ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Synthesis and Antimicrobial Evaluation of Some New Heterocyclic Compounds from Thienopyridine and Pyrazolopyridine Derivatives

Nora M. Rateb, Shiamaa H. Abdelaziz and Hussein F. Zohdi*
Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt.

Manuscript Info

Manuscript History:

Received: 14 November 2013
Final Accepted: 24 December 2013
Published Online: January 2013

Key words:
Antimicrobial evaluation, pyrazolopyridine, pyridothienopyrimidine, thienopyridine, triazine, ylidene exchange.

Abstract

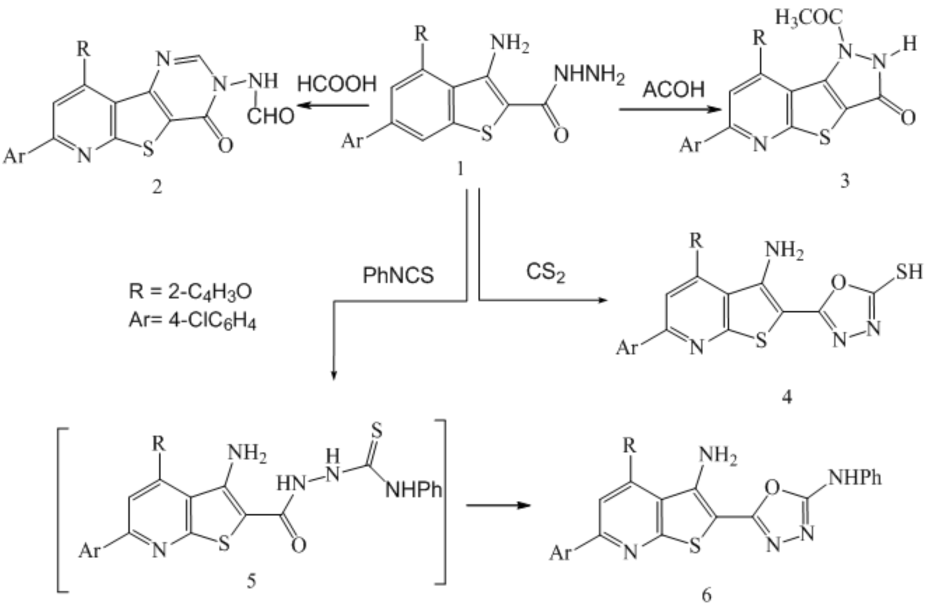
Synthesis and antimicrobial activities of a variety of functionalized pyrido[3`,2`:4,5]thieno[3,2-d]pyrimidine, pyrazolo[3`,2`:4,5]thieno[2,3-b]pyridin-3-one and thieno[2,3-b]pyridine derivatives are illustrated. Structures of the newly synthesized compounds were elucidated based on their elemental analyses, spectral data and alternative synthesis whenever possible.

Introduction

The reported biological activities of thienopyridines[1,2] as well as pyrazolopyridines[3,4] stimulated our interest to investigate the reactions of such ring systems with different reagents for the synthesis of several new heterocyclic derivatives with anticipated biological activities. Thienopyridines are of special importance due to their biological activities such as antimicrobial[5-7], anti-inflamatory[8] and neurotropic activity[9]. Also, pyrazolopyridines are a very interesting class of heterocyclic compounds because of their significant and versatile biological and pharmacological activities such as antimalarial[10], antiproliferative[11], cardiovascular[12-14], and antileishmanial[15] activities. Thus, in continuation of our interest in the synthesis of heterocyclic compounds with expected biologically activity [16-21], we report herein a convenient routes for the synthesis of some novel heterocyclic compounds, utilizing thienopyridine and pyrazolopyridine derivatives.

Results and Discussion

It has been found that the reaction of 3-amino-6-(4-chlorophenyl)-(4-furan-2-yl)-thieno[2,3-b]pyridine-2-carbohydrazide **(1)** [19] with boiling formic acid gave the corresponding derivative **2**. The IR spectrum of **2** showed absorption bands for carbonyl and aldehydic groups at 1678 cm^{-1} and 1705 cm^{-1}, respectively. The mass spectrum of **2** showed the molecular ion peak at m/z 422. The above mentioned data along with elemental analysis confirmed the proposed structure **2**. Similarly, 1-acetyl-6-(4-chlorophenyl)-8-furan-2-yl-1,2-dihydro-pyrazolo[3`,4`:4,5]-thieno[2,3-b]pyrimidin-3-one derivative **(3)** was obtained *via* treatment of **1** with glacial acetic acid under reflux. The structure of the latter compound was elucidated based on the elemental analysis and spectral data, (Scheme 1).

