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Total Synthesis of Indole-Derived Allocolchicine Analogues Exhibiting Strong Apoptosis-Inducing Activity

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Abstract: A series of novel pyrrolo-allocolchicine derivatives (containing a 1-methyl-1*H*-indol-5-yl moiety replacing ring C) was synthesized. The tetracyclic ring system was constructed by Suzuki–Miyaura cross-coupling of a 1-methylindole-5-boronate with an *ortho*-iodo-dihydrocinnamic acid derivative and subsequent intramolecular Friedel–Crafts acylation. After reduction of the resulting ketone, the nitrogen functionality was introduced in a Mitsunobu-type reaction by using zinc azide followed by $LiAlH_4$ reduction. Structural assignments were supported by X-ray crystallography. The compounds synthesized were then tested against BJAB tumor cells and found to exhibit pronounced cytotoxic activity (proliferation inhibition and apoptosis induction). The ketone **24b** was even active at sub-nanomolar concentration. In addition, the antitumor potential of the compounds was confirmed by using B lymphoid cell lines.

Keywords: allocolchicinoids · atropisomerism · cross-coupling · heterocycles · antitumor agents

Introduction

Due to the essential role of the mitotic spindle in cell division, the microtubule dynamic is still regarded as one of the most relevant drug targets for the treatment of cancer.[1] Colchicine (**1**)[2] (Figure 1), isolated from *Colchicum autumnale*, is a cytostatic drug that strongly binds to tubulin, the main constitutive protein of microtubules.[3] Although **1** is frequently employed in the treatment of acute gout and familial Mediterranean fever, a high general toxicity has prevented its use in cancer chemotherapy. Nevertheless, **1** and its structural analogues, such as allocolchicine (**2**),[4] combretastatin A-4 (**3**),[5,6] and 4-arylcoumarins (for example, **4**[7]), represent promising lead structures for the development of new anticancer agents addressing the colchicine-binding site of tubulin.[7,8] The structural similarity of these compounds arises from the presence of two neighboring polyoxygenated aromatic rings, **A** and **C**, arranged in a *syn* non-coplanar fashion within an appropriate distance.[3,7,9]

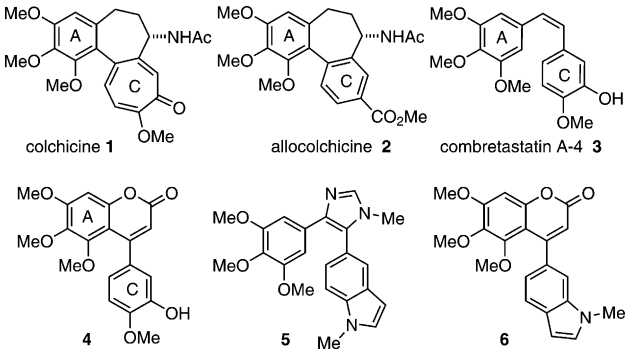

Figure 1. Chemical structure of the natural colchicinoids **1**–**4** and the highly active analogues **5** and **6**, which display an *N*-methylindole substructure.

Although a molecular-modeling study[8a] has suggested certain key structural features as being responsible for strong tubulin binding, the search for novel colchicinoids with improved antitumor properties is still a rather empiric enterprise and relies on the "educated intuition" of synthetic chemists, in combination with biological screening.

Some years ago, it was found that replacement of the 3-hydroxy-4-methoxyphenyl fragment in molecules **3** and **4** by a hydrophobic 1-methyl-1*H*-indol-5-yl group led to highly cytotoxic compounds **5**[10] and **6**,[11] respectively, which were found to inhibit microtubule assembly in vitro at nanomolar

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concentrations. The presence of the hydrophobic *N*-methyl group in **5** and **6** turned out to be crucial for the biological activity, because the corresponding compounds with a free N–H group did not exhibit notable degrees of cytotoxicity.[10,11] It is noteworthy that the geometric parameters of the neoflavonoid **6** (the torsion angle and the distances between the centroids of rings **A** and **C** and the heteroatom substituents) were shown to closely resemble those of **1** and **3**.[11]

The remarkable activity of compounds **5** and **6** suggested that allocolchicine analogues with an *N*-methylindole substructure might also exhibit interesting biological properties. After examining molecular models, we considered **7a** and its isomer **7b** (Figure 2), in which the indole N atom takes a

