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Benzamide Bioisosteres Incorporating Dihydroheteroazole Substructures: EPC Synthesis and SAR Leading to a Selective Dopamine D4 Receptor Partial Agonist (FAUC 179)

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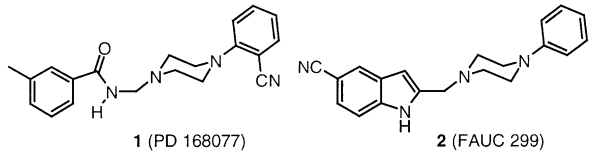
Abstract—Conformationally restricted benzamide bioisosteres were investigated when the chiral phenyldihydroimidazole derivative **4e** (FAUC 179) showed strong and highly selective dopamine D4 receptor binding ($K_{i\text{high}} = 0.95$ nM). Mitogenesis experiments indicated partial agonist properties (42%). EPC syntheses of the target compounds of type **4** were performed starting from α-amino acids.

Besides psychotic, addictive and personality disorders, the dopamine D4 receptor *DRD4* gene[1] has been implicated with ADHD (attention deficit hyperactivity disorders).[2] A genetic association between a 5′ 120-bp tandem duplication polymorphism and an exon 3 48-bp variable number of tandem repeats with ADHD leads to the hypothesis that selective dopamine D4 receptor agonists or partial agonists might be beneficial for an effective pharmacotherapy.[3]



During the last years, a number of SAR studies giving insights into the molecular properties that are responsible for dopamine D4 receptor affinity and selectivity were reported.[4] Furthermore, ligand efficacy was described for a few *N*-arylpiperazines including PD 168077 (**1**),[5] FAUC 299 (**2**),[6] NGD 94-1 (**3**)[7] as well as L-745,870[8] and U-101,958.[9] As a complement to our previous studies on heterocyclic benzamide bioisosteres,[10] we herein report EPC syntheses and biological investigations of dihydroheteroazoles of type **4** designed by a structural hybridization of the lead compounds **1**–**3**. For studying the influence of chirality on the receptor binding profiles, we decided to prepare the target products optically pure starting from the α-amino acids serine, threonine and cysteine in natural and unnatural absolute configuration.

The preparation of the phenyloxazolines **4a**–**d** and the phenylthiazoline **4f** was envisioned by building up the basic scaffolds from the corresponding α-amino esters and suitable activated benzoic acid derivatives. Phenylpiperazine should be introduced by reduction of the ester functionality, activation and subsequent S_N2-displacement. For the synthesis of the highly polar and basic imidazoline derivative **4e**, we decided to perform the cyclization as the last reaction step after preparing an appropriate 1,2-diamine building block.

In detail, (*R*)-serine methyl ester hydrochloride (**5a**)[11] and (2*R*,3*S*)-threonine methyl ester hydrochloride (**5b**)[12] were cyclized with methyl benzimidate[13] to give the oxazoline derivatives **6a**[14,15] and **6b**, respectively (Scheme 1).[16,17] In order to obtain high yields, it was advantageous to perform the reactions in MeOH. Low

