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Synthesis and biological evaluation of a series of flavone derivatives as potential radioligands for imaging the multidrug resistance-associated protein 1 (ABCC1/MRP1)

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Abstract—Multidrug resistance (MDR) is one of the major problems affecting the treatment of cancer. In vivo visualization and quantification of MDR proteins would be of great value to better select the therapeutic strategy. Six flavone-based compounds were synthesized and evaluated for their cytotoxic activity and MDR-reversing capacity using hMRP1 or hMDR1 overexpressing cell lines for in vitro assays. All the flavone derivatives were highly selective for hMRP1-expressing cell lines. These derivatives each used at 4 μM (a non-cytotoxic concentration) enhance significantly the sensitivity of hMRP1-mediated MDR cell line toward doxorubicin toxicity. Their MDR-reversing capacity suggests that, in particular, the 4′-fluoroalkyloxy and 4′-iodo apigenin derivatives are potential new radiopharmaceuticals to visualize in vivo MRP1-mediated MDR phenomenon by PET or SPECT.
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1. Introduction

Multidrug resistance (MDR) is a major problem in the treatment of cancer. This phenomenon is in part mediated by the overexpression of plasma membrane transporters like the P-glycoprotein[1] (MDR1 or ABCB1) or the multidrug resistance-associated protein[2] (MRP1 or ABCC1) which both possess a broad capacity to transport compounds of chemical diversity. It is of great interest to identify MDR cells and the development of radiopharmaceuticals targeted toward MDR1 and/or MRP1 should be useful for improving chemotherapeutic strategies. A great number of compounds belonging to various chemical structural classes have been demonstrated to interact with these two membrane transporters. In particular, flavone-based compounds have been shown in vitro to modulate the binding affinity of nucleotide on cytosolic domains of MDR1.[3,4] However, differences in binding affinities were observed between the various classes of flavonoid compounds. It seems that halogen or long alkyl chain on the B-ring of chalcones or flavones increases the binding affinities toward the NBD2 recombinant domain of hMDR1.[5,6] In addition, flavone-based compounds have been shown in vitro to modulate the hMRP1 transport and ATPase activities.[7–10] In this respect, apigenin is among the most potent flavone-based modulator. Therefore, we anticipated that apigenin-based compounds might be good candidates to target hMDR1 or hMRP1 and have synthesized a series of apigenin derivatives, introducing iodine or fluorine atoms in order to be potentially used as imaging agents to monitor MDR phenotype in tumors by single-photon emission tomography (SPECT) or positron emission tomography (PET). At this moment, most studies are performed with ^{99m}Tc-labeled substrates[11] as SPECT radiotracers. However, the interest in studying

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MDR with PET is increasing, and thus the development of a new radiopharmaceutical labeled with ^{11}C or ^{18}F is being pursued.

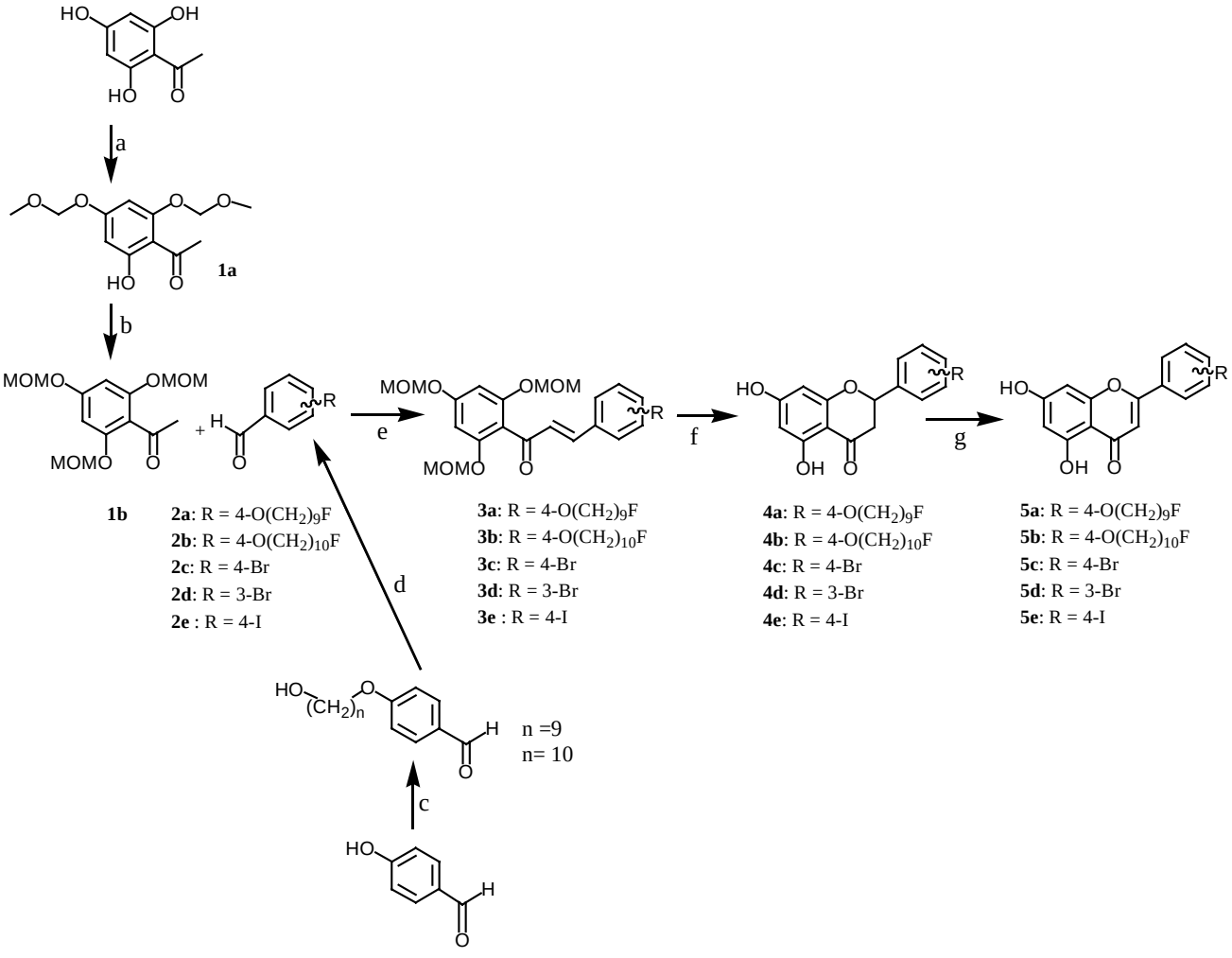
Thereafter, we evaluate their putative interaction with either hMDR1 or hMRP1 through their respective ability to reverse the transporter-mediated MDR phenotype using cell cytotoxic assays performed on well-characterized cell lines expressing either human MDR1 (hMDR1-NIH3T3) or human MRP1 (GLC4/Adr). We found that among the apigenin derivatives studied most of them appear to modulate selectively the hMRP1-associated MDR.

