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Unexpected cytotoxicity of a triisopropylsilylated syringaldehyde derived cinnamic acid amide

Björn A. Weber [1], Ratna Kancana Wolfram [1], Sophie Hoenke [1], Lucie Fischer [1], Ahmed Al-Harrasi [2] and René Csuk [1,*]

[1] Full Address: Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

[2] Full Address: University of Nizwa, Chair of Oman's Medicinal Plants and Marine Natural Products, P.O. Box 33, PC 616, Birkat Al-Mauz, Nizwa, Sultanate of Oman

Abstract: A small series of substituted cinnamic acid amides was prepared and screened for their cytotoxic activity. As a rather astonishing and unprecedented result, compound **5** holding a triisopropylsilyl (TIPS) protecting group at position 4 of the aromatic ring was highly cytotoxic (EC_{50} = 3.2 μM for HT29 human colon adenocarcinoma cells) while analogs with a methoxy or hydroxyl group at this position were of low cytotoxicity or not cytotoxic at all.

Keywords: cinnamic acid amides; cytotoxicity; SRB assay; HT29 cells.

1. Introduction

Cinnamic acid derivatives are widely used as flavors and fragrance [1, 2]. During the last years, compounds holding a cinnamoyl scaffold attracted attention because of their biological activities combined with low toxicity [3-8]. However, several of these compounds have also shown cytotoxic [9-14] as well as antimicrobial [15-20] and anti-oxidant properties [21-26]. Also, cinnamic acid derivatives have been used as valuable starting materials for the synthesis of peroxisome proliferator-activated receptors (PPAR). This class of compounds is of high scientific and commercial interest for the therapy of patients suffering from diabetes mellitus type II [27, 28].

2. Results and discussion

During our research on analogs of piplartine [13, 29], we became interested in the synthesis of several amides of 4-hydroxy-3,5-dimethoxy-cinnamic acid and their biological properties, especially their cytotoxic impact on human tumor cell lines.

Reaction (Scheme 1) of syringaldehyde (**1**, 4-hydroxy-3,5-dimethoxy-benzaldehyde) with triisopropylsilyl chloride (TIPS-Cl) gave 93% of TIPS-protected **2** [30]. Its reaction with malonic acid under Knoevenagel-Doebner conditions furnished (*E*) configurated cinnamic acid **3**. This compound is characterized in its ^{1}H NMR spectrum by the presence of two doublets at δ = 7.61 and 6.38 ppm with a coupling constant ^{3}J = 15.9 Hz being typical for (*E*) configurated olefins. The corresponding carbons were detected in the ^{13}C NMR spectrum at δ = 147.1 and 117.1 ppm, respectively. The reaction of piperidine with 2-chloro-ethanol gave **4** [31-34] whose reaction with **3** afforded **5** as a colorless solid in 92% isolated yield. Desilylation of **5** with tetra-*n*-butylammonium fluoride trihydrate in THF yielded **6**. Mono-bocylation of piperazine (**7**) gave mono-*N*-bocylated **8** [35] whose reaction with 2-bromo-ethanol yielded **9**. From the reaction of **9** with **3** compounds **10** was obtained. The reaction of (*E*) 3,4,5-trimethoxy-cinnamic acid (**11**) with **4** gave compound **12** in 90% isolated yield while its reaction with **9** afforded **13**. For comparison (*E*) cinnamic acid **14** was transformed into esters **15** and **16**, respectively.

The compounds were subjected to sulforhodamine B assays (SRB) to determine their cytotoxic activity employing several human tumor cell lines (FaDu pharynx carcinoma, A2780 ovarian carcinoma, HT29 colon adenocarcinoma, MCF-7 breast adenocarcinoma and SW1736 thyroid gland carcinoma); the results of this assays are summarized in Table 1.

