

Supporting Information

for

4-Benzyl-1H-imidazoles with oxazoline termini as histamine H₃ receptor agonists

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Purity table.

Compound #	HPLC Purity (%)	Criterion ^a
10	98.28	- LC System 2 - Elem. Anal.
11	97.45	LC System 2
12.maleate	99.43	LC System 2
13	97.55	- LC System 2 - Analyzed by Elem. Anal. as hydrogen oxalate by us before. ¹
14	100	LC System 1 acidic
15.maleate	94	¹ H-NMR
16	>95	¹ H-NMR ^b
17	99.05	LC System 1 acidic
18	98.41	LC System 2
19	98.39	LC System 1 acidic
20.maleate	99.78	LC System 1 acidic
21.maleate	98.62	LC System 1 acidic
22.maleate	98.58	LC System 2
23.maleate	98.93	LC System 2
24	100	LC System 2
25.2HBr	satisfactory	Elem. Anal. (See Ref. 2)
26	98.40	LC System 2

Table S1. Purities of all compounds prepared by classical chemistry. a) For LC-systems, see below. b) For the ¹H-NMR spectrum, see section 'Representative spectra'

LC system 1

Equipment:

A ZQ Water single quadrupole mass spectrometer is used for LC-MS analysis. This spectrometer is equipped with an ESI source and an HPLC Waters 2795 quaternary pump with diode array detector (210 to 400 nm.) *Source parameters.* ESI capillary voltage: 3.2kV. Cone and Extractor voltage: 25 and 2 V, respectively. Source block temperature: 120°C. Desolvation temperature: 320°C. Cone gaz flow: 160 L/Hr (Nitrogen). Desolvation Gas flow: 560 L/Hr. *Detector parameter.* Multiplier voltage: 500 V. Data are acquired in a full MS scan from m/z 90 to 1000 in positive mode with an acidic elution and both in positive and negative modes with a basic elution. The reverse phase separation is carried out at 45°C on a Sunfire MS C18 column (3.5µm, 150 x 4.6 mm) for acidic elution and on a XBridge MS C18 column (3.5µm, 150 x 4.6 mm) for basic elution. Gradient elution is done with Water (solvent A), Acetonitrile (solvent B), Water/Acetonitrile/TFA : 49.5/49.5/1, v/v/v (solvent C, acidic elution), NH₄HCO₃ in water 15.8g/4L + 2 ml NH₄OH 30% (solvent D, basic elution).

Acidic gradient program

HPLC flow rate: 1.8 ml/min to 2.3 ml/min, injection volume: 5 µl

The splitting ratio is set at +/- 150 µl to MS.

Basic gradient program

HPLC flow rate: 1.8 ml/min to 2.3 ml/min, injection volume: 5 µl

The splitting ratio is set at +/- 150 μ l to MS.

LC system 2

Equipment

A LCQ Finnigan ion trap mass spectrometer is used for LC-MS analysis. This spectrometer is equipped with an APCI source and an HPLC Agilent 1100 quaternary pump with diode array detector (205 to 400 nm.). *Source parameters.* APCI capillary voltage: 31 V. Capillary temperature: 160 °C. Discharge voltage: 4 kV. Vaporizer temperature: 450 °C. Sheat gas flow rate (arb. unit): 55. Auxiliary gas flow rate (arb. unit): 2. *Detector parameter.* Tube Lens Offset: 10 V. Electron Multiplier: -1150. Octapole 1 Offset: -3.25 V. Lens Voltage: -18 V. Octapole 2 Offset: -6 V. Octapole Rf amplitude: 400. Scan Time: 3 μ scans, 200 msec. Data are acquired in a full MS scan from m/z 50 to 1500 in positive mode with an acidic elution. The reverse phase separation is carried out at 40°C on a Inertsil ODS3 column (5 μ m, 250 x 4.6 mm). Gradient elution is done with Water (solvent A), Acetonitrile (solvent B), Water/Acetonitrile/TFA; 49.5/49.5/1, v/v/v (solvent C)

Acidic gradient program

HPLC flow rate: 2.0 ml/min to 3.0 ml/min, injection volume: 10 μ l

The splitting ratio is set at +/- 150 μ l to MS.

Synthetic methods.

General synthetic methods. Reagents were obtained from commercial suppliers and used without further. Solvents used were either AR or HPLC grade. Dry THF and CH_2Cl_2 were freshly distilled from lithium aluminium hydride and calcium hydride, respectively. Thin-layer chromatography was carried out on Merck Kieselgel 60 F254 on aluminium sheets and flash chromatography was performed using J. T. Baker Kieselgel 60 under pressure. Mass spectrometry was performed on Finnigan LCQDECA ion-trap mass spectrometer using APCI technique. The ^1H - and ^{13}C -spectra were recorded on a Bruker 200 MHz spectrometer. Melting points were measured on a Mettler FP-5 + FP-52 instrument and are uncorrected. Systematic names for molecules according to IUPAC rules were generated using the Beilstein AutoNom program. Elemental analyses were performed in-house at UCB Pharma.

4(5)-Iodo-1*H*-imidazole (32). A solution of iodine (100 g, 0.394 mol) and potassium iodide (125 g, 0.753 mol) in water (400 mL) was added dropwise to a solution of imidazole (20.0 g, 0.294 mol) in NaOH (1 L, 2.0 M solution). After stirring overnight, the solution was neutralised with glacial acetic acid to pH 7. The resulting suspension was filtered and the residue was dispersed in ethanol (400 mL). Sodium sulfite (100 g, 0.794 mol) dissolved in water (350 mL) was added and this was then refluxed for 72 h. After hot filtration the filtrate was concentrated until no more precipitation of 4,5-diiodo-imidazole occurred. Subsequently, the suspension was filtered and the filtrate was concentrated *in vacuo*. The resulting solid was recrystallised from EtOAc, yielding white crystals: yield 20%. NMR data matched previously reported spectra.³

4-Iodo-1-trityl-1*H*-imidazole (33). Triethylamine (8.6 mL, 62 mmol) and chlorotriphenylmethane (10.35 g, 37.12 mmol) were added to a solution of **32** (6.00 g, 30.9 mmol) in THF (250 mL). After refluxing for 1.5 h, the mixture was cooled and the suspension was filtered. The filtrate was evaporated *in vacuo*, the residue was dissolved in chloroform (100 mL) and this was then washed with sodium thiosulfate (100 mL, 5% solution in water). The water layer was extracted three times with chloroform, the combined organic layers were dried over MgSO_4 and then the solvent was evaporated *in vacuo*. The residue was recrystallised with hot filtration from EtOAc: yield 64%. NMR data matched previously reported spectra.³

3-[Hydroxy-(1-trityl-1*H*-imidazol-4-yl)-methyl]-benzonitrile (34a). Ethylmagnesium bromide (1.38 mL, 3M solution in diethylether) was added to a solution of **33** (1.50 g, 3.43 mmol) in CH_2Cl_2 (15 mL). After stirring for 30 min a solution of 3-cyanobenzaldehyde (0.451 g, 3.43 mmol) in CH_2Cl_2 (3 mL) was added slowly. After stirring overnight, NH_4Cl (50 mL, half-saturated solution in water) was added. The organic layer was separated and the water layer was extracted three times with CH_2Cl_2 . The combined organic layers were dried over MgSO_4 and the solvent was removed *in vacuo*. The residue was triturated with 10% acetone/diethylether: yield 85%. $^1\text{H-NMR}$ (CDCl_3) δ 7.4 (app d, 2H, ImH + PhH), 7.4 – 7.0 (m, 18H, PhH), 6.5 (s, 1H, ImH), 5.8 (s, 1H, ArCH).

4-[Hydroxy-(1-trityl-1*H*-imidazol-4-yl)-methyl]-benzonitrile (34b). Used the same method as for compound **34a**, starting from **33** and 4-cyanobenzaldehyde. Yield = 70 %. $^1\text{H-NMR}$ (CDCl_3) δ 7.5 – 7.0 (several m, 20H, ImH + PhH), 6.5 (s, 1H, ImH), 5.8 (s, 1H, ArCH).

Acetic acid (3-cyano-phenyl)-(1-trityl-1H-imidazol-4-yl)-methyl ester (35a). Acetic anhydride (288 μ L, 3.06 mmol) was added to a solution of **34a** (900 mg, 2.038 mmol), DMAP (25 mg, 0.20 mmol) and triethylamine (568 μ L, 4.08 mmol) in CH_2Cl_2 (40 mL) at 0 °C. After 15 min, cooling was stopped and after 1 h the reaction was complete (TLC; 10% acetone in CH_2Cl_2). Water was added, the organic layer was separated, after which the water layer was extracted three times with CH_2Cl_2 . The combined organic layers were dried over MgSO_4 and the solvent was removed *in vacuo*. The title compound was obtained by flash column chromatography (silica; 2-4% acetone in CH_2Cl_2). Yield = 89%. $^1\text{H-NMR}$ (CDCl_3) δ 7.6 – 7.0 (several m, 20H), 6.8 (app s, 2H), 2.1 (s, 3H, Me).

Acetic acid (4-cyano-phenyl)-(1-trityl-1H-imidazol-4-yl)-methyl ester (35b). Used the same method as for compound **35a**, starting from **34b**. Yield = 87 %. $^1\text{H-NMR}$ (CDCl_3) δ 7.6 (app q, 4H), 7.5 – 7.0 (several m, 16H, ImH + PhH), 6.8 (app s, 2H), 2.1 (s, 3H, Me).

3-(1-Trityl-1H-imidazol-4-ylmethyl)-benzonitrile (36a). Compound **35a** (2.39 g, 4.94 mmol) was dissolved in ethanol (250 mL) and was hydrogenated with 5% Pd/C and hydrogen at atmospheric pressure and room temperature. After 3 h the catalyst was filtered off and the solvent of the filtrate was removed *in vacuo*. The residue was dissolved in EtOAc and washed with water, then basified with some NaHCO_3 . The organic layer was separated after which the water layer was extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The title compound was obtained by flash column chromatography (silica; 10-20% EtOAc in CH_2Cl_2). Yield = 62%. $^1\text{H-NMR}$ (CDCl_3) δ 7.5 – 7.0 (several m, 20H), 6.8 (s, 1H, ImH), 3.9 (s, 2H, ArCH_2).

4-(1-Trityl-1H-imidazol-4-ylmethyl)-benzonitrile (36b). Used the same method as for compound **36a**, starting from **35b**. Yield = 78 %. $^1\text{H-NMR}$ (CDCl_3) δ 7.5 – 7.0 (several m, 20H), 6.6 (s, 1H, ImH), 3.9 (s, 2H, ArCH_2).