2. Results

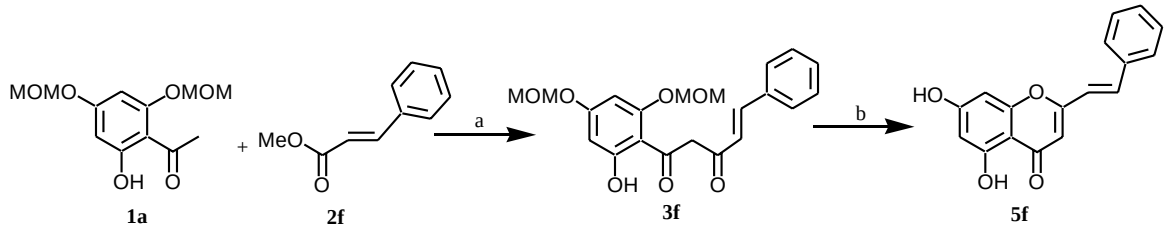
2.1. Synthesis

To prepare the desired compounds in high yields, the hydroxyl groups in the starting acetophenone were protected as the MOM-ether derivatives (**1**) in a step-wise manner with methoxymethyl (MOM) chloride. Regioselective methoxymethylation of the 2- and 4-hydroxyls was achieved with the use of the weak base K_2CO_3 to give the bis-MOM ether,[12] whereas the triMOM-ether **1b** required the addition of the stronger base, NaOH, and the phase transfer catalyst tetrabutyl ammonium chloride.[13] The chalcones **3a**–**e** were prepared in high yield by base-catalyzed condensation of the protected acetophenone **1b** with the substituted benzaldehydes **2a**–**e**. Hydrolysis of the MOM-protecting groups with dilute HCl in methanol followed by cyclization with solid sodium acetate under reflux gave the desired dihydro-flavonoid derivatives **4a**–**e**. Oxidation of the crude intermediate with iodine in pyridine[14] led to the target flavones **5a**–**e** (Scheme 1).

The styrylchromone was prepared by a Claisen condensation of the 2-hydroxyacetophenone **1a** and the cinnamic ester **2f**, giving the β-diketone **3f** as a non-purified intermediate (Scheme 2). The ^1H NMR spectra of this intermediate **3f** revealed that it was a mixture of tautomers.[15,16] Cyclodehydration and simultaneous deprotection of dimethoxymethylated diketone with amberlyst acidic cation exchange resin in refluxing propan-2-ol[16] gave **5f** in 82% yield.



Scheme 1. Synthesis of flavones **5a**–**e**. Reagents and conditions: (a) MOMCl, K_2CO_3, acetone, reflux; (b) tetrabutyl ammonium chloride, NaOH, MOMCl; (c) K_2CO_3, $HO(CH_2)_nBr$ ($n = 9$, 10), DMF, reflux; (d) PBSF/$NEt_3(HF)_3$/iPr$_2$NEt (1.2/1.3/3.8 equiv, respectively), MeCN, rt; (e) KOH, DMF, 0 °C; (f) HCl, MeOH, reflux, NaOAc, reflux; (g) I_2, pyridine, reflux.



Scheme 2. Synthesis of the styrene derivative **5f**. Reagents and conditions: (a) NaH, THF, reflux; (b) amberlyst, iC_3H_5OH, reflux.

2.2. In vitro evaluation

Tables 1 and 2 summarize the cytotoxicity data from dose–response curves obtained for each of the flavone derivatives and from chemosensitization experiments in which these compounds were tested at a non-toxic concentration in combination with several concentrations of doxorubicin or colchicine depending on the cell

Table 1. Determination of compounds **5a**–**f** toxicity for parental GLC4 and hMRP1-expressing GLC4/Adr cells

Compound added	IC_{50}[a] (μM)			
	GLC4	RR[c]	GLC4/Adr	RR[c]
Doxorubicin (DOX) (clog P[d] 2.29)	0.009 ± 0.003[b]	1	3.1 ± 0.3	340
MK571 (clog P[d] 6.13)	25 ± 2		37 ± 14	
5a (clog P[d] 6.99)	12 ± 4		17 ± 3	
5b (clog P[d] 7.52)	>100		>100	
5c (clog P[d] 4.42)	22 ± 2		36 ± 13	
5d(clog P[d] 4.42)	29 ± 2.5		26 ± 7	
5e (clog P[d] 4.69)	23 ± 2		30 ± 10	
5f (clog P[d] 3.76)	>50		>50	
DOX + MK571 (10 μM)	0.009 ± 0.003	1	0.70 ± 0.09	78
DOX + **5a** (4 μM)	0.0095 ± 0.001	1	1.5 ± 0.1	167
DOX + **5b** (4 μM)	0.009 ± 0.001	1	1.1 ± 0.1	122
DOX + **5c** (4 μM)	0.0085 ± 0.002	1	0.70 ± 0.06	78
DOX + **5d** (4 μM)	0.0095 ± 0.001	1	1.1 ± 0.1	122
DOX + **5e** (4 μM)	0.011 ± 0.002	1	1.0 ± 0.1	111
DOX + **5f** (4 μM)	0.009 ± 0.001	1	1.4 ± 0.1	156

[a] Cell viability was determined by the MTT assay, IC_{50} is the compound concentration value (μM) required to kill 50% of cells tested.
[b] Values are means ± SD of at least three experiments.
[c] RR: relative resistance (doxorubicin IC_{50} values for GLC4 or CLC4/Adr cells in the absence or presence of a nontoxic concentration of another compound were divided by the doxorubicin IC_{50} value for GLC4).
[d] Calculated log P (clog$P$, ChemDraw Ultra 6.0.1, 2000).

Table 2. Determination of compound **5a**–**f** toxicity for parental NIH3T3 and hMDR1-expressing NIH3T3 cells

Compound added	IC_{50}[a] (μM)			
	NIH3T3	RR[c]	hMDR1-NIH3T3	RR[c]
Colchicine (COL) (clog P[d] 1.03)	0.0017 ± 0.0002[b]	1	0.036 ± 0.002	21
Cyclosporine A (clog P[d] 14.0)	6 ± 0.4		9.1 ± 0.35	
5a (clog P[d] 6.99)	9 ± 1		9 ± 1	
5b (clog P[d] 7.52)	10 ± 1		10 ± 1	
5c (clog P[d] 4.42)	20 ± 2		20 ± 2	
5d(clog P[d] 4.42)	30 ± 3		30 ± 3	
5e (clog P[d] 4.69)	20 ± 2		20 ± 2	
5f (clog P[d] 3.76)	40 ± 4		40 ± 4	
COL + cyclosporine A (1 μM)	0.0006 ± 0.00003	0.3	0.0035 ± 0.0001	6
COL + **5a** (0.3 μM)	0.0019 ± 0.0001	1	0.036 ± 0.003	21
COL + **5b** (0.3 μM)	0.0017 ± 0.0001	1	0.036 ± 0.002	21
COL + **5c** (1 μM)	0.0017 ± 0.0001	1	0.046 ± 0.004	27
COL + **5d** (1 μM)	0.0019 ± 0.0001	1	0.042 ± 0.003	25
COL + **5e** (1 μM)	0.0017 ± 0.0002	1	0.042 ± 0.003	25
COL + **5f** (1 μM)	0.0015 ± 0.0001	1	0.036 ± 0.002	21

