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Synthesis of non-racemic pyrrolo-allocolchicinoids exhibiting potent cytotoxic activity.

Ekaterina S. Shchegravina,[a] Dmitry I. Knyazev,[a] Irina P. Beletskaya,[b] Elena V. Svirshchevskaya,[c] Hans-Günther Schmalz,[d] and Alexey Yu. Fedorov[a]*

Abstract: An efficient eight-step semi-synthetic approach towards non-racemic pyrrolo-allocolchicinoids starting from natural colchicine was developed exploiting a Pd-catalyzed domino Sonogashira coupling/*5-endo-dig* cyclization of a 2-iodo-trifluoroacetanilide intermediate to build up the heterocyclic ring system. The *N-Me* substitution of the pyrrole ring enhances the antitumoral activity of the prepared molecules by 2-3 orders of magnitude. Among the active compounds, the *N*-methylated colchicinoid **14** exhibits powerful cytotoxic and anti-proliferative properties at concentrations below 1 nM.

Introduction

Colchicine (**1**), an alkaloid isolated from *Colchicum autumnale*, was the first discovered tubulin-destabilizing agent (Figure 1).[1] On a molecular level, it blocks the self-assembly of tubulin to microtubules by interacting at the interface between α- and β-tubulin heterodimers (colchicine binding site of tubulin).[2] This results in both in an antimitotic activity and the suppression of cell motility. Clinically, colchicine is approved for the treatment of Familial Mediterranean fever, Behcet's disease, as well as acute gout, chondrocalcinosis and other types of microcrystalline arthritis.[3-6] Other therapeutical indications include primary biliary cirrhosis, psoriasis, amyloidosis, various forms of dermatitis, relapsing polychondritis, necrotizing vasculitis, Sweet's syndrome and leukocytoclastic vasculitis.[7-10] Colchicine can be useful for the treatment of cardiovascular diseases,[11] such as particular pericarditis, atrial fibrillation caused by inflammation, and ischemic episodes.[12] Besides, it is considered as an anticancer agent, although its clinical use in sufficient doses is hampered by relatively high general toxicity, resulting from its accumulation in the gastro-intestinal tract as well as neurotoxicity.[13]

While colchicine (**1**) itself cannot be used in cancer chemotherapy, it still represents an important lead structure in the search for new anticancer agents. Several structurally related compounds, such as allocolchicine (**2**),[14] Z-stilbenes,[15] 4-arylcoumarins[16] and related compounds[17] were shown to exhibit pronounced cytotoxic effects (Figure 1). In this context, we recently demonstrated that compounds **3** and *rac*-**4**, with an extra furan or pyrrole unit annealed to ring C of the allocolchicine skeleton, exhibit unique antitumoral activity.[18-20]

Notably, the furano-allocolchicinoid **3** inhibits tumor growth in mice without noticeable negative neurological symptoms, weights loss or lethality of experimental animals.[19] The (racemic) pyrrolo-allocolchicinoid (*rac*-**4**) demonstrated high level of proliferation inhibition and apoptosis induction against different lymphoma cells, while its unspecific cytotoxicity is relatively low.[18]

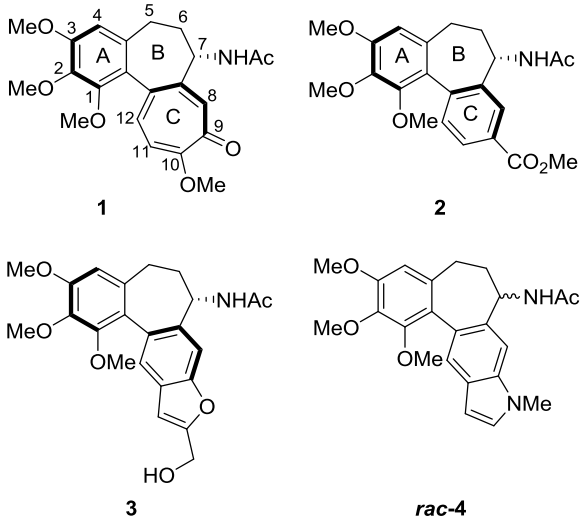

Figure 1. Colchicine (1), allocolchicine (2) and their analogs **3** and **4**.

