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ARTICLE *in* EUROPEAN JOURNAL OF ORGANIC CHEMISTRY · JANUARY 2014

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Synthesis and Biological Evaluation of 7-Deoxy Analogues of the Human Rhinovirus 3C Protease Inhibitor Thysanone

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Keywords: Viruses / Oxa-Pictet–Spengler reaction / Quinones / Biological activity / Inhibitors

The synthesis of (±)-7-deoxythysanone and three analogues has been developed. The key oxa-Pictet–Spengler reaction enabled the synthesis of the naphthopyran precursor, which could be readily converted to 7-deoxythysanone and three related analogues. The biological activity of these compounds was also evaluated against HRV 3C protease, the results of which suggest that inhibition of the enzyme requires the presence of the 7-oxa functionality.

Introduction

The human rhinovirus (HRV) is the major cause for the common cold in the western world and yet, only symptomatic treatment is available to medicate rhinovirus infections.[1] It is estimated that the total economic impact of cold-related work loss exceeded $40 billion in 2003 in the United States alone.[2] Therefore, the discovery of an effective therapeutic agent to cure HRV infections continues to be of major interest to the pharmaceutical industry.[3]

Rhinoviruses are non-enveloped, single-stranded positive-sense RNA viruses with an icosahedral capsid.[4] Upon replication of the viral RNA genome by virally encoded RNA-dependent RNA polymerase (RDRP) through a double-stranded RNA intermediate, translation of the RNA is undertaken by host cell ribosomes.[5] However, translation is not initiated by a (5′)G-cap as usual, but rather is initiated by one single internal ribosome entry site (IRES) and, thus, one single polyprotein precursor is produced. This polyprotein precursor is self-processed by the encoded viral proteases 2A and 3C to generate functional proteins and enzymes.[6] This renders HRV 3C protease an interesting target in the quest to discover an effective therapeutic agent for the treatment of rhinovirus infections.

During a screening of fermentation broths of the fungus *Thysanophora penicilloides* for lead structures for HRV 3C protease inhibition, chemists at Merck isolated the pyranonaphthoquinone antibiotic (–)-thysanone (**1**, Figure 1), which inhibits HRV 3C protease with an IC_{50} value of 13 μg/mL.[7]

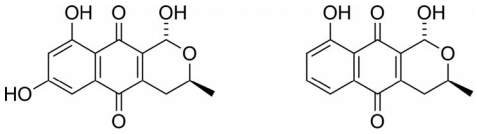
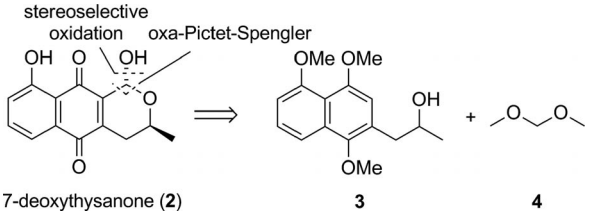


Figure 1. (–)-Thysanone (**1**) and (±)-7-deoxythysanone (**2**).

Currently, two total syntheses and one formal synthesis of the natural product **1** have been published.[8] We have an ongoing interest in the synthesis of **1** and its analogues. Over the past decade, we have published the total synthesis of **1** as well as the syntheses of a series of analogues.[8e,8f,9] In this current study, we report the synthesis of 7-deoxythysanone (**2**) and analogues to identify the role of the 7-oxa function in the mechanism of inhibition of HRV 3C protease by **1**.

Results and Discussion

We planned to synthesize **2** by using a key oxa-Pictet–Spengler reaction[10] of naphthalene **3** with dimethoxymethane (**4**) to provide the complete carbon framework of the target compound **2** (Scheme 1). This approach comple-



Scheme 1. Retrosynthetic analysis of (±)-7-deoxythysanone (**2**).

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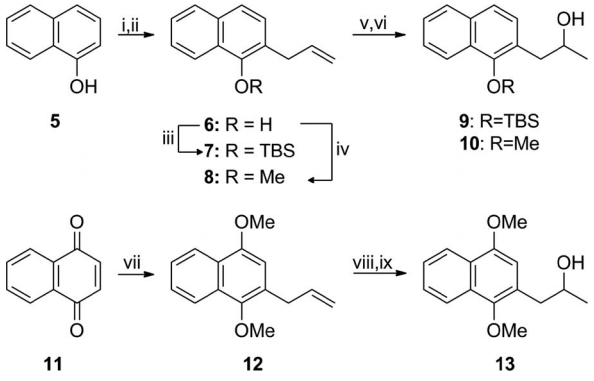
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201301515.

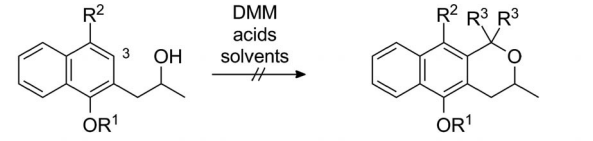
ments a related oxa-Pictet–Spengler strategy employed during a formal synthesis of thysanone.[8c,8d]

With this strategy in mind, we decided to start with an investigation of the key oxa-Pictet–Spengler reaction by using the easily accessible homobenzylic alcohols **9**, **10** and **13** with dimethoxymethane (Scheme 2). The homobenzylic alcohols **9** and **10** were synthesized from the common intermediate naphthol **6**, which is itself readily available from 1-naphthol (**5**) in two steps by allylation[11] followed by Claisen rearrangement at 180 °C.[12] Naphthol **6** was protected as either *tert*-butyldimethylsilyl (TBS) ether **7** by using sodium hydride and TBSCl in 99% yield[13] or as methyl ether **8** by using dimethyl sulfate and potassium carbonate in 97% yield.[14] The Wacker oxidation of both **7** and **8** followed by reduction gave homobenzylic alcohols **9** and **10**. Homobenzylic alcohol **13** was synthesised in three steps from commercially available naphthoquinone (**11**), which was converted to allylnaphthalene **12** by slight modification to the conditions described by Ray et al.[15] Allylindium iodide, prepared in situ from allyl bromide, indium and sodium iodide, was treated with **11** to afford the corresponding hydroquinone.[9b,16] A one-pot methylation with dimethyl sulfate and potassium hydroxide afforded naphthalene **12** in 80% over two steps. The Wacker oxidation of **12** followed by reduction afforded homobenzylic alcohol **13** in 76% yield over two steps.



