NEW APPROACHES FOR THE SYNTHESIS OF HYDRAZONE DERIVATIVES AND THEIR ANTITUMOR EVALUATION

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ABSTRACT

The hydrazide-hydrazone **3** reacted with benzenediazonium chloride to give the phenylhydrazone derivative **5**. The latter underwent a series of hetero-cyclization reactions to give pyridazine, 1,2,3-triazole and pyrazole derivatives. The antitumor evaluation of the newly synthesized products against the three cancer cells lines namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) showed that some of them have higher inhibitory effects towards the three cell lines compared to the standard.

Key word: hydrazide-hydrazone, pyridazine, 1,2,3-triazole, pyrazole, anti-tumor.

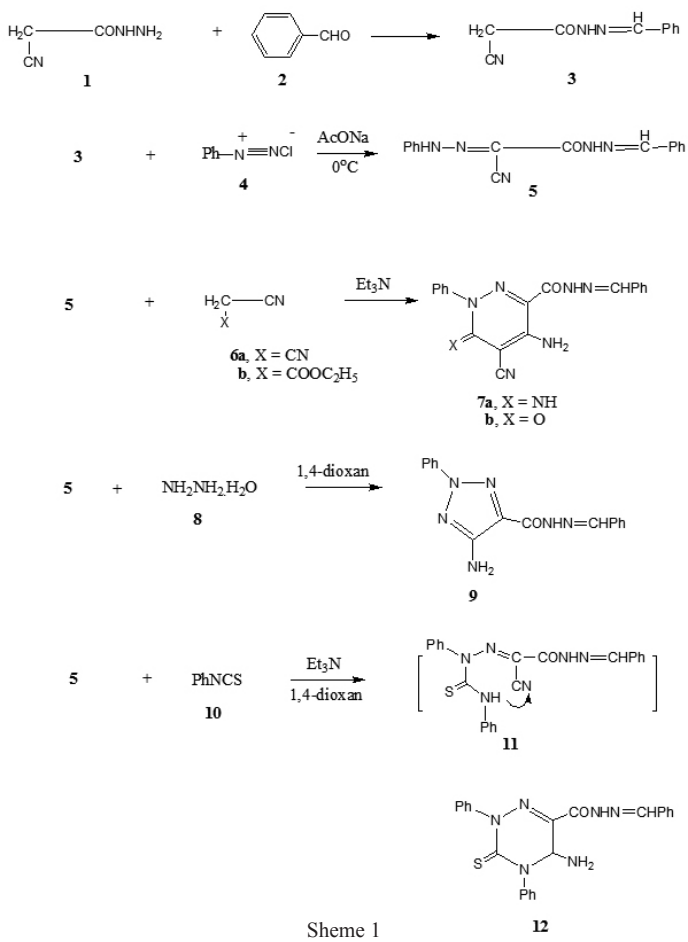
INTRODUCTION

Hydrazide-/hydrazones have been demonstrated to possess antibacterial,[1-7] anticonvulsant[8-11] and antitubercular[12-16] activities. These observations led us to synthesize novel hydrazide-hydrazones and to investigate their possible antitumor activities. It has been reported in the literature[17,18] that hydrazide-hydrazones can give the corresponding hydrazide and aldehyde metabolites whereas the related hydrazides are known to yield carboxylic acids via hydrolytic route. Based on this knowledge, hydrazide-hydrazones derivative **3** was prepared easily from the reaction of cyanoacetyl hydrazine with benzaldehyde and subsequently used for the synthesis of pyridazine, 1,2,3-triazole and pyrazole derivatives. The newly synthesized compounds were tested to evaluate their *in vitro* anti-tumor activities against three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268). It was found that some of these compounds showed inhibitory effects on the three cell lines, indicating their potential use in the development of anti-cancer agents.

