Rationally Based Efficacy Tuning of Selective Dopamine D4 Receptor Ligands Leading to the Complete Antagonist 2-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-*a*]pyridine (FAUC 213)

Stefan Löber, Harald Hübner, Wolfgang Utz, and Peter Gmeiner*

Department of Medicinal Chemistry, Emil Fischer Center, Friedrich-Alexander University, Schuhstrasse 19, D-91052 Erlangen, Germany

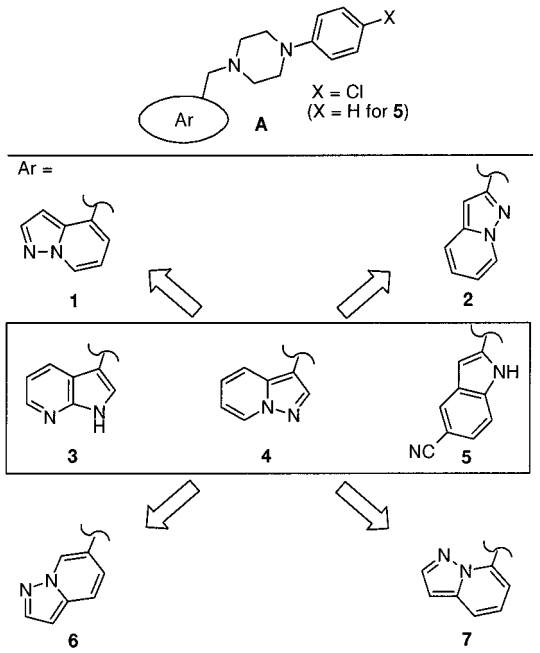
Received April 6, 2001

Abstract: Structure dependent efficacy studies in the field of selective D4 ligands led to the 2-aminomethyl substituted azaindole **2** (FAUC 213) that displayed strong D4 binding, high subtype selectivity, and complete antagonist properties in ligand-induced mitogenesis experiments. According to our schematic molecular model, the intrinsic activity of the regioisomers investigated is controlled by the ability of the heterocyclic unit to interact with both elements of the D4 binding-site crevice, the aromatic microdomain in TM6, and a serine residue in TM5.

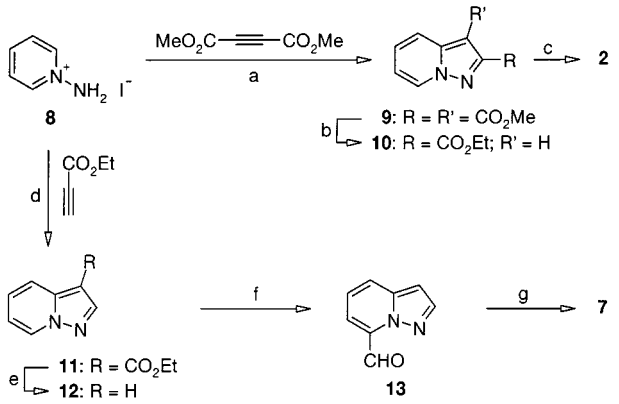
Introduction. Dopamine D4 receptors have been attributed to involvement in the pathogenesis of neuropathological diseases, dependencies, and normal personality trait.[1] Due to previous studies, indicating an increase in the density of D4 receptors of schizophrenia brains[2] and high D4 affinity for the atypical antipsychotic clozapine,[3] selective dopamine D4 receptor antagonists have been pursued as potential antipsychotic agents. The azaindole L-745,870 (**3**) and further drug candidates were entered into clinical trials but failing to demonstrate clinical efficacy.[4,5] However, the ineffectiveness can be explained by the partial agonist properties that were found very recently.[6] To find a complete antagonist, structure dependent efficacy studies in the field of selective D4 ligands are of particular relevance.

