



NOTE

Synthesis and Antitumour Activity of Novel Colchicine C-10 Derivatives

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A series of new colchicine C-10 derivatives (**2a-i**, **3a-h**) were synthesized by replacement of the 10-methoxy with NR₂ and SCH₃ in order to determine their cytotoxic activity. The compounds were synthesized in good yield and the structures of all newly synthesized compounds were established on the basis of their IR, ¹H NMR and elemental analysis. The synthesized compounds were tested *in vitro* antitumor activity against four human cancer cell lines by MTT assay. It was found that many of the derivatives displayed significant activity, particularly, compound **2a** and **2b** showed more potent cytotoxic activities than colchicine.

Keywords: Colchicine, Derivatives, Cytotoxic activity.

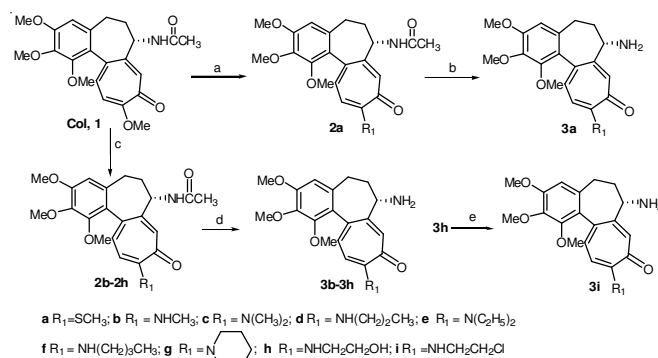
Colchicine (Col, **1**), the major alkaloid extracted from the seed of *Colchicum autumnale* Liliaceae and *Gloriosa superba*, is a potent drug that interferes with microtubules both *in vitro* and *in vivo*, thereby causing cells to accumulate in obvious mitotic arrest during cell cycle¹⁻³. Colchicine and many of its derivatives are powerful mitotic poisons, antiinflammatories and inhibitors of tumor growth⁴⁻⁶. Although colchicine is a potent antimetabolic agent, its medical uses in cancer chemotherapy are limited due to its high toxicity⁴. Structure-activity relationship studies suggest that demethylation of 10-methoxy to the 10-OH forms colchicine and destroys activity; however, replacement of the 10-methoxy with NR₂ or SCH₃ (**2**, **Scheme-I**) leads to increased potency⁷. Structure-activity studies indicate that the C-10 substituent of colchicine is involved in its biological activity. However, only a small variety of C-10 analogues of colchicine have been synthesized⁸⁻¹⁰.

We reported previously some derivatives of N-methyl colchiceinamide, which replacement of the 10-methoxy with NHCH₃, have proved that the derivatives of C-10 could really improve colchicine's antitumor activity *in vitro*^{11,12}. In view of this, to search for antitumor drugs with high potency and selectivity, we recently synthesized a series of new analogues of colchicine which replacement of the 10-methoxy with NR₂ or SCH₃, hoping that these derivatives might be transported to target site where they would release active compounds to selectively kill tumor cells without affecting normal cells.

The synthetic route of these target compounds is outlined in **Scheme-I**. Colchicine (**1**) was purchased from Taiyuan Xingyuan Biochemical Co. Ltd China, with an over 98 % purity.

The compound **2a** was prepared from **1**, according to the literature¹³ in 90 % yield and then **2a** was refluxed with 2 mol L⁻¹ HCl in CH₃OH to give the compound **3a**.

Colchicine (**1**) was treated with amines in the presence of C₂H₅OH at room temperature to generate important intermediates **2b-2h** in 90-95 % yields. Compounds **2b-2h** were further converted to the corresponding target compounds **3b-3h** with



Scheme-I: Reagents and conditions: (a) NaSCH₃, H₂O, r.t., 72 h, 65 %. (b) 2 mol L⁻¹, HCl, MeOH, reflux, 24 h, 50 %. (c) Amines, r.t., 48 h, 90-95 %. (d) conc H₂SO₄, H₂O, reflux, 5 h, 60-80 %. (e) SO₂Cl₂, reflux, 5 h, 90 %

concentrated H₂SO₄ in water in good yields (60-85 %). Compound **3h** was refluxed with SO₂Cl₂ to give the compound **3i**.

The resulting products were purified by column chromatography and their structures were shown in **Scheme-I** and the data of yield, MS, IR and ¹H NMR spectra and elemental analysis of selected compounds were shown in reference¹⁴.

The cytotoxic activity of all target compounds **2a-2h** and **3a-3i** *in vitro* was determined by MTT assay¹⁵, using colchicine as positive control and the result is summarized in Table-1. Four different cell lines were used: A2780 (human ovary cancer), A549 (human lung cancer), BEL7402 (human hepatoma), MCF7 (human breast carcinoma).

TABLE-1
CYTOTOXICITY DATA OF THE TARGET COMPOUNDS

Compound	IC ₅₀ (μM)/cell line			
	A2780	A549	BEL7402	MCF7
2a	0.095	0.080	0.042	0.085
2b	0.098	0.088	0.086	0.105
2c	0.120	0.115	0.118	0.109
2d	0.195	0.198	0.123	0.135
2e	0.207	0.208	0.126	0.192
2f	0.226	0.232	0.203	0.217
2g	0.245	0.234	0.238	0.241
2h	0.230	0.224	0.225	0.238
3a	0.102	0.108	0.112	0.125
3b	0.168	0.178	0.156	0.145
3c	0.258	0.298	0.276	0.295
3d	0.224	0.315	0.318	0.305
3e	0.285	0.258	0.293	0.312
3f	0.207	0.208	0.126	0.192
3g	0.326	0.312	0.323	0.316
3h	0.335	0.324	0.328	0.411
3i	0.232	0.234	0.265	0.278
Colchicine	0.094	0.078	0.080	0.084

The study results indicate that these new C-10 derivatives showed superior or comparable cytotoxic activity to colchicine *in vitro*. For all four tumor cell lines, compounds **2a** and **2b** have better cytotoxicity than colchicine. In human hepatoma cell line (BEL7402), compounds **2b** has similar cytotoxicity as colchicine, whereas compounds **2a** exhibited almost twofold potent activities than colchicine. As to human breast carcinoma cell line (MCF7), compound **2a** exhibited similar cytotoxicity as colchicine. The results demonstrated that new C-10 derivatives could really improve colchicine's antitumor activity *in vitro*.

