European Journal of Medicinal Chemistry 85 (2014) 127–138

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Towards the development of chromone-based MEK1/2 modulators

Itedale Namro Redwan [a], Christine Dyrager [a], Carlos Solano [a], Guillermo Fernández de Trocóniz [a], Laure Voisin [b], David Bliman [a], Sylvain Meloche [b], Morten Grøtli [a, *]

[a] Department of Chemistry and Molecular Biology, Medicinal Chemistry, University of Gothenburg, SE-41296 Göteborg, Sweden
[b] Institute of Research in Immunology and Cancer, Université de Montréal, Montreal, Quebec H3C 3J7, Canada

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form
30 June 2014
Accepted 6 July 2014
Available online 7 July 2014

Keywords:
Mitogen-activated protein kinase kinases
Chromones
Molecular modeling
Biochemical activity
Whole-cell assay
Antiproliferative activity
Kinase selectivity profile

ABSTRACT

Inhibition or allosteric modulation of mitogen-activated protein kinase kinases MEK1 and MEK2 (MEK1/2) represent a promising strategy for the discovery of new specific anticancer agents. In this paper, structure-based design, beginning from the lead compound PD98059, was used to study potential structural modifications on the chromone structure in order to obtain highly potent derivatives that target the allosteric pocket in MEK1. Subsequently, a small series of PD98059 analogs were synthesized to provide a first generation of chromone-based derivatives that inhibit the activation of MEK1 with IC_{50} values as low as 30 nM *in vitro*. Complementary cellular studies also showed that two of the compounds in the series inhibit the activity of MEK1/2 with IC_{50} values in the nanomolar range (73–97 nM). In addition, compounds in this series were found to inhibit the proliferation of a small panel of human cancer cell lines.

1. Introduction

The highly conserved mitogen-activated protein kinases (MAPKs) are essential components of signal transduction pathways that play crucial roles in cellular processes such as transcription, proliferation, differentiation and apoptosis [1–3]. Dysregulation of MAPKs, in particular the extracellular-signal regulated kinases 1 and 2 (ERK1/2), the effector kinases of the Ras/Raf/MEK/ERK1/2 pathway, is strongly associated with human cancers and this pathway therefore offers attractive targets for the development of anticancer agents [4,5]. The dual specificity MEK1 and MEK2 kinases (MEK1/2) are of special interest since they only have two known substrates ERK1 and ERK2 (ERK1/2). Hence, the interest in MEK1/2 has generated several small molecule inhibitors, e.g. highly specific allosteric MEK1/2 modulators such as CI-1040 (PD184352), PD0325901, U0126, PD98059, GSK1120212 and AZD2644 (Fig. 1.) [4–9]. These allosteric MEK1/2 inhibitors are particularly discriminating kinase inhibitors that bind to the inactive (dephosphorylated) form of MEK1/2 and are thus non-ATP competitive modulators also called type III kinase inhibitors [10].

We have for a long time been working on the synthesis and functionalization of chromone derivatives [11–19]. Hence, in this study we have investigated the use of PD98059 as a starting point for designing chromone-based MEK1/2 inhibitors. Molecular modeling was used to identify potential structural modifications on the chromone scaffold and a series of chromone derivatives were synthesized and evaluated using biochemical and cell-based assays for their activity against MEK1/2.

2. Results and discussion

2.1. Molecular modeling

The docking study was performed using the Schrödinger Package (Glide XP Mode) [20]. The structure of MEK1 bound to ATPMg in complex with the allosteric modulator PD0325901 (PDB 1S9J) was used for the study [21,22]. The X-ray structure showed that PD0325901 binds into the allosteric pocket of MEK1 via one hydrogen bond between the hydroxamic acid oxygen and the side chain of Lys97 (Fig. 2A and B). Additionally, the dihydroxypropoxy moiety utilizes a hydrophobic cavity that connects the allosteric pocket and the ATP-binding site. Thus, the diol fragment forms two hydrogen bonds with the triphosphate group in ATP, one between the primary alcohol and the α-phosphate group (O–H···O=P) and

* Corresponding author.
E-mail address: grotli@chem.gu.se (M. Grøtli).

http://dx.doi.org/10.1016/j.ejmech.2014.07.018
0223-5234/

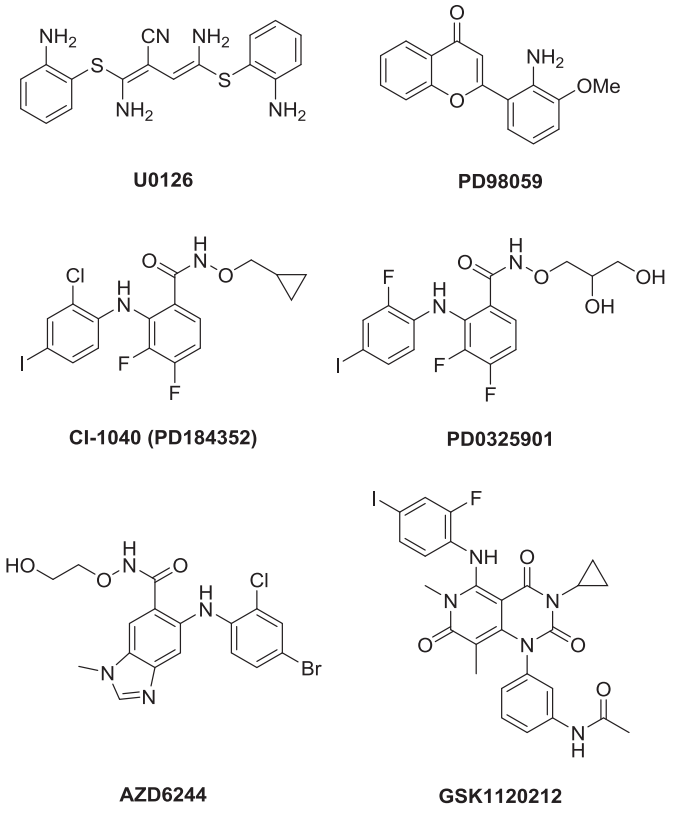


Fig. 1. Examples of non-ATP competitive MEK1/2 modulators that bind to an allosteric pocket, adjacent to the ATP-binding site.

one between the secondary alcohol and the γ-phosphate (O–H···O=P). Furthermore, the docking of PD98059 suggested that it forms three hydrogen bonds with the protein backbone; one between the chromone carbonyl oxygen and NH of Val211 in the activation loop (C=O···H–N), a second hydrogen bond between the chromone carbonyl oxygen and NH of Ser212 in the activation loop (C=O···H–N) and a third between the aniline NH_2 and the carbonyl oxygen of Phe209 (N–H···O=C) (Fig. 2C and D). PD98059 does not utilize the hydrophobic tunnel that links to the ATP-binding site (Fig. 2C), as shown for PD0325901 (Fig. 2A). Nevertheless, the docking study suggested that substituents in the 8-position on the chromone structure could extend toward the ATP-binding site. For example, introduction of an aminopropyl group in the 8-position (e.g. via Pd-mediated cross coupling of the halogenated chromone derivative) could tentatively reach down to the ATP-binding site. According to the docking model, compound **19** can achieve similar hydrogen bonding interactions as PD98059 (Val211, Ser212 and Phe209) in addition to the positively charged amino functionality which can bind to the γ-phosphate group in ATP (N–H···O=P) (Fig. 2E and F). Furthermore, compound **19** interacts via an additional hydrogen bond to the side chain of Asp190 (N–H···O=C). Compound **32**, can also achieve similar hydrogen bonding interactions as PD98059 (Val211, Ser212 and Phe209) and via an additional hydrogen bond between the hydroxyl functionality which can bind to the γ-phosphate group in ATP (O–H···O=P) (Fig. 2G and H). Based on these results, we wanted to explore the use of 3′-bromo-5′-chloro-2′-hydroxyacetophenone as a starting material for the synthesis of 8-substituted PD98059 analogs, due to its commercial availability and low price in comparison with 3′-halogenated 2′-hydroxyacetophenones. However, this required that the chlorine atom in the 6-position on the chromone structure did not affect the activity or the binding negatively. The chlorine atom in the 6-position, as depicted in Fig. 2E, did not seem to affect the docking or binding mode into the allosteric pocket. In fact, the chlorine atom could possibly act as a handle for further modifications (e.g. via Pd-mediated coupling reactions) since the allosteric pocket extends in the corresponding direction. We also observed that the methoxy group in the 3′-position of PD98059 could be replaced with an ethoxy group, which could utilize the area more efficiently (not shown).

2.2. Synthesis

The synthetic strategy was based on earlier work toward functionalized chromone derivatives in our laboratory [11,12,17]. The target compounds were prepared from 2′-hydroxyacetophenones **1**–**3** and the appropriate acid chlorides, which were prepared in situ from the corresponding carboxylic acids, **4** or **5**, using oxalyl chloride or thionyl chloride as chlorinating agents, which gave esters **6**–**8** in 70–92% yield (Scheme 1) [17,23,24]. The 2-nitrobenzoic acids **4**–**5** were used with the aim to later convert the nitro group to the corresponding amine under reductive reaction conditions. Base-promoted rearrangement of **6**–**8** to obtain diketones **9**–**11** was carried out in pyridine using conventional heating at 50 °C or microwave heating at 100 °C for 30 min [11,12,17]. Subsequently, acid-promoted cyclization gave the flavones **12**–**14** in high yields (68–99%). Thereafter, the nitro functionality in **12**–**13** was successfully converted to the corresponding amine under acidic conditions using tin as a reducing agent in ethanol to afford **15**–**16** in 80% and 68% yield, respectively [25].

Regioselective introduction of *N*-Boc-protected allylamine in the 8-position of the dihalogenated flavone **14** was achieved using the Heck reaction under inert conditions (Scheme 2). Compound **14** was treated with *N*-Boc-allylamine, $PdCl_2[P(o\text{-}Tol)_3]_2$, and triethylamine in acetonitrile at 75 °C overnight to give an isomeric mixture of **17** in 45% yield [27]. Subsequent reduction of the nitro group to the corresponding amine and reduction of the olefin in **17** was performed simultaneously by catalytic hydrogenation over Pd/C (10%) using an H-Cube® Continuous-flow Hydrogenation Reactor. The product **18** was obtained in low yields (33%), which could be explained by the observation of the formation of at least two byproducts; dehalogenated product together with a product where the olefin had been reduced but not the nitro group. Finally, acid-promoted deprotection of the Boc-group using TFA in dichloromethane gave the target compound **19** in 78% yield.

Regioselective introduction of amines in the 8-position of the dihalogenated flavone **14** was achieved using the Buchwald–Hartwig reaction under inert conditions (Table 1). Compound **14** was treated with the appropriate amine, $Pd_2(dba)_3$, *R*-(+)-BINAP and cesium carbonate in THF at 80 °C overnight to give compounds **20**–**25** in yields varying from 55 to 94% [27]. Subsequent reduction of the nitro group using tin in ethanol (and *in-situ* deprotection of **R** groups for compounds **23**, **24** and **26**) afforded **29**–**37** in good yields [25]. Dechlorination of **32** and **33** with $Pd(OAc)_2$, 2-(di-*t*-butylphosphino)biphenyl and HCOONa in MeOH, using microwave assisted heating (160 °C, 40 min) afforded **38** and **39** in 73% and 77% yields respectively [26].

