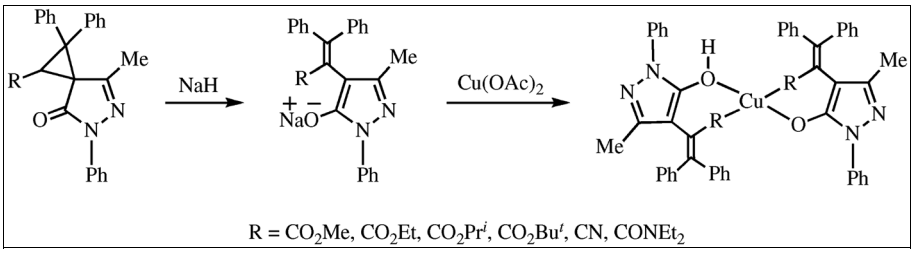
Synthesis and Biological Activity of Some New Pyrazole Copper(II) Complexes

Hiroshi Maruoka,* Sho Nishida, Nobuhiro Kashige, Yuki Yoshimura, Masahiko Omori, Ryoko Tomita, Eiichi Masumoto, Fumi Okabe, Fumio Miake, Kenji Yamagata, and Toshihiro Fujioka

Laboratory of Instrumental Analysis, Faculty of Pharmaceutical Sciences, Fukuoka University, Jonan-ku, Fukuoka 814-0180, Japan
*E-mail: maruoka@fukuoka-u.ac.jp

Received December 15, 2010
DOI 10.1002/jhet.919
Published online 29 October 2012 in Wiley Online Library (wileyonlinelibrary.com).



As a part of systematic investigation of synthesis and biologically active compounds of pyrazole derivatives containing transition metal, several new pyrazole copper(II) complexes **3a**–**f** were synthesized from pyrazole sodium salts **2a**–**f**, which were produced from spiro-pyrazoles **1a**–**f** and sodium hydride by a ring-opening reaction. All the synthesized compounds were characterized by spectroscopic analysis. Pyrazole copper(II) complexes **3a**–**d** and **3f** exhibited high DNA cleavage activity *in vitro*. Furthermore, compounds **3a**–**f** were tested for their growth inhibitory activity in A549 lung cancer, B16F10 murine melanoma, and HeLa human uterine carcinoma cells. Compounds **3c**,**d** displayed moderate B16F10 and HeLa inhibitory activity levels (**3c**: IC_{50} = 45 μ*M* in B16F10 cells and 34 μ*M* in HeLa cells, **3d**: IC_{50} = 50 μ*M* in B16F10 cells and 32 μ*M* in HeLa cells).

J. Heterocyclic Chem., **49**, 1218 (2012).

INTRODUCTION

The cleaving agents of nucleic acid have attracted extensive attention due to their potential applications in the fields of molecular biological technology and drug development [1,2]. DNA is an important cellular receptor and many chemicals exert their antitumor effects through binding to DNA thereby changing the replication of DNA and inhibiting the growth of tumor cells [3,4]. Then discussing the mechanism of compounds cleaving and/or binding to DNA possesses significant meanings. As well-known, the interaction of small molecular ligands and its transition metal complexes with DNA has been the main subject of intense investigations for almost half of a century [5]. Many transition metals, such as copper, zinc, and nickel, are important trace metals in human body. There has been considerable interest in developing new synthetic reagents to manipulate DNA, with selectivity different from the naturally occurring system. Simple metal complexes have been successfully employed to accelerate the rate of double-stranded DNA hydrolysis and those metal complexes with intrinsically high affinity for DNA are the most effective reagent [6–10]. In this context, the design of small complexes that can bind to DNA becomes more and more important.

Bleomycin is a glycopeptide containing several unusual amino acids, sugars, a pyrimidine, and a planar bithiazole ring system. The bleomycin molecule may be formally divided between its DNA-binding domain and its metal-chelating domain. Bleomycin is believed to act by degrading cellular DNA in a reaction requiring iron(II) and oxygen cofactors [11–14]. The evidence strongly suggests that the ultimate agent of DNA damage is some form of activated reduced oxygen, produced as a consequence of oxidation of bleomycin-chelated iron(II) to iron(III) in a quaternary DNA-bleomycin-iron-oxygen complex. On the other hand, copper(II) complexes are known to be effective DNA interaction and cleavage agents [15–22]. These complexes are structurally well-defined and thus suitable for mechanistic studies. Metal coordination complexes have been described as good models of many natural enzymes that mediate this important cleavage reaction.

Pyrazole derivatives are also well established in the literatures as important biologically effective heterocyclic compounds. These derivatives are the subject of many research studies due to their widespread potential pharmacological activities such as analgesic, antidepressant, antibacterial, plant growth regulatory, anti-inflammatory, and antihyperglycemic activities [23–28]. Hence, the

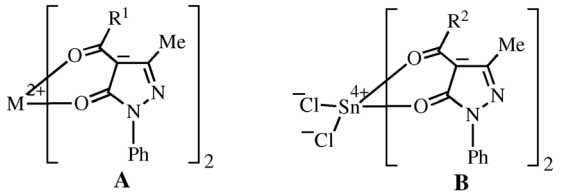


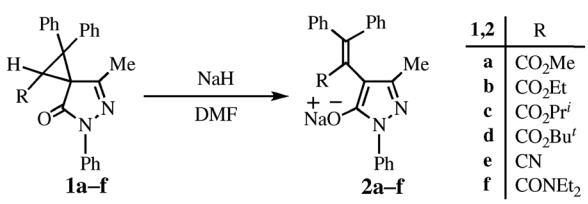
Figure 1. Structures of pyrazole metal complexes **A** and **B**.