In summary, a series of novel colchicine C-10 conjugates were synthesized and evaluated for their *in vitro* cytotoxicity against four human tumor cell lines. It was found that **2a** and **2b** showed significant cytotoxic activities. Further biological evaluations are currently in progress and will be reported in due course.

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- Data of selected compounds: **2b**: yield 83 %, m.p. 165-167 °C, IR (KBr, ν_{max}, cm⁻¹): 3370, 3280, 1665, 1613, 1491, 1456; ¹H NMR (CDCl₃, 300 MHz, δ ppm): 1.79 (s, 3H, COCH₃), 3.13 (d, 3H, NCH₃), 3.64 (s, 3H, MeO-1), 3.87 (s, 3H, MeO-2), 3.93 (s, 3H, MeO-3), 4.70 (m, 1H, H-7), 6.50 (s, 1H, H-4), 6.65 (d, 1H, 11.2 Hz, H-11), 7.52 (d, 1H, 11.2 Hz, H-12), 7.60 (s, 1H, H-8); MS (ESI, m/z): 398.4 [M]⁺; Anal. Calcd. for C₂₂H₂₆O₅N₂: C 66.32, H 6.58, N 7.03; Found: C 67.08, H 6.65, N 7.06. **2e**: yield 90 %, m.p. 128-130 °C, IR (KBr, ν_{max}, cm⁻¹): 3420, 3280, 1655, 1528, 1476; ¹H NMR (CDCl₃, 300 MHz, δ ppm): 1.25 [t, 6H, N(CH₂CH₃)₂], 1.98 (s, 3H, COCH₃), 1.92-2.58 (m, 4H, 2 × CH₂), 3.26-3.42 [m, 4H, N(CH₂CH₃)₂], 3.65 (s, 3H, MeO-1), 3.89 (s, 3H, MeO-2), 3.95 (s, 3H, MeO-3), 4.65 (m, 1H, H-7), 6.51 (s, 1H, H-4), 6.83 (d, 1H, 10.4 Hz, H-11), 7.30 (d, 1H, 10.4 Hz, H-12), 7.64 (s, 1H, H-8); MS (ESI, m/z): 412.3 [M]⁺; Anal. Calcd. for C₂₃H₂₈O₅N₂: C 66.97, H 6.84, N 6.79; Found: C 67.08, H 6.89, N 7.01. **2h**: yield 90 %, m.p. 166-168 °C, IR (KBr, ν_{max}, cm⁻¹): 3456, 2985, 1739, 1605, 1560, 1447; ¹H NMR (CDCl₃, 300 MHz, δ ppm): 1.95 (s, 3H, COCH₃), 1.90-2.58 (m, 4H, 2 × CH₂), 3.45 (m, 2H, NCH₂), 3.66 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.94 (s, 3H, MeO-3), 4.15 (t, 2H, CH₂OH), 4.65-4.72 (m, 1H, H-7), 6.52 (s, 1H, H-4), 7.18 (m, 2H, H-11 and H-12), 7.54 (s, 1H, H-8); MS (ESI, m/z): 428.2 [M]⁺; Anal. Calcd. for C₂₃H₂₈O₆N₂: C 64.47, H 6.59, N 6.54; Found: C 64.58, H 6.56, N 6.61. **3d**: yield 63 %, IR (KBr, ν_{max}, cm⁻¹): 3420, 2920, 1663, 1568, 1450; ¹H NMR (CDCl₃, 300 MHz, δ ppm): 0.99-1.17 (t, 3H, NCH₂CH₂CH₃), 1.50-1.60 (m, 2H, NCH₂CH₂CH₃), 1.90-2.48 (m, 4H, 2 × CH₂), 3.25-3.40 (m, 2H, NCH₂), 3.60 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.94 (s, 3H, MeO-3), 4.70 (m, 1H, H-7), 6.52 (s, 1H, H-4), 7.20 (m, 2H, H-11 and H-12), 7.66 (s, 1H, H-8); MS (ESI, m/z): 384.3 [M]⁺; Anal. Calcd. for C₂₂H₂₈O₄N₂: C 68.73, H 7.34, N 7.29; Found: C 68.87, H 7.49, N 7.37. **3g**: yield 72 %, IR (KBr, ν_{max}, cm⁻¹): 3400, 3280, 1663, 1510; ¹H NMR (CDCl₃, 300 MHz, δ ppm): 1.50-1.90 [m, 6H, N-CH₂(CH₂)₃CH₂], 1.70-2.45 (m, 4H, 2 × CH₂), 3.35-3.48 [m, 4H, N-CH₂(CH₂)₃CH₂], 3.65 (s, 3H, MeO-1), 3.88 (s, 3H, MeO-2), 3.92 (s, 3H, MeO-3), 4.67 (m, 1H, H-7), 6.52 (s, 1H, H-4), 7.20 (m, 2H, H-11 and H-12), 7.70 (s, 1H, H-8); MS (ESI, m/z): 410.2 [M]⁺; Anal. Calcd. for C₂₄H₃₀O₄N₂: C 70.22, H 7.37, N 6.82; Found: C 70.97, H 7.39, N 6.87.
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