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SYNTHESIS OF COLCHICINE C-10-AMINO-ACID DERIVATIVES

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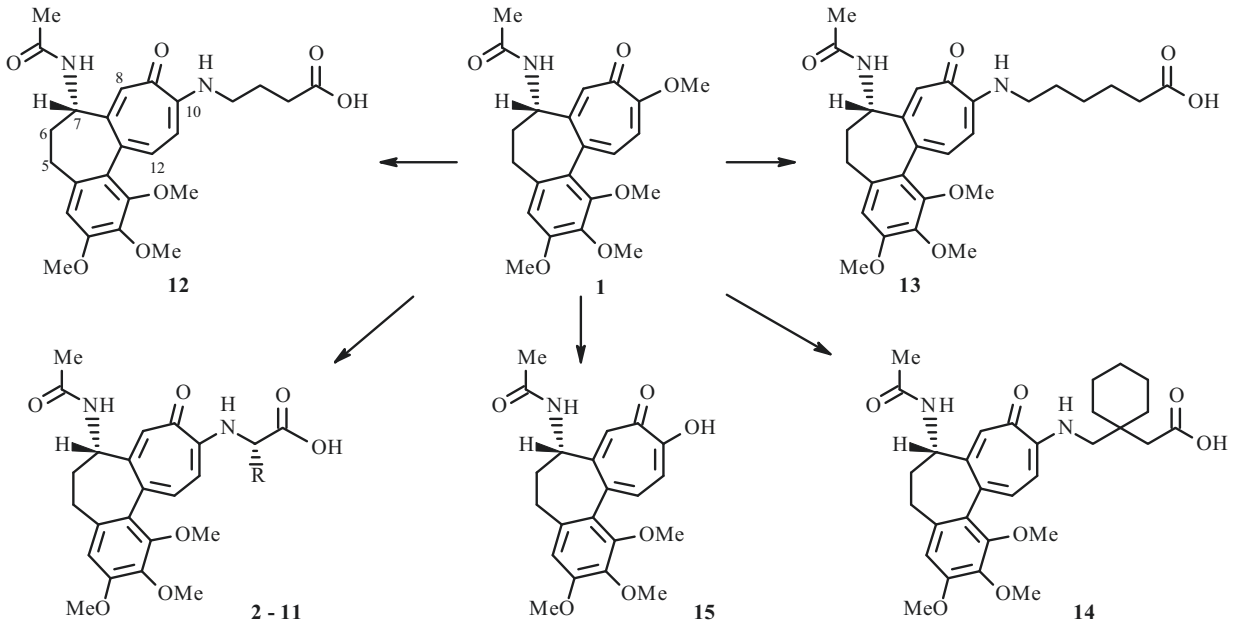
Colchicine derivatives modified on C-10 by natural and synthetic amino acids were synthesized.

Keywords: colchicine, amino-acid derivatives, colchicine C-10-amine derivatives.

Chemical modification of naturally-occurring biologically active compounds is one of the most promising and effective methods for discovering new drugs. Colchicine, a tropolone alkaloid and the principal representative of the colchicine family (homomorphinanes), is especially interesting as this type of starting material. This secondary metabolite was first isolated from bulbs of *Colchicum autumnale* L. (Liliaceae) in 1811 and has attracted attention ever since [1]. Colchicine is a potent anti-mitotic that binds to the protein tubulin, which forms microtubules and blocks cell division in the metaphase stage. Colchicine is one of the most cytotoxic compounds. Its use in chemotherapy is limited because of its relatively high toxicity despite that fact that it is a potent growth inhibitor for many types of malignant cells. However, it has been used recently to treat gout, acute pericarditis, and familial Mediterranean fever [2].

One of the most common methods for modifying colchicine is regioselective replacement of the 10-methoxy on the tropolone ring by an amine through the reaction of colchicine with the corresponding amines [3]. Biologically active colchicine derivatives with significantly less toxicity were prepared by such transformations using several amino acids as the amines [4, 5].



