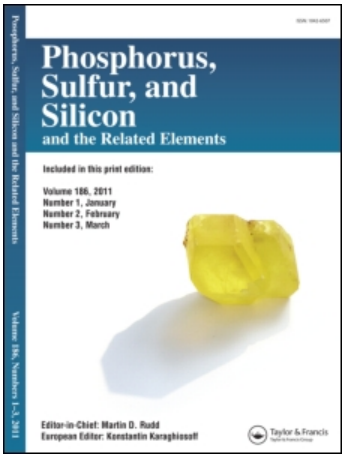

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Synthesis, Characterization, and Antiviral Activities of Pyridopyrazolotriazines

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Synthesis, Characterization, and Antiviral Activities of Pyridopyrazolotriazines

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A pyrazolo[3,4-b]pyridine-5-carbonitrile derivative was diazotized to give the corresponding diazonium salt, which was used as a good synthon to synthesize pyrido[2′,3′:3,4]pyrazolo[5,1-c]triazines via its coupling with several active hydrogen-containing reagents, e.g., 2,4-pentandione, ethyl 3-oxo-butanoate, diethylmalonate, malononitrile, 2-cyanoethanethioamide, and ethyl cyanoacetate. Also, it reacted with phenylisothiocyanate to afford the corresponding pyrazolo[3,4-b]pyridin-3-ylphenylthiourea derivative, which, in turn, was used for further chemical transformations. The data of IR, ^{1}H NMR, mass spectra, and chemical analyses elucidated the structures of all newly synthesized heterocyclic compounds. Cytotoxicity, anti–HSV1 and anti–HAV, and MBB activities were evaluated for all newly synthesized heterocyclic compounds.

Keywords 2-cyanoethanethioamide; phenylisothiocyanate; Pyrazolo[3,4-*b*]pyridine-5-carbonitrile; pyrazolo-[3,4-b]pyridin-3-ylphenylthiourea; pyrido[2′,3′:3,4]pyrazolo[5,1-c]triazines

INTRODUCTION

We were interested here to investigate the chemical reactivity and position of the NH_2 group on the pyrazolo[3,4-*b*]pyridine-5-carbonitrile ring system as well as the preparation of several derivatives of this ring system required for a medicinal chemistry program. In continuation to our previous work[1–18] and the reported biological activities of

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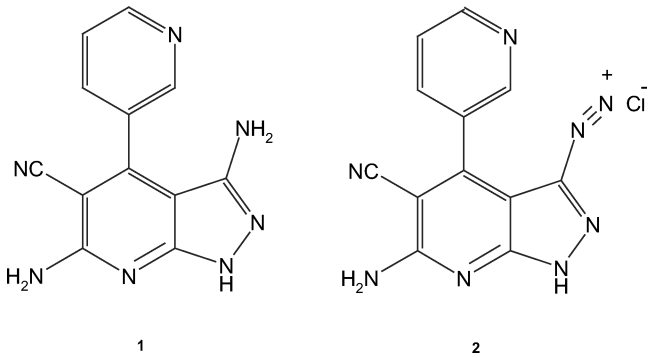


SCHEME 1

triazines,[19–22] pyrazolo-pyridines,[23,24] and thiazoles,[25–27] we were interested in synthesizing several derivatives of this ring system required for several chemical transformations as well as evaluating cytotoxicity, anti–HSV1, and anti–HAV, and MBB activities.

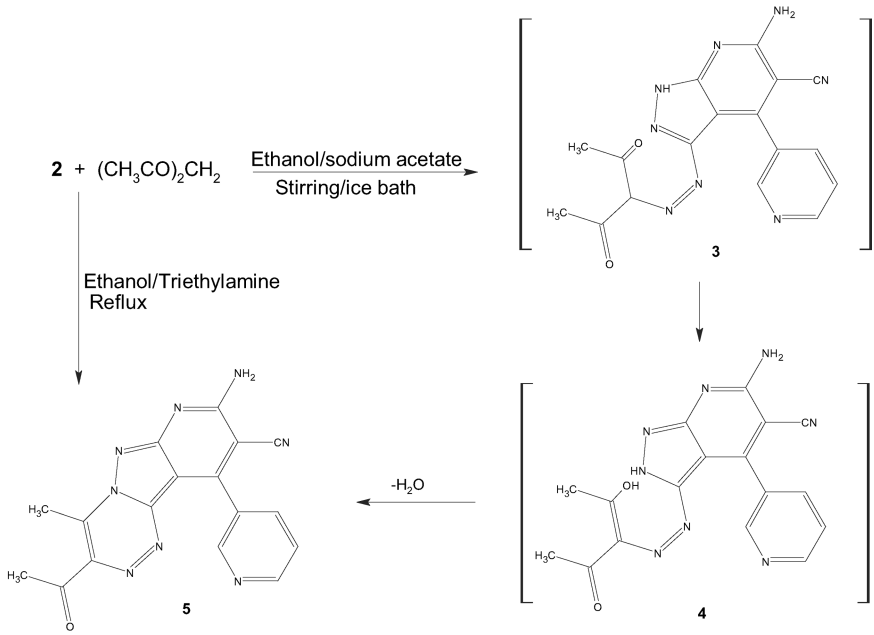
RESULTS AND DISCUSSION

3,6-diamino-4-pyridin-3-yl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile **1** was obtained according to literature procedure[28] and used as a good starting material for the present study. Thus, it has been found that **1** diazotized with sodium nitrite and hydrochloric acid to give the corresponding diazonium salt **2** (Scheme 1). All trials to isolate 2 failed while its structure was confirmed via its coupling with active $-CH_2-$ containing reagents.

Thus, diazonium salt **2** reacted with 2,4-pentandione in stirred ethanol containing sodium acetate (2–3 g) at r.t. to give a reaction product formed through dehydrochlorination. The IR of this reaction product showed bands of NH_2, CN, and CO acetyl; its ^{1}H NMR spectrum revealed the signals of CH_3, $\underline{CH_3}CO$, NH_2, and pyridine protons. Moreover, its mass spectrum gave m/z = 344, which corresponded to the molecular weight of the molecular formula $C_{17}H_{12}N_8O$ of the assigned structure (cf. Experimental Section and Scheme 2).

