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Review

Dopamine D3 receptor ligands—Recent advances in the control of subtype selectivity and intrinsic activity

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Abstract

Various pharmacological studies have implicated the dopamine D_3 receptor as an interesting therapeutic target in the treatment of different neurological disorders. Because of these putative therapeutic applications, D_3 receptor ligands with diverse intrinsic activities have been an active field of research in recent years. Separation of purely D_3-mediated drug effects from effects produced by interactions with similar biogenic amine receptors allows to verify the therapeutic impact of D_3 receptors and to reduce possible side-effects caused by "promiscuous" receptor interactions. The requirement to gain control of receptor selectivity and in particular subtype selectivity has been a challenging task in rational drug discovery for quite a few years. In this review, recently developed structural classes of D_3 ligands are discussed, which cover a broad spectrum of intrinsic activities and show interesting selectivities.

Keywords: Dopamine D3 receptor; Agonist; Antagonist; Structure activity relationship; Rational drug discovery; G protein-coupled receptor

Contents

1. Introduction

In more than 15 years of research since the discovery of the D_3 receptor by Sokoloff et al. [1], enormous progress has been made in improving our understanding of its physiological

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function and pharmacological impact. Being preferentially located in brain regions which have an impact on emotional and cognitive functions, such as e.g. the nucleus accumbens and the islands of Calleja [2–4], the D_3 receptor is capable of affecting behavioral properties, such as locomotor activity, reinforcement and reward. Thus, various pharmacological studies have investigated the D_3 system as an interesting therapeutic target for the treatment of schizophrenia [5,6], Parkinson's disease [7], drug-induced dyskinesia [8] and drug abuse (in particular cocaine addiction) [6,9]. Moreover, D_3 might be involved in the cortical development during gestation obviously orchestrating neuronal migration and differentiation [10].

Neuroprotective effects during the induction phase of Parkinson's disease have been reported for D_3 receptor agonists, such as pramipexole (**2**). Recently, the selective D_3 partial agonist FAUC 329 (**30**) has been evaluated in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease [11]. FAUC 329 showed most pronounced neuroprotective effects on dopamine (DA) depletion in the nucleus accumbens. reflecting the preferential abundance of D_3 receptors in this region [12]. Moreover, it has been demonstrated that the D_3 selective partial agonist BP 897 (**27**) inhibits cocaine-seeking behavior caused by the presentation of drug-associated cues, however, in the absence of any intrinsic, primary rewarding effects [9]. While D_3 agonists have already been established as valuable treatment alternatives in PD [13,14], the more recently discovered selective D_3 partial agonists and antagonists are currently evaluated for their clinical relevance.

2. Homology-based difficulty to gain selectivity among D_2-like receptors

Parallel to gaining a more detailed insight into D_3 receptor pharmacology and to the evaluation of respective treatment opportunities, the available D_3 ligands have undergone a structural evolution: mostly driven by rational drug discovery, small, dopamine-related agonists have evolved into structurally diverse agents with high affinity, selectivity over the closely related biogenic amine receptors and a broad range of intrinsic activities. However, gaining selectivity for D_3 versus the other D_2-like receptors (in particular D_2) has been a challenge in medicinal chemistry for quite a few years. This challenge was predominantly based on a distinct structural homology between the D_2-like receptors and the absence of direct structural data about the dopaminergic or even any aminergic receptors.

As outlined in Fig. 1, an elaborate sequence alignment of the D_2-like *wild type* receptors [15], which is largely consistent with previous investigations [16,17] reveals a moderate overall sequence identity between D_2 and D_4 (~32%) or D_3 and D_4 receptors (~34%). However, the overall sequence identity for D_2 and D_3 receptors is significantly higher (~50%). Extending the comparison criteria from identity to similarity (as defined by the use of a Gonnet250 similarity matrix [18]) confirms the trend observed for identity comparison. The similarity scores are: 48% for D_2/D_4, 49% for D_3/D_4 and 63% for D_2/D_3. Focusing on the predicted transmembrane regions as a rough estimation for the relevant interaction sites for ligand recognition, sequence identities are increased to 51% for D_2/D_4, 53% for D_3/D_4 and 79% for D_2/D_3. Representing an analog trend at a higher level, the sequence similarities for the predicted transmembrane regions are: 72% for D_2/D_4, 73% for D_3/D_4 and 90% for D_2 versus D_3 receptors. Based on the substantial sequence homology between D_2 and D_3 receptors in particular in the binding-relevant regions, it becomes obvious that the improvement of D_3:D_2 selectivity is a challenging task and that recent success is still hardly explainable on a structural molecular level.

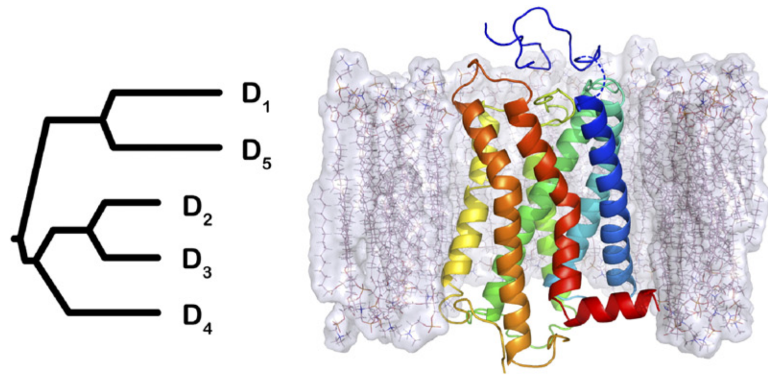
3. Recent progress in controlling subtype selectivity and intrinsic activity of D_3 ligands

Because of the various putative therapeutic applications, D_3 receptor ligands with diverse intrinsic activities have been an active field of research in recent years. Separation of purely D_3-mediated drug effects from effects produced by interactions with similar biogenic amine receptors allows to improve the significance of insights into the therapeutic impact of D_3 receptors and to reduce possible side-effects caused by "promiscuous" receptor interactions. Thus, there is the necessity to gain control of receptor selectivity and in particular subtype selectivity, which has been a challenging task in rational drug discovery for quite a few years. In this review, we present some recently developed structural families of D_3 ligands covering a broad spectrum of intrinsic activities and showing interesting selectivities.

3.1. Heterocyclic agonists

Similarity replacement of atoms, functions or moieties based on physicochemical or topological aspects has lead to numerous bioisosters of the genuine neurotransmitter dopamine (**1**). For instance, exchange of the catechol substructure of DA into a heterocyclic aminothiazole moiety and rigidization of the flexible aminoethyl side chain (Scheme 1) has yielded pramipexole (**2**), which has become a reference D_3 agonist for *in vitro* and *in vivo* studies, as well as a standard therapeutic

