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7-Deacetyl-10-alkylthiocolchicine derivatives – new compounds with potent anticancer and fungicidal activity

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A series of new semi-synthetic 7-deacetyl-10-alkylthiocolchicne derivatives with ethyl, *n*-propyl, *i*-propyl and *n*-butyl substituents were synthesised and characterised by spectroscopic method, elemental analysis, DFT calculations and molecular docking simulations. All synthesized compounds have been tested for fungicidal and anticancer activities against SKOV-3, LoVo, MFC-7, MDA-MB-231 and lung-derrieved fibroblasts CCD39Lu. All new colchicine derivatives exhibit significantly higher cytotoxicity towards SKOV-3 tumour cell line than that of the natural product - colchicine. The most effective cytotoxic agents were 7-deacetyl-10-*n*-buthylthiocolchicine and 7-deacetyl-10-*i*-propylthiocolchicine. Among all compounds tested, 7-deacetyl-10-*n*-buthylthiocolchicine revealed the highest fungicidal activity. Molecular modeling indicated that several mutations found in the β-tubulin unit of tested fungal strains are crucial for antifungal activity and selectivity of 7-deacetyl-10-*n*-buthylthiocolchicine. Obtained results may be useful for the development of selective colchicine derivatives as effective fungicidal and / or anticancer drugs.

Introduction

Colchicine **(1)** Figure 1., is an alkaloid isolated from *Colchicum autumnale* and *Gloriosa superba* and is known to show notable anti-inflammatory, anti-mitotic, and anti-fibrotic effects.[1,2] The colchicine behaviour in human body, its anti-inflammatory and anticancer properties as well as its interaction with amino acids in tubulin have been studied for many years.[3-5] Although colchicine shows significant *in vitro* antitumor effects, it has limited applications in medicine because of high toxicity.[6] One of the best known colchicine derivatives is 10-methylthiocolchicine (known as thiocolchicine), **2** Figure 1[7,8] – the compound with higher biological activity than colchicine **1** and with confirmed anti-cancer properties but almost insoluble in water.[9,10] Other 10-alkilthiocolchicine derivatives **3-6** with alkyl chain C2 to C4 (-C_2H_5, *n*-C_3H_7, *i*-C_3H_7 and *n*-C_4H_9) were also obtained and their cytotoxic activity was tested.[8] Unfortunately these compounds were less active than **2**.[8] Insolubility compound **2** in water limits the interest of this compound as a drug candidate. 10-Methylthiocolchicine, however, has been so far scientific interest as a starting compound to obtain other biologically active derivatives.[10-19] One of them is 7-deacetyl-10-methylthiocolchicine **7**, Figure 1, which inhibitory effect has been tested on tubulin assembly.[13,20-23] Both 10-methylthiocolchicine **2** and its derivative **7** have been screened as conjugate compounds for tubulin.[23] Many derivatives of **7** have been also obtained and tested as anticancer agents.[14,15,17,25] e.g. thiocolchicoside (3-*O*-glycosyl-3-demethylthiocolchicine) is a muscle relaxant agent and an anti-inflammatory drug.[26] Despite the large number of known colchicine derivatives there remains a need for new derivatives with better biological activity than that of starting compound. Hence, in the present communication we report the synthesis and characterization of new thiocolchicine analogues showing good water solubility and better fingicidal and anticancer activity than that of colchicine **1** and thiocolchicine **2**.

Results and discussion

The aim of the current studies is to determine cytotoxic and fungicidal activity of new colchicine **1** derivatives. The synthesis of new alkylthiocolchicine derivatives **7-11** with -CH_3, -C_2H_5, *n*-C_3H_7, *i*-C_3H_7 and *n*- C_4H_9 substituents at C-10 carbon atom are presented in Figure 1. The experimental and full spectral data for compounds **7-11** are given in Supplementary Data. The obtained compounds were characterized by spectroscopic methods (^{13}C NMR, ^{1}H NMR, FT IR, EI MS, UV-vis, elemental analysis) as well as by DFT and molecular docking calculations.

The cytotoxic activity of all 7-deacetyl-10-alkylthiocolchicines **7-11** against SKOV-3 (human cancer ovarian cell) LoVo (human colon carcinoma), estrogen-dependent MFC-7, estrogen-independent MDA-MB-231 breast cancer cells and lung derived fibroblasts CCD39Lu as well as their antifungal activity against selected molds

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Electronic Supplementary Information (ESI) available: [experimental details, spectroscopic data (NMR, FT IR,EI MS), DFT and docking studies, details of cytotoxic and fungicidal data] See DOI: 10.1039/x0xx00000x