Considering the previously discussed data, it is possible to conclude that the dehydrochlorination product spontaneously underwent cyclization to afford the corresponding triazine derivative **5** via the nonisolable intermediates **3** and **4**.

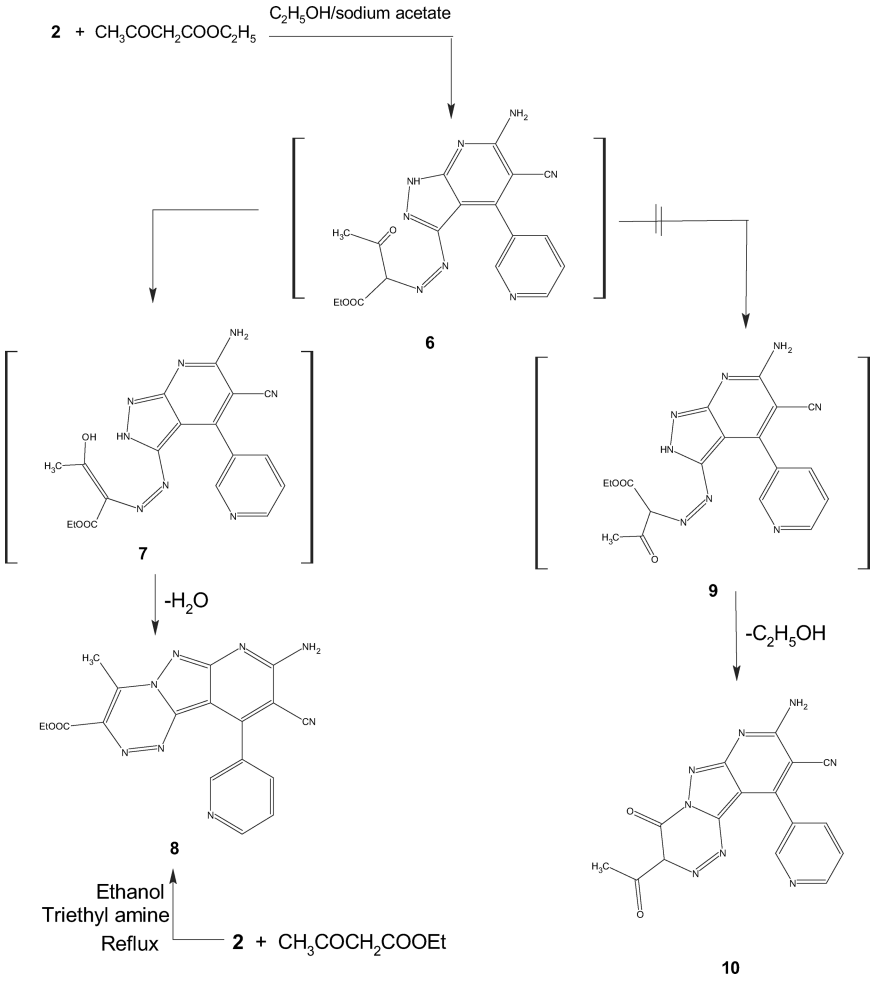
Similarly, **2** reacted with ethyl 3-oxobutanoate under the same experimental conditions to give a reaction product formed via dehydrochlorination followed by spontaneous cyclization. The ^{1}H NMR spectrum of



SCHEME 2

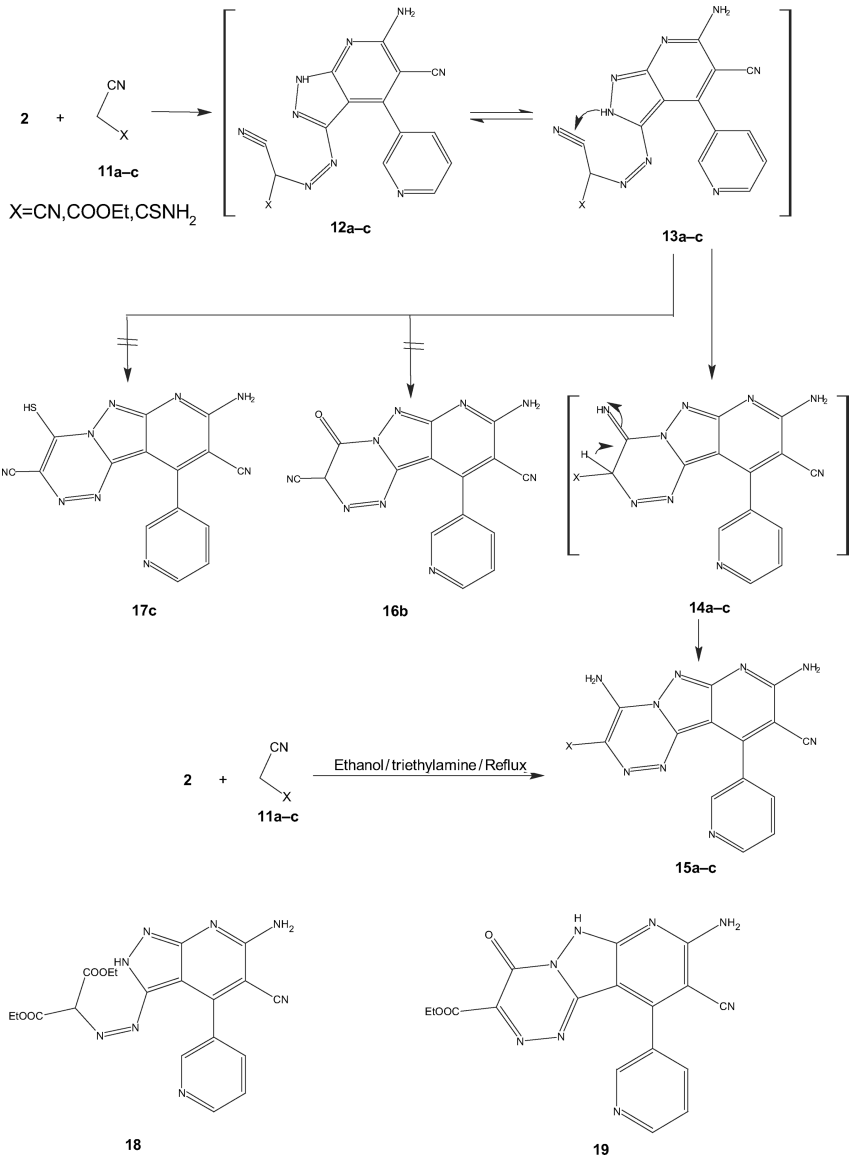
this reaction product revealed 1.9 (t, 3H, J = 7.1, $COOCH_2\underline{CH_3}$) and 4.2 (q, 2H, J = 7.1, $COO\underline{CH_2}CH_3$), and this confirmed the presence of $COOCH_2CH_3$. Therefore, this reaction product formed via water-molecule removal, not by ethanol molecule removal, so it was represented as **8**, not as **10**. Moreover, the mass spectrum of this reaction product gave m/z = 374, which corresponded to the molecular weight of the molecular formula $C_{18}H_{14}N_8O_2$ of the assigned structure **8** (cf. Scheme 3). Considering the previously mentioned data in addition to elemental analyses data, the structure **10** rolled out. Authentically, both **5** and **8** were obtained through the reaction of **2** with 2,4-pentandione and ethyl 3-oxobutanoate, respectively, in ethanol containing a catalytic amount of triethyl amine under reflux for 4–6 h.

