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Fancy bioisosteres: synthesis and dopaminergic properties of the endiyne FAUC 88 as a novel non-aromatic D3 agonist

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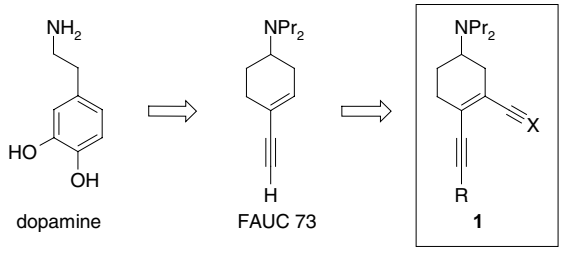
Abstract—Enlargement of the π-electronic system of the non-aromatic D3 agonist FAUC 73 led to dopaminergic endiynes of type **1** being synthesized via the bromovinyl triflate **7a** as a key intermediate when palladium catalyzed coupling reactions were exploited for the introduction of the (aza)alkyne substituents. As the first neuroreceptor active endiyne, FAUC 88 (**1c**) displayed high and selective dopamine D3 receptor affinity ($K_{i\,high}$ = 3.2 nM) and substantial ligand efficacy (72%, EC_{50} = 2.5 nM). Similarities between molecular electrostatic potentials induced by the catechol subunit of the genuine neurotransmitter and those of its non-aromatic endiyne bioisostere are discussed.

1. Introduction

Bioisosteric replacement of benzene derived structural moieties by heteroarenes has proven to be a valuable methodology in drug discovery providing access to drug candidates with excellent pharmacodynamic or pharmacokinetic properties.[1] As an extension of this means, we recently disclosed that 2,2-dicyanovinyl as a non-aromatic π-substituent as well as [2.2]paracyclophanes can serve as competitive aryl bioisosteres.[2,3] Moreover, we could show that a conjugated enyne system can simulate the catechol moiety of dopamine exerting agonist effects and D3 subtype selectivity. Thus, the dopaminergic FAUC 73 was evaluated as the first non-aromatic biogenic amine surrogate activating type 1 GPCRs.[4]



In order to investigate scope and limitations of these fancy aryl bioisostere and to develop a D3 selective antipsychotic drug candidate, we regarded further lead structure modifications as a highly interesting project. Our main focus lay on the enlargement of the π-electronic system in order to mimic the aromatic shape more closely. For this reason we envisioned to introduce a further conjugated triple bond leading to conjugated (aza)endiynes of type **1**.[5]

2. Results and discussion

To build up the endiyne moiety, we intended to use a cyclohexenediyl-1,2-bis-triflate of type **4** as a central intermediate (Scheme 1). Aside from aryl bis-triflates, this structural element was only known in diarylvinylenes.[6,7] We planned to synthesize the functionalized cyclohexene precursor via a *Rühlmann*-type acyloin condensation.[8] To find suitable conditions for the reaction sequence, we started first from the commercially available dimethyl adipate (**2a**) as a representative model building block, which was reacted with disperse sodium in the presence of chlorotrimethylsilane to furnish **3a** in 82% yield. Subsequent removal of the trimethylsilyl groups followed by sulfonylation turned out to be difficult when fluoride induced desilylation and subsequent reaction with trifluoromethanesulfonic anhydride[5] or *N*-phenyltrifluoromethane sulfonimide did not result in

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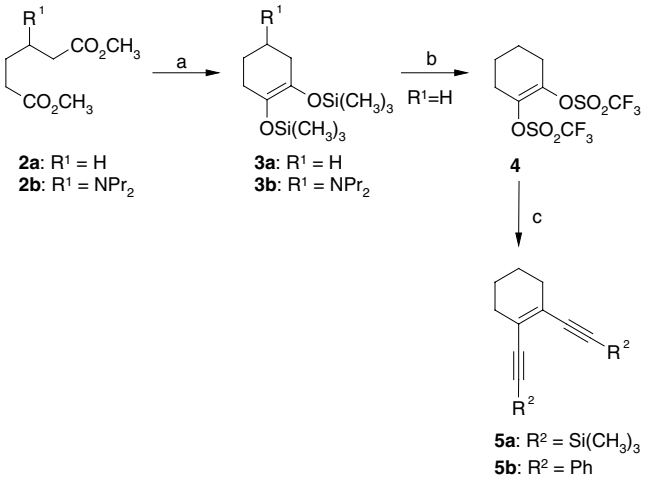
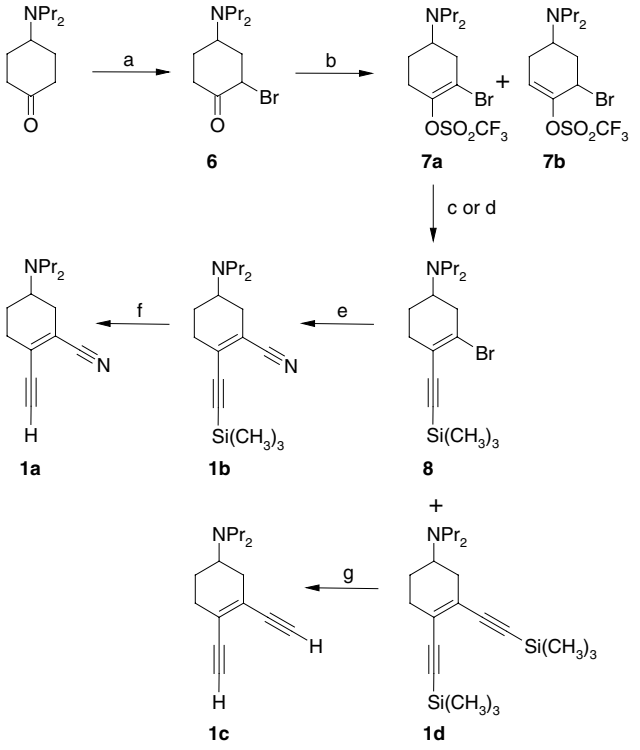


