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Synthesis of Novel 4-Substituted Phenazone Derivatives as Potential Antibacterial and Antineoplastic Agents

M.M. Girges, M.M. Abou El-Zahab and M.A. Hanna*§

*Chemistry Department, Mansoura and*Damiatta Faculties of Science, Mansoura University, Mansoura, Egypt.*

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Abstract □ A new series of substituted phenazone derivatives has been prepared through a series of reactions that are illustrated in Scheme I. The antibacterial and antineoplastic activities of the prepared compounds were evaluated. While none of the synthesized products showed marked antibacterial activity, all of them possessed a significant antitumor effect.

Keywords □ Substituted phenazone derivatives, synthesis, antibacterial, antineoplastic.

Diverse biological properties have been shown to be associated with numerous pyrazole derivatives. These include antineoplastic[1,2], antidiabetic[3-5] and CNS depressant[6] activities. In addition, antipyrine possesses high pharmacological properties[7]. Thus, in continuation of our earlier interest in poly-substituted 4-pyrazolone[8] and promptive by these facts, we report here the synthesis of ethyl 5-arylazo-4-hydroxy-1-(phenazon-4-yl)pyrazole-3-carboxylate and their precursors (scheme I).

Moreover we have examined the course of some nucleophilic substitution reactions that might lead to production of new different phenazone derivatives with the hope of extending and/or improving its pharmaceutical activity. The antibacterial as well as antitumor effect of the prepared compounds have been determined.

EXPERIMENTAL METHODS

All M. p's were determined in open-glass capillaries and are uncorrected. IR spectra were recorded for Nujol mulls on an SP 2000 Pye Unicam spectrophotometer. PMR spectra were carried out on Varian EM 360 NMR spectrometer 60 MHz. MS were performed on Varian 111 (80 eV) spectrometer and peaks of relative intensity below 5% of the base peak were ommitted except of the parent peaks.

Ethyl γ -bromo- α -(phenazon-4-yl)hydrazono- β -oxobutyrate (2)

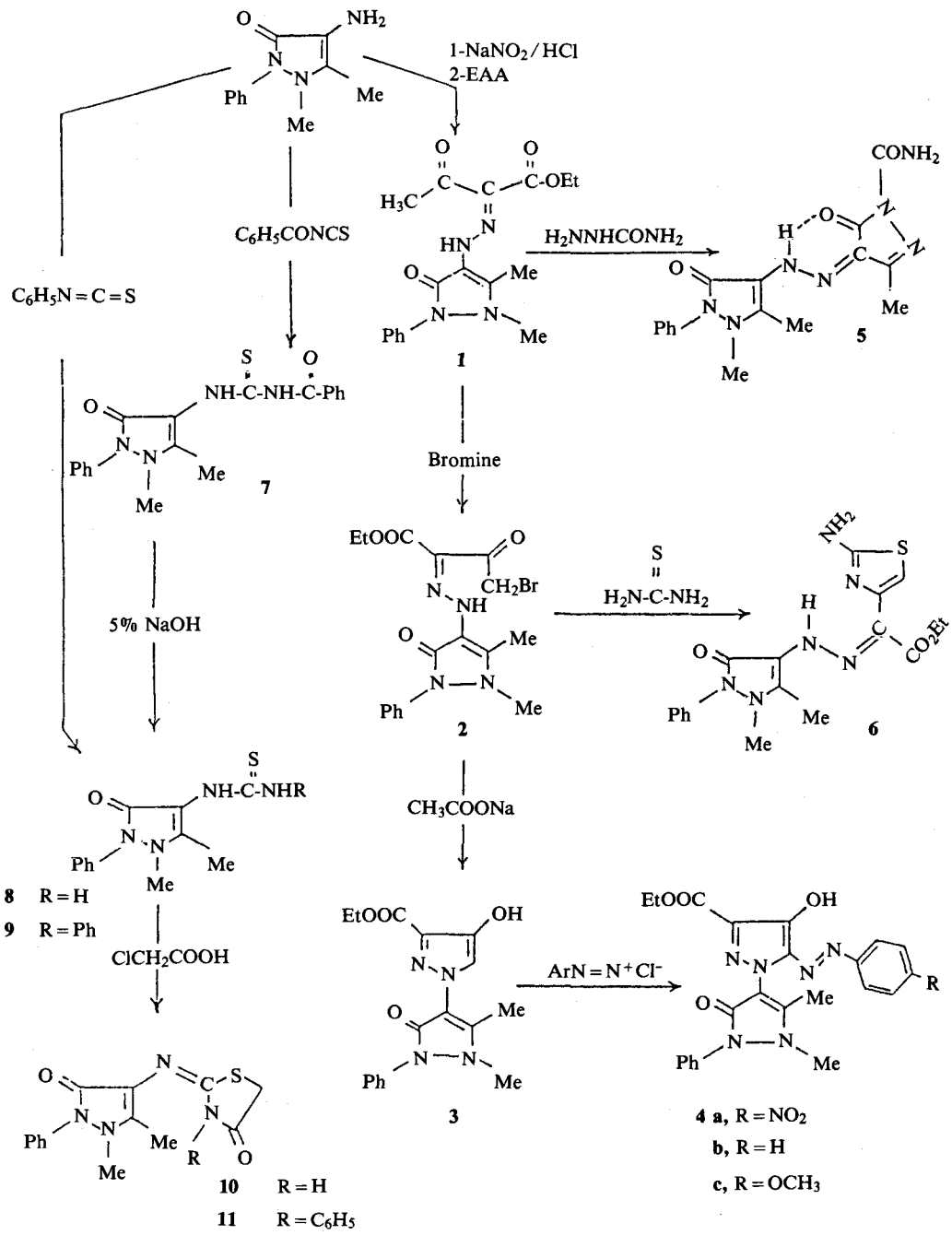
Ethyl α -(phenazon-4-yl)hydrazono- β -oxobutyrate (**1**), (1 g, 0.003 mol), suspended in dry ether (20 m*l*) was treated gradually with a solution of bromine (0.5 g, 0.003 mol) in ether (10 m*l*) in sun light while stirring. The suspended material went into solution with evolution of hydrogen bromide. After two hours, the ether was evaporated off and the crude product was washed with water to remove hydrobromic acid and dried. Recrystallization from ethanol gave the product as yellow crystals in 70% yield. M.p. 138 °C. Found: C 48.36, H 4.58, N 13.76, Br 19.32, $C_7H_{19}N_4O_4Br$ (423.26) req.: C 48.24, H 4.52, N 13.24, Br 18.88. IR: 3240 cm^{-1} (NH), 1700 cm^{-1} (CO of ester group), 1680 cm^{-1} (CO of bromoacetyl moiety) and 1660 cm^{-1} (cyclic t-amide).

