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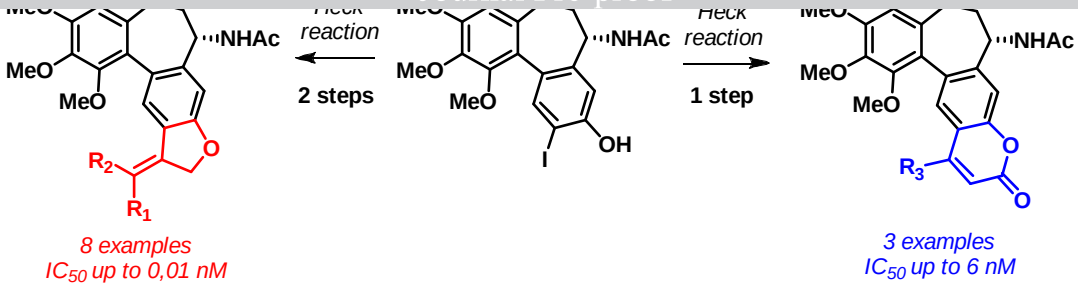

8 examples
IC_{50} up to 0,01 nM

3 examples
IC_{50} up to 6 nM

Discovery of dihydrofuranoallocolchicinoids - highly potent antimitotic agents with low acute toxicity

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Abstract: Two series of heterocyclic colchicinoids bearing β-methylenedihydrofuran or *2H*-pyran-2-one fragments were synthesized by the intramolecular Heck reaction. Methylenedihydrofuran compounds **9a** and **9h** were found to be the most cytotoxic among currently known colchicinoids, exhibiting outstanding antiproliferative activity on tumor cell lines in picomolar (0.01-2.1 nM) range of concentrations. Compound **9a** potently and substoichiometrically inhibits microtubule formation *in vitro*, being an order of magnitude more active in this assay than colchicine. Derivatives **9a** and **9h** revealed relatively low acute toxicity in mice (LD_{50} ≥10 mg/kg i.v.). The X-Ray structure of colchicinoid **9a** bound to tubulin confirmed interaction of this compound with the colchicine binding site of tubulin.

Keywords: colchicine, tubulin, antiproliferative activity, antimitotics, X-ray structure

Introduction

Development of multidrug resistance (MDR) [1], and severe side effects of anticancer agents are among the most significant drawbacks of modern anticancer therapy. . Emergence of MDR is mostly associated with the drug efflux mediated by ABC-protein family [2,3]. Alterations of *β3*-tubulin isotype is one of the causes of MDR development [4]. Several modern antimitotic agents such as taxanes and *Vinca* alkaloids are subject of MDR-mediating efflux

mechanisms [5–8]. Numerous studies in this area have revealed evidences that colchicine-binding site ligands are able to overcome MDR mediated by ABCB1-protein (*P-gp*) [9–12]. Recently, colchicine has been approved by FDA for the treatment of patients with acute gout. It has also been found promising in the therapy of amyloidosis [13], Behcet's disease [14], familial Mediterranean fever [15] and some other autoimmune and inflammatory diseases [16–19]. However, unpredictable severe side effects occurring in some patients and significant systemic toxicity prevent its application as a chemotherapeutic agent, where a higher dosage is required [20,21].

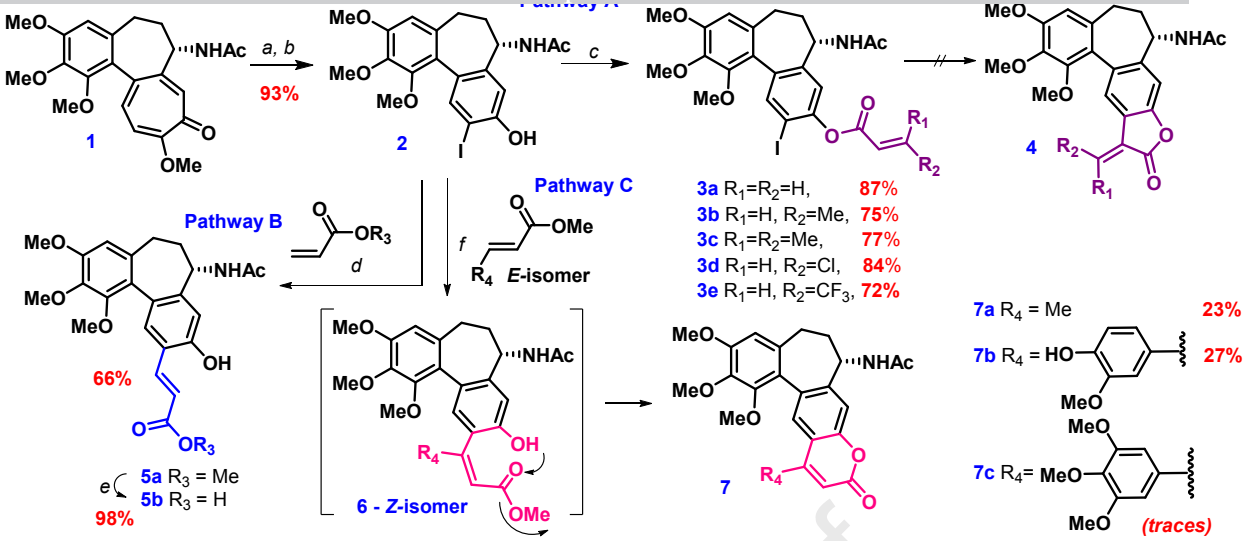
Colchicine binds tubulin in a slow and poorly reversible manner. The half-live of tubulin-colchicine complex was found to be about 12.5 hours [22]. An increase of affinity, or even covalent binding to tubulin could be realized *via* Michael addition of nucleophilic groups in Cys, Lys and Tyr residues to tubulin-binding molecules, possessing an activated double bonds [23,24]. Covalent modifications of various approved drugs and clinical candidates [25] such as telaprevir-based covalent HCV-protease inhibitor [26], irreversible EGFR inhibitors afatinib [27,28], osimertinib [29] and rociletinib [30], covalent Btk and PI3Kα-inhibitors ibrutinib [31,32] and CNX1351 [33] lead to an increase in their affinity to the target protein, and thus to improved efficacy.

We considered that the introduction of an activated double bond into a molecule of a colchicinoid may significantly facilitate and strengthen its binding to tubulin *via* irreversible bond formation. Herein, we report a straightforward synthesis and biological evaluation of two types of heterocyclic colchicinoids, potentially capable of covalent interactions with tubulin: those possessing a *2H*-pyran-2-one scaffold, and a series of compounds containing a methylenedihydrofuran fragment with an aryl-conjugated exocyclic double bond [34]. Two of them were active against tumor cell cultures in low picomolar concentrations, displaying at the same time relatively low acute toxicity in mice.

Results and discussion

Chemistry

First, we attempted to synthesize colchicinoids of type **4**, bearing a conjugated 5-membered lactone ring (D) with an *exo*-double bond potentially active as a Michael acceptor (Scheme 1). Such compounds were supposed to interact with cysteine residues in the colchicine site of tubulin [34].

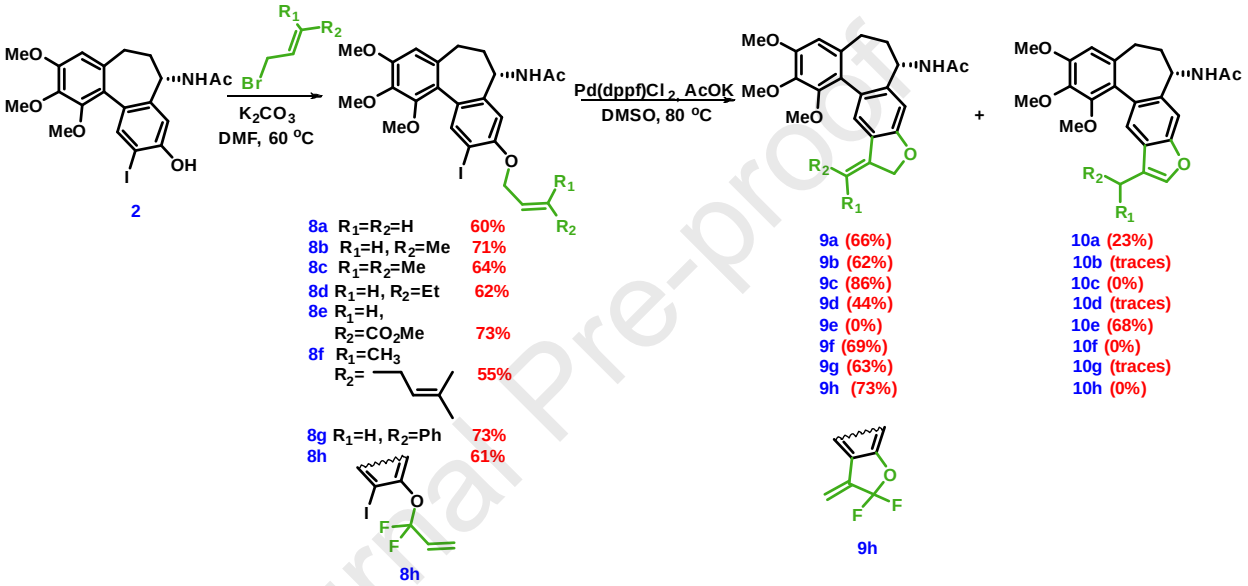


Scheme 1. Synthesis of allocolchicinoids bearing a conjugated lactone as a *D*-cycle. *Reagents and conditions*: *a) HCl, AcOH, 100 °C, 3 h, reflux; b) NaOH, I_2, KI, H_2O, 0-5 °C, 1 h; c) α,β-unsaturated acid, DCC, DMAP, DCM, rt, overnight; d) methyl acrylate, $Pd(OAc)_2$, Et_3N, MeCN, 80 °C, 20 h; e) NaOH, H_2O, rt, 2 h; f) methyl or ethyl ester of α,β-unsaturated acid acid, $Pd(OAc)_2$, Et_3N, H_2O, 80 °C, overnight.*

Naturally occurring (*aR,7S*)-colchicine (**1**) was converted into iodocolchinol (**2**) [35] using 2-step reaction sequence. It was acylated by several α,β-unsaturated acids under Steglich reaction conditions (Scheme 1, Pathway A). Corresponding phenolic esters **3a-d** were supposed to undergo intramolecular Heck reaction leading to conjugated lactones **4**. However, under the tested conditions only hydrolysis of ester groups took place. When iodo-colchinol **2** was reacted with *E*-forms of α,β-unsaturated esters, the formation of coumarine derivatives **7a-c** was observed, albeit in low yields (Scheme 1, Pathway C). Obviously, the Heck coupling afforded *Z*-intermediate **6**, which underwent *in situ* lactonization with formation of allocolchicinocoumarins **7** [36]. Several 4-arylcoumarins are known to exhibit potent antitumor activity [37]. Regarding this, we have also synthesized 4-arylcoumarins **7b** and **7c**. On the other hand, our recent studies [38–42] have shown that the size of substituents in cycle *D* of heterocyclic allocolchicinoids dramatically affects their antiproliferative activity. Therefore, methyl crotonate was used as a reagent, leading to the formation of methyl-substituted coumarin **7a**. Synthesis of the unsubstituted analog failed, as the cross-coupling of iodo-colchinol **2** with methyl acrylate lead to product **5** possessing *E*-configuration that prevented its cyclization to coumarin (Scheme 1, Pathway B).

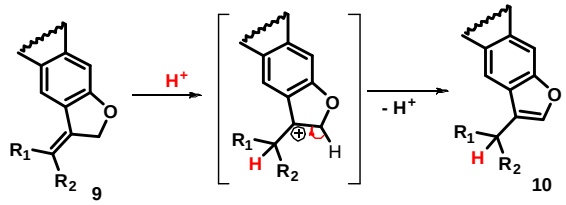
Thus, despite the failure to obtain conjugated lactones of type **4**, we synthesized a number of new colchicinoids **3**, **5** and **7** as potential Michael acceptors, supposedly capable for covalent binding to cysteine residues of the colchicine site of tubulin.