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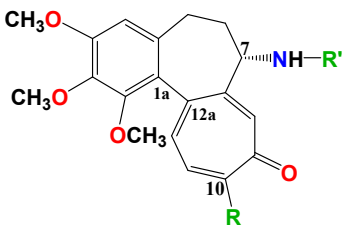
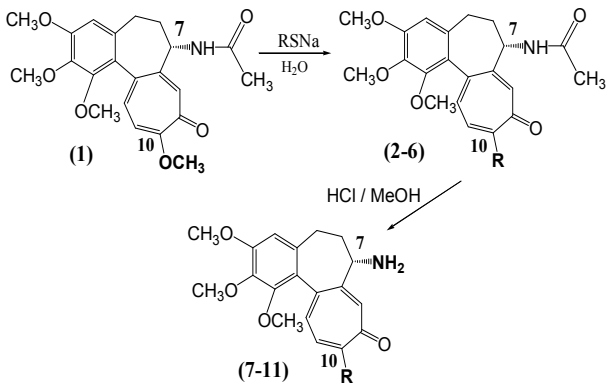
were also studied. All used in experiments cell lines were obtained from The *European Collection* of Cell Cultures (ECACC) Salisbury UK via Sigma-Aldrich Poland. For experiments two types of cell culture were used: proliferating and growth arrested cells.

Figure 1. Colchicine **1** and its derivatives **2-11**



	R	R'
1	OCH_3	$COCH_3$
2	SCH_3	$COCH_3$
3	SCH_2CH_3	$COCH_3$
4	$SCH_2CH_2CH_3$	$COCH_3$
5	$SCH(CH_3)_2$	$COCH_3$
6	$SCH_2CH_2CH_2CH_3$	$COCH_3$
7	SCH_3	H
8	SCH_2CH_3	H
9	$SCH_2CH_2CH_3$	H
10	$SCH(CH_3)_2$	H
11	$SCH_2CH_2CH_2CH_3$	H

Scheme 1. Synthesis of 10-alkilthiocolchicines[a] and 7-deacetyl-10-alkylthiocolchicines[b]



Reagents and conditions: [a] Colchicine (**1**), RSNa (R: Me, Et, *i*Pr, *n*Pr, *n*Bu), water, ~40°C for 2-14 h, [b]: 10-alkylthiocolchicine (**2-6**), HCl (aq)/MeOH, reflux at ~70°C for 6-16 h. Time for completion of all the reaction was indicated by TLC.

The UV-Vis spectra of compounds **7-11** revealed bathochromic shifts with respect spectra of compounds **2-6**, which were explained by the disappearance of acetyl substituent at C-7 position in coupled trimethoxyphenyl and tropolonic chromophores, from 255-258 for 2-6 to 245-250 nm for **7-11** and from 385-388 to 360-375 nm for trimethoxyphenol and tropolonic chromophores, respectively. ^1H and ^{13}C NMR spectra of 7-deacetyl-10-alkylthiocolchicine derivatives **7-11** were recorded in two different solvents: DMSO-d_6 and $CDCl_3$, details are given in Supplementary Data. In the EI MS mass spectra (see Supplementary Data, Figures S2-S5) of all new colchicine derivatives signals of molecular ions are observed at: *m/z* = 372 (for 7), *m/z* = 387 (for 8), *m/z* = 401 (for **9** and **10**) and at *m/z* = 415 (for 11). In the FT IR spectra (KBr pellets) as a result of the deacetylation of the acetamide substituent at C-7 position, the bands assigned to stretching vibration of C=O for compounds **2-6** in the region 1681-1678 cm^{-1} disappeared (see Supplementary Data, Figures S6-S10). The second band assigned to stretching vibration of C=O assigned to C-9 of the tropolone ring which appeared in the region of 1603-1606 cm^{-1} for **2-6** is shifted to the lower wave numbers: 1598-1596 cm^{-1} for compounds **7-11**.

The natural (-)-(7S) colchicine has two aromatic moieties arranged counterclockwise. Colchicine displays molecular asymmetry derived from a non-planar arrangement of the tropolonic ring C and trimetoxyphenyl ring A. Rings A and C are twisted around the C1a-C12a bond, the helicity around this bond is *aR* and the torsion angle is close to 53° in colchicine and its biologically active derivatives.[27] Additional information about colchicine and its derivatives **2-11** were obtained at the theoretical level. The geometry optimization in gas-phase of molecules **1-11** were performed using DFT method B3LYP in the combination with 6-31G basis set using Gaussian03 program package (see Supplementary Data p. S19). Calculated dihedral angles for compounds **2-5**, **7**, **8** and **11** are close to this for **1**.

Table 1. Calculated dihedral angle [1-1a-12a-12] and energies of colchicine **1** and its derivatives **2-11**. Total energies are given in Hartrees (H = 627.5 kcal mol^{-1})

Compound	Energy	Δ Energy[a]	Dihedral angle
1	-1397.57135849	-	52.75057
2	-1680.05840199	-190.593435	53.64795
3	-1681.38806998	-152.619133	53.95637
4	-1720.69238336	-152.619024	53.66950
5	-1720.69364176	-152.619006	53.82392
6	-1759.99516748	-152.619676	54.01425
7	-1489.46496671	-	53.97105
8	-1528.76893614	-	53.71845
9	-1568.07335993	-	54.33612
10	-1568.07463599	-	54.39321
11	-1607.37549128	-	53.30232

[a]calculated as a difference between 10-alkylthiocolchicines (**2-6**) and 7-deacetyl-10-alkylthiocolchicines (**7-11**).

