Bioorg. Med. Chem. Lett. 47 (2021) 128197

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and antiproliferative screening of novel doubly modified colchicines containing urea, thiourea and guanidine moieties

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ARTICLE INFO

Keywords:
Anticancer agents
Antiproliferative activity
Colchicine derivatives

ABSTRACT

A new series of 10-demethoxy-10-methylaminocolchicines bearing urea, thiourea or a guanidine moieties at position C7 has been designed, synthesized and evaluated for *in vitro* anticancer activity against different cancer cell lines (A549, MCF-7, LoVo, LoVo/DX). The majority of the new derivatives were active in the nanomolar range and were characterized by lower IC_{50} values than cisplatin or doxorubicin. Two ureas (**4** and **8**) and thioureas (**19** and **25**) were found to be good antiproliferative agents (low IC_{50} values and high SI) and could prove to be promising candidates for further research in the field of anticancer drugs based on the colchicine skeleton.

Colchicine **1** is the main alkaloid isolated from *Colchicum autumnale* and *Gloriosa superba*. It is used for the treatment of gout, familial Mediterranean fever or Behcet's disease.[1–6] Although colchicine is not used as an antitumor agent due to its toxic effects,[7–14] it does exert a significant inhibitory effect on cancer cells proliferation. The biological activity of colchicine is associated with its ability to bind to the tubulin, inhibit its assembly and microtubule polymerization and finally arresting cell division at metaphase.[7,15–20] Therefore, it is an interesting scaffold for designing of new anticancer compounds based on its skeleton.

Chemical compounds containing urea, thiourea or guanidine moieties in their structure show a broad range of biological activities and therefore, are widely used in the search for new anticancer, antimicrobial, antibacterial, antituberculosis, antimalarial or antiviral drugs candidates.[21,22,31–34,23–30] Pharmacological activity of (thio)ureas is possible thanks to certain interactions between proteins, receptor targets and drugs. For example, the protons on the two nitrogens act as hydrogen bond donors, capable of providing a few hydrogen bonds, depending on substituents, while the C═O fragment of the urea act as a hydrogen bond acceptors.[35,36] Such derivatives (ureas, thioureas) play an important role in regulation of various pharmacological activities such as the ability to improve potency and selectivity or modulation of physiochemical properties. In turn, the biochemical and biophysical properties of the guanidine/guanidinium groups may be attributed to the specific pattern of hydrogen bonding and the high basicity in comparison to their parent amines. Guanidine is considered to be one of the strongest organic bases, which allows this moiety to bind tightly to carboxylates,[37] phosphates and metals. The guanidinium cation can engage in the unique interactions ligand-receptor or enzyme-substrate and therefore, the guanidine group is also one of the moieties of interest in drug development.

Taking into account the above-mentioned attributes and in continuation of our interest in the design and synthesis of biologically active new doubly-modified colchicines with a methylamino group at carbon C10 [38–40], together with the reports on the improvement of colchicine activity by introducing a thio(urea) moiety at position C7,[41,42] herein, we decided to check the effects of the incorporation of urea, thiourea and guanidine moieties into 10-demethoxy-10-*N*-methylaminocolchicine (compound **2**, Scheme 1).

We designed a series of 10-*N*-methylaminocolchicines with various substituents at the urea or thiourea group attached to the C7 carbon: monoalkyl and dialkyl chains of various lengths, straight (**5**, **7**–**8**, **16**–**19**, **21**), branched (**6**, **9**) or unsaturated (**20**), non-aromatic cyclic chains (**10**, **22**), polyhydroxy chains (**25**, **26**) and aromatic moieties with (**12**–**14**, **23**–**24**) or without (**11**) substituents. For comparison, we also synthesized guanidines (**27**, **28**) due to the structural similarity to the

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https://doi.org/10.1016/j.bmcl.2021.128197
Received 23 April 2021; Received in revised form 1 June 2021; Accepted 7 June 2021
Available online 8 June 2021

simplest urea (**4**) and thiourea (**15**) analogues. A variety of side chains have been selected to facilitate the preliminary structure–activity relationship (SAR) analysis, which should help in designing colchicines showing improved biological properties.