Further, iodo-colchinol **2** was subjected to the alkylation using various allyl bromides (Scheme 2), followed by intramolecular Heck reaction leading to ethers **8a-h**. According to this method, dihydrofuranoallocolchicinoids **9a-h** containing *exo*-double bond in *D*-ring were obtained in only four preparative steps starting from commercially available colchicine with conservation of the absolute configuration at C-7.



Scheme 2. Synthesis of dihydrofuran-containing allocolchicinoids

Dihydrofuranoallocolchicinoids **9**, containing *exo*-double C=C bond can easily undergo acid-catalyzed isomerization into corresponding benzofurans **10** with aromatization (**Scheme 3**).



Scheme 3. Acid-catalyzed isomerization of dihydrofuranoallocolchicinoids **9** into benzofurans **10**.

Compound **9e** completely isomerized into benzofuran **10e** during the Heck reaction, while colchicinoids **9a** and **10a** were initially obtained in 3:1 ratio. The degree of isomerization of **9a** into corresponding benzofuran **10a** could not be reduced by temperature decrease, shortening of the reaction time or varying of the amount of bases. Screening of additives to the

reaction mixture in order to suppress this undesired reaction revealed that the addition of B_2Pin_2 decreased the formation of **10a** from 23% to 7%. We presume that the isomerization takes places via the deprotonation of the allylic α-carbon in the dihydrofuran ring followed by the double bond migration. In this case, B_2Pin_2 might act as a buffer, decreasing the basicity of acetate anion in the reaction media by complexation. For the compounds **9b-d**, and **9f-g** the isomerization into benzofuranoallocolchicinoids **10b-d** and **10f-g** was not observed under the Heck reaction conditions. Compound **9h** was designed to be incapable of isomerizing into **10h** due to the absence of α-hydrogen atoms on the dihydrofuran ring *D*.

Biology

Antiproliferative activity of New Compounds against Cancer Cell Lines

Antiproliferative activity of all synthesized compounds against human cell lines was investigated by MTT assay. Among new colchicinoids **3**, **5**, and **7**, containing fragments of Michael acceptors, none exceeded the colchicine level of cytotoxicity (Table 1). Most of the compounds exhibited activity in 2-4 orders of magnitude lower than colchicine. Compound **5a** with a tricyclic skeleton was found to be the least active. No correlation between the activity and the electronic features of the substituents was observed.

Table 1. Antiproliferative activity of colchicinoids **3** and **7**(IC_{50}*, nM)

	3a	**3b**	**3c**	**3d**	**3e**	**5a**	**7a**	**7b**	**Colchicine (1)**
COLO357	150	161	160	810	30	>4000	160	870	1.3
SW620	30	4	32	800	6	>4000	140	160	2.4
HEK-293	158	>4000	805	810	170	>4000	32	4000	16
Raji	32	139	160	825	180	>4000	16	837	16

*Data are shown as the averages of 5 experiments. Standard deviations were within 10% and not shown for the easier reading of the results

The *in vitro* cytotoxic activity of the compounds **9a-d**, **9f-h**, **10a** and **10e** was tested on a broader panel of tumor cell lines of different origin: pancreatic (PANC-1, COLO357), cervical (HeLa) andcolon (SW620) cancers, Burkitt's lymphoma (Raji), immortalized kidney cells (HEK-293), and keratinocytes (HaCaT). The calculated IC_{50} values are summarized in Table 2. Among these compounds, **9a** and fluorinated colchicinoid **9h** with *exo*-double bonds exhibited the most prominent antiproliferative activity known for colchicinoids, with IC_{50} values in the low picomolar range for some cell lines. Methyl-substituted furan **10a**, isomeric to dihydrofurane **9a**, was substantially less active, but also cytotoxic already in subnanomolar concentrations. The

cytotoxicity significantly decreases with an increase of a substituent size. Thus, colchicinoid **9b** with a monomethylated *exo*-double bond is drastically less cytotoxic than its analogs **9a** and **9h**. A double-methylated analog **9c** or ethyl-substituted colchicinoid **9d** revealed the activity one or two orders of magnitude lower than **9b** (Table 2). Compounds **9f** and **9g** with more bulky substituents, as well as **10e,** were not cytotoxic. Therefore, the substitution of the *exo*-double bond in the dihydrofuran fragment was found to be an important factor affecting the activity of colchicinoids **9**. However, it cannot be excluded that the presence of substituents on the exocyclic double bond also facilitates its isomerization in the cell media to less cytotoxic benzofurans.

Table 2. Antiproliferative activity of colchicinoids with dihydrofuran *D*-cycle (IC_{50}* nM)

	9a	**9b**	**9c**	**9d**	**9f**	**9g**	**9h**	**10a***	**10e**	**1**
COLO357	**0.04**	5	860	800	4330	>20000	**0.02**	1.3	830	1.3
SW620	**0.02**	6	780	810	>20000	>20000	**0.04**	1.3	4100	2.4
HaCaT	**0.3**	14	390	135	4500	4370	**0.03**	0.5	810	0.3
HeLa	**1.7**	32	410	150	4250	4025	**0.03**	0.8	805	1.3
HEK-293	**0.08**	3.8	400	166	4280	>20000	**0.03**	5	4420	16
PANC-1	**0.08**	32	850	80	810	>20000	**0.01**	2.1	820	0.1
Raji	**0.03**	3.8	420	90	800	4600	**0.05**	1.3	3900	16

*Compound **10a** was obtained from **9a** by the treatment with trifluoroacetic acid in DCM with a quantitative yield. Most potent compounds **9a** and **9h** are shown in bold.

*Data are shown as the averages of 4 experiments. Standard deviations were within 10% and not shown for the easier reading of the results

Cell cycle analysis

The impact of colchicinoid **9a** on cell cycle and its comparison with the action of colchicine was established (**Fig. 1**). Both compounds decreased the population of cells in G_1-phase, accumulating cells in G_2/M phase during incubation for 48 hours (**Fig. 1**). As expected, both colchicinoids exhibited the same manner of cell cycle arrest and apoptosis induction (**Fig. 1**). Notably, in COLO357 culture the apoptotic cells were effectively eliminated, oppositely to SW620, where percentage of apoptotic cells increased 5-10-fold over the control (**Fig. 1**). Similar results were found for **9h** (data not shown).

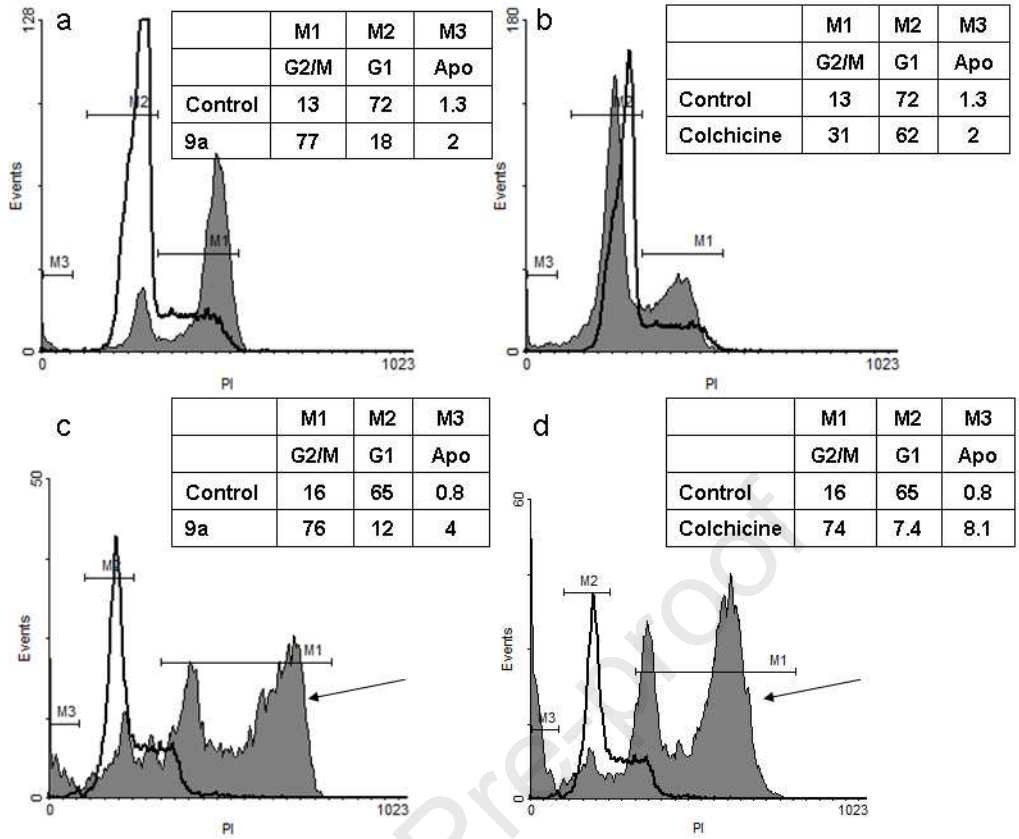


Figure 1. Cell cycle analysis in COLO357 and SW620 cells. COLO357 (a, b) or SW620 (c, d) cells were incubated without (black lines) or with 5 nM of **9a** or colchicine (**1)** for 48 h (grey histograms), fixed, perforated, stained with propidium iodide (PI), and analyzed by flow cytometry. SW620 cells formed diploids and tetraploids (arrows). M1, M2, M3 correspond to G2/M, G1, and apoptosis (Apo) phases of cell cycle. Table data are shown as percentages of cells in each phase.

Notably, 48 h incubation of these cells in the presence of the colchicinoids did not result in significant apoptosis due to a low number of dying cells at this time point. In case of a longer incubation, the number of apoptotic cells increases significantly (SI Figure 1) [38].

Destabilization of microtubules and inhibition of assembly

Compound **9a**, as well as colchicine, disrupted the mitotic spindle and induced tubulin dissipation and chromosomes disturbance (Fig. 2). In the control images (Fig. 2 a, d) a well-developed microtubules network and mitotic spindles were observed. In contrast, total microtubules depolymerization and chromosomes disordering were recognized after cells incubation with **9a** within 24 hours (Fig. 2 b, c, e, f). This clearly indicates that synthesized compound **9a** inhibits cell division by influencing the tubulin assembly.