Scheme 2. Reagents and conditions: (i) allyl bromide, TBAI, K_2CO_3, acetone, reflux, quantitative; (ii) neat, 180 °C, 20 min, 96%; (iii) NaH, TBSCl, THF, 99%; (iv) Me_2SO_4, K_2CO_3, acetone, reflux, 97%; (v) $PdCl_2$, CuCl, O_2, R = TBS: 71%, R = Me: 76%; (vi) $NaBH_4$, MeOH, **9**: quantitative, **10**: quantitative; (vii) a: In, allyl bromide, NaI, THF; b: Me_2SO_4, KOH, THF/H_2O, 80%; (viii) $PdCl_2$, CuCl, O_2, 76%; (ix) $NaBH_4$, MeOH, quantitative.

With homobenzylic alcohols **9**, **10** and **13** in hand, we next investigated the oxa-Pictet–Spengler reaction with different solvents, acids and reaction temperatures. Disappointingly, although we attempted a plethora of reaction conditions to effect the oxa-Pictet–Spengler reaction, none of the desired product was observed (Scheme 3). The decomposition of starting materials **9**, **10** and **13** was observed with various Brønsted and Lewis acids (boron trifluoride diethyl etherate, TBS trifluoromethanesulfonate and *para*-toluenesulfonic acid). Furthermore, only the methoxy methyl ether (MOM) protected homobenzylic alcohols were isolated when Amberlyst-15® was used. These disappointing results suggested that further electronic activation of the C-3 position of the homobenzylic alcohol is required to facilitate electrophilic substitution at C-3 by the intermediate oxonium ion.

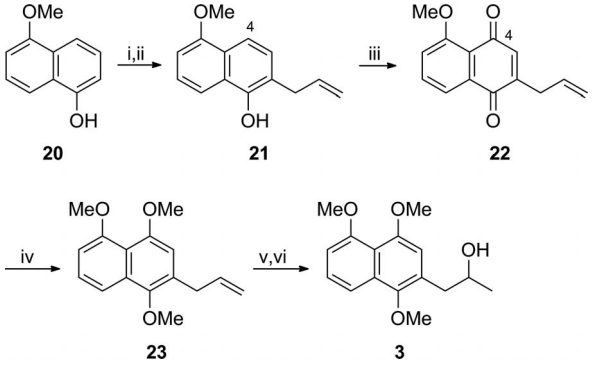


9: R^1 = TBS, R^2 = H
10: R^1 = Me, R^2 = H
13: R^1 = Me, R^2 = OMe

14: R^1 = TBS, R^2 = H, R^3 = H
15: R^1 = TBS, R^2 = H, R^3 = Me
16: R^1 = Me, R^2 = H, R^3 = H
17: R^1 = Me, R^2 = H, R^3 = Me
18: R^1 = Me, R^2 = OMe, R^3 = H
19: R^1 = Me, R^2 = OMe, R^3 = Me

Scheme 3. Failed oxa-Pictet–Spengler reactions.

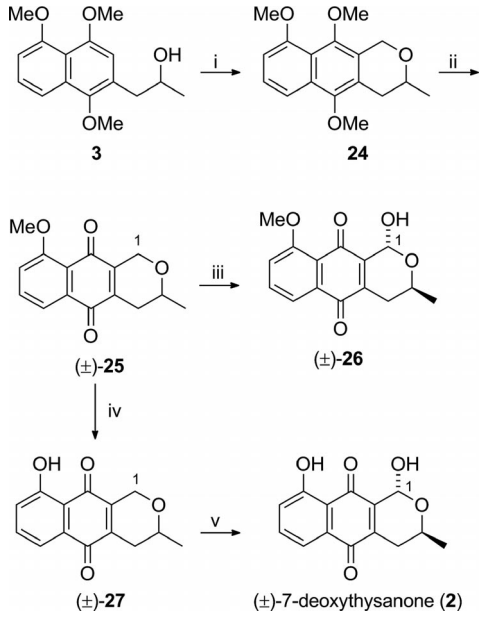
Despite the failure of these model studies, we next pursued the synthesis of **2** from homobenzylic alcohol **3** starting from 5-methoxy-1-naphthol (**20**; Scheme 4).[17] O-Allylation of **20** by using potassium carbonate, allyl bromide and tetrabutylammonium iodide (TBAI)[18] followed by Claisen rearrangement at 180 °C afforded naphthol **21** in 89% yield over two steps (Scheme 4).[19] To introduce the C-4 oxa functionality, naphthol **21** was oxidized to naphthoquinone **22** in 55% yield by using freshly prepared salcomine and oxygen.[20] The reductive methylation of **22** then afforded homobenzylic alcohol precursor **23** in 67% yield. Wacker oxidation followed by sodium borohydride reduction then gave homobenzylic alcohol **3** in 86% yield over two steps.[21]



Scheme 4. Reagents and conditions: (i) allyl bromide, TBAI, K_2CO_3, acetone, reflux, 99%; (ii) neat, 180 °C, 3 h, 90%; (iii) salcomine, O_2, MeCN, 55%; (iv) a: $Na_2S_2O_4$, TBAI, THF, H_2O, 1 h; b: KOH, Me_2SO_4, 67%, (v) $PdCl_2$, CuCl, O_2, DMF/H_2O, 96%; (vi) $NaBH_4$, MeOH, 90%.

Gratifyingly, the oxa-Pictet–Spengler reaction of homobenzylic alcohol **3** with dimethoxymethane (DMM) and boron trifluoride diethyl etherate proceeded smoothly and delivered naphthopyran **24** in 89% yield (Scheme 5). The oxidative demethylation of **24** by using silver(II) oxide afforded the first 7-deoxythysanone analogue pyrano-naphthoquinone (±)-**25** in an excellent 97% yield. The stereoselective oxidation at C-1 according to the established procedure[8a,8b,8e,8f] afforded a second analogue, pyrano-naphthoquinone (±)-**26**, in 56% yield.[8a] The methyl ether of (±)-**25** was also cleaved by using boron trichloride to

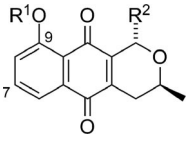
afford a third analogue, pyranonaphthoquinone (±)-**27**, in excellent yield. The stereoselective oxidation of (±)-**27** gave (±)-7-deoxythysanone (**2**) in 84% yield.



