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Synthesis of Bifunctional Furano-Allocolchicinoids

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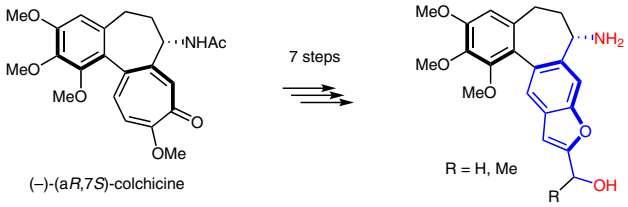

(–)-(a*R*,7*S*)-colchicine

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Abstract An efficient seven-step semisynthetic approach towards non-racemic bifunctional furano-allocolchicinoids, starting from naturally occurring colchicine is presented. The Pd-catalyzed domino Sonogashira coupling/5-*endo-dig* cyclization was employed as the key step. The prepared compounds exhibited substantial cytotoxicity against T3M4, MiaPaCa-2, Colo-357, and PANC-1 cell lines. The presence of two functionalities with different reactivity (hydroxyl and amino groups) in the target molecules allows for an easy conjugation of furano-allocolchicinoids with drug delivery carriers, and opens promising opportunities for their further exploitation in the search of therapeutics.

Key words antitumor agents, tubulin, colchicine, allocolchicinoids, heterocycles

The key role of the mitotic spindle in cell division has made it a promising target for cancer chemotherapy.[1] Colchicine (**1**), an alkaloid found in the plants of the genus *Colchicum*, *Merendera*, *Androcymbium*, *Gloriosa*, and *Littonia*,[2] effectively inhibits microtubule polymerization via binding to tubulin, one of the main constituent elements of spindle microtubules.[3] For a long time, colchicine was considered a perspective antitumor agent. However, its significant general toxicity in therapeutic doses prevents its usage in cancer therapy.[4] Apart from functioning as a 'mitotic poison', colchicine also inhibits neutrophile motility and activity, leading to a net anti-inflammatory effect.[5] This unique set of biological properties of colchicine can explain its efficacy in clinical treatment of familial Mediterranean fever, Behcet's disease, acute gout, chondrocalcinosis, and other types of microcrystalline arthritis.[6] Recently, colchicine was suggested as a drug for the treatment of cardiovascular diseases such as acute pericarditis, atrial fibrillation caused by inflammation, and ischemic diseases.[7] Potentially, colchicinoids may be effective in the case of autoimmune and neurodegenerative diseases, as well as for the treatment of chronic infections.[8]

Among the compounds, structurally related to colchicine,[9] allocolchicine (**2**)[10] and its analogues proved to be suitable candidates for further elaboration due to their promising biological activities.[11] Recently, our group described the synthesis and biological evaluation of a series of heterocyclic allocolchicine congeners – pyrrolo-allocolchicines **3**–**5**,[12] with the different orientation of the pyrrole fragment and the corresponding furano-allocolchicines **6** and **7**.[13,14] Derivatives **3**–**7**, containing a hydroxyl group in the benzylic or pseudobenzylic position demonstrated high antimitotic and apoptosis-inducing activity in nano- and subnanomolar concentrations, and promising in vivo activity with less pronounced toxic effects in comparison with parent colchicine (**1**) (Figure 1).

One way to reduce the general toxicity of a drug and to improve its biodistribution upon the systemic administration is the design of carrier-linked drug delivery systems.[15] Conjugation of therapeutic agents with a wide spectrum of macromolecules[16] including antibodies, polysaccharides, lectins, serum proteins, peptides, growth factors, and synthetic polymers, as well as their incorporation into micelles or liposomes[17] can seriously extend the therapeutic potential of the drug. Indeed, when colchicinoid **6** was linked to chitosan, the resulting conjugate demonstrated significantly better tumor growth inhibition than the intact molecule **6**, possibly, as a result of better accumulation in the tumor.[18] On the other hand, the linkage of colchicinoid with a target carrier via a very liable ester bond placed in the pseudobenzylic (in the case of **6**) or in the benzylic (compound **7**) positions of the molecule led to instability of the obtained conjugates during the storage and administration. Herein, we report the synthesis of furano-allocolchicinoids **8**, con-