Scheme 1. Reagents and conditions: (a) Na, chlorotrimethylsilane, toluene, for **2a**: rf, 1.5 h, 82%; for **2b**: rt, 5 h, rf, 30 min, 61%; (b) (1) MeLi, DME, −60 °C to −20 °C, 2 h; (2) Tf_2O, −78 °C to 10 °C, 15 h, 15%; (c) trimethylsilylacetylene for **5a**, phenylacetylene for **5b**, $Pd(PPh_3)_4$, CuI, $EtMe_2N$, THF, rt, for **5a**: 3 h, 50%; for **5b**: 10 h, 65%.

formation of the vicinal bistriflate **4**. However, one-pot desilylation with MeLi and reaction with trifluoromethanesulfonic anhydride furnished the desired bistriflate **4**. Transition-metal catalyzed cross-coupling reactions employing trimethylsilylacetylene or phenylacetylene in the presence of $Pd(PPh_3)_4$, CuI and $EtMe_2N$[9,10] led to formation of the endiynes **5a**,**b** in 50–65% yield, respectively. Unfortunately, we could not successfully apply this methodology for the synthesis of our target compounds of type **1** since the silyl sulfonyl exchange reaction of the dipropylamine substituted intermediate **3b**, being readily available from the aminoadipate **2b**, led to complete decomposition when we added MeLi and trifluoromethanesulfonic anhydride.

To develop an alternative synthetic pathway, we decided to utilize a 2-bromo substituted vinyltriflate[11] as a central intermediate that should be available from the respective α-bromoketone. Bromination of the 4-dipropylaminocyclohexanone[4] by deprotonation with LDA and subsequent reaction with dibromotetrachloroethane furnished the α-bromoketone **6** in 66% yield (Scheme 2). Further deprotonation with LiHMDS and sulfonylation by 2-[*N*,*N*-bis-(trifluoromethylsulfonyl)-amino]-5-chloropyridine afforded the bromovinyltriflate **7a** in 36% yield besides 15% of the regioisomer **7b** being separated by flash chromatography. It is interesting to note that both reactive positions could be reacted consecutively. Thus, treatment of the key intermediate **7a** with trimethylsilylacetylene in the presence of $Pd(PPh_3)_4$, CuI and piperidine at 40 °C resulted in monosubstitution of the triflate group affording bromoenyne **8** in 67% yield, besides 7% of the endiyne **1d**. On the other hand, treatment of the bromovinyltriflate **7a** at 95 °C with the same mixture of reagents afforded the endiyne **1d** as the main product. Cleavage of the trimethylsilyl groups with Bu_4NF led to the terminal endiyne **1c**. To investigate the biological effect of the introduction of a nitrogen atom into the pharmacophore, the aza-analog **1a** was approached. Thus, Pd-catalyzed cross-coupling of the bromoenyne **8** with CuCN[12] led to the cyano substituted enyne **1b** that was transformed into the final product **1a** by fluoride induced desilylation.

Receptor binding experiments were established to evaluate the binding properties of the target compounds **1a**–**d** and the intermediate **8** in comparison to the references FAUC 73 and dopamine (Table 1). D1 receptor affinities were determined utilizing porcine striatal membranes and the D1 selective radioligand [^{3}H]SCH 23390. D2, D3 and D4 affinities were investigated employing the cloned human dopamine receptors D2long, D2short,[13] D3[14] and D4.4[15] stably expressed in Chinese hamster ovary cells (CHO) and the radioligand [^{3}H]spiperone. The competition data were analyzed according to a sigmoid model by non-linear regression. If the dose–response curves showed biphasic properties and the calculated Hill coefficients (n_H) were between −0.50 and −0.75 with a better fit of equation indicating a two-site model and if the amount of calculated high affinity binding sites was greater than 15% of the total receptor population, K_i values for the high and low affinity binding sites of the receptor were derived. The $K_{i\,high}$ values representing the ternary complex of ligand,



Scheme 2. Reagents and conditions: (a) (1) LDA, THF, −78 °C, 1 h; (2) dibromotetrachloroethane, THF, −90 °C, 30 min, 66%; (b): (1) LiHMDS, THF, −78 °C, 1.75 h; (2) 2-[*N*,*N*-bis-(trifluoromethylsulfonyl)amino]-5-chloropyridine, THF, −78 °C to −20 °C, 2.25 h, rt, 1 h, 36% **7a**, 15% **7b**; (c) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, piperidine, THF, 40 °C, 3.5 h, 67% **8**; 7% **1d**; (d) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, piperidine, THF, 95 °C, 1 h, 24% **8**, 42% **1d**; (e) CuCN, $Pd_2(dba)_3$, dppf, dioxane, reflux, 2.75 h, 54%; (f) Bu $_4$NF, THF, −20 °C, 45 min, 60%; (g) Bu_4NF, THF, −20 °C, 20 min, 82%.