Scheme 5. Reagents and conditions: i) DMM, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 89%; (ii) AgO, HNO_3, THF/H_2O, 97%; (iii) a: Br_2, $(BzO)_2$, CCl_4; b: H_2O/THF, 56% over two steps; (iv) BCl_3, CH_2Cl_2, 0 °C → room temp., 98%; (v) a: Br_2, $(BzO)_2$, CCl_4; b: H_2O/THF, 84% over two steps.

All four 7-deoxyanalogues of thysanone (**1**), namely, (±)-**25**, (±)-**26**, (±)-**27** and (±)-7-deoxythysanone (**2**) were subjected to HRV 3C protease screening as well as to cytotoxicity assays (Table 1). For HRV 3C protease screening, our previously reported assay that uses a solid-phase-based fluorescent substrate was used.[22] The cytotoxicity (LD_{50}) was detected by using a cell-proliferation assay. The human lung adenocarcinoma epithelia cell line NCI-H441 was grown in the presence of the synthesised inhibitors (±)-**25**, (±)-**26**, (±)-**27** and (±)-**2** and treated with ^{3}H-thymidine for detection of cell proliferation. Unfortunately, all analogues lacked activity against HRV 3C protease, which suggests that the inhibition of HRV 3-C protease by thysanone (**1**) is very reliant on the 7-oxa functionality. Interestingly, both analogues that contain a free hydroxy function at C-9, namely, (±)-**27** and (±)-**2**, exhibit greater cytotoxicity than the analogues (±)-**25** and (±)-**26** for which the C-9 hydroxy group is protected as a methyl ether.

Table 1. Determination of the IC_{50} and LD_{50} values of 7-deoxythysanone analogues.



Compound	R^1	R^2	IC_{50} [μM]	LD_{50} [μM]
(±)-7-deoxythysanone (**2**)	H	OH	n.d.	13.2 ± 0
(±)-**25**	Me	H	n.d	107.3 ± 2
(±)-**26**	Me	OH	n.d.	155.3 ± 0
(±)-**27**	H	H	n.d.	8.1 ± 2

Conclusions

We have achieved an efficient synthesis of (±)-7-deoxythysanone (**2**) and three analogues (±)-**25**, (±)-**26** and (±)-**27** by the oxa-Pictet–Spengler reaction of homobenzylic alcohol **3** with dimethoxymethane **4** to afford pyranonaphthalene **24**. Subsequent screening of (±)-7-deoxythysanone (**2**) and analogues (±)-**25**, (±)-**26** and (±)-**27** established that all 7-deoxyanalogues of thysanone (**1**) do not inhibit HRV 3C protease; this suggests that the C-7 oxa function is an important structural feature, which possibly anchors **1** to HRV 3C protease by hydrogen bonding. The structure activity results reported herein are significant for the future design of HRV 3C protease inhibitors based on the thysanone (**1**) scaffold.

Experimental Section

General Details: Unless otherwise stated, all nonaqueous reactions and distillations were performed under a nitrogen or argon atmosphere in oven- or flame-dried glassware. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. Dichloromethane (CH_2Cl_2) and toluene were freshly distilled from calcium hydride. Tetramethylethylenediamine (TMEDA) was distilled from calcium hydride and stored under a nitrogen atmosphere over magnesium sulfate. Reactions performed at low temperature were either cooled in an acetone–dry-ice bath for temperatures below 0 °C or a water-ice bath for 0 °C. Flash chromatography was performed with 0.063–0.1 mm silica gel (Davisil R LC60A 40–63 Micron) with the indicated solvent. Preparatory TLC was performed on 500 μm UniplateTM (Analtech) silica gel (20 × 20 cm) thin layer chromatography plates. Infrared spectra were recorded with a Perkin–Elmer R Spectrum 1000 Fourier transform infrared spectrometer. Values are expressed in wavenumbers (cm^{-1}) and were recorded in the range 4000 to 450 cm^{-1}. NMR spectra were recorded at 21 °C in $CDCl_3$ or C_6D_6 with either a Bruker® Avance 300 spectrometer operating at 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei or a Bruker® DRX400 or Bruker® 400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei. All chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS, δ = 0 ppm) and were measured relative to the solvent in which the sample was analysed ($CDCl_3$/TMS δ = 0.00 ppm for ^{1}H NMR and $CDCl_3$ δ = 77.16 ppm for ^{13}C NMR; [D_6]acetone: δ = 2.05 ppm for ^{1}H NMR and δ = 29.84 ± 0.01 ppm for ^{13}C NMR). Coupling constants (J) are reported in Hertz (Hz). ^{1}H NMR spectroscopic data is reported as chemical shift in ppm, followed by multiplicity (s" singlet, "d" doublet, "dd" doublet of doublets, "ddd", doublet of doublets of doublets, "dt" doublet of triplets, "t" triplet, "q" quartet, "quint." quintuplet, "sept." septet, "m" multiplet, "br" broad), coupling constant where applicable and relative integral. ^{13}C NMR spectroscopic data is reported as chemical shift in ppm. High-resolution mass spectra were recorded with a Bruker micrOTOF-QII mass spectrometer.

3-{1′-[(*tert*-Butyldimethylsilyl)oxy]naphthalen-2′-yl}propan-2-ol (9): Copper(I) chloride (80 mg, 0.80 mmol) and palladium(II) chloride (23 mg, 0.13 mmol) were dissolved in DMF/water (6:1, 12 mL).

