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Synthesis and biological investigations of dopaminergic partial agonists preferentially recognizing the D4 receptor subtype

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Abstract—Aminomethyl-substituted biaryls bearing a pyrazole or triazole moiety were synthesized and investigated for dopamine and serotonin receptor binding. The *N*-arylpyrazoles **3b**,**f**,**g** and **4** revealed K_i values in the subnanomolar range (0.28–0.70 nM) for the dopamine D4 receptor subtype. Employing both mitogenesis and GTPγS assays, ligand efficacy was evaluated indicating partial agonist properties. Interestingly, the tetrahydropyrimidine **4** (FAUC 2020) displayed significant intrinsic selectivity for $D2_{long}$ over $D2_{short}$.

The dopamine D4 receptor subtype received much attention as a pharmacological target for the treatment of schizophrenia, Parkinson's disease, depression, and attention deficit hyperactivity disorder (ADHD).[1] As a consequence, ongoing efforts have been made to find selective ligands revealing high affinity for the D4 receptor. Thus, an *N*-arylpiperazine framework proved to be a privileged structural unit,[2] which can be labeled as an integral part of a majority of potent D4 receptor ligands. The second nitrogen atom of the piperazine moiety is preferably attached to a benzylic CH_2 position of a fused heteroaryl or a biaryl substructure (Chart 1). Starting from the highly selective D4 ligand L-745,870 displaying weak partial agonist properties,[3,4] structural variations proved to be highly beneficial when ligand efficacy could be tuned. Thus, FAUC 213 turned out to be a complete antagonist exhibiting atypical antipsychotic properties in behavioral and neurochemical models of schizophrenia.[5,6] Very recently, the D4 agonist ABT-724 was discovered as a drug candidate for the treatment of erectile dysfunction (ED).[7]

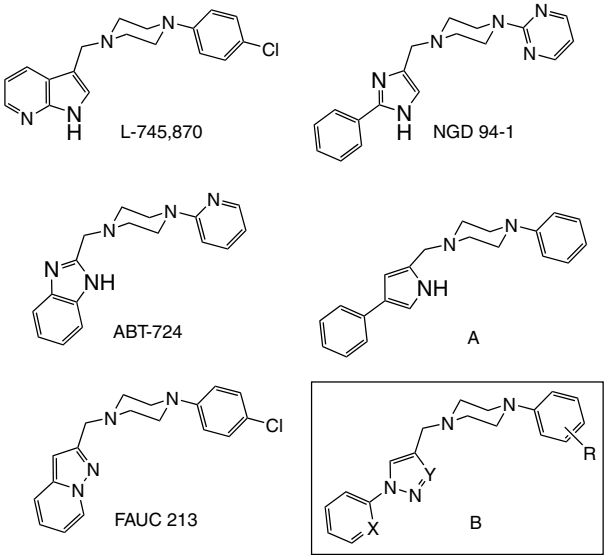

Chart 1. Structures of D4 selective lead and target compounds.

The phenylimidazole NGD 94-1 is a prominent member of the biaryl type class of D4 selective ligands being reported to act as a full antagonist.[8] Following studies indicated partial agonist effects.[9] Recently, we reported on the synthesis and receptor binding of dehydroimidazole and pyrrole analogs of type A,[10,11] demonstrating that the phenyl nucleus can be displaced by a 1,1-dicyanovinyl substituent.[12] Having in mind that a negative molecular electrostatic potential (MEP) exerted by the lone pair of an endocyclic sp^2 nitrogen proved to be beneficial for preferential D4 binding of the fused