**Corresponding author: René Csuk*
Email address: rene.csuk@chemie.uni-halle.de
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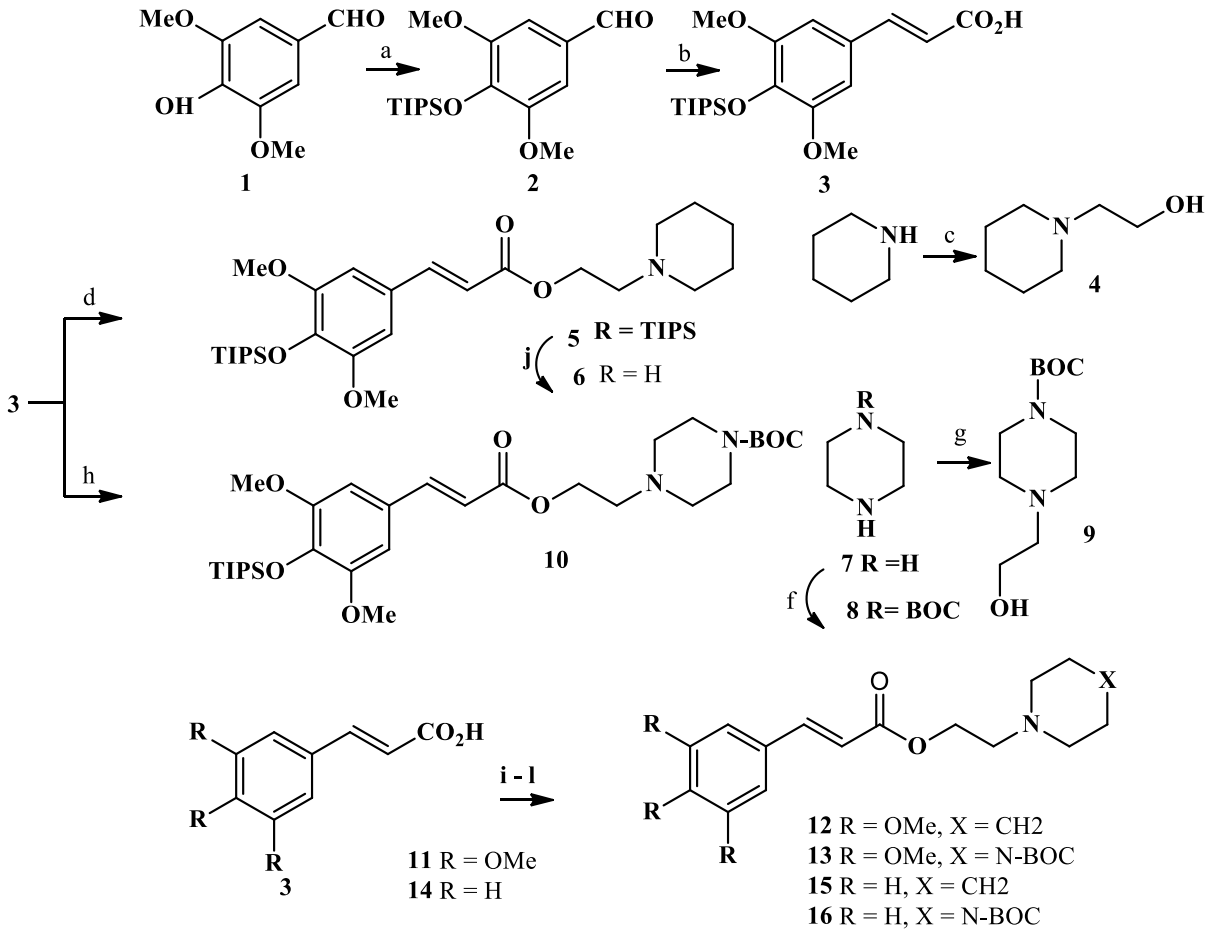

Scheme 1. Synthesis of **2-16**

Reactions and conditions: a) TIPS-Cl, triethylamine, cat. DMAP, DCM, 25°C, 1 h, 93%; b) malonic acid, pyridine, cat. piperidine, 120°C, 1h, 62%; c) Cl-CH_2-CH_2-OH, piperidine, toluene, 70°C, 5 h, 92%; d) EDC, **4**, cat. DMAP, DCM, 25°C, 1 day, 92%; e) *n*-Bu_4NF trihydrate, THF, 25°C, 6 h, 87%; f) according to lit. [35], 71%; g) Br-CH_2-CH_2-OH, tritehylamine, DCM, 60°C, 12 h, 70%; h) EDC, **9**, cat. DMAP, DCM, 25°C, 1 day, 73%; i) **12** from **11**: EDC, EDC, **4**, cat. DMAP, DCM, 25°C, 1 day, 86%; j) **13** from **11**: EDC, EDC, **9**, cat. DMAP, DCM, 25°C, 1 day, 61%; k) **15** from **14**: EDC, EDC, **4**, cat. DMAP, DCM, 25°C, 1 day, 90%; **16** from **14**: EDC, **9**, cat. DMAP, DCM, 25°C, 1 day, 62%.

Table 1. Cytotoxicity of compounds **1-3, 5, 6, 11-16**.

