ICC
Iranian Chemical Communication
Original Research Article
Payame Noor University http://icc.journals.pnu.ac.ir

Design, synthesis and anticancer evaluation of novel thiazine, pyrimidine and pyridine derivatives

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Received: 11 April 2016, Accepted: 16 August 2016, Published: 16 August 2016

Abstract

Acylisothiocyanate (**1**) was allowed to react with benzylidenemalononitrile producing oxazine derivative (**2**). Also, compound (**1**) was reacted with sodium azide followed by refluxing with sodium ethoxide affording thiazinotetrazole derivative (**4**). The reaction of acetylacetone with compound (**1**) gave pyridine derivative (**5**) by intramolecularcycloaddition while it was reacted with *N*-methyl aniline affording thiazine derivative (**7**). In addition, it was reacted with cyanoacetamide producing mercaptopyrimidine derivative (**9**). Finally, compound (**1**) was refluxed with phenylhydrazine, urea, guanidinum carbonate and anthranilic acid in the presence of dry acetone affording triazole derivative (**11**), N-substituted pyrimidine (**15**), compound (**18**) and thiopyrimidine derivative (**19**) respectively. The structures of the new compounds were confirmed on the basis of elemental and spectral data. Some of the synthesized compounds were screened as anticancer.

Keywords: Thiazine; acetyl pyridine; mercapto pyrimidine; triazole derivative; anticancer.

Introduction

Isothiocyanates are important building units for the preparation of several classes of nitrogen, sulfur and oxygen heterocycles and organometallic compounds. Isothiocyanates are versatile synthetic intermediates in organic chemistry due to their availability and their tendency to undergo nucleophilic additions and cycloadditions [1-3]. The pyrimidine nucleus is present in a wide variety of biologically active natural products. In addition, pharmaceutical and biological activities of pyrimidine derivatives are well documented [4-6]. Pyrimidine derivatives are inhibitors of platelet aggregation and anticonceptive and antiparkinson's disease [7-9]. Also, pyrimidine derivatives have other activities as antimicrobial and analgesic [[10-12]. On the other hand, a variety of systems of heterocyclic compounds were condensed, especially those related to pyrimidine ring, playing an important role in medical, cancer and virus research [13,14]. Recent studies have shown the synthesis of some new thiazole derivatives used as antimicrobial agents and anticancer agents [15-17] in these notes; and to continue our previous work in heterocyclic chemistry, we have synthesized some new thiazolopyrimidine and tested their anticancer activities.

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Results and discussion

Acylisothiocyante**1** underwent [4+2] cycloaddition followed by elimination of HCN to produce oxazine derivative **2** (Scheme 1). The structure of compound **٢** was deduced from the IR spectrum which showed bands at 2207, 1584cm^{-1} due to CN and C=S groups, respectively. The ^{1}H NMR spectrum showed a signal at δ 7.69-8.13 attributed to Ar-H proton and C-H protons.

