Synthesis and Antitumor Activity of Novel Pyrazolylenaminone and Bis(Pyrazolyl)ketones *via* Hydrazonoyl Halides

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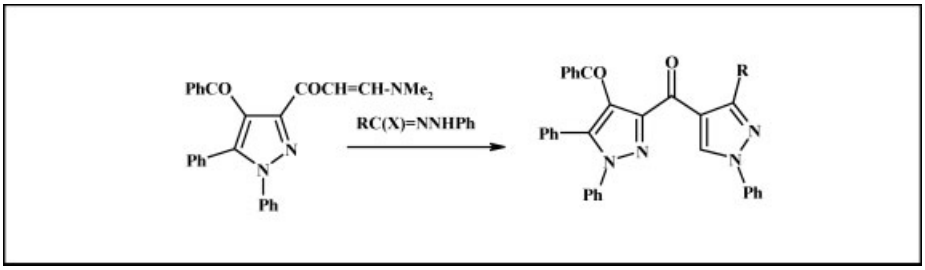

3-Acetyl-4-benzoyl-1,5-diphenylpyrazole reacts with DMF-DMA to give the novel enaminone **2**. The reaction of the latter with various hydrazonoyl halides afforded regioselectively the respective substituted (3-pyrazolyl)(4-pyrazolyl)ketones **4** in good over all yield. The preliminary screening for the antitumor activity of the synthesized compounds **2** and **4a-g** against human breast cancer cell line (MCF-7) revealed that both compounds **2** and **4b** have high-antitumor activity. SAR is discussed.

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INTRODUCTION

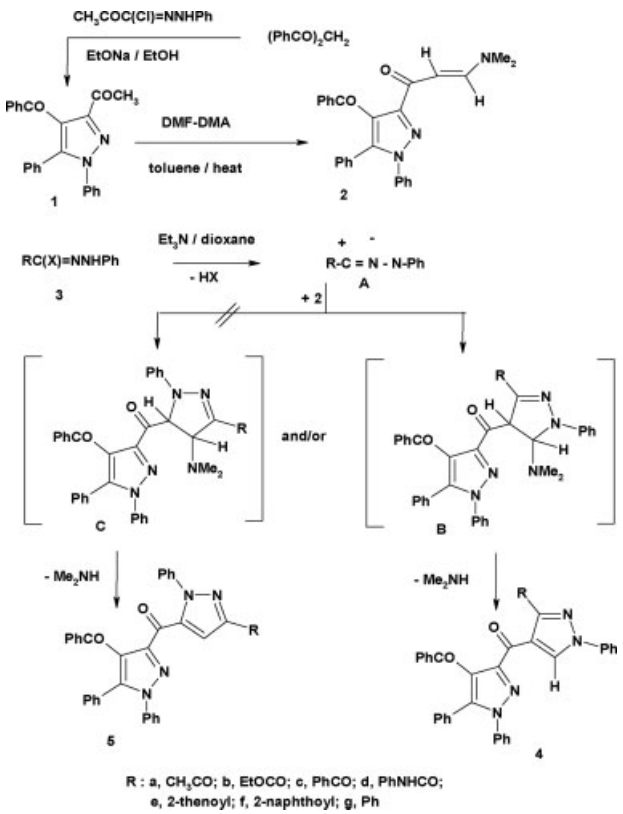
Within the past 10 years, we have been engaged on the utility of hydrazonoyl halides for synthesis of various heterocyclic systems [1–10]. Furthermore, literature cites many reports on valuable biological activity of various pyrazole derivatives [11–21]. On the basis of these facts, it was thought interesting to explore the utility of hydrazonoyl halides for synthesis of the hitherto unreported (3-pyrazolyl)(4-pyrazolyl) ketones and evaluate their antitumor activity against human breast cell line MCF-7. For this purpose, we investigated the reactions of a series of hydrazonoyl halides **3** with the hitherto unreported 3-pyrazolyl-enaminone **2** (Scheme 1). We carried out such reactions to explore also their regiochemistry as they can lead to (3-pyrazolyl)(4-pyrazolyl) ketones **4** and/or their isomers (3-pyrazolyl)(5-pyrazolyl) ketones **5** (Scheme 1).