R = H (**2**); CH_3 (**3**); $CH(CH_3)_2$ (**4**); $CH_2CH(CH_3)_3$ (**5**); $CH(CH_3)CH_2CH_3$ (**6**); $CH_2CH_2SCH_3$ (**7**)
C_6H_5 (**8**); CH_2CH_3 (**9**); $CH_2CH_2CH_3$ (**10**); $CH_2CH_2CH_2CH_3$ (**11**)

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Herein, modification of colchicine by addition of natural and synthetic amino acids is reported. Condensation of colchicine and sodium salts of amino acids in EtOH–H_2O mixtures with heating followed by acidolysis of the resulting salts produced in 62–76% yields colchicine amino-acid derivatives **2**–**14** with a free carboxylic acid that contained glycine (**2**), L-alanine (**3**), L-valine (**4**), L-leucine (**5**), L-isoleucine (**6**), L-methionine (**7**), L-phenylglycine (**8**), DL-norvaline (**10**), DL-norleucine (**11**) DL-2-aminobutanoic (**9**), 4-aminobutanoic (**12**), 6-aminohexanoic (**13**), and 1-(aminomethyl)cyclohexaneacetic (gabapentin, Neurontin) (**14**) acids. PMR spectra of **2**–**14** contained resonances for the colchicine ring and amino-acid moiety and lacked the resonance of the colchicine C-10 methoxy. The amine resonance was observed in the range 7.44–8.12 ppm as a triplet with SSCC 5.4–6.3 Hz (for **2** and **12**–**14**) or a doublet with SSCC 7.2–8.4 Hz (for **3**–**11**).

A compound that was identified as colchicein (10-*O*-demethylcolchicine or *N*-acetyltrimethylcolchincinic acid) (**15**) based on PMR spectroscopy was isolated as a side product [6–8]. Obviously, **15** was formed in low yields (5–10%) via hydrolysis of colchicine (**1**) under the alkaline conditions for synthesizing the amino-acid derivatives.

The obtained colchicine C-10-amino-acid derivatives **2**–**14** were interesting as multi-purpose organic synthons because they contained an active carboxylic functional group and opened new possibilities for further modification of colchicine derivatives.

EXPERIMENTAL

The course of reactions and purity of products were monitored by TLC on Merck 60 F254 plates using $CHCl_3$–MeOH (9:1 and 95:5). Melting points were determined on a Kofler apparatus. PMR spectra were measured vs. TMS (internal standard) on a Varian VXR-300 spectrometer at 300 MHz. Elemental analyses of all compounds agreed with those calculated.

General Method for Synthesizing Colchicine C-10-Amino-Acid Derivatives 2–14. A solution of colchicine (**1**, 4.00 g, 10 mmol) in EtOH (10 mL) was treated with a solution of the appropriate amino acid (20 mmol) and NaOH (0.80 g, 20 mmol) in distilled H_2O (10 mL), stirred vigorously, and heated (60–70°C) for 5–7 h (end of reaction determined by TLC). After the reaction was finished, the EtOH was removed *in vacuo* in a rotary evaporator. The residue was extracted with EtOAc (3 × 10 mL). The aqueous phase was acidified to pH 5. The resulting precipitate of **2**–**14** was filtered off and crystallized from aqueous EtOH. The organic phase was dried over anhydrous Na_2SO_4. The EtOAc was removed *in vacuo* in a rotary evaporator. The residue was triturated with Et_2O to isolate **15**.

10-Colchincidylglycine (2). Yield 2.74 g (62%), mp 186–187°C (lit.: 226–227°C), $C_{23}H_{26}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.78–1.94 (1H, m, H-6α), 1.86 (3H, s, NAc), 1.96–2.22 (2H, m, H-5α, 6β), 2.45–2.55 (1H, m, H-5β), 3.48 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.16 (2H, d, J = 6.3, H_2 glycine), 4.33–4.42 (1H, m, H-7), 6.55 (1H, d, J = 11.1, H-11), 6.75 (1H, s, H-4), 7.14 (1H, s, H-8), 7.17 (1H, d, J = 11.1, H-11), 7.73 (1H, t, J = 5.4, NH-10), 8.58 (1H, d, J = 7.5, NH-7), 13.04 (1H, br.s, COOH).

