European Journal of Medicinal Chemistry 67 (2013) 115–126

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of flavones and benzoflavones as inhibitors of BCRP/ABCG2

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ARTICLE INFO

Article history:
Received 31 October 2012
Received in revised form
12 June 2013
Accepted 13 June 2013
Available online 25 June 2013

Keywords:
Flavonoids
Benzoflavones
Breast cancer resistance protein (BCRP)
P-gp
MRP1
Multidrug resistance (MDR)

ABSTRACT

Multidrug resistance (MDR) often leads to a failure of cancer chemotherapy. Breast Cancer Resistance Protein (BCRP/ABCG2), a member of the superfamily of ATP binding cassette proteins has been found to confer MDR in cancer cells by transporting molecules with amphiphilic character out of the cells using energy from ATP hydrolysis. Inhibiting BCRP can be a solution to overcome MDR. We synthesized a series of flavones, 7,8-benzoflavones and 5,6-benzoflavones with varying substituents at positions 3, 3′ and 4′ of the (benzo)flavone structure. All synthesized compounds were tested for BCRP inhibition in Hoechst 33342 and pheophorbide A accumulation assays using MDCK cells expressing BCRP. All the compounds were further screened for their P-glycoprotein (P-gp) and Multidrug resistance-associated protein 1 (MRP1) inhibitory activity by calcein AM accumulation assay to check the selectivity towards BCRP. In addition most active compounds were investigated for their cytotoxicity. It was observed that in most cases 7,8-benzoflavones are more potent in comparison to the 5,6-benzoflavones. In general it was found that presence of a 3-OCH_3 substituent leads to increase in activity in comparison to presence of OH or no substitution at position 3. Also, it was found that presence of 3′,4′-OCH_3 on phenyl ring lead to increase in activity as compared to other substituents. Compound **24**, a 7,8-benzoflavone derivative was found to be most potent being 50 times selective for BCRP and showing very low cytotoxicity at higher concentrations.

1. Introduction

Cancer is the main cause of millions of deaths worldwide. Since decades chemotherapy has been a major form of the treatment for different types of cancers. Unfortunately, the majority of cancers are either resistant to chemotherapy or acquire multidrug resistance (MDR) during treatment. MDR is an acquired drug resistance by tumour cells that consists of simultaneous emergence of cellular resistance to toxic action of chemotherapeutic drugs originally used and to other chemicals having different chemical structure and mechanism of action. As a result of MDR, chemotherapeutic agents fail to target the tumour cells and cancer becomes untreatable by chemotherapy [1,2].

One of the mechanisms by which tumours develop multidrug resistance is over expression of efflux transport proteins, especially certain ATP-binding cassette (ABC) transporters in the plasma membrane of cancer cells. ABC transporters utilize energy obtained from hydrolysis of ATP to efflux chemically unrelated compounds [3–5]. Until now 48 ABC transporters have been identified in humans.

P-glycoprotein (P-gp) and the multidrug resistance associated protein 1 (MRP1) belonging to subfamily ABCC have been shown to confer resistance to a broad spectrum of chemotherapeutic agents. More recently BCRP (breast cancer resistance protein) which is also known as ABCG2 has been discovered and proved to cause resistance in tumour cells too.

Although BCRP was first cloned in doxorubicin resistant MCF-7 breast cancer cells [6], later it has been also found to confer resistance to mitoxantrone (hence named MXR) [7] and subsequently it was found in the placenta (hence named ABCP) [8]. BCRP is an ABC transporter protein consisting of 655 amino acids and having a molecular weight of 72 kDa. It is a half transporter containing a single NH_2-terminal nucleotide binding domain (NBD) followed by six transmembrane domains (TMD). It has been proposed that, to achieve its functionality, BCRP needs to be homo- or hetero-dimerized [6,7]. BCRP has been found to be present in many normal tissues other than in tumour cells, including apical membrane of placental syncytiotrophoblasts, endocrine cells of the pancreas, epithelial cells in the small intestine and colon, liver caniculi, blood–brain and blood–testis barrier, gallbladder epithelium and

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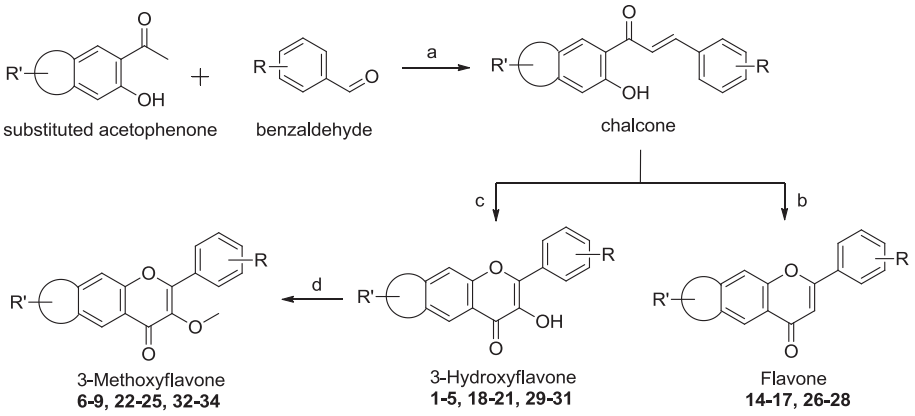
http://dx.doi.org/10.1016/j.ejmech.2013.06.035



Scheme 1. General scheme for the synthesis of target compounds. Reagents and conditions: a) EtOH, NaOH or LiOH, rt, 5–72 h; b) DMSO, I_2, reflux, 7–8 h; c) EtOH, 25% H_2O_2, NaOH, rt, 24–48 h; d) acetone, CH_3I, K_2CO_3, reflux, 24 h.

stem cells [8–10]. In normal tissues BCRP protects the body from various endogenous and exogenous toxins.

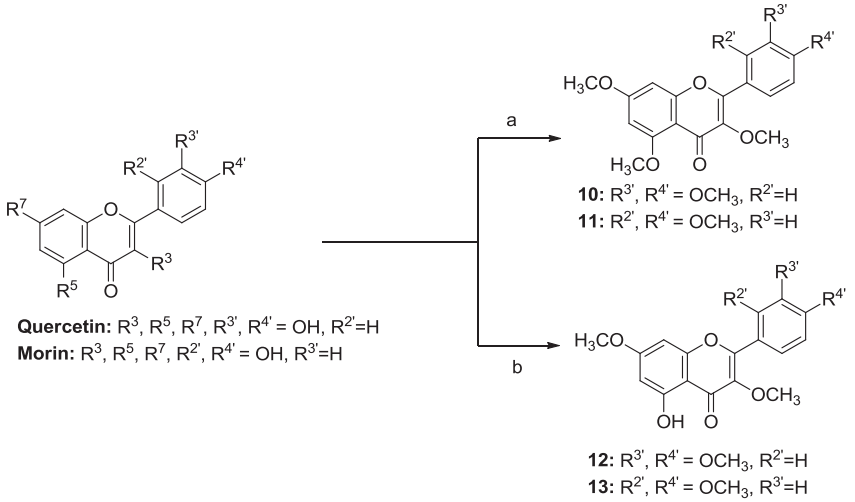
Several substrates of BCRP have been identified, which include anticancer drugs such as mitoxantrone [11], podophyllotoxins etoposid and teniposid, flavopiridol [12], topotecan, irinotecan [13] and its active metabolite SN-38. To overcome MDR acquired by over expression of BCRP several efforts have been made by investigating inhibitors of BCRP. These structurally unrelated compounds include naturally occurring flavonoids [14–17], broad spectrum inhibitors like tariquidar [18] and elacridar, chromons [19] and methoxystilbenes [20]. Several research groups have investigated chalcones (precursor of flavonoids) for their inhibitory effect [21–24]. Fumitremorgin C was found to be a very potent inhibitor of BCRP [13], but its use was limited due to neurotoxicity. Its non-toxic analogue Ko143 is the most potent and selective inhibitor of BCRP known today.

Recently a flavonoid, the 7,8-benzoflavone was identified as very potent inhibitor of BCRP [15,25]. To further investigate benzoflavones for their potential to inhibit BCRP, we synthesized several 7,8- and 5,6-benzoflavones bearing varying substitutions at positions 3, 3′ and 4′. Also several substituted synthetic flavones were investigated to allow comparison of their inhibitory potential with that of the benzoflavones. These compounds were checked for their inhibitory effect on BCRP in Hoechst 33342 and pheophorbide A accumulation assays. The compounds were also investigated for their effect on P-gp and MRP1. Compound **24**, a 7,8-benzoflavone bearing three methoxy substituents at positions 3, 3′,4′ was found to be most potent having an inhibitory concentration only about 2 fold less than Ko143 which is the most potent specific BCRP inhibitor [26], while being almost 3 fold more potent than the unsubstituted 7,8-benzoflavone.

2. Result and discussion

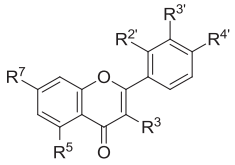
2.1. Chemistry

In the current study we synthesized and investigated several flavones (**1**–**13**), 7,8-benzoflavones (**14**–**25**) and 5,6-benzoflavones (**26**–**34**). Flavones (**1**–**9**) and both type of benzoflavones were synthesized from their precursor chalcones and benzochalcones. The precursors were synthesized by Claisen–Schmidt condensation using substituted acetophenones and benzaldehydes in presence of NaOH or LiOH as base in ethanol as reported earlier [23]. The synthesis of the studied compounds is depicted in Scheme 1 and Scheme 2. Synthesis of benzoflavones occurred via one of two routes. The first route involved cyclization of a precursor benzochalcone to the corresponding benzoflavone in presence of iodine in DMSO to give benzoflavones with no substituent at



Scheme 2. General scheme for the synthesis of compounds **10**–**13**. Reagents and conditions: a) acetone, CH_3I, K_2CO_3, reflux, 24 h; b) acetone/water (2:1), dimethyl sulphate, KOH, reflux, 3 h.

Table 1
Synthesized flavonoids and their inhibitory potencies against MDCK BCRP cells in Hoechst 33342 assay and pheophorbide A assay. Data are expressed as mean ± SD ($n = 3$).