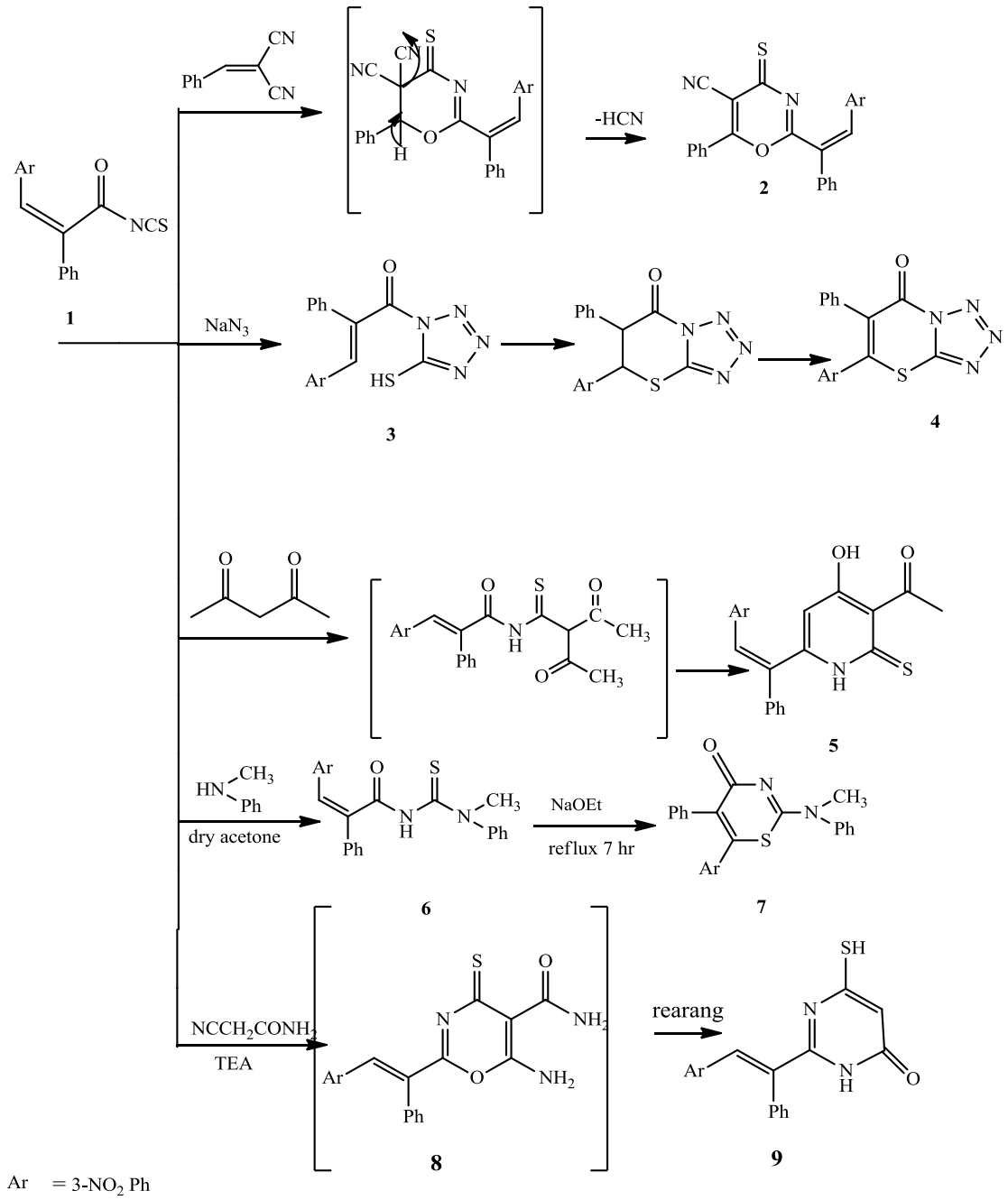
Heteroallene**1** underwent [3+2] cycloaddition to give tetrazole derivative **3**. The ring closure of compound **3** was achieved by refluxing in sodium ethoxide to produce thiazinotetrazole that underwent dehydrogenation to give the final product **4**.

Activated methylene of acetylacetone was added to α,β-unsaturated acylisothiocyanate to produce pyridine derivative **5**presumably *via* the formation of non-isolated acyclic thioamide.

Reaction of compound **1** with *N*-methylaniline afforded thiourea derivative **6** that underwent base induced intramolecular cyclization producing Michael adduct and subsequent dehydrogenation affording thiazine derivative **7**.

Also, compound **1** reacted with cyanoacetamide to give pyrimidine derivative **9**; presumably *via* the formation adduct **8** and subsequent dimorth rearrangement, and subsequent hydrolysis followed by decarboxylation (Scheme 1).

The structure of this product was proved by its spectroscopic data. Thus, the IR spectrum of compound **9** displayed NH and C=O absorbtions at 3440 cm^{-1} and 1677cm^{-1} respectively. ^{1}HNMR spectrum showed signals at δ 13.45 and 12.08 ppm attributed to SH and NH.