Fig. 1. Multiple sequence alignment of the D_2, D_3, and D_4 receptors created with ClustalX [75]. Only marginal manual improvements were necessary using the alignment editor GeneDoc [76]. A consensus sequence was generated by applying standard criteria from MULTALIGN [77]: uppercase is identity, lowercase is consensus level >0.6, ! is any one of the amino acid groups IV, $ is any one of LM, % is any one of FY, # is any one of NDQEBZ. Residues contributing to this consensus are drawn as white letters on black background, when strictly conserved throughout all 3 sequences, or shaded in gray when conserved in terms of any other MULTALIGN rules. The transmembrane domains TM1 to TM7 and the adjacent helix H8 are denoted above the sequences. The cylinders are color-coded corresponding to the D_3 receptor model depicted above the sequence alignment. Filled areas of the cylinder reflect parts of the sequence, where all 3 receptors show helical structures in a recent comparative modeling study [15]. Cylinder areas without filling represent parts of the sequence that are found to be helical only in some of the dopamine receptor models. Both sequence numbers as well as residue numbers according to the Ballesteros and Weinstein numbering scheme [78] are given. The phylogenetic tree illustrates sequence homologies among dopamine receptors.




```
                                                  TM1
                                            1.30        1.40
hD2    1   MDPLNLSWYDDDLERQNWSRPFNGSDGKADR--PHYNYYATLLTLLIAVI   48
hD3    1   --MASLSQLSSHL---NYTCGAENSTGASQA--RPHAYYALSYCALILAI   43
hD4    1   --MGNRSTADADGLLAGRGPAAGASAGASAGLAGQGAAALVGGVLLIGAV   48
cons.      ..m.nlS..d.dl...n....a..S.Gas.......ayya....lLI.a!

                                    TM2
              1.50       1.60     2.40       2.50       2.60
hD2   49   VFGNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVG   98
hD3   44   VFGNGLVCMAVLKERALQTTTNYLVVSLAVADLLVATLVMPWVVYLEVTG   93
hD4   49   LAGNSLVCVSVATERALQTPTNSFIVSLAAADLLLALLVLPLFVYSEVQG   98
cons.      vfGN.LVCmaV..ErALQTtTNyl!VSLAvADLLvAtLV$PwvVYlEV.G

                              TM3
                       3.30       3.40       3.50
hD2   99   -EWKFSRIHCDIFVTLDVMMCTASILNLCAISIDRYTAVAMPMLYN--TR  145
hD3   94   GVWNFSRICCDVFVTLDVMMCTASILNLCAISIDRYTAVVMPVHYQHGTG  143
hD4   99   GAWLLSPRLCDALMAMDVMLCTASIFNLCAISVDRFVAVAVPLRYN---R  145
cons.      g.W.fSri.CD.fvt$DVM$CTASIlNLCAIS!DR%tAVamP..Y#..tr

                      TM4
             4.40       4.50       4.60                 5.40
hD2  146   YSSKRRVTVMISIVWVLSFTISCPLLFGLNN---ADQNECIIANPAFVVY  192
hD3  144   QSSCRRVALMITAVWVLAFAVSCPLLFGFNTT--GDPTVCSISNPDFVIY  191
hD4  146   QGGSRRQLLLIGATWLLSAAVAAPVLCGLNDVRGRDPAVCRLEDRDYVVY  195
cons.      qss.RRv.l$I.avWvLsfa!scPlLfGlN.....Dp.vC.i.#pd%V!Y

              TM5
                  5.50       5.60
hD2  193   SSIVSFYVPFIVTLLVYIKIYIVLR----RRRKRVN---TKRSSRAFRAH  235
hD3  192   SSVVSFYLPFGVTVLVYARIYVVLKQ---RRRKRIL---TRQNSQCNSVR  235
hD4  196   SSVCSFFLPCPLMLLLYWATFRGLQRWEVARRAKLHGRAPRRPSGPGPPS  245
cons.      SS!vSF%lPf.vtlLvY..i%.vL.....rRRkr.....trr.S......

hD2  236   LRAPLKGNCTHPEDMKLCTVIMKSNGSFPVNRRRVEAARRAQELEMEMLS  285
hD3  236   PGFPQQTLSPDPAHLEL-------KRYYSICQDTALGGPGFQERGGELKR  278
hD4  246   PTPPAPRLPQDPCGPDCAPP-APGLPRGPCGPDCAPAAPGLPPDPCGPDC  294
cons.      p..P...l..dP....l...........p...d.a.aapg.qe...e...

hD2  286   STSPPERTRYSPIPPSHHQLTLPDPSHHGLHSTPDSPAKPEKNGHAKDHP  335
hD3  279   ----EEKTRNSLSPTIAPKLSL----------------------------  296
hD4  295   APPAPGLPQDPCGPDCAPPAPGLPRGPCGPDCAPPAPGLPQDPCGPDCAP  344
cons.      ....pe.tr.s..P..ap.l.l.....g....p..p..p.........p

                                                     6.30
hD2  336   KIAKIFEIQTMPNGKTRTSLKTMSRR-KLS-----------QQKEKKATQ  373
hD3  297   ------EVRKLSNGRLSTSLKLGPLQPRGV-----------PLREKKATQ  329
hD4  345   PAPGLPPDPCGSNCAPPDAVRAAALPPQTPPQTRRRRRAKITGRERKAMR  394
cons.      .......e....sNg...tslk...l.p...............rEkKAtq

              TM6                                 TM7
             6.40       6.50       6.60           7.40
hD2  374   MLAIVLGVFIICWLPFFITHILNIHCD-CNIPPVLYSAFTWLGYVNSAVN  422
hD3  330   MVAIVLGAFIVCWLPFFLTHVLNTHCQTCHVSPELYSATTWLGYVNSALN  379
hD4  395   VLPVVVGAFLLCWTPFFVVHITQALCPACSVPPRLVSAVTWLGYVNSALN  444
cons.      mla!VlGaFi.CWlPFF.tH!l#.hC..C.!pP.LySA.TWLGYVNSAlN

                        H8
           7.50       7.60       7.70
hD2  423   PIIYTTFNIEFRKAFLKILHC--                             443
hD3  380   PVIYTTFNIEFRKAFLKILSC--                             400
hD4  445   PVIYTVFNAEFRNVFRKALRACC                             467
cons.      P!IYTtFNiEFRkaFlKiL.c..
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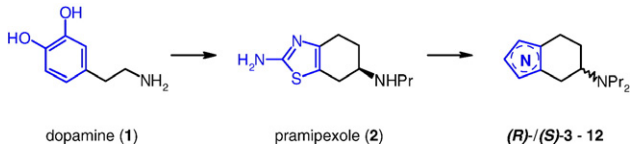


Scheme 1.

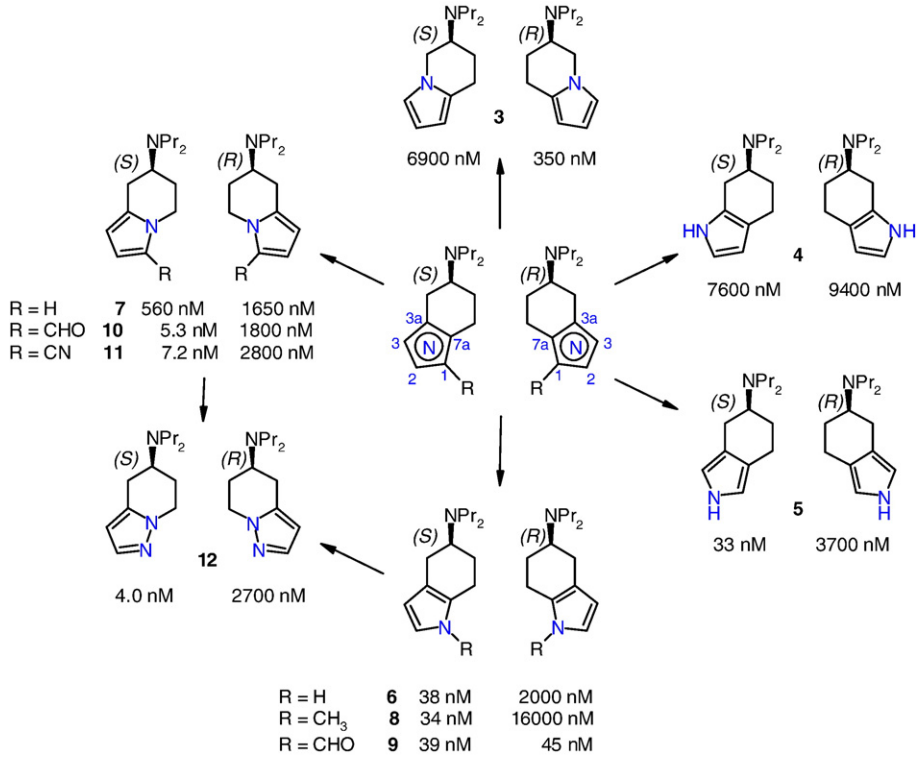
agent for Parkinson's Disease. Being a valuable alternative to the "gold standard" L-Dopa, pramipexole is frequently used as an adjunct therapy to reduce L-Dopa doses in later stages of PD or, particularly, in early-onset patients to delay the begin of L-Dopa therapy. The D_3 affinity of **2** is consistently high (K_i=0.5 to 8.5 nM), but D_3:D_2-selectivity varies from weak (~8-fold) to strong (~193-fold) [19–21] being largely depended on the assay conditions. Measured in mitogenesis assay, the intrinsic activity of pramipexole is ~100% at D_2 and ~80% at D_3 receptors as compared to the effect of quinpirole [21], which is frequently used as a reference for full agonism [22] in this functional assay. The ~8-fold functional selectivity for D_3 (EC_{50}=0.29 nM) versus D_2 receptors (EC_{50}=2.4 nM) is in agreement with previous studies that reported a ~15-fold selectivity [19].

Rationalizing that a pyrrole moiety can act as a catechol bioisostere as well, a novel series of ligands was constructed by diversifying the position of the nitrogen throughout the five-membered aromatic ring. Thus, various regioisomeric azabicyclo[4.3.0]nonanes (Table 1, Scheme 2) were generated including 6- and 7-aminotetrahydroindolizines [23–25], 5-aminotetrahydroisoindoles and 5- and 6-aminotetrahydroindoles [26]. The highest affinity for D_3 among the unsubstituted derivatives (R=H) is found for the 1-aza (K_i=38 nM for (***S***)-**6**) and 2-aza analogs (K_i=33 nM for (***S***)-**5**), showing both considerable eutomer:distomer differences to their (*R*)-enantiomers [26]. In contrast to (***S***)-**5**, a high-affinity binding site for (***S***)-**6** can only be detected at the D_3, but not at the D_2 or D_4 receptor subtypes. Comparing the *N*-methyl (**8**) or *N*-formyl (**9**) with the unsubstituted (*S*)-enantiomer of the 1-aza-series (**6**) indicates that these substitutions hardly affect D_3 receptor binding. It is notable that (***R***)-**9** exhibits similar affinity for the D_3 receptor as (***S***)-**9** giving an eudismic (eutomer:distomer) ratio of ~0.9 and even higher affinities than (***S***)-**9** for the D_2 and D_4 subtypes. Despite the weak binding of the unsubstituted 7a-aza derivative (**7**) to D_3 receptors, introduction of a formyl (**10**) or cyano substituent (**11**) enhances the affinities substantially (K_i=5.3 nM for (***S***)-**10** also known as FAUC 54 and K_i=7.2 nM (***S***)-**11**). Again, (***S***)-**10** and (***S***)-**11** are clearly the eutomers exhibiting substantial eudismic ratios of 0.0029 and 0.0026, respectively. The ~10-fold preference of FAUC 54 for D_3 over D_2 receptors increases by a factor of ~26 for (***S***)-**11**, while the ~6-fold

Table 1
Binding affinities of regioisomeric azabicyclo[4.3.0]nonanes including 6- and 7-aminotetrahydroindolizines (3 and 7), 5-aminotetrahydroisoindoles (5), 5- and 6-aminotetrahydroindoles (6 and 4), 5-aminotetrahydro-pyrazolo[1,5-*a*]pyridines (12) and analogs at D_1, D_2, D_3, and D_4 dopamine receptors

Compound	Nitrogen position [a]	*R* [a]	K_i [nM]				
			bD_1 [b]	hD_2 [c]	$DA_{autorec.}$ [d]	hD_3 [c]	hD_4 [c]
(***S***)-**3** [e]	3a	H	>100,000	>100,000	6900		
(***R***)-**3** [e]	3a	H	>100,000	72,000	350		
