Triazole Bridged Flavonoid Dimers as Potent, Nontoxic and Highly Selective Breast Cancer Resistance Protein (BCRP/ABCG2) Inhibitors

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ABSTRACT

The present work describes the syntheses of diverse triazole bridged flavonoid dimers and identifies potent, nontoxic and highly selective BCRP inhibitors. A homodimer, **Ac22(Az8)$_2$**, with *m*-methoxycarbonylbenzyloxy substitution at C-3 of the flavone moieties and a *bis*-triazole-containing linker (21 atoms between the two flavones) showed low toxicity (IC_{50} towards L929, 3T3 and HFF-1 > 100 μM), potent BCRP-inhibitory activity (EC_{50} = 1-2 nM) and high BCRP selectivity (BCRP selectivity over MRP1 and P-gp > 455-909). **Ac22(Az8)$_2$** inhibits BCRP-ATPase activity, blocks the drug efflux activity of BCRP, elevates the intracellular drug accumulation, and finally restores the drug sensitivity of BCRP-overexpressing cells. It does not down-regulate the surface BCRP protein expression to enhance the drug retention. Therefore, **Ac22(Az8)$_2$** and similar flavonoid dimers appear to be promising candidates for further development into combination therapy to overcome MDR cancers with BCRP overexpression.

Keywords: CuAAC reaction, multidrug resistance, breast cancer resistance transporter, BCRP modulators, flavonoids

1. INTRODUCTION

Cancer causes significant number of human deaths in the world.[1] For cancer patients, chemotherapy remains one of the critical treatments. However, multidrug resistance (MDR) often emerges to become a major impediment to successful chemotherapy. Overexpression of an active ATP-binding cassette (ABC) transporter protein on the plasma membrane of cancer cells is a common mechanism for causing drug resistance.[2] Three major ABC transporter proteins including P-glycoprotein (P-gp; ABCB1), multidrug resistance-associated protein 1 (MRP1; ABCC1), and breast cancer resistance protein (BCRP; ABCG2) have been associated with MDR in cancer.[3-6] They can pump many structurally and mechanistically diverse anticancer drugs out of the cancer cells and render these drugs ineffective at a therapeutic dosage. Using the mutliplatform approach analysis (https://www.carislifesciences.com/wp-content/uploads/2015/05/Drug-Efflux-Pump-Expression-in-50000-Molecularly-Profiled-Cancer-Patients_ASCO-2015.pdf), across all tumors profiled (n=51,939), MRP1 positivity was highest at 81%, BCRP at 66% and P-gp the lowest at 23%. About 29% of the patients tested exhibits co-expression of all three drug transporters. The ABC transporter proteins are also expressed in many normal tissues and are believed to perform a protective role. For ABC transporters expressed in the brain, testis and placenta, they protect these 'sanctuaries' from cytotoxins; and for those expressed in the liver, gastrointestinal tract and kidney they are used to excrete toxins, protecting the entire organism.[7]

BCRP, unlike P-gp and MRP1, is a 72kDa half transporter and has a distinct structure which consists of only one cytosolic nucleotide-binding domain (NBD) and one transmembrane domain (TMD).[8] Several investigations have indicated that it would form a dimeric or tetrameric structure when it functions as a transporter.[9-11,12, 13] BCRP was found to be overexpressed in a number of

human cancers and associated with clinical drug resistance and lower survival rate.[14-21] Moreover, it is highly overexpressed in normal and putative cancer stem cells.[22-24] Since its identification in 1998,[25-27] numerous anticancer drugs including methotrexate,[28] mitoxantrone,[29] topotecan,[30] irinotecan[31] and its active metabolite SN-38,[32] as well as some tyrosine kinase inhibitors (TKIs),[33] have been identified as substrates of BCRP. Co-administration of potent inhibitor of ABC transporter such as P-gp with an anticancer drug has been evaluated in several clinical trials to overcome MDR but led to disappointing outcome.[34] One factor which may account for the lack of success is likely the effect of the inhibitor on the pharmacokinetics of the anticancer drug.[7] It is suggested that further improvement of inhibitors of ABC transporters should focus on potency and specificity to minimize unexpected pharmacokinetic effects.[7] In the case of BCRP, the first identified inhibitor was fumitremorgin C (**Figure 1**) with EC_{50} round 1 - 5 μM.[35, 36] It was found to be neurotoxic and unsuitable for clinical development.[36] In subsequent studies, one of the analogues of fumitremorgin C, Ko143 (**Figure 1**)[37] was identified as a potent and selective inhibitor of BCRP with EC_{50} around 10 nM.[38] It is not stable in mouse plasma, even though when administered p.o. to inhibit intestinal BCRP. Ko143 markedly increased the oral bioavailability of topotecan in mice.[39] Tariquidar (**Figure 1**),[40] a third-generation P-gp inhibitor, has also been reported to inhibit BCRP with EC_{50} around 100 nM.[41] Recently, Wiese and his group reported that 2,4,6-substituted quinazolines (with EC_{50} = 20 - 71 nM),[42] 2,4-disubstituted pyridopyrimidines (with EC_{50} = 37 nM) [43] and 4-anilino-2-pyridylquinazolines and -pyrimidines (with EC_{50} = 21 nM), are highly potent and nontoxic inhibitors of BCRP.[44] Recently, the crystal structure of human BCRP has been reported and provides insight about the binding of BCRP with its substrates and inhibitors.[45, 46] These binding results may provide the essential structural basis for discovery of novel BCRP modulators.

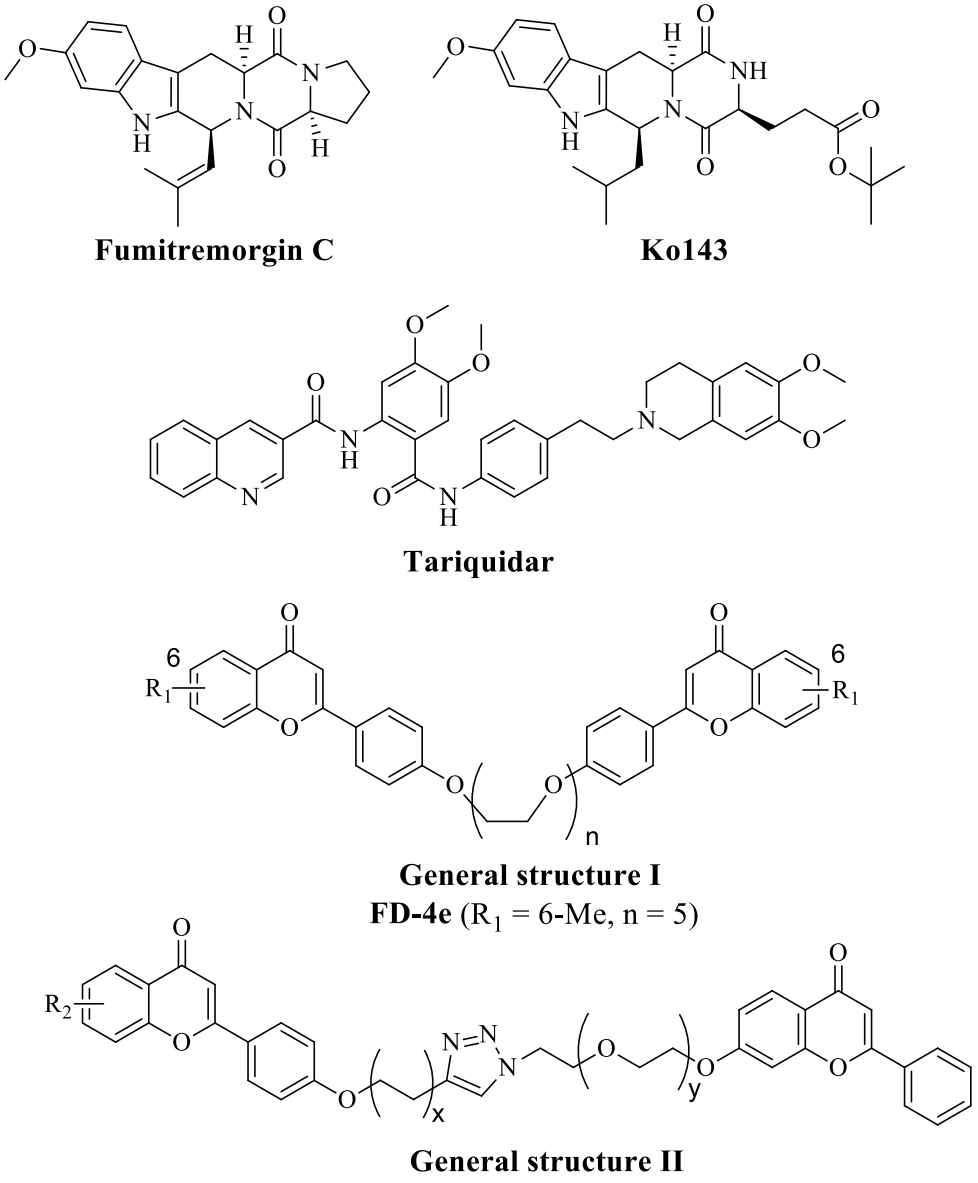


Figure 1. Chemical structures of fumitremorgin C, Ko143, tariquidar and general structure I & II.

Flavonoids are abundantly present in fruits and vegetables and commonly regarded as safe substances for human consumption. Many natural flavonoids are found to exhibit moderate activity in modulating ABC transporters.[47] Because of the pseudo-dimeric structure of ABC transporters, we reasoned that a bivalent approach by coupling two flavonoid moieties together may provide

potent and non-toxic inhibitors of ABC transporters. Previously, we synthesized a series of flavonoid dimers of general structure I (**Figure 1**) with polyethylene glycol (PEG) linkers and some of them showed promising P-gp- and MRP1-modulating activities with nanomolar EC_{50} values (70 to 170 nM).[48-53] The inhibitory selectivity of I towards P-gp or MRP-1 depends critically on the linker length, with four PEG groups in I (n=4) selective for P-gp[48-51] and with five to six PEG groups (I, n=5-6) selective for MRP1.[52] Recently, we have successfully employed the copper (I) catalyzed Huisgen 1,3-dipolar cycloaddition reaction between azide and alkyne to efficiently synthesize triazole-linked flavonoid dimers of general structure II (**Figure 1**) as potent MRP1 inhibitors.[54] Interestingly, out of the 300-member synthetic library, the 21 most active compounds (EC_{50} = 53-298 nM for reversing DOX resistance in 2008/MRP1) discovered in these triazole-linked flavonoid dimers, all have linkers with 13−17 atoms between the two flavonoid moieties, similar in linker length to I with n=5-6.[52] In the process, we also found that some of the compounds showed inhibitory activities against BCRP.[54] In this study, we further construct more triazole-linked flavonoid dimers to discover potent and nontoxic inhibitors of BCRP, to explore the structure activity relationship and, on that basis, discover specific inhibitor of BCRP over P-gp and MRP1.

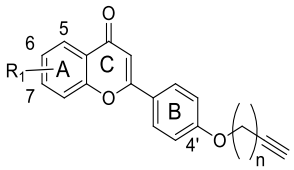
2. RESULTS

2.1. Design and synthesis of triazole and *bis*-triazole bridged flavonoid dimers

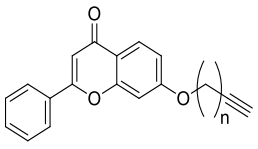
The copper(I) catalyzed Huisgen 1,3-dipolar cycloaddition reaction between azides and alkynes (commonly known as CuAAC reaction)[55] was used to prepare the triazole and *bis*-triazole bridged flavonoid dimers. The alkynes **Ac1-3, Ac5, Ac12, Ac13** and **Ac16** and the azides **Az1-3**, **Az11-13** and **Az17** were conveniently prepared according to the procedure described in the previous study.[54] To further expand structural diversity, we synthesized additional flavonoid bearing azides **Az8**, **Az9, Az14** and **Az15**. The azides **Az8-9** were prepared starting from compounds **1a-b** which was followed by debenzylation and alkylation with methyl 3-(bromomethyl)benzoate to furnish compounds **1c-d**. Azides **Az8-9** were realized after the conversion of the hydroxyl group in **1c-d** to azido group (**Scheme 1**). For **Az14-15**, the hydroxylated flavone **2b-c** were coupled with 2,2'-(benzylazanediyl)diethanol under Mitsunobu condition and the hydroxyl group of the coupling product was converted to azido group to furnish the desired azides (**Scheme 2**). With one flavonoid bearing an acetylene group **AcN** and another flavonoid bearing an azido group **AzM**, a triazole bridged flavonoid dimer **AcNAzM** could be easily obtained by employing the CuAAC reaction. (**Scheme 3**). Treatment of the diacetylenes **Ac15** with two mole equivalents of azides (**Az1-3, Az8, Az9, Az11-13, Az16** and **Az17**) in the presence of catalytic amount of $Cu(PPh_3)_3Br$ under THF refluxing temperature afforded the *bis*-triazole bridged flavonoid dimers **Ac15(AzM)$_2$** (**Scheme 3**). Their dimeric nature was confirmed by their high-resolution mass and NMR spectra. Altogether, a library of 74-member of pure triazole and *bis*-triazole bridged homo- and hetero-flavonoid dimers was constructed and tested with biological assays for inhibitory activity against BCRP, MRP1 and P-gp.

After biological assays of these flavonoid dimers, **Ac15(Az8)$_2$** and **Ac15(Az9)$_2$** exhibited not only high BCRP-modulating activity but also good selectivity for BCRP. These promising results encouraged us to carry out further structural optimization of these compounds with various diacetylenes including **Ac22-31**. The diacetylenes **Ac22-25** are commercially available. The diacetylenes **Ac26, Ac29** and **Ac31** were prepared according to the previous procedure.[54-56] Treatment of amine **3a-b** with propargyl bromide furnished the diacetylenes **Ac28** and **Ac30** (**Scheme 4)**. The desired *bis*-triazole bridged flavonoid dimers were furnished by treatment of azides **Az8** and **Az9** with various diacetylenes in the presence of catalytic amount of $Cu(PPh_3)_3Br$ in reasonably good yields (**Scheme 5**). The chemical structures of all alkynes, azides and clicked dimers were summarized in **Figures 2** and **3** and **Table 1,** respectively.

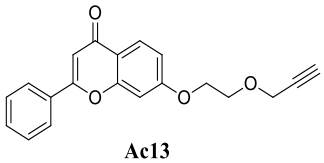
Mono-acteylenes



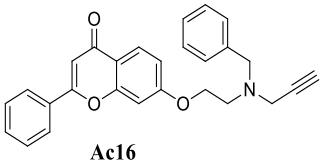
Ac1 R_1 = H, n=3
Ac3 R_1 = 7-F, n=3
Ac5 R_1 = 6-Me, n= 4



Ac2 n=3
Ac12 n=4

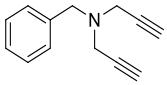


Ac13

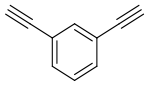


Ac16

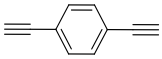
Diacetylenes



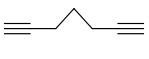
Ac15



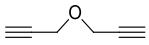
Ac22



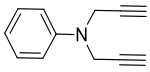
Ac23



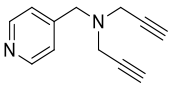
Ac24



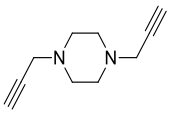
Ac25



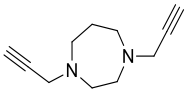
Ac26



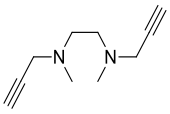
Ac28



Ac29



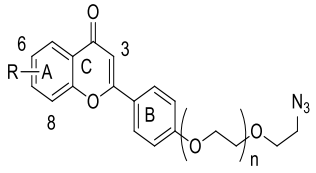
Ac30



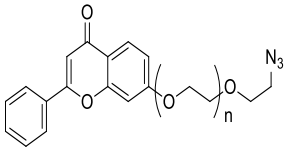
Ac31

Figure 2. Structures of alkynes.

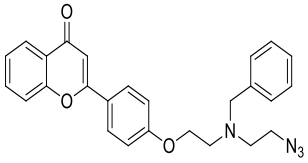
Azide-bearing flavonoids



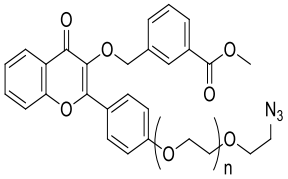
Az1 R = H, n = 1
Az2 R = H, n = 2
Az3 R = 6-Me, n = 2
Az4 R = 6-F, n = 2
Az5 R = 3-OBn, n =2
Az7 R = 6-F, n = 1
Az10 R = 3-OBn, n = 1



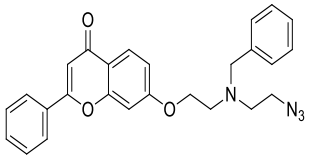
Az11 n = 1
Az12 n = 2
Az13 n = 0



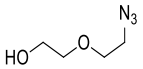
Az14



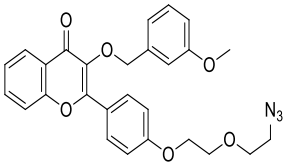
Az8 n = 1
Az9 n = 2



Az15



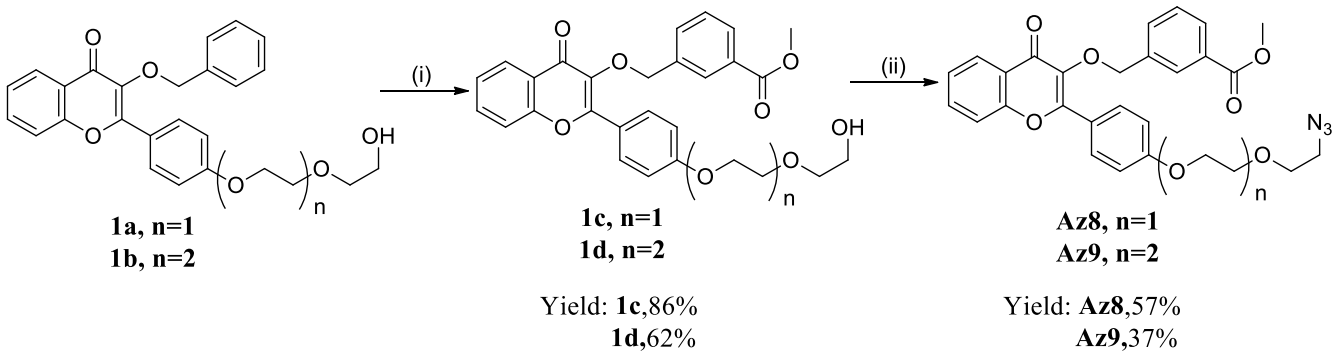
Az16



Az17

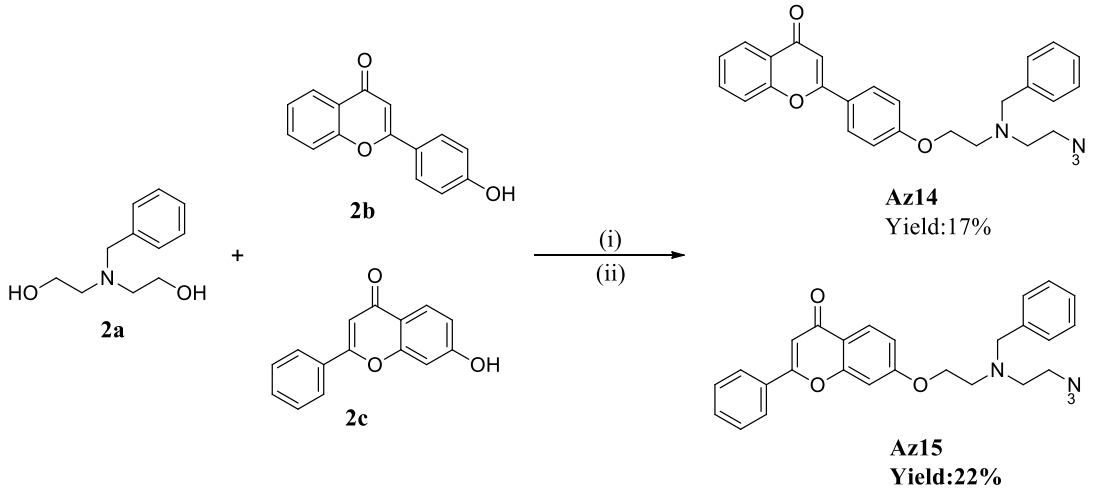
Figure 3. Structures of azides.

Scheme 1. Synthesis of azides **Az8** and **Az9**.[a]



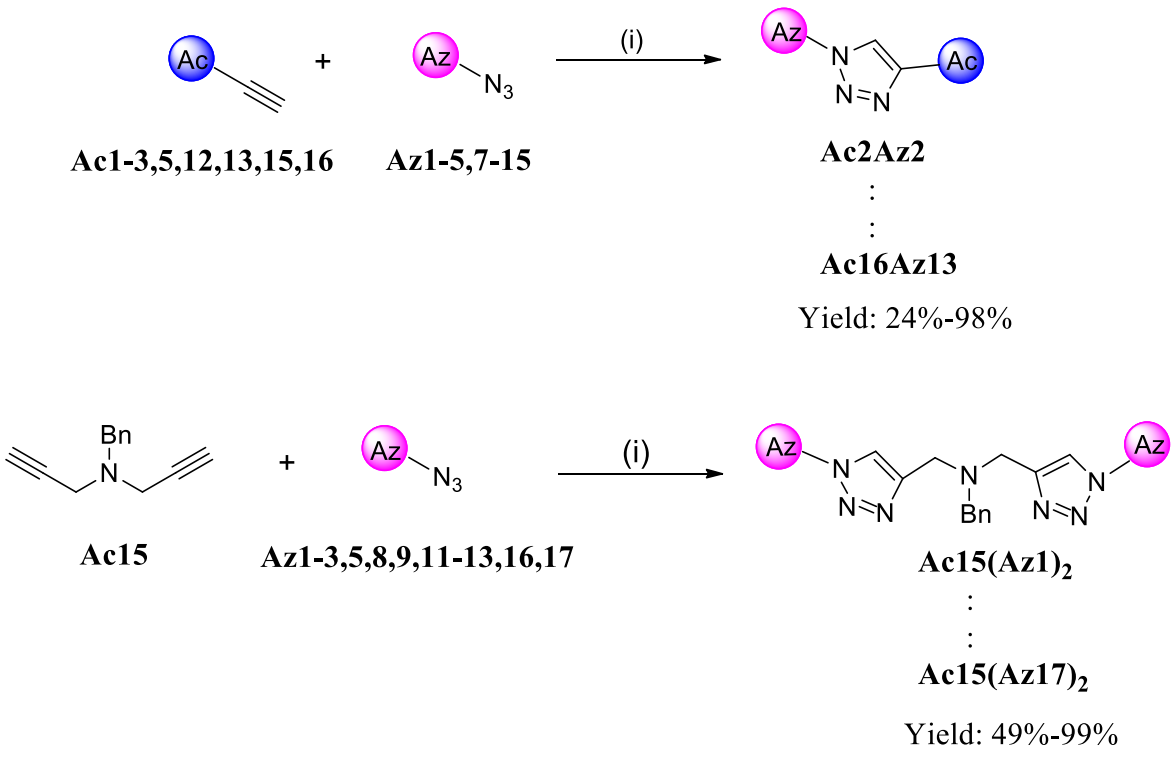
[a] Reagents and condition: (i) (a) H_2, Pd/C, MeOH, rt; (b) K_2CO_3, methyl 3-(bromomethyl)benzoate, acetone, reflux; (ii) (a) methanesulfonyl chloride, NEt_3, DCM, 0°C; (b) NaN_3, ACN.

Scheme 2. Synthesis of azides **Az14** and **Az15**.[a]



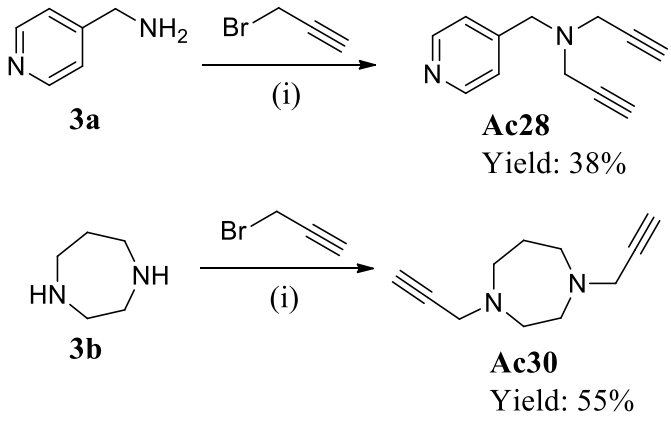
[a] Reagents and condition: (i) PPh_3, DIAD, THF; (ii) (a) methanesulfonyl chloride, NEt_3, DCM, 0°C; (b) NaN_3, ACN.

Scheme 3. Synthesis of triazole and *bis*-triazole bridged flavonoid dimers [a]



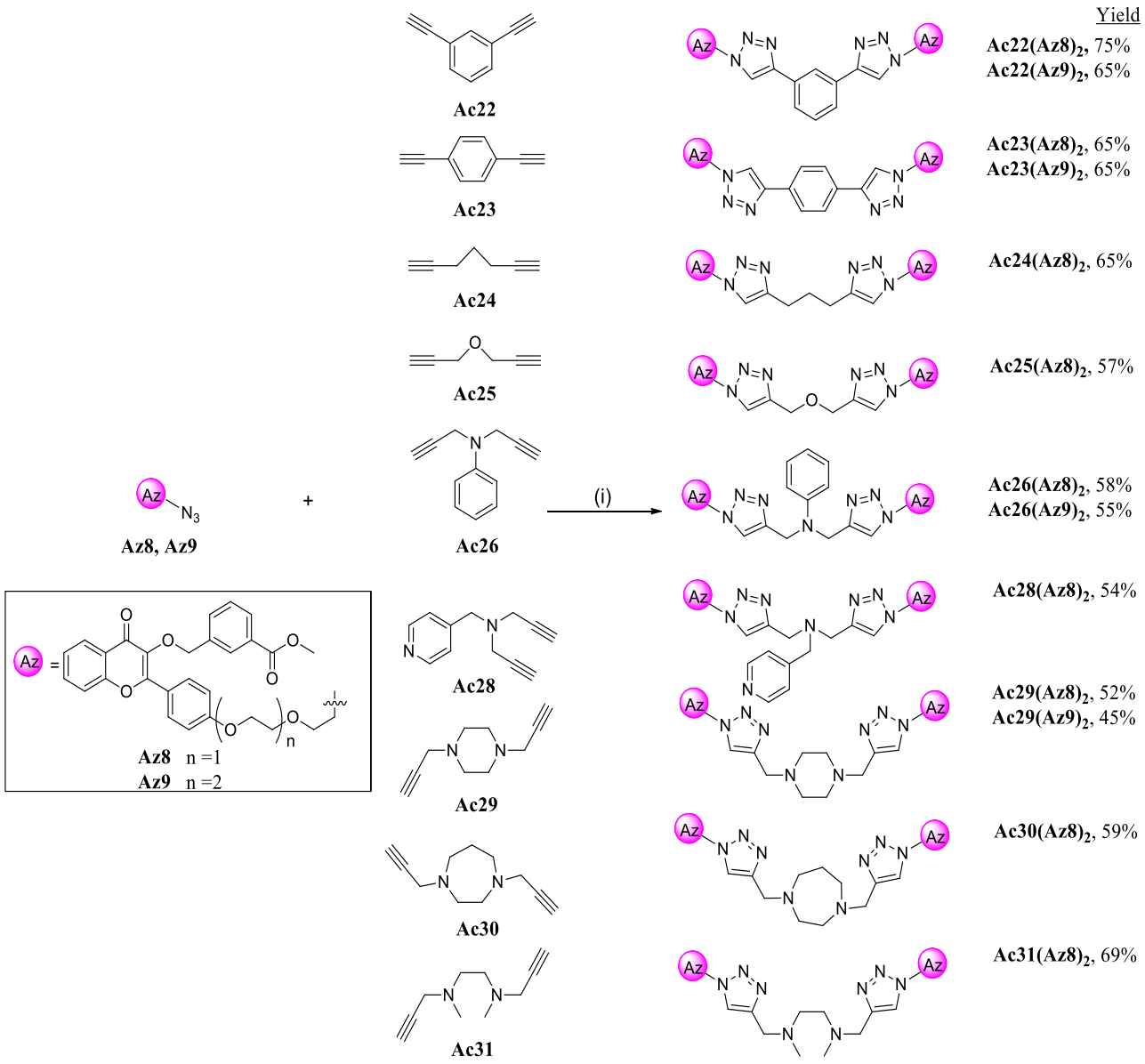
[a] Reagents and condition: (i) cat. $Cu(PPh_3)_3Br$, THF, reflux, 12hr.

Scheme 4. Synthesis of diacetylenes **Ac28 and Ac30** [a]



[a] Reagents and condition: (i) propargyl bromide, acetone, rt.

Scheme 5. Synthesis of analogues of **Ac15(Az8)$_2$** and **Ac15(Az9)$_2$**.[a]



[a] Reagents and condition: (i) cat. $Cu(PPh_3)_3Br$, THF, reflux, 12hr.

2.2. Biological assay results

2.2.1. BCRP-modulating activity of triazole and *bis*-triazole bridged flavonoid dimers

For the biological assays, four different cell lines were employed in this study including: (i) BCRP-transfected human embryonic kidney cell line HEK293/R2; (ii) mitoxantrone-selected breast cancer cell line MCF7-MX100 in which the BCRP transporter protein was found to be overexpressed; (iii) MRP1-transfected ovarian cancer cell line 2008/MRP1 and (iv) P-gp-transfected human breast cancer cell line LCC6MDR (**Table 1**). Effective concentration (EC_{50}), at which the modulator can reduce the IC_{50} of an anticancer drug towards a cell line by half, was used to differentiate the inhibitory potency of the flavonoid dimers. A lower EC_{50} value implies that the flavonoid dimer displays higher potency to reverse BCRP-, MRP1- and P-gp-mediated drug resistance. For positive controls in the biological assays, Ko143 (EC_{50} = 9 nM in HEK293/R2 and MCF7-MX100), a BCRP-specific modulator, cyclosporine A (EC_{50} = 32 nM in LCC6MDR), a known P-gp inhibitor, and the flavonoid dimer, **FD-4e** (**Figure 1**, EC_{50} = 111 nM in 2008/MRP1), previously reported to possess promising MRP1-modulating activity were used.[52] The triazole bridged flavonoid dimers would be considered as potent MDR chemosensitizers if they exhibit EC_{50} values lower than that of positive controls. In addition, the cytotoxicity (IC_{50}) of these compounds towards L929 cells, a normal mouse fibroblast cell line, was also determined as an indication of their potential toxicity. The results are summarized in **Table 1**.

Some of these flavonoid dimers exhibited potent MDR inhibitory activity against BCRP for the two cell lines HEK293/R2 and MCF7-MX100 in restoring topotecan cytotoxicity (**Table 1**). As reported in **Table 1**, among the 74 compounds tested, 11 compounds, **Ac2Az9**, **Ac3Az4**, **Ac3Az9**, **Ac3Az11**, **Ac5Az8**, **Ac5Az9**, **Ac12Az8**, **Ac12Az9**, **Ac13Az9**, **Ac15(Az8)$_2$** and **Ac15(Az9)$_2$**, display a more potent activity than Ko143 in both cell lines with EC_{50} in the range of

0.9-7.9 nM. Equally important is the fact that these potent compounds are essentially nontoxic towards L929 with IC_{50}>100 μM. In contrast, Ko143 has an IC_{50} = 31 μM. These triazole bridged flavonoid dimers are therefore potent and nontoxic inhibitors of BCRP.

2.2.2. Structure Activity Relationship (SAR) of flavonoid dimers and BCRP-inhibitory activity

In order to understand the SAR for inhibiting BCRP activity, we have (1) introduced various substituents at C-6 or C-7 on A-ring or at C-3 on C-ring of the flavonoid moiety; (2) synthesized different linker length between the two flavonoids; (3) located the linker at different positions of the flavonoid; and (4) designed the linker with a N-atom bearing benzyl substituent. For easier analysis of the results, we reformatted the EC_{50} data of **Table 1** into **Table 2A** and **2B**. Each cell in **Table 2** represents the EC_{50} of **AcNAzM** in reversing topotecan resistance in HEK293/R2 (**Table 2A**) or MCF7-MX100 (**Table 2B**) with different color coding for different potency. First, the potency is attributed to the presence of the two flavonoid moieties. The compound **Ac15(Az16)$_2$** contains no flavonoid and is essentially nonactive (EC_{50} >1000 nM) whereas all other compounds in **Table 2** with two flavonoid moieties are active (EC_{50} 1-543 nM). Secondly, it is clear from **Table 2** that flavonoid dimers derived from **Az8** or **Az9** are highly potent irrespective of the Ac component to which they are coupled with EC_{50} ranged from 1 to 24 nM. For dimers not containing **Az8** or **Az9**, the most potent compound is **Ac3Az4** with EC_{50} = 3.1- 4.0 nM, suggesting that a fluorine substitution in ring A of the azide component can enhance potency. Comparing **Az2** with **Az12** where the difference is the location of the linker, suggesting that placing the linker at C-7 of the A ring in the azide component may be preferred. On the other hand, replacing an oxygen atom in the linker by a benzylamine group did not lead to lower EC_{50} as we compare **Ac5Az14** with **Ac5Az1** or **Ac5Az15** with **Ac5Az11**.