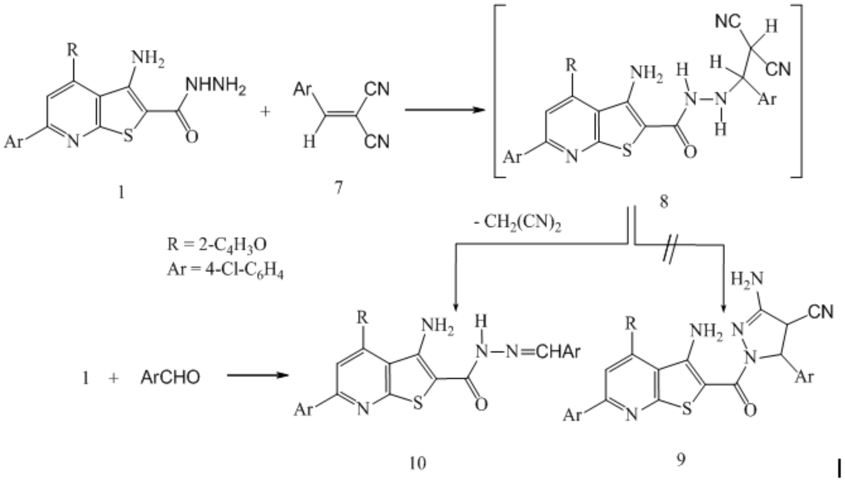


Scheme 1: Reactions of 1 with some sulfur compounds

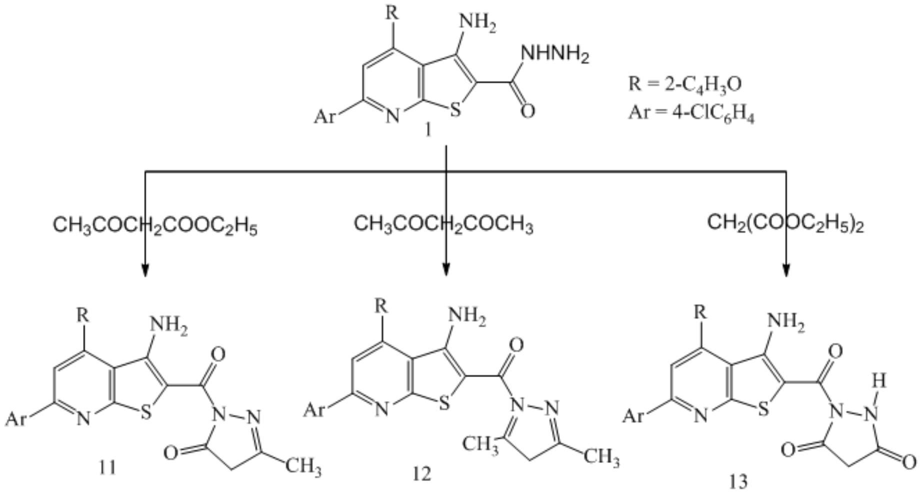
Compound **1** reacted with sulfur containing compounds, such as carbon disulphide and phenylisothiocyanate to synthesize new heterocyclic derivatives such as 5-[3-amino-6-(4-chlorophenyl)-4-(furan-2-yl)-thieno[2,3-b]pyridin-2-yl-][1,3,4] oxadiazole-2-thiol **(4)** and 6-(4-chlorophenyl)-4-(furan-2-yl)-2-(5-phenylamino-[1,3,4]oxadiazol-2-yl)-thieno[2,3-b]pyridin-3-ylamine**(6)**, respectively. Structure **4** was confirmed based on its spectral data and elemental analysis. Thus, its IR spectrum showed absorption bands at 3467, 3348, 2954 cm^{-1} for NH_2 and SH groups and its mass spectrum showed its molecular ion at m/z 426. Also, compound **1** reacted with phenyl isothiocyanate to give the corresponding 6-(4-chlorophenyl)-4-furan-2-yl-2-(5-phenylamino-[1,3,4]-oxadiazol-2-yl)-thieno[2,3-b]pyridine-3-ylamine derivative **6** *via* loss of hydrogen sulfide through the formation of the intermediate **5**, (Scheme 1). Structure **6** was elucidated based on its elemental analysis and spectral data.

On the other hand, compound **1** reacted with 2-(4-chlorobenzylidene)malononitrile (**7**) to afford the corresponding N`-(4-chlorobenzylidene)-3-amino-6-(4-chlorophenyl)-4-(furan-2-yl)thieno[2,3-b]pyridine-2-carbohydrazide derivative **10**. The IR spectrum of **10** showed absorption bands of NH and NH_2 at 3483, 3309, 3244 cm^{-1} and hydrazidic carbonyl at 1645 cm^{-1}. The mass spectrum of **10** showed its molecular ion peak at m/z 506 which, along with elemental analysis confirmed the corresponding structure **10**. The reaction seemed to proceed via ylidene exchange with the elimination of one molecule of malononitrile. This mechanism was confirmed by the preparation of **9** by alternative route through the reaction of **1** with p-chlorobenzaldehyde and was found to be identical in all aspects (m. p., mixed m. p., TLC), (Scheme 2).

Scheme 2: Reaction of compound 1 with cinnamonitrile derivatives.



Compound **1** reacted also with different β-dicarbonyl compounds to afford 2-(pyrazol-2-yl)-thieno[2,3-b]pyridine derivatives **11**, **12** and **13**, respectively (Scheme 3).

