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Bicyclic isothioureas for conjugation with tubulin targeted anticancer agents

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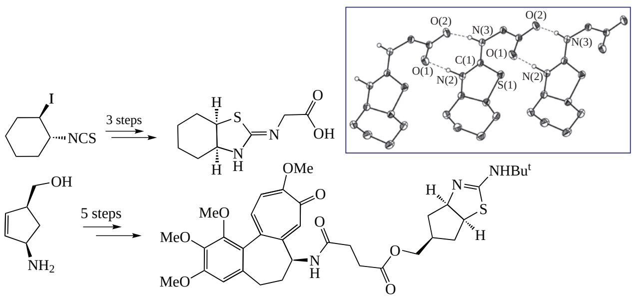
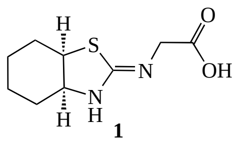
Two bicyclic annulated isothiourea derivatives were synthesized using as a key stage either the reaction of isothiocyanate halide with sodium sulfide or cyclization of unsaturated thiourea in the presence of bromine. X-ray molecular structure of *N*-[(3a*SR*,7a*RS*,*Z*)-hexahydro-1,3-benzothiazol-2(3*H*)-ylidene]glycine was determined. The conjugate of colchicine with [(3a*R*,5*S*,6a*S*)-2-(*tert*-butylamino)-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]thiazol-5-yl]methanol obtained demonstrated pronounced cytotoxic effect on cancer cells.



Keywords: non-aromatic heterocycles, annulated bicyclic isothiourea, thioureas, cyclization, zwitterions, tubulin, podophyllotoxin, colchicine.

In the course of drug design, it is often necessary to introduce a bulky, lipophilic and non-flat group into the structure of a lead molecule in order to provide interaction with hydrophobic site of the target protein. The presence of such group, however, leads to the essential decrease in the water solubility. To compensate this, heterocyclic fragments capable of protonation (at least partially) under physiological conditions can be inserted into the lipophilic moiety. Among non-aromatic heterocycles, piperidine, morpholine, pyrrolidone or piperazine are often used for this purpose. Our recent analysis of the literature showed that it was also convenient to use cyclic isothiourea residues possessing good balance lipophilicity–water solubility and other drug-like properties, that have allowed some substances with such groups to reach the clinical trials.[1]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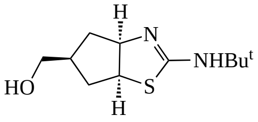
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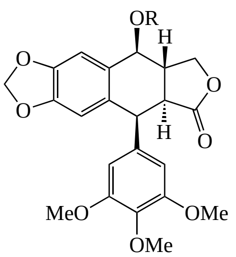
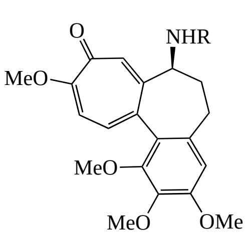
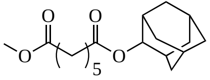

3a,**b**


4a,**b**

a R = H Podophyllotoxin
b R = $C(O)CH_2NH_2$

a R = Ac Colchicine
b R =

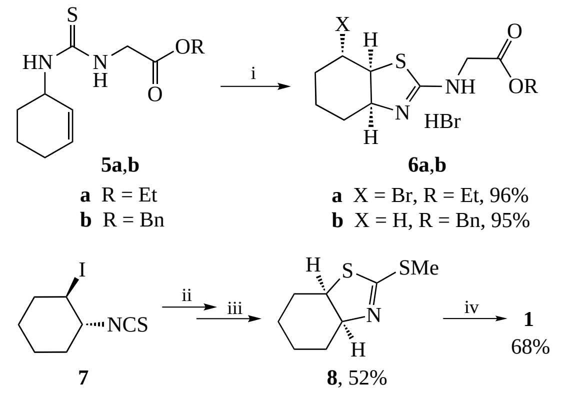

Tubuloclustin

In the present work we synthesized two cyclic isothioureas **1** and **2** for the purpose of their further conjugation with tubulin-targeted anticancer agents podophyllotoxin **3a** and colchicine **4a**. Interest in the synthesis of these conjugates was due to the previously obtained data on the selectivity of certain C^4-podophyllotoxin and C^7-colchicine derivatives with bulky lipophilic residues to tumor cells[2–6] and on unusual effect of some conjugates on the microtubule network of cancer cells.[7–15] In addition, for a series of analogues of tubuloclustin **4b**, which are derivatives of colchicine with voluminous non-flat lipophilic groups at C^7, a correlation of unusual (clustering) effect on microtubules with activity and an improvement in toxicological profile *in vivo* (ref. 12 and citations therein) compared to the natural molecule were shown. Note that so far numerous attempts to solve the problem of high toxicity of colchicine[16,17] have met the limited success.[18–22]

Since the molecule of podophyllotoxin contains an alcohol group at C^4, we obtained acid **1** for the subsequent preparation of the corresponding ester. For the synthesis of tubuloclustin-like C^7-colchicine derivative, we have chosen the structure **2** with a branched lipophilic *tert*-butyl substituent and an alcohol group, which should allow one to attach a linker of a certain length to the parent molecule.