#	FaDu	A2780	HT29	MCF-7	SW1736
1-3	> 30	> 30	> 30	> 30	> 30
5	6.0 ± 0.2	5.8 ± 0.3	3.2 ± 0.7	4.4 ± 0.4	9.3 ± 0.9
6	27.1 ± 1.7	28.3 ± 2.3	29.9 ± 2.4	25.1 ± 3.2	29.1 ± 3.0
11-16	> 30	> 30	> 30	> 30	> 30
STS	0.14 ± 0.01	0.12 ± 0.01	0.15 ±0.02	0.10 ± 0.02	0.12 ± 0.02

EC_{50} values from SRB assays after 96 h of treatment are given in μM; the values are averaged from three independent experiments each performed in triplicate; confidence interval CI = 95%; cut-off 30 μM. Human tumor cell lines: FaDu pharynx carcinoma, A2780 ovarian carcinoma, HT29 colon adenocarcinoma, MCF-7 breast adenocarcinoma and SW1736 thyroid gland carcinoma. Staurosporine (**STS**) was used as a positive control.

As a result, **11-16** were not cytotoxic within the limits of the assay (cut-off 30 μM). The highest (and rather unexpected) cytotoxicity was established for **5** showing EC_{50} values in the low μM range for the human tumor cell lines. This compound was shown to be most cytotoxic for human colon adenocarcinoma cells HT29, and an EC_{50} = 3.2 μM was determined. Interestingly, most of the cytotoxicity is lost for desilylated **6**. The reason for this loss of cytotoxicity cannot be explained by the mere presence of a free phenolic hydroxy group in compound **6**. Compounds **12** (with a methoxy group on the phenyl substituent) and **15** (only hydrogen substituents on the ring) have no cytotoxicity either. Thus, the reason for the

enhanced cytotoxicity of **5** remains unclear and will be subject to further studies concerning also the mode-of-action for this class of compounds.

3. Conclusion

Several cinnamic acid derived amides were prepared and screened for their cytotoxic activity employing five human tumor cell lines. Interestingly, a piperidinyl-ethyl substituted 3,5-dimethoxy cinnamic acid amide **5** carrying a TIPS protecting group at position 4 was highly cytotoxic for all tumor cell lines in the low µM concentration range. While analogs holding a hydroxyl group (as exemplified in **6**) or a methoxy substituent (as exemplified in **12**) or no substituents (as exemplified in **15**) at this position were of significantly reduced cytotoxicity or were not cytotoxic at all.

4. Acknowledgments

Many thanks are due to Dr. D. Ströhl and his team for the NMR spectra as well as to Dr. R. Kluge for numerous ESI-MS spectra. The IR and UV/Vis spectra were recorded by Ms. V. Simon. Additional help in the lab has been provided by Ms. J. Wiese, Ms T.L.T. Luong, and especially by Ms T. Zeitz. The cell lines were kindly provided by Dr. Th. Müller (Dep. of Haematology/Oncology, Martin-Luther-Universität Halle-Wittenberg). The authors declare no conflict of interest.

5. Experimental

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometer Gemini 2000 (δ given in ppm, *J* in Hz), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554); elemental analyses were performed on a Vario EL (CHNS). The solvents were dried according to usual procedures (DCM by distillation over K_2CO_3, pyridine over KOH, toluene over Na). The purity of the compounds was determined by HPLC and found to be > 97%.

Sulforhodamine B assay (SRB)

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (Kiton-Red S, ABCR) micro culture colorimetric assay. Cells were seeded into 96-well plates on day 0 at appropriate cell densities to prevent confluence of the cells during the period of the experiment. After 24 hours, the cells were treated with six different concentrations (1, 3, 7, 12, 20 and 30 µM) minimum. The final concentration of DMSO/DMF never exceeded 0.5%, which was non-toxic to the cells. After a 96 h treatment, the supernatant medium from the 96-well plates was discarded, the cells were fixed with 10% trichloroacetic acid (TCA) and allowed to rest at 4°C. After 24 h fixation, the cells were washed in a strip washer and dyed with SRB solution (100 µL, 0.4% in 1 % acetic acid) for about 20 min. After dying, the plates were washed four times with 1% acetic acid to remove the excess of the dye and allowed to air-dry overnight. Tris base solution (200 µL, 10 mM) was added to each well and absorbance was measured at λ = 570 nm using a 96 well plate reader (Tecan Spectra, Crailsheim, Germany). The EC_{50} values were averaged from three independent experiments performed each in triplicate calculated from semi-logarithmic dose-response curves applying a non-linear 4P Hills-slope equation (GraphPad Prism5; variables top and bottom were set to 100 and 0, respectively).

