

Supplementary Information

Analogues of morphanthridine and the tear gas CR as extremely potent activators of the human TRPA1 receptor

Harrie J. M. Gijzen^{*1} D. Berthelot,¹ M. Zaja,^{1,a} B. Brône,^{2,b} I. Geuens,² M. Mercken.²

¹Medicinal Chemistry Department and ²Neuroscience- Johnson&Johnson Pharmaceutical Research&Development, Turnhoutseweg 30, 2340 Beerse, Belgium.

Contents

- I. Synthetic Procedures
- II. Electrophysiological experiments with **6**.
- III. Metabolic stability and reactive metabolite experiments.

^{*}To whom correspondence should be addressed. Phone: +32-14-606830. Fax: + 32-14-5344 E-mail: hgijzen@its.jnj.com.

^aCurrent affiliation: 4SC AG, Am Klopferspitz 19a, 82152 Planegg - Martinsried, Germany

^bCurrent affiliation: BIOMED Research Institute, Hasselt University, Agoralaan, Bld C, B-3590 Diepenbeek, Belgium.

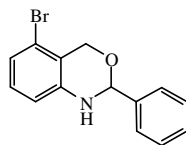
I. Synthetic Procedures

General. Melting points were determined with a DSC823e (Mettler-Toledo) and were measured with a temperature gradient of 30 °C/minute. The reported values are peak values. ¹H NMR spectra were recorded with Bruker Avance DPX 400 and 360 spectrometers, and chemical shifts (δ) are expressed in parts per million (ppm) with TMS as internal standard. Mass spectral data were obtained via GC-MS analysis, using an Agilent 6890 series GC combined with a 5973N Mass selected detector. High resolution mass spectra were acquired on a Acquity UPLC/QToF Premier system (Waters). The mass spectrometer was equipped with an ESI source and was operated in the positive ion mode. Leucine Enkephalyn was used as external lockspray. The source temperature was 120 °C, desolvation temperature 350 °C and the cone voltage was set at 30 V. Elemental analyses were performed with a Carlo-Erba EA1110 analyzer. Combustion Analytical results were within 0.4% of the theoretical values, except when noted otherwise. For all tested and final compounds where no analytical purity is mentioned, compounds were confirmed >95% pure via HPLC methods: The analytical LC measurement was performed using an Acquity UPLC (Waters) system comprising a binary pump, a sample organizer, a column heater (set at 55 °C), a diode-array detector (DAD) and a bridged ethylsiloxane/silica hybrid (BEH) C18 column (1.7 μ m, 2.1 x 50 mm; Waters Acquity) with a flow rate of 0.8 ml/min. Two mobile phases (mobile phase A: 0.1 % formic acid in H₂O/methanol 95/5; mobile phase B: methanol) were used to run a gradient condition from 95 % A and 5 % B to 5 % A and 95 % B in 1.3 minutes and hold for 0.2 minutes. An injection volume of 0.5 μ l was used. Cone voltage was 10 V for positive ionization mode and 20 V for negative ionization mode. Flow from the column was split to a MS spectrometer. The MS detector was configured with an electrospray ionization source. Mass spectra were acquired by scanning from 100 to 1000 in 0.18 seconds using a dwell time of 0.02 seconds. The capillary needle voltage was 3.5 kV and the source temperature was maintained at 140 °C. Nitrogen was used as the nebulizer gas. Sensitive reactions were performed under nitrogen. Commercial solvents were used without any pretreatment. Preparative HPLC purifications were carried out using a Shandon Hyperprep[®] column: C18 BDS (Base Deactivated Silica), 8 μ m, 250 g, I.D. 5 cm, and eluting with a gradient of three subsequent mobile phases: (phase A: a 0.25 % NH₄HCO₃ solution in water; phase B: CH₃OH; phase C: CH₃CN).

In general, final compounds obtained as solids were triturated with DIPE. Alternatively, some compounds were precipitated and isolated as HCl salt, by dissolving them in diethylether, followed by the addition of a 1N HCl solution in diethylether.

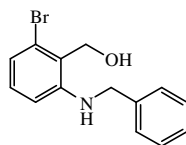
Synthesis of 11H-Dibenzo[b,e]azepine-1-carboxylic acid methyl ester (19).

Step 1. 5-Bromo-2-phenyl-1,4-dihydro-2H-benzo[d][1,3]oxazine (14c).



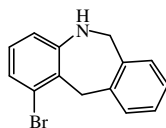
A mixture of (2-amino-6-bromo-phenyl)-methanol (7.1 g, 35 mmol) and benzaldehyde (3.9 g, 37 mmol) in 2-propanol (120 mL) was stirred overnight at room temperature. The solvent was evaporated, yielding intermediate **14c**, which was used as such in the next step. ^1H NMR (360 MHz, DMSO- d_6) δ ppm 4.73 (d, $J=15.0$ Hz, 1 H), 4.86 (d, $J=15.0$ Hz, 1 H), 5.57 (d, $J=2.6$ Hz, 1 H), 6.70 (dd, $J=8.1, 0.7$ Hz, 1 H), 6.82 (d, $J=2.6$ Hz, 1 H), 6.89 (dd, $J=8.1, 0.7$ Hz, 1 H), 6.98 (t, $J=8.1$ Hz, 1 H), 7.38 - 7.46 (m, 3 H), 7.50 - 7.56 (m, 2 H).

Step 2. (2-Benzylamino-6-bromo-phenyl)-methanol (15c).



NaBH_4 (2.6 g, 70 mmol) was added slowly to a mixture of **14c** (10.2 g, 35 mmol) in ethanol (200 mL) under nitrogen atmosphere. The reaction mixture was stirred and refluxed for 2 h. The mixture was cooled on an ice-water bath, quenched with a 20 % aqueous NH_4Cl solution (200 mL) followed by water (200 mL). The resulting precipitate was filtered off and dried, yielding 9.2 g (90 %) of intermediate **15c**, mp 97.3 °C. ^1H NMR (360 MHz, DMSO- d_6) δ ppm 4.37 (d, $J=5.9$ Hz, 2 H), 4.72 (d, $J=5.1$ Hz, 2 H), 5.29 (t, $J=5.1$ Hz, 1 H), 6.20 (t, $J=5.9$ Hz, 1 H), 6.47 (dd, $J=8.1, 1.1$ Hz, 1 H), 6.76 (dd, $J=8.1, 1.1$ Hz, 1 H), 6.92 (t, $J=8.1$ Hz, 1 H), 7.20 - 7.26 (m, 1 H), 7.30 - 7.37 (m, 4 H).

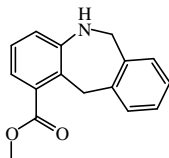
Step 3. 1-Bromo-6,11-dihydro-5H-dibenzo[b,e]azepine (16c).



A H_2SO_4 solution (80 mL) was cooled to -10 °C on an ice-salt bath. A solution of **15c** (9.1 g, 31 mmol) in CH_2Cl_2 (60 mL) was added dropwise. The ice-bath was removed, and stirring continued for 90

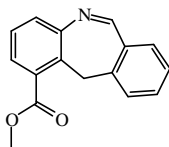
minutes at room temperature. The reaction mixture was cooled again, and a potassium hydroxide solution (10 M) was added carefully dropwise till the pH became basic. A precipitate was filtered off and washed with CH₂Cl₂ and the aqueous filtrate was extracted with CH₂Cl₂ (twice 300 mL). The organic layers were combined, dried over MgSO₄ and concentrated under reduced pressure, yielding 8.4 g (99 %) of intermediate **16c**. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.35 (s, 2 H), 4.44 (d, *J*=5.1 Hz, 2 H), 6.25 (t, *J*=5.1 Hz, 1 H), 6.38 (dd, *J*=7.3, 2.2 Hz, 1 H), 6.65 - 6.74 (m, 2 H), 7.18 - 7.30 (m, 4 H).

Step 4. 6,11-Dihydro-5H-dibenzo[b,e]azepine-1-carboxylic acid methyl ester (18c).



