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Acryloylphenylcarboxamides: A New Class of Breast Cancer Resistance Protein (ABCG2) Modulators

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Chalcones are easily synthesized natural precursors of secondary plant metabolites, and their derivatives show various biological activities including inhibition of ABC transporters. Especially, their role as inhibitors of ABCG2, the most recently discovered ABC transporter involved in multidrug resistance, inspired the synthesis of new structurally diverse derivatives. Therefore, we combined the typical chalcone moiety with several acid chlorides by using an amide linker at position 2′, 3′, or 4′ on ring A of the chalcone. The resulting 35 compounds covered a wide spectrum of substitution patterns, which allowed development of structure–activity relationships and to find the optimal structural features for further investigations. Synthesized acryloylphenylcarboxamides were investigated for their inhibitory activity against ABCG2 and their behavior toward ABCB1 and ABCC1. Furthermore, for the most promising compounds, their intrinsic cytotoxicity and their ability to reverse ABCG2-mediated multidrug resistance were determined.

Introduction

Multidrug resistance (MDR) of cancer cells is an insidious clinical problem of cancer chemotherapy. The most prominent mechanism generating MDR is the overexpression of ATP-binding cassette (ABC) transporters.[1,2] These transport proteins perform diverse functions in organisms and are present in both prokaryotes and eukaryotes.[3] Whereas importers and exporters can be found in prokaryotes, in mammals only export transporters are found. Currently, 48 human ABC proteins have been isolated and identified. They have been divided into seven subfamilies, ABCA through ABCG.[4] ABC efflux transporters generally possess two nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs) as minimal functional units. The NBDs are responsible for the hydrolysis of ATP to ADP, which delivers the necessary energy for transporting substrates across the membrane against a concentration gradient.[5] Three important ABC transporters involved in MDR are ABCB1 (P-glycoprotein, P-gp), ABCC1 (multidrug resistance-associated protein 1, MRP1), and ABCG2 (breast cancer resistance protein, BCRP).

First discovered in 1998, ABCG2 is the most recently described ABC transporter participating in MDR. The protein is composed of 655 amino acids and has a molecular weight of 72 kDa.[6,7] ABCG2 is a half-transporter with one TMD and one NBD, and it forms dimers or higher oligomers to generate a functional transporter.[8,9] It is widely expressed in organs with barrier functions, such as the placenta, blood–brain barrier, gastrointestinal tract, liver, and kidney, and in many types of cancer as well.[10,11] The structural diversity of substrates ranges from chemotherapeutic drugs such as mitoxantrone over organic anion conjugates to fluorescent chlorophyll degradation products such as pheophorbide A.[12,13]

Several approaches have been explored to overcome MDR, including, for example, application of antisense DNA[14,15] or downregulation of the expression of the transporters.[16] However, the most commonly applied approach is the direct inhibition of the transport function. In this way, the reduced intracellular concentration of the anticancer drug can be restored.[1] During the last decade, a variety of ABCG2 inhibitors were identified. Fumitremorgin (FTC), a natural product isolated from *Aspergillus fumigatus*, was found to completely reverse mitoxantrone resistance in ABCG2 expressing cell lines. Owing to the high neurotoxicity of FTC, the structurally related nontoxic inhibitor Ko143 was developed.[17–21]

Natural products such as flavonoids are becoming more and more interesting in anticancer therapy. More than 6000 naturally occurring flavonoids have been identified to date.[22] They are important constituents of the human diet and have been shown to possess health-optimizing activities, including cancer prevention as a result of several modes of action. More recently, chalcones, the bioprecursors of flavonoids, were also shown to possess a variety of biological activities that may benefit human health.[23] Among their beneficial properties are also anticancer activities, as reported for flavonoids.[24] Similar to flavonoids, chalcones were found to be potent ABCG2 inhibitors with IC_{50} values in the low micromolar range.[25] Owing to the relatively poor knowledge about the structural dependence of inhibition of ABC transporters by chalcones, it is important to develop new structurally deviating derivatives to gain more insight into structure–activity relationships to optimize the scaffold.

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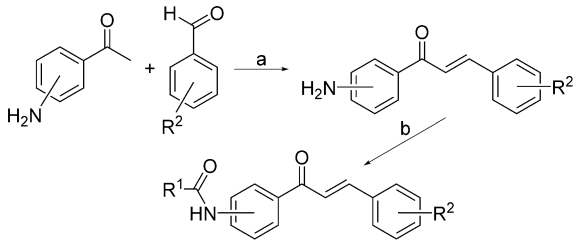
In this study, we combined the chalcone scaffold with different substituted acid chlorides through an amide linker to establish acryloylphenylcarboxamides as a new class of ABCG2 inhibitors. The rationale for selecting an amide linker was the presence of amide groups in a number of ABCG2 inhibitors, including Ko143, the dual ABCB1 and ABCG2 inhibitors elacridar and tariquidar, and others. The amide group could allow hydrogen-bonding interactions with the protein and, in this way, contribute to binding. A series of 35 compounds were synthesized, including structural modifications at position 2′, 3′, and 4′ on ring A and positions 3 and 4 on ring B of the chalcone. All compounds were investigated for their inhibitory effect in ABCG2 overexpressing cell lines by using the pheophorbide A and Hoechst 33342 assays. For determination of selectivity toward ABCG2, the effect on ABCB1 and ABCC1 was additionally examined by using the calcein accumulation assay. The intrinsic cytotoxicity of the most potent derivatives was determined in 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays.

Results and Discussion

Chemistry

The synthesis of target compounds **6**–**41** is shown in Scheme 1. The initial step involved the formation of the chalcone scaffold. For this purpose, Claisen–Schmidt condensation between 2′-, 3′-, or 4′-aminoacetophenone and variably substituted benzaldehydes was performed by using LiOH as the base catalyst. Afterward, the obtained chalcones were transformed into acryloylphenylcarboxamides by amide coupling. Different substituted acid chlorides were added in the presence of triethylamine. All synthesized compounds were characterized by NMR spectroscopy, and their purities were confirmed by elemental analysis.



Scheme 1. General synthesis of acryloylphenylcarboxamides **6**–**41**. *Reagents and conditions*: a) LiOH, MeOH, ultrasonic bath, 4–6 h; b) acid chloride, triethylamine, THF, RT, overnight.

Biological testing

Inhibition of ABCG2

The inhibitory effects of final compounds **6**–**41** were investigated in the pheophorbide A assay and partially in the Hoechst 33342 assay by using the MDCK II BCRP cell line. Pheophorbide A and Hoechst 33342 are fluorescent dyes and substrates of ABCG2. These assays were performed as previously described with minor modifications.[26–32] The determined IC_{50} values for all compounds are given in Table 1.

For comparison of these two assays, the pIC_{50} values were plotted against each other in a scatterplot (Figure 1). The correlation yielded a squared correlation coefficient (r^2) of 0.90,

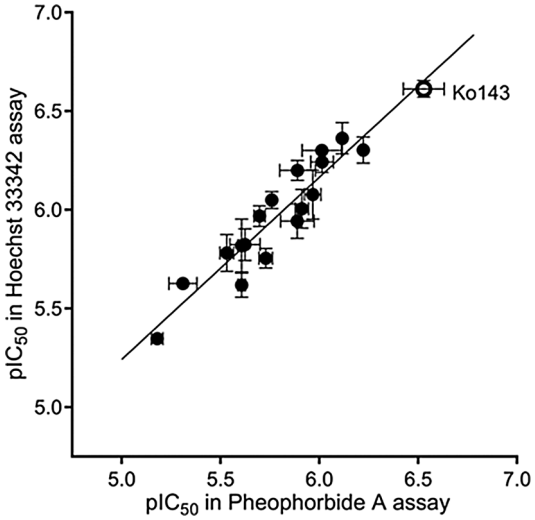


Figure 1. Scatterplot of pIC_{50} values for ABCG2 inhibition obtained in the pheophorbide A assay and Hoechst 33342 assay by using MDCK II BCRP cells. Ko143 (○) was used as positive control. Each point indicates mean of pIC_{50} values, and error bars indicate standard deviation (SD) obtained in at least three independent experiments with duplicate measurements. Squared correlation coefficient of $r^2 = 0.90$.

which indicated that inhibitory activity was independent of substrate with regard to pheophorbide A and Hoechst 33342.

Altogether, the substance library consisted of three different series with varying amide linker positions, that is, either *ortho*, *meta*, or *para* substitution. Substitution on ring B of the chalcone moiety was kept almost constant. On the basis of previous studies, a 3,4-dimethoxy group for substitution on ring B was selected, as it had proven to be beneficial for inhibition of ABCG2.[25] Within the series, structural variability was mostly present at R^1 located at the carboxamide substructure.

The first series included three compounds (i.e., compounds **6**–**8**) bearing the amide linker in the *meta* position and containing a phenyl (see compound **6**), 4-nitrophenyl (see compound **7**), or 3-bromophenyl (see compound **8**) substituent. Unsubstituted compound **6** showed the highest activity with an IC_{50} value of 2.18 μM, followed by compound **8** with slightly less potency, whereas the 4-nitro derivative was almost inactive. Owing to the low activities of these three derivatives, no further compounds with this substitution pattern were synthesized.

The second series (i.e., compounds **9**–**25**) contained compounds with an amide linker in the *para* position. The three compounds that could be directly compared with the *meta* series showed higher activities. Therefore, more different substituents were selected. They were subdivided into halogen atoms, methoxy groups, and strong electron-withdrawing groups, such as nitro or cyano groups. Upon comparing the

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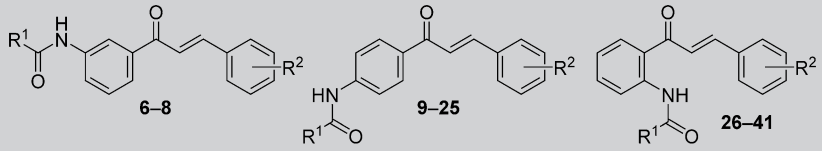
Table 1. Inhibitory potencies of compounds **6–41** against MDCK II BCRP cells by using pheophorbide A and Hoechst 33342 assays.[a]



Compd	R^1	R^2	$IC_{50} \pm SD$ [μM]	
			Pheo. A	Hoechst 33342
6	Ph	3,4-$(MeO)_2$	2.18 ± 0.13	n.t.
7	4-$O_2NC_6H_4$	3,4-$(MeO)_2$	> 20	n.t.
8	3-BrC_6H_4	3,4-$(MeO)_2$	2.99 ± 0.14	n.t.
9	Ph	3,4-$(MeO)_2$	1.30 ± 0.17	1.15 ± 0.15
10	2-$O_2NC_6H_4$	3,4-$(MeO)_2$	2.98 ± 0.40	n.t.
11	3-$O_2NC_6H_4$	3,4-$(MeO)_2$	2.82 ± 0.19	n.t.
12	4-$O_2NC_6H_4$	3,4-$(MeO)_2$	> 20	n.t.
13	3-BrC_6H_4	3,4-$(MeO)_2$	1.74 ± 0.04	0.893 ± 0.059
14	4-BrC_6H_4	3,4-$(MeO)_2$	2.94 ± 0.16	1.67 ± 0.24
15	3-ClC_6H_4	3,4-$(MeO)_2$	2.39 ± 0.28	1.51 ± 0.18
16	4-ClC_6H_4	3,4-$(MeO)_2$	1.87 ± 0.10	1.76 ± 0.13
17	3,4-$Cl_2C_6H_3$	3,4-$(MeO)_2$	2.95 ± 0.20	n.t.
18	2-FC_6H_4	3,4-$(MeO)_2$	4.93 ± 0.53	2.36 ± 0.09
19	4-NCC_6H_4	3,4-$(MeO)_2$	2.80 ± 0.16	n.t.
20	4-$F_3CC_6H_4$	3,4-$(MeO)_2$	15.3 ± 3.5	n.t.
21	2-$MeOC_6H_4$	3,4-$(MeO)_2$	2.48 ± 0.07	2.42 ± 0.22
22	3-$MeOC_6H_4$	3,4-$(MeO)_2$	2.18 ± 0.14	n.t.
23	4-$MeOC_6H_4$	3,4-$(MeO)_2$	6.61 ± 0.29	4.49 ± 0.14
24	3,4-$(MeO)_2C_6H_3$	3,4-$(MeO)_2$	3.15 ± 0.20	1.55 ± 0.32
25	3,4,5-$(MeO)_3C_6H_2$	3,4-$(MeO)_2$	3.04 ± 0.32	n.t.
26	Ph	3,4-$(MeO)_2$	0.982 ± 0.149	0.500 ± 0.009
27	3-ClC_6H_4	3,4-$(MeO)_2$	0.767 ± 0.011	0.436 ± 0.052
28	4-ClC_6H_4	3,4-$(MeO)_2$	1.86 ± 0.16	n.t.
29	2-$O_2NC_6H_4$	3,4-$(MeO)_2$	> 20	n.t.
30	3-$O_2NC_6H_4$	3,4-$(MeO)_2$	17.4 ± 6.4	n.t.
31	2-$MeOC_6H_4$	3,4-$(MeO)_2$	1.23 ± 0.06	0.997 ± 0.149
32	3-$MeOC_6H_4$	3,4-$(MeO)_2$	1.12 ± 0.11	0.629 ± 0.046
33	3-quinolinyl	3,4-$(MeO)_2$	0.971 ± 0.084	0.573 ± 0.046
34	1-naphthyl	3,4-$(MeO)_2$	1.08 ± 0.07	0.851 ± 0.162
35	3-pyridyl	3,4-$(MeO)_2$	2.01 ± 0.09	1.08 ± 0.08
36	7-methoxy-2-oxo-3-chromenyl	3,4-$(MeO)_2$	> 20	n.t.
37	2-thienyl	3,4-$(MeO)_2$	0.600 ± 0.010	0.501 ± 0.050
38	3-ClC_6H_4	3-Cl	> 20	n.t.
39	3,4-$(MeO)_2C_6H_3$	3-Cl	3.32 ± 0.18	n.t.
40	2-thienyl	3-MeO, 4-F	3.37 ± 0.05	n.t.
41	3-ClC_6H_4	3-MeO, 4-F	10.6 ± 0.6	n.t.
Ko143			0.276 ± 0.040	0.221 ± 0.024