The synthetic potential of **2** was further investigated through its reaction with malononitrile, ethyl cyanoacetate, and 2-cyanoethanethioamide **11a–c** to afford the reaction products formed via dehydrochlorination. The IR (cm^{-1}) of these reaction products showed in respective manner the bands of NH_2, CN, CO ester, and CS function, while the ^1H NMR (δppm) in the case of ethyl cyanoacetate revealed signals of $COOCH_2CH_3$ as triplet and quartet signals. Moreover, their mass spectra gave m/z = 328, 375, and 362, respectively, and these corresponded to the molecular weights of the molecular formulas $C_{15}H_8N_{10}$,



SCHEME 3

$C_{17}H_{13}N_9O_2$, and $C_{15}H_{10}N_{10}S$ of the assigned structures **15a–c** (cf. Scheme 4). It is important to report that the structures of **15a–c** formed through the intramolecular cyclization via the CN groups in **11a–c** and the proton of the pyrazole ring in **2** involving the non-isolable intermediates **13a–c** and **14a–c**. Depending on the data of IR, ^{1}H NMR, and mass spectra, both **16b** given from the reaction of **2** with **11b**; **17c** given from the reaction of **2** and **11c** was rejected. Authentic samples of **15a–c** were obtained via the reflux of **2** with all of **11a–c** in ethanol containing a catalytic amount of triethyl amine (cf. Scheme 4). Synthon **2**



SCHEME 4

also reacted with diethylmalonate in stirred ethanol containing sodium acetate at r.t. to afford structure **19** via the non-isolable intermediate **18**. The structure of **19** was established depending on the data of IR, ^{1}H NMR, and elemental analyses. Moreover, its mass spectrum gave

m/z = 376, which corresponded to the molecular weight of the molecular formula $C_{17}H_{12}N_8O_3$ of the assigned structure.

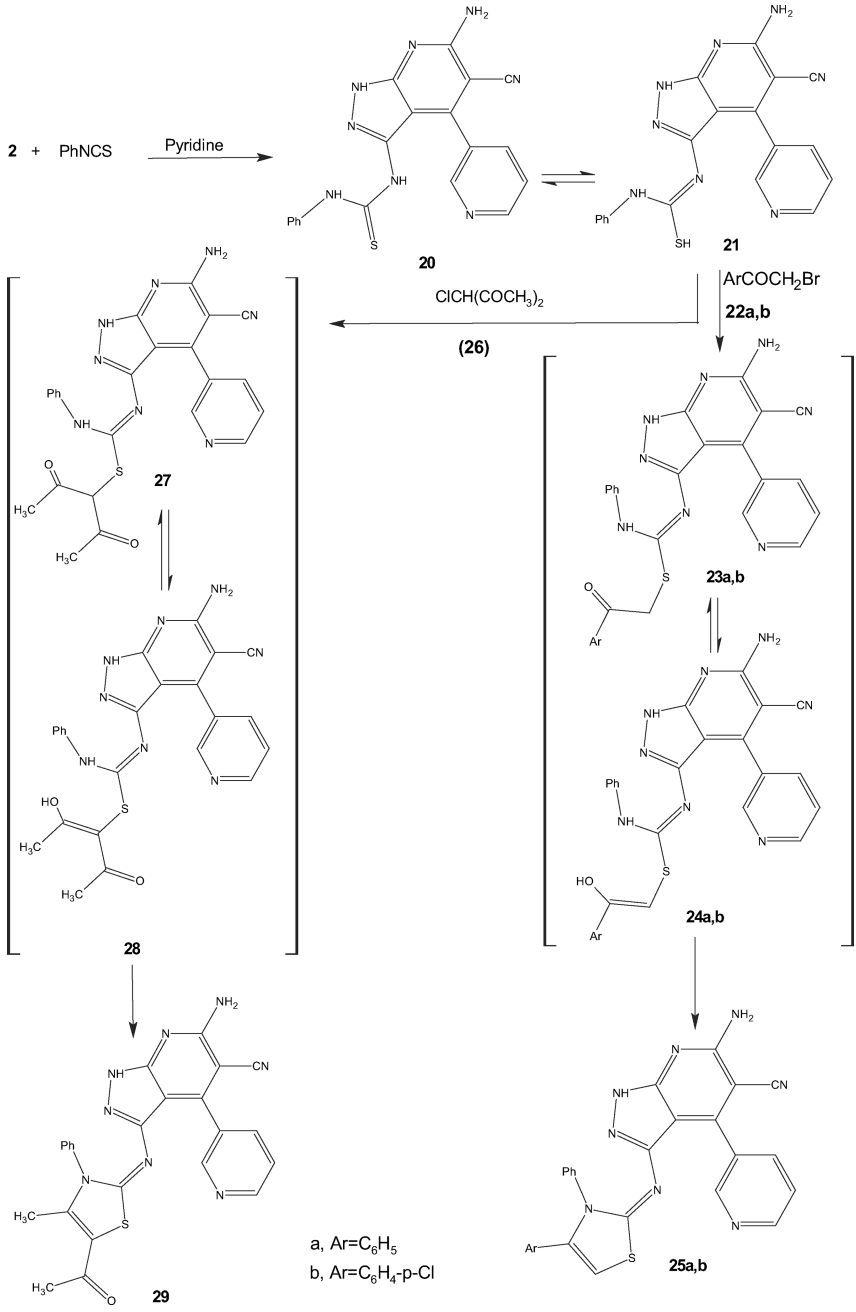
The work was extended to shed more light on the position and chemical reactivity of NH_2 in compound **2**. Thus, it has been found that **2** reacted with phenylisothiocyanate in pyridine to give a reaction product that formed through the addition of the NH_2-proton of **2** on the —N=C- bond of phenylisothiocyanate. Considering the data of IR, ^{1}H NMR, and elemental analyses, the structure of this reaction product was elucidated (cf. Experimental section). Moreover, its mass spectrum gave m/z = 386, which corresponded to the molecular weight of the molecular formula $C_{19}H_{14}N_8S$ of the assigned structure, so it formulated as 1-(6-amino-5-cyano-4-pyridin-3-yl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-3-phenylthiourea **20**. In addition to the previously mentioned physical tools, chemical reactions were used to elucidate the structure of this reaction product. Thus, it has been found that **20** reacted with 2-bromo-1-arylethanones **22a,b** in ethanol containing a catalytic amount of triethyl amine to afford the corresponding triazole derivatives **25a,b**, whose structures were elucidated based on the data of IR, ^{1}H NMR, and elemental analyses (cf. Experimental section). Moreover, their mass spectra gave m/z = 486 and 521, which corresponded to the molecular weights of the molecular formulas $C_{27}H_{18}N_8S$ and $C_{27}H_{17}N_8SCl$ of the assigned structures (cf. Scheme 5 and the Experimental section).

