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SHORT COMMUNICATION

Synthesis and dopamine receptor binding affinity of 4*H*-thiochromenoapomorphines

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Abstract The synthesis of 4*H*-thiochromene derivatives of apomorphines, a novel class of isoquinoline alkaloid-related compounds, has been achieved by different *O*-dealkylation methods applied on previously published heteroring-fused aporphinoids. Detailed DFT study has been presented regarding the mechanism of the L-selectride-mediated multiple *O*-dealkylation of a seven-ring aporphine analogue. Dopamine-binding tests confirmed the essential function of 11-hydroxy moiety of the aporphine skeleton and revealed a remarkable D_1 over D_2 specificity for the derivative having the 4*H*-thiochromene ring system attached to positions 2 and 3 of the aporphine backbone.

Keywords Alkaloids · Heterocycles · Density functional calculations · Molecular modeling · Structure-activity relationships

Introduction

Since the approval of apomorphine hydrochloride as a drug substance by the FDA for the management of both Parkinson′s disease and erectile dysfunction, the development of potent and selective dopamine D_2 agonists gained a special emphasis [1–7].

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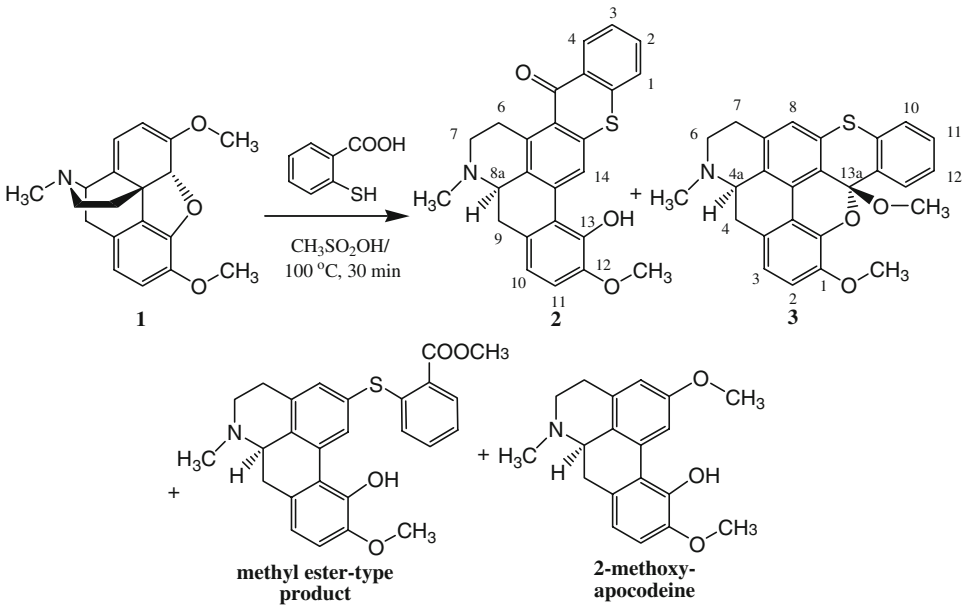
Our research group has been examining the possibilities of new heteroring formations on aporphine backbone for the last decade. Two protocols have been developed to achieve this goal: the first one involves the acid-catalyzed rearrangement of morphinandienes in the presence of O- and S-nucleophiles [3]; while the second one is based on the rearrangement of morphinans on which the heteroring was previously formed [4–7]. The pharmacological curiosity of apomorphines fused with novel five- or six-membered aromatic rings at the A ring of the aporphine skeleton originates from the steric similarity to 2- and 3-alkyl/arylapomorphines [8, 9] having superior dopamine receptor-binding affinity. This remarkable D_2 dopamine receptor-binding property is assigned to a specific lipophilic-lipophilic interaction between substituents at positions 2 or 3 and a cavity present on the peptide surface of D_2 subtype of dopamine receptors in the proximity of the binding site [1, 2].