Flavons (**1-13**)

Compound	R^3	R^5	R^7	$R^{2'}$	$R^{3'}$	$R^{4'}$	Hoechst 33342 IC_{50} ± SD (μM)	Pheophorbide A IC_{50} ± SD (μM)
1	OH	H	H	H	H	H	n.e.	n.e.
2	OH	H	H	H	H	OCH_3	n.e.	n.e.
3	OH	H	OCH_3	H	H	OCH_3	n.e.	n.e.
4	OH	H	OCH_3	H	OCH_3	OCH_3	1.01 ± 0.10	1.77 ± 0.14
5	OH	OCH_3	OCH_3	H	H	OCH_3	12.4 ± 0.47	8.95 ± 0.65
6	OCH_3	H	H	H	H	H	1.21 ± 0.12	1.28 ± 0.17
7	OCH_3	H	H	H	H	OCH_3	7.74 ± 0.35	6.50 ± 0.58
8	OCH_3	H	H	H	OCH_3	OCH_3	1.18 ± 0.29	1.34 ± 0.19
9	OCH_3	H	OCH_3	H	OCH_3	OCH_3	1.18 ± 0.18	2.14 ± 0.31
10	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	0.822 ± 0.169	1.88 ± 0.24
11	OCH_3	OCH_3	OCH_3	OCH_3	H	OCH_3	5.98 ± 0.45	5.94 ± 0.74
12	OCH_3	OH	OCH_3	H	OCH_3	OCH_3	0.540 ± 0.079	0.570 ± 0.093
13	OCH_3	OH	OCH_3	OCH_3	H	OCH_3	3.27 ± 0.15	3.89 ± 0.16

*n.e. = no inhibitory effect up to 10 μM.

position 3. In second route chalcones or benzochalcones were converted to 3-OH (benzo)flavones using the Algar–Flynn–Oyamada reaction [27], by treatment with H_2O_2 and NaOH for 24 h. 3-OH flavones or benzoflavones were further converted to 3-OCH_3 flavones or benzoflavones using methyl iodide and potassium carbonate in acetone. Compounds **12** and **13**, the tetramethoxy derivatives of quercetin and morin were synthesized by a method described earlier and purified by column chromatography [27]. All the synthesized compounds are listed in Tables 1 and 2.

All synthesized compounds were characterized by ^1H NMR, ^{13}C NMR and elemental analysis. Characterization and purity data of precursor benzochalcones have been reported previously [23].

2.2. Biological investigation

The synthesized compounds were investigated for their BCRP inhibition ability by measuring accumulation of Hoechst 33342 and pheophorbide A in MDCK BCRP cells. All compounds were additionally checked for their P-gp and MRP1 inhibition in calcein AM assay. Cytotoxicity of representative most active compounds was assessed by MTT-cytotoxicity assay.

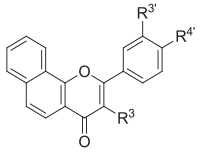
2.2.1. Hoechst 33342 and pheophorbide A assay to determine BCRP inhibition

Hoechst 33342 and pheophorbide A are substrates of BCRP and have been used to determine the effect of BCRP inhibitors on their accumulation in BCRP overexpressing cells. All the compounds were investigated in BCRP overexpressing MDCK BCRP cells. The accumulation of Hoechst 33342 and pheophorbide A in non-BCRP expressing MDCK cells was also determined and was considered as maximum accumulation, as a result of absence of BCRP expression.

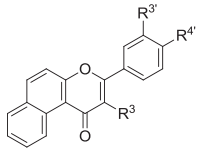
In the current study two types of flavonoids were investigated: A) flavones and B) 7,8- and 5,6-benzoflavones. For the calculation of IC_{50} values of these compounds, concentration response curves were generated from plots of averages of fluorescence values obtained in Hoechst 33342 and pheophorbide A assays against the logarithmic concentrations of the inhibitor. Fig. 1 depicts representative concentration–response curves obtained in the Hoechst 33342 assay for compound **24** compared to the standard Ko143. The inhibitory concentration of all the compounds is given in Tables 1 and 2.

In case of flavones, two types of compounds were investigated 1) those having hydroxy substituents at position 3 of the flavone

Table 2
Synthesized 7,8- and 5,6-benzoflavones and their inhibitory potencies against MDCK BCRP cells in Hoechst 33342 assay and Pheophorbide A assay. Data are expressed as mean ± SD ($n = 3$).



7,8-Benzoflavones (**14-25**)



5,6-benzoflavones (**26-34**)

Compound	R^3	$R^{3'}$	$R^{4'}$	Hoechst 33342 IC_{50} ± SD (μM)	Pheophorbide A IC_{50} ± SD (μM)
14	H	H	H	1.31 ± 0.12	1.40 ± 0.18
15	H	H	OCH_3	1.28 ± 0.07	1.29 ± 0.09
16	H	OCH_3	OCH_3	1.23 ± 0.08	1.13 ± 0.19
17	H	OCH_3	F	0.522 ± 0.083	0.457 ± 0.124
18	OH	H	H	2.89 ± 0.73	1.59 ± 0.14
19	OH	H	OCH_3	6.93 ± 1.58	4.97 ± 0.88
20	OH	OCH_3	OCH_3	0.724 ± 0.049	1.52 ± 0.08
21	OH	OCH_3	F	22.5 ± 4.90	17.9 ± 0.29
22	OCH_3	H	H	2.71 ± 0.41	2.31 ± 0.04
23	OCH_3	H	OCH_3	1.06 ± 0.26	3.07 ± 0.38
24	OCH_3	OCH_3	OCH_3	0.426 ± 0.019	0.468 ± 0.034
25	OCH_3	OCH_3	F	2.44 ± 0.25	4.01 ± 0.77
26	H	H	H	8.32 ± 0.61	6.05 ± 1.62
27	H	H	OCH_3	2.46 ± 0.15	1.69 ± 0.07
28	H	OCH_3	OCH_3	0.590 ± 0.064	0.458 ± 0.053
29	OH	H	H	11.0 ± 2.98	13.6 ± 2.40
30	OH	H	OCH_3	8.93 ± 2.37	6.12 ± 1.54
31	OH	OCH_3	OCH_3	6.12 ± 0.35	4.84 ± 0.99
32	OCH_3	H	H	11.1 ± 1.37	12.5 ± 0.65
33	OCH_3	H	OCH_3	3.07 ± 0.04	8.02 ± 1.39
34	OCH_3	OCH_3	OCH_3	4.27 ± 0.64	5.25 ± 0.67
Ko143	–			0.215 ± 0.017	0.354 ± 0.042

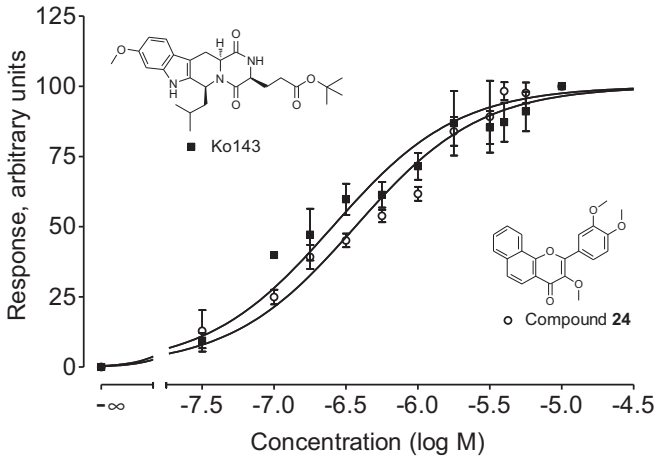


Fig. 1. Representative concentration–response curves of compound **24** (open circles, $IC_{50} = 0.43 \pm 0.02$ μM) and Ko143 (closed squares, $IC_{50} = 0.22 \pm 0.02$ μM) obtained in the Hoechst 33342 assay. Each data point shown represents the average of 3 independent experiments and error bars indicate standard deviation.

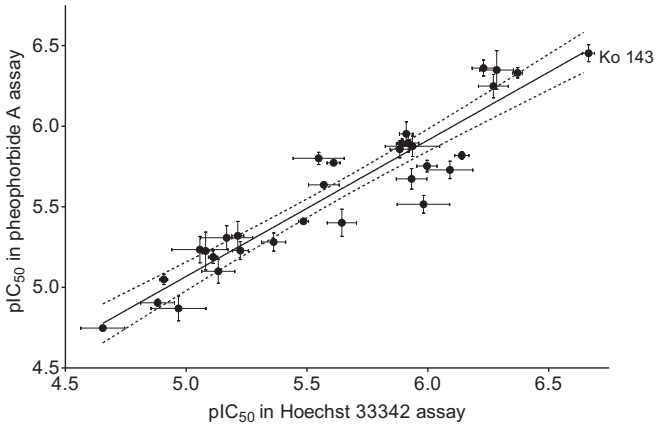


Fig. 2. Scatterplot of pIC_{50} values for ABCG2 inhibition obtained in the Hoechst 33342 assay and the pheophorbide A assay. Each point indicates mean of IC_{50} values obtained in three independent experiments and error bars indicate standard deviation. The squared correlation coefficient is $r^2 = 0.89$.

nucleus (**1**–**5**) and 2) those having methoxy substituents at the same position (**6**–**13**). Generally compounds possessing a 3-OH substituent were very less active or even inactive when compared to compounds bearing a 3-OCH_3 substituent as can be seen from Table 1. But this difference in activity was much smaller for compounds bearing an unsubstituted phenyl ring at position 2 of benzoflavones, as is shown by the negligible differences of IC_{50} values for compounds **18** and **22** or **29** and **32**. The results obtained from our functional assays are in accordance with the structure activity relationship proposed earlier by several investigators [15,16,28]. It was also observed that presence of methoxy groups on side ring of flavones lead to an increase in activity. Presence of 3′,4′-OCH_3 substitution was found to be optimum for BCRP inhibition. The comparison of IC_{50} values for compounds **10**–**13** shows the importance of the presence of a 5-OH substituent (in compounds **12** and **13**) for BCRP inhibition. Replacement of 5-OH with 5-OCH_3 leads to a decrease in activity (compare compounds **10** and **11**). Compound **12**, bearing 3-OCH_3 and 5-OH substituents on the flavone scaffold was found to be most potent in the flavone series with an IC_{50}-value of 0.54 μM.

The second series includes 7,8-benzoflavones and 5,6-benzoflavones. Compound **14**, the unsubstituted benzoflavone has been shown to be a potent inhibitor of BCRP by Zhang et al. [15] and was included in the current study for comparison purpose. As can be seen from Table 2 also in case of 7,8- and 5,6-benzoflavones the presence of a 3-OH substituent (in compounds **18**–**20** and **29**–**31**) led to a decrease in activity. While, particularly in case of 7,8-benzoflavones the presence of a 3-OCH_3 group (compounds **22**–**24**) led to a strong increase in BCRP inhibition. As for the flavones 3′,4′-OCH_3 substituted benzoflavones were more potent BCRP inhibitors as compared to unsubstituted compounds and those bearing a 4′-OCH_3 substituent only. Compound **17** having a 3′-OCH_3, 4-F substitution was found to be most active ($IC_{50} = 0.52 \pm 0.08$ μM in Hoechst 33342 assay) amongst the benzoflavones having no substitution at position 3. However, other derivatives of this compound (**21** and **25**) showed less activity. From Table 2, it can be seen that 5,6-benzoflavones are less potent as compared to 7,8-benzoflavones with exception of compound **28** which showed strong BCRP inhibition ($IC_{50} = 0.59 \pm 0.06$ μM in Hoechst 33342 assay).

