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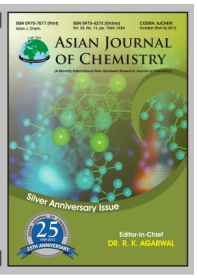

Synthesis and *in Vitro* Cytotoxic Activity of Some New Colchicine Analogues

JIAN SEN WANG[1], BO WANG[1], HONG YU LI[1], LI HONG SHEN[1,*], XU ZHANG[2] and LI JIE LIU[3]

[1]Handan Key Laboratory of Organic Small Molecule Materials, Handan College, Handan 056005, Hebei Province, P.R. China
[2]Department of Chemistry, Handan College, Handan 056005, Hebei Province, P.R. China
[3]Hebei YiLing Academy of Medicine, Shijiazhuang 050035, Hebei Province, P.R. China

*Corresponding author: E-mail: lhshen1974@gmail.com

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A series of new colchicine analogues (**6a-f**, **8a-b**) were synthesized by coupling nitrates with C-7 and replacement of the 10-methoxy with $N(CH_3)_2$ 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 **6c** showed more potent cytotoxic activities than colchicine.

Key Words: Colchicine, Nitrates, Cytotoxic activity.

INTRODUCTION

Colchicine (**1**), a naturally alkaloid extracted from the seed of *Colchicum autumnale Liliaceae* and *Gloriosa superba*, is a powerful antimitotic agent that blocks microtubule polymerization, thereby causing cells to accumulate in obvious mitotic arrest during cell cycle[1-3]. Colchicine and many of its derivatives could not be used due to its high toxicity[4]. 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 $N(CH_3)_2$ (**2**, **Scheme-I**) leads to increased potency[5].

Nitric oxide is a small, diffusible, highly reactive molecule involved in the regulation of many physiological processes including blood vessel dilatation, neurotransmission and events of the immune system. NO can also be generated from synthetic NO-releasing agents, such as nitrate, furoxan, hydroxyguanidine, diazeniumdiolate and others[6-8]. Studies showed that high levels of NO were cytotoxic and could promote the apoptosis of tumor cells[9,10].

