Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 6 pp. 1193–1200, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND ANTITUMOR SCREENING OF SOME NEW 2,6-BIS PYRIDINES FUNCTIONALIZED WITH PYRAZOLE-BASED HETEROCYCLES

KORANY A. ALI[1], MOHAMED A. ELSAYED[1], SALWA M. ELHALLOUTY[2], KHALED MAHMOUD[2] and AHMAD M. FARAG[3]*

[1]Department of Applied Organic Chemistry, [2]Department of Pharmacognosy, National Research Centre, Dokki, Giza 12622, Egypt
[3]Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt

Abstract: Several new pyrazole, 1,3,4-oxadiazole, 1,2,4-triazole, 1,3,4-thiadiazole and thiazol-2-ylidene derivatives attached to pyridine ring at 2,6-positions have been synthesized starting from the versatile 3,3'-(pyridine-2,6-diyl)bis(1*H*-pyrazole-4-carbohydrazide). The newly synthesized compounds were evaluated for their *in vitro* anticancer activity against HEPG2, A549 and MCF-7 human cancer cell lines. The results showed that the newly synthesized compounds displayed low to moderate activity against the tested human cancer cell lines.

Keywords: pyridine, thiazole, bis-pyrazole, 1,2,4-triazole, 1,3,4-thiadiazole, anticancer screening

Several nitrogen-containing heterocyclic compounds incorporating pyridine nucleus were found to possess interesting pharmacological activities. For example, several pyridine derivatives were found to have potential anticancer activity (1-4). Moreover, 2-pyridone derivatives have considerable pharmacological importance as cardiotonic agents, such as milrinone, and as potential HIV-1 specific transcriptase inhibitors (5, 6). On the other hand, diverse pharmacological activities have been associated with pyrazole derivatives that include: antitumor, anti-inflammatory, analgesic, anti-rheumatic and antipyretic properties (7-10).

In view of these observations and in continuation of our current interest in the synthesis of a variety of substituted heterocycles for biological screening and our interest in the chemistry of 2,6-di-substituted pyridine derivatives (11-20), the present work was undertaken to synthesize some new 2,6-disubstituted pyridines bearing different heterocycles and to evaluate their antitumor activity.

RESULTS AND DISCUSSION

Chemistry

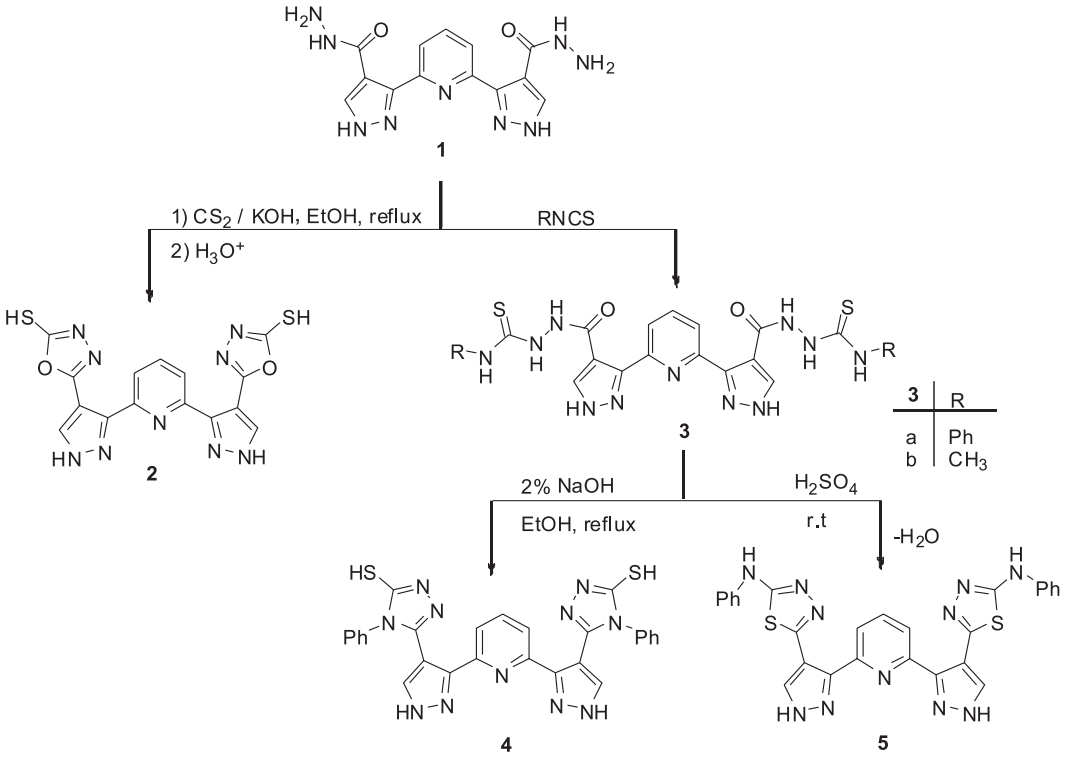
Recently, we have reported the preparation of the starting material for this study: 3,3'-(pyridine-2,6-diyl)bis(1*H*-pyrazole-4-carbohydrazide) (**1**) by hydrazinolysis of 2,6-bis(4-ethoxycarbonyl-1*H*-pyrazol-5-yl)pyridine (12). Treatment of **1** with carbon disulfide and potassium hydroxide, followed by acidification using HCl solution, afforded 2,6-bis[4-(5-mercapto-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-3-yl]pyridine (**2**) (Scheme 1). The bis-carbohydrazide **1** was treated also with phenyl isothiocyanate and with methyl isothiocyanate, in refluxing ethanol and afforded the corresponding thiosemicarbazide derivatives **3a** and **3b**, respectively (Scheme 1).