The BCRP inhibition analysis done by two different assays gave comparable results with good correlation ($r^2 = 0.89$) between both assays as shown in Fig. 2. Also the Hill slopes of the dose–response curves were similar and not significantly different from one, except for a few experiments. This indicates that the inhibitory activity of these compounds is not substrate specific. From the activity data of all compounds, it was found that 7,8-benzoflavones are more active as compared to flavones, while 5,6-benzoflavones are the least active class with exception of compound **28**, which was found to be a potent inhibitor of BCRP. The effect of different substituents on BCRP activity is summarized in Fig. 3.

To determine the type of interaction of flavones and benzoflavones with BCRP, enzyme kinetic studies were performed with pheophorbide A as substrate and compounds **6** and **24** as inhibitors. Fig. 4 presents the Lineweaver–Burk plots for both compounds, which shows that both studied compounds are non-competitive inhibitors of BCRP in presence of pheophorbide A as substrate.

2.2.2. Calcein AM assay to determine P-gp and MRP1 inhibition

All the compounds were screened for their P-gp and MRP1 inhibition to investigate selectivity towards BCRP. Screening was performed at 10 μM final inhibitor concentration using the calcein AM assay. Cyclosporine A, a P-gp and MRP1 inhibitor, was used as standard. Most compounds showed no or very weak inhibition of P-gp and MRP1. Fig. 5 illustrates the effect of the compounds on calcein accumulation in P-gp overexpressing A2780 adr cells. Fig. 6 shows the effect of the compounds on accumulation of calcein in MRP1 overexpressing 2008 MRP1 cells. In case of flavones only compound

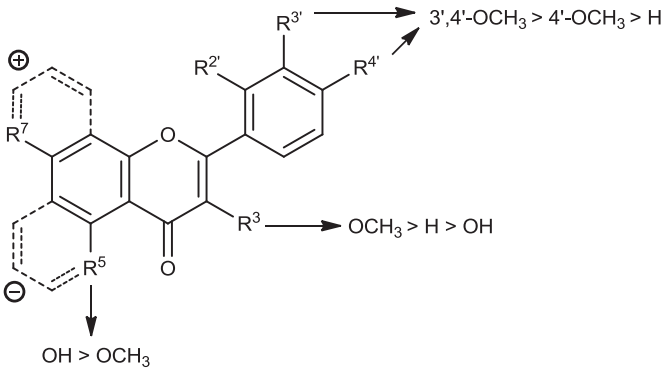


Fig. 3. Effect of different substituents on activity of flavonoids. Dotted lines indicate 7,8- or 5,6-benzo substitution for benzoflavones.

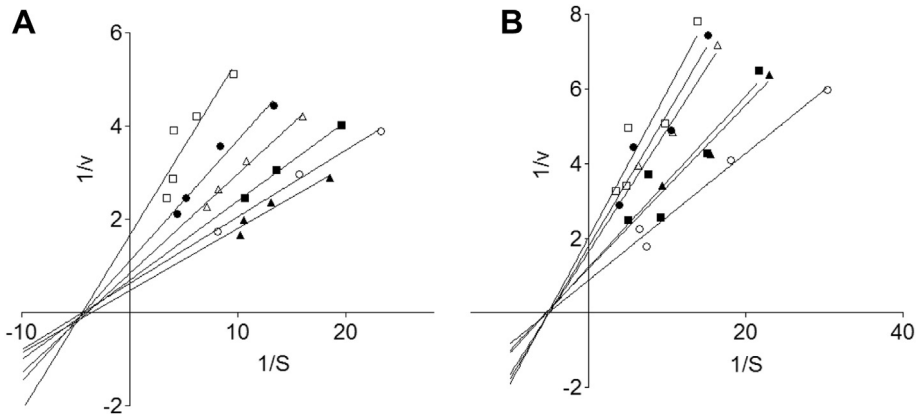


Fig. 4. Lineweaver–Burk plot for BCRP inhibitors **6** (panel A) and **24** (panel B) at various concentrations with the BCRP substrate pheophorbide A. Inhibitor concentrations used were, 0 μM (open circles), 0.031 μM (closed triangles), 0.1 μM (closed squares), 0.18 μM (open triangles), 0.31 μM (closed circles) and 0.56 μM (open squares).

10 was found to show substantial effect on both P-gp and MRP1. While in case of benzoflavones, only 7,8-benzoflavones showed considerable effect. Compounds showing more than 25% of response when compared to control (non P-gp or MRP1 expressing sensitive cells) were further investigated with a range of different concentrations to calculate their IC_{50} values. P-gp and MRP1 inhibition data of selected compounds are given in Table 3. Compound **16**, a 7,8-benzoflavone bearing 3′,4′-OCH_3 substituents was found to be most potent and active for both P-gp and MRP1 inhibition. It was even more potent than cyclosporine A in MRP1 inhibition. (Fig. 6)

Compound **24**, the most active flavonoid in the current study showed very weak P-gp and MRP1 inhibition with almost 50 times less activity compared to BCRP inhibition.

2.2.3. MTT cytotoxicity assay

To investigate the cytotoxicity of flavones and benzoflavones, the most potent compounds (**4**, **12**, **24** and **28**) from each class were selected. These compounds were studied for their cytotoxic effect in the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay. For this purpose both MDCK BCRP and sensitive MDCK cells were used. Very little toxicity could be observed at 100 μM and none of the compound showed cytotoxicity at lower concentrations. For most of the compounds GI_{50} could be estimated to be more than 100 μM and only for compound **12** GI_{50} was estimated to be around 85 μM (Fig. 7).

The MTT assay was also used to investigate the effect of selected flavones (**12**) and benzoflavones (**24**) on the antiproliferative effect of cytotoxic agents in presence of the BCRP transporter. Cytotoxicity of SN-38, the active metabolite of irinotecan, and Hoechst 33342 was evaluated in MDCK BCRP cells. The cytotoxic effect of both drugs was determined in the absence and presence of 5 μM and 10 μM concentrations of the selected compounds. Both compounds were found to be able to reverse the resistance of the BCRP expressing cell lines, proving their functional efficacy. Fig. 8 shows representative dose–response curves of SN-38 in presence of compound **12** and of Hoechst 33342 in presence of compound **24**. The shift in the dose–response curve of MDCK BCRP in presence of these compounds towards lower concentrations indicates sensitization of MDCK BCRP cells to cytotoxicity of SN-38 and Hoechst 33342.

3. Conclusion

Naturally occurring as well as synthetic flavonoids have been shown to inhibit BCRP and P-gp in several studies. In the current study we synthesized and investigated several 7,8-benzoflavones, 5,6-benzoflavones along with comparison to multisubstituted flavones for their BCRP inhibition potential. All the compounds were evaluated using two different substrates of BCRP. From the activity data obtained in the Hoechst 33342 and pheophorbide A assays we were able to propose the effect of substituents as well as the effect of attachment of a benzo-ring at position 7,8 or 5,6 of the flavone moiety. 7,8-benzoflavones were found to be more potent than flavones and 5,6-benzoflavones. The inhibitory effect of these compounds was further confirmed by evaluating the influence of selected compounds on the cytotoxicity of SN-38 and Hoechst 33342 in BCRP overexpressing cells. We also investigated all

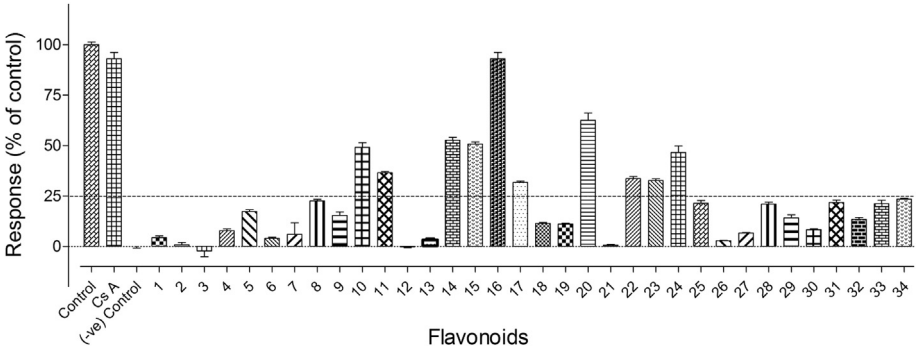


Fig. 5. Effect of flavones and benzoflavones on accumulation of calcein in P-gp overexpressing A2780 adr cells. The compounds were investigated at 10 μM final concentration. Cyclosporine A (CsA, 10 μM) was used as a standard. Response in absence of any compound was used as negative control. Data are expressed as response in percentage of control (accumulation of calcein in non P-gp expressing sensitive A2780 cells) and presented as the mean ± SD of three independent experiments.

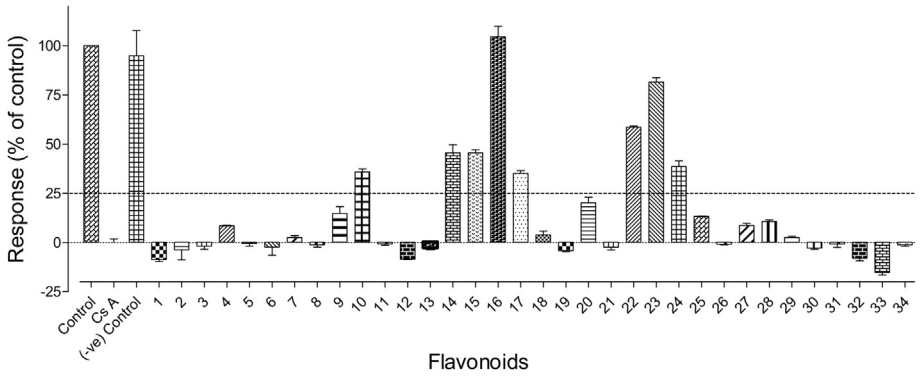


Fig. 6. Effect of flavones and benzoflavones on accumulation of calcein in MRP1 overexpressing 2008 MRP1 cells. The compounds were investigated at 10 μM final concentration. Cyclosporine A (CsA, 10 μM) was used as a standard. Response in absence of any compound was used as negative control. Data are expressed as response in percentage of control (accumulation of calcein in non MRP1 expressing sensitive 2008 cells) and presented as the mean ± SD of three independent experiments.

compounds for their P-gp and MRP1 inhibition. Most of the compounds were found to be selective towards BCRP. Cytotoxicity of most active compounds was determined in MTT cytotoxicity assay. Compound **24** was found to be a non-toxic potent BCRP inhibitor having 50 times selectivity towards BCRP. Compound **16**, a substituted 7,8-benzoflavone could be a good broad spectrum MDR modulator as it produced low IC_{50} values for all BCRP, P-gp and MRP1.