10-Colchincidyl-L-alanine (3). Yield 3.10 g (68%), mp 166–167°C, $C_{24}H_{28}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.48 and 1.50 (3H, two d, J = 7.2, CH_3 alanine), 1.78–1.92 (1H, m, H-6α), 1.86 (3H, s, NAc), 1.97–2.23 (2H, m, H-5α, 6β), 2.45–2.55 (1H, m, H-5β), 3.48 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.30–4.51 (2H, m, H-7, 2 alanine), 6.65 (1H, d, J = 11.1, H-11), 6.75 (1H, s, H-4), 7.16 (1H, s, H-8), 7.19 (1H, d, J = 11.1, H-11), 7.56 and 7.58 (1H, two d, J = 7.2, NH-10), 8.58 (1H, d, J = 7.5, NH-7), 13.16 (1H, br.s, COOH).

10-Colchicidyl-L-valine (4). Yield 3.36 g (70%), mp 161–162°C, $C_{26}H_{32}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.96 and 1.03 (6H, two d, J = 6.6, $2CH_3$ valine), 1.81–1.93 (1H, m, H-6α), 1.86 (3H, s, NAc), 1.98–2.23 (2H, m, H-5α, 6β), 2.23–2.29 (1H, m, H-3 valine), 2.50–2.58 (1H, m, H-5β), 3.47 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.26–4.42 (2H, m, H-7, 2 valine), 6.71 (1H, d, J = 11.1, H-11), 6.75 (1H, s, H-4), 7.17 (1H, s, H-8), 7.20 (1H, d, J = 11.1, H-11), 7.49 (1H, d, J = 7.8, NH-10), 8.59 (1H, d, J = 7.8, NH-7), 13.27 (1H, br.s, COOH).

10-Colchicidyl-L-leucine (5). Yield 3.64 g (74%), mp 155–156°C, $C_{27}H_{34}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.87 and 0.95 (6H, two d, J = 5.4, $2CH_3$ leucine), 1.81–1.93 (4H, m, H-6α, 4, H_2-3 leucine), 1.86 (3H, s, NAc), 1.96–2.23 (2H, m, H-5α, 6β), 2.45–2.55 (1H, m, H-5β), 3.48 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.26–4.44 (2H, m, H-7, 2 leucine), 6.63 (1H, d, J = 11.1, H-11), 6.77 (1H, s, H-4), 7.15 (1H, s, H-8), 7.18 (1H, d, J = 11.1, H-11), 7.44 (1H, d, J = 7.8, NH-10), 8.54 (1H, d, J = 7.8, NH-7), 13.17 (1H, br.s, COOH).

10-Colchicidyl-L-isoleucine (6). Yield 3.49 g (71%), mp 158–159°C, $C_{27}H_{34}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.93 (3H, t, J = 7.2, CH_3-5 isoleucine), 0.95 (3H, d, J = 6.8, CH_3-3 isoleucine), 1.22–1.37 (1H, m, H-4α isoleucine), 1.53–1.67 (1H, m, H-4β isoleucine), 1.80–1.91 (1H, m, H-6α), 1.86 (3H, s, NAc), 1.96–2.23 (3H, H-5α, 6β, 3 isoleucine), 2.52–2.62 (1H, m, H-5β), 3.47 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.30–4.42 (2H, m, H-7, 2 isoleucine), 6.68 (1H, d, J = 11.1, H-11), 6.75 (1H, s, H-4), 7.18 (1H, s, H-8), 7.21 (1H, d, J = 11.1, H-11), 7.54 (1H, t, J = 8.1, NH-10), 8.60 (1H, d, J = 7.5, NH-7), 13.19 (1H, br.s, COOH).