Also, **20** reacted with 3-chloropentan-2,4-dione (**26**) under the same mentioned experimental conditions to give a reaction product formed through dehydrochlorination. The IR spectrum of this reaction product showed bands or NH, NH_2, CN, and CO acetyl function, and its ^{1}H NMR revealed signals of CH_3, CH_3CO, NH, NH_2, and pyridine protons. Moreover, its mass spectrum gave m/z = 451, which corresponded to the molecular weight of the formula $C_{24}H_{17}N_7OS$ of the assigned structure **29** (cf. Experimental section and Scheme 5). In further investigation, compound **20** reacted with ethyl 2-chloro-3-oxo-butanoate (**30**) under similar experimental conditions to afford **31**, which tautomerized to the more stable **32** whose structure was established by considering the data of IR, ^{1}H NMR, and elemental analyses (cf. Experimental section). It is important to report that **20** in solution converted to the more reactive isomer **31**.

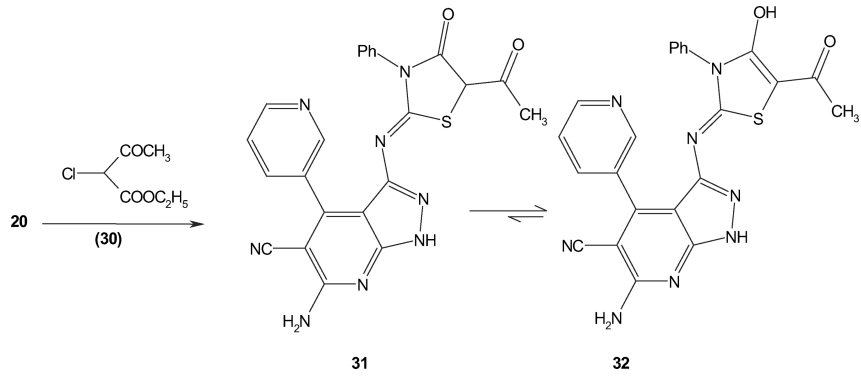
Biological Evaluation

Cytotoxicity Assay

As shown in Table I, all newly synthesized heterocyclic compounds were safe at 5, 10, 15, and 20 μg, while these compounds were less safe at doses of more than 20 μg.



SCHEME 5



SCHEME 5 ***(Continued)***

Antivirus Bioassay

Screening for Anti–HSV1 Activity

Plaque-reduction assay showed that compound **15a** showed 89% of the virus reduction at dose 25 μg, and this was considered highly promising to be an anti–HSV1 agent. Compounds **5**, **20**, and **25a** showed 62%, 71%, and 68% of virus reduction at dose 25 μg, and the other compounds showed either low or moderate anti–HSV1 activity at dose 25 μg. (Figure 1).

TABLE I Cytotoxicity Assay for the Synthetic Compound in the Vero Cell Line

	Cytotoxicity Grade of the Tested Materials (μg)							
Compound No.	5	10	15	20	25	30	35	40
5	—	—	—	—	+2	+3	+2	+3
8	—	—	—	—	+3	+3	+4	+4
15a	—	—	—	—	+2	+3	+3	+4
15b	—	—	—	—	+3	+2	+3	+4
15c	—	—	—	—	+2	+2	+2	+4
19	—	—	—	—	+1	+3	+2	+4
20	—	—	—	—	+3	+3	+4	+3
25a	—	—	—	—	+2	+3	+4	+4
25b	—	—	—	—	+2	+2	+2	+2
25c	—	—	—	—	+3	+3	+4	+4
29	—	—	—	—	+2	+1	+3	+4
32	—	—	—	—	+2	+2	+2	+2

Note: Cytotoxicity grades are divided into four grades: +1 = 25%; +2 = 50%; +3 = 75%; +4 = 100% (of the cell monolayer that showed CPE).