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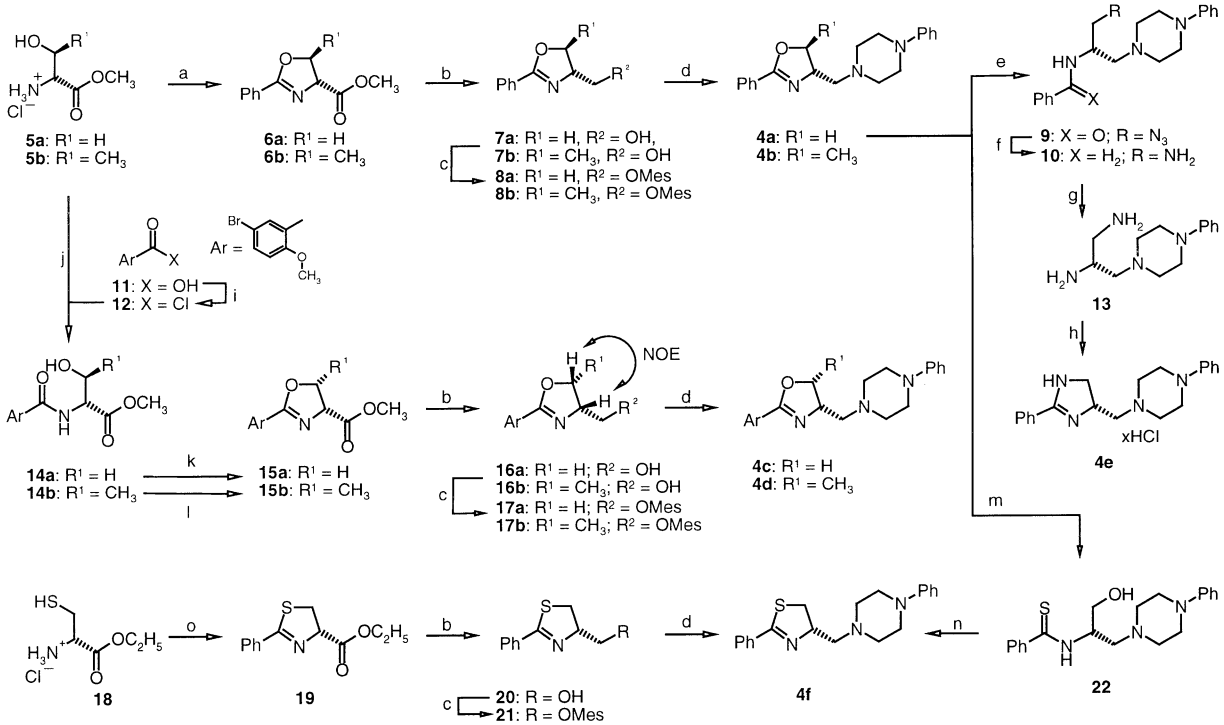
temperature $LiAlH_4$ reduction afforded the hydroxymethyl derivatives **7a** and **7b**, which could be activated to furnish the mesylates **8a,b** in over 90% yield. Nucleophilic displacement with phenylpiperazine resulted in formation of the target compounds **4a,b**. Comparison of the optical rotation data of the intermediates **7a,b** with those reported in the literature for **ent7a,b** did not give any evidence for partial racemization.[16,18,19] For the threonine derivative **4b**, formation of a *cis*-diastereomer as a side product could not be observed in the ^1H NMR spectra, indicating configurational integrity. The 5-bromo-2-methoxy-phenyl analogues were synthesized by *N*-acylation of the serine and threonine ester hydrochlorides **5a,b** with 5-bromo-2-methoxy-benzoyl chloride **12** (available by $SOCl_2$-activation of the corresponding benzoic acid **11**[20]) to give the *N*-acylamino acids **14a,b**. Subsequent intramolecular *O*-alkylation resulted in formation of the oxazoline derivatives **15a,b**. For the *N*-acylthreonine derivative **14b**, $SOCl_2$ induced cyclization occurred under complete inversion when the *cis*-oxazoline **15b** was obtained in 78% yield. On the other hand, activation of the *N*-acylserine derivative **14a** with $SOCl_2$ gave disappointing results concerning yield and purity of the oxazoline **15a**. However, smooth and high yielding ring closure could be performed by application of the Burgess reagent.[21] Chemoselective reduction of the carboxylic esters **15a,b** furnished the primary alcohols **16a,b** that were converted into the mesylates **17a,b** and subsequently reacted with phenylpiperazine to give the target compounds **4c,d**. The *cis*-disposition between the hydroxymethyl and the methyl group in the positions 4 and 5 of the oxazoline **16b** was proved by NOE experiments when irradiation of H-4 showed a strong positive enhancement at H-5 and vice versa.

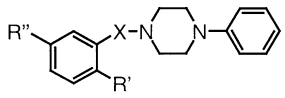
The thiazoline **4f** was synthesized starting from the unnatural (*S*)-cysteine ethyl ester hydrochloride (**18**).[22] In detail, coupling with methyl benzimidate afforded the cyclization product **19**[23] that could be transformed chemoselectively into the alcohol **20** when low temperature $LiAlH_4$ reduction was employed.[24] The hydroxymethyl group was activated to give the mesylate **21** and subsequently subjected to phenylpiperazine. Because of the high costs of (*S*)-cysteine, we established an alternative route starting from the readily available oxazoline **4a**. Thus, thiolysis of **4a** with NEt_3/H_2S/MeOH afforded the thioamide **22** that was cyclized to give thiazoline **4f** by intramolecular *S*-alkylation induced by Burgess reagent.[25]

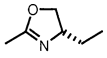
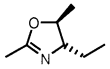
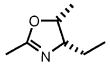
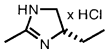
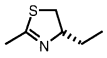
The oxazoline derivative **4a** should also be exploited for the synthesis of the chiral imidazoline derivative **4e**. Our initial attempts involving reductive ring opening by $LiAlH_4$, *N*-protection, *O*-activation and subsequent nucleophilic amination failed. When using *N,N*-dibenzyl protection, we observed rearrangement via an aziridinium intermediate and low yield of the cyclization precursor. On the other hand, reduction of the nucleophilicity by *N*-benzyl-*N*-Cbz protection precluded the formation of aziridinium intermediates and facilitated a



