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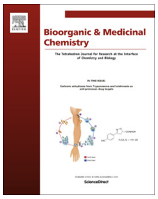

3-Phenylalkyl-2*H*-chromenes and -chromans as novel rhinovirus infection inhibitors

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ABSTRACT

Following our studies on structure-activity relationships of anti-rhinovirus chromene and chroman derivatives, we designed and synthesized new series of 3-phenylalkyl-2*H*-chromenes and -chromans bearing differently sized, aliphatic linker chains between the two cycles. The cytotoxicity and the antiviral activity of the new compounds on human rhinovirus (HRV) serotype 1B and 14 infection were evaluated in HeLa cell cultures. Most of the tested compounds interfered with HRV1B multiplication in the micromolar or submicromolar concentrations while HRV14 was less susceptible. 3-[3-(4-Chlorophenyl) propyl]chroman (**9c**) was selected for preliminary mechanism of action studies due to its potent activity against both serotypes (IC_{50} of 0.48 μM and 1.36 μM towards HRV1B and 14, respectively) coupled with high selectivity (SI = 206.18 and 73.26, respectively). Results of time of addition/removal studies suggest that **9c**, similarly to related derivatives, behaves as a capsid binder interfering with some early events of the HRV1B infectious cycle.

1. Introduction

Human Rhinoviruses (HRVs) belong to the genus Enterovirus of the Picornaviridae family. The over 160 serotypes of HRVs are responsible for common cold, a mild illness of the upper respiratory tract. Although not important in terms of clinical morbidity, the high incidence of the disease in adults as well as in children produces significant clinical and economic burden worldwide.[1] Beside cold, HRV infection has been clinically associated with different respiratory tract complications such as bronchiolitis, pneumonia, sinusitis and acute otitis media, and has been implicated in exacerbations of chronic respiratory disorders, such as asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD).[2] Recently, experimental HRV infection in COPD patients established a causative relationship between virus infection and exacerbations,[3] moreover COPD exacerbations were predicted by the World Health Organization to become one of the major cause of death in the next decades worldwide.[4]

Due to the large number of serotypes, vaccination against HRVs is not feasible at present. Despite intensive efforts in antiviral search and development, so far specific drugs have not been approved for clinical use, although several compounds progressed into clinical trials. For example, clinical studies demonstrated that intranasal prophylaxis with Pirodavir lowered HRV replication and shedding rates,[5] and double-blind placebo-controlled natural cold trials with oral administration of Pleconaril documented a reduction in illness duration and severity.[6,7] However, because of safety concerns due to adverse effects,[8] further investigations have been discontinued.[9] Vapendavir, a new 1,2-benzisoxazole analog of Pirodavir, significantly reduced viral load in experimental HRV infection studies with healthy volunteers.[10] It is still under clinical evaluation for treatment of HRV-induced exacerbations of asthma.[11,12] Therefore, to date, the search for antiviral compounds is still open.

Viral attachment to the host cell membrane and uncoating of the RNA genome represent suitable targets of the HRV life cycle for antiviral intervention and prevention of infection. Based on receptor usage, HRVs can be distinguished into a major and a minor group. Major group HRVs bind and gain entry into the cell cytoplasm through a complex interaction with intercellular adhesion molecule-1 (ICAM-1).[13–15] Minor group HRVs bind to low density lipoprotein receptor (LDLR) family[16] and enter the cells by an endocytotic pH-dependent pathway.[17]

Several small molecules, belonging to different chemical classes, were found to effectively interfere with the early stages of HRV infection.[18] Among these, the so-called capsid-binders are able to directly interact with virion shell proteins thus hindering

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virus adsorption and/or hampering genome uncoating, probably through an alteration of capsid flexibility. Interference by capsid-binding molecules with RNA uncoating was observed for all studied serotypes independent of receptor grouping. On the contrary, inhibition of virus binding to the cell surface was found to be dependent on individual serotype or compound tested.[19]

During our long-lasting research program, we explored the anti-enterovirus properties of synthetic analogues of natural flavanoids[20–23] and flavonoids.[24–30] Several derivatives showed a potent and broad antipicornavirus spectrum of efficacy and behaved as capsid-binders preventing virus adsorption to the host cell and/or uncoating of the viral genome.[23,31,32] Starting from the promising activity of isoflavanoids[20–23] and homoisoflavonoids,[25,30] more recently, we designed and synthesized structurally related 3-benzyl-2*H*-chromenes, 3-benzylchromans and (*Z*)-3-benzylidenechromans.[33] These capsid-binder derivatives showed activity within micro or submicromolar range against HRV serotype 1B, while only weakly affected the replication of serotype 14.[33] The different sensibility of the two serotypes could be explained according to the different size of compound-binding site within VP1 capsid protein. X-ray crystallographic studies on a variety of HRV serotypes confirmed the diverse size of the hydrophobic pocket into the capsid protein VP1 and revealed that larger molecules occupy more efficiently the HRV14 binding site.[34–41] Therefore, in order to obtain analogues with a broader anti-HRV spectrum of activity, we increased the length of the spacer between the heterocycle (chromone, chroman-4-one, chroman or 2*H*-chromene) and the phenyl or pyridine moiety.[42–44] A linear, unsaturated chain containing from two to four carbon atoms was introduced as a linker between the two rings.[42–44] Optimum activity against both HRV serotypes was achieved by introducing an unsaturated two carbon chain joining the 2*H*-chromene to the benzene ring.[42] Mechanism of action studies on (*E*)-3-styryl-2*H*-chromene confirmed an interference with the early stages of HRV1B replication.[42]

In this paper, we describe the synthesis and the anti–HRV activity of new series of 3-phenylalkyl-2*H*-chromenes and -chromans designed to further explore the structure-activity relationships of 2*H*-chromene and chroman HRV inhibitors. Starting from the hypothesis that more flexible molecules could be capable to insert more easily into the canyon structure and then to occupy more efficiently the binding site of different HRV serotypes, linear, saturated polymethylene chains ranging from two to five carbon atoms were introduced between heterocyclic and phenyl rings (**3a**,**d-f** and **4a**,**d-f**). In addition, maintaining the 2-carbon bridge between 2*H*-chromene and benzene ring, a small series of branched-chain linker compounds (**3c**, **3b** and **13**) was synthesized and tested for anti-HRV activity in cell cultures.

2. Results and discussion

2.1. Chemistry

The designed 3-phenylalkyl-2*H*-chromenes (**3a-f**) were obtained modifying the synthetic strategy developed to prepare 3-benzyl-2*H*-chromene derivatives[45] (Scheme 1). For this purpose, the required bromomethyl-phenylalkylketones (**2a-f**) were prepared in good overall yields starting from the treatment of the appropriate acids (**1a-f**) with oxalyl chloride in dry dichloromethane. The obtained acyl chlorides were reacted with a solution of trimethylsilyldiazomethane (TMSCHN$_2$), a stable and safe substituted for hazardous diazomethane, in dry acetonitrile. The intermediate diazoketones were treated in situ with 48% aqueous HBr to afford the desired bromomethylketones (**2a-f**) in excellent yields. Finally, the reaction of **2a-f** with (2-hydroxybenzyl)triphenylphosphonium bromide in the presence of *tert*-BuOK allowed the alkylation of the phenolic hydroxyl group and the cyclization by an intramolecular Wittig reaction to give the desired 2*H*-chromenes (**3a-f**).

Subsequent catalytic hydrogenation of 3-phenylalkyl-2*H*-chromenes **3a**,**d-f** afforded the corresponding 3-(3-phenylalkyl) chromans **4a**,**d-f** with high yields (Scheme 2).

As shown in Scheme 3, the chloro substituted 3-(3-phenylpropyl)chromans (**9a-c**) were prepared by catalytic hydrogenation of the appropriate mixture of (*Z*)-3-[(*E*)-3-phenylallylidene]-chroman, (*E*)-3-(3-phenyl) prop-1-en-1-yl)-2*H*-chromene, and 3-cinnamyl-2*H*-chromene (**6**, **7**, **8a**; **6**, **7**, **8b** or **6**, **7**, **8c**). As previously reported, these mixtures were obtained by reduction of the corresponding (*E*)-3-[(*E*)-3-phenylallylidene]chroman-4-one (**5a-c**) with lithium aluminium hydride.[43]

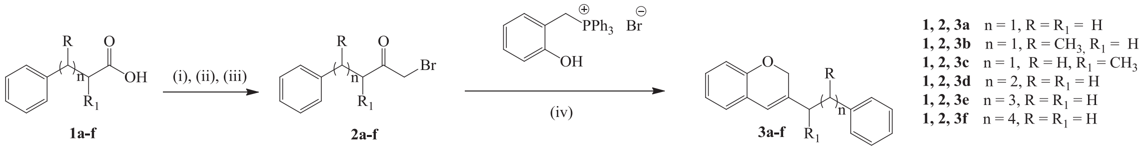
The synthesis of (*E*)-3-(1-phenylpropen-2-yl)-2*H*-chromene (**13**) started from the preparation of 3-acetyl-2*H*-chromene (**10**) by reaction of 2-hydroxybenzaldehyde and 3-buten-2-one in the presence of 1,4-diazabicyclo-[2.2.2]-octane (DABCO) in chloroform and water (1:1), at room temperature. The reduction of the obtained 3-acetyl-2*H*-chromene (**10**) with sodium borohydride in methanol provided the corresponding alcohol (**11**). This intermediate was treated with triphenylphosphine hydrobromide in dry acetonitrile to yield [1-(2*H*-chromen-3-yl)ethyl]triphenylphosphonium bromide (**12**) with good yield. Finally, the target compound **13** was obtained by the Wittig reaction with benzaldehyde (Scheme 4).

