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Novel azulene derivatives for the treatment of erectile dysfunction

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ABSTRACT

Based on the dopamine D_4 receptor partial agonist FAUC 3019, a series of azulenylmethylpiperazines was synthesized and affinities for the monoaminergic GPCRs including dopamine, serotonin, histamine and α-adrenergic receptor subtypes were determined. Ligand efficacies of the most promising test compounds revealed the *N*,*N*-dimethylaminomethyl substituted azulene **11** to be the most potent D_4 partial agonist (EC_{50} = 0.41 nM). This candidate was investigated for its ability to promote penile erection. Applying an in vivo animal model, test compound **11** turned out to stimulate penile erection in male rats with superior potency in low concentrations when compared to apomorphine.

The disability to initiate or maintain penile erection sufficient to permit sexual intercourse is a prevalent ailment among men. It has been estimated that erectile dysfunction (ED) affects 150 million individuals worldwide.[1] Since the 1970s, substantial progress has been made to understand the biochemical basis for the physiology of penile erection and the pathophysiology of erectile dysfunction.[2] It is now generally believed that the majority of patients with ED have an underlying vascular or neurological impairment that causes insufficient penile erection.[3] Starting from the early 1980s, non-selective inhibitors of the phosphodiesterase (PDE) like papaverine have been used as penile erecting agents,[4] but turned out to cause unpleasant side effects like prolonged erection and penile fibrosis.[5] In the following years, selective PDE5 inhibitors including sildenafil or tadalafin were launched to the market and turned out to be highly efficient drugs allowing oral administration. These agents act through a stimulation of NO release in the penile smooth muscle inducing its relaxation.[6]

Recent findings indicated that ED therapy needs not necessarily be based on peripheral modulation of endogenous mediators.[7] The non-selective dopamine receptor agonist apomorphine (Fig. 1) proved to be able to induce penile erection in rats, rabbits, monkeys and men.[8–10] Initially, it was hypothesized that the proerectile effect of apomorphine was mediated by the stimulation of D_2 receptors. The discovery of subtypes within the D_2 receptor family (D_2, D_3 and D_4) led to studies aiming to identify the subtype involved in this response to apomorphine and other dopaminergics.[11,12] Interestingly, selective (partial) agonists of the D_4 subtype displayed proerectile activity resulting in a new approach to the treatment of ED. These findings led to the development of the novel D_4-selective partial agonists ABT-724[13,14] and ABT-670[15] that have been under investigation in clinical trials for the treatment of erectile dysfunction. Recently, we could demonstrate that PIP3EA and PD-168,077, two dopamine D_4 receptor (partial) agonists induce penile erection when injected into the paraventricular nucleus of the hypothalamus (PVN) in rats by increasing

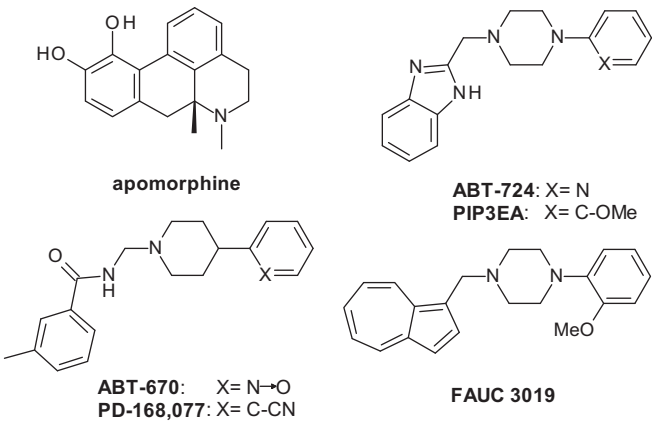

Figure 1. Dopamine D_4 (partial) agonists showing proerectile effects.