Heating of the thiosemicarbazide derivative **3a** in 2% sodium hydroxide solution, afforded 2,6-bis[4-(5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1*H*-pyrazol-3-yl]pyridine (**4**). When **3a** was treated with sulfuric acid, it afforded the corresponding 2,6-bis[4-(5-phenylamino-1,3,4-thiadiazol-2-yl)-1*H*-pyrazol-3-yl]pyridine (**5**) (Scheme 1). The structure of compounds **3a,b**, **4** and **5** was established on the basis of their elemental analysis and spectral data.

The thiosemicarbazide derivative **3a** was treated with phenacyl chloride or chloroacetone, in the presence of catalytic amount of triethylamine, and afforded the corresponding 3*H*-thiazol-2-ylidene-3-carboxylic acid hydrazide derivatives **7a** or **7b**, respectively (Scheme 2).

Similarly, **3a** reacted also with ethyl chloroacetate, in the presence of catalytic amount of triethylamine to afford pyridine-2,6-bis[1*H*-pyrazol-3-yl-4-

* Corresponding author: e-mail: afarag49@yahoo.com



Scheme 1. Synthetic pathways for the formation of compounds **2**-**5**

(3-phenyl-4-oxo-3*H*-thiazol-2-ylidene)-3-carboxylic acid hydrazide] (**9**) *via* the non-isolable intermediate **8** (Scheme 2). The structures of the isolated products were established and confirmed on the bases of their elemental analyses and spectroscopic data (see Experimental).

Treatment of the acid hydrazide (**1**) with pentane-2,4-dione and with ethyl 3-oxobutanoate, in refluxing ethanol, afforded products identified as 2,6-bis[4-(3,5-dimethyl-1-carbonyl)-1*H*-pyrazol-3-yl]pyridine (**10**) and 2,6-bis[4-(3-methylpyrazol-5-one-1-carbonyl)-1*H*-pyrazol-3-yl]pyridine (**11**), respectively (Scheme 3).

Treatment of the acid hydrazide **1** with acetone and acetophenone, in refluxing ethanol, in the presence of few drops of glacial acetic acid, afforded the corresponding hydrazone derivatives **12a** and **12b**, respectively, on the basis of elemental analysis and spectral data (see Experimental). Finally, the acid hydrazide **1** undergoes condensation also with aromatic aldehydes *viz*. benzaldehyde, 4-methylbenzaldehyde and 4-methoxybenzaldehyde, in refluxing EtOH, in the presence of glacial acetic acid, to afford the corresponding hydrazone derivatives **13a-c** (Scheme 3).

The structure of the product **12a**,**b** and **13a-c** were established on the basis of their elemental analysis and spectral data (see Experimental).

***In vitro* anticancer screening**

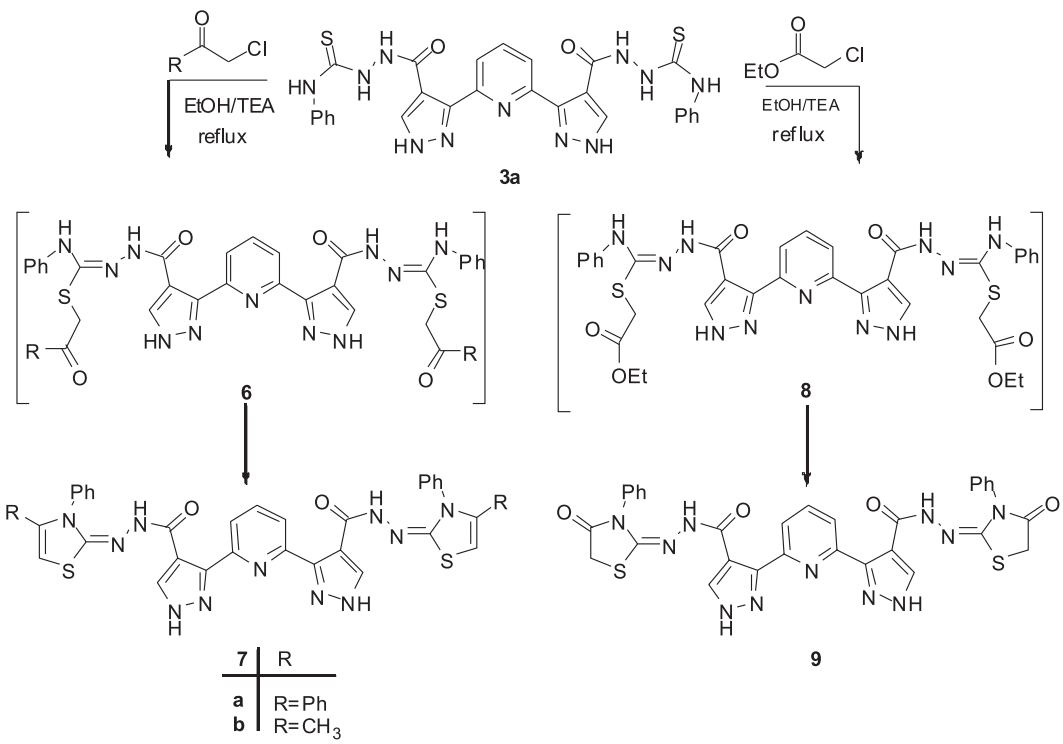
The newly synthesized compounds were preliminarily screened for their *in vitro* cytotoxic activity against 3 human tumor cell lines such as human lung adenocarcinoma (A549), hepatocellular carcinoma (HEPG2) and breast adenocarcinoma (MCF7). Table 1 shows the results of *in vitro* cytotoxic activity of the newly synthesized compounds at concentration of 100 µM where two compounds revealed anticancer activity percentage > 75% against human lung adenocarcinoma (A549).

