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Synthesis and nicotinic binding studies on enantiopure pinnamine variants with an 8-azabicyclo[3.2.1]octane moiety

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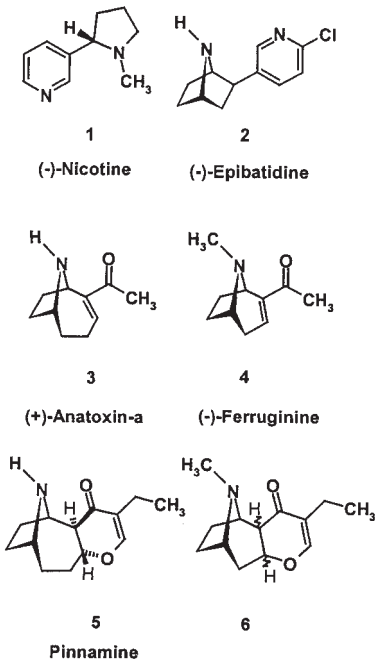
Bioisosteric replacement of the 9-azabicyclo[4.2.1]nonane pharmacophoric element of the novel alkaloidal marine toxine pinnamine (**5**) by the 8-azabicyclo[3.2.1]octane moiety resulted in conformationally restricted analogues **6a** and **6c** of (−)-ferruginine (**4**). Key step in the diastereoselective synthesis of these pyranotropanes was the condensation of enantiopure ecgonine methyl ester (**9**) from the "chiral pool" with the lithium anion of *N-tert*-butylbutyraldimin and subsequent cyclisation with TFA. The potential nAChR ligands were tested for their *in vitro* affinity for the $(\alpha4)_2(\beta2)_3$ and the $\alpha7^*$ nAChR subtypes. Despite obvious structural similarities with the potent alkaloids pinnamine (**5**) and (−)-ferruginine (**4**) the pyranotropanes **6a** and **6c** exhibited distinctly lower nAChR affinities.

1. Introduction

Over recent years steadily increasing interest in nicotinic acetylcholine receptors (nAChR) has arisen because they represent attractive targets for drug discovery [1−10]. Studies in humans and animals with (−)-nicotine (**1**) exhibiting the ability of this alkaloid **1** to upregulate nAChRs and demonstrating the properties of **1** as a cognitive-enhancing agent with additionally neuroprotective effects supported the attractiveness of this research field [3, 11, 12]. Moreover, worldwide interest in nAChR agonists as potential analgesics has emerged, representing an attractive area of research in pain control since the discovery of the potent, broad spectrum antinociceptive actions of (−)-epibatidine (**2**) [13] mediated via a neuronal nAChR mechanism [3]. Besides (−)-nicotine (**1**) and (−)-epibatidine (**2**) several other alkaloidal nAChR ligands e.g. (+)-anatoxin-a (**3**) [14] and (−)-ferruginine (**4**) [15, 16] interact more or less selective with the multifarious nAChR subtypes. Consequently all of them are rather toxic. Mainly ganglionic-type nAChRs are believed to at least partially mediate the toxic effects of the natural ligands **1**–**4** [3]. Thus, an important objective in the research field targeting the neuronal nAChRs would be to achieve selectivity for central versus ganglionic nAChRs. This prompted a world wide search for novel subtype-selective nAChR ligands as potential therapeutic agents with potent activity possibly improved safety profile and satisfactory bioavailability compared to the alkaloidal agents.

Our recent efforts aim at the development of novel semisynthetic variants of highly potent nAChR ligands with a distinct subtype profile towards the central, heteropentameric $(\alpha4)_2(\beta2)_3$ nAChR (the main subtype found in brain tissue) eliminating interactions with the ganglionic subtype [17].