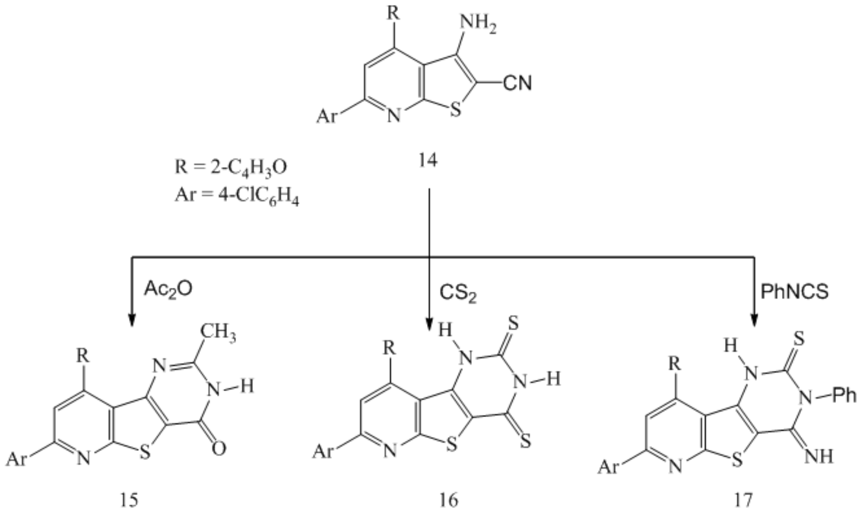


Scheme 3: Reaction of 1 with different β-dicarbonyl compounds

Thus, compound **1** reacted with ethyl acetoacetate in boiling glacial acetic acid to give the corresponding derivative **11**. The IR spectrum of the reaction product showed absorption bands at 3321, 3128 cm^{-1} for the NH_2 and 1674, 1612 cm^{-1} for CO and (C=N) groups. Its ^1H NMR revealed signals at δ 2.81 (s, 3H, CH_3), 5.22 (s, 2H, CH_2), 6.65 (s, 1H, 5H thienopyridine), 7.51-8.45 (m, 7H, Ar`H), 11.2 (b, 2H, NH_2). Based on the previously obtained data, the reaction product was formulated as 2-[3-amino-6-(4-chlorophenyl)-4-furan-2-yl-thieno[2,3-b]pyridine-2-carbonyl]-5-methyl-2,4-dihydro-pyrazol-3-one (**11**). In the same manner, compound **1** reacted with acetylacetone and diethyl malonate to give the corresponding 2-(pyrazol-2-yl)thieno[2,3-b]pyridine derivatives **12**, **13**, respectively (Scheme 3).

On the other hand, compound **14** [19] reacted with acetic anhydride under reflux for 6 hours to give 7-(4-chloro-phenyl)-9-furan-2-yl-2-methyl-3H-pyrido[3`,2`:4,5]thieno[3,2-d]pyrimidin-4-one (**15**). The IR spectrum of the reaction product showed no absorption band for cyano group and revealed the appearance of the absorption bands for the carbonyl group (CO) and NH groups at 1654 and 3379 cm^{-1}. ^1H NMR spectrum of this product showed signals at δ 2.28 **(**s, 3H, CH_3), δ 6.61 (s, 1H, 5-H of thienopyridine ring), δ 7.27-8.09 (m, 7H, Ar' H) and 10.45 (s, 1H, NH).

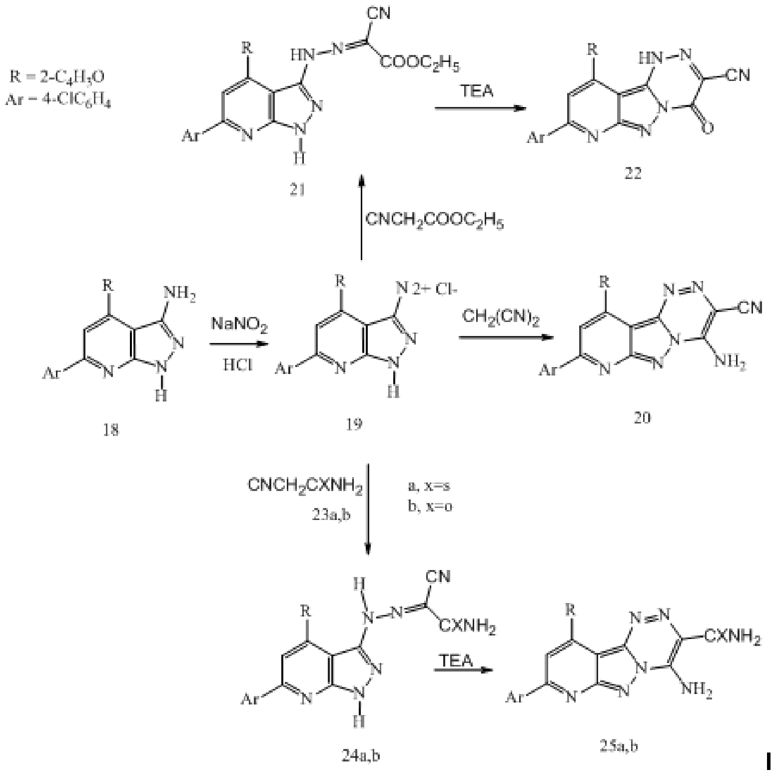
Also, compound **14** reacted with each of carbon disulfide and phenyl isothiocyanate to give compounds **16** and **17** respectively. Structures **16** and **17** were elucidated on the basis of elemental analysis and spectral data. The IR spectrum of compound **16** showed no absorption band for cyano group and the appearance of absorption bands for NH groups at 3199, 3066 cm^{-1}. The mass spectrum of this product showed molecular ion peak at m/z 427 which, along with elemental analysis, confirmed the corresponding structure **16**, (Scheme 4).



Scheme 4: Synthesis of some pyridothienopyrimidine derivatives.

