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Fancy bioisosteres: Synthesis, SAR, and pharmacological investigations of novel nonaromatic dopamine D3 receptor ligands

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Dedicated to Professor Claus Herdeis on the occasion of his 60th birthday

Abstract—Structural variations of the lead compound FAUC 88 led to dopaminergic enynes with an extended π-system when Pd-catalyzed cross coupling reactions were employed for the key reaction steps. The dienyne **9b** displayed substantial affinity for the dopamine receptor subtype D3 and remarkable selectivity over D4. Compared to FAUC 88, the novel fancy bioisostere **9b** displayed reduced ligand efficacy. DFT-based conformational analysis of the test compound **9b**, including the calculation of diagnostic magnetic shielding properties and their comparison with experimentally derived NMR data, indicated a clear energetic preference for the *s*-trans geometry of the diene substructure.

1. Introduction

Considering the huge number of drugs situated in the worldwide market, it is striking that only very few substances involving the oral contraceptive ethynylestradiol and the CNS active MAO inhibitor selegiline contain a carbon–carbon triple bond. To the best of our knowledge, the modern antifungal terbinafine is the single representative for a drug displaying a conjugated enyne substructure (Fig. 1).[1]

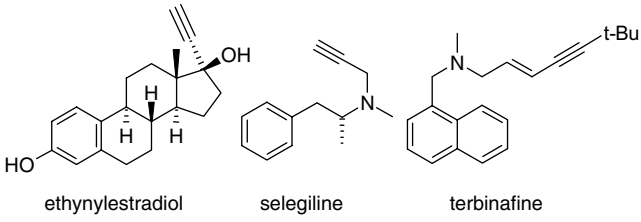

Figure 1.

We have recently presented FAUC 73 and FAUC 88 as the first nonaromatic dopamine receptor agonists when the conjugated enyne and endiyne units serve as fancy bioisosteres simulating the aromatic substructure of dopamine.[2,3] Interestingly, both dopaminergics, being of potential interest for the treatment of Parkinson's disease, revealed substantial selectivity for the D3 subtype when compared to D2 and moderate selectivity over D4. As a complement to our FAUC 73 based SAR studies indicating that structural modification of the ethyne subunit can be used for a fine tuning of D3/D4 selectivity,[4] we herein report the synthesis, receptor binding experiments, and computational investigations of FAUC 88 derivatives when the alkyne group in position 3 is replaced by C═C and C═O derived double bond moieties as alternative π-systems (Fig. 2).

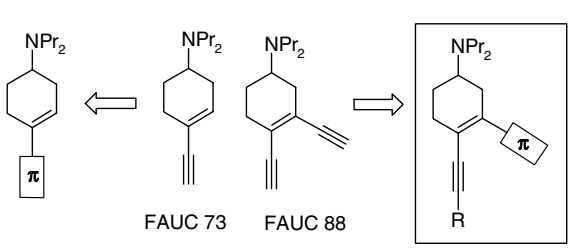

Figure 2.

Keywords: Fancy bioisosteres; Dopamine; D3; Palladium catalyzed cross coupling.
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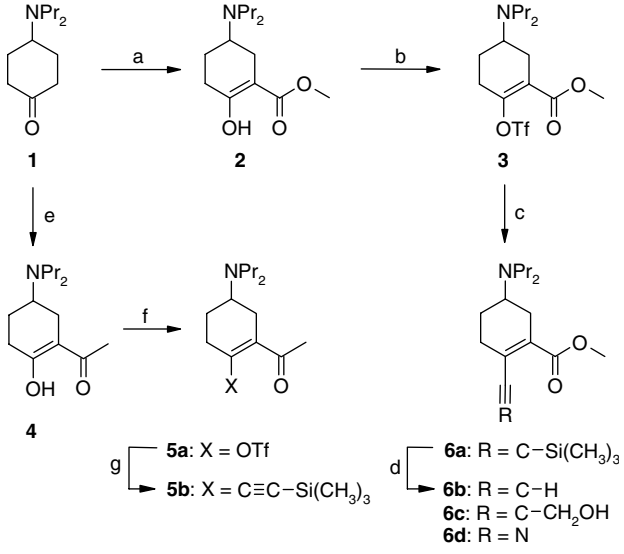
2. Results and discussion

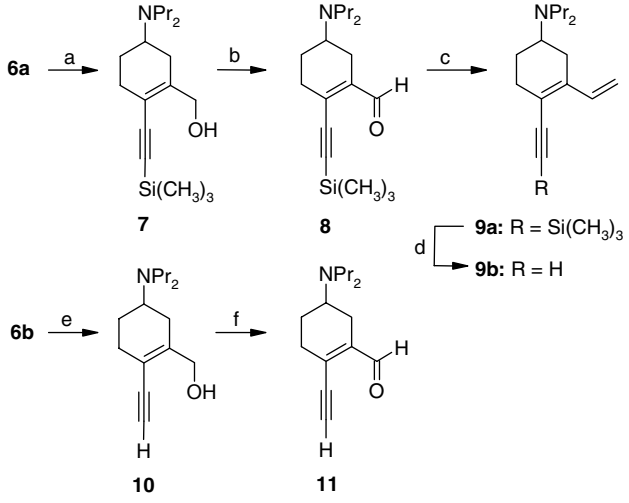
Our initial efforts were directed to the synthesis of 3-methoxycarbonyl and 3-acetyl substituted enynes **6a–c** and **5b**, respectively, and to the carbonitrile **6d** (Scheme 1). Deprotonation of 4-dipropylaminocyclohexanone (**1**)[2] with a mixture of sodium hydride and potassium hydride,[5] and subsequent trapping of the thus formed enolate with dimethylcarbonate gave access to the β-keto ester **2** in 85% yield that predominantly exists as an enol tautomer (in $CDCl_3$). Formation of the enol triflate **3** could be accomplished in 74% yield upon deprotonation of the enol **2** with sodium hydride and subsequent reaction with trifluoromethanesulfonic anhydride.[6] To attach the (aza)alkyne moiety, the central intermediate **3** was subjected to transition-metal catalyzed cross coupling reactions. Thus, utilization of trimethylsilylacetylene or propargyl alcohol in the presence of $Pd(PPh_3)_4$, CuI, and piperidine[7,8] afforded the methoxycarbonyl substituted enynes **6a** and **c** in 88% and 77% yield, respectively. Pd-catalyzed reaction of the enol triflate **3** with potassium cyanide in the presence of 18-crown-6 furnished the cyano derivative **6d** in 61% yield. Cleavage of the trimethylsilyl group of the enyne **6a** with Bu_4NF[9] led to the terminal alkyne **6b**. For the synthesis of the acetyl substituted enyne **5b**, C-acetylation could be done only in 9% yield when applying the conditions described above for the methoxycarbonylation. Thus, 4-dipropylaminocyclohexanone (**1**) was reacted with acetic anhydride in the presence of boron trifluoride diacetic acid complex[10] leading to the desired β-diketone **4** in 79% yield. According to the NMR spectra, **4** completely exists as an enol tautomer when dissolved in $CDCl_3$. The position of the C–C double bond was identified by CH correlation when a cross peak between the exocyclic methyl protons and the carbonyl C-atom unambiguously indicated the endocyclic position of the enol double bond. Formation of the triflate **5a** was accomplished by deprotonation of the synthetic precursor **4** with sodium hydride and subsequent reaction with N-phenyltrifluoromethanesulfonimide.[11] Pd-catalyzed coupling of **5a** with trimethylsilylacetylene furnished the acetyl substituted enyne **5b** in 58% yield. Unless a variety of reaction conditions, including Bu_4NF,[9] KF/crown ether,[12] LiOH,[13] $AgNO_3$, and lutidine,[14] were investigated for the removal of the trimethylsilyl group of **5b**, the respective terminal alkyne could not be isolated.