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central oxytocinergic neurotransmission.[16] This effect is associated by an increased NO production in the PVN. The findings boosted the search for new D_4 ligands, which has been an emerging field of drug discovery within the last two decades.[17]

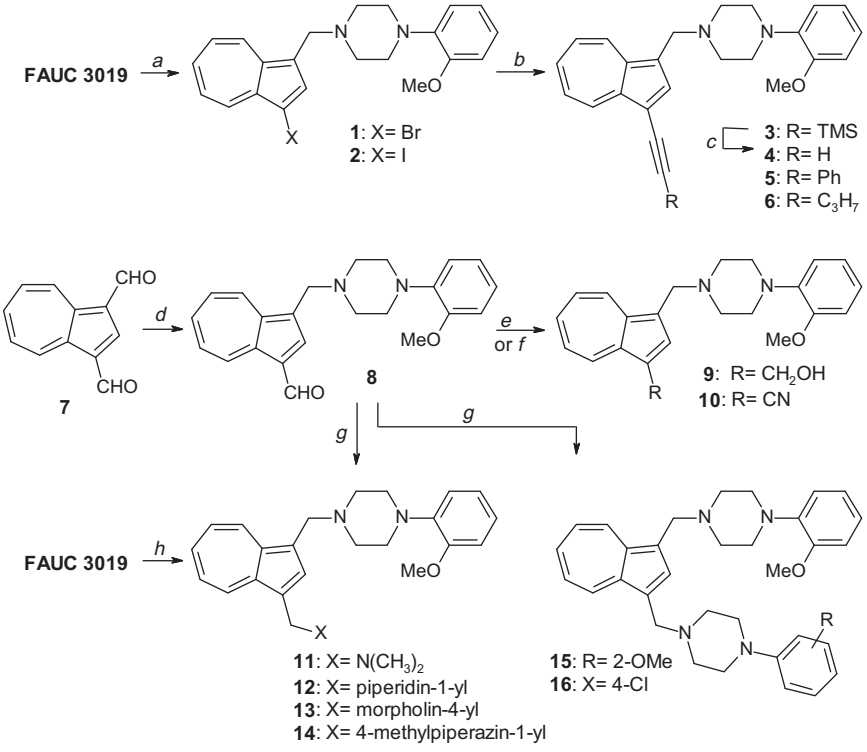
On the course of our investigations on subtype selective dopamine receptor agonists and antagonists,[18–20] we reported on the piperazinylmethyl substituted azulene FAUC 3019 showing subnanomolar affinity and partial agonism at the D_4 subtype.[21] FAUC 3019 revealed a strong pro-erectile effect in rats superior to the in vivo efficacy of apomorphine. The effect could be prevented in a dose-dependent manner by L-745,870,[22] a highly selective D_4 receptor antagonist. The rationale for the incorporation of the quite uncommon azulene moiety has been based on its non-uniform charge distribution with a significant negative molecular electrostatic field (MEP) below and above the five-membered ring and a positive MEP map at the seven-membered ring. The large dipole moment of azulenes accounts not only for an intense charge-transfer absorption in the visible region and the tendency to undergo electrophilic aromatic substitution at the five-membered ring, but also leads to receptor recognition properties similar to that of the pyrazolo[1,5-*a*]pyridine nucleus which has proved to be a potent recognition element in dopaminergic D_4 ligands.[23–25]

To further validate scope and limitations of the azulene scaffold for the design of potent dopamine D_4 partial agonists, we herein present the synthesis and SAR investigations on a series of FAUC 3019 derivatives bearing additional substituents in position 3 of the azulene moiety.

Our initial chemical investigations were directed to the introduction of a bromo or iodo substituent into the electron rich position 3 of the azulene ring system. Thus, FAUC 3019 was converted to the halogenated derivatives **1** and **2** in 52% and 49% yield, respectively (Scheme 1). The iodo azulene **2** was employed as a synthetic intermediate for an introduction of ethynyl substituents. Thus, *Sonogashira* cross-coupling with TMS-acetylene, phenylacetylene and 1-pentyne led to the test compounds **3**, **5** and **6**. The trimethylsilyl group of **3** could be easily removed with tetrabutylammonium fluoride to obtain the unprotected alkyne **4**. We also planned to introduce functional groups with H-bond donor or acceptor properties. Starting from the readily available azulenedicarbaldehyde **7**,[26] reductive amination yielded the *N*-phenylpiperazine derivative **8**. Subsequent reduction of the formyl group resulted in formation of the alcohol **9**. As a further structural variation, the carbaldehyde group of **8** was converted into a nitrile function upon reaction with NH_3 in the presence of iodine. The attachment of a third tertiary amine moiety was performed by reductive amination of the aldehyde **8** with secondary amines to give the respective bis-aminomethyl azulenes **11**–**16**. Alternatively, the dimethylaminomethyl derivative **11** could be synthesized by treatment of FAUC 3019 with *Eschenmoser's* salt.