(***S***)-**4** [f]	3	H	21,000	27,000		7600	20,000
(***R***)-**4** [f]	3	H	42,000	36,000		9400	14,000
(***S***)-**5** [f]	2	H	24,000	94+11,000 [g]		33+1100 [g]	28+2500 [g]
(***R***)-**5** [f]	2	H	43,000	28,000		3700	5400
(***S***)-**6** [f]	1	H	35,000	12,000		38+1900 [g]	1700
(***R***)-**6** [f]	1	H	28,000	28,000		2000	3000
(***S***)-**7** [h]	7a	H	>100,000	4100	150	560	
(***R***)-**7** [i]	7a	H	>100,000	15,000	1500	1650	
(***S***)-**8** [f]	1	CH_3	16,000	13,000		34+1800	610
(***R***)-**8** [f]	1	CH_3	16,000	20,000		16,000	3100
(***S***)-**9** [j]	1	CHO	>20,000	230+19,000 [g]		39+580 [g]	210+4600 [g]
(***R***)-**9** [j]	1	CHO	>20,000	58+>20,000 [g]		45+2500 [g]	68+5600 [g]
(***S***)-**10** [j]	7a	CHO	>20,000	52+6900 [g]	21 [h]	5.3+150 [g]	32+2500 [g]
(***R***)-**10** [j]	7a	CHO	47,000	21,000	1700 [h]	1800	
(***S***)-**11** [j]	7a	CN	>20,000	190+6500 [g]		7.2+140 [g]	40+9700 [g]
(***R***)-**11** [j]	7a	CN	>20,000			2800	
(***S***)-**12** [k]	1, 7a	H	>20,000	180+15,000 [g]		4.0+110 [g]	58+1700 [g]
(***R***)-**12** [k]	1, 7a	H	>20,000	>20,000		2700	13,000

[a] Corresponding to the depiction in Scheme 2.
[b] Determined with [^{3}H]SCH23390.
[c] Determined with [^{3}H]spiperone.
[d] Determined with the agonist radioligand [^{3}H]pramipexole.
[e] IC_{50} *[nM]* determined in rat brain striatum [23].
[f] D_1 binding determined with porcine D_1 receptors [26].
[g] K_i values of the high and low affinity binding state of the receptor, when analysis of the binding data resulted in a biphasic dose–response curve.
[h] [24].
[i] Hübner and Gmeiner, unpublished results.
[j] [25].
[k] [27].



Scheme 2.

preference over D_4 is maintained. In mitogenesis assay [25], both are full agonists at D_2 receptors, whereas only FAUC 54 shows near full agonism at D_3 (89% relative to quinpirole) and (***S***)-**11** is a partial agonist (59%). The potencies of these ligands (EC_{50}=2.4 nM for (***S***)-**11** and EC_{50}=1.1 nM for FAUC 54) are comparable to quinpirole (EC_{50}=2.6 nM) in this functional assay. At D_4 receptors, FAUC 54 has relatively strong partial agonistic properties (67%), however, a weak potency (4200 nM) and (***S***)-**11** is a pure antagonist.

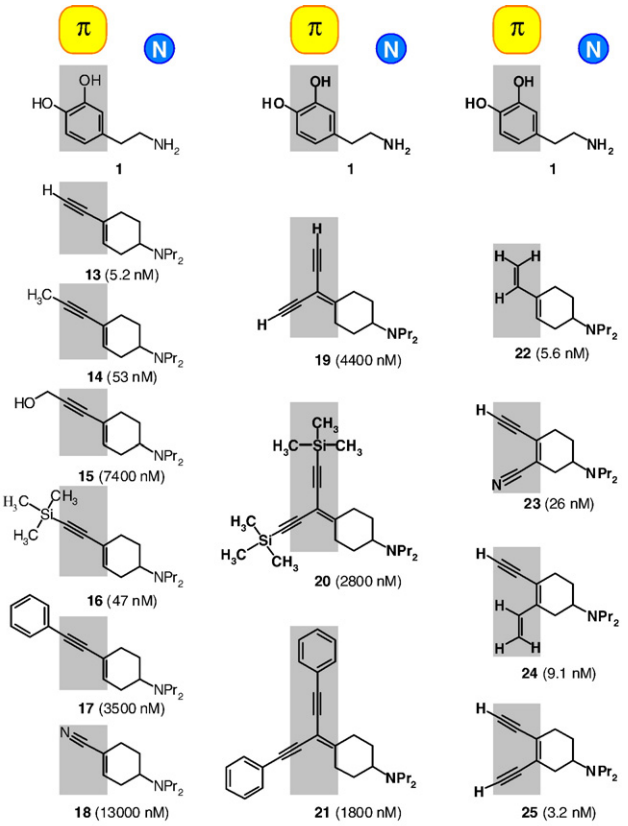
As a very recent extension of this series of ligands, the aminotetrahydropyrazolo[1,5-*a*]pyridine **12** was reported [27], bearing nitrogens in positions 1 and 7a. In this structure, the hydrogen-bond accepting formyl (**10**) or cyano functions (**11**) are truncated to the lone pair of the sp^2-nitrogen. Thus, this structure can be regarded as a hybrid molecule of FAUC 54 (***S***)-**10** and pramipexole (**2**). Similar to FAUC 54, **12** shows substantial D_3 affinity and only weak to moderate receptor binding to D_1, D_2 and D_4. Again, the biological activity and molecular recognition at the D_3 receptor resides exclusively in the (*S*)-enantiomer ($K_{i\ (high)}$=4.0 nM). (***S***)-**12** exhibits high potency (EC_{50}=3.4 nM) and high intrinsic activity (82%) in stimulation of mitogenesis at D_3 receptors.

3.2. Non-aromatic agonists

For the ligands presented in the previous section, the close relation to the natural ligand DA is still easily visible due to containing an aromatic system at a set distance from a protonatable amine. For quite a long time aromatic or heteroaromatic substructures have been regarded as an essential pharmacophore requirement. However, recently several types of non-aromatic, but conjugated π-systems proved to be able to mimic the catechol nucleus of DA. This appears to be even more remarkable, as most of the potent derivatives are lacking any heteroatomic functions, which could putatively substitute for the polar hydroxyl functions of DA. While polar hydroxyls of their equivalents are expected to be involved in receptor binding [28–30], increasing evidence suggests that optimized hydrophobic effects can compensate for the attractive forces resulting from hydrogen bonds (reviewed by [31]).

In the class of conformationally restrained enynes (Scheme 3), the methyl- (**14**) and trimethylsilyl-substituted (**16**) acetylene derivatives exhibit modest D_3 binding (Table 2), while its unsubstituted analog (**13** also known as FAUC 73) has low nanomolar D_3 affinity (5.2 nM) and ~52-fold preference for D_3 over D_2 receptors [32,33]. In contrast to these favorable properties of FAUC 73, several other members of this structural class, such as the hydroxymethyl-substituted (**15**) or the phenyl-substituted acetylenes (**17**), the nitrile analog (**18**) and the whole class of enediynes (**19–21**) are inactive at the D_2-like receptors. Interestingly, none of the ligands with putative hydrogen-bonding properties (e.g. **15** or **18**) shows any significant receptor binding.

Exchanging the acetylene in **13** by a vinyl function produced the diene FAUC 206 (**22**), which exhibits notable D_3 affinity (5.6 nM) and preference over D_2 (41-fold). Additionally, FAUC 206 displays improved preference of 64-fold for D_3 over D_4 receptors [33]. Experimental and computational investigations suggest that the *s-trans*-isomer should be considerably favored. The high affinity of FAUC 206 for D_3 implies that specific hydrogen bond-like interactions of the acetylene proton in



Scheme 3.

FAUC 73 are of no significance in the anticipated receptor-binding mode of these ligands. Subsequent structure activity relationship studies focusing on the enlargement of the π-electronic system, have led to the introduction of a further triple bond yielding conjugated (aza)endiynes [34]. In addition, a vinyl substituent was alternatively introduced giving a structure of the dienyne type (**24**) [35]. D_3 affinity is improved by the different substituents in the order: cyano (K_i=26 nM for **23**)<vinyl (K_i=9.1 nM for **24**)<ethynyl (K_i=3.2 nM for **25**). In fact, the *cis*-hexendiyne functionality of FAUC 88 (**25**) is superior to the butenyne moiety of the lead compound FAUC 73 (**13**) making it the most potent non-aromatic dopamine receptor ligand yet investigated (Table 2). From a comparison of the molecular electrostatic potentials (Fig. 2), it becomes obvious that the extended conjugated π-system of FAUC 88 is able to mimic efficiently the molecular electrostatic potential (MEP) of the α-rotamer of dopamine.

FAUC 88 is a mixed D_3/D_4 agonist with only ~2-fold preference for D_3 over D_4, but ~29-fold preference over D_2. Like FAUC 73, FAUC 88 does not exhibit significant binding to 5-HT_{1A} (K_i=1000 nM for FAUC 73 and K_i=150 nM for FAUC 88 using the radioligand [^{3}H]8-OH-DPAT) or 5-HT_2 receptors (K_i=9000 nM for FAUC 73 and K_i=3100 nM for FAUC 88 using the radioligand [^{3}H]ketanserin). Moreover, both ligands have substantial ligand efficacy (>85% for both at D_{2L}, 72% for FAUC 88 and 74% for FAUC 73 at D_3, and >60% for both at $D_{4.2}$) relative to the maximal effect of quinpirole as determined in mitogenesis assays. Consistent with affinity values from binding experiments, the potency of FAUC 88 (EC_{50}=3.2 nM) indicates that it has a superior activity profile compared to FAUC 73 (EC_{50}=4.4 nM).

The high affinities and potencies of compounds presented in this section indicate that aromatic or heteroaromatic systems, although being commonly present in DA agonists, appear to be not essential for molecular recognition or intrinsic activity at the D_3 receptor. Thus, this uncommon ("fancy") bioisosteric replacement of the aromatic by conjugated, but non-aromatic π-systems has proven to be a powerful strategy for the generation of preferential D_3 agonists and could be further exploited for probing the nature of ligand–receptor complexation between π-systems.

3.3. 4-Phenylpiperazines and analogs with partial agonistic and antagonistic properties