3-(1H-Imidazol-4-ylmethyl)-benzonitrile, maleate salt (12). Hydrochloric acid (5 mL, 1 M) was added to a solution of **36a** (327 mg, 0.768 mmol) in ethanol (10 mL). After refluxing for 1 h the mixture was extracted three times with diethyl ether. The water layer was basified with NaHCO_3 and then extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The title compound was obtained by flash column chromatography (silica; 5-10% methanol in CHCl_3). Yield = 79%. This was then converted to the maleate salt and recrystallised from ethanol/EtOAc.

M.p. = 140-141 °C. $^1\text{H-NMR}$ (CD_3OD) δ 8.7 (s, 1H, ImH), 7.6-7.4 (m, 4H, Ph), 7.3 (s, 1H, ImH), 6.7 (s, 2H, maleate), 4.2 (s, 2H, Ph-CH_2). $^{13}\text{C-NMR}$ (CD_3OD) δ 170.75, 140.68, 136.46, 135.66, 134.58, 133.81, 133.32, 132.10, 131.01, 119.56, 118.03, 113.96, 30.92. $M + 1$ (ESI-MS) = 184.5.

4-(1H-Imidazol-4-ylmethyl)-benzonitrile, maleate salt (22). Used the same method as for compound **12**, starting with **36b**. Yield = 78 %. M.p. = 138-140 °C. $^1\text{H-NMR}$ (CD_3OD) δ 8.7 (s, 1H, ImH), 7.7 (d, 2H, PhH), 7.4 (d, 2H, PhH), 7.3 (s, 1H, ImH), 6.3 (s, 2H, maleate), 4.2 (s, 2H, Ph-CH_2). $^{13}\text{C-NMR}$ (CD_3OD) δ 170.75, 144.14, 136.43, 135.66, 133.81, 133.63, 130.78, 119.50, 118.13, 112.18, 32.44. $M + 1$ (ESI-MS) = 184.4.

4-Benzyl-1H-imidazole, maleate salt (13). This compound was prepared according to the literature procedure, except that a maleate salt was prepared.¹ Spectral data of the free base matched. M.p. = 129-130 °C. ¹H-NMR (CD₃OD) δ 8.5 (s, 1H, ImH), 7.4-7.3 (m, 5H, Ph), 7.2 (s, 1H, ImH), 6.7 (s, 2H, maleate), 4.0 (s, 2H, Ph-CH₂). ¹³C-NMR (CD₃OD) δ 170.65, 138.81, 136.00, 135.32, 129.86, 129.67, 128.03, 117.73, 31.96. M + 1 (ESI-MS) = 159.2.

4-(3-Bromo-benzyl)-imidazole-1-sulfonic acid dimethylamide (38). P₁-protected 4-iodoimidazole **37**⁴ (8.5 g, 28.2 mmol) was dissolved in 130 mL of dry DCM under a nitrogen atmosphere. EtMgBr (11.3 mL, 3M in ether) was added and the mixture was stirred for 80 min. Now, CuCN.2LiCl (28.3 mL, 1M solution) was added and a yellow precipitated was formed that gradually dissolved. Finally, 3-bromobenzylbromide was added and the suspension was stirred at r.t. over the weekend. Next, ammoniumchloride solution containing 2% ammonia was added and the mixture was stirred for 30 min. Some more DCM was added and the layers were separated. The DCM layer was washed twice more with the ammoniumchloride solution, dried on MgSO₄ and evaporated *in vacuo*. Flash chromatography using a gradient of DCM to DCM/EA (3:1) afforded the product. Yield = 4.5 g (13.1 mmol, 46%). ¹H-NMR (CDCl₃) δ 7.87 (s, 1H, Im-2H), 7.45-7.15 (m, 4H, Ph), 6.90 (s, 1H, Im-5H), 3.90 (s, 2H, CH₂), 2.85 (s, 6H, CH₃).

4-(3-Bromo-benzyl)-1H-imidazole (39). Imidazole **38** (2.3 g, 6.7 mmol) was dissolved in EtOH. Next, KOH (10 g) was dissolved in 50 mL of water and this solution was added to the solution of **38**. This mixture was refluxed overnight. Some HCl was added (pH = 14) and the product mixture was extracted with DCM. The organic layers were dried on Na₂SO₄ and evaporated *in vacuo*. This gave the product in nearly quantitative yield and of sufficient purity to be carried on to subsequent steps without a problem. ¹H-NMR (CDCl₃) δ 8.15 (s, 1H, NH), 7.50 (s, 1H, Im-2H), 7.45-7.02 (m, 4H, Ph), 6.75 (s, 1H, Im-5H), 3.95 (s, 2H, CH₂).

Imidazole-1-sulfonic acid dimethylamide (40). Imidazole (63 g, 926 mmol) was dissolved in DCM (400 mL) and TEA (153 mL) and *N,N*-dimethylsulfamoylchloride (100 mL, 926 mmol) was added dropwise to this mixture. After stirring at r.t. overnight, a potassium carbonate solution (10 % in water) was added, the layers were separated and the organic layer was dried on Na₂SO₄ and evaporated *in vacuo*. Yield = 147 g (843 mol, 91%). ¹H-NMR (CDCl₃) δ 7.82 (s, 1H, Im-2H), 7.28 (s, 1H, Im-5H), 7.05 (s, 1H, Im-4H), 2.79 (s, 6H, CH₃).

4-(4-Bromo-benzyl)-2-(1-methyl-1-trimethylsilyl-ethyl)-imidazole-1-sulfonic acid dimethylamide (41). Compound **40** (10 g, 57 mmol) was dissolved in dry THF (200 mL) and cooled to -70 °C. Next, n-BuLi (37.5 mL 1.6 M in n-hexane, 60 mmol) was added drop-wise while maintaining a temperature of -65 °C. After stirring for 15 min at this temperature, t-butyldimethylsilylchloride (8.6 g, 57 mmol) was added drop-wise while maintaining a temperature of -60 °C. The mixture was slowly warmed to room temperature and stirred for 1 h. The mixture was now cooled to -70 °C again and n-BuLi (34 mL 1.6 M in n-hexane, 55 mmol) was added drop-wise. After stirring at -65 °C for 30 min 4-bromobenzylbromide (14.25 g, 57 mmol) dissolved in 15 mL of dry THF was added at -60 °C. The mixture was allowed to warm to room temperature and stirred overnight. Water was added and the layers were separated. The water layer was extracted with diethylether twice and the combined organic layers were dried on Na₂SO₄ and evaporated *in vacuo*. This way, 29.2 g of material was obtained that was of enough purity level to be carried on to the next step (Yield > 95 %). ¹H-NMR (CDCl₃) δ 7.33 (d, J =

7.3Hz, 2H), 6.96 (d, J = 7.3 Hz, 2H), 6.62 (s, 1H), 3.97 (s, 2H), 2.60 (s, 6H), 0.93 (s, 9H), 0.29 (s, 6H).

4-(4-Bromo-benzyl)-1H-imidazole, maleate salt (23). Compound **41** (1.4 g, 3.05 mmol) was dissolved in 50 mL of hydrobromic acid (30%) and refluxed overnight. The mixture was washed with diethylether and evaporated. A 20% solution of NaOH in water was added to the remaining residue and the suspension was extracted with DCM (3 x 50 mL). The combined organic layers were dried on Na₂SO₄ and evaporated *in vacuo*. Yield = 700 mg (2.95 mmol, 97%). ¹H-NMR (CDCl₃) δ 7.93 (s, 1H, Im-2H), 7.78 (d, 2H, o-Ph (tosyl), 7.38-7.24 (m, 4H, Ph (Br)), 7.03 (d, 2H, m-Ph (tosyl), 6.89 (s, 1H, Im-5H), 3.79 (s, 2H, CH₂). The maleate salt was prepared by dissolving the compound in EtOAc and adding maleic acid in EA/EtOH. After filtration, heating and cooling white needles appeared. Yield: 110 mg (31 mmol, 32%). M.p.= 115 °C. ¹H-NMR (DMSO-d6) δ 8.85 (s, 1H, Im-2H), 7.55 (d, 2H, o-Ph), 7.38 (s, 1H, Im-5H), 7.25 (d, 2H, m-Ph), 6.07 (s, 2H, CH), 3.99 (s, 2H, Ph-CH₂). ¹³C-NMR (DMSO-d6) δ 166.9, 137.0, 135.2, 134.2, 132.2, 131.2, 130.6, 119.7, 116.3, 29.3. M + 1 (ESI-MS) = 237.2.

4-Biphenyl-3-ylmethyl-imidazole-1-sulfonic acid dimethylamide (42a). Compound **38** (600 mg, 1.8 mmol) was dissolved in DMF (15 mL) and sodium carbonate (460 mg, 4.3 mmol) dissolved in 1 mL water was added. This mixture was degassed by sonication for 1 h, while bubbling nitrogen gas through. Phenylboronic acid (0.23 g, 1.9 mmol) and Pd(PPh₃)₄ (60 mg, 3 mol%) were added under a nitrogen atmosphere and the black mixture was heated to 90 °C for 16 h. Next, the mixture was diluted with EA and washed with water (4x). The organic layer was dried on Na₂SO₄ and evaporated *in vacuo*. The compound was dissolved in DCM, 2 M HCl was added and the layers were separated. The water layer was washed with DCM twice more. The water layer was now basified with sodium carbonate and extracted with DCM (3x). The combined organic layers were dried on MgSO₄ and evaporated in vacuo. Yield = 550 mg (1.6 mmol, 91%). ¹H-NMR (CDCl₃) δ 7.85 (s, 1H, Im-2H), 7.39-7.20 (m, 9H, Ph), 6.91 (s, 1H, Im-5H), 3.99 (s, 2H, Ph-CH₂), 2.81 (s, 6H, CH₃).

4-(3-Pyridin-2-yl-benzyl)-imidazole-1-sulfonic acid dimethylamide (42b). Compound **38** (600 mg, 1.8 mmol) dissolved in DMF (25 mL) was degassed by sonication for 1 h, while bubbling nitrogen gas through. Stannane **43** (0.46 mL, 1.1 equiv) and Pd(PPh₃)₄ (100 mg, 3 mol%) were added under a nitrogen atmosphere and the black mixture was heated to 90 °C for 16 h. Next, the mixture was diluted with EA and washed with water (4x). The organic layer was dried on Na₂SO₄ and evaporated *in vacuo*. Flash chromatography on silicagel and EA as the eluent afforded the product. Yield = 180 mg (0.52 mmol, 30%). ¹H-NMR (CDCl₃) δ 8.68 (d, 1H, Pyr-2H), 7.91 (s, 1H, Im-2H), 7.88-7.20 (m, 7H, Ph + Pyr-3H + Pyr-4H + Pyr-5H), 6.95 (s, 1H, Im-5H), 4.05 (s, 2H, Ph-CH₂), 2.85 (s, 6H, CH₃).