2.3. Biological evaluation

The biochemical activity of the synthesized chromone-based MEK1/2 modulators was first evaluated by measuring the ability of recombinant active human MEK1 Ser218D/Ser222D (MEK1DD) to increase the myelin basic protein (MBP) kinase activity of ERK2 (Table 2). The study showed that the tested compounds have IC_{50} values ranging from 0.03 μM (**32**) to 10.3 μM (**35**), which

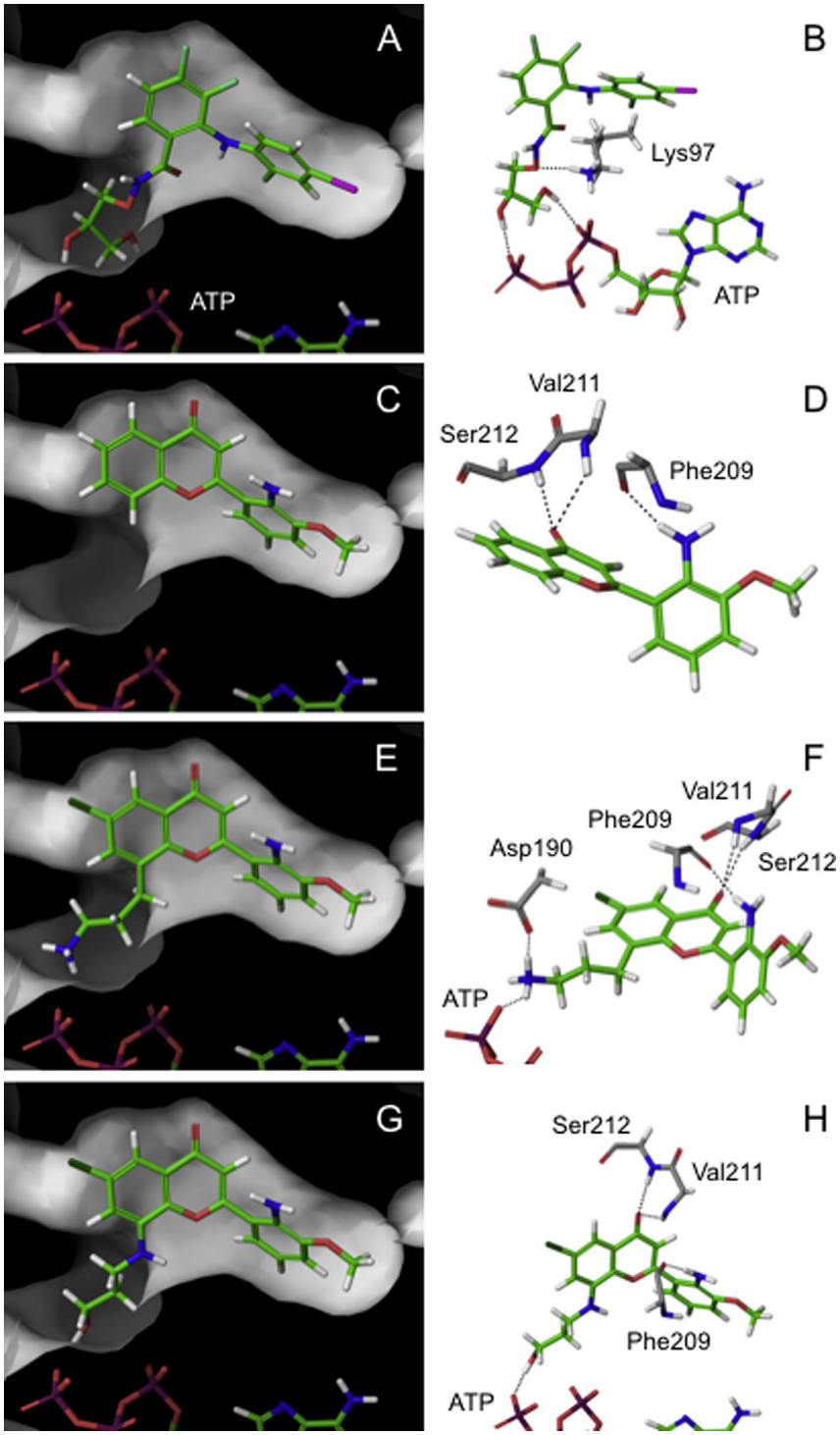
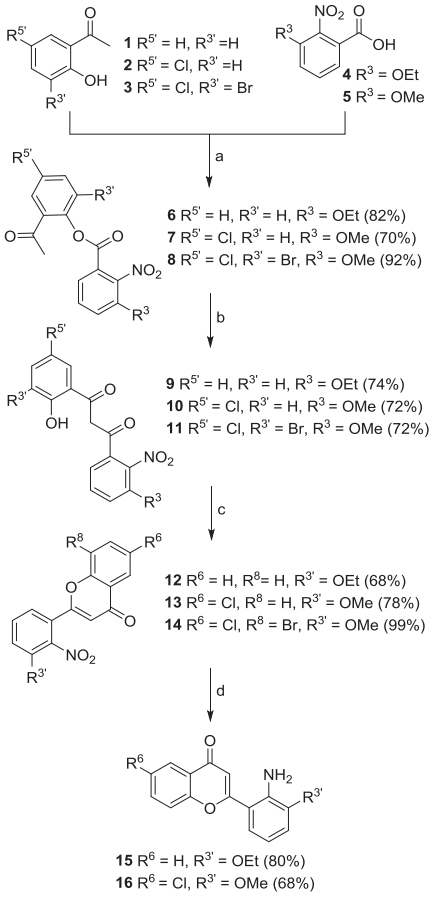


Fig. 2. Comparison of PD0325901 (A), PD98059 (C), compound **19** (E), and compound **32** (G) docked into the allosteric pocket of MEK1. Panels B, D, F and H show potential hydrogen bonding interactions (black dashed lines) of PD0325901, PD98059, **19**, or **32** respectively, with ATP and/or amino acid residues in the allosteric pocket.

corresponds up to 100 fold increased potency over **PD98059** (IC_{50} 2.0 μM).

The results indicate that the replacement of the methoxy group in the 3′-position (PD98059) on the chromone structure to an ethoxy group, as in **15**, did not have any noticeable impact on the *in vitro* activity (IC_{50} 2.0 vs. 1.9 μM). Compound **16** having a chloride in the 6-position, showed a slight decrease in the activity compared to PD98059, which indicates that it seems to have minor effect on the activity (PD98059 vs. **16**, IC_{50} 2.0 and 6.4 μM, respectively). Furthermore, introduction of terminal amino groups in the 8-position as in **19**, **34** and **35** did not increase the activity as expected. However, going back to the modeling study, this could be explained by the positioning of the carbonyl and aniline amine of **19** (Fig. 2F) in the allosteric pocket. The structure is placed somewhat further down towards the ATP-binding site, which could potentially prevent the favored interaction between the chromone carbonyl



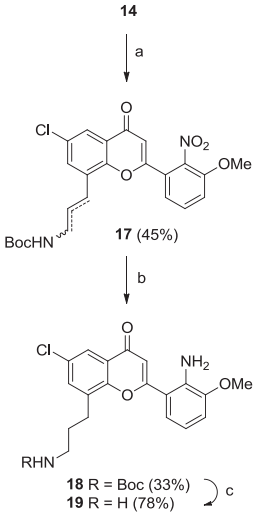
[a]Reagents and conditions: (a) (i) oxalyl chloride, DMF, dichloromethane, rt, 2 h or $SOCl_2$, toluene, reflux, 20 h; (ii) pyridine, 0 °C → rt, 1–2.5 h. (b) KOH, pyridine, 50 °C, 30 min, conventional heating or 100 °C, 30 min, microwave heating. (c) H_2SO_4, acetic acid, reflux, 45 min or H_2SO_4, EtOH, 100 °C, 30 min, microwave heating. (d) Sn powder, conc. HCl, EtOH, reflux, 1 h.

Scheme 1. Synthesis of chromone-based allosteric MEK1/2 modulators **15** and **16**.[a]

and Ser212. Alternatively, positively charged terminal amino groups in the 8-position could be unfavorable for the inhibitory activity due to charge repulsion of the adjacent Lys97 (Fig. 2B). Interestingly, the introduction of an isopropyl, n-butyl, benzyl or a tetrahydropyranyl substituent in the 8-position resulted in similar activity for **29**, **30**, **36** and **37** respectively compared to PD98059 (IC_{50} 2.0 vs. 1.1–2.7 μM). Strikingly, having terminal hydroxyl groups in the 8-position as in **32** and **33** resulted in a remarkable increase in the activity (IC_{50} 2.0 vs. 0.03 and 0.22 μM respectively). Using the modeling study, these results can be explained by the ability for these compounds to achieve the hydrogen bonding interactions between the chromone carbonyl and Ser212, and also by achieving an additional hydrogen bond to the γ-phosphate group in ATP (Fig. 2H). However, having two terminal hydroxyl groups in the 8-position (compound **33**) did not increase the activity as suspected. In contrast, the activity for compound **32**, with only one terminal hydroxyl group, was 10 fold higher (IC_{50} 0.03 μM) than for **33** (IC_{50} 0.22 μM). The reason for this is to our knowledge unknown and the results could not directly be explained by our modeling study.

In order to design an efficient inhibitor, in addition to strong and fast inhibition of the kinase, the inhibitor should also bind selectively to the target kinase. The selectivity of compound **32** (at a concentration of 1.0 μM) was evaluated towards a panel of 97 kinases distributed throughout the AGC, CAMK, CMGC, CK1, STE, TK, TKL, lipid, and atypical kinase families, including important mutant forms (Fig. A10) [28]. Only MEK1 was efficiently inhibited at 1 μM compound concentration. These results suggest that compound **32** has a very good selectivity profile towards MEK1 amongst other human kinases and it is reasonable to believe that the selectivity for the compounds are even higher at lower concentrations (below 1.0 μM).

The whole cell activity of the analogs towards MEK1/2 was investigated by monitoring the activating phosphorylation of their downstream substrates ERK1/2 by immunoblotting analysis in IEC-6 intestinal epithelial cells (Table 2). As hypothesized using our



[a]Reagents and conditions: (a) *N*-Boc-allylamine, $PdCl_2[P(o\text{-Tol})_3]_2$, Et_3N, acetonitrile, 75 °C, overnight. (b) H_2 (H-cube®), Pd/C (10%), THF, 20 °C, 30 bar. (c) trifluoroacetic acid, dichloromethane, rt, overnight.

Scheme 2. Synthesis of chromone-based allosteric MEK1/2 modulator **19**.[a]

modeling study, the results obtained for compounds **32** and **33** indicates an increase, although moderate, in the activity compared to PD98059 in the whole cell assay (IC_{50} 4.2 vs. 1.6 and 3.1 μM respectively). A representative immunoblot is shown for compound **33** (Fig. 3A). Unfortunately, the whole cell activity for compounds **29**–**31** and **34**–**37** were either approximately or more than 100 μM. Tentatively, these results indicate that lipophilic substituents in the 8-position interfere with the cellular uptake of these compounds and that substituents with terminal hydroxyl groups are favorable for cell permeability. Notably, permeability issues with the present scaffold have previously been reported. Hence, clinical trials using PD98059 as an MEK1 inhibitor was abandoned due to poor solubility and bioavailability of the compound [29].