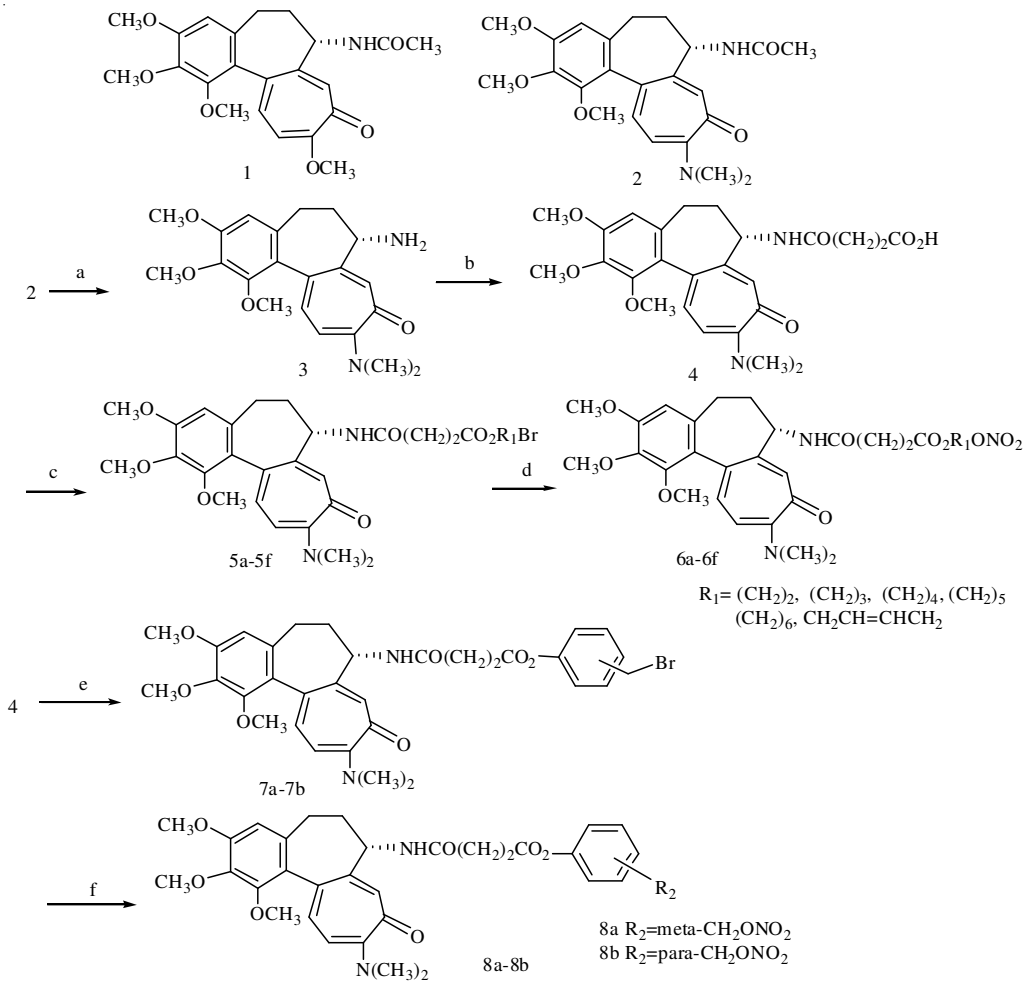
We reported previously a group of nitrate derivatives of colchicine, which have proved that NO-donating derivatives could really improve colchicine's antitumor activity *in vitro*[11]. In view of this, to search for antitumor drugs with high potency and selectivity, we recently synthesized a series of new analogues of colchicine which replacement of the 10-methoxy with $N(CH_3)_2$ by connecting nitrate to the C-7, hoping that these derivatives might be transported to target site where they would release active compounds and high concentrations of NO 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 Nanjing Tianzun Chemicals Co. Ltd. China, with an over 98 % purity. The lead compound **2** was prepared from **1**, according to the literature[12] in 90 % yield.

The lead compound **2** was refluxed with concentrated H_2SO_4 in water to give the intermediate **3**. Compound **3** was acylated by succinic anhydride in dry pyridine at 60 ºC to give succinate **4** in 90 % yield. **4** was treated with dibromoalkanes in the presence of K_2CO_3 and DMF at room temperature to generate important intermediates **5a-5f** in 70-85 % yields. Compounds **5a-5f** were further converted to the corresponding target compounds **6a-6f** with $AgNO_3$ in CH_3CN in good yields (80-95 %).

In a similar way, compounds **7a** and **7b** were prepared in good yields from **4** *via* chlorination by $SOCl_2$ to form acid chloride, followed by condensation with *m*-hydroxybenzyl bromide and *p*-hydroxybenzyl bromide respectively in the presence of CH_2Cl_2 at room temperature, subsequent reaction with $AgNO_3$ in CH_3CN gave target compounds **8a** and **8b**.

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 reference[13].



Scheme-I: Reagents and conditions: a. conc. H_2SO_4, H_2O, reflux, 5 h; b. succinic anhydride, pyridine, 60 ºC, 5 h, 90 %; c. BrR_1Br, K_2CO_3, DMF, rt, 6-8 h, 70-85 %; d. $AgNO_3$, CH_3CN, reflux, 15 h, 80-95 %; e. (i) $SOCl_2$, DMF (cat), reflux, 5 h; (ii) *m*- or *p*-hydroxybenzyl bromide, CH_2Cl_2, rt, 12 h, 70 and 75 %; f. $AgNO_3$, CH_3CN, reflux, 15 h, 45 and 43 %