4-(3-Pyridin-4-yl-benzyl)-imidazole-1-sulfonic acid dimethylamide (42c). This procedure was carried out according to the synthesis of **42a** using 420 mg (1.22 mmol) of **38** and pyridine-4-boronic acid pinacol cyclic ester. Yield = 110 mg (0.32 mmol, 26%). ¹H-NMR (CDCl₃) δ 8.65 (d, 2H, Pyr-2H + Pyr-6H), 7.85 (s, 1H, Im-2H), 7.55-7.26 (m, 6H, Ph + Pyr-3H + Pyr-4H), 6.92 (s, 1H, Im-5H), 4.02 (s, 2H, Ph-CH₂), 2.83 (s, 6H, CH₃).

4-Biphenyl-3-ylmethyl-1H-imidazole (14). This procedure was carried out according to the synthesis of **39** using 550 mg (1.6 mmol) of **42a**. Flashchromatography with EA as eluent and crystallization from EA gave the product as a solid. Yield = 200 mg (0.93 mmol, 58%). M.p. = 202-203 °C. ¹H-NMR (CDCl₃) δ 8.75 (s, 1H, Im-2H), 7.40-7.13 (m, 9H, Ph), 6.95 (s, 1H, Im-5H), 4.09 (s, 2H, Ph-CH₂). ¹³C-NMR (CDCl₃) δ 133.5, 129.2, 128.5, 127.3, 127.2, 127.2, 126.8, 126.0, 115.6, 30.7 30.6. M+1 (ESI-MS) = 235.3.

2-[3-(1H-Imidazol-4-ylmethyl)-phenyl]-pyridine, maleate salt (15). This procedure was carried out according to the synthesis of **39** using 180 mg (0.52 mmol) of **42b**. Flashchromatography with EA as eluent afforded the free base. Yield = 120 mg (0.51 mmol, 98%). The maleate salt was prepared as follows. The free base was dissolved in EA. Maleic acid was dissolved in EA/EtOH and added drop-wise to the free base. A precipitate was formed, which was filtered and recrystallized from EA/EtOH. Yield = 35 mg. M.p. = 128 °C. ¹H-NMR (CD₃OD) δ 8.82 (s, 1H, Im-2H), 8.67 (d, 1H, Pyr-2H), 8.00-7.37 (m, 8H, Ph + Pyr-3H + Pyr-4H + Pyr-5H + Im-5H), 6.32 (s, 2H, CH), 4.22 (s, 2H, Ph-CH₂). ¹³C-NMR (CD₃OD) δ 169.9, 158.0, 149.6, 140.2, 139.0 134.5, 134.1, 130.9, 130.7, 128.6, 127.3, 124.6, 123.4, 117.7, 31.4. M + 1 (ESI-MS) = 236.4.

4-[3-(1H-Imidazol-4-ylmethyl)-phenyl]-pyridine (16). This procedure was carried out according to the synthesis of **39** using 110 mg (0.32 mmol) of **42c**. Flashchromatography with EA as eluent and crystallization from EA gave the product as a solid. Yield = 55 mg (0.23 mmol, 73%). M.p.= 131 °C. ¹H-NMR (CDCl₃) δ 8.59 (d, 2H, Pyr-2H + Pyr-6H), 7.55-7.20 (m, 7H, Im-2H + Ph + Pyr-3H + Pyr-4H), 6.75 (s, 1H, Im-5H), 6.53 (s, 1H, NH), 4.02 (s, 2H, Ph-CH₂). ¹³C-NMR (CDCl₃) δ 150.7, 150.5, 142.6, 138.9, 137.8, 136.3, 132.1, 130.9, 130.6, 130.4, 128.4, 126.0, 123.2, 118.1, 34.0. M+1 (ESI-MS) = 236.1.

4-(4-Bromo-benzyl)-1-(toluene-4-sulfonyl)-1H-imidazole (44). Compound **23** (700 mg, 2.95 mmol) was dissolved in 50 mL of dry DCM. TEA (1 mL, 7.2 mmol) was added at once and tosylchloride (590 mg, 3.3 mmol) was added drop-wise. After 4 d, a saturated solution of NaHCO₃ was added, the layers were separated and the DCM layer was washed twice more with the same solution. Finally, the DCM was dried on Na₂SO₄ and evaporated *in vacuo*. Yield = 1.07 g (2.8 mmol, 94%); ¹H-NMR (CDCl₃) δ 7.93 (s, 1H, Im-2H), 7.78 (d, 2H, o-Ph (tosyl), 7.38-7.24 (m, 4H, Ph (Br)), 7.03 (d, 2H, m-Ph (tosyl), 6.89 (s, 1H, Im-5H), 3.79 (s, 2H, CH₂), 2.44 (s, 3H, CH₃).

4-[4-(3,3-Dimethyl-but-1-ynyl)-benzyl]-1-(toluene-4-sulfonyl)-1H-imidazole (45). DMF (15 mL) and TEA (2 mL) were added to a small flask with septum. Nitrogen gas was bubbled through while sonicating for 1 h. In another small flask, **44** (500 mg, 1.31 mmol), CuI (25 mg, 10 mol%) and Pd(PPh₃)₄ (70 mg (5 mol%)) were weighed and a cooler with septum was placed on top. After nitrogen gas was led through, the DMF/TEA mixture and 3,3-dimethyl-1-butyne (4 mL, 36.6 mmol) were added. The black mixture was heated to 70 °C for 3 h and stirred at r.t. overnight. Next, the mixture was diluted with 60 mL of ether. This mixture was washed with water (50 mL x 3). The organic layer was dried on Na₂SO₄ and evaporated *in vacuo*. Flash chromatography of the remaining black oil using EA/n-hexane (1:1) as the eluent, yielded the expected product. Yield = 250 mg. (0.64 mmol, 49%); ¹H-NMR (CDCl₃) δ 7.93 (s, 1H, Im-2H),

7.76 (d, 2H, o-Ph (tosyl), 7.36-7.20 (m, 4H, Ph (Br)), 7.08 (d, 2H, m-Ph (tosyl), 6.80 (s, 1H, Im-5H), 3.82 (s, 2H, CH₂), 2.40 (s, 3H, CH₃), 1.27 (s, 9H, CH₃).

4-[4-(3,3-dimethyl-but-1-ynyl)-benzyl]-1H-imidazole (24). Imidazole **45** (250 mg, 0.64 mmol) was dissolved in 15 mL methanol and 2 mL of a 1M NaOH solution. The remaining suspension was stirred for 2 h while gently heating with a water bath. Water was added, the methanol was evaporated *in vacuo* and more NaOH solution and EA were added. The layers were separated and the EA layer was washed with 0.2M NaOH twice more and with brine. Finally, the organic layer was dried on Na₂SO₄ and evaporated *in vacuo*. Yield: 190 mg (light green crystals). To remove the green color (copper), the compound was taken up in EA and washed with NH₄Cl solution and with an imidazole solution. Unfortunately, the green color remained, but the yield was decreased as a result of this procedure. Yield: 40 mg (0.17 mmol, 27%); M.p.= 179 °C. ¹H-NMR (CDCl₃/CD₃OD) δ 7.70 (s, 1H, Im-2H), 7.28-7.10 (m, 4H, Ph), 6.82 (s, 1H, Im-5H), 3.95 (s, 2H, CH₂), 1.30 (s, 9H, CH₃). ¹³C-NMR (CDCl₃) δ 138.7, 131.4, 128.3, 121.7, 98.0, 78.6, 30.8, 27.7. M + 1 (ESI-MS) = 239.2.

4-[3-(3,3-Dimethyl-but-1-ynyl)-benzyl]-imidazole-1-sulfonic acid dimethylamide (46a). This procedure was carried out according to the synthesis of **45** starting from 500 mg (1.45 mmol) of **38**. Yield = 280 mg (0.81 mmol = 56%). ¹H-NMR (CDCl₃) δ 7.85 (s, 1H, Im-2H), 7.30-7.12 (m, 4H, Ph), 6.83 (s, 1H, Im-5H), 3.87 (s, 2H, CH₂), 2.82 (s, 6H, CH₃), 1.29 (s, 9H, CH₃).

4-(3-Phenylethynyl-benzyl)-imidazole-1-sulfonic acid dimethylamide (46b). This procedure was carried out according to the synthesis of **45** starting from 490 mg (1.42 mmol) of **38**. Yield = 200 mg (0.55 mmol = 39%). ¹H-NMR (CDCl₃) δ 7.85 (s, 1H, Im-2H), 7.55-7.20 (m, 10H, Ph + Im-5H), 3.92 (s, 2H, CH₂), 2.85 (s, 6H, CH₃).

4-[3-(3,3-Dimethyl-but-1-ynyl)-benzyl]-1H-imidazole (18). This procedure was carried out according to the synthesis of **39**, starting from 280 mg (0.81 mmol) of **46a**. Flashchromatography with EA as eluent and crystallization from EA gave the product as a solid. Yield 70 mg (0.29 mmol, 36%). M.p. = 148 °C. ¹H-NMR (CDCl₃) δ 7.55 (s, 1H, Im-2H), 7.30-7.05 (m, 4H, Ph), 6.75 (s, 1H, Im-5H), 3.91 (s, 2H, CH₂), 1.28 (s, 9H, CH₃). ¹³C-NMR (CDCl₃) δ 139.3, 134.7, 131.7, 129.4, 128.2, 127.8, 124.0, 117.5, 98.3, 78.8, 77.5, 76.9, 76.2, 33.0, 30.9, 27.7. M + 1 (ESI-MS) = 239.3.

4-(3-Phenylethynyl-benzyl)-1H-imidazole (19). This procedure was carried out according to the synthesis of **39**, starting from 200 mg (0.55 mmol) of **46b**. Flashchromatography with EA as eluent and crystallization from EA gave the product as a solid. Yield 134 mg = 0.52 mmol, 95%). M.p.= 124 °C. ¹H-NMR (CDCl₃) δ 7.55-7.20 (m, 10H, Ph + Im-2H), 6.75 (s, 1H, Im-2H), 3.95 (s, 2H, CH₂). ¹³C-NMR (CDCl₃) δ 139.6, 131.7, 131.4, 129.5, 128.7, 128.4, 128.2, 128.1, 123.1, 123.0, 89.2, 89.2. M + 1 (ESI-MS) = 268.1.

