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2,4-Disubstituted Pyrroles: Synthesis, Traceless Linking and Pharmacological Investigations Leading to the Dopamine D4 Receptor Partial Agonist FAUC 356

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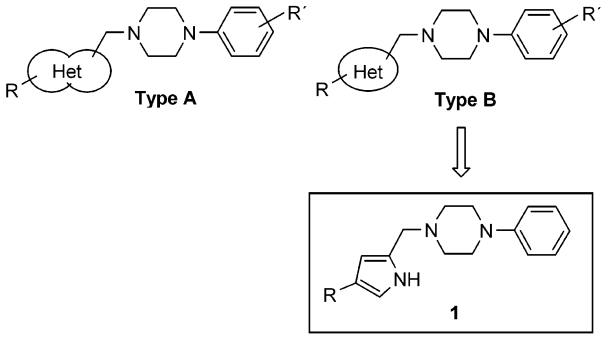
Abstract—Solution-phase synthesis and a solid-phase supported approach to piperazinylmethyl substituted pyrroles are described. Receptor binding studies and the measurement of D4 ligand efficacy led to the ethynylpyrrole **1d** (FAUC 356) exerting selective D4 binding and substantial ligand efficacy (66%, $EC_{50} = 1.9$ nM). This activity profile might be of interest for the treatment of ADHD.

Recent investigations using molecular cloning technology have led to the characterization of a number of dopamine receptor subtypes that can be divided into two classes, D1-like (D1 and D5) and D2-like (D2, D3, D4).[1] D4 receptors have been attributed to involvement in the pathogenesis of neuropathological diseases,[2] when a polymorphism of the dopamine D4 receptor gene has been implicated with ADHD (attention deficit hyperactivity disorders).[3] Due to these findings, selective agonists or partial agonists might offer a chance for an effective treatment of ADHD.

SAR studies in the field of selective D4 ligands led to a variety of piperazine derivatives of type A containing bicyclic heteroaromatic moieties, when 1H-pyrrolo[2,3-*b*]pyridines,[4] pyrazolo[1,5-*a*]pyridines[5] or 5-cyanoindoles[6] turned out to be superior. Interestingly, mitogenesis assays with compounds of type A showed that the intrinsic activity of the ligands strongly depends on the nature and the spatial orientation of the heterocyclic system.[7] Piperazines of type B, which are characterized by imidazole, imidazoline, thiazoline or oxazoline substructures[8,9] as monocyclic heteroaromatic systems, also proved to have preferential binding to the D4 receptor when substituted by a phenyl residue extending the π-system of the heteroarene unit. Previously, we developed D4 active pyrrole derivatives of type B involving an associated non-aromatic π-system being α-positioned (adjacent to the pyrrole nitrogen).[10] Projecting analogous binding modes of the structural families of type A and B, modification of the substituted pyrrole moiety should influence not only receptor binding but also ligand efficacy. As a consequence, we planned to prepare and pharmacologically investigate the pyrroles **1** containing different aromatic and non-aromatic π-systems in the pyrrole β-position. Besides exploiting solution-phase synthesis involving DEM-protection of the pyrrole nitrogen,[11] we envisioned to demonstrate our recently described solid-phase supported approach to substituted pyrroles.[12]



As a common starting material for the selected target compounds, we chose the DEM-protected 4-iodopyrrole-2-carbaldehyde **2** which was obtained by our previously described methodology.[11] Applying Pd-catalyzed

