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# Fused Azaindole Derivatives: Molecular Design, Synthesis and In Vitro Pharmacology Leading to the Preferential Dopamine D3 Receptor Agonist FAUC 725

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**Abstract**—Computational studies based on the similarity of molecular electrostatic potential maps initiated the synthesis of the tricyclic target compounds **1** (FAUC 725) and **2**. Receptor binding studies at the dopamine receptor subtypes D1, $D2_{long}$, $D2_{short}$, D3 and D4 showed that the azaindole **1** revealed D3 affinity ($K_i = 0.54$ nM) comparable to the lead pramipexole and enhanced selectivity over D2 and D4. Mitogenesis experiments indicated substantial intrinsic activity for the D3 selective dipropylamine **1**. Based on the structure of (*S*)-3-PPP, bioisosteric replacement and conformational restriction leading to the test compound **2** was not fruitful.


Dopamine neurotransmission is intimately involved in the pathophysiology of Parkinson's disease and schizophrenia.[1] There is strong evidence that D2 and D3 receptors exist both postsynaptically and as autoreceptors controlling dopamine synthesis, release and neuronal firing. The D3 receptor subtype, which is preferentially located in the mesolimbic region of the brain, can be considered as particularly related to affective disorders.[2] Thus, the discovery of selective dopamine D3 receptor agonists is of current interest for the treatment of unipolar major depression and for affecting the negative symptoms of schizophrenia.

Conformationally restricted dopamine analogues including (*R*)-7-OH-dipropylaminotetralin (7-OH-DPAT) and (*S*)-3-PPP are known for their high affinity to the D2 and D3 receptor subtypes.[3] Although substantial in vitro activity is reported, these compounds are crucial for an application in vivo which is due to their instability towards metabolic processes. Bioisosteric replacement of the hydroxyphenyl substructure of 7-OH-DPAT by an aminothiazole moiety led to the DA receptor agonist pramipexole.[4] We recently reported that a conjugated enyne moiety can serve as nonaromatic catechol bioisostere leading to the potent D3 receptor agonist FAUC 73.[5] As an extension of these structure–activity relationship studies we were intrigued by the question whether the pyrazolo[1,5-*a*]pyridine nucleus, which proved to be a valuable pharmacophoric element in the field of dopamine D4 receptor ligands,[6] could also serve as a potent structural framework for the construction of novel D3 receptor agonists.

For the molecular design of our target compounds, we exploited molecular electrostatic potentials (MEPs), being derived from MOPAC ESP charges of geometrically optimized structures, as markers of similarity. Calculations were conducted by the SYBYL program package using the AM1-algorithm as implemented in MOPAC 6.0. Based on the comparison of molecular electrostatic potential maps of a series of dopamine D2/D3 agonists with special focus on pramipexole and (*S*)-3-PPP, we approached the tetrahydropyrido[1,2-*b*]-indazole **1** and the tetrahydrotriazafluorene **2**, respectively. The 10π-system of **1** and **2** requires more steric demand and the six-membered rings are forced to adopt an approximately coplanar orientation for **2** which is different to energetically favorable conformations of (*S*)-3-PPP. However, both target compounds show high conformity with their lead structures with respect to the size, shape and direction of the negative isopotentials (−2 kcal/mol), indicated in red (Fig. 1). We supposed this feature to be essential for high D3 affinity.

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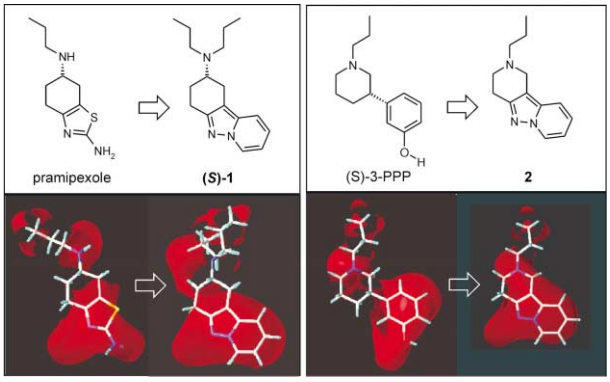
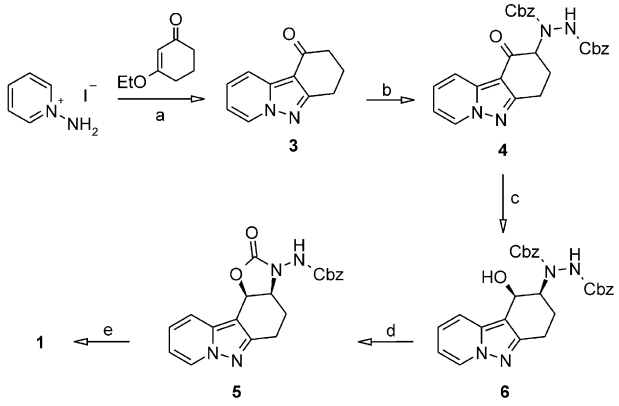