When rationally approaching this goal we studied previous work on the molecular mechanisms of agonist−receptor interaction clearly indicating the involvement of a cluster of conserved serine residues in the fifth transmembrane domain cooperating with aromatic side chains in TM6.[7−9] On the basis of site-directed mutagenesis data and docking studies with the D4 ligand **3**, the Ar subunit of the lead structure **A** simulating the catechole fragment of dopamine interacts with the TM5-TM6 microdomain of the D4 receptor controlling receptor activation.[10,11] On the other hand, the (chloro)phenyl moiety interacts with a divergent microdomain in TM2-TM3-TM7 which is responsible for D4/D2 selectivity. As a consequence, we envisioned manipulating ligand efficacy by subtle structural modification of Ar when employing the D4 selective partial agonists L-745,870 (**3**), FAUC 113 (**4**),[12] and FAUC 299 (**5**)[13] as lead compounds (Chart 1). In this paper, we describe the synthesis, receptor binding, and ligand efficacy of the regioisomeric azaindole derivatives **1**,**2**, **6**, and **7**, gaining insights to the question whether the relative orientation of Ar can determine the amount of receptor activation.

Chart 1



Scheme 1[a]



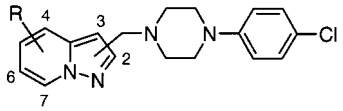
[a] Reagents and conditions: (a) K_2CO_3, air−O_2, DMF, rt, 2 h (45%); (b) (1) H_2SO_4 (v/v 50%), 80 °C, 3 h, (2) EtOH, H_2SO_4, reflux, 16 h (72%); (c) 1-(4-chlorophenyl)piperazine, $LiAlH_4$, THF, rt to 60 °C, 45 min (35%); (d) K_2CO_3, air-O_2, DMF, rt, 1.5 h (72%); (e) H_2SO_4 (v/v 40%), reflux, 3 h (86%); (f) *n*-BuLi, HCOOEt, THF, −78 °C, 0.5 h (82%); (g) 1-(4-chlorophenyl)piperazine, $Na(OAc)_3BH$, CH_2Cl_2, rt, 16 h (87%).

Results and Discussion. The synthesis of the 2-aminomethyl pyrazolo[1,5-*a*]pyridine derivative **2** and its 7-substituted regioisomer **7** started from *N*-aminopyridinium iodide **8**[14] when 1,3-dipolar cycloaddition with dimethyl acetylenedicarboxylate afforded the pyrazolo[1,5-*a*]pyridine-2,3-dicarboxylate **9** (Scheme 1).[15] Hydrolysis and regioselective decarboxylation with sulfuric acid (50%) followed by esterification gave the ethyl azaindole-2-carboxylate **10** in 72% yield. Employing a reductive amination protocol reported by Wright,[16] this

* To whom correspondence should be addressed. Tel: +49(9131)8529383. Fax: +49(9131)8522585. E-mail: gmeiner@pharmazie.uni-erlangen.de.

10.1021/jm015522j CCC: $20.00
Published on Web 07/24/2001

Table 1. Receptor Binding Data of the Pyrazolo[1,5-*a*]pyridines **1**, **2**, **4**, **6**, **7**, **17**, and **18** to Human and Bovine Dopamine Receptors[a]



	positions		K_i (nM) ± SEM[b]				
			[^{3}H]SCH 23390	[^{3}H]spiperone			
compd	R	$CH_2N<$	bovine D1	human $D2_{long}$	human $D2_{short}$	human D3	human D4.4
1	H	4	32000 ± 9500	16000 ± 2000	21000 ± 1500	12000 ± 3900	64 ± 7.0 ($n = -1.0$)
2	H	2	5500 ± 1300	3400 ± 450	6300 ± 1900	5300 ± 720	2.2 ± 0.23 ($n = -1.1$)
4	H	3	12000 ± 500	3200 ± 400	4300 ± 650	5000 ± 650	3.6 ± 0.87 ($n = -0.9$)
6	H	6	7500 ± 950	13000 ± 500	15000 ± 2500	4900 ± 1100	3.1 ± 0.12 ($n = -0.9$)
7	H	7	18000 ± 5000	31000 ± 8000	67000 ± 33000	10000 ± 2100	1300 ± 150 ($n = -0.9$)
17	3-CO_2Et	4	19000 ± 2000	37000 ± 9000	45000 ± 1500	12000 ± 500	6800 ± 3200 ($n = -1.0$)
18	3-CH_2OH	4	70000 ± 14000	39000 ± 11000	53000 ± 1500	23000 ± 4500	3600 ± 550 ($n = -0.9$)
clozapine			420 ± 50	41 ± 1.5	28 ± 0.50	960 ± 45	16 ± 0.50 ($n = -0.9$)

[a] K_i values are the means of two to four independent experiments ± SEM using eight different concentrations each as triplicates. [b] Steepness of the competition curves and the derived hill slopes indicate a one-site binding model typical for antagonists; the results of the functional test characterizing **4** and **6** as partial agonists led to the conclusion that the binding data at least for the $D_{4.4}$ receptor represent a two-state model and should be expressed as $K_{0.5}$ values.