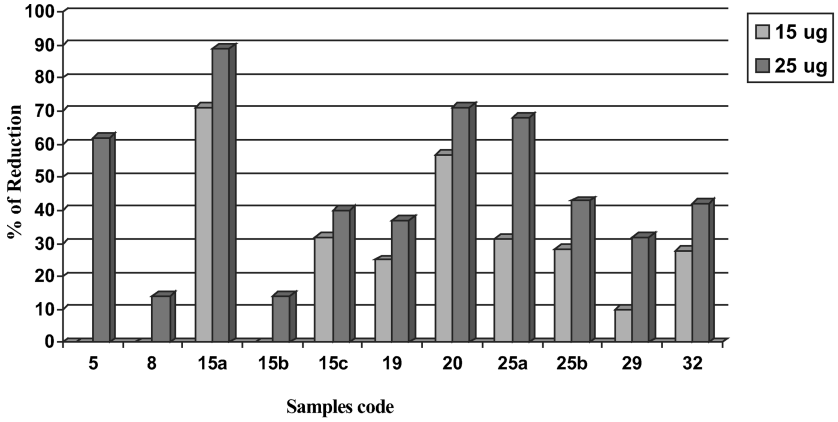


FIGURE 1 An anti–HSV1 bioassay of synthetic compounds in HepG2 cells.

Screening for Anti–HAV and MBB Strain Activity

All compounds were tested for anti–HAV activity in HepG2 cells by plaque-reduction assay. The results showed that compounds **5** and **25a,b** exhibited a moderate percentage of virus reduction at dose 25 μg, while the other newly synthesized heterocyclic compounds exhibited no activity against HAV (Figure 2).

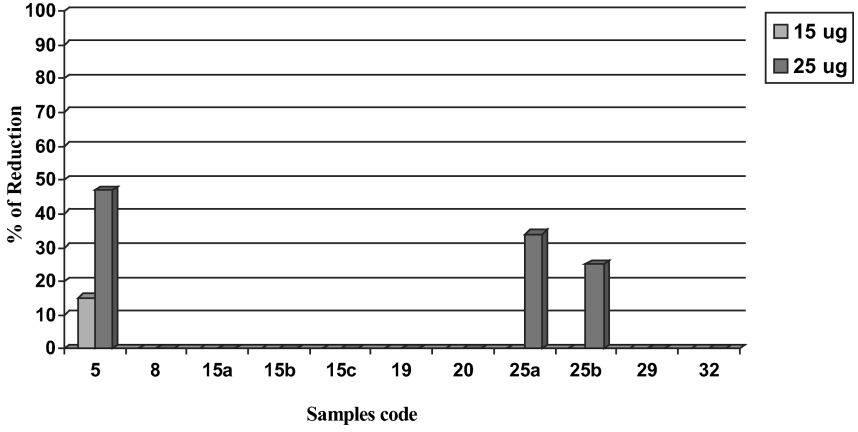


FIGURE 2 An anti–HAV bioassay of synthetic compounds in the Vero cell line.

EXPERIMENTAL

All melting points were uncorrected. IR (KBr discs) spectra were recorded on a Shimadzu FTIR-8201PC spectrophotometer. ^{1}H NMR spectra were recorded on Varian Mercury 300 MHz and Varian Gemini 200 MHz spectrometers using TMS as an internal standard and $CDCl_3$, DMSO-d_6, and $(CD_3)_2CO$ as solvents. Chemical shifts were expressed as δ (ppm) units. Mass spectra were recorded on Shimadzu GCMS-QP1000EX using an inlet type at 70 eV. The Microanalytical Center of Cairo University performed the microanalyses.

Synthesis of 5, 8, 15a–c, and 19: General Procedure

A solution of **2** (0.01 mole), prepared by stirring of 2.51 g of **1** with 2 mL of HCL followed by a dropwise addition of 0.69 g $NaNO_2$ in 5 mL of H_2O, in ethanol (50 mL) in the presence of sodium acetate (1 g) was treated with pentan-2,4-dione (1 g), ethyl 3-oxobutanoate (1.3 g), malonitrile (0.66 g), ethyl cyanoacetate (1.13 g), 2-cyanoethanethioamide (1 g; **11a–c**), and diethyl malonate (1.6 g; 0.01 mole of each), respectively. The reaction mixture was stirred in an ice-cold bath for 1 h. The solid product obtained in each case was filtered off, washed with water, and crystallized from ethanol as **5**, **8**, **15a–c**, and **19**, respectively.

3-Acetyl-8-amino-4-methyl-10-pyridin-3-ylpyrido[2′,3′:3,4]pyrazolo-[5,1-*c*][1,2,4]triazine-9-carbonitrile (5)

Crystallized from ethanol as brown crystals (68%) m.p. 300°C; **IR** (cm^{-1}): 3452, 3930, 3165 (NH_2), 3079 (pyridine-CH), 2878 (sat. CH), 2217 (CN) and 1712 (acetyl-CO), **^{1}H NMR** (δ): 1.2 (s, 3H, CH_3), 2.5 (s, 3H, $\underline{CH_3}CO$), 5.4 (s, br., 2H, NH_2) and 7.0–8.2 (m, 4H, Pyridine H's); anal. for $C_{17}H_{12}N_8O$ (344): calcd./found: C, 59.30/59.1%; H, 3.49/3.5%; N, 32.56/32.2%.

Ethyl 9-amino-8-cyano-4-methyl-10-pyridin-3-ylpyrido[2′,3′:3,4]-pyrazolo[5,1-*c*][1,2,4]triazine-3-carboxylate (8)

Crystallized from ethanol as brown crystals (59%) m.p. 300°C; **IR** (cm^{-1}): 3452, 3386, 3167 (NH_2), 3052 (pyridine-CH), 2874 (sat. CH), 2213 (CN) and 1718 (ester-CO), **^{1}H NMR** (δ): 1.1 (s, 3H, CH_3), 1.9 (t, 3H, J = 7.1, $COOCH_2\underline{CH_3}$), 4.3 (q, 2H, J = 7.1, $COO\underline{CH_2}CH_3$), 5.3 (s,

br., 2H, NH_2) and 7.1–8.5 (m, 4H, Pyridine H's); anal. for $C_{18}H_{14}N_8O_2$ (374): calcd./found: C, 57.75/57.4%; H, 3.74/3.9%; N, 29.95/30.1%.