Tow compounds that gave cytotoxic activity > 75% inhibition of cell viability at concentration (100 µM) were submitted to calculate their IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability, using MTT method (21, 22) (Table 2). Doxorubicin (one of the most effective anticancer agents) was used as reference drug. From the results obtained (Table 2), it was observed that the tested compounds showed low to moderate activity against the tested human cancer cell lines.

Table 1. Anticancer screening of the newly synthesized compounds against human tumor cell lines (A549, MCF7 and HEPG2) at concentration of 100 µM.

Compound	*In-vitro* cytotoxic activity percentage at 100 µM (mean ± SE)		
	A549	MCF7	HEPG2
1	35.3 ± 0.12	*NA*	28.8 ± 0.1
2	30.1 ± 0.41	*NA*	*NA*
3	76.5 ± 0.57	*NA*	44.5 ± 0.5
4a	*NA*	*NA*	19.3 ± 0.8
4b	64.5 ± 0.2	*NA*	15.5 ± 0.2
5	NA	*NA*	*NA*
6	29.9 ± 0.7	*NA*	*NA*
8a	*NA*	*NA*	*NA*
8b	*NA*	*NT*	19.3 ± 0.8
11	*NA*	13.2 ± 0.2	39.6 ± 0.2
12	60.9 ± 0.5	*NA*	27.7 ± 0.5
13a	*NA*	*NA*	*NT*
13b	49.7 ± 0.3	*NA*	25.3 ± 0.2
15a	86.1 ± 0.3	12.9 ± 0.3	52.1 ± 0.3
15b	19.2 ± 0.4	*NA*	21.4 ± 0.4
15c	25.6 ± 0.2	*NA*	13.8 ± 0.2
Doxorubicin	99.9 ± 0.2	91.5 ± 0.1	95.7 ± 0.2

NA = compounds having cytotoxic activity percentage less than 10%; *NT* = not tested.



Scheme 2. Synthetic pathway for the formation of compounds **7a**,**b** and **9**