Oxygen was bubbled through the mixture for 2 h, followed by the addition of [(2′-allylnaphthalen-1′-yl)oxy](*tert*-butyl)dimethylsilane (**7**, 200 mg, 0.67 mmol) in DMF (10 mL). Oxygen was bubbled through the mixture for further 2 h, and the reaction mixture was stirred overnight under an atmosphere of oxygen. Aqueous hydrochloric acid (1 M, 30 mL) was added, and the aqueous layer was extracted with ethyl acetate (3× 50 mL). The combined organic extracts were washed with water (3× 50 mL) and brine (50 mL) and then dried with magnesium sulfate. The solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford 3-{1′-[(*tert*-butyldimethylsilyl)oxy]-naphthalen-2′-yl}propan-2-one (149 mg, 0.47 mmol, 71%) as a yellow oil. R_f (hexanes/EtOAc 9:1): 0.29. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.92–7.85 (m, 1 H), 7.62–7.55 (m, 1 H), 7.29 (d, J = 8.5 Hz, 1 H), 7.27–7.21 (m, 2 H), 7.03 (d, J = 8.5 Hz, 1 H), 3.66 (s, 2 H), 1.89 (s, 3 H), 0.94 (s, 9 H), –0.03 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 207.0, 149.1, 134.5, 128.5, 128.3, 127.8, 126.0, 125.2, 123.3, 122.2, 120.1, 46.2, 29.2, 26.2, 18.8, –3.1 ppm. HRMS (ESI): calcd. for $C_{19}H_{27}O_2Si$ $[M + H]^+$ 315.1775; found 315.1779; calcd. for $C_{19}H_{26}NaO_2Si$ $[M + Na]^+$ 337.1594; found 337.1606; calcd. for $C_{19}H_{26}KO_2Si$ $[M + K]^+$ 353.1334; found 353.1351. MS (EI): *m*/*z* (%) = 314 (10), 257 (100), 239 (15), 215 (15), 199 (10), 185 (10), 75 (35). To a mixture of 3-{1′-[(*tert*-butyldimethylsilyl)oxy]naphthalen-2′-yl}propan-2-one (0.50 g, 1.6 mmol) in methanol (10 mL) was added sodium borohydride (72 mg, 1.9 mmol). The mixture was stirred for 1 h and then quenched with water (10 mL). The aqueous layer was extracted with ethyl acetate (3× 10 mL), and the combined organic extracts were washed with brine (10 mL) and then dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford the title compound (0.51 g, 1.6 mmol, quant.) as a colourless oil. R_f (hexanes/EtOAc 9:1): 0.09. IR (film): $\tilde{\nu}_{max}$ = 3412 (OH), 2966 (Ar), 2929 (Ar), 2839 (Ar), 1598, 1572, 1446, 1368, 1258, 1245, 1085, 989, 807, 748 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10–8.04 (m, 1 H), 7.80–7.34 (m, 1 H), 7.49–7.40 (m, 3 H), 7.30 (d, J = 8.5 Hz, 1 H), 4.15–4.05 (m, 1 H), 3.00–2.86 (m, 2 H), 1.22 (d, J = 6.0 Hz, 3 H), 1.14–1.10 (m, 9 H), 0.21–0.18 (m, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 149.2, 134.2, 129.2, 128.3, 127.7, 125.6, 125.0, 123.5, 123.3, 122.0, 68.7, 40.8, 26.3, 26.2, 23.1, –2.9 ppm. HRMS (ESI): calcd. for $C_{19}H_{28}NaO_2Si$ $[M + Na]^+$ 339.1751; found 339.1750; calcd. for $C_{19}H_{28}KO_2Si$ $[M + K]^+$ 355.1490; found 355.1493.

1-(1′-Methoxynaphthalen-2′-yl)propan-2-ol (10): Copper(I) chloride (0.87 g, 8.8 mmol) and palladium(II) chloride (0.25 g, 1.4 mmol) were dissolved in DMF/water (6:1, 130 mL). Oxygen was bubbled through the mixture for 2 h, followed by the addition of 2-allyl-1-methoxynaphthalene **8** (1.45 g, 7.3 mmol) in DMF (40 mL). Oxygen was bubbled through the mixture for a further 2 h, and the reaction mixture was stirred overnight under an atmosphere of oxygen. Aqueous hydrochloric acid (1 M, 100 mL) was added, and the aqueous layer was extracted with ethyl acetate (3× 100 mL). The combined organic extracts were washed with water (3× 100 mL) and brine (100 mL) and dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford 1-(1-methoxynaphthalen-2-yl)propan-2-one (1.19 g, 5.6 mmol, 76%) as a yellow oil. R_f (hexanes/EtOAc 4:1): 0.38. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11–8.07 (m, 1 H), 7.85–7.82 (m, 1 H), 7.61 (d, J = 8.4 Hz, 1 H), 7.54–7.45 (m, 2 H), 7.26 (d, J = 8.4 Hz, 1 H), 3.90 (s, 5 H), 2.20 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.7, 154.2, 134.7, 128.5, 128.3, 128.1, 126.3, 126.2, 124.5, 123.4, 122.2, 62.1, 45.5, 29.5 ppm. HRMS (ESI): calcd. for $C_{14}H_{15}O_2$ $[M + H]^+$ 215.1067; found 215.1061; calcd. for $C_{14}H_{14}NaO_2$ $[M + Na]^+$ 237.0886; found 237.0890. MS (EI): *m*/*z* (%) = 214 (60), 171 (100), 156 (50) 141 (50), 128 (50), 115 (30). To a mixture of 1-(1-methoxynaphthalen-2-yl)propan-2-one (0.85 g, 4.0 mmol) in methanol (40 mL) was added sodium borohydride (185 mg, 4.9 mmol). The mixture was stirred for 1 h and then quenched with water (40 mL). The aqueous layer was extracted with ethyl acetate (3× 40 mL), and the combined organic extracts were washed with brine (40 mL) and then dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford the title compound (0.87 g, 4.0 mmol, quant.) as a colourless oil. R_f (hexanes/EtOAc 4:1): 0.12. IR (film): $\tilde{\nu}_{max}$ = 3213 (OH), 3053 (Ar), 2967 (Ar), 2931 (Ar), 2841 (Ar), 1598, 1572, 1507, 1446, 1368, 1341, 1258, 1245, 1195, 1114, 1085, 989, 949, 931, 808, 750, 707 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, J = 8.6 Hz, 1 H), 7.82 (d, J = 7.7 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 1 H), 7.53–7.42 (m, 2 H), 7.32 (d, J = 8.4 Hz, 1 H), 4.21–4.08 (m, 1 H), 3.94 (s, 3 H), 2.97–2.95 (m, 2 H), 2.26 (br s, 1 H), 1.27 (d, J = 6.1 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.1, 134.3, 129.0, 128.1, 128.1, 127.1, 126.2, 125.8, 124.4, 122.2, 68.8, 62.0, 40.1, 23.4 ppm. HRMS (ESI): calcd. for $C_{14}H_{16}O_2$ $[M + H]^+$ 239.1043; found 239.1046. MS (EI): *m*/*z* (%) = 216 (65), 172 (100), 157 (80), 141 (65), 128 (65), 115 (20).

