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3,4-Dihydroxy- and 3,4-methylenedioxy- phenanthrene-type alkaloids with high selectivity for D_2 dopamine receptor

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ABSTRACT

Dopamine-mediated neurotransmission plays an important role in relevant psychiatric and neurological disorders. Nowadays, there is an enormous interest in the development of new drugs acting at the dopamine receptors (DR) as potential new targets for the treatment of schizophrenia or Parkinson's disease. Previous studies have revealed that isoquinoline compounds such as tetrahydroisoquinolines (THIQs) can behave as selective D_2 dopaminergic alkaloids. In the present study we have synthesized five aporphine compounds and five phenanthrene alkaloids and evaluated their potential dopaminergic activity. Binding studies on rat striatal membranes were used to evaluate their affinity and selectivity towards D_1 and D_2 DR. Phenanthrene type alkaloids, in particular the 3,4-dihydroxy- and 3,4-methylenedioxy derivatives, displayed high selectivity towards D_2 DR. Therefore, they are potential candidates to be used in the treatment of schizophrenia (antagonists) or Parkinson's disease (agonists) due to their scarce D_1 DR-associated side effects.

Dopamine-mediated neurotransmission plays an important role in several psychiatric and neurological disorders which affect several million people worldwide. Researchers have focused on various approaches towards modulating dopaminergic activity via the dopamine receptors (DR) as a potential means of treating schizophrenia and Parkinson's diseases. The consequences of an activation or blockade of DR are wide-ranging, and a perturbation of dopamine neurotransmission may result in profound neurological, psychiatric, or physiological signs and symptoms. For these reasons, much research has focused on the discovery of novel dopaminergic ligands as potential drug candidates.[1] From a therapeutic point of view, drugs acting at D_2-like DR have become very relevant since most used antipsychotics in schizophrenia or bipolar disease treatment are D_2 antagonists and they are also involved in dopamine's release.[2]

Isoquinoline alkaloids are a large family of natural products with a variety of powerful biological activities,[3] including serotoninergic and dopaminergic.[4] Tetrahydroisoquinolines (THIQs), the most numerous naturally occurring alkaloids, include 1-benzyl-THIQs and aporphines, both of which share structural similarities with dopamine and can interact with DR.[5,6] Aporphine alkaloids constitute one of the largest groups of isoquinolines,[7] and many of them display pharmacological activities such as antioxidant, antiplatelet, antitumor, anticonvulsant, antineoplastic, cytotoxic, and antiparkinsonian.[8] In addition, aporphines' Hofmann degradation products, phenanthrene alkaloids, have also attracted researchers' attention since they can exert varied and powerful biological activities including α_1-adrenoceptor antagonism,[9] inhibition of acetylcholinesterase[10] and intestinal glucose uptake[11], antioxidant activity[12,13] or impairment of leukocyte–endothelial cells interactions.[14] Moreover, since they have been reported to produce effects associated with DR stimulation,[15] interaction with DR is therefore expected but barely explored.

Our group has long been interested in finding new and potent dopaminergic ligands. Previous observations reported by us have demonstrated that some natural and synthetic 1-benzyl-THIQs alkaloids can bind to DR.[16–20] In the present study, we have carried out the total synthesis of phenanthrene alkaloids, and take advantage of the diversity generated over the synthetic route of compounds which might display dopaminergic activity. Phenanthrene alkaloids are good candidates to bind to DR with high affinities due to their structural similarity to dopamine. Furthermore, as opposed to 1-benzyl-tetrahydroisoquinolines (BTHIQ) or aporphines where the amino function is held into a ring system, the flexible disposition