Our synthetic strategy to obtain colchicine derivatives **2**–**28** is illustrated in Scheme 1 and a detailed description of the synthetic procedures is provided in the Supplementary Materials. The key intermediate **3** was available from colchicine **1** by treatment with methylamine followed by hydrolysis with 2 M HCl.[38,43] New analogs were synthesized from **3** by treatment with respective isocyanate (**6**, **11**–**14**), isothiocyanate (**17**, **23**–**24**) or carbamoyl chloride in the presence of triethylamine (**7**–**10**). The thioureas **15**–**16**, **18**–**22** and **25** were prepared by compound **3**, thiophosgene and corresponding primary or secondary amine, based on the method described earlier.[42] Compounds **5** and **27** were obtained in the reaction with *N*-succinimidyl *N*-methylcarbamate and *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine, respectively. Protecting groups from compounds **25** and **27** were removed with HCl in MeOH or EtOAc, giving derivatives **26** and **28**, respectively. All compounds were isolated in pure form after column flash chromatography on silica gel.

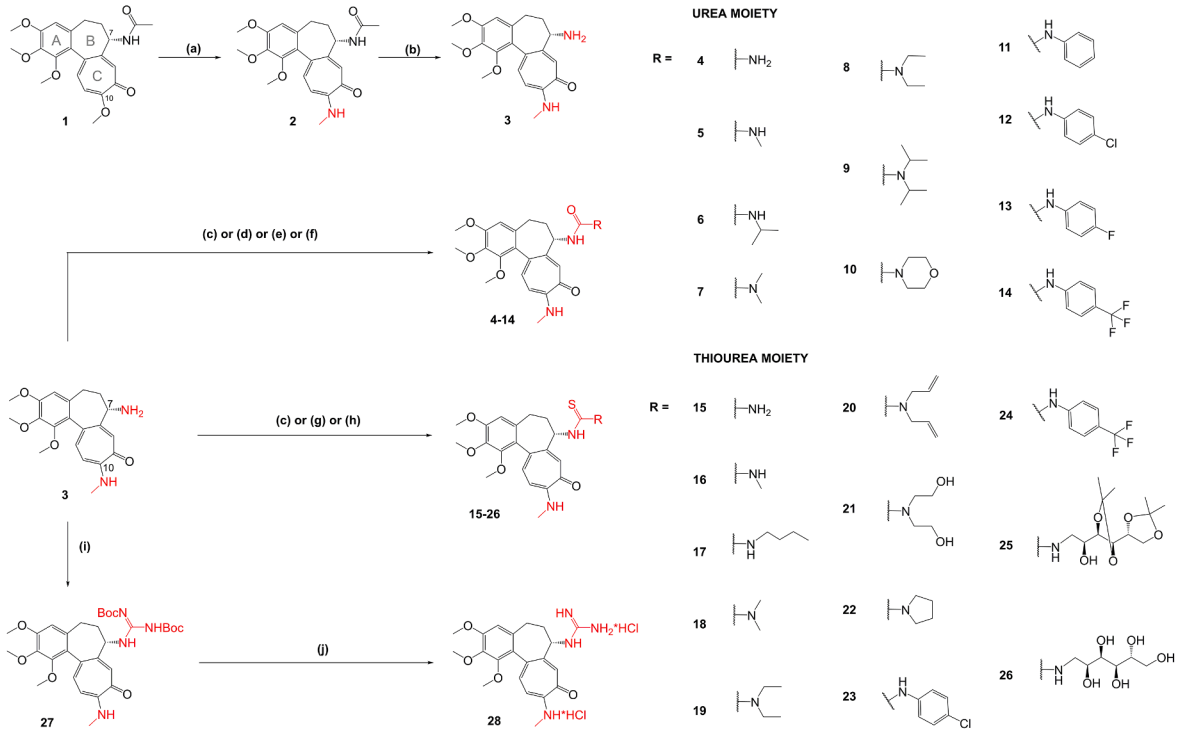
The purity and structures of the obtained compounds **2–28** were determined using LC-MS, ^{1}H and ^{13}C NMR methods and are shown in the Supplementary Materials. The characteristic signals of the –OCH_3 group of the tropolone C ring of colchicine **1** in the ^{1}H NMR and ^{13}C NMR spectra were observed at 4.0 ppm and at 56.5 ppm, respectively. After the reaction with methylamine, these signals were no longer visible, but new ones appeared: approx. at 3.1 ppm (–CH_3-) and approx. at 7.3 ppm (–NH-) in ^{1}H NMR and approx. at 29.5 ppm in ^{13}C NMR, corresponding to the –$NHCH_3$ group. The chemical shifts of the amide moiety of the starting compounds **1** and **2** can be found at 1.9 ppm (–CH_3) and 8.6 ppm (–NH–) in ^{1}H NMR and at 23.0 ppm (–CH_3) and at 170.0 ppm (C═O) in ^{13}C NMR and also were not visible in the spectra of new analogs **4**–**28**. In the NMR spectra of derivatives **4**–**14**, the signals corresponding to the urea moiety were observed in the range 7.0–8.5 ppm (–NH(C═O)N–) in ^{1}H NMR and approx. at 157.0 ppm (C═O) in ^{13}C NMR spectra. In the NMR spectra of derivatives **15**–**26**, the signals corresponding to the thiourea moiety were observed in the range 7.4–9.4 ppm (–NH(C═S)N–) in ^{1}H NMR and approx. at 182.0 ppm (C═S) in ^{13}C NMR ones. The ESI mass spectrometry confirmed the structure of the synthesized compounds by the presence of *m/z* signals assigned to the corresponding pseudomolecular ions of these analogs.