(*E*)-3-(2*H*-Chromen-3-yl)-1-phenylprop-2-en-1-one **15** was synthesized according to the two step procedure shown in Scheme 5. 2*H*-Chromene-3-carbaldehyde **14** was prepared as previously reported by refluxing 2-hydroxybenzaldehyde and acrolein in the presence of potassium carbonate.[42] The subsequent Horner–Wadsworth–Emmons reaction of **14** with commercially available diethyl (2-oxo-2-phenylethyl)phosphonate, carried out using sodium hydride as base in dry THF, provided only the (*E*) isomer **15**. The stereochemistry of compounds was established on the basis of the coupling constant value of the protons in the chain ($J_{\alpha\text{-}\beta}$ = 16.0 Hz).

2.2. Antiviral activity

The results on cytotoxicity and anti-rhinovirus evaluation of the new synthesized compounds (**3a-f**, **4a**, **4d-f**, **9a-c**, **13** and **15**) are reported in Table 1.

Cytotoxicity is indicated as maximum non-cytotoxic concentration (MNCC) and 50% cytotoxic concentration (CC$_{50}$). Starting from MNCC, the dose-dependent effect of compounds on HRV serotype 1B and HRV serotype 14 infection was evaluated in HeLa cell cultures by a plaque reduction test. HRV 1B and 14 were taken as representative serotypes for antiviral group B and A of HRVs, respectively. Antiviral group A and B were defined on the basis of



Scheme 1. Reagents and conditions: (i) CO_2C1_2, dry CH_2C1_2, 0 °C, rt, 3 h; (ii) TMSCHN$_2$, dry CH_3CN, 0 °C, 2 h; (iii) HBr 48%, 0 °C, rt, 18 h; (iv) *tert*-BuOK, dry THF, rt, 23 h.

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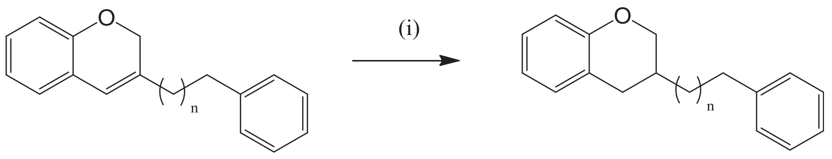
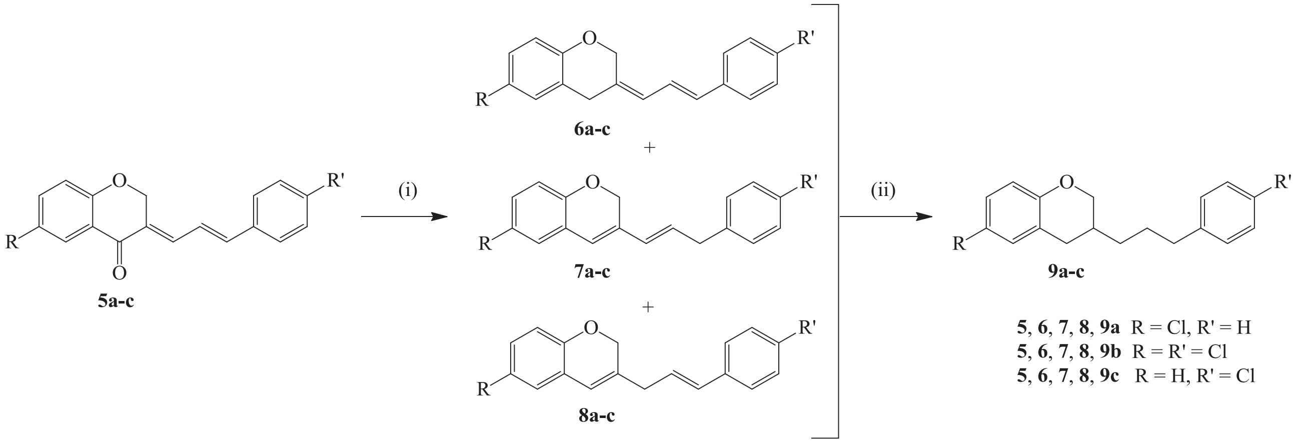


3a, d-f **4a, d-f**

3a, **4a** n = 1; **3d**, **4d** n = 2; **3e**, **4e** n = 3; **3f**, **4f** n = 4

Scheme 2. Reagents and conditions: (i) Pd/C 10%, dry dioxane, rt, 30 min.



5a-c

6a-c

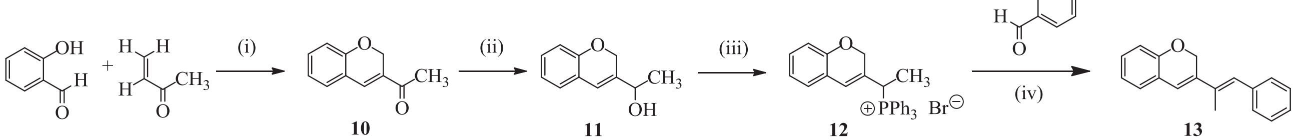
7a-c

8a-c

9a-c

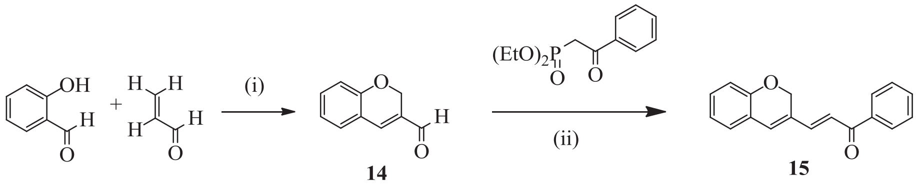
5, 6, 7, 8, 9a R = Cl, R' = H
5, 6, 7, 8, 9b R = R' = Cl
5, 6, 7, 8, 9c R = H, R' = Cl

Scheme 3. Reagents and conditions: (i) LiAlH$_4$, AlCl$_3$ dry Et$_2$O, rt 1 h and reflux 2.5 h; (i) Pd/C 10%, dry dioxane, rt, 15 min.



10 **11** **12** **13**

Scheme 4. Reagents and conditions: (i) DABCO, CHC1$_3$/H$_2$O (1:1), rt, 7 days; (ii) NaBU, MeOH, rt, 6 h; (iii) triphenylphosphine hydrobromide, dry CH$_3$CN, reflux, 5 h; (iv) *tert*-BuOK, dry THF, rt, 23 h.



14 **15**

Scheme 5. Reagents and conditions: (i) K$_2$CO$_3$, dry dioxane, reflux, 4 h; (ii) NaH, dry THF, r.t. 20 h.

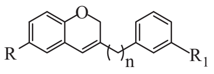
contrasting susceptibilities of HRVs to a selected panel of capsid-binding compounds of different length.[46,47]

The antiviral activity is expressed as the compound concentration required to produce a 50% reduction of plaque number with respect to mock-treated virus-infected cultures (IC$_{50}$) (Table 1). When a 50% reduction of plaque count is not achieved, the percentage inhibition obtained at the MNCC is reported in parentheses. The selectivity index (SI), calculated by the CC$_{50}$/IC$_{50}$ ratio, is also reported in Table 1. 4′,6-Dichloroflavan (**BW683C**), an inhibitor of group B serotypes, has been utilized as a control for activity.

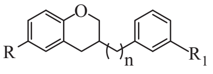
Generally, the new compounds were able to dose-dependently inhibit the replication of both serotypes, although HRV14 was always less susceptible than HRV1B. For several compounds, the percent reduction of HRV14 plaque number was found lower than 50% at the MNCC while they exhibited an anti-HRV1B effect within the micromolar or submicromolar range. When compared to **BW683C**, all the synthesized compounds were less potent against HRV1B; instead some of them were active against serotype 14, differently from **BW683C** which was shown ineffective against this serotype.[48]

Within chromene derivatives, compounds containing a spacer of two carbon atoms between heterocyclic and phenyl moieties, 3-phenylethyl-2*H*-chromene **3a** showed high potency (IC$_{50}$ = 0.48 μM) and good selectivity against HRV1B (SI = 103.09) but low efficacy against serotype 14. The introduction of a methyl group in the α-position of the linker chain between the two cycles (**3b**) resulted

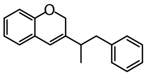
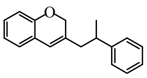
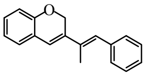
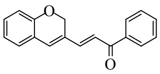
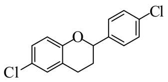
Table 1
Cytotoxicity and anti-rhinovirus activity of 3-phenylalkyl-2*H*-chromenes (**3a-f**), 3-(3-phenylalkyl)chromans (**4a,d-f**, **9a-c**), (*E*)-3-(1-phenylpropen-2-yl)-2*H*-chromene (**13**) and (*E*)-3-(2*H*-chromen-3-yl)-1-phenylprop-2-en-1-one (**15**).