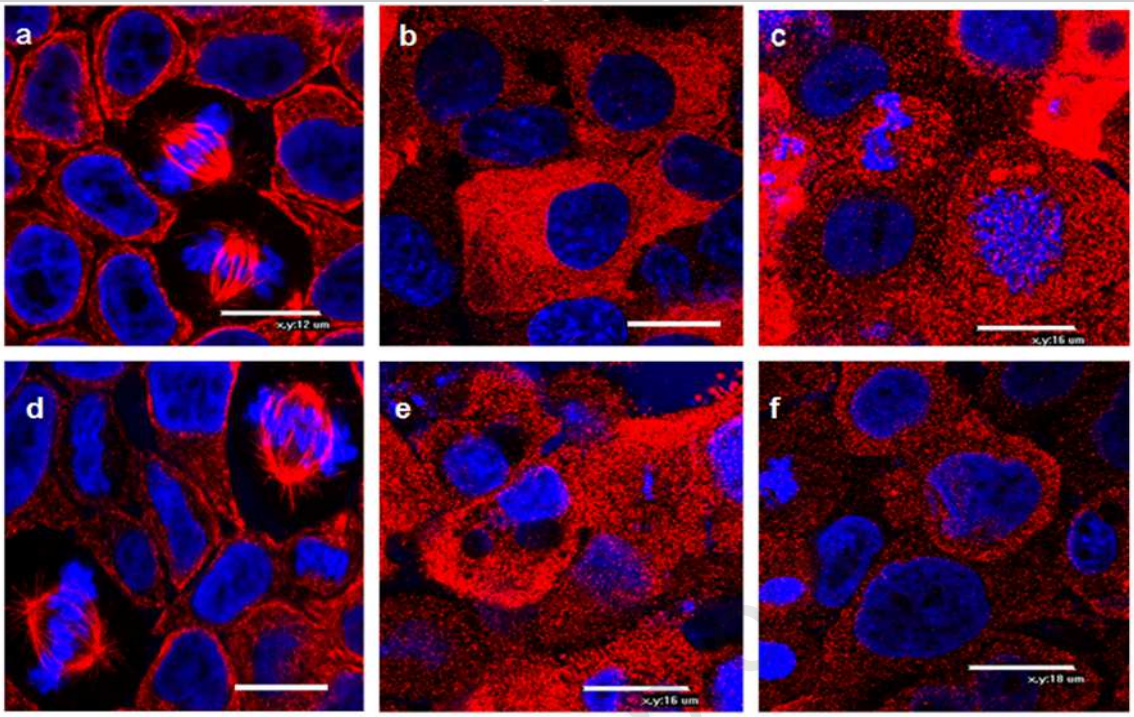


Figure 2. Disruption of mitotic spindle by **9a** and colchicine. SW620 (a-c) or COLO387 (d-f) were incubated without (a, d) or with 5 nM **9a** (b, e) or colchicine (c, f) for 24 h, fixed, and stained with anti β-tubulin antibody (red). Note the loss of mitotic spindles as well as tubulin disassembling, and chromosome scattering (b, c, e, f) after cell incubation with colchicinoids. Nuclei are stained with Hoechst 33342 (blue). Objective bars correspond to 12-16 μM.

Figure 3 shows the effects of colchicine (panel A) as a positive control, and **9a** (panel B), on the turbidimetry time course of microtubule assembly from pure tubulin. Clear inhibition was noted, and the rate of assembly as well as the final amount of microtubules was lower in the presence of ligands than in the control experiment. When the samples were cooled to 15 °C, the polymers depolymerized (data not shown). The graphs A and B of Figure 3 show that the extent of inhibition by colchicine and **9a**, respectively, increased monotonically with the mole ratio of the total ligand to total tubulin in the solution (R). In these figures, 50% inhibition occurred at ligand/tubulin mole ratio of 0.27 mol/mol for colchicine and 0.06 mol/mol for **9a.**. Thus, compound **9a** exhibits more potent inhibition of tubulin polymerization as well as stronger binding to colchicine site on microtubules than colchicine itself. . Interestingly, combretastatin A-4 (CA4), similar to colchicine in the mode of action on tubulin, but possessing also antivascular activity, revealed R=0.09 in similar experiments [38]. The difference in R is likely a reflexion of a difference in binding affinity rather than kinetics parameters, suggesting that ring D and/or *exo*-bond of compound **9a** makes contribution to the induction of assembly inhibition.

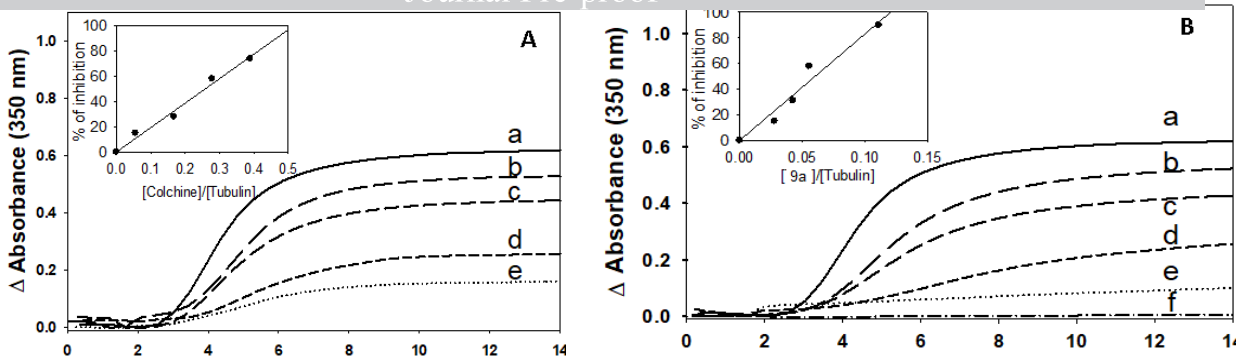
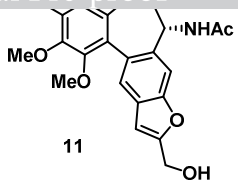


Figure 3. Effect of ligands on the turbidity time course of *in vitro* microtubule assembly. The aliquots were incubated for 40 min at 4 °C prior to start the reaction by warming the samples to 37 °C **(A).** Effect of various concentrations of colchicine (*a*: 0 μM; *b*: 1 μM; *c*: 3 μM; *d*: 5 μM; and *e*: 7 μM) on tubulin aggregation at 18 μM in polymerization buffer. **(B)** Effect of various concentrations of **9a** (*a*: 0 μM; *b*: 0.5 μM; *c*: 0.75 μM; *d*: 1 μM; *e*: 2 μM, and *f*: 5 μM) on tubulin aggregation at 18 μM in polymerization buffer. The inserts on the graphs represent the percentage of assembly inhibition as a function of the mole ratio of the total ligand to total tubulin in the solution (R).

*In vivo toxicity of the compounds **9a** and **9h***

Determination of the acute *in vivo* toxicity was performed in C57BL/6, BALB/c, CBA, A/Sn, and outbred CD1 mice (3 mice of each strain per group). Several genetically different strains were used to cover a variety of genotypes [43]. Compounds **9a**, **9h**, and colchicine were dissolved in DMSO to 7.9 and 8.6 mg/mL respectively, diluted by saline, and 200 μl of this solution per mouse was injected intravenously. Pure DMSO was diluted in the same way and was used as a control (detailed description is in Experimental Section). Preparations were injected at 5 and 10 mg/kg of body weight. All the mice injected with 5 mg/kg colchicine died 14 h after the injections. None of the mice in DMSO, **9a**, and **9h** groups died at 5 mg/kg dose. When the mice were injected with 10 mg/kg DMSO, **9a**, and **9h**, only one mouse of three C57BL/6 died in **9a** group. Consequently, LD_{50} for **9a** can be estimated as 10 mg/kg and LD_{50}>10 mg/kg for **9h**. Estimated LD_{50} for colchicine (mice, i. v.) is 1.6 mg/kg [44], which corresponds well to the numbers given above. Interestingly, structurally similar furan derivative of allocolchicine **11**, previously synthesized by us [38], demonstrated much higher acute toxicity (LD_{50} = 2 mg/kg for C57BL/6 mice, i. v.).



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Apparently, the shift of small substituents from position 2 to position 3 of the furan or dihydrofuran ring of heterocyclic allocolchicinoids has a profound effect on antiproliferative activity and systemic toxicity. Lower acute toxicity can be translated into expansion of therapeutic window of the novel colchicinoids. To better understand the structure-activity relationship of allocolchicine derivatives, the structure of selected compound **9a** in tubulin complex was resolved by X-ray crystallography and is discussed below.

*Crystal structure of **9a**-T2R*

To gain an insight into the mechanism of microtubule assembly inhibition by colchicinoids **9**, we determined the 2.3 Å resolution the crystal structure of compound **9a** bound to T2R, a complex of two tubulin heterodimers stabilized by the stathmin-like domain of the RB3 protein [45] (**Fig. 4**). The structure shows that **9a** binds to the colchicine site of tubulin in a mode very similar to that of colchicine, interacting with the T5 loop of the α-subunit and with β-strands S8 and S9, loop T7 and helices H7 and H8 of β-tubulin (**Fig. 5**). Therefore, the most likely mechanism of action of **9a** is preventing the curved-to-straight tubulin structural changes that occur during microtubule assembly, same to colchicine [46].

The *exo*-C=C bond is directed toward a narrow pocket, in which bulky substituents on the dihydrofuran fragment could not be easily accommodated, giving a reason why compounds with larger substituents are less active than **9a** and **9h** (Table 2). This bond is located in the vicinity of the Thr314 and Met259 residues of β-tubulin, but is too far for a covalent interaction (**Fig. 4**). We concluded, therefore, that compound **9a**, contrarily to our anticipations, does not bind covalently with tubulin.

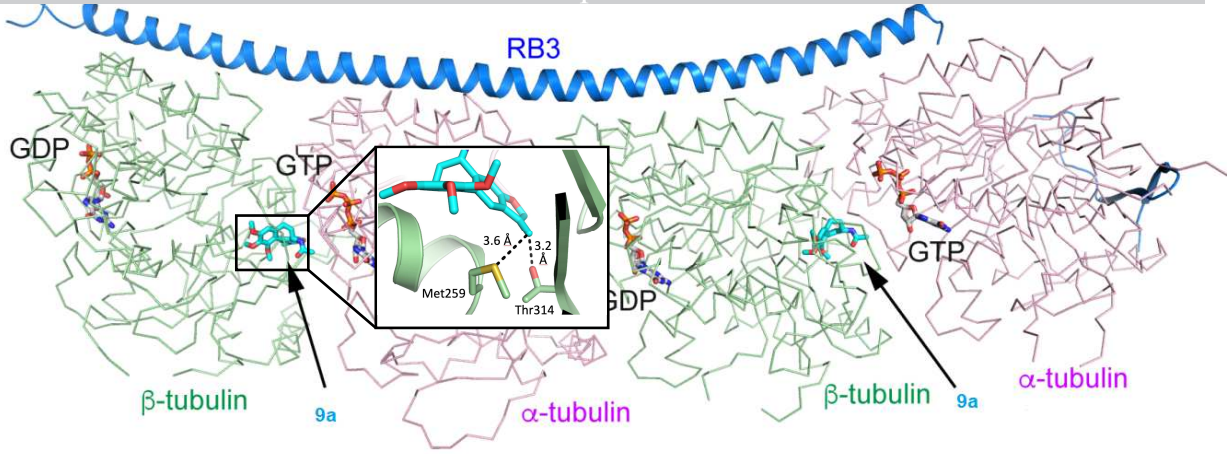

Figure 4. Tubulin binding of compound **9a**. The T2R:**9a** complex comprises two αβ-tubulin heterodimers stabilized by the stathmin-like domain of the RB3 protein (blue). Colchicinoid **9a** (cyan) binds to β-tubulin (green) at the interface with the α-subunit (pink).

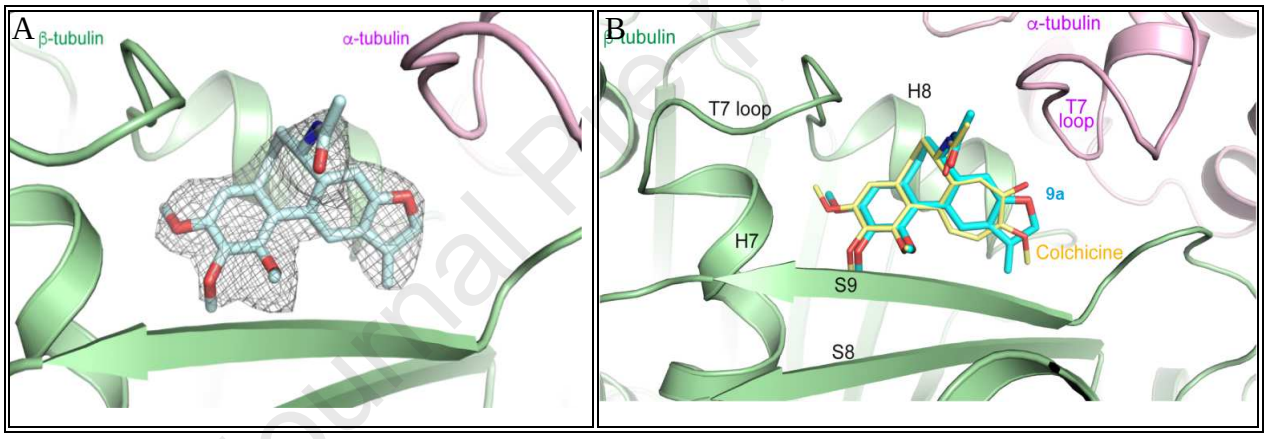

Figure 5. **A**. F_{obs}-F_{calc} electron density omit map contoured at the 3σ level of **9a** bound to tubulin. **B**. Close-up of **9a** bound to tubulin and comparison with colchicine. The tubulin secondary structural elements that contact **9a** are labeled. Colchicine (yellow) from the tubulin:colchicine complex [47] (pdb id 5EYP) is shown after superposition of β-tubulin from that complex to that of tubulin:**9a** (root mean square deviation, 0.53 Å (423 Cαs compared)). Only tubulin from T2R:**9a** is shown.