4-(3-Naphtylethynyl-benzyl)-1H-imidazole, maleate salt (20). NMP (6 mL) was added to a small flask with septum. Nitrogen gas was bubbled through while sonicating for 1 h. To this solution was added **39** (396 mg, 1.67 mmol), CuI (32 mg, 0.167 mmol) and Pd(PPh₃)₄ (123 mg, 0.1068 mmol). Nitrogen gas was bubbled through and a cooler with septum was placed on top. Then, TEA (2.3 mL) and ethynylnaphtalene (3.8 g, 25.05 mmol) were added. The mixture was

heated at 80 °C overnight. Next, the mixture was diluted with 60 mL of ether. This was washed with 2 N HCl, and the aqueous layer back-extracted twice with ether. Then, the aqueous layer was basified with Na₂CO₃ and washed once with EtOAc. The organic layer was washed with satd. NH₄Cl-soln, water (3x) and brine (1x). The organic layer was dried on Na₂SO₄ and evaporated *in vacuo*. Flash chromatography of the remaining oil using 4 % MeOH / 4 % TEA / EtOAc as the eluent yielded the expected product. Yield = 326 mg (63%). The maleate salt was prepared by addition of maleic acid, after which instant precipitation of the salt occurred. M.p. = 136.6 – 136.9 °C. ¹H-NMR (DMSO-d6) δ 8.9 (s, 1H, ImH), 8.4 (d, 1H, naphthH), 8.0 (d, 2H, NapthH), 7.85-7.20 (m, 8H, Ph +NapthH), 7.4 (s, 1H, ImH), 6.0 (s, 2H, maleate), 4.0 (s, 2H, CH₂). ¹³C-NMR (DMSO-d6) δ 166.96, 138.30, 135.23, 123.24, 132.59, 132.18, 132.12, 131.29, 130.25, 129.71, 129.01, 128.95, 128.34, 127.14, 126.58, 125.42, 125.12, 122.28, 119.29, 116.33, 93.86, 87.10, 29.56. M + 1 (ESI-MS) = 309.1.

4-(3-Pent-1-ynyl-benzyl)-1H-imidazole, maleate salt (21). This procedure was carried out according to the synthesis of **45** starting from 350 mg (1.48 mmol) of **39**. Flash chromatography on silicagel with 2% TEA and 2% MeOH in chloroform as the solvent mixture yielded the product as an oil. Yield = 121 mg (0.54 mmol, 36%). ¹H-NMR (CDCl₃) δ 7.55 (s, 1H, Im-2H), 7.45-7.05 (m, 4H, Ph), 6.75 (s, 1H, Im-5H), 6.15 (m, 1H, CH), 3.91 (s, 2H, CH₂), 2.3-1.55 (m, 4H, cyclohexyl). The maleate salt was prepared by addition of maleic acid, after which instant precipitation of the salt occurred. M.p. = 108.6 – 110.0 °C. ¹H-NMR (DMSO-d6) δ 8.9 (s, 1H, ImH), 7.4 (s, 1H, ImH), 7.3 – 7.1 (m, 4H, PhH), 6.0 (s, 2H, maleate), 4.0 (s, 2H, ArCH₂), 2.4 (t, 2H, C≡CCH₂), 1.5 (p, 2H, C≡CCH₂CH₂), 0.9 (t, Me). ¹³C-NMR (DMSO-d6) δ 166.96, 137.95, 135.26, 134.18, 132.15, 131.05, 129.34, 128.65, 127.91, 123.26, 116.27, 90.49, 80.26, 29.47, 21.37, 20.26, 13.10. M + 1 (ESI-MS) = 225.2.

1-[3-(1H-Imidazol-4-ylmethyl)-phenyl]-3,3-dimethyl-butan-1-one (17). Compound **18** (200 mg, 0.84 mmol) was dissolved in TFA (15 mL), water (1 mL) was added and this mixture was heated to 75 °C for 4 h. More water was added and the mixture was basified with sodium carbonate. The water layer was extracted with EA twice. 2 M HCl was added to the combined EA layers and the layers were separated. The water layer was basified and extracted with EA (3x). The EA was dried on MgSO₄ and evaporated *in vacuo*. Yield 100 mg (0.39 mmol, 47%). M.p.= 101 °C. ¹H-NMR (CDCl₃) δ 7.80-7.71 (m, 2H, Im-2H + o-Ph), 7.55-7.30 (m, 3H, o, m, p-Ph), 6.72 (s, 1H, Im-5H), 3.99 (s, 2H, Ph-CH₂), 2.85 (s, 2H, COCH₂), 1.03 (s, 9H, CH₃). ¹³C-NMR (CDCl₃) δ 200.7, 140.3, 138.4, 135.0, 133.1, 128.4, 128.2, 126.1, 116.8, 49.9, 33.2, 31.2, 29.9. M + 1 (ESI-MS) = 257.2.

2-(3-bromophenyl)-4,4,6-trimethyl-5,6-dihydro-4H-1,3-oxazine (47a). 3-Bromobenzonitrile (64 g, 0.36 mol) is added portionwise to sulfuric acid (100 mL, 0.227 N) at 0°C. The mixture is warmed to room temperature for dissolution, then 2-methyl-2,4-pentanediol (42 g, 0.36 mol) is added dropwise at 0°C. The solution is stirred for 3 d at r.t. After cooling to 0°C, 500 g of ice is added and the solution is basified with aqueous NaOH (50%). The mixture is extracted with toluene. The organic phases are dried over Na₂SO₄, filtered and concentrated under reduced pressure. Distillation under reduced pressure (95-120°C/0.005-0.001 mmHg) afforded, after taking up with benzene, 43.9 g (68 %) of **47a**, which is a known compound.⁵

2-(3-((1H-imidazol-4-yl)methyl)phenyl)-4,4,6-trimethyl-5,6-dihydro-4H-1,3-oxazine (26). A solution of bromide **47a** (43.23 g, 0.154 mol) in THF (50 mL) is added dropwise to a solution of magnesium turnings (4.1 g, 0.170 mole, activated with dibromoethane) at r.t. The mixture is stirred at 66°C (internal temperature) for 30 min, then cooled to room temperature before adding a solution of aldehyde **48**⁶ (52.0 g, 0.154 mole) in THF (500 mL). The reaction mixture is stirred at r.t. for 1.5 h, treated with 8.25 g of solid NH₄Cl and stirred for an additional 1 h. Water (200 mL) and toluene (300 mL) are added successively and the mixture is concentrated under reduced pressure. The residue is taken up in DCM and washed with aqueous NaCl-solution. The organic phases are dried over Na₂SO₄ and concentrated under reduced pressure. The residue is recrystallised from acetonitrile (100 mL) while concentrating under reduced pressure. Filtration gave 59.19 g (71 %) of intermediate (3-(4,4,6-trimethyl-5,6-dihydro-4H-1,3-oxazin-2-yl)phenyl)(1-trityl-1H-imidazol-4-yl)methanol (**49a**) which was carried on without extensive analysis. To a solution of **49a** (15.0 g, 27.7 mmol) in acetic acid (200 mL) is added Pd/C 10% (3 g). The mixture is stirred at 60°C for 4 h under 135 Psi H₂. The mixture is then filtered, concentrated, dissolved in toluene and concentrated under reduced pressure to remove residual acetic acid. The residue is dissolved in 40 mL of MeOH, filtered and the filtrate is concentrated under reduced pressure. Purification by chromatography over silica gel (DCM:MeOH 90:10) provided 4.76 g (65 %) of the title compound **26**. ¹H-NMR (CDCl₃) δ 7.76 (s, 1H), 7.71 (m, 1H), 7.38 (s, 1H), 7.25 (m, 1H), 7.23 (m, 1H), 6.59 (s, 1H), 4.30 (m, 1H), 3.90 (s, 2H), 1.82 (dd, 13.3, J=2.5 Hz, 1H), 1.44 (m, 1H), 1.39 (d, J=6 Hz, 3H), 1.29 (s, 3H), 1.25 (s, 3H). ¹³C-NMR (CDCl₃) δ 154.7, 139.4, 134.7, 134.4, 130.8, 128.4, 128.3, 127.9, 125.6, 117.8, 68.5, 50.6, 42.3, 42.3, 33.4, 32.0, 29.9, 21.6. M + 1 (LR-MS) = 284.

(3-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl)(1-trityl-1H-imidazol-4-yl)methanol (49b). This procedure was carried out as described for **49a** in the procedure for **26**, starting with 2-(3-bromophenyl)-4,4-dimethyl-4,5-dihydrooxazole⁷ (78 g, 0.308 mol) and aldehyde **48** (103.8 g, 0.307 mol) to provide the title compound (90 g, 57 %). M.p.= 95-105°C. ¹H-NMR (CDCl₃) δ 8.0 (s, 1H), 7.8 (d, 1H), 7.6 (d, 1H), 7.4 – 7.0 (m, 18H), 6.6 (s, 1H), 5.8 (s, 1H), 4.0 (s, 2H), 1.3 (s, 6H).

2-(3-((1H-imidazol-4-yl)methyl)phenyl)-4,4-dimethyl-4,5-dihydrooxazole (10). This procedure was carried out as described for **26**, starting with alcohol **49b** (15.8 g, 31 mmol), catalyst (3 g) and 80 Psi H₂ to provide the title compound (1.52 g, 19.4%). M.p. 135-140°C. ¹H-NMR (CDCl₃) δ 7.75 (s, 1H), 7.7 (m, 1H), 7.4 (s, 1H), 7.35 – 7.20 (m, 2H), 6.6 (s, 1H), 4.1 (s, 2H), 3.9 (s, 2H), 1.3 (s, 6H). ¹³C-NMR (CDCl₃) δ 162.28, 140.09, 136.57, 134.89, 131.71, 128.34, 128.29, 127.75, 125.98, 116.72, 78.91, 67.29, 33.24, 28.24. M + 1 (LR-MS) = 256.2. C₁₂H₁₄N: calc. 70.51, 6.87, 15.58. Found: 71.48, 6.85, 15.51. DSC 104.6°C.