4,8-Diamino-10-pyridin-3-ylpyrido[2′,3′:3,4]pyrazolo[5,1-*c*][1,2,4]tri-zine-3,9-dicarbonitrile (15a)

Crystallized from ethanol as pale brown crystals (68%) m.p. 300°C; IR (cm^{-1}): 3453, 3351, 3177(NH_2), 3069 (pyridine-CH), 2878 (sat. CH) and 2218 (CN); ^{1}H NMR (δ): 5.2 (s, br., 4H, two NH_2) and 7.2–8.4 (m, 4H PyridineH's); anal. for $C_{15}H_8N_{10}$ (328): calcd./found: C, 54.88/54.7%; H, 2.44/2.5%; N, 42.68/43.1%.

Ethyl 4,8-diamino-9-cyano-10-pyridin-3-ylpyrido[2′,3′:3,4]pyrazolo[5,1-*c*]-[1,2,4]triazine-3-carboxylate (15b)

Crystallized from ethanol as brown crystals (72%) m.p. 300°C; IR (cm^{-1}): 3442, 3335, 3145 (NH_2), 3065 (pyridine-CH), 2874 (sat. CH), 2209 (CN) and 1719 (ester-CO); ^{1}H NMR (δ): 1.4 (t, 3H, J = 7.2, $COOCH_2\underline{CH_3}$), 4.2 (q, 2H, J = 7.2, $COO\underline{CH_2}CH_3$), 5.6 (s, br., 4H, two NH_2) and 7.0–8.4 (m, 4H, PyridineH's); anal. for $C_{17}H_{13}N_9O_2$ (375): calcd./found: C, 54.40/54.4%; H, 3.47/3.6%; N, 33.60/33.9%.

4,8-Diamino-3-thiocarboxamido-10-pyridin-3-ylpyrido[2′,3′:3,4]pyrazolo-[5,1-*c*][1,2,4]triazine-9-carbonitrile (15c)

Crystallized from ethanol as black crystals (60%) m.p. 300°C; **IR** (cm^{-1}): 3450, 3312, 3159 (NH_2), 3048 (pyridine-CH), 2877 (sat. CH), 2213 (CN) and 1552 (CS), **^{1}H NMR** (δ): 5.6 (m, 6H, three NH_2) and 7.2–8.5 (m, 4H, Pyridine H's); anal. for $C_{15}H_{10}N_{10}S$ (362): calcd./found: C, 49.72/50.0%; H, 2.76/2.5%; N, 38.67/37.4%; S, 8.84/8.7%.

Ethyl 9-amino-8-cyano-4-oxo-10-pyridin-3-yl-3,4-dihydropyrido[2′,3′:3,4]-pyrazolo[5,1-*c*][1,2,4]triazine-3-carboxylate (19)

As pale dark brown crystals (67%) m.p. °C; **IR** (cm^{-1}): 3422, 3325, 3198 (NH_2 and NH), 3069 (pyridine-CH), 2867 (sat. CH), 1721 (ester-CO) and 1665 (ring-CO); **^{1}H NMR** (δ): 1.5 (t, 3H, $COOCH_2\underline{CH_3}$), 4.2 (q, 2H, $COO\underline{CH_2}CH_3$), 4.5 (s, br., 1H, NH), 5.5 (s, br., 2H, NH_2) and

7.0–8.4 (m, 4H, PyridineH's); anal. for $C_{17}H_{12}N_8O_3$ (376): calcd./found: C, 54.26/54.4%; H, 3.19/3.3%; N, 29.79/30.4%.

The Synthesis of 20

A solution of **2** (0.01 mole), prepared by stirring of 2.51 g of **1** with 2 mL of HCL followed by a dropwise addition of 0.69 g $NaNO_2$ in 5 mL of H_2O, in pyridine (35 mL) was treated with phenyl isothiocyanate 1.35 g (0.01 mole). The reaction mixture was heated under reflux for 5 h and then cooled, poured onto ice-cold water, and acidified with diluted HCl. The solid product formed was filtered off, washed with water, and crystallized from ethanol to give **20**.

1-(6-Amino-5-cyano-4-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridin-3-yl)-3-phenylthiourea (20)

Crystallized from ethanol as brown crystals, (77%), m.p. 150–152°C; **IR** (cm^{-1}): 3422, 3277, 3205 (NH_2 and NH), 3042 (pyridine-CH), 2976 (sat. CH) and 2215 (CN); **^{1}H NMR** (δ): 4.3 (s, br., 2H, NH_2), 5.5–6.2 (m, 3H, three NH) and 7.2–8.8 (m, 9H, ArH's and Pyridine H's); anal. for $C_{19}H_{14}N_8S$ (386): calcd./found: C, 59.07/60.0%; H, 3.63/3.8%; N, 29.02/30.1%; S, 8.29/8.1%.

The Synthesis of 25a,b

A solution of **20** (1.93 g, 0.005 mole) in ethanol (50 mL) in the presence of sodium acetate (1 g) was heated under reflux with 2-bromo-1-phenylethanone (0.99 g, 0.005 mole) and 2-bromo-1-(4′-chlorophenyl)ethanone (1.16 g, 0.005 mole) **22a,b** for 5 h. After cooling, the solids that formed were filtered off, washed with water, and crystallized with ethanol to give **25a,b** respectively.

6-Amino-3[(3,4-diphenyl-1,3-thiazol-2(3H)-ylidene]amino]-4-pyridin-3-yl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (25a)

Crystallized from ethanol as yellow crystals (56%) m.p. 190–192°C; **IR** (cm^{-1}): 3422, 3214, 3142 (NH_2 and NH), 3069 (aromatic-CH), 2873 (sat. CH) and 2215 (CN); **^{1}H NMR** (δ): 4.0 (s, br., 2H, NH_2), 5.3 (s, 1H, thiazole H-5), 5.9 (s, 1H, NH) and 7.1–8.2 (m, 14H, ArH's and Pyridine H's); anal. for $C_{27}H_{18}N_8S$ (486): calcd./found: C, 66.67/66.8%; H, 3.70/3.7%; N, 23.05/23.2%; S, 6.58/6.4%.