Further structural modifications of the methoxycarbonyl substituted enyne **6b** and its trimethylsilyl protected derivative **6a** are displayed in Scheme 2. Thus, reduction of the ester functions of **6a** and **b** with $LiAlH_4$ gave the primary alcohols **7** and **10**, which could be oxidized by MnO_2 resulting in the formation of the formyl substituted enynes **8** and **11**, respectively. Besides its relevance as a putative dopaminergic, the carbaldehyde **8** was exploited as a valuable synthetic intermediate when a Wittig-type methenylation gave access to the dienyne **9a**. Finally, C-deprotection resulted in formation of the desilylation product **9b**.

Receptor binding experiments were established to evaluate the binding properties of the target compounds **5b**, **6a–d**, **8**, **9a**,**b**, and **11** in comparison to the reference agent FAUC 88 (Table 1). D1 receptor affinities were determined utilizing porcine striatal membranes and the D1 selective radioligand [^{3}H]SCH23390. D2, D3, and D4 affinities were investigated employing the cloned



Scheme 1. Reagents and conditions: (a) (1) KH, NaH, THF, rt, 30 min; (2) dimethylcarbonate, THF, rt, 2 h, 85%; (b) (1) NaH, Et_2O, CH_2Cl_2, 0 °C, 1 h; (2) Tf_2O, Et_2O, CH_2Cl_2, 0 °C, 20 min, rt, 4.75 h, 74%; (c) for **6a**: trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, CuI, piperidine, THF, rt, 30 min, 88%; for **6c**: propargyl alcohol, $Pd(PPh_3)_4$, CuI, piperidine, THF, rt, 30 min, 77%; for **6d**: KCN, 18-crown-6, $Pd(PPh_3)_4$, benzene, rt, 1.5 h, 61%; (d) Bu_4NF, THF, −20 °C, 40 min, 86%; (e) (1) boron trifluoride diacetic acid complex, acetic anhydride, 0 °C, 30 min, rt, 16 h; (2) NaOAc, H_2O, rt, 5 h, 79%; (f) (1) NaH, THF, rt, 30 min; (2) *N*-phenyltrifluoromethanesulfonimide, rt, 3.75 h, 54%; (g) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, piperidine, THF, rt, 1 h, 58%.



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, −50 °C, 3.25 h; (b) MnO_2, CH_2Cl_2, 40 °C, 62 h, 50% overall; (c) methyltriphenylphosphonium bromide, *n*-BuLi, THF, −78 °C to rt, 1.5 h, rt, 2.5 h, 62%; (d) Bu_4NF, THF, −15 °C, 1 h, 83%; (e) $LiAlH_4$, THF, −50 °C, 1.75 h; (f) MnO_2, CH_2Cl_2, rt, 24 h, 32% overall.

Table 1. Receptor binding data for **5b**, **6a–d**, **8**, **9a+b**, **11** and FAUC 88 employing porcine D1 as well as human $D2_{long}$, $D2_{short}$, D3, and D4.4 receptors[a]

Compound	K_i values (nM)				
	[^{3}H]SCH23390	[^{3}H]Spiperone			
	pD1	$hD2_{short}$	$hD2_{short}$	hD3	hD4.4
5b	29,000	16,000	18,000	85 + 3200[b]	18,000
6a	22,000	61,000	100 + 25,000[b]	19 + 2800[b]	250 + 372,000[b]
6b	48,000	500 + 93,000[b]	120 + 27,000[b]	27 + 3400[b]	230 + 31,000[b]
6c	15,000	30,000	57,000	7200	56,000
6d	15,000	44,000	130 + 64,000	9800	42 + 89,000
8	29,000	140 + 52,000[b]	600 + 50,000[b]	2700	21 + 5400[b]
9a	30,000	170 + 19,000[b]	420 + 32,000[b]	1100	1500 + 34,000[b]
9b	26,000	260 + 9700[b]	110 + 4800[b]	9.1 + 500[b]	250 + 6100[b]
11	69,000	260 + 16,000[b]	260 + 12,000[b]	49 + 3000[b]	13,000
FAUC 88	12,000	94 + 10,000[b]	54 + 2600[b]	3.2 + 49[b]	6.3 + 420[b]

[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.

human dopamine receptors $D2_{long}$, $D2_{short}$,[15] D3,[16] and D4.4[17] 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 nonlinear 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, 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, receptor, and G-protein thus indicating agonist properties were compared for further SAR studies.

As observed for FAUC 88, the nonaromatic bioisosteres showed only weak affinity for the D1 receptor. All test compounds investigated displayed significant affinity for the receptors of the D2 family with a preference for the D3 subtype. Moderate affinity was observed for the ternary complex when the acetyl substituted TMS-alkyne **5b** was investigated for D3 binding (K_i = 85 nM). Interestingly, the methoxycarbonyl function was tolerated well at the binding site crevice of the D3 receptor indicated by K_i values of 19 and 27 nM for the TMS-protected alkyne **6a** and the terminal alkyne **6b**, respectively. Different observations were made for the formyl substituted analogs when the binding of the terminal alkyne **11** was comparable to **6b** but the C-silyl derivative **8** displayed a monophasic curve with a low affinity binding site. On the other hand, receptor recognition is strongly reduced for the hydroxymethyl substituted alkyne **6c** and the aza-analog **6d**, which is in good agreement with our previous results on FAUC 73 derived enynes.[2,4] The most interesting binding profile revealed the dienyne **9b** combining high D3 binding (K_i = 9.1 nM) with a substantial selectivity, especially over the D4 subtype which is superior to FAUC 73.

