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4-Chlorocolchicine derivatives bearing a thiourea side chain at the C-7 position as potent anticancer agents†

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A series of 4-substituted colchicine derivatives were synthesized and evaluated with an eye toward developing new anticancer agents. As a result, 4-chlorocolchicine derivatives bearing a thioureide side chain at the C-7 position were found to exhibit significant cytotoxicities to three human cancer cell lines (A549, HT-29, and HCT116). In particular, compound **26** having an ethylthioureide group at the C-7 had high antitumor activity *in vivo* and a broad effective dosage range. Furthermore, compound **58**, which has a (5-methylpyrazol-3-yl)thioureide group at the C-7 side chain, exhibited strong cytotoxicity and desirable metabolic stability *in vitro*.

Introduction

Colchicine (**1**), the major alkaloid isolated from *Colchicum autumnale*, is a well-known antimitotic agent that acts by binding to tubulin.[1] A number of research groups have poured much effort into the development of potential anticancer agents derived from colchicine.[2–15] However, the inherent adverse effects of alkaloids have hampered the development of colchicine-derived anticancer drugs.[16–19]

We reported that 4-chlorocolchicine (**2**) exhibited potent activities *in vitro* and *in vivo*.[20] Furthermore, we found that derivatives **3** and **4**, which possess an α-hydroxyalkanamide side chain at the C-7 position, had significant antitumor activities *in vivo* with broad effective dosage ranges.[21] Therefore, we looked into the potency of C-4 substituted colchicines in greater detail. In this paper, we report the synthesis of 4-substituted colchicines having newly designed side chains at the C-7 position and their antitumor activities (Fig. 1).

Results and discussion

We initially synthesized 4-(mono-, di-, and tri-)fluoromethyl derivatives **5**, **6**, and **7** (Scheme 1), because, it was reported that the biological activity of a chloro-containing compound was improved when the chlorine group was substituted with a fluorinated methyl group.[22–24] First, colchicine (**1**) was converted into 4-formylcolchicine (**8**) by reacting with Cl_2CHOMe in the presence of $SnCl_4$. The reduction of **8** with $NaBH_4$ gave 4-hydroxymethyl derivative **9** and subsequent fluorination with DAST gave 4-fluoromethyl derivative **5**. In turn, compound **8** was converted into 4-difluoromethylcolchicine (**6**) *via* 4-(1,3-dithian-2-yl)colchicine (**10**). 4-(Trifluoromethyl)colchicine (**7**) could be synthesized in good yield by treatment of 4-iodocolchicine (**11**) with FSO_2CF_2COOMe and CuI in a sealed tube.[25,26]

The cytotoxicities of derivatives **5–7** were evaluated in three human cancer cell lines (A549, HT-29, and HCT116) (Table 1).

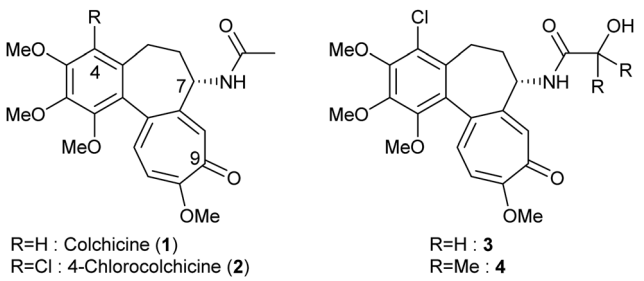

Fig. 1 Structures of colchicine (**1**), 4-chlorocolchicine (**2**), and their analogues **3** and **4** with an α-hydroxyalkylamide function at the C-7 position.

