Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3191–3193

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Mechanism of Action of Novel Pyrimidinyl Pyrazole Derivatives Possessing Antiproliferative Activity

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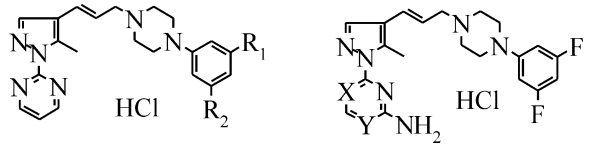
Received 27 May 2002; accepted 17 July 2002

Abstract—Pyrimidinyl pyrazole derivatives **1**–**4**, prepared as a new scaffold of an anti-tumor agent, showed antiproliferative activity against human lung cancer cell lines and inhibited tubulin polymerization. Furthermore, it was found that compound **2** bound at the colchicine site on tubulin, but the tubulin binding pattern was different from that of colchicine. Here, we describe the synthesis of the derivatives and the differences of the action mechanism on tubulin polymerization inhibition between compound **2** and colchicine.

In our previous paper, we reported that pyrimidinyl pyrazole **1** showed antiproliferative and inhibitory activities on tubulin polymerization.[1,2] As a result of focusing on further modification of the pyrimidine ring in order to find better compounds, we obtained new analogues **2**–**4** which showed more potent cytotoxicity in vitro than **1**. It has been known that there are two major binding sites on tubulin, which are called the colchicine (CLC) and vincristine (VCR) sites for tubulin polymerization inhibitors.[3] The binding site of pyrimidinyl pyrazole derivatives was investigated by the use of compound **2**. In this paper, we describe the synthesis, in vitro cytotoxicity, the tubulin polymerization inhibitory activity of compounds **2**–**4**, and the binding site of compound **2** on tubulin (Fig. 1).

Compounds **2** and **3** having a difluoro group on the phenyl ring were prepared as described for the synthesis of compound **1** via the Mannich reaction of **7** or **8** with **9**.[1,4] Compound **10** was treated with trifluoroacetic acid (TFA), $POCl_3$, and concd NH_4OH to give **3** as shown in Scheme 1. The Mannich reaction of compounds having an amino group on the pyrimidine ring with **9**, however, caused the cleavage of the C–N bond between the pyrimidine and pyrazole rings. Therefore, compound **4** bearing an amino group on the pyrimidine ring was prepared via reductive amination of formyl derivative **16** with **9** in the presence of $NaBH_3CN$, and deprotection of the benzyl group by the treatment with TFA. To perform this reductive amination, compound **11** derived from **5**[5] was cyclized with **12** instead of **6** to give **13**. The ester of **13** was reduced by diisobutylaluminum hydride (DIBAL), then successively oxidized with MnO_2, and treated with **14** to give **15**. The ester **15** was reduced and oxidized again to afford the formyl derivative **16**, which was led to **4** through reductive amination with **9**.

In vitro cytotoxicity and the tubulin inhibitory activity of compounds **2**–**4** were assessed in comparison with **1**, vinblastine (VBL), and CLC as shown in Table 1. The cytotoxicity (MTT assay[6]) of **2** bearing a difluoro group



1 R_1=Cl, R_2=H
2 R_1=R_2=F
3 X=N, Y=CH
4 X=CH, Y=N

Figure 1. Structure of pyrimidinyl pyrazole derivatives.

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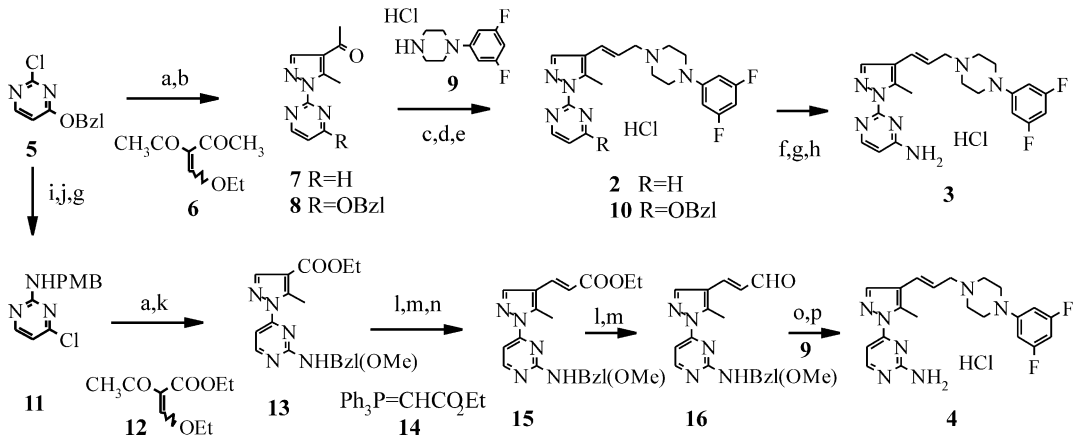
PII: S0960-894X(02)00568-1