Results and Discussion

In our previous studies we prepared pyrrolo-allocolchicines of types **5a/5b** in racemic form by total synthesis (11 steps, 3-18% overall yield) starting from 3,4,5-trimethoxyphenylpropionic acid and exploiting the Suzuki coupling and the Friedel-Crafts cyclization as the key C-C bond forming steps (Scheme 1).[18] Herein, we report a conceptionally different synthesis of enantiomerically pure pyrrolo-allocolchicines of type **5'** bearing a 4-bromo and a 2''-oxymethyl substituent. Starting from commercially available natural (-)-(aR,7S)-colchicine (**1**) the semi-synthetic pathway proceeds in only 8 steps under the conservation of the chirality center at C-7. This specific substitution pattern was selected because we had previously observed, that the presence of a hydroxylmethyl substituent either at C-7 of ring ***B*** [18a,18c] or on the heterocyclic ring (like in compound **3**)[19] significantly increases the anti-proliferative activity, as does a halogen atom at ring ***A***.[21]

For the construction of the indolyl moiety in **5'** we devised the strategy depicted in Scheme 2, which relies on a Sonogashira cross-coupling with subsequent *in situ* transition-metal mediated

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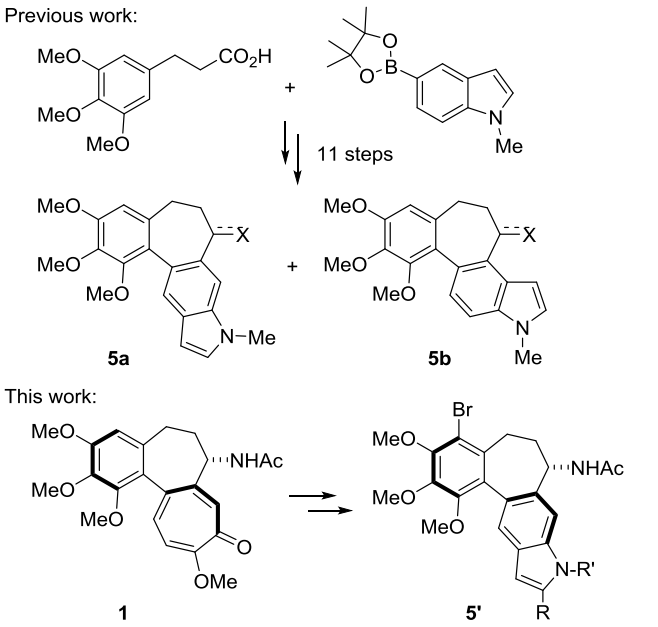
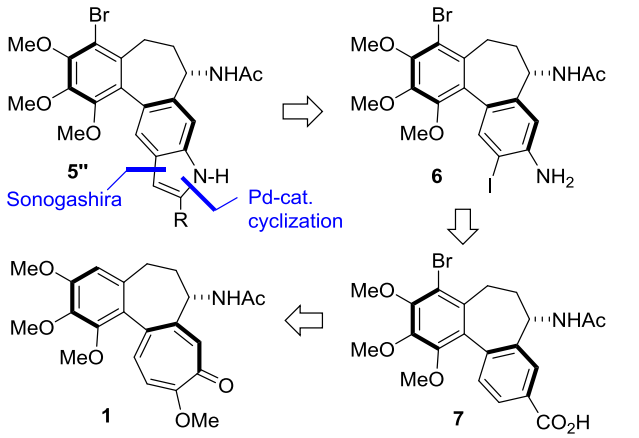
cyclization.[22] The required *ortho*-iodoaniline precursor **6** could be prepared through Curtius degradation and iodination from 4-bromo-allocolchicinic acid (**7**), which, in turn, is readily accessible from colchicine (**1**) by the known ring contraction and bromination.[19, 23]



Scheme 1. Total- and semi-synthetic approaches towards pyrrolo-allocolchicines.