During the last decade, 4-phenylpiperazine has been recognized as a "privileged structure" for biogenic amine receptors. In particular with respect to the application as potent and selective D_3 ligands, the class of 4-phenylpiperazines and its close derivatives have become quite popular. Basically, most of these ligands comprise the following structural features (Scheme 4): An aromatic or heteroaromatic carboxamide (π_1) that is connected through a conformationally flexible or (partly) rigidized alkyl-spacer of variable length (*spacer*) to a piperazine bearing an aromatic or heteroaromatic moiety in position 4 (π_2).

Table 2
Binding affinities of non-aromatic D_3 ligands including (aza)enynes (**13–18**), endiynes (**19–21**), a diene (**22**), a dienyne (**24**) and (aza)endiynes (**23** and **25**) at bD_1, hD_{2L}, hD_3, and $hD_{4.4}$ dopamine receptors

Compound	K_i [nM]				Ratio	
	bD_1 [a]	hD_{2L} [b]	hD_3 [b]	$hD_{4.4}$ [b]	D_2/D_3	D_4/D_3
13 [c,d]	>20,000	270+14,000 [e]	5.2+590 [e]	22+380 [e]	52	4.2
14 [f]	44,000 [g]	110+16,000	53+2600	4100	2.1	(77)
15 [f]	38,000 [g]	16,000	7400	2200	2.2	0.30
16 [c]	>20,000	160+>20,000	47+1600	160+3800	3.4	3.4
17 [c]	>20,000	15,000	3500	16,000	4.3	4.6
18 [c]	>20,000	>20,000	13,000	>20,000	>1.5	>1.5
19 [c]	16,000	12,000	4400	3900	2.7	0.89
20 [c]	3900	11,000	2800	1900	3.9	0.68
21 [c]	2200	5200	1800	1400	2.9	0.27
22 [f,h]	40,000 [g]	230+12,000	5.6+430	360+3100	41	64
23 [i]	21,000 [g]	42+5300	26+910	77+5600	1.6	3.0
24 [j]	26,000 [g]	260+9700	9.1+500	250+6100	29	27
25 [i,k]	12,000 [g]	94+10,000	3.2+49	6.3+420	29	2.0

[a] Determined with [^{3}H]SCH23390.
[b] Determined with [^{3}H]spiperone.
[c] [32].
[d] Ste
[e] Ki_{high} and Ki_{low} values derived from a biphasic curve, if data analysis fitted better with the equations for a two-site binding mode.
[f] [33].
[g] Determined using porcine D_1 receptors (pD_1), instead of bovine (bD_1).
[h] FAUC 206.
[i] [34].
[j] [35].
[k] FAUC 88.

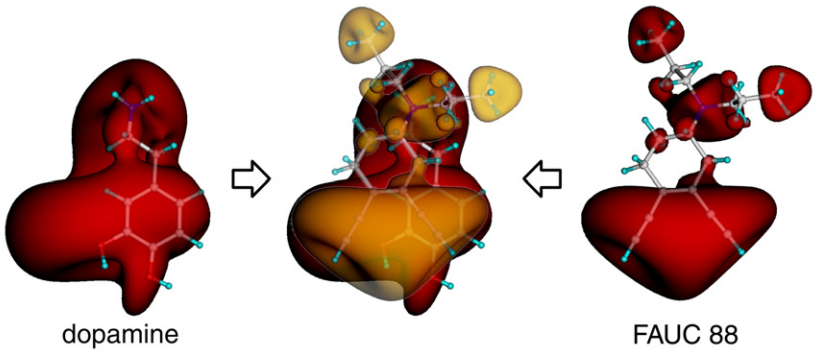


Fig. 2. Isopotential surfaces of dopamine and FAUC 88 contouring negative molecular electrostatic potentials (MEP; −1.0 kcal/mol). To facilitate easier comparison both molecules are directly superimposed fitting the protonatable amine as their common interaction point.

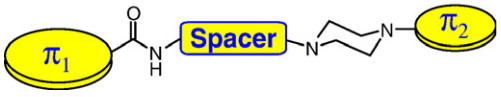
Across different laboratories diverse in vitro binding affinities and functional potencies have been reported involving some ligands cited in this section. These differences appear to concern predominantly the measurement of D_2 receptor affinities, whereas D_3 affinities are found to be rather consistent. However, D_3:D_2 selectivity ratios are of course affected. Possible underlying reasons for these discrepancies have been recently discussed [36].

3.3.1. Modifications of the π_1 moiety

The 2-methoxyphenylpiperazines GR 103691 (**26**) and BP 897 (**27**), as well as the 2,3-dichlorophenylpiperazine NGB 2904 (**28**) can be regarded as early lead structures of this family of ligands (Scheme 5). The 4′-acetyl-biphenyl-4-carboxamide GR 103691 shows subnanomolar D_3 affinity (0.32 nM) and ~130-fold selectivity for D_3 versus D_2 receptors [37]. Although the selectivities against the other DA receptors subtypes are substantial (e.g. ~1300-fold over D_1), distinct affinities for 5-HT_{1A} (K_i=3.2 nM) and α_1 receptors (K_i=13 nM) have been reported. However, replacement of the 4′-acetyl with a 4′-methylsulphone or a 4′-amino group improves the D_3:5-HT_{1A} selectivity.

Variation of the arylcarbamide moiety yielded the 9*H*-fluoren-3-carboxamide **28** (NGB 2904), a tricyclic D_3 receptor antagonist [38]. High D_3 affinity (K_i=1.4 nM), a >160-fold selectivity for D_3 versus all other DA receptor subtypes and potent antagonism in mitogenesis assays (EC_{50}=5.0 nM) has been reported for **28**. More recently, the 9*H*-fluorene-9-carboxamide **29**, a less linear regioisomer of **28**, has been synthesized, but showed only a moderate and nonselective binding profile (K_i=19 nM at D_3 and K_i=10 nM at D_2) [39].

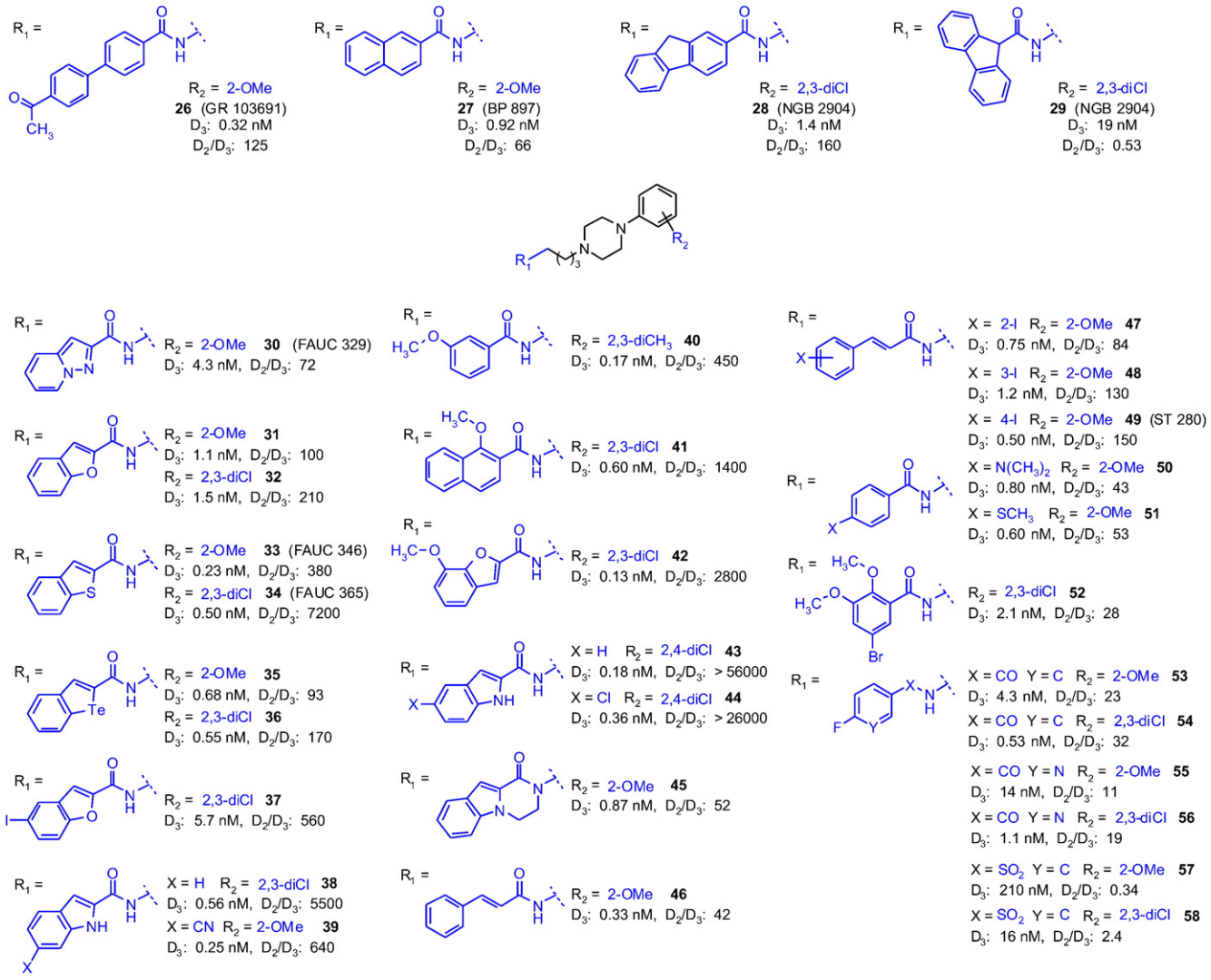
Particular interest in this ligand family has been sparked by the discovery that the naphthamide derivative BP 897 (**27**) can reduce cocaine-seeking behavior in rats, while it does not produce reinforcement on its own [9,40]. BP 897 is a high affinity D_3 receptor ligand (K_i=0.92 nM) with proper selectivity



Scheme 4.

(~66-fold) over D_2 receptors, but also moderate affinity for 5-HT_{1A} (84 nM), α_1 (60 nM) and α_2 receptors (83 nM). The affinities for 5-HT_7, histamine, muscarinic and opiate receptors have been found to be very low or even negligible. Other investigators [41] report its D_3 affinity and selectivity over D_2 to be slightly weaker (1.6 nM; ~38-fold). BP 897 is a partial agonist at the human D_3 receptor measured by the decrease in forskolin-induced cAMP synthesis (max. effect relative to quinpirole=59% and EC_{50}=1 nM) or by stimulating mitogenesis (max. effect=55% and EC_{50}=3 nM) [9,42]. However, in more recent studies BP 897 has been characterized as an antagonist, as it potently (pK_b=9.43) inhibits the acidification response of quinpirole, while alone it has no effect [41]. Moreover, BP 897 does not modify the basal levels of [^{35}S]GTPγS, whereas the ligand dose-dependently (IC_{50}=0.31 nM) inhibits the effects of DA [43]. Mitogenesis as well as microphysiometry experiments indicate that BP 897 is an antagonist at D_2 receptors [9,41].

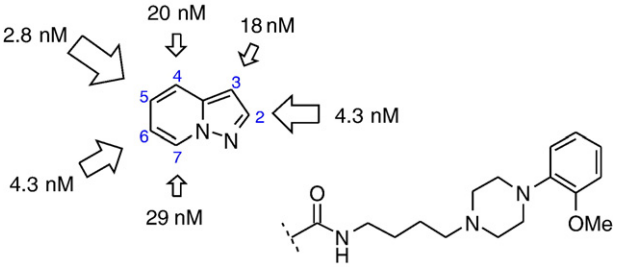
Based on the lead structures of BP 897 and NGB 2904, more selective D_3 partial agonists and antagonist have been discovered. As the pyrazolo-[1,5-*a*]pyridine moiety acts as a heterocyclic bioisostere of the naphthamide substructure of BP 897, it provides an excellent opportunity for the fine-tuning of selectivity and intrinsic efficacy [44]. Exploiting all possible attachment points of the pyrazolo-[1,5-*a*]pyridine scaffold, six regioisomeric carboxamides connected to a *o*-methoxyphenyl-piperazine through a butyl chain linker have been obtained (Scheme 6). The resulting D_3 affinities (2.8 to 29 nM) increase in the order of the attachment points at the various positions: 5>2=6>3>4>7. The pyrazolo-[1,5-*a*]pyridine-2-carboxamide (**30** also known as FAUC 329) exhibits the highest selectivity versus D_2 receptors (~72-fold) in this series and binds with nanomolar affinity (4.3 nM) to D_3 receptors. FAUC 329 has partial agonist activity (52% compared to quinpirole) and low nanomolar potency (EC_{50}=1.4 nM) in mitogenesis assays. Throughout this whole series of regioisomers only moderate affinity is observed for 5-HT_{1A} and α_1 receptors. FAUC 329 exerts neuroprotective effects in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease, as it dose-dependently attenuates MPTP-induced DA reduction in the nucleus accumbens [12]. Moreover, FAUC 329 is able to protect in part against DA depletion



Scheme 5.

in the dorsal striatum and against loss of DA transporter immunoreactivity in the substantia nigra pars compacta.

A tetramethylene spacer, as compared to the penta-, tri- or dimethylene spacer, yields superior D_3 affinities and selectivities versus D_2 receptors. These data reinforce findings obtained during early stages of structure–activity relationship investigations in the class of 4-phenylpiperazines. Replacement of the pyrazolopyridine moiety by a benzo[*b*]furan (**31** and **32**), benzo[*b*]thiophene (**33** also known as FAUC 346 and **34** also known as FAUC 365) or a benzo[*b*]tellurophene ring system (**35** and **36**) results in a marked increase in D_3 binding (K_i=0.23 nM to 1.5 nM) [44]. What is more, the selectivities for D_3 over D_2 receptors are considerably enhanced. Thus, the benzo[*b*]thiophene FAUC 365 is one of most selective D_3 antagonists, reported to date. Interestingly, only the *o*-methoxyphenyl-derivatives (**31**, **33**, **35**) exhibit notable affinities at 5-HT_{1A} and α_1 receptors, whereas the 2,3-dichlorophenyl-derivatives (**32**, **34**, **36**) display reduced 5-HT_{1A} binding and are almost inactive at 5-HT_2 or α_1 receptors. Except for the *o*-methoxyphenyl-analogs **31** and **33**, all other derivatives (**32**, **34–36**) are full antagonists in mitogenesis experiments. Thus, FAUC 346 (**33**) is a high affinity (K_i=0.23 nM), superpotent (EC_{50}=0.36 nM) and highly selective D_3 partial agonist (~50% maximal intrinsic activity), while FAUC 365 (**34**) is a full antagonist with subnanomolar affinity (K_i=0.50 nM) and extraordinary subtype selectivity (~7200-fold vs. D_2). While other laboratories have been able to corroborate the high D_3 affinities of these two ligands (**33** and **34**), interesting differences in D_2 receptor binding have been reported [45–47]. Similar measuring inconsistencies have also