Conclusion

We synthesized a series of heterocyclic colchicinoids bearing dihydrofuran fragments of type **9** as a novel class of antiproliferative analogs of the naturally occurring colchicine (**1**). These compounds were obtained in four preparative steps starting from colchicine, under conservation of the absolute configuration at C(7). Dihydrofuran **9a** and its fluorinated analog **9h** exhibited cytotoxicity in the picomolar concentration range for all studied tumor cell lines.

isomeric benzofuran **10a** was 1-2 orders of magnitude less cytotoxic. Compared to colchicine (**1**), and combretastatin A-4, allocolchicinoid **9a** proved to be a more efficient tubulin assembly inhibitor, acting in a substoichiometric mode. First *in vivo* experiments demonstrated significant decrease in acute toxicity for the compounds **9a** and **9h** (an order of magnitude) compared to colchicine, so that LD_{50} for **9a** was estimated as 10 mg/kg and for **9h** even higher, with LD_{50} = 1.6 mg/kg for colchicine under the same conditions. The localization of the compounds **9** in the colchicine site of tubulin has been unambiguously proven by X-ray diffraction.

The synthetic availability and strong cytotoxic activity exhibited by the new colchicinoids make them perspective objects for in-depth biological investigations. We consider the compounds **9a** and **9h** as promising leads for future development of water-soluble prodrugs for *in vivo* experiments.

Acknowledgements

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Experimental part

Chemistry

General information.

^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent DDR2 400 spectrometer at 25 °C. Chemical shifts (δ) are reported in ppm for the solution of compound in CD_3OD, $CDCl_3$ and DMSO-d_6 with internal reference TMS and *J* values in Hertz. Atomic numeration is given only for NMR assignment. MALDI spectra were recorded on Bruker Microflex LT spectrometer. Elemental analysis was performed using an Elementar (Vario Micro Cube) apparatus, all the compounds were found to have purity >95%. Column chromatography was performed using *Merck Kieselgel 60* (70 – 230 mesh). All reactions were performed with commercially available reagents. Solvents were purified according to standard procedures. The petroleum ether used corresponds fraction 40 – 70 °C. Synthesis of iodocolchinol **2** was performed as described earlier [35,38].

*Synthesis of colchicinoids **3a-e.***

*General procedure for synthesis of esters **3a-e.*** Iodo-colchinol **2** (1.00 equiv.) was treated with the corresponding α,β-unsaturated carboxylic acid (1.00 equiv.) and 4-DMAP (0.10 equiv.) in ca. 1 M dichloromethane solution under inert atmosphere. DCC (1.20 equiv.) was added to the solution in portions, and the reaction was stirred at room temperature for 20 hours. Solvent was removed under reduced pressure, and the residue purified by column chromatography.

(*5S*)-5-acetamido-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cycloheptene-3-yl acrylate (**3a)**

Eluent: petroleum ether-ethyl acetate-ethanol (5 : 1 : 1). Pale-yellow solid, yield 87%.

M.p. = 139 – 141 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (d, *J* = 8.5 Hz, *NH*, 1H), 7.77 (s, *C8-H*, 1H), 7.17 (s, *C11-H*, 1H), 6.82 (s, *C4-H*, 1H), 6.64 (dd, *J* = 17.2, 1.0 Hz, C_α*-H*, 1H), 6.48 (dd, *J* = 17.2, 10.4 Hz, C_β*-H*, 1H), 6.25 (dd *J* = 10.4, 1.0 Hz, C_β*-H'*, 1H), 4.55 – 4.45 (m, *C7-H*, 1H), 3.85 (s, *OMe*, 3H), 3.79 (s, *OMe*, 3H), 3.56 (s, *OMe*, 3H), 2.58 – 2.52 (m, *C6-H*, 1H), 2.21 – 2.13 (m, *C5-H*, 1H), 2.11 – 2.03 (m, *C5-H*, 1H), 1.93 – 1.87 (m, *C6-H*, 1H), 1.86 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO -d_6): δ = 168.50, 163.31, 152.99, 150.16, 149.71, 142.65, 140.48, 139.53, 134.90, 134.49, 133.64, 127.44, 122.15, 117.97, 108.22, 88.09, 60.78, 60.55, 55.86, 47.91, 38.30, 33.35, 22.64.

Elemental analysis: for $C_{23}H_{24}INO_6$ calcld.: C, 51.41; H, 4.50; found: C, 51.53; H, 4.64.

MALDI: (pos. mode): 537.2 (M^+) 68%.

(*5S*)-5-acetamido-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cycloheptene-3-yl (*E*)-but-2-enoate (3b)

Eluent: petroleum ether-ethyl acetate-ethanol (8 : 1 : 1). Pale-yellow solid, yield 75%.

M.p. = 130 – 131 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (d, *J* = 8.1 Hz, *NH*, 1H), 7.76 (s, *C8-H*, 1H), 7.23 (dd, *J* = 15.4, 6.9 Hz, C_α*-H*, 1H), 7.13 (s, *C11-H*, 1H), 6.82 (s, *C4-H*, 1H), 6.21 (d *J* = 15.5 Hz, C_β*-H*, 1H), 4.57 – 4.42 (m, *C7-H*, 1H), 3.85 (s, *OMe*, 3H), 3.79 (s, *OMe*, 3H), 3.56 (s, *OMe*, 3H), 2.60 – 2.53 (m, *C6-H*, 1H), 2.19 (dd, *J* = 11.6, 6.2 Hz, *C5-H*, 1H), 2.09 (dd, *J* = 12.3, 7.2 Hz, *C5-H*, 1H), 1.99 (d, *J* = 6.7 Hz, C_β*-CH*$_3$, 3H), 1.92 – 1.86 (m, *C6-H*, 1H), 1.85 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO -d_6): δ = 168.47, 163.34, 152.96, 150.16, 149.88, 148.80, 142.55, 140.48, 139.45, 134.89, 133.45, 122.19, 121.27, 118.05, 108.22, 88.30, 60.77, 60.54, 55.85, 47.88, 38.30, 29.85, 22.63, 18.06.

Elemental analysis: for $C_{24}H_{26}INO_6$ calcld.: C, 52.28; H, 4.75; found: C, 52.40; H, 4.53.

MALDI (DCTB, pos. mode): 574.0 ($M+Na^+$) 41%, 552.0 ($M+H^+$) 20%, 590.0 ($M+K^+$) 13%.

(*5S*)-5-acetamido-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cycloheptene-3-yl 3-methylbut-2-enoate (3c)

Eluent: petroleum ether-ethyl acetate-ethanol (8 : 1 : 1). Beige solid, yield 77%.

M.p. = 118 – 120 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.37 (d, *J* = 8.5 Hz, *NH*, 1H), 7.75 (s, *C8-H*, 1H), 7.11 (s, *C11-H*, 1H), 6.81 (s, *C4-H*, 1H), 6.05 (s, C_α-*H*, 1H), 4.50 (dd, *J* = 19.3, 8.0 Hz, *C7-H*, 1H), 3.84 (s, *OMe*, 3H), 3.79 (s, *OMe*, 3H), 3.55 (s, *OMe*, 3H), 2.55 (dd, *J* = 12.6, 5.5 Hz, *C6-H*, 1H), 2.20 (s, C_β-CH_3, 3H), 2.19 – 2.12 (m, *C5-H*, 1H), 2.12 – 2.04 (m, *C5-H*, 1H), 2.02 (s, C_β-CH_3, 3H), 1.89 (d, *J* = 12.1 Hz, *C6-H*, 1H), 1.85 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO -d_6): δ = 168.46, 163.31, 161.63, 152.93, 150.16, 149.92, 142.46, 140.48, 139.39, 134.89, 133.30, 122.25, 118.16, 114.35, 108.22, 88.61, 60.76, 60.54, 55.85, 47.86, 38.32, 33.34, 27.14, 22.63, 20.32.

Elemental analysis: для $C_{25}H_{28}INO_6$ calcld.: C, 53.11; H, 4.99; found: C, 53.24; H, 4.86.

MALDI: (pos. mode): 588.0 ($M+Na^+$) 28%, 603.9 ($M+K^+$) 34%.

(*5S*)-5-acetamido-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cycloheptene-3-yl (*E*)-3-chloroacrylate (3d)

Eluent: petroleum ether-ethyl acetate-ethanol (10 : 1 : 1). Pale-yellow powder, yield 84%.

M.p. = 121 – 122 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.39 (d, *J* = 8.4 Hz, *NH*, 1H), 8.02 (d, *J* = 13.4 Hz, C_α-*H*, 1H), 7.77 (s, *C8-H*, 1H), 7.17 (s, *C11-H*, 1H), 6.82 (s, *C4-H*, 1H), 6.79 (d, *J* = 13.4 Hz, C_β-*H*, 1H), 4.48 (dt, *J* = 12.1, 7.8 Hz, *C7-H*, 1H), 3.84 (s, *OMe*, 3H), 3.79 (s, *OMe*, 3H), 3.56 (s, *OMe*, 3H), 2.55 (dd, *J* = 12.8, 5.6 Hz, *C6-H*, 1H), 2.24 – 2.14 (m, *C5-H*, 1H), 2.11 – 2.03 (m, *C5-H*, 1H), 1.89 (dd, *J* = 11.5, 6.6 Hz, *C6-H*, 1H), 1.86 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.50, 161.28, 156.59, 153.01, 150.15, 149.50, 142.72, 141.14, 140.48, 139.54, 134.90, 133.79, 123.93, 122.11, 117.97, 108.23, 88.11, 60.77, 60.55, 55.86, 47.49, 33.35, 25.32.

Elemental analysis: for $C_{23}H_{23}ClINO_6$ calcld.: C, 48.31; H, 4.05; found: C, 48.39; H, 4.16.

(*5S*)-5-acetamido-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cycloheptene-3-yl (*E*)-4,4,4-trifluorobut-2-enoate (3e)

Eluent: petroleum ether-ethyl acetate-ethanol (9 : 1 : 1) Beige powder, yield 72%.

M.p. = 115 – 117 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (d, *J* = 8.2 Hz, *NH*, 1H), 7.78 (s, *C8-H*, 1H), 7.45 (dd, *J* = 15.9, 6.7 Hz, C_α*-H*, 1H), 7.23 (s, *C11-H*, 1H), 7.08 (dd, *J* = 15.9, 2.1 Hz, C_β*-H*, 1H), 6.82 (s, *C4-H*, 1H), 4.48 (dt, *J* = 12.0, 7.7 Hz, *C7-H*, 1H), 3.85 (s, *OMe*, 3H), 3.79 (s, *OMe*, 3H), 3.56 (s, *OMe*, 3H), 2.56 (dd, *J* = 12.4, 5.4 Hz, *C6-H*, 1H), 2.18 (dd, *J* = 12.3, 6.4 Hz, *C5-H*, 1H), 2.09 (dd, *J* = 12.8, 6.7 Hz, *C5-H*, 1H), 1.91 (dd, *J* = 11.8, 6.9 Hz, *C6-H*, 1H), 1.86 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO -d_6): δ = 168.53, 161.53, 156.59, 153.04, 150.16, 149.41, 142.86, 140.48, 139.61, 134.91, 134.01, 132.69, 128.88, 122.07, 117.80, 108.22, 87.82, 60.78, 60.55, 55.86, 48.05, 33.35, 25.32, 22.64.