Receptor binding experiments were established to evaluate the binding properties of the azulene derivatives **4**–**6** and **9**–**16** to the most relevant monoaminergic GPCRs. D_1 receptor affinities were determined utilizing porcine striatal membranes and the D_1 selective radioligand [^{3}H]SCH23390.[27] D_{2long}, D_{2short}, D_3, and D_4 receptor affinities were investigated employing the cloned human dopamine receptor subtypes D_{2long}, D_{2short},[28] D_3,[29] and $D_{4.4}$[30] stably expressed in Chinese hamster ovary cells (CHO) and the radioligand [^{3}H]spiperone.[27] Affinities to further aminergic GPCRs were determined to check the selectivities over connatural receptor families. Therefore, binding affinities to the porcine serotonin receptors 5-HT_{1A}, 5-HT_2 and the adrenoceptors α_1, α_2 as well as to the human histamine receptor subtypes H_1, H_2 H_3, H_4 were determined utilizing the radioligands [^{3}H]WAY100,635, [^{3}H]ketanserin, [^{3}H]prazosin, [^{3}H]RX821002, [^{3}H]mepyramine, [^{9}H]UR-DE257, [^{3}H]N^{α}-methylhistamine and [^{3}H]UR-PI294, respectively. The binding data was analyzed according to a sigmoid model by nonlinear regression.

The competition experiments clearly showed that the introduction of an alkyne side chain to the five-membered ring of the azulene core results in a decrease of dopamine D_4 receptor binding when compared to FAUC 3019 (Table 1). Thus, K_i values of approximately 10–20 nM were determined for the *Sonogashira* products **4**, **5** and **6**, whereas the affinity for D_{2long} and D_{2short} was more or less



Scheme 1. Reagents and conditions: (a) NBS or NIS, benzene, 5 °C, 3 h; (b) R-CCH, CuI, $Pd(PPh_3)_2Cl_2$, THF, rt, 16 h; (c) TBAF, THF, 0 °C, 1 h; (d) 4-(2-methoxyphenyl)piperazine, $Na(OAc)_3BH$, CH_2Cl_2, rt, 1 h; (e) $NaBH_4$, THF, rt, 1.5 h; (f) NH_4OH, I_2, H_2O, THF, rt, 16 h; (g) secondary amine, $Na(OAc)_3BH$, CH_2Cl_2, rt, 16 h; (h) $CH_2{=}N(CH_3)_2I$, CH_2Cl_2, rt, 4 h.

Table 1
Receptor binding data of compounds **4**–**6**, **9**–**16** and FAUC 3019 at the porcine dopamine D_1, the human dopamine D_{2long}, D_{2short}, D_3 and $D_{4.4}$, the porcine 5-HT_{1A} and 5-HT_2, the procine adrenergic α_1 and α_2 and the human histamine H_1, H_2, H_3 and H_4 receptors