RESULTS AND DISCUSION

The reaction of the cyanoacetylhydrazine **1** with benzaldehyde **2** gives the hydrazide-hydrazone **3**.[19] Reaction of the latter product with benzenediazonium chloride **4** afforded the phenylhydrazone derivative **5**. The structure of compound **5** was confirmed on the basis of analytical and spectral data. Heterocyclization of compound **5** to form different heterocyclic derivatives with potential pharmaceutical applications was studied. Thus, compound **5** reacted with either malononitrile **6a** or ethyl cyanoacetate **6b** in 1,4-dioxan containing a catalytic amount of triethylamine, gave the pyridazine derivatives **7a** and **7b**, respectively. The structures of compounds **7a & 7b** were established on the basis of their analytical and spectral data. Thus, the ^{1}H NMR spectrum of **7a** showed the presence of a singlet at δ 4.87 ppm corresponding to NH_2 group, a singlet at δ 6.69 ppm for the CH=N group, a multiplet at δ 7.28-7.58 ppm for the two phenyl groups and two singlets (D_2O exchangeable) at δ 8.23 and 8.30 ppm for the two NH groups. On the other hand, reaction of compound **5** with hydrazine hydrate gave the triazole derivative **9**. Moreover, the reaction of compound **5** with phenylisothiocyanate **10** gave the 1,2,4-triazine derivative **12**. The structures of compounds **9** and **12** were established on the basis of their analytical and spectral data (see experimental section).

Next, the reaction of compound **3** with phenylisothiocyanate in DMF/KOH solution was studied. This reaction led to the formation of the nonisolable intermediate potassium sulphide salt **13** which subsequently allowed to react with hydrazine hydrate to afford the hydrazino derivative **14** was formed. The structure of the latter product was confirmed on the basis of its analytical and spectral data. Thus, the ^{1}H NMR spectrum showed the presence of a singlet at δ 5.02 ppm corresponding to NH_2 group, a singlet at δ 6.99 for the CH=N group, a multiplet at δ 7.28-7.49 ppm corresponding to the two phenyl group and three singlets at δ 8.02, 8.90, 9.21 ppm corresponding to the three NH groups. Compound **14** was cyclized readily in sodium ethoxide solution affording the 3-amino-5-(phenylamino)-1H-pyrazole-4-N-benzalcarbohydrazide derivative **15**. The structure of the latter product was established based on its analytical and spectral data (see experimental sections). Diazotization of the 3-amino group of compound **15** using sodium nitrite solution in the presence of acetic/hydrochloric acids resulted in the formation of intermediate pyrazole-3-diazonium salt **16**. Coupling of the latter diazonium salt with malononitrile in ethanol containing sodium acetate afforded the hydrazo derivative **17**. The analytical and spectral data of the obtained product were consistence with the proposed structure (Scheme 2).



Sheme 1

The reaction of compound **14** with either acetylacetone **18a** or ethyl acetoacetate **18b** gave the pyrazole derivatives **19a** and **19b**, respectively. In a similar manner, the reaction of **14** with either malononitrile **6a** or ethyl cyanoacetate **6b** gave the pyrazole derivatives **20a** and **20b**, respectively. Moreover, the reaction of **14** with either a-cyanocinnamonitrile **21a** or ethyl a-cyanocinnamate **21b** gave the 5-phenylpyrazole derivatives **22a** and **22b**,

