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Synthesis of Tricyclic Derivatives of Antitumor Alkaloid Ellipticine

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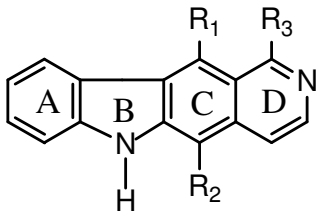
The synthesis of tricyclic analogue of ellipticine, 3-[N-(2-hydroxy-ethyl)-aminomethyl]-4-methyl-9*H*-carbazole (**10**) has been achieved. In this study, new carbazole and tetrahydrocarbazole derivatives (**3, 4, 7** and **9**) have also been synthesized.

Key Words: Carbazole, Ellipticine, Olivacine, Pyridocarbazoles.

INTRODUCTION

Ellipticine (**1a**) and olivacine (**1b**) are two naturally occurring tetracyclic 6*H*-pyrido[4,3-b]carbazole alkaloids isolated from the leaves of *Ochrasia elliptica* Labill[1]. The discovery of the antitumoral activity of the ellipticine and olivacine has stimulated many synthetic studies of analogous 6*H*-pyrido[4,3-b]carbazoles[2-4]. Antitumoral activity of alkaloid ellipticine is based on the intercalation effect[5]. Ellipticine and its derivatives have planar structure which penetrate between poly-nucleotide chains of DNA double helix. As a result ellipticine and derivatives demolish the matrix activity and inhibit the growth of tumor cells. For this purpose, tricyclic ellipticine analogues were synthesized first by Alakseeva *et al.*[6].



1a R_1:CH_3, R_2:CH_3, R_3:H Ellipticine
1b R_1:H, R_2:CH_3, R_3:CH_3 Olivacine

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EXPERIMENTAL

All melting points were measured in sealed tubes using an electrothermal digital melting point apparatus (Gallenkamp) and are uncorrected. IR spectra were recorded on a Hitachi 270-30 infrared spectrometer. ^{1}H NMR spectra were obtained on a high resolution fourier transform Bruker WH-400 NMR spectrometer with tetramethylsilane as an internal standard. Mass spectra were determined on the electron impact mode by direct insertion at 70 eV with a Micromass UK Platform II LC-MS spectrometer. Combustion analysis of compounds was obtained on a CHNS-932-LECO. Analytical and preparative thin layer chromatography (TLC) was carried out using silica gel 60 HF-254 (Merck). Column chromatography was carried out by using 70-230 mesh silica gel (0.063-0.2 mm, Merck) and aluminum oxide 90 active neutral (Merck).

4-Methyl-3-hydroxymethyl-1,2,3,4-tetrahydro-9*H*-carbazole (3): A solution of 1 g (3.89 mmol) of **2** in anhydrous tetrahydrofuran was added to a stirred solution of 1 g lithium aluminum hydride in 50 mL of tetrahydrofuran. The mixture was refluxed under nitrogen for 5 h and then cooled to 0 °C and the excess of lithium aluminum hydride destroyed with water and extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The residue was recrystallized from diethyl ether to yield 0.59 g (70%) of **3**. IR (KBr, ν_{max}, cm^{-1}): 3399 (NH), 2927(CH). ^{1}H NMR (DMSO-d_6): δ 1.03 (d, 3H, J = 7.80 Hz, CH_3), 1.74-1.77 (m, 1H, CH) 1.89-1.93 (m, 1H, CH), 2.06-2.09 (m, 2H, CH_2), 2.66-2.71 (m, 1H, CH), 3.13-3.16 (m, 1H, CH), 3.43-3.50 (m, 2H, CH_2), 5.74 (s, 1H, OH), 6.91 (t, 2H, J = 7.20 Hz, ArH), 6.97 (t, 2H, J = 7.20 Hz, ArH), 7.23 (d, 1H, J = 7.60 Hz, ArH), 7.36 (d, 1H, J = 7.60 Hz, ArH), 10.55 (s, 1H, NH).

4-Methyl-1,2,3,4-tetrahydro-9*H*-carbazole-3-carbaldehyde (4): To a solution of 1 g (4.65 mmol) of **3** in dichloromethane was added 1.5 g (6.98 mmol) of pyridinium chlorochromate and 1 g molecular sieve (5 Å) under nitrogen atmosphere and stirred for 6 h at room temperature. After the reaction had finished, the solution passed to filter paper. The mixture was extracted with dichloromethane. The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The compound **4** was obtained as an oil (55 %). IR (KBr, ν_{max}, cm^{-1}): 3319 (NH), 2928 (CH), 1665 (C=O).

3-[N-(2-Hydroxyethyl)aminomethyl]-4-methyl-1,2,3,4-tetrahydro-9*H*-carbazole (7): Method A: A solution of 1 g (4.69 mmol) of **4**, 0.29 g (4.69 mmol) 2-aminoethanol and 0.1 g *p*-toluene sulphonic acid as a catalyst in benzene was refluxed for 12 h in Dean Stark apparatus. The solvent was evaporated and crude imine **5** was reduced with sodium borohydride immediately.

A solution of 1 g (3.91 mmol) of **5** in 50 mL methanol was cooled to 0 ºC and treated with 0.72 g (19.5 mmol) sodium borohydride portion wise and the reaction mixture was stirred for 6 h at room temperature. The solution was extracted with chloroform and washed with sodium bicarbonate (10 %), hydrochloric acid (10 %)

and saturated sodium chloride. The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. After purification of the residue by column chromatography using silica gel and ethyl acetate-*n* hexane (1:1) and crystallized from diethyl ether, yielded 0.79 g (80 %) of **7**.

Method B: A solution of 1 g (3.33 mmol) of **6** in 25 mL of anhydrous tetrahydrofuran was added to a stirred solution of 1 g lithium aluminum hydride in 50 mL of tetrahydrofuran at 0 °C. The mixture was refluxed under nitrogen for 5 h. Then the reaction mixture was cooled to 0 °C and the excess of lithium aluminum hydride destroyed with water. 100 mL of water was added to the mixture and then the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The residue was recrystallized from diethyl ether yielded 0.61 g (71 %) of **7**, m.p. 164 ºC. R_f: 0.35 (ethyl acetate). IR (KBr, ν_{max}, cm^{-1}): 3312-3100 (NH, OH, NH), 2919 (CH); ^1H NMR ($CDCl_3$): δ 1.06 (d, 3H, *J* = 6.90 Hz, $CHCH_3$), 1.58-1.74 (m, 1H, CH), 1.80-1.81 (m, 1H, CH), 1.89-1.94 (m, 1H, CH), 2.51-2.54 (m, 2H, CH_2), 2.60-2.64 (m, 2H, CH_2), 2.69-2.71 (m, 2H, CH_2), 3,11 (t, 1H, *J* = 6.20 Hz, CH), 3.34 (bs, 1H, NH), 3.50 (t, 2H, *J* = 5.69 Hz, CH_2), 3.51 (s, 1H, OH), 6.91 (t, 1H, *J* = 7.02 Hz, ArH) 6.97 (t, 1H, *J* = 7.40 Hz, ArH), 7.25 (d, 1H, *J* = 7.88 Hz, ArH), 7.37 (d, 1H, *J* = 7.58 Hz, ArH), 10.56 (s, 1H, NH). MS (70 eV): m/z % 260 ($M+2^+$, 2.6), 259 ($M+1^+$, 21.8), 258 (M^+, 40.7), 227(1.6), 169(17.6), 167(45), 43(100). Anal. calcd. (%) for $C_{16}H_{22}N_2O$: C, 74.38; H, 8.58; N, 10.84. Found (%): C, 74.41; H, 8.55; N, 10.82.

4-Methyl-9*H*-carbazole-3-carboxylic acid (8): A mixture of 2.5 g (10.9 mmol) of 4-methyl-1,2,3,4-tetrahydro-9*H*-carbazole-3-carboxylic acid (**2**), 50 mg palladium-charcoal and 50 mL of decalin was refluxed for 6 h under nitrogen atmosphere. Then the mixture was filtrated immediately. The filtrate was cooled to room temperature. Compound **8** was obtained as a white powder and recrystallization from diethyl ether yielded 1.72 g (70 %) of **8** (lit.[7]).

3-[N-(2-Methoxycarbonylmethyl)-carboxamide]-4-methyl-9*H*-carbazole (9): To a solution of 0.96 g (8.88 mmol) of ethyl chloroformate in 10 mL of dichloromethane at 0 °C was added dropwise a solution of 2 g (8.88 mmol) of acid **8** followed by 2.69 g (26.64 mmol) triethyl amine in 25 mL of anhydrous chloroform. The solution was stirred for 2 h and 1.11 g (8.88 mmol) of methyl glycinate hydrochloride was added. After 12 h at room temperature, the mixture diluted with chloroform and washed with 25 mL of 10 % hydrochloric acid and then with 25 mL of 10 % sodium bicarbonate. The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate-hexane (1:1). The solvent was evaporated under reduced pressure and crystallized from diethyl ether yielded 1.9 g (65 %) of compound **9**, m.p.: 182 ºC. R_f: 0.74 (ethyl acetate). IR (KBr, ν_{max}, cm^{-1}): 3408 (NH), 3278 (NH), 2960 (CH), 1743 (C=O, ester), 1739 (C=O, amide). ^1H NMR ($CDCl_3$): δ 3.00 (s, 3H, $ArCH_3$), 3.82 (s, 3H, OCH_3), 4.30 (d, 2H, *J* = 5.33 Hz,

$NHCH_2CO_2CH_3$), 6.33 (bs, 1H, NH), 7.22-7.31 (m, 2H, ArH), 7.43-7.52 (m, 3H, ArH), 8.22 (d, 1H, *J* = 7.55 Hz, ArH), 8.32 (s, 1H, NH). MS (70 eV): m/z % 297 ($M+1^+$, 14.3), 296 (M^+, 37.4), 265 (46.5), 237 (24.8), 208 (100), 180 (62.4), 165 (22.4). Anal. calcd. (%) for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found (%): C, 68.97; H, 5.38; N, 9.39.

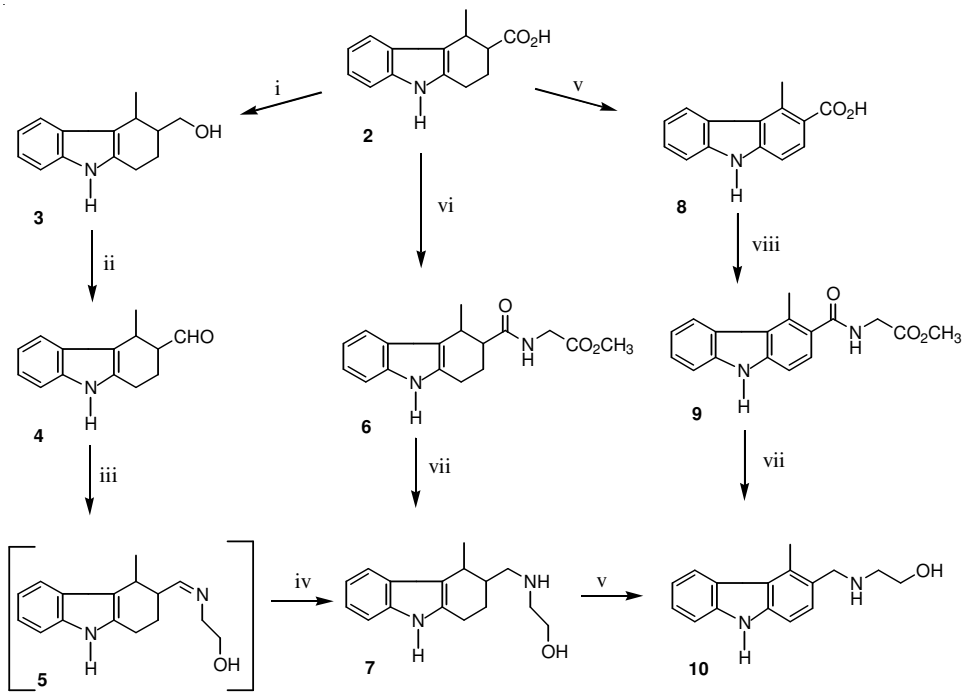
3-[N-(2-Hydroxyethyl)-aminomethyl]-4-methyl-9*H*-carbazole (10): Method A: A solution of 1 g (3.38 mmol) of **9** in 25 mL of anhydrous tetrahydrofuran was added to a stirred solution of 1 g lithium aluminum hydride in 25 mL of tetrahydrofuran at 0 °C. The mixture was refluxed under nitrogen for 5 h. Then the reaction mixture was cooled to 0 °C and the excess lithium aluminum hydride was destroyed with water and then extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The residue was recrystallized from diethyl ether yielded 0.57 g (67 %) of **10**.