4. Experimental section

4.1. Chemistry

The general synthetic route to target flavones and benzoflavones is depicted in Scheme 1 and Scheme 2. Syntheses and characterization of precursor chalcones and benzochalcones have been described earlier [23]. Synthesis of final flavones and benzoflavones was carried out as reported earlier with small modifications [27,29].

All chemicals were purchased from Acros Organics, Alfa Aesar or Sigma–Aldrich. During the synthesis reaction progress was monitored using analytical thin layer chromatography (TLC) on silica gel plates (Silica Gel 60 F_{254} from Merck). Purity of all compounds was confirmed by NMR and elemental analysis. NMR spectra were recorded in DMSO-d_6 or $CDCl_3$. ^1H NMR spectra were obtained on Bruker Advance 500 (500 MHz); ^{13}C NMR on Bruker Advance 500 (126 MHz); chemical shifts are expressed in δ values (ppm) using the solvent peak as an internal standard; multiplicity of resonance peaks is indicated as singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). The ^{13}C signals were assigned with the aid of distortion less enhancement by polarization transfer (DEPT) and attached proton test (APT), the J values are in Hertz. Elemental analyses were performed on a Vario EL of Elementar. Found values

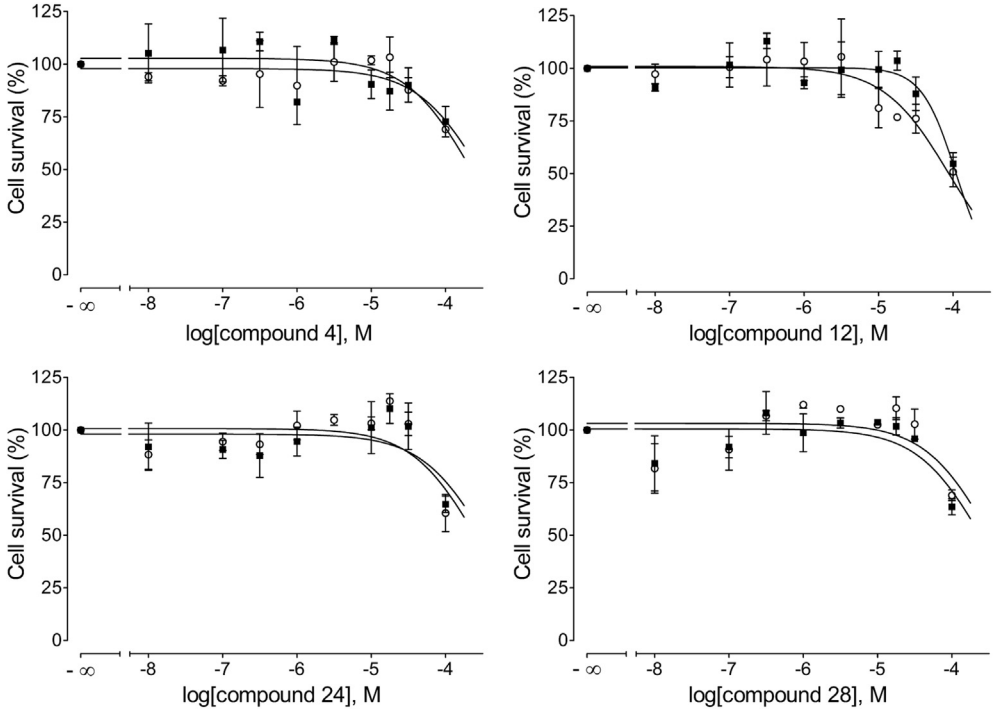


Fig. 7. Toxicity of selected compounds was determined in the MTT assay using MDCK BCRP and sensitive MDCK cells. Compounds **4**, **12**, **24** and **28** were investigated up to 100 μM concentration, for 72 h. Open circles indicate sensitive MDCK cells and closed squares indicate MDCK BCRP cells. Data are expressed as mean ± SD obtained from least three independent experiments.

Table 3
Inhibitory potencies of selected flavonoids using A2780 adr and 2008 MRP1 cells in calcein AM assay. Data are expressed as mean ± SD ($n = 3$). Cyclosporine A was used as standard.

Compound	A2780 adr IC_{50} ± SD (μM)	2008 MRP1 IC_{50} ± SD (μM)
10	23.4 ± 2.78	26.35 ± 2.65
11	27.1 ± 3.53	n.a.[a]
14	24.6 ± 3.98	11.3 ± 2.49
15	19.7 ± 2.82	9.86 ± 1.05
16	4.00 ± 0.22	1.76 ± 0.04
17	n.a.	15.1 ± 3.83
20	4.27 ± 0.59	n.a.
22	n.a	27.5 ± 4.44
23	n.a	19.0 ± 2.48
24	21.9 ± 2.33	21.8 ± 2.74
Cyclosporine A	0.99 ± 0.08	2.81 ± 0.62

[a] n.a. = not active, showing a response of less than 25% of control in screening at 10 μM concentration.

were all within ±0.4% of the theoretical values except when indicated. All the compounds were analyzed for purity by HPLC using reversed phase column Eurospher II 100-5 C18H (50 × 4 mm) connected with a pre-column Vertex-Plus Eurospher II 100-5 C18H (5 × 4 mm) obtained from Knauer, Germany. Eluent flow-rate was maintained at 1.5 ml/min. The capacity factor (k') is given for each compound.

4.2. General procedure for synthesis of 7,8- and 5,6-benzoflavones

The appropriate benzochalcone (2 mmol), I_2 (0.14 g, 0.55 mmol) was dissolved in DMSO (10 ml). The solution was refluxed for 7–8 h. After completion of the reaction as indicated by TLC, the solution was cooled to room temperature and poured onto crushed ice. 1 N HCl solution (50 ml) was added and stirred for 15 min. This solution was extracted with ethyl acetate and washed 3 times with brine. The organic phase was evaporated under reduced pressure to get crude product. It was further purified by column chromatography using silica gel as solid phase and dichloromethane:methanol (9.5:0.5) as eluent to get compounds **14**–**17** and **26**–**28**.

4.2.1. 2-Phenyl-4H-benzo[h]chromen-4-one (**14**)

Synthesized from (*E*)-1-(1-hydroxynaphthalen-2-yl)-3-phenylprop-2-en-1-one, beige solid, 68% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.63–8.57 (m, 1H), 8.21–8.15 (m, 2H), 8.07–8.02 (m, 1H), 7.95 (d, $J = 8.7$ Hz, 1H), 7.87 (d, $J = 8.6$ Hz, 1H), 7.78–7.74 (m, 2H), 7.63–7.58 (m, 3H), 7.13 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 176.92, 162.00, 152.86, 135.55, 131.82, 131.34, 129.63, 129.35, 128.34, 127.78, 126.46, 125.47, 123.60, 122.36, 120.08, 119.74, 108.25. Anal. Calcd for $C_{19}H_{12}O_2$: C, 83.81; H, 4.44; O, 11.75. Found: C, 83.72; H, 4.52. P_{HPLC} (MeOH/H_2O, 35/65) 99.9%, $k' = 3.85$.

4.2.2. 2-(4-Methoxyphenyl)-4H-benzo[h]chromen-4-one (**15**)

Synthesized from (*E*)-1-(1-hydroxynaphthalen-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one, yellow solid, 72% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.70–8.66 (m, 1H), 8.22–8.18 (m, 2H), 8.13–8.09 (m, 1H), 8.00 (d, $J = 8.7$ Hz, 1H), 7.93 (d, $J = 8.6$ Hz, 1H), 7.84–7.80 (m, 2H), 7.18–7.15 (m, 2H), 7.09 (s, 1H), 3.88 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 176.83, 162.22, 152.78, 135.54, 129.60, 128.38, 128.35, 127.80, 125.37, 123.63, 123.52, 122.37, 120.17, 119.68, 114.83, 106.80, 55.65. Anal. Calcd for $C_{20}H_{14}O3$: C, 79.46; H, 4.67; O, 15.88. Found: C, 79.33; H, 4.73. P_{HPLC} (MeOH/H_2O, 35/65) 99.56%, $k' = 4.48$.

4.2.3. 2-(3,4-Dimethoxyphenyl)-4H-benzo[h]chromen-4-one (**16**)

Synthesized from (*E*)-3-(3,4-dimethoxyphenyl)-1-(1-hydroxynaphthalen-2-yl)prop-2-en-1-one, yellowish brown solid, 64% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.66–8.62 (m, 1H), 8.12–8.08 (m, 1H), 7.99 (d, $J = 8.6$ Hz, 1H), 7.92 (d, $J = 8.6$ Hz, 1H), 7.85 (dd, $J = 8.5$, 2.2 Hz, 1H), 7.83–7.79 (m, 2H), 7.66 (d, $J = 2.2$ Hz, 1H), 7.18 (s, 2H), 3.93 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 176.89, 162.22, 152.79, 152.06, 149.27, 135.55, 129.58, 128.39, 127.84, 125.35, 123.63, 122.31, 120.16, 119.98, 119.67, 112.01, 109.58, 107.16, 56.00, 55.86. Anal. Calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85; O, 19.26. Found: C, 75.56; H, 5.02. P_{HPLC} (MeOH/H_2O, 35/65) 97.97%, $k' = 4.06$.

4.2.4. 2-(4-Fluoro-3-methoxyphenyl)-4H-benzo[h]chromen-4-one (**17**)

Synthesized from (*E*)-3-(4-fluoro-3-methoxyphenyl)-1-(1-hydroxynaphthalen-2-yl)prop-2-en-1-one, pale yellow solid, 76% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.70–8.64 (m, 1H), 8.12–8.04 (m, 3H), 7.98 (d, $J = 8.7$ Hz, 1H), 7.94–7.89 (m, 1H), 7.83–7.78 (m, 2H), 7.40–7.33 (m, 1H), 7.15 (s, 1H), 3.95 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 176.83, 160.98, 152.80, 152.71, 150.76, 135.57, 129.67, 128.36, 127.84, 125.51, 123.99, 123.74, 123.57, 122.53, 120.10, 119.69, 114.33, 113.98, 107.63, 56.47. Anal. Calcd for $C_{20}H_{13}FO_3$: C, 74.99; H, 4.09; O, 14.98. Found: C, 74.68; H, 4.25. P_{HPLC} (MeOH/H_2O, 35/65) 96.18%, $k' = 4.02$.