The calculated energies of optimized structures as well as the value of dihedral angleare presented in Table 1. The results clearly indicate that the all thiocolchicines present the most stable compounds in the gas phase than the colchicine. Regarding the data collected in Table 1 it reveals thatthe calculated energy difference between compounds **2** and **7** is -190.593435 H and the difference in the computed energy between the thiocolchicine derivatives **3-6** and their deacetyl counterparts **8-11** are lower and almost the same (approx. -152 H) irrespective to the length of thioalkyl chain at C-10 position.

The new colchicine derivatives have been studied mainly as potential anticancer agents because of the antimitotic properties of colchicine. The cytotoxic activity of **7-11** 10-alkylthio analogues was screened on human ovarian cancer cell lines SKOV-3, human colon cancer cell line LoVo, two human breast cancer cell lines: MFC-7, MDA-MB-231 and lung fibroblasts CCD39Lu (Table 2). In the cytotoxicity studies, the tested compounds were dissolved in DMSO (final conentration 0,1%) as a positive control Doxorubicine was used, while as negative control DMSO alone was administerd.

The IC_{50} values were calculated from concentration–effect curves obtained in GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA. Statistical analyses were carried

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out using one-way ANOVA with Dunnett's multiple comparison tests. Moreover, the statistically significant differences ($p<0.005$) between the values for proliferating and growth arrested cells are marked with asterisks (for SKOV-3 cell line). As we can see from Table 2 the cytotoxic activity of tested compounds towards SKOV-3 cell line was significantly higher than that activity of colchicine **1**. Surprisingly except compound **7** (whose high activity was expected) also derivatives with longer alkylthio chain **10** and **11** showed high activity. Moreover tested compounds were significantly higher active against proliferating cells when compared to growth arrested cells what suggested their interaction with cellular mitotic mechanisms. Further studies are necessary in order to fully explain their antimitotic activity (Table. 2).

Table 2. The IC_{50}[a] values (nM) of compounds **7-11** tested against selected cancer cell line after 72 hours of incubation. Data were obtained from triplicate experiments. **Doxorubicin** was used as positive control

Cell line	MCF-7	MDA-MB-231	DLD1	LoVo	CCD39Lu
Doxorubicin	9.84	1240	145	78.3	850

	IC_{50} [nM] proliferating cells					
	Compound					
Cell line	**1**	**7**	**8**	**9**	**10**	**11**
SKOV-3	198	1.3	305	22	4	9
MCF-7	41	670	1620	2080	4900	1460
MDA-MB-231	25	1260	3550	4240	5000	4180
DLD1	43	1180	510	3420	6260	720
LoVo	119	1020	390	3830	130	1870
CCD39Lu	586	1920	150	670	1680	2170

	SKOV-3 cell line	
Compound	IC_{50} [nM] proliferating cells	IC_{50} [nM] non-proliferating cells
1	198	210*
7	1.3	9.6*
8	305	409*
9	22	46*
10	4	27*
11	9	62*
doxorubicin	32.6	128

[a] compound concentration to inhibit tumor cell proliferation by 50%

Colchicine binding to fungal tubulins is characterized with lower affinity as compared with that to mammalian tubulins.[28] Colchicine has been tested previously for antifungal activity against some microfungi species.[29,30] The results tests shown that **1** inhibited the growth of *Candida*.[30] Colchicine significantly inhibited the fungicidal activity of neutrophils against *P.marneffei* in dose-dependent manner and this inhibition was not due to its cytotoxic effect.[31] Chronic human exposure to different microfungi species can cause symptoms of disease (*A. pullulans* can lead to hypersensitivity pneumonitis or "humidifier lung").[32.33] In our previous study colchicine complexes exhibited some fungicidal activity.[34] In this study the fungicidal activity of new colchicine derivatives **7-11** as well as their 10-alkylthiocolchicines counterpart **2-6** were tested against eight fungal strains (screening test are given in Table S2). Two known fungicides: IPBC and chalkone were tested as a reference. As a positive control, Amphotericin B (1μg/mL) and as a negative control untreated agar medium were used. The results of MIC and MFC values obtained for all tested compounds are presented in Table 3 and Table S2. Colchicine **1** showed good activity against *A. pullulans* only but compounds **2-6** were completely inactive against all tested microfungi species. The derivatives with longer alkylthio chain: 7-deacetyl-10-*n*-buthylthiocolchicine **11** and 7-deacetyl-10-*n*-propylthiocolchicine **9** were the most active compounds against 5 among the 8 tested fungus (Table 3). Among the compounds with propylthio substituent less active was this with unbranched alkylthio chain **9** than the one with *i*-propylthio substituent **10**. Derivatives **9-11** showed to be active againstthe following fungi strains: *P. variotti,P. funiculosum, T. viride, P. cyclopium*and **10-11** especially against *A. pullulans* and *Ch. Globosum*. The results permit a conclusion that the antifungal properties of some new colchicine derivatives may be potentially useful for partly controlling strains.

