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Investigation of chalcones and benzochalcones as inhibitors of breast cancer resistance protein

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ABSTRACT

Breast cancer resistance protein (BCRP/ABCG2) belongs to the ATP binding cassette family of transport proteins. BCRP has been found to confer multidrug resistance in cancer cells. A strategy to overcome resistance due to BCRP overexpression is the investigation of potent and specific BCRP inhibitors. The aim of the current study was to investigate different multi-substituted chalcones for their BCRP inhibition. We synthesized chalcones and benzochalcones with different substituents (viz. OH, OCH_3, Cl) on ring A and B of the chalcone structure. All synthesized compounds were tested by Hoechst 33342 accumulation assay to determine inhibitory activity in MCF-7 MX and MDCK cells expressing BCRP. The compounds were also screened for their P-glycoprotein (P-gp) and Multidrug resistance-associated protein 1 (MRP1) inhibitory activity in the calcein AM accumulation assay and were found to be selective towards inhibition of BCRP. Substituents at position 2′ and 4′ on chalcone ring A were found to be essential for activity; additionally there was a great influence of substituents on ring B. Presence of 3,4-dimethoxy substitution on ring B was found to be optimal, while presence of 2- and 4-chloro substitution also showed a positive effect on BCRP inhibition.

1. Introduction

Chemotherapy is a major form of treatment for cancers. But unfortunately the majority of cancers are either resistant to chemotherapy or acquire resistance during treatment. A frequent mechanism by which human cancers develop resistance is overexpression of certain ATP-binding cassette (ABC) transporters conferring resistance to structurally unrelated anticancer agents, the so called multidrug resistance (MDR). Among these transporters especially P-glycoprotein (P-gp),[1] Multidrug Resistance-associated Protein 1 (MRP1)[2] and the breast cancer resistance protein (BCRP) are found to be involved in MDR.[3]

Transfection studies from various laboratories have confirmed that overexpression of BCRP cDNA in different cell types confers resistance to a variety of anticancer agents and decrease drug accumulation in the cells.[3,4] BCRP belongs to subfamily G of the ABC-transporter superfamily and is designated as ABCG2. BCRP has been found to transport large molecules with amphiphilic character using energy from ATP hydrolysis.[5,6] BCRP substrates include the anticancer drugs mitoxantrone,[7] topotecan,[7] irinotecan[8] and its active metabolite SN-38, etoposide and flavopiridol.[9] Several inhibitors of BCRP have been developed during the last decade in an attempt to overcome the resistance mediated by this transporter. These involve specific inhibitors like fumitremorgin C[10] and its synthetic analog Ko143, broad spectrum modulators like XR9576 (tariquidar),[11] naturally occurring flavonoids and their derivatives.[12]

Chalcones, bioprecursors of flavonoids, have also been reported to inhibit P-gp and BCRP.[13] It was shown by Liu et al., that functionalized basic chalcones are inhibitors of P-gp,[14] while Han et al. reported that non-basic chalcones showed no effect on P-gp while inhibiting BCRP.[15] Recently Zoldakova et al. reported the inhibition of BCRP and P-gp by a combretastin A4 analogous chalcone and its Pt-complex.[16]

In the current study we investigated the effect of different substitution patterns on the inhibitory activity of chalcones. For this reason we synthesized several multi-substituted derivatives with varying substituents on ring A and ring B of chalcones (Table 1). Synthesis of selected chalcones was done by modified Claisen–Schmidt condensation reaction. The compounds were investigated for BCRP inhibition in MCF-7 MX and MDCK BCRP cell lines using the Hoechst 33342 assay.[17] To check selectivity the chalcones were also tested for their P-gp and MRP1 inhibition.[18] Reversal of resistance due to BCRP inhibition was investigated using the two anticancer agents mitoxantrone and SN-38 in presence of selected compounds.

Substitution at position 2′ and 4′ in ring A of chalcones was found to be essential for the activity. On ring B presence of 3,4-dimethoxy substitution yielded maximum inhibitory effect on BCRP, while presence of 2-chloro or 4-chloro substituents on ring B also showed a positive effect. Compounds **8** and **25** were found

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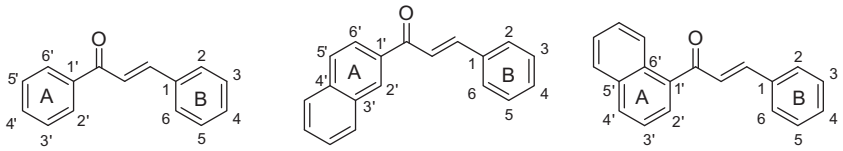
Table 1
Synthesized chalcones and their inhibitory potencies against MCF-7 MX and MDCK BCRP cells using Hoechst 33342 assay



1-30 31-37 38-44

Chalcone	Substituents on ring A	Substituents on ring B	MCF-7 MX IC_{50} ± SD [μM]	MDCK BCRP IC_{50} ± SD [μM]
1	H	3,4-OCH_3	n.a.	n.a.
2	H	2-Cl	n.a.	n.a.
3	2′-OH	H	n.a.	n.a.
4	2′-OH	4-OCH_3	n.a.	n.a.
5	2′-OH	3,4-OCH_3	5.07 ± 0.27	4.97 ± 0.31
6	2′,4′-OH	H	n.a.	n.a.
7	2′,4′-OH	4-OCH_3	33.32 ± 7.09	12.05 ± 1.69
8	2′,4′-OH	3,4-OCH_3	0.93 ± 0.03	0.85 ± 0.13
9	2′,4′-OH	3- OCH_3	2.80 ± 0.17	1.94 ± 0.17
10	2′,4′-OH	2-Cl	3.21 ± 0.69	3.28 ± 0.50
11	2′,4′-OH	4-Cl	6.07 ± 2.37	6.37 ± 0.70
12	2′-OH,4′-OCH_3	H	31.02 ± 6.39	30.95 ± 1.34
13	2′-OH,4′-OCH_3	4-OCH_3	4.20 ± 0.76	2.20 ± 0.75
14	2′-OH,4′-OCH_3	3,4-OCH_3	2.17 ± 0.41	1.06 ± 0.17
15	2′-OH,4′-OCH_3	3-OCH_3	3.15 ± 0.59	1.47 ± 0.31
16	2′-OH,4′-OCH_3	2-Cl	6.85 ± 0.76	3.32 ± 0.57
17	2′-OH,4′-OCH_3	4-Cl	13.51 ± 1.02	10.72 ± 2.82
18	2′-OH,4′-OCH_3	3,4-Cl	n.a.	n.a.
19	2′,4′-OCH_3	4-OCH_3	2.34 ± 0.62	1.91 ± 0.78
20	2′,4′-OCH_3	3,4-OCH_3	2.20 ± 0.40	1.03 ± 0.29
21	2′,4′-OCH_3	2-Cl	3.13 ± 0.57	2.63 ± 0.29
22	2′,4′-OCH_3	4-Cl	3.09 ± 0.44	1.13 ± 0.21
23	2′,4′-OCH_3	3,4-Cl	n.a.	n.a.
24	2′-OH,4′,6′-OCH_3	4-OCH_3	5.83 ± 0.32	3.00 ± 0.50
25	2′-OH,4′,6′-OCH_3	3,4-OCH_3	0.75 ± 0.03	0.53 ± 0.22
26	2′-OH,4′,6′-OCH_3	2-Cl	5.27 ± 0.35	2.82 ± 0.26
27	3′,4′-OCH_3	4-OCH_3	2.19 ± 0.43	1.16 ± 0.34
28	3′,4′-OCH_3	3,4-OCH_3	3.28 ± 0.64	3.80 ± 0.13
29	3′,4′-OCH_3	2-Cl	11.04 ± 2.32	7.80 ± 2.01
30	3′,4′-OCH_3	4-Cl	2.72 ± 0.42	1.73 ± 0.13
31	2′-OH	H	24.32 ± 3.11	9.23 ± 1.00
32	2′-OH	4-OCH_3	16.92 ± 1.02	9.83 ± 0.72
33	2′-OH	3,4-OCH_3	2.50 ± 0.45	1.95 ± 0.39
34	2′-OH	3-OCH_3	11.89 ± 1.97	5.68 ± 0.50
35	2′-OH	2-Cl	n.a.	n.a.
36	2′-OH	4-Cl	n.a.	n.a.
37	2′-OH	3,4-Cl	26.12 ± 3.77	22.39 ± 6.88
38	2′-OH	H	2.77 ± 0.41	2.58 ± 0.94
39	2′-OH	4-OCH_3	3.81 ± 0.62	2.89 ± 0.73
40	2′-OH	3,4-OCH_3	1.84 ± 0.87	0.72 ± 0.14
41	2′-OH	3-OCH_3	1.93 ± 0.15	1.93 ± 0.15
42	2′-OH	2-Cl	5.21 ± 0.75	5.58 ± 0.60
43	2′-OH	4-Cl	n.a.	n.a.
44	2′-OH	3,4-Cl	8.78 ± 1.66	7.26 ± 1.10
Ko143			0.33 ± 0.05	0.26 ± 0.02

Data are expressed as mean ± SD (n = 3). n.a. = compounds showed no inhibitory effect. Ko143 was used for comparison.

to be the most active ones with IC_{50} values in the high nanomolar range, being only about threefold less active than Ko143 the most potent BCRP inhibitor.