3,5-Dimethoxy-4-[(triisopropyl)silyloxy]-benzaldehyde (2)

To a solution of 4-hydroxy-3,5-dimethoxy-benzaldehyde (**1**, 9.0 g, 49.4 mmol) and triethylamine (9.0 mL, 64.6 mmol) in dry DCM (50 mL) triisopropyl chloride (11.80 g, 0.06 mol) was slowly added at 25°C. A catytic amount of DMAP was added, and stirring at 25°C was continued for 1 hour. Usual aqueous workup (water, brine, $MgSO_4$) followed by column chromatography (silica gel, hexane/ethyl acetate, 5:1) gave **2** (15.6 g, 93%) as a slightly yellowish oil; R_F = 0.48 (silica gel, hexane/ethyl acetate, 5:1); b.p. 340°C (1 bar);
IR (film): ν = 3449*br*, 2945*m*, 2868*m*, 2360*w*, 1695*m*, 1585*m*, 1507*s*, 1465*m*, 1425*w*, 1389*w*, 1338*s*, 1268*w*, 1230w, 1130*s* cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 232 (4.09), 311 (4.09) nm;
^{1}H NMR (500 MHz, CDCl3): d = 9.81 (*s*, 1H, 1-H, CHO), 7.08 (*s*, 2H, arom.), 3.85 (*s*, 6H, 2 x OCH_3), 1.25 (*m*, 3H, 3 x CH-Si), 1.07 (*d*, *J* = 7.4 Hz, 18H, 6 x CH_3) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): *δ* = 191.5 (CH, C=O), 151.8 (C, 2 x arom. *C*-OCH_3), 141.3 (C, arom. *C*-OSi), 128.8 (C, arom.), 106.6 (2 x CH, arom.), 55.6 (2 x OCH_3), 17.8 (6 x CH_3), 13.9 (CH, 3 x CH-Si) ppm;
MS (ESI, MeOH): m/z (%) = 339.1 ($[M+H]^+$, 100);
analysis calcd for $C_{18}H_{30}O_4Si$ (338.52): C 63.87, H 8.93; found: C 63.55, H 9.15.

(*E*) 3-[(3,5-dimethoxy-4-[(triisopropylsilyl)oxy]-phenyl]-acrylic acid (3)

Reaction of malonic acid (3.2 g, 30.75 mmol) with **2** (8.0 g, 23.6 mmol) in dry pyridine (100 mL) in the presence of a cat. amount of piperidine at 120°C for 1 hour followed by usual work-up (10% aq. HCl, extraction with DCM) and column chromatography (silica gel, hexane/ethyl acetate/acetic acid, 75:25:1) gave **3** (5.58 g, 62%) as a colorless solid; m.p. 172-176°C; R_F = 0.36 (silica gel, hexane/ethyl acetate, 4:1);
IR (KBr): ν = 3447*br*, 2943*m*, 2862*m*, 1685*m*, 1630*m*, 1582*m*, 1508*s*, 1458*m*, 1287*s*, 1133*s*, 908*m*, 883*m*, 671*m* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 336 (4.16), 322 (4.19) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.61 (*d*, *J* = 15.9 Hz, 1H, 3-H), 6.88 (*s*, 2H, arom.), 6.38 (*d*, *J* = 15.9 Hz,

1H, 2-H), 3.83 (*s*, 6H, 2 x OCH_3), 1.26 (*m*, 3H, 3 x CH-Si), 1.09 (*d*, *J* = 7.4 Hz, 18H, 3 x CH_3) ppm;
^{13}C NMR (125 MHz, CD_3OD): *δ* = 170.8 (C-1, C=O), 153.1 (C, 2 x arom. *C*-OCH_3), 147.1 (CH, C-3), 138.5 (C, arom. *C*-OSi), 128.6 (C, arom.), 117.1 (CH, C-2), 106.5 (CH, arom.), 56.2 (2 x OCH_3), 18.7 (6 x CH_3), 15.0 (3 x CH-Si) ppm;
MS (ESI, MeOH): m/z (%) = 381.1 ([M+H]$^+$, 100);
analysis calcd for $C_{20}H_{32}O_5Si$ (380.55): C 63.12, H 8.48; found: 62.95, H 8.69.