Diazonium chloride **19;** obtained by diazotization of 6-(4-chlorophenyl)-4-furan-2-yl-1H-pyrazolo[3,4-b]pyridin-3-ylamine (**18**) [19]; was coupled with malononitrile to give the triazine derivative **20**. The IR spectrum of **20** revealed absorption bands at 3394, 3236, 2229 cm^{-1} due to the amino and the cyano groups respectively. Also, the mass spectrum of **20** showed a peak corresponding to the molecular ion at m/z 387. In Contrast, diazonium **19** reacted with ethyl cyanoacetate under the same conditions to give the corresponding 5-hydrazono derivative **21**. The IR spectrum of **21** revealed an absorption bands at 1720, 2133 cm^{-1} due to the CO ester and the cyano groups respectively. Its mass spectrum showed a peak corresponding to the molecular ion at m/z 434 which corresponds to the molecular formula $C_{21}H_{15}ClN_6O_3$. Its ^1H NMR spectrum revealed signals $\delta = 1.46$ (t, 3H, $CH_2\underline{CH_3}$), 4.52 (q, 2H, $\underline{CH_2}CH_3$), 6.87 (s, 1H H-5 pyridine), 7.47-8-77 (m, 7H, Ar\`H), 9.79 (s, 1H, NH). Compound **21** was cyclized under basic conditions to give the triazine derivative **22** (Scheme 5).

Scheme 5: Synthesis of some triazine derivatives



Also, diazonium **19** reacted with each of cyanothioacetamide and cyanoacetamide to afford compounds **24a,b** respectively, which cyclized to give the corresponding triazine derivatives **25a,b** . The IR spectrum of **24a** revealed absorption bands at 3417, 3344 for the amino group, and 2133 for the cyano group cm^{-1}. Its mass spectrum showed a peak corresponding to the molecular ion at m/z = 421.

The IR spectrum of **25a** revealed absorption bands at 3483, 3301 cm^{-1} due to the amino group and no absorption band for the (CN) group was detected. Its mass spectrum showed a peak corresponding to the molecular ion at m/z = 421, which corresponds to the molecular formula $C_{19}H_{12}ClN_7OS$. Thus, structure **25a** assigned to tricycle showed in scheme 5. Similarly, under the same conditions, triazine derivative **25b** was obtained by the reaction of cyanoacetamide with **19**, (Scheme 5).

Antimicrobial Activity

The antibacterial and antifungal activities were carried out at the Microbiology Division of the Microanalytical Center at Cairo University, using the diffusion plate method[22-24]. A bottomless cylinder containing a measured quantity (1 mL, 20 mg/mL) of the sample is placed on a plate (7 cm diameter) containing a solid bacterial medium (nutrient agar broth) or a fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the test organism. After incubation (24 hrs for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism (% inhibition = sample inhibition zone (cm)/plate diameter x 100). All measurements were done in DMSO as a solvent which has zero inhibition activity. The obtained results were compared with some reference antibiotics that were purchased from Egyptian markets. As shown in Table 1, all the tested compounds were found to exhibit moderate to high activity against both *Escherichia coli* and *Staphylococcus aureus* microorganisms with respect to the used reference tetracyclin. Compounds **3, 4, 13, 15** and **20** were found to exhibit much higher antifungul activity than the used reference Amfoterisin B.

Table 1: Antibacterial and antifungal activities of some of the synthesized compounds

Sample	***Escherichia coli Inhibition (%)***	***Staphylococcus aureus Inhibition (%)***	***Aspergillus Flavus Inhibition (%)***	***Candida albicans Inhibition (%)***
Control: DMSO	***0.0***	***0.0***	***0.0***	***0.0***
2	***36.4***	***40.0***	***0.0***	***0.0***
3	***27.5***	***30.0***	***0.0***	***52.6***
4	***36.4***	***40.0***	***62.5***	***63.2***
10	***0.0***	***0.0***	***0.0***	***0.0***
13	***36.4***	***40.0***	***0.0***	***52.6***
15	***30.3***	***33.3***	***0.0***	***52.6***
16	***30.3***	***40.0***	***0.0***	***0.0***
17	***0.0***	***33.3***	***0.0***	***0.0***
20	***33.3***	***36.7***	***0.0***	***47.5***
Tetracyclin	***33.0***	***30.0***	***-***	***--***
Amfoterisin B	***--***	***--***	***16.0***	***19.0***

Table 2: Minimum inhibitory concentration (MIC) determination

	MIC (μg/ml)
Sample	*Candida albicans*
4	198
Amphotericin B	84

Experimental

All melting point were measured on an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. The ^{1}H NMR spectra were recorded on a Varian Mercury at 300 MHz spectrometer using TMS as internal standard and DMSO-d6 as solvent and chemical shift are expressed as δ ppm units. Mass spectra were recorded on GCMS-QP 1000 EX spectrometer using inlet type at 70 ev. Elemental analyses were carried out at the Microanalytical center of Cairo University. Compound **1, 14** and **18** were prepared as previously reported [19].

Synthesis of N-[7-(4-chlorophenyl)-9-(furan-2-yl)-4-oxo-4H-pyrido[3`,2`:4,5]thieno[3,2-d]pyrimidin-3-yl-] formamide (2).

A mixture of **1** (0.38g, 1mmole) and formic acid (15 ml) was heated under reflux for 6 hours. The reaction mixture was cooled; the so formed precipitate was filtered off, washed with ethanol, dried and recrystallized from acetic acid as brown crystals (78%); m.p= 299-302^{o}C; IR (υ cm^{-1})**:** 1678, 1705 (CO), 3193 (NH); **MS(**m/z): (M+ = 422, M+2 = 424) Anal for $C_{20}H_{11}ClN_4O_3S$ (422.85); Calcd/Found (%) C (56.81/56.90), H (2.62/2.75), N (13.25/13.30), S (7.58/7.60).