Among the different acetylene-derived dimers, **Ac3AzM** dimers displayed the most promising BCRP inhibitory potency if we exclude the dominant effect of **Az8** and **Az9** on activity mentioned above (**Table 2A** and **2B**), suggesting that 7-fluoro substitution on A-ring in acetylene-containing flavone may play an important role in enhancing the BCRP inhibitory activity. In contrast, a 6-methyl substitution (**Ac5AzM**) on A-ring did not increase the potency as the EC_{50} ranged from 15 nM to 543 nM, excluding **Ac5Az8** and **Ac5Az9**. Comparing **Ac1Az1** with **Ac2Az1**, their EC_{50} values are similar, suggesting that the location of the linker for the Ac component, either at A or B ring, does not make a substantial difference on the activity.

Ac15 is different from all other **AcN** considered thus far. It is a diacetylene with no flavonoid by itself. It generated flavonoid homodimers when it was coupled with 2 mole equivalents of azido monomers, **AzM**, to form **Ac15(AzM)$_2$**. Their BCRP-modulating activity appear to be similar to other flavonoid dimers **AcNAzM** (**Table 2**). However structurally, **Ac15(AzM)$_2$** contain two triazole groups in the linker whereas the other **AcNAzM** dimers have only one triazole group in the linker. The effect of such structural difference on MRP1- and P-gp-modulating activity would be further explored.

Table 1. BCRP-, MRP1- and P-gp-modulating activity of flavonoid dimers.

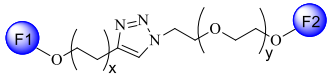
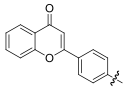
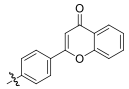
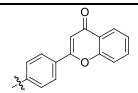
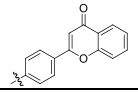
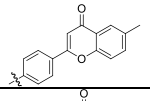
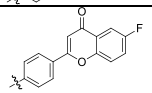
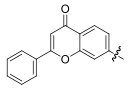
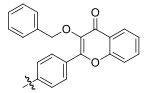
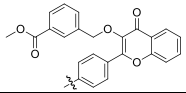
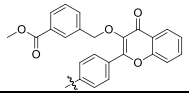
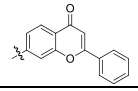
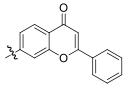
Compounds	F1	F2	x	y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
						BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
Ac1Az1*			3	1	60.2 ± 16.3*	12.3 ± 6.7	23.1 ± 15.2	127.5 ± 32.5*	201.0 ± 1.4
Ac2Az1*			3	1	>100*	66.7 ± 7.6	29.5 ± 26.5	151.3 ± 14.2*	300.0 ± 113.1
Ac2Az2			3	2	12.0 ± 6.2	51.7 ± 20.2	202.7 ± 101.3	170.0 ± 17.3	384.0 ± 15.1
Ac2Az3			3	2	>50	34.7 ± 18.2	185.0 ± 7.1	200.0 ± 62.4	349.5 ± 74.2
Ac2Az4*			3	2	>100*	38.3 ± 32.8	97.5 ± 59.4	81.4 ± 8.5*	328.0 ± 87.6
Ac2Az5			3	2	>100	59.7 ± 9.0	78.3 ± 1.5	207.3 ± 63.1	258.8 ± 77.2
Ac2Az8			3	1	>100	7.2 ± 3.1	23.6 ± 29.2	212.0 ± 69.3	616.3 ± 167.2
Ac2Az9			3	2	>100	3.5 ± 2.0	7.0 ± 5.0	181.4 ± 43.8	414.3 ± 168.8
Ac2Az11*			3	1	>100*	42.0 ± 15.6	22.3 ± 43.2	297.8 ± 44.3*	489.7 ± 61.8
Ac2Az12			3	2	>50	57.0 ± 8.5	176.7 ± 86.2	205.0 ± 55.2	515.0 ± 106.1

Table 1…….continued

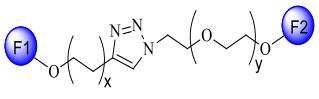
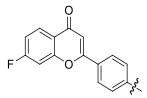
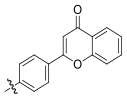
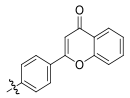
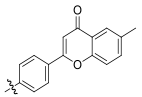
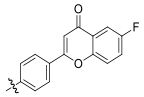
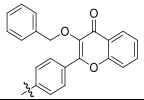
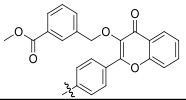
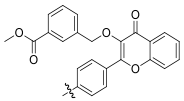
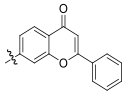
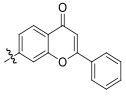
Compounds	F1	F2	x	y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
						BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
Ac3Az1*			3	1	>100*	7.2 ± 4.4	17.5 ± 11.4	137.0 ± 16.2*	155.0 ± 56.6
Ac3Az2*			3	2	>100*	24.0 ± 15.6	7.4 ± 8.0	208.0 ± 48.1*	255.0 ± 9.9
Ac3Az3*			3	2	>100*	14.0 ± 1.4	19.7 ± 17.4	114.5 ± 10.9*	218.5 ± 99.7
Ac3Az4*			3	2	>100*	3.1 ± 2.1	4.0 ± 3.2	131.7 ± 19.8*	161.0 ± 50.9
Ac3Az5			3	2	>100	25.2 ± 18.9	47.3 ± 35.6	111.0 ± 13.9	344.4 ± 91.8
Ac3Az8			3	1	>100	9.6 ± 3.9	6.5 ± 3.5	137.0 ± 7.2	480.7 ± 183.1
Ac3Az9			3	2	>100	2.0 ± 1.2	2.3 ± 1.1	139.3 ± 21.5	245.5 ± 157.7
Ac3Az11*			3	1	>100*	6.4 ± 0.2	7.9 ± 6.8	53.0 ± 1.5*	68.7 ± 20.4
Ac3Az12			3	2	>100	27.5 ± 19.1	27.1 ± 16.5	155.8 ± 46.8	88.5 ± 27.6

Table 1…….continued

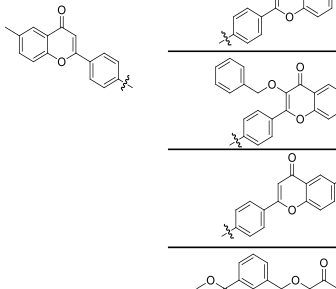
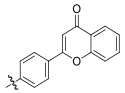
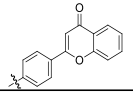
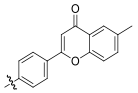
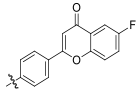
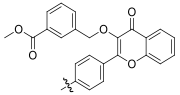
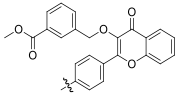
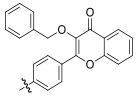
Compounds	F1	F2	x	y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
						BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
Ac5Az1*			4	1	>57*	66.5 ± 9.2	32.5 ± 17.7	97.2 ± 11.8*	370.0 ± 41.0
Ac5Az2			4	2	>50	285.0 ± 77.8	160.0 ± 84.9	425.0 ± 106.1	>1000
Ac5Az3*			4	2	>50*	54.0 ± 1.4	230.0 ± 14.1	136.7 ± 11.7*	526.0 ± 272.1
Ac5Az4*			4	2	>100*	57.5 ± 55.9	320.0 ± 14.1	217.4 ± 25.0*	746.7 ± 128.6
Ac5Az5			4	2	>100	24.3 ± 7.5	19.7 ± 0.6	225.0 ± 91.9	421.7 ± 75.2
Ac5Az7*			4	1	>100*	14.8 ± 1.8	64.0 ± 4.2	250.0 ± 30.8*	155.7 ± 30.0
Ac5Az8			4	1	>100	3.6 ± 0.4	4.0 ± 3.3	245.0 ± 35.4	430.0 ± 42.4
Ac5Az9			4	2	>100	2.8 ± 0.9	0.9 ± 0.4	220.0 ± 14.1	420.0 ± 141.4
Ac5Az10			4	1	>100	32.5 ± 23.3	23.6 ± 26.3	>1000	605.0 ± 35.4

Table 1…….continued

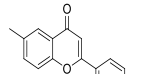
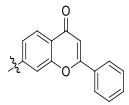
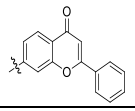
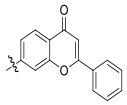
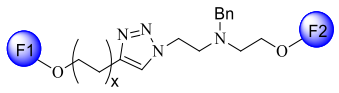
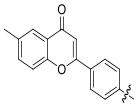
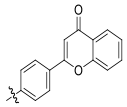
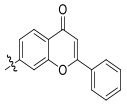
Compounds	F1	F2	x	y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
						BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
Ac5Az11*			4	1	>100*	28.0 ± 2.8	135.0 ± 35.4	175.0 ± 17.6*	159.7 ± 35.9
Ac5Az12			4	2	>100	32.0 ± 5.7	150.8 ± 129.9	133.8 ± 54.4	141.0 ± 1.4
Ac5Az13			4	0	9.2 ± 6.7	60.0 ± 7.1	223.3 ± 86.2	348.3 ± 197.5	204.0 ± 33.9
			x						
Ac5Az14			4		>100	69.5 ± 7.8	38.7 ± 46.9	238.3 ± 102.5	678.8 ± 100.5
Ac5Az15			4		>100	542.5 ± 293.4	371.7 ± 184.7	616.7 ± 125.8	>1000

Table 1…….continued

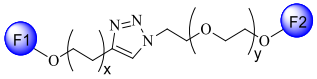
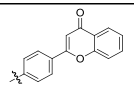
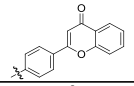
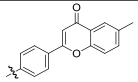
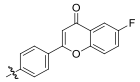
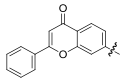
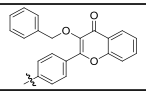
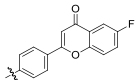
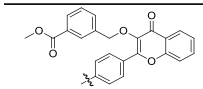
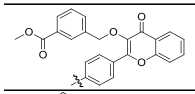
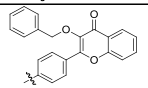
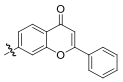
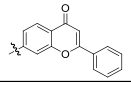
Compounds	F1	F2	x	y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
						BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
Ac12Az1*			4	1	>100*	51.5 ± 4.9	24.5 ± 12.0	86.5 ± 16.6*	321.0 ± 43.8
Ac12Az2			4	2	>50	47.0 ± 19.8	18.0 ± 0.0	230.0 ± 71.9	360.0 ± 56.6
Ac12Az3			4	2	>50	32.8 ± 24.3	45.5 ± 44.5	171.0 ± 41.9	198.3 ± 7.6
Ac12Az4			4	2	>100	43.5 ± 2.1	30.5 ± 20.5	219.0 ± 40.7	325.0 ± 7.1
Ac12Az5			4	2	>100	35.0 ± 7.1	15.5 ± 3.5	233.3 ± 115.9	277.5 ± 81.3
Ac12Az7			4	1	>100	43.0 ± 4.2	17.5 ± 2.1	172.3 ± 53.7	264.0 ± 79.2
Ac12Az8			4	1	>100	5.2 ± 1.8	5.6 ± 2.4	255.0 ± 63.6	325.0 ± 63.6
Ac12Az9			4	2	>100	0.9 ± 0.1	1.4 ± 1.0	165.0 ± 35.4	285.0 ± 35.4
Ac12Az10			4	1	>100	20.0 ± 7.1	34.2 ± 20.8	650.0 ± 348.7	650.0 ± 495.0
Ac12Az11			4	1	>100	34.1 ± 32.4	39.0 ± 39.6	114.0 ± 15.6	365.0 ± 91.9
Ac12Az12			4	2	>100	47.0 ± 1.4	94.0 ± 36.8	135.0 ± 14.1	287.5 ± 24.7

Table 1…….continued

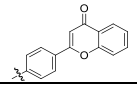
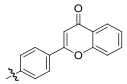
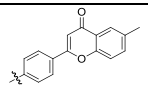
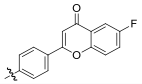
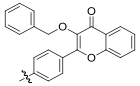
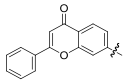
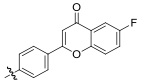
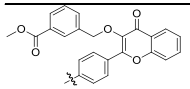
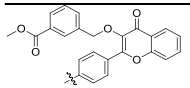
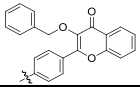
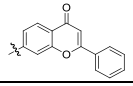
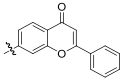
Compounds	F1	F2	y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
					BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
Ac13Az1*			1	>50*	302.5 ± 53.0	235.0 ± 184.8	235.0 ± 35.5*	960.0 ± 49.0
Ac13Az2			2	22.0 ± 15.6	302.5 ± 24.7	340.0 ± 172.8	276.3 ± 120.6	962.5 ± 47.9
Ac13Az3			2	>100	90.0 ± 7.1	145.0 ± 97.1	246.7 ± 92.4	773.3 ± 20.8
Ac13Az4			2	>50	230.0 ± 84.9	264.3 ± 199.0	243.3 ± 55.1	966.7 ± 57.7
Ac13Az5*			2	>100	38.0 ± 18.4	24.9 ± 22.6	180.0 ± 19.5*	383.3 ± 32.1
Ac13Az7			1	>100	285.0 ± 49.5	195.0 ± 102.6	292.7 ± 65.1	>1000
Ac13Az8			1	>100	4.1 ± 0.6	17.7 ± 12.0	135.0 ± 35.4	436.7 ± 47.3
Ac13Az9			2	>100	1.7 ± 0.8	2.0 ± 1.3	180.0 ± 56.6	313.3 ± 49.3
Ac13Az10			1	>100	16.5 ± 3.5	118.3 ± 61.7	184.0 ± 33.9	345.0 ± 5.0
Ac13Az11			1	>100	195.0 ± 61.4	308.0 ± 209.8	333.3 ± 119.3	>1000
Ac13Az12			2	12.1 ± 4.1	145.0 ± 21.2	59.0 ± 58.0	272.0 ± 116.0	927.5 ± 31.8

Table 1…….continued

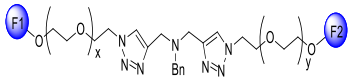
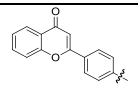
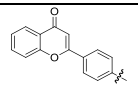
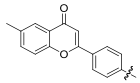
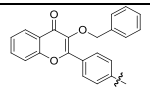
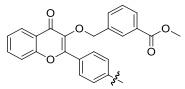
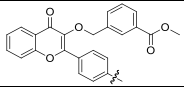
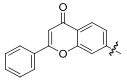
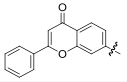
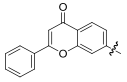
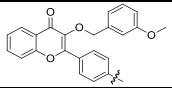
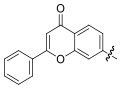
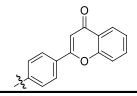
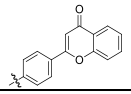
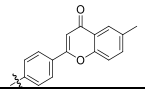
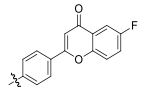
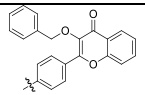
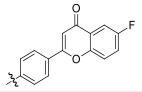
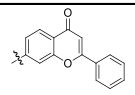
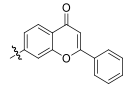
Compounds	F1 F2	x y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
				BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
$Ac15(Az1)_2$		1 1	>100	111.0 ± 12.7	205.0 ± 63.6	950.0 ± 70.7	670.0 ± 130.8
$Ac15(Az2)_2$		2 2	>100	77.5 ± 31.8	64.0 ± 31.1	530.0 ± 169.7	893.3 ± 110.2
$Ac15(Az3)_2$		2 2	>100	36.0 ± 5.7	46.0 ± 33.9	475.0 ± 35.4	763.3 ± 47.3
$Ac15(Az5)_2$		2 2	>100	35.5 ± 27.1	25.5 ± 12.0	579.0 ± 111.7	560.0 ± 165.2
$Ac15(Az8)_2$		1 1	>100	5.3 ± 2.7	3.3 ± 4.1	457.5 ± 10.6	>1000
$Ac15(Az9)_2$		2 2	>100	3.3 ± 2.1	4.3 ± 4.6	441.7 ± 14.4	>1000
$Ac15(Az11)_2$		1 1	>100	152.5 ± 123.7	278.3 ± 133.6	760.0 ± 28.3	>1000
$Ac15(Az12)_2$		2 2	>100	82.5 ± 17.7	250.0 ± 70.7	750.0 ± 0.0	>1000
$Ac15(Az13)_2$		0 0	>100	242.5 ± 3.5	485.0 ± 21.2	>1000	>1000
$Ac15(Az16)_2$	H	/ /	>100	>1000	>1000	>1000	>1000
$Ac15(Az17)_2$		1 1	>100	18.5 ± 9.3	10.9 ± 4.7	>1000	>1000

Table 1…….continued

Compounds	F1	F2	y	IC_{50} towards L929 (μM)	EC_{50} (nM) for reversing MDR			
					BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR
Ac16Az1*			1	>100*	86.1 ± 40.8	205.0 ± 7.1	77.7 ± 16.5*	630.0 ± 42.4
Ac16Az2*			2	>100*	36.5 ± 2.1	17.0 ± 1.4	98.8 ± 18.3*	275.0 ± 35.4
Ac16Az3			2	>100	54.5 ± 17.1	15.8 ± 1.6	137.7 ± 37.0	210.0 ± 56.6
Ac16Az4*			2	>100*	26.5 ± 6.4	19.9 ± 15.1	156.0 ± 26.9*	333.5 ± 3.5
Ac16Az5			2	>100	156.3 ± 50.9	69.3 ± 47.2	301.7 ± 161.0	983.3 ± 28.9
Ac16Az7*			1	>100*	48.0 ± 15.9	53.5 ± 51.6	123.0 ± 13.0*	285.0 ± 63.6
Ac16Az12			2	>100	54.0 ± 5.7	58.0 ± 38.2	208.0 ± 45.3	403.3 ± 55.1
Ac16Az13			0	>100	190.0 ± 127.3	280.0 ± 141.4	670.0 ± 113.1	896.7 ± 274.7
Ko143				31.4 ± 5.0	8.7 ± 4.9	9.0 ± 2.1	1950.0 ± 353.6	1060.0 ± 207.8
FD-4e				>100	64.8 ± 28.1	63.2 ± 73.6	110.7 ± 42.6	264.0 ± 19.8
Cyclosporine A				21.9 ± 9.1	1750.0 ± 150.0	ND	1700.0 ± 141.4	32.0 ± 1.4

A total of 74 compounds were synthesized and their BCRP-, MRP1- and P-gp-modulating activities were determined. EC_{50} value was used to differentiate the MDR reversal potency of these flavonoid dimers. EC_{50} value was presented as mean ± standard deviation. N = 2-7 independent experiments. L929 is a normal mouse fibroblast cell line. The cytotoxicity of these compounds towards L929 was tested. Ko143, **FD-4e** and cyclosporine A are BCRP, MRP1 and P-gp inhibitors, respectively. They were included in the assay as positive controls *The chemical structure, cytotoxicity, EC_{50} values for reversing MRP1-mediated drug resistance have been previously reported.[54] Here, we included them in order to make a comparison. ND = not determined. The chemical structures of these compounds are shown in Supporting Information. / = not applicable.

Table 2. Comparison of EC_{50} of triazole bridged flavonoid dimers for reversing BCRP-, MRP1- and P-gp-mediated drug resistance.

A

BCRP / HEK293/R2 (EC_{50} nM)	Az1	Az2	Az3	Az4	Az5	Az7	Az8	Az9	Az10	Az11	Az12	Az13	Az14	Az15	Az16	Az17
Ac1	12															
Ac2	67	52	35	38	60		7	4		42	57					
Ac3	7	24	14	3	25		10	2		6	28					
Ac5	67	285	54	58	24	15	4	3	33	28	32	60	70	543		
Ac12	52	47	33	44	35	43	5	1	20	34	47					
Ac13	303	303	90	230	38	285	4	2	17	195	145					
Ac15	111	78	36		36		5	3		153	83	243			>1000	19
Ac16	86	37	55	27	156	48					54	190				
Ko143	9															

B

BCRP / MCF7-MX100 (EC_{50} nM)	Az1	Az2	Az3	Az4	Az5	Az7	Az8	Az9	Az10	Az11	Az12	Az13	Az14	Az15	Az16	Az17
Ac1	23															
Ac2	30	203	185	98	78		24	7		22	177					
Ac3	18	7	20	4	47		7	2		8	27					
Ac5	33	160	230	320	20	64	4	1	24	135	151	223	39	372		
Ac12	25	18	46	31	16	18	6	1	34	39	94					
Ac13	235	340	145	264	25	195	18	2	118	308	59					
Ac15	205	64	46		26		3	4		278	250	485			>1000	11
Ac16	205	17	16	20	69	54					58	280				
Ko143	9															

Table 2…….continued.

C

MRP1 / 2008/MRP1 (EC_{50} nM)	Az1	Az2	Az3	Az4	Az5	Az7	Az8	Az9	Az10	Az11	Az12	Az13	Az14	Az15	Az16	Az17
Ac1	128															
Ac2	151	170	200	81	207		212	181		298	205					
Ac3	137	208	115	132	111		137	139		53	156					
Ac5	97	425	137	217	225	250	245	220	>1000	175	134	348	238	617		
Ac12	87	230	171	219	233	172	255	165	650	114	135					
Ac13	235	276	247	243	180	293	135	180	184	333	272					
Ac15	950	530	475		579		458	442		760	750	>1000			>1000	>1000
Ac16	78	99	138	156	302	123					208	670				

FD-4e	111

D

Pgp / LCC6MDR (EC_{50} nM)	Az1	Az2	Az3	Az4	Az5	Az7	Az8	Az9	Az10	Az11	Az12	Az13	Az14	Az15	Az16	Az17
Ac1	201															
Ac2	300	384	350	328	259		616	414		490	515					
Ac3	155	255	219	161	344		481	246		69	89					
Ac5	370	>1000	526	747	422	156	430	420	605	160	141	204	679	>1000		
Ac12	321	360	198	325	278	264	325	285	650	365	288					
Ac13	960	962	773	967	383	>1000	437	313	345	>1000	928					
Ac15	670	893	763		560		>1000	>1000		>1000	>1000	>1000			>1000	>1000
Ac16	630	275	210	334	983	285					403	897				

Cyclosporine A	32

The mean EC_{50} (nM) value of each flavonoid dimer for reversing BCRP-, MRP1- and P-gp mediated drug resistance was listed. A red color gradient was used to quantitatively discriminate from high-to-low MDR reversal potency. The EC_{50} values lower than or equal to 10 nM was in red scale, bolded and underlined. N = 2-7 independent experiment. (A) BCRP-modulating activity of HEK293/R2 cell line, (B) BCRP-modulating activity of MCF7-MX100 cell line, (C) MRP1-modulating activity of 2008/MRP1 cell line and (D) P-gp-modulating activity of LCC6MDR cell line. The box in grey indicated that the flavonoid dimers have not been synthesized.

2.2.3. MRP1 and P-gp inhibitory activity of flavonoid dimers

The inhibitory activities against MRP1 and P-gp of these 74 compounds were also tested (**Table 1** and **Table 2C** and **2D**). Compounds **Ac2Az4**, **Ac3Az11**, **Ac5Az1**, **Ac12Az1**, **Ac16Az1** and **Ac16Az2** displayed the highest MRP1-inhibitory potency with EC_{50} less than 100 nM, more potent than the positive control **FD-4e** with EC_{50} =110.7 nM (**Table 2C**), as previously reported.[52] In contrast, all 74 compounds are less potent than the positive control, cyclosporine A (EC_{50}=32.0 nM), in their P-gp inhibitory activity. Compounds **Ac3Az11** and **Ac3Az12** showed the highest P-gp inhibitory potency with EC_{50} at 68.7 and 88.5 nM respectively (**Table 2D**). The linker length effect of flavonoid dimers to modulate P-gp and MRP1 transporters has been previously reported for compounds of general structure I.[48-53] A good inhibitor of P-gp or MRP1 should have 4 PEG linker or 5-6 PEG linker respectively to make the two flavone moieties binding to transporters selectively.[49,52] Shorter or longer linkers would lead to less inhibition towards these two transporters. For the triazole-bridged flavonoid dimers in the present study, all the potent MRP1 inhibitors have linkers with 13−17 atoms between the two flavonoid moieties, similar in linker length to I with n=5-6.[54] For the most active P-gp inhibitors in **Table 2D**, **Ac3Az11** and **Ac3Az12** contain 13-16 atoms between the two flavonoids, slightly longer than the optimal length of I with n=4 (12 atoms in between the two flavonoids). In this context, the 11 compounds, **Ac2Az9, Ac3Az4, Ac3Az9, Ac3Az11, Ac5Az8, Ac5Az9, Ac12Az8, Ac12Az9, Ac13Az9, Ac15(Az8)$_2$** and **Ac15(Az9)$_2$,** with the most potent activity against BCRP, (**Table 1**, **Table 2A** and **2B**) contain 13-27 atoms between the two flavonoids. The linker length requirement for inhibitory activity against BCRP thus appears to be less stringent. This offers an opportunity to design inhibitors which are more selective for BCRP. Specifically, of the 11 most active compounds, the two *bis*-triazole bridged dimers, **Ac15(Az8)$_2$** and **Ac15(Az9)$_2$** have the longest linker length with 21 and 27 atoms

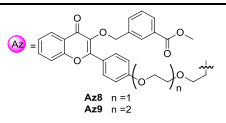
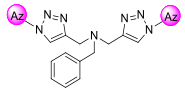
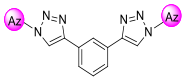
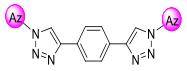
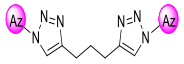
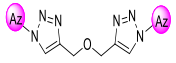
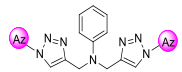
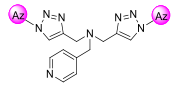
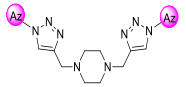
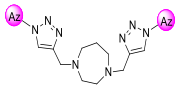
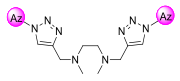
respectively. Indeed, both compounds showed only modest activity towards MRP1 (EC_{50} = 442-458 nM) and essentially no activity towards P-gp (EC_{50} >1000 nM) as expected from their longer than the optimal linker length for MRP1 and P-gp inhibition. They are potent (EC_{50} = 3-5 nM), nontoxic (IC_{50} >100 μM towards L929 cells) and selective inhibitors of BCRP.

2.2.4. Analogues of Ac15(Az8)$_2$ and Ac15(Az9)$_2$ and BCRP selectivity relationship

We define the selectivity index of an inhibitor for transporter A over transporter B as the inverse ratio of EC_{50} of the inhibitor for transporter A over the EC_{50} of the same inhibitor for transporter B (**Table 3**). The selectivity index of **Ac15(Az8)$_2$** and **Ac15(Az9)$_2$** is about 86-139 for BCRP over MRP1 and >189-303 for BCRP over P-gp depending on the cell lines used. (**Table 3**). In order to further improve the BCRP selectivity, several analogues of **Ac15(Az8)$_2$** and **Ac15(Az9)$_2$** have been synthesized by varying the structure of the diacetylene **Ac15** as shown in **Scheme 4**. Their biological data are shown in **Table 3**. We examine first the effect of replacing the *N*-benzyl group in **Ac15** with *N*-phenyl (**Ac26**) or *N*-4-pyridinylmethyl (**Ac28**). **Ac26(Az8)$_2$** and **Ac26(Az9)$_2$** are about as potent as the **Ac15** counterparts, so both *N*-benzyl and *N*-phenyl have significant effect on BCRP-modulation. On the other hand, replacing *N*-benzyl with *N*-4-pyridinylmethyl leads to diminished activity because **Ac28(Az8)$_2$** is much less potent than **Ac15(Az8)$_2$**. In fact, the *N*-group does not appear to be essential at all for BCRP inhibitory activity. **Ac24(Az8)$_2$** and **Ac25(Az8)$_2$** are as potent as **Ac15(Az8)$_2$** with CH_2 or O replacing N-benzyl respectively. The difference between **Ac24** and **Ac22** is that the conformationally flexible three carbon $CH_2CH_2CH_2$ chain in **Ac24** is replaced by an aromatic ring with conformationally rigid three carbon chain (*m*-substitution) in **Ac22**. Interestingly, **Ac22(Az8)$_2$** is slightly more active than **Ac24(Az8)$_2$.** The reason may not be due to the aromatic ring because **Ac23(Az8)$_2$** is less potent

than either **Ac22(Az8)$_2$** or **Ac24(Az8)$_2$.** Compounds **Ac29(Az8)$_2$, Ac30(Az8)$_2$** and **Ac31(Az8)$_2$** are all less potent, suggesting that the presence of two nitrogen amines in the linker did not enhance BCRP inhibition. Of the compounds in **Table 3**, five dimers, **Ac22(Az8)$_2$**, **Ac24(Az8)$_2$**, **Ac25(Az8)$_2$**, **Ac26(Az8)$_2$** and **Ac26(Az9)$_2$** display a more potent activity than Ko143 in inhibiting BCRP in both cell lines. All five compounds are essentially nontoxic towards L929, 3T3 and HFF-1 cells (IC_{50} >100 μM) **(Table 3).** Importantly, all five compounds are essentially devoid of inhibitory activity against MRP1 and P-gp (EC_{50} >1000 nM) and thus are highly selective inhibitors of BCRP (**Table 3**). For the moment, **Ac22(Az8)$_2$** (EC_{50} = 1.1 – 2.2 nM), being the most potent BCRP inhibitors among the five, has been chosen for further biological characterization.

Table 3. EC_{50} (nM) of $Ac15(Az8)_2$ and $Ac15(Az9)_2$ analogues for reversing MDR and their BCRP-selectivity.

Compounds		IC_{50} (μM) towards			EC_{50} (nM) needed for reversing MDR				BCRP-selectivity			
					BCRP-mediated topotecan resistance HEK293/R2	BCRP-mediated topotecan resistance MCF7-MX100	MRP1-mediated DOX resistance 2008/MRP1	P-gp mediated paclitaxel resistance LCC6MDR	Relative to MRP1-inhibitory activity		Relative to P-gp-inhibitory activity	
		L929	3T3	HFF-1	HEK293/R2	MCF7-MX100	2008/MRP1	LCC6MDR	HEK293/R2	MCF7-MX100	HEK293/R2	MCF7-MX100
$Ac15(Az8)_2$		>100	>100	>100	5.3 ± 2.7	3.3 ± 4.1	457.5 ± 10.6	>1000	86	139	189	303
$Ac15(Az9)_2$		>100	>100	>100	3.3 ± 2.1	4.3 ± 4.6	441.7 ± 14.4	>1000	134	103	303	233
$Ac22(Az8)_2$		>100	>100	>100	2.2 ± 1.5	1.1 ± 0.4	>1000	>1000	455	909	455	909
$Ac22(Az9)_2$		>100	>100	>100	2.8 ± 1.7	12.8 ± 1.1	>1000	>1000	357	78	357	78
$Ac23(Az8)_2$		>100	ND	ND	13.5 ± 0.1	26.5 ± 9.2	>1000	>1000	74	38	74	38
$Ac23(Az9)_2$		>100	>100	>100	2.4 ± 1.1	13.7 ± 4.0	>1000	>1000	417	73	417	73
$Ac24(Az8)_2$		>100	>100	>100	6.1 ± 2.5	2.4 ± 1.0	>1000	>1000	164	417	164	417
$Ac25(Az8)_2$		>100	>100	>100	4.2 ± 1.6	2.1 ± 1.6	>1000	>1000	238	476	238	476
$Ac26(Az8)_2$		>100	>100	>100	6.8 ± 2.2	2.2 ± 2.3	>1000	>1000	147	455	147	455
$Ac26(Az9)_2$		>100	>100	>100	5.8 ± 2.0	3.9 ± 1.6	>1000	>1000	172	256	172	256
$Ac28(Az8)_2$		>50	ND	ND	48.8 ± 6.8	24.5 ± 7.6	>1000	>1000	20	41	20	41
$Ac29(Az8)_2$		>100	ND	ND	17.8 ± 11.4	18.7 ± 11.6	>1000	683.2 ± 301.4	56	53	38	37
$Ac29(Az9)_2$		>100	ND	ND	30.7 ± 8.3	39.5 ± 20.5	>1000	>1000	33	25	33	25
$Ac30(Az8)_2$		>100	ND	ND	108.3 ± 28.4	55.4 ± 23.5	>1000	573.3 ± 23.1	9	18	5	10
$Ac31(Az8)_2$		>100	ND	ND	133.3 ± 20.8	84.0 ± 27.5	>1000	>1000	8	12	8	12
Ko143		31.4 ± 5.0	>100	56.5 ± 5.1	8.7 ± 4.9	9.0 ± 2.1	1950.0 ± 354	1060.0 ± 207.8	224	217	122	118

BCRP-selectivity > 400

BCRP-selectivity = 301 - 400

BCRP-selectivity = 201 - 300

BCRP-selectivity = 101 - 200

BCRP-selectivity < 100

Thirteen **Ac15(Az8)$_2$** and **Ac15(Az9)$_2$** analogues were synthesized and their BCRP-, MRP1- and P-gp-modulating activities determined. EC_{50} value was used to differentiate the MDR reversal potency of analogues. EC_{50} value was presented as mean ± standard deviation. N = 2-4 independent experiments. L929 and 3T3 are normal mouse fibroblast cell lines. HFF-1 is a normal human skin fibroblast. The cytotoxicity of these compounds towards normal fibroblasts was tested. A specific BCRP inhibitor Ko143 was included as a positive control. A red color gradient was used to quantitatively discriminate from high-to-low BCRP selectivity over MRP1 and P-gp. The selectivity index for BCRP over MRP1 = EC_{50} for reversing MRP1-mediated DOX resistance in 2008/MRP1 cells / EC_{50} for reversing BCRP-mediated topotecan resistance in HEK293/R2 cells or MCF7-MX100 cells. The selectivity index for BCRP over P-gp = EC_{50} for reversing P-gp mediated paclitaxel resistance in LCC6MDR cells / EC_{50} for reversing BCRP-mediated topotecan resistance in HEK293/R2 cells or MCF7-MX100 cells. ND = not determined. The chemical structures of these analogues are shown in Supporting Information.