[a] Cell viability was determined by the MTT assay, IC_{50} is the compound concentration value (μM) required to kill 50% of cells tested.
[b] Values are means ± SD of at least three experiments.
[c] RR: relative resistance (colchicine IC_{50} values for NIH3T3 or hMDR1-NIH3T3 cells in the absence or presence of a nontoxic concentration of another compound were divided by the colchicine IC_{50} value for NIH3T3).
[d] Calculated log P (clog$P$, ChemDraw Ultra 6.0.1, 2000).

line. For each flavone derivative tested sensitive and resistant cells of each cell type share similar chemosensitive profiles (see Tables 1 and 2). The simplest explanation for these results is that the flavone derivatives tested are probably not transported by hMDR1 or hMRP1 because if they were one would expect to observe a relative resistance in MDR cells compared to the sensitive cells which is not the case (see Tables 1 and 2). Since the binding of a molecule to a MDR transporter can take place without necessarily the bound molecule being transported, as exemplified by many MDR modulators, we first examined the effect of each flavone derivative on colchicine sensitivity of hMDR1-NIH3T3 cells that express human MDR1 but not MRP1. For this purpose, hMDR1-NIH3T3 cells were cultured in the continuous presence of a non-toxic concentration of each flavone derivative and treated for three days with increasing concentrations of colchicine. The results presented in Table 2 indicate that none of the flavone derivatives tested was able to significantly sensitize hMDR1-NIH3T3 cells to colchicine. Moreover, control experiments demonstrate that none of these flavone derivatives had any significant effect on the colchicine chemosensitivity of the parental NIH3T3 cells (Table 2). In contrast, we observed that each flavone derivative added to the culture medium at a non-toxic concentration was able to sensitize GLC4/Adr cells to doxorubicin, with the 4′-bromo flavone being the most potent modulator (**5c**, Table 1). Incubation of GLC4/Adr cells with the 4′-bromo flavone **5c** (at 4 μM) caused a fourfold decrease in the doxorubicin IC_{50} value (see Table 1). This enhancement of doxorubicin toxicity is similar to the one observed when a non-toxic concentration of MK571 (at 10 μM) is added to the cell culture medium (see Table 1). None of the flavone derivatives tested neither MK571 (at 10 μM) had any significant effect on the doxorubicin toxicity toward the parental GLC4 cells (see Table 1), suggesting a role for hMRP1 in determining the capacity of these apigenin derivatives to enhance the toxicity of doxorubicin.

3. Discussion

In the present study, we have investigated the effects of a series of new synthesized flavone derivatives on membrane-mediated drug resistance. Growth inhibition assays with continuous exposure to various compounds were performed using two well-characterized MDR cell lines to test whether the hMDR1 or hMRP1 membrane proteins can efflux these compounds and therefore confer resistance to these cells when compared with their sensitive counterpart. We found that hMDR1 as well as hMRP1 was unable to confer cross-resistance to the six synthesized compounds which suggests that these flavone derivatives are likely not effluxed by either of these two transporters. Flavonoids have long been investigated as a class of MDR1 modulators and molecules belonging to various flavonoid chemical subclasses were demonstrated to bind with high affinity to MDR1.[17–19] However, to our knowledge, the flavone apigenin has not been reported to modulate drug accumulation in hMDR1 cancer cells. The only data available regarding apigenin, in the field of MDR, are its binding to the cytosolic C-terminal nucleotide-binding domain (NBD2) of mouse MDR1 or *Leishmania* ltrmdr1.[3,4] Thus even if we cannot rule out the possibility that some of the apigenin derivatives investigated in the present study might interact with hMDR1, it remains that this putative interaction is likely not interfering with either colchicine binding or efflux since colchicine resistance of hMDR1-NIH3T3 cells is not perturbed by the presence of either of these compounds. Concerning MRP1-mediated resistance, our results are reminiscent of a recent study performed on hMRP1 transfected HeLa cells in which it was found that hMRP1 does not confer resistance to a series of bioflavonoids and in particular to apigenin.[20] The fact that these two studies were performed on different hMRP1-expressing cell types gives strength to the common conclusion that apigenin and the apigenin derivatives tested in the present study are likely not transported by hMRP1. In this respect, direct transport studies using radiolabeled flavone derivatives would certainly be helpful in drawing a definitive conclusion. Interestingly, we found a reduction in long-term viability of hMRP1-expressing cells treated with apigenin derivatives (4 μM) when compared to their sensitive counterpart. Evidently, this potentiation of doxorubicin toxicity was not a result of non-specific membrane perturbation induced by either of these flavone derivatives, since it was not observed for sensitive cell lines or hMDR1-expressing cell line. In particular, compound **5c** (at 4 μM) appeared to sensitize hMRP1-expressing cells to doxorubicin as efficiently as MK571 (at 10 μM). It seems clear that growth inhibition was brought about by modulation of hMRP1 function by each of the various flavone derivatives studied and raises questions concerning the mechanism(s) involved. It can be noticed that, independently of the chemical modifications introduced in the B ring of compounds **5a**–**e**, it seems that the common apigenin backbone of these five molecules represents a structural pattern which precludes the transport of these compounds by either hMDR1 or hMRP1. This is an interesting structural property common to these halogenated apigenin derivatives because if they turn out to bind selectively to hMRP1 they will stay associated longer than if they were expelled from the cell by hMRP1 therefore making easier the observation of hMRP1-related MDR by SPECT or PET.

4. Conclusion

In conclusion, according to cytotoxicity experiments, we found that halogenated apigenin derivatives constituted a class of hMRP1-related MDR modulators. These data open the way for investigation in new potential radiopharmaceuticals (**5a**,**b** as potent radiofluorinated, **5e** can be radioiodinated) in the field of MDR imaging. The relatively short and easy synthesis of these molecules makes them potential candidates for further development.

5. Experimental

General details: NMR spectra were recorded on a Bruker 400 MHz spectrometer. Mass spectra were

performed on a Waters Micromass 2MD Quadropole. Elemental analysis was performed by Microanalytical and the analytical results obtained were within ±0.4% of the theoretical values.

All chemicals were obtained from Sigma Aldrich unless stated otherwise. Doxorubicin was dissolved at 5 mM in ethanol:PBS (v/v). All the drug stock solutions were stored at 4 °C and diluted in culture medium before use.

5.1. 2-Hydroxy-4,6-di-*O*-methoxymethylacetophenone 1a and 2,4,6-tri-*O*-methoxymethylacetophenone 1b

Compounds **1a**,**b** were synthesized as previously described.[12,21]

5.2. 4-(Hydroxyalkyloxy)benzaldehydes. General method

To 9-bromononan-1-ol (20.9 g, 0.1 mol) or 10-bromodecan-1-ol (20.9 g, 0.1 mol) in dry DMF (40 mL) were added 4-hydroxybenzaldehyde (12.2 g, 0.1 mol) and dry K_2CO_3 (20.7 g, 0.15 mol) and refluxed overnight under N_2. The DMF was evaporated under reduced pressure, diluted with H_2O (50 mL), extracted with DCM (5 × 20 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was evaporated to dryness and the crude product was purified by column chromatography (silica gel, ethyl acetate–hexane: 30/70) to afford a white solid.