Scheme 1. (a) Methyl benzimidate, MeOH, refl, 2 h (**6a**: 82%, **6b**: 80%); (b) $LiAlH_4$, Et_2O, −30 °C, 15 min (**7a**: 85%, **7b**: 67%, **16a**: 45%, **16b**: 53%, **20**: 82%); (c) MesCl, NEt_3, THF, −23 °C, 30 min (**8a**, **8b**: 100%, **17a,b**: 82%, **21**: 96%); (d) phenylpiperazine, DMF, 70 °C, **4a,b**: 12 h, **4c,d**: 24 h, **4f**: 6 h (**4a**: 82%, **4b**: 62%, **4c**: 64%, **4d**: 38%, **4f**: 40%); (e) TMS-N_3, *t*-BuOH, 120 °C, 36 h (86%); (f) $LiAlH_4$, THF, 0 °C–refl (crude: 100%); (g) H_2, $Pd(OH)_2$/C, RT, 8 h (92%); (h) methyl benzimidate–HCl, MeOH, refl, 45 min (68%); (i) $SOCl_2$, toluene, $CHCl_3$, cat DMF, 60 °C, 15 h (crude); (j) (1) NEt_3, $CHCl_3$, −5 °C, 5 min; (2) NEt_3, **12**, 0 °C–rt, 15 min (**14a**: 94%, **14b**: 78%); (k) (1) $SOCl_2$, −5 °C, 12 h; (2) $CHCl_3$, aq Na_2CO_3, 0 °C (78%); (l) $MeO_2CNSO_2NEt_3$ (Burgess reagent), THF, 70 °C, 45 min (70%); (m) NEt_3, MeOH, H_2S, rt, 45 min (100%); (n) $MeO_2CNSO_2NEt_3$, THF, rt, 45 min (47%); (o) methyl benzimidate, EtOH, rt, 45 min (91%).

Table 1. Receptor binding data [K_i values (nM) based on the means of 2–4 experiments each performed in triplicate]



Compd	-X-	R′	R″	D1	$D2_{long}$	$D2_{short}$	D3	D4
4a		H	H	9700	1700	1100	3300	340
ent4a		H	H	4000	690	330	690	410
4c		OMe	Br	9000	2200	900	5200	1600
ent4c		OMe	Br	2000	400	110	1000	390
4b		H	H	20000	3200	1900	2700	700
ent4b		H	H	2000	820	320	710	440
4d		OMe	Br	3500	3700	2400	2500	290
ent4d		OMe	Br	480	21,000	18,000	10,000	3300
4e		H	H	12,000	10,000	11,000	7100	0.95/51
ent4e		H	H	nd	14,000	10,000	9000	72
4f		H	H	16,000	1500	1100	1200	1800
ent4f		H	H	4500	1100	720	1200	1200
quinpirole				nd	64/31,000	52/4000	24/420	1.8/53

smooth amination by NaN_3. However, we clearly observed complete racemization which is obviously due to the formation of an azetidinium intermediate via the nucleophilic piperazine nitrogen. Inspired by a recently reported amination sequence giving access to neuraminidase inhibitors,[26] we tried to initiate a nucleophilic ring opening of **4a** by TMS-N_3. In practice, utilization of *t*-butanol as a solvent afforded an 86% yield of the azide **9**. Subsequently, it was possible to reduce both the amide and the azide function in one step to give the benzylamine derivative **10**. Hydrogenolytic debenzylation afforded the 1,2-diamine **13** in 92% yield. Derivatization of both amino functions with (*R*)-phenylethyl isocyanate and subsequent HPLC-analysis indicated a satisfactory configurational integrity of the synthesis.[27] The final product **4e**[28] was obtained by cyclization of the diamine **13** with methyl benzimidate hydrochloride.[29]

The enantiomers **ent4a**–**f** were prepared analogously starting from the respective natural α-amino acids.

The test compounds **ent4a**–**f** and the dopamine receptor agonist quinpirole were evaluated in vitro for their abilities to displace [^{3}H]spiperone from the cloned human dopamine receptors $D2_{long}$, $D2_{short}$,[30] D3[31] and D4.4[32] being stably expressed in CHO cells (see Table 1).[33] D1 affinity was determined by employing bovine striatal membrane preparations and the D1 selective antagonist [^{3}H]SCH 23390.[33] The oxazolines **ent4a**–**d** and the thiazolines **ent4f** showed only moderate affinity in the radioligand binding experiments. However, the K_i value of the *(S)*-imidazoline **ent4e** (72 nM) indicated substantial D4 affinity. It turned out that receptor binding was strongly dependent on the spatial orientation of the phenylpiperazinylmethyl side chain. Thus, the (*R*)-enantiomer **4e** (FAUC 179) showed high D4 affinity. Careful analysis of the D4 binding experiments employing a large number of test concentrations showed a biphasic curve ($n_H = 0.55$). The calculation provided a K_i of 0.95 nM for the high affinity binding site and 51 nM for the low affinity binding site. These data are comparable to the D4 binding properties of the unselective dopamine receptor agonist quinpirole. However, FAUC 179 showed high selectivity over D1, $D2_{long}$, $D2_{short}$ and D3. Emloying porcine brain homogenates and the radioligands [^{3}H]8-OH-DPAT and [^{3}H]ketanserine, substantial selectivity over 5-$HT1_A$ ($K_i = 45$ nM) and 5-HT2 ($K_i = 870$ nM), respectively, was observed.