Agonist activation of dopamine receptors can be determined by measuring the rate of [^{3}H]thymidine incorporation into growing heterologously transfected cell lines.[18] To investigate the intrinsic effects of the most promising compound **9b** at the D3 receptor, a mitogenesis assay was performed employing CHO dhfr$^-$ cells stably expressing human D3 receptor.[19] Dose–response curves for the lead compound FAUC 88 clearly indicated substantial ligand efficacy (75%) for the endiyne FAUC 88 when compared to the effect of the nonselective full agonist quinpirole (Fig. 3). Interestingly, the dienyne **9b** revealed a partial agonist effect when stimulating [^{3}H]thymidine incorporation in a range of 26%. It is worthy of note that the EC_{50} values of the functional assay and the K_i data for the high affinity binding site correspond well for both test compounds.

Due to the finding that not only an endiyne but also a dienyne moiety can efficiently simulate the aromatic substructure of dopamine, we tried to characterize the structure of the dienyne **9b** in comparison to the lead compound FAUC 88. Thus, conformational properties including the consequences on magnetic shielding and molecular electrostatic potential maps were calculated. Initially, we performed a semiempirical AM1 optimization with VAMP[20] on the *s*-trans conformer, followed by two steps of DFT calculations in Gaussian98.[21] We used a B3LYP density functional analysis with a 3-21G basis set in order to produce a reasonable geometry

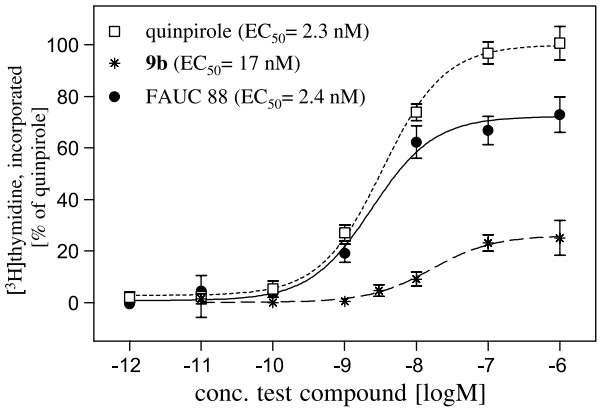


Figure 3. Stimulation of mitogenesis as a functional assay to determine the intrinsic effect of **9b** and the lead compound FAUC 88 at the human D3 receptor.

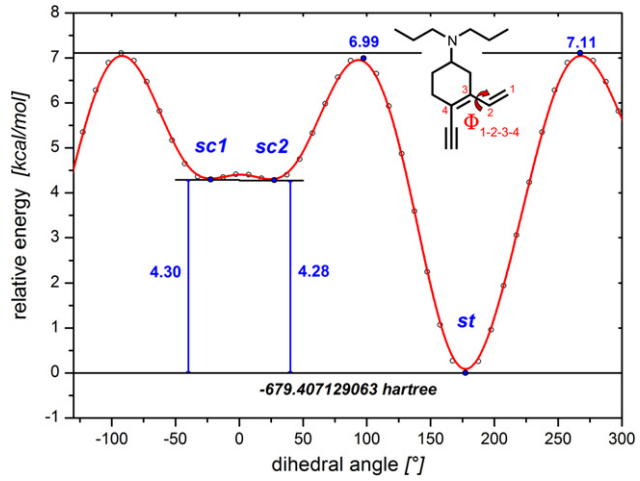


Figure 4. DFT grid-calculation of the rotational barriers and minima of the diene **9b** with B3LYP/6-31G(d). For better readability the relative energies in kcal/mol applied to the lowest energy calculated (- 679.40713 hartree) are denoted.

in appropriate time. Then, we increased the basis set to the double-valence level 6-31G(d) to enhance the quality of the structure. At the same level, we accomplished a grid calculation varying the dihedral angle $\Phi_{1\text{-}2\text{-}3\text{-}4}$ in steps of 10°. With this dihedral frozen, the rest of the system was allowed to freely adapt to the new geometry by minimization of energy. As depicted in Figure 4, we obtained two *s*-cis-like local minima (further called 'sc1' and 'sc2') and one *s*-trans-like local minimum ('st'), separated by a rotational barrier of at least 2.69 kcal/mol for sc1, 2.71 kcal/mol for sc2 and 6.99 kcal/mol for st, respectively.

Subsequently, we subjected all three conformers to a series of higher-level calculations (Table 2), a method we already applied successfully to confirm a dominant conformer in a similar study before.[4] These calculations were found to give a highly consistent ranking of the conformers with almost identical relative energy differences. A marginal energy gap of 0.10–0.15 kcal/mol in favor of sc2 compared to sc1 was found, as well as a considerably larger gap of 4.10–4.23 kcal/mol for st compared to sc2 and 4.20–4.38 kcal/mol for st compared to sc1, respectively. According to the Boltzmann equation, the ratio of structures in the sc1- and st-conformation at room temperature (298.15 K) is about 0.07% and the ratio sc2/st is 0.08%, indicating that the most relevant structure for the bioactive conformation is *s*-trans.

Table 2. Energy differences [kcal/mol] of the local minima

Density functional/basis set	$\Delta E_{sc2-sc1}$	ΔE_{st-sc2}	ΔE_{st-sc1}
B3LYP/6-311G(d)[a]	−0.10	−4.10	−4.20
B3LYP/6-311+G(d,p)[a]	−0.14	−4.14	−4.28
B3PW91/6-311+G(2d,p)[b]	−0.15	−4.19	−4.34
B3LYP/6-311+G(2df,2p)[a]	−0.13	−4.19	−4.32
B3PW91/6-311+G(2df,2p)[b]	−0.15	−4.23	−4.38

[a] Full optimization.
[b] Single point calculation on previously minimized structure.

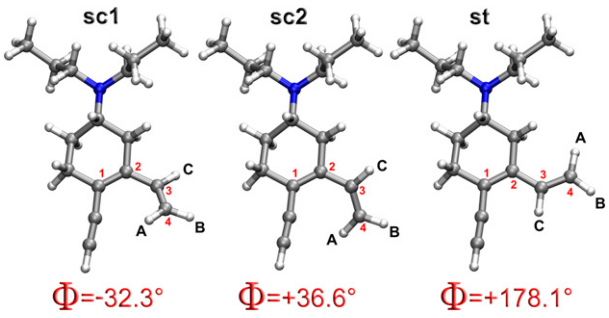


Figure 5. Final geometries obtained for B3LYP/6-311 + G(2df,2p) with the dihedral angle Φ and the labeling of the olefinic protons used in subsequent ^{1}H NMR calculations (see Table 3).

Additionally, the structure of the transition state of the rotation around $\Phi_{1\text{-}2\text{-}3\text{-}4}$ was calculated at the 6-311G(d) level of theory. The fact that a real transition state was found was verified by frequency calculation, which yielded only one negative frequency corresponding to the examined rotational motion. The energy of the transition state was found to be 2.25 kcal/mol higher than the sc1-state, 2.34 kcal/mol higher than the sc2-state, and 6.44 kcal/mol higher than the st-state, demonstrating that a pronounced rotational barrier exists between the *s*-cis-like states and the *s*-trans-like state.