Table 1. Receptor binding data for **1a–d**, **8**, FAUC 73 and dopamine employing porcine D1 as well as human D2long, D2short, D3 and D4.4 receptors[a]

Compound	K_i values (nM)				
	[^{3}H]SCH23390	[^{3}H]spiperone			
	pD1	hD2long	hD2short	hD3	hD4.4
8	19,000	520 + 18,000[b]	260 + 8800[b]	110 + 1200[b]	3100
1b (R = TMS, X = N)	21,000	49 + 9700[b]	96 + 13,000[b]	38 + 950[b]	120 + 5500[b]
1a (R = H, X = N)	21,000	42 + 5300[b]	72 + 6500[b]	26 + 910[b]	77 + 5600[b]
1d (R = TMS, X = CH)	4400	5200	8600	23 + 720[b]	3600
1c (R = H, X = CH)	12,000	94 + 10000[b]	54 + 2600[b]	3.2 + 49[b]	6.3 + 420[b]
FAUC 73	49,000	270 + 14,000[b]	250 + 12,000[b]	5.2 + 590[b]	22 + 380[b]
Dopamine	7.0 + 650	20 + 1900	17 + 1100	50 + 1600	1.2 + 62

[a] K_i values are the means of two to six experiments each done in triplicate.
[b] $K_{i\,high}$ and $K_{i\,low}$ values derived from a biphasic curve, if data analysis fits better with the equations for a two-site binding mode.

receptor and G-protein thus indicating agonist properties were chosen for the comparison of agonist affinities.

After a D1 binding assay, indicating only weak affinities of the non-aromatic test compounds, our initial SAR investigations were directed to a comparison of the 4-trimethylsilylethynyl substituted cyclohexenylamines involving the 3-bromo, 3-cyano and 3-trimethylsilylethynyl derivatives **8**, **1b** and **1d**. In fact, substantial binding affinities were determined for the D3 subtype leading to K_i values between 23 and 110 nM. Strong subtype selectivity over D2long, D2short and D4 was observed, especially for the bis-trimethylsilyl protected endiyne **1d** combining high D3 affinity (K_i = 23 nM) and selectivity ratios greater 150. At least for the D3 subtype, the binding data corroborate earlier observations that an H-bond donor activity of the terminal sp-hybridized CH proton is not essential for the receptor recognition.[4,16] However, desilylation led to a significant increase of affinity when the terminal alkyne **1c** and its aza-analog **1a** revealed K_i values of 3.2 and 26 nM, respectively, at the D3 receptor. Whereas deprotection of the nitrile **1b** caused a similar increase of binding for all subtypes of the D2 family, the bis-desilylation of the endiyne **1d** had much higher influence onto the D2long, D2short and D4 affinity. In detail, a 55-, 160- and 570-fold increase of affinities was observed for the D2long, D2short and D4 subtypes whereas the K_i values for the D3 subtype improved only by a factor of 7. Nevertheless, the *cis*-hexendiyne functionality of the test compound **1c** (FAUC 88) was superior to the butenyne moiety of the lead compound FAUC 73 and, thus, proved to be the most potent non-aromatic dopamine receptor ligand investigated, yet. In order to further characterize the binding properties of FAUC 88 (**1c**) and the reference agent FAUC 73, 5-HT1A and 5-HT2 receptor recognition was evaluated (Table 2). 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 (150–1000 nM) and 5-HT2 affinities (3100–9000 nM).

Table 2. K_i values [nM] of **1c** and FAUC 73 for the porcine 5-HT1A and 5-HT2 receptor[a]

Compound	5-HT1A	5-HT2
	[^{3}H]8-OH-DPAT	[^{3}H]ketanserin
1c	150	3100
FAUC 73	1000	9000

[a] K_i values are the means of two to four experiments each done in triplicate.

To investigate the intrinsic effects of FAUC 88 (**1c**) compared to the lead compound FAUC 73 and the full agonist quinpirole, a mitogenesis assay was performed employing CHO 10001 cells stably expressing human D2long, D2short and D4.2 receptors as well as D3 expressing CHO dhfr$^-$ cells.[19] Agonist activation of dopamine receptors can be determined by measuring the rate of [^{3}H]thymidine incorporation into growing heterologously transfected cell lines.[20] Intrinsic activities and EC_{50} values are depicted in Table 3 clearly indicating substantial ligand efficacy for all subtypes investigated and significant D3 preference. In consistence to

Table 3. Intrinsic activity of **1c**, FAUC 73 and the reference quinpirole at the D2long, D2short, D3 and D4.4 receptor determined by measuring the stimulation of mitogenesis[a]