development of improved methods for the synthesis of substituted pyrazole derivatives has acquired relevance to current research. Furthermore, the coordination chemistry of pyrazole derivatives such as 4-acyl-pyrazolones **A**, wherein M represents Cu(II), Zn(II), Co(II), Ni(II), Mn(II), Mg(II), or Hg(II), is well developed with respect to their wide spectrum of applications, e.g., metal extractants, NMR shift reagents, and in laser technology [29–36] (Fig. 1). In connection with our current research interests in the synthesis and reactivity of pyrazole derivatives [37,38], we have reported the synthesis of pyrazole tin(IV) complexes **B** [39]. Based on these properties, it can be reasonably supposed that the development of synthetic strategies for new pyrazole metal complexes might provide additional lead molecules for drug discovery. For these reasons, we have been interested in the preparation of pyrazole copper(II) complexes to evaluate their biological activity and now report the results of our investigation, *in vitro* their DNA cleavage and antitumor activities.

RESULTS AND DISCUSSION

Initially, the synthesis of pyrazole sodium salts **2a**−**f** has been accomplished as outlined in Scheme 1. In earlier studies, we have demonstrated the ring-opening reaction [38] of spiro-pyrazole compounds, which were prepared by treatment of 2,4-dihydro-5-methyl-2-phenyl-4-(diphenylmethylene)-3*H*-pyrazol-3-one and α-chloro esters according to our previous investigation [37]. Based on the ring-opening reaction, we hypothesized if the spiro-pyrazole compounds would be treated with an appropriate basic condition, the isolation of pyrazole sodium salts would be possible through a ring-opening reaction. Hence, we tried the synthesis of pyrazole sodium salts **2a**−**f**. In fact, when a mixture of spiro-pyrazole compounds **1a**−**f** and sodium hydride in *N,N*-dimethylformamide was stirred at room temperature for 1 h and then the solvent wad removed *in vacuo*, the expected pyrazole sodium salts **2a**−**f** were isolated by the recrystallization in good yields. The results are summarized in Table 1. Elemental analyses and spectral data of **2a**−**f** are consistent with the assigned structures (see "Experimental" section).

Scheme 1



1,2	R
a	CO_2Me
b	CO_2Et
c	CO_2Pr^i
d	CO_2Bu^t
e	CN
f	$CONEt_2$

In the next step, a metal exchange reaction of pyrazole sodium salts **2a**−**f** was examined. After some optimization, we found the reaction condition under which pyrazole copper(II) complexes **3a**−**f** could be isolated in the presence of copper(II) acetate. When a mixture of **2a**−**f** and copper(II) acetate monohydrate in methylene chloride and water was stirred at room temperature for 24 h, pyrazole copper(II) complexes **3a**−**f** were obtained in moderate yields (Scheme 2 and Table 2). By comparison of the IR, NMR, mass spectra, and elemental analyses of **3a**−**f** it seems that the structural assignments given to these compounds are correct (see Experimental section). For example, the IR spectrum of **3a** displays bands at 3447 cm^{-1} due to a hydroxyl group and at 1717 cm^{-1} due to two ester carbonyl groups. The ^1H NMR spectrum of **3a** in deuteriochloroform exhibits two three-proton singlets at δ 1.85 attributable to two methyl protons and two three-proton singlets at δ 3.58 due to two methyl ester protons. The ^{13}C NMR spectrum of **3a** shows a signal at δ 13.6 due to two methyl carbons, a signal at δ 51.8 due to two methyl carbons of the methyl ester function, two signals at δ 132.5 and 159.6 due to the olefin carbons, and a signal at δ 170.4 due to two carbonyl carbons of the methyl ester function.

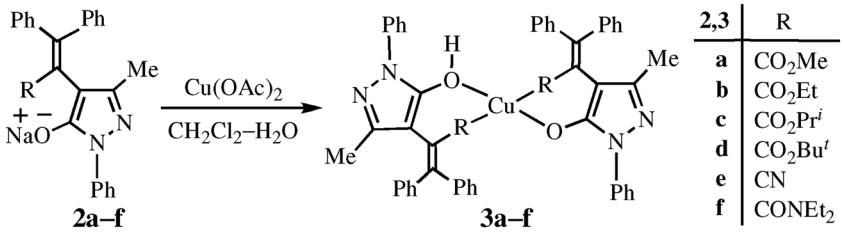
Finally, we have tested *in vitro* DNA cleavage activity of all the synthesized compounds **2a**−**f** and **3a**−**f**. The values obtained for activity were based on the remaining amounts of covalently closed circular duplex DNA of plasmid pBR322 (ccc-DNA) [40–42]. The DNA cleavage activity data are summarized in Table 3. The activity is accelerated to a remarkable degree by the addition of cupric ion (Cu^{2+}), which may stimulate the production of active radicals such as hydroxyl radical, resulting in DNA damage. Unfortunately, it was found that pyrazole sodium salts **2a**−**f** exhibited no DNA damage, with or without Cu^{2+} (entries 2−7). Interestingly, in the absence of Cu^{2+},

Table 1

Synthesis of pyrazole sodium salts **2a-f.**

Entry	Substrate	Product	Yield (%)
1	**1a**	**2a**	99
2	**1b**	**2b**	99
3	**1c**	**2c**	93
4	**1d**	**2d**	99
5	**1e**	**2e**	99
6	**1f**	**2f**	99

Scheme 2



pyrazole copper(II) complexes **3a**−**d** and **3f** showed moderate activity (entries 8−11, 13, and Fig. 2) and these activities were obviously accelerated by addition of 1 mM Cu^{2+} (entries 8−11, 13, and Fig. 3).