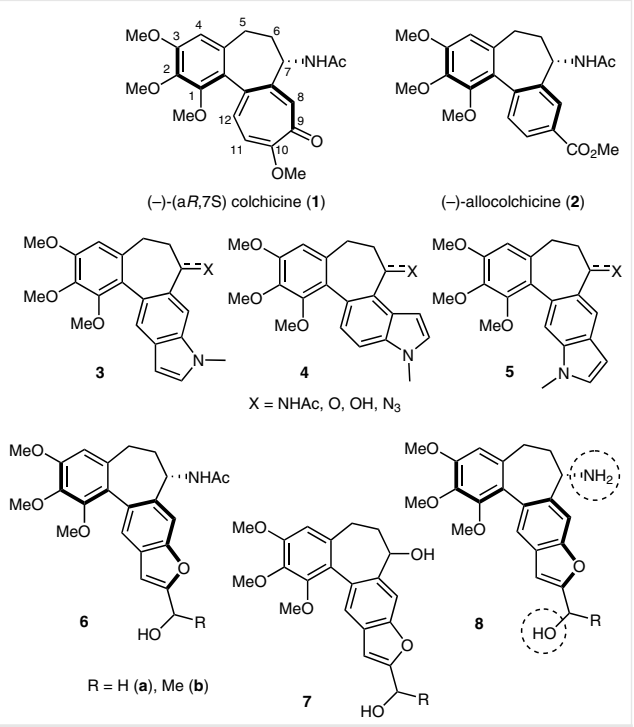


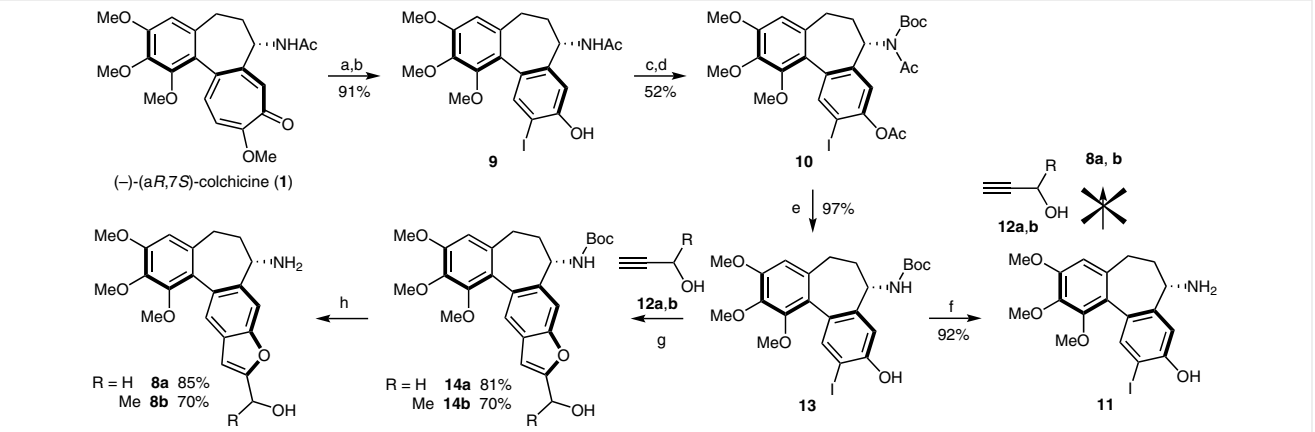
Figure 1 Structures of colchicine (**1**), allocolchicine (**2**), pyrrolo-allocolchicinoids **3–5**, furano-allocolchicinoids **6**,**7**, and the target molecules **8**

taining simultaneously two functionalities, that is, amino and hydroxyl groups, which can simplify the conjugation and improve the stability of the linker between the therapeutic molecule and the targeted vector or carrier.

