Kojic Acid Derivatives as Histamine H_3 Receptor Ligands

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The histamine H_3 receptor (H_3R) is a promising target in the development of new compounds for the treatment of mainly centrally occurring diseases. However, emerging novel therapeutic concepts have been introduced and some indications in the H_3R field, *e.g.* migraine, pain or allergic rhinitis, might take advantage of peripherally acting ligands. In this work, kojic acid-derived structural elements were inserted into a well established H_3R antagonist/inverse agonist scaffold to investigate the bioisosteric potential of γ-pyranones with respect to the different moieties of the H_3R pharmacophore. The most affine compounds showed receptor binding in the low nanomolar concentration range. Evaluation and comparison of kojic acid-containing ligands and their corresponding phenyl analogues (3—7) revealed that the newly integrated scaffold greatly influences chemical properties (S Log P, topological polar surface area (tPSA)) and hence, potentially modifies the pharmacokinetic profile of the different derivatives. Benzyl-1-(4-(3-(piperidin-1-yl)propoxy)phenyl)methanamine ligands 3 and 4 belong to the centrally acting diamine-based class of H_3R antagonist/inverse agonist, whereas kojic acid analogues 6 and 7 might act peripherally. The latter compounds state promising lead structures in the development of H_3R ligands with a modified profile of action.

Key words histamine; G protein-coupled receptor; topological polar surface area; kojic acid; medicinal chemistry

Histamine is one of the most important chemical messengers in the human (*h*) organism. It mediates its pleiotropic effects *via* four known G protein-coupled receptors (GPCR), the histamine receptors (H_1R—H_4R). H_1R and H_2R are mainly associated with the development of allergy and ulcer, respectively, and the corresponding antagonists have reached blockbuster status in the therapy of these widespread diseases.[1)] The youngest member of the histamine receptor family, H_4R, is involved in inflammatory processes and takes an immunomodulatory role. The first ligands are currently evaluated in an early stage of clinical studies.[2)] The H_3R takes an exceptional role as an auto- and heteroreceptor that is mainly but not exclusively expressed in the central nervous system (CNS). It controls the synthesis and liberation of the endogenous ligand histamine and hence, regulates its concentration in the synaptic cleft. Located on non-histaminergic neurons, the H_3R modulates the release of various other neurotransmitters and neuropeptides.[3)] By keeping a certain neurotransmitter balance in brain compartments with co-localized neurons the H_3R ensures different physiological functions like vigilance, learning, attention, or feeding behaviour. In the peripheral nervous system (PNS) the receptor influences *e.g.* nerve and C fibers involved in the regulation of sympathetic effector systems and pain sensation, respectively. Antagonists/inverse agonists of the H_3R are able to increase the neurotransmitter content and may find their application in the therapy of cognitive diseases, sleep/wake disorders, epilepsy, obesity, pain or allergic rhinitis. Several substances are progressing in clinical trials.[4,5)]

H_3R antagonists/inverse agonists (Fig. 1) follow a general structural blueprint consisting of a basic moiety coupled *via* an alkyl spacer to a central core. This element constitutes a hydrogen bond acceptor, which in most cases is connected to an aromatic ring system, and might be substituted with a variety of polar, lipophilic, basic or acidic moieties or their combination.[6)] Whereas the amino-alkyl-acceptor fragment is required as basic pharmacophore to interact with the western part of the H_3R binding pocket, the target allows for broader variation in the eastern part of the molecule. A second basic moiety usually boosts H_3R affinity as has been shown with many compounds of the class of diamine-based ligands.[7—9)] Computational models support an additional ionic interaction with transmembrane domain 5.[10,11)] However, due to the strong receptor binding and their lipophilicity diamine-based ligands tend to accumulate centrally, which as a result might induce severe side effects and/or phospholipidosis.[12)] One aim of this study was to establish new lead structures that combine the second basic centre with increased hydrophilicity to avoid potentially occurring side effects in an early state of compound development.

Due to high polarity and electron density γ-pyranone derivatives were tested as substitutes of the different elements in the H_3R pharmacophore. Kojic acid, 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one, served as starting point for the preparation of a series of H_3R ligands. This interesting scaffold was first isolated from various fungi strains, *e.g. Penicillum*, *Gluconoacetobacter* and especially *Aspergillus oryzae*, that is commonly called *koji* in Japanese. Koji malt has been used in the production of sake or soya sauce for a long time. A broad field of application is found in food and cosmetic industries. Due to its antioxidant properties it is used to decolourize food or as depigmenting agent. The latter is supported by a strong inhibitory effect on the pigment-generating enzyme tyrosinase.[13—15)] For chemical purposes kojic acid consists of a 4*H*-pyran-4-one scaffold that is substituted with a hydroxyl and a hydroxymethyl group in positions C-3 and C-5, respectively. The resulting enol moiety exhibits a pK_a value of 7.9—8.0 allowing for similar reactions as described for phenols. Kojic acid is a chelating agent that enables bidentate complexes with various metals that have been reported as radical scavengers (Fig. 2).[16—18)] Physicochemical properties of kojic acid and its application in consumer

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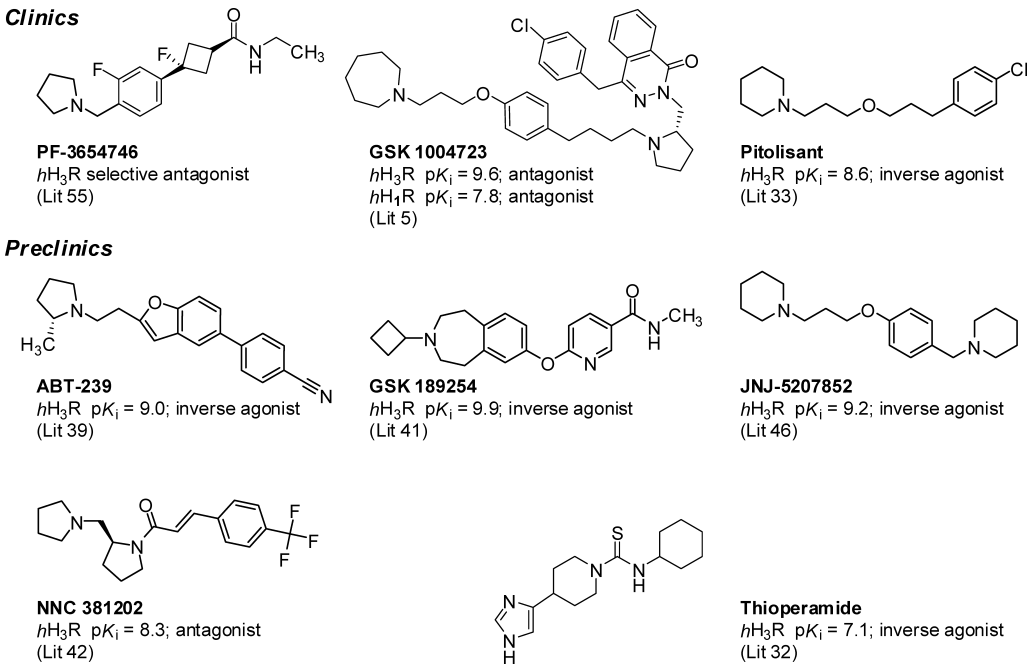


Fig. 1. Reference H_3R Antagonists/Inverse Agonists

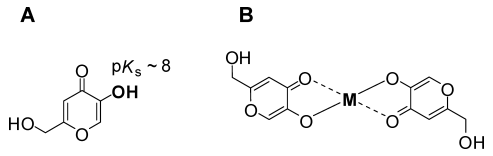


Fig. 2. Acidic (A) and Chelate Complexing (B) Properties of Kojic Acid

goods emphasize the safety of this scaffold.

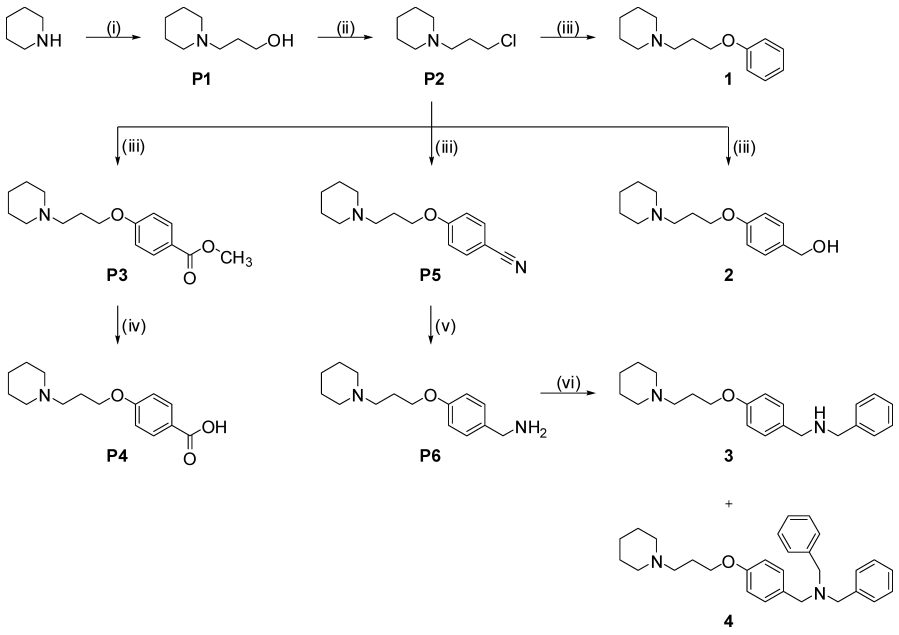
The goal of this work was to combine the H_3R antagonist/inverse agonist pharmacophore with kojic acid-derived elements by integrated and spacer approaches to take advantage of the physicochemical and pharmacological properties of the γ-pyranone scaffold and to modify the pharmacokinetic properties of an existing H_3R pharmacophore.