Scheme 1. Synthesis of oxazine 2, thiazinotetrazole 4, pyridine 5, thiazine 7 and pyrimidine 9 derivatives

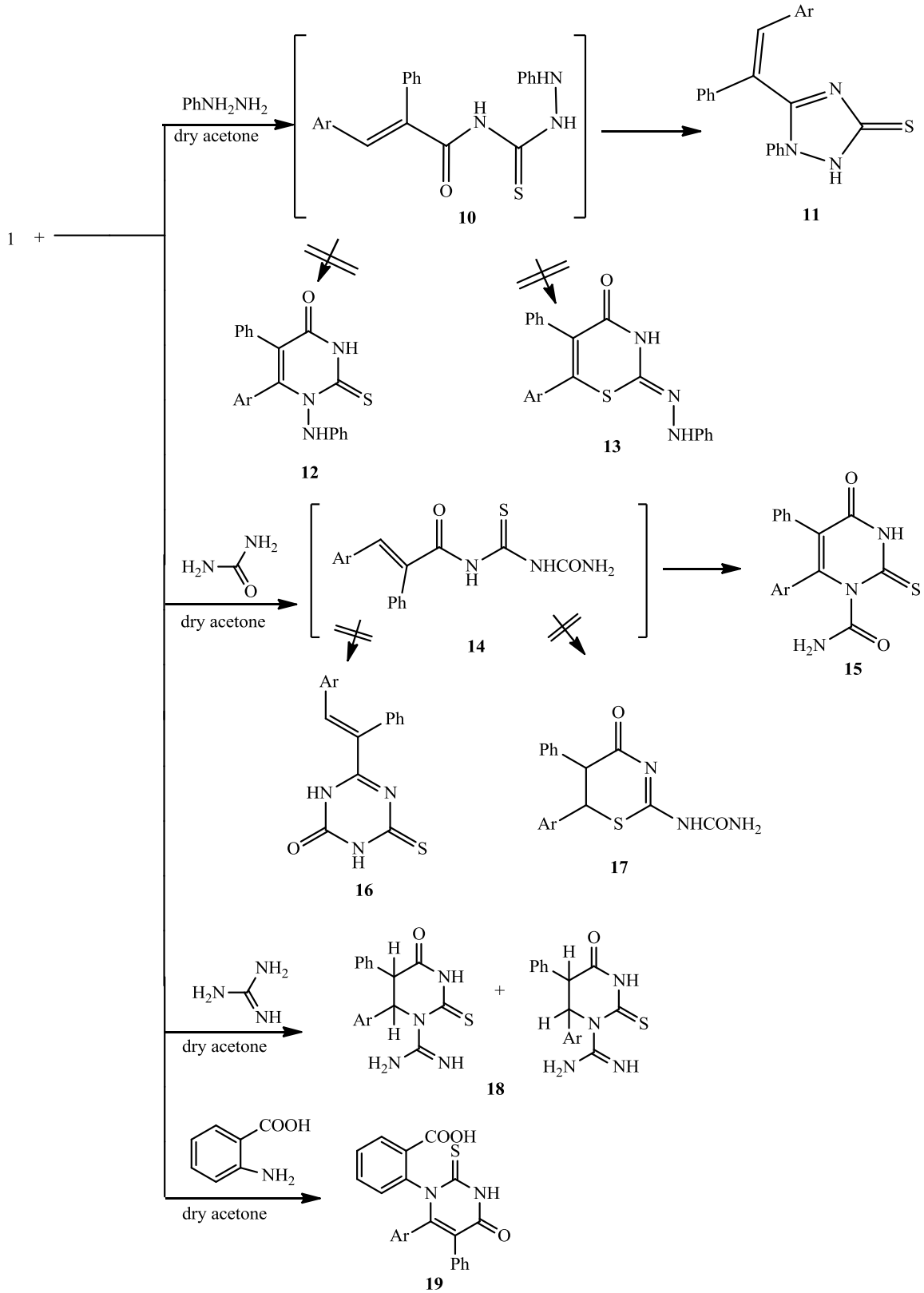
Phenyl hydrazine was reacted with α, β-unsaturated acylisothiocyanate**1** to produce triazole derivative **11**. Based on spectroscopic analysis, the expected pyrimidine **12** and thiazine **13** have ruled out. The formation of 10 from **1**and phenylhydrazine may be proceeding *via* the intermediacy of thiosemicabazide derivative **10** and subsequent intramolecularcyclodehydration.

