4H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.43 (dd, *J* = 2.0, 8.0 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 4H), 7.93 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 20.9, 48.9, 67.5, 69.4, 70.2, 105.9, 114.9, 117.6, 123.4, 124.1, 124.9, 127.8, 134.8, 134.9, 154.3, 161.5, 163.1, 178.4; LRMS (ESI) *m/z* 662 (M^+ + H, 100), 684 (M^+ + Na, 5); HRMS (ESI) calcd for $C_{40}H_{40}NO_8$ (M^+ + H) 662.2754, found 662.2758.

1,13-Bis[4′-((5-methoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,10,13-tetraoxa-7-azatridecane (14d). The titled compound **14d** was obtained from **13d** (62 mg, 0.08 mmol), TFA (4 mL), and CH_2Cl_2 (4 mL) as a pale brown oil (47 mg, 87%) according to the general procedure II described above: ^{1}H NMR ($CDCl_3$) δ 2.19 (br, 1H), 2.80 (t, *J* = 4.6 Hz, 4H), 3.62 (t, *J* = 4.6 Hz, 4H), 3.77 (t, *J* = 4.2 Hz, 4H), 3.91 (s, 6H), 4.08 (t, *J* = 4.6 Hz, 4H), 6.53 (s, 2H), 6.71 (d, *J* = 8.0 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 4H), 6.99 (d, *J* = 8.4 Hz, 2H), 7.45 (dd, *J* = 8.0, 8.4 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 48.9, 56.5, 67.5, 69.4, 70.2, 106.4, 107.7, 110.1, 114.5, 115.0, 123.9, 127.7, 133.6, 158.2, 159.7, 161.0, 161.3, 178.3; LRMS

(ESI) m/z 694 (M^+ + H, 100), 716 (M^+ + Na, 20); HRMS (ESI) calcd for $C_{40}H_{40}NO_{10}$ (M^+ + H) 694.2652, found 694.2653.

1,13-Bis[4′-((3-benzyloxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,10,13-tetraoxa-7-azatridecane (14e). The titled compound **14e** was obtained from **13e** (90 mg, 0.10 mmol), TFA (4 mL), and CH_2Cl_2 (4 mL) as a pale brown oil (75 mg, 93%) according to the general procedure II described above: ^{1}H NMR ($CDCl_3$) δ 2.19 (br, 1H), 2.86 (t, J = 4.6 Hz, 4H), 3.67 (t, J = 4.2 Hz, 4H), 3.82 (t, J = 4.6 Hz, 4H), 4.13 (t, J = 4.6 Hz, 4H), 5.08 (s, 4H), 6.92 (d, J = 9.2 Hz, 4H), 7.22−7.36 (m, 12H), 7.42 (d, J = 8.4 Hz, 2H), 7.58 (dd, J = 8.0, 8.4 Hz, 2H), 7.98 (d, J = 8.8 Hz, 4H), 8.22 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 49.2, 67.4, 69.3, 70.9, 73.8, 114.3, 117.9, 123.4, 124.1, 124.5, 125.6, 128.0, 128.2, 128.8, 130.4, 133.2, 136.7, 139.2, 155.0, 156.1, 160.6, 174.8; LRMS (ESI) m/z 846 (M^+ + H, 100), 868 (M^+ + Na, 8); HRMS (ESI) calcd for $C_{52}H_{48}NO_{10}$ (M^+ + H) 846.3278, found 846.3268.

1,13-Bis[4′-((6-fluoro)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,10,13-tetraoxa-7-azatridecane (14f). The titled compound **14f** was obtained from **13f** (2.30 g, 2.99 mmol), TFA (10 mL), and CH_2Cl_2 (10 mL) as a pale brown oil (1.99 g, 99%) according to the general procedure II described above: ^{1}H NMR ($CDCl_3$) δ 2.48 (br, 1H), 2.91 (t, J = 4.6 Hz, 4H), 3.72 (t, J = 4.6 Hz, 4H), 3.87 (t, J = 4.6 Hz, 4H), 4.18 (t, J = 4.6 Hz, 4H), 6.68 (s, 2H), 7.00 (d, J = 8.8 Hz, 4H), 7.39 (ddd, J = 2.0, 8.4, 8.4 Hz, 2H), 7.50 (dd, J = 4.4, 9.2 Hz, 2 H), 7.79 (d, J = 8.8 Hz, 4H), 7.81 (dd, J = 4.4, 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 49.3, 67.6, 69.3, 70.7, 105.4, 110.6 (d, J = 23.6 Hz, C5), 115.1, 119.9 (d, J = 7.8 Hz, C10), 121.7 (d, J = 25.2 Hz, C7), 123.8, 125.0 (d, J = 7.8 Hz, C8), 127.9, 152.2 (d, J = 1.5 Hz, C9), 159.5 (d, J = 245.3 Hz, C6), 161.7, 163.5, 177.4; LRMS (ESI) m/z 670 (M^+ + H, 100), 692 (M^+ + Na, 13); HRMS (ESI) calcd for $C_{38}H_{34}NO_8F_2$ (M^+ + H) 670.2252, found 670.2232.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(ethyl)-1,4,10,13-tetraoxa-7-azatridecane (15). A round-bottom flask was charged with compound **14a** (80 mg, 0.13 mmol), bromoethane (25 mg, 0.23 mmol), K_2CO_3 (40 mg), and ACN (10 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **15** as a pale brown oil (56 mg, 67%): ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.2 Hz, 3H), 2.77 (q, J = 5.2 Hz, 2H), 2.84 (t, J = 5.2 Hz, 4H), 3.65 (t, J = 4.8 Hz, 4H), 3.82 (t, J = 4.8 Hz, 4H), 4.15 (t, J = 4.8 Hz, 4H), 6.68 (s, 2H), 6.99 (d, J = 8.8 Hz, 4H), 7.36 (dd, J = 7.6, 7.6 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.65 (dd, J = 8.0, 8.4 Hz, 2H), 7.82 (d, J = 8.8 Hz, 4H), 8.17 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 49.2, 53.3, 67.6, 69.3, 69.5, 106.1, 114.9, 117.9, 123.8, 124.1, 125.0, 125.5, 127.9, 133.5, 156.1, 161.5, 163.2, 178.3; LRMS (ESI) m/z 661 (M^+ + H, 100); HRMS (ESI) calcd for $C_{40}H_{40}NO_8$ (M^+ + H) 661.7396, found 661.7386.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(2-hydroxyethyl)-1,4,10,13-tetraoxa-7-azatridecane (16). A round-bottom flask was charged with compound **14a** (70 mg, 0.11 mmol), bromoethanol (25 mg, 0.20 mmol), K_2CO_3 (35 mg), and ACN (15 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **16** as a pale brown oil (45 mg, 56%): ^{1}H NMR ($CDCl_3$) δ 2.17 (br, OH), 2.75 (t, J = 5.2 Hz, 2H), 2.84 (t, J = 5.2 Hz, 4H), 3.58 (t, J = 5.2 Hz, 2H), 3.65 (t, J = 4.8 Hz, 4H), 3.84 (t, J = 4.8 Hz, 4H), 4.15 (t, J = 4.8 Hz, 4H), 6.66 (s, 2H), 7.02 (d, J = 8.8 Hz, 4H), 7.39 (dd, J = 7.6, 7.6 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.65 (dd, J = 8.0, 8.4 Hz, 2H), 7.84 (d, J = 8.8 Hz, 4H), 8.19 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 54.3, 56.7, 59.3, 67.5, 69.2, 69.8, 105.9, 114.9, 117.9, 123.7, 123.9, 125.0, 125.4, 127.8, 133.5, 156.0, 161.5, 163.1, 178.1; LRMS (ESI) m/z 677 (M^+ + H, 100); HRMS (ESI) calcd for $C_{40}H_{40}NO_9$(M^+ + H) 677.7390, found 677.7382.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(3-ethoxy-3-oxopropyl)-1,4,10,13-tetraoxa-7-azatridecane (17). A round-bottom flask was charged with compound **14a** (90 mg, 0.14 mmol), ethyl 3-bromopropionate (40 mg, 0.22 mmol), K_2CO_3 (40 mg), and ACN (15 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **17** as a pale brown oil (65 mg, 62%): ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J = 6.8 Hz, 3H), 2.48 (t, J = 6.8 Hz, 2H), 2.79 (t, J = 6.0 Hz, 4H), 2.94 (t, J = 7.2 Hz, 2H), 3.64 (t, J = 6.0 Hz, 4H), 3.83 (t, J = 4.8 Hz, 4H), 4.08−4.17 (m, 6H), 6.71 (s, 2H), 7.01 (d, J = 8.8 Hz, 4H), 7.38 (dd, J = 7.6, 7.6 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.65 (dd, J = 8.0, 8.4 Hz, 2H), 7.84 (d, J = 8.8 Hz, 4H), 8.19 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 50.7, 54.0, 60.4, 67.6, 69.4, 106.2, 115.0, 117.9, 123.9, 124.2, 125.1, 125.6, 128.0, 133.6, 156.1, 161.6, 163.3, 178.3; LRMS (ESI) m/z 733 (M^+ + H, 100); HRMS (ESI) calcd for $C_{43}H_{44}NO_{10}$ (M^+ + H) 733.8023, found 733.8016.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(benzyl)-1,4,10,13-tetraoxa-7-azatridecane (18). A round-bottom flask was charged with compound **14a** (1.30 g, 2.05 mmol), benzyl bromide (0.45 g, 2.63 mmol), K_2CO_3 (0.40 g), and ACN (40 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the hot reaction mixture was filtered to remove solid K_2CO_3. The filtrate was cooled in an ice bath, and a large amount of white precipitate was formed. The titled compound **18** was obtained after suction filtration as a white solid (1.05 g, 71%): mp 112−114 °C; ^{1}H NMR ($CDCl_3$) δ 2.80 (t, J = 6.0 Hz, 4H), 3.64 (t, J = 6.0 Hz, 4H), 3.72 (s, 2H), 3.78 (t, J = 4.8 Hz, 4H), 4.11 (t, J = 4.8 Hz, 4H), 6.67 (s, 2H), 6.97 (d, J = 8.8 Hz, 4H), 7.25−7.36 (m, 7H), 7.48 (d, J = 8.4 Hz, 2H), 7.62 (dd, J = 8.0, 8.4 Hz, 2H), 7.79 (d, J = 8.8 Hz, 4H), 8.17 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 53.9, 59.8, 67.6, 69.2, 70.2, 106.1, 115.0, 117.9, 123.8, 124.0, 125.0, 125.5, 126.9, 127.8, 128.1, 128.7, 133.5, 139.6, 156.0, 161.6, 163.2, 178.2; LRMS (ESI) m/z 724 (M^+ + H, 100), 746 (M^+ + Na, 14); HRMS (ESI) calcd for $C_{45}H_{42}NO_8$ (M^+ + H) 724.2910, found 724.2917.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(4-nitrobenzyl)-1,4,10,13-tetraoxa-7-azatridecane (19). A round-bottom flask was charged with compound **14a** (100 mg, 0.16 mmol), 4-nitrobenzyl bromide (52 mg, 0.24 mmol), K_2CO_3 (45 mg), and ACN (15 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **19** as pale brown solid (85 mg, 70%): mp 95−97 °C; ^{1}H NMR ($CDCl_3$) δ 2.82 (t, J = 5.6 Hz, 4H), 3.64 (t, J = 6.0 Hz, 4H), 3.78 (t, J = 4.8 Hz, 4H), 3.85 (s, 2H), 4.14 (t, J = 4.8 Hz, 4H), 6.70 (s, 2H), 6.99 (d, J = 8.8 Hz, 4H), 7.35 (d, J = 5.2 Hz, 2H), 7.49 (dd, J = 8.0, 8.4 Hz, 4H), 7.65 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 8.8 Hz, 4H), 8.11 (d, J = 8.0 Hz, 2H), 8.18 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 54.1, 59.1, 67.6, 69.3, 70.1, 106.1, 114.9, 117.9, 123.4, 123.8, 124.2, 125.0, 125.6, 127.9, 129.0, 133.6, 146.9, 148.2, 156.1, 161.5, 163.2, 178.3; LRMS (ESI) m/z 769 (M^+ + H, 100), 791 (M^+ + Na, 13); HRMS (ESI) calcd for $C_{45}H_{41}N_2O_{10}$ (M^+ + H) 769.2761, found 769.2762.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(4-methoxycarbonylbenzyl)-1,4,10,13-tetraoxa-7-azatridecane (20). A round-bottom flask was charged with compound **14a** (120 mg, 0.19 mmol), methyl 4-(bromomethyl)benzoate (60 mg, 0.26 mmol),