Chemistry Precursors containing the 1-(3-phenoxypropyl)piperidine pharmacophore were prepared as described before.[9,19,20] Briefly, piperidine was alkylated with 3-chloropropan-1-ol under basic conditions (Chart 1). The resulting alcohol precursor **P1** was chlorinated (**P2**) and subsequently transferred to phenol ether synthesis. Precursor compounds **P3** and **P5** as well as final compounds **1** and **2** were prepared by applying Williamson-like conditions combined with a Finkelstein exchange.[21,22] Derivatization of ester **P3** and nitrile **P5** led to carboxylic acid **P4** and benzyl amine **P6**, respectively.[23] Hence, a series of precursor compounds consisting of the H_3R affine pharmacophore but differing functional moieties was designed as a molecular toolbox. Amine precursor **P6** was the starting point in the preparation of diamine-based ligands. Benzyl amines **3** and **4** were prepared in a one-pot approach by reductive amination of benzaldehyde using sodium triacetoxyborohydride as reducing agent.[24]

Kojic acid (**P7**) was functionalized to obtain a variety of γ-pyranone-containing precursors (Charts 2, 3). Benzyl kojic acid **P8** was synthesized under basic conditions using high-boiling solvents (cesium carbonate in anhydrous *N*,*N*-dimethylformamide (DMF) (a)[25] and soda lye in methanol (b),[26,27] respectively). Although side processes like ring-opening of the vinylogous lactone caused by the attack of the aqueous alkali solution were expected,[28] yields ranged between 50% and 60% in both approaches. Method (b) resulted to be the more practicable one due to faster purification. Precursor **P9** was prepared by chlorination in pure thionyl chloride resulting in 75% conversion to the product.[29] The addition of solvents like toluene to improve a usually quantitative reaction minimized yields even further. **P9** was subsequently reduced using zinc/hydrochloric acid to obtain the methyl derivative **P10**.[30]

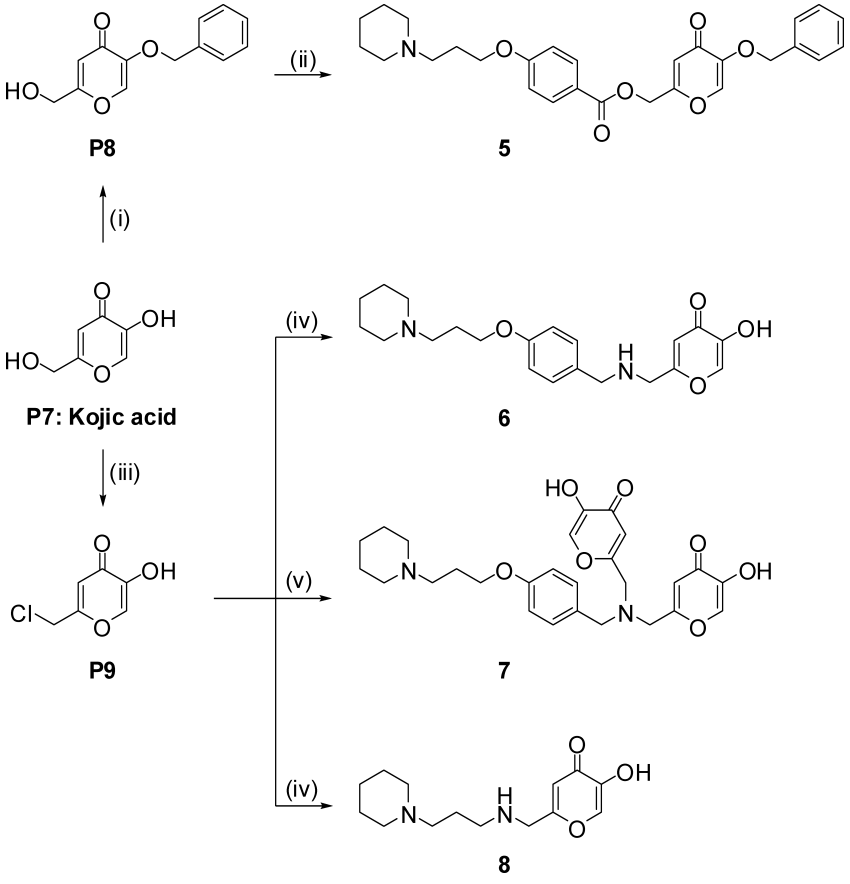
Benzyl kojic acid **P8** was treated with acid **P4** in a Mitsunobu reaction[31] giving compound **5**. Diamine-containing compounds **6**—**8** were obtained by nucleophilic substitution of chlorokojic acid **P9**. Secondary amines **6** and **8** were synthesized in tetrahydrofuran (THF) at ambient temperature, whereas stronger conditions were required for the quantitative conversion into the tertiary amine **7**. Microwave irradiation of the reagents in DMF led to the desired product formation. Purification, especially of the diamine-based compounds, by column chromatography had to occur under harsh conditions. Due to the high polarity of the compounds equivoluminous mixtures of methylene chloride and ammoniacal methanol were regularly used. Compounds were usually crystallized as salts of oxalic acid. Polarity and hydrophilicity frequently resulted in the enclosure of water requiring repeated re-crystallization steps, which minimized overall reaction yields.

Williamson-like conditions, as described before, were applied to get methyl derivative **9**. In contrast, the synthesis of compounds **10** and **11** as well as the corresponding precursors **P11** and **P12** was realized starting from kojic acid (**P7**) due to a more convenient preparation and higher overall yields (Chart 3). After alkylation of the enol moiety of **P7**



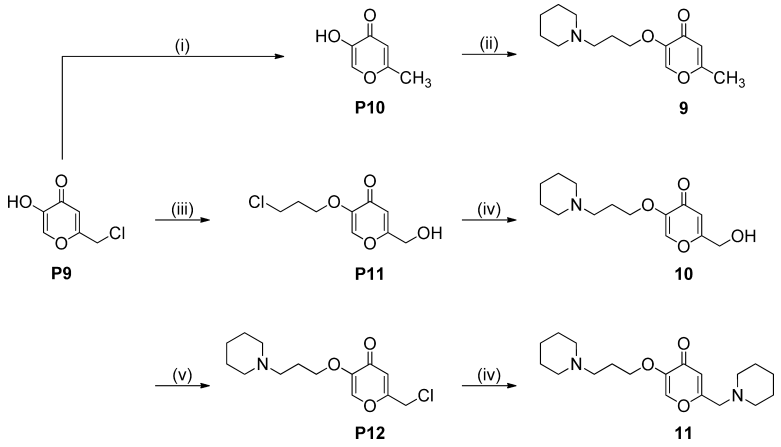
Reagents and conditions: (i) 1. K_2CO_3, KI, acetone, reflux, 3 d; 2. HCl, 2-propanol; 79%. (ii) $SOCl_2$, toluene, 0 °C→60 °C, 3 h; quantitative conversion. (iii) Phenol (**1**)/4-(hydroxymethyl)phenol (**2**)/methyl 4-hydroxybenzoate (**P3**)/4-hydroxybenzonitrile (**P5**), K_2CO_3, KI, acetone, reflux, 2—3 d; 47—61%. (iv) KOH, H_2O, THF, MeOH, μW, 70 °C, 15 min; quantitative conversion. (v) Raney-Ni, MeOH/NH_3, rt, 12 h, 10 bar; 94%. (vi) Benzaldehyde, $Na(OAc)_3$, DCE, rt, 3.5 h; 1—20.5%.

Chart 1. Synthesis of Precursors **P1**—**P6** as well as Final Compounds **1**—**4**



Reagents and conditions: (i) a) Benzyl bromide, Cs_2CO_3, DMF, rt, 24 h; 52%; b) Benzyl bromide, NaOH, MeOH, rf, 12 h; 57%. (ii) **P4**, DEAD, PPh_3, THF, rt, 12 h; 4%. (iii) $SOCl_2$, 0 °C→rt, 1 h; 75%. (iv) **P6** (**6**)/3-(piperidin-1-yl)propan-1-amine (**8**), TEA, THF, rt, 12 h; 5—15%. (v) **P6**, DMF, μW, 30 min, 130 °C; 10%.

Chart 2. Synthesis of Precursors **P8** and **P9** as well as Final Compounds **5**—**8**



Reagents and conditions: (i) Zn, HCl, 70 °C, 3 h; 31%. (ii) **P2**, Cs_2CO_3, KI, DMF, 110 °C, 2 h; 11%. (iii) 1-Bromo-3-chloropropane, Cs_2CO_3, KI, DMF, 110 °C, 2 h; 72%. (iv) Piperidine, K_2CO_3, KI, acetone, 60 °C→rt, 14 h; 3—12%. (v) $SOCl_2$, toluene, 70 °C, 3 h; quantitative conversion.

Chart 3. Synthesis of Precursors **P10**—**P12** and Final Compounds **9**—**11**

the resulting 3-chloropropoxy derivative **P11** was substituted with piperidine to obtain a H_3R pharmacophore-like scaffold with exchanged central core (**10**). The hydroxymethyl group of **10** was subsequently chlorinated (**P12**) and coupled with piperidine resulting in a diamine-containing final compound (**11**). First approaches to insert the two piperidine moieties in one step by applying the activated dialcohol 3-(6-((methylsulfonyloxy)methyl)-4-oxo-4*H*-pyran-3-yloxy)propyl methanesulfonate failed due to compound polymerization.