Compound	D2long		D2short		D3		D4.2	
	EC_{50}[b]	Eff[c]	EC_{50}[b]	Eff[c]	EC_{50}[b]	Eff[c]	EC_{50}[b]	Eff[c]
1c	4.2	87	12	86	3.2	72	180	60
FAUC 73	6.5	85	25	76	4.4	74	280	67
quinpirole	2.4	100	12	100	2.7	100	14	100

[a] Incorporation of [^{3}H]thymidine in CHO10001 cells expressing the rat D2long, D2short and the human D4.2 receptor or in CHO dhfr$^-$ cells expressing the human D3 receptor.
[b] EC_{50} values in [nM] derived from the mean curves of 6–11 experiments each done in quadruplicate.
[c] Ligand efficacy in [%] compared to the full agonist qunipirole.

the K_i high values of the binding experiments, the EC_{50} data of the endiyne FAUC 88 (**1c**) indicated a superior activity profile compared to the enyne FAUC 73.

In order to understand the molecular necessities for the receptor recognition of dopamine receptor agonists, we investigated the molecular electrostatic potentials (MEPs) of the catechol moiety (**A**) of dopamine and the *cis*-hexene-1,5-diyne fragment (**B**) of the non-aromatic agonist FAUC 88 (**1c**) both being calculated quantum chemically. In detail, the pharmacophoric elements **A** and **B** were pre-optimized with B3LYP/3-21G and subsequently optimized with B3LYP/6-311G(d).

The charges used to contour the MEPs were calculated on the resulting structures applying Breneman's CHelpG charge distribution scheme. The negative isopotential surfaces were contoured with MOLCAD implemented in Sybyl 6.9.1[18] at −1.0 kcal/mol. Interestingly, shape and size of the MEP of the catechol unit (**A**) in dopamine revealed strong similarity to the respective isopotentials of the non-aromatic bioisostere (**B**) (Fig. 1). Obviously the endiyne **B** efficiently mimics the aromatic moiety of dopamine. According to the receptor binding profile of FAUC 88, the non-covalent intermolecular π-interactions[19] will best stabilize the protein–ligand association at the dopamine D3 receptor binding site crevice.

In conclusion, we were able to demonstrate that the π-electronic system of the non-aromatic endiyne FAUC 88 can be used as an efficient bioisostere for the catechol fragment of dopamine. Combined with conformational rigidization, the approach led to dopamine receptor agonists with high affinity for the subtypes of the D2-family, especially for D3. Further investigations giving mechanistic insights into the π-interactions responsible for the receptor recognition of the novel bioisostere are currently in progress.

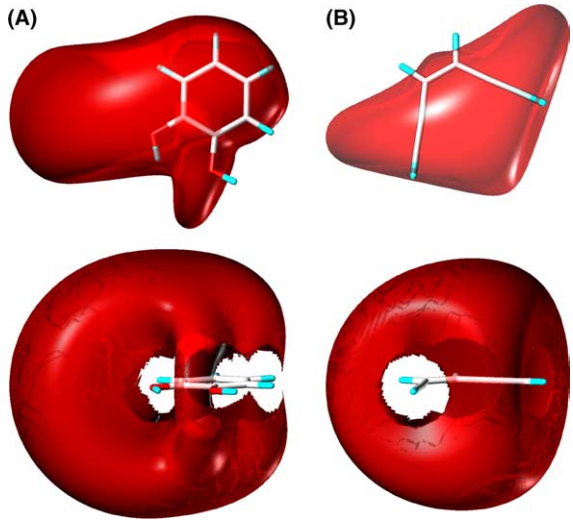


Figure 1. Isopotential surfaces of the catechol fragment (A) of dopamine and the endiyne core unit (B) of the non-aromatic dopamine receptor agonist FAUC 88 contouring negative electrostatic potentials (−1 kcal/mol) viewed from the front side (top) and from the bottom side (below).

3. Experimental

Reactions were performed under dry N_2. Solvents were purified and dried under standard procedures. All reagents were of commercial quality and used as purchased. Flash chromatography was carried out with silica gel 60 (4.0–6.3 μm) eluting with appropriate solution in the stated v:v proportions. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ on Bruker AM 360 (360 MHz) and Bruker AC 250 (90 MHz) spectrometers, respectively. MS and HRMS were run on Finnigan MAT TSQ 70 and 8200 spectrometers, respectively, by EI (70 eV). IR spectra were recorded on a Jasco FT/IR 410 spectrometer.

3.1. [Cyclohexene-1,2-diyl-bisoxy]-bis(trimethylsilane) (3a)

Sodium (4.06 g, 175 mmol) was stirred vigorously in refluxing toluene (100 mL) until sodium sand was obtained. After being cooled to room temperature, chlorotrimethylsilane (22.7 mL, 179 mmol) and dimethyl adipate (**2a**) (7.29 mL, 44 mmol) were added to the mixture. After being refluxed for 1.5 h, the mixture was filtrated immediately and evaporated. The residue was purified by flash chromatography (petroleum ether–CH_2Cl_2 6:4) to give **3a** as a colourless liquid (9.33 g, 82%): IR (film) 2956, 2841, 1694, 1446, 1265, 1213, 1122, 951 cm^{-1}; ^1H NMR δ 0.00 (s, 18H, 2×$Si(CH_3)_3$), 1.40–1.44 (m, 4H, 4-H, 5-H), 1.88–1.90 (m, 4H, 3-H, 6-H); EIMS 258 (M^+).

