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CONCISE ARTICLE

Synthesis of indole-derived allocolchicine congeners exhibiting pronounced anti-proliferative and apoptosis-inducing properties†

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Nikolay S. Sitnikov,[a,b] Alexander V. Sinzov,[a] Diane Allegro,[c] Pascale Barbier,[c] Sebastien Combes,[c] Liliane Abodo Onambele,[d] Aram Prokop,[d] Hans-Günther Schmalz[b] and Alexey Yu. Fedorov[a]*

Basing on the natural antimitotic agent allocolchicine as a lead structure, a series of novel indole-based allocolchicine congeners was synthesized and assessed *in vitro* for cytostatic properties. Several compounds exhibited potent anti-proliferative and apoptosis-inducing activity towards lymphoma cells along with low unspecific cytotoxicity. The observed activity is supposed to result from the inhibition of microtubule assembly, as indicated by the tubulin polymerisation assay.

Introduction

Colchicine (**1**),[1,2] an alkaloid isolated from plants of the genus *Colchiceae*, *Merendera* and *Gloriosa*, is a long-known natural product exhibiting high levels of cytotoxicity towards proliferating cells. The origin of its biological effect lies in the ability to inhibit polymerisation of tubulin, the main constitutive protein of microtubules.[3-7] This effect leads to the disruption of the mitotic spindle formation, arrest of the cell cycle in G2/M phase and, eventually, to apoptotic cell death. The intriguing biological properties of colchicine as well as its unique structural features[8] became a motivation for a number of total syntheses (resulting in one of the most fascinating endeavours in the history of organic synthesis),[8] as well as several studies concerning structure-activity relationships (SAR) of colchicine structural analogs.[3,9-11] While high systemic toxicity[12,13] (resulting in strong gastrointestinal upset, neuropathy, bone marrow) has prevented its use in the treatment of cancer, colchicine (**1**) became a lead structure in the design of novel tubulin polymerisation inhibitors. Along with several classes of structurally related compounds (e.g. combretastatins[14-17] and 4-arylcoumarins[18]), allocolchicine (**2**)[19] and its analogues[9-11,20-27] were identified as promising candidates for further development. Recently, our group reported on the synthesis and biological evaluation of a series of heterocyclic allocolchicine congeners (for instance **3** and **4**; Figure 1), in which ring C of the parent compound **2** is replaced by an indole[28,29] or a benzofurane[30] pharmacophore. Allo-colchicinoids **3** and **4** showed high levels of proliferation inhibition and apoptosis induction at nanomolar concentrations against different lymphoma cells, although their unspecific cytotoxicity was found to be particularly low.[28] Herein, we report on the synthesis of pyrrolo-allocolchicinoids of type **5**, i.e. the constitutional isomers of **3** and **4**, and present primary results of their biological assessment using a human lymphoma cell line. In addition, the influence of the compounds on tubulin polymerisation was determined *in vitro*.

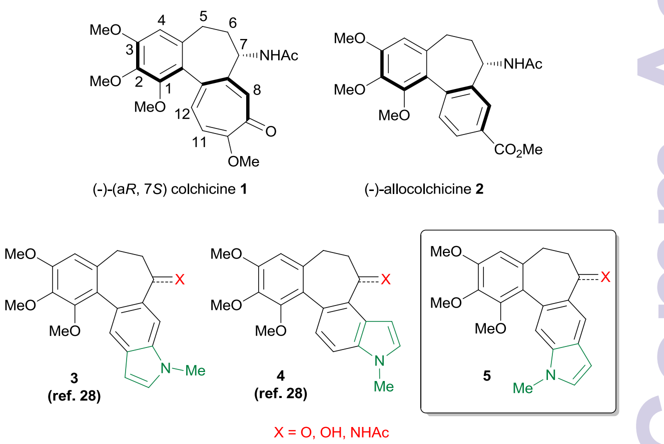


Figure 1 Structures of colchicine (**1**), allocolchicine (**2**), known pyrrolo-allocolchicinoids (**3**, **4**) and their target constitutional isomers of type **5**.

