Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 633–636

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Di- and Trisubstituted Pyrazolo[1,5-*a*]pyridine Derivatives: Synthesis, Dopamine Receptor Binding and Ligand Efficacy

Stefan Löber, Tarek Aboul-Fadl, Harald Hübner and Peter Gmeiner*

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

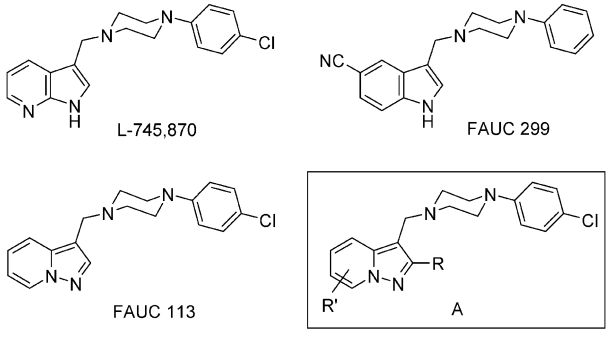
Received 29 October 2001; revised 28 November 2001; accepted 30 November 2001

Abstract—Based on the lead molecule FAUC 113, a series of di- and trisubstituted pyrazolo[1,5-*a*]pyridine derivatives was synthesized and investigated for their dopamine receptor binding profile. The carbonitrile **11a** (FAUC 327) showed excellent pharmacological properties combining high D4 affinity ($K_i = 1.5$ nM) and selectivity with significant intrinsic activity (31%) in low nanomolar concentrations ($EC_{50} = 1.5$ nM). © 2002 Elsevier Science Ltd. All rights reserved.

The use of classical neuroleptics is frequently associated with acute movement disorders, tardive dyskinesia and hyperlactinemia, which are due to an unselective blockade of the D2-like dopamine receptor family.[1] In contrast, the atypical neuroleptic clozapine, which does not induce these side effects, reveals significant selectivity for the D4 subtype.[2–4] On the other hand, selective D4 receptor agonists or partial agonists might be of interest for the treatment of attention deficit hyperactivity disorders (ADHD), mood disorders, and Parkinson's disease.[5,6]

A number of SAR studies including piperazinylmethyl substituted indoles and azaindoles led to the partial D4 agonists L-745,870, FAUC 299 and FAUC 113.[7–9] According to molecular orbital calculations, their preference for the D4 subtype strongly depends on the size and shape of the negative molecular electrostatic potential (MEP) formed by the heteroaromatic moiety when a large negative region extending the area of the heteroarene obviously is not tolerated by the D1, D2 and D3 subtypes.

In order to further improve the pharmacological properties, we tried to develop structural derivatives combining high D4 affinity and selectivity with significant ligand efficacy in low nanomolar concentrations. Employing the 7a-azaindole FAUC 113 as a major lead, we herein report on di- and trisubstituted analogues of type A when an extension of the π-system of the pyrazolo[1,5-*a*]pyridine moiety turned out advantageous.

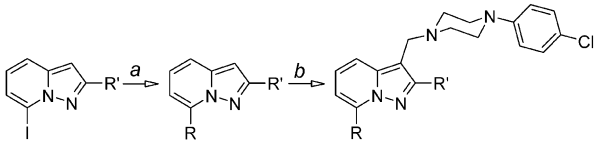


Taking into account the binding properties of our recently described iodine labeled radioligand,[10] our synthetic investigations were initiated by site-directed transformations facilitating the incorporation of substituents into position 7 of the pyrazolo[1,5-*a*]pyridine moiety. Taking advantage of the ability of N1 to direct metalation into position 7,[11] the 7-iodo pyrazolo[1,5-*a*]pyridine **1a** and its 2-methyl derivative **1b** were readily synthesized by treatment of the corresponding lithiated heterocycles with diiodoethane. Subsequent palladium catalyzed coupling reactions resulted in formation of the 7-alkynyl, -aryl, -amino or -cyano derivatives **2a**–**6a** and **2b**–**6b**.[12] Employing these valuable building blocks as educts, we synthesized the piperazinylmethyl substituted azaindoles **7a**–**11a** and **7b**–**11b** by Mannich aminomethylation (Table 1).[13,14]