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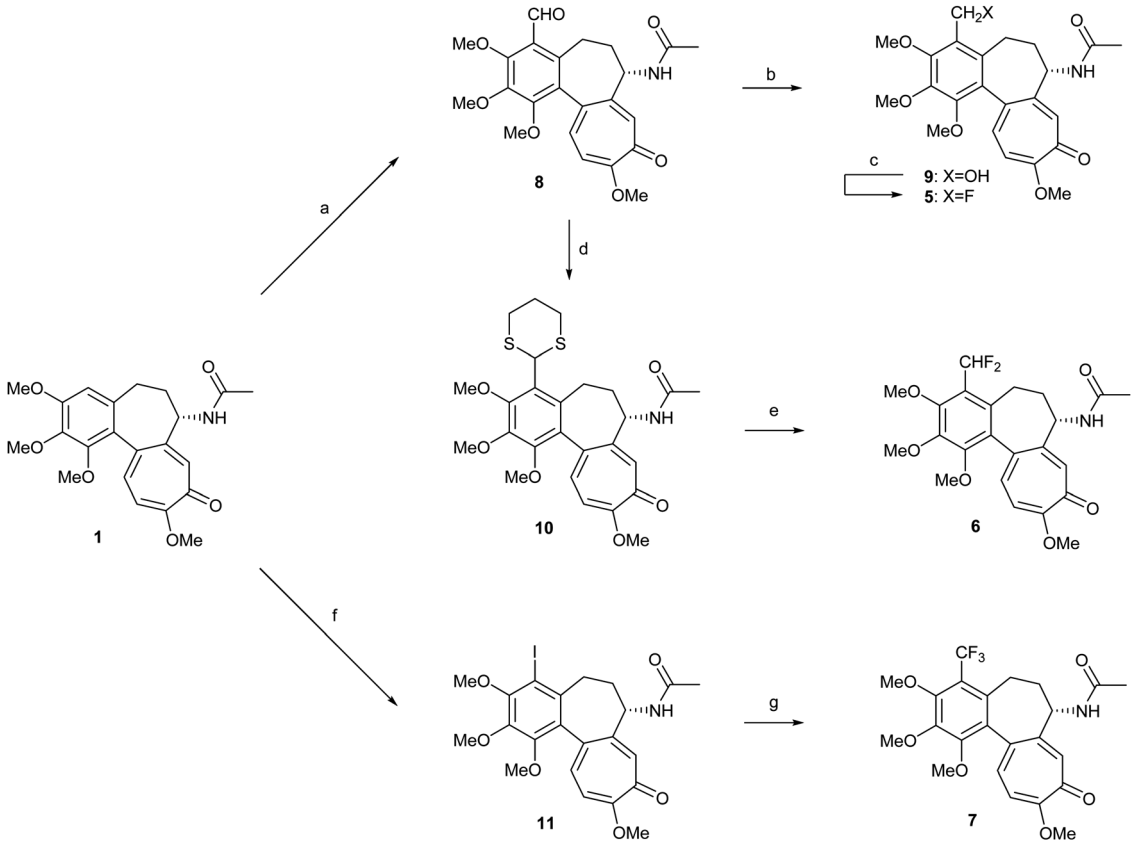
† Electronic supplementary information (ESI) available: Synthetic procedures and characterization data of all compounds, as well as the methodology used in cytotoxicity evaluation, antitumor experiments, metabolic stability experiments, and docking simulations. See DOI: 10.1039/c3md00287j

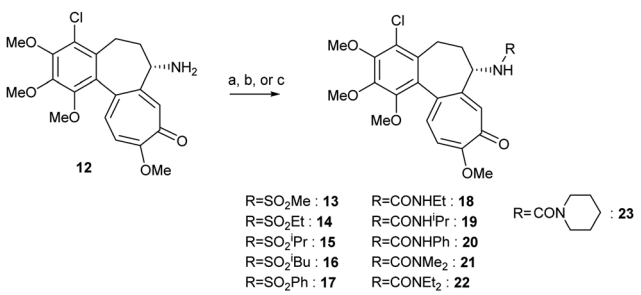


Scheme 1 *Reagents and conditions*: (a) Cl_2CHOMe, $SnCl_4$, CH_2Cl_2, rt, overnight; (b) $NaBH_4$, MeOH, rt, 3 h; (c) DAST, CH_2Cl_2, 0 °C, 2 h; (d) 1,3-propanedithiol, I_2, $CHCl_3$, rt, overnight; (e) NIS, HF·Py, CH_2Cl_2, −78 °C to rt, then $NaBH_4$, MeOH, rt, 0.5 h; (f) NIS, AcOH, 70 °C, 7 h and (g) FSO_2CF_2COOMe, CuI, NMP, in a sealed tube, 120 °C, overnight.

Table 1 Cytotoxicities and *S* log *P* values of 4-substituted colchicine derivatives

Compound no.	IC_{50} value (nM) A549	HT-29	HCT116	*S* log *P*[a]	Metabolic stability (mL min^{-1} mg^{-1})
1	54.4	8.2	10.6	2.59	0.001
2	9.9	7.9	8.1	3.24	0.028
5	8793	2615	4753	3.32	NT[b]
6	55.4	48.1	45.8	3.62	NT
7	55.2	35.0	37.9	3.92	NT

[a] *S* log *P*: the index of hydrophobicity was estimated by MOE. [b] Not tested.