Synthesis of 1-Acetyl-6-(4-chlorophenyl)-8-(furan-2-yl)-2-methyl-1,2-dihydro-pyrazolo[3`,2`:4,5]thieno[2,3-b]pyridin-3-one (3):

A mixture of **1** (0.38g, 1mmole) and acetic acid (15 ml) was heated under reflux for 5 hours. The excess solvent was evaporated, the so formed precipitate was filtered off and washed with ethanol, dried and recrystallized from acetic acid as brown crystals; (83%); m.p = 307-310^{o}C; **IR** (υ cm^{-1}): 1670, 1690 (2 CO), 3433 (NH); **^{1}H NMR** (DMSO-d6) (δ ppm): 2.67 (s, 3H, CH_3), 6.81 (s, 1H, 5-H of the pyridinethione ring), 7.58-8.38 (m, 7H, Ar`H), 11.32 (s, 1H, NH); Anal for $C_{20}H_{12}ClN_3O_3S$ (409.85) Calcd/Found (%): C (58.61/58.80), H (2.95/3.00), N (10.25/10.25), S (7.82/7.99).

Synthesis of 5-[3-Amino-6-(4-chlorophenyl)-4-(furan-2-yl)-thieno[2,3-b]pyridin-2-yl-][1,3,4] oxadiazole-2-thiol (4):

A mixture of **1** (0.38g, 1mmole) and carbon disulphide (5 ml) in pyridine (15 ml) was heated under reflux for 6 hours. The reaction mixture was cooled, poured onto ice-cold water, the so formed precipitate was filtered off, washed with ethanol, dried and recrystallized from dioxane as Brown crystals (99%); m.p = 176-180^{o}C; **IR** (υ cm^{-1})**:** 3467, 3348, 3151 (NH_2, NH); **MS(**m/z): (M+ = 426, M+2 = 428) . Anal for $C_{19}H_{11}ClN_4O_2S_2$ (426.91); Calcd/Found (%) C (53.46/53.50), H (2.60/2.75), N (13.12/13.24), S (15.02/15.00).

Synthesis of 6-(4-chlorophenyl)-4-(furan-2-yl)-2-(5-phenylamino-[1,3,4]oxadiazol-2-yl)-thieno[2,3-b]pyridin-3-ylamine (6):

A mixture of **1** (0.38g, 1mmole) and phenyl isothiocyanate (0.14g, 1mmole) in pyridine (15 ml) was heated under reflux for 6 hours. The reaction mixture was cooled, poured onto ice-cold water, the so formed precipitate was filtered off, washed with ethanol, dried and recrystallized from dioxane as brown crystals (98%); m.p. >300^{o}C; **IR** (υ cm^{-1})**:** 3213, 3151, 3035 (NH_2, NH); **MS(**m/z) : (M+ = 485, M+2 = 487) Anal for $C_{25}H_{16}ClN_5O_2S$ (485.96); Calcd/Found (%) C (61.79/61.99), H (3.32/3.35), N (14.41/14.51), S (6.60/6.80).

Synthesis of N`-(4-chlorobenzylidene)-3-amino-6-(4-chlorophenyl)-4-(furan-2-yl)thieno[2,3-b]pyridine-2-carbohydrazide (10):

A mixture of **1** (0.38g, 1mmole) and (4-chlorobenzylidene)malonitrile **(7)** (0.19g, 1 mmole) or 4-chlorobenzaldhyde (0.14g, 1 mmole) in pyridine (15 ml) and ethanol (20 ml) was heated under reflux for 2 hours. The reaction mixture was cooled, the so formed precipitate was filtered off, washed with ethanol, dried and recrystallized from acetic acid as orange crystals (92%); m.p = 250-252^{o}C; **IR** (υ cm^{-1})**:** 1602 (CO), 3483, 3309, 3244 (NH_2, NH); **MS(**m/z): (M+ = 506, M+2 = 508, M+4 = 510). Anal for $C_{25}H_{16}Cl_2N_4O_2S$ (507.39); Calcd/Found (%) C (59.18/59.30), H (3.18/3.18), N (11.04/11.10), S (6.32/6.55).

Synthesis of 11, 12 and 13: (General method)

A mixture of **1** (0.38g, 1mmole) and each of ethyl acetoacetate, acetylacetone and diethylmalonate (1 mmole) in acetic acid (15 ml) was heated under reflux for 5 hours. The excess solvent was evaporated, the so formed precipitate was filtered off, washed with ethanol, dried and recrystallized from acetic acid to give **11**, **12** and **13** respectively.

2-[3-Amino-6-(4-chlorophenyl)-4-(furan-2-yl)-thieno[2,3-b]pyridin-2-carbonyl]-5-methyl-2,4-dihydro-pyrazol-3-one (11): Brown crystals; (43%); m.p. > 300^{o}C; **IR** (υ cm^{-1}): 1674, 1612 (2 CO), 3435, 3321, 3128 (NH_2, NH); **^{1}H NMR** ($CHCl_3$) (δ ppm): 2.80 (s, 3H, CH_3), 5.20 (s, 2H, CH_2) 6.65 (s, 1H, 5-H of the pyridinethione ring), 7.51-8.45 (m, 7H, Ar`H), 11.2 (b, 2H, NH_2); Anal for $C_{22}H_{15}ClN_4O_3S$ (409.85) Calcd/Found (%): C (58.60/58.90), H (3.35/3.35), N (12.43/12.45), S (7.11/7.12).