*Corresponding author. Tel.: +49-9131-852-9383; fax: +49-9131-852-2585; e-mail: gmeiner@pharmazie.uni-erlangen.de

PII: S0960-894X(01)00814-9

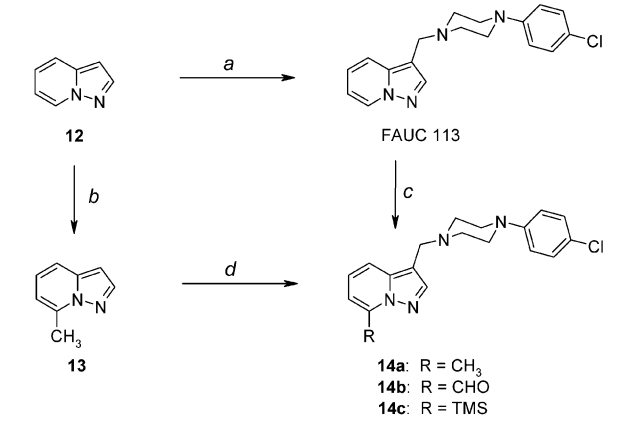
Table 1. (a) See ref 12; (b) 4-chlorophenylpiperazine dihydrochloride, $Et(Me)_2N$, CH_2O, CH_2Cl_2, CH_3COOH, rt, 16 h



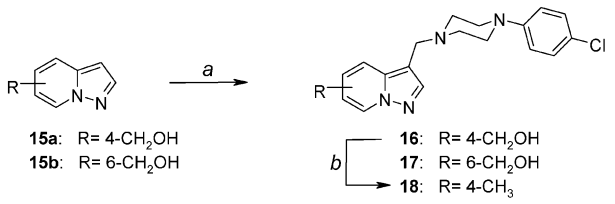
		R	R′	Yield (%)
2a	**7a**	CCH	H	82
3a	**8a**	Phe	H	77
4a	**9**	4-F-Phe	H	67
5a	**10a**	$N(CH_2CH_2)_2NBn$	H	56
6a	**11a**	CN	H	60
2b	**7b**	CCH	CH_3	75
3b	**8b**	Phe	CH_3	60
4b	**9b**	4-F-Phe	CH_3	63
5b	**10b**	$N(CH_2CH_2)_2NBn$	CH_3	53
6b	**11b**	CN	CH_3	59

In order to extend the variety of substituents, introduction of electrophiles via an organolithium intermediate was envisioned. Thus, lithiation of the pyrazolopyridine **12** followed by trapping with MeI resulted in formation of the 7-methylazaindole **13** as outlined in Scheme 1. Subsequent Mannich reaction furnished the corresponding 3-aminomethyl analogue **14a**. Regioselective lithiation of the heteroarene also proved possible in the presence of the piperazinylmethyl functionality. Thus, treatment of FAUC 113 with *n*-BuLi at dry ice temperature followed by addition of ethyl formate or TMSCl resulted in the corresponding test compounds **14b** and **14c**, respectively.

Finally, we envisioned synthesizing 4- and 6-substituted analogues. Starting from the (hydroxymethyl)pyrazolo[1,5-*a*]pyridines **15a**,**b**[15] we obtained the aminomethylation products **16** and **17** in 54 and 47% yield, respectively. O-Activation of the regioisomer **16** followed by $LiAlH_4$ reduction gave access to the 4-methyl derivative **18**. In contrast to the lead structure FAUC 113, the resulting 4-methylpyrazolo[1,5-*a*]pyridine **18** is expected to be conformationally restricted when looking at the rotation of the piperazinylmethyl side chain, whereas the electrostatic properties of both molecules should be very similar (Scheme 2).



Scheme 1. (a) See ref 9; (b) (1) *n*-BuLi, THF, −78 °C, 20 min; (2) MeI, THF, −78 °C, 30 min (84%); (c) (1) *n*-BuLi, THF, −78 to 0 °C, 1 h; (2) HCOOEt, THF, −78 °C to rt, 1 h (**14b**: 78%); (2) TMSCl, THF, −78 °C to rt, 1 h (**14c**: 51%); (d) 4-chlorophenylpiperazine dihydrochloride, $Et(Me)_2N$, CH_2O, DCM, CH_3COOH, rt, 4 h (**14a**: 78%).