Pharmacology All final compounds **1**—**11** as well as reference inverse agonists thioperamide[32] and pitolisant (formerly known as BF2.649 or tiprolisant)[33] (*cf.* Fig. 1) were evaluated with regard to their hH_3R affinity using a membrane preparation from HEK-293 cells stably expressing hH_3R. In a competition binding assay the displacement of [^{3}H]N^α-methylhistamine by the respective test compound was determined.[33,34] Thioperamide and pitolisant exhibited hH_3R affinities in the nanomolar concentration range (pK_i=7.1 and 7.9, respectively), which is in accordance with results from other experiments.[33,35—37] The antagonist/inverse agonist efficacy of the 1-(3-phenoxypropyl)piperidine scaffold was proven in several investigations of different research groups, including ours.[38]

Results and Discussion

1-(3-Phenoxypropyl)piperidine (**1**) is the receptor-binding domain of many H_3R antagonists/inverse agonists and incorporates the most important structural elements that ensure receptor interactions, namely the tertiary amine and in a certain distance a polar moiety coupled to an aryl group. In the [^{3}H]N^α-MeHA displacement assay **1** showed moderate hH_3R affinity (pK_i=6.6; Table 1). Affinity was slightly improved by insertion of the benzylic hydroxyl group in compound **2** (pK_i=6.9). The flexible benzyl amine **3** combines lipophilic with hydrogen bond donor properties of a second basic moiety in the eastern molecule part leading to a highly affine compound with binding strength in the low nanomolar concentration range (pK_i=8.6). The trisubstituted analogue **4** maintained affinity in the nanomolar concentration range (pK_i=7.1) showing comparable receptor binding to thioperamide. Probably, affinity differences between **3** and **4** result from the improved accessibility of the second basic moiety in compound **3**, whereas the tertiary amine in ligand **4** is sterically shielded by the benzyl rests.

Table 1. hH_3R Affinities and Selected Chemical Properties of Compounds **1**—**11** and References

Compound	hH_3R affinity K_i [nM][a]	Chemical Properties[b]	
		S Log P[c]	tPSA[c]
Thioperamide	72.0±12.0		
Pitolisant	11.7± 2.5		
1	307	1.52	13.7
2	123	1.28	33.9
3	2.3± 1.0	4.72	30.3
4	73.5± 5.1	4.11	18.1
5	34.3±12.5	3.49	75.5
6	31.5± 9.7	0.74	76.8
7	10.5± 0.4	0.94	111.2
8	49671	−1.11	67.6
9	11017	0.81	40.0
10	21975	−0.22	60.2
11	596	−0.14	44.4

a) [^{3}H]N^α-MeHA binding assay (HEK-293 cells expressing hH_3R); values are means of at least two experiments, each in triplicates, ±S.D. (for K_i values <100 nM). *b*) Values calculated for (di-)protonated compounds. *c*) Calculation with MOE software suite (Molecular Operating Environment, version 2007.09, Chemical Computing Group, Montreal, Canada, www.chemcomp.com).

Kojic acid derivatives were inserted into the pharmacophoric blueprint of H_3R ligands aiming at the substitution of either the benzyl moieties of compounds **3** and **4** in the eastern molecule part or the central phenoxy core of the pharmacophore **1**. In a first attempt, kojic acid was coupled to carboxylic acid **P4** and benzyl amine **P6**, respectively, resulting in compounds **5**—**7**. γ-Pyranone analogues of compounds **3** and **4**, the diamines **6** and **7**, exhibited good hH_3R affinities in the nanomolar concentration range (pK_i=7.5 and 8.0, respectively) indicating that the hH_3R binding pocket accepts these highly polar and electron withdrawing moieties in the eastern part of the ligand. The binding strength of the disubstituted derivative **7** is comparable to that of reference inverse agonist pitolisant. Compared to its phenyl analogue **4** compound **7** showed improved affinity (Fig. 3A). This may be caused by additional polar interactions. Lipophilic, nonaromatic residues with fixed points of high electron density, as seen in benzyl ester **5**, were also well tolerated (pK_i=7.5).

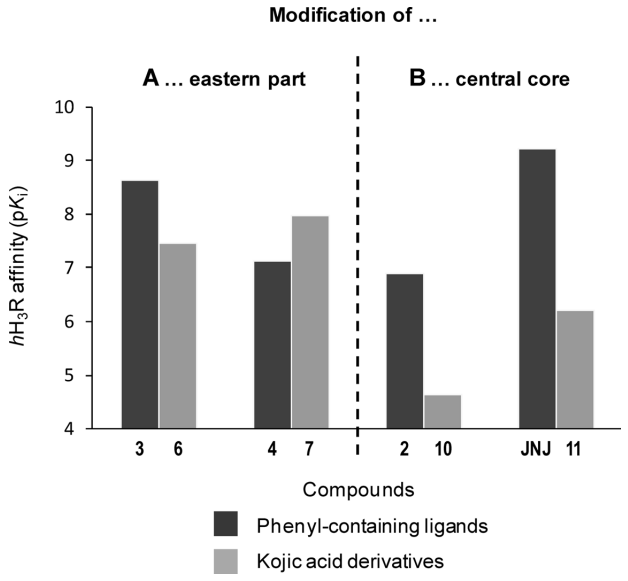


Fig. 3. Comparison of Phenyl and Pyran-4-one Moieties in the Eastern Part (A) and in the Central Core (B) of H_3R Ligands

Abbreviation: JNJ=JNJ-5207852.

Surprisingly, this compound exhibited receptor binding in the same range as diamine **6** did indicating that π interactions evoked by the benzyl residue in **5** compensate ionic interactions resulting from the second basic moiety in **6**. A smaller diamine-based ligand was designed with compound **8**. However, probably due to the missing hydrogen bond acceptor or π donor in the central core of the ligand this approach led to a pronounced loss of receptor binding (pK_i=4.3).

The direct implementation of kojic acid instead of the central phenyl ring of pharmacophore **1** was realized with compounds **9**—**11**. Related scaffold-hopping approaches were followed by several research groups aiming at the discovery of new leads or at the differentiation of the pharmacodynamic properties of H_3R ligands (*cf.* Fig. 1). Abbott chemists focused on the rigidification of the phenoxy core (*e.g.* ABT-239),[39,40] whereas researchers from Glaxo Smith Kline (GSK) published an 'inverse' aromatic core (*e.g.* GSK 189254).[41] Scientists at Novo Nordisk increased anti-obesity efficacy by using urea and cinnamic amide moieties as central elements (*e.g.* NNC 38-1202).[42,43] Comparable functionalities had already been used in imidazole-containing ligands like thioperamide or clobenpropit and derivatives.[44,45] The advanced clinical candidate, pitolisant, contains an alkyl ether core.[33] However, the application of this strategy, *i.e.* the direct implementation of kojic acid derivatives, did not succeed: compound **11** showed weak receptor binding in the submicromolar concentration range (pK_i=6.2), whereas **9** and **10** hardly exhibited hH_3R affinity (pK_i=5.0 and 4.7, respectively). The comparison between affinities of pyran-4-ones **10** and **11** and the corresponding phenyl-substituted ligands **2** and JNJ-5207852,[37,46] respectively, is diagrammed in Fig. 3B. Results clearly show the limitations of kojic acid derivatives as bioisosteric phenyl substitutes. Whereas kojic acid-derived moieties as central core hardly show receptor interactions, they are well accepted as extension of the eastern molecule part resulting in ligands with affinities in the low nanomolar concentration range. Receptor binding strength of compounds **6** and **7** is comparable to that of phenyl-derived ligands **3** and **4**.

In a first approximation of a compound's drug-likeness Lipinski's rule of five considering size, number of donor and acceptor groups and lipophilicity of a ligand is frequently applied.[47] Extended guidelines include *e.g.* molar refractivity[48] and the topological polar surface area (tPSA) as helpful parameters to assess drug transport properties.[49] The size of the most affine compounds **3**—**7** and the arrangement of donor and acceptor systems are in accordance with the general rules. Physicochemical parameters calculated to evaluate the CNS accessibility of these ligands are shown in Table 1. Estimated SLogP values were calculated using the MOE software suite (MOE, Molecular Operating Environment, version 2007.09, Chemical Computing Group, Montreal, Canada, www.chemcomp.com). This parameter reflects the lipophilicity of the virtually protonated compounds. Determined values differ from the optimum of *ca.* 4.[50,51] Only phenyl derivatives **3** and **4** as well as kojic acid ester **5** fulfil this requirement exhibiting S Log P values between 3.5 and 4.7, whereas S Log P values of the γ-pyranones **6** and **7** (0.7 and 0.9, respectively) hint at a peripheral bioavailability. The tPSA, which is based on the summation of surface contributions of polar fragments, should not exceed 120 $Å^2$ to ensure bioavailability. Compounds with tPSA values below 70 $Å^2$ potentially pass the blood/brain barrier (BBB).[52] Within the presented series of compounds phenyl derivatives **3** and **4** exhibit tPSA values of 18.1 $Å^2$ and 33.9 $Å^2$, respectively. Regarding the kojic acid analogues **5**—**7** this parameter ranges between 75.5 $Å^2$ and 111.2 $Å^2$. The calculated chemical properties indicate that phenyl derivatives **3** and **4** but not γ-pyranones **5**—**7** are able to pass the BBB. Hence, the newly designed kojic acid derivatives offer new perspectives for the development of H_3R ligands with a modified pharmacokinetic and pharmacodynamic profile. At least partially peripherally acting H_3R antagonists/inverse agonists could be effective in the therapy of different states of pain, obesity, or allergic rhinitis.[53] Clinical studies (phase II for allergic rhinitis)[54] of compounds like the H_3R antagonist PF-3654746[55] or the dual acting H_1R/H_3R antagonist GSK-1004723[5] corroborate this approach.

