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Hybrid approach for the design of highly affine and selective dopamine D_3 receptor ligands using privileged scaffolds of biogenic amine GPCR ligands

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Abstract—A series of compounds containing privileged scaffolds of the known histamine H_1 receptor antagonists cetirizine, mianserin, ketotifen, loratadine, and bamipine were synthesized for further optimization as ligands for the related biogenic amine binding dopamine D_3 receptor. A pharmacological screening was carried out at dopamine D_2 and D_3 receptors. In the preliminary testing various ligands have shown moderate to high affinities for dopamine D_3receptors, for example, *N*-(4-{4-[benzyl(phenyl)amino]piperidin-1-yl}butylnaphthalen-2-carboxamide (**19a**) (hD_3 K_i = 0.3 nM; hD_2 K_i = 703 nM), leading to a selectivity ratio of 2343.

1. Introduction

All dopamine receptor subtypes belong to class A of G-protein coupled receptors (GPCRs) and are subdivided into two subfamilies: D_1-like receptors, with its subtypes D_1 (D_{1a}) and D_5 (D_{1b}), activating adenylyl cyclase, and D_2-like receptors, including D_2, D_3, and D_4 receptor subtypes, inhibiting adenylyl cyclase.[1–3] Imbalance of the dopaminergic system is implicated in various neurological and neuropsychiatric disorders, including Parkinson's disease, schizophrenia, Tourette's syndrome, and drug abuse.[1–4]

The dopamine D_3 receptor subtype has been discovered by Sokoloff and colleagues in 1990.[5] Subsequent identification of its distinct distribution in the limbic regions of the striatum, predominant in the islands of Calleja and the nucleus accumbens, allowed to deduce that it may be a therapeutic target for antipsychotic and antiparkinsonian drugs.[4,6–8] Selective antagonism at D_3 receptors reduces negative and cognitive symptoms of schizophrenia, additionally prevents from undesirable extrapyramidal side effects, including tardive dyskinesia, parkinsonism, and dystonic reactions, which are associated with dopamine D_2 receptor antagonism in the caudate putamen.[4,9–13] It has been reported that dopamine D_3 receptors play an important role in mediating the reinforcing effects of psychostimulants, such as cocaine.[14] Therefore selective dopamine D_3 receptor antagonists and partial agonists are under investigation to prove their therapeutic potential in the treatment of drug addiction.[15,16] Additionally, D_3 receptor antagonists might improve cognitive deficits due to enhancing frontocortical cholinergic transmission.[17]

Identification of selective dopamine D_3 receptor ligands bearing subnanomolar affinities to lower the risk of motor extrapyramidal syndrome and to understand the pharmacological role of dopamine D_3 receptors has been and still is a great topical challenge in drug development. To date some series of compounds with these requirements have been achieved and are actually in ongoing clinical development as potential therapeutics for the aforementioned disorders.[18,19] Some representative ligands showing D_3 receptor-preference with antagonist and partial agonist properties are shown in Figure 1.[20–22]

Keywords: Privileged structures; Class A GPCR; Dopamine; D_3 receptor; Histamine; H_1 receptor; Radioligand competition binding assay.
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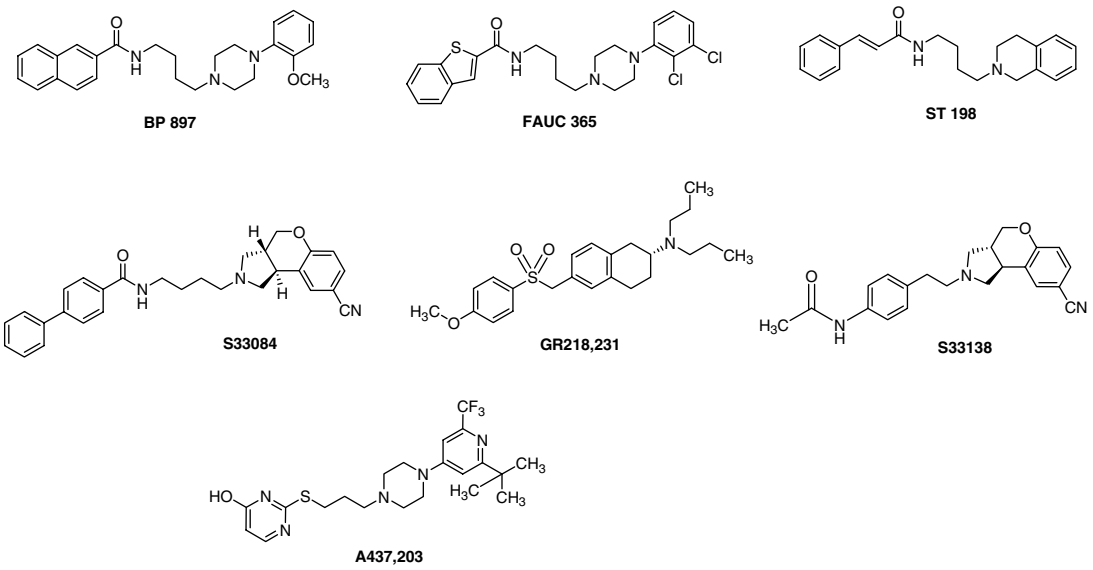


Figure 1. Dopamine D_3 receptor selective antagonists and partial agonists.

Compound BP 897 (Fig. 1) was identified as a selective dopamine D_3 receptor partial agonist with high affinity binding at human D_3 (hD_3) (K_i = 0.92 nM) and 70-fold selectivity over human D_2 (hD_2) (K_i = 61 nM) behaving as an antagonist at this subtype.[23,24] Further evaluation of in vitro models showed that accordingly BP 897 demonstrated in many models antagonist D_3 receptor profile in addition to its partial agonist properties.[25,26] BP 897 attenuated the behavioral and reinforcing effects of cocaine, showing a promising property for the treatment of drug abuse. Regarding the interactions of BP 897 at multiple classes of monoaminergic receptors, the only pivotal role of D_3 receptors in the mechanism of reduced cocaine-seeking behavior is not confirmed yet.[4] The treatment of levodopa-induced dyskinesias in patients with Parkinson's disease is under investigation.[27] To date BP 897 is ongoing phase II clinical studies.[28]

Another related development on this lead structure is FAUC 365 (Fig. 1), an antagonist bearing a heteroatom substituted bicyclic ring system and a (2,3-dichlorophenyl)piperazine substructure. The compound demonstrated high affinity for hD_3 (K_i = 0.5 nM), while the affinity for hD_2 was dependent on different assay conditions, but provided an impressive high selectivity for hD_3 over hD_2 (7200)[29] although this could not be confirmed by other groups.[30] Further development is represented by the 1,2,3,4-tetrahydroisoquinoline compound ST 198 (Fig. 1) (K_i = 12 nM (hD_3); K_i = 780 nM (hD_2)). It has been reported that this antagonist normalizes dopamine D_3 receptor function and subsequently attenuates levodopa-induced dyskinesia.[27]

The benzopyranopyrrole derivative S33084 (Fig. 1) also behaves as an antagonist and has displayed high hD_3 binding affinity (pK_i = 9.5) and >100-fold selectivity ratio for hD_3 receptors.[31] Furthermore GR218,231 (Fig. 1), an aminotetraline derivative with less selectivity for D_3 receptors compared to S33084 has been described. This ligand preferentially interacts as an antagonist at hD_3 (pK_i = 9.0) over hD_2 (pK_i = 7.2).[32,33] S33138 (Fig. 1) and A437,203 (Fig. 1) are promising agents which are under clinical evaluation. Both compounds behave as selective dopamine hD_3 over hD_2 receptor antagonists. A437,203 has an influence on brain dopamine activity and has demonstrated antipsychotic properties without clear extrapyramidale effects.[34,35] In vitro data have confirmed the former as a highly potent D_3 receptor antagonist having a K_i value of 2.9 nM.[36]

Antipsychotic drugs have become first line treatment of schizophrenia despite their interactions with several neurotransmitter receptors, including histamine H_1 receptors, serotonin 5-HT_{2a} receptors, and α_1/α_2 adrenergic receptors.[37] This multireceptor affinity has been considered to effect both therapeutic advantages but also adverse effects.[38] The antagonist binding profile of antipsychotics for central histamine H_1 receptors has been well demonstrated by chlorpromazine, a phenothiazine derivative, initially developed for its antiallergic properties by Delay and Deniker in 1952.[39] Additionally, histamine H_1 receptor antagonists containing tri- and tetracyclic structures display high affinity for diverse catecholamine receptors, due to the highly conserved ligand–receptor interaction of biogenic amine receptors by an aspartate (Asp) residue in transmembrane (TM) domain 3.[40–42]

Lipophilic/aromatic moieties connected to a basic nitrogen atom[41] are often claimed as 'privileged structures' for GPCRs and are supposed to be important for both dopamine D_2-like receptor and histamine H_1 receptor binding sites. In order to elucidate structure–activity relationships (SAR) of the D_2-like receptor profile showing similar structural requirements than that for H_1 receptor binding, we investigated in a novel hybrid structure development to identify highly affine dopamine D_3 receptor selective ligands. An approach was undertaken by synthesizing hybrids containing privileged scaffolds of histamine H_1 receptor antagonists,

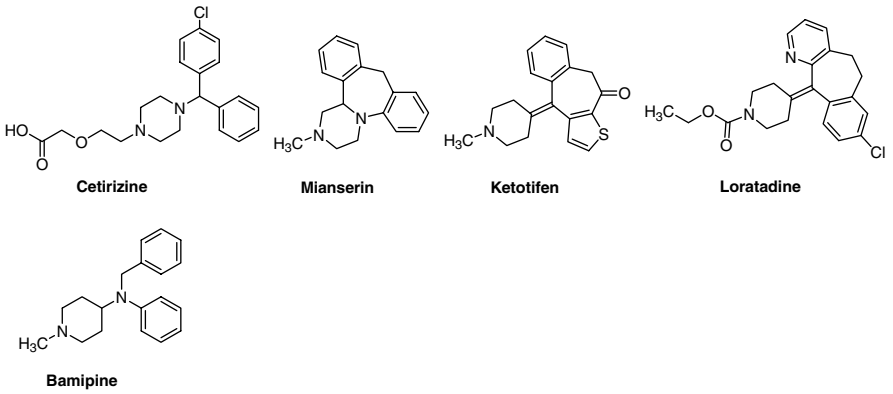


Figure 2. Histamine H_1 receptor antagonists containing privileged scaffolds.

so-called 'antihistamines'[43] (Fig. 2) and fragments of dopamine D_3 receptor-preferring ligands. The components of histamine H_1 receptor antagonists comprise basic substructures of cetirizine ([(4-chlorophenyl)phenylmethyl]piperazine, **S1**), mianserin (4-(2,3,4,5,10,15-hexahydro-1*H*-dibenzo[*b*:*e*]pyrazino[2,1-*g*])azepine, **S2**), ketotifen (4-[4-(10-oxo-9,10-dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen)-4-yliden]piperidine, **S3**), loratadine (4-[(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin)-11-yliden]piperidine, **S4**), and bamipine (*N*-benzyl-*N*-piperidin-4-ylaniline, **S5**).[43] These residues were connected via a tetramethylene chain to naphtalen-2-carboxamide, cinnamide, benzo[*b*]thiophen-2-carboxamide and its diminished bioisostere thiophen-2-carboxamide, or phthalimide moiety, recognized as the structural elements of BP 897, ST 198, FAUC 365 (Fig. 1), and NAN 190, respectively. NAN (*N*-(4-[4-(2-methoxyphenyl)piperazinyl)butyl)isoindolin-1,3-dion) has demonstrated less binding affinity and selectivity for dopamine D_3 receptors compared to BP 897, ST 198 and FAUC 365. It has shown high affinity binding at serotonin 5-HT_{1A} receptors and this pharmacological profile might enhance existing antipsychotic treatment.[44] The phthalimide residue combines two amide functions rigidly incorporated into the heteroaromatic moiety and provides a promising novel scaffold. Moreover, the necessity of an aromatic amide residue has been investigated by introducing a cyclohexylamide.