Although lipophilicity as a property facilitating passive drug transport should increase the toxicity of compounds tested, it is known that lipophilicity may be also a significant factor modulating molecular interactions resulting in changes in ligand-receptor binding or enzyme inhibition potency.[35] Calculated values of LogP (Table 4) of tested compounds showed that in comparison to the starting compounds **2-6** the new one **7-11** showed to be less hydrophobic. As expected, with extension of alkyl chain in C-10 position the lipophilicity of the molecules increases. Compounds **2-6** have lipophilicity at the range of 2.17-3.60. To be able to make some comparison between tested derivatives with the shortest (**2** and **7**) and the longest alkylthio chain **6** and **11** their molecules (the molecular surface for space-filling CPK models) were visualized in Figure.S11-S12. Because the most potent derivatives occurred to be **9-11**, both as cytotoxic and fungicidal agents, we suggested that amino NH_2 substituent at C7, longer alkylthio chain at C10 and lipophilicity at the range of 2.19-2.89 are optimal for their biological activity.

The molecular docking was performed to study the molecular mechanism of binding for synthesized series of compounds. The compound **11**, which globally showed the best anticancer and antifungal profile was selected to visualize the key molecular interactions within protein targets responsible for anticancer and antifungal activity. The colchicine–*β*-tubulin complex (PDB ID: 1SA0) was used to assess the potential of the compounds **2-11** as an anticancer agents and as a template to homology modeling of the β-tubulin of tested fungus. Docking studies revealed that binding modes of the compounds with the highest anticancer activity are coherent with the co-crystallized colchicine (Figure 2A). The trimethoxyphenyl ring of **11** is buried in the *β*-subunit occupying the same position as the corresponding ring of the colchicine. To evaluate the antifungal activity, a homology models of β-tubulin for all fungus were generated and used in molecular docking study. It should be noted that adding an alkylthio chain at C_{10} led to rotation of the colchicine ring in the binding site and exposing the alkylthio substituent toward the hydrophobic cavity formed by Lys254β, Val181α and Thr179α residues. Six out of eight tested strains showed more than 80% similarity to human β-tubulin (Table S1), whereas, one (*A. pullulans*) showed only approx. 41% of similarity. In general, the binding mode of **11** in species, where it showed the highest antifungal activity (i.e. *P.cyclopium*, *A.pullulans*, *Ch.globosum*) was coherent, however, different than in human β-tubulin (Figure 2B).

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Table 3. Antifungal activity of compounds **1** and **2-11** the results of MIC, MFC [μg/mL] and [mMol/mL] and lipophilicity

Compounds	MIC [μg/mL] and [mMol/mL] [a] Fungi								LogP
	A. niger	*A. versicolor*	*P. variotti*	*P. funiculosum*	*T. viride*	*P. cyclopium*	*A. pullulans*	*Ch. globosum*	
1	>4000	>4000	>4000	>4000	>4000	>4000	1±0.0 [$2.5\cdot10^{-12}$]	>4000	1.10
2	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000	2.17
3	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000	2.54
4	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000	3.04
5	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000	2.90
6	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000	3.60
7	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000	1.45
8	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000	1.83
9	>4000	>4000	1000±0.0 [$2.5\cdot10^{-10}$]	2000±600 [$4.9\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	>4000	>4000	2.33
10	>4000	>4000	1000±0.0 [$2.5\cdot10^{-10}$]	1000±0.0 [$2.5\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	500±0.0 [$1.2\cdot10^{-10}$]	260±0.0 [$6.5\cdot10^{-11}$]	130±0.0 [$3.2\cdot10^{-11}$]	2.19
11	>4000	>4000	1000±0.0 [$2.4\cdot10^{-10}$]	1000±0.0 [$2.4\cdot10^{-10}$]	2000±0.0 [$4.8\cdot10^{-10}$]	260±0.0 [$6.3\cdot10^{-11}$]	260±0.0 [$6.3\cdot10^{-11}$]	130±0.0 [$3.1\cdot10^{-11}$]	2.89
chalcone (fungicide)*	65±0.0 [$3.1\cdot10^{-11}$]	2000±0.0 [$9.6\cdot10^{-10}$]	1000±0.0 [$4.8\cdot10^{-10}$]	130±0.0 [$6.2\cdot10^{-11}$]	500±0.0 [$2.4\cdot10^{-10}$]	260±0.0 [$1.2\cdot10^{-10}$]	500±0.0 [$2.4\cdot10^{-10}$]	130±0.0 [$6.2\cdot10^{-11}$]	3.81
IPBC [b] (fungicide)*	2±0.0 [$7\cdot10^{-12}$]	2±0.0 [$7\cdot10^{-12}$]	2±0.0 [$7\cdot10^{-11}$]	2±0.0 [$7\cdot10^{-12}$]	100±0.0 [$3.5\cdot10^{-8}$]	2±0.0 [$7\cdot10^{-12}$]	1±0.0 [$3.5\cdot10^{-12}$]	5±0.0 [$1.7\cdot10^{-11}$]	2.84

