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

Nikolay S. Sitnikov,ab Alexander V. Sinzov,a Diane Allegro,c Pascale Barbier,c Sebastien Combes,d Liliane Abodo Onambele,e Aram Prokop,e Hans-Günter Schmalzb and Alexey Yu. Fedorov*a

Based 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 their 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 genera Colchicaceae, 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 constituent protein of microtubules.3–7 This effect leads to the disruption of mitotic spindle formation, arrest of the cell cycle in G2/M phase and, eventually, apoptotic cell death. The intriguing biological properties of colchicine as well as its unique structural features8 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 the structure–activity relationship (SAR) of colchicine structural analogs.3,9–11 While high systemic toxicity12,13 (resulting in strong gastrointestinal upset, neuropathy, and bone marrow suppression) 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. combretastatins14–17 and 4-arylcoumarins18), allocolchicine (2)19 and its analogues9–11,20–27 were identified as promising candidates for further development. Recently, our group reported the synthesis and biological evaluation of a series of heterocyclic allocolchicine congeners (for instance 3 and 4, Fig. 1), in which ring C of the parent compound 2 is replaced with an indole26,29 or a benzofuran10 pharmacophore. Allocolchicinoids 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 the synthesis of pyrrolo.allocolchicinoids of type 5, i.e. the constitutional isomers of 3 and 4, and present the 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.

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

* Department of Organic Chemistry, Lobachevsky State University of Nizhni Novgorod, Gagarina Av. 23, Nizhni Novgorod 603950, Russia. E-mail: afnn@rambler.ru
† 5 Department für Chemie, Universität zu Köln, Greinstr. 4, 50939, Köln, Germany
‡ Aix-Marseille Université, INSERM, CRO2 UMR_S 911, F-13005, Marseille, France
§ Laboratory of Integrative Structural and Chemical Biology, Institut Paoli-Calmettes, Aix-Marseille Université, UM 105, F-13009, Marseille, France
# 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/c5md00320b
Results and discussion

Synthesis

Multiple strategies to access the tricyclic fused ring system of allocolchicine (2) and its analogues have been developed to date.\textsuperscript{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.

First, the halogen-selective Suzuki–Miyaura reaction between methyl (iodoaryl)proponate 7 (ref. 30) and indolyboronate 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)\textsuperscript{44,45} resulted in the formation of acyl chloride with (1-chloro-2-methylpropenyl)dimethylamine (hydrolysis of the methyl ester yielded acid acylation. Under the previously reported\textsuperscript{30} cyclisation conditions (ZnCl\textsubscript{2}, 0.02 M 11 in DCM), 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\textsubscript{2}AlCl or EtAlCl\textsubscript{2} as proton-scaevenging Lewis acids\textsuperscript{46} also gave only low yields of 12 due to competing nucleophilic addition of the 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, \textit{in situ} reduction of the carbonyl group\textsuperscript{47,48} (via β-hydride transfer). This way, tetracyclic alcohol rac-13 was obtained in 68% yield over three steps in a one-pot procedure.‡ After cyclization, the bromine in rac-13 was removed \textit{via} halogen–lithium exchange/protonation to give rac-5a in 96% yield (32% overall from 7) (Scheme 2).

Alcohol rac-5a further served as the substrate for the synthesis of allocolchcinoids with various functionalities at C(7)§ (Scheme 3). Thus, oxidation of rac-5a with N-methylmorpholine-N-oxide in the presence of catalytic Pr\textsubscript{4}NRuO\textsubscript{4} (Ley oxidation)\textsuperscript{49} gave ketone 5b in 90% yield. Quantitative conversion of rac-5a to the corresponding acetate rac-5e was achieved \textit{via} transesterification with ethyl acetate. The reaction of rac-5a with Zn(N\textsubscript{2})\textsubscript{2}·2Py under Mitsunobu conditions\textsuperscript{50} resulted in the formation of azide rac-5d (91% yield) which was subsequently reduced with lithium aluminium hydride to amine rac-5e (93% yield). Finally, acylation of rac-5e with acetic anhydride in pyridine provided acetamide rac-5f in 94% yield.