**Figure 1.** Molecular design of the potential dopamine D3 receptor agonists **1** and **2** based on the similarity of negative MEPs depicted as isopotential surfaces (−2 kcal/mol).




**Scheme 1.** (a) $K_2CO_3$, $HOCH_2CH_2OH$, 130 °C, 16 h (38%); (b) LiHMDS, DBAD, THF, −78 °C, 0.5 h (54%); (c) $LiBEt_3H$, THF, −78 °C, 2 h (not isolated); (d) THF, rt, 1 h [(c) + (d) 82%]; (e) (1) $H_2$, Ra–Ni, Pd/C, MeOH, rt, 18 h; (2) $CH_3CH_2CHO$, $NaCNBH_3$, rt, 6 h (34%).

As the key reaction step for an effective synthesis of the conformationally restricted β-arylethylamine **1** in racemic form, we planned to exploit our previously reported methodology involving electrophilic amination and subsequent reductive degradation.[7] Since our efforts to synthesize the pyridoindazolone framework according to ref 8 failed, the tricyclic ring system was constructed by cycloaddition reaction of *N*-aminopyridinium iodide and 3-ethoxycyclohexenone, resulting in formation of the ketone **3** in 38% yield.[9]

Deprotonation with LiHMDS and trapping of the resulting enolate by addition of dibenzyl azodicarboxylate (DBAD) afforded the Cbz-protected hydrazine **4** (Scheme 1).[10] Diastereoselective *cis*-reduction with $LiBEt_3H$ at −78 °C gave the protected hydrazino alcohol **6**. Warming up of the reaction mixture to room temperature furnished the oxazolidinone **5** in 82% yield.[11] Finally, reductive degradation involving removal of the protecting group as well as *N,N*-cleavage and hydrogenolysis of the benzylic C–O bond followed by reductive alkylation with propanal afforded the final product **1** in analytically pure form.[12]

For the preparation of the 3-PPP analogue **2**, we started from the pyrazolo[1,5-*a*]pyridine derivative **7**, which was synthesized by cyclization of *N*-aminopyridinium iodide with dimethyl acetonedicarboxylate following the protocol of Awano and Suzue,[13] which was slightly modified to give **7** in 75% yield (Scheme 2). Hydrolysis, regioselective decarboxylation and subsequent esterification resulted in formation of the ethyl carboxylate **8**, which was reduced to the corresponding hydroxyethyl derivative **10** in almost quantitative yield. The alcohol function was activated with MesCl and transformed into the secondary amine **9** by treatment with *n*-$PrNH_2$. Finally, ring closure could be accomplished by intramolecular Mannich reaction, leading to the desired tetrahydrotriazafluorene **2** in 71% yield.[14]

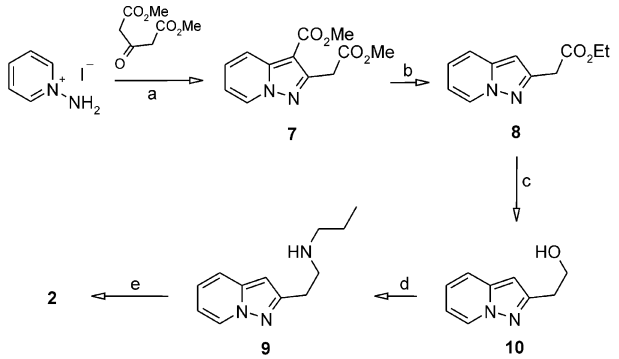
Receptor binding profiles of the test compounds **1** and **2** were established in vitro by measuring their ability to compete with [$^3$H]spiperone for the cloned human dopamine receptor subtypes $D2_{long}$, $D2_{short}$,[15] D3[16] and D4.4[17] stably expressed in Chinese hamster ovary cells (CHO).[5] D1 receptor affinities were measured employing bovine striatal membranes and the D1 selective radioligand [$^3$H]SCH 23390.[5] Bovine striatal membranes labeled by [$^3$H]pramipexole selectively recognizing the high affinity ternary complex were utilized for investigating D2 agonist properties. The resulting $K_i$ values are listed in Table 1 compared to the dopaminergic lead compounds pramipexole and (*S*)-3-PPP.