Compound	K_i Values[a] [nM]												
	pD_1	hD_{2long}	hD_{2short}	hD_3	$hD_{4.4}$	p5-HT_{1A}	p5-HT_2	$p\alpha_1$	$p\alpha_2$	hH_1[b]	hH_2[b]	hH_3[b]	hH_4[b]
4	6400	95	76	370	9.5	680	2100	230	n.d.	n.d.	n.d.	n.d.	n.d.
5	1300	65	67	210	16	770	1000	370	n.d.	n.d.	n.d.	n.d.	n.d.
6	1400	76	60	370	19	410	1300	210	n.d.	n.d.	n.d.	n.d.	n.d.
9	860	150	68	580	0.71	120	990	57	44	1400	>10,000	>10,000	>10,000
10	410	8.3	39	530	2.4	110	380	31	n.d.	n.d.	n.d.	n.d.	n.d.
11	4500	210	140	390	1.5	110	1800	33	23	2600	3200	>10,000	>10,000
12	4100	51	37	160	1.9	65	1300	32	140	5500	4200	>10,000	>10,000
13	5000	130	92	350	4.6	99	1000	57	220	6500	6100	>10,000	>10,000
14	6400	60	57	460	3.8	91	2100	66	170	>10,000	5500	5500	>10,000
15	740	17	34	33	0.79	100	560	8.3	120	1000	530	>10,000	>10,000
16	1100	95	160	85	6.0	760	1600	27	1200	490	460	>10,000	>1000
FAUC 3019	500	33	24	83	0.40	54	92	20	39	n.d.	n.d.	n.d.	n.d.

n.d. = not determined.
[a] K_i Values in nM are based on the means of 2–11 experiments each done in triplicate.
[b] K_i Values in nM are based on one orientating experiment done in triplicate.

unchanged. Substitution of a hydroxymethyl group in position 3 of the azulene moiety led to the ligand **9** displaying D_4 ligand affinity that was similar to the lead compound FAUC 3019. Significant binding to the adrenoreceptors α_1 and α_2 (K_i: 57 nM and 44 nM, respectively) could be observed while selectivity over 5-HT_{1A}, 5-HT_2 and the histamine receptors H_1–H_4 was very high. The cyano derivative **10** showed K_i values in the single digit nanomolar range for D_4 and D_{2short} (K_i: 2.4 nM and 8.3 nM, respectively). Attachment of an aminomethyl substituent appeared to be an effective strategy to obtain suitable D_4 ligands with pronounced selectivity over related GPCR subtypes. Thus, the tertiary amines **11**–**14** showed K_i between 1.5 and 4.6 nM for D_4, when the dimethylaminomethyl derivative **11** displayed the best selectivity over D_1, D_2 and D_3. Moreover, the selectivity of **11** over the hallucinogenic anti-target 5-HT_2 (1200-fold) was superior when compared to the reference compound FAUC 3019 (230-fold). Interestingly, the symmetric bis-(2-methoxyphenylpiperazinyl methyl) substituted azulene **15** exhibited subnanomolar D_4 affinity (0.79 nM) as well as strong α_1 (8.3 nM) affinity and moderate H_2 binding (550 nM). The respective 2-chlorophenypiperazine **16** showed significantly weaker affinities to all tested aminergic GPCRs, but displayed the highest affinities to H_1 (490 nM) and H2 (460 nM) within the tested group. Due to compound instability, no reliable receptor binding data could be determined for the halogenated derivatives **1** and **2**.

To evaluate the efficacy of our D_4 ligands, intrinsic activities of the most promising target compounds were determined in vitro in a mitogenesis assay by measuring the rate of [^{3}H]thymidine incorporation into growing CHO cells stably expressing the human $D_{4.2}$ receptor (Table 2). In comparison with the reference agonist quinpirole (efficacy = 100%), the test compounds **9**, **11** and **15** showed partial agonist activity with ligand efficacies of 41%, 45% and 37%, respectively, which is similar to FAUC 3019 (39%). However, the dimethylamine **11** displayed D_4 potency (EC_{50} = 0.41 nM). Thus, the D_4 partial agonist **11** was chosen to be investigated for its ability to promote penile erection in an animal model.

