Novel Synthesis of Thieno[2,3-*b*]pyridine and Substituted 2-Thienylthiourea Derivatives as Antibiotic Agents

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Derivatives of thieno[2,3-*b*]pyridine, 2-thienylthiourea, 2-thienylurea, 2-thienylacetamide incorporating the 2-phthalimidomethyl moiety have been synthesized. The synthesized compounds were then investigated for antibacterial activity against *Escherichia coli*, *Bacillus subtilis and Staphylococcus aureus*. The compounds were also investigated for antifungal activity against *Aspergillus niger* and *Fusarium oxysporium*. The structures of the newly synthesized compounds have been established on the basis of their analytical and spectral data.

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The emergence of bacterial strains that are resistant to all available antibacterial agents, has created a public health problem [1-3], especially for people with impaired immune systems, such as AIDS and cancer patients. About 90% of the AIDS patients who are infected with multiple drug resistance TB dies. In order to overcome these emerging resistance problems, there is an urgent need to discover new antibiotics-agents with novel modes of action.

In light of the above reports, and the importance of the biological activities of *N*-alkylphthalimide derivatives [4,5] as well as the recent study of the thiourea compound which exhibits significant antioxidant activity with potent anti-HIV activity [6]. Also to the recent reports showing that thiphene-containing substances act as analgesic anticonvulsant agents [7], and antirheumatic drugs [8]. Moreover, they are also known to be human GnRH receptor antagonists to treat reproductive diseases [9] and have been used in the treatment of protazoal parasitic diseases [10].

The applications mentioned lead the authors to combine the above mentioned rings together to synthesize 2-(2'-amino-3'-cyanothien-4'-ylmethyl)phthalimide and to study the behavior of these compounds towards carbon and nitrogen nucleophiles in order to produce polyfunctional substituted thiophens in the hope of discovering potential antibiotics agents. Therefore, in a continuation of our efforts to generate new synthetic routes to different polyfunctional thiophenes [11-14] we have used Gewald's reaction [15] for the synthesis of 2-(2'-amino-3'-cyanothien-4'-ylmethyl)phthalimide (**2**). Hence, compound **2** is obtained *in situ via* a one step process by treatment of **1** with malononitrile in refluxing dimethylformamide in the presence of elemental sulfur and a catalytic amount of piperidine.

The structure of **2** was established on the basis of elemental analysis and spectral data. Thus the ir spectrum of the reaction product shows the presence of NH_2 and nitrile groups due to absorptions at ν_{max} 3412 and 3332 cm^{-1} and 2204 cm^{-1} respectively, in addition to strong phthalimide carbonyl bands at ν_{max} 1772 and 1714 cm^{-1}. Moreover, in the ^1H nmr spectrum a signal corresponding to the acetyl group is not observed and three singlets at δ_H 4.56, 6.21 and 7.21ppm due to methylene, thiophene 5'-H protons and the amino group, respectively are observed.

The 2-aminothiophene derivative **2** underwent nucleophilic addition with a number of reagents under mild conditions, and it was found that the C-N bond of the phthalimide moiety was unaffected in all cases. Thus, **2** reacted with arylidenemalononitriles **3a-e** in refluxing ethanol to afford products with the possible structures **4**, **5** or **6**. 4,5-Dihydrothieno[2,3-*b*]pyridine **6** was established as the structure on the basis of elemental analysis and spectral data. The ^1H nmr for **6a** revealed the presence of broad signal (D_2O-exchangeable) at δ_H 8.77 ppm due to the amino group. Multiplets corresponding to CH's, in the range of δ_H 2.00-4.00 ppm other than signlet for H-4 at δ_H 2.32 ppm, were not observed, which ruled out structure **4**. Two singlets were observed at δ_H 4.58 and 6.67 ppm due to methylene and H-2 of the thiophene ring respectively, in addition to an aromatic multiplet and a broad signal for amino group. Moreover, the ^{13}C nmr of the reaction product **6b,d-e** is characterized by two signals at δ_C 116 and 114 ppm corresponding to the two nitrile groups.

The formation of **6** from treatment of **2** with **3a-e** is assumed to occur *via* initial formation of Michael adduct **4**. A similar mechanism has been proposed earlier to account for the formation of the aminothieno[2,3-*b*]pyridine derivative *via* the reaction of 2-aminothiophene-3-carbonitrile with benzylidenemalononitrile [14,16]. In contrast to this report, the formed adduct did not aromatize by losing hydrogen cyanide. Perhaps, in this case **6** is more stable than the product that would be obtained by elimination of hydrogen cyanide.