A library of newly synthesized derivatives (**4**–**28**), starting compounds (**1**–**3**) and commonly used anticancer agents doxorubicin and cisplatin were screened for their antiproliferative activity against four human cancer cell lines (A549, MCF-7, LoVo, LoVo/DX) and normal cells (BALB/3T3) following the previously published procedures.[38] Detailed information concerning the biological assay can be found in the Supplementary Materials. The results are collected in Table 1.

Resistance indexes (RI) were calculated (ratio IC_{50} value for LoVo/DX cell line to IC_{50} value for LoVo cell line) for evaluation of the activity of the studied compounds against the cells with MDR (multidrug resistance) phenotype.[44] RI values are shown in Table 1. According to the RI value, the cells can be classified to one of the three categories: drug-sensitive for RI ranging from 0 to 2, moderate drug-sensitive for RI 2–10 and strong drug resistant for RI above 10.[44,45]

The effects of the obtained compounds were studied also toward the non-cancerous murine embryonic fibroblasts (BALB/3T3) in order to estimate the therapeutic potential. Selectivity indexes were calculated as the ratio of IC_{50} value for normal cell line (BALB/3T3) to IC_{50} value for a respective cancer cell line.[46] High SI values (at least greater than 2) mean that cancer cells will be killed at a higher rate than normal (healthy) ones (see Figure 1).

The majority of new derivatives **4**–**28** showed cytotoxicity against cancerous cells in the nanomolar range and were characterized by lower IC_{50} values than the conventional chemotherapeutics: cisplatin (except **10** and **28** against LoVo/DX cells) and doxorubicin (except **10** and **26**–**28** against A549, **26**–**28** against MCF-7 and LoVo cells and only **28**



Scheme 1. Synthesis of doubly modified colchicine derivatives (**2**–**28**), changes at C7 and C10 positions are highlighted in red. Reagents and conditions: (a) NH_2CH_3/EtOH, reflux; (b) 2 M HCl, reflux; (c) 1) $C(O)Cl_2/PhCH_3$ or $C(S)Cl_2$, Et_3N, THF, 0 °C, 2) $NH_{3(gas)}$, THF, 0 °C to RT for **4** or **15**; (d) *N*-succinimidyl *N*-methylcarbamate, Et_3N, DCM, RT for **5**; (e) RNCO, THF, RT for **6, 11**–**14**; (f) $R^1R^1C(O)Cl$, Et_3N, DCM, reflux for **7**–**10**; (g) 1) $C(S)Cl_2$, Et_3N, DCM, 0 °C to RT, 2) R^1R^2NH, DCM, RT for **16**, **18**–**22** and **25**; (h) RNCS, THF, RT for **17**, **23**–**24**; (i) *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine, Et_3N, DCM/MeOH, RT for **27**; (j) 4 M HCl/EtOAc for **28**.