[a] Data are the mean ± SD of at least three independent measurements with duplicate measurements; n.t.: not tested.

position-dependent effect in all cases except for a chloro substituent, *ortho* and *meta* substitutions were similar and better than *para* substitution or disubstitution. In most cases, these effects were small, which led to compounds with similar potencies. However, in the whole series with *para* benzamide groups, unsubstituted compound **9** was the most potent modulator with an IC_{50} value of 1.30 μM in the pheophorbide A assay and 1.15 μM in the Hoechst 33342 assay. Representatives bearing a chlorine atom showed slightly decreased activity, followed by compounds with a fluorine atom. Compounds with electron-withdrawing substituents behaved differently. Whereas the activities of the nitro and cyano derivatives were similar to those with compounds with halogen substituents, trifluoromethyl derivative **20** was by far the least active with an IC_{50} value of only 16.1 μM.

The effects of nitro substituents on the benzamide moiety were generally deactivating but to varying degrees. A 4-nitro substituent led to inactivity, whereas a 3- or 2-nitro group was tolerated in the *para* substituted series. Finally, among the methoxy substituted compounds the contribution of the methoxy groups seemed to be additive. Again, 2-methoxy (IC_{50} = 2.48 μM) and 3-methoxy (IC_{50} = 2.18 μM) derivatives **21** and **22** were most potent, and 4-methoxy derivative **23** was considerably worse (IC_{50} = 6.60 μM). 3,4-Dimethoxy substitution yielded an IC_{50} value of 3.15 μM, and this value lies between those for *meta*-methoxy substituted and *para*-methoxy substituted compounds **22** and **23**. 3,4,5-Trimethoxy derivative **25**, containing two *meta*-methoxy groups, was slightly more active than 3,4-dimethoxy derivative **24** (**25**: IC_{50} = 3.04 vs. **24**: IC_{50} = 3.15 μM). In summary, in the second series the effects of substituents at

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the benzamide ring were mostly small, which led to derivatives with similar potencies, with the exception of the 2-fluoro (see compound **18**), 4-methoxy (see compound **23**), and 4-trifluoromethyl (see compound **20**) derivatives that were considerably less active. Also, all other substituents decreased the activity, and the 3-bromo (see compound **13**) and 4-chloro (see compound **15**) groups led to the smallest decreases relative to unsubstituted compound **9**.

The third series contained compounds **26**–**41** with the amide linker in the *ortho* position. Compounds **26**–**37** also had 3,4-dimethoxy substituents on ring B and contained the best substituents from the second series on the benzamide group. Furthermore, the benzamide moiety was replaced by selected heterocyclic residues. Upon comparing the unsubstituted phenyl derivatives an increase in activity was apparent. Compound **26** had IC_{50} values of 0.959 and 0.500 μM in the pheophorbide A and Hoechst 33342 assays, respectively, and these values are considerably lower than those of compounds **9** and **6**. A pairwise comparison of the substituted derivatives showed that the IC_{50} values in the *ortho*-substituted series were generally lower than those in the *para*-substituted series, except for the nitro derivatives that showed lower potencies. A 3-chloro substituent led to a slight increase in activity, and this derivative (i.e., compound **27**) was the most potent among the benzamides with IC_{50} values of 0.766 and 0.430 μM in the pheophorbide A and Hoechst 33342 assays, respectively. Also, 3-methoxy derivative **32** was almost as active as the unsubstituted one, having IC_{50} values of 1.12 and 0.629 μM in the pheophorbide A and Hoechst 33342 assays, respectively. Interestingly, 1-naphthyl derivative **34** was also very potent (IC_{50} = 1.08 and 0.851 μM, respectively). Among the derivatives in which the phenyl ring was replaced by a heterocycle, 2-thienyl derivative **37** was the most active, followed by 3-quinolinyl derivative **33**. The latter compound was two times more potent than its 3-pyridyl counterpart. Unfortunately, the chromenyl derivative that was prepared as a possible fluorescent inhibitor proved to be inactive.

Figure 2 shows representative concentration–response curves for best compounds **27** and **37** obtained in the pheophorbide A and Hoechst 33342 assays by using MDCK II BCRP and MDCK II wild-type cells. As depicted in the graphs, both compounds, in addition to the Ko143 standard, demonstrated no significant inhibitory or other side effects in the MDCK II wild-type cell line serving as a negative control.

In the second part of the third series (i.e., compounds **38**–**41**), the substitution pattern of the chalcone on ring B was varied, and the 3,4-dimethoxy substituents were replaced by either 3-chloro or 3-methoxy-4-fluoro substituents. Both alternatives led to drastically decreased activity toward ABCG2, which indicated the necessity of the 3,4-dimethoxy groups on ring B of the chalcone.

Figure 3 summarizes the structural features of the investigated acryloylphenylcarboxamides, whereof structure–activity relationships could be derived. The *ortho* substitution of the amide linker and the 3,4-dimethoxy groups on ring B was beneficial for inhibitory activity. Furthermore, the phenyl ring could be replaced by heterocyclic residues.

Screening for ABCB1 and ABCC1 inhibition by calcein AM assay

To examine the selectivities of the acryloylphenylcarboxamides, all compounds were additionally screened for their inhibition of ABCB1 and ABCC1 at a concentration of 10 μM. The assay was performed as previously described by using cyclosporine A (CsA, 10 μM) as a standard inhibitor for both ABC transporters.[27,29–32] For the calcein AM assays, the ABCB1 overexpressing A2780 adr cell line and the ABCC1 overexpressing H69 AR cell line were used. The results are depicted in Figures 4a and 5a for the A2780 adr and H69 AR cells, respectively. None of the compounds showed significant activity against ABCC1 (Figure 5)

As could be expected by the presence of the 3,4-dimethoxy groups on ring B of the chalcone, almost all compounds demonstrated some ABCB1 inhibition (Figure 4a).[33,34] For the most active ABCG2 inhibitors that also showed a high ABCB1 inhibition in the screening (i.e., compounds **11**, **13**, **19**, **32**, and **33**), complete dose–response curves were measured and IC_{50} values were calculated (Table 2).

The screening for ABCB1 and ABCC1 inhibition was additionally performed in the transfected MDCK II MDR1 (Figure 4b) and MDCK II MRP1 (Figure 5b) cell lines for selected compounds to exclude cell-line-specific effects. As can be seen from the figures, no significant differences in the amount of inhibition relative to cyclosporine A was observed for both ABC transporters, except for compound **19**, which showed much less inhibition of ABCB1 in the MDCK II MDR1 cells.

Compounds **13** and **33** showed the highest inhibitory potency against ABCB1 with IC_{50} values of 0.529 and 0.494 μM, respectively. As already observed for the quinazoline chalcones, the compounds did not reach the maximum fluorescence obtained with cyclosporine A.[34] Instead, the dose–response curves leveled off to give maximum inhibition values in the range of 40 to 60% of that of cyclosporine A. A representative concentration–response curve for compound **33** versus cyclosporine A is given in Figure 6. For the five derivatives, no correlation between ABCB1 and ABCG2 inhibition was observed.

MTT assay for determining cell toxicity

Several potent modulators of ABCG2 were furthermore investigated for their intrinsic cytotoxicity in the MTT assay by using MDCK II BCRP and MDCK II wild-type cells. The GI_{50} values are summarized in Table 3.

Compounds **9**, **13**, and **31** showed GI_{50} values < 10 μM and, consequently, have high intrinsic cytotoxicity, which limits their usability in future in vivo studies. However, there was a minor difference between the *para* substituted and *ortho* substituted series. Compounds **9** and **13** as representatives of the *para* series were more cytotoxic than compounds of the *ortho* series, although the concentrations were eightfold higher than the obtained IC_{50} values for ABCG2 inhibition. Two potent compounds containing a heterocyclic residue proved to be almost nontoxic. Compound **33** with a 3-quinoline residue had a GI_{50} value of 66 μM and 2-thienyl derivative **37**, which was

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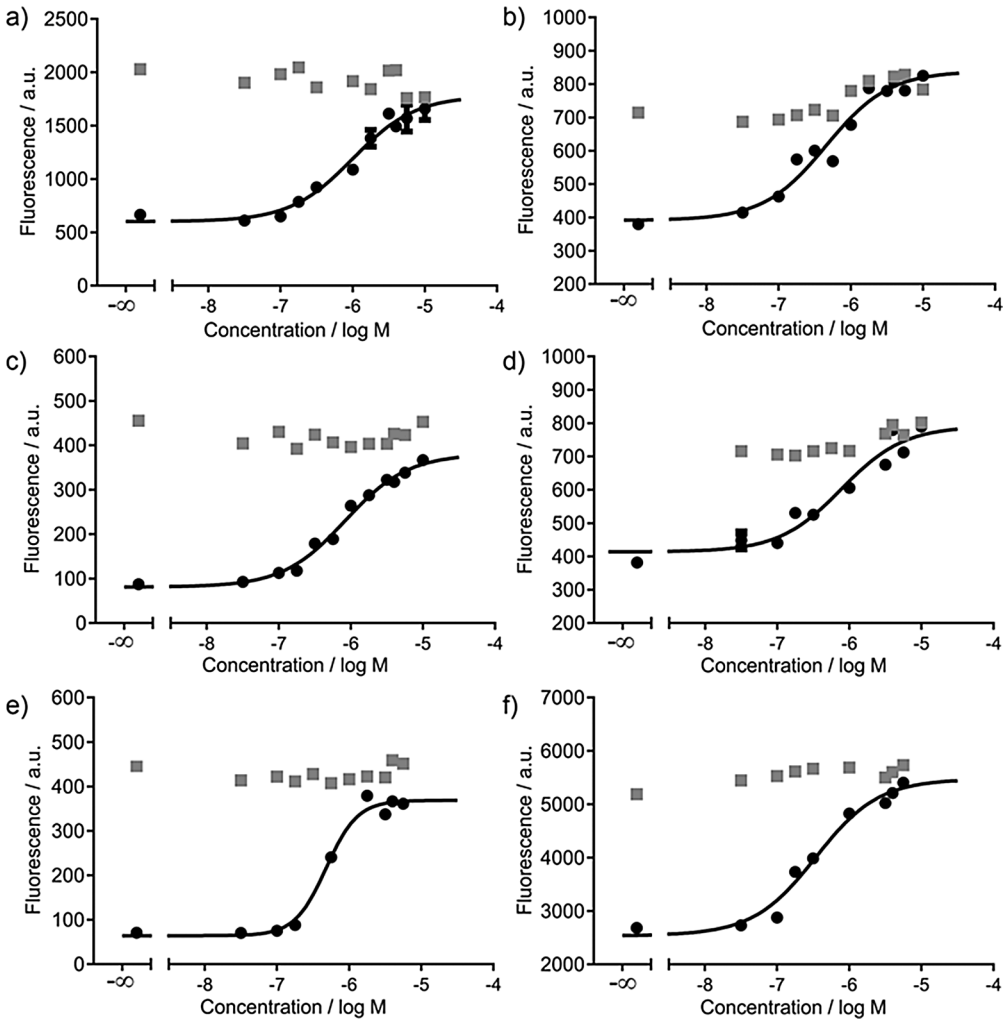

Figure 2. Representative concentration–response curves of compounds **27** (a, b) and **37** (c, d) as well as Ko143 (e, f) in the pheophorbide A (a, c, e) and Hoechst 33342 (b, d, f) assays by using MDCK II BCRP (●) and MDCK II wild-type cells (■). Data shown represent one experiment out of a series of at least three independent experiments with duplicate measurements.