Scheme 1. Reagents and conditions: (a) NH_2NH_2, K_2CO_3, EtOH, reflux, 18 h; (b) **6**, EtOH, 80 °C, 1 h; (c) **9**, (HCHO)*n*, *p*-TsOH, EtOH, reflux, 24 h; (d) $NaBH_4$, EtOH, THF, rt, 4 h; (e) *p*-TsOH, dioxane, THF, reflux, 3 h; (f) TFA, thioanisole, 60 °C, 30 min; (g) $POCl_3$, 60 °C, 2 h; (h) concd NH_4OH, THF, 80 °C, sealed, 2 h; (i) *p*-methoxybenzylamine [$H_2NBzl(OMe)$], K_2CO_3, THF, reflux, 2 days; (j) TFA, anisole, rt, 2 days; (k) **12**, EtOH, 80 °C, 1 h; (l) DIBAL, CH_2Cl_2, −78 °C, 4 h; (m) MnO_2, dioxane, rt, 24 h; (n) **14**, toluene, 80 °C, 15 h; (o) **9**, $NaBH_3CN$, AcOH, EtOH, rt, 2 days; (p) TFA, anisole, reflux, 4 days.

was stronger than that of **1** bearing a chloro group; and compounds **3** and **4** possessing an amino group on the pyrimidine ring showed more potent cytotoxicity than **2**. Introduction of an amino group was considered to enhance the cytotoxicity. Inhibitory activity of tubulin polymerization was examined by measurement of the turbidity of an aqueous solution of porcine brain tubulin.[7,8] Among the compounds, 4 showed high tubulin polymerization inhibitory activity ($IC_{50} = 3.72$ μM), which was more potent than that of CLC ($IC_{50} = 29.3$ μM); but the activity was lower than that of VBL. There was not a clear correlation between cytotoxicity and tubulin polymerization inhibitory activity, but compounds showing strong cytotoxicity tended to show stronger tubulin polymerization inhibitory activity. The time-dependent changes in the turbidity of **2** were shown in Figure 2. Compound **2** inhibited tubulin polymerization in a concentration-dependent manner. Furthermore, the inhibitory pattern of **2** on microtubule assembly was compared with colchicine. After tubulin was polymerized at 37 °C for 20 min, compound **2** was added to the assembled microtubulin. As shown in Figure 3, microtubule disassembly was observed at higher doses, whereas CLC showed no such disassembly activity (data not shown). A repeated tubulin polymerization-depolymerization assay (i.e., tubulin was assembled in the presence of CLC at 37 °C for 25 min; then it was disassembled at 4 °C for 10 min, and reincubated at 37 °C again) indicated that CLC-treated tubulin was more sensitive toward the polymerization inhibiting

Table 1. Inhibition of tubulin polymerization (IC_{50}) and in vitro cytotoxic activity (growth inhibition: GI_{50}) of compounds **1**–**4**, vinblastine, and colchicine against human cancer cell lines

Compd	Inhibition of microtubule assembly (%) IC_{50} (μM)	In vitro cytotoxic activity GI_{50} (ng/mL)	
		PC-12	HCT116
1	35.96	181.0	254.0
2	9.08	62.5	70.1
3	11.05	21.6	20.8
4	3.72	19.9	32.9
Vinblastine	0.60	10.2	1.0
Colchicine	29.30	31.5	8.4

Microtubule proteins (1 mg/mL) were incubated at 37 °C in the absence or presence of the compound, and the turbidity was monitored at 400 nm.[8,9] The IC_{50} values of compounds were calculated from the turbidity at 20 min after incubation. In vitro cytotoxic activity (a growth inhibition of 50%: GI_{50}) was calculated according to the published procedure.[7]

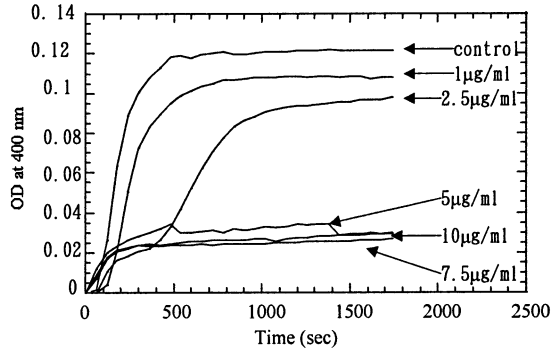


Figure 2. Effect of compound **2** on tubulin polymerization. Microtubule proteins (1.5 mg/mL) were incubated at 37 °C in the absence or presence (1–10 μg/mL) of the compound, and the turbidity was monitored at 400 nm. The IC_{50} value of compound **2** was calculated from the turbidity at 20 min after incubation.

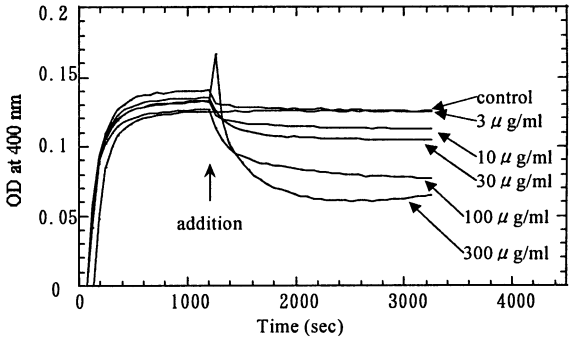


Figure 3. Microtubule disassembly by compound **2**. Microtubule proteins (1.5 mg/mL) were incubated at 37 °C without a compound. Compound **2** (3–300 μg/mL) was added 20 min later, and the turbidity was monitored at 400 nm.