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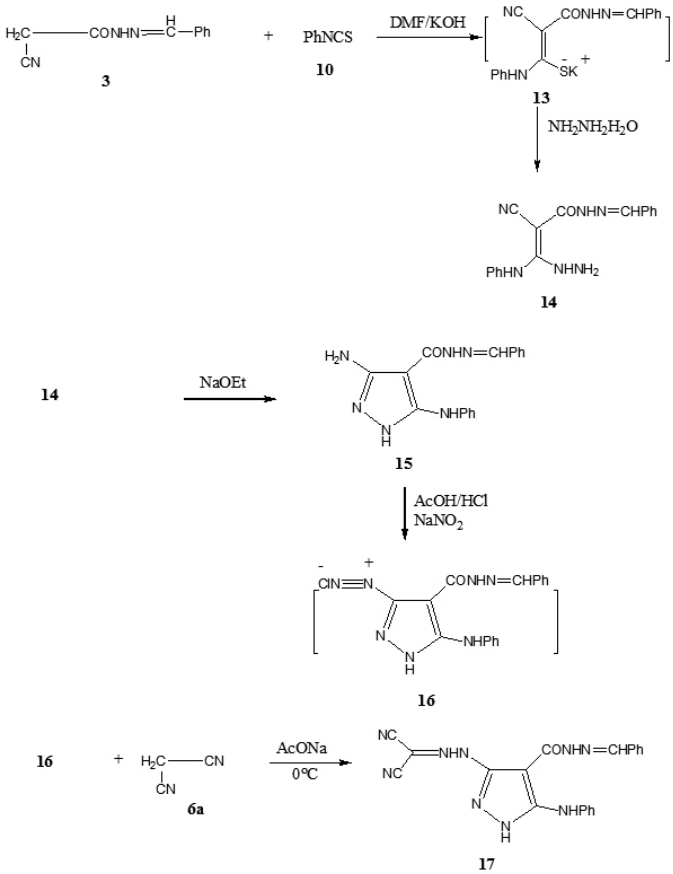
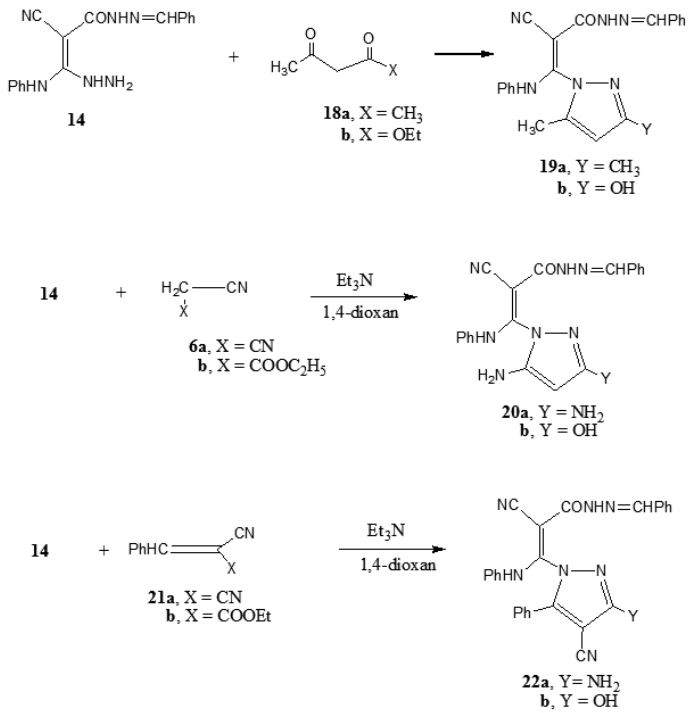
respectively (Scheme 3). The analytical and spectral data of compounds **19a,b-22a,b** were in agreement with their structures (see experimental section).



Scheme 2

Material, methods & Reagents: Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) from Sigma Chemical Co. (Saint Louis, USA). Samples: Stock solutions of compounds **5-22b** were prepared in DMSO and kept at -20 ºC. Appropriate dilutions of the compounds were freshly prepared just prior the assays. Final concentrations of DMSO did not interfere with the cell growth.

Cell cultures: Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 mg/mL), at 37 ºC in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 X 10^5 cells/mL for MCF-7 and SF-268 and 0.75 X 10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.



Sheme 3

Table I. Effect of compounds 5-22b on the growth of three human tumor cell lines.