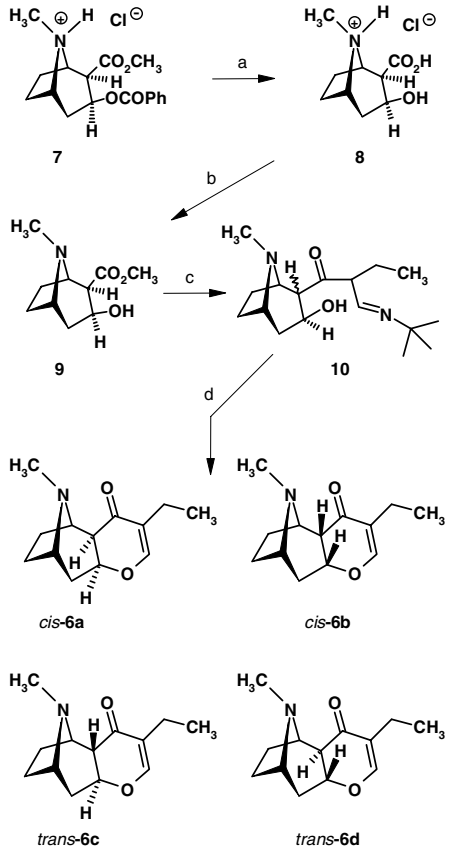
Recently Uemara et al. [18] described a novel alkaloidal marine toxin pinnamine (**5**). This was isolated from the Okinawan bivalve *Pinna muricata* and resembles the toxic symptoms and the structure of (+)-anatoxin-a (**3**), referred to as "very fast death factor" and well known as a potent



nAChR ligand with strong agonist actions. Because of this evident similarity to (+)-anatoxin-a (**3**) and its interesting biological properties, pinnamine (**5**) provides an attractive lead for the design of intriguing structural analogues.

The novel marine alkaloidal toxin **5** can be regarded as a conformationally restricted variant of (+)-anatoxin-a (**3**). In Schmitt's (based on the structure) classification scheme [8] pinnamine (**5**) belongs to class E of nAChR ligands where both the cationic and the HBA/π sites (hydrogen bond acceptor π-moiety) are contained within a fused polycyclic ring system. The structural features of the toxin **5** are a 9-azabicyclo[4.2.1]nonane moiety trans-annulated with a dihydro-4-pyrone ring. The absolute stereochemistry of the four stereogenic centers in the pyrano-anatoxinoid core of **5** were determined to be 4R, 5R, 8S and 11R [18]. A total synthesis of enantiopure pinnamine (**5**) was published in 2001 [19]. This gave rise to tackle the synthesis and biological evalutation of enantiopure pinnamine variants containing a γ-pyranotropane skeleton as illustrated with structure **6**. The ligands of type **6** can be regarded as conformationally restricted analogues of (−)-ferruginine (**4**). Herein we report an efficient strategy for the synthesis of novel pinnamine modifications in which the 9-azabicyclo[4.2.1]nonane pharmacophoric element of toxin **5** is bioisosterically [20] replaced by the 8-azabicyclo[3.2.1]octane moiety. Additionally, the preliminary biological profile of these potential nAChR ligands is described.

Scheme 1



Reagents and conditions: (a) 1 M HCl, reflux, 20 h (91%); (b) 1. 32% HCl/MeOH = 1 : 3, reflux, 5 h; 2. H_2O, conc. NH_3, CH_2Cl_2 (79%); (c) t-BuN=CH$(CH_2)_2CH_3$, LDA, THF, 0 °C, 1 h; (d) 70% TFA, 55 °C, 3 h (23% overall yield in two steps)