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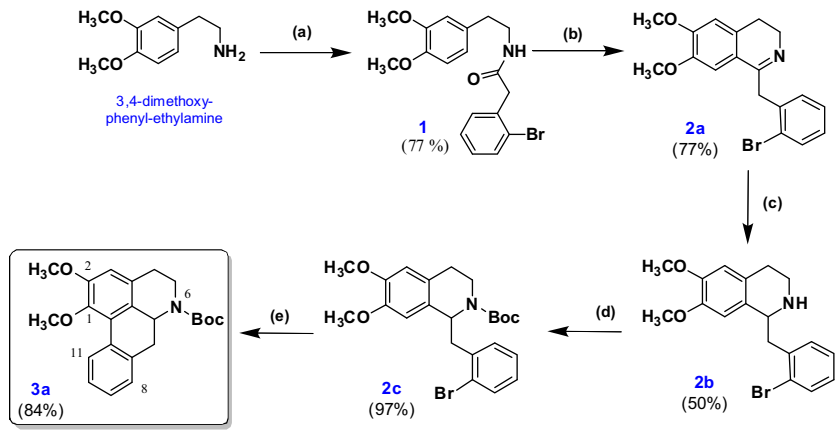
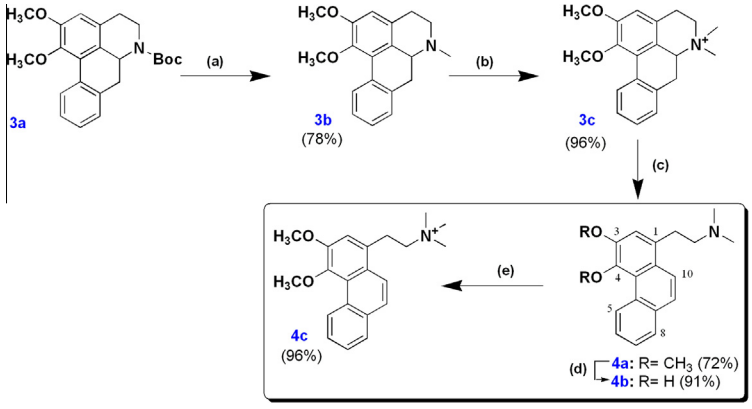


Scheme 1. Synthesis of aporphine **3a**. Reagents and conditions: (a) 2-bromophenylacetyl chloride, CH_2Cl_2, 5% aq NaOH, rt, 16 h; (b) $POCl_3$, CH_3CN, N_2, reflux, 1 h; (c) $NaBH_4$, CH_3OH, N_2, rt, 2 h; (d) di-*tert*-butyl dicarbonate, CH_2Cl_2, rt, 2 h; (e) 2-diphenylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, $Pd(OAc)_2$, K_2CO_3, DMA, 130 °C, 4 h.

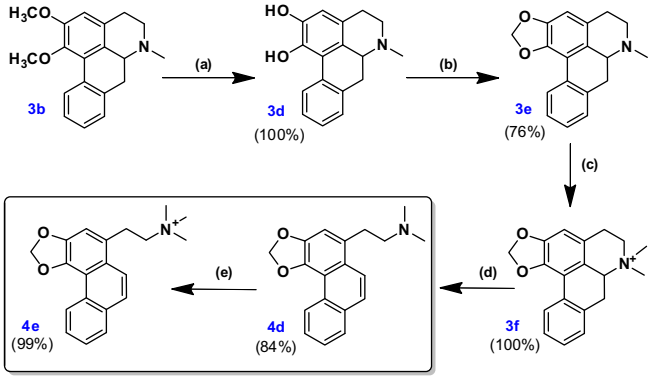


Scheme 2. Synthesis of phenathrenes **4a**–**4c**. Reagents and conditions: (a) $LiAlH_4$, THF, reflux, 4 h; (b) CH_3I, CH_3OH, Et_2O, rt, 3 h; (c) KOH 3 N, CH_3OH, 45 °C, 6 h; (d) BBr_3, CH_2Cl_2, N_2, rt, 2 h; (e) CH_3I, CH_3OH, Et_2O, rt, 1 h.

of the aminoethyl chain in phenanthrene alkaloids might reach the required conformation for an optimal DR interaction.