The IR spectrum of compound **11** showed a strong absorption at 3382 cm^{-1} due to the NH group in addition to absorption band at 1529 cm^{-1} attributed to C=S group.

Cyclocondensation of urea with α, β-unsaturated acylisothiocyanate**1** afforded N-substituted pyrimidine **15** and none of the expected thiazine **17** or triazine**16** was obtained. The formation of **15** may be proceeded *via* the formation of non-isolated adduct **14** followed by a cyclization involving the (imino) nucleophilic nitrogen to the activated double bond and subsequent dehydrogenation.

Acylisothiocyanate**1** was refluxed with guanidine in cycloaddition affording the cis and trans pyrimidine derivative **18**. The IR analysis showed that **18** displayed absorption bands at 3385 cm^{-1} and 1721 cm^{-1} that were assigned to the amino and carbonyl group.

Cyclization of the activated heteroallene **1** with anthranilic acid afforded thiopyrimidine derivative **19**. The structure of this product was confirmed through its spectroscopic data.



Scheme 2. Synthesis of compounds 11, 15, 18, and 19

Anticancer activity

Cell survival will be determined using Sulforhodamine B (SRB) method as previously described by Skehan*et al*. The sulforhodamine B (SRB) assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation for large-scale drug-screening applications. Its principle is based on the ability of the protein dye sulforhodamine B to bind electrostatically and pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to and under mild basic conditions, it can be extracted from cells and solubilized for measurement.

Results of the SRB assay were linear with cell number and cellular protein measured at cellular densities ranging from 1 to 100% of confluence. Its sensitivity is comparable with that of several fluorescence assays and superior to that of Lowry or Bradford. The signal-to-noise ratio is favorable and the resolution is 1000-2000 cells/well.

The cytotoxic and antitumor activities of prepared compounds **2**, **5**, **7**, **11** and **19** were evaluated for cytotoxic activity against A549 and HePG2 cell lines according to the method of [**18**]. The inhibitory activities against lung carcinoma cells(A549 cell line) and hepatocellular carcinoma cells (HePG2 cell line) were detected using different concentrations of the tested compounds (100, 50, 25, 12.5 and 6.25 µg/mL) and the viability of cells (%) were determined by colorimetric method. Also, the (IC_{50}) was calculated from Tables 1 and 2 and Figures 1 and 2.

sample conc. (µg/mL)	**viability %**				
	2	5	7	11	19
0	100	100	100	100	100
6.25	92.8	82.7	50.1	88.0	73.2
12.5	88.9	76.2	48.6	86.3	72.2
25	63.3	73.3	48.3	84.5	70.2
50	53.0	61.3	46.1	82.6	68.9
100	32.5	46.3	42.0	37.4	67.9

Table1. Evaluation of cytotoxicity of compounds **2**, **5**, **7**, **11** and **19** against A549 cell line