4.2.5. 3-Phenyl-1H-benzo[f]chromen-1-one (**26**)

Synthesized from (*E*)-1-(2-hydroxynaphthalen-1-yl)-3-phenylprop-2-en-1-one, yellowish solid, 59% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.98–9.90 (m, 1H), 8.35 (d, $J = 9.0$ Hz, 1H), 8.16–8.12 (m, 2H), 8.11–8.07 (m, 1H), 7.87 (d, $J = 9.0$ Hz, 1H), 7.77 (ddd, $J = 8.5$, 6.9,

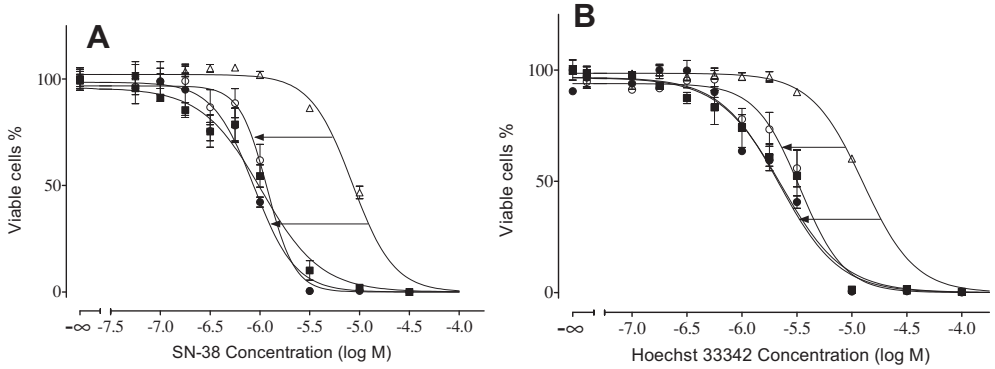


Fig. 8. Representative shift in dose–response curves of SN-38 and Hoechst 33342 cytotoxicity. Panel A depicts the effect of compound **12** on SN-38 cytotoxicity in BCRP cells and panel B shows the effect to compound **24** on cytotoxicity of Hoechst 33342. Both compounds were investigated at 5 μM (open circles) and 10 μM (closed circles) concentrations. BCRP cells without inhibitor (open triangles) are more resistant than sensitive cells (closed squares).

1.5 Hz, 1H), 7.67 (ddd, $J = 8.1$, 6.9, 1.2 Hz, 1H), 7.63–7.58 (m, 3H), 7.18 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 179.32, 160.38, 157.15, 135.90, 131.78, 130.88, 130.51, 129.81, 129.29, 129.17, 128.71, 126.72, 126.31, 126.21, 118.28, 116.37, 109.90. Anal. Calcd for $C_{19}H_{12}O_2$: C, 83.81; H, 4.44; O, 11.75. Found: C, 83.72; H, 4.52. P_{HPLC} (MeOH/H_2O, 35/65) 97.06%, $k' = 3.72$.

4.2.6. 3-(4-Methoxyphenyl)-1H-benzo[f]chromen-1-one (**27**)

Synthesized from (*E*)-1-(2-hydroxynaphthalen-1-yl)-3-(4-methoxyphenyl)prop-2-en-1-one, yellowish brown solid, 65% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.99–9.95 (m, 1H), 8.35 (d, $J = 9.0$ Hz, 1H), 8.12 (d, $J = 9.0$ Hz, 2H), 8.11–8.08 (m, 1H), 7.88 (d, $J = 9.0$ Hz, 1H), 7.77 (ddd, $J = 8.5$, 6.9, 1.5 Hz, 1H), 7.67 (ddd, $J = 8.1$, 6.9, 1.2 Hz, 1H), 7.14 (d, $J = 9.0$ Hz, 2H), 7.09 (s, 1H), 3.87 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 179.24, 162.17, 160.52, 157.01, 135.67, 130.49, 129.87, 129.07, 128.67, 128.15, 126.62, 126.23, 123.01, 118.27, 116.24, 114.76, 108.45, 55.67. Anal. Calcd for $C_{20}H_{14}O_3$: C, 79.46; H, 4.67; O, 15.88. Found: C, 79.51; H, 4.59. P_{HPLC} (MeOH/H_2O, 35/65) 96.88%, $k' = 5.60$.

4.2.7. 3-(3,4-Dimethoxyphenyl)-1H-benzo[f]chromen-1-one (**28**)

Synthesized from (*E*)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one,yellow solid, 77% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.98–9.94 (m, 1H), 8.33 (d, $J = 9.0$ Hz, 1H), 8.07 (dd, $J = 8.3$, 1.0 Hz, 1H), 7.88 (d, $J = 9.0$ Hz, 1H), 7.78–7.72 (m, 2H), 7.69–7.62 (m, 2H), 7.16 (s, 1H), 7.13 (d, $J = 8.6$ Hz, 1H), 3.91 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 179.30, 160.51, 157.01, 151.97, 149.26, 135.60, 130.48, 129.89, 129.04, 128.66, 126.60, 126.26, 123.10, 119.78, 118.33, 116.23, 111.94, 109.46, 108.72, 56.03, 55.86. Anal. Calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85; O, 19.26. Found: C, 76.03; H, 4.67. P_{HPLC} (MeOH/H_2O, 35/65) 99.9%, $k' = 2.85$.

4.3. General procedure for synthesis of 3-hydroxy flavones and benzoflavones

The appropriate chalcone or benzochalcone (5 mmol) was dissolved in EtOH (25 ml). NaOH 25% (10 ml) and H_2O_2 25% (10 ml) were added in small portions. The solution was stirred overnight at room temperature. After completion of the reaction as indicated by TLC, the reaction mixture was poured onto crushed ice and was acidified with dilute HCl. Yellow–brown precipitate was filtered under suction, washed with water. The crude product was recrystallized from ethanol to give compounds **1**–**5**, **18**–**21** and **29**–**31**.

4.3.1. 3-Hydroxy-2-phenyl-4H-chromen-4-one (**1**)

Synthesized from (*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one, white solid, 59% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.86 (s, 0H), 8.30–8.21 (m, 2H), 7.69 (ddd, $J = 1.6$, 7.1, 8.6, 1H), 7.58 (d, $J = 8.5$, 1H), 7.53 (dd, $J = 4.7$, 10.3, 2H), 7.49–7.43 (m, 1H), 7.40 (t, $J = 7.5$, 1H), 7.07 (dd, $J = 2.8$, 5.9, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.49, 155.44, 144.98, 138.46, 133.64, 131.07, 130.20, 128.60, 128.31, 127.77, 126.30, 125.47, 124.53, 120.66, 118.28. Anal. Calcd for $C_{15}H_{10}O_3{}^*1.0H_2O$: C, 70.31; H, 4.72; O, 24.97. Found: C, 70.22; H, 4.83. P_{HPLC} (MeOH/H_2O, 50/50) 95.72%, $k' = 5.92$.

4.3.2. 3-Hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**2**)

Synthesized from (*E*)-1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, pale yellow solid, 61% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.39 (s, 1H), 8.20 (d, $J = 9.2$ Hz, 2H), 7.91–7.86 (m, 1H), 7.82–7.69 (m, 2H), 7.45 (ddd, $J = 8.0$, 6.8, 1.3 Hz, 1H), 7.12 (d, $J = 9.2$ Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.77, 160.60, 154.59, 145.74, 138.28, 133.61, 131.45, 129.54, 124.87, 123.73, 121.50, 118.44, 114.19, 55.50. Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51; O, 23.86. Found: C, 71.53; H, 4.64. P_{HPLC} (MeOH/H_2O, 50/50) 95.83%, $k' = 4.71$.

4.3.3. 3-Hydroxy-7-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**3**)

Synthesized from (*E*)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, pale yellow solid, 66% yield. ^{1}H NMR (500 MHz, DMSO) δ 12.58 (s, 1H), 7.90–7.86 (m, 2H), 7.69 (d, $J = 8.7$ Hz, 1H), 7.02–6.99 (m, 2H), 6.48 (dt, $J = 5.9$, 2.4 Hz, 2H), 3.81 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.97, 167.12, 165.28, 163.56, 162.98, 131.76, 131.47, 123.13, 113.95, 107.17, 105.78, 100.86, 55.69, 55.57. Anal. Calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73; O, 26.82. Found: C, 68.19; H, 4.91. P_{HPLC} (MeOH/H_2O, 35/65) 99.56%, $k' = 4.48$.

4.3.4. 2-(3,4-Dimethoxyphenyl)-3-hydroxy-7-methoxy-4H-chromen-4-one (**4**)

Synthesized from (*E*)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one, yellow solid, 62% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.27 (s, 1H), 7.98 (d, $J = 8.9$, 1H), 7.85 (dd, $J = 2.1$, 8.6 Hz, 1H), 7.79 (d, $J = 2.1$ Hz, 1H), 7.28 (d, $J = 2.3$ Hz, 1H), 7.13 (d, $J = 8.7$, 1H), 7.03 (dd, $J = 2.4$, 8.9 Hz, 1H), 3.92 (s, 3H), 3.84 (d, $J = 3.1$ Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.18, 163.66, 156.47, 150.32, 148.59, 144.94, 138.06, 126.20, 123.94, 121.41, 115.29, 114.64, 111.70, 111.10, 100.49, 56.22, 55.90, 55.76. Anal. Calcd for $C_{18}H_{16}O_6{}^*0.5H_2O$: C, 64.09; H, 5.08; O, 30.83. Found: C, 63.94; H, 5.17. P_{HPLC} (MeOH/H_2O, 35/65) 99.56%, $k' = 4.48$.

4.3.5. 3-Hydroxy-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**5**)

Synthesized from (*E*)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, yellow solid, 70% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.12 (d, $J = 1.7$ Hz, 1H), 7.66 (d, $J = 8.9$ Hz, 2H), 6.90 (d, $J = 8.9$ Hz, 2H), 6.35 (s, 2H), 3.75 (s, 6 H), 3.69 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 191.14, 162.18, 159.23, 158.24, 148.88, 131.44, 127.52, 116.02, 114.13, 108.89, 91.27, 55.99, 55.62, 55.29. Anal. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91; O, 29.24. Found: C, 65.67; H, 5.11. P_{HPLC} (MeOH/H_2O, 35/65) 96.17%, $k' = 3.32$.

4.3.6. 3-Hydroxy-2-phenyl-4H-benzo[h]chromen-4-One (**18**)

Synthesized from (*E*)-1-(1-hydroxynaphthalen-2-yl)-3-phenylprop-2-en-1-one, white solid, 57% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.78 (s, 1H), 8.66 (dd, $J = 6.1$, 3.4 Hz, 1H), 8.34 (dd, $J = 8.5$, 1.2 Hz, 2H), 8.10 (dd, $J = 6.0$, 3.3 Hz, 1H), 8.05 (d, $J = 8.7$ Hz, 1H), 7.89 (d, $J = 8.7$ Hz, 1H), 7.84–7.79 (m, 2H), 7.74 (d, $J = 8.7$ Hz, 1H), 7.64–7.60 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 172.80, 151.96, 144.74, 140.52, 135.24, 131.61, 129.92, 129.73, 128.87, 128.48, 127.90, 127.68, 125.04, 124.89, 123.76, 123.21, 122.46, 120.28, 117.66. Anal. Calcd for $C_{19}H_{12}O_3$: C, 79.16; H, 4.20; O, 16.65. Found: C, 79.28; H, 4.15. P_{HPLC} (MeOH/H_2O, 35/65) 98.85%, $k' = 1.13$.

4.3.7. 3-Hydroxy-2-(4-methoxyphenyl)-4H-benzo[h]chromen-4-one (**19**)

Synthesized from (*E*)-1-(1-hydroxynaphthalen-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one, pale yellow solid, 59% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.60 (s, 1H), 8.68–8.62 (m, 1H), 8.32 (d, $J = 8.9$ Hz, 2H), 8.14–8.07 (m, 1H), 8.04 (d, $J = 8.7$ Hz, 1H), 7.89 (d, $J = 8.7$ Hz, 1H), 7.81 (dd, $J = 6.0$, 3.3 Hz, 2H), 7.18 (d, $J = 9.0$ Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.46, 160.52, 151.70, 145.18, 139.53, 135.14, 129.58, 129.39, 128.46, 127.83, 124.75, 123.91, 123.73, 122.37, 120.30, 117.64, 114.38, 55.50. Anal. Calcd for $C_{20}H_{14}O_4$: C, 75.46; H, 4.43; O, 20.10. Found: 75.37; H, 4.54. P_{HPLC} (MeOH/H_2O, 35/65) 97.51%, $k' = 2.46$.

4.3.8. 2-(3,4-Dimethoxyphenyl)-3-hydroxy-4H-benzo[h]chromen-4-one (**20**)

Synthesized from (*E*)-3-(3,4-dimethoxyphenyl)-1-(1-hydroxynaphthalen-2-yl)prop-2-en-1-one, pale yellow solid, 61% yield.

^{1}H NMR (500 MHz, DMSO) δ 9.61 (s, 1H), 8.61 (s, 1H), 8.10 (d, J = 9.1, 1H), 8.04 (d, J = 8.7, 1H), 7.95 (d, J = 8.5, 1H), 7.91 (d, J = 2.0, 1H), 7.89 (d, J = 8.8, 1H), 7.83–7.79 (m, 2H), 7.20 (d, J = 8.6, 1H), 3.88 (d, J = 12.2, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.44, 151.70, 150.44, 148.68, 145.14, 139.66, 135.17, 129.60, 128.49, 127.92, 124.00, 123.72, 117.63, 111.96, 111.19, 55.83, 55.80. Anal. Calcd for $C_{21}H_{16}O_5$*0.5H_2O: C, 72.41; H, 4.63; O, 22.96. Found: C, 72.35; H, 4.71. P_{HPLC} (MeOH/H_2O, 35/65) 95.75%, k' = 2.85.

4.3.9. 2-(4-Fluoro-3-methoxyphenyl)-3-hydroxy-4H-benzo[h] chromen-4-one (**21**)

Synthesized from (*E*)-3-(4-fluoro-3-methoxyphenyl)-1-(1-hydroxy-naphthalen-2-yl)prop-2-en-1-one, white solid, 54% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.85 (s, 1H), 8.72–8.63 (m, 1H), 8.22–8.08 (m, 3H), 8.04 (d, J = 8.7 Hz, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.86–7.77 (m, 2H), 7.41 (t, J = 8.9 Hz, 1H), 3.95 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.56, 151.77, 150.32, 148.47, 144.01, 143.78, 140.07, 135.22, 129.70, 128.46, 127.90, 124.90, 124.64, 124.29, 123.68, 122.52, 120.26, 117.67, 115.10, 114.13, 56.36. Anal. Calcd for $C_{20}H_{13}FO_4$: C, 71.43; H, 3.90; O, 19.03. Found: C, 71.54; H, 3.78. P_{HPLC} (MeOH/H_2O, 35/65) 97.42%, k' = 3.48.

4.3.10. 2-Hydroxy-3-phenyl-1H-benzo[f]chromen-1-one (**29**)

Synthesized from (*E*)-1-(2-hydroxynaphthalen-1-yl)-3-phenyl-prop-2-en-1-one, white solid, 63% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.49 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 9.0 Hz, 1H), 7.86–7.82 (m, 2H), 7.78 (d, J = 9.0 Hz, 1H), 7.71 (td, J = 7.5, 1.4 Hz, 1H), 7.65 (d, J = 9.0 Hz, 1H), 7.61 (dt, J = 13.4, 4.6 Hz, 1H), 7.55–7.48 (m, 2H), 7.35 (ddd, J = 8.0, 6.8, 1.1 Hz, 1H), 7.16 (d, J = 8.9 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 172.28, 165.73, 159.67, 156.12, 142.48, 135.82, 134.73, 134.56, 129.88, 128.77, 127.98, 127.94, 124.59, 123.33, 119.04, 113.08. Anal. Calcd for $C_{19}H_{12}O_3$*1.0H_2O: C, 74.50; H, 4.61; O, 20.89. Found: C, 74.57; H, 4.88. P_{HPLC} (MeOH/H_2O, 35/65) 98.15%, k' = 3.85.

4.3.11. 2-Hydroxy-3-(4-methoxyphenyl)-1H-benzo[f]chromen-1-One (**30**)

Synthesized from (*E*)-1-(2-hydroxynaphthalen-1-yl)-3-(4-meth oxyphenyl)prop-2-en-1-one, pale yellow solid, 57% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.33 (s, 1H), 8.67 (dd, J = 7.2, 1.7 Hz, 1H), 7.79–7.71 (m, 2H), 7.54 (d, J = 7.5 Hz, 1H), 7.49 (d, J = 7.5 Hz, 2H), 7.42 (dtd, J = 19.9, 7.4, 1.6 Hz, 2H), 6.97 (d, J = 7.5 Hz, 2H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.28, 167.89, 161.06, 159.95, 143.82, 134.65, 131.57, 131.44, 130.02, 128.79, 128.03, 127.00, 126.96, 124.54, 123.41, 118.94, 116.64, 114.47, 55.42. Anal. Calcd for $C_{20}H_{14}O_4$: C, 75.46; H, 4.43; O, 20.10. Found: C, 75.27; H, 4.67. P_{HPLC} (MeOH/H_2O, 35/65) 95.28%, k' = 4.03.

4.3.12. 3-(3,4-Dimethoxyphenyl)-2-hydroxy-1H-benzo[f]chromen-1-one (**31**)

Synthesized from (*E*)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-naphthalen-1-yl)prop-2-en-1-one,yellowish brown solid, 66% yield. ^{1}H NMR (500 MHz, DMSO) δ 9.83 (s, 1H), 7.55 (dd, J = 8.4, 2.0 Hz, 2H), 7.43 (d, J = 2.0 Hz, 2H), 7.29 (d, J = 2.0 Hz, 1H), 7.18 (ddd, J = 14.2, 7.5, 4.5 Hz, 1H), 7.03 (d, J = 8.5 Hz, 2H), 6.42 (d, J = 15.9 Hz, 1H), 3.81 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 167.98, 167.22, 152.78, 150.95, 149.15, 148.47, 127.22, 123.32, 123.12, 122.74, 116.86, 112.11, 111.74, 111.47, 111.17, 110.55, 55.80, 55.62. Anal. Calcd for $C_{21}H_{16}O_5$: C, 72.41; H, 4.63; O, 22.96. Found: C, 72.18; H, 4.92. P_{HPLC} (MeOH/H_2O, 35/65) 96.01%, k' = 4.22.

4.4. General procedure for synthesis of 3-methoxy flavones and benzoflavones

To a solution of 3-hydroxyflavone or 3-hydroxybenzoflavone (2 mmol) in acetone (25 ml), K_2CO_3 (2 mmol) and methyl iodide (3 mmol) were added. The resulting mixture was refluxed for 24 h. After completion of the reaction, acetone and methyl iodide were removed under reduced pressure. To the resulting solid water was added, the precipitated product was filtered and washed with water to remove traces of K_2CO_3. The product was dried at room temperature and recrystallized from ethanol to yield compounds **6**–**9**, **22**–**25** and **32**–**34**.

4.4.1. 3-Methoxy-2-phenyl-4H-chromen-4-one (**6**)

Synthesized from 3-hydroxy-2-phenyl-4*H*-chromen-4-one, pale yellow solid, 74% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (dd, J = 8.0, 1.7 Hz, 1H), 8.12–8.06 (m, 2H), 7.66 (ddd, J = 8.5, 7.1, 1.7 Hz, 1H), 7.55–7.48 (m, 4H), 7.39 (ddd, J = 8.0, 7.1, 1.0 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.14, 155.60, 155.28, 141.53, 133.45, 130.72, 128.53, 128.50, 125.83, 124.66, 117.98, 60.13. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79; O, 19.03. Found: C, 75.92; H, 4.96. P_{HPLC} (MeOH/H_2O, 30/70) 98.39%, k' = 1.17.

4.4.2. 3-Methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**7**)

Synthesized from 3-hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one, pale yellow solid, 64% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.09 (d, J = 1.3 Hz, 1H), 8.08–8.06 (m, 1H), 8.06–8.04 (m, 1H), 7.80 (ddd, J = 8.6, 7.0, 1.7 Hz, 1H), 7.75–7.71 (m, 1H), 7.47 (ddd, J = 8.0, 7.0, 1.1 Hz, 1H), 7.16–7.11 (m, 2H), 3.85 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.80, 161.38, 155.10, 154.76, 140.22, 134.01, 130.20, 125.08, 125.01, 123.68, 122.75, 118.47, 114.35, 59.61, 55.58. Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00; O, 22.67. Found: C, 72.47; H, 5.38. P_{HPLC} (MeOH/H_2O, 50/50) 99.74%, k' = 6.36.

4.4.3. 2-(3,4-Dimethoxyphenyl)-3-methoxy-4H-chromen-4-one (**8**)

Synthesized from 2-(3,4-dimethoxyphenyl)-3-hydroxy-4*H*-chromen-4-one, yellow solid, 73% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.08 (dd, J = 7.9, 1.6 Hz, 1H), 7.81 (ddd, J = 8.5, 6.9, 1.6 Hz, 1H), 7.78–7.75 (m, 1H), 7.72 (dd, J = 8.5, 2.1 Hz, 1H), 7.68 (d, J = 2.1 Hz, 1H), 7.48 (ddd, J = 8.0, 6.9, 1.2 Hz, 1H), 7.16 (d, J = 8.6 Hz, 1H), 3.86 (d, J = 1.8 Hz, 6H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.81, 155.07, 154.75, 151.26, 148.62, 140.35, 134.00, 125.10, 125.00, 123.65, 122.76, 122.14, 118.57, 111.78, 111.68, 59.64, 55.82. Anal. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16; O, 25.61. Found: C, 69.28; H, 5.13. P_{HPLC} (MeOH/H_2O, 50/50) 99.33%, k' = 3.95.