On the basis of the above results, to elucidate *in vitro* the DNA damage activity of pyrazole copper(II) complexes **3a**−**f**, the antitumor activity of **3a**−**f** was measured in A549 lung cancer, B16F10 murine melanoma, and HeLa human uterine carcinoma cells using MTT assay [43,44]. In general, the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) cell proliferation assay has been widely accepted as a reliable way to measure the cell proliferation rate. The growth inhibitory properties (IC_{50}) for the compounds **3a**−**f** are listed in Table 4. Interestingly, the data obtained by MTT assay showed that all the synthesized compounds **3a**−**f** had inhibitory effects on the growth of B16F10 and HeLa cells in dosage-dependent manners. However, compounds **3a**−**f** could not almost inhibit the cell growth of A549 at 100 μ*M* after 48 h of the treatment. Taken altogether, pyrazole copper(II) complex **3d** was the most potent compound in this series, having an IC_{50} value of 32 μ*M* in suppressing HeLa cell growth (entry 4).

In conclusion, we have demonstrated a novel method for the synthesis of pyrazole copper(II) complexes **3a**−**f**, proceeding by a metal exchange reaction when pyrazole sodium salts **2a**−**f** were treated with copper(II) acetate. Furthermore, we found that compounds **3a**−**f** could suppress B16F10 and HeLa cancer cell growth. Pyrazole copper(II) complex **3d** was the most effective small molecule in inhibiting HeLa cell growth and might perform its action through including DNA damage. Functionalized pyrazole metal complexes are important for the preparation of biologically active compounds with interest in medicinal chemistry. Further studies on the synthesis of new substituted pyrazole metal complexes are under way.

Table 2

Synthesis of pyrazole copper(II) complexes **3a-f**.

Entry	Substrate	Product	Yield (%)
1	**2a**	**3a**	88
2	**2b**	**3b**	72
3	**2c**	**3c**	68
4	**2d**	**3d**	48
5	**2e**	**3e**	60
6	**2f**	**3f**	74

EXPERIMENTAL

All melting points are uncorrected. The IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-A500 spectrometer at 500 and 125 MHz, respectively. The ^1H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative

Table 3

DNA cleavage by **2a-f** and **3a-f** in the absence and/or presence of Cu^{2+}.

Entry	Compound	DNA type	Relative amounts of DNA (%) Without Cu^{2+}[a]	With Cu^{2+}[b]
1	Control[c]	ccc-	100	100
		Oc-	0	0
2	**2a**[d]	ccc-	100	100
		Oc-	0	0
3	**2b**[d]	ccc-	97	99
		Oc-	3	1
4	**2c**[d]	ccc-	95	98
		Oc-	5	2
5	**2d**[d]	ccc-	99	100
		Oc-	1	0
6	**2e**[d]	ccc-	100	100
		Oc-	0	0
7	**2f**[d]	ccc-	97	100
		Oc-	3	0
8	**3a**[e]	ccc-	74	60
		Oc-	26	40
9	**3b**[e]	ccc-	52	31
		Oc-	48	69
10	**3c**[e]	ccc-	56	28
		Oc-	44	72
11	**3d**[e]	ccc-	52	28
		Oc-	48	72
12	**3e**[e]	ccc-	94	100
		Oc-	6	0
13	**3f**[e]	ccc-	75	71
		Oc-	25	29

[a]Incubation for 3 h.
[b]Incubation for 1 h.
[c]Amount: 0 m*M*.
[d]Amount: 1 m*M*.
[e]Amount: 0.1 m*M*.
As activity was accelerated upon addition of Cu^{2+}, the quantity of compounds and the incubation time were minimized until differences in activity could be observed.

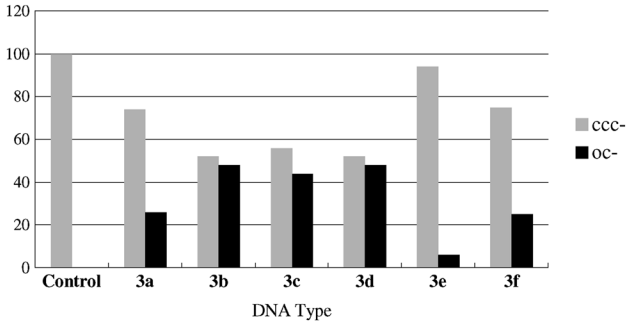


Figure 2. DNA cleavage by **3a–f** in the absence of Cu^{2+}.

Table 4

Antitumor activity of **3a-f** against A549, B16F10, and HeLa cells *in vitro*.

		IC_{50} values (μ*M*)		
Entry	Compound	A549	B16F10	HeLa
1	**3a**	>100	65	36
2	**3b**	No effect	70	36
3	**3c**	>100	45	34
4	**3d**	86	50	32
5	**3e**	90	69	36
6	**3f**	>100	92	51

to tetramethylsilane as internal standard. The positive FAB mass spectra were obtained on a JEOL JMS-700T spectrometer. The elemental analyses were performed on a YANACO MT-6 CHN analyzer.

General procedure for the preparation of pyrazole sodium salts 2a−f from 1a−f and sodium hydride. To an ice-cooled and stirred solution of **1a−f** [37] (1 mmol) in *N,N*-dimethylformamide (5 mL) was added 60% sodium hydride (0.04 g, 1 mmol). After the mixture was stirred at room temperature for 1 h, the solvent was removed *in vacuo*. The residue was recrystallized from chloroform-petroleum ether to afford **2a−f**.

Methyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate sodium salt (2a). This compound was obtained as colorless needles, mp 200–202°C; IR (potassium bromide): ν 1699 cm^{-1} (CO); ^1H NMR (deuterium oxide): δ 1.56 (s, 3H, pyrazole 3−Me), 3.56 (s, 3H, CO_2Me), 7.23–7.30 (m, 9H, Ph−H), 7.40−7.48 (m, 4H, Ph−H), 7.57–7.59 ppm (m, 2H, Ph−H); ^{13}C NMR (deuterium oxide): δ 15.5 (pyrazole 3−Me), 55.2 (CO_2Me), 102.5 (pyrazole C−4), 125.1 (Ph−C), 128.1 ($Ph_2C{=}C{-}CO_2Me$), 128.2, 130.3, 130.6, 130.8, 131.3, 131.2, 131.7, 131.8, 132.2, 132.4, 133.3, 133.4, 142.1, 145.1, 145.6 (Ph−C), 150.2 ($Ph_2C{=}C{-}CO_2Me$), 152.0 (pyrazole C−3), 164.5 (pyrazole C−5), 177.4 ppm (CO); ms: *m/z* 433 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{21}N_2O_3Na{\cdot}1H_2O$: C, 69.32; H, 5.15; N, 6.22. Found: C, 69.36; H, 5.36; N, 6.10.

Ethyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate sodium salt (2b). This compound was obtained as colorless needles, mp 76−79°C; IR (potassium bromide): ν 1705 cm^{-1} (CO); ^1H NMR (deuterium oxide): δ 0.92 (t, *J* = 7.3 Hz, 3H, CO_2CH_2Me), 1.57 (s, 3H, pyrazole 3−Me), 4.01 (q, *J* = 7.3 Hz, 2H, CO_2CH_2Me), 7.22−7.29 (m, 8H, Ph−H), 7.39−7.48 (m, 5H, Ph−H), 7.57−7.59 ppm (m, 2H, Ph−H); ^{13}C NMR (deuterium oxide): δ 15.6, 15.7 (pyrazole 3−Me, CO_2CH_2Me), 64.9 (CO_2CH_2Me), 102.5 (pyrazole C−4), 125.1, 128.2 (Ph−C), 128.6 ($Ph_2C{=}C{-}CO_2CH_2Me$), 130.5, 130.8, 131.0, 131.2, 131.8, 132.2, 133.4, 142.2, 145.1, 145.7 (Ph−C), 149.7 ($Ph_2C{=}C{-}CO_2CH_2Me$), 152.1 (pyrazole C−3), 164.5 (pyrazole C−5), 177.0 ppm (CO); ms: *m/z* 447 $[M+H]^+$. Anal. Calcd. for $C_{27}H_{23}N_2O_3Na{\cdot}0.1H_2O$: C, 72.05; H, 5.24; N, 6.22. Found: C, 72.05; H, 5.53; N, 6.18.

Isopropyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate sodium salt (2c). This compound was obtained as colorless needles, mp 216−218°C; IR (potassium bromide): ν 1695 cm^{-1} (CO); ^1H NMR (deuterium oxide): δ 0.97 (d, *J* = 6.4 Hz, 6H, CO_2CHMe_2), 1.61 (s, 3H, pyrazole 3−Me), 4.85 (sep, *J* = 6.4 Hz, 1H, CO_2CHMe_2), 7.24−7.30 (m, 8H, Ph−H), 7.41−7.47 (m, 5H, Ph−H), 7.55−7.57 ppm (m, 2H, Ph−H); ^{13}C NMR (deuterium oxide): δ 15.6 (pyrazole 3−Me), 23.3 (CO_2CHMe_2), 73.1 (CO_2CHMe_2), 102.4 (pyrazole C−4), 125.1, 128.2 (Ph−C), 129.0 ($Ph_2C{=}C{-}CO_2CHMe_2$), 130.5, 130.8, 131.0, 131.2, 131.8, 132.4, 133.4, 142.2, 145.2, 145.7 (Ph−C), 149.0 ($Ph_2C{=}C{-}CO_2CHMe_2$), 152.3 (pyrazole C−3), 164.5 (pyrazole C−5), 176.6 ppm (CO); ms: *m/z* 461 $[M+H]^+$. Anal. Calcd. for $C_{28}H_{25}N_2O_3Na{\cdot}0.7H_2O$: C, 71.08; H, 5.62; N, 5.92. Found: C, 71.05; H, 5.70; N, 5.88.

tert-Butyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate sodium salt (2d). This compound was obtained as colorless needles, mp 197−199°C; IR (potassium bromide): ν 1691 cm^{-1} (CO); ^1H NMR (deuterium oxide): δ 1.21 (s, 9H, CO_2CMe_3), 1.70 (s, 3H, pyrazole 3−Me), 7.20−7.30 (m, 8H, Ph−H), 7.41−7.47 (m, 5H, Ph−H), 7.54−7.56 ppm (m, 2H, Ph−H); ^{13}C NMR (deuterium oxide): δ 15.6 (pyrazole 3−Me), 29.7 (CO_2CMe_3), 86.0 (CO_2CMe_3), 102.4 (pyrazole C−4), 125.2, 128.2 (Ph−C), 130.0 ($Ph_2C{=}C{-}CO_2CMe_3$), 130.3, 130.7, 130.8, 131.2, 131.7, 132.5, 133.1, 142.2, 145.3, 145.8 (Ph−C), 149.3 ($Ph_2C{=}C{-}CO_2CMe_3$), 152.4 (pyrazole C−3), 164.3 (pyrazole C−5), 175.8 ppm (CO); ms: *m/z* 475 $[M+H]^+$; high-resolution ms: Calcd. for $C_{29}H_{28}N_2O_3Na$ 475.1998, found 475.1991.