2.2.5 Potent flavonoid dimers showed no synergistic effect with topotecan towards normal L929 cells

Our flavonoid dimers were found to enhance the cancer killing activity of topotecan in BCRP-overexpressing HEK293/R2 and MCF7-MX100 cells. We would like to know whether such drug combination also shows synergistic effect towards normal cells. Normal mouse fibroblast L929 or BCRP-overexpressing HEK293/R2 cells were incubated with different doses of topotecan and 1 μM of clicked flavonoid dimers together. A strong synergistic effect was only observed in BCRP-overexpressing HEK293/R2 cells with RF = 21.6 – 36.7 (**Table 4**). Remarkably, no potentiation effect was observed in normal L929 cells with RF = 1.1 – 1.7 (**Table 4**), indicating that combination of topotecan and the flavonoid dimers is safe and does not enhance the killing activity of topotecan towards normal cells.

Table 4. Potent clicked flavonoid dimers showed no synergistic effect with topotecan towards normal L929 cells

	L929		HEK293/R2	
Compounds	**Mean IC_{50} of topotecan (nM)**	**RF**	**Mean IC_{50} of topotecan (nM)**	**RF**
0.1% DMSO	1168 ± 109	1.0	531.5 ± 180	1.0
1 μM Ko143	1040 ± 27	1.1	24.0 ± 6.7	22.1
1 μM Ac15(Az8)$_2$	668 ± 72	1.7	14.5 ± 1.6	36.7
1 μM Ac22(Az8)$_2$	783 ± 77	1.5	24.6 ± 2.2	21.6
1 μM Ac24(Az8)$_2$	877 ± 78	1.3	19.1 ± 4.2	27.8
1 μM Ac25(Az8)$_2$	851 ± 130	1.4	20.2 ± 4.8	26.3

Normal mouse fibroblast L929 cells or HEK293/R2 cells were incubated together with different dosage of topotecan and 1 μM of potent clicked flavonoid dimers. Relative fold (RF) was

determined by dividing IC_{50} of topotecan of a cell line without modulators by that with modulators. N = 3 independent experiments. The IC_{50} value was presented as mean ± standard deviation.

2.2.6 Ac22(Az8)$_2$ selectively reverses mitoxantrone resistance in BCRP-overexpressed cell line but not in MRP1- or P-gp-overexpressed cell lines.

Mitoxantrone is an overlap substrate between BCRP, MRP1 and P-gp transporters.[57] In order to further confirm the BCRP-selectivity of **Ac22(Az8)$_2$**, mitoxantrone sensitization assay with or without **Ac22(Az8)$_2$** was carried out using BCRP-, MRP1- and P-gp-overexpressed cell lines. Overexpression of BCRP, MRP1 or P-gp transporter resulted in 77-, 3.5- and 40-fold higher mitoxantrone resistance (**Table 5A**). **Ac22(Az8)$_2$** and Ko143 markedly modulated BCRP-mediated mitoxantrone resistance in HEK293/R2 with EC_{50} of 10.5 nM and 5.4 nM respectively (**Table 5B**). **Ac22(Az8)$_2$** did not exhibit any MRP1- or P-gp modulating activities as EC_{50} greater than 2000 nM (**Table 5B**). However, Ko143 showed weak MRP1- and P-gp modulating activities with EC_{50} of 1800 nM and 943 nM (**Table 5B**). Once again, mitoxantrone sensitization assay demonstrated that **Ac22(Az8)$_2$** was highly selective towards BCRP over MRP1 or P-gp.

Table 5. Ac22(Az8)$_2$ selectively reverses mitoxantrone resistance in BCRP-overexpressed cell line but not MRP1- or P-gp overexpressed cell lines.

A

Cell lines	IC_{50} of mitoxantrone (nM)	Resistance factor
HEK293/R2	903.8 ± 517.4	77.2
HEK293/pcDNA3.1	11.7 ± 7.9	1.0
2008/MRP1	27.5 ± 16.8	3.5
2008	7.8 ± 5.7	1.0
LCC6MDR	1501.0 ± 750.9	40.1
LCC6	37.4 ± 23.5	1.0

B

Compounds	EC_{50} (nM) for reversing mitoxantrone resistance		
	BCRP-overexpressed HEK293/R2	MRP1-overexpressed 2008/MRP1	P-gp-overexpessed LCC6MDR
Ac22(Az8)$_2$	10.5 ± 5.6	>2000	>2000
Ko143	5.4 ± 1.8	1800.0 ± 282.8	942.5 ± 465.9

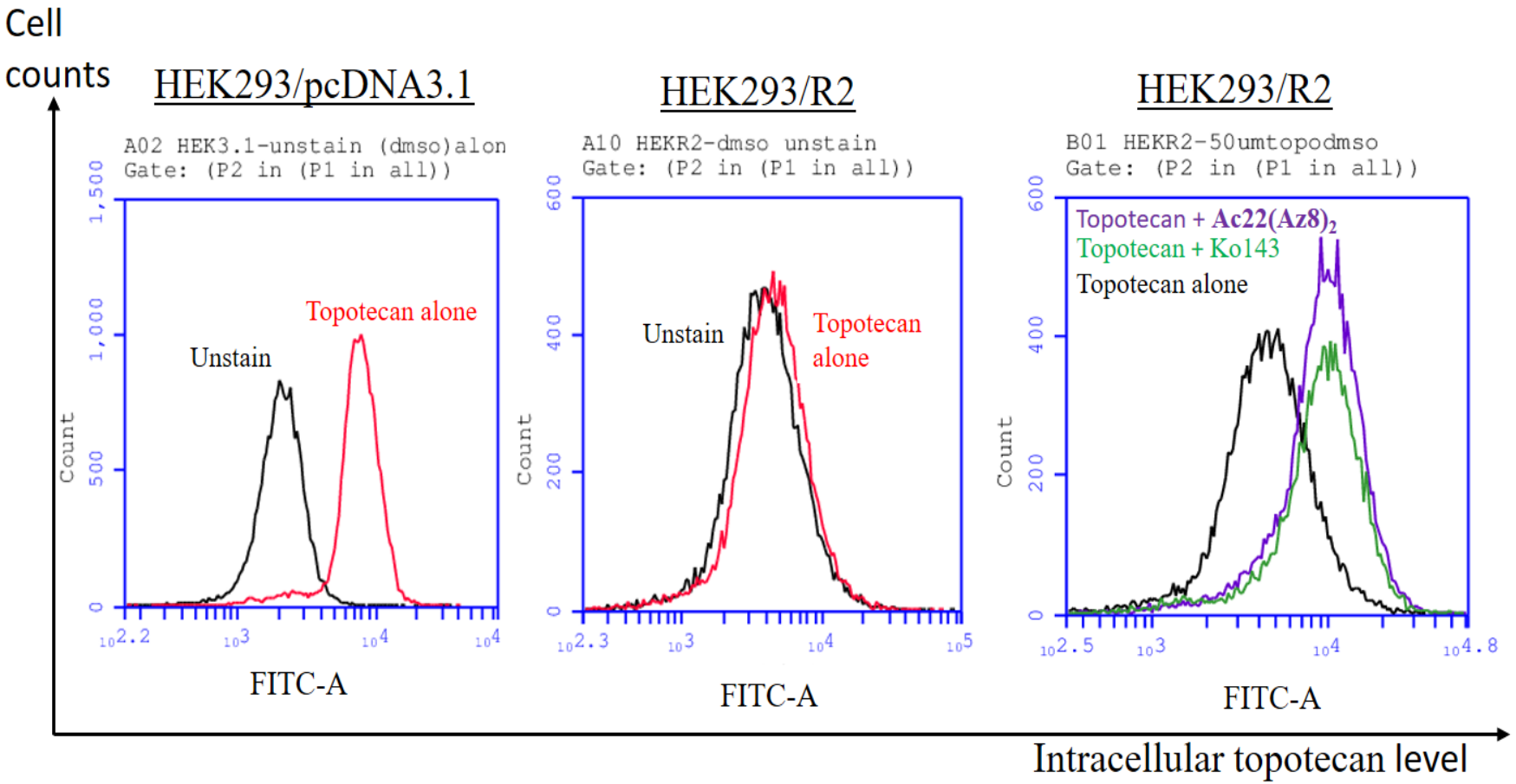
Mitoxantrone is an overlap substrate between BCRP, MRP1 and P-gp transporters. (A) The IC_{50} of mitoxantrone towards HEK293/R2, 2008MRP1 and LCC6MDR and their parental cells were determined. Resistance-fold was calculated by dividing mean IC_{50} of mitoxantrone of transporter-overexpressing cell lines by its parental cells. (B) The EC_{50} of **Ac22(Az8)$_2$** and Ko143 for reversing mitoxantrone resistance in different cell lines was also determined. N=2-4 independent experiments. The IC_{50} and EC_{50} values were presented as mean ± standard deviation.

2.2.7. BCRP inhibitor Ac22(Az8)$_2$ increases topotecan accumulation in HEK293/R2 cells

The above results showed that these triazole linked flavonoid dimers were potent, nontoxic and highly selective BCRP modulators. We determined whether the modulation of BCRP-mediated topotecan resistance was associated with a concomitant increase in drug accumulation. **Ac22(Az8)$_2$** was selected for further characterization. As shown in **Figure 4**, BCRP-

overexpressing cell line HEK293/R2 significantly accumulated 11-fold ($P<0.01$) less topotecan accumulation as compared to its wild type. Treatment of HEK293/R2 cells with 1 μM of compounds **Ac22(Az8)$_2$** ($P<0.01$) or Ko143 ($P<0.005$) significantly resulted in 11-12 fold increase in intracellular topotecan accumulation. The result suggests that **Ac22(Az8)$_2$** inhibits transport activity of BCRP and restores the intracellular topotecan concentration to a level similar to that of parental HEK293/pcDNA3.1.

A)


Cell counts
HEK293/pcDNA3.1
A02 HEK3.1-unstain (dmso)alon
Gate: (P2 in (P1 in all))
Unstain
Topotecan alone
Count
FITC-A
HEK293/R2
A10 HEKR2-dmso unstain
Gate: (P2 in (P1 in all))
Unstain
Topotecan alone
Count
FITC-A
HEK293/R2
B01 HEKR2-50umtopodmso
Gate: (P2 in (P1 in all))
Topotecan + Ac22(Az8)2
Topotecan + Ko143
Topotecan alone
Count
FITC-A
Intracellular topotecan level

B)

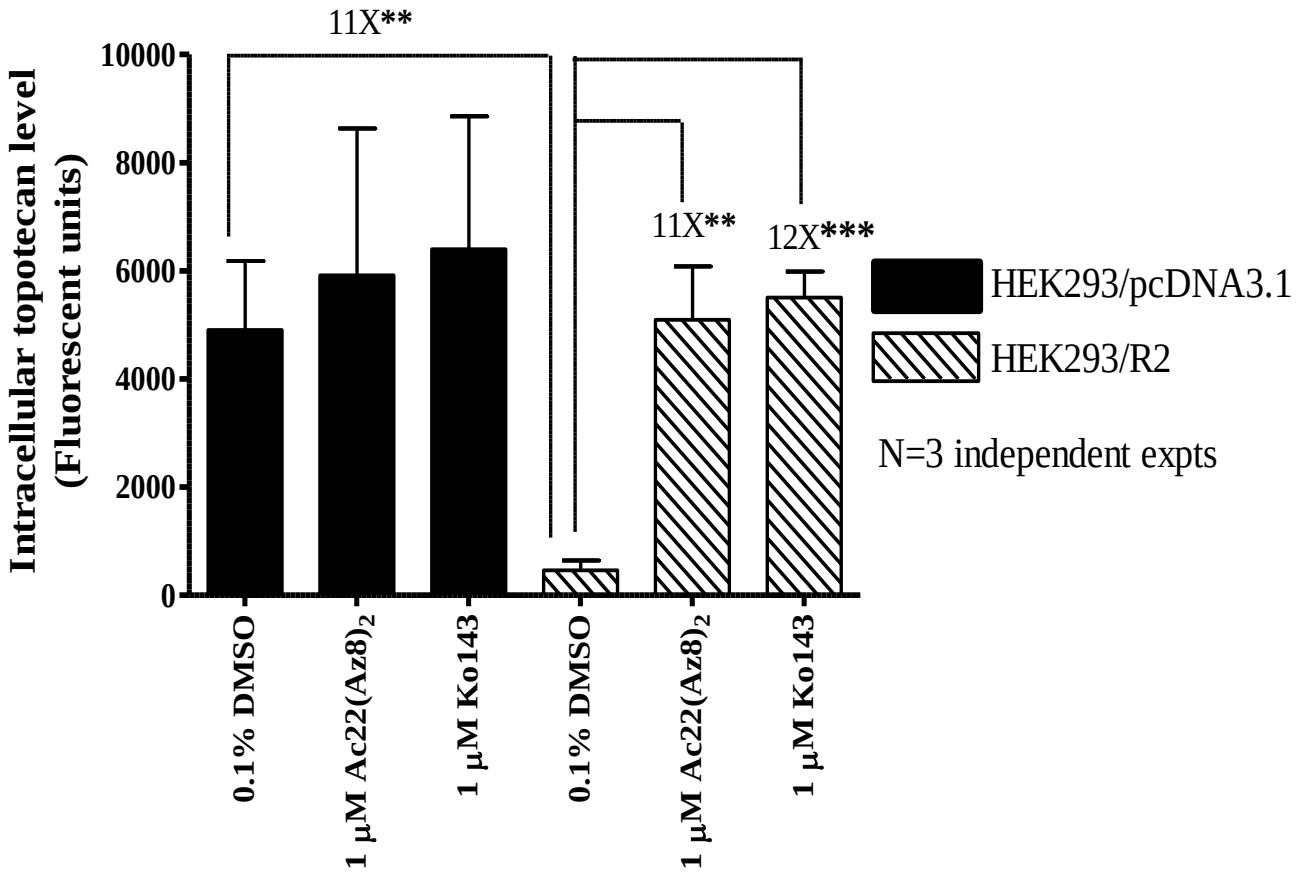


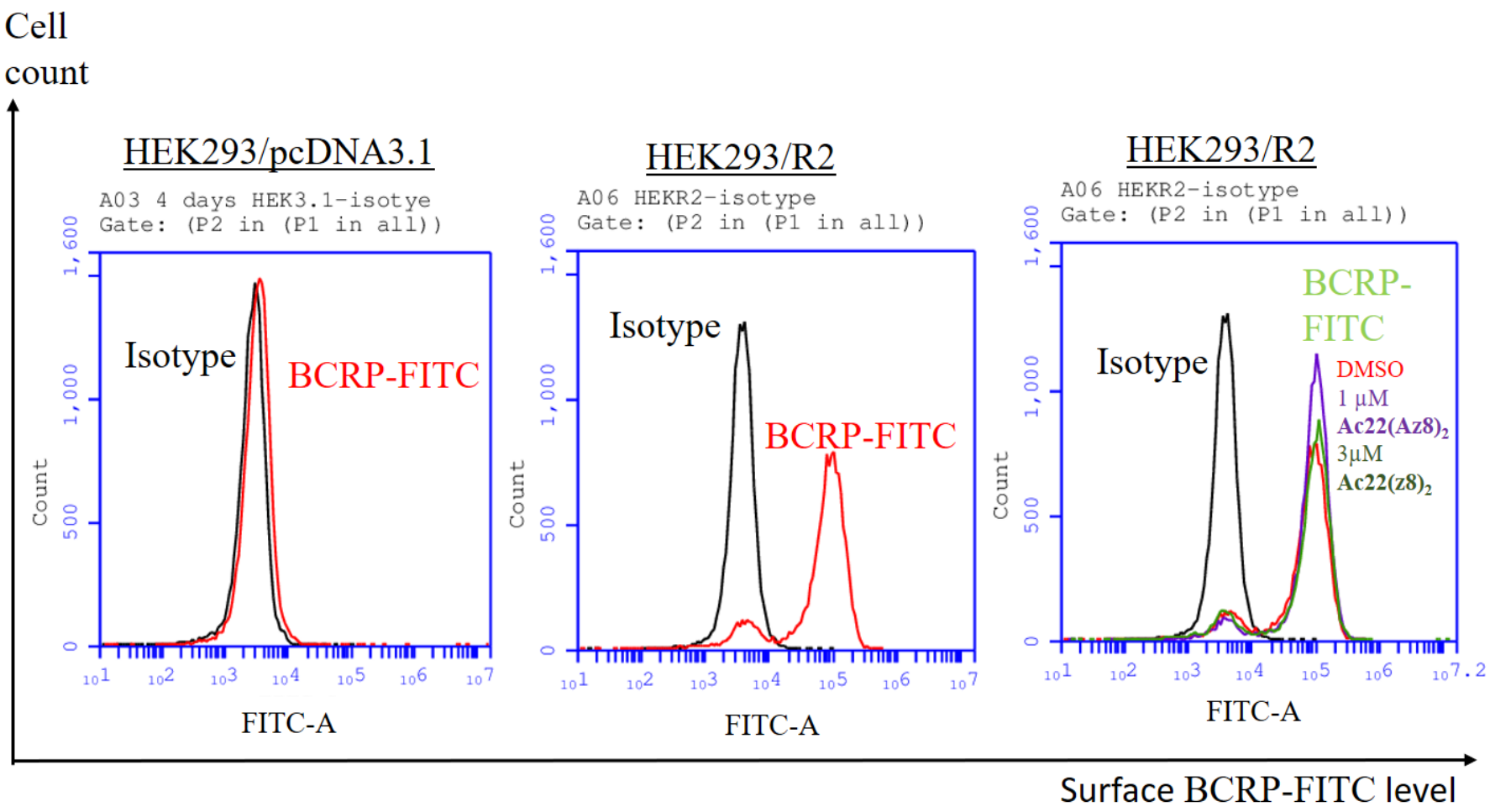
Figure 4. Effect of **Ac22(Az8)$_2$** on intracellular topotecan accumulation in HEK293/pcDNA3.1 and HEK293/R2 cells. The cells were incubated with 50 μM topotecan for 120 minutes at 37 ^{o}C with or without 1 μM of **Ac22(Az8)$_2$** and Ko143. 0.1% of DMSO was used as negative control. After incubation, cells were washed and intracellular accumulation of topotecan was measured by flow cytometry at FL1 channel. (A) The flow cytometric histogram of HEK293/pcDNA3.1 and HEK293/R2 cells after incubating with topotecan in absence or presence of modulators. (B) The fluorescence level of intracellular topotecan of cells after different treatments was plotted. Experiments were performed in duplicate and repeated thrice. The fluorescence units were

presented as mean ± standard deviation. Student paired t test was conducted relative to HEK293/R2 cells incubated with 0.1% DMSO. **P<0.01 and *** P<0.005.

2.2.8. BCRP inhibitor Ac22(Az8)$_2$ does not affect BCRP protein expression levels in HEK293/R2 cells

We characterized the effect of **Ac22(Az8)$_2$** on surface BCRP protein expression by flow cytometric detection (**Figure 5A and 5B**). HEK293/R2 cells highly overexpressed surface BCRP protein with 42-fold higher than the wild type HEK293/pcDNA3.1 (**Figure 5B**). After incubating with 1 or 3 μM of **Ac22(Az8)$_2$** for 4 days, the high BCRP protein expression in HEK293/R2 cells remained more or less unchanged, indicating that **Ac22(Az8)$_2$** did not down-regulate the surface BCRP protein expression to sensitize the cells to topotecan. After co-incubating with **Ac22(Az8)$_2$**, the increased topotecan accumulation level observed in HEK293/R2 (**Figure 4**) might be due to the loss of functionality of BCRP.

A)


Cell count
HEK293/pcDNA3.1
A03 4 days HEK3.1-isotye
Gate: (P2 in (P1 in all))
Isotype
BCRP-FITC
Count
FITC-A
HEK293/R2
A06 HEKR2-isotype
Gate: (P2 in (P1 in all))
Isotype
BCRP-FITC
Count
FITC-A
HEK293/R2
A06 HEKR2-isotype
Gate: (P2 in (P1 in all))
Isotype
BCRP-FITC
DMSO
1 µM
Ac22(Az8)2
3µM
Ac22(z8)2
Count
FITC-A
Surface BCRP-FITC level

B)

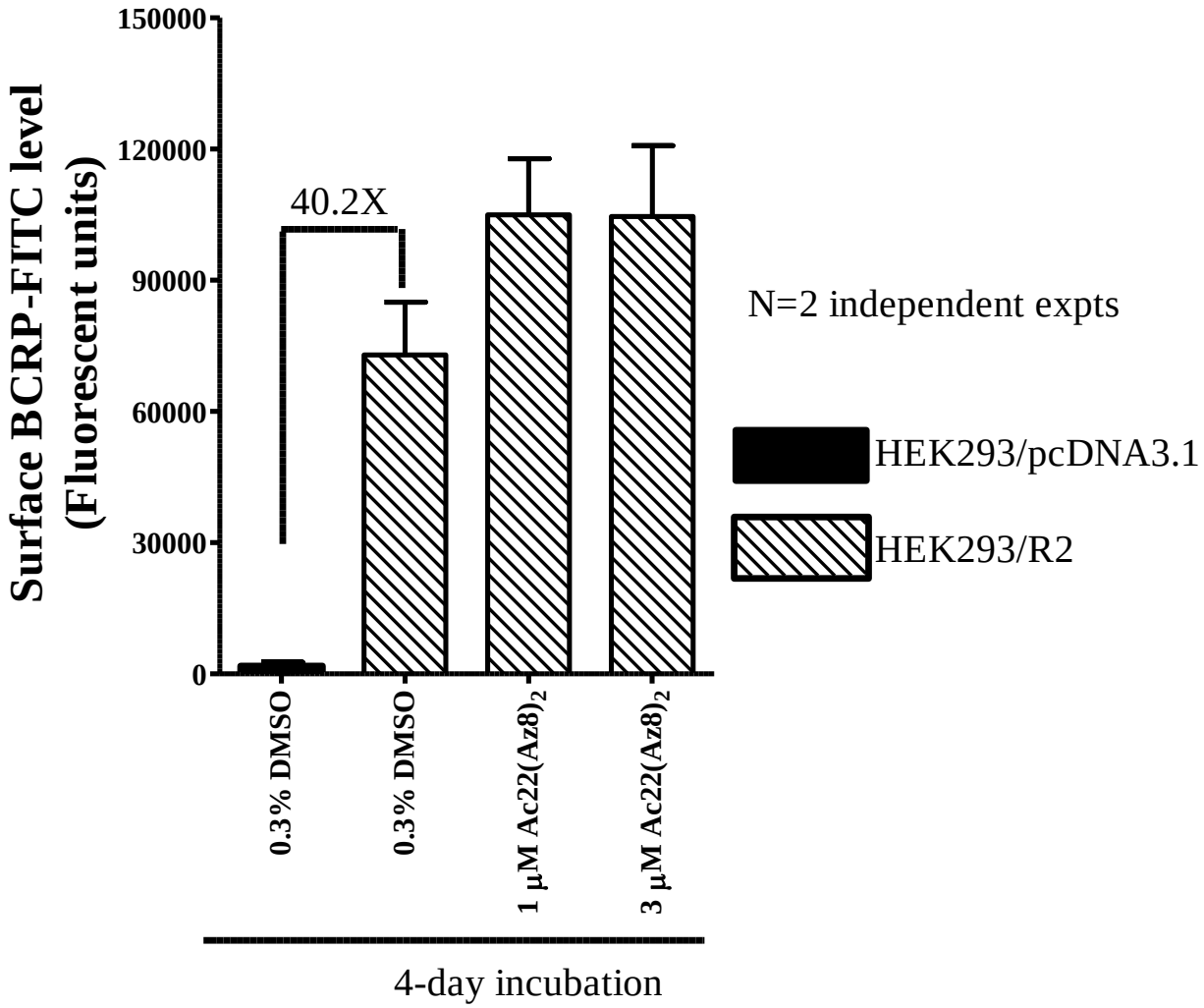


Figure 5. Effect of **Ac22(Az8)$_2$** on surface BCRP protein expression. The HEK293/R2 cells were incubated with 1 or 3 µM of **Ac22(Az8)$_2$** for 4 days, respectively. The surface BCRP protein level was measured by flow cytometer at FL1 channel. (A) The flow cytometric histogram of BCRP protein expression in HEK293/pcDNA3.1 and HEK293/R2 cells after incubating with 0.3% of DMSO. (B) The surface BCRP protein level of HEK293/R2 cells was detected after incubating with 1 or 3 µM of **Ac22(Az8)$_2$** for 4 days. N = 2 independent experiments. The fluorescence units were presented as mean ± standard deviation. 0.3% of DMSO was used as a solvent control.

2.2.9. Ac22(Az8)$_2$ inhibits topotecan efflux in HEK293/R2 cells

We then performed experiment to determine whether the increased topotecan retention in HEK293/R2 cells caused by **Ac22(Az8)$_2$** was due to inhibition of topotecan efflux (**Figure 6**). In the efflux experiments, the topotecan pre-loaded cells were incubated with or without **Ac22(Az8)$_2$**. After 0, 5, 10 and 15 min, the amount of topotecan remained inside the cells was measured by flow cytometry. In the absence of **Ac22(Az8)$_2$**, the intracellular topotecan level of wild type HEK293/pcDNA3.1 cells was gradually diminished from 100% at 0 min to 67% at 15 min (**Figure 6**). In contrast, the topotecan level of HEK293/R2 cells was reduced from 100% at 0 min to 44% at 15 min (**Figure 6**), indicating that the efflux rate of HEK293/R2 cells was higher than the wild type. This difference in efflux rate may explain why HEK293/R2 cells had less accumulation and were resistant to topotecan as compared to the wild type. In the presence of 5 μM of **Ac22(Az8)$_2$**, topotecan efflux rate kept unchanged in wild type, whereas in HEK293/R2 cells, the topotecan efflux rate was changed. After 10 min and 15 min, the intracellular topotecan levels still retained 73% ($P<0.05$) and 58% ($P<0.01$) in HEK293/R2 cells, respectively (**Figure 6**). The above results demonstrate that reversal of topotecan resistance by **Ac22(Az8)$_2$** is due to an inhibition of BCRP-mediated drug efflux, leading to an increased drug accumulation and thus restoring the drug sensitivity.

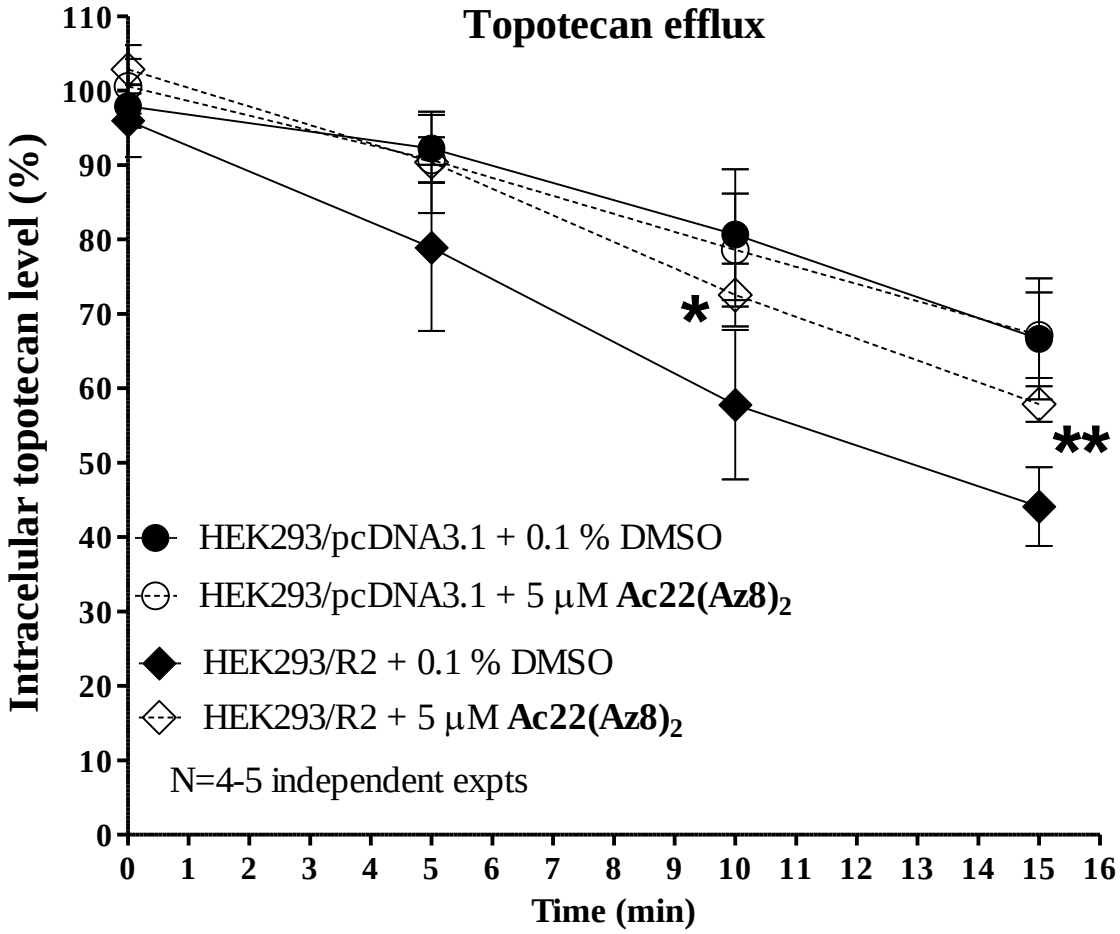


Figure 6. Effect of **Ac22(Az8)$_2$** on topotecan efflux in HEK293/pcDNA3.1 and HEK293/R2 cells**.** Topotecan pre-loaded cells were incubated with or without compound **Ac22(Az8)$_2$** (5 μM) at 37^{o}C. At 0, 5, 10, and 15 min, cells were harvested and intracellular topotecan concentration was measured by flow cytometer at FL-1 channel. The values were presented as mean ± standard deviation. Student paired t test was conducted at each time point in HEK293/R2 cells after incubating with or without **Ac22(Az8)$_2$**. *P<0.05 and ** P<0.01.

2.2.10 Ac22(Az8)$_2$ inhibits BCRP-ATPase activity

In order to study whether **Ac22(Az8)$_2$** can inhibit ATPase activity of BCRP and then block transporter efflux, membrane microsome from BCRP-overexpressed S1M180 was purified and the effect of **Ac22(Az8)$_2$** on vanadate-sensitive BCRP-ATPase activity was investigated. It was found that both Ko143 and **Ac22(Az8)$_2$** inhibited vanadate-sensitive BCRP-ATPase activity in a dose-dependent manner (**Figure 7**). The higher concentration of **Ac22(Az8)$_2$**, the lower BCRP-ATPase activity was observed. These results suggested that **Ac22(Az8)$_2$** depletes energy supply for BCRP-mediated drug efflux via BCRP-ATPase inhibition.