[3-Amino-6-(4-chlorophenyl)-4-(furan-2-yl)-thieno[2,3-b]pyridin-2-yl]-(3,5-dimethyl-pyrazol-1-yl)methanone (12): Orange crystals; (50%); m.p. > 300°C; **IR** (υ cm^{-1}): 1653, (CO), 3483, 3301, (NH_2); **^{1}H NMR** ($CHCl_3$) (δ ppm): 2.34 (s, 3H, CH_3), 2.64 (s, 3H, CH_3) 6.01 (s, 1H, 5-H of the pyridinethione ring), 6.69-8.11 (m, 7H, Ar`H), 9.98 (b, 2H, NH_2); Anal for $C_{23}H_{17}ClN_4O_2S$ (448.93) Calcd/Found (%): C (61.54/61.70), H (3.82/3.90), N (12.48/12.50), S (7.14/7.15**)**.

1[3-Amino-6-(4-chlorophenyl)-4-(furan-2-yl)-thieno[2,3-b]pyridine-2-carbonyl]-pyrazolidine-3,5-dione. (13): Brown crystals (40%); m.p = 315-320°C; IR (υ cm^{-1}): 1670 (CO) 3445, 3213, 3035, (NH_2, NH); **MS(**m/z): (M+ = 452, M+2 = 454). Anal for $C_{21}H_{13}ClN_4O_4S$ (452.88); Calcd/Found (%) C (55.70/56.00), H (2.89/3.00), N (12.37/12.50), S (7.08/7.00).

Synthesis of 7-(4-Chlorophenyl)-9-(furan-2-yl0-2-methyl-3H-pyrido[3`,2`:4,5]thieno[3,2-d]pyrimidin-4-one (15):
A mixture of **14** (0.35g, 1mmole) and acetic anhydride (15 ml) was heated under reflux for 6 hours. The reaction mixture was cooled, the so formed precipitate was filtered off, washed with ethanol, dried and recrystallized from dioxane as green crystals; (43%); m.p. > 300°C; **IR** (υ cm^{-1}): 1654(CO), 3379 (NH); **^{1}H NMR** ($CHCl_3$) (δ ppm): 2.28 (s, 3H, CH_3), 6.61 (s, 1H, 5-H of the pyridinethione ring), 7.27-8.90 (m, 7H, Ar`H), 10.45 (s, 1H, NH); Anal for $C_{20}H_{12}ClN_3O_2S$ (393.85) Calcd/Found (%): C(60.99/61.00), H(3.07/3.10), N(10.67/10.80), S(8.14/8.30).

Synthesis of 16 and 17:
A mixture of **14** (0.35g, 1mmole) with each of carbon disulphide and phenyl isothiocyanate (5 ml) in pyridine (15 ml) was heated under reflux for 6 hours. The reaction mixture was cooled, the so formed precipitate was filtered off, washed with ethanol, dried and recrystallized from acetic acid to give **16** and **17** respectively.

7-(4-chlorophenyl)-9-(furan-2-yl)-1H-pyrido[3`,2`:4,5]thieno[3,2-d]pyrimidin-2,4-dithione (16): Orange crystals (67%); m.p. >300°C; **IR** (υ cm^{-1}): 3199, 3066 (2 NH); **MS**(m/z): (M+ = 427, M+2 = 429). Anal; for $C_{19}H_{10}ClN_3OS_3$ (427.96); Calcd/found (%) C (53.33/53.55), H (2.36/2.40), N (9.82/10.00), S (22.48/22.50).

7-(4-chlorophenyl)-9-(furan-2-yl)-4-imino-3-phenyl-3,4-dihydro-1H-pyrido[3`,2`:4,5]thieno[3,2-d]pyrimidin-2-thione (17) : Yellow crystals (42%); m.p. >300°C; **IR** (υ cm^{-1}): 3398, 3344 (2 NH); **MS**(m/z): (M+= 486, M+2 = 488). Anal; for $C_{25}H_{15}ClN_4OS_2$ (487.01); Calcd/Found (%) C (61.66/61.71), H (3.10/3.30), N (11.50/10.70), S (13.17/13.30).

Synthesis of 20, 21, 24a,b (General method):
A Solution of **18** (0.31g, 1 mmole) in concentrated hydrochloric acid (5ml) and glacial acetic acid (10ml) was heated until dissolved and then allowed the mixture to cool. Sodium nitrite solution (0.14g, 2 mmole) was added drop wise with stirring in ice bath for 5 minutes, then the clear diazonium solution was added to each of malononitrile, ethylcyanoacetate, cyanothioacetamide and cyanoacetamide in sodium acetate/ethanol solution with stirring for 30 minutes. The solid so formed was collected by filtration, washed with water and recrystallized from the proper solvent to give **20, 21 24a,b** respectively.

4-Amino-8-(4-chlorophenyl)-10-(furan-2-yl)pyrido[2`,3`:3,4]pyrazolo[5,1-c][1,2,4]triazine-3-carbonitrile (20): Deep brown crystals (83 %); from ethanol/DMF; m.p. > 300°C; **IR** (υ cm^{-1}): 2229 (CN), 3394, 3236 (NH_2); **MS** (m/z): (M+ = 387, M+2 = 389); Anal. For $C_{19}H_{10}ClN_7O$ (387.79) Calcd/Found (%) C (58.85/ 58.80), H (2.60/ 2.58), N (25.28/25.30).