Table 2. Agonist effects of the imidazoline **4e**, quinpirole and clozapine at the D4.2 receptor investigated by measuring the stimulation of mitogenesis

	Test compounds		
	4e	Quinpirole	Clozapine
Agonist effect (%)[a]	42	100	0
EC_{50} (nM)[b]	31	9.5	nd

[a]Rate of incorporation of [^{3}H]thymidine (in %) relative to the maximal effect of the full agonist quinpirole (100%); the results are the means of quadruplicates of 10–14 experiments.
[b]EC_{50} values derived from the mean curves of 10–14 experiments; nd, not determined.

To investigate the intrinsic effect of FAUC 179 (**4e**), an in vitro functional assay measuring the [^{3}H]thymidine uptake in growing CHO cells stably expressing the dopamine D4.2 receptor was performed[6] when a 42% stimulation of mitogenesis (compared to the full agonist effect of quinpirole and the D4 antagonist clozapine) was determined ($EC_{50} = 31$ nM) (Table 2).[34]

In conclusion, SAR investigations on conformationally restricted benzamide bioisosteres led to the highly selective dopamine D4 receptor partial agonist **4e** (FAUC 179) incorporating a chiral imidazoline substructure. The biological activity was found to be strongly enantiospecific. Replacement of the imidazoline moiety by oxazoline or thiazoline was not successful. This leads to the assumption that the NH function is essential for D4 receptor recognition of the investigated family of compounds.

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27. To a solution of 15 mg (0.064 mmol) of **13** in dry CH_2Cl_2 was added dropwise 10 μL (0.064 mmol) of (*R*)-phenylethyl isocyanate at 0 °C. After 30 min, the solution was evaporated. HPLC (Nucleodex beta-PM; $MeOH/HNEt_3OAc_{1\%,pH4}$ 98:2; 1 mL/min; 250 nm; *RR* (rt = 21.78 min)/*SR* (rt = 19.28 min) = 96.4:3.6.
28. **4e**: $\alpha_D^{20} = -51.5°$ (*c* 0.93, $CHCl_3$); ^1H NMR (CD_3OD, 360 MHz): δ 2.66 (dd, *J* = 12.8, 6.4 Hz, 1H, $CH_2N(CH_2)_2$), 2.70–2.78 (m, 4H, $CH_2N(CH_2)_2$), 2.80 (dd, *J* = 12.8, 6.8 Hz, 1H, $CH_2N(CH_2)_2$), 3.18–3.21 (m, 4H, $PhN(CH_2)_2$), 3.86 (dd, *J* = 11.7, 7.4 Hz, 1H, H-5a), 4.16 (dd, *J* = 11.7, 11.2 Hz, 1H, H-5b), 4.63 (dddd, *J* = 11.2, 7.4, 6.8, 6.4 Hz, 1H, H-4), 6.81–6.86 (m, 1H, p-Ph), 6.95–6.98 (m, 2H, o-Ph), 7.20–7.26 (m, 2H, m-Ph), 7.59–7.65 (m, 2H, m-Ph′), 7.72–7.77 (m, 1H, p-Ph′), 7.88–7.91 (m, 2H, o-Ph′). ^{13}C NMR (CD_3OD, 90 MHz): δ 50.5 ($PhN(CH_2)_2$), 50.7 (C-5), 54.8 ($CH_2N(CH_2)_2$), 57.1 (C-4), 63.0 ($CH_2N(CH_2)_2$), 117.4 (o-Ph), 121.1 (p-Ph), 124.6 (i-Ph), 129.3 (o-Ph′), 130.1 (m-Ph), 130.5 (m-Ph′), 135.5 (p-Ph′), 152.7 (i-Ph′), 167.1 (C-2). HR-MS (EI) calcd for $C_{20}H_{24}N_4$ (M^+): 320.2001. Found: 320.2004.
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34. Ligand efficacy was also observed for the optical antipode **ent4e**, when the stimulation of [^{3}H]thymidine uptake was comparable for both enantiomers.