Scheme 2 *Reagents and conditions*: (a) $R'SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 2 h; (b) R′N═C═O, CH_2Cl_2 or MeOH−H_2O, 0 °C; and (c) R'_2NCOCl, Et_3N, CH_2Cl_2, reflux.

Fluorinated derivatives **6** and **7** exhibited moderate activities but were disappointingly inferior to 4-chlorocolchicine (**2**). 4-Fluoromethyl derivative **5** showed markedly diminished activity.

Consequently, we shifted our attention again to the 4-chloro derivatives having a newly designed side chain, such as sulfonamide, urea, or thiourea, at the C-7 position. The synthesis of compounds **13–23** is shown in Scheme 2. Sulfonamides (**13–17**) were obtained by treatment of 4-chlorodeacetylcolchicine (**12**) with the corresponding sulfonyl chlorides. Compounds **18–20** were prepared from **12** by employing the corresponding isocyanates. Similarly, ureides (**21–23**) were synthesized by acylation using the corresponding carbamoyl chlorides.

Table 2 enumerates the cytotoxicities of sulfonamides (**13–17**) and ureides (**18–23**). Sulfonamides (**13–17**) and urea-type compounds **21–23** showed modest IC_{50} values regardless of the length and type of the group (cyclic or acyclic), whereas the metabolic stability *in vitro* of compound **14** was comparable to the favorable stability of colchicine (**1**) (see Tables 1 & 2). The cytotoxicities of urea compounds **18** and **19** were low. On the other hand, compounds **17** and **20** possessing an aromatic ring exhibited slightly improved cytotoxicities.

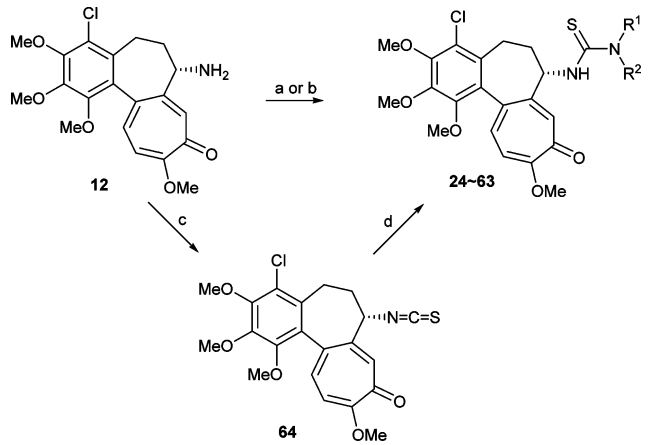
Table 2 Cytotoxicities, *S* log *P* values, and metabolic stabilities of 4-chlorocolchicine derivatives

Compound no.	IC_{50} value (nM) A549	HT-29	HCT116	*S* log *P*[a]	Metabolic stability (mL min^{-1} mg^{-1})
13	45.4	38.6	36.9	2.65	NT[b]
14	48.1	42.6	39.9	3.04	0.005
15	44.9	42.2	40.7	3.43	NT
16	40.8	36.4	33.2	3.68	NT
17	36.8	8.7	25.8	4.08	NT
18	247	81.8	134	3.42	NT
19	289	181	189	3.81	NT
20	48.1	35.2	36.0	4.93	NT
21	192	53.2	50.4	3.37	NT
22	48.3	43.9	44.3	4.15	0.021
23	26.6	42.6	42.4	4.30	0.043

[a] Estimated by MOE. [b] Not tested.

As a matter of course, thiourea-type derivatives were synthesized next (Scheme 3). Derivatives **24–63** were prepared from 4-chlorodeacetylcolchicine (**12**) by condensation with the corresponding isothiocyanates or substitution of the homologous imidazole-1-carbothioamides. In another experiment, deacetyl compound **12** was converted into isothiocyanate (**64**) with thiophosgene, and this was followed by the addition of amines to afford 4-chlorocolchicines having various thiourea side chains.