The pathways for the synthesis of target compound **1** are depicted in Scheme 1. The attempts to obtain it *via* cyclization of cyclohex-3-en-1-ylthioureas **5a**,**b** by the action of bromine or HBr[23] were not successful: although the cyclization products **6a**,**b** were accessed (Scheme 1, stage i), further removal of the protective groups could not be accomplished (for details, see Online Supplementary Materials).

Another pathway to obtain isothiourea **1** (see Scheme 1, stages ii, iii) was fulfilled using modified method.[23] It included the cyclization of isothiocyanate halide **7** (synthesized from cyclohexene) with sodium sulfide and led to desired bicyclic



Scheme 1 *Reagents and conditions*: i, Br_2, CH_2Cl_2, 22 °C, 8 h (for **6a**) or AcBr, MeOH, CH_2Cl_2, 22 °C, 12 h (for **6b**); ii, $Na_2S\cdot 9H_2O$, acetone, H_2O, room temperature, 45 min; iii, MeI, acetone, reflux, 3 h, then K_2CO_3; iv, glycine, MeOH, H_2O, reflux, 5 h. Relative configuration is indicated, since the compounds were obtained as racemic mixtures.

template with a thiocarbonyl group. Further methylation and displacement of the thiomethyl group in intermediate **8** by glycine gave the desired 2-aminoacetic acid *N*-substituted heterocycle **1**. Its structure as tautomer **1** was unambiguously proved by X-ray analysis (the compound crystallizes as hydrate with 0.33 water molecules, Figure 1).[†]

Bond length distribution indicates that thiourea **1** crystallizes in the zwitterionic form with pronounced delocalization in cationic $(NH)_2CS$ and anionic CO_2 fragments. According to optimization of **1** with the account of non-specific solvation (SCRF, solvent is water) the zwitterionic form is more stable than the neutral form according to PBE1PBE/def-2-TZVP level of theory, but the gain due to one-fold increase of dipole moment of zwitterionic form (19.14 D) in comparison to neutral one (1.77 D) is only 1.8 kcal mol^{-1}. Therefore, the decrease in medium polarity may be sufficient to shift the equilibrium in the crystal towards the neutral form. In addition to non-specific solvation, the zwitterionic form can, of course, be stabilized due to non-covalent interaction. Indeed, expected in crystal **1** strong N–H···OC hydrogen bonds are formed with the N···O separation equal to 2.684(1) and 2.772(1) Å. These N–H···O hydrogen bonds assemble molecules into the chains directed along axis *a* (Figure 2).

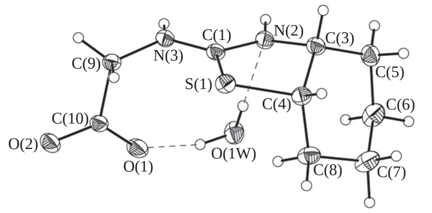


Figure 1 General view of *N*-[(3a*RS*,7a*SR*,*Z*)-hexahydro-1,3-benzothiazol-2(3*H*)-ylidene]glycine **1** in representation of atoms by thermal ellipsoids (p = 50%). The water molecule has occupancy 0.33. The principal bond lengths (Å): S(1)–C(1) 1.7499(8), C(1)–N(2) 1.323(1), C(1)–N(3) 1.318(1), C(10)–O(1) 1.254(1), C(10)–O(2) 1.251(1) and bond angles (°): N(2)C(1)N(3) 122.70(7), N(3)C(1)S(1) 123.90(6), N(2)C(1)S(1) 113.30(6).

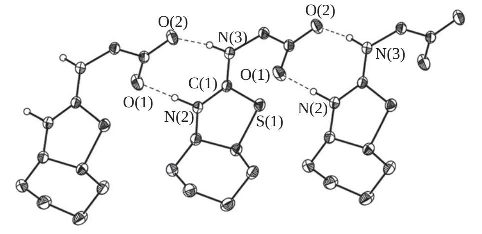
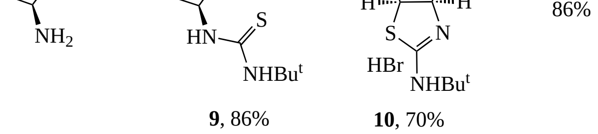


Figure 2 The N–H···O bonded chains in crystal **1** (the partly occupied position of water molecule is omitted for clarity).

Compound **1** was introduced in the esterification with podophyllotoxin, however, the target C^4 ester was not isolated. Probably, the zwitterionic nature of acid **1** is the reason for its low reactivity under standard esterification conditions. Note that the use of the reverse synthetic strategy also turned out to be unsuccessful: the reaction of podophyllotoxin glycine ester **3b** (obtained from the parent molecule in two steps as described[2]) with compound **8** did not afford the target ester (see Online Supplementary Materials for details).