Interestingly the difference in activity between **15** (1.0 μM) and **16** (>100 μM) suggested that the chlorine atom might affect the compounds solubility and permeability. In order to test this hypothesis the whole cell activity of the dechlorinated analogs of **32** and **33** (**38** and **39**) were determined. Compounds **38** and **39** gave significant improvement of the inhibitory effect in the whole-cell assay (IC_{50} 0.097 and 0.073 μM respectively) compared with the chlorinated counterparts **32** and **33** (IC_{50} 1.6 and 3.1 μM respectively).

To determine whether the inhibition of ERK1/2 phosphorylation translates into an inhibitory effect on cell proliferation, we tested the effect of compounds **32** and **33** on the proliferation of a small panel of human cancer cell lines bearing either activated *KRAS* (HCT 116 and A549 cells) or *BRAF* (HT-29 and A-375 cells) mutations. Compounds **32** and **33** were found to markedly inhibit the proliferation of all cancer cell lines with IC_{50} values comparable to the concentrations required to inhibit ERK1/2 phosphorylation (Fig. 4).

3. Conclusions

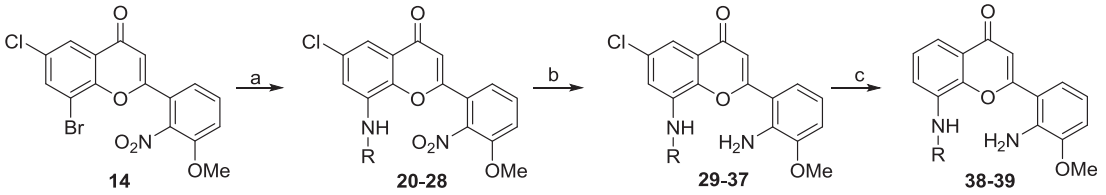
In conclusion, we have explored the use of chromone-based PD98059 analogs as allosteric MEK1 modulators. Molecular modeling and efficient synthesis provided a small series of chromone derivatives that showed good to moderate biochemical activities (IC_{50} 0.03–10.3 μM) against MEK1. The most potent derivative in the series (compound **32**, IC_{50} 30 nM) also showed a high selectivity profile against MEK1 in a panel of 97 different kinases. Two of the synthesized compounds (**38** and **39**) efficiently inhibited the MEK-ERK1/2 pathway in a whole cell assay with IC_{50} below 100 nM. Furthermore, compounds **32** and **33** demonstrated antiproliferative activity against both *RAS* and *BRAF* activated cancer cell lines. The syntheses of chromone-based compounds represent a promising starting point for the development of potent small molecule inhibitors of the MEK1/2 kinases.

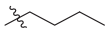
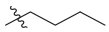
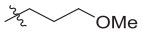
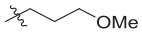
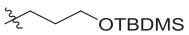
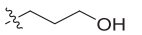
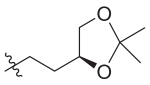
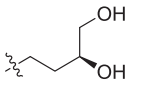
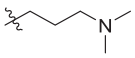
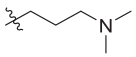
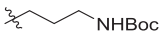
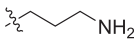
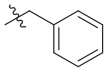
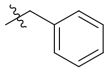
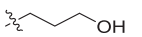
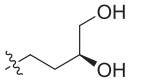
4. Experimental section

4.1. Chemistry

General All reagents and solvents were of analysis or synthesis grade. PD98059 was purchased from a commercial supplier. ^1H- and ^{13}C NMR-spectra were recorded on a Varian 400/54 spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$, CD_3OD or DMSO-d_6. Chemical shifts are reported in ppm with the solvent residual peak as reference; $CDCl_3$ (δ_H 7.26, δ_C 77.0) DMSO-d_6 (δ_H 2.50, δ_C 39.5) and CD_3OD (δ_H 3.31, δ_C 49.0). The reactions were monitored by thin-layer chromatography (TLC), on silica plated (Silica gel 60 F_{254}, E. Merck) aluminum sheets, detecting spots by UV (254 and 365 nm). Flash chromatography was performed manually on Merck Silica gel 60 (0.040–0.063 mm) or using a Biotage SP4 Flash instrument with prepacked columns. Analytical high-performance liquid chromatography (HPLC) analysis was carried out on a Waters separation module 2690 connected to a Waters photodiode array detector 996 using an Atlantis® 5 μm C18 AQ (250*4.6 mm) column. Preparative HPLC was carried out on a Waters 600 controller connected to a Waters 2487 Dual λ Absorbance detector using an Atlantis® Prep T3 5 μm C-18 (250*19 mm) column, unless otherwise stated. Dry solvents: THF and toluene were refluxed over sodium/benzophenone and distilled into 4 Å MS. Microwave reactions were carried out in a Biotage Initiator instrument with a fixed hold time using capped vials. Catalytic

Table 1
Synthesis of chromone-based allosteric MEK1/2 modulators **29**–**39**.[a]



Compound	R	Yield (%)	Compound	R	Yield (%)
20		93	**29**		74
21		94	**30**		83
22		80	**31**		74
23		81	**32**		51
24		76	**33**		77
25		82	**34**		67
26		55	**35**		61
27		78	**36**		58
28		59	**37**		54
			38		73
			39		77

[a] Reagents and conditions: (a) $Pd_2(dba)_3$, *R*-(+)- BINAP, Cs_2CO_3, THF, 80 °C, overnight. (b) Sn powder, conc. HCl, EtOH, reflux, 1 h; (c) $Pd(OAc)_2$, 2-(di-*t*-butylphosphino) biphenyl, HCOONa, MeOH, 160 °C (MW), 40 min.

Table 2
Biochemical and whole cell inhibitory activity against MEK1/2 of compounds **PD98059**, **15**, **16**, **19** and **29**–**39**.

Compound	Biochemical assay IC_{50} (μM)[a] MEK1	Whole cell assay IC_{50} (μM)[b] MEK1/2
PD98059	2.0	4.2
15	1.9	1.0
16	6.4	>100
19	4.4	ND[c]
29	2.7	>100
30	1.9	>100
31	9.1	>100
32	0.03	1.6
33	0.22	3.1
34	6.8	>100
35	10.3	>100
36	1.4	>100
37	1.1	>100
38	ND[c]	0.097
39	ND[c]	0.073

[a] Duplicate determination.
[b] Triplicate determination.
[c] ND = Not determined.

hydrogenation was performed in a H-Cube® Continuous-flow Hydrogenation Reactor from *ThalesNano* using sealed cartridges (Thales'CarCart®) with a heterogeneous palladium catalyst. A purity of more than 95% was determined by analytical HPLC for all compounds evaluated for biological activity.

4.1.1. 3-Ethoxy-2-nitrobenzoic acid (*4*)

Ethyl iodide (1.1 mL, 13.7 mmol) was added to a suspension of 3-hydroxy-2-nitrobenzoic acid (0.5 g, 2.73 mmol) and K_2CO_3 (2.45 g, 17.75 mmol) in DMF (6 mL). The reaction mixture was stirred at 60 °C for 2 h, then diluted with water (50 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic layers was washed with water (3 × 50 mL) and brine (50 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography (heptane/ethyl acetate, gradient 20 → 40% ethyl acetate) to give ethyl 3-ethoxy-2-nitrobenzoate (0.61 g, 93%) as a white solid. ^1H NMR ($CDCl_3$): δ 1.35 (t, J = 7.1 Hz, 3H), 1.42 (t, J = 6.0 Hz, 3H), 4.16 (q, J = 7.0 Hz, 2H), 4.36 (q, J = 7.1 Hz, 2H), 7.23 (d, J = 8.4 Hz, 1H), 7.44–7.49 (m, 1H), 7.58 (d, J = 7.6 Hz, 1H). Ethyl 3-ethoxy-2-nitrobenzoate (0.59 g, 2.47 mmol) was suspended in aqueous NaOH (0.1 M) (100 mL) and the mixture was refluxed for 1 h. The solution was acidified with

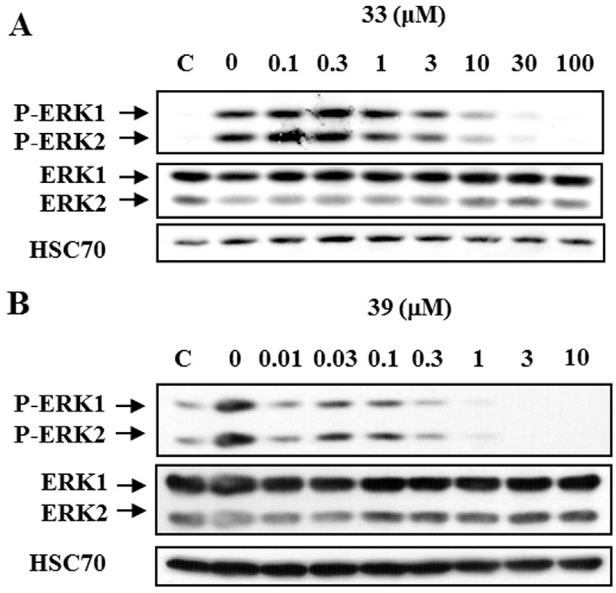


Fig. 3. Compounds **33** and **39** inhibits the activating phosphorylation of ERK1/2 in intact cells. Quiescent IEC-6 cells were incubated with the indicated concentration of compound **33** or **39** for 30 min prior to stimulation with serum for 5 min. Total lysates were analyzed by immunoblotting using antibodies specific for phospho-ERK1/2, total ERK1/2 and HSC70. The results are representative of two independent experiments.

HCl (4 M) and extracted with ethyl acetate (3 × 60 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure to give **4** (0.46 g, 88%) as a pale brown solid. ^{1}H NMR (CD_3OD): δ 1.37 (t, J = 7.0 Hz, 3H), 4.18 (q, J = 7.0 Hz, 2H), 7.44 (dd, J = 1.4, 8.1 Hz, 1H), 7.51–7.60 (m, 2H). Notably, the carboxylic acid hydrogen was not observed in the spectrum due to solvent exchange.

*4.1.2. General procedure for the synthesis of compounds **6**–**7***

Oxalyl chloride (1.2 equiv) followed by DMF (0.1 equiv) were added to an ice-cooled stirred suspension of the appropriate carboxylic acid (1.0 equiv) in DCM (1.5 mL/mmol carboxylic acid). The reaction mixture was allowed to reach room temperature, and was stirred for 2 h. It was then concentrated under reduced pressure to give a crude residue of the corresponding acid chloride. The appropriate 2′-hydroxyacetophenone (1 equiv) was added to an ice-cooled solution of the crude residue dissolved in pyridine (4 mL/mmol). The reaction mixture was stirred at 0 °C for 15 min and was then stirred at room temperature for 45 min. The mixture was poured over aqueous HCl (1 M) and ice and the formed precipitate was filtered off, washed with water and dried under reduced pressure.