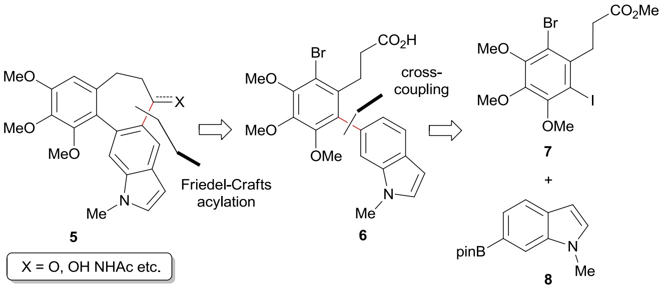
Results and Discussion

Synthesis

Multiple strategies to access the tricyclic fused ring system of allocolchicine (**2**) and its analogues have been developed to date.[31-43] For the construction of the carbocyclic skeleton of **5** we followed the strategy depicted in Scheme 1, which relies on intramolecular Friedel-Crafts acylation and Pd-catalysed cross-coupling as the C-C bond forming steps.

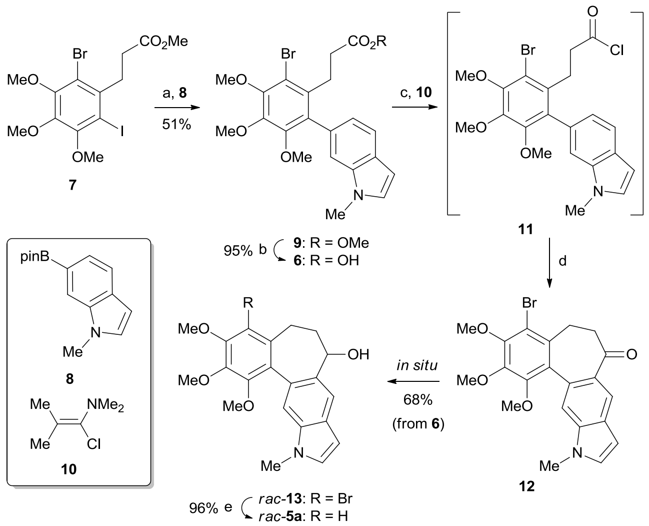
[a.] Department of Organic Chemistry, Lobachevsky State University of Nizhni Novgorod, Gagarina av. 23, Nizhni Novgorod 603950 (Russia); afnn@rambler.ru
[b.] Department für Chemie, Universität zu Köln, Greinstr. 4, 50939, Köln (Germany).
[c.] Laboratory of Integrative Structural and Chemical Biology, Institut Paoli-Calmettes, Aix-Marseille Université, UM105, F-13009, Marseille (France)
[d.] Department of Pediatric Hematology/Oncology, Children's Hospital Köln, Amsterdamer Str. 59, 50735 Köln (Germany).

† Electronic Supplementary Information (ESI) available: [synthetic procedures and characterisation data for all new compounds; details of biological experiments]. See DOI: 10.1039/x0xx00000x



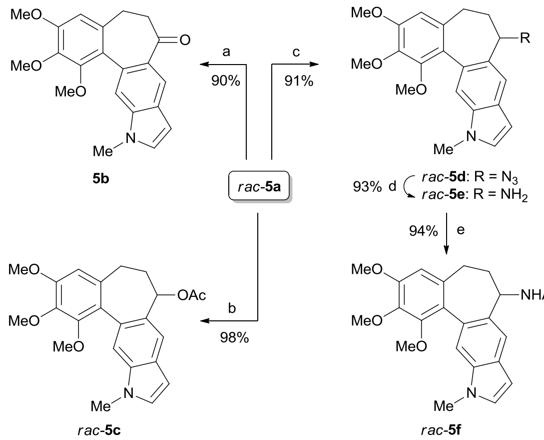
Scheme 1 Retrosynthetic analysis of pyrrolo-allocolchicinoids of type **5**

