Discovery of novel flavonoid dimers to reverse multidrug resistance protein 1 (MRP1; ABCC1)-mediated drug resistance in cancers using a high throughput platform with "click chemistry"

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ABSTRACT

A 300-member flavonoid dimer library of multidrug resistance-associated protein 1 (MRP1; ABCC1) modulators was rapidly assembled using "click chemistry". Subsequent high-throughput screening has led to the discovery of highly potent (EC_{50} ranging from 53 to 298 nM) and safe (selective indexes ranging from >190 to >1887) MRP1 modulators. Some dimers have potency about 6.5- to 36-fold and 64- to 358-fold higher than the well-known MRP1 inhibitors, verapamil and MK571, respectively. They inhibited DOX efflux and restored intracellular DOX concentration. The most potent modulator, **Ac3Az11**, was predicted to bind to the bipartite substrate-binding site of MRP1 in a competitive manner. Moreover, it provided sufficient concentration to maintain its plasma level above its *in vitro* EC_{50} (53 nM for DOX) for about 90 minutes. Overall, we demonstrate that "click chemistry" coupled with high throughput screening is a rapid, reliable and efficient tool in the discovery of compounds having potent MRP1-modualting activity.

Keywords: Click chemistry, CuAAC reaction, multidrug resistance, multidrug resistance-associated protein 1, MRP1 modulators, flavonoids

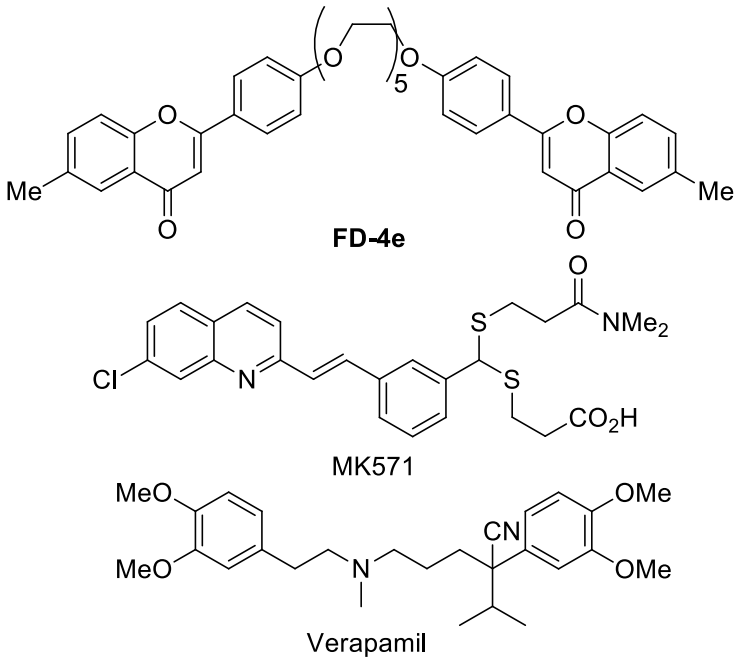
1. INTRODUCTION

Multidrug resistance (MDR) is a major impediment to successful treatment of many forms of malignant cancers. MDR is often associated with overexpression of an ATP-binding cassette (ABC) transporter which can extrude a wide variety of structurally-unrelated anticancer drugs and decrease intracellular drug accumulation below toxic levels. P-gp/ABCB1, MRP1/ABCC1 and BCRP/ABCG2 are the three major ABC members that confer cancer MDR. Multidrug resistance–associated protein 1 (MRP1/ABCC1) was first identified in 1992 in a drug-selected human small lung cancer cell line H69AR.[1, 2] It pumps out organic anions, glutathione-, glucuronate- or sulfate-conjugated drugs, or unconjugated drugs in concert with free glutathione, including the chemotherapeutic agents, vincristine, doxorubicin and etoposide.[3-8] Its elevated protein and mRNA levels have been reported in many tumors and is correlated with poor patient outcome including non-small-cell lung cancer, gastrointestinal carcinoma, melanoma, neuroblastoma and cancers of the breast, ovary and prostate.[9-12] Similar to P-gp/ABCB1, MRP1 contains an internally duplicated structure of two transmembrane domains (TMD1 and TMD2) and two cytosolic nucleotide binding domains (NBD). However, MRP1 has an extra N-terminal transmembrane domain containing five membrane-spanning helices (TMD0). The functional role of this third TMD0 is currently unclear. [3, 4, 7, 8]

Considerable efforts have been placed to overcome MDR by designing modulators which can inhibit the function of ABC transporter and restore intracellular accumulation of drugs. A large number of modulators have been identified for P-gp/ABCB1 (verapamil, cyclosporine A, PSC-833, biricodar, zosuquidar, tariquidar, elacridar and ontogen)[13-15] and BCRP/ABCG2 (elacridar, ko143, pantoprazole, tariquidar and biricodar), [16-20] some with high potencies having low nM range of EC_{50} (effective concentration that can lower the IC_{50} of a drug to resistant cancer cells by 50%)

63 *in vitro*. In contrast, there are fewer MRP1 inhibitors including probenecid and MK-571.[21, 22] These
64 MRP1 inhibitors are far from satisfactory because of their relatively high EC_{50} which may cause
65 toxicity and side effects in clinical trials. Therefore, development of modulators which are potent
66 and safe against MRP1 is highly desirable.

67 We have previously synthesized some potent and safe P-gp and MRP1 modulators using
68 flavonoid as the structural motif.[23-28] Many natural flavonoids are known to be modest modulators
69 of these ABC transporters.[39] Flavonoids are commonly found in fruits, vegetables, and plant-
70 derived products of human diet and generally considered as safe compounds. Because of the
71 pseudo-dimeric structure of many ABC transporters, we reasoned that a bivalent approach by
72 combining two flavonoid moieties linked with different polyethylene glycol linkers would yield
73 selective and potent modulators. Indeed, we discovered that some flavonoid dimers showed
74 effectiveness to inhibit P-gp and MRP1 transporters with nanomolar EC_{50} values (<170 nM).[23-28]
75 The flavonoid dimer **FD-4e,** with PEG linker length n=5, displayed a low EC_{50} value of 73 nM in
76 reversing DOX resistance in a MRP1 overexpressing ovarian (2008/MRP1) cell line (**Figure 1**).[27]
77 However, its relatively low aqueous solubility rendered it a poor candidate for further *in vivo*
78 animal study.



79

80 **Figure 1.** Chemical structures of **FD-4e**, MK571 and verapamil used in this study.

81 Triazoles are amphoteric in nature, acting as both acids and bases. Such properties make them
82 usually soluble in aqueous medium. In order to rapidly generate a large number of diverse and
83 dimeric MRP1 modulators which may have better physiochemical properties as potential drug
84 candidates, we employed the copper (I) catalyzed Huisgen 1,3-dipolar cycloaddition reaction
85 between azides and alkynes to yield 1,4-disubstituted 1,2,3 triazoles (commonly known as CuAAC
86 reaction).[29] The reaction belongs to a class of reactions known as "click chemistry". The features
87 of these reactions include high efficiency (~100% reaction yield), chemoselectivity, and
88 modularity. They have been found to be useful as a rapid method to assemble compound libraries
89 and allow direct *in vitro* screening of the clicked products without the need of purification.[30, 31]
90 Click-chemistry based high throughput screening platform has been developed in cases where the
91 target enzyme is available as reported for the Ras palmitoylation[32] and various other enzymes.[33-36]
92 Click-chemistry has also been employed to synthesize bivalent ligands in the study of P-gp-

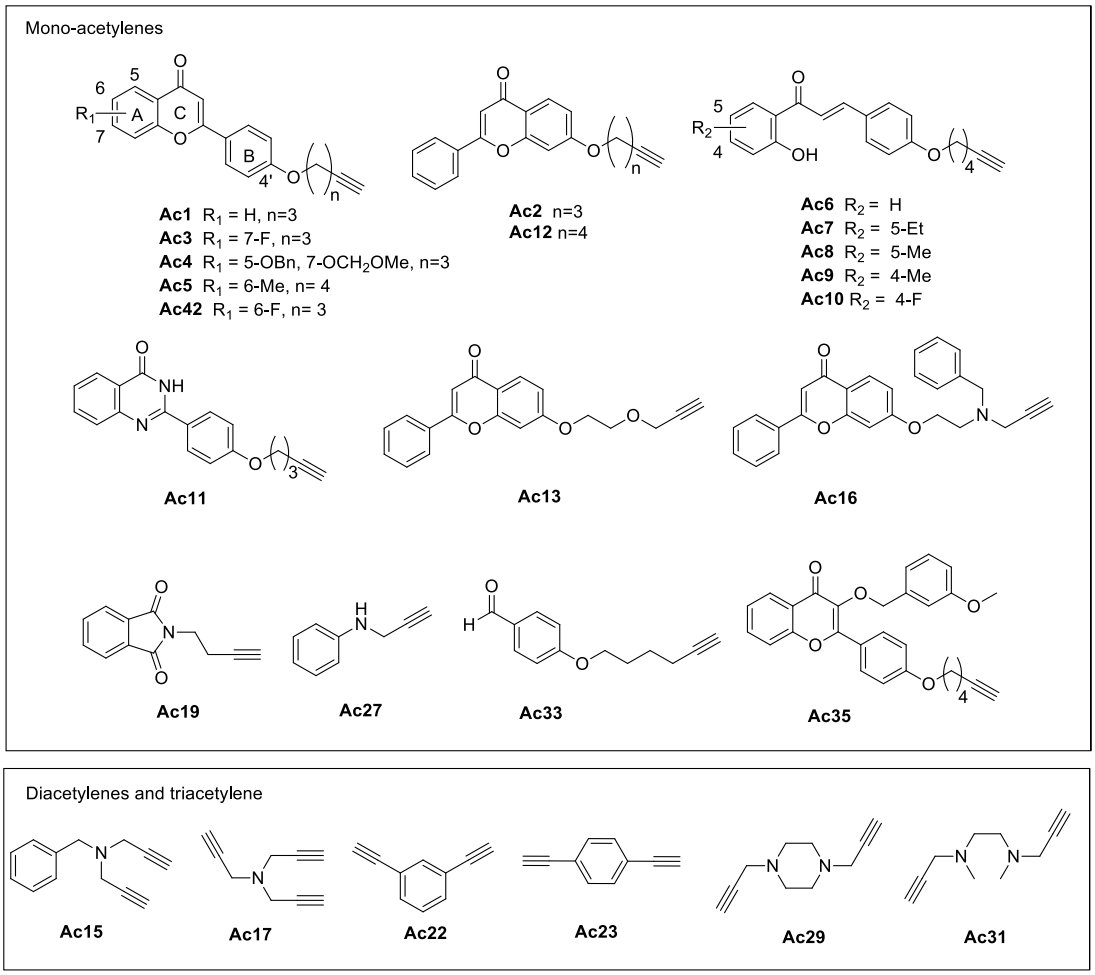
mediated cellular efflux.[37-39] Because whole cells, instead of a pure enzyme, have to be used for the bioassay of ABC transporter-mediated cellular efflux, no high throughput screening platform was developed and only a small library of pure click products was prepared and studied. Herein, we report a 300-member flavonoid dimer library synthesized using the "click chemistry" approach and coupled that with a high throughput screening platform to discover novel MRP1/ABCC1 modulators with favorable physiochemical properties.

2. RESULTS

2.1 Library Generation by Click Chemistry

Previously, we synthesized a series of bivalent ligands by coupling two flavonoid moieties with PEG linkers. **FD-4e** was demonstrated to be a potent MPR1 inhibitor for reversing DOX resistance with EC_{50} of 73 nM.[27] In terms of chemical structures, our previous dimers contain mainly symmetrical structures with two identical flavonoid moieties. Based on the previous synthetic routes, it was difficult to generate a large number of unsymmetrical dimers with two different flavonoids.

In this work, we take advantage of the appealing ease and chemo-selectivity of the Cu (I)-catalyzed azide-alkyne cycloaddition as the key dimerizing approach for the construction of a library of triazole bridged flavonoid bearing molecules. At first, we designed twenty-five terminal alkynes comprising of mono-acetylenes **(Ac1-13, Ac16, Ac19, Ac27, Ac33, Ac35, Ac42**), diacetylenes (**Ac15**, **Ac22**, **Ac23**, **Ac29**, **Ac31**) and the triacetylene **Ac17** (**Figure 2**) and twelve flavonoids bearing azido groups (**Az1-5, Az7, Az10-13**, **Az17** and **Az18**) (**Figure 3**).



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114 **Figure 2**. Structures of various alkynes.

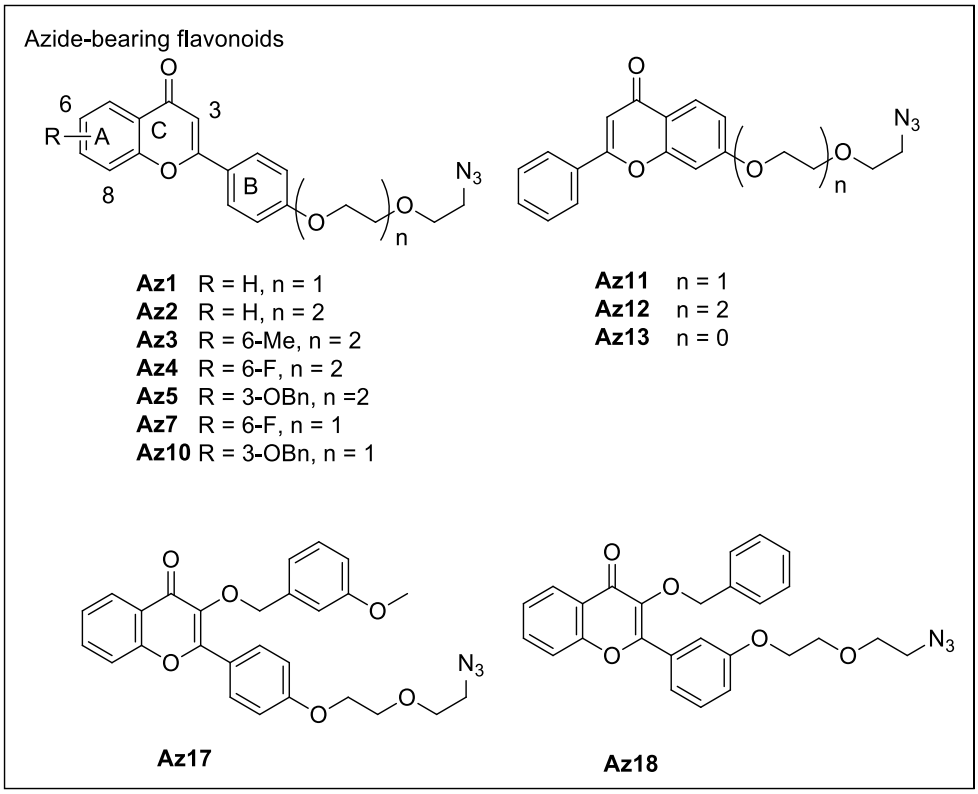


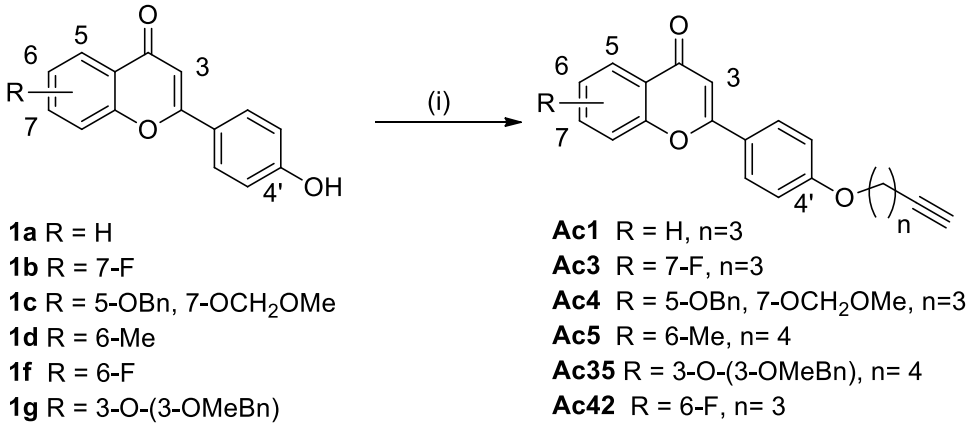
Figure 3. Structures of various azides.

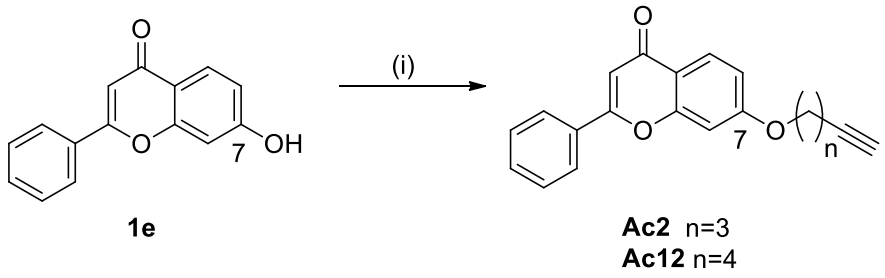
2.1.1 Synthesis of alkynes

The flavonoids bearing alkynes **Ac1-5, Ac12, Ac35** and **Ac42** were prepared by treating 4' or 7-hydroxyflavones **1a-g** with various haloalkynes in high yield (Scheme 1). **Ac33** was easily prepared by treating *p*-hydroxybenzaldehyde **4d** with 6-chloro-1-hexyne (Scheme 4). Base-catalyzed aldol condensation of aldehyde **Ac33** with various 2-hydroxyl acetophenones **3a-e** afforded the chalcones **Ac6-10** (Scheme 2). 2-Phenylquinazolin-4(3*H*)-one derivative **Ac11** was prepared by treatment of 2-aminobenzamide with aldehyde **2a** in the presence of catalytic amount of iodine (Scheme 2). Acetylene **Ac13** was simply obtained in two steps: (1) alkylation of hydroxyl flavone **1e** with bromoethanol; (2) alkylation of the hydroxyl group with propargyl bromide in the presence of sodium hydride (Scheme 3). Acetylenes bearing flavonoid **Ac16** were easily prepared

through ligation of flavone **1e** with 2-(benzyl(prop-2-yn-1-yl)amino)ethanol under Mitsunobu condition (Scheme 3). Treatment of aniline (**4a**), piperazine (**4b**) and N^1, N^2-dimethylethane-1,2-diamine (**4c**) with propargyl bromide easily furnished mono-acetylene **Ac27**, diacetylene **Ac29** and **Ac31** (Scheme 4). Acetylene **Ac15**, triacetylene **Ac17**, mono-acetylene **Ac19**, di-acetylene **Ac22** and **Ac23** are commercially available.

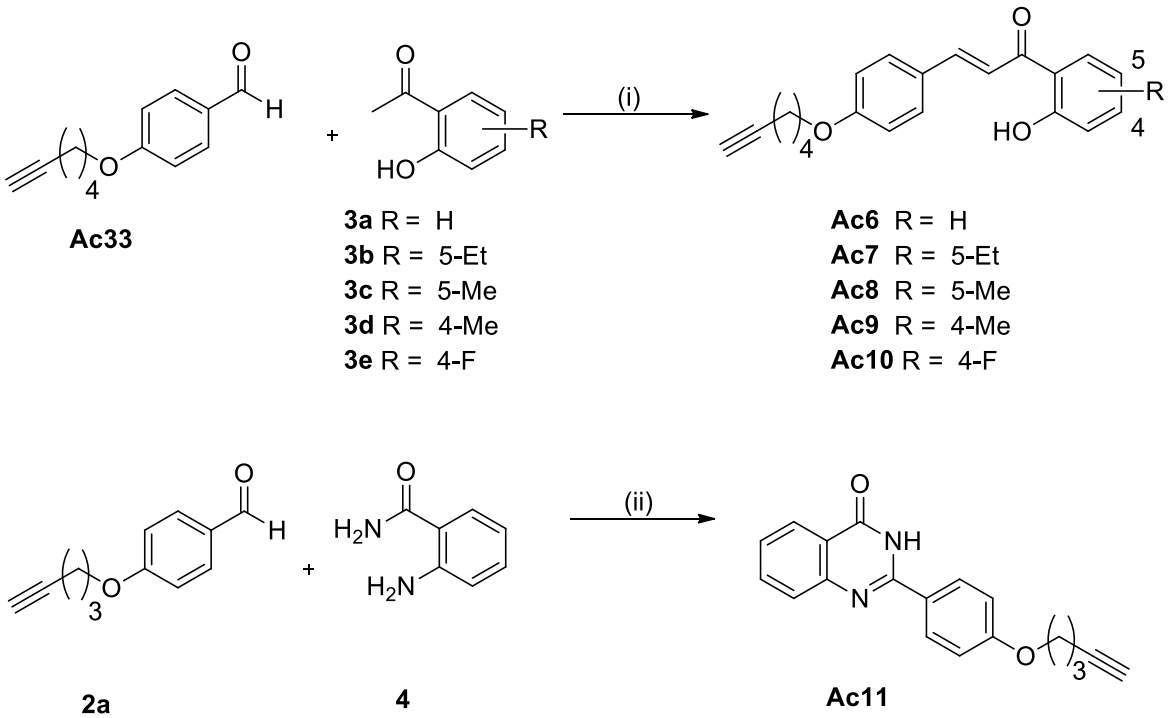
Scheme 1. Synthesis of acetylenes **Ac1-5, Ac12 and Ac42**.[a]





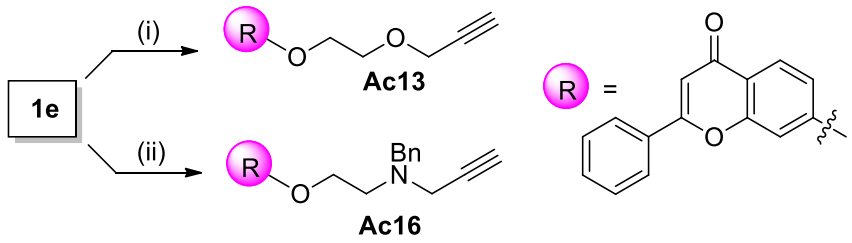
[a] Reagents and condition: (i) K_2CO_3, 6-chloro-1-hexyne or 5-chloro-1-pentyne, DMF, reflux;

Scheme 2. Synthesis of acetylenes **Ac6-Ac11**.[a]