1-(1′,4′-Dimethoxynaphthalen-2′-yl)propan-2-ol (13): Copper(I) chloride (525 mg, 5.3 mmol) and palladium(II) chloride (148 mg, 0.8 mmol) were dissolved in DMF/water (6:1, 70 mL). Oxygen was bubbled through the mixture for 2 h, followed by the addition of **12** (1.0 g, 4.4 mmol) in DMF (40 mL). Oxygen was bubbled through the mixture for a further 2 h, and the reaction was stirred overnight under an atmosphere of oxygen. Aqueous hydrochloric acid (1 M, 100 mL) was added, and the aqueous layer was extracted with ethyl acetate (3× 100 mL). The combined organic extracts were washed with water (3× 100 mL) and brine (100 mL) and then dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford 1-(1′,4′-dimethoxynaphthalen-2′-yl)-propan-2-one (0.82 g, 3.4 mmol, 76%) as a yellow oil. R_f (hexanes/EtOAc 4:1): 0.16. IR (film): $\tilde{\nu}_{max}$ = 2929, 2832, 1672, 1589, 1584, 1461, 1438, 1398, 1263, 1125, 1069, 1002 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.25–8.21 (m, 1 H), 8.05–8.00 (m, 1 H), 7.56–7.51 (m, 1 H), 7.49–7.44 (m, 1 H), 6.54 (s, 1 H), 3.95 (s, 3 H), 3.87 (s, 2 H), 3.85 (s, 3 H), 2.20 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.9, 152.2, 147.6, 128.6, 126.9, 126.3, 125.5, 122.7, 122.6, 121.9, 105.6, 67.1, 55.8, 45.8, 29.4 ppm. HRMS (ESI): calcd. for $C_{15}H_{17}O_3$ $[M + H]^+$ 245.1172; found 245.1170; calcd. for $C_{15}H_{16}NaO_3$ $[M + Na]^+$ 267.0992; found 267.0998; calcd. for $C_{15}H_{16}KO_3$ $[M + K]^+$ 283.0731; found 283.0745. MS (EI) *m*/*z* (%) = 244 (100), 229 (20), 201 (25), 169 (25), 141 (20), 128 (19), 115 (18), 55 (19). To a mixture of 1-(1′,4′-dimethoxynaphthalen-2′-yl)-propan-2-one (0.80 g, 3.3 mmol) in methanol (50 mL) was added sodium borohydride (149 mg, 3.9 mmol). The mixture was stirred for 1 h and then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate (3× 50 mL), and the combined organic extracts were washed with brine (50 mL) and then dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford the title compound (0.81 g, 3.3 mmol, quant.) as a colourless oil. R_f (hexanes/EtOAc 4:1): 0.02. IR (film): $\tilde{\nu}_{max}$ = 3403, 2963, 2932, 2838, 1627, 1595, 1508, 1460, 1417, 1394, 1345, 1263, 1225, 1161, 1119, 1093, 1030, 1000, 978, 829, 767, 712 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24–8.19 (m, 1 H), 8.03–7.99 (m, 1 H), 7.57–7.40 (m, 2 H), 6.62 (s, 1 H), 4.23–4.10 (m, 1 H), 3.97 (s, 3 H), 3.89 (s, 3 H), 2.94 (d, J = 6.2 Hz, 2 H), 2.29 (br s, 1 H), 1.28 (d, J = 6.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$):

δ = 162.5, 152.1, 128.7, 126.8, 126.6, 125.9, 125.2, 122.5, 121.9, 106.3, 68.9, 62.0, 55.8, 40.6, 23.4 ppm. HRMS (ESI): calcd. for $C_{15}H_{18}NaO_3$ $[M + Na]^+$ 269.1148; found 269.1146; calcd. for $C_{15}H_{18}KO_3$ $[M + K]^+$ 285.0888; found 285.0892. MS (EI): *m*/*z* (%) = 246 (60), 201 (25), 187 (100), 170 (10), 150 (10) 128 (15), 115 (13).

2-Allyl-5-methoxynaphthalene-1,4-dione (22): To a mixture of 2-allyl-5-methoxynaphthalen-1-ol (**21**, 250 mg, 1.17 mmol) in acetonitrile (5 mL) was added salcomine (50 mg, 0.15 mmol). Oxygen was bubbled through the mixture for 4 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford the title compound (145 mg, 0.64 mmol, 55%) as a yellow oil. R_f (hexanes/EtOAc 7:3): 0.52. IR (film): $\tilde{\nu}_{max}$ = 3073, 3010, 2980, 2848, 1663, 1649, 1629, 1584, 1478, 1449, 1438, 1402, 1370, 1280, 1256, 1171, 1063, 1051, 977, 921, 882, 775 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.79–7.74 (m, 1 H), 7.70–7.63 (m, 1 H), 7.32–7.27 (m, 1 H), 6.70 (t, *J* = 1.5 Hz, 1 H), 5.97–5.81 (m, 1 H), 5.25–5.15 (m, 2 H), 4.00 (s, 3 H), 3.32–3.26 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.3, 184.7, 159.6, 147.3, 137.6, 134.9, 134.6, 133.2, 120.1, 119.6, 118.9, 117.9, 56.6, 33.2 ppm. HRMS (ESI): calcd. for $C_{14}H_{13}O_3$ $[M + H]^+$ 229.0859; found 229.0848; calcd. for $C_{14}H_{12}NaO_3$ $[M + Na]^+$ 251.0679; found 251.0678; calcd. for $C_{14}H_{12}KO_3$ $[M + K]^+$ 267.0418; found 267.0425. MS (EI): *m*/*z* (%) = 228 (100), 213 (85), 199 (20), 181 (50), 157 (30), 141 (50), 121 (55), 104 (30), 76 (60), 63 (20).