3.2. Dipropyl-[3,4-bis(trimethylsilyloxy)cyclohex-3-en-1-yl]amine (3b)

(a) After treatment of sodium (1.22 g, 53 mmol), having reacted in MeOH (300 mL) at 0 °C, with a solution of dipropylamine (200 mL) and dimethyl *trans*-3-hexenedioate (52.8 g, 307 mmol) in MeOH (100 mL), the mixture was being stirred at room temperature for 15 h and evaporated. HCl (2 N) was added to the residue at 0 °C and the mixture was extracted with Et_2O. The aqueous layer was then alkalized with 2 N NaOH and extracted with Et_2O. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether–EtOAc 8:2) to give **2b** as a colourless oil (5.84 g, 7%); (b) Sodium (703 mg, 30.5 mmol) was stirred vigorously in refluxing toluene (50 mL) until sodium sand was obtained. After being cooled to room temperature chlorotrimethylsilane (3.9 mL, 30.8 mmol) and **2b** (2.09 g, 7.64 mmol) were added to the mixture, stirred at room temperature for 5 h, then refluxed for 1.5 h and then immediately filtrated and evaporated. The residue was purified by bulb-to-bulb distillation to give **3b** as a colourless oil (1.68 g, 61%): IR (film) 2958, 2865, 2807, 1690, 1250, 1210, 1125 cm^{-1}; ^1H NMR δ 0.18 (s, 18H, 2×$Si(CH_3)_3$), 0.83 (t, $J = 7.3$ Hz, 6H, 2×$NCH_2CH_2CH_3$), 1.32–1.56 (m, 5H, 2×$NCH_2CH_2CH_3$, 6-H), 1.69–1.85 (m, 1H, 6-H),

1.96–2.17 (m, 4H, 2-H; 5-H), 2.31–2.45 (m, 4H, 2×NC$H_2$$CH_2CH_3$), 2.67–2.84 (m, 1H, 1-H).

3.3. Cyclohexene-1,2-diyl bis(trifluoromethanesulfonate) (4)

To a solution of **3a** (3.1 g, 12 mmol) in DME (30 mL) at −60 °C was added MeLi (16 mL, 1.6 M in Et_2O) and the solution was allowed to warm up to −20 °C during 2 h. After being recooled to −78 °C trifluoromethanesulfonic anhydride (4 mL, 24.3 mmol) was added drop by drop and the mixture was warmed to 10 °C slowly. After 15 h aqueous $NaHCO_3$ solution (5%) was added and the mixture was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (neutral Al_2O_3, petroleum ether–CH_2Cl_2 9:1) to give **4** as a colourless liquid (675 mg, 15%): IR (film) 2960, 2870, 1712, 1425, 1260, 1220, 1050, 920, 875 cm^{-1}; ^1H NMR δ 1.68–1.79 (m, 4H, 4-H, 5-H), 2.41–2.50 (m, 4H, 3-H, 6-H); ^{13}C NMR δ 21.8 (C-4, C-5), 27.3 (C-3, C-6), 113.4, 116.6, 119.8, 122.9 (CF_3), 140.2 (C=C); EIMS 378 (M^+).

3.4. (Cyclohexene-1,2-diyl-bisethynyl)-bis(trimethylsilane) (5a)

To a solution of **4** (52 mg, 0.13 mmol) in THF (4 mL) were added $EtMe_2N$ (130 μL, 1.2 mmol), trimethylsilylacetylene (75 μL, 0.53 mmol), $Pd(PPh_3)_4$ (10 mg, 6 mol%) and CuI (5 mg, 20 mol%). After being stirred at room temperature for 3 h, aqueous $NaHCO_3$ (5%) was added and the mixture was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–CH_2Cl_2 9:1) to give **5a** as colourless crystals (18 mg, 50%): ^1H NMR δ 0.19 (s, 18H, 2×Si$(CH_3)_3$), 1.50–1.63 (m, 4H, 4-H, 5-H), 2.13–2.27 (m, 4H, 3-H, 6-H); EIMS 274 (M^+); Anal. Calcd for $C_{16}H_{26}Si_2$: C, 69.99; H, 9.54. Found: C, 69.88; H, 9.64.

3.5. (Cyclohexene-1,2-diyl-bisethynyl)-bisbenzene (5b)

To a solution of **4** (52 mg, 0.13 mmol) in THF (4 mL) were added $EtMe_2N$ (130 μL, 1.2 mmol), phenylacetylene (57 μL, 0.51 mmol), $(Ph_3P)_4Pd$ (10 mg, 6 mol%) and CuI (5 mg, 20 mol%). After being stirred at room temperature for 10 h, aqueous $NaHCO_3$ (5%) was added and the mixture was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether) to give **5b** as a slightly yellowish oil (24 mg, 65%): ^1H NMR δ 1.62–1.74 (m, 4H, 4-H, 5-H), 2.30–2.43 (m, 4H, 3-H, 6-H), 7.21–7.54 (m, 10H, Ph); EIMS 282 (M^+).