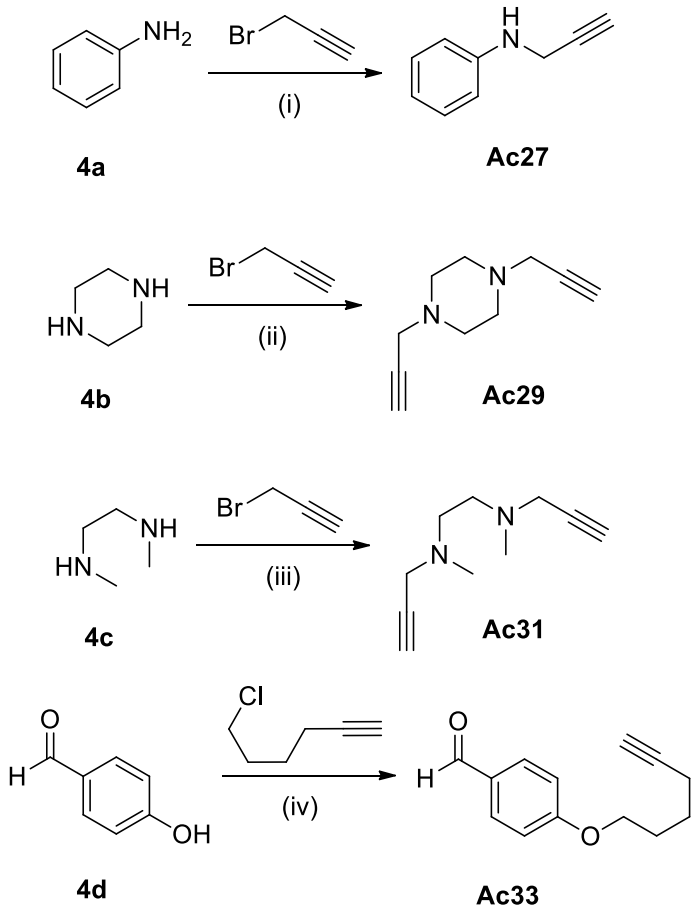
[a] Reagents and condition: (i) KOH, EtOH, rt; (ii) I_2, DMSO, 150°C;

Scheme 3. Synthesis of acetylenes **Ac13** and **Ac16**.[a]



[a] Reagents and condition: (i) (a) K_2CO_3, 2-bromoethanol, DMF, reflux; (b) NaH, propargyl bromide solution, THF; (ii) 2-(benzyl(prop-2-yn-1-yl)amino)ethanol, PPh_3, DIAD, THF;

147 **Scheme 4**. Synthesis of acetylenes **Ac27, Ac29, Ac31** and **Ac33**.[a]



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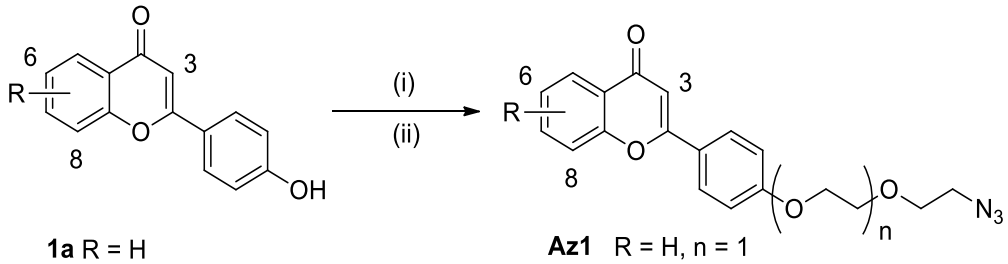
149 [a] Reagents and condition: (i) K_2CO_3, propargyl bromide, DMF, rt; (ii) K_2CO_3, propargyl bromide,
150 acetone, rt; (iii) K_2CO_3, propargyl bromide, DMF, 10°C; (iv) K_2CO_3, 6-chloro-1-hexyne, KI, DMF,
151 80°C;

152 ***2.1.2 Synthesis of azides***

153 The synthesis of the required azide bearing flavonoids is shown in Scheme 5. The azides **Az1-**
154 **7**, **Az10-13**, **Az17** and **Az18** were conveniently prepared from 4' or 7-hydroxyflavones (**1a**, **d**, **f**,
155 **g**, **h**, **i**) with high yield by the following three steps: (1) treatment of 4' or 7-hydroxyflavones with
156 various hydroxyl halides such as bromoethanol, 2-(2-chloroethoxy)ethanol and 2-(2-(2-

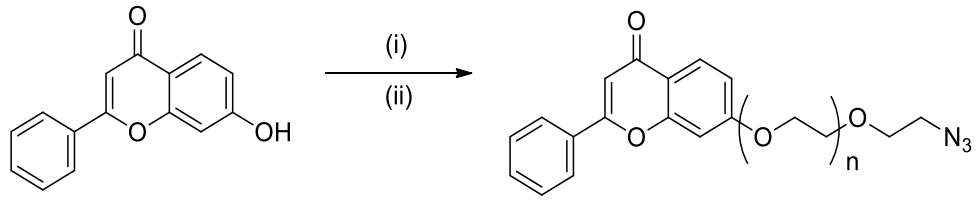
chloroethoxy)ethoxy)ethanol in the presence of sodium carbonate; (2) conversion of hydroxyl to methanesulfonate group by methanesulfonyl chloride; and (3) treatment of the mesylated flavones with excess sodium azide.

Scheme 5. Synthesis of azides **Az1-5, Az7-13, Az17** and **Az18**.[a]



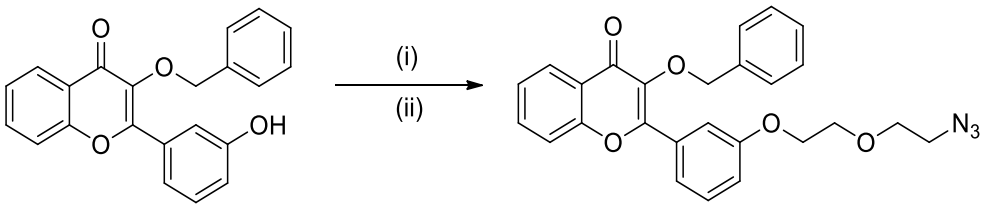
1a R = H
1d R = 6-Me
1f R = 6-F
1h R = 3-OBn
1g R = 3-O-(3-OMeBn)

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
Az17 R = 3-O-(3-OMeBn), n =2



1e

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



1i **Az18**

[a] Reagents and condition: (i) K_2CO_3, 2-bromoethanol (n = 0) or 2-(2-chloroethoxy)ethanol (n = 1) or 2-(2-(2-chloroethoxy)ethoxy)ethanol (n = 2), DMF, reflux; (ii) (a) methanesulfonyl chloride, NEt_3, DCM, 0°C; (b) NaN_3, ACN;

2.1.3 The click reaction

Using the CuAAC reactions, a 300-member "clicked" flavonoid dimers library was constructed in microtiter plates and in millimolar scale. In general, one millimolar of each acetylene (AcN) was mixed with each of 1 mM azides (AzM) in 100 μL of THF solvent, followed by addition of catalytic amount of bromotris(triphenylphosphine)copper(I) to yield about 1 mM of triazole bridged products (AcN-AzM) without purification. For diacetylenes **Ac15**, **Ac22**, **Ac23**, **Ac29**, **Ac 31** and triacetylene **Ac17**, two millimolar and three millimolar of azides (AzM) were used respectively. The reactions were carried out overnight in microplate heater (70 °C). We generated 300 triazole compounds in such identical reaction condition. After overnight reaction, all THF solvent in each well was removed by evaporation and the 300 triazole compounds were then dissolved in DMSO. Without any further purification of the assembled products, they were directly screened for MRP1 modulating activity in 96-well plates containing 2008/MRP1 cells and anticancer drug, doxorubicin.

2.2 Biological assay results

2.2.1 Primary and secondary screening of MRP1-modulating activity of clicked flavonoid dimers

Each of the un-purified 300-member of the clicked homo- and hetero-flavonoid dimer library contained at least four components: (1) the alkyne AcN, (2) the azide AzM (3) the catalyst bromo*tris*(triphenylphosphine)copper(I) and (4) the product AcN-AzM. This library was used for

primary screening (**Table 1**) at 2 μM of the product AcN-AzM by assuming a nearly 100% yield of the click reaction. A hundred nanomolar of DOX was employed for the assay, a concentration at which it showed no toxicity towards 2008/MRP1 cells (100% of survival, data not shown). The relative potency of MRP1-modulating activity of the clicked dimers was determined by MTS proliferation assay and presented as % of survival normalized to those with DOX but without un-purified clicked dimer. The active clicked dimers would result in a relatively lower % of cell survival. Controlled experiments with the pure alkyne, or with the pure azide, or with the catalyst were also performed (**Table 2**). At 2 μM, all pure alkynes, azides and catalyst showed no or low MRP1-modulating activity with % of survival ranged from 57% to 103% (**Table 2**).

In the primary screening, the relative MRP1-modulating activity of each clicked dimer was measured and presented as in **Table 1**. Out of the 300-member library, 53 members were considered as "hit" compounds with % of survival ranging from 20% to 49% (**Table 1**, bolded and underlined). Among these 53 flavonoid dimer "hits", 18 of them exhibited cytotoxicity towards 2008/MRP1 cells at 2 μM with % of survival less than 70% (**Table 3**). Interestingly, 7 out of the 12 dimers in the **Ac19** sub-library showed cytotoxic effect, suggesting that **Ac19** is a poor building block for making MRP1 modulators (**Table 3**). After excluding these 18 cytotoxic flavonoid dimers, the remaining 35 "hit" compounds were further differentiated by testing them at a lower concentration of 1 μM. A total of 21 compounds consistently maintained promising activity with < 51% of survival (**Table 4**).

Table 1. Primary screening of clicked flavonoid dimers to reverse DOX resistance in 2008/MRP1 cells.[a]

> 90 % survival | 90 – 70% survival | 69 – 50% survival | < 50% survival

	Az1	Az7	Az12	Az11	Az4	Az3	Az2	Az18	Az5	Az10	Az13	Az17
Ac5	20	30	33	34	34	38	39	55	59	73	75	78
Ac3	21	55	52	41	42	45	44	45	37	60	77	61
Ac2	23	55	57	45	48	55	54	65	63	51	56	78
Ac16	26	36	51	46	44	53	45	73	65	49	80	79
Ac1	29	39	42	39	46	51	56	54	45	50	69	68
Ac12	30	52	61	53	47	51	57	62	63	79	60	86
Ac13	31	54	60	60	53	54	59	77	45	46	68	47
Ac19	37	39	41	51	28	81	28	62	34	57	37	75
Ac6	39	72	72	58	56	61	65	83	61	72	85	74
Ac4	39	63	76	54	58	53	66	74	89	88	81	79
Ac35	40	65	78	56	60	46	58	75	33	68	51	57
Ac7	40	80	79	68	67	63	60	85	77	69	86	76
Ac10	42	70	67	64	53	63	55	68	68	69	73	75
Ac15	49	70	74	76	54	52	29	87	87	62	96	80
Ac9	57	71	72	62	51	61	55	79	71	66	75	78
Ac11	58	76	60	49	51	72	76	67	68	64	67	67
Ac8	60	82	84	73	72	67	70	78	76	68	77	75
Ac33	75	78	81	82	74	72	70	82	75	59	83	76
Ac22	79	74	74	76	72	77	85	89	80	87	90	82
Ac23	81	77	88	79	83	74	83	82	86	82	87	88
Ac17	82	76	77	83	77	71	77	91	81	77	87	86
Ac31	87	73	88	99	79	76	71	83	84	76	90	64
Ac27	90	81	90	88	90	86	88	92	87	91	85	89
Ac42	93	74	40	111	76	82	79	79	75	97	89	86
Ac29	116	86	89	99	85	103	100	84	96	58	93	85

[a] A total of 300 clicked dimers was used to screen for the MRP1-modulating activity. 2008/MRP1 cells were incubated with 100 nM DOX and 2 μM of crude clicked dimers together. The % of

211 survival was determined using MTS assay after 72 h incubation and normalized to the control
212 containing no DOX and any clicked dimer. Each clicked dimer was tested in triplicate in each
213 experiment. N = 3 independent experiment. The % of survivors was presented as mean here.
214 Percentage survival less than 50% was bolded and underlined. These dimers displaying potential
215 MRP1-modulating activity were picked up for further cytotoxicity test.

216

217 **Table 2**. MRP1-modulating activity of pure alkyne, pure azide or Cu(I) catalyst in 2008/MRP1

218 cells. [a]

Compounds	% Survival
Ac1	74 ± 3
Ac2	85 ± 1
Ac3	79 ± 3
Ac4	67 ± 5
Ac5	88 ± 8
Ac6	102 ± 2
Ac7	103 ± 4
Ac8	100 ± 1
Ac9	100 ± 2
Ac10	99 ± 0
Ac11	86 ± 3
Ac12	99 ± 2
Ac13	98 ± 4
Ac15	81 ± 2
Ac16	86 ± 2
Ac17	84 ± 3
Ac19	83 ± 2
Ac22	84 ± 3
Ac23	86 ± 2
Ac27	87 ± 2
Ac29	81 ± 3
Ac31	87 ± 1
Ac33	86 ± 1
Ac35	71 ± 3
Ac42	81 ± 3
Az1	78 ± 2
Az2	85 ± 7
Az3	84 ± 8
Az4	85 ± 6
Az5	74 ± 8
Az7	90 ± 5
Az10	72 ± 9
Az11	91 ± 7
Az12	90 ± 8
Az13	79 ± 4
Az17	57 ± 4
Az18	86 ± 2
Cu (I) catalyst	103 ± 3

219

[a] A total of 25 pure alkynes, 12 pure azides and Cu(I) catalyst were tested for their MRP1-modulating activity. 2008/MRP1 cells were incubated with 100 nM DOX and 2 μM of each monomer or catalyst together. Percentage survival was determined using MTS assay after 5-day incubation and normalized to the control containing no DOX or monomers. Each monomer was tested in triplicate in each experiment. N = 3 independent experiment. Percentage survival was presented as mean ± SEM here. Percentage survival below 50% indicates that the compound showed promising MRP1-modulating activity.

Table 3. Cytotoxicity of 53 clicked dimers alone towards 2008/MRP1 cells. [a]

Compounds Ac	Compounds Az	% of survival 2 µM dimers + 100 nM DOX 2008/MRP1	% of survival 2 µM dimers alone 2008/MRP1
5	1	20 ± 1	85
3	1	21 ± 2	91
2	1	23 ± 3	92
16	1	26 ± 3	97
19	2	28 ± 19	4
19	4	28 ± 18	10
1	1	29 ± 0	81
15	2	29 ± 3	49
5	7	30 ± 2	99
12	1	30 ± 6	95
13	1	31 ± 1	97
35	5	33 ± 12	53
5	12	33 ± 1	89
19	5	34 ± 13	16
5	4	34 ± 1	100
5	11	34 ± 2	93
16	7	36 ± 17	87
3	5	37 ± 3	97
19	1	37 ± 15	18
19	13	37 ± 20	11
5	3	38 ± 2	100
1	7	39 ± 2	43
1	11	39 ± 2	39
19	7	39 ± 13	14
4	1	39 ± 4	95
6	1	39 ± 3	83
5	2	39 ± 3	68
7	1	40 ± 2	95
35	1	40 ± 13	63
42	12	40 ± 9	105
3	11	41 ± 3	93
19	12	41 ± 21	23
1	12	42 ± 3	49
3	4	42 ± 2	95
10	1	42 ± 2	90
3	2	44 ± 3	93
16	4	44 ± 7	72
1	5	45 ± 5	39
2	11	45 ± 5	98
3	3	45 ± 2	95
3	18	45 ± 3	99
16	2	45 ± 5	74
13	5	45 ± 6	95
1	4	46 ± 3	44
16	11	46 ± 6	80
35	3	46 ± 13	50
13	10	46 ± 5	85
13	17	47 ± 8	88
12	4	47 ± 3	92
2	4	48 ± 2	88
11	11	49 ± 5	65
16	10	49 ± 2	82
15	1	49 ± 1	92

[a] After primary screening, a total of 53 promising clicked dimers were tested for their cytotoxicity towards 2008/MRP1 cells. The clicked dimers were ranked from highest to the lowest according to their MRP1-modulating activity from the primary screening. Each clicked dimer was tested in triplicates and performed in 3 independent experiments. In cytotoxicity towards 2008/MRP1 cells, the % of survival was presented as mean. The % of survival below 70% was highlighted in grey color, indicating that these dimers alone showed cytotoxicity towards 2008/MRP1 cells and were excluded from the secondary screening.

Table 4. Secondary screening of 35 promising clicked dimers in reversing DOX resistance in 2008/MRP1 cells.[a]

Compounds		% of survival 1 μM dimers + 100 nM DOX
Ac	Az	2008/MRP1
5	1	25 ± 0
3	1	29 ± 2
16	1	29 ± 1
2	1	30 ± 3
5	7	38 ± 3
5	4	41 ± 1
12	1	42 ± 4
5	11	46 ± 1
13	1	46 ± 4
16	7	46 ± 14
3	4	48 ± 3
16	2	48 ± 2
13	5	48 ± 2
2	4	49 ± 2
2	11	49 ± 1
3	11	49 ± 2
3	3	50 ± 2
16	4	50 ± 2
5	3	50 ± 3
3	2	51 ± 2
1	1	51 ± 3
3	5	53 ± 8
16	10	53 ± 3
1	12	54 ± 14
16	11	54 ± 2
3	18	55 ± 6
6	1	56 ± 2
13	10	56 ± 3
12	4	59 ± 1
1	5	62 ± 7
16	2	63 ± 3
4	1	64 ± 7
5	12	65 ± 4
7	1	66 ± 4
10	1	66 ± 3
11	11	70 ± 4
13	17	71 ± 5
15	1	79 ± 3

[a] A total of 35 dimers showing low cytotoxicity towards 2008/MRP1 cells was selected for secondary screening of MRP1-modulating activity. The cells were incubated with 100 nM DOX and 1 μM of clicked dimers together. These dimers were ranked from the highest to the lowest according to the MRP1-modulating activity as determined by % of survival. Each dimer was tested in triplicate and performed in 3 independent experiments. The % of survival was presented as mean here. In the presence of 100 nM DOX, the clicked dimers showing 25% to 51% of survival were selected for determining EC_{50} and cytotoxicity towards L929 cells.

2.2.2 Effective concentration (EC_{50}) and therapeutic indexes of potent clicked flavonoid dimers.

After the secondary screening, the top 21 "hit" compounds (% of survival < 51% at 1 μM) were re-synthesized, purified and tested for their MRP1-modulating activity. EC_{50} values were found to be in nanomolar range from 53 nM to 298 nM and did not show cytotoxicity towards L929 with IC_{50} > 50 μM (**Table 5**). Among them, **Ac3Az11** has the lowest EC_{50} in reversing DOX resistance in 2008/MRP1 cells (**Figure 4**). In contrast, under identical protocol, the EC_{50} of well-established MRP1 inhibitors verapamil (EC_{50} = 1925 nM) and MK571 (EC_{50} = 19000 nM) were in micromolar range (**Table 5**). The potencies of these clicked dimers were about 6.5- to 36-fold and 64- to 358-fold higher than verapamil and MK571, respectively. Moreover, our compounds are relatively non-toxic towards L929 cells. Their selective indexes ranged from >190 to >1887. Selective index was calculated by dividing the IC_{50} of modulators towards L929 cells by the EC_{50} of modulators in reversing DOX resistance. EC_{50} values of pure dimers are in general consistent with the data from the primary and secondary screening using un-purified clicked dimers, indicating that the CuAAC reaction coupled with the high throughput screening is a reliable platform for rapid discovery of MRP1 modulators.

Table 5. Effective concentrations (EC_{50}) of 21 potent pure clicked dimers and their selective indexes. [a]

Pure clicked dimers	L929 (IC_{50}, μM)	EC_{50} (nM) for reversing Dox resistance in 2008/MRP1	Selective indexes	Linker conjugation site	No. of carbon atom in linker
Ac3Az11	>100	53 ± 2	>1887	A	9
Ac16Az1	>100	78 ± 17	>1282	A	9
Ac12Az1	>100	87 ± 17	>1149	A	10
Ac2Az4	>100	95 ± 5	>1053	A	11
Ac16Az2	>100	99 ± 18	>1010	A	11
Ac5Az1	>57	103 ± 15	>553	B	10
Ac3Az4	>100	103 ± 6	>971	B	11
Ac1Az1	60±16	113 ± 49	531	B	9
Ac3Az3	>100	121 ± 13	>826	B	11
Ac5Az3	>50	137 ± 12	>365	B	12
Ac3Az1	>100	140 ± 40	>714	B	9
Ac2Az1	>100	151 ± 14	>662	A	9
Ac16Az7	>100	155 ± 33	>645	A	9
Ac16Az4	>100	156 ± 27	>641	A	11
Ac5Az11	>100	175 ± 18	>571	A	10
Ac13Az5	>100	193 ± 13	>518	A	11
Ac3Az2	>100	208 ± 48	>481	B	11
Ac5Az4	>100	217 ± 25	>461	B	12
Ac5Az7	>100	250 ± 31	>400	B	10
Ac13Az1	>50	263 ± 7	>190	A	9
Ac2Az11	>100	298 ± 44	>336	C	9
FD-4e	>100	73 ± 13	>1370	B	10
Verapamil	88±7	1925 ± 677	46	/	/
MK571	>100	19000 ± 1000	>5	/	/

[a] A total of 21 pure clicked dimers was re-synthesized and determined their EC_{50} values. EC_{50} values were presented as mean ± standard error of mean. N = 3-7 independent experiments. Selective index = (IC_{50} of modulators towards L929)/ (EC_{50} of modulators for reversing DOX

resistance). L929 is normal mouse fibroblast cell line. These pure clicked dimers can be divided into three classes according to the linker conjugation site: (A) linker conjugated at C7 of A-ring and C4' of B-ring of flavones, (B) linker conjugated at C4' of both B-rings of flavones and (C) linker conjugated at C7 of both A-rings of flavones. "/" means verapamil and MK571 do not contain linker. The chemical structures of these potent pure clicked dimers are shown in supporting information (**Figure S7 – S27**).