2. Results and discussion

2.1. Chemistry

Based on the results obtained in earlier studies[14,15] we synthesized different chalcones with varying substituents on ring A and ring B. These chalcones could be divided into three different subtypes: chalcones (**1–30**), 3′,4′-benzochalcones (**31–37**) and 5′,6′-benzochalcones (**38–44**). All the compounds were synthesized by modified Claisen–Schmidt condensation of different substituted acetophenones and benzaldehydes. Compounds **1–30** were synthesized by the classical method in which aqueous alkaline base (NaOH) was used as catalyst and reaction was carried out at room temperature with stirring. Compounds **31–44** were synthesized under ultrasound bath using LiOH as catalyst. Ultrasonication led to much smaller reaction time and products obtained were almost always pure as compared to classical method. All the synthesized compounds are listed in Table 1.

2.2. Hoechst 33342 assay: inhibition of BCRP by chalcones

All the synthesized chalcones were tested for BCRP inhibition by the Hoechst 33342 assay using two different cell lines MCF-7 MX and MDCK BCRP showing resistance due to presence of BCRP.

Hoechst 33342 is a substrate of BCRP[3,19] and has been used as a tool for investigation of BCRP inhibitors.[17,20] To generate the concentration response curves averages of fluorescence values at plateau of fluorescence–time curves of Hoechst 33342 were plotted against the corresponding logarithmic concentrations. The activity data of investigated compounds are presented in Table 1. A scatter plot of the pIC_{50} values for the investigated chalcones obtained in the Hoechst 33342 assay with MCF-7 MX and MDCK BCRP cells gave a good correlation ($r^2 = 0.87$) and is shown in Figure 1.

It was observed that compounds with no substituents on ring A (**1**, **2**) were inactive and presence of single substitution at position 2′ (**3–5**) did not lead to improved inhibition. But compounds with multiple substituents at positions 2′, 3′, 4′ and 6′ of ring A showed increased BCRP inhibition. In case of chalcones with 3,4-dimethoxy substituents on ring B (**8**, **14**, **20**, **25**), it was observed that compounds with hydroxyl group at position 2′ had slightly increased activity as compared to compounds with methoxy substituents. But no substantial difference was noted for position 4′ of ring A. Presence of a methoxy group at position 6′ had only very slight effect on activity. Compounds with 3′,4′-dimethoxy substituent (**27–30**) showed comparable activity to that having 2′,4′-dimethoxy substituents (**19–23**).

In the study by Han et al., effects of chloro substituents at different positions of ring B were investigated.[15] It was observed that presence of 2-chloro and 4-chloro substituents led to increased BCRP inhibition. This encouraged us to study chalcones with varying methoxy and chloro substituents on ring B, allowing to compare the effect of methoxy and chloro substituents.

In case of all types of chalcones it was clearly observed that substitution was needed on ring B, as unsubstituted chalcones were found to be inactive. Chalcones with 3,4-dimethoxy substitution showed maximum efficacy of BCRP inhibition, with the chalcones **8**, **14**, **20**, **25** being most potent. A 3-methoxy substituent in case of compounds (**9**, **15**) was found to be slightly better compared to 4-methoxy substituent on ring B (**7**, **13**), having the same substituents at ring A. Chalcones with 2-chloro and 4-chloro substituents were found to be active, while presence of a 3,4-dichloro substituent (**18**, **23**) led to inactivity.

We also investigated some benzochalcones to study the effect of variation of ring A structure. In general 5′,6′-benzochalcones (**38–44**) were found to be more potent as compared to 3′,4′-benzochalcones (**31–37**). Among all three subtypes of chalcones studied, 3′,4′-benzochalcones (**31–37**) were found to be least active.

The effect of different substituents on BCRP inhibitory potency of chalcones is summarized in Figure 2.

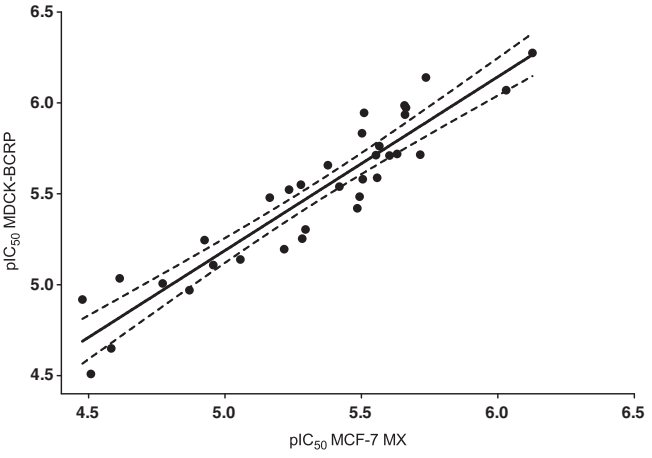


Figure 1. Scatterplot of the pIC_{50} values determined by the Hoechst 33342 assay with MDCK BCRP and MCF-7 MX cells. Each point is an average of at least three independent experiments. The squared correlation coefficient $r^2 = 0.87$.

2.3. Screening of chalcones for P-gp and MRP1 inhibition

To check the selectivity of the chalcones towards BCRP inhibition it was important to investigate their effect on the other major ABC transporters P-gp and MRP1. For this purpose we tested all chalcones at 10 μM final concentration using calcein AM accumulation assay. Figure 3 illustrates the effects of chalcones on accumulation of calcein in P-gp overexpressing A2780adr cells and Figure 4 illustrates their effect on accumulation of calcein in MRP1 overexpressing 2008 MRP1 cells. For comparison purpose cyclosporine A (10 μM) was used as a standard. All the chalcones showed only very weak effect on P-gp, while none of the chalcones showed any inhibition of MRP1. This indicates that the investigated chalcones are selective for BCRP inhibition.

2.4. MTT cytotoxicity assay

To investigate the influence of chalcones on the antiproliferative effect of cytotoxic agents in presence of the BCRP transporter, cytotoxicity of mitoxantrone and SN-38 was evaluated in both MDCK BCRP and MCF-7 MX cells. For this purpose, cell viability was determined using MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide) assay for the most potent chalcone (**25**) and benzochalcone (**40**). EC_{50}-values of the two anticancer agents were determined in the absence and presence of three different concentrations of the selected compounds. Both compounds were able to reverse the resistance of the BCRP expressing cell lines, proving their functional efficacy. Figure 5 shows a representative dose–response curve of SN-38 in presence of compound **40**. The shift in the dose–response curve of MDCK BCRP towards lower concentration indicates the dose dependent BCRP inhibition by compound **40**. Figure 6 illustrates the effect of compound **25** on the EC_{50} of mitoxantrone in MCF-7 cells. Even the lowest concentration of compound **25** (1 μM) led to a reversal of resistance and the higher concentrations were able to reverse the resistance completely.

3. Conclusion

Different types of chalcones have been previously shown to be inhibitors of BCRP, P-gp and MRP1. In the current study we investigated chalcones, 3′,4′-benzochalcones and 5′,6′-benzochalcones for their inhibitory effects and selectivity towards BCRP. The aim of the current work was to study the effect of different substituents on both rings A and B. Also we determined IC_{50} values of chalcones against BCRP using two different cell lines MCF-7 MX and MDCK BCRP, which allowed us to compare the inhibitory potential of these compounds. It was clearly observed that among all chalcones, compounds with 3,4-dimethoxy substituents at ring B were most active. The inhibitory effect of the most active compounds was confirmed by MTT cytotoxicity assay. Compounds **8** and **25** were found to be most potent, being only 3 fold less active as compared to the most active BCRP inhibitor Ko143. We also investigated all chalcones for their inhibitory effect on P-gp and

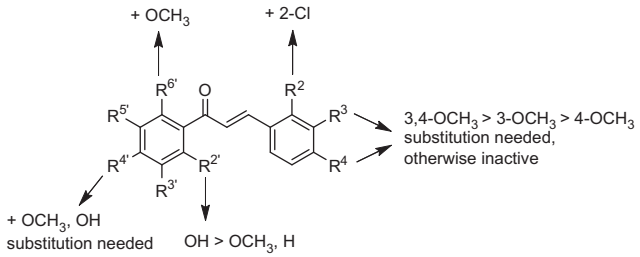


Figure 2. Effect of different substituents on BCRP inhibition by chalcones (**1–30**).

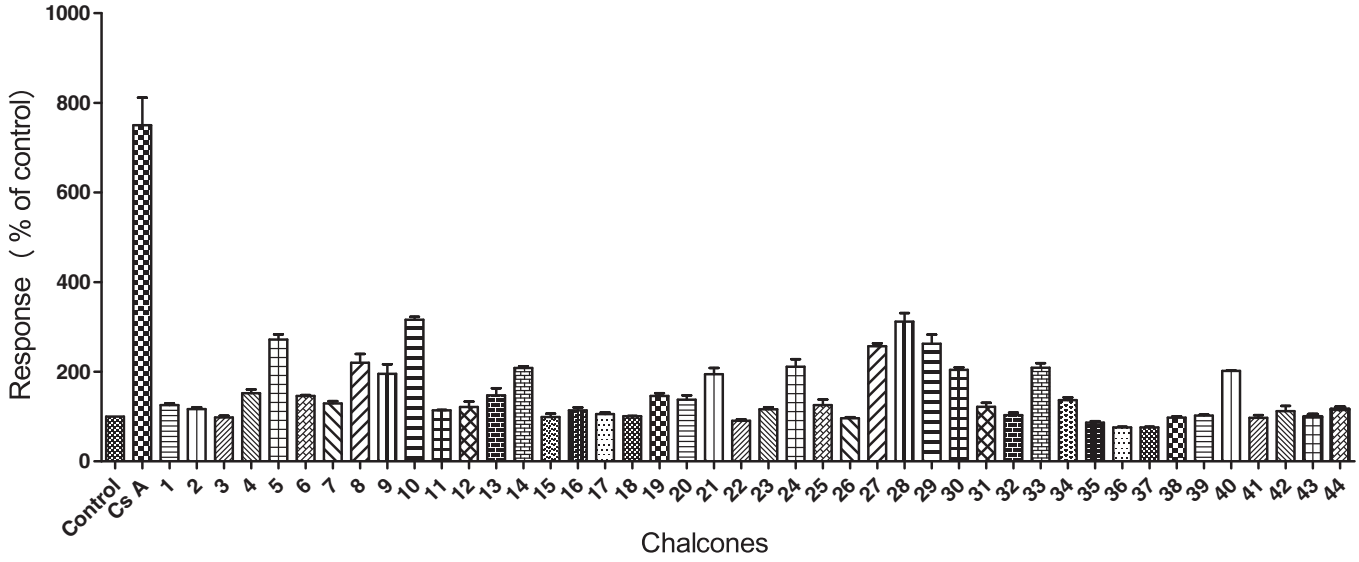


Figure 3. Effect of chalcones **1–44** on the accumulation of calcein AM in P-gp overexpresssing A2780adr cells. All the chalcones were investigated at 10 μM concentration (n = 3). Cyclosporine A (Cs A), 10 μM, was used as a positive control leading to total inhibition. Data is expressed as response in percentage of control (vehicle).