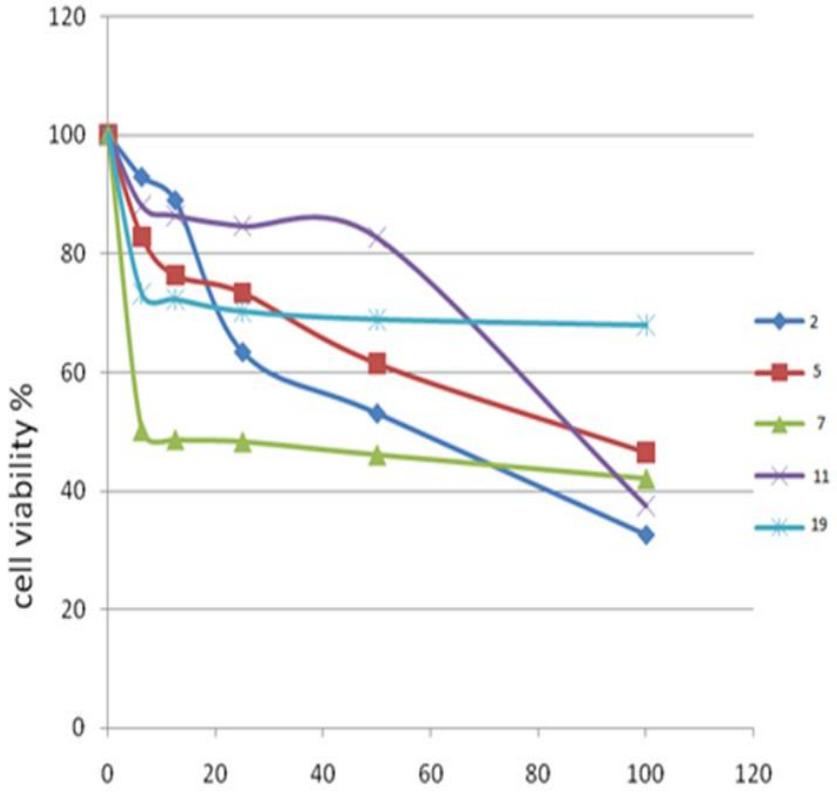


Figure 1. The inhibitory activities against lung carcinoma cells (A549)

Table 2. Evaluation of cytotoxicity of compounds 2, 5, 7, 11 and 19 against HePG2 cell line

sample (µg/mL)conc	**viability %**				
	2	5	7	11	19
0	100	100	100	100	100
6.25	93.6	84.9	88.5	83.3	90.6
12.5	90.6	78.6	54.8	81.2	87.9
25	78.2	71.2	29.8	78.6	85.4
50	58.4	64.0	26.8	77.6	82.6
100	43.9	37.4	17.9	41.6	79.5

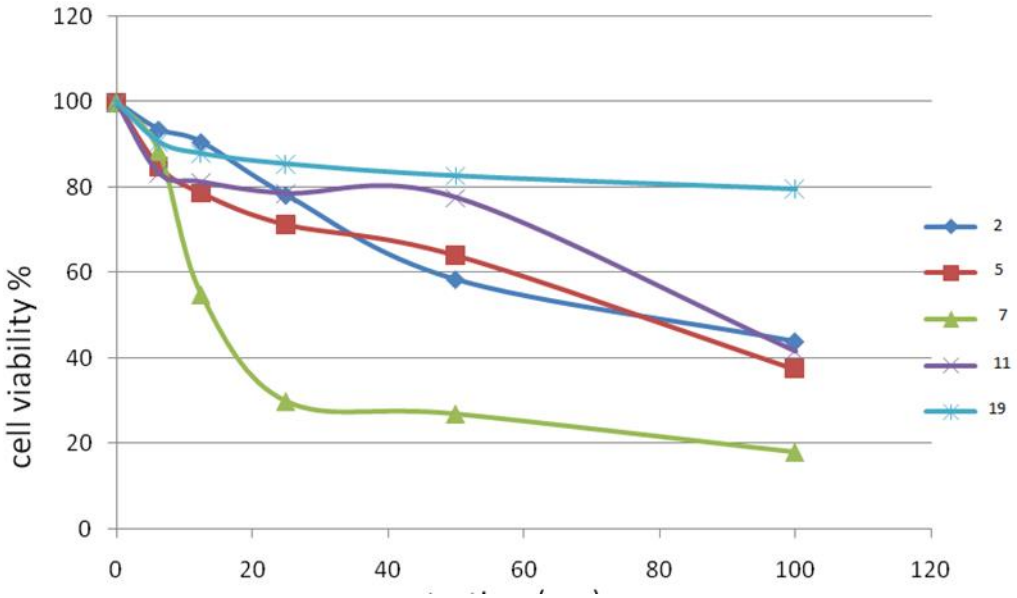


Figure 2. The inhibitory activities against Hepatocellular carcinoma cells (HePG2)
Concentration (µg/mL)

Table 3. The results of cytotoxicity testing against lung carcinoma cells lines and Hepatocellular carcinoma cells lines (IC_{50})

IC50 (µg/mL) values of tumor cell lines after 72h continuous exposure to test compounds.

Compound	**Tumor type / Cell line**	
	A549(IC50)	**HePG2**(IC50)
2	56. 81	78. 125
5	67.89	66. 3
7	8. 22	13.5 3
11	82. 16	86. 125
19	116.89	128. 3

IC50is the concentration that induces 50 % growth inhibition compared with treated control cells.
A549: Human lung adenocarcinoma epithelial cell line.
HePG2: Human hepatocellular carcinoma cell line.