Scheme 2. (a) 4-Chlorophenylpiperazine dihydrochloride, $Et(Me)_2N$, CH_2O, DCM, CH_3COOH, rt, 4 h (54% for **16**; 47% for **17**); (b) (1) MsCl, $Et(Me)_2N$, THF, rt, 2 h; (2) $LiAlH_4$, THF, rt, 30 min (47%).

Receptor binding profiles of the test compounds **7a**,**b**–**11a**,**b**, **14a**–**c** and **16**–**18** were determined in vitro by measuring their ability to compete with [^{3}H]spiperone for the cloned human dopamine receptor subtypes $D2_{long}$, $D2_{short}$,[16] D3[17] and D4.4[18] stably expressed in Chinese hamster ovary cells (CHO).[19] D1 receptor affinities were measured employing bovine striatal membranes and the D1 selective radioligand [^{3}H]SCH 23390.[19]

The resulting K_i values of the test compounds are listed in Table 2 in comparison to FAUC 113, L-745,870 and the atypical neuroleptic clozapine. In fact, a variety of saturated and unsaturated substituents in position 7 of the heteroarene is tolerated by the D4 subtype when incorporation of an alkynyl- (**7a**,**b**), cyano- (**11a**,**b**), methyl- (**14a**), formyl- (**14b**) or TMS-substituent (**14c**) provided K_i values between 1.2 and 2.8 nM. On the other hand, introduction of an aryl- (**8a**,**b**, **9a**,**b**) or benzylpiperazine-substituent (**10a**,**b**) led to a strong reduction of D4 affinity. Binding data of all the target compounds investigated showed remarkable D4 selectivity, being superior to that of clozapine. Whereas the test compound **7a** displayed superior D4 affinity, the 2-methyl-7-alkynyl-derivative **7b** as well as the 7-cyanopyrazolo[1,5-*a*]pyridines **11a**,**b** revealed an outstanding

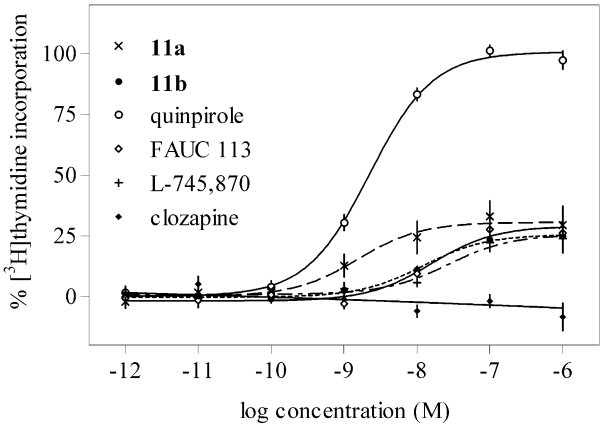
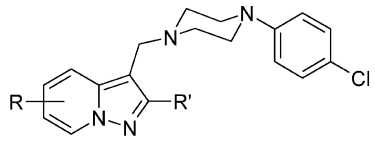


Figure 1. Stimulation of mitogenesis as a functional assay to investigate the agonist effect of **11a** and **11b** at the human dopamine D4.2 receptor in comparison with the full agonist quinpirole, the partial agonists FAUC 113 and L-745,870 and the antagonist clozapine.

Table 2. Binding data of target compounds to human and bovine dopamine receptor ligands[a]



Compd	R	Pos. of R	R′	D1	$D2_{long}$	$D2_{short}$	D3	D4
7a	CCH	7	H	810	3700	6200	4600	1.2
7b	CCH	7	CH_3	15,000	21,000	25,000	17,000	1.5
8a	Ph	7	H	930	5400	2800	840	7.6
8b	Ph	7	CH_3	3300	28 000	24,000	3100	37
9a	4-F-Ph	7	H	7000	15,000	15,000	5700	29
9a	4-F-Ph	7	CH_3	1000	14,000	16,000	3800	87
10a	$N(CH_2CH_2)_2NBn$	7	H	1500	640	400	410	180
10b	$N(CH_2CH_2)_2NBn$	7	CH_3	1500	300	120	270	26
11a	CN	7	H	2500	44,000	45,000	26,000	1.5
11b	CN	7	CH_3	11,000	59,000	58,000	21,000	2.3
14a	CH_3	7	H	2200	4900	5000	3800	2.7
14b	CHO	7	H	1200	9500	9200	5900	2.6
14c	TMS	7	H	7100	3100	2800	2000	2.8
16	CH_2OH	4	H	4800	56,000	79,000	> 100,000	110
17	CH_2OH	6	H	1400	28,000	22,000	2600	20
18	CH_3	4	H	7000	13,000	12,000	9400	50
FAUC 113	H	—	H	12,000	3200	4300	5000	3.1
L-745,870				9900	54,000	38,000	22,000	0.62
Clozapine				420	41	28	960	16

