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Synthesis and biological evaluation of B-ring modified colchicine and isocolchicine analogs

Michael Cifuentes,[a] Brett Schilling,[b] Rudravajhala Ravindra,[b] Jacquelyn Winter[a] and Mark E. Janik[a,*]

[a]*Department of Chemistry, SUNY Fredonia, Fredonia, NY 14063, USA*
[b]*Department of Chemistry, Binghamton University, Binghamton, NY 13902-6016, USA*

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Abstract—A series of modified colchicine and isocolchicine analogs (C-7 substituent) were synthesized and evaluated in vitro against a PC3 cancer cell line and for inhibition of microtubule polymerization. The colchicine analogs all displayed strong inhibition of tubulin polymerization, while compounds **6** and **20** also possessed an increased cytotoxic activity as compared to colchicine. More importantly, isocolchicine analogs **7**, **15**, and **17** showed inhibition of microtubule polymerization with IC_{50} values ranging from 58 to 68 μM. In addition, **7** displayed strong cytotoxic activity with an IC_{50} = 93 nM which was more potent than colchicine analog **12**.

The compound colchicine (**1**) (Fig. 1) is a highly potent antimitotic agent that derives its therapeutic benefit by binding to the protein tubulin.[1,2] The mechanistic understanding of colchicine–tubulin binding has been highly investigated using both structure–activity relationship (SAR) studies and thermodynamic analyses.[3–5] These studies suggest that the A- and C-rings of the parent molecule comprise the minimum structural feature necessary for high affinity drug–tubulin binding. An example illustrating the importance of the C-ring is revealed by isocolchicine (**2**), which is virtually inactive in binding to tubulin.[6,7] Recently, however, the discovery of the first active isocolchicine analog (**3**) (B-ring substituent modified) has prompted renewed interest in the role of the B-ring in colchicine–tubulin binding.[8] Therefore, in a continuing effort to identify potent tubulin binding isocolchicine analogs and to investigate the SAR of the B-ring substituent we decided to synthesize and biologically evaluate a series of colchicine and isocolchicine analogs modified at the C-7 position of the B-ring.

Deacetylcolchicine (**4**) and deacetylisocolchicine (**5**) obtained from colchicine (Scheme 1) [9,10] were reacted with lithium perchlorate and propylene oxide in acetonitrile to afford their respective β-amino alcohols in 95%

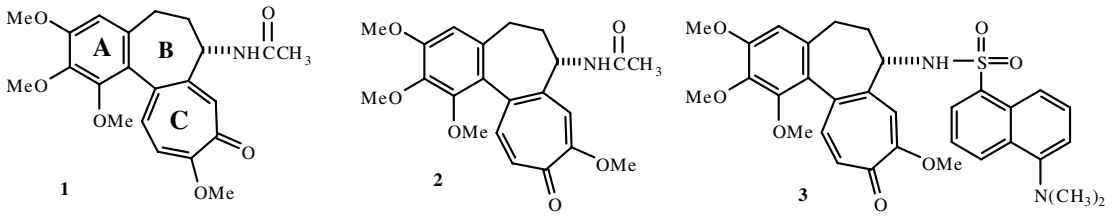

Figure 1.

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* Corresponding author. Tel.: +1 716 673 3508; fax: +1 716 673 3347; e-mail: janikm@fredonia.edu