2-(Piperidin-1-yl)-ethan-1-ol (4)
To a solution of 2-chloro-ethanol (8.0 g, 99.4 mmol) in dry toluene (20 mL), piperidine (17.0 g, 199.6 mmol) was slowly added. The mixture was stirred at 70 °C for an additional 5 hours. The precipitate was filtered off, the volatiles were removed under reduced pressure, and the remaining oil was distilled under reduced pressure to afford **4** (11.8 g, 92%) as a colorless oil; b.p. 72-76°C (11 mbar) [lit.: [31] 204-208°C (706 Torr)]; R_F = 0.35 (silica gel, DCM/MeOH/triethylamine, 98:2:1);
IR (film): ν = 3384*br*, 2935*s*, 2855*m*, 2803*m*, 21659*w*, 1443*m*, 1303*m*, 1155*m*, 1123*m*, 1049*m*, 757*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.02 (OH), 3.57 (*m*, 2H, CH_2OH), 2.46 (*m*, 2H, N-CH_2), 2.41 (*m*, 4H, N-CH_2), 1.16 (*m*, 4H, CH_2), 1.43 (*m*, 2H, CH_2) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): □ = 59.9 (N-CH_2), 57.7 (O-CH_2), 54.3 (N-CH_2), 26.0 (CH_2), 24.3 (CH_2) ppm;
ESI-MS (MeOH): m/z (%)) 130.1 ([M+H]$^+$, 100);
analysis calcd for $C_7H_{15}NO$ (129.20): C 65.07, H 11.70, N 10.84; found: C 64.85, H 11.95, N 10.63.

(*E*) 2-(Piperidin-1-yl)-ethyl 3-[3,5-dimethoxy-4-[(triisopropylsilyl)oxy]phenyl]-acrylate (5)
Following the procedure given for the preparation of **15**, from **3** (2.69 g, 7.07 mmol) and **4** (1.29 g, 9.98 mmol), **5** (3.2 g, 92%) was obtained as a colorless solid; m.p. 64-66°C; R_F = 0.34 (silica gel, hexane/ethyl acetate/triethylamine, 100:20:2.5);
IR (KBr): ν = 3448*br*, 2940*m*, 2864*m*, 1697*s*, 1578*m*, 1507*s*, 1466*m*, 1422*m*, 1264*s*, 1128*s*, 1085*m*, 900*m*, 459*m* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 248 (4.10), 360 (4.17) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (*d*, *J* = 15.9 Hz, 1H, =CH), 6.71 (*s*, 2 H, arom.), 6.32 (*d*, *J* = 15.9 Hz, 1H, =CH), 4.32 (*t*, *J* = 6.1 Hz, 2H, O-CH_2), 3.81 (*s*, 6H, OMe), 2.67 (*t*, *J* = 6.1 Hz, 2H, N-CH_2), 2.47 (*t*, *J* = 5.4 Hz, 4H, N-CH_2), 1.60 (*m*, 4H, CH_2), 1.44 (*m*, 2H, CH_2), 1.25 (*sept*, *J* = 15.6 Hz, 3H, CH), 1.06 (*d*, *J* = 15.6 Hz, 18H, Me) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.1 (CO_2R), 151.4 (arom.), 145.3 (=CH), 137.5, 126.6 (arom.), 115.7 (=CH), 105.1 (arom.), 61.9 (N-CH_2), 57.5 (O-CH_2), 55.5 (OMe), 54.8 (N-CH_2), 25.9 (CH_2), 24.2 (CH_2), 17.8 (Me), 13.3 (CH) ppm;
ESI-MS (MeOH): m/z (%) = 492.3 ([M+H|+, 100);
analysis calcd for $C_{27}H_{45}SiNO_5$ (491.74): C 66.95, H 9.22, N 2.85; found: C 66.72, H 9.49, N 2.69.