Scheme 6.

been observed for several other ligands (see also the short discussion at the beginning of Section 3.3). In order to develop suitable radiopharmaceuticals for investigating the CNS located dopamine D_3 receptors in vivo, 5-iodo derivatives of the benzo[*b*]furan and the benzo[*b*]thiophene have been synthesized as new [^{131}I]-labeled SPECT (single photon emission computed tomography) ligands [48]. The most beneficial combination of D_3 affinity (5.7 nM) and selectivity over D_2 (~560-fold) is ascribed to the 5-iodo-benzo[*b*]furan containing a 2,3-dichlorophenylpiperazine (**37**).

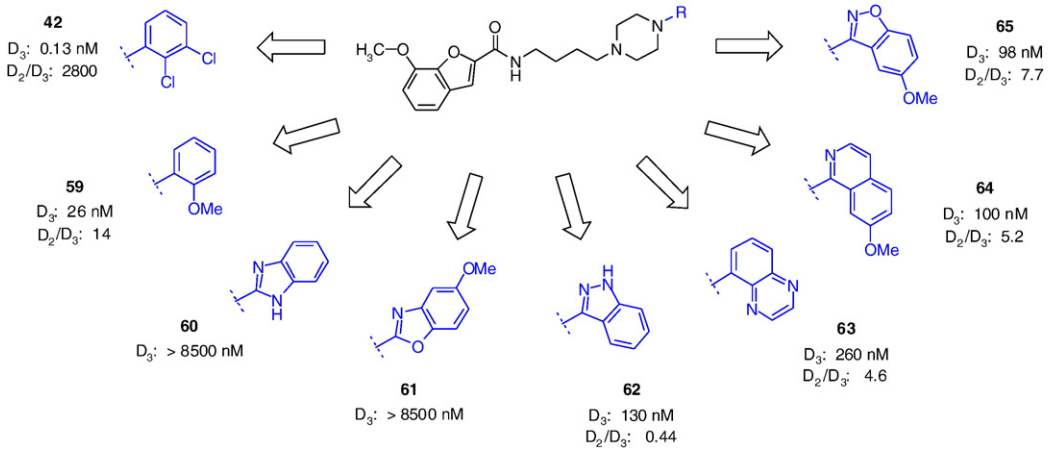
Two other very interesting members of these series of heteroaromatic bioisosteres of BP 897 and NGB 2904, the indol-2-carboxamide (**38**) and 6-cyano-indol-2-carboxamide (**39**), have been disclosed in the patent literature [49]. Both have subnanomolar D_3 affinity (K_i=0.56 nM for **38** and K_i=0.25 nM for **39**) and high selectivity versus D_2 receptors (~5500-fold for **38** and ~640-fold for **39**). The indole carboxamide **38** also is more than 1000-fold selective over D_1, D_2, D_4, 5-HT_{1A} and α_1 receptors.

Structure–activity studies starting from D_4 selective ligands have also yielded **32**, **34** and **38**, which all have high D_3 affinity and selectivity over other related receptors [45]. Variation of the spacer length again produced the rank order: butyl>pentyl>propyl>ethyl, which is in agreement with previous results regarding the optimal spacer length. Variations of the π_2 moiety for the 3-methoxyphenylcarboxamides revealed that the replacement of the commonly used 2,3-dichlorophenyl by a 2,3-dimethylphenyl group (**40**) maintains D_3 affinity and increases selectivity to at least 300-fold over D_4, 5-HT_{1A} and α_1 receptors. However, the selectivity over D_2 receptors is attenuated from 5200-fold for the 2,3-dichlorophenyl to 450-fold for the 2,3-dimethylphenyl derivative. Other methoxy-substituted compounds obtained by variation of π_1 are the 1-methoxy-2-naphthamide (**41**) and the 7-methoxybenzo[*b*]furan-2-carboxamide analog (**42**), which exhibit both subnanomolar D_3 affinities (K_i=0.60 nM for **41** and K_i=0.13 nM for **42**) and substantial selectivities over D_2, D_4, 5-HT_{1A} and α_1 (>950-fold for **41** and >840-fold for **42**). It should be noted that in this preceding study dopamine receptor subtypes from different species were compared (human D_2, rat D_3, and human $D_{4.4}$) and, thus, it has been argued that selectivities might be influenced by species differences [36]. **41** and **42** have been recently evaluated as [^{11}C]-labeled PET ligands [50].

A further series of heteroaromatic BP 897/NGB 2904 bioisosteres has been generated, yielding compounds, such as **43**–**45** [51]. The 2-indolcarboxamide **43** is closely related to **38**, except that the 2,4-dichlorophenyl replaces the 2,3-dichlorophenyl. The 5-chloro-indole-2-carboxamide derivative **44** shows almost the same binding profile as **43**, but **44** acts as a partial agonist in [^{35}S]GTPγS assays, whereas **43** is an antagonist. For **43** a partial reduction of cocaine-seeking behavior has been observed in rats, while **44** fails to show any effect in this animal model. Interestingly, the 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole-1(2*H*)-one **45** retains high affinity for D_3 (0.87 nM), even though it lacks both the indole-NH and the carboxamide-NH hydrogen bonding capacity. However, the selectivity for D_3 over other receptors was reduced for **45** (~52-fold over D_2, ~38-fold over α_1, and ~5.6-fold over 5-HT_{1A}). Hence, the hydrogen bond donor function of the carboxamides may have an impact on subtype selectivity, but certainly not on D_3 receptor recognition.

A new series of *o*-methoxyphenylpiperazine analogs has been developed by replacing the naphthamide in BP 897 with (*E*)-cinnamide derivatives [52]. The iodo-substituted analogs **47**–**49** possess increased selectivity for D_3 over D_2 receptors in comparison to the unsubstituted (*E*)-cinnamide **46**, while they maintain high D_3 affinity. The selectivity increases from 42-fold (**46**) to 84-fold for the 2-iodo-cinnamide (**47**), 130-fold for the 3-iodo-cinnamide (**48**) and 150-fold for the 4-iodo-cinnamide (**49**). The 4-iodo-cinnamide (**49** also known as ST 280) exhibits the best pharmacological profile in this series and thus has been ascribed to be a promising radioligand candidate. Exploiting the benzamide structure and some heteroaromatic bioisosteres in further structural modifications of the π_1 moiety has led to the 4-dimethylaminobenzamide derivative **50**, the 4-methylsulfanylbenzamide **51** and the 5-bromo-2,3-dimethoxybenzamide **52** [47,53]. All three ligands feature high affinity binding to D_3 receptors (K_i=0.8 nM for **50**, K_i=0.6 nM for **51**, and K_i=2.1 nM for **52**) and a moderate preference for D_3 versus D_2 receptors (43-fold for **50**, 53-fold for **51**, and 28-fold for **52**). While **52** has been reported to bind with appreciable affinity at σ_1 (809 nM) and σ_2 receptors (75 nM), **50** and **51** both show considerable affinity for 5-HT_{1A} receptors (K_i=6.0 nM for **50** and K_i=1.5 nM for **51**). Measuring the inhibition of forskolin-induced adenylyl cyclase activity, **50** has been determined to be a weak partial agonist (21% of the maximal effect).

As the development of potent and selective positron emission tomography (PET) tracers for D_3 receptors has been deemed an important step to investigate the role of this receptor subtype in the pathophysiology of numerous diseases, a series of [^{18}F]-labeled PET tracers has been synthesized taking advantage of 4-bromophenyl carboxamide as a lead structure [54]. When combined with 2-methoxyphenyl as π_2 moiety, the resulting 4-fluorophenyl carboxamide **53** and 6-fluoropyridin-3-yl carboxamide **55** exhibit nanomolar D_3 affinities (4.3 nM for **53** and 14 nM for **55**), while in combination with 2,3-dichlorophenyl the D_3 affinities are further increased (0.53 nM for the 4-fluorophenyl carboxamide **54** and 1.1 nM for the 6-fluoropyridin-3-yl carboxamide **56**). All of these ligands share a moderate preference for D_3 over D_2 receptors (11 to 32-fold), whereas the 2,3-dichloro substitution pattern clearly enhances the selectivity for D_3 over D_4 (83-fold for **54** and 91-fold for **56**), 5-HT_{1A} (110-fold for **54** and 25-fold for **56**) and α_1 (32-fold for **54** and 15-fold for **56**) at least by a factor of 10 as compared to the 2-methoxy derivatives **53** and **55**, respectively. For the 6-fluoropyridin-3-yl carboxamides **55** and **56** a significantly higher radiochemical yield (RCY) of >80% has been reported. Exchange of the carboxamide in **53** and **54** in the respective sulfonamides **57** and **58** gives a most interesting insight into the nature of ligand receptor recognition. Upon this modification the D_3 affinity is substantially impaired (49-fold for **57** and 30-fold for **58**), while the D_2 affinity is approximately preserved and the D_4 affinity is even slightly improved. This suggests that an extended planar π system might contribute



Scheme 7.

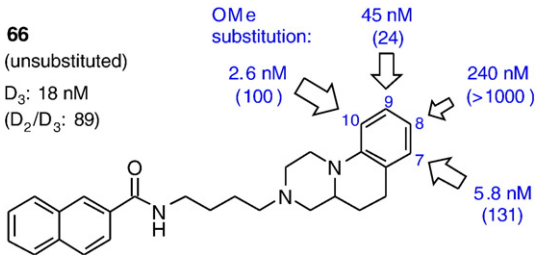
more to efficient D_3 receptor binding, than hydrogen bonding capabilities of the carboxamide or sulfonamide can do.