Table 1
Antiproliferative activity (IC_{50}) [nM] of colchicine (**1**) and its derivatives (**2–28**) compared with that of standard anticancer drugs doxorubicin and cisplatin and the calculated values of the resistance index (RI) of tested compounds.

Compound	A549	MCF-7	LoVo	LoVo/DX		BALB/3T3
	IC_{50} [nM]	IC_{50} [nM]	IC_{50} [nM]	IC_{50} [nM]	RI	IC_{50} [nM]
1	76.0 ± 26.0	13.0 ± 3.1	9.7 ± 4.1	2400 ± 1000	250	100 ± 20
2	12.0 ± 0.8	14.0 ± 5.4	4.2 ± 1.5	610 ± 70	150	11.0 ± 1.6
3	14.0 ± 1.0	22.0 ± 5.5	11.0 ± 0.9	190 ± 20	17	14.0 ± 1.8
4	38.0 ± 14.0	16.0 ± 9.8	10.0 ± 2.8	6300 ± 2600	630	150 ± 30
5	79.0 ± 14.0	12.0 ± 5.1	17.0 ± 11.0	7300 ± 2900	430	21.0 ± 12.0
6	34.0 ± 14.0	16.0 ± 1.3	24.0 ± 1.6	2400 ± 1000	100	34.0 ± 13.0
7	33.0 ± 11.0	16.0 ± 2.2	27.0 ± 12.0	1600 ± 350	59	42.0 ± 12.0
8	110 ± 11	23.0 ± 13.0	12.0 ± 2.2	1200 ± 100	100	89.0 ± 13.0
9	74.0 ± 38.0	42.0 ± 4.0	46.0 ± 5.8	730 ± 420	16	100 ± 10
10	190 ± 13	120 ± 48	53.0 ± 28.0	10000 ± 440	190	110 ± 20
11	12.0 ± 2.8	11.0 ± 2.6	10.0 ± 1.9	1100 ± 300	110	20.0 ± 9.3
12	4.0 ± 1.0	4.2 ± 1.0	7.3 ± 2.9	1000 ± 30	140	14.0 ± 2.3
13	4.9 ± 2.2	4.0 ± 0.5	4.8 ± 2.5	1000 ± 170	210	15.0 ± 6.6
14	82.0 ± 4.2	130 ± 50	19.0 ± 8.8	1000 ± 190	53	73.0 ± 23.0
15	12.0 ± 5.5	16.0 ± 4.0	11.0 ± 3.1	1400 ± 250	130	15.0 ± 6.0
16	2.0 ± 1.2	16.0 ± 2.4	9.6 ± 2.3	1000 ± 110	100	8.2 ± 8.0
17	13.0 ± 2.6	17.0 ± 2.7	9.4 ± 1.1	700 ± 70	74	6.3 ± 2.8
18	13.0 ± 1.5	8.2 ± 1.8	13.0 ± 3.6	200 ± 50	15	13.0 ± 1.3
19	78.0 ± 15.0	10.0 ± 4.8	16.0 ± 3.2	860 ± 200	54	91.0 ± 12.0
20	23.0 ± 9.7	17.0 ± 4.2	36.0 ± 13.0	140 ± 10	4	32.0 ± 9.3
21	27.0 ± 9.2	14.0 ± 0.6	27.0 ± 12.0	140 ± 30	5	31.0 ± 1.3
22	15.0 ± 3.7	9.5 ± 4.5	16.0 ± 6.8	160 ± 70	10	18.0 ± 6.9
23	12.0 ± 1.8	15.0 ± 2.2	23.0 ± 11.0	940 ± 150	41	36.0 ± 3.3
24	86.0 ± 2.0	120 ± 20	54.0 ± 19.0	2700 ± 550	50	67.0 ± 26.0
25	70.0 ± 5.6	15.0 ± 7.7	32.0 ± 7.6	5300 ± 990	170	440 ± 140
26	930 ± 190	1200 ± 340	680 ± 270	6600 ± 1100	10	1300 ± 280
27	850 ± 110	940 ± 150	500 ± 110	5900 ± 870	12	690 ± 60
28	740 ± 130	760 ± 330	920 ± 230	86000 ± 18000	93	1800 ± 110
Doxorubicin	190 ± 20	240 ± 70	160 ± 60	11000 ± 2100	69	200 ± 30
Cisplatin	5700 ± 970	7100 ± 1200	7100 ± 1600	8300 ± 1100	1	5700 ± 630