6-Amino-3[(3-phenyl-4-(4-chlorophenyl)-1,3-thiazol-2(3H)-ylidene]-amino]-4-pyridin-3-yl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (25b)

Crystallized from ethanol as yellow crystals (61%) m.p. 182–184°C; IR (cm^{-1}): 3400, 3250, 3150 (NH_2 and NH), 3077 (aromatic-CH), and 2213 (CN); **^{1}H NMR** (δ): 4.2 (s, br., 2H, NH_2), 5.3 (s, 1H, thiazole H-5), 6.1 (s, 1H, NH) and 7.1–8.4 (m, 13H, ArH's and Pyridine H's); anal. for $C_{27}H_{17}N_8SCl$ (521): calcd./found: C, 62.19/62.3%; H, 3.26/3.3%; N, 21.50/22.0%; S, 6.14/6.1%; Cl, 6.81/6.9.

The Synthesis of 29

A solution of **20** (1.93 g, 0.005 mole) in ethanol (50 mL) in the presence of sodium acetate (1 g) was heated under reflux with 3-chloropentan-2,4-dione **26** (0.66 g, 0.005 mole) for 5 h. After cooling, the solid that formed was filtered off, washed with water, and crystallized with ethanol to give **29**.

3-{5-Acetyl-3-phenyl-4-methyl-1,3-thiazol-2(3H)-ylidine]amino}-6-amino-4-pyridin-3-yl-1H-pyrazolo[3,4-b]pyridin-5-carbonitrile (29)

Crystallized from ethanol as yellow crystals (69%) m.p. 260–262°C; **IR** (cm^{-1}): 3420, 3240, 3128 (NH_2 and NH), 3077 (aromatic-CH), 2869 (sat. CH) 2214 (CN) and 1701 (acetyl-CO); **^{1}H NMR** (δ): 1.2 (s, 3H, CH_3), 2.4 (s, 3H, CH_3CO), 4.3 (s, br., 2H, NH_2), 6.1 (s, 1H, NH) and 7.1–8.3 (m, 9H, ArH's and Pyridine H's); anal. for $C_{24}H_{18}N_8OS$ (466): calcd./found: C, 61.80/62.1%; H, 3.86/4.0%; N, 24.03/25.1%; S, 6.87/6.7%.

The Synthesis of 32

A solution of **20** (1.93 g, 0.005 mole) in ethanol (50 mL) in the presence of sodium acetate (1 g) was heated under reflux with ethyl 2-chloro-3-oxobutanoate (0.82 g, 0.005 mole) for 5 h. After cooling, the solid that formed was filtered off, washed with water, and crystallized with ethanol to give **32**.

3-[(5-Acetyl-4-hydroxy-3-phenyl-1,3-thiazol-2(3*H*)-ylidene)amino]-6-amino-4-pyridin-3-yl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (32)

Crystallized from ethanol as pale yellow crystals (69%) m.p. 220–222°C; **IR** (cm^{-1}): 3450, (OH), 3452, 3230 and 3180 (NH_2 and NH), 3059 (aromatic-CH), 2978 (sat. CH), 2218 (CN) and 1704 (acetyl CO); **^{1}H NMR** (δ): 2.3 (s, 3H, $\underline{CH_3}CO$), 4.2 (s, br., 2H, NH_2), 5.2 (s, 1H, OH) 6.4 (s, br., 1H, NH) and 7.0–8.1, (m, 9H, ArH's and Pyridine H's); anal. for $C_{23}H_{16}N_8OS$ (452): calcd./found: C, 61.06/61.1%; H, 3.54/3.5%; N, 24.78/25.1%; S, 7.08/7.2%.

Biological Evaluation

Two types of cell lines were used for the propagation of Herpes Simplex Virus type 1 (HSV-1) and hepatitis A Virus (HAV-MBB strain). These cell lines: African geen monkey kidney cells (Vero) and human hepatoma cell line (Hep G2).

Viruses

Two models of DNA and RNA viruses were used for the bioassay. These viruses were Herpes simplex virus type 1 (HSV-1) and Hepatitis A virus, MBB strain (HAV-MBB)

Media and Supplements

Cell Culture Medium

Minimum Essential Media (MEM with Hank's balanced salt solution, GIBCO-BRL) was prepared and sterilized by filtration through a 0.22-μm pore-size nitrocellulose membrane. The pH value was adjusted at 7.4 by sodium bicarbonate.

Foetal Bovine Serum: (Sigma)

Foetal Bovine Serum was inactivated at 56°C for 30 min and used at a 10% final concentration for a growth medium and at 2% for a maintenance medium.

Antibiotic–Antimycotic Mixture (GIBCO-BRL)

100 X antibiotic–antimycotic mixtures consisted of 10,000 U of penicillin G sodium, 10,000 μg, of streptomycin sulfate, and 25 μg of amphotericin B.

Cell Dissociation Solution (Trypsin–Versene Mixture)

Phosphate-buffered Saline

(PBS, pH 7.5, 0.15 M) The buffer was prepared at the following concentrations: NaCl (8.9 g/L), KCl (0.2 g/L), KH_2PO_4 (0.12 g/L), Na_2HPO_4 (0.91 g/L), Deionized H_2O (up to 1 L). Ingredients were mixed gently in the order previously shown, and the pH value was adjusted at 7.5. The buffer was sterilized by filtration through a 0.22-μm nitrocellulose membrane. The solution was used in washing cell monolayer sheets and in preparing a cell dissociation solution as follows.

Trypsin 1:250 (Sigma)

1.5 gm of trypsin powder (1:250) was disolved in 500 mL of PBS and digested at 4°C overnight with stirring.

Versene Solution (0.04%)

Tetrasodium salt of ethylenediamine tetraacetic acid was dissolved in 500 mL of 0.15 M of PBS (pH 7.5) to prepare a 2-mM solution (0.04 g), which was mixed with an equal volume of trypsin solution. The pH value of the trypsin-versene mixture was adjusted to 8.4 by 7.5% sodium bicarbonate solution and sterilized by filtration through a 0.22-μm pore-size nitrocellulose membrane, and the mixture was aliquoted and stored at −20°C until used.

Methods

The Preparation of Synthetic Compounds for Bioassay

Compounds were dissolved as 100 mg each in 1 mL of 10% DMSO in water. The final concentration was 100 μg/μL (stock solution). The dissolved stock solutions were sterilized by the addition of an antibiotic-antimycotic mixture: 10,000 U penicillin G sodium or Gentamicin (50 μg/mL), 10,000 μg streptomycin sulfate, and 250 μg of amphotericin B. Sterility tests were carried out in a nutrient agar.