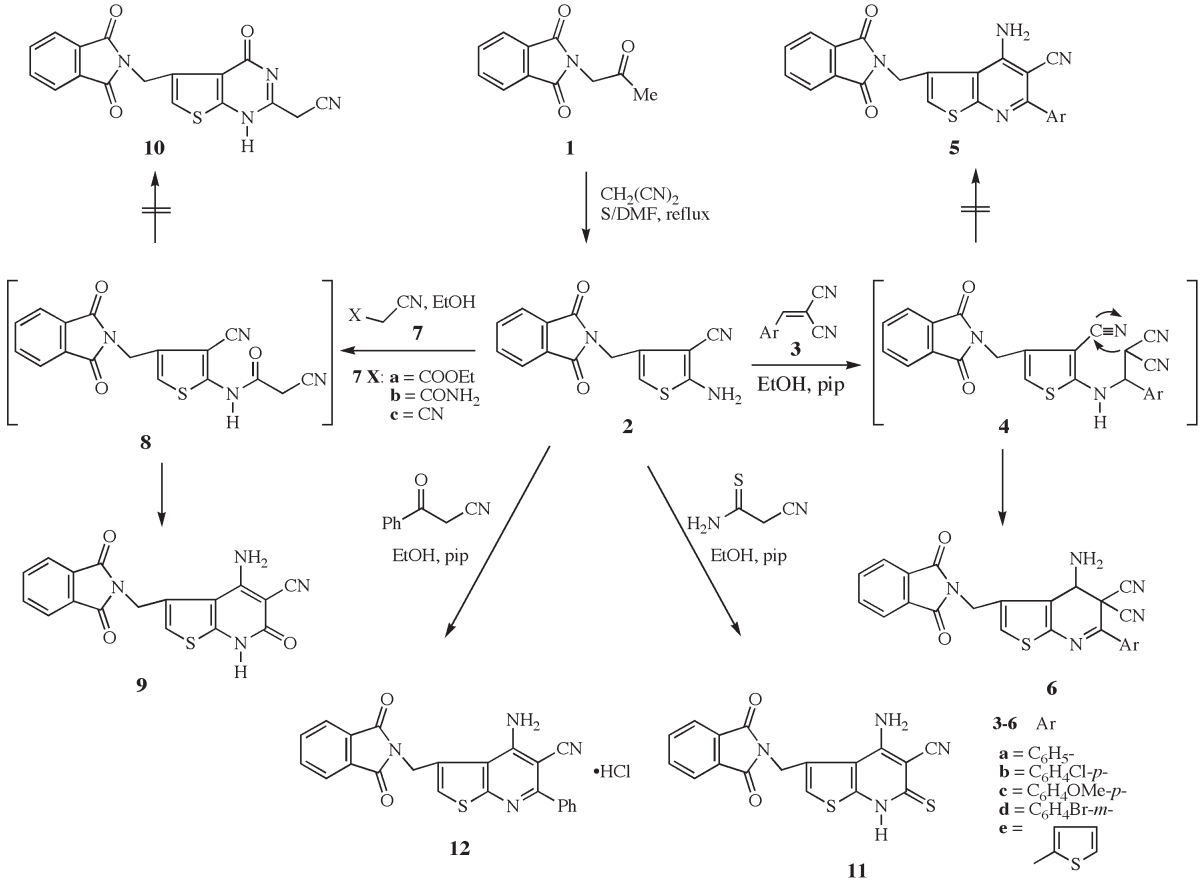
Treatment of compound **2** with ethyl cyanoacetate (**7a**) in refluxing ethanol may produce either 6-oxo-thieno[2,3-*b*]-pyridine (**9**) or the thieno[2,3-*d*]pyrimidine (**10**) similar to that which has recently been reported from our laboratories [14]. In fact, only a single product was obtained and confirmed by TLC. Structure **10** was ruled out on the basis of its elemental analysis and spectroscopic data. The ^1H nmr

spectrum of reaction product **9** revealed the presence of two D_2O-exchangeable protons at δ_H 7.27 and 8.69 ppm due to NH_2 and NH groups, respectively. Also two singlets corresponding to the N-methylene and thiophene H-2 protons at δ_H 4.55 and 6.20 ppm respectively were observed. The absence of any singlet signal corresponding to CH_2CN protons, which would be expected to appear at approximately δ_H 4.13 ppm [14], supports structure **9** and again discounts structure **10**. Moreover, the ir spectrum revealed the presence of NH_2 and NH stretching bands at ν_{max} 3394-3206 cm^{-1} and CN group stretching at 2205 cm^{-1} in addition to three carbonyl stretching bands at 1771, 1714 and 1629 cm^{-1}, respectively which can be assigned to the symmetric and asymmetric phthalimide carbonyl and the pyridine carbonyl stretching bands. The formation of **9** is assumed to take place *via* intermediate **8**, which cannot be isolated, and cyclizes into **9** under these reaction conditions. In a similar manner, compound **2** reacts with cyanoacetamide (**7b**) and malononitrile (**7c**) in refluxing ethanol to afford, in each case, a product indentical in all respects (mp, TLC and spectra) with that obtained previously form the reaction **2** with **7a** (Scheme 1).

amount of piperidine gave a brown crystalline product identified as 4-amino-6,7-dihydro-3-(2-phthalimidomethyl)-6-thioxothieno[2,3-*b*]pyridine-5-carbonitrile (**11**) and thieno[2,3-*b*]pyridine derivative **12**, respectively. The ^1H nmr spectrum of (**12**) exhibited two singlets at δ 4.56 and 6.23 ppm due to methylene and H-2 thiophene protons, respectively in addition to NH_2 and an aromatic multiplet in the region at δ_H 7.56-7.94 ppm. The signal at δ_H 7.87 ppm underwent a facile hydrogen exchange upon addition of deuterium oxide.