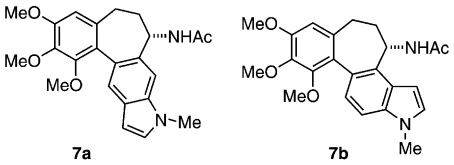
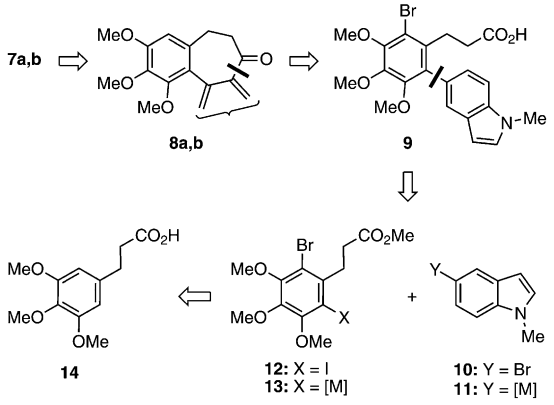


Figure 2. Indole-derived allocolchicinoids **7a** and **7b**, the "designed" target molecules of this study.

para position with respect to the biaryl bond, as particularly promising structures. We report herein the total synthesis of these "designed" natural product analogues. Moreover, we disclose the results of a primary biological study, which revealed compounds *rac*-**7a**, *rac*-**7b**, and some of their synthetic precursors to exhibit powerful cytotoxic and apoptosis-inducing activities against two different tumor cells lines.

Results and Discussion

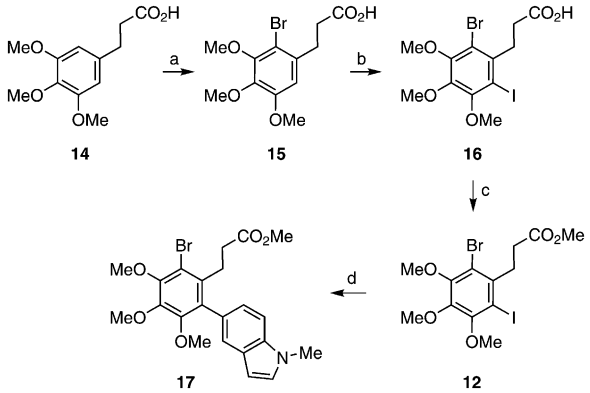
The strategy of our synthesis of **7a** and **7b** is illustrated in Scheme 1. By using ketones **8a** and **8b**, respectively, as pretarget compounds, we envisioned that the formation of the seven-membered B ring, which is the key challenge in any allocolchicine synthesis,[12] could be achieved by intramolecular Friedel–Crafts acylation of precursor **9**, in which the



Scheme 1. Retrosynthetic analysis of the target compounds **7a** and **7b**.

"unsubstituted" position in ring A is protected by a bromine atom. Biaryl derivative **9**, in turn, could be prepared by cross-coupling of suitable precursors, for instance, 5-bromoindole **10** and metalated A-ring synthon **13**. Of course, **9** could alternatively be assembled from building blocks with a reversed polarity, for example, from a metalated indole of type **11** and iodoarene **12**. Compounds **10** and **11** could be prepared from commercial 5-bromoindole, whereas 3,4,5-trimethoxyphenylpropionic acid (**14**) represents a well-accessible starting material for the ring-A building block **12** and metalated derivatives of type **13** (Scheme 1).

We started our investigation with the conversion of **14** into the dihalosubstituted ester **12** through bromination (Br_2 in AcOH), silver-assisted iodination ($AgO(O)CCF_3/I_2$),[13] and subsequent esterification with diazomethane (Scheme 2).



Scheme 2. Synthesis of biaryl compound **17**. Reagents and conditions: a) Br_2, AcOH, 5 °C to RT, 2 h; b) I_2, $AgO(O)CCF_3$, CH_2Cl_2, RT, 6 h; c) CH_2N_2, Et_2O, RT, 72 % (3 steps); d) **19b**, $Pd(OAc)_2$ (3 mol %), Ph_3P (9 mol %.), Cs_2CO_3 (1.5 equiv), toluene, 110 °C, 24 h, 96 %.