For the novel ligands, the calculated clog*P* values are in the range of 3.18–6.53 (Table 1) (ChemOffice Ultra 7.0) which show higher lipiphilicity than of most CNS active compounds with an log*P* value of about 2.5 as standard orientation. The hybrid approach provided compounds with physicochemical properties allowing them to penetrate the blood–brain barrier although they are more voluminous and more lipophilic than optimal for marketed drugs.[45]

The revealed benzhydrylpiperazine derivatives, tri- and tetracyclic ring system containing compounds and bamipine analogues were preliminary screened for binding affinities at dopamine hD_2 and hD_3 receptors in radioligand competition experiment carrying out six-point measurements (Table 1). The aim was to develop highly and affine dopamine D_3 receptor selective ligands and to elucidate the SAR of dopamine D_3 and D_2 receptors by employing privileged scaffolds of histamine H_1 receptor ligands.

2. Chemistry

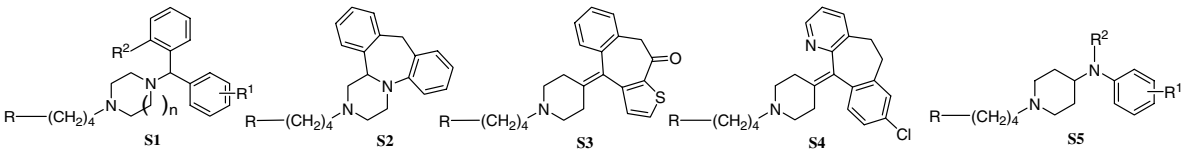
Benzhydrylpiperazine compounds (**3a**–**d**) were prepared starting from monocarbamate-protected piperazine and either benzhydryl chlorides (**1a**, **1b**) or benzhydryl alcohols (**1c**, **1d**) (Scheme 1).

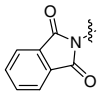
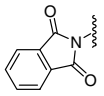
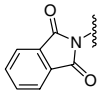
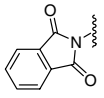
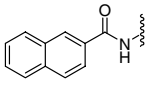
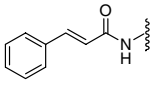
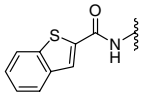
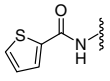
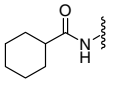
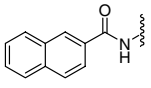
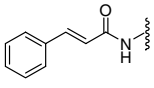
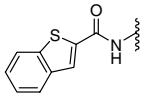
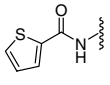
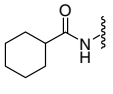
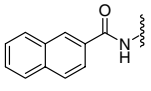
In the latter case, we utilized a method to couple secondary amines and primary, aliphatic alcohols.[46] This convenient method, which is here applied for the first time on secondary benzylic alcohols, allowed the preparation of benzhydrylpiperazines in remarkable yields about 95%. The protective group was subsequently cleaved under alkaline conditions. Comparable to the preparation of **3a**–**d**, monoacetylated 1,4-diazepane was substituted with benzhydryl chloride, the acetyl group was cleaved under strong basic conditions in moderate yields (not shown).[47] Preparation of the final compounds followed two different routes. A first straightforward approach involved the alkylation of appropriate secondary amines with *N*-(4-bromobutyl)phthalimide (**4a**–**d**). Subsequent hydrazinolysis led to primary amines (**5a**–**c**), which on treatment with different arylcarboxylic acid chlorides resulted in the corresponding amides **6a**–**8d** (Scheme 2).[48]

A more elegant way of preparation was adopted from the published procedure as mentioned below (**9a**–**11c**) (Scheme 3).[46] *N*-(4-Hydroxybutyl) substituted arylcarboxamides, phenylvinylcarboxamides, and benzo[*b*]thiophen-2-carboxamides were coupled to benzhydrylpiperazines in one-pot procedures. This method allowed the versatile preparation of numerous potentially active compounds (**10**, **11a**–**c**).

A series of (semi-)rigid analogues closely related to the benzhydrylpiperazine scaffold **15a**, **b**, **16a**–**e**, **17a**, **18a**–**b**, and **19a**–**b** were prepared, starting from commercially available compounds, for example, mianserin, ketotifen or bamipine (Scheme 4).

Table 1. Chemical structures, physical data, and pharmacological screening results for human dopamine D_2 and D_3 receptor binding affinities



Compound	Structure	R	R^1	R^2	n	K_i (nM)		D_2/D_3[b]	M_W[e]	$c\log P$
						D_2[a]	D_3[a]			
4a	**S1**		H	H	1	598	47.3	13	453.6	4.75
4b	**S1**		4-Cl	H	1	559	28.0	20	488.1	5.31
4c	**S1**		H	H	2	1180	133	9	467.7	4.85
4d	**S1**		4-Cl	H	2	226	42.9	5	502.1	5.41
6a	**S1**		H	H	1	457	11.1	41	477.7	5.93
6b	**S1**		H	H	1	652	6.2	105	453.7	5.28
6c	**S1**		H	H	1	590	30.3	20	483.7	5.97
6d	**S1**		H	H	1	621	28.4	22	433.6	4.92
6e	**S1**		H	H	1	435	30.2	14	433.7	5.01
7a	**S1**		4-Cl	H	1	587	2.82[c] ± 0.4	208	512.1	6.49
7b	**S1**		4-Cl	H	1	963	2.4[d] ± 1.1	401	488.1	5.84
7c	**S1**		4-Cl	H	1	1000	17.7	57	518.2	6.53
7d	**S1**		4-Cl	H	1	160	38.9	4	468.1	5.48
7e	**S1**		4-Cl	H	1	189	12.3	15	467.1	5.57
8a	**S1**		H	H	2	136	32.6	4	491.7	6.04

(*continued on next page*)

Table 1 (*continued*)

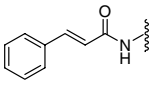
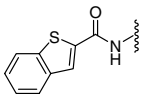
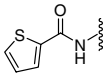
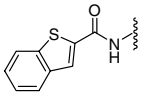
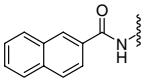
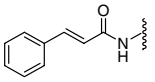
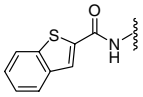
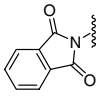
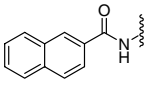
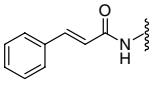
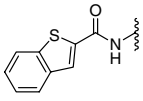
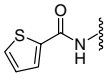
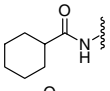
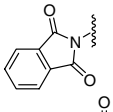
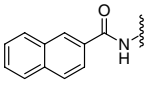
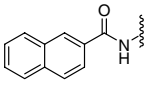
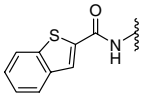
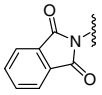
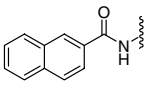
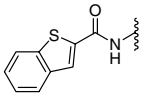
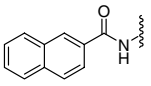
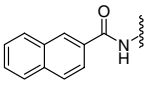
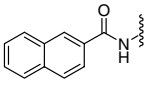
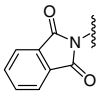
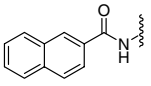
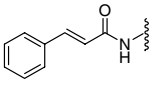
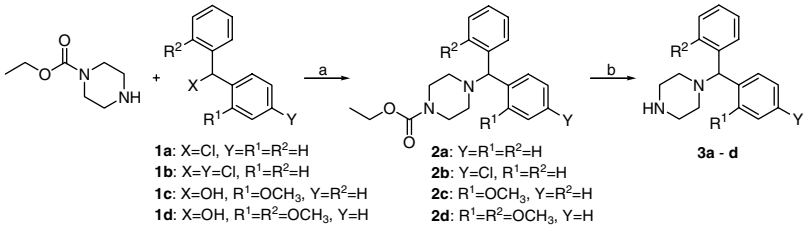
Compound	Structure	R	R^1	R^2	*n*	K_i (nM)		D_2/D_3[b]	M_W[e]	*c*log *P*
						D_2[a]	D_3[a]			
8b	**S1**		H	H	2	161	40.9	4	467.7	5.38
8c	**S1**		H	H	2	633	36.9	17	497.8	6.08
8d	**S1**		H	H	2	663	340	2	447.7	5.02
10	**S1**		2-OCH_3	H	1	626	3.60	174	513.8	5.84
11a	**S1**		2-OCH_3	OCH_3	1	313	15.2	21	537.8	5.68
11b	**S1**		2-OCH_3	OCH_3	1	972	15.2	64	513.8	5.02
11c	**S1**		2-OCH_3	OCH_3	1	619	22.8	27	543.8	5.72
15a	**S2**					808	500	2	451.6	5.15
16a	**S2**					1,324	>1000	1	475.7	6.33
16b	**S2**					429	50.2	9	451.6	5.68
16c	**S2**					4,925	>1000	5	481.7	6.37
16d	**S2**					497	500	1	431.7	5.32
16e	**S2**					258	>1000	0.3	431.7	5.41
15b	**S3**					1,149	99.1	12	496.7	4.22
17a	**S3**					>1000	10.9	92	546.8	5.41
18a	**S4**					252	6.01	41	581.2	5.57
18b	**S4**					849	2.7	314	551.2	6.46
15c	**S5**		H			128	>1000	0.1	467.7	5.30

Table 1 (*continued*)

Compound	Structure	R	R^1	R^2	*n*	K_i (nM)		D_2/D_3[b]	M_W[e]	*c*log *P*
						D_2[a]	D_3[a]			
19a	**S5**		H			703	0.3	2343	491.7	6.49
19b	**S5**		H			1570	31.1	51	497.7	6.53
23a	**S5**		2-OCH_3	Acetyl		13,350	2340	6	473.6	3.49
23b	**S5**		2-OCH_3	Propionyl		>1000	>1000	1	457.7	4.14
23c	**S5**		2-OCH_3	Benzoyl		4620	>1000	5	535.7	5.38
24	**S5**		2-OCH_3	H		2288	>1000	2	407.5	2.65
25a	**S5**		2-OCH_3	H		163	18.4	9	431.6	3.84
25b	**S5**		2-OCH_3	H		>1000	10.8	93	407.6	3.18
BP 897						52 ± 12	0.91 ± 0.2	57	417.5	4.44
ST 198						1272 ± 99	8.72 ± 0.2	146	334.5	3.79

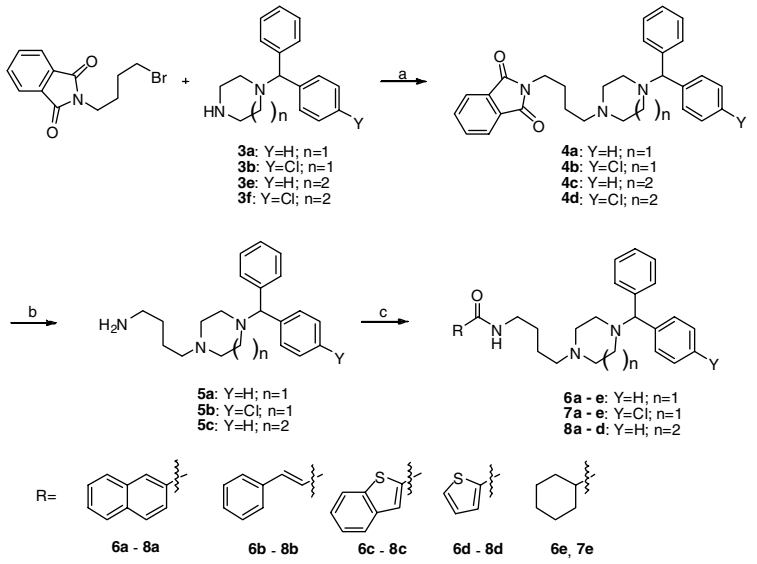
[a] Values (K_i) received by one experiment performed in duplicates and 6 concentrations: 0.1–10,000 nM.
[b] Ratio is calculated from corresponding K_i values.
[c] Values (K_i) received by 6 experiments performed in duplicates and 6 concentrations: 0.1–10,000 nM.
[d] Values (K_i) received by 5 experiments performed in duplicates and 6 concentrations: 0.1–10,000 nM.
[e] Molecular weights of the analytically tested salt form is given.