5.2.1. 4-(9-Hydroxynonyloxy)benzaldehyde. Yield 64% ^{1}H NMR ($CDCl_3$): δ 1.38 (m, $4CH_2$), 1.49 (m, CH_2), 1.61 (m, CH_2), 1.84 (m, CH_2), 3.67 (t, J = 6.6 Hz, 2H, CH_2), 4.07 (t, J = 6.5 Hz, 2H, CH_2), 7.02 (d, J = 8.75 Hz, $2H_{ar}$), 7.85 (d, J = 8.75 Hz, $2H_{ar}$), 9.91 (s, CHO). ^{13}C NMR ($CDCl_3$): 26.9, 27.1, 30.2, 30.4, 30.5, 30.7, 33.9, 64.2, 69.6 ($9CH_2$), 116.0 ($2CH_{ar}$), 131.0 (C_{ar}), 133.2 ($2CH_{ar}$), 165.5 (C_{ar}), 192.0 (CHO). MS: (ES+): 265.0.

5.2.2. 4-(10-Hydroxydecyloxy)benzaldehyde. Yield 55% ^{1}H NMR ($CDCl_3$): δ 1.28 (m, $5CH_2$), 1.48 (m, CH_2), 1.59 (m, CH_2), 1.80 (m, CH_2), 3.67 (t, J = 6.6 Hz, 2H, CH_2), 4.07 (t, J = 6.6 Hz, 2H, CH_2), 7.02 (d, J = 8.5 Hz, $2H_{ar}$), 7.86 (d, J = 8.5 Hz, $2H_{ar}$), 9.91 (s, CHO). ^{13}C NMR ($CDCl_3$): δ 26.9, 27.1, 30.2, 30.5, 30.6, 30.6, 30.7, 34.0, 64.3, 69.6 ($10CH_2$), 115.96 (C_{ar}), 130.9 ($2CH_{ar}$), 133.2 (C_{ar}), 165.5 ($2CH_{ar}$), 192.0 (CHO). MS: (ES+): 279.0.

5.3. 4-(Fluoroalkyloxy)benzaldehydes 2a,b. General method

4-(9-Fluorononyloxy)benzaldehyde **2a** and 4-(10-fluorodecyloxy)benzaldehyde **2b** were synthesized as per the method of Yin et al.[22]

5.3.1. 4-(9-Fluorononyloxy)benzaldehyde 2a. Compound **2a** was purified by column chromatography (silica gel, ethyl acetate–hexane: 30/70) to afford a white solid. Yield (88%). ^{1}H NMR (MeOH-d_4): δ 1.38–1.51 (m, $5CH_2$), 1.68 (m, CH_2), 1.81 (m, CH_2), 4.08 (t, J = 6.1 Hz, CH_2), 4.41 (dt, J = 47.6, 6.1 Hz, CH_2F), 7.07 (d, J = 8.7 Hz, $2H_{ar}$), 7.85 (d, J = 8.7 Hz, $2H_{ar}$), 9.83 (s, CHO). ^{13}C NMR ($CDCl_3$): 25.1 (d, J = 5.3 Hz, CH_2), 25.8, 28.9, 29.0, 29.1, 29.3 ($5CH_2$), 30.3 (d, J = 19 Hz, CH_2), 68.3 (CH_2), 84.0 (d, J = 164 Hz, CH_2), 114.7 ($2CH_{ar}$), 129.7 (C_{ar}), 131.9 ($2CH_{ar}$), 164.2 (C_{ar}), 190.7 (CHO). MS: (ES+): 267.37.

5.3.2. 4-(10-Fluorodecyloxy)benzaldehyde 2b. Compound **2b** was purified by column chromatography (silica gel, ethyl acetate–hexane: 30/70) to afford a white solid. Yield (81%). ^{1}H NMR (CD_3CN): δ 1.36–1.50 (m, $6CH_2$), 1.68–1.75 (m, CH_2), 1.80 (m, CH_2), 4.09 (t, J = 6.2 Hz, CH_2), 4.46 (dt, J = 47.6, 6.2 Hz, CH_2F), 7.08 (d, J = 8.75 Hz, $2H_{ar}$), 7.86 (d, J = 8.75 Hz, $2H_{ar}$), 9.89 (s, CHO). ^{13}C NMR (CD_3CN): 26.1 (d, J = 5 Hz, CH_2), 26.8, 29.9, 30.1, 30.2, 30.4, 30.4 ($6CH_2$), 31.3 (d, J = 19 Hz, CH_2), 69.6 (CH_2), 84.3 (d, J = 162 Hz, CH_2), 116.0 ($2CH_{ar}$), 131.1 (C_{ar}), 132.9 ($2CH_{ar}$), 165.4 (C_{ar}), 192.1 (CHO). MS: (ES+): 281.43.

5.4. 1-(2,4,6-Tris(methoxymethyl)phenyl)-3-(substituted-phenyl)prop-2-enones 3a–e. General method

To the 4-substituted benzaldehydes **2a**–**e** (0.1 mol) was added 2,4,6-tri-*O*-methoxymethylacetophenone **1b** (28.63, 0.1 mol) in DMF (100 mL) at 0 °C followed by powdered KOH (8.42 g, 0.15 mol). After stirring for 4 h at 0 °C, the reaction mixture was diluted with H_2O (250 mL) and extracted with DCM (100 mL × 3). The organic layer was washed with H_2O (50 mL × 2), dried over anhydrous $MgSO_4$, and filtered. The product was purified by column chromatography (silica gel, ethyl acetate–hexane: 30/70) to afford a pale yellow solid.

5.4.1. 1-(2,4,6-Tris(methoxymethyl)phenyl)-3-(4-(9-fluorononyloxy)phenyl)prop-2-en-1-one 3a. Yield (88%). ^{1}H NMR (CD_3CN): δ 1.33–1.43 (m, $5CH_2$), 1.70–1.75 (m, $2CH_2$), 3.32 (s, 6H, OCH_3), 3.46 (s, 3H, OCH_3), 3.99 (t, J = 6.6 Hz, 2H, CH_2), 4.41 (dt, J = 47.5, 6.2 Hz, 2H, CH_2F), 5.10 (s, 4H, CH_2O), 5.18 (s, 2H, CH_2O), 6.52 (s, $2H_{ar}$), 6.83 (d, J = 16.2 Hz, 1H, HC═C), 6.91 (d, J = 8.8 Hz, $2H_{ar}$), 7.27 (d, J = 16.2 Hz, 1H, Hβ), 7.52 (d, J = 8.8 Hz, $2H_{ar}$). ^{13}C NMR (CD_3CN): δ 26.0 (d, J = 5 Hz, CH_2), 26.8, 30.0, 30.1, 30.1, 30.3 ($5CH_2$), 31.3 (d, J = 19 Hz, CH_2), 56.8 ($2OCH_3$), 56.8 (OCH_3), 69.3 (CH_2), 85.3 (d, J = 164 Hz, CH_2), 95.7 ($2OCH_2$), 95.7 (OCH_2), 98.2 ($2CH_{ar}$), 116.1 ($2CH_{ar}$), 116.2 (C_{ar}), 127.8 (HC═C), 128.2 (C_{ar}), 131.4 ($2CH_{ar}$), 146.3 (CHβ), 156.7 (C_{ar}), 160.5 ($2C_{ar}$), 162.5 (C_{ar}), 194.8 (C═O). MS: (ES+): 549.37.

