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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

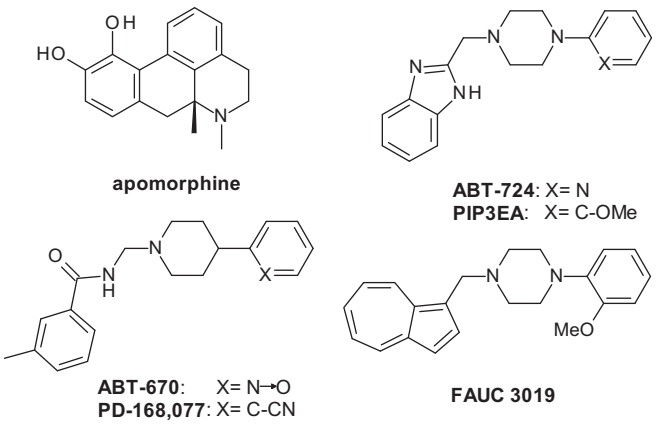

Figure 1. Dopamine D_4 (partial) agonists showing proerectile effects.

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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]

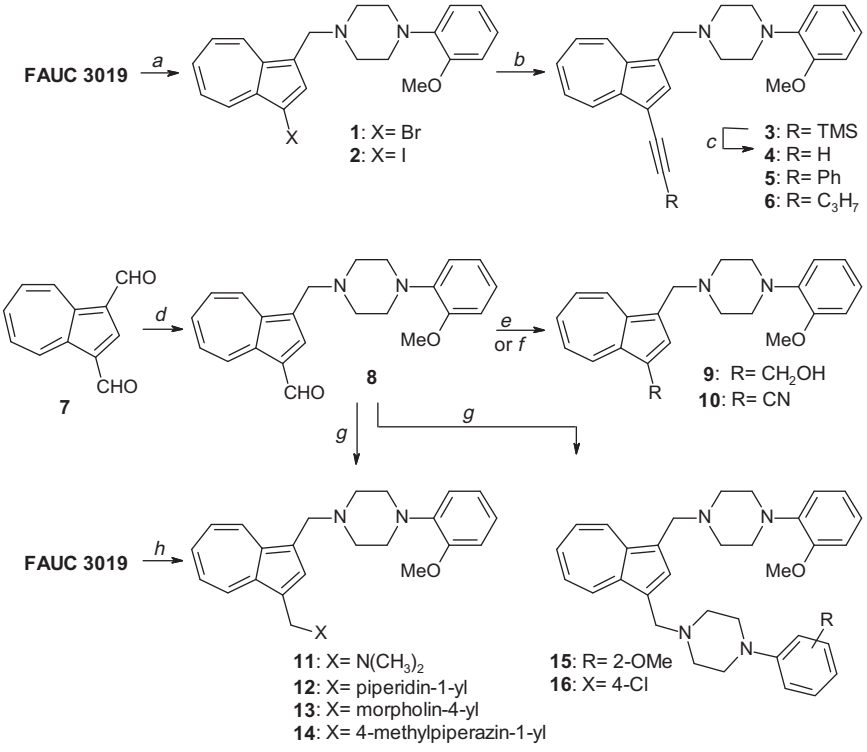
On the course of our investigations on subtype selective dopamine receptor agonists and antagonists,[18–20] we reported on the piperazinylmethyl substituted azulene FAUC 3019 showing subnanomolar affinity and partial agonism at the D_4 subtype.[21] FAUC 3019 revealed a strong pro-erectile effect in rats 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.