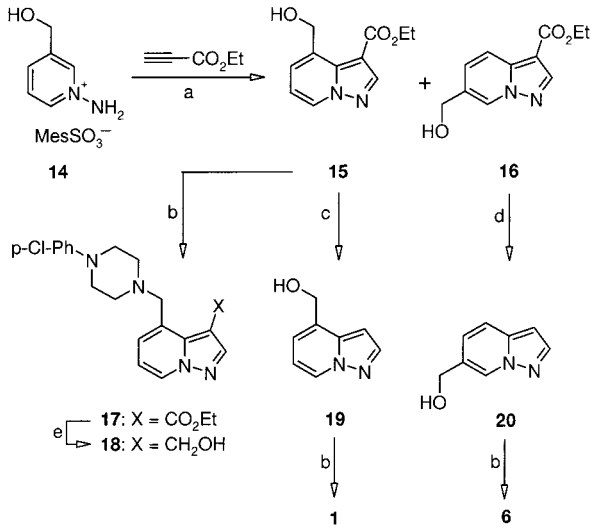
ester could be transformed into the piperazinylmethyl derivative **2** within a one-step process using $LiAlH_4$ in THF.

Formation of the unsubstituted pyrazolo[1,5-*a*]pyridine (**12**)[17] was achieved by cycloaddition of the aminopyridinium salt **8** with ethyl propiolate in the presence of O_2[18] followed by hydrolysis and decarboxylation of the ethylester **11** in 62% overall yield. Regioselective lithiation of the azaindole **12** by BuLi at dry ice temperature followed by treatment with ethyl formiate and acid workup to avoid Cannizzaro reaction resulted in formation of the aldehyde **13**.[19] Finally, reductive amination with 1-(4-chlorophenyl)piperazine gave the 7-aminomethyl substituted final product **7** in 87% yield.

The target compounds **1** and **6** were synthesized starting from *N*-amino-3-(hydroxymethyl)pyridinium mesityl sulfonate **14** which was converted into the regioisomeric (hydroxymethyl)pyrazolo[1,5-*a*]pyridine carboxylates **15** and **16**.[20] Subsequent hydrolysis and decarboxylation under strong acidic conditions furnished the (hydroxymethyl)pyrazolo[1,5-*a*]pyridines **19** and **20**, respectively (Scheme 2). Activation of these benzylic alcohols by MsCl followed by nucleophilic substitution with 1-(4-chlorophenyl)piperazine gave the (azaindolylmethyl)piperazines **1** and **6** in 63% and 61% yield, respectively. To synthesize 3-substituted analogues of **1**, the alcohol functionality of **15** was activated and transformed into the tertiary amine without former removing of the ethoxycarbonyl side chain. Subsequent reduction of the ester derivative **17** gave the corresponding 3-(hydroxymethyl)pyrazolo[1,5-*a*]pyridine **18**.

Radioligand binding assays were employed to characterize the dopamine receptor affinity profiles of the pyrazolo[1,5-*a*]pyridines **1**, **2**, **6**, and **7** representing regioisomers of the recently described D4 selective ligand **4** (FAUC 113). Comparative data were collected for the 3,4-disubstituted derivatives **17** and **18** and for the atypical neuroleptic agent clozapine. The selected compounds were investigated for their ability to displace [^{3}H]spiperone from the cloned human dopamine receptor subtypes $D2_{long}$, $D2_{short}$,[21] D3,[22] and D4.4[23] stably expressed in Chinese hamster ovary cells (CHO).[24] D1 affinities were assessed via competition experiments using bovine striatal membrane preparations and the

Scheme 2[a]



[a] Reagents and conditions: (a) K_2CO_3, air−O_2, DMF, rt, 1.5 h (25% for **15**; 17% for **16**); (b) (1) MsCl, Et_3N, THF, rt, 2 h; (2) 1-(4-chlorophenyl)piperazine, DMF, K_2CO_3, rt, 16 h (63% for **1**; 61% for **6**; 61% for **17**); (c) H_2SO_4 (v/v 40%), 100 °C, 1.5 h (83%); (d) H_2SO_4 (v/v 40%), 100 °C, 3 h (78%); (e) $LiAlH_4$, THF, 0 °C to rt, 5 h (86%).