*4.1.2.1. 2-Acetylphenyl 3-ethoxy-2-nitrobenzoate (**6**).* The title compound was synthesized according to the general procedure. 2′-Hydroxyacetophenone **1** (255 mg, 1.87 mmol) gave **6** (508 mg, 82%) as a beige powder. ^{1}H NMR ($CDCl_3$): δ 1.44 (t, J = 7.0 Hz, 3H), 2.54 (s, 3H), 4.20 (q, J = 7.0 Hz, 2H), 7.22 (dd, J = 1.2, 8.0 Hz, 1H), 7.31 (dd, J = 1.2, 8.4 Hz, 1H), 7.36–7.41 (m, 1H), 7.53–7.61 (m, 2H), 7.80 (dd, J = 1.0, 7.8 Hz, 1H), 7.85 (dd, J = 1.7, 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 14.4, 29.2, 65.8, 118.7, 122.5, 123.3, 123.7, 126.6, 130.4, 130.6, 131.0, 133.6, 136.5, 148.4, 150.6, 161.7, 197.1.

*4.1.2.2. 2-Acetyl-4-chlorophenyl 3-methoxy-2-nitrobenzoate (**7**).* The title compound was synthesized according to the general procedure. 5′-Chloro-2′-hydroxyacetophenone **2** (285 mg, 1.17 mmol)

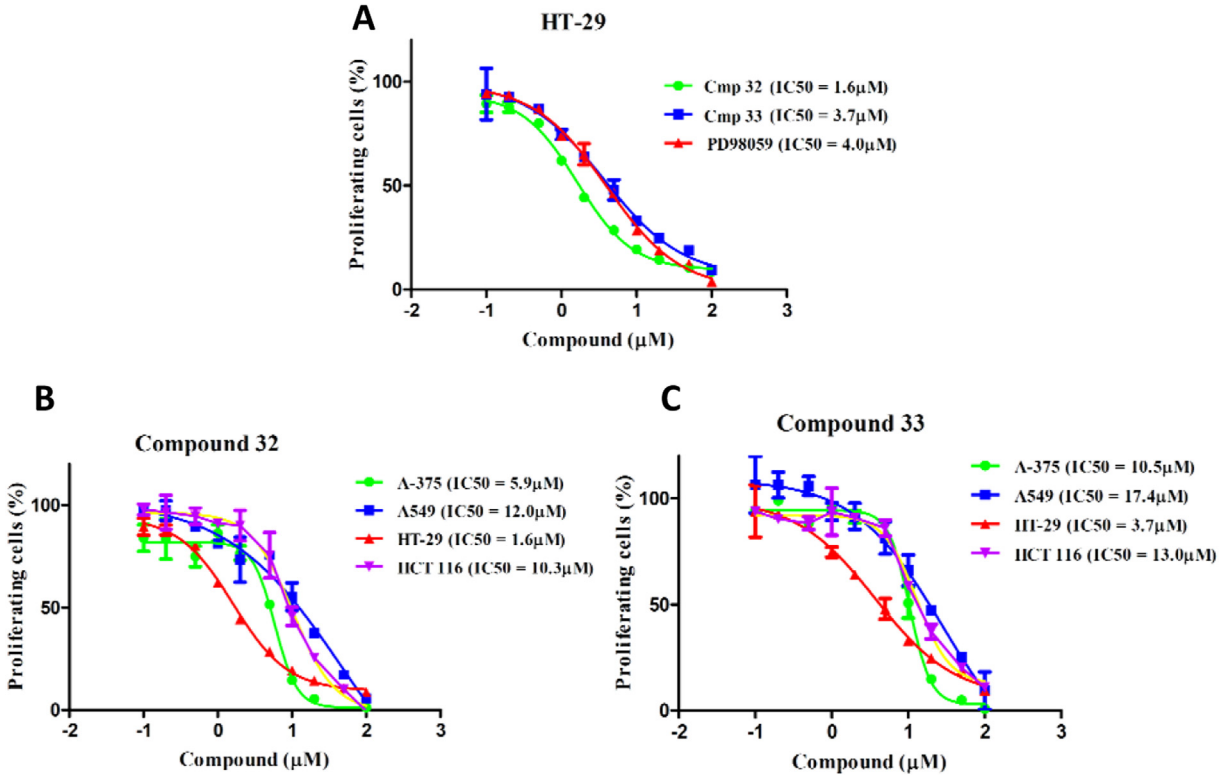


Fig. 4. Antiproliferative activity of compounds **PD98059**, **32** and **33** in four different human cancer cell lines. (A) Antiproliferation activity of **PD98059**, **32** and **33** in HT-29 cells. (B) Antiproliferative activity of compound **32** in A-375, A549, HT-29 and HCT 116 cells. (C) Antiproliferative activity of compound **33** in A-375, A549, HT-29 and HCT 116 cells. The cancer cell lines were incubated with increasing concentrations of the given compounds for 5 days. Cell proliferation was measured using a colorimetric WST-1 assay (mean ± SEM values from triplicate wells are presented and are expressed as percentage of the DMSO control.

gave **7** (480 mg, 70%) as white crystals. ^{1}H NMR ($CDCl_3$): δ 2.52 (s, 3H), 3.96 (s, 3H), 7.17 (d, J = 8.6 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.49–7.64 (m, 2H), 7.74–7.82 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 29.4, 57.0, 118.0, 122.8, 123.2, 125.3, 130.4, 131.4, 132.0, 133.5, 146.9, 151.4, 161.7, 196.0.

4.1.3. 2-Acetyl-6-bromo-4-chlorophenyl 3-methoxy-2-nitrobenzoate (*8*)

$SOCl_2$ (5 mL, 68.8 mmol) was added to a solution of 3-methoxy-2-nitrobenzoic acid (0.14 g, 0.71 mmol) in toluene (5 mL). The reaction mixture was refluxed for 20 h, allowed to reach room temperature and was then concentrated under reduced pressure. The crude residue was dissolved in pyridine (0.5 mL) and the mixture was added to an ice-cooled solution of 2′-hydroxyacetophenone **3** (90 mg, 0.355 mmol) in pyridine (2.5 mL). The reaction mixture was allowed to reach room temperature and was stirred for 2.5 h. The crude mixture was concentrated under reduced pressure and purified by column chromatography (toluene/ethyl acetate 4:1) to give **8** (0.14 g, 92%) as offwhite crystals. ^{1}H NMR ($CDCl_3$): δ 2.53 (s, 3H), 3.96 (s, 3H), 7.34 (dd, J = 0.7, 8.4 Hz, 1H), 7.37–7.64 (m, 1H), 7.73 (d, J = 2.2 Hz, 1H), 7.79 (d, J = 2.2 Hz, 1H), 7.88 (dd, J = 1.0, 7.9 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 29.3, 57.0, 118.5, 119.4, 121.8, 123.0, 128.3, 129.3, 131.2, 133.0, 133.5, 136.4, 144.8, 151.3, 160.3, 195.1.

4.1.4. 2-(3-Ethoxy-2-nitrophenyl)chromone (*12*)

Pulverized KOH (97 mg, 1.75 mmol) was added to a solution of **6** (385 mg, 1.17 mmol) in pyridine (8 mL). The reaction mixture was stirred at 50 °C for 30 min. The mixture was allowed to reach room temperature and was then acidified with aqueous acetic acid (5%). The precipitate was filtered off, washed with water dried which gave 1-(3-Ethoxy-2-nitrophenyl)-3-(2-hydroxyphenyl)propane-1,3-dione (**9**). (285 mg, 74%) as a solid powder. **9** was used in the next reaction step without any further purification. ^{1}H NMR ($CDCl_3$): δ 1.44 (t, J = 7.0 Hz, 3H), 4.19 (q, J = 7.0 Hz, 2H), 6.61 (s, 1H), 6.88–6.94 (m, 1H), 7.00 (dd, J = 1.2, 8.4 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.31 (dd, J = 1.2, 7.8 Hz, 1H), 7.45–7.54 (m, 2H), 7.66 (dd, J = 1.2, 8.4 Hz, 1H), 11.81 (s, 1H). Concentrated H_2SO_4 (70 μl, 1.31 mmol) was added to a suspension of **9** (250 mg, 0.76 mmol) in acetic acid (4 mL). The reaction mixture was refluxed for 45 min and was then allowed to reach room temperature. The generated precipitate was filtered off and washed with water. The crude residue was purified by column chromatography (heptane/ethyl acetate 3:2) and **12** (160 mg, 68%) was isolated as a beige solid. ^{1}H NMR ($CDCl_3$): δ 1.46 (t, J = 6.9 Hz, 3H), 4.22 (q, J = 7.0 Hz, 2H), 6.65 (s, 1H), 7.20–7.31 (m, 2H), 7.41–7.46 (m, 2H), 7.51–7.58 (m, 1H), 7.69 (dd, J = 7.2, 8.4 Hz, 1H), 8.20 (d, J = 8.1 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 14.6, 66.0, 111.4, 116.6, 118.3, 120.6, 120.7, 123.9, 125.8, 125.9, 126.6, 131.7, 134.4, 151.2, 156.3, 160.4, 178.0.

4.1.5. 6-Chloro-2-(3-methoxy-2-nitrophenyl)chromone (*13*)

Pulverized KOH (92 mg, 1.67 mmol) was added to a solution of **7** (390 mg, 1.11 mmol) in pyridine (7 mL). The reaction mixture was stirred at 50 °C for 30 min. The mixture was allowed to reach room temperature and was then acidified with aqueous acetic acid (5%). The precipitate was filtered off, washed with water dried which gave 1-(5-Chloro-2-hydroxyphenyl)-3-(3-methoxy-2-nitrophenyl) propane-1,3-dione (**10**). (280 mg, 72%). as a solid powder. **10** was used in the next reaction step without any further purification. ^{1}H NMR ($CDCl_3$): δ 3.97 (s, 3H), 6.55 (s, 1H), 6.90–7.06 (m, 1H), 7.09–7.79 (m, 5H), 11.73 (s, 1H). Concentrated H_2SO_4 (30 μl, 0.32 mmol) was added to a suspension of the **10** (95 mg, 0.27 mmol) in acetic acid (1.9 mL). The reaction mixture was refluxed for 45 min and was then allowed to reach room temperature. The generated precipitate was filtered off and washed which gave **13** (70 mg, 78%) as a white solid after drying. ^{1}H NMR (DMSO-d_6) δ 3.97 (s, 3H), 6.92 (s, 1H), 7.40–7.79 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 14.6, 66.0, 76.8, 77.2, 77.5, 111.4, 116.6, 118.3, 120.6, 123.9, 125.8, 125.9, 126.6, 131.7134.4, 151.2, 156.3, 160.4, 178.0.