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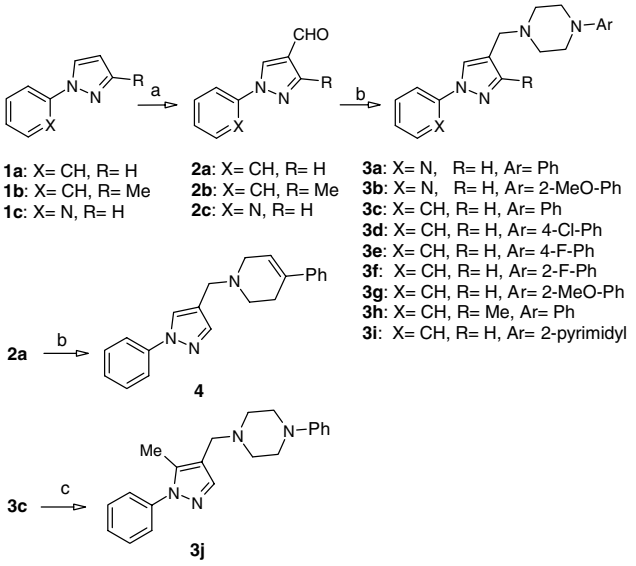
heteroarene derivatives,[13] we envisioned to prepare heterocyclic biaryl analogs of type B revealing a pyrazole or triazole nucleus in combination with a benzene or pyridine ring.[14] Further structural manipulations were envisaged including the exchange of the positions of the five- and the six-membered heteroarene units and the introduction of a tetrahydropyridine moiety replacing the piperazine ring.[15]

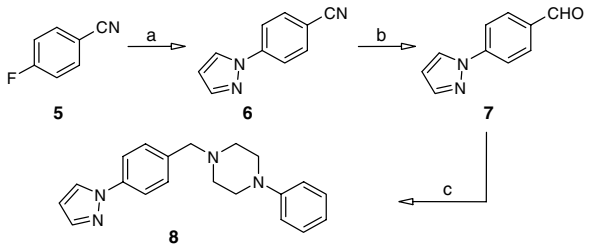
Starting from *N*-phenylpyrazoles **1a**,**b**[16] and the pyridine analog **1c**,[17] we employed a Vilsmeier formylation reaction to obtain the respective pyrazole carbaldehydes **2a**–**c**, which could be transformed into the piperazinylmethylpyrazoles **3a**–**i** by reductive amination in 78–88% yield (Scheme 1). Regiodirected lithiation of the azole moiety of **3c** followed by alkylation with MeI gave access to the 5-methyl derivative **3j**. Treatment of **2a** with 4-phenyl-1,2,5,6-tetrahydropyridine in the presence of $Na(OAc)_3BH$ resulted in formation of the aminomethylpyrazole **4**.

To prove our hypothesis that the relative orientation of the negative MEP is crucial for preferential D4 binding, we synthesized the regioisomer of the phenylpyrazole **3c** featuring an exchange of the rings within the biaryl moiety. Thus, 4-fluorobenzonitrile (**5**) was reacted with pyrazole to give the *N*-arylazole **6**.[18] DIBAL-H reduction furnished the respective carbaldehyde **7**, which was converted into the desired phenylpiperazine **8** by reductive amination (Scheme 2).

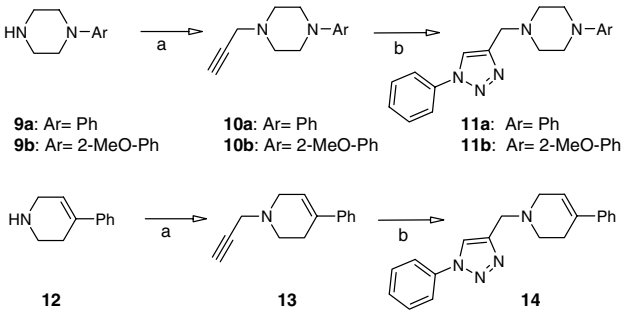
As an extension, we synthesized triazole analogs of the above-mentioned pyrazoles **3c**,**g** and **4**. Thus, alkylation of the secondary amines **9a**,**b** and **12** with propargyl bromide gave the 3-aminopropynes **10a**,**b** and **13**, respectively. Employing a slightly modified 'click chemistry' protocol,[19] we transformed the respective intermediates into the triazoles **11a**,**b** and **14** employing 1,3-dipolar



Scheme 1. Reagents and conditions: (a) Ref. 18; (b) arylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, rt, 16 h (78–88%); (c) 1—*n*-BuLi, THF, −78 to −30 °C, 2 h; 2—MeI, −78 to 0 °C, 1.5 h (62%).