Results and discussion

In one of our previous papers, we reported the acid-catalyzed rearrangement of thebaine (**1**) in the presence of thiosalicylic acid [3]. From the multi-component product mixture, two heteroring-fused aporphines were isolated and characterized with a variety of spectoscopic procedures in order to unambiguously prove their structure (Scheme 1).

We aimed to study the *O*-demethylation of compounds **2** and **3** in order to obtain apomorphine derivatives with potential pharmacological interest. From numerous useful procedures for the conversion of apocodeines into apomorphines in our laboratory, the methanesulfonic acid/methionine reagent mixture has been applied for over 15 years [10]. With the application of the conventional

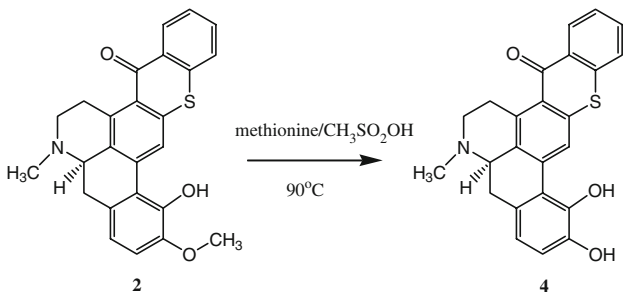
Scheme 1



conditions [10], we isolated the expected apomorphine **4** from the 4*H*-thiochromene derivative **2** (Scheme 2).

However, starting from the cyclic acetal **3**, the same procedure gave rise to a partially *O*-demethylated product **5** without the presence of the characteristic catechol structure of apomorphines (Scheme 3).

L-Selectride was successfully applied earlier in the *O*-demethylation of thebaine (**1**) and other morphinans by several research groups [11–14]. We decided to subject cyclic ketal **3** to a 2-week-long, room-temperature *O*-demethylation procedure with the use of L-selectride (5 eqv.) according to Coop's method elaborated for morphinans [11]. With the slightly modified workup reported by our group [12, 13], a one-component crude product was obtained. The detailed analytical characterization of compound **6** confirmed the presence of the free catechol motif and the absence of methoxy and aliphatic hydroxyl moieties. The proposed mechanism involves the L-selectride-assisted multiple *O*-dealkylations of the starting ketal **3** (Scheme 4).