4.1.6. 8-Bromo-6-chloro-2-(3-methoxy-2-nitrophenyl)chromone (*14*)

KOH (0.36 g, 6.43 mmol) was added to a solution of **8** (0.92 g, 2.14 mmol) in pyridine (20 mL). The reaction was heated in a microwave cavity at 100 °C for 30 min. The solution was poured into water (20 mL) and neutralized with HCl (1 M). The yellow precipitate was filtered off and dried in vacuo to give **1**-(3-Bromo-5-chloro-2-hydroxyphenyl)-3-(3-methoxy-2-nitrophenyl)propane-1,3-dione (**11**) (0.66 g, 72%) as a yellow powder. **11** was used in the next reaction step without any further purification. ^{1}H NMR ($CDCl_3$): δ 3.96 (s, 3H), 6.55 (s, 1H), 7.22–7.28 (m, 1H), 7.30–7.34 (m, 1H), 7.52–7.62 (m, 2H), 7.74 (d, J = 2.2 Hz, 1H), 12.43 (s, 1H). Concentrated H_2SO_4 (0.01 mL, 0.21 mmol) was added to a solution of **11** (0.03 g, 0.07 mmol) in EtOH (2 mL). The reaction was heated in a microwave cavity at 100 °C for 30 min. The solution was allowed to reach room temperature and the white precipitate was filtered off to give **14** (28 mg, 99%) as white fluffy crystals. ^{1}H NMR ($CDCl_3$): δ 3.99 (s, 3H), 6.64 (s, 1H), 7.27–7.32 (m, 2H), 7.59–7.69 (m, 1H), 7.89 (d, J = 2.5 Hz, 1H), 8.12 (d, J = 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 56.9, 11.7, 113.0, 116.0, 121.2, 124.5, 125.5, 126.6, 131.9, 132.0, 137.4, 139.2, 151.7, 152.0, 161.0, 176.

4.1.7. General procedure for the synthesis of chromones *15*–*16*

Tin powder (5 equiv) was added to a suspension of the appropriate nitroflavone (**12** or **13**) (1 equiv) in EtOH (9 mL/mmol) and the reaction mixture was heated to reflux. Concentrated HCl (6 equiv) was added to the heated mixture and the reaction was refluxed for one hour. The reaction was allowed to reach room temperature and was then basified with aqueous NaOH (5%). The mixture was diluted with water and extracted with ethyl acetate. The combined organic phases were dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the pure product.

*4.1.7.1. 2-(2-Amino-3-ethoxyphenyl)chromone (**15**).* The title compound was synthesized according to the general procedure described above. Compound **12** (0.1 g, 0.32 mmol) gave **15** (72 mg, 80%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.47 (t, J = 7.0 Hz, 3H), 4.10 (q, J = 7.0 Hz, 2H), 4.70 (bs, 2H), 6.66 (s, 1H), 6.72–6.79 (m, 1H), 6.86 (d, J = 7.9 Hz, 1H), 7.09 (d, J = 7.9 Hz, 1H), 7.37–7.44 (m, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.62–7.70 (m, 1H), 8.22 (d, J = 7.9 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 14.9, 64.2, 110.3, 112.9, 116.3, 117.4, 117.8, 120.9, 123.8, 125.2, 125.7, 133.6, 136.0, 146.7, 156.2, 165.0, 178.2. HRMS (ESI): m/z calculated for $C_{17}H_{15}NO_3$: 282.1046. Found: 282.1128.

*4.1.7.2. 2-(2-Amino-3-methoxyphenyl)-6-chlorochromone (**16**).* The title compound was synthesized according to the general procedure. Compound **13** (67 mg, 0.20 mmol) gave **16** (41 mg, 68%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 3.91 (s, 3H), 4.67 (bs, 2H), 6.68 (s, 1H), 6.76–6.82 (m, 1H), 6.90 (d, J = 7.9 Hz, 1H), 7.10 (d, J = 7.9 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.62 (dd, J = 2.5, 8.9 Hz, 1H), 8.19 (d, J = 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 55.9, 110.2, 112.1, 115.9, 117.5, 119.5, 121.0, 124.8, 125.2, 131.2, 133.8, 136.0, 147.5, 154.5, 165.1, 176.9. HRMS (ESI): m/z calculated for $C_{16}H_{12}ClNO_3$: 302.0584. Found: 302.0568.

4.1.8. (E)-8-(3-tert-Butoxycarbonylamino-1-propenyl)-6-chloro-2-(3-methoxy-2-nitrophenyl)chromone (*17*)

The reaction was performed under inert conditions using dry evacuated glassware, which were backfilled with nitrogen. *N*-Boc-allylamine (191 mg, 1.20 mmol), $PdCl_2[P(o\text{-}Tol)_3]_2$ (48 mg, 0.1 mmol) and triethylamine (0.17 mL, 1.22 mmol) were added to a

solution of **14** (250 mg, 0.61 mmol) in acetonitrile (1.1 mL) and the reaction mixture was heated at 75 °C overnight. The mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography (heptane/ethyl acetate 6:4) to give **17** (32 mg, 11%) as a pure product. In addition, fractions containing **17** and the *E/Z* isomers of the corresponding enamine (105 mg, 34%) were combined and used in the next reaction step. ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H), 3.93–4.04 (m, 5H), 4.96 (bs, 1H), 6.23–6.32 (m, 1H), 6.63–6.65 (m, 1H), 6.77 (d, J = 15.9 Hz, 1H), 7.22–7.25 (m, 2H), 7.54–7.60 (m, 1H), 7.68–7.72 (m, 1H), 8.00–8.03 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 28.3 (3C), 42.5, 56.8, 79.4, 111.4 (2C), 115.7, 121.0, 121.4, 123.8, 124.9, 126.7, 129.8, 130.7, 131.7, 132.0, 132.5, 151.4, 151.8, 155.8, 160.5, 176.6.

4.1.9. 2-(2-Amino-3-methoxyphenyl)-8-(3-tert-butoxycarbonyl-aminopropyl-6-chlorochromone (**18**)

An isomeric mixture of **17** (75 mg, 0.15 mmol) in THF (6 mL) was injected through a cartridge of Pd/C (10%) using an H-cube reactor with a flow rate of 1 mL/min at 20 °C (atm. pressure). The hydrogen gas pressure was increased to 30 bar and the mixture was looped again (twice) through the catalyst. The solution was concentrated and the crude residue was purified by column chromatography (heptane/ethyl acetate 65:35) to give **18** (23 mg, 33%). ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H), 1.83–1.95 (m, 2H), 2.88–2.95 (m, 2H), 3.13–3.30 (m, 2H), 3.91 (s, 3H), 4.60–4.82 (m, 3H), 6.67 (s, 1H), 6.77–6.83 (m, 1H), 6.90 (dd, J = 1.4, 8.0 Hz, 1H), 7.09 (dd, J = 1.4, 8.0 Hz, 1H), 7.47 (d, J = 2.5 Hz, 1H), 8.02 (d, J = 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 27.1, 28.5 (3C), 30.4, 40.3, 56.0, 79.4, 110.3, 112.2, 116.3, 117.8, 121.1, 123.2, 125.0, 131.0, 133.3, 133.8, 136.2, 147.7, 153.0, 156.1, 164.8, 177.4.

4.1.10. 2-(2-Amino-3-methoxyphenyl)-8-(3-aminopropyl)-6-chloro-chromone (**19**)

Trifluoroacetic acid (0.2 mL, 2.61 mmol) was added to a solution of **18** (23 mg, 0.05 mmol) in dichloromethane (0.5 mL) and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography (heptane/ethyl acetate 4:6) to give **19** (14 mg, 78%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.64 (bs, 2H), 1.79–1.91 (m, 2H), 2.70–2.85 (m, 2H), 2.89–3.01 (m, 2H), 3.92 (s, 3H) 4.68 (bs, 2H), 6.69 (s, 1H), 6.75–6.83 (m, 1H), 6.90 (dd, J = 1.4, 7.9 Hz, 1H), 7.10 (dd, J = 1.4, 7.9 Hz, 1H), 7.48 (d, J = 2.5 Hz, 1H), 8.03 (d, J = 2.5 Hz, 1H): ^{13}C NMR ($CDCl_3$): δ 26.8, 29.7, 41.5, 55.8, 110.2, 112.0, 116.2, 117.6, 120.9, 122.8, 124.9, 130.8, 133.6, 133.7, 136.0, 147.5, 152.9, 164.5, 177.3. HRMS (ESI): m/z calculated for $C_{19}H_{19}ClN_2O_3$: 359.1162. Found: 359.1146.

4.1.11. General procedure for the synthesis of chromones **20**–**28**

The reactions were performed under inert conditions using dry evacuated glassware, which were backfilled with nitrogen. Appropriate amine (1.10 mmol), $Pd_2(dba)_3$ (11 mg, 0.05 mmol), *R*-(+)-BINAP (18 mg, 0.12 mmol), and Cesium carbonate (111 mg, 1.40 mmol) were added to a solution of **14** (100 mg, 0.24 mmol) in THF (3 mL) and the reaction mixtures were heated at 80 °C overnight. The mixtures were concentrated under reduced pressure and the crude residues purified by column chromatography (heptane/ethyl acetate 7:3).

4.1.11.1. 6-Chloro-8-(isopropylamino)-2-(3-methoxy-2-nitrophenyl)-4H-chromen-4-one (**20**).

The title compound was synthesized according to the general procedure using isopropyl amine (23 μl, 0.27 mmol) gave **20** (88 mg, 93%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 1.33 (bs, 3H), 1.35 (bs, 3H), 3.60–3.70 (m, 1H), 3.99 (s, 3H), 4.53 (bs, 1H), 6.69, (s, 1H), 6.76 (dd, J = 2.4, 0.5 Hz, 1H), 7.24 (m, 2H), 7.29 (dd, J = 7.9, 1.0 Hz, 1H), 7.33 (d, J = 2.4 Hz, 1H), 7.59 (t, J = 8.3 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 22.4, 44.5, 57.0, 109.7, 111.3, 113.2, 115.6, 120.8, 124.7, 126.3, 132.0, 132.7, 143.6, 151.6, 159.4, 177.3.

4.1.11.2. 8-(Butylamino)-6-chloro-2-(3-methoxy-2-nitrophenyl)-4H-chromen-4-one (**21**).

The title compound was synthesized according to the general procedure using n-butylamine (27 μl, 0.27 mmol) gave **21** (92 mg, 94%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, J = 7.4 Hz, 3H), 1.42–1.55 (m, 2H), 1.68–1.79 (m, 2H), 3.07–3.18 (m, 2H), 3.95 (s, 3H), 4.56 (t, J = 5.0 Hz, 1H), 6.63 (s, 1H), 6.69 (d, J = 2.4 Hz, 1H), 7.20–7.27 (m, 3H), 7.53 (t, J = 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 13.9, 20.3, 30.9, 43.5, 56.9, 109.7, 111.1, 112.6, 115.6, 120.6, 124.4, 126.0, 131.9, 132.6, 139.7, 143.4, 151.5, 159.3, 177.1.

4.1.11.3. 6-Chloro-2-(3-methoxy-2-nitrophenyl)-8-((3-methoxypropyl)amino)-4H-chromen-4-one (**22**).

The title compound was synthesized according to the general procedure using 3-Methoxypropylamine (27 μl, 0.27 mmol) gave **22** (82 mg, 80%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 1.92–2.08 (m, 2H), 3.17–3.42 (m, 5H), 3.45–3.62 (m, 2H), 3.90–3.99(m, 3H), 4.67 (bs, 1H), 6.61–6.85 (m, 2H), 7.18–7.35 (m, 3H), 7.56 (t, J = 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 26.8, 42.2, 45.6, 57.0, 57.4, 110.0, 111.3, 112.9, 115.6, 120.7, 124.5, 126.3, 131.9, 132.7, 139.2, 139.7, 143.6, 151.6, 159.3, 177.2.

4.1.11.4. 8-((3-((Tert-butyldimethylsilyl)oxy)propyl)amino)-6-chloro-2-(3-methoxy-2-nitrophenyl)-4H-chromen-4-one (**23**).

