Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Reactivity Assessment of Chalcones by a Kinetic Thiol Assay

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General Experimental Information

All reactions were carried out under N_2 atmosphere in oven-heated glassware (110 °C) when dry conditions were required and monitored by TLC on silica gel plates 60 F_{254} by Merck. Spots were detected under UV light (λ = 254 and 366 nm) or visualized by staining with vanillin/H_2SO_4 (6.0 g vanillin in 100 mL 95% EtOH/conc. H_2SO_4 100:1). Column chromatography was performed on silica gel Geduran Si 60 (0.063-0.200 mm) by Merck. Preparative plates were prepared using silica gel 60 GF_{254} by Merck. Melting points are determined with a Büchi SMP 20 apparatus and are uncorrected. IR spectroscopy was carried on a Specac Golden Gate Diamond Single Reflection ATR System Excalibur Series FTS3000MX by BIO-RAD. NMR spectra were recorded on Brucker Avance 300, Brucker Avance 400, and Brucker Avance III 600 spectrometers. ^{1}H NMR spectra are referenced to $CDCl_3$ (7.26 ppm), ^{13}C NMR spectra to $CDCl_3$ (77.0 ppm). The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; dd, doublet of doublets; sept, septet; m, multiplet. Mass spectra were obtained on Agilent Tech. 6540 UHD, Finnigan MAT 95 or Thermo Quest Finnigan TSQ 7000 instruments.
All reagents were purchased from commercial sources and were used without further purification. Solvents were distilled before using and dried if water-free conditions were necessary. Ethylene glycol was used in spectrophotometric grade from Sigma-Aldrich.

Known isopropoxy compounds **9**,[1] **10**,[2] **11**,[3] **12**,[4] and **13**[5] were prepared by using the corresponding phenols, 2-bromopropane and K_2CO_3 in DMF.[6] Their NMR spectral data were in agreement with the literature.

1-(2-Isopropoxy-4-methoxyphenyl)ethanone (10)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (d, *J* = 8.7 Hz), 6.47 (d, *J* = 8.7 Hz), 6.42 (d, *J* = 2.3 Hz), 4.64 (sept, *J* = 6.0 Hz), 3.82 (s), 2.57 (s), 1.39 (d, *J* = 6.1 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 198.1 (C=O), 164.2, 159.3, 132.6 (CH), 122.0, 104.8 (CH), 99.8 (CH), 70.6 (*C*H$(CH_3)_2$), 55.4 (OCH_3), 32.1 (CO*C*H_3), 22.0 (CH_3) ppm.

General procedure for the synthesis of chalcones by Claisen-Schmidt condensations[7]

Benzaldehydes (**11**, **12** or **13**) (1.0 eq.) and the corresponding acetophenones (**9** or **10**) (1.0 eq.) were dissolved in MeOH (0.2 M) and stirred together with $Ba(OH)_2 \cdot 8\ H_2O$ (1.0 eq.) at 40-60 °C. The reaction mixture was subsequently concentrated *in vacuo*. Then H_2O was added, the resulting solution neutralized with 1 M HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by column chromatography or on preparative TLC plates to afford the corresponding chalcones **14-18**.

Known chalcones **2**,[8] **19**,[9] **20**,[10] **22**[11] were also prepared according to this general procedure without protection of the OH-group in **2**, **20** and **22**. Chalcone (**21**) was purchased from Merck (Darmstadt) and xanthohumol (**4**) from Carl Roth (Karlsruhe).

2',3,4,4'-Tetraisopropoxychalcone (14)

The reaction mixture was heated at 40 °C. **14** is a yellow oil, yield: 87%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, J = 8.7 Hz, 1H), 7.61 (d, J = 15.7 Hz, 1H, β-H), 7.50 (d, J = 15.7 Hz, 1H, α-H), 7.19 (d, = 2.0 Hz, 1H), 7.15 (dd, J = 8.3, 2.0 Hz, 1H), 6.89 (d, J = 8.3 Hz, 1H), 6.52 (dd, J = 8.7, 2.2 Hz, 1H), 6.45 (d, J = 2.2 Hz, 1H), 4.71 – 4.36 (m, 4H, 4 C*H*(CH_3)$_2$), 1.39 – 1.33 (m, 24H, 8 CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 190.5 (C=O), 162.3, 158.7, 151.2, 148.8, 141.3 (CH), 132.9 (CH), 128.9, 125.5, 123.3 (CH), 123.1 (CH), 117.4 (CH), 116.6 (CH), 106.8 (CH), 102.3 (CH), 72.5 (*C*H(CH_3)$_2$), 71.8 (*C*H(CH_3)$_2$), 71.1 (*C*H(CH_3)$_2$), 70.1 (*C*H(CH_3)$_2$), 22.2 (CH_3), 22.1 (CH_3), 22.0 (CH_3) ppm; MS (EI-MS): *m/z* (%) = 441 (100) $[M+H]^+$; HRMS (EI-MS): calcd. for $C_{27}H_{36}O_5$ $[M^{+\cdot}]$ 440.2563; found 440.2561.