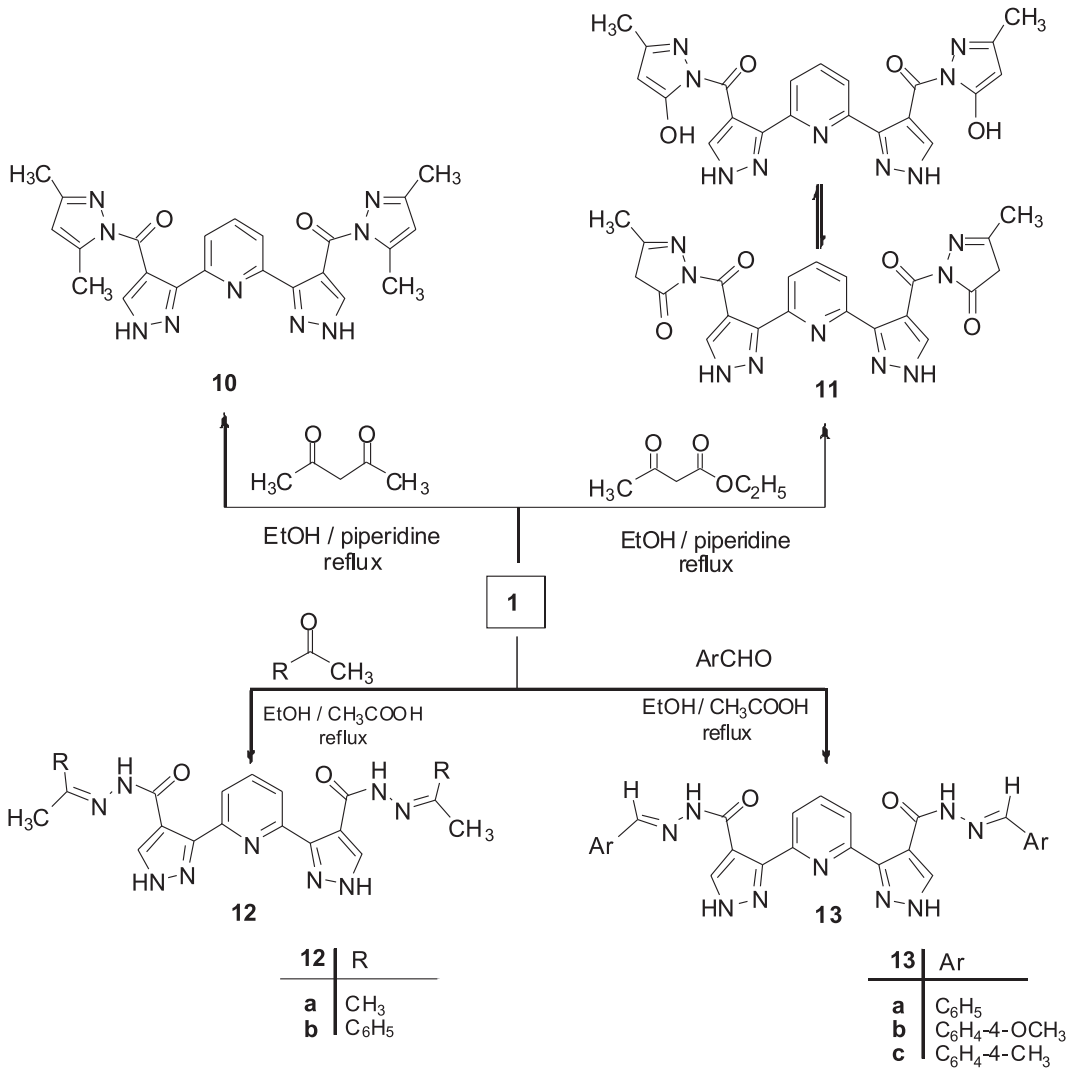
EXPERIMENTAL

Chemistry

All melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded in potassium bromide discs on a Pye Unicam SP 3-300 and a Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VXR-300 NMR spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.46 MHz) determinations were run in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Micro-analytical Centre of Cairo University, Giza, Egypt and recorded on Elementar-Vario EL (Germany) automatic analyzer. All reactions were followed by TLC (silica gel, aluminum sheets 60 F_{254}, Merck). 3,3'-(Pyridine-2,6-diyl)bis(1*H*-pyrazole-4-carbohydrazide) (**1**) (12) was prepared as reported in the given reference.

2,6-Bis[4-(5-mercapto-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-3-yl]pyridine (2)

To a stirred solution of the acid hydrazide **1** (0.71 g, 2 mmol) and potassium hydroxide (0.34 g, 6 mmol) in EtOH (20 mL), carbon disulfide (0.46 g, 6 mmol) was added gradually. The reaction mixture was stirred at room temperature for 1/2 h then refluxed for 2 h. A yellow precipitate of the corresponding potassium salt was separated. Dry ether (30 mL) was added to complete the precipitation of the formed salt which was fil-



Scheme 3. Synthetic pathways for the formation of compounds **10**, **11**, **12a**,**b**, **13a-c**

Table 2. Anticancer activity (IC_{50}[a], μM) of compounds **2** and **13a** against human cancer cell line (A549).

Compound No.	Cytotoxicity [b]						IC_{50}
	100 (μM)	50 (μM)	25 (μM)	12.5 (μM)	6.25 (μM)	3.13 (μM)	
3	76.3	29.4	10.7	2.1	0	0	23.2
15a	86.1	38.7	26.7	15.7	8.6	3.4	27.8
Doxorubicin	100	100	100	100	100	65.7	12.6

[a] IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50%. [b] Values are means of three experiments.

tered off and washed with dry ether (50 mL). The precipitated product was dissolved in cold water then acidified with HCl solution (2 M) to pH 5. The precipitated solid was filtered off, washed with water, dried, and finally recrystallized from EtOH to afford yellow crystals of 2,6-bis(4-(5-mercapto-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-3-yl)pyridine (**2**). Yield (0.63 g, 77%); yellow crystals; m.p.: 238-240°C. IR (KBr, cm^{-1}): 3425-3300 (NH). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.95-8.07 (m, 3H, pyridine), 8.12 (s, 2H, pyrazole H-5), 10.04 (br s, 4H, D_2O-exchangeable, 4NH), 13.51 (s, 2H, D_2O-exchangable, 2SH), MS *m*/*z* (%): 413 (12), 411 [M^+] (5), 229 (5), 188 (12), 171 (23), 135 (5), 129 (51), 75 (25), 60 (100). Analysis: calcd. for $C_{15}H_9N_9O_2S_2$ (411.42): C, 43.79; H, 2.20; N, 30.64%; found: C, 43.72; H, 2.29; N, 30.63%.

Synthesis of the thiosemicarbazide derivatives (3a,b)

General procedure

A mixture of (**1**) (0.65 g, 2 mmol) and phenyl isothiocyanate or methyl isothiocyanate (4 mmol) in EtOH (20 mL) was refluxed for 8-10 h and then left to cool to room temperature. Fine crystals of the thiosemicarbazide derivatives **3a** and **3b** were separated out, filtered off, washed with EtOH, dried and finally recrystallized from EtOH. The synthesized compounds together with their physical and spectral data are listed below:

2,6-Bis[4-(3-phenylthioureidocarbamoyl)-1*H*-pyrazol-3-yl]pyridine (3a)

Yield (0.92 g, 77%); yellow crystals; m.p.: 178-179°C. IR (KBr, cm^{-1}): 3227-3112 (NH, overlapped), 1670 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 5.37 (br s, 2H, D_2O-exchangeable, 2NH), 7.65-8.05 (m, 3H, pyridine and 10 Ar-H), 8.11 (s, 2H, pyrazole-CH-5), 10.51, 12.5, 13.7 (br s, 6H, D_2O-exchangeable 6NH). MS *m*/*z* (%): 597 [M^+] (36), 149 (14), 106 (44), 83 (57), 69 (100). Analysis: calcd. for $C_{27}H_{23}N_{11}O_2S_2$ (597.67): C, 54.26; H, 3.88; N, 25.78%; found: C, 54.33; H, 3.81; N, 25.72%.