Compound	GI50 (m mol L^{-1})		
	MCF-7	NCI-H460	SF-268
5	10.2 ± 0.6	12.3 ± 1.2	20.3 ±2.5
7a	20.3 ± 0.4	24.3 ± 0.8	30 ± 0.8
7b	60.6 ± 12.8	31.3 ± 6.4	44.2 ± 3.8
9	32.8 ± 8.2	30.6 ± 8.6	40.4 ± 4.6
12	35.4 ± 10.2	22.1 ± 0.8	6.7 ± 4.7
14	14.8 ± 3.6	10.3 ± 0.9	16.7 ± 1.6
15	60.7 ± 12.5	33.2 ± 10.6	66.0 ± 9. 1
17	50.1 ± 0.7	23.2 ± 4.8	10.4 ± 1.8
19a	22.0 ± 0.2	30.6 ± 1.4	32.6 ± 2.9
19b	32.0 ± 4.5	41.0 ± 2.4	22.5 ± 1.6
20a	22.0 ± 3.7	20.0 ± 4.4	30.5 ± 8.0
20b	6.4 ± 2.6	10.1 ± 2.8	4.2 ± 1.5
22a	70.9 ± 0.9	48.6 ± 4.8	50.8 ± 0.8
22b	30.4 ± 2.6	22.8 ± 2.8	56.2 ± 3.8
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

Effect on the growth of human tumor cell lines

The effect of compounds **5-22b** was evaluated on the in vitro growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268), after a continuous exposure of 48 h. The results are summarized in Table 1.

All the compounds were able to inhibit the growth of the human tumor cell lines in a dose-dependent manner (data not shown). Compounds **5** and **20b** showed the best results, exhibiting an equivalent potency in all the three tumor cell lines which is still much lower than the control doxorubicin, On the other hand, compounds **7a**, **14**, **19a** and **20a** showed moderated growth inhibitory effect. Comparing the activities of **20a** and **20b** it is observed that OH group present in **20b** presents a stronger growth inhibitory effect than compound **20a**

with the NH_2 group. It is obvious that compounds **7b**, **15**, **17**, **19b**, **22a** showed the lowest inhibitory effect towards the three cell lines.

EXPERIMENTAL SECTION

All melting points were determined in open capillaries and are uncorrected. IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H-NMR spectra were measured on a Varian EM390-200 MHz instrument in CD_3SOCD_3 as solvent using TMS as internal standard, and chemical shifts are expressed as δ in units of parts per million (ppm). Analytical data were obtained from the Micro analytical data unit at Cairo University.

2- Cyano-2-phenylhydrazo-N-benzalacetylhydrazide (5):

To a cold solution (0-5 ˚C) of compound **3** (1.87 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (4.0 g), benzenediazonium chloride [prepared by adding sodium nitrite solution (0.70 g in 10 mL water) to a cold solution (0-5 ˚C) of aniline (0.94 g, 0.01 mol) in concentrated hydrochloric acid (10 mL) with continuous stirring] was added with continuous stirring. The reaction mixture was then stirred at room temperature for additional 1 h and the formed solid product was collected by filtration.

Orange crystals from ethanol, yield (2.03 g, 70 %), m.p. 70 ˚C . IR (KBr): υ/cm^{-1} = 3522-3332 (2NH), 3057 (CH aromatic), 2222 (CN), 1690 (CO), 1645 (C=C), 1637 (C=N). ^{1}H NMR (DMSO) δ = 6.56 (s, 1H, CH=N), 7.30-7.39 (m, 10H, $2C_6H_5$), 8.30, 8.37 (2s, 2H, D_2O exchangeable, 2NH). Calcd for $C_{16}H_{13}N_5O$ (291.31): C, 65.97; H, 4.50; N, 24.04 %. Found: C, 66.01; H, 4.48; N, 24.33 %. MS m/z: 291 (M^+).