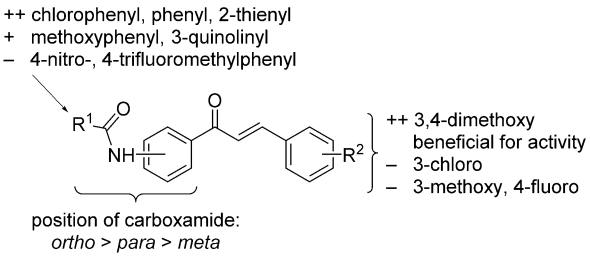

Figure 3. Structural features of acryloylphenylcarboxamides as ABCG2 inhibitors. ++: very high activity, +: good activity, –: no positive effect on activity.

Table 2. Inhibitory effects of selected compounds toward ABCB1 overexpressing A2780 adr cells determined by the calcein AM assay.

Compd	$IC_{50} \pm SD$ [μM][a]	$I_{max} \pm SD$ [%][a]
11	1.13 ± 0.02	45 ± 3
13	0.529 ± 0.048	43 ± 3
19	1.45 ± 0.08	47 ± 7
32	1.01 ± 0.06	47 ± 6
33	0.494 ± 0.029	62 ± 5
CsA	1.21 ± 0.16	100

[a] Data are then mean ± SD of at least three independent experiments with duplicate measurements; I_{max}: maximum response relative to cyclosporine A (CsA).

the most promising inhibitor in this study, showed no intrinsic cytotoxicity (GI_{50} = 93 μM), as shown in Figure 7.

MTT assay for determining the ability to reverse MDR

The most promising inhibitor in this study was further analyzed for its efficacy to reverse MDR in ABCG2 overexpressing cells by using the MTT assay. For this purpose, MDCK II BCRP and MDCK II wild-type cells were exposed to different concentrations of SN-38 in the presence or absence of compound **37** (0.1 and 0.5 μM) for 72 h. The left shift of the dose–response curve seen in Figure 8 indicates increased cytotoxicity of SN-38 and, therefore, sensitization of the MDCK II BCRP cells. This

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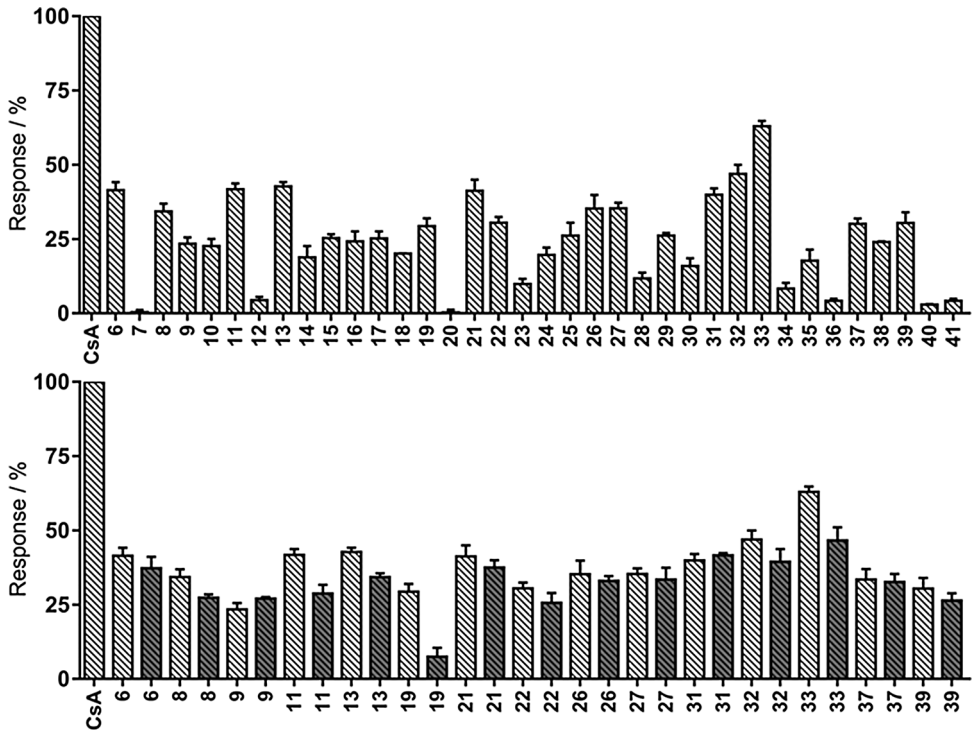

Figure 4. a) Summary of ABCB1 screening of acryloylphenylcarboxamides **6**–**41** (10 μM) obtained in the calcein AM assay in ABCB1 overexpressing A2780 adr cells in comparison with the standard inhibitor cyclosporine A (CsA, 10 μM). b) Comparison of ABCB1 screening of selected compounds in A2780 adr cells (light gray) and MDCK II MDR1 cells (dark gray). Data shown is mean ± SD of at least three independent experiments with duplicate measurements.

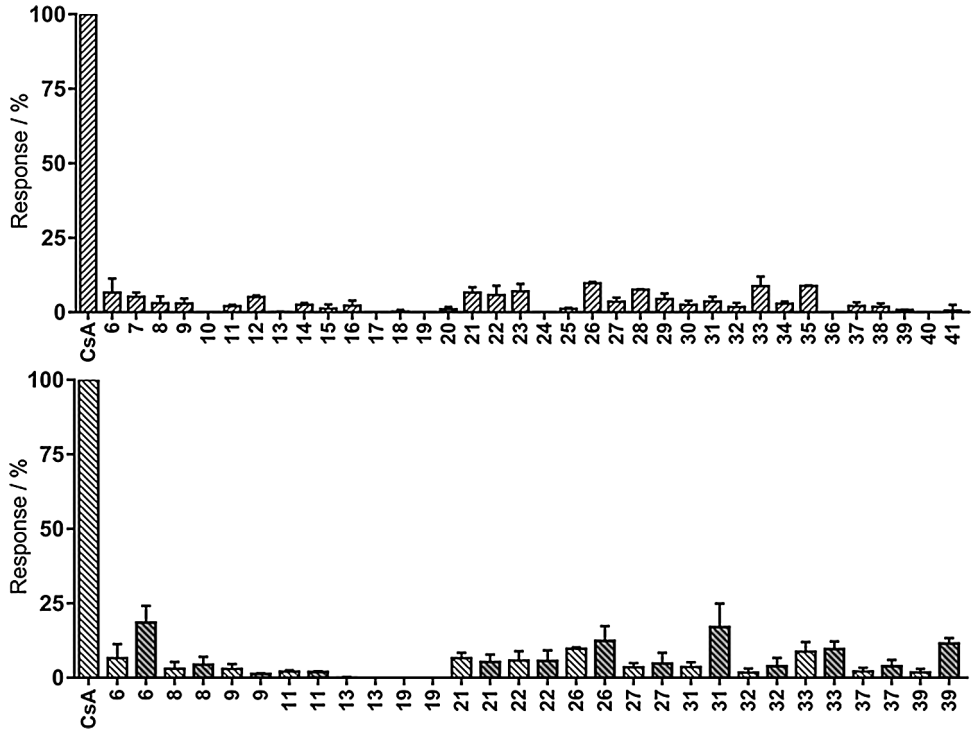

Figure 5. a) Summary of ABCC1 screening of acryloylphenylcarboxamides **6**–**41** (10 μM) obtained in the calcein AM assay with ABCC1 overexpressing H69 AR cells in comparison with the standard inhibitor cyclosporine A (CsA, 10 μM). b) Comparison of ABCC1 screening of selected compounds in H69 AR cells (light gray) and MDCK II MRP1 cells (dark gray). Data shown is mean ± SD of at least three independent experiments with duplicate measurements.

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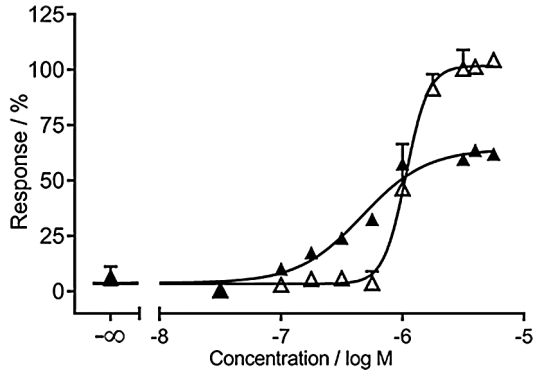

Figure 6. Representative concentration–response curves of compound **33** (▲) and the standard inhibitor cyclosporine A (△) obtained in the calcein AM assay by using A2780 adr cells. Data shown represent one experiment out of a series from at least three independent experiments with duplicate measurements.

Table 3. Cytotoxicity of selected compounds in MDCK II BCRP and MDCK II wild-type cells determined by MTT assay.

Compd	$GI_{50} \pm SD$ [μM][a]	
	MDCK II BCRP	MDCK II wild-type
9	7.08 ± 1.01	7.88 ± 1.57
13	8.69 ± 1.99	7.91 ± 1.74
26	20.5 ± 3.1	17.4 ± 5.5
27	17.8 ± 1.0	21.6 ± 4.0
31	8.29 ± 0.73	8.78 ± 0.49
33	65.9 ± 12.0	69.3 ± 15.7
37	93.2 ± 10.6	89.4 ± 6.7

[a] Data are the mean ± SD of at least three independent experiments with duplicate measurements.

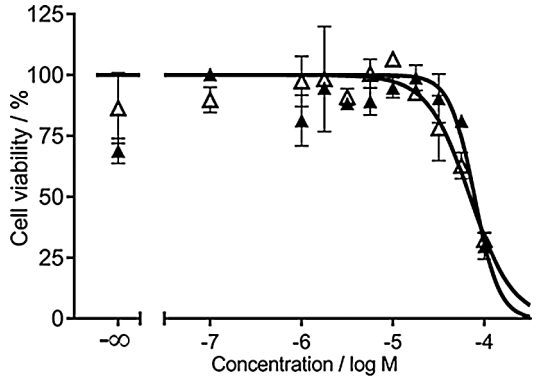

Figure 7. Cytotoxicity of compound **37** determined in the MTT assay by using the MDCK II BCRP (▲) and MDCK II wild-type (△) cell line. The compound was investigated up to a final concentration of 100 μM for 72 h. Data shown represent one experiment out of a series from at least three independent experiments with duplicate measurements.

shows the ability of **37** to reverse MDR and, consequently, the inhibition of SN-38 transport through ABCG2.

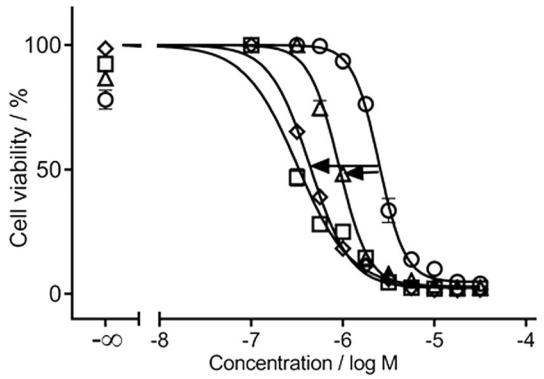

Figure 8. Representative dose–response curve of SN-38 cytotoxicity in presence of compound **37**. Arrows indicate dose-dependent sensitization of MDCK II BCRP cells toward SN-38 by compound **37**. The compound was investigated at 0.1 μM (△) and 0.5 μM (◇) concentrations. MDCK wild-type cells: □; MDCK II BCRP cells without modulator: ○. Data shown represent one experiment out of a series from at least three independent experiments with duplicate measurements.