3a,d-f



4a,d-f; 9a-c

Comp.	n	R	R_1	MNCC (μM)[a]	CC_{50} (μM)[b]	IC_{50} (μM)[c] HRV 1B	SI[d]	IC_{50} (μM)[c] HRV 14	SI[d]
3a	2	H	H	25.00	50.00	0.48 ± 0.03	104.17	>25.00 (26%)	–
4a	2	H	H	3.12	6.25	0.49 ± 0.05	12.75	>3.12 (16%)	–
3d	3	H	H	100.00	200.00	2.37 ± 0.18	84.39	40.47 ± 0.66	4.94
4d	3	H	H	100.00	200.00	9.59 ± 0.43	20.85	>100 (37%)	–
9a	3	Cl	H	100.00	200.00	1.79 ± 0.14	111.73	25.00 ± 1.21	8.00
9b	3	Cl	Cl	100.00	200.00	0.56 ± 0.03	357.14	30.00 ± 1.08	6.67
9c	3	H	Cl	50.00	100.00	0.48 ± 0.02	208.33	1.36 ± 0.09	73.53
3e	4	H	H	50.00	100.00	0.54 ± 0.04	185.18	12.70 ± 0.15	7.87
4e	4	H	H	50.00	100.00	0.71 ± 0.08	140.84	>50.00 (25%)	–
3f	5	H	H	50.00	100.00	0.56 ± 0.07	178.57	>50.00 (37%)	–
4f	5	H	H	100.00	200.00	4.74 ± 0.37	42.19	33.03 ± 1.11	6.05
3b				12.50	25.00	IN		>12.50 (31%)	–
3c				6.25	12.50	1.17 ± 0.51	10.68	>6.25 (5%)	–
13				12.50	25.00	IN		IN	
15				25.00	50.00	IN		NT	
BW683C				25.00	>25.00[e]	0.025 ± 0.001	>1000	NA[e]	–

Values shown in this table are the means ± the standard deviations of two independent assays done in triplicate.

[a] The maximum non-cytotoxic concentration (MNCC) was the highest dose tested that did not produce any cytotoxic effect and reduction in viability of HeLa cells, or on cell growth after 3 days of incubation at 37 °C.

[b] The CC_{50} value was the concentration of compound which reduced the cell viability by 50%, as compared with the control.

[c] The IC_{50} value was the dose of compound reducing the plaque number by 50% and was calculated by plotting the log of drug concentration versus the percentage of plaque reduction. When a 50% reduction was not achieved, the percent of inhibition obtained at the MNCC was reported in parentheses.

[d] The selectivity index (SI) value was equal to CC_{50}/IC_{50}.

[e] Not active up to the MNCC.

in a loss of activity against HRV1B. In contrast, the shift of the methyl group to the β-position led to the isomer **3c** endowed with good activity against HRV1B (IC_{50} = 1.17 μM) but low selectivity (SI = 10.68). Both analogues exhibited a modest inhibitory effect on HRV14 replication. In addition, the presence of α-methyl substituent coupled with a double bound in the chain (**13**) abolished the efficacy towards both serotypes. For these reasons, the impact on activity of branched chains was not further explored. Instead, the reduction of the chromene ring to give the 3-phenylethylchromans (**4a**) maintained the anti-HRV1B efficacy within the submicromolar range (IC_{50} = 0.49 μM), although **4a** showed only a modest selectivity index (SI = 12.75). The activity towards serotype 14 remained modest for both phenylethyl derivatives **3a** and **4a**.

The elongation up to 5 carbon atoms of the linear, polymethylene spacer connecting 2*H*-chromene with phenyl ring resulted in analogues **3d-f** showing low cytotoxicity and potent inhibitory activity against HRV1B. The corresponding chroman analogues (**4d-f**), although exhibiting a low cytotoxicity, demonstrated a lower potency than chromenes. Both series of compounds were endowed with a weak activity against HRV14.

The introduction of chlorine at the 4′ and/or 6 position of the 3-(3-phenylpropyl)chroman **4d** led to derivatives **9a-c** showing improved potency and selectivity when compared to **4d**. In particular, 3-[3-(4-chlorophenyl)propyl]chroman (**9c**) displayed the highest level of activity against both serotypes (IC_{50} of 0.48 μM and 1.36 μM towards HRV 1B and 14, respectively) coupled with high selectivity (SI = 206.18 and 73.26, respectively). Therefore, this chroman emerged as a good candidate for further studies.

2.3. Mechanism of action studies

The mechanism of action of the most powerful synthesized analog (**9c**) within the new series has been studied under one cycle of HRV1B growth in HeLa cells. The concentration of compound utilized was 48.5 μM, a hundred times the IC_{50} value towards HRV1B.

Maximal inhibition of virus production (94%) was achieved when **9c** was present during the entire time of virus infection (Fig. **1**, **bar T**). The same level of inhibition was observed when **9c** was present only during the time of virus adsorption (1 h, 4 °C) (Fig. **1**, **bar B**) indicating a successful interference with virus binding to the host cells. Also treatment of cells with **9c** for 1 h (4 °C) prior to infection reduced by 90% the virus yield (Fig. **1**, **bar P**) suggesting an ability of compound to adsorb to the cell surface in a suitable position to prevent infection. This result could be

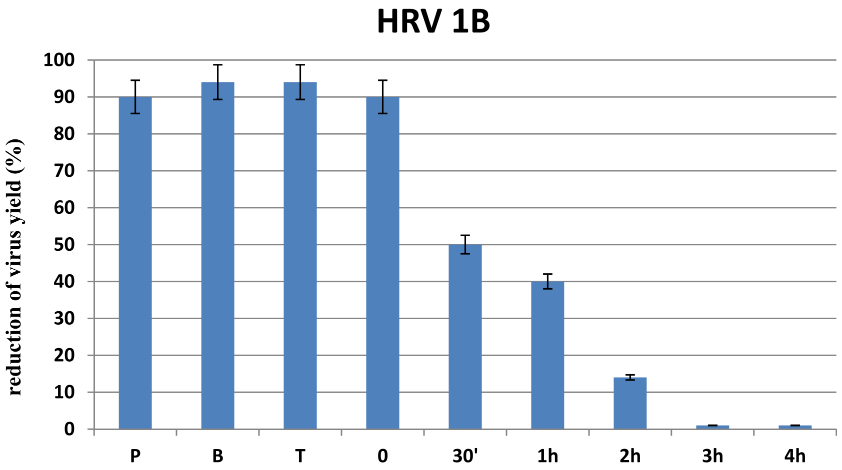


Fig. 1. Effect of addition of **9c** at different times during HRV1B one-step growth cycle in HeLa cells. Virus yield was determined by plaque assay after 10 h of incubation at 33 °C. Virus yield of mock-treated HRV1B infected cultures was 4.1×10^4 PFU/mL. P: Cells were treated with **9c** for 1 h at 4 °C before virus binding (1 h at 4 °C). B: Cells were treated with **9c** during virus binding (1 h at 4 °C). T: Cells were treated with **9c** during virus binding and virus multiplication (10 h at 33 °C). 0, 30′, 1 h, 2 h, 3 h, 4 h: **9c** was added to infected cells at different times after virus binding (time 0) and maintained for the remaining infection time (up to 10 h at 33 °C).

due to the ability of membrane-bound compound to interact with HRV particles at the cell membrane level thus inhibiting viral canyon function(s). Alternatively, binding of **9c** to the cell surface could hinder cell receptor recognition by the virus either through a specific interaction with receptor molecules or by a steric hindrance effect. Similarly to our results, Tisdale and Selway[49] reported that pretreatment of cultures with **BW683C** produced an inhibition of HRV1B yield equal to that obtained when the compound was added with virus.

Addition of compound immediately after virus adsorption (time 0) until the end of virus growth still resulted in 90% reduction of HRV1B progeny yield (Fig. **1**, **bar 0**). This result indicates that **9c**, beside an action on virus attachment to the cell membrane, is also able to interfere with some event(s) of virus multiplication. Addition of **9c** to the infected culture medium after only 30 min-1 h post binding resulted less effective (50% and 40% reduction, respectively) and the inhibition was lost after a 2 h delay or more (Fig. **1**, **bar 2h**, **3h**, **4h**). All these results indicate that **9c** acts on the early stages of rhinovirus multiplication.

In a parallel set of experiments **9c** was added to the cells immediately after virus adsorption (time 0) and removed after different times of incubation at 33 °C. Removal of compound from 15 min up to 1 h post infection (p.i.) caused a reduction of virus yield ranging from 47% to 69% indicating that the antiviral effect of **9c** was partially reversible (Fig. **2**). Removal of compound at later times p.i. (2 h, 4 h, and 6 h) did not significantly affect virus yield confirming an action of **9c** on the early stages of HRV infection (Fig. **2**).

A virucidal test was set up to verify an effect on virus particles. After a 1 h exposure (33 °C) of HRV1B to **9c**, the infectivity titers of treated and mock-treated virus suspensions were found similar (mock-treated virus 1.63×10^7 PFU/mL, **9c**-treated virus 1.38×10^7 PFU/mL). On the contrary, in stabilization studies, **9c** protected HRV1B infectivity against inactivation by both mild acid and heat treatments. Exposure to mild acid produced a drop in infectivity of 3.1 log. In the presence of **9c**, the reduction in infectivity was only 0.7 log (Fig. **3**). In a similar manner, heat treatment significantly lowered virus infectivity (by 5.4 log) while in the presence of **9c** the reduction was 4.2 log (Fig. **4**). All together these results suggest that **9c** behaves as a capsid binder and that the inhibitory effect is reversible by dilution.