2-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylonitrile sodium salt (2e). This compound was obtained as colorless needles, mp >280°C; IR (potassium bromide): ν 2216 cm^{-1} (CN); ^1H NMR (deuterium oxide): δ 1.65 (s, 3H, pyrazole 3−Me), 7.20−7.32 (m, 6H, Ph−H), 7.46−7.50 (m, 7H, Ph−H), 7.57−7.59 ppm (m, 2H, Ph−H); ^{13}C NMR (deuterium oxide): δ 15.3 (pyrazole 3−Me), 98.5 (pyrazole C−4), 106.2 (CN), 123.7 ($Ph_2C{=}C{-}CN$), 125.4, 128.5, 131.0, 131.3, 131.6, 131.8, 132.3, 132.8, 132.9, 141.9, 142.9, 143.4 (Ph−C), 151.3 (pyrazole C−3), 160.1 ($Ph_2C{=}C{-}CN$), 164.0 ppm (pyrazole C−5); ms: *m/z* 400 $[M+H]^+$. Anal. Calcd. for

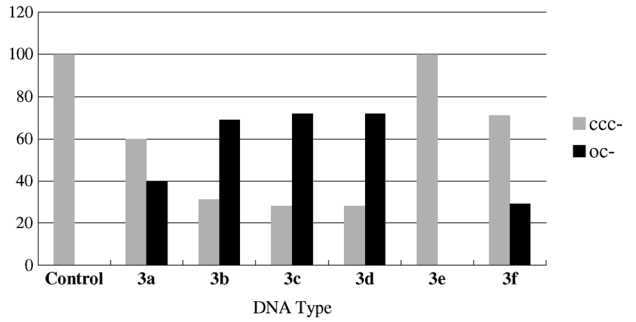


Figure 3. DNA cleavage by **3a–f** in the presence of Cu^{2+}.

$C_{25}H_{18}N_3ONa·2.7H_2O$: C, 67.01; H, 5.26; N, 9.38. Found: C, 66.91; H, 4.97; N, 9.27.

N,N-Diethyl-2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylamide sodium salt (2f). This compound was obtained as colorless needles, mp 169−172°C; IR (potassium bromide): ν 1674 cm^{-1} (CO); ^1H NMR (deuterium oxide): δ 0.77, 0.99 [t, J = 7.3 Hz, 6H, $CON(CH_2\mathit{Me})_2$], 1.88 (s, 3H, pyrazole 3−Me), 3.07, 3.36, 3.54, 3.73 [br s, 4H, $CON(\mathit{CH_2}Me)_2$], 7.22−7.27 (m, 8H, Ph−H), 7.36−7.37 (m, 3H, Ph−H), 7.40−7.47 ppm (m, 4H, Ph−H); ^{13}C NMR (deuterium oxide): δ 13.8, 14.2 [$CON(CH_2\mathit{Me})_2$], 15.8 (pyrazole 3−Me), 41.5, 46.1 [$CON(\mathit{CH_2}Me)_2$], 101.8 (pyrazole C−4), 118.4, 125.5, 128.3, 129.9, 130.6, 131.0 (Ph−C), 131.4 [$Ph_2C{=}\mathit{C}{-}CON(CH_2Me)_2$], 131.7, 132.4, 133.4, 142.2, 145.4 (Ph−C), 145.6 (pyrazole C−3), 152.5 [$Ph_2\mathit{C}{=}C{-}CON(CH_2Me)_2$], 163.3 (pyrazole C−5), 176.2 ppm (CO); ms: *m/z* 474 $[M+H]^+$. Anal. Calcd. for $C_{29}H_{28}N_3O_2Na·0.7H_2O$: C, 71.65; H, 6.10; N, 8.64. Found: C, 71.68; H, 6.23; N, 8.69.

General procedure for the preparation of pyrazole copper(II) complexes 3a–f from 2a–f and copper(II) acetate. To a stirred solution of **2a**−**f** (1 mmol) in methylene chloride (10 mL) and water (10 mL) was added copper(II) acetate monohydrate (0.10 g, 0.5 mmol). After the mixture was stirred at room temperature for 24 h, the precipitate was removed by filtration and washed with methylene chloride. The combined filtrates were extracted with methylene chloride (60 mL). The extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude products **3a**−**f** were sufficiently pure to be used without further purification.

Methyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate copper(II) complex (3a). This compound was obtained as brown solid, mp 107−110°C; IR (potassium bromide): ν 3447 (OH), 1717 cm^{-1} (CO); ^1H NMR (deuteriochloroform): δ 1.85 (s, 6H, 2× pyrazole 3−Me), 3.28 (br s, 1H, OH), 3.58 (s, 6H, 2× CO_2Me), 7.10−7.54 (m, 26H, Ph−H), 7.96−7.98 ppm (m, 4H, Ph−H); ^{13}C NMR (deuteriochloroform): δ 13.6 (2× pyrazole 3−Me), 51.8 (2× $CO_2\mathit{Me}$), 118.5 (2× pyrazole C−4), 119.2, 122.3, 124.4, 125.2, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.9, 129.1, 129.2, 129.3, 129.4, 129.9 (Ph−C), 132.5 (2× $Ph_2C{=}\mathit{C}{-}CO_2Me$), 138.4, 140.6 (Ph−C), 144.7 (2× pyrazole C−3), 159.4 (pyrazole C−5), 159.6 (2× $Ph_2\mathit{C}{=}C{-}CO_2Me$), 162.9 (pyrazole C−5), 170.4 ppm (2× CO); ms: *m/z* 883 $[M+H]^+$. Anal. Calcd. for $C_{52}H_{43}N_4O_6Cu·1H_2O$: C, 69.28; H, 5.03; N, 6.21. Found: C, 69.27; H, 4.78; N, 6.15.

Ethyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate copper(II) complex (3b). This compound was obtained as brown solid, mp 90−93°C; IR (potassium bromide): ν 3446 (OH), 1716 cm^{-1} (CO); ^1H NMR (deuteriochloroform): δ 0.98 (t, J = 7.0 Hz, 6H, 2× $CO_2CH_2\mathit{Me}$), 1.85 (s, 6H, 2× pyrazole 3−Me), 3.82 (br s, 1H, OH), 4.02−4.08 (m, 4H, 2× $CO_2\mathit{CH_2}Me$), 7.06−7.55 (m, 26H, Ph−H), 7.97−8.00 ppm (m, 4H, Ph−H); ^{13}C NMR (deuteriochloroform): δ 13.6, 13.7 (2× pyrazole 3−Me, 2× $CO_2CH_2\mathit{Me}$), 60.8 (2× $CO_2\mathit{CH_2}Me$), 118.9, 122.3, 124.3, 125.1, 127.6, 127.8, 127.9, 128.1 (Ph−C), 128.2 (2× pyrazole C−4), 128.9, 129.0, 129.1, 129.2, 129.9 (Ph−C), 132.6 (2× $Ph_2C{=}\mathit{C}{-}CO_2CH_2Me$), 138.5, 140.6 (Ph−C), 144.8 (2× pyrazole C−3), 159.5 (pyrazole C−5, 2× $Ph_2\mathit{C}{=}C{-}CO_2CH_2Me$), 162.3 (pyrazole C−5), 170.4 ppm (2× CO); ms: *m/z* 911 $[M+H]^+$. Anal. Calcd. for $C_{54}H_{47}N_4O_6Cu$: C, 71.15; H, 5.20; N, 6.15. Found: C, 71.43; H, 5.17; N, 6.10.

Isopropyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate copper(II) complex (3c). This compound was obtained as brown solid, mp 115−118°C; IR (potassium bromide): ν 3439 (OH), 1708 cm^{-1} (CO); ^1H NMR (deuteriochloroform): δ 0.98−1.05 (m, 12H, 2× $CO_2CH\mathit{Me_2}$), 1.85 (s, 6H, 2× pyrazole 3−Me), 4.94 (br s, 2H, 2× $CO_2\mathit{CH}Me_2$), 7.16−7.53 (m, 27H, Ph−H and OH), 8.00 ppm (br s, 4H, Ph−H); ^{13}C NMR (deuteriochloroform): δ 13.6, 13.7 (2× pyrazole 3−Me), 21.1, 21.3, 21.48, 21.54 (2× $CO_2CH\mathit{Me_2}$), 68.5 (2× $CO_2\mathit{C}HMe_2$), 118.5, 118.8, 122.0, 122.3, 124.2, 125.0, 127.3, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4 (Ph−C), 128.5 (2× pyrazole C−4), 128.9, 129.0, 129.1, 129.5, 129.8, 130.5 (Ph−C), 132.6 (2× $Ph_2C{=}\mathit{C}{-}CO_2CHMe_2$), 138.6, 140.5 (Ph−C), 144.9 (2× pyrazole C−3), 159.4 (pyrazole C−5), 159.7 (2× $Ph_2\mathit{C}{-}C{-}CO_2CHMe_2$), 161.7 (pyrazole C−5), 170.4 ppm (2× CO); ms: *m/z* 939 $[M+H]^+$. Anal. Calcd. for $C_{56}H_{51}N_4O_6Cu·1.2H_2O$: C, 69.98; H, 5.60; N, 5.83. Found: C, 70.04; H, 5.32; N, 5.75.

tert-Butyl 2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylate copper(II) complex (3d). This compound was obtained as brown solid, mp 85−88°C; IR (potassium bromide): ν 3446 (OH), 1716 cm^{-1} (CO); ^1H NMR (deuteriochloroform): δ 1.26 (s, 18H, 2× $CO_2C\mathit{Me_3}$), 1.86 (s, 6H, 2× pyrazole 3−Me), 2.94 (s, 1H, OH), 7.16−7.53 (m, 26H, Ph−H), 8.00−8.02 ppm (m, 4H, Ph−H); ^{13}C NMR (deuteriochloroform): δ 13.6 (2× pyrazole 3−Me), 27.5, 27.6, 27.7, 27.9 (2× $CO_2C\mathit{Me_3}$), 71.5 (2× pyrazole C−4), 81.9 (2× $CO_2\mathit{C}Me_3$), 118.5, 118.7, 122.2, 124.0, 124.9, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.7, 128.8, 128.9, 129.1, 129.5, 129.6, 129.8 (Ph−C), 132.8 (2× $Ph_2C{=}\mathit{C}{-}CO_2CMe_3$), 138.6, 140.4 (Ph−C), 144.9 (2× pyrazole C−3), 158.7 (pyrazole C−5), 159.8 (2× $Ph_2\mathit{C}{=}C{-}CO_2CMe_3$), 161.4 (pyrazole C−5), 170.4 ppm (2× CO); ms: *m/z* 967 $[M+H]^+$. Anal. Calcd. for $C_{58}H_{55}N_4O_6Cu·1H_2O$: C, 70.68; H, 5.83; N, 5.68. Found: C, 70.64; H, 5.99; N, 5.59.