(*E*) 2-(Piperidin-1-yl) 3-(4-hydroxy-3,5-dimethoxyphenyl) acrylate (6)
To a solution of **5** (250 mg, 0.51 mmol) in THF (10 mL) *n*-Bu_4NF * $3H_2O$ (160 mg, 0.51 mmol) was added, and the mixture was stirred at 25°C for 6 hours. Usual aqueous workup (water, brine, $MgSO_4$) followed by column chromatography (silica gel, $CHCl_3$/MeOH, 97:3) gave **6** (148 mg, 87%) as a colorless oil; R_F = 0.17 (silica gel, $CHCl_3$/MeOH, 97:3);
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05 (*br*, 1H, OH), 7.51 (*d*, *J* = 16.0 Hz, 1H, =CH), 6.95 (*s*, 2H, arom.), 6.35 (*d*, *J* = 16.0 Hz, 1H, =CH), 4.24 (*t*, *J* = 5.9 Hz, 2H, O-CH_2), 3.92 (*s*, 6H, OMe), 2.78 (d, *J* = 5.9 Hz, 2H, N-CH_2), 2.50 (*m*, 4H, N-CH_2), 1.50 (*m*, 2H, CH_2) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.1 (CO_2R), 147.5 (arom.), 145.1 (=CH), 137.1 128.3 (arom.), 115.9 (=CH), 105.9 (arom.), 62.7 (O-CH_2), 56.1 (OMe), 54.7 (N-CH_2), 54.2 (N-CH_2), 23.5 (CH_2), 22.9 (CH2) ppm;
ESI-MS (MeOH): m/z (%) = 352-2 ([M+H]$^+$, 100), 374.2 ([M+Na]$^+$, 25);
analysis calcd for $C_{18}H_{25}NO_6$ (335.40): C 64.46, H 7.51; found: C 64.03, H 7.81.

Piperazine (7)
This compound was commercially obtained from Merck and used as received.

N-Boc-piperazine (8)
This compound was prepared from **7** in 71% yield according to literature; m.p. 46-48°C (lit.: [35] 46-47°C)

***tert*-Butyl 4-(2-hydroxyethyl)-piperazine-1-carboxylate (9)**
To an ice-cold solution of N-Boc-piperazine (**8**, 1.0 g, 5.37 mmol) in dry DCM (20 mL), triethylamine (1.09 g, 10.77 mmol) and 2-bromo-ethanol (95%, 1.34 g, 10.19 mmol) were slowly added. The mixture was stirred at 60°C overnight. Usual aqueous work-up (water, brine, $MgSO_4$) followed by column chromatography (silica gel, $CHCl_3$/MeOH, 97:3) gave **9** (865 mg, 70%) as a colorless oil; R_F = 0.32 (silica gel, $CHCl_3$/MeOH, 97:3);
^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.53 (*t*, *J* = 5.2 Hz, N-CH_2), 3.33 (*t*, *J* = 5.2 Hz, 4 H, N-CH_2), 3.15 (*s*, 1H, OH), 2.44 (*t*, *J* = 5.2 Hz, 2H, N-CH_2), 2.35 (*t*, *J* = 5.2 Hz, 4H, N-CH_2), 1.34 (*s*, 9H, Me) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.5 (C=O), 79.6 (C_q), 59.9, 57.8, 52.7 (CH_2), 28.3 (Me) ppm;
ESI-MS (MeOH): m/z (%) = 231.0 ([M+H]$^+$, 100), 131.1 ([M+H-Boc]$^+$, 11);
analysis calcd for $C_{11}H_{22}N_2O_3$ (230.31): C 57.31, H 9.63, N 12.16; found: C 57.11, H 9.97, N 11.97.

(*E*) tert-Butyl 4-[2-[(3-(3,5-dimethoxy-4-(triisopropylsilyl)oxy)phenyl]acryloyl)oxy)-ethyl)piperazine-1-carboxylate (10)

Following the procedure given for the preparation of **15**, from **3** (1.0 g, 2.95 mmol) and **9** (0.908 g, 3.94 mmol) **10** (1.28 g, 73%) was obtained as a colorless solid; m.p. 112-114°C; R_F = 0.66 (silica gel, hexane/ethyl acetate/triethylamine, 100:50:3);
IR (KBr): ν = 3448*br*, 2944*w*, 2865*m*, 1702*s*, 1633*m*, 1581*m*, 1511*s*, 1461*m*, 1424*m*, 1285*m*, 1246*m*, 1159*s*, 1128*s*, 1006*m*, 906*m* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 249 (4.29), 364 (4.38) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 (*d*, *J* = 15.8 Hz, =CH), 6.71 (*s*, 2H, arom.), 6.30 (*d*, *J* = 15.8 Hz, =CH), 4.33 (*m*, 2 H, O-CH_2), 3.81 (*s*, 6H, OMe), 3.44 (*m*, 4H, N-CH_2), 2.71 (*m*, 2H, N-CH_2), 2.48 (*m*, 4H, N-CH_2), 1.46 (*s*,9H, Me), 1.31-1.18 (*sept.*, *J* = 15.8 Hz, 3H, CH), 1.08 (*d*, *J* = 15.8 Hz, 18H, Me) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.0 (CO_2R), 154.7 (NCO), 151.5 (arom.), 145.5 (=CH), 137.6, 126.5 (arom.), 115.5 (=CH), 105.1 (arom.), 79.6 (C_q), 61.6 (O-CH_2), 56.8 (N-CH_2), 55.5 (OMe), 53.1 (N-CH_2), 28.4 (Me), 17.8 (CH), 13.3 (Me) ppm;
ESI-MS (MeOH): m/z (%): 593.2 ($[M+H]^+$, 100), 615.2 ($[M+Na]^+$, 4);
analysis calcd for $C_{31}H_{52}N_2O_7Si$ (592.85): C 62.81, H 8.84, N 4.73; found: C 62.66, H 9.03, N 4.47.