Ethyl 4-hydroxy-1-(phenazon-4-yl)pyrazole-3-carboxylate (3)

To (1.3 g, 0.003 mol) of bromo-dye **2** in ethanol (30 m*l*), sodium acetate (1.5 g) in water (10 m*l*) was added. The mixture was refluxed for three hours and cooled. The solution was extracted with chloroform and the organic layer was dried over magnesium sulfate. Evaporation of solvent in vacuo followed by recrystallization of residue from light petrol (60/80) gave the product as red crystals in 40% yield. M.p. 150 °C. Found: C 59.54, H 5.36, N 16.52, $C_{17}H_{18}N_4O_4$ (342.34) req.: C 59.64, H 5.30, N 16.37. IR (KBr): 3450 cm^{-1} (hydrogen bonded OH), 1690 cm^{-1} (CO of ester moiety, hydrogen bonded) and 1660 cm^{-1} (cyclic t-amide). PMR spectra ($CDCl_3$): δ 1.4 (3H, t, CH_2CH_3), 2.95 (3H, s, C-CH_3), 3.15 (3H, s, N-CH_3), 4.4 (2H, q, CH_2CH_3) and 7.2-7.7 (7H, m, Ar-H and -$COCH_2N$-).

§ To whom inquires should be addressed.

(scheme I)



Ethyl 5-arylazo-4-hydroxy-1-(phenazon-4-yl)pyrazole-3-carboxylate (4a-c)

A solution of the pyrazole carboxylic ester **3** (1 g, 0.003 mol) in ethanol (20 m*l*) was cooled to 0 °C in an ice-salt bath. To the resulting solution, (0.003 mol) of the appropriate diazonium salt solution (prepared as usuall) was added slowly over a period of 20-30 minutes with continuous stirring. During the addition a dark colored solid separated. The reaction mixture was stirred for an additional 30

min. then left over-night in an ice-box. The crude product was collected, washed with water and finally recrystallized from dil. ethanol.

Compound **4a** was obtained as orange crystals in 60% yield. M.p. 168 °C. Found: C 56.35, H 4.44, N 19.62, $C_{23}H_{21}N_7O_6$ (491.45) req.: C 56.21, H 4.31, N 19.95.