Ethylthioureide (**26**) exhibited more potent cytotoxicity than ethylureide (**18**) (see Tables 2 & 3). The results prompted us to investigate the antitumor potency of thiourea-type derivatives in detail (Table 3). *N*-Monosubstituted thiourea-type derivatives **24–28** exhibited comparable activities to 4-chlorocolchicine (**2**). However, *n*-hexylthioureide (**29**) showed decreased activity. Similarly, in *N*,*N*-disubstituted derivatives **31–33**, compounds **31** and **32** showed potent antitumor activities, whereas the activity of *N*,*N*-dipropylthioureide (**33**) was decreased. Moreover,



Scheme 3 *Reagents and conditions*: (a) RN=C=S, CH_2Cl_2 or MeOH–H_2O, 0 °C, 1.5 h; (b) *N*-R-1*H*-imidazole-1-carbothioamide; (c) thiophosgene, Et_3N, CH_2Cl_2, 0 °C, 2 h; and (d) RNH_2 or R_2NH, CH_2Cl_2 or MeOH–H_2O, rt, overnight.

all cyclic thioureides (**35–38**) possessed good potencies. On the other hand, *N*-(hydroxyethyl)thioureides (**30** and **34**) resulted in diminished activities in spite of the fact that 4-chlorocolchicine derivatives bearing a hydroxyalkanamide side chain at the C-7 position showed high antitumor activities and favorable metabolic stabilities.[21]

Docking simulations *in silico* were carried out to estimate why the thiourea-type derivatives showed higher activities than the urea-type derivatives. The thioureide–NH group of derivative **26** interacts with Thr179 of the tubulin α-subunit through a hydrogen bond. In addition, the C-9 carbonyl group in compound **26** and Lys254 of the tubulin β-subunit are held together by a hydrogen bond (Fig. 2b). On the other hand, urea derivative **18** has no such favorable interaction with tubulin (Fig. 2a). The reason is inferred as follows. It is known that the hydrogen-bond donating ability of a thioureide–NH group is generally higher than that of the corresponding ureide–NH group, because the pK_a value of a thioureide–NH group is lower than that of the homologous ureide–NH group.[27] Therefore, the interaction of Thr179 of tubulin with the thioureide group of compound **26** presumably determines the orientation of compound **26** in the binding pocket, and compound **26** is better for accepting hydrogen-bonding with Lys254 of tubulin than compound **18**.

Table 4 presents the cytotoxicities of phenylthioureides (**39–47**). Phenyl compound **39**, and *meta*- and *para*-methoxyphenyl compounds **41** and **42** showed good potencies but *ortho*-methoxyphenyl derivative **40** exhibited weaker activity than **41** and **42**. A similar tendency was observed in the cytotoxicity of cyanophenyl derivatives (*ortho*: **43**, *meta*: **44**, and *para*: **45**). *para*-(Dimethylamino)phenyl derivative **47** showed stronger activity than *meta*-substituted compound **46**.

Next, we selected four compounds **26**, **32**, **38**, and **47** with potent cytotoxicities *in vitro* to examine antitumor activity *in vivo* using HCT116 transplanted nude mice. The results are summarized in Table 5. All of the tested compounds *in vivo* were found to possess significant antitumor activities at half of the maximum dose (1/2MD) and no signs of toxicity, such as weight loss and mortality, were noted in the mice.[28] Thioureide-type 4-chlorocolchicines (**26**, **32**, **38**, and **47**) exhibited broad effective dosage ranges, similar to our previously reported amide-type derivatives.[21] It should be stressed that compound **26** showed high potency (44.4% IR) at even 1/4MD.

Aromatic thioureide (**47**) showed significant antitumor activity in *in vivo* experiments, in contrast to the corresponding aromatic amide-type derivative that had no activity *in vivo*.[21] However, the metabolic stability of **47** was lower than that of colchicine (**1**) (see Tables 1 & 4). Therefore, heteroaromatic thioureides (**48–63**) having lower *S* log *P* values than compound **47** were synthesized and evaluated *in vitro* with a view to improving metabolic stabilities (Scheme 3 and Table 6). Six-membered heteroaromatic-type compounds **48–53** exhibited moderate cytotoxicities compared to compound **47**. On the other hand, 2-thiazolylthioureide (**54**) demonstrated potent activity. All of the five-membered derivatives **55–63**, except compound **61**, showed strong activities. Notably, 3-pyrazolylthiourea (**56**), 5-methyl-3-pyrazolylthiourea (**58**), 3-triazolylthiourea (**59**), and 5-methyl-3-triazolylthiourea (**62**) exhibited