2. Investigations, results and discussion

2.1. Chemistry

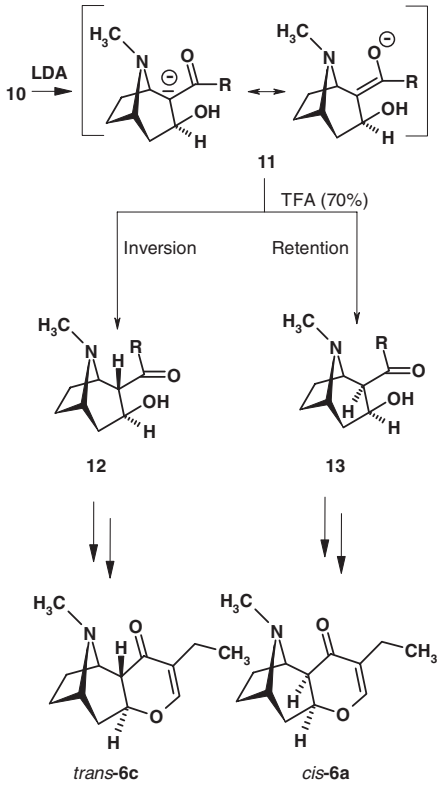
A particularly attractive feature for the synthesis of enantiomerically pure γ-pyranotropanes of type **6** was the opportunity for their ready preparation from (−)-ecgonine hydrochloride (**8**) [21]. This represents a chiral building block with the required 8-azabicyclo[3.2.1]octane skeleton easily available by degradation of (−)-cocaine hydrochloride (**7**) in a high yielding one step protocol. As illustrated in Scheme 1 esterification of compound **8** with an excess of methanol in the presence of aqueous hydrochloric acid (32%) yielded the corresponding ecgonine methyl ester **9** after treatment with aqueous ammonia in 79% yield. The conversion of the ester **9** to the requisite ketone **10** could be accomplished in one step utilizing Uemara's procedure [19] with the lithium anion of *N-tert*-butylbutyraldimin in THF as nucleophile, originally developed by Corey et al. [22].

Purification of the reaction product was complicated due to a mixture of tautomeric species being formed containing the keto imine **10** in equilibrium with the corresponding enamine and enol. Thus, the resultant mixture was submitted to cyclisation without further purification. After treatment with 70% aqueous TFA two of the four possible stereoisomeric γ-pyranotropanes **6a**–**d** [23] were obtained in a diastereomeric ratio of 2 : 1 in 23% overall yield starting from cocaine hydrochloride (**7**).

Analysis of the 1D and 2D NMR spectra (including H,C- and H,H-COSY and NOESY) of the two isolated stereoisomers *cis*-**6a** and *trans*-**6c** allowed the unambiguous assignment of the structure and stereochemistry of the novel pinnamine variants with a γ-pyranotropane skeleton.

The relative stereochemical orientation (*cis*- or *trans*-annulation) of the stereoisomer with the greater R_f is evident

Scheme 2



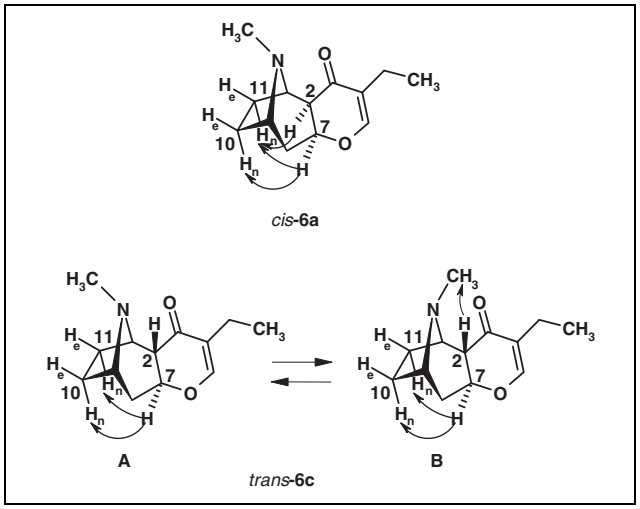
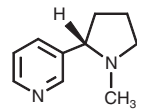
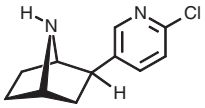
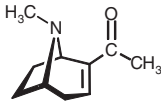
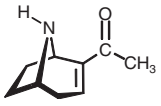
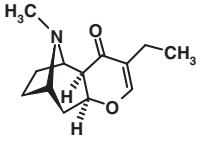
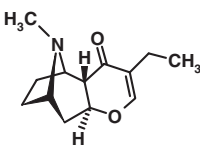