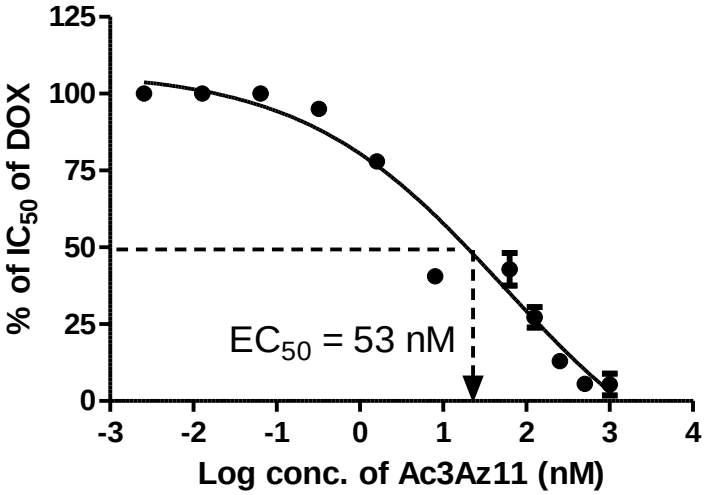


Figure 4. EC_{50} of **Ac3Az11** for reversing DOX resistance in 2008/MRP1 cells

2.2.3 Dimeric clicked alkyne-azide modulator is much more potent than monomeric alkyne or azide in reversing DOX resistance in 2008/MRP1 cells

To illustrate the effect of dimerization in improving the MRP1-modulating activity, we determine the potency of the dimers and monomers. One μM of clicked dimers **Ac3Az11** and **Ac12Az1** showed promising MRP1-modulating activity with RF = 13.5 and 12.2, respectively (**Table 6**). In contrary, their constituent monomers **Ac3**, **Az11**, **Ac12** or **Az1** were not active with

RF values below 1.8 even when they were used at double the concentration (**Table 6**). A combination of **Ac3** and **Az11** or **Ac12** and **Az1** also displayed no activity with RF = 1.3 and 1.5, respectively (**Table 6**). This observation of MRP1 being strongly inhibited by dimeric AcN-AzM modulator, but not by the constituent monomeric AcN or AzM highlights the importance of dimerization of constituent flavonoid monomers in modulating MRP1.

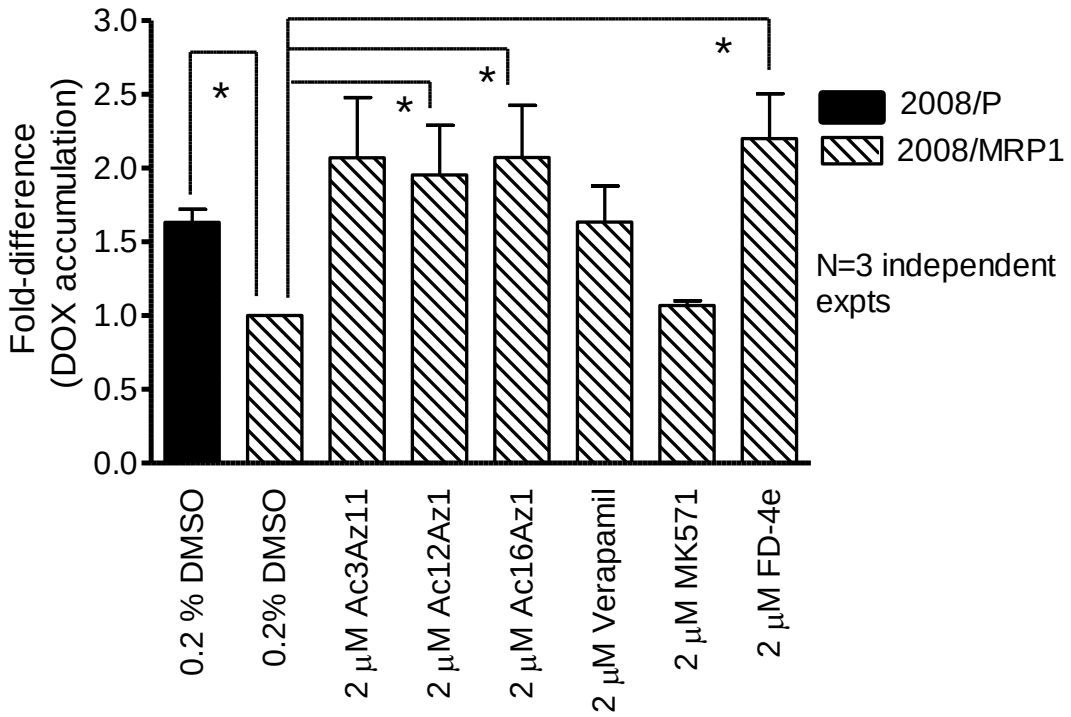
Table 6. Comparing MRP1-modulating activity of pure **Ac3Az11**, **Ac12Az1** and their respective monomers in 2008/MRP1 cells.[a]

Compounds	Mean IC_{50} of DOX (nM) in 2008/MRP1 cells	RF
1 μM Ac3Az11	31 ± 10	13.5
2 μM Ac3	541 ± 189	1.3
2 μM Az11	487 ± 108	1.1
1 μM Ac3 + 1 μM Az11	444 ± 101	1.3
1 μM Ac12Az1	51 ± 16	12.2
2 μM Ac12	566 ± 132	1.0
2 μM Az1	338 ± 87	1.8
1 μM Ac12 + 1 μM Az1	400 ± 105	1.5
0.1% DMSO	597 ± 165	1.0
0.1% DMSO	63 ± 5*	9.5

[a] 2008/MRP1 cells were incubated with either 1 μM of clicked dimer or 2 μM of alkyne or azide monomers or a mixture of 1 μM each of alkyne and azide monomers in the presence of DOX for 5 days. After incubation, percentage survival was determined by MTS proliferation assay. Relative fold (RF) was determined by dividing the IC_{50} value without modulators / IC_{50} value with modulators. *2008 wild type ovarian cancer cells were used. 2008/MRP1 is MRP1 overexpressing ovarian cancer cell line. Each compound was tested in triplicate and performed in 3 independent experiments. 0.1% DMSO was used as a solvent control.

2.2.4 Effect on intracellular DOX accumulation in 2008/MRP1 cells

The above results showed that these triazole linked flavonoid dimers are effective MRP1 modulators. DOX is a fluorescent MRP1substrate that can be used to monitor intracellular drug accumulation. We determined whether the modulation of MRP1-mediated drug resistance is associated with a concomitant increase in drug accumulation. Since **Ac3Az11, Ac12Az1 and Ac16Az1** are effective MRP1 modulators according to their EC_{50} (**Table 5**), we selected these compounds for further characterization. As shown in **Figure 5**, treatment of 2008/MRP1 cells with 2 μM of compounds **Ac3Az11**, **Ac12Az1** and **Ac16Az1** resulted in 2.0-fold increase in intracellular DOX accumulation. Verapamil, being a less potent MRP1 modulator, only increased the DOX accumulation by about 1.6-fold (**Figure 5**). MK571, an even less potent modulator, did not increase DOX accumulation in 2008/MRP1 cells at 2 μM (**Figure 5**). The result suggests that these flavonoid dimers inhibit transport activity of MRP1 and restore the intracellular DOX concentration to a level similar to that of parental 2008/P cells.



336

337 **Figure 5.** Effect of pure clicked flavonoid dimers on intracellular DOX accumulation in
338 2008/MRP1 cells. 2008/MRP1 cells were incubated with 5 μM DOX for 120 minutes at 37 ºC
339 with or without 2 μM of **Ac3Az11, Ac12Az1, Ac16Az1, FD-4e**, verapamil or MK571. 0.2% of
340 DMSO was used as negative control. After incubation, cells were washed and intracellular
341 accumulation of DOX was measured by flow cytometry. Experiments were performed in duplicate
342 and repeated thrice. The florescence level of each sample was normalized to the 0.2% DMSO
343 negative control and presented as a fold-difference. The results were presented as mean ± standard
344 error of mean. Student paired t test was conducted relative to 2008/MRP1 cells incubated with
345 0.2% DMSO. * $P < 0.05$.

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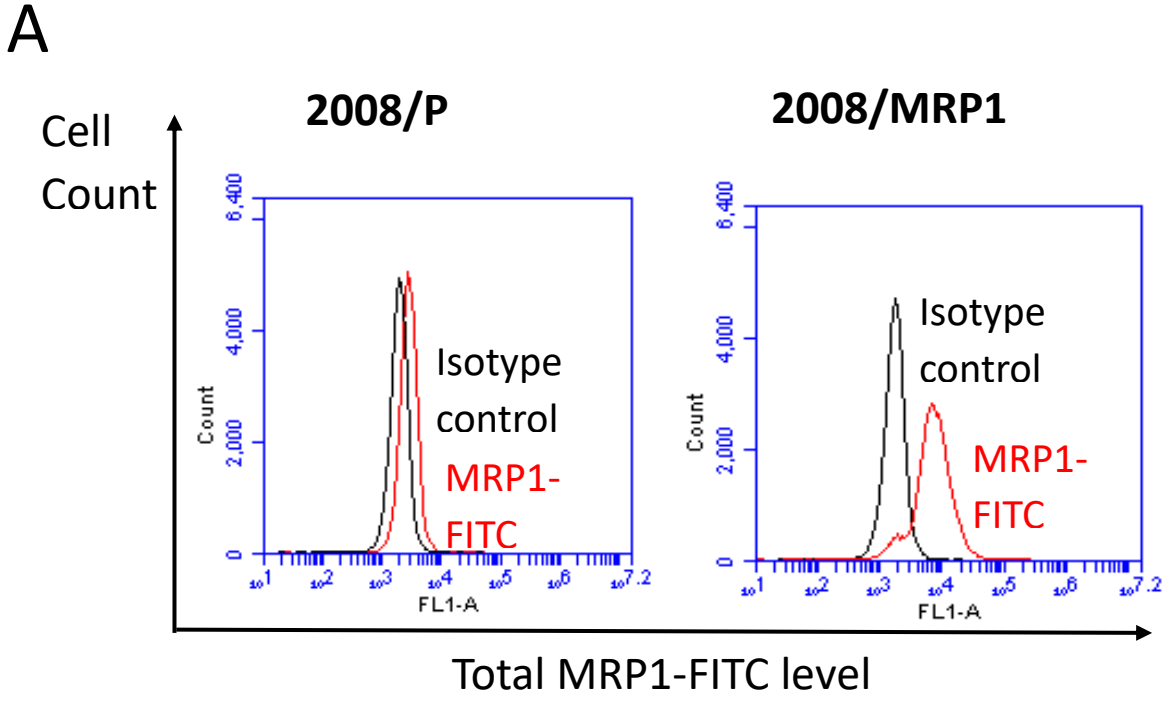
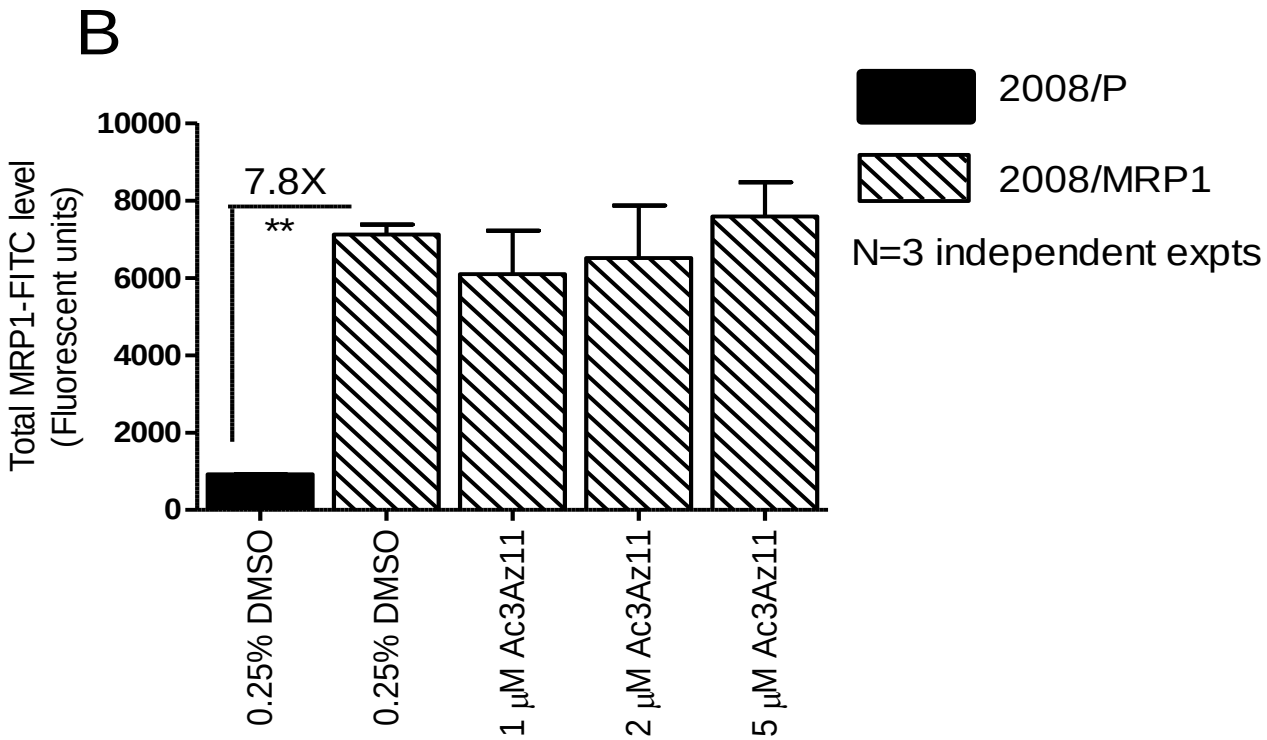
347

2.2.5 *Effect on MRP1 protein expression level*

We characterized the effect of **Ac3Az11** on MRP1 protein expression by flow cytometry (**Figure 6A and 6B**). 2008/MRP1 cells has 7.8-fold ($P<0.001$) higher levels of MRP1 than the parental 2008/P cells (**Figure 6B**). After incubating with 1, 2 or 5 μM of **Ac3Az11** for 3 days, the high expression level of MRP1 in 2008/MRP1 cells remained unchanged, indicating that **Ac3Az11** does not affect the protein expression. After co-incubating with **Ac3Az11**, the increased DOX accumulation level observed in 2008/MRP1 cells (**Figure 5**) might be due to the loss of functionality of MRP1.





364

365 **Figure 6.** Effect of **Ac3Az11** on MRP1 protein expression. The 2008/MRP1 cells were incubated
366 with 1, 2 or 5 μM of **Ac3Az11** for 3 days. Total MRP1 level was measured by flow cytometer at
367 FL1 channel. (A) Flow cytometry result of total MRP1 protein expression in 2008/P or
368 2008/MRP1 cells after incubating with 0.25% DMSO. (B) Total MRP1 protein level of
369 2008/MRP1 cells after incubating with 1, 2 or 5 μM of **Ac3Az11** for 3 days. N = 3 independent
370 experiments. The fluorescence units were presented as mean ± standard error of mean. 0.25%
371 DMSO was used as a solvent control. Student paired t test was conducted between 2008/P and
372 2008/MRP1 cells after incubating with 0.25% of DMSO for 3 days. ** $P<0.001$.

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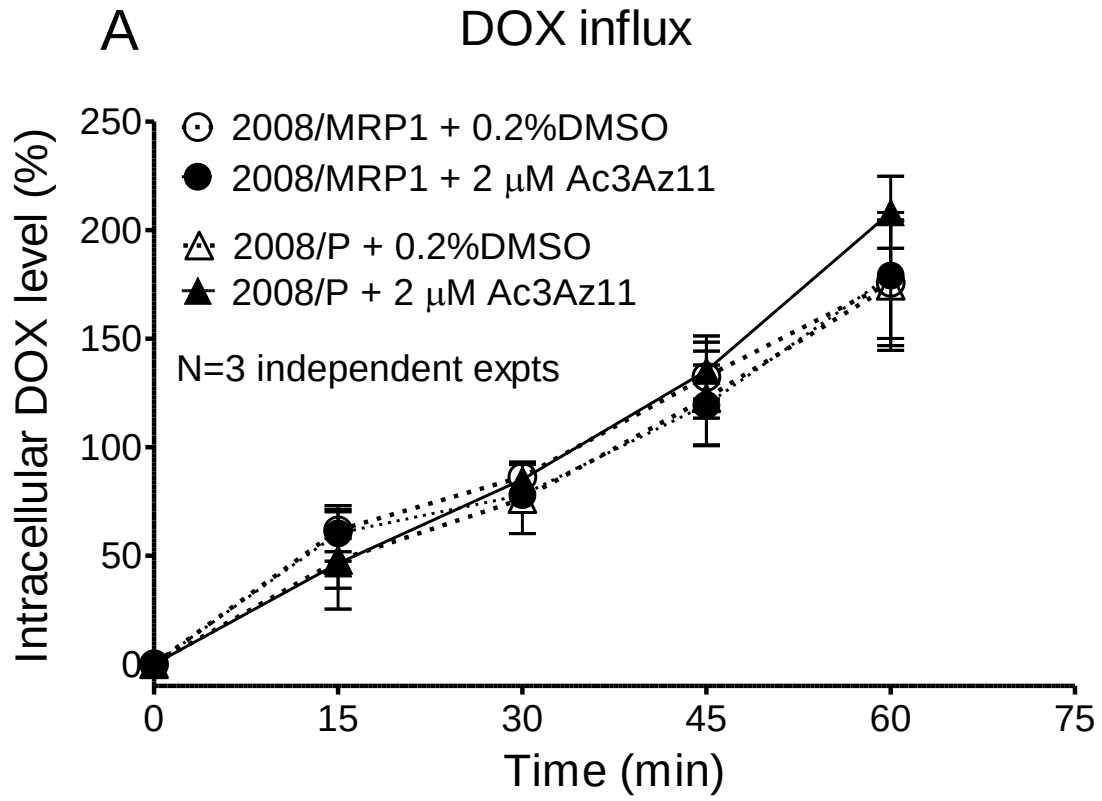
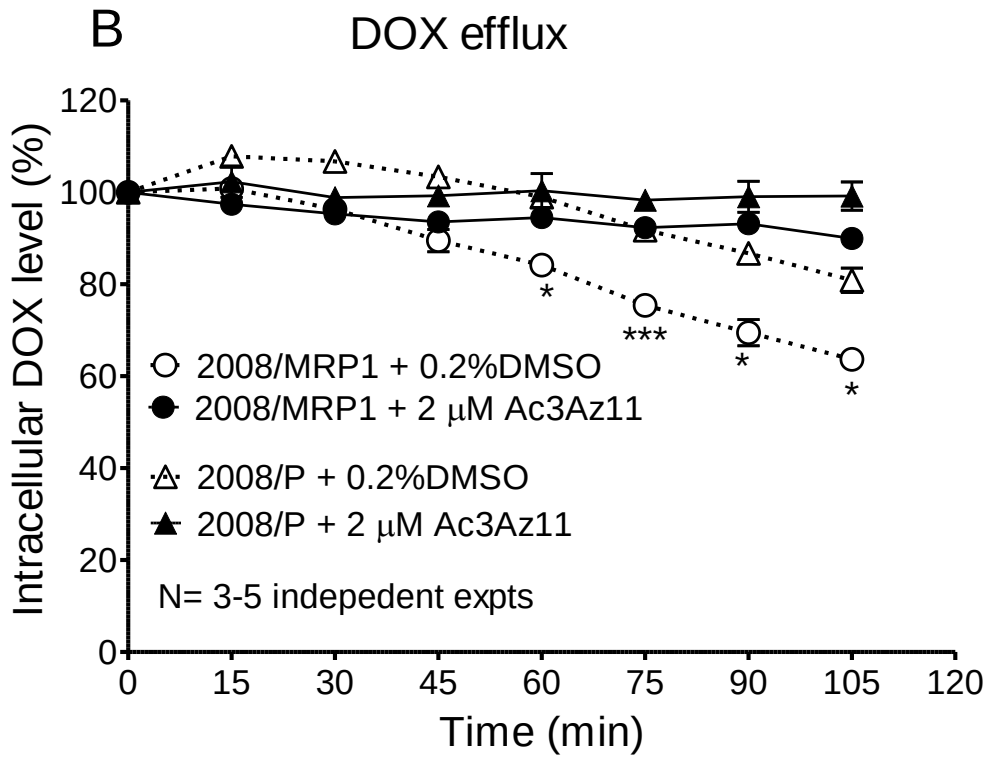
375

2.2.6 *Effect on DOX efflux and DOX influx*

We studied if **Ac3Ac11** increased DOX accumulation in 2008/MRP1 cells by increasing DOX influx or inhibiting DOX efflux. When influx was measured, both 2008/P and 2008/MRP1 cells can influx DOX at almost identical rate with or without **Ac3Az11** (**Figure 7A**). When efflux was measured, 2008/MRP1 cells showed significantly higher efflux rate of DOX than 2008/P cells, with DOX fluorescence dropped to 64% of original level in 105 minutes compared to 81% in 2008/P (**Figure 7B**). Importantly, addition of **Ac3Ac11** completely inhibited DOX efflux in 2008/MRP1 cells (**Figure 7B**). This result demonstrates that reversal of DOX resistance by **Ac3Az11** is due to an inhibition of MRP1-mediated drug efflux, leading to an increased drug accumulation and thus restoring the drug sensitivity.





387

388 **Figure 7.** Effect of **Ac3Az11** on DOX influx and DOX efflux in 2008/MRP1 cells. In the influx
389 experiments (A), cells were co-incubated with DOX (5 μM) and **Ac3Az11** (2 μM) in supplemented
390 RPMI1640 media at 37ºC. 0.25% of DMSO was used as a negative control. The cells were
391 harvested after 0, 15, 30, 45 and 60 min for determining the intracellular DOX concentration. To
392 measure DOX efflux (B), cells were incubated in supplemented RPMI1640 containing 20 μM
393 DOX for 1 hr at 37ºC. Cells were then washed and further incubated with or without compound
394 **Ac3Az11** (2 μM). At 0, 15, 30, 45, 60, 75, 90 and 105 min, cells were harvested and intracellular
395 DOX concentration was measured. The values were presented as mean ± standard error of mean.
396 Student paired t test was conducted at each time point in 2008/MRP1 cells after incubating with
397 or without **Ac3Az11**. ***P<0.0001 and * P<0.01.

398

2.2.7 *Preliminary pharmacokinetics study of Ac3Az11 in mice*

The previous generation of flavonoid dimer **FD-4e** has low solubility in many formulations, therefore hampering it from *in vivo* efficacy study despite its high potency. The present series of flavonoid dimers contain a basic triazole ring and their hydrochloride salts can be readily prepared. Here we have dissolved **Ac3Az11.HCl** in a formulation (NMP: Cremorphol: Tween 80: H_2O = 5 : 5 : 4.5 : 85.5) at 1.5 mg/mL and used it to study intravenous (i.v.) pharmacokinetics in Balb/c mice (**Figure 8**). **Ac3Az11** was dosed at 10mg/kg and its plasma level in Balb/c mice was monitored up to 360 minutes post administration. **Ac3Az11** could be detected in plasma and its plasma level was maintained above its *in vitro* EC_{50} (53 nM for DOX) for about 90 minutes (**Figure 8**). In contrast, **FD-4e** was completely insoluble in the same formulation. When **FD-4e** was dissolved in DMSO and administered to mice by i.v. injection, it precipitated very quickly and the concentration of **FD-4e** in plasma was below the detection limit. Further *in vivo* efficacy studies of these compounds are in progress.

