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The Azulene Framework as a Novel Arene Bioisostere: Design of Potent Dopamine D4 Receptor Ligands Inducing Penile Erection

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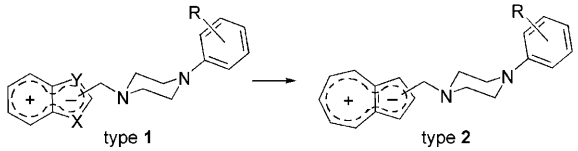
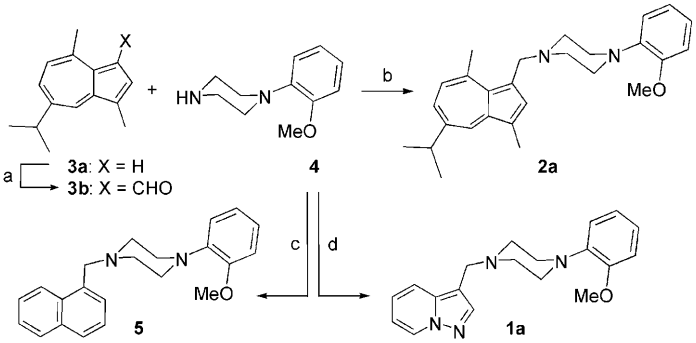
Heteroarene moieties are of primary importance in medicinal chemistry, as their conjugated π-electron systems can serve as both excellent bioisosteres for benzene-derived natural ligands and pharmacophoric appendages leading to an increase in binding affinity and subtype selectivity.[1] In the field of G-protein-coupled receptor (GPCR)-directed lead optimization, azaindole derivatives of type **1** (Scheme 1) could be developed as highly selective dopamine D4 receptor ligands, as the side chain of a particular amino acid in transmembrane helix 2 (F 2.61) clearly interacts with the heteroarene unit.[2] Fine-tuning these lead compounds is of particular interest because D4 up-regulation seems to be one of the biomolecular origins of schizophrenia, as in vivo pharmacological experiments with D4 antagonists have indicated antipsychotic activity.[3] Alternatively, selective D4 agonists have been shown to facilitate penile erection in rats and thus might be used for the treatment of erectile dysfunction.[4] The displacement of heterocyclic π-electron systems in D4 receptor ligands by benzoid carbocyclic analogues usually leads to a decrease in biological activity.[5] Because target binding can often be regained by the introduction of substituents, the effect might be due to the loss of polarization-inducing ring heteroatoms.[6] In contrast to the generally used alternant benzene and naphthalene units, the non-alternant azulene system reveals a non-uniform charge distribution with a significant buildup of electronic charge in the five-membered ring at the expense of the seven-membered ring which might lead to heteroarene-type recognition properties.[7] Although azulenes are known for their biological activity in natural-product-derived plant extracts,[8] the non-alternant scaffold has never been used as a pharmacophoric element in dopamine D4 receptor ligands.



Scheme 1. Molecular design of azulene-derived D4 agonists.

As an extension of our recent studies on unusual bioisosteres,[9] we present herein the chemical synthesis, receptor binding, functional assays, and in vivo pharmacological investigations of azulene-based D4 agonists of type **2**. By using site-directed mutagenesis an analogy of the binding modes of the lead compounds and the newly developed bioisosteres was investigated. Our initial studies were directed at the incorporation of the natural product derivative guaiazulene[10] into the pharmacophore of interest focusing on (2-methoxyphenyl)piperazines as potential D4 agonists. Thus, the non-benzoid building block **3a** was subjected to a Vilsmeier-type formylation to give the carbaldehyde **3b** (Scheme 2).[11] Subsequent reductive amination with 2-methoxyphenylpiperazine (**4**) resulted in the formation of the guaiazulene-based test compound **2a**.



Scheme 2. Reagents and conditions: a) *N*,*N*-dimethylformamide (DMF), $POCl_3$, 0 °C, 30 min (95%); b) $Na(OAc)_3BH$, CH_2Cl_2, 20 °C, 2 h (58%); c) 1-chloromethylnaphthalene, *N*,*N*-diisopropylethylamine (DIPEA), DMF, 60 °C, 30 min (90%); d) pyrazolo[1,5-*a*]pyridine, HCHO, AcOH, 20 °C, 4 h (92%).