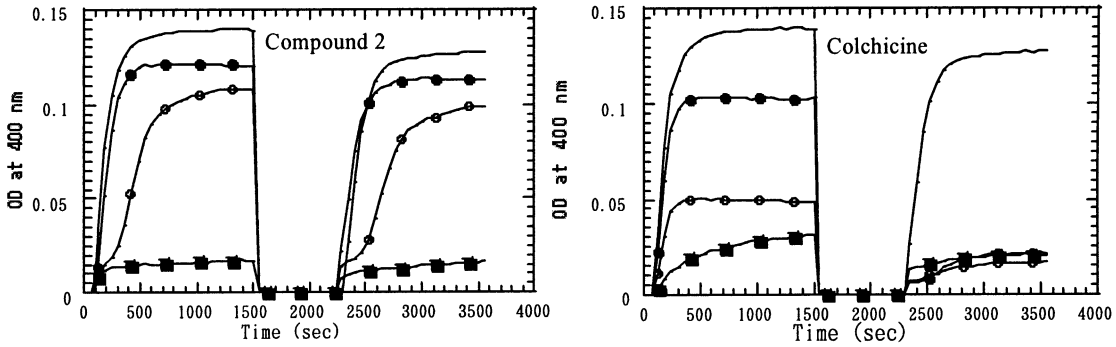


Figure 4. Comparison of compound **2** with colchicine on tubulin. Microtubule proteins were incubated at 37 °C for 25 min in the absence or presence of compound **2** (●: 0.5, ○: 2.5, ■: 20 μg/mL) or colchicines (●: 5, ○: 20, ■: 100 μg/mL). Microtubule proteins were disassembled at 5 °C for 10 min. Microtubule proteins were incubated at 37 °C again, and the turbidity was monitored at 400 nm (– control).

Table 2. Effect of compound **2** and colchicine on tubulin-[^{3}H]colchicine binding

Compd	Added concentration (μM)	Inhibitory binding[a] (%)
2	100	82
2	200	93
Colchicine	100	98
Colchicine	200	100

Tubulin (2 μM) was incubated with [^{3}H]colchicine (2 μM) at 37 °C for 20 min. Then compound **2** (100 or 200 μM) or colchicine (100 or 200 μM) was added at 37 °C.

[a]The inhibitory binding was determined by comparing the radioactivity under the compound-free condition with that in the presence of the compound on the tubulin-[^{3}H]colchicine binding portion after the treatment by Sephadex-G25 chromatography.[9]

activity of CLC, while **2** showed no such a pattern (Fig. 4). Therefore, it was suggested that our pyrimidinyl pyrazole derivatives possess characteristics different from that of CLC.

Then we examined the binding site of compound **2** on tubulin. As a result, compound **2** was found to bind at the CLC site, because compound **2** at 100 and 200 μM inhibited 82 and 93% of the CLC binding competitively (Table 2).

In conclusion, this new pharmacophore showed potent cytotoxicity against two tumor cell lines including the P-gp-associated multi-drug resistant (MDR) cell line PC-12 and tubulin polymerization inhibition. Although compound **2** was found to bind at the CLC site, the pattern of tubulin polymerization inhibitory effect elicited by **2** was different from that of CLC. These results suggest that our pyrimidinyl pyrazole derivatives could be novel leading compounds for the development of MDR-overcoming antitumor agents targeting tubulin.

References and Notes

1. Naito, H.; Sugimori, M.; Mitsui, I.; Nakamura, Y.; Iwahana, M.; Ishii, M.; Hirotani, K.; Ejima, A. *Chem. Pharm. Bull.* **1999**, *47*, 1679.
2. Iwahana, M.; Ochi, Y.; Ejima, A. *Anticancer Res.* **2000**, *20*, 785.
3. Iwasaki, S. *Med. Res. Rev.* **1993**, *13*, 183.
4. Ejima, A.; Sugimori, M.; Mitsui, I. *Japan Kokai Tokkyo Koho* JP 97 48776, 1997.
5. Mitchell, M. B.; Wallbank, P. J. *Tetrahedron Lett.* **1991**, *32*, 2273.
6. Mitsui, I.; Kumazawa, E.; Hirota, Y.; Aonuma, M.; Sugimori, M.; Ohsuki, S.; Uoto, K.; Ejima, A.; Terasawa, H.; Sato, K. *Jpn. J. Cancer Res.* **1995**, *86*, 776.
7. Shelanski, M. L.; Gaskin, F.; Cantor, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 765.
8. Peyot, V.; Briand, C.; Andreu, J. M. *Arch. Biochem. Biophys.* **1990**, *249*, 611.
9. Cortese, F.; Bhattacharyya, B.; Wolff, J. *J. Biol. Chem.* **1977**, *252*, 1134.