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Molecular design and synthesis of new heterobivalent compounds based on chlorambucil and colchicine

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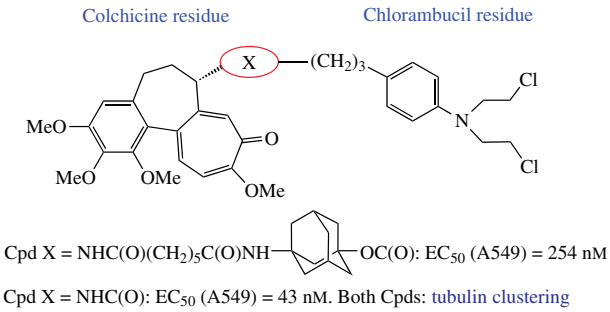
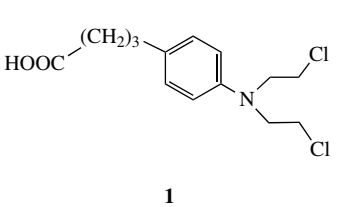
Two new heterobivalent molecules were designed by linking a DNA-alkylating agent chlorambucil with tubulin-targeting compound colchicine or its adamantyl containing C(7)-derivative. Target compounds were synthesized *via* acid coupling with *N*-deacetylcolchicine. According to preliminary biotests, both conjugates caused microtubule depolymerization and strong tubulin clustering, and colchicine–chlorambucil conjugate was found to be one order of magnitude more potent and to exhibit some selectivity to A549 carcinoma cells.



Keywords: heterobivalent compounds, dual target profile, twin drugs, chlorambucil, colchicine, adamantane, tubulin, lung carcinoma A549.

Design of heterobivalent compounds (non-identical twin drugs) is a common strategy used in medicinal chemistry.[1] These compounds typically comprise two pharmacophore moieties which interact either with two different molecular targets or with two sites of the same molecular target in the cells. An ability to interact simultaneously with multiple cellular targets may lead to improvement of efficacy, selectivity, toxicological profile or pharmacokinetic properties of therapeutic agents. Moreover, twin drugs can exhibit an activity profile different from those of the parent molecules. We report herein the molecular design, synthesis and preliminary biotesting of new heterobivalent compounds based on well-known antimitotic agents chlorambucil (**1**) and colchicine (**2a**).

Molecules **1** and **2a** interact with different molecular targets: chlorambucil alkylates guanosine residue in DNA[2] and colchicine binds with α,β-dimeric protein tubulin and inhibits its polymerization to microtubules (MTs).[3,4] Both compounds demonstrate low selectivity to cancer cells and are consequently rather toxic. However, certain analogues of **1** and **2a** with improved toxicological profile have been reported. They comprise, for example, twin- or prodrugs of chlorambucil[5,6] and C(7)-substituted colchicine derivatives such as tubuloclustin (**2b**) or **2c** and related compounds (see ref. 7 and references therein). Action of tubuloclustin on cancer cells also differs as compared to colchicine by an ability to stimulate the formation of long tubulin clusters after MTs disassembly. Recent studies revealed that clustering could be promoted by C(7) colchicine derivatives with aromatic moieties.[8–10] This led to an idea to synthesize a heterobivalent conjugate **4** (Scheme 1) and to check if it can induce tubulin clustering and thus be interesting for toxicological studies.



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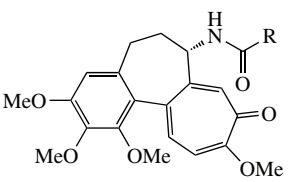


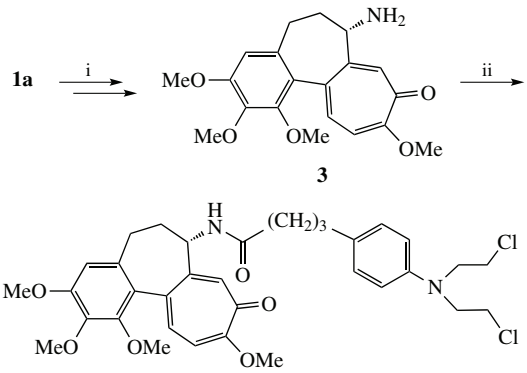
2a–c

a R = Me

b, **c** R = (CH2)5 C(O) OAd

Ad = 2-adamantyl (**b**)

Ad = 1-adamantyl (**c**)



Scheme 1 *Reagents and conditions*: i, (1) Boc_2O, NEt_3, DMAP, MeCN, reflux, 8 h; (2) MeONa, MeOH, room temperature, 2 h; (3) CF_3COOH, CH_2Cl_2, room temperature, 1 h, then NaOH, H_2O; ii, **1**, EEDQ, CH_2Cl_2, room temperature, 24 h.

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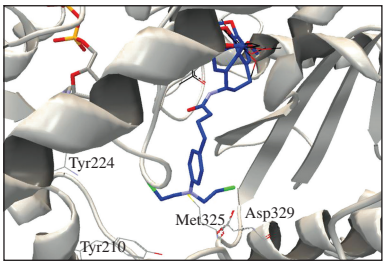

Figure 1 Complex of compound **4** (in blue) in colchicine binding site in tubulin dimer (PDB ID 4O2B) with highest docking score as calculated by AutoDock Vina 1.1.2 program[12,13] (α-subunit is shown on the left, β-subunit – on the right, hydrogen atoms are omitted for clarity).