RESULTS AND DISCUSSION

The hitherto unreported 3-acetyl-4-benzoyl-1,5-diphenylpyrazole 1 was prepared *via* reaction of *N*-phenyl 2-oxopropanehydrazonoyl chloride 3a with dibenzoylmethane in ethanol in the presence of sodium ethoxide. Its structure was confirmed by its spectra (IR, MS, ^{1}H NMR and ^{13}C NMR) and its elemental analysis (see Experimental section). For example, its IR spectrum showed two bands at υ 1692 and 1659 cm^{-1} assignable to two C=O groups. Its ^{1}H NMR (DMSO-d_6) displayed signals at δ 3.34 (s, 3H, CH_3), 7.15–7.80 (m, 15H, ArH). Reaction of compound 1 with dimethylformamide-dimethylacetal (DMF-DMA) in refluxing toluene furnished the required new enaminone 2. The IR spectrum of the latter showed two carbonyl absorption bands at 1678 and 1649 cm^{-1}. Its ^{1}H NMR spectrum revealed, in addition to the aromatic protons multiplet, two doublet signals at δ 5.84 and 7.62 with $J = 19$ Hz assignable to the two olefinic protons and two methyl protons singlet signals at δ 2.86 and 3.09. This finding indicates that this enaminone 2 exists in the indicated trans-configuration (Scheme 1).

Reaction of **2** with each of the hydrazonoyl halides **3a-g** in dioxane in the presence of triethylamine gave, in each case, one isolable product as evidenced by TLC analysis. Both mass spectra and elemental analysis data of the isolated products were consistent with either one of the two isomeric structures **4** or **5** (Scheme 1). On the basis of their ^{1}H NMR spectra, the isolated products were assigned structure **4** and the isomeric structure **5** was discarded. This is because their ^{1}H NMR spectra revealed, in each case, a singlet signal for the pyrazole ring proton in the region δ 9.20–9.33 assignable to pyrazole-5H. Literature reports [22,23] indicate that the ^{1}H NMR spectra of 5- and 4-unsubstituted pyrazoles exhibit the characteristic singlet signals of 5-CH and 4-CH

Scheme 1



protons at δ 8.35 and 7.30, respectively. Furthermore, spectral simulation by ACD/H NMR Predictor (v.6.12) for 5- and 4-unsubstituted pyrazoles showed the 5-H and 4-H signals at d 8.30 and 7.63, respectively. On the basis of such data, it is not unreasonable to conclude that the studied reactions are completely regioselective and the structure of the isolated products is **4** and not **5**. This conclusion is consistent with literature reports that indicate that reactions of hydrazonoyl halides with various enaminones are regioselective and lead to the formation of the respective 5-unsubtituted pyrazole derivatives [8].

To account for the formation of products **4**, it is suggested that the reaction starts with a regioselective 1,3-dipolar cycloaddition of the nitrilimine intermediate **A**, generated *in situ* by base-catalyzed dehydrohalogenation of the hydrazonoyl halide **3**, to the carbon–carbon double bond of the enaminone **2** to afford the nonisolable cycloadduct intrermediate **B**. The latter then undergoes *in situ* elimination of dimethylamine to yield the corresponding pyrazole derivative **4** as end product (Scheme 1).