Table 3 Cytotoxicities, *S* log *P* values, and metabolic stabilities of 4-chlorocolchicine derivatives

Compound			IC_{50} value (nM)				
No.	R^1	R^2	A549	HT-29	HCT116	*S* log *P*[a]	Metabolic stability (mL min^{-1} mg^{-1})
24	H	H	45.0	6.1	9.0	2.94	NT[b]
25	H	Me	10.9	2.8	6.3	3.20	NT
26	H	Et	37.9	8.6	7.9	3.59	0.124
27	H	^{n}Pr	9.9	1.6	4.3	3.98	NT
28	H	^{n}Bu	7.4	1.0	1.8	4.37	NT
29	H	$^n C_6H_{13}$	212	176	171	5.15	NT
30	H	CH_2CH_2OH	251	52.7	89.7	2.56	NT
31	Me	Me	10.7	8.8	8.4	3.54	NT
32	Et	Et	10.1	6.1	6.9	4.32	0.541
33	^{n}Pr	^{n}Pr	40.3	38.2	34.4	5.10	NT
34	Me	CH_2CH_2OH	44.9	30.4	32.6	2.90	NT
35	$-CH_2CH_2CH_2-$		33.3	5.8	8.4	3.68	NT
36	$-CH_2CH_2CH_2CH_2-$		12.2	7.5	7.2	4.07	NT
37	$-CH_2CH_2CH_2CH_2CH_2-$		9.6	6.1	6.9	4.46	NT
38	$-CH_2CH_2OCH_2CH_2-$		44.9	7.2	9.1	3.31	0.061

[a] Estimated by MOE. [b] Not tested.

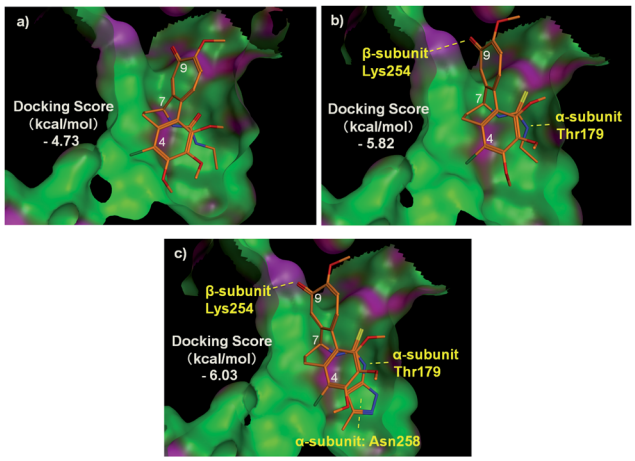


Fig. 2 Docking modes of compounds **18** (a), **26** (b), and **58** (c) at the colchicine binding site of tubulin (green: hydrophobic regions and pink: polar regions).

Table 4 Cytotoxicities and *S* log *P* values of 4-chlorocolchicine derivatives

Compound[a]		IC_{50} value (nM)				
No.	R^1	A549	HT-29	HCT116	*S* log *P*[b]	Metabolic stability (mL min^{-1} mg^{-1})
39	Ph	24.4	0.4	1.2	5.09	NT[c]
40	Ph-2-OMe	77.6	15.0	29.4	5.10	NT
41	Ph-3-OMe	23.3	0.5	1.4	5.10	NT
42	Ph-4-OMe	8.0	0.3	1.4	5.10	NT
43	Ph-2-CN	3795	866	2204	4.96	NT
44	Ph-3-CN	126	6.7	25.3	4.96	NT
45	Ph-4-CN	67.8	6.4	25.0	4.96	NT
46	Ph-3-NMe_2	38.4	4.4	24.2	5.16	NT
47	Ph-4-NMe_2	1.8	0.3	1.1	5.16	0.152

[a] R^2 = H. [b] Estimated by MOE. [c] Not tested.