Conclusions

In the current work, we investigated a new type of chalcone derivatives, namely, acryloylphenylcarboxamides, for their inhibitory effect toward ABCG2. The aim of this study was to explore the best position of the amide linker and to optimize the substitution pattern of the carboxamide. For this purpose, 35 compounds were synthesized, including structural modifications in positions 2′, 3′, and 4′ on ring A (amide linker) and positions 3 and 4 on ring B of the chalcone. We demonstrated that the *ortho* position of the amide linker and the 3,4-dimethoxy groups on ring B were important for inhibitory activity. Furthermore, the highest potencies were found for an unsubstituted phenyl, thiophene, and quinoline ring. Compound **37** ((*E*)-*N*-{2-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}thiophene-2-carboxamide) was found to have the best combination, that is, 3,4-dimethoxy groups on ring B of the chalcone, an amide linker in the *ortho* position, and a 2-thienyl residue. Besides a high inhibitory potency against ABCG2, the compound showed less affinity toward ABCB1 and was almost inactive against ABCC1. It also showed no intrinsic cytotoxicity in the MTT assay.

Experimental Section

Chemistry

Materials: The used chemicals were purchased from Sigma–Aldrich, Acros Organics, or Alfa Aesar and were used without further purification. To examine reaction progress, thin-layer chromatography (TLC) on aluminum sheets coated with silica gel 60 F_{254} (Merck) and cyclohexane/ethyl acetate (2:8) as eluent was performed. Melting points were measured with a Stuart melting point apparatus model SMP3 (Barloworld Scientific Limited Stone, Staffordshire, ST15 0SA, UK). The structures of the target compounds were approved by NMR spectroscopy. ^{13}C NMR and ^{1}H NMR spectra were processed with a Bruker Advance 500 MHz NMR spectrometer at 500 MHz (^{1}H) or 126 MHz (^{13}C) or a Bruker Advance 600 MHz (^{1}H,

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600 MHz/^{13}C, 151 MHz) NMR spectrometer. [D$_6$]DMSO was used as solvent at 303 K. Chemical shifts are reported as parts per million (ppm) and the signal of DMSO was used as internal standard: $\delta(^1H) = 2.54$ ppm; $\delta(^{13}C) = 40.45$ ppm. Coupling constants, *J*, are given in Hertz and spin multiplicities as s (singlet), d (doublet), doublet of doublets (dd), triplet of doublets (td), t (triplet), doublet of triplets (dt), q (quartet), and m (multiplet). The ^{13}C signals were allocated with the aid of distortionless enhancement by polarization transfer (DEPT) and attached proton test (APT). The purities of all target compounds were assigned to be >95% by elemental analyses with a Vario EL V24 CHN Elemental Analyzer. Found values were within ±0.4% of the theoretical values except when indicated.

General procedure for the preparation of substituted chalcones: Chalcones **1**–**5** were synthesized by Claisen–Schmitt condensation. Therefor, a mixture of the selected aminoacetophenone (1 equiv) and different substituted benzaldehyde (1 equiv) in methanol (25 mL) was kept in an ultrasonic bath for 4–6 h by using LiOH (7 equiv) as the base. Upon completion of the reaction, the mixture was poured onto crushed ice. Through acidification with dilute HCl, a precipitate was formed, which was filtered off and washed with water. The solid was then recrystallized from EtOH/H_2O (1:1).

(*E*)-1-(3-Aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (1): The title compound was synthesized from 3′-aminoacetophenone (2.3 mmol) and 3,4-dimethoxybenzaldehyde (2.3 mmol) to yield a yellow solid (52%). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.64 (d, *J* = 2.7 Hz, 2H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.34 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.32–7.29 (m, 1H), 7.26–7.23 (m, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.85–6.80 (m, 1H), 5.31 (s, 2H), 3.84 (s, 3H), 3.80 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 189.74, 151.31, 149.20 (2C), 143.90, 138.89, 129.18, 127.75, 123.68, 120.33, 118.52, 116.43, 113.09, 111.79, 110.96, 55.89, 55.76 ppm.

(*E*)-1-(4-Aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (2): The title compound was synthesized from 4′-aminoacetophenone (10 mmol) and 3,4-dimethoxybenzaldehyde (10 mmol) to yield a yellow solid (69%). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.91 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J* = 15.5 Hz, 1H), 7.56 (d, *J* = 15.4 Hz, 1H), 7.47 (d, *J* = 1.9 Hz, 1H), 7.30 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.62 (d, *J* = 8.7 Hz, 2H), 6.06 (s, 2H), 3.85 (s, 3H), 3.80 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 186.05, 153.81, 150.89, 149.17, 141.96, 131.11 (2C), 128.17, 125.74, 123.33, 120.18, 112.83 (2C), 111.75, 110.74, 55.88, 55.72 ppm.

(*E*)-1-(2-Aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3): The title compound was synthesized from 2′-aminoacetophenone (10 mmol) and 3,4-dimethoxybenzaldehyde (10 mmol) to yield an orange solid (57%). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.08 (d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 15.4 Hz, 1H), 7.58 (d, *J* = 15.4 Hz, 1H), 7.49 (d, *J* = 1.8 Hz, 1H), 7.32 (dd, *J* = 8.0, 1.9 Hz, 3H), 7.29–7.24 (m, 1H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.78 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.62–6.55 (m, 1H), 3.85 (s, 3H), 3.80 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 190.75, 152.04, 151.01, 149.18, 142.56, 134.14, 131.45, 128.03, 123.53, 121.09, 117.86, 116.98, 114.52, 111.73, 110.81, 55.90, 55.72 ppm.

(*E*)-1-(2-Aminophenyl)-3-(3-chlorophenyl)prop-2-en-1-one (4): The title compound was synthesized from 2′-aminoacetophenone (5 mmol) and 3-chlorobenzaldehyde (5 mmol) to yield an orange solid (75%). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.11 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.04–7.99 (m, 2H), 7.78–7.75 (m, 1H), 7.58 (d, *J* = 15.5 Hz, 1H), 7.47–7.44 (m, 2H), 7.39 (s, 2H), 7.31–7.25 (m, 1H), 6.80 (dd, *J* = 8.4, 1.1 Hz, 1H), 6.61–6.55 ppm (m, 1H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 190.45, 152.28, 140.31, 137.48, 134.55, 133.90, 131.72, 130.72, 129.73, 127.84, 127.69, 125.16, 117.48, 117.01, 114.57 ppm.

(*E*)-1-(2-Aminophenyl)-3-(4-fluoro-3-methoxyphenyl)prop-2-en-1-one (5): The title compound was synthesized from 2′-aminoacetophenone (5 mmol) and 3,4-difluorobenzaldehyde (5 mmol) to yield a yellow solid (39%). During the reaction, one fluorine atom (position 3) was replaced by a methoxy group by Williamson ether synthesis that occurred under the reaction conditions. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.08 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.87 (dd, *J* = 12.9, 2.1 Hz, 3H), 7.85 (d, *J* = 15.4 Hz, 3H), 7.59–7.54 (m, 4H), 7.34 (s, 4H), 7.29–7.24 (m, 2H), 7.20 (t, *J* = 8.8 Hz, 2H), 6.78 (dd, *J* = 8.4, 1.1 Hz, 2H), 6.60–6.55 (m, 2H), 3.88 ppm (s, 6H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 190.56, 152.14, 151.78 (d, *J* = 244.1 Hz, 1C), 148.89 (d, *J* = 10.9 Hz, 1C), 140.98, 134.31, 131.55, 128.52 (d, *J* = 6.8 Hz, 1C), 126.76, 122.50, 117.70, 116.99, 115.00 (d, *J* = 18.5 Hz, 1C), 114.55, 113.90, 56.29 ppm.

General procedure for the preparation of substituted acryloylphenylcarboxamides: A mixture of the selected chalcone (0.25 mmol) and substituted acid chloride (0.25 mmol) was dissolved in tetrahydrofuran (15 mL) and an excess amount of triethylamine. The solution was stirred overnight at room temperature. Upon completion of the reaction, precipitated triethylamine hydrochloride was filtered off, and the solvent was removed under reduced pressure. Finally, the oily residue was recrystallized from ethanol.

(*E*)-*N*-{3-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}benzamide (6): The title compound was synthesized from **1** and benzoyl chloride to yield an ochre solid (42%), mp: 166–167 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 10.43 (d, *J* = 9.8 Hz, 1H), 8.41 (t, *J* = 1.8 Hz, 1H), 8.11 (dd, *J* = 8.1, 1.3 Hz, 1H), 8.01–7.98 (m, 2H), 7.95–7.92 (m, 1H), 7.77–7.70 (m, 2H), 7.63–7.58 (m, 1H), 7.57–7.51 (m, 4H), 7.39 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.85 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 189.52, 166.16, 151.82, 149.51, 145.16, 140.09, 138.87, 135.09, 132.20, 129.47, 128.88, 128.15, 127.94, 125.13, 124.42, 124.25, 120.44, 120.26, 112.11, 111.43, 56.24, 56.10 ppm. Elemental analysis calcd (%) for $C_{24}H_{21}NO_4$ (387.43): C 74.40, H 5.46, N 3.62; found: C 74.02, H 5.44, N 3.56.

(*E*)-*N*-{3-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-4-nitrobenzamide (7): The title compound was synthesized from **1** and 4-nitrobenzoyl chloride to yield a yellow solid (43%), mp: 239–240 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.74 (s, 1H), 8.42–8.35 (m, 3H), 8.26–8.20 (m, 2H), 8.11 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.99–7.96 (m, 1H), 7.78–7.69 (m, 2H), 7.59 (t, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 2.0 Hz, 1H), 7.39 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.85 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 189.08, 164.15, 151.51, 149.40, 149.17, 144.90, 140.37, 139.27, 138.58, 129.37 (2C), 129.25, 127.57, 124.88, 124.57, 123.93, 123.68 (2C), 120.22, 119.85, 111.78, 111.12, 55.89, 55.75 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}N_2O_6$ (432.43): C 66.66, H 4.66, N 6.48; found: C 66.72, H 4.82, N 6.27.

(*E*)-3-Bromo-*N*-{3-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (8): The title compound was synthesized from **1** and 3-bromobenzoyl chloride to yield a yellow solid (43%), mp: 172–174 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO) 10.53 (s, 1H), 8.38 (t, *J* = 1.8 Hz, 1H), 8.19 (t, *J* = 1.8 Hz, 1H), 8.11 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.82–7.80 (m, 1H), 7.77–7.70 (m, 2H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.54–7.49 (m, 2H), 7.39 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.85 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 189.11, 164.21, 151.48, 149.16, 144.86, 139.45, 138.53, 136.86, 134.57, 130.80, 130.38, 129.18, 127.57, 127.03, 124.81, 124.33, 123.92, 121.82, 120.13, 119.86,

