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Synthesis and evaluation of nitrate derivatives of colchicine as anticancer agents

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Abstract

To search for more potent antitumor agent, a series of novel nitric oxide-donating colchicine (Col) derivatives (**6a–f**, **8a** and **b**) were synthesized by coupling nitrates with *N*-methyl colchiceinamide. Their cytotoxicity against four human cancer cell lines *in vitro* were evaluated by MTT assay. It was found that many of the derivatives displayed significant activity, particularly, compounds **6c**, **8a** and **8b** showed more potent cytotoxic activities than Col.

Keywords: Colchicine; *N*-methyl colchiceinamide; Nitrates; Cytotoxicity

Colchicine (Col, **1**), the major alkaloid in *Colchicum autumnale* and *Gloriosa superba* is best known for its antimitotic effects, and is a potent drug that interferes with microtubules both *in vitro* and *in vivo*, thereby causing cells to accumulate in apparent mitotic arrest during the cell cycle [1,2]. Col has been used in the treatment of acute gout, familial Mediterranean fever, scleroderma, amyloidosis, Behcet's disease, liver disorder, tumor growth, particularly cirrhosis [3–5]. Although Col is a potent antimitotic agent, its medicinal uses are limited due to its high toxicity. Much interest has focused on structural modifications in the hope of improving the antitumor activity [6]. *N*-methyl colchiceinamide (**2**, Scheme 1), a synthetic derivative of Col, showed considerably higher stability toward acid hydrolysis and is a slightly less active antitumor and toxicity agent than Col [3].

Nitric oxide (NO) is a ubiquitous free radical in cells and tissues. It is produced in the body by an enzyme called the NO synthases (NOS)[0], which converts the amino acid L-arginine to NO and L-citrulline. NO displays diverse potent physiological actions such as vascular relaxation, neurotransmission, and events of the immune system. NO can also be generated from synthetic NO-releasing agents, such as nitrate, furoxan, S-nitrosothiol, and diazeniumdiolate [7,8]. Studies showed that high levels of NO were cytotoxic and could induce the apoptosis of tumor cells [9]. In addition, it was reported that NO conjugates of certain cytotoxic agents showed selective cytotoxicity and could be developed as potential antitumor drugs [10,11].

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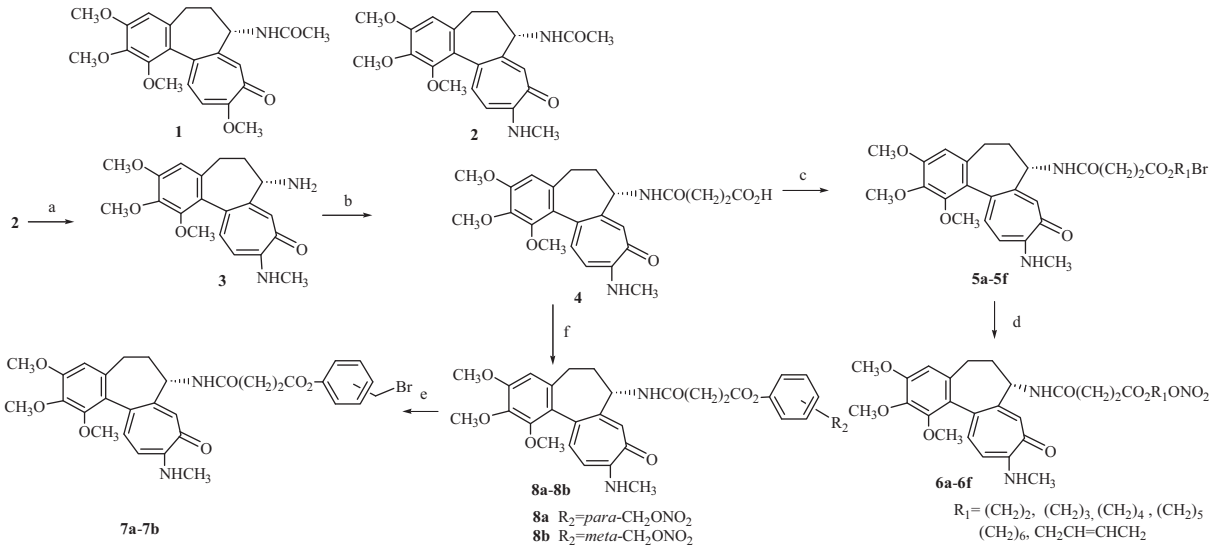

Scheme 1. Reagents and conditions: a, conc. H_2SO_4, H_2O, reflux, 5 h; b, succinic anhydride, pyridine, 60 °C, 5 h, 95%; c, BrR_1Br, K_2CO_3, DMF, rt, 5 h, 80–85%; d, $AgNO_3$, CH_3CN, reflux, 15 h, 88–95%; e, (i) $SOCl_2$, DMF (cat), reflux, 5 h; (ii) *p*- or *m*-hydroxybenzyl bromide, CH_2Cl_2, rt, 12 h, 71% and 78%; f, $AgNO_3$, CH_3CN, reflux, 15 h, 45% and 50%.