5.4.2. 3-(4-(10-Fluorodecyloxy)phenyl)-1-(2,4,6-tris(methoxymethy)phenyl)prop-2-enone 3b. Yield (81%). ^{1}H NMR (CD_3CN): δ 1.38–1.44 (m, $6CH_2$), 1.60–1.75 (m, $2CH_2$), 3.32 (s, 6H, OCH_3), 3.46 (s, 3H, OCH_3), 3.99 (t, J = 6.8, 2H, CH_2), 4.41 (dt, J = 47.6, 6.0 Hz, 2H, CH_2F), 5.09 (s, 4H, CH_2O), 5.18 (s, 2H, CH_2O), 6.53 (s, $2H_{ar}$), 6.83 (d, J = 16.2 Hz, 1H, HC═C), 6.90 (d, J = 8.8 Hz, $2H_{ar}$), 7.27 (d, J = 16.2 Hz, 1H, C═CHβ), 7.52 (d, J = 8.8 Hz, $2H_{ar}$). ^{13}C NMR (CD_3CN): δ 26.51 (d, J = 6Hz, CH_2), 27.2, 30.4, 30.5, 30.6, 30.8, 30.8 ($6CH_2$), 31.7 (d, J = 19 Hz, CH_2), 57.3 ($2OCH_3$), 57.3 (OCH_3), 69.7 (CH_2), 85.8 (d, J = 162 Hz, CH_2F), 96.1 ($2CH_2O$), 96.2 (CH_2O), 98.6 ($2CH_{ar}$), 116.5 ($2CH_{ar}$), 116.6 (C_{ar}), 128.2 (HC═C), 128.6 (C_{ar}), 131.8 ($2CH_{ar}$), 146.7 (CHβ), 157.1

(C_{ar}), 161.0 ($2C_{ar}$), 162.9 (C_{ar}), 195.27 (C=O). MS: (ES+): 563.38.

5.4.3. 1-(2,4,6-Tris(methoxymethoxy)phenyl)-3-(4-bromophenyl)prop-2-enone 3c. Yield (63%). ^{1}H NMR (CD_3CN): δ 3.36 (s, 6H, OCH_3), 3.49 (s, 3H, OCH_3), 5.13 (s, 4H, OCH_2), 5.23 (s, 2H, OCH_2), 6.56 (s, $2H_{ar}$), 7.01 (d, J = 16.2 Hz, 1H, HC=C), 7.34 (d, J = 16.2 Hz, 1H, C=CHβ), 7.55 (d, J = 8.6 Hz, $2H_{ar}$), 7.6 (d, J = 8.6 Hz, $2H_{ar}$). ^{13}C NMR (CD_3CN): δ 56.4 ($2OCH_3$), 56.3 (OCH_3), 95.4 ($2OCH_2$), 95.6 (OCH_2), 98.0 ($2CH_{ar}$), 115.6 (C_{ar}), 125.1 (C_{ar}), 130.5 (HC=C), 131.1 ($2CH_{ar}$), 133.1 ($2CH_{ar}$), 135.0 (C_{ar}), 144.4 (CHβ), 156.6 ($2C_{ar}$), 160.6 (C_{ar}), 194.6 (C=O). MS: (ES+): 467.20/470.22.

5.4.4. 1-(2,4,6-Tris(methoxymethoxy)phenyl)-3-(3-bromophenyl)prop-2-enone 3d. Yield (63%). ^{1}H NMR ($CDCl_3$): δ 3.36 (s, 6H, OCH_3), 3.49 (s, 3H, OCH_3), 5.15 (s, 4H, OCH_2), 5.23 (s, 2H, OCH_2), 6.56 (s, $2H_{ar}$), 7.02 (d, J = 8.5 Hz, 1H, CHα), 7.33 (d, J = 8.5 Hz, 1H, CHβ), 7.36 (t, J = 7.6 Hz, $1H_{ar}$), 7.60 (t, J = 6.8 Hz, $1H_{ar}$), 7.65 (t, J = 6.8 Hz, $1H_{ar}$), 7.84 (s, $1H_{ar}$). ^{13}C NMR (CD_3CN): δ 56.3 ($2OCH_3$), 56.4 (OCH_3), 95.4 (OCH_2), 95.6 ($2OCH_2$), 97.9 ($2CH_{ar}$), 115.03 (C_{ar}), 123.85 (C_{ar}), 128.0 (CH_{ar}), 131.0 (CHα), 131.6 (CH_{ar}), 131.9 (CH_{ar}), 134.2 (CH_{ar}), 138.1 (C_{ar}), 145.0 (CHβ), 157.1 ($2C_{ar}$), 161.2 (C_{ar}), 196.3 (C=O). MS: (ES+): 467.19/469.19.

5.4.5. 1-(2,4,6-Tris(methoxymethoxy)phenyl)-3-(4-iodophenyl)prop-2-enone 3e. Yield (57%). ^{1}H NMR (DMSO-d_6): δ 3.28 (s, 6H, OCH_3), 3.43 (s, 3H, OCH_3), 5.15 (s, 4H, OCH_2), 5.22 (s, 2H, OCH_2), 6.54 (s, $2H_{ar}$), 7.07 (d, J = 16.2 Hz, 1H, CHα), 7.24 (d, J = 16.2 Hz, 1H, CHβ), 7.50 (d, J = 8.4 Hz, $2H_{ar}$), 7.78 (d, J = 8.4 Hz, $2H_{ar}$). ^{13}C NMR (DMSO-d_6): δ 55.8 ($2OCH_3$), 55.9 (OCH_3), 94.1 ($2OCH_2$), 94.0 (OCH_2), 96.6 ($2CH_{ar}$), 97.66 (C_{ar}), 114.2 (C_{ar}), 129.3 (CHα), 130.3 ($2CH_{ar}$), 133.8 ($2CH_{ar}$), 137.7 (C_{ar}) 143.2 (CHβ), 155.1 ($2C_{ar}$), 158.9 (C_{ar}), 193.1 (C=O). MS: (ES+): 515.15.

5.5. 1-(2-Hydroxy-4,6-di-*O*-methoxymethylphenyl)-5-phenyl-1,3-pent-4-enedione 3f

A solution of the ester **2f** (4.05 g, 25 mmol) in anhydrous THF (10 mL) was added to a solution of NaH (60% in mineral oil 1.6 g, 40 mmol) in anhydrous THF (10 mL) under nitrogen. After stirring under reflux for 15 min, the mixture was treated dropwise with a solution of 2-hydroxy-4,6-di-*O*-methoxymethylacetophenone **1a** (229 mg, 10 mmol) in anhydrous THF (15 mL) over 30 min. The mixture was heated to reflux and monitored by TLC until the reaction was complete (4 h). The cooled mixture was hydrolyzed with H_2O (200 mL), then acidified with HCl diluted and extracted with CH_2Cl_2. The organic phase was dried and evaporated to dryness. The diketone **3f** was recrystallized from methanol in 72% yield.