The synthesis of colchicine conjugate with isothiourea **2** was more successful. The target isothiourea was synthesized in three step procedure (Scheme 2). First, [(1*S*,4*R*)-4-aminocyclopent-2-en-1-yl]methanol was treated with *tert*-butyl isothiocyanate to give the corresponding thiourea **9**. Its cyclization by reacting with HBr did not take place, but in the presence of bromine it proceeded smoothly and led to the substituted bicyclic isothiourea **10** as individual stereoisomer with *cis*-ring junction. The relative configuration of the ring junction hydrogen atoms and C^6H–Br atom was assigned in accordance with our earlier studies.[23,24] Reductive debromination of compound **10** provided target compound **2**. In the ^1H NMR spectrum of **2**, two resonances at 4.09 and 4.61 ppm correspond to the protons at the ring junction of the fused system, while the signals at 64.5 and 67.9 ppm in ^{13}C NMR spectrum refer to the appropriate carbon atoms. The peak of C^2 atom of hexahydrothiazole part is observed at 166.6 ppm. Spectral data of all cyclic isothioureas obtained indicate the presence of one tautomer but does not allow assigning it to the specific form.

Next, we obtained the target colchicine conjugate with isothiourea **2**. Succinic acid linker was chosen to ensure that the overall length of the C^7-substituent was approximately equal to that in tubuloclustin **4b**. Therefore, alcohol **2** was reacted with succinic anhydride and converted to monoester **11** (Scheme 3). The reaction proceeded chemoselectively, and the possible bis-ester was not formed. Finally, acid coupling of compound **11** with *N*-deacetylcolchicine (obtained in three steps as described[25,26]) in the presence of EEDQ gave the target conjugate

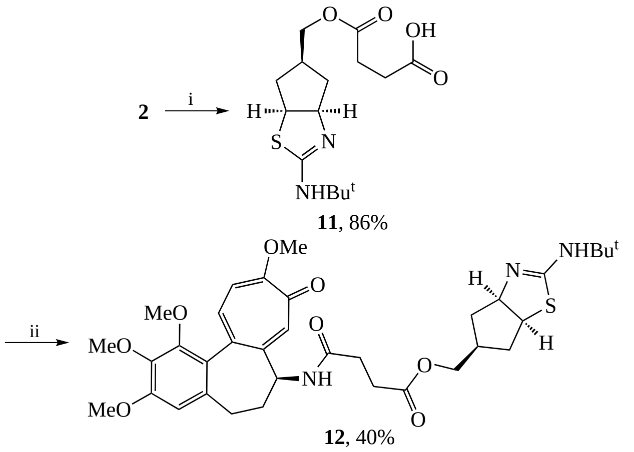


9, 86% **10**, 70%

Scheme 2 *Reagents and conditions*: i, Bu^tNCS, DIPEA, CH_2Cl_2, room temperature, 12 h; ii, Br_2, CH_2Cl_2, room temperature, 12 h; iii, Bu_3SnH, PhMe, AIBN, 100 °C, 5 h.

[†] *Crystal data for* **1** (from MeOH). $C_9H_{14}N_2O_2S\cdot{}^1/_3H_2O$ (M = 220.23), orthorhombic, space group $Pca2_1$, at 106 K, a = 10.48090(1), b = 11.1095(1) and c = 9.3370(1) Å, V = 1087.177(18) Å^3, Z = 4, (Z' = 1), d_{calc} = 1.345 g cm^{-3}. Total of 136041 reflections were collected (12634 independent reflections, θ_{max} = 52.5°, R_{int} = 0.0728). The refinement converged to R_1 = 0.0381 (for 10426 observed reflections), GOF = 1.031. The X-ray diffraction analysis was carried out on a Bruker D8 QUEST diffractometer (graphite monochromated MoKα radiation, ω-scanning). Collection, editing and refinement of unit cell parameters, as well as accounting for absorption were carried out using APEXII and SAINT PLUS programs. All calculations were carried using SHELXTL PLUS software. The structure was solved by dual methods and refined by least squares methods in the anisotropic approximation for non-hydrogen atoms.

CCDC 2168373 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.



Scheme 3 *Reagents and conditions*: i, succinic anhydride, DMAP, CH_2Cl_2, room temperature, 8 h; ii, *N*-deacetylcolchicine, EEDQ, CH_2Cl_2, room temperature, 24 h.

12 with the total yield of 21% [from ((1*S*,4*R*)-4-aminocyclopent-2-en-1-yl)methanol]. Mass spectrometry ($[M]^+ = 668$, $[M+H]^+ = 669$) and NMR spectral data confirm the structure of target compound. The estimation of solubility of conjugate **12** compared to tubuloclustin **4b** indicates that the calculated value for the latter is almost independent on pH and lies in the range of 0.6–0.7 μM, while for compound **12** it, as expected, depends on pH of the solution (for details see Online Supplementary Materials). At physiological pH 7.4, the predicted solubility value for compound **12** is 80 μM and is therefore three orders higher than that of tubuloclustin.

Preliminary bioscreening of the effect of conjugate **12** on cell viability performed using described[27,28] procedure revealed that at a concentration of 10 μM it demonstrated pronounced cytotoxic effect on human lung carcinoma cells A549. This makes it interesting to further test the obtained conjugate, especially to study its action on microtubules of cancer cells.

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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.2022.11.019.

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