K_2CO_3 (50 mg), and ACN (15 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **20** as a pale brown oil (98 mg, 66%): ^{1}H NMR ($CDCl_3$) δ 2.90 (t, J = 5.6 Hz, 4H), 3.67 (t, J = 6.0 Hz, 4H), 3.78 (t, J = 4.8 Hz, 4H), 3.82 (s, 2H), 3.87 (s, 3H), 4.14 (t, J = 4.8 Hz, 4H), 6.70 (s, 2H), 6.99 (d, J = 8.8 Hz, 4H), 7.37 (dd, J = 8.0, 8.4 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.65 (dd, J = 8.0, 8.4 Hz, 2H), 7.83 (d, J = 8.8 Hz, 4H), 7.95 (d, J = 8.0 Hz, 2H), 8.18 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 54.0, 67.6, 69.2, 106.1, 115.0, 117.9, 123.8, 124.1, 125.0, 125.6, 127.9, 128.6, 129.5, 133.5, 156.1, 161.5, 163.2, 178.3; LRMS (ESI) m/z 782 (M^+ + H, 100), 804 (M^+ + Na, 8); HRMS (ESI) calcd for $C_{47}H_{44}NO_{10}$ (M^+ + H) 782.2965, found 782.2959.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(4-pyridylmethyl)-1,4,10,13-tetraoxa-7-azatridecane (21). A round-bottom flask was charged with compound **14a** (210 mg, 0.33 mmol), 4-(bromomethyl)pyridine hydrobromide salt (85 mg, 0.34 mmol), K_2CO_3 (110 mg), and ACN (15 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **21** as a pale brown oil (130 mg, 54%): ^{1}H NMR ($CDCl_3$) δ 2.81 (t, J = 5.6 Hz, 4H), 3.63 (t, J = 6.0 Hz, 4H), 3.78 (t, J = 4.8 Hz, 4H), 3.84 (s, 2H), 4.14 (t, J = 4.8 Hz, 4H), 6.70 (s, 2H), 6.99 (d, J = 8.8 Hz, 4H), 7.30 (d, J = 5.2 Hz, 2H), 7.37 (dd, J = 8.0, 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.62 (dd, J = 8.0, 8.4 Hz, 2H), 7.83 (d, J = 8.8 Hz, 4H), 8.17 (d, J = 8.0 Hz, 2H), 8.49 (d, J = 5.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 54.2, 58.7, 67.6, 69.3, 70.0, 106.2, 115.0, 117.9, 123.7, 123.9, 124.2, 125.1, 125.6, 127.9, 133.6, 149.6, 156.1, 161.5, 163.2, 178.3; LRMS (ESI) m/z 725 (M^+ + H, 25); HRMS (ESI) calcd for $C_{44}H_{41}N_2O_8$ (M^+ + H) 725.2863, found 725.2859.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(2-pyridylmethyl)-1,4,10,13-tetraoxa-7-azatridecane (22). A round-bottom flask was charged with compound **14a** (110 mg, 0.17 mmol), 2-(bromomethyl)pyridine hydrobromide salt (49 mg, 0.19 mmol), K_2CO_3 (60 mg), and ACN (10 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **22** as an off white solid (73 mg, 58%): mp 107−109 °C; ^{1}H NMR ($CDCl_3$) δ 2.87 (t, J = 5.6 Hz, 4H), 3.66 (t, J = 6.0 Hz, 4H), 3.78 (t, J = 4.8 Hz, 4H), 3.89 (s, 2H), 4.14 (t, J = 4.8 Hz, 4H), 6.68 (s, 2H), 6.98 (d, J = 8.8 Hz, 4H), 7.13 (dd, J = 8.0, 8.4 Hz, 1H), 7.47 (dd, J = 8.0, 8.4 Hz, 2H), 7.48−7.63 (m, 6H), 7.81 (d, J = 8.8 Hz, 4H), 8.17 (d, J = 8.8 Hz, 2H), 8.49 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 54.2, 61.3, 67.6, 69.2, 70.0, 106.1, 115.0, 117.9, 121.9, 123.0, 123.8, 124.0, 125.0, 125.5, 127.9, 133.5, 136.3, 148.9, 156.1, 160.0, 161.6, 163.2, 178.3; LRMS (ESI) m/z 725 (M^+ + H, 23), 747 (M^+ + Na, 8); HRMS (ESI) calcd for $C_{44}H_{41}N_2O_8$ (M^+ + H) 725.2863, found 725.2849.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]-1,4,10,13-tetraoxa-7-azatridecane (23). A round-bottom flask was charged with compound **14a** (90 mg, 0.14 mmol), 1-bromo-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethane (65 mg, 0.26 mmol), K_2CO_3 (50 mg), and ACN (10 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **23** as a pale brown oil (72 mg, 63%): ^{1}H NMR ($CDCl_3$) δ 2.88 (s, 6H), 3.63 (t, J = 5.2 Hz, 4H), 3.78 (t, J = 4.8 Hz, 6H), 4.11 (t, J = 4.4 Hz, 4H), 6.68 (s, 2H), 6.99 (d, J = 8.8 Hz, 4H), 7.37 (dd, J = 8.0, 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.63−7.67 (m, 4H), 7.77−7.81 (m, 6H), 8.19 (dd, J = 1.2, 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 52.5, 54.2, 67.6, 69.3, 70.1, 106.1, 115.0, 117.9, 123.1, 123.9, 124.0, 125.0, 125.6, 127.9, 132.1, 133.5, 133.8, 156.1, 161.6, 163.2, 168.3, 178.3; LRMS (ESI) m/z 807 (M^+ + H, 20), 829 (M^+ + Na, 4); HRMS (ESI) calcd for $C_{48}H_{43}N_2O_{10}$ (M^+ + H) 807.2918, found 807.2920.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(3-phenylpropyl)-1,4,10,13-tetraoxa-7-azatridecane (24). A round-bottom flask was charged with compound **14a** (110 mg, 0.17 mmol), 3-bromo-1-phenylpropane (60 mg, 0.30 mmol), K_2CO_3 (50 mg), and ACN (15 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **24** as a pale brown oil (86 mg, 66%): ^{1}H NMR ($CDCl_3$) δ 1.81 (m, 2H), 2.58−2.62 (m, 4H), 2.79 (t, J = 5.2 Hz, 4H), 3.63 (t, J = 4.8 Hz, 4H), 3.81 (t, J = 4.8 Hz, 4H), 4.14 (t, J = 4.8 Hz, 4H), 6.70 (s, 2H), 7.00 (d, J = 8.8 Hz, 4H), 7.15−7.26 (m, 5H), 7.37 (dd, J = 7.6, 7.6 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.65 (dd, J = 8.0, 8.4 Hz, 2H), 7.83 (d, J = 8.8 Hz, 4H), 8.19 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ28.8, 33.4, 33.5, 54.1, 54.9, 67.7, 69.3, 70.1, 106.1, 115.0, 117.9, 123.9, 124.1, 125.1, 125.6, 125.8, 127.9, 128.3, 128.4, 133.6, 142.2, 156.1, 161.6, 163.3, 178.3; LRMS (ESI) m/z 752 (M^+ + H, 55); HRMS (ESI) calcd for $C_{47}H_{46}NO_8$ (M^+ + H) 751.8621, found 751.8612.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(4-fluorobenzyl)-1,4,10,13-tetraoxa-7-azatridecane (25). A round-bottom flask was charged with compound **14a** (0.31 g, 0.49 mmol), 4-fluorobenzyl methanesufonate (0.12 g, 0.59 mmol), K_2CO_3 (90 mg), and ACN (20 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the hot reaction mixture was filtered to remove solid K_2CO_3. The filtrate was cooled in an ice bath, and a large amount of white precipitate was formed. The titled compound **25** was obtained after suction filtration as a white solid (0.25 g, 69%): mp 119−122 °C; ^{1}H NMR ($CDCl_3$) δ 2.83 (s, 4H), 3.67 (t, J = 4.6 Hz, 4H), 3.79 (t, J = 4.6 Hz, 4H), 3.81 (s, 2H), 4.14 (t, J = 4.6 Hz, 4H), 6.69 (s, 2H), 6.98 (d, J = 8.6 Hz, 4H), 7.36 (dd, J = 7.2, 8.0 Hz, 2H), 7.47−7.54 (m, 6H), 7.64 (dd, J = 7.2, 8.0 Hz, 2H), 7.82 (d, J = 8.6 Hz, 4H), 8.16 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 54.0, 59.3, 67.7, 69.3, 70.2, 106.1, 115.0, 117.9, 123.9, 124.1, 125.0, 125.1, 125.6, 127.9, 128.8, 133.5, 144.3, 156.1, 161.6, 163.2, 178.2; LRMS (ESI) m/z 742 (M^+ + H, 100); HRMS (ESI) calcd for $C_{45}H_{41}NO_8F$ (M^+ + H) 742.2816, found 742.2787.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(4-trifluoromethylbenzyl)-1,4,10,13-tetraoxa-7-azatridecane (26). A round-bottom flask was charged with compound **14a** (210 mg, 0.33 mmol), 4-trifluoromethylbenzyl chloride (90 mg, 0.46 mmol), K_2CO_3 (70 mg), and ACN (15 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **26** as a pale brown oil (190 mg, 72%): ^{1}H NMR ($CDCl_3$) δ 2.83 (s, 4H), 3.68 (s, 4H), 3.79 (s, 4H), 3.83 (s, 2H), 4.17 (s, 4H), 6.73 (s, 2H), 6.96−7.03 (m, 6H), 7.34−7.42 (m, 4H), 7.55