2-Allyl-1,4,5-trimethoxynaphthalene (23): To a mixture of 2-allyl-5-methoxynaphthalene-1,4-dione (**22**, 450 mg, 2.0 mmol) in THF (20 mL) were added TBAI (cat.) and sodium dithionate (2.0 g, 11.8 mmol) in water (10 mL). The mixture was stirred for 30 min. Potassium hydroxide (2.5 g, 45.3 mmol) in water (10 mL) and dimethyl sulfate (4.3 mL, 45.3 mmol) were then added, and the mixture was stirred for 2 h. Aqueous ammonia (10 mL) was added, and the mixture was stirred for a further 30 min. The phases were separated, and the aqueous layer was extracted with ethyl acetate (3× 30 mL). The combined organic extracts were washed with aqueous HCl (1 M, 50 mL), water (50 mL) and then brine (50 mL). The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 19:1) to afford the title compound (346 mg, 1.34 mmol, 67%) as a yellow oil. R_f (hexanes/EtOAc 7:3): 0.72. IR (film): $\tilde{\nu}_{max}$ = 3076, 2933, 2838, 1724, 1618, 1599, 1583, 1508, 1461, 1448, 1379, 1346, 1262, 1237, 1127, 1069, 1006, 913, 847, 810, 754 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.69–7.65 (m, 1 H), 7.40 (t, *J* = 8.0 Hz, 1 H), 6.86–6.83 (m, 1 H), 6.66 (s, 1 H), 6.09–5.97 (m, 1 H), 5.16–5.07 (m, 2 H), 3.97 (s, 3 H), 3.93 (s, 3 H), 3.84 (s, 3 H), 3.57 (dt, *J* = 6.5, 1.5 Hz, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.5, 153.5, 147.2, 137.2, 131.7, 128.5, 126.7, 116.2, 114.9, 108.5, 106.3, 62.0, 57.1, 56.6, 34.2 ppm. HRMS (ESI): calcd. for $C_{16}H_{19}O_3$ $[M + H]^+$ 259.1329; found 259.1330; calcd. for $C_{16}H_{18}NaO_3$ $[M + Na]^+$ 281.1148; found 281.1147. MS (EI): *m*/*z* (%) = 258 (100) $[M]^+$, 243 (60), 228 (20), 212 (40).

1-(1′,4′,5′-Trimethoxynaphthalen-2′-yl)propan-2-ol (3): A mixture of freshly prepared copper(I) chloride (46 mg, 0.47 mmol) and palladium(II) chloride (13 mg, 74 μmol) in DMF/H_2O (6:1.7 mL) was stirred for 1 h as oxygen was bubbled through the mixture. Naphthalene (**23**, 100 mg, 0.39 mmol) was added in DMF (3 mL). Oxygen was bubbled through the mixture for 4 h, and the reaction mixture was then stirred overnight under an atmosphere of oxygen. The mixture was hydrolysed with 1 M aqueous HCl (20 mL), and the aqueous layer was extracted with EtOAc (3× 20 mL). The combined organic extracts were washed with water (3× 20 mL) and then brine (20 mL). The organic layer was dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 7:3) to afford the title compound (103 mg, 0.38 mmol, 96%), which was directly transferred to the next step. To a mixture of 1-(1′,4′,5′-trimethoxynaphthalen-2′-yl)propan-2-one (0.50 g, 1.8 mmol) in methanol (20 mL) was added sodium borohydride (100 mg, 2.6 mmol). The mixture was stirred for 1 h and then quenched with water (20 mL). The aqueous layer was extracted with ethyl acetate (3× 20 mL), and the combined organic extracts were washed with brine (20 mL) and then dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford the title compound (0.45 g, 1.63 mmol, 90%) as a colourless oil. R_f (hexanes/EtOAc 7:3): 0.21. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.63 (dd, *J* = 8.0, 0.8 Hz, 1 H), 7.40 (t, *J* = 8.0 Hz, 1 H), 6.84 (dd, *J* = 8.0, 0.8 Hz, 1 H), 6.66 (s, 1 H), 4.19–4.11 (m, 1 H), 3.95 (s, 3 H), 3.92 (s, 3 H), 3.84 (s, 3 H), 2.92–2.88 (m, 2 H), 2.47 (br s, 1 H), 1.27 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.6, 153.7, 147.8, 131.5, 127.4, 126.9, 117.8, 114.8, 109.0, 106.4, 68.8, 61.7, 57.1, 56.6, 40.5, 23.4 ppm. The spectroscopic data was in agreement with that reported in the literature.[21]

5,9,10-Trimethoxy-3-methyl-3,4-dihydro-1*H*-benzo[*g*]isochromene (24): To a mixture of **3** (180 mg, 0.64 mmol) and dimethoxymethane (170 μL, 1.96 mmol) in dry Et_2O (5 mL) was added $BF_3{\cdot}OEt_2$ at –78 °C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature over 18 h and then quenched with saturated aqueous ammonium chloride solution (10 mL). The aqueous layer was extracted with ethyl acetate (3× 20 mL), and the combined organic extracts were washed with brine (20 mL) and then dried with magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 9:1) to afford the title compound (167 mg, 0.58 mmol, 89%) as a colourless oil. R_f (hexanes/EtOAc 7:3): 0.31. IR (film): $\tilde{\nu}_{max}$ = 2930, 2837, 1654, 1619, 1597, 1573, 1502, 1460, 1446, 1362, 1337, 1281, 1262, 1222, 1179, 1127, 1115, 1094, 1065, 1044, 1005, 991, 970, 838, 811, 761 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.66 (dd, *J* = 8.0, 0.7 Hz, 1 H), 7.36 (t, *J* = 8.0 Hz, 1 H), 6.83 (d, *J* = 8.0 Hz, 1 H), 5.25 (d, *J* = 15.8 Hz, 1 H), 4.87 (d, *J* = 15.8 Hz, 1 H), 4.00 (s, 3 H), 3.86 (s, 3 H), 3.79 (s, 3 H), 3.79–3.73 (m, 1 H), 3.06 (dd, *J* = 16.6, 2.9 Hz, 1 H), 2.65 (dd, *J* = 16.6, 10.9 Hz, 1 H), 1.43 (d, *J* = 6.1 Hz, 3 H, Me) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.2, 149.2, 147.9, 130.0, 125.8, 125.8, 124.9, 119.2, 114.7, 105.6, 70.6, 65.2, 61.8, 61.0, 56.2, 30.9, 21.9 ppm. HRMS (ESI): calcd. for $C_{17}H_{21}O_4$ $[M + H]^+$ 289.1434; found 289.1434; calcd. for $C_{17}H_{20}NaO_4$ $[M + Na]^+$ 311.1254; found 311.1262; calcd. for: $C_{17}H_{20}KO_4$ $[M + K]^+$ 327.0993; found 327.1014. MS (EI): *m*/*z* (%) = 288 (100), 273 (10), 257 (14), 244 (15), 229 (82), 214 (12), 199 (10), 128 (10), 115 (12).