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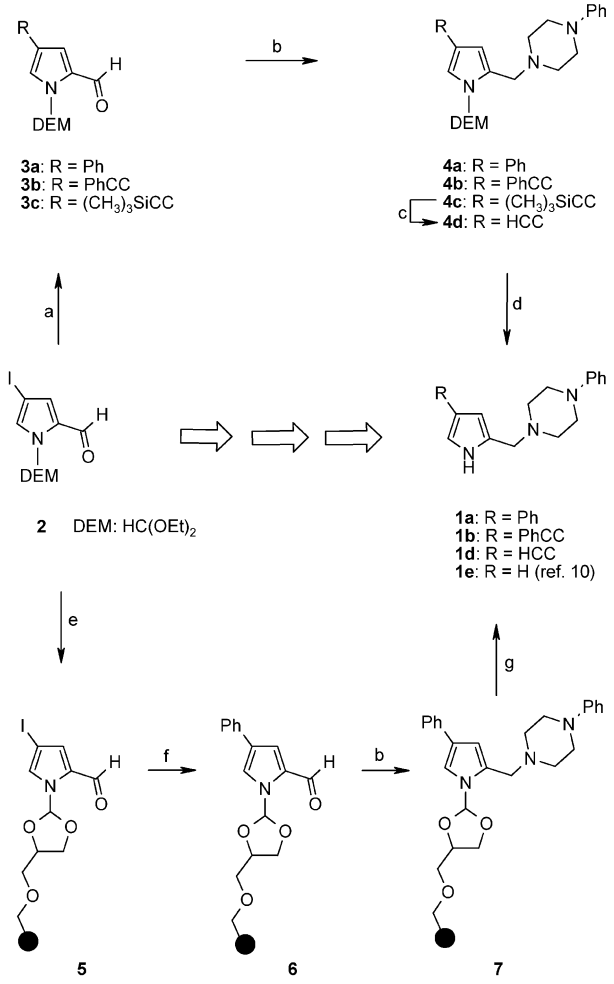
coupling reactions, the introduction of pharmacophoric elements into position 4 could be accomplished (Scheme 1). Thus the phenyl derivative **3a** and the alkynes **3b**,**c** were synthesized according to Suzuki[13] and Sonogashira[14] using the catalysts $Pd(PPh_3)_4$ and $Pd(PPh_3)_2Cl_2$/CuI, respectively (Scheme 1). Reductive amination of the pyrrolecarbaldehydes **3a–c** with phenylpiperazine and sodium triacetoxyborohydride furnished the piperazines **4a–c** in 63–88% yield. The trimethylsilyl group of the alkyne **4c** was removed using tetrabutylammonium fluoride to give **4d** (82%). After deprotection of the DEM-pyrroles **4a**, **4b**, and **4d** with TFA and subsequent treatment with NaOH, we obtained the final products **1a**,**b**,**d**[15] in 56–94% yield.

Choosing the phenylpyrrole **1a** as a representative target compound, we elaborated a reaction protocol on solid phase being suitable for two-dimensional parallel



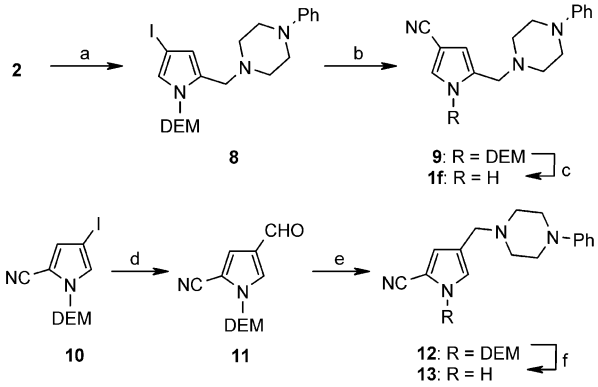
Scheme 1. (a) **3a**, **3c**: see ref 11; PhCCH, $Pd(PPh_3)_2Cl_2$, CuI, 1,4-dioxane/NEt_3 (2:1), rt, 15 min (**3b**: 85%); (b) phenylpiperazine, $NaBH(OAc)_3$, 1,2-dichloroethane, rt (**4a**: 19 h, 88%; **4b**: 22 h, 63%; **4c**: 19 h, 77%; **7**: 22 h); (c) tetrabutylammonium fluoride, THF, rt, 1 h (82%); (d) **4a**: TFA (3 equiv), CH_3CN, rt, 2 days; 2 N NaOH (6 equiv), rt, 16 h (**1a**: 69%); **4b**: TFA (4 equiv), CH_3CN, rt, 0.5 h; 2 N NaOH (8 equiv), rt, 0.5 h (**1b**: 94%); **4d**: TFA (3 equiv), CH_3CN, rt, 24 h; 2 N NaOH (6 equiv), rt, 1 h (**1d**: 56%); (e) 1,4-dioxane, *p*-TsOH, rt;[12] (f) phenylboronic acid, 15% $Pd_2(dba)_3$, $P(t\text{-}Bu)_3$, Cs_2CO_3, 1,4-dioxane, 80 °C, 24 h; (g) (1) 1,4-dioxane/CH_3CN/TFA (2:6:1), 60 °C, 48 h; (2) 2 N NaOH, rt, 3 h (53%; purity 70%, indicated by ^1H NMR spectroscopy).