(d, J = 8.0 Hz, 2H), 7.68 (dd, J = 8.0, 8.4 Hz, 2H), 7.87 (d, J = 8.4 Hz, 4H), 8.22 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 67.7, 69.3, 106.2, 115.0, 117.9, 123.9, 124.2, 125.1, 125.7, 128.0, 133.6, 156.2, 161.6, 163.3, 178.3; LRMS (ESI) m/z 792 (M^+ + H, 100); HRMS (ESI) calcd for $C_{46}H_{41}NO_8F_3$ (M^+ + H) 792.2784, found 792.2764.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(2-fluorobenzyl)-1,4,10,13-tetraoxa-7-azatridecane (27). A round-bottom flask was charged with compound **14a** (190 mg, 0.30 mmol), 2-fluorobenzyl bromide (90 mg, 0.48 mmol), K_2CO_3 (70 mg), and ACN (20 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the hot reaction mixture was filtered to remove solid K_2CO_3. The filtrate was cooled in an ice bath, and a large amount of white precipitate was formed. The titled compound **27** was obtained after suction filtration as a white solid (153 mg, 69%): mp 82−84 °C; ^{1}H NMR ($CDCl_3$) δ 2.87 (s, 4H), 3.71 (s, 4H), 3.82 (s, 4H), 3.84 (s, 2H), 4.15 (t, J = 4.6 Hz, 4H), 6.72 (s, 2H), 7.01−7.23 (m, 7H), 7.39 (dd, J = 8.0, 8.4 Hz, 2H), 7.49 (m, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.67 (dd, J = 8.0, 8.4 Hz, 2H), 7.82 (d, J = 8.4 Hz, 4H), 8.18 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 52.1, 53.9, 67.7, 69.3, 70.0, 106.1, 115.0, 115.3, 117.9, 123.9, 124.1, 125.1, 125.6, 127.9, 131.4, 133.6, 156.1, 160.1, 161.6, 162.5, 163.3, 178.3; LRMS (ESI) m/z 742 (M^+ + H, 100), 764 (M^+ + Na, 4); HRMS (ESI) calcd for $C_{45}H_{41}NO_8F$ (M^+ + H) 742.2816, found 742.2852.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(3-fluorobenzyl)-1,4,10,13-tetraoxa-7-azatridecane (28). A round-bottom flask was charged with compound **14a** (190 mg, 0.30 mmol), 3-fluorobenzyl bromide (90 mg, 0.48 mmol), K_2CO_3 (70 mg), and ACN (20 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the hot reaction mixture was filtered to remove solid K_2CO_3. The filtrate was cooled in an ice bath, and a large amount of white precipitate was formed. The titled compound **28** was obtained after suction filtration as a white solid (160 mg, 72%): mp 91−93 °C; ^{1}H NMR ($CDCl_3$) δ 2.85 (s, 4H), 3.69 (s, 4H), 3.78 (s, 2H), 3.83 (t, J = 4.6 Hz, 4H), 4.17 (t, J = 4.6 Hz, 4H), 6.72 (s, 2H), 6.94 (dd, J = 8.0, 8.4 Hz, 1H), 7.02 (J = 8.8 Hz, 4H), 7.13−7.24 (m, 3H), 7.39 (dd, J = 8.0, 8.4 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H), 7.67 (dd, J = 8.0, 8.4 Hz, 2H), 7.85 (d, J = 8.4 Hz, 4H), 8.21 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 54.0, 59.3, 67.7, 69.3, 70.2, 106.2, 115.0, 117.9, 123.9, 124.2, 125.1, 125.6, 127.9, 129.6, 133.6, 156.1, 161.6, 161.8, 163.3, 164.2, 178.3; LRMS (ESI) m/z 742 (M^+ + H, 100), 764 (M^+ + Na, 5); HRMS (ESI) calcd for $C_{45}H_{41}NO_8F$ (M^+ + H) 742.2816, found 742.2828.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(3,4-difluorobenzyl)-1,4,10,13-tetraoxa-7-azatridecane (29). A round-bottom flask was charged with compound **14a** (210 mg, 0.33 mmol), 3,4-difluorobenzyl bromide (90 mg, 0.43 mmol), K_2CO_3 (70 mg), and ACN (20 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the hot reaction mixture was filtered to remove solid K_2CO_3. The filtrate was cooled in an ice bath, and a large amount of white precipitate was formed. The titled compound **29** was obtained after suction filtration as a white solid (160 mg, 64%): mp 96−98 °C; ^{1}H NMR ($CDCl_3$) δ 2.83 (s, 4H), 3.67 (s, 4H), 3.72 (s, 2H), 3.82 (t, J = 4.6 Hz, 4H), 4.16 (t, J = 4.6 Hz, 4H), 6.72 (s, 2H), 6.98−7.06 (m, 6H), 7.28 (m, 1H), 7.39 (dd, J = 8.0, 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.67 (dd, J = 8.0, 8.4 Hz, 2H), 7.85 (d, J = 8.8 Hz, 4H), 8.21 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 53.8, 58.7, 67.7, 69.3, 70.2, 106.2, 115.0, 116.6, 116.8, 117.9, 123.9, 124.2, 125.1, 125.6, 127.9, 133.6, 156.1, 161.6, 163.2, 178.3; LRMS (ESI) m/z 760 (M^+ + H, 100); HRMS (ESI) calcd for $C_{45}H_{40}NO_8F_2$ (M^+ + H) 760.2722, found 760.2757.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(3,4,5-trifluorobenzyl)-1,4,10,13-tetraoxa-7-azatridecane (30). A round-bottom flask was charged with compound **14a** (210 mg, 0.33 mmol), 3,4,5-trifluorobenzyl bromide (100 mg, 0.42 mmol), K_2CO_3 (75 mg), and ACN (20 mL). The reaction mixture was stirred at refluxing temperature for 3 h. When TLC indicated complete consumption of starting material, the hot reaction mixture was filtered to remove solid K_2CO_3. The filtrate was cooled in an ice bath, and a large amount of white precipitate was formed. The titled compound **30** was obtained after suction filtration as a white solid (145 mg, 56%): mp 78−80 °C; ^{1}H NMR ($CDCl_3$) δ 2.83 (s, 4H), 3.68 (s, 4H), 3.73 (s, 2H), 3.83 (s, 4H), 4.17 (s, 4H), 6.72 (s, 2H), 7.01 (d, J = 8.4 Hz, 4H), 7.04 (s, 2H), 7.39 (dd, J = 8.0, 8.4 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H), 7.67 (dd, J = 8.0, 8.4 Hz, 2H), 7.85 (d, J = 8.4 Hz, 4H), 8.21 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 53.9, 58.6, 67.6, 69.4, 70.2, 106.2, 115.0, 117.9, 123.9, 124.2, 125.1, 125.6, 127.9, 133.6, 156.1, 161.6, 163.2, 178.3; LRMS (ESI) m/z 778 (M^+ + H, 100); HRMS (ESI) calcd for $C_{45}H_{39}NO_8F_3$(M^+ + H) 778.2628, found 778.2657.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(*tert*-butylacetyl)-1,4,10,13-tetraoxa-7-azatridecane (31). A round-bottom flask was charged with compound **14a** (100 mg, 0.16 mmol), *tert*-butylacetyl chloride (40 mg, 0.30 mmol), and pyridine (10 mL). The reaction mixture was stirred at room temperature for 2 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing 1 M hydrochloric acid solution. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **31** as a pale brown oil (81 mg, 70%): ^{1}H NMR ($CDCl_3$) δ 1.01 (s, 9H), 3.59 (s, 2H), 3.59−3.71 (m, 8H), 3.79 (t, J = 4.8 Hz, 4H), 4.14 (t, J = 4.8 Hz, 4H), 6.71 (s, 1H), 6.72 (s, 1H), 6.98 (dd, J = 8.0, 8.4 Hz, 4H), 7.34 (dd, J = 7.2, 8.0 Hz, 2H), 7.51 (dd, J = 8.0, 8.4 Hz, 2H), 7.63 (dd, J = 7.2, 8.0 Hz, 2H), 7.82 (d, J = 8.6 Hz, 4H), 8.17 (dd, J = 8.0, 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 29.9, 31.4, 44.6, 46.4, 49.5, 67.6, 69.3, 69.6, 69.8, 69.9, 106.1, 106.1, 114.9, 117.9, 117.9, 123.8, 124.1, 124.1, 125.1, 125.6, 127.9, 133.6, 156.1, 161.5, 161.6, 163.2, 163.3, 172.4, 178.2; LRMS (ESI) m/z 732 (M^+ + H, 100), 754 (M^+ + Na, 90); HRMS (ESI) calcd for $C_{44}H_{46}NO_9$ (M^+ + H) 732.3173, found 732.3147.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(benzoyl)-1,4,10,13-tetraoxa-7-azatridecane (32). A round-bottom flask was charged with compound **14a** (90 mg, 0.14 mmol), benzoyl chloride (42 mg, 0.30 mmol), and pyridine (10 mL). The reaction mixture was stirred at room temperature for 2 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing 1 M hydrochloric acid solution. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **32** as a pale brown oil (76 mg, 73%): ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 4H), 3.73 (s, 4H), 3.88 (s, 4H), 4.18 (s, 4H), 6.74 (s, 2H), 7.01 (d, J = 8.8 Hz, 4H), 7.31−7.41 (m, 7H), 7.52 (d, J = 8.4 Hz, 2H), 7.66 (dd, J = 8.0, 8.4 Hz, 2H), 7.84 (d, J = 8.8 Hz, 4H), 8.19 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 67.6, 69.4, 69.5, 106.1, 115.0, 118.0, 123.8, 124.1, 125.1, 125.6, 126.8, 128.0, 128.3, 129.3, 133.6, 136.7, 156.1, 161.6, 163.3, 172.4, 178.2; LRMS (ESI) m/z 738 (M^+ + H, 100), 760 (M^+ + Na, 91); HRMS (ESI) calcd for $C_{45}H_{40}NO_9$ (M^+ + H) 738.2703, found 738.2681.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(4-hydroxy-1,4-dioxobutyl)-1,4,10,13-tetraoxa-7-azatridecane (33). A round-bottom flask was charged with compound **14a** (70 mg, 0.11 mmol), succinic anhydride (22 mg, 0.22 mmol), and pyridine (10 mL). The reaction mixture was stirred at room temperature for 14 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing 1 M hydrochloric acid solution. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 20−40% acetone in CH_2Cl_2 as eluent to furnish the titled compound **33** as a pale brown oil (48 mg, 59%): ^{1}H NMR ($CDCl_3$) δ 2.61−2.77 (m, 4H), 3.62 (t, J = 5.2 Hz, 4H), 3.69 (t, J = 4.8 Hz, 4H), 3.78 (s, 4H), 4.13 (t, J = 4.8 Hz, 4H), 6.70 (s, 1H), 6.72 (s,1H), 6.99 (d, J = 8.8 Hz, 4H), 7.36 (dd, J = 7.6, 7.6 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.65 (dd, J = 8.0, 8.4 Hz, 2H),