A review of literature revealed that reaction of aminonitrile derivatives with isothiocyanates gave a variety of product depending on the experimental conditions [17,18]. Thus, treatment of compound **2** with phenyl isothiocyanates in refluxing pyridine, a crystalline brown colored product was formed. The thieno[2,3-*d*]pyrimidine structure **13** would seem to be a reasonable possibility, however the N,N-disubstituted thiourea **14** was actually assigned for this product on the basis of its ir, ^1H nmr and elemental analysis. The latter compound was refluxed in pyridine for a longer time period in an attempt to obtain 4-amino-2-thioxothieno[2,3-*d*]pyrimidine derivative **13** was unsuc-

Scheme 1



Treatment of **2** with cyanothioacetamide or benzoylacetonitrile in refluxing ethanol and in the presence of a catalytic

cessful, however, again the ir spectra of the reaction product was found to have a cyano absorption band. In contrast

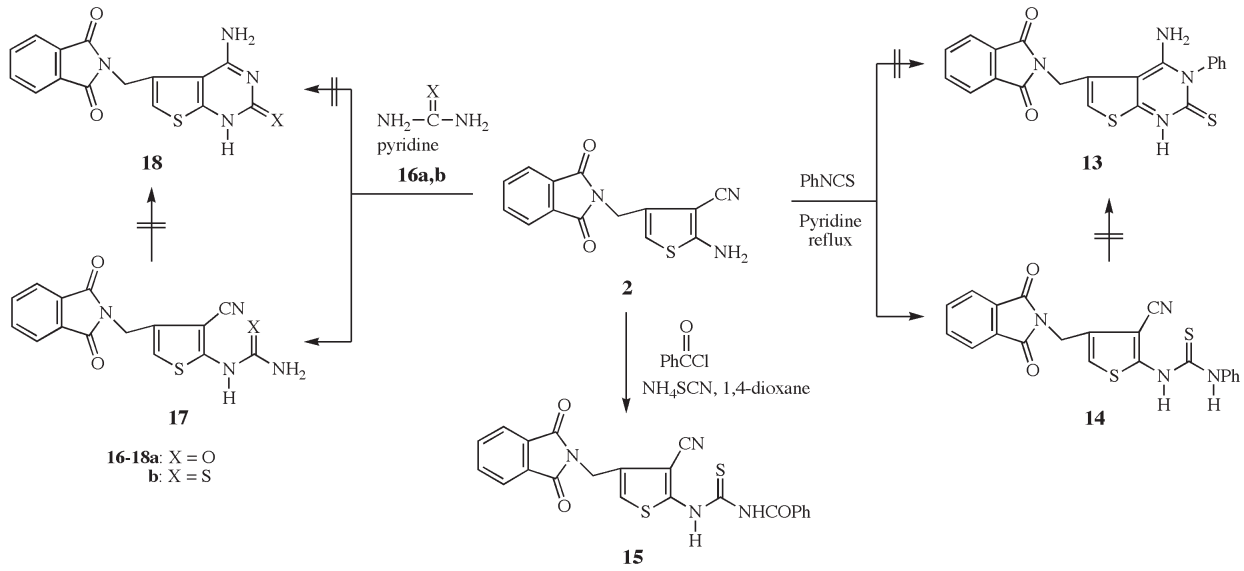
to this, treatment of compound **2** with benzoyl chloride in refluxing 1,4-dioxane as solvent and in the presence of ammonium thiocyanate afforded disubstituted thiourea derivatives **15**.

Compound **2** reacts with urea **16a** or thiourea **16b** in refluxing pyridine to afford the monosubstituted urea or thiourea structures **17a** and **17b**, respectively. The structural assignment is based on analytical data. The presence of the cyano group absorption in the ir spectra of the products indicates that the cyano group is not involved in the reaction. It is worth reporting here that all attempts to convert compound **17a,b** into the corresponding 4-aminothieno[2,3-*b*]-pyrimidine derivatives **18a,b** were unsuccessful (Scheme 2).

Biological Activity.

The biological activities of some newly synthesized compounds were screened for their antifungal activity against *Aspergillus niger* and *Fusarium oxysporiurn*. Most tested compounds showed strong activity against *Aspergillus niger* and moderate activity against *Fusarium oxysporium*, while the antibacterial activity was tested against *Staphylococcus aureus*, *Bacillus subtilis and Escherichia Coli*, respectively. All the 4,5-dihydrothieno[2,3-*b*]pyridine derivatives **6a,c,d** as well as the 2-aminothiophene derivative **2** showed stronger activity against antibacterial and fungicidal activity (Table 1). It was interesting that 2-(2'amino-3-cyanothien-4'-ylmethyl)-phthalimide **2** showed stronger activity against some types of bacteria than that of 2-amino-5-benzotriazol-1-yl-4-methylthiophene-3-carbonitrile, which has been reported recently from our laboratories [14].