The title compound was synthesized according to the general procedure using 3-((*tert*-butyldimethylsilyl)oxy)propan-1-amine (51 mg, 0.27 mmol) gave **23** (102 mg, 81%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 0.03 (s, 6H), 0.87 (s, 9H),1.89–2.03 (m, 2H), 3.21–3.32 (m, 2H), 3.80 (t, J = 5.9 Hz, 2H), 3.95 (s, 3H), 4.62 (bs, 1H), 6.64 (s, 1H), 6.75 (d, J = 2.4 Hz, 1H), 7.19–7.30 (m, 3H), 7.54 (t, J = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 5.3 (2C), 18.4, 26.0 (3C), 31.9, 40.6, 56.9, 60.6, 109.8, 111.1, 112.7, 115.6, 120.6 (2C), 124.4, 126.1, 131.9, 132.6, 139.7, 143.4, 1515, 159.3, 177.1.

4.1.11.5. (S)-6-Shloro-8-((2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)-2-(3-methoxy-2-nitrophenyl)-4H-chromen-4-one (**24**).

The title compound was synthesized according to the general procedure using (*S*)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethan-1-amine (70 μl, 0.27 mmol) gave **24** (87 mg, 76%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 1.33 (s, 3H), 1.39 (s, 3H), 1.99 (dd, J = 13.5, 6.7 Hz, 2H), 3.23–3.37 (m, 2H), 3.55–3.66 (m, 1H), 3.95 (s, 3H), 4.04–4.15 (m, 1H), 4.20–4.35 (m, 1H), 4.68 (t, J = 5.1 Hz, 1H), 6.63 (s, 1H), 6.76 (d, J = 2.3 Hz, 1H), 7.15–7.33 (m, 3H), 7.55 (t, J = 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 25.7, 27.0, 33.0, 40.7, 56.9, 69.4, 73.9, 109.2, 110.1, 111.2, 112.9, 115.6, 120.6, 124.4, 126.1, 131.9, 132.6, 139.0, 139.4, 143.4, 151.5, 159.2, 177.0.

4.1.11.6. 6-Chloro-8-((3-(dimethylamino)propyl)amino)-2-(3-methoxy-2-nitrophenyl)-4H-chromen-4-one (**25**).

The title compound was synthesized according to the general procedure using *N,N*-Dimethyl-1,3-propanediamine (34 μl, 0.27 mmol) gave **25** (86 mg, 82%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 1.87–2.06 (m, 2H), 2.29 (s, 6H), 2.53 (t, J = 7.0 Hz, 2H), 3.14–3.37 (m, 2H), 3.97 (s, 2H), 4.85 (bs, 1H), 6.64 (s, 1H), 6.77 (d, J = 2.3 Hz, 1H), 7.20–7.34 (m, 3H), 7.57 (t, J = 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 26.5, 42.1, 45.3 (2C), 57.0, 57.3, 110.0, 111.3, 112.9, 115.7, 120.7, 124.5, 126.2, 131.9, 132.7, 139.6, 143.5, 151.6, 159.2, 177.1.

4.1.11.7. Tert-butyl (3-((6-chloro-2-(3-methoxy-2-nitrophenyl)-4-oxo-4H-chromen-8-yl)amino)propyl)carbamate (**26**).

The title compound was synthesized according to the general procedure using *tert*-butyl (3-aminopropyl)carbamate (47 μl, 0.27 mmol) gave **26** (67 mg, 55%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H), 1.88–2.11 (m, 2H), 3.14–3.39 (m, 4H), 3.98 (s, 3H), 4.60 (bs, 1H),

4.75 (bs, 1H), 6.69 (s, 1H), 6.75 (d, $J = 2.1$ Hz, 1H), 7.21–7.38 (m, 3H), 7.58 (t, $J = 8.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 28.5, 29.2, 41.0, 57.0, 110.4, 111.3, 112.9, 115.7, 120.7, 124.6, 126.2, 128.5, 129.1, 130.3, 132.0, 132.7, 139.4, 143.7, 151.7, 156.2, 159.2, 177.1.

*4.1.11.8. 8-(Benzylamino)-6-chloro-2-(3-methoxy-2-nitrophenyl)-4H-chromen-4-one (**27**).* The title compound was synthesized according to the general procedure using Benzylamine (29 μl, 0.27 mmol) gave **27** (83 mg, 78%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 3.94 (s, 3H), 4.45 (d, $J = 5.8$ Hz, 2H), 5.16 (t, $J = 5.7$ Hz, 1H), 6.66 (s, 1H), 6.73 (d, $J = 2.4$ Hz, 1H), 7.19–7.47 (m, 8H), 7.56 (t, $J = 8.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 47.7, 56.9, 110.7, 111.4, 113.4, 115.7, 120.7, 124.6, 126.2, 127.5 (2C), 127.7, 128.9 (2C), 131.9, 132.6, 137.6, 139.1, 143.6, 151.6, 159.4, 177.0.

*4.1.11.9. 6-Chloro-2-(3-methoxy-2-nitrophenyl)-8-((tetrahydro-2H-pyran-4-yl)amino)-4H-chromen-4-one (**28**).* The title compound was synthesized according to the general procedure using Benzylamine (28 μl, 0.27 mmol) gave **28** (62 mg, 59%) as a beige solid. ^{1}H NMR ($CDCl_3$): δ 1.65–1.84 (m, 2H), 2.00–2.19 (m, 2H), 3.56 (t, $J = 11.3$ Hz, 3H), 3.99 (s, 2H), 4.02–4.11 (m, 2H), 4.62 (d, $J = 7.3$ Hz, 1H), 6.69 (s, 1H), 6.78 (d, $J = 1.8$ Hz, 1H), 7.21–7.38 (m, 3H), 7.59 (t, $J = 8.2$ Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 32.7 (2C), 49.1, 57.0, 66.7 (2C), 110.4, 111.3, 113.1, 115.7, 120.7 (2C), 124.8, 126.2, 132.0, 138.2, 143.6, 151.7, 159.4, 177.1.

*4.1.12. General procedure for the synthesis of chromones **29**–**37***

Tin powder (5 equiv) was added to a suspension of the appropriate nitroflavone (**29**–**37**) (1 equiv) in EtOH (9 mL/mmol) and the reaction mixture was heated to reflux. Concentrated HCl (6 equiv) was added to the heated mixture and the reaction was refluxed for one hour. The reaction was allowed to reach room temperature and was then basified with aqueous NaOH (5%). The mixture was diluted with water and extracted with ethyl acetate. The combined organic phases were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified using preparative HPLC (H_2O:AcCN (0.1%TFA) 100:0 to 0:100 for 45 min, then at 0:100 for 15 min, with a flow of 14 ml/min).

*4.1.12.1. 2-(2-Amino-3-methoxyphenyl)-6-chloro-8-(isopropylamino)-4H-chromen-4-one (**29**).* The title compound was synthesized according to the general procedure described above. Compound **20** (80 mg, 0.21 mmol) gave **29** (55 mg, 75%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.29 (s, 3H), 1.30 (s, 3H), 3.61–3.74 (m, 1H), 3.92 (s, 3H), 4.36 (s, 3H), 6.64 (s, 1H), 6.91 (dd, $J = 8.0$, 1.3 Hz, 1H), 7.06 (dd, $J = 8.0$, 1.3 Hz, 1H), 7.26 (s, 1H), 7.38 (d, $J = 2.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 22.8, 44.4, 56.0, 110.4, 110.5, 112.2, 112.8, 116.5, 117.9, 121.1, 124.5, 132.1, 135.9, 138.4, 143.6, 147.7, 164.0, 177.8. HRMS (ESI): m/z calculated for $C_{19}H_{19}ClN_2O_3$: 356.1162. Found: 356.1174.

*4.1.12.2. 2-(2-Amino-3-methoxyphenyl)-8-(butylamino)-6-chloro-4H-chromen-4-one (**30**).* The title compound was synthesized according to the general procedure described above. Compound **21** (70 mg, 0.17 mmol) gave **30** (54 mg, 83%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 0.99 (t, $J = 7.3$ Hz, 3H), 1.40–1.53 (m, 2H), 1.63–1.75 (m, 2H), 3.21 (t, $J = 6.8$ Hz, 2H), 3.94 (s, 3H), 6.95–6.71 (m, 6H), 7.09 (d, $J = 7.2$ Hz, 1H), 7.32 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 13.9, 20.4, 31.1, 43.4, 56.2, 109.6, 110.4, 112.8, 113.1, 116.9, 119.2, 121.2, 123.5, 132.6, 134.6, 139.2, 143.9, 148.3, 165.1, 179.0. HRMS (ESI): m/z calculated for $C_{20}H_{21}ClN_2O_3$: 373.1319. Found: 373.1321.

*4.1.12.3. 2-(2-Amino-3-methoxyphenyl)-6-chloro-8-((3-methoxypropyl)amino)-4H-chromen-4-one (**31**).* The title compound was synthesized according to the general procedure described above. Compound **22** (50 mg, 0.12 mmol) gave **31** (34 mg, 73%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.86–2.02 (m, 2H), 3.21–3.38 (m, 5H), 3.54 (t, $J = 5.4$ Hz, 2H), 3.91 (s, 3H), 5.10 (bs, 2H), 6.65 (s, 1H), 6.72–6.94 (m, 3H), 7.08 (d, $J = 7.8$ Hz, 1H), 7.35 (d, $J = 1.9$ Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 28.9, 42.2, 56.0, 58.9, 71.7, 110.4, 110.5, 112.2, 112.3, 116.9, 118.0, 121.1, 124.3, 132.0, 135.4, 139.5, 143.8, 147.8, 163.7, 177.8. HRMS (ESI): m/z calculated for $C_{20}H_{21}ClN_2O_4$: 389.1268. Found: 389.1259.

*4.1.12.4. 2-(2-Amino-3-methoxyphenyl)-6-chloro-8-((3-hydroxypropyl)amino)-4H-chromen-4-one (**32**).* The title compound was synthesized according to the general procedure described above. Compound **23** (90 mg, 0.17 mmol) gave **32** (33 mg, 51%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.98 (m, 2H), 3.37 (t, $J = 6.4$ Hz, 2H), 3.85–3.94 (m, 5H), 4.67 (bs, 2H), 6.66 (s, 1H), 6.73–6.80 (m, 2H), 6.86 (d, $J = 7.1$ Hz, 1H), 7.04 (d, $J = 8.0$ Hz, 1H), 7.34 (d, $J = 2.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 31.1, 41.9, 56.0, 61.6, 110.1, 110.6, 112.3, 112.6, 116.4, 118.1, 121.2, 124.0, 132.2, 135.8, 139.4, 143.8, 147.8, 178.2. HRMS (ESI): m/z calculated for $C_{19}H_{19}ClN_2O_4$: 375.1111. Found: 375.1099.