7.81 (d, J = 8.8 Hz, 4H), 8.16 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 28.1, 29.7, 46.7, 49.0, 67.5, 69.2, 69.3, 69.6, 105.9, 114.9, 117.9, 123.7, 124.0, 124.1, 125.1, 125.5, 127.9, 133.6, 156.1, 161.4, 161.6, 163.3, 172.8, 175.9, 178.5; LRMS (ESI) m/z 734 (M^+ + H, 100), 756 (M^+ + Na, 65); HRMS (ESI) calcd for $C_{42}H_{40}NO_{11}$(M^+ + H) 734.2601, found 734.2602.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-[(*S*)-2-amino-1-oxopropyl]-1,4,10,13-tetraoxa-7-azatridecane (34). A stirred solution of the compound **14a** (190 mg, 0.30 mmol) in CH_2Cl_2 (15 mL) at 0 °C was treated successively with 1-hydroxybenzotriazole (60 mg, 0.44 mmol), Boc-Ala-OH (68 mg, 0.36 mmol), and dicyclohexylcarbodiimide (82 mg, 0.40 mmol). The mixture was allowed to warm to room temperature, and stirring was continued for 14 h. The reaction mixture was then evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the compound 1,13-bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-[(*S*)-2-(*tert*-butyloxycarbonylamino)-1-oxopropyl]-1,4,10,13-tetraoxa-7-azatridecane as a white foam (170 mg, 70%): ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J = 6.8 Hz, 3H), 1.35 (s, 9H), 3.45−3.75 (m, 12H), 4.06 (s, 4H), 4.67 (m, 1H), 5.39 (d, J = 8.0 Hz, 1H), 6.62 (s, 1H), 6.63 (s, 1H), 6.92 (dd, J = 8.0, 8.4 Hz, 4H), 7.30 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.51 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.0, 8.4 Hz, 4H), 8.09 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 19.3, 28.3, 46.1, 46.7, 48.6, 67.4, 67.5, 69.3, 69.4, 69.5, 79.4, 105.8, 110.4, 114.9, 117.9, 118.2, 123.6, 123.8, 125.0, 125.2, 125.4, 126.7, 127.9, 128.5, 133.6, 155.0, 155.9, 161.5, 161.5, 163.3, 163.4, 173.7, 178.4; LRMS (ESI) m/z 805 (M^+ + H, 100), 827 (M^+ + Na, 46); HRMS (ESI) calcd for $C_{46}H_{49}N_2O_{11}$ (M^+ + H) 805.3336, found 805.3340. The titled compound **34** was then obtained from the compound 1,13-bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-[(*S*)-2-(*tert*-butyloxycarbobylamino)-1-oxopropyl]-1,4,10,13-tetraoxa-7-azatridecane (170 mg, 0.21 mmol), TFA (5 mL), and CH_2Cl_2 (5 mL) as a pale brown oil (130 mg, 87%) according to the general procedure II described above: ^{1}H NMR ($CDCl_3$) δ 1.22 (d, J = 6.8 Hz, 3H), 2.01 (s, NH_2), 3.44−3.81 (m, 12H), 3.93 (d, J = 6.8 Hz, 1H), 4.14 (t, J = 4.8 Hz, 4H), 6.67 (s, 1H), 6.68 (s, 1H), 6.98 (dd, J = 8.0, 8.4 Hz, 4H), 7.34 (m, 2H), 7.49 (d, J = 8.0 Hz, 2H), 7.63 (m, 2H), 7.79−7.82 (m, 4H), 8.15 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.3, 46.5, 48.4, 67.5, 69.3, 69.4, 69.6, 69.7, 106.1, 114.9, 117.9, 117.9, 123.8, 124.1, 124.2, 125.0, 125.5, 127.9, 133.5, 156.0, 161.3, 161.5, 163.1, 177.1, 178.2; LRMS (ESI) m/z 705 (M^+ + H, 100), 727 (M^+ + Na, 3); HRMS (ESI) calcd for $C_{41}H_{41}N_2O_9$ (M^+ + H) 705.2812, found 705.2815.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(methanesulfonyl)-1,4,10,13-tetraoxa-7-azatridecane (35). A round-bottom flask was charged with compound **14a** (80 mg, 0.13 mmol), methanesulfonyl chloride (30 mg, 0.26 mmol), and pyridine (10 mL). The reaction mixture was stirred at room temperature for 14 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing 1 M hydrochloric acid solution. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **35** as a pale brown oil (56 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 3H), 3.53 (t, J = 5.2 Hz, 4H), 3.72 (t, J = 4.8 Hz, 4H), 3.81 (t, J = 4.8 Hz, 4H), 4.13 (t, J = 4.8 Hz, 4H), 6.68 (s, 2H), 6.97 (d, J = 8.8 Hz, 4H), 7.35 (dd, J = 7.6, 7.6 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.63 (dd, J = 8.0, 8.4 Hz, 2H), 7.82 (d, J = 8.8 Hz, 4H), 8.19 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 38.8, 47.5, 67.4, 69.3, 70.0, 106.1, 114.8, 117.9, 123.8, 124.2, 125.1, 125.5, 128.0, 133.6, 156.1, 161.4, 163.1, 178.2; LRMS (ESI) m/z 712 (M^+ + H, 62), 734 (M^+ + Na, 90); HRMS (ESI) calcd for $C_{39}H_{38}NO_{10}S$ (M^+ + H) 712.2216, found 712.2224.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-*N*-(4-methylbenzenesulfonyl)-1,4,10,13-tetraoxa-7-azatridecane (36). A round-bottom flask was charged with compound **14a** (80 mg, 0.13 mmol), *p*-toluenesulfonyl chloride (40 mg, 0.21 mmol), and pyridine (10 mL). The reaction mixture was stirred at room temperature for 14 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing 1 M hydrochloric acid solution. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **36** as a pale brown oil (61 mg, 66%): ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H), 3.42 (t, J = 5.2 Hz, 4H), 3.72 (t, J = 4.8 Hz, 4H), 3.77 (t, J = 4.8 Hz, 4H), 4.08 (t, J = 4.8 Hz, 4H), 6.68 (s, 2H), 6.98 (d, J = 8.8 Hz, 4H), 7.25 (d, J = 7.6 Hz, 2H), 7.35 (dd, J = 7.6, 7.6 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.63 (dd, J = 8.0, 8.4 Hz, 2H), 7.71 (d, J = 7.6 Hz, 2H), 7.82 (d, J = 8.8 Hz, 4H), 8.17 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.5, 49.0, 67.5, 69.3, 70.5, 106.1, 114.9, 117.9, 123.8, 124.1, 125.0, 125.5, 127.1, 129.6, 133.6, 136.6, 143.3, 156.0, 161.5, 163.1, 178.3; LRMS (ESI) m/z 788 (M^+ + H, 55), 810 (M^+ + Na, 67); HRMS (ESI) calcd for $C_{45}H_{42}NO_{10}S$ (M^+ + H) 788.2529, found 788.2540.