First, the halogen-selective Suzuki-Miyaura reaction between methyl (iodoaryl)propionate **7**[30] and indolylboronate **8** generated biaryl **9**, which upon basic hydrolysis of the methyl ester yielded acid **6**. Treatment of **6** with (1-chloro-2-methylpropenyl)dimethylamine (**10**) (Ghosez reagent)[44,45] resulted in the formation of acyl chloride **11** which was used *in situ* in the intramolecular Friedel-Crafts acylation. Under the previously reported[30] cyclisation conditions ($ZnCl_2$, 0.02M **11** in DCM) the tetracycle **12** was formed as a single regioisomer, however, in only 17% yield (as a consequence of the acid-catalysed oligomerisation of the starting material). Application of Et_2AlCl or $EtAlCl_2$ as proton-scavenging Lewis acids[46] also gave only low yields of **12** due to competing nucleophilic addition of Al-alkyl reagent to acid chloride **11**. However, treatment of **11** with an excess of bulky diisobutylaluminum chloride resulted in efficient seven membered ring closure and, in addition, an *in situ* reduction of the carbonyl group[47,48] (via β-hydride transfer). This way, the tetracyclic alcohol *rac*-**13** was obtained in 68% yield over three steps in a one-pot procedure.[‡] After the cyclization, the bromine in *rac*-**13** was removed via halogen-lithium exchange/protonation to give *rac*-**5a** in 96% yield (32% overall from **7**).



Scheme 2 Construction of the carbocyclic scaffold of the target allocolchicinoids. *Reagents and conditions*: (a) $Pd(OAc)_2$ (0.05 equiv.), PPh_3 (0.1 equiv.), Cs_2CO_3, toluene, reflux, 24 h; (b) LiOH aq., THF/MeOH, 40 °C, 1 h; (c) CH_2Cl_2, 0 °C, 12 h; (d) $(i\text{-Bu})_2AlCl$ (2 equiv.), CH_2Cl_2, 0 °C to r.t., 30 min; e) *t*-BuLi, THF, -78 °C, 30 min, then MeOH.

Alcohol *rac*-**5a** further served as the substrate for the synthesis of allocolchicinoids with various functionalities at C(7)[§] (scheme 3). Thus, oxidation of *rac*-**5a** with *N*-methylmorpholine-*N*-oxide in the presence of catalytic Pr_4NRuO_4 (Ley oxidation)[49] gave ketone **5b** in 90% yield. Quantitative conversion of *rac*-**5a** to the corresponding acetate *rac*-**5c** was achieved via transesterification with ethyl acetate. The reaction of *rac*-**5a** with $Zn(N_3)_2$×2Py under Mitsunobu conditions[50] resulted in the formation of azide *rac*-**5d** (91% yield), which was subsequently reduced with lithium aluminum hydride to the amine *rac*-**5e** (93% yield). Finally, acylation of *rac*-**5e** with acetic anhydride in pyridine provided acetamide *rac*-**5f** in 94% yield.



Scheme 3 Synthesis of pyrrolo-allocolchicinoids **5a-f** with various functionalities at C(7). Reagents and conditions: (a) *N*-methylmorpholine-*N*-oxide, Pr_4NRuO_4 (0.05 equiv.), molecular sieves 4 Å, CH_2Cl_2/MeCN, r.t., 1 h; (b) EtOLi, EtOAc, 40 °C, 50 torr, 30 min; (c) $Zn(N_3)_2$×2Py, PPh_3, diisopropyl azodicarboxylate, toluene, r.t., 5 h; (d) $LiAlH_4$, THF, r.t., 24 h; (e) Ac_2O, pyridine, CH_2Cl_2, 0 °C, 10 min.

Biological assessment

The cytostatic activity of the target pyrrolo-allocolchicinoids **5a-f** against BJAB (Burkitt-type lymphoma) cells was evaluated using colchicine (**1**) as a standard (Table 1). All compounds exhibited a clear dose-dependent effect on cell proliferation and apoptosis (see ESI for detailed information). As a general tendency, compounds bearing an oxygen-based functionality at C(7) possessed higher cytostatic activity and lower unspecific cytotoxicity as compared to the corresponding analogues with a C(7)-N bond. Acetate *rac*-**5c** was identified as a particularly potent antimitotic agent, as it caused virtually complete inhibition of cell proliferation at low nanomolar concentrations (Figure 2), while no necrosis was detected (in a lactate dehydrogenase (LDH) release assay after 1 h) at concentrations of up to 5 µM. Noteworthy, the novel pyrrolo-allocolchicinoids **5a-f** possess biological activity in the same concentration range as the previously reported isomeric series **3** and **4** (Figure 1). This indicates that the mode of the pyrrole ring fusion to the allocolchicine scaffold does not excert a profound influence on the cytostatic properties.