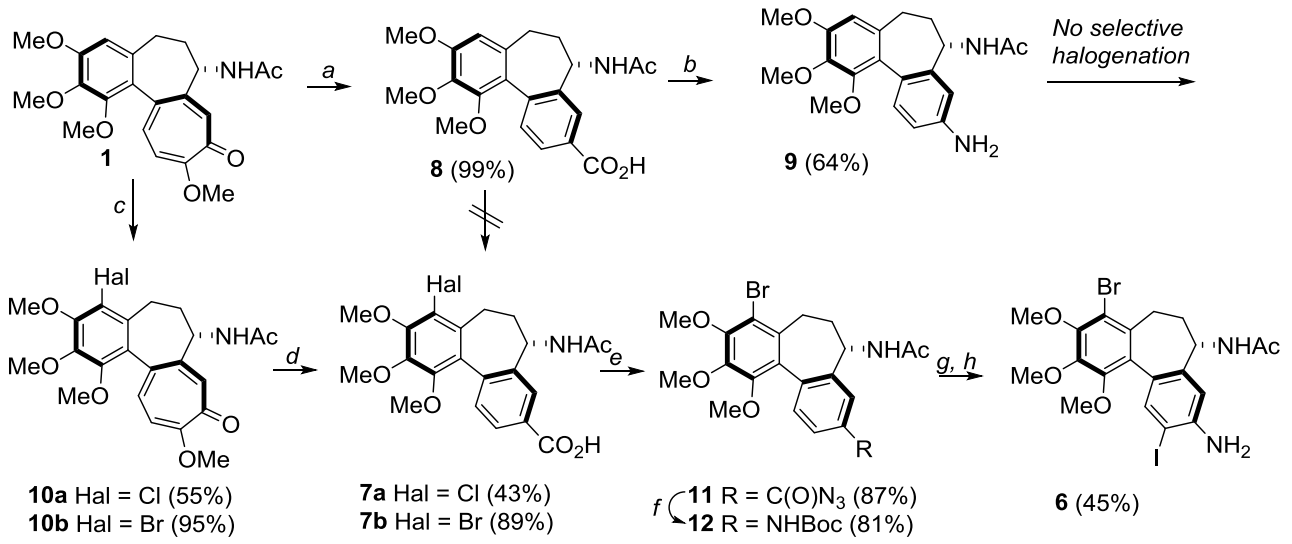
Scheme 2. Strategy for the semi-synthesis of pyrrolo-allocolchicinoids of type 5' starting from colchicine (1).

The synthesis starts with the base-induced tropolone ring contraction[23] of **1** (in wet MeOH) to give allocolchicinic acid **8** in excellent yield (Scheme 3). After a careful optimization, the subsequent Curtius degradation proceeded smoothly using NaN_3 in combination with Boc_2O as an activating agent to afford the amine **9** in 64% yield. However, all attempts to achieve a selective iodination of **9** failed, and only complex and inseparable mixtures of iodinated products were obtained, due to a similar reactivity of three aromatic positions. Similarly, we were not able to achieve a selective C-4 halogenation of allocolchicinic acid **(8)**.

Therefore, we decided to perform the halogenation at C-4 prior to the C-ring contraction. The described protocol for this transformation[21] failed in our hands. However, the reaction of **1** with either NCS or NBS works well in the presence of TFA to give the 4-halogenated colchicines **10a** and **10b** in 55% and 95% yield, respectively. Interestingly, these two compounds behave quite differently in the base-induced ring contraction. While **10a** reacted rather sluggishly to give **7a** (43% yield) the corresponding bromide **10b** cleanly afforded the allocolchicinic acid **7b** in 89% isolated yield. To our surprise, the C-4 halogenation proved to have a dramatic effect on the tendency of the allocolchicinic acids (**7**) to undergo Curtius degradation. Under the proven conditions, used formerly for the one-pot conversion of **8** into **9**, only trace amounts of the expected aniline products were obtained. Instead, the stepwise treatment of bromide **7b** with NaN_3 in the presence of Boc_2O afforded the acylazide **11** in 87% yield. Interestingly, compound **7a** did not afford the corresponding product at all. Nevertheless, the rearrangement of the acylazide **11** could be achieved by heating with an excess of Boc_2O in *tert*-BuOH to afford the Boc-protected amine **12** in 81% yield. Finally, the Boc-group was cleaved, and on treatment with NIS/AcOH the resulting aniline gave the desired *ortho*-iodinated product **6** in 45% yield (over two steps).

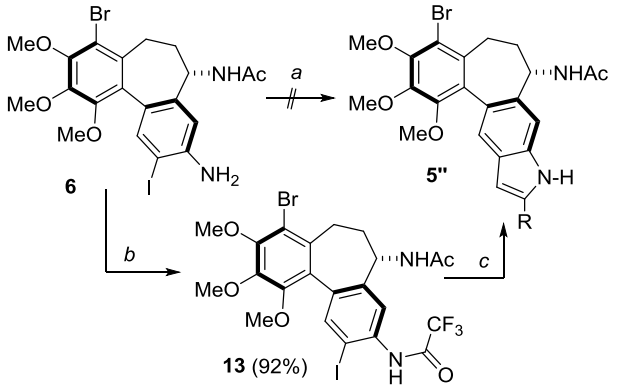
Our attempts to perform the Pd/Cu-catalyzed Sonogashira coupling of unprotected *ortho*-iodoaniline **6** with several terminal alkynes failed. Instead, trifluoroacetamide **13**, prepared from **6** (Scheme 4) readily underwent the Larock annulation to afford the desired pyrrolo-allocolchicines **5''** in a good yield. Notably, the *N*-trifluoroacetamide group is cleaved under the reaction conditions. The choice of alkynes was based on our previous studies,[19] which indicated that the presence of a CH_2OH, $CH(CH_3)OH$ or CH_2OAc group in the side chain of the heterocyclic ring leads to higher *in vitro* antitumor activity. In order to determine the influence of substituents at the nitrogen atom on the biological activity, *N*-methylated pyrrolo-allocolchine **14** was prepared in 3 steps from amide **13**. In the first step, the indole **5f''** was obtained through tandem Sonogashira/cyclization sequence, using TBDMS-protected propargyl alcohol. Then, after the *N*-methylation (NaH/MeI), and the selective cleavage of the silyl group, compound **14** was isolated in 28% overall yield (over 3 steps).