Antitumor screening. The cytotoxic effects of the new enaminone **2** and bis(pyrazolyl) ketones **4a-g** against human breast cell line MCF-7 were evaluated at the National Institute of Cancer, Cairo, Egypt. Doxorubicin was used as a reference to evaluate the potency of the tested compounds. Five different concentrations of each compound and the reference were used in such screening tests and determination of IC_{50} values. The results are given in Table 1. As shown in Table 1, compound **2** has unpredictable antitumor activity against MCF-7 cell line as its IC_{50} value is 0.87 μg/mL which is close to that of the reference doxotrubicin (IC_{50} = 0.70). Compounds **4a-g** showed, however, less activity than that of compound **2**. This finding indicates that substitution of *N*,*N*-dimethylaminovinyl group in **2** by the 1,3-disubstituted pyrazol-4-yl moiety in **4** decreased the activity. Such a decrease might be due to the fact that the pyrazolyl group is more complex than *N*,*N*-dimethylaminovinyl group. This complexity limits penetration of compounds **4** into the cell or interferes with their metabolism or both and as a result, the antitumor activity decreases. Furthermore, a comparison of the IC_{50} values of compounds **4a-g** indicates further that their activity depends on the nature of the R group. The order of activity is: EtOCO > C_6H_5 > CH_3CO > C_6H_5NHCO > C_6H_5CO = 2-thenoyl > 2-naphthoyl.

In conclusion, a facile synthesis of each of both the novel enaminone **2** and bis(pyrazolyl)ketones **4a-g** is demonstrated. Evaluation of the antitumor activity of these new compounds revealed that compounds **2** (IC_{50} = 0.87 μg/mL) and **4b** (IC_{50} = 1.81 μg/mL) showed good inhibitory activity of human breast cancer cell (MCF-7).

Table I

In vitro cytotoxic activity of the new compounds **2** and **4a-g**.

	Surviving fraction × 10^3, conc. (μg/mL)							Surviving fraction × 10^3, conc. (μg/mL)					
No.	0.0	1.0	2.5	5.0	10.0	IC_{50}	No.	0.0	1.0	2.5	5.0	10.0	IC_{50}
2	1000	444	168	760	700	0.87	**4e**	1000	836	554	240	245	2.68
4a	1000	904	418	234	233	2.21	**4f**	1000	900	671	232	255	2.89
4b	1000	674	471	197	223	1.81	**4g**	1000	716	523	240	232	2.08
4c	1000	828	566	292	222	2.68	Dox[a]	1000	417	260	261	267	0.70
4d	1000	832	501	218	294	2.42							

[a] Doxotrobicin, an antitumor reference.

EXPERIMENTAL

All melting points are uncorrected. IR spectra were recorded in KBr using Pye Unicam SP-1000 Spectrophotometer. ^{1}H NMR spectra were recorded in $DCCl_3$ and DMSO-d_6 using a Varian Em-200 MHz Spectrometer, and TMS as internal reference. Mass spectra were recorded on AEI MS 30 mass spectrometer operating at 70 eV. Elemental analyses were carried out at the Microanalytical Centre of Cairo University. The hydrazonoyl halides **3** were prepared following literature procedures [24].

3-Acetyl-4-benzoyl-1,5-diphenyl-1*H*-pyrazole (1). To an ethanolic sodium ethoxide solution, prepared by dissolving sodium metal (0.12 g, 0.005 g-atom) and absolute ethanol (20 mL), was added dibenzoylmethane (1.12 g, 5 mmol). To the resulting solution *N*-phenyl 2-oxopropanehydrazonoyl chloride **3a** (5 mmol) was added to the solution portionwise while stirring the reaction mixture at room temperature. After complete addition, the reaction mixture was stirred for further 3 h during which the hydrazonoyl chloride dissolved and a new solid precipitated. The latter was filtered off, washed and crystallized from ethanol to give 3-acetyl-4-benzoyl-1,5-diphenyl-1*H*-pyrazole (**1**) as yellow crystals (yield 80%), mp 170–171°C. IR (KBr) ν 1692, 1659 (2C=O); ^{1}H NMR (DMSO-d_6): δ 3.34 (s, 3H, CH_3), 7.15–7.80 (m, 15H, ArH); ^{13}C NMR (DMSO-d_6): δ 26.3, 120.8, 125.8, 127.5, 128.4, 128.6, 128.9, 129.1, 129.3, 129.5, 133.3, 137.3, 138.5, 143.2, 148.5, 177.8, 190.9, 192.2; ms: *m/z* (%): 367 (M^+ + 1, 25), 366 (M^+, 54), 323 (20), 289 (100), 247 (20), 180 (13), 77 (64). *Anal*. Calcd. for $C_{24}H_{18}N_2O_2$ (366.42): C, 78.67; H, 4.95; N, 7.65%. Found: C, 78.37; H, 5.06; N, 7.66%.