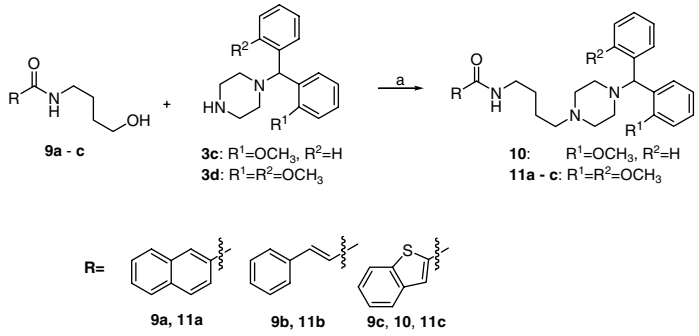
Scheme 1. Synthesis of *N*-(diphenylmethyl)piperazine derivatives. Reagents and condition: (a) acetonitrile, K_2CO_3 for X = Cl; $(CH_3)_3PCH_2CN^+I^-$, DIPEA, propionitrile for X = OH; (b) KOH, MeOH, H_2O, reflux.

The *N*-methylated compounds **12a**, **12b**, **12d** were converted to the corresponding carbamates **13a**, **13b**, **13d** employing ethyl chloroformate. The carbamates (including loratadine **13c**) were cleaved under alkaline conditions to release the secondary amines **14a**–**d**.[49,50] These amine precursors were subsequently coupled with *N*-(4-bromobutyl)phthalimide, followed by the cleavage of the phthalimide and acylation with the activated arylcarboxylic acid, or directly coupled with an *N*-(4-hydroxybutyl)arylcarboxamide employing cyanomethyl(trimethyl)phosphonium iodide to give the final compounds.[43]

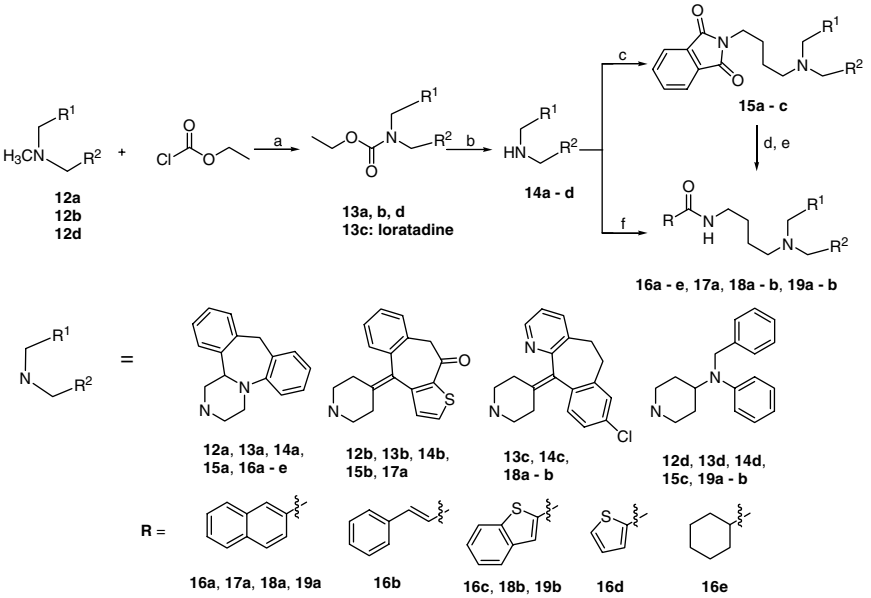
A third series of compounds involved the preparation of piperidine analogues of BP 897 (**23a**–**c**, **24**, **25a**, **25b**) (Scheme 5). Starting material was methoxy-substituted aniline, which was condensed with *N*-benzylpiperidone under Dean–Stark-conditions and hydrogenated over PtO_2 (**20**).[51] Debenzylation and alkylation of compound **20** led to compound **24**, which is both a final compound and the starting material for the phthalimide procedure described above. This deprotection followed by acylation sequence resulted in compounds **25a**, **25b**. Alternatively, the acylation of intermediate **20** with different acylchlorides provided compounds **21a**– **c**, which were



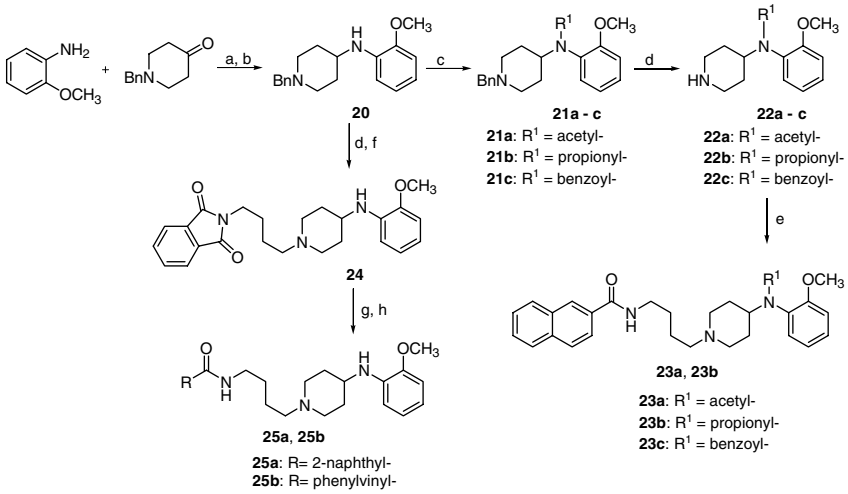
Scheme 2. Synthesis of substituted benzhydrylpiperazine derivatives. Reagents and condition: (a) acetonitrile, K_2CO_3; (b) N_2H_4, MeOH, reflux; (c) ArCOCl, CH_2Cl_2, K_2CO_3.



Scheme 3. Alkylation of *N*-(4-hydroxybutyl)arylcarboxamides with benzhydrylpiperazine derivatives. Reagents and condition: $(CH_3)_3PCH_2CN^+I^-$, DIPEA; propionitrile; 90 °C, 3 h.



Scheme 4. Synthesis of rigidized analogues of benzhydrylpiperazines. Reagents and conditions: (a) toluene, reflux; (b) KOH, MeOH, reflux; (c) *N*-(4-bromobutyl)isoindolin-1,3-dione, K_2CO_3, acetonitrile; (d) N_2H_4, MeOH, reflux; (e) ArCOCl, K_2CO_3, CH_2Cl_2; (f) *N*-(4-hydroxybutyl)arylcarboxamide, $(CH_3)_3PCH_2CN^+I^-$, DIPEA, propionitrile; 90 °C.



Scheme 5. Synthesis of piperidine analogues of BP 897. Reagents: (a) toluene, Dean–Stark-conditions; (b) H_2/PtO_2, MeOH; (c) acylchloride, K_2CO_3, CH_2Cl_2; (d) $H_2/Pd(OH)_2$, MeOH; (e) *N*-(4-oxobutyl)naphthalen-2-carboxamide, $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$; (f) *N*-(4-bromobutyl)isoindolin-1,3-dione; K_2CO_3, acetonitrile; (g) N_2H_4, MeOH; (h) ArCOCl, K_2CO_3, CH_2Cl_2.

subsequently debenzylated (**22a**–**c**) and reductively alkylated to the final compounds **23a**–**c**.[52]

3. Results and discussion

We investigated in forty-one newly prepared compounds and determined binding affinities in a competition binding experiment with six-point measurements in duplicates. Binding assays were carried out using HEK-cells transfected with human D_{2S} and CHO-cells transfected with human D_3 dopamine receptor cDNAs and [^{3}H]spiperone (Table 1).[53] Data received from one single experiment can only give preliminary estimates of structure–activity relationships and further evaluation need to be carried out to provide variability and reliability within statistical limits.

The benzhydrylpiperazine residue (**S1**) based on the structural element of the antihistaminergic cetirizine (Fig. 2) has been combined with diverse (hetero)aryl amides, cinnamides, phthalimides, and cyclohexyl amides via a butyl spacer (**4**–**11**).

Furthermore, the benzhydrylpiperazine element has been substituted either with a chlorine at 4-position (**4b**, **4d**, and **7**) or methoxy group(s) at 2-position(s) of the phenyl ring(s) (**10** and **11**), related to BP 897. For some compounds, the piperazine ring has been replaced by 1,4-diazepane (**4c**, **4d**, **8**).

Among the series of benzhydrylpiperazine derivatives, compounds have revealed moderate nanomolar to low nanomolar affinities for hD_3 receptors and micromolar to moderate nanomolar binding affinities for hD_2 receptors.

The influence of diverse dopamine D_3 receptor-preferring residues on the binding profile at dopamine D_3 and D_2 receptors has been investigated within compounds **4a** and **6**. The replacement of the phthalimide residue (**4a**) by a naphthalen-2-carboxamide (**6a**), cinnamide (**6b**), benzo[*b*]thiophen-2-carboxamide (**6c**), thiophen-2-carboxamide (**6d**) or cyclohexyl carboxamide (**6e**) resulted in similar binding affinities at dopamine D_2 receptors within the series. Alterations led to improved affinity binding data and dopamine D_3 receptor-preference for the naphthalen-2-carboxamide **6a** and a distinct enhanced selectivity for the cinnamide derivative **6b** (K_i (D_2/D_3) = 105). The cinnamide structure provides an amide in a precise steric space relative to the inflexible aromatic ring. This exact spacial orientation seems to contribute to the selectivity due to beneficial interactions with the dopamine D_3 receptor binding site. Comparing the affinity profiles of amides (**6**) with data received from the imide containing compound **4a** led to the assumption that the amide NH function as a hydrogen bond donor is not essential as a pharmacophoric element for receptor binding, whereas the phthalimide moiety neither improves dopamine D_3 receptor binding nor selectivity compared to that of the amides. As demonstrated by the cyclohexyl amide derivate **6e**, an aromatic system in this position is not necessarily required for affinity binding at both receptor subtypes.