The *in vitro* cytotoxicity of the synthesized compounds toward Colo (human pancreatic adenocarcinoma), HEK (human em bryonic kidney) and Mia (human pancreatic carcinoma) cell lines was investigated. A tetrazolium-based assay was used to determine the drug concentration required to inhibit cell growth by 50% after incubation in the culture medium for 72 h. The calculated IC_{50} values are summarized in Table 1. Compound **5a''** was found to be completely inactive, while a modest cytotoxic effect was observed for compounds **5b-e''**.



Reaction conditions: *a*: MeONa (solid, 17 eq.), $MeOH/H_2O$, 65 °C; *b*: NaN_3 (3 eq.), Boc_2O (6 eq.), DME, 75 °C, then CF_3COOH (3 M in DCM), rt; *c*: NCS or NBS, TFA-AcOH (3:1), rt; *d*: NaOMe, MeOH, 65 °C; *e*: NaN_3 (6 eq.), Boc_2O (8 eq.), THF, rt; *f*: Boc_2O (6 eq.), *t*-BuOH, 90 °C; *g*: 3 M HCl-H_2O/ DCM, rt; *h*: NIS (1,1 eq.), AcOH, rt.

Scheme 3. Conversion of colchicines (1) into iodo-aniline (6)

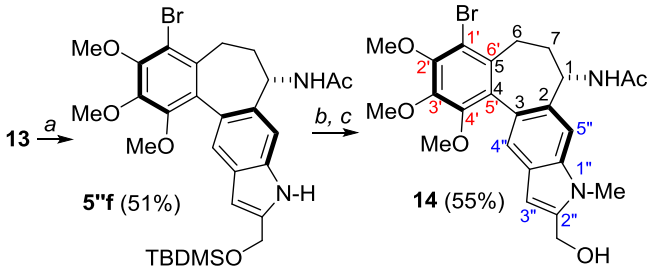


5a" R = Ph (50%); **5b"** R = CH_2OH (40%);
5c" R = $CH(CH_3)OH$ (48%); **5d"** R = CH_2OAc (52%)
5e" R = CH_2CH_2OH (65%)

Reaction conditions: *a*: alkyne (1,2 eq.), [Pd]-source (0,05 eq.), CuI (0,1 eq.), base, solvent; *b:* $(CF_3CO_2)_2O$ (1,8 eq.), pyridine (2,6 eq.), DCM, 0 °C; *c:* alkyne (1,2 eq.), $Pd(OAc)_2$ (0,05 eq.), CuI (0,1 eq.), PPh_3 (0,15 eq.), AcOK, (3 eq.), MeCN, 80 °C.

Scheme 4. Final steps of the synthesis of pyrrolo-allocolchicines of type **5**"

In contrast, compound **14** inhibited cell proliferation at nanomolar or even subnanomolar concentrations. We presume that the presence of an alkyl on the N-1 of the pyrrolo-allocolchicine skeleton is essential for the antitumor activity of the prepared compounds .[2]



Reaction conditions: *a*: $HC{\equiv}CCH_2OTBDMS$ (1,2 eq.), $Pd(dppf)Cl_2$ (0,05 eq.), CuI (0,1 eq.), DIPEA (3 eq.), MeCN, 90 °C, 6 h; *b*: NaH (60% in mineral oil, 2,3 eq.), MeI (1,5 eq.), THF, 0→65 °C; *c*: 1,0 M TBAF in THF.

Scheme 5. Synthetic route to *N*-methylated pyrrolo-allocolchicinoids.

Table 1. Anti-proliferative properties (IC_{50}, μM)[a] of the prepared compounds.

	Cell line	Colo	HEK	Mia
Compound	Colchicine	0.02	0.007	0.008
	5a''	>4	20	4
	5b''	0.1	0.8	0.04
	5c''	0.32	0.1	0.2
	5d''	0.09	0.8	0.01
	5e''	0.5	0.8	0.16
	14	0.004	0.001	<0.001

[a] Drug concentration that inhibits the growth of the represented cells by 50% after incubation in a liquid medium for 72 h. Each drug concentration was tested in triplicate, and the SE of each point is <10%.