The enol derivative: ^{1}H NMR ($CDCl_3$): δ 3.52 (s, CH_3), 3.60 (s, CH_3), 5.22 (s, CH_2), 5.33 (s, CH_2), 6.32 (d, J = 2.4 Hz, H-3), 6.34 (d, J = 2.4 Hz, H-5), 6.58 (d, J = 15.6 Hz, Hβ), 6.81 (s, CHenol), 7.41–7.45 (m, $3H_{ar}$), 7.58–7.69 (m, 3H, Hα, $2H_{ar}$), 13.24 (s, OH), 13.50 (s, OH). ^{13}C NMR ($CDCl_3$): 56.2, 56.5 ($2CH_3$), 93.9 (CH_2), 94.9 (CHenol), 94.9 (CH_2), 97.6, 102.9 (C-3, C-5), 106.8 (C_{ar}), 122.8 (Cα), 127.7 (C-2′, C-6′), 128.7 (C-3′, C-5′), 130.7 (C-4′), 135.1 (C_{ar}), 138.6 (Cβ), 159.1 (C_{ar}), 162.8 (C_{ar}), 166.2 (C_{ar}), 173.4 (C_{ar}), 193.9 (C_{ar}).

5.6. 2-(Substituted-phenyl)-2,3-dihydro-5,7-dihydroxychromen-4-ones 4a–e. General method

To the substituted 1-(2,4,6-Tris(methoxymethyl)phenyl)-3-(substituted-phenyl)prop-2-enone **3a**–**e** (0.0032 mol) in MeOH (60 mL) was added 10% HCl (6 mL) and the mixture was refluxed for 1 h. Then NaOAc (5.3 g, 0.064 mol) was added and then the resulting mixture was refluxed for 3 h. The mixture was cooled and H_2O (50 mL) added and extracted with EtOAc (50 mL × 2), dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The solid was purified by column chromatography (silica gel, ethyl acetate–hexane: 30/70) to afford a white solid.

5.6.1. 2-(4-(9-Fluorononyloxy)phenyl)-2,3-dihydro-5,7-dihydroxychromen-4-one 4a. Yield 55%. ^{1}H NMR (DMSO-d_6): δ 1.3–1.41 (m, $5CH_2$), 1.57–1.71 (m, $2CH_2$), 2.71 (dd, J = 17.1, 3.1 Hz, 1H, H-3), 3.25 (dd, J = 17.1, 12.6 Hz, 1H, H-3), 3.97 (t, J = 6.6 Hz, CH_2), 4.42 (dt, J = 44.6, 6.1 Hz, CH_2F), 5.50 (dd, J = 12.7, 3.0 Hz, 1H, H-2), 5.89 (s, $1H_{ar}$), 5.89 (s, $1H_{ar}$) 6.95 (d, J = 8.7 Hz, $2H_{ar}$), 7.41 (d, J = 8.7 Hz, $2H_{ar}$), 10.78 (s, OH). ^{13}C NMR (DMSO-d_6): 24.5 (d, J = 5 Hz, CH_2), 25.3, 28.4, 28.5, 28.5, 28.7 ($5CH_2$), 29.7 (d, J = 19 Hz, CH_2), 41.9 (C-3), 67.4 (CH_2), 78.1 (C-2), 83.7 (d, J = 162 Hz, CH_2F), 94.9 (CH_{ar}), 95.7 (CH_{ar}), 101.7 (C_{ar}), 114.2 ($2CH_{ar}$), 128.1 ($2CH_{ar}$), 130.3 (C_{ar}), 158.7 (C_{ar}), 162.7 (C_{ar}), 163.4 (C_{ar}), 166.5 (C_{ar}), 196.1 (CO). MS: (ES+): 431.3.

5.6.2. 2-(4-(10-Fluorodecyloxy)phenyl)-2,3-dihydro-5,7-dihydroxychromen-4-one 4b. Yield 50%. ^{1}H NMR (DMSO-d_6): δ 1.22–1.45 (m, $6CH_2$), 1.56–1.71 (m, $2CH_2$), 2.71 (dd, J = 17.2, 3.2 Hz, 1H, H-3), 3.26 (dd, J = 17.2, 12.6 Hz, 1H, H-3), 3.97 (t, J = 6.3 Hz, CH_2), 4.41 (dt, J = 47.6, 6.1 Hz, CH_2F), 5.50 (dd, J = 12.6, 2.9 Hz, H-2), 5.89 (s, $1H_{ar}$), 5.89 (s, $1H_{ar}$), 6.95 (d, J = 8.7 Hz, $2H_{ar}$), 7.41 (d, J = 8.7 Hz, $2H_{ar}$), 10.78 (s, OH). ^{13}C NMR (DMSO-d_6): δ 24.6 (d, J = 5 Hz, CH_2), 25.4, 28.6, 28.6, 28.7, 28.8, 28.8 ($6CH_2$), 29.8 (d, J = 19 Hz, CH_2), 42.0 (C-3), 67.5 (CH_2), 78.2 (C-2), 83.7 (d, J = 162 Hz, CH_2F), 95.0 (CH_{ar}), 95.8 (CH_{ar}), 101.8 (C_{ar}), 114.3 ($2CH_{ar}$), 128.2 ($2CH_{ar}$), 130.4 (C_{ar}), 158.9 (C_{ar}), 162.8 (C_{ar}), 163.4 (C_{ar}), 166.6 (C_{ar}), 196.2 (CO). MS: (ES+): 417.3.

5.6.3. 2-(4-Bromophenyl)-2,3-dihydro-5,7-dihydroxychromen-4-one 4c. Yield 55%. ^{1}H NMR (DMSO-d_6): δ 2.78 (dd, J = 17.1, 3.2 Hz, 1H, H-3), 3.21 (dd, J = 17.1, 12.6 Hz, 1H, H-3), 5.57 (dd, J = 12.6, 3.1 Hz, 1H, H-2), 5.90 (s, $1H_{ar}$), 5.92 (s, $1H_{ar}$), 7.48 (d, J = 8.5 Hz, $2H_{ar}$), 7.63 (d, J = 8.5 Hz, $2H_{ar}$), 10.83 (br s, OH). ^{13}C NMR (DMSO-d_6): δ 41.8 (CH_2-3), 77.5 (CH-2), 94.9 (CH_{ar}), 95.9 (CH_{ar}), 101.7 (C_{ar}), 121.6 (C–Br), 128.7 ($2CH_{ar}$), 131.4 ($2CH_{ar}$), 138.0 (C_{ar}), 162.4 (C_{ar}), 163.4 (C_{ar}), 166.6 (C_{ar}), 195.6 (CO). MS: (ES−): 335.1/336.1.