Scheme 2



Heating compound **2** with acetic acid or acetic anhydride under reflux, yielded a product with the potential structures *N*-acetamido derivative **19** or thienopyrimidine derivative **20**. However, the presence of cyano absorption band in the ir spectrum of the reaction product at ν_{max} 2213 cm^{-1} readily excluded the possibility of **20**. Compound **19** was refluxed for a longer time in an attempt to obtain the pyrimidine derivative **20**, however again it was found to be unsuccessful as the cyano absorption band was observed in the ir spectrum.

In a similar manner, compound **2** heated with phthalic anhydride in the presence of a glacial acetic acid under reflux afford **21** and not **22**. The ir spectrum of the reaction product showed absorption bands at ν_{max} 2229 cm^{-1} for the cyano group and the absorption corresponding to NH_2 group was not observed. Moreover, its mass spectrum gave m/z = 413 (M^+) which corresponds to a molecular weight consistent with a formula of $C_{22}H_{11}N_3O_4S$ like that of **21** (Scheme 3).

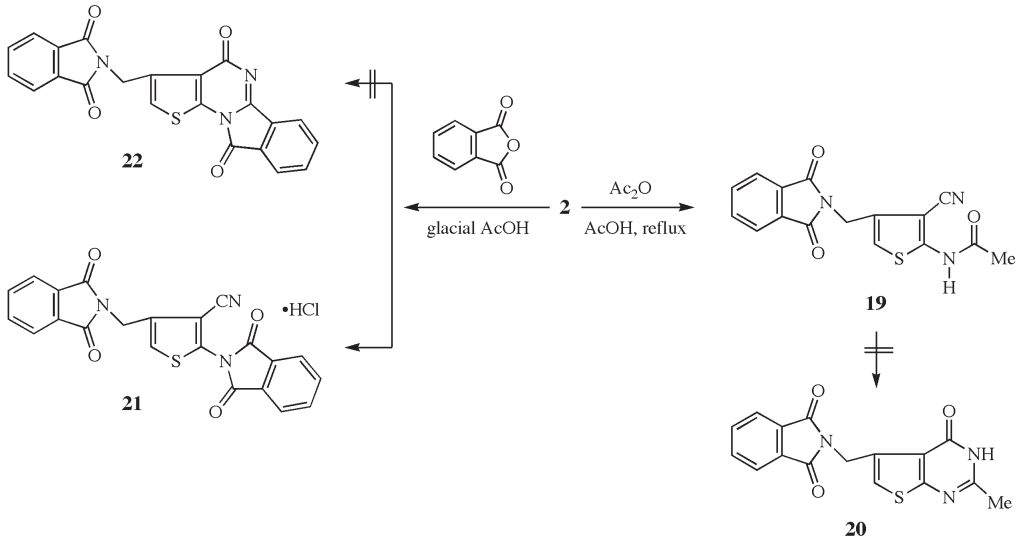
Table 1

In *vitro* Bactericidal and Fungicidal Activity of Newly Synthesized Compounds

Compound	*E-coli*	*B-subtilis*	*S-aureus*	*A-niger*	*F-oxysporium*
2	+++	++	+++	++++	++++
6a	+++	++	+++	++++	+
6d	+++	++	+++	++++	+
6e	+++	+++	+++	++++	+++
12	+	+	++	++	+
13	++	+	++	++++	++
15	++	++	++	++++	++
17b	++	++	++	++++	++

slight effect = + ; Moderate effect = ++ ; strong effect = +++, ++++

Scheme 3



EXPERIMENTAL

All melting points are uncorrected. The ir spectra (KBr) were recorded on a Shimadzu 2000 FT-IR spectrophotometer. ^{1}H and ^{13}C nmr spectra were recorded on a Bruker 400 MHz spectrometer with dimethyl-d_6-sulfoxide or deuteriochloroform as solvent and tetramethylsilane (TMS) as an internal standard; chemical shifts are reported as δ units (ppm). Mass spectra were measured on GS/MS INCOL XL Finningan MAT. Microanalyses were performed on a Leco CHNS 932 analyzer.

2-(2'-Amino-3'-cyanothien-4'-yl-methyl)phthalimide (**2**).

A mixture of **1** (2.03 g, 10 mmol) malononitrile (0.66 g, 10 mmol) elemental sulfur (0.32 g, 10 mmol) in DMF (20 mL) and few drops of piperidine was refluxed for 3 hours. The reaction mixture was left to cool at room temperature then poured into ice-cold water. The solid product so formed was collected by filtration and recrystallized from a mixture of EtOH/DMF ratio 2:1 as brown crystals in 83% yield, m.p. 103-105 °C; ir: ν_{max} 3412 & 3327 (NH_2), 2204 (CN) and 1772 and 1714 cm^{-1} (phthalimide CO); ^{1}H nmr (dimethyl-d_6-sulfoxide); δ_H 4.56 (s, 2H, CH_2), 6.21 (s,1H,H-5'), 7.21 (bs, 2H, NH_2) and 7.85-7.92 (m, 4H, phthalimide-H); ^{13}C nmr (dimethyl-d_6-sulfoxide) δ_C 168.46, (phthalimide CO), 166.64 (C-2'), 134.69, 134.53, 134.33, 132.53, 124.43 (phthalimide carbons C-4' & C-5'), 116.26 (CN), 105.51 (C-3') and 45.70 ppm (CH_2).