2-(4-((1H-imidazol-4-yl)methyl)phenyl)-4,4-dimethyl-4,5-dihydrooxazole (11). Compound **11** was retrieved from UCB's compound collection. It can be prepared in four steps from aldehyde **48** and 2-(4-bromophenyl)-4,4-dimethyl-4,5-dihydrooxazole⁷ utilizing a Grignard addition (as in **49a**, see synthesis **26**), acetylation (see synthesis **35a**), hydrogenation (see synthesis **36a**) and acidic deprotection (see synthesis **12**). M.p. = 112-116 °C. ¹H-NMR (CDCl₃) δ 7.80 (d, 2H), 7.45 (s, 1H), 7.20 (d, 2H), 6.55 (s, 1H), 4.1 (s, 1H), 3.9 (s, 2H), 1.3 (s, 6H). ¹³C-NMR (CDCl₃) δ 162.16, 143.32, 136.63, 134.82, 128.56, 128.20, 125.70, 16.73, 78.90, 67.29, 33.47, 28.27. M + 1 (LR-MS) = 256.3.

3-((1H-imidazol-4-yl)methyl)benzoic acid hydrochloride (51). KOH (3.37 g, 60.0 mmol) and water (20 mL) were added to a solution of protected nitrile **36a** (1.42 g, 3.34 mmol) in ethanol (30 mL). This was refluxed overnight. Hydrochloric acid (2.0 M, 20 mL) was added and the mixture was extracted three times with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo* to give intermediate carboxylic acid **50**. Hydrochloric acid (1.0 M, 25 mL) was added to the residue and this was refluxed for 1 h. The mixture was extracted three times with diethyl ether, after which the water was evaporated *in vacuo*. The title compound was obtained as a solid (437 mg, 55 %). $^1\text{H-NMR}$ (DMSO-d_6) δ 14.5 (br s, 1H), 9.0 (s, 1H), 7.8 (m, 2H), 7.6 – 7.4 (m, 3H), 4.1 (s, 2H).

3-((1-(4-methoxyphenylsulfonyl)-1H-imidazol-4-yl)methyl)benzoic acid (52). To a stirred solution of acid **51** (1.7g, 7.1 mmol) in DCM (35 mL) was added triethylamine (3 mL, 21.6 mmol). The solution was cooled to 0°C in an ice/water bath and 4-methoxybenzenesulfonyl chloride (1.48 g, 7.2 mmol) was added in portions. The reaction mixture was stirred overnight at r.t. The mixture was diluted with a solution of KHSO_4 (1 M, 25 mL) and subsequently extracted with DCM (2 x 40 mL). The combined organic layers were washed with brine (40 mL) and dried over MgSO_4 . The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (DCM/MeOH 9/1) to afford the title compound (750 mg, 28 %). $^1\text{H-NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 8.0 – 7.8 (m, 5H), 7.5 – 7.3 (m, 2H), 7.1 – 6.9 (m, 3H), 3.9 (s, 2H), 3.9 (s, 3H).

Coupling of acid 52 and polystyrene resin to afford resin 53. To a solution of the acid **52** (1.07 g, 2.9 mmol) in DCM (25 mL) and DMF (1 mL) was added the tetrafluorophenyl-polystyrene resin (2 g, 1 eq) followed by the addition of 4-dimethylaminopyridine (212 mg, 1.74 mmol) and $\text{N,N}'$ -diisopropylcarbodiimide (2.04 mL, 13.05 mmol). The mixture was stirred overnight at r.t. The resin was filtered and rinsed twice with DMF, DCM, MeOH, DCM and dried *in vacuo* to afford the product resin.

Reaction of resin 53 to afford amino-alcohols 54. In case of hydrochloride amino alcohol starting materials, the salts were removed prior to the reaction with polymer supported carbonate (90 mg, 4 eq) and $\text{N,N}'$ -diisopropylamine (0.6 mL, 0.05 eq) in DCM (0.5mL). The mixture was stirred 1 h, filtered and the filtrate was used for the aminolysis reaction.

To resin **53** (50 mg, 1 eq) was added a solution of the respective amino alcohol (0.8 eq) in DCM (0.5 mL). In case of poor solubility in DCM, DMF (100 μL) was added. The mixture was stirred overnight at r.t. The resin was then filtered and rinsed with a solution of DCM (2 x 0.5 mL) to get the products **54**.

Cyclisation of amino alcohols 54 and deprotection. To a concentrated solution of **54** (0.032 mmol) in DCM (0.5 mL) was added a solution of diethylaminosulfur trifluoride (DAST) in DCM (100 μL , 0.7 M, 2 eq). The mixture was stirred for 1.5 h at r.t. The solvent was removed under N_2 stream. To a solution of the residue (0.032 mmol) in MeOH (0.6 mL) was added a solution of NaOH (1.0 M, 0.4 mL). The mixture was stirred overnight at r.t. The solvent was removed under N_2 stream and the residue was purified by preparative LC/MS (basic conditions). Forty-two compounds, amongst which **27-31**, were prepared this way in typical yields of 6 – 14 % over all steps.

Compounds **10** and **26** were also prepared this way, but their synthesis was repeated on larger scale as described elsewhere in this section.

Pharmacological methods.

[³H]-N^α-methylhistamine displacement studies on H₃R. Confluent 182-cm² flasks of CHO cells stably expressing the human histamine H₃ receptor were harvested and centrifuged (3 min, 500 g). The pellets were stored at -20 °C until the day of the experiment. Before use the pellets were dissolved in distilled water and homogenized for 2 s by sonication (40 Watt, Labsonic 1510). The cell homogenates (30-100 µg) were incubated for 2 h at 25 °C with ~1 nM [³H]-N^α-methylhistamine (85.0 Ci/mmol) in 50 mM Tris-HCl buffer containing 2 mM MgCl₂, pH 7.4, with or without competing ligands. The mixture was washed with icecold washing buffer (3 x 250 µL, 50 mM Tris-HCl, pH 7.4). The combined washings were filtered over 0.1 % polyethylenimine-pretreated Whatmann GF/C filters. Retained radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined with 1 µM thioperamide as competing ligand. Protein concentrations were determined spectrometrically (Packard Argus 400 Microplate Reader) using the Bradford reagent with bovine serum albumin as standard.

[³H]-histamine displacement studies on H₄R. The assays were carried out at 25°C for 3h in a 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM MgCl₂, 100 µg of membrane proteins and 4 nM [³H]-histamine (23 Ci/mmol; PerkinElmer, Belgium). Non specific binding was determined in the presence of 10 µM histamine.

[³⁵S]GTPγS binding assay. Stimulation (agonist) or inhibition (inverse agonist) of [³⁵S]GTPγS binding to membrane expressing human H₃ histamine receptors was measured as described by Lorenzen et al⁸ with a few modifications. Briefly, membranes (10-20 µg proteins) expressing human H₃ histamine receptors were incubated at 25°C in 0.2 mL of a 50 mM Tris-HCl buffer (pH 7.4) containing 3 mM MgCl₂, 50 mM NaCl, 1 µM GDP, 2 µg saponin and increasing concentrations of drugs. After 15 min preincubation, 0.2 nM of [³⁵S]GTPγS (1250 Ci / mmol; purchased from Perkin Elmer (Belgium)) were added and the samples were further incubated for 30 min. The non specific binding (NSB) was defined as the residual binding observed in the presence of 100 µM Gpp(NH)p. Membrane-bound and free radioligand were separated by rapid filtration through glass fiber filters. Samples and filters were rinsed by at least 6 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The entire filtration procedure did not exceed 10 seconds per sample. Radioactivity trapped onto the filters was counted by liquid scintillation in a β-counter.

cAMP assays. Transfected cells seeded in 12-well plates (basal levels) or 24-well plates (forskolin-induced levels) were incubated for 10 min at 37 °C with the appropriate drug in DMEM supplemented with 25 mM HEPES (pH=7.4 at 37°C) and 300 µM of the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX). The incubation was stopped by aspiration of the medium and the addition of 200 µL of 0.1 N cold HCl. The cells were kept on ice and disrupted by sonication (2 sec, 40 % output, Sonifier, Branson). The resulting homogenate was frozen at -20 °C or directly neutralized with 1 N NaOH and assayed. The amount of cAMP was determined using a competitive protein kinase A binding assay according to Nordstedt and Fredholm⁹ with some minor modifications.¹⁰ Briefly, 200 µL of protein kinase A was mixed with 25-200 µL of the cell homogenate or cAMP standards and 30.000 dpm [³H]-cAMP. After incubation for 150 min at 4 °C the mixture was rapidly diluted with 3 mL ice-cold 50 mM Tris/HCl (pH=7.4 at 4 °C) and filtered through Whatman GF/B filters using a Brandel cell-

harvester (Semat). The radioactivity retained on the filters was measured by liquid scintillation counting.

Effect of compounds on guinea pig isolated paced ileal myenteric plexus stimulated with R- α -methylhistamine. The method was developed according to Menkveld *et al.*¹¹ Longitudinal myenteric plexus was prepared from the isolated guinea pig ileum. Tissues were mounted in 20-mL organ baths containing modified Krebs' solution with 10^{-6} M mepyramine, 10^{-5} M ranitidine, 10^{-5} M propranolol and 10^{-6} M yohimbine. The bathing solution was maintained at 37 °C and gassed with 95 % O₂- 5 % CO₂. Tissues were allowed to equilibrate for a 60-min period under a resting tension of 0.5 g and an electrical field stimulation (pulses of 5-20 V, 1 ms and 0.1 Hz applied during the whole experiment). Such a stimulation induces stable and reproducible twitch contractions. Isometric contractions were measured by force-displacement transducers coupled to an amplifier connected to a computer system (EMKA Technologies) controlling (i) automatic data acquisition, (ii) bath washout by automatic fluid circulation through electrovalves at predetermined times or signal stability and (iii) automatic dilution/injection of drug in the bath at predetermined times or signal stability. After a 60 min-stabilisation period, paced tissues were stimulated twice with 10^{-6} M (R)-(-)- α -methylhistamine at 30-min interval. After a 60-min incubation period in the presence of solvent or antagonist test compound, a cumulative concentration-response to (R)-(-)- α -methylhistamine was constructed (10⁻¹⁰ to maximum 10⁻⁴ M). Only one antagonist concentration was tested on each tissue. When agonist effect of a compound was tested, (R)-(-)- α -methylhistamine was replaced by this compound in order to construct the concentration-response curve. The pA₂ and pD₂ values describing the potency of an antagonist and pD₂ value describing the potency of an agonist were calculated according to Van Rossum *et al.*¹² The pK_b value was calculated according to Arunlakshana and Schild.¹³

Dextromethorphan O-demethylase assay for CYP2D6 inhibition. Dextromethorphan O-demethylase activity was analyzed by a modification of the method of Wu *et al.*¹⁴ The assays were carried out in a 500 μ l incubate containing human liver microsomes (batch H71, H72 and H81, ca. 0.4 mg microsomal protein/mL), 10 μ mol/l dextromethorphan, 1 mmol/l NADPH, 5 mmol/l MgCl₂ and tested compound at different final concentrations (final DMSO concentration of 1% v/v) in 100 mmol/l phosphate buffer pH 7.4. After a 30 min incubation at 37°C, the reaction was stopped by the addition of 50 μ l of a 50% aqueous trichloroacetic acid solution (v/v). The proteins were pelleted by centrifugation 10 min at 3,000 g. The supernatants were analyzed by reverse phase HPLC using fluorescence detection with an excitation wavelength at 270 nm, an emission wavelength at 312 nm and an isocratic elution with 1 mL/min of 10 mmol/l KH₂PO₄:CH₃OH:CH₃CN:TEMED 69:5:22:1 (v/v/v/v). A Lichrospher 100 RP18, 5 μ m, 125x4 mm (Merck, Darmstadt, Germany) preceded by a guard column was used. Activities are expressed as percentage of control activity measured in the presence of tested compound vehicle.