D1 selective radioligand [^{3}H]SCH 23390.[24] The K_i values of the 2- and 6-aminomethyl derivatives **2** and **6** depicted in Table 1 indicate strong D4 binding ($K_i = 2.2$, 3.1 nM) and substantial selectivity over D1, $D2_{long}$, $D2_{short}$, and D3 (>1000). Significant loss of D4 receptor recognition was observed for the 4-aminomethyl pyrazolo[1,5-*a*]pyridine **1** and for the 3,4-disubstituted derivatives **17** and **18**. Migration of the aminomethyl substituent into position 7 resulted in strong reduction of D4 binding. The steepness of the competition curves and the derived Hill slopes gave one-site binding models.

Agonist activation of dopamine receptors is known to increase mitogenesis in heterologously transfected cell lines that can be determined by observing the rate of [^{3}H]thymidine incorporation into growing cells.[25] The functional experiment can be quantified by determination of the effective test compound concentration (EC_{50}) and by comparing the stimulating effect to the [^{3}H]thymidine incorporation that is caused by a full agonist.

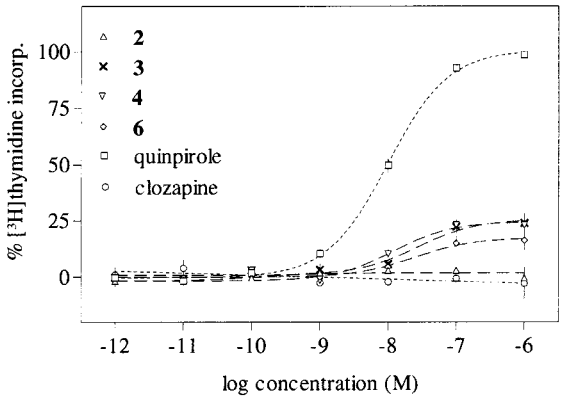


Figure 1. Stimulation of mitogenesis as a functional assay to assess the agonist effects of **2**, **3**, **4**, and **6** at the human dopamine D4.2 receptor in relation to the full agonist quinpirole and the antagonist clozapine, derived from 16, 7, 11, 8, 10, and 6 experiments, respectively.

Table 2. Intrinsic Activity of the Azaindoles **2**, **3**, **4**, and **6** in Relation to the Reference Compounds Quinpirole and Clozapine at the Dopamine D4 Receptor Established by Measuring the Stimulation of Mitogenesis

	test compounds					
	2	**3**	**4**	**6**	quinpirole	clozapine
agonistic effect[a]	<3%	23%	26%	17%	100%	<3%
EC_{50} (nM)	nd	31	12	24	10	nd

[a] Rate of incorporation of [3H]thymidine (percentage) as evidence for mitogenetic activity related to the full agonistic effect of quinpirole (100%) as the means of quadruplicates from 6 to 16 experiments. EC_{50} values are derived from the mean curves of the experiments. nd = not determined.

To investigate whether the relative orientation of the azaindole substructure can determine the amount of receptor activation, the intrinsic effects of the pyrazolo-[1,5-*a*]pyridines **2**, **4**, and **6**, which emphasized the most promising D4 ligands in the series of tested regioisomers, were determined. CHO10001 cells stably expressing the human D4.2 receptor were established for the mitogenesis assay.[13,26] Comparative experiments were performed with the full agonist quinpirole, the antagonist clozapine, and the 7-azaindole **3** (L-745,870) that is known as a partial agonist.[6] Figure 1 shows dose response curves clearly indicating partial agonist effects for the 3-substituted azaindoles **3** (L-745,870) and **4** (FAUC 113) with an efficacy of approximately one-fourth (23% and 26%) of the effect of quinpirole. The ligand efficacy of the 6-aminomethyl derivative **6** was slightly reduced (17%). The results including the EC_{50} values for **3**, **4**, and **6** (31, 12, and 24 nM) are shown in Table 2. As outlined in Figure 1, the most interesting biological properties were observed for the 2-aminomethyl substituted regioisomer. As a matter of fact, the highly selective D4 ligand **2** (FAUC 213) proved complete antagonist properties, comparable to those of the atypical neuroleptic agent clozapine. Thus, ligand efficacy of the investigated family of drug candidates seems really to be a function of the orientation of the heteroarene subunit.