Table 1 Anti-proliferative and apoptosis-inducing activity of pyrrolo-allocolchicinoids **5a-f** (in comparison with colchicine (**1**)) against BJAB Burkitt-type lymphoma cells

	IC_{50} [a] [μM]	AC_{50} [b] [μM]	Proliferation inhibition at AC_{50} [%]	Necrosis at AC_{50} [%] [c]
colchicine (**1**)	0.02	0.03	n.d.	n.d.
5a (X = **OH**)[d]	0.001	0.001	52	7
5b (X = **O**)	0.01-0.05	0.05	98	4
5c (X = **OAc**)	0.01-0.05	0.05	89	0
5d (X = N_3)	50	>100	30	1
5e (X = NH_2)	0.5-1	5	90	29
5f (X = **NHAc**)	>100	>100	-	-

Each experiment was performed in a triplicate; n.d. = not determined. [a] IC_{50}: concentration of the compound causing 50% cell growth inhibition after 24 h, as determined by CASY cell counting. [b] AC_{50}: concentration of the compound causing 50% cell apoptosis after 72 h, as determined by a DNA-fragmentation assay. [c] Necrosis level caused by the compound at AC_{50} concentration after 1 h, measured by the LDH-release assay. [d] X corresponds to the functional group at C(7) (Fig 1).

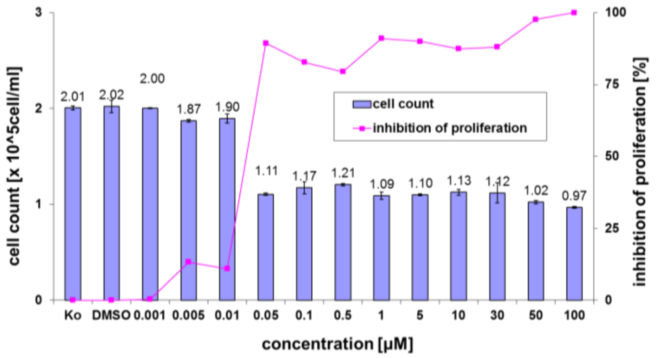


Figure 2 Concentration-dependent inhibition of BJAB lymphoma cell proliferation by **5c** as determined by CASY cell counting after 24 h.

To probe whether the cytostatic activity of the pyrrolo-allocolchicinoids might be a consequence of tubulin binding, acetate *rac*-**5c** as well as *rac*-**3** (X = OH) and *rac*-**4** (X = OH) (as the most active of the previously reported compounds) were tested in a fluorescence-based tubulin polymerisation assay (Figure 3). The depicted turbidimetry curves reflect the effect of all three compounds on the microtubule assembly from purified tubulin. A clear inhibition was noted, as the rate of assembly as well as the final amount of microtubules were clearly lower in the presence of allocolchicinoids than in the control experiment. The extent of inhibition increased steadily with the mole ratio of the total ligand to total tubulin in the solution (R). All three compounds demonstrated sub-stoichiometric mode of action.[51] Half-inhibition of tubulin polymerisation was achieved at a molar ratio (compound/tubulin) of 0.125 for *rac*-**5c**, 0.264 for of *rac*-**3** (X=OH) and 0.228 for *rac*-**4** (X = OH) (the corresponding value for colchicine (**1**) is 0.375,[52] for combretastatin A-4 – 0.09 (own data)). Thus, the high cytostatic activity of pyrrolo-allocolchicinoids **3-5** appears to be a direct consequence of efficient tubulin binding.