Molecular docking of compound **4** into the tubulin model (PDB ID 4O2B)[11] demonstrated that colchicine residue of **4** fits well in the colchicine binding site. Bis(2-chloroethyl)amino-phenyl moiety of **4** is located at the interface of α- and β-subunits close to amino acid residues αTyr210, βMet325 and βAsp329 (Figure 1). Although orientation of C(7)-substituent in **4** differs from that in tubuloclustin **2b** (adamantane moiety of the latter is located close to αTyr224[7]), the difference is not significant given the conformational flexibility of C(7)-substituents in both molecules.

Following our attempts to enhance tubulin clustering ability of colchicine derivatives,[8,14] we have also designed the structure **8** (Scheme 2) by linking tubuloclustin analogue with a chlorambucil residue to enable additional interactions with α- and β-subunits of tubulin.

Compound **4** was obtained in four steps from colchicine according to Scheme 1. *N*-Deacetylcolchicine **3** synthesized as described[15] was coupled with chlorambucil **1** in the presence of EEDQ to produce target heterobivalent conjugate **4** (for the detailed procedure description and novel compound characteristics, see Online Supplementary Materials).

Scheme 2 describes synthesis of compound **8**. Steglich esterification of 3-(*N*-*tert*-butoxycarbonylamino)adamantanol **5** with chlorambucil **1** provided ester **6a**, which was then deprotected under standard conditions to produce amine **6b**. Further coupling with pimelic acid polyanhydride yielded amide intermediate **7**. Finally, acid coupling with *N*-deacetylcolchicine **3** gave target compound **8** in a total yield of 21%.

Primary biological evaluation performed on human lung carcinoma cells A549 using procedures described in refs. 16–19 revealed that at a concentration of 2 μM both conjugates **4** and **8** induced full MTs depolymerization and a formation of strong tubulin clusters (Figure 2). The effect was the same as for compounds **2b** and **2c**, and no further enhancement of clustering intensity (expected for compound **8**) was detected at 10 μM.

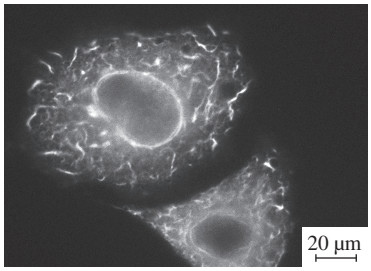

Figure 2 Tubulin clusters in A549 cells treated with 2 μM of **8** (analogous results are produced with compound **4**).

Cytotoxicity of conjugate **8** was noticeable: EC_{50} = 254 nM (MTT), IC_{50} = 300 nM (cell growth inhibition), but inferior compared to colchicine (EC_{50} = 30 nM) or bridged tubuloclustin analogue **2c** (EC_{50} = 11 nM). Contrary to **8**, heterobivalent compound **4** was highly cytotoxic (EC_{50} = 43 nM). Moreover, in the primary screening assay carried out as described in refs. 20, 21 at concentrations of 0.015, 0.6 and 3 μM it has demonstrated some selectivity to A549 cancer cells compared to non-cancer line lung fibroblasts VA13 (viability of VA13 cells after 72 h treatment was 4.6, 3.1 and 6.5 times higher than that for A549). Reasonable activity and selectivity profiles of new heterobivalent compound **4** and its tubulin clustering effect (unusual for the parent molecules **1** and **2**) make it interesting to perform additional studies of the mechanism of antimitotic action of **4**, *e.g.* its ability to inhibit DNA synthesis. This work is now in progress and will be published in due course.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.11.005.

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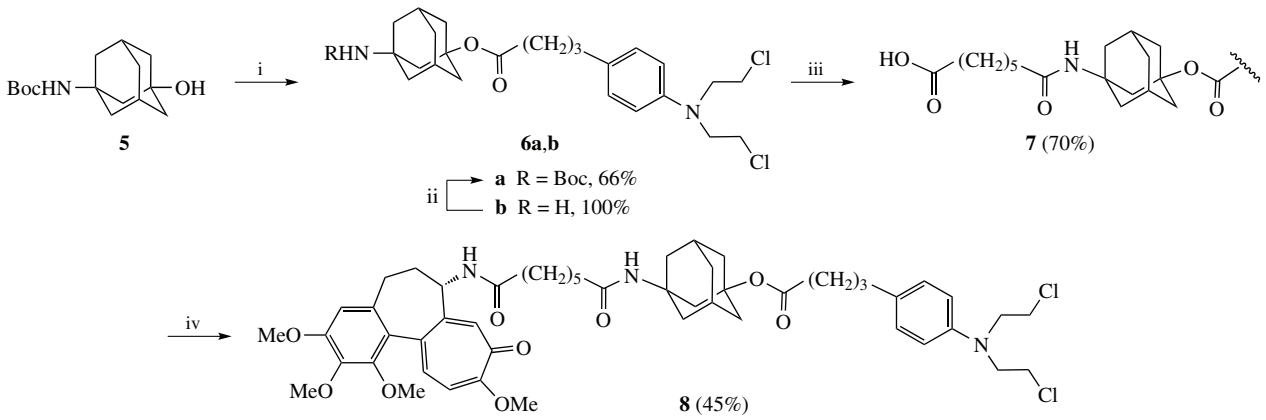

Scheme 2 *Reagents and conditions*: i, **1**, DCC, DMAP, CH_2Cl_2, room temperature, 66%; ii, TFA, CH_2Cl_2, room temperature, 24 h; iii, pimelic acid polyanhydride, DMAP, CH_2Cl_2, room temperature, 24 h; iv, **3**, EEDQ, CH_2Cl_2, room temperature, 24 h.

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