On the basis of a pharmacophore model derived from a CoMFA study of a series of D4 receptor ligands, a specific spatial orientation of two π-systems and a cationic nitrogen seems to be necessary for receptor binding.[27] Taking into account recently reported site-directed mutagenesis studies and docking experiments, the protonated aliphatic amine functionality of the D4 ligands described herein is expected to interact with the conserved Asp115 (3.32) in the third membrane-spanning segment (TM3) whereas the chlorophenyl moiety is supposed to contribute to D4 affinity and selectivity by recognizing a phenylalanine at position 89 (2.61) in the second transmembrane-spanning domain.[10,11] As a consequence, the azaindole subunit is located next to an aromatic cluster in TM6 that is in proximity to the conserved serine residues in TM5 being known as the molecular determinants for agonist-induced signaling.[7,8] We propose that the intrinsic activity of the D4 ligands investigated is controlled by the ability of the heterocyclic unit to interact with both elements: the aromatic microdomain in TM6 and a serine residue in TM5. A schematic molecular model illustrating the potential binding situation of the partial agonist **4** and the antagonist **2** at the human D4 receptor indicates

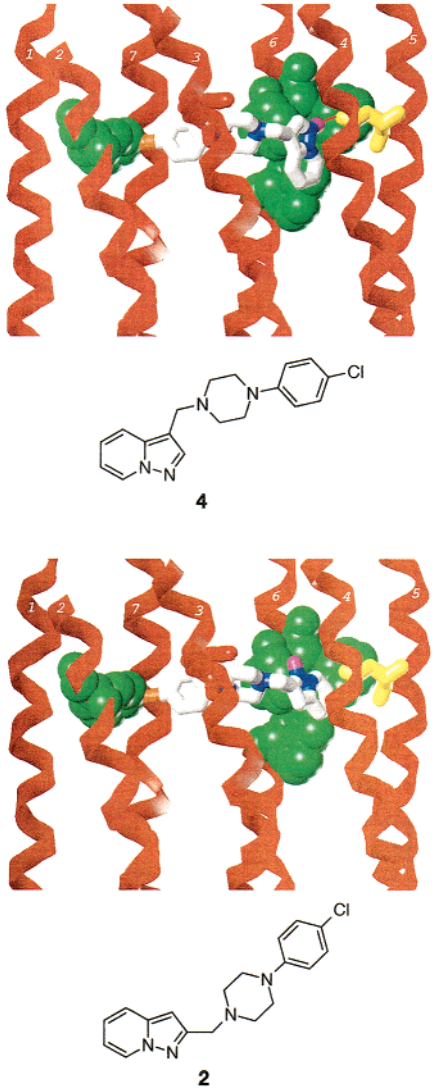


Figure 2. Schematic molecular model illustrating the potential D4 receptor binding of the partial agonist **4** (top) and the antagonist **2** (bottom). In contrast to the binding situation of **4** (FAUC 113) involving an H-bond (approximate distance = 2.3 Å) between the lone pair in position 1 and the HO function of Ser-196 (red line), analogous docking of **2** (FAUC 213) results in a significantly higher N−HO distance (approximately 4.1 Å).

hydrogen bonding between Ser196 (5.46) in helix 5 and the 3-substituted azaindole **4** (Figure 2).[28] Due to the higher distance between the lone pair in position 1 of the heterocycle and the Ser-OH function, activation of the receptor by the 2-substituted regioisomer **2** is unlikely, resulting in complete antagonism.

In conclusion, the 2-aminomethyl substituted azaindole **2** (FAUC 213) showed potent D4 binding, high subtype selectivity, and complete antagonist properties in ligand-induced mitogenesis experiments. Behavioral pharmacological studies investigating the potential use of the D4 antagonist **2** as an atypical neuroleptic agent are in progress.