Scheme 2. Reagents and conditions: (a) pyrazole, K_2CO_3, DMSO, 120 °C, 16 h (84%); (b) DIBAL-H, toluene, −60 °C (55%); (c) phenylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, rt, 16 h (80%).



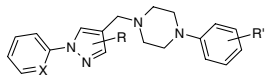
Scheme 3. Reagents and conditions: (a) Ref. 20; (b) phenylazide, $CuCl_2$, Na-ascorbate, *i*-PrOH, rt, 24 h (62–68%).

cycloaddition with phenyl azide in the presence of Cu(II) and Na-ascorbate (Scheme 3).

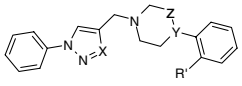
The final products **3a**–**j**, **4**, **8**, **11a**,**b**, and **14**[20] and the phenylpyrrole A as a reference compound were evaluated in vitro for their abilities to displace [^{3}H]spiperone from the cloned human dopamine receptors $D2_{long}$, $D2_{short}$,[21] D3,[22] and D4.4[23] being stably expressed in CHO cells.[24] The D1 affinities were determined by employing porcine striatal membrane preparations and the D1 selective antagonist [^{3}H]SCH 23390.

Receptor binding studies clearly indicated that the target compounds reveal only poor affinity to the D1 subtype. Depending on the substitution pattern of the aryl piperazine group, K_i values ranged from 11 to 6000 nM, 8 to 14,000 nM, and 33 to 4900 nM for $D2_{long}$, $D2_{short}$, and D3, respectively (Table 1). Interestingly, incorporation of a halogen atom in *para*-position (**3d**,**e**) strongly decreases D2 and D3 binding whereas *ortho*-substituents bearing a negative electrostatic potential caused an increase of affinity (**3b**,**f**,**g** and **11b**). In contrast, the 2-pyrimidyl-substituted NGD 94-1 analog **3i** displayed poor receptor binding for all GPCRs investigated. With the exception of the pyrimidine **3i** and the reversed regioisomer **8**, all target compounds displayed extraordinarily high D4 affinity with K_i values in the subnanomolar and single digit nanomolar range. Thus, the arylpyrazole and aryltriazole scaffold causes a substantial increase of affinity when compared to the phenylpyrrole substructure of the reference D4 ligand of type A. SAR observations indicate that the introduction of a further sp^2 nitrogen into the six- or five-membered arenes resulting in formation of the pyridylpyrazoles

Table 1. Receptor binding data (K_i values[a] of the arylazoles **3a–j**, **4**, **8**, **11a**,**b**, and **14**, and the reference agent A based on means of 3–14 experiments each performed in triplicate) (nM)



Compound	X	R	R′	D1	$D2_l$	$D2_s$	D3	D4
3a	N	H	H	1600	340[b]	190	160	2.6
3b	N	H	2-MeO	1900	40	31	72	0.44
3c	CH	H	H	1600	140	75	140	1.0
3d	CH	H	4-Cl	7500	2900	1900	1900	2.9
3e	CH	H	4-F	1900	450	400	540	2.9
3f	CH	H	2-F	680	63	37	67	0.70
3g	CH	H	2-MeO	1000	11	8.0	35	0.28[b]
3h	CH	3-CH_3	H	3900	71	43	360[b]	1.0
3i				7100	6000	14,000	2900[b]	310[b]
3j	CH	5-CH_3	H	560	630	250	320	5.3



Compound	X	Y	Z	R′	D1	$D2_l$	$D2_s$	D3	D4
11a	N	N	CH_2	H	21,000	1500	860	1200	9.9
11b	N	N	CH_2	OMe	27,000	120	65	340	2.7
4	CH	C	CH	H	3000	38	28	33	0.59
8					7600	3000	2400	2100	20
14	N	C	CH	H	10,000	220	110	94	3.2
A					2100	230[b]	170	514	12

[a] All standard deviations (±SEM) are within <25% of the appropriate K_i values.
[b] Standard deviation in the range of 25–35%.