For the preparation of biaryl **17**, we then explored different cross-coupling methodologies. Initially, we thought that the electron-rich and sterically-hindered aryliodide **12** might not be a particularly good substrate for oxidative addition.[14] Therefore, we converted **12** into the organozinc reagent **18** (Figure 3) following either the procedure of Dexter and Jackson[15] or the protocol of Knochel and co-workers.[16] Unfortunately, all attempts to achieve the Pd-catalyzed Negishi coupling of **18** with bromoindole **10** (even with the conditions recently proposed by Knochel and co-workers)[17] failed and the desired product (**17**) was not formed to any

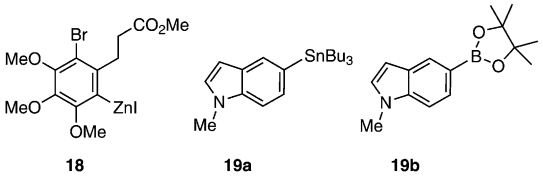
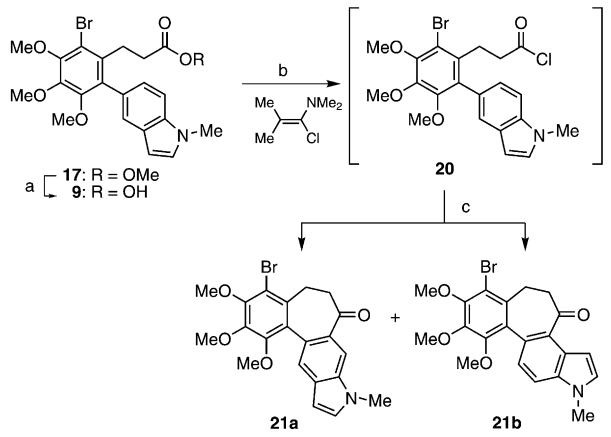


Figure 3. Possible organometallic reagents for the synthesis of biaryl compound **9**.

significant extent. We thus returned to the aryliodide **12** and tried to react it with an appropriate indole-derived metalated reagent under Stille or Suzuki cross-coupling conditions.[14a] Accordingly, the organotin compound **19a** and the boronate **19b** (Figure 3) were prepared from *N*-methyl-5-bromoindole (**10**) by following common protocols. Disappointingly, the Stille coupling of **12** with **19a** could not be achieved under a variety of conditions with different palladium sources ($Pd[PPh_3]_4$, Pd_2(*trans*,*trans*-dibenzylideneacetone)$_3$, $Pd(OAc)_2$), ligands (phosphine ligands, Ph_3As),[18] bases (K_2CO_3, Cs_2CO_3), additives (CuI),[19] and solvents (toluene, MeCN, *N*-methylpyrrolidine). Only a side product resulting from reductive dehalogenation of **12** was isolated, which at least indicated the occurrence of the oxidative addition step. Encouraged by this observation, we finally tried the Suzuki coupling between **12** and 1-methylindole-5-boronic acid pinacol ester (**19b**). We were pleased to find that the desired product **17** was formed in high yield when the reaction was performed with $Pd(OAc)_2$ (3 mol%) and Ph_3P (9 mol%) in the presence of Cs_2CO_3 in toluene at 110°C (Scheme 2). In this reaction, the choice of base turned out to be crucial, because no reaction was observed with K_2CO_3 instead of Cs_2CO_3. By following this protocol, the synthesis of **17** could be easily performed on a multigram scale.

With compound **17** in our hands, the next goal was to close ring B by means of Friedel–Crafts acylation. Although a number of protocols employing esters as substrates in such reactions have been reported,[20] our initial attempts to directly cyclize ester **17** by treatment with a Lewis acid ($AlCl_3$, $BF_3{\cdot}Et_2O$, or $ZnCl_2$) were not successful. Instead of the desired products **21a**/**21b** (Scheme 3), only complex product mixtures were formed, presumably as a consequence of the high reactivity and sensitivity of the electron-rich indole system. The carboxylic acid **9**, obtained from **17** by basic hydrolysis, also failed to give the desired cyclization products **21a**/**21b** upon treatment with either polyphosphoric acid or $Sc(OTf)_3$ as the catalyst (Tf: trifluoromethanesulfonyl).[21]



Scheme 3. Synthesis of intermediates **21a** and **21b**. Reagents and conditions: a) 1M LiOH (aq), THF/MeOH/H_2O, 50°C; b) Ghosez reagent (1.1 equiv), CH_2Cl_2, 0°C, 12 h; c) $ZnCl_2$ (2 equiv), CH_2Cl_2, 0°C to RT, 2 h. THF: tetrahydrofuran.