To gain further evidences for the bioactive conformation, we calculated the magnetic shielding tensor using gauge invariant atomic orbitals[22] (GIAO) within a B3PW91/6-311+G(2d,p) or B3PW91/6-311+G(2df,2p) single point calculation. The chemical shifts for all olefinic protons (A, B, and C as depicted in Fig. 5) were calculated by subtraction of the total shielding (average isotropic value) of the respective proton from the total shielding of a TMS-proton:

$$\delta_{x\in\{A,B,C\}} = \sigma_{TMS} - \sigma_x;$$

TMS as a reference was optimized and subjected to NMR single point calculations on the same levels as the compared structures utilizing its T_d-symmetry. The chemical shifts of A, B, and C were obtained with an average deviation from experimental values of 0.80 ppm (sc1) or 0.72 ppm (sc2) for the *s*-cis-like and 0.27 ppm for the *s*-trans-like structures at the 6-311+G(2d,p)-level. Likewise, they were determined at the 6-311+G(2df,2p)-level with average deviations of 0.79 ppm (sc1) or 0.69 ppm (sc2) for the *s*-cis-like and 0.22 ppm for the *s*-trans-like structures. What is more, the comparison of similar and dissimilar olefinic protons revealed marked differences between sc1/sc2 and st. While the chemical shifts for H^A and H^C in the *s*-cis-like structures are separated by 0.05–0.24 ppm (with H^A and H^C being in the wrong order), in the *s*-trans structure the separation is 2.02 or 2.19 ppm, respectively, which reflects the correct order and is much closer to the experimental value of 1.76 ppm (Table 3). Similarly, the difference of the chemical shifts for H^B and H^C is found to be considerably higher (0.69–0.80 ppm) in the *s*-cis-like structures than in the *s*-trans structure, where the difference is almost identical with the experimental data

Table 3. Differences in chemical shifts [ppm]

	B3PW91/6-311+G(2d,p)		B3PW91/6-311+G(2df,2p)	
	$\delta(H^C)-\delta(H^A)$	$\delta(H^C)-\delta(H^B)$	$\delta(H^C)-\delta(H^A)$	$\delta(H^C)-\delta(H^B)$
sc1	−0.19	0.80	−0.24	0.79
sc2	−0.05	0.72	−0.05	0.69
st	2.02	0.27	2.19	0.22
exp.	**1.76**	**0.18**	**1.76**	**0.18**

(0.27/0.22 ppm compared to 0.18 ppm). Thus, comparison between the theory-based calculations and the experimental ^{1}H NMR data clearly suggests a strong preference of the *s*-trans-conformer. Renouncing effects of the receptor binding cavity, >99% of the substance should be in the *s*-trans-state.

In order to understand the structural requirements for receptor recognition, we decided to take a closer look at the molecular electrostatic potentials (MEPs). The dienyne **9b** and the endiyne FAUC 88 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 surface was contoured with MOLCAD, implemented in Sybyl 6.9.1[23] (−1.0 kcal/mol). Because of the strong preference for the *s*-trans conformer, the negative electrostatic potential surrounding the dienyne substructure in **9b** shows significant volume and shape similarity (Fig. 6) to the endiyne substructure in FAUC 88. This structural feature obviously mimics the aromatic moiety of dopamine, as previously suggested.[3]

In conclusion, structural variation of the extended π-system of FAUC 88 demonstrated that replacement of its 3-ethynyl moiety by carbonyl containing functional groups (ester, ketone, or aldehyde) decreases D3 affinity. However, replacement by a vinyl group produces good D3 binding combined with an enhanced D3/D4 selectivity. Compared to FAUC 88, the novel fancy bioisostere **9b** displayed reduced ligand efficacy. Because of the high similarity of the electrostatic potential between the dienyne **9b** and the endiyne FAUC 88, this selectivity increase is presumably a result of the different steric requirements.

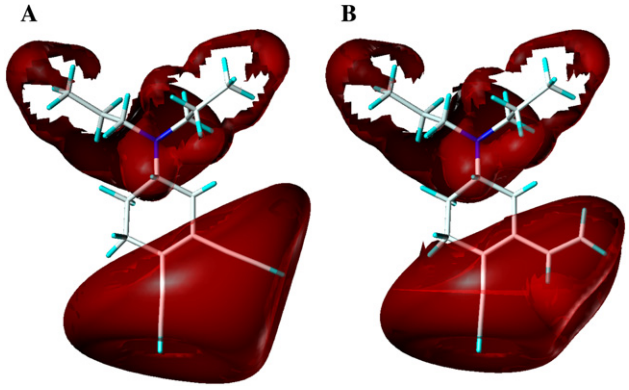


Figure 6. Isopotential surfaces of FAUC 88 (A) and the dienyne **9b** (B) contouring negative (−1.0 kcal/mol) electrostatic potentials.

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. ^{1}H 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. Quantum chemical calculations were performed on a four-nodes Linux Cluster consisting of 2 Intel Xeon 2.6 GHz processors each and running SuSE Linux 8.1. Visualization of molecular electrostatic potentials was prepared on a SGI Octane2.

3.1. Methyl 2-hydroxy-5-(dipropylamino)-cyclohex-1-ene-1-carboxylate (2)

To a suspension of KH (203 mg, 5.07 mmol) and NaH (30 mg, 1.27 mmol) in THF (19 mL), a solution of **1** (312 mg, 1.58 mmol) in THF (1 mL) was added. After being stirred for 30 min at room temperature, dimethylcarbonate (440 μL, 5.32 mmol) was added and the mixture was refluxed for 2 h. Then, the mixture was cooled to room temperature and saturated aqueous $NaHCO_3$ was added. After the aqueous layer was extracted with EtOAc, the combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 95:5) to give **2** as a colorless oil (345 mg, 85%): IR (film) 2958, 2810, 1744, 1719, 1658, 1616, 1464, 1209, 827 cm^{-1}; ^{1}H NMR δ 0.87 (t, J = 7.5 Hz, 6H, 2 × $NCH_2CH_2CH_3$), 1.39–1.49 (m, 4H, 2 × $NCH_2CH_2CH_3$), 1.51–1.61 (m, 1H, 4-H_{ax}), 1.85–1.89 (m, 1H, 4-H_{eq}), 2.03–2.12 (m, 1H, 3-H or 6-H), 2.36–2.44 (m, 7H, 2 × $NCH_2CH_2CH_3$, 3-H, 6-H), 2.74 (dddd, J = 11.9, 10.9, 5.0, 2.8 Hz, 1H, 5-H_{ax}), 3.76 (s, 3H, CO_2Me), 12.11 (s, 1H, C=COH); ^{13}C NMR δ 11.8 ($NCH_2CH_2CH_3$), 22.3 ($NCH_2CH_2CH_3$), 24.3, 24.6, 29.5 (C-3, C-4, C-6), 51.4 (CO_2CH_3), 52.8 ($NCH_2CH_2CH_3$), 57.1 (C-5), 96.6 (C-1), 171.5 (C-2), 173.0 (CO_2CH_3); EIMS 255 (M^+); Anal. Calcd for $C_{14}H_{25}NO_3$: C, 65.85; H, 9.87; N, 5.49. Found: C, 65.76; H, 9.87; N, 5.49.