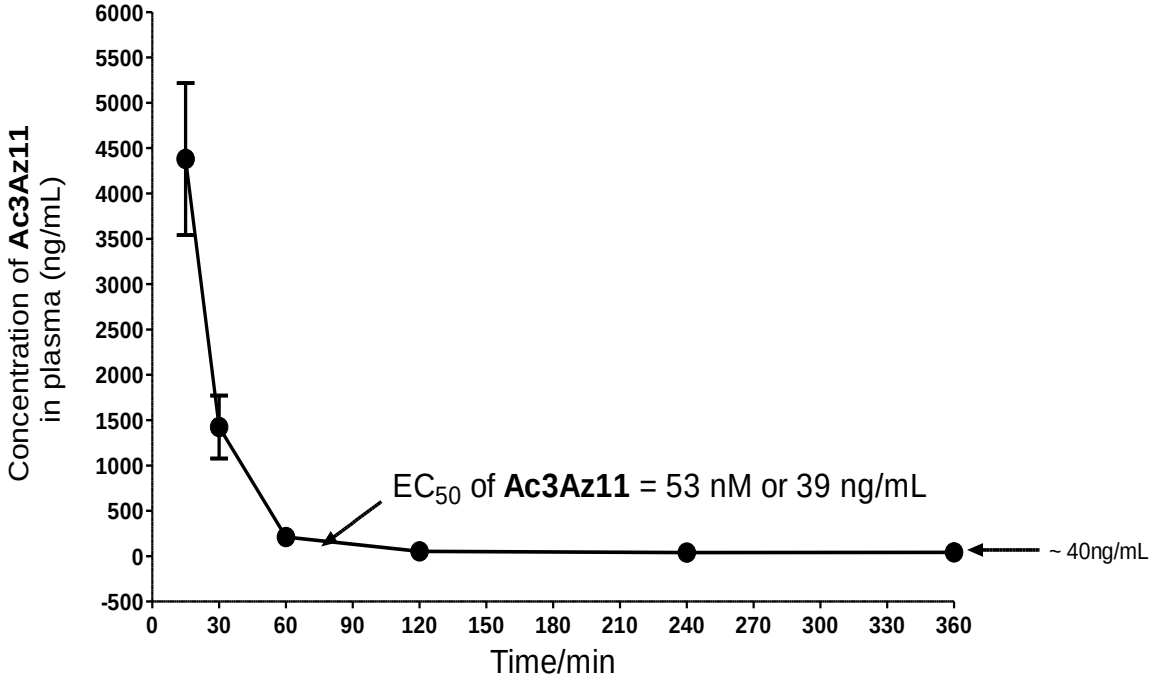


Figure 8. Pharmacokinetics study of **Ac3Az11.HCl** in Balb/c mice. **Ac3Az11.2.HCl** at 10 mg/kg was injected intravenously to the Balb/c mice. At each time point indicated (15, 30, 60, 120, 240 and 360 minutes), 3 mice were sacrificed and blood was collected. The plasma level of **Ac3Az11** was quantified by LC-MS/MS. The data was presented as mean ± standard error of mean. *In vitro* EC_{50} (nM) for reversing DOX resistance was 53 nM.

2.2.8 *In silico docking studies*

The electron cryo-microscopy (cryo-EM) structure of bovine MRP1 in two different inward-facing conformations, including an apo form at 3.5 Å resolution without any substrate and a complex form at 3.3 Å resolution with one substrate leukotriene C_4 (LTC_4), and an outward-facing conformation with an ATP-bound at 3.1 Å resolution have been determined recently. [40,41] These cryo-EM structures suggest that the bovine MRP1 recognizes a wide range of chemicals by forming a single bipartite substrate binding site of higher substrate-binding affinity and extrudes

them through reconfiguring substrate binding site to a lower substrate-binding affinity upon ATP binding. More importantly, both bovine MRP1 and human MRP1 share a high level of similarity not only in their physiological functions of xenobiotics extrusion but also in their amino acid sequence identities (91% identical, **Figure S27**). Therefore, these cryo-EM structures of bovine MRP1 are good homology models that can be employed to perform *in silico* docking studies for providing more insights into the potential molecular interactions between selected compounds and human MRP1. In the present docking studies, the bovine MRP1 in the inward-facing conformation with LTC4-bound (PDB ID: 5UJA) was selected as the docking model. Flavonoid dimers **Ac3Az11**, **FD-4e** and DOX, a MRP1 substrate, were docked into this cryo-EM structure respectively. As shown in **Figure 9A1**, the highest docking scores of **Ac3Az11**, **FD-4e** and DOX predicted that they all bind to the bipartite binding site of MRP1 and occupy the central translocation pathway, in which they share similar interactions with bovine MRP1 as LTC_4 does. The predicted binding poses for **Ac3Az11**, **FD-4e** and DOX are shown in **Figure 9A2**, **9A3** and **9A4** respectively. Docking results suggested that extensive networks of hydrogen bonding interactions, π-π interactions and van der Waals contacts are formed inside the bipartite binding site between these ligands and several amino acid residues including His335, Lys332, Leu381, Phe385, Asn1244, Trp1245, Met1092, Ser1096, Thr550, Tyr1242, Trp553, Phe594, Arg1248 and Arg1196. In particular, the "tryptophan sandwich" formed by Trp1245 and Trp553 in the bipartite binding site is potentially the major interacting amino acid residues as flavonoid dimers **Ac3Az11**, **FD-4e** and DOX were predicted to have strong π-π interactions between the aromatic moieties of these compounds and the indole moieties of Trp. Such π-π interactions were also predicted to occur between the aromatic moieties of these compounds and the phenyl rings of Phe385 and Phe594 respectively. Moreover, extensive networks of hydrogen bonding interactions were predicted to

449 exist between these compounds and the amino acid residues of bovine MRP1. For example, the
450 carbonyl groups of flavonoid dimer **Ac3Az11** formed hydrogen bonding interactions with Tyr1242
451 and Ser1096 respectively (**Figure 9A2**); the carbonyl groups and oxygen atoms of flavonoid dimer
452 **FD-4e** formed hydrogen bonding interactions with Gln1238, Thr1241 and Tyr440 respectively
453 (**Figure 9A3**); the amine group and hydroxyl group of DOX formed hydrogen bonding interactions
454 with Tyr1242 and Tyr440 respectively (**Figure 9A4**). In addition, the linker portion of both
455 flavonoid dimers may form van der Waals contacts with amino acid residues including Tyr1242,
456 Met1092, Glu1088, Thr550, Val554 and Trp553. From the docking studies, the highest docking
457 scores of **Ac3Az11** (score -114) and **FD4e** (score -110) were more energetically favorable than
458 that of DOX (score -82) or LTC_4 (score - 95), implying that both flavonoid dimers may have
459 stronger binding affinity to bovine MRP1 than that of the substrate DOX or LTC_4, therefore
460 capable of inhibiting their transportation.

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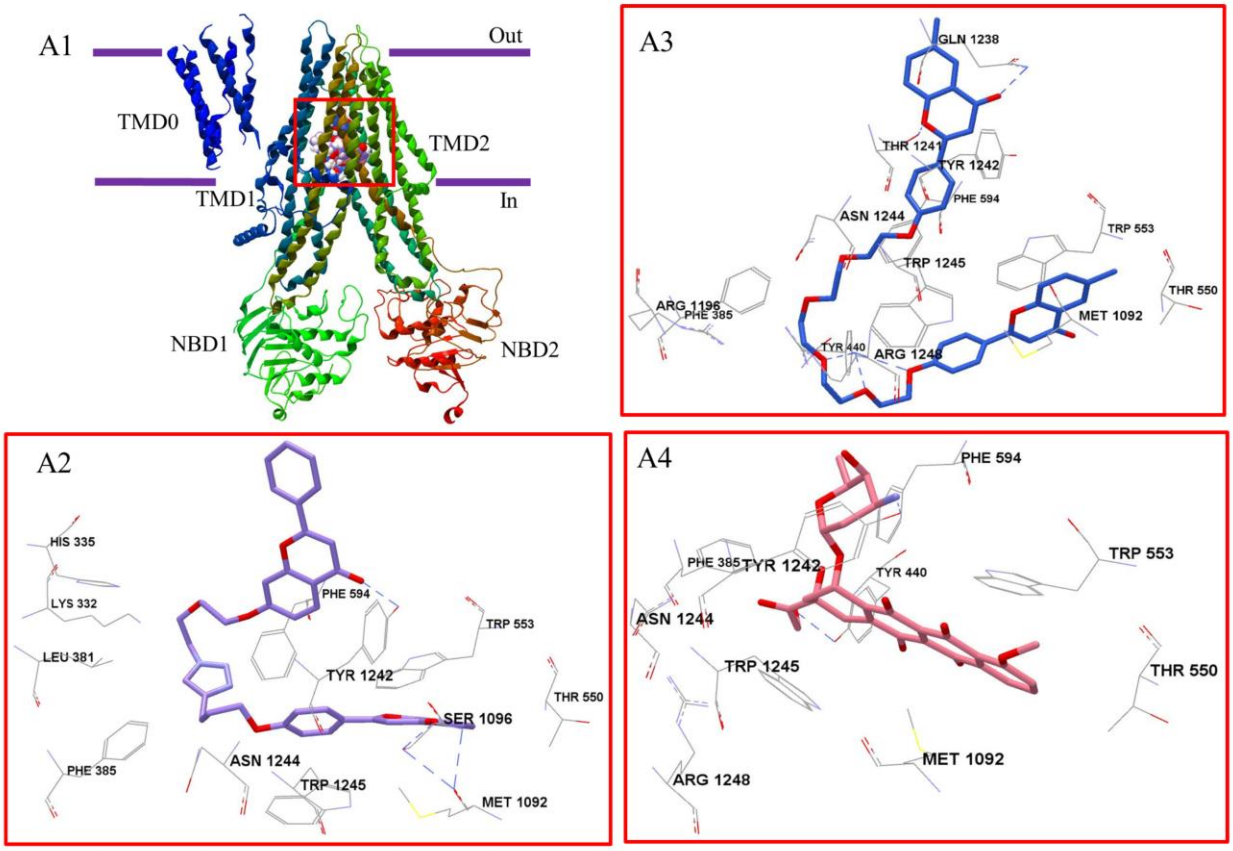

Figure 9. *In silico* docking. (A1) Representation of bovine MRP1 with labeled TMD0, TMD1, TMD2, NBD1 and NBD2 (PDB ID: 5UJA). The red square indicates the bipartite binding site with zoomed in view of (A2) **Ac3Az11** (violet color), (A3) **FD-4e** (blue color) and (A4) DOX (brown color) in the same binding site with important amino acids highlighted in black and hydrogen bonding interactions indicated as dotted blue lines

2.2.9 *Modulation activity of promising flavonoid dimers towards P-gp and BCRP transporters*

Other than MRP1-modulating activity, we also examined the ability of promising clicked flavonoid dimers (listed in **Table 5**) to reverse P-gp and BCRP-mediated drug resistance. It was found that the P-gp-mediated paclitaxel resistance in LCC6MDR cells and BCRP-mediated topotecan resistance in HEK293/R2 cells were substantially reversed by the clicked dimers at 1

476 μM, respectively (**Table 7**). Compared to cyclosporine A (RF = 79.4), **Ac3Az11** (RF = 40.7) was
477 about 2-fold lower in P-gp modulating activity (**Table 7**). On the other hand, the BCRP-
478 modulating activity of **Ac3Az11** (RF = 18.2) was as potent as Ko143 (RF = 20.7) in reversing
479 topotecan resistance in HEK293/R2 cells. It demonstrates that dimer **Ac3Az11** can modulate
480 MRP1-, P-gp- and BCRP-mediated drug resistance.

481 **Table 7.** Modulating activity of promising clicked flavonoid dimers towards P-gp and BCRP
482 transporters.[a]

Compounds	P-gp-expressing LCC6MDR Mean IC_{50} of paclitaxel (nM)	RF	BCRP-expressing HEK293/R2 Mean IC_{50} of toptoecan (nM)	RF
1 μM Ac3Az11	3.9 ± 1.3	40.7	27.3 ± 7.3	18.2
1 μM Ac16Az1	4.1 ± 0.8	38.7	39.9 ± 4.0	12.4
1 μM Ac12Az1	8.0 ± 2.1	19.8	25.8 ± 4.5	19.3
1 μM Ac2Az4	9.9 ± 0.7	16.0	32.6 ± 6.6	15.2
1 μM Ac16Az2	5.0 ± 0.4	31.7	47.9 ± 2.5	10.4
1 μM Ac5Az1	2.3 ± 0.2	69.0	47.9 ± 1.8	10.4
1 μM Ac3Az4	7.0 ± 1.4	22.7	27.0 ± 5.5	18.4
1 μM Ac1Az1	5.0 ± 1.1	31.7	23.1 ± 6.1	21.5
1 μM Ac3Az3	4.3 ± 1.1	36.9	30.6 ± 3.3	16.2
1 μM Ac5Az3	2.7 ± 0.7	58.8	41.5 ± 2.1	12.0
1 μM Ac3Az1	2.5 ± 0.5	63.5	24.1 ± 9.5	20.6
1 μM Ac2Az1	8.3 ± 1.1	19.1	32.0 ± 11.3	15.5
1 μM Ac16Az7	6.0 ± 1.0	26.5	40.0 ± 5.7	12.4
1 μM Ac16Az4	9.4 ± 2.5	16.9	32.8 ± 12.4	15.1
1 μM Ac5Az11	2.9 ± 0.2	54.7	20.3 ± 0.8	24.5
1 μM Ac13Az5	3.4 ± 0.3	46.7	18.4 ± 3.2	27.0
1 μM Ac3Az2	40.1 ± 5.7	4.0	39.3 ± 5.6	12.6
1 μM Ac5Az4	1.6 ± 0.3	99.2	28.8 ± 3.7	17.2
1 μM Ac5Az7	1.6 ± 0.2	99.2	32.0 ± 5.8	15.5
1 μM Ac13Az1	48.7 ± 9.8	3.3	47.9 ± 3.7	10.4
1 μM Ac2Az11	39.9 ± 3.5	4.0	42.6 ± 7.5	11.7
1 μM Cyclosporine A	2.0 ± 0.2	79.4	ND	
1 μM Ko143	ND		24.0 ± 1.9	20.7
0.1% DMSO	158.7 ± 6.1	1.0	496.7 ± 31.1	1.0
LCC6 + 0.1 % DMSO	1.6 ± 0.3	99.2	ND	
HEK293/pcDNA3.1 + 0.1% DMSO	ND		15.8 ± 1.5	31.4

483

[a] All promising clicked flavonoid dimers were tested for their modulating activity towards P-gp and BCRP. LCC6MDR is a P-gp transfected breast cancer cell line and was incubated with different concentrations of paclitaxel (0, 1.6, 5, 15, 44, 133, 400 nM) and 1 μM of dimers or the known P-gp inhibitor cyclosporine A. HEK293/R2 is a BCRP-transfected human embryonic kidney cell line. It was co-incubated with different doses of topotecan (0, 12, 37, 111, 333, 1000, 3000 nM) and 1 μM of dimers or known BCRP inhibitor Ko143. After 5-day incubation, percentage of survivors was determined by MTS proliferation assay and the IC_{50} of drug was determined. Relative fold (RF) was determined by dividing the IC_{50} value without modulators / IC_{50} value with modulators. LCC6 and HEK293/pcDNA3.1 were parental cell line of LCC6MDR and HEK293/R2, respectively. Each compound was tested in triplicate and performed in 3 independent experiments. 0.1% DMSO was used as a solvent control. ND = not determined.

3. DISCUSSION and CONCLUSION

Overexpression of MRP1 transporter has been associated with tumor MDR and poor patient outcome. To circumvent MRP1-mediated MDR, combination of MRP1 modulator and anticancer drugs has been considered as a potential treatment. To date, no potent and safe MRP1 modulator has been developed. Herein, we have successfully applied "click chemistry" to construct a 300-member homo- and hetero-flavonoid dimer library without the need for purification. With the use of high throughput screening of the unpurified flavonoid dimer library, we were able to rapidly identify the active members of the library. Of the 300 members, we only needed to synthesize and purify 21 members of the flavonoid dimers. The EC_{50} of and the selective index of these 21 pure dimers can be further characterized to confirm the validity of such high throughput platform. It is also possible to draw some information regarding the structure activity relation for MRP1 modulation. Our preliminary conclusion regarding the pharmacophore of active MRP1 modulator

is that it should have: (1) dimeric structure, (2) un-substitution or fluoro or methyl substitution at A-ring of flavone, (3) un-substitution at C-ring of flavone, (4) flavone phenyl A, B and C rings on both flavonoid moieties, (5) one triazole ring on the linker and (6) linker length of n = 4-9 atoms on either side of the triazole ring.

Among the 21 active pure clicked dimers, all of them possess above six criteria. The much higher potency of the dimer over the corresponding monomeric units (**Table 6**) is in conformity with the divalent approach of design of modulators due to the pseudo-dimeric structures of MRP1 and ABC transporters in general.[23-28] Previously, we found that the flavonoid dimer **FD-4e,** with PEG linker length n=5 having 15 atoms between the two flavonoid moieties, displayed a low EC_{50} value of 73 nM in reversing DOX resistance in a MRP1 overexpressing ovarian (2008/MRP1) cell line, whereas flavonoid dimers with shorter (n≤4,) or longer (n≥6) PEG linkers were less potent.[27] It is interesting to note that of the 21 active compounds discovered in here, all have linkers with 13-17 atoms between the two flavonoid moieties. Linker conjugation site at the phenyl rings appears to influence the MRP1-modulating activity (**Table 5**). The top 5 "hit" compounds, **Ac3Az11**, **Ac16Az1**, **Ac12Az1**, **Ac2Az4 and Ac16Az2** showed the highest potency (EC_{50} ranged from 53 – 99 nM). Their triazole-bridged linkers were conjugated at C7 position of A-ring of one flavonoid moiety and the C4' position of B-ring of the other flavonoid moiety. Compounds with linkers conjugated at C4' position of B-rings of both flavonoid moieties showed slightly weaker potency with EC_{50} ranged from 103 – 250 nM. Compounds with linker conjugated at C7 position of both A-rings displayed the lowest potency with EC_{50} of 298 nM among the 21 compounds. On the other hand, the presence of the triazole group confers different physiochemical properties to the active compounds. Thus, compound **Ac3Az11** and its hydrochloride salt have much better aqueous solubility than **FD-4e**. Preliminary pharmacokinetics study showed that **Ac3Az11** can be

administered to mice with sufficient plasma concentration above its EC_{50} level (**Figure 8**). In an effort to understand the mechanism of action of these compounds, we demonstrated that **Ac3Az11** did not affect the protein expression level of MRP1 when 2008/MRP1 cells were incubated with 1, 2 and 5 μM of **Ac3Az11** for 3 days (**Figure 6**). On the other hand, **Ac3Az11** was found to inhibit DOX efflux in 2008/MRP1 cells (**Figure 7**), leading to an increased drug accumulation (**Figure 5**) and thus restoring the drug sensitivity.

Recently, the electron cryo-microscopy structures of bovine MRP1 in the apo form, the substrate added form, and an ATP-bound outward facing form have been reported.[40, 41] The high similarities in amino acid sequences and functional properties between bovine and human MRP1 implied that the structure of bovine MRP1 would be a reasonable starting point for structural studies of human MRP1. By comparing the two inward facing structures, it is possible to conclude that the substrate LTC_4 binds to two pockets (H and P) of bMRP1, with one pocket in TMD1 and the other pocket in TMD2.[40] Furthermore, large conformational change is induced by substrate binding, bringing the two halves of the transporter together. Such a bipartite substrate binding is consistent with the fact that MRP1 can recognize a spectrum of substrates with different chemical structures. [40] It is likely that our flavonoid dimer modulators bind to the same bipartite substrate binding site.

In our *in silico* study, the bovine MRP1 in complex with substrate LTC_4 was used to perform the docking studies with flavonoid dimers **Ac3Az11, FD-4e** and DOX using CLC Drug Discovery Workbench software. The physiological substrate LTC_4 was used as reference compound to compare the molecular interactions of flavonoid dimers with the bovine MRP1. Compound **Ac3Az11**, **FD-4e,** LTC_4 and DOX interact with bovine MRP1 in the same binding site with a score of -114, -110, -89 and -82 respectively (**Figure 9**). Considering the scores, both flavonoid dimers

553 are expected to bind stronger than LTC_4 or DOX and function as competitive inhibitor. Previously,
554 we demonstrated that **FD-4e** is a competitive inhibitor of DOX transport by MRP1, presumably
555 by binding to the same binding site as the substrate.[27]

556 Other than MRP1-modulating activity, we also examined the ability of **Ac3Az11** to reverse P-
557 gp and BCRP-mediated drug resistance. It appeared that dimer **Ac3Az11** can modulate MRP1- as
558 well as P-gp- and BCRP-mediated drug resistance (**Table 7**). In the literature, some modulators
559 are known to have activity against two ABC transporters. For example, Ko143 has been reported
560 to have inhibitory effect on the transport activity of both P-gp and MRP1 at the concentrations of >
561 1μM[49] and HM30181 was reported to be potent inhibitors of both P-gp and the breast cancer
562 resistance protein (BCRP/ABCG2).[50] As far as we are aware, there are few compounds that can
563 inhibit all three ABC transporters. We will further investigate such pan-transporter activities and
564 report in due course.

565 To our knowledge, this is the first click-chemistry derived library coupled with high throughput
566 screening platform for transmembrane transporters. It appears that discovery of potent MRP1
567 modulators can be achieved effectively and with much less synthetic effort required. The size of
568 the library can be easily expanded by simply increasing the number of monomeric units. We are
569 cognizant of the possibility that such an approach could lead to false negatives, in that potentially
570 active compounds may have been missed because the CuAAC reaction is inefficient for some
571 specific alkynes or azides. The platform however can be readily modified by changing the reaction
572 conditions if necessary. This approach can be easily applied to screen for active modulators of
573 other membrane transporters and this will be pursued.

574

4. EXPERIMENTAL SECTION

4.1 Materials and Methods

All NMR spectra were recorded on a Bruker Advance-III 400 MHz spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, Varian Unity Inova 500 MHz NMR Spectrometer at 500 MHz for ^{1}H and 125 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). Substituted 4' or 7-hydroxyflavones **1a-i** were prepared as reported previously.[25] 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 320 nm (reference at 450 nm) with isocratic elution of hexane (50%)/ethyl acetate (25%)/methanol (25%) at a flow rate of 1.0 mL/min. All tested compounds were shown to have >95% purity according to HPLC.