Method B: A solution of 1 g (3.88 mmol) of **7** and 50 mg palladium-charcoal and 50 mL of decalin was refluxed for 6 h under nitrogen atmosphere. Then the mixture was filtrated immediately. The filtrate was cooled to room temperature. White powder was collected and purified by column chromatography using silica gel and ethyl acetate-hexane (1:1) and recrystallized from diethyl ether, yield 0.63 g (64 %) of **10**, m.p.: 161 ºC. R_f: 0.32 (ethyl acetate). IR (KBr, ν_{max}, cm^{-1}): 3398-3232 (NH, OH, NH), 2910 (CH). ^1H NMR ($CDCl_3$): δ 2.15 (bs, 2H, NH and OH, disappeared with D_2O), 2.88 (s, 3H, $ArCH_3$), 2.89 (t, 2H, *J* = 5.41 Hz, CH_2), 3.67-3.71 (t, 2H, *J* = 5.27 Hz, CH_2), 4.00 (s, 2H, CH_2), 7.19-7.38 (m, 2H, ArH), 7.39-7.45 (m, 3H, ArH), 8.13 (s, 1H, NH), 8.24 (d, 1H, *J* = 7.94 Hz, ArH). MS (70 eV): m/z % 255 ($M+1^+$, 7.2), 254 (M^+, 11.2), 237 (24.7), 209 (37.4), 180 (17.5), 165 (54.5), 141 (27.8), 115 (100). Anal. calcd. (%) for $C_{16}H_{18}N_2O$: C, 75.56; H, 7.13; N, 11.01. Found (%): C, 75.59; H, 7.11; N, 10.96.

RESULTS AND DISCUSSION

In this work, a new tricyclic analogue of ellipticine (or 3-substituted aminomethyl carbazole derivative) has been synthesized. 3-Substituted aminomethyl carbazole derivatives have also showed as NPY Y1 and Y5 antagonists[8,9]. For this aim, tetrahydrocarbazole **2** was selected as the starting material which was synthesized previously[10]. Firstly, compound **2** was reduced with lithium aluminum hydride and gave alcohol **3**[11]. Alcohol **3** was reacted with pyridinium chlorochromate and gave aldehyde **4**[12]. Imine **5** was reduced with sodium borohydride which obtained from condensation of aldehyde **4** and ethanolamine, gave amino alcohol **7**[13]. Alternatively, amino alcohol **7** was produced *via* different way. First compound **2** was converted to amide **6** according to the literature using ethyl chloroformate, triethyl amine and methyl glycinate hydrochloride[10]. Then reduction of amide **6** with lithium aluminum hydride yielded tetrahydrocarbazole amino alcohol **7**[6]. Compound **7** was dehydrogenated with Pd/C (10 %) in decalin to give tricyclic ellipticine analogue **10**[14]. Compound **10** was also synthesized from a different way. First compound **2**

was dehydrogenated with Pd/C (10 %) in decalin to give compound **8** which was synthesized with different way[7,14]. Then compound **8** was converted to amide **9** using ethyl chloroformate, triethyl amine and methyl glycinate hydrochloride[10]. Reduction of amide **9** with lithium aluminum hydride yielded compound **10**[6].



Reagents and conditions: (i) $LiAlH_4$, THF, N_2, reflux, 5 h; (ii) PCC, dichloromethane, stirred, 12 h; (iii) *p*-TsOH, ethanol amine, benzene, reflux, 12 h; (iv) $NaBH_4$, methanol, stirred, 6 h; (v) Pd/C, decalin, reflux, 6 h; (vi) ethyl chloroformate, triethyl amine, $NH_2CH_2CO_2CH_3$.HCl, room temp., 12 h; (vii) $LiAlH_4$, tetrahydrofuran, N_2, reflux, 6 h

Scheme-I

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