Anal. Calcd. for $C_{14}H_9N_3SO_2$: C, 59.36; H, 3.20; N, 14.84. Found: C, 59.08; H, 3.46; N, 14.62.

General Procedure for the Preparation of **6a-c**.

A mixture of **2** (2.83 g, 10 mmol) and benzylidinemalononitrile (**3a**) (1.54 g, 10 mmol) or *p*-chlorobenzylidinemalononitrile (**3b**) (1.89 g, 10 mmol) or *p*-methoxybenzylidinemalononitrile (**3c**) (1.84 g, 10 mmol) in ethanol (20 mL) and few drops of piperidine was refluxed for 3-5 hours and then left to cool at room temperature. The solid product so formed was collected by filtration and recrystallized from a mixture of EtOH/DMF ratio 2:1.

4-Amino-4,5-dihydro-3-(2'-phthalimidomethyl)-6-phenyl-thieno[2,3-*b*]pyridine-5,5-dicarbonitrile (**6a**).

This compound was obtained as deep brown crystals in 78% yield; m.p. 120-122 °C ir: ν_{max}: 3333-3213 (NH_2), 2200 (2CN), 1772 and 1715 cm^{-1} (phthalimide CO); ^{1}H nmr (diemthyl-d_6-sulfoxide); δ_H 2.32 (s, 1H, H-4), 4.58 (s, 2H, CH_2), 6.67 (s, 1H, H-2) and 7.48-8.77 (m, 9H, Ar-H), 8.77 ppm (bs, 2H, NH_2, D_2O exchangeable).

Anal. Calcd. for $C_{24}H_{15}N_5O_2S$: C, 65.90; H, 3.46; N, 16.01. Found: C, 65.83; H, 3.58; N, 16.22.

4-Amino-6-(*p*-chlorophenyl)-4,5-dihydro-3-(2'-phthalimidomethyl)thieno[2,3-*b*]pyridine-5,5-dicarbonitrile (**6b**).

This compound was obtained as green crystals in 76% yield; m.p. 148-151°C ir: ν_{max}: 3444, 3345 (NH_2), 2201 (2CN), 1772 and 1717 cm^{-1} (phthalimide CO); ^{1}H nmr (diemthyl-d_6-sulfoxide); δ_H 2.31 (s, 1H, H-4), 4.58 (s, 2H, CH_2), 6.57 (s, 1H, H-2), 7.40-7.90 (m, 8H, Ar-H) and 8.54 ppm (bs, 2H, NH_2, D_2O exchangeable). ^{13}C nmr (dimethyl-d_6- sulfoxide); δ_C 168.40 (phthalimide CO), 166.70, 153.83, 149.50, 146.45, 135.48, 134.31, 132.40, 131.28, 130.34, 129.72, 127.21, 124.43 (aromatic carbons), 116.99 and 115.67 (2CN), 47.91 (CH_2), 44.58 C-4) and 27.83 ppm (C-5).

Anal. Calcd. for $C_{24}H_{14}N_5O_2SCl$: C, 61.09; H, 2.99; N, 14.84. Found: C, 60.89; H, 2.79; N, 14.65.

4-Amino-4,5-dihydro-6-(*p*-methoxyphenyl)-3-(2'-phthalimidomethyl)thieno[2,3-*b*]pyridine-5,5-dicarbonitrile (**6c**).

This compound was obtained as brown crystals in 73% yield; mp 94-96%; ir, ν_{max} 3336, 3212 (NH_2), 2222 (b, 2CN) and 1772, 1715 cm^{-1} (phthalimide CO); ^{1}H nmr (dimethyl-d_6-sulfoxide); δ_H 2.32 (s, 1H, H-4); 3.84 (s, 3H, OMe), 4.58 (s, 2H, CH_2), 6.21 (s, 1H, H-2), 7.83-8.41 (m, 8H, Ar-H) and 8.40 ppm (bs, 2H, NH_2).

Anal. Calcd. for $C_{25}H_{17}N_5O_3S$: C, 59.59; H, 3.66; N, 13.89. Found: C, 59.73; H, 3.74; N, 14.10.

General Procedure for the Preparation of **6d,e**.

A mixture of **2** (2.83 g, 10 mmol) and *m*-bromobenzylidinemalononitrile (2.33 g, 10 mmol) or 2-thienylbenzylidinemalononitrile (1.60 g, 10 mmol) in pyridine (20 mL) was refluxed for 4-5 hours. The reaction mixture was left to cool at room temperature and acidified with 10% HCl. The solid product so formed was collected by filtration and recrystallized from a mixture of EtOH/DMF ratio 2:1.

4-Amino-6-(*m*-bromophenyl)-4,5-dihydro-3-(2'-phthalimidomethyl)thieno[2,3-*b*]pyridine-5,5-dicarbonitrile (**6d**).