Moreover, when we tried to convert **9** into the corresponding acid chloride by treatment with oxalyl chloride, rapid decomposition was observed, even at −78°C. However, from the reaction of **9** with (1-chloro-2-methylpropenyl)dimethylamine (Ghosez reagent)[22] in CH_2Cl_2, a clear solution containing acid chloride **20** (as proven by sample aminolysis with $HNEt_2$) was obtained (Scheme 3).

In a first attempt to induce the projected Friedel–Crafts cyclization, we added $ZnCl_2$ to a crude solution of **20** in CH_2Cl_2 at room temperature. However, the desired ketones **21a** and **21b** were obtained in only 5% yield (as a 4:1 mixture), whereas the major products, formed in more than 50% combined yield (in a ratio of 1:3.5), turned out to be compounds **22a** and **22b**, as unambiguously confirmed by means of X-ray crystallography (Figure 4).

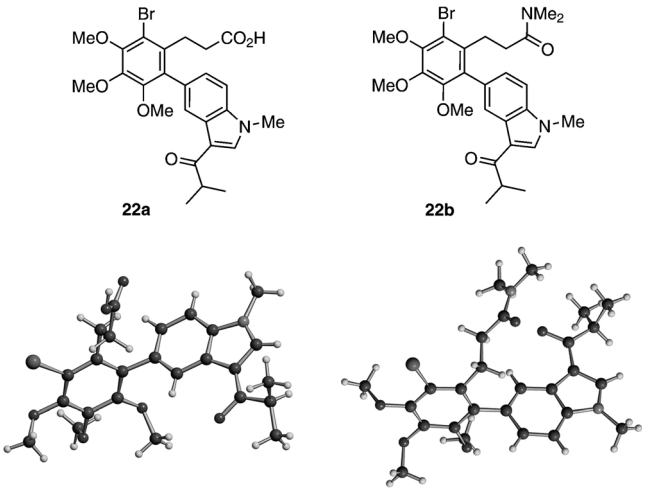
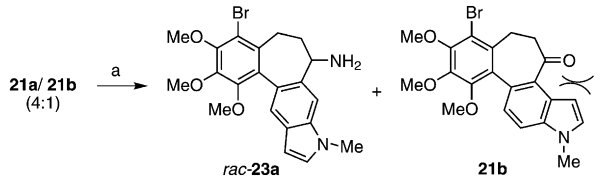


Figure 4. Structures of compounds **22a** and **22b** in the crystalline state.

As compounds **22a** and **22b** probably result from reaction of the indole part of **20** with unreacted Ghosez reagent in a Vilsmeier/Haack type process (and concomitant hydrolysis or aminolysis of the acid chloride function), we reduced the amount of Ghosez reagent in the acid chloride forming step to 1.1 equivalents and extended the reaction time to 12 h. In addition, the resulting solution of **20** was diluted to a concentration of 0.02 molL^{-1} prior to addition of $ZnCl_2$ at 0°C and further stirring for 2 h at room temperature. In this way, ketones **21a** and **21b** were obtained (as a 4:1 mixture) in 40% yield on a gram scale. Other solvents ($MeNO_2$, $PhNO_2$) and Lewis acids ($AlCl_3$, $BF_3{\cdot}Et_2O$, $Sc(OTf)_3$, $Ti(OiPr)_4$, $EtAlCl_2$, Et_2AlCl) proved to be much less efficient. Only $ZnBr_2$ led to a comparable result. As we did not succeed in separating isomers **21a** and **21b** (either by column chromatography or by preparative HPLC), we used the mixture in the subsequent transformations.

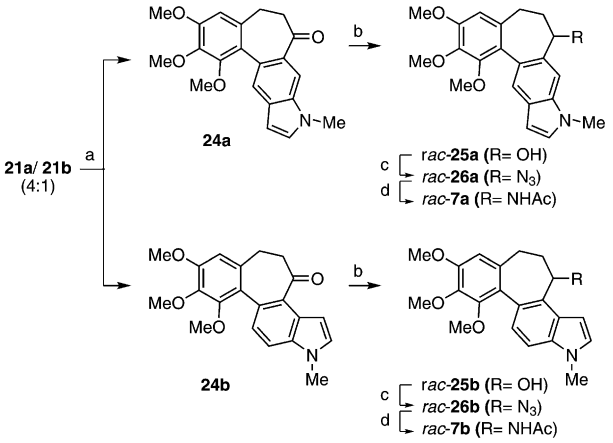
Reductive amination with $NaBH_3CN/NH_4OAc$ in MeOH[23] afforded *rac*-**23a** in 45% yield (56% with respect to **21a**) as the only amine formed under the reaction conditions (Scheme 4). The interesting fact that ketone **21b** did not react to form the corresponding amine (even at elevated