Ethyl{2-(6-(4-chlorophenyl)-4-(furan-2-yl)-1H-pyrazolo[3,4-b]pyridine-3-yl]hydrazinylidene}(cyano) acetate (21): Green crystals (80 %); from ethanol/DMF; m.p. > 300°C ; **IR** (υ cm^{-1}): 2133 (CN), 3120 (NH), 1720 (CO ester); **MS** (m/z): (M+ = 434, M+2 = 436); **^{1}H NMR** (DMSO-d6) (δ ppm): 1.46 (t, 3H, $CH_2\underline{CH_3}$), 4.52 (q, 2H, $\underline{CH_2}CH_3$), 6.87 (s, 1H H-5 pyridine), 7.47-8-77 (m, 7H, Ar`H), 9.79 (s, 1H, NH). Anal. For $C_{21}H_{15}ClN_6O_3$ (434.85) Calcd/Found (%) C (58.01/58.00), H (3.48/3.45), N (19.33/19.35).

2-{2-[6-(4-chlorophenyl)-4-(furan-2-yl)-1H-pyrazolo[3,4-b]pyridine-3-yl]hydrazinylidene}-2-cyanoethanethioamide (24a):Deep brown crystals (88 %); from ethanol/DMF; m.p. > 300°C; **IR** (υ cm^{-1}): 2135(CN), 3344, 3417 (NH_2), 3097 (NH); **MS** (m/z): (M+ = 421, M+2 = 423); Anal. For $C_{19}H_{12}ClN_7OS$ (421.87) Calcd/Found (%) C (54.10/54.16), H (2.87/2.85), N (23.24/23.27), S (7.60/7.65).

2-{2-[6-(4-chlorophenyl)-4-(furan-2-yl)-1H-pyrazolo[3,4-b]pyridine-3-yl]hydrazinylidene}-2-cyanoacetamide (24b):Brown crystals (85 %); from acetic acid; m.p. > 300°C; **IR** (υ cm^{-1}): 2129(CN), 1670 (CO), 3344, 3417 (NH_2), 3136 (NH); **MS** (m/z): (M+ = 405, M+2 = 407); Anal. For $C_{19}H_{12}ClN_7O_2$ (405.81) Calcd/Found (%) C (56.24/ 54.29), H (2.98/2.95), N (24.16/24.19).

Synthesis of 22, 25a,b (General method):
Compounds **21**, **24a,b** were refluxed in boiling ethanol containing triethylamine for 4 hours. The so formed solid was collected by filtration, crystallized from acetic acid to afford **22, 25a,b** respectively.

8-(4-chlorophenyl)-10-(furan-2yl)-4-oxo-3,4-dihydropyrido[2`,3`:3,4]pyrazolo[5,1-c][1,2,4]triazine-3-carbonitrile (22):Green cystals (81 %); m.p= > 300°C ; **IR** (υ cm^{-1}): 2117 (CN), 1643 (CO); **MS** (m/z): (M+ = 388, M+2 =

400); ^{1}**H NMR** (DMSO-d6) (δ ppm): 3.4 (s, 1H, CH), 7.76 (s, 1H H-5 pyridinethione), 6.35-7.93 (m, 7H, Ar`H), 12.98 (s, 1H, NH). Anal. For $C_{19}H_9ClN_6O_2$ (388.78) Calcd/Found (%) C (58.70/58.75), H (2.33/2.35), N (21.62/21.65).

4-Amino-8-(4-chlorophenyl)-10-(furan-2-yl)pyrido[2`3`:3,4]pyrazolo[5,1-c][1,2,4]triazine-3-carbothioamide (25a):Red crystals (75 %); m.p. > 300^0C; **IR** (υ cm $^{-1}$): 3483, 3301 (NH_2); **MS** (m/z): (M+ = 421, M+2 = 423); Anal. For $C_{19}H_{12}ClN_7OS$ (421.87) Calcd/Found (%) C (54.10/ 54.34), H (2.87/2.80), N (23.24/23.19) S (7.60/780).

4-Amino-8-(4-chlorophenyl)-10-(furan-2-yl)pyrido[2`3`:3,4]pyrazolo[5,1-c][1,2,4]triazine-3-carboxamide (25b):Brown crystals (80 %); m.p. > 300^0C; **IR** (υ cm $^{-1}$): 1670 (CO), 3433, 3151 (NH_2); **MS** (m/z): (M+ =405, M+2 = 407); Anal. For $C_{19}H_{12}ClN_7O_2$ (405.81) Calcd/Found (%) C (56.24/ 56.34), H (2.98/2.90), N (24.16/24.19).

References

[1] Gachet, C.; Cattaneo, M.; Ohlmann, P.; Hechler, B.; Lecchi, A.; Chevalier, J.; Cassel, D.; Mannvcci, P.M.; Cazenave, P. Purinoceptors on blood platelets: further pharmacological and clinical evidence to suggest the presence of two ADP receptors: Br. J. Haematol, **1995**, 91, 434-444.

[2] Umemura, K.; Kawai, H.; Ishihara, H.; Nakashima, M. Inhibitory effect of clopidogrel, vapiprost and argatroban on the middle cerebral artery thrombosis in the rat: Jpn. J. Pharmacol, **1995**, 67, 253-258.

[3] Tolf, B.R.; Dahloom, R.; Theorell; Akenson, A. Synthetic inhibitors of alcohol dehydrogenase. Pyrazoles containing an unsaturated hydrocarbon residue in the 4-position: Acta. Chem. Scand. Ser. B., **1982**, 36, 101-107.

[4] Komuro, M.; Ishida, R.; and Uchida, H. Identification of urinary metabolites of 2-methyl-3-(1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-a]pyridine in rat, rabbit and dog: Azneim-Forch, 1992, 42, 48-45.

[5] Hussin, A.M.; Abu-Shanab, F.A.; Ishak, E.A. Polycyclic pyridines: synthesis of pyridothienopyrimidines pyridothienotriazines and pyridothienotriazepines:

Phosphorus, Sulfur, Silicon, Relat. Elem., **2000**, 159, 55-68.