5-Amino-4-cyano-3-imino-1-phenylpyridazin-6-N-benzalcarbohydrazide (7a) and 5-Amino-4-cyano-3-oxo-1-phenylpyridazin-6-N-benzalcarbohydrazide (7b)

General procedure: To a solution of compound **5** (1.91 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (0.50 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.25 g, 1.18 mL, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **7a**: Yellow crystals from 1,4-dioxan, yield (2.74 g, 77 %), m.p. 90 ˚C. IR (KBr): υ/cm^{-1} = 3480-3330 (NH_2, 2NH), 3060 (CH aromatic), 2223 (CN), 1688 (CO), 1638 (C=C), 1628 (C=N). ^{1}HNMR (DMSO) δ = 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.69 (s, 1H, CH=N), 7.28-7.58 (m, 10H, $2C_6H_5$), 8.23, 8.30 (2s, 2H, D_2O exchangeable, 2NH). Calcd for $C_{19}H_{15}N_7O$ (357.37): C, 63.86; H, 4.23; N, 27.44 %. Found: C, 64.08; H, 4.47; N, 27.72 %. MS m/z: 357 (M^+).

Compound **7b**: Pale orange crystals from 1,4-dioxan, yield (2.74 g, 77 %), m.p.160 ˚C. IR (KBr): υ/cm^{-1} = 3477-3328 (NH_2, NH), 3055 (CH aromatic), 2220 (CN), 1686 (2 CO), 1636 (C=C), 1625 (C=N). ^{1}H NMR (DMSO) δ = 4.85 (s, 2H, D_2O exchangeable, NH_2), 6.66 (s, 1H, CH=N), 7.29-7.49 (m, 10H, $2C_6H_5$), 8.26 (s, 1H, D_2O exchangeable, NH). Calcd for $C_{19}H_{14}N_6O_2$ (358.35): C, 63.68; H, 3.94; N, 23.45 %. Found: C, 63.84; H, 4.02; N, 23.66 %. MS m/z: 358 (M^+).

4-Amino-2-phenyl-5-N-benzalcarbohydrazido-1,2,3-triazole (9)

General procedure: To a solution of compound **5** (1.91 g, 0.01 mol) in 1,4-dioxan (40 mL), hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

Yellow crystals from 1,4-dioxan, yield (2.45 g, 80 %), m.p. 60 ˚C. IR (KBr): υ/cm^{-1} = 3467-3338 (NH_2, NH), 3059 (CH aromatic), 1689 (CO), 1632 (C=C), 1620 (C=N). ^{1}H NMR (DMSO) δ = 4.89 (s, 2H, D_2O exchangeable, NH_2), 6.68 (s, 1H, CH=N), 7.20-7.36 (m, 10H, $2C_6H_5$), 8.22 (s, 1H, D_2O exchangeable, NH). Calcd for $C_{16}H_{14}N_6O$ (306.32): C, 62.74; H, 4.61; N, 27.44 %. Found: C, 62.44; H, 4.52; N, 27.71 %. MS m/z: 306 (M^+).

5-Amino-2,4-diphenyl-3-thioxo-6-N-benzalcarbohydrazido-1,3,4-triazine (12)

To a solution of compound **5** (1.91 g, 0.01 mol) in 1,4-dioxan (40 mL), phenylisothiocyanate (1.35 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

Yellow crystals from 1,4-dioxan, yield (3.42 g, 80 %), m.p. 40 ˚C. IR (KBr): υ/cm^{-1} = 3438-3322 (NH_2, NH), 3052 (CH aromatic), 1685 (CO), 1630 (C=C), 1628 (C=N). ^{1}H NMR (DMS): δ = 4.68 (s, 2H, D_2O exchangeable, NH_2), 6.72 (s, 1H, CH=N), 7.28-7.42 (m, 16H, $3C_6H_5$, pyridazine CH), 8.29 (s, 1H, D_2O exchangeable, NH). Calcd for $C_{23}H_{20}N_6OS$ (428.51): C, 64.47; H, 4.70; N, 19.61; S, 7.48 %. Found: C, 64.55; H, 4.66; N, 19.55; S, 7.71 %. MS m/z: 428 (M^+).