Fig.: Relevant NOE correlations from NOESY spectra of the diastereomers *cis*-**6a** and *trans*-**6c**

from the characteristic vicinal ^3J coupling of $J_{2,7} = 7.9$ Hz between the two angular protons 2-H and 7-H, indicating *cis*-annulation consistent with **6a** or **6b**. In contrast, the second isolated stereoisomer with the lower R_f exhibited a vicinal coupling of $J_{2,7} = 14.3$ Hz between the protons at the annulation bridge confirming *trans*-stereochemistry compatible with **6c** or **6d**. Application of NOE difference spectroscopy allowed a conclusive decision between the two *cis*- and the two *trans*-annulated diastereoisomers **6a**, **6b** and **6c**, **6d** respectively. In the case of **6a/6b** the stereochemistry has been proven by significant NOEs of 2-H ($\delta = 2.67$) with 7-H ($\delta = 4.78$), 1-H ($\delta = 3.89$) and 11-H_{endo} ($\delta = 1.54-1.59$ ppm) on the one hand, and of 7-H ($\delta = 4.78$) with 2-H ($\delta = 2.67$), 8-H_{endo} ($\delta = 1.71-1.75$), 10-H_{endo} ($\delta = 1.41-1.46$ ppm) and 11-H_{endo} ($\delta = 1.54-1.59$) on the other hand. These results are consistent only with the structure of *cis*-**6a**. In the NOE experiment with the second isolated diastereoisomer irradiation of the signal at 2-H ($\delta = 2.62$) enhanced the signals for 1-H ($\delta = 3.67$) and that for the protons of the N-methyl group ($\delta = 2.29$). This revealed that the N-methyl group was at least partly axially oriented towards the pyrane ring, in agreement with the experimental nitrogen inversion barrier of N-methylated tropanes $\Delta G^{\neq} = 7.8$ kcal/mol [24]. This low barrier indicated that at room temperature conversion of the **A** form to the **B** form of **6c** can easily occur (Fig.). Additionally, irradiation of the signal at 7-H ($\delta = 4.10-4.18$) caused significant enhancements of the signals of 8-H ($\delta = 1.84-1.89$), 10-H_{endo} and 11-H_{endo} ($\delta = 1.35-1.45$). This applies especially to structure *trans*-**6c**.

Mechanistically, the formation of only two of four possible diastereomeric pyranotropanes of type **6** is interesting and needs some explanatory comments. Looking at the stereogenic centers C-2 and C-3 of the starting material ecgonine methyl ester (**9**) and those of the pyranotropanes *cis*-**6a** and *trans*-**6c** it is obvious that only the reaction sequence to *cis*-**6a** is stereoconservative with conservation of chirality and retention of configuration at both stereogenic centers. For *trans*-**6c** inversion at the stereogenic center C-7 is observed (Scheme 2).

Table: Radioligand binding affinities[a] of the novel pinnamine variants 6a and 6c to $(\alpha4)_2(\beta2)_3$ and $\alpha7^*$ nAChRs in comparison with (−)-nicotine, (−)-ferruginine and (−)-norferruginine.

Structure	Compound	$(\alpha4)_2(\beta2)_3$[b] (±)-[^{3}H]-epibatidine rat brain K_i (nM)	$\alpha7^*$[b] [^{3}H]MLA rat brain K_i (nM)
	(−)-Nicotine	0.838 ± 0.132	127 ± 5 [^{125}I] α-BTX
	(±)-Epibatidine	0.008 ± 0.0002	4 ± 0.5 [^{125}I] α-BTX
	(−)-Ferruginine	120 ± 5	330 ± 21
	(−)-Nor-ferruginine HCl	94 ± 2.1	>100,000
	Pyranotropane *cis*-**6a**	4,800 ± 1,630	22,470 ± 680
	Pyranotropane *trans*-**6c**	976 ± 62	1,360 ± 675

[a] Values represent mean ±SEM obtained from n independent experiments where n = 3–5. [b] Naturally expressed nAChRs

A reasonable explanation is that the electron-withdrawing ketone group of the intermediate keto imine **10** confers considerable lability to the H-bond also at C−2. It is, therefore, not surprising that the action of LDA on **10** easily brings about deprotonation to furnish the resonance stabilised enolate anion **11** (Scheme 3). Protonation with 70% aqueous TFA – necessary for the cyclisation process – occurs diastereoselectively providing a 2 : 1 mixture of the diastereomeric intermediates **12** and **13**. These are cyclised subsequently to the pyranotropanes *cis*-**6a** (produced in a larger measure) and *trans*-**6c**.