Results revealed that all tested compounds have cytotoxic and antitumor activity against lung carcinoma cell line and hepatocellular carcinoma cell line. The highest cytotoxic potency was shown by compound 2 with IC50 values of 56. 81 and 78. 125 µg/mL against A549 and HePG2 respectively.

Experimental Section

General Procedures

Melting points were determined on Electro Thermal IA 9,100 series digital melting point apparatus in capillaries and are uncorrected. IR spectra were obtained in the solid state as potassium bromide discs using a Perkin-Elmer model 1430 Spectrometer. ^{1}HNMR spectra were recorded on a Varian/Gemini 200/ MHz spectrometer in DMSO-d_6 as a solvent and TMS as an internal standard (chemical shift in δ, ppm). Mass spectra were measured on an instrument "VG-7035". Spectra were recorded at 70 or 15 eV. Elemental analysis was performed at the Microanalytical Centre, Cairo University, and Giza, Egypt.

2-(3-Nitrophenyl)-1-phenylvinyl)-6-phenyl-4-thioxo-*4H*-1,3-oxazine-5-carbonitrile (2)

A mixture of compound **1** (3.3 gm, 0.01 mol), and benzylidenmalononitrile (1.54 gm, 0.01 mol) was refluxed for 12 h in the presence of triethylamine (3 drops) in dry acetone (20 mL). The separated solid was formed upon dilution with water and then filtrated, dried and recrystallized from butanol to give dark brown crystals of **2**, yield (5.32 gm, 82 %);mp 270 -272°C. IR spectrum (υ_{max}, cm^{-1}): 2207 (CN) and 1584(C=S).^{1}H NMR (δ, ppm):7.69-8.13 (m, 15H, CH and Ar-H). Mass (m/z value): 437 (12), 224 (17), 201 (7), 197 (85), 77 (100) Anal. Calcd. for $C_{25}H_{15}N_3O_3S$: C, 68.64; H, 3.46; N, 9.61. Found: C, 68.61; H, 3.43; N, 9.59.

1-(5-Mercapto-1H-tetrazol-1-yl)-3-(3-nitrophenyl)-2-phenylprop-2-en-1-one (3)

A mixture of compound **1** (3.3 gm, 0.01 mol) and sodium azide (0.65 gm, 0.01 mol) was refluxed for 3 h in dry acetone (20 mL). The separated solid was formed upon dilution with water and then filtered, dried and recrystallized from ethanol to give light brown crystals of **3**, yield (3.87 gm, 91 %; mp 156-158 °C. IR spectrum (υ_{max}, cm^{-1}): 3354 (NH), 1681 (C=O) and 1395 (SH).^{1}H NMR (δ, ppm): 13.06 (s, 1H, SH);7.45-7.98 (m, 10H, CH and Ar-H). Anal. Calcd. for $C_{16}H_{11}N_5O_3S$ (353.47): C, 54.38; H, 3.14; N, 19.82. Found: C, 54.39; H, 3.16; N, 19.80.

5-(3-Nitrophenyl)-6-phenyl-*7H*-tetrazolo[5,1-*b*][1,3]thiazin-7-one (4)

A mixture of compound **3** (3.5 gm, 0.01 mol) and sodium ethoxide (0.01 mol) was refluxed for 3 h in ethanol (20 mL). The separated solid was formed upon acidification with HCl (10 mL, 20%) and diluted with water and then filtered, dried and recrystallized from ethanol to give light brown crystals of **4**, yield 0.33 gm, 90 %;mp>360 °C. IR spectrum (υ_{max}, cm^{-1}): 1685(C=O).^{1}H NMR (δ, ppm):7.63-8.01 (m, 9H, Ar-H). Mass (m/z value): 351 (10), 323(15), 309 (70), 229 (11) 122(20), 95 (100). Anal. Calcd. For $C_{16}H_9N_5O_3S$: C, 54.70; H, 2.58; N, 19.93. Found: C, 54.69; H, 2.56; N, 19.94.