Despite an ambitious synthetic procedure the preparation of a series of kojic acid derivatives (**5**—**11**) was successfully realized. Additionally, for reasons of comparison and evaluation, the corresponding phenyl analogues were prepared (**1**—**4**). Kojic acid as a scaffold was for the first time associated with H_3R medicinal chemistry. It was integrated into a well established H_3R antagonist/inverse agonist pharmacophore showing possibilities and limitations of kojic acid derivatives as bioisosters of the different phenyl moieties existent in compounds **3** and **4**. The comparison of H_3R affinities and calculated chemical properties of ligands **3** and **4** with those of compounds **6** and **7** indicates that the scaffold modification allows for a new pharmacokinetic profile within the class of diamine-based H_3R antagonist/inverse agonists and might determine the site of action. A comparable approach that was recently performed with lipophilic sandwich complex-containing residues resulted in the design of centrally acting H_3R pharmacological tools.[56] In contrast, kojic acid derivatives **6** and **7** represent novel lead compounds offering promising possibilities for the development of peripherally

acting H_3R antagonist/inverse agonists.

Experimental

Chemistry. General Remarks Reagents and solvents were commercially obtained from Merck, Sigma-Aldrich and ABCR. Melting points were determined on a Büchi 510 melting point apparatus (Büchi, Switzerland) and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AMX 300 (300 MHz) spectrometer (Bruker, Germany). ^{1}H-NMR data are reported in the following order: chemical shift (δ) in ppm downfield from tetramethylsilane as internal reference; multiplicity (br, broad; s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; q, quintet); approximate coupling constants (J) in Hertz (Hz). ^{13}C-NMR spectra were recorded on a Bruker AC 200 (50 MHz) spectrometer (Bruker, Germany); all peaks resulted to be singlets. Electrospray ionization (ESI)-MS was performed on a Fisons Instruments VG Platform II (Manchester, Great Britain) in positive polarity. Data (m/z) are listed as mass number ($[M+H]^+$). Elemental analyses (C, H, N) were measured on a CHN-Rapid (Heraeus, Germany) or on a 'vario MICRO cube' (Elementar Analysensysteme GmbH, Germany) and were within $\pm$0.4% of the theoretical values for all final compounds. Preparative column chromatography was performed on silica gel 63—200 μM (Merck, Germany). Hydrogenations were carried out on an autoclave model IV, 500 ml (Roth, Germany). The microwave oven used was a Biotage Initiator 2.0 (Biotage, Sweden).

Reaction yields were not optimized and refer to the pure final products, which in most cases were crystallized as salts.

3-(Piperidin-1-yl)propan-1-ol Hydrochloride (P1)[9,19,20] Piperidine (51.1 g, 0.6 mol), 3-chloropropan-1-ol (47.3 g, 0.5 mol), potassium carbonate (74.3 g, 0.75 mol) and potassium iodide (8.3 g, 0.05 mol) were refluxed in absolute acetone (300 ml) during 72 h. The mixture was allowed to cool to room temperature, inorganic components were removed by filtration and the filtrate was concentrated to dryness. Purification of the crude oil was achieved by means of distillation (20 mbar, 95 °C). The oily product was crystallized as hydrochloride from 2-propanol (white solid, 54.6 g, 79%).

Chemical Formula: $C_8H_{17}NO\cdot HCl$. ^{1}H-NMR (DMSO-d_6) δ: 1.30—1.43 (1H, m), 1.64—1.88 (7H, m), 2.78—2.82 (2H, m), 2.95—3.02 (2H, m), 3.33—3.38 (2H, m), 3.44 (2H, t, J=5.9 Hz), 4.42 (1H, s), 10.54 (1H, br s). ^{13}C-NMR (DMSO-d_6) δ: 21.45, 22.27, 26.38, 51.95, 53.57, 57.98. ESI-MS m/z: 143.8 (Calcd for $C_8H_{17}NO$: 143.13).

1-(3-Chloropropyl)piperidine Hydrochloride (P2)[9,19,20] Alcohol **P1** (13.4 g, 0.08 mol) was suspended in toluene (100 ml). Under inert atmosphere and at 0 °C an excess of thionyl chloride (11.6 ml, 0.16 mol) was added dropwise. Once the exothermic reaction had decayed the mixture was stirred for 3 h at 60 °C. Upon completion of the reaction thionyl chloride and toluene were distilled off. The crude product was re-crystallized from ethanol (beige solid, 14.8 g, quantitative conversion).

Chemical Formula: $C_8H_{16}ClN\cdot HCl$. ^{1}H-NMR (DMSO-d_6) δ: 1.31—1.41 (1H, m), 1.65—1.87 (5H, m), 2.15—2.25 (2H, m), 2.77—2.89 (2H, m), 3.03—3.10 (2H, m), 3.35—3.40 (2H, m), 3.73 (2H, t, J=6.4 Hz), 10.73 (1H, br s). ^{13}C-NMR (DMSO-d_6) δ: 21.34, 22.19, 26.13, 42.50, 51.94, 53.56. ESI-MS m/z: 161.6 (Calcd for $C_8H_{16}ClN$: 161.10).

Methyl 4-(3-(Piperidin-1-yl)propoxy)benzoate (P3)[9,20] Chloride **P2** (3.0 g, 15.1 mmol), methyl 4-hydroxybenzoate (2.6 g, 16.8 mmol), potassium carbonate (9.0 g, 90.6 mmol) and potassium iodide (2.5 g, 15.1 mmol) were refluxed in absolute acetone (100 ml) during 3 d. The crude was taken up in soda lye (2 N) and purified as described for compound **P3**. The resulting product was a brown oil (2.6 g, 61%).

Chemical Formula: $C_{16}H_{23}NO_3$. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (2H, br s), 1.45—1.58 (4H, m), 1.87 (2H, q), 2.31 (4H, br s), 2.36 (2H, t, J=7.2), 3.81 (3H, s), 4.07 (2H, t, J=6.4 Hz), 7.04 (2H, d, J=8.8 Hz), 7.89 (2H, d, J=8.8 Hz). ^{13}C-NMR (DMSO-d_6) δ: 24.11, 25.58, 26.12, 51.73, 54.07, 54.96, 66.31, 114.39, 121.65, 131.18, 162.54, 165.85. ESI-MS m/z: 277.9 (Calcd for $C_{16}H_{23}NO_3$: 277.17).

4-(3-(Piperidin-1-yl)propoxy)benzoic Acid (P4)[23] Ester **P3** (2.0 g, 7.2 mmol) was dissolved in a mixture of THF (8 ml) and methanol (8 ml); potassium hydroxide pellets (1.2 g, 21.6 mmol) were dissolved in water (16 ml). Both solutions were transferred into a sealed vial and heated in the microwave oven for 15 min at 70 °C. After cooling the organic solvents were removed under reduced pressure and the aqueous solution neutralized with hydrochloric acid (10 N). Reaching the isoelectric point of the product (close to neutral pH value) it crystallized as a white solid (1.9 g, quantitative conversion).

Chemical Formula: $C_{15}H_{21}NO_3$. ^{1}H-NMR (DMSO-d_6) δ: 1.53 (2H, br s), 1.77—1.81 (4H, m), 2.18—2.25 (2H, m), 3.08—3.12 (6H, m), 4.13 (2H, t, J=6.1 Hz), 7.03 (2H, d, J=8.8 Hz), 7.88 (2H, d, J=8.8 Hz), 11.66 (1H, br s). ^{13}C-NMR (DMSO-d_6) δ: 21.53, 22.40, 23.24, 52.00, 53.27, 65.40, 114.24, 123.20, 131.30, 161.81, 166.91. ESI-MS m/z: 264.0 (Calcd for $C_{15}H_{21}NO_3$: 263.15).

4-(3-(Piperidin-1-yl)propoxy)benzonitrile (P5)[9,20] Chloride **P2** (3.0 g, 15.1 mmol), 4-hydroxybenzonitrile (2.0 g, 16.8 mmol), potassium carbonate (9.0 g, 90.6 mmol) and potassium iodide (2.5 g, 15.1 mmol) were refluxed in absolute acetone (100 ml) during 3 d. After cooling the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in soda lye (5 N) and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulphate. The organic solvent was removed under reduced pressure. The product was obtained as yellowish oil (1.7 g, 47%).

Chemical Formula: $C_{15}H_{20}N_2O$. ^{1}H-NMR (DMSO-d_6) δ: 1.35—1.37 (2H, m), 1.43—1.50 (4H, m), 1.85 (2H, q), 2.30—2.37 (6H, m), 4.07 (2H, t, J=6.4 Hz), 7.10 (2H, dd, J=11.5 Hz), 7.72 (2H, dd, J=11.5 Hz). ^{13}C-NMR (DMSO-d_6) δ: 21.46, 22.61, 23.28, 52.12, 65.56, 102.99, 115.53, 119.05, 134.17, 164.42. ESI-MS m/z: 245.0 (Calcd for $C_{15}H_{20}N_2O$: 244.16).

(4-(3-(Piperidin-1-yl)propoxy)phenyl)methanamine (P6)[23] The nitrile **P5** (2.2 g, 8.8 mmol) was hydrogenated in an autoclave (10 bar) using Raney nickel (nickel–aluminium alloy activated with sodium hydroxide pellets) in ammoniacal methanol. After 12 h stirring at room temperature the catalyst was removed by means of a filtration aid (Celite®) and the filtrate concentrated under reduced pressure to give a yellowish oil (2.1 g, 94%).