syntheses of pyrrole derived compound libraries. In detail, traceless linking was facilitated by transacetalization of the DEM-protected iodopyrrolecarbaldehyde **2** and the 1,3-diol linker of Leznoff resin[16] to give the polymer-bound dioxolane **5**. Subsequent Suzuki coupling gave access to the arylation product **6** when a catalyst based on $Pd_2(dba)_3$ and $P(t\text{-}Bu)_3$ was utilized. Finally, reductive amination gave the resin-bound piperazine **7** that was hydrolytically cleaved to afford the final product **1a**.

Stimulated by the high D4 affinity of our previously described indolecarbonitrile derivatives of type A, we envisioned to introduce a cyano function enlarging the π-system and inducing a negative electrostatic region around the sp-nitrogen. Thus, we approached the test compound **1f** as well as its reversed substituted regioisomer **13** (Scheme 2).

In detail, the aldehyde **2** was reacted with phenylpiperazine and sodium triacetoxyborohydride to give the amination product **8** in 83% yield. Sakamoto et al.[17] described an effective protocol for the palladium catalyzed cyanation of bromo- and iodo-substituted benzenes, indoles and pyrroles. Employing these conditions ($CuCN/Pd_2(dba)_3$/dppf in 1,4-dioxane) gave the desired cyano pyrrole **9** in low yield (15%). Alternatively, we tried a halogen–metal exchange reaction using *n*-BuLi and subsequent trapping with TsCN. This reaction sequence resulted in formation of the carbonitrile **9** in 28% yield. *N*-Deprotection with TFA/NaOH gave the piperazine **1f** (86%). The reversed functionalized isomer **13** was synthesized starting from the cyano pyrrole **10**[11] when iodine–magnesium exchange and reaction with DMF gave the aldehyde **11** that was subjected to reductive amination with phenylpiperazine and hydrolytic deprotection to furnish 81% of the final product **13**.

Receptor binding profiles of the test compounds **1a,b, 1d–f**, and **13** were determined in vitro by measuring their ability to displace [^{3}H]spiperone from the cloned human dopamine receptor subtypes $D2_{long}$, $D2_{short}$,[18]



Scheme 2. (a) Phenylpiperazine, $NaBH(OAc)_3$, 1,2-dichloroethane, rt, 4 h (83%); (b) (1) *n*-BuLi (1.1 equiv), THF, −78 °C, 1 h; (2) TsCN (1.5 equiv), −78 °C, 30 min (28%); (c) TFA (4 equiv), CH_3CN, 60 °C, 22 h; 2 N NaOH (8 equiv), rt, 1 h (86%); (d) ref 11; (e) phenylpiperazine, $NaBH(OAc)_3$, rt, 14 h (83%); (f) (1) TFA (4 equiv), CH_3CN, 60 °C, 22 h; (2) 2 N NaOH (8 equiv), rt, 2 h (81%).