2.2. *In vitro receptor binding*

The potential nAChR ligands *cis*-**6a** and *trans*-**6c** were tested for their *in vitro* affinity for $(\alpha4)_2(\beta2)_3$ and $\alpha7^*$ nAChRs subtypes by radioligand binding assays. To determine the affinities for the $(\alpha4)_2(\beta2)_3$ and $\alpha7^*$ nAChR subtype previously described competition assays were used with (±)-[^{3}H]epibatidine and [^{3}H]MLA, respectively, and P2 membrane fraction of Sprague-Dawley rat forebrain [17, 25, 26, 27]. The specific binding of (±)-[^{3}H]epibatidine to crude synaptic membranes of rat forebrain, at concentrations up to 800 pM, is characterized by a single population of binding sites with $K_d = 8 \pm 0.3$ pM. It has been previously found that the predominant receptor with high affinity for (±)-[^{3}H]epibatidine in rat brain is composed of α4 and β2 subunits [25, 26]. Regarding the $\alpha7^*$ nAChR subtype, [^{3}H]MLA bound to a single population of binding sites exhibited a K_d value of 1.2 ± 0.2 nM [17] with regional distribution characteristic of α-BTX-sensitive, putative $\alpha7^*$ subunit-containing nAChRs [27].

As shown in the Table, the above characterized competition assays yielded K_i values of 0.838 nM for (−)-nicotine and 0.008 nM for (±)-epibatidine for the $(\alpha4)_2(\beta2)_3$ nAChR subunit. These findings are consistent with recently reported *in vitro* measurements of the natural alkaloids [25].

To our surprise the conformationally restricted variants *cis*-**6a** and *trans*-**6c** of (−)-ferruginine (**4**) don't retain not even the moderate affinity of the lead compounds (−)-ferruginine or (−)-norferruginine. Like (−)-norferruginine the pyranotropane *cis*-**6a** is devoid of affinity at the $\alpha7^*$ subtype whereas the *trans*-**6c** binds with significant affinity in the micromolar range.

2.3. *Conclusion*

Starting from the versatile chiral building block (−)-ecgonine hydrochloride (**8**) we developed an efficient synthesis of two pinnamine variants **6a** and **6c**. These are characterized by a pyranotropane skeleton and can be regarded as conformationally restricted (−)-ferruginine analogs. Despite obvious structural similarities to the marine toxin pinnamine (**5**) and to the nAChR agonist (−)-ferruginine (**4**) both variants **6a** and **6c** exhibit nAChR affinities only in the lower micromolar range for both the $(\alpha4)_2(\beta2)_3$ and the $\alpha7^*$ nAChRs.

3. Experimental

3.1. *General procedures*

Standard vacuum techniques were used in handling of air sensitive materials. Solvents were dried and freshly distilled before use according to literature procedures. IR spectra were recorded on a Perkin-Elmer 257, 398 and a Nicolet FT-IR spectrometer 510-P. Liquids were run as films. ^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol JNM-GX 400 and LA 500 spectrometers; δ/ppm = 0 for tetramethylsilane, and δ/ppm = 7.24 for chloroform. MS were measured on a Vacuum Generators 707 (70 eV; ^{11}B) spectrometer. For column chromatography, purifications were carried out on Merck silica gel 60 [70–260 (flash chromatography) or 200–400 mesh]. Reactions were monitored by TLC using plates of silica gel (0.063–0.200 mm, Merck) or silicagel−60F_{254} microcards (Riedel de Haen).