The IC_{50} value is defined as the concentration of a compound at which 50% growth inhibition is observed. The IC_{50} values shown are mean ± SD. Human lung carcinoma (A549), human breast adenocarcinoma (MCF-7), human colon adenocarcinoma cell line (LoVo) and doxorubicin-resistant subline (LoVo/DX), normal murine embryonic fibroblast cell line (BALB/3T3).

against LoVo/DX cells) (see Table 1).

Sixteen of the twenty-five new colchicine derivatives were more active against the A549 cell line than the unmodified parent compound **1**. The most cytotoxic towards these cells turned out to be thiourea **16** (IC_{50} = 2.0 nM) with a methyl group and ureas with a *p*-chlorophenyl **12** (IC_{50} = 4.0 nM) or a *p*-fluorophenyl **13** (IC_{50} = 4.9 nM) substituent. As far as MCF-7 cells are concerned, seven of the twenty-five new analogs presented here showed higher antiproliferative activity than the starting amides **1** and **2**. Again, urea derivatives **12** and **13** showed the lowest IC_{50} values (IC_{50} = 4.0–4.2 nM). More toxic than colchicine **1** towards the LoVo line were two of the newly designed ureas and thioureas (**12**–**13**), while only urea with a *p*-fluorophenyl moiety **13** with IC_{50} = 4.8 nM had comparable activity to 10-methylaminocolchicine **2** (IC_{50} = 4.2 nM). Additionally, two thioureas — **20** with a diallyl and **21** with a dihydroxyethyl moiety – showed good activity against the doxorubicin-resistant subline LoVo/DX (IC_{50} < 150 nM), approx. 17 times more potent than unmodified colchicine **1** and approx. 4 times more potent than initial amide **2**. The compounds **26**–**28** (thioureas with polyhydroxyl group and guanidines) exhibited significantly lower antiproliferative activity than other derivatives towards three of the four cell lines tested (see Table 1).

Below the results of *in vitro* tests and the therapeutic potential of compounds with various side chains attached to 10-*N*-methylaminocolchicine *via* a carbonyl (ureas) or thiocarbonyl (thioureas) group are compared. Attempts were also made to draw preliminary conclusions about the relationship between the structure and biological activity (SAR) of the presented compounds.

Antiproliferative activity of the structurally simplest urea **4** and thiourea **15** against the MCF-7 and LoVo lines was comparable, characterized by the IC_{50} values in the range 10.0–16.0 nM, while **15** was about 3–4 times more cytotoxic towards to A549 and LoVo/DX cells than **4** (see Table 1). It is noteworthy that urea **4** inhibited the proliferation of non-cancerous BALB/3T3 cells 10 times weaker than thiourea **15** (IC_{50} = 150.0 nM *versus* 15.0 nM), which is reflected in the favorable selectivity coefficients for **4** (SI = 3.9, 9.4 and 15.0 for A549, MCF-7 and LoVo tumor cells, respectively). SI values of **4** were also higher than those obtained for the starting compounds **1**–**3** and the other derivatives presented in this work (except for compound **25**) and doxorubicin or cisplatin, that is why **4** is concluded to be a potential pharmacophore for further extended biological research.

In order to assess the significance of the presence of carbonyl/thiocarbonyl group in the designed compounds, the derivatives with a guanidine at position C7 (**27** and **28**) were also synthesized and tested *in vitro*. The results showed that the introduction of this group negatively affects the biological properties - analogs **27** and **28** turned out to be weaker cytostatics than C7-ureas (**4**–**14**), C7-thioureas (**15**–**25**) and starting compounds (**1**–**3**) towards A549, MCF-7 and LoVo cells (Table 1). However, 7-guanidino-10-*N*-methylaminocolchicine **28** showed some selectivity (SI about 2) towards three of the four tumor lines (see Figure 1).