Cell Culture

African green monkey kidney-derived cells (VERO) were used. The cells were propagated in Hanks' MEM and supplemented with 10% Foetal bovine serum, and 1% antibiotic-antimycotic mixture. The pH was adjusted to 7.2–7.4 by 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through a 0.2-μm pore-size nitrocellulose membrane.

Viruses

Herpes Simplex virus type 1 was obtained from Environmental Virology Laboratory, Department of Water Pollution Res., National Research Centre, Dokki, Egypt.

Antiviral Assay

Cytotoxicity Assay. Cytotoxicity was assayed for both dimethyl sulfoxide (DMSO) and the test compounds. Serial dilutions were prepared and inoculated on Vero and HepG2 cells grown in 96 well tissue culture plates. The maximum tolerated concentration for each compound was determined by both cell morphology and cell viability by straining with trypan blue dye.

Plaque Reduction Assay. A six-well plate was cultivated with Vero cell culture (10^5 cell/mL) and incubated for 2 days at 37°C. HSV-1 was diluted to give 10^4 PFU/mL as a final concentration, mixed with the plant extract at the previous concentration, and incubated overnight at 4°C. The growth medium was removed from the multiwell plate, and the virus-compound mixture was inoculated (100 μL/well). After 1 hr of contact time, the inoculum was aspirated, and 3 mL of MEM with 1% agar rose was overlaid the cell sheets. The plates were left to solidify and incubated at 37°C until the development of virus plaques. Cell sheets were fixed in 10% formaline solution for 2 h and stained with crystal violet stain. Control virus and cells were treated identically without chemical compounds. Virus plaques were counted, and the percentage of reduction was calculated.

REFERENCES

[1] F. A. Attaby, A. H. H. Elghandour, M. A. Ali, and Y. M. Ibrahem, *Phosphorus, Sulfur, and Silicon*, **181,** 1 (2006).
[2] F. A. Attaby, M. A. Ali, A. H. H. Elghandour, and Y. M. Ibrahem, *Phosphorus, Sulfur, and Silicon*, **181,** 1087 (2006).
[3] F. A. Attaby, S. M. Eldin, M. A. A. Elneairy, and A. K. K. Elouh, *Phosphorus, Sulfur, and Silicon*, **179,** 2205 (2004).
[4] F. A. Attaby, A. H. H. Elghandour, H. M. Mustafa, and Y. M. Ibrahem, *J. C. C. S.* (China), **49,** 561 (2002).
[5] F. A. Attaby, H. M. Mustafa, A. H. H. Elghandour, and Y. M. Ibrahem, *Phosphorus, Sulfur, and Silicon*, **177,** 2753 (2002).
[6] F. A. Attaby, M. A. A. Elneairy, S. M. Eldin, and A. K. K. El-Louh, *J. C. C. S.* (China), **48,** 893 (2001).
[7] M. A. A. Elneairy, F. A. Attaby, and M. S. Elsayed, *Phosphorus, Sulfur, and Silicon*, **167,** 161 (2000).
[8] M. A. A. Elneairy, S. M. Eldin, F. A. Attaby, and A. K. K. El-Louh, *Phosphorous, Sulfur, and Silicon*, **167,** 289 (2000).

[9] F. A. Attaby and A. M. Abdel-Fattah, *Phosphorous, Sulfur, and Silicon*, **155,** 253 (1999).
[10] F. A. Attaby, M. A. A. Elneairy, and M. S. Elsayed, *Phosphorous, Sulfur, and Silicon*, **149,** 230 (1999).
[11] F. A. Attaby, S. M. Eldin, and M. A. A. Elneairy, *J. Chem. Res. (M)*, **10,** 2754, (1998); *(S)* **10** (1998).
[12] F. A. Attaby, S. M. Eldin, and M. A. A. Elneairy, *Heteroatom Chemistry*, **9,** 571 (1998).
[13] F. A. Attaby, *Phosphorous, Sulfur, and Silicon*, **139,** 1 (1998).
[14] F. A. Attaby, *Phosphorous, Sulfur, and Silicon*, **126,** 27 (1997).
[15] F. A. Attaby and A. M. Abdel-Fattah, *Phosphorous, Sulfur, and Silicon*, **119,** 257 (1996).
[16] F. A. Attaby, S. M. Eldin, and M. Abdel Razik, *Phosphorous, Sulfur, and Silicon*, **106,** 21 (1994).
[17] F. A. Attaby, L. I. Ibrahim, S. M. Eldin, and A. K. K El-louh, *Phosphorous, Sulfur, and Silicon*, **73,** 127 (1992).
[18] F. A. Attaby, *Arch. Pharmcol. Res.*, **13,** 342 (1990).
[19] D. R. Rao, S. P. Raychaudhuri, and V. S. Verma, *International Journal of Tropical Plant Diseases*, **12,** 177 (1994).
[20] F. Szurdki, L. Jaerger, A. Harris, H. Kido, I. Wengatz, and M. H. Goodrow, *Journal of Environmental Science and Health Part B*, **31,** 451 (1996).
[21] I. F. F. Benzie and J. J. Strain, *Anal. Biochem.*, **15,** 239 (1996).
[22] P. G. Baraldi, B. Cacciari, A. Dalpiaz, and S. Dionisotti, *Arzneium-Forsch*, **46,** 365 (1996).
[23] B. R. Tolf, R. Dahlbom, H. Theroell, and A. Akeson, *Acta Chem. Scand., Ser. B.*, **36,** 101 (1982).
[24] M. Komuro, R. Ishida, and H. Uchida, *Arzneium-Forsch*, **42,** 48 (1992); *C. A.*, **116,** 98851q (1992).
[25] W. Wieniowski, *Roczniki Chem.*, **32,** 545 (1958); *C. A.*, **53,** 1416 (1959).
[26] F. C. Brown and C. K. Bradsher, *Nature*, **168,** 171 (1951).
[27] A. A. Abou-Zeid and M. Y. Shata, *Indian J. Pharm.*, **31,** 72 (1969).
[28] F. A. Attaby, M. A. Ali, A. H. H. Elghandour, and Y. M. Ibrahem, *Phosphorus, Sulfur, and Silicon*, accepted (2006).