1,13-Bis[4′-(4*H*-chromen-4-on-2-yl)phenyl]-7-(dansyl)-1,4,10,13-tetraoxa-7-azatridecane (37). A round-bottom flask was charged with compound **14a** (120 mg, 0.19 mmol), dansyl chloride (80 mg, 0.30 mmol), and pyridine (10 mL). The reaction mixture was stirred at room temperature for 14 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing 1 M hydrochloric acid solution. The mixture was continuously extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture, which was subjected to purification by flash column chromatography on silica gel with 10−20% acetone in CH_2Cl_2 as eluent to furnish the titled compound **37** as a pale yellow solid (120 mg, 73%): mp 72−74 °C; ^{1}H NMR ($CDCl_3$) δ 2.81 (s, 6H), 3.64−3.67 (m, 12H), 3.98 (t, J = 4.8 Hz, 4H), 6.63 (s, 2H), 6.90 (d, J = 8.8 Hz, 4H), 7.12 (d, J = 7.6 Hz, 1H), 7.32 (dd, J = 7.6, 8.0 Hz, 2H), 7.41−7.50 (m, 4H), 7.56 (dd, J = 7.6, 8.0 Hz, 2H), 7.74 (d, J = 8.8 Hz, 4H), 8.10 (d, J = 8.0 Hz, 2H), 8.12 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 45.3, 48.1, 67.4, 69.2, 70.2, 106.0, 114.9, 115.1, 117.9, 119.5, 123.1, 123.7, 123.9, 125.0, 125.4, 127.8, 128.0, 128.6, 130.0, 130.0, 130.2, 133.5, 135.6, 151.7, 156.0, 161.4, 163.1, 178.2; LRMS (ESI) m/z 867 (M^+ + H, 85), 889 (M^+ + Na, 18); HRMS (ESI) calcd for $C_{50}H_{47}N_2O_{10}S$ (M^+ + H) 867.2951, found 867.2927.