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111.76, 111.09, 55.88, 55.74 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}BrNO_4$ (466.32): C 61.82, H 4.32, N 3.00; found: C 61.42, H 4.69, N 2.94.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}benzamide (9): The title compound was synthesized from **2** and benzoyl chloride to yield a pale-yellow solid (42%), mp: 180–181 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.55 (s, 1H), 8.21–8.17 (m, 2H), 8.02–7.96 (m, 4H), 7.84 (d, J = 15.5 Hz, 1H), 7.69 (d, J = 15.5 Hz, 1H), 7.65–7.59 (m, 1H), 7.57–7.52 (m, 3H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.87 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 187.65, 166.14, 151.37, 149.20, 144.05, 143.65, 134.76, 133.07, 132.02, 129.72 (2C), 128.59 (2C), 127.93 (2C), 127.79, 123.98, 119.71, 119.66 (2C), 111.77, 110.95, 55.93, 55.76 ppm. Elemental analysis calcd (%) for $C_{24}H_{21}NO_4$ (387.43): C 74.40, H 5.46, N 3.62; found: C 74.15, H 5.82, N 3.51.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-2-nitrobenzamide (10): The title compound was synthesized from **2** and 2-nitrobenzoyl chloride to yield a pale-yellow solid (54%), mp: 121–123 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 11.00 (s, 1H), 8.21–8.17 (m, 3H), 7.89 (td, J = 7.5, 0.9 Hz, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.84–7.80 (m, 2H), 7.80–7.77 (m, 1H), 7.69 (d, J = 15.5 Hz, 1H), 7.53 (d, J = 1.9 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 188.02, 165.05, 151.73, 149.53, 146.80, 144.55, 143.46, 134.72, 133.72, 132.82, 131.69, 130.28 (2C), 129.80, 128.08, 124.81, 124.33, 120.02, 119.47 (2C), 112.08, 111.27, 56.25, 56.09 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}N_2O_6$ (432.43): C 66.66, H 4.66, N 6.48; found: C 66.42, H 4.78, N 6.42.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-3-nitrobenzamide (11): The title compound was synthesized from **2** and 3-nitrobenzoyl chloride to yield a pale-yellow solid (36%), mp: 198–199 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 10.88 (s, 1H), 8.81 (t, J = 1.9 Hz, 1H), 8.47–8.45 (m, 1H), 8.44–8.41 (m, 1H), 8.22 (d, J = 8.8 Hz, 2H), 8.00 (d, J = 8.8 Hz, 2H), 7.88–7.82 (m, 2H), 7.70 (d, J = 15.5 Hz, 1H), 7.54 (d, J = 1.9 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.87 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 187.67, 163.96, 151.40, 149.20, 147.92, 144.21, 143.12, 136.12, 134.47, 133.49, 130.41, 129.76 (2C), 127.75, 126.58, 124.04, 122.71, 119.95 (2C), 119.65, 111.75, 110.93, 55.93, 55.76 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}N_2O_6$ (432.43): C 66.66, H 4.66, N 6.48; found: C 66.54, H 4.74, N 6.31.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-4-nitrobenzamide (12): The title compound was synthesized from **2** and 4-nitrobenzoyl chloride to yield a yellow solid (65%), mp: 224–225 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.85 (s, 1H), 8.40–8.36 (m, 2H), 8.23–8.18 (m, 4H), 8.01–7.97 (m, 2H), 7.84 (d, J = 15.5 Hz, 1H), 7.69 (d, J = 15.5 Hz, 1H), 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), 3.87 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 187.69, 164.51, 151.42, 149.49, 149.22, 144.21, 143.12, 140.39, 133.54, 129.77 (2C), 129.52 (2C), 127.77, 124.03, 123.73 (2C), 119.89 (2C), 119.68, 111.78, 110.98, 55.94, 55.78 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}N_2O_6$ (432.43): C 66.66, H 4.66, N 6.48; found: C 66.72, H 4.77, N 6.29.

(*E*)-3-Bromo-*N*-{4-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (13): The title compound was synthesized from **2** and 3-bromobenzoyl chloride to yield a pale-yellow solid (50%), mp: 169–170 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.64 (s, 1H), 8.21–8.18 (m, 2H), 8.17 (t, J = 1.8 Hz, 1H), 8.00–7.96 (m, 3H), 7.83 (d, J = 15.5 Hz, 1H), 7.83–7.79 (m, 1H), 7.69 (d, J = 15.5 Hz, 1H), 7.53 (d, J = 1.5 Hz, 1H), 7.51 (d, J = 7.9 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.87 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 187.65, 164.55, 151.38, 149.19, 144.12, 143.31, 136.89, 134.72, 133.29, 130.84, 130.49, 129.71 (2C), 127.76, 127.14, 123.98, 121.84, 119.78 (2C), 119.67, 111.76, 110.95, 55.92, 55.75 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}BrNO_4$ (466.32): C 61.82, H 4.32, N 3.00; found: C 61.52, H 4.72, N 3.07.

(*E*)-4-Bromo-*N*-{4-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (14): The title compound was synthesized from **2** and 4-bromobenzoyl chloride to yield a yellow solid (33%), mp: 189–191 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 10.61 (s, 1H), 8.19 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 7.95–7.92 (m, 2H), 7.84 (d, J = 15.5 Hz, 1H), 7.77 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 15.5 Hz, 1H), 7.54 (d, J = 1.9 Hz, 1H), 7.38 (dd, J = 8.3, 1.8 Hz, 1H), 7.02 (d, J = 8.3 Hz, 1H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 187.65, 165.16, 151.39, 149.20, 144.13, 143.44, 133.80, 133.22, 131.63 (2C), 130.09 (2C), 129.75 (2C), 127.78, 125.86, 124.02, 119.75 (2C), 119.67, 111.75, 110.93, 55.93, 55.77 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}BrNO_4$ (466.32): C 61.82, H 4.32, N 3.00; found: C 61.86, H 4.32, N 3.00.

(*E*)-3-Chloro-*N*-{4-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (15): The title compound was synthesized from **2** and 3-chlorobenzoyl chloride to yield an ochre solid (42%), mp: 161–162 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 10.64 (s, 1H), 8.21–8.19 (m, 2H), 8.03 (t, J = 1.8 Hz, 1H), 8.00–7.97 (m, 2H), 7.95–7.92 (m, 1H), 7.84 (d, J = 15.5 Hz, 1H), 7.71–7.66 (m, 2H), 7.59 (t, J = 7.9 Hz, 1H), 7.54 (d, J = 1.9 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 187.64, 164.65, 151.37, 149.18, 144.13, 143.32, 136.70, 133.39, 133.28, 131.83, 130.60, 129.73 (2C), 127.75, 127.67, 126.78, 124.01, 119.77 (2C), 119.65, 111.73, 110.91, 55.91, 55.74 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}ClNO_4$ (421.87): C 68.33, H 4.78, N 3.32; found: C 68.55, H 4.87, N 3.21.

(*E*)-4-Chloro-*N*-{4-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (16): The title compound was synthesized from **2** and 4-chlorobenzoyl chloride to yield a yellow solid (51%), mp: 192–193 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 10.61 (s, 1H), 8.19 (d, J = 8.8 Hz, 2H), 8.03–8.00 (m, 2H), 7.98 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 15.5 Hz, 1H), 7.69 (d, J = 15.5 Hz, 1H), 7.64–7.61 (m, 2H), 7.54 (d, J = 1.9 Hz, 1H), 7.38 (dd, J = 8.3, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 187.66, 165.05, 151.40, 149.22, 144.14, 143.46, 136.92, 133.46, 133.23, 129.95 (2C), 129.76 (2C), 128.70 (2C), 127.79, 124.04, 119.76 (2C), 119.69, 111.77, 110.94, 55.94, 55.78 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}ClNO_4$ (421.87): C 68.33, H 4.78, N 3.32; found: C 68.20, H 5.09, N 3.41.

(*E*)-3,4-Dichloro-*N*-{4-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (17): The title compound was synthesized from **2** and 3,4-dichlorobenzoyl chloride to yield a pale-yellow solid (31%), mp: 164–165 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.67 (s, 1H), 8.24 (d, J = 2.1 Hz, 1H), 8.22–8.18 (m, 2H), 7.99–7.95 (m, 3H), 7.85–7.81 (m, 2H), 7.69 (d, J = 15.5 Hz, 1H), 7.53 (d, J = 2.0 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.86 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 187.67, 163.76, 151.41, 149.21, 144.17, 143.17, 135.02, 134.84, 133.41, 131.51, 130.97, 129.89, 129.75 (2C), 128.33, 127.77, 124.02, 119.84 (2C), 119.67, 111.77, 110.97, 55.94, 55.77 ppm. Elemental analysis calcd (%) for $C_{24}H_{19}Cl_2NO_4$ (456.32): C 63.17, H 4.20, N 3.07; found: C 63.16, H 4.30, N 3.20.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-2-fluorobenzamide (18): The title compound was synthesized from **2** and 2-fluorobenzoyl chloride to yield a yellow solid (38%), mp: 142–143 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 10.75 (s, 1H), 8.21–8.17 (m, 2H),

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7.91 (d, J=8.7 Hz, 2H), 7.83 (d, J=15.5 Hz, 1H), 7.71–7.66 (m, 2H), 7.63–7.58 (m, 1H), 7.53 (d, J=1.9 Hz, 1H), 7.40–7.33 (m, 3H), 7.02 (d, J=8.4 Hz, 1H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ=187.63, 163.37, 159.06 (d, J=249.1 Hz, 1C), 158.23, 151.39, 149.20, 144.16, 143.18, 133.28, 132.97 (d, J=8.3 Hz, 1C), 130.09, 129.89 (2C), 124.86, 124.77 (d, J=2.8 Hz, 1C), 124.02, 119.67, 119.22 (2C), 116.36 (d, J=21.7 Hz, 1C), 111.75, 110.91, 55.91, 55.76 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}FNO_4$ (405.42): C 71.10, H 4.97, N 3.45; found: C 71.26, H 5.34, N 3.49.