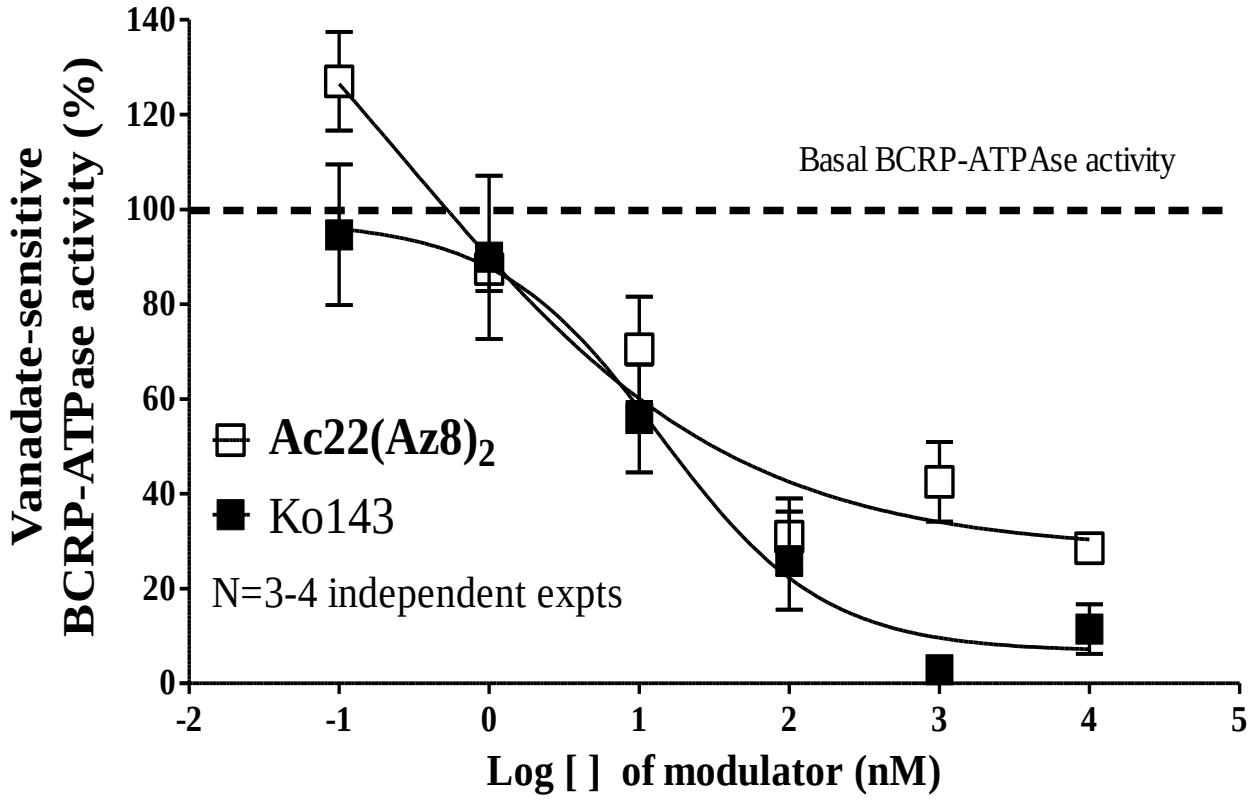


Figure 7. Effect of **Ac22(Az8)$_2$** on vanadate-sensitive BCRP-ATPase activity. Membrane fraction of S1M180 was collected after sonication and ultracentrifugation. Ouabain (Na+/K+-ATPase

inhibitor) and sodium azide were added to microsome fraction to inhibit non-ABC transporter ATPase activities. Ko143 and **Ac22(Az8)$_2$** were pre-incubated at 37˚C for 30 minutes, followed by 1-hour incubation of 2.5 mM ATP. After stopping reaction, phosphate level was determined using colorimetry method. These data were shown as mean ± standard error of mean. N=3-4 independent experiments.

2.2.11. *In Silico* docking studies

Several high-resolution cryo-electron microscopy (cryo-EM) structures of human BCRP in complex with its substrate estrone-3-sulfate (PDB ID: 6HCO)[58] and with potent inhibitors MZ29 (PDB ID: 6ETI) and MB136 (PDB ID: 6FEQ)[46] have been reported recently, allowing a better understanding of how drug binding occurs in BCRP. It was observed that the drug-binding site in the central translocation pathway of inward-facing BCRP was optimally suited to bind flat, hydrophobic and polyphenolic molecules of either BCRP substrates or inhibitors. It has been proposed that binding of an inhibitor blocks access for substrate and prevents conformational changes necessary for ATP hydrolysis, thus accounts for the inhibitory activity.[58] In this study, flavonoid dimer **Ac22(Az8)$_2$** is the most promising BCRP inhibitor in term of its high potency, low cytotoxicity and high specificity. We were therefore interested to interrogate its interaction with BCRP with *in silico* molecular docking studies to see if these BCRP models may account for its inhibitory activity. BCRP substrates topotecan and mitoxantrone were similarly examined. In agreement with the cryo-EM observations,[46,58] the binding poses with the top-ranked scores positioned the inhibitor **Ac22(Az8)$_2$** and the two substrates into the same drug-binding site, which was located at the central translocation pathway of BCRP and involved mainly six transmembrane helices 1b, 1b', 2, 2', 5a and 5a' (**Figure 8A**). Top-ranked binding poses of **Ac22(Az8)$_2$**,

mitoxantrone and topotecan using the BCRP model (6ETI) are shown in **Figure 8B-8D** respectively. For others BCRP models (6HCO and 6FEQ), top-ranked binding poses are described in **Figure S68-S69** of the Supporting Information. As described in **Table 6**, the top-ranked scores of flavonoid dimer **Ac22(Az8)$_2$** with all three BCRP models were generally much lower than that of mitoxantrone and topotecan, suggesting that **Ac22(Az8)$_2$** may bind preferentially to BCRP and block the access for the substrates topotecan or mitoxantrone.

Detailed analysis of binding poses of **Ac22(Az8)$_2$** with BCRP model (6ETI) revealed that strong π-π interactions were formed between the linker part 1,3-*bis*-(triazol-4-yl)benzene of **Ac22(Az8)$_2$** and Phe439 of helix 2' as well as between one of the benzyl groups of **Ac22(Az8)$_2$** and Phe547 of helix 5a (**Figure 8B**). Although there was no hydrogen bonding interaction predicted to occur between **Ac22(Az8)$_2$** and BCRP, there were, however, extensive networks of van der Waals contacts with many amino acid residues inside the drug-binding site of BCRP, thus providing strong interaction with BCRP. Particularly, this interaction involved amino acid residues of helix 5a (Phe551, Met549, Val546, Ile543, Thr542, Thr538, Val534), helix 2 (Phe432, Ser440, Phe439, Thr435, Ser443, Val442, Glu446, Val445), helix 1b (Val401, Gln398), helix 2' (Phe432, Asn436, Thr435, Ser440), helix 1b' (Ile412, Val408, Val404, Leu405, Val401, Ile400) and helix 5a' (Met549, Val546, Ile543, Thr542, Thr538, Leu539). It has been proposed by Locher's group that a "leucine plug" (Leu554 and Leu555), which was located in a flexible loop between helix 5a and 5b, acts as a gate to allow BCRP substrate moving outside (**Figure 8A**).[58] Interestingly, one of the flavonoid moieties of **Ac22(Az8)$_2$** is predicted to insert into a cavity between the helix 5a and 5b and form van der Waals contacts with amino acid residues of helix 5a and 5b (Leu568, Leu565). We, therefore, believed that by occupying the cavity, this flavonoid moiety might reduce the flexibility of this loop and allow only very limited movement of the "leucine plug". This may

obstruct the gate-opening function of the "leucine plug" and account for its inhibitory activity. More importantly, structural comparison between transporters P-gp, MRP1 and BCRP revealed that such cavity exists only in BCRP but not in P-gp or MRP1.[59-61] We, therefore, reasoned that the high BCRP specificity of **Ac22(Az8)$_2$** over P-gp and MRP1 may be attributed to this structural difference. Careful evaluation of binding poses of BCRP substrates topotecan and mitoxantrone revealed that both ligands occupied exactly the same drug-binding area as the linker part 1,3-*bis*-(triazol-4-yl)benzene of **Ac22(Az8)$_2$** (**Figure 8C-D**). As both ligands are flat and polyaromatic molecules, strong π-π interactions were predicted to form between the ligands and Phe439 of helix 2' and helix 2. As shown in **Figure 8C**, multiple hydrogen bonding interactions were also observed between the mitoxantrone (amino-alcohol side chains and phenol group) and amino acid residues (Thr435 of helix 2 and 2', Phe439 of helix 2') of BCRP. On the contrary, only one hydrogen bonding interaction was observed between the phenyl group of topotecan and Thr435 of helix 2 of BCRP (**Figure 8D**).

Table 6. The top-ranked scores of ligands using different cryo-EM structures of BCRP.

PDB ID	Ligands docked		
	Ac22(Az8)$_2$	Topotecan	Mitoxantrone
6HCO	-126	-80	-82
6ETI	-137	-88	-92
6FEQ	-122	-81	-91

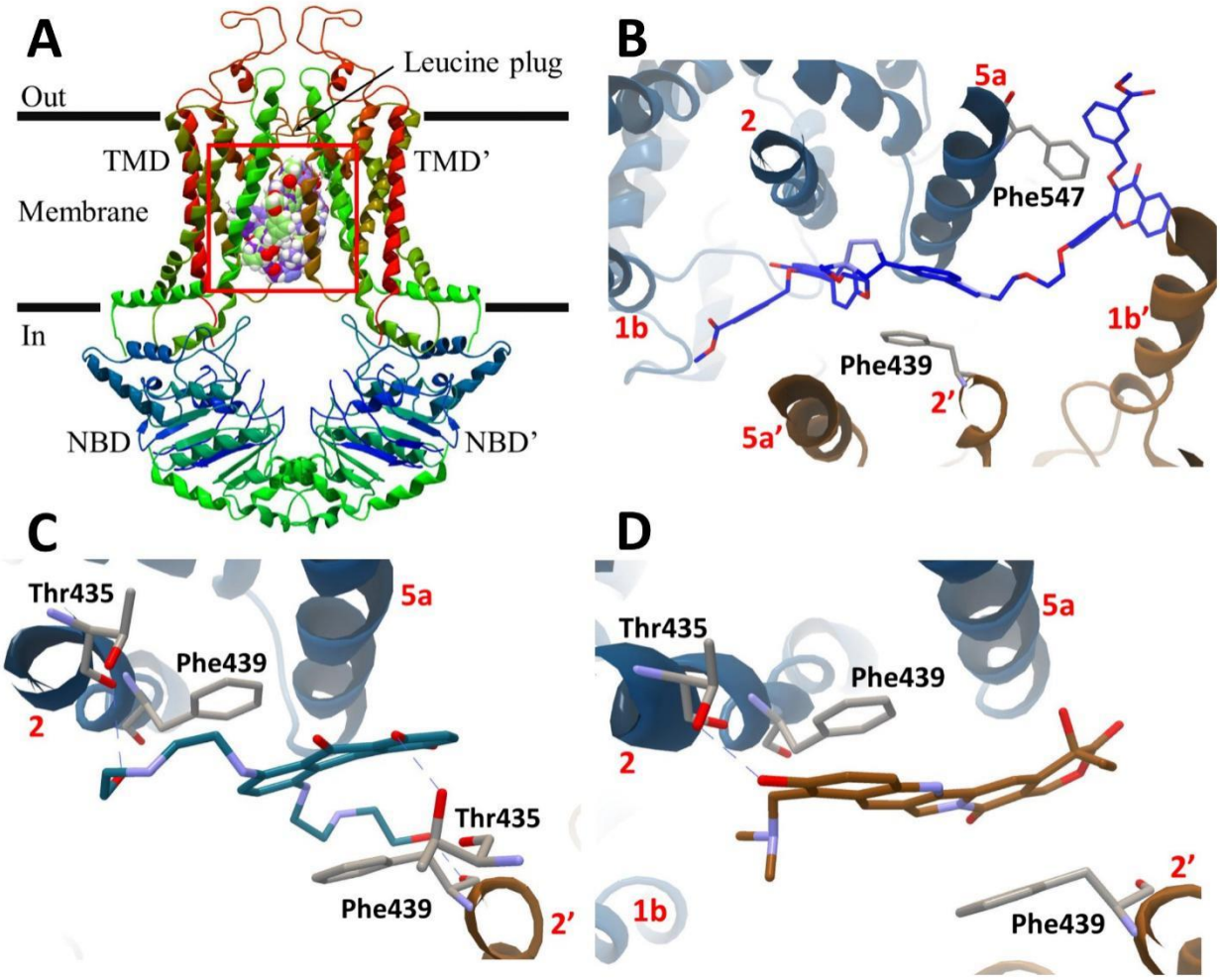


Figure 8. *In silico* docking study using cryo-EM structure of BCRP (PDB ID: 6ETI). (A) Ribbon diagram of the overall human BCRP homodimer with labeled TMD, NBD and "leucine plug". The red square indicates the location of binding pocket of estrone-3-sulfate, MZ29, MB136, mitoxantrone, topotecan and **Ac22(Az8)**$_2$; Top-ranked binding poses of (B) inhibitor **Ac22(Az8)**$_2$ (blue sticks), (C) substrate mitoxantrone (deep green sticks) and (D) substrate topotecan (brown sticks). Transmembrane helices 1b, 1b', 2, 2', 5a and 5a' were labeled in red. Important amino acid residues (grey sticks) are highlighted in black and hydrogen bonding interactions are indicated as dotted blue lines.

3. DISCUSSIONS AND CONCLUSION

Several clinical trials using P-gp inhibitors together with anticancer drug had been evaluated to overcome MDR but led to disappointing outcomes,[34] possibly due to the effect of the inhibitor on the pharmacokinetics of the anticancer drug.[7] Therefore, there is still need to develop MDR inhibitors for clinical use to reverse MDR in cancer patient. Further improvement of inhibitors of ABC transporters should not only focus on potency but also improve specificity to minimize such potential drug-drug interaction.[7]

In this study, we have successfully used the CuAAC reaction to rapidly synthesize various flavonoid dimers. In a 74-member homo- and hetero-flavonoid dimer library, we found that many of these dimers generally displayed no cytotoxicity and strong inhibition against BCRP (**Table 1** and **2**). Fifteen of them displayed higher BCRP inhibitory potency than Ko143, the most potent BCRP-specific inhibitor thus far reported,[37-39] with EC_{50} values less than 10 nM for reversing topotecan resistance (**Tables 1** and **2**).

Some information was drawn regarding the SAR for BCRP-inhibition and selectivity. Structural features which can enhance BCRP-inhibition activity are: (1) two flavonoid moieties, (2) a benzyloxy substitution at the C-3 of the C-ring of flavone (**Az5**-, **Az8**-, **Az9**- and **Az10**-dimers), (3) 7-fluoro substitution on A-ring of flavone (**Ac3**-clicked dimers). To make highly selective BCRP inhibitor, we demonstrated that extending the linker length using *bis*-triazole (**Ac15**-clicked dimers) between two flavones is a simple and promising approach. We previously showed that potent P-gp and MRP1 inhibitors required an optimal linker length between the two flavones, with about 13-15 atoms and 13-17 atoms, respectively. In contrast, the linker length needed for active BCRP inhibitor appeared to be more flexible, with about 13-27 atoms. Thus, **Ac15(Az8)$_2$** (linker length with 21 atoms) and **Ac15(Az9)$_2$** (linker length with 27 atoms) with *bis*-

triazole in linker resulted in very low inhibitory activity towards P-gp and MRP1, therefore high BCRP selectivity (**Table 1** and **2**). Further modification of the *bis*-triazole structure led to several compounds with high potency, low cytotoxicity and high specificity. In particular, **Ac22(Az8)$_2$** was found to have low toxicity towards L929, 3T3 and HFF-1 cells (IC_{50} >100 μM), high potency in BCRP inhibitory activity (EC_{50} = 1-2 nM) and high BCRP selectivity (BCRP selectivity over MRP1 and P-gp > 455 – 909) (**Table 3**). It had about 5- to 8-fold and 2- to 8-fold higher BCRP-modulating activity and selectivity than Ko143, respectively (**Table 3**). It was found to inhibit the BCRP-ATPase activity **(Figure 7)**, block the efflux activity of BCRP (**Figure 6**), thus elevate the intracellular drug accumulation (**Figure 4**) and finally restore the drug sensitivity of the BCRP-overexpressing cells (**Table 1** and **Table 3**). It did not down-regulate the surface BCRP protein expression to enhance the drug retention (**Figure 5**). Interestingly, *in silico* docking study shows that it binds to the same drug-binding site, consistent with the cryo-EM structures revealed for BCRP in complex with substrate/inhibitor.

Therefore, these flavonoid dimers appear to be promising candidates for further investigation including pharmacokinetics and *in vivo* efficacy study. We demonstrated previously that **FD-18**, a PEG-linked flavonoid dimer, inhibited P-gp modulation in breast cancer xenograft model.[53] Here, these triazole-bridged flavonoid dimers may possess higher aqueous solubility and potency than **FD-18** and might have better pharmacokinetics and *in vivo* efficacy. All in all, they have the potential to be developed into combination therapy to overcome MDR cancers with BCRP overexpression.[62]

4. EXPERIMENTAL SECTION

4.1. Materials and Methods

4.1.1. General. All NMR spectra were recorded on a Bruker Advance-III 400 MHz spectrometer at 400 MHz for ^{1}H and 101 MHz for ^{13}C, Varian Unity Inova 500 MHz NMR Spectrometer at 500 MHz for ^{1}H and 101 MHz for ^{13}C or Bruker Advance-III 600 MHz spectrometer at 600 MHz for ^{1}H and 151 MHz for ^{13}C. All NMR measurements were carried out at room temperature and the chemical shifts are reported as parts per million (ppm) in unit 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, respectively). Low-resolution and high-resolution mass spectra were obtained on a Micromass Q-TOF-2 by electron spray ionization (ESI) mode or on Finnigan MAT95 ST by electron ionization (EI) mode. 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). The purity of tested compounds was determined by HPLC, which was performed by using Agilent 1100 series installed with an analytic column of Agilent Prep-Sil Scalar column (4.6 mm x 250 mm, 5-µm) at UV detection of 254 nm (reference at 450 nm) with gradient elution of dichloromethane(10%)/methanol (90%) to dichloromethane(1%)/methanol (99%) at a flow rate of 1.0 mL/min. All tested compounds were shown to have >95% purity according to HPLC.

4.2. Procedure for the synthesis of Ac1, **Ac2**, **Ac3**, **Ac5**, **Ac12**, **Ac13**, **Ac15, Ac16, Ac22, Ac23, Ac24, Ac25, Ac26, Ac28, Ac29, Ac30** and **Ac31**

4.2.1. Synthesis of 2-(4-(Pent-4-yn-1-yloxy)phenyl)-4*H*-chromen-4-one (**Ac1**): This compound was obtained according to the procedure as described.[54]

4.2.2. Synthesis of 7-(Pent-4-yn-1-yloxy)-2-phenyl-4*H*-chromen-4-one (**Ac2**): This compound was obtained according to the procedure as described.[54]

4.2.3. Synthesis of 7-Fluoro-2-(4-(pent-4-yn-1-yloxy)phenyl)-4*H*-chromen-4-one (**Ac3**)**:** This compound was obtained according to the procedure as described.[54]

4.2.4. Synthesis of 7-(Hex-5-yn-1-yloxy)-2-phenyl-4*H*-chromen-4-one (**Ac12**)**:** This compound was obtained according to the procedure as described.[54]

4.2.5. Synthesis of 2-Phenyl-7-(2-(prop-2-yn-1-yloxy)ethoxy)-4*H*-chromen-4-one (**Ac13**)**:** This compound was obtained according to the procedure as described.[54]

4.2.6. *N*-Benzyl-*N*,*N*-di(prop-2-yn-1-yl)amine (Ac15): This compound was commercially available.

4.2.7. Synthesis of 7-(2-(Benzyl(prop-2-yn-1-yl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac16**)**:** This compound was obtained according to the procedure as described.[54]

4.2.8. -*m*-Diethynylbenzene (Ac22): This compound was commercially available.

4.2.9. 1,4-Diethynylbenzene (Ac23): This compound was commercially available.

4.2.10. Hepta-1,6-diyne (Ac24): This compound was commercially available.

4.2.11. 3-(Prop-2-yn-1-yloxy)prop-1-yne (Ac25): This compound was commercially available.

4.2.12. Synthesis of *N*,*N*-di(Prop-2-yn-1-yl)aniline (Ac26): This compound was obtained according to the procedure as described.[56]

4.2.13. Synthesis of *N*-(prop-2-yn-1-yl)-*N*-(pyridin-4-ylmethyl)prop-2-yn-1-amine (Ac28): To a solution of **3a** (1.00 g, 9.26 mmol) in acetone (25 ml) at room temperature, was added excess propargyl bromide (2.20 g, 18.52 mmol) solution. The reaction mixture was then stirred at room temperature for 12 h. The solution was rotary evaporated followed by column chromatography on silica gel using hexane and EA in 3:1 to afford the desired compound (0.652g, 3.37mmol, 38.3%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (t, *J* = 2.20 Hz, 1 H), 3.43 (d, *J* = 2.45 Hz, 2 H), 3.72 (s, 1 H), 7.32 (d, *J* = 5.38 Hz, 1 H), 8.57 (d, *J* = 5.62 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 42.1, 55.8, 73.5, 78.3, 123.8, 147.0, 149.9; LRMS (ESI) m/z 185 $[M+H]^+$; HRMS (ESI) calcd for $C_{12}H_{13}N_2$ $[M+H]^+$ 185.2450, found 185.2469.

4.2.14. Synthesis of 1,4-Di(prop-2-yn-1-yl)piperazine (Ac29): This compound was obtained according to the procedure as described.[54]

4.2.15. Synthesis of 1,4-di(Prop-2-yn-1-yl)-1,4-diazepane (Ac30): To a solution of **3b** (1.00g, 10.00mmol) in acetone (25ml) at room temperature, was added excess propargyl bromide (3.57g, 30.00mmol) solution. The reaction mixture was then stirred at room temperature for 12 h. The solution was rotary evaporated followed by column chromatography on silica gel using hexane and EA in 100:1 to afford the desired compound (0.972 g, 5.52 mmol, 55.2%); ^{1}H NMR (400 MHz, CHLOROFORM-d) d 1.87 (quin, *J* = 5.99 Hz, 2 H), 2.21 (t, *J* = 2.32 Hz, 2 H), 2.75 - 2.85 (m, 8 H), 3.40 (d, *J* = 2.45 Hz, 4 H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 27.7, 47.6, 53.5, 54.4, 72.3, 79.8; LRMS (ESI) m/z 177 $[M+H]^+$; HRMS (ESI) calcd for $C_{11}H_{17}N_2$ $[M+H]^+$ 177.2661, found 177.2678.

4.2.16. Synthesis of N^1,N^2-Dimethyl-N^1,N^2-di(prop-2-yn-1-yl)ethane-1,2-diamine (Ac31): This compound was obtained according to the procedure as described.[54]

4.3. Procedure for the synthesis of Az1, Az2, Az3, Az4, Az5, Az7, Az10, Az11, Az12, Az13, Az14, Az16 and **Az17**

4.3.1. Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Az1**)**:** This compound was obtained according to the procedure as described.[54]

4.3.2. Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Az2**)**:** This compound was obtained according to the procedure as described.[54]

4.3.3. Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-6-methyl-4*H*-chromen-4-one (**Az3**)**:** This compound was obtained according to the procedure as described. [54]

4.3.4. Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-6-fluoro-4*H*-chromen-4-one (**Az4**)**:** This compound was obtained according to the procedure as described. [54]

4.3.5. Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-3-(benzyloxy)-4*H*-chromen-4-one (**Az5**): This compound was obtained according to the procedure as described. [54]

4.3.6. Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-6-fluoro-4*H*-chromen-4-one (**Az7**): This compound was obtained according to the procedure as described.[54]

4.3.7. Synthesis of Methyl 3-(((2-(4-(2-(2-azidoethoxy)ethoxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)methyl)benzoate (**Az8**): The starting material **1a** was prepared according the procedure as described.[54] A round-bottom flask was charged with **1a** (5 mmol, 2.2 g), a catalytic amount of $Pd(OH)_2$ and THF/MeOH (1:1 - 10 ml). The reaction mixture was stirred vigorously under H_2

atmosphere at balloon pressure and room temperature for 14 h. When TLC indicated complete consumption of the starting material, the charcoal was removed by suction filtration. The pale-yellow filtrate was purified by passing through a short pad of silica gel to furnish debenzylated product 3-hydroxy-2-(4-(2-(2-hydroxyethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (76%, 1.3 g). To a round-bottom flask was charged with the debenzylated product, methyl 3-(bromomethyl)benzoate (4 mmol, 0.92 g), K_2CO_3 (4 mmol, 0.55 g) and acetone (10 ml). The reaction mixture was stirred at refluxing temperature for 12 h. When TLC indicated complete consumption of starting material, Solvent was rotary evaporated to dryness. Purification was performed by flash column chromatography on silica gel with acetone in DCM as eluent to furnish **1c** (86%, 1.6 g). The hydroxylated flavone **1c** was then dissolved in a solution of DCM (10 mL) and triethylamine (2 mL) at 0 ^oC. Methanesulfonyl chloride (5 mmol, 0.56 g) was then added dropwise and stirred for 1 hr at room temperature. When TLC indicated complete consumption of the starting material, the white precipitate formed was removed by passing through a short pad of silica gel to furnish the mesylated product which was sufficiently pure for the next step. To a solution of the mesylate in ACN (15 mL) was added excess of sodium azide (15 mmol, 0.98 g). The solution was kept for reflux at 80 °C for 15 h. The resulting solution was treated with water and then extracted with DCM. The combined organic layer was dried over $MgSO_4$ and concentrated at reduced pressure to give pale yellow viscous liquid. Purification was performed by flash column chromatography on silica gel with acetone in DCM as eluent to furnish desired product **Az8** (0.29 g, 57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.44 (t, *J* = 4.88 Hz, 2 H), 3.76 - 3.79 (m, 2 H), 3.89 (s, 3 H), 3.90 - 3.94 (m, 2 H), 4.20 - 4.25 (m, 2 H), 5.15 (s, 2 H), 6.99 (d, *J* = 8.79 Hz, 2 H), 7.35 (t, *J*=7.81 Hz, 1 H), 7.41 (t, *J* = 7.57 Hz, 1 H), 7.52 (d, *J* = 8.30 Hz, 1 H), 7.59 (d, *J*=7.32 Hz, 1 H), 7.65 - 7.71 (m, 1 H), 7.93 (d, *J*=7.81 Hz, 1 H), 7.96 - 8.01 (m, 3 H), 8.29 (dd,

J=7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.73, 52.03, 67.52, 69.64, 70.31, 73.36, 114.41, 117.93, 123.46, 124.19, 124.65, 125.77, 128.29, 129.27, 129.81, 130.13, 130.54, 133.24, 133.30, 137.18, 139.07, 155.22, 156.51, 160.62, 166.82, 174.87; LRMS (ESI) m/z 516 $[M+H]^+$, 538 $[M+Na]^+$; HRMS (ESI) calcd for $C_{28}H_{26}N_3O_7$ $[M+H]^+$ 516.1771, found 516.1783; calcd for $C_{28}H_{25}N_3O_7Na$ $[M+Na]^+$ 538.1590, found 538.1583.

4.3.8. Synthesis of Methyl 3-(((2-(4-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)methyl)benzoate (**Az9**): The starting material **1b** was prepared according the procedure as described.[54] The titled compound **Az9** (0.62g, 37%) was obtained from **1b** (3mmol, 1.5g) according to the procedure for the synthesis of **Az8** described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.40 (t, *J*=4.39 Hz, 2 H), 3.67 - 3.74 (m, 5 H), 3.74 - 3.79 (m, 2 H), 3.89 (s, 3 H), 3.92 (t, *J*=4.15 Hz, 2 H), 4.22 (t, *J*=4.15 Hz, 2 H), 5.15 (s, 2 H), 6.98 (d, *J*=8.79Hz, 2 H), 7.35 (t, *J*=7.81 Hz, 1 H), 7.42 (t, *J*=7.57 Hz, 1 H), 7.52 (d, *J*=8.79 Hz, 1 H), 7.59 (d, *J*=6.34 Hz, 1 H), 7.65 - 7.70 (m, 1 H), 7.93 (d, *J*=7.50 Hz, 1 H), 7.96 - 8.01 (m, 3 H), 8.29 (d, *J*=8.30 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.71, 52.04, 67.55, 69.71, 70.12, 70.78, 70.94, 73.35, 114.41, 117.92, 123.36, 124.20, 124.64, 125.78, 128.29, 129.28 , 129.80, 130.14, 130.52, 133.23, 133.30, 137.19, 139.07, 155.23, 156.53, 160.73 166.82, 174.87; LRMS (ESI) m/z 560 $[M+H]^+$, 582 $[M+Na]^+$; HRMS (ESI) calcd for $C_{30}H_{30}N_3O_8$ $[M+H]^+$ 560.2033, found 560.2028; calcd for $C_{30}H_{29}N_3O_8Na$ $[M+Na]^+$ 582.1852, found 582.1831.

4.3.9. Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-3-(benzyloxy)-4*H*-chromen-4-one (**Az10**)**:** This compound was obtained according to the procedure as described. [54]

4.3.10. Synthesis of 7-(2-(2-Azidoethoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Az11**)**:** This compound was obtained according to the procedure as described.[54]

4.3.11. Synthesis of 7-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Az12**)**:** This compound was obtained according to the procedure as described.[54]

4.3.12. Synthesis of 7-(2-Azidoethoxy)-2-phenyl-4*H*-chromen-4-one (**Az13**)**:** This compound was obtained according to the procedure as described.[54]

4.3.13. Synthesis of 2-(4-(2-((2-Azidoethyl)(benzyl)amino)ethoxy)phenyl)-4*H*-chromen-4-one (**Az14**): The starting material **2b** was prepared according the procedure as described.[50] To a well stirred solution of **2b** (3mmol, 0.71g), *N*-benzyl-*N*,*N*-di(2-hydroxyethyl)amine (3mmol, 0.6g) and PPh_3 (3mmol, 0.79g) in THF (20ml) at room temperature was added DIAD (3mmol, 0.59ml) dropwise. The reaction mixture was then stirred for 12 h at room temperature. When TLC indicated complete consumption of starting material, the reaction mixture was evaporated to give a brown crude reaction mixture. Purification was performed by flash column chromatography on silica gel with acetone in DCM (1:10) as eluent to furnish intermediate compound 2-(4-(2-(benzyl(2-hydroxyethyl)amino)ethoxy)phenyl)-4*H*-chromen-4-one (0.13g, 10.4%). The hydroxylated flavone was then dissolved in a solution of DCM (2 mL) and triethylamine (0.5 mL) at 0 ^oC. Methanesulfonyl chloride (0.5 mmol, 0.06 g) was then added dropwise and stirred for 1 hr at room temperature. When TLC indicated complete consumption of the starting material, the white precipitate formed was removed by passing through a short pad of silica gel to furnish the mesylated product which was sufficiently pure for the next step. To a solution of the mesylate in ACN (2 mL) was added excess of sodium azide (5 mmol, 0.32 g). The solution was kept for reflux at 80 °C for 15 h. The resulting solution was treated with water and then extracted with DCM. The combined organic layer was dried over $MgSO_4$ and concentrated at reduced pressure to give pale yellow viscous liquid. Purification was performed by flash column chromatography on silica gel

with acetone in DCM as eluent to furnish desired product **Az14** (10mg, 17%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.91 (br. s., 2 H), 3.03 (br. s., 2 H), 3.32 (br. s., 2 H), 3.80 (br. s., 2 H), 4.15 (br. s., 2 H), 6.77 (s, 1 H), 6.92 - 6.96 (m, 2 H), 7.27 - 7.37 (m, 5 H), 7.49 - 7.56 (m, 3 H), 7.89 - 7.93 (m, 2 H), 8.13 (d, *J*=8.30 Hz, 1 H); LRMS (ESI) m/z 441 $[M+H]^+$; HRMS (ESI) calcd for $C_{26}H_{25}N_4O_3$ $[M+H]^+$ 441.1927, found 441.1909.

4.3.14. 7-(2-((2-Azidoethyl)(benzyl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Az15**): The starting material **2c** was commercially available. The titled compound **Az15** (12mg, 22%) was obtained from **2c** according to the procedure for the synthesis of **Az14** described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.90 (br. s., 2 H), 3.00 (br. s., 2 H), 3.32 (br. s., 2 H), 3.79 (s, 2 H), 4.11 (br. s., 2 H), 6.75 (s, 1 H), 6.98 (m, *J*=8.79 Hz, 2 H), 7.26 - 7.44 (m, 6 H), 7.54 - 7.58 (m, 1 H), 7.66 - 7.71 (m, 1 H), 7.84 - 7.91 (m, 2 H), 8.23 (dd, *J*=7.81, 1.46 Hz, 1 H); LRMS (ESI) m/z 441 $[M+H]^+$; HRMS (ESI) calcd for $C_{26}H_{25}N_4O_3$ $[M+H]^+$ 441.1927, found 441.1908.

4.3.15. Synthesis of 2-(2-azidoethoxy)ethanol (Az16): This compound was obtained according to the procedure as described.[63]

4.3.16. Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-3-((3-methoxybenzyl)oxy)-4H-chromen-4-one(Az17): This compound was obtained according to the procedure as described.[54]

Synthesis of *anti*-triazole bridged flavonoid dimers.