Chemical Formula: $C_{15}H_{24}N_2O$. ^{1}H-NMR (DMSO-d_6) δ: 1.35—1.37 (2H, m), 1.43—1.51 (4H, m), 1.74—1.86 (2H, m), 2.30—2.37 (6H, m), 3.72 (2H, s), 4.07 (2H, t, J=6.4 Hz), 6.88 (2H, d, J=8.6 Hz), 7.24 (2H, d, J=8.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 24.12, 25.58, 26.32, 45.05, 54.09, 55.16, 65.81, 113.99, 128.02, 136.22, 157.02. ESI-MS m/z: 249.9 (Calcd for $C_{15}H_{24}N_2O$: 248.19.

1-(3-Phenoxypropyl)piperidine Hydrogenoxalate (1)[9,20] Chloride **P2** (2.2 g, 11.1 mmol), phenol (1.2 g, 12.2 mmol), potassium carbonate (9.2 g, 66.6 mmol) and potassium iodide (1.8 g, 11.1 mmol) were refluxed in absolute acetone (100 ml) during 3 d. The crude was purified as described for compound **P3**. The resulting yellow oil was crystallized as oxalic salt from ethanol (1.6 g, 47%).

Chemical Formula: $C_{15}H_{21}NO_3\cdot(COOH)_2$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.54 (2H, s), 1.75 (4H, s), 2.13 (2H, s), 3.13 (6H, s), 4.03 (2H, s), 6.95 (3H, s), 7.30 (2H, s). ^{13}C-NMR (DMSO-d_6) δ: 21.48, 22.59, 23.51, 52.10, 53.47, 64.90, 114.44, 120.72, 129.62, 158.24, 164.62. ESI-MS m/z: 219.9 (Calcd for $C_{15}H_{21}NO_3$: 219.16). mp 185 °C. *Anal*. Calcd for $C_{15}H_{21}NO_3\cdot(COOH)_2$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.39; H, 7.75; N, 4.64.

(4-(3-(Piperidin-1-yl)propoxy)phenyl)methanol Hydrochloride (2)[9,20] Chloride **P2** (2.1 g, 10.5 mmol), 4-hydroxybenzyl alcohol (2.6 g, 21.0 mmol), potassium carbonate (8.7 g, 63.0 mmol) and potassium iodide (1.7 g, 10.5 mmol) were refluxed in absolute acetone (100 ml) during 2 d. After purification (as described for compound **P3**) the resulting light solid was crystallized as hydrochloride from 2-propanol (1.5 g, 50%).

Chemical Formula: $C_{15}H_{23}NO_2\cdot HCl$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.34—1.38 (1H, m), 1.66—1.84 (5H, m), 2.13—2.22 (2H, m), 2.80—2.90 (2H, m), 3.09—3.16 (2H, m), 3.36—3.43 (2H, m), 4.01 (2H, t, J=6.0 Hz), 4.40 (2H, s), 5.10 (1H, br s), 6.89 (2H, d, J=8.6 Hz), 7.20 (2H, d, J=8.6 Hz), 10.70 (1H, br s). ^{13}C-NMR (DMSO-d_6) δ: 21.39, 22.26, 23.47, 51.93, 53.36, 62.46, 65.06, 114.10, 127.88, 134.88, 157.11. ESI-MS m/z: 250.0 (Calcd for $C_{15}H_{23}NO_2$: 249.17). mp 167 °C. *Anal.* Calcd for $C_{15}H_{23}NO_2\cdot HCl$: C, 63.04; H, 8.46; N, 4.90. Found: C, 62.92; H, 8.59; N, 4.68.

Benzyl-1-(4-(3-(piperidin-1-yl)propoxy)phenyl)methanamine Ligands (3, 4)[24] Under inert atmosphere at ambient temperature benzaldehyde (0.6 g, 6.0 mmol) and amine **P6** (1.0 g, 4.0 mmol) were coupled in 1,2-dichloroethane (DCE) (20 ml) for 3 h. The resulting imine was reduced with sodium triacetoxyborohydride (1.3 g, 6.0 mmol) during 30 min of stirring. The reaction was quenched by adding a saturated solution of sodium carbonate. The product was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulphate, filtered and concentrated under reduced pressure. Further purification was carried out by column chromatography (methylene chloride : ammoniacal methanol = 9.8 : 0.2 → 9.5 : 0.5). Two fractions were eluted. Both colourless oils were crystallized as salts of maleic and oxalic acid, respectively, resulting in white solids after re-crystallization (**3**: 0.3 g, 20.5%; **4**: 0.02 g, 1%). Especially 4 had to be re-crystallized several times from acetonitrile due to incorporation of water and stayed quite hygroscopic.

***N*-Benzyl-1-(4-(3-(piperidin-1-yl)propoxy)phenyl)methanamine Dihydrogenmaleate (3)** Chemical Formula: $C_{22}H_{30}N_2O\cdot 2C_4H_4O_4$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.43—1.78 (6H, m), 2.07—2.16 (2H, m), 2.91 (2H, br s), 3.19 (2H, t, J=7.95 Hz), 3.45 (2H, br s), 4.05 (2H, t, J=5.88 Hz), 4.35 (4H,

d, J=5.73 Hz), 6.03 (4H, s), 7.00 (2H, d, J=8.62 Hz), 7.26—7.45 (7H, m). ^{13}C-NMR (DMSO-d_6) δ: 21.21, 22.63, 23.45, 49.65, 52.20, 53.36, 64.93, 114.53, 123.85, 128.68, 128.97, 129.87, 131.60, 131.84, 135.73, 158.70, 167.17. ESI-MS m/z: 339.1 (Calcd for $C_{22}H_{30}N_2O$: 338.24). mp 133 °C. *Anal.* Calcd for $C_{22}H_{30}N_2O \cdot 2C_4H_4O_4$: C, 63.14; H, 6.71; N, 4.91. Found: C, 62.89; H, 6.63; N, 4.77.

***N*,*N*-Dibenzyl-1-(4-(3-(piperidin-1-yl)propoxy)phenyl)methanamine Dihydrogenoxalate Hydrate (4)** Chemical Formula: $C_{29}H_{36}N_2O \cdot 2(COOH)_2 \cdot 1.5H_2O$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.38 (1H, br s), 1.73 (5H, br s), 2.06—2.12 (2H, m), 2.72 (2H, br s), 3.14 (2H, t, J=7.91 Hz), 3.54 (4H, br s), 3.50 (4H, s), 4.00 (2H, t, J=5.92 Hz), 6.92 (2H, d, J=8.57 Hz), 7.21—7.39 (12H, m). ^{13}C-NMR (DMSO-d_6) δ: 21.27, 22.48, 23.39, 52.04, 53.41, 56.16, 56.69, 64.82, 114.26, 126.99, 128.38, 129.78, 130.64, 138.69, 157.31, 162.53. ESI-MS m/z: 429.4 (Calcd for $C_{29}H_{36}N_2O$: 428.28). mp 97 °C. *Anal.* Calcd for $C_{29}H_{36}N_2O \cdot 2(COOH)_2 \cdot 1.5H_2O$: C, 62.35; H, 6.82; N, 4.41. Found: C, 62.64; H, 6.79; N, 4.07%.

5-(Benzyloxy)-2-(hydroxymethyl)-4*H*-pyran-4-one (P8) (a)[25] Under inert atmosphere kojic acid (**P7**, 5 g, 35.2 mmol), cesium carbonate (11.5 g, 35.2 mmol), and benzyl bromide (12.5 ml, 105 mmol) were suspended in absolute DMF (25 ml). The mixture was refluxed for 2 h. After cooling it was purified by dry load column chromatography (methylene chloride : ammoniacal methanol=9.5 : 0.5) giving a light brown solid (4.2 g, 52%).

(b)[26,27] Alternatively, a solution of P7 (5 g, 35.2 mmol) in methanol (40 ml) was added to a solution of sodium hydroxide (1.6 g, 38.7 mmol) in water (5 ml). This mixture was heated to reflux, benzyl bromide was added dropwise and reflux was continued for 12 h. The solution was allowed to cool to ambient temperature, the solvent removed by reduced pressure and the resulting brown solid was washed with water (15 ml) and methanol (10 ml). Re-crystallization in methanol led to beige crystal needles, which were collected by filtration (4.6 g, 57%).

Chemical Formula: $C_{13}H_{12}O_4$. ^{1}H-NMR (DMSO-d_6) δ: 4.28 (2H, d, J=6.0 Hz), 4.93 (2H, s), 5.67 (1H, t, J=6.1 Hz), 6.31 (1H, s), 7.24—7.42 (5H, m), 8.16 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 59.31, 70.61, 111.19, 128.04, 128.11, 128.38, 136.17, 141.35, 146.59, 167.98, 173.20. ESI-MS m/z: 232.8 (Calcd for $C_{13}H_{12}O_4$: 232.07).

(5-(Benzyloxy)-4-oxo-4*H*-pyran-2-yl)methyl 4-(3-(Piperidin-1-yl)propoxy)benzoate Hydrogenoxalate (5)[31] A solution of benzyl kojic acid **P8** (0.56 g, 2.4 mmol) and triphenylphosphine (0.42 g, 1.6 mmol) in THF (5 ml) was added dropwise to another solution consisting of benzoic acid **P4** (0.42 g, 1.6 mmol) and diethyl azodicarboxylate (DEAD) (0.28 g, 1.6 mmol) in THF (5 ml). Under inert atmosphere the mixture was stirred overnight at room temperature, filtered and purified by column chromatography (methylene chloride : ammoniacal methanol=9.5 : 0.5). The product is crystallized with oxalic acid in ethanol and re-crystallized twice in acetonitrile (0.035 g, 4%).