(*E*)-4-Cyano-*N*-{4-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (19): The title compound was synthesized from **2** and 4-cyanobenzoyl chloride to yield a yellow solid (51%), mp: 190–192 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=10.78 (s, 1H), 8.20 (d, J=8.8 Hz, 2H), 8.14–8.11 (m, 2H), 8.06–8.03 (m, 2H), 7.98 (d, J=8.8 Hz, 2H), 7.84 (d, J=15.5 Hz, 1H), 7.69 (d, J=15.5 Hz, 1H), 7.53 (d, J=1.9 Hz, 1H), 7.38 (dd, J=8.3, 1.9 Hz, 1H), 7.02 (d, J=8.4 Hz, 1H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ=187.67, 164.79, 151.41, 149.21, 144.21, 143.18, 138.77, 133.46, 132.66 (2C), 129.78 (2C), 128.83 (2C), 127.76, 124.05, 119.84 (2C), 119.65, 118.41, 114.27, 111.76, 110.93, 55.93, 55.77 ppm. Elemental analysis calcd (%) for $C_{25}H_{20}N_2O_4$ (412.44): C 72.80, H 4.89, N 6.79; found: C 72.43, H 5.21, N 6.56.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-4-(trifluoromethyl)benzamide (20): The title compound was synthesized from **2** and 4-(trifluoromethyl)benzoyl chloride to yield an ochre solid (56%), mp: 194–195 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=10.77 (s, 1H), 8.21 (d, J=8.8 Hz, 2H), 8.17 (d, J=8.1 Hz, 2H), 7.99 (dd, J=6.6, 4.8 Hz, 2H), 7.93 (t, J=6.2 Hz, 2H), 7.84 (d, J=15.5 Hz, 1H), 7.69 (d, J=15.5 Hz, 1H), 7.54 (d, J=1.9 Hz, 1H), 7.38 (dd, J=8.4, 1.9 Hz, 1H), 7.02 (d, J=8.4 Hz, 1H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ=187.69, 165.03, 151.43, 149.23, 144.21, 143.29, 138.60, 133.42, 131.78 (q, J=31.9 Hz), 129.80 (2C), 128.93 (2C), 127.79, 125.62 (d, J=3.5 Hz, 2C), 124.16 (m), 124.06 (q, J=272.4 Hz), 119.84 (2C), 119.68, 111.78, 110.95, 55.95, 55.79 ppm. Elemental analysis calcd (%) for $C_{25}H_{20}F_3NO_4$ (455.43): C 65.93, H 4.43, N 3.08; found: C 66.07, H 4.76, N 3.07.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-2-methoxybenzamide (21): The title compound was synthesized from **2** and 2-methoxybenzoyl chloride to yield an ochre solid (32%), mp: 140–141 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=10.44 (s, 1H), 8.17 (d, J=8.7 Hz, 2H), 7.92 (d, J=8.6 Hz, 2H), 7.83 (d, J=15.5 Hz, 1H), 7.68 (d, J=15.5 Hz, 1H), 7.63 (dd, J=7.5, 1.7 Hz, 1H), 7.53 (d, J=1.8 Hz, 1H), 7.52–7.50 (m, 1H), 7.38 (dd, J=8.3, 1.8 Hz, 1H), 7.19 (d, J=8.4 Hz, 1H), 7.07 (td, J=7.5, 0.6 Hz, 1H), 7.02 (d, J=8.4 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ=187.62, 165.29, 156.67, 151.38, 149.21, 144.07, 143.45, 132.96, 132.41, 129.88 (2C), 129.76, 127.80, 125.03, 123.97, 120.67, 119.73, 119.15 (2C), 112.21, 111.77, 110.97, 56.09, 55.93, 55.78 ppm. Elemental analysis calcd (%) for $C_{25}H_{23}NO_5$ (417.45): C 71.93, H 5.55, N 3.36; found: C 71.54, H 5.93, N 3.33.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-3-methoxybenzamide (22): The title compound was synthesized from **2** and 3-methoxybenzoyl chloride to yield a beige solid (50%), mp: 161–162 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=10.51 (s, 1H), 8.21–8.16 (m, 2H), 8.01–7.97 (m, 2H), 7.84 (d, J=15.5 Hz, 1H), 7.69 (d, J=15.5 Hz, 1H), 7.58–7.55 (m, 1H), 7.54 (d, J=2.0 Hz, 1H), 7.51 (dd, J=2.4, 1.7 Hz, 1H), 7.46 (t, J=7.9 Hz, 1H), 7.38 (dd, J=8.4, 1.9 Hz, 1H), 7.20–7.17 (m, 1H), 7.02 (d, J=8.4 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=187.64, 165.85, 159.37, 151.38, 149.21, 144.07, 143.58, 136.14, 133.10, 129.77, 129.71 (2C), 127.79, 123.98, 120.14, 119.73 (2C), 119.71, 117.77, 113.23, 111.77, 110.96, 55.93, 55.76, 55.53 ppm. Elemental analysis calcd (%) for $C_{25}H_{23}NO_5$ (417.45): C 71.93, H 5.55, N 3.36; found: C 71.94, H 5.58, N 3.58.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-4-methoxybenzamide (23): The title compound was synthesized from **2** and 4-methoxybenzoyl chloride to yield a pale-yellow solid (37%), mp: 187–189 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=10.40 (s, 1H), 8.18 (d, J=8.8 Hz, 2H), 8.00–7.97 (m, 4H), 7.84 (d, J=15.5 Hz, 1H), 7.69 (d, J=15.5 Hz, 1H), 7.54 (d, J=1.9 Hz, 1H), 7.38 (dd, J=8.4, 1.9 Hz, 1H), 7.10–7.06 (m, 2H), 7.02 (d, J=8.4 Hz, 1H), 3.86 (s, 3H), 3.84(s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ=187.61, 165.45, 162.34, 151.36, 149.20, 144.01, 143.91, 132.83, 129.96 (2C), 129.72 (2C), 127.80, 126.73, 123.99, 119.71, 119.58 (2C), 113.84 (2C), 111.75, 110.92, 55.93, 55.76, 55.63 ppm. Elemental analysis calcd (%) for $C_{25}H_{23}NO_5$ (417.45): C 71.93, H 5.55, N 3.36; found: C 71.99, H 5.56, N 3.32.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-3,4-dimethoxybenzamide (24): The title compound was synthesized from **2** and 3,4-dimethoxybenzoyl chloride to yield a yellow solid (31%), mp: 93–96 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=10.37 (s, 1H), 8.19 (d, J=8.8 Hz, 2H), 7.98 (d, J=8.8 Hz, 2H), 7.84 (d, J=15.5 Hz, 1H), 7.69 (d, J=15.5 Hz, 1H), 7.66 (dd, J=8.4, 2.0 Hz, 1H), 7.55 (dd, J=8.8, 1.9 Hz, 2H), 7.38 (dd, J=8.3, 1.8 Hz, 1H), 7.10 (d, J=8.5 Hz, 1H), 7.02 (d, J=8.4 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.82 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=187.62, 165.47, 152.13, 151.37, 149.21, 148.55, 144.01, 143.84, 132.88, 129.70 (2C), 127.81, 126.76, 123.97, 121.47, 119.71, 119.69 (2C), 111.77, 111.40, 111.13, 110.97, 55.94, 55.88, 55.85, 55.76 ppm. Elemental analysis calcd (%) for $C_{26}H_{25}NO_6$·0.5 H_2O (456.49): C 68.41, H 5.74, N 3.07; found: C 68.34, H 5.88, N 3.07.

(*E*)-*N*-{4-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-3,4,5-trimethoxybenzamide (25): The title compound was synthesized from **2** and 3,4,5-trimethoxybenzoyl chloride to yield a yellow solid (39%), mp: 184–185 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=10.42 (s, 1H), 8.20 (d, J=8.7 Hz, 2H), 7.96 (d, J=8.7 Hz, 2H), 7.85 (d, J=15.5 Hz, 1H), 7.69 (d, J=15.4 Hz, 1H), 7.54 (d, J=1.7 Hz, 1H), 7.38 (dd, J=8.3, 1.7 Hz, 1H), 7.30 (s, 2H), 7.02 (d, J=8.3 Hz, 1H), 3.88 (s, 6H), 3.86 (s, 3H), 3.81 (s, 3H), 3.74 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ=187.64, 165.51, 152.83 (2C), 151.40, 149.22, 144.12, 143.59, 140.81, 133.10, 129.83, 129.74 (2C), 127.80, 124.02, 119.87 (2C), 119.68, 111.77, 110.97, 105.71, 60.31, 56.34 (3C), 55.95, 55.78 ppm. Elemental analysis calcd (%) for $C_{27}H_{27}NO_7$ (477.51): C 67.91, H 5.70, N 2.93; found: C 67.63, H 5.71, N 2.99.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}benzamide (26): The title compound was synthesized from **3** and benzoyl chloride to yield a yellow solid (48%), mp: 154–155 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.45 (s, 1H), 8.61 (d, J=8.3 Hz, 1H), 8.33 (d, J=8.0 Hz, 1H), 8.02–7.96 (m, 2H), 7.85–7.76 (m, 2H), 7.73–7.68 (m, 1H), 7.67–7.62 (m, 1H), 7.62–7.57 (m, 2H), 7.52 (d, J=1.8 Hz, 1H), 7.40 (dd, J=8.3, 1.9 Hz, 1H), 7.35–7.31 (m, 1H), 7.02 (d, J=8.3 Hz, 1H), 3.84 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=193.04, 165.12, 151.71, 149.17, 145.85, 140.17, 134.58, 134.30, 132.27, 131.42, 129.04 (2C), 127.49, 127.24 (2C), 125.31, 124.47, 123.46, 121.16, 120.81, 111.75, 111.06, 55.88, 55.76 ppm. Elemental analysis calcd (%) for $C_{24}H_{21}NO_4$ (387.43): C 74.40, H 5.46, N 3.62; found: C 74.06, H 5.58, N 3.71.

(*E*)-3-Chloro-*N*-{2-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (27): The title compound was synthesized from **3** and 3-chlorobenzoyl chloride to yield a yellow solid (26%), mp: 140–141 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.23 (s, 1H), 8.44 (dd,

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J=8.3, 1.0 Hz, 1H), 8.25 (dd, J=7.9, 1.4 Hz, 1H), 7.95 (t, J=1.8 Hz, 1H), 7.93–7.89 (m, 1H), 7.74 (s, 2H), 7.71–7.67 (m, 2H), 7.61 (t, J=7.9 Hz, 1H), 7.49 (d, J=2.0 Hz, 1H), 7.39–7.33 (m, 2H), 7.01 (d, J=8.4 Hz, 1H), 3.83 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=192.91, 163.84, 151.70, 149.19, 145.68, 139.35, 136.67, 134.00, 133.82, 132.03, 131.18, 130.99, 127.47, 127.28, 126.64, 125.88, 124.38, 124.03, 121.88, 121.09, 111.76, 111.01, 55.87, 55.77 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}ClNO_4$ (421.87): C 68.33, H 4.78, N 3.32; found: C 68.15, H 4.76, N 3.37.