3.6. 2-Bromo-4-(dipropylamino)cyclohexanone (6)

To a solution of 4-dipropylaminocyclohexanone[4] (1.18 g, 6.0 mmol) in THF (60 mL), was added a freshly prepared solution of LDA (17.4 mL, 0.40 M in THF) at −78 °C. After being stirred at −78 °C for 1 h, the mixture was cooled to −90 °C and dibromotetrachlorethane (2.13 g, 6.5 mmol) was added. After 30 min at −90 °C saturated aqueous $NaHCO_3$ solution was added and the mixture was allowed to warm to room temperature. After extraction with EtOAc the combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 1:1) to give **6** as a colourless oil (1.11 g, 66%): IR (film) 2958, 2871, 2810, 1721, 1462, 1190, 1076 cm^{-1}; ^1H NMR δ 0.87 (t, J = 7.3 Hz, 6H, 2×NC$H_2CH_2CH_3$), 0.88 (t, J = 7.3 Hz, 6H, 2×NC$H_2CH_2CH_3$), 1.37–1.51 (m, 8H, 4×NC$H_2CH_2CH_3$), 1.67–1.82 (m, 2H, 2×5-H_{ax}), 1.98–2.48 (m, 15H, 4×NC$H_2CH_2CH_3$, 2×5-H_{eq}, 2×6-H, 3-H), 2.63–2.73 (m, 2H, 2×4-H_{ax}), 3.03–3.16 (m, 2H, 3-H_2), 3.35 (dd, J = 10.6, 3.4 Hz, 1H, 3-H), 4.43–4.47 (m, 1H, 2-H_{eq}), 4.65 (dd, J = 13.1, 6.4 Hz, 1H, 2-H_{ax}); EIMS 276 (M^+); HREIMS calcd for $C_{12}H_{22}{}^{81}BrNO$: 277.0864; Found: 277.0866 (M^+); HREIMS calcd for $C_{12}H_{22}{}^{79}BrNO$: 275.0885; Found: 275.0887 (M^+).

3.7. 2-Bromo-4-(dipropylamino)cyclohex-1-en-1-yl trifluoromethanesulfonate (7a). 6-Bromo-4-(dipropylamino)-cyclohex-1-en-1-yl trifluoromethanesulfonate (7b)

To a solution of **6** (660 mg, 2.4 mmol) in THF (60 mL) was added LiHMDS (2.99 mL, 1.06 M in hexane) at −78 °C. After being stirred at −78 °C for 1.75 h, 2-[*N*,*N*-di-(trifluoromethylsulfonyl)amino]-5-chloropyridine (1.01 g, 2.56 mmol) in THF (6 mL) was added and the mixture was allowed to warm to −20 °C during 2.25 h and then stirred at room temperature for 1 h. Then saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 95:5) to give **7a** as a slightly yellowish oil (348 mg, 36%) as well as **7b** as a slightly yellowish oil (146 mg, 15%): **7a**: IR (film) 2960, 2813, 1677, 1422, 1212, 1140, 860 cm^{-1}; ^1H NMR δ 0.86 (t, J = 7.3 Hz, 6H, 2×NC$H_2CH_2CH_3$), 1.42 (m, 4H, 2×NC$H_2CH_2CH_3$), 1.70 (dddd, J = 12.6, 12.4, 10.1, 6.7 Hz, 1H, 5-H_{ax}), 1.95 (dddd, J = 12.6, 6.2, 3.0, 1.5 Hz, 1H, 5-H_{eq}), 2.35–2.41 (m, 4H, 2×NC$H_2CH_2CH_3$), 2.44–2.69 (m, 4H, 3-H, 6-H), 2.95 (dddd, J = 12.4, 9.5, 5.6, 3.0 Hz, 1H, 4-H_{ax}); EIMS 409, 407 (M^+); HREIMS calcd for $C_{13}H_{21}{}^{81}BrF_3O_3S$: 409.0357; Found: 409.0356 (M^+); HREIMS calcd for $C_{13}H_{21}{}^{79}BrF_3NO_3S$: 407.0378; Found: 407.037 (M^+). **7b**: IR (film) 2961, 2813, 1671, 1464, 1420, 1246, 1222, 1141, 862 cm^{-1}; ^1H NMR δ 0.88 (t, J = 7.4 Hz, 6H, 2×NC$H_2CH_2CH_3$), 1.38–1.51 (m, 4H, 2×NC$H_2CH_2CH_3$), 2.14 (ddd, J = 14.4, 12.1, 4.1 Hz, 1H, 5-H_{ax}), 2.28–2.47 (m, 7H, 2×NC$H_2CH_2CH_3$, 3-H, 5-H_{eq}), 3.37 (dddd, J = 12.1, 9.7, 6.7, 2.8 Hz, 1H, 4-H_{ax}), 4.82–4.86 (m, 1H, 6-H), 5.94 (dd, J = 5.1, 3.3 Hz, 1H, 2-H); EIMS 407, 409 (M^+).