5.6.4. 2-(3-Bromophenyl)-2,3-dihydro-5,7-dihydroxychromen-4-one 4d. Yield 50%. ^{1}H NMR (DMSO-d_6): δ 2.79 (dd, J = 17.1, 12.7 Hz, 1H, H-3), 3.26 (dd, J = 17.1, 3.2 Hz, 1H, H-3), 5.59 (dd, J = 12.7, 3.2 Hz, 1H, H-2), 5.91 (d, J = 2.1 Hz, $1H_{ar}$), 5.95 (d, J = 2.1Hz, $1H_{ar}$), 7.39 (t, J = 7.9 Hz, $1H_{ar}$), 7.52 (d, J = 7.9, $1H_{ar}$), 7.58 (d, J = 8.0 Hz, $1H_{ar}$), 7.74 (s, $1H_{ar}$), 10.85 (br s, OH). ^{13}C NMR (DMSO-d_6): δ 41.8 (C-3), 77.4 (C-2), 95.0 (CH_{ar}), 96.0 (CH_{ar}), 101.6 (C_{ar}), 121.7 (C–Br), 125.5 (CH_{ar}), 129.2 (CH_{ar}), 130.7 (CH_{ar}), 131.3 (CH_{ar}), 141.3 (C_{ar}), 162.3 (C_{ar}), 162.4 (C_{ar}), 166.6 (C_{ar}), 195.5 (CO). MS: (ES−): 335.1/336.1.

5.6.5. 2-(4-Iodophenyl)-2,3-dihydro-5,7-dihydroxychromen-4-one 4e. Yield 61%. ^{1}H NMR (DMSO-d_6): δ 2.78 (dd, J = 17.1, 3.2 Hz, 1H, H-3), 3.21 (dd, J = 17.2, 12.5 Hz, H1, H-3), 5.57 (dd, J = 12.5, 3.2 Hz, 1H, H-2), 5.90 (s, $1H_{ar}$), 5.92 (s, $1H_{ar}$), 7.33 (d, J = 8.4 Hz, $2H_{ar}$), 7.80 (d, J = 8.4 Hz, $2H_{ar}$), 10.84 (br s, OH). ^{13}C NMR (DMSO-d_6): δ 41.9 (C-3), 77.7 (C-2), 94.7 (CH_{ar}), 95.0 (CH_{ar}), 96.0 (C_{ar}), 101.7 (C_{ar}), 128.8 ($2CH_{ar}$), 137.3 ($2CH_{ar}$), 138.5 (C_{ar}), 162.5 (C_{ar}), 163.4 (C_{ar}), 166.7 (C_{ar}), 195.7 (CO). MS: (ES−): 381.1.

5.7. 4′-Substituted-5,7-dihydroxyflavones 5a–e. General method

To a solution of 2-(substituted-phenyl)-2,3-dihydro-5,7-dihydroxychromen-4-one **4a**–**e** (0.02 mol) in anhydrous pyridine (4 mL) was added iodine (0.53 g, 0.0021 mol) under N_2 and heated to reflux for 4 h. The solution was then cooled and diluted with H_2O (50 mL) and was extracted with ethyl acetate (100 mL × 3). The organic layer was washed with 10% HCl (50 mL × 2), dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The solid was purified by column chromatography (silica gel, ethyl acetate–hexane: 30/70) to afford a pale white solid.

5.7.1. 4′-(9-Fluorononyloxy)-5,7-dihydroxyflavone 5a. Yield 54%. ^{1}H NMR (DMSO-d_6): δ 1.30 (m, $5CH_2$), 1.57–1.71 (m, $2CH_2$), 4.03 (t, J = 6.5 Hz, CH_2), 4.41 (dt, J = 47.6 Hz, J = 6.1 Hz, CH_2F), 6.19 (d, J = 1.4 Hz, H-6), 6.48 (d, J = 1.4 Hz, H-8), 6.82 (s, H-3), 7.07 (d, J = 8.9 Hz, 2H, H-2′, 6′), 7.98 (d, J = 8.9 Hz, 2H, H-3′, 5′), 10.83 (br s, 7-OH), 12.90 (s, 5-OH). ^{13}C NMR (DMSO-d_6): 24.6 (d, J = 5 Hz, CH_2), 25.4, 28.5, 28.5, 28.7, 28.9 ($5CH_2$), 29.8 (d, J = 19 Hz, CH_2), 67.8 (CH_2), 83.8 (d, J = 162 Hz, CH_2F), 94.0 (C-8), 98.9 (C-6), 103.4, 103.7 (C-3, C-4a), 114.9 (C-3′, C-5′), 122.6 (C-1′), 128.2 (C-2′-C-6′), 157.3 (C-4′), 161.4, 161.7, 163.3, 164.2 (C-8a, C-5, C-2, C-7,), 181.7 (C-4). MS/EI m/z 413 [M^+−1, 100%], 399 [16%], 395 [9%], 333 [10%], 314, 312 [13%].

5.7.2. 4′-(10-Fluorodecyloxy)-5,7-dihydroxyflavone 5b. Yield 50%. ^{1}H NMR (DMSO-d_6): δ 1.29–1.41 (m, $6CH_2$), 1.56 (m, CH_2), 1.75 (m, CH_2), 3.37 (t, J = 6.4 Hz, CH_2), 4.04 (dt, J = 47.6 Hz, J = 6.2 Hz, CH_2F), 6.20 (d, J = 1.4 Hz, H-6), 6.50 (d, J = 1.4 Hz, H-8), 6.85 (s, H-3), 7.76 (d, J = 8.7 Hz, 2H, H-2′, 6′), 8.00 (d, J = 8.7 Hz, 2H, H-3′, 5′), 10.90 (br s, 7-OH), 12.90 (s, 5-OH). ^{13}C NMR (DMSO-d_6): 27.1, 30.2, 30.6, 34.2 ($8CH_2$), 64.2, 69.6 ($2CH_2$), 95.7 (C-8), 100.6 (C-6), 105.1, 105.4 (C-3, C-4a), 116.7 (C-3′, C-5′), 124.3 (C-1′), 130.0 (C-2′–C-6′), 159.0, 163.1, 163.3, 165.0, 166.0 (C-8a, C-5, C-2, C-7, C-4′), 183.4 (C-4). MS: (ES+): 429.42.

5.7.3. 4′-Bromo-5,7-dihydroxyflavone 5c. Yield 47%. ^{1}H NMR (DMSO-d_6): δ 6.21 (s, H-6), 6.51 (s, H-8), 6.99 (s, H-3), 7.76 (d, J = 8.6 Hz, H-3′, 5′), 8.00 (d, J = 8.6 Hz, H-2′, 6′), 10.95 (br s, 7-OH), 12.75 (s, 5-OH). ^{13}C NMR (DMSO-d_6): 94.1 (C-8), 99.1 (C-6), 104.0 (C-3), 105.5 (C-4a), 125.7 (C–Br), 128.3 (C-2′, C-6′), 129.9 (C-1′), 132.1 (C-3′, C-5′), 157.3, 161.4, 162.0, 164.5 (C-8a, C-5, C-2, C-7), 181.8 (C-4). MS: (ES−): 333.15/334.16.