4.4. General procedure for the synthesis of *anti*-triazole bridged flavonoid dimers catalyzed by Cu(I)

The $Cu(PPh_3)_3Br$ catalyst (MW=929) (0.05 mmol), prepared according to literature,[64] was added to a THF solution (2 mL) containing the azide (**Az,** 0.1 mmol for Ac with one acetylene or 0.2

mmol for Ac with two acetylenes) and the alkyne (**Ac**, 0.1 mmol). The reaction mixture was stirred overnight under reflux condition. The crude residue was purified by flash chromatography on silica gel using gradient of 10-50% of acetone with CH_2Cl_2 to afford the desired compound.

4.4.1. Synthesis of 2-(4-(3-(1-(2-(2-(4-(4-Oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)propoxy)phenyl)-4*H*-chromen-4-one (**Ac1Az1**): This compound was obtained according to the procedure as described.[54]

4.4.2. Synthesis of 7-(3-(1-(2-(2-(4-(4-Oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)propoxy)-2-phenyl-4*H*-chromen-4-one (**Ac2Az1**): This compound was obtained according to the procedure as described.[54]

4.4.3. Synthesis of 7-(3-(1-(2-(2-(2-(4-(4-oxo-4H-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-2-phenyl-4H-chromen-4-one (Ac2Az2): This compound (50 mg) was obtained from **Ac2** and **Az2** in 72% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.17 – 2.26 (m, 2 H), 2.90 (t, *J* = 7.5 Hz, 2 H), 3.59 – 3.70 (m, 4 H), 3.78 – 3.90 (m, 4 H), 4.07 (t, *J* = 6.3 Hz, 2 H), 4.12 – 4.19 (m, 2 H), 4.52 (t, *J* = 5.0 Hz, 2 H), 6.70 (s, 1 H), 6.72 (s, 1 H), 6.87 – 7.03 (m, 4 H), 7.32 – 7.41 (m, 1 H), 7.42 – 7.56 (m, 5 H), 7.60 – 7.69 (m, 1 H), 7.78 – 7.90 (m, 4 H), 8.08 (d, *J* = 8.8 Hz, 1 H), 8.17 (dd, *J* = 7.9, 1.7 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 22.14, 28.77, 50.28, 67.70, 67.71, 69.64, 69.73, 70.64, 70.85, 100.98, 106.30, 107.56, 114.88, 115.08, 117.85, 118.05, 122.35, 123.97, 124.37, 125.23, 125.74, 126.24, 127.09, 128.10, 129.12, 131.56, 131.86, 133.73, 146.82, 156.22, 158.06, 161.59, 163.09, 163.29, 163.63, 177.96, 178.46. LRMS (ESI) m/z 700 $[M+H]^+$, 722 $[M+Na]^+$; HRMS (ESI) calcd for $C_{41}H_{38}N_3O_8$ $[M+H]^+$ 700.2659, found 700.2637; calcd for $C_{41}H_{37}N_3O_8Na$ $[M+Na]^+$ 722.2478, found 722.2461.

4.4.4. Synthesis of 6-methyl-2-(4-(2-(2-(2-(4-(3-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4H-chromen-4-one (Ac2Az3): This compound (63 mg) was obtained from **Ac2** and **Az3** in 88% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.14 – 2.24 (m, 2 H), 2.38 (s, 3 H), 2.87 (t, *J* = 7.5 Hz, 2 H), 3.57 – 3.68 (m, 4 H), 3.79 (t, *J* = 4.6 Hz, 2 H), 3.84 (t, *J* = 5.0 Hz, 2 H), 4.03 (t, *J* = 6.2 Hz, 2 H), 4.12 (t, *J* = 4.6 Hz, 2 H), 4.50 (t, *J* = 5.0 Hz, 2 H), 6.63 (s, 1 H), 6.67 (s, 1 H), 6.85 (d, *J* = 2.3 Hz, 1 H), 6.89 (dd, *J* = 8.8, 2.4 Hz, 1 H), 6.92 – 6.98 (m, 2 H), 7.33 (d, *J* = 8.5 Hz, 1 H), 7.40 (s, 1 H), 7.42 – 7.49 (m, 3 H), 7.53 (s, 1 H), 7.74 – 7.80 (m, 2 H), 7.80 – 7.85 (m, 2 H), 7.90 (s, 1 H), 8.04 (d, *J* = 8.8 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.05, 28.68, 50.16, 67.60, 67.64, 69.54, 69.62, 70.53, 70.74, 100.86, 105.99, 107.38, 114.76, 114.94, 117.69, 117.72, 122.23, 123.48, 124.32, 124.94, 126.10, 126.91, 127.92, 129.00, 131.44, 131.71, 134.81, 135.03, 146.71, 154.35, 157.91, 161.42, 162.91, 163.00, 163.51, 177.76, 178.38; LRMS (ESI) m/z 714 $[M+H]^+$, 736 $[M+Na]^+$; HRMS (ESI) calcd for $C_{42}H_{40}N_3O_8$ $[M+H]^+$ 714.2815, found 714.2808; calcd for $C_{42}H_{39}N_3O_8Na$ $[M+Na]^+$ 736.2635, found 736.2615.

4.4.5. Synthesis of 6-Fluoro-2-(4-(2-(2-(2-(4-(3-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4H-chromen-4-one (Ac2Az4): This compound was obtained according to the procedure as described.[54]

4.4.6. Synthesis of 3-(benzyloxy)-2-(4-(2-(2-(2-(4-(3-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4H-chromen-4-one (Ac2Az5): This compound (51 mg) was obtained from **Ac2** and **Az5** in 63% yield according to the general procedure described above. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.16 – 2.30 (m, 2H), 2.91 (t, *J* = 7.5 Hz, 2H), 3.55 – 3.73 (m, 4H), 3.79 – 3.95 (m, 4H), 4.08 (t, *J* = 6.2 Hz,

2H), 4.17 (t, *J* = 4.6 Hz, 2H), 4.53 (t, *J* = 5.0 Hz, 2H), 5.10 (s, 2H), 6.73 (s, 1H), 6.94 (m, 4H), 7.28 (dt, *J* = 7.0, 2.3 Hz, 3H), 7.37 (td, *J* = 6.3, 5.1, 3.1 Hz, 3H), 7.44 – 7.52 (m, 4H), 7.56 (s, 1H), 7.61 – 7.67 (m, 1H), 7.83 – 7.91 (m, 2H), 7.99 – 8.06 (m, 2H), 8.08 (d, *J* = 8.7 Hz, 1H), 8.24 (dd, *J* = 8.0, 1.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 22.04, 28.67, 50.17, 67.50, 67.64, 69.60, 69.63, 70.54, 70.73, 73.92, 100.88, 107.42, 114.31, 114.83, 117.71, 117.92, 122.29, 123.59, 124.12, 124.66, 125.71, 126.15, 126.94, 128.14, 128.28, 128.82, 129.03, 130.56, 131.47, 131.74, 133.34, 136.78, 139.30, 146.73, 155.12, 156.09, 157.96, 160.55, 163.01, 163.55, 174.96, 177.88. LRMS (ESI) m/z 806 $[M+H]^+$, 828 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{44}N_3O_9$ $[M+H]^+$ 806.3078, found 806.3089; calcd for $C_{48}H_{43}N_3O_9Na$ $[M+Na]^+$ 828.2897, found 828.2889.

4.4.7. Synthesis of methyl 3-(((4-oxo-2-(4-(2-(2-(4-(3-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4H-chromen-3-yl)oxy)methyl)benzoate (Ac2Az8): This compound (58 mg) was obtained from **Ac2** and **Az8** in 71% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.23 (s, 2 H), 3.00 (s, 2 H), 3.78 – 3.96 (m, 5 H), 3.95 – 4.17 (m, 6 H), 4.65 (s, 2 H), 5.10 (s, 2 H), 6.72 (s, 1 H), 6.96 – 6.83 (m, 4 H), 7.28 – 7.38 (m, 2 H), 7.39 -7.52 (m, 4 H), 7.56 (d, *J* = 7.5 Hz, 1 H), 7.59 – 7.65 (m, 1 H), 7.80 – 7.87 (m, 3 H), 7.89 (d, *J* = 7.7 Hz, 1 H), 7.92 – 8.10 (m, 4 H), 8.21 (dd, *J* = 8.0, 1.5 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 21.80, 28.49, 52.22, 67.38, 67.71, 69.49, 69.66, 73.41, 101.01, 107.23, 114.35, 114.92, 117.99, 123.54, 124.09, 124.82, 125.75, 126.27, 126.91, 128.43, 129.09, 129.36, 129.88, 130.11, 130.65, 131.59, 131.61, 133.35, 133.51, 137.17, 139.08, 155.17, 156.36, 157.95, 160.48, 163.24, 163.58, 166.89, 174.91, 177.98. LRMS (ESI) m/z 820 $[M+H]^+$, 842 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{42}N_3O_{10}$ $[M+H]^+$ 820.2870, found 820.2883; calcd for $C_{48}H_{41}N_3O_{10}Na$ $[M+Na]^+$ 842.2690, found 842.2665.

4.4.8. Synthesis of methyl 3-(((4-oxo-2-(4-(2-(2-(2-(4-(3-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4H-chromen-3-yl)oxy)methyl)benzoate (Ac2Az9): This compound (24 mg) was obtained from **Ac2** and **Az9** in 68% yield according to the general procedure described above. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 8.27 (d, *J* = 8.80 Hz, 1H), 8.11 (d, *J* = 8.80 Hz, 1H), 7.97 - 8.01 (m, 3H), 7.94 (d, *J* = 8.80 Hz, 1H), 7.90 (d, *J* = 7.34 Hz, 2H), 7.65 - 7.70 (m, 1H), 7.61 (d, *J* = 7.34 Hz, 1H), 7.46 - 7.58 (m, 5H), 7.41 (t, *J* = 7.34 Hz, 1H), 7.37 (t, *J* = 7.34 Hz, 1H), 6.92 - 7.00 (m, 4H), 6.76 (s, 1H), 5.15 (s, 2H), 4.57 (br. s., 2H), 4.19 (t, *J* = 4.40 Hz, 2H), 4.12 (br. s., 2H), 3.89 - 3.94 (m, 5H), 3.85 - 3.88 (m, 2H), 3.71 (d, *J* = 2.93 Hz, 2H), 3.66 (d, *J* = 2.93 Hz, 2H), 2.95 (br. s., 2H), 2.27 (br. s., 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 21.89, 29.20, 30.91, 31.71, 52.06, 53.70, 67.38, 67.52, 69.44, 69.52, 70.46, 70.64, 73.25, 100.81, 107.37, 114.24, 114.73, 117.86, 123.36, 124.02, 124.65, 125.66, 126.10, 126.90, 128.29,128.96, 129.22, 129.72, 130.00, 130.48, 131.39, 131.68, 133.21, 133.34, 137.09, 138.98, 155.07, 156.26, 157.89, 160.48, 162.94, 163.44, 166.79, 174.78, 177.78; LRMS (ESI) m/z 864 $[M+H]^+$, 886 $[M+Na]^+$; HRMS (ESI) calcd for $C_{50}H_{46}N_3O_{11}$ $[M+H]^+$ 864.3132, found 864.3145; calcd for $C_{50}H_{45}N_3O_{11}Na$ $[M+Na]^+$ 886.2952, found 886.2927.

4.4.9. Synthesis of 7-(3-(1-(2-(2-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-2-phenyl-4H-chromen-4-one (Ac2Az11): This compound was obtained according to the procedure as described.[54]

4.4.10. Synthesis of 7-(3-(1-(2-(2-(2-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-2-phenyl-4H-chromen-4-one (Ac2Az12): This compound (62 mg) was obtained from **Ac2** and **Az12** in 89% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.14 – 2.26 (m, 2 H),

2.89 (t, *J* = 7.4 Hz, 2 H), 3.58 – 3.70 (m, 4 H), 3.79 – 3.90 (m, 4 H), 4.05 (t, *J* = 6.1 Hz, 2 H), 4.18 (t, *J* = 4.6 Hz, 2 H), 4.51 (t, *J* = 5.0 Hz, 2 H), 6.69 (d, *J* = 2.3 Hz, 2 H), 6.84 – 6.98 (m, 4 H), 7.40 – 7.56 (m, 7 H), 7.78 – 7.88 (m, 4 H), 8.06 (dd, *J* = 11.8, 8.7 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 22.07, 28.68, 50.23, 67.65, 68.09, 69.43, 69.64, 70.57, 70.81, 100.92, 101.15, 107.44, 107.50, 114.72, 114.77, 117.77, 118.02, 126.13, 126.16, 126.97, 127.11, 129.04, 131.47, 131.51, 131.71, 131.78, 157.88, 157.96, 162.98, 163.04, 163.27, 163.53, 177.75, 177.82; LRMS (ESI) m/z 700 $[M+H]^+$, 722 $[M+Na]^+$; HRMS (ESI) calcd for $C_{41}H_{38}N_3O_8$ $[M+H]^+$ 700.2659, found 700.2651; calcd for $C_{41}H_{37}N_3O_8Na$ $[M+Na]^+$ 722.2478, found 722.2490.

4.4.11. Synthesis of 7-Fluoro-2-(4-(3-(1-(2-(2-(4-(4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)propoxy)phenyl)-4*H*-chromen-4-one (**Ac3Az1**): This compound was obtained according to the procedure as described.[54]

4.4.12. Synthesis of 7-Fluoro-2-(4-(3-(1-(2-(2-(2-(4-(4-oxo-4H-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-4H-chromen-4-one (Ac3Az2): This compound was obtained according to the procedure as described.[54]

4.4.13. Synthesis of 7-Fluoro-2-(4-(3-(1-(2-(2-(2-(4-(6-methyl-4-oxo-4H-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-4H-chromen-4-one (Ac3Az3): This compound was obtained according to the procedure as described.[54]

4.4.14. Synthesis of 7-Fluoro-2-(4-(3-(1-(2-(2-(2-(4-(6-fluoro-4-oxo-4H-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-4H-chromen-4-one (Ac3Az4): This compound was obtained according to the procedure as described.[54]

4.4.15. Synthesis of 3-(benzyloxy)-2-(4-(2-(2-(2-(4-(3-(4-(7-fluoro-4-oxo-4H-chromen-2-yl)phenoxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4H-chromen-4-one (Ac3Az5): This compound (49 mg) was obtained from **Ac3** and **Az5** in 60% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.13 – 2.26 (m, 2 H), 2.89 (t, *J* = 7.6 Hz, 2 H), 3.60 – 3.78 (m, 4 H), 3.82 – 3.92 (m, 4 H), 4.02 (t, *J* = 6.2 Hz, 2 H), 4.15 – 4.22 (m, 2 H), 4.54 (t, *J* = 5.1 Hz, 2 H), 5.11 (s, 2 H), 6.65 (s, 1 H), 6.91 – 7.00 (m, 4 H), 7.09 – 7.15 (m, 1 H), 7.18 – 7.23 (m, 1 H), 7.24 – 7.31 (m, 3 H), 7.32 – 7.41 (m, 3 H), 7.47 (d, *J* = 8.4 Hz, 1 H), 7.56 (s, 1 H), 7.63 (t, *J* = 7.8 Hz, 1 H), 7.77 (d, *J* = 8.4 Hz, 2 H), 8.02 (d, *J* = 8.5 Hz, 2 H), 8.16 – 8.27 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 22.14, 28.84, 50.17, 67.24, 67.53, 69.63, 70.56, 70.75, 73.92, 104.64, 104.89, 106.02, 113.68, 113.90, 114.31, 114.97, 117.91, 120.75, 120.78, 122.22, 123.40, 123.61, 124.15, 124.65, 125.72, 127.93, 128.03, 128.14, 128.16, 128.29, 128.84, 130.57, 133.33, 136.80, 139.32, 146.89, 155.13, 156.05, 157.02, 157.16, 160.57, 161.93, 163.66, 164.33, 166.86, 174.95, 177.37; LRMS (ESI) m/z 824 $[M+H]^+$, 846 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{43}FN_3O_9$ $[M+H]^+$ 824.2983, found 824.3005; calcd for $C_{48}H_{42}FN_3O_9Na$ $[M+Na]^+$ 846.2803, found 846.2829.

4.4.16. Synthesis of methyl 3-(((2-(4-(2-(2-(4-(3-(4-(7-fluoro-4-oxo-4H-chromen-2-yl)phenoxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4-oxo-4H-chromen-3-yl)oxy)methyl)benzoate (Ac3Az8): This compound (54 mg) was obtained from **Ac3** and **Az8** in 65% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.17 (m, 2 H), 2.90 (m, 2 H), 3.91 – 3.80 (m, 5 H), 3.96 (t, *J* = 4.7 Hz, 2 H), 4.05 (t, *J* = 5.6 Hz, 2 H), 4.14 (t, *J* = 4.4 Hz, 2 H), 4.57 (s, 2 H), 5.13 (s, 2 H), 6.66 (s, 1 H), 7.01 – 6.90 (m, 4 H), 7.12 (td, *J* = 8.5, 2.4 Hz, 1 H), 7.21 (dd, *J* = 9.0, 2.4 Hz, 1 H), 7.42 – 7.30 (m, 2 H), 7.47 (d, *J* = 8.5 Hz, 1 H), 7.50 – 7.56 (m, 1 H), 7.59 (d, *J* = 7.6 Hz, 1 H), 7.64 (td, *J* = 7.7, 7.1, 1.7 Hz, 1 H), 7.76 (d, *J*

= 8.3 Hz, 2 H), 7.88 – 8.01 (m, 4 H), 8.16 – 8.28 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 22.18, 22.82, 29.83, 30.30, 31.56, 52.21, 67.32, 67.40, 69.71, 69.90, 73.45, 104.70, 104.95, 106.16, 113.77, 114.00, 114.37, 115.03, 118.01, 123.54, 123.67, 124.21, 124.84, 125.87, 128.03, 128.15, 128.25, 128.45, 129.40, 129.94, 130.18, 130.69, 133.39, 133.51, 137.25, 139.17, 155.25, 156.36, 157.12, 157.25, 160.52, 161.99, 163.76, 164.42, 166.93, 166.95, 174.94, 177.49; LRMS (ESI) m/z 838 $[M+H]^+$, 860 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{41}FN_3O_{10}$ $[M+H]^+$ 838.2776, found 838.2755; calcd for $C_{48}H_{40}FN_3O_{10}Na$ $[M+Na]^+$ 860.2595, found 860.2590.

4.4.17. Synthesis of methyl 3-(((2-(4-(2-(2-(2-(4-(3-(4-(7-fluoro-4-oxo-4H-chromen-2-yl)phenoxy)propyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4-oxo-4H-chromen-3-yl)oxy)methyl)benzoate (Ac3Az9): This compound (24 mg) was obtained from **Ac3** and **Az9** in 75% yield according to the general procedure described above. ^{1}H NMR (600 MHz, $CDCl_3$) □ ppm 2.21 - 2.25 (m, 2H), 2.96 (t, *J* = 7.47 Hz, 2H), 3.65 - 3.69 (m, 2H), 3.71 (dd, *J* = 3.11, 5.61 Hz, 2H), 3.84 - 3.88 (m, 2H), 3.89 (s, 3H), 3.92 (t, *J* = 4.98 Hz, 2H), 4.04 (t, *J* = 5.61 Hz, 2H), 4.17 - 4.22 (m, 2H), 4.59 (t, *J* = 4.98 Hz, 2H), 5.14 (s, 2H), 6.67 (s, 1H), 6.96 (dd, *J* = 4.98, 8.72 Hz, 4H), 7.11 - 7.16 (m, 1H), 7.20 - 7.24 (m, 1H), 7.33 - 7.41 (m, 2H), 7.49 (d, *J* = 7.47 Hz, 1H), 7.61 (d, *J* = 7.47 Hz, 1H), 7.63 - 7.71 (m, 2H), 7.78 (d, *J* = 8.72 Hz, 2H), 7.93 (d, *J* = 7.47 Hz, 1H), 7.95 - 8.00 (m, 3H), 8.21 (dd, *J* = 6.23, 8.72 Hz, 1H), 8.25 (d, *J* = 6.23 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) □ ppm 21.54, 28.49, 29.17, 52.04, 66.96, 67.37, 69.14, 69.49, 70.42, 70.56, 73.21, 104.56, 104.73, 105.89, 113.64, 113.79, 114.19, 114.82, 117.83, 120.58, 123.35, 123.97, 124.64, 125.60, 127.84, 127.88, 127.93, 128.00, 128.27, 129.20, 129.70, 129.97, 130.47, 133.19, 133.33, 137.05, 138.94, 155.03, 156.19, 156.92, 157.01, 160.44, 161.68, 163.55, 164.65, 166.34, 166.76, 174.75, 177.29; LRMS (ESI) m/z 882 $[M+H]^+$, 904 $[M+Na]^+$; HRMS (ESI) calcd for $C_{50}H_{45}N_3O_{11}F$

$[M+H]^+$ 882.3038, found 882.3051; calcd for $C_{50}H_{44}N_3O_{11}FNa$ $[M+Na]^+$ 904.2858, found 904.2833.

4.4.18. Synthesis of 7-Fluoro-2-(4-(3-(1-(2-(2-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-4H-chromen-4-one (Ac3Az11): This compound was obtained according to the procedure as described.[54]

4.4.19. Synthesis of 7-fluoro-2-(4-(3-(1-(2-(2-(2-((4-oxo-3-phenyl-4H-chromen-7-yl)oxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-4H-chromen-4-one (Ac3Az12): This compound (26 mg) was obtained from **Ac3** and **Az12** in 65% yield according to the general procedure described above.^{1}H NMR (600 MHz, $CDCl_3$) □ ppm 2.20 (br. s., 2H), 2.91 (br. s., 2H), 3.65 (d, *J* = 3.74 Hz, 2H), 3.67 - 3.71 (m, 2H), 3.83 - 3.88 (m, 2H), 3.90 (br. s., 2H), 4.01 (br. s., 2H), 4.17 - 4.23 (m, 2H), 4.56 (br. s., 2H), 6.60 - 6.65 (m, 1H), 6.70 (s, 1H), 6.88 - 6.97 (m, 4H), 7.09 (t, *J* = 8.10 Hz, 1H), 7.17 (dd, *J* = 2.49, 8.72 Hz, 1H), 7.43 - 7.50 (m, 3H), 7.57 - 7.69 (m, 1H), 7.74 (d, *J* = 8.72 Hz, 2H), 7.83 (d, *J* = 7.47 Hz, 2H), 8.07 (d, *J* = 8.72 Hz, 1H), 8.14 - 8.20 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) □ ppm 21.74, 28.45, 29.11, 66.95, 67.87, 69.20 (s), 69.25 (s), 70.34 (s), 70.55 (s), 100.88 (s), 104.44 (s), 104.61 (s), 105.71 (s), 107.16 (s), 113.51 (s), 113.66 (s), 114.55 (s), 114.71 (s), 117.71 (s), 120.49 (s), 123.14 (s), 125.91 (s), 126.83 (s), 127.72 (s), 127.80 (s), 127.87 (s), 128.82 (s), 131.34 (s), 131.39 (s), 156.79 (s), 156.88 (s), 157.63 (s), 161.65 (s), 162.83 (s), 163.05 (s), 163.44 (s), 164.52 (s), 166.20 (s), 177.15 (s), 177.55 (s); LRMS (ESI) m/z 718 $[M+H]^+$, 740 $[M+Na]^+$; HRMS (ESI) calcd for $C_{41}H_{37}N_3O_8F$ $[M+H]^+$ 718.2565, found 718.2540; calcd for $C_{41}H_{36}N_3O_8FNa$ $[M+Na]^+$ 740.2384, found 740.2410.

4.4.20. Synthesis of 6-Methyl-2-(4-(4-(1-(2-(2-(4-(4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az1**): This compound was obtained according to the procedure as described. [54]

4.4.21. Synthesis of 6-Methyl-2-(4-(4-(1-(2-(2-(2-(4-(4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az2**): This compound (63 mg) was obtained from **Ac5** and **Az2** in 86% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.86 (br. s., 3 H), 2.42 - 2.47 (m, 2 H), 2.78 (br. s., 1 H), 3.63 - 3.66 (m, 2 H), 3.68 - 3.72 (m, 2 H), 3.86 (dt, *J*=17.81, 4.76 Hz, 4 H), 3.99 - 4.03 (m, 1 H), 4.15 - 4.19 (m, 2 H), 4.52 (t, *J*=5.12 Hz, 1 H), 6.68 (s, 1 H), 6.71 (s, 1 H), 6.94 (d, *J*=8.79 Hz, 2 H), 7.00 (d, *J*=8.79 Hz, 2 H), 7.34 - 7.40 (m, 1 H), 7.40 - 7.43 (m, 1 H), 7.44 - 7.48 (m, 1 H), 7.48 - 7.53 (m, 2 H), 7.63 - 7.68 (m, 1 H), 7.80 (d, *J*=8.79 Hz, 2 H), 7.85 (d, *J*=9.27 Hz, 2 H), 7.97 (s, 1 H), 8.19 (dd, *J*=7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.73, 25.18, 25.77, 28.48, 49.94, 67.44, 67.65, 69.35, 69.44, 70.36, 70.45, 70.55, 71.15, 105.66, 105.93, 114.63, 114.80, 117.52, 117.76, 121.80, 123.35, 123.66, 123.70, 123.99, 124.73, 124.87, 125.36, 127.68, 127.76, 133.38, 134.55, 134.77, 147.38, 154.19, 155.91, 161.33, 161.57, 162.97, 163.00, 178.16; LRMS (ESI) m/z 728 $[M+H]^+$, 750 $[M+Na]^+$; HRMS (ESI) calcd for $C_{43}H_{42}N_3O_8$ $[M+H]^+$ 728.2972, found 728.2955; calcd for $C_{43}H_{41}N_3O_8Na$ $[M+Na]^+$ 750.2791, found 750.2815.

4.4.22. Synthesis of 6-Methyl-2-(4-(2-(2-(2-(4-(4-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az3**): This compound was obtained according to the procedure as described.[54]

4.4.23. Synthesis of 6-Fluoro-2-(4-(2-(2-(2-(4-(4-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az4**): This compound was obtained according to the procedure as described.[54]

4.4.24. Synthesis of 3-(Benzyloxy)-2-(4-(2-(2-(2-(4-(4-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az5**): This compound (56 mg) was obtained from **Ac5** and **Az5** in 63% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.81 (br. s., 4 H), 2.38 (s, 3 H), 2.74 (br. s., 2 H), 3.59 - 3.63 (m, 2 H), 3.78 - 3.82 (m, 2 H), 3.84 (t, *J*=5.07 Hz, 2 H), 3.91 - 3.97 (m, 2 H), 4.09 - 4.15 (m, 2 H), 4.48 (t, *J*=4.88 Hz, 2 H), 5.07 (s, 2 H), 6.62 (s, 1 H), 6.85 - 6.93 (m, 4 H), 7.20 - 7.26 (m, 3 H), 7.28 - 7.37 (m, 4 H), 7.38 - 7.44 (m, 2 H), 7.50 (br. s., 1 H), 7.58 (ddd, *J*=8.59, 7.03, 1.56 Hz, 1 H), 7.70 - 7.77 (m, 2 H), 7.91 (br. s., 1 H), 7.98 (d, *J*=8.0, 2 H), 8.19 (dd, *J*=8.20, 1.56 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.74, 25.21, 25.76, 28.49, 49.98, 67.36, 67.66, 69.44, 70.38, 70.56, 73.70, 105.70, 114.14, 114.66, 117.54, 117.71, 121.80, 123.37, 123.40, 123.70, 123.97, 124.40, 124.75, 125.48, 127.69, 127.91, 128.06, 128.60, 130.34, 133.08, 134.54, 134.76, 136.65, 139.14, 154.21, 154.93, 155.81, 160.39, 161.58, 163.01, 174.69, 178.18; LRMS (ESI) m/z 834 $[M+H]^+$, 856 $[M+Na]^+$; HRMS (ESI) calcd for $C_{50}H_{48}N_3O_9$ $[M+H]^+$ 834.3391, found 834.3367; calcd for $C_{50}H_{47}N_3O_9Na$ $[M+Na]^+$ 856.3210, found 856.3195.

4.2.25. Synthesis of 6-Fluoro-2-(4-(2-(2-(4-(4-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az7**): This compound was obtained according to the procedure as described.[54]

4.4.26. Synthesis of Methyl 3-(((2-(4-(2-(2-(4-(4-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4-oxo-4*H*-chromen-3-

yl)oxy)methyl)benzoate (**Ac5Az8**): This compound (44 mg) was obtained from **Ac5** and **Az8** in 51% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.84 (br. s., 4 H), 2.43 (s, 3 H), 2.77 (br. s., 2 H), 3.82 – 3.84 (m, 2 H), 3.86 (s, 3 H), 3.94 (t, *J*=4.88 Hz, 2 H), 3.98 (br. s., 2 H), 4.11 - 4.17 (m, 2 H), 4.54 (t, *J*=4.88 Hz, 2 H), 5.13 (s, 2 H), 6.65 (s, 1 H), 6.92 (t, *J*=8.30 Hz, 4 H), 7.32 – 7.37 (m, 2 H), 7.40 (d, *J*=5 Hz, 1 H), 7.45 (d, *J*=8.79 Hz, 2 H), 7.50 (br. s., 1 H), 7.58 (d, *J*=6.83 Hz, 1 H), 7.60 - 7.64 (m, 1 H), 7.77 (d, *J*=8.30 Hz, 2 H), 7.86 - 8.01 (m, 5 H), 8.22 (d, *J*=7.81 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.86, 25.29, 25.83, 28.58, 50.07, 51.98, 67.29, 67.76, 69.56, 69.79, 73.26, 105.86, 114.25, 114.77, 117.65, 117.81, 121.88, 123.51, 123.86, 124.07, 124.61, 124.91, 125.66, 127.81, 128.25, 129.20, 129.73, 130.07, 130.49, 133.15, 133.29, 134.65, 134.90, 137.15, 139.03, 147.58, 154.35, 155.08, 156.15, 160.40, 161.68, 163.13, 166.72, 174.72, 178.33; LRMS (ESI) m/z 848 $[M+H]^+$, 870 $[M+Na]^+$; HRMS (ESI) calcd for $C_{50}H_{46}N_3O_{10}$ $[M+H]^+$ 848.3183, found 848.3145; calcd for $C_{50}H_{45}N_3O_{10}Na$ $[M+Na]^+$ 870.3003, found 870.2966.

4.4.27. Synthesis of Methyl 3-(((2-(4-(2-(2-(2-(4-(4-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)methyl)benzoate (**Ac5Az9**): This compound (83 mg) was obtained from **Ac5** and **Az9** in 93% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.86 (br. s., 4 H), 2.44 (s, 3 H), 2.78 (br. s., 2 H), 3.62 - 3.67 (m, 2 H), 3.68 - 3.72 (m, 2 H), 3.81 - 3.90 (m, 7 H), 4.01 (br. s., 2 H), 4.17 (t, *J*=4.39 Hz, 2 H), 4.52 (t, *J*=4.88 Hz, 2 H), 5.14 (s, 2 H), 6.69 (s, 1 H), 6.91 - 6.97 (m, 4 H), 7.34 (t, *J*=7.81 Hz, 1 H), 7.36 - 7.40 (m, 1 H), 7.42 (d, *J*=10 Hz, 1 H), 7.44 - 7.50 (m, 2 H), 7.51 (br. s., 1 H), 7.60 (d, *J*=5 Hz, 1 H), 7.61 - 7.68 (m, 1 H), 7.80 - 7.82 (d, *J*=10 Hz, 2 H), 7.87 - 8.02 (m, 5 H), 8.25 (d, *J*=10, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.76, 25.22, 25.79, 28.51, 29.17, 49.99, 51.91, 67.36, 67.69, 69.45, 69.49, 70.41,

70.59 , 73.15, 105.74, 114.20, 114.68, 117.56, 117.77, 121.81, 123.26, 123.40, 123.74, 123.97, 124.50, 124.79, 125.53, 127.72, 128.17, 129.12, 129.61, 129.99, 130.36, 133.05, 133.19, 134.56, 134.80, 137.08, 138.92, 154.24, 154.99, 156.11, 160.46, 161.61, 163.04, 166.63, 174.62, 178.20; LRMS (ESI) m/z 892 $[M+H]^+$, 914 $[M+Na]^+$; HRMS (ESI) calcd for $C_{52}H_{50}N_3O_{11}$ $[M+H]^+$ 892.3445, found 892.3410; calcd for $C_{52}H_{49}N_3O_{11}Na$ $[M+Na]^+$ 914.3265, found 914.3301.