3.8. Dipropyl-[3-bromo-4-(trimethylsilylethynyl)cyclohex-3-en-1-yl]amine (8)

To suspension of **7a** (24 mg, 0.059 mmol), $Pd(PPh_3)_4$ (10 mg, 0.009 mmol) and CuI (2 mg, 0.01 mmol) in THF (2.5 mL) were added trimethylsilylacetylene

(37 μL, 0.26 mmol) and piperidine (75 μL, 0.76 mmol) and the reaction mixture was heated at 40 °C for 3.5 h. After being cooled to room temperature saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 95:5) to give **1d** (1.5 mg, 7%) (see below) and **8** as a colourless oil (14 mg, 67%): IR (film) 2958, 2810, 2147, 1249, 859 cm^{-1}; ^1H NMR δ 0.20 (s, 9H, $Si(CH_3)_3$), 0.85 (t, J = 7.2 Hz, 6H, 2×$NCH_2CH_2CH_3$), 1.34–1.55 (m, 5H, 2×$NCH_2CH_2CH_3$, 6-H_{ax}), 1.81–1.89 (m, 1H, 6-H_{eq}), 2.20–2.44 (m, 6H, 2×$NCH_2CH_2CH_3$, 5-H), 2.45–2.58 (m, 1H, 2-H), 2.59–2.69 (m, 1H, 2-H), 2.88 (dddd, J = 12.2, 10.4, 5.2, 2.9 Hz, 1H, 1-H); EIMS 355, 357 (M^+); HREIMS calcd for $C_{17}H_{30}{}^{79}BrNSi$: 355.1331; Found: 355.1331 (M^+); HREIMS calcd for $C_{17}H_{30}{}^{81}BrNSi$: 357.1310; Found: 357.1323 (M^+).

3.9. Dipropyl-[3,4-di(trimethylsilylethynyl)cyclohex-3-en-1-yl]amine (1d)

To a suspension of $Pd(PPh_3)_4$ (20 mg, 0.018 mmol) and CuI (5 mg, 0.02 mmol) in THF (9 mL) were added **7a** (72 mg, 0.18 mmol) in THF (1 mL), trimethylsilylacetylene (150 μL, 1.06 mmol) and piperidine (170 μL, 1.72 mmol). After being stirred at 95 °C for 1 h, the mixture was allowed to cool to room temperature, saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 95:5) to give **8** (15 mg, 24%) and **1d** as a slightly yellowish oil (28 mg, 42%): IR (film) 2958, 2900, 2871, 2809, 2142, 1249, 842 cm^{-1}; ^1H NMR δ 0.20 (s, 9H, $Si(CH_3)_3$), 0.21 (s, 9H, $Si(CH_3)_3$), 0.85 (t, J = 7.4 Hz, 6H, 2×$NCH_2CH_2CH_3$), 1.33–1.47 (m, 5H, 2×$NCH_2CH_2CH_3$, 6-H_{ax}), 1.76–1.86 (m, 1H, 6-H_{eq}), 2.08–2.45 (m, 8H, 2×$NCH_2CH_2CH_3$, 5-H, 2-H), 2.66–2.78 (m, 1H, 1-H); EIMS 373 (M^+); HREIMS calcd for $C_{22}H_{39}NSi_2$: 373.2621; Found: 373.2622 (M^+).

3.10. Dipropyl(3,4-diethynylcyclohex-3-en-1-yl)amine (1c)

To a solution of **1d** (12 mg, 0.032 mmol) in THF (3 mL) was added Bu_4NF (70 μL, 1 M solution in THF) at −20 °C. After being stirred for 20 min saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 2:1) to give **1c** as a slightly yellowish oil (6 mg, 82%): IR (film) 3291, 2958, 2933, 2871, 2809, 2096, 1463, 1380 cm^{-1}; ^1H NMR δ 0.86 (t, J = 7.3 Hz, 6H, 2×$NCH_2CH_2CH_3$), 1.36–1.51 (m, 5H, 2×$NCH_2CH_2CH_3$, 6-H_{ax}), 1.85 (dddd, J = 12.6, 5.0, 5.0, 2.3 Hz, 1H, 6-H_{eq}), 2.15–2.45 (m, 8H, 2×$NCH_2CH_2CH_3$, 5-H, 2-H), 2.78 (dddd, J = 12.1, 10.5, 5.0, 2.8 Hz, 1H, 1-H), 3.24 (s, 2H, 2×C≡CH); ^{13}C NMR δ 11.8 ($NCH_2CH_2CH_3$), 22.2 ($NCH_2CH_2CH_3$), 24.8, 30.9, 32.4 (C-2, C-5, C-6), 52.5 ($NCH_2CH_2CH_3$), 55.2 (C-1), 80.9, 81.4 (2×C≡CH), 83.4, 83.6 (2×CCH), 126.4 (C=C); EIMS 229 (M^+); HREIMS calcd for $C_{16}H_{23}N$: 229.1830; Found: 229.1834 (M^+).