3.2. Methyl 2-(trifluoromethylsulfonyloxy)-5-(dipropylamino)-cyclohex-1-ene-1-carboxylate (3)

A suspension of NaH (135 mg, 3.37 mmol, 60% oil dispersion) in Et_2O (22 mL) was cooled to 0 °C and **2** (477 mg, 1.87 mmol) in CH_2Cl_2 (2 mL) was added. The reaction mixture was allowed to stir at 0 °C for 1 h and trifluoromethanesulfonate anhydride (552 μL, 3.37 mmol) was then added. After being stirred for 20 min at 0 °C and 4.75 h at room temperature, saturated aqueous $NaHCO_3$ was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 95:5) to give **3** as a colorless oil (540 mg, 74%): IR (film)

2959, 2812, 1730, 1670, 1423, 1270, 1210, 835 cm^{-1}; ^1H NMR δ 0.87 (t, $J = 7.4$ Hz, 6H, $2 \times NCH_2CH_2CH_3$), 1.38–1.50 (m, 4H, $2 \times NCH_2CH_2CH_3$), 1.65 (dddd, $J = 12.5$, 12.1, 10.4, 5.5 Hz, 1H, 4-H_{ax}), 1.92–1.96 (m, 1H, 4-H_{eq}), 2.35 (ddd, $J = 17.3$, 10.4, 4.2 Hz, 1H, 3-H_{ax}), 2.38–2.44 (m, 4H, $2 \times NCH_2CH_2CH_3$), 2.47–2.56 (m, 2H, 6-H_2), 2.62 (ddd, $J = 17.3$, 5.5, 3.6 Hz, 1H, 3-H_{eq}), 2.83 (dddd, $J = 12.1$, 10.3, 4.9, 2.9 Hz, 1H, 5-H_{ax}), 3.81 (s, 3H, CO_2Me); ^{13}C NMR δ 11.7 ($NCH_2CH_2CH_3$), 22.1 ($NCH_2CH_2CH_3$), 25.0, 27.7, 28.9 (C-3, C-4, C-6), 52.2 (CO_2CH_3), 52.5 ($NCH_2CH_2CH_3$), 54.6 (C-5), 118.3 (q, CF_3), 122.2 (C-1), 151.0 (C-2), 164.9 (CO_2CH_3); EIMS 387 (M^+); Anal. Calcd for $C_{15}H_{24}F_3NO_5S$: C, 46.50; H, 6.24; N, 3.62. Found: C, 46.80; H, 6.37; N, 3.61.

3.3. Methyl 2-(trimethylsilylethynyl)-5-(dipropylamino)-cyclohex-1-ene-1-carboxylate (6a)

To a suspension of $PdCl_2(PPh_3)_2$ (43 mg, 0.06 mmol) and CuI (17 mg, 0.09 mmol) in THF (15 mL) were added **3** (295 mg, 0.76 mmol), trimethylsilylacetylene (428 μL, 3.0 mmol), and piperidine (449 μL, 4.5 mmol). After being stirred at room temperature for 30 min, saturated aqueous $NaHCO_3$ was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 97:3) to give **6a** as a slightly yellowish oil (224 mg, 88%): IR (film) 2958, 2807, 2144, 1708, 1608, 1250, 844 cm^{-1}; ^1H NMR δ 0.20 (s, 9H, $Si(CH_3)_3$), 0.85 (t, $J = 7.3$ Hz, 6H, $2 \times NCH_2CH_2CH_3$), 1.36–1.46 (m, 5H, $2 \times NCH_2CH_2CH_3$, 4-H_{ax}), 1.83 (dddd, $J = 12.6$, 5.0, 4.6, 2.6 Hz, 1H, 4-H_{eq}), 2.21 (dddd, $J = 18.3$, 10.8, 4.6, 2.3 Hz, 1H, 3-H_{ax}), 2.36–2.43 (m, 5H, $2 \times NCH_2CH_2CH_3$, 6-H_{ax}), 2.44–2.52 (m, 1H, 6-H_{eq}), 2.57 (dddd, $J = 18.3$, 5.0, 1.9, 1.9 Hz, 1H, 3-H_{eq}), 2.75 (dddd, $J = 12.0$, 10.7, 4.9, 2.6 Hz, 1H, 5-H_{ax}), 3.78 (s, 3H, CO_2CH_3); EIMS 355 (M^+); Anal. Calcd for $C_{19}H_{33}NO_2Si$: C, 68.01; H, 9.91; N, 4.17. Found: C, 67.99; H, 9.66; N, 4.14.

3.4. Methyl 2-ethynyl-5-(dipropylamino)-cyclohex-1-ene-1-carboxylate (6b)

To a solution of **6a** (28 mg, 0.08 mmol) in THF (3 mL) was added Bu_4NF (100 μL, 1 M solution in THF) at −20 °C. After being stirred at this temperature for 40 min, saturated aqueous $NaHCO_3$ was added, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 6:4) to give **6b** as a slightly yellowish oil (19 mg, 86%): IR (film) 3288, 2958, 2809, 2090, 1725, 1614, 1238, 765 cm^{-1}; ^1H NMR δ 0.87 (t, $J = 7.4$ Hz, 6H, $2 \times NCH_2CH_2CH_3$), 1.36–1.52 (m, 5H, $2 \times NCH_2CH_2CH_3$, 4-H_{ax}), 1.85 (dddd, $J = 12.6$, 5.0, 4.7, 2.6 Hz, 1H, 4-H_{eq}), 2.23 (dddd, $J = 18.3$, 10.8, 4.7, 2.4 Hz, 1H, 3-H_{ax}), 2.35–2.49 (m, 6H, $2 \times NCH_2CH_2CH_3$, 6-H_2), 2.57 (dddd, $J = 18.3$, 5.0, 2.1, 2.1 Hz, 1H, 3-H_{eq}), 2.77 (dddd, $J = 12.0$, 10.7, 5.0, 2.6 Hz, 1H, 5-H_{ax}), 3.38 (s, 1H, C≡CH), 3.78 (s, 3H, CO_2Me); EIMS 263 (M^+); Anal. Calcd for $C_{16}H_{25}NO_2$: C, 72.97; H, 9.57; N, 5.32. Found: C, 72.64; H, 9.87; N, 5.25.