10-Colchicidyl-L-methionine (7). Yield 3.56 g (69%), mp 153–152°C (lit. [5]: 147°C, dec.), $C_{26}H_{32}N_2O_7S$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.81–1.92 (1H, m, H-6α), 1.87 (3H, s, NAc), 2.05 (3H, s, CH_3S methionine), 1.98–2.25 (4H, H-5α, 6β, CH_2S methionine), 2.55–2.62 (1H, m, H-5β), 3.48 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.33–4.43 (1H, m, H-7), 4.50–4.56 (1H, m, H-2 methionine), 6.69 (1H, d, J = 11.1, H-11), 6.76 (1H, s, H-4), 7.17 (1H, s, H-8), 7.19 (1H, d, J = 11.1, H-11), 7.60 (1H, t, J = 8.1, NH-10), 8.60 (1H, d, J = 7.5, NH-7), 13.29 (1H, br.s, COOH).

10-Colchicidyl-L-phenylglycine (8). Yield 3.94 g (76%), mp 203–204°C, $C_{29}H_{30}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.79–1.93 (1H, m, H-6α), 1.85 (3H, s, NAc), 1.96–2.20 (2H, m, H-5α, 6β), 2.45–2.55 (1H, m, H-5β), 3.348 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.33–4.38 (1H, m, H-7), 5.50 (1H, d, J = 6.6, H-2 phenylglycine), 6.45 (1H, d, J = 11.1, H-11), 6.73 (1H, s, H-4), 7.04 (1H, d, J = 11.1, H-11), 7.19 (1H, s, H-8), 7.33–7.49 (5H, m, Ph), 8.12 (1H, t, J = 6.6, NH-10), 8.58 (1H, d, J = 7.5, NH-7), 13.35 (1H, br.s, COOH).

10-Colchicidyl-DL-2-aminobutanoic Acid (9). Yield 3.06 g (65%), mp 184–185°C, $C_{25}H_{30}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.89 and 0.91 (3H, two t, J = 7.5, CH_3 amino-acid), 1.86 (3H, s, NAc), 1.81–2.33 (5H, m, H-5α, 6α, 6β, CH_2 amino-acid), 2.50–2.60 (1H, m, H-5β), 3.48 and 3.50 (3H, two s, OCH_3), 3.79 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.31–4.46 (2H, m, H-7, 2 amino-acid), 6.68 (1H, d, J = 11.1, H-11), 6.75 (1H, s, H-4), 7.17 (1H, s, H-8), 7.20 (1H, d, J = 11.1, H-11), 7.53 and 7.56 (1H, two d, J = 8.4, NH-10), 8.58 (1H, d, J = 7.5, NH-7), 13.10 (1H, br.s, COOH).

10-Colchicidyl-DL-norvaline (10). Yield 3.48 g (72%), mp 167–168°C, $C_{26}H_{32}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.89 and 0.92 (3H, two t, J = 7.5, CH_3 norvaline), 1.34 (2H, q, J = 7.5, CH_2-4 norvaline), 1.86 (3H, s, NAc), 1.80–2.23 (5H, m, H-5α, 6α, 6β, CH_2-3 norvaline), 2.50–2.60 (1H, m, H-5β), 3.48 and 3.50 (3H, two s, OCH_3), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.31–4.46 (2H, m, H-7, 2 norvaline), 6.66 (1H, d, J = 11.1, H-11), 6.74 (1H, s, H-4), 7.16 (1H, s, H-8), 7.18 (1H, d, J = 11.1, H-11), 7.49 and 7.53 (1H, two d, J = 8.4, NH-10), 8.55 (1H, d, J = 7.5, NH-7), 13.01 (1H, br.s, COOH).

10-Colchicidyl-DL-norleucine (11). Yield 3.24 g (65%), mp 145–146°C, $C_{27}H_{34}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.86 (3H, t, J = 7.5, CH_3 norleucine), 1.22–1.40 (4H, m, CH_2-4, 5 norleucine), 1.86 (3H, s, NAc), 1.78–2.22 (5H, m, H-5α, 6α, 6β, CH_2-3 norleucine), 2.50–2.60 (1H, m, H-5β), 3.47 and 3.48 (3H, two s, OCH_3), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.32–4.47 (2H, m, H-7, 2 norleucine), 6.66 (1H, d, J = 11.1, H-11), 6.75 (1H, s, H-4), 7.15 (1H, s, H-8), 7.18 (1H, d, J = 11.1, H-11), 7.52 and 7.55 (1H, two d, J = 8.1, NH-10), 8.59 (1H, d, J = 7.5, NH-7), 13.20 (1H, br.s, COOH).

