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Highly Potent Fluorescence-Tagged Nonimidazole Histamine H_3 Receptor Ligands

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Different (3-phenoxypropyl)piperidine derivatives have been coupled to fluorescent moieties (5-dimethylaminonaphthalene-1-sulfonyl, carbazol-9-ylcarbonyl, 2-cyanoisoindol-1-yl, 2-cyanobenzo[f]isoindol-1-yl, 2,4-dinitrobenzen-1-yl, 2,4-diaminophenyl, 7-nitrobenzofurazan-4-yl, 7-aminosulfonylbenzofurazan-4-yl, 4-methylcoumarin-6-yl) as novel histamine H_3 receptor ligands. They have been synthesised starting from piperidine in a few steps. The compounds display good to excellent histamine hH_3 receptor affinities with K_i values ranging from 13.4 to 0.048 nM. Some of the new compounds belong to the most potent ligands known so far and may act as tools for identification and understanding of the binding site on the histamine H_3 receptor. In vivo screening on selected derivatives of Sanger's reagent showed antagonist potencies with ED_{50} values from 7.9 to 0.39 mg kg^{-1}, p.o.

Introduction

G-protein-coupled receptors (GPCRs) are seven-transmembrane domain (heptahelical) receptors that control or influence a large range of physiological functions, and represent one of the largest families of membrane-bound signalling systems found in the human genome.[1] Recent analysis demonstrates that approximately 45% of all known pharmaceutical drugs are directed at transmembrane receptors, with GPCRs being the predominant target.[2] Histamine elicits a variety of physiological responses that are mediated by the four GPCRs (H_1, H_2, H_3, and H_4). Within these four known histamine receptors the H_3 subtype is predominantly expressed in the brain.[3] The synthesis and release of histamine is regulated by presynaptic H_3 receptors as part of a negative feedback mechanism.[4] As a heteroreceptor, the H_3 receptor also modulates the release of several other neurotransmitters.[5] Actually there is a great effort in the development potential of highly potent and selective antagonists because of their discussed importance in the treatment of various central diseases, for example, schizophrenia, Alzheimer's disease, obesity, and attention-deficit hyperactivity disorder (ADHD).[3]

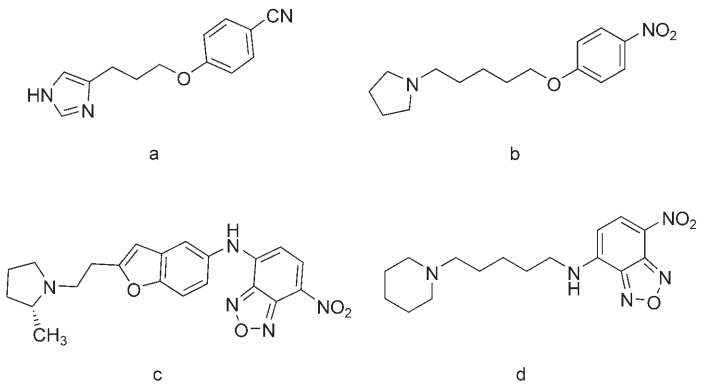

Figure 1. Structurally related lead structures for histamine H_3 receptor antagonists.[6–9]

Nowadays the development of H_3 receptor antagonists results in a wide range of compounds differentiated into two main classes. Almost all potent antagonists published before 1999 were imidazole derivatives monosubstituted in the 4(5)-position of the imidazole ring (Figure 1; a).[6] Most imidazole-containing compounds interact with the cytochrome P450 system[10,11] which is unfavourable for further drug development. By exchange of the imidazole moiety to nonaromatic *N*-containing hetereocycles, for example, piperidine, pyrrolidine, piperazine, and related structures, an additional class of antagonists has been described, the so-called nonimidazole ligands (Figure 1; b).[3,5,7] This class offers the possibility of numerous structural variations without losing the H_3 receptor affinity. Most current candidates for potential clinical development belong to this important class.

Fluorescently tagged drug molecules for GPCRs are rarely described, but they may be useful research tools for nonradioactive binding assays and for investigations on structural properties of receptor–ligand interactions amongst other uses.[12,13] They can give hints on the environment of receptor binding sites because some fluorophores show excitation and

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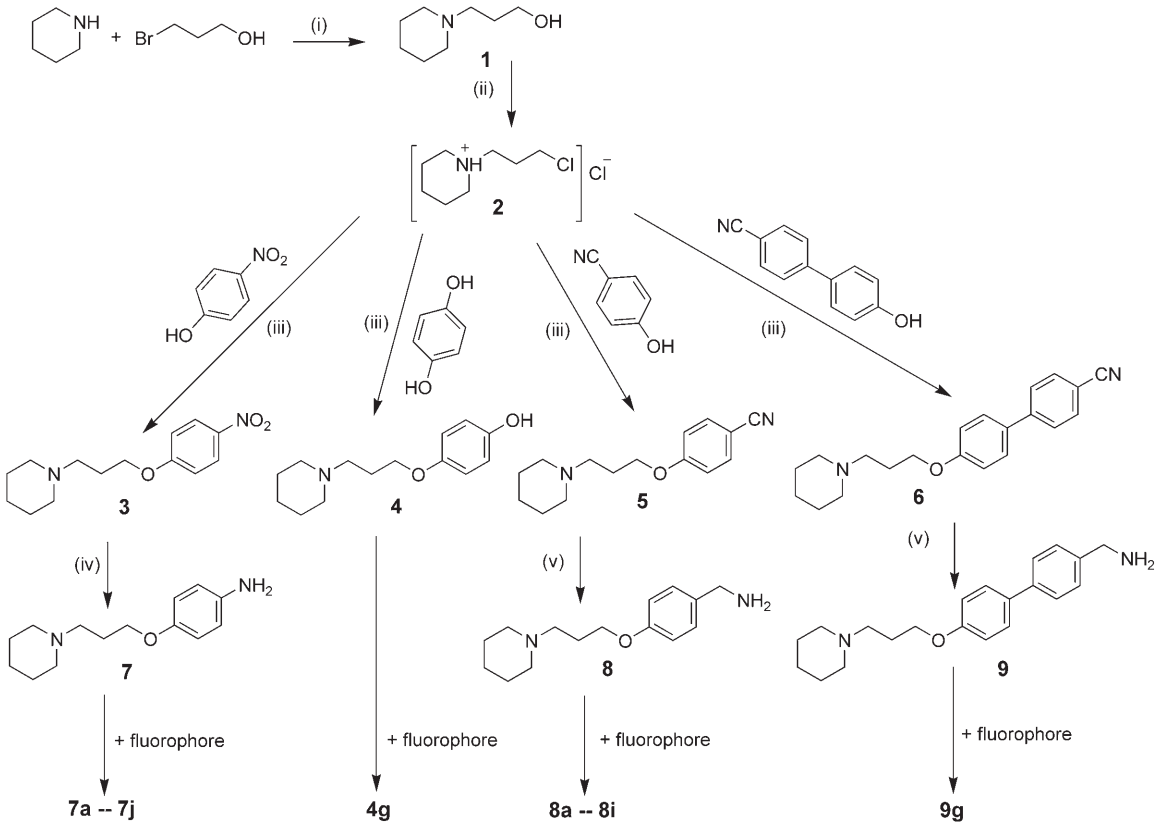
emission wavelengths depending on the surrounding lipophilicity, pH, temperature, solvent etc.[14,15] With modern spectroscopic methods such as scanning confocal spectroscopy (SCM) and fluorescence correlation spectroscopy (FCS) it is possible to exploit assays at the single cell[16] and single molecule[17] levels. If the binding is reversible, one can now obtain detailed pharmacological information such as affinity using single-molecule detection techniques. They may be used as a starting point for fluorescent resonance energy transfer (FRET) or bioluminescence resonance energy transfer (BRET) investigations.[18] Such ligands may provide the chance to see a multiplicity of information. In most cases the steric demands of the fluorescence moieties hinder the ligand–receptor interaction with small ligands which is not a problem with fluorescently tagged proteins or polypeptides. Few fluorescent ligands have been described for the histamine receptors.[19–21] A commercially available fluorescent histamine derivate (Bodipy FL histamine; Molecular Probes) is able to show lysosomal localisation,[22] but to our knowledge receptor binding properties have not been described.