(*E*)-4-Chloro-*N*-{2-[3-(3,4-dimethoxyphenyl)acryloyl]phenyl}benzamide (28): The title compound was synthesized from **3** and 4-chlorobenzoyl chloride to yield a yellow solid (63%), mp: 176–177 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.37 (s, 1H), 8.52 (d, J=8.3 Hz, 1H), 8.30 (d, J=7.1 Hz, 1H), 8.00–7.96 (m, 2H), 7.81–7.73 (m, 2H), 7.71–7.64 (m, 3H), 7.50 (d, J=1.8 Hz, 1H), 7.38 (dd, J=8.3, 1.7 Hz, 1H), 7.33 (t, J=7.6 Hz, 1H), 7.01 (d, J=8.3 Hz, 1H), 3.83 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=193.00, 164.11, 151.73, 149.19, 145.81, 139.79, 137.14, 134.19, 133.34, 131.34, 129.18 (2C), 129.13 (2C), 127.47, 125.83, 124.46, 123.74, 121.46, 120.92, 111.76, 111.04, 55.89, 55.77 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}ClNO_4$ (421.87): C 68.33, H 4.78, N 3.32; found: C 68.00, H 4.77, N 3.29.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-2-nitrobenzamide (29): The title compound was synthesized from **3** and 2-nitrobenzoyl chloride to yield a yellow solid (56%), mp: 189–190 °C.^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=11.70 (s, 1H), 8.18 (d, J=8.1 Hz, 1H), 8.14–8.10 (m, 2H), 7.86–7.82 (m, 1H), 7.80–7.74 (m, 2H), 7.71–7.66 (m, 1H), 7.63 (s, 2H), 7.48 (d, J=2.0 Hz, 1H), 7.40–7.35 (m, 1H), 7.34 (dd, J=8.3, 2.0 Hz, 1H), 7.01 (d, J=8.4 Hz, 1H), 3.82 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=192.60, 163.99, 151.61, 149.16, 147.03, 145.48, 137.99, 134.08, 133.42, 132.11, 131.61, 130.63, 128.65, 128.22, 127.39, 124.60, 124.58, 124.23, 122.39, 121.57, 111.73, 110.93, 55.84, 55.73 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}N_2O_6$ (432.43): C 66.66, H 4.66, N 6.48; found: C 66.88, H 4.69, N 6.35.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-3-nitrobenzamide (30): The title compound was synthesized from **3** and 3-nitrobenzoyl chloride to yield a yellow solid (65%), mp: 185–186 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.26 (s, 1H), 8.73–8.71 (m, 1H), 8.48–8.43 (m, 1H), 8.36 (dd, J=7.0, 6.5 Hz, 2H), 8.22 (dd, J=7.9, 1.3 Hz, 1H), 7.87 (t, J=8.0 Hz, 1H), 7.72–7.68 (m, 3H), 7.46 (d, J=1.9 Hz, 1H), 7.38 (d, J=7.8 Hz, 1H), 7.36–7.33 (m, 1H), 6.99 (d, J=8.3 Hz, 1H), 3.81 (s, 3H), 3.80 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=192.88, 163.21, 151.67, 149.16, 148.16, 145.58, 138.84, 136.04, 133.83, 133.42, 131.04, 130.78, 127.48, 127.43, 126.66, 124.42, 124.29, 122.37, 122.26, 121.30, 111.74, 110.96, 55.85, 55.76 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}N_2O_6$ (432.43): C 66.66, H 4.66, N 6.48; found: C 66.23, H 4.57, N 6.51.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-2-methoxybenzamide (31): The title compound was synthesized from **3** and 2-methoxybenzoyl chloride to yield an ochre solid (53%), mp: 138–139 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.13 (s, 1H), 8.67 (dd, J=8.4, 0.9 Hz, 1H), 8.15 (dd, J=7.9, 1.5 Hz, 1H), 7.98 (dd, J=7.8, 1.8 Hz, 1H), 7.70 (d, J=1.8 Hz, 2H), 7.66–7.62 (m, 1H), 7.60–7.55 (m, 1H), 7.50 (d, J=2.0 Hz, 1H), 7.38 (dd, J=8.4, 2.0 Hz, 1H), 7.30–7.26 (m, 1H), 7.25 (d, J=7.8 Hz, 1H), 7.13–7.08 (m, 1H), 7.02 (d, J=8.4 Hz, 1H), 4.10 (s, 3H), 3.83 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=192.58, 163.69, 157.30, 151.65, 149.19, 145.60, 138.96, 133.70, 133.38, 131.49, 130.74, 127.46, 126.66, 124.22, 123.18, 122.07, 121.96, 121.84, 120.94, 112.41, 111.76, 111.14, 56.06, 55.89, 55.77 ppm. Elemental analysis calcd (%) for $C_{25}H_{23}NO_5$ (417.45): C 71.93, H 5.55, N 3.36; found: C 71.84, H 5.67, N 3.39.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-3-methoxybenzamide (32): The title compound was synthesized from **3** and 3-methoxybenzoyl chloride to yield a yellow solid (31%), mp: 153–154 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.37 (s, 1H), 8.56 (dd, J=8.3, 1.0 Hz, 1H), 8.31 (dd, J=8.0, 1.5 Hz, 1H), 7.82–7.74 (m, 2H), 7.71–7.67 (m, 1H), 7.57–7.54 (m, 1H), 7.53–7.48 (m, 3H), 7.39 (dd, J=8.4, 2.0 Hz, 1H), 7.35–7.30 (m, 1H), 7.22–7.19 (m, 1H), 7.02 (d, J=8.4 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=193.03, 164.93, 159.69, 151.72, 149.19, 145.80, 140.02, 136.10, 134.25, 131.40, 130.25, 127.51, 125.59, 124.45, 123.57, 121.28, 120.91, 119.21, 117.86, 112.88, 111.77, 111.09, 55.89, 55.79, 55.50 ppm. Elemental analysis calcd (%) for $C_{25}H_{23}NO_5$ (417.45): C 71.93, H 5.55, N 3.36; found: C 71.99, H 5.75, N 3.32.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}quinoline-3-carboxamide (33): The title compound was synthesized from **3** and quinolone-3-carbonyl chloride to yield a yellow solid (33%), mp: 165–167 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.34 (s, 1H), 9.38 (d, J=2.3 Hz, 1H), 8.92 (d, J=2.2 Hz, 1H), 8.43 (dd, J=8.3, 1.1 Hz, 1H), 8.24 (dd, J=7.9, 1.4 Hz, 1H), 8.16–8.10 (m, 2H), 7.93–7.88 (m, 1H), 7.74 (s, 2H), 7.74–7.70 (m, 2H), 7.48 (d, J=2.0 Hz, 1H), 7.39–7.34 (m, 2H), 6.99 (d, J=8.5 Hz, 1H), 3.80 (s, 3H), 3.79 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=192.88, 163.94, 151.66, 149.18, 148.84, 148.54, 145.64, 139.15, 136.06, 133.90, 131.82, 131.08, 129.43, 128.98, 127.81, 127.47, 127.38, 127.26, 126.63, 124.33, 124.23, 122.25, 121.26, 111.75, 110.97, 55.85, 55.76 ppm. Elemental analysis calcd (%) for $C_{27}H_{22}N_2O_4$ (438.47): C 73.96, H 5.06, N 6.39; found: C 73.83, H 5.15, N 6.40.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-1-naphthamide (34): The title compound was synthesized from **3** and 1-naphthoyl chloride to yield a pale-yellow solid (58%), mp: 150–152 °C. ^{1}H NMR (500 MHz, DMSO) δ: 11.86 (s, 1H), 8.44 (dd, J=8.2, 0.9 Hz, 1H), 8.35–8.32 (m, 1H), 8.19 (dd, J=7.9, 1.5 Hz, 1H), 8.11 (d, J=8.3 Hz, 1H), 8.04–8.01 (m, 1H), 7.86 (dd, J=7.1, 1.2 Hz, 1H), 7.73–7.67 (m, 2H), 7.64 (d, J=15.6 Hz, 1H), 7.61–7.55 (m, 3H), 7.47 (d, J=2.0 Hz, 1H), 7.39–7.34 (m, 1H), 7.33 (dd, J=8.4, 2.0 Hz, 1H), 6.99 (d, J=8.4 Hz, 1H), 3.81 (s, 3H), 3.80 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=192.79, 167.30, 151.63, 149.20, 145.48, 139.08, 134.22, 133.68, 133.49, 131.09, 130.86, 129.83, 128.55, 127.52, 127.43, 127.31, 126.67, 125.71, 125.31, 125.21, 124.27, 124.07, 122.08, 121.48, 111.77, 111.01, 55.89, 55.78 ppm. Elemental analysis calcd (%) for $C_{28}H_{23}NO_4$ (437.49): C 76.87, H 5.30, N 3.20; found: C 76.89, H 5.27, N 3.34.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}nicotinamide (35): The title compound was synthesized from **3** and nicotinoyl chloride to yield a yellow solid (22%), mp: 192–194 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=12.30 (s, 1H), 9.14 (dd, J=2.3, 0.8 Hz, 1H), 8.79 (dd, J=4.8, 1.6 Hz, 1H), 8.45 (dd, J=8.3, 1.1 Hz, 1H), 8.31–8.28 (m, 1H), 8.27 (dd, J=7.9, 1.4 Hz, 1H), 7.75 (s, 2H), 7.73–7.67 (m, 1H), 7.63–7.59 (m, 1H), 7.50 (d, J=2.0 Hz, 1H), 7.39–7.34 (m, 2H), 7.01 (d, J=8.4 Hz, 1H), 3.83 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ=192.92, 163.81, 152.75, 151.71, 149.18, 148.43, 145.79, 139.29, 135.12, 134.02, 131.18, 130.24, 127.47, 126.66, 124.43, 124.10, 124.02, 121.89, 121.03, 111.76, 110.99, 55.88, 55.77 ppm. Elemental analysis calcd (%) for $C_{23}H_{20}N_2O_4$ (388.42): C 71.12, H 5.19, N 7.21; found: C 70.90, H 5.31, N 6.94.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}-7-methoxy-2-oxo-2*H*-chromene-3-carboxamide (36): The title compound was

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synthesized from **3** and 7-methoxycumarine-3-carbonyl chloride to yield a pale-yellow solid (50%), mp: 213–214 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.19 (s, 1H), 8.94 (s, 1H), 8.50 (d, J = 8.2 Hz, 1H), 8.08 (dd, J = 7.8, 1.3 Hz, 1H), 7.92 (d, J = 8.7 Hz, 1H), 7.69–7.59 (m, 3H), 7.48 (d, J = 1.7 Hz, 1H), 7.38–7.29 (m, 2H), 7.13 (d, J = 2.1 Hz, 1H), 7.04 (dd, J = 8.7, 2.4 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 3.91 (s, 3H), 3.83 (s, 3H), 3.80 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 192.02, 164.97, 160.79, 160.31, 156.68, 151.60, 149.25, 149.18, 145.56, 137.80, 132.92, 131.96, 130.42, 128.17, 127.46, 124.17, 123.92, 122.96, 121.97, 114.99, 113.89, 112.35, 111.74, 111.00, 100.43, 56.47, 55.87, 55.75 ppm. Elemental analysis calcd (%) for $C_{28}H_{23}NO_7$ (485.48): C 69.27, H 4.78, N 2.89; found: C 68.93, H 4.85, N 3.01.

(*E*)-*N*-{2-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}thiophene-2-carboxamide (37): The title compound was synthesized from **3** and thiophene-2-carbonyl chloride to yield a yellow solid (59%), mp: 198–199 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.40 (s, 1H), 8.46 (dd, J = 8.4, 1.0 Hz, 1H), 8.31 (dd, J = 8.0, 1.4 Hz, 1H), 7.92 (dd, J = 5.0, 1.1 Hz, 1H), 7.85 (dd, J = 3.8, 1.2 Hz, 1H), 7.82–7.75 (m, 2H), 7.71–7.66 (m, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.39 (dd, J = 8.4, 2.0 Hz, 1H), 7.34–7.30 (m, 1H), 7.27 (dd, J = 5.0, 3.7 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.84 (s, 3H), 3.81 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 193.01, 159.89, 151.75, 149.20, 145.88, 139.78, 139.72, 134.30, 132.64, 131.42, 129.09, 128.56, 127.50, 125.43, 124.53, 123.58, 121.29, 120.84, 111.76, 111.01, 55.89, 55.79 ppm.. Elemental analysis calcd (%) for $C_{22}H_{19}NO_4S$ (393.46): C 67.16, H 4.87, N 3.56; found: C 66.87, H 4.77, N 3.95.

(*E*)-3-Chloro-*N*-{2-[3-(3-chlorophenyl)acryloyl]phenyl}benzamide (38): The title compound was synthesized from **4** and 3-chlorobenzoyl chloride to yield a pale-yellow solid (72%), mp: 142–143 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.04 (s, 1H), 8.38 (dd, J = 8.3, 1.1 Hz, 1H), 8.25 (dd, J = 7.9, 1.5 Hz, 1H), 8.00 (t, J = 1.8 Hz, 1H), 7.94 (dd, J = 2.8, 1.1 Hz, 1H), 7.92–7.87 (m, 2H), 7.77 (dt, J = 7.3, 1.2 Hz, 1H), 7.72 (d, J = 2.3 Hz, 1H), 7.71–7.67 (m, 2H), 7.60 (t, J = 7.9 Hz, 1H), 7.50–7.47 (m, 1H), 7.37–7.32 (m, 1H) ppm. ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 192.82, 163.95, 142.99, 139.23, 136.87, 136.55, 134.28, 133.93, 133.79, 132.03, 131.36, 130.94, 130.78, 130.38, 128.13, 127.97, 127.28, 126.70, 125.91, 125.25, 124.16, 122.07 ppm. Elemental analysis calcd (%) for $C_{22}H_{15}Cl_2NO_2$ (396.27): C 66.68, H 3.82, N 3.53; found: C 66.59, H 3.86, N 3.75.

(*E*)-*N*-{2-[3-(3-Chlorophenyl)acryloyl]phenyl}-3,4-dimethoxybenzamide (39): The title compound was synthesized from **4** and 3,4-dimethoxybenzoyl chloride to yield a pale-yellow solid (50%), mp: 167–168 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.15 (s, 1H), 8.52 (dd, J = 8.4, 1.1 Hz, 1H), 8.31 (dd, J = 8.0, 1.5 Hz, 1H), 8.02 (t, J = 1.8 Hz, 1H), 7.96 (d, J = 15.6 Hz, 1H), 7.78 (dt, J = 7.3, 1.3 Hz, 1H), 7.73 (d, J = 15.5 Hz, 1H), 7.70–7.68 (m, 1H), 7.59 (dd, J = 8.4, 2.1 Hz, 1H), 7.52 (d, J = 2.1 Hz, 1H), 7.50–7.43 (m, 2H), 7.32–7.27 (m, 1H), 7.13 (d, J = 8.5 Hz, 1H), 3.85 (s, 3H), 3.84 ppm (s, 3H). ^{13}C NMR (126 MHz, [D$_6$]DMSO): δ = 193.01, 164.85, 152.26, 148.86, 142.99, 140.33, 136.93, 134.58, 133.93, 131.64, 130.80, 130.39, 128.14, 128.05, 126.77, 125.35, 125.15, 123.35, 121.29, 120.40, 111.47, 110.88, 55.89, 55.73 ppm. Elemental analysis calcd (%) for $C_{24}H_{20}ClNO_4$ (421.87): C 68.33, H 4.78, N 3.32; found: C 68.49, H 4.80, N 3.41.

(*E*)-*N*-{2-[3-(4-Fluoro-3-methoxyphenyl)acryloyl]phenyl}thiophene-2-carboxamide (40): The title compound was synthesized from **5** and thiophene-2-carbonyl chloride to yield a yellow solid (50%), mp: 167–168 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 12.45 (s, 1H), 8.53 (dd, J = 8.3, 0.9 Hz, 1H), 8.39 (dd, J = 8.0, 1.4 Hz, 1H), 8.00–7.95 (m, 2H), 7.92–7.88 (m, 2H), 7.82 (d, J = 15.5 Hz, 1H), 7.77–7.73 (m, 1H), 7.70 (dd, J = 8.5, 1.4 Hz, 1H), 7.39–7.36 (m, 1H), 7.34 (dd, J = 4.9, 3.7 Hz, 1H), 7.29 (t, J = 8.7 Hz, 1H), 3.96 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 192.94, 159.89, 151.72 (d, J = 244.3 Hz, 1C), 149.58 (d, J = 10.9 Hz, 1C), 144.21, 139.89, 139.67, 134.49, 132.65, 131.56, 129.10, 128.54, 127.92 (d, J = 6.9 Hz, 1C), 127.53 (d, J = 2.6 Hz, 1C), 125.08, 123.54, 122.19, 121.17, 115.36 (d, J = 18.5 Hz, 1C), 113.96, 56.35 ppm. Elemental analysis calcd (%) for $C_{21}H_{16}FNO_3S$ (381.42): C 66.13, H 4.23, N 3.67; found: C 65.95, H 4.24, N 3.66.