α-Cyano-b-phenylamino-β-hydrazinoylidino-N-benzalacrylohydrazide (14)

To a stirred solution of compound 5 (1.91 g, 0.01 mol) in dimethylformamide (20 mL) containing potassium hydroxide (0.56 g, 0.01 mol) phenylisothiocyanate (1.35 g, 0.01 mol) was added. The whole reaction mixture was kept at room temperature overnight then hydrazine hydrate (0.50 g, 0.01 mol) was added. The formed solid product was collected by filtration.

White crystals from ethanol, yield (2.81 g, 88 %), m.p. 110 ˚C. IR (KBr): υ/cm^{-1} = 3459-3331 (NH_2, 3NH), 3056 (CH aromatic), 1689 (CO), 1631 (C=C), 1623 (C=N). H^{1}NMR (DMSO) δ = 4.77 (s, 2H, D_2O exchangeable, NH_2), 6.59 (s, 1H, CH=N), 7.22-7.38 (m, 10H, $2C_6H_5$), 8.24, 8.44, 8.50 (3s, 3H, D_2O exchangeable, 3NH). Calcd for $C_{17}H_{16}N_6O$ (320.35): C, 63.74; H, 5.03; N, 26.23 %. Found: C, 64.01; H, 4.89; N, 26.51 %. MS m/z: 320 (M^+).

3-Amino-5-phenylamino-3-N-benzalcarbohydrazido-pyrazole (15)

A suspension of compound 14 (3.20 g, 0.01 mol) in sodium ethoxide solution [prepared by dissolving sodium metal (0.64 g, 0.02 mol) in absolute ethanol (40 mL) was heated in a boiling water bath for 3 h. The reaction mixture was left to cool and the formed solid product upon pouring onto ice/water containing few hydrochloric acid (till pH 6) was collected by filtration.

Pale yellow crystals from ethanol, yield (2.21 g, 69 %), m.p. 60 ˚C. IR (KBr): υ/cm^{-1} = 3446-3323 (NH_2, 3NH), 3051 (CH aromatic), 1686 (CO), 1630 (C=C), 1626 (C=N). ^{1}H NMR (DMSO) δ = 4.82 (s, 2H, NH_2), 6.61 (s, 1H, CH=N), 7.29-7.39 (m, 10H, $2C_6H_5$), 8.20, 8.76, 8.82 (3s, 3H, D_2O exchangeable, 3NH). Calcd for $C_{17}H_{16}N_6O$ (320.35): C, 63.74; H, 5.03; N, 26.23 %. Found: C, 63.71; H, 5.24; N, 26.49 %. MS m/z: 320 (M^+).

3-Hydrazodicyanoylideno-5-phenylamino-3-N-benzalcarbohydrazidopyrazole (17)

A solution of the 3-diazo-5-phenylamino-3-N-benzalcarbohydrazidopyrazole (0.01 mol) [prepared by adding sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution (0 ˚C) of compound **15** (3.20 g, 0.01 mol) in acetic acid (20 mL) and concentrated hydrochloric acid (10 mL) with continuous stirring] was added to a cold solution of malononitrile (0.66 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (4.0 g) with continuous stirring for 2 h. The formed solid product was collected by filtration.

Orange crystals from ethanol, yield (3.02 g, 76 %), m.p. 160 ˚C. IR (KBr): υ/cm^{-1} = 3560-3328 (4NH), 3046 (CH aromatic), 2227, 2220 (2CN), 1691 (CO), 1633 (C=C), 1626 (C=N). ^{1}H NMR (DMSO) δ = 6.70 (s, 1H, CH=N), 7.31-7.40 (m, 10H, $2C_6H_5$), 8.14, 8.23, 8.40, 8.66 (4s, 4H, D_2O exchangeable, 4NH). Calcd for $C_{20}H_{15}N_9O$ (397.39): C, 60.45; H, 3.80; N, 31.72 %. Found: C, 60.53; H, 4.48; N, 32.06 %. MS m/z: 397 (M^+).