Compounds	MFC [μg/mL] and [mMol/mL] [a]							
	A. niger	*A. versicolor*	*P. variotti*	*P. funiculosum*	*T. viride*	*P. cyclopium*	*A. pullulans*	*Ch. globosum*
1	>4000	>4000	>4000	>4000	>4000	>4000	1±0.0 [$2.5\cdot10^{-12}$]	>4000
9	>4000	>4000	1000±0.0 [$2.5\cdot10^{-10}$]	2000±600 [$4.9\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	>4000	>4000
10	>4000	>4000	1000±0.0 [$2.5\cdot10^{-10}$]	1000±0.0 [$2.5\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	500±0.0 [$1.2\cdot10^{-10}$]	260±0.0 [$6.5\cdot10^{-11}$]	130±0.0 [$3.2\cdot10^{-11}$]
11	>4000	>4000	1000±0.0 [$2.4\cdot10^{-10}$]	1000±0.0 [$2.4\cdot10^{-10}$]	2000±0.0 [$4.8\cdot10^{-10}$]	260±0.0 [$6.3\cdot10^{-11}$]	260±0.0 [$6.3\cdot10^{-11}$]	130±0.0 [$3.1\cdot10^{-11}$]
chalcone (fungicide)*	65±0.0 [$3.1\cdot10^{-11}$]	2000±0.0 [$9.6\cdot10^{-10}$]	1000±0.0 [$4.8\cdot10^{-10}$]	130±0.0 [$6.2\cdot10^{-11}$]	500±0.0 [$2.4\cdot10^{-10}$]	260±0.0 [$1.2\cdot10^{-10}$]	500±0.0 [$2.4\cdot10^{-10}$]	130±0.0 [$6.2\cdot10^{-11}$]
IPBC [b] (fungicide)*	2±0.0 [$7\cdot10^{-12}$]	2±0.0 [$7\cdot10^{-12}$]	2±0.0 [$7\cdot10^{-11}$]	2±0.0 [$7\cdot10^{-12}$]	100±0.0 [$3.5\cdot10^{-8}$]	2±0.0 [$7\cdot10^{-12}$]	1±0.0 [$3.5\cdot10^{-12}$]	5±0.0 [$1.7\cdot10^{-11}$]

MIC – minimal inhibition concentration, MFC – minimal fungal concentration

[a] *Values are mean SD± standard deviation of three replicates;* [b] **IPBC*(3-Iodo-2-propynyl butyl carbamate) Antimicrobial Preventol®MP100from Lanxess

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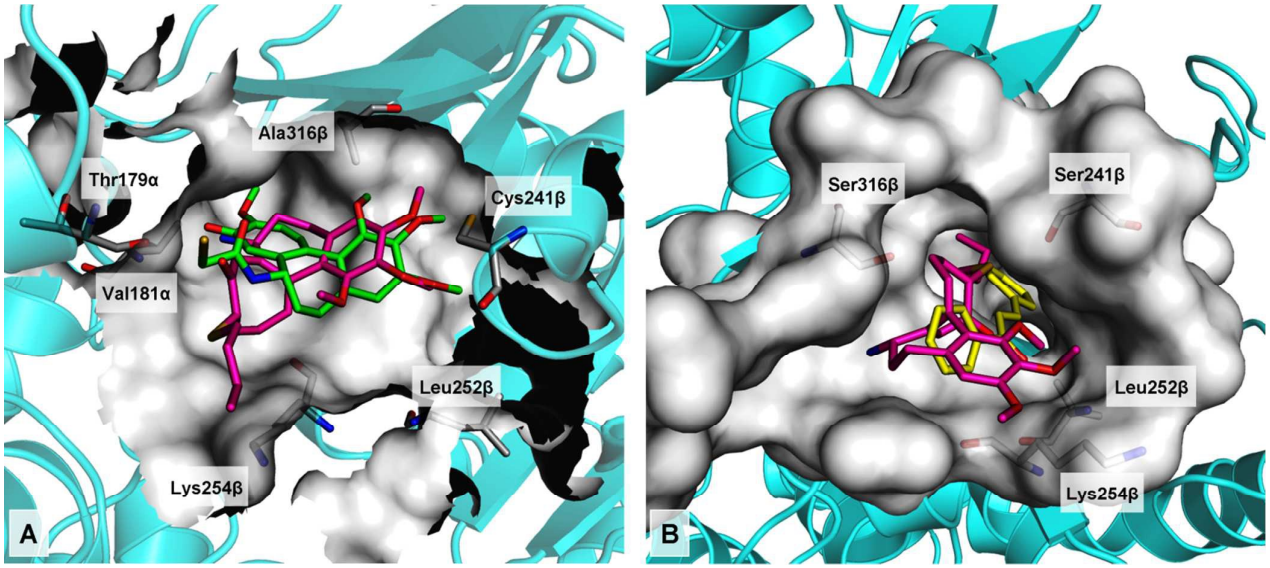

Figure 2. The comparison of the binding mode of **11** (magenta) with colchicine (green) in human β-tubulin binding site (A) and with chalcone (yellow) in the *Ch. globosum* β-tubulin binding site (B).

The closest inspection of the binding sites indicated that several mutations occurred between human and fungal tubulins. The common mutations are: Ala316Ser, Val318Ile and Asn167Ala, whereas two specific (Cys241Ser and Ile378Val) occurred only in species where **11** showed the highest activity.