Our initial investigations involved the observation of [$^3$H]pramipexole competition, clearly indicating poor receptor recognition for the test compound **2** ($K_i$ = 3100 nM) but substantial high affinity binding of the dipropylamine **1** ($K_i$ = 8.6 nM). Both azaindoles showed weak

**Table 1.** Binding data of the test compounds **1** (FAUC 725) and **2** for the human $D2_{long}$, $D2_{short}$, D3 and D4 receptors and the bovine D1 and D2 receptors in correlation to the reference compounds pramipexole and (*S*)-3-PPP [$K_i$ values (nM) based on the means of 2–5 experiments each performed in triplicate]

| Test compd | $K_i$ values in (nM)[a] | | | | | |
|---|---|---|---|---|---|---|
| | [$^3$H]SCH23390 | [$^3$H]Spiperone | | | | [$^3$H]Pramipexole |
| | bD1 | $hD2_{long}$ | $hD2_{short}$ | hD3 | hD4.4 | bD2 |
| **1** | 18,000 | 85/6400 | 35/3700 | 0.54/59 | 50/2200 | 8.6 |
| **2** | 21,000 | 230/53,000 | 130/50,000 | 16/16,000 | 87/23,000 | 3100 |
| Pramipexole | nd | 27/5400 | 40/3600 | 0.87/44 | 8.5/130 | 1.6 ($K_d$) |
| (*S*)-3-PPP | nd | 9.0/1800 | 27/1800 | 25/1600 | 16/1300 | 52 |

nd, values not determined.
[a]High/low affinity binding sites as indicated by nonlinear regression analysis and further fitting of the calculated curves using PRISM (Graph Pad).



**Scheme 2.** (a) $K_2CO_3$, DMF, 50 °C, 2 h (75%); (b) (1) $H_2SO_4$ (40%), 100 °C, 3 h; (2) $EtOH/H_2SO_4$, reflux, 6 h (83%); (c) $LiAlH_4$, THF, 0 °C, 1 h (99%); (d) (1) MesCl, TEA, THF, 0 °C, 0.5 h; (2) $PrNH_2$, 50 °C, 12 h (86%); (e) $CH_2O$, HOAc, rt, 0.5 h (71%).

**Table 2.** Intrinsic activities of the fused azaindoles **1** (FAUC 725) and **2** and the reference compounds pramipexole and (*S*)-3-PPP in relation to the full agonist quinpirole at the rat $D2_{long}$, rat $D2_{short}$, human D3 and human D4.2 receptors established by measuring the stimulation of mitogenesis

| Test compd | $D2_{long}$ | $D2_{short}$ | D3 | D4.2 |
|---|---|---|---|---|
| | $EC_{50}$ (nM)[a] | $EC_{50}$ (nM)[a] | $EC_{50}$ (nM)[a] | $EC_{50}$ (nM)[a] |
| **1** | 16 (101%) | 47 (92%) | 2.8 (82%) | 430 (73%) |
| **2** | >1000 | >1000 | >1000 | >1000 |
| Pramipexole | 9.2 (85%) | 12 (103%) | 1.5 (93%) | 15 (84%) |
| (*S*)-3-PPP | 19 (54%) | 19 (82%) | 9.8 (68%) | 60 (65%) |

[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 4 to 10 experiments. $EC_{50}$ values in nM are derived from the mean curves of the experiments.

D1 affinity. Employing the cloned human dopamine receptor subtypes, careful analysis of the competition experiments employing a large number of test concentrations of **1** (FAUC 725) clearly displayed biphasic curves for the members of the D2 family when the respective Hill coefficients and a better fit of equations indicated a two-site competition. We determined $K_i$ values of 85, 35, 0.54 and 50 nM for the high affinity binding sites of $D2_{long}$, $D2_{short}$, D3 and D4, respectively, representing the G-protein coupled ternary complexes. Furthermore, D3 selectivity proved to be superior when compared to pramipexole, especially over $D2_{long}$ and D4. In contrast, the triazafluorene derivative **2** displayed disappointing dopamine receptor binding. Due to common structural features of **1** with 8-OH-DPAT and derivatives thereof, $5HT1_A$ recognition was evaluated with the aid of [$^3$H]8-OH-DPAT-labeled porcine brain homogenates when the resulting $K_i$ values indicated only moderate $5\text{-}HT1_A$ affinity ($K_i = 2500$ nM).