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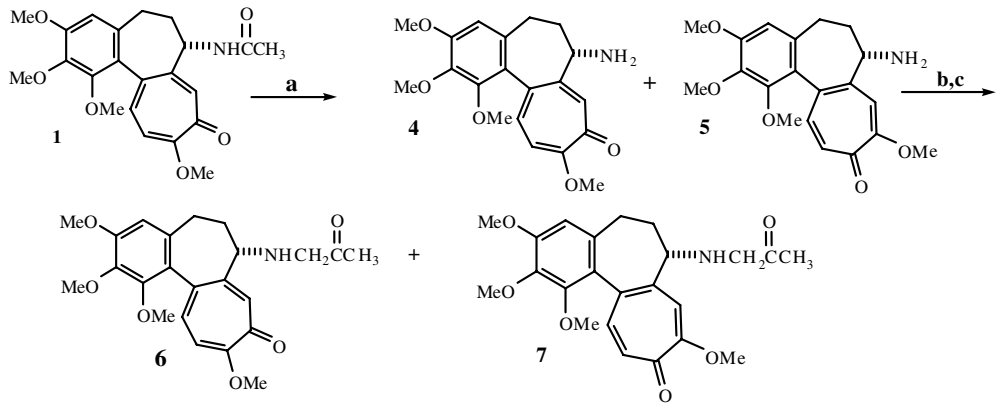
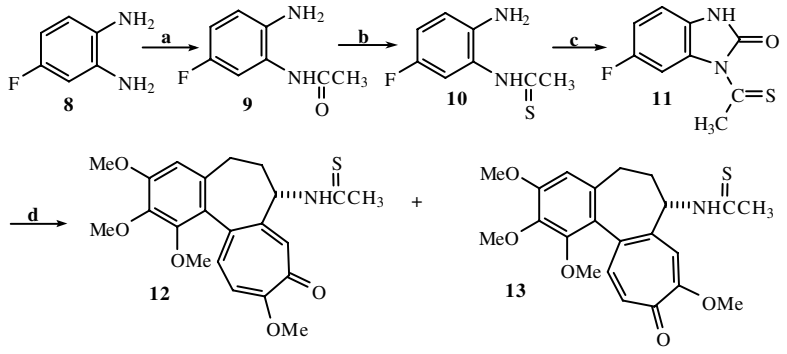


Scheme 1. Reagents and conditions: (a) 1—2 N HCl/CH_3OH, reflux; 2—CH_2N_2/CH_2Cl_2; (b) propylene oxide, $LiClO_4$, CH_3CN; (c) DMSO, $(COCl)_2$, CH_2Cl_2, TEA, −60 °C.



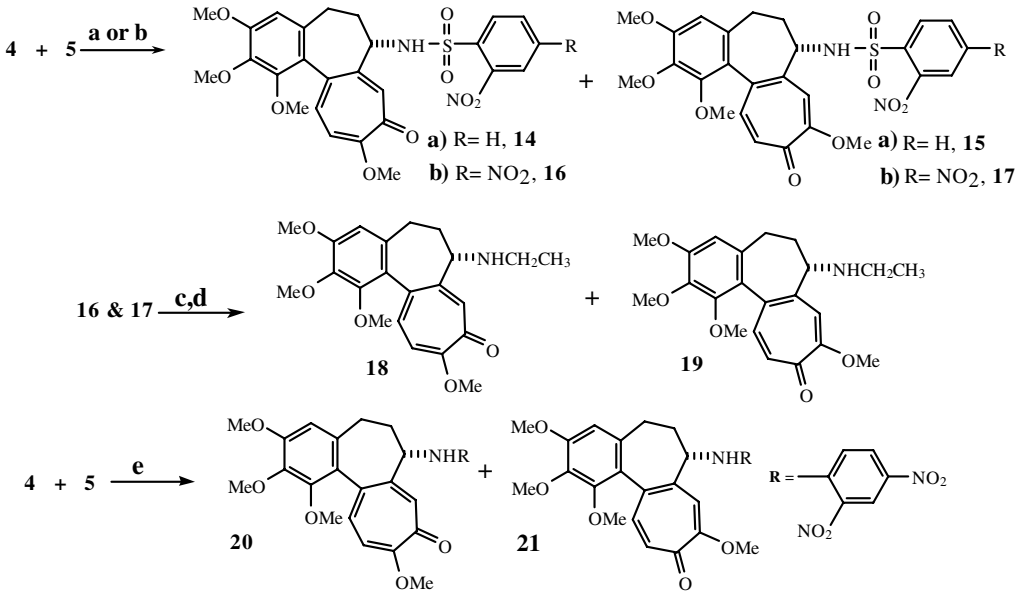
Scheme 2. Reagents and conditions: (a) $(CH_3CO)_2O$, CH_2Cl_2, 0 °C; (b) P_4S_{10}, Na_2CO_3, THF, 0 °C; (c) $COCl_2$, THF; (d) **4** and **5**, TEA, CH_3CN.

yield.[11] It should be noted that various metal salts (LiOTf, $ZnCl_2$, and $CoCl_2$) were initially used prior to lithium perchlorate; however, they all resulted in non-regioselective attack of the amine on the epoxide ring.[12,13] Initial attempts at alcohol oxidation utilized both pyridinium chlorochromate and pyridinium dichromate, however, no product formation was observed.[14] Thus, we alternatively employed Swern oxidation whereby the β-amino alcohols were added to a solution of DMSO/oxalyl chloride at −60 °C to afford compounds **6** and **7** in 88% yield.[15] The two isomers were separated using radial chromatography.