From among the analogues of doubly-modified colchicines with straight, branched or cyclic alkyl side chains located in urea (**5**–**10**) and thiourea (**16**–**22**) moiety, the highest IC_{50} values were reported for compound **10** containing a morpholine substituent (see Table 1). Probably, the large volume ring that can adopt different conformations prevents effective interaction with the colchicine-binding pocket in β-tubulin and therefore, the compound has a slightly lower cytotoxicity. The lowest IC_{50} = 2.0 nM (for the A549 cells) was found for the 10-*N*-

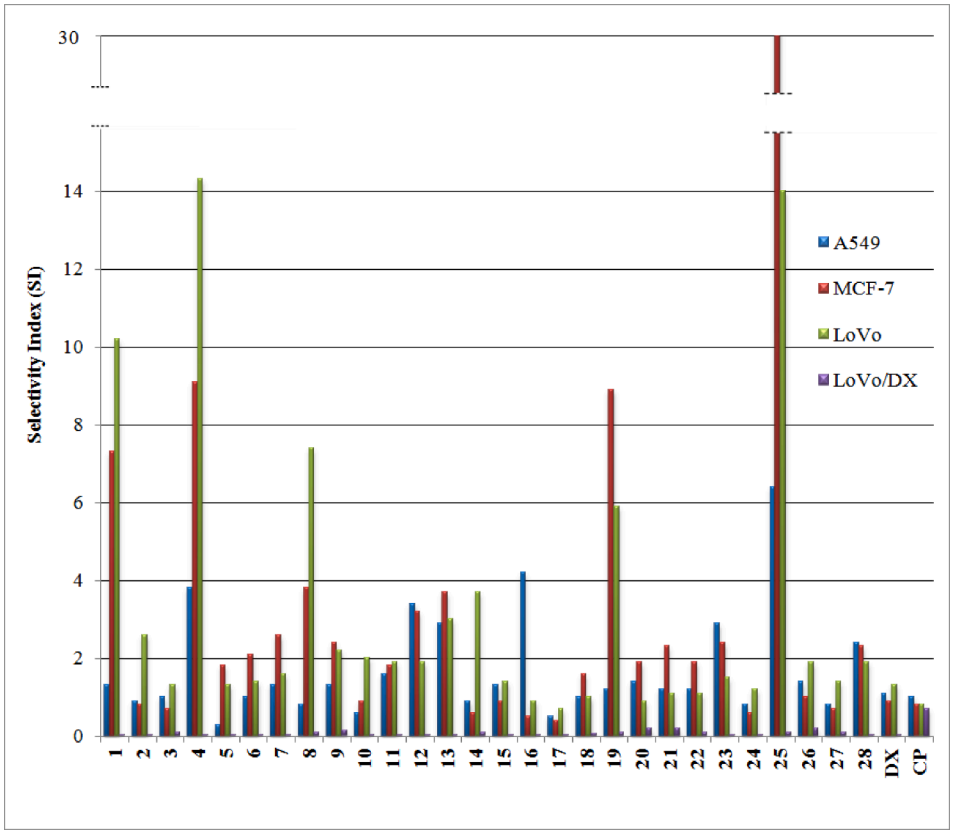


Fig. 1. Comparison of selectivity index (SI) values of the tested compounds. The SI (Selectivity Index) was calculated for each compound using the formula: SI = (IC_{50} for normal cell line BALB/3T3)/(IC_{50} for respective cancerous cell line). A favorable SI greater than 1.0 indicates a drug with efficacy against tumor cells greater than the toxicity against normal cells.

methylaminocolchicine thiourea **16** with the –NH(C═S)NHMe substituent at the C7 position. The selectivity coefficient of compound **16** for the aforementioned A549 line is also favorable (SI = 4.1, Figure 1), which means that tumor cells will be attacked first and normal cells only secondarily. An outstanding selectivity towards MCF-7 and LoVo cells (from **5 to 10** and **16**–**22**) was obtained for urea **8** and thiourea **19** with a diethyl substituent (SI in the range 3.9–9.1). The mentioned SI values are also higher than those of commonly used chemotherapy drugs (doxorubicin and cisplatin). The diethyl fragment in the (thio)urea group is thus concluded to be a good starting point for further research on colchicine derivatives that may be useful in cancer therapy.