This compound was obtained as brown crystals, m.p. 178-180 °C; ir ν_{max}: 3435-3346 (NH_2), 2211 (2CN), 1774, 1718 (phthalimide CO); ^{1}H nmr (dimethyl-d_6-sulfoxide); δ_H 2.31 (s, 1H, H-4), 4.57 (s, 2H, CH_2), 6.56 (s, 1H, H-2), 7.29-8.64 (m, 8H, Ar-H), 8.79 ppm (bs, 2H, NH_2, D_2O-exchangeable); ^{13}C nmr (dimethyl-d_6-sulfoxide); δ_C 168.64, (phthalimide CO), 166.85, 154.00, 145.39, 135.55, 134.55, 132.77, 132.58, 132.48, 131.98, 131.77, 127.26, 124.59, 124.38, 124.33 (aromatic carbons), 115.25 and 114.28 (2CN), 48.29 (CH_2), 45.29 (C-4), 27.00 ppm (C-5).

Anal. Calcd. for $C_{24}H_{14}N_5O_2SBr$: C, 55.83; H, 2.73; N, 13.56. Found: C, 55.63; H, 2.84; N, 13.36.

4-Amino-4,5-dihydro-3-(2'-phthalimidomethyl)-6-(2'-thienyl)-thieno[2,3-*b*]pyridine-5,5-dicarbonitrile (**6e**).

This compound was obtained as deep brown crystals in 81% yield, m.p. 140-142 °C; ir, ν_{max}: 3445, 3346 (NH_2), 2212 (2CN) and 1771, 1716 cm^{-1} (phthalimide CO); ^{1}H nmr (dimethyl-d_6-sulfoxide); δ_H 2.34 (s, 1H, H-4), 4.58 (s, 2H, CH_2), 6.59 (s, 1H, H-2) and 7.41-8.84 (m, 7H, Ar-H), 8.74 ppm (bs, 2H, NH_2); ^{13}C nmr (dimethyl-d_6-sulfoxide); δ_C 168.40, (phthalimide CO), 166.30, 154.30, 141.41, 139.49, 135.60, 134.30, 132.42, 130.07, 127.22, 124.43, 124.29, 124.16 (aromatic carbons), 115.21 and 114.48 (2CN), 47.89 (CH_2), 45.61 (C-4) and 27.80 ppm (C-5).

Anal. Calcd. for $C_{22}H_{13}N_5O_2S_2$: C, 59.58; H, 2.95; N, 15.79. Found: C, 59.60; H, 3.00; N, 16.01.

4-Amino-6,7-dihydro-3-(2'-phthalimidomethyl)-6-oxo-thieno[2,3-*b*]pyridine-5-carbonitrile (**9**).

A solution of **2** (2.83 g, 10 mmol) in ethanol (20 mL) was treated with ethyl cyanoacetate (**7a**) (1.13 g, 10 mmol) or cyanoacetamide (**7b**) (0.84 g, 10 mmol) or malononitrile (**7c**) (1.13 g, 10 mmol). The reaction mixture was refluxed for 3 hours and left to cool at room temperature. The solid product so formed was collected by filtration and recrystallized from ethanol as brown crystals in 72%, 69%, 73% yield, respectively; m.p. 205-207 °C; ir, ν_{max}: 3394, 3306, 3209 (NH_2 and NH), 2205 (CN), 1771 and 1714 (phthalimide CO) and 1629 cm^{-1} (amide CO); ^{1}H nmr (dimethyl-d_6-sulfoxide); δ_H 4.55 (s, 2H, CH_2), 6.20 (s, 1H, H-6), 7.27 (bs, 2H, NH_2, D_2O-exchangeable) and 7.74-7.96 (m, 4H, Ar-H), 8.69 ppm (bs, 1H, NH, D_2O exchangeable); ^{13}C nmr (dimethyl-d_6-sulfoxide); δ_C 168.44, (phthalimide CO), 166.88 (CO), 150.80, 140.86, 135.77, 140.86, 135.77, 134.48, 132.42, 124.34, 116.43, 105.72 (aromatic carbons & CN), 48.07 ppm (CH_2).

Anal. Calcd. for $C_{17}H_{10}N_4O_3S$: C, 58.29; H, 2.87; N, 15.99. Found: C, 58.10; H, 3.02; N, 15.83.

4-Amino-6,7-dihydro-3-(2'-phthalimidomethyl)-6-thioxo-thieno[2,3-*b*]pyridine-5-carbonitrile (**11**).

A mixture of **2** (2.83 g, 10 mmol) and cyanothioacetamide (1.0 g, 10 mmol) in ethanol (30 mL) and few drops of piperidine was added. The reaction mixture was refluxed for 3 hours. The solvent was then evaporated under reduced pressure. The solid product so formed was collected by filtration and recrystallized from ethanol as pale brown crystals (78%); m.p. 170-172 °C; ir, ν_{max}: 3445, 3339 and 3212 (NH_2 & NH), 2205 (CN), 1772 and 1714 cm^{-1} (phthalimide CO); ^{1}H nmr (deuteriochloroform), δ_H 4.79 (s, 2H, CH_2), 6.25 (s, 1H, H-6), 7.76-7.91 (m, 6H, phthalimide-H and NH_2) and 11.00 ppm (bs, 1H, NH, D_2O-exchangeable).