Ligand efficacy of FAUC 725 (**1**) and **2** was confirmed by mitogenesis assays measuring the rate of [$^3$H]thymidine incorporation into growing CHO10001 cells stably expressing $D2_{long}$, $D2_{short}$ and D4.2[18] and D3 receptor expressing CHOdhfr$^-$ cells, respectively.[16] Intrinsic activity was 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.[4,19] The data listed in Table 2 clearly show substantial ligand efficacy of FAUC 725 (**1**). According to the ratio of $EC_{50}$ values, the selectivity profile being determined by the binding experiments could be confirmed. By contrast, the conformationally restricted (*S*)-3-PPP analogue **2** did not exert significant intrinsic activity within the investigated dose range.

In conclusion, molecular design based on the similarity of characteristic MEP maps proved successful for the development of the preferential D3 agonist FAUC 725 (**1**). Obviously, the increased steric demand compared to the lead structure can be tolerated by the receptor excluded volume. On the other hand, the conformational restriction involved in the design of the (*S*)-3-PPP analogue **2** led to a strong reduction of biological activity.

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9. **3**: A mixture of *N*-aminopyridinium iodide (2.0 g; 9 mmol), $K_2CO_3$ (2.5 g; 18 mmol) and ethane-1,2-diol (30 mL) was treated with 3-ethoxycyclohexenone (3.0 g; 21 mmol) and stirred for 16 h at 130 °C. After cooling to rt, satd $NaHCO_3$ and $Et_2O$ were added. The organic layer was dried ($Na_2SO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether/EtOAc 1:1) to yield pure **3**[8] (0.92 g, 38%) as a colorless solid.
10. **4**: LiHMDS (1.0 M in hexane; 3.3 mL; 3.3 mmol) was added to a solution of **3** (0.60 g; 3.2 mmol) in THF (30 mL) at −78 °C. After 30 min, the mixture was transferred to a precooled solution of dibenzyl azodicarboxylate (1.32 g; 4.4 mmol) in THF (15 mL) and stirred at −78 °C for additional 5 min. After addition of satd $NaHCO_3$ and EtOAc, the organic layer was dried ($Na_2SO_4$) and evaporated and the residue was purified by flash chromatography (EtOAc/$CH_2Cl_2$/MeOH 25:25:1) to yield pure **4** (0.84 g, 54%) as a colorless solid. $^1$H NMR ($CDCl_3$, 360 MHz): δ (ppm) 2.30–2.47 (m, 1H), 2.56–2.69 (m, 1H), 3.05–3.28 (m, 2H), 4.94–5.30 (m, 5H), 6.92 (br s, 1H), 7.03 (ddd, $J$ = 7.0, 7.0, 1.5 Hz, 1H), 7.21–7.37 (m, 10H), 7.49 (dd, $J$ = 8.5, 7.0 Hz, 1H), 8.11 (br d, $J$ = 8.5 Hz, 1H), 8.49 (br d, 7.0 Hz, 1H); EI–MS: $m/z$ 484 ($M^+$). Anal. calcd for $C_{27}H_{24}N_4O_5 \cdot 1/4H_2O$: C 66.30; H 5.05; N 11.46. Found: C 66.20; H 5.16 N 11.30.