A mixture of **16c** (2.0 g, 7.3 mmol), Pd(OAc)₂ (34 mg, 0.15 mmol), potassium acetate (2.2 g, 22 mmol) and 1,3-bis(diphenylphosphino)propane (120 mg, 0.29 mmol) in methanol (20 mL) and THF (20 mL) was reacted in a pressure reactor at 125 °C for 16 h at 50 bar carbonmonoxide. The reaction mixture was cooled and filtered over dicalite, then concentrated *in vacuo*. The residue was partitioned between CH₂Cl₂ and water. The organic layer was dried over MgSO₄, filtered, then the filtrate was concentrated. The residue was purified by flash chromatography over silica gel (eluent: CH₂Cl₂). The desired fractions were collected and the solvent was evaporated, yielding 1.54 g (83 %) of intermediate **18c** as an oil. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.86 (s, 3 H) 4.22 (s, 2 H) 4.46 (d, *J*=4.76 Hz, 2 H) 6.15 (t, *J*=4.94 Hz, 1 H) 6.57 (d, *J*=8.05 Hz, 1 H) 6.69 (d, *J*=7.32 Hz, 1 H) 6.89 (t, *J*=7.87 Hz, 1 H) 7.14 - 7.30 (m, 4 H).

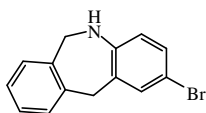
Step 5. 11H-Dibenzo[b,e]azepine-1-carboxylic acid methyl ester (19).



Compound **19** was prepared from **18c** according to the general MnO₂ oxidation procedure as described for **6**. Yield 35 % (final step); mp 86.9 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.86 (br. s., 2 H), 3.95 (s, 3 H), 7.33 - 7.43 (m, 2 H), 7.45 - 7.56 (m, 3 H), 7.63 - 7.68 (m, 2 H), 9.02 (s, 1 H); Anal. (C₁₆H₁₃NO₂) C, H, N.

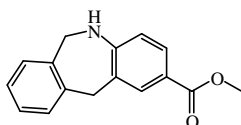
Synthesis of 11H-dibenzo[b,e]azepine-2-carboxylic acid methyl ester (20).

Step 1. 2-Bromo-6,11-dihydro-5H-dibenzo[b,e]azepine (16g).



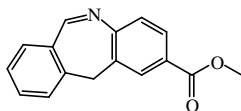
Bromine (1.3 mL, 25 mmol) was added dropwise to a mixture of 6,11-dihydro-5H-dibenzo[b,e]azepine (5 g, 25 mmol) in acetic acid (12 mL) and the reaction mixture was stirred for 4 h at room temperature. The mixture was evaporated and the residue was taken up in CH₂Cl₂ and washed with a 10 % aqueous NH₄OH solution. The organic layer was dried over Na₂SO₄, filtered off and evaporated. The residue was purified by flash chromatography over silica gel (eluent: hexane/EtOAc 9/1). The desired fractions were collected and the solvent was evaporated, yielding 4 g (56 %) of **16g**; ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.10 (s, 2 H) 4.40 (d, *J*=5.1 Hz, 2 H) 6.16 (t, *J*=5.1 Hz, 1 H) 6.31 (d, *J*=8.4 Hz, 1 H) 6.94 (dd, *J*=8.6, 2.4 Hz, 1 H) 7.08 (d, *J*=2.2 Hz, 1 H) 7.18 - 7.29 (m, 4 H).

Step 2. 6,11-Dihydro-5H-dibenzo[b,e]azepine-2-carboxylic acid methyl ester (18g).



A mixture of 2-bromo-6,11-dihydro-5H-dibenz[b,e]azepine (**16g**) (13.7 g, 50 mmol), potassium acetate (9.8 g, 0.1 mol), Pd(OAc)₂ (112 mg, 0.5 mmol) and 1,3-bis(diphenylphosphino)propane (412 mg, 1.0 mmol) in methanol (150 mL) was placed in a pressure reactor and pressurized with carbonmonoxide up to 50 bar. The reaction mixture was heated at 150 °C for 24 h, then cooled and the solvent was evaporated. The residue was partitioned between NH₄OH/H₂O and CH₂Cl₂. The organic layer was separated, dried, filtered and the solvent was evaporated. The residue was triturated with DIPE, filtered off and dried *in vacuo* yielding 10.5 g (83 %) of intermediate **18g**, mp 195.8 °C. ¹H NMR (360 MHz, CDCl₃) δ ppm 3.83 (s, 3 H), 4.20 (s, 2 H), 4.52 - 4.59 (m, 3 H), 6.31 (d, *J*=8.4 Hz, 1 H), 7.17 - 7.26 (m, 4 H), 7.58 (dd, *J*=8.4, 2.2 Hz, 1 H), 7.70 (d, *J*=2.2 Hz, 1 H); Anal. (C₁₆H₁₅NO₂) C, H, N.

Step 3. 11H-Dibenzo[b,e]azepine-2-carboxylic acid methyl ester (20).

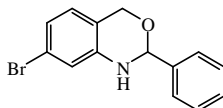


Compound **20** was prepared from **18g** according to the general MnO₂ oxidation procedure as described for **6**. Yield 33 % yield; mp 111.9 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.79 (s, 2 H), 3.85 (s, 3 H), 7.36 - 7.42 (m, 2 H), 7.46 - 7.56 (m, 2 H), 7.64 (dd, *J*=7.7, 1.1 Hz, 1 H), 7.83 (dd, *J*=8.1, 1.8 Hz, 1

H), 7.99 (d, $J=1.8$ Hz, 1 H), 8.99 (s, 1 H); Anal. ($C_{16}H_{13}NO_2$) C, H, N.

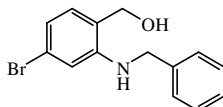
Synthesis of 11H-Dibenzo[b,e]azepine-3-carboxylic acid methyl ester (21).

Step 1. 3-Bromo-2-phenyl-1,4-dihydro-2H-benzo[d][1,3]oxazine (14d).



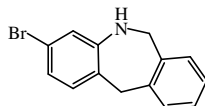
A mixture of (2-amino-4-bromo-phenyl)-methanol (7.5 g, 37 mmol) and benzaldehyde (4.33 g, 40.8 mmol) in toluene (120 mL) was refluxed under Dean-Stark conditions for 3h. The solvent was evaporated, yielding 10.7 g of crude intermediate **14d**, which was used as such in the next step.

Step 2. (2-Benzylamino-4-bromo-phenyl)-methanol (15d).



$NaBH_4$ (2.8 g, 74 mmol) was added slowly to a mixture of **14d** (10.7 g, 37 mmol) in ethanol (120 mL) under nitrogen atmosphere. The reaction mixture was stirred and refluxed for 1 h. The mixture was cooled on an ice-water bath, quenched with a 20 % aqueous NH_4Cl solution (150 mL) followed by water (100 mL). The mixture was extracted with CH_2Cl_2 (2 x 250 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure, yielding 10.8 g (~100 %) of crude intermediate **15d** which was used as such in the next step.

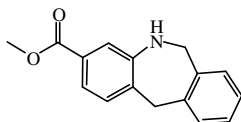
Step 3. 3-Bromo-6,11-dihydro-5H-dibenzo[b,e]azepine (16d).



Sulfuric acid (100 mL) was cooled to -10 °C on an ice-salt bath. Then **15d** (10.8 g, 37 mmol) in CH_2Cl_2 (30 mL) was added portionswise. The ice-bath was removed, and stirring continued for 1.5 hour at room temperature. The reaction mixture was cooled again, and an aqueous potassium hydroxide solution (10M) was added carefully dropwise till the pH became basic. To the salt mixture CH_2Cl_2 (500 mL) was added and the mixture was stirred for 1 hour at room temperature. The salts were filtered off and washed with CH_2Cl_2 . The combined organic layer was dried on $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (eluent:

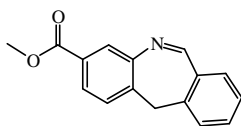
CH₂Cl₂/heptane/EtOAc 10/86/4 to 7/63/30). The pure fractions were collected and the solvent was evaporated, yielding 6.4 g (63 %) of intermediate **16d** as a white solid. mp 143.2 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.07 (s, 2 H), 4.41 (d, *J*=5.1 Hz, 2 H), 6.26 (t, *J*=5.1 Hz, 1 H), 6.49 (dd, *J*=8.1, 2.2 Hz, 1 H), 6.53 (d, *J*=2.2 Hz, 1 H), 6.84 (d, *J*=8.1 Hz, 1 H), 7.19 - 7.25 (m, 4 H); Anal. (C₁₄H₁₂BrN) C, H, N.

Step 4. 6,11-Dihydro-5H-dibenzo[b,e]azepine-3-carboxylic acid methyl ester (18d).