The exchange of the cysteine 241 to serine leads to the formation of a stronger hydrogen bond with methoxyl group of compound 11 , while mutation to alanine 167 causes, mainly, deepening of the binding pocket (the comparison of binding pocket shapes is presented on Figure S14).

Conclusions

In conclusion, we showed that a simple two-step reaction leads to a new deacetyl alkylthiocolchicine derivatives with biological properties. Deacetylation of colchicine derivatives increased their biological activity, both in cytotoxic and fungicidal properties.7-Deacetyl-10-*i*-propylthiocolchine and 7-deacetyl-10-*n*-buthyl-thiocolchicine show the cytotoxic activity against SKOV-3 ovarian cell line compared to that of known 7-deacetyl-10-methylthiocolchinine. Moreover, in contrast to 10-alkylthiocolchicines which were completely inactive against all tested fungi species, the all new deacetyl derivatives showed to be active against *A. pullulans* and *Ch. globosum*. Additionally, derivatives with longer alkyl chain: 7-deacetyl-10-*n*-propylthiocolchicine and 7-deacetyl-10-*n*-buthylthiocolchicine were active against four additional fungi strains. Among the compounds with propylthio substituent this with unbranched alkylthio chain was more active than the one with *i*-propylthio substituent. All these findings have suggested that further modifications of this group of colchicine derivatives could lead to more active compounds with greater potential as drug candidates.

Conflicts of interest

There are no conflicts to declare.

References

1 O.Boyé, A. Brossi, *The Alkaloids, Chemistry andpharmacology*. Brossi, A. Cordell, GA., Eds, Academic Press, New York, 1992, **41**, 125-176.
2 A. Brossi, Bioactive alkaloids. 4. Results of recent investigations with colchicine and physostigmine, *J. Med. Chem.* 1990, **33**, 2311-2314.
3 S.H. Zhang, J. Feng, S.C. Kuo, A. Brossi, E. Hamel, A. Tropsha, K.H. Lee. Antitumor Agents. 199. Three-Dimensional Quantitative Structure−Activity Relationship Study of the Colchicine Binding Site Ligands Using Comparative Molecular Field Analysis, *J. Med. Chem*. 2000, **43**, 167-176.
4 Y. Dong, Q. Shi, H.C. Pai, C.Y. Peng, S.L. Pan, C.M. Teng, K. Nakagawa-Goto, D. Yu, Y.N. Liu, P.C. Wu, K.F. Bastow, S.L. Morris-Natschke, A. Brossi, J.Y. Lang, J.L. Hsu, M.C. Hung, E.Y. Lee, K.H. Lee, Antitumor Agents. 272. Structure−Activity Relationships and In Vivo Selective Anti-Breast Cancer Activity of Novel Neo-tanshinlactone Analogues, *J. Med. Chem.,* 2010, **53**, 2299-2308.