4.2 General procedure for the synthesis of Ac1-Ac5, Ac12, Ac35 and Ac42 (Scheme 1)

To a round-bottom flask was charged with corresponding 4'-hydroxyflavones or 7-hydroxyflavones **1a**-**e** (1 equiv.), 5-chloropent-1-yne or 6-chlorohex-1-yne (1.2 equiv.), K_2CO_3 (1.5 equiv.) and DMF (3 mL per equiv (mmol)). The reaction mixture was stirred at refluxing temperature for 2 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with DCM. If the mixture could not be separated into two layers, small amount of 1M HCl was added. The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a brown crude reaction mixture. Purification was performed by flash column chromatography on silica gel with acetone in DCM as eluent to furnish desired product.

4.2.1 Synthesis of 2-(4-(Pent-4-yn-1-yloxy)phenyl)-4*H*-chromen-4-one (**Ac1**): This compound (0.53 g, 82%) was obtained from 2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1a**) and 5-chloropent-1-yne according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, *J*=7.60, 1.60 Hz, 1H), 7.85 (d, *J*=8.80 Hz, 2H), 7.65 (ddd, *J*=7.60, 7.20, 1.60 Hz, 1H), 7.52 (d, *J*=8.40 Hz, 1H), 7.38 (dd, *J*=7.60, 7.20 Hz, 1H), 7.00 (d, *J*=8.80 Hz, 2H), 6.71 (s, 1H), 4.13 (t, *J*=6.40 Hz, 2H), 2.40 - 2.44 (m, 2H), 1.98 - 2.06 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 163.3, 161.6, 156.1, 133.5, 127.9, 125.5, 125.0, 123.9, 123.8, 117.9, 114.8, 106.0, 83.1, 69.1, 66.3, 27.9, 15.0; LRMS (ESI) m/z 305 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{17}O_3$ $[M+H]^+$ 305.1178, found 305.1180.

4.2.2 Synthesis of 7-(Pent-4-yn-1-yloxy)-2-phenyl-4*H*-chromen-4-one (**Ac2**): This compound (0.33 g, 79%) was obtained from 7-hydroxy-2-phenyl-4*H*-chromen-4-one (**1e**) and 5-chloropent-1-yne according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J*=7.20, 2.80 Hz, 1H), 7.84 - 7.86 (m, 2H), 7.47 - 7.49 (m, 3H), 6.93 - 6.95 (m, 2H), 6.71 (s, 1H), 4.16 (t, *J*=6.40 Hz, 2H), 2.40 - 2.44 (m, 2H), 1.99 - 2.07 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ

177.7, 163.3, 162.9, 157.8, 131.7, 131.3, 128.9, 126.9, 126.0, 117.7, 114.6, 107.3, 100.8, 82.9, 69.2, 66.7, 27.7, 15.0; LRMS (ESI) m/z 305 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{17}O_3$ $[M+H]^+$ 305.1178, found 305.1181.

4.2.3 Synthesis of 7-Fluoro-2-(4-(pent-4-yn-1-yloxy)phenyl)-4*H*-chromen-4-one (**Ac3**): This compound (0.31 g, 89%) was obtained from 7-fluoro-2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1b**) and 5-chloropent-1-yne according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, *J*=6.40, 6.40 Hz, 1H), 7.81 (t, *J*=8.80 Hz, 2H), 7.20 (dd, *J*=9.20, 2.40 Hz, 1H), 7.08 - 7.13 (m, 1H), 6.99 (d, *J*=8.80 Hz, 2H), 6.68 (s, 1H), 4.14 (t, *J*=6.00 Hz, 2H), 2.40 - 2.44 (m, 2H), 1.98 - 2.06 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 166.7, 164.2, 163.6, 161.8, 157.1, 156.9, 127.8, 123.5, 120.7, 114.9, 113.7, 113.5, 106.0, 104.7, 104.5, 83.0, 69.1, 66.3, 27.9, 15.0; LRMS (ESI) m/z 323 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{16}FO_3$ $[M+H]^+$ 323.1083, found 323.1086.

4.2.4 Synthesis of 5-(Benzyloxy)-7-(methoxymethoxy)-2-(4-(pent-4-yn-1-yloxy)phenyl)-4*H*-chromen-4-one (**Ac4**): This compound (0.11 g, 71%) was obtained from 5-(benzyloxy)-2-(4-hydroxyphenyl)-7-(methoxymethoxy)-4*H*-chromen-4-one (**1c**) and 5-chloropent-1-yne according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J*=8.40 Hz, 2H), 7.62 (d, *J*=7.20 Hz, 2H), 7.26 - 7.40 (m, 3H), 6.95 (d, *J*=8.80 Hz, 2H), 6.73 (d, *J*=1.60 Hz, 1H), 6.54 (s, 1H), 6.47 (d, *J*=1.60 Hz, 1H), 5.21 (s, 2H), 5.20 (s, 2H), 4.09 (t, *J*=6.00 Hz, 2H), 3.47 (s, 3H), 2.38 - 2.42 (m, 2H), 1.97 - 2.03 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 161.3, 161.1, 160.6, 159.5, 159.3, 136.4, 128.5, 127.5, 126.6, 123.7, 114.7, 110.1, 107.4, 98.6, 95.9, 94.2, 83.1, 70.6, 69.1, 66.2, 56.3, 27.9, 15.0; LRMS (ESI) m/z 471 $[M+H]^+$; HRMS (ESI) calcd for $C_{29}H_{27}O_6$ $[M+H]^+$ 471.1808, found 471.1815.

4.2.5 Synthesis of 2-(4-(Hex-5-yn-1-yloxy)phenyl)-6-methyl-4*H*-chromen-4-one (**Ac5**): This compound (0.22 g, 73%) was obtained from 2-(4-hydroxyphenyl)-6-methyl-4*H*-chromen-4-one (**1d**) and 6-chloropent-1-yne according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (s, 1 H), 7.80 (d, *J*=8.79 Hz, 2 H), 7.37 - 7.46 (m, 2 H), 6.95 (d, *J*=8.30 Hz, 2 H), 6.67 (s, 1 H), 4.02 (t, *J*=6.10 Hz, 2 H), 2.41 (s, 3 H), 2.23 - 2.31 (m, 2 H), 1.97 (br. s., 1 H), 1.86 - 1.96 (m, 2 H), 1.67 - 1.76 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3, 163.1, 161.6, 154.3, 134.8, 134.6, 127.8, 124.8, 123.8, 123.4, 117.6, 114.7, 105.8, 83.8, 68.7, 67.4, 28.0, 24.8, 20.8, 18.0; LRMS (ESI) m/z 333 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{21}O_3$ $[M+H]^+$ 333.1491, found 333.1495.

4.2.6 Synthesis of 7-(Hex-5-yn-1-yloxy)-2-phenyl-4*H*-chromen-4-one (**Ac12**): This compound (0.13 g, 69%) was obtained from 7-hydroxy-2-phenyl-4*H*-chromen-4-one (**1e**) and 6-chloropent-1-yne according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J*=8.79 Hz, 1 H), 7.89 - 7.94 (m, 2 H), 7.49 - 7.55 (m, 3 H), 6.95 - 7.01 (m, 2 H), 6.77 (s, 1 H), 4.12 (t, *J*=6.34 Hz, 3 H), 2.31 (td, *J*=7.08, 2.44 Hz, 2 H), 1.95 - 2.04 (m, 3 H), 1.71 - 1.81 (m, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.63, 163.44, 162.78, 157.81, 131.72, 131.25, 128.86, 126.83, 125.99, 117.63, 114.58, 107.34, 100.78, 83.71, 68.81, 67.91, 27.87, 24.79, 18.01; LRMS (ESI) m/z 319 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{19}O_3$ $[M+H]^+$ 319.1334, found 319.1328.

4.2.7 Synthesis of 2-(4-(Hex-5-yn-1-yloxy)phenyl)-3-((3-methoxybenzyl)oxy)-4H-chromen-4-one (Ac35) :

This compound (0.84 g, 54%) was obtained from 2-(4-hydroxyphenyl)-3-((3-methoxybenzyl)oxy)-4H-chromen-4-one (**1g**) according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (dd, *J* = 7.95, 1.35 Hz, 1 H), 7.99 - 8.06 (m, 2 H), 7.59 - 7.68

(m, 1 H), 7.49 (d, *J* = 8.56 Hz, 1 H), 7.38 (t, *J* = 7.58 Hz, 1 H), 7.13 - 7.21 (m, 1 H), 6.89 - 6.98 (m, 4 H), 6.77 - 6.83 (m, 1 H), 5.10 (s, 2 H), 4.04 (t, *J* = 6.24 Hz, 2 H), 3.71 (s, 3 H), 2.29 (td, *J* = 7.03, 2.57 Hz, 2 H), 2.00 (t, *J* = 2.69 Hz, 1 H), 1.90 - 1.98 (m, 2 H), 1.68 - 1.79 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.8, 160.8, 159.4, 156.2, 155.0, 139.1, 138.2, 133.1, 130.4, 129.1, 125.6, 124.4, 124.1, 123.1, 120.9, 117.8, 114.2, 114.1, 113.5, 83.8, 73.7, 68.7, 67.3, 55.0, 28.1, 24.9,18.0; LRMS (ESI) m/z 455 $[M+H]^+$; HRMS (ESI) calcd for $C_{29}H_{26}O_5$ $[M+H]^+$ 455.1858, found 455.1853.

4.2.8 Synthesis of 6-Fluoro-2-(4-(pent-4-yn-1-yloxy)phenyl)-4H-chromen-4-one (Ac42): This compound (0.11 g, 29%) was obtained from 6-fluoro-2-(4-hydroxyphenyl)-4H-chromen-4-one (**1c**) and 5-chlorohex-1-yne according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 - 7.87 (m, 3H), 7.53 (dd, *J* = 4.16, 9.05 Hz, 1H), 7.33 - 7.43 (m, 1H), 7.00 (d, *J* = 8.80 Hz, 2H), 6.70 (s, 1H), 4.14 (t, *J* = 6.11 Hz, 2H), 2.43 (dt, *J* = 2.45, 6.85 Hz, 2H), 2.01 - 2.07 (m, 2H), 2.00 (d, *J* = 2.45 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.5, 177.4, 163.6, 161.8, 160.7, 158.2, 152.4, 152.3, 128.0, 125.1, 125.0, 123.6, 121.7, 121.5, 120.0, 119.9, 114.9, 110.6, 110.4, 105.4, 83.1, 69.1, 66.4, 27.9, 15.1; LRMS (ESI) m/z 323 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{16}FO_3$ $[M+H]^+$ 323.1005, found 323.1007.

4.3 General procedure for the synthesis of Ac6-Ac11 (Scheme 2)

Excess KOH (3M solution in 96% EtOH, 3–4 equiv) was added to a mixture of 4-(hex-5-yn-1-yloxy)benzaldehyde (**Ac33**) (1.0 equiv) and the substituted 2'-hydroxyacetophenone **3a-e** (1.0 equiv). The mixture was stirred at room temperature for 16 h. When TLC indicated complete consumption of starting material, the reaction mixture was acidified to pH 5 with 1M HCl at ice-bath temperature. The yellow precipitate formed was collected by suction filtration. The yellow

685 solid was washed with n-hexane and subjected to crystallization from MeOH to afford the desired
686 chalcones. If no precipitate was formed after the addition of 1M HCl, then the mixture was
687 continuously extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered,
688 and evaporated under reduced pressure to give a crude mixture, which was subjected to flash
689 column chromatography using 15% EtOAc in hexane as eluent to furnish the desired chalcones.

690 **4.3.1 Synthesis of (*E*)-3-(4-(Hex-5-yn-1-yloxy)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one**
691 (**Ac6**): This compound (0.36 g, 75%) was obtained from 4-(hex-5-yn-1-yloxy)benzaldehyde (**Ac33**)
692 and 2'-hydroxyacetophenone (**3a**) according to the general procedure described above. ^{1}H NMR
693 (400 MHz, $CDCl_3$) δ 12.97 (s, 1H), 7.64 - 7.95 (m, 2H), 7.48 - 7.62 (m, 2H), 6.94 - 7.05 (m, 3H),
694 4.09 (t, *J*=6.00 Hz, 2H), 2.31 - 2.35 (m, 2H), 1.93 - 2.01 (m, 3H), 1.82 - 1.85 (m, 2H); ^{13}C NMR
695 (100 MHz, $CDCl_3$) δ 193.6, 163.5, 161.5, 145.4, 136.1, 131.9, 130.5, 129.5, 127.2, 120.1, 118.7,
696 118.5, 117.5, 114.9, 114.7, 83.9, 68.8, 67.7, 28.1, 24.9, 18.1; LRMS (ESI) m/z 321 $[M+H]^+$;
697 HRMS (ESI) calcd for $C_{21}H_{21}O_3$ $[M+H]^+$ 321.1491, found 321.1492.

698 **4.3.2 Synthesis of (*E*)-1-(5-Ethyl-2-hydroxyphenyl)-3-(4-(hex-5-yn-1-yloxy)phenyl)prop-2-**
699 **en-1-one** (**Ac7**): This compound (0.23 g, 61%) was obtained from 4-(hex-5-yn-1-
700 yloxy)benzaldehyde (**Ac33**) and 2'-hydroxy-5'-ethylacetophenone (**3b**) according to the general
701 procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.84 (s, 1H), 7.91 (d, *J*=7.20 Hz,1H),
702 7.53 - 7.71 (m, 3H), 7.35 (dd, *J*=2.00, 7.20 Hz, 1H), 6.94 - 6.98 (m, 3H), 4.09 (t, *J*=6.00 Hz, 2H),
703 2.63 - 2.69 (m, 2H), 1.93 - 2.01 (m, 3H), 1.74 - 1.78 (m, 2H), 1.26 (t, *J*=6.00 Hz, 3H); ^{13}C NMR
704 (100 MHz, $CDCl_3$) δ 193.5, 161.6, 161.4, 145.1, 136.0, 134.3, 130.5, 128.1, 127.3, 119.8, 118.4,
705 117.6, 114.9, 83.9, 68.8, 67.5, 28 .1, 24.9, 18.1, 15.9; LRMS (ESI) m/z 349 $[M+H]^+$; HRMS (ESI)
706 calcd for $C_{23}H_{25}O_3$ $[M+H]^+$ 349.1804, found 349.1806.

4.3.3 Synthesis of (*E*)-3-(4-(Hex-5-yn-1-yloxy)phenyl)-1-(2-hydroxy-5-methylphenyl)prop-2-en-1-one (**Ac8**): This compound (0.25 g, 70%) was obtained from 4-(hex-5-yn-1-yloxy)benzaldehyde (**Ac33**) and 2'-hydroxy-5'-methylacetophenone (**3c**) according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75 - 1.79 (m, 2H), 1.94 - 2.01 (m, 3H), 2.29 (t, *J*=6.00 Hz, 2H), 2.43 (s, 3H), 4.08 (t, *J*=6.00 Hz, 2H), 6.95 (d, *J*=8.70 Hz, 2H), 7.46 (d, *J*=15.40 Hz, 1H), 7.64 (d, *J*=8.70 Hz, 2H), 7.89 - 8.01 (m, 3H), 13.45 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$) δ 192.3, 161.9, 154.7, 146.9, 137.2, 136.0, 131.0, 130.9, 128.0, 126.8, 124.2, 118.0, 115.0, 83.9, 68.8, 67.5, 28.1, 24.9, 20.3, 18.1; LRMS (ESI) m/z 335 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}O_3$ $[M+H]^+$ 335.1647, found 335.1649.

4.3.4 Synthesis of (*E*)-3-(4-(Hex-5-yn-1-yloxy)phenyl)-1-(2-hydroxy-4-methylphenyl)prop-2-en-1-one (**Ac9**): This compound (0.31 g, 65%) was obtained from 4-(hex-5-yn-1-yloxy)benzaldehyde (**Ac33**) and 2'-hydroxy-4'-methylacetophenone (**3d**) according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.02 (s, 1H), 7.92 (d, *J*=7.20 Hz, 2H), 7.82 (d, *J*=7.20 Hz, 1H), 7.64 (d, *J*=8.00 Hz, 2H), 7.55 (d, *J*=7.20 Hz, 1H), 6.97 (d, *J*=8.00 Hz, 2H), 6.85 (s, 1H), 6.78 (d, *J*=7.20 Hz, 1H), 4.08 (t, *J*=6.00 Hz, 2H), 2.39 (s, 3H), 2.30 - 2.34 (m, 2H), 1.93 - 2.01 (m, 3H), 1.74 - 1.78 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.1, 163.7, 161.3, 147.7, 144.8, 130.4, 129.4, 127.4, 120.0, 118.6, 117.9, 117.7, 114.9, 83.9, 68.7, 67.5, 28.1, 24.9, 21.9, 18.1; LRMS (ESI) m/z 335 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}O_3$ $[M+H]^+$ 335.1647, found 335.1650.

4.3.5 Synthesis of (*E*)-1-(4-Fluoro-2-hydroxyphenyl)-3-(4-(hex-5-yn-1-yloxy)phenyl)prop-2-en-1-one (**Ac10**): This compound (0.33 g, 69%) was obtained from 4-(hex-5-yn-1-yloxy)benzaldehyde (**Ac33**) and 2'-hydroxy-5'-fluoroacetophenone (**3e**) according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.37 (s, 1H), 7.89 - 7.96 (m, 2H), 7.63

(d, *J*=8.00 Hz, 2H), 7.47 (d, *J*=15.40 Hz, 1H), 6.94 (d, *J*=8.00 Hz, 2H), 6.64 - 6.73 (m, 4H), 4.07 (t, *J*=6.00 Hz, 2H), 2.30 - 2.33 (m, 2H), 1.93 - 2.01 (m, 3H), 1.74 - 1.78 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 166.2, 166.1, 166.0, 161.6, 145.7, 131.9, 131.7, 130.6, 127.1, 117.2, 115.0, 114.7, 107.1, 106.9, 105.2, 105.0, 84.0, 68.8, 67.6, 28.1, 25.0, 18.2; LRMS (ESI) m/z 339 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{20}FO_3$ $[M+H]^+$ 339.1396, found 339.1398.

4.3.6 Synthesis of 2-(4-(Pent-4-yn-1-yloxy)phenyl)quinazolin-4(3*H*)-one (**Ac11**): To a well stirred solution of 4-(pent-4-yn-1-yloxy)benzaldehyde (**2a**) and 2-aminobenzamide (**4**) in DMSO at 150 ºC, was added catalytic amount of iodine. The reaction mixture was further heated for 3 h. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a beaker containing water ice-bath temperature. The white precipitate formed was collected by suction filtration. The white solid was washed with n-hexane and subjected to crystallization from MeOH to afford the desired compound **Ac11** (0.33 g, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 8.10 - 8.17 (m, 3H), 7.78 (dd, *J*=7.60, 7.60Hz, 1H), 7.68 (d, *J*=7.60 Hz, 1H), 7.46 (dd, *J*=7.60, 7.60Hz, 1H), 7.06 (d, *J*=8.80 Hz, 2H), 4.10 (t, *J*=6.00 Hz, 2H), 2.81 (s, 1H), 2.31 - 2.35 (m, 2H), 1.87 - 1.93 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 161.5, 152.3, 134.9, 129.9, 127.6, 126.5, 126.2, 125.3, 121.1, 114.8, 84.0, 72.1, 66.7, 28.0, 14.9; LRMS (ESI) m/z 305 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{17}N_2O_2$ $[M+H]^+$ 305.1290, found 305.1296.

4.3.7 Synthesis of 2-Phenyl-7-(2-(prop-2-yn-1-yloxy)ethoxy)-4*H*-chromen-4-one (**Ac13**): To a round-bottom flask was charged with corresponding 7-hydroxyflavones **1e** (0.021 mol, 5 g), 2-bromoethanol (0.022 mol, 1.6 mL), K_2CO_3 (0.021 mol, 2.9 g) and anhydrous DMF (20 mL). The reaction mixture was stirred at refluxing temperature for 3 h. The reaction mixture was poured into a beaker containing ice water followed by filtration and washing (50 mL hexane). This (3.2 g, 54%) was used without further purification. The obtained compound (7.1 mmol, 2 g) was then dissolved

753 in anhydrous THF (10 mL). To this solution at room temperature, was added excess sodium
754 hydride (8.5 mmol, 0.2 g) and propargyl bromide (80% in xylene) (7.1 mmol, 0.79 mL) solution
755 successively at 0 ºC for 1 hr. The reaction mixture was then stirred for 3 h at RT. When TLC
756 indicated complete consumption of starting material, the reaction mixture was poured into a
757 separating funnel containing water. The mixture was continuously extracted with DCM. The
758 combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a brown crude
759 reaction mixture. Purification was performed by flash column chromatography on silica gel with
760 acetone in DCM (1:10) as eluent to furnish titled compound (1.7 g, 75%). ^{1}H NMR (500 MHz,
761 $CDCl_3$) δ 8.15 (d, *J*=8.79 Hz, 1 H), 7.88 - 7.94 (m, 2 H), 7.49 - 7.57 (m, 3 H), 7.03 (dd, *J*=8.79,
762 2.44 Hz, 1 H), 7.00 (d, *J*=2.44 Hz, 1 H), 6.78 (s, 1 H), 4.24 - 4.33 (m, 4 H), 3.97 - 3.99 (m, 2 H),
763 2.49 (t, *J*=2.44 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.5, 163.1, 162.8, 157.7, 131.6, 131.2,
764 128.8, 126.8, 126.0, 117.8, 114.6, 107.3, 101.0, 79.1, 74.9, 67.7, 58.5; LRMS (ESI) m/z 321
765 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{17}O_4$ $[M+H]^+$ 321.1127, found 321.1121.

766 **4.3.8 *N*-Benzyl-*N*,*N*-di(prop-2-yn-1-yl)amine** (**Ac15**): This compound was commercially
767 available.

768 **4.3.9 Synthesis of 7-(2-(Benzyl(prop-2-yn-1-yl)amino)ethoxy)-2-phenyl-4*H*-chromen-4-one**
769 (**Ac16**): To a well stirred solution of 7-hydroxyflavones **1e** (2.9 mmol, 0.7 g), 2-(benzyl(prop-2-
770 yn-1-yl)amino)ethanol (2.9 mmol, 0.56 g) and PPh_3 (0.77 g, 1equiv.) in THF (10 mL) at room
771 temperature, was added DIAD (0.58 mL, 1 equiv.) dropwise. The reaction mixture was then stirred
772 for 12 h. The reaction mixture was evaporated to give a brown crude reaction mixture. Purification
773 was performed by flash column chromatography on silica gel with acetone in DCM (1:50) as eluent
774 to furnish titled compound (0.42g, 35%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J*=8.78 Hz, 1 H),
775 7.88 - 7.93 (m, 2 H), 7.50 - 7.55 (m, 3 H), 7.37 - 7.40 (m, 2 H), 7.31 - 7.35 (m, 2 H), 7.27 - 7.29

(m, 1 H), 6.95 - 7.01 (m, 2 H), 6.77 (s, 1 H), 4.21 (t, *J*=5.61 Hz, 2 H), 3.79 (s, 2 H), 3.48 (d, *J*=2.44 Hz, 2 H), 3.07 (t, *J*=5.61 Hz, 2 H), 2.30 (t, *J*=2.20 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.8, 163.2, 163.0, 157.9, 131.8, 131.3, 129.1, 128.9, 128.4, 127.4, 127.0, 126.1, 117.9, 114.7, 107.5, 101.1, 70.0, 67.2, 58.5, 51.7, 42.6; LRMS (ESI) m/z 410 $[M+H]^+$; HRMS (ESI) calcd for $C_{27}H_{24}NO_3$ $[M+H]^+$ 410.1756, found 410.1750.