In conclusion, new series of 3-phenylalkyl-2*H*-chromenes and -chromans bearing aliphatic spacers ranging from two to five C atoms between the two cycles have been synthesized and tested for cytotoxicity and antiviral activity on HRV serotype 1B and 14 infection in HeLa cell cultures. 3-[3-(4-Chlorophenyl)propyl]chroman (**9c**) showed potent activity against both serotypes and high selectivity. Preliminary mechanism of action studies, carried out on HRV serotype 1B as a model, suggest that **9c** affect functions associated with the viral capsid, including virus attachment to cell membrane and the uncoating of viral genome.

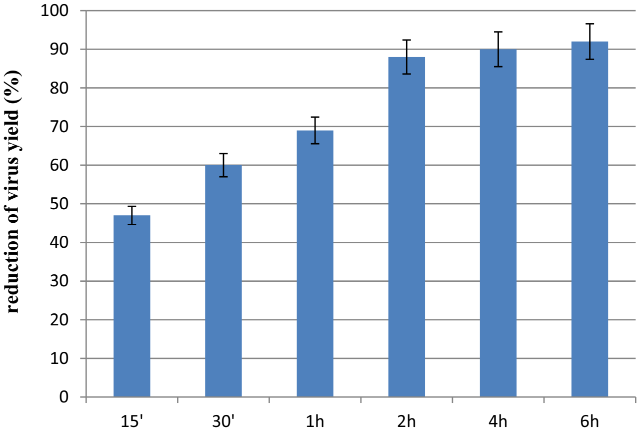


Fig. 2. Effect of removal of **9c** at different times during HRV1B one-step growth cycle in HeLa cells. Virus yield was determined by plaque assay after 10 h of incubation at 33 °C. Virus yield of mock-treated HRV1B infected cultures was 4.1×10^4 PFU/mL. 15′, 30′, 1 h, 2 h, 4 h, 6 h: **9c** was added to cells after virus binding (1 h at 4 °C, time 0) and removed after different incubation times at 33 °C. The cultures were further incubated in maintenance medium without drug up to 10 h (33 °C).

3. Experimental

3.1. Chemistry

Chemicals were purchased from Sigma-Aldrich and used without further purification. Melting points were determined on a Stanford Research Systems OptiMelt (MPA-100) apparatus and are uncorrected. NMR spectra were detected with a Bruker AM-400 spectrometer, using TMS as internal standard. IR spectra were recorded on a FT-IR PerkinElmer Spectrum 1000. All compounds were routinely checked by thin-layer chromatography (TLC) and

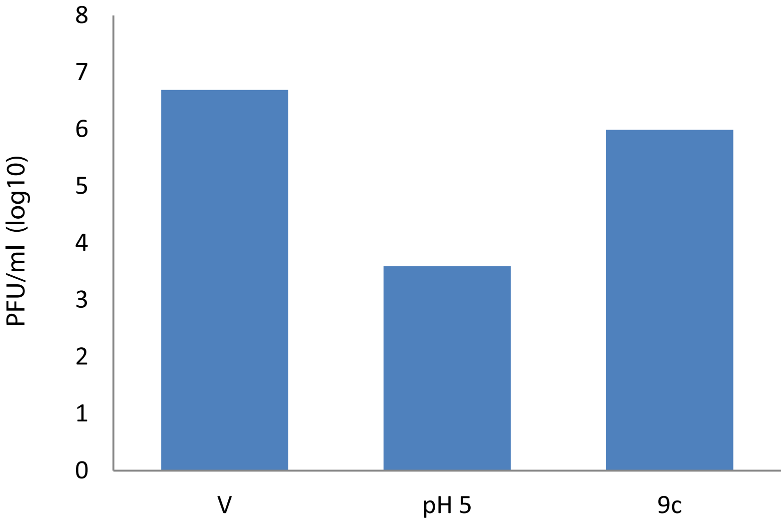


Fig. 3. Protective effect of **9c** on acid inactivation of HRV1B infectivity. V: Untreated virus. pH 5: Virus exposed to pH 5.0 for 30 min. 9c: Virus exposed to pH 5.0 in the presence of **9c**.

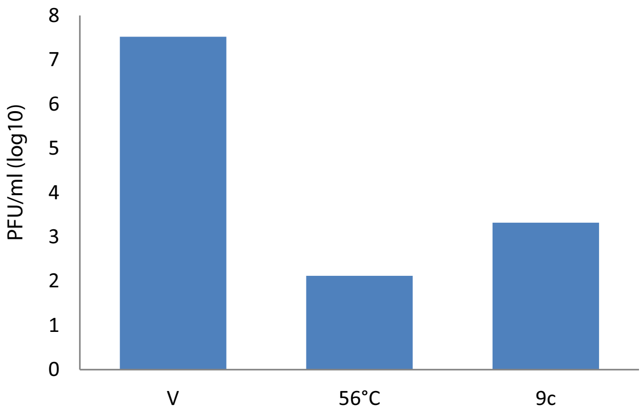


Fig. 4. Protective effect of **9c** on thermal inactivation of HRV1B infectivity. V: Untreated virus. 56 °C: Virus incubated at 56 °C for 20 min. 9c: Virus incubated at 56 °C for 20 min in the presence of **9c**.

^{1}H NMR. TLC was performed on silica gel or aluminium oxide fluorescent coated plates (Merck, Kieselgel or Aluminium oxide 60 F254). Components were visualised by UV light. Elemental analyses (C, H, N, Cl) of new compounds were within ±0.4% of theoretical values. 2*H*-Chromene-3-carbaldehyde was synthesized following the procedure previously described.[42]

3.1.1. General procedure for the synthesis of the 1-bromomethylphenylalkyl-2-ones (*2a-f*)

Oxalyl chloride (23 mmol) was added dropwise to a solution of the appropriate phenylalkyl acid (**1a-f**) (10 mmol) in dry CH_2Cl_2 (18.5 mL) cooled at 0 °C. After complete addition, the mixture was stirred at room temperature for 3 h. After this period, the mixture was evaporated to dryness and the obtained phenylalkyl acyl chloride was used for the next reaction without further purification. 2N trimethylsilyldiazomethane in dry Et_2O (27.3 mL) was added dropwise to a solution of the appropriate phenylalkyl acyl chloride (10 mmol) in dry CH_3CN (13.5 mL) cooled at 0 °C. After stirring at 0 °C for 2 h, 48% aqueous HBr (15.3 mL) was added dropwise to the mixture cooled at 0 °C. After complete addition, the mixture was stirred at room temperature overnight, then diluted with water and extracted with ethyl acetate. The organic layer was washed with brine and $NaHCO_3$ solution, then dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with a mixture of ethyl acetate/light petroleum (1:2).

*3.1.1.1. 1-Bromo-4-phenylbutan-2-one (**2a**).* Yield: 72%, mp = 43–44 °C. IR (KBr): 1718 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.29 (t, 2H, H3′, H5′ $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.22–7.17 (m, 3H, H2′, H4′, H6′), 3.84 (s, 2H, 2H1), 2.98–2.94 (m, 4H, 2H3, 2H4). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 200.63, 136.23, 130.29, 128.96, 128.60, 40.82, 33.85, 27.10.

*3.1.1.2. 1-Bromo-4-phenylpentan-2-one (**2b**).* Yield: 76%, mp = 46–47 °C. IR (KBr): 1718 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.19–7.14 (m, 3H, H2′, H4′, H6′), 3.70 (s, 2H, 2H1), 3.32–3.27 (m, 1H, H4), 2.89 (dd, 1H, 1H3, $J_{gem} = 16.4$ Hz, $J_{3\text{-}4} = 6.8$ Hz), 2.82 (dd, 1H, 1H3, $J_{gem} = 16.4$ Hz, $J_{3\text{-}4} = 7.6$ Hz), 1.25 (d, 3H, CH_3, $J_{CH3\text{-}CH} = 6.8$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 200.50, 145.44, 128.55, 126.66, 126.45, 47.93, 35.46, 34.95, 21.79.

*3.1.1.3. 1-Bromo-3-methyl-4-phenylbutan-2-one (**2c**).* Yield: 75%, mp = 48–49 °C. IR (KBr): 1713 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.24 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.16 (t, 1H, H4′, $J_{4'-5'} = 7.6$ Hz), 7.10 (d, 2H, H2′, H6′, $J_{2'-3'} = 7.6$ Hz), 3.76 (s, 2H, 2H1), 3.14–3.07 (m, 1H, H3), 2.92 (dd, 1H, 1H4, $J_{gem} = 13.6$ Hz, $J_{3\text{-}4} = 7.2$ Hz), 2.82 (dd, 1H, 1H4, $J_{gem} = 13.6$ Hz, $J_{3\text{-}4} = 7.2$ Hz), 1.09 (d, 3H, CH_3, $J_{CH3\text{-}CH} = 6.8$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 204.95, 139.01, 128.89, 128.63, 126.60, 45.68, 39.73, 34.31, 16.94.