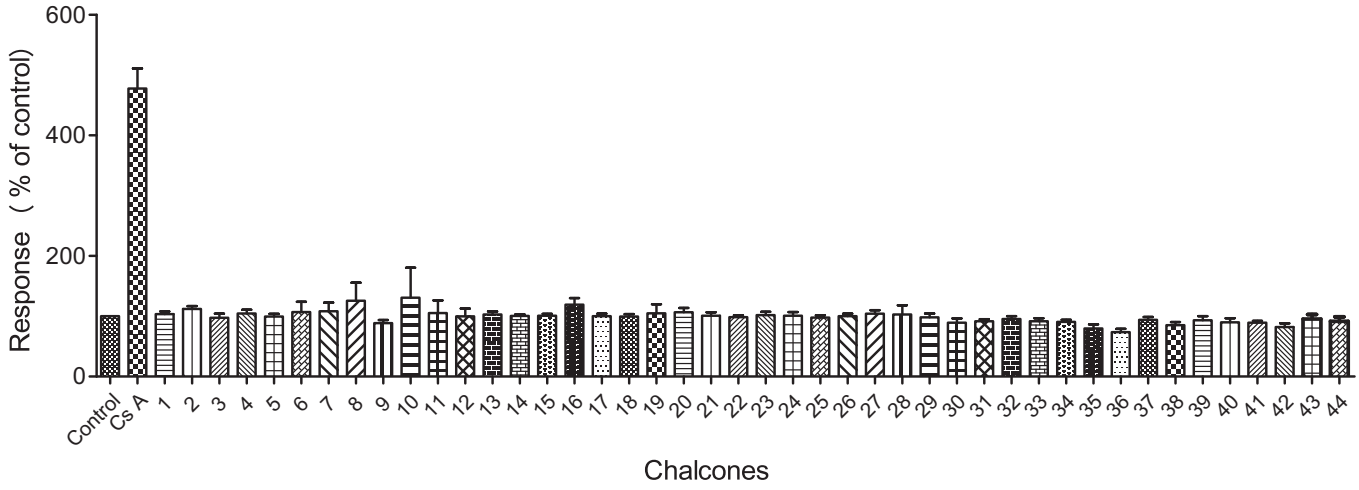


Figure 4. Effect of chalcones **1–44** on the accumulation of calcein AM in MRP1 overexpresssing 2008 MRP1 cells. All the chalcones were investigated at 10 μM concentration (n = 3). Cyclosporine A (Cs A), 10 μM, was used as standard. Data is expressed as response in percentage of control (vehicle).

MRP1, showing selectivity of these chalcones towards BCRP inhibition. The outcome of this study may be useful for the further modifications and investigation of chalcones as BCRP inhibitors.

4. Experimental

4.1. Chemistry

The general synthetic scheme of target chalcones is depicted in Scheme 1. All the chalcones were synthesized by base catalyzed Claisen–Schmidt condensation, from selected benzaldehydes and acetophenones. Syntheses and characterization of chalcones **1–36** and **38–43** have been described earlier.[21–26]

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). Melting points of newly synthesized compounds **37** and **44** were determined by Stuart open capillary melting point instrument and are uncorrected. 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, 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 distortionless 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 were all within ±0.4% of the theoretical values except when indicated.

4.2. General procedures for synthesis of chalcones

For synthesis of chalcones we have used 2 different methods. Chalcones **1–30** were synthesized by method A and chalcones **31–44** were synthesized by method B. For Method B we used LiOH instead of usual base NaOH for Claisen–Schmidt synthesis and the

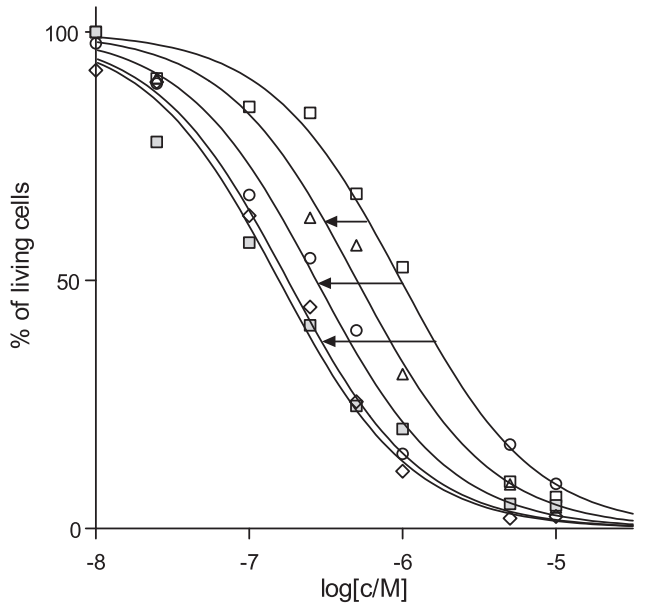


Figure 5. Representative dose–response curve of SN-38 in presence of compound **40**. Arrows indicate dose dependent effect of chalcone on sensitisation of MDCK BCRP cells towards SN-38. Closed squares: MDCK wild type cells, open square: MDCK BCRP cells, triangle: MDCK BCRP cells + 1 μM **40**, circle: MDCK BCRP cells + 3 μM **40**, diamond: MDCK BCRP cells + 5 μM **40**.

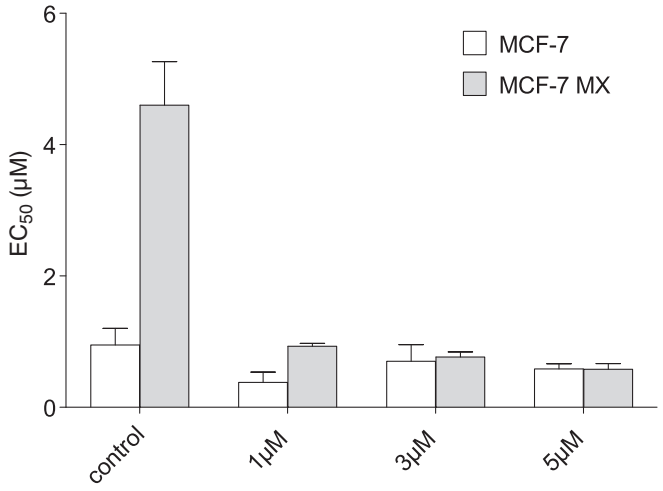


Figure 6. Effect of compound **25** on the EC_{50} of mitoxantrone in MCF-7 cells was investigated at 1, 3, and 5 μM concentrations. Untreated cells were used as control. The figure shows average and standard deviation obtained from three independent experiments.

reaction was carried out in an ultrasonic bath. The general reaction scheme is depicted in Scheme 1.

Method A: 20% NaOH (5 ml) was added dropwise to a previously cooled mixture of 5 mmol of selected acetophenone and 5 mmol of selected benzaldehyde in 25 ml ethanol under vigorous stirring. The mixture was stirred at room temperature for 24–72 h. After completion of the reaction as indicated by TLC, the mixture was poured onto crushed ice and acidified with dilute HCl. Precipitated product was filtered by suction and washed to neutral. The solid was recrystallised from dilute ethanol to get crystalline chalcone.

Method B: To a mixture of 5 mmol of selected acetophenone and 5 mmol of selected benzaldehyde in 50 ml round bottom flask, 20 ml methanol and 35 mmol of LiOH was added. The reaction mixture was kept in an ultrasonic bath for 1–5 h, until reaction completion was indicated by TLC. The reaction was worked up as described for method A.

4.2.1. (*E*)-3-(3,4-Dimethoxyphenyl)-1-phenylprop-2-en-1-one (1)

Synthesized from acetophenone and 3,4-dimethoxybenzaldehyde, yield 61%. ^{1}H NMR ($CDCl_3$) δ: 7.90 (d, J = 15.1 Hz, 1H), 7.80 (dd, J = 7.4, 1.4 Hz, 2H), 7.54 (dd, J = 13.6, 11.3 Hz, 2H), 7.46 (t, J = 7.4 Hz, 2H), 7.21–7.16 (m, 2H), 6.99 (d, J = 7.4 Hz, 1H), 3.82 (d, J = 21.9 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ: 191.02, 150.98, 150.02, 144.78, 139.01, 133.69, 130.20, 129.13, 128.52, 123.94, 123.01, 112.69, 111.32, 55.94, 56.15. Anal. Calcd for $C_{17}H_{16}O_3$:*0.2 H_2O: C, 65.03; H, 9.30; O, 25.67. Found: C, 65.21; H, 9.16.