4.4.28. Synthesis of 3-(Benzyloxy)-2-(4-(2-(2-(4-(4-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az10**): This compound (77 mg) was obtained from **Ac5** and **Az10** in 98% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.83 (br. s., 3 H), 2.41 (s, 2 H), 2.67 - 2.81 (m, 2 H), 3.78 - 3.85 (m, 2 H), 3.89 - 3.98 (m, 4 H), 4.09 - 4.17 (m, 2 H), 4.53 (t, *J*=5.07 Hz, 2 H), 5.09 (s, 2 H), 6.63 (s, 1 H), 6.92 (d, *J*=9.37 Hz, 2 H), 6.89 (d, *J*=8.98 Hz, 2 H), 7.22 - 7.28 (m, 3 H), 7.29 - 7.46 (m, 6 H), 7.48 (s, 1 H), 7.59 (ddd, *J*=8.59, 7.03, 1.56 Hz, 1 H), 7.75 (d, *J*=10 Hz, 2 H), 7.95 (s, 1 H), 8.0 (d, *J*=10 Hz, 2 H), 8.21 (d, *J*=10.0 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.84, 25.27, 25.81, 28.56, 50.05, 67.29, 67.73, 69.53, 69.76, 73.81, 105.83, 114.20, 114.75, 117.63, 117.76, 121.89, 123.47, 123.67, 123.82, 124.07, 124.51, 124.87, 125.62, 127.79, 128.01, 128.15, 128.68, 130.46, 133.18, 134.63, 134.87, 136.74, 139.27, 147.55, 154.32, 155.02, 155.81, 160.34, 161.66, 163.11, 174.78, 178.30; LRMS (ESI) m/z 790 $[M+H]^+$, 812 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{44}N_3O_8$ $[M+H]^+$ 790.3128, found 790.3140; calcd for $C_{48}H_{43}N_3O_8Na$ $[M+Na]^+$ 812.2948, found 812.2961.

4.4.29. Synthesis of 6-Methyl-2-(4-(4-(1-(2-(2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az11**): This compound was obtained according to the procedure as described.[54]

4.4.30. Synthesis of 6-Methyl-2-(4-(4-(1-(2-(2-(2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)phenyl)-4*H*-chromen-4-one (**Ac5Az12**): This compound (33 mg) was obtained from **Ac5** and **Az12** in 46% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.83 - 1.89 (m, 4 H), 2.45 (s, 3 H), 2.76 - 2.81 (m, 2 H), 3.62 - 3.67 (m, 2 H), 3.68 - 3.73 (m, 2 H), 3.86 - 3.90 (m, 4 H), 4.00 - 4.02 (m, 2 H), 4.19 - 4.24 (m, 2 H), 4.52 (t, *J*=4.88 Hz, 2 H), 6.70 (s, 1 H), 6.74 (s, 1 H), 6.91 - 7.02 (m, 4 H), 7.40 - 7.54 (m, 6 H), 7.82 (d, *J*=9.0 Hz, 2 H), 7.87 (dd, *J*=7.57, 1.71 Hz, 2 H), 7.99 (s, 1 H), 8.13 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.90, 25.21, 25.89, 28.62, 50.29, 67.80, 68.07, 69.41, 69.55, 70.56, 70.78, 101.19, 105.94, 107.53, 114.59, 114.84, 117.68, 118.04, 122.03, 123.54, 123.97, 125.00, 126.12, 127.15, 127.91, 128.99, 131.44, 131.76, 134.73, 135.00, 154.44, 157.87, 161.74, 163.06, 163.23, 163.27, 177.70, 178.44; LRMS (ESI) m/z 728 $[M+H]^+$; HRMS (ESI) calcd for $C_{43}H_{42}N_3O_8$ $[M+H]^+$ 728.2972, found 728.3006.

4.4.31. Synthesis of 6-methyl-2-(4-(4-(1-(2-((4-oxo-2-phenyl-4H-chromen-7-yl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-4H-chromen-4-one (Ac5Az13): This compound (23mg) was obtained from **Ac5** and **Az13** in 67% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.82 (br. s., 4 H), 2.36 (s, 3 H), 2.79 (t, *J* = 4.58 Hz, 2 H), 3.94 (t, *J* = 4.60, 2 H), 4.42 (t, *J* = 4.68 Hz, 2 H), 4.75 (t, *J* = 4.68 Hz, 2 H), 6.63 (s, 1 H), 6.59 (s, 1 H), 6.81 - 6.89 (m, 4 H), 7.29 - 7.37 (m, 1 H), 7.37 - 7.46 (m, 4 H), 7.51 (s, 1 H), 7.72 (d, *J* = 8.20 Hz, 2 H), 7.77 (d, *J* = 7.81 Hz, 2 H), 7.89 (s, 1 H), 8.01 (d, *J* = 8.98 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.8, 25.1, 25.7, 28.4, 49.2, 66.8, 67.6, 101.2, 105.7, 107.3, 114.1, 114.7, 117.6, 118.3, 121.9, 123.3, 123.7, 124.7, 125.9, 127.1, 127.7, 128.9, 131.4, 134.6, 134.8, 147.8, 154.2, 157.5, 161.6, 162.1, 162.9, 163.1, 177.4, 178.2; LRMS (ESI) m/z 640.3 base peak: 640.3 $[M+H]^+$; HRMS (ESI) calcd for $C_{39}H_{34}N_3O_6[M+H]^+$ 640.2448 , found 640.2471.

4.4.32. Synthesis of 2-(4-(4-(1-(2-(Benzyl(2-(4-(4-oxo-4*H*-chromen-2-yl)phenoxy)ethyl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)phenyl)-6-methyl-4*H*-chromen-4-one (**Ac5Az14**): This compound (38 mg) was obtained from **Ac5** and **Az14** in 49% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.81 (br. s., 4 H), 2.44 (s, 3 H), 2.71 (br. s., 2 H), 2.97 - 3.00 (m, 2 H), 3.11 (br. s., 2 H), 3.76 (s, 2 H), 3.89 - 4.03 (m, 4 H), 4.40 (br. s., 2 H), 6.66 (s, 1 H), 6.72 (s, 1 H), 6.84 - 6.94 (m, 4 H), 7.20 - 7.32 (m, 5 H), 7.33 - 7.53 (m, 6 H), 7.74 - 7.81 (m, 2 H), 7.82 - 7.88 (m, 2 H), 7.97 (d, *J*=0.78 Hz, 1 H), 8.09 (d, *J*=8.98 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.9, 25.3, 25.9, 28.6, 48.7, 52.9, 54.8, 59.8, 67.1, 67.8, 100.9, 105.9, 107.5, 114.5, 114.8, 117.7, 117.9, 121.5, 123.6, 123.9, 124.9, 126.1, 127.1, 127.5, 127.9, 128.5, 128.7, 128.9, 131.4, 131.7, 134.7, 134.9, 138.4, 154.4, 157.9, 161.7, 162.9, 163.0, 163.2, 177.6, 178.4; LRMS (ESI) m/z 773 $[M+H]^+$; HRMS (ESI) calcd for $C_{48}H_{45}N_4O_6$ $[M+H]^+$ 773.3339, found 773.3314.

4.4.33. Synthesis of 2-(4-(4-(1-(2-(Benzyl(2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethyl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)phenyl)-6-methyl-4*H*-chromen-4-one (**Ac5Az15**): This compound (41 mg) was obtained from **Ac5** and **Az15** in 53% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.84 (br. s., 4 H), 2.46 (s, 3 H), 2.73 (br. s., 2 H), 2.97 (br. s., 2 H), 3.11 (br. s., 2 H), 3.77 (br. s., 2 H), 3.89 - 4.05 (m, 4 H), 4.40 (br. s., 2 H), 6.68 (s, 1 H), 6.72 (s, 1 H), 6.88 - 6.97 (m, 4 H), 7.21 - 7.33 (m, 5 H), 7.33 - 7.40 (m, 2 H), 7.40 - 7.44 (m, 1 H), 7.45 - 7.52 (m, 2 H), 7.61 - 7.67 (m, 1 H), 7.76 - 7.81 (m, 2 H), 7.84 (d, *J*=8.79 Hz, 2 H), 7.99 (s, 1 H), 8.18 (dd, *J*=8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.9, 25.3, 25.9, 28.7, 48.7, 53.1, 54.8, 59.9, 66.8, 67.8, 105.9, 106.3, 114.8, 114.9, 117.7, 117.9, 123.6, 123.9, 123.9, 124.2, 125.0, 125.1, 125.7, 127.5, 127.9, 128.0, 128.5,

128.7, 133.6, 134.7, 134.9, 154.4, 156.1, 161.3, 161.7, 163.2, 163.2, 178.3, 178.4; LRMS (ESI) m/z 773 $[M+H]^+$; HRMS (ESI) calcd for $C_{48}H_{45}N_4O_6$ $[M+H]^+$ 773.3339, found 773.3353.

4.4.34. Synthesis of 7-(4-(1-(2-(2-(4-(4-Oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)-2-phenyl-4*H*-chromen-4-one (**Ac12Az1**): This compound was obtained according to the procedure as described.[54]

4.4.35. Synthesis of 7-(4-(1-(2-(2-(2-(4-(4-Oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)butoxy)-2-phenyl-4*H*-chromen-4-one (**Ac12Az2**): This compound (70 mg) was obtained from **Ac12** and **Az2** in 98% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.89 (br. s., 4 H), 2.80 (br. s., 2 H), 3.62 - 3.68 (m, 2 H), 3.68 - 3.75 (m, 2 H), 3.85 (t, *J*=4.15 Hz, 2 H), 3.89 (t, *J*=5.12 Hz, 2 H), 4.07 (br. s., 2 H), 4.18 (t, *J*=4.39 Hz, 2 H), 4.53 (t, *J*=4.88 Hz, 2 H), 6.72 (s, 1 H), 6.74 (s, 1 H), 6.91 (s, 1 H), 6.94 (dd, *J*=9.03, 1.22 Hz, 1 H), 7.01 (d, *J*=8.30 Hz, 2 H), 7.39 (t, *J*=7.57 Hz, 1 H), 7.52 (d, *J*=4.88 Hz, 5 H), 7.66 (t, *J*=7.57 Hz, 1 H), 7.82 - 7.93 (m, 4 H), 8.10 (d, *J*=8.79 Hz, 1 H), 8.20 (d, *J*=7.81 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.13, 25.73, 28.31, 49.93, 67.44, 68.13, 69.35, 69.44, 70.35, 70.55, 100.65, 105.92, 107.19, 114.52, 114.79, 117.48, 117.74, 121.76, 123.67, 123.98, 124.86, 125.35, 125.89, 126.67, 127.74, 128.78, 131.20, 131.56, 133.36, 147.28, 155.89, 157.71, 161.33, 162.68, 162.94, 163.36, 177.50, 177.98; LRMS (ESI) m/z 714 $[M+H]^+$, 736 $[M+Na]^+$; HRMS (ESI) calcd for $C_{42}H_{40}N_3O_8$ $[M+H]^+$ 714.2815, found 714.2804; calcd for $C_{42}H_{39}N_3O_8Na$ $[M+Na]^+$ 736.2635, found 736.2625.

4.4.36. Synthesis of 6-Methyl-2-(4-(2-(2-(2-(4-(4-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac12Az3**): This compound (62 mg) was obtained from **Ac12** and **Az3** in 85% yield according to

the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.83 (br. s., 4 H), 2.37 (s, 3 H), 2.74 (br. s., 2 H), 3.61 (br. s., 2 H), 3.63 - 3.70 (m, 2 H), 3.80 (br. s., 2 H), 3.84 (t, *J*=4.88 Hz, 2 H), 4.00 (br. s., 2 H), 4.12 (br. s., 2 H), 4.48 (t, *J*=4.88 Hz, 2 H), 6.63 (s, 1 H), 6.67 (s, 1 H), 6.83 (s, 1 H), 6.86 (d, *J*=9.27 Hz, 1 H), 6.94 (d, *J*=8.30 Hz, 2 H), 7.33 (d, *J*=8.30 Hz, 1 H), 7.39 (d, *J*=8.79 Hz, 1 H), 7.41 - 7.48 (m, 3 H), 7.50 (s, 1 H), 7.77 (d, *J*=8.30 Hz, 2 H), 7.81 (d, *J*=6.83 Hz, 2 H), 7.90 (s, 1 H), 8.02 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.76, 25.18, 25.77, 28.36, 49.96, 67.48, 68.17, 69.40, 69.48, 70.39, 70.59, 100.70, 105.85, 107.24, 114.55, 114.81, 117.53, 121.80, 123.36, 124.21, 124.79, 125.95, 126.74, 127.75, 128.82, 131.23, 131.62, 134.61, 134.86, 147.32, 154.22, 157.77, 161.28, 162.75, 162.85, 163.41, 177.57, 178.18; LRMS (ESI) m/z 728 $[M+H]^+$, 750 $[M+Na]^+$; HRMS (ESI) calcd for $C_{43}H_{42}N_3O_8$ $[M+H]^+$ 728.2972, found 728.2949; calcd for $C_{43}H_{41}N_3O_8Na$ $[M+Na]^+$ 750.2791, found 750.2790.

4.4.37. Synthesis of 6-Fluoro-2-(4-(2-(2-(2-(4-(4-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac12Az4**): This compound (53 mg) was obtained from **Ac12** and **Az4** in 73% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.89 (br. s., 4 H), 2.79 (br. s., 2 H), 3.61 - 3.67 (m, 2 H), 3.67 – 3.71 (m, 2 H), 3.81 - 3.86 (m, 2 H), 3.88 (t, *J*=5.12 Hz, 2 H), 4.06 (br. s., 2 H), 4.13 - 4.20 (m, 2 H), 4.52 (t, *J*=4.39 Hz, 2 H), 6.69 (s, 1 H), 6.73 (s, 1 H), 6.90 (s, 1 H), 6.93 (d, *J*=8.79Hz, 1 H), 6.99 (d, *J*=8.79Hz, 2 H), 7.34 - 7.40 (m, 1 H), 7.46 - 7.55 (m, 5 H), 7.80 - 7.85 (m, 3 H), 7.85 - 7.90 (m, 2 H), 8.09 (d, *J*=8.79Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.07, 25.63, 28.22, 49.94, 67.38, 68.05, 69.24, 69.31, 70.26, 70.47, 100.56, 105.04, 107.03, 110.13 (d, *J*=23.63Hz, C5), 114.42, 114.73, 117.37, 119.77 (d, *J*=8.08Hz, C8), 121.30 (d, *J*=25.15Hz, C7), 123.49, 124.75 (d, *J*=6.67Hz, C10), 125.77, 126.52, 127.67, 128.68, 131.11, 131.40, 151.94 (d, *J*=2.02Hz, C9), 157.59, 159.18 (d, *J*=247.85 Hz, C6), 161.39, 162.54, 163.11,

163.26, 176.94 (d, *J*=3.03Hz, C4), 177.32; LRMS (ESI) m/z 732 $[M+H]^+$, 754 $[M+Na]^+$; HRMS (ESI) calcd for $C_{42}H_{39}N_3O_8F$ $[M+H]^+$ 732.2721, found 732.2712; calcd for $C_{42}H_{38}N_3O_8FNa$ $[M+Na]^+$ 754.2541, found 754.2524.

4.4.38. Synthesis of 3-(Benzyloxy)-2-(4-(2-(2-(2-(4-(4-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac12Az5**): This compound (58 mg) was obtained from **Ac12** and **Az5** in 71% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.83 - 1.94 (m, 4 H), 2.77 – 2.80 (m, 2 H), 3.62 - 3.68 (m, 2 H), 3.68 - 3.74 (m, 2 H), 3.84 – 3.86 (m, 2 H), 3.88 (t, *J*=5.12 Hz, 2 H), 4.01 - 4.08 (m, 2 H), 4.17 – 4.19 (m, 2 H), 4.52 (t, *J*=5.12 Hz, 2 H), 5.11 (s, 2 H), 6.74 (s, 1 H), 6.89 - 6.97 (m, 4 H), 7.25 - 7.30 (m, 3 H), 7.34 - 7.41 (m, 3 H), 7.45 - 7.54 (m, 5 H), 7.61 - 7.67 (m, 1 H), 7.85 - 7.91 (m, 2 H), 8.00 - 8.05 (m, 2 H), 8.09 (d, *J*=8.79 Hz, 1 H), 8.26 (dd, *J*=8.30, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.13, 25.73, 28.32, 49.94, 67.35, 68.14, 69.42, 69.44, 70.36, 70.56, 73.71, 100.68, 107.24, 114.14, 114.55, 117.49, 117.71, 121.79, 123.40, 123.95, 124.41, 125.48, 125.94, 126.71, 127.91, 128.05, 128.58, 128.79, 130.33, 131.21, 131.60, 133.08, 136.64, 139.13, 147.30, 154.91, 155.84, 157.75, 160.38, 162.74, 162.76, 163.39, 174.71, 177.58; LRMS (ESI) m/z 820 $[M+H]^+$, 842 $[M+Na]^+$; HRMS (ESI) calcd for $C_{49}H_{46}N_3O_9$ $[M+H]^+$ 820.3234, found 820.3246; calcd for $C_{49}H_{45}N_3O_9Na$ $[M+Na]^+$ 842.3054, found 842.3068.

4.4.39. Synthesis of 6-Fluoro-2-(4-(2-(2-(4-(4-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac12Az7**): This compound (63 mg) was obtained from **Ac12** and **Az7** in 91% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.82 - 1.94 (m, 5 H), 2.78 – 2.80 (m, 2 H), 3.80 - 3.86 (m, 2 H), 3.95 (t, *J*=5.00, 2 H), 4.01 - 4.09 (m, 2 H), 4.15 – 4.16 (m, 2 H), 4.55 (t,

J=5.12 Hz, 2 H), 6.70 (s, 1 H), 6.73 (s, 1 H), 6.88 - 6.93 (m, 2 H), 6.97 - 7.02 (m, 2 H), 7.37 (ddd, *J*=9.15, 7.69, 3.17 Hz, 1 H), 7.47 - 7.54 (m, 5 H), 7.78 - 7.89 (m, 5 H), 8.09 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.07, 25.64, 28.24, 49.89, 67.28, 68.08, 69.28, 69.61, 100.58, 105.15, 107.08, 110.16 (d, *J*=23.23Hz, C5), 114.48, 114.76, 117.39, 119.78 (d, *J*=8.08Hz, C8), 121.38 (d, *J*=25.25Hz, C7), 121.77, 123.67, 124.78 (d, *J*=6.06Hz, C10), 125.82, 126.57, 127.73, 128.73, 131.17, 131.44, 147.29, 151.97, 157.63, 159.23 (d, *J*=248.46Hz, C6), 161.31, 162.61, 163.09, 163.30, 177.00, 177.41; LRMS (ESI) m/z 688 $[M+H]^+$, 710 $[M+Na]^+$; HRMS (ESI) calcd for $C_{40}H_{35}N_3O_7F$ $[M+H]^+$ 688.2459, found 688.2454; calcd for $C_{40}H_{34}N_3O_7FNa$ $[M+Na]^+$ 710.2278, found 710.2261.

4.4.40. Synthesis of Methyl 3-(((4-oxo-2-(4-(2-(2-(4-(4-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-3-yl)oxy)methyl)benzoate (**Ac12Az8**): This compound (63 mg) was obtained from **Ac12** and **Az8** in 76% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.84 - 1.92 (m, 4 H), 2.77 – 2.80 (m, 2 H), 3.82 - 3.86 (m, 2 H), 3.87 (s, 3 H), 3.96 (t, *J*=4.88 Hz, 2 H), 4.03 - 4.07 (m, 2 H), 4.14 - 4.18 (m, 2 H), 4.55 (t, *J*=4.88 Hz, 2 H), 5.13 (s, 2 H), 6.73 (s, 1 H), 6.89 - 6.96 (m, 4 H), 7.34 (t, *J*=7.57 Hz, 1 H), 7.36 - 7.41 (m, 1 H), 7.45 - 7.53 (m, 5 H), 7.59 (d, *J*=7.81 Hz, 1 H), 7.64 (ddd, *J*=8.42, 6.95, 1.71 Hz, 1 H), 7.85 - 7.90 (m, 2 H), 7.92 (dd, *J*=7.56, 1.22 Hz, 1 H), 7.95 - 8.00 (m, 3 H), 8.08 (d, *J*=8.79 Hz, 1 H), 8.25 (dd, *J*=8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.18, 25.74, 28.37, 50.02, 51.93, 67.25, 68.18, 69.50, 69.73, 73.21, 100.73, 107.33, 114.21, 114.61, 117.57, 117.77, 121.88, 123.44, 124.00, 124.57, 125.59, 126.00, 126.79, 128.19, 128.86, 129.14, 129.66, 130.01, 130.42, 131.25, 131.69, 133.08, 133.24, 137.10, 138.97, 147.44, 155.02, 156.08, 157.81, 160.36, 162.80, 163.44, 166.67, 174.65,

177.61; LRMS (ESI) m/z 834 $[M+H]^+$, 856 $[M+Na]^+$; HRMS (ESI) calcd for $C_{49}H_{44}N_3O_{10}$ $[M+H]^+$ 834.3027, found 834.3041; calcd for $C_{49}H_{43}N_3O_{10}Na$ $[M+Na]^+$ 856.2846, found 856.2834.

4.4.41. Synthesis of Methyl 3-(((4-oxo-2-(4-(2-(2-(2-(4-(4-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-3-yl)oxy)methyl)benzoate (**Ac12Az9**): This compound (78 mg) was obtained from **Ac12** and **Az9** in 89% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.84 - 1.93 (m, 4 H), 2.74 - 2.82 (m, 2 H), 3.63 - 3.67 (m, 2 H), 3.68 - 3.73 (m, 2 H), 3.84 - 3.87 (m, 2 H), 3.87 - 3.90 (m, 5 H), 4.04 - 4.09 (m, 2 H), 4.15 - 4.20 (m, 2 H), 4.52 (t, *J*=5.12 Hz, 2 H), 5.14 (s, 2 H), 6.74 (s, 1 H), 6.89 - 6.97 (m, 4 H), 7.34 (t, *J*=7.56 Hz, 1 H), 7.39 (t, *J*=7.57 Hz, 1 H), 7.46 - 7.54 (m, 5 H), 7.59 (d, *J*=7.81 Hz, 1 H), 7.65 (ddd, *J*=8.54, 7.08, 1.46 Hz, 1 H), 7.86 - 7.90 (m, 2 H), 7.92 (d, *J*=7.81 Hz, 1 H), 7.95 - 8.01 (m, 3 H), 8.09 (d, *J*=8.79 Hz, 1 H), 8.26 (dd, *J*=7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.19, 25.80, 28.39, 50.00, 51.93, 67.39, 68.20, 69.49, 69.52, 70.44, 70.62, 73.19, 100.75, 107.33, 114.23, 114.60, 117.60, 117.79, 121.81, 123.31, 124.00, 124.55, 125.59, 126.01, 126.80, 128.20, 128.86, 129.14, 129.64, 130.01, 130.40, 131.26, 131.69, 133.08, 133.23, 137.10, 138.96, 147.36, 155.03, 156.17, 157.83, 160.49, 162.83, 163.47, 166.68, 174.68, 177.63; LRMS (ESI) m/z 878 $[M+H]^+$, 900 $[M+Na]^+$; HRMS (ESI) calcd for $C_{51}H_{48}N_3O_{11}$ $[M+H]^+$ 878.3289, found 878.3313; calcd for $C_{51}H_{47}N_3O_{11}Na$ $[M+Na]^+$ 900.3108, found 900.3151.

4.4.42. Synthesis of 3-(Benzyloxy)-2-(4-(2-(2-(4-(4-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac12Az10**): This compound (74 mg) was obtained from **Ac12** and **Az10** in 96% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.82 - 1.94 (m, 4 H), 2.78 – 2.80

(m, 2 H), 3.81 - 3.87 (m, 2 H), 3.96 (t, *J*=4.88 Hz, 2 H), 4.01 - 4.08 (m, 2 H), 4.13 - 4.20 (m, 2 H), 4.55 (t, *J*=4.88 Hz, 2 H), 5.12 (s, 2 H), 6.73 (s, 1 H), 6.88 - 6.98 (m, 4 H), 7.25 - 7.30 (m, 3 H), 7.34 - 7.41 (m, 3 H), 7.44 - 7.54 (m, 5 H), 7.61 - 7.67 (m, 1 H), 7.85 - 7.91 (m, 2 H), 8.01 - 8.06 (m, 2 H), 8.09 (d, *J*=8.75, 1 H), 8.25 (dd, *J*=8.54, 1.22 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.22, 25.79, 28.40, 50.05, 67.30, 68.20, 69.55, 69.77, 73.82, 100.76, 107.40, 114.21, 114.64, 117.63, 117.77, 121.87, 123.70, 124.07, 124.53, 125.64, 126.05, 126.85, 128.00, 128.15, 128.66, 128.90, 130.46, 131.28, 131.76, 133.18, 136.75, 139.28, 147.47, 155.03, 155.80, 157.86, 160.33, 162.85, 163.48, 174.78, 177.66; LRMS (ESI) m/z 776 $[M+H]^+$, 798 $[M+Na]^+$; HRMS (ESI) calcd for $C_{47}H_{42}N_3O_8$ $[M+H]^+$ 776.2972, found 776.2946; calcd for $C_{47}H_{41}N_3O_8Na$ $[M+Na]^+$ 798.2791, found 798.2767.

4.4.43. Synthesis of 7-(2-(2-(4-(4-((4-Oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac12Az11**): This compound (30 mg) was obtained from **Ac12** and **Az11** in 45% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.87 (br. s., 4 H), 2.78 (br. s., 2 H), 3.85 - 3.87 (m, 2 H), 3.96 (t, *J*=4.88 Hz, 2 H), 4.04 (br. s., 2 H), 4.18 - 4.22 (m, 2 H), 4.55 (t, *J*=4.39 Hz, 2 H), 6.72 (s, 1 H), 6.73 (s, 1 H), 6.86 - 7.00 (m, 4 H), 7.45 - 7.55 (m, 7 H), 7.83 - 7.90 (m, 4 H), 8.07 (d, *J*=8.79 Hz, 1 H), 8.13 (d, *J*=8.78 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.17, 25.71, 28.36, 50.10, 67.75, 68.18, 69.28, 69.74, 100.73, 101.08, 107.31, 107.36, 114.41, 114.62, 117.58, 117.97, 125.97, 126.02, 126.77, 127.01, 128.87, 128.89, 131.28, 131.37, 131.55, 131.70, 157.71, 157.81, 162.81, 162.92, 162.99, 163.45, 177.50, 177.64; LRMS (ESI) m/z 670 $[M+H]^+$; HRMS (ESI) calcd for $C_{40}H_{36}N_3O_7$ $[M+H]^+$ 670.2553, found 670.2565.

4.4.44. Synthesis of 7-(2-(2-(2-(4-(4-((4-Oxo-2-phenyl-4*H*-chromen-7-yl)oxy)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac12Az12**): This compound (17 mg) was obtained from **Ac12** and **Az12** in 24% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.85 - 1.91 (m, 4 H), 2.80 (br. s., 2 H), 3.62 - 3.68 (m, 2 H), 3.68 - 3.72 (m, 2 H), 3.86 - 3.90 (m, 4.70 Hz, 4 H), 4.06 (s, 2 H), 4.19 - 4.23 (m, 2 H), 4.52 (t, *J*=5.12 Hz, 2 H), 6.73 (s, 1 H), 6.74 (s, 1 H), 6.88 - 7.01 (m, 4 H), 7.46 - 7.55 (m, 7 H), 7.84 - 7.91 (m, 4 H), 8.09 (d, *J*=8.79 Hz, 1 H), 8.12 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.26, 25.90, 28.48, 50.16, 68.05, 68.27, 69.41, 69.61, 70.54, 70.79, 100.84, 101.18, 107.48, 107.54, 114.59, 114.72, 117.73, 118.04, 121.92, 126.12, 126.14, 126.97, 127.15, 128.98, 128.99, 131.37, 131.44, 131.77, 131.85, 157.86, 157.97, 162.97, 163.05, 163.22, 163.57, 177.70, 177.80; LRMS (ESI) m/z 714 $[M+H]^+$; HRMS (ESI) calcd for $C_{42}H_{40}N_3O_8$ $[M+H]^+$ 714.2815, found 714.2818.

4.4.45. Synthesis of 7-(2-((1-(2-(2-(4-(4-Oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac13Az1**): This compound was obtained according to the procedure as described.[54]

4.4.46. Synthesis of 7-(2-((1-(2-(2-(2-(4-(4-Oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac13Az2**): This compound (54 mg) was obtained from **Ac13** and **Az2** in 76% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.60 - 3.69 (m, 4 H), 3.80 – 3.82 (m, 2 H), 3.86 – 3.88 (m, 2 H), 3.92 – 3.93 (m, 2 H), 4.13 – 4.15 (m, 2 H), 4.20 – 4.22 (m, 2 H), 4.53 (t, *J*=4.88 Hz, 2 H), 4.73 (s, 2 H), 6.69 (d, *J*=11.2 Hz, 2 H), 6.90 (s, 1 H), 6.92 - 7.00 (m, 3 H), 7.36 (t, *J*=7.57 Hz, 1 H), 7.43 - 7.51 (m, 4 H), 7.61 - 7.67 (m, 1 H),

7.76 - 7.87 (m, 5 H), 8.07 (d, *J*=8.79, 1 H), 8.17 (d, *J*=8.0, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.22, 64.76, 67.54, 67.93, 68.40, 69.40, 69.48, 70.50, 70.66, 101.06, 106.13, 107.42, 114.65, 114.94, 117.86, 117.92, 123.77, 123.85, 124.19, 125.00, 125.56, 126.05, 126.96, 127.88, 128.92, 131.35, 131.70, 133.48, 144.57, 156.06, 157.77, 161.43, 162.93, 163.12, 163.19, 177.63, 178.18; LRMS (ESI) m/z 716 $[M+H]^+$, 738 $[M+Na]^+$; HRMS (ESI) calcd for $C_{41}H_{38}N_3O_9$ $[M+H]^+$ 716.2608, found 716.2574; calcd for $C_{41}H_{37}N_3O_9Na$ $[M+Na]^+$ 738.2427, found 738.2396.

4.4.47. Synthesis of 6-Methyl-2-(4-(2-(2-(2-(4-((2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac13Az3**): This compound (47 mg) was obtained from **Ac13** and **Az3** in 65% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.60 - 3.69 (m, 4 H), 3.78 - 3.83 (m, 2 H), 3.87 (t, *J*=4.88 Hz, 2 H), 3.90 – 3.92 (m, 2 H), 4.11 - 4.16 (m, 2 H), 4.18 - 4.22 (m, 2 H), 4.53 (t, *J*=4.88 Hz, 2 H), 4.73 (s, 2 H), 6.65 (s, 1 H), 6.69 (s, 1 H), 6.88 - 6.99 (m, 4 H), 7.34 - 7.39 (m, 1 H), 7.41 - 7.51 (m, 4 H), 7.76 - 7.86 (m, 5 H), 7.94 (s, 1 H), 8.06 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.83, 50.21, 64.75, 67.52, 67.91, 68.37, 69.39, 69.47, 70.49, 70.64, 101.05, 105.97, 107.39, 114.63, 114.90, 117.60, 117.90, 123.46, 123.76, 124.30, 124.90, 126.03, 126.93, 127.83, 128.90, 131.33, 131.68, 134.67, 134.94, 144.55, 154.32, 157.75, 161.34, 162.91, 162.97, 163.18, 177.62, 178.29; LRMS (ESI) m/z 730 $[M+H]^+$, 752 $[M+Na]^+$; HRMS (ESI) calcd for $C_{42}H_{40}N_3O_9$ $[M+H]^+$ 730.2765, found 730.2753; calcd for $C_{42}H_{40}N_3O_9Na$ $[M+Na]^+$ 752.2584, found 752.2604.

4.4.48. Synthesis of 6-Fluoro-2-(4-(2-(2-(2-(4-((2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac13Az4**): This compound (48 mg) was obtained from **Ac13** and **Az4** in 65% yield according

to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.60 - 3.70 (m, 4 H), 3.78 - 3.83 (m, 2 H), 3.88 – 3.89 (m, 2 H), 3.95 (br. s., 2 H), 4.11 - 4.17 (m, 2 H), 4.22 (br. s., 2 H), 4.50 - 4.58 (m, 2 H), 4.73 (br. s., 2 H), 6.66 (s, 1 H), 6.71 (s, 1 H), 6.88 - 6.99 (m, 4 H), 7.32 - 7.38 (m, 1 H), 7.43 - 7.51 (m, 4 H), 7.76 - 7.86 (m, 6 H), 8.07 (d, *J*=8.78 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 64.80, 64.82, 67.57, 67.94, 68.40, 69.36, 69.47, 70.50, 70.67, 101.05, 105.42, 107.41, 110.50 (d, *J*=23.23Hz, C5), 114.66, 114.99, 119.92 (d, *J*=8.08Hz, C8), 121.56 (d, *J*=25.25Hz, C7), 123.86, 126.04, 126.95, 127.93, 128.92, 131.37, 131.67, 152.24 (d, *J*=2.22 Hz, C9), 157.78, 159.46 (d, *J*=247.45 Hz, C6), 161.58, 162.92, 163.19, 163.41, 177.31 (d, *J*=2.53 Hz, C4), 177.61; LRMS (ESI) m/z 734 $[M+H]^+$; HRMS (ESI) calcd for $C_{41}H_{37}N_3O_9F$ $[M+H]^+$ 734.2514, found 734.2546.