Compound **4b** was obtained as orange crystals in 66% yield. M.p. 163 °C. Found: C 61.66, H 4.99, N 18.43, $C_{23}H_{22}N_6O_4$ (446.46) req.: C 61.87, H 4.97, N 18.83. IR: 3400 cm^{-1} (hydrogen bonded OH group), 1685 cm^{-1} (CO of ester moiety, hydrogen bonded), 1665 cm^{-1} (cyclic t-amide moiety) and 1620 cm^{-1} (C=N).

Compound **4c** was obtained as yellowish orange crystals in 56% yield. M.p. 190 °C. Found: C 60.63, H 5.29, N 17.47, $C_{24}H_{24}N_6O_5$ (476.48) req.: C 60.49, H 5.08, N 17.64.

1-Carbamoyl-3-methyl-4-(phenazon-4-yl)hydrazono-5-pyrazolone (5)

To a mixture of ethyl α -(phenazon-4-yl)hydrazono-β-oxobutyrate (**1**), (1.7 g, 0.005 mol) and semicarbazide hydrochloride (0.7 g, 0.006 mol) in ethanol (30 m*l*) was added sodium acetate trihydrate (0.2 g) in water (2 m*l*). The mixture was refluxed for three hours then cooled. The product that precipitated was collected, washed with water and recrystallized from ethanol as yellow crystals in 73% yield. M.p. above 250 °C. Found: C 53.97, H 4.65, N 27.83, $C_{16}H_{17}N_7O_3$ (355.35) req.: C 54.08, H 4.82, N 27.59. IR: 3500-3250 cm^{-1} (NH of $CONH_2$ & hydrazone moieties), 1675 cm^{-1} (CO of 5-pyrazolone moiety), 1660 cm^{-1} (cyclic t-amide) and 1725 & 1760 cm^{-1} ($CONH_2$ group in the two possible conformations).

Ethyl (2-aminothiazol-4-yl)glyoxalate (phenazon-4-yl)hydrazone (6)

A mixture of γ -bromo derivative **2** (4.2 g, 0.01 mol) and thiourea (1 g, 0.013 mol) was refluxed in ethanol (30 m*l*) for three hours and cooled. The reaction mixture was made alkaline by adding ammonium hydroxide solution. The crude product was filtered and recrystallized from ethanol as yellow crystals in 55% yield. M.p. 117 °C. Found: C 54.17, H 4.94, N 21.33, S 7.88; $C_{18}N_{20}N_6O_3S$ (400.45) req.: C 53.98, H 5.03, N 20.99, S 8.01. IR: 3300 cm^{-1} (NH_2 group), 1710 cm^{-1} (CO of α ,β -unsaturated ester), 1665 cm^{-1} (CO of phenazone moiety) and 1640 cm^{-1} (C=N).

N_1-(phenazon-4-yl)-N_2-benzoylthiourea (7)

Benzoyl chloride (14.1 g, 0.1 mol) was added dropwisely with stirring to a solution of ammonium thiocyanate (7.6 g, 0.1 mol) in acetone (20 m*l*) and the mixture was refluxed for 30 min., then 4-aminoantipyrine (10.2 g, 0.05 mol) in acetone (75 m*l*) was added with stirring during 15 min. The benzoyl derivative, that separated as orange precipitate was filtered, washed with acetone and recrystallized from ethanol to give the product as orange crystals in 71% yield. M.p. 194 °C. Found: C 62.57, H 5.12, N 15.72, S 8.93; $C_{19}H_{18}N_4O_2S$ (366.42) req.: C 62.28, H 4.95, N 15.29, S 8.75. IR: 3050-3100 cm^{-1} (NH groups), 1670 cm^{-1} (benzoyl CO), 1660 cm^{-1} (cyclic t-amide) and 1200 cm^{-1} (-CS-N-).

N_1-(phenazon-4-yl)thiourea (8)

The benzoyl derivative **7**, (3.7 g, 0.01 mol) was hydrolysed by boiling in sodium hydroxide solution (20 m*l*, 10%) for 15 min, and cooled. Acidification with hydrochloric acid yielded a yellowish white precipitate which was filtered off, washed with water and recrystallized from methanol as yellow crystals in 53% yield. M.p. 117 °C. Found: C 54.78, H 5.11, N 21.54, S 11.93; $C_{12}H_{14}N_4OS$ (262.32) req.: C 54.94, H 5.38, N 21.36, S 12.22. IR: 3080 cm^{-1} (NH), 1660 cm^{-1} (cyclic t-amide) and 1200 cm^{-1} (-CS-N-).