*4.1.12.5. (S)-2-(2-Amino-3-methoxyphenyl)-6-chloro-8-((3,4-dihydroxybutyl)amino)-4H-chromen-4-one (**33**).* The title compound was synthesized according to the general procedure described above. Compound **24** (50 mg, 0.11 mmol) gave **33** (33 mg, 77%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 1.49–1.63 (m, 1H), 1.77–1.89 (m, 1H), 3.20–3.45 (m, 4H), 3.53–3.63 (m, 1H), 3.84 (s, 3H), 6.03 (bs, 1H), 6.56 (s, 1H), 6.71 (t, $J = 8.0$ Hz, 1H), 6.89 (d, $J = 2.4$ Hz, 1H), 7.00 (d, $J = 7.1$ Hz, 1H), 7.08 (d, $J = 2.4$ Hz, 1H), 7.11 (d, $J = 1.1$ Hz, 1H), 7.13 (d, $J = 1.1$ Hz, 1H): ^{13}C NMR (DMSO-d_6): δ 31.9, 55.3, 55.8, 65.8, 69.9, 107.9, 109.9, 111.0, 112.4, 116.4, 121.3, 124.0, 130.5, 136.5, 140.2, 143.6, 147.1, 150.1, 163.6, 176.2. HRMS (ESI): m/z calculated for $C_{20}H_{21}ClN_2O_5$: 405.1217. Found: 405.1199.

*4.1.12.6. 2-(2-Amino-3-methoxyphenyl)-6-chloro-8-((3-(dimethylamino)propyl)amino)-4H-chromen-4-one (**34**).* The title compound was synthesized according to the general procedure described above. Compound **25** (80 mg, 0.19 mmol) gave **34** (50 mg, 67%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 1.80–2.02 (m, 2H), 3.05–3.19 (m, 2H), 3.31 (t, $J = 6.5$ Hz, 2H), 3.85 (s, 3H), 6.06 (bs, 1H), 6.56 (s, 1H), 6.67–6.76 (m, 1H), 6.96 (d, $J = 2.3$ Hz, 1H), 7.01 (d, $J = 8.0$ Hz, 1H), 7.08–7.16 (m, 1H); ^{13}C NMR (DMSO-d_6): δ 23.0, 40.2, 42.3, 54.7, 55.8, 108.4, 110.1, 111.3, 112.4, 115.9, 116.3, 121.5, 124.2, 130.5, 136.5, 139.7, 143.8, 147.1, 163.7, 176.2. HRMS HRMS (ESI): m/z calculated for $C_{21}H_{24}ClN_3O_3$: 402.1584. Found: 402.1565.

*4.1.12.7. 2-(2-Amino-3-methoxyphenyl)-8-((3-aminopropyl)amino)-6-chloro-4H-chromen-4-one (**35**).* The title compound was synthesized according to the general procedure described above. Compound **26** (40 mg, 0.08 mmol) gave **35** (18 mg, 61%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 1.74–1.94 (m, 2H), 2.82–3.03 (m, 2H), 3.24–3.39 (m, 2H), 3.84 (s, 3H), 6.04 (bs, 1H), 6.55 (s, 1H), 6.96 (d, $J = 2.4$ Hz, 1H), 7.00 (dd, $J = 8.1$, 1.3 Hz, 1H), 7.16–7.04 (m, 1H), 7.66 (bs, 2H); ^{13}C NMR (DMSO-d_6): δ 25.8, 36.9, 40.2, 55.8, 108.3, 110.1, 111.3, 112.3, 115.8, 116.2, 121.4, 124.2, 130.5, 136.6, 139.8, 143.8, 147.0, 163.7, 176.2. HRMS (ESI): m/z calculated for $C_{19}H_{20}ClN_3O_3$: 374.1271. Found: 374.1259.

*4.1.12.8. 2-(2-Amino-3-methoxyphenyl)-8-(benzylamino)-6-chloro-4H-chromen-4-one (**36**).* The title compound was synthesized according to the general procedure described above. Compound **27** (100 mg, 0.23 mmol) gave **36** (54 mg, 58%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 4.03 (s, 2H), 4.40 (s, 2H), 6.39 (d, $J = 2.2$ Hz, 1H), 6.98 (d, $J = 2.2$ Hz, 1H), 7.24 (d, $J = 7.2$ Hz, 1H), 7.29–7.40 (m, 7H), 7.49 (d, $J = 8.0$ Hz, 1H), 7.71 (d, $J = 8.3$ Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 46.0, 56.1, 99.9, 109.5, 110.0, 111.3, 113.4, 116.3, 120.5, 122.2, 122.2,

125.8, 126.7, 127.0, 128.35, 128.35, 136.4, 139.7, 140.0, 147.8, 152.9, 156.1, 162.9. 174.0. HRMS (ESI): *m/z* calculated for $C_{23}H_{19}ClN_2O_3$: 407.1162. Found: 407.1163.

*4.1.12.9. 2-(2-Amino-3-methoxyphenyl)-6-chloro-8-((tetrahydro-2H-pyran-4-yl)amino)-4H-chromen-4-one (**37**).* The title compound was synthesized according to the general procedure described above. Compound **28** (30 mg, 0.07 mmol) gave **37** (15 mg, 54%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 1.35–1.64 (m, 2H), 1.77–2.01 (m, 2H), 3.45 (t, J = 10.9 Hz, 2H), 3.56 (s, 2H), 3.88 (d, J = 11.3 Hz, 3H), 4.02 (s, 3H), 6.70 (s, 1H), 7.05 (s, 1H), 7.31 (d, J = 7.9 Hz, 1H), 7.40 (s, 1H), 7.49 (t, J = 8.1 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 32.2, 48.0, 55.8, 65.8, 108.5, 109.5, 111.9, 112.4, 115.7, 116.3, 121.4, 124.2, 130.4, 138.9, 143.6, 147.2, 156.2, 164.1, 176.2. HRMS (ESI): *m/z* calculated for $C_{21}H_{21}ClN_2O_4$: 401.1268. Found: 401.1254.

*4.1.13. General procedure for the synthesis of chromones **38**–**39***

Using a modified literature procedure [26], a stock solution in MeOH (100 μl) of the catalyst complex containing palladiumacetate (0.04 eqviv) and 2-(di-t-butylphosphino)biphenyl (0.075 equiv) was diluted in MeOH (1.4 ml) and sodium formate (3 equiv) was added. The vial was flushed with nitrogen and stirred at room temperature for 5 min followed by addition of starting material (1 equiv). The vial was capped, flushed again with nitrogen and heated in a microwave reactor at 160 °C for 40 min. Full consumption of starting material was confirmed by TLC (10% MeOH in $CHCl_3$) and the reaction mixture was diluted with ethyl acetate (2 ml) and filtered through a plug of celite. The celite plug was washed with ethyl acetate (8 ml) and the organic solution was washed with water (4 ml). The aqueous phase was extracted with ethyl acetate (2 × 4 ml), the organic phases were pooled, washed with brine and dried over Na_2SO_4. The solvents were removed under reduced pressure and the crude product purified by flash column chromatography (2–5% MeOH in CH_2Cl_2).

*4.1.13.1. 2-(2-Amino-3-methoxyphenyl)-8-((3-hydroxypropyl) amino)-4H-chromen-4-one (**38**).* The title compound was synthesized from **32** according to the general procedure described above and was obtained as a yellow solid (8 mg, 73%). ^{1}H NMR ($CDCl_3$): 1.64 (br s, 1H), 2.02–1.92 (m, 2H), 3.39 (t, J 6.4 Hz, 2H), 3.86 (t, J 5.7 Hz, 2H), 3.91 (s, 3H), 4.63 (br s, 2H), 4.95 (br s, 1H), 6.61 (s, 1H), 6.79 (t, J 8.0 Hz, 1H), 6.92–6.86 (m, 2H), 7.07 (dd, J 8.0, 1.4 Hz, 1H), 7.24 (t, J 7.9 Hz, 1H), 7.47 (dd, J 8.0, 1.4 Hz, 1H); ^{13}C NMR (CDCl3): 31.6, 41.7, 56.0, 61.4, 110.4, 111.9, 112.0, 112.8, 116.9, 117.8, 121.3, 123.7, 125.7, 135.8, 138.2, 145.2, 147.7, 163.9, 179.0. HRMS (ESI): *m/z* calculated for $C_{19}H_{20}N_2O_4$: 340.1423. Found: 340.1429.

*4.1.13.2. (S)-2-(2-Amino-3-methoxyphenyl)-8-((3,4-dihydroxybutyl) amino)-4H-chromen-4-one (**39**).* The title compound was synthesized from **33** according to the general procedure described above and was obtained yellow solid (6 mg, 77%). ^{1}H NMR ($CDCl_3$): 1.90–1.79 (m, 2H), 3.47–3.33 (m, 2H), 3.54 (dd, J 11.0, 7.2 Hz, 1H), 3.70 (dd, J 11.0, 3.3 Hz, 1H), 3.90 (s, 3H), 3.99–3.91 (m, 1H), 4.62 (br s, 2H), 5.02 (br s, 1H), 6.59 (s, 1H), 6.78 (t, J 8.0 Hz, 1H), 6.87 (ddd, J 8.0, 4.7, 1.4 Hz, 2H), 7.06 (dd, J 7.9, 1.4 Hz, 1H), 7.21 (t, J 7.9 Hz, 1H), 7.44 (dd, J 8.0, 1.4 Hz, 1H); ^{13}C NMR ($CDCl_3$): 32.0, 41.1, 56.0, 66.9, 71.2, 110.5, 111.9, 112.1, 112.9, 117.0, 117.9, 121.3, 123.7, 125.7, 135.7, 138.1, 145.2, 147.7, 164.0, 179.0. HRMS (ESI): *m/z* calculated for $C_{20}H_{22}N_2O_5$: 370.1529. Found: 370.1534.

4.2. Molecular modeling

The docking study was performed using the Schrödinger Package, MAESTRO interface [6]. The structure of MEK1 in complex with the allosteric modulator PD0325901 (PDB 1S9J) was used for the study [7,22]. The MEK1-ATPMg complex, PD0325901, PD98059 and various chromone derivatives were prepared and energy minimized using ligand preparation. Molecular docking was performed using GLIDE with extra precision (XP) settings and standard parameters for ligand docking.

4.3. Biological assays

4.3.1. Biochemical analysis of MEK1 kinase activity

Recombinant purified active human GST-tagged MEK1DD (S218D/S22D) protein was purchased from Jena Bioscience, Germany. Recombinant His_6-ERK2 (plasmid kindly provided by Dr Melanie Cobb, University of Texas Southwestern Medical Center) was expressed and purified from *E. coli* [30]. The enzymatic activity of MEK1 was assayed by measuring its ability to increase the MBP kinase activity of ERK2 *in vitro*. GST-MEK1DD (50 ng) was incubated for 30 min at 30 °C with vehicle or indicated MEK1/2 inhibitor and ATP (mix of 50 μM ATP and 5 μCi [γ-^{32}P]ATP) in kinase assay buffer (20 mM Hepes, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.4) [31]. Then, recombinant His_6-ERK2 (300 ng) was added and incubated for 30 min. Finally, bovine myelin basic protein (MBP) (0.2 mg/mL) was added to the reaction and the incubation was continued for an additional 10 min. Control incubations were performed in the absence of ERK2. The reaction was stopped by addition of 5× Laemmli's sample buffer. The samples were analyzed by SDS-gel electrophoresis on 12% acrylamide gels and the band corresponding to MBP was excised and counted in a liquid scintillation counter. Dose-response curves were analyzed according to a three- or four-parameter logistic equation using the SigmaPlot software.

DiscoveRx scan EDGE selectivity profiling was conducted by DiscoveRx Bioscience with KinomeScan™ Technology.

4.3.2. Cell culture

All cell lines [IEC-6 (rat epithelial), A-375 (human malignant melanoma), A549 (human lung carcinoma), HCT 116 and HT-29 (human colorectal adenocarcinoma)] were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, 2 mM glutamine and antibiotics. Quiescent IEC-6 were obtained by incubation of 95% confluent cell cultures in serum-free DMEM-Ham's F-12 (1:1) supplemented with 15 mM Hepes (pH 7.4) and 0.1% bovine serum albumin for 24 h.

4.3.3. Whole cell analysis of MEK1/2 activity

The cellular activity of MEK1/2 was assayed by monitoring the activation loop phosphorylation of ERK1/2. Compounds dissolved in DMSO were added to the culture medium 30 min prior to stimulation of quiescent IEC-6 cells with 10% serum for 5 min. Cell were lysed as previously reported and the activation loop phosphorylation of ERK1/2 was analyzed by immunoblotting with anti-phospho-ERK1/2(Thr202/Tyr204) (Cell Signaling Technology) [31]. Total ERK1/2 expression and protein loading were controlled by immunoblotting with anti-ERK1/2 (Cell Signaling Technology) and anti-HSC70 (Santa Cruz Biotechnology) antibodies. Immunoblotting results were quantified by densitometry analysis using Multi Gauge software.

4.3.4. Cell proliferation assays

Cell proliferation was measured by the colorimetric WST-1 assay. Briefly, cells were seeded in 96-well plates at 2 × 10^3 (HCT 116 and HT-29) or 1.2 × 10^3 (A-375 and A549) cells per well in 100 μl of medium (DMEM containing 10% fetal bovine serum, 2 mM glutamine and antibiotics). The compounds dissolved in DMSO were added to the plates and the culture medium was changed daily for 5 days. Then, 5 μl of WST-1 (Roche) was added to each well,

the plates were incubated for 1 h, and absorbance was measured at 450 nm with reference at 620 nm. Proliferation assays were performed on triplicate wells.

Acknowledgments

We thank Edith Giasson and Maude David for technical assistance during the biological experiments. For the financial support we thank the Knut and Alice Wallenberg Foundation, the Swedish Research Council (Grant 62120083533) and the Cancer Research Society (S.M.).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.018.

References

[1] G. Pearson, F. Robinson, T.B. Gibson, B.E. Xu, M. Karandikar, K. Berman, M.H. Cobb, Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions, Endocr. Rev. 22 (2001) 153–183.
[2] Z. Chen, T.B. Gibson, F. Robinson, L. Silvestro, G. Pearson, B.E. Xu, A. Wright, C. Vanderbilt, M.H. Cobb, MAP kinases, Chem. Rev. 101 (2001) 2449–2476.
[3] J.M. Kyriakis, J. Avruch, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation, Physiol. Rev. 81 (2001) 807–869.
[4] J.L. Yap, S. Worlikar, A.D. MacKerell, P. Shapiro, S. Fletcher, Small-molecule inhibitors of the ERK signaling pathway: towards novel anticancer therapeutics, ChemMedChem 6 (2011) 38–48.
[5] C. Fremin, S. Meloche, From basic research to clinical development of MEK1/2 inhibitors for cancer therapy, J. Hematol. Oncol. 3 (2010) 8.
[6] J.M. English, M.H. Cobb, Pharmacological inhibitors of MAPK pathways, Trends Pharmacol. Sci. 23 (2002) 40–45.
[7] J.S. Sebolt-Leopold, R. Herrera, Targeting the mitogen-activated protein kinase cascade to treat cancer, Nat. Rev. Cancer 4 (2004) 937–947.
[8] H. Abe, S. Kikuchi, K. Hayakawa, T. Iida, N. Nagahashi, K. Maeda, J. Sakamoto, N. Matsumoto, T. Miura, K. Matsumura, N. Seki, T. Inaba, H. Kawasaki, T. Yamaguchi, R. Kakefuda, T. Nanayama, H. Kurachi, Y. Hori, T. Yoshida, J. Kakegawa, Y. Watanabe, A.G. Gilmartin, M.C. Richter, K.G. Moss, S.G. Laquerre, Discovery of a highly potent and selective MEK inhibitor: GSK1120212 (JTP-74057 DMSO solvate), ACS Med. Chem. Lett. 2 (2011) 320–324.
[9] J. Carretero, T. Shimamura, K. Rikova, A.L. Jackson, M.D. Wilkerson, C.L. Borgman, M.S. Buttarazzi, B.A. Sanofsky, K.L. McNamara, K.A. Brandstetter, Z.E. Walton, T.L. Gu, J.C. Silva, K. Crosby, G.I. Shapiro, S.M. Maira, H.B. Ji, D.H. Castrillon, C.F. Kim, C. Garcia-Echeverria, N. Bardeesy, N.E. Sharpless, N.D. Hayes, W.Y. Kim, J.A. Engelman, K.K. Wong, Integrative genomic and proteomic analyses identify targets for Lkb1-deficient metastatic lung tumors, Cancer Cell. 17 (2010) 547–559.
[10] A.C. Dar, K.M. Shokat, The evolution of protein kinase inhibitors from antagonists to agonists of cellular signaling, Annu. Rev. Biochem. 80 (2011) 769–795.
[11] K. Dahlen, M. Grøtli, K. Luthman, A scaffold approach to 3,6,8-trisubstituted flavones, Synlett (2006) 897–900.
[12] K. Dahlen, E.A.A. Wallen, M. Grøtli, K. Luthman, Synthesis of 2,3,6,8-tetrasubstituted chromone scaffolds, J. Org. Chem. 71 (2006) 6863–6871.
[13] E.A.A. Wallen, K. Dahlen, M. Grøtli, K. Luthman, Synthesis of 3-aminomethyl-2-aryl-8-bromo-6-chlorochromones, Org. Lett. 9 (2007) 389–391.
[14] C. Dyrager, A. Friberg, K. Dahlen, M. Friden-Saxin, K. Borjesson, L.M. Wilhelmsson, M. Smedh, M. Grøtli, K. Luthman, 2,6,8-trisubstituted 3-hydroxychromone derivatives as fluorophores for live-cell imaging, Chem. Eur. J. 15 (2009) 9417–9423.
[15] M. Friden-Saxin, N. Pemberton, K.D. Andersson, C. Dyrager, A. Friberg, M. Grøtli, K. Luthman, Synthesis of 2-Alkyl-Substituted chromone derivatives using microwave irradiation, J. Org. Chem. 74 (2009) 2755–2759.
[16] T. Ankner, M. Friden-Saxin, N. Pemberton, T. Seifert, M. Grøtli, K. Luthman, G. Hilmersson, KHMDS enhanced SmI2-mediated reformatsky type alpha-cyanation, Org. Lett. 12 (2010) 2210–2213.
[17] C. Dyrager, L.N. Möllers, L.K. Kjäll, J.P. Alao, P. Diner, F.K. Wallner, P. Sunnerhagen, M. Grøtli, Design, synthesis, and biological evaluation of chromone-based p38 MAP kinase inhibitors, J. Med. Chem. 54 (2011) 7427–7431.
[18] C. Dyrager, M. Wickstrom, M. Friden-Saxin, A. Friberg, K. Dahlen, E.A.A. Wallen, J. Gullbo, M. Grøtli, K. Luthman, Inhibitors and promoters of tubulin polymerization: synthesis and biological evaluation of chalcones and related dienones as potential anticancer agents, Bioorg. Med. Chem. 19 (2011) 2659–2665.
[19] D.A. Horton, G.T. Bourne, M.L. Smythe, The combinatorial synthesis of bicyclic privileged structures or privileged substructures, Chem. Rev. 103 (2003) 893–930.
[20] Maestro [9.0], Schrödinger, LLC, Portland, 2009. Glide [2.1], Schrödinger, LLC, Portland, 2009.
[21] http://www.pdb.org.
[22] J.F. Ohren, H.F. Chen, A. Pavlovsky, C. Whitehead, E.L. Zhang, P. Kuffa, C.H. Yan, P. McConnell, C. Spessard, C. Banotai, W.T. Mueller, A. Delaney, C. Omer, J. Sebolt-Leopold, D.T. Dudley, I.K. Leung, C. Flamme, J. Warmus, M. Kaufman, S. Barrett, H. Tecle, C.A. Hasemann, Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition, Nat. Struct. Mol. Biol. 11 (2004) 1192–1197.
[23] B. Ye, D.O. Arnaiz, Y.L. Chou, B.D. Griedel, R. Karanjawala, W. Lee, M.M. Morrissey, K.L. Sacchi, S.T. Sakata, K.J. Shaw, S.C. Wu, Z.C. Zhao, M. Adler, S. Cheeseman, W.P. Dole, J. Ewing, R. Fitch, D. Lentz, A. Liang, D. Light, J. Morser, J. Post, G. Rumennik, B. Subramanyam, M.E. Sullivan, R. Vergona, J. Walters, Y.X. Wang, K.A. White, M. Whitlow, M.J. Kochanny, Thiophene-anthranilamides as highly potent and orally available factor Xa inhibitors, J. Med. Chem. 50 (2007) 2967–2980.
[24] W.K. Anderson, D.C. Dean, T. Endo, Synthesis, chemistry, and antineoplastic activity of alpha-halopyridinium salts – potential pyridone prodrugs of acylated vinylogous carbinolamine tumor inhibitors, J. Med. Chem. 33 (1990) 1667–1675.
[25] P. Kahnberg, E. Lager, C. Rosenberg, J. Schougaard, L. Camet, O. Sterner, E.O. Nielsen, M. Nielsen, T. Liljefors, Refinement and evaluation of a pharmacophore model for flavone derivatives binding to the benzodiazepine site of the GABA(A) receptor, J. Med. Chem. 45 (2002) 4188–4201.
[26] M.F. Logan, M.E. Oinen, Dechlorination of aryl chlorides with sodium formate using a homogeneous palladium catalyst, Organometallics 25 (2006) 1052–1054.
[27] Y. Dong, C.A. Busacca, Indoles from *O*-haloanilines: syntheses of tryptamines and tryptophols via regioselective hydroformylation of functionalized anilines, J. Org. Chem. 62 (1997) 6464–6465.
[28] http://www.discoverx.com/services/drug-discovery-development-services/kinase-profiling/kinomescan/scanedge.
[29] D. Wang, S.A. Boerner, J.D. Winkler, P.M. LoRusso, Clinical experience of MEK inhibitors in cancer therapy, Bba-Mol. Cell. Res. 1773 (2007) 1248–1255.
[30] D.J. Robbins, E.Z. Zhen, H. Owaki, C.A. Vanderbilt, D. Ebert, T.D. Geppert, M.H. Cobb, Regulation and properties of extracellular signal-regulated protein kinase-1 and kinase-2 invitro, J. Biol. Chem. 268 (1993) 5097–5106.
[31] M.J. Servant, P. Coulombe, B. Turgeon, S. Meloche, Differential regulation of p27(Kip1) expression by mitogenic and hypertrophic factors: involvement of transcriptional and posttranscriptional mechanisms, J. Cell. Biol. 148 (2000) 543–556.