Based on recent structure–activity relationships, we have designed and prepared novel nonimidazole histamine H_3 receptor ligands which possess a fluorescent chromophore in the lipophilic part of a general blueprint for antagonist structures.[3] Some related examples of fluorescent H_3 receptor ligands in a benzofuran series have also been presented very recently (Figure 1; c and d).[8,9]

Chemistry

The compounds were mostly synthesised in five steps according to Scheme 1: 1) Alkylation of piperidine with 3-bromopropanol (87% yield).[23] 2) Chlorination of the alcohol group with thionylchloride (85% yield).[24] 3) Reaction with different phenols to the asymmetrical aromatic ether structures **3**–**6** (65–85% yields).[25] 4) Catalytic reduction: 4a—Reduction of the nitro group with palladium/carbon hydrogen at room temperature (95% yield).[9] 4b—Reduction of the cyano group with Raney-Nickel/hydrogen at room temperature (90–95% yields).[26] 5) Coupling to different fluorescent moieties or precursors to the final compounds.

The key synthetic intermediate of numerous nonimidazole histamine H_3 receptor ligands is 1-(3-chloropropyl)piperidine (**2**, $n=3$), which was obtained by standard reaction of piperidine with 3-bromopropanol and followed by chlorination in nucleophilic substitution (S_Ni). The classical Williamson ether synthesis could be applied without strong heating and the product was easily obtained by extraction. The reduction of aromatic nitro groups with hydrogen under Pd/C catalysis has been optimised for final yields of 95%. The application of tin/HCl as reductive media has caused purification problems which strongly reduced yields. The reduction of cyano compounds with hydrogen under freshly prepared Raney-Nickel catalysis in ammonia has proven to be a time-sparing and cost-effective method, giving good yields (90–95%). The most



Scheme 1. Synthesis of precursors **3**–**9** and final compounds. (i) K_2CO_3, KI, abs. acetonitrile, 18 h, RT; (ii) Thionylchloride, abs. THF, 2 h, 50 °C; (iii) K_2CO_3, KI, abs. acetone, 48 h, reflux; (iv) 10% Pd/C, H_2, 12 h, RT; (v) MeOH/NH_3, Raney-Nickel, H_2, 12 h, RT.

crucial step has been the coupling with the fluorophores. In our hands it required the use of an excess of the amine compounds (**7**, **8**, **9**) as other basic additives such as K_2CO_3, Et_3N, or NaH did not yield the final compounds or caused some decomposition. The excess of the amine can be recycled by column chromatography. The final compounds are instable in strong UV-light.

All final compounds were characterised by ^{1}H NMR, ^{13}C NMR, ESI–MS, elemental analysis, and fluorescent emission and absorption spectra.

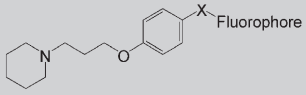
Pharmacology

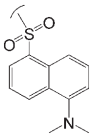
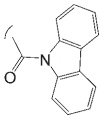
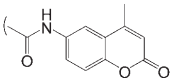
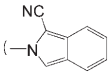
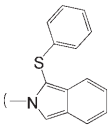
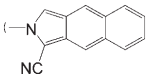
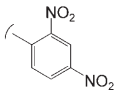
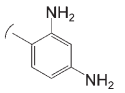
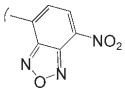
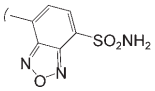
The affinity of the compounds was determined by measuring the displacement of the [^{125}I]iodoproxyfan binding to human histamine H_3 receptors stably expressed in CHO-K1 cells.[27,28]

Results and Discussion

All compounds tested could be obtained conveniently by the synthetic methods described using related synthons with the pharmacophoric elements for the histamine H_3 receptor affinity. The introduction of the fluorescent moieties was performed at the final reaction step. Despite the structural diversity of the fluorescent moieties, the compounds showed histamine H_3 receptor affinities from the nanomolar to the picomolar concentration range (Table 1 and Figures 2–4).

Table 1.
Binding affinities and physicochemical properties of fluorescent histamine H_3 receptor ligands.



Compd.	X	Fluorophore	$K_i \pm$S.E.M[a] [nM]	Clog P[b]	Ex. λ_{max} [nm]	Em. $\lambda_{max.}$ [nm]
7a	NH		9.9 ± 0.2	5.76	300	355
8a	CH_2NH		8.3 ± 0.3	5.75	302	357
7b	NH		2.9 ± 0.1	6.04	322	342
8b	CH_2NH		13.4 ± 1.4	5.90	323	337
7c	NH		1.7 ± 0.6	5.25	360	387
7d			2.6 ± 0.8	5.56	343	389
8d	CH_2		10.0 ± 3.4	5.28	328	389
7e			1.0 ± 0.3	8.22	314	328
7f			2.0 ± 0.2	5.22	347	476
8f	CH_2		9.3 ± 3.5	6.45	341	479
7g	NH		0.14 ± 0.04	5.70	396	449
4g	O		0.048 ± 0.013	5.00	397	527
8g	CH_2NH		0.603 ± 0.151	5.16	396	496
9g	$(C_6H_4)CH_2NH$		0.706 ± 0.175	7.04	452	531
7h	NH		2.15 ± 0.425	2.91	338	398
7i	NH		0.066 ± 0.014	5.63	481	531
8i	CH_2NH		0.572 ± 0.082	5.10	415	533
7j	NH		0.273 ± 0.032	5.63	468	579
a			12 ± 3[c]	1.89		
b			39[d]	3.82		
c			0.10[e]	5.76	465[e]	535[e]
d			11 ± 1[f]	4.37	467[f]	526[f]

[a] [^{125}I]Iodoproxyfan binding on CHO-K1 cells stably expressing the hH_3 receptor.[27,28] [b] *n*-Octanol/water partition coefficient based on established chemical interactions; calculated with ChemDraw Ultra 7.0.[29] [c] Ref. [6]. [d] Ref. [7]. [e] Ref. [8]. [f] Ref. [9].