4.4.4. 2-(3,4-Dimethoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one (**9**)

Synthesized from 2-(3,4-dimethoxyphenyl)-3-hydroxy-7-methoxy-4*H*-chromen-4-one, yellow solid, 71% yield. ^{1}H NMR (500 MHz, DMSO) δ 7.96 (d, J = 8.9 Hz, 1H), 7.71 (dd, J = 8.5, 2.1 Hz, 1H), 7.66 (d, J = 2.1 Hz, 1H), 7.27 (d, J = 2.3 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 7.05 (dd, J = 8.9, 2.4 Hz, 1H), 3.91 (s, 3H), 3.86 (s, 6H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.21, 163.86, 156.57, 154.50, 151.11, 148.60, 140.10, 126.36, 122.83, 121.93, 117.44, 114.71, 111.73, 111.56, 100.73, 59.62, 56.26, 55.84, 55.80. Anal. Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30; O, 28.04. Found: C, 66.38; H, 5.64. P_{HPLC} (MeOH/H_2O, 50/50) 97.28%, k' = 3.51.

4.4.5. 3-Methoxy-2-phenyl-4H-benzo[h]chromen-4-one (**22**)

Synthesized from 3-hydroxy-2-phenyl-4*H*-benzo[*h*]chromen-4-one, yellowish brown solid, 62% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.59 (dd, J = 6.1, 3.4 Hz, 1H), 8.21–8.18 (m, 2H), 8.14–8.10 (m, 1H), 8.04 (d, J = 8.7 Hz, 1H), 7.94 (d, J = 8.7 Hz, 1H), 7.85–7.79 (m, 2H), 7.68–7.61 (m, 3H), 3.90 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.83, 154.36, 152.10, 142.14, 135.39, 130.99, 130.70, 129.82, 129.02, 128.44, 128.40, 127.97, 125.34, 123.56, 122.40, 120.22, 120.05, 59.82. Anal. Calcd for $C_{20}H_{14}O_3$: C, 79.46; H, 4.67; O, 15.88. Found: C, 79.29; H, 4.81. P_{HPLC} (MeOH/H_2O, 35/65) 99.31%, k' = 9.87.

4.4.6. 3-Methoxy-2-(4-methoxyphenyl)-4H-benzo[h]chromen-4-one (**23**)

Synthesized from 3-hydroxy-2-(4-methoxyphenyl)-4*H*-benzo[*h*]chromen-4-one, yellowish brown solid, 59% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.59–8.54 (m, 1H), 8.20–8.16 (m, 2H), 8.11–8.07 (m, 1H), 8.01 (d, J = 8.7 Hz, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.80 (ddd, J = 4.7, 2.2, 0.8 Hz, 2H), 7.19 (d, J = 9.1 Hz, 2H), 3.88 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 173.58, 161.33, 154.37, 151.85, 141.34, 135.31, 130.13, 129.67, 128.41, 127.87, 125.16, 123.53, 122.87, 122.31, 120.23, 119.96, 114.52, 59.58, 55.56. Anal. Calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85; O, 19.26. Found: C, 75.58; H, 5.04. P_{HPLC} (MeOH/H_2O, 35/65) 98.20%, k' = 6.54.

4.4.7. 2-(3,4-Dimethoxyphenyl)-3-methoxy-4H-benzo[h]chromen-4-one (**24**)

Synthesized from 2-(3,4-dimethoxyphenyl)-3-hydroxy-4*H*-benzo[*h*]chromen-4-one, yellowish brown solid, 69% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.58 (dd, J = 3.5,6.2 Hz, 1H), 8.12 (dd, J = 3.1,6.2 Hz, 1H), 8.03 (d, J = 8.7 Hz, 1H), 7.92 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.83–7.82 (m, 1H), 7.81 (d, J = 3.3 Hz, 1H), 7.77 (d, J = 2.1 Hz, 1H), 7.22 (d, J = 8.6 Hz, 1H), 3.92 (d, J = 11.4 Hz, 6H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.61, 154.45, 151.90, 151.22,148.72, 135.37, 129.72, 128.47, 128.00, 125.24, 123.57, 122.89, 122.33, 121.99, 120.27, 119.99, 111.98, 111.62, 59.67, 55.85, 55.81 Anal. Calcd for $C_{22}H_{18}O_5$: C, 72.92; H, 5.01; O, 22.08. Found: C, 72.68; H, 5.25. P_{HPLC} (MeOH/H_2O, 35/65) 98.74%, k' = 5.82.

4.4.8. 2-(4-Fluoro-3-methoxyphenyl)-3-methoxy-4H-benzo[h]chromen-4-one (**25**)

Synthesized from 2-(4-fluoro-3-methoxyphenyl)-3-hydroxy-4*H*-benzo[*h*]chromen-4-one, yellow solid, 74% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.41 (dd, J = 7.5, 1.6 Hz, 1H), 7.75 (dt, J = 8.1, 1.4 Hz, 2H), 7.61 (d, J = 7.1 Hz, 1H), 7.44 (dtd, J = 25.6, 7.2, 1.5 Hz, 2H), 6.88–6.81 (m, 3H), 3.61 (s, 3H), 3.55 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 173.68, 158.40, 155.41, 154.67, 151.95, 151.02, 150.80, 136.86, 134.25, 129.19, 129.16, 128.22, 128.27, 127.72, 125.19, 124.54, 124.15, 123.62, 123.57, 123.22, 121.35, 115.26, 115.04, 114.78, 114.72, 61.45, 59.80, 57.76. Anal. Calcd for $C_{21}H_{15}FO_4$: C, 71.99; H, 4.32; O, 18.27. Found: C, 72.18; H, 4.24. P_{HPLC} (MeOH/H_2O, 35/65) 97.83%, k' = 4.49.

4.4.9. 2-Methoxy-3-phenyl-1H-benzo[f]chromen-1-one (**32**)

Synthesized from 2-hydroxy-3-phenyl-1*H*-benzo[*f*]chromen-1-one, yellow solid, 54% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.78 (dd, J = 7.5, 1.6 Hz, 1H), 7.82–7.76 (m, 2H), 7.58–7.54 (m, 1H), 7.49 (td, J = 7.5, 1.5 Hz, 1H), 7.42 (td, J = 7.5, 1.5 Hz, 1H), 7.32 (dd, J = 7.5, 1.3 Hz, 2H), 7.24 (t, J = 7.3 Hz, 2H), 7.21–7.15 (m, 1H), 3.49 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 172.01, 156.52, 152.44, 136.05, 133.83, 131.19, 130.92, 130.85, 130.59, 128.69, 128.47, 128.01, 127.98, 126.47, 125.09, 119.04, 116.89, 59.51. Anal. Calcd for $C_{20}H_{14}O_3$: C, 79.46; H, 4.67; O, 15.88. Found: C, 79.14; H, 4.88. P_{HPLC} (MeOH/H_2O, 35/65) 96.51%, k' = 4.18.

4.4.10. 2-Methoxy-3-(4-methoxyphenyl)-1H-benzo[f]chromen-1-one (**33**)

Synthesized from 2-hydroxy-3-(4-methoxyphenyl)-1*H*-benzo[*f*]chromen-1-one, pale yellow solid, 63% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.75 (dd, J = 7.5, 1.7 Hz, 1H), 7.81–7.75 (m, 2H), 7.66–7.58 (m, 1H), 7.51 (dtd, J = 25.1, 7.3, 1.7 Hz, 2H), 7.32 (d, J = 7.5 Hz, 2H), 6.89 (d, J = 7.5 Hz, 2H), 3.77 (s, 3H), 3.59 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 173.14, 160.42, 155.62, 152.44, 138.05, 133.73, 130.92, 130.85, 129.87, 128.01, 127.98, 126.47, 125.09, 123.08, 119.04, 114.85, 113.56, 59.97, 58.03. Anal. Calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85; O, 19.26. Found: C, 75.68; H, 5.03. P_{HPLC} (MeOH/H_2O, 35/65) 98.17%, k' = 4.87.

4.4.11. 3-(3,4-Dimethoxyphenyl)-2-methoxy-1H-benzo[f]chromen-1-one (**34**)

Synthesized from 3-(3,4-dimethoxyphenyl)-2-hydroxy-1*H*-benzo[*f*]chromen-1-one, yellowish brown solid, 68% yield. ^{1}H NMR (500 MHz, DMSO) δ 8.79 (dd, J = 7.4, 1.6 Hz, 1H), 7.85–7.78 (m, 2H), 7.68–7.62 (m, 1H), 7.45 (dtd, J = 25.1, 7.4, 1.5 Hz, 2H), 7.08–6.91 (m, 2H), 6.79 (d, J = 7.8 Hz, 1H), 3.59 (s, 6H), 3.51 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 176.04, 155.52, 153.59, 152.27, 149.41, 137.83, 133.83, 130.92, 130.85, 128.01, 127.98, 126.47, 125.09, 123.58, 122.40, 119.04, 114.85, 112.34, 111.89, 61.45, 56.78. Anal. Calcd for $C_{22}H_{18}O_5$: C, 72.92; H, 5.01; O, 22.08. Found: C, 73.05; H, 5.12. P_{HPLC} (MeOH/H_2O, 35/65) 96.83%, k' = 4.92.

4.5. General procedure for the synthesis of pentamethyl ethers of quercetin and morin

To a solution of quercetin hydrate or morin (10 mmol) in acetone (50 ml), K_2CO_3 (30 mmol) and methyl iodide (70 mmol, excess) were added. The resulting mixture was refluxed for 24 h. After completion of the reaction, acetone and methyl iodide were removed under reduced pressure. To the resulting solid water was added, the precipitated product was filtered and washed with water to remove traces of K_2CO_3. The product was dried at room temperature and recrystallized from ethanol to yield compounds **10** and **11**.

4.5.1. 2-(3,4-Dimethoxyphenyl)-3,5,7-trimethoxy-4H-chromen-4-one (**10**)

Synthesized from quercetin, yellow solid, 82% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.67 (d, J = 2.1 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 6.48 (d, J = 2.3 Hz, 1H), 6.33 (d, J = 2.3 Hz, 1H), 3.94 (s, 9H), 3.88 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.00, 163.87, 161.02, 158.78, 152.52, 150.79, 148.65, 141.18, 123.41, 121.60, 111.26, 110.76, 109.49, 95.72, 92.42, 59.93, 56.39, 56.05, 55.95, 55.75. Anal. Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41; O, 30.08. Found: C, 64.34; H, 5.62. P_{HPLC} (MeOH/H_2O, 50/50) 97.46%, k' = 2.69.