3.5. Methyl 2-(3-hydroxyprop-1-yn-1-yl)-5-(dipropylamino)-cyclohex-1-ene-1-carboxylate (6c)

To a suspension of $Pd(PPh_3)_4$ (17 mg, 0.015 mmol) and CuI (3.7 mg, 0.019 mmol) in THF (3.5 mL) were added a solution of **3** (38 mg, 0.1 mmol) in THF (0.5 mL), propargyl alcohol (40 μL, 0.68 mmol) and piperidine (75 μL, 0.58 mmol). After being stirred at room temperature for 30 min, saturated aqueous $NaHCO_3$ was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 95:5) to give **6c** as a yellowish oil (22 mg, 77%): IR (film) 3425, 2958, 2869, 2210, 1704, 1612, 1435, 1249, 1030, 764 cm^{-1}; ^1H NMR δ 0.87 (t, $J = 7.3$ Hz, 6H, $2 \times NCH_2CH_2CH_3$), 1.40–1.50 (m, 5H, $2 \times NCH_2CH_2CH_3$, 4-H), 1.70–1.90 (m, 2H, 4-H, CH_2OH), 2.20–2.28 (m, 1H, 3-H_{ax}), 2.32–2.52 (m, 6H, $2 \times NCH_2CH_2CH_3$, 3-H and/or 6-H), 2.56–2.61 (m, 1H, 3-H or 6-H), 2.78 (m, 1H, 5-H), 3.77 (s, 3H, CO_2Me), 4.46 (s, 2H, CH_2OH); ^{13}C NMR 11.8 ($NCH_2CH_2CH_3$), 21.9 ($NCH_2CH_2CH_3$), 24.7, 29.7, 32.9 (C-3, C-4, C-6), 51.7, 51.8, 52.5 (CH_2OH, CO_2CH_3, $NCH_2CH_2CH_3$), 55.6 (C-5), 85.4, 95.3 (C≡C), 128.2, 128.6 (C-1, C-2), 167 (CO_2CH_3); EIMS 293 (M^+); HREIMS calcd for $C_{17}H_{27}NO_3$: 293.1991; Found: 293.1993 (M^+).

3.6. Methyl 2-cyano-5-(dipropylamino)cyclohex-1-ene-1-carboxylate (6d)

To a solution of **3** (34 mg, 0.09 mmol) in benzene (4 mL) were added potassium cyanide (23 mg, 0.35 mmol), 18-crown-6 (46 mg, 0.17 mmol), and $Pd(PPh_3)_4$ (10 mg, 0.009 mmol). After being refluxed for 1.5 h, the mixture was cooled to room temperature, saturated aqueous $NaHCO_3$ was added, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 7:3) to give **6d** as a yellowish oil (14 mg, 61%): IR (film) 2958, 2811, 2217, 1727, 1631, 1249 cm^{-1}; ^1H NMR 0.87 (t, $J = 7.4$ Hz, 6H, $2 \times NCH_2CH_2CH_3$), 1.39–1.45 (m, 4H, $2 \times NCH_2CH_2CH_3$), 1.49 (dddd, $J = 12.7$, 11.7, 10.5, 5.4 Hz, 1H, 4-H_{ax}), 1.93 (dddd, $J = 12.7$, 5.2, 4.7, 2.5 Hz, 1H, 4-H_{eq}), 2.30 (dddd, $J = 19.2$, 10.5, 4.7, 2.5 Hz, 1H, 3-H_{ax}), 2.38–2.46 (m, 4H, $2 \times NCH_2CH_2CH_3$), 2.42–2.54 (m, 1H, 3-H_{eq} or 6-H), 2.58–2.70 (m, 2H, 3-H_{eq} or 6-H), 2.79 (dddd, $J = 11.7$, 10.4, 5.2, 2.9 Hz, 1H, 5-H_{ax}), 3.85 (s, 3H, CO_2CH_3); EIMS 264 (M^+); HREIMS calcd for $C_{15}H_{24}N_2O_2$: 264.1838; Found: 264.1841 (M^+).

3.7. 1-[2-Hydroxy-5-(dipropylamino)-cyclohex-1-en-1-yl]-ethanone (4)

A mixture of **1** (425 mg, 2.2 mmol) and Ac_2O (1.428 mL, 15.1 mmol) was added to $BF_3{\cdot}2CH_3COOH$ (4.161 mL, 10.8 mmol, 36%) at 0 °C. After being stirred at this temperature for 30 min and at room temperature for 16 h, a

solution of NaOAc (2.476 g, 30.2 mmol) in H_2O (15 mL) was added and the mixture was refluxed for 5 h. After the mixture was cooled to room temperature, pH was adjusted to 8 with saturated aqueous $NaHCO_3$, the aqueous layer was extracted with EtOAc, and the organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 95:5) to give **4** as a yellowish oil (409 mg, 79%): IR (film) 2958, 2811, 1744, 1704, 1612, 1461, 1419, 952 cm^{-1}; ^1H NMR δ 0.89 (t, J = 7.3 Hz, 6H, $2\times NCH_2CH_2CH_3$), 1.41–1.48 (m, 4H, $2\times NCH_2CH_2CH_3$), 1.55 (dddd, J = 12.9, 11.8, 11.1, 6.8 Hz, 1H, 4-H_{ax}), 1.88 (dddd, J = 12.9, 5.1, 5.0, 2.7 Hz, 1H, 4-H_{eq}), 2.14 (s, 3H, $COCH_3$), 2.22–2.30 (m, 1H, 3-H or 6-H), 2.37–2.47 (m, 7H, $2\times NCH_2CH_2CH_3$, 3-H, 6-H), 2.81 (dddd, J = 11.8, 10.8, 5.1, 2.8 Hz, 1H, 5-H_{ax}), 15.86 (s, 1H, C═COH); ^{13}C NMR δ 11.8 ($NCH_2CH_2CH_3$), 22.3 ($NCH_2CH_2CH_3$), 23.6 (C-4), 25.0 ($COCH_3$), 26.8, 31.3 (C-3, C-6), 52.8 ($NCH_2CH_2CH_3$), 56.5 (C-5), 106.0 (C-1), 181.4 (C-2), 199.1 ($COCH_3$); EIMS 239 (M^+); Anal. Calcd for $C_{14}H_{25}NO_2$: C, 70.25; H, 10.53; N, 5.85; Found: C, 70.15; H, 10.46; N, 5.84.