3.3.2. Modifications of the π_2 moiety

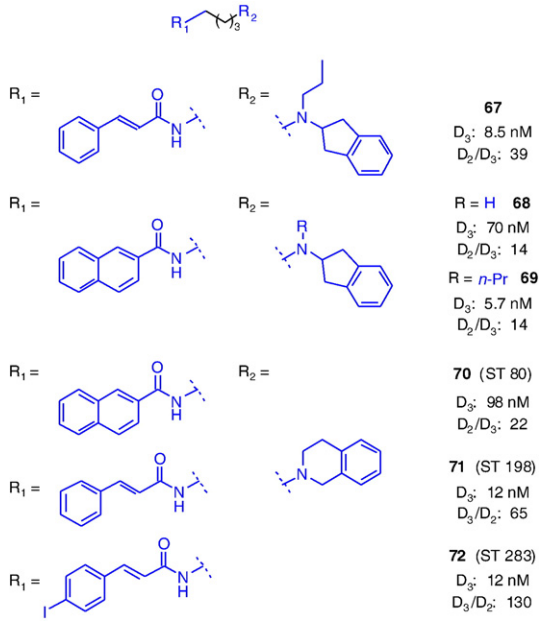
As a balanced lipophilicity is know to be crucial for drug bioavailability and permeation of the blood–brain barrier, tuning of the calculated logP (clogP) by evaluating different π_2 moieties has been performed in a recent study aiming to identify potential PET radioligands [55]. Based on the 7-methoxybenzo[*b*]furan-2-carboxamide derivative **42** bearing a 2,3-dichloro substituted phenylpiperazine moiety, alternative (hetero)aromatic systems have been evaluated (**59–65**, Scheme 7). Replacement of the 2,3-dichloro substitution pattern by a 2-methoxy substituent (**59**) leads to a 200-fold attenuated D_3 affinity and selectivity exceeding the regular reduction frequently observed upon this replacement. Linear prolongation of the π-system by exchange of the phenyl into a 2-benzoimidazolyl (**60**) or a 5-methoxy-2-benzoxazolyl moiety (**61**) has a fairly detrimental effect on ligand recognition by the D_3 receptor (K_i > 8500 nM). Lateral prolongation of π_2 by introduction of a 3-indazolyl (**62**), 5-quinoxalinyl (**63**), 7-methoxy-1-isoquinolinyl (**64**) or 5-methoxy-2-benzisoxazolyl (**65**) replacing the phenyl moiety yields moderate D_3 binding (98 nM–260 nM) for all resulting ligands **62–65** and some D_3 versus D_2 preference for **63–65** (4.6-fold to 7.7-fold). A broader comparison between 2-methoxyphenyl and 5-methoxy-2-benzisoxazolyl as π_2 moieties with similar lipophilicity (clogP = 3.30 or 3.42, respectively) involving 7 different carboxamides reveals that the 2-methoxyphenyl derivatives show higher D_3 affinity, but tend to be less selective over D_2 receptors.

Trying to increase the chemical diversity in D_3 ligand design, a computational 3D database screening strategy has helped to identify the hexahydropyrazinoquinoline as a rigidized replacement of the phenylpiperazine moiety [17]. The naphthamide derivative **66** (Scheme 8) binds with a K_i value of 18 nM to D_3 receptors and shows 87-fold selectivity over D_2-like receptors (measured in rat brain homogenate using [^{3}H]spiperone), as well as 44-fold selectivity over D_1-like receptors (measured in rat brain homogenate using [^{3}H]SCH23390) [56]. Based on the hypothesis that introduction of a methoxy function attached to positions 7 to 10 of the hexahydropyrazinoquinoline core might be able to form hydrogen bonds toward the conserved serine residues in TM5, respective structural variations of **66** have been performed (Scheme 8). Methoxy functions in position 7 or 10 are able to increase both D_3 affinity, as well as selectivity over D_2 receptors, whereas a methoxy substituent in position 9 impairs both affinity and selectivity. Interestingly, when introducing a methoxy group in position 8, the D_3 affinity is considerably decreased, however, the selectivity over D_2 receptors increases significantly indicating that this modification is far more detrimental for D_2 than for D_3 receptor binding.

Going beyond modifications of π_2, in a recent study the entire phenylpiperazine has been structurally reduced to the essential requirements of a basic nitrogen connected to an aryl group through an aliphatic linker [57]. Higher degrees of rigidity with varying geometry and hydrogen-bonding capabilities were introduced to diversify this phenylalkylamine scaffold. Despite the fact that it has turned out to be not trivial to find a valid bioisostere for the "privileged structure" phenylpiperazine, two scaffolds have been retrieved in this study: the 2-aminoindan and the 1,2,3,4-tetrahydroisoquinoline (Scheme 9). Combining (*E*)-cinnamoylcarboxamide with the N-*n*-propyl-2-aminoindan results in a nanomolar D_3 ligand (**67**: K_i = 8.5 nM) showing a 39-fold preference for D_3 over D_2 receptors. Exchange to a naphthylamide (**69**) yields slightly increased D_3 affinity (K_i = 5.7 nM), but at the expense of a reduced preference over D_2 (selectivity 14-fold). The corresponding secondary amine (**68**) shows a marked decrease in D_2 and D_3 affinity by a factor of



Scheme 8.



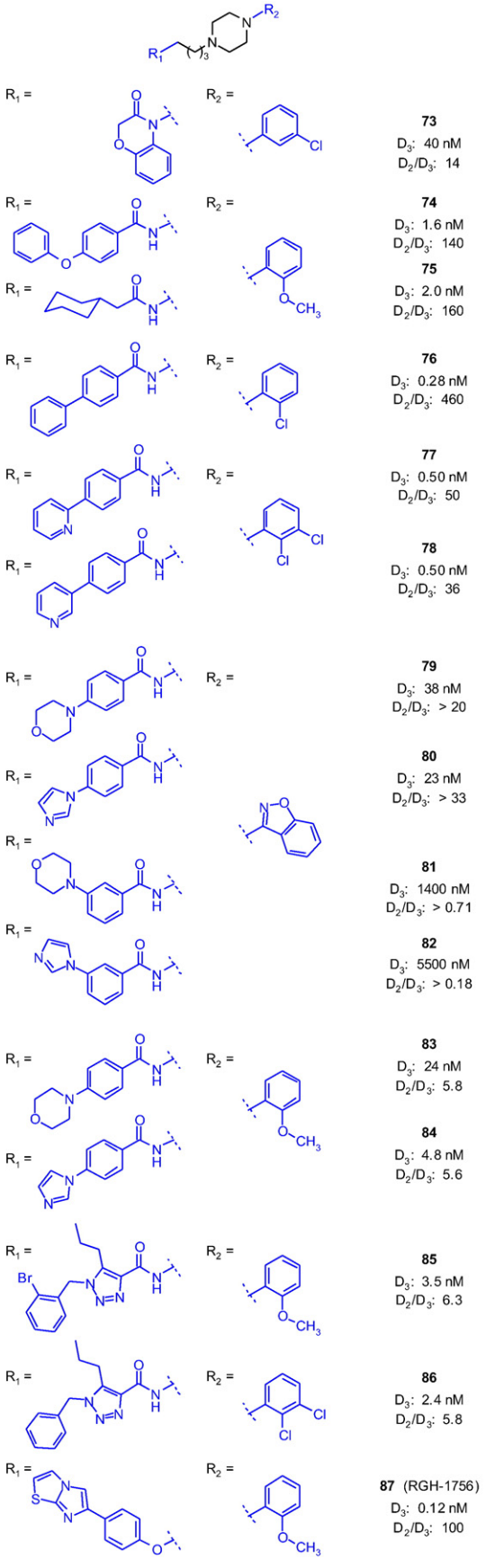
Scheme 9.

13 and 12, respectively, when compared to **69**. Combination of a naphthamide moiety with 1,2,3,4-tetrahydroisoquinoline as the second identified scaffold yields ST 80 (**70**). Replacement of the naphthamide moiety by cinnamide improves the moderate binding profile of **70** (K_i=98 nM at D_3 and K_i=2200 nM at D_2) resulting in a K_i of 12 nM at D_3 receptors for **71** (ST 198) and also an increased 65-fold D_3:D_2-selectivity ratio. Furthermore, ST 198 displays >400-fold selectivity over the other dopamine receptor subtypes and is devoid of intrinsic activity in mitogenesis experiments [8]. ST 198 has been employed in pharmacological studies investigating dopamine autoreceptors in guinea pigs [58] and exhibits attenuation of L-DOPA-induced dyskinesias in monkeys simultaneous with a deterioration of PD-like symptoms and ablation of locomotor activity below the 'on-time' threshold [8]. Iodination of the *para*-position of the (*E*)-cinnamide leading to the (*E*)-3-(4-iodophenyl)acryl-derivative **72** (ST 283) preserves D_3 binding (12 nM) and doubles its selectivity over D_2 receptors (130-fold). ST 283 has been suggested as a useful D_3-selective radioligand with putative applicability in single-photon emission computed tomography (SPECT) [57].

3.3.3. Further extensions of the π_1 moiety

Using a ligand-based virtual screening approach, a ligand (**73**) comprising a shortened π_1 moiety attached to a 3-chlorophenylpiperazine though a *n*-butylene spacer was identified [59]. In the 3-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl (π_1) moiety the aromatic ring is shifted to a lateral position and thus should fail to mimic the interactions of the "regular" (hetero-) aromatic substructure (Scheme 10). However, **73** was demonstrated to retain still a D_3 affinity of 40 nM, as well as a 14-fold preference over D_2. Therefore, **73** may indeed be a novel lead structure representing a distinct mode of interaction.

In contrast to this "truncation" of the π_1 moiety in **73**, considerably more efforts have been made to extend it similar to



Scheme 10.

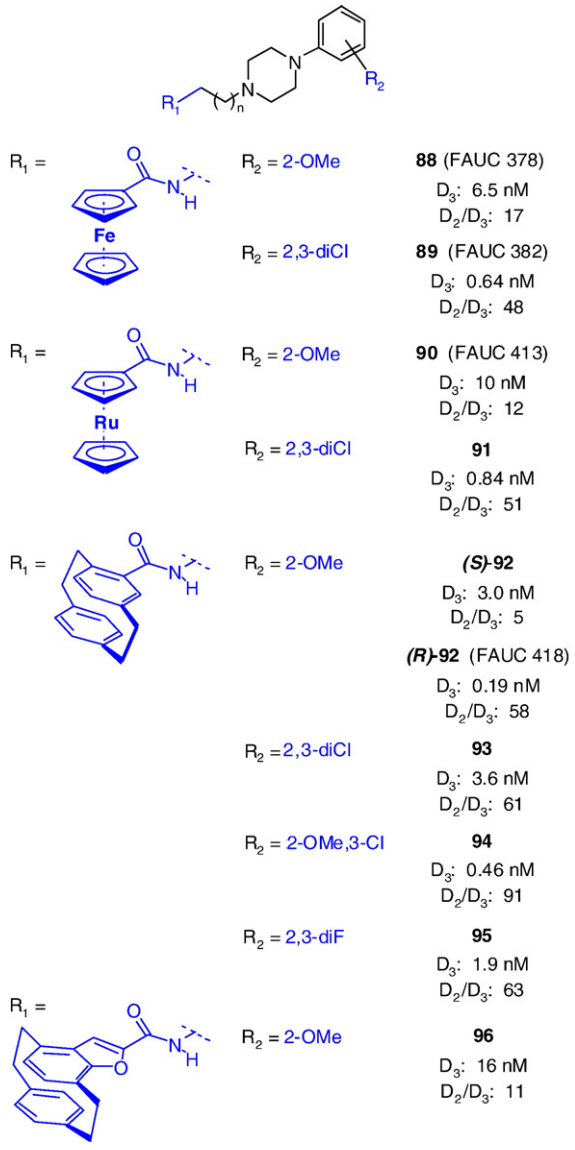
the enlarged aromatic systems of GR 103691 (**26**) or NGB 2904 (**28**). Employing a parallel derivatization method the 4-phenoxyphenyl derivative **74** and the 1-cyclohexylmethyl derivative **75** have been obtained [60]. Both **74** and **75** exhibit low nanomolar affinity to D_3 receptors (1.6 nM and 2.0 nM, respectively) and a good selectivity over D_2 receptors (140-fold and 160-fold, respectively). Taking advantage of a click chemistry based BAL linker, a solid phase supported parallel synthesis of a focused library of arylcarboxamides has led to the biphenyl-4-carboxamide **76**, which is attached to a 2-chlorophenylpiperazine moiety by a tetramethylene spacer [61]. This compound has subnanomolar affinity for D_3 (0.28 nM), a pronounced D_3:D_2-selectivity (460-fold) and it is more than 850-fold selective over other dopamine receptors. Although having just a moderate selectivity for D_3 over α_1 receptors (~39-fold), **76** shows the best receptor binding profile of this focused library. Upon introduction of an aza function into the terminal benzene ring the resulting 4-(2-pyridinyl)-phenylcarboxamide (**77**) and 4-(3-pyridinyl)-phenylcarboxamide derivative (**78**) maintain both subnanomolar D_3 receptor binding (K_i=0.50 nM each) [46]. With a 50-fold preference over D_2 receptors **77** only slightly exceeds the 36-fold D_3:D_2 preference of its regioisomer **78**. In contrast to **76**, both ligands bear a 2,3-dichloro substitution instead of a 2-chloro substituent at the phenyl ring. Further heterocyclic and heteroaromatic analogs of the biphenyl carboxamide have been prepared attempting to identify potent derivatives with