Acknowledgment. The authors thank Dr. H. H. M. Van Tol (Clarke Institute of Psychiatry, Toronto), Dr. J.-C. Schwartz, and Dr. P. Sokoloff (INSERM, Paris) as well as Dr. J. Shine (The Garvan Institute of Medical Research, Sydney) for providing dopamine D4, D3, and D2 receptor expressing cell lines, respectively. Dr. R. Huff (Pharmacia & Upjohn, Inc., Kalamazoo, MI) is acknowledged for providing a D4 expressing cell line employed for mitogenesis. Thanks are also due to Mrs. H. Szczepanek, Mrs. B. Linke, and Mrs. P. Schmitt for skillful technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

Supporting Information Available: Complete Experimental Section including detailed information on the synthesis, analytical characterization, and biological investigations.

References

(1) Hrib, N. J. The dopamine D4 receptor: a controversial therapeutic target. *Drugs Future* **2000**, *25*, 587−611 and references therein.
(2) Seeman, P.; Guan, H. C.; Van Tol, H. H. M. Dopamine D4 receptors elevated in schizophrenia. *Nature* **1993**, *365*, 441−445.
(3) Van Tol, H. H. M.; Bunzow, J. R.; Guan, H.-C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* **1991**, *350*, 610−614.
(4) Kulagowski, J. J.; Broughton, H. B.; Curtis, N. R.; Mawer, I. M.; Ridgill, M. P.; Baker, R.; Emms, F.; Freedman, S. B.; Marwood, R.; Patel, S.; Patel, S.; Ragan, C. I.; Leeson, P. D. 3-[[4-(4-Chlorophenyl)piperazin-1-yl]methyl]-1H-pyrrolo[2,3-*b*]pyridine: an antagonist with high affinity and selectivity for the human dopamine D_4 receptor. *J. Med. Chem.* **1996**, *39*, 1941−1942.
(5) Rowley, M.; Bristow, L. J.; Hutson, P. H. Current and novel approaches to the drug treatment of schizophrenia. *J. Med. Chem.* **2001**, *44*, 477−501.
(6) Gazi, L.; Bobirnac, I.; Danzeisen, M.; Schüpbach, E.; Langenegger, D.; Sommer, B.; Hoyer, D.; Tricklebank, M.; Schoeffter, P. Receptor density as a factor governing the efficacy of the dopamine D_4 receptor ligands, L-745,870 and U-101958 at human recombinant $D_{4.4}$ receptors expressed in CHO cells. *Br. J. Pharmacol.* **1999**, *128*, 613−620.
(7) Wiens, B. L.; Nelson, C. S.; Neve, K. A. Contribution of serine residues to constitutive and agonist-induced signaling via the D2S dopamine receptor: evidence for multiple, agonist-specific active conformations. *Mol. Pharmacol.* **1998**, *54*, 435−444.
(8) Javitch, J. A.; Ballesteros, J. A.; Weinstein, H.; Chen, J. A cluster of aromatic residues in the sixth membrane spanning segment of the dopamine D2 receptor is accessible in the binding-site crevice. *Biochemistry* **1998**, *37*, 998−1006.
(9) Sansom, S. P.; Weinstein, H. Hinges, swivels and switches: the role of prolines in signaling via transmembrane α-helices. *Trends Pharmacol. Sci.* **2000**, *21*, 445−451.
(10) Simpson, M. M.; Ballestros, J. A.; Ciappa, V.; Chen, J.; Suehiro, M.; Hartman, D. S.; Godel, T.; Snyder, L. A.; Sakmar, T. P.; Javitch, J. A. Dopamine D4/D2 receptor selectivity is determined by a divergent aromatic microdomain contained within the second, third, and seventh membrane spanning segments. *Mol. Pharmacol.* **1999**, *56*, 1116−1126.
(11) Schetz, J. A.; Benjamin, P. S.; Sibley, D. R. Nonconserved residues in the second tansmembrane-spanning domain of the D4 dopamine receptor are molecular determinants of D4-selective pharmacology. *Mol. Pharmacol.* **2000**, *57*, 144−152.
(12) Löber, S.; Hübner, H.; Gmeiner, P. Azaindole derivatives with high affinity for the dopamine D4 receptor: synthesis, ligand binding studies and comparison of molecular electrostatic potential maps. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 97−102.