2-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylonitrile copper(II) complex (3e). This compound was obtained as brown solid, mp 157−160°C; IR (potassium bromide): ν 3438 (OH), 2209 cm^{-1} (CN); ^1H NMR (deuteriochloroform): δ 1.93, 1.94, 1.95, 2.26 (s, 6H, 2× pyrazole 3−Me), 3.18 (br s, 1H, OH), 7.00−7.97 ppm (m, 30H, Ph−H); ^{13}C NMR (deuteriochloroform): δ 13.6, 13.7 (2× 3−Me), 78.9 (2×pyrazole C−4), 114.4, 115.1 (2× CN), 118.5, 119.1, 122.8, 123.0, 124.3, 125.3, 125.7, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 128.8, 128.9, 129.0, 129.1, 129.2, 129.3, 129.4, 129.5, 129.7, 130.0, 130.1, 130.2, 130.2, 130.5, 130.6, 130.9 (Ph−C), 135.9 (2× $Ph_2C{=}\mathit{C}{-}CN$), 136.5, 137.1, 137.9, 138.8, 139.0 (Ph−C), 140.8, 141.7 (2× pyrazole C−3), 156.9, 158.4 (2× $Ph_2\mathit{C}{=}C{-}CN$), 162.6, 163.1 ppm (2× pyrazole C−5); ms: *m/z* 817 $[M+H]^+$. Anal. Calcd. for $C_{50}H_{37}N_6O_2Cu·0.7H_2O$: C, 72.35; H, 4.66; N, 10.12. Found: C, 72.40; H, 4.70; N, 10.01.

N,N-Diethyl-2-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3,3-diphenylacrylamide copper(II) complex (3f). This compound was obtained as brown solid, mp 171−174°C; IR (potassium bromide): ν 3446 (OH), 1657 cm^{-1} (CO); ^1H NMR (deuteriochloroform): δ 0.55−1.27 [m, 12H, 2× $CON(CH_2\mathit{Me})_2$], 1.62, 1.68 (s, 6H, 2× pyrazole 3−Me), 2.20−3.50 [m, 9H, OH and 2× $CON(\mathit{CH_2}Me)_2$], 6.86−7.97 ppm (m, 30H, Ph−H); ^{13}C NMR (deuteriochloroform): δ 11.8, 13.2 [2× $CON(CH_2\mathit{Me})_2$], 14.1 (2× 3−Me), 38.0, 42.3 [2× $CON(\mathit{CH_2}Me)_2$], 73.0 (2× pyrazole C−4), 118.9, 122.2, 122.5, 125.0, 127.3, 127.4, 127.5, 127.7, 127.8, 127.9, 128.1, 128.2, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.2, 129.4, 129.5, 129.6, 129.8, 130.1, 130.2 (Ph−C) 132.7, 133.5 [2× $Ph_2C{=}\mathit{C}{-}CON(CH_2Me)_2$], 138.3, 140.0, 143.8 (Ph−C), 145.8 (2× pyrazole C−3), 161.0 [pyrazole C−5, 2× $Ph_2\mathit{C}{=}C{-}CON(CH_2Me)_2$], 165.0 (pyrazole C−5), 169.9 ppm

(2× CO); ms: *m/z* 965 $[M+H]^+$. Anal. Calcd. for $C_{58}H_{57}N_6O_4Cu$: C, 72.14; H, 5.95; N, 8.70. Found: C, 72.34; H, 6.12; N, 8.70.

DNA cleavage activity assay. The method of assaying the DNA cleavage activity, using a covalently closed circular duplex DNA of plasmid pBR322 (ccc-DNA) as a substrate, was described in our previous investigation [40–42].

Tumor cell culture and MTT assay. A549 and HeLa cells were obtained from DS Pharma Biomedical (Japan). B16F10 cells were purchased from American Type Culture Collection. Tumor cells were cultured in Roswell Park Memorial Institute 1640 medium or Dulbecco's Modified Eagle Medium at 37°C with 5% carbon dioxide and 95% air, supplemented with fetal bovine serum and/or penicillin, streptomycin, or kanamycin. The cells were seeded onto 96-well plates. The inhibition of the cellular growth was estimated using MTT assay according to Mosmann [43] and our previous methods [44].

Acknowledgments. The authors thank Mr. Hiroshi Hanazono and Ms. Yukiko Iwase for obtaining Mass and NMR spectra and to Ms. Junko Honda for her valuable help with elemental analyses.