Materials for Biological Studies. Dimethyl sulfoxide (DMSO), paclitaxel, DOX, vinblastine, vincristine, verapamil, cyclosporine A, mitoxantrone, daunorubicin, topotecan, Ko143, and phenazine methosulfate (PMS) were purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, trypsin/ethylenediaminetetraacetic acid (EDTA), and penicillin/streptomycin were purchased from Gibco BRL. The fetal bovine serum (FBS) was purchased from HyClone Laboratories. 3-(4,5-Dimethylthiazol-2-yl)-5-[3-(carboxymethoxy)-phenyl]-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) was purchased from Promega. The human breast cancer cell lines MDA435/LCC6 and MDA435/LCC6MDR were kindly provided by Dr. Robert Clarke (Georgetown University, U.S.).The human ovarian carcinoma cell lines 2008/P and 2008/MRP1 were generous gifts from Prof. P. Borst (The Netherlands Cancer Institute, Amsterdam, The Netherlands). The human embryonic kidney (HEK) 293 cell lines, HEK293/pcDNA3.1 (empty vector-transfected), and HEK293/R2 (BCRP-transfected) were kindly provided by Dr. Kenneth To (The Chinese University of Hong Kong, China). Agilent Prep-SIL Scalar column (440905-901, 4.6 mm × 250 mm, 5 μm) was purchased from Tegent Technology. All other reagents or solvents used were either analytical or high-performance liquid chromatography (HPLC) grade and were purchased from Tedia.

Cell Culture. MDA435/LCC6 and MDA435/LCC6MDR cell lines were cultured in supplemented DMEM with 10% heat inactivated FBS and 100 U/mL penicillin and 100 μg/mL streptomycin. 2008/P and 2008/MRP1 cells or HEK293/pcDNA3.1 and HEK293/R2 were cultured in RPMI 1640 medium containing heat inactivated 10% FBS and 100 U/mL penicillin and 100 μg/mL streptomycin. They were maintained at 37 °C 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.

Cell Proliferation Assay. Six-thousand cells of LCC6 or LCC6MDR and paclitaxel were mixed with or without 0.5 and 1.0 μM modulator to a final volume of 200 μL in each well of 96-well plates. Four-thousand cells of 2008/P or 2008/MRP1 and DOX were co-incubated with or without 0.5 μM **18** or **8** to a final volume of 200 μL. Seven-thousand-five-hundred cells of HEK293/pcDNA3.1 or HEK293/R2 and topotecan were co-incubated with or without 0.5 μM **18** or **9** to a final volume of 200 μL. The plates were then incubated for 5 days at 37 °C. After 5 days, the % of survival or viability was determined by MTS according to procedures reported previously.[36] These results were represented as the mean ± standard error of mean. IC_{50} values were calculated from the dose−response curves of MTS assays (Prism 4.0).

Cytotoxicity Assay. The cytotoxicity assay of flavonoid dimers was performed against LCC6, LCC6MDR, and L929 cell lines in order to determine the relative toxicity. The cells were grown in supplemented DMEM in an atmosphere of 95% air with 5% CO_2 at 37 °C. In brief, LCC6, LCC6MDR, and L929 cells were seeded into 96-well flat bottom microtiter plate at 1×10^4 cells per well in a final volume of 100 μL of medium, respectively. A graded dose of flavonoid dimers was added into the wells. The plate was incubated at 37 °C for 72 h in an atmosphere of 5% CO_2 in air. The % of survivors was determined as described previously.

DOX Accumulation. DOX accumulation assay was carried out according to the reported procedures.[36] Briefly, 1×10^6 cells of LCC6 and LCC6MDR were added in an Eppendorf and incubated with 20 μM DOX and different concentrations of **18** or **1** for 150 min at 37 °C. A 0.4% of DMSO was used as a negative control. After incubation, the cells were washed and lysed with lysis buffer (0.75 M HCl, 0.2% Triton-X100 in isopropanol). The fluorescence level of DOX in the lysate was determined by fluorescence spectrophotometer (BMG Technologies) using an excitation and an emission wavelength pair of 460 and 610 nm.

For the accumulation test, the log of the agonist versus response variable-slope model was used to determine the Hill coefficient by nonlinear regression to the equation Y = bottom + (top − bottom)/$1 + 10^{(\log EC_{50} - X)\text{Hill coefficient}}$, where "bottom" is the baseline and "top" is the maximum effect, EC_{50} is the dose giving half the maximum effect, and X is the modulator concentration (log). The cells were incubated with 20 μM DOX and various dosages of **18** or **1** (40, 30, 20, 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 μM). After incubation, the intracellular DOX level was measured by cell lysis followed by fluorescence spectrophotometry as described previously.

Kinetic Characterization for P-gp Inhibition. Kinetic parameters for P-gp inhibition by modulators were determined by incubating the 1×10^6 LCC6MDR cells with various concentrations of DOX (1.5, 3, 4.4, 13.3, 20, 30, and 40 μM) in the presence of **18** (0, 0.006, 0.025, 0.05, 0.075, and 0.1 μM) or **1** (0, 0.06, 0.25, 0.5, 0.75, and 1 μM) for 150 min at 37 °C. The cell lysis and fluorescence intensity measurements were carried out as described previously. The relationship between DOX and modulators for P-gp inhibition was analyzed by Lineweaver−Burk and Dixon plots as reported previously.[36,49]

P-gp-ATPase Assay. P-gp-ATPase activity was measured with the P-gp-Glo assay system (Promega) with human P-gp membrane.[34] The assay relies on the ATP dependence of the light-generating reaction of firefly luciferase. First, about 25 μg of human P-gp membrane fraction was incubated with (1) 100 μM sodium vanadate and 0.5% DMSO, (2) 0.5% DMSO, (3) 200 μM **1**, and (4) 200 μM **18**, respectively. Second, the reaction was initiated by addition of 5 mM MgATP and then the plate was incubated at 37 °C for 1 h. Third, after incubation, the remaining unmetabolized ATP was determined as a luciferase-generated luminescence signal by addition of ATP detection reagent. Fourth, the plate was further incubated at room temperature for 20 min for signal stabilization and then the luminescence signal was measured with GloMax-20/20 luminometer (Promega). ATPase activity was presented as a drop in luminescence of samples compared to that treated with sodium vanadate.