4.3.10 *Tris*(prop-2-yn-1-yl)amine (**Ac17**): This compound was commercially available.

4.3.11 2-(But-3-yn-1-yl)isoindoline-1,3-dione (**Ac19**): This compound was commercially available.

4.3.12 1,3-Diethynylbenzene (**Ac22**): This compound was commercially available.

4.3.13 1,4-Diethynylbenzene (**Ac23**): This compound was commercially available.

4.3.14 *N*-(Prop-2-yn-1-yl)aniline (Ac27): This compound was obtained from aniline (**4a**) as described.[42]

4.3.15 1,4-Di(prop-2-yn-1-yl)piperazine (Ac29): This compound was obtained from piperazine (**4b**) as described.[43]

4.3.16 N^1,N^2-Dimethyl-N^1,N^2-di(prop-2-yn-1-yl)ethane-1,2-diamine (Ac31): This compound was obtained from N^1,N^2-dimethylethane-1,2-diamine (**4c**) as described.[44]

4.3.17 4-(Hex-5-yn-1-yloxy)benzaldehyde (Ac33): This compound was obtained from 4-hydroxybenzaldehyde (**4d**) as described.[45]

4.4 General procedure for synthesis of Az1-Az5, Az7, Az10, Az11-Az13, Az17 and Az18 (Scheme 5).

To a round-bottom flask was charged with 4'-hydroxyflavones (**1a, d, f, g, h, i**) or 7-hydroxyflavones (**1e**) (1 equiv.), 2-bromoethanol or 2-(2-chloroethoxy)ethanol or 2-(2-(2-chloroethoxy)ethoxy)ethanol (1.2 equiv.), K_2CO_3 (1.5 equiv.) and DMF (3 mL per equiv.). The reaction mixture was stirred at refluxing temperature. When TLC indicated complete consumption of starting material, the reaction mixture was poured into a separating funnel containing water. The mixture was continuously extracted with DCM. If the mixture could not be separated into two layers, small amount of 1M HCl was added. The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a brown crude reaction mixture. Purification was performed by flash column chromatography on silica gel with acetone in DCM as eluent to furnish desired product.

The hydroxylated flavone obtained from above was then dissolved in a solution of DCM (1 mL per equiv.) and triethylamine (1 mL per equiv.) at 0 ^oC. Methanesulfonyl chloride (1.2 equiv.) 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 per equiv.) was added excess of sodium azide (3 equiv.). 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.

4.4.1 Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Az1**): This compound (0.62 g, 45%) was obtained from 2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1a**) and 2-

(2-chloroethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (dd, *J*=7.60, 1.60 Hz, 1H), 7.68 (d, *J*=8.80 Hz, 2H), 7.53 (ddd, *J*=7.60, 7.20, 1.60 Hz, 1H), 7.37 (d, *J*=8.40 Hz, 1H), 7.26 (dd, *J*=7.60, 7.20 Hz, 1H), 6.86 (d, *J*=8.80 Hz, 2H), 6.56 (s, 1H), 4.06 (t, *J*=4.80 Hz, 2H), 3.77 (t, *J*=4.80 Hz, 2H), 3.65 (t, *J*=4.80 Hz, 2H), 3.32 (t, *J*=4.80 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 163.0, 161.4, 155.9, 133.4, 127.7, 125.3, 124.9, 123.8, 123.6, 117.8, 114.8, 105.8, 70.1, 69.3, 67.4, 50.5; LRMS (ESI) m/z 352 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{18}N_3O_4$ $[M+H]^+$ 352.1297, found 352.1295.

4.4.2 Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-4*H*-chromen-4-one (**Az2**): This compound (0.36 g, 41%) was obtained from 2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1a**) and 2-(2-(2-chloroethoxy)ethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J*=7.81 Hz, 1 H), 7.88 (d, *J*=10 Hz, 2 H), 7.66 - 7.71 (m, 1 H), 7.55 (d, *J*=8.30 Hz, 1 H), 7.41 (t, *J*=7.57 Hz, 1 H), 7.05 (d, *J*=10 Hz, 2 H), 6.75 (s, 1 H), 4.20 - 4.25 (m, 2 H), 3.91-3.93 (m, 2 H), 3.74 - 3.78 (m, 2 H), 3.68 - 3.73 (m, 4 H), 3.40 (t, *J*=5.12 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.1, 163.2, 163.1, 161.5, 156.0, 133.4, 127.8, 125.4, 124.9, 123.9, 123.7, 117.8, 114.9, 106.0, 70.7, 70.6, 69.9, 69.5, 67.5, 50.5; LRMS (ESI) m/z 396 $[M+H]^+$, 418 $[M+Na]^+$; HRMS (ESI) calcd for $C_{21}H_{22}N_3O_5$ $[M+H]^+$ 396.1559, found 396.1544; calcd for $C_{21}H_{21}N_3O_5Na$ $[M+Na]^+$ 418.1379, found 418.1378.

4.4.3 Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-6-methyl-4*H*-chromen-4-one (**Az3**): This compound (0.21 g, 36%) was obtained from 2-(4-hydroxyphenyl)-6-methyl-4*H*-chromen-4-one (**1d**) and 2-(2-(2-chloroethoxy)ethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (s, 1 H), 7.71 (d, *J*=10.0 Hz, 2 H), 7.26 - 7.38 (m, 2 H), 6.90 (d, *J*=10.0 Hz, 2 H), 6.57 (s, 1 H), 4.09 (t, *J*=4.64 Hz, 2 H), 3.80 (t, *J*=4.64 Hz, 2 H), 3.64 - 3.69 (m, 2 H), 3.58 - 3.64 (m, 4 H), 3.30 (t, *J*=4.88 Hz, 2 H), 2.33

(s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.9, 162.7, 161.2, 154.0, 134.6, 134.3, 127.5, 124.5, 123.8, 123.1, 117.4, 114.6, 105.5, 77.2, 76.9, 76.7, 70.5, 70.4, 69.7, 69.3, 67.3, 50.3, 20.5; LRMS (ESI) m/z 410 $[M+H]^+$, 432 $[M+Na]^+$; HRMS (ESI) calcd for $C_{22}H_{24}N_3O_5$ $[M+H]^+$ 410.1716, found 410.1709; calcd for $C_{22}H_{23}N_3O_5Na$ $[M+Na]^+$ 432.1535, found 432.1544.

4.4.4 Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-6-fluoro-4*H*-chromen-4-one (**Az4**): This compound (0.23 g, 31%) was obtained from 6-fluoro-2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1f**) and 2-(2-(2-chloroethoxy)ethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 - 7.89 (m, 3 H), 7.53 - 7.58 (m, 1 H), 7.40 (ddd, *J*=9.03, 7.57, 2.93 Hz, 1 H), 7.05 (d, *J*=10.0 Hz, 2 H), 4.20 - 4.24 (m, 2 H), 6.73 (s, 1 H), 3.90 - 3.93 (m, 2 H), 3.74 - 3.78 (m, 2 H), 3.67 - 3.72 (m, 4 H), 3.39 (t, *J*=4.88 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.8 (d, *J*=2.50Hz, C4), 163.1, 161.4, 159.6 (d, *J*=244.88 Hz, C6), 151.9 (d, *J*=1.25Hz, C9), 127.5, 124.7 (d, *J*=7.25Hz, C10), 123.2, 121.2 (d, *J*=25.63Hz, C7), 119.7 (d, *J*=8.25Hz, C8), 114.7, 110.0 (d, *J*=23.25Hz, C5), 104.9, 70.5, 70.3, 69.7, 69.7, 69.2, 67.3, 50.3; LRMS (ESI) m/z 414 $[M+H]^+$, 436 $[M+Na]^+$; HRMS (ESI) calcd for $C_{21}H_{21}N_3O_5F$ $[M+H]^+$ 414.1465, found 414.1472; calcd for $C_{21}H_{20}N_3O_5FNa$ $[M+Na]^+$ 436.1285, found 436.1299.

4.4.5 Synthesis of 2-(4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)phenyl)-3-(benzyloxy)-4*H*-chromen-4-one (**Az5**): This compound (0.17 g, 32%) was obtained from 3-(benzyloxy)-2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1g**) and 2-(2-(2-chloroethoxy)ethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, *J*=10.0 Hz, 1 H), 7.95 (d, *J*=10.0 Hz, 2 H), 7.50 - 7.55 (m, 1 H), 7.38 (d, *J*=8.30 Hz, 1 H), 7.32-7.34 (m, 2 H), 7.28 (t, *J*=7.50 Hz, 1 H), 7.17 - 7.24 (m, 3 H), 6.89 (d, *J*=10.0 Hz, 2 H), 5.05 (s, 2 H), 4.06 - 4.12 (m, 2 H), 3.77 - 3.83 (m, 2 H), 3.64 - 3.69 (m, 2 H), 3.56 - 3.64 (m, 4 H), 3.29 (t, *J*=4.88 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.3, 160.2, 155.6, 154.6, 138.8, 136.4, 132.7, 130.0, 128.3,

127.8, 127.6, 125.1, 124.1, 123.7, 122.9, 117.4, 113.9, 73.3, 70.4, 70.2, 69.6, 69.1, 67.1, 50.2; LRMS (ESI) m/z 502 $[M+H]^+$, 524 $[M+Na]^+$; HRMS (ESI) calcd for $C_{28}H_{28}N_3O_6$ $[M+H]^+$ 502.1978, found 502.1989; calcd for $C_{28}H_{27}N_3O_6Na$ $[M+Na]^+$ 524.1798, found 524.1797.

4.4.6 Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-6-fluoro-4*H*-chromen-4-one (**Az7**): This compound (0.18 g, 37%) was obtained from 6-fluoro-2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1f**) and 2-(2-chloroethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 - 7.88 (m, 3 H), 7.54 (dd, *J*=9.03, 4.15 Hz, 1 H), 7.35 - 7.42 (m, 1 H), 7.03 (d, *J*=9.0, 2 H), 6.71 (s, 1 H), 4.19 - 4.24 (m, 2 H), 3.88 - 3.93 (m, 2 H), 3.73 - 3.79 (m, 2 H), 3.42 (t, *J*=4.88 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.1, 177.0, 163.2, 161.4, 159.2 (d, *J*=244.88 Hz, C6), 152.0, 127.7, 124.8 (d, *J*=7.83Hz, C10), 123.5, 121.3 (d, *J*=25.13Hz, C7), 119.8 (d, *J*=8.25Hz, C8), 114.8, 110.2 (d, *J*=23.75Hz, C5), 105.1, 70.0, 69.3, 67.4, 50.4; LRMS (ESI) m/z 370 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{17}N_3O_4F$ $[M+H]^+$ 370.1203, found 370.1218.

4.4.7 Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-3-(benzyloxy)-4*H*-chromen-4-one (**Az10**): This compound (0.23 g, 31%) was obtained from 3-(benzyloxy)-2-(4-hydroxyphenyl)-4*H*-chromen-4-one (**1g**) and 2-(2-chloroethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (d, *J*=7.81 Hz, 1 H), 8.04 (d, *J*=8.79 Hz, 2 H), 7.67 (t, *J*=7.81 Hz, 1 H), 7.52 (d, *J*=8.30 Hz, 1 H), 7.34 - 7.44 (m, 3 H), 7.26-7.28 (m, 3 H), 6.99 (d, *J*=8.79 Hz, 2 H), 5.12 (s, 2 H), 4.22-4.24 (m, 2 H), 3.91-3.93 (m, 2 H), 3.77-3.79 (m, 2 H), 3.43-3.45 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.6, 160.3, 155.9, 154.9, 139.1, 136.6, 133.0, 130.3, 128.6, 128.0, 127.8, 125.4, 124.3, 123.9, 123.3, 117.7, 114.1, 73.7, 70.0, 69.3, 67.3, 50.4; LRMS (ESI) m/z 458 $[M+H]^+$, 480 $[M+Na]^+$; HRMS (ESI) calcd for $C_{26}H_{24}N_3O_5$ $[M+H]^+$ 458.1716, found 458.1738; calcd for $C_{26}H_{23}N_3O_5Na$ $[M+Na]^+$ 480.1535, found 480.1527.

888 **4.4.8 Synthesis of 7-(2-(2-Azidoethoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one** (**Az11**): This
889 compound (0.12 g, 32%) was obtained from 7-hydroxy-2-phenyl-4*H*-chromen-4-one (**1e**) and 2-
890 (2-chloroethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR
891 (500 MHz, $CDCl_3$) δ 8.14 (d, *J*=8.30 Hz, 1 H), 7.88 - 7.93 (m, 2 H), 7.49 - 7.56 (m, 3 H), 6.99 -
892 7.04 (m, 2 H), 6.79 (s, 1 H), 4.27 (t, *J*=5.0 Hz, 2 H), 3.93 (t, *J*=5.0 Hz, 2 H), 3.78 (t, *J*=5.0 Hz, 2
893 H), 3.43 (t, *J*=5.0 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.2, 162.8, 162.5, 157.3, 131.2,
894 131.0, 128.6, 126.4, 125.6, 117.5, 114.3, 106.9, 100.7, 69.9, 69.0, 67.6, 50.3; LRMS (ESI) m/z
895 352 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{18}N_3O_4$ $[M+H]^+$ 352.1297, found 352.1288.

896 **4.4.9 Synthesis of 7-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-2-phenyl-4*H*-chromen-4-one**
897 (**Az12**): This compound (0.14 g, 38%) was obtained from 7-hydroxy-2-phenyl-4*H*-chromen-4-one
898 (**1e**) and 2-(2-(2-chloroethoxy)ethoxy)ethanol according to the general procedure (i) and (ii)
899 described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J*=8.78 Hz, 1 H), 7.88 - 7.93 (m, 2 H),
900 7.49 - 7.56 (m, 3 H), 6.98 - 7.04 (m, 2 H), 6.77 (s, 1 H), 4.26 (t, *J*=5.0 Hz, 2 H), 3.93 - 3.95 (m, 2
901 H), 3.75 - 3.78 (m, 2 H), 3.67 - 3.72 (m, 4 H), 3.39 (t, *J*=4.88 Hz, 2 H); ^{13}C NMR (101 MHz,
902 $CDCl_3$) δ 177.6, 163.2, 162.8, 157.7, 131.6, 131.2, 128.8, 126.8, 125.9, 117.7, 114.6, 107.2, 101.0,
903 70.7, 70.5, 69.9, 69.3, 67.9, 50.5; LRMS (ESI) m/z 396 $[M+H]^+$; HRMS (ESI) calcd for
904 $C_{21}H_{22}N_3O_5$ $[M+H]^+$ 396.1559, found 396.1544.

905 **4.4.10 Synthesis of 7-(2-Azidoethoxy)-2-phenyl-4*H*-chromen-4-one** (**Az13**): This compound
906 (0.11 g, 29%) was obtained from 7-hydroxy-2-phenyl-4*H*-chromen-4-one (**1e**) and 2-
907 bromoethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR (500 MHz,
908 $CDCl_3$) δ 8.11 - 8.19 (m, 1 H), 7.85 - 7.93 (m, 2 H), 7.47 - 7.56 (m, 3 H), 6.96 - 7.05 (m, 2 H),
909 6.74 - 6.80 (m, 1 H), 4.26 (t, *J*=4.64 Hz, 2 H), 3.68 (t, *J*=4.88 Hz, 2 H); ^{13}C NMR (101 MHz,
910 $CDCl_3$) δ 177.7, 163.1, 162.6, 157.8, 131.7, 131.4, 129.0, 127.3, 126.1, 118.3, 114.3, 107.5, 101.3,

911 67.4, 49.9; LRMS (ESI) m/z 308 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{14}N_3O_3$ $[M+H]^+$ 308.1035,
912 found 308.1037.

913 **4.4.11 Synthesis of 2-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-3-((3-methoxybenzyl)oxy)-4H-**
914 **chromen-4-one (Az17) :** The titled compound (0.84 g, 54%) was obtained from 2-(4-
915 hydroxyphenyl)-3-((3-methoxybenzyl)oxy)-4H-chromen-4-one (**1g**) and 2-(2-
916 chloroethoxy)ethanol according to the general procedure (i) and (ii) described above. ^{1}H NMR
917 (500 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 1.71, 8.05 Hz, 1H), 8.00 - 8.06 (m, 2H), 7.66 (ddd, *J* = 1.95,
918 7.08, 8.54 Hz, 1H), 7.51 (d, *J* = 8.79 Hz, 1H), 7.40 (ddd, *J* = 0.98, 7.08, 8.05 Hz, 1H), 7.18 (t, *J* =
919 7.81 Hz, 1H), 6.99 - 7.01 (m, 1H), 6.97 - 6.99 (m, 1H), 6.90 - 6.94 (m, 2H), 6.78 - 6.83 (m, 1H),
920 5.11 (s, 2H), 4.20 - 4.23 (m, 2H), 3.91 (dd, *J* = 4.15, 5.61 Hz, 2H), 3.75 - 3.79 (m, 2H), 3.72 (s,
921 3H), 3.44 (t, *J* = 4.88 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.8, 160.4, 159.4, 156.1, 155.0,
922 139.1, 138.1, 133.1, 130.4, 129.0, 125.5, 124.4, 124.0, 123.4, 120.9, 117.8, 114.2, 114.1, 113.6,
923 73.7, 70.2, 69.5, 67.4, 55.0, 50.6; LRMS (ESI) m/z 488 $[M+H]^+$; HRMS (ESI) calcd for
924 $C_{27}H_{26}N_3O_6$ $[M+H]^+$ 488.1822, found 488.1819.

925 **4.4.12 Synthesis of 2-(3-(2-(2-Azidoethoxy)ethoxy)phenyl)-3-(benzyloxy)-4H-chromen-4-one**
926 **(Az18):** This compound (0.26 g, 26%) was obtained from 3-(benzyloxy)-2-(3-hydroxyphenyl)-
927 4H-chromen-4-one (**1i**) and 2-(2-chloroethoxy)ethanol according to the general procedure (i) and
928 (ii) described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (dd, *J* = 7.81, 1.46 Hz, 1 H), 7.66 - 7.72
929 (m, 1 H), 7.63 (dt, *J* = 8.05, 1.10 Hz, 1 H), 7.60 (dd, *J* = 2.44, 1.46 Hz, 1 H), 7.53 (d, *J* = 7.81 Hz,
930 1 H), 7.39 - 7.45 (m, 1 H), 7.36 - 7.39 (m, 1 H), 7.31 - 7.36 (m, 2 H), 7.24 - 7.28 (m, 4 H), 7.06
931 (dt, *J* = 8.30, 1.46 Hz, 1 H), 5.14 (s, 2 H), 4.03 - 4.06 (m, 2 H), 3.82 - 3.85 (m, 2 H), 3.73 - 3.76
932 (m, 2 H), 3.40 - 3.44 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 158.4, 155.8, 155.1, 140.0,
933 136.6, 133.3, 132.0, 129.2, 128.7, 128.1, 128.0, 128.0, 125.6, 124.6, 124.0, 121.3, 117.9, 117.5,

114.5, 74.1, 70.1, 69.5, 67.4, 50.6; LRMS (ESI) m/z 458 $[M+H]^+$; HRMS (ESI) calcd for $C_{26}H_{24}N_3O_5$ $[M+H]^+$ 458.1716, found 458.1731.

4.5 General procedure for the synthesis of triazole bridged flavonoid dimers catalyzed by Cu(I). The $Cu(PPh_3)_3Br$ catalyst (MW=929) (0.05 mmol), prepared according to literature,[46] was added to a THF solution (2 mL) containing the azide (**Az,** 0.1 mmol) 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.

4.5.1 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 (90 mg) was obtained from **Ac1** and **Az1** in 81% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J*=7.20, 7.20 Hz, 2H), 7.74 (d, *J*=8.40 Hz, 2H), 7.69 (d, *J*=8.40 Hz, 2H), 7.55 - 7.56 (m, 2H), 7.48 (s, 1H), 7.39 (dd, *J*=7.20, 7.20 Hz, 2H), 7.25 - 7.28 (m, 2H), 6.89 (d, *J*=8.40 Hz, 2H), 6.85 (d, *J*=8.40 Hz, 2H), 6.60 (s, 1H), 6.57 (s, 1H), 4.50 (t, *J*=6.40 Hz, 2H), 4.04 (t, *J*=6.40 Hz, 2H), 3.87 - 3.96 (m, 4H), 3.75 (t, *J*=6.40 Hz, 2H), 2.82 (t, *J*=6.40 Hz, 2H), 2.08 (t, *J*=6.40 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 178.0, 163.1, 162.9, 161.6, 161.2, 155.9, 146.8, 133.4, 127.8, 127.8, 125.3, 124.9, 124.0, 123.7, 123.5, 122.1, 117.8, 117.8, 114.8, 114.7, 105.9, 105.8, 69.7, 69.4, 67.3, 67.0, 50.0, 28.6, 21.9; LRMS (ESI) m/z 656 $[M+H]^+$; HRMS (ESI) calcd for $C_{39}H_{34}N_3O_7$ $[M+H]^+$ 656.2397, found 656.2394.

4.5.2 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 (82 mg) was obtained from **Ac2** and **Az1** in 85% yield according to the general procedure described above. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J*=7.20 Hz, 1H), 7.99 (d, *J*=7.20 Hz, 1H), 7.74 - 7.79 (m, 4H), 7.50 (dd, *J*=7.20, 7.20 Hz, 1H), 7.49 (s, 1H), 7.38 - 7.42 (m, 4H), 7.28 (dd, *J*=7.20, 7.20 Hz, 1H), 6.81 - 6.91 (m, 4H), 6.63 (s, 1H), 6.61 (s, 1H), 4.51 (t, *J*=6.40 Hz, 2H), 3.99 - 4.06 (m, 4H), 3.89 (t, *J*=6.40 Hz, 2H), 3.76 (t, *J*=6.40 Hz, 2H), 2.84 (t, *J*=6.40 Hz, 2H), 2.13 (t, *J*=6.40 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 177.6, 163.3, 162.9, 162.8, 161.2, 157.7, 155.9, 146.6, 133.5, 131.5, 131.3, 128.9, 127.8, 126.7, 125.9, 125.3, 125.0, 124.1, 123.7, 122.1, 117.8, 117.5, 114.8, 114.5, 107.2, 106.0, 100.8, 69.7, 69.4, 67.5, 67.3, 50.0, 28.5, 21.9; LRMS (ESI) m/z 656 $[M+H]^+$; HRMS (ESI) calcd for $C_{39}H_{34}N_3O_7$ $[M+H]^+$ 656.2397, found 656.2401.