The influence of different substitution patterns on the phenyl rings of the benzhydrylpiperazine system has been validated in the following series. Analogues of **4a** and **6** have been synthesized by introducing a chloro atom at 4-position of the phenyl ring in the benzhydrylpiperazine residue (**4b**, **7**). The chlorine substitution induced inhomogeneous binding profiles at dopamine D_2 and D_3 receptors when compared to the unsubstituted series. Binding affinities of the ligands at D_3 receptors have been improved with regard to their chlorine unsubstituted analogues with the exception of the thiophen-2-carboxamide **7d**. As for dopamine D_2 receptors, affinity binding data did not change for the phthalimide (**4a** → **4b**) and naphthalen-2-carboxamide (**6a** → **7a**) derivatives. The modification led to a deterioration of affinity binding for the cinnamide (**6b** → **7b**) and

benzo[*b*]thiophen-2-carboxamide (**6c** → **7c**) derivatives, while K_i values of the thiophen-2-carboxamide (**6d** → **7d**) and cyclohexyl carboxamide (**6e** → **7e**) ligands demonstrated an improvement of affinities at dopamine D_2 receptors. The chlorine substituent accounts for an additional hydrophobic interaction in the binding pocket of the dopamine D_3 receptor. As previously mentioned for the unsubstituted ligands **6a** and **6b**, the naphthalen-2-carboxamide **7a** and the cinnamide **7b** derivatives possessed the highest affinities for dopamine D_3 receptors in this series. A superior selectivity for the dopamine D_3 receptor have been revealed for **7b** (K_i (D_2/D_3) = 401). The affinity and selectivity profiles of benzhydrylpiperazine derivatives which are methoxy disubstituted at 2-positions of the phenyl rings have been investigated within compounds **11a**–**11c**. Compared to the chlorine substituted analogues **7a**–**7c** the modification yielded compounds with slightly declined affinity binding and selectivity for D_3 receptors. In this series, introducing a cinnamide residue (**11b**) has been beneficial for selectivity binding for D_3 over D_2 receptor as previously demonstrated by the unsubstituted compound **6b** as well as for the chlorine substituted benzhydrylpiperazine derivative **7b**. Replacing the second methoxy substituent of benzo[*b*]thiophen-2-carboxamide derivative **11c** resulted in the methoxy monosubstituted analogue **10** with advanced affinity and selectivity for the dopamine D_3 receptor. In addition, this modification revealed superior affinity binding and selectivity for dopamine D_3 receptors when compared to its monosubstituted chlorine analogue **7c**. Disubstitution at 2-positions of the phenyl rings causes a steric restriction of the aromatic diphenyl residues in relation to the basic nitrogen. By introducing a substituent in *ortho*- or *para*-position, the phenyl rings are more flexible and capable to adopt a favorable orientation for dopamine D_3 receptor binding.

The piperazine ring extension of unsubstituted benzhydrylpiperazine derivatives **4a** and **6a**–**6d** to a 1,4-diazepane (**4c** and **8a**–**8d**) resulted in a reduction of affinity binding for D_3 receptors excluding the benzo[*b*]thiophen-2-carboxamide **8c** demonstrating similar data as seen for **6c**. Affinity bindings of the naphthalen-2-carboxamide **8a** and the cinnamide **8b** have been enhanced for D_2 receptors, while a reduced binding affinity was obtained for **4c**. The modification in compounds **8c** and **8d** did not alter the binding behavior for this receptor subtype. The ring expansion of the chloro substituted compounds **4b**–**4d** led to improved affinity binding at D_2 receptors but resulted in deterioration of data for dopamine D_3 receptors. Ring extension changes the position of the basic amine piperazine relative to the lipophilic aromatic residue. This alteration generally impairs the dopamine D_3 receptor–ligand interaction. In contrast, the interference of dopamine D_2 receptor–ligand additionally depends on the carboxamide and carboximide residue, respectively.

Introduction of the hexahydrodibenzopyrazinoazepine residue (**S2**), the tetracyclic substructure of mianserin (Fig. 2), has generated ligands (**15a** and **16a**–**e**) with modest nanomolar to micromolar affinity binding values at dopamine D_2 and D_3 receptors. A nanomolar binding result at dopamine D_3 receptors has been obtained only for the cinnamide bearing compound **16b** with a modest D_3 receptor-preference. A dopamine D_2 receptor-preference has been received for the cyclohexyl amide derivative **16e**.

Benzothienylcycloheptadienpiperidine (**S3**), the substructure of ketotifen (Fig. 2), was incorporated in **15b** and **17a** and the modification has resulted in compounds with nanomolar binding affinity for D_3 receptors and micromolar affinity binding for D_2 receptors. Compared to the phthalimide analogue **15b**, the naphthalen-2-carboxamide **17a** has an improved selectivity ratio for dopamine D_3 over D_2 by enhancing the affinity for dopamine D_3 receptors. The loratadine ring system (**S4**) was introduced in compounds **18a** and **18b** and this has resulted in molecules with moderate nanomolar affinities for D_2 binding. Introducing this bulky cyclic system has been well tolerated by dopamine D_3 receptors affecting low nanomolar binding affinities. High affinity binding and selectivity for the dopamine D_3 receptor has been revealed for the benzo[*b*]thiophen-2-carboxamide substituted compound **18b** (K_i (D_2/D_3) = 314).

For a comprehensive series of potentially privileged structures of H_1 receptor antagonists, derivatives containing the phenylaminopiperidine substructure (**S5**) of bamipine (Fig. 2) were synthesized (**15c**, **19a**, **19b**, **23a**–**23c**, **24**, and **25a**–**b**). In the first series, a benzyl-*N*-piperidin-4-ylaniline has been combined with phthalimide (**15c**), naphthalen-2-carboxamide (**19a**), or benzo[*b*]thiophen-2-carboxamide (**19b**) connected via a butyl linker. Introducing the phthalimide residue resulted in a compound with a preference for the dopamine D_2 receptor. The naphthalen-2-carboxamide containing compound (**19a**) displayed subnanomolar affinity binding for hD_3 (K_i (D_3) = 0.3) and moderate affinity binding for hD_2 and represents the most selective ligand in this hybrid approach (K_i (D_2/D_3) = 2343). The molecule presents a promising affinity profile regarding the dopamine D_3 receptor, but due to the preliminary testing further investigations for reliability need to be carried out.

Encouraged by the results of **19a**, it was of interest to validate the influence of an additional hydrogen-bond acceptor on affinity binding. Consequently, the benzyl residue has been exchanged by a benzoyl moiety and the molecule has been sterically restricted by a methoxy substitution in 2-position of the phenyl ring (**23c**). Introducing the oxo functionality and methoxy substituent led to a compound with micromolar affinities at both receptor subtypes. By retaining the added oxo hydrogen-bond acceptor function, but derogating the benzoyl residue to an acetyl and propionyl moiety, compounds **23a** and **23b** were synthesized. As seen for **23c**, binding affinity values were in the micromolar range at both receptor subtypes and no further improvement concerning the binding profiles has been observed.

In the following compounds **24**, **25a**, **25b**, the phenylaminopiperidine moiety with methoxy substitution in

2-position on the phenyl ring has been maintained. Introducing a phthalimide residue (**24**) resulted in a compound with micromolar affinities at dopamine D_2 and D_3 receptors. The exchange of the imide by a naphthalen-2-carboxamide moiety (**25a**) led to a clear increase in affinity binding at dopamine D_2 and D_3 receptors. Further variation included the exchange of the naphthoyl moiety by a cinnamide residue (**25b**). This ligand presents a pharmacological binding profile with low nanomolar binding data for dopamine D_3 receptors and a 93-fold selectivity for dopamine D_3 over D_2 receptors. The phenylaminopiperidine residue is an analogue of the 4-(2-methoxyphenyl)piperazino moiety as seen in BP 897 (Fig. 1) and has been connected via a butyl linker to the cinnamide substructure of ST 198 (Fig. 1). This structural combination gives an explanation for the high affinity and selectivity for the dopamine D_3 receptor.

The existence of highly conserved residues in the ligand binding pocket of class A family of GPCRs, predominantly possessing hydrophobic and aromatic properties, is well established.[54] Other regions of the binding site among this receptor family have variable residues and strongly influence the selectivity of ligands toward a receptor subtype.[54] In this hybrid approach, we employed privileged scaffolds of histamine H_1 receptor antagonists expecting the recognition of this structural element by the highly conserved receptor region in the related biogenic amine binding dopamine D_3 receptor. It was assumed that the introduction of the dopamine D_3 receptor-preferring substructures has a refined impact on the affinity and selectivity for the dopamine D_3 receptor. The hybrid approach using these privileged structures provided ligands mostly with nanomolar affinity and selectivity for dopamine D_3 receptors. The histamine H_1 receptor antagonist fragment containing basic nitrogen connected to an aromatic/lipophilic residue interfered with the binding pocket of D_2 and D_3 receptors, assumingly interacting with the highly conserved Asp in TM3.[12] The binding site in the pocket of the dopamine D_3 receptor tolerated bulky flexible as well as bulky rigid aromatic elements. Exchange of the dopamine D_3 receptor-preferring elements influenced binding affinities and selectivity profile.

Since the moieties introduced into the novel dopamine ligands are well-known histamine H_1 receptor antagonists, a future evaluation on their histamine H_1 receptor binding properties is of absolute necessity. Additionally, the histamine H_1 receptor antagonists have to be pharmacologically tested for their binding affinities at dopamine D_2 and D_3 receptors to assess the impact of the dopamine D_3 substructures on the parent ligands.

4. Conclusion

A rational hybrid design of novel ligands has been sucessfully employed using privileged structures of ligands for biogenic amine binding GPCRs. The combination of privileged scaffolds of H_1 receptor antagonists with dopamine D_3 receptor-preferring substructures generated ligands with remarkable affinity and selectivity for dopamine D_3 receptors. Incorporation of flexible residues such as phenylaminopiperidine and benzhydrylpiperazine, the substructures of histamine H_1 receptor antagonists, has been well-tolerated as demonstrated by the naphthalen-2-carboxamide containing compound **19a** and cinnamide derivative **7b**. The former is most promising compound with a K_i (hD_3) = 0.3 nM and a remarkable selectivity ratio of 2343, while the latter possesses high affinity for dopamine D_3 receptors and a selectivity ratio of 401 for dopamine D_3 over D_2 receptors. Most of the rigid and bulky tricyclic or tetracyclic ring systems have been tolerated by dopamine D_2 and D_3 receptors as exemplified by the loratadine derivative **18b** with high affinity binding (K_i (D_3) = 2.7) and selectivity for the dopamine D_3 receptor (K_i (D_2/D_3) = 314).

The influence of diverse dopamine D_3 receptor-preferring residues on the binding profile at dopamine D_3 and D_2 receptors has been investigated and the results indicate an impact on affinity binding for both receptor subtypes but a clear effect on selectivity ratios.

Although the data have to be confirmed by repetitive competition binding assays, the preliminary data demonstrate that by the presented hybrid approach we designed potent and selective drugs with an optimized binding profile for dopamine D_3 receptors. A refined SAR for dopamine D_3 and D_2 receptors has been discussed and improves the understanding of ligand–receptor interactions.