10-Colchicidyl-4-aminobutanoic Acid (12). Yield 3.58 g (76%), mp 182–183°C, $C_{25}H_{30}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.80–1.98 (3H, m, H-6α, CH_2-3 amino-acid), 1.86 (3H, s, NAc), 1.97–2.24 (2H, m, H-5α, 6β), 2.33 (2H, t, J = 7.5, CH_2-2 amino-acid), 2.45–2.55 (1H, m, H-5β), 3.38 (2H, q, J = 6.6, CH_2-4 amino-acid), 3.49 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.34–4.42 (1H, m, H-7), 6.69 (1H, d, J = 11.1, H-11), 6.74 (1H, s, H-4), 7.10 (1H, s, H-8), 7.19 (1H, d, J = 11.1, H-11), 7.69 (1H, t, J = 6.0, NH-10), 8.52 (1H, d, J = 7.5, NH-7), 12.08 (1H, br.s, COOH).

10-Colchicidyl-6-aminohexanoic Acid (13). Yield 3.56 g (72%), mp 155–156°C, $C_{27}H_{34}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.35–1.40 (2H, m, CH_2-4 amino-acid), 1.52–1.66 (4H, m, CH_2-3, 5 amino-acid), 1.80–1.92 (1H, m, H-6α), 1.85 (3H, s, NAc), 1.97–2.22 (2H, m, H-5α, 6β), 2.22 (2H, t, J = 7.5, CH_2-2 amino-acid), 2.45–2.55 (1H, m, H-5β), 3.35 (2H, q, J = 6.6, CH_2-6 amino-acid), 3.47 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.30–4.44 (1H, m, H-7), 6.67 (1H, d, J = 11.1, H-11), 6.74 (1H, s, H-4), 7.08 (1H, s, H-8), 7.19 (1H, d, J = 11.1, H-11), 7.61 (1H, t, J = 6.0, NH-10), 8.54 (1H, d, J = 7.5, NH-7), 11.98 (1H, br.s, COOH).

10-Colchicidyl-1-(aminomethyl)cyclohexaneacetic Acid (14). Yield 4.03 г (75%), mp 161–162°C, $C_{30}H_{38}N_2O_7$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.26–1.58 (10H, m, cyclohexane amino-acid protons), 1.80–1.91 (1H, m, H-6α), 1.85 (3H, s, NAc), 1.97–2.23 (2H, m, H-5α, 6β), 2.32 (2H, s, CH_2-2 amino-acid), 2.45–2.55 (1H, m, H-5β), 3.44 (2H, t, J = 6.6, CH_2-4 amino-acid), 3.48 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.29–4.44 (1H, m, H-7), 6.74 (1H, s, H-4), 6.83 (1H, d, J = 11.1, H-11), 7.12 (1H, s, H-8), 7.19 (1H, d, J = 11.1, H-11), 7.61 (1H, t, J = 6.3, NH-10), 8.54 (1H, d, J = 7.5, NH-7), 12.06 (1H, br.s, COOH).

Colchicein (15). Mp 174–175°C (lit. 150°C [8], 169–172°C [10], 177–178°C [11], 178–180°C [12]), $C_{21}H_{32}NO_6$. PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.84–1.99 (1H, m, H-6α), 1.87 (3H, s, NAc), 2.04–2.22 (2H, m, H-5α, 6β), 2.54–2.62 (1H, m, H-5β), 3.52 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.30–4.40 (1H, m, H-7), 6.80 (1H, s, H-4), 7.16 (1H, d, J = 12.0, H-11), 7.32 (1H, s, H-8), 7.34 (1H, d, J = 12.0, H-11), 8.63 (1H, d, J = 6.9, NH-7).

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