4.2.2. (*E*)-3-(2-Chlorophenyl)-1-phenylprop-2-en-1-one (2)

Synthesized from acetophenone and 2-chlorobenzaldehyde, yield 64%. ^{1}H NMR ($CDCl_3$) δ: 1H NMR (500 MHz, Chloroform) δ 7.97 (d, J = 14.9 Hz, 1H), 7.69 (dd, J = 7.3, 1.5 Hz, 2H), 7.52–7.49 (m, 1H), 7.48–7.39 (m, 4H), 7.37 (d, J = 14.8 Hz, 1H), 7.34–7.25 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 191.56, 140.83, 138.01, 132.95, 132.04, 131.48, 130.91, 129.14, 128.18, 127.85, 128.15, 127.08, 125.72. Anal. Calcd for $C_{15}H_{11}ClO$: C, 74.23; H, 4.57; Cl, 14.61; O, 6.59. Found: C, 74.08; H, 4.62.

4.2.3. (*E*)-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (3)

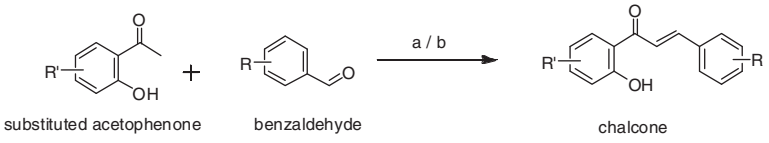
Synthesized from 2-hydroxyacetophenone and benzaldehyde, yield 69%. ^{1}H NMR (DMSO-d_6) δ: 12.45 (s, 1H), 8.24 (dd, 1H), 8.02 (d, J = 15.6 Hz, 1H), 7.92–7.88 (m, 2H), 7.84 (d, J = 15.6 Hz, 1H), 7.56 (ddd, J = 8.4, 7.2, 1.7 Hz, 1H), 7.48–7.45 (m, 3H), 7.03–6.98 (m, 2H). ^{13}C NMR (DMSO-d_6) δ: 193.75, 161.94, 144.87, 136.42, 134.58, 131.07, 131.00, 129.26, 129.08, 121.99, 120.93, 119.29, 117.85. Anal. Calcd for $C_{15}H_{12}O_2$: C, 80.34; H, 5.39. Found: C, 80.51; H, 5.28.

4.2.4. (*E*)-1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (4)

Synthesized from 2-hydroxyacetophenone and 4-methoxybenzaldehyde, yield 78%. ^{1}H NMR (DMSO-d_6) δ: 12.92 (s, 1H), 7.92–7.88 (m, 2H), 7.61 (d, J = 8.7 Hz, 2H), 7.52 (d, J = 15.4 Hz, 1H), 7.47 (ddd, J = 8.6, 7.2, 1.6 Hz, 1H), 7.01 (dd, J = 8.4, 1.1 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.93–6.90 (m, 1H), 3.85 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 193.64, 163.52, 162.00, 145.32, 136.11, 130.52, 129.50, 127.33, 120.10, 118.72, 118.56, 117.58, 114.50, 55.43. Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55. Found: C, 75.24; H, 5.72.

4.2.5. (*E*)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (5)

Synthesized from 2-hydroxyacetophenone and 3,4-dimethoxybenzaldehyde, yield 75%. ^{1}H NMR (DMSO-d_6) δ: 12.89 (s, 1H),



Scheme 1. General scheme for synthesis of chalcones **1**–**44**. (a) For compounds **1–30**: reaction was carried out by stirring at rt for 24–72 h using ethanol as solvent and NaOH as catalyst. (b) For compounds **31–44**: reaction was carried out in an ultrasonic bath at rt for 1–5 h using methanol as solvent and LiOH as catalyst.

7.91 (dd, J = 8.1, 1.6 Hz, 1H), 7.87 (d, J = 15.4 Hz, 1H), 7.50–7.46 (m, 1H), 7.27–7.23 (m, 1H), 7.15 (d, J = 2.0 Hz, 1H), 7.02 (d, J = 1.1 Hz, 1H), 7.00 (d, J = 1.1 Hz, 1H), 6.95–6.88 (m, 2H), 3.94 (d, J = 12.6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ: 193.54, 163.51, 151.79, 149.29, 145.62, 136.13, 129.49, 127.57, 123.56, 120.06, 118.69, 118.58, 117.77, 111.14, 110.26, 56.00, 55.98. Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.79; H, 5.48.

4.2.6. (*E*)-1-(2,4-Dihydroxyphenyl)-3-phenylprop-2-en-1-one (6)

Synthesized from 2,4-dihydroxyacetophenone and benzaldehyde, yield 64%. ^{1}H NMR (DMSO-d_6) δ: 13.35 (s, 1H), 8.19 (d, J = 9.0 Hz, 1H), 7.96 (d, J = 15.4 Hz, 1H), 7.88 (dd, J = 6.7, 3.2 Hz, 2H), 7.79 (d, J = 15.5 Hz, 1H), 7.46 (dd, J = 5.0, 1.9 Hz, 3H), 6.42 (dd, J = 8.9, 2.4 Hz, 1H), 6.30 (d, J = 2.4 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 191.61, 165.93, 165.41, 143.75, 134.75, 133.28, 130.78, 129.36, 129.11, 129.03, 128.65, 121.46, 113.18, 108.41, 102.73. Anal. Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03. Found: C, 75.06; H, 5.16.

4.2.7. (*E*)-1-(2,4-Dihydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (7)

Synthesized from 2,4-dihydroxyacetophenone and 4-methoxybenzaldehyde, yield 79%. ^{1}H NMR (DMSO-d_6) δ: 13.51 (s, J = 3.0 Hz, 1H), 10.64 (s, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.85 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 14.4 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 6.41 (dd, J = 8.9, 2.4 Hz, 1H), 6.28 (d, J = 2.4 Hz, 1H), 3.82 (s, J = 1.8 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 191.66, 165.93, 165.17, 161.62, 143.86, 133.09, 131.97, 131.09, 127.43, 118.74, 114.66, 114.58, 113.17, 108.27, 102.73, 55.56. Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 71.50; H, 4.96.

4.2.8. (*E*)-1-(2,4-Dihydroxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (8)

Synthesized from 2,4-dihydroxyacetophenone and 3,4-dimethoxybenzaldehyde, yield 72%. ^{1}H NMR (DMSO-d_6) δ: 13.55 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.79 (dd, J = 40.4, 15.4 Hz, 2H), 7.54 (d, J = 2.0 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.42 (dd, J = 8.9, 2.4 Hz, 1H), 6.28 (d, J = 2.4 Hz, 1H), 3.84 (d, J = 22.4 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ: 191.68, 165.97, 165.18, 151.59, 149.22, 144.41, 133.12, 127.59, 124.40, 118.73, 113.15, 111.74, 111.02, 108.22, 102.74, 55.93, 55.77. Anal. Calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.73; H, 5.51.

4.2.9. (*E*)-1-(2,4-Dihydroxyphenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (9)

Synthesized from 2,4-dihydroxyacetophenone and 3-methoxybenzaldehyde, yield 67%. ^{1}H NMR (DMSO-d_6) δ: 13.11 (s, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 15.3 Hz, 1H), 7.61 (d, J = 15.3 Hz, 1H,), 7.54–6.58 (m, 6H), 3.89 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 191.66, 165.93, 165.17, 161.62, 143.86, 133.09, 131.97, 131.09, 127.43, 118.74, 114.66, 114.58, 113.17, 108.27, 102.73, 55.56. Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 70.89; H, 5.37.

4.2.10. (*E*)-3-(2-Chlorophenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (10)

Synthesized from 2,4-dihydroxyacetophenone and 2-chlorobenzaldehyde, yield 81%. ^{1}H NMR (DMSO-d_6) δ: 13.17 (s, 1H), 10.79 (s, 1H), 8.21 (dd, J = 7.3, 2.2 Hz, 1H), 8.18 (d, J = 9.0 Hz, 1H), 8.09 (d, J = 15.5 Hz, 1H), 8.01 (d, J = 15.5 Hz, 1H), 7.56 (dd, J = 7.7, 1.6 Hz, 1H), 7.46 (ddd, J = 6.2, 4.4, 1.2 Hz, 2H), 6.43 (dd, J = 8.9, 2.4 Hz, 1H), 6.31 (d, J = 2.4 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 191.15, 165.99, 165.70, 138.16, 134.53, 133.49, 132.41, 132.18, 130.21, 128.88, 127.84, 124.40, 113.24, 108.61, 102.80. Anal. Calcd for $C_{15}H_{11}ClO_3$: C, 65.58; H, 4.04. Found: C, 65.39; H, 4.19.

4.2.11. (*E*)-3-(4-Chlorophenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (11)

Synthesized from 2,4-dihydroxyacetophenone and 4-chlorobenzaldehyde, yield 84%. ^{1}H NMR (DMSO-d_6) δ: 12.58 (s, 1H), 10.58 (s, 1H), 7.95–7.90 (m, 2H), 7.73 (dd, J = 8.9, 5.2 Hz, 1H), 7.55 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.39–7.29 (m, 2H), 6.36 (dd, J = 8.8, 2.4 Hz, 1H), 6.23 (d, J = 2.3 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 202.78, 165.00, 164.33, 142.26, 137.90, 133.80, 131.25, 129.81, 128.85, 122.28, 113.01, 108.23, 102.43. Anal. Calcd for $C_{15}H_{11}ClO_3$: C, 65.58; H, 4.0. Found: C, 65.52; H, 4.21.

4.2.12. (*E*)-1-(2-Hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (12)

Synthesized from 2-hydroxy-4-methoxyacetophenone and benzaldehyde, yield 62%. ^{1}H NMR (DMSO-d_6) δ: 13.49 (s, 1H), 7.95 (d, J = 15.1 Hz, 1H), 7.63 (d, J = 1.7 Hz, 1H), 7.61 (d, J = 9.3 Hz, 1H), 7.56–7.53 (m, 2H), 7.43–7.35 (m, 3H), 6.65 (dd, J = 7.6, 1.4 Hz, 1H), 6.61 (d, J = 1.6 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 192.78, 165.22, 164.48, 144.23, 135.03, 130.98, 129.17, 128.88, 129.87, 128.07, 128.07, 122.22, 114.91, 106.23, 101.47, 56.04. Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55. Found: C, 75.62; H, 5.39.

4.2.13. (*E*)-1-(2-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (13)

Synthesized from 2-hydroxy-4-methoxyacetophenone and 4-methoxybenzaldehyde, yield 71%. ^{1}H NMR (DMSO-d_6) δ: 13.56 (s, 1H), 8.26 (d, J = 9.1 Hz, 1H), 7.87 (d, J = 8.8 Hz, 2H), 7.85 (s, 1H), 7.81 (s, 1H), 7.02 (d, J = 8.8 Hz, 2H), 6.55 (dd, J = 9.0, 2.5 Hz, 1H), 6.50 (d, J = 2.5 Hz, 1H), 3.83 (d, J = 8.1 Hz, 6H). ^{13}C NMR (DMSO) δ: 192.04, 165.99, 165.85, 161.72, 144.38, 132.67, 131.22, 127.36, 118.65, 114.59, 114.01, 107.43, 101.08, 55.87, 55.55. Anal. Calcd for $C_{17}H_{16}O_4$*0.2 H_2O: C, 71.20; H, 5.91. Found: C, 71.14; H, 5.69.

4.2.14. (*E*)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (14)

Synthesized from 2-hydroxy-4-methoxyacetophenone and 3,4-dimethoxybenzaldehyde, yield 89%. ^{1}H NMR (DMSO-d_6) δ: 13.61 (s, 1H), 8.28 (d, J = 9.1 Hz, 1H), 7.87 (d, J = 15.3 Hz, 1H), 7.79 (d, J = 15.3 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.41 (dd, J = 8.4, 1.9 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.56 (dd, J = 9.0, 2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 3.87–3.81 (m, 9H). ^{13}C NMR (DMSO-d_6) δ: 192.07, 166.00, 165.94, 151.72, 149.23, 144.93, 132.69, 127.54, 124.55, 118.66, 113.98, 111.75, 111.14, 107.46, 101.04, 55.95, 55.88, 55.78. Anal. Calcd for $C_{18}H_{18}O_5$*0.25 H_2O: C, 67.81; H, 5.81. Found: C, 67.71; H, 5.77.

4.2.15. (*E*)-1-(2-Hydroxy-4-methoxyphenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (15)

Synthesized from 2-hydroxy-4-methoxyacetophenone and 3-methoxybenzaldehyde, yield 68%. ^{1}H NMR (DMSO-d_6) δ: 13.40 (s, 1H), 8.29 (d, J = 9.1 Hz, 1H), 8.00 (d, J = 15.5 Hz, 1H), 7.79 (d, J = 15.5 Hz, 1H), 7.51–7.48 (m, 1H), 7.45 (d, J = 7.6 Hz, 1H), 7.37 (t, J = 7.9 Hz, 1H), 7.03 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 6.56 (dd, J = 9.0, 2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 3.84 (d, J = 8.2 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ: 192.04, 166.19, 165.88, 159.80, 144.24, 136.06, 132.90, 130.05, 122.06, 121.64, 117.01, 114.01, 113.67, 107.55, 101.06, 55.89, 55.45. Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.66; H, 5.486.

4.2.16. (*E*)-3-(2-Chlorophenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (16)

Synthesized from 2-hydroxy-4-methoxyacetophenone and 2-chlorobenzaldehyde, yield 74%. ^{1}H NMR (DMSO-d_6) δ: 13.19 (s, 1H), 8.26 (d, J = 9.1 Hz, 1H), 8.22 (dd, J = 7.4, 2.1 Hz, 1H), 8.11 (d, J = 15.5 Hz, 1H), 8.04 (d, J = 15.4 Hz, 1H), 7.57 (dd, J = 7.7, 1.6 Hz,

1H), 7.47 (ddd, J = 14.5, 7.1, 1.8 Hz, 2H), 6.57 (dd, J = 9.0, 2.5 Hz, 1H), 6.52 (d, J = 2.5 Hz, 1H), 3.85 (s, J = 6.4 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 191.55, 166.38, 165.83, 138.54, 134.58, 133.01, 132.30, 132.26, 130.18, 128.92, 127.82, 124.31, 114.08, 107.72, 101.13, 55.96. Anal. Calcd for $C_{16}H_{13}ClO_3$*0.2 H_2O: C, 65.74; H, 4.62. Found: C, 65.71; H, 4.388.

4.2.17. (*E*)-3-(4-Chlorophenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (17)

Synthesized from 2-hydroxy-4-methoxyacetophenone and 4-chlorobenzaldehyde, yield 79%. ^{1}H NMR (DMSO-d_6) δ: 13.34 (s, 1H), 8.27 (d, J = 9.1 Hz, 1H), 8.02 (d, J = 15.5 Hz, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 15.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H), 6.56 (dd, J = 9.0, 2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 191.87, 166.24, 165.85, 142.72, 135.40, 133.66, 132.90, 130.88, 129.07, 122.19, 114.01, 107.59, 101.08, 55.91. Anal. Calcd for $C_{16}H_{13}ClO_3$: C, 66.56; H, 4.54. Found: C, 66.31; H, 4.54.

4.2.18. (*E*)-3-(3,4-Dichlorophenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (18)

Synthesized from 2-hydroxy-4-methoxyacetophenone and 3,4-dichlorobenzaldehyde, yield 67%. ^{1}H NMR (DMSO-d_6) δ: 13.29 (s, 1H), 8.31–8.28 (m, 2H), 8.09 (d, J = 15.5 Hz, 1H), 7.87 (dd, J = 8.4, 2.0 Hz, 1H), 7.76 (d, J = 15.5 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 6.57 (dd, J = 9.0, 2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 191.75, 166.39, 165.96, 141.37, 135.58, 133.05, 133.02, 131.99, 131.13, 130.40, 129.48, 123.59, 114.03, 107.66, 101.08, 55.96. Anal. Calcd for $C_{16}H_{12}Cl_2O_3$: C, 59.46; H, 3.74. Found: C, 59.41; H, 3.79.

4.2.19. (*E*)-1-(2,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (19)

Synthesized from 2,4-dimethoxyacetophenone and 4-methoxybenzaldehyde, yield 85%. ^{1}H NMR (DMSO-d_6) δ: 7.68–7.64 (m, 2H), 7.58 (d, J = 8.6 Hz, 1H), 7.50 (d, J = 15.8 Hz, 1H), 7.39 (dd, J = 15.8, 0.7 Hz, 1H), 7.01–6.96 (m, 2H), 6.67 (d, J = 2.2 Hz, 1H), 6.65–6.61 (m, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.79 (d, J = 3.7 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 189.51, 163.84, 161.15, 160.16, 141.45, 131.97, 130.23, 127.58, 124.92, 121.82, 114.59, 106.03, 98.80, 56.05, 55.69, 55.44. Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.58; H, 5.95.

4.2.20. (*E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (20)

Synthesized from 2,4-dimethoxyacetophenone and 3,4-dimethoxybenzaldehyde, yield 76%. ^{1}H NMR (DMSO-d_6) δ: 7.56 (d, J = 8.5 Hz, 1H), 7.47 (d, J = 15.8 Hz, 1H), 7.39 (d, J = 15.7 Hz, 1H), 7.30 (d, J = 1.9 Hz, 1H), 7.26 (dd, J = 8.3, 2.0 Hz, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.68 (d, J = 2.2 Hz, 1H), 6.63 (dd, J = 8.6, 2.2 Hz, 1H), 3.88 (s, 3H), 3.84 (s, J = 2.4 Hz, 3H), 3.80 (d, J = 7.4 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ: 189.79, 163.74, 160.08, 151.03, 149.12, 142.03, 131.85, 127.81, 125.21, 122.70, 121.93, 111.91, 110.95, 105.99, 98.85, 56.03, 55.73, 55.70,55.70. Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 69.29; H, 6.01.

4.2.21. (*E*)-3-(2-Chlorophenyl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (21)

Synthesized from 2,4-dimethoxyacetophenone and 2-chlorobenzaldehyde, yield 69%. ^{1}H NMR (DMSO-d_6) δ: 7.92 (dd, J = 7.2, 2.3 Hz, 1H), 7.82 (d, J = 15.8 Hz, 1H), 7.64 (d, J = 8.6 Hz, 1H), 7.59 (d, J = 15.8 Hz, 1H), 7.56–7.53 (m, 1H), 7.43 (ddd, J = 6.7, 5.7, 1.8 Hz, 2H), 6.69 (d, J = 2.3 Hz, 1H), 6.65 (dd, J = 8.6, 2.3 Hz, 1H), 3.90 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 189.06, 164.39, 160.57, 135.96, 134.07, 132.74, 132.30, 131.61, 130.13, 129.92, 128.23, 127.93, 121.14, 106.31, 98.75, 56.13, 55.76. Anal. Calcd for $C_{17}H_{15}ClO_3$: C, 67.44; H, 4.99. Found: C, 67.73; H, 5.183.

4.2.22. (*E*)-3-(4-Chlorophenyl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (22)

Synthesized from 2,4-dimethoxyacetophenone and 4-chlorobenzaldehyde, yield 72%. ^{1}H NMR (DMSO-d_6) δ: 7.95 (dd, J = 7.1, 2.3 Hz, 1H), 7.79 (d, J = 15.6 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), (d, J = 7.4 Hz, 2H), 7.43 (dd, J = 11.2, 7.7 Hz, 3H), 6.69 (d, J = 2.3 Hz, 1H), 6.65 (dd, J = 8.6, 2.3 Hz, 1H), 3.91 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 189.08, 164.42, 161.22, 135.76, 133.99, 132.84, 132.26, 131.57, 130.78, 130.03, 128.74, 128.02, 121.12, 106.45, 98.64, 56.23, 55.68. Anal. Calcd for $C_{17}H_{15}ClO_3$: C, 67.44; H, 4.99. Found: C, 67.25; H, 5.02.

4.2.23. (*E*)-3-(3,4-Dichlorophenyl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (23)

Synthesized from 2,4-dimethoxyacetophenone and 3,4-dichlorobenzaldehyde, yield 67%. ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, J = 2.0 Hz, 1H), 7.73 (dd, J = 8.4, 2.0 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.63–7.57 (m, 2H), 7.49 (d, J = 15.9 Hz, 1H), 6.68 (d, J = 2.3 Hz, 1H), 6.64 (dd, J = 8.6, 2.3 Hz, 1H), 3.89 (s, J = 2.1 Hz, 3H), 3.85 (s, J = 1.9 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 189.23, 164.32, 160.56, 138.40, 136.02, 132.40, 132.24, 131.90, 131.17, 130.32, 129.27, 128.14, 121.33, 106.22, 98.80, 56.17, 55.78. Anal. Calcd for $C_{17}H_{14}Cl_2O_3$: C, 60.55; H, 4.18. Found: C, 60.72; H, 4.23.

4.2.24. (*E*)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (24)

Synthesized from 2-hydroxy-4,6-dimethoxyacetophenone and 4-methoxybenzaldehyde, yield 55%. ^{1}H NMR (DMSO-d_6) δ: 13.75 (s, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 16.1 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 16.1 Hz, 1H), 6.31–6.27 (m, 2H), 3.78 (s, 3H), 3.70 (s, 6H). ^{13}C NMR (DMSO-d_6) δ: 193.33, 161.93, 161.32, 158.12, 143.76, 130.40, 127.55, 127.06, 127.02, 125.04, 114.58, 111.45, 93.81, 91.26, 90.97, 55.93, 55.57, 55.46. Anal. Calcd for $C_{18}H_{18}O_5$*0.5 H_2O: C, 67.64; H, 6.27. Found: C, 67.47; H, 6.00.

4.2.25. (*E*)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4,6-dimethoxy-phenyl)prop-2-en-1-one (25)

Synthesized from 2-hydroxy-4,6-dimethoxyacetophenone and 3,4-dimethoxybenzaldehyde, yield 71%. ^{1}H NMR (DMSO-d_6) δ: 13.40 (s, 1H), 7.62 (d, J = 7.4 Hz, 2H), 7.29 (s, J = 6.1 Hz, 2H), 7.01 (d, J = 8.7 Hz, 1H), 6.14 (d, J = 2.3 Hz, 1H), 6.11 (d, J = 2.3 Hz, 1H), 3.88 (s, J = 2.9 Hz, 3H), 3.82 (s, J = 4.8 Hz, 3H), 3.81 (d, J = 2.1 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ: 192.41, 165.39, 165.36, 161.88, 151.28, 149.15, 143.13, 127.77, 125.38, 122.92, 111.97, 111.02, 106.63, 94.06, 91.18, 56.27, 56.17, 55.76, 55.67. Anal. Calcd for $C_{19}H_{20}O_6$*0.33 H_2O: C, 65.13; H, 5.95. Found: C, 65.00; H, 6.12.

4.2.26. (*E*)-3-(2-Chlorophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one (26)

Synthesized from 2-hydroxy-4,6-dimethoxyacetophenone and 2-chlorobenzaldehyde, yield 68%. ^{1}H NMR (DMSO-d_6) δ: 13.22 (s, 1H), 7.92–7.88 (m, 2H), 7.77 (d, J = 15.7 Hz, 1H), 7.57–7.54 (m, 1H), 7.46–7.42 (m, 2H), 6.15 (d, J = 2.3 Hz, 1H), 6.13 (d, J = 2.3 Hz, 1H), 3.88 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 192.04, 165.94, 165.61, 162.08, 136.79, 134.10, 132.65, 131.79, 130.47, 130.20, 128.36, 128.01, 106.51, 94.06, 91.26, 56.38, 55.83. Anal. Calcd for $C_{17}H_{15}ClO_4$: C, 64.06; H, 4.74. Found: C, 63.69; H, 4.80.

4.2.27. (*E*)-1-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (27)

Synthesized from 3,4-dimethoxyacetophenone and 4-methoxybenzaldehyde, yield 81%. ^{1}H NMR (DMSO-d_6) δ: 7.87 (dd, J = 8.4, 1.9 Hz, 1H), 7.83 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 15.5 Hz, 1H), 7.67

(d, J = 15.5 Hz, 1H), 7.59 (d, J = 1.9 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 8.7 Hz, 2H), 3.86 (d, J = 5.6 Hz, 6H), 3.81 (s, J = 3.5 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 187.41, 161.34, 153.22, 148.93, 143.15, 130.91, 130.78, 127.62, 123.27, 119.64, 114.51, 111.06, 110.97, 55.91, 55.76, 55.50. Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.12; H, 6.20.

4.2.28. (*E*)-1,3-Bis(3,4-dimethoxyphenyl)prop-2-en-1-one (28)

Synthesized from 3,4-dimethoxyacetophenone and 3,4-dimethoxybenzaldehyde, yield 81%. ^{1}H NMR (DMSO-d_6) δ: 7.80 (d, J = 15.5 Hz, 1H), 7.66–7.63 (m, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 7.04 (d, J = 2.3 Hz, 1H), 7.02 (d, J = 3.5 Hz, 1H), 6.76 (d, J = 1.0 Hz, 1H), 3.86–3.80 (m, 12H). ^{13}C NMR (DMSO-d_6) δ: 197.32, 153.17, 153.09, 149.10, 148.61, 143.59, 130.88, 129.90, 123.59, 123.33, 122.74, 119.73, 111.72, 110.93, 110.43, 55.86, 55.81, 55.57, 55.54. Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 69.34; H, 6.23.

4.2.29. (*E*)-3-(2-Chlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (29)

Synthesized from 3,4-dimethoxyacetophenone and 2-chlorobenzaldehyde, yield 73%. ^{1}H NMR (DMSO-d_6) δ: 7.99 (d, J = 4.4 Hz, 1H), 7.65 (dd, J = 8.5, 2.0 Hz, 1H), 7.52 (dd, J = 7.8, 1.7 Hz, 1H), 7.42 (d, J = 2.0 Hz, 1H), 7.36 (dd, J = 7.9, 1.3 Hz, 1H), 7.25–7.22 (m, 1H), 7.18–7.14 (m, 1H), 7.04 (d, J = 8.5 Hz, 2H), 3.83 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 196.75, 153.22, 148.65, 141.75, 137.78, 133.08, 129.66, 129.41, 128.37, 127.75, 127.29, 124.94, 122.77, 110.98, 110.47, 55.85, 55.61. Anal. Calcd for $C_{17}H_{15}ClO_3$*0.25 H_2O: C, 66.45; H, 5.08. Found: C, 66.67; H, 5.293.

4.2.30. (*E*)-3-(4-Chlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (30)

Synthesized from 3,4-dimethoxyacetophenone and 4-chlorobenzaldehyde, yield 77%. ^{1}H NMR (DMSO-d_6) δ: 7.95 (d, J = 15.6 Hz, 1H), 7.92 (s, J = 1.9 Hz, 1H), 7.90 (dd, J = 8.5, 2.0 Hz, 2H), 7.68 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.51 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 8.5 Hz, 1H), 3.86 (d, J = 7.8 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ: 187.38, 153.50, 148.99, 141.70, 134.99, 133.96, 130.63, 130.63, 130.55, 129.03, 129.03 123.62, 122.90, 111.08, 110.99, 55.95, 55.78. Anal. Calcd for $C_{17}H_{15}ClO_3$: C, 67.44; H, 4.99. Found: C, 67.19; H, 5.01.

4.2.31. (*E*)-1-(1-Hydroxynaphthalen-2-yl)-3-phenylprop-2-en-1-one (31)

Synthesized from 2-acetyl-1-hydroxynaphthalene and benzaldehyde, yield 88%. ^{1}H NMR (DMSO-d_6) δ: 15.00 (s, 1H), 8.37 (dd, J = 8.3, 0.7 Hz, 1H), 8.30 (d, J = 9.0 Hz, 1H), 8.18 (d, J = 15.5 Hz, 1H), 7.97 (dd, J = 6.2, 3.4 Hz, 2H), 7.93 (d, J = 8.3 Hz, 1H), 7.73 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.60 (ddd, J = 8.2, 6.9, 1.1 Hz, 1H), 7.51–7.48 (m, 3H), 7.46 (d, J = 8.9 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 193.72, 163.37, 145.42, 137.22, 134.59, 131.19, 130.60, 129.45, 129.08, 127.74, 126.30, 125.17, 124.58, 123.78, 121.18, 118.38, 113.47. Anal. Calcd for $C_{19}H_{14}O_2$: C, 83.19; H, 5.14. Found: C, 82.87; H, 5.26.

4.2.32. (*E*)-1-(1-Hydroxynaphthalen-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (32)

Synthesized from 2-acetyl-1-hydroxynaphthalene and 4-methoxybenzaldehyde, yield 84%. ^{1}H NMR (DMSO-d_6) δ: 15.18 (s, 1H), 8.36 (d, J = 8.2 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H), 8.04 (d, J = 15.4 Hz, 1H), 7.94 (d, J = 9.2 Hz, 3H), 7.92 (d, J = 3.9 Hz, 1H), 7.72 (ddd, J = 8.1, 6.9, 1.3 Hz, 1H), 7.59 (ddd, J = 8.2, 5.0, 1.1 Hz, 1H), 7.45 (d, J = 8.9 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 193.59, 163.26, 161.97, 145.58, 137.11, 131.53, 130.45, 127.71, 127.29, 126.22, 125.13, 123.73, 118.42, 118.25, 114.63, 113.47, 55.57. Anal. Calcd for $C_{20}H_{16}O_3$*0.25 H_2O: C, 77.95; H, 5.58. Found: C,77.83; H, 5.37.

4.2.33. (*E*)-3-(3,4-Dimethoxyphenyl)-1-(1-hydroxynaphthalen-2-yl)prop-2-en-1-one (33)

Synthesized from 2-acetyl-1-hydroxynaphthalene and 3,4-dimethoxybenzaldehyde, yield 79%. ^{1}H NMR (DMSO-d_6) δ: 14.08 (s, 1H), 8.36 (d, J = 8.2 Hz, 1H), 8.25 (d, J = 9.0 Hz, 1H), 8.14 (d, J = 15.4 Hz, 1H), 7.94 (d, J = 9.2 Hz, 3H), 7.92 (d, J = 3.9 Hz, 1H), 7.72 (m, 2H), 7.59 (d, J = 8.2, 1H), 7.47 (d, J = 8.9 Hz, 1H), 3.83 (d, 6H). ^{13}C NMR (DMSO-d_6) δ: 193.60, 158.12, 150.2, 149.90, 144.56, 135.26, 130.93, 129.67, 127.68, 127.35, 126.52, 124.99, 123.25, 122.24, 121.93, 121.12, 120.83, 113.57, 110.67, 55.92, 55.57. Anal. Calcd for $C_{21}H_{18}O_4$: C, 75.43; H, 5.43. Found: C, 75.37; H, 5.51.

4.2.34. (*E*)-1-(1-Hydroxynaphthalen-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (34)

Synthesized from 2-acetyl-1-hydroxynaphthalene and 3-methoxybenzaldehyde, yield 67%. ^{1}H NMR (DMSO-d_6) δ: 14.05 (s, 1H), 8.33 (d, J = 8.7 Hz, 2H), 8.18 (d, J = 15.4 Hz, 1H), 7.93 (dd, J = 15.4, 7.3 Hz, 3H), 7.86 (d, J = 8.8 Hz, 1H), 7.72 (dddd, J = 14.0, 8.2, 6.9, 1.3 Hz, 2H), 7.63–7.60 (m, 1H), 7.44–7.41 (m, 1H), 3.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 219.48, 193.80, 163.41, 159.87, 147.20, 145.50, 137.26, 136.00, 130.36, 127.78, 126.34, 125.95, 124.60, 124.36, 123.81, 123.71, 121.45, 117.39, 113.88, 55.54. Anal. Calcd for $C_{20}H_{16}O_3$*0.33 H_2O: C, 77.64; H, 5.68. Found: C, 77.59; H, 5.44.

4.2.35. (*E*)-3-(2-Chlorophenyl)-1-(1-hydroxynaphthalen-2-yl)prop-2-en-1-one (35)

Synthesized from 2-acetyl-1-hydroxynaphthalene and 2-chlorobenzaldehyde, yield 72%. ^{1}H NMR (DMSO-d_6) δ: 14.81 (s, 1H), 8.39–8.35 (m, 1H), 8.32 (dd, J = 7.5, 2.1 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H), 8.23 (d, J = 3.0 Hz, 2H), 7.93 (d, J = 8.1 Hz, 1H), 7.73 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H), 7.63–7.57 (m, 2H), 7.51–7.44 (m, 3H). ^{13}C NMR (DMSO-d_6) δ: 193.37, 163.51, 139.61, 137.33, 134.79, 132.53, 132.17, 130.78, 130.23, 129.12, 127.85, 127.78, 126.39, 125.17, 124.55, 123.97, 123.87, 118.52, 113.49. Anal. Calcd for $C_{19}H_{13}ClO_2$: C, 73.91; H, 4.24. Found: C, 74.05; H, 4.19.

4.2.36. (*E*)-3-(4-Chlorophenyl)-1-(1-hydroxynaphthalen-2-yl)prop-2-en-1-one (36)

Synthesized from 2-acetyl-1-hydroxynaphthalene and 4-chlorobenzaldehyde, yield 76%. ^{1}H NMR (DMSO-d_6) δ: 14.95 (s, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.31 (d, J = 9.0 Hz, 1H), 8.20 (d, J = 15.5 Hz, 1H), 8.02 (d, J = 8.6 Hz, 2H), 7.95 (d, J = 5.2 Hz, 1H), 7.93 (d, J = 1.6 Hz, 1H), 7.74–7.70 (m, 1H), 7.64–7.61 (m, 1H), 7.56 (d, J = 8.5 Hz, 2H), 7.46 (d, J = 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 193.63, 163.40, 143.92, 137.25, 135.72, 133.57, 131.14, 130.67, 129.14, 127.76, 126.34, 125.17, 124.55, 123.80, 121.96, 118.43, 113.48. Anal. Calcd for $C_{19}H_{13}ClO_2$: C, 73.91; H, 4.24. Found: C, 73.85; H, 4.33.

4.2.37. (*E*)-3-(3,4-Dichlorophenyl)-1-(1-hydroxynaphthalen-2-yl)prop-2-en-1-one (37)

Synthesized from 2-acetyl-1-hydroxynaphthalene and 3,4-dichlorobenzaldehyde, yield 64%, mp 161 °C. ^{1}H NMR (DMSO-d_6-d_6) δ: 14.89 (s, J = 1.5 Hz, 1H), 8.37 (d, J = 1.9 Hz, 1H), 8.33 (d, J = 9.1 Hz, 1H), 8.27 (d, J = 15.5 Hz, 1H), 7.94 (dd, J = 8.4, 2.3 Hz, 2H), 7.74 (d, J = 8.3 Hz, 2H), 7.71 (ddd, J = 8.2, 5.0, 1.3 Hz, 1H), 7.60 (m, J = 11.3, 8.2, 6.9, 1.2 Hz, 2H), 7.47 (d, J = 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 193.57, 163.53, 142.58, 137.36, 135.49, 133.35, 132.09, 131.21, 130.81, 130.63, 129.78, 127.82, 126.43, 125.27, 124.56, 123.87, 123.38, 118.49, 113.54. Anal. Calcd for $C_{19}H_{12}Cl_2O_2$: C, 66.49; H, 3.52. Found: C, 66.23; H, 3.61.

4.2.38. (*E*)-1-(2-Hydroxynaphthalen-1-yl)-3-phenylprop-2-en-1-one (38)

Synthesized from 1-acetyl-2-hydroxynaphthalene and benzaldehyde, yield 59%. ^{1}H NMR (DMSO-d_6) δ: 8.23 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 15.7 Hz, 1H), 7.58 (d, J = 7.3 Hz, 2H), 7.45–7.39 (m, 3H), 7.37 (t, J = 7.4 Hz, 2H), 7.33–7.28 (m, 1H), 7.16 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 6.94–6.85 (m, 1H), 6.61 (d, J = 9.2 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 190.38, 175.79, 136.69, 135.95, 134.84, 133.55, 132.31, 129.58, 128.85, 128.66, 127.87, 127.69, 126.22, 124.34, 123.14, 118.88, 116.93. Anal. Calcd for $C_{19}H_{14}O_2$: C, 83.19; H, 5.14. Found: C, 83.04; H, 5.28.

4.2.39. (*E*)-1-(2-Hydroxynaphthalen-1-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (39)

Synthesized from 1-acetyl-2-hydroxynaphthalene and 4-methoxybenzaldehyde, yield 62%. ^{1}H NMR (DMSO-d_6) δ: 10.15 (s, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.56 (dd, J = 8.4, 0.9 Hz, 1H), 7.41 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H), 7.31 (dt, J = 8.0, 3.9 Hz, 1H), 7.28–7.22 (m, 2H), 7.10 (d, J = 16.1 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 3.78 (s, J = 1.5 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 196.35, 161.46, 152.86, 144.30, 131.66, 131.11, 130.57, 128.40, 128.30, 127.79, 127.19, 127.04, 126.70, 123.47, 123.19, 120.40, 118.53, 114.61, 114.08, 55.48. Anal. Calcd for $C_{20}H_{16}O_3$: C, 78.93; H, 5.30. Found: C, 78.76; H, 5.30.