4.5.3 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 (64 mg) was obtained from **Ac2** and **Az4** in 72% yield according to the general procedure described above. ^1H NMR (600 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.80 Hz, 1H), 7.88 (d, *J* = 5.87 Hz, 2H), 7.80 - 7.85 (m, 3H), 7.49 - 7.56 (m, 5H), 7.36 - 7.42 (m, 1H), 7.01 (d, *J* = 8.80 Hz, 2H), 6.90 - 6.98 (m, 2H), 6.75 (s, 1H), 6.70 (s, 1H), 4.57 (br. s., 2H), 4.18 (br. s., 2H), 4.11 (br. s., 2H), 3.92 (br. s., 2H), 3.82 - 3.87 (m, 2H), 3.68 - 3.72 (m, 2H), 3.62 - 3.68 (m, 3H), 2.94 (br. s., 2H), 2.27 (br. s., 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.7, 177.4, 163.4, 163.4, 162.9, 161.5, 160.3, 158.6, 157.9, 152.2, 131.6, 131.4, 128.9, 128.0, 126.9, 126.1, 125.0, 125.0, 123.8, 121.7, 121.5, 120.0, 119.9, 117.7, 114.9, 114.7, 110.6, 110.4, 107.3, 105.4, 100.8, 70.7, 70.4, 69.4, 67.5, 53.7, 31.7, 30.9, 29.2, 21.9; LRMS (ESI) m/z 740 $[M+Na]^+$; HRMS (ESI) calcd for $C_{41}H_{37}N_3O_8F$ $[M+H]^+$ 718.2559, found 718.2556; calcd for $C_{41}H_{36}N_3O_8Na$ $[M+Na]^+$ 740.2379, found 740.2381.

4.5.4 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 (50 mg)

was obtained from **Ac2** and **Az11** in 69% yield according to the general procedure described above. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (d, *J* = 7.34 Hz, 1H), 8.11 (d, *J* = 8.80 Hz, 1H), 7.89 (d, *J* = 7.34 Hz, 4H), 7.49 - 7.66 (m, 7H), 7.00 (d, *J* = 7.34 Hz, 1H), 6.94 (s, 1H), 6.96 (s, 2H), 6.75 - 6.82 (m, 2H), 4.63 (br. s., 2H), 4.21 (br. s., 2H), 4.14 (br. s., 2H), 4.02 (br. s., 2H), 3.89 (br. s., 2H), 3.00 (br. s., 2H), 2.28 (br. s., 2H);^{13}C NMR (151 MHz, $CDCl_3$) δ 177.8, 177.7, 163.4, 163.1, 163.1, 163.0, 157.9, 157.8, 131.7, 131.7, 131.6, 131.6, 131.5, 131.5, 129.0, 127.3, 127.0, 126.2, 126.2, 126.1, 118.1, 117.7, 114.8, 114.8, 114.5, 107.5, 107.4, 101.2, 101.0, 101.0, 69.7, 69.5, 67.8, 67.6, 28.5, 21.8; LRMS (ESI) m/z 678 $[M+Na]^+$; HRMS (ESI) calcd for $C_{39}H_{34}N_3O_7$ $[M+H]^+$ 656.2390, found 656.2412; calcd for $C_{39}H_{33}N_3O_7Na$ $[M+Na]^+$ 678.2216, found 678.2238.

4.5.5 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 (92 mg) was obtained from **Ac3** and **Az1** in 91% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 - 8.09 (m, 2H), 7.74 (d, *J*=8.20 Hz, 2H), 7.65 (d, *J*=8.20 Hz, 2H), 7.55 (dd, *J*=7.20, 7.20 Hz, 1H), 7.48 (s, 1H), 7.48 (d, *J*=7.40 Hz, 1H), 7.25 - 7.27 (m, 2H), 6.98 - 7.02 (m, 2H), 6.89 (d, *J*=8.20 Hz, 2H), 6.84 (d, *J*=8.20 Hz, 2H), 6.59 (s, 1H), 6.53 (s, 1H), 4.51 (t, *J*=6.40 Hz, 2H), 4.05 (t, *J*=6.40 Hz, 2H), 3.95 (t, *J*=6.40 Hz, 2H), 3.89 (t, *J*=4.80 Hz, 2H), 3.76 (t, *J*=4.80 Hz, 2H), 2.82 (t, *J*=6.40 Hz, 2H), 2.09 (t, *J*=6.40 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 177.1, 166.6, 164.1, 163.4, 162.9, 161.7, 161.2, 156.9, 156.8, 155.9, 146.8, 133.5, 127.8, 127.7, 125.3, 124.9, 124.0, 123.7, 123.1, 122.1, 120.5, 117.8, 114.8, 114.7, 105.9, 105.7, 69.7, 69.4, 67.3, 67.1, 50.0, 28.6, 21.9; LRMS (ESI) m/z 674 $[M+H]^+$; HRMS (ESI) calcd for $C_{39}H_{33}N_3O_7$ $[M+H]^+$ 674.2303, found 674.2309.

4.5.6 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 (70 mg) was obtained from **Ac3** and **Az2** in 90% yield according to the general procedure described above. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.22 (dd, *J* = 6.23, 8.72 Hz, 1H), 8.18 (d, *J* = 9.96 Hz, 1H), 7.86 (d, *J* = 8.72 Hz, 2H), 7.79 (d, *J* = 8.72 Hz, 3H), 7.65 - 7.69 (m, 1H), 7.53 (d, *J* = 8.72 Hz, 1H), 7.39 (t, *J* = 7.47 Hz, 1H), 7.23 (dd, *J* = 2.49, 8.72 Hz, 1H), 7.12 - 7.16 (m, 1H), 7.01 - 7.06 (m, *J* = 8.72 Hz, 2H), 6.94 - 6.98 (m, *J* = 8.72 Hz, 2H), 6.75 (s, 1H), 6.68 (s, 1H), 4.62 (br. s., 2H), 4.19 - 4.24 (m, 2H), 4.03 - 4.07 (m, 2H), 3.94 (br. s., 2H), 3.85 - 3.88 (m, 2H), 3.72 (d, *J* = 4.98 Hz, 2H), 3.69 (d, *J* = 4.98 Hz, 2H), 3.01 (br. s., 2H), 2.26 (br. s., 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.3, 177.3, 166.4, 164.7, 163.5, 163.2, 161.7, 161.4, 157.0, 157.0, 156.0, 133.7, 129.5, 128.0, 127.9, 125.5, 125.1, 124.2, 123.7, 123.4, 120.6, 117.9, 115.4, 114.9, 114.9, 114.8, 113.8, 113.7, 106.1, 105.9, 104.8, 104.6, 70.6, 70.5, 69.5, 69.0, 67.6, 66.9, 61.8, 28.4, 21.4; LRMS (ESI) m/z 718 $[M+H]^+$, 740 $[M+Na]^+$; HRMS (ESI) calcd for $C_{41}H_{37}N_2O_8F$ $[M+H]^+$ 718.2565, found 718.2588; calcd for $C_{41}H_{36}N_3O_8FNa$ $[M+Na]^+$ 740.2384, found 740.2397.

4.5.7 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 (55 mg) was obtained from **Ac3** and **Az3** in 79% yield according to the general procedure described above. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 6.23, 8.72 Hz, 1H), 7.88 (s, 1H), 7.77 (d, *J* = 8.72 Hz, 2H), 7.68 - 7.73 (m, *J* = 8.72 Hz, 2H), 7.37 - 7.42 (m, 1H), 7.34 (d, *J* = 8.72 Hz, 1H), 7.15 (d, *J* = 7.47 Hz, 1H), 7.05 - 7.11 (m, 1H), 6.93 - 6.99 (m, *J* = 8.72 Hz, 2H), 6.89 (d, *J* = 8.72 Hz, 2H), 6.63 (s, 1H), 6.59 (s, 1H), 4.54 (br. s., 2H), 4.12 - 4.16 (m, 2H), 3.97 (br. s., 2H), 3.88 (br. s., 2H), 3.82 (t, *J* = 4.36 Hz, 2H), 3.66 - 3.70 (m, 2H), 3.61 - 3.66 (m, 2H), 2.88 (br. s., 2H), 2.36 (s, 3H), 2.15 - 2.21 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.1, 177.1, 166.2, 164.5, 163.3, 162.8, 161.6, 161.2, 156.8, 156.7, 154.1, 134.8, 134.6, 127.8, 127.7, 127.7, 127.6, 124.7, 124.0, 123.2, 123.0, 120.5, 117.5, 114.7, 114.7, 113.6, 113.4, 105.7, 105.6,

104.6, 104.4, 70.5, 70.3, 69.3, 69.3, 67.4, 67.0, 53.7, 29.1, 28.5, 20.7; 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 732.2744; calcd for $C_{42}H_{38}N_3O_8FNa$ $[M+Na]^+$ 754.2541, found 754.2554.

4.5.8 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 (60 mg) was obtained from **Ac3** and **Az4** in 86% yield according to the general procedure described above. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 6.23, 8.72 Hz, 1H), 7.83 (d, *J* = 8.72 Hz, 2H), 7.77 - 7.82 (m, 4H), 7.53 (dd, *J* = 3.74, 8.72 Hz, 1H), 7.38 (dt, *J* = 3.11, 8.41 Hz, 1H), 7.19 - 7.24 (m, 1H), 7.14 (dt, *J* = 2.49, 8.72 Hz, 1H), 7.02 (d, *J* = 7.47 Hz, 2H), 6.96 (d, *J* = 8.72 Hz, 2H), 6.70 (s, 1H), 6.68 (s, 1H), 4.63 (br. s., 2H), 4.20 (br. s., 2H), 4.05 (br. s., 2H), 3.94 (br. s., 2H), 3.83 - 3.90 (m, 2H), 3.70 (dd, *J* = 4.36, 14.32 Hz, 4H), 3.01 (br. s., 2H), 2.27 (br. s., 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.4, 177.4, 177.3, 166.4, 164.7, 163.5, 163.4, 161.6, 161.5, 160.2, 158.6, 157.0, 156.9, 152.2, 131.1, 131.0, 128.0, 128.0, 128.0, 127.9, 125.0, 124.9, 123.9, 123.4, 121.8, 121.6, 120.6, 120.6, 120.0, 119.9, 114.9, 114.8, 113.8, 113.7, 110.5, 110.4, 105.9, 105.4, 104.7, 104.6, 70.6, 70.4, 69.5, 68.9, 67.6, 66.9, 28.4, 21.4; LRMS (ESI) m/z 736 $[M+H]^+$, 758 $[M+Na]^+$; HRMS (ESI) calcd for $C_{41}H_{34}N_3O_8F_2$ $[M+H]^+$ 736.2392, found 736.2380; calcd for $C_{41}H_{35}N_3O_8F_2Na$ $[M+Na]^+$ 758.2290, found 758.2313.

4.5.9 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 (70 mg) was obtained from **Ac3** and **Az11** in 81% yield according to the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 6.85, 8.80 Hz, 1H), 8.13 (d, *J* = 8.80 Hz, 1H), 7.88 (d, *J* = 6.85 Hz, 2H), 7.79 (d, *J* = 8.80 Hz, 2H), 7.40 - 7.69 (m, 4H), 7.17 - 7.25 (m, 1H), 7.13 (t, *J* = 8.31 Hz, 1H), 6.92 - 7.00 (m, 4H), 6.75 (s, 1H), 6.68 (s, 1H), 4.60 (br.

s., 2H), 4.20 (br. s., 2H), 4.07 (br. s., 2H), 3.99 (br. s., 2H), 3.87 (br. s., 2H), 2.18 - 2.27 (m, 2H), 1.97 (br. s., 2H);^{13}C NMR (151 MHz, $CDCl_3$) δ 177.7, 177.3, 166.9, 164.3, 163.7, 163.1, 161.9, 157.9, 131.7, 131.5, 129.0, 128.1, 128.0, 127.9, 127.2, 126.1, 123.5, 115.0, 114.5, 113.9, 113.6 107.5, 106.0, 104.8, 104.6, 101.2, 77.4, 77.1, 76.7, 69.8, 69.4, 67.8, 67.2, 28.4, 22.0; LRMS (ESI) m/z 674 $[M+H]^+$, 696 $[M+Na]^+$; HRMS (ESI) calcd for $C_{39}H_{33}N_3O_7F$ $[M+H]^+$ 674.6936, found 674.6842; calcd for $C_{39}H_{32}N_3O_7FNa$ $[M+Na]^+$ 696.2122, found 696.2258. Compound **Ac3Az11.HCl** was prepared by adding excess conc. hydrochloric acid to a solution of **Ac3Az11** in chloroform at room temperature and stirred for 30 mins. The solvents were then evaporated to dryness under high vacuum to obtain **Ac3Az11.HCl** for PK study.

4.5.10 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 (52 mg) was obtained from **Ac5** and **Az1** in 76% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (dd, *J*=5.0 Hz, 1 H), 7.91 (s, 1 H), 7.78 (d, *J*=10.0 Hz, 2 H), 7.73 (d, *J*=10.0 Hz, 2 H), 7.59 (t, *J*=7.5 Hz, 1 H), 7.47 (s, 1 H), 7.44 (d, *J*=8.30 Hz, 1 H), 7.41 (dd, *J*=8.54, 2.20 Hz, 1 H), 7.35 (d, *J*=8.79 Hz, 1 H), 7.31 (t, *J*=10.0 Hz,1 H), 6.94 (d, *J*=10.0 Hz, 2 H), 6.87 (d, *J*=10.0 Hz, 2 H), 6.65 (s, 1 H), 6.61 (s, 1 H), 4.52 (t, *J*=5.12 Hz, 2 H), 4.08 - 4.14 (m, 2 H), 3.89 - 3.97 (m, 4 H), 3.77 - 3.82 (m, 2 H), 2.71 - 2.77 (m, 2 H), 2.39 (s, 3 H), 1.82 (br. s., 4 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.1, 178.0, 163.0, 162.9, 161.6, 161.2, 155.9, 154.2, 147.4, 134.7, 134.5, 133.4, 127.8, 127.7, 125.4, 124.9, 124.7, 124.1, 123.7, 123.3, 121.8, 117.7, 117.5, 114.8, 114.6, 106.0, 105.7, 69.7, 69.4, 67.6, 67.3, 49.9, 28.5, 25.7, 25.2, 20.7; LRMS (ESI) m/z 684 $[M+H]^+$; HRMS (ESI) calcd for $C_{41}H_{38}N_3O_7$ $[M+H]^+$ 684.2710, found 684.2727.

4.5.11 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 (68 mg) was obtained from **Ac5** and **Az3** in 92% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1 H), 7.97 (s, 1 H), 7.81 (d, *J*=8.79 Hz, 2 H), 7.84 (d, *J*=8.79 Hz, 2 H), 7.51 (s, 1 H), 7.47 (t, *J*=8.79 Hz, 2 H), 7.41 (t, *J*=9.03 Hz, 2 H), 7.00 (m, *J*=7.81 Hz, 2 H), 6.94 (d, *J*=8.79 Hz, 2 H), 6.70(s, 1 H), 6.69 (s, 1 H), 4.52 (t, *J*=4.88 Hz, 2 H), 4.17 (t, *J*=4.64 Hz, 2 H), 3.99 - 4.03 (m, 2 H), 3.88 (t, *J*=4.88 Hz, 2 H), 3.85 (t, *J*=4.39 Hz, 2 H), 3.67 - 3.72 (m, 2 H), 3.63 - 3.67 (m, 2 H), 2.77 – 2.79 (m, 2 H), 2.43 (s, 3 H), 2.45 (s, 3 H), 1.85 – 1.87 (m, 4 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1, 162.9, 161.6, 161.3, 154.3, 134.9, 134.9, 134.7, 134.66, 127.8, 127.8, 124.9, 124.3, 123.8, 123.5, 121.8, 117.7, 117.6, 114.8, 114.7, 106.0, 105.8, 70.6, 70.5, 69.6, 69.5, 67.7, 67.5, 50.0, 29.2, 28.6, 25.9, 25.3, 20.8; LRMS (ESI) m/z 742 $[M+H]^+$; HRMS (ESI) calcd for $C_{44}H_{44}N_3O_8$ $[M+H]^+$ 742.3128, found 742.3103.

4.5.12 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 (59 mg) was obtained from **Ac5** and **Az4** in 79% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1 H), 7.78 - 7.85 (m, 5 H), 7.49 - 7.53 (m, 2 H), 7.47 (dd, *J*=8.54, 2.20 Hz, 1 H), 7.41 (d, *J*=8.30 Hz, 1 H), 7.37 (ddd, *J*=9.15, 7.69, 3.17 Hz, 1 H), 6.70 (d, *J*=10 Hz, 2 H), 6.94 (d, *J*=10 Hz, 2 H), 6.69 (s, 1 H), 6.69 (s, 1 H), 4.52 (t, *J*=5.12 Hz, 2 H), 4.13 - 4.18 (m, 2 H), 3.99 - 4.04 (m, 2 H), 3.88 (t, *J*=5.12 Hz, 2 H), 3.82 - 3.86 (m, 2 H), 3.67 - 3.72 (m, 2 H), 3.62 - 3.67 (m, 2 H), 2.75 - 2.81 (m, 2 H), 2.44 (s, 3 H), 1.84 - 1.89 (m, 4 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ178.1, 177.1, 163.2, 162.9, 161.5, 161.4, 159.3 (d, *J*=244.88 Hz, C6), 154.1, 152.0, 147.3, 134.7, 134.5, 127.7, 127.6, 124.8 (d, *J*=7.38Hz, C10),

124.7, 123.6, 123.3, 121.7, 121.4 (d, *J*=25.63Hz, C7), 119.8 (d, *J*=7.75Hz, C8), 117.5, 114.8, 114.6, 110.2 (d, *J*=23.38Hz, C5), 105.6, 105.2, 70.5, 70.3, 69.4, 69.3, 67.6, 67.4, 49.9, 28.4, 25.7, 25.2, 20.7; LRMS (ESI) m/z 746 $[M+H]^+$, 768 $[M+Na]^+$; HRMS (ESI) calcd for $C_{43}H_{41}N_3O_8F$ $[M+H]^+$ 746.2878, found 746.2845; calcd for $C_{43}H_{40}N_3O_8FNa$ $[M+Na]^+$ 768.2697, found 768.2685.

4.5.13 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 (61 mg) was obtained from **Ac5** and **Az7** in 87% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (s, 1 H), 7.74 - 7.82 (m, 5 H), 7.43 - 7.50 (m, 3 H), 7.37 - 7.41 (m, 1 H), 7.30 - 7.36 (m, 1 H), 6.97 (d, *J*=8.79 Hz, 2 H), 6.91 (d, *J*=8.79 Hz, 2 H), 6.67 (s, 1 H), 6.65 (s, 1 H), 4.54 (t, *J*=4.88 Hz, 2 H), 4.11 - 4.16 (m, 2 H), 3.99 (br. s., 2 H), 3.94 (t, *J*=4.88 Hz, 2 H), 3.81 - 3.85 (m, 2 H), 2.77 (br. s., 2 H), 2.43 (s, 3 H), 1.85 (br. s., 4 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3, 177.2, 163.3, 163.0, 161.6, 161.4, 159.4 (d, *J*=245.25 Hz, C6), 154.3, 152.2, 152.2, 147.6, 134.9, 134.6, 127.9, 127.8, 125.0 (d, *J*=7.38Hz, C10), 124.9, 124.0, 123.8, 123.4, 121.8, 121.5 (d, *J*=25.75Hz, C7), 119.8 (d, *J*=7.75Hz, C8), 117.6, 114.9, 114.7, 110.5 (d, *J*=23.75Hz, C5), 105.8, 105.4, 69.8, 69.4, 67.7, 67.4, 50.0, 28.5, 25.8, 25.2, 20.8; LRMS (ESI) m/z 702 $[M+H]^+$; HRMS (ESI) calcd for $C_{42}H_{37}N_3O_8F$ $[M+H]^+$ 702.2503, found 702.2534.

4.5.14 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 (40 mg) was obtained from **Ac5** and **Az11** in 59% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J*=8.79 Hz, 1 H), 7.97 - 8.00 (m, 1 H), 7.83 - 7.90 (m, 2 H), 7.81 (d, *J*=9.0 Hz, 2 H), 7.40 - 7.54 (m, 6 H), 6.90 - 7.00 (m, 4 H), 6.75 (s, 1 H), 6.69 (s, 1 H), 4.55 - 4.75 (m, 2 H), 4.17 - 4.21 (m, 2 H), 3.95 - 4.01 (m, 4 H), 3.84 - 3.88 (m, 2 H), 2.78 (br. s., 2 H), 2.45 (s, 3 H), 1.85 (br. s., 4 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ

178.3, 177.5, 163.1, 163.0, 162.9, 161.6, 157.7, 154.3, 134.8, 134.6, 131.5, 131.4, 128.9, 127.8, 127.0, 126.0, 124.8, 123.8, 123.4, 118.0, 117.6, 114.7, 114.4, 107.4, 105.7, 101.1, 69.7, 69.3, 67.8, 67.7, 50.1, 28.5, 25.7, 25.2, 20.8; LRMS (ESI) m/z 684 $[M+H]^+$; HRMS (ESI) calcd for $C_{41}H_{38}N_3O_7$ $[M+H]^+$ 684.2710, found 684.2692.

4.5.15 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 (63 mg) was obtained from **Ac12** and **Az1** in 91% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.79 - 1.91 (m, 4 H), 2.74 – 2.77 (m, 2 H), 3.77 - 3.83 (m, 2 H), 3.92 (t, *J*=4.88 Hz, 2 H), 3.98 - 4.04 (m, 2 H), 4.08 - 4.15 (m, 2 H), 4.52 (t, *J*=4.88 Hz, 2 H), 6.66 (s, 1 H), 6.67 (s, 1 H), 6.82 - 6.89 (m, 2 H), 6.92 - 6.98 (m, 2 H), 7.33 (t, *J*=7.32 Hz, 1 H), 7.41 - 7.50 (m, 5 H), 7.59 – 7.62 (m, 1 H), 7.77 - 7.85 (m, 4 H), 8.03 (d, *J*=8.75 Hz, 1 H), 8.13 (dd, *J*=7.75, 1.45 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 25.17, 25.74, 28.36, 50.00, 67.36, 68.17, 69.43, 69.73, 100.70, 106.08, 107.29, 114.58, 114.84, 117.57, 117.78, 121.84, 123.74, 124.23, 124.96, 125.45, 125.97, 126.76, 127.83, 128.84, 131.25, 131.66, 133.45, 147.41, 155.96, 157.79, 161.25, 162.77, 162.93, 163.42, 177.58, 178.04; LRMS (ESI) m/z 670 $[M+H]^+$, 692 $[M+Na]^+$; HRMS (ESI) calcd for $C_{40}H_{36}N_3O_7$ $[M+H]^+$ 670.2553, found 670.2525; calcd for $C_{40}H_{35}N_3O_7Na$ $[M+Na]^+$ 692.2373, found 692.2357.