A 75-mL stainless steel autoclave was charged under nitrogen atmosphere with **16d** (2.0 g, 7.3 mmol), Pd(OAc)₂ (10 mg, 0.04 mmol), 1,3-bis(diphenylphosphino)propane (40 mg, 0.1 mmol), potassium acetate (1.5 g, 15.3 mmol) in methanol (20 mL) and THF (20 mL). The autoclave was closed and pressurized to 50 bar carbonmonoxide and the reaction was carried out for 16 h at a temperature of 125 °C. The reaction mixture was filtered and concentrated *in vacuo*. The residue was partitioned between CH₂Cl₂ and water. The organic layer was dried on MgSO₄ and concentrated. The residue was purified by flash chromatography over silica gel (eluent heptane/ CH₂Cl₂ 50/50 to 0/100). The pure fractions were collected and the solvent was evaporated, yielding 1.1 g (60 %) of **18d**, mp 153.4 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.75 (s, 3 H), 4.18 (s, 2 H), 4.44 (d, *J*=5.1 Hz, 2 H), 6.31 (t, *J*=5.1 Hz, 1 H), 6.96 (dd, *J*=7.7, 1.5 Hz, 1 H), 7.01 - 7.05 (m, 2 H), 7.20 - 7.26 (m, 4 H); Anal. (C₁₆H₁₅NO₂) C, H, N.

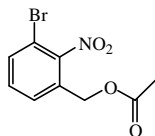
Step 5. 11H-Dibenzo[b,e]azepine-3-carboxylic acid methyl ester (21).



Compound **21** was prepared from **18d** according to the general MnO₂ oxidation procedure as described for **6**. Yield 2 %; mp 106 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.80 (s, 2 H), 3.85 (s, 3 H), 7.41 (td, *J*=7.7, 1.5 Hz, 1 H), 7.46 (dd, *J*=7.7, 1.5 Hz, 1 H), 7.51 - 7.57 (m, 2 H), 7.66 (dd, *J*=7.7, 1.5 Hz, 1 H), 7.81 - 7.85 (m, 2 H), 8.98 (s, 1 H); Anal. (C₁₆H₁₃NO₂) C, H, N.

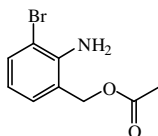
Synthesis of 11H-dibenzo[b,e]azepine-4-carboxylic acid methyl ester (22).

Step 1. Acetic acid 3-bromo-2-nitro-benzyl ester.



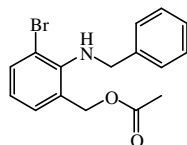
1-Bromo-3-methyl-2-nitro-benzene (9.9 g, 46 mmol), dibenzoyl peroxide (250 mg, 1.0 mmol), tetrachloromethane (100 mL) and NBS (8.2 g, 46 mmol) were refluxed overnight. Extra dibenzoyl peroxide (250 mg, 1.0 mmol) was added and refluxing continued overnight. The reaction mixture was concentrated *in vacuo* and the residue partitioned between EtOAc and water. The organic layer was washed with water and brine, dried on MgSO_4 and concentrated. The residue was dissolved in DMF (200 mL) and KOAc (31.6 g, 0.3 mol) was added. The reaction mixture was stirred at 70 °C for 2 h., cooled, poured onto icewater, and subsequently extracted with EtOAc (2 x 300 mL). The combined organic layer was washed with water and brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified via SiO_2 flash chromatography (eluent heptane/EtOAc 90/10 to 60/40). The pure fractions were collected and the solvent was evaporated, yielding 5.9 g (47 %) of Acetic acid 3-bromo-2-nitro-benzyl ester.

Step 2. Acetic acid 2-amino-3-bromo-benzyl ester.



Pt/C 5 % (1 g) and V_2O_5 (50 mg) was suspended in THF under nitrogen flow. A 4 % thiophene solution in DIPE (0.5 mL) was added followed by acetic acid 3-bromo-2-nitro-benzyl ester (5.8 g, 21 mmol). The reaction mixture was stirred under hydrogen atmosphere until 3 equivalents of hydrogen were absorbed. The catalyst was removed by filtration over dicalite. The mixture was concentrated, yielding 5.2 g of acetic acid 2-amino-3-bromo-benzyl ester which was used as such in the next step. ^1H NMR (360 MHz, $\text{DMSO}-d_6$) δ ppm 2.05 (s, 3 H), 5.00 (s, 2 H), 5.22 (s, 2 H), 6.51 (t, $J=7.7$ Hz, 1 H), 7.13 (dd, $J=7.7$, 1.5 Hz, 1 H), 7.38 (dd, $J=7.7$, 1.5 Hz, 1 H).

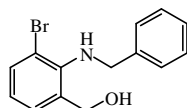
Step 3. Acetic acid 2-benzylamino-3-bromo-benzyl ester.



Acetic acid 2-amino-3-bromo-benzyl ester (1.7 g, 6.8 mmol), benzyl bromide (1.3 g, 7.4 mmol), sodium

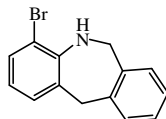
iodide (1.1 g, 7.4 mmol) in DMF (50 mL) were stirred at 80 °C for 6 h. The reaction mixture was cooled and water (500 mL) was added. The mixture was extracted twice with 250 mL EtOAc. The combined organic layer was washed with water and brine, dried on MgSO₄, and concentrated *in vacuo*. The residue was purified via reversed phase HPLC, yielding 1.9 g (84%) of acetic acid 2-benzylamino-3-bromo-benzyl ester. ¹H NMR (360 MHz, CDCl₃) δ ppm 0.89 (t, *J*=6.2 Hz, 1 H), 1.76 (s, 3 H), 3.80 (d, *J*=13.1 Hz, 1 H), 3.98 (d, *J*=13.9 Hz, 1 H), 4.06 (dd, *J*=13.1, 6.2 Hz, 1 H), 5.69 (d, *J*=13.9 Hz, 1 H), 7.18 - 7.30 (m, 6 H), 7.44 (d, *J*=7.7 Hz, 1 H), 7.66 (d, *J*=7.7 Hz, 1 H).

Step 4. (2-Benzylamino-3-bromo-phenyl)-methanol (**15b**).



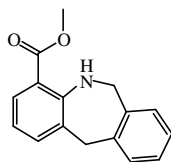
Acetic acid 2-benzylamino-3-bromo-benzyl ester (1.9 g, 5.7 mmol), methanol (20 mL) and sodium methylate in methanol (30 %, 10 mL) were heated in a microwave at 160 °C for 20 minutes. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between CH₂Cl₂ and water. The organic layer was dried on MgSO₄, concentrated under reduced pressure, yielding 1.7 g of crude **15b** (~quantitative) which was used as such in the next step. ¹H NMR (360 MHz, CDCl₃) δ ppm 3.89 (br. s., 1 H), 4.00 (br. s., 1 H), 4.17 (s, 2 H), 4.76 (br. s., 2 H), 6.92 (d, *J*=8.1 Hz, 1 H), 7.20 (dd, *J*=8.1, 1.5 Hz, 1 H), 7.29 - 7.42 (m, 5 H), 7.50 (dd, *J*=8.1, 1.5 Hz, 1 H).

Step 5. 4-Bromo-6,11-dihydro-5H-dibenzo[be]azepine (**16f**).



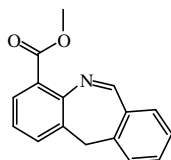
Sulfuric acid (25 mL) was cooled to -10 °C on an ice-salt bath. Then (2-benzylamino-3-bromo-phenyl)-methanol (**15b**) (1.7 g, 5.8 mmol) in CH₂Cl₂ (20 mL) was added dropwise. The ice-bath was removed, and stirring continued for 1.5 h at room temperature. The reaction mixture was cooled again, and KOH (10M) solution was added carefully dropwise till the pH became basic. The formed salt was filtered off and washed with CH₂Cl₂. The aqueous filtrate was extracted with CH₂Cl₂ (2 x 300 mL). The combined organic layer was dried on MgSO₄ and concentrated under reduced pressure, yielding 1.36 g (86 %) of **16f**, mp 91.8 °C. ¹H NMR (360 MHz, CDCl₃) δ ppm 4.19 (s, 2 H), 4.61 (d, *J*=4.8 Hz, 2 H), 4.76 (br. s., 1 H), 6.42 (t, *J*=7.7 Hz, 1 H), 6.94 (dd, *J*=7.7, 0.7 Hz, 1 H), 7.18 - 7.27 (m, 5 H).

Step 6. 6,11-Dihydro-5H-dibenzo[b,e]azepine-4-carboxylic acid methyl ester (**18f**).



A 75-mL stainless steel autoclave was charged under nitrogen atmosphere with **16f** (850 mg, 3.1 mmol), Pd(OAc)₂ (14 mg, 0.06 mmol), 1,3-bis(diphenylphosphino)propane and potassium acetate (618 mg, 6.3 mmol) in methanol (20 mL) and THF (20 mL). The autoclave was closed and pressurized to 50 bar carbonmonoxide and the reaction was carried out for 16 h at a temperature of 125 °C. The reaction mixture was filtered over dicalite, concentrated *in vacuo*, and the residue was partitioned between saturated aqueous NaHCO₃ and CH₂Cl₂. The organic layer was dried on MgSO₄ and concentrated, yielding 545 mg (69 %) of **18f**. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.74 (s, 3 H), 4.21 (s, 2 H), 4.69 (d, *J*=5.1 Hz, 2 H), 6.40 (t, *J*=7.7 Hz, 1 H), 7.17 - 7.29 (m, 5 H), 7.60 (dd, *J*=8.1, 1.5 Hz, 1 H), 8.42 (t, *J*=5.1 Hz, 1 H).

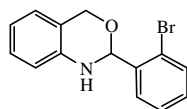
Step 7. 11H-dibenzo[b,e]azepine-4-carboxylic acid methyl ester (**22**).



Compound **22** was prepared from **18f** according to the general MnO₂ oxidation procedure as described for **6**. Yield 30 %; ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.71 (s, 2 H), 3.80 (s, 3 H), 7.28 (t, *J*=7.7 Hz, 1 H), 7.37 - 7.44 (m, 3 H), 7.48 - 7.54 (m, 2 H), 7.65 (dd, *J*=7.7, 1.1 Hz, 1 H), 8.96 (s, 1 H); Anal. (C₁₆H₁₃NO₂·HCl) C, H, N.

Synthesis of 11H-Dibenzo[b,e]azepine-7-carboxylic acid methyl ester (**23**).

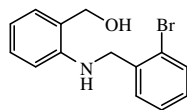
Step 1. 2-(2-Bromo-phenyl)-1,4-dihydro-2H-benzo[d][1,3]oxazine (**14e**).



2-Amino-benzenemethanol (5.0 g, 41 mmol) and 2-bromo-benzaldehyde (7.6 g, 41 mmol) in isopropanol (60 mL) were stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* yielding intermediate **14e** which was used as such in the next step. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.77 (d, *J*=15.0 Hz, 1 H), 5.02 (d, *J*=15.0 Hz, 1 H), 5.79 (d, *J*=2.6 Hz, 1 H), 6.40 (d,

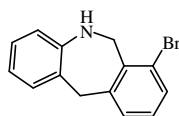
$J=2.6$ Hz, 1 H), 6.65 - 6.70 (m, 2 H), 6.93 (dd, $J=8.1$, 1.1 Hz, 1 H), 7.02 (td, $J=7.7$, 1.1 Hz, 1 H), 7.36 (td, $J=7.7$, 1.8 Hz, 1 H), 7.46 (td, $J=7.7$, 1.1 Hz, 1 H), 7.65 - 7.69 (m, 2 H).

Step 2. [2-(2-Bromo-benzylamino)-phenyl]-methanol (15e).



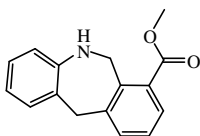
Under a nitrogen atmosphere, NaBH_4 (3.0 g, 80 mmol) was added portionwise to **14e** (11.6 g, 40 mmol) in ethanol (120 mL). The mixture was stirred at reflux for 1 hour, cooled on an ice-bath and quenched with 20 % aqueous NH_4Cl . The aqueous layer was extracted with CH_2Cl_2 (2 x 250 mL). The organic layer was dried on MgSO_4 , and concentrated *in vacuo*, yielding 11.7 g (~100 %) of crude intermediate **15e**, which was used as such in the next step.

Step 3. 7-Bromo-6,11-dihydro-5H-dibenzo[b,e]azepine (16e).



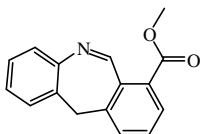
Sulfuric acid (100 mL) was cooled to -10 °C on an ice-salt bath. Then **15e** (11.7 g, 40 mmol) was added portionwise. The ice-bath was removed, and stirring continued for 1.5 hour at room temperature. The reaction mixture was cooled again, and an aqueous KOH solution (10M) was added carefully dropwise till the pH became basic. The solids were filtered off and washed with diethylether. The aqueous filtrate was extracted twice with 300 mL diethylether. The combined organic layer was dried on MgSO_4 and concentrated under reduced pressure to yield a first residue. The solids obtained before extraction were stirred with CH_2Cl_2 , and filtered again. The organic layer was dried, concentrated under reduced pressure, and combined with the first residue. The combined residues were purified via flash chromatography over silica gel (eluent: heptane/EtOAc 96/4 to 70/30). The pure fractions were collected and the solvent was evaporated, yielding 5.4 g (49 %) of intermediate **16e**. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 4.18 (s, 2 H), 4.65 (d, $J=5.5$ Hz, 2 H), 6.04 (t, $J=5.5$ Hz, 1 H), 6.37 - 6.43 (m, 2 H), 6.84 (td, $J=7.7$, 1.5 Hz, 1 H), 6.89 (dd, $J=7.7$, 1.5 Hz, 1 H), 7.13 (t, $J=7.7$ Hz, 1 H), 7.26 (dd, $J=7.7$, 1.5 Hz, 1 H), 7.48 (dd, $J=8.1$, 1.1 Hz, 1 H).

Step 4. 6,11-Dihydro-5H-dibenzo[b,e]azepine-7-carboxylic acid methyl ester (18e).



A 75-mL stainless steel autoclave was charged under nitrogen atmosphere with **16e** (1.9 g, 7.1 mmol), Pd(OAc)₂ (10 mg, 0.04 mmol), 1,3-bis(diphenylphosphino)propane (40 mg, 0.1 mmol) and potassium acetate (1.5 g, 15.3 mmol) in methanol (20 mL) and THF (20 mL). The autoclave was closed and pressurized to 50 bar carbonmonoxide and the reaction was carried out for 16 h at a temperature of 125 °C. The reaction mixture was filtered and concentrated *in vacuo*. The residue was partitioned between CH₂Cl₂ and water. The organic layer was dried on MgSO₄ and concentrated. The residue was purified over flash chromatography over silica gel (eluent CH₂Cl₂). The pure fractions were collected and the solvent was evaporated, yielding 1.5 g (83 %) of intermediate **18e**, mp 99.2 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.86 (s, 3 H), 4.21 (s, 2 H), 4.82 (d, *J*=5.5 Hz, 2 H), 5.91 (t, *J*=5.5 Hz, 1 H), 6.34 - 6.40 (m, 2 H), 6.81 (td, *J*=7.7, 1.5 Hz, 1 H), 6.90 (dd, *J*=7.7, 1.5 Hz, 1 H), 7.31 (t, *J*=7.7 Hz, 1 H), 7.47 (dd, *J*=7.7, 1.5 Hz, 1 H), 7.64 (dd, *J*=7.7, 1.5 Hz, 1 H); Anal. (C₁₆H₁₅NO₂) C, H, N.

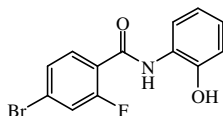
Step 5. 11H-Dibenzo[b,e]azepine-7-carboxylic acid methyl ester (**23**).



Compound **23** was prepared from **18e** according to the general MnO₂ oxidation procedure as described for **6**. Yield 55 % (final step); mp 123.9 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.67 (s, 2 H), 3.88 (s, 3 H), 7.13 - 7.21 (m, 1 H), 7.22 - 7.29 (m, 2 H), 7.33 (dd, *J*=7.3, 0.7 Hz, 1 H), 7.58 (t, *J*=7.7 Hz, 1 H), 7.68 (dd, *J*=7.7, 1.1 Hz, 1 H), 7.83 (dd, *J*=7.7, 1.1 Hz, 1 H), 9.17 (s, 1 H); Anal. (C₁₆H₁₃NO₂) C, H, N.

Synthesis of Dibenzo[b,f][1,4]oxazepine-3-carboxylic acid methyl ester (**31**).

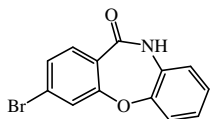
Step 1. 4-Bromo-2-fluoro-N-(2-hydroxy-phenyl)-benzamide (**27b**).



A mixture of 4-bromo-2-fluoro-benzoyl chloride (5.7 g, 24 mmol) in THF (25 mL) was added dropwise to a mixture of 2-amino-phenol (2.6 g, 24 mmol) and triethylamine (4.9 g, 48 mmol) in THF (75 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight, then poured

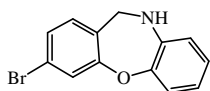
into water (400 mL) and acidified to pH 4-5 with a 1N aqueous HCl solution. The precipitate was filtered off and washed with 1 N HCl and water, dried *in vacuo*, yielding 6.6 g (89 %) of intermediate **27b**, which was used as such in the next step.

Step 2. 3-Bromo-10H-dibenzo[b,f][1,4]oxazepin-11-one (28b).



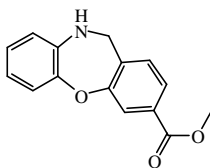
A mixture of **27b** (6.5 g, 21 mmol) and sodium hydroxide (0.84 g, 21 mmol) in DMF (100 mL) was refluxed for 5 h. The reaction mixture was poured onto 800 mL ice water and the resulting precipitate was filtered off and washed with a 1N aqueous NaOH solution and water, then dried *in vacuo*, yielding 5.6 g (92 %) of crude intermediate **28b** which was used as such in the next step.

Step 3. 3-Bromo-10,11-dihydro-dibenzo[b,f][1,4]oxazepine (29b).



To a suspension of **28b** (3.1 g, 11 mmol) in THF (100 mL) was added borane-dimethyl sulfide complex (1:1) (2M in THF; 14.7 mL, 29 mmol) at room temperature. The reaction mixture was stirred further at room temperature for two days. The reaction mixture was cooled on ice and 100 mL of a 1N aqueous HCl solution was added. The mixture was partly concentrated *in vacuo*, then alkalized with solid NaHCO₃ (pH ~7). The aqueous layer was extracted twice with 200 mL CH₂Cl₂. The organic layer dried over MgSO₄ and concentrated. The residue was purified by flash chromatography over silica gel (eluent heptane/CH₂Cl₂ 70/30 to 20/80). The product fractions were collected and the solvent was evaporated, yielding 1.7 g (56 %) of intermediate **29b**, mp 132.1 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.36 (d, *J*=4.0 Hz, 2 H), 6.07 (t, *J*=4.0 Hz, 1 H), 6.49 - 6.55 (m, 1 H), 6.59 (dd, *J*=8.1, 1.5 Hz, 1 H), 6.78 - 6.84 (m, 1 H), 7.00 (dd, *J*=8.1, 1.5 Hz, 1 H), 7.29 (d, *J*=8.1 Hz, 1 H), 7.33 (dd, *J*=8.1, 1.8 Hz, 1 H), 7.41 (d, *J*=1.8 Hz, 1 H); Anal. (C₁₃H₁₀BrNO) C, H, N.

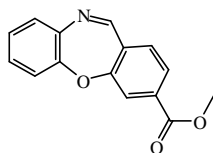
Step 4. 10,11-Dihydro-dibenzo[b,f][1,4]oxazepine-3-carboxylic acid methyl ester (30b).



A 75-mL stainless steel autoclave was charged under nitrogen atmosphere with **29b** (1.6 g, 5.9 mmol),

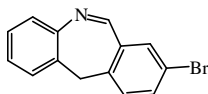
(Pd(OAc)₂ (10 mg, 0.04 mmol), 1,3-bis(diphenylphosphino)propane (40 mg, 0.1 mmol), potassium acetate (1.5 g, 15 mmol), methanol (20 mL) and THF (20 mL). The autoclave was closed and pressurized to 50 bar with carbonmonoxide and the reaction was carried out for 16 h at a temperature of 125 °C. The reaction mixture was filtered and concentrated *in vacuo*. The residue was partitioned between CH₂Cl₂ and water. The organic layer was dried over MgSO₄ and concentrated. The residue was purified by silica gel flash chromatography (eluent CH₂Cl₂), yielding 1.4 g (93 %) of intermediate **30b**, mp 156.7 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.85 (s, 3 H), 4.44 (d, *J*=4.0 Hz, 2 H), 6.11 (t, *J*=4.0 Hz, 1 H), 6.53 (td, *J*=7.7, 1.5 Hz, 1 H), 6.60 (dd, *J*=8.1, 1.5 Hz, 1 H), 6.82 (td, *J*=7.7, 1.5 Hz, 1 H), 7.05 (dd, *J*=8.1, 1.5 Hz, 1 H), 7.48 (d, *J*=7.7 Hz, 1 H), 7.68 (d, *J*=1.8 Hz, 1 H), 7.72 (dd, *J*=7.7, 1.8 Hz, 1 H); Anal. (C₁₅H₁₃NO₃) C, H, N.

Step 5. Dibenzo[b,f][1,4]oxazepine-3-carboxylic acid methyl ester (31).



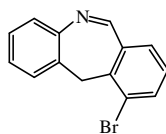
Starting from **30b**, standard MnO₂ oxidation gave compound **31** in 7 % yield: mp 105.4 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.89 (s, 3 H), 7.25 - 7.38 (m, 4 H), 7.74 (d, *J*=8.1 Hz, 1 H), 7.77 (d, *J*=1.5 Hz, 1 H), 7.89 (dd, *J*=8.1, 1.5 Hz, 1 H), 8.73 (s, 1 H); Anal. (C₁₅H₁₁NO₃) C, H, N.

8-Bromo-11H-dibenzo[b,e]azepine (34).



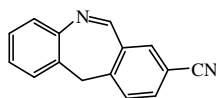
Starting from **16b**, standard MnO₂ oxidation gave compound **34** in 39 % yield; mp 115.8 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.67 (2 H, s), 7.21 - 7.26 (1 H, m), 7.27 - 7.30 (2 H, m), 7.34 (1 H, br. d, *J*=7.0 Hz), 7.38 (1 H, d, *J*=8.1 Hz), 7.68 (1 H, dd, *J*=8.1, 2.0 Hz), 7.86 (1 H, d, *J*=2.0 Hz), 8.86 (1 H, s); Anal. (C₁₀H₁₀BrN) C, H, N.

10-Bromo-11H-dibenzo[b,e]azepine, HCl salt (35).



Starting from **16a**, standard MnO₂ oxidation gave compound **34** in 77 % yield.; mp 191.8 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.00 (s, 2 H), 7.36 - 7.47 (m, 4 H), 7.49 - 7.55 (m, 1 H), 7.85 (d, *J*=7.3 Hz, 1 H), 7.96 (d, *J*=8.1 Hz, 1 H), 9.21 (s, 1 H); Anal. (C₁₀H₁₀BrN·HCl) C, H, N.

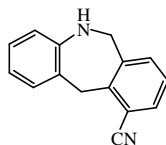
Synthesis of 11H-dibenzo[b,e]azepine-8-carbonitrile (**36**).



A mixture of 8-bromo-6,11-dihydro-5H-dibenzo[b,e]azepine (**16b**) (500 mg, 1.8 mmol) and copper cyanide (320 mg, 3.6 mmol) in DMF (40 mL) was stirred at 125 °C for 7 d. The mixture was evaporated, the residue was taken up in CH₂Cl₂ and washed with water. The organic layer was dried over MgSO₄, filtered and evaporated. The crude residue, containing 6,11-dihydro intermediate **33c** was purified via reversed phase HPLC yielding 44 mg (11 %) of compound **36**. ¹H NMR (360 MHz, CDCl₃) δ ppm 3.75 (s, 2 H) 7.20 - 7.36 (m, 3 H) 7.42 (d, *J*=8.05 Hz, 2 H) 7.69 (dd, *J*=7.87, 1.65 Hz, 1 H) 7.77 (d, *J*=1.83 Hz, 1 H) 8.87 (s, 1 H).

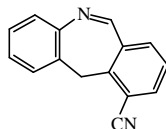
Synthesis of 11H-Dibenzo[b,e]azepine-10-carbonitrile, HCl-salt (**37**).

Step 1. 6,11-Dihydro-5H-dibenzo[b,e]azepine-10-carbonitrile (**33b**).



A mixture of **16a** (800 mg, 2.9 mmol) and CuCN (0.660 mg, 7.3 mmol) in DMF (15 mL) was degassed and then shaken under nitrogen atmosphere at 140 °C for 3 days. The reaction mixture was cooled. An 0.2 N aqueous NaOH solution (200 mL) was added. This mixture was extracted with ethyl acetate (2 x 100 mL). The organic layer was separated, washed with water and brine, dried over MgSO₄, filtered and the filtrate was evaporated. The residue was purified by silica gel flash chromatography (eluent: CH₂Cl₂). The product fractions were collected and the solvent was evaporated, yielding 0.530 mg (83 %) of **33b**; mp 139.8 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.34 (s, 2 H), 4.49 (d, *J*=5.3 Hz, 2 H), 6.04 (t, *J*=5.3 Hz, 1 H), 6.39 (dd, *J*=8.0, 1.2 Hz, 1 H), 6.44 (td, *J*=7.3, 1.2 Hz, 1 H), 6.84 - 6.91 (m, 2 H), 7.41 (t, *J*=7.7 Hz, 1 H), 7.60 (dd, *J*=7.6, 1.3 Hz, 1 H), 7.70 (dd, *J*=7.8, 1.3 Hz, 1 H); Anal. (C₁₅H₁₂N₂) C, H, N.

Step 2. 11H-Dibenzo[b,e]azepine-10-carbonitrile, HCl-salt (**37**).

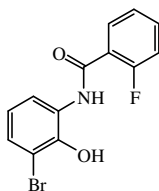


A mixture of **33b** (0.4 g, 1.8 mmol) and MnO₂ (790 mg, 9.0 mmol) in toluene (15 mL) was stirred at 90

°C for 4 h. The reaction mixture was filtered over a dicalite pad and washed with CH₂Cl₂. The organic layer was evaporated. The residue was dissolved in diethyl ether and a 1N HCl solution in diethyl ether was added. The precipitate was filtered off, washed with diethyl ether and dried *in vacuo*, yielding 350 mg (76 %) of **37**; mp 194.9 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 3.96 (s, 2 H), 7.37 - 7.46 (m, 4 H), 7.62 (t, *J*=7.9 Hz, 1 H), 8.05 - 8.11 (m, 2 H), 9.13 (s, 1 H).

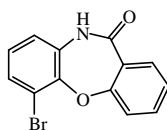
Synthesis of dibenzo[b,f][1,4]oxazepine-6-carbonitrile (**39**).

Step 1. N-(3-Bromo-2-hydroxy-phenyl)-2-fluoro-benzamide (**27c**).



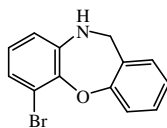
2-Fluoro-benzoyl chloride (2.5 g, 16 mmol) in THF (25 mL) was added dropwise to a mixture of 2-amino-6-bromo-phenol (3.0 g, 16 mmol) and triethylamine (3.1 g, 31 mmol) in THF (75 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was poured into water (400 mL) and acidified to pH 4-5 with 1N aqueous HCl. The aqueous layer was extracted twice with 200 mL of CH₂Cl₂. The organic layer was dried on MgSO₄ and concentrated *in vacuo*. The residue was purified via reversed phase HPLC, yielding 2.8 g (56 %) of intermediate **27c**, mp 146.3 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 6.86 (t, *J*=8.1 Hz, 1 H), 7.31 - 7.46 (m, 3 H), 7.58 - 7.69 (m, 1 H), 7.77 (d, *J*=8.7 Hz, 1 H), 7.88 (t, *J*=7.3 Hz, 1 H), 9.80 (s, 1 H), 9.90 (d, *J*=5.1 Hz, 1 H).

Step 2. 6-Bromo-10H-dibenzo[b,f][1,4]oxazepin-11-one (**28c**).



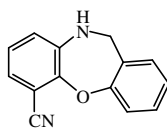
NaOH (powder, 356 mg, 8.9 mmol) and **27c** (2.8 g, 8.9 mmol) in DMF (60 mL) were refluxed for 5 h. The reaction mixture was poured onto ice water (800 mL) and the resulting precipitate filtered off and washed with 1N aqueous NaOH and water, then dried *in vacuo*, yielding 2.4g (94%) of intermediate **28c**, mp 263.3 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 7.16 - 7.29 (m, 4 H), 7.44 (dd, *J*=8.1, 1.5 Hz, 1 H), 7.76 (dd, *J*=7.7, 1.5 Hz, 1 H), 7.94 (dd, *J*=7.7, 1.5 Hz, 1 H), 10.72 (s, 1 H).

Step 3. 6-Bromo-10,11-dihydro-dibenzo[b,f][1,4]oxazepine (**29c**).



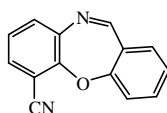
To a suspension of **28c** (2.4 g, 8.4 mmol) in THF (80 mL) was added borane 1M in THF (25.3 mL, 25.3 mmol) at room temperature. The reaction mixture was stirred further at room temperature over the weekend. The reaction mixture was cooled on ice and 100 mL 1N aqueous HCl was added. The mixture was partly concentrated *in vacuo*, then basified with solid NaHCO₃ (pH about 7). The aqueous layer was extracted twice with 150 mL CH₂Cl₂. The organic layer was dried on MgSO₄ and concentrated. The residue was purified via reversed phase HPLC, yielding 1.4 g (60 %) of intermediate **29c**. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.43 (d, *J*=4.3 Hz, 2 H), 6.40 (t, *J*=4.3 Hz, 1 H), 6.54 (dd, *J*=8.1, 1.8 Hz, 1 H), 6.69 (t, *J*=7.7 Hz, 1 H), 6.75 (dd, *J*=8.1, 1.8 Hz, 1 H), 7.17 (td, *J*=7.3, 1.5 Hz, 1 H), 7.28 (dd, *J*=8.1, 1.5 Hz, 1 H), 7.31 - 7.37 (m, 2 H).

Step 4. 10,11-Dihydro-dibenzo[b,f][1,4]oxazepine-6-carbonitrile (**33d**).



Intermediate **29c** (745 mg, 2.7 mmol), and copper cyanide (609 mg, 6.8 mmol) in DMF (40 mL) were degassed and then shaken under nitrogen at 140 °C overnight. The reaction mixture was cooled to room temperature and an aqueous NaOH solution (200 mL, 0.2N) was added. The mixture was extracted twice with 100 mL EtOAc. The combined organic layer was washed with water and brine and dried on MgSO₄, concentrated *in vacuo*, yielding 500 mg of a ~1:1 mixture of **33d** and oxidized product dibenzo[b,f][1,4]oxazepine-6-carbonitrile (**39**), which was used as such in the next step.

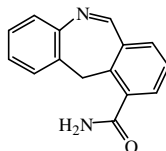
Step 5. Dibenzo[b,f][1,4]oxazepine-6-carbonitrile (**39**).



A mixture of **29c** (already partially oxidized to **39**) (500 mg, 2.25 mmol) and manganese dioxide (587 mg, 6.75 mmol) in toluene (15 mL) was stirred at 80 °C for 3 h. The reaction mixture was filtered over a pad of dicalite (eluent: toluene, then CH₂Cl₂). The CH₂Cl₂ layer was evaporated and the residue was purified via reversed phase HPLC yielding 310 mg (62 %) of **39**, mp 123.3 °C. ¹H NMR (360 MHz,

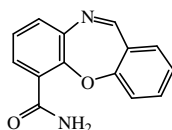
DMSO-*d*₆) δ ppm 7.28 (dd, *J*=8.1, 1.1 Hz, 1 H), 7.41 - 7.46 (m, 2 H), 7.67 - 7.72 (m, 3 H), 7.82 (dd, *J*=7.7, 1.5 Hz, 1 H), 8.76 (s, 1 H); Anal. (C₁₄H₈N₂O) C, H, N.

11H-Dibenzo[b,e]azepine-10-carboxylic amide (40).



A mixture of **37** (100 mg, 0.4 mmol) and conc. H₂SO₄ (3 mL) was stirred at a temperature between 0 and 5 °C for 2 h. The mixture was kept stirring at room temperature for 4 days. The reaction mixture was poured into 50 mL ice water, and basified with a concentrated aqueous NH₃ solution. The formed precipitate was filtered off, dissolved in methanol and concentrated. The residue was purified via reversed phase HPLC, yielding 24 mg (26 %) of **40**; ¹H NMR (360 MHz, CDCl₃) δ ppm 3.88 (s, 2 H), 5.89 (br. s., 1 H), 5.96 (br. s., 1 H), 7.23 - 7.42 (m, 5 H), 7.56 - 7.60 (m, 2 H), 8.93 (s, 1 H).

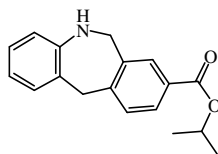
Dibenzo[b,f][1,4]oxazepine-6-carboxylic acid amide (42).



Compound **42** was prepared from **39** according to the procedure as described for **40** and **41**. Yield: 45%; mp 193.2 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 7.21 - 7.29 (m, 2 H), 7.31 - 7.38 (m, 3 H), 7.66 (dd, *J*=7.7, 1.8 Hz, 1 H), 7.69 (dd, *J*=7.7, 1.8 Hz, 1 H), 7.74 (br. s., 1 H), 7.96 (br. s., 1 H), 8.68 (s, 1 H); Anal. (C₁₄H₁₀N₂O₂) C, H, N.

Synthesis of 11H-dibenzo[b,e]azepine-8-carboxylic acid isopropyl ester (43).

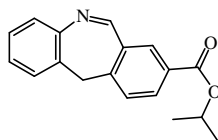
Step 1. 6,11-Dihydro-5H-dibenzo[b,e]azepine-8-carboxylic acid isopropyl ester (18h).



A 75-mL stainless steel autoclave was charged under nitrogen atmosphere with 8-bromo-6,11-dihydro-5H-dibenzo[b,e]azepine (**16b**) (1.65 g, 6.0 mmol), Pd(OAc)₂ (10 mg, 0.04 mmol), 1,3-

bis(diphenylphosphino)propane (40 mg, 0.1 mmol), potassium acetate (1.5 g, 15.3 mmol), isopropanol (20 mL) and THF (20 mL). The autoclave was closed and pressurized to 50 bar carbonmonoxide and the reaction was carried out for 16 h at a temperature of 125 °C. The reaction mixture was filtered and concentrated *in vacuo*. The residue was partitioned between CH₂Cl₂ and water. The separated organic layer was dried over MgSO₄, filtered and the filtrate was concentrated. The residue was purified by flash chromatography over silica gel (eluent: heptane/EtOAc). The product fractions were collected and the solvent was evaporated, yielding 375 mg (22 %) of intermediate **18h**. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 1.31 (d, *J*=6.2 Hz, 6 H), 4.19 (s, 2 H), 4.47 (d, *J*=5.3 Hz, 2 H), 5.07 - 5.17 (m, *J*=6.2, 6.2, 6.2, 6.2, 6.2, 6.2 Hz, 1 H), 5.93 (t, *J*=5.3 Hz, 1 H), 6.35 - 6.42 (m, 2 H), 6.83 (td, *J*=7.6, 1.6 Hz, 1 H), 6.91 (dd, *J*=7.4, 1.6 Hz, 1 H), 7.38 (d, *J*=8.3 Hz, 1 H), 7.77 - 7.83 (m, 2 H).

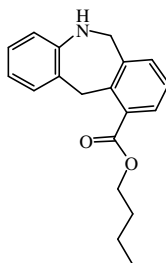
Step 2. 11H-dibenzo[b,e]azepine-8-carboxylic acid isopropyl ester (**43**).



Compound **43** was prepared from **18h** according to the general MnO₂ oxidation procedure as described for **6**. Yield 14%; ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 1.31 (d, *J*=6.2 Hz, 6 H), 3.77 (s, 2 H), 5.09 - 5.19 (m, *J*=6.2, 6.2, 6.2, 6.2, 6.2, 6.2 Hz, 1 H), 7.22 - 7.32 (m, 3 H), 7.37 (dd, *J*=6.6, 1.1 Hz, 1 H), 7.57 (d, *J*=8.0 Hz, 1 H), 8.04 (dd, *J*=8.0, 1.8 Hz, 1 H), 8.18 (d, *J*=1.8 Hz, 1 H), 9.01 (s, 1 H).

Synthesis of 11H-dibenzo[b,e]azepine-10-carboxylic acid butyl ester, HCl salt (**44**).

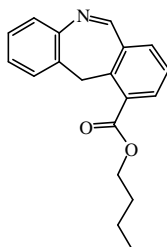
Step 1. 6,11-Dihydro-5H-dibenzo[b,e]azepine-10-carboxylic acid butyl ester (**18i**).



A 75-mL stainless steel autoclave was charged under nitrogen atmosphere with 10-bromo-6,11-dihydro-5H-dibenzo[b,e]azepine (**16a**) (1.6 g, 6 mmol), Pd(OAc)₂ (10 mg, 0.04 mmol), 1,3-bis(diphenylphosphino)propane (40 mg, 0.1 mmol), potassium acetate (1.5 g, 15.3 mmol), n-butanol (20 mL) and THF (20 mL). The autoclave was closed and pressurized to 50 bar carbonmonoxide and the reaction was carried out for 16 h at a temperature of 125 °C. The reaction mixture was filtered and

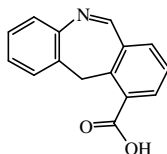
concentrated *in vacuo*. The residue was partitioned between CH₂Cl₂ and water. The separated organic layer was dried over MgSO₄, filtered and the filtrate was concentrated. The residue was purified by flash chromatography over silica gel (eluent: heptane/EtOAc 100/0 to 80/20). The product fractions were collected and the solvent was evaporated, yielding 760 mg (43 %) of intermediate **18i**. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 0.93 (t, *J*=7.4 Hz, 3 H), 1.35 - 1.46 (m, 2 H), 1.66 - 1.74 (m, 2 H), 4.31 (t, *J*=6.5 Hz, 2 H), 4.43 (s, 2 H), 4.49 (d, *J*=5.3 Hz, 2 H), 5.95 (t, *J*=5.3 Hz, 1 H), 6.32 - 6.40 (m, 2 H), 6.81 (td, *J*=7.6, 1.6 Hz, 1 H), 6.86 (dd, *J*=7.5, 1.6 Hz, 1 H), 7.31 (t, *J*=7.6 Hz, 1 H), 7.46 (dd, *J*=7.5, 1.4 Hz, 1 H), 7.63 (dd, *J*=7.9, 1.4 Hz, 1 H).

Step 2. 11H-dibenzo[b,e]azepine-10-carboxylic acid butyl ester, HCl salt (44).



Compound **44** was prepared from **18i** according to the general MnO₂ oxidation procedure as described for **6**. Yield 79%; Mp 173 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 0.95 (t, *J*=7.3 Hz, 3 H), 1.39 - 1.50 (m, 2 H), 1.71 - 1.81 (m, 2 H), 4.04 (br. s., 2 H), 4.40 (t, *J*=6.6 Hz, 2 H), 7.36 - 7.59 (m, 5 H), 8.05 (t, *J*=7.5 Hz, 2 H), 9.25 (s, 1 H); Anal. (C₁₉H₁₉NO₂·HCl) C, H, N.

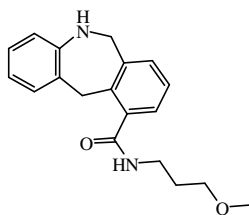
11H-Dibenzo[b,e]azepine-10-carboxylic acid (46).



A mixture of **18a** (250 mg, 1.0 mmol) in THF (30 mL) and LiOH (1N aqueous solution, 10 mL) was stirred at room temperature for 2 h. The reaction mixture was heated overnight at 50 °C, then allowed to stand over the weekend at room temperature. The mixture was concentrated *in vacuo*. The residue was purified via reversed phase HPLC, yielding 100 mg (42 %) of **46**; ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 4.01 (br. s., 2 H), 7.18 - 7.32 (m, 5 H), 7.38 (d, *J*=6.6 Hz, 1 H), 7.55 (d, *J*=7.7 Hz, 1 H), 7.75 (d, *J*=7.0 Hz, 1 H), 8.91 (s, 1 H).

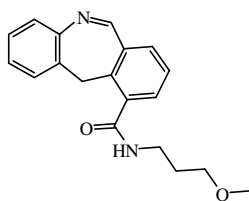
Synthesis of 11H-Dibenzo[b,e]azepine-10-carboxylic acid (3-methoxy-propyl)-amide, HCl salt (49).

Step 1. 6,11-Dihydro-5H-dibenzo[b,e]azepine-10-carboxylic acid (3-methoxy-propyl)-amide **47.**



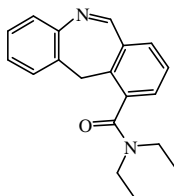
A mixture of **16a** (800 mg, 3.0 mmol), 1,3-bis(diphenylphosphino)propane (80 mg, 0.2 mmol), Pd(OAc)₂ (10 mg, 0.04 mmol) and triethylamine (1 g, 10 mmol) in THF (50 mL) and water (1 mL) was stirred in an autoclave at 125 °C for 16 h under 50 atm. of CO pressure. The reaction mixture was cooled and filtered over dicalite. 3-Methoxypropylamine (437 mg, 49 mmol) and HBTU (1.5 g, 40 mmol) were added and the reaction mixture was stirred overnight at room temperature. The organic layer was washed with water and with a 1N aqueous sodium hydroxide solution, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified via reversed phase HPLC, yielding 300 mg (32 %) of **47**, mp 144.1 °C. Anal. (C₁₉H₂₂N₂O₂) C, H, N.