Chemical Formula: $C_{28}H_{31}NO_6 \cdot (COOH)_2 \cdot 0.5H_2O$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.51 (2H, br s), 1.62—1.78 (4H, m), 2.12 (2H, br s), 2.98—3.18 (6H, m), 4.12 (2H, t, J=5.46 Hz), 4.94 (2H, s), 5.18 (2H, s), 6.53 (1H, s), 7.08 (2H, d, J=8.78 Hz), 7.33—7.41 (5H, m), 7.94 (2H, d, J=8.69 Hz), 8.27 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 21.63, 22.82, 23.54, 52.27, 53.38, 61.51, 65.50, 70.56, 113.67, 114.65, 128.09, 128.19, 128.41, 131.60, 141.55, 146.89, 161.78, 162.60, 164.41, 164.57, 172.85. ESI-MS m/z: 478.4 (Calcd for $C_{28}H_{31}NO_6$: 477.22). mp 176 °C. *Anal.* Calcd for $C_{28}H_{31}NO_6 \cdot (COOH)_2 \cdot 0.5\ H_2O$: C, 62.49; H, 5.94; N, 2.43. Found: C, 62.27; H, 5.94; N, 2.37.

2-(Chloromethyl)-5-hydroxy-4*H*-pyran-4-one (P9)[29] Kojic acid (**P7**, 10 g, 70.4 mmol) was dissolved in thionyl chloride (40 ml) and stirred at room temperature for 1 h. The product crystallized during this time and was collected by filtration. The resulting residue was washed with petroleum ether and re-crystallized from water giving a beige solid (8.4 g, 75%).

Chemical Formula: $C_6H_5ClO_3$. ^{1}H-NMR (DMSO-d_6) δ: 4.65 (2H, s), 6.56 (1H, s), 8.11 (1H, s), 9.30 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 41.23, 113.23, 140.12, 146.02, 161.68, 173.72. ESI-MS m/z: 160.0 (Calcd for $C_6H_5ClO_3$: 159.99).

5-Hydroxy-2-((4-(3-(piperidin-1-yl)propoxy)benzylamino)methyl)-4*H*-pyran-4-one Dihydrogenoxalate (6) Benzylamine **P6** (2.2 g, 9.0 mmol), kojic acid chloride **P9** (0.48 g, 3.0 mmol) and TEA (0.4 ml, 3.0 mmol) were stirred in absolute THF (20 ml) overnight under inert atmosphere and at ambient temperature. After evaporation of the solvent the crude product was purified by column chromatography (methylene chloride : ammoniacal methanol=9.0 : 1.0→8.5 : 1.5). By adding oxalic acid the product was crystallized as a brown solid and re-crystallized several times from acetonitrile (0.1 g, 5%).

Chemical Formula: $C_{21}H_{28}N_2O_4 \cdot 3(COOH)_2$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.35—1.90 (6H, m), 2.11 (2H, m), 3.14 (2H, t, J=6.29 Hz), 2.87 (2H, br s), 3.44 (2H, br s), 3.96—4.10 (6H, m), 6.51 (1H, s), 6.95 (2H, d, J=8.07 Hz), 7.36 (2H, d, J=7.89 Hz), 8.07 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 21.31, 22.46, 23.30, 49.91, 52.02, 53.30, 59.94, 64.98, 114.04, 114.42, 125.56, 131.15, 139.88, 146.05, 158.34, 160.20, 163.25, 173.47. ESI-MS m/z: 373.3 (Calcd for $C_{21}H_{28}N_2O_4$: 372.20). mp 190 °C. *Anal.* Calcd for $C_{21}H_{28}N_2O_4 \cdot 3(COOH)_2$: C, 50.47; H, 5.33; N, 4.36. Found: C, 50.74; H, 5.48; N, 4.09.

6,6′-(4-(3-(Piperidin-1-yl)propoxy)benzylazanediyl)bis(methylene)bis-(3-hydroxy-4*H*-pyran-4-one) (7) Benzylamine **P6** (0.5 g, 2.0 mmol) and kojic acid chloride **P9** (1.3 g, 8.0 mmol) were dissolved in DMF and heated under microwave irradiation for 30 min. After cooling the solvent was removed under reduced pressure. Purification was performed by column chromatography (methylene chloride : ammoniacal methanol=9.0 : 1.0⟨1.0 : 1.0). The product is a brown solid (0.1 g, 10%).

Chemical Formula: $C_{27}H_{32}N_2O_7 \cdot 2H_2O$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.37—1.39 (2H, m), 1.48—1.52 (4H, m), 1.82—1.89 (2H, m), 2.42 (6H, br s), 3.54 (4H, s), 3.62 (2H, s), 3.96 (2H, t, J=6.20 Hz), 6.35 (2H, s), 6.88 (2H, d, J=8.60 Hz), 7.20 (2H, d, J=8.54 Hz), 8.01 (2H, s). ^{13}C-NMR (DMSO-d_6) δ: 23.67, 25.05, 25.74, 53.75, 54.22, 54.86, 57.22, 65.61, 112.34, 114.29, 129.57, 129.83, 139.60, 145.77, 157.74, 165.03, 173.66. ESI-MS m/z: 497.6 (Calcd for $C_{27}H_{32}N_2O_7$: 496.22). mp 90 °C. *Anal.* Calcd for $C_{27}H_{32}N_2O_7 \cdot 2H_2O$: C, 60.89; H, 6.81; N, 5.26. Found: C, 60.85; H, 6.38; N, 5.25.

5-Hydroxy-2-((3-(piperidin-1-yl)propylamino)methyl)-4*H*-pyran-4-one Dihydrogenoxalate (8) Under inert atmosphere kojic acid chloride **P9** (1.0 g, 6.2 mmol), 3-(piperidin-1-yl)propan-1-amine (1.8 g, 12.4 mmol), TEA (0.8 ml, 6.2 mmol) and THF (20 ml) were stirred overnight at ambient temperature. During this time the yellow solution turned brown. The solvent was removed under reduced pressure and the crude product purified by column chromatography (methylene chloride : ammoniacal methanol=8.0 : 2.0→6.0 : 4.0). The crystallization of the product was performed with oxalic acid in ethanol, while re-crystallization took place in acetonitrile to give a light brown solid (0.4 g, 15%).

Chemical Formula: $C_{14}H_{22}N_2O_3 \cdot 2(COOH)_2$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.51 (2H, s), 1.71 (4H, s), 1.94 (2H, s), 2.82 (2H, s), 3.07 (6H, s), 3.90 (2H, s), 6.49 (1H, s), 8.06 (1H, s), 8.23 (1H, br s). ^{13}C-NMR (DMSO-d_6) δ: 21.29, 22.41, 40.29, 47.56, 51.96, 53.30, 113.50, 139.83, 146.00, 161.42, 164.11, 173.55. ESI-MS m/z: 267.1 (Calcd for $C_{14}H_{22}N_2O_3$: 266.34). mp 153—160 °C (carbonization). *Anal.* Calcd for $C_{14}H_{22}N_2O_3 \cdot 2(COOH)_2$: C, 48.43; H, 5.87; N, 6.28. Found: C, 48.63; H, 6.14; N, 6.20.

5-Hydroxy-2-methyl-4*H*-pyran-4-one (P10)[30] Chlorokojic acid **P9** (1.0 g, 6.2 mmol) was suspended in water (5 ml) and heated to 50 °C. Zinc powder (0.8 g, 12.45 mmol) and hydrochloric acid (2 ml) were added. Whereas the mixture was kept at 70 °C for 3 h, the solids mostly dissolved. After hot filtration the product was extracted with methylene chloride. The combined organic extracts were dried over sodium sulphate and evaporated to dryness. The crude was re-crystallized from 2-propanol affording a white solid (0.24 g, 31%).

Chemical Formula: $C_6H_6O_3$. ^{1}H-NMR (DMSO-d_6) δ: 2.23 (3H, s), 6.23 (1H, s), 7.96 (1H, s), 8.96 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 19.03, 111.96, 139.26, 145.29, 165.20, 173.74. ESI-MS m/z: 126.7 (Calcd for $C_6H_6O_3$: 126.03).

2-Methyl-5-(3-(piperidin-1-yl)propoxy)-4*H*-pyran-4-one Hydrogenoxalate (9) Methyl derivative **P10** (0.23 g, 1.8 mmol), chloride **P2** (1.8 g, 9.0 mmol), cesium carbonate (2.9 g, 9.0 mmol) and potassium iodide (0.3 g, 1.8 mmol) were heated in DMF for 2 h. The crude product was purified by means of column chromatography (methylene chloride : ammoniacal methanol=9.5 : 0.5→9.0 : 1.0) and crystallized with oxalic acid in ethanol. Re-crystallization of the beige solid was performed in acetonitrile (0.064 g, 11%).

Chemical Formula: $C_{14}H_{21}NO_3 \cdot (COOH)_2$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.51 (2H, br s), 1.70—1.74 (4H, m), 2.06 (2H, q), 2.24 (3H, s), 3.07—3.12 (6H, m), 3.88 (2H, t, J=5.79 Hz), 6.24 (1H, s), 8.11 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 18.96, 21.45, 22.49, 23.31, 52.14, 53.60, 67.15, 113.34, 141.74, 146.39, 164.45, 165.53, 173.22. ESI-MS m/z: 252.2 (Calcd for $C_{14}H_{21}NO_3$: 251.15). mp 178 °C. *Anal.* Calcd for $C_{14}H_{21}NO_3 \cdot (COOH)_2$: C, 56.30; H, 6.79; N, 4.10. Found: C, 56.07; H, 6.79; N, 4.27.