4.5.2. 2-(2,4-Dimethoxyphenyl)-3,5,7-trimethoxy-4H-chromen-4-one (**11**)

Synthesized from morin, yellowish brown solid, 76% yield. ^{1}H NMR (500 MHz, DMSO) δ 7.34 (d, J = 8.4 Hz, 1H), 6.69 (d, J = 2.3 Hz, 1H), 6.64 (dd, J = 8.5, 2.3 Hz, 1H), 6.61 (d, J = 2.3 Hz, 1H), 6.47 (d, J = 2.3 Hz, 1H), 3.83 (d, J = 2.1 Hz, 9H), 3.79 (s, 3H), 3.60 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.12, 163.71, 162.44, 160.64, 158.85, 158.38, 152.86, 141.48, 131.46, 114.37, 112.26, 109.11, 105.34, 98.78, 96.01, 93.05, 59.57, 56.30, 56.10, 55.91, 55.63. Anal. Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41; O, 30.08. Found: C, 64.67; H, 5.34. P_{HPLC} (MeOH/H_2O, 50/50) 95.69%, k' = 4.64.

4.6. General procedure for synthesis of tetramethyl ethers of quercetin and morin

Quercetin hydrate or morin (10 mmol) was added to a mixture of acetone (150 ml) and water (75 ml). To the resulting mixture, 30% aqueous KOH (6 ml) was added and refluxed for 10 min. Dimethyl sulphate (2.4 ml) was added and refluxed for 20 min. More amount of KOH (3 ml) was added producing dark brown solution. To this solution an additional amount of dimethyl sulphate (2.4 ml) was added and refluxed for 20 min. Finally, again KOH (3.0 ml) and dimethyl sulphate (3.5 ml) were added, refluxed further for 1.5 h and allowed to cool to room temperature. Evaporation of acetone gave a dark yellow to brown residue. This residue was purified by column chromatography using hexan:ethylacetate (1:3) as mobile phase. The first two fractions were combined to give pure **12** or **13**.

*4.6.1. 2-(3,4-Dimethoxyphenyl)-5-hydroxy-3,7-dimethoxy-4H-chromen-4-one (**12**)*

Synthesized from quercetin, yellow solid, 51% yield. ^{1}H NMR (500 MHz, DMSO) δ 12.61 (s, 1H), 7.71 (dd, J = 8.6, 2.1 Hz, 1H), 7.65 (d, J = 2.1 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 6.77 (d, J = 2.1 Hz, 1H), 6.37 (d, J = 2.2 Hz, 1H), 3.87–3.84 (m, 9H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 178.20, 165.34, 160.81, 156.51, 155.59, 151.52, 148.67, 138.51, 122.25, 122.22, 111.79, 111.52, 105.39, 97.95, 92.70, 59.94, 56.27, 55.85. Anal. Calcd for $C_{19}H_{18}O_7$*0.5H_2O: C, 62.12; H, 5.21; O, 32.67. Found: C, 62.27; H, 5.32. P_{HPLC} (MeOH/H_2O, 50/50) 96.32%, k' = 3.82.

*4.6.2. 2-(2,4-Dimethoxyphenyl)-5-hydroxy-3,7-dimethoxy-4H-chromen-4-one (**13**)*

Synthesized from morin, yellowish brown solid, 48% yield. ^{1}H NMR (500 MHz, DMSO) δ 12.62 (s, 1H), 7.39 (d, J = 8.5 Hz, 1H), 6.72 (d, J = 2.3 Hz, 1H), 6.66 (dd, J = 8.5, 2.3 Hz, 1H), 6.61 (d, J = 2.2 Hz, 1H), 6.38 (d, J = 2.2 Hz, 1H), 3.84 (d, J = 7.2 Hz, 6H), 3.81 (s, 3H), 3.69 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 178.24, 165.31, 162.85, 161.27, 158.56, 157.10, 156.82, 139.54, 131.58, 111.72, 105.77, 105.53, 98.80, 97.98, 92.48, 60.12, 56.24, 55.99, 55.67. Anal. Calcd for $C_{19}H_{18}O_7$*0.5H_2O: C, 62.12; H, 5.21; O, 32.67. Found: C, 61.99; H, 5.36. P_{HPLC} (MeOH/H_2O, 50/50) 97.25%, k' = 4.04.

4.7. Biology

4.7.1. Materials for biological studies

Chemicals: Ko143 was purchased from Tocris Bioscience (Bristol, United Kingdom). All other chemicals were purchased from Sigma–Aldrich Chemicals (Taufkirchen, Germany) unless otherwise specified. Stock solutions (10 mM) of investigated compounds were prepared in DMSO.

4.7.2. Cell culture

The cell line MDCK wt and MDCK BCRP were received as a generous gift from Dr. A. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands). MDCK BCRP cells were generated by transfection of the canine kidney epithelial cell line MDCKII with the human wild-type cDNA C-terminally linked to the cDNA of the green fluorescent protein (GFP) [30]. These cells were cultured in Dulbecco's modified eagle medium (DMEM) with 10% foetal calf serum (FCS), streptomycin (50 μg/mL) and penicillin (50 U/mL). Human ovarian carcinoma cell lines A2780 and the corresponding MDR1 overexpressing doxorubicin resistant A2780 adr cell line were purchased from ECACC (Nos. 93112519 and 93112520). The cell lines were grown in RPMI-1640 medium supplemented with 10% FBS, 50 μl/mL streptomycin, 50 U/mL penicillin G, and 300 mg/L L-glutamine. The human ovarian cancer cell line 2008 MRP1 stably expressing MRP1 was a gift from Prof. Dr. Piet Borst (NCI, Amsterdam, The Netherlands). The cell line 2008 MRP1 was grown in the same medium as described for A2780 cells with addition of 400 μg/mL G-418 (Geneticin) for maintaining ABCC1 over expression.

All the cells were maintained in a 5% CO_2 humidified atmosphere at 37 °C. After confluence of 80–90%, subculturing was performed with 0.05% trypsin and 0.02% EDTA.

4.7.3. Drug accumulation assays

4.7.3.1. Hoechst 33342 assay. To investigate the inhibitory effect of synthesized compounds on BCRP, a Hoechst 33342 accumulation assay was performed as described earlier with small modifications [16,23,31]. In brief, after reaching a confluence of 80–90%, cells were harvested by gentle trypsination (0.05% trypsin/0.02% EDTA) and then transferred to a 50 ml tube followed by centrifugation (266×g, 4 °C, 4 min). The cell pellet obtained was re-suspended in fresh culture medium and the cell density was determined using a Casy I Modell TT cell counter device (Schaerfe System GmbH, Reutlingen, Germany). Followed by another centrifugation cells were washed three times with Krebs-HEPES buffer (KHB) and seeded into black 96 well plates (Greiner, Frickenhausen, Germany) at a density of approximately 20,000 cells per well in a volume of 90 μl. 10 μl of various test compounds in different concentrations were added to a total volume of 100 μl. The prepared 96 well plate was kept under 5% CO_2 and 37 °C for 30 min. After this preincubation period, 20 μl of a 6 μM Hoechst 33342 solution (protected from light) was added to each well yielding a final concentration of Hoechst 33342 of 1 μM. Fluorescence was measured immediately in constant intervals (60 s) up to 120 min at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a 37 °C tempered BMG POLARstar microplate reader (BMG Labtech, Offenburg, Germany). For the analysis of the data obtained from the assay, first fluorescence of KHB was subtracted from the fluorescence reading obtained from MDCK cells. Average of fluorescence values in the steady state (from 100 min to 109 min) were calculated for each concentration and from these data, concentration response curves were generated by nonlinear regression using the four-parameter logistic equation with variable Hill slope (GraphPad Prism v. 5.0, San Diego, CA, USA).

4.7.3.2. Pheophorbide A assay. The synthesized compounds were additionally investigated for BCRP inhibition in pheophorbide A assay as described earlier using MDCK wt and MDCK BCRP cells [32,33]. To perform the pheophorbide A assay, cells were prepared as described for the Hoechst 33342 assay. Approximately 45,000 cells per well were seeded into U-shaped clear 96 well plates (Greiner, Frickenhausen, Germany) in a volume of 160 μl. To this 20 μl of various test compounds in different concentrations were added. The prepared 96 well plate was kept under 5% CO_2 and 37 °C for 30 min. After this preincubation period, 20 μl of a 5 μM pheophorbide A solution (protected from light) was added to each well and plates were further incubated for 120 min. Fluorescence was measured by flow cytometry (FACSCalibur, Becton Dickinson Biosciences, Heidelberg, Germany). For the measurement, pheophorbide A was excited at an excitation wavelength of 488 nm and emission was detected in the FL3 channel (670 nm). Concentration response curves were generated by nonlinear regression using the four-parameter logistic equation with variable Hill slope.

4.7.3.3. Calcein-AM assay. For checking the selectivity of synthesized compounds towards BCRP, these were further tested for their P-gp and MRP1 inhibition in the calcein-AM assay [23,32]. For this purpose A2780 adr cells for P-gp and 2008 MRP1 cells for MRP1 were used. After confluence of 80–90%, subculturing was performed with 0.05% trypsin and 0.02% EDTA. Cells were prepared as described above and washed three times with Krebs-HEPES buffer and then seeded into colourless 96 well plates (Greiner, Frickenhausen, Germany) at a density of approximately 30,000 cells in a volume of 90 μl per well. Then, 10 μl of the test compounds were added, resulting in a final volume of 100 μl per well. The prepared 96 well plates were preincubated for 30 min. After the preincubation period, 33 μl of a 1.25 μM calcein AM solution, which was protected from light, were added to each well. The fluorescence was measured immediately in constant time intervals (60 s) up to 90 min using an excitation wavelength of 485 nm and an emission wavelength of 520 nm with a BMG POLARstar microplate reader tempered at 37 °C. For calculation of inhibitory effects the first linear part of the fluorescence time curves was used.

4.7.4. MTT cytotoxicity assay

Cytotoxicity of the selected active flavones (**4**, **12**) and benzoflavones (**24**, **28**) was studied in MDCK BCRP and sensitive MDCK

cells using the MTT cytotoxicity assay. The assay was performed as described earlier with minor modifications [34,35]. In brief, cells were seeded into 96-well tissue culture plates (Sarstedt, Newton, USA) at a density of 2500 cells per well in a volume of 180 μl and kept under 5% CO_2 at 37 °C for 6 h. Attachment of cells was controlled under microscope and the test compounds were added to the cells to achieve the required final concentration in a final volume of 200 μl. Control experiments were performed with medium containing 0.1% (v/v) of DMSO. After an incubation period of 72 h, the MTT reagent was added (20 μl of a 5 mg/ml solution) to each well. Plates were further incubated for 1 h and after that MTT was terminated by removing supernatants and lysing the cells with 100 μl DMSO per well. Viability of cells was measured spectrophotometrically by measuring absorbance at 544 nm and background corrected at 710 nm using BMG POLARstar microplate reader.

Acknowledgements

We would like to thank Dr. A. H. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands) for kindly providing the MDCK BCRP cell line. K.J. thanks to DAAD (Germany) for doctoral fellowship.

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