4.4.49. Synthesis of 3-(Benzyloxy)-2-(4-(2-(2-(2-(4-((2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac13Az5**): This compound was obtained according to the procedure as described.[54]

4.4.50. Synthesis of 6-Fluoro-2-(4-(2-(2-(2-(4-((2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac13Az7**): This compound (30 mg) was obtained from **Ac13** and **Az7** in 44% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.83 – 3.83 (m, 2 H), 3.95 - 3.97 (m, 4 H), 4.14 – 4.15 (m, 2 H), 4.22 (br. s., 2 H), 4.59 (t, *J*=4.88 Hz, 2 H), 4.75 (br. s., 2 H), 6.69 (s, 1 H), 6.72 (s, 1 H), 6.90 - 7.01 (m, 4 H), 7.36 (ddd, *J*=9.15, 7.44, 2.93 Hz, 1 H), 7.47 - 7.52 (m, 4 H), 7.78 - 7.87 (m, 6 H), 8.08 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.37, 64.85, 67.43, 67.96, 68.44, 69.55, 69.66, 101.09, 105.57, 107.48, 110.59 (d, *J*=24.24Hz, C5), 114.72, 115.01, 119.94 (d, *J*=8.08Hz, C8), 121.63 (d, *J*=26.26 Hz, C7), 124.14, 126.09,

127.01, 128.01, 128.98, 131.41, 131.74, 152.28 (d, *J*=1.46 Hz, C9), 157.81, 159.53 (d, *J*=247.45 Hz, C6), 161.45, 162.98, 163.23, 163.36, 177.34, 177.35 (d, *J*=2.02 Hz, C4), 177.66; LRMS (ESI) m/z 690 $[M+H]^+$; HRMS (ESI) calcd for $C_{39}H_{33}N_3O_8F$ $[M+H]^+$ 690.2252, found 690.2220.

4.4.51. Synthesis of Methyl 3-(((4-oxo-2-(4-(2-(2-(4-((2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-3-yl)oxy)methyl)benzoate (**Ac13Az8**): This compound (78 mg) was obtained from **Ac13** and **Az8** in 94% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.80 - 3.84 (m, 2 H), 3.85 (s, 3 H), 3.89 - 3.93 (m, 2 H), 3.95 (t, *J*=4.88 Hz, 2 H), 4.10 - 4.16 (m, 2 H), 4.16 - 4.21 (m, 2 H), 4.57 (t, *J*=4.88 Hz, 2 H), 4.73 (s, 2 H), 5.11 (s, 2 H), 6.70 (s, 1 H), 6.88 - 6.96 (m, 4 H), 7.32 (t, *J*=7.57 Hz, 1 H), 7.37 (t, *J*=7.57 Hz, 1 H), 7.43 - 7.51 (m, 4 H), 7.57 (d, *J*=7.81 Hz, 1 H), 7.63 (ddd, *J*=8.54, 7.08, 1.95 Hz, 1 H), 7.78 (br. s., 1 H), 7.81 - 7.86 (m, 2 H), 7.90 (d, *J*=7.81 Hz, 1 H), 7.92 - 7.98 (m, 3 H), 8.06 (d, *J*=9.27 Hz, 1 H), 8.23 (dd, *J*=8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.51, 52.23, 65.01, 67.50, 68.15, 68.64, 69.83, 69.87, 73.51, 76.95, 77.26, 77.47, 77.58, 101.32, 107.70, 114.52, 114.94, 118.09, 123.78, 124.33, 124.88, 125.93, 126.32, 127.18, 128.51, 129.18, 129.44, 129.96, 130.33, 130.73, 131.59, 131.99, 133.40, 133.54, 137.42, 139.29, 155.35, 156.40, 158.02, 160.62, 163.18, 163.46, 166.99, 174.97, 177.87; LRMS (ESI) m/z 836 $[M+H]^+$, 858 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{42}N_3O_{11}$ $[M+H]^+$ 836.2819, found 836.2792; calcd for $C_{48}H_{42}N_3O_{11}Na$ $[M+Na]^+$ 858.2639, found 858.2606.

4.4.52. Synthesis of Methyl 3-(((4-oxo-2-(4-(2-(2-(2-(4-((2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-3-yl)oxy)methyl)benzoate (**Ac13Az9**): This compound (47 mg) was obtained from **Ac13** and **Az9** in 53% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ

ppm 3.62 - 3.66 (m, 2 H), 3.66 - 3.70 (m, 2 H), 3.81 - 3.85 (m, 2 H), 3.87 (s, 3 H), 3.88 (t, *J*=4.75 Hz, 2 H), 3.91 - 3.95 (m, 2 H), 4.14 - 4.17 (m, 2 H), 4.19 - 4.23 (m, 2 H), 4.54 (t, *J*=5.12 Hz, 2 H), 4.74 (s, 2 H), 5.13 (s, 2 H), 6.72 (s, 1 H), 6.90 - 6.99 (m, 4 H), 7.33 (t, *J*=7.57 Hz, 1 H), 7.36 - 7.41 (m, 1 H), 7.45 - 7.52 (m, 4 H), 7.58 (d, *J*=7.32 Hz, 1 H), 7.64 (ddd, *J*=8.42, 6.95, 1.71 Hz, 1 H), 7.79 (br. s., 1 H), 7.83 - 7.87 (m, 2 H), 7.91 (d, *J*=7.81 Hz, 1 H), 7.93 - 7.98 (m, 3 H), 8.09 (d, *J*=8.79 Hz, 1 H), 8.25 (dd, *J*=7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.29, 52.00, 64.76, 67.44, 67.93, 68.40, 69.42, 69.56, 70.53, 70.68, 73.27, 101.10, 107.48, 114.31, 114.69, 117.86, 123.39, 124.11, 124.62, 125.70, 126.09, 126.99, 128.27, 128.95, 129.21, 129.70, 130.09, 130.46, 131.36, 131.77, 133.16, 133.28, 137.18, 139.04, 155.12, 156.26, 157.81, 160.55, 162.97, 163.22, 166.76, 174.76, 177.66; LRMS (ESI) m/z 880 $[M+H]^+$; HRMS (ESI) calcd for $C_{50}H_{46}N_3O_{12}$ $[M+H]^+$ 880.3081, found 880.3043.

4.4.53. Synthesis of 3-(Benzyloxy)-2-(4-(2-(2-(4-((2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Ac13Az10**): This compound (39 mg) was obtained from **Ac13** and **Az10** in 50% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.79 - 3.84 (m, 2 H), 3.88 - 3.93 (m, 2 H), 3.95 (t, *J*=4.88 Hz, 2 H), 4.10 - 4.15 (m, 2 H), 4.15 - 4.21 (m, 2 H), 4.57 (t, *J*=4.88 Hz, 2 H), 4.73 (s, 2 H), 5.10 (s, 2 H), 6.71 (s, 1 H), 6.87 - 6.96 (m, 4 H), 7.22 - 7.29 (m, 3 H), 7.33 - 7.39 (m, 3 H), 7.43 - 7.51 (m, 4 H), 7.59 - 7.65 (m, 1 H), 7.77 (br. s., 1 H), 7.80 - 7.86 (m, 2 H), 7.98 - 8.04 (m, 2 H), 8.06 (d, *J*=8.75, 1 H), 8.23 (dd, *J*=8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.27, 64.76, 67.26, 67.89, 68.40, 69.57, 69.61, 73.83, 101.07, 107.45, 114.23, 114.70, 117.80, 123.73, 124.11, 124.55 , 125.67, 126.07, 126.94, 128.02, 128.18, 128.69, 128.92, 130.48, 131.34, 131.74, 133.20, 136.77, 139.30, 155.07, 155.84, 157.78, 160.31, 162.95,

163.21, 174.82, 177.63; LRMS (ESI) m/z 778 $[M+H]^+$; HRMS (ESI) calcd for $C_{46}H_{40}N_3O_9$ $[M+H]^+$ 778.2765, found 778.2791.

4.4.54. Synthesis of 7-(2-((1-(2-(2-((4-Oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac13Az11**): This compound (38 mg) was obtained from **Ac13** and **Az11** in 57% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.80 - 3.85 (m, 2 H), 3.91 (br. s., 2 H), 3.94 (t, *J*=4.88 Hz, 2 H), 4.12 - 4.19 (m, 4 H), 4.57 (t, *J*=4.88 Hz, 2 H), 4.72 (s, 2 H), 6.65 (s, 1 H), 6.67 (s, 1 H), 6.85 (dd, *J*=7.32, 2.44 Hz, 2 H), 6.90 (dd, *J*=9.03, 2.20 Hz, 1 H), 6.93 (dd, *J*=8.79, 1.95 Hz, 1 H), 7.41 - 7.51 (m, 6 H), 7.80 (ddd, *J*=7.69, 3.29, 1.71 Hz, 5 H), 8.03 (d, *J*=8.78 Hz, 1 H), 8.07 (d, *J*=9.27 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.24, 64.76, 67.69, 67.87, 68.41, 69.28, 69.59, 100.97, 101.03, 107.34, 107.37, 114.51, 114.68, 117.82, 117.97, 126.00, 126.02, 126.82, 127.00, 128.88, 128.90, 131.33, 131.37, 131.58, 131.64, 157.70, 162.86, 162.92, 162.95, 163.14, 177.52, 177.59; LRMS (ESI) m/z 672 $[M+H]^+$; HRMS (ESI) calcd for $C_{39}H_{34}N_3O_8$ $[M+H]^+$ 672.2346, found 672.2317.

4.4.55. Synthesis of 7-(2-((1-(2-(2-(2-((4-Oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac13Ac12**): This compound (32 mg) was obtained from **Ac13** and **Az12** in 45% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.60 - 3.66 (m, 2 H), 3.66 - 3.70 (m, 2 H), 3.81 - 3.86 (m, 2 H), 3.88 - 3.90 (m, 2 H), 3.94 (br. s., 2 H), 4.17 - 4.25 (m, 4 H), 4.53 - 4.55 (m, 2 H), 4.73 (br. s., 2 H), 6.71 (s, 2 H), 6.87 - 6.98 (m, 4 H), 7.43 - 7.54 (m, 6 H), 7.80 - 7.88 (m, 5 H), 8.08 (t, *J*=8.54 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.45, 64.80, 67.93, 67.99, 68.43, 69.36, 69.37, 70.51, 70.71, 101.07, 101.16, 107.44,

107.45, 114.58, 114.68, 118.01, 126.08, 126.96, 127.04, 128.94, 131.37, 131.72, 157.79, 157.80, 162.97, 163.18, 163.20, 177.63; LRMS (ESI) m/z 716 $[M+H]^+$; HRMS (ESI) calcd for $C_{41}H_{38}N_3O_9$ $[M+H]^+$ 716.2608, found 716.2577.

4.4.56. Synthesis of 2,2'-((((((4,4'-((Benzylazanediyl)bis(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4*H*-chromen-4-one) (**Ac15(Az1)$_2$**): This compound (88 mg) was obtained from **Ac15** and **Az1** in 99% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.62 - 3.74 (m, 2 H), 3.75 - 3.81 (m, 2 H), 3.93 (br. s., 2 H), 4.06 - 4.12 (m, 2 H), 4.52 – 4.54 (m, 2 H), 6.65 (s, 1 H), 6.91 (d, *J*=9.27 Hz, 2 H), 7.11 - 7.18 (m, 1 H), 7.22 (t, *J*=7.08 Hz, 1 H), 7.29 - 7.39 (m, 2 H), 7.48 (d, *J*=8.30 Hz, 1 H), 7.63 (ddd, *J*=8.54, 7.08, 1.46 Hz, 1 H), 7.68 - 7.79 (m, 3 H), 8.15 (dd, *J*=7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.11, 67.34, 69.37, 69.55, 106.00, 114.84, 117.83, 123.74, 124.08, 124.94, 125.41, 126.89, 127.83, 127.89, 128.13, 128.73, 133.44, 155.97, 161.22, 163.06, 178.10; LRMS (ESI) m/z 886 $[M+H]^+$; HRMS (ESI) calcd for $C_{51}H_{48}N_7O_8$ $[M+H]^+$ 886.3564, found 886.3524.

4.4.57. Synthesis of 2,2'-(((((((((4,4'-((Benzylazanediyl)bis(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4*H*-chromen-4-one) (**Ac15(Az2)$_2$**): This compound (48 mg) was obtained from **Ac15** and **Az2** in 49% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.60 - 3.90 (m, 10 H), 4.10 - 4.18 (m, 2 H), 4.53 (br. s., 2 H), 6.72 (s, 1 H), 6.99 (d, *J*=8.79 Hz, 2 H), 7.17 - 7.33 (m, 2 H), 7.33 - 7.43 (m, 2 H), 7.50 - 7.56 (m, 1 H), 7.64 - 7.70 (m, 1 H), 7.72 - 7.89 (m, 3 H), 8.20 (dd, *J*=7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 67.62, 69.53, 70.59, 70.72, 106.19, 115.01, 117.93, 124.19, 125.04,

125.60, 127.12, 127.94, 128.32, 133.52, 156.14, 161.50, 163.25, 178.26; LRMS (ESI) m/z 974 $[M+H]^+$; HRMS (ESI) calcd for $C_{55}H_{56}N_7O_{10}$ $[M+H]^+$ 974.4089, found 974.4064.

4.4.58. Synthesis of 2,2'-(((((((((4,4'-((Benzylazanediyl)bis(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))bis(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(6-methyl-4*H*-chromen-4-one) (**Ac15(Az3)$_2$**): This compound (99 mg) was obtained from **Ac15** and **Az3** in 99% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.41 (s, 3 H), 3.59 - 3.70 (m, 5 H), 3.73 (br. s., 1 H), 3.76 - 3.81 (m, 2 H), 3.87 (br. s., 2 H), 4.10 – 4.12 (m, 2 H), 4.51 (br. s., 2 H), 6.66 (s, 1 H), 6.95 (d, *J*=8.79 Hz, 2 H), 7.14 - 7.23 (m, 1 H), 7.23 - 7.31 (m, 1 H), 7.31 - 7.47 (m, 3 H), 7.70 - 7.82 (m, 3 H), 7.95 (s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.80, 67.50, 69.37, 69.43, 70.50, 70.62, 105.90, 114.87, 117.60, 123.43, 124.16, 124.83, 126.95, 127.80, 128.20, 128.84, 134.64, 134.89, 154.29, 161.33, 163.01, 178.27; LRMS (ESI) m/z 1002 $[M+H]^+$; HRMS (ESI) calcd for $C_{57}H_{60}N_7O_{10}$ $[M+H]^+$ 1002.4402, found 1002.4353.

4.4.59. Synthesis of 2,2'-(((((((((4,4'-((Benzylazanediyl)bis(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))bis(oxy))*bis*(4,1-phenylene))*bis*(3-(benzyloxy)-4*H*-chromen-4-one) (**Ac15(Az5)$_2$**): This compound (110 mg) was obtained from **Ac15** and **Az5** in 92% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.61 - 3.66 (m, 2 H), 3.66 - 3.72 (m, 2 H), 3.76 (br. s., 2 H), 3.80 - 3.86 (m, 2 H), 3.88 (t, *J*=5.12 Hz, 2 H), 4.12 - 4.19 (m, 2 H), 4.53 (t, *J*=4.88 Hz, 2 H), 5.11 (s, 2 H), 6.94 (d, *J*=9.27 Hz, 2 H), 7.24 - 7.30 (m, 4 H), 7.35 - 7.42 (m, 4 H), 7.51 (d, *J*=7.81 Hz, 1 H), 7.66 (ddd, *J*=8.54, 7.08, 1.95 Hz, 1 H), 7.74 (br. s., 1 H), 7.99 - 8.05 (m, 2 H), 8.28 (dd, *J*=8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.10, 67.44,

69.47, 69.54, 70.56, 70.68, 73.83, 114.26, 117.83, 123.47, 124.11, 124.52, 125.64, 126.98, 127.99, 128.14 , 128.23, 128.71, 128.88, 130.44, 133.17, 136.71, 139.23, 155.08, 156.09, 160.50, 174.85; LRMS (ESI) m/z 1186 $[M+H]^+$; HRMS (ESI) calcd for $C_{69}H_{68}N_7O_{12}$ $[M+H]^+$ 1186.4926, found 1186.4880.

4.4.60. Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-((benzylazanediyl)*bis*(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4*H*-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (**Ac15(Az8)$_2$**): This compound (120 mg) was obtained from **Ac15** and **Az8** in 98% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.74 (br. s., 2 H), 3.80 - 3.82 (m, 2 H), 3.86 (s, 3 H), 3.94 (t, *J*=5.12 Hz, 2 H), 4.10 - 4.15 (m, 2 H), 4.54 (t, *J*=5.12 Hz, 2 H), 5.12 (s, 2 H), 6.87 - 6.93 (m, 2 H), 7.17 (d, *J*=7.32 Hz, 1 H), 7.24 (t, *J*=7.57 Hz, 1 H), 7.32 (t, *J*=7.81 Hz, 1 H), 7.35 - 7.42 (m, 2 H), 7.49 (d, *J*=7.81 Hz, 1 H), 7.56 - 7.60 (m, 1 H), 7.65 (ddd, *J*=8.54, 7.08, 1.46 Hz, 1 H), 7.75 (br. s., 1 H), 7.89 - 7.98 (m, 4 H), 8.26 (dd, *J*=8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 47.53, 50.11, 51.98, 57.43, 67.30, 69.55, 69.69, 73.27, 114.28, 117.88, 123.43, 124.10, 124.22, 124.63, 125.68, 126.99 , 128.21, 128.25, 128.89, 129.20, 129.72, 130.07, 130.48, 133.16, 133.30, 137.14, 139.02, 144.38, 155.13, 156.31, 160.39, 166.73, 174.78; LRMS (ESI) m/z 1214 $[M+H]^+$; HRMS (ESI) calcd for $C_{69}H_{64}N_7O_{14}$ $[M+H]^+$ 1214.4511, found 1214.4476.

4.4.61. Synthesis of Dimethyl 3,3'-(((2,2'-(((((((((4,4'-((benzylazanediyl)*bis*(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))bis(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4*H*-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate

(**Ac15(Az9)$_2$**): This compound (120 mg) was obtained from **Ac15** and **Az9** in 90% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.60 - 3.65 (m, 3 H), 3.65 - 3.70 (m, 3 H), 3.77 (br. s., 2 H), 3.80 - 3.84 (m, 2 H), 3.85 - 3.90 (m, 5 H), 4.14 (t, *J*=4.64 Hz, 2 H), 4.52 (t, *J*=5.12 Hz, 2 H), 5.13 (s, 2 H), 6.93 (d, *J*=8.79 Hz, 2 H), 7.20 - 7.22 (d, *J*=6.83 Hz, 1 H), 7.29 (t, *J*=7.08 Hz, 1 H), 7.33 (t, *J*=7.81 Hz, 1 H), 7.40 (t, *J*=7.57 Hz, 2 H), 7.50 (d, *J*=8.79 Hz, 1 H), 7.58 (d, *J*=7.32 Hz, 1 H), 7.63 - 7.69 (m, 1 H), 7.75 (br. s., 1 H), 7.91 (d, *J*=7.81 Hz, 1 H), 7.93 - 7.99 (m, 3 H), 8.27 (d, *J*=7.81 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.20, 52.03, 67.49, 69.56, 69.61, 70.64, 70.75, 73.32, 114.36, 117.92, 123.38, 124.16, 124.66, 125.75, 128.29, 129.25 , 129.76 , 130.12, 130.51, 133.21, 133.31, 137.19, 139.07, 155.19, 156.42, 160.62, 166.80, 174.84; LRMS (ESI) m/z 1302 $[M+H]^+$; HRMS (ESI) calcd for $C_{73}H_{72}N_7O_{16}$ $[M+H]^+$ 1302.4980, found 1302.5036.

4.4.62. Synthesis of 7,7'-(((((4,4'-((Benzylazanediyl)bis(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl)) *s* (ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(2-phenyl-4*H*-chromen-4-one) (**Ac15(Az11)$_2$**): This compound (88 mg) was obtained from **Ac15** and **Az11** in 99% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.61 - 4.17 (m, 8 H), 4.56 (br. s., 2 H), 6.73 (s, 1 H), 6.92 (br. s., 2 H), 7.16 (br. s., 1 H), 7.26 (d, *J*=1.46 Hz, 1 H), 7.44 - 7.56 (m, 3 H), 7.86 (d, *J*=7.81 Hz, 2 H), 8.07 (d, *J*=8.30 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 67.89, 69.36, 101.25, 107.51, 114.56, 126.16, 127.10, 128.99, 131.42, 131.78, 157.83, 163.05, 177.68; LRMS (ESI) m/z 886 $[M+H]^+$; HRMS (ESI) calcd for $C_{51}H_{48}N_7O_8$ $[M+H]^+$ 886.3564, found 886.3521.

4.4.63. Synthesis of 7,7'-(((((((4,4'-((Benzylazanediyl)*bis*(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-

diyl))*bis*(oxy))*bis*(2-phenyl-4*H*-chromen-4-one) (**Ac15(Az12)$_2$**): This compound (58 mg) was obtained from **Ac15** and **Az12** in 60% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.59 - 3.65 (m, 2 H), 3.65 - 3.71 (m, 2 H), 3.77 (br. s., 2 H), 3.81 - 3.86 (m, 2 H), 3.87 - 3.89 (m, 2 H), 4.16 - 4.22 (m, 2 H), 4.52 (t, *J*=4.88 Hz, 2 H), 6.74 (s, 1 H), 6.91 - 6.99 (m, 2 H), 7.19 - 7.22 (m, 1 H), 7.28 (t, *J*=7.08 Hz, 1 H), 7.39 (br. s., 1 H), 7.46 - 7.55 (m, 3 H), 7.77 (br. s., 1 H), 7.85 - 7.91 (m, 2 H), 8.10 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.21, 68.06, 69.42, 69.52, 70.64, 70.79, 101.20, 107.53, 114.65, 117.98, 126.15, 127.05, 128.33, 128.98, 131.40, 131.83, 157.86, 163.04, 163.26, 177.74; LRMS (ESI) m/z 974 $[M+H]^+$; HRMS (ESI) calcd for $C_{55}H_{56}N_7O_{10}$ $[M+H]^+$ 974.4089, found 974.4063.

4.4.64. Synthesis of 7,7'-(((4,4'-((Benzylazanediyl)*bis*(methylene))*bis*(1*H*-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(2-phenyl-4*H*-chromen-4-one) (**Ac15(Az13)$_2$**): This compound (69 mg) was obtained from **Ac15** and **Az13** in 87% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.75 (br. s., 2 H), 4.50 (br. s., 2 H), 4.83 (br. s., 2 H), 6.73 (s, 1 H), 6.94 (br. s., 2 H), 7.31 (br. s., 1 H), 7.46 - 7.56 (m, 3 H), 7.86 (d, *J*=7.81 Hz, 2 H), 8.10 (d, *J*=8.30 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 53.84, 69.47, 101.42, 107.51, 114.28, 126.14, 127.36, 128.48, 128.99, 131.49, 131.61, 157.71, 162.13, 163.14, 177.52; LRMS (ESI) m/z 798 $[M+H]^+$; HRMS (ESI) calcd for $C_{47}H_{40}N_7O_6$ $[M+H]^+$ 798.3040, found 798.3013.

4.4.65. Synthesis of 2,2'-(((4,4'-((Benzylazanediyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))diethanol (**Ac15(Az16)$_2$**): This compound (28.5 mg) was obtained from **Ac15** and **Az16** in 64% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.54 - 3.59 (m, 4 H) 3.68 - 3.75 (m, 10 H) 3.82 - 3.88 (m, 4 H)

4.51 (t, *J*=4.88 Hz, 4 H) 7.19 - 7.24 (m, 1 H) 7.30 (t, *J*=7.57 Hz, 2 H) 7.40 (d, *J*=7.32 Hz, 2 H) 7.84 (s, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 47.55 (s) 50.14 (s) 58.38 (s) 61.40 (s) 69.13 (s) 72.61 (s) 124.55 (s) 127.06 (s) 128.27 (s) 128.90 (s) 138.67 (s) 144.44 (s). LRMS (ESI) m/z 446 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{32}N_7O_4$ $[M+H]^+$ 446.5153, found 446.5258.

4.4.66. Synthesis of 2,2'-((((((4,4'-((Benzylazanediyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(3-((3-methoxybenzyl)oxy)-4H-chromen-4-one) (Ac15(Az17)$_2$): This compound (30.6 mg) was obtained from **Ac15** and **Az17** in 49% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.63 (br. s., 1 H), 3.70 (s, 3 H), 3.74 (br. s., 2 H), 3.81 (t, *J* = 4.88 Hz, 2 H), 3.94 (t, *J* = 4.88 Hz, 2 H), 4.11 (t, *J* = 4.89 Hz, 2 H), 4.53 (t, *J* = 4.89 Hz, 2 H), 5.09 (s, 2 H), 6.76 - 6.81 (m, 1 H), 6.88 - 6.95 (m, 4 H), 7.16 (t, *J* = 7.70 Hz, 2 H), 7.23 (t, *J* = 7.34 Hz, 1 H), 7.31 - 7.43 (m, 2 H), 7.50 (d, *J* = 8.56 Hz, 1 H), 7.65 (ddd, *J* = 8.50, 7.03, 1.59 Hz, 1 H), 7.73 (br. s., 1 H), 8.00 (d, *J* = 8.80 Hz, 2 H), 8.27 (dd, *J* = 8.07, 1.47 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 47.6, 50.2, 55.1, 57.6, 67.3, 69.6, 69.7, 73.8, 113.7, 114.2, 114.3, 117.9, 121.0, 123.7, 124.2, 124.6, 125.7, 127.0, 128.2, 128.9, 129.2, 130.5, 133.2, 138.3, 139.3, 155.1, 156.1, 159.5, 160.4, 174.9**;** LRMS (ESI) m/z 1158.5 base peak: 1158.5 $[M+H]^+$; HRMS (ESI) calcd for $C_{67}H_{64}N_7O_{12}[M+H]^+$ 1158.4613, found 1158.4630.

4.4.67. Synthesis of 7-(2-(Benzyl((1-(2-(2-(4-(4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac16Az1**): This compound was obtained according to the procedure as described.[54]

4.4.68. Synthesis of 7-(2-(Benzyl((1-(2-(2-(2-(4-(4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4*H*-

chromen-4-one (**Ac16Az2**): This compound was obtained according to the procedure as described.[54]

4.4.69. Synthesis of 7-(2-(Benzyl((1-(2-(2-(2-(4-(6-methyl-4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac16Az3**): This compound (28 mg) was obtained from **Ac16** and **Az3** in 34% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.38 (s, 3 H), 2.94 (br. s., 2 H), 3.45 - 3.68 (m, 6 H), 3.68 - 3.76 (m, 3 H), 3.82 (br. s., 3 H), 4.05 (m, 2 H), 4.11 (br. s., 2 H), 4.48 (br. s., 2 H), 6.62 (s, 1 H), 6.67 (s, 1 H), 6.81 - 6.92 (m, 4 H), 7.16 - 7.21 (m, 2 H), 7.26 (br. s., 2 H), 7.31 - 7.37 (m, 2 H), 7.37 - 7.48 (m, 5 H), 7.74 (d, *J*=8.79 Hz, 2 H), 7.78 - 7.83 (m, 2 H), 7.91 (s, 1 H), 8.02 (d, *J*=8.79 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.90, 67.58, 69.52, 69.55, 70.59, 70.76, 101.05, 106.10, 107.48, 114.66, 114.95, 117.67, 124.41, 125.01, 126.13, 127.03, 127.91, 128.44, 128.98, 131.40, 131.78, 134.75, 135.02, 154.42, 157.91, 161.37, 162.99, 163.05, 177.72, 178.39; LRMS (ESI) m/z 819 $[M+H]^+$, 841 $[M+Na]^+$; HRMS (ESI) calcd for $C_{49}H_{47}N_4O_8$ $[M+H]^+$ 819.3394, found 819.3392; calcd for $C_{49}H_{46}N_4O_8Na$ $[M+Na]^+$ 841.3213, found 841.3220.

4.4.70. Synthesis of 7-(2-(Benzyl((1-(2-(2-(2-(4-(6-fluoro-4-oxo-4H-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4H-chromen-4-one (Ac16Az4): This compound was obtained according to the procedure as described.[54]

4.4.71. Synthesis of 7-(2-(Benzyl((1-(2-(2-(2-(4-(3-(benzyloxy)-4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac16Az5**): This compound (29 mg) was obtained from **Ac16** and **Az5** in 31%

yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.00 (br. s., 2 H), 3.57 - 3.70 (m, 4 H), 3.70 - 3.85 (m, 4 H), 3.88 - 3.98 (m, 4 H), 4.09 - 4.23 (m, 4 H), 4.53 (t, *J*=4.88 Hz, 2 H), 5.10 (s, 2 H), 6.73 (s, 1 H), 6.87 - 6.95 (m, 4 H), 7.20 - 7.42 (m, 11 H), 7.45 - 7.52 (m, 4 H), 7.64 (td, *J*=7.81, 1.46 Hz, 1 H), 7.70 (br. s., 1 H), 7.86 - 7.59 (m, 2 H), 8.00 (d, *J*=8.75 Hz, 2 H), 8.08 (d, *J*=8.79 Hz, 1 H), 8.25 (dd, *J*=8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.33, 67.44, 69.51, 69.57, 70.55, 70.72, 73.85, 100.98, 107.45, 114.26, 114.67, 117.83, 123.58, 124.14, 124.55, 125.70 , 126.11, 126.94, 127.18, 128.02, 128.17, 128.34, 128.72, 128.79, 128.94, 130.47, 131.36, 131.77, 133.19, 136.77, 139.29, 155.10, 156.01, 157.89, 160.46, 162.95, 163.28, 174.87, 177.70; LRMS (ESI) m/z 911 $[M+H]^+$, 933 $[M+Na]^+$; HRMS (ESI) calcd for $C_{55}H_{51}N_4O_9$ $[M+H]^+$ 911.3656, found 911.3662; calcd for $C_{55}H_{50}N_4O_9Na$ $[M+Na]^+$ 933.3475, found 933.3487.

4.4.72. Synthesis of 7-(2-(Benzyl((1-(2-(2-(4-(6-fluoro-4-oxo-4*H*-chromen-2-yl)phenoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac16Az7**): This compound was obtained according to the procedure as described.[54]

4.4.73. Synthesis of 7-(2-(Benzyl((1-(2-(2-(2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac16Az12**): This compound (33 mg) was obtained from **Ac16** and **Az12** in 41% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.99 (br. s., 2 H), 3.58 - 3.69 (m, 4 H), 3.71 - 3.84 (m, 4 H), 3.88 (t, *J*=5.12 Hz, 2 H), 3.93 (br. s., 2 H), 4.11 - 4.20 (m, 4 H), 4.53 (t, *J*=5.12 Hz, 2 H), 6.72 (s, 1 H), 6.72 (s, 1 H), 6.87 - 6.96 (m, 4 H), 7.20 - 7.25 (m, 1 H), 7.30 (t, *J*=7.57 Hz, 2 H), 7.33 - 7.41 (m, 2 H), 7.45 - 7.54 (m, 6 H), 7.68 (br.

s., 1 H), 7.82 - 7.89 (m, 4 H), 8.08 (dd, *J*=8.79, 2.93 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 49.28, 50.25, 51.69, 58.83, 67.24, 67.99, 69.38, 69.55, 70.57, 70.78, 100.97, 101.14, 107.45, 107.51, 114.56, 114.67, 117.83, 118.00, 123.80, 126.11, 126.96, 127.06, 127.20, 128.36, 128.78, 128.96, 131.37, 131.39, 131.77, 138.96, 157.80, 157.88, 162.95, 162.97, 163.16, 163.25, 177.65, 177.69; LRMS (ESI) m/z 805 $[M+H]^+$, 827 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{45}N_4O_8$ $[M+H]^+$ 805.3237, found 805.3265; calcd for $C_{48}H_{44}N_4O_8Na$ $[M+Na]^+$ 827.3057, found 827.3078.

4.4.74. Synthesis of 7-(2-(Benzyl((1-(2-((4-oxo-2-phenyl-4*H*-chromen-7-yl)oxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one (**Ac16Az13**): This compound (18 mg) was obtained from **Ac16** and **Az13** in 25% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.00 (br. s., 2 H), 3.75 (br. s., 2 H), 3.97 (br. s., 2 H), 4.16 (br. s., 2 H), 4.47 (t, *J*=4.88 Hz, 2 H), 4.81 (t, *J*=4.64 Hz, 2 H), 6.99 (s, 1 H), 6.70 (s, 1 H), 6.84 - 6.93 (m, 4 H), 7.20 - 7.25 (m, 1 H), 7.30 (t, *J*=7.32 Hz, 2 H), 7.36 (br. s., 2 H), 7.44 - 7.53 (m, 6 H), 7.73 (br. s., 1 H), 7.85 (dd, *J*=7.56, 1.71 Hz, 2 H), 7.82 (dd, *J*=8.05, 1.22 Hz, 2 H), 8.06 (dd, *J*=9.03, 2.20 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 49.48, 51.83, 58.83, 66.84, 67.21, 101.02, 101.34, 107.44, 107.51, 114.08, 114.59, 117.84, 118.54, 124.00, 126.09, 126.11, 126.96, 127.28, 127.39, 128.38, 128.73, 128.95, 128.98, 131.38, 131.49, 131.58, 131.76, 138.71, 157.67, 157.85, 162.08, 162.93, 163.09, 163.21, 177.44, 177.66; LRMS (ESI) m/z 717 $[M+H]^+$, 739 $[M+Na]^+$; HRMS (ESI) calcd for $C_{44}H_{37}N_4O_6$ $[M+H]^+$ 717.2713, found 717.2729; calcd for $C_{44}H_{36}N_4O_6Na$ $[M+Na]^+$ 739.2533, found 739.2541.