(*E*) 3,4,5-Trimethoxy-cinnamic acid (11)
This compound was commercially obtained from Merck and used as received.

(*E*) 2-(Piperidin-1-yl)ethyl 3-(3,4,5-trimethoxy-phenyl)-acrylate (12)
Following the procedure given for the preparation of **15**, from **11** (2.0 g, 8.39 mmol) and **4** (1.83 g, 14.16 mmol) **12** (2.52 g, 86%) was obtained as a colorless solid; m.p. 49-51°C; R_F = 0.37 (silica gel, hexane/ethyl acetate/triethylamine, 100:50:3);
IR (KBr): ν = 3442*br*, 2938*m*, 2784*w*, 1708*s*, 1581*m*, 1506*m*, 1464*m*, 1244*s*, 1129*s*, 1004*m* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 242 (4.31), 333 (4.29) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 (*d*, *J* = 15.9 Hz, =CH), 6.74 (*s*, 2H, arom.), 6.36 (*d*, *J* = 15.9 Hz, 1H, =CH), 4.32 (*t*, *J* = 6.1 Hz, 2H, O-CH_2), 3.88 (*s*, 6H, OMe), 3.87 (*s*, 3H, OMe), 2.67 (*t*, *J* = 6.1 Hz, 2H, N-CH_2), 2.47 (*t*, *J* = 5.4 Hz, 4H, N-CH_2), 1.60 (*m*, 4H, CH_2), 1.43 (*m*, 2H, CH_2) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.8 (CO2R), 153.4, 144.7, 140.1, 129.8 (arom.), 117.3 (=CH), 105.2 (arom.), 62.0 (N-CH_2), 60.9 (OMe), 57.4 (O-CH_2), 56.1 (OMe), 54.7 (N-CH_2), 25.8 (CH_2), 24.1 (CH_2) ppm; ESI-MS (MeOH): m/z (%) = 350.1 ([M+H]+, 100);
analysis calcd for $C_{19}H_{27}NO_5$ (349.43): C 65.31, H 7.79, N 4.01; found: C 65.04, H 7.50, N 3.83.

(*E*) *tert*-Butyl 4-[2-[(3-(3,4,5-trimethoxyphenyl)-acryloyl)oxy]ethyl]piperazine-1-carboxylate (13)
Following the procedure given for the preparation of **15**, from **11** (500 mg, 2.1 mmol) and **9** (725 mg, 3.15 mmol) **13** (577 mg, 61%) was obtained as a colorless liquid; b.p. 203°C (1 bar); R_F = 0.32 (silica gel, hexane/ethyl acetate/triethylamine, 100:50:3);
IR (film): ν = 3446*br*, 2939*w*, 1698*s*, 1636*m*, 1583*m*, 1505*m*, 1420*m*, 1276*s*, 1246*s*, 1171*s*, 1128*s*, 1004*m* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 242 (4.33), 340 (4.32) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56 (*d*, *J* = 16.0 Hz, 1H, =CH), 6.72 (*s*, 2H, arom.), 6.33 (*d*, *J* = 16.0 Hz, 1H, =CH), 4.30 (*m*, 2H, O-CH_2), 3.85 (*s*, 6H, OMe), 3.84 (*s*, 3H, OMe), 3.41 (*m*, 4H, N-CH_2), 2.68 (*m*, 2H, N-CH_2), 2.45 (*m*, 4H, N-CH_2), 1.42 (*s*, 9H, Me) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.7 (CO_2R), 154.6 (NCO), 153.4 (arom.), 145.0 (=CH), 140.1, 129.7 (arom.), 117.0 (=CH), 105.2 (arom.), 79.6 (C_q), 61.7 (O-CH_2), 60.9 (OMe), 56.8 (N-CH_2), 56.1 (OMe), 53.1 (N-CH_2), 28.4 (Me) ppm;
ESI-MS (MeOH): m/z (%) = 451.1 ($[M+H]^+$, 100), 473.1 ($[M+Na]^+$, 11);
analysis calcd for $C_{23}H_{34}N_2O_7$ (450.53): C 61.32, H 7.61, N 6.22; found: C 61.06, H 7.87, N 5.94.