1,7-Dideoxythysanone-9-methylether [(±)-25]: To a mixture of **24** (30 mg, 0.10 mmol) in THF (3 mL) were added freshly prepared AgO (124 mg, 1.14 mmol) and aqueous nitric acid (6 N, 0.35 mL, 2.10 mmol). The reaction mixture was stirred for 5 min and then filtered through Celite®. The filtrate was diluted with H_2O (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3× 10 mL). The combined organic extracts were washed with brine and dried with $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 7:3→1:1) to afford the title compound (25 mg, 97 μmol, 97%) as a yellow oil. R_f (hexanes/EtOAc 7:3): 0.24. IR (film): $\tilde{\nu}_{max}$ = 2922, 2851, 1654, 1644, 1584, 1574, 1474, 1445, 1332, 1291, 1276, 1261, 1209, 1200, 1187, 1137, 1124, 1091, 1034, 954, 917, 780 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.76 (dd, *J* = 7.8, 1.0 Hz, 1 H), 7.66 (t, *J*

= 7.8 Hz, 1 H), 7.28 (dd, *J* = 7.8, 1.0 Hz, 1 H), 4.84 (dd, *J* = 18.7, 2.2 Hz, 1 H), 4.50 (ddd, *J* = 18.7, 4.0, 3.2 Hz, 1 H), 4.00 (s, 3 H), 3.71–3.62 (m, 1 H), 2.74–2.67 (m, 1 H), 2.32–2.22 (m, 1 H), 1.37 (d, *J* = 6.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.1, 183.2, 159.8, 144.3, 139.8, 135.0, 134.4, 119.7, 119.4, 117.9, 69.7, 63.8, 56.6, 29.3, 21.4 ppm. HRMS (ESI): calcd. for $C_{15}H_{15}O_4$ [M + H]$^+$ 259.0965; found 259.0967; calcd. for $C_{15}H_{14}NaO_4$ [M + Na]$^+$ 281.0784; found 281.0787. MS (EI): *m*/*z* (%) = 258 (100), 243 (30), 225 (30), 187 (20), 128 (35).

7-Deoxythysanone-9-methylether (±)-26: To a mixture of (±)-**25** (10 mg, 39 μmol) in CCl_4 (8 mL) were added bromine (1 M in CCl_4, 39 μL, 39 μmol) and Bz_2O_2 (cat.). The reaction mixture was irradiated with a desk lamp and heated to reflux for 20 min. The solvent was removed in vacuo, and THF (2 mL) and H_2O (1 mL) were added. The reaction mixture was stirred for 1 h and then diluted with H_2O (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3× 10 mL). The combined organic extracts were dried with $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by preparative TLC (SiO_2, hexanes/EtOAc 1:1) to afford the title compound (6 mg, 22 μmol, 56%) as a yellow oil. R_f (hexanes/EtOAc 1:1): 0.21. IR (film): $\tilde{\nu}_{max}$ = 3468, 2974, 2844, 1656, 1645, 1585, 1472, 1448, 1435, 1413, 1331, 1289, 1263, 1194, 1122, 1084, 1069, 1054, 1029, 1012, 955, 922, 856, 849, 795, 749, 735, 674, 554 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (dd, *J* = 8.0, 1.0 Hz, 1 H), 7.68 (t, *J* = 8.0 Hz, 1 H), 7.31 (dd, *J* = 8.0, 1.0 Hz, 1 H), 6.05 (s, 1 H), 4.38–4.28 (m, 1 H), 4.01 (s, 3 H), 3.40 (br s, 1 H), 2.72 (dd, *J* = 19.2, 3.3 Hz, 1 H), 2.25 (ddd, *J* = 19.2, 11.2, 0.9 Hz, 1 H), 1.40 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.7, 183.2, 160.0, 142.1, 141.2, 135.2, 134.2, 128.6, 119.4, 118.3, 87.2, 63.0, 56.6, 29.1, 21.1 ppm. HRMS (ESI): calcd. for $C_{15}H_{14}NaO_5$ [M + Na]$^+$ 297.0733; found 297.0745.

1,7-Dideoxythysanone (±)-27: To a mixture of (±)-**25** (12 mg, 46 μmol) in CH_2Cl_2 (0.2 mL) was added BCl_3 (1 M in CH_2Cl_2, 0.14 μL, 0.14 mmol) at –78 °C. The cooling bath was removed, and the reaction mixture was stirred for 30 min. Aqueous $NaHCO_3$ (5 mL) was slowly added, and the aqueous layer was extracted with CH_2Cl_2 (3× 5 mL). The combined organic extracts were dried with $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by preparative TLC (SiO_2, hexanes/EtOAc 1:1) to afford the title compound (11 mg, 45 μmol, 98%) as a yellow oil. R_f (hexanes/EtOAc 1:1): 0.65. IR (film): $\tilde{\nu}_{max}$ = 2975, 7919, 2850, 1663, 1647, 1623, 1580, 1466, 1386, 1366, 1346, 1278, 1249, 1088, 743 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.90 (s, 1 H), 7.66–7.57 (m, 2 H), 7.24 (dd, *J* = 7.6, 1.8 Hz, 1 H), 4.90–4.82 (m, 1 H), 4.52 (ddd, *J* = 18.8, 4.1, 3.2 Hz, 1 H), 3.75–3.62 (m, 1 H), 2.81–2.70 (m, 1 H), 2.37–2.34 (m, 1 H), 1.39 (d, *J* = 6.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 188.7, 183.1, 161.6, 143.4, 142.6, 136.5, 132.1, 124.5, 119.4, 114.8, 69.8, 63.0, 29.8, 21.3 ppm. HRMS (ESI): calcd. for $C_{14}H_{13}O_4$ [M + H]$^+$ 245.0808; found 245.0806; calcd. for $C_{14}H_{12}NaO_4$ [M + Na]$^+$ 267.0628; found 267.0641; calcd. for $C_{14}H_{11}O_4$ [M – H]$^-$ 243.0663; found 243.0677. MS (EI): *m*/*z* (%) = 244 (100), 229 (10), 214 (20), 200 (95), 172 (55), 144 (20), 115 (30), 92 (27), 63 (13).