Considering derivatives with an aromatic urea moiety, **11** and **14**, it is apparent that the introduction of trifluromethyl group –CF_3 into the *para* position in the benzene ring decreases the cytotoxicity of the compound (towards A549, MCF-7 and LoVo cells). The compounds with a chlorine (**12**) or fluorine (**13**) atom at this position showed comparable antiproliferative activity (see Table 1). Comparing the IC_{50} values obtained for urea and thiourea with the *p*-chlorophenyl ring (**12** and **23**, respectively), we note that the compound with the carbonyl moiety (C═O) **12** is more active against three out of four tested cancer cell lines (A549, MCF-7, LoVo) than compound **23** containing the thiocarbonyl fragment (C═S). On the other hand, compounds having a *p*-triflоromethylphenyl ring attached to 10-methylaminocolchicine *via* urea (**14**) or thiourea (**24**) moieties at position C7 did not show such significant differences in cytotoxicity. The obtained results indicate that these compounds may have therapeutic potential in treatment of cancers, but it is necessary to synthesize and examine other 10-*N*-methylaminocolchicine ureas and thioureas with more diverse substituents at the benzene ring.

The series of colchicine analogues (**4**–**28**) included also two glucitol derivative-containing compounds (**25** and **26**). Compound **25** having hydroxyl groups protected with isopropylidenes showed higher antiproliferative activity against three cancer cell lines (A549, MCF-7 and LoVo) than the derivative with free hydroxyl groups **26** (IC_{50} in the range 15.0 – 70.0 nM for **25** and IC_{50} > 680 nM for **26**). The activities of these two analogs against the LoVo/DX line were comparable (Table 1). Thiourea **25** showed also the highest selectivity against three cancer cell lines (SI = 6.3 for A549 cells, SI = 29.3 for MCF-7 cells and SI = 13.8 for LoVo) of all tested compounds **1**–**28** as well as doxorubicin and cisplatin (see Fig. 1). Compound **25** is therefore the most promising derivative of those analyzed in this study in terms of therapeutic potential in clinical use, and further *ex vivo/in vivo* studies will be useful to confirm or rule out good *in vitro* test results.

The calculated RI values indicated that the two obtained thioureas (**20** with a diallyl and **21** with a dihydroxyethyl substituent) were able to break the drug-resistance of cancer cell line LoVo/DX (RI = 4.0 for compound **20** and RI = 5.0 for compound **21**). The RI values (see Table 1) characterizing these two derivatives were significantly lower than those obtained for the reference compounds: doxorubicin (RI = 69), unmodified colchicine **1** (RI = 250) or starting compounds **2** and **3** (RI = 150 and 17, respectively). It should be noted that compounds **20** and **21** also showed low IC_{50} values, in the range 14.0–36.0 nM relative to three of the four tumor lines investigated (A549, MCF-7, LoVo) and IC_{50} = 140.0 nM for LoVo/DX subline.

In conclusion, we have designed and synthesized a series of new colchicine derivatives with urea, thiourea and guanidine moieties and evaluated their antiproliferative activity against different drug-sensitive as well as drug-resistant cancer cells. From among all double-modified colchicine ureas, the most interesting (low IC_{50} values and favorable SI values) seems to be **4** (the simplest urea), **8** (with a diethyl group) and **12**–**13** (with a *p*-chlorophenyl or *p*-fluorophenyl substituent). Out of colchicine thioureas the most promising are thiourea **19** (with a diethyl

fragment), **23** (with a *p*-chlorophenyl group) and **25** (with a glucitol derivative having hydroxyls protected with acetonides). Compound **25** found to be the most potent antiproliferative agent amongst the series of compounds **4**–**28**, could prove to be a promising candidate for drug discovery.

The above results confirm that chemical modification of colchicine can lead to compounds with improved biological properties compared to unmodified **1** and other commonly used chemotherapeutic agents. In addition, changes in the structure of colchicine (**1**) allow obtaining compounds with reduced toxicity to normal cells or reduce the problem of resistance of cancer cells to known cytostatics - therefore, these studies can help in the rational design of colchicines that can be used in the future as anticancer drugs.

Declaration of Competing Interest

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128197. These data include MOL files and InChiKeys of the most important compounds described in this article.

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