Table 1. Binding data of the test compounds for human dopamine D2, D3 and D4 receptors, bovine D1 and porcine 5-HT1A and 5-HT2A receptors [K_i values (nM) based on the means of 2–5 experiments each performed in triplicate]

	K_i values (nM)						
	[^{3}H]SCH23390		[^{3}H]Spiperone			[^{3}H]8-OH-DPAT	[^{3}H]Ketanserin
	bD1	$hD2_{short}$	$hD2_{short}$	hD3	hD4	p5-HT1A	p5-HT2A
1a	2100	230	170	540	12	790	1300
1b	2700	760	550	670	83	nd	nd
1d	2000	770	580	850	5.9	270	380
1e	5400	3100	3000	570	100	nd	nd
1f	4600	3700	8000	1800	21	450	610
13	1200	2400	3400	720	18	510	450
FAUC 113	12,000	3200	4300	5000	3.1	nd	nd
Clozapine	420	41	28	960	16	nd	nd
Quinpirole[a]	nd	64/3100	52/4000	24/420	1.6/49	nd	nd

nd, values not determined.
[a]High/low affinity binding sites.

Table 2. Intrinsic activity of the piperazines **1a, 1d, 1f** and **13** in relation to the full agonist quinpirole, the partial agonist FAUC 113 and the antagonist clozapine at the human D4.2 receptor established by measuring the stimulation of mitogenesis

	Test compounds						
	1a	**1d**	**1f**	**13**	FAUC 113	Quinpirole	Clozapine
Intrinsic effect[a] (%)	43	66	38	36	27	100	0
EC_{50} (nM)	6.8	1.9	5.6	7.8	16	2.2	—

[a]Rate of incorporation of [^{3}H]thymidine as evidence for mitogenetic activity relative to the maximal effect of the full agonist quinpirole (100%) as the means of quadruplicates from 6 to 12 experiments. EC_{50} values in nM are derived from the mean curves of the experiments.

D3[19] and D4.4[20] stably expressed in CHO (Chinese hamster ovary) cells.[21] D1 receptor affinities were investigated employing bovine striatal membranes and the D1 selective radioligand [^{3}H]SCH 23390.[21] The resulting K_i values of the test compounds are listed in Table 1 in comparison to quinpirole, the D4 partial agonist FAUC 113 and the atypical neuroleptic clozapine.

The test compounds displayed substantial D4 selectivity. The highest affinities for the D4 binding site were determined for the phenylpyrrole **1a** and its alkynyl substituted bioisostere **1d** with K_i values of 12 and 5.9 nM, respectively. Within the D2 receptor family, the alkyne **1d** showed a significantly stronger D4 preference than the phenyl derivative **1a** when the selectivity over $D2_{long}$, $D2_{short}$ and D3 was 7 and 3 times higher, respectively. Increase of the π-system by joining together the acetylene unit and the phenyl moiety resulted in significant reduction of D4 affinity for **1b** ($K_i = 83$ nM). Substitution of the ethynyl group of **1d** with the more polarized cyano function led to a slight reduction of D4 affinity when the position of the NH-function exerted only a minor influence onto the binding profiles of the regioisomers **1f** and **13**. The binding data of the unsubstituted aminomethylpyrrole **1e**[10] show that an extension of the pyrrole π-system is, in fact, necessary to provide substantial D4 affinity. In order to further characterize the binding properties of the potent D4 ligands **1a**,**d**,**f** and **13**, 5-HT1A and 5-HT2 receptor recognition was evaluated. Employing porcine brain homogenates and the radioligands [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively, the resulting K_i values indicated only moderate 5-HT1A (270–790 nM) and 5-HT2 affinities (380–1300 nM).