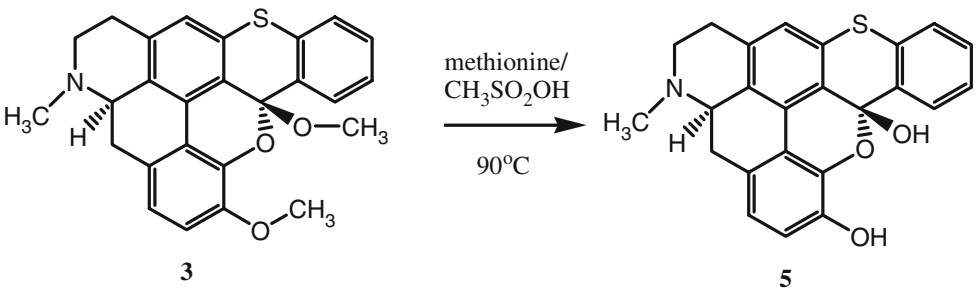


Scheme 2

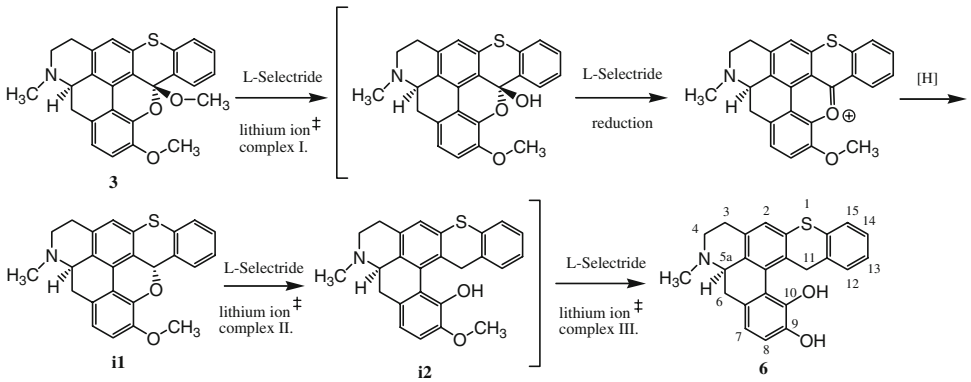
We established a computational model to determine the order of the possible steps and to characterize the intrinsic lithium ion complexes to discover the steric properties of the fundamental steps. This model was based on the mechanistic considerations of Coop et al. regarding the L-selectride-mediated *O*-demethylation [15], previous scientific works on quantum mechanical calculations and models for lithium ion complexes of ethylene glycol building blocks [16, 17] as well as the knowledge of the conformation of the aporphine skeletons of the cyclic ketal and the thioxanthylium ion [3]. In light of the energetic and steric details of the starting complex of Li^+-ketal **3**, two feasible mechanisms were identified. The first one involved the deprotection of the phenolic hydroxyl as an initiating step and then included the multistep *O*-dealkylation of the ketal center. The second one (Scheme 4) comprised the cleavage of the aliphatic methoxy moiety, involving an initial *O*-demethylation of the ketal and the fast reduction of the hemiketal structure, in accordance with the mechanism described by Czernecki and Ville [18], and resulted in **i1**, the cleavage of the O–C bridge bond between aromatic and aliphatic rings leading to **i2**, and finally the deprotection of the phenolic hydroxyl function forming the free catechol moiety of compound **6**. DFT calculations were carried out using the B3LYP [19, 20] exchange correlation functionals, together with the standard 6-31G* basis set [21][1]. The stationary points were characterized by frequency calculations in order to verify that the transition states have one, and only one, imaginary frequency. The intrinsic reaction coordinate (IRC) [22] path was traced in

[1] Calculation results using B3LYP/6-31G* level for the prediction of the structure of compound **3** showed satisfactory conformity with available X-ray data (Ref. [3]).

Scheme 3



Scheme 4



order to check the energy profiles connecting each transition structure to the two associated minima of the proposed mechanism.

After processing the details the two mechanisms, they were evaluated in terms of energetic and steric parameters applying also the Curtin–Hammett principle, and it was found that the second one is significantly favorable in all aspects.

The structure and key geometry data for determining lithium ion complexes I–III are presented in Fig. 1 and Table 1, respectively.

All three *O*-dealkylation products **4–6** were screened for their binding affinities for the human cloned D_1 and D_{2L} receptor subtypes by in vitro radioligand binding studies. The results are summarized in Table 2. With the exception of **5**, all of the compounds displayed low affinities compared to apomorphine, reaching the maximum for compound **4** at the D_1 binding site. It could be concluded that compounds **5** and **6** containing the novel 4*H*-thiochromene moiety at positions 1 and 2 of the aporphine backbone showed significantly lower affinity to both D_1- and D_2-subtypes; furthermore, the inactivity of compound **5** is another direct proof of the generally accepted theory pointing out the crucial role of 11-hydroxy function in the dopaminergic activity of aporphines. In compound **5** the hemiketal moiety in the proximity of position 11 does not only block the phenolic hydroxyl, but hinders any advantageous interactions. The aliphatic hydroxyl group is not able to effectively replace the determining function of aromatic hydroxyl.

In order to obtain detailed pharmacological profile for the most active compound, the binding affinities of compound **4** were extended to all five human dopamine receptor subtypes and the results summarized in Table 3.