[a]K_i values are the means of two to four independent experiments using eight different concentrations each in triplicate.

Table 3. Intrinsic activity of the azaindoles **11a,b** in relation to the reference compounds FAUC 113, L-745,870, quinpirole and clozapine at the human D4.2 receptor established by measuring the stimulation of mitogenesis

	Test compounds					
	11a	**11b**	FAUC 113	L-745,870	Quinpirole	Clozapine
Intrinsic effect[a] (%)	31	26	26	24	100	< 3
EC_{50} (nM)	1.5	12	12	27	2.0	nd

[a]Rate of incorporation of [^{3}H]thymidine as evidence for mitogenetic acivity related to the full agonist quinpirole as the means of quadruplicates from four to 11 experiments. EC_{50} values in nM are derived from the mean curves of the experiments. nd, not determined.

D4 binding profile, including a 10,000–30,000-fold selectivity over the D2 and D3 subtypes. It is interesting to note that the 2-methyl substitution of **7b**, **8b** and **11b** significantly reduced D4 affinity. Hydroxymethyl or methyl substituents in the positions 4 or 6 of the pyrazolo[1,5-*a*]pyridine moiety (**16**–**18**) caused substantially reduced D4 receptor binding.

In order to further characterize the binding properties of the most potent and selective D4 ligands **11a,b**, 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 (2200 nM for **11a** and 4500 nM for **11b**) and 5-HT2 affinities (180 nM for **11a** and 1000 nM for **11b**). In order to investigate potential ligand efficacy, the highly selective test compounds **11a**,**b** were evaluated for their mitogenetic activity. Agonist activation of dopamine receptors is known to increase mitogenesis in heterologously transfected cell lines. This can be determined by measuring the rate of [^{3}H]thymidine incorporation into growing cells.[20] Intrinsic activity can be quantified by determination of the effective concentration (EC_{50}) of a test compound and by comparing the maximal effect to that of a full agonist. CHO10001 cells stably expressing the human D4.2 receptor were established for this mitogenesis assay.[8,21] Comparative experiments were performed with the full agonist quinpirole, the partial agonists FAUC 113 and L-745,870 and the antagonist clozapine. Figure 1 shows dose–response curves clearly indicating partial agonist effects of **11a** and **11b** (31 and 26% relative to quinpirole, respectively) comparable to those of FAUC 113 and L-745,870.

Interestingly, the dose–response curve and the EC_{50} value for the 7-cyanopyrazolopyridine **11a** (FAUC 327) demonstrate that the partial agonist effect is exerted at substantially lower concentrations than for L-745,870 and FAUC 113 (Table 3), possibly indicating superior properties in vivo.

In summary, SAR studies on analogues of the D4 partial agonist FAUC 113 resulted in the finding that an extension of the π-system of the pyrazolo[1,5-*a*]pyridine moiety by the introduction of suitable substituents into position 7 is tolerated well by the D4 receptor. Extraordinary high selectivity was observed when a cyano group was selected, leading to the partial agonists **11a**

(FAUC 327) and **11b**. Functional studies of FAUC 327 showed an 8–18-fold lower EC_{50} value when compared to L-745,870 and FAUC 113.

Acknowledgements

The authors wish to 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, USA) is acknowledged for providing a D4 expressing cell line employed for mitogenesis. Thanks are also due to Mrs. H. Szczepanek, Mrs. P. Schmitt and Mrs. P. Hübner for skillful technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft, the BMBF, and the Fonds der Chemischen Industrie.