(*E*) Cinnamic acid (14)
This compound was commercially obtained from Merck and used as received.

(*E*) 2-(Piperidin-1-yl)-ethyl cinnamate (15)
To a solution of cinnamic acid (**14**, 2.0 g, 13.5 mmol) in dry DCM (40 mL) **4** (2.6 g, 20.12 mmol) and EDC (3.14 g, 20.23 mmol) and cat. amounts of DMAP were added, and the mixture was stirred for 1 day at room temperature. Usual aqueous workup followed by column chromatography (silica gel, hexane/ethyl acetate/triethylamine, 100:50:3) gave **15** (3.16 g, 90%) as a slightly yellowish liquid; R_F = 0.49 (silica gel, hexane/ethyl acetate/triethylamine, 100:50:3); b.p. 308°C (1 bar);
IR (film): ν = 3408*br*, 3061*w*, 3028*w*, 2934*s*, 2883*m*, 2753*m*, 1713*s*, 1637*s*, 1450*m*, 1309*s*, 1169*s*, 1020*m*, 980*m*, 767*s*, 684*m* cm-1; UV/vis (MeOH): λ_{max} (log ε) = 299 (4.16) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (*d*, *J* = 16.0 Hz, 1H, =CH), 7.51 (*dd*, *J* = 6.7, 2.9 Hz, 2H, arom.), 7.41-7.34 (*m*, 3H, arom.), 6.46 (*d*, *J* = 16.0 Hz, 1H, =CH), 4.33 (*t*, *J* = 6.1 Hz, 2H, O-CH_2), 2.68 (*t*, *J* = 6.1 Hz, 2H, N-CH_2), 2.47 (*t*, *J* = 5.4 Hz, 4 H, N-CH_2), 1.59 (*m*, 4H, CH_2), 1.43 (*m*, 2H, CH_2) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.9 (CO_2R), 144.7 (=CH), 134.1, 130.2, 128.8, 128.0 (arom.), 118.1 (=CH), 62.1 (N-CH_2), 57.4 (OCH_2), 54.8 (N-CH_2), 52.9 (CH_2), 24.2 (CH_2) ppm;
ESI-MS (MeOH): m/z (%) = 260.2 ($[M+H]^+$, 100);
analysis calcd for $C_{16}H_{21}NO_2$ (259.35): C 74.10, H 8.16, N 5.40; found: C 73.84, H 8.32, N 5.11.

(*E*) *tert*-Butyl 4-[2-(cinnamoyloxy)ethyl]piper-azine-1-carboxylate (16)
Following the procedure given for the preparation of **15**, from **14** (200 mg, 1.35 mmol) and **9** (466 mg, 2.02 mmol), **16** (301 mg, 62%) was obtained as a colorless oil; b.p. 153°C (1 bar); R_F = 0.25 (hexane/ethyl acetate/triethylamine, 100:50:3);

IR (film): ν = 3448*br*, 2975*w*, 1696*s*, 1421*m*, 1247*m*, 1171*s*, 1004*w* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 298 (4.2) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (*d*, *J* = 16 Hz, 1H, =CH), 7.52, 7.39-7.36 (*m*, 5H, arom.), 6.44 (*d*, *J* = 16.0 Hz, 1H, =CH), 4.33 (*m*, 2H, O-CH_2), 3.43 (*m*, 4H, N-CH_2), 2.71 (*m*, 2H, N-CH_2), 2.48 (*m*, 4H, N-CH_2), 1.45 (*s*, 9H, Me) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.8 (CO_2R), 154.7 (NCO), 145.0 (=CH), 134.3, 130.3, 128.8, 128.0 (arom.), 117.9 (=CH), 79.6 (C_q), 61.8 (OCH_2), 56.7 (N-CH_2), 53.7 (N-CH_2), 28.4 (Me) ppm;
ESI-MS (MeOH): m/z (%) = 361.1 ([M+H]+, 100), 383.1 ([M+Na]$^+$, 22);
analysis calcd for $C_{20}H_{28}N_2O_4$ (360.45): C 66.64, H 7.83, N 7.77; found: C 66.41, H 7.96, N 7.45.

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