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6,7,14,15-Tetrahydro-*15aH*-azocino[1,2-*a*:6,5-*b*′]diindole. Synthesis of a novel pentacyclic ring system☆

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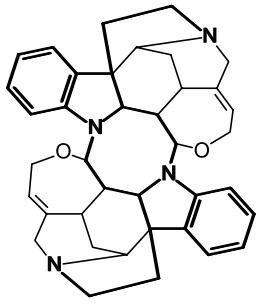
Abstract—In search of new lead structures for potent allosteric enhancers of antagonist binding to muscarinic M_2 receptors, a novel heterocyclic ring system, 6,7,14,15-tetrahydro-*15aH*-azocino[1,2-*a*:6,5-*b*′]diindole, has been synthesized. The new ring skeleton was obtained from indol-2-yl-acetic acid in three steps.

Novel heterocyclic ring systems derived from the *Strychnos* alkaloid caracurine V are of great pharmacological interest as potential ligands for the allosteric binding site of muscarinic M_2 receptors.[1,2] The very rigid caracurine V ring skeleton comprises the pharmacophore of two positively charged nitrogens at a distance of about 10 Å surrounded by two aromatic ring systems.[2,3] Recently, we reported the synthesis of 6,7,14,15-tetrahydro[1,5]diazocino[1,2-*a*:6,5-*a*′]diindole, a new caracurine V derived pentacyclic ring system.[4] In this novel heterocycle, the caracurine V ring system is reduced to the essential pharmacophoric elements (Fig. 1). The key step of the synthesis involved the intermolecular double *N*-alkylation of ethylamine-substituted bromoethylindole.

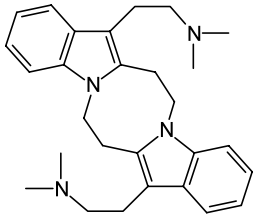
In order to examine the influence of the length of the side-chains on muscarinic activity, we were interested in exchanging the ethylamine moieties by methylamine groups. This should be accomplished by the dimerization of the unsubstituted 2-bromoethylindole **1** employing the double alkylation strategy mentioned above, and a subsequent Mannich aminomethylation of the resulting pentacyclic ring.

The starting material for the synthesis of **1** was the commercially available indole-2-carboxylic acid which could be converted to the homologous indol-2yl-acetic acid **2** using the reaction sequence described previously by Kutney et al.[5] Reduction of indole-2 carboxylic acid using lithium aluminium hydride in THF gave indol-2-yl methanol, which was converted to the corresponding benzoate by reaction with benzoyl chloride in the presence of triethylamine in THF. Heating of the resulting indolylmethyl benzoate with KCN in DMSO at 60°C led to indol-2-yl acetonitrile, which was hydrolyzed in refluxing methanolic NaOH to give acid **2** in an overall yield of 39%.

Since **2** easily decarboxylates to 2-methylindole, it was immediately reduced to indol-2-yl ethanol **3** using lithium aluminium hydride in THF (56%). Finally, alcohol **3** was converted to the corresponding bromide



Caracurine V



Tetrahydro-diazocino[1,2-a:6,5-a´]diindole

Figure 1.

Keywords: 6,7,14,15-tetrahydro-*15aH*-azocino[1,2-*a*:6,5-*b*′]diindole; novel pentacyclic ring system; ligands for the allosteric binding site of muscarinic M2 receptors; dimerization of 2-(indol-2-yl)-ethyl tosylate; intermolecular eight-ring formation via double alkylation.

☆ Supplementary data associated with this article can be found at doi:10.1016/S0040-4039(03)01796-9

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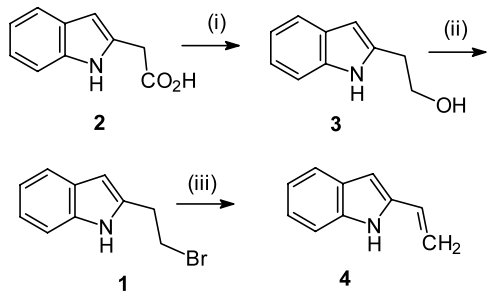


Scheme 1. *Reagents and conditions*: (i) $LiAlH_4$, dry ether, rt, 3 h; (ii) CBr_4, PPh_3, dry CH_2Cl_2, rt, 16 h; (iii) NaH (1.2 equiv.), dry DMF, 0°C, 15 min, rt, 20 min.

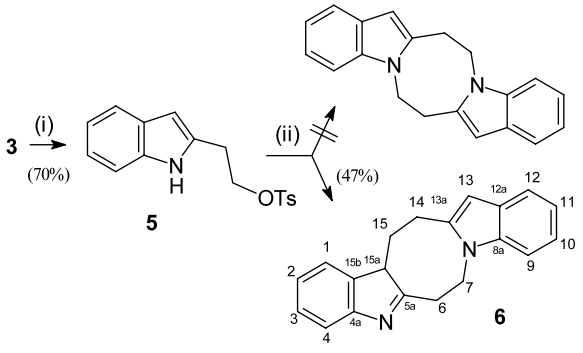
1 by treatment with carbon tetrabromide and tris-(dimethylamino)phosphine (24%).

For the dimerization of **1**, we applied the same procedure as for the synthesis of the substituted diazocinodiindole.[4] Unfortunately, after treatment of **1** with sodium hydride in DMF, only alkene **4** was isolated, as a result of the HBr-elimination (31%) (Scheme 1).

In order to suppress the undesired side-reaction, we used the tosylate of alcohol **3** as a starting material for the crucial double-alkylation step. Interestingly, instead of the expected diazocinodiindole, an isomeric pentacyclic ring system: 6,7,14,15-tetrahydro-*15aH*-azocino[1,2-*a*:6,5-*b'*]diindole, was exclusively built (Scheme 2).

Formation of **6** is due to an ambident nucleophilic character of the indolyl anion, which can be alkylated either at N or at C-3.[6] (Fig. 2). In contrast, when the 3-position of the indole ring was blocked, for instance by an ethylamine substituent, the dimerization gave the symmetrical double *N*-alkylation product.[4]

The structure elucidation of the new heterocyclic ring system required an intensive analysis of NMR spectra. The HREIMS exhibited the expected $[M-1]^+$-ion at m/z 285.1393 ($C_{20}H_{17}N_2$). Inspection of the 400 MHz ^1H NMR spectrum and COSY data established the presence of four independent ^1H spin systems, two aromatic (2×indole) and two aliphatic, in addition to an isolated proton at δ 6.15, which could be assigned as H-13. COSY correlations starting from the triplet at δ 4.73 revealed the first aliphatic spin system as a CH–CH_2–CH_2 chain (H-15a, CH_2-15 and CH_2-14). The remaining aliphatic signals corresponded to a CH_2–CH_2 group (CH_2-6 and CH_2-7). The assignment of all protons could be confirmed by HMQC and HMBC experiments which can be found in the supplementary material.



Scheme 2. *Reagents and conditions*: (i) TsCl (1.2 equiv.), NEt_3 (1.1 equiv.), dry CH_2Cl_2, rt, 14 h; (ii) NaH (1.2 equiv.), dry DMF, 0°C, 15 min, rt, 20 min.

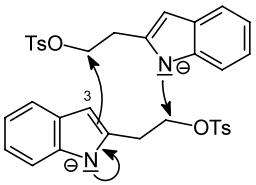
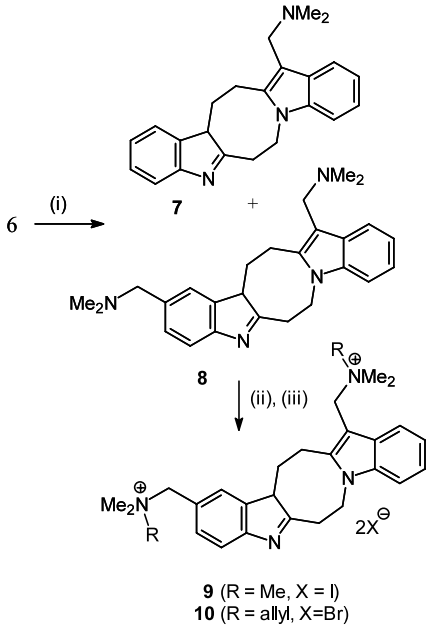


Figure 2. Mechanism of the formation of compound **6**.

In order to obtain potential muscarinic active compounds, the new ring system was subjected to a Mannich reaction. Treatment of **6** with dimethylamine/formaldehyde in acetic acid provided two products, **7** and **8**, which could be separated by column chromatography on silica-gel with $CHCl_3$:MeOH:25% NH_3/100:10:1 as eluent. The more polar compound **8** (43%) was the desired double-aminoalkylation product with the 2,13-disubstitution pattern. The presence of small amounts of the 13-monosubstituted product **7** (8%) indicated that the first aminomethylation occurred at C-13 (Scheme 3).

The position of the second aminomethyl group at the aromatic ring C-1–C-15b of **8** could be determined by NMR. HMBC correlations from C-4a and C-15a revealed H-1 as a narrow doublet at δ 6.93 with a typical *meta* coupling constant $J_{1H-3H} = 1.5$ Hz. This coupling pattern is only possible when proton H-2 is absent. The C-2 substitution could be confirmed by HMBC-correlations from C-1 and C-3 to the methylene protons of the aminomethyl group. The NMR spectra of **8** are given in the supplementary material. Since double *N*-quaternization of caracurine V with methyl and allyl groups, respectively caused the highest increase of allosteric potency,[2] we used the same substituents for alkylation of **8**. Double quaternization of **8** could be best accomplished using pure methyl iodide and allyl bromide, respectively, as alkylation agents, to give the respective ammonium salts **9** and **10**, which could be precipitated from the reaction mixture by adding diethyl ether.

In order to determine the allosteric potency of **9** and **10**, their ability to inhibit the dissociation of the antagonist [^3H]-*N*-methylscopolamine ([^3H]NMS) from porcine cardiac M_2 receptors was determined.[7] The concentration which retarded [^3H]NMS dissociation by a factor of 2 ($EC_{50,\ diss}$) served as a measure of binding affinity to the allosteric binding site of M_2 receptors whose orthosteric site was blocked by [^3H]NMS. Compounds



Scheme 3. *Reagents and conditions*: (i) 40% aq. dimethylamine (3 equiv.), 40% aq. formaldehyde (3 equiv.), acetic acid, 4 h; (ii) methyl iodide and allyl bromide, respectively, rt, 1 h; (iii) diethyl ether.

9 and **10** exhibited an approximately 4-fold lower M_2 binding affinity ($EC_{50,\ diss}$ 35 and 48 nM, respectively) than the corresponding caracurine V analogues (dimethylcaracurinium diiodide: 8 nM, diallylcaracurinium dibromide: 11 nM), which is probably due to different spatial arrangements of the aromatic rings, as well as to different internitrogen distances in both ring systems.

In conclusion, dimerization of 2-(indol-2-yl)-ethyl tosylate provided a novel pentacycyclic ring system: 6,7,14,15 - tetrahydro - *15aH* - azocino[1,2 - *a*:6,5-*b′*]-diindole. The new heterocycle was formed via asymmetrical double alkylation of 2-(indol-2-yl)-ethyl tosylate at nitrogen and C-3-carbon. The *N*-methyl and *N*-allyl salts of the double aminomethylated new ring skeleton are potent ligands of the allosteric site of muscarinic M_2 receptors with an approximately 4-fold lower binding affinity than the corresponding caracurine V analogues.

Analytical data for compounds 6–8

6: mp 148–149°C; TLC $R_f = 0.43$ (SiO_2, $CHCl_3$:hexane 1:3); FT-IR (ATR) ν (cm^{-1}) 3042, 2992, 2967, 2946, 2874, 1607, 1557, 1480, 1454, 1387, 1351, 1320, 1245, 1225, 1199, 1145, 1906, 1018, 915, 867, 772, 739; ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, 1H, $J = 8.1$ Hz, H-9), 7.53 (d, 1H, $J = 7.8$ Hz, H-12), 7.17 (m, 1H, H-10), 7.11 (m, 1H, H-11), 7.09 (m, 1H, H-3), 6.99 (d, 1H, $J = 7.1$ Hz, H-1), 6.64 (m, 1H, H-2), 6.61 (d, 1H, $J = 7.8$ Hz, H-4), 6.15 (d, 1H, $J = 0.8$ Hz, H-13), 4.73 (t, 1H, $J = 8.3$ Hz, H-15a), 3.92 (dd, 1H, $J = 14.3$, 5.4 Hz, H^b-6), 3.51 (ddd, 1H, $J = 14.3$, 12.8, 3.3 Hz, H^a-6), 3.41 (m, 1H, H^b-14), 3.11 (m, 1H, H^b-7), 2.87 (dd, 1H, $J = 15.7$, 2.6 Hz, H^a-7), 2.64 (m, 2H, H^a-14 and H^b-15), 2.05 (m, 1H, H^a-15); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.2 (C-4a), 135.3 (C-13a), 134.2 (C-8a), 132.2 (C-15b), 129.0 (C-12a), 127.7 (C-3), 124.2 (C-1), 120.5 (C-10), 120.3 (C-12), 119.8 (C-11), 118.2 (C-2), 110.9 (C-9), 105.7 (C-4), 98.5 (C-13), 83.4 (C-5a), 48.1 (C-15a), 36.6 (C-6), 34.2 (C-14), 21.7 (C-7), 21.5 (C-15); MS (EI, 70 eV) m/z (rel. int.), 286 (3), 285 (3), 258 (100), 257 (26), 129 (15), 128 (16), HREIMS m/z 285.1393 $[M-1]^+$ (calcd for $C_{20}H_{17}N_2$ 285.1392).

7: TLC $R_f = 0.36$ (SiO_2, $CHCl_3$:MeOH:25%NH_3/100:10:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, 1H, $J = 7.9$ Hz, H-9), 7.62 (d, 1H, $J = 7.9$ Hz, H-12), 7.18 (m, 1H, H-10), 7.12 (m, 1H, H-11), 7.07 (dd, 1H, $J = 8.0$, 7.1 Hz, H-3), 6.98 (d, 1H, $J = 7.1$ Hz, H-1), 6.63 (dd, 1H, $J = 8.0$, 7.1 Hz, H-2), 6.60 (d, 1H, $J = 8.0$ Hz, H-4), ^1H chemical shifts of the remaining protons coincide with the δ values of the corresponding atoms of **8** within ±0.03 ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.1 (C-4a), 127.7 (C-3), 124.2 (C-1), 118.2 (C-4), 105.7 (C-2), ^{13}C chemical shifts of the remaining carbon atoms coincide with the δ-values of the corresponding atoms of **8** within ±0.2 ppm.

8: mp 75–76°C; TLC $R_f = 0.14$ (SiO_2, $CHCl_3$:MeOH: 25%NH_3/100:10:1); FT-IR (ATR) ν (cm^{-1}) 3047, 2940, 2853, 2809, 2716, 1612, 1566, 1485, 1455, 1404, 1320, 1239, 1197, 1173, 1146, 1041, 1013, 842, 802, 737; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (m, 2H, H-9 and H-12), 7.19 (t, 1H, $J = 7.6$ Hz, H-10), 7.12 (t, 1H, $J = 7.6$ Hz, H-11), 6.98 (dd, 1H, $J = 8.0$, 1.5 Hz, H-3), 6.93 (d, 1H, $J = 1.5$ Hz, H-1), 6.54 (d, 1H, $J = 8.0$ Hz, H-4), 4.68 (t, 1H, $J = 8.2$ Hz, H-15a), 3.92 (ddd, 1H, $J = 14.2$, 5.1, 2.1 Hz, H^b-6), 3.48 (m, 1H, H^a-6), 3.49 (d, 1H, $J = 13.1$ Hz, N-CH^aH^b at C-13), 3.41 (m, 1H, H^b-14), 3.40 (d, 1H, $J = 13.1$ Hz, N-CH^aH^b at C-13), 3.30 (d, 1H, $J = 12.5$ Hz, N-CH^aH^b at C-2), 3.20 (d, 1H, $J = 12.5$ Hz, N-CH^aH^b at C-2), 2.99 (m, 1H, H^b-7), 2.95 (m, 1H, H^a-7), 2.60 (m, 2H, H^a-14 and H^b-15), 2.19 (s, 6H, 2×N-CH_3), 2.17 (s, 6H, 2×N-CH_3), 2.03 (m, 1H, H^a-15); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.3 (C-4a), 134.0 (C-8a), 133.7 (C-13a), 132.3 (C-15b), 129.5 (C-12a), 128.8 (C-2), 128.6 (C-3), 125.3 (C-1), 120.6 (C-10), 119.6 (C-11), 119.0 (C-12), 110.7 (C-9), 107.4 (C-13), 105.1 (C-4), 83.5 (C-5a), 64.1 (N-CH_2 at C-2), 52.9 (N-CH_2 at C-13), 48.1 (C-15a), 45.5 and 45.1 (N-CH_3), 36.6 (C-6), 34.3 (C-14), 21.6 (C-15), 20.1 (C-7), MS (EI, 70 eV) m/z (rel. int.) 400 $[M^+]$ (2), 372 (60), 328 (100), 284 (34), 142 (34).

Supplementary material: COSY, HMQC and HMBC spectra of compound **6**, ^1H and HMBC spectra of compound **8**.

Acknowledgements

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7. Dissociation assays were conducted in a buffer composed of 4 mM Na_2HPO_4 and 1 mM KH_2PO_4 (pH 7.4) at 23°C. Cardiac membranes were preincubated with [^{3}H]NMS (0.2 nM) for 30 min; radioligand dissociation was then revealed by the addition of 1 μM atropine, in the presence or absence of the allosteric modulator. The time course of dissociation was observed by withdrawing aliquots at various times over a period of 120 min. Membranes were separated by vacuum filtration and membrane bound radioactivity was determined by liquid scintillation counting. Experimental results were analyzed by nonlinear regression analysis (Prism 2.01, Graph Pad®). Dissociation data were fitted using a monoexponential decay function that yielded the apparent rate constant of dissociation k_{-1}. To obtain concentration–effect curves for the retardation of radioligand dissociation, curve fitting was based on a four parameter logistic function.