*3.1.1.4. 1-Bromo-5-phenylpentan-2-one (**2d**).* Yield: 30%, mp = 51–50 °C. IR (KBr): 1715 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.20 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.18–7.15 (m, 3H, H2′, H6′, H4′), 3.83 (s 2H, 2H1), 2.66–2.61 (m, 4H, 2H3, 2H5), 1.94 (quin, 2H, 2H4 $J_{3\text{-}4} = J_{4\text{-}5} = 7.6$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 201.74, 141.19, 128.45, 128.08, 126.08, 38.91, 34.83, 33.37, 25.23.

*3.1.1.5. 1-Bromo-6-phenylhexan-2-one (**2e**).* Yield: 58%, mp = 48–49 °C. IR (KBr): 1715 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.19 (m, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.17–7.08 (m, 3H, H2′, H6′, H4′), 3.73 (s, 2H, 2H′), 2.52 (t, 2H, 2H3, $J_{3\text{-}4} = 7.2$ Hz), 2.46 (t, 2H, 2H6, $J_{5\text{-}6} = 7.2$ Hz), 1.57–1.48 (m, 4H, 2H4, 2H5). ^{13}C NMR

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($CDCl_3$, 100 MHz): δ (ppm) 201.25, 141.93, 128.27, 128.23, 125.69, 39.28, 35.47, 34.71, 30.55, 23.27.

*3.1.1.6. 1-Bromo-7-phenylheptan-2-one (**2f**).* Yield: 47%, mp = 47–48 °C. IR (KBr): 1717 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.25 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.15–7.13 (m, 3H, H2′, H6′, H4′), 3.82 (s, 2H, 2H1), 2.61–2.57 (m, 4H, 2H3, 2H7), 1.66–1.57 (m, 4H, 2H4, 2H6), 1.36–1.28 (m, 2H, 2H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 201.95, 142.35, 128.34, 128.24, 125.66, 39.61, 35.64, 34.35, 31.09, 28.54, 23.61.

*3.1.2. General procedure for the synthesis of the phenylalkyl-2H-chromenes (**3a-f**)*

Tert-BuOK (10 mmol) was added to a solution of (2-hydroxybenzyl)triphenylphosphonium bromide (10 mmol) in dry THF (43 mL) at room temperature. After 10 min, a solution of the appropriate bromomethylphenylketone (**2a-f**) (10 mmol) in dry THF (44 mL) was added dropwise. After 2 h, a new portion of *tert*-BuOK (10 mmol) was added and the mixture was stirred for 24 h at room temperature under nitrogen. After this period, ice and water were added and the mixture was neutralized and extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The solid product was chromatographed on silica gel column eluting with a mixture of $CHCl_3$/light petroleum (1:1) and further purified by crystallization by *n*-hexane.

*3.1.2.1. 3-Phenethyl-2H-chromene (**3a**).* Yield: 22%, mp = 39–40 °C from *n*-hexane. IR (KBr): 1604 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.22 (t, 2H, H3′, H5′, $J_{2'-3'} = 7.6 = J_{3'-4'} = 7.6$ Hz), 7.21–7.17 (m, 3H, H2′, H6′, H4′), 7.06 (dt, 1H, H7, $J_{6-7} = J_{7-8} = 7.4$ Hz, $J_{5-7} = 1.6$ Hz), 7.03 (dd, 1H, H5, $J_{5-6} = 7.4$ Hz, $J_{5-7} = 1.6$ Hz), 6.90 (dt, 1H, H6, $J_{5-6} = J_{6-7} = 7.4$ Hz, $J_{6-8} = 1.2$ Hz), 6.83 (dd, 1H, H8, $J_{7-8} = 7.4$ Hz, $J_{6-8} = 1.2$ Hz), 6.15 (s, 1H, H4), 4.66 (s, 2H, H2), 2.80 (t, 2H, H10, $J_{9-10} = 8.0$ Hz), 2.36 (t, 2H, H9, $J_{9-10} = 8.0$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 152.98, 141.24, 134.44, 128.48, 128.31, 128.25, 126.14, 126.03, 122.87, 121.31, 119.22, 115.29, 68.31, 33.52, 29.71. Anal. Calcd for $C_{17}H_{16}O$: C, 86.40; H, 6.82. Found: C, 86.73; H, 6.79.

*3.1.2.2. 3-(2-Phenylpropyl)-2H-chromene (**3b**).* Yield: 58%, mp = 63–64 °C from *n*-hexane. IR (KBr): 1604 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.24 (t, 2H, H3′, H5′, $J_{2'-3'} = 7.6 = J_{3'-4'} = 7.6$ Hz), 7.16–7.11 (m, 3H, H2′, H6′, H4′), 6.98 (ddd, 1H, H7, $J_{6-7} = 7.6$ Hz, $J_{7-8} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz), 6.84 (dd, 1H, H5, $J_{5-6} = 7.6$ Hz, $J_{5-7} = 1.6$ Hz), 6.77 (t, 1H, H6, $J_{5-6} = J_{6-7} = 7.6$ Hz), 6.73 (d, 1H, H8, $J_{7-8} = 8.0$ Hz), 6.05 (s, 1H, H4), 4.56 (s, 2H, H2), 2.90–2.87 (m, 1H, H10), 2.34 (dd, 1H, 1H9, $J_{gem} = 14.4$ Hz, $J_{9-10} = 7.0$ Hz), 2.26 (dd, 1H, 1H9, $J_{gem} = 14.4$ Hz, $J_{9-10} = 8.0$ Hz), 1.24 (d, 3H, CH_3, $J_{CH3-CH} = 6.8$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 152.89, 146.32, 133.35, 128.40, 128.13, 126.70, 126.21, 125.91, 122.84, 121.17, 120.61, 115.20, 68.22, 42.58, 37.99, 21.73. Anal. Calcd for $C_{18}H_{18}O$: C, 86.36; H, 7.25. Found: C, 86.02; H, 7.28.

*3.1.2.3. 3-(1-Phenylpropan-2-yl)-2H-chromene (**3c**).* Yield: 38%, mp = 65–68 °C from *n*-hexane. IR (KBr): 1604 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.23 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.16–7.10 (m, 3H, H2′, H6′, H4′), 7.01 (dt, 1H, H7, $J_{6-7} = J_{7-8} = 7.6$ Hz, $J_{5-7} = 1.6$ Hz), 6.89 (dd, 1H, H5, $J_{5-6} = 7.6$ Hz, $J_{5-7} = 1.6$ Hz), 6.81 (dt, 1H, H6, $J_{5-6} = J_{6-7} = 7.6$ Hz, $J_{6-8} = 1.2$ Hz), 6.76 (d, 1H, H8, $J_{7-8} = 7.6$ Hz), 6.11 (s, 1H, H4), 4.64 (s, 2H, H2), 2.81 (dd, 1H, H10, $J_{gem} = 13.2$ Hz, $J_{9-10} = 6.0$ Hz), 2.45 (dd, 1H, H10, $J_{gem} = 13.2$ Hz, $J_{9-10} = 8.0$ Hz), 2.47–2.38 (m, 1H, H9), 1.05 (d, 3H, CH_3, $J_{CH3-CH} = 7.2$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 153.18, 140.01, 138.97, 129.00, 128.24, 128.18, 126.15, 126.08, 123.09, 121.29, 118.18, 115.27, 67.21, 41.37, 39.41, 18.04. Anal. Calcd for $C_{18}H_{18}O$: C, 86.36; H, 7.25. Found: C, 86.57; H, 7.29.

*3.1.2.4. 3-(3-Phenylpropyl)-2H-chromene (**3d**).* Yield: 43%, mp = 43–45 °C from *n*-hexane. IR (KBr): 1604 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 7.24 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.17–7.12 (m, 3H, H2′, H6′, H4′), 7.05 (dt, 1H, H7, $J_{7-8} = J_{6-7} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz), 6.87 (dd, 1H, H5, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz), 6.79 (dt, 1H, H6, $J_{6-7} = J_{5-6} = 8.0$ Hz, $J_{6-8} = 1.2$ Hz), 6.74 (dd, 1H, H8, $J_{7-8} = 8.0$ Hz, $J_{6-8} = 1.2$ Hz), 6.10 (s, 1H, H4), 4.60 (s, 1H, H2), 2.59 (t, 2H, 2H9, $J_{9-10} = 7.6$ Hz), 2.02 (t, 1H, 2H11, $J_{10-11} = 7.6$ Hz), 1.77 (quin, 2H, 2H10, $J_{9-10} = J_{10-11} = 7.6$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 153.16, 142.02, 134.97, 128.62, 128.58, 128.35, 126.14, 126.10, 123.12, 121.47, 119.04, 115.48, 68.51, 35.59, 32.81, 28.60. Anal. Calcd for $C_{18}H_{18}O$: C, 86.36; H, 7.25. Found: C, 86.67; H, 7.23.