Table 5 Antitumor activities of 4-chlorocolchicine derivatives

Compound no.	Dose (mg kg^{-1} day^{-1})	Total dose[a] (mg kg^{-1})	IR (%)	Mortality
26	20	60	44.4	0/4
	40[b]	120	62.6	0/4
32	25	75	29.5	0/4
	50[b]	150	54.6	0/4
38	25	75	31.3	0/4
	50[b]	150	52.7	0/4
47	10	30	32.6	0/4
	20[b]	60	52.5	0/4

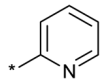
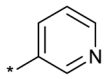
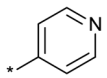
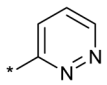
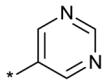
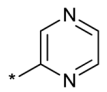
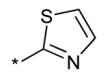
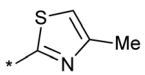
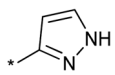
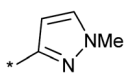
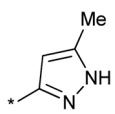
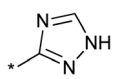
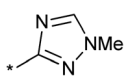
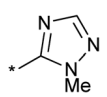
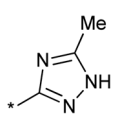
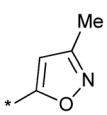
[a] Dosing schedule on days 1, 5, and 9. [b] Half of the maximum dose.

potent activities of sub-nanomole order against two or all three of the human cancer cell lines examined. Of particular interest was that the metabolic stability of compound **58** was comparable to that of colchicine (**1**).

Table 7 shows the data for inhibition of tubulin polymerization by **1**, **26**, and **58**. As expected, **58** showed the most potent antitubulin activity among the three assay samples, and compounds **26** and **58** were revealed to have same mode of action as **1**. The antitubulin activities of three compounds almost paralleled those of the cytotoxic activities. On the other hand, big differences between the IC_{50} values of cytotoxicities and of anti-tubulin activity on compound **58** were observed. Almost the same result has been reported in the study of tubulin polymerization inhibitors.[29] Possibly, this result is due to the difference in experimental conditions, *i.e.*, the cell-based assay (cytotoxicity) or the cell-free assay (tubulin assembly) and/or due to the cytotoxic effect of the compound through some additional target besides tubulin.

In silico simulation was conducted to uncover the factors responsible for the quite strong antitubulin activity *in vitro* of compound **58**. Compound **58** interacted not only with Thr179

Table 6 Cytotoxicities, *S* log *P* values, and metabolic stabilities of 4-chlorocolchicine derivatives

Compound[a]		IC_{50} value (nM)				
No.	R^1	A549	HT-29	HCT116	*S* log *P*[b]	Metabolic stability (mL min^{-1} mg^{-1})
48		170	34.6	61.7	4.48	NT[c]
49		186	31.6	41.7	4.48	NT
50		247	23.5	34.1	3.90	NT
51		39.6	6.3	13.2	3.88	NT
52		941	154	177	3.88	NT
53		37.4	19.4	33.0	3.88	NT
54		10.1	1.1	6.5	4.55	NT
55		7.6	0.4	2.4	4.85	NT
56		7.8	0.3	0.9	3.81	0.188
57		5.8	0.7	1.3	4.18	0.037
58		2.0	0.3	0.3	4.12	0.004
59		0.5	0.2	0.3	3.21	0.088
60		34.8	6.0	8.8	3.58	NT
61		40.3	18.0	33.9	3.58	NT
62		1.7	0.3	0.5	3.52	0.223
63		42.5	5.6	7.3	4.39	NT

[a] R^2 = H. [b] Estimated by MOE. [c] Not tested.

Table 7 Inhibition of tubulin assembly

Compound no.	IC_{50} value (μM)
1	8.12
26	2.36
58	1.11

and Lys254 of tubulin through the formation of hydrogen bonds with the C-7 thioureide side chain and the C-9 carbonyl group, respectively, but also Asn258 of the tubulin β-subunit through CH–π interaction with the terminal pyrazole ring at the C-7 side chain (Fig. 2c). It is speculated that the CH–π interaction with the pyrazole ring in compound **58** confers potent activity relative to compound **26**.

Conclusions

4-Chlorocolchicine derivative **26**, which has an ethylthioureide side chain at the C-7 position, was found to have high antitumor activity *in vivo* and a broad effective dosage range. Furthermore, we developed a new derivative, 5-methylpyrazol-3-ylthioureide (**58**), which exhibited the strongest cytotoxicity to HCT116 cell line in our series of colchicine studies and possessed desirable metabolic stability *in vitro*. Further evaluation of compounds **26** and **58** is in progress to examine their potential as anticancer agents.

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