Anal. Calcd. for $C_{17}H_{10}N_4O_2S_2$: C, 55.74; H, 2.74; N, 15.29. Found: C, 55.51; H, 2.62; N, 15.34.

4-Amino-3-(2'-phthalimidomethyl)-6-phenylthieno[2,3-*b*]pyridine-5-carbonitrile Hydrochloride (**12**).

A mixture of **2** (2.83 g, 10 mmol) and benzoylacetonitrile (1.45 g, 10 mmol) in ethanol (20 mL) and few drops of piperidine was refluxed for 3 hours. The reaction mixture was left to cool at room temperature. The solid product so formed was collected by filtration and recrystallized from a mixture of EtOH/DMF ratio 1:2 as brown crystals in 71% yield; m.p. 117-119 °C; ir, ν_{max}: 3442, 3328 (NH_2), 2204 (CN) and 1772, 1715 cm^{-1} (phthalimide CO); ^{1}H nmr (dimethyl-d_6-sulfoxide): δ_H 4.56 (s, 2H, CH_2), 6.23 (s, 1H, H-2), 7.56 - 7.94 ppm (m, 11H, Ar-H & NH_2); ^{13}C nmr (dimethyl-d_6-sulfoxide): δ_C 168.59, (phthalimide CO), 167.70, 164.98, 154.01, 149.79, 146.66, 141.80, 135.65, 135.32, 132.67, 129.94, 129.63, 129.14, 124.61, 124.36, 117.10 (aromatic carbons & CN) and 48.08 ppm (CH_2).

Anal. Calcd. for $C_{23}H_{15}N_4O_2SCl$: C, 61.81; H, 3.38; N, 12.53. Found: C, 61.62; H, 3.74; N, 12.48.

N-[3-Cyano-4-(2'-phthalimidomethyl)thien-2-yl)]-*N*'-phenylthiourea (**14**).

A mixture of **2** (2.83 g, 10 mmol) and phenyl isothiocyanate (1.35 g, 10 mmol) in pyridine (10 mL) was refluxed for 2 hours. The reaction mixture left to cool at room temperature, then poured into ice-cold water and neutralized with HCl (10%). The solid product so formed was collected by filtration and recrystallized from DMF/EtOH ratio 2:1 as brown crystal, m.p. 149-151 °C; ir, ν_{max}: 3325, 3213 (2NH), 2210 (CN) and 1771, 1714 cm^{-1} (phthalimide CO); ^{1}H nmr (deuteriochloroform); δ_H 4.52 (s, 2H, CH_2), 5.14 (bs, 1H, NH, D_2O-exchangeable),6.50 (s, 1H, H-5), 7.15 (bs, 1H, NH, D_2O-exchangeable), 7.28-8.62 (m, 9H, Ar-H).

Anal. Calcd. for $C_{21}H_{14}N_4O_2S_2$: C, 60.29; H, 3.37; N, 13.39. Found: C, 60.33; H, 3.43; N, 13.54.

N'-Benzoyl-*N*-[3-cyano-4-(2'-phthalimidomethyl)thien-2-yl]thiourea (**15**).

A mixture of benzoyl chloride (1.40 g, 10 mmol) and ammonium thiocyanate (0.76 g, 10 mmol) in 10 mL of 1,4-dioxane was heated under reflux for 20 minutes. Compound **2** (2.83 g, 10 mmol) was added to the reaction mixture. The reaction mixture was refluxed for 4 hours then poured into ice-cold water. The solid product so formed was collected by filtration and recrystallized from ethanol as deep brown crystals in 76% yield; m.p. 138-140 °C; ir, ν_{max}: 3342, 3147 (2NH), 2213 (CN), 1720, 1716 (phthalimide CO) and 1640 cm^{-1} (amide CO); ^{1}H nmr (deuteriochloroform): δ_H 4.52 (s, 2H, CH_2), 6.83 (s, 1H, H-5), 7.28-7.97 (m, 9H, Ar-H), 9.27 (bs, 1H, NH, D_2O-exchangeable); 14.38 ppm (bs, 1H, NH, D_2O-exchangeable); ^{13}C nmr (dimethyl-d_6-sulfoxide): δ_C 176.85 (CS), 170.47 (CO), 168.55, (phthalimide CO), 167.68, 154.00, 151.59, 146.61, 135.92, 134.61, 132.67, 130.05, 128.48, 124.37, 117.55 and 114.06 (aromatic carbons & CN), 48.09 ppm (CH_2).

Anal. Calcd. for $C_{22}H_{14}N_4O_3S_2$: C, 59.19; H, 3.16; N, 12.55. Found: C, 59.40; H, 3.09; N, 12.40.

General Reaction for the Synthesis of **17a,b**.