Colchicine (**1**) underwent the two-step oxidative ring contraction according to modified Windaus procedure[11c] to afford iodocolchinol **9** in 91% yield (Scheme 1). Then, the phenolic hydroxyl group and the NH fragment of the amide in **9** were protected with acetic anhydride and Boc_2O, respectively, to afford colchicinoid **10** in 52% yield over two steps. Simultaneous deacylation of two acetate groups in compound **10** by NaOMe, followed by Boc-deprotection under acidic conditions gave deacetyliodocolchinol **11** in good yield. Unfortunately, we have not succeeded in using the intermediate **11** for the preparation of target furano-allocolchicinoids **8**, as its reaction with propargylic alcohols **12a**,**b** under Sonogashira reaction conditions[13,14] yielded a complex and inseparable mixture of products. However, when Boc-protected iodoallocolchinol **13**, prepared in one step from diacetate **10** was subjected to the cascade Sonogashira cross-coupling/Larock-type cyclization sequence with corresponding alkynes **12**, furans **14a** and **14b** were obtained in 81 and 70% yield, respectively. The choice of alkynes was based on our previous studies,[13] which indicated that the presence of CH_2OH or CH(Me)OH groups in the side chain of the heterocyclic ring leads to higher in vitro antitumor activity. Finally, the cleavage of the Boc-function was achieved by treatment of **14** with aqueous 3 M HCl in CH_2Cl_2 in the presence of a scavenger to give the target products **8** in good yields (Scheme 1).

The in vitro cytotoxicity of the synthesized compounds toward Colo-357, PANC-1, MiaPaCa-2, and T3M4 cell lines was investigated. A tetrazolium-based assay was used to determine the drug concentration required to inhibit cell growth by 50% after incubation in the culture medium for 72 hours. The calculated IC_{50} values are summarized in Table 1. Compound **8a** inhibited cell proliferation at low nanomolar concentrations, while its analogue **8b** demonstrated a modest cytotoxic effect.

In conclusion, we have reported a short synthetic route to a new type of bifunctional furano-allocolchicinoids containing two different reacting groups. The synthesized compounds retain their antimitotic properties and can be



Scheme 1 Synthesis of bifunctional furano-allocolchicinoids **8**. *Reagents and conditions*: (a) 0.1 M HCl, AcOH, 100 °C, 3 h; (b) NaOH, I_2, KI, H_2O, 0–5 °C, 2 h; (c) Ac_2O, TBAH, NaOH, 1,4-dioxane, r.t., 1 h; (d) Boc_2O, DMAP, Et_3N, MeCN, reflux, 24 h; (e) NaOMe (20 mol%), MeOH, r.t., 1.5 h; (f) HCl, EtOH, r.t., 20 h; (g) $Pd(OAc)_2$ (5 mol%), CuI (10 mol%), Ph_3P (15 mol%), KOAc (3 equiv), MeCN, 70 °C, 12 h; (h) aq 3 M HCl in CH_2Cl_2, 1,3,5-trimethoxybenzene, r.t., 1 h.

Table 1 In Vitro Cytotoxic Activity (IC_{50}, nM)[a] of Furano-Allocolchicinoids **8a**,**b** (vs **7a**,**b**)[14]

Compound	8a	8b	7a	7b
Colo-357	160	800	16	64
PANC-1	160	800	16	30
MiaPaCa-2	32	–[b]	16	64
T3M4	32	–	–	–

[a] IC_{50} (nM) concentration inducing 50% inhibition of cell growth.
[b] Not determined.

conjugated with various substrates via the amine group on the B-ring to modify their physicochemical and cytotoxicity profiles. This opens new promising options for therapeutic intervention in cancer chemotherapy. Further work in this direction is currently underway.

Commercially available reagents were used without additional purification. Column chromatography was performed using Macherey-Nagel Kieselgel 60 (70–230 mesh). All ^{1}H and ^{13}C NMR spectra were recorded at r.t. in DMSO-d_6 or CD_3OD on Bruker Avance DRX 500 or Agilent DD2 400 instruments. Chemical shifts (δ) are reported in parts per million (ppm) from TMS using the residual solvent resonance (DMSO-d_6: 2.50 ppm for ^{1}H NMR, 39.52 ppm for ^{13}C NMR; CD_3OD: 3.31 ppm for ^{1}H NMR). Standard abbreviations for multiplicities are used. EI mass spectra (70 eV) were obtained on a DSQ II mass spectrometer (Thermo Electron Corporation) with a quadrupole mass analyzer. Combustion analysis was performed using an Elementar (Vario Micro Cube) apparatus. High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher LTQ Orbitrap XL – FTMS Analyzer (HR-ESI-MS). Solvents were purified according to the standard procedures. Petroleum ether (PE) used had bp 40–70 °C. Iodocolchinol **9** was prepared according to the previously proposed procedure.[11c]

(5*S*)-5-[*N*-(*tert*-Butoxycarbonyl)acetamido]-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-5-yl] Acetate (10)

To a well-stirred mixture of iodocolchinol **9**[11c] (0.5 g, 1.035 mmol), 1,4-dioxane (3.0 mL), TBAH (0.014 g, 0.041 mmol), and powdered NaOH (0.104 g, 2.588 mmol) was added dropwise a solution of Ac_2O (0.117 mL, 1.241 mmol) in 1,4-dioxane (3.0 mL) at r.t. The resulting mixture was stirred for 1 h under an inert atmosphere, then filtered, washed with 1,4-dioxane, and evaporated. The residue was dissolved in CH_2Cl_2, washed with H_2O, and dried (Na_2SO_4). The solvent was removed under reduced pressure. The pure *N*-[(5*S*)-2-iodo-9,10,11-trimethoxy-3-(acetyloxy)-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-5-yl]acetamide was obtained as yellowish crystals, and was used directly in the following step; yield: 0.480 g (88%); mp 158 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.38 (d, *J* = 8.3 Hz, 1 H), 7.75 (s, 1 H), 7.12 (s, 1 H), 6.81 (s, 1 H), 4.52–4.44 (m, 1 H), 3.84 (s, 3 H), 3.79 (s, 3 H), 3.56 (s, 3 H), 2.58–2.52 (m, 1 H), 2.36 (s, 3 H), 2.25–1.95 (m, 3 H), 1.86 (s, 3 H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 168.36, 168.29, 152.90, 150.08, 149.91, 142.55, 140.46, 139.38, 134.79, 133.44, 122.13, 117.93, 108.19, 88.04, 60.67, 60.45, 55.82, 47.88, 38.19, 29.76, 22.54, 20.91.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{22}H_{24}INO_6$ + H: 526.0721; found: 526.0723; *m*/*z* [M + Na]$^+$ calcd for $C_{22}H_{24}INO_6$ + Na: 548.0541; found: 548.0539.

To a stirred mixture of *N*-[(5*S*)-2-iodo-9,10,11-trimethoxy-3-(acetyloxy)-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-5-yl]acetamide (0.48 g, 0.914 mmol) and DMAP (0.112 g, 0.914 mmol) in MeCN (5.3 mL) were added a half of the solution of Boc_2O (0.916 g, 4.203 mmol) in MeCN (5.3 mL) and Et_3N (0.255 mL) under an inert atmosphere. The resulting solution was stirred at 100 °C for 1 h. Then, the second half of the solution of Boc_2O was added, the mixture was stirred for 2 h at 90 °C, and overnight at r.t. After completion of the reaction (TLC monitoring), sat. aq solution of citric acid (50 mL) was added, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and the crude product obtained after solvent removal was purified by column chromatography (eluent: PE–EtOAc–EtOH, 6:1:1) to afford compound **10** as a reddish foam; yield: 0.340 g (59%); mp 88 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.76 (s, 1 H), 7.11 (s, 1 H), 6.82 (s, 1 H), 5.19–5.13 (m, 1 H), 3.84 (s, 3 H), 3.78 (s, 3 H), 3.49 (s, 3 H), 2.70–2.61 (m, 2 H), 2.34 (s, 3 H), 2.30 (s, 3 H), 2.21–2.13 (m, 1 H), 2.11–2.02 (m, 1 H), 1.46 (s, 9 H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 168.34, 153.05, 153.03, 150.35, 149.76, 140.57, 140.10, 139.45, 134.79, 133.55, 122.19, 119.69, 108.11, 88.55, 83.89, 60.74, 60.57, 60.57, 55.83, 34.79, 29.97, 27.37, 21.00.

MS (EI, 70 eV): *m*/*z* (%) = 625 (12), 583 (32), 483 (66), 424 (100), 409 (22), 381 (10), 297 (22), 282 (16), 238 (12), 139 (12).

Anal. Calcd for $C_{27}H_{32}INO_8$: C, 51.85; H, 5.16. Found: C, 51.62; H, 5.31.

tert-Butyl [(5*S*)-3-Hydroxy-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl)carbamate (13)

MeOH (8.0 mL) and a 2 M solution of freshly prepared NaOMe in MeOH (0.066 mL) were added to a flask containing compound **10** (0.395 g, 0.632 mmol) under an inert atmosphere. The resulting solution was stirred for 1.5 h at r.t. After evaporating the solvent, the product was partitioned between CH_2Cl_2 and H_2O (1:1, 70 mL), the organic layer was washed with brine, and dried (Na_2SO_4). After removal of all volatiles, the pure product **13** was obtained as yellow crystals and was used directly in the next step; yield: 0.333 g (97%); mp 123 °C.

^{1}H NMR (DMSO-d_6, 500 MHz): δ = 10.26 (s, 1 H), 7.56 (s, 1 H), 7.41 (d, *J* = 8.3 Hz, 1 H), 6.91 (s, 1 H), 6.74 (s, 1 H), 4.12 (dt, *J* = 11.5, 7.7 Hz, 1 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 3.52 (s, 3 H), 2.46 (dd, *J* = 12.5, 5.6 Hz, 1 H), 2.15–2.07 (m, 1 H), 2.07–2.00 (m, 1 H), 1.91 (dd, *J* = 16.9, 10.9 Hz, 1 H), 1.34 (s, 9 H).

^{13}C NMR (DMSO-d_6, 126 MHz): δ = 155.54, 154.57, 152.16, 150.04, 142.82, 140.48, 139.05, 134.72, 126.45, 123.12, 110.03, 108.03, 81.32, 77.70, 60.48, 60.44, 55.78, 49.85, 37.88, 29.94, 28.16.

HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{23}H_{28}INO_6$ + Na: 564.0854; found: 564.0853.

(5*S*)-6,7-Dihydro-3-hydroxy-2-iodo-9,10,11-trimethoxy-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl-amine (11)

Compound **13** (0.2 g, 0.369 mmol) was placed in a 50 mL flask and dissolved in a minimum amount of EtOH. Concd HCl (10 mL) was added to the solution, and the reaction mixture was stirred at r.t. for 1 h. Then, an additional amount of concd HCl (5 mL) was added, and the temperature was raised to 40 °C. After completion of the reaction (TLC monitoring), the solution was neutralized with aq NaOH. Vacu-