These tests pointed out that the novel apomorphine having the 4*H*-thiochromene moiety fused to positions 2

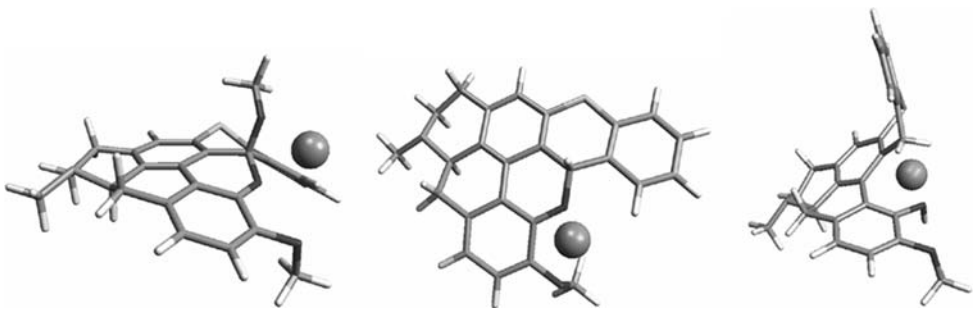


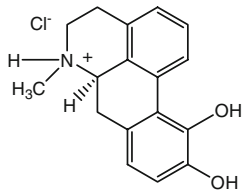
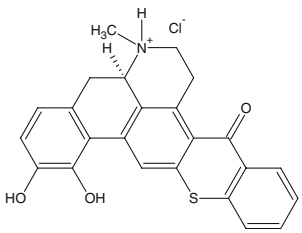
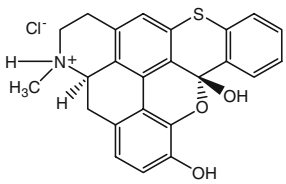
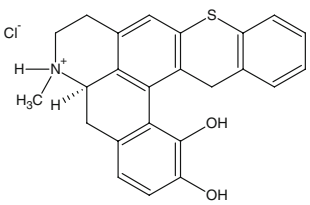
Fig. 1 Presumed lithium ion complexes I–III involved in the *O*-dealkylation of cyclic ketal **3**. The coordination of THF molecules was omitted for clarity

Table 1 Important parameters of lithium ion complexes

	Distances (Å)			Bond angles (°)		Dihedral (°)
	C1-O···Li^+	C14-O···Li^+	C13a-O···Li^+	C1-O–Li^+–C14-O	C14-O–Li^+–C13a-O	C14-C1-O-Li^+
Li^+ complex I	2.270	2.013	2.245	59.31	57.29	59.31
Li^+ complex II	2.317	1.998	–	56.69	–	51.45
Li^+ complex III	2.216	2.334	–	50.81	–	75.77

Numbering is in accordance with the Scheme 1

Table 2 Affinities for dopamine receptor subtypes measured by radioligand binding studies

Compound		K_i [nM] Average ± SD or SEM (experiments in triplicate)		D_1/D_2 selectivity
		hD_1	hD_{2L}	
APOMORPHINE.HCl		210*	13*	16.15
4.HCl		252 ± 30 (2)	1,025 ± 169 (2)	0.25
5.HCl		>10,000 (3)	>10,000 (3)	NA
6.HCl		3,237 ± 226 (3)	2,096 ± 886 (2)	1.54

SD standard deviation, *SEM* standard error of the mean; the SEM was used when the number of values was less than three
*Data from [1, 2]

and 3 of the aporphine backbone **4** had a significantly stronger affinity to D_1 subtype referring to the calculated specificities.

In conclusion we have presented the formation of 4*H*-thiochromene derivatives of apomorphines by different *O*-dealkylation methods applied on previously published

Table 3 Detailed dopamine activity data for compound **4**

Compound	K_i [nM] Average ± SD or SEM (experiments in triplicate)		hD_1/hD_x specificity
4.HCl	hD_1	252 ± 30 (2)	–
	hD_{2L}	1,025 ± 169 (2)	4.1
	hD_3	1,152 ± 630 (2)	4.6
	$hD_{4.4}$	7,443 ± 1,184 (2)	29.5
	hD_5	117 ± 51 (2)	0.46

SD standard deviation, *SEM* standard error of the mean

The SEM was used, when the number of values was less than three

heteroring-fused aporphinoids. Detailed DFT study has been presented regarding the mechanism of the L-selectride-mediated multiple *O*-dealkylation of a seven-ring aporphine analogue including the structures and geometry details of the involved Li^+ complexes. Dopamine-binding tests confirmed the importance of 11-hydroxy moiety of the aporphine skeleton.