Scheme 4. Synthesis of **23a** by reductive amination. Reagents and conditions: a) $NaBH_3CN$, NH_4OAc, MeOH, 3 Å molecular sieves, 50 °C, 4 days, 56 % (with respect to **21a**).

temperatures) presumably results from the steric shielding of its carbonyl group.

Radical dehalogenation of the 4:1 mixture of **21a**/**21b** by using tributylstannane in the presence of azobisisobutyronitrile (AIBN) afforded a mixture of isomers **24a**/**24b**, which could be separated by column chromatography on SiO_2/KF[24] to yield the pure ketones **24a** (73 %) and **24b** (18 %; Scheme 5).



Scheme 5. Synthesis of various pyrrolo-allocolchicinoids. Reagents and conditions: a) AIBN, $HSnBu_3$, then separation by chromatography; b) $NaBH_4$, THF/MeOH/H_2O, RT, 3 h; c) $Zn(N_3)_2 \cdot 2py$, PPh_3, DIAD, toluene, RT, 0.5 h; d) 1 M $LiAlH_4$ in Et_2O, THF, RT, 24 h; then Ac_2O, pyridine, CH_2Cl_2, RT, 0.5 h. Yields: **24a**: 73 %; **24b**: 18 %; **25a**: 92 %; **25b**: 93 %; **26a**: 89 %; **26b**: 68 %; *rac*-**7a**: 87 %; *rac*-**7b**: 89 %. py: pyridine; DIAD: diisopropylazodicarboxylate.

As the reductive amination proved to be troublesome with this kind of substrate (see above), ketones **24a** and **24b**, respectively, were transformed into the target compounds **7a** and **7b** by using an S_N2 amination approach (Scheme 5).[12b] For this reason, the carbonyl functions in **24a** and **24b** were first reduced with $NaBH_4$ to give the alcohols *rac*-**25a** and *rac*-**25b**, respectively, in almost quantitative yield. Subsequent nucleophilic displacement of the hydroxy groups by azides under Mitsunobu conditions[25] afforded *rac*-**26a** and *rac*-**26b** in 89 % and 68 % yield, respectively. Finally, reduction of the azide group with $LiAlH_4$ in Et_2O and subsequent acetylation of the resulting amino function with acetic anhydride in the presence of pyridine led to the indole-derived allocolchicinoids *rac*-**7a** and *rac*-**7b** in 87 % and 89 % yield, respectively (Scheme 5).

We briefly also explored the possibility of enantioselectively introducing the stereogenic center at the C7 position through asymmetric reduction of ketones **24a** or **24b**. Both the Noyori transfer hydrogenation[26] and the Corey–Bakshi–Shibata (CBS) reduction[27] were reported to give low selectivities with related allocolchicine-type substrates,[12h] so we tested the more promising[12b,f,h] method of Singaram and co-workers, that is, the use of $LiBH_4$ (2.5 equiv) in the presence of 2-(3-nitrophenyl)-1,3,2-dioxaborolane-4*R*,5*R*-dicarboxylic acid ((+)-TarB-NO_2, **27**; 2.0 equiv)[28] as a chiral Lewis acid (Figure 5). However, the reduction of either **21a/21b** or **24a** under these conditions proceeded only with moderate yields (56–59 %) and rather low enantioselectivities (53 % and 37 % *ee*, respectively), as determined by means of HPLC on a chiral stationary phase.

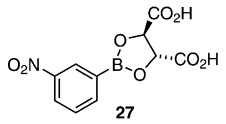


Figure 5. Structure of (+)-TarB-NO_2, which was used for the enantioselective $LiBH_4$ reduction of ketones **21a**/**21b** and **24a**.

The constitution of the various pyrrolo-allocolchicinoids prepared was proven by NMR spectroscopy, and the assignments were additionally confirmed by X-ray crystal structure analysis of ketones **21a** and **24b** (Figure 6).