Midazolam 1'-hydroxylase assay for CYP3A4 inhibition. Midazolam 1'-hydroxylase activity was analyzed by a modification of the method of Ma and Lau.¹⁵ The assays were carried out in a 500 μ l incubate containing human liver microsomes (batch H71, H72 and H80, ca. 0.7 mg microsomal protein/mL), 7.5 μ mol/l midazolam (final methanol concentration of 0.3 % v/v), 2.5 mmol/l NADPH, 5 mmol/l MgCl₂ and the compound of interest at different final concentrations (final DMSO concentration of 1% v/v) in 100mmol/l phosphate buffer pH 7.4. After a 8 min incubation at 37°C, the reaction was stopped by the addition of 250 μ l of CH₃CN. The proteins

were pelleted by a 20 min centrifugation at 1,000 g. The supernatant was used for reverse phase HPLC analysis of 1'-hydroxymidazolam, using UV detection at 235 nm and an isocratic elution with 1 mL/min of CH₃OH:CH₃CN:15 mmol/l sodium acetate at pH 3.0 10:23:67 (v/v/v). A Symmetry C18 5 μ m, 150 x 4.6 mm I.D column (Waters, Milford, MA 01757 USA) preceded by a guard column was used. Activities are expressed as a percentage of control activity measured in the presence of tested compound vehicle.

Selected spectral data.

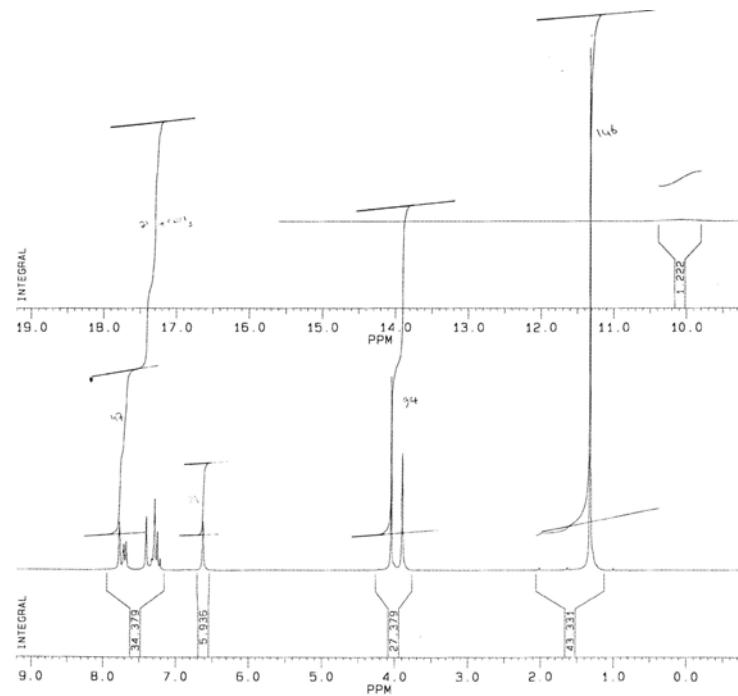


Figure S1. ^1H -NMR spectrum of compound **10** in CDCl_3 .

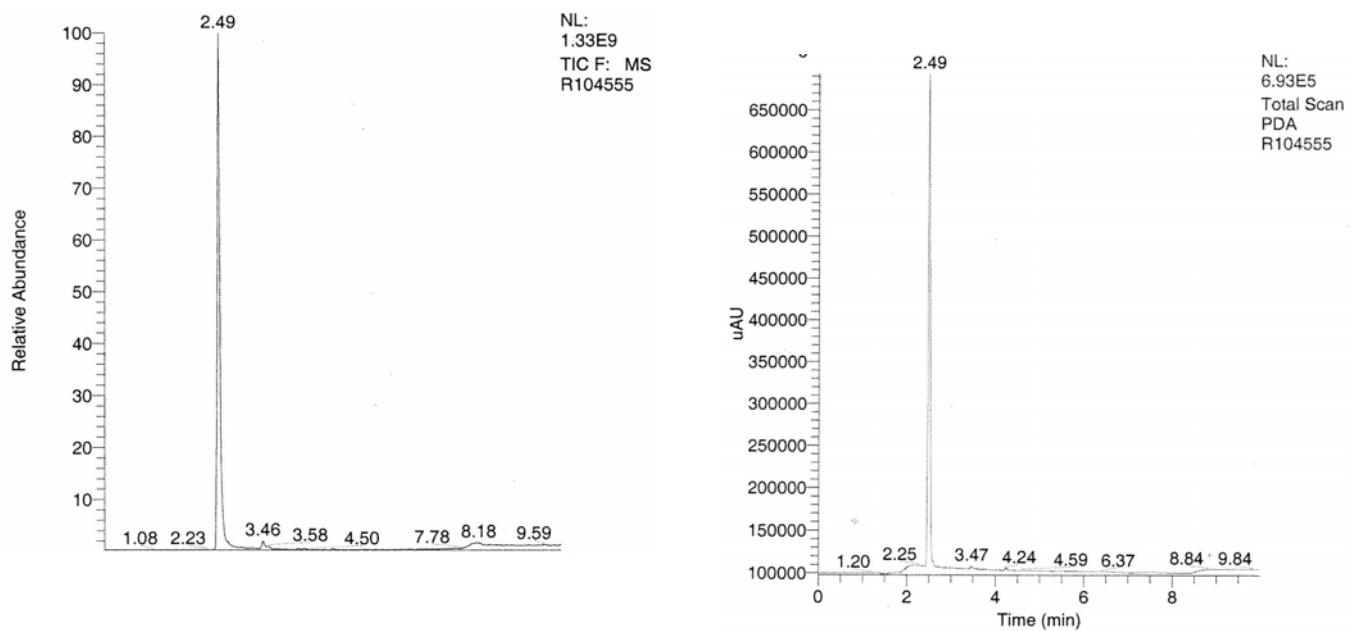


Figure S2. LC-chromatogram of compound **10**.

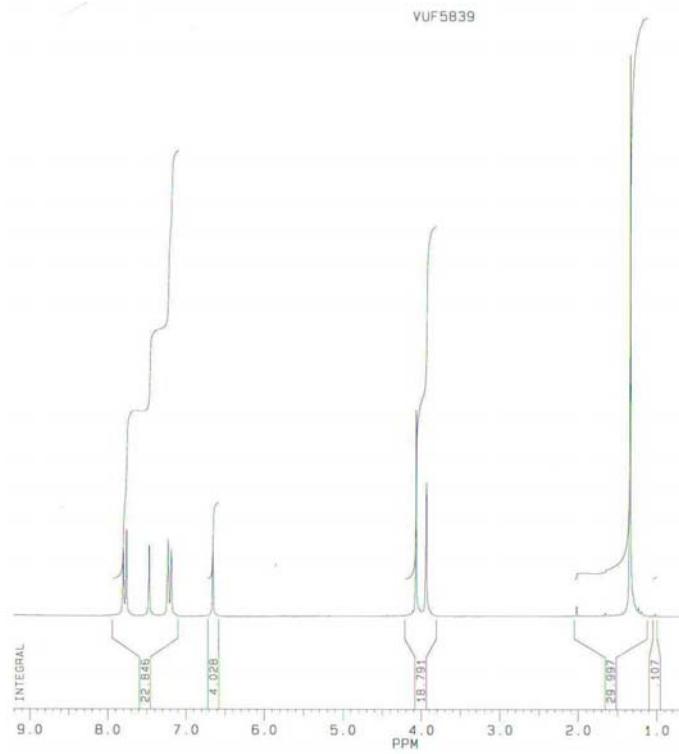


Figure S3. ^1H -NMR spectrum of compound **11** in CDCl_3 .

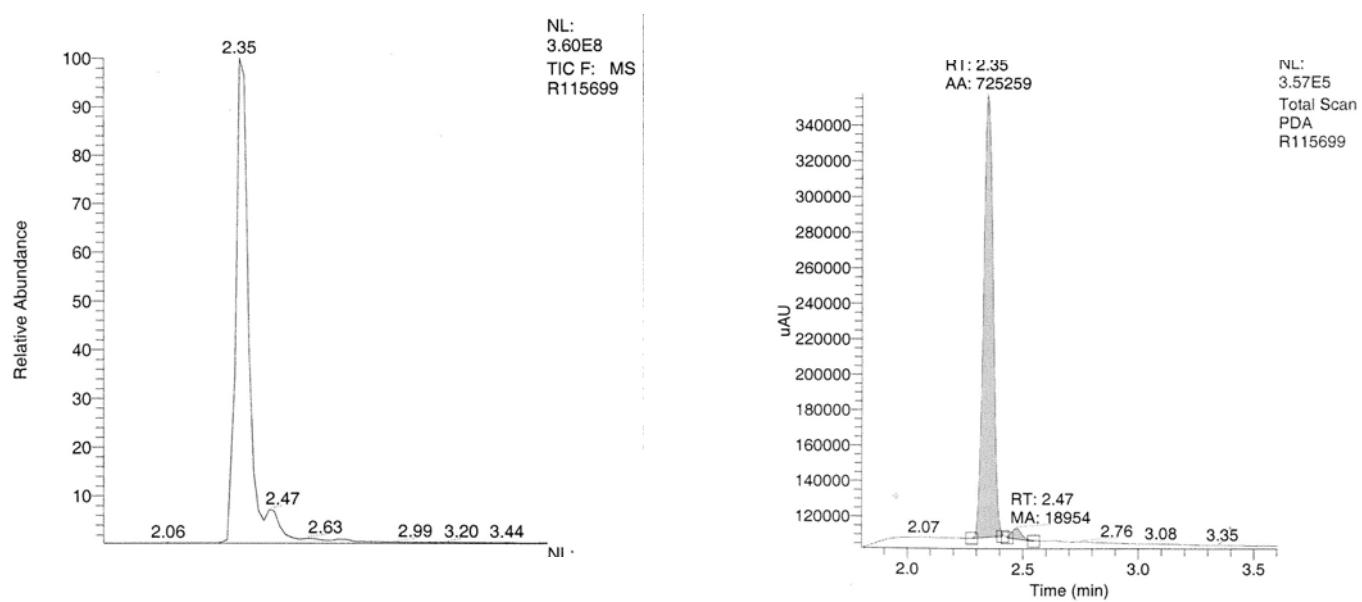


Figure S4. LC-chromatogram of compound **11**.