3.2. *Chemistry: (1R,2R,7S,9S)- and (1R,2S,7S,9S)-4-Ethyl-12-methyl-6-oxa-12-azatricyclo-[7.2.1.0^{2,7}]dodec-4-en-3-one* (**6a**) *and* (**6c**)

To a solution of *n*-butyllithium in hexane (1.6 M, 9 ml, 14.4 mmol) under argon at room temperature a solution of diisopropylamine (1 ml, 14.4 mmol) in dried THF (6 ml) was added dropwise. After 5 min this freshly prepared LDA solution was cooled to 0 °C and added dropwise to a stirred solution of *N-tert*-butylbutyraldimin (1.1 g, 8.6 mmol) in dry THF (6 ml) under argon. After 10 min a solution of ester **9** [21] (1.2 g, 5.8 mmol) in dry THF was added. The mixture was stirred at 0 °C for 1 h and after warming to room temperature water (20 ml) was carefully added. The mixture was extracted with CH_2Cl_2 (2 × 30 ml), the combined organic phase dried (Na_2SO_4), filtered and the filtrate concentrated in vacuo. The residue was dissolved in aqueous TFA (70%, 15 ml) and stirred for 3 h at 55 °C. After removal of the volatile components *in vacuo*, a saturated Na_2CO_3-solution was added to the residue and the resulting solution was extracted with CH_2Cl_2 (3 × 30 ml). The combined organic phase was dried with $MgSO_4$, filtered, the filtrate concentrated under reduced pressure and the crude product purified by column chromatography (silica gel, column, 3 × 15 cm, eluent, ethyl acetate). Two diastereomers were obtained as colourless oils with a ratio of **6a** : **6c** = 2 : 1 (overall yield: 23%; yield of **6a** = 280 mg; yield of **6c** = 135 mg).

6a: $R_f = 0.24$ (eluent: ethyl acetate); $[\alpha]_D^{20} = -32.1°$ (c = 0.49, CH_2Cl_2); IR (film): ν (cm^{-1}) = 2961, 2878, 2770, 1737, 1662; UV (CH_2Cl_2): λ_{max} (lg ε) = 273 nm (3.89); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.96 (t, J = 7.5 Hz, 3 H), 1.41–1.46 (m, 1 H), 1.54–1.59 (m, 1 H), 1.71–1.75 (m, 1 H), 2.00–2.16 (m, 5 H), 2.18 (s, 3 H), 2.67 (dd, J = 3.2 Hz, J = 7.8 Hz, 1 H), 3.14 (d, J = 6.7 Hz, 1 H), 3.89 (dd, J = 2.1 Hz, J = 7.1 Hz, 1 H), 4.73–4.78 (m, 1 H), 6.91 (s, 1 H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 13.3, 18.4, 24.7, 25.4, 34.9, 40.7, 50.2, 61.0, 61.1, 73.6, 118.0, 156.3, 191.2. EI-MS (70 eV): m/z (%) = 221 (100, M^+), HR-MS (M^+) calcd. for $C_{13}H_{19}NO_2$: 221.1416, found 221.1415.

6c: $R_f = 0.11$ (eluent: ethyl acetate); $[\alpha]_D^{20} = -122.6°$ (c = 0.60, CH_2Cl_2); IR (film): ν (cm^{-1}) = 2963, 1672, 1609, 1451; UV (CH_2Cl_2): λ_{max} (lg ε) = 269 nm (3.81). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.96 (t, J = 7.5 Hz, 3 H), 1.35–1.45 (m, 2 H), 1.84–1.89 (m, 1 H), 1.95–2.16 (m, 5 H), 2.29 (s, 3 H), 2.62 (dd, J = 2.8 Hz, J = 14.3 Hz, 1 H), 3.24 (d, J = 6.7 Hz, 1 H), 3.67 (dd, J = 2.1 Hz, J = 7.1 Hz, 1 H), 4.10–4.18 (m, 1 H), 7.11 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.8, 18.6, 24.6, 26.6, 35.2, 39.1, 51.0, 58.9, 60.5, 76.3, 120.4, 158.8, 193.8. EI-MS (70 eV): m/z (%) = 272 (100, M^+); HR-MS (M^+) calcd. for $C_{13}H_{19}NO_2$: 221.1416, found 221.1409.

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