3-[3-(*N*,*N*-Dimethylamino)acryloyl]-4-benzoyl-1,5-diphenyl-1*H*-pyrazole (2). A mixture of 3-acetyl-4-benzoyl-1,5-diphenyl-1*H*-pyrazole (**1**) (3.66 g, 10 mmol) and dimethylformamide-dimethylacetal (DMF-DMA) (2.4 g, 20 mmol) was refluxed for 5 h then left to cool. To the cold mixture was added ether. The resulting yellow solid was filtered, washed with ether, dried and finally crystallized from ethanol to afford the enaminone **2**. This compound was obtained as yellow crystals (yield 70 %), mp 190–192°C; IR (KBr) ν 1678, 1649 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.86 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 5.84 (d, 1H, J = 19 Hz, —CO—CH=), 7.15–7.55 (m, 15H Ar—H), 7.62 (d, 1H, J = 19 Hz, =CH—N—); ms: *m/z* (%): 422 (M^+ + 1, 11), 421 (M^+, 12), 404 (50), 316 (80), 180 (23), 98 (78), 77 (100). *Anal*. Calcd. for $C_{27}H_{23}N_3O_2$ (421.49): C, 76.94; H, 5.50; N, 9.97%. Found: C, 76.70; H, 5.32; N, 10.14%.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1-phenyl-3-substituted-pyrazol-4-yl)ketones (4a-g): General procedure. To a stirred solution of the appropriate hydrazonoyl halide **3** (1 mmol) and the enaminone **2** (0.42 g, 1 mmol) in dry dioxane (50 mL), was added triethylamine (0.5 mL) and the mixture was refluxed for 12 h. The precipitated triethylamine hydrochloride was filtered off, and the filtrate was evaporated under reduced pressure. The residue was triturated with ethanol. The solid product so formed was filtrated, washed with water, and dried. Crystallization from ethanol afforded the corresponding pyrazole derivatives **4**. The physical constants of the products 4a-g are listed in the subsequent sections.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1-phenyl-3-acetyl-pyrazol-4-yl)ketone (4a). This compound was obtained as pale yellow crystals (yield 75%), mp 150–152°C; IR (KBr) ν 1693, 1655, 1620 (3C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H, CH_3), 7.24–7.93 (m, 20H, Ar—H), 9.15 (s, 1H, pyrazole); ms: *m/z* (%) 537 (M^+ + 1, 25), 536 (M^+, 38), 493 (23), 417 (29), 180 (17), 105 (100). *Anal*. Calcd. for $C_{34}H_{24}N_4O_3$ (536.58): C, 76.11; H, 4.51; N, 10.44%. Found: C, 76.41; H, 4.85; N, 10.77%.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1-phenyl-3-ethoxy-carbonyl-pyrazol-4-yl)ketone (4b). This compound was obtained as pale orange crystals (yield 68%), mp 105–107°C; IR (KBr) ν 1725, 1663, 1615 (3C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 7.5 Hz, CH_3), 4.14 (q, 2H, J = 7.5 Hz, CH_2), 7.21–7.90 (m, 20H, Ar—H), 9.2 (s, 1H, pyrazole); ms: *m/z* (%) 567 (M^+ + 1, 32), 566 (M^+, 74), 493 (82), 417 (74), 316 (42), 260 (42), 215 (22), 180 (25), 77 (100). *Anal*. Calcd. for $C_{35}H_{26}N_4O_4$ (566.61): C, 74.19; H, 4.63; N, 9.89%. Found: C, 73.98; H, 4.78; N, 10.11%.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1-phenyl-3-benzoyl-pyrazol-4-yl)ketone (4c). This compound was obtained as pale brown crystals (yield 70%), mp 110–112°C; IR (KBr) ν 1690, 1663, 1620 (3C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.1–7.97 (m, 25H, Ar—H), 9.33 (s, 1H, pyrazole); ms: *m/z* (%) 599 (M^+ + 1, 21), 598 (M^+, 34), 493 (62), 404 (74), 351 (24), 316 (72), 105 (100). *Anal*. Calcd. for $C_{39}H_{26}N_4O_3$ (598.65): C, 78.25; H, 4.38; N, 9.36%. Found: C, 78.46; H, 4.20; N, 9.55%.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1-phenyl-3-phenyl-aminocarbonyl-pyrazol-4-yl)ketone (4d). This compound was obtained as yellow crystals (yield 66%), mp 120–122°C; IR (KBr) ν 3236 (NH), 1699, 1664, 1618 (3C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.19–8.00 (m, 25H, Ar—H), 9.25 (s, 1H, pyrazole), 10.8 (s, 1H, NH, D_2O-exchangeable); ms: *m/z* (%) 614 (M^+ + 1, 10), 613 (M^+, 37), 521 (46), 260 (8), 222 (9), 180 (10), 105 (100). *Anal*. Calcd. for $C_{39}H_{27}N_5O_3$ (613.66): C, 76.33; H, 4.43; N, 11.41%. Found: C, 76.16; H, 4.58; N, 11.27%.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1-phenyl-3-thenoyl-pyrazol-4-yl)ketone (4e). This compound was obtained as white buff solid (yield 65%), mp 118–120°C; IR (KBr) ν 1694, 1658, 1615 (3C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ, 7.15–8.14 (m, 23H, Ar—H, Het-H), 9.29 (s, 1H, pyrazole); ms: *m/z* (%) 605 (M^+ + 1, 15), 604 (M^+, 32), 499 (54), 281 (13), 111 (100), 77 (12.5). *Anal*. Calcd. for $C_{37}H_{24}N_4O_3S$ (604.68): C, 73.49; H, 4.00; N, 9.27; S, 5.30%. Found: C, 73.67; H, 3.86; N, 9.10; S, 5.18%.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1-phenyl-3-(2-naphthoyl)-pyrazol-4-yl)ketone (4f). This compound was obtained as white buff crystals (yield 68%), mp 100–102°C; IR (KBr) ν 1688, 1662, 1616 (3C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.01–8.18 (m, 27H, Ar—H), 9.30 (s, 1H, pyrazole); ms: *m/z* (%) 649 (M^+ + 1, 71), 648 (M^+, 81), 544 (71), 494 (31), 127 (100). *Anal.* Calcd. for $C_{43}H_{28}N_4O_3$ (648.71): C, 79.61; H, 4.35; N, 8.64%. Found: C, 79.47; H, 4.50; N, 8.40%.