To investigate the role of the amide carbonyl group, we sought to synthesize analogs of **1** and **2** whereby the amide was replaced with a thioamide functionality.

Our initial attempts at thioamide formation involved the use of Lawesson's reagent;[16] however, this resulted in conversion of the C-ring carbonyl into a thiocarbonyl. To synthesize our thioamide analogs (**12** and **13**), we instead utilized a procedure that relied on the use of thioacylating reagent **11**, which was synthesized as shown in (Scheme 2).[17] Compound **8** was chosen as our benzimidazolone source and it was reacted with acetic anhydride to afford compound **9** in 89% yield. Conversion of the amide in **9** to a thioamide was accomplished using phosphorus pentasulfide to give **10** in 72% yield. Compound **10** was reacted with two equivalents of phosgene for 4 h at room temperature to yield thiobenzimidazolone **11** (71%). Thiobenzimidazolone **11** was then reacted with a mixture containing isomers **4** and **5** in acetonitrile. The reaction was monitored by TLC and after stirring for 48 h at room temperature compounds **12** and **13** were afforded in a combined 76% yield. The two isomeric compounds were separated using radial chromatography.

Additional investigation of the amide carbonyl was sought through conversion of the carbonyl moiety into a methylene (CH_2) group. Initial attempts at the synthesis of compounds **18** and **19** (Scheme 3) focused on the use of reducing agents such as $LiAlH_4$ and borane-dimethyl sulfide; however, no formation of the desired secondary amine occurred.[18] Thus, we instead attempted to synthesize the target amines utilizing a Mitsunobu alkylation of a 2-nitrobenzenesulfonamide intermediate (**14** and **15**).[19] The alkylation reactions proceeded in moderate yield but reaction times tended to be of the order of 2–3 days. We therefore alternatively employed the use of compounds **16** and **17** (2,4-dinitrobenzenesulfonamide), as shown in (Scheme 3), which were more efficiently alkylated using Mitsunobu conditions in 8 h.[20] The dinitrobenzenesulfonamide-protecting group was removed under mild reaction conditions to yield compounds **18** and **19** (82%) which were separated using radial chromatography. It is of interest to note that an attempt to form



Scheme 3. Reagents and conditions: (a) 2-nitrobenzenesulfonyl chloride, TEA, CH_2Cl_2; (b) 2,4-dinitrobenzenesulfonyl chloride, 2,6-lutidine, CH_2Cl_2; (c) CH_3CH_2I, K_2CO_3, CH_3CN; (d) $HSCH_2CO_2H$, TEA, CH_2Cl_2; (e) 2,4-dinitrobenzenesulfonyl chloride, TEA, CH_2Cl_2.

Table 1. Inhibition of microtubule polymerization and cytotoxic activity against PC3 cell lines for compounds **6**, **7**, and **12**–**21**

Colchicine isomer	IC_{50}, MTP assembly[a] (μM)	IC_{50}, PC3[b] (nM)	Isocolchicine isomer	IC_{50}, MTP assembly (μM)	IC_{50}, PC3[b] (nM)
1	1.7 ± 0.04[c]	11 ± 0.65[c]	**2**	Inactive[d]	4,600 ± 0.19
6	8.3 ± 0.37	7.6 ± 2.1	**7**	68 ± 2.4	93 ± 10.3
12	2.2 ± 0.10	97.5 ± 28.7	**13**	Inactive[e]	1,752 ± 435
14	5.6 ± 0.14	19.8 ± 11.8	**15**	58 ± 2.4	905 ± 117
16	9.7 ± 0.27	50 ± 16.6	**17**	59 ± 4.8	10,250 ± 1,639
18	5.1 ± 0.16	17.6 ± 13.2	**19**	Inactive[e]	1,722 ± 819
20	4.6 ± 0.17	9.0 ± 2	**21**	Not soluble	5,700 ± 754

[a] Concentration of ligand required to effect a 50% reduction in microtubule polymerization.
[b] Human prostate cancer cells were used for cytotoxicity measurements.
[c] Values represent means ± SD of at least three determinations (assay procedure described in supplementary materials section).
[d] Inhibition of polymerization measured up to a concentration of 300 μM drug.
[e] Inhibition of polymerization measured up to a concentration of 150 μM drug.

16 and **17** using triethylamine (2 equiv.) rather than lutidine as the base resulted in the formation of compounds **20** and **21**, respectively, as the major reaction products.