To compare the potency of azulene **2a** with the biological properties of heterocyclic and alternant carbocyclic analogues, the 7a-azaindole **1a** and the naphthalene derivative **5** were prepared from (2-methoxyphenyl)piperazine **4** via a Mannich-type reaction with pyrazolo[1,5-*a*]pyridine and alkylation with naphth-1-ylmethylchloride, respectively (Scheme 2). To differentiate between interactions due to the non-alternant π-electron system and those of the *C*-alkyl substituents, unnatural azulenes were prepared and treated with various phenylpiperazine derivatives. Starting from 2,5-dihydrothiophene-1,1-dioxide, Br_2 addition gave the respective dibromide, which underwent elimination of HBr followed by [6+4]-cycloaddition with 6-di-

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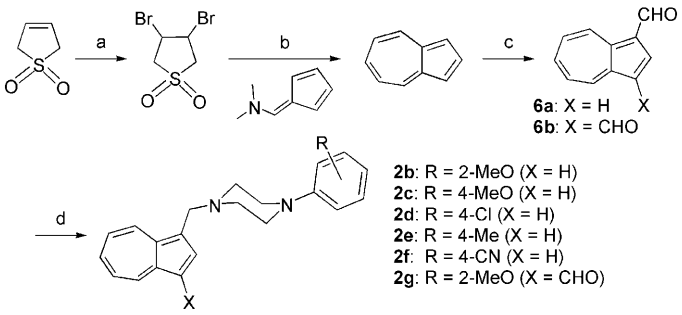
methylaminofulvene to give azulene in 34% overall yield.[12] Subsequent Vilsmeier formylation led to the respective mono- and diformylazulenes **6a** and **6b**, depending on the reaction conditions.[11] Finally, reductive amination resulted in the *N*-phenylpiperazine derivatives **2b**–**g** (Scheme 3).



Scheme 3. Reagents and conditions: a) Br_2, $CHCl_3$, 60 °C, 2 h (75%); b) 1. NaOH, THF, 0 °C, 2 h; 2. dimethylaminofulvene, THF, 0→65 °C, 4 h (34%); c) DMF, $POCl_3$, 20/70 °C, 1.5 h (**6a**: 61%, **6b**: 33%); d) phenylpiperazine derivative, $Na(OAc)_3BH$, CH_2Cl_2, 20 °C, 1 h (**2b**: 95%, **2c**: 98%, **2d**: 98%, **2e**: 83%; **2f**: 75%, **2g**: 45%).

Employing the dopaminergic reference agent quinpirole, receptor binding of the test compounds **1a**, **2a**–**g**, and **5** was determined subtype specifically by measuring their ability to compete in vitro with [^{3}H]spiperone for the cloned human dopamine receptors $D2_{long}$, $D2_{short}$, D3, and D4.4 stably expressed in Chinese hamster ovary (CHO) cells.[9c] D1 affinities were assessed by competition experiments using porcine striatal membrane preparations and the D1-selective radioligand [^{3}H]SCH23390 (Table 1).

Initially, we evaluated the formal replacement of the heteroarene moiety of lead compound **1a** by the alternant and non-alternant carbocyclic analogues **5** and **2a**, respectively. According to previous SAR studies on structurally related derivatives, the 7a-azaindole **1a** displayed significant and selective D4 recognition with a K_i value in the single-digit nanomolar range.[2a,c]

As expected, the uniform charge distribution of the benzoid carbocyclic analogue **5** led to a greater than tenfold decrease in affinity (K_i = 14 nM). Disappointingly, D4 receptor binding of the guaiazulene derived test compound was even worse; a K_i value of 230 nM indicated poor target recognition, which could be attributed to steric clashes of the alkyl substituents. However, evaluation of the unsubstituted azulene **2b** demonstrated excellent binding properties, with a K_i value of 0.40 nM. The superior binding energy is likely to be caused by the polarized charge distribution of the non-alternant carbocyclic bioisostere, as steric demand and lipophilicity are not significantly different from the naphthalene scaffold. Structural variations at the phenylpiperazine moiety showed decreased binding affinities, and SARs as described for heterocyclic D4 ligands.[2] Introduction of a formyl substituent into position 1 of the azulene led to a 3.3-fold decrease in affinity, corroborating our hypothesis that the non-uniform distribution with a negatively charged five-membered ring is highly beneficial for an attractive receptor–ligand interaction.

In contrast to 1,4-disubstituted aromatic piperazine/piperidines with longer spacer arms, D4 ligand–receptor recognition of heterocyclic lead compounds of type **1** with a one-carbon linker depends on favorable interactions with the side chain of a phenylalanine at position 2.61.[2b, 13] To determine if azulene-derived bioisosteres adopt an analogous binding mode, a transiently transfected mutant D4 receptor was constructed by substituting the non-conserved amino acid F2.61 with valine as the respective amino acid at the D2 subtype. In fact, comparison of the binding affinities of **1a** and **2b** toward D4 F2.61V and the D4 wild-type background revealed a 100-fold loss of affinity (Figure 1). On the other hand, identical K_d values

Table 1. Binding data for **1a**, **2a**–**g**, and **5** relative to the reference quinpirole at the human $D2_{long}$, $D2_{short}$, D3, and D4.4 receptors and the porcine D1 receptor.