2',3,4-Triisopropoxy-4'-methoxychalcone (15)

The reaction mixture was heated at 50 °C. **15** is a yellow solid, yield: 95%, mp 85 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, J = 8.7 Hz, 1H), 7.60 (d, J = 15.7 Hz, 1H, β-H), 7.48 (d, J = 15.7 Hz, 1H, α-H), 7.20 – 7.13 (m, 2H), 6.90 (d, J = 8.3 Hz, 1H), 6.55 (dd, J = 8.7, 2.3 Hz, 1H), 6.48 (d, J = 2.3 Hz, 1H), 4.62 (sept, J = 6.1 Hz, 1H, C*H*(CH_3)$_2$), 4.54 (sept, J = 6.1 Hz, 1H, C*H*(CH_3)$_2$), 4.46 (sept, J = 6.1 Hz, 1H, C*H*(CH_3)$_2$), 3.85 (s, 3H, OCH_3), 1.41 – 1.32 (m, 18H, 6 CH_3) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.7 (C=O), 163.9, 158.6, 151.3, 148.9, 141.6 (CH), 133.0 (CH), 129.0, 125.5 (CH), 123.6, 123.4 (CH), 117.5 (CH), 116.7 (CH), 105.5 (CH), 100.9 (CH), 72.6 (*C*H(CH_3)$_2$), 71.9 (*C*H(CH_3)$_2$), 71.3 (*C*H(CH_3)$_2$), 55.5 (OCH_3), 22.2 (2 CH_3), 22.2 (2 CH_3), 22.2 (2 CH_3) ppm; MS (EI-MS): *m/z* (%) = 412 (25) $[M^{+\cdot}]$, 151 (100); HRMS (EI-MS): calcd. for $C_{22}H_{32}O_5$ $[M^{+\cdot}]$ 412.2250; found 440.2246.

2',4,4'-Triisopropoxychalcone (16)

The reaction mixture was heated at 50 °C. **16** is a yellow oil, yield: 63%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, J = 8.7 Hz, 1H), 7.63 (d, J = 15.8 Hz, 1H, β-H), 7.53 – 7.49 (m, 3H), 6.92 – 6.85 (m, 2H), 6.53 (dd, J = 8.7, 2.2 Hz, 1H), 6.45 (d, J = 2.2 Hz, 1H), 4.65 – 4.57 (m, 3H, 3 C*H*(CH_3)$_2$), 1.39 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.35 (s, 3H, CH_3) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.8 (C=O), 162.3, 159.6, 158.8, 141.2 (CH), 132.9 (CH), 129.9 (CH), 128.0, 125.2 (CH), 123.3, 115.9 (CH), 106.9 (CH), 102.5 (CH), 71.3 (*C*H(CH_3)$_2$), 70.1(*C*H(CH_3)$_2$), 70.0 (*C*H(CH_3)$_2$), 22.2 (CH_3), 22.0 (CH_3) ppm; MS (ESI): *m/z* (%) = 383 (100) $[M+H]^+$; HRMS (ESI-MS): calcd. for $C_{24}H_{31}O_4$ $[M+H]^+$ 383.2217; found 383.2223.

2',3,4'-Triisopropoxy-4-methoxychalcone (17)

The reaction mixture was heated at 50 °C. **17** is a yellow oil, yield: 73%.

^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.76 (d, *J* = 8.7 Hz, 1H), 7.62 (d, *J* = 15.8 Hz, 1H, β-H), 7.50 (d, *J* = 15.7 Hz, 1H, α-H), 7.18 – 7.16 (m, 2H), 6.88 (d, *J* = 8.8 Hz, 1H), 6.53 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.45 (d, *J* = 2.2 Hz, 1H), 4.66 – 4.51 (m, 3H, 3 C*H*$(CH_3)_2$), 3.89 (s, 3H, OCH_3), 1.40 – 1.36 (m, 18H, 6 CH_3) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.4 (C=O), 162.3, 158.6, 152.2, 147.3, 141.3 (CH), 132.8 (CH), 128.4, 125.4 (CH), 123.1, 122.9(CH), 114.4 (CH), 111.7 (CH), 106.8 (CH), 102.2 (CH), 71.4 (*C*H$(CH_3)_2$), 71.0 (*C*H$(CH_3)_2$), 70.0 (*C*H$(CH_3)_2$), 55.9 (OCH_3), 22.1 (2 CH_3), 22.0 (2 CH_3), 21.9 (2 CH_3) ppm; MS (ESI): *m/z* (%) = 413 (100) $[M+H]^+$; HRMS (ESI-MS): calcd. for $C_{25}H_{33}O_5$ $[M+H]^+$ 413.2323; found 413.2334.