11. **5**: To a solution of **4** (0.60 g; 1.24 mmol) in THF (12 mL) was added Super-hydride© (1 M in THF; 1.30 mL; 1.30 mmol) at −78 °C. The mixture was stirred for 2 h and warmed to rt. After 1 h, satd $NaHCO_3$ and $CH_2Cl_2$ were added. The organic layer was dried ($Na_2SO_4$) and evaporated and the residue was purified by flash chromatography (EtOAc/$CH_2Cl_2$/MeOH 10:10:1) to yield 0.39 g (82%) as a colorless solid. IR ($cm^{-1}$): 3185, 2986, 1781, 1637, 1537, 1509, 1238; $^1$H NMR ($CDCl_3$, 360 MHz): δ (ppm) 1.93–2.06 (m, 1H), 2.25–2.36 (m, 1H), 2.75–3.03 (m, 2H), 4.46–4.64 (m, 1H), 5.10–5.32 (m, 2H), 5.92 (d, $J$ = 7.5 Hz, 1H), 6.78 (ddd, $J$ = 7.0, 7.0, 1.5 Hz, 1H), 7.04 (br s, 0.5H rotamers), 7.06 (br s, 0.5H rotamers), 7.21 (dd, $J$ = 8.5, 7.0 Hz, 1H), 7.30–7.42 (m, 5H), 7.55 (d, $J$ = 8.5 Hz, 1H), 8.41 (d, 7.0 Hz, 1H); EI–MS: $m/z$ 378 ($M^+$). Anal. calcd for $C_{20}H_{18}N_4O_4 \cdot 1/3H_2O$: C 62.49; H 4.89; N 14.58. Found: C 62.45; H 4.87 N 14.65.
12. **1**: To a solution of **5** (150 mg; 0.4 mmol) in MeOH (10 mL) was added moist Raney–Ni (200 mg) and Pd/C (10%; 50 mg). After stirring for 18 h under $H_2$-atmosphere at ambient temperature, the mixture was filtered and the solvent was evaporated (*Caution*: The crude primary amine is extremely sensitive to air!). The residue was resolved in $CH_2Cl_2$ (10 mL), treated with propionaldehyde (35 μL; 0.6 mmol) and $NaCNBH_3$ (50 mg; 0.8 mmol) and stirred for 6 h at rt. After addition of 2N NaOH and $CH_2Cl_2$, the organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (EtOAc/$CH_2Cl_2$/MeOH 10:10:1) to yield pure **1** (0.36 g, 34%) as a colorless oil. $^1$H NMR ($CDCl_3$, 360 MHz): δ (ppm) = 0.91 (t, $J$ = 7.0 Hz, 6H), 1.50 (tt, $J$ = 7.0, 7.0 Hz, 4H), 1.72–1.86 (m, 1H), 2.10–2.19 (m, 1H), 2.52 (t, $J$ = 7.0 Hz, 4H), 2.58–2.66 (m, 1H), 2.81–2.93 (m, 2H), 3.01–3.12 (m, 2H), 6.62 (ddd, $J$ = 7.0, 7.0/1.5 Hz, 1H), 7.01 (dd, $J$ = 8.5/7.0 Hz, 1H), 7.32 (d, $J$ = 8.5 Hz, 1H), 8.32 (d, $J$ = 7.0 Hz, 1H). EI–MS: $m/z$ 271 ($M^+$). Anal. calcd for $C_{17}H_{25}N_3 \cdot 1/3H_2O$: C 73.60; H 9.33; N 15.15. Found: C 73.45; H 9.24; N 15.17.
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14. A mixture of **9** (40 mg; 0.2 mmol), formaldehyde (40% m/m in $H_2O$; 20 μL; 0.27 mmol) and HOAc (2 mL) was stirred at rt for 30 min. The solution was neutralized with satd $NaHCO_3$ and extracted with $CH_2Cl_2$. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography ($CH_2Cl_2$/MeOH 10:1) to yield pure **2** (30 mg, 71%) as a colorless oil. $^1$H NMR ($CDCl_3$, 360 MHz): δ (ppm) 0.97 (t, $J$ = 7.0 Hz, 3H), 1.66 (tt, $J$ = 7.0, 7.0 Hz, 2H), 2.58 (t, $J$ = 7.0 Hz, 2H), 2.89 (t, $J$ = 6.0, 2H), 3.02 (t, $J$ = 6.0, 2H), 3.71 (s, 2H), 6.64 (ddd, $J$ = 7.0, 7.0, 1.5 Hz, 1H), 7.03 (dd, $J$ = 8.5, 7.0 Hz, 1H), 7.27 (d, $J$ = 8.5 Hz, 1H), 8.36 (d, $J$ = 7.0 Hz, 1H). EI–MS: $m/z$ 215 ($M^+$). Anal. calcd for $C_{13}H_{17}N_3 \cdot 1/2H_2O$: C 69.61; H 8.09; N 18.73. Found: C 69.98; H 7.77; N 18.77.
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