2,6-Bis[4-(3-methylthioureidocarbamoyl)-1*H*-pyrazol-3-yl]pyridine (3b)

Yield (0.70 g, 74%); colorless crystals; m.p.: 214-216°C. IR (KBr, cm^{-1}): 3228-3209 (4NH, overlapped), 1654 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.41 (s, 6H, $2CH_3$), 6.31 (br s, 2H, D_2O-exchangeable, 2NH), 7.85-8.11 (m, 3H, pyridine), 8.14 (s, 2H, pyrazole-H-5), 10.48, 13.2, 14.2 (br s, 6H, 6NH, D_2O-exchangeable). MS *m*/*z* (%): 473 [M^+] (5), 196 (14), 166 (16), 149 (100), 121 (35), 105 (30), 77 (15), 65 (40). Analysis: calcd. for $C_{17}H_{19}N_{11}O_2S_2$ (473.54): C, 43.12; H, 4.04; N, 32.54%; found: C, 43.21; H, 4.14; N, 32.52%.

2,6-Bis[4-(5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1*H*-pyrazol-3-yl]pyridine (4)

The thiosemicarbazide derivative **3a** (0.6 g, 1 mmol) was refluxed in sodium hydroxide solution (2%, 4 mL) for 4 h and the resulting solution was treated with charcoal then filtered. The cold filtrate was acidified with hydrochloric acid (2 M) to pH 5 and the formed solid was filtered off, washed several times with distilled water, dried and finally recrystallized from EtOH/dioxane to afford 2,6-bis(4-(5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1*H*-pyrazol-3-yl)pyridine (**4**) in 71% yield (0.39 g); m.p. > 300°C. IR (KBr, cm^{-1}): 3461-3200 (2NH,). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.69-8.07 (m, 13H, pyridine and Ar-H), 8.12 (s, 2H, pyrazole-H-5), 10.51 (br, s, 2H, D_2O-exchangeable, 2NH), 14.21 (s, 2H, D_2O-exchangeable, 2SH). MS *m*/*z* (%): 561 [M^+] (2), 495 (15), 176 (11), 104 (22), 77 (100). Analysis: calcd. for $C_{27}H_{19}N_{11}S_2$ (561.64): C, 57.74; H, 3.41; N, 27.43%; found: C, 57.83; H, 3.32; N, 27.42%.

2,6-Bis[4-(5-phenylamino-1,3,4-thiadiazol-2-yl)-1*H*-pyrazol-3-yl]pyridine (5)

To an ice-cold stirred solution of the thiosemicarbazide **3a** (0.60 g, 1 mmol) in EtOH (5 mL), sulfuric acid (3 mL) was carefully added over a period of 15 min. Stirring was maintained at room temper-

ature for 4 h, then the reaction mixture was poured onto an equal volume of ice water. The formed precipitate was filtered off, washed with water several times, dried and finally recrystallized from EtOH. Yield (0.39 g, 70%); green powder; m.p.: 158-159°C. IR (KBr, cm^{-1}): 3410, 3215 (NH). ^1H NMR (DMSO-d_6, δ, ppm): 6.99-8.11 (m, 13H, pyridine and Ar-H), 8.35 (s, 2H, pyrazole-H-5), 10.40 and 11.43 (br s, 4H, D_2O-exchangeable, 4NH). MS *m/z* (%): 561 [M^+] (13), 215 (10), 85 (12), 77 (19), 63 (100). Analysis: calcd. for $C_{27}H_{19}N_{11}S_2$ (561.64): C, 57.74; H, 3.41; N, 27.43%; found: C, 57.69; H, 3.45; N, 27.45%.

Reaction of the thiosemicarbazide 3a with α-halo compounds

General procedure

To a solution of the thiosemicarbazide derivative **3a** (0.6 g, 1 mmol) and the appropriate α-halo compounds (phenacyl chloride, chloroacetone and ethyl chloroacetate) (2 mmol) in absolute EtOH (20 mL), a catalytic amount of triethylamine was added. The reaction mixture was refluxed for 4-7 h, and then allowed to cool. The precipitated solid was filtered off, washed with ethanol, dried, and finally recrystallized from EtOH to afford the corresponding thiazol-2-ylidene derivatives **7a**, **7b** and **9**, respectively. The synthesized compounds together with their physical and spectral data are listed below:

2,6-Bis[4-(3,4-diphenylthiazol-2(3*H*)-ylideneaminocarbamoyl)-1*H*-pyrazole-3-yl]pyridine (7a)

Yield (0.67 g, 84%); yellow crystals, m.p.: 230-232°C. IR (KBr, cm^{-1}): 3358-3210 (2NH, overlapped), 1687 (C=O). ^1H NMR ($CDCl_3$, δ, ppm): 6.44 (s, 2H, thiazole-H) 6.75-7.23 (m, 20H, Ar-H), 7.75-8.10 (m, 3H, pyridine-H), 8.15 (s, 2H, 2CH pyrazole), 8.43, 11.45 (br s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, δ, ppm): 103.4, 105.1, 114.6, 123.7, 123.6, 124.3, 126.7, 127.9, 128.9, 129.6, 130.4, 130.7, 138.6, 137.5, 140.1, 141.1, 147.1, 164.2. MS *m/z* (%): 797 [M^+] (1), 665 (5), 415 (7), 252 (44), 135 (85), 93 (17), 77 (100). Analysis: calcd. for $C_{43}H_{31}N_{11}O_2S_2$ (797.91): C, 64.73; H, 3.92; N, 19.31%; found: C, 64.61; H, 3.87; N, 19.36%.