α-Cyano-β-phenylamino-β-(3,5-dimethylpyrazol-1-yl)-N-benzalacrylo-hydrazide (19a) and α-Cyno-β-phenylamino-β-(3-hydroxy-5-methylpyrazol-1-yl)-N-benzalacrylohydrazide (19b)

General procedure: To a solution of compound 14 (3.20 g, 0.01 mol) in 1,4-dioxan (30 mL) either acetylacetone (1.0 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then the solvent was evaporated under reduced pressure. The remaining residue was triturated with ethanol and the formed solid product was collected by filtration.

Compound **19a**: Orange crystals from 1,4-dioxan, yield (2.76 g, 72 %), m.p. 70 ˚C. IR (KBr): υ/cm^{-1} = 3487-3335 (2NH), 3055 (CH aromatic), 2225 (CN), 1685 (CO), 1630 (C=C), 1623 (C=N). ^{1}H NMR (DMSO) δ = 2.85, 3.03 (2s, 6H, $2CH_3$), 5.99 (s, 1H, pyrazole H-4), 6.73 (s, 1H, CH=N), 7.29-7.37 (m, 10H, $2C_6H_5$), 8.19, 8.25 (2s, 2H, D_2O exchangeable, 2NH). Calcd for $C_{22}H_{20}N_6O$ (384.43): C, 68.73; H, 5.24; N, 21.86 %. Found: C, 68.52; H, 5.46; N, 22.04%. MS m/z: 384 (M^+).

Compound **19b**: Pale yellow crystals from 1,4-dioxan, yield (2.56 g, 66 %), m.p.100 ˚C. IR (KBr): υ/cm^{-1} = 3592-3330 (OH, 2NH), 3058 (CH aromatic),

2223 (CN), 1688 (CO), 1633 (C=C), 1620 (C=N). ^{1}H NMR (DMSO) δ = 2.89 (s, 3H, CH_3), 5.97 (s, 1H, pyrazole H-4), 6.71 (s, 1H, CH=N), 7.26-7.39 (m, 10H, $2C_6H_5$), 8.15, 8.22 (2s, 2H, D_2O exchangeable, 2NH), 10.22 (s, 1H, D_2O exchangeable, OH). Calcd for $C_{21}H_{18}N_6O_2$ (386.41): C, 65.27; H, 4.70; N, 21.75 %. Found: C, 65.31; H, 5.02; N, 22.01%. MS m/z: 386 (M^+).

α-Cyano-b-phenylamino-β-(3,5-diaminopyrazol-1-yl)-N-benzalacrylo-hydrazide (20a) and α-Cyano-β-phenylamino-β-(3-hydroxy-5-aminopyrazol-1-yl)-N-benzalacrylohydrazide (20b)

General procedure: To a solution of compound **14** (3.20 g, 0.01 mol) in 1,4-dioxan (30 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.25 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then the solvent was evaporated under reduced pressure. The remaining residue was triturated with ethanol and the formed solid product was collected by filtration.

Compound **20a**: Orange crystals from 1,4-dioxan, yield (2.76 g, 66 %), m.p. 60 °C. IR (KBr): υ/cm^{-1} = 3487-3335 (2NH), 3058 (CH aromatic), 2228 (CN), 1689 (CO), 1633 (C=C), 1622 (C=N). ^{1}H NMR (DMSO) δ = 4.66, 5.23 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.63 (s, 1H, pyrazole H-4), 6.80 (s, 1H, CH=N), 7.26-7.39 (m, 10H, $2C_6H_5$), 8.20, 8.29 (2s, 2H, D_2O exchangeable, 2NH). Calcd for $C_{20}H_{18}N_8O$ (386.41): C, 62.17; H, 4.70; N, 29.00 %. Found: C, 60.93; H, 5.83; N, 28.37%. MS m/z: 386 (M^+).