(13) Hübner, H.; Kraxner, J.; Gmeiner, P. Cyanoindole derivatives as highly selective dopamine D4 receptor partial agonists: solid-phase synthesis, binding assays, and functional experiments. *J. Med. Chem.* **2000**, *43*, 4563−4569.
(14) Gösl, R.; Meuwsen, A. 1-Aminopyridinium iodide. *Org. Synth.* **1963**, *43*, 1−3.
(15) Anderson, P. L.; Hasak, J. P.; Kahle, A. D.; Paolella, N. A.; Shapiro, M. J. 1,3-Dipolar addition of pyridine *N*-imine to acetylenes and the use of 13-C NMR in several structural assignments. *J. Heterocycl. Chem.* **1981**, *18*, 1149−1152.
(16) Wright, W. B. Alkylation of amines by esters and lithium aluminium hydride. *J. Org. Chem.* **1962**, *27*, 1042−1045.
(17) Boekelheide, V.; Fedoruk, N. A. Syntheses of fused aromatic heterocycles by 1,3-dipolar addition reactions. 3-azapyrrocolines. *J. Org. Chem.* **1968**, *33*, 2062−2064.
(18) Gmeiner, P.; Sommer, J. Azaindole Derivatives I: Asymmetric Synthesis of a Novel α-Amino Acid. *Arch. Pharm. (Weinheim)* **1988**, *321*, 505−507.
(19) For similar lithiation reactions of **12**, see: Aboul-Fadl, T.; Löber, S.; Gmeiner, P. Effective and variable functionalization of pyrazolo[1,5-*a*]pyridines involving palladium-catalyzed coupling reactions. *Synthesis* **2000**, 1727−1732 and references therein.
(20) Gmeiner, P.; Sommer, J. Selective Dopamine D2 Autoreceptor Agonists with 8-Azaindole Substructure: Synthesis and Theoretical Investigations. *Arch. Pharm. (Weinheim)* **1994**, *327*, 435−443.
(21) Hayes, G.; Biden, T. J.; Selbie, L. A.; Shine J. Structural subtypes of the dopamine D2 receptor are functionally distinct: expression of the cloned $D2_A$ and $D2_B$ subtypes in a heterologous cell line. *Mol. Endocrinol.* **1992**, *6*, 920−926.
(22) Sokoloff, P.; Andrieux, M.; Besançon, R.; Pilon, C.; Martres, M.-P.; Giros, B.; Schwartz, J.-C. Pharmacology of human dopamine D_3 receptor expressed in a mammalian cell line: comparison with D_2 receptor. *Eur. J. Pharmacol.* **1992**, *225*, 331−337.
(23) Asghari, V.; Sanyal, S.; Buchwaldt, S.; Paterson, A.; Jovanovic, V.; Van Tol, H. H. M. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J. Neurochem.* **1995**, *65*, 1157−1165.
(24) Hübner, H.; Haubmann, C.; Utz, W.; Gmeiner, P. Conjugated enynes as nonaromatic catechol bioisosteres: synthesis, binding experiments, and computational studies of novel dopamine receptor agonists recognizing preferentially the D_3 subtype. *J. Med. Chem.* **2000**, *43*, 756−762.
(25) Chio, C.; Lajiness, M. E.; Huff, R. M. Activation of heterologously expressed D3 dopamine receptors: comparison with D2 dopamine receptors. *Mol. Pharmacol.* **1994**, *45*, 51−60.
(26) Mierau, J.; Schneider, F. J.; Ensinger, H. A.; Chio, C. L.; Lajiness, M. E.; Huff, R. M. Pramipexole binding and activation of cloned and expressed dopamine D_2, D_3 and D_4 receptors. *Eur. J. Pharmacol.* **1995**, *290*, 29−36.
(27) Lanig, H.; Utz, W.; Gmeiner, P. Comparative molecular field analysis of dopamine D4 receptor antagonists including 3-[4-(4-chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-*a*]pyridine (FAUC 113), 3-[4-(4-chlorophenyl)piperazin-1-ylmethyl]-1*H*- pyrrolo[2,3-*b*]pyridine (L-745,870), and clozapine. *J. Med. Chem.* **2001**, *44*, 1151−1157.
(28) The receptor partial structure was obtained from www.gpcr.org/7tm/models/vriend/D4DR_HUMAN.ent. For visualization and manual docking, the software package SYBYL 6.7 was used.

JM015522J