Compd	K_i [nM][a] D1[b]	$D2_{long}$[c]	$D2_{short}$[c]	D3[c]	D4.4[c]
1a	4100	280	230	1200	1.2
2a	3800	830	780	620	230[d]
2b	500	33	24	82	0.40
2c	1400	6900	3500	3000	5.9
2d	3100	4700	2800	2200	49
2e	730	2300	300	2100	8.5[d]
2f	2100	35000	32000	4500	30
2g	860	42	33	880	1.3
5	2700	100	62	530	14
quinpirole	72000	950[e]	870[e]	49[e]	16[e]

[a] K_i values represent the mean of 2–13 experiments, each done in triplicate; standard deviation (±SEM) was <35%. [b] Dopamine D1 binding at porcine striatal receptors with the radioligand [^{3}H]SCH23390. [c] D2, D3, and D4 binding at human receptors stably expressed in CHO cells with the radioligand [^{3}H]spiperone. [d] Standard deviation (±SEM) <45%. [e] $K_{0.5}$ values (in nM) derived from competition curves displaying Hill slopes in the range of −0.52 to −0.71.

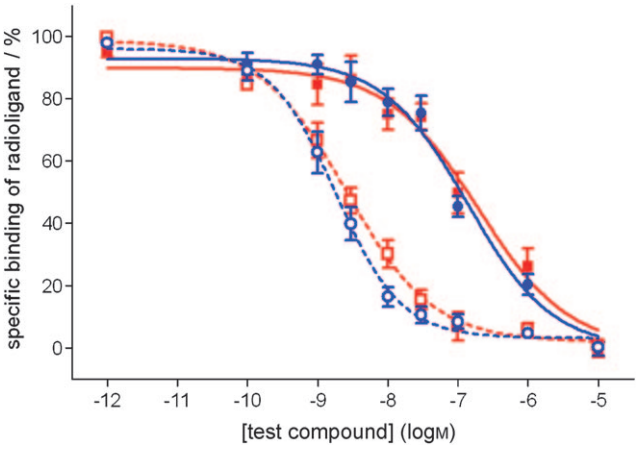


Figure 1. Radioligand displacement curves of **1a** and **2b** at the wild-type dopamine D4 and mutant D4 F2.61V receptors, indicating identical binding properties for heteroarene and azulene derivatives. □: **1a** + D4 wild-type (K_i = 1.1 nM); ■: **1a** + D4 F2.61V (K_i = 98 nM); ○: **2b** + D4 wild-type (K_i = 0.74 nM); ●: **2b** + D4 F2.61V (K_i = 70 nM).

indicated that the F 2.61 V mutation is insensitive to the long-chain reference agent spiperone ($K_d = 0.18$ nM).

Thus, the non-alternant carbocyclic ring of type **2** ligands acts as a superior surrogate for strongly polarized heteroarene-based scaffolds as incorporated in lead structure **1**, which adopts a highly similar binding mode; both ligand types interact with phenylalanine 2.61 in transmembrane helix 2 (Figure 2). The indicated binding mode was corroborated by

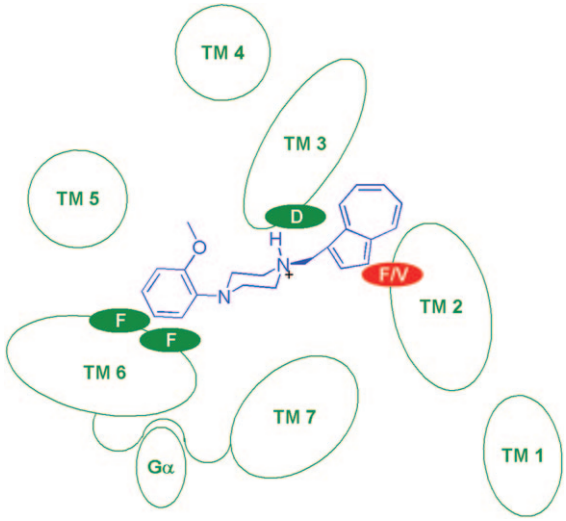


Figure 2. Conceptual visualization of the binding mode of the azulene-based D4 receptor ligand **2b** in which an in-house 3D homology model, based on the X-ray crystal structure of the β2 adrenergic receptor, was used as a template.[13]

docking studies on a D4 homology model that was built on the X-ray crystal structure of the β2 receptor.