*3.1.2.5. 3-(4-Phenylbutyl)-2H-chromene (**3e**).* Yield: 42%, mp = 45–46 °C from *n*-hexane. IR (KBr): 1604 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 7.27 (t, 2H,, $J_{2'-3'} = J_{3'-4'} = 7.8$ Hz), 7.19–7.15 (m, 3H, H2′, H6′, H4′), 7.02 (dt, 1H, H7, $J_{6-7} = J_{7-8} = 7.6$ Hz, $J_{7-5} = 2.0$ Hz), 6.90 (dd, 1H, H5, $J_{5-6} = 7.6$ Hz, $J_{7-5} = 2.0$ Hz), 6.82 (dt, 1H, H6, $J_{5-6} = J_{6-7} = 7.6$ Hz, $J_{6-8} = 1.2$ Hz), 6.74 (dd, 1H, H8, $J_{7-8} = 7.6$ Hz, $J_{6-8} = 1.2$ Hz), 6.10 (s, 1H, H4), 4.60 (s, 2H, H2), 2,63 (t, 2H, 2H9, $J_{9-10} = 7.8$ Hz), 2.08 (t, 2H, 2H12, $J_{11-12} = 7.8$ Hz), 1.68–1.62 (m, 2H, 2H11), 1.55–1.49 (m, 2H, 2H10). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 152.88, 142.26, 135.01, 128.38, 128.31, 128.07, 125.87, 125.75, 122.94, 121.25, 118.69, 115.22, 68.32, 35.69, 33.07, 30.99, 26.36. Anal. Calcd for $C_{19}H_{20}O$: C, 86.32; H, 7.63. Found: C, 86.01; H, 7.66.

*3.1.2.6. 3-(5-Phenylpentyl)-2H-chromene (**3f**).* Yield: 55%, mp = 45–46 °C from *n*-hexane. IR (KBr): 1604 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.25 (t, 2H, H3′, H5′ $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.16–7.14 (m, 3H, H2′, H6′, H4′), 7.02 (dt, 1H, H7, $J_{7-6} = J_{7-8} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz), 6.88 (dd, 1H, H5, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz), 6.81 (dt, 1H, H6, $J_{5-6} = J_{7-6} = 8.0$ Hz, $J_{6-8} = 1.2$ Hz), 6.75 (dd, 1H, H8, $J_{7-8} = 8.0$ Hz, $J_{6-8} = 1.2$ Hz), 6.10 (s, 1H, H4), 4.63 (s, 2H, H2), 2.59 (t, 2H, 2H9, $J_{9-10} = 7.6$ Hz), 2.03 (t, 2H, 2H13, $J_{12-13} = 7.6$ Hz), 1.63 (quin, 2H, 2H10, $J_{9-10} = J_{10-11} = 7.6$ Hz), 1.49 (quin, 2H, 2H12, $J_{11-12} = J_{12-13} = 7.6$ Hz), 1.36 (quin, 2H, 2H11, $J_{10-11} = J_{11-12} = 7.6$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 152.87, 142.52, 135.19, 128.35, 128.25, 128.03, 125.86, 125.45, 122.96, 121.23, 118.56, 115.21, 68.32, 35.82, 33.11, 31.23, 28.88, 26.68. Anal. Calcd for $C_{20}H_{22}O$: C, 86.29; H, 7.97. Found: C, 86.58; H, 7.96.

*3.1.3. General procedure for the synthesis of 3-phenylalkylchromans (**4a**,**d-f**, **9a-c**)*

A solution of the appropriate phenylalkyl-2*H*-chromene (**3a**,**d-f**) or the appropriate mixture of (*Z*)-3-[(*E*)-3-phenylallylidene]chroman, (*E*)-3-(3-phenyl)prop-1-en-1-yl)-2*H*-chromene, and 3-cinnamyl-2*H*-chromene (**6**, **7**, **8a**; **6**, **7**, **8b** or **6**, **7**, **8c**)[43] (10 mmol) in dry dioxane (80 mL) was hydrogenated over 10% Pd/C (15.2 mmol) at room temperature for 30 min, under 40 psi pressure. After this period, the catalyst was filtered off and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel eluting with a mixture of chloroform/light petroleum (1:1). The product was further purified by crystallization from *n*-hexane.

*3.1.3.1. 3-Phenethylchroman (**4a**).* Yield: 69%, mp = 42–43 °C from *n*-hexane. IR (KBr): 1607 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.27 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'} = 7.6$ Hz), 7.19–7.15 (m, 3H, H2′, H6′, H4′), 7.06 (ddd, 1H, H7, $J_{6-7} = 7.4$ Hz, $J_{7-8} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz), 7.02 (dd, 1H, H5, $J_{5-6} = 7.4$ Hz, $J_{5-7} = 1.6$ Hz), 6.82 (dt, 1H, H6, $J_{5-6} = J_{6-7} = 7.4$ Hz, $J_{6-8} = 1.2$ Hz), 6.78 (dd, 1H, H8, J_{7-}

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$_{8}$ = 8.0 Hz, J_{6-8} = 1.2 Hz), 4.20 (m, 1H, 1H2, J_{gem} = 10.8 Hz, J_{2-3} = 3.2 Hz), 3.76 (dd, 1H, 1H2, J_{gem} = 10.8 Hz, J_{2-3} = 9.2 Hz), 2.88 (dd, 1H, 1H4, J_{gem} = 16.2 Hz, J_{3-4} = 5.2 Hz), 2.78–2.66 (m, 2H, H10), 2.49 (dd, 1H, H4, J_{gem} = 16.2 Hz, J_{3-4} = 9.6 Hz), 2.07–1.97 (m, 1H, H3), 1.75–1.56 (m, 2H, H9). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.67, 141.84, 129.88, 128.45, 128.32, 127.23, 125.96, 121.57, 120.25, 116.44, 70.38, 33.50, 33.03, 31.67, 31.49. Anal. Calcd for $C_{17}H_{18}O$: C, 85.67; H, 7.61. Found: C, 85.41; H, 6.63.

*3.1.3.2. 3-(3-Phenylpropyl)chroman (**4d**).* Yield: 34%, mp = 41–42 °C from *n*-hexane. IR (KBr): 1606 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.27 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'}$ = 7.8 Hz), 7.18–7.15 (m, 3H, H2′, H6′, H4′), 7.05 (t, 1H, H7, $J_{6-7} = J_{7-8}$ = 7.8 Hz), 6.99 (d, 1H, H5, J_{5-6} = 7.2 Hz), 6.83–6.76 (m, 2H, H6, H8), 4.17 (dd, 1H, 1H2, J_{gem} = 10.4 Hz, J_{2-3} = 3.2 Hz), 3.69 (dd, 1H, 1H2, J_{gem} = 10.4 Hz, J_{2-3} = 9.2 Hz), 2.82 (dd, 1H, 1H4, J_{gem} = 16.0 Hz, J_{3-4} = 4.0 Hz), 2.62 (t, 2H, 2H11, J_{10-11} = 7.6 Hz), 2.41 (dd, 1H, 1H4, J_{gem} = 16.0 Hz, J_{3-4} = 9.6 Hz), 2.03–1.97 (m, 1H, H3), 1.77–1.66 (m, 2H, 2H10), 1.45–1.36 (m, 2H, 1H9), 1.36–1.27 (m, 2H, 1H9). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.65, 142.14, 129.83, 128.34, 128.32, 127.16, 125.79, 121.67, 120.18, 116.39, 70.49, 35.95, 32.03, 31.48, 31.36, 28.58. Anal. Calcd for $C_{18}H_{20}O$: C, 85.67; H, 7.99. Found: C, 86.85; H, 8.02.

*3.1.3.3. 3-(4-Phenylbutyl)chroman (**4e**).* Yield: 86%, mp = 43–44 °C from *n*-hexane. IR (KBr): 1607 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.24 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'}$ = 7.6 Hz), 7.16–7.13 (m, 3H, H2′, H6′, H4′), 7.04 (t, 1H, H7, $J_{7-6} = J_{7-8}$ = 7.6 Hz), 6.97 (d, 1H, H5, J_{5-6} = 7.2 Hz), 6.81–6.77 (m, 2H, H6, H8), 4.14 (dd, 1H, 1H2, J_{gem} = 10.4 Hz, J_{2-3} = 3.2 Hz), 3.67 (dd, 1H, 1H2, J_{gem} = 10.4 Hz, J_{2-3} = 9.6 Hz), 2.78 (dd, 1H, 1H4, J_{gem} = 16.0 Hz, J_{3-4} = 4.8 Hz), 2.59 (t, 2H, 2H12, J_{11-12} = 7.6 Hz) 2.38 (dd, 1H, 1H4, J_{gem} = 16.0 Hz, J_{3-4} = 9.6 Hz), 1.99–1.88 (m, 1H, H3), 1.62 (quin, 2H, 2H11, $J_{10-11} = J_{11-12}$ = 7.6 Hz), 1.46–1.22 (m, 4H, 2H9, 2H10). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.61, 142.38, 129.81, 128.32, 128.24, 127.10, 125.65, 121.68, 120.11, 116.34, 70.48, 35.77, 31.98, 31.55, 31.48, 31.46, 26.31. Anal. Calcd for $C_{19}H_{22}O$: C, 85.67; H, 8.32. Found: C, 85.98; H, 8.29.