Compound 18 Accumulation. A quantity of 7.5×10^6 LCC6 and LCC6MDR cells suspended in 5 mL of DMEM was incubated with 10 or 100 μM **18** for 120 min at 37 °C. After incubation, the cells were washed and resuspended with 500 μL of lysis buffer (acetonitrile with 20% formic acid). The cells were lysed by repeated freeze−thaw cycles. The level of **18** in the lysate was determined by high performance liquid chromatography (HPLC). The HPLC system consisted of an Agilent 1100 pump, UV−visible detector, and an automated sample injector with a 100 μL loop. Chromatographic separation of **18** was carried out in a 17 min run by Agilent Prep-sil column (440905-901, 4.6 mm i.d. × 250 mm, 5 μm). The elution profile consisted of hexane−methanol−ethyl acetate (45:15:40, v/v/v) from 0 to 7 min. Then a gradient elution of hexane−methanol−ethyl acetate (45:15:40, v/v/v) to hexane−methanol−ethyl acetate (10:80:10, v/v/v) was applied from 7 to 10 min following, and the condition was gradually changed back to hexane−methanol−ethyl acetate (45:15:40, v/v/v) from 10 to 11 min to equilibrate the column for the next injection. The flow rate was 1.0 mL/min. Eluent was recorded at 315 nm with reference to 450 nm. Then 500 μL of cell lysate was alkalized by 100 μL of sodium hydroxide (15M) and dried in an 80 °C dry bath. The dried samples were reconstituted with 100 μL of chloroform and immediately passed through a 13 mm × 0.22 μm nylon syringe filter (Chelleson Scientific Instruments Company, Hong Kong, China). About 20 μL of the sample was injected into the HPLC-DAD for quantitative analysis. Standard stock solution of **18** was prepared at 0.5 mg/mL by accurately weighing the required amount and dissolving in chloroform. The calibration curve was constructed by serial dilution of **18** in chloroform from 25 to 0.25 μg/mL. All prepared solutions were immediately injected into the HPLC instrument for calibration curve construction.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR spectra, ^{13}C NMR spectra of all compounds, and HPLC chromatogram of compound **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For L.M.C.C.: phone, (852)-34008662; fax, (852)-23649932; e-mail, bclchow@polyu.edu.hk. For T.H.C.: phone, (852)-34008670; fax, (852)-23649932; e-mail, bcchanth@polyu.edu.hk.

Author Contributions

‖These two authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful for funding support from the Hong Kong Polytechnic University (Grants G-YE45, G-U383, and 1-BB8S) and the Hong Kong Research Grants Council (Grants B-Q423, B-Q762, and B-Q16G).

■ ABBREVIATIONS USED

MDR, multidrug resistance; P-gp, P-glycoprotein; ABC, ATP binding cassette; MRP1, multidrug resistance protein 1; BCRP, breast cancer resistance protein; PEG, polyethylene glycol; RF, relative fold; DOX, doxorubicin

■ REFERENCES

(1) Germann, U. A. P-glycoprotein: a mediator of multidrug resistance in tumour cells. *Eur. J. Cancer* **1996**, *32A*, 927−944.

(2) Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. Targeting multidrug resistance in cancer. *Nat. Rev. Drug Discovery* **2006**, *5*, 219−234.

(3) Aller, S. G.; Yu, J.; Ward, A.; Weng, Y.; Chittaboina, S.; Zhuo, R.; Harrell, P. M.; Trinh, Y. T.; Zhang, Q.; Urbatsch, I. L.; Chang, G. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* **2009**, *323*, 1718−1722.
(4) Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res.* **1981**, *41*, 1967−1972.
(5) Hollt, V.; Kouba, M.; Dietel, M.; Vogt, G. Stereoisomers of calcium antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein. *Biochem. Pharmacol.* **1992**, *43*, 2601−2608.
(6) Tsuruo, T.; Iida, H.; Nojiri, M.; Tsukagoshi, S.; Sakurai, Y. Circumvention of vincristine and Adriamycin resistance in vitro and in vivo by calcium influx blockers. *Cancer Res.* **1983**, *43*, 2905−2910.
(7) Ganapathi, R.; Grabowski, D.; Turinic, R.; Valenzuela, R. Correlation between potency of calmodulin inhibitors and effects on cellular levels and cytotoxic activity of doxorubicin (adriamycin) in resistant P388 mouse leukemia cells. *Eur. J. Cancer Clin. Oncol.* **1984**, *20*, 799−806.
(8) Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. *Cancer Res.* **1982**, *42*, 4730−4733.
(9) Beck, W. T.; Cirtain, M. C.; Glover, C. J.; Felsted, R. L.; Safa, A. R. Effects of indole alkaloids on multidrug resistance and labeling of P-glycoprotein by a photoaffinity analog of vinblastine. *Biochem. Biophys. Res. Commun.* **1988**, *153*, 959−966.
(10) Tsuruo, T.; Iida, H.; Kitatani, Y.; Yokota, K.; Tsukagoshi, S.; Sakurai, Y. Effects of quinidine and related compounds on cytotoxicity and cellular accumulation of vincristine and adriamycin in drug-resistant tumor cells. *Cancer Res.* **1984**, *44*, 4303−4307.
(11) Chao, N. J.; Aihara, M.; Blume, K. G.; Sikic, B. I. Modulation of etoposide (VP-16) cytotoxicity by verapamil or cyclosporine in multidrug-resistant human leukemic cell lines and normal bone marrow. *Exp. Hematol.* **1990**, *18*, 1193−1198.
(12) Slater, L. M.; Sweet, P.; Stupecky, M.; Gupta, S. Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. *J. Clin. Invest.* **1986**, *77*, 1405−1408.
(13) Slater, L. M.; Sweet, P.; Stupecky, M.; Wetzel, M. W.; Gupta, S. Cyclosporin A corrects daunorubicin resistance in Ehrlich ascites carcinoma. *Br. J. Cancer* **1986**, *54*, 235−238.
(14) Twentyman, P. R.; Fox, N. E.; White, D. J. Cyclosporin A and its analogues as modifiers of adriamycin and vincristine resistance in a multi-drug resistant human lung cancer cell line. *Br. J. Cancer* **1987**, *56*, 55−57.
(15) Solary, E.; Velay, I.; Chauffert, B.; Bidan, J. M.; Caillot, D.; Dumas, M.; Guy, H. Sufficient levels of quinine in the serum circumvent the multidrug resistance of the human leukemic cell line K562/ADM. *Cancer* **1991**, *68*, 1714−1719.
(16) Barnes, K. M.; Dickstein, B.; Cutler, G. B. Jr.; Fojo, T.; Bates, S. E. Steroid treatment, accumulation, and antagonism of P-glycoprotein in multidrug-resistant cells. *Biochemistry* **1996**, *35*, 4820−4827.
(17) Gruol, D. J.; Zee, M. C.; Trotter, J.; Bourgeois, S. Reversal of multidrug resistance by RU 486. *Cancer Res.* **1994**, *54*, 3088−3091.
(18) Ueda, K.; Okamura, N.; Hirai, M.; Tanigawara, Y.; Saeki, T.; Kioka, N.; Komano, T.; Hori, R. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.* **1992**, *267*, 24248−24252.
(19) Pirker, R.; FitzGerald, D. J.; Raschack, M.; Frank, Z.; Willingham, M. C.; Pastan, I. Enhancement of the activity of immunotoxins by analogues of verapamil. *Cancer Res.* **1989**, *49*, 4791−4795.
(20) Hofmann, J.; Wolf, A.; Spitaler, M.; Bock, G.; Drach, J.; Ludescher, C.; Grunicke, H. Reversal of multidrug resistance by B859-35, a metabolite of B859-35, niguldipine, verapamil and nitrendipine. *J. Cancer Res. Clin. Oncol.* **1992**, *118*, 361−366.
(21) Twentyman, P. R.; Bleehen, N. M. Resistance modification by PSC-833, a novel non-immunosuppressive cyclosporin. *Eur. J. Cancer* **1991**, *27*, 1639−1642. Erratum: *Eur. J. Cancer* **1992**, *28*, 616.
(22) Germann, U. A.; Ford, P. J.; Shlyakhter, D.; Mason, V. S.; Harding, M. W. Chemosensitization and drug accumulation effects of VX-710, verapamil, cyclosporin A, MS-209 and GF120918 in multidrug resistant HL60/ADR cells expressing the multidrug resistance-associated protein MRP. *Anti-Cancer Drugs* **1997**, *8*, 141−155.
(23) Germann, U. A.; Shlyakhter, D.; Mason, V. S.; Zelle, R. E.; Duffy, J. P.; Galullo, V.; Armistead, D. M.; Saunders, J. O.; Boger, J.; Harding, M. W. Cellular and biochemical characterization of VX-710 as a chemosensitizer: reversal of P-glycoprotein-mediated multidrug resistance in vitro. *Anti-Cancer Drugs* **1997**, *8*, 125−140.
(24) Krishna, R.; Mayer, L. D. Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. *Eur. J. Pharm. Sci.* **2000**, *11*, 265−283.
(25) Thomas, H.; Coley, H. M. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting P-glycoprotein. *Cancer Control* **2003**, *10*, 159−165.
(26) Harborne, J. B. Nature, distribution and function of plant flavonoids. *Prog. Clin. Biol. Res.* **1986**, *213*, 15−24.
(27) Castro, A. F.; Altenberg, G. A. Inhibition of drug transport by genistein in multidrug-resistant cells expressing P-glycoprotein. *Biochem. Pharmacol.* **1997**, *53*, 89−93.
(28) Chieli, E.; Romiti, N.; Cervelli, F.; Tongiani, R. Effects of flavonols on P-glycoprotein activity in cultured rat hepatocytes. *Life Sci.* **1995**, *57*, 1741−1751.
(29) Comte, G.; Daskiewicz, J. B.; Bayet, C.; Conseil, G.; Viornery-Vanier, A.; Dumontet, C.; Di Pietro, A.; Barron, D. C-Isoprenylation of flavonoids enhances binding affinity toward P-glycoprotein and modulation of cancer cell chemoresistance. *J. Med. Chem.* **2001**, *44*, 763−768.
(30) Critchfield, J. W.; Welsh, C. J.; Phang, J. M.; Yeh, G. C. Modulation of adriamycin accumulation and efflux by flavonoids in HCT-15 colon cells. Activation of P-glycoprotein as a putative mechanism. *Biochem. Pharmacol.* **1994**, *48*, 1437−1445.
(31) Jodoin, J.; Demeule, M.; Beliveau, R. Inhibition of the multidrug resistance P-glycoprotein activity by green tea polyphenols. *Biochim. Biophys. Acta* **2002**, *1542*, 149−159.
(32) Scambia, G.; Ranelletti, F. O.; Panici, P. B.; De Vincenzo, R.; Bonanno, G.; Ferrandina, G.; Piantelli, M.; Bussa, S.; Rumi, C.; Cianfriglia, M.; Mancuso, S. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. *Cancer Chemother. Pharmacol.* **1994**, *34*, 459−464.
(33) Zhang, S.; Morris, M. E. Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1258−1267.
(34) Chan, K. F.; Zhao, Y.; Burkett, B. A.; Wong, I. L.; Chow, L. M.; Chan, T. H. Flavonoid dimers as bivalent modulators for P-glycoprotein-based multidrug resistance: synthetic apigenin homodimers linked with defined-length poly(ethylene glycol) spacers increase drug retention and enhance chemosensitivity in resistant cancer cells. *J. Med. Chem.* **2006**, *49*, 6742−6759.
(35) Chan, K. F.; Zhao, Y.; Chow, T. W.; Yan, C. S.; Ma, D. L.; Burkett, B. A.; Wong, I. L.; Chow, L. M.; Chan, T. H. Flavonoid dimers as bivalent modulators for P-glycoprotein-based multidrug resistance: structure−activity relationships. *ChemMedChem* **2009**, *4*, 594−614.
(36) Wong, I. L.; Chan, K. F.; Tsang, K. H.; Lam, C. Y.; Zhao, Y.; Chan, T. H.; Chow, L. M. Modulation of multidrug resistance protein 1 (MRP1/ABCC1)-mediated multidrug resistance by bivalent apigenin homodimers and their derivatives. *J. Med. Chem.* **2009**, *52*, 5311−5322.
(37) Wong, I. L.; Chan, K. F.; Burkett, B. A.; Zhao, Y.; Chai, Y.; Sun, H.; Chan, T. H.; Chow, L. M. Flavonoid dimers as bivalent modulators for pentamidine and sodium stiboglucanate resistance in leishmania. *Antimicrob. Agents Chemother.* **2007**, *51*, 930−940.
(38) Wong, I. L.; Chan, K. F.; Zhao, Y.; Chan, T. H.; Chow, L. M. Quinacrine and a novel apigenin dimer can synergistically increase the