The antimicrotubule activities of compounds **6**, **7**, and **12**–**21** were assayed by their ability to inhibit in vitro assembly of microtubule protein (Table 1) using colchicine and isocolchicine as reference compounds.[21] As expected, the nature of the C-7 substituent did not greatly affect the in vitro polymerization activity of colchicine analogs (**6**, **12**, **14**, **16**, **18**, and **20**) which all displayed strong microtubule inhibition. The most active compound was the thioamide analog **12** (IC_{50} = 2.2 μM) possessing an activity nearly identical to that of colchicine (IC_{50} = 1.7). Concerning the aromatic analogs (**14**, **16**, and **20**), compound **20** possessed the strongest inhibition with an IC_{50} = 4.6 μM. Introduction of a methylene group between the amide nitrogen and carbonyl of colchicine resulted in compound **6** which displayed nearly a 5-fold reduction in activity (IC_{50} = 8.3 μM) as compared to colchicine. Interestingly though, conversion of the carbonyl in colchicine into a methylene group resulted in the more active compound **18** (IC_{50} = 5.1 μM) as compared to **6**.

Of significant importance was the antimicrotubule activity displayed by select isocolchicine analogs. The most active isocolchicine analog was compound **15** with an IC_{50} value of 58 μM. This analog contained an aromatic nitrobenzenesulfonamide substituent at the C-7 position. Addition of a second nitro group on the C-7 aromatic ring resulted in compound **17**, which displayed similar activity (IC_{50} = 59 μM) as compared to **15**. Removal of the sulfonyl group, however, resulted in poor compound solubility beyond 30 μM as seen in **21**. Insertion of a methylene unit between the amide nitrogen and carbonyl in inactive isocolchicine **2** (Table 1) resulted in the active compound **7** (IC_{50} = 68 μM). Interestingly, the thioamide isocolchicine analog **13** displayed no activity, which is in contrast to **12** the most active analog in the colchicine series. Compound **19**, conversion of the carbonyl into a methylene group, was inactive as well.

In Table 1, we have also reported the cytotoxic activities of the analogs against a human prostate cancer cell line (PC3).[22] The most active colchicine analogs were compounds **6** (IC_{50} = 7.6 nM) and **20** (IC_{50} = 9.0 nM) which were both more potent than the parent compound colchicine (IC_{50} = 11 nM). Additional colchicine analogs (**12**, **14**, **16**, and **18**) all displayed strong cytotoxic activities as well. Concerning the isocolchicine analogs, compounds **7**, **13**, **15**, and **19** were significantly more active than the parent isocolchicine. Most noteworthy was compound **7** (IC_{50} = 93 nM) which displayed strong antiproliferative activity. Remarkably, compound **7** possessed stronger activity than the colchicine analog **12**. The remaining isocolchicines (**17** and **21**) possessed cytotoxicities of 10,250 and 5700 nM, respectively.

In conclusion, a series of B-ring C-7 substituent modified colchicine and isocolchicine analogs were synthesized and evaluated for antimicrotubule and cytotoxic activity. The colchicine derivatives all displayed strong potencies with **12** possessing the strongest antimicrotubule inhibition and **6** and **20** displaying a more potent cytotoxicity against PC3 than colchicine. Interestingly, compound **6** was 5-fold less active in the MTP assay as compared to colchicine. Previous studies have indicated that the association rate of colchicine analogs binding to tubulin is affected by the C-7 substituent.[23] Thus, **6** may bind slower to tubulin than colchicine which could explain its lower potency in the MTP assay (fixed/short incubation time) as compared to the cytotoxicity experiments (3 days incubation time) where kinetics should not be a factor. A most noteworthy finding was the potency displayed by isocolchicine compounds **15** and **17**. Previous studies have indicated that select isocolchicine analogs (aromatic C-7 side chains) may be able to interact with α-tubulin through their B-ring substituent thereby increasing their overall binding affinity for the protein.[8] Compounds **15** and **17** could likely interact with tubulin in a similar manner which would explain their improved activity as compared to inactive isocolchicine. A most unexpected result in the iso series was the relatively high activity of **7** in both the MTP and cytotoxic assays. In fact, compound **7** surprisingly displayed a stronger inhibition against PC3 cell lines as compared to colchicine derivative **12**. In further studies, we will focus on determining the association rate of **6** with tubulin as compared to colchicine. In addition, we will synthesize analogs of **7** (–$NHCH_2CH_2COCH_3$, etc.) to further explore the SAR of isocolchicinoids of this type.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.02.010.

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