Elemental analysis: for $C_{24}H_{23}F_3INO_6$ calcld.: C, 47.62; H, 3.83; found: C, 47.74; H, 3.92.

MALDI: (pos. mode) 627.9 ($M+Na^+$) 87%, 643.9 ($M+K^+$) 73%.

*Synthesis of colchicinoids **5a,b** and **7a-c.***

Methyl (*E*)-3-((5S)-5-acetamido-3-hydroxy-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo-[a,c]cycloheptene-2-yl)acrylate (5a)

To the mixture of iodocolchinol **2** (100.0 mg, 0.206 mmol, 1.00 equiv.) and $Pd(OAc)_2$ (4.6 mg, 0.02 mmol, 0.10 equiv.), MeCN (10 mL) was added under inert atmosphere. Et_3N (66 μL, 0.620 mmol, 3.00 equiv.) and methyl acrylate (90 μL, 0.309 mmol, 1.50 equiv.) were subsequently added. The reaction was run at 80 °C for 20 hours, solvent removed under reduced pressure and the product isolated by column chromatography (petroleum ether – ethyl acetate – ethanol 4 : 1 : 1). White powder, yield 66%.

M.p. = 186 – 188 °C

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.32 (s, *OH*, 1H), 8.40 (d, *J* = 7.9 Hz, *NH*, 1H), 7.87 (d, *J* = 16.1 Hz, =$\underline{CH}$-CO_2*Me*, 1H), 7.47 (s, *C8-H*, 1H), 6.90 (s, *C11-H*, 1H), 6.76 (s, *C4-H*, 1H), 6.55 (d, *J* = 16.1 Hz, *C10-*$\underline{CH}$, 1H), 4.41 (dt, *J* = 14.3, 7.2 Hz, *C7-H*, 1H), 3.82 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.70 (s, $CO_2\underline{Me}$, 3H), 3.52 (s, *OMe*, 3H), 2.21 – 2.01 (m, *C6-H*, *C5-H*, 3H), 1.94 – 1.91 (m, *C6-H*, 1H), 1.88 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO -d_6): δ = 168.36, 167.20, 155.99, 152.17, 150.35, 144.89, 140.59, 140.41, 134.68, 129.86, 125.09, 123.88, 118.59, 116.22, 111.14, 108.00, 60.57, 60.51, 55.84, 51.28, 48.53, 37.77, 29.99, 22.63.

Elemental analysis: for $C_{24}H_{27}NO_7$ calcld.: C, 65.29; H, 6.16; found: C, 65.43; H, 6.28.

(*E*)-3-((*5S*)-5-acetamido-3-hydroxy-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]-cyclohepta-2-yl)acrylic acid (5b)

Compound **5a** (200.0 mg, 0.433 mmol) was suspended in 2.5M NaOH (15 mL). The reaction was carried out for 1 hour at 20 °C. The solution was then acidified to pH 2, extracted with AcOEt (3x40 mL), organic layer dried over Na_2SO_4 and concentrated under reduced pressure. The product **5b** was obtained as white solid in 98% yield.

M.p. = 178-179 °C

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.27 (br.s, *OH*, 1H), 8.42 (d, *J* = 8.0 Hz, *NH*, 1H), 7.79 (d, *J* = 16.1 Hz, =*CH*-*CO$_2$H*, 1H), 7.41 (s, *C8-H*, 1H), 6.90 (s, *C11-H*, 1H), 6.74 (s, *C4-H*, 1H), 6.43 (d, *J* = 16.1 Hz, *C10-CH*, 1H), 4.39 (dt, *J* = 14.0, 7.2 Hz, *C7-H*, 1H), 3.80 (s, *OMe*, 3H), 3.76 (s, *OMe*, 3H), 3.49 (s, *OMe*, 3H), 2.55 – 2.49 (m, *C6-H*, 1H), 2.17 – 2.04 (m, *C5-H*, 2H), 1.96 – 1.87 (m, *C6-H*, 1H), 1.86 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6): δ 168.40, 168.11, 155.84, 152.14, 150.35, 144.53, 140.59, 139.86, 134.72, 129.66, 125.01, 123.97, 118.82, 117.64, 111.19, 108.02, 60.58, 60.53, 55.85, 48.52, 37.80, 30.03, 22.65.

Elemental analysis: for $C_{23}H_{25}NO_7$ calcld.: C, 64.63; H, 5.90; found: C, 64.79; H, 6.18.

MALDI: (neg. mode) 426.3 (M-H) 100%, 449.2 ($M+Na^+$) 76%, 465.1 ($M+K^+$) 68%.

*General procedure for synthesis of compounds **7a-b***

A Schlenk flask was charged with iodo-colchinol **2** (100.0 mg, 0.207 mmol, 1.00 equiv.) and $Pd(OAc)_2$ (4.7 mg, 0.021 mmol, 0.10 equiv.) under inert atmosphere. Distilled water (5 mL) and the corresponding unsaturated ester (1.50 equiv.) followed by Et_3N (62.7 mg, 0.621 mmol, 86 µL, 3.00 equiv.) were added and the reaction was run for 30 hours at 85 °C. The crude product was extracted with AcOEt and purified using column chromatography.

_N_-((_7S_)-1,2,3-trimethoxy-12-methyl-10-oxo-5,6,7,10-tetrahydrobenzo-[6,7]cyclohepta[1,2-g]chromen-7-yl)acetamide (7a)

Eluent: petroleum ether-ethyl acetate-ethanol (4 : 1 : 1). White powder, yield 23%.

M.p. = 177 – 179 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.48 (d, *J* = 8.3 Hz, *NH*, 1H), 7.65 (s, *C8-H*, 1H), 7.29 (s, *C12-H*, 1H), 6.81 (s, *C4-H*, 1H), 6.38 (d, *J* = 1.1 Hz, *C10-H*, 1H), 4.55 (dt, *J* = 11.8, 7.6 Hz, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.58 (s, *OMe*, 3H), 2.54 (dd, *J* = 13.0, 6.0 Hz, *C6-H*, 1H), 2.20 (dd, *J* = 12.5, 6.6 Hz, *C5-H*, 1H), 2.09 – 2.01 (m, *C5-H*, *C6-H*, 2H), 1.89 (s, *CO*$\underline{CH_3}$, 3H), 1.85 (br.s, *C11-*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.62, 159.81, 153.10, 152.87, 152.03, 150.30, 145.56, 140.61, 134.74, 130.20, 126.05, 122.92, 117.76, 113.95, 111.20, 108.21, 60.85, 60.53, 55.90, 48.40, 37.97, 29.83, 22.61, 18.12.

Elemental analysis: for $C_{24}H_{25}NO_6$ calcld: C, 68.07; H, 5.95; found: C, 68.19; H, 6.04.

MALDI: (pos. mode) 446.1 ($M+Na^+$) 40%, 462.0 ($M+K^+$) 63%.

***N*-((*7S*)-12-(4-hydroxy-3-methoxyphenyl)-1,2,3-trimethoxy-10-oxo-5,6,7,10-tetrahydro-benzo[6,7]cyclohepta[1,2-g]chromen-7-yl)acetamide (7b)**

Eluent: petroleum ether-ethyl acetate-ethanol (4 : 1 : 1) White powder, yield 27%.

M.p. = 186 - 188°C

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.58 (s, *OH*, 1H), 8.57 (d, *J* = 8.2 Hz, *NH*, 1H), 7.62 (s, *C8-H*, 1H), 7.40 (s, *C12-H*, 1H), 7.16 (d, *J* = 1.9 Hz, *C5'-H*, 1H), 7.00 (d, *J* = 2.0 Hz, *C6-H*, 1H), 6.94 (s, *C10-H*, 1H), 6.81 (s, *C2'-H*, 1H), 6.39 (s, *C4-H*, 1H), 4.57 (dt, *J* = 11.5, 7.6 Hz, *C7-H*, 1H), 3.87 (s, *OMe*, 3H), 3.82 (s, *OMe*, 3H), 3.76 (s, *OMe*, 3H), 3.45 (s, *OMe*, 3H), 2.57 – 2.53 (m, *C6-H*, 1H), 2.24 – 2.18 (m, *C5-H*, 1H), 2.12 – 2.05 (m, *C6-H*, *C5-H*, 2H), 1.93 (s, *CO*$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.69, 159.93, 154.92, 152.84, 152.80, 150.20, 148.34, 147.71, 145.83, 140.54, 134.84, 129.91, 128.00, 125.51, 122.83, 121.70, 116.58, 115.65, 113.12, 112.71, 111.73, 108.35, 62.79, 60.71, 60.59, 55.86, 55.54, 48.46, 37.93, 22.63.

Elemental analysis: for $C_{30}H_{29}NO_8$ calcld.: C, 67.79; H, 5.50; found: C, 67.45; H, 5.67.

MALDI: 571.1 (M+K) 35%, 554.2 (M+Na) 15%, 532.2 (M+H) 13%

*General procedure for synthesis of compounds **8a-h***

Into a Schlenk flask under inert atmosphere were placed iodo-colchinol **2** (100.0 mg, 0.207 mmol, 1.00 equiv.) and Na_2CO_3 (65.8 mg, 0.620 mmol, 3.00 equiv.). Dry DMF (12 mL) was added. The mixture was stirred at room temperature for 10 minutes and corresponding allyl bromide (2.00 equiv.) was added dropwise. The resulting solution was stirred at 60° C for 20 hours. The solvent was removed under reduced pressure and the corresponding allyl ether was isolated using column chromatography.

***N*-((*5S*)-3-(allyloxy)-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cycloheptene-5-yl)acetamide (8a)**

Eluent: petroleum ether–ethyl acetate–ethanol (7 : 1 : 1). Pale-beige solid, yield 60%.

M.p. = 82 – 83 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, *J* = 8.5 Hz, *NH*, 1H), 7.68 (s, *C8-H*, 1H), 6.98 (s, *C11-H*, 1H), 6.78 (s, *C4-H*, 1H), 6.08 (ddd, *J* = 15.5, 10.5, 5.1 Hz, *OCH*$_2$-$\underline{CH}$, 1H), 5.56 (d, *J* = 17.2 Hz, C_{vinyl}-*H*, 1H), 5.33 (d, *J* = 10.5 Hz, C_{vinyl}-*H'*, 1H), 4.66 (s, *O*$\underline{CH_2}$-*CH*, 2H), 4.49 (dt, *J* = 12.0, 7.9 Hz, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.55 – 2.50

(m, C6-*H*, 1H), 2.23 – 2.12 (m, C5-*H*, 1H), 2.12 – 2.00 (m, C5-*H*, 1H), 1.89 (s, CO$\underline{CH_3}$, 3H), 1.87 – 1.76 (m, *C6-H*, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.48, 155.69, 152.46, 150.15, 142.51, 140.51, 139.43, 134.82, 133.20, 128.32, 122.85, 117.55, 108.20, 107.79, 83.24, 69.13, 60.60, 60.55, 55.82, 48.16, 38.39, 30.00, 22.64.

Elemental analysis: for $C_{23}H_{26}INO_5$ calcd.: C, 52.78; H, 5.01; found: C, 52.55; H, 5.14.

MALDI (DCTB, pos. mode): 523.1 (M^+) 100%, 332.2 (29%), 242.3 (23%).

***N*-((*5S*)-3-(((E)-but-2-en-1-yl)oxy)-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]-cycloheptene-5-yl)acetamide (8b)**

Eluent: petroleum ether–ethyl acetate–ethanol (8 : 1 : 1). Pale-beige solid, yield 71%.