To investigate the intrinsic effects of the most interesting test compounds **1a**,**d**,**f** and **13**, a mitogenesis assay was performed employing CHO10001 cells stably expressing the human D4.2 receptor.[6,22] Agonist activation of dopamine receptors can be determined by measuring the rate of [^{3}H]thymidine incorporation into growing heterologously transfected cell lines.[23] Comparative experiments were done with the full agonist quinpirole, the partial agonist FAUC 113 and the full antagonist clozapine. Intinsic activities and EC_{50} values are depicted in Table 2, clearly indicating ligand efficacy for the phenylpyrrole **1a** (43%) and the cyano substituted regioisomers **1f** and **13** (36–38%) that is comparable to the D4 partial agonist FAUC 113 (27%). Both these

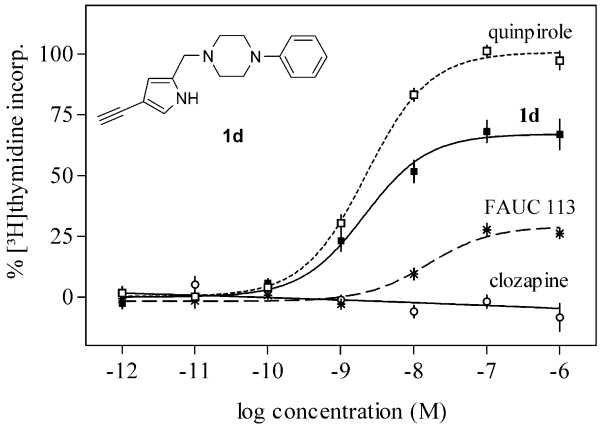


Figure 1. Stimulation of mitogenesis as a functional assay to estimate the effect of **1d** at the human dopamine D4.2 receptor in relation to the full agonist quinpirole, the antagonist clozapine and the D4 ligand FAUC 113.

parameters, however, are substantially improved for the alkyne **1d** (66%, $EC_{50} = 1.9$ nM), which might be due to the ability of the acetylene substructure to act as a hydrogen bond donating functionality, possibly stabilizing active receptor conformations.[24] Figure 1 shows dose–response curves for **1d** (FAUC 356) in comparison to the full agonist quinpirole, the full antagonist clozapine and the previously described D4 ligand FAUC 113.

In conclusion, solution-phase synthesis and a solid-phase supported approach to piperazinylmethyl substituted pyrroles are described. Receptor binding studies and the measurement of D4 ligand efficacy indicated analogous binding modes for the general compound families of type A and B. The most interesting activity profile was discovered for the ethynylpyrrole **1d** (FAUC 356) exerting selective D4 recognition and substantial ligand efficacy (66%), which might be of interest for the treatment of ADHD.

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15. **1d**: ^{1}H NMR ($CHCl_3$, 360 MHz): δ 2.58 (t, $J = 5.0$ Hz, 4H, $CH_2NC\underline{H_2}CH_2$), 2.92 (s, 1H, HCC), 3.17 (t, $J = 5.0$ Hz, 4H, CH_2NPh), 3.49 (s, 2H, pyrrole–CH_2), 6.17 (m, 1H, H-3), 6.99 (dd, $J = 2.4$, 1.7 Hz, 1H, H-5), 6.8–7.0, 7.2–7.3 (m, 5H, Ph), 8.58 (s, 1H, NH); ^{13}C NMR ($CHCl_3$, 90 MHz): 49.11 ($CH_2N\underline{C}H_2CH_2$), 53.02 ($CH_2NPh$), 54.97 (pyrrole–$CH_2$), 75.31 ($\overline{H}\underline{C}C$), 79.48 ($H\underline{C}\overline{C}$), 102.98 (C-4), 111.23 (C-3), 116.05 (Ph–C-2/6), 119.80 (Ph–C-4), 122.82 (C-5), 128.58 (C-2), 129.18 (Ph–C-3/5), 151.17 (Ph–C-1); EIMS: 265 (M^+). Anal. calcd for $C_{17}H_{19}N_3$ (265.36): C, 76.95; H, 7.22; N, 15.84. Found: C, 76.74; H, 7.07; N, 15.53.
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