As depicted in Figure 2, the test compound **11** proved to induce penile erection in male rats after both systemic (subcutaneous, sc) administration and injection into the paraventricular nucleus (PVN). The determined minimal effective dose has been 5 μg/kg sc and 5 ng when injected into the PVN, respectively. When compared to the reference agent apomorphine, the azulene **11** showed superior potency in concentrations up to 25 μg/kg (sc) or 25 ng/rat (into PVN), respectively. This is a substantial improvement when compared to FAUC 3019, which required a higher dose than apomorphine to reach the same efficacy.[21] The penile erection

Table 2
Intrinsic activities of the test compounds **9**, **11**, **15**, the reference compounds FAUC 3019 and quinpirole obtained from the stimulating effect on mitogenesis of D_4 receptor expressing cells

Compound	[^{3}H]thymidine incorporation (mitogenesis)	
	Agonist effect[a]	EC_{50}[b] (nM)
9	41%	0.94
11	45%	0.41
15	37%	0.98
FAUC 3019	39%	1.2
Quinpirole	100%	1.4

[a] Rate of incorporation of [^{3}H]thymidine as evidence for mitogenesis activity relative to the maximal effect of the full agonist quinpirole (=100%) used as a reference.
[b] EC_{50} values derived from a mean curve of 4–8 independent experiments each done in hexaplicates.

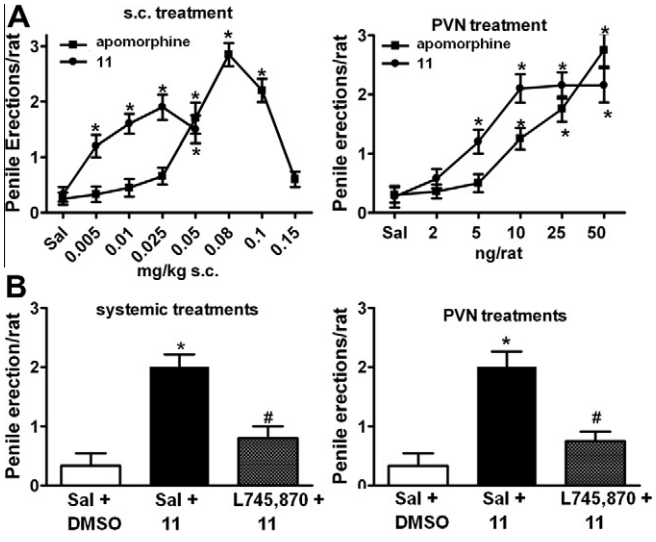


Figure 2. (A) Effect of **11** injected sc (left) or into the PVN (right) on penile erections in male rats: dose-response curves in comparison with apomorphine. Values are means ± SEM of 8 and 6 rats per group for sc and PVN treatment, respectively. (B) Effect of the D_4 antagonist L-745,870 given sc (1 mg/kg) (left) or into the PVN (1 μg) (right) on penile erections induced by **11** given sc (0.025 mg/kg) (left) or into the PVN (10 ng) (right). Values are means ± SEM of 10 and 8 rats per group for systemic and PVN treatments, respectively. $^*P$ <0.01 with respect to vehicle-treated rats; $^\#P$ <0.01 with respect to the corresponding group of vehicle + **11**-treated rats (One-Way ANOVA followed by Tukey's multicomparison test).

promoting effect of **11** reached 60–65% of the maximal response of apomorphine. In order to prove that this effect is attributed to the dopamine D_4 receptor agonism of **11**, the highly selective D_4 antagonist L-745,870 was used to block the observed response. Accordingly, administration of L-745,870 sc (1 mg/kg) or into the PVN (1 μg) antagonized almost completely the effect of **11** indicating a D_4 receptor mediated penile erection promoting effect.

In summary, we could demonstrate that the introduction of an additional substituent into the azulene moiety of our D4 partial agonist FAUC 3019 allows an effective tuning of affinity, subtype selectivity and in vivo potency. The *N,N*-dimethylaminomethyl substituted azulene **11** combines excellent D_4 affinity and good selectivity over a collection of relevant monaminergic GPCRs with high ligand efficacy. Applying an in vivo model, **11** turned out to stimulate penile erection in male rats with superior potency in low concentrations when compared to apomorphine, whereas the penile erection promoting effect of **11** reached 60–65% of the maximal response of apomorphine. Compared to FAUC 3019, which required a higher dose than apomorphine to reach the same efficacy, the test compound **11** displayed higher potency than apomorphine.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.064.

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