(*E*)-3-Chloro-*N*-{2-[3-(4-fluoro-3-methoxyphenyl)acryloyl]phenyl}benzamide (41): The title compound was synthesized from **5** and 3-chlorobenzoyl chloride to yield a yellow solid (75%), mp: 191–192 °C. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 12.22 (s, 1H), 8.45 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 7.8 Hz, 1H), 7.96 –7.87 (m, 3H), 7.80 (d, J = 15.5 Hz, 1H), 7.74–7.68 (m, 3H), 7.65–7.60 (m, 2H), 7.35 (t, J = 7.5 Hz, 1H), 7.23 (t, J = 8.7 Hz, 1H), 3.91 ppm (s, 3H). ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 193.16, 164.20, 152.04 (d, J = 244.5 Hz, 1C), 149.87 (d, J = 10.9 Hz, 1C), 144.35, 139.79, 136.98, 134.53, 134.16, 132.37, 131.65, 131.32, 128.24 (d, J = 6.7 Hz, 1C), 127.79 (d, J = 2.5 Hz, 1C), 127.61, 126.68, 126.21, 124.35, 122.77, 122.13, 115.64 (d, J = 18.5 Hz, 1C), 114.27, 56.68 ppm. Elemental analysis calcd (%) for $C_{23}H_{17}ClFNO_3 \cdot H_2O$ (427.85): C 64.57, H 4.48, N 3.27; found: C 64.49, H 4.14, N 3.24.

Biological methods

Chemicals: The reference compounds Ko143 and cyclosporine A were purchased from Tocris Bioscience (Bristol, UK). The fluorescent dye pheophorbide A was delivered by Frontier Scientific, Inc. (Logan, UT, USA) and calcein AM by Merck KGaA (Darmstadt, Germany). The cell culture material was supplied by Sarstedt (Newton, USA), and all other chemicals were purchased from Sigma–Aldrich (Taufkirchen, Germany), unless otherwise specified.

Cell culture: The MDCK II BCRP cell line was a generous gift of Dr. A. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands). Those cells were generated by transfection of the canine kidney epithelial cell line MDCK II with the human wild-type cDNA C-terminally linked to the cDNA of the green fluorescent protein (GFP). The MDCK II BCRP cells were cultured in DMEM (Dulbeccos's modified Eagle's medium) supplemented with 10% FCS (fetal calf serum), 50 μg mL^{-1} streptomycin, 50 U mL$^-$ penicillin G, and 2 mM L-glutamine. MDCK II cell lines transfected with human MDR1 (ABCB1) or MRP1 (ABCC1) were kindly provided by Prof. Dr. P. Borst (The Netherlands Cancer Institute, Amsterdam, The Netherlands) and cultured in DMEM with 10% FCS, 50 μg mL^{-1} streptomycin, 50 U mL$^-$ penicillin G, and 2 mM L-glutamine. The cell culture medium was supplemented with 2 mL G418 to maintain the overexpression of ABCB1 and ABCC1. Another used cell line was the ABCB1 (P-glycoprotein, P-gp) overexpressing doxorubicin resistant A2780 adr cell line, a human ovarian carcinoma cell line. The cells were purchased from European collection of animal cell cultures (ECACC, no. 93112519 and 93112520, UK) and cultured in RPMI-1640 medium, supplemented with 10% FCS, 50 μg mL^{-1} streptomycin, 50 U mL$^-$ penicillin G and 2 mM L-glutamine. The small cell lung cancer cell line, H69 AR, expressing ABCC1 (multidrug resistance-associated protein 1, MRP1) was purchased from American Type Culture Collection (ATCC, CRL-11351). The cell line was grown in RPMI-1640 medium with 20% FCS, 50 μg mL^{-1} streptomycin, 50 U mL$^-$ penicillin G and 2 mM L-glutamine.

Cells were incubated in a 5% CO_2 humidified atmosphere at 37 °C. After reaching 80–90% confluency, cells were harvested for subculturing with 0.05% trypsin and 0.02% EDTA.

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Pheophorbide A assay: To analyze the inhibitory effect of the compounds against ABCG2, the pheophorbide A assay was performed as described earlier with small modifications.[26–32] In the first step, different dilutions of each compound were prepared in Krebs-HEPES buffer (KHB). For the highest concentration (10 μM), a 10^{-2} stock solution in DMSO (1%) was used and methanol (not more than 50%) was added. In the assay, the concentration of DMSO was not more than 0.1% and that of methanol not more than 5%. Afterward, 20 μL of each dilution was placed on an F-shaped colorless 96-well plate (Greiner, Frickenhausen, Germany). Next, the preparation of the MDCK II cell line took place. The cells were harvested after reaching a confluence of 80–90% by gentle trypsination (0.05% trypsin/0.02% EDTA). After addition of culture medium, the cells were transferred into 50 mL tubes and centrifuged (266 *g*, 4 °C; 4 min). The obtained cell pellet was resuspended in fresh culture medium and the cell count determined using a CASY1 model TT cell counter device (Schaerfe System GmbH, Reutlingen, Germany). Cell suspension was then washed with KHB (3×) by centrifugation. Approximately 45000 cells were seeded per well in a total volume of 160 μL. The plate was kept under 5% CO_2 at 37 °C for 20–30 min. Following this pre-incubation period, 20 μL of a 5 μM pheophorbide A solution (protected from light) was added to each well and the plate was incubated for 120 min. After this period, cells in all wells were resuspended. Fluorescence was measured by flow cytometry (FACS Calibur, Becton Dickinson Biosciences, Heidelberg, Germany). Pheophorbide A was excited at a wavelength of 488 nm and emission detected in the FL3 channel ($\lambda \geq 670$ nm). Concentration–response curves were generated by nonlinear regression by using a four-parameter logistic equation with variable or fixed (=1) Hill slope. The statistically preferred model was chosen for calculating IC_{50} values (GraphPad Prism, version 5.0, San Diego, CA, USA). IC_{50} values and standard deviations were calculated from the pIC_{50} values and their standard deviation according to the equation for log-normal distributed values.[35]

Hoechst 33342 accumulation assay: To investigate substrate specificity, selected compounds were additionally tested for ABCG2 inhibition in the Hoechst 33342 accumulation assay. Cells and compound dilutions were prepared as described for the pheophorbide A assay. First, 20 μL of each concentration of the serial dilution was placed into black 96-well plates (Greiner, Frickenhausen, Germany). Approximately 30000 cells per well were added in a volume of 160 μL. The plates were incubated for 30 min under 5% CO_2 at 37 °C. After this pre-incubation period, 20 μL of a 10 μM Hoechst 33342 solution (protected from light) was placed in each well. Immediately, the fluorescence was measured at constant intervals (60 s) for a period of 120 min at an excitation wavelength of 355 nm and an emission wavelength of 460 nm at 37 °C. For measurement, a BMG POLARstar microplate reader (BMG Labtech, Offenburg, Germany) was used. The average of fluorescence values in the steady state (100–109 min) was calculated for all concentrations. The concentration–response curves were generated by nonlinear regression by using a four-parameter logistic equation with variable or fixed (=1) Hill slope. The statistically preferred model was chosen for calculating IC_{50} values (GraphPad Prism, version 5.0, San Diego, CA, USA).[26–31] IC_{50} values and standard deviations were calculated from the pIC_{50} values and their standard deviation according to the equation for log-normal distributed values.[35]

Calcein AM assay: Furthermore, all compounds were analyzed for their selectivity toward ABCB1 and ABCC1 by using the calcein AM assay as described earlier with minor modifications.[27,29–32] The procedure was similar to that of the Hoechst 33342 assay. From each dilution series, 20 μL was pipetted into colorless 96-well plates (Greiner, Frickenhausen, Germany). Afterward, A2780 adr cells (or MDCK II MDR1 cells) for ABCB1 and H69 AR cells (or MDCK II MRP1 cells) for ABCC1 were placed into the plates with a density of approximately 30000 cells to a total volume of 180 μL. After 30 min pre-incubation, under 5% CO_2 at 37 °C, 20 μL of a 3.125 μM calcein AM solution (protected from light) was added to each well. Immediately, the fluorescence was measured at constant time intervals (60 s) for a period of 60 min at 37 °C at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. For measurement, a BMG POLARstar microplate reader (BMG Labtech, Offenburg, Germany) was used. The first linear part of the fluorescence-time curves was used for calculating slopes. These slopes were plotted against logarithmic concentrations of tested compounds. Data analysis was done as described above.

MTT assay for determining cytotoxicity: The intrinsic cytotoxicity of selected compounds in MDCK II BCRP and MDCK II wild-type cells was determined by using the MTT colorimetric assay. Mitochondrial dehydrogenases of viable cells reduced MTT to the purple formazan, which was quantified spectrophotometrically. The assay was performed as described earlier with minor modifications.[25,26,31,36,37] Cells were harvested and seeded into 96-well tissue-culture treated plates (Starlab GmbH, Hamburg, Germany) at a density of approximately 3000 cells per well in a total volume of 180 μL. The plates were kept under 5% CO_2 atmosphere and 37 °C for 6 h for attachment of cells. Different concentrations of selected compounds were made in culture medium. The concentration of DMSO was not more than 0.1% and that of methanol not more than 5%. Then, 20 μL of each dilution was added to reach the final concentration in a volume of 200 μL. Furthermore, two wells per row were prepared with 10% (*v/v*) DMSO (positive control) and pure culture medium (negative control). To reduce evaporation of the solvent during an incubation time of 72 h, PBS (phosphate buffered saline) was added to the interspaces of the plates. After 3 days, a solution of MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] in PBS (5 mg mL^{-1}) was added to each well in a volume of 20 μL. During the incubation of 1 h, MTT was reduced to purple formazan. The liquid was removed to stop the reaction, and the cells were lysed by adding DMSO (100 μL) to each well. Absorbance was measured at $\lambda = 570$ nm and background correction at $\lambda = 690$ nm by using a Multiscan Ex microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA). The obtained data sets were normalized and GI_{50} values calculated by nonlinear regression analysis, assuming a sigmoidal concentration response curve with variable Hill slope (GraphPad Prism, version 5.0, San Diego, CA, USA). From the pIC_{50} values and their standard deviation IC_{50} values and standard deviations were calculated according to the equation for log-normal distributed values.[35]

MTT assay for determining the ability to reverse MDR: Another application of the MTT assay was to determine the effect of inhibitors on the reversal of resistance against cytotoxic compounds such as SN-38 (7-ethyl-10-hydroxycamptothecin). The principle was to measure the cytotoxic effect of SN-38 in the absence and presence of different concentrations of selected inhibitors. MDCK II wild-type and MDCK II BCRP cells were seeded into 96-well tissue-culture treated plates in a volume of 160 μL with a density of approximately 3000 cells per well. Plates were incubated under 5% CO_2 atmosphere and 37 °C for 6 h. After preparation of a dilution series of SN-38 in culture medium, 20 μL of each concentration was added to the cells. Afterward, 20 μL of culture medium (negative control) and 1 or 5 μM of test compound (dissolved in culture medium) were added to the wells containing MDCK II BCRP cells to achieve the required final concentration in a volume of 200 μL. In

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the end, 20 μL of culture medium was added to the MDCK II wild-type cells, which attended as positive control. The following steps were performed as described before.

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Notes: The authors declare no competing financial interest.

Abbreviations: ABC, ATP-binding cassette; BCRP, breast cancer resistance protein (ABCG2); MDCK, Madin Darby Canine Kidney; CsA, cyclosporine A; FTC, fumitremorgin C; I_{max}, maximal inhibition; GI_{50}, half-maximal growth inhibition.

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Keywords: acryloylphenylcarboxamides · amides · chalcones · inhibitors · multidrug resistance

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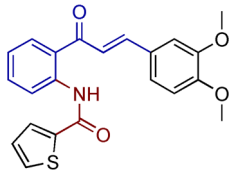
Busted! Acryloylphenylcarboxamides represent a new class of ABCG2 inhibitors. We combined the chalcone moiety with an additional aromatic residue by an amide linker. The position of the aromatic residue as well as that of the dimethoxy substituent on ring B have a large impact on activity. The most promising compound shows high potency and no cytotoxicity.

Acryloylphenylcarboxamide



ABCG2: IC_{50} = 0.5 μM
(Hoechst 33342 assay)

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Acryloylphenylcarboxamides: A New Class of Breast Cancer Resistance Protein (ABCG2) Modulators

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