improved lipophilicity [55]. In a series containing 1,2-benzisoxazolyl as π_2 moiety, the 4-(4-morpholinyl)benzamide **79** and the 4-(1-imidazolyl)benzamide **80** have been prepared as heterocyclic and heteroaromatic variations. Both ligands show moderate affinity at D_3 receptors (K_i=38 nM for **79** and K_i=23 nM for **80**), as well as moderate preference of D_3 over D_2 receptors (>20-fold for **79** and >33-fold for **80**). However, when comparing these ligands with their bend isomers, the 3-(4-morpholinyl)benzamide **81** and the 3-(1-imidazolyl)benzamide **82**, it is quite obvious that deviations from a linear arrangement have substantial impact on affinity and selectivity. For **81** the D_3 affinity is decreased by a factor of 37 to a K_i of 1400 nM, while for **82** even a 240-fold reduction of D_3 affinity to 5500 nM is found. At the same time the moderate preference for D_3 over D_2 is lost at all in **81** and **82**. The 2-methoxyphenyl derivatives (**83** and **84**) show higher D_3 affinities than their 1,2-benzisoxazolyl counterparts **79** and **80**, but their D_3:D_2 preference is attenuated. Thus, **84** is a nanomolar (K_i=4.8 nM), but only weakly preferential D_3 ligand (5.6-fold vs. D_2).

Employing click chemistry-based 1,3-dipolar cycloaddition to synthesize a focused library of *N*-benzyl-1,2,3-triazole carboxamides has yielded a series of superior picomolar α_1 ligands. Some of these show also high D_3 affinity. For instance the 1-(2-bromo)benzyl-5-propyl-1,2,3-triazole-4-carboxamide **85** binds with low nanomolar affinity to D_3 receptors (K_i=3.5 nM) and also with subnanomolar affinity (K_i=0.15 nM) to α_1 receptors. This very high α_1 affinity appears to be related to the presence of a 2-methoxyphenylpiperazine moiety, as this picomolar affinity is strongly impaired when changing to a 2,3-dichlorophenylpiperazine. The 1-benzyl-5-propyl-1,2,3-triazole-4-carboxamide derivative **86** for example bears a 2,3-dichloro substitution pattern at the phenylpiperazine and binds with only 5.5 nM to α_1, while it preserves a low nanomolar binding to D_3 receptors (K_i=2.4 nM).

Aiming to develop new atypical antipsychotics, RGH-1756 (**87**) has emerged from a series of 2-methoxyphenylpiperazines [62,63]. Although it is rather uncommon in D_3 receptor ligands, the extended π_1 moiety consisting of a 4-(6-imidazo[2,1-*b*]thiazolyl)phenyl partial structure is attached to the butylene spacer by an ether group instead of an amide, thus enlarging the flexible spacer and shortening the conjugated π_1-system on this side. However, despite of the missing carboxamide the linear extension of the π_1-system to the other side yields a subnanomolar D_3 ligand (K_i=0.12 nM) with high affinity at 5-HT_{1A} (K_i=0.96 nM) and α_{2C} receptors (K_i=4.0 nM) and a selectivity of at least 91-fold over α_{2A}, D_{2L}, 5-HT_7, D_{2S}, D_5 and D_1 receptors [64]. In GTPγS binding assay **87** inhibits the stimulatory effect of dopamine at human D_3 receptors with nanomolar potency (IC_{50}=8.5 nM) [65]. RGH-1756 has been radiolabeled using [^{11}C]methyl triflate [66] and applied to cynomolgus monkeys for PET imaging of the monkey brain [64]. In a recent study on spatial learning performance of rats in a water labyrinth test, RGH-1756 together with other D_3 antagonists improved FG7142-induced learning deficits and scopolamine-induced amnesia [67]. Thus, it has been concluded that the cognition-enhancing effect of D_3 antagonists may be beneficial in the treatment of cognitive dysfunction associated with several psychiatric disorders.

3.3.4. 3-Dimensional extensions of the π_1 moiety

Proceeding beyond these two dimensional variations, the thin, "single-layer" π_1 system has very recently been extended to the third dimension by introducing metallocene carboxamides (Scheme 11) [49,68]. As these bilayered aromatic systems are sterically quite demanding (Fig. 3), the high D_3 affinities found for the ferrocene derivative **89** (FAUC 382; K_i=0.64 nM) and the ruthenocene derivative **91** (K_i=0.84 nM) indicate that the binding site of the D_3 receptor tolerates rather bulky systems such as these metallocene carboxamides quite well. Both compounds also show high affinity for the D_4 receptors (K_i=0.63 nM for **89** and K_i=0.60 nM for **91**) and their selectivities for D_3 and D_4 over D_2 receptors are moderate (D_3:D_2 selectivity=~48-fold for **89** and ~51-fold for **91**, D_4:D_2 selectivity=~49-fold for **89** and ~72-fold for **91**). Moreover, both have moderate to high selectivities for D_3 over α_1 (~114-fold for **89** and ~31-fold for **91**), 5-HT_{1A} (~42-fold for **89** and ~92-fold for **91**), 5-HT_2, and D_1 (both >390-fold for **89** and **91**). In mitogenesis assays **89** and **91** are both partial agonists at D_3 receptors with 28% (EC_{50}=3.5 nM) and 50% (EC_{50}=9.1 nM) relative maximal effect, respectively. Thus, **89** and **91** can be regarded as "fancy bioisosteres" of the benzamides or naphthamides, showing a highly unusual mixed subnanomolar D_3/D_4 binding profile. Their 2-methoxyphenylpiperazine analogs FAUC 378 (**88**) and FAUC 413 (**90**) exhibit both an impaired D_3 receptor binding (K_i=6.5 nM for **88** and K_i=10 nM for **90**), but the subnanomolar affinity of **89** and **91** for D_4 receptors is retained or even further improved in **88** and **90** (K_i=0.52 nM for **88** and K_i=0.37 nM for **90**). The



Scheme 11.

selectivities for D_4 over D_1 and D_2 receptors are enhanced for **88** ($D_1/D_4=2900$ and $D_2/D_4=210$) and **90** ($D_1/D_4=1900$ and $D_2/D_4=320$), while the preference for D_4 over α_1 receptors is reduced to 19-fold for **88** and 15-fold for **90**. It should be noticed that FAUC 378 (**88**) binds to 5-HT_{1A} receptors with a subnanomolar K_i of 0.50 nM, thus representing a superpotent mixed D_4/5-HT_{1A} ligand. In mitogenesis assay FAUC 378 is a strong partial agonist (67% relative maximal effect, $EC_{50}=0.55$ nM), which nicely corresponds to the intrinsic activity obtained from [^{35}S]GTPγS binding (74% relative maximal effect, $EC_{50}=2.5$ nM). Remarkably, the ruthenocene carboxamide analog FAUC 413 (**90**) does not show appreciable 5-HT_{1A} affinity ($K_i=20$ nM) and thus has been classified as a D_4-selective ligand. Evaluation in mitogenesis assay shows a potent partial agonist profile at D_4 receptors (60% relative maximal effect, $EC_{50}=1.2$ nM), however [^{35}S]GTPγS binding indicates almost full agonistic properties (94% relative maximal effect, $EC_{50}=1.9$ nM) of FAUC 413.

As an even more structurally challenging three-dimensional extension of the π_1 system, a series of [2.2]paracyclophanecarboxamide derivatives (**92–94**) has been synthesized [69]. To investigate the structural effects of the planar chirality of [2.2]paracyclophane, the pure enantiomers (***R***)-**92** (FAUC 418) and (***S***)-**92** have been prepared and tested. Interestingly, the structural difference caused by this planar chirality leads to an eudismic ratio of more than 15 ($K_i=3.0$ nM for (***S***)-**92** and $K_i=0.19$ nM for (***R***)-**92**). To evaluate the sensitivity of the binding profiles toward the substitution pattern of the phenyl substituent, the 2,3-dichloro, 3-chloro-2-methoxy, and 2,3-difluoro analogs **93**, **94**, and **95**, respectively, have been synthesized. In contrast to the metallocenes **88–91**, D_3 receptor binding considerably decreases for all GPCRs when displacing the 2-methoxy group ($K_i=3.6$ nM for **93** and 1.9 nM for **95**). The structural hybrid **94** bearing an ortho positioned methoxy group and a chloro atom in meta position displays an interesting binding pattern with both high D_3 affinity ($K_i=0.46$ nM) and considerable selectivity over the potential anti-target α_1 ($K_i=80$ nM).

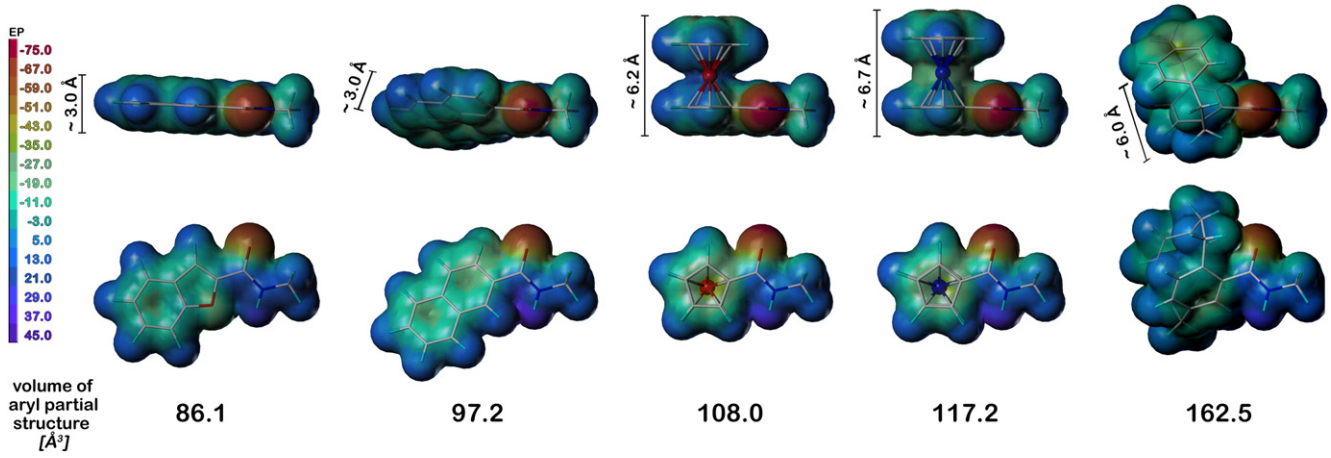


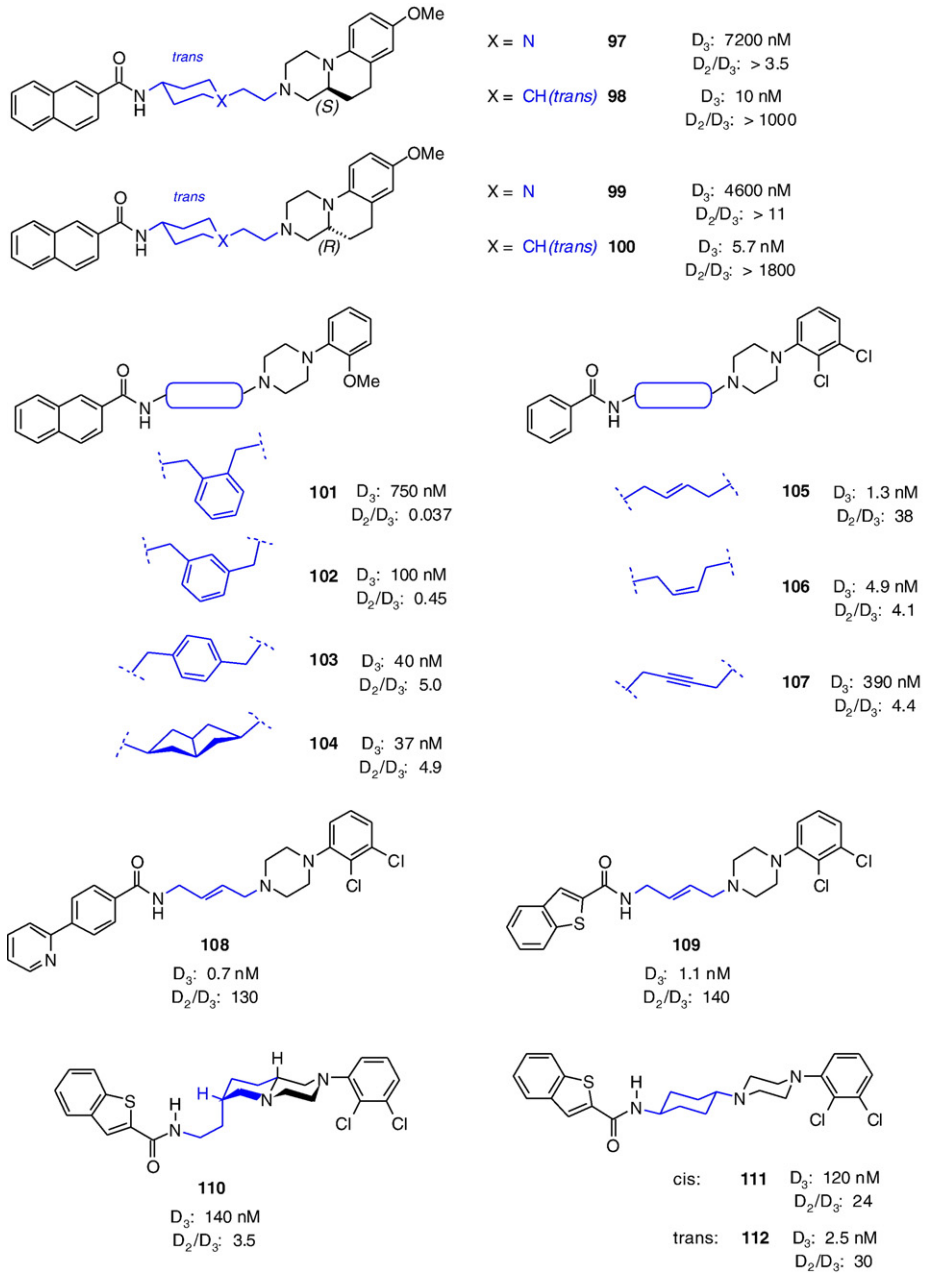