um evaporation of the volatiles gave a dark-yellow oily residue that was extracted with CH_2Cl_2, washed with brine, and dried (Na_2SO_4). After removal of the solvent, compound **11** was obtained as white crystals; yield: 0.150 g (92%); mp 155 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.57 (s, 1 H), 7.16 (s, 1 H), 6.75 (s, 1 H), 3.82 (s, 3 H), 3.75 (s, 3 H), 3.57 (dd, *J* = 11.3, 7.0 Hz, 1 H), 3.51 (s, 3 H), 2.30–2.25 (m, 2 H), 2.06 (td, *J* = 12.9, 7.4 Hz, 1 H), 1.69 (td, *J* = 11.8, 7.8 Hz, 1 H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 155.62, 152.15, 150.06, 140.42, 139.15, 135.10, 126.62, 123.09, 110.51, 107.96, 81.38, 60.52, 60.49, 55.82, 50.06, 40.45, 30.22.

MS (EI, 70 eV): *m*/*z* (%) = 441 (100), 424 (100), 409 (100), 393 (74), 366 (42), 314 (52), 297 (41), 282 (40), 267 (34), 254 (25), 212 (12), 207 (25), 181 (22).

Anal. Calcd for $C_{18}H_{20}INO_4$: C, 48.99; H, 4.57. Found: C, 50.26; H, 4.69.

Compounds 14a,b; General Procedure

Compound **13** (1 equiv), $Pd(OAc)_2$ (5 mol%), CuI (10 mol%), PPh_3 (15 mol%), and KOAc (3 equiv) were placed in a two-necked flask under an inert atmosphere. Anhyd MeCN (16.2 mL per 1 mmol of **13**) and alkyne **12** (1 equiv) were added. The mixture was stirred at 60 °C for 1 h, then the temperature was raised to 80 °C, and stirring was continued until completion of the reaction (TLC monitoring). The solvent was removed under reduced pressure and the solid residue was purified by column chromatography (eluent: PE–EtOAc–EtOH) to obtain pure **14**.

tert-Butyl *N*-[(1*S*)-2″-(1-Hydroxymethyl)-1′,2′,3′-trimethoxy-6,7-dihydro-1*H*-benzo[5′,6′:5,4]cyclohepta[3,2-*f*]benzofuran-1-yl]carbamate (14a)

Compound **14a** was prepared according to the general procedure from **13** (0.035 g, 0.065 mmol) and propargyl alcohol (**12a**; 0.004 mL, 0.065 mmol). Column chromatography with PE–EtOAc–EtOH (5:1:1) as eluent gave product **14a** as yellow crystals; yield: 0.025 g (81%); mp 121 °C.

^{1}H NMR (DMSO-d_6, 500 MHz): δ (mixture of conformers) = 7.54 (d, *J* = 8.5 Hz, 1 H), 7.50 (s, 1 H), 7.45 (s, 1 H), 6.78 (s, 1 H), 6.75 (s, 1 H), 5.39 (t, *J* = 5.8 Hz, 1 H), 4.57 (d, *J* = 5.8 Hz, 2 H), 4.34–4.26 (m, 1 H), 3.84 (s, 3 H), 3.80 (s, 3 H), 3.46 (s, 3 H), 2.49–2.45 (m, 1 H), 2.16 (m, 1 H), 2.02–1.95 (m, 1 H), 1.89 (dd, *J* = 19.0, 11.9 Hz, 1 H), 1.35 (s, 9 H).

^{13}C NMR (DMSO-d_6, 126 MHz): δ (mixture of conformers) = 158.33, 154.70, 153.67, 152.18, 150.24, 140.56, 137.79, 134.69, 131.42, 131.34, 128.69, 128.60, 128.53, 126.10, 124.61, 121.70, 107.96, 105.34, 103.14, 77.74, 60.47, 60.43, 56.17, 55.79, 50.22, 38.23, 29.98, 28.16.

HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{26}H_{31}NO_7$ + Na: 492.1993; found: 492.1990.

tert-Butyl *N*-[(1*S*)-2″-(1-Hydroxyethyl)-1′,2′,3′-trimethoxy-6,7-dihydro-1*H*-benzo[5′,6′:5,4]cyclohepta[3,2-*f*]benzofuran-1-yl]carbamate (14b)

Compound **14b** was prepared according to the general procedure from **13** (0.2 g, 0.369 mmol) and but-3-yn-2-ol (**12b**; 0.029 mL, 0.369 mmol). Column chromatography with PE–EtOAc–EtOH (5:1:1) as eluent gave **14b** as yellowish crystals; yield: 0.125 g (70%); mp 145 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ (mixture of diastereomers) = 7.58 (d, *J* = 8.2 Hz, 1 H), 7.49 (s, 1 H), 7.44 (s, 1 H), 6.78 (s, 1 H), 6.70 (s, 1 H), 5.49 (s, 1 H), 4.85 (dd, *J* = 11.8, 6.2 Hz, 1 H), 4.30 (dd, *J* = 18.3, 9.4 Hz, 1 H), 3.84 (s, 3 H), 3.79 (s, 3 H), 3.45 (s, 3 H), 2.22–1.80 (m, 4 H), 1.48 (d, *J* = 6.2 Hz, 3 H), 1.35 (s, 9 H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ (mixture of diastereomers) = 161.97, 161.90, 154.76, 153.45, 152.24, 150.29, 140.58, 137.69, 134.78, 128.53, 126.14, 124.68, 121.76, 107.97, 105.43, 101.26, 101.22, 77.81, 62.27, 60.56, 56.56, 55.82, 50.23, 38.32, 30.06, 28.22, 22.02.

MS (EI, 70 eV): *m*/*z* (%) = 483 (58), 427 (50), 383 (27), 382 (92), 366 (55), 354 (46), 338 (64), 335 (56), 308 (43), 293 (48), 292 (42), 265 (40), 249 (34), 205 (34), 193 (26), 165 (34), 152 (34), 139 (20), 127 (12), 115 (10), 57 (100).

Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.06; H, 6.88. Found: C, 67.29; H, 6.52.

Compounds 8a,b; General Procedure

Compound **14** (1 equiv) and 1,3,5-trimethoxybenzene (2 equiv) were dissolved in aq 3 M HCl in CH_2Cl_2 (24 mL per 1 mmol of **14**). The mixture was stirred at r.t. for 1 h. After completion of the reaction, solid NaOH was added till pH 8 and the product was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the solid residue was purified by column chromatography (eluent: PE–EtOAc–EtOH) to afford compound **8**.

(1*S*)-[1′,2′,3′-Trimethoxybenzo[5′,6′:5,4]-1*H*-1-amino-6,7-dihydrocyclohepta[3,2-*f*]-2″-(1-hydroxymethyl)]benzofuran (8a)

Compound **8a** was prepared according to the general procedure from **14a** (0.055 g, 0.117 mmol). Column chromatography with PE–EtOAc–EtOH (2:1:1) as eluent gave **8a** as white crystals; yield: 0.037 g (85%); mp 265 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.79 (s, 1 H), 7.59 (s, 1 H), 7.04 (s, 1 H), 6.81 (s, 1 H), 5.01 (s, 2 H), 3.84 (s, 3 H), 3.82–3.80 (m, 1 H), 3.78 (s, 3 H), 3.49 (s, 3 H), 2.40 (m, 2 H), 2.02 (td, *J* = 12.4, 7.4 Hz, 2 H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 153.66, 153.58, 152.78, 150.39, 140.79, 134.04, 132.75, 129.20, 126.59, 123.61, 123.18, 108.24, 106.76, 106.26, 60.70, 60.57, 55.91, 50.68, 37.94, 36.84, 29.31.

MS (EI, 70 eV): *m*/*z* (%) = 369 (56), 252 (46), 323 (100), 322 (58), 307 (53), 293 (88), 291 (54), 262 (50), 248 (39), 235 (36), 209 (42), 207 (63), 205 (46), 183 (38), 176 (30), 155 (24), 152 (36), 135 (34), 115 (26), 83 (24).