1-(1,2-Dihydro-4-hydroxy-6-((*Z*)-2-(3-nitrophenyl)-1-phenylvinyl)-2-thioxopyridin-3-yl)ethanone (5)

A mixture of compound **1** (3.3 gm, 0.01 mol), acetylacetone (1 gm, 0.01 mol) and triethylamine (3drops) was refluxed for 6 h in dry acetone (20 mL). The separated solid was formed upon dilution with water then filtered, dried and recrystallized from ethanol to give

white crystals of **5**, yield 3.2 gm, 81 %;mp 112-114 °C. IR (υ_{max}, cm^{-1}): 3449 (OH), 3380 (NH), 1713 (C=O), 1351 (SH).^{1}H NMR (δ, ppm): 13.09 (s, 1H, SH) and 11.62 (s, 1H, OH); 7.52-8.01 (m, 11H, ArH, CH); 2.52 (s, 3H, CH_3).Anal. Calcd. for $C_{21}H_{16}N_2O_4S$: C, 64.27; H, 4.11; N, 7.14. Found: C, 64.26; H, 4.13, N, 7.13.

1-Methyl-3-((*Z*)-3-(3-nitrophenyl)-2-phenylacryloyl)-1-phenylthiourea (6)

A mixture of compound **1** (3.3 gm, 0.01 mol) and *N*-methylbenzenamine (1.07 gm, 0.01 mol) was stirred for 7 h in dry acetone (20 mL). The separated solid was formed upon diluted with water, dried and recrystallized from ethanol to give light brown crystals of **6**, yield 3.74 gm, 90 %;m.p. 124-128 °C. IR spectrum (υ_{max}, cm^{-1}): 3382 (NH), 1675 (C=O), 1582 (C=S). Anal. Calcd. For $C_{23}H_{19}N_3O_3S$: C, 66.17; H, 4.59; N, 10.07. Found: C, 66.15; H, 4.55; N, 10.04.

2-(N-methyl-N-phenylamino)-6-(3-nitrophenyl)-5-phenyl-*4H*-1,3-thiazin-4-one (7)

A mixture of compound **6** (3.7 gm, 0.01 mol) and sodium ethoxide (0.01 mol) was stirred for 7 h in ethanol (20 mL). The separated solid was formed upon acidification with HCl (10 mL, 20%) and diluted with water, filtered, dried and recrystallized from ethanol to give dark brown crystals of **7**, yield 3.6 gm, 88 %;m.p. 260 °C. IR (υ_{max}, cm^{-1}): 1689 (C=O). ^{1}H NMR: (δ, ppm): 7.23-8.56 (m, 14H, Ar-H), 3.42 (s, 3H, CH_3). Anal. Calcd. For $C_{23}H_{17}N_3O_3S$: C, 66.49; H, 4.12; N 10.11. Found: C, 66.48; H, 4.10; N, 10.10

6-Mercapto-2-((*E*)-2-(3-nitrophenyl)-1-phenylvinyl)pyrimidin-4(*3H*)-one (9):

A mixture of compound **1** (3.3 gm, 0.01 mol), cyanoacetamide (1.04 gm, 0.01 mol) and triethylamine (3drops) was refluxed for 6 h in dry acetone (20 mL). The separated solid was formed upon dilution with water, filtered, dried and recrystallized from ethanol gave brown crystals of **9**, yield 3.1 gm, 89%;mp 136-140 °C. IR (υ_{max}, cm^{-1}): 3440 (NH), 1677 (C=O)..^{1}HNMR: (δ, ppm): 13.45 (s, 1H, SH); 12.08 (s, 1H, NH); 7.65-8.00 (m, 11H, Ar-H); 6.90 (s, 1H, CH). Mass (m/z value): 351 (20), 224 (15), 127 (100). Anal. Calcd. for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.73, ; N, 11.96. Found: C, 61.5; H, 3.71; N, 11.90.

1,2-Dihydro-5-((*Z*)-2-(3-nitrophenyl)-1-phenylvinyl)-1-phenyl-1,2,4-triazole-3-thione (11).