The general synthetic plan for these compounds consisted in preparing the appropriate β-phenyl acetamide **1** under Shotten–Baumann conditions to be then cyclized by Bischler–Napieralski cyclodehydration followed by imine reduction.[18,19] This sequence led us to obtain expected 1-(2′-bromobenzyl)-tetrahydroisoquinoline **2b**. The BTHIQ derivatives with a bromine atom placed over the phenyl ring (after protection of amino function with a BOC group) were then subjected to direct arylation to generate aporphine **3a** (Scheme 1).[21] After reduction of the carbamate protecting group, aporphine **3b** was *N*-methylated to obtain ammonium salt **3c** which, under basic Hofmann's degradation conditions, gave the corresponding phenanthrene derivative **4a** (Scheme 2).[22] Thereafter, these phenanthrene alkaloids were again *N*-methylated in order to further explore the influence of a quaternary ammonium group over dopaminergic activity (**4c**). The deprotection of dimethoxy groups from aporphine and phenanthene compounds was performed using BBr_3 to afford catechols **3d** and **4b**. Methylenedioxy derivative **3e** was formed by the reaction of **3d** with CsF and dichloromethane (Scheme 3).[17–19] Using this sequence, several known alkaloids were obtained, aporphines (±)-nuciferine (**3b**), (±)-roemerine (**3e**), (±)-roemrefidine (**3f**), and phenanthrenes atherosperminine (**4a**) and stephenanthine (**4d**).[8]

All the aporphines and phenanthrenes were assayed in vitro for their ability to displace selective D_1 and D_2·DR ligands from their respective specific binding sites in the striatal membranes



Scheme 3. Synthesis of phenanthrenes **4d**, **4e**. Reagents and conditions: (a) BBr_3, CH_2Cl_2, N_2, rt, 2 h; (b) CH_2Cl_2, CsF, DMF, reflux, 2 h; (c) CH_3I, CH_3OH, Et_2O, rt, 3 h; (d) KOH 3 N, CH_3OH, 45 °C, 6 h; (e) CH_3I, CH_3OH, Et_2O, rt, 1 h.

(Table 1).[17–19] Many of these compounds were able to displace both ^{3}H-SCH 23390 and ^{3}H-raclopride from their specific binding sites in rat striatum at micromolar or nanomolar concentrations.

In general, all the tested compounds followed the same tendency: (a) The nonquaternary aporphine compounds displayed similar potency against D_1 receptor (in the low micromolar range) regardless whether the oxygenated functions over the A-ring were protected or not. However, the affinity was substantially increased

Table 1
Affinity values (K_i, pK_i) and selectivity (ratio K_i·D_1/K_i·D_2) determined in binding experiments to D_1 and D_2·DR of compounds 3b, 3d, 3e, and 4a, 4b, 4d

Compounds	Specific ligand D_1 [^{3}H]-SCH 23390		Specific ligand D_2 [^{3}H]-raclopride		K_i D_1/D_2
	K_i (μM)	pK_i	K_i (μM)	pK_i	
Aporphines					
3b	0.630 ± 0.149	6.231 ± 0.123[f]	0.209 ± 0.086	6.761 ± 0.195	
3d	0.401 ± 0.044	6.401 ± 0.047[g]	0.045 ± 0.019	7.413 ± 0.174[a,d]	8.9
3e	0.377 ± 0.148	6.489 ± 0.167[h]	0.084 ± 0.012	7.080 ± 0.061[b,d]	4.4
Phenanthrenes					
4a	2.944 ± 0.304	5.535 ± 0.04	0.323 ± 0.026	6.494 ± 0.037[b]	9.1
4b	7.699 ± 0.330	5.208 ± 0.212	0.049 ± 0.018	7.414 ± 0.243[c,f]	**157**
4d	6.347 ± 0.415	5.383 ± 0.274	0.066 ± 0.009	7.189 ± 0.063[c,e]	**96**

Data were displayed as mean ± SEM for 3–5 experiments.
ANOVA, post Newmann–Keuls multiple comparison tests.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$, versus D_1-like DR.
[d] $p < 0.05$ versus 3b.
[e] $p < 0.05$ versus 4a.
[f] $p < 0.01$ versus 4a.
[g] $p < 0.001$ versus 4b.
[h] $p < 0.001$ versus 4d.