3.8. 2-Acetyl-4-(dipropylamino)cyclohex-1-en-1-yl trifluoro-methanesulfonate (5a)

To a suspension of NaH (187 mg, 4.7 mmol, 60% oil dispersion) in THF (14 mL) at 0 °C was added **4** (249 mg, 1.04 mmol) in THF (0.5 mL). The reaction mixture was allowed to stir at room temperature for 30 min, cooled to 0 °C again, and a solution of *N*-phenyltrifluoromethanesulfonimide (1.189 g, 3.3 mmol) in THF (1.5 mL) was then added. After being stirred at room temperature for 3.75 h, saturated aqueous $NaHCO_3$ was added and the aqueous layer 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 **5a** as a yellowish oil (210 mg, 54%): IR (film) 2962, 2815, 1704, 1662, 1419, 1211, 1141, 867 cm^{-1}; ^1H NMR δ 0.87 (t, J = 7.4 Hz, 6H, $2\times NCH_2CH_2CH_3$), 1.37–1.47 (m, 4H, $2\times$ $NCH_2CH_2CH_3$), 1.65 (dddd, J = 12.5, 12.0, 10.8, 6.0 Hz, 1H, 5-H_{ax}), 1.95 (dddd, J = 12.5, 5.2, 5.0, 2.5 Hz, 1H, 5-H_{eq}), 2.27 (dddd, J = 17.4, 10.4, 4.3, 2.5 Hz, 1H, 6-H_{ax}), 2.36–2.43 (m, 4H, $2\times NCH_2CH_2CH_3$), 2.38 (s, 3H, $COCH_3$), 2.48–2.56 (m, 3H, 3-H, 6-H_{eq}), 2.82 (dddd, J = 12.0, 10.3, 5.0, 2.9 Hz, 1H, 4-H_{ax}); ^{13}C NMR 11.8 ($NCH_2CH_2CH_3$), 22.1 ($NCH_2CH_2CH_3$), 25.0 (C-5), 27.7, 28.6 (C-3, C-6), 30.1 ($COCH_3$), 52.8 ($NCH_2CH_2CH_3$), 54.7 (C-4), 118.7 (q, CF_3), 130.1 (C-2), 148.3 (C-1), 198.2 ($COCH_3$); EIMS 238 (M-133), no molpeak found.

3.9. 1-[2-(Trimethylsilylethynyl)-5-(dipropylamino)-cyclohex-1-en-1-yl]ethanone (5b)

To a suspension of $Pd(PPh_3)_4$ (12 mg, 0.01 mmol) and CuI (3 mg, 0.016 mmol) in THF (2 mL) were added a solution of **5a** (50 mg, 0.13 mmol) in THF (0.5 mL), trimethylsilylacetylene (76 μL, 0.5 mmol), and piperidine (93 μL, 0.9 mmol). After being stirred at room temperature for 1 h, saturated aqueous $NaHCO_3$ was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 98:2) to give **5b** as a slightly yellowish oil (25 mg, 58%): IR (film) 2958, 2811, 2136, 1662, 1597, 1250, 844 cm^{-1}; ^1H NMR δ 0.20 (s, 9H, $Si(CH_3)_3$), 0.86 (t, J = 7.4 Hz, 6H, $2\times NCH_2CH_2CH_3$), 1.38–1.51 (m, 5H, $2\times NCH_2CH_2CH_3$, 4-H_{ax}), 1.86 (dddd, J = 10.0, 7.6, 4.9, 2.3 Hz, 1H, 4-H_{eq}), 2.13 (dddd, J = 18.3, 10.8, 3.9, 2.3 Hz, 1H, 3-H_{ax}), 2.37–2.59 (m, 7H, $2\times NCH_2CH_2CH_3$, 3-H_{eq}, 6-H), 2.56 (s, 3H, $COCH_3$), 2.74 (dddd, J = 11.9, 10.7, 4.9, 2.9 Hz, 1H, 5-H_{ax}); EIMS 319 (M^+); HREIMS calcd for $C_{19}H_{33}NOSi$: 319.2332; Found: 319.2328 (M^+).

3.10. [2-(Trimethylsilylethynyl)-5-(dipropylamino)-cyclohex-1-en-1-yl]methanol (7)

To a solution of **6a** (158 mg, 0.47 mmol) in THF (14 mL) was added $LiAlH_4$ (0.847 mL, 1 M in THF) at −50 °C. After being stirred at −50 °C for 3.25 h, saturated aqueous $NaHCO_3$ was added and the solution allowed to warm to room temperature. After filtration over Celite, the solvent was evaporated. A small sample of the residue was purified by flash chromatography (CH_2Cl_2–MeOH 9:1) to give the analytical data: IR (film) 3351, 2958, 2873, 2140, 1708, 1461, 1249, 844 cm^{-1}; ^1H NMR δ 0.19 (s, 9H, $Si(CH_3)_3$), 0.91 (t, J = 7.5 Hz, 6H, $2\times NCH_2CH_2CH_3$), 1.60 (m, 6H, $2\times NCH_2CH_2CH_3$, 4-H), 2.00–2.05 (m, 1H, 3-H or 6-H), 2.25–2.37 (m, 3H, 3-H, 6-H), 2.45–2.66 (m, 4H, $2\times NCH_2CH_2CH_3$), 2.97 (m, 1H, 5-H), 4.32 (s, 2H, CH_2OH); EIMS 307 (M^+).

3.11. 2-(Trimethylsilylethynyl)-5-(dipropylamino)-cyclohex-1-ene-1-carbaldehyde (8)

To a solution of **7** (120 mg crude) in CH_2Cl_2 (5 mL) in a pressure vial was added MnO_2 (245 mg, 2.8 mmol) and the reaction mixture was allowed to stir at 40 °C for 62 h. After being cooled to room temperature, the mixture was filtrated over Celite and the solvent was evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 97:3) to give **8** as a slightly yellowish oil (72 mg, 50% overall yield): IR (film) 2958, 2869, 2136, 1678, 1600, 1434, 1249, 848 cm^{-1}; ^1H NMR δ 0.22 (s, 9H, $Si(CH_3)_3$), 0.86 (t, J = 7.4 Hz, 6H, $2\times NCH_2CH_2CH_3$), 1.38–1.52 (m, 5H, $2\times NCH_2CH_2CH_3$, 4-H_{ax}), 1.89 (dddd, J = 12.8, 5.3, 4.5, 2.7 Hz, 1H, 4-H_{eq}), 2.01 (dddd, J = 18.2, 10.8, 4.5, 2.2 Hz, 1H, 3-H_{ax}), 2.38–2.44 (m, 4H, $2\times NCH_2CH_2CH_3$), 2.46–2.59 (m, 3H, 3-H_{eq}, 6-H), 2.73 (dddd, J = 11.7, 10.9, 5.0, 2.7 Hz, 1H, 5-H_{ax}), 10.19 (s, 1H, CHO); EIMS 305 (M^+): HREIMS calcd for $C_{18}H_{31}$ NOSi: 305.2175; Found: 305.2172 (M^+).