3a,**b** and the phenyltriazoles **11a**,**b**, respectively, leads to a significant reduction of dopamine receptor binding. C-Methylation in position 3 or 5 of the pyrazole ring resulting in the test compounds **3h** and **j** was not crucial for D4 affinity and selectivity. It is interesting to note that the replacement of the piperazine ring by a tetrahydropyridine unit proved to be possible without significant reduction of receptor binding for the bioisosteres **4** and **14**.

To investigate the intrinsic effect of the most active D4 ligands of the piperazine and tetrahydropyridine class of compounds, respectively, in vitro functional assays with **3g** and **4** were envisaged measuring the [^{3}H]thymidine uptake in growing CHO cells stably expressing the dopamine $D2_{long}$, $D2_{short}$, D3, and D4.2[25] receptor, respectively, and GTPγS binding for D2 and D4.[26]

Employing the mitogenesis assay, the aminomethylpyrazoles **3g** and **4** proved to be partial agonists for $D2_{long}$, $D2_{short}$, D3, and D4.2 displaying intrinsic activities between 30% and 66% (Table 2). The partial agonist effect of **4** at the D4 subtype was further confirmed when antagonizing the effect of 100 nM quinpirole in a dose-dependent manner (Fig. 1). Interestingly, the D4 preference demonstrated for the binding experiments could not be observed in the functional assay. Having in mind that ligand binding and functional properties of the two isoforms $D2_{long}$ and $D2_{short}$ have been described to be more or less identical, it was very surprising that the data indicated an intrinsic selectivity of the tetrahydropyridine **4** (FAUC 2020). In detail, ligand efficacy of 54% relative to the dopaminergic full agonist quinpirole significantly exceeded a 25% relative efficacy upon the $D2_{short}$ mediated transduction system. To confirm these results, a GTPγS assay was performed when FAUC 2020 (**4**) led to 45% intrinsic activity via $D2_{long}$ and only 17% via $D2_{short}$ corroborating the results of the mitogenesis assay. To our knowledge, FAUC 2020 is the first example of a dopamine receptor ligand revealing nearly strong partial agonist properties at the $D2_{long}$ splice variant in combination with an only weak agonist effect at the $D2_{short}$ isoform and, thus might be of great interest as a pharmacological tool.

Due to the structural similarity of the test compounds to the atypical neuroleptic agent bifeprunox[27] displaying a combination of dopaminergic properties and single digit nanomolar 5-HT_{1A} receptor affinity, porcine striatal membranes were employed for serotonin receptor binding studies. In fact, the phenylpyrazoles **3g** and **4** have been capable to efficiently displace the radioligand [^{3}H]WAY100635, whereas 5-HT_2 binding was only weak.

In conclusion, SAR experiments on dopaminergic arylazole derivatives lead to highly active D4 partial agonist showing substantial 5-HT_{1A} binding, as well. The tetrahydropyridine analog FAUC 2020 (**4**) indicated significant intrinsic selectivity for $D2_{long}$ over the isoform $D2_{short}$.

Table 2. Agonist effects of the arylpyrazoles **3g**, **4** and quinpirole at the $D2_{long}$, $D2_{short}$, D3, and D4.2 receptor investigated by measuring the stimulation of mitogenesis and GTPγS binding as well as serotonin receptor binding