5.7.4. 3′-Bromo-5,7-dihydroxyflavone 5d. Yield 50%. ^{1}H NMR (DMSO-d_6): δ 6.23 (s, H-6), 6.57 (s, H-8), 7.06 (s, H-3), 7.52 (m, H-5′), 7.80 (d, J = 8.1 Hz, H-6′), 8.08 (d, J = 7.8 Hz, H-4′), 8.27 (s, H-2′), 10.94 (s, 7-OH), 12.75 (s, 5-OH). ^{13}C NMR (DMSO-d_6): 94.2 (C-8), 99.5 (C-6), 103.4 (C-4a), 110.3 (C-3), 121.0 (C–Br), 128.2, 131.4, 132.7, 133.1 (C-2′, C-4′, C-5′, C-6′), 133.5 (C-1′), 157.8, 161.5, 163.8, 165.9 (C-8a, C-5, C-2, C-7), 181.2 (C-4). MS: (ES−): 333.17/334.18.

5.7.5. 4′-Iodo-5,7-dihydroxyflavone 5e. Yield 44%. ^{1}H NMR (DMSO-d_6): δ 6.21 (d, J = 1.8 Hz, H-6), 6.49 (d, J = 1.8 Hz, H-8), 6.97 (s, H-3), 7.82 (d, J = 8.6 Hz, 2H, H-3′, 5′), 7.93 (d, J = 8.6 Hz, 2H, H-2′, 6′), 10.92 (br s, 7-OH). ^{13}C NMR (DMSO-d_6): 94.1 (C-8), 99.0 (C-6), 99.8 (C–I), 104.0 (C-4a), 105.4 (C-3), 128.1 (C-2′, C-4′), 130.2 (C-1′), 138.0 (C-5′, C-6′), 157.4, 161.4, 162.3, 164.5 (C-8a, C-5, C-2, C-7), 181.7 (C-4). MS: (ES−): 379.12.

5.8. 5,7-Dihydroxy-2-styrylchromen-4-one 5f

A mixture of the diketone **3f** (1.15 g, 3 mmol) and amberlyst-15 resin (750 mg) in propan-2-ol (15 mL) was stirred under reflux, whilst the reaction was monitored by TLC until completion (4 h). After cooling, the mixture was diluted with propan-2-ol (40 mL), filtered, evaporated under vacuum, and the crude product was purified by column chromatography (silica gel, ethyl acetate–hexane: 30/70) to afford white/yellow crystals of the flavone **5f** (yield 82%).

^{1}H NMR (DMSO-d_6): δ 6.15 (s, 1H, H-3), 6.32 (d, J = 1.8 Hz, 1H, H-6), 6.45 (d, J = 1.8 Hz, 1H, H-8), 6.74 (d, J = 14.6 Hz, 1H, Hβ), 7.40–7.61 (m, 6H, $5H_{ar}$, Hα), 9.95 (br s, 1H, 7-OH), 12.75 (s, 1H, 5-OH). ^{13}C NMR (DMSO-d_6): 93.7 (C-8), 99.0 (C-6), 104.4 (C-4a), 108.2 (C-3), 119.3 (Cβ), 127.2 (C-3′, C-5′), 128.5 (C-2′-C-6′), 129.4 (C-4′), 134.4 (C-1′), 136.6 (Cα), 157.2, 161.5, 161.7, 164.1, 181.8 (C-8a, C-5, C-2, C-7, C-4). MS (EI) m/z 280 [M^+, 100%], 262 (19%), 153 (21%), 127 (16%), 124 (20%), 96 (12%).

5.9. Biological evaluation: in vitro testing

5.9.1. Cell lines and culture conditions. The human GLC4 and GLC4/Adr small cell lung cancer cell lines have

been previously characterized[23,24] and were kindly provided by Dr. H. J. Broxterman (Vrije Universiteit Medical Centre, Amsterdam, The Netherlands) and maintained in an exponential growth in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin G (100 U/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere of 6% CO_2. The GLC4/Adr cell line was cultured in the presence of 1 μM doxorubicin. Before use in cytotoxic experiments GLC4/Adr cells were cultured in a doxorubicin-free medium for 11 days.

The parental Swiss mouse embryo NIH3T3 cell line and resistant hMDR1-NIH3T3 cell line transfected with the human MDR1 were provided by Dr. M. M. Gottesman (National Cancer Institute, Bethesda, MD). Both cell lines were maintained in Dulbecco's modified Eagle's medium with 4.5 g of glucose per liter supplemented with 10% FCS, penicillin G (50 U/mL), streptomycin (50 μg/mL), and sodium pyruvate 1 mM at 37 °C in a humidified atmosphere of 6% CO_2. The resistant cell line was cultured in the presence of 60 ng/mL colchicine.

5.9.2. Cell survival assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) was used to determine the cytotoxicity of each compound in 96-well plates.[25] This assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product that can be determined spectrophotometrically. The wells situated at the periphery of the microtiter plates are more subjected to evaporation and were not used for the cytotoxic MTT assay but filled with 200 μL PBS. For each cell line, equal numbers of cells were seeded into each well in a volume of 200 μL of their respective culture media (800 cells for GLC4; 5000 cells for GLC4/Adr; 200 cells for NIH3T3; 300 cells for hMDR1-NIH3T3). After 12 h incubation (37 °C in a humidified atmosphere with 6% CO_2), a dose–response curve was generated for each compound tested by adding 20 μL of compound stock solution to each well and incubated for a further 72 h. Nine compound concentrations were used to determine a dose–response curve. At the end of drug exposure, 20 μL of MTT (0.5 mg/mL final concentration) was added to each well and the plates were put back in the incubator for an additional 4 h. Thereafter, the microtiter plates were centrifuged (45 min at 600*g*) and the culture medium was removed by aspiration. To solubilize the resulting formazan crystals, 200 μL of dimethylsulfoxide (DMSO) was added in each well and the plates were incubated at 23 °C on a plate shaker for 1 h. Then the absorbance at 540 nm was determined on a scanning microplate reader (Labsystems Multiskan Bichromatic). The mean absorbance of six wells for each compound concentration was measured. IC_{50} was defined as being the compound concentration that reduced this absorbance to 50% of control values. IC_{50} values were derived by non-linear regression analysis assuming a sigmoidal dose–response curve using SigmaPlot 2001 V.7.101 software. Similarly, to investigate the ability of the various compounds to chemosensitize MDR cells, each compound was added at a concentration that produces less than 5% cytoxicity and tested in combination with several concentrations of either doxorubicin or colchicine, the choice of the drug depending on the cell line. Flavone derivatives were dissolved at 10 mM in DMSO and dilutions prepared in culture medium. Final concentration of DMSO was lower than 1% and without effect on cell growth. To examine the effects on drug sensitivity of either cyclosporin A (CsA) or MK571, cells were preincubated with CsA (10 μM) or MK571 (10 μM) for 1 h and then incubated with various concentrations of drugs. Data represent means of six replicate determinations and standard deviation from at least two independent experiments.

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