M.p. = 124 - 126 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, *J* = 8.6 Hz, *NH*, 1H), 7.66 (s, *C8-H*, 1H), 6.95 (s, *C11-H*, 1H), 6.78 (s, *C4-H*, 1H), 5.95 (dd, *J* = 15.3, 6.5 Hz, OCH_2-$\underline{CH}$=, 1H), 5.77 – 5.66 (m, =$\underline{CH}$-CH_3, 1H), 4.58 (dd, *J* = 4.2, 1.6 Hz, OCH_2, 2H), 4.53 – 4.44 (m, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.77 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.55 – 2.51 (m, *C6-H*, 1H), 2.22 – 2.11 (m, *C5-H*, 1H), 2.10 – 2.00 (m, *C5-H*, 1H), 1.89 (s, CO$\underline{CH_3}$, 3H), 1.88 – 1.81 (m, *C6-H*, 1H), 1.79 – 1.71 (m, =CH-$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.42, 155.82, 152.44, 150.13, 142.43, 140.50, 139.42, 134.81, 129.89, 128.15, 125.95, 125.38, 122.88, 108.20, 107.79, 83.33, 69.03, 60.61, 60.54, 55.82, 48.14, 38.46, 22.63, 17.63.

Elemental analysis: for $C_{24}H_{28}INO_5$ calcld.: C, 53.64; H, 5.25; found: C, 53.49; H, 5.32.

MALDI: (pos. mode) 537.3 (M+) 100%, 596.1 ($M+Na^+$) 26%.

***N*-((*5S*)-2-iodo-9,10,11-trimethoxy-3-((3-methylbut-2-en-1-yl)oxy)-6,7-dihydro-5H-dibenzo-[a,c]cycloheptene-5-yl)acetamide (8c)**

Eluent: petroleum ether–ethyl acetate–ethanol (8 : 1 : 1). White solid, yield 64%.

M.p. = 77 – 79 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (d, *J* = 8.5 Hz, *NH*, 1H), 7.65 (s, *C8-H*, 1H), 6.96 (s, *C11-H*, 1H), 6.78 (s, *C4-H*, 1H), 5.47 (t, *J* = 6.7 Hz, C_{vinyl}-*H*, 1H), 4.62 (d, *J* = 6.5 Hz, $O\underline{CH_2}$, 2H), 4.48 (dt, *J* = 12.0, 7.9 Hz, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.77 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.16 (dt, *J* = 12.1, 8.6 Hz, *C6-H*, 1H), 2.10 – 2.01 (m, *C5-H*, 2H), 1.88 (s, CO$\underline{CH_3}$, 3H), 1.88 – 1.80 (m, *C6-H*, 1H), 1.77 (s, C_{vinyl}-CH_3, 3H), 1.76 (s, C_{vinyl}-CH_3', 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 156.01, 152.44, 150.14, 142.46, 140.52, 139.42, 137.64, 134.83, 128.18, 122.91, 119.54, 108.21, 107.92, 83.57, 65.56, 60.61, 60.56, 55.83, 48.21, 39.52, 38.36, 30.01, 25.50, 22.65, 18.16.

Elemental analysis: for $C_{25}H_{30}INO_5$ calcld.: C, 54.45; H, 5.48; found: C, 54.59; H, 5.37.

***N*-((*5S*)-2-iodo-9,10,11-trimethoxy-3-(((E)-pent-2-en-1-yl)oxy)-6,7-dihydro-5H-dibenzo-[a,c]cycloheptene-5-yl)acetamide (8d)**

Eluent: petroleum ether–ethyl acetate–ethanol (4 : 1 : 1). Pale-beige solid, yield 62%.

M.p. = 102 – 104 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 8.5 Hz, *NH*, 1H), 7.66 (s, *C8-H*, 1H), 6.96 (s, *C11-H*, 1H), 6.78 (s, *C4-H*, 1H), 6.02 – 5.94 (m, OCH_2-$\underline{CH}$, 1H), 5.69 (dt, *J* = 15.4, 5.7 Hz, CH_2-CH=$\underline{CH}$, 1H), 4.58 (d, *J* = 8.9 Hz, $O\underline{CH_2}$-CH, 2H), 4.53 – 4.44 (m, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.51 (s, *OMe*, 3H), 2.26 – 1.97 (m, *C6-H*, *C5-H*, $\underline{CH_2}$-CH_3, 5H), 1.89 (s, $CO\underline{CH_3}$, 3H), 1.87 – 1.81 (m, *C6-H*, 1H), 0.99 (t, *J* = 7.5 Hz, CH_2-$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 155.86, 152.46, 150.15, 142.44, 140.52, 139.43, 136.45, 134.83, 128.22, 123.60, 122.89, 108.20, 107.93, 83.41, 69.16, 60.61, 60.55, 55.83, 48.17, 38.42, 30.02, 24.77, 22.64, 13.17.

Elemental analysis: for $C_{25}H_{30}INO_5$ calcld.: C, 54.45; H, 5.48; found: C, 54.61; H, 5.59.

Methyl (*E*)-4-(((*5S*)-5-acetamido-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]-cycloheptene-3-yl)oxy)but-2-enoate (8e)

Eluent: petroleum ether–ethyl acetate–ethanol (8 : 1 : 1). White solid, yield 73%.

M.p. = 93 – 95 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 8.5 Hz, *NH*, 1H), 7.71 (s, *C8-H*, 1H), 7.05 (s, *C11-H*, 1H), 6.79 (s, *C4-H*, 1H), 6.77 (dt, *J* = 6.0, 1.6 Hz, $\underline{CH}$-CO_2Me, 1H), 5.13 (dd, *J* = 13.1, 7.2 Hz, $CH_2\underline{CH}$=, 1H), 4.49 (dt, *J* = 12.1, 7.8 Hz, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.64 (s, CO_2Me, 3H), 3.51 (s, *OMe*, 3H), 3.34 (s, OCH_2, 1H), 3.32 (s, OCH_2, 1H), 2.55 – 2.51 (m, *C6-H*, 1H), 2.17 (td, *J* = 12.2, 6.4 Hz, *C5-H*, 1H), 2.06 (dd, *J* = 12.6, 6.7 Hz, *C5-H*, 1H), 1.88 (s, $COCH_3$, 3H), 1.84 (dd, *J* = 11.2, 4.7 Hz, *C6-H*, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.18, 168.53, 154.29, 152.69, 150.14, 142.92, 142.06, 140.51, 139.70, 134.84, 130.53, 122.48, 110.12, 108.24, 104.67, 83.55, 60.67, 60.56, 55.83, 51.67, 48.10, 39.52, 38.28, 29.89, 29.40, 24.77, 22.66.

Elemental analysis: for $C_{25}H_{28}INO_7$ calcld.: C, 51.65; H, 4.85; found: C, 51.52; H, 4.77.

MALDI: (CHCA, pos. mode): 603.9 ($M+Na^+$) 100%, 581.0 (M^+) 14%.

_N_-((5*S*)-3-(((*E*)-2,7-dimethylocta-2,6-dien-1-yl)oxy)-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cycloheptene-5-yl)acetamide (8f)

Eluent: petroleum ether–ethyl acetate–ethanol (5 : 1 : 1). Pale-yellow oil, yield 55%. Atomic numeration for NMR assignment is given in SI.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, *J* = 8.5 Hz, *NH*, 1H), 7.65 (s, *C8-H*, 1H), 6.98 (s, *C11-H*, 1H), 6.78 (s, *C4-H*, 1H), 5.46 (t, *J* = 6.1 Hz, *C3'-H*, 1H), 5.11 – 5.04 (m, *C6'-H*, 1H), 4.65 (d, *J* = 6.4 Hz, *C1'-H*, 2H), 4.48 (dt, *J* = 11.9, 7.9 Hz, *C7-H*, 1H), 3.82 (s, *OMe*, 3H), 3.77 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.22 – 2.09 (m, *C6-H*, *C5-H*, 2H), 2.09 – 2.04 (m, *C4'-H*, *C5'-H*, 4H), 2.02 – 1.90 (m, *C6-H*, *C5-H*, 2H), 1.88 (s, *COCH$_3$*, 3H), 1.75 (s, *C2'-CH$_3$*, 3H), 1.62 (s, *C7'-CH$_3$*, 3H), 1.56 (s, *C7'-CH$_3$*, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.49, 155.99, 152.45, 150.16, 142.45, 140.96, 140.53, 139.40, 134.84, 131.06, 128.21, 123.71, 122.94, 119.26, 108.21, 108.15, 83.65, 65.59, 60.62, 60.56, 55.84, 48.23, 39.52, 38.98, 38.40, 30.02, 25.84, 25.51, 22.65, 17.60, 16.53.

Elemental analysis: for $C_{30}H_{38}INO_5$ calcld.: C, 58.16; H, 6.18; found: C, 58.28; H, 6.31.

_N_-((5*S*)-3-(cinnamyloxy)-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]-cycloheptene-5-yl)acetamide (8g)

Eluent: petroleum ether–ethyl acetate–ethanol (6 : 1 : 1). Beige solid, yield 73%.

M.p. = 181 – 183 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 8.5 Hz, *NH*, 1H), 7.69 (s, *C8-H*, 1H), 7.50 (d, *J* = 7.2 Hz, *C2'-H*, *C6'-H*, 2H), 7.37 (t, *J* = 7.5 Hz, *C3'-H*, *C5'-H*, 2H), 7.29 (t, *J* = 7.3 Hz, *C4'-H*, 1H), 7.04 (s, *C11-H*, 1H), 6.90 (d, *J* = 16.0 Hz, *CH=<u>CH</u>-Ph*, 1H), 6.78 (s, *C4-H*, 1H), 6.54 (dt, *J* = 16.0, 5.6 Hz, *<u>CH</u>=CH-Ph*, 1H), 4.83 (d, *J* = 5.5 Hz, *O<u>CH$_2$</u>*, 2H), 4.54 – 4.44 (m, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.51 (s, *OMe*, 3H), 2.55 – 2.51 (m, *C6-H*, 1H), 2.17 (dt, *J* = 18.0, 5.8 Hz, *C5-H*, 1H), 2.06 (td, *J* = 12.5, 5.9 Hz, *C5-H*, 1H), 1.94 – 1.85 (m, *C6-H*, 1H), 1.83 (s, *CO<u>CH$_3$</u>*, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 155.88, 152.47, 150.15, 142.56, 140.52, 139.48, 136.07, 134.82, 132.54, 128.69, 128.40, 127.96, 126.51, 124.52, 122.85, 108.22, 107.99, 83.45, 69.28, 60.62, 60.55, 55.83, 48.17, 38.41, 30.01, 22.61.

Elemental analysis: for $C_{29}H_{30}INO_5$ calcld.: C, 58.10; H, 5.04; found: C, 58.23; H, 5.19.

_N_-((5*S*)-3-((1,1-difluoroallyl)oxy)-2-iodo-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]-cycloheptene-5-yl)acetamide (8h)

The reaction was carried out in a closed vial due to the low boiling point of 3-bromo-3,3-difluoropropene. Eluent: petroleum ether–ethyl acetate–ethanol (7 : 1 : 1). White solid, yield 61%.

M.p. = 109 – 111 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, *J* = 8.1 Hz, *NH*, 1H), 7.77 (s, *C8-H*, 1H), 7.28 (s, *C11-H*, 1H), 6.81 (s, *C4-H*, 1H), 6.26 (ddt, *J* = 17.5, 10.8, 6.7 Hz, CF_2-CH=, 1H), 6.04 (dd, *J* = 17.2, 2.1 Hz, -CH=CH_2, 1H), 5.80 (d, *J* = 10.8 Hz, -CH=CH_2,1H), 4.44 (dt, *J* = 12.0, 7.6 Hz, *C7-H*, 1H), 3.84 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.55 (s, *OMe*, 3H), 2.55 (dd, *J* = 13.3, 5.9 Hz, *C6-H*, 1H), 2.23 – 2.01 (m, *C6-H*, *C5-H*, 3H), 1.86 (s, $COCH_3$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.52, 152.99, 150.16, 148.73, 142.80, 140.48, 139.93, 134.91, 133.41, 128.67, 123.64, 122.04, 116.90, 108.25, 88.11, 76.38, 60.77, 60.55, 55.86, 48.17, 37.92, 29.83, 22.48.