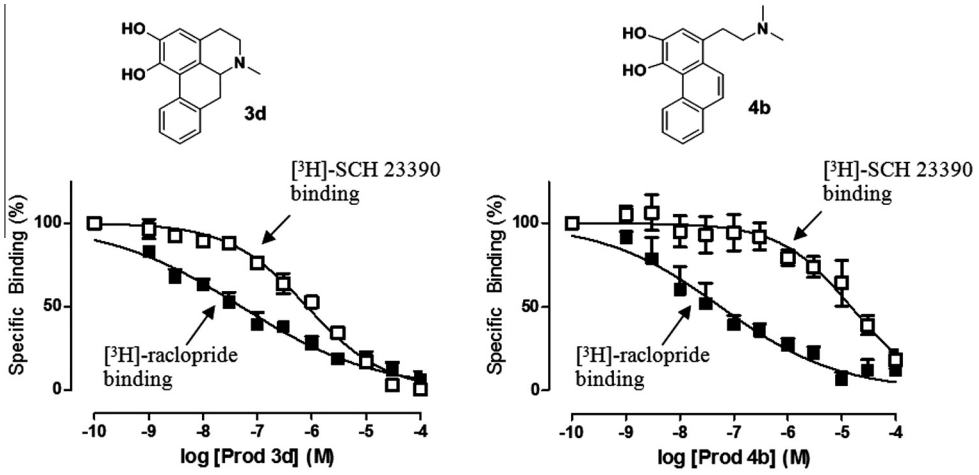


Figure 1. Selectivity of compounds **3d** and **4b** at the D_2-like dopaminergic receptors ([^{3}H]-raclopride binding). Data were displayed as mean ± SEM for 3–5 experiments.

towards D_2 receptor, with K_i values reaching the nanomolar range, when the oxygenated functions were in the catechol or the methylenedioxy form. The highest affinity of compounds with free phenol groups has been previously described in several isoquinolines.[17–20] (b) A similar behavior was observed in the non-quaternary phenanthrene alkaloids. Both, the methylenedioxy and the catechol groups improved the affinity towards D_2·DR (showing potent affinities within the nanomolar range). In contrast, their interaction with the D_1·DR was clearly undermined. (c) Regarding the quaternary ammonium compounds, both aporphines (**3c**, **3f**) and phenanthrenes (**4c**, **4e**) were inactive, probably due to the nitrogen atom in the quaternary form hindering its role as anchoring group with Asp86 which is essential for DA binding to D_2·DR.[23]

When both series were compared, they displayed similar D_2 affinities, but the conformationally restricted aporphines bound better to D_1 receptor than phenanthrenes (Table 1, Fig. 1). Therefore, it seems that the disposition of all carbon and hydrogen atoms lying in one plane (the phenanthrene ring) was slightly unfavorable towards D_1·DR interaction but not to D_2·DR. Indeed, phenanthrenes showed increased selectivity towards D_2·DR (compound **4b** D_1/D_2 ratio: 157) which may be of relevance from a pharmacological point of view. As previously mentioned, drugs acting at D_2-like DR are of special therapeutic potential since while the antagonists are used in the treatment of schizophrenia (antipsychotics), the agonists are employed in the treatment of Parkinson's disease.[24,25] Of note, other studies have even revealed the potential antidepressant effect of D_2 agonist.[3,18] Therefore, the therapeutic potential of these compounds is noticeable given their selectivity on D_2-related activities and their minimal D_1-associated collateral effects.

In summary, we have carried out the total synthesis of five aporphine compounds and five phenanthrene alkaloids and evaluated their potential dopaminergic activity towards D_1 and D_2 DR. Our results have demonstrated that phenanthrene alkaloids, in particular the 3,4-dihydroxy- and 3,4-methylenedioxy derivatives, display high selectivity towards D_2·DR. Taking into account that D_1·DR interaction may lead to undesirable side effects, including dyskinesia and cardiovascular disturbances, the D_2 selective compounds synthesized may constitute new and useful candidates for the treatment of schizophrenia (antagonists) or Parkinson's disease (agonists).

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