pentamidine susceptibility of the protozoan parasite Leishmania. *J. Antimicrob. Chemother.* **2009**, *63*, 1179−1190.
(39) Leonessa, F.; Green, D.; Licht, T.; Wright, A.; Wingate-Legette, K.; Lippman, J.; Gottesman, M. M.; Clarke, R. MDA435/LCC6 and MDA435/LCC6MDR1: ascites models of human breast cancer. *Br. J. Cancer* **1996**, *73*, 154−161.
(40) Mistry, P.; Stewart, A. J.; Dangerfield, W.; Okiji, S.; Liddle, C.; Bootle, D.; Plumb, J. A.; Templeton, D.; Charlton, P. In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res.* **2001**, *61*, 749−758.
(41) Iseki, K.; Sugawara, M.; Sato, K.; Naasani, I.; Hayakawa, T.; Kobayashi, M.; Miyazaki, K. Multiplicity of the H^+-dependent transport mechanism of dipeptide and anionic beta-lactam antibiotic ceftibuten in rat intestinal brush-border membrane. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 66−71.
(42) Wang, E. J.; Casciano, C. N.; Clement, R. P.; Johnson, W. W. Two transport binding sites of P-glycoprotein are unequal yet contingent: initial rate kinetic analysis by ATP hydrolysis demonstrates intersite dependence. *Biochim. Biophys. Acta* **2000**, *1481*, 63−74.
(43) Burns, J. M.; Dairaghi, D. J.; Deitz, M.; Tsang, M.; Schall, T. J. Comprehensive mapping of poxvirus vCCI chemokine-binding protein. Expanded range of ligand interactions and unusual dissociation kinetics. *J. Biol. Chem.* **2002**, *277*, 2785−2789.
(44) Ayesh, S.; Shao, Y. M.; Stein, W. D. Co-operative, competitive and non-competitive interactions between modulators of P-glycoprotein. *Biochim. Biophys. Acta* **1996**, *1316*, 8−18.
(45) Litman, T.; Zeuthen, T.; Skovsgaard, T.; Stein, W. D. Competitive, non-competitive and cooperative interactions between substrates of P-glycoprotein as measured by its ATPase activity. *Biochim. Biophys. Acta* **1997**, *1361*, 169−176.
(46) Wang, Y.; Hao, D.; Stein, W. D.; Yang, L. A kinetic study of rhodamine123 pumping by P-glycoprotein. *Biochim. Biophys. Acta* **2006**, *1758*, 1671−1676.
(47) Bordunov, A. V. H., P.C.; Bradshaw, J. S.; Dalley, N. K.; Kou, X.; Zhang, X. X.; Izatt, R. M. Synthesis of new pyridinoazacrown ethers containing aromatic and heteroaromatic proton ionizable substituents. *J. Org. Chem.* **1995**, *60*, 6097−6102.
(48) Maeda, H. F., S.; Nakatsuji, Y.; Okahara, M. Intramolecular cyclization of *N*,*N*-di(oligooxyethylene)amines: a new synthesis of monoaza crown ethers. *Tetrahedron* **1982**, *38*, 3359−3362.
(49) Zhang, P. Y.; Wong, I. L.; Yan, C. S.; Zhang, X. Y.; Jiang, T.; Chow, L. M.; Wan, S. B. Design and syntheses of permethyl ningalin B analogues: potent multidrug resistance (MDR) reversal agents of cancer cells. *J. Med. Chem.* **2010**, *53*, 5108−5120.