^{19}F NMR (377 MHz, DMSO-d_6) δ -65.69

Elemental analysis: for $C_{23}H_{24}F_2INO_5$ calcld.: C, 49.39; H, 4.33; found: C, 49.56; H, 4.24.

*General procedure for synthesis of compounds **9a-h, 10e***

Corresponding allyl ether **8a-h** (1.00 equiv.), Pd(dppf)Cl_2 (0.05 equiv.) and KOAc (3.00 equiv.) were placed into a Schlenk flask. The flask was filled with inert atmosphere and dry DMSO (15 mL) was added. The mixture was stirred for 18 hours at 80° C, poured into distilled water (100 mL), and extracted with AcOEt (3x100 mL). The extract was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified using column chromatography.

***N*-((*7S*)-1,2,3-trimethoxy-11-methylene-6,7,10,11-tetrahydro-5H-benzo[6,7]cyclohepta[1,2-f]benzofuran-7-yl)acetamide (9a)**

Eluent: petroleum ether–ethyl acetate–ethanol (8 : 1 : 1). Pale-beige solid, yield 66%.

M.p. = 198 – 200 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.37 (d, *J* = 8.4 Hz, *NH*, 1H), 7.41 (s, *C8-H*, 1H), 6.86 (s, *C11-H*, 1H), 6.77 (s, *C4-H*, 1H), 5.46 (s, C_{vinyl}-*H*, 1H), 5.14 (s, CH_2-*O*, 2H), 5.02 (s, C_{vinyl}-*H*, 1H), 4.51 – 4.43 (m, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.79 (s, *OMe*, 3H), 3.48 (s, *OMe*, 3H), 2.19 – 1.99 (m, *C6-H*, *C5-H*, 3H), 1.87 (s, $COCH_3$, 3H), 1.85 – 1.78 (m, *C6-H*, 1H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.38, 162.52, 152.19, 150.31, 143.89, 143.25, 140.56, 134.74, 126.57, 124.31, 123.61, 122.08, 108.03, 105.23, 99.63, 74.91, 60.55, 55.82, 48.50, 38.16, 29.94, 22.62.

Elemental analysis: for $C_{23}H_{25}NO_5$ calcld.: C, 69.86; H, 6.37; found: C, 69.73; H, 6.24.

MALDI: (CHCA, pos. mode) 394.3 (M^+) 100%.

***N*-((*7S*,*E*)-11-ethylidene-1,2,3-trimethoxy-6,7,10,11-tetrahydro-5H-benzo[6,7]-cyclohepta[1,2-f]benzofuran-7-yl)acetamide (9b)**

Eluent: petroleum ether–ethyl acetate–ethanol (7 : 1 : 1). Pale-beige solid, yield 62%.

M.p. = 163 – 165 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, *J* = 8.6 Hz, *NH*, 1H), 7.66 (s, *C8-H*, 1H), 6.96 (s, *C11-H*, 1H), 6.75 (s, *C4-H*, 1H), 5.95 – 5.88 (m, C_{vinyl}-*H*, 1H), 4.64 – 4.54 (m, $\underline{CH_2}$-*O*, 2H), 4.49 (dd, *J* = 7.6, 3.7 Hz, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.78 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.48 – 2.44 (m, *C6-H*, 1H), 2.19 – 2.10 (m, *C5-H*, 2H), 2.06 (dd, *J* = 13.0, 7.6 Hz, *C6-H*, 1H), 1.89 (s, *CO*$\underline{CH_3}$, 3H), 1.74 (d, *J* = 6.2 Hz, C_{vinyl}-$\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.30, 158.72, 152.01, 150.31, 141.29, 140.55, 138.28, 134.75, 126.89, 126.11, 125.76, 124.61, 116.36, 108.06, 104.29, 75.74, 60.56, 60.50, 55.81, 48.25, 45.75, 38.49, 30.09, 22.63.

Elemental analysis: for $C_{24}H_{27}NO_5$ calcld: C, 70.40; H, 6.65; found: C, 70.53; H, 6.72.

MALDI: (CHCA, pos. mode): 409.4 (M^+) 100%.

***N*-((*7S*)-1,2,3-trimethoxy-11-(propan-2-ylidene)-6,7,10,11-tetrahydro-5H-benzo[6,7]-cyclohepta[1,2-f]benzofuran-7-yl)acetamide (9c)**

Eluent: petroleum ether–ethyl acetate–ethanol (7 : 1 : 1). Yellow solid, yield 86%.

M.p. = 166 – 167 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (d, *J* = 8.5 Hz, *NH*, 1H), 7.65 (s, *C8-H*, 1H), 6.96 (s, *C11-H*, 1H), 6.78 (s, *C4-H*, 1H), 4.62 (d, *J* = 6.5 Hz, $\underline{CH_2}$-*O*, 2H), 4.48 (dd, *J* = 7.9, 4.1 Hz, *C7-H*, 1H), 3.83 (s, *OMe*, 3H), 3.77 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.21 – 2.01 (m, *C6-H*, *C5-H*, 3H), 1.88 (s, *CO*$\underline{CH_3}$, 3H), 1.87 – 1.84 (m, *C6-H*, 1H), 1.77 (s, C_{vinyl}-CH_3, 3H), 1.75 (s, C_{vinyl}-CH_3, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 156.01, 152.45, 150.15, 142.46, 140.52, 139.43, 137.65, 134.83, 128.18, 122.91, 119.54, 108.21, 107.93, 83.58, 65.57, 60.62, 60.56, 55.83, 48.21, 38.37, 30.01, 25.50, 22.65, 18.17.

Elemental analysis: for $C_{25}H_{29}NO_5$ calcld.: C, 70.90; H, 6.90; found: C, 70.76; H, 6.81.

MALDI: (pos. mode): 423.2 (M^+) 100%.

***N*-((*7S*)-1,2,3-trimethoxy-11-propylidene-6,7,10,11-tetrahydro-5H-benzo[6,7]-cyclohepta[1,2-f]benzofuran-7-yl)acetamide (9d)**

Eluent: petroleum ether–ethyl acetate–ethanol (4 : 1 : 1). Beige solid, yield 44%.

M.p. = 125 – 127 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, *J* = 3.4 Hz, *NH*, 1H), 7.31 (s, *C8-H*, 1H), 6.99 (s, *C11-H*, 1H), 6.77 (s, *C4-H*, 1H), 5.89 – 5.78 (m, C_{vinyl}-*H*, 1H), 5.15 (br.s., $\underline{CH_2}$-*O*, 2H), 4.49 – 4.46 (m, *C7-H*, 1H), 4.17 (q, *J* = 7.5 Hz, $\underline{CH_2}CH_3$, 2H), 3.82 (s, *OMe*, 3H), 3.77 (s, *OMe*, 3H), 3.44 (s, *OMe*, 3H), 2.48 – 2.46 (m, *C6-H*, 1H), 2.14 – 2.09 (m, *C5-H*, 2H), 2.03 – 2.01 (m, *C6-H*, 1H), 1.86 (s, $CO\underline{CH_3}$, 3H), 1.02 (t, *J* = 7.5 Hz, $CH_2\underline{CH_3}$, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.36, 161.90, 158.71, 152.02, 150.32, 142.51, 141.12, 140.58, 134.78, 131.05, 127.63, 126.82, 126.07, 124.69, 104.25, 76.25, 60.60, 60.51, 55.84, 48.27, 38.51, 30.12, 22.65, 17.61, 13.61.

Elemental analysis: for $C_{25}H_{29}NO_5$ calcld.: C, 70.90; H, 6.90; found: C, 70.78; H, 7.03.

MALDI (DCTB, pos. mode): 423.2 (M^+) 85%.

Methyl 2-((*7S*)-7-acetamido-1,2,3-trimethoxy-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-f]benzofuran-11-yl)acetate (10e)

Eluent: petroleum ether–ethyl acetate–ethanol (7 : 2 : 2). Yellow solid, yield 68%.

M.p. = 151 – 152 °C.

^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (s, *C8-H*, 1H), 7.62 (s, *C11-H*, 1H), 7.46 (s, C_{furan}-*H*, 1H), 6.77 (s, *C4-H*, 1H), 4.77 (dd, *J* = 11.9, 6.5 Hz, *C7-H*, 1H), 3.92 (s, CO_2Me, 3H), 3.91 (s, *OMe*, 3H), 3.78 (d, *J* = 0.5 Hz, $\underline{CH_2}CO_2Me$, 2H), 3.73 (s, *OMe*, 3H), 3.49 (s, *OMe*, 3H), 2.54 (dd, *J* = 12.1, 5.0 Hz, *C6-H*, 1H), 2.29 (ddd, *J* = 16.0, 11.8, 6.2 Hz, *C5-H*, 2H), 2.05 (s, $CO\underline{CH_3}$, 3H), 2.00 – 1.94 (m, *C6-H*, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 173.08, 172.48, 156.32, 154.11, 152.20, 144.49, 142.43, 138.67, 136.62, 130.48, 127.42, 126.51, 122.33, 114.81, 109.01, 106.67, 61.61, 61.44, 56.59, 52.60, 50.75, 39.83, 31.42, 30.04, 22.64.

Elemental analysis: for $C_{25}H_{27}NO_7$ calcld.: C, 66.21; H, 6.00; found: C, 66.45; H, 6.13.

***N*-((*7S*)-1,2,3-trimethoxy-11-(6-methylhept-5-en-2-ylidene)-6,7,10,11-tetrahydro-5H-benzo[6,7]cyclohepta[1,2-f]benzofuran-7-yl)acetamide (9f)**

Eluent: petroleum ether–ethyl acetate–ethanol (4 : 1 : 1). Pale-beige oil, yield 69%. For atomic numeration in NMR assignment see SI.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, *J* = 8.5 Hz, *NH*, 1H), 6.98 (s, *C8-H*, 1H), 6.78 (s, *C11-H*, 1H), 6.75 (s, *C4-H*, 1H), 4.99 (t, *J* = 6.4 Hz, *C4'-H*, 1H), 4.84 (d, *J* = 9.2 Hz, OCH_2, 2H), 4.50 – 4.47 (m, *C7-H*, 1H), 3.81 (s, *OMe*, 3H), 3.76 (s, *OMe*, 3H), 3.44 (s, *OMe*, 3H), 2.47 – 2.42 (m, *C5-H*, 1H), 2.13 – 2.01 (m, *C5-H*, *C6-H*, *C2'-H*, *C3'-H*, 7H), 1.87 (s, $COCH_3$, 3H), 1.63 (s, *C1'*-CH_3, 3H), 1.55 (s, *C5'*-CH_3, 3H), 1.46 (s, *C5'*-CH_3, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.33, 159.24, 152.02, 150.29, 149.01, 141.23, 140.58, 134.77, 131.03, 126.63, 126.12, 126.05, 124.72, 123.91, 123.86, 108.05, 104.20, 75.82, 60.57, 60.50, 55.83, 48.53, 48.28, 38.49, 32.58, 25.98, 25.35, 23.30, 22.66, 17.46.

Elemental analysis: for $C_{30}H_{37}NO_5$ calcld.: C, 73.29; H, 7.59; found: C, 73.47; H, 7.75.

MALDI (pos. mode): 491.2 (M^+) 83%.