A solution of **2** (2.83 g, 10 mmol) in pyridine (10 mL) was treated with urea **16a** (0.56 g, 10 mmol) or thiourea **16b** (0.76 g, 10 mmol). The reaction mixture was refluxed for 3 hours and poured into ice-cold water and acidified with 10% HCl. The solid product so formed was collected by filtration and recrystallized from a mixture of (DMF/EtOH) ratio 2:1.

N-[3-Cyano-4-(2'-phthalimidomethyl)thien-2-yl)]urea (**17a**).

This compound was obtained as white crystals, m.p. 198-200 °C; ir, ν_{max}: 3445 and 3336, 3212 (NH_2 & NH), 2209 (CN), 1772 and 1715 (phthalimide CO) and 1629 cm^{-1} (amide CO); ^1H nmr ($CDCl_3$): δ_H 4.52 (s, 2H, CH_2), 6.24 (s, 1H, H-5), 7.28-7.96 (m, 6H, phthalimide-H & NH_2), 8.65 ppm (bs, 1H, NH).

Anal. Calcd. for $C_{15}H_{10}N_4O_3S$: C, 55.22, H, 3.09; N, 17.17. Found: C, 55.08; H, 3.34; N, 17.18.

N-[3-Cyano-4-(2'-phthalimidomethyl)thien-2-yl]thiourea (**17b**).

This compound was obtained as brown crystals, 69% yield, m.p. 145-147 °C; ν_{max}: 3440, 3335 and 3212 (NH_2 & NH), 2207 (CN), 1772 and 1713 cm^{-1} (phthalimide CO); ^1H nmr (deuteriochloroform): δ_H 4.52 (s, 2H, CH_2), 6.50 (s, 1H, H-5), 7.28-7.96 ppm (m, 6H, phthalimide-H & NH_2), 8.73 ppm (br, 1H, NH, D_2O-exchangeable).

Anal. Calcd. for $C_{15}H_{10}N_4O_2S_2$: C, 52.64, H, 2.95; N, 16.37. Found: C, 52.39; H, 3.29; N, 16.68.

N-[3-Cyano-4-(2'-phthalimidomethy)thien-2-yl]acetamide (**19**).

A solution of **2** (2.83 g, 10 mmol) in acetic acid (20 mL) or acetic anhydride (20 mL) was refluxed for 2 hours and then left to cool at room temperature. The reaction mixture was poured into ice-cold water. The solid product, so formed, was collected by filtration and recrystallized from ethanol as brown crystals in 78% yield, m.p.14-146 °C; ir, ν_{max}: 3335 (NH), 2213 (CN), 1772 & 1718 (phthalimide CO) and 1627 cm^{-1} (amide CO); ^1H nmr (dimethyl-d_6-sulfoxide): δ_H 2.50 (s, 3H, Me), 4.57 (s, 2H, CH_2), 6.96 (s, 1H, H-5), 7.81-7.79 (m, 4H, phthalimide-H) and 11.67 ppm (bs, 1H, NH, D_2O-exchangeable).

Anal. Calcd. for $C_{16}H_{11}N_3O_2S$: C, 59.08; H, 3.40; N, 12.91. Found: C, 59.01; H, 3.58; N, 12.99.

2[3'-Cyano-4'(2''-phthalimidomethyl)thien-2'-yl)]phthalimide Hydrochloride (**21**).

A mixture of **2** (2.83 g, 10 mmol) in glacial acetic acid (20 mL) and phthalic anhydride (1.48 g, 10 mmol) was refluxed for 6 hours and left to cool at room temperature. The reaction mixture was poured into ice-cold water. The solid product so formed was collected by filtration and recrystallized from ethanol as brown crystals in 73% yield; m.p. 205-207 °C; ir, ν_{max}: 2229 (CN), 1784, 1774, 1736 and 1716 cm^{-1} (phthalimide CO); ^1H nmr (dimethyl-d_6-sulfoxide); δ_H 4.87 (s, 2H, CH_2), 7.71 (s, 1H, H-5), 7.89-8.04 ppm (m, 8H, Ar-H), MS (EI); m/z = 413 (M^+).

Anal. Calcd. for $C_{22}H_{12}N_3O_4SCl$: C, 58.74; H, 2.68; N, 9.34. Found: C, 58.86, H, 2.76; N, 9.33.

Biological Testing.

The newly synthesized compounds were tested against the specified microorganism, using 400 μg/mL (w/v) solutions in sterile dimethyl-d_6-sulfoxide (DMSO). A solution of the tested compound (1.0 mL) was poured aseptically in a well of 6 mm diameter made by a Cork borer in the nutrient agar medium for bacterial test and sabourand agar for fungal test. After placing the same volume in wells of all tested microorganism, nutrient agar plates were incubated at 37 °C for 24 hours and sabourand dextrose agar plates were incubated at 25 °C for 48 hours. The activities were expressed as inhibition zones (mm, diameter, as clear areas) as antibacterial and antifungal effect. The least concentration, which showed inhibitory effect on any specific microorganism, was considered as the minimum inhibitory concentration (MIC) which was determined using streptomycin (50 μg/ml) as the references.

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