4.4.75 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-(1,3-phenylene)*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))bis(oxy))*bis*(ethane-2,1-diyl))bis(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac22(Az8)$_2$): This

compound (72.0 mg) was obtained from **Ac22** and **Az8** in 75% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.78 - 3.87 (m, 5 H), 3.92 - 3.97 (m, 2 H), 4.09 - 4.15 (m, 2 H), 4.55 (t, *J* = 4.88 Hz, 2 H), 5.08 (s, 2 H), 6.84 - 6.91 (m, 1 H), 6.85 - 6.90 (m, 2 H), 7.27 - 7.39 (m, 2 H), 7.46 (d, *J* = 8.30 Hz, 1 H), 7.54 (d, *J* = 7.81 Hz, 1 H), 7.63 (ddd, *J* = 8.54, 7.08, 1.46 Hz, 1 H), 7.70 (dd, *J* = 7.81, 1.46 Hz, 1 H), 7.84 - 7.91 (m, 3 H), 7.92 - 8.00 (m, 2 H), 8.24 (dd, *J* = 8.30, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.3, 51.9, 67.2, 69.4, 69.5, 69.6, 73.2, 114.2, 117.9, 121.1, 122.7, 123.3, 124.0, 124.6, 125.2, 125.6, 128.2, 129.1, 129.7, 130.0, 130.4, 131.1, 133.1, 133.3, 137.1, 138.9, 147.2, 155.1, 156.3, 160.4, 166.7, 174.7; LRMS (ESI) m/z 1157 $[M+H]^+$; HRMS (ESI) calcd for $C_{66}H_{57}N_6O_{14}$ $[M+H]^+$ 1157.3933, found 1157.3907.

4.4.76 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-(1,4-phenylene)*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))bis(oxy))*bis*(methylene))dibenzoate (Ac23(Az8)$_2$): This compound (52.0 mg) was obtained from **Ac23** and **Az8** in 65% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.83 - 3.89 (m, 5 H), 3.96 - 4.02 (m, 2 H), 4.14 (dd, *J* = 5.12, 3.66 Hz, 2 H), 4.60 (t, *J* = 4.88 Hz, 2 H), 5.12 (s, 2 H), 6.87 - 6.91 (m, 2 H), 7.32 (t, *J* = 7.81 Hz, 1 H), 7.37 - 7.44 (m, 1 H), 7.50 (d, *J* = 8.30 Hz, 1 H), 7.55 - 7.58 (m, 1 H), 7.62 - 7.69 (m, 1 H), 7.76 (s, 2 H), 7.89 - 7.94 (m, 4 H), 7.95 - 7.98 (m, 1 H), 8.26 (dd, *J* = 8.05, 1.71 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.4, 52.0, 67.4, 69.7, 69.8, 73.3, 114.3, 118.0, 120.9, 123.5, 124.1, 124.6, 125.6, 126.0, 128.3, 129.2, 129.8, 130.1, 130.3, 130.5, 133.2, 133.3, 137.1, 139.0, 147.3, 155.2, 156.3, 160.4, 166.8, 174.8; LRMS (ESI) m/z 1157 $[M+H]^+$; HRMS (ESI) calcd for $C_{66}H_{57}N_6O_{14}$ $[M+H]^+$ 1157.3933, found 1157.3942.

4.4.77 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-(propane-1,3-diyl)*bis*(1H-1,2,3-triazole-4,1-diyl))bis(ethane-2,1-diyl))bis(oxy))*bis*(ethane-2,1-diyl))bis(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac24(Az8)$_2$) : This compound (55.0 mg) was obtained from **Ac24** and **Az8** in 65% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.99 - 2.07 (m, 1 H), 2.74 (t, *J* = 7.57 Hz, 2 H), 3.80 - 3.84 (m, 2 H), 3.87 (s, 3 H), 3.91 - 3.95 (m, 2 H), 4.13 - 4.16 (m, 2 H), 4.51 (t, *J* = 5.12 Hz, 2 H), 5.14 (s, 2 H), 6.92 - 6.95 (m, 2 H), 7.34 (t, *J* = 7.81 Hz, 1 H), 7.41 (ddd, *J* = 8.05, 7.08, 0.98 Hz, 1 H), 7.47 (s, 1 H), 7.52 (d, *J* = 8.30 Hz, 1 H), 7.58 - 7.61 (m, 1 H), 7.65 - 7.69 (m, 1 H), 7.92 (dt, *J* = 7.81, 1.46 Hz, 1 H), 7.95 - 8.00 (m, 3 H), 8.28 (dd, *J* = 7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 25.0, 29.1, 50.0, 52.0, 67.3, 69.6, 69.8, 73.3, 114.3, 117.9, 122.0, 123.5, 124.1, 124.7, 125.7, 128.3, 129.2, 129.8, 130.1, 130.5, 133.2, 133.3, 137.2, 139.1, 147.4, 155.2, 156.3, 160.4, 166.8, 174.8; LRMS (ESI) m/z 1123 $[M+H]^+$; HRMS (ESI) calcd for $C_{63}H_{59}N_6O_{14}$ $[M+H]^+$ 1123.4089, found 1123.4125

4.4.78 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-(oxy*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))bis(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac25(Az8)$_2$): This compound (45.0 mg) was obtained from **Ac25** and **Az8** in 57% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.81 (dd, *J* = 5.37, 3.90 Hz, 3 H), 3.84 - 3.88 (m, 3 H), 3.93 (t, *J* = 5.12 Hz, 2 H), 4.13 (dd, *J* = 5.12, 3.66 Hz, 2 H), 4.54 (t, *J* = 5.12 Hz, 2 H), 4.68 (s, 2 H), 5.13 (s, 2 H), 6.93 (d, *J* = 8.79 Hz, 2 H), 7.33 (t, *J* = 7.57 Hz, 1 H), 7.37 - 7.41 (m, 1 H), 7.51 (d, *J* = 7.81 Hz, 1 H), 7.56 - 7.60 (m, 1 H), 7.62 - 7.69 (m, 1 H), 7.76 (s, 1 H), 7.91 (d, *J* = 7.81 Hz, 1 H), 7.94 - 7.99 (m, 3 H), 8.26 (dd, *J* = 7.81, 1.46 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 51.3, 53.2, 64.7, 68.4, 70.7, 70.8, 74.4, 106.7, 115.4, 119.1, 124.6, 125.3,

125.8, 126.8, 129.4, 130.4, 130.9, 131.2, 131.7, 134.3, 134.5, 138.3, 140.2, 156.3, 157.5, 161.6, 167.9, 176.0; LRMS (ESI) m/z 1125 $[M+H]^+$ (ditto); HRMS (ESI) calcd for $C_{62}H_{57}N_6O_{15}$ $[M+H]^+$ 1125.3882, found 1125.3889.

4.4.79 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-((phenylazanediyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))bis(ethane-2,1-diyl))bis(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac26(Az8)$_2$): This compound (34.0 mg) was obtained from **Ac26** and **Az8** in 58% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.69 - 3.80 (m, 4 H), 3.85 - 3.96 (m, 10 H), 4.02 - 4.11 (m, 4 H), 4.42 - 4.53 (m, 4 H), 4.64 (s, 4 H), 5.14 (s, 4 H), 6.61 - 6.72 (m, 1 H), 6.83 (d, *J* = 8.31 Hz, 2 H), 6.91 (d, *J* = 9.05 Hz, 4 H), 7.13 (t, *J* = 7.82 Hz, 2 H), 7.34 (t, *J* = 7.70 Hz, 2 H), 7.41 (t, *J* = 7.58 Hz, 2 H), 7.49 - 7.62 (m, 6 H), 7.63 - 7.71 (m, 2 H), 7.88 - 8.03 (m, 8 H), 8.25 - 8.31 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 46.8, 50.2, 52.0, 67.3, 69.6, 69.7, 73.3, 113.7, 114.3, 117.7, 117.9, 123.1, 123.5, 124.1, 124.7, 125.7, 128.3, 129.1, 129.2, 129.8, 130.1, 130.5, 133.2, 133.3, 137.2, 139.0, 147.9, 155.2, 156.3, 160.4, 166.8, 174.8; LRMS (ESI) m/z 1200 $[M+H]^+$; HRMS (ESI) calcd for $C_{68}H_{62}N_7O_{14}$ $[M+H]^+$ 1200.4355, found 1200.4362.

4.4.80 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-(((pyridin-4-ylmethyl)azanediyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))bis(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac28(Az8)$_2$) : This compound (23.0 mg) was obtained from **Ac28** and **Az8** in 54% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.65 (s, 1 H), 3.74 (s, 2 H), 3.81 - 3.86 (m, 2 H), 3.88 (s, 3 H),

3.93 - 3.99 (m, 2 H), 4.11 - 4.19 (m, 2 H), 4.56 (t, *J* = 5.14 Hz, 2 H), 5.15 (s, 2 H), 6.93 (d, *J* = 9.05 Hz, 2 H), 7.29 - 7.37 (t, *J* = 7.90 Hz 2 H), 7.39 - 7.44 (t, *J* = 8.28 Hz 1 H), 7.52 (d, *J* = 8.56 Hz, 1 H), 7.60 (d, *J* = 7.58 Hz, 1 H), 7.63 - 7.72 (m, 1 H), 7.74 (s, 1 H), 7.87 - 8.01 (m, 4 H), 8.29 (d, *J* = 6.60 Hz, 1 H), 8.49 (br. s., 1 H) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 50.6, 52.1, 67.4, 69.2, 69.6, 73.3, 114.3, 118.0, 123.5, 124.1, 124.8, 125.7, 128.4, 129.3, 129.8, 130.0, 130.5, 133.3, 133.5, 137.1, 139.0, 155.1, 156.3, 160.3, 166.8, 174.8; LRMS (ESI) m/z 1215 $[M+H]^+$ (ditto) ; HRMS (ESI) calcd for $C_{68}H_{63}N_8O_{14}$ $[M+H]^+$ 1215.4467, found 1215.4521.

4.4.81 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-(piperazine-1,4-diyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac29(Az8)$_2$): This compound (25.0 mg) was obtained from **Ac29** and **Az8** in 52% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.91 (br. s., 4 H), 3.79 - 3.93 (m, 7 H), 3.96 (t, *J* = 5.01 Hz, 2 H), 4.13 - 4.21 (m, 2 H), 4.56 (t, *J* = 5.01 Hz, 2 H), 5.15 (s, 2 H), 6.95 (d, *J* = 9.05 Hz, 2 H), 7.30 - 7.48 (m, 2 H), 7.52 (d, *J* = 8.31 Hz, 1 H), 7.59 (d, *J* = 7.34 Hz, 1 H), 7.63 - 7.73 (m, 1 H), 7.86 - 7.95 (m, 2 H), 7.95 - 8.02 (m, 3 H), 8.28 (d, *J* = 7.82 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.1, 52.5, 67.3, 69.6, 73.3, 114.3, 117.9, 124.1, 125.7, 128.3, 129.9, 133.3, 137.1, 139.0, 143.8, 155.2, 156.4, 160.4, 166.8, 174.8; LRMS (ESI) m/z 1193 $[M+H]^+$ (ditto); HRMS (ESI) calcd for $C_{66}H_{65}N_8O_{14}$ $[M+H]^+$ 1193.4620 , found 1193.4635.

4.4.82 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-((1,4-diazepane-1,4-diyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-

diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac30(Az8)$_2$): This compound (30.0 mg) was obtained from **Ac30** and **Az8** in 59% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.95 (br. s., 2 H), 2.91 (br. s., 4 H), 3.83 - 3.91 (m, 6 H), 3.97 (t, *J* = 5.14 Hz, 2 H), 4.14 - 4.21 (m, 2 H), 4.57 (t, *J* = 5.01 Hz, 2 H), 5.15 (s, 2 H), 6.96 (d, *J* = 8.80 Hz, 2 H), 7.33 - 7.38 (t, *J* = 7.60 Hz 1 H), 7.39-7.44 (t, *J* = 7.60 Hz 1 H), 7.53 (d, *J* = 8.31 Hz, 1 H), 7.60 (d, *J* = 7.83 Hz, 1 H), 7.63 - 7.72 (m, 1 H), 7.87 - 7.95 (m, 2 H), 7.95 - 8.03 (m, 3 H), 8.28 (d, *J* = 7.34 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.3, 52.0, 52.5, 53.2, 67.4, 69.6, 73.3, 114.3, 117.9, 123.5, 124.1, 124.7, 125.0, 125.7, 129.6, 133.3, 137.0, 139.1, 155.0, 160.4, 166.8, 174.8; LRMS (ESI) m/z 1207 $[M+H]^+$; HRMS (ESI) calcd for $C_{67}H_{67}N_8O_{14}$ $[M+H]^+$ 1207.4777, found 1207.4761.

4.4.83 Synthesis of Dimethyl 3,3'-(((2,2'-((((((4,4'-((ethane-1,2-diyl)*bis*(methylazanediyl))bis(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac31(Az8)$_2$): This compound (32.0 mg) was obtained from **Ac31** and **Az8** in 69% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.22 (s, 3 H), 2.54 (s, 2 H), 3.68 (s, 2 H), 3.80 - 3.85 (m, 2 H), 3.88 (s, 3 H), 3.92 - 4.00 (m, 2 H), 4.09 - 4.18 (m, 2 H), 4.55 (t, *J* = 5.01 Hz, 2 H), 5.15 (s, 2 H), 6.94 (d, *J* = 8.80 Hz, 2 H), 7.32 - 7.37 (t, *J* = 7.60 Hz, 1 H), 7.38 - 7.43 (t, *J* = 6.80 Hz, 1 H), 7.51 (d, *J* = 8.31 Hz, 1 H), 7.59 (d, *J* = 7.82 Hz, 1 H), 7.63 - 7.71 (m, 2 H), 7.92 (d, *J* = 7.82 Hz, 1 H), 7.95 - 8.01 (m, 3 H), 8.28 (d, *J* = 8.07 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 42.4, 50.1, 52.0, 52.6, 54.4, 67.3, 69.6, 69.7, 73.3, 114.3, 117.9, 123.5, 123.7, 124.1, 124.7, 125.7, 128.3, 129.2, 129.8, 130.1, 130.5, 133.2, 133.3, 137.2, 139.1, 144.6, 155.2, 156.4, 160.4, 166.8, 174.8; LRMS

(ESI) m/z 1195 $[M+H]^+$; HRMS (ESI) calcd for $C_{66}H_{67}N_8O_{14}$ $[M+H]^+$ 1195.4777, found 1195.4814.

4.4.84 Synthesis of Dimethyl 3,3'-(((2,2'-(((((((((4,4'-(1,3-phenylene)*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))bis(oxy))*bis*(methylene))dibenzoate (Ac22(Az9)$_2$): This compound (22.0 mg) was obtained from **Ac22** and **Az9** in 65% yield according to the general procedure described above.^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.61 - 3.70 (m, 4 H), 3.81 (t, *J* = 5.08 Hz, 2 H), 3.85 (s, 3 H), 3.89 (t, *J* = 5.08 Hz, 2 H), 4.11(t, *J* = 4.96 Hz, 2 H), 4.55 (t, *J* = 4.96 Hz, 2 H), 5.10 (s, 2 H), 6.83 - 6.88 (m, 2 H), 7.31 (t, *J* = 7.61 Hz, 1 H), 7.34 - 7.40 (m, 2 H), 7.48 (d, *J* = 8.20 Hz, 1 H), 7.55 (d, *J* = 7.42 Hz, 1 H), 7.61 - 7.67 (m, 1 H), 7.75 (dd, *J* = 7.81, 1.56 Hz, 1 H), 7.86 - 7.92 (m, 3 H), 7.95 (s, 1 H), 8.02 (s, 1 H), 8.22 - 8.27 (m, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.4, 52.0, 67.5, 69.5, 69.5, 69.6, 70.6, 70.7, 73.3, 114.3, 117.9, 121.2, 122.7, 123.2, 124.1, 124.6, 125.2, 125.7, 128.3, 129.2, 129.3, 129.7, 130.1, 130.4, 131.3, 133.2, 133.3, 137.1, 139.0, 147.3, 155.1, 156.4, 160.6, 166.8, 174.8; LRMS (ESI) m/z 1245.5$[M+H]^+$ base peak: 1267.5 $[M+Na]^+$; HRMS (ESI) calcd for $C_{70}H_{65}N_6O_{16}[M+H]^+$ 1245.4457, found 1245.4473.

4.4.85 Synthesis of Dimethyl 3,3'-(((2,2'-(((((((((4,4'-(1,4-phenylene)*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))bis(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac23(Az9)$_2$): This compound (22.0 mg) was obtained from **Ac23** and **Az9** in 65% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.57 - 3.67 (m, 4 H), 3.76 (t, *J* = 4.68 Hz, 2 H), 3.85 (s, 3 H), 3.86 (t, *J*

= 4.68 Hz, 2 H), 4.05(t, *J* = 4.88 Hz, 2 H), 4.51 (t, *J* = 4.88 Hz, 2 H), 5.08 (s, 2 H), 6.84 (dd, *J* = 8.98, 1.17 Hz, 2 H), 7.26 - 7.32 (m, 1 H), 7.35 (t, *J* = 7.42 Hz, 1 H), 7.46 (d, *J* = 8.20 Hz, 1 H), 7.50 - 7.55 (m, 1 H), 7.58 - 7.65 (m, 1 H), 7.79 (d, *J* = 1.56 Hz, 2 H), 7.84 - 7.91 (m, 3 H), 7.91 - 7.95 (m, 2 H), 8.22 (d, *J* = 8.20 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.2, 51.9, 67.3, 69.3, 69.4, 70.5, 70.5, 73.2, 114.2, 117.9, 120.9, 123.2, 124.0, 124.6, 125.5, 125.9, 128.2, 129.2, 129.7, 130.0, 130.3, 130.4, 133.1, 133.2, 137.1, 138.9, 147.0, 155.1, 156.4, 160.5, 166.7, 174.7; LRMS (ESI) m/z 1245.5 $[M+H]^+$ base peak: 1267.5 $[M+Na]^+$; HRMS (ESI) calcd for $C_{70}H_{65}N_6O_{16}[M+H]^+$ 1245.4457, found 1245.4454.

4.4.86 Synthesis of Dimethyl 3,3'-(((2,2'-(((((((((4,4'-((phenylazanediyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac26(Az9)$_2$): This compound (21.0 mg) was obtained from **Ac26** and **Az9** in 55% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.50 - 3.60 (m, 4 H), 3.75 (t, *J* = 4.48 Hz, 2 H), 3.80 (t, *J* = 4.48 Hz, 2 H), 3.84 (s, 3 H), 4.08 (t, *J* = 4.46 Hz, 2 H), 4.43 (t, *J* = 4.46 Hz, 2 H), 4.63 (br. s., 2 H), 5.10 (s, 2 H), 6.67 (t, *J* = 7.22 Hz, 1 H), 6.82 (d, *J* = 7.81 Hz, 1 H), 6.90 (d, *J* = 7.81 Hz, 2 H), 7.06 - 7.16 (m, 1 H), 7.30 (t, *J* = 7.61 Hz, 1 H), 7.33 - 7.41 (m, 1 H), 7.47 (d, *J* = 8.20 Hz, 1 H), 7.55 (d, *J* = 6.25 Hz, 2 H), 7.59 - 7.67 (m, 1 H), 7.86 - 7.91 (m, 1 H), 7.91 - 7.99 (m, 3 H), 8.23 (d, *J* = 7.81 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 46.6, 50.2, 52.0, 67.4, 69.4, 69.5, 70.5, 70.6, 73.2, 113.6, 114.3, 117.7, 117.9, 123.1, 123.3, 124.0, 124.6, 125.6, 128.2, 129.1, 129.2, 129.7, 130.0, 130.4, 133.1, 133.3, 137.1, 139.0, 147.9, 155.1, 156.4, 160.5, 166.7, 174.8; LRMS (ESI) m/z 1288.5$[M+H]^+$ base peak: 1310.5 $[M+Na]^+$; HRMS (ESI) calcd for $C_{72}H_{70}N_7O_{16}[M+H]^+$ 1288.4879, found 1288.4938.

4.4.87 Synthesis of Dimethyl 3,3'-(((2,2'-(((((((((4,4'-(piperazine-1,4-diyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy))*bis*(4,1-phenylene))*bis*(4-oxo-4H-chromene-3,2-diyl))*bis*(oxy))*bis*(methylene))dibenzoate (Ac29(Az9)$_2$): This compound (41.0 mg) was obtained from **Ac29** and **Az9** in 45% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.48 (br. s., 4 H), 3.59 (s, 4 H), 3.64 (t, *J* = 4.59 Hz, 2 H), 3.75 - 3.86 (m, 7 H), 4.12 (t, *J* = 4.86 Hz, 2 H), 4.46 (t, *J* = 5.07 Hz, 2 H), 5.09 (s, 2 H), 6.87 - 6.95 (m, 2 H), 7.29 (t, *J* = 7.81 Hz, 1 H), 7.35 (t, *J* = 7.61 Hz, 1 H), 7.46 (d, *J* = 8.20 Hz, 1 H), 7.54 (d, *J* = 7.81 Hz, 1 H), 7.57 - 7.66 (m, 2 H), 7.87 (d, *J* = 7.81 Hz, 1 H), 7.90 - 7.97 (m, 3 H), 8.17 - 8.26 (m, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 50.1, 51.9, 52.5, 53.0, 67.4, 69.4, 69.5, 70.4, 70.6, 73.2, 114.3, 117.9, 123.3, 123.7, 124.0, 124.6, 125.6, 128.2, 129.2, 129.7, 130.0, 130.4, 133.1, 133.3, 137.1, 138.9, 143.9, 155.1, 156.4, 160.5, 166.7, 174.7; LRMS (ESI) m/z 1281.6 $[M+H]^+$ base peak: 1303.5 $[M+Na]^+$; HRMS (ESI) calcd for $C_{70}H_{73}N_8O_{16}[M+H]^+$ 1281.5145 , found 1281.5099.

4.5. Materials for Biological Studies. Dimethyl sulfoxide (DMSO), paclitaxel, topotecan, DOX, mitoxantrone, cyclosporine A, Ko143 and phenazine methosulfate (PMS) were purchased from Sigma-Aldrich. Dulbecco's Modified Eagle's Medium (DMEM), trypsin-ethylenediaminetetraacetic acid (EDTA) and penicillin/streptomycin were purchased from Gibco BRL. Roswell Park Memorial Institute (RPMI) 1640 medium and fetal bovine serum (FBS) was purchased from HyClone Laboratories. 3-(4,5-Dimethylthiazol-2-yl)-5-[3-(carboxymethoxy)phenyl]-2-(4-sulfo-phenyl)-2H-tetrazolium (MTS) was purchased from Promega. The human breast cancer cell lines LCC6 and P-gp transfectant LCC6MDR were kindly provided by Prof. R. Clarke (Georgetown University Medical School, USA). The human ovarian

carcinoma cell line 2008/MRP1 was generous gift from Prof. P. Borst (The Netherlands Cancer Institute, Amsterdam, Netherlands). The human embryonic kidney cell lines HEK293/pcDNA3.1, BCRP-transfectant HEK293/R2, MCF7-MX100 and S1M180 cells were generously provided by Dr. Kenneth To (The Chinese University of Hong Kong, Hong Kong). L929, 3T3 and HFF-1 cell lines were purchased from ATCC.

4.6. Cell Culture. HEK293/R2, HEK293/pcDNA3.1, MCF7-MX100, 2008/MRP1 and S1M180 cells were cultured in RPMI 1640 medium with 10% FBS and 100 U/mL penicillin and 100 μg/mL of streptomycin and maintained at 37 ^{o}C in a humidified atmosphere with 5% CO_2. The L929, 3T3, HFF-1 and LCC6MDR cells were cultured in DMEM supplemented with 10% FBS and 100 U/mL penicillin and 100 μg/mL of streptomycin and maintained at 37 ^{o}C in a humidified atmosphere with 5% CO_2. For each passage of HEK293pcDNA3.1 or HEK293/R2, 1 mg/mL G418 was added to culture. The cells were split constantly after a confluent monolayer had been formed. To split cells, the plate was washed briefly with phosphate-buffered saline (PBS), treated with 0.05% trypsin-EDTA and harvested by centrifugation.

4.7. EC_{50} determination. 5,000 cells of HEK293/R2 in each well of 96-well plate were incubated with different concentrations of topotecan (0, 8, 25, 74, 222, 667 and 2000 nM) and modulators (0, 1.6, 8, 40, 200, 1000 nM). 7,500 cells of MCF7-MX100 were incubated with different concentrations of topotecan (0, 0.41, 1.2, 3.7, 11, 33 and 100 μM) and modulators (0, 1.6, 8, 40, 200, 1000 nM). 4,000 cells of 2008/MRP1 were incubated with various doses of DOX (0, 8, 25, 74, 222, 667 and 2000 nM) and modulators (0, 62.5, 125, 250, 500, 1000 nM). 6,500 cells of LCC6MDR were incubated with various doses of paclitaxel (0, 1.6, 5, 15, 15, 44, 133 and 400

nM) and modulators (0, 62.5, 125, 250, 500, 1000 nM). The final volume in each well of 96-well plates was 200 µL. The plates were then incubated for 5 days at 37 ºC.

The CellTiter 96 AQ_{ueous} Assay (Promega) was used to measure the cell proliferation according to the manufacturer's instructions. MTS (2 mg/mL) and PMS (0.92 mg/mL) were mixed in a ratio of 20:1. An aliquot (10 µL) of the freshly prepared MTS/PMS mixture was added into each well, and the plate was incubated for 2 hours at 37 ºC. Optical absorbance at 490 nm was recorded with microplate absorbance reader (Bio-Rad). All experiments were performed in triplicate and repeated at least twice and the results were represented as mean ± standard error of mean. The EC_{50} value was determined by PRISM software.

4.8. Mitoxantrone sensitization assay. 5,000 cells of HEK293/R2, 4,000 cells of 2008MRP1 or 6,500 cells of LCC6MDR cells were incubated with different doses of mitoxantrone with or without 1 µM **Ac22(Az8)$_2$** or Ko143 for 5 days. The final volume in each well of 96-well plates was 200 µL. For determining EC_{50} value, the cells were incubated with different doses of mitoxantrone and modulators together. The % of survival and EC_{50} values was determined as mentioned previously.

4.9. Topotecan accumulation assay. Topotecan accumulation assay was done in 1 mL volume. A $5x10^5$ cells of HEK293/pcDNA3.1 or HEK293/R2 cells were added in an Eppendorf tube and incubated with 50 µM topotecan and 1 µM of **Ac22(Az8)$_2$** or Ko143 at 37 ºC for 120 min. A 0.1% DMSO was used as a negative control. After incubation, the cells were spun down and washed with cold PBS, pH 7.4 for one time and then resuspended with 200 µL of cold FACS buffer (1% BSA and 1 mM EDTA in PBS). The intracellular topotecan level was analyzed by BD C6 Accuri

flow cytometer using FL1 channel EX 480 nm and EM 533/30 nm. For each sample, a total of 30,000 events was collected.

4.10. Determination of surface BCRP protein expression. 40,000 cells of HEK293/pcDNA3.1 and HEK293/R2 cells were seeded in a 6-well plate and incubated with 0, 1 or 3 µM of **Ac22(Az8)$_2$** for 4 days, respectively. After 4 days, the cells were trypsinized and washed once with 1X PBS. After spinning, the cells were resuspended in 50 µL FACS buffer (1% BSA and 1 mM EDTA in PBS) and stained with 2.5 µL FITC mouse anti-human BCRP antibody (Miltenyi Biotec) at 4 ^{o}C for 45 min. After staining, the cells were washed once with 500 µL cold FACS buffer and resuspended in 200 µL FACS buffer. The BCRP-FITC level was analyzed by BD C6 Accuri flow cytometer using FL1 channel at EX 480 nm and EM 533/30 nm. For each sample, a total of 30,000 events was collected.

4.11. Topotecan efflux study. To measure the topotecan efflux, HEK293/pcDNA3.1 or HEK293/R2 cells were pre-incubated with 5 µM of **Ac22(Az8)$_2$** for 2 hrs at 37 ^{o}C with 5% CO_2. After pre-incubation, the cells were trypsinized and the number of cells was counted using haemocytometer. The cells were then co-incubated with 50 µM of topotecan and 5 µM of **Ac22(Az8)$_2$** in supplemented RPMI1640 media for 2 hrs at 37 ^{o}C. After 2 hrs, the cells were spun down and washed once with cold PBS. Then the cells were further incubated with or without compound **Ac22(Az8)$_2$** (5 µM). At 0, 5, 10 and 15 min, $1x10^5$ cells in 1 mL volume were harvested for measuring the intracellular topotecan concentration. The % of topotecan reduction was calculated = [(topotecan level at final time point / topotecan level at 0 min) * 100%]. The topotecan level was determined by C6 Accuri flow cytometer at FL1 channel as described previously.

4.12.Vanadate-sensitive BCRP-ATPase activity. 5x 10^7 cells of S1M180 were resuspended in 5 mL homogenization buffer (0.33M sucrose, 300mM Tris pH7.4, 1mM EDTA, 1mM EGTA, 2mM DTT, 100mM 6-aminocaproic acid, 1mM PMSF and 1x protease inhibitor (cOmplete™ Protease Inhibitor Cocktail Tablets, Roche) and lysed using a Branson SFX550 sonicator for 10 cycles at 50% amplitude with 30 seconds on / 30 seconds off . Lysate was centrifuged at 3,500 x g for 10 minutes at 4°C. Membrane fraction of cells was collected by ultracentrifugation of cell lysate at 45,000 rpm using Himac CP70G (Hitachi) for 1.5 hours. Membrane fraction pellet was re-suspended in 300 µL of ATPase assay buffer (50 mM Tris at pH7.5, 2 mM EGTA at pH 7.0, 2 mM DTT, 50 mM KCl, 10 mM $MgCl_2$, 5mM sodium azide, and 1mM ouabain). Protein concentration was determined by Bradford assay. Membrane fraction was incubated with or without 0.3 mM sodium orthovanadate and 2.5 mM ATP for 1 hour at 37°C. Reactions were stopped by adding 200 µL freshly prepared cold stop buffer (0.2% ammonium molybdate, 1.4% sulphuric acid, 0.9% SDS and 1% ascorbic acid) and incubated at room temperature for 15 minutes. Absorbance of 655nm was measured by CLARIOstar® microplate reader (BMG).

4.13. *In Silico* Docking Study. CLC Drug Discovery Workbench (version 2.5, QIAGEN) software was used. Three high-resolution cryo-EM structures of BCRP (PDB ID: 6FEQ, 6HCO and 6ETI) were downloaded from Protein Data Bank (https://www.rcsb.org/) and used directly without any changes. Briefly, the 2-D structures of different ligands were generated from SIMLES and imported individually into the software for docking study. Employing the function of "Find Binding Pockets", the software was able to identify the central translocation pathway of BCRP as one of the potential binding pockets in all BCRP models. The binding sites of each cryo-EM structure of BCRP (PDB ID: 6FEQ, 6HCO and 6ETI) was individually validated using the co-crystalized ligands MB136, estrone-3-sulfate and MZ29, providing reliable RMSD values of 1.2

Å, 0.3 Å and 0.5 Å respectively. The identification of ligand binding modes was done iteratively by evaluating 3,000 random ligand conformations and estimating the binding energy of their interactions with the potential binding pockets. The 10 top-ranked binding poses of ligands were returned for visual inspection. The highest scores positioned the ligands into the central cavity of BCRP, which was mainly formed by the transmembrane helices 1b, 1b', 2, 2', 5a and 5a'. Important amino acid residues involved in the putative interactions with ligands are highlighted in black and potential hydrogen bonding interactions are indicated as dotted blue lines. These docking results were illustrated in **Figure 8**, **S68-S69** and **Table 6** respectively.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

HPLC chromatogram of **Ac22(Az8)$_2$;** ^{1}H NMR and ^{13}C NMR spectra of all representative compounds listed in Table 1 and Table 3; *in silico* docking studies and ^{1}H & ^{13}C-NMR assignment of **Ac2Az2** and **Ac22(Az8)$_2$**.

SMILES molecular strings formulas (CSV).

Binding **Ac22(Az8)$_2$**, mitoxantrone and topotecan to BCRP (PDB ID: 6ETI) (PDB).

Authors will release the atomic coordinates upon article publication.

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[#]Xuezhen Zhu, [#]Iris L. K. Wong and [#]Kin-Fai Chan contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest. The patent US 9611256 B1 associated with this manuscript has been licensed to Athenex Inc.

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ABBREVIATIONS USED

BCRP, Breast cancer resistance protein; MRP1, multidrug resistance protein-1; P-gp, P-glycoprotein; MDR, multidrug resistance; ABC, ATP-binding cassette; DOX, doxorubicin; EC_{50}, effective concentration; RPMI1640, Roswell Park Memorial Institute 1640; MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt.

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