Conclusions

We have described an efficient semi-synthetic approach towards non-racemic pyrrolo-allocolchicines starting from natural colchicine. As key steps we used a Curtius degradation of an allo-colchicinic acid followed by a Larock annulation to build-up the indole ring system. The developed synthetic methodology may find future applications in the synthesis of various pyrrolo-allocolchicines. The *N*-methylated compound **14** may serve as a leading structure for the development of anticancer compounds, possessing significant cytotoxic activity already in subnanomolar concentration.

Experimental Section

Synthesis of compound 14. Into a Schlenk flask with a stirring bar, compound **13** (80 mg, 0,12 mmol), $Pd(dppf)_2Cl_2$ (0,05 eq., 4,4 mg, 0,006 mmol), and CuI (0,1 eq., 2,3 mg, 0,012 mmol) were placed. Acetonitrile was added under inert atmosphere. DIPEA (3 eq., 95 mcl, 0,366 mmol) and alkyne (1,2 eq., 30 mcl, 0,146 mmol) were added to the reaction mixture, the temperature was risen to 90 °C, and the mixture stirred for 8 hours. The solvent was removed in vacuum and the residue was purified using column chromatography on silica with petroleum ether-ethyl acetate-ethanol (5:1:1) as eluent. The product **5f** was obtained as pale-beige oil in 51% yield. It was mixed with NaH (60% in mineral oil, 2,3 eq., 0,19 mmol, 7,6 mg) and MeI (1,5 eq., 0,125 mmol, 17,7 mg) in THF at 0 °C, and the temperature was rised to 65 °C. The reaction was carried out for 6 hours, the mixture filtered through a short pad of silica, and the solvent was removed. The crude product was dissolved in 4 ml of THF, 45 mcl of 1*N* TBAF in THF was added, and the mixture was stirred for 3 hours. The product was purified by column on silica (petroleum ether – ethyl acetate – ethanol 15:1:1). Compound **14** was obtained as a white solid in 55% yield (over two steps). M.p. = 175-177 °C. ^{1}H NMR (400 MHz, dmso-d_6): δ 8.51 (d, *J* = 8.3 Hz, 1H, N**H**Ac), 7.46 (s, 1H, C(8)-H), 7.37 (s, 1H, C(12)-H), 6.38 (s, 1H, C(11)-H), 5.22 (t, J = 5.0 Hz, 1H, O**H**), 4.64 (d, *J* = 5.1 Hz, 2H, C**H$_2$**OH), 4.60 – 4.56 (m, 1H, C(7)-H), 3.91 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.34 (s, 3H, NMe), 3.04 – 2.99 (m, 1H, C(5)-H), 2.10 – 2.05 (m, 1H, C(5)-H), 2.02 – 1.97 (m, 1H, C(6)-H), 1.93 (s, 3H, C(O)C**H$_3$**), 1.84 – 1.79 (m, 1H, C(6)-H). ^{13}C NMR (101 MHz, dmso-d_6): δ 168.5, 150.1, 149.1, 145.8, 140.7, 137.2, 133.8, 133.7, 130.2, 125.2, 124.3, 121.2, 112.5, 103.6, 100.1, 61.2, 60.7, 60.2, 55.5, 48.3, 37.0, 29.8, 29.7, 22.80. For the experimental details see Supporting Information.

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Keywords: alkaloids • cross-coupling • antitubulin agents • cytotoxicity • rearrangements

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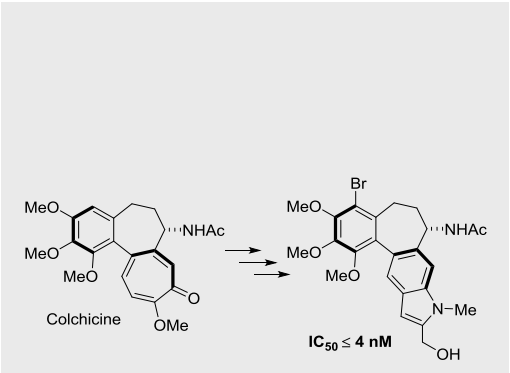

Alkaloids, anti-tubulin agents*

*Ekaterina S. Shchegravina, Dmitry I. Knyazev, Irina P. Beletskaya, Elena V. Svirshchevskaya, Hans-Günther Schmalz, Alexey Yu. Fedorov**

Page No. – Page No.

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