4.5.16 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 (56 mg) was obtained from **Ac13** and **Az1** in 84% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (dd, *J*=7.81, 1.46 Hz, 1 H), 8.07 (d, *J*=8.79 Hz, 1 H), 7.80 - 7.87 (m, 4 H), 7.76 (s, 1 H), 7.64 (ddd, *J*=8.42, 6.95, 1.71 Hz, 1 H), 7.44 - 7.53 (m, 4 H), 7.35 - 7.40 (m, 1 H), 6.96 - 7.00 (m, 2 H), 6.94 (dd, *J*=8.79, 2.44 Hz, 1 H), 6.91 (d,

J=2.44 Hz, 1 H), 6.70 (d, *J*=3.90 Hz, 2 H), 4.74 (s, 2 H), 4.57 (t, *J*=4.88 Hz, 2 H), 4.18 - 4.23 (m, 2 H), 4.11 - 4.16 (m, 2 H), 3.92 – 3.96 (m, 4 H), 3.82 – 3.84 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.2, 177.6, 163.2, 163.0, 162.9, 161.3, 157.8, 156.1, 144.7, 133.5, 131.7, 131.3, 128.9, 127.9, 126.9, 126.0, 125.6, 125.0, 124.4, 123.8, 123.8, 117.9, 117.8, 114.9, 114.7, 107.4, 106.2, 101.0, 69.6, 69.5, 68.4, 67.9, 67.4, 64.8, 50.2; LRMS (ESI) m/z 672 $[M+H]^+$; HRMS (ESI) calcd for $C_{39}H_{34}N_3O_8$ $[M+H]^+$ 672.2346, found 672.2334.

4.5.17 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 (54 mg) was obtained from **Ac13** and **Az5** in 66% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (dd, *J*=8.30, 1.46 Hz, 1 H), 8.09 (d, *J*=8.79 Hz, 1 H), 7.99 - 8.03 (m, 2 H), 7.83 - 7.88 (m, 2 H), 7.78 (s, 1 H), 7.63 (ddd, *J*=8.54, 7.08, 1.95 Hz, 1 H), 7.45 - 7.51 (m, 4 H), 7.34 - 7.40 (m, 3 H), 7.23 - 7.30 (m, 3 H), 6.91 - 6.99 (m, 4 H), 6.72 (s, 1 H), 5.10 (s, 2 H), 4.74 (s, 2 H), 4.54 (t, *J*=5.12 Hz, 2 H), 4.18 - 4.24 (m, 2 H), 4.13 - 4.18 (m, 2 H), 3.90 - 3.95 (m, 2 H), 3.88 (t, *J*=5.12 Hz, 2 H), 3.80 - 3.85 (m, 2 H), 3.61 - 3.71 (m, 4 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.6, 174.8, 163.2, 162.9, 160.5, 157.8, 155.9, 155.1, 144.5, 139.2, 136.7, 133.1, 131.7, 131.3, 130.4, 128.9, 128.7, 128.1, 128.0, 126.9, 126.0, 125.7, 124.5, 124.1, 123.7, 123.5, 117.9, 117.8, 114.6, 114.2, 107.4, 101.1, 73.8, 70.7, 70.5, 69.5, 69.4, 68.4, 67.9, 67.4, 64.7, 50.2; LRMS (ESI) m/z 822 $[M+H]^+$, 844 $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{44}N_3O_{10}$ $[M+H]^+$ 822.3027, found 822.3003; calcd for $C_{48}H_{44}N_3O_{10}Na$ $[M+Na]^+$ 844.2846, found 844.2825.

4.5.18 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 (69 mg) was obtained from **Ac16** and **Az1** in 90% yield according to the general

procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 - 8.20 (m, 1 H), 8.06 (d, *J*=8.79, 1 H), 7.82 - 7.87 (m, 2 H), 7.75 - 7.81 (m, 2 H), 7.71 (br. s., 1 H), 7.62 - 7.67 (m, 1 H), 7.43 - 7.52 (m, 4 H), 7.36 - 7.39 (m, 3 H), 7.29 (t, *J*=7.32 Hz, 2 H), 7.20 - 7.25 (m, 1 H), 6.83 - 6.95 (m, 4 H), 6.71 (s, 1 H), 6.67 (s, 1 H), 4.57 (t, *J*=5.12 Hz, 2 H), 4.14 (br. s., 2 H), 4.06 - 4.12 (m, 2 H), 3.95 (t, *J*=5.12 Hz, 4 H), 3.71 - 3.85 (m, 4 H), 2.98 (br. s., 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.1, 177.6, 163.2, 163.0, 162.8, 161.2, 157.8, 156.0, 133.5, 131.6, 131.3, 128.9, 128.7, 128.3, 127.8, 127.2, 126.9, 126.0, 125.5, 125.0, 124.3, 123.8, 117.8, 117.7, 114.8, 114.6, 107.3, 106.1, 100.9, 69.7, 69.5, 67.4, 67.1, 58.8, 51.6, 50.2, 49.3; LRMS (ESI) m/z 761 $[M+H]^+$, 783 $[M+Na]^+$; HRMS (ESI) calcd for $C_{46}H_{41}N_4O_7$ $[M+H]^+$ 761.2975, found 761.2980; calcd for $C_{46}H_{40}N_4O_7Na$ $[M+Na]^+$ 783.2795, found 783.2794.

4.5.19 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 (19 mg) was obtained from **Ac16** and **Az2** in 24% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (dd, *J*=7.81, 1.46 Hz, 1 H), 8.08 (d, *J*=8.79 Hz, 1 H), 7.85 - 7.90 (m, 2 H), 7.81 (d, *J*=9.25 Hz, 2 H), 7.74 (br. s., 1 H), 7.66 (ddd, *J*=8.66, 6.95, 1.46 Hz, 1 H), 7.45 - 7.54 (m, 4 H), 7.35 - 7.44 (m, 3 H), 7.32 (t, *J*=7.57 Hz, 2 H), 7.22 - 7.28 (m, 1 H), 6.88 - 6.98 (m, 4 H), 6.73 (s, 1 H), 6.70 (s, 1 H), 4.54 (t, *J*=5.12 Hz, 2 H), 4.19 (br. s., 2 H), 4.07 - 4.14 (m, 2 H), 3.96 (br. s., 2 H), 3.88 (t, *J*=5.0 Hz, 2 H), 3.73 - 3.84 (m, 4 H), 3.59 - 3.69 (m, 4 H), 3.02 (br. s., 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.2, 177.7, 163.2, 163.1, 162.9, 161.4, 157.8, 156.1, 133.5, 131.7, 131.3, 128.9, 128.8, 128.3, 127.9, 127.2, 126.9, 126.1, 125.6, 125.0, 124.2, 123.8, 117.8, 117.8, 114.9, 114.6, 107.4, 106.1, 100.9, 70.7, 70.5, 69.5, 69.5, 67.5, 67.2, 58.8, 51.6, 50.2, 49.2; 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 805.3260; calcd for $C_{48}H_{44}N_4O_8Na$ [M+Na]$^+$ 827.3057, found 827.3070.

4.5.20 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 (57 mg) was obtained from **Ac16** and **Az4** in 75% yield according to the general procedure described above. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.80 Hz, 1H), 7.89 (d, *J* = 8.80 Hz, 2H), 7.81 - 7.85 (m, 3H), 7.50 - 7.55 (m, 5H), 7.42 (t, *J* = 8.80 Hz, 3H), 7.34 (t, *J* = 8.07 Hz, 2H), 7.25 - 7.28 (m, 1H), 6.90 - 7.00 (m, 5H), 6.76 (s, 1H), 6.71 (s, 1H), 4.57 (t, *J* = 5.14 Hz, 2H), 4.18 - 4.25 (m, 2H), 4.11 - 4.16 (m, 2H), 3.99 (br. s., 2H), 3.91 (t, *J* = 5.14 Hz, 2H), 3.82 (br. s., 2H), 3.67 - 3.71 (m, 3H), 3.64 - 3.67 (m, 2H), 3.04 (br. s., 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.8, 177.4, 163.4, 163.0, 161.5, 160.3, 158.6, 157.8, 152.2, 131.6, 131.4, 128.9, 128.4, 127.9, 126.9, 126.1, 125.0, 124.9, 123.8, 121.7, 121.5, 120.0, 119.9, 114.9, 114.7, 110.6, 110.4, 107.3, 105.4, 100.9, 70.7, 70.5, 69.5, 69.4, 67.5, 58.7, 53.7, 51.5, 50.2, 49.1; LRMS (ESI) m/z 823 [M+H]$^+$, 845 [M+Na]$^+$; HRMS (ESI) calcd for $C_{48}H_{44}N_4O_8F$ [M+H]$^+$ 823.3143, found 823.3166; calcd for $C_{48}H_{43}N_4O_8FNa$ [M+Na]$^+$ 845.2963, found 845.2976.

4.5.21 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 (20 mg) was obtained from **Ac16** and **Az7** in 25% yield according to the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, *J*=8.79 Hz, 1 H), 7.82 - 7.88 (m, 2 H), 7.80 (dd, *J*=8.05, 3.17 Hz, 1 H), 7.77 (d, *J*=8.79 Hz, 2 H), 7.45 - 7.53 (m, 4 H), 7.21 - 7.44 (m, 7 H), 6.85 - 6.96 (m, 4 H), 6.72 (s, 1 H), 6.66 (s, 1 H), 4.58 (br. s., 2 H), 4.08 - 4.27 (m, 4 H), 3.92 - 4.05 (m, 4 H), 3.68 - 3.92 (m, 4 H), 2.99 (br. s., 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.6, 177.3, 163.3, 162.9, 161.4, 159.5 (d, *J*=247.45 Hz, C6), 157.8,

152.2, 152.2, 131.6, 131.4, 128.9, 127.9, 127.0, 126.0, 124.0, 121.6 (d, *J*=25.25Hz, C7), 119.9 (d, *J*=8.08Hz, C8), 114.9, 114.6, 110.5 (d, *J*=24.24Hz, C5), 107.4, 105.5, 101.0, 69.7, 69.5, 67.5; LRMS (ESI) m/z 779 $[M+H]^+$, 801 $[M+Na]^+$; HRMS (ESI) calcd for $C_{46}H_{40}N_4O_7F$ $[M+H]^+$ 799.2881, found 799.2916; calcd for $C_{46}H_{39}N_4O_7FNa$ $[M+Na]^+$ 801.2700, found 801.2738.

4.6 Purity determination by HPLC. HPLC equipped with an Agilent Prep-Sil Scalar column 4.6 × 250 mm, 5-micron; flow rate, 1 mL/min; detection: 254 or 365 nm with reference at 450 nm. The compounds were weighted and dissolved in DCM solution. 5 µL of the samples were injected into the HPLC system. The chromatographic separation was performed using a linear gradient (1% dichloromethane +99% methanol changed to 10% dichloromethane +90% methanol in 20 min).

4.7 Construction of clicked flavonoid dimers library. In a 96-well PCR-plate, 1 mM alkyne monomer, 1 mM azide (AzM) monomer and 1 mM Cu(I) catalyst were added into each well. For diacetylenes **Ac15**, **Ac22**, **Ac23**, **Ac29**, **Ac 31** and triacetylene **Ac17**, two millimolar and three millimolar of azides (AzM) were used respectively. Each reaction was topped up with THF to a final volume of 100 µL. The plate was capped and an ice pad was placed on the top of the plate in order to reduce evaporation of reagents. The plate was placed over 96-well hot plate and heated for overnight at 70 ºC. In the next morning, the lid of 96-well plate was removed and the reagents inside the well were completely removed by incubating at 70 ºC for 1 hr. After drying up, each clicked product in each well was dissolved in 100 µL of 100% DMSO. The concentration of clicked product was around 1 mM because the clicked reaction has been reported to be highly efficient (~ 100% reaction yield).

4.8 Materials for Biological Studies. Dimethyl sulfoxide (DMSO), paclitaxel, topotecan, DOX, verapamil, MK571, 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 lines 2008 and 2008/MRP1 were generous gift from Prof. P. Borst (The Netherlands Cancer Institute, Amsterdam, Netherlands). The human embryonic kidney cell lines HEK293/pcDNA3.1 and BCRP-transfectant HEK293/R2 were generously provided by Dr. Kenneth To (The Chinese University of Hong Kong, Hong Kong). L929 was purchased from ATCC.

4.9 Cell Culture. 2008/MRP1 was the stable MRP1 transfectant generated by retroviral transduction using pCMV-Neo-MRP1.[47] pCMV-Neo-MRP1 was constructed by inserting a *Sal*I-*Not*I DNA fragment containing the complete human MRP1 cDNA as a blunt-end fragment in pCMVneo.[47] 2008/MRP1 and 2008/P, HEK293/R2 and HEK293/pcDNA3.1 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 LCC6 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. The cells were split constantly after a confluent monolayer has been formed. To split cells, the plate was washed briefly with phosphate-buffered saline (PBS), treated with 0.05% trypsin-EDTA and harvested by centrifugation.

4.10 High throughput screening of MRP1 modulating activity. 4,000 cells of 2008/MRP1 and 100 nM doxorubicin (DOX) were mixed with 2 μM (primary screening) or 1 μM (secondary screening) of crude clicked products to a final volume of 200 μL in each well of 96-well plates. The plates were then incubated for 5 days at 37 ºC. Three controls were involved in which (1) cancer cells incubated with 2 μM of each pure alkyne and DOX, (2) cancer cells incubated with 2 μM of each pure azide and DOX and (3) cancer cells incubated with Cu(I) catalyst and DOX. 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). The % of survivors was calculated: (OD_{490nm} in the presence of DOX and dimers)/ (OD_{490nm} in the absence of DOX and dimers) x 100%. All experiments were performed in triplicate and repeated at least thrice and the results were represented as mean ± standard error of mean.

4.11 Cell proliferation assay of LCC6MDR and HEK293/R2. 6500 cells of LCC6MDR or 5000 cells of HEK293/R2 were seeded into each well of 96-well plate in a total volume of 200 μL. LCC6MDR cells were incubated with a range of paclitaxel (0, 4.1, 12, 37, 111, 333 and 400 nM) and 1 μM of modulator. HEK293/R2 cells were incubated with different concentration of topotecan (0, 12, 37, 111, 333, 1000, 3000 nM) and 1 μM of modulator. The plates were incubated at 37ºC with 5 % CO_2 for 5 days. After incubation, the cell survival in each well was determined by MTS assay as described above.

4.12 DOX accumulation assay. DOX accumulation assay was done in 1 mL volume. A $5x10^5$ cells of 2008/MRP1 cells were added in an Eppendorf tube and incubated with 5 μM DOX and 2

μM of modulators (**Ac3Az11, Ac12Az1, Ac16Az1,** verapamil and MK571) at 37 ºC for 120 min. A 0.2% DMSO was used as a negative control. After incubation, the cells were spinned down and washed with cold PBS, pH7.4 for 2 times and finally resuspended with 300 μL of cold PBS, pH7.4. The intracellular DOX level was analyzed by BD C6 Accuri flow cytometer using FL2 channel at EX 480 nm and EM 590 nm. For each sample, a total of 20,000 events was collected.

4.13 Determination of MRP1 protein expression. 20,000 cells of 2008/P and 2008/MRP1 cells were seeded in a 6-well plate and incubated with 0, 1, 2, 5 μM of **Ac3Az11** for 3 days, respectively. After 3 days, the cells were trypsinized and washed once with 1X PBS. After spinning down the cells, they were fixed with 4% paraformaldehyde at room temperature for 15 min and then permeabilized with 0.5% Tween 20 at room temperature for 15 min. The cells were resuspended in 100 μL FACS buffer (1% BSA, 1 mM EDTA, 0.1% Tween 20 in PBS) and stained with 2.5 μL FITC mouse anti-human MRP1 antibody (BD bioscience) at 4ºC for 45 min. After staining, the cells were washed once with 500 μL cold FACS buffer and resuspended in 200 μL FACS buffer. The MRP1-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 20,000 events was collected.

4.14 Dox influx and efflux studies. To measure the DOX influx, 2008/P and 2008/MRP1 cells were co-incubated with DOX (5 μM) and **Ac3Az11** (2 μM) in the supplemented RPMI1640 media at 37ºC. 0.25% of DMSO acted as a negative control. The cells were harvested after 0, 15, 30, 45 and 60 min for determining the intracellular DOX concentration as described previously. The DOX level was determined by C6 Accuri flow cytometer as described previously. The % of DOX increase was calculated = [(DOX level at final time point – DOX

level at 0 min) / DOX level at 0 min * 100%]. To measure DOX efflux, 2008/P and 2008/MRP1 cells were incubated in supplemented RPMI1640 containing 20 μM DOX for 1 hr at 37°C. Then the cells were washed and further incubated with or without compound **Ac3Az11** (2 μM). At 0, 15, 30, 45, 60, 75, 90 and 105 min, the cells were harvested for measuring the intracellular DOX concentration. The % of DOX reduction was calculated = [(DOX level at final time point / DOX level at 0 min) * 100%].

4.15 Pharmacokinetic studies. This animal study was conducted in full compliance with the standard protocol approved by the Animal Subjects Ethics Sub-committee (ASESC) of The Hong Kong Polytechnic University (ASESC Case No. 14-15/02-ABCT-R-GRF). Female Balb/c mice of weight 18 to 23 grams were obtained from the Centralised Animal Facilities of The Hong Kong Polytechnic University. They were kept in a temperature and humidity controlled environment with 12-hour light-dark cycle with the provision of standard diet and water throughout the experiment. A **Ac3Az11.HCl** solution was prepared in a formulation (NMP: Cremorphol: Tween80: H_2O = 5 : 5 : 4.5 : 85.5). **Ac3Az11.HCl** at dosage of 10 mg/kg was administered to female Balb/c mice through the intravenous (i.v.) injection by using a 25G needle. Blood samples were collected in heparinized tubes by cardiac puncture after deep anesthesia by ethyl ether at 15, 30, 60, 90, 120, 240, and 360 min post-administration of **Ac3Az11.HCl**. Blood samples were centrifuged at 16,000 *g* for 10 minutes immediately after collection to obtain blood plasma. Blood plasma was stored at -20 °C until analysis.

4.16 Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Plasma samples collected were thawed in room temperature. Ten microliter of internal standard ([^{13}C6] paclitaxel, 10 μg/mL) was spiked into 90 μL of each plasma samples.[48] Three hundred microliter of methanol

was added, followed by strong vortex for 30 seconds. After a centrifugation at 7500 rpm for 10 minutes at 4°C, supernatant of each tube was collected and filtered. Filtered supernatants were transferred into glass vials with micro-volume inserts for LC-MS/MS analysis.

The concentration of **Ac3Az11** was determined by LC-MS/MS. Ten microliter of each sample was injected into liquid chromatography system (AcQuity, Waters) by auto-sampler (4ºC), separated by a BEH C18 column (2.1 X 50 mm, 1.7µm; AcQuity UPLC, Waters) fitted with a BEH C18 guard column (2.1 X 5 mm, 1.7µm; VanGuard, AcQuity UPLC, Waters). The mobile phase was composed of MilliQ water (containing 0.1% formic acid) (A) and methanol (containing 0.1% formic acid) (B). The flow rate of mobile phase was 0.3 mL/min. And the gradient elution program is: at 0 min 90% A / 10% B, at 1 min 90% A / 10% B, at 6 min 15% A / 85% B, at 7 min 15% A / 85% B, at 8 min 90% A / 10% B, at 9 min 90% A / 10% B. Effluent was detected by a triple-quadrupole mass spectrometer (Waters Quattro Ultima). For data acquisition, the capillary voltage was set as 3.0 kV, and the cone voltage was set at 30 V. After the electrospray ionization (ESI), **Ac3Az11** was ionized to a precursor ion with positive charge ([**Ac3Az11.H**]$^+$). [**Ac3Az11.H**]$^+$ (m/z 674) was allowed to pass the first quadrupole (Q1) to get into the collision cell (Q2). Precursor ions were derived into many fragment ions under a collision energy of 34 eV. Only desired product ion (m/z 418) were detected and recorded through third quadrupole (Q3). The analysis of quantification was processed by Mass Lynx Mass Spectrometry Software (Waters).

4.17 *In silico* docking study

CLC Drug Discovery Workbench (Version 2.5, QIAGEN) software was used to predict how a ligand binds to its target protein of bMRP1. The ligands were flavonoid dimers **Ac3Az11**and **FD-4e** as well as MRP1 substrates LTC_4 and DOX. The 2D structures of these ligands were generated

from SIMLES and imported into the software for docking study. The electron cryo-microscopy structure of bMRP1 (PDB ID: 5UJA) bound to LTC_4 was downloaded from Protein Data Bank (https://www.rcsb.org/) and used directly for docking without any changes. Using the software function of "Find Binding Pockets", the software was able to identify the central translocation pathway of MRP1 as one of the potential binding pockets. The identification of ligand binding modes was done iteratively by evaluating 10,000 ligand conformations and estimating the binding energy of their interactions with the binding pocket. The binding pose with the top 5% highest scores were returned for further visual inspection. The highest scores positioned the ligand **Ac3Az11**, **FD-4e** and DOX into the binding site of LTC_4. Amino acid residues involved in the interaction with ligands are shown in **Figure 9**. All ligands are surrounded by the similar residues in the central translocation pathway of MRP1, therefore it can be concluded that these residues play an important functional role.

ASSOCIATED CONTENT

Supporting Information. The supporting information is available free of charge via the Internet at http://pubs.acs.org. HPLC chromatogram of compounds **Ac3Az11**, **Ac3Az2**, **Ac3Az4** and **Ac16Az4;** ^{1}H NMR and ^{13}C NMR spectra of representative compounds listed in Table 5, superimposition of reported co-complex structure of LTC4 (green) and its predicted binding pose and alignment of hMRP1 and bMRP1 (PDF).

SMILES molecular strings formulas (CSV).

Binding LTC_4, **Ac3Az11**, **FD-4e** and DOX to bMRP1 (PDB ID: 5UJA) (PDB).

Accession Codes

Authors will release the atomic coordinates upon article publication.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

MDR, multidrug resistance; P-gp, P-glycoprotein; MRP1, multidrug resistance-associated protein 1; TMD, BCRP, breast cancer resistance protein; transmembrane domain; ABC, ATP-binding cassette; CuAAC, copper-catalyzed alkyne azide cycloaddition; PEG, polyethylene glycol; DOX, doxorubicin; LTC_4, leukotriene C_4; NMP, N-methyl-2-pyrrolidone

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