3.11. 5-(Dipropylamino)-2-(trimethylsilylethynyl)cyclohex-1-ene-1-carbonitrile (1b)

To a solution of **8** (31 mg, 0.09 mmol) in dioxane (4 mL) were added CuCN (35 mg, 0.4 mmol), $Pd_2(dba)_3$ (7.3 mg, 0.008 mmol) and dppf (18 mg, 0.03 mmol) and the reaction mixture was refluxed for 2.75 h. After being cooled to room temperature the mixture was filtrated over Celite, saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 9:1) to give **1b** as a colourless oil (14 mg, 54%): IR (film) 2958, 2811, 2214, 2148, 1461, 1249, 845 cm^{-1}; ^1H NMR δ 0.23 (s, 9H, $Si(CH_3)_3$), 0.86 (t, J = 7.3 Hz, 6H, 2×$NCH_2CH_2CH_3$), 1.36–1.55 (m, 5H, 2×$NCH_2CH_2CH_3$, 4-H_{ax}), 1.84 (dddd, J = 10.4, 7.6, 5.0, 2.6 Hz, 1H, 4-H_{eq}), 2.19–2.49 (m, 8H, 2×$NCH_2CH_2CH_3$, 3-H, 6-H), 2.86 (dddd, J = 12.0, 10.4, 5.0, 2.8 Hz, 1H, 5-H); EIMS 302 (M^+); HREIMS calcd for $C_{18}H_{30}N_2Si$: 302.2178; Found: 302.2177 (M^+).

3.12. 5-(Dipropylamino)-2-ethynylcyclohex-1-ene-1-carbonitrile (1a)

To a solution of **1b** (7 mg, 0.023 mmol) in THF (1 mL) was added Bu_4NF (28 μL, 1 M solution in THF) at −20 °C. After being stirred at this temperature for 45 min saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 8:2) to give **1a** as a slightly yellowish oil (3 mg, 60%): IR (film) 3297, 2816, 2214, 2098, 1605, 1462, 1249, 1099 cm^{-1}; ^1H NMR δ 0.87 (t, J = 7.4 Hz, 6H, 2×$NCH_2CH_2CH_3$), 1.37–1.54 (m, 5H, 2×$NCH_2CH_2CH_3$, 4-H_{ax}), 1.90–1.94 (m, 1H, 4-H_{eq}), 2.21–2.52 (m, 8H, 2×$NCH_2CH_2CH_3$, 3-H, 6-H), 2.85 (dddd, J = 12.0, 10.4, 5.0, 2.8 Hz, 1H, 5-H), 3.43 (s, 1H, C≡CH); EIMS 230 (M^+); HREIMS calcd for $C_{15}H_{22}N_2$: 230.1783; Found: 230.1780 (M^+).

3.13. Receptor binding experiments and data analysis

Receptor binding studies utilizing dopamine receptors were carried out as described in Ref. 4. In brief, the dopamine D1 receptor assay was done with porcine striatal membranes at a final protein concentration of 40 μg/assay tube and the radioligand [^{3}H]SCH 23390 at 0.3 nM (K_d = 0.35–0.70 nM). Competition experiments with the human D2long, D2short, D3 and D4.4 receptors were run with preparations of membranes from CHO cells expressing the corresponding receptor and [^{3}H]spiperone at a final concentration of 0.5 nM. The assays were carried out at a protein concentration of 6–20 μg/assay tube and K_d values of 0.10–0.12 nM for D2long, 0.10 nM for D2short, 0.10–0.30 nM for D3 and 0.10–0.50 nM for D4.4.

Receptor binding experiments with 5-HT1A and 5-HT2 receptors were done according to literature with porcine cortical membranes (at 330 μg/tube and 150 μg/tube for 5-HT1A and 5-HT2, respectively) and the radioligand [^{3}H]8-OH-DPAT (for 5-HT1A; $K_d = 1.4$ nM) or [^{3}H]ketanserin (for 5-HT2; $K_d = 1.6$ nM) both at 0.5 nM.[17]

The resulting competition curves were analyzed by non-linear regression using the algorithms in PRISM (GraphPad Software, San Diego, USA). The data were initially fit using a sigmoid model to provide a slope coefficient (n_H) and an IC_{50} value, representing the concentration corresponding to 50% of maximal inhibition. Data were then calculated for a one-site ($n_H \sim 1$) or a two-site model ($n_H < 1$) depending on the slope factor. Calculating a two-site model IC_{50} values for a high and low affinity binding site and the amount of both receptor populations could be obtained. Finally, IC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[20]

3.14. Mitogenesis experiments

Determination of the ligand efficacy of representative compounds was carried out by measuring the incorporation of [^{3}H]thymidine into growing cells after stimulation with the test compound as described in literature.[21,22] For this assay CHO10001 cells stably expressing the ratD2long, ratD2short and humanD4.2 receptor and D3 expressing CHO dhfr$^-$ cells have been incubated with 0.02 μCi [^{3}H]thymidine per well (specific activity 25 Ci/mmol). Dose–response curves of 6–11 experiments have been normalized and summarized to get a mean curve from which the EC_{50} value and the maximum intrinsic activity of each compound could be derived compared to the effects of the full agonist quinpirole.

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