View Article Online
DOI: 10.1039/C8MD00352A

5 S. Sharma, B. Poliks, C. Chiauzzi, R. Ravindra, A. Balden, S. Bane, Characterization of the Colchicine Binding Site on Avian Tubulin Isotype βVI, *Biochemistry,* 2010,**49**, 2932-2942.
6 K. Nakagawa-Goto; C.X. Chen, E. Hamel, C.-C. Wu, K. F. Bastow, K.-H. Lee, A. Brossi, Antitumor agents. Part 236: Synthesis of water-soluble colchicine derivatives, *Bioorg. Med. Chem. Let*. 2005, **15**, 235-238.
7 L. Velluz, G. Muller, No224. La thiocolcicine II – Produitsd'hydrolyse, de reduction etd'oxydation, avec exemples de soufreasymetrique, *Bull. Soc. Chim. Fr*. 1954, 1072.
8 J. Kurek, Wł. Boczoń, M. Murias, K. Myszkowski, T. Borowiak, I. Wolska, Synthesis of sulfur containing colchicine derivatives and their biological evaluation as cytotoxic agents, *Let. Drug Des. Disc*. 2014**, 11**, 279-289.
9 A. Brossi, H.J.C. Yeh, M. Chrzanowska, J. Wolff, E. Hamel, C.M. Lin,F. Quin, M. Suffness, Silverton, Colchicine and its analogues: recent findings,*J. Med. Res. Rev*. 1988, **8**, 77-94.
10 D. Batrusik, B. Tomanek, E. Latova, H. Perreault, J. Tuszynski, G. Fallone,Derivatives of thiocolchicine and its applications to CEM cell treatments using ^{19}FMagnetic Resonance ex *vivo*, *Bioorg. Chem*. 2010, **38**, 1-6.
11 A. Muzaffar, A. Brossi, C.M. Lin, E. Hamel,Antitubulin effects of derivatives of 3-demethylthiocolchicine, methylthio ethers of natural colchicinoids, and thioketones derived from thiocolchicine. Comparison with colchicinoids, *J. Med. Chem*. 1990, **33**, 567-571.
12 S.H. Zhang, J. Feng, S.C. Kuo, A. Brossi, E. Hamel, A. Tropsha, K.H. Lee,Antitumor Agents. 199. Three-Dimensional Quantitative Structure−Activity Relationship Study of the Colchicine Binding Site Ligands Using Comparative Molecular Field Analysis, *J. Med. Chem*. 2000, **43**, 167-176.
13 Q. Shi, P. Verdier-Pinard, A. Brossi, E. Hamel, C.C. Wu, K.H. Lee,Antitumor agents—CLXXV. Anti-tubulin action of (+)-thiocolchicine prepared by partial synthesis, *Bioorg. Med. Chem.* 1997, **5**, 2277-2288.
14 T. Kozaka, K. Nakagawa-Goto, Q. Shi, C.-Y. Lai, E. Hamel, K.F. Bastow, A. Brossi, K.-H. Lee,Antitumor agents 273. Design and synthesis of *N*-alkylthiocolchicinoids as potential antitumor agents, *Bioorg. Med. Chem. Lett*. 2010, **20**, 4091-4094.
15 M.L. Gelmi, S. Mottadelli, D. Pocar, *N*-Deacetyl-*N*-aminoacylthiocolchicine Derivatives: Synthesis and biological evaluation on MDR-Positive and MDR-Negative human cancer cell lines, *J. Med. Chem*. 1999, **42**, 5272-5276.
~~16~~ M.L. Gelmi, D. Pocar, G. Pontremoli, S. Pellegrino, E. Bombardelli, G. Fontana, A. Riva, W. Balduini, S. Carloni, M. Cimino, F. Johnson,3-Demetoxy-3-glycosylaminothiocolchicines: synthesis of a new class of putative muscle relaxant compounds,*J. Med. Chem*. 2006, **49**, 5571-5577.
17 S.H. Lee, S.K. Park, J.M. Kim, M.H. Kim, K.H. Kim, K.W. Chum, K.H. Cho, J.Y. Youn, S.K. Namgoong,New Synthetic Thiocolchicine Derivatives as Low toxic Anticancer Agents, *Arch. Pharm. Chem. Life Sci*. 2005, **338**, 582-586.
18 P. Ferri, C. Bruno, T. Ceccheni, S. Ciaroni, P. Ambrogini, L. Guidi, R. Cuppini, E. Bombardelli, P. Morazzoni, A. Riva, P. Grande,Effects of thiocolchicine on axonal cytoskeleton of the rat peroneus nerve,*Exp. Toxic Pathol*. 2002,**54**, 211-216.
19 M. Cavazza, M. Zandomeneghi,F. Pietra, Synthesis and chiroptical properties of pseudocolchicine and neocolchicine, novel unnatural regioisomers of colchicine, *J. Chem. Soc. Perkin Trans. 1* 2002, 560-564.
20 P. Kerkes, P.N. Sharma, A. Brossi, C.F. Chignell, F.R. Quinn,Synthesis and biological effects of novel thiocolchicines. 3. evaluation of *N*-acyldeacetylthiocolchicines, *N*-(alkoxycarbonyl)deacetylthiocolchicines, and *O*-ethyldemethylthiocolchicines. New synthesis of thiodemecolcine and antileukemic effects of 2-demethyl- and 3-demethylthiocolchicine, *J. Med. Chem*. 1985, **28**, 1204-1208.
21 Q. Shi, P. Verdier-Pinard, A. Brossi, E. Hamel, A.T. McPhail, K.H.Lee, Synthesis and biological evaluation of novel deacetamidothiocolchicin-7-ols and ester analogs as antitubilin agents*, J. Med. Chem*. 1997, **40**, 961-966.
22 L. Sun, E. Hamel, Lin C.M., S.B. Hastie, A. Pyluck, K.H. Lee,Synthesis and biological evaluation of novel thiocolchicineanalogs: *N*-acyl, *N*-aroyl and *N*-(substituted benzyl)deacetylthiocolchicines as potent cytotoxic and antimitotic compounds*, J. Med. Chem*. 1993, **36**, 1474-1479.
23 G. Cappelletti, D. Cartelli, B. Peretto, M. Ventura, M. Riccioli, E. Colombo, J.S. Snaith, S. Borrelli, D. Passarella, Tubulin-guided dynamic combinatorial library of thiocolchicine-podophyllotoxin conjugates, *Tetrahedron*, 2011, **67**,7354-7357.
24 B. Danieli, G. Lesma, D. Passarella, D. Prosperi, A.E. Silvani, Bombardelli, Oxidative deamination of *N*-deacetylcolchinoids with 3,5-di(tert-butyl)-1,2-benzoquinone: synthesis of 2H-1,4-benzoxazine-type adducts, *Helv. Chim. Acta*1999, **82**, 1502-1508.
25 D. Passarella, A. Giardini, B. Peretto, G. Fontana, A. Sacchetti, A. Silvani, C. Ronchi, G. Cappelletti, D. Cartelli, J.B. Borlak, Danielli,Inhibitors of tubulin polymerization: Synthesis and biological evaluation of hybrids of vindoline, anhydrovinblastine and vinorelbine with thiocolchicine, podophyllotoxin and baccatin III*, Bioorg. Med. Chem*. 2008, **16**, 6269-6285.
26 J. Guan, A. Brossi, X.-K. Zhu, H.-K. Wang, K.-H. Lee, *Synt. Commun*.Oxidation Products of Phenolic Thiocolchicines: Ring a Quinones and Dienones, 1998, **28**, 1585-1591.
27 S. Budavari, The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, White House Station, New York, **1989**.
28 V.P. Gullo, Newnes, Discovery of Novel Natural Products with Therapeutic Potential 2013, 544
29 K. Mikanagi, Purine and Pyrimidine Metabolism in Man VI: Part A: Clinical and Molecular Biology, Springer Science & Business Media, 2012, 560
30 A.V. Costantini, The fungal etiology of gout and hyperuricemia**:** the antifungal mode of action of colchicine, *Bio. Med. Rev*. 1992, 47-52.
31 N. Kudeken, K. Kawakami, A. Saito, Mechanisms of the in vitro fungicidal effects of human neutrophils against Penicillium marneffei induced by granulocyte-macrophage colony-stimulating factor (GM-CSF), *Clin. Exp. Immunol*. 2000, **11**, 9472-9478.
32 C. Gostinčar, R.A. Ohm, T. Kogej, S. Sonjak, M. Turk, J. Zajc, P. Zalar, M. Grube, H. Sun, J. Han, A. Sharma, J. Chiniquy, C.Y. Ngan, A. Lipzen, K. Barry, I.V. Grigoriev, N. Gunde-Cimerman, Genome sequencing of four A. pullulans varieties: biotechnological potential, stress tolerance, and description of new species. BMC *Genomics.* 2014, **15**, 1, 549-554.
33 J.H. Andrews, R.N. Spear, E.V. Nordheim, *A. pullulans, Can. J. Microbiol*. 2002, **48**, 6, 500-507.
34 J. Kurek, G. Bartkowiak, W. Jankowski, P. Kwaśniewska-Sip, G. Schroeder, M. Hoffmann, G. Cofta, P. Barczyński, Spectroscopic, DFT Studies and Fungicidal Activity of colchicine complexes with Sodium, Potassium, Magnesium and Calcium Carbonates and Sulphates, *IOSR J. Pharm*. 2016, **6**, 8, 40-55.
35 K. Roy, K. K. Roy, Exploring QSAR and QAAR for inhibitors of cytochrome P450 2A6 and 2A5 enzymes using GFA and G/PLS techniques. *Eur. J. Med. Chem*. 2009, **44**, 1941-1951.