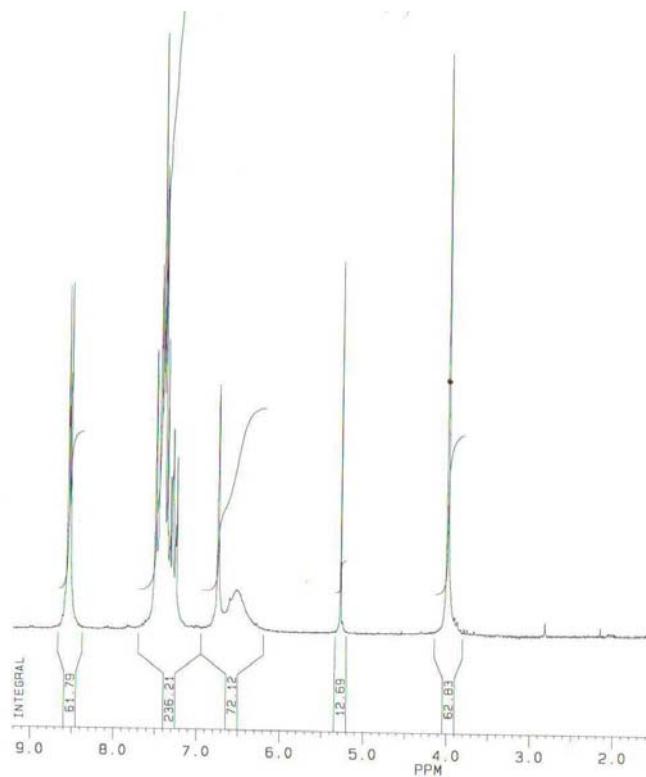


Figure S5. ^1H -NMR spectrum of compound **16** in CDCl_3 .

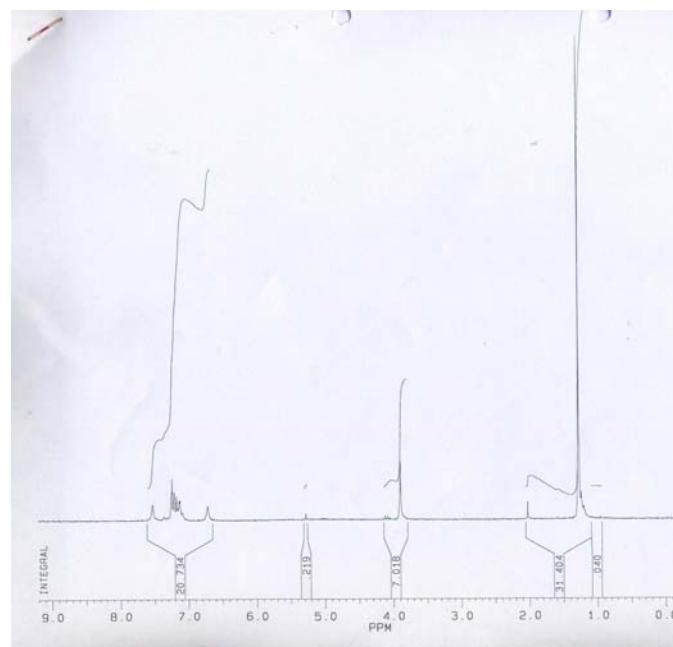


Figure S6. ^1H -NMR spectrum of compound **18** in CDCl_3 . Traces of EtOAc and DCM are visible.

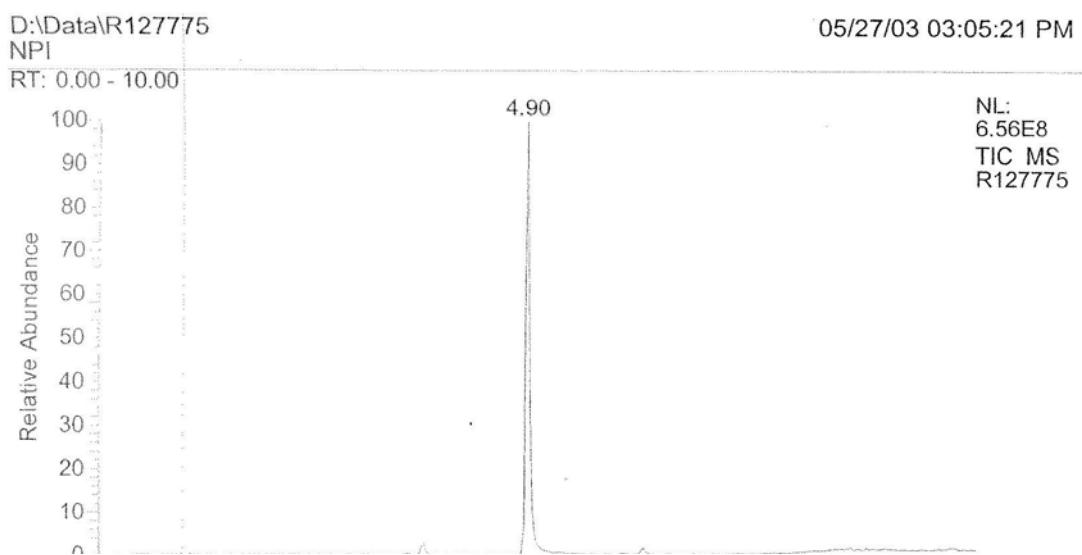


Figure S7. LC-chromatogram of compound **18**.

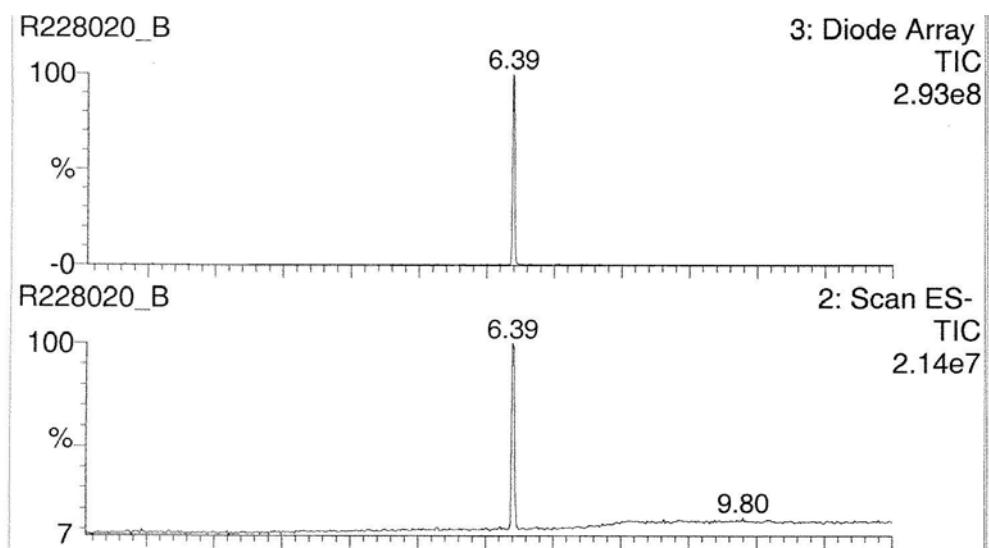


Figure S8. LC-chromatogram of compound **19**. Run time = 12.0 min.

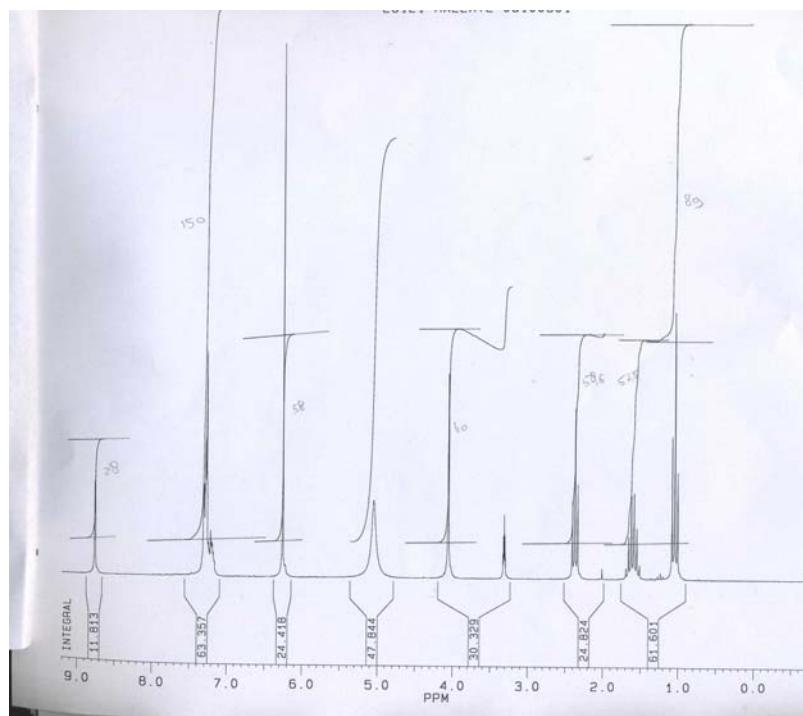


Figure S9. ^1H -NMR spectrum of compound **21.maleate** in CD_3OD .