2,6-Bis[4-(4-methyl-3-phenylthiazol-2(3*H*)-ylideneaminocarbamoyl)-1*H*-pyrazol-3-yl]pyridine (7b)

Yield (0.61 g, 91%); brown crystals; m.p.: 97-99°C. IR (KBr, cm^{-1}): 3439, 3181 (2NH, overlapped), 1673 (C=O). ^1H NMR ($CDCl_3$, δ, ppm): 2.23 (s, 6H, $2CH_3$), 6.25 (s, 2H, thiazole-H), 6.65-7.21 (m, 10H, Ar-H), 7.65-8.22 (m, 3H, pyridine, 2H, 2CH pyrazole), 8.57, 10.52 (br s, 2H, 2NH, D_2O-exchangeable). MS *m/z* (%): 673 [M^+] (2), 654 (8), 313 (18), 296 (13), 190 (7), 149 (100), 58 (91). Analysis: calcd. for $C_{33}H_{27}N_{11}O_2S_2$ (673.77): C, 58.83; H, 4.04; N, 22.87%; found: C, 58.76; H, 4.01; N, 22.79%.

2,6-Bis[4-(4-oxo-3-phenylthiazolidin-2-ylideneaminocarbamoyl)-1*H*-pyrazol-3-yl]pyridine (9)

Yield (0.55 g, 81%); yellow crystals; m.p.: 152-153°C. IR (KBr, cm^{-1}): 3193, 3141 (2NH), 1734, 1644 (2C=O). ^1H NMR ($CDCl_3$, δ, ppm): 4.23 (s, 4H, $2CH_2$), 6.98-7.23 (m, 10H, Ar-H), 7.55-8.02 (m, 3H, pyridine-H), 8.10 (s, 2H, 2CH, pyrazole), 8.55, 10.52 (br s, 2H, 2NH, D_2O-exchangeable). MS *m/z* (%): 677 [M^+] (5), 204 (8), 165 (10), 149 (31), 93 (7), 77 (23), 58 (100). Analysis: calcd. for $C_{31}H_{23}N_{11}O_4S_2$ (677.72): C, 54.94; H, 3.42; N, 22.73%; found: C, 54.88; H, 3.41; N, 22.76%.

2,6-Bis[4-(3,5-dimethyl-1-carbonyl)-1*H*-pyrazol-3-yl]pyridine (10)

To a mixture of the acid hydrazide **1** (0.33 g, 1 mmol) and acetylacetone (0.20 g, 2 mmol) in EtOH (10 mL), few drops of piperidine were added. The reaction mixture was refluxed for 6 h and then allowed to cool. The precipitated solid was filtered off, washed with water, dried and finally recrystallized from EtOH to afford compound **10**. Yield (0.39 g, 86%); buff powder (EtOH); m.p.: 137-138°C. IR (KBr, cm^{-1}): 3228 (NH), 1687 (C=O). ^1H NMR (DMSO-d_6, δ, ppm): 2.32 (s, 6H, $2CH_3$), 2.46 (s, 6H, $2CH_3$), 5.59 (s, 2H, pyrazole-H), 7.66-8.01 (m, 3H, pyridine-H), 8.13 (s, 2H, pyrazole-H), 12.95 (s, 2H, 2NH, D_2O-exchangeable). MS *m/z* (%): 455 [M^+] (2), 449 (9), 356 (30), 289 (14), 249 (14), 211 (16), 172 (40), 97 (100), 76 (41). Analysis: calcd. for $C_{23}H_{21}N_9O_2$ (455.47): C, 60.65; H, 4.65; N, 27.68%; found: C, 60.72; H, 4.58; N, 27.61%.

2,6-Bis[4-(3-methylpyrazol-5-one-1-carbonyl)-1*H*-pyrazol-3-yl]pyridine (11)

To a mixture of the acid hydrazide **1** (0.33 g, 1 mmol) and ethyl acetoacetate (0.26 g, 2 mmol) in EtOH (20 mL), a few drops of piperidine were added. The reaction mixture was refluxed for 5 h. The precipitated solid was filtered off, washed with water, dried and finally recrystallized from EtOH to afford compound **11**. Yield (0.32 g, 71%); brown powder (EtOH); m.p.: 163-164°C. IR (KBr, cm^{-1}): 3310 (NH), 1716, 1656 (C=O). ^1H NMR (DMSO-d_6,

δ, ppm): 2.32 (s, 6H, $2CH_3$), 4.21 (s, 2H, $2CH_2$), 5.79 (s, 2H, pyrazole-H), 7.86-8.11 (m, 3H, pyridine), 8.17 (s, 2H, pyrazole-H), 10.95 (s, 2H, 2NH, D_2O-exchangeable). MS *m*/*z* (%): 459 [M^+] (3), 431 (42), 358 (45), 284 (39), 180 (95), 76 (100). Analysis: calcd. for $C_{21}H_{17}N_9O_4$ (459.42): C, 54.90; H, 3.73; N, 27.44%; found: C, 55.02; H, 3.71; N, 27.52%.