(4-Benzoyl-1,5-diphenyl-pyrazol-3-yl)-(1,3-diphenyl-py-razol-4-yl)ketone (4g). This compound was obtained as pale yellow crystals (yield 72%), mp 130–132°C; IR (KBr) ν 1668, 1647 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.16–7.74 (m, 25H, Ar—H), 9.23 (s, 1H, pyrazole); ms: *m/z* (%) 571 (M^+ + 1, 49), 570 (M^+, 100), 405 (93), 317 (51), 247 (42), 84 (38). *Anal*. Calcd. for $C_{38}H_{26}N_4O_2$ (570.64): C, 79.98; H, 4.59; N, 9.82%. Found: C, 80.14; H, 4.79; N, 9.68%.

Pharmacology. ***Cytotoxic activity against human breast cancer (MCF-7) in vitro.*** The method applied is similar to that reported by Skehan and Storeng [25] using Sulfo-Rhodamine-B stain (SRB). Cells were plated in 96-multiwill plate (10^4 cells/well) for 24 h before treatment with the test compound to allow attachment of cell to the wall of the plate, different concentration of the compound under test (0, 1.0, 2.5, 5, and 10 μg/mL) were added to the cell monolayer in triplicate wells individual dose, monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with tris-EDTA buffer, color intensity was measured in an ELISA reader, the relation between surviving fraction and drug concentration is plotted to get the survival curve of tumor cell line and the IC_{50} was calculated. The results are summarized in Table 1.

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