2',4'-Diisopropoxy-3,4-dimethoxychalcone (18)

The reaction mixture was heated at 50 °C. **18** is a yellow oil, yield: 74%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, *J* = 8.7 Hz, 1H), 7.63 (d, *J* = 15.7 Hz, 1H, β-H), 7.54 (d, *J* = 15.7 Hz, 1H, α-H), 7.16 – 7.14 (m, 2H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.54 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 4.67 – 4.57 (m, 2H, 2 C*H*$(CH_3)_2$), 3.92 (d, *J* = 1.6 Hz, 6H, 2 OCH_3), 1.40 – 1.38 (m, 12H, 4 CH_3) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.5 (C=O), 162.4, 158.8, 150.8, 149.2, 141.3 (CH), 133.0 (CH), 128.7, 125.7 (CH), 123.1, 122.8 (CH), 111.1 (CH), 109.6 (CH), 107.0 (CH), 102.4 (CH), 71.2 (*C*H$(CH_3)_2$), 70.2 (*C*H$(CH_3)_2$), 56.0 (OCH_3), 55.8 (OCH_3), 22.2 (2 CH_3), 22.0 (2 CH_3) ppm; MS (ESI): *m/z* (%) = 385 (100) $[M+H]^+$; HRMS (ESI-MS): calcd. for $C_{23}H_{29}O_5$ $[M+H]^+$ 385.2010; found 385.2017.

General procedure for the deprotection of isopropoxy ethers

To a 0.06 M solution of isopropoxychalcones (1.0 eq) in dry CH_2Cl_2 was added at –78 °C dropwise a 1 M solution of BCl_3 (3.0 eq per isopropoxy group) in hexane. Upon addition the yellow solution turned immediately deep-red. After 30 min at –78 °C the solution was allowed to warm to 0 °C and the solution was stirred for 3 h at this temperature. Then brine is added to the dark-violet solution and the resulting mixture extracted with EtOAc/EtOH 10:1 (6×). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification was performed either first with FC on silica gel (CH_2Cl_2/EtOH 60:1) and then recrystallization with EtOAc/CH_2Cl_2 or acetone/petroleum ether, or alternatively, a direct recrystallization to yield bright yellow solids.

All received compounds are known and the spectral data corresponded to the literature. Individual yields for the deprotection step were as follows: isoliquiritigenin (**3**)[12]: 68%; butein (**5**)[12]: 68%; calythropsin (**6**)[13]: 96%, 2',3,4'-trihydroxy-4-methoxychalcone (**7**)[14]: 83%, and 2',4'-dihydroxy-3,4-methoxychalcone (**8**)[15]: 66%.

Kinetic thiol assay

The thiol assay was performed in 96 well plates (Elisa Microplates, F-bottom, MICROLON 200, Greiner Bio-one, Germany) and PCR foils (Viewseal transparent, 80x140 mm, Greiner Bio-one, USA) were used to cover the plate during the measurements. As a solvent system 100 mM TRIS-HCl buffer pH 7.4 with 2 mM EDTA/ethylene glycol 20:80 was used that was filtered and degassed prior to use. All measurements were done in a Multiscan Spectrum Photometer (Thermo, Finnland) at 25 °C.

To perform the assay 10 mM stock solutions of α,β-unsaturated carbonyl compounds in DMSO were diluted in the buffer-ethylene glycol mixture to give a concentration of 80 µM. 100 mM thiol (cysteamine HCl) stock solutions were directly prepared in the buffer-ethylene glycol mixture and diluted accordingly to give 12-500fold dilutions compared to the α,β-unsaturated carbonyl compounds. Electrophile and thiol solutions were incubated separately in the instrument for 10 min at 25 °C just prior to the measurement. Then, equal amounts of both solutions are combined, mixed thoroughly, the wells covered with foil and the kinetic measurement is started immediately. Measurements are done in duplicates. 380 data points are collected each time with varying time intervals (Δt).

Table S1 Wavelengths, fold thiol and time intervals (Δt) used in the kinetic assay.