For the pyrazolo[1,5-*a*]pyridine **1a** and the most promising azulene compound **2b**, functional activity at the dopamine D4 receptor was investigated in vitro in a mitogenesis assay that measures the rate of [^{3}H]thymidine incorporation into growing CHO cells stably expressing the human D4.2 receptor.[14] To understand the data at the molecular level, both **1a** and **2b** were also subjected to a GTPγS assay, which allows specific determination of agonist-stimulated coupling of G-protein to its receptor by inducing the binding of [^{35}S]GTPγS at membranes containing human D4 receptors.[2d,15] In comparison with the reference agonist quinpirole (efficacy = 100%), compounds **1a** and **2b** showed partial agonist activity in both the mitogenesis assay, with respective efficacies of 50 and 39%, and in the GTPγS experiments (40 and 54%) with potencies of 2.9 and 1.4 nM for **1a** and 1.2 and 3.7 nM for **2b**, respectively. These data are in good accordance with the intrinsic effects determined for structurally related analogues[2c,d] and confirm our observation that the polarized non-alternant carbocyclic moiety of the azulenes is able to substitute for the heteroarene pharmacophore not only in binding at the receptor but also in stimulating signal transduction.

Because of the interest in the treatment of erectile dysfunction, the role of D4 agonists in facilitating penile erection has been extensively investigated.[4] Employing the nonselective dopamine receptor agonist apomorphine as a reference agent, the D4-selective azulene derivative **2b** was tested in rats to study its ability to promote erection after intracerebroventricular (i.c.v.) or systemic (subcutaneous, s.c.) administration (Figure 3). Owing to its poor subtype selectivity, apomorphine

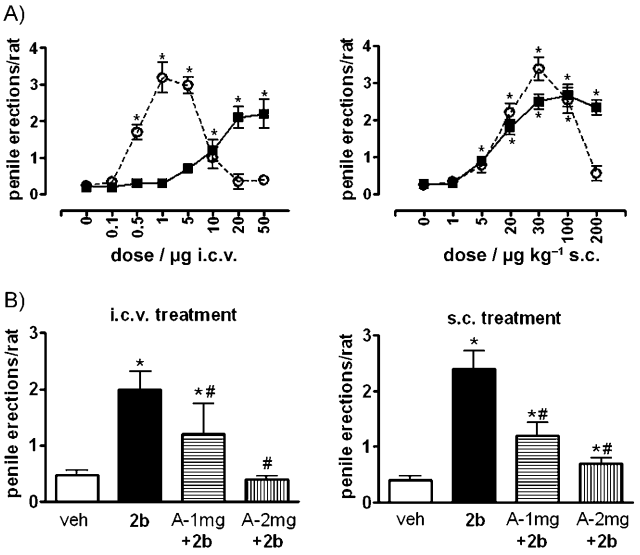


Figure 3. A) Effect of azulene **2b** (——■——), given i.c.v. (left) or s.c. (right), on penile erection in male rats in comparison with apomorphine (-----○-----): after administration of the test compounds, penile erection episodes per rat were counted for 60 min. B) Inhibition of **2b**-induced penile erection by the D4-selective antagonist L-745,870: L-745,870 was given i.p. at 1 mg kg^{-1} (A-1mg) or 2 mg kg^{-1} (A-2mg) 15 min before administration of test compound **2b** (50 µg i.c.v. (left) and 100 µg kg^{-1} s.c. (right)). Values represent the mean ± SEM of eight rats per group. *$P < 0.001$ with respect to vehicle-treated rats (veh); #$P < 0.01$ with respect to rats treated with azulene **2b** alone.

gives a bell-shaped curve, which is due to the drug exerting stereotypy and hypermotility at high doses, masking penile erection. Furthermore, systemic (s.c.) application requires a significantly higher effective dose, which indicates low bioavailability. In fact, azulene **2b** given either i.c.v. (0.1–50 µg) or s.c. (1–200 µg) induced penile erection in a dose-dependent manner with a potency similar to that of apomorphine (when given s.c.). An attenuation of efficacy at high doses was not observed. Although the heterocyclic surrogate **1a** showed a dose–response curve for concentrations ≤ 20 µg, similar to **2b**, significantly worse effects on penile erection were observed after s.c. treatment (Supporting Information). The pro-erectile effect of azulene **2b** was prevented in a dose–dependent manner by L-745,870, a selective D4 receptor antagonist,[4d,16] given intraperitoneally (i.p., 1–2 mg kg^{-1}) 15 min before azulene **2b.**

Based on D4 receptor agonists as a representative family of drug candidates, it can be concluded that the non-uniform charge distribution of the azulene framework is highly suitable for the bioisosteric replacement of bicyclic heteroarene moieties. Showing a binding mode analogous to that of lead com-

pounds of type **1**, the induction of penile erection in male rats over a greater range of doses indicates a putative advantage of the azulene derivative **2b** over apomorphine.

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Keywords: agonists · azulenes · bioisosteres · dopamine D4 receptors · GPCR · penile erection

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