Fig. 3. Electrostatic potentials mapped onto the van der Waals surfaces of the following *N*-methylcarboxamide fragments: benzo[*b*]furan (as in 31 and 32), naphthalene (as in BP 897), ferrocene (as in FAUC 378 and FAUC 382), ruthenocene (as in FAUC 413 and 91) or paracyclophane (as in FAUC 418 and 93–95). The metallocene structures were derived from X-ray data of suitable precursors. Electrostatic potential charges were calculated using the PM3(tm) Hamiltonian in the program package Spartan. The distribution of charge on the molecular surfaces was visualized with MOLCAD implemented in SYBYL 6.9.1.

In mitogenesis assay, all paracyclophane derivatives **92–95** revealed an approximately neutral antagonism at the D_3 receptor [69]. This finding appears to be rather surprising, as it is complementary to the group of metallocenes **88–91**, where all ligands had partial agonist properties, and differs from the group of monolayered dopaminergics such as BP 897, FAUC 346 and 365, where the intrinsic activity strongly depends on the nature of both the π_1 system and the phenyl substituents.

Further extension of the π_1 moiety was performed though creation of a "chimera" of the benzofuranylcarboxamide **31** and the paracyclophanecarboxamide FAUC 418 (**92**) resulting in the 1(4,7)benzofurano-4(1,4)benzenehexaphanyl-1^2-carboxamide **96** [69]. This shift of the bilayered paracyclophane to a more distal position results in a 15- and 84-fold decrease of D_3 affinity as compared to **31** and FAUC 418, respectively. Consequently, **96** points out the limitations of enlarging π_1.

3.3.5. Rigidization of the spacer

In addition to the modifications already presented concerning the structure of the π_1 or π_2 moieties and the length of the spacer connecting π_1 and π_2, another strategy towards establishing novel D_3 ligands is the introduction of rigid partial structures into the highly flexible alkyl spacer (Scheme 12). Based on previous studies indicating that the introduction of a cyclohexylethyl spacer was capable of inducing high D_3 affinity [70], the butylene chain of the 8-methoxy-substituted **66** has been replaced by this rigidized *trans*-cyclohexylethyl spacer and its piperidine analog. This has been combined with the

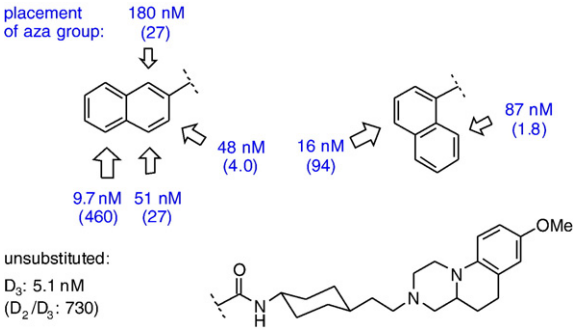


Scheme 12.

discrimination of the enantiomerically pure hexahydropyrazinoquinolines. The (*R*)-enantiomer (**99** and **100**) turns out to be slightly superior to the (*S*)-enantiomer (**97** and **98**) for both rigidized spacers, however, the corresponding eudismic ratio is just <2. Using a *trans*-cyclohexyl-ethyl spacer yields good D_3 affinity (K_i=10 nM for **98** and 4.7 nM for **100**) and high selectivity versus D_2 receptors (>1000-fold for **98** and >1800-fold for **100**). Exchange of the *trans*-cyclohexyl into a piperidine causes a severe loss of D_3 affinity (K_i=7200 nM for **97** and 4600 nM for **99**) and D_2:D_3 selectivity (>3.5. for **97** and >11 for **99**). In order to achieve a more suitable lipophilicity, heteroaromatic derivatives of the racemate of **98**/**100** have been prepared (Scheme 13) [71]. "Placement" of an aza atom in different positions of the naphthyl moiety yields a number of quinolinyl and isoquinolinyl carboxamides. Only the quinoline-6-carboxamide shows D_3 binding (K_i=9.7 nM) and selectivity over D_2 receptors (460-fold) similar to the naphthyl lead structure.

Other rigid structural elements, such as an *o*-, *m*- or *p*-xylenylene spacer (**101**–**103**) or a *cis*-octahydropentalen-2,5-diyl spacer (**104**) integrated into the BP 897 scaffold [52] result in low to moderate affinities only. This clearly demonstrates that these spacers are hardly able to rigidize the bioactive conformation of BP 897. However, an interesting rank order is found for the D_3 binding of the ligands **101**–**104**. While the *o*-xylenylene spacer has the most deleterious effect (750 nM), the *m*-xylenylene is significantly better (100 nM) and the *p*-xylenylene is the best of these three spacers (K_i=40 nM). Thus, the most linear structural element is favored over the more bent elements. Further supporting this observation, also the similarly extended octahydropentalene spacer is found to have comparable D_3 affinity (37 nM).

Introduction of a double or a triple bond into the butylene spacer produces the *trans*-butenylene derivative **105**, the *cis*-butenylene derivative **106** and the butynylene derivative **107** [72]. While **107** shows a strong decrease in D_3 affinity (390 nM), both **105** and **106** have nanomolar D_3 affinities (1.3 nM and 4.9 nM). Combining the *trans*-butenylene spacer with established π_1 and π_2 moieties generates partly rigidized structures with considerable D_3 affinities [46]. Both, the 4-pyridin-2-yl-benzamide derivative **108** and the 2-benzothiophene carboxamide derivative **109**, retain affinities at D_3 receptors (K_i=0.70 nM for **108** and 1.1 nM for **109**) which almost match the affinities of their butylene analogs (K_i=0.50 nM for **77** and for FAUC 365).



Scheme 13.

Taking advantage of the structure–activity information of previously invented D_3 ligands, novel rigidized FAUC 365 analogs have been designed facilitated by the predictivity of ligand-based 3D-QSAR models. Introduction of a cyclohexyl ring annelated to the piperazine gives the [(*7S,9aS*)-2-(2,3-dichlorophenyl)octahydro-pyrido[1,2-*a*]pyrazine-7-yl] ethyl analog (**110**) of FAUC 365. The specific stereochemistry of the annelated system implies a bend conformation which might be the reason for its weak to moderate D_3 receptor binding (K_i=140 nM). Replacement of the butylene by a *cis*-cyclohexylene spacer (**111**) also induces a bend conformation, which may explain that **111** shows a similar D_3 affinity (K_i=120 nM) as **110**. Introduction of a trans-cyclohexylene spacer connecting the benzo[*b*]thiophene-2-carboxamide to the 2,3-dichlorophenylpiperazine moiety allows for a stretched conformation of the ligand (**112**), which exhibits a low nanomolar D_3 affinity (K_i=2.5 nM), a medium preference over D_2 receptors (30-fold) and a pronounced selectivity over D_4 receptors (3200-fold).

4. Conclusion

Careful affinity, selectivity and potency "tuning" has resulted in the development of a variety of agonists, partial agonist, and neutral antagonists selective for the dopamine D_3 receptor. Thus, strong D_3 affinities and potencies, considerable selectivities over the other dopamine receptor subtypes, as well as adjusted intrinsic activities have been achieved up to date. Selectivity profiles towards other biogenic GPCRs and pharmacokinetic properties such as bioavailability, blood–brain distribution or drug clearance may represent further dimensions of ligand optimization.

With such a broad variety of ligands available, elucidation of the therapeutic impact of D_3 receptors will most likely further proceed during the forthcoming years. However, the successful treatment of multifactorial CNS diseases might require drugs with balanced receptor binding and efficacy profiles recognizing more than one molecular target. Thus, a paradigm shift from "magic bullets", which are aimed with optimizing selectivity at a single target, towards "magic shotguns" that interact with an optimized spectrum of several targets may be necessary [73].

Further challenges in D_3 drug discovery may be to find ligands that are able to discriminate between different functional states of the D_3 receptor, which appears to relate to the ability of these receptors to couple to different G proteins. Moreover, there is strong evidence for the ability of rhodopsin-like (type I) G-protein coupled receptors to form homo- or heterodimers (or even higher order oligomers) [74]. In the near future, it may be possible for ligands to distinguish between these higher order oligomerization states of the receptor, which may have an impact on their clinical utility in the treatment of specific central nervous systems diseases.

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