In 2009, Chang *et al*. reported a group of nitrate derivatives of Col, which have proved that the structure modification of the 10 positions does not interfere with the molecular recognition of Col [12]. Succinic acid is a low toxicity agent and can help in the compounds forming prodrug effectively by ester bond [13,14]. With these in mind, and also based on the diverse bioactivities of the above-mentioned Col, we recently conjugated *N*-methyl colchiceinamide with nitrate using succinic acid as a linker to synthesize a series of novel NO-releasing derivatives of Col, 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 1. Col **1** was purchased from Nanjing Tianzun Chemicals Co., Ltd., China, with over 98% purity. The lead compound **2** was prepared from **1**, according to the literature [15] in 90% yield.

N-methyl colchiceinamide **2** was refluxed with concentrated H_2SO_4 in water to give *N*-methyl deacetylcolchiceinamide **3**. Compound **3** was acylated by succinic anhydride in dry pyridine at 60 °C to give succinate **4** in 95% 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 80–85% yields. Compounds **5a–5f** were further converted to the corresponding target compounds **6a–6f** with $AgNO_3$ in CH_3CN in good yields (88–95%).

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 *p*-hydroxybenzyl bromide and *m*-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 are shown in Scheme 1 and the data of yield, MS, IR and ^1H NMR spectra and elemental analysis (EA) of selected compounds are shown in Ref. [16].

The cytotoxic activity of all target compounds *in vitro* was determined by MTT assay [17], using Col as a 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).

The study results indicate that these novel nitrate derivatives showed superior or comparable cytotoxic activity to Col *in vitro*. For human ovary cancer cell line (A2780) and human lung cancer cell line (A549), compounds **6c**, **8a** and **8b** have better cytotoxicity than Col, and compounds **6b** and **6d** have similar cytotoxicity as Col. In human hepatoma cell line (BEL7402), compounds **6b**, **6d** and **6e** have similar cytotoxicity as Col, whereas compounds **6c**, **8a** and **8b** have more potent cytotoxicity than Col. As to human breast carcinoma cell line (MCF7), compound **6c** exhibited almost tenfold potent activities than Col. The results demonstrated that NO-donating Col derivatives could really improve colchicine's antitumor activity *in vitro*.

Table 1
The cytotoxicity data of the target compounds.

Compd.	IC_{50} (μmol/L)/cell line			
	A2780	A549	BEL7402	MCF7
6a	0.104	0. 106	0.202	0.103
6b	0.099	0.088	0.085	0.135
6c	0.010	0.012	0.013	0.008
6d	0.096	0.086	0.085	0.100
6e	0.109	0.205	0.086	0.095
6f	0.205	0.135	0.120	0.202
8a	0.027	0.038	0.029	0.036
8b	0.036	0.049	0.037	0.058
Col	0.094	0.078	0.080	0.084

In summary, a series of Col–nitrate 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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[16] The data of selected compounds: **6a**: yield 90.0%, IR (KBr, *ν*): 3444, 2907, 1733, 1630, 1490, 1455, 1414 cm^{-1}; 1H NMR ($CDCl_3$, 300 MHz): *δ* 1.50–3.40 (m, 9H), 3.07 (d, 3H, N–CH_3), 3.67 (s, 3H, MeO-1), 3.91 (s, 3H, MeO-2), 3.95 (s, 3H, MeO-3), 4.25–4.29 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.44–4.48 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.60–4.72 (m, 1H, H-7), 6.54 (s, 1H, H-4), 7.34 (m, 2H, Ar-H), 7.49 (s, 1H), 8.71 (bs, 1H, NHCO); MS (ESI, *m/z*): 545.3 $[M]^+$; Anal. Calcd. for $C_{26}H_{31}O_{10}N_3$: C 57.24, H 5.73, N 7.70; Found: C 57.28, H 5.78, N 7.63. **6c**: yield 92.0%, IR (KBr, *ν*): 3450, 2925, 1738, 1630, 1598, 1506, 1447 cm^{-1}; 1H NMR ($CDCl_3$, 300 MHz):*δ* 1.50–3.38 (m, 13H), 3.07 (d, 3H, N–CH_3), 3.66 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.94 (s, 3H, MeO-3), 4.24–4.28 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.44–4.48 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.65–4.72 (m, 1H, H-7), 6.52 (s, 1H, H-4), 7.27 (m, 2H, Ar-H), 7.49 (s, 1H), 8.93 (bs, 1H, NHCO); MS (ESI, *m/z*): 573.4 $[M]^+$; Anal. Calcd. for $C_{28}H_{35}O_{10}N_3$: C 58.63, H 6.15, N 7.33; Found: C 58.65, H 6.26, N 7.39. **8a**: yield 45%, IR (KBr, *ν*): 3445, 2920, 1739, 1630, 1597, 1510, 1455, 1322 cm^{-1}; 1H NMR ($CDCl_3$, 300 MHz): *δ* 1.50–3.37 (m, 9H), 3.07 (d, 3H, N–CH_3), 3.65 (s, 3H, MeO-1), 3.89 (s, 3H, MeO-2), 3.93 (s, 3H, MeO-3), 4.68 (m, 1H, H-7), 5.47 (s, 2H, OCH2), 6.52 (s, 1H, H-4), 6.93 (m, 4H, Ar-H), 7.30 (m, 2H, Ar-H), 7.49 (s, 1H), 9.07 (bs, 1H, NHCO); MS (ESI, *m/z*): 607.2 $[M]^+$; Anal. Calcd. for $C_{31}H_{33}O_{10}N_3$: C 61.28, H 5.47, N 6.92; Found: C 61.35, H 5.52, N 6.89.
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