Step 2. 11H-Dibenzo[b,e]azepine-10-carboxylic acid (3-methoxy-propyl)-amide, HCl salt (49**).**



Standard MnO₂ oxidation of **47** gave **49** in 79% yield as HCl-salt. ¹H NMR (360 MHz, DMSO-*d*₆) δ ppm 1.78 - 1.86 (m, 2 H), 3.27 (s, 3 H), 3.37 (q, *J*=6.6 Hz, 2 H), 3.44 (t, *J*=6.4 Hz, 2 H), 3.88 (s, 2 H), 7.35 - 7.60 (m, 5 H), 7.73 (d, *J*=7.3 Hz, 1 H), 7.97 (d, *J*=7.3 Hz, 1 H), 8.62 (t, *J*=5.7 Hz, 1 H), 9.32 (s, 1 H); Anal. (C₁₉H₂₀N₂O₂·HCl·0.7 H₂O) C, H, N.

11H-Dibenzo[b,e]azepine-10-carboxylic acid diethylamide (50**).**



A mixture of **16a** (800 mg, 3 mmol), diethylamine (1 g, 13.7 mmol), Pd(OAc)₂ (10 mg, 0.04 mmol) and 1,3-bis(diphenylphosphino)propane (40 mg, 0.1 mmol) in THF (50 mL) was reacted in an autoclave at 150 °C for 16 h under 50 atm. of CO pressure. The reaction mixture was filtrated through dicalite and the filtrate was concentrated. The residue was purified by reversed phase HPLC. The desired fraction was collected and the solvent was evaporated, yielding 6 mg (1 %) of **50**; ¹H NMR (360 MHz, CDCl₃) δ

ppm 1.00 (t, $J=7.1$ Hz, 3 H), 1.42 (t, $J=7.1$ Hz, 3 H), 2.96 - 3.14 (m, 2 H), 3.35 - 3.48 (m, 1 H), 3.65 (s, 2 H), 3.87 - 4.02 (m, 1 H), 7.16 (dd, $J=7.3$, 1.5 Hz, 1 H), 7.21 (td, $J=7.3$, 1.3 Hz, 1 H), 7.27 - 7.31 (m, 1 H), 7.31 - 7.36 (m, 2 H), 7.40 (dd, $J=7.7$, 1.4 Hz, 1 H), 7.50 (t, $J=4.4$ Hz, 1 H), 8.91 (s, 1 H).

II. Electrophysiological experiments

The effect of **6** on the hTRPA1 current is given as a representative example of the electrophysiological effect of the agonists (Fig. 1A). Cells were clamped at a holding potential of -30 mV and every 4 seconds the hTRPA1 current was measured during a voltage ramp. In the control period of two minutes almost no hTRPA1 current was measured. After the application of 10 nM **6**, the hTRPA1 current was activated showing a clear outward rectification and the inward holding current increased. Five minutes after the compound application, the compound was washed out for 2 minutes. At the end of the experiment 50 μ M of the TRPA1 blocker ruthenium red (RR) was applied to evaluate the non-TRPA1 component of the holding current and as a consequence the quality of the experiment. The current amplitudes measured at +75 mV and -75 mV (see arrows and dotted lines in Fig. 1A) were plotted as a function of time in Fig. 1B, and this was done for 3 different concentrations of **6**. Fig. 1B shows a concentration dependent increase in current amplitude and decrease in time to peak after application of **6**.

Figure 1.

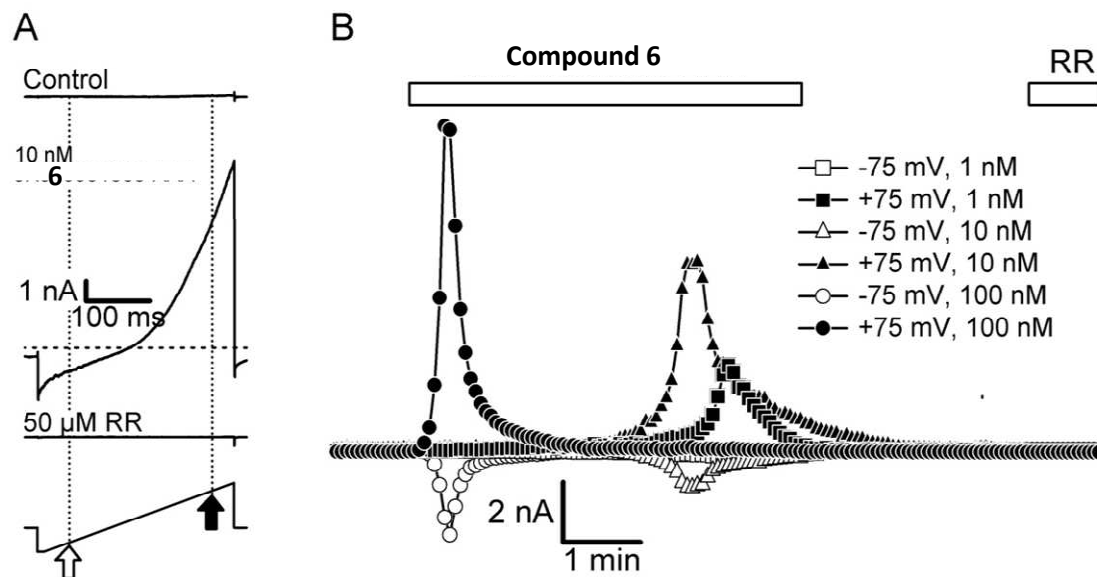


Fig. 1. Currents activated by **6** in hTRPA1-HEK293 cells were measured in the whole cell voltage clamp configuration. A. Representative current traces recorded from 1 cell are shown. The cells were kept at a holding potential of -30 mV and stimulated every 4 seconds with a voltage ramp (see lower

panel in panel A): first, the cells were clamped at -100 mV for 10 ms and subsequently the potential was gradually increased to $+100$ mV during 400 ms. The upper current trace is recorded in control solutions. 10 nM **6** activated an outward rectifying current that was completely abolished in the presence of ruthenium red (RR; 50 μ M). B. The current amplitude, measured at -75 mV and at 75 mV (as indicated by the arrows and dotted lines in panel A), was plotted as a function of time for the indicated concentrations.

III Metabolic stability assay and reactive metabolite experiments.

Hepatic metabolic stability was determined as described by Kantharaj et al.¹ Briefly, all incubations were conducted by shaking reaction mixtures (250 μ L) in duplicate containing test compound (5 μ M), microsomal protein (1 mg/mL), phosphate buffer (0.5 M, pH 7.4), and NADPH generating system (1.6 mM MgCl_2 , 1.6 mM glucose-6-phosphate, and 0.125 U/mL glucose-6-phosphate-dehydrogenase). The mixtures were pre-incubated for 5 min at 37 $^{\circ}\text{C}$, followed by the addition of NADP (0.16 mM) to initiate the biochemical reaction. Reactions were terminated after 15 min by addition of 2 volumes of DMSO. The precipitated material was removed by centrifugation, and the supernatant was analyzed by LC-MS on a ThermoFinnigan Ion Trap instrument. Metabolic stability was determined by comparing the peak areas of the parent compound measured at 15 min with that at 0 min.

Reactive Metabolite Assay

Incubations were performed in 100 mM KPi buffer (pH=7.4) with 1 mM MgCl_2 and 1 mM EDTA. Test incubation mixtures contained 1 mg/ml protein from human liver microsomes (HLMs), 2 mM NADPH and 2 mM L-glutathione (GSH). 500 nl from a 5 mM compound DMSO stock was directly spiked in 250 μ L incubation mixture to obtain a final compound concentration of 10 μ M. Control incubations were incubated without NADPH, without GSH and with NADPH and GSH but without compound. Samples were incubated in a square deep well plate for 1 h at 37 $^{\circ}\text{C}$ while shaking at 500 rpm on a vortex plate shaker. The reaction was quenched by addition of 200 μ L CH_3CN and was mixed for 10 min at 800 rpm on a vortex plate shaker. Subsequently the mixture was centrifuged at 5600 rpm for 10 min at 4 $^{\circ}\text{C}$, and the supernatant analysed via standard LC-MS/MS analysis.

To investigate direct conjugation of GSH to the compounds and to obtain an indication of their chemical stability, incubations were performed under identical conditions with or without GSH, and without protein from liver microsomes or NADPH, and the mixtures were analyzed after 1, 4, and 7h. At all

timepoints, no glutathione adducts could be detected for compounds **6**, **8**, and **32**, and the compounds appeared to be stable under the incubation conditions, with or without the presence of GSH.

References

1. Kantharaj E., Tuytelaars A., Proost P., Ongel Z., van Assouw H., Gilissen R. Simultaneous measurement of drug metabolic stability and identification of metabolites using ion-trap mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2661-2668