REFERENCES AND NOTES

[1] Pogozelski, W. K.; Tullius, T. D. Chem Rev 1998, 98, 1089.
[2] Liu, C.; Wang, M.; Zhang, T.; Sun, H. Coord Chem Rev 2004, 248, 147.
[3] Pyle, A. M.; Morii, T.; Barton, J. K. J Am Chem Soc 1990, 112, 9432.
[4] Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. Nucleic Acids Res 1991, 19, 2595.
[5] Dervan, P. B. Bioorg Med Chem 2001, 9, 2215.
[6] Kong, D.-Y.; Meng, L.-H.; Ding, J.; Xie, Y.-Y.; Huang, X.-Y. Polyhedron 2000, 19, 217.
[7] Nomura, A.; Sugiura, Y. J Am Chem Soc 2004, 126, 15374.
[8] Liang, F.; Wang, P.; Zhou, X.; Li, T.; Li, Z.; Lin, H.; Gao, D.; Zheng, C.; Wu, C. Bioorg Med Chem Lett 2004, 14, 1901.
[9] Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. Chem Commun 2005, 2540.
[10] Murugavel, R.; Choudhury, A.; Walawalkar, M. G.; Pothiraja, R.; Rao, C. N. R. Chem Rev 2008, 108, 3549.
[11] Farrell, P. J.; Sen, G. C.; Dubois, M. F.; Ratner, L.; Slattery, E.; Lengyel, P. Proc Natl Acad Sci USA 1978, 75, 5893.
[12] Povirk, L. F. In Molecular Aspects of Anti-Cancer Drug Action; Neidle, S., Waring, M. J., Eds.; Macmillan Press: London, 1983, p 157.
[13] Burger, R. M. Chem Rev 1998, 98, 1153.
[14] Paz, M. M. In Anticancer Therapeutics; Missailidis, S., Ed.; Wiley: Oxford, UK, 2008, p 115.
[15] Deck, K. M.; Tseng, T. A.; Burstyn, J. N. Inorg Chem 2002, 41, 669.
[16] Baldini, M.; Belicchi-Ferrari, M.; Bisceglie, F.; Pelosi, G.; Pinelli, S.; Tarasconi, P. Inorg Chem 2003, 42, 2049.
[17] Gupta, T.; Dhar, S.; Nethaji, M.; Chakravarty, A. R. Dalton Trans 2004, 1896.
[18] Sissi, C.; Mancin, F.; Gatos, M.; Palumbo, M.; Tecilla, P.; Tonellato, U. Inorg Chem 2005, 44, 2310.
[19] Arya, D. P. Top Heterocycl Chem 2006, 2, 129.
[20] Nunes, N.; Amaro, R.; Costa, F.; Rombi, E.; Carvalho, M. A.; Neves, I. C.; Fonseca, A. M. Eur J Inorg Chem 2007, 1682.
[21] Liu, C.; Wang, L. Dalton Trans 2009, 227.
[22] Patra, A. K.; Bhowmick, T.; Roy, S.; Ramakumar, S.; Chakravarty, A. R. Inorg Chem 2009, 48, 2932.
[23] Kees, K. L.; Fitzgerald, J. J.; Steiner, K. E.; Mattes, J. F.; Mihan, B.; Tosi, T.; Mondoro, D.; McCaleb, M. L. J Med Chem 1996, 39, 3920.
[24] Ono, S.; Okazaki, K.; Sakurai, M.; Inoue, Y. J Phys Chem A 1997, 101, 3769.
[25] Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Iwai, N.; Hiyama, Y.; Suzuki, K.; Ito, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. J Med Chem 2004, 47, 3693.
[26] Singh, P.; Paul, K.; Holzer, W. Bioorg Med Chem 2006, 14, 5061.
[27] Ismail, M. M. F.; Ammar, Y. A.; EI-Zahaby, H. S. A.; Eisa, S. I.; Barakat, S. E.-S. Arch Pharm Chem Life Sci 2007, 340, 476.
[28] Kimata, A.; Nakagawa, H.; Ohyama, R.; Fukuuchi, T.; Ohta, S.; Suzuki, T.; Miyata, N. J Med Chem 2007, 50, 5053.
[29] Okafor, E. C. Spectrochim Acta 1981, 37A, 939.
[30] Okafor, E. C. Spectrochim Acta 1981, 37A, 945.
[31] Prasad, K. M.; Rao, N. R.; Ganorkar, M. C.; Chary, C. M. J Organomet Chem 1989, 376, 53.
[32] Buono-Core, G. E.; Leon, G. J Coord Chem 1990, 21, 323.
[33] Mahon, M. F.; Molloy, K. C.; Omotowa, B. A.; Mesubi, M. A. J Organomet Chem 1996, 525, 239.
[34] Joshi, K. T.; Dabhi, H. R.; Pancholi, A. M.; Rana, A. K. Orient J Chem 1996, 12, 287.
[35] Pettinari, C.; Marchetti, F.; Pettinari, R.; Cingolani, A.; Rivarola, E.; Phillips, C.; Tanski, J.; Rossi, M.; Caruso, F. Eur J Inorg Chem 2004, 3484.
[36] Pettinari, C.; Pettinari, R.; Fianchini, M.; Marchetti, F.; Skelton, B. W.; White, A. H. Inorg Chem 2005, 44, 7933.
[37] Maruoka, H.; Kashige, N.; Eishima, T.; Okabe, F.; Tanaka, R.; Fujioka, T.; Miake, F.; Yamagata, K. J Heterocycl Chem 2008, 45, 1883.
[38] Maruoka, H.; Masumoto, E.; Eishima, T.; Okabe, F.; Nishida, S.; Yoshimura, Y.; Fujioka, T.; Yamagata, K. J Heterocycl Chem 2009, 46, 782.
[39] Maruoka, H.; Yamagata, K.; Okabe, F.; Tomioka, Y. J Heterocycl Chem 2006, 43, 859.
[40] Watanabe, K.; Kashige, N.; Nakashima, Y.; Hayashida, M.; Sumoto, K. Agric Biol Chem 1986, 50, 1459.
[41] Kashige, N.; Yamaguchi, T.; Mishiro, N.; Hanazono, H.; Miake, F.; Watanabe, K. Biol Pharm Bull 1995, 18, 653.
[42] Maruoka, H.; Kashige, N.; Miake, F.; Yamaguchi, T. Chem Pharm Bull 2005, 53, 1359.
[43] Mosmann, T. J Immunol Methods 1983, 65, 55.
[44] Yoshida, M.; Fuchigami, M.; Nagao, T.; Okabe, H.; Matsunaga, K.; Takata, J.; Karube, Y.; Tsuchihashi, R.; Kinjo, J.; Mihashi, K.; Fujioka, T. Biol Pharm Bull 2005, 28, 173.