*3.1.3.4. 3-(5-Phenylpentyl)chroman (**4f**).* Yield: 81%, mp = 42–43 °C from *n*-hexane. IR (KBr): 1607 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.24 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'}$ = 7.6 Hz), 7.15–7.13 (m, 3H, H2′, H6′, H4′), 7.04 (t, 1H, H7, $J_{7-6} = J_{7-8}$ = 7.8 Hz), 6.98 (d, 1H, H5, J_{5-6} = 7.4 Hz), 6.82–6.77 (m, 2H, H6, H8), 4.15 (dd, 1H, 1H2, J_{gem} = 10.4 Hz, J_{2-3} = 3.2 Hz), 3.67 (dd, 1H, 1H2, J_{gem} = 10.4 Hz, J_{2-3} = 9.6 Hz), 2.78 (dd, 1H, 1H4, J_{gem} = 16.4 Hz, J_{3-4} = 4.8 Hz), 2.58 (t, 2H, H13, J_{12-13} = 7.6 Hz), 2.38 (dd, 1H, 1H4, J_{gem} = 16.4 Hz, J_{3-4} = 9.6 Hz), 1.96–1.91 (m, 1H, H3), 1.61 (quin, 2H, 2H12, $J_{11-12} = J_{12-13}$ = 7.6 Hz), 1.46–1.18 (m, 6H, 2H9, 2H10, 2H11). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.62, 142.56, 129.81, 128.32, 128.21, 127.09, 125.60, 121.72, 120.10, 116.34, 70.52, 35.88, 32.00, 31.66, 31.49, 31.35, 29.33, 26.57. Anal. Calcd for $C_{20}H_{24}O$: C, 85.67; H, 8.63. Found: C, 86.98; H, 6.60.

*3.1.3.5. 6-Chloro-3-(3-phenylpropyl)chroman (**9a**).* Yield: 79%, mp = 42–43 °C from *n*-hexane. IR (KBr): 1484 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.28 (t, 2H, H3′, H5′, $J_{2'-3'} = J_{3'-4'}$ = 7.6 Hz), 7.20–7.16 (m, 3H, H2′, H6′, H4′), 7.00 (dd, 1H, H7, J_{6-7} = 8.4 Hz, J_{5-7} = 2.4 Hz), 6.97 (d, 1H, H5, J_{5-7} = 2.4 Hz), 6.70 (d, 1H, H8, J_{7-8} = 8.4 Hz), 4.17 (dd, 1H, 1H2, J_{gem} = 10.8 Hz, J_{2-3} = 3.2 Hz), 3.70 (dd, 1H, 1H2, J_{gem} = 10.8 Hz, J_{2-3} = 9.2 Hz), 2.80 (dd, 1H, 1H4, J_{gem} = 16.4 Hz, J_{3-4} = 4.0 Hz), 2,61 (t, 2H, 2H11, J_{10-11} = 7.6 Hz), 2.39 (dd, 1H, 1H4, J_{gem} = 16.4 Hz, J_{3-4} = 9.2 Hz), 2.01–1.95 (m, 1H, H3), 1.76–1.68 (m, 2H, 2H10), 1.45–1.36 (m, 1H, 1H9), 1.36–1.28 (m, 1H, 1H9). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 153.29, 142.05, 129.36, 128.37, 127.15, 125.87, 124.83, 123.29, 117.70, 70.57, 35.94, 31.75, 31.34, 31.19, 28.55. Anal. Calcd for $C_{18}H_{19}ClO$: C, 75.38; H, 6.68; Cl, 12.36. Found: C, 75.65; H, 6.70; Cl, 12.42.

*3.1.3.6. 6-Chloro-3-[3-(4-chlorophenyl)propyl]chroman (**9b**).* Yield: 85%, mp = 41–42 °C from *n*-hexane. IR (KBr): 1484 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.20 (d, 2H, H3′, H5′, $J_{2'-3'}$ = 8.0 Hz), 7.05 (d, 2H, H2′, H6′, $J_{2'-3'}$ = 8.0 Hz),6.96 (dd, 1H, H7, J_{7-8} = 8.8 Hz, J_{5-7} = 2.4 Hz), 6.92 (d, 1H, H5, J_{7-5} = 2.4 Hz), 6.67 (d, 1H, H8, J_{7-8} = 8.8 Hz), 4.11 (dd, 1H, H2, J_{gem} = 10.8 Hz, J_{2-3} = 3.4 Hz), 3.63 (dd, 1H, H2, J_{gem} = 10.8 Hz, J_{2-3} = 9.4 Hz), 2.71 (dd, 1H, 1H4, J_{gem} = 16.4 Hz, J_{3-4} = 4.8 Hz), 2.54 (t, 2H, 2H11, J_{10-11} = 7.6 Hz), 2,31 (dd, 1H, 1H4, J_{gem} = 16.4 Hz, J_{3-4} = 9.6 Hz), 1.91–1.87 (m, 1H, H3), 1.70–1.59 (m, 2H, 2H10), 1.37–1.28 (m, 1H, 1H9), 1.28–1.19 (m, 1H, 1H9). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 153.19, 140.41, 131.44, 129.64, 128.28, 128.36, 127.05, 124.69, 123.15, 117.64, 70.34, 35.16, 31.58, 31.18, 30.98, 28.34. Anal. Calcd for $C_{18}H_{18}Cl_2O$: C, 67.30; H, 5.65; Cl, 22.07. Found: C, 67.12; H, 5.66; Cl, 21.99.

*3.1.3.7. 3-[3-(4-Chlorophenyl)propyl]chroman (**9c**).* Yield: 16%, mp = 42–43 °C from *n*-hexane. IR (KBr): 1489 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.23 (d, 2H, H3′, H5′, $J_{2'-3'}$ = 8.4 Hz), 7.10–7.04 (m, 3H, H2′, H6′, H7), 7.00 (d, 1H, H5, J_{5-6} = 7.2 Hz), 6.84–6.77 (m, 2H, H6, H8), 4.18 (dd, 1H, 1H2, J_{gem} = 10.8 Hz, J_{2-3} = 3.2 Hz), 3.71 (dd, 1H, 1H2, J_{gem} = 10.8 Hz, J_{2-3} = 9.2 Hz), 2.83 (dd, 1H, 1H4, J_{gem} = 16.2 Hz, J_{3-4} = 4.4 Hz), 2.59 (t, 2H, 2H11, J_{10-11} = 8.0 Hz), 2,43 (dd, 1H, 1H4, J_{gem} = 16.2 Hz, J_{3-4} = 9.4 Hz), 2,04–1.95 (m, 1H, H3), 1.76–1.63 (m, 2H, 2H10), 1.45–1.36 (m, 2H, 1H9), 1.36–1.28 (m, 2H, 1H9). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.62, 140.56, 131.54, 129.86, 129.71, 128.44, 127.21, 121.58, 120.24, 116.41, 70.43, 35.28, 32.03, 31.47, 31.23, 28.51. Anal. Calcd for $C_{18}H_{19}ClO$: C, 75.38; H, 6.68; Cl, 12.36. Found: C, 75.63; H, 6.66; Cl, 12.32.

*3.1.3.8. 1-(2H-Chromen-3-yl)ethanone (**10**).* 3-Buten-2-one (40 mmol) was added dropwise to a suspension of 2-hydroxybenzaldehyde (20 mmol) and 1,4-diazabicyclo-[2.2.2]-octane (DABCO) (20 mmol) in $CHCl_3$ (14 mL) and H_2O (14 mL) at room temperature. The mixture was stirred, under nitrogen atmosphere, at room temperature and for a week. After this period, the separated solid was filtered and washed with water. The organic layer was washed with 2 N NaOH, 2 N HCl and brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The obtained solid was purified by column chromatography on silica gel eluting with a mixture of chloroform/light petroleum (1:1). Yield: 46%. mp = 44–45 °C IR (KBr): 1637 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.30 (d, 1H, H4, J_{2-4} = 1.6 Hz), 7.27–7.23 (m, 1H, H7, J_{6-7} = 7.6 Hz, J_{7-8} = 8.0 Hz, J_{5-7} = 1.6 Hz), 7.16 (dd, 1H, H5, J_{5-6} = 7.6 Hz, J_{5-7} = 1.6 Hz), 6.93 (dt, 1H, H6, $J_{5-6} = J_{6-7}$ = 7.6 Hz, J_{6-8} = 1.6 Hz), 6.85 (d, 1H, H8, J_{7-8} = 8.0 Hz), 5.00 (d, 2H, H2, J_{2-4} = 1.6 Hz), 2.40 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 194.65, 154.43, 132.72, 131.29, 129.56, 128.10, 120.65, 119.63, 115.13, 63.10, 23.80.

*3.1.3.9. 1-(2H-Chromen-3-yl)ethanol (**11**).* A solution of $NaBH_4$ (10 mmol) in 2 N NaOH (5 mL) was added, in a single portion, to a stirred solution of **10** in dry EtOH (50 mL). After stirring at room temperature for 2 h, the solvent was evaporated to dryness under vacuum and the residue was taken up with H_2O and extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The white oil was used for the next reaction without further purification. Yield: 89%, oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.08 (dt, 1H, H7, $J_{7-8} = J_{6-7}$ = 8.0 Hz, J_{5-7} = 1.6 Hz), 6.96 (dd, 1H, H5, J_{5-6} = 7.6 Hz, J_{5-7} = 1.6 Hz), 6.85 (m, 1H, H6, J_{5-6} = 7.6 Hz, J_{6-7} = 8.0 Hz, J_{6-}

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$_{8}$ = 1.2 Hz), 6.78 (dd, 1H, H8, $J_{7\text{-}8}$ = 8.0 Hz, $J_{6\text{-}8}$ = 1.2 Hz), 6.32 (s, 1H, H4), 4.77 (s, 2H, H2), 4.36 (q, 1H, CH, $J_{CH\text{–}CH3}$ = 6.4 Hz), 2.12 (bs, 1H, OH), 1.34 (d, 3H, CH_3, $J_{CH\text{-}CH3}$ = 6.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 153.55, 137.48, 128.98, 126.75, 122.49, 121.49, 118.46, 115.54, 68.62, 65.51, 21.30.