A mixture of compound **1** (3.3 gm, 0.01 mol) and phenylhydrazine (1.1 gm, 0.01 mol) was refluxed for 6 h in dry acetone (20 mL). The separated solid was formed upon dilution with water, filtered, dried and recrystallized from toluene to give brown crystals of **11**, yield 3.7 gm, 89 %;m.p. 240-245 °C. IR spectrum (υ_{max}, cm^{-1}): 3382 (NH), 1529 (C=S).^{1}HNMR (δ, ppm): 7.69-8.12 (m, 15H, ArH, CH); 9.33 (s, 1H, NH). Anal. Calcd. for $C_{22}H_{16}N_4O_2S$: C 65.98, H 4.03, N 13.99. Found C 65.97, H 4.02, N 13.97.

3,4-Dihydro-6-(3-nitrophenyl)-4-oxo-5-phenyl-2-thioxopyrimidine-1(*2H*)-carboxamide (15)

A mixture of compound **1** (3.3 gm, 0.01 mol) and urea (0.48 gm, 0.01 mol) was refluxed for 6 h in dry acetone (20 mL). The separated solid formed upon dilution with water, filtered, dried and crystallized from ethanol to give light yellow crystals of **15**, yield 3.29 gm, 90 %;mp 256-258 °C. IR (υ_{max}, cm^{-1}): 3425 (NH_2), 3233 (NH), 1706 (C=O).^{1}HNMR: (δ, ppm): 10.23 (s, 2H, NH_2), 9.32 (s, 1H, NH), 7.69-8.32 (m, 9H, Ar-H). Mass (m/z value): 368 (15), 324 (19), 246 (30), 169 (100), 77 (24). Anal. Calcd. For $C_{17}H_{12}N_4O_4S$: C,

55.43; H, 3.28; N, 15.21. Found: C, 55.41; H, 3.27; N, 15.20.

Tetrahydro-6-(3-nitrophenyl)-4-oxo-5-phenyl-2-thioxopyrimidine-1(*2H*)-carboxamidine (18)

A mixture of compound **1** (3.3 gm, 0.01 mol) and guanidinium carbonate (0.47 gm, 0.01 mol) was refluxed for 6 h in dry acetone (20 mL). The separated solid formed upon dilution with water, filtered, dried and crystallized from acetic acid to give light yellow crystals of **18**, yield 3.24 gm, 88 %;mp 129-132 °C. IR (υ_{max}, cm^{-1}): 3385 (NH_2), 1721 (C=O).^{1}H NMR (δ, ppm): 11.71 (s, 1H, NH), 10.43 (s, 1H, NH), 10.42 (s, 1H, NH), 9.63 (s, 2H, NH_2), 9.52 (s, 2H, NH_2), 7.72-8.03 (m, 9H, ArH), 5.82 and 5.43(d, d ,1H, CH-CH, J_{AB} = 6.34 Hz), 4.92 and 4.25(d, d ,1H, CH-CH, J_{AB} = 4.32Hz). Anal. Calcd. for $C_{17}H_{15}N_5O_3S$: C, 55.27; H, 7.09; N, 18.96. Found: C. 55.26; H, 7.08; N, 18.94.

2-(3,4-Dihydro-6-(3-nitrophenyl)-4-oxo-5-phenyl-2-thioxopyrimidin-1(*2H*)-yl)benzoic acid (19)

A mixture of compound **1** (0.01 mol) and anthranilic acid (1.40 gm, 0.01 mol) was refluxed for 2 h in dry acetone (20 mL). The separated solid formed upon dilution with water, filtered, dried and crystallized from acetic acid to give yellow crystals of **19**, yield 2.69 gm, 61 %;mp 220 - 222°C. IR spectrum (υ_{max}, cm^{-1}): 3412 (OH), 3135 (NH), 1706, 1670 (C=O). Mass (m/z value): 445 (20), 401 (50), 323 (5), 203 (100). ^{1}HNMR: (δ, ppm): 13.06 (s, 1H, OH), 11.53 (s, 1H, NH), 7.73-8.31 (m, 13H, Ar-H). Anal. Calcd. for $C_{23}H_{15}N_3O_5S$: C, 62.02; H, 3.39; N, 9.43. Found: C, 62.01; H, 3.37; N, 9.41.

Acknowledgments

The authors are thankful to Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt.

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