5-(3-Chloropropoxy)-2-(hydroxymethyl)-4*H*-pyran-4-one (P11) Kojic acid (P7, 5 g, 35.2 mmol), cesium carbonate (11.5 g, 35.2 mmol), and potassium iodide (5.8 g, 35.2 mmol) were suspended in absolute DMF (100 ml). 1-Bromo-3-chloropropane (17.3 ml, 176 mmol) was added *via* dropping funnel. After refluxing (110 °C) the mixture for 2 h it was allowed to cool to room temperature, filtered, and the dark brown solution was concentrated. The residue was dissolved in methylene chloride (100 ml) and concentrated

on silica gel to be submitted to dry load column chromatography (methylene chloride : ammoniacal methanol = 9.5 : 0.5). The product was evaporated to dryness to give an orange solid (5.5 g, 72%).

Chemical Formula: $C_9H_{11}ClO_4$. ^{1}H-NMR (DMSO-d_6) δ: 2.07—2.15 (2H, m), 3.74 (2H, t, J=6.43 Hz), 3.93 (2H, t, J=6.02 Hz), 4.28 (2H, s), 5.69 (1H, br s), 6.30 (1H, s), 8.16 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 31.63, 41.82, 59.33, 65.87, 111.05, 140.78, 146.88, 168.02, 173.08. ESI-MS m/z: 218.6 (Calcd for $C_9H_{11}ClO_4$: 218.03).

2-(Hydroxymethyl)-5-(3-(piperidin-1-yl)propoxy)-4*H*-pyran-4-one Hydrogenoxalate (10) Chloride **P11** (2 g, 9.2 mmol), piperidine (1.1 ml, 11 mmol), potassium carbonate (1.9 g, 13.8 mmol) and potassium iodide (0.15 g, 0.9 mmol) were heated in absolute acetone (20 ml) for 2 h and stirred overnight at room temperature. After cooling, filtration and concentration steps the crude was purified by column chromatography (methylene chloride : ammoniacal methanol = 9.5 : 0.5). 0.2 g of the isolated brown oil (0.66 g, 27%) were crystallized with oxalic acid in ethanol and re-crystallized from acetonitrile (beige solid, 0.4 g, 12%).

Chemical Formula: $C_{14}H_{21}NO_4 \cdot (COOH)_2$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.51 (2H, br s), 1.71—1.74 (4H, m), 2.03—2.12 (2H, m), 3.07—3.12 (6H, m), 3.89 (2H, t, J=5.92 Hz), 4.29 (2H, s), 6.31 (1H, s), 7.26 (1H, br s), 8.15 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 21.44, 22.58, 23.32, 52.14, 53.57, 59.28, 67.20, 111.09, 141.56, 146.69, 164.58, 168.31, 173.26. ESI-MS m/z: 268.0 (Calcd for $C_{14}H_{21}NO_4$: 267.15). mp 143 °C. *Anal.*. Calcd for $C_{14}H_{21}NO_4 \cdot (COOH)_2$: C, 53.78; H, 6.49; N, 3.92%. Found: C, 53.52; H, 6.35; N, 3.89.

2-(Chloromethyl)-5-(3-(piperidin-1-yl)propoxy)-4*H*-pyran-4-one (P12) Under inert atmosphere alcohol **10** (0.35 g, 1.0 mmol) was chlorinated with thionyl chloride (0.21 ml, 3.0 mmol) in toluene (10 ml) as described for compound **P2** to give a yellow solid (0.32 g, quantitative conversion).

Chemical Formula: $C_{14}H_{20}ClNO_3 \cdot HCl$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.41—1.48 (1H, m), 1.74—1.96 (5H, m), 2.17—2.28 (2H, m), 2.90—2.95 (2H, m), 3.15—3.23 (2H, m), 3.41—3.53 (2H, m), 4.01 (2H, t, J=5.91 Hz), 4.75 (2H, s), 6.65 (1H, s), 8.36 (1H, s), 10.37 (1H, br s). ^{13}C-NMR (DMSO-d_6) δ: 21.32, 22.30, 23.04, 41.08, 51.98, 53.38, 67.05, 114.43, 141.93, 146.89, 161.92, 172.94. ESI-MS m/z: 286.0 (Calcd for $C_{14}H_{20}ClNO_3$: 285.11).

5-(3-(Piperidin-1-yl)propoxy)-2-(piperidin-1-ylmethyl)-4*H*-pyran-4-one (11) Chloride **P12** (0.3 g, 0.93 mmol), piperidine (0.14 ml, 1.4 mmol), potassium carbonate (0.64 g, 4.7 mmol) and potassium iodide (0.16 g, 0.93 mmol) were refluxed in absolute acetone (20 ml) for 8 h. After filtration of the inorganic salts and concentration of the filtrate the remaining residue was dissolved in soda lye (2 N) and the product extracted with methylene chloride. The resulting oil was purified by column chromatography (methylene chloride : ammoniacal methanol = 9.0 : 1.0) and crystallization with oxalic acid. The white solid was re-crystallized in acetonitrile (0.015 g, 3%).

Chemical Formula: $C_{19}H_{30}N_2O_3 \cdot 2(COOH)_2 \cdot 0.5H_2O$. ^{1}H-NMR (DMSO-$d_6$) δ: 1.40 (2H, s), 1.54 (6H, s), 1.74 (4H, s), 2.08 (2H, m), 2.53 (8H, m), 3.14 (2H, t, J=7.40 Hz), 3.53 (2H, s), 3.91 (2H, s), 6.40 (1H, s), 8.18 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 21.30, 22.52, 23.05, 23.20, 24.80, 52.13, 53.39, 53.62, 58.20, 67.11, 114.70, 141.87, 146.79, 163.16, 163.60, 173.01. ESI-MS: 335.1 (Calcd for $C_{19}H_{30}N_2O_3$: 334.23). mp 166 °C. *Anal.* Calcd for $C_{19}H_{30}N_2O_3 \cdot 2(COOH)_2 \cdot 0.5H_2O$: C, 52.77; H, 6.74; N, 5.35. Found: C, 52.42; H, 6.37; N, 5.21.

Pharmacology. *h*H_3R [^{3}H]N^α-MeHA Binding Assay on HEK-293 Cell Membrane Preparation[33] Before starting experiments cell membranes were thawed, homogenized by sonication at 4 °C and kept in ice-cold binding buffer (12.5 mM $MgCl_2$, 100 mM NaCl and 75 mM Tris/HCl, pH 7.4). Competition binding experiments were carried out as follows: membranes (20—25 μg/well in a final volume of 0.2 ml binding buffer) were incubated with [^{3}H]N^α-MeHA (2 nM; 85 Ci/mmol) and different concentrations of test ligand. Assays were run at least in duplicates with seven appropriate concentrations between 0.01 nM and 100 μM of the test compound. Incubations were performed for 90 min at 25 °C and shaking at 250 rpm. Non-specific binding was determined in the presence of 10 μM unlabelled HA. In saturation binding experiments the maximal binding concentration (B_{max}) was found to be 0.89 pmol·mg^{-1} and the K_d value of [^{3}H]N^α-MeHA resulted to be 2.98 nmol·l^{-1} (pK_d=8.53±0.01). The bound radioligand was separated from free radioligand by filtration through GF/B filters pre-treated with 0.3% (m/v) polyethyleneimine using an Inotech cell harvester. Unbound radioligand was removed by three washing steps with 0.3 ml/well of ice-cold 50 mM Tris–HCl buffer, pH 7.4, containing 120 mM NaCl. The filters were soaked in a 9 ml scintillator and counted using a PerkinElmer MicroBeta® Trilux scintillation counter. Competition binding data were analyzed by the software GraphPad Prism 3.02 using non-linear least squares fit. Affinity values (K_i) were expressed as mean±standard deviation (S.D.). K_i values were calculated from the IC_{50} values according to the Cheng–Prusoff equation.[57]