[6] Eldin, S.M. Cyanothioacetamide and its Derivatives in Heterocyclic Synthesis: A New Route for the Synthesis of Several Pyridine and Thieno [2,3-b]pyridine Derivatives and their Biological Evaluation: Z. Naturforsch, **1999**, 45b, 674-680.

[7] Abdel-Rahman, A. E.; Bakhite, E. A.; Al-Taifi, E. A. Synthesis and Antimicrobial Activity of New Pyridothienopyrimidines and Pyridothienotriazines: J. Chin. Chem. Soc., **2002**, 49, 223-231.

[8] Moloney, G.P. Methyl 3-Hydroxythieno[2,3-b]pyridine-2-carboxylate: Molecules, **2001**, 6(3), M203.

[9] Krauze, A.; Germame, S.; Eberlins, O.; Sturms, I.; Klusa, V.; Duburs, G. Derivatives of 3-cyano-6-phenyl-4-(3`-pyridyl)-pyridine-2(1H)-thione and their neurotropic activity: Eur. J. Med. Chem., **1999**, 34, 301-310.

[10] Menezes, C.M.S; Sant`Anna, C.M.R.; Rodrigues, C.R.; Barreiro, E.J.J. Molecular modeling of novel 1H-pyrazolo[3,4-b]pyridine derivatives designed as isosters of the antimalarial mefloquine: THEOCHEM., **2002**, 579(1), 31-39.

[11] Poreba, K.; Oplski, A.; Wietrzky, J. Synthesis and antiproliferative activity in vitro of new 3-substituted aminopyrazolo[3,4-b]pyridines: Acta Pol. Pharm., **2002**, 59 (3), 215-222.

[12] Stasch, J.P.; Dembowsky, K.; Perzborn, E.; Stahl, E.; Schramm, M. Br. Cardiovascular actions of a novel NO-independent guanylyl cyclase stimulator, BAY 41-8543: *in vivo* studies: J. Pharmacol., **2002**, 135(2), 344-355.

[13] Boerrigter, G.; Costello-Boerrigter, L.C.; Cataliotti, A.; Tsuruda, T.; Harty, G.J.; Lapp, H.; Stasch, J.P.; Burnett, J.C. Cardiorenal and Humoral Properties of a Novel Direct Soluble Guanylate Cyclase Stimulator BAY 41-2272 in Experimental Congestive Heart Failure: Circulation, **2003**, 107(5), 686-689.

[14] Bawankule, D.U.; Stathishkumar, K.; Sardar, K.K.; Chanda, D.; Krishna, A.V.; Prakash, V.R.; Mishra, S.K. Bay 41-2272[5-cyclopropyl-2-[1-(2-fluoro-benzyl)-]-1-H-pyrazolo[3,4-b]pyridine-3-yl]pyrimidine-4-ylamine] induced Dilation in Ovine Pulmo: J. Pharmacol. Exp. Ther., **2005**, 314, 207-213.

[15] De Mello, H.; Echevarria, A.; Bernardino, A.M.; Canto-Cavalheiro, M.; Leon, L.L. Antileishmamial pyrazolopyridine derivatives: Synthesis and structure activity relationship analysis: J. Med. Chem., **2004**, 47, 5427-5432.

[16] Abdou, I.M.; Saleh, A.M.; Zohdi, H.F. Synthesis and Antitumor Activity of 5-Trifluoromethyl-2,4-dihydropyrazol-3-one Nucleosides: Molecules, **2004**, 9, 109-116.

[17] Abdou, I.M.; Merghani, S.; Zohdi, H.F.; Al-Marzouqi, A. Synthesis and biological evaluation of novel fluorinated pyrazolone nucleosides as anti-tumor agents Ninth: Tetrahedron Symposium, **2008**, July 22-25,; Berkeley, CA, USA

[18] Rateb, N.M. Convenient synthesis and antimicrobial evaluation of multicyclic thienopyridines: Phosphorus, Sulfur, Silicon, Relat. Elem., **2007**, 182, 2393-2407.

[19] Rateb, N.M.; Abdelaziz, Sh.H.; Zohdi, H.F. Synthesis and antimicrobial evaluation of some new thienopyridine, pyrazolopyridine and pyridothienopyrimidine

derivatives: Journal of Sulfur Chemistry, **2011**, 32(4), 345-354.

[20] Zohdi, H.F.; Rateb, N.M.; Elnagdy, S.M. Green synthesis and antimicrobial evaluation of some new trifluoromethyl-substituted hexahydropyrimidines by grinding: Eur. J. Med. Chem., **2011**, 46, 5636-5640.

[21] Rateb, N.M. Synthesis and reactions of 4-trifluoromethyl-3-cyano

pyridine-2(*1H*)-thione/one derivatives: Journal of Sulfur Chemistry, **2011**, 32(6), 611.

[22] Muanz, D.N.; Kim, B.W.; Euler, K.L.; William, L. Antibacterial and Antifungal activity of Nine medical plants from Zaire: Int. J. Pharmacog., **1994,** 32(4), 337–345.

[23] Grayer, R.J.; Harborne, J.B. A Survey of antifungal compounds from higher plants: Phytochemistry, **1994,** 37, 19–42.

[24] Irob, O.N.; Young, M.M.; Anderson, W.A. Antimicrobial Activity of Annatto (Bixaorellano) Extract: Int. J. Pharmacog., **1996,** 34(2), 87–90.