Condensation of acid hydrazide (1) with ketones

To a solution of the acid hydrazide **1** (1 mmol) and the appropriate ketones (acetophenone or acetone) (2 mmol) in EtOH (10 mL), few drops of glacial acetic acid were added. The reaction mixture was refluxed for 5-9 h then allowed to cool to room temperature. The precipitated solid was filtered off, washed with ethanol, dried and finally recrystallized from DMF-EtOH to give the corresponding products **12a**,**b**, respectively. The synthesized compounds together with their physical and spectral data are listed below:

Pyridine-2,6-bis[(*Z*)-*N*'-(1-methylethylidene)-3-yl-2*H*-pyrazole-4-carbohydrazide] (12a)

Yield (0.39 g, 85%); white powder (EtOH), m.p.: 250-252°C. IR (KBr, cm^{-1}): 3231-3201 (2NH), 1645 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.40, 2.50 (2s, 12H, $4CH_3$), 7.89-8.33 (m, 3H, pyridine, 2H, pyrazole-H), 10.21 (s, 2H, 2NH, D_2O-exchangeable), 12.51 (s, 2H, 2NH, D_2O exchangeable). MS *m*/*z* (%):407 [M^+] (11). Analysis: calcd. for $C_{19}H_{21}N_9O_2$ (407.43): C, 56.01; H, 5.20; N, 30.94%; found: C, 56.11; H, 5.26; N, 30.87%.

Pyridine-2,6-bis[(*Z*)-*N*'-(1-phenylethylidene)-3-yl-2*H*-pyrazole-4-carbohydrazide] (12b)

Yield (0.41 g, 77%); white powder (EtOH); m.p.: 245-247°C. IR (KBr, cm^{-1}): 3224 (NH), 1667 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.10 (s, 6H, $2CH_3$), 7.36-7.71 (m, 10H Ar-H), 7.75-8.35 (m, 3H, pyridine and pyrazole CH-protons), 10.53 (br s, 2H, 2NH, D_2O-exchangeable), 12.46 (br s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, δ, ppm): 15.2, 111.1, 121.2, 126.6, 127.7, 128.8, 128.9, 129.1, 138.3, 149.1, 157.8, 159.9, 163.5. MS *m*/*z* (%): 531 [M^+] (2), 464 (5), 398 (69), 297 (11), 238 (72), 171 (33), 118 (47), 77 (100). Analysis: calcd. for $C_{29}H_{25}N_9O_2$ (531.57): C, 65.53; H, 4.74; N, 23.71%; found: C, 65.61; H, 4.69; N, 23.67.

Condensation of the acid hydrazide (1) with aromatic aldehydes

To a mixture of the acid hydrazide **1** (0.33 g, 1 mmol) and the appropriate aldehyde (2 mmol), in EtOH (10 mL), few drops of glacial acetic acid were added. The reaction mixture was refluxed for 4-7 h then left to cool. The precipitated product was filtered off, washed with EtOH, dried and finally recrystallized from the appropriate solvent to afford the corresponding Schiff bases **13a-d**.

Pyridine-2,6-bis[(*N*'-benzylidene-2*H*-pyrazol-3-yl)-4-carbohydrazide] (13a)

Yield (0.35 g, 69%); white powder (EtOH), m.p.: 266-268°C. IR (KBr, cm^{-1}): 3201 (NH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.10-7.21 (m, 10H, Ar-H), 7.63-8.18 (m, 3H, pyridine-H, 2H, pyrazole-H, 2CH, imine-CH), 10.85 (br s, 2H, 2NH, D_2O-exchangeable), 12.41 (br s, 2H, 2NH, D_2O-exchangeable). MS *m*/*z* (%): 503 [M^+] (14), 352 (9), 290 (11), 230 (15), 188 (91), 171 (100), 89 (67), 77 (42). Analysis: calcd. for $C_{27}H_{21}N_9O_2$ (503.51): C, 64.41; H, 4.20; N, 25.04%; found: C, 64.49; H, 4.27; N, 25.11%.

Pyridine-2,6-bis[(*N*'-4-methoxybenzylidene-2*H*-pyrazol-3-yl)-4-carbohydrazide] (13b)

Yield (0.41 g, 73%); white powder (EtOH); m.p.: 235-237°C. IR (KBr, cm^{-1}): 3260-3116 (2NH), 1663 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.73 (s, 6H, $2CH_3$), 7.05-7.25 (m, 8H, Ar-H), 7.79-8.10 (m, 3H, pyridine-H, 2CH, imine-CH), 8.21 (s, 2H, pyrazole-H), 11.71 (br s, 2H, 2NH, D_2O-exchangeable), 12.41 (br s, 2H, 2NH, D_2O-exchangeable). MS *m*/*z* (%):563 [M^+] (5), 379 (20), 262 (17), 230 (30), 171 (100), 116 (61), 77 (37). Analysis: calcd. for $C_{29}H_{25}N_9O_4$ (563.57): C, 61.80; H, 4.47; N, 22.37%; found: C, 61.71; H, 4.41; N, 22.43%.

Pyridine-2,6-bis[(*N*'-4-methylbenzylidene-2*H*-pyrazol-3-yl)-4-carbohydrazide] (13c)

Yield (0.43 g, 81%); white powder (EtOH); m.p.: 270-272°C. IR (KBr, cm^{-1}): 3176, 3106 (2NH), 1647 (C=O).^{1}H NMR (DMSO-d_6, δ, ppm): 2.60, 2.69 (s, 6H, $2CH_3$), 7.17-7.31 (m, 8H, Ar-H), 7.63-8.05 (m, 3H, pyridine-H, 2CH, imine-CH), 8.13 (s, 2H, pyrazole-H), 11.76 (br s, 2H, 2NH, D_2O-exchangeable), 12.42 (br s, 2H, 2NH, D_2O-exchangeable). MS *m*/*z* (%): 531 [M^+] (2), 505 (10), 92 (100), 77 (42). Analysis: calcd. for $C_{29}H_{25}N_9O_2$ (531.57): C, 65.53; H, 4.74; N, 23.71%; found: C, 65.57; H, 4.72; N, 23.79%.