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References

1) Hill S. J., Ganellin C. R., Timmerman H., Schwartz J. C., Shankley N. P., Young J. M., Schunack W., Levi R., Haas H. L., *Pharmacol. Rev.*, **49**, 253—278 (1997).
2) Engelhardt H., Smits R. A., Leurs R., Haaksma E., de Esch I. J., *Curr. Opin. Drug Discov. Devel.*, **12**, 628—643 (2009).
3) Brown R. E., Stevens D. R., Haas H. L., *Prog. Neurobiol.*, **63**, 637—672 (2001).
4) Sander K., Kottke T., Stark H., *Biol. Pharm. Bull.*, **31**, 2163—2181 (2008).
5) Raddatz R., Tao M., Hudkins R. L., *Curr. Top. Med. Chem.*, **10**, 153—169 (2010).
6) Sander K., von Coburg Y., Camelin J. C., Ligneau X., Rau O., Schubert-Zsilavecz M., Schwartz J. C., Stark H., *Bioorg. Med. Chem. Lett.*, **20**, 1581—1584 (2010).
7) Apodaca R., Dvorak C. A., Xiao W., Barbier A. J., Boggs J. D., Wilson S. J., Lovenberg T. W., Carruthers N. I., *J. Med. Chem.*, **46**, 3938—3944 (2003).
8) Sasho S., Seishi T., Kawamura M., Hirose R., Toki S., Shimada J. I., *Bioorg. Med. Chem. Lett.*, **18**, 2288—2291 (2008).
9) Isensee K., Amon M., Galaparti A., Ligneau X., Camelin J. C., Capet M., Schwartz J. C., Stark H., *Bioorg. Med. Chem. Lett.*, **19**, 2172—2175 (2009).
10) Axe F. U., Bembenek S. D., Szalma S., *J. Mol. Graph. Model.*, **24**, 456—464 (2006).
11) Schlegel B., Laggner C., Meier R., Langer T., Schnell D., Seifert R., Stark H., Höltje H. D., Sippl W., *J. Comput. Aided Mol. Des.*, **21**, 437—453 (2007).
12) Ratcliffe A. J., *Curr. Med. Chem.*, **16**, 2816—2823 (2009).
13) Kobayashi K., Kayahara H., Tadasa K., Tanaka H., *Bioorg. Med. Chem. Lett.*, **6**, 1303—1308 (1996).
14) Kim D. H., Hwang J. S., Baek H. S., Kim K. J., Lee B. G., Chang I., Kang H. H., Lee O. S., *Chem. Pharm. Bull.*, **51**, 113—116 (2003).
15) Rho H. S., Baek H. S., You J. W., Kim S., Lee J. Y., Kim D. H., Chang I. S., *Bull. Korean Chem. Soc.*, **28**, 471—473 (2007).
16) Niwa Y., Akamatsu H., *Inflammation*, **15**, 303—315 (1991).
17) Vajragupta O., Boonchoonga P., Sumanonta Y., Watanabe H., Wongkrajang Y., Kammasud N., *Bioorg. Med. Chem.*, **11**, 2329—2337 (2003).
18) Emami S., Hosseinimehra S. J., Taghdisia S. M., Akhlaghpoor S., *Bioorg. Med. Chem. Lett.*, **17**, 45—48 (2007).
19) Meier G., Apelt J., Reichert U., Grassmann S., Ligneau X., Elz S., Leurquin F., Ganellin C. R., Schwartz J. C., Schunack W., Stark H., *Eur. J. Pharm. Sci.*, **13**, 249—259 (2001).
20) Amon M., Ligneau X., Camelin J. C., Berrebi-Bertrand I., Schwartz J. C., Stark H., *ChemMedChem*, **2**, 708—716 (2007).
21) Williamson A., *Ann. Chem. Pharm.*, **77**, 37—49 (1851).
22) Finkelstein H., *Ber. Dtsch. Chem. Ges.*, **43**, 1528—1532 (1910).
23) Proschak E., Sander K., Zettl H., Tanrikulu Y., Rau O., Schneider P., Schubert-Zsilavecz M., Stark H., Schneider G., *ChemMedChem*, **4**, 45—48 (2009).
24) Abdel-Magid A. F., Carson K. G., Harris B. D., Maryanoff C. A., Shah R. D., *J. Org. Chem.*, **61**, 3849—3862 (1996).
25) Leblond B., Petit S., Picard V., Taverne T., Schweighoffer F., WO 2004/076445 A2 (2004).
26) Öztürk G., Erol D. D., Uzbay T., Aytemir M. D., *Farmaco*, **56**, 251—256 (2001).
27) Ma Y., Luo W., Quinn P. J., Liu Z., Hider R. C., *J. Med. Chem.*, **47**, 6349—6362 (2004).
28) Collie J. N., Wilsmore N. T. M., *J. Chem. Soc.*, **69**, 293—304 (1896).
29) Chen Y.-L., Chen P.-H., Chung C.-H., Li K.-C., Jeng H.-Y., Tzeng C.-C., *Helv. Chim. Acta*, **86**, 778—786 (2003).
30) Liu Z. D., Piyamongkol S., Liu D. Y., Khodr H. H., Lu S. L., Hider R.

C., *Bioorg. Med. Chem.*, **9**, 563—573 (2001).
31) Mitsunobu O., *Synthesis*, **1981**, 1—28 (1981).
32) Stark H., Kathmann M., Schlicker E., Schunack W., Schlegel B., Sippl W., *Mini Rev. Med. Chem.*, **4**, 965—977 (2004).
33) Ligneau X., Perrin D., Landais L., Camelin J. C., Calmels T. P., Berrebi-Bertrand I., Lecomte J. M., Parmentier R., Anaclet C., Lin J. S., Bertaina-Anglade V., la Rochelle C. D., d'Aniello F., Rouleau A., Gbahou F., Arrang J. M., Ganellin C. R., Stark H., Schunack W., Schwartz J. C., *J. Pharmacol. Exp. Ther.*, **320**, 365—375 (2007).
34) Ligneau X., Garbarg M., Vizuete M. L., Diaz J., Purand K., Stark H., Schunack W., Schwartz J. C., *J. Pharmacol. Exp. Ther.*, **271**, 452—459 (1994).
35) Esbenshade T. A., Krueger K. M., Miller T. R., Kang C. H., Denny L. I., Witte D. G., Yao B. B., Fox G. B., Faghih R., Bennani Y. L., Williams M., Hancock A. A., *J. Pharmacol. Exp. Ther.*, **305**, 887—896 (2003).
36) Lim H. D., van Rijn R. M., Ling P., Bakker R. A., Thurmond R. L., Leurs R., *J. Pharmacol. Exp. Ther.*, **314**, 1310—1321 (2005).
37) Bonaventure P., Letavic M., Dugovic C., Wilson S., Aluisio L., Pudiak C., Lord B., Mazur C., Kamme F., Nishino S., Carruthers N., Lovenberg T., *Biochem. Pharmacol.*, **73**, 1084—1096 (2007).
38) Celanire S., Lebon F., Stark H., "The Third Histamine Receptor: Selective Ligands as Potential Therapeutic Agents in CNS Disorders," ed. by Vohora D. S., Taylor & Francis CRC Press Inc., Boca Raton, 2008.
39) Cowart M., Pratt J. K., Stewart A. O., Bennani Y. L., Esbenshade T. A., Hancock A. A., *Bioorg. Med. Chem. Lett.*, **14**, 689—693 (2004).
40) Black L. A., Nersesian D. L., Sharma P., Ku Y. Y., Bennani Y. L., Marsh K. C., Miller T. R., Esbenshade T. A., Hancock A. A., Cowart M., *Bioorg. Med. Chem. Lett.*, **17**, 1443—1446 (2007).
41) Medhurst A. D., Atkins A. R., Beresford I. J., Brackenborough K., Briggs M. A., Calver A. R., Cilia J., Cluderay J. E., Crook B., Davis J. B., Davis R. K., Davis R. P., Dawson L. A., Foley A. G., Gartlon J., Gonzalez M. I., Heslop T., Hirst W. D., Jennings C., Jones D. N., Lacroix L. P., Martyn A., Ociepka S., Ray A., Regan C. M., Roberts J. C., Schogger J., Southam E., Stean T. O., Trail B. K., Upton N., Wadsworth G., Wald J. A., White T., Witherington J., Woolley M. L., Worby A., Wilson D. M., *J. Pharmacol. Exp. Ther.*, **321**, 1032—1045 (2007).
42) Peschke B., Bak S., Hohlweg R., Pettersson I., Refsgaard H. H., Viuff D., Rimvall K., *Bioorg. Med. Chem.*, **12**, 2603—2616 (2004).
43) Lau J. F., Jeppesen C. B., Rimvall K., Hohlweg R., *Bioorg. Med. Chem. Lett.*, **16**, 5303—5308 (2006).
44) Ganellin C. R., Hosseini S. K., Khalaf Y. S., Tertiuk W., Arrang J. M., Garbarg M., Ligneau X., Schwartz J. C., *J. Med. Chem.*, **38**, 3342—3350 (1995).
45) Sasse A., Ligneau X., Rouleau A., Elz S., Ganellin C. R., Arrang J. M., Schwartz J. C., Schunack W., Stark H., *J. Med. Chem.*, **45**, 4000—4010 (2002).
46) Barbier A. J., Berridge C., Dugovic C., Laposky A. D., Wilson S. J., Boggs J., Aluisio L., Lord B., Mazur C., Pudiak C. M., Langlois X., Xiao W., Apodaca R., Carruthers N. I., Lovenberg T. W., *Br. J. Pharmacol.*, **143**, 649—661 (2004).
47) Lipinski C. A., Lombardo F., Dominy B. W., Feeney P. J., *Adv. Drug Deliv. Rev.*, **46**, 3—26 (2001).
48) Ghose A. K., Viswanadhan V. N., Wendoloski J. J., *J. Comb. Chem.*, **1**, 55—68 (1999).
49) Ertl P., Rohde B., Selzer P., *J. Med. Chem.*, **43**, 3714—3717 (2000).
50) Wildman S. A., Crippen G. M., *J. Chem. Inf. Comput. Sci.*, **39**, 868—873 (1999).
51) Summerfield S. G., Stevens A. J., Cutler L., del Carmen Osuna M., Hammond B., Tang S. P., Hersey A., Spalding D. J., Jeffrey P., *J. Pharmacol. Exp. Ther.*, **316**, 1282—1290 (2006).
52) Schneider G., Baringhaus K. H., "Molecular Design-Concepts and Applications," Weinheim, New York, 2008.
53) Tiligada E., Zampeli E., Sander K., Stark H., *Expert Opin. Investig. Drugs*, **18**, 1519—1531 (2009).
54) www.ClinicalTrials.gov (access date May 2010).
55) Drahl C., *Chem. Eng. News*, **87**, 31—33 (2009).
56) Sander K., Kottke T., Hoffend C., Walter M., Weizel L., Camelin J. C., Ligneau X., Schneider E. H., Seifert R., Schwartz J. C., Stark H., *Org. Lett.*, **12**, 2578—2581 (2010).
57) Cheng Y. C., Prusoff W. H., *Biochem. Pharmacol.*, **22**, 3099—3108 (1973).