Experimental

Melting points were determined with a Kofler hot-stage apparatus. Thin-layer chromatography was performed on pre-coated Merck 5554 Kieselgel 60 F_{254} foils using chloroform: methanol = 8:2 mobile phase. The spots were visualized with Dragendorff's reagent. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DMX 400 spectrometer; chemical shifts are reported in ppm (δ) from internal TMS, and coupling constants (J) are measured in Hz. High-resolution mass spectral measurements were performed with a Bruker micrOTOF-Q instrument in the ESI mode. Optical rotation was determined with a Perkin Elmer Model 241 polarimeter. Elemental analyses (C, H, N, S) were conducted using the Elemental Analyser Carlo Erba 1106; their results were found to be in good agreement (±0.2%) with the calculated values.

*8aR)-12,13-Dihydroxy-8-methyl-5,6,7,8,8a,9-hexahydronaphto[3,2,1-ij] thiochromeno-[3′,2′-f] isoquinolin-5-one.HCl (**4**.HCl)*

The title compound was obtained from compound **2** (1,000 mg, 2.41 mmol) in line with the procedure described in [10]. Yield for the HCl salt 692 mg (85%), yellow, cubic crystals; M.p.: >250 °C (ether); $[\alpha]_D^{25}$ −156 cm^2 g^{-1} (c 0.1, DMSO); R_f base (chloroform : methanol = 8:2) 0.18; HR-MS (ESI) *m/z* (%) calculated for $C_{24}H_{20}NO_3S^+$: 402.1158 ($M^+ + 1$), Found: 402.1174 ($M^+ + 1$, 100); ^{1}H-NMR (400 MHz DMSO-d_6) δ = 7.43–7.12 (m, H1–H4, H14), 6.63, 6.58 (2d, $J_{10\text{-}11}$ 8.0 Hz, H10, H11), 6.34, 6.27 (2 br s, 2 OH), 4.14 (td, $J_{8a\text{-}9a}$ 9.0 Hz, $J_{8a\text{-}9b}$ 3.1 Hz, H8a), 3.12–2.34 (m, H6a, H6b, H7a, H7b, H9a, H9b, NCH_3) ppm; ^{13}C-NMR (100 MHz DMSO-d_6) δ = 187.1 (C5), 145.1 (C12), 144.7 (C13), 143.2 (C6a), 137.2–116.2 (15C, aromatic), 60.7 (C8a), 52.6 (C7), 41.0 (NCH_3), 36.2 (C9), 23.6 (C6) ppm.

*(4aR,13bS)-1,13b-Dihydroxy-5-methyl-4,4a,5,6,7,13b-hexahydrobenzo [8′,9′] thiochromeno[2″,3″,4″f′g′-] isochromeno[6,5,4-def] quinoline.HCl (**5**.HCl)*

The title compound was obtained from compound **3** (1,000 mg, 2.33 mmol) in line with the procedure described in [10]. Yield for the HCl salt 683 mg (67%), orange needles, M.p.: 218 °C (decomp.); $[\alpha]_D^{25}$ +96 cm^2 g^{-1} (c 0.1, DMSO); R_f base (chloroform : methanol = 8:2) 0.26; HR-MS (ESI) *m/z* (%) calculated for $C_{24}H_{20}NO_3S^+$: 402.1164 ($M^+ + 1$), Found: 402.1155 ($M^+ + 1$, 100); ^{1}H-NMR (400 MHz DMSO-d_6) δ = 7.35-7.02 (m, H8, H10-H13), 6.57, 6.52 (2d, $J_{2\text{-}3}$ 8.2 Hz, H2, H3), 6.44 (br s, C1–OH), 4.05 (td, $J_{4a\text{-}3a}$ 8.4 Hz, $J_{4a\text{-}3b}$ 2.5 Hz, H4a), 3.23–2.12 (m, H3a, H3b, H5a, H5b, H6a, H6b, C14-OH, NCH_3) ppm; ^{13}C-NMR (100 MHz DMSO-d_6) δ = 151.7 (C14), 145.7 (C13a), 143.4 (C1), 139.4–114.6 (15C, aromatic), 99.9 (C13b), 60.2 (C4a), 51.8 (C5), 43.0 (NCH_3), 35.1 (C3), 27.3 (C6) ppm.