***In vitro* assay for anti-cancer activity**

The synthesized compounds were supplied to the Bioassay-Cell Culture Laboratory, National Research Centre, Cairo, Egypt for *in vitro* primary antitumor screening on lung adenocarcinoma

(A549), hepatocellular carcinoma (HEPG2) and caucasian breast adenocarcinoma (MCF7) (American Type Culture Collection). Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan (21, 22).

Procedure

The following procedures were done in a sterile area using a laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). HEPG-2 cell line was cultured in RPMI-1640 and MCF-7 and A549 cell line were cultured in DMEM. Cells were applied in 96-well plates (having about 10000 cells /well). The plates were then incubated for 24 h in 37^{o}C incubation and 5% CO_2 atmosphere before treatment with the tested compounds to allow attachment of cells to the wall of the plate. The tested compounds were dissolved in DMSO. Different concentrations of the compounds under test were added to the cell monolayer. Triplicate wells were prepared for each individual concentration, and then the plate was incubated for 48 h in 37^{o}C incubator. Forty microliters of MTT solution (2.5 mg/mL) was added into each well for additional 4 h. Formazan was dissolved in 200 µL (10%) sodium dodecyl sulfate and then measured at absorbance at $\lambda = 495$ nm. Cell viability at given compound concentration was calculated as the percentage of absorbance in wells with the compound-treated cells to that of vehicle control cells (100%). The active compounds that gave > 75% at 100 µM, were submitted to calculate their IC_{50} values (the concentration that inhibited cell viability by 50%) (22).

Acknowledgments

Financial support for this work was provided by the program of the Science and Technology Development Fund (STDF), Egypt (Project No.170). The program is financed through the Ministry of State for Scientific Research and is administered by Higher Council for Science & Technology.

REFERENCES

1. Davari A. S., Abnous K., Mehri S, Ghandadi M., Hadizadeh F.: Bioorg. Chem. 57, 83 (2014).
2. Lu T., Goh A.W., Yu M., Adams J., Lam F. et al.: J. Med. Chem. 57, 2275, (2014).
3. Kamal A., Reddy J.S., Ramaiah M.J., Dastagiri D., Bharathi E.V. et al.: Med. Chem. Commun. 1, 355 (2010).
4. Kuźmin V.E., Lozitsky V.P., Kamalov G.L., Lozitskaya R.N, Zheltvay A.I. et al.: Acta Biochim. Pol. 47, 867 (2000).
5. Deshang P., Cipollina J.A., Lowmoster N.K.: J. Org. Chem. 53, 1356 (1988).
6. Beckman R.K., Potenza J.C., Enholm E.J.: J. Org. Chem. 52, 469 (1987).
7. Kleemann A., Engel J., Kutscher B., Reichert D.: "Pharmaceutical Substances", 3rd edn., George Thieme Verlag, Stuttgart/New York 1999.
8. El-Sharive A. M.sh, Zahran M.A., Elgaby M.A.: Afinidad 60, 81 (2003).
9. Dominguez J., Charris J.E.: Arzeimittelforschung 52, 482 (2002).
10. Farag A.M, Mayhoub A.S., Abdalla T.M., Amr A.E., Ali K.A. et al.: Arch. Pharm. 343, 384 (2010).
11. Ali K.A., Ragab E.A., Farghaly T.A., Abdalla M.M.: Acta Pol. Pharm. Drug Res. 68, 237 (2011).
12. Ali K.A., Elsayed M.A., Farag A.M.: Heterocycles 85, 1913 (2012).
13. Ali K.A.: Heterocycles 85, 1975 (2012).
14. Ali K.A., Hosni H.M., Ragab E.A., Abd El-Moez S.I.: Arch. Pharm. 345, 231 (2012).
15. Ghozlan S.A., Al-Omar M.A., Amr A.E., Ali K.A., Abd El-Wahab A.A.: J. Heterocycl. Chem. 48, 1103 (2011).
16. Ali K.A., Elsayed M.A., Abdalghfar H.S.: Arkivoc ii, 103 (2011).
17. Farag A.M., Ali K.A., Mayhoub A.S., Abdalla T.M., Amr A.E. et al.: Eur. J. Med. Chem. 45, 5887 (2010).
18. Amr A.E., Ali K.A., Abdalla M.M., Eur. J. Med. Chem. 44, 901 (2009).
19. Shaaban M.R., Saleh T.S., Mayhoub A.S., Mansour A., Farag A.M.: Bioorg. Med. Chem., 16, 6344 (2008)
20. Farag A.M., Mayhoub A.S., Barakat S.E., Bayomi A.H.: Bioorg. Med. Chem. 16, 4569 (2008).
21. Mosmann T.: J. Immunol. Methods 65, 55 (1983).
22. El-Menshawi B.S., Fayad W., Mahmoud K., El-Hallouty S.M., El-Manawaty M. et al.: Indian J. Exp. Biol. 48, 258 (2010).

Received: 4. 03. 2015