#	Wavelength [nm]	Fold thiol[a]	Δt [s]
2	375	60-108	11
3	390	60-108	17
4	390	60-108	50
5	390	60-108	15
6	390	100-300	11
7	390	60-108	18
8	390	60-108	15
14	365	60-108	35
15	365	60-108	32
16	360	60-108	35
17	360	36-84	40
18	365	60-108	40
19	360	60-108	11
20	390	60-108	11
21	320	12-60	11
22	295	100-500	Stopped flow
24	310	100-200	11
25	435	300-500	11

[a] **12-60**: 12, 18, 24, 36, 48, 60; **36-84**: 36, 48, 60, 72, 84; **60-108**: 60, 72, 84, 96, 108; **100-200**: 100, 125, 150, 175, 200; **100-300**: 100, 150, 200, 250, 300; **300-500**: 300, 350, 400, 450, 500; **100-500**:100, 200, 300, 400, 500.

The raw data was corrected vs blank to give individual curves for each measurement. These curves are fit to a 1st order exponential decay function with the software OriginPro 8. All compounds were measured with 3 – 7 independent experiments. Individual conditions are given in Table S1.

Stopped-flow technique was applied to study the kinetics of the reaction of compound **22** with cysteamine. The measurements were performed at 25 °C in the same solvent system utilized in the microtiter plate assay (100 mM TRIS-HCl buffer pH 7.4 with 2 mM EDTA/ethylene glycol 20:80 = B/EG), using the SX20 stopped-flow instrument (Applied Photophysics). The decrease in absorbance at 295 nm was recorded overtime. Multiple turnover kinetics of **22** with cysteamine were measured by mixing a 80 μM solution of chalcone **22** in B/EG and cysteamine solutions in B/EG (different concentrations, 100-500fold) in a 1:1 volume ratio. The final concentration of the chalcone is 40 μM. Three to five traces were recorded at each thiol concentration and averaged. These curves were fit to a 1st order exponential decay function using the software ProData SX. Three independent experiments were performed.

References

[1] Iwasaki, M.; Watanabe, T.; Ishikawa, T.; Chansakaow, S.; Higuchi, Y.; Tahara, S. *Heterocycles* **2004**, *63*, 1375-1392.

[2] Kawamura, Y.; Takatsuki, H.; Torii, F.; Horie, T. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 511-515.

[3] Hasse, K.; Willis, A. C.; Banwell, M. G. *Eur. J. Org. Chem.* **2011**, *2011*, 88-99.

[4] Dabak, K. *Turk. J. Chem.* **2002**, *26*, 955-964.

[5] Solladié, G.; Pasturel-Jacopé, Y.; Maignan, J. *Tetrahedron* **2003**, *59*, 3315-3321.

[6] Sala, T.; Sargent, M. V. *J. Chem. Soc., Perkin Trans. 1* **1979**, *10*, 2593-2598.

[7] Sogawa, S.; Nihro, Y.; Ueda, H.; Izumi, A.; Miki, T.; Matsumoto, H.; Satoh, T. *J. Med. Chem.* **1993**, *36*, 3904-3909.

[8] Aponte, J. C.; Verástegui, M.; Málaga, E.; Zimic, M.; Quiliano, M.; Vaisberg, A. J.; Gilman, R. H.; Hammond, G. B. *J. Med. Chem.* **2008**, *51*, 6230-6234.

[9] Bianco, A.; Cavarischia, C.; Guiso, M. *Eur. J. Org. Chem.* **2004**, *2004*, 2894-2898.

[10] Doan, T. N.; Tran, D. T. *Pharmacol. Pharm.* **2011**, *2*, 282-288.

[11] Karki, R.; Thapa, P.; Kang, M. J.; Jeong, T. C.; Nam, J. M.; Kim, H.-L.; Na, Y.; Cho, W.-J.; Kwon, Y.; Lee, E.-S. *Bioorg. Med. Chem.* **2010**, *18*, 3066-3077.

[12] Namikoshi, M.; Nakata, H.; Nuno, M.; Ozawa, T.; Saitoh, T. *Chem. Pharm. Bull.* **1987**, *35*, 3568-3575.

[13] Beutler, J. A.; Cardellina, J. H.; Gray, G. N.; Prather, T. R.; Shoemaker, R. H.; Boyd, M. R.; Lin, C. M.; Hamel, E.; Cragg, G. M. *J. Nat. Prod.* **1993**, *56*, 1718-1722.

[14] Mostahar, S.; Alam, S.; Islam, A. *Indian J. Chem., Sec B* **2006**, *45B*, 1478-1486.

[15] Matin, A.; Gavande, N.; Kim, M. S.; Yang, N. X.; Salam, N. K.; Hanrahan, J. R.; Roubin, R. H.; Hibbs, D. E. *J. Med. Chem.* **2009**, *52*, 6835-6850.

NMR spectra