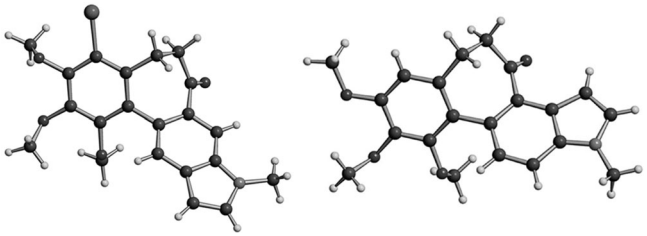


Figure 6. Structures of ketones **21a** (left) and **24b** (right) in the crystalline state.

An interesting fact is that, in contrast to colchicine (**1**),[29] two diastereomers are observed in the ^1H and ^{13}C NMR spectra of compounds *rac*-**25a**, *rac*-**25b**, *rac*-**26a**, *rac*-**26b**, *rac*-**7a**, and *rac*-**7b** under ambient conditions. This can be attributed to a less pronounced energy difference between the atrop-diastereomers although the rotational barrier around the chiral biaryl axis is still high enough to prevent rapid interconversion on the NMR timescale (Figure 7).[12,30] The ratio of the atrop-diastereomers varies strongly with the polarity of the solvent used ($CDCl_3$ or CD_3OD), possibly as a consequence of subtle solvation effects.[31] In the case of *rac*-**7a** and *rac*-**7b**, the NMR spectra are additionally complicated by the presence of *Z*/*E* isomers of the acetamido group.

The relative configuration of the preferred atrop-diastereomer of *rac*-**7b** was found to be (*aR,7S*) by means of an X-ray structure analysis of a crystal obtained from $CDCl_3$ (Figure 8). The dihedral angle along the chiral axis between

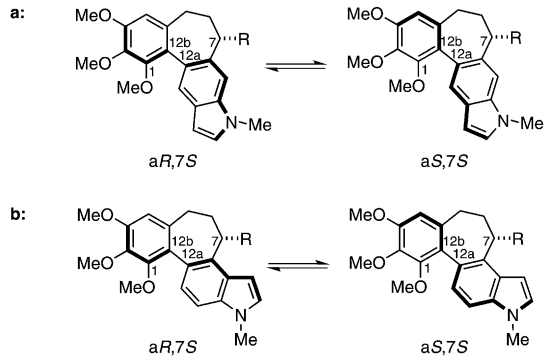


Figure 7. Equilibrium between atrop-diastereomers of pyrrolo-allocolchicinoids (R: OH, N_3, or NHAc).

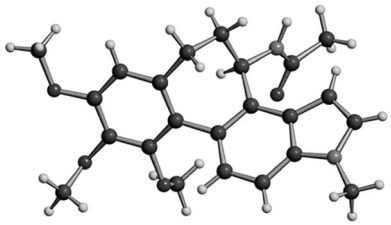


Figure 8. Structure of *rac*-**7b** in the crystalline state.

the benzene and the indole ring (approximately 50°) was found to be very similar to that of natural colchicine (**1**) and combretastatin A-4 (**3**). Thus, the structural requirements for efficient interaction with tubulin should be fulfilled.

The antitumor properties of compounds **24a/b**, *rac*-**25a/b**, *rac*-**26a**, and *rac*-**7a/b** were first investigated by using BJAB tumor cells (Burkitt-like lymphoma cells).[32] The induction of apoptosis (AC_{50}) was determined by using a DNA fragmentation assay, whereas proliferation inhibition (IC_{50}) was determined by cell counting (see Table 1). All compounds were found to exhibit strong antitumor activity at nanomolar or even sub-nanomolar (in the case of **24b**) concentrations and to display a particularly low unspecific cytotoxicity, as determined by lactate dehydrogenase (LDH) release measurements (see the Supporting Information). Compounds of the **b** series (for example, **24b**) displayed a higher cytotoxicity and apoptosis-inducing activity than their **a**-type isomers. Also, the functional group at the C7 position significantly affected the biological properties. Among all of the compounds investigated, ketone **24b** exhibited the most powerful activity by causing 83% inhibition of cell proliferation and 64% apoptosis induction (after 72 h) at 1 nm concentration, whereas virtually no unspecific cytotoxicity was detected after 1 h in a control experiment (LDH assay).

Table 1. Antitumor activity of pyrrolo-allocolchicinoids *rac*-**7a/b** and the synthetic precursors **24a/b**, *rac*-**25a/b**, and *rac*-**26a** on the BJAB tumor cell line.