3.12. Dipropyl[4-(trimethylsilylethynyl)-3-vinylcyclohex-3-en-1-yl]amine (9a)

A suspension of methyltriphenylphosphonium bromide (450 mg, 1.3 mmol) in THF (13 mL) was cooled to −78 °C and *n*-BuLi (716 μL, 1.6 N in hexane) was added drop by drop. After being stirred at room temperature

for 1 h, the mixture was cooled to −78 °C again and a solution of **8** (38 mg, 0.12 mmol), in THF (0.5 mL) was added. After that, the reaction mixture was allowed to warm to room temperature in 1.5 h and stirred for another 2.5 h at that temperature. After that, saturated aqueous $NaHCO_3$ was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 7:3) to give **9a** as a colorless oil (24 mg, 62%): IR (film) 2958, 2807, 2134, 1616, 1461, 1249, 844 cm^{-1}; ^1H NMR δ 0.20 (s, 9H, $Si(CH_3)_3$), 0.87 (t, J = 7.4 Hz, 6H, 2 × $NCH_2CH_2CH_3$), 1.37–1.49 (m, 5H, 2 × $NCH_2CH_2CH_3$, 6-H_{ax}), 1.85 (dddd, J = 9.8, 7.4, 5.0, 2.4 Hz, 1H, 6-H_{eq}), 2.05–2.13 (m, 1H, 5-H_{ax}), 2.33–2.54 (m, 7H, 2 × $NCH_2CH_2CH_3$, 2-H, 5-H_{eq}), 2.78 (dddd, J = 12.0, 11.1, 5.0, 2.7 Hz, 1H, 1-H_{ax}), 5.13 (d, J = 11.0 Hz, 1H, HC=CH_2), 5.30 (d, J = 17.5 Hz, 1H, HC=CH_2), 7.08 (dd, J = 17.5, 11.0 Hz, 1H, HC=CH_2); EIMS 303 (M^+): HREIMS calcd for $C_{19}H_{33}NSi$: 303.2382; Found: 303.2380 (M^+).

3.13. Dipropyl-(4-ethynyl-3-vinylcyclohex-3-en-1-yl)-amine (9b)

To a solution of **9a** (19 mg, 0.06 mmol) in THF (2 mL) at −15 °C was added Bu_4NF (72 μL, 1 M solution in THF). After being stirred at this temperature for 1 h, saturated aqueous $NaHCO_3$ was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 9:1) to give **9b** as a colorless oil (12 mg, 83%): IR (film) 3309, 2957, 2808, 2087, 1618, 1464, 1249, 904, 600 cm^{-1}; ^1H NMR δ 0.88 (t, J = 7.4 Hz, 6H, 2 × $NCH_2CH_2CH_3$), 1.40–1.55 (m, 5H, 2 × $NCH_2CH_2CH_3$, 6-H_{ax}), 1.89 (dddd, J = 9.9, 7.2, 5.0, 2.3 Hz, 1H, 6-H_{eq}), 2.09–2.18 (m, 1H, 5-H_{ax}), 2.36–2.50 (m, 7H, 2 × $NCH_2CH_2CH_3$, 5-H_{eq}, 2-H), 2.78–2.88 (m, 1H, 1-H_{ax}), 3.24 (s, 1H, C≡CH), 5.14 (d, J = 11.0 Hz, 1H, HC=CH_2), 5.32 (d, J = 17.6 Hz, 1H, HC=CH_2), 7.08 (dd, J = 17.6, 11.0 Hz, 1H, HC=CH_2); EIMS 231 (M^+): HREIMS calcd for $C_{16}H_{25}N$: 231.1986; Found: 231.1987 (M^+).

3.14. [2-Ethynyl-5-(dipropylamino)-cyclohex-1-en-1-yl] methanol (10)

To a solution of **6b** (35 mg, 0.13 mmol) in THF (2.5 mL) was added $LiAlH_4$ (164 μL, 1 M in THF) at −50 °C. After being stirred at −50 °C for 1.75 h, saturated aqueous $NaHCO_3$ was added and the solution allowed to warm to room temperature. After filtration over Celite, the solvent was evaporated. A small sample of the residue was purified by flash chromatography (CH_2Cl_2–MeOH 9:1) to give the analytical data: IR (film) 3309, 2958, 2871, 2090, 1708, 1459, 1014 cm^{-1}; ^1H NMR δ 0.86 (t, J = 7.5 Hz, 6H, 2 × $NCH_2CH_2CH_3$), 1.37–1.50 (m, 5H, 2 × $NCH_2CH_2CH_3$, 4-H_{ax}), 1.82–1.88 (m, 2H, 4-H_{eq}, CH_2OH), 2.11–2.20 (m, 1H, 3-H or 6-H), 2.26–2.35 (m, 3H, 3-H, 6-H), 2.38–2.45 (m, 4H, 2 × $NCH_2CH_2CH_3$), 2.78 (dddd, J = 12.1, 10.7, 5.0, 2.8 Hz, 1H, 5-H_{ax}), 3.11 (s, 1H, C≡CH), 4.32 (s, 2H, CH_2OH).

3.15. 2-Ethynyl-5-(dipropylamino)-cyclohex-1-ene-1-carbaldehyde (11)

To a solution of **10** (29 mg crude) in CH_2Cl_2 (2 mL) in a pressure vial was added MnO_2 (113 mg, 1.3 mmol) and the reaction mixture was allowed to stir at room temperature for 24 h. After that, the mixture was filtrated over Celite and the solvent was evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH 97:3) to give **11** as a slightly yellowish oil (10 mg, 32% overall yield): IR (film) 3297, 2956, 2871, 2090, 1677, 1604, 1461, 1222, 848 cm^{-1}; ^1H NMR δ 0.87 (t, J = 7.4 Hz, 6H, 2 × $NCH_2CH_2CH_3$), 1.39–1.49 (m, 4H, 2 × $NCH_2CH_2CH_3$), 1.53 (dddd, J = 12.8, 11.9, 10.9, 6.0 Hz, 1H, 4-H_{ax}), 1.93 (dddd, J = 12.8, 5.2, 5.1, 2.6 Hz, 1H, 4-H_{eq}), 2.04 (m, 1H, 3-H_{ax}), 2.38–2.47 (m, 4H, 2 × $NCH_2CH_2CH_3$), 2.49–2.64 (m, 3H, 3-H_{eq}, 6-H), 2.77 (dddd, J = 11.9, 10.9, 4.8, 2.6 Hz, 1H, 5-H_{ax}), 3.47 (s, 1H, C≡CH), 10.18 (s, 1H, CHO); EIMS 233 (M^+): HREIMS calcd for $C_{15}H_{23}NO$: 233.1780; Found: 233.1779 (M^+).

3.16. Receptor binding experiments and data analysis

Receptor binding studies were carried out as described in the literature.[2] 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]SCH23390 at 0.3 nM (K_d = 0.7–1.1 nM). Competition experiments with the human $D2_{long}$, $D2_{short}$, 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 3–30 μg/assay tube and K_d values of 0.10 nM for $D2_{long}$ and $D2_{short}$, 0.10–0.30 nM for D3, and 0.39–0.98 nM for D4.4.

The resulting competition curves were analyzed by nonlinear 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. IC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[24]

3.17. 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 the literature.[19] For this assay 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 10 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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