References and Notes

1. Andersen, P. H.; Nielsen, E. B. *Drugs News Perspect*. **1991**, *4*, 150.
2. Van Tol, H. H. M.; Bunzow, J. R.; Guan, H.-C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. *Nature* **1991**, *350*, 610.
3. Seeman, P.; Guan, H. C.; Van Tol, H. H. M. *Nature* **1993**, *365*, 441 –445.
4. Hrib, N. J. *Drugs Future* **2000**, *25*, 587.
5. McCracken, J. T.; Smalley, S. L.; McGough, J. J.; Crawford, L.; Del'Homme, M. A.; Cantor, R. M.; Liu, A.; Nelson, S. F. *Mol. Psychiatry* **2000**, *5*, 531.
6. Lichter, J. B.; Barr, C. L.; Kennedy, J. L.; Van Tol, H. H.; Kidd, K. K.; Livak, K. J. *Hum. Mol. Genet*. **1993**, *2*, 767.
7. 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. *Med. Chem*. **1996**, *39*, 1941.
8. Hübner, H.; Kraxner, J.; Gmeiner, P. *J. Med. Chem*. **2000**, *43*, 4563.
9. Löber, S.; Hübner, H.; Gmeiner, P. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 97.
10. Prante, O.; Löber, S.; Hübner, H.; Gmeiner, P.; Kuwert, T. *J. Label. Compd. Radiopharm*. **2001**, *44*, 849.
11. Finkelstein, B. L. *J. Org. Chem*. **1992**, *57*, 5539.
12. Aboul-Fadl, T.; Löber, S.; Gmeiner, P. *Synthesis* **2000**, 1727.
13. Typical procedure: A mixture of pyrazolo[1,5-*a*]pyridine derivative (0.50 mmol), 4-chlorophenylpiperazine dihydrochloride (0.55 mmol), $Et(Me)_2N$ (1.1 mmol), formaldehyde (37% solution in H_2O; 2.0 mmol) and acetic acid (4 mL) was stirred at room temp. for 16 h. The reaction mixture was neutralized with 2 N NaOH and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (silica gel, EtOAc).
14. Analytical data for **11a**: IR (cm^{-1}) 3090 (arom. C–H), 2821 (aliph. C–H), 2239 (C≡N), 1624 (C=N), 1496 (C=C), 1233 (C–N); ^1H NMR (360 MHz) δ 2.61 (br t, 4H, 2 × CH_2–N–CH_2–CH_2), 3.15 (br t, 4H, 2 × –CH_2–CH_2–N–Ph), 3.77 (s, 2H, Het–CH_2–N), 6.79–6.82 (m, 2H, 4-Cl-Ph), 7.12–7.26 (m, 3H, 4-Cl–Ph and H-5), 7.33 (dd, J = 7.0, 1.0 Hz, 1H, H-4), 7.96 (dd, J = 9.0, 1.0 Hz, 1H, H-6), 8.06 (s, 1H, H-2); EI–MS: m/z 351 (M^+). Anal. calcd for $C_{19}H_{18}N_5Cl$: C 65.73; H 5.52; N 19.17. Found: C 65.57; H 5.60; N 18.97.
15. Löber, S.; Hübner, H.; Utz, W.; Gmeiner, P. *J. Med. Chem*. **2001**, *44*, 2691.
16. Hayes, G.; Biden, T. J.; Selbie, L. A.; Shine, J. *Mol. Endocrinol*. **1992**, *6*, 920.
17. Sokoloff, P.; Andrieux, M.; Besançon, R.; Pilon, C.; Martres, M.-P.; Giros, B.; Schwartz, J.-C. *Eur. J. Pharmacol*. **1992**, *225*, 331.
18. Asghari, V.; Sanyal, S.; Buchwaldt, S.; Paterson, A.; Jovanovic, V.; Van Tol, H. H. M. *J. Neurochem*. **1995**, *65*, 1157.
19. Hübner, H.; Haubmann, C.; Utz, W.; Gmeiner, P. *J. Med. Chem*. **2000**, *43*, 756.
20. Chio, C.; Lajiness, M. E.; Huff, R. M. *Mol. Pharmacol*. **1994**, *45*, 51.
21. Mierau, J.; Schneider, F. J.; Ensinger, H. A.; Chio, C. L.; Lajiness, M. E.; Huff, R. M. *Eur. J. Pharmacol*. **1995**, *290*, 29.