N_1-(phenazon-4-yl)-N_2-phenylthiourea (9)

Phenyl isothiocyanate (3 g, 0.022 mol) was added with stirring to a solution of 4-aminoantipyrine (4 g, 0.02 mol) in dry benzene (30 m*l*). The reaction mixture was refluxed on water bath where, a yellowish mass separated out within 15 min., the latter was filtered off, washed with benzene and recrystallized from ethanol to give the product in 65% yield. M.p. 199 °C. Found: C 63.48, H 5.48, N 16.23, S 9.66; $C_{18}H_{18}N_4OS$ (338.41) req.: C 63.88, H 5.36, N 16.56, S 9.47. IR: 3080 cm^{-1} (NH), 1660 cm^{-1} (cyclic t-amide) and 1200 cm^{-1} (-CS-N-).

2-(phenazon-4-yl)imino-4-thiazolidinone (10)

A mixture of N_1-(phenazon-4-yl)thiourea (**8**), (2.6 g, 0.01 mol), monochloroacetic acid (0.95 g, 0.01 mol) and anhydrous sodium acetate (0.5 g) in absolute ethanol (50 m*l*) was refluxed on water bath for six hours. The solvent was distilled off and the residue was triturated with water. The product that separated was washed several times with cold water to make it free from sodium acetate, dried and recrystallized from ethanol as yellowish white crystals in 40% yield. M.p. 122 °C. Found: C 55.53, H 4.14, N 18.59, S 10.12; $C_{14}H_{14}N_4O_2S$ (302.34) req.: C 55.61, H 4.67, N 18.53, S 10.60. IR: 3355 cm^{-1} (broad, -NH-), 2895 cm^{-1} (-CH_2- stretch), 1755 cm^{-1}

(CO, thiazolidinone moiety), 1665 cm^{-1} (cyclic t-amide) and 1650 cm^{-1} (C=N). PMR spectra ($CDCl_3$): δ 2.9 (3H,s,C-CH_3), 3.05 (3H,s,N-CH_3), 4.35 (2H,s,CH_2), 5.6 (broad singlet, -NH-, exchanged with D_2O) and 7.25 (5H,m,Ar-H).

2-(phenazon-4-yl)imino-3-phenyl-4-thiazolidinone (11)

This was prepared by following the above general work up procedure using N_1-(phenazon-4-yl)-N_2-phenylthiourea (1.7 g, 0.005 mol) and monochloroacetic acid (0.5 g, 0.005 mol) and the other required reagents. The product was obtained as white crystals in 42% yield. M.p. 237 °C. Found: C 63.64, H 4.94, N 15.05, S 9.02; $C_{20}H_{18}N_4O_2S$ (378.43) req.: C 63.47, H 4.79, N 14.81, S 8.47. PMR ($CDCl_3$): δ 2.85 (3H,s,C-CH_3), 3.15 (3H,s, N-CH_3), 4.20 (2H,s,-CH_2-) and 7.4-7.6 (10H, m,aromatic moieties). MS (m/z, relative abundance %): 378 (M^+,4), 259 (32), 245 (44), 203 (16), 147 (5), 135 (43), 91 (50), 77 (34) and 56 (100).

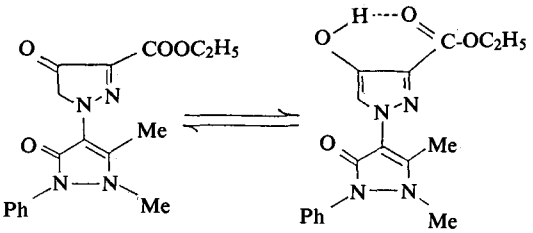
Biological screening

The prepared compounds were screened for their antibacterial activity against *E. coli, Staph. albus, Staph. aureus* and *N. catarrhalis* using nutrient agar pour plate method[12]. None of the prepared compounds showed marked antibacterial activity. For antitumor activity, compounds **2,5,7,9** and **11** were tested in vivo against Ehrlich-ascites carcinoma in Swiss albino mice using the previously reported techniques[13,14].

RESULTS AND DISCUSSION

Ethyl α -(phenazon-4-yl)hydrazono-β-oxobutyrate (**1**) was previously prepared in good yield[9]. According to (Scheme I), bromination of **1** in ether afforded the corresponding ethyl γ-bromo-α-(phenazon-4-yl)hydrazono-β-oxobutyrate (**2**) in quantitative yield. The latter was cyclized by boiling in ethanolic buffered solution to give ethyl 4-hydroxy-1-(phenazon-4-yl)-pyrazole-3-carboxylate (**3**). This product can exist in two possible tautomeric structures.