Compound	IC_{50}[a] [μM]	AC_{50}[b] [μM]
1	0.02	0.03
24a	0.0025	0.005
24b	< 0.001	< 0.001
rac-**25a**	0.03	0.1
rac-**25b**	0.008	0.01
rac-**26a**	0.2	0.5
rac-**7a**	0.08	0.5
rac-**7b**	0.03	0.05

[a] The proliferation inhibition (IC_{50}) after 24 h was determined by using a CASY cell counter. [b] Apoptosis induction (AC_{50}) was measured after 72 h by DNA fragmentation of BJAB cells on a single cell level.

In a second series of biological investigations, the activity of the indole-derived allocolchicinoids against lymphoid cell lines was assessed. Initially, Mec-1 cells were incubated for 48 h with the different compounds at a standard concentration of 50 nm and the metabolic activity of the cells was then determined through a 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) assay (Table 2). For colchicine (**1**, used as a reference) and *rac*-**25b** (the most active sample), flow cytometric analysis (after 48 h, staining with annexin V and 7-aminoactinomycin D (7-ADD)) revealed comparable levels of apoptosis induction (IC_{50} values of 13 nm for **1** and 11 nm for *rac*-**25b**, respectively). In order to detect possible differences in the biological effects on two distinct B lymphoid cell lines, the dose-dependent apoptosis induction assay was repeated with the human B cell lymphoma cell line SU-DHL-5 (Table 2, values given in brackets). The effect of 50 nm of colchicine and *rac*-**25b** on the distribution of cell-cycle phases was examined by analysis of the DNA content of the SU-DHL-5 cells and was found to be equivalent.

Table 2. Cytotoxicity of various pyrrolo-allocolchicinoids on lymphoid Mec-1 cells.

Compound	Metabolic activity [%][a]	IC_{50}[b] [μM]
1	10	0.013 (0.010)
24a	100	–
24b	32	–
rac-**25a**	35	–
rac-**25b**	26	0.011 (0.011)
rac-**26a**	98	–
rac-**7a**	77	–
rac-**7b**	82	–

[a] Mec-1 cells were incubated with 50 nm concentration of the compounds and the metabolic activity (relative to an untreated control) was determined by means of an XTT assay. [b] Apoptosis induction (IC_{50}) was determined by flow cytometric cell-cycle analysis after 48 h (values in brackets refer to SU-DHL-5 cells).

Conclusion

In conclusion, we have designed, synthesized, and biologically assessed a new class of indole-containing allocolchicine analogues. By starting from commercial 3,4,5-trimethoxyphenylpropionic acid (**14**), the target compounds *rac*-**7a** and *rac*-**7b** were obtained in 11 steps with overall yields of 14% and 3%, respectively, by using a Suzuki coupling and a Friedel–Crafts cyclization as the key C–C bond-forming reac-

tions. Structural assignments were proven by X-ray crystallography of various intermediates.[33] Biological tests with BJAB (Burkitt-like lymphoma) and two distinct B lymphoid cell lines revealed that all compounds exhibited cytotoxic and apoptosis-inducing activity. Ketone **24b** and alcohol *rac*-**25b** were identified as the most active compounds, with the former acting against BJAB cells even at sub-nanomolar concentration. The new type of highly active indole-derived antimitotic compounds[34] discovered in the course of this study challenges additional synthetic and biological investigations in the future.

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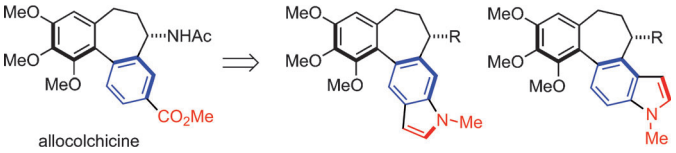
Natural Product Analogues

N. Sitnikov, J. Velder, L. Abodo, N. Cuvelier, J. Neudörfl, A. Prokop, G. Krause, A. Y. Fedorov, H.-G. Schmalz** ..... ▮▮▮▮–▮▮▮▮

Total Synthesis of Indole-Derived Allocolchicine Analogues Exhibiting Strong Apoptosis-Inducing Activity



Induced suicide of tumor cells: Analogues of the natural product allocolchicine containing an *N*-methylindole substructure (see scheme) were synthesized and found to exhibit pronounced cytotoxic and apoptosis-inducing activity against different tumor cell lines at low nanomolar or even sub-nanomolar concentrations.