Anal. Calcd for $C_{21}H_{23}NO_5$: C, 68.28; H, 6.28. Found: C, 68.02; H, 6.01.

(1*S*)-[1′,2′,3′-Trimethoxybenzo[5′,6′:5,4]-1*H*-1-amino-6,7-dihydrocyclohepta[3,2-*f*]-2″-(1-hydroxyethyl)]benzofuran (8b)

Compound **8b** was prepared according to the general procedure from **14b** (0.25 g, 0.517 mmol). Column chromatography with the eluent PE–EtOAc–EtOH (2:1:1) gave product **8b** as yellowish crystals; yield: 0.139 g (70%); mp 252 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ (mixture of diastereomers) = 7.77 (s, 1 H), 7.53 (s, 1 H), 6.84 (s, 1 H), 6.79 (s, 1 H), 4.65 (q, *J* = 6.6 Hz, 1 H), 3.84 (s, 3 H), 3.78 (s, 3 H), 3.75–3.71 (m, 1 H), 3.47 (s, 3 H), 2.37 (td, *J* = 12.0, 5.9 Hz, 1 H), 1.91 (m, 3 H), 1.50 (d, *J* = 6.5 Hz, 3 H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ (mixture of diastereomers) = 158.51, 158.47, 153.53, 152.28, 150.25, 150.24, 140.54, 134.89, 128.81, 125.83, 124.43, 122.02, 107.95, 106.28, 103.64, 103.62, 70.02, 63.43, 60.51, 55.83, 50.66, 30.12, 19.81.

MS (EI, 70 eV): *m*/*z* (%) = 383 (84), 366 (70), 351 (33), 339 (48), 338 (100), 323 (50), 292 (50), 264 (50), 236 (47), 235 (50), 220 (58), 208 (62), 193 (58), 181 (44), 165 (52), 154 (44), 152 (38), 129 (21), 115 (26), 105 (20), 85 (18), 83 (12).

Anal. Calcd for $C_{22}H_{25}NO_5$: C, 68.91; H, 6.57. Found: C, 69.22; H, 6.84.

Cell Cultures

Pancreatic cells (T3M4, PANC-1, Colo-357, MiaPaCa-2) were grown in DMEM medium supplemented with 10% fetal calf serum (FCS), penstrep-glut (all from PanEco, Moscow, Russian Federation). All cell lines used were routinely tested for mycoplasma. Adherent cells were detached using 0.05% trypsin-EDTA (PanEco, Moscow), counted and sub-cultured. Twenty-four hours before assays, cells were seeded in the appropriate plates (96- or 24-well plates), adjusted to 3 × 105 cells/mL, and incubated overnight to achieve standardized growth conditions.

MTT-Assay

Cytotoxic effect of the furano-allocolchicinoids **8a**,**b** was estimated by a standard 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Sigma) test as described earlier.[19] All the compounds were dissolved in DMSO to 20 mM concentration and stored at –20 °C until the assay. Different dilutions of the new compounds from 20 µM to 0.1 nM were prepared separately and transferred in 100 µL to the plates with the cells. Non-treated cells served as controls. Plates were incubated for 72 h. For the last 6 h, 5 mg/mL of MTT were added in the amount of 10 µL to each well. After the incubation, culture medium was removed and 100 µL of DMSO was added to each well. Plates were incubated by shaking for 15 min to dissolve the formed formazan product. Optical density was read on spectrophotometer Titertek (UK) at 540 nm. Results were analyzed by Excel package (Microsoft). Cytotoxic concentration giving 50% of the maximal toxic effect (IC_{50}) was calculated from the titration curves. The inhibition of proliferation (inhibition index, II) was calculated as [1 – ($OD_{experiment}$/$OD_{control}$)], where OD was MTT optical density.

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Supporting Information

Supporting information for this article is available online at https://doi.org /10.1055/s-0036-1589060.

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