*3.1.4. [1-(2H-Chromen-3-yl)ethyl]triphenylphosphonium bromide (**12**)*

Triphenylphosphine hydrobromide (10 mmol) was added at room temperature to a solution of the 1-(2*H*-chromen-3-yl)ethanol (**11**) (10 mmol) in dry CH_3CN (24 mL). The reaction mixture was refluxed for 5 h. After cooling to room temperature, Et_2O was added. The white precipitate was filtered and used for the next reaction without further purification. Yield: 45%. Mp > 200 °C ^1H NMR (CD_3OD, 400 MHz): δ (ppm) 7.96–7.88 (m, 15H, 3H2′, 3H6′, 3H3′, 3H5′, 3H4′), 7.17–6.76 (m, 3H, H5, H6, H7), 6.24 (d, 1H, H4, *J* = 4.8 Hz), 4.91–4.83 (m, 1H, H9), 4.41 (dd, 1H, 1H2, *J* = 14.4 Hz, *J* = 3.6 Hz), 4.27 41 (dd, 1H, 1H2, *J* = 14.4 Hz, *J* = 3.6 Hz), 1.72 (dd, 3H, CH_3, *J* = 7.2 Hz, *J* = 18.4 Hz).

*3.1.4.1. (E)-3-(1-Phenylprop-1-en-2-yl)-2Hchromene (**13**).* Tert-BuOK (10 mmol) was added to a suspension of [1-(2*H*-chromen-3-yl)ethyl]triphenylphosphonium bromide (**12**) (10 mmol) in dry THF (43 mL) at room temperature. After 30 min, a solution of benzaldehyde (10 mmol) in dry THF (45 mL) was added dropwise. The mixture was stirred for 24 h at room temperature under nitrogen. After this period, ice and water was added and the mixture was neutralized with 2 N HCl. The obtained suspension was extracted with $CHCl_3$ and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The obtained solid was chromatographed on silica gel column eluting with a mixture of $CHCl_3$/light petroleum (1:1), and further purified by crystallization from *n*-hexane. Yield: 24%, mp = 56–57 °C from *n*-hexane. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.34 (t, 2H, H2′, H6, $J_{2'\text{-}3'}$ = 7.6 Hz), 7.29–7.21 (m, 3H, H3′, H5′, H4′), 7.11 (dt, 1H, H7, $J_{7\text{-}6} = J_{7\text{-}8}$ = 8.0 Hz, $J_{5\text{-}7}$ = 1.6 Hz), 7.04 (dd, 1H, H5, $J_{5\text{-}6}$ = 8.0 Hz, $J_{5\text{-}7}$ = 1.6 Hz), 6.88 (t, 1H, H6, $J_{7\text{-}6} = J_{5\text{-}6}$ = 8.0 Hz), 6.83 (d, 1H, H8, $J_{7\text{-}8}$ = 8.0 Hz) 6.61 (s, 1H, H4), 6.45 (s, 1H, Hβ), 5.10 (s, 2H, H2), 2.12 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 153.54, 137.71, 133.44, 133.24, 129.40, 128.96, 128.17, 127.13, 126.79, 126.47, 122.91, 121.46, 120.41, 115.35, 66.48, 14.88. Anal. Calcd for $C_{18}H_{16}O$: C, 87.06; H, 6.49. Found: C, 87.32; H, 6.51.

*3.1.4.2. (E)-3-(2H-Chromen-3-yl)-1-phenylprop-2-en-1-one (**15**).* A solution of diethyl (2-oxo-2-phenethyl)phosphonate (11 mmol) in dry THF (30 mL) was added dropwise to a stirred suspension of NaH (11 mmol) in dry THF (15 mL) under nitrogen. The mixture was stirred for 10 min at room temperature, then a solution of 2*H*-chromene-3-carbaldehyde (**14**) (10 mmol) in dry THF (15 mL) was added dropwise. After stirring for 20 h at room temperature, water was added to the mixture and THF was removed under reduced pressure. The solid was collected by filtration, washed with water, and chromatographed on silica gel column, eluting with a mixture of dichloromethane and light petroleum (1:1). The product was further purified by crystallization from *n*-hexane. Yield: 72%, mp = 150–151 °C (lit. 148 °C)[50] from *n*-hexane. IR (KBr): 1651 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz): δ (ppm) 8.02 (d, 2H, H2′, H6′, $J_{2'\text{-}3'}$ = 7.4 Hz), 7.64 (t, 1H, H4′), 7.60–7.52 (m, 3H, H3′, H5′, Hα), 7.22–7.16 (m, 2H, H5, H7), 7.10 (d, 1H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.0 Hz), 7.00 (s, 1H, H4), 6.93 (t, 1H, H6, $J_{6\text{-}7} = J_{5\text{-}6}$ = 7.2 Hz), 6.84 (d, 1H, H8, $J_{7\text{-}8}$ = 8.0 Hz), 5.13 (s, 2H, H2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 190.09, 154.66, 141.69, 135.70, 132.80, 132.25, 131.03, 129.01, 128.65, 128.41, 128.13, 121.96, 121.83, 120.63, 115.88, 65.28. Anal. Calcd for $C_{18}H_{14}O_2$: C, 82.42; H, 5.38. Found: C, 82.19; H, 5.40.

3.2. Virology

3.2.1. Cells and virus

HeLa cells (Ohio strain) were grown in Eagle's Minimum Essential Medium (MEM) with 1% non essential aminoacids, 100 μg/mL streptomycin, 100 U/mL penicillin G and 8% heat-inactivated foetal calf serum (growth medium). The serum concentration was reduced to 2% for cell maintenance (maintenance medium). Cell monolayers were kept at 37 °C in a humidified atmosphere with 5% CO_2.

HRV type 1B and type 14 were purchased from the American Type Culture Collection (ATCC). Virus stocks were prepared infecting HeLa cells at a multiplicity of infection of 0.1 PFU/cell. Infected cells were incubated in maintenance medium at 33 °C. When the viral-induced cytopathic effect involved most of the cells, working stocks were prepared as cellular lysates and the clarified supernatants titrated by plaque assay as previously described.[44]

3.2.2. Compounds

Stock solutions of compounds were made up in ethanol (1, 0.5 or 0.1 mg/mL) and further diluted in cell culture medium shortly before use.

3.2.3. Cytotoxicity studies

The cytotoxicity of compounds was evaluated with a tetrazolium-based (XTT) colorimetric assay and by microscopical evaluation, as previously described.[44] The highest concentration of compound that did not affect morphology and viability in 100% of cells was recorded as the maximum non-cytotoxic concentration (MNCC). The 50% cytotoxic concentration (CC_{50}) was the dose of compound reducing the cell viability by 50% of the control samples.

3.2.4. Determination of the 50% inhibitory concentration (IC_{50})

The IC_{50} values were determined as described previously.[44] Briefly, HeLa cells in 6-well plates were infected with a virus suspension producing approximately 100 plaques per well. After incubation for 1 h at 33 °C, the cells were overlaid with medium for plaques in the presence or absence of fourfold dilutions of drugs, starting from the MNCC. After incubation at 33 °C for 72 h, the cells were stained and the plaques were counted. The compound concentration reducing virus plaque formation by 50% was the IC_{50}. It was calculated by plotting the percentage of plaque reduction, with respect to the control plaque count, versus the logarithm of compound dose.

3.2.5. Virus inactivation and stabilization

In inactivation studies, HRV1B was exposed or not to **9c** (48.5 μM) for 1 h at 33 °C and titrated by plaque assay after serial ten-fold dilutions. In stabilization studies, HRV1B was incubated with or without **9c** (48.5 μM) for 1 h at 33 °C before mild acid or thermal treatment. For mild acid treatment, the pH of the mixtures was lowered to 5 by addition of 0.2 M acetate buffer (pH 5). After incubation at 33 °C for 30 min, the mixtures were neutralized with 0.85 M Tris base. For thermal treatment, the mixtures were incubated for 20 min at 56 °C (pH 7.2) and then refrigerated on ice. Samples were diluted ten-fold serially and titrated by plaque assay.

3.2.6. Time-reduction studies of virus yield

HeLa cells were infected at a multiplicity of 5 in the presence or absence of **9c** (48.5 μM) for 1 h in the cold (4 °C). The end of virus binding was taken as 0 time. Then, MEM with or without 9c (48.5 μM) was added and the temperature raised to 33 °C. Single-cycle conditions were achieved by incubating the cells at 33 °C for 10 h post infection (phi.). The cultures were freeze-thawed three times and virus yield from clarified supernatants detected

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by plaque assay. To determine which stage of virus replication was affected by **9c**, the compound was added or removed from HRV-infected cultures at different times p.i. (0, 15′, 30′, 1 h, 2 h, 4 h, 6 h) and the cultures incubated at 33 °C up to 10 h p.i. The virus yield was determined by plaque assay.

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