4.2.40. (*E*)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (40)

Synthesized from 1-acetyl-2-hydroxynaphthalene and 3,4-dimethoxybenzaldehyde, yield 68%. ^{1}H NMR (DMSO-d$_6$) δ: 10.12 (s, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.54 (dd, J = 8.4, 0.9 Hz, 1H), 7.41 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H), 7.33–7.30 (m, 1H), 7.29 (d, J = 2.2 Hz, 1H), 7.25 (dd, J = 12.5, 9.5 Hz, 2H), 7.21–7.17 (m, 1H), 7.15 (d, J = 16.0 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 3.77 (d, J = 2.8 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ: 196.59, 152.73, 151.32, 149.16, 144.94, 131.69, 130.95, 128.29, 127.77, 127.28, 127.15, 126.94, 123.45, 123.26, 123.16, 120.50, 118.55, 111.76, 110.92, 55.75, 55.72. Anal. Calcd for $C_{21}H_{18}O_4$: C, 75.43; H, 5.43. Found: C, 75.50; H, 5.35.

4.2.41. (*E*)-1-(2-Hydroxynaphthalen-1-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (41)

Synthesized from 1-acetyl-2-hydroxynaphthalene and 3-methoxybenzaldehyde, yield 63%. ^{1}H NMR (DMSO-d_6) δ: 8.29 (d, J = 8.5 Hz, 1H), 7.72 (d, J = 15.7 Hz, 1H), 7.41 (d, J = 9.3 Hz, 2H), 7.36 (d, J = 15.7 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 7.16 (dd, J = 6.9, 1.4 Hz, 2H), 7.13–7.11 (m, 1H), 6.92–6.85 (m, 2H), 6.60 (d, J = 9.2 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (DMSO) δ: 190.67, 159.70, 140.32, 138.24, 137.75, 136.00, 134.67, 133.48, 132.71, 129.84, 128.83, 127.84, 126.24, 124.34, 123.06, 120.13, 116.89, 114.46, 112.75, 55.23. Anal. Calcd for $C_{20}H_{16}O_3$: C, 78.93; H, 5.30. Found: C, 78.67; H, 5.46.

4.2.42. (*E*)-3-(2-Chlorophenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (42)

Synthesized from 1-acetyl-2-hydroxynaphthalene and 2-chlorobenzaldehyde, yield 56%. ^{1}H NMR (DMSO-d_6) δ: 8.53 (d, J = 8.6 Hz, 1H), 7.82 (d, J = 15.6 Hz, 1H), 7.76 (dd, J = 7.3, 2.2 Hz, 1H), 7.65 (d, J = 15.6 Hz, 1H), 7.48 (dd, J = 7.0, 2.3 Hz, 1H), 7.40 (d, J = 9.1 Hz, 2H), 7.34–7.30 (m, 2H), 7.15 (ddd, J = 8.5, 6.8, 1.5 Hz, 1H), 6.87 (ddd, J = 7.9, 6.9, 1.2 Hz, 1H), 6.57 (d, J = 9.2 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 190.19, 176.91, 136.23, 135.64, 134.82, 133.80, 133.20, 129.85, 129.77, 129.73, 128.76, 127.80, 127.67, 127.60, 126.46, 124.33, 122.92, 118.89, 116.50. Anal. Calcd for $C_{19}H_{13}ClO_2$: C, 73.91; H, 4.24. Found: C, 73.84; H, 4.31.

4.2.43. (*E*)-3-(4-Chlorophenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (43)

Synthesized from 1-acetyl-2-hydroxynaphthalene and 4-chlorobenzaldehyde, yield 68%. ^{1}H NMR (DMSO-d_6) δ: 10.00 (s, 1H), 8.16 (d, J = 8.9 Hz, 1H), 7.92 (d, J = 8.6 Hz, 1H), 7.67 (dd, J = 6.5, 5.1 Hz, 3H), 7.62 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 9.0 Hz, 1H), 7.12 (d, J = 9.0 Hz, 1H), 5.83 (dd, J = 13.2, 3.1 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 192.74, 163.20, 137.79, 134.31, 133.31, 130.95, 129.68, 129.04, 129.00, 128.83, 128.74, 128.65, 128.13, 125.12, 124.92, 119.11, 112.01. Anal. Calcd for $C_{19}H_{13}ClO_2$: C, 73.91; H, 4.24. Found: C, 73.68; H, 4.26.

4.2.44. 44(*E*)-3-(3,4-Dichlorophenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (44)

Synthesized from 1-acetyl-2-hydroxynaphthalene and 4-chlorobenzaldehyde, yield 71%, mp 123 °C. ^{1}H NMR (DMSO-d_6) δ: 8.42 (d, J = 8.5 Hz, 1H), 7.82 (dd, J = 12.2, 8.7 Hz, 2H), 7.59 (dd, J = 11.2, 5.1 Hz, 2H), 7.41 (dd, J = 5.3, 4.1 Hz, 2H), 7.31 (d, J = 15.7 Hz, 1H), 7.15 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 6.92–6.86 (m, 1H), 6.60 (d, J = 9.2 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 190.07, 176.41, 138.00, 136.07, 134.86, 133.78, 131.60, 131.40, 130.92, 130.44, 129.58, 129.13, 127.83, 127.54, 126.43, 124.40, 122.99, 119.00, 116.65. Anal. Calcd for $C_{19}H_{12}Cl_2O_2$: C, 66.49; H, 3.52. Found: C, 66.60; H, 3.42.

4.3. Biological testing

4.3.1. Chemicals

Ko143 was purchased from Tocris Bioscience (Bristol, United Kingdom). Mitoxantrone hydrochloride was purchased from Molekula (Gillingham, United Kingdom). All other chemicals were purchased from Sigma–Aldrich Chemicals (Taufkirchen, Germany) unless otherwise specified.

4.3.2. Cell culture

The breast cancer cell line MCF-7 MX was kindly provided by Dr. E. Schneider (Wadsworth Center, Albany, NY, USA). The cells were grown in RPMI-1640 medium supplemented with 20% fetal bovine serum (FBS), 50 μg/ml streptomycin, 50 U/ml penicillin G, and 2 mM L-glutamine.

The cell line MDCK BCRP was 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. The cell line was grown in DMEM medium supplemented with 10% FBS, 50 μg/ml streptomycin, 50 U/ml penicillin G. Human ovarian carcinoma cell lines A2780 and its corresponding MDR1 overexpressing doxorubicin resistant A2780adr cell line were purchased from European collection of animal cell cultures (ECACC, Nos. 93112519 and 93112520, UK). The cell lines were grown in RPMI-1640 medium supplemented with 10% FBS, 50 μg/ml streptomycin, 50 U/ml penicillin G, and 365 μg/ml L-glutamine. The human ovarian cancer cell line 2008 stably expressing MRP1was used for MRP1 testing. The cell line 2008MRP1 was grown in same medium as described for A2780 with addition of 400 μg/ml G-418 (Geneticin).

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.

4.3.3. Accumulation assays

4.3.3.1. Hoechst 33342 assay. The Hoechst 33342 assay was performed as described earlier with small modification.[17] The final concentration of the Hoechst was kept at 1 μM. Cell lines were cultivated under standard conditions in T75- or T175-flasks. When

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 resuspended 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 when using MCF-7 MX or MDCK BCRP cells. The non-BCRP overexpressing counter parts of these two cell lines MCF-7 and MDCK were used as controls to identify unspecific interactions of the investigated substances which could influence the detected fluorescence intensities. 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.

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 applying a 37 °C tempered BMG POLARstar microplate reader (BMG LABTECH, Offenburg, Germany).

For the analyses of the data obtained from the assay, first fluorescence of KHB was subtracted from the fluorescence reading obtained from MCF-7 or MDCK cells. Average of fluorescence values from 100 min to 109 min was calculated for each concentration and from this 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).

4.3.3.2. Screening for P-gp and MRP1 inhibition by Calcein-AM uptake assay. Calcein-AM uptake assay was performed as described earlier with small modifications.[27] Cells (A2780adr for P-gp and 2008 MRP1) were grown under standard conditions in T75-flasks. After reaching confluence of approximately 80%, cells were harvested by short trypsination (0.05% trypsin/0.02% EDTA). Pelleted cells were resuspended in fresh culture medium and counted with a Casy I Modell TT cell counter (Schaerfe System GmbH, Reutlingen, Germany). Cells were washed three times with Krebs-HEPES buffer, and then seeded into colorless 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.

4.3.3.3. MTT cytotoxicity assay. The influence of chalcones on the cytotoxic effect of mitoxantrone and SN-38 was studied in MDCK and MCF-7 cells by MTT cytotoxicity assay. Assays were performed as described earlier with minor modifications.[28] Briefly, cells were seeded into 96-well tissue culture plates (Sarstedt, Newton, USA) in the appropriate density evaluated for each cell line (1250 cells per well for MDCK and 10,000 cells per well for MCF-7 cells) in a volume of 100 μl and kept under 5% CO_2 at 37 °C for 6 h. Attachment of cells was controlled by microscopy and the test compound as well as the mitoxantrone or SN-38 were added to achieve the required final concentration in a final volume of 200 μl per well. After an incubation period of 72 h, the MTT reagent was added (40 μl of a 5 mg/mL solution per well). Incubation with MTT was terminated after 1 h by removing the supernatants and lysing the cells with 100 μl DMSO per well. Viability of the cells was measured spectrophotometrically by absorbance at 544 nm and background corrected at 710 nm using a BMG POLARstar microplate reader.

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