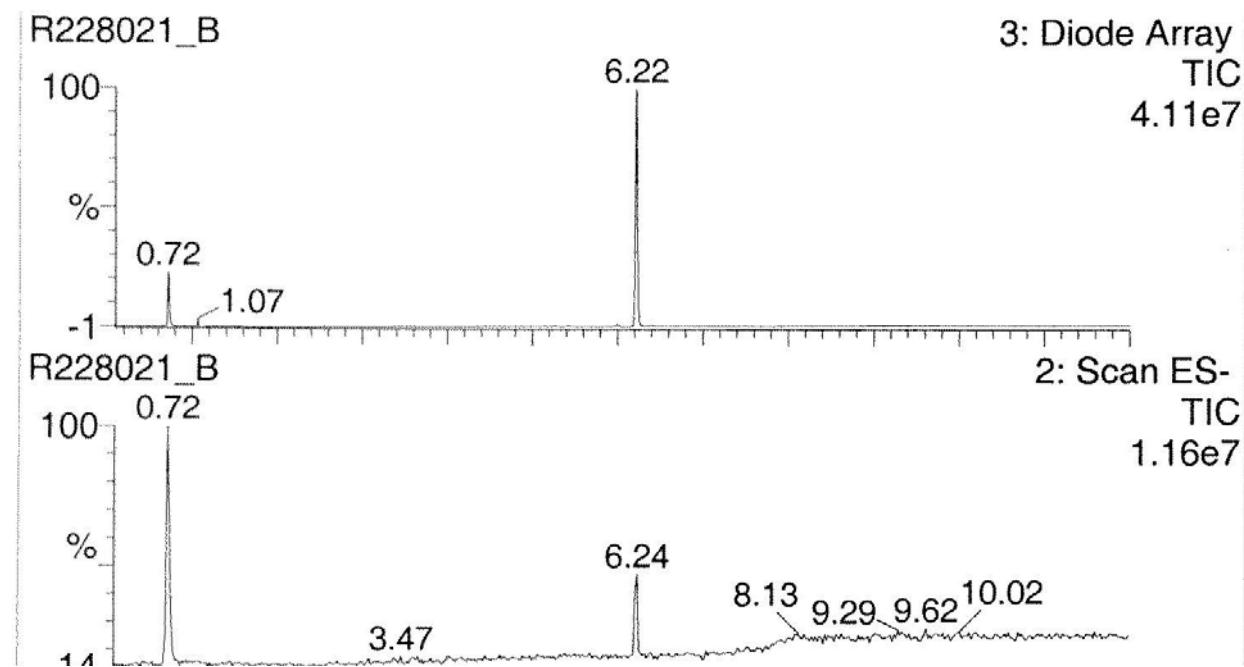


Figure S10. LC-chromatogram of compound **21.maleate**. Run time = 12.0 min. The maleate portion is visible at the front.

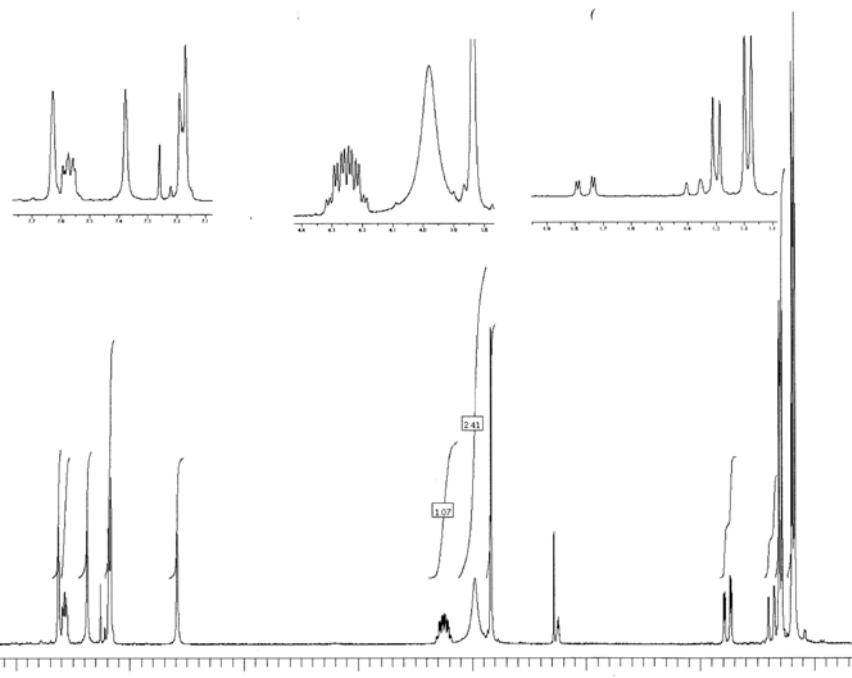


Figure S11. ^1H -NMR spectrum of compound **26** in $\text{CD}_3\text{OD}/\text{CDCl}_3$. A trace of MeOH solvate is visible.

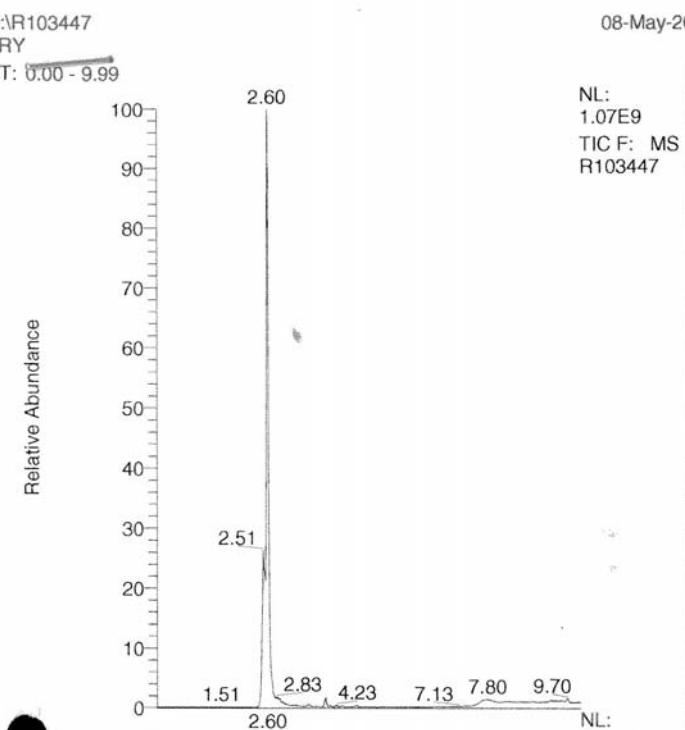


Figure S12. LC-chromatogram of compound **26**. Run time = 12.0 min.

References.

- (1) De Esch, I. J. P.; Gaffar, A.; Menge, W. M. P. B.; Timmerman, H. Synthesis and histamine H-3 receptor activity of 4-(n-alkyl)-1H-imidazoles and 4-(omega-phenylalkyl)-1H-imidazoles. *Bioorg. Med. Chem.* **1999**, *7*, 3003-3009.
- (2) Kitbunnadaj, R.; Hoffmann, M.; Fratantoni, S. A.; Bongers, G.; Bakker, R. A.; Wieland, K.; el Jilali, A.; De Esch, I. J. P.; Menge, W. M. P. B.; Timmerman, H.; Leurs, R. New high affinity H-3 receptor agonists without a basic side chain. *Bioorg. Med. Chem.* **2005**, *13*, 6309-6323.
- (3) Panosyan, F. B.; Still, I. W. J. An efficient route to 5-iodo-1-methylimidazole: synthesis of exstomanzamine A. *Can. J. Chem.* **2001**, *79*, 1110-1114.
- (4) De Esch, I. J.; Vollinga, R. C.; Goubitz, K.; Schenk, H.; Appelberg, U.; Hacksell, U.; Lemstra, S.; Zuiderveld, O. P.; Hoffmann, M.; Leurs, R.; Menge, W. M.; Timmerman, H. Characterization of the binding site of the histamine H3 receptor. 1. Various approaches to the synthesis of 2-(1H-imidazol-4-yl)cyclopropylamine and histaminergic activity of (1R,2R)- and (1S,2S)-2-(1Himidazole- 4-yl)cyclopropylamine. *J. Med. Chem.* **1999**, *42*, 1115-1122.
- (5) Meyers, A. I.; Politzer, I. R.; Bandlish, B. K.; Malone, G. R. Syntheses via dihydro-1,3-oxazines. VI. A carboxyl protecting group stable to the Grignard reagent. A new synthesis of carboxylic acids. *J. Am. Chem. Soc.* **1969**, *91*, 5886-5887.
- (6) Ohkanda, J.; Lockman, J. W.; Kothare, M. A.; Qian, Y.; Blaskovich, M. A.; Sebti, S. M.; Hamilton, A. D. Design and synthesis of potent nonpeptidic farnesyltransferase inhibitors based on a terphenyl scaffold. *J. Med. Chem.* **2002**, *45*, 177 - 188.
- (7) Meyers, A. I.; Temple, D. L.; Haidukewych, D.; Mihelich, E. D. Oxazolines. XI. Synthesis of functionalized aromatic and aliphatic acids. Useful protecting group for carboxylic acids against Grignard and hydride reagents. *J. Org. Chem.* **1974**, *39*, 2787-2793.
- (8) Lorenzen, A.; Fuss, M.; Vogt, H.; Schwabe, U. Measurement of guanine nucleotide-binding protein activation by A1 adenosine receptor agonists in bovine brain membranes: stimulation of guanosine-5'-O-(3-[³⁵S]thio)triphosphate binding. *Mol. Pharm.* **1993**, *44*, 115-123.
- (9) Nordstedt, C.; Fredholm, B. B. A modification of a protein-binding method for rapid quantification of cAMP in cell-culture supernatants and body fluid. *Anal. Biochem.* **1990**, *189*, 231-234.
- (10) Smit, M. J.; Leurs, R.; Alewijne, A. E.; Blauw, J.; Amerongen, G. P. V.; VandeVrede, Y.; Roovers, E.; Timmerman, H. Inverse agonism of histamine H-2 antagonists accounts for upregulation of spontaneously active histamine H-2 receptors. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6802-6807.
- (11) Menkveld, G. J.; Timmerman, H. Inhibition of electrically evoked contractions of guinea-pig ileum preparations mediated by the histamine H3-receptor. *Eur. J. Pharmacol.* **1990**, *186*, 343-347.
- (12) Rossum, J. M. V.; Hurkmans, J. A. T. M.; Wolters, C. J. J. Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn. Ther.* **1963**, *143*, 299-330.
- (13) Arunlakshana, O.; Schild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **1959**, *14*, 48-58.

- (14) Wu, D.; Otton, S. V.; Sproule, B. A.; Busto, U.; Inaba, T.; Kalow, W.; Sellers, E. M. Inhibition of human cytochrome P450 2d6 (Cyp2d6) by methadone. *Br. J. Clin. Pharmacol.* **1993**, *35*, 30-34.
- (15) Ma, F.; Lau, C. E. Determination of midazolam and its metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats. *J. Chromatogr. B Biomed. Appl.* **1996**, *682*, 109-113.