		Test compounds		
		3g	**4**	Quinpirole
$D2_l$	Efficacy mitogenesis[a] (%)	56	54	100
	EC_{50}[b] (nM)	0.28	3.0	7.4
	Efficacy GTPγS (%)[a]	nd	45	100
	EC_{50}[b] (nM)	—	25	330
$D2_s$	Efficacy mitogenesis[a] (%)	66	30	100
	EC_{50}[b] (nM)	0.22	1.7	10
	Efficacy GTPγS (%)[a]	nd	17	100
	EC_{50}[b] (nM)	—	32	230
D3	Efficacy mitogenesis[a] (%)	62	44	100
	EC_{50}[b] (nM)	0.21	3.3	3.4
D4	Efficacy mitogenesis[a] (%)	43	39	100
	EC_{50}[b] (nM)	0.60	5.6	11
	Efficacy GTPγS (%)[a]	nd	38	100
	EC_{50}[b] (nM)	—	9.5	23
$5\text{-}HT_{1A}$	K_i[c] (nM)	6.8	37	nd
$5\text{-}HT_2$	K_i[c] (nM)	1300	2100	nd

[a] Rate of incorporation of [^{3}H]thymidine (in %) relative to the maximal effect of the full agonist quinpirole (100%); the results are means of quadruplicate from 2 to 14 experiments.
[b] EC_{50} values derived from the mean curves of 2–14 experiments; nd, not determined.
[c] K_i values for $5\text{-}HT_{1A}$ and $5\text{-}HT_2$ binding were derived from two experiments each done in triplicate.

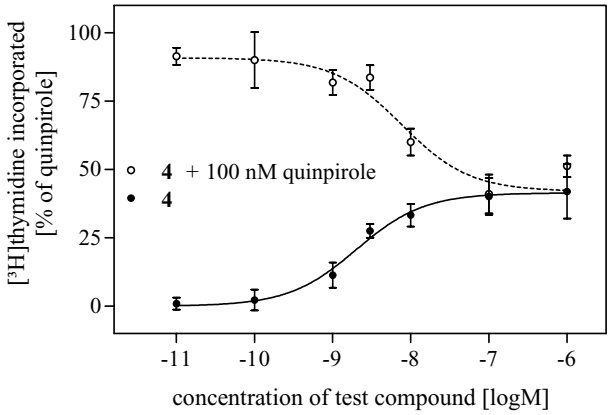


Figure 1. Partial agonism of the tetrahydropyridine **4** at the D4 receptor indicated by stimulation of [^{3}H]thymidine incorporation and confirmed by partially antagonizing the effect of quinpirole.

Acknowledgments

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20. Analytical data of the most potent test compounds. Compound **3b**: mp: 94 °C. Anal. Calcd for $C_{20}H_{23}N_5O$: C, 68.75; H, 6.63; N, 20,04. Found: C, 68.78; H, 6.62; N, 19.99.
Compound **3c**: mp: 109 °C. Anal. Calcd for $C_{20}H_{22}N_4$ (+0.1H_2O): C, 75.44; H, 6.96; N, 17.60. Found: C, 75.49; H, 6.87; N, 17.92.
Compound **3f**: mp: 98 °C. Anal. Calcd for $C_{20}H_{21}FN_4$ (+0.1H_2O): C, 71.41; H, 6.29; N, 16.65. Found: C, 70.85; H, 6.30; N, 16.57.
Compound **3g**: mp: 128 °C. Anal. Calcd for $C_{21}H_{24}N_4O$: C, 72.39; H, 6.94; N, 16.08. Found: C, 72.44; H, 6.94; N, 16.01.
Compound **4**: mp: 93 °C. ^{1}H NMR ($CDCl_3$, 360 MHz): δ = 2.60–2.66 (m, 2H), 2.81 (t, 6.0 Hz, 2H), 3.27 (dd, 3.0 Hz, 3.0 Hz, 2H), 3.68 (s, 2H), 6.09–6.13 (m, 1H), 7.23–7.52 (m, 8H), 7.70–7.76 (m, 2H), 7.74 (s, 1H), 7.97 (s, 1H). FTIR: λ (cm^{-1}) 752, 1500, 1599, 2735, 2821, 3053, 3095. APCI-MS: 316.2 (M^++1). Anal. calcd for $C_{21}H_{21}N_3$: C, 79.97; H, 6.71; N, 13.32. Found: C, 80.02; H, 6.68; N, 13.26.
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