(±)-*trans*-7-Deoxythysanone (2): To a mixture of (±)-**27** (11 mg, 45 μmol) in CCl_4 (9 mL) were added bromine (1 M in CCl_4, 50 μL, 50 μmol) and Bz_2O_2 (cat.). The reaction mixture was irradiated with a desk lamp and heated to reflux for 20 min. The solvent was removed in vacuo, and THF (3 mL) and H_2O (1.5 mL) were added. The reaction mixture was stirred for 1 h and then diluted with H_2O (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3× 10 mL). The combined organic extracts were dried with $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by preparative TLC (SiO_2, hexanes/EtOAc 1:1) to afford the title compound (9 mg, 38 μmol, 84%) as a yellow oil. R_f (hexanes/EtOAc 1:1): 0.55. IR (film): $\tilde{\nu}_{max}$ = 3350, 2976, 2923, 2853, 1650, 1623, 1596, 1579, 1447, 1405, 1393, 1360, 1311, 1276, 1231, 1207, 1179, 1167, 1142, 1120, 1081, 1071, 1050, 1021, 1008, 984, 875, 857, 836, 722 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.94 (s, 1 H), 7.66–7.59 (m, 2 H), 7.27 (dd, *J* = 7.8, 2.0 Hz, 1 H), 6.07 (d, *J* = 3.8 Hz, 1 H), 4.41–4.31 (m, 1 H), 3.27 (d, *J* = 2.8 Hz, 1 H), 2.79 (dd, *J* = 19.6, 3.4 Hz, 1 H), 2.31–2.23 (m, 1 H), 1.41 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 188.5, 183.7, 161.8, 145.1, 140.4, 136.6, 131.9, 119.5, 114.8, 86.7, 62.7, 29.6, 21.1 ppm. HRMS (ESI): calcd. for $C_{14}H_{12}NaO_5$ [M + Na]$^+$ 283.0577; found 283.0584.

HRV 3C Protease Assay:[22] A typical protease assay was performed at room temperature for 1 h in a 50 μL reaction containing rHRV 3C protease (50 μg/mL), substrate (52 μM) and tris(2-carboxyethyl)-phosphine (TCEP, 5 μM) in phosphate buffered saline (PBS) pH 6.8 under continuous rotation. The reaction mixture was centrifuged, and a portion of the supernatant (10 μL) was added to PBS pH 6.8 (200 μL) in a 96-well opti plate. The fluorescence intensity (FI) was measured with a Perkin–Elmer EnSpire multimode plate reader with an excitation wavelength of 495 nm and an emission wavelength of 516 nm and 100 excitation flashes. A blank was measured that contained enzyme only in PBS at pH 6.8, and an assay with (–)-thysanone (**1**) was run as a control (detected IC_{50} for **1**: 53 ± 0 μM).

Cytotoxicity Assay: Materials: Complete RPMI 1640: Roswell Park Memorial Institute media 1640 pH 7.4 (Invitrogen, USA) supplemented with sodium hydrogen carbonate (1.5 mg/mL), penicillin (Gibco, Invitrogen, USA; 50 U/mL), streptomycin (Gibco, Invitrogen, USA; 50 μg/mL), L-glutamine (Gibco, Invitrogen, USA; 2 mM), sodium pyruvate (Gibco, Invitrogen, USA; 110 μg/mL) and 10% foetal calf serum (FCS). Foetal calf serum: heat-inactivated for 30 min at 56 °C and filtered (Invitrogen, USA). Cells: NCI-H441 (human lung adenocarcinoma epithelial cell line). Thymidine solution: [methyl-^{3}H] thymidine, 12.5 μCi/mL, GE Healthcare TRK 120. Assays: The NCI-H441 human lung adenocarcinoma epithelial cell line was used for cytotoxicity assays. The cell line was maintained in complete RPMI 1640 supplemented with sodium hydrogen carbonate (1.5 mg/mL), penicillin (50 U/mL), streptomycin (50 μg/mL), L-glutamine (2 mM), sodium pyruvate (110 μg/mL) and 10% heat-inactivated FCS (cRPMI; all from Gibco, Invitrogen, USA). Cells were harvested with 0.5% v/v trypsin/EDTA, washed in cRPMI and incubated at 37 °C, 5% CO_2 with serial twofold dilutions of inhibitor (80 μg/mL → 0.63 μg/mL) in cRPMI. For measurement of cytotoxicity, 40000 cells/well were added to flat-bottom 96-well plates for 36 h, and the cytotoxicity was quantified by adding 0.25 μCi/well methyl-[^{3}H] thymidine (GE Healthcare, TRK 120) to the cells for an additional 6 h. The plates were harvested onto glass filter mats by using a Tomtec Harvester 96 and dried, and Betaplate scint was added prior to the measurement of radioactivity with a Microbeta TriLux counter (all materials from Perkin–Elmer).

Supporting Information (see footnote on the first page of this article): Details of synthesis and spectroscopic data for intermediates, ^{1}H and ^{13}C NMR spectra.

Acknowledgments

The authors are grateful to the Maurice Wilkins Centre for Molecular Biodiscovery for financial support.

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Received: September 25, 2013
Published Online: November 7, 2013