Compound **6** was synthesized in accordance with the room-temperature procedure described in [11] starting from 1 g of ketal **3**; however, the workup was performed in line with our modified methodology [12, 13]. The product was immediately transformed into stable HCl salt form.

*(5aR)-9,10-Dihydroxy-5-methyl-3,4,6,7,7a,8-hexahydronaphto[3,2,1-ij] thiochromeno-[2′,1′-f] isoquinolin.HCl (**6**.HCl)*

The title compound was obtained from compound **3** (1,000 mg, 2.33 mmol). Yield for the HCl salt 721 mg (73%),off-white, cubic crystals; M.p.: >250 °C (ether); $[\alpha]_D^{25}$ −12 cm^2 g^{-1} (c 0.1, DMSO); R_f base (chloroform : methanol = 8:2) 0.31; HR-MS (ESI) *m/z* (%) calculated for $C_{24}H_{22}NO_2S^+$: 388.1371 ($M^+ + 1$), Found: 388.1389 ($M^+ + 1$, 100); ^{1}H-NMR (400 MHz DMSO-d_6) δ = 7.11–6.88 (m, H2, H12-H15), 6.59, 6.52 (2d, $J_{7\text{-}8}$ 8.1 Hz, H7, H8), 6.41, 6.32 (2 br s, 2 OH), 4.21 (td, $J_{5a\text{-}6a}$ 8.2 Hz, $J_{5a\text{-}6b}$ 2.9 Hz, H5a), 3.77 (s, H11a, H11b), 3.32-2.53 (m, H3a, H3b, H4a, H4b, H6a, H6b, NCH_3) ppm; ^{13}C-NMR (100 MHz DMSO-d_6) δ = 145.7 (C9), 143.8 (C10), 138.5–116.3 (15C, aromatic), 61.5 (C5a), 51.5 (C4), 41.1 (NCH_3), 35.8 (C6), 22.7 (C3) ppm.

Pharmacological protocol

Human D_1, D_{2L} or D_5 receptors were stably expressed in human embryonic kidney cells (HEK293). Stable cell lines of HEK 293 cells were generated by transfecting the plasmids coding for hD_3 using Polyfect® transfection

reagent (Qiagen, Hilden, Germany) according to manufacturer's instructions and were selected using G-418 (400 μg/ml medium). The human $D_{4.4}$ receptor was stabily expressed in CHO cells. The density of D_1-like receptors measured with [^{3}H]-SCH23390 was 3,139 fmol/mg protein. For D_2-like receptors, the density of receptors was 186.53 fmol/mg protein measured with [^{3}H]-spiperone. The binding studies were performed following the protocol described previously [23], but in 96- well format. The assays with the whole-cell suspension were carried out in triplicate in a volume of 550 μl (final concentration): TRIS-Mg^{2+} buffer (345 μl), [^{3}H] ligand (50 μl), whole-cell suspension (100 μl), and appropriate drugs (55 μl). Non-specific binding was determined using fluphenazine (100 μM) in hD_1 tests and haloperidol (10 μM) in hD_{2L} tests. The incubation was initiated by addition of the radioligand [^{3}H]SCH23390 for hD_1-like receptors and [^{3}H]spiperone for hD_{2L}-like receptors (both Amersham Biosiences, Little Chalfront, UK). For determining the K_i values, at least two independent experiments each in triplicate were performed. For a detailed description of the pharmacology methods, see [6].

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