The cytotoxic activity of all target compounds *in vitro* was determined by MTT assay[14], using colchicine and compound **2** 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
6a	0.204	0. 186	0.202	0.163
6b	0.096	0.087	0.082	0.115
6c	0.020	0.015	0.018	0.009
6d	0.095	0.098	0.103	0.105
6e	0.207	0.208	0.126	0.192
6f	0.226	0.232	0.203	0.217
8a	0.045	0.034	0.038	0.041
8b	0.030	0.024	0.025	0.038
2	0.090	0.070	0.078	0.080
Colchicine	0.094	0.078	0.080	0.084

The study results indicate that these new nitrates derivatives showed superior or comparable cytotoxic activity to colchicine and **2** *in vitro*. For all four tumor cell lines, compounds **6c**, **8a** and **8b** have better cytotoxicity than colchicine and **2**. In human hepatoma cell line (BEL7402), compounds **6b** has similar cytotoxicity as colchicine, whereas compounds **6c** exhibited almost fourfold potent activities than **2**. As to human breast carcinoma cell line (MCF7), compound **6c** exhibited almost ninefold potent activities than **2**. The results demonstrated that new NO-donating derivatives could really improve colchicine's antitumor activity *in vitro*.

In summary, a series of novel nitrate-colchicine conjugates were synthesized and evaluated for their *in vitro* cytotoxicity against four human tumor cell lines. It was found that **6c** showed significant cytotoxic activities. Further biological evaluations are currently in progress and will be reported in due course.

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13. The data of selected compounds: **6a**: yield 83.0 %, IR (KBr, ν_{max}, cm^{-1}): 3400, 3207, 1735, 1633, 1491, 1455; ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.60-2.80 (m, 8H), 3.16 (s, 6H, $N(CH_3)_2$), 3.64 (s, 3H, MeO-1), 3.87 (s, 3H, MeO-2), 3.93 (s, 3H, MeO-3), 4.24-4.28 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.45-4.49 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.64 (m, 1H, H-7), 6.50 (s, 1H, H-4), 7.16 (m, 2H, H-11 and H-12), 7.52 (s, 1H, H-8), 8.95 (brs, 1H, NHCO); MS (ESI, m/z): 559.3 $[M]^+$; anal. calcd. (%) for $C_{27}H_{33}O_{10}N_3$: C 57.95, H 5.94, N 7.51; found (%): C 57.78, H 6.05, N 7.62. **6c**: yield 90.0 %, IR (KBr, ν_{max}, cm^{-1}): 3410, 3125, 1736, 1635, 1528, 1476; ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.57-2.88 (m, 12H), 3.18 (s, 6H, $N(CH_3)_2$), 3.66 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.94 (s, 3H, MeO-3), 4.23-4.27 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.46-4.50 (t, 2H, *J* = 6.0Hz, OCH_2), 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), 8.90 (bs, 1H, NHCO); MS (ESI, m/z): 587.4 $[M]^+$; anal. calcd. (%) for $C_{29}H_{37}O_{10}N_3$: C 59.27, H 6.35, N 7.15; found (%): C 59.38, H 6.46, N 7.21. 8b: yield 43 %, IR (KBr, ν_{max}, cm^{-1}): 3405, 3220, 1738, 1633, 1596, 1510; ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.57 (m, 4H), 2.64 (m, 4H, $OCH_2 \times 2$), 3.15 (s, 6H, $N(CH_3)_2$), 3.68 (s, 3H, MeO-1), 3.91 (s, 3H, MeO-2), 3.95 (s, 3H, MeO-3), 4.71 (m, 1H, H-7), 5.48 (s, 2H, OCH_2), 6.54 (s, 1H, H-4), 6.96 (m, 4H, Ar-H), 7.20 (m, 2H, H-11 and H-12), 7.56 (s, 1H, H-8), 9.01 (bs, 1H, NHCO); MS (ESI, m/z): 621.3 $[M]^+$; anal. calcd. (%) for $C_{32}H_{35}O_{10}N_3$: C 61.83, H 5.68, N 6.76; found (%): C 61.97, H 5.59, N 6.87.
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