\[\text{Scheme 1} \quad \text{Retrosynthetic analysis of pyrrolo-allocolchcinoids of type 5.}\]

\[\text{Scheme 2} \quad \text{Construction of the carbocyclic scaffold of the target allocolchcinoids. Reagents and conditions: (a) Pd(OAc)\textsubscript{2} (0.05 equiv.), PPh\textsubscript{3} (0.1 equiv.), Cs\textsubscript{2}CO\textsubscript{3}, toluene, reflux, 24 h; (b) LiOH aq., THF/MeOH, 40 °C, 1 h; (c) CH\textsubscript{2}Cl\textsubscript{2}, 0 °C, 12 h; (d) (i-Bu)\textsubscript{2}AlCl (2 equiv.), CH\textsubscript{2}Cl\textsubscript{2}, 0 °C to r.t., 30 min; (e) t-BuLi, THF, −78 °C, 30 min, then MeOH.}\]

\[\text{Scheme 3} \quad \text{Synthesis of pyrrolo-allocolchcinoids 5a–f with various functionalities at C(7). Reagents and conditions: (a) N-methylmorpholine-N-oxide, Pr\textsubscript{4}NRuO\textsubscript{4} (0.05 equiv.), molecular sieves 4 Å, CH\textsubscript{2}Cl\textsubscript{2}/MeCN, r.t., 1 h; (b) EtOAc, EIOAc, 40 °C, 50 torr, 30 min; (c) Zn(N\textsubscript{2})\textsubscript{2}·2Py, PPh\textsubscript{3}, disopropyl azodicarboxylate, toluene, r.t., 5 h; (d) LiAlH\textsubscript{4}, THF, r.t., 24 h; (e) Ac\textsubscript{2}O, pyridine, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C, 10 min.}\]

‡ To our best knowledge, this is the first example demonstrating the feasibility of the tandem Friedel–Crafts acylation-carbonyl group reduction using (i-Bu)\textsubscript{2}AlCl as an activator (Lewis acid) and an \textit{in situ} reducing agent.

§ Colchicine numbering (Fig. 1) is used throughout the manuscript.
Burkitt-type lymphoma cells determined. 5f (X = NHAc)

Concentration-dependent inhibition of BJAB lymphoma cell proliferation by 5a

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>IC50</th>
<th>AC50</th>
<th>Proliferation inhibition at AC50</th>
<th>Necrosis at AC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine (1)</td>
<td>0.02</td>
<td>0.03</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>5a (X = OH)</td>
<td>0.001</td>
<td>0.001</td>
<td>52</td>
<td>7</td>
</tr>
<tr>
<td>5b (X = O)</td>
<td>0.01–0.05</td>
<td>0.05</td>
<td>98</td>
<td>4</td>
</tr>
<tr>
<td>5c (X = OAc)</td>
<td>0.01–0.05</td>
<td>0.05</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>5d (X = NH3)</td>
<td>50</td>
<td>&gt;100</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>5e (X = NHAc)</td>
<td>0.5–1</td>
<td>5</td>
<td>90</td>
<td>29</td>
</tr>
<tr>
<td>5f (X = NHAc)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Each experiment was performed in triplicate; n.d. = not determined. * IC50: concentration of the compound causing 50% cell growth inhibition after 24 h, as determined by CASY cell counting. 6* AC50: concentration of the compound causing 50% cell apoptosis after 72 h, as determined by a DNA fragmentation assay. 7 Necrosis level caused by the compound at AC50 concentration after 1 h, measured by the LDH release assay. 8 X corresponds to the functional group at C(7) (Fig. 1).

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 the 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 compared to the corresponding analogues with a C(7)–N bond. Acetate rac-5c was identified as a particularly potent antimitic agent, as it caused virtually complete inhibition of cell proliferation at low nanomolar concentrations (Fig. 2), while no necrosis was detected (in a lactate dehydrogenase (LDH) release assay after 1 h) at concentrations of up to 5 µM. It is noteworthy that the novel pyrrolo allocolchicinoids 5a–f possess biological activity in the same concentration range as the previously reported isomeric series 3 and 4 (Fig. 1). This indicates that the mode of pyrrole ring fusion to the allocolchicine scaffold does not induce a profound influence on the cytostatic properties.

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 (Fig. 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 and the final amount of microtubules were clearly lower in the presence of allocolchicinoids than those in the control experiment. The extent of inhibition increased steadily with the molar ratio of the total ligand to the total tubulin in the solution (R). All three compounds demonstrated a 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 rac-3 (X = OH) and 0.228 for rac-4 (X = OH) (the corresponding value for colchicine (1) is 0.375,52 and that for combretastatin A-4 is 0.09 (own data)). Thus, the high cytostatic activity of pyrrolo allocolchicinoids 3–5 appears to be a direct consequence of efficient tubulin binding.
Conclusions

A synthetic route to a new structural type of pyrroloallocolchicinoids was developed. The cytostatic properties of target compounds 5a–f bearing different functional groups were evaluated employing Burkitt-like lymphoma cells (BJAB). Allocolchicinoids 5a–e 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 effects of pyrroloallocolchicinoids most probably result from the disruption of the mitotic spindle formation and subsequent cell cycle arrest.

Acknowledgements

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Notes and references