The assignement of the highly hydrogen bonded hydroxy pyrazole structure **3B** rather than the possible keto-pyrazole structure **3A** is based on its spectral data. The solid IR (KBr) spectra revealed a broad band near 3450 cm^{-1} for hydrogen bonded OH group and a strong absorption band near 1690 cm^{-1} for hydrogen bonded carbonyl group of ester moiety. Absorption band near 1680 cm^{-1}, that is characteristic for carbonyl group of the keto form, was completely absent. This behaviour finds support from the work of Chattaw *et al.*[10] PMR spectra in $CDCl_3$ solution displayed characteristic signals as a multiplet at 7.2-7.7 ppm for methylene protons of -$COCH_2N$- moiety together with protons of phenyl group. The predominance of the **3A** form in solutions can be rationalized in terms of the greater stability of **3A** form than the tautomeric structure **3B** in polar solvents.

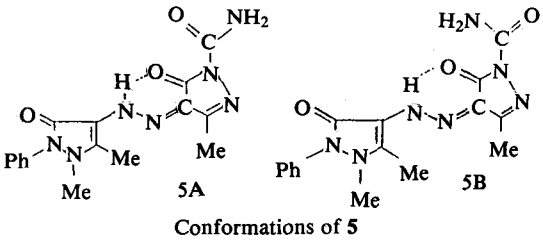


3A 3B

Structure-3

Coupling of **3** with different arene diazonium salts gave ethyl 5-arylazo-4-hydroxy-1-(phenazon-4-yl)pyrazole-3-carboxylate (**4a-c**). Structure of these dyes was confirmed by both of elemental and spectral data.

When compound **1** was allowed to react with semicarbazide hydrochloride in ethanolic solution containing sodium acetate at the reflux temperature, 1- carbamoyl -3-methyl-4-(phenazon-4-yl) hydrazono-5-pyrazolone (**5**) was obtained. Chemical structure of this product was confirmed by spectral and elemental analysis. Beside normal absorption bands for different functional groups in the product, its IR spectra revealed the presence of two absorption bands at 1725 and 1760 cm^{-1} which were ascribed to stretching vibrations of the carbamoyl moiety in the two possible conformations (**5A** & **5B**) respectively. The latter two absorption bands of $CONH_2$ group are due to dipolar repulsion between the two CO groups which causes an increase in force constant of CO group and thus shifts its $\bar{\nu}$ to higher frequency value[11].



5A 5B

Conformations of **5**

Reaction of bromo-dye **2** with thiourea in ethanol afforded ethyl (2-aminothiazol-4-yl)glyoxalate (phenazon-4-yl)hydrazone (**6**) in good yield.

Both elemental and spectral data are in agreement with the assigned structure.

The antipyrinyl-thiourea derivatives **8** and **9** were obtained either by reacting 4-aminoantipyrine with benzoyl isothiocyanate followed by hydrolysis of the product **7** with 5% NaOH or by direct reaction with phenyl isothiocyanate respectively. Treatment of thiourea derivatives **8** and **9** with chloroacetic acid yielded 2-(phenazon-4-yl) imino-4-thiazolidinone (**10**) and 2-(phenazon-4-yl)-imino-3-phenyl-4-thiazolidinone (**11**) respectively. Both of IR, PMR and mass spectral data are consistent with the proposed structures. Compound **11** revealed in its mass spectrum a weak molecular and (M-231) ions and the base peak occured at m/z = 56. The latter fragment can be formulated as ($CH_3N=C^+CH_3$).

All of the tested compounds exhibited a significant antitumor activity at different concentrations (Table I). While some of the tested compounds prolonged the life span of mice, that were previously treated with Ehrlich ascites carcinoma, beyond that of untreated animals, other compounds completely suppressed the tumor growth in these animals*

Table I. Antitumor activity of substituted phenazone derivatives (2,5,7,9 and 11) against Ehrlich ascites carcinoma in Swiss albino mice

Compound No.	Dose[a] mg/Kg body weight	MST[b]	T/C (%)[c]
control	—	25.1	
2	2	37	147.4
	10	37	147.4
	50	38.2	152.0
5	2	22.8	90.8
	10	31.7	126.3
	50	34.9	138.8
7	2	44.6	177.7
	10	34.8	138.4
	50	— [d]	— [d]
9	2	19.3	77
	10	43.6	173.7
	50	— [d]	— [d]
11	2	50.3	176
	10	26.5	128.1
	50	29.0	115.5

a) Doses of different compounds were administered by intraperitoneal (i.p.) injection.

b) Mean survival time (days).

c) T/C (%) > 125 denotes significant activity.

d) Death due to drug-related cytotoxicity.

*) The toxicological study of the hitherto synthesized compounds together with their effect on the immune cells will be published separately.

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