***N*-((*7S*)-11-((*Z*)-benzylidene)-1,2,3-trimethoxy-6,7,10,11-tetrahydro-5H-benzo[6,7]-cyclohepta[1,2-f]benzofuran-7-yl)acetamide (9g)**

Eluent: petroleum ether–ethyl acetate–ethanol (4 : 1 : 1). Light-brown solid, yield 63%.

M.p. = 135 – 137 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 8.4 Hz, *NH*, 1H), 7.58 (s, *C8-H*, 1H), 7.41 (t, *J* = 7.6 Hz, *C2$_{Ar}$-H*, *C6$_{Ar}$-H*, 2H), 7.32 (d, *J* = 7.4 Hz, *C3$_{Ar}$-H*, C5$_{Ar}$-H, 2H), 7.25 (t, *J* = 7.3 Hz, *C4$_{Ar}$-H*, 1H), 6.99 (t, *J* = 2.9 Hz, *C$_{vinyl}$-H*, 1H), 6.93 (s, *C11-H*, 1H), 6.79 (s, *C4-H*, 1H), 5.53 (qd, *J* = 16.3, 3.0 Hz, *CH$_2$-O*, 2H), 4.52 (dd, *J* = 17.0, 9.5 Hz, *C7-H*, 1H), 3.84 (s, *OMe*, 3H), 3.81 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.56 – 2.51 (m, *C6-H*, 1H), 2.19 – 2.06 (m, *C5-H*, 2H), 1.89 (s, *COCH$_3$*, 3H), 1.87 – 1.83 (m, *C6-H*, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.43, 161.42, 152.21, 150.37, 143.77, 140.60, 136.72, 136.27, 134.78, 128.73, 127.99, 126.87, 126.51, 124.91, 124.39, 121.52, 115.95, 108.07, 105.02, 74.69, 60.62, 60.61, 55.83, 48.56, 38.17, 29.97, 22.64.

Elemental analysis: for $C_{29}H_{29}NO_5$ calcld.: C, 73.87; H, 6.20; found: C, 73.72; H, 6.34.

MALDI (DCTB, pos. mode): 471.1 (M^+) 87%.

***N*-((*7S*)-10,10-difluoro-1,2,3-trimethoxy-11-methylene-6,7,10,11-tetrahydro-5H-benzo-[6,7]cyclohepta[1,2-f]benzofuran-7-yl)acetamide (9h)**

Eluent: petroleum ether–ethyl acetate–ethanol (6 : 1 : 1). White solid, yield 73%.

M.p. = 126 – 128 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, *J* = 8.1 Hz, *NH*, 1H), 7.67 (s, *C8-H*, 1H), 7.11 (s, *C11-H*, 1H), 6.81 (s, *C4-H*, 1H), 6.35 (br.s, *C$_{vinyl}$-H*, 1H), 5.93 (br.s, *C$_{vinyl}$-H'*, 1H), 4.50 (dt, *J* = 14.6, 7.4 Hz, *C7-H*, 1H), 3.84 (s, *OMe*, 3H), 3.80 (s, *OMe*, 3H), 3.50 (s, *OMe*, 3H), 2.59 – 2.52 (m, *C6-H*, 1H), 2.19 – 2.05 (m, *C5-H*, 2H), 1.97 – 1.93 (m, *C6-H*, 1H), 1.89 (s, *COCH$_3$*, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.64, 154.71, 152.66, 150.26, 145.59, 140.55, 135.98 ($J_{C\text{-}F}$= 25.5 Hz), 134.80, 129.87, 127.82, 123.34, 123.24, 119.30, 113.93, 108.14, 105.52, 60.68, 60.59, 55.84, 48.75, 37.97, 29.73, 22.60.

^{19}F NMR (377 MHz, DMSO) δ -66.04 ($J_{H, F}$= 372.5, $J_{H', F}$= 165.2 Hz).

Elemental analysis: for $C_{23}H_{23}F_2NO_5$ calcld: C, 64.03; H, 5.37; found: C, 64.12; H, 5.45.

MALDI: (pos. mode): 431.1 (M^+) 74%, 454.1 ($M+Na^+$) 11%, 470.0 ($M+K^+$) 20%.

***N*-((*7S*)-1,2,3-trimethoxy-11-methyl-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-f]benzofuran-7-yl)acetamide (10a)**

Compound **9a** (100.0 mg, 0.253 mmol) was dissolved in dry DCM (15 mL). Trifluoroacetic acid (3 mL) was added, the mixture was stirred at room temperature for 1 hour, neutralized with sat. aq. $NaHCO_3$, and extracted with AcOEt (50 mL x 3). The extract was dried over Na_2SO_4 and evaporated under reduced pressure. Yellow solid, yield 97.7 mg (98%).

M.p. = 202-204 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, *J* = 8.5 Hz, *NH*, 1H), 7.74 (d, *J* = 1.3 Hz, *C8-H*, 1H), 7.49 (s, *C11-H*, 1H), 7.47 (s, *C9-H*, 1H), 6.79 (s, *C4-H*, 1H), 4.62 (dt, *J* = 11.9, 7.8 Hz, *C7-H*, 1H), 3.84 (s, *OMe*, 3H), 3.80 (s, *OMe*, 3H), 3.45 (s, *OMe*, 3H), 2.21 (d, *J* = 1.1 Hz, *C10-CH$_3$*, 3H), 2.19 – 2.11 (m, *C6-H*, 1H), 2.02 (td, *J* = 12.6, 6.9 Hz, *C5-H*, 1H), 1.90 (s, *CO<u>CH$_3$</u>*, 3H), 1.89 – 1.82 (m, *C6-H*, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.40, 154.00, 152.28, 150.38, 141.94, 140.60, 137.67, 134.75, 128.54, 126.78, 124.74, 120.36, 115.19, 108.00, 105.78, 60.59, 60.57, 55.83, 48.37, 38.44, 30.69, 22.71, 7.66.

Elemental analysis: for $C_{23}H_{25}NO_5$ calcld.: C, 69.86; H, 6.37; found: C, 69.59; H, 6.24.

MALDI: (pos. mode): 395.2 (M^+) 89%, 418.1 ($M+Na^+$) 14%.

Biology

Cell cultures

Human cell lines: pancreatic (PANC-1, COLO357), cervical (HeLa), colon (SW620) cancers, Burkitt's lymphoma (Raji), and immortalized kidney (HEK-293) and keratinocytes (HaCaT) cells were grown in DMEM medium supplemented with 10% fetal calf serum (FCS), pen-strep-glut (all from PanEco, Moscow, Russian Federation). Adherent cells were detached using 0.05% trypsin-EDTA (PanEco, Moscow), counted and sub-cultured. For the assay cells were seeded in the appropriate plates (96- or 24-well plates), adjusted to $3x10^5$ cells/mL.

MTT-assay

Cytotoxic effect of colchicine and the compounds was estimated by a standard 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma) test. All the compounds were dissolved in dimethyl sulfoxide to 20 mM concentration and stored at -20^{o}C until use. Different dilutions of the new compounds from 20 μM to 0.001 nM were prepared separately and transferred in 100 μl to the plates. Cell were added at 5-10*10^3 per well. Non-treated cells served

as controls. Plates were incubated for 72 hrs. For the last 4 hours, 10 µl MTT (5 mg/ml) were added to each well. After the incubation, culture medium was removed and 100 µl dimethyl sulfoxide was added to each well. Plates were incubated in a shaker 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.

Confocal analysis

For confocal analysis, cells were grown overnight on sterile cover slips immersed into 6-well plates (Costar) in 200 µl of complete culture medium. Colchicine or new compounds **9a** and **9h** were dissolved in 4 ml of complete medium and added to the wells. β-tubulin was identified by anti-β-tubulin antibody (SantaCruz, USA) followed by anti-mouse IgG-AlexaFluor555 (Molecular Probes, Invitrogen, USA) in cells permeabilized by 0.1% Triton X100 in PBS. Slides were analyzed using Eclipse TE2000 confocal microscope (Nikon, Japan).

Flow cytometry

Cell cycle was analyzed using PI-stained DNA. Cells from colchicinoid-treated cultures were collected at indicated time, trypsinized, washed in ice-cold PBS, fixed by the addition of 70% ethanol and left for 2 hours at -20 ^{o}C. Thereafter, the cells were washed twice in PBS, stained with 50 µg/ml of propidium iodide (Sigma, USA) in PBS, treated with 10 µg/ml of RNAse and analyzed by flow cytometry using FACScan device (BD, USA). Total 2000 events were collected. The results were analyzed using WinMDI 2.8.

Acute toxicity in vivo

Determination of the acute *in vivo* toxicity was performed in C57BL/6, BALB/c, CBA, A/Sn, and outbred CD1 mice (6 mice of each strain per group). All experimental procedures were approved by Animal Care and Use Committee of Nizhny Novgorod State University, Russia. Several genetically different strains were used to cover various genotypes. Preparations **9a**, **9h**, and colchicine were dissolved in DMSO to 20 mM, diluted with saline, and injected intravenously in 200 µl per mouse. Total volume of stack solution of colchicinoids in DMSO varied from 30 to 43 µl depending on the mouse body weight. DMSO was diluted in the same way (40 µl of DMSO in 160 µl saline) and was used as a control. Preparations were injected 5 (n=3 per strain, 15 total mice for preparation) and 10 mg/kg (n=3 per strain, 15 total mice for

preparation) of body weight. Mice were monitored during a week, lethality was detected only at days 1-2. All studies were conducted in an AAALAC accredited facility in compliance with the PHS Guidelines for the Care and Use of Animals in Research.

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Microtubule assembly assay

Tubulin was purified from the lamb brain by ammonium sulfate fractionation and ion exchange chromatography. The pure protein was stored in liquid nitrogen and prepared as described[38]. Protein concentrations were determined spectrophotometrically with a PerkinElmer spectrophotometer Lambda 800 and an extinction coefficient at 275 nm of 1.07 L·g−1·cm−1 in 0.5% SDS in neutral aqueous buffer or 1.09 L·g−1·cm−1 in 6 M guanidine hydrochloride. Microtubule assembly was performed in 20 mM sodium phosphate buffer, 1 mM EGTA, 10 mM $MgCl_2$, 3.4 M glycerol, and 1 mM GTP at pH 6.5. The aliquots were incubated for 40 min at 4 °C prior to start the reaction by warming the samples to 37 °C in thermostated cuvettes. The mass of polymer formed was monitored by turbidimetry at 350 nm with a Jasco V-750 spectrophotometer. Samples containing the compound and their controls had less than 3% residual DMSO.

Crystallization, data collection, and structure refinement of 9a bound to the T_2R complex.

Compound **9a** was added to the T2R complex using a 5-fold excess of the ligand over tubulin (purified from ovine brain). Crystals were grown from T_2R seeds obtained from subtilisin-treated tubulin[45]. X-ray data were collected on the PROXIMA 1 beamline at SOLEIL Synchrotron (Saint-Aubin, France), and were processed with autoPROC[48] which implements the STARANISO treatment for anisotropy (http://staraniso.globalphasing.org/). Molecular replacement was done with Phaser[49] using the T_2R complex (pdb id 3RYC) as the search model. The structural model was refined by BUSTER[50] with iterative model building in Coot[51]. Data collection and refinement statistics are reported in Table 1 in Supplementary Information. The atomic coordinates and structure factors have been deposited in the Protein Data Bank under accession code 6TDE. Figures of structural models were generated with PyMOL (www.pymol.org).

Associated content

Supporting Information

Supplementary data to this article can be found online at www.sciencedirect.com.

Accession Codes

PDB code for T2R with 9a is 6TDE.

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Notes

The authors declare no competing financial interest.

Author contributions

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Highlights

- Synthesis of two novel types of heterocyclic colchicinoids is described
- Selected compounds exhibited antiproliferative activity in picomolar range
- Inhibition of microtubules polymerization is observed in sub-stoichiometric mode
- Acute toxicity of novel colchicnoids is decreased in comparison with natural colchicine

Declaration of interests

v The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: