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SYNTHESIS AND DNA CLEAVAGE ACTIVITY OF FUNCTIONALIZED PYRAZOL-3-ONES CONTAINING OXIME ESTER

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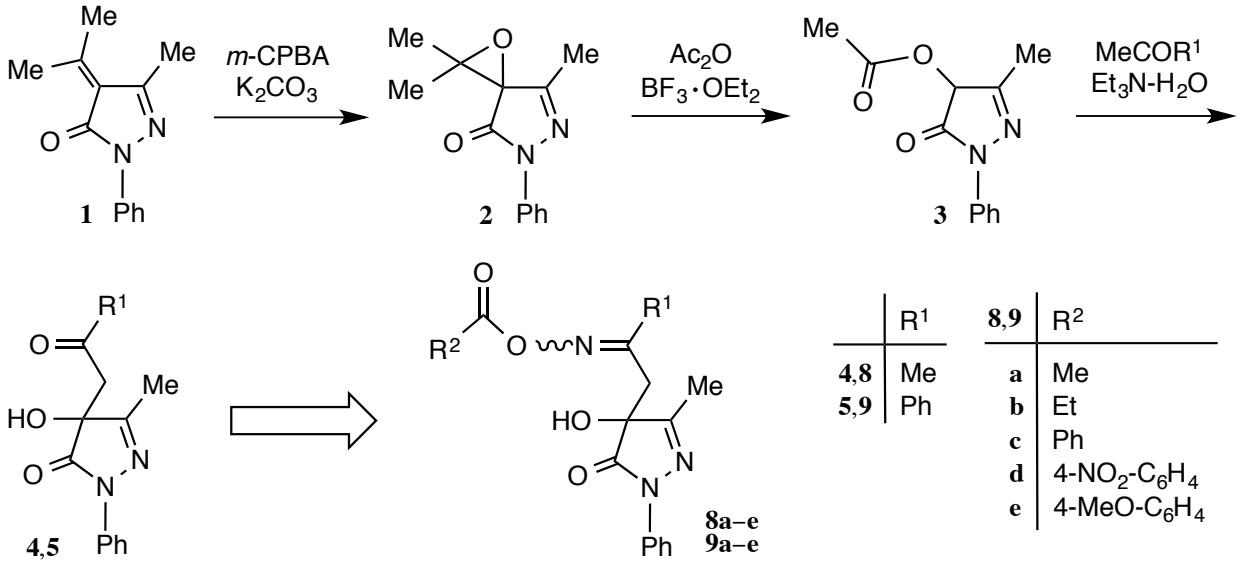
Abstract – A facile access to the synthesis of functionalized pyrazol-3-ones containing oxime ester from 4-hydroxypyrazol-3-ones, which were prepared starting from 4-alkylidenepyrazol-3-one, in moderate to good yields is reported. The structures of all products were identified by spectral data and some synthesized compounds were tested for their DNA cleavage activity.

Heterocyclic compounds with pyrazol-3-one and pyrazole moiety were found to be valuable intermediates for medicinal drugs. Pyrazole derivatives are highly essential heterocyclic structures prevalent in biologically active products used widely throughout various disciplines of chemical industry-including medicine and agriculture.[1] They are now essential to the fields of medicinal chemistry and material science as well as the agrochemical and food industry.[2] Pyrazole derivatives have wide-ranging collection of conventional biological and pharmaceutical activities, such as antitumor,[3] anti-inflammatory, analgesic, antipyretic,[4] antiviral,[5] antimicrobial,[6] anti-ischemic effects,[7] hypoglycemic,[8] antihypertensive,[9] antiangiogenic,[10] and antioxidant[11] activity. They are also inhibitors of human 15-lipoxygenase.[12] In this context, a large number of general methods for the preparation of pyrazol-3-one and pyrazole derivatives have recently been reported.[13]

On the other hand, oxime esters are a small, but valuable intermediates for the synthesis of heterocyclic compounds.[14] They are also an important class of biologically useful compounds for the synthesis of fragrances[15] and therapeutic studies,[16] and useful building blocks in peptide synthesis.[17] In addition, oxime esters are selective covalent inhibitors of serine hydrolase retinoblastoma-binding protein 9 (RBBP9)[18] and cleave DNA under photolytic conditions.[19] Hence, their synthesis continues to attract attention and provides an interesting challenge.[20]

In connection with the synthesis and reactivity of pyrazol-3-one and pyrazole derivatives,[21] we have reported the novel synthesis of 4-hydroxypyrazol-3-ones **4** and **5** starting from 4-alkylidenepyrazol-3-one

1 (Scheme 1).[22] Owing to the importance of oxime esters, we decided to extend our previous studies to synthesize the functionalized pyrazol-3-ones **8a–e** and **9a–e** containing oxime ester from **4** and **5**, which might have useful biological and therapeutic activities, especially *in vitro* their DNA cleavage activities. 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.[23] 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.[24] Then discussing the mechanism of compounds cleaving and/or binding to DNA possesses significant meanings. For these reasons, we have been interested in the preparation of functionalized pyrazol-3-ones containing oxime ester to evaluate their DNA cleavage activity and now report the results of our investigation.



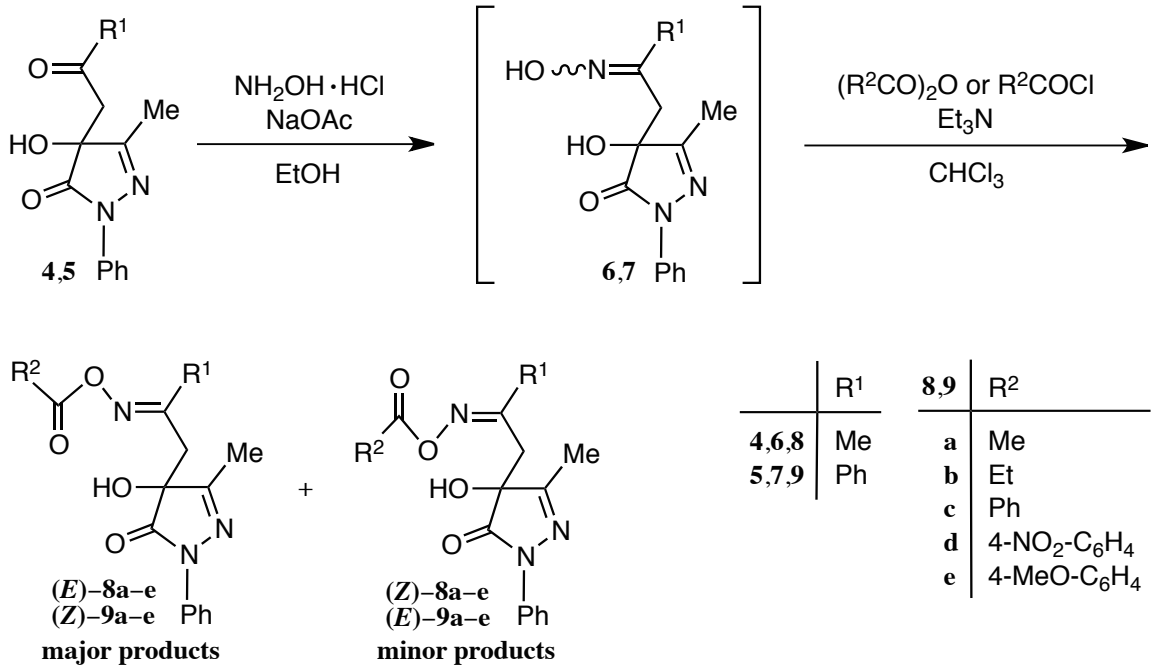
	R^1
4,**8**	Me
5,**9**	Ph

8,**9**	R^2
a	Me
b	Et
c	Ph
d	4-NO_2-C_6H_4
e	4-MeO-C_6H_4

Scheme 1

The starting materials, 4-hydroxypyrazol-3-ones **4** and **5**, were prepared by the treatment of 4-acetyloxypyrazol-3-one **3** with acetone and/or acetophenone according to our previous procedure.[22] In the first step, we examined the conversion of compounds **4** and **5** into the oxime derivatives **6** and **7** (Scheme 2). In this reaction, the base NaOAc was investigated because of its ease of handling. Indeed, when **4** and **5** were treated with hydroxylamine hydrochloride in the presence of NaOAc in refluxing EtOH, the expected pyrazol-3-one oxime derivatives **6** and **7** were produced in good yields as crude products. On the basis of this result, in the next step, we have tried to directly construct pyrazol-3-one oxime esters **8a–e** and **9a–e** starting from **4** and/or **5** and acid anhydride or chloride in a one-step process, without isolation of the intermediate oxime derivatives **6** and **7**. The results are summarized in Table 1. When a mixture of **4** and/or **5** and hydroxylamine hydrochloride in the presence of NaOAc in refluxing

EtOH for 1 h and then the reaction mixture was treated with acid anhydride or chloride in the presence of Et_3N in refluxing $CHCl_3$ for 1 h, the desired pyrazol-3-ones (*E*)-**8a–e** and (*Z*)-**9a–e** containing oxime ester were obtained together with *(Z)*-**8a–e** and (*E*)-**9a–e** as minor products.



Scheme 2

Table 1. Synthesis of pyrazol-3-ones **8a–e** and **9a–e** containing oxime ester

Entry	Substrate	R^1	R^2	Product	Yield (%)	Ratio of *E*/*Z*
1	**4**	Me	Me	**8a**	72	7/1[a]
2	**4**	Me	Et	**8b**	80	9/1[a]
3	**4**	Me	Ph	**8c**	45	5.4/1.0[b]
4	**4**	Me	4-NO_2-C_6H_4	**8d**	62	6.8/1.0[b]
5	**4**	Me	4-MeO-C_6H_4	**8e**	47	1/0[b]
6	**5**	Ph	Me	**9a**	60	1.0/2.3[b]
7	**5**	Ph	Et	**9b**	67	1.0/4.2[b]
8	**5**	Ph	Ph	**9c**	69	1.0/1.5[b]
9	**5**	Ph	4-NO_2-C_6H_4	**9d**	65	1.0/2.8[b]
10	**5**	Ph	4-MeO-C_6H_4	**9e**	62	0.9/1.0[b]

[a] The ratio of *E* and *Z* was determined based on the ^{1}H NMR data.
[b] The ratio of *E* and *Z* was determined based on the isolated yield.

These products **8a–e** and **9a–e** gave satisfactory elemental analyses and spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, and MS) consistent with their assigned structures (see experimental section). For example, IR spectrum of (*Z*)-**9a** displays a band at 3383 cm^{-1} because of a OH group and two bands at 1770 and 1720 cm^{-1} because of two C=O groups, whereas that of (*E*)-**9a** shows a band at 3414 cm^{-1} because of a OH group and two bands at 1759 and 1730 cm^{-1} because of two C=O groups. The ^{1}H NMR spectrum of (*Z*)-**9a** in $CDCl_3$ exhibits a three-proton singlet at δ 1.98 assignable to the acetyl protons, a two-proton singlet at δ 3.29 assignable to the methylene protons, and a D_2O exchangeable one-proton broad singlet at δ 4.43 assignable to the OH proton, whereas that of (*E*)-**9a** exhibits a three-proton singlet at δ 2.14 assignable to the acetyl protons, a two-proton singlet at δ 3.48 assignable to the methylene protons, and a D_2O exchangeable a one-proton singlet at δ 3.89 assignable to the OH proton. In general, the signals of methylene bonded to pyrazole moiety in (*Z*)-**9a** isomer are observed in high magnetic field, whereas those of (*E*)-**9a** appear at lower field.[25] It seems that the methylene protons of (*E*)-**9a** are found in downfield because of the deshielding effect of the acetyloxy group. The ^{13}C NMR spectrum of (*Z*)-**9a** in $CDCl_3$ shows a signal at δ 40.8 because of the methylene carbon and a signal at δ 160.5 because of the imino carbon, whereas that of (*E*)-**9a** shows a signal at δ 35.0 because of the methylene carbon and a signal at δ 159.7 because of the imino carbon. Fortunately, the heteronuclear multiple bond correlation (HMBC) experiment of (*E*)-**9a** isomer gave the correlation between the OH proton and the pyrazole C-4 carbon, which provides further evidence for the positional relationship of the OH group. In addition, elemental analyses of compounds (*Z*)-**9a** and (*E*)-**9a** point to the same elemental composition $C_{20}H_{19}N_3O_4$.

Finally, we have tested *in vitro* DNA cleavage activity of the synthesized compounds **1–5**, (*E*)-**8c–e**, and (*Z*)-**9a–e**. The values obtained for activity were based on the remaining amounts of covalently closed circular duplex DNA, namely ccc-DNA, of plasmid pBR322.[26] The data of DNA cleavage activity is summarized in Table 2. Indeed, in the absence of Cu^{2+}, all the tested compounds showed no DNA cleavage activity. These activities of compounds **1**, **2**, **4**, and (*Z*)-**9a**, however, were obviously accelerated by the addition of 1 mM Cu^{2+} (entries 2, 3, 5, and 10). Furthermore, it was found that compounds (*E*)-**8c–e** and (*Z*)-**9b–e** have moderate activity with Cu^{2+} (entries 7–9 and 11–14).

In conclusion, we have prepared new functionalized pyrazol-3-ones **8a–e** and **9a–e** containing oxime ester and found that (*Z*)-**9a** showed high DNA cleavage activity *in vitro* with Cu^{2+}. In addition, (*E*)-**8c–e** and (*Z*)-**9b–e** exhibited moderate DNA cleavage activity. Our results suggest that these new compounds may play a role *in vivo*. Pyrazol-3-one oxime esters are important building blocks in organic synthesis and for the preparation of biologically active compounds with interest in medicinal chemistry. Further synthetic applications for novel functionalized pyrazole derivatives are in progress.

Table 2. DNA cleavage by **1–5**, (*E*)-**8c–e**, and (*Z*)-**9a–e** 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- oc-	100 0	100 0
2	**1**[d]	ccc- oc-	100 0	0 89
3	**2**[d]	ccc- oc-	100 0	28 72
4	**3**[d]	ccc- oc-	100 0	100 0
5	**4**[d]	ccc- oc-	100 0	24 76
6	**5**[d]	ccc- oc-	100 0	80 20
7	(***E***)-**8c**[d]	ccc- oc-	100 0	89 11
8	(***E***)-**8d**[d]	ccc- oc-	100 0	87 13
9	(***E***)-**8e**[d]	ccc- oc-	100 0	91 9
10	(***Z***)-**9a**[d]	ccc- oc-	100 0	61 39
11	(***Z***)-**9b**[d]	ccc- oc-	100 0	84 16
12	(***Z***)-**9c**[d]	ccc- oc-	100 0	88 12
13	(***Z***)-**9d**[d]	ccc- oc-	100 0	88 12
14	(***Z***)-**9e**[d]	ccc- oc-	100 0	88 12

[a] Incubation for 3 h. [b] Incubation for 3 h. [c] Amount: 0 mM. [d] Amount: 10 mM.
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.

EXPERIMENTAL

All melting points are uncorrected. The IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. The ^{1}H and ^{13}C NMR spectra were measured with a JEOL JNM-A500 spectrometer at 500.00 and 125.65 MHz, respectively. The ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative to TMS as internal standard. Positive FAB MS spectra were obtained on a JEOL JMS-700T spectrometer. Elemental analyses were performed on YANACO MT-6 CHN analyzer. The starting compounds **4** and **5** were prepared in this laboratory according to the procedure reported in literature.[22]

General procedure for the preparation of pyrazol-3-one oxime esters 8a–e and 9a–e from 4 and 5. A mixture of **4** (0.246 g, 1 mmol) and/or **5** (0.308 g, 1 mmol), hydroxylamine hydrochloride (0.139 g, 2 mmol), and NaOAc (0.164 g, 2 mmol) in EtOH (10 mL) was refluxed for 1 h. After removal of the solvent *in vacuo*, cold H_2O was added to the residue with stirring and ice cooling. The resulting mixture was extracted with EtOAc (60 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give crude products **6** and **7**. To an ice-cooled and stirred mixture of **6** and/or **7** and Et_3N (0.122 g, 1.2 mmol) in $CHCl_3$ (10 mL), acetic anhydride (0.123 g, 1.2 mmol), propionic anhydride (0.156 g, 1.2 mmol), benzoyl chloride (0.169 g, 1.2 mmol), 4-nitrobenzoyl chloride (0.223 g, 1.2 mmol), or 4-methoxybenzoyl chloride (0.205 g, 1.2 mmol) was added. After the mixture was refluxed for 1 h, cold H_2O was added to the reaction mixture with stirring and ice cooling. The resulting mixture was extracted with $CHCl_3$ (60 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with $CHCl_3$ as the eluent to afford **8a–e** and **9a–e**.

(*E*/*Z*)-4-[2-[(Acetyloxy)imino]propyl]-2,4-dihydro-4-hydroxy-5-methyl-2-phenyl-3*H*-pyrazol-3-one (8a): Pale yellow oil (0.219 g, 72% as a 7:1 mixture of *E* and *Z* configurations by ^{1}H NMR spectrum); IR (neat): ν 3382 (OH), 1769, 1722 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): (*E*)-isomer: δ 2.00 [s, 3H, $CH_2C(Me)$=N-OCOMe], 2.08 [s, 3H, CH_2C(Me)=N-OCO*Me*], 2.19 (s, 3H, pyrazole 5-Me), 2.87 [AB q, *J* = 14.6 Hz, 2H, C*H*$_2$C(Me)=N-OCOMe], 4.42 (s, 1H, OH), 7.17–7.20 (m, 1H, Ph-H), 7.36–7.39 (m, 2H, Ph-H), 7.79–7.84 (m, 2H, Ph-H); (*Z*)-isomer: δ 2.04 [s, 3H, CH_2C(Me)=N-OCO*Me*], 2.14 [s, 3H, $CH_2C(Me)$=N-OCOMe], 2.17 (s, 3H, pyrazole 5-Me), 2.95 [AB q, *J* = 13.4 Hz, 2H, C*H*$_2$C(Me)=N-OCOMe], 4.16 (br, 1H, OH), 7.17–7.20 (m, 1H, Ph-H), 7.36–7.39 (m, 2H, Ph-H), 7.79–7.84 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): (*E*)-isomer: δ 13.4 (pyrazole 5-Me), 17.1 [$CH_2C(Me)$=N-OCOMe], 19.5 [CH_2C(Me)=N-OCO*Me*], 40.8 [*C*H_2C(Me)=N-OCOMe], 77.8 (pyrazole C-4), 118.8, 125.4, 128.9, 137.5 (Ph-C), 161.15 (pyrazole C-5), 161.20 [CH_2*C*(Me)=N-OCOMe], 168.4 [CH_2C(Me)=N-O*C*OMe], 172.1 (pyrazole C-3); (*Z*)-isomer: δ 13.2 (pyrazole 5-Me), 17.0 [CH_2C(Me)=N-OCO*Me*], 21.8 [$CH_2C(Me)$=N-OCOMe], 36.7 [*C*H_2C(Me)=N-OCOMe], 78.3 (pyrazole C-4), 118.7, 125.6, 129.0, 137.3 (Ph-C), 160.0 [CH_2*C*(Me)=N-OCOMe], 161.7 (pyrazole C-5), 168.0 [CH_2C(Me)=N-O*C*OMe], 172.4 (pyrazole C-3); MS: *m*/*z* 304 [M+H]$^+$. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85. Found: C, 59.31; H, 5.87; N, 13.70.

(*E*/*Z*)-2,4-Dihydro-4-hydroxy-5-methyl-2-phenyl-4-[2-[(propionyloxy)imino]propyl]-3*H*-pyrazol-3-one (8b): Pale yellow oil (0.255 g, 80% as a 9:1 mixture of *E* and *Z* configurations by ^{1}H NMR spectrum); IR (neat): ν 3382 (OH), 1762, 1722 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): (*E*)-isomer: δ 1.12 [t, *J* = 7.6 Hz, 3H, CH_2C(Me)=N-OCOCH_2*Me*], 2.00 [s, 3H, $CH_2C(Me)$=N-OCOCH_2Me], 2.19 (s, 3H, pyrazole

5-Me), 2.31–2.43 [m, 2H, $CH_2C(Me)$=N-OCOCH_2Me], 2.87 [AB q, J = 15.0 Hz, 2H, CH_2C(Me)=N-OCOCH_2Me], 4.41 (s, 1H, OH), 7.17–7.20 (m, 1H, Ph-H), 7.36–7.39 (m, 2H, Ph-H), 7.82–7.84 (m, 2H, Ph-H); (*Z*)-isomer: δ 1.20 [t, J = 7.6 Hz, 3H, $CH_2C(Me)$=N-OCOCH_2*Me*], 2.15 [s, 3H, CH_2C(*Me*)=N-OCOCH_2Me], 2.16 (s, 3H, pyrazole 5-Me), 2.31–2.43 [m, 2H, $CH_2C(Me)$=N-OCOCH_2Me], 2.94 [AB q, J = 13.4 Hz, 2H, CH_2C(Me)=N-OCOCH_2Me], 4.07 (s, 1H, OH), 7.17–7.20 (m, 1H, Ph-H), 7.36–7.39 (m, 2H, Ph-H), 7.82–7.84 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): (*E*)-isomer: δ 8.7 [$CH_2C(Me)$=N-OCOCH_2*Me*], 13.4 (pyrazole 5-Me), 17.1 [CH_2C(*Me*)=N-OCOCH_2Me], 26.1 [$CH_2C(Me)$=N-OCO*C*H_2Me], 40.8 [*C*$H_2C(Me)$=N-OCOCH_2Me], 77.8 (pyrazole C-4), 118.7, 125.4, 128.9, 137.6 (Ph-C), 161.16 (pyrazole C-5), 161.22 [CH_2*C*(Me)=N-OCOCH_2Me], 171.8 [$CH_2C(Me)$=N-O*C*OCH_2Me], 172.1 (pyrazole C-3); (*Z*)-isomer: δ 8.8 [$CH_2C(Me)$=N-OCOCH_2*Me*], 13.2 (pyrazole 5-Me), 21.9 [CH_2C(*Me*)=N-OCOCH_2Me], 26.2 [$CH_2C(Me)$=N-OCO*C*H_2Me], 36.7 [*C*$H_2C(Me)$=N-OCOCH_2Me], 78.3 (pyrazole C-4), 118.7, 125.6, 129.0, 137.4 (Ph-C), 160.1 [CH_2*C*(Me)=N-OCOCH_2Me], 161.6 (pyrazole C-5), 171.3 [$CH_2C(Me)$=N-O*C*OCH_2Me], 172.4 (pyrazole C-3); MS: *m*/*z* 318 $[M+H]^+$. Anal. Calcd for $C_{16}H_{19}N_3O_4$ 0.15H_2O: C, 60.05; H, 6.08; N, 13.13. Found: C, 60.00; H, 6.18; N, 13.02.

(*E*)-4-[2-[(Benzoyloxy)imino]propyl]-2,4-dihydro-4-hydroxy-5-methyl-2-phenyl-3*H*-pyrazol-3-one (8c): Colorless prisms (0.137 g, 38%), mp 152–154 °C (acetone/petroleum ether); IR (KBr): ν 3382 (OH), 1722 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.15 [s, 3H, CH_2C(*Me*)=N-OCOPh], 2.25 (s, 3H, pyrazole 5-Me), 2.95 [AB q, J = 14.6 Hz, 2H, CH_2C(Me)=N-OCOPh], 4.47 (s, 1H, OH), 7.17–7.20 (m, 1H, Ph-H), 7.36–7.46 (m, 4H, Ph-H), 7.57–7.60 (m, 1H, Ph-H), 7.84–7.86 (m, 2H, Ph-H), 8.01–8.04 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.6 (pyrazole 5-Me), 17.4 [CH_2C(*Me*)=N-OCOPh], 41.0 [*C*H_2C(Me)=N-OCOPh], 78.0 (pyrazole C-4), 118.9, 125.4, 128.56, 128.62, 128.9, 129.6, 133.5, 137.6 (Ph-C), 161.3 (pyrazole C-5), 162.6 [CH_2*C*(Me)=N-OCOPh], 163.3 [$CH_2C(Me)$=N-O*C*OPh], 172.1 (pyrazole C-3); MS: *m*/*z* 366 $[M+H]^+$. Anal. Calcd for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50. Found: C, 65.51; H, 5.23; N, 11.43.

(*Z*)-4-[2-[(Benzoyloxy)imino]propyl]-2,4-dihydro-4-hydroxy-5-methyl-2-phenyl-3*H*-pyrazol-3-one (8c): Colorless prisms (0.026 g, 7%), mp 163–165 °C (acetone/petroleum ether); IR (KBr): ν 3418 (OH), 1724 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.16 (s, 3H, pyrazole 5-Me), 2.29 [s, 3H, CH_2C(*Me*)=N-OCOPh], 3.02 [AB q, J = 13.4 Hz, 2H, CH_2C(Me)=N-OCOPh], 4.15 (s, 1H, OH), 7.13–7.17 (m, 1H, Ph-H), 7.26–7.32 (m, 4H, Ph-H), 7.47–7.50 (m, 1H, Ph-H), 7.75–7.77 (m, 2H, Ph-H), 7.88–7.90 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.2 (pyrazole 5-Me), 22.1 [CH_2C(*Me*)=N-OCOPh], 36.8 [*C*H_2C(Me)=N-OCOPh], 78.5 (pyrazole C-4), 118.6, 125.5, 128.4, 128.5, 128.9, 129.6, 133.4, 137.4 (Ph-C), 161.7 [CH_2*C*(Me)=N-OCOPh], 162.0 (pyrazole C-5), 163.5 [$CH_2C(Me)$=N-O*C*OPh], 172.6 (pyrazole C-3); MS: *m*/*z* 366 $[M+H]^+$. Anal. Calcd for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50. Found: C, 65.65; H, 5.29;

N, 11.32.

(*E*)-2,4-Dihydro-4-hydroxy-5-methyl-4-[2-[[(4-nitrobenzoyl)oxy]imino]propyl]-2-phenyl-3*H*-pyrazol-3-one (8d): Yellow prisms (0.220 g, 54%), mp 165–167 °C (acetone/petroleum ether); IR (KBr): ν 3376 (OH), 1745, 1730, 1721 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 2.17 [s, 3H, $CH_2C(Me)$=N-OCO-4-NO_2-C_6H_4], 2.25 (s, 3H, pyrazole 5-Me), 2.97 [AB q, *J* = 14.6 Hz, 2H, CH_2C(Me)=N-OCO-4-NO_2-C_6H_4], 4.34 (s, 1H, OH), 7.17–7.21 (m, 1H, Ph-H), 7.36–7.39 (m, 2H, Ph-H), 7.83–7.84 (m, 2H, Ph-H), 8.15–8.18 (m, 2H, Ph-H), 8.27–8.29 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.4 (pyrazole 5-Me), 17.5 [$CH_2C(Me)$=N-OCO-4-NO_2-C_6H_4], 40.9 [CH_2C(Me)=N-OCO-4-NO_2-C_6H_4], 77.9 (pyrazole C-4), 118.9, 123.7, 125.5, 128.9, 130.7, 134.1, 137.5, 150.8 (Ph-C), 161.1 (pyrazole C-5), 161.4 [CH_2C(Me)=N-O*C*O-4-NO_2-C_6H_4], 163.6 [CH_2C(Me)=N-OCO-4-NO_2-C_6H_4], 172.0 (pyrazole C-3); MS: *m*/*z* 411 $[M+H]^+$. Anal. Calcd for $C_{20}H_{18}N_4O_6$: C, 58.54; H, 4.42; N, 13.65. Found: C, 58.31; H, 4.52; N, 13.39.

(*Z*)-2,4-Dihydro-4-hydroxy-5-methyl-4-[2-[[(4-nitrobenzoyl)oxy]imino]propyl]-2-phenyl-3*H*-pyrazol-3-one (8d): Yellow prisms (0.034 g, 8%), mp 175–177 °C (acetone/petroleum ether); IR (KBr): ν 3394 (OH), 1751, 1731 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 2.20 (s, 3H, pyrazole 5-Me), 2.34 [s, 3H, $CH_2C(Me)$=N-OCO-4-NO_2-C_6H_4], 2.99 [AB q, *J* = 14.6 Hz, 2H, CH_2C(Me)=N-OCO-4-NO_2-C_6H_4], 4.20 (s, 1H, OH), 7.07–7.10 (m, 1H, Ph-H), 7.18–7.22 (m, 2H, Ph-H), 7.64–7.66 (m, 2H, Ph-H), 7.93–7.99 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.1 (pyrazole 5-Me), 22.3 [$CH_2C(Me)$=N-OCO-4-NO_2-C_6H_4], 36.9 [CH_2C(Me)=N-OCO-4-NO_2-C_6H_4], 78.5 (pyrazole C-4), 118.1, 123.5, 125.6, 128.9, 130.5, 133.8, 137.2, 150.6 (Ph-C), 161.7 [CH_2C(Me)=N-O*C*O-4-NO_2-C_6H_4], 162.1 (pyrazole C-5), 162.6 [CH_2C(Me)=N-OCO-4-NO_2-C_6H_4], 172.4 (pyrazole C-3); MS: *m*/*z* 411 $[M+H]^+$. Anal. Calcd for $C_{20}H_{18}N_4O_6$ 0.3H_2O: C, 57.77; H, 4.51; N, 13.47. Found: C, 57.77; H, 4.41; N, 13.51.

(*E*)-2,4-Dihydro-4-hydroxy-4-[2-[[(4-methoxybenzoyl)oxy]imino]propyl]-5-methyl-2-phenyl-3*H*-pyrazol-3-one (8e): Colorless prisms (0.186 g, 47%), mp 143–145 °C (acetone/petroleum ether); IR (KBr): ν 3365 (OH), 1725, 1604 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 2.13 [s, 3H, $CH_2C(Me)$=N-OCO-4-MeO-C_6H_4], 2.25 (s, 3H, pyrazole 5-Me), 2.93 [AB q, *J* = 14.6 Hz, 2H, CH_2C(Me)=N-OCO-4-MeO-C_6H_4], 3.86 [s, 3H, CH_2C(Me)=N-OCO-4-*Me*O-C_6H_4], 4.52 (s, 1H, OH), 6.91–6.94 (m, 2H, Ph-H), 7.16–7.26 (m, 1H, Ph-H), 7.36–7.39 (m, 2H, Ph-H), 7.83–7.86 (m, 2H, Ph-H), 7.96–7.99 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.6 (pyrazole 5-Me), 17.4 [$CH_2C(Me)$=N-OCO-4-MeO-C_6H_4], 41.0 [CH_2C(Me)=N-OCO-4-MeO-C_6H_4], 55.0 [CH_2C(Me)=N-OCO-4-*Me*O-C_6H_4], 78.1 (pyrazole C-4), 113.9, 118.9, 120.8, 125.4, 128.9, 131.8, 137.6 (Ph-C), 161.4 (pyrazole C-5), 162.2 [CH_2C(Me)=N-OCO-4-MeO-C_6H_4], 163.1 [CH_2C(Me)=N-O*C*O-4-MeO-C_6H_4], 163.9 (Ph-C), 172.1 (pyrazole C-3); MS: *m*/*z* 396 $[M+H]^+$. Anal.

Calcd for $C_{21}H_{21}N_3O_5$: C, 63.79; H, 5.35; N, 10.63. Found: C, 63.64; H, 5.38; N, 10.52.

(*E*)-4-[2-[(Acetyloxy)imino]-2-phenylethyl]-2,4-dihydro-4-hydroxy-5-methyl-2-phenyl-3*H*-pyrazol-3-one (9a): Pale yellow prisms (0.067 g, 18%), mp 102–104 °C (Et_2O/petroleum ether); IR (KBr): ν 3414 (OH), 1759, 1730 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 2.04 (s, 3H, pyrazole 5-Me), 2.14 [s, 3H, $CH_2C(Ph)$=N-OCO*Me*], 3.48 [AB q, *J* = 13.1 Hz, 2H, C*H*$_2$C(Ph)=N-OCOMe], 3.89 (s, 1H, OH), 7.13–7.17 (m, 1H, Ph-H), 7.26–7.37 (m, 5H, Ph-H), 7.55–7.60 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.2 (pyrazole 5-Me), 19.6 [$CH_2C(Ph)$=N-OCO*Me*], 35.0 [*C*H_2C(Ph)=N-OCOMe], 78.5 (pyrazole C-4), 118.7, 125.5, 127.8, 128.6, 128.7, 130.9, 133.4, 137.1 (Ph-C), 159.7 [CH_2*C*(Ph)=N-OCOMe], 161.2 (pyrazole C-5), 168.2 [$CH_2C(Ph)$=N-O*C*OMe], 172.3 (pyrazole C-3); MS: *m/z* 365 [M^+]. Anal. Calcd for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50. Found: C, 65.81; H, 5.30; N, 11.49.

(*Z*)-4-[2-[(Acetyloxy)imino]-2-phenylethyl]-2,4-dihydro-4-hydroxy-5-methyl-2-phenyl-3*H*-pyrazol-3-one (9a): Pale yellow oil (0.155 g, 42%); IR (neat): ν 3383 (OH), 1770, 1720 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 1.98 [s, 3H, $CH_2C(Ph)$=N-OCO*Me*], 2.22 (s, 3H, pyrazole 5-Me), 3.29 [AB q, *J* = 15.0 Hz, 2H, C*H*$_2$C(Ph)=N-OCOMe], 4.43 (br, 1H, OH), 7.12–7.15 (m, 1H, Ph-H), 7.25–7.32 (m, 7H, Ph-H), 7.60–7.62 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.5 (pyrazole 5-Me), 19.4 [$CH_2C(Ph)$=N-OCO*Me*], 40.8 [*C*H_2C(Ph)=N-OCOMe], 78.1 (pyrazole C-4), 118.7, 125.2, 127.7, 128.3, 128.6, 130.3, 131.1, 137.3 (Ph-C), 160.5 [CH_2*C*(Ph)=N-OCOMe], 160.9 (pyrazole C-5), 168.0 [$CH_2C(Ph)$=N-O*C*OMe], 171.9 (pyrazole C-3); MS: *m/z* 366 [M+H]$^+$. Anal. Calcd for $C_{20}H_{19}N_3O_4$ $0.2H_2O$: C, 65.10; H, 5.30; N, 11.39. Found: C, 65.10; H, 5.53; N, 11.12.

(*E*)-2,4-Dihydro-4-hydroxy-5-methyl-2-phenyl-4-[2-phenyl-2-[(propionyloxy)imino]ethyl]-3*H*-pyrazol-3-one (9b): Colorless needles (0.049 g, 13%), mp 116–118 °C (Et_2O/petroleum ether); IR (KBr): ν 3423, 3359 (OH), 1752, 1732, 1722 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 1.15 [t, *J* = 7.6 Hz, 3H, $CH_2C(Ph)$=N-OCOCH_2*Me*], 2.04 (s, 3H, pyrazole 5-Me), 2.37–2.49 [m, 2H, $CH_2C(Ph)$=N-OCOC*H*$_2$Me], 3.47 [AB q, *J* = 13.4 Hz, 2H, C*H*$_2$C(Ph)=N-OCOCH_2Me], 3.81 (s, 1H, OH), 7.13–7.16 (m, 1H, Ph-H), 7.25–7.36 (m, 5H, Ph-H), 7.55–7.61 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 8.79 [$CH_2C(Ph)$=N-OCOCH_2*Me*], 13.2 (pyrazole 5-Me), 26.3 [$CH_2C(Ph)$=N-OCO*C*H_2Me], 34.9 [*C*H_2C(Ph)=N-OCOCH_2Me], 78.5 (pyrazole C-4), 118.6, 125.5, 127.8, 128.6, 128.7, 130.9, 133.5, 137.1 (Ph-C), 159.8 [CH_2*C*(Ph)=N-OCOCH_2Me], 161.2 (pyrazole C-5), 171.5 [$CH_2C(Ph)$=N-O*C*OCH_2Me], 172.3 (pyrazole C-3); MS: *m/z* 379 [M^+]. Anal. Calcd for $C_{21}H_{21}N_3O_4$: C, 66.48; H, 5.58; N, 11.08. Found: C, 66.45; H, 5.65; N, 11.07.

(*Z*)-2,4-Dihydro-4-hydroxy-5-methyl-2-phenyl-4-[2-phenyl-2-[(propionyloxy)imino]ethyl]-3*H*-pyrazol-3-one (9b): Colorless needles (0.203 g, 54%), mp 100–102 °C (Et_2O/petroleum ether); IR (KBr): ν 3238 (OH), 1759, 1681 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 1.03 [t, *J* = 7.6 Hz, 3H,

$CH_2C(Ph)$=N-OCOCH$_2$*Me*], 2.23 (s, 3H, pyrazole 5-Me), 2.22–2.28 [m, 2H, $CH_2C(Ph)$=N-OCOC*H*$_2$Me], 3.30 [AB q, *J* = 13.4 Hz, 2H, C*H*$_2$C(Ph)=N-OCOCH$_2$Me], 4.39 (s, 1H, OH), 7.12–7.15 (m, 1H, Ph-H), 7.26–7.32 (m, 7H, Ph-H), 7.60–7.62 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 8.60 [$CH_2C(Ph)$=N-OCOCH$_2$*Me*], 13.6 (pyrazole 5-Me), 26.1 [$CH_2C(Ph)$=N-OCO*C*H$_2$Me], 40.8 [*C*H$_2$C(Ph)=N-OCOCH$_2$Me], 78.2 (pyrazole C-4), 118.7, 125.2, 127.8, 128.3, 128.6, 130.3, 131.1, 137.4 (Ph-C), 160.6 [CH$_2$*C*(Ph)=N-OCOCH$_2$Me], 161.0 (pyrazole C-5), 171.4 [$CH_2C(Ph)$=N-O*C*OCH$_2$Me], 171.8 (pyrazole C-3); MS: *m*/*z* 380 [M+H]$^+$. Anal. Calcd for $C_{21}H_{21}N_3O_4$: C, 66.48; H, 5.58; N, 11.08. Found: C, 66.47; H, 5.65; N, 11.09.

(*E*)-4-[2-[(Benzoyloxy)imino]-2-phenylethyl]-2,4-dihydro-4-hydroxy-5-methyl-2-phenyl-3*H*-pyrazol-3-one (9c): Colorless needles (0.121 g, 28%), mp 160–162 °C (acetone/petroleum ether); IR (KBr): ν 3375 (OH), 1727 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 3H, pyrazole 5-Me), 3.57 [AB q, *J* = 13.4 Hz, 2H, C*H*$_2$C(Ph)=N-OCOPh], 3.61 (s, 1H, OH), 7.11–7.14 (m, 1H, Ph-H), 7.25–7.39 (m, 7H, Ph-H), 7.51–7.60 (m, 3H, Ph-H), 7.72–7.73 (m, 2H, Ph-H), 8.00–8.02 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.3 (pyrazole 5-Me), 34.8 [*C*H$_2$C(Ph)=N-OCOPh], 78.5 (pyrazole C-4), 118.5, 125.4, 128.0, 128.4, 128.6, 128.7, 129.7, 131.0, 133.50, 133.53, 137.2 (Ph-C), 161.21 (pyrazole C-5), 161.23 [CH$_2$*C*(Ph)=N-OCOPh], 163.3 [CH$_2$C(Ph)=N-O*C*OPh], 172.2 (pyrazole C-3); MS: *m*/*z* 427 [M$^+$]. Anal. Calcd for $C_{25}H_{21}N_3O_4$: C, 70.25; H, 4.95; N, 9.83. Found: C, 70.26; H, 5.05; N, 9.83.

(*Z*)-4-[2-[(Benzoyloxy)imino]-2-phenylethyl]-2,4-dihydro-4-hydroxy-5-methyl-2-phenyl-3*H*-pyrazol-3-one (9c): Pale yellow solid (0.177 g, 41%), mp 39–41 °C (Et_2O/petroleum ether); IR (KBr): ν 3392 (OH), 1747, 1723 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, pyrazole 5-Me), 3.40 [AB q, *J* = 14.6 Hz, 2H, C*H*$_2$C(Ph)=N-OCOPh], 4.69 (br, 1H, OH), 7.11–7.14 (m, 1H, Ph-H), 7.25–7.37 (m, 9H, Ph-H), 7.48–7.52 (m, 1H, Ph-H), 7.58–7.61 (m, 2H, Ph-H), 7.77–7.79 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.7 (pyrazole 5-Me), 41.0 [*C*H$_2$C(Ph)=N-OCOPh], 78.4 (pyrazole C-4), 118.7, 125.2, 127.8, 128.29, 128.32, 128.5, 128.6, 129.67, 129.71, 130.4, 131.2, 133.4, 137.3 (Ph-C), 161.2 (pyrazole C-5), 161.5 [CH$_2$*C*(Ph)=N-OCOPh], 163.3 [CH$_2$C(Ph)=N-O*C*OPh], 171.9 (pyrazole C-3); MS: *m*/*z* 428 [M+H]$^+$. Anal. Calcd for $C_{25}H_{21}N_3O_4$ 0.5H_2O: C, 68.80; H, 5.08; N, 9.63. Found: C, 68.81; H, 5.03; N, 9.42.

(*E*)-2,4-Dihydro-4-hydroxy-5-methyl-4-[2-[[(4-nitrobenzoyl)oxy]imino]-2-phenylethyl]-2-phenyl-3*H*-pyrazol-3-one (9d): Yellow needles (0.079 g, 17%), mp 177–179 °C (acetone/petroleum ether); IR (KBr): ν 3404 (OH), 1739, 1719, 1703 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.03 (s, 3H, pyrazole 5-Me), 3.53 [AB q, *J* = 13.7 Hz, 2H, C*H*$_2$C(Ph)=N-OCO-4-NO_2-C_6H_4], 3.65 (s, 1H, OH), 7.10–7.13 (m, 1H, Ph-H), 7.23–7.27 (m, 2H, Ph-H), 7.37–7.46 (m, 3H, Ph-H), 7.58–7.60 (m, 2H, Ph-H), 7.75–7.78 (m, 2H, Ph-H), 8.06–8.10 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.2 (pyrazole 5-Me), 35.2 [*C*H$_2$C(Ph)=N-OCO-4-NO_2-C_6H_4], 78.4 (pyrazole C-4), 118.2, 123.6, 125.5, 128.0, 128.8, 130.8, 131.4,

133.3, 133.8, 137.1, 150.7 (Ph-C), 161.3 (pyrazole C-5), 161.6 [CH_2C(Ph)=N-OCO-4-NO_2-C_6H_4], 161.8 [CH_2C(Ph)=N-OCO-4-NO_2-C_6H_4], 172.1 (pyrazole C-3); MS: *m/z* 472 [M^+]. Anal. Calcd for $C_{25}H_{20}N_4O_6$: C, 63.56; H, 4.27; N, 11.86. Found: C, 63.51; H, 4.24; N, 11.92.

(*Z*)-2,4-Dihydro-4-hydroxy-5-methyl-4-[2-[[(4-nitrobenzoyl)oxy]imino]-2-phenylethyl]-2-phenyl-3*H*-pyrazol-3-one (9d): Yellow needles (0.226 g, 48%), mp 172–174 °C (acetone/petroleum ether); IR (KBr): ν 3418 (OH), 1736, 1721 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, pyrazole 5-Me), 3.40 [AB q, *J* = 14.6 Hz, 2H, CH_2C(Ph)=N-OCO-4-NO_2-C_6H_4], 4.22 (s, 1H, OH), 7.12–7.16 (m, 1H, Ph-H), 7.26–7.37 (m, 7H, Ph-H), 7.58–7.61 (m, 2H, Ph-H), 7.90–7.92 (m, 2H, Ph-H), 8.16–8.19 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.6 (pyrazole 5-Me), 41.0 [CH$_2$C(Ph)=N-OCO-4-NO_2-C_6H_4], 78.2 (pyrazole C-4), 118.7, 123.6, 125.3, 127.6, 128.5, 128.6, 130.70, 130.72, 130.9, 133.8, 137.3, 150.7 (Ph-C), 160.8 (pyrazole C-5), 161.4 [CH_2C(Ph)=N-OCO-4-NO_2-C_6H_4], 162.8 [CH_2C(Ph)=N-OCO-4-NO_2-C_6H_4], 171.7 (pyrazole C-3); MS: *m/z* 473 [M+H]$^+$. Anal. Calcd for $C_{25}H_{20}N_4O_6$: C, 63.56; H, 4.27; N, 11.86. Found: C, 63.56; H, 4.28; N, 11.84.

(*E*)-2,4-Dihydro-4-hydroxy-4-[2-[[(4-methoxybenzoyl)oxy]imino]-2-phenylethyl]-5-methyl-2-phenyl-3*H*-pyrazol-3-one (9e): Colorless needles (0.139 g, 30%), mp 177–179 °C (acetone/petroleum ether); IR (KBr): ν 3323 (OH), 1735, 1604 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.05 (s, 3H, pyrazole 5-Me), 3.56 [AB q, *J* = 13.7 Hz, 2H, CH_2C(Ph)=N-OCO-4-MeO-C_6H_4], 3.62 (s, 1H, OH), 3.81 [s, 3H, CH_2C(Ph)=N-OCO-4-*Me*O-C_6H_4], 6.78–6.80 (m, 2H, Ph-H), 7.11–7.14 (m, 1H, Ph-H), 7.25–7.37 (m, 5H, Ph-H), 7.60–7.62 (m, 2H, Ph-H), 7.72–7.74 (m, 2H, Ph-H), 7.94–7.97 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.3 (pyrazole 5-Me), 34.8 [CH$_2$C(Ph)=N-OCO-4-MeO-C_6H_4], 55.4 [CH_2C(Ph)=N-OCO-4-*Me*O-C_6H_4], 78.5 (pyrazole C-4), 114.0, 118.6, 120.5, 125.3, 128.0, 128.66, 128.68, 130.9, 131.9, 133.7, 137.2 (Ph-C), 160.7 [CH_2C(Ph)=N-OCO-4-MeO-C_6H_4], 161.2 (pyrazole C-5), 163.1 [CH_2C(Ph)=N-OCO-4-MeO-C_6H_4], 163.9 (Ph-C), 172.3 (pyrazole C-3); MS: *m/z* 457 [M^+]. Anal. Calcd for $C_{26}H_{23}N_3O_5$: C, 68.26; H, 5.07; N, 9.19. Found: C, 68.40; H, 5.12; N, 9.17.

(*Z*)-2,4-Dihydro-4-hydroxy-4-[2-[[(4-methoxybenzoyl)oxy]imino]-2-phenylethyl]-5-methyl-2-phenyl-3*H*-pyrazol-3-one (9e): Pale yellow solid (0.146 g, 32%), mp 49–51 °C (Et_2O/petroleum ether); IR (KBr): ν 3382 (OH), 1744, 1725, 1604 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, pyrazole 5-Me), 3.37 [AB q, *J* = 14.6 Hz, 2H, CH_2C(Ph)=N-OCO-4-MeO-C_6H_4], 3.81 [s, 3H, CH_2C(Ph)=N-OCO-4-*Me*O-C_6H_4], 4.40 (s, 1H, OH), 6.81–6.83 (m, 2H, Ph-H), 7.11–7.14 (m, 1H, Ph-H), 7.25–7.37 (m, 7H, Ph-H), 7.59–7.61 (m, 2H, Ph-H), 7.72–7.75 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$): δ 13.8 (pyrazole 5-Me), 40.9 [CH$_2$C(Ph)=N-OCO-4-MeO-C_6H_4], 55.4 [CH_2C(Ph)=N-OCO-4-*Me*O-C_6H_4], 78.3 (pyrazole C-4), 113.8, 118.7, 120.5, 125.1, 127.8, 128.3, 128.6, 130.3, 131.3, 131.8, 137.4 (Ph-C), 161.06 [CH_2C(Ph)=N-OCO-4-MeO-C_6H_4], 161.11 (pyrazole C-5), 163.0 [CH_2C(Ph)=N-OCO-4-MeO-C_6H_4],

163.8 (Ph-C), 171.8 (pyrazole C-3); MS: *m/z* 458 $[M+H]^+$. Anal. Calcd for $C_{26}H_{23}N_3O_5$ $0.15H_2O$: C, 67.86; H, 5.10; N, 9.13. Found: C, 67.84; H, 5.33; N, 9.04.

Reaction of plasmid pBR322 with compounds 1–5, (*E*)-8c–e, and (*Z*)-9a–e. The method of assaying the DNA cleavage activity, using a covalently closed circular duplex DNA (ccc-DNA) of plasmid pBR322 as a substrate, was described in our previous investigation.[26] The results are listed in Table 2. The reaction mixture (100 μL) containing 1 μg of ccc-DNA of plasmid pBR322, 10 mM of **1–5**, (*E*)-**8c–e**, and (*Z*)-**9a–e**, and 50 mM Tris-HCl buffer (pH7.4), was incubated at 37 °C. At interval, 20 μL of the reaction was mixed with 2 μL of 10 × Loading Buffer (TAKARA BIO INC. Shiga, Japan). The resulting mixture was directly by 1.0% agarose gel electrophoresis. After electrophoresis, the gels were stained with ethidium bromide (0.5 μg/mL) for 20 min. Under these conditions the order of anodal migration for the tree topological forms of the DNA was ccc-DNA, full-length linear duplex DNA (linear-DNA), and nicked open circular duplex DNA (oc-DNA). The ccc-DNA produced oc-DNA after single strand scission and linear-DNA after double-strand scission. They were all detected as clearly separated bands in agarose gels. The stained DNA bands were made visible using BioDoc-It™ Imaging Systems (UVP, Upland, CA) and then took the JPEG image file. For quantitative analysis of DNA on the gels, densitometric analyses of the images file were carried out using QuantiScan densitometry software (BIOSOFT, Cambridge, U.K.). The area under the ccc-DNA was multiplied by a factor of 1.42 to correct for its reduced binding of ethidium bromide as indicated by Lloyd and coworkers.[26a]

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