Compound **20b**: Buff crystals from ethanol, yield (2.56 g, 66 %), m.p. 70 °C. IR (KBr): υ/cm^{-1} = 3569-3324 (OH, 2NH & NH_2), 3056 (CH aromatic), 2222 (CN), 1690 (CO), 1630 (C=C), 1622 (C=N). ^{1}H NMR (DMSO) δ = 4.66 (s, 2H, D_2O exchangeable, NH_2), 5.89 (s, 1H, pyrazole H-4), 6.69 (s, 1H, CH=N), 7.24-7.40 (m, 10H, $2C_6H_5$), 8.18, 8.26 (2s, 2H, D_2O exchangeable, 2NH), 10.09 (s, 1H, D_2O exchangeable, OH). Calcd for $C_{20}H_{17}N_7O_2$ (387.39): C, 62.01; H, 4.42; N, 25.31 %. Found: C, 61.89; H, 4.63; N, 25.44%. MS m/z: 387 (M^+).

α-Cyano-b-phenylamino-β-(3-amino-4-cyano-5-phenylpyrazol-1-yl)-N-benzalacrylohydrazide (22a) and α-Cyno-b-phenylamino-β-(3-hydroxy-3-cyano-5-phenylpyrazol-1-yl)-N-benzalacrylohydrazide (22b)

General procedure: To a solution of compound **14** (3.20 g, 0.01 mol) in 1,4-dioxan (30 mL) either a-cyanocinnamonitrile (1.54 g, 0.01 mol) or ethyl a-cyanocinnamate (2.01 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then evaporated under vacuum. The remaining product was triturated with ethanol and the formed solid product was collected by filtration.

Compound **22a**: Pale yellow crystals from 1,4-dioxan, yield (4.17 g, 88 %), m.p. 60 °C IR (KBr): υ/cm^{-1} = 3463-3324 (2NH& NH_2), 3056 (CH aromatic), 2224 (CN), 1689 (CO), 1631 (C=C), 1620 (C=N). ^{1}H NMR (DMSO) δ = 4.69 (s, 2H, D_2O exchangeable, NH_2), 6.77 (s, 1H, CH=N), 7.30-7.42 (m, 15H, $3C_6H_5$), 8.21, 8.32 (2s, 2H, D_2O exchangeable, 2NH). Calcd for $C_{27}H_{20}N_8O$ (472.18): C, 68.63; H, 4.27; N, 23.72 %. Found: C, 68.41; H, 4.08; N, 23.68 %. MS m/z: 472 (M^+).

Compound **22b** : Pale brown crystals from ethanol, yield (3.83 g, 60 %), m.p.70 °C. IR (KBr): υ/cm^{-1} = 3584-3348 (OH, 2NH), 3051 (CH aromatic), 2220 (CN), 1690 (CO), 1630 (C=C), 1622 (C=N). ^{1}H NMR (DMSO) δ = 6.59 (s, 1H, CH=N), 7.27-7.37 (m, 15H, $3C_6H_5$), 8.22, 8.29 (2s, 2H, D_2O exchangeable, 2NH), 10.32 (s, 1H, D_2O exchangeable, OH). Calcd for $C_{27}H_{19}N_7O_2$ (473.49): C, 68.49; H, 4.04; N, 20.71 %. Found: C, 68.72; H, 4.28; N, 20.94%. MS m/z: 473 (M^+).

CONCLUSIONS

The work described showed the uses of the phenylhydrazone derivative **5** in a series of heterocyclic transformations to form pyridazine, 1,2,4-triazine and pyrazole derivatives. The antitumor evaluation of the synthesized products showed that the pyrazole derivative **20b** has the maximum inhibitory effect towards the cancer cell lines.

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