As the fluorescent moieties showed quite different steric (for example, **7a**, **7b**, **7c**, **7g**, **7h**, **7i**; **7d**, **7e**, **7f**; **8a**, **8b**, and **8g**) and hydrogen bonding acceptor and donor properties (for example, **4g**, **7b**, **7c**, **7g**, **7h**, **7i**, **7j**, **8i**), it is amazing that the compound with the lowest affinity showed a K_i value of less than 15 nM (**8b**). As the number of compounds is too small to draw general conclusions, one finds the tendency that compounds with fluorophores directly connected to the pharmacophore showed higher affinities than the related compound with an additional methylene spacer (**7b**→**8b**; **7d**→**8d**; **7f**→**8f**; **7g**→**8g**; **7i**→**8i**), with the exception of **7a**→**8a** which showed comparable affinities. On the other hand, it should be stressed that compound **9g** containing a biphenyl structure also showed subnanomolar affinity. Therefore, it may be the case that additional binding pockets at a greater distance to the common pharmacophoric binding element may contribute to the ligand–receptor binding. Generally, the fluorophoric group did not diminish the ligand–receptor binding as is often the case in other structural ligand classes of aminergic receptors. Here, some fluorophoric groups even increase the binding

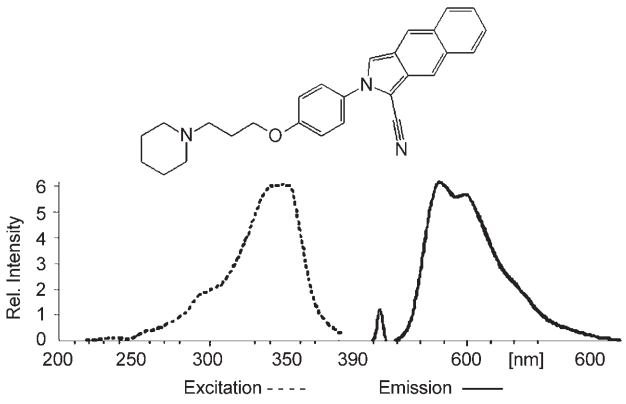


Figure 2. Excitation and emission spectra of the prominent 2-cyanobenzo-[f]isoindole substituted compound **7f**.

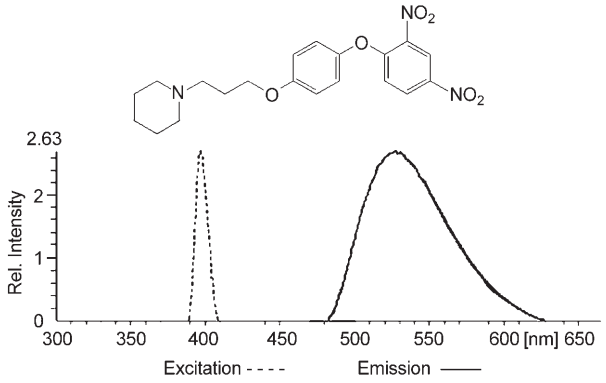


Figure 3. Excitation and emission spectra of the prominent 2,4-dinitrobenzen substituted compound **4g**.

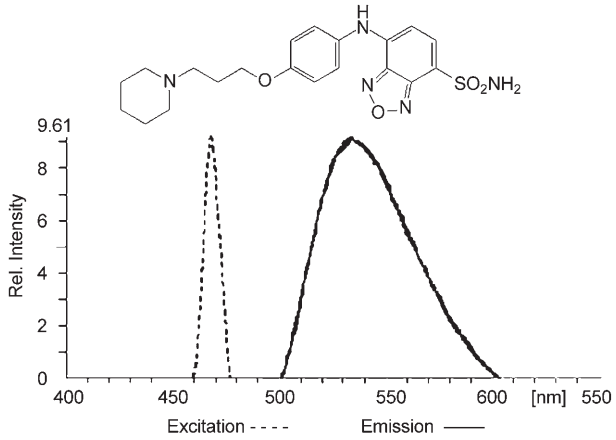


Figure 4. Excitation and emission spectra of the prominent 4-sulfonamid-benzofuran substituted compound **7j**.

as impressively shown with compounds **4g**, **7g**, and **7i**. For the derivatives of Sanger's reagent (**4g**, **7g**, **8g**, and **9g**) different linker groups maintained or increased affinities. The only compound with an ether linker in this series showed the highest affinity (**4g**), but other compounds demonstrated similar affinities (**7i**). Compounds **4g** and **7i** belong to the most potent compounds for this receptor subtype described so far.

From a physicochemical point of view, all compounds showed an acceptable difference in excitation and emission maximal wavelengths. Stoke shifts varied from 50 to 138 nm, with compound **4g** (one of the most potent compounds), showing one of the highest Stoke shifts, 130 nm. Calculated lipophilicities of all compounds ranged within acceptable Clog*P* values; from 5.0 to 8.2.[29] As these compounds will not be used as drugs but as pharmacological tools, drug-like properties (for example, Lipinski's Rule of Five) are of minor interest. Once more, the most potent compound with the highest Stoke shift showed one of the lowest lipophilicity (**4g**).

This series of different histamine H_3 receptor ligands with fluorophore properties demonstrated some possibilities for structural variations of the previously developed pharmacophore. The application of a general blue print for H_3 receptor ligands and strong knowledge of structure–activity relationships in different leads allowed the development of fluorescent receptor ligands which may be useful for further nonradioactive assays. The small compound synthons for the preparation of the fluorescent moieties are commercially available at a reasonable price. Therefore, it is easily possible to make a series of differently fluorophore-tagged compounds. In our hands they are more stable and robust on chemical experiments and light (laser, sun) than most of bulky Bodipy-like compounds. Some of the small fluorescent compounds employed increase both in vitro and in vivo efficiencies, proceeding from a well known lead structure. Some compounds selected from the Sanger's reagent series showed rather good H_3-receptor antagonist potencies as shown in the standard in vivo screening assay in Swiss mice after oral administration, observing the modulation of the N^{τ}-methylhistamine level in brain cortex (**7h**: $ED_{50} = 7.9 \pm 4.0\ mg\,kg^{-1}$ p.o.; **9g**: $ED_{50} = 1.1. \pm 0.1\ mg\,kg^{-1}$ p.o.; **7g**: $ED_{50} = 0.39 \pm 13\ mg\,kg^{-1}$ p.o.).[30] The most potent compound **4g** has an in vitro affinity of 0.048 nM and a good in vivo efficacy with an ED_{50} value of $0.96 \pm 0.15\ mg\,kg^{-1}$, p.o.[30]

The fluorescent compounds represent a new series of potent compounds, which may be utilized for further in vitro, in vivo, or cell studies using modern imaging techniques such as FRET, SCM, and FCS. They have the potential to be useful pharmacological tools for investigating receptor–ligand interactions, for example, in fluorimetric binding assays or functional studies. The good to high affinities and different maximal emission wavelengths present a wide area for further optimization and assignment of these fluorescent histamine H_3 receptor ligands to new pharmacological assays.

Experimental Section

Chemistry

All materials were obtained from commercial suppliers and used without further purification. Melting points were determined on a Büchi Melting Point B-510 apparatus, CH-9230 Flawil, Switzerland. ^{1}H- and ^{13}C NMR were recorded on Bruker AC 300 (^{1}H NMR 300 MHz) and Bruker AC 200 (^{13}C NMR 50 MHz). Mass spectra (ESI) were obtained with a Fisons Instruments VG Platform II, Manchester, UK. Elemental analyses were determined on a CHN-Rapid (Her-

aeus, Germany) and were within ±0.4%. Purification with Chromatotron: Version 7924T, Harrison Research, USA. All excitation and emission data were measured with an Amico-Bowman Series 2 Spectrometer. The fluorescent compounds were measured at a concentration of 10^{-5} M in absolute spectroscopic ethanol at room temperature. Emission spectra were recorded at the maximal excitation wavelength.

1: 3-(Hydroxypropyl)piperidine was synthesised from 3-bromopropan-1-ol (20 mmol) with piperidine (30 mmol) and sodium carbonate(30 mmol) in 50 mL of abs. acetonitrile. After stirring for over 24 h at room temperature, the reaction mixture was filtered and purified by distillation at 11 mbar. The product was obtained as a colourless oil.[31] Yield 83%, bp. 103 °C/11 mbar; ESI–MS $C_8H_{17}NO$ calculated: 143.23, found: 143.3; ^{1}H NMR (300 MHz, DMSO): δ = 4.48 (s, 1H), 3.40 (t, J = 6.3 Hz, 2H), 2.25 (t, J = 6.9 Hz,6H), 1.45 (t, J = 5.4 Hz, 6H), 1.35 ppm (dd, J = 5.1 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 59.73, 56.04, 54.14, 29.55, 25.62, 24.14 ppm.

2: 3-(Chloropropyl)piperidine hydrochloride was synthesised from 3-(hydroxypropyl)piperidine (**1**) (20 mmol) with thionyl chloride (25 mmol) in 100 mL of abs. tetrahydrofuran at 0 °C. After stirring for 2 h the reaction mixture was heated at 50 °C over 3 h. Following evaporation in vacuum the solid product with light-coloured yellow crystals was formed.[32] Yield 71%; ESI–MS $C_8H_{16}ClN$ calculated: 161.1, found: 161.3; ^{1}H NMR (300 MHz, DMSO): δ = 3.72 (t, J = 6.4 Hz, 2H), 3.34 (t, J = 10.0 Hz, 2H), 3.03 (t, J = 3.3 Hz, 2H), 2.81 (t, J = 6.4 Hz, 2H), 2.23 (dd, J = 6.4 Hz, 2H), 1.64–1.82 ppm (sb, J = 6H); ^{13}C NMR (50 MHz, DMSO): δ = 53.56, 51.94, 42.52, 26.15, 22.17, 21.36 ppm.

3: 4-(3-Piperidin-1-ylpropoxy)nitrobenzene was synthesised from 3-(chloropropyl)piperidine hydrochloride (**2**) (20 mmol) with 4-nitrophenol (30 mmol), KI (20 mmol) and sodium carbonate (50 mmol) in 50 mL of abs. acetone. After 48 h stirring at reflux temperature the reaction mixture is filtered and the solvent is evaporated in vacuum. The solid reaction products were partitioned in CH_2Cl_2 and 1 M NaOH. The organic layers were dried with $MgSO_4$. Evaporation of the solvent in vacuum gave the final product as a brown oil.[33] Yield 91%; ESI–MS $C_{14}H_{20}N_2O_3$ calculated: 264.15, found: 264.4; ^{1}H NMR (300 MHz, DMSO): δ = 8.16 (d, J = 5.0 Hz, 2H), 7.09 (d, J = 5.0 Hz, 2H), 4.11 (t, J = 6.4 Hz, 2H), 2.31–2.36 (s, 6H), 1.78 (dd, J = 7.3 Hz, 2H), 1.46 (dd, J = 5.3 Hz, 4H), 1.37 ppm (dd, J = 5.0 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 164.0, 140.7, 125.8, 114.9, 67.1, 54.8, 54.1, 26.0, 25.6, 24.1 ppm.

4: 4-Hydroxy-4-(3-piperidin-1-ylpropoxy)benzene was synthesised from 3-(chloropropyl)piperidine hydrochloride (**2**) (20 mmol) with 1,4-dihydroxybenzen (30 mmol), KI (20 mmol) and sodium carbonate (50 mmol) in 50 mL of abs. acetone. After 48 h stirring at reflux temperature the reaction mixture is filtered and the solvent evaporated in vacuum. Purification by column chromatography using CH_2Cl_2-MeOH, NH_3 saturated (95:5) resulted in the final product (white crystals). Yield 53%; ESI–MS $C_{14}H_{21}NO_2$ calculated: 235.32, found: 235.5; ^{1}H NMR (300 MHz, DMSO): δ = 6.71 (d, J = 6.7 Hz, 2H), 6.64 (d, J = 6.6 Hz, 2H), 3.85 (t, J = 6.4 Hz, 2H), 3.37 (s, 1H), 2.33 (t, J = 7.2 Hz, 6H), 1.85 (dd, J = 6.8 Hz, 2H), 1.47 (dd, J = 5.9 Hz, 4H), 1.35 ppm (dd, J = 5.4 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 151.4, 151.0, 115.6, 115.4, 66.4, 55.2, 54.1, 26.4, 25.5, 24.1 ppm.

5: 4-(3-Piperidin-1-ylpropoxy)benzonitrile was synthesised from 3-(chloropropyl)piperidine hydrochloride (**2**) (20 mmol) with 4-cyanophenol (30 mmol), KI (20 mmol) and sodium carbonate (50 mmol) in 50 mL of abs. acetone. After 48 h stirring at reflux temperature the reaction mixture is filtered and the solvent evaporated in vacuum. The solid reaction products were partitioned in CH_2Cl_2 and 1 M NaOH. The solution was extracted with 1 M NaOH to eliminate the excess of phenol compound. The organic layer was dried with $MgSO_4$ and evaporated in vacuum to give the product as a light coloured red oil.[34] Yield 88%; ESI–MS $C_{15}H_{20}N_2O$ calculated: 244.33, found: 244.6; ^{1}H NMR (300 MHz, DMSO): δ = 7.69 (d, J = 5.0 Hz, 2H), 7.04 (d, J = 5.1 Hz, 2H), 4.02 (t, J = 6.4 Hz, 2H), 2.30 (t, J = 7.0 Hz, 6H), 1.81 (dd, J = 6.9 Hz, 2H), 1.45 (dd, J = 5.7 Hz, 4H), 1.32 ppm (dd, J = 5.0 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 162.1, 134.0, 119.0, 115.4, 102.7, 66.48, 54.9, 54.1, 26.1, 25.6, 24.1 ppm.

6: 4′-(3-Piperidin-1-ylpropoxy)biphenylnitrile was synthesised from 3-(chloropropyl)piperidine hydrochloride (**2**) (20 mmol) with 4′-hydroxybiphenyl-4-carbonitrile (30 mmol), KI (20 mmol) and sodium carbonate (50 mmol) in 50 mL of abs. acetone. After 48 h stirring at reflux temperature the reaction mixture is filtered and the solvent evaporated in vacuum. The solid reaction products were partitioned in CH_2Cl_2 and 1 M NaOH. The organic layer was dried with $MgSO_4$ and evaporated in vacuum to give the product (white crystals). Yield 73%; ESI–MS $C_{21}H_{24}N_2O$ calculated: 320.43, found: 320.9; ^{1}H NMR (300 MHz, DMSO): δ = 7.85 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 4.04 (t, J = 6.3 Hz, 2H), 2.30 (t, J = 7.0 Hz, 6H), 1.81 (dd, J = 6.9 Hz, 2H), 1.45 (dd, J = 5.7 Hz, 4H), 1.32 ppm (dd, J = 5.0 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 162.1, 134.0, 130.0, 128.6, 128.4 126.9, 119.0, 115.4, 102.7, 66.5, 54.9, 54.1, 26.1, 25.6, 24.1 ppm.

7: 4-(3-Piperidin-1-ylpropoxy)aniline was synthesised from 4-(3-piperidin-1-ylpropoxy)-1-nitrobenzene (**3**) (30 mmol) by reduction with 10% Pd/C, H_2 in methanol. After 10 h of stirring at room temperature the reaction mixture is filtered. Evaporation the solvent in vacuum gives the product (dark oil). Yield 98%; ESI–MS $C_{14}H_{22}N_2O$ calculated: 234.35, found: 234.6; ^{1}H NMR (300 MHz, DMSO): δ = 6.64 (d, J = 3.5 Hz, 2H), 6.51 (d, J = 3.5 Hz, 2H), 4.56–4.59 (s, 2H, N) 3.82 (t, J = 6.4 Hz, 2H), 2.34 (t, J = 7.5 Hz, 6H), 1.76 (dd, J = 6.6 Hz, 2H), 1.50 (dd, J = 5.7 Hz, 4H), 1.38 ppm (dd, J = 5.0 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 149.9, 142.2, 115.3, 114.9, 66.4, 55.2, 54.0, 26.5, 25.5, 24.1 ppm.

8: 4-(3-Piperidin-1-ylpropoxy)benzylamine was synthesised from 4-(3-piperidin-1-ylpropoxy)benzonitrile by reduction with Raney-Nickel/H_2 in methanol/ammonia at room temperature over 12 h. The reaction mixture is filtered and purified by extraction with CH_2Cl_2. The organic layers were dried with $MgSO_4$ and evaporated in vacuum to give the product (colourless oil).[35] Yield 87%; ESI–MS $C_{15}H_{24}N_2O$ calculated: 248.37, found: 248.8; ^{1}H NMR (300 MHz, DMSO): δ = 7.22 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 3.92 (t, J = 6.4 Hz, 2H), 6.64 (s, 2H), 2.35 (t, J = 7.0 Hz, 6H), 1.85 (dd, J = 7.0 Hz, 2H), 1.48 (dd, J = 5.2 Hz, 4H), 1.36 ppm (dd, J = 4.8 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 157.2, 136.2, 128.9, 128.0, 114.0, 65.9, 55.2, 54.1, 45.1, 26.4, 25.6, 24.1 ppm.

9: 4′-(3-Piperidin-1-ylpropoxy)biphenyl-4-ylmethylamine was synthesised from 4′-(3-piperidin-1ylpropoxy)benzonitrile by reduction with Raney-Nickel/H_2 in methanol/ammonia at room temperature over 12 h. The reaction mixture was filtered and purified by extraction with CH_2Cl_2. The organic layers were dried with $MgSO_4$ and evaporated in vacuum to give the final product (white powder). Yield 66%; ESI–MS $C_{21}H_{28}N_2O$ calculated: 324.46, found: 324.8; ^{1}H NMR (300 MHz, DMSO): δ = 7.39 (d, J = 7.9 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 3.88 (t, J = 6.2 Hz, 2H), 3.79 (s, 2H), 2.31 (t, J = 6.6 Hz, 6H), 1.80 (dd, J = 6.9 Hz, 2H), 1.41 (dd, J = 5.3 Hz, 4H), 1.30 ppm (dd, J = 5.0 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 158.1, 141.3, 134.2, 128.3, 128.1, 126.9, 114.7, 68.5, 54.9, 52.1, 47.5, 26.1, 25.6, 24.1 ppm.

4g: 1-{3-[4-(2,4-Dinitrophenoxy)phenoxy]propyl}piperidine was synthesised from 4-(3-piperidin-1-ylpropoxy)phenol (**4**) (1.6 mmol) with 2,4-dinitrofluorobenzene (1.6 mmol), KI (1.6 mmol) and sodium carbonate (4.8 mmol) in 50 mL of abs. acetone. After 48 h stirring at reflux temperature the reaction mixture was filtered and the solvent evaporated in vacuum. Two purifications by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) and CH_2Cl_2:MeOH (9:1) resulted in the final product (pale white powder). Yield 58%; ESI–MS $C_{20}H_{23}N_3O_6$ calculated: 401.42, found: 401.8; ^{1}H NMR (300 MHz, DMSO): δ = 8.87 (d, J = 6.9 Hz, 1H), 8.44 (d, J = 6.5 Hz, 1H), 7.24 (s, 1H), 7.08 (d, J = 8.9 Hz, 2H), 7.05 (d, J = 6.7 Hz, 2H), 4.06 (t, J = 5.4 Hz, 2H), 3.05–3.18 (s, 6H), 2.10–2.14 (s, 2H), 1.71–1.73 (s, 4H), 1.39–1.40 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 164.3, 156.3, 155.7, 146.8, 141.0, 139.0, 129.5, 121.8, 118.3, 116.3, 65.6, 53.4, 52.2, 23.6, 22.7, 21.5 ppm.

7a: 5-Dimethylaminonaphthalene-1-sulfonic acid [4-(3-piperidin-1-ylpropoxy)phenyl]amide was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (**7**) (1.8 mmol) and 5-dimethylaminonaphthalene-1-sulfonylchloride (Dansyl chloride) (0.9 mmol) in 60 mL of abs. dioxane. After 48 h stirring at room temperature the solvent was evaporated in vacuum. Four purifications by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (yellow oil). Yield 32%; ESI–MS $C_{26}H_{33}N_3O_3S$ calculated: 467.62, found: 467.8; ^{1}H NMR (300 MHz, DMSO): δ = 8.40 (d, J = 8.5 Hz, 1H), 8.35 (d, J = 8.7 Hz, 1H), 8.06 (d, J = 7.1 Hz, 1H), 7.58 (dd, J = 7.7 Hz, 2H), 7.23 (d, J = 7.5 Hz, 1H), 6.88 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.8 Hz, 2H), 3.82–3.86 (s, 2H), 2.88–2.98 (s, 6H), 2.80 (s, 6H), 1.94–1.98 (s, 2H), 1.62–1.66 (s, 4H), 1.44–1.48 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 164.5, 155.1, 151.4, 135.0, 130.3, 129.9, 129.5, 129.1, 128.9, 128.0, 123.4, 122.2, 118.8, 115.2, 114.9, 65.6, 53.7, 52.5, 45.0, 24.0, 21.9 ppm.

7b: *N*-[4-(3-Piperidin-1-ylpropoxy)phenyl]carbazole-9-carboxamide was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (**7**) (1.50 mmol) and carbazole-9-carbonyl chloride (0.65 mmol) in 50 mL of abs. dioxane. After 48 h stirring at room temperature the solvent was evaporated in vacuum. Purification by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (grey powder). Yield 97%; ESI–MS $C_{27}H_{29}N_3O_2$ calculated: 427.54, found: 427.4; ^{1}H NMR (300 MHz, DMSO): δ = 8.21 (d, J = 7.6 Hz, 2H), 7.89 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.51 (dd, J = 7.5 Hz, 2H), 7.36 (dd, J = 7.4 Hz, 2H), 6.98 (d, J = 8.5 Hz, 2H), 5.74 (s, NH, 1H) 3.95–4.05 (s, 2H), 3.08–3.19 (s, 6H), 2.10–2.14 (s, 2H), 1.66–1.78 (s, 4H), 1.50–1.54 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 164.2, 154.8, 150.0, 137.9, 131.6, 126.7, 124.0, 121.9, 121.6, 120.3, 114.8, 113.6, 65.3, 53.5, 52.2, 23.6, 21.5 ppm.

7c: 1-(4-Methyl-2-oxo-2*H*-chromen-7-yl)-3-[4-(3-piperidin-1-ylpropoxy)phenyl]urea was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (**7**) (2.50 mmol) and 7-isocyanato-4-methyl-chromen-2-one (1.00 mmol) in 50 mL of abs. dioxane. After 70 h stirring at room temperature the solvent was evaporated in vacuum. Purification by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the product (white powder). Yield 12%; ESI–MS $C_{25}H_{29}N_3O_4$ calculated: 435.52, found: 435.7; ^{1}H NMR (300 MHz, DMSO): δ = 9.11 (s, NH, 1H), 8.64 (s, NH, 1H), 7.67 (d, J = 8.7 Hz, 1H), 7.61 (s, 1H), 7.36 (d, J = 5.0 Hz, 2H), 7.32 (s, 1H), 6.88 (d, J = 6.9 Hz, 2H), 6.20 (s, 1H), 3.96 (t, J = 6.4 Hz, 2H), 2.48 (s, 3H) 2.33 (t, J = 6.4 Hz, 6H), 1.83 (t, J = 7.3, 2H), 1.50 (t, J = 5.8 Hz 4H), 1.37 ppm (t, J = 5.1 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 161.1, 154.2, 154.0, 153.2, 152.3, 143.6, 132.0, 125.8, 120.4, 114.6, 114.2, 113.6, 111.3, 104.1, 66.1, 55.1, 54.1, 26.3, 25.6, 24.1, 17.9 ppm.

7d: 2-[4-(3-Piperidin-1-ylpropoxy)phenyl]-2*H*-isoindole-1-carbonitrile was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (0.6 mmol) (**7**) with phthalaldehyde (0.6 mmol), NaCN (9 mmol) and sodium tetraborate (10 mmol) in 40 mL of methanol. After stirring in the dark for 18 h at room temperature, the reaction mixture was added to ice/water. Filtration, drying, and purification by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (beige powder). Yield 63%; ESI–MS $C_{23}H_{25}N_3O$ calculated: 359.20, found: 359.8; ^{1}H NMR (300 MHz, DMSO): δ = 8.06 (s, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 8.6 Hz, 3H), 7.33 (t, J = 6.9 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 7.15 (s, 1H), 4.10–4.20 (s, 2H), 2.95–3.20 (s, 6H), 2.10–2.20 (s, 2H), 1.65–1.80 (s, 4H), 1.50–1.60 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 158.8, 134.6, 131.8, 128.8, 126.4, 124.7, 124.0, 123.3, 122.9, 121.7, 117.4, 115.4, 114.3, 92.9, 65.7, 53.4, 52.2, 23.5, 22.7 ppm.

7e: 1-Phenylsulfanyl-2-[4-(3-piperidin-1-ylpropoxy)phenyl]-2*H*-isoindole was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (0.6 mmol) (**7**) and phthalaldehyde (0.6 mmol) with thiophenol (0.9 mmol) in 30 mL of methanol. After stirring in the dark for 42 h at room temperature, the reaction mixture was added to ice/water. Filtration, drying, and two purifications by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (brown oil). Yield 16%; ESI–MS $C_{28}H_{30}N_2OS$ calculated: 442.21, found: 442.6; ^{1}H NMR (300 MHz, DMSO): δ = 7.93 (dd, J = 8.0 Hz, 1H), 7.75 (s, 1H), 7.63 (d, J = 7.9 Hz, 2H), 7.49 (dd, J = 6.9 Hz, 2H), 7.34 (d, J = 5.4 Hz, 2H), 7.22 (d, J = 7.6 Hz, 1H), 7.10 (d, J = 4.0 Hz, 2H) 6.95 (s, 1H), 6.86 (d, J = 7.2 Hz, 2H), 4.10 (t, J = 5.3 Hz, 2H), 2.90–3.20 (s, 6H), 2.10–2.20 (s, 2H), 1.65–1.80 (s, 4H), 1.50–1.60 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 158.5, 155.7 134.7, 132.4, 131.6, 128.9, 128.4, 125.7, 125.0, 124.0, 123.3, 123.0, 122.7, 121.3, 119.5, 114.8, 113.7, 65.3, 52.2, 50.7, 23.6, 22.7 ppm.

7f: 2-[4-(3-Piperidin-1-ylpropoxy)phenyl]-2*H*-benzo[*f*]isoindole-1-carbonitrile was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (0.4 mmol)(**7**) with napthalene-2,3-dicarbaldehyde (0.4 mmol), NaCN (0.8 mmol) and sodium tetraborate (1 mmol) in 30 mL of methanol. After stirring in the dark for 18 h at room temperature, the reaction mixture was added to ice/water. The reaction mixture was filtered and purified by extraction with CH_2Cl_2. The organic layers were dried with $MgSO_4$ and evaporated in vacuum giving clean product (grey powder).Yield 98%; ESI–MS $C_{27}H_{27}N_3O$ calculated: 409.22, found: 409.8; ^{1}H NMR (300 MHz, DMSO): δ = 8.59 (s, 1H), 8.50 (s, 1H), 8.40 (s, 1H), 8.30 (s, 1H) 7.95 (dd, J = 8.0 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.34 (dd, J = 6.4 Hz, 1H), 7.21 (d, J = 8.9 Hz, 2H), 4.17 (t, J = 5.8 Hz, 2H), 3.15 (t, J = 4.9 Hz, 6H), 2.18 (t, J = 9.4 Hz, 2H), 1.71–1.76 (s, 4H), 1.51–1.55 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 156.7, 155.7, 135.1, 133.2, 131.8, 129.6, 129.3, 128.7, 124.4, 124.1, 124.0, 122.9, 121.4, 120.0, 115.4, 114.7, 90.83, 65.7, 53.6, 52.3, 23.7, 22.8, 21.6 ppm.

7g: (2,4-Dinitrophenyl)[4-(3-piperidin-1-ylpropoxy)phenyl]amine was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (**7**) (2.50 mmol) with 1-fluoro-2,4-dinitrobenzene (2.50 mmol) and potassium carbonate (5 mmol) in 50 mL of abs. acetonitrile. After stirring in the dark for 42 h at room temperature, the reaction mixture was filtered, dried, and purified by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulting in the final product (yellow powder). Yield 76%; ESI–MS $C_{20}H_{24}N_4O_5$ calculated: 400.43, found: 401.2; ^{1}H NMR (300 MHz, DMSO): δ = 8.87 (s, 1H), 8.19 (d, J = 9.6 Hz, 1H), 7.28 (d, J = 9.4 Hz, 2H), 7.06 (d, J = 8.9 Hz, 2H), 6.93 (d, J = 8.2 Hz, 1H), 5.75 (s, NH, 1H), 4.02 (t, J = 6.2 Hz, 2H) 3.39 (d, J = 6.6 Hz, 2H), 2.30–2.38 (s, 6H), 1.92 (t, J = 6.5 Hz, 2H), 1.72–1.75 (s, 4H), 1.52–1.55 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 157.2,

147.5, 143.1, 136.0, 130.7, 129.7, 126.1, 123.5, 116.5, 115.6, 65.4, 53.4, 52.2, 23.6, 22.7, 21.5 ppm.

7h: N^1-[4-(-Piperidin-1-ylpropoxy)phenyl]benzene-1,2,4-triamine was synthesised from (2,4-dinitrophenyl)-[4-(3-piperidin-1-ylpropoxy)phenyl]amine (**7g**) (30 mmol) by reduction with 10% Pd/C, H_2 in 30 mL of methanol. After 6 h stirring at room temperature the reaction mixture is filtered. Purification by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (9:1) and three crystallisations gave the final product (dark powder). Yield 8%; ESI–MS $C_{20}H_{28}N_4O$ calculated: 340.44, found: 340.9; ^{1}H NMR (300 MHz, DMSO): δ = 7.53 (d, J = 9.6 Hz, 1H), 7.29 (s, 1H), 7.16 (s, 1H), 6.71 (d, J = 10.3 Hz, 2H), 6.56 (d, J = 8.6 Hz, 2H), 4.85–5.45 (s, NH, 5H), 4.11 (t, J = 6.8 Hz, 2H) 3.80–3.95 (s, 2H), 3.05–3.18 (s, 4H), 1.90–2.05 (s, 2H), 1.70–1.75 (s, 4H), 1.50–1.54 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 163.9, 128.7, 124.7, 115.5, 114.8, 65.4, 62.6, 56.0, 53.5, 52.1, 37.7, 23.6, 23.4, 22.5, 21.3, 20.1 ppm.

7i: (7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)[4-(3-piperidin-1-ylpropoxy)phenyl]amine was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (**7**) (1.0 mmol) with 4-chloro-7-nitro-benzo[*c*][1,2,5]oxadiazole (0.5 mmol) in 50 mL of abs. dioxane in the dark over 17 h at room temperature. Purification was performed after evaporation in vacuum by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulting in the final product (red powder). Yield 80%; ESI–MS $C_{20}H_{23}N_5O_4$ calculated: 397.18, found: 398.0; ^{1}H NMR (300 MHz, DMSO): δ = 8.50 (d, J = 8.9 Hz, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 6.53 (d, J = 9.1 Hz, 1H), 4.09 (t, J = 5.6 Hz, 2H), 3.15 (t, J = 7.0 Hz, 6H), 2.10–2.20 (s, 2H), 1.70–1.80 (s, 4H), 1.50–1.60 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 156.4, 144.9, 144.3, 143.4, 137.6, 131.0, 125.7, 122.0, 115.4, 101.2, 65.4, 53.5, 52.2, 23.6, 22.7, 21.5 ppm.

7j: 7-[4-(3-Piperidin-1-ylpropoxy)phenylamino]benzo[*c*]-[1,2,5]oxadiazol-4-sulfonamide was synthesised from 4-(3-piperidin-1-ylpropoxy)aniline (**7**) (1.0 mmol) with 7-chloro-benzo[*c*]-[1,2,5]oxadiazole-4-sulfonic acid amide (0.23 mmol) in 50 mL of abs. dioxane in the dark over 17 h at room temperature. Purification by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (yellow powder). Yield 81%; ESI–MS $C_{20}H_{25}N_5O_4$ calculated: 431.51, found: 431.9; ^{1}H NMR (300 MHz, DMSO): δ = 9.81 (s, NH, 2H), 7.82 (d, J = 8.0 Hz, 1H), 7.44 (s, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 8.8 Hz, 2H), 6.57 (d, J = 8.0 Hz, 1H), 4.00–4.10 (s, 2H), 3.08–3.18 (s, 6H), 2.10–2.18 (s, 2H), 1.70–1.74 (s, 4H), 1.48–1.55 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 155.47 145.4, 144.8, 138.1, 136.3, 132.0, 125.0, 116.5, 115.3, 100.7, 65.3, 53.5, 52.3, 23.7, 22.7, 21.5 ppm.

8a: *N*-4-(3-Piperidin-1-ylpropoxy)benzyl-5-dimethylaminonaphthalene-1-sulfonamide was synthesised from 4-(3-piperidin-1-ylpropoxy)benzylamine (**8**) (1.5 mmol) and 5-dimethylaminonaphthalene-1-sulfonyl chloride (Dansyl chloride) (0.5 mmol) in 50 mL of abs. dioxane. After 48 h stirring at room temperature the solvent was evaporated in vacuum. Two purifications by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (yellow oil). Yield 83%; ESI–MS $C_{27}H_{35}N_3O_3S$ calculated: 481.65, found: 482.1; ^{1}H NMR (300 MHz, DMSO): δ = 8.42 (d, J = 8.5 Hz, 1H), 8.31 (d, J = 8.6 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.58 (dd, J = 6.5 Hz, 2H), 7.25 (d, J = 7.5 Hz, 1H), 7.00 (d, J = 8.5 Hz, 2H), 6.68 (d, J = 8.6 Hz, 2H), 4.10 (s, 2H), 3.89 (t, J = 6.4 Hz, 2H), 2.81 (s, 6H), 2.32 (t, J = 7.2 Hz, 6H), 1.78 (t, J = 6.9 Hz, 2H), 1.47 (t, J = 5.1 Hz, 4H), 1.37 ppm (d, J = 5.0 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 157.6, 151.2, 136.3, 129.3, 129.2, 129.0, 128.9, 128.7, 128.2, 127.6, 123.4, 119.2, 114.9, 113.8, 65.8, 55.0, 54.0, 45.4, 45.0, 26.2, 25.6 24.0 ppm.

8b: *N*-4-(3-Piperidin-1-ylpropoxy)benzylcarbazole-9-carboxamide was synthesised from 4-(3-piperidin-1-ylpropoxy)benzylamine (**8**) (1.3 mmol) and carbazole-9-carbonyl chloride (0.65 mmol) in 50 mL of abs. dioxane. After 48 h stirring at room temperature the solvent was evaporated in vacuum. Purification by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (colourless crystals). Yield 85%; ESI–MS $C_{28}H_{31}N_3O_2$ calculated: 441.57, found: 442.0; ^{1}H NMR (300 MHz, DMSO): δ = 7.91 (d, J = 7.4 Hz, 2H), 7.63 (d, J = 8.0 Hz, 2H), 7.54 (d, J = 8.7 Hz, 2H), 7.33 (dd, J = 7.6 Hz, 2H), 7.26 (dd, J = 7.6 Hz, 2H), 6.88 (d, J = 8.3 Hz, 2H), 5.01 (s, NH, 1H) 4.15 (s, 2H), 3.85–3.95 (s, 2H), 3.05–3.20 (s, 6H), 1.90–2.05 (s, 2H), 1.54–1.66 (s, 4H), 1.38–1.48 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 164.1, 155.0, 150.8, 138.2, 131.8, 125.7, 123.5, 121.9, 121.2, 120.6, 115.2, 114.2, 65.3, 54.5, 52.2, 45.8, 23.8, 21.7 ppm.

8d: 2-[4-(3-Piperidin-1-ylpropoxy)benzyl]-2*H*-isoindole-1-carbonitrile was synthesised from 4-(3-piperidin-1-ylpropoxy)benzylamine (0.6 mmol)(**8**) with phthalaldehyde (0.6 mmol), NaCN (0.6 mmol) and sodium tetraborate (2 mmol) in 50 mL of methanol. After stirring in the dark for 18 h at room temperature, the reaction mixture was added to ice/water. Filtration, drying, and purification by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulted in the final product (beige powder). Yield 75%; ESI–MS $C_{24}H_{27}N_3O$ calculated: 373.22, found: 373.9; ^{1}H NMR (300 MHz, DMSO): δ = 7.97 (s, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.5 Hz, 3H), 6.93 (t, J = 8.6 Hz, 1H), 5.51 (s, 2H), 3.95 (t, J = 6.4 Hz, 2H), (s, 2H), 2.30 (t, J = 6.3 Hz, 6H), 1.81 (t, J = 6.8 Hz, 2H), 1.47 (t, J = 5.7 Hz, 4H), 1.36 ppm (t, J = 4.9 Hz, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 158.6, 131.2, 129.2, 128.1, 125.4, 123.5, 122.2, 121.1, 117.1, 114.7, 114.3, 91.7, 66.0, 55.0, 54.0, 52.6, 26.2, 25.5, 24.1 ppm.

8f: 2-[4-(3-Piperidin-1-ylpropoxy)benzyl]-2*H*-benzo[*f*]isoindole-1-carbonitrile was synthesised from 4-(3-piperidin-1-ylpropoxy)benzylamine (0.4 mmol)(**8**) with napthalene-2,3-dicarbaldehyde (0.4 mmol), NaCN (0.8 mmol) and sodium tetraborate (1 mmol) in 40 mL of methanol. After stirring in the dark for 18 h at room temperature, the reaction mixture was added to ice/water. The product was filtered and dried in vacuum (yellow powder). Yield 92%; ESI–MS $C_{28}H_{29}N_3O$ calculated: 423.23, found: 423.8; ^{1}H NMR (300 MHz, DMSO): δ = 7.83 (s, 1H), 7.68 (d, J = 7.3 Hz, 2H), 7.20 (s, 1H), 7.17 (d, J = 7.4 Hz, 2H), 7.15 (s, 1H), 7.12 (s, 1H), 6.83 (dd, J = 7.5 Hz, 2H), 5.56 (s, 2H), 3.91 (t, J = 5.8 Hz, 2H), 2,95–3,10 (s, 6H), 1.95–2.10 (s, 2H), 1.68–1.72 (s, 4H), 1.46–1.53 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 157.4, 137.0, 135.0, 130.1, 129.3, 128.6, 127.8, 126.0, 125.8, 123.9, 119.3, 114.4, 111.9, 65.0, 53.5, 52.3, 48.4, 23.6, 22.8, 21.6 ppm.

8g: (2,4-Dinitrophenyl)[4-(3-piperidin-1-ylpropoxy)benzyl]amine was synthesised from 4-(3-piperidin-1-ylpropoxy)benzylamine (**8**) (2.50 mmol) with 1-fluoro-2,4-dinitrobenzene (2.50 mmol) and potassium carbonate (7.5 mmol) in 50 mL of abs. acetonitrile. After stirring in the dark for 78 h at room temperature, the reaction mixture was filtered, dried, and purified by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulting in the final product (yellow powder). Yield 68%; ESI–MS $C_{21}H_{26}N_4O_5$ calculated: 414.46, found: 415.0; ^{1}H NMR (300 MHz, DMSO): δ = 9.16 (d, J = 2.6 Hz, 1H), 8.82 (s, 1H), 8.25 (d, J = 8.8 Hz, 1H), 7.27 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 7.6 Hz, 2H), 4.57 (d, J = 5.5 Hz, 2H), 4.05 (t, J = 6.0 Hz, 2H), 3.49 (s, NH, 1H), 2.60–2.85 (s, 6H), 2.10–2.20 (s, 2H), 1.77–1.85 (s, 4H), 1.50–1.60 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): δ = 158.8, 148.1, 136.4, 130.8, 130.3, 128.6, 127.7, 124.2, 115.2, 114.3, 66.0, 55.6, 54.1, 47.1, 25.5, 24.4, 23.4 ppm.

8i: (7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)[4-(3-piperidin-1-ylpropoxy)benzyl]amine was synthesised from 4-(3-piperidin-1-ylpropoxy)-benzylamine (**8**) (2.5 mmol) with 4-chloro-7-nitrobenzo[*c*]-[1,2,5]oxadiazole (1.0 mmol) in 50 mL of abs. dioxane in the dark for 48 h at room temperature. Purification was performed after evaporation in vacuum by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulting in the final product (red powder). Yield 41%; ESI–MS $C_{21}H_{25}N_5O_4$ calculated: 411.46, found: 412.2; ^{1}H NMR (300 MHz, DMSO): $\delta = 8.48$ (d, $J = 8.9$ Hz, 1H), 7.35 (d, $J = 8.3$ Hz, 2H), 6.92 (d, $J = 8.3$ Hz, 2H), 6.37 (d, $J = 8.7$ Hz, 1H), 4.62 (s, 2H), 3.95–4.02 (s, 2H), 2.90–3.10 (s, 6H), 2.00–2.12 (s, 2H), 1.65–1.73 (s, 4H), 1.45–1.55 ppm (s, 2H); ^{13}C NMR (50 MHz, DMSO): $\delta = 157.6$, 144.8, 144.4, 137.6, 131.7, 129.0, 128.7, 114.9, 114.6, 99.6, 65.0, 53.5, 52.2, 45.7, 23.6, 22.7, 21.5 ppm.

9g: (2,4-Dinitrophenyl)[4′-(3-piperidin-1-ylpropoxy)biphenyl-4-ylmethyl]amine was synthesised from 4′-(3-piperidin-1-ylpropoxy)biphenyl-4-ylmethylamine (**9**) (0.89 mmol) with 1-fluoro-2,4-dinitrobenzene (1.07 mmol) and potassium carbonate (1.78 mmol) in 50 mL of abs. acetonitrile. After stirring in the dark for 18 h at room temperature, the reaction mixture was filtered, dried, and purified by column chromatography using CH_2Cl_2:MeOH, NH_3 saturated (95:5) resulting in the final product (yellow powder). Yield 69%; ESI–MS $C_{27}H_{30}N_4O_5$ calculated: 490.55, found: 491.1; ^{1}H NMR (300 MHz, DMSO): $\delta = 8.87$ (s, 1H), 8.20 (d, $J = 6.9$ Hz, 1H), 7.59 (2d, $J = 8.5$ Hz, 4H), 7.43 (d, $J = 8.2$ Hz, 2H), 7.09 (d, $J = 9.6$ Hz, 1H), 6.98 (d, $J = 8.7$ Hz, 2H), 4.78 (s, 2H), 4.00 (t, $J = 6.4$ Hz, 2H), 3.34 (s, NH, 1H), 2.35 (dt, $J = 9.0$ Hz, 6H), 1.84 (t, $J = 7.0$ Hz, 2H), 1.47 (t, $J = 5.3$ Hz, 4H), 1.37 ppm (d, $J = 5.0$ Hz, 2H); ^{13}C NMR (50 MHz, DMSO): $\delta = 158.3$, 148.0, 138.9, 135.7, 131.9, 130.1, 129.8, 127.6, 127.4, 126.3, 123.5, 115.6, 114.8, 66.0, 55.1, 54.1, 45.7, 26.3, 25.6, 24.1 ppm.

Pharmacology

In brief CHO-K1 cells stably transfected with the human H_3 receptor were washed and harvested with a PBS medium.[27,28] Then, they were centrifuged (140 g, 10 min, +4 °C) and homogenized with a Polytron in the ice-cold binding buffer (Na_2HPO_4/KH_2PO_4, 50 mM, pH 7.5). The homogenate was centrifuged (23000 g, 30 min, +4 °C) and the pellet obtained resuspended in the binding buffer to constitute the membrane preparation used for the binding assays. Aliquots of the membrane suspension were incubated for 60 min at 25 °C with [^{125}I]iodoproxyfan (25–30 pM) alone or together with competing drugs dissolved in the same buffer to give a final volume of 200 μL. Incubations were performed in triplicate and stopped by four additions of 5 mL of ice-cold phosphate buffer, followed by rapid filtration through glass microfibre filters (GF/B Whatman, Clifton, NJ) presoaked in 0.3% polyethylenimine. Radioactivity trapped on the filters was measured with a LKB (Rockville, MD) gamma counter (82% efficiency). Specific binding was defined as that inhibited by 1 μM imetit, a specific H_3 receptor agonist. K_i values were determined according to Cheng–Prusoff equation (Cheng and Prusoff 1973).[27,28,36]

The pharmacological in vivo assay was performed on male Swiss mice after oral administration measuring the modulation of N^τ-methylhistamine as described before.[26]

Keywords: fluorescence · GPCR · histamine · ligands · medicinal chemistry

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