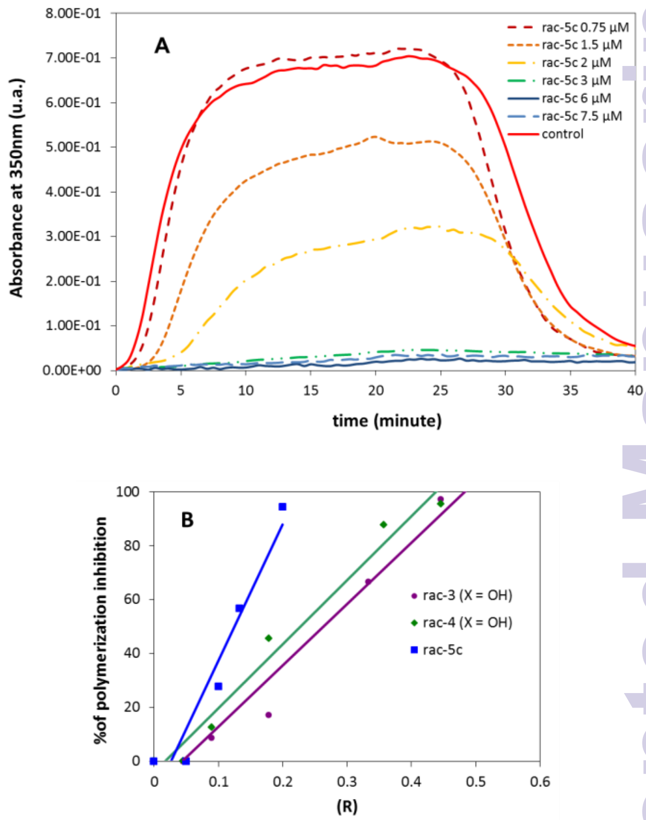


Figure 3 Effect of pyrrolo-allocolchicinoids *rac*-**5c**, *rac*-**3** (X = OH) and *rac*-**4** (X = OH) on the polymerisation of tubulin. A: Turbidimetry curves of tubulin assembly in the presence of different concentrations of *rac*-**5c**. B: Comparative polymerisation inhibition efficiency of *rac*-**5c** and previously reported allocolchicinoids *rac*-**3** (X = OH), *rac*-**4** (X = OH); R = ligand to tubulin molar ratio.

Conclusions

A synthetic route to a new structural type of pyrrolo-allocolchicinoids was developed. The cytostatic properties of target compounds **5a-f** bearing different functional groups were evaluated employing Burkitt-like lymphoma cells (BJAB). Allocolchicinoids **5a-c** exhibited potent anti-proliferative and apoptosis-inducing activity with IC_{50} and AC_{50} values in the low nanomolar concentration range along with low unspecific cytotoxicity (according to LDH-release measurements). The *in vitro* tubulin polymerisation assay revealed that compound **5a** as well as the previously reported structural isomers *rac*-**3** (X = OH) and *rac*-**4** (X = OH) inhibit the assembly of tubulin into microtubules. This indicates that, similarly to colchicine, the anti-proliferative and pro-apoptotic effect of pyrrolo-allocolchicinoids most probably results from the disruption of the mitotic spindle formation and subsequent cell-cycle arrest.

Acknowledgements

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Notes and references

‡ To our best knowledge this is the first example demonstrating the feasibility of the tandem Friedel-Crafts acylation - carbonyl group reduction using (i-Bu)$_2$AlCl as an activator (Lewis acid) and an *in situ* reducing agent.

§ Colchicine numbering (Figure 1) is used throughout the manuscript.

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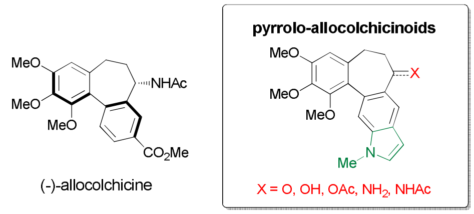

inhibition of tubulin assembly
IC_{50} & AC_{50} in nanomolar concentration range
(Burkitt-like lymphoma cells)

The synthesis and biological assessment of indole-based allocolchicine congeners with potent anti-proliferative and apoptosis-inducing activity towards lymphoma cells is reported.