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7-Deacetyl-10-alkylthiocolchicine derivatives – new compounds with potent anticancer and fungicidal activity

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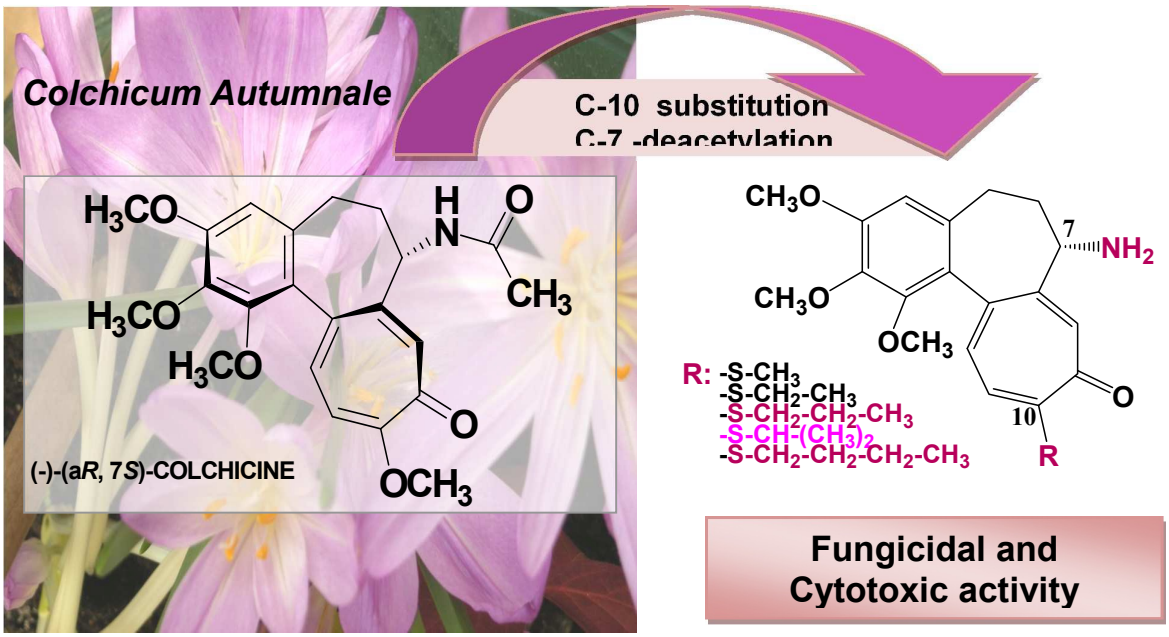

Cytotoxic and fungicidal activity of 7-deacetyl-10-alkylthiocolchicine derivatives.