5. Experimental

5.1. General experimental

Melting points were determined on a Büchi 510 melting point apparatus (Büchi, Flawil, Switzerland) and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance DPX 400 (^{1}H NMR 400 MHz) or Bruker AC 300 (^{1}H NMR 300 MHz). Chemical shifts are expressed in parts per million (ppm) downfield from internal tetramethylsilane (TMS) as reference. The following abbreviations are used for multiplicity of NMR signals: br, broad, s, singlet, d, doublet, t, triplet; m, multiplet; approximate coupling constants in Hertz (Hz); number of protons. Elemental analyses (C, H, N) were measured on Elementaranalysator 240C, Vario EL (Perkin-Elmer) or CHN-Rapid (Heraeus) and were within ± 0.4% of the theoretical values for all compounds. Preparative column chromatography was performed on silica gel 63–200 μM, mobile phase usually dichloromethane/methanol. Thin-layer chromatography (TLC) was performed on silica gel PF_{254} plates (Merck). Spectral data and elemental analyses are shown only for intermediates and parent compounds, which were obtained by different reactions or methods, and additionally for the most potent compounds (**2b**, **4**, **6–8**, **15**, **16**, **19**, **25b**). EI-MS was performed on a Finnigan Varian MATCH/A or a MAT 171; FAB-MS was performed on a Finnigan MAT CH5DF (Xe, DMSO, Glycerole, Cu-Target, Plus-Mode); ESI-MS was performed on a Fisons Instru-

mentsVG Platform II. Data are listed as mass number (*m*/*z*) and relative intensity (%). Descriptions for general procedure for the preparation have been exemplified with the first compound mentioned if not stated otherwise.

5.2. General procedure for the preparation of 2a, 2b. Method A1

A suspension of the monoprotected piperazine derivative (12 mmol), the appropriately substituted chloro(diphenyl)methane (9 mmol) and K_2CO_3 (1.4 g, 10 mmol) and dry acetonitrile (40 mL) was stirred for 24 h at rt. After filtration, the solvent was evaporated under vacuum. The residue was purified by column chromatography.

5.3. General procedure for the preparation of 2c, 2d. Method A2

Cyanomethyl(trimethyl)phosphonium iodide (1.8 g, 7.5 mmol) was added to a mixture of the appropriate benzhydryl alcohol (6.6 mmol), ethyl(piperazino)formiate (0.98 g, 6.2 mmol), diisopropylethylamine (DIPEA) (1.01 g, 7.8 mmol), and propionitrile (10 mL), and the mixture was stirred at 90 °C for 3 h under argon atmosphere. The mixture was allowed to cool to room temperature and a solution of potassium carbonate (3 g) in water (30 mL) was added, and the product was extracted with dichloromethane (3× 30 mL). The combined organic layers were dried with sodium sulfate, and concentrated under vacuum to yield 2.51 g of a brown oil. Purification by column chromatography (CH_2Cl_2/MeOH (99:1) yielded 1.18 g (46%) of the product as a white solid.

5.4. General procedure for the preparation of 13a, 13b, 13d. Method A3

To a suspension the *N*-methylated compound (7.1 mmol) in dry toluene (60 mL) was added ethyl chloroformiate (16 g, 147 mmol). The mixture was refluxed for 5 h. After completion of the reaction monitored by TLC, the excess of the ethyl chloroformiate and the solvent were removed under reduced pressure. The product was used in the next step without further purification.

5.5. General procedure for the preparation of 3a–d. Method B

The *N*-ethoxycarbonyl protected compound (2.9 mmol) was refluxed in methanol (20 mL) and water (7 mL) in the presence of KOH (2.5 g) for 24 h. Methanol was evaporated and to the residue was added water (30 mL). The mixture was extracted with dichloromethane and purified by flash chromatography (CH_2Cl_2/MeOH (95:5)).

5.6. General procedure for the preparation of 4 and 24. Method C1

A suspension of the secondary amine (10 mmol), *N*-(bromobutyl)isoindoline-1,3-dione (2.8 g, 10 mmol) and K_2CO_3 (1.4 g, 10 mmol) and dry acetonitrile (40 mL) was stirred for 24 h. After filtration, the solvent was evaporated under vacuum. The residue was purified by column chromatography (CH_2Cl_2/MeOH (9:1)) and crystallized as the oxalate from ethanol/diethylether.

5.7. General procedure for the preparation of 6–8, 16–19, and 25. Method C2

The purified compound (6.9 mmol) was dissolved in dry methanol (30 mL) and hydrazine hydrate (0.6 g, 12 mmol) was added and refluxed for 3 h. In cases where the reaction was not complete, more hydrazine hydrate (0.3 g, 6 mmol) was added and again refluxed for 2 h. After evaporation of hydrazine and methanol under vacuum, ether was added and filtrated. The filtrate was concentrated under vacuum; the crude product was used in the next reaction without further purification. To an ice-cooled suspension of the amine component (5 mmol) and K_2CO_3 (0.69 g, 5 mmol) in dry dichloromethane (10 mL) was added a solution of the arylcarboxylic acid chloride (5 mmol) in dry dichloromethane (5 mL) in a dropwise manner. After the addition was complete, the solution was stirred for 2–6 h at room temperature. The mixture was washed with saturated $NaHCO_3$ (10 mL), water (5 mL) and brine (5 mL). The organic phase was dried ($MgSO_4$), freed from volatiles under vacuum and purified by column chromatography and crystallized from ethanol/diethylether as the oxalate salt.

5.8. General procedure for the preparation of 10, 11, and 16–19. Method C3

Cyanomethyl(trimethyl)phosphonium iodide (1.8 g, 7.5 mmol) was added to a mixture of the appropriate secondary amine (0.98 g, 6.2 mmol), *N*-(4-hydroxybutyl)arylcarboxamide (6.6 mmol), DIPEA (1.01 g, 7.8 mmol), and propionitrile (10mL), and the mixture was stirred at 90 °C for 3 h under argon atmosphere. The mixture was allowed to cool to room temperature and a solution of potassium carbonate (3 g) in water (30 mL) was added, and the product was extracted with dichloromethane (3× 30 mL). The combined organic layers were dried with sodium sulfate, and concentrated under vacuum to yield 2.51 g of brown oil. The crude product was purified by column chromatography (CH_2Cl_2/MeOH (9:1)) and precipitated with oxalic acid from ethanol/diethylether.

5.9. General procedure for the preparation of 23. Method C4

5.9.1. *N*-(4-Oxobutyl)naphthalen-2-carboxamide. A solution of *N*-(4,4-diethoxybutyl)naphthalen-2-carboxamide (0.5 g, 1.6 mmol), acetic acid (2 mL) and HCl (1 N, 1 mL) in ethanol (5 mL) was stirred for 16 h at room temperature. After completion of the reaction was confirmed by TLC, the solvent was evaporated under vacuum ($T < 40$ °C). The residue was dissolved in dichloromethane (10 mL) and washed with saturated $NaHCO_3$ solution (5 mL). The organic phase was dried with Na_2SO_4. The solvent was evaporated under vacuum. The crude product was dissolved in 1,2-dichloroethane (9 mL). The secondary amine (0.6 mmol), acetic acid (0.3 mL) and $NaHB(OAc)_3$ (0.6 g, 3 mmol) were added. The suspension was stirred under exclusion of

light for 24 h at room temperature. The organic phase was washed with saturated $NaHCO_3$ solution (10 mL) and water (15 mL), dried ($MgSO_4$) and freed from solvents under vacuum. The crude product was purified by column chromatography. The pure compound was precipitated with oxalic acid from ethanol/diethylether.

5.10. Preparation and analytical data of intermediate and final compounds

5.10.1. Ethyl {4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl}carboxylate (2b). Method A1 with ethyl(piperazino)formate and chloro-(4-chlorophenyl)(phenyl)methane. Yield 96%. White solid, mp 78 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (t, J = 7.1 Hz, 3H), 2.44–2.56 (m, 4H), 3.27–3.55 (m, 4H), 4.10–4.34 (m, 2H), 4.39 (s, 1H), 7.11–7.50 (m, 9H).

5.10.2. [4-(Diphenylmethyl)-1,4-diazepan-1-yl]ethanone. Method A1 with 1-(1,4-diazepan-1-yl)ethanone and chloro (diphenyl)methane. Yield 71%. Yellowish solid, mp 149 C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78–2.04 (m, 5H), 3.09–3.58 (m, 8H), 4.71 (s, 1H), 7.01–7.61 (m, 10 H).

5.10.3. *N*-{4-[4-(*N*′-Benzyl-*N*′-phenylamino)piperidin-1-yl]butyl}isoindolin-1,3-dione (15c). Method A3, method B, method C1. Yield (all steps) 54%; white solid; mp 145 °C; R_f 0.3 (CH_2Cl_2/MeOH (9:1)). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55–1.75 (m, 4H), 1.78–2.05 (m, 4H), 2.89–3.14 (m, 4H), 3.32–3.50 (m, 2H), 3.53–3.64 (s, br, 2H), 4.09–4.22 (m, 1H), 4.42 (s, 2H), 6.57–6.78 (m, 3H), 7.07–7.37 (m, 7H), 7.78–7.95 (m, 4H). Anal. ($C_{30}H_{32}N_3O_2$·0.8 $C_2H_2O_4$·H_2O) calcd: C, 68.06; H, 6.62; N, 7.54; found: C, 67.76; H, 6.88; N, 7.27. ESI-MS: 467 (M+H$^+$, 100).

5.10.4. *N*-[4-(2,3,4,5,10,15-Hexahydro-1*H*-dibenzo[*b*: *e*]pyrazino[2,1-*g*]azepin-1-yl)butyl]isoindolin-1,3-dione (15a). Method A3, method B, method C1. Yield (all steps) 62%; white solid; mp 238 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.66 (s, br, 4H), 2.84–3.15 (m, 4H), 3.25–3.53 (m, 5H), 3.53–3.69 (m, 2H), 4.25 (d, J = 10.1 Hz, 1H), 4.64 (d, J = 12.4 Hz, 1H), 6.87 (t, J = 7.3Hz, 1H), 6.95–7.32 (m, 7H), 7.82–7.93 (m, 4H). Anal. ($C_{29}H_{29}N_3O_2$·$C_2H_2O_4$) calcd: C, 68.75; H, 5.77; N, 7.76; found: C, 68.51; H, 5.71; N, 7.88. ESI-MS: 452 (M+H$^+$, 100).

5.10.5. *N*-{4-[4-(10-Oxo-9,10-dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*] thiophen)-4-ylidenpiperidin-1-yl]butyl}isoindolin-1,3-dione (15b). Method A3, method B, method C1. Yield (all steps) 9.5%; yellowish solid; mp 204 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.63 (s, br, 4H), 2.33–2.45 (m, 2H), 2.45–3.1 (m, 6H), 3.17–3.42 (m, 2H), 3.57–3.78 (m, 3H), 4.38 (d, J = 13.6 Hz, 1H), 7.12–7.43 (m, 5H), 7.80–7.93 (m, 4H), 7.98 (d, J = 5.0 Hz, 1H). Anal. ($C_{30}H_{28}N_2O_3$·$C_2H_2O_4$) calcd: C, 65.52; H, 5.15; N, 4.78; found: C, 65.22; H, 5.20; N, 4.98. ESI-MS: 497 (M+H$^+$, 100).

5.10.6. *N*-{4-[4-(Diphenylmethyl)piperazin-1-yl]butyl}isoindolin-1,3-dione (4a). Method C1. Yield 75%. White solid; mp 208 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.52–1.68 (m, 4H), 2.35–2.65 (m, 4H), 2.84–3.26 (m, 6H), 3.5–3.65 (s, br, 2H), 4.40 (s, 1H), 7.13–7.48 (m, 10H), 7.78–7.90 (m, 4H). Anal. ($C_{29}H_{31}N_3O_2$·$C_2H_2O_4$) calcd: C, 68.49; H, 6.12; N, 7.73; found: C, 68.63; H, 6.02; N, 7.86. ESI-MS: 454 (M+H$^+$, 100), 228(M+2H$^+$, 15).

5.10.7. *N*-(4-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}butyl)isoindolin-1,3-dione (4b). Method A3, method B, method C1. Yield (all steps) 61%; white solid; mp 189 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.6 (s, br, 4H), 2.50 (s, br, 4H), 2.96 (s, br, 2H), 3.08 (s, br, 4H), 3.57 (s, br, 2H), 4.38 (s, br, 1H), 7.12–7.53 (m, 9H), 7.78–7.93 (m, 4H). Anal. ($C_{29}H_{30}ClN_3O_2$·$C_2H_2O_4$) calcd: C, 64.41; H, 5.58; N, 7.27; found: C, 64.46; H, 5.51; N, 7.46. ESI-MS: 489 (M+H$^+$, 100).

5.10.8. *N*-{4-[4-(Diphenylmethyl)-1,4-diazepan-1-yl]butyl}isoindolin-1,3-dione (4c). Method A3, method B, method C1. Yield (all steps) 37%; white solid; mp 102 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50–1.85 (m, 4H), 1.85–2.08 (m, 2H), 2.52–2.65 (m, 2H), 2.68–2.93 (m, 2H), 2.95–3.63 (m, 8H), 4.71 (s, 1H), 7.05–7.65 (m, 10H), 7.7–7.98 (m, 4H). Anal. ($C_{30}H_{33}N_3O_2$· $C_2H_2O_4$· 2 H_2O) calcd: C, 64.74; H, 6.62; N, 7.08; found: C, 64.79; H, 6.47; N, 7.18. ESI-MS: 467 (M+H$^+$, 100).

5.10.9. N-(4-{4-[(4-Chlorophenyl)phenylmethyl]-1,4-diazepan-1-yl}butyl)isoindolin-1,3-dione (4d). Method A3, method B, method C1. Yield (all steps) 20%; white solid; mp 104 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.47–1.82 (m, 4H), 1.87–2.12 (m, 2H), 2.52–2.65 (m, 2H), 2.67–2.90 (m, 2H), 2.91–3.67 (m, 8H, H-9), 4.71 (s, 1H), 7.05–7.67 (m, 9H), 7.7–7.98 (m, 4H). Anal. ($C_{30}H_{32}ClN_3O_2$·$C_2H_2O_4$·H_2O) calcd: C, 63.00; H, 5.95; N, 6.89; found: C, 62.89; H, 5.68; N, 6.62. ESI-MS: 503 (M+H$^+$, 100).

5.10.10. N-(4-{4-[Benzyl(phenyl)amino]piperidin-1-yl}butyl)naphthalen-2-carboxamide (19a). Starting with the free base of **15c**, method C2. Yield 80%; yellowish solid; mp 166 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.52–1.88 (m, 4H), 1.89–2.07 (m, 4H), 2.93–3.12 (m, 4H), 3.27–3.52 (m, 4H), 4.11–4.24 (m, 1H), 4.41 (s, 2H), 6.58–6.64 (m, 1H), 6.70–6.74 (m, 2H), 7.07–7.36 (m, 7H), 7.55–7.65 (m, 2H), 7.87–8.05 (m, 4H), 8.44 (s, 1H), 8.70 (t, J = 5.5 Hz, 1H). Anal. ($C_{33}H_{37}N_3O$· $C_2H_2O_4$) calcd: C, 72.27; H, 6.76; N, 7.22; found: C, 72.18; H, 6.75; N, 7.45.

5.10.11. *N*-[4-(2,3,4,5,10,15-Hexahydro-1*H*-dibenzo[*b*:*e*]pyrazino[2,1-*g*]azepin-1-yl)butyl] naphthalen-2-carboxamide (16a). Starting with the free base of **15a**, method C2. Yield 79%; mp 134 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40–1.57 (m, 2H), 1.63–1.78 (m, 2H), 2.88–3.13 (m, 4H), 3.13–3.55 (m, 7H), 4.25 (d, J = 10.3 Hz, 1H), 4.64 (d, J = 12,3 Hz, 1H), 6.82–6.88 (m, 1H), 6.94–7.23 (m, 7H), 7.56 (m, 2H), 7.86–8.05 (m, 4H), 8.35 (s, 1H). Anal. ($C_{32}H_{33}N_3O$·0.5$C_2H_2O_4$·H_2O) calcd: C, 73.58; H, 6.74; N, 7.80; found: C, 73.65; H, 6.79; N, 8.00.

5.10.12. *N*-{4-[4-(Diphenylmethyl)piperazin-1-yl]butyl}naphthalen-2-carboxamide (6a). Starting with the free base

of **4a**, method C2. Yield 90%; mp 118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.52–1.78 (m, 4H), 2.45–2.71 (m, 4H), 3.01 (s, br, 2H), 3.13 (s, br, 4H), 3.34 (s, br, 2H), 4.40 (s, 1H), 7.16–7.37 (m, 6H), 7.40–7.50 (m, 4H), 7.53–7.65 (m, 2H), 7.78–8.08 (m, 4H), 8.45 (s, 1H), 8.71 (s, br, 1H). Anal. ($C_{32}H_{35}N_3O\cdot C_2H_2O_4$) calcd: C, 71.94; H, 6.57; N, 7.40; found: C, 71.78; H, 6.47; N, 7.50. ESI-MS: 478 (M+H$^+$, 100).

5.10.13. *N*-(4-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}butyl)naphthalen-2-carboxamide (7a). Starting with the free base of **4b**, method C2. Yield 85%; mp 118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54–1.82 (m, 4H), 2.43–2.81 (m, 4H), 3.02 (s, br, 2H), 3.14 (s, br, 4H), 3.34 (s, br, 2H), 4.41 (s, 1H), 7.13–7.67 (m, 11H), 7.78–8.08 (m, 4H), 8.45 (s, 1H), 8.71 (s, br, 1H). Anal. ($C_{32}H_{34}ClN_3O\cdot C_2H_2O_4$) calcd: C, 67.82; H, 6.03; N, 6.98; found: C, 67.57; H, 5.95; N, 7.00. ESI-MS: 497 (M+H$^+$, 100).

5.10.14. *N*-{4-[4-(Diphenylmethyl)-1,4-diazepan-1-yl]butyl}-naphthalen-2-carboxamide (8a). Starting with the free base of **4c**, method C2. Yield 70%; mp 76 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55–1.80 (m, 4H), 1.92 (s, br, 2H), 2.55–2.79 (m, 2H), 2.75 (s, br, 2H), 3.10–3.28 (m, 4H), 3.28–3.45 (m, 4H), 4.68 (s, 1H), 7.18 (t, J = 7.3 Hz, 2H), 7.29 (t, J = 7.5 Hz, 4H), 7.6 (d, J = 7.3 Hz, 4H), 7.58–7.64 (m, 2H), 7.91–8.05 (m, 4H), 8.45 (s, 1H), 8.73 (s, br, 1H). Anal. ($C_{33}H_{37}N_3O\cdot C_2H_2O_4\cdot H_2O$) calcd: C, 70.10; H, 6.89; N, 7.01; found: C, 69.98; H, 6.74; N, 7.17. ESI-MS: 492 (M+H$^+$, 100).

5.10.15. *N*-(4-{4-[Benzyl(phenyl)amino]piperidin-1-yl}butyl)-benzo[*b*]thiophen-2-carboxamide (19b). Starting with the free base of **15c**, method C2. Yield 78%; mp 182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.49–1.90 (m, 4H), 1.91–2.13 (m, 4H), 2.90–3.09 (m, 4H), 3.21–3.52 (m, 4H), 4.13–4.27 (m, 1H), 4.43 (s, 2H), 6.53–6.68 (m, 1H), 6.73–6.77 (m, 2H), 7.02–7.33 (m, 7H), 7.38–7.45 (m, 2H), 7.80–7.98 (m, 2H), 8.32 (s, 1H), 8.87 (s, br, 1H). Anal. ($C_{31}H_{35}N_3OS\cdot C_2H_2O_4$) calcd: C, 67.44; H, 6.35; N, 7.15; found: C, 67.18; H, 6.42; N, 7.23. ESI-MS: 498 (M+H$^+$, 100).

5.10.16. *N*-[4-(2,3,4,5,10,15-Hexahydro-1*H*-dibenzo[*b*:*e*]-pyrazino[2,1-*g*]azepin-1-yl)butyl]cinnamide (16b). Starting with the free base of **15a**, method C2. Yield 87%; mp 138 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41–1.58 (m, 2H), 1.58–1.79 (m, 2H), 2.85–3.55 (m, 11H), 4.25 (d, J = 10.31 Hz, 1H), 4.65 (d, J = 12.34 Hz, 1H), 6.63 (d, J = 15.82 Hz, 1H), 6.88 (t, J = 7.31 Hz, 1H), 6.97–7.28 (m, 7H), 7.32–7.47 (m, 4H), 7.48–7.60 (m, 2H), 8.22 (t, J = 5.47 Hz, 1H). Anal. ($C_{30}H_{33}N_3O\cdot C_2H_2O_4$) calcd: C, 70.96; H, 6.51; N, 7.76; found: C, 70.75; H, 6.55; N, 7.69. ESI-MS: 452 (M+H$^+$, 100).

5.10.17. *N*-[4-(2,3,4,5,10,15-Hexahydro-1*H*-dibenzo[*b*:*e*]-pyrazino[2,1-*g*]azepin-1-yl)butyl]thiophen-2-carboxamide (16d). Starting with the free base of **15a**, method C2. Yield 92%; mp 186 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45–1.86 (m, 4 H), 2.92–3.63 (m, 11H), 4.26 (d, J = 9.93 Hz, 1H), 4.65 (d, J = 12.24 Hz, 1H), 6.89 (t, J = 7.29 Hz, 1H), 7.02–7.36 (m, 8H), 7.80 (d, J = 4.38 Hz, 2H), 8.56 (t, J = 5.51 Hz, 1H). Anal. ($C_{26}H_{29}N_3O$-$S\cdot 0.8C_2H_2O_4\cdot 1.8\ H_2O$) calcd: C, 60.70; H, 6.29; N, 7.58; found: C, 60.73; H, 6.10; N, 7.29. ESI-MS: 432 (M+H$^+$, 100).

5.10.18. *N*-[4-(2,3,4,5,10,15-Hexahydro-1*H*-dibenzo[*b*:*e*]-pyrazino[2,1-*g*]azepin-1-yl)butyl]benzo[*b*]thiophen-2-carboxamide (16c). Starting with the free base of **15a**, method C2. Yield 93%; mp 171 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48–1.82 (m, 4H), 2.76–3.12 (m, 4H), 3.18–3.55 (m, 7H), 4.20 (d, J = 10.1 Hz, 1H), 4.64 (d, J = 12.4 Hz, 1H), 6.88 (t, J = 7.29 Hz, 1H), 6.96–7.30 (m, 7H), 7.26–7.54 (m, 2H), 7.88–8.03 (m, 3H), 8.83 (t, J = 5.4 Hz, 1H). Anal. ($C_{30}H_{31}N_3OS\cdot C_2H_2O_4$) calcd: C, 67.23; H, 5.78; N, 7.35; found: C, 67.02; H, 5.78; N, 7.32. ESI-MS: 482 (M+H$^+$, 100).

5.10.19. *N*-[4-(2,3,4,5,10,15-Hexahydro-1*H*-dibenzo[*b*:*e*]-pyrazino[2,1-*g*]azepin-1-yl)butyl]cyclohexancarboxamide (16e). Starting with the free base of **15a**, method C2. Yield 86%; mp 142 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05–1.51 (m, 8H), 1.51–1.82 (m, 6H), 2.02–2.15 (m, 1H) 2.87–3.12 (m, 4H), 3.18–3.55 (m, 7H), 4.20 (d, J = 10.1 Hz, 1H), 4.64 (d, J = 12.4 Hz, 1H), 6.88 (t, J = 7.29 Hz, 1H), 6.96–7.30 (m, 7H), 7.72 (t, J = 5.4 Hz, 1H). Anal. ($C_{28}H_{37}N_3O\cdot C_2H_2O_4\cdot 0.25\ H_2O$) calcd: C, 68.48; H, 7.57; N, 7.99; found: C, 68.25; H, 7.77; N, 8.20. ESI-MS: 432 (M+H$^+$, 100).

5.10.20. *N*-[4-(4-Diphenylmethylpiperazin-1-yl)butyl]cinnamide (6b). Starting with the free base of **4a**, method C2. Yield 89%; mp 182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39–1.74 (m, 4H), 2.50–2.72 (m, 4H), 2.88–3.33 (m, 8H), 4.40 (s, 1H), 6.64 (d, J = 15.8 Hz, 1H), 7.12 (t, J = 4.3 Hz, 1 H), 7.21–7.51 (m, 13H), 7.51–7.62 (m, 2H), 8.53–8.64 (m, 1H). Anal. ($C_{30}H_{35}N_3O\cdot C_2H_2O_4$) calcd: C, 70.70; H, 6.86; N, 7.73; found: C, 70.53; H, 6.70; N, 7.83.

5.10.21. *N*-[4-(4-Diphenylmethylpiperazin-1-yl)butyl]thiophen-2-carboxamide (6d). Starting with the free base of **4a**, method C2. Yield 89%; mp 182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45–1.72 (m, 4H), 2.50–2.72 (m, 4H), 2.93–3.33 (m, 8H), 4.40 (s, 1H), 7.12 (t, J = 4.3 Hz, 1H), 7.16–7.25 (m, 2H), 7.31 (t, J = 7.1 Hz, 4H), 7.38–7.51 (m, 4H), 7.69–7.81 (m, 2H), 8.53–8.64 (m, 1H). Anal. ($C_{26}H_{31}N_3OS\cdot C_2H_2O_4$) calcd: C, 64.22; H, 6.35; N, 8.02; found: C, 64.06; H, 6.39; N, 7.96.

5.10.22. *N*-[4-(4-Diphenylmethylpiperazin-1-yl)butyl]benzo-[*b*]thiophen-2-carboxamide (6c). Starting with the free base of **4a**, method C2. Yield 87%; mp 144 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.47–1.78 (m, 4H), 2.50–2.72 (m, 4H), 2.91–3.42 (m, 8H), 4.39 (s, 1H), 7.14–7.23 (m, 2H), 7.30 (t, J = 7.4 Hz, 4H), 7.35–7.51 (m, 6H), 7.88–8.06 (m, 2H), 8.11 (s, 1H), 8.80–8.92 (m, 1H). Anal. ($C_{30}H_{33}N_3OS\cdot C_2H_2O_4$) calcd: C, 66.99; H, 6.15; N, 7.32; found: C, 67.03; H, 6.14; N, 7.35. ESI-MS: 484 (M+H$^+$, 100).

5.10.23. *N*-[4-(4-Diphenylmethylpiperazin-1-yl)butyl]cyclohexancarboxamide (6e). Starting with the free base of **4a**, method C2. Yield 90%; mp 128 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05–1.45 (m, 6H), 1.45–1.73 (m, 8H), 2.04 (t, J = 5.6 Hz, 1H), 2.51 (s, br, 4H), 2.87–3.22 (m, 8H), 4.41 (s, 1H), 7.21 (t, J = 7.3 Hz, 2H), 7.28–7.36 (m, 4H), 7.36–7.45 (m, 4H), 7.70 (t, J = 5.5 Hz, 1H). Anal. ($C_{28}H_{39}N_3O{\cdot}C_2H_2O_4{\cdot}0.75\ H_2O$) calcd: C, 67.08; H, 7.97; N, 7.82; found: C, 67.02; H, 7.74; N, 8.03. ESI-MS: 434 ($M+H^+$, 100).

5.10.24. *N*-(4-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}butyl)cinnamide (7b). Starting with the free base of **4b**, method C2. Yield 84%; mp 158 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35–1.73 (m, 4H), 2.48 (s, br, 4H), 2.90–3.35 (m, 8H), 4.42 (s, 1H), 6.64 (d, J = 15.6 Hz, 1H), 7.13–7.65 (m, 14H), 8.25 (s, br, 1H). Anal. ($C_{30}H_{34}ClN_3O{\cdot}C_2H_2O_4$) calcd: C, 66.48; H, 6.28; N, 7.27; found: C, 66.24; H, 6.31; N, 7.13.

5.10.25. *N*-(4-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}butyl)thiophen-2-carboxamide (7d). Starting with the free base of **4b**, method C2. Yield 87%; mp 159 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35–1.73 (m, 4H), 2.48 (s, br, 4H), 2.90–3.35 (m, 8H), 4.42 (s, 1H), 7.05–7.59 (m, 10H), 7.59–7.80 (m, 2H), 8.57 (s, br, 1H). Anal. ($C_{26}H_{30}ClN_3OS{\cdot}C_2H_2O_4$) calcd: C, 60.26; H, 5.78; N, 7.53; found: C, 60.02; H, 5.79; N, 7.53.

5.10.26. *N*-(4-{4-[(4-Chlorophenyl)phenylmethyl)piperazin-1-yl]butyl}benzo[*b*]thiophen-2-carboxamide (7c). Starting with the free base of **4b**, method C2. Yield 63%; mp 128 °C ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35–1.73 (m, 4H), 2.48 (s, br, 4H), 2.90–3.35 (m, 8H), 4.42 (s, 1H), 7.05–7.59 (m, 11H), 7.90–8.05 (m, 2H), 8.09 (s, 1H), 8.57 (s, br, 1H). Anal. ($C_{28}H_{32}ClN_3OS{\cdot}C_2H_2O_4$) calcd: C, 63.20; H, 5.63; N, 6.91; found: C, 63.18; H, 5.66; N, 6.84. ESI-MS: 495 ($M+H^+$, 100).

5.10.27. *N*-(4-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}butyl)cyclohexancarboxamide (7e). Starting with the free base of **4b**, method C2. Yield 79%; mp 109 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05–1.73 (m, 14H), 1.95–2.1 (m, 1H), 2.48 (s, br, 4H), 2.90–3.35 (m, 8H), 4.42 (s, 1H), 7.05–7.59 (m, 9H), 7.73 (s, br, 1H). Anal. ($C_{26}H_{38}ClN_3O{\cdot}C_2H_2O_4{\cdot}0.5\ H_2O$) calcd: C, 63.54; H, 7.29; N, 7.41; found: C, 63.71; H, 7.31; N, 7.35. ESI-MS: 445 ($M+H^+$, 100).

5.10.28. *N*-{4-[4-(Diphenylmethyl)-1,4-diazepan-1-yl]butyl}-cinnamide (8b). Starting with the free base of **4c**, method C2. Yield 79%; mp 89 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42–1.57 (m, 2H), 1.60–1.79 (m, 2H), 1.85–2.01 (m, 2H), 2.51–2.61 (m, 2H), 2.71–2.83 (m, 2H), 3.04–3.46 (m, 8H), 4.68 (s, 1H), 6.64 (d, J = 15.81 Hz, 1H), 7.12–7.24 (m, 2H), 7.24–7.63 (m, 14H), 8.25 (t, J = 5.47 Hz, 1H). Anal. ($C_{31}H_{37}N_3O{\cdot}1.2C_2H_2O_4{\cdot}2\ H_2O$) calcd: C, 65.58; H, 7.15; N, 6.87; found: C, 65.79; H, 6.91; N, 7.18. ESI-MS: 468 ($M+H^+$, 100).

5.10.29. *N*-{4-[4-(Diphenylmethyl)-1,4-diazepan-1-yl]butyl}-thiophen-2-carboxamide (8d). Starting with the free base of **4c**, method C2. Yield 65%; mp 84 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45–1.61 (m, 2H), 1.63–1.83 (m, 2H), 1.87–2.1 (m, 2H), 2.53–2.67 (m, 2H), 2.74–2.89 (m, 2H), 3.05–3.52 (m, 8H), 4.76 (s, 1H), 7.07–7.47 (m, 7H), 7.48–7.58 (d, J = 7.32 Hz, 4H), 7.70–7.86 (m, 2H), 8.64 (t, J = 5.52 Hz, 1H). Anal. ($C_{27}H_{33}N_3OS{\cdot}1.5\text{-}C_2H_2O_4{\cdot}1.5\ H_2O$) calcd: C, 58.92; H, 6.46; N, 6.87; found: C, 58.75; H, 6.64; N, 6.80. ESI-MS: 448 ($M+H^+$, 100).

5.10.30. *N*-{4-[4-(Diphenylmethyl)-1,4-diazepan-1- yl]butyl}-benzo[*b*]thiophen-2-carboxamide (8c). Starting with the free base of **4c**, method C2. Yield 71%; mp 148 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48–1.64 (m, 2H), 1.65–1.81 (m, 2H), 1.84–1.98 (m, 2H), 2.50–2.60 (m, 2H), 2.67–2.83 (m, 2H), 3.06–3.46 (m, 8H), 4.67 (s, 1H), 7.12–7.38 (m, 6H), 7.39–7.53 (m, 6H), 7.87–8.05 (m, 2H), 8.09 (s, 1H), 8.86 (t, J = 5.40 Hz, 1H). Anal. ($C_{31}H_{35}N_3OS{\cdot}C_2H_2O_4{\cdot}H_2O$) calcd: C, 65.43; H, 6.49; N, 6.94; found: C, 65.59; H, 6.23; N, 7.02.

5.10.31. *N*-(4-{4-[(2-Methoxyphenyl)amino]piperidin-1-yl}butyl)cinnamide (25b). Starting with the free base of **24**, method C2. Yield 65%; mp 82 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45–1.80 (m, 6H), 2.03–2.13 (m, 2H), 2.96–3.06 (m, 4H), 3.18–3.27 (m, 2H), 3.31–3.58 (m, 2H), 3.73 (s, 3H), 6.5–6.86 (m, 5H), 7.32–7.45 (m, 4H), 7.55–7.60 (m, 2H), 8.19 (s, br, 1H). Anal. ($C_{25}H_{33}N_3O_2{\cdot}C_2H_2O_4$) calcd: C, 65.17; H, 7.09; N, 8.44; found: C, 64.92; H, 7.00; N, 8.47. ESI-MS: 408 ($M+H^+$, 100).

5.11. Pharmacology experiments

5.11.1. Dopamine receptors binding studies. Cell culture was carried out using standard procedures. Human D_{2S} and D_3 receptors were expressed in stably transfected Human Embryonic Kidney (HEK) and Chinese hamster ovary (CHO) cells, respectively. The CHO-D_3 cells were cultured at 37 °C in Dulbecco's Modified Eagle Medium (DMEM, Cambrex Bio Sciences, Rockland Inc) supplemented with 10% dialysed fetal calf serum (Invitrogen,Co, Carlsbad, CA), 100 Units/ml penicillin-steptomycin (Cambrex, Bio Sciences, Rockland Inc), Hepes 20 mM, pH 7.4 and 2 mM glutamine (Cambrex, Bio Sciences, Rockland Inc) in an atmosphere of 5% CO_2.[53] The HEK-D_{2S} cell line was obtained following transfection by pCDNA3.1-D2S expressing vector. HEK-D_{2S} were cultured at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM-NUT.F-12, Cambrex, Bio Sciences, Rockland Inc) supplemented with 10% fetal calf serum (Cambrex, Bio Sciences, Rockland Inc), 100 U/ml penicillin-steptomycin (Cambrex, Bio Sciences, Rockland Inc), Hepes 20 mM, pH 7.4, 400 μg/ml geneticin (Cambrex, Bio Sciences, Rockland Inc) and 2 mM glutamine (Cambrex, Bio Sciences, Rockland Inc) in an atmosphere of 5% CO_2.

Incubations containing 2 nM [^{3}H] spiperone (specific activity 15 Ci/mmol, Perkin-Elmer Life Sciences, Boston, MA) were run in duplicate in 0.1% polyethylenimine (PEI) (Sigma–Aldrich, Inc, St. Louis, MI) coated multiscreen GF/B 96 wells microplates (Milli-

pore, Billerica, MA). Incubations were started by adding per well 250 µl membrane suspension diluted to 10 µg protein/ml. Two microliters of tested compounds diluted in dimethyl sulfoxide (Sigma–Aldrich, Inc, St. Louis, MI) were added in increasing final concentrations, at 0.1, 1, 10, 100, 1000 or 10,000 nM. Non specific binding was measured in the presence of 5 µM haloperidol (Sigma–Aldrich, Inc, St. Louis, MI). Incubations were run 1 h at room temperature and stopped by vacuum filtration. Filters were washed 4 times by 250 µl of ice-cold binding buffer. Then 50 µl of Optiphase Supermix scintillation cocktail (Perkin- Elmer, Boston, MA) was added and the filters were counted by liquid scintillation on the 14.50 microbeta Trilux counter (Wallac-Perkin Elmer, Boston, MA). IC_{50} values representing the concentrations to 50% of maximal inhibition were calculated by nonlinear regression using the Origin 6.0 software (microcal software, Inc, Northampton, MA). K_i values were derived from the formula $K_i = IC_{50}/(1 + L/K_d)$ where L is the concentration of [^{3}H]spiperone and K_d its dissociation constant.[55]

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References and notes

1. Levant, B. *Pharmacol. Rev.* **1997**, *49*, 231.
2. Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. *Physiol. Rev.* **1998**, *78*, 189.
3. Carlson, A. *Neuropsychopharmacology* **1988**, *1*, 179.
4. Sokoloff, P.; Diaz, J.; Le Foll, B.; Guillin, O.; Leriche, L.; Bezard, E.; Gross, C. *CNS Neurol. Disord. Drug Targets* **2006**, *5*, 25.
5. Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J.-C. *Nature* **1990**, *347*, 146.
6. Gurevich, E. V.; Joyce, J. N. *Neuropsychopharmacology* **1999**, *20*, 60.
7. Sealfon, S. C.; Olanow, C. W. *Trends Neurosci.* **2000**, *23*, S34.
8. Joyce, J. N. *Pharmacol. Ther.* **2001**, *90*, 231.
9. Sokoloff, P.; Martres, M. P.; Giros, B.; Bouthenet, M. L.; Schwartz, J.-C. *Biochem. Pharmacol.* **1992**, *43*, 659.
10. Griffon, N.; Sokoloff, P.; Diaz, J.; Levesque, D.; Sautel, F.; Schwartz, J.-C.; Simon, P.; Costentin, J.; Garrido, F.; Mann, A.; Wermuth, C. *Eur. Neuropsychopharmacol.* **1995**, *5*, 3.
11. Richtand, N. M.; Woods, S. C.; Berger, S. P.; Strakowski, S. M. *Neurosci. Biobehav. Rev.* **2001**, *25*, 427.
12. Strange, P. G. *Pharmacol. Rev.* **2001**, *53*, 119.
13. Schwartz, J-C.; Diaz, J.; Pilon, C.; Sokoloff, P. *Brain Res. Brain Res. Rev.* **2000**, *31*, 277.
14. Le Foll, B.; Diaz, J.; Sokoloff, P. *Neuroreport* **2005**, *16*, 175.
15. Le Foll, B.; Goldberg, S. R.; Sokoloff, P. *Expert Opin. Investig. Drugs* **2007**, *16*, 45.
16. Foll, B.; Sokoloff, P.; Stark, H.; Goldberg, S. R. *Neuropsychopharmacology* **2005**, *30*, 720.
17. Dekeyne, A. *Soc. Neurosi. Abstr.* **2004**, *29*, 776.4.
18. Joyce, J. N.; Millan, M. J. *Drug Discov. Today* **2005**, *10*, 917.
19. Newman, A. H.; Grundt, P.; Nader, M. A. *J. Med. Chem.* **2005**, *48*, 3663.
20. Luedtke, R. R.; Mach, R. H. *Curr. Pharm. Des.* **2003**, *9*, 643.
21. Hackling, A. E.; Stark, H. *Chem. Bio. Chem.* **2002**, *3*, 946.
22. Boeckler, F.; Gmeiner, P. *Pharmacol. Ther.* **2006**, *112*, 281.
23. Pilla, M.; Perachon, S.; Sautel, F.; Garrido, F.; Mann, A.; Wermuth, C. G.; Schwartz, J.-C.; Everitt, B. J.; Sokoloff, P. *Nature* **1999**, *401*, 371.
24. Wermuth, C. G.; Mann, A.; Garrido, F.; Lecomte, J.; Schwartz, J. -C.; Sokoloff, P. Eur. Pat. 0779284, 1997, *Chem. Abstr.*, **1997**, *127*, 108937.
25. Wood, M. D.; Boyfield, I.; Nash, D. J.; Jewitt, F. R.; Avenell, K. Y.; Riley, G. J. . *Eur. J. Pharmacol.* **2000**, *407*, 47.
26. Wicke, K.; Garcia-Ladona, F. J. *Eur. J. Pharmacol.* **2001**, *424*, 85.
27. Bezard, E.; Ferry, S.; Mach, U.; Stark, H.; Leriche, L.; Boraud, T.; Gross, C.; Sokoloff, P. *Nat. Med.* **2003**, *9*, 762.
28. Garcia- Ladona, F. J.; Cox, B. F. *CNS Drug Rev.* **2003**, *9*, 141.
29. Bettinetti, L.; Schlotter, K.; Huebner, H.; Gmeiner, P. *J. Med. Chem.* **2002**, *45*, 4594.
30. Leopoldo, M.; Berardi, F.; Colabufo, N. A.; De Giorgio, P.; Lacivita, E.; Perrone, R.; Tortorella, V. *J. Med. Chem.* **2002**, *45*, 5727.
31. Dubuffet, T.; Newman- Tancredi, A.; Cussac, D.; Audinot, V.; Loutz, A.; Millan, M. J.; Lavielle, G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2059.
32. Millan, M. J.; Gobert, A.; Newman-Tancredi, A.; Lejeune, F.; Cussac, D.; Rivet, J.; Audinot, V.; Dubuffet, T.; Lavielle, G. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 1048.
33. Cussac, D.; Newman-Tancredi, A.; Sezgin, L.; Millan, M. J. *Eur. J. Pharmacol.* **2000**, *394*, 47.
34. Drescher, K. U.; Garcia-Ladona, F. J.; Teschendorf, H. J.; Traut, M.; Unger, L.; Wicke, K. M.; Weddige, F. K.; Freeman, A. S.; Gross, G. *Am. Soc. Neurosci.* **2002**, 894.6.
35. Unger, L.; Garcia-Ladona, F. J.; Wernet, W.; Sokoloff, P.; Wicke, K. M.; Gross, G. *Am. Soc. Neurosci.* **2002**, 894.5.
36. Brocco, M. *Int. J. Neuropsychopharm.* **2004**, *7*, S265.
37. Kroeze, W. K.; Hufeisen, S. J.; Popadak, B. A.; Renock, S. M.; Steinberg, S.; Ernsberg, P.; Jayathilake, K.; Meltzer, H. Y.; Roth, B. L. *Neuropsychopharmacology* **2003**, *3*, 519.
38. Richelson, E. *J. Clin. Psychiatry* **1999**, *60*, 5.
39. Shen, W. W. *Compr. Psychiatry* **1999**, *40*, 407.
40. Cusack, B.; Nelson, A.; Richelson, E. *Psychopharmacology* **1994**, *114*, 559.
41. Nonaka, H.; Otaki, S.; Ohshima, E.; Kono, M.; Kase, H.; Ohta, K.; Fukui, H.; Ichimura, M. *Eur. J. Pharmacol.* **1998**, *345*, 111.
42. Strange, P. G. *Trend Pharmacol. Sci.* **1996**, *17*, 238.
43. Leurs, R.; Smit, M. J.; Timmerman, H. *Pharmacol. Ther.* **1995**, *66*, 413.
44. Hjorth, S.; Sharp, T. *Life Sci.* **1990**, *46*, 955.
45. Begley, D. J. *Pharmacol. Ther.* **2004**, *104*, 29.
46. Zaragoza, F.; Stephensen, H. *J. Org. Chem.* **2001**, *66*, 2518.
47. Sommers, A. H.; Michaels, R. J.; Weston, A. W. *J. Am. Chem. Soc.* **1954**, *76*, 5805.
48. Russell, R. K.; Rampulla, R. A.; vanNievelt, C. E.; Klaubert, D. H. *J. Heterocycl. Chem.* **1990**, *27*, 1761.
49. Waldvogel, E.; Schwarb, G.; Bastian, J.-M.; Bourquin, J.-P. *Helv. Chim. Acta* **1976**, *59*, 866.
50. Kaspersen, F. M.; Favier, J. S.; Wagenaars, G.; Wallaart, J.; Funke, C. W. *JR. Neth. Chem. Soc.* **1983**, *102*, 457.
51. Mensonides-Harsema, M. M.; Liao, Y.; Boettcher, H.; Bartoszyk, G. D.; Greiner, H. E.; Harting, J.; deBoer, P.; Wikstroem, H. V. *J. Med. Chem.* **2000**, *43*, 432.

52. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.
53. Sokoloff, P.; Andrieux, M.; Besancon, R.; Pilon, C.; Martres, M. P.; Giros, B.; Schwartz, J.-C. *Eur. J. Pharmacol.* **1992**, *225*, 331.
54. Bondensgaard, K.; Ankersen, M.; Thøgersen, H.; Hansen, B. S.; Wulff, B.; Bywater, R. P. *J. Med. Chem.* **2004**, *47*, 888.
55. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.