Synthesis and Biological Effects of New Derivatives of Benzotriazole as Antimicrobial and Antifungal Agents

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Received December 26, 2001

Derivatives of 2-aminothiophene-3-carbonitrile, 2-thioxopyridine-3-carbonitrile, 1,8-naphthyridine-2-one, thieno[2,3-*b*]pyridine-5-carbonitrile and thieno[2,3-*d*]pyrimidine incorporating with a 1*H*-benzotriazole moiety or 1,3,4-thiadiazole derivatives incorporating with a benzotriazol-1-ylmethyl group have been synthesized and tested for antimicrobial and antifungal activities. The structures of the newly synthesized compounds have been established on the basis of their analytical and spectral data.

J. Heterocyclic Chem., **39**, 877(2002).

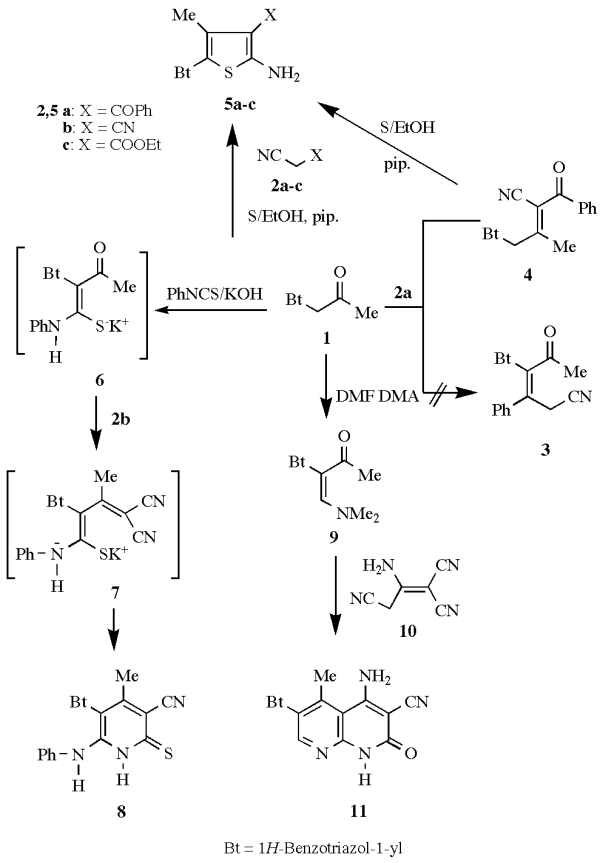
Pharmacological studies of the 1*H*-benzotriazole nucleus have been shown to posses a variety of pharmacological activities such as anti-inflammatory [1], antimicrobial [2-4], anticonvulsant and CNS depressant [5] effects. Furthermore, several pharmacological studies have also pointed out the value of 2-aminothiophene [6,7], naphthyridine [8], thieno[2,3-*d*]pyrimidine [9,10], thieno[2,3-*b*]pyridine [11] and thiadiazole [12] as biologically active nuclei. These findings focused on incorporating 2-aminothiophene-3-carbonitrile, 2-thioxopyridine-3-carbonitrile, 4-amino-2-oxo-1,8-naphthyridine-3-carbonitrile, 4-aminothieno[2,3-*b*]pyridine-5-carbonitrile and thieno[2,3-*d*] pyrimidine with 1*H*-benzotriazole or 1,3,4-thiadiazole derivatives incorporating with a benzotriazol-1-ylmethyl group in the hope of obtaining compounds of potential antimicrobial and antifungal activities.

Thus, treatment of benzotriazol-1-yl acetone (**1**) with benzoylacetonitrile (**2a**) in refluxing ethanol, and in the presence of a catalytic amount of piperidine gave a product that could be formulated as **3** or its isomers **4**. Spectral data seemed to be little help in discriminating **3** or **4**. However, the structure **3** was ruled out on the basis of its reaction with elemental sulfur in refluxing ethanol and in the presence of a catalytic amount of piperidine the Gewald reaction [7,13] and gave a product identified as the 2-aminothiophene derivative **5a**. The structure of the latter product was established on the basis of its elemental analysis and spectral data. Thus, the ir spectrum of the reaction product showed absorption bands at 3352 and 3244 cm^{-1} corresponding to NH_2 group in addition to the strong absorption band at 1661 cm^{-1}, which can be assigned to the carbonyl absorption. Compound **5a** could be obtained *in situ*, *via* a one step process by treatment of **1** with benzoylacetonitrile (**2a**) in refluxing ethanol and in the presence of elemental sulfur and a catalytic amount of piperidine. This affords a product identical in all respects (mp and spectra) with that obtained previously from the reaction **4** with elemental sulfur.

In a similar manner, compound (**1**) reacted with malononitrile (**2b**) or ethyl cyanoacetate (**2c**) in refluxing ethanol in the presence of elemental sulfur and a catalytic amount of piperidine to afford the 2-aminothiophene derivatives **5b-c**. The structures of **5b-c** were established on the basis of their elemental analysis and spectral data. On the other hand, treatment of benzotriazol-1-yl acetone (**1**) with phenyl isothiocyanate in ethanol and in the presence of potassium hydroxide at room temperature gave the non-isolable intermediate **6**. Such an intermediate reacts with an equimolar amount of malononitrile (**2b**) to afford the corresponding thioxopyridine (**8**) in good yield. The formation of **8** is assumed to proceed *via* initial condensation of **6** with malononitrile (**2b**) to form the non-isolable intermediate **7** that readily undergo cyclization to **8**. The structure of the latter product was established on the basis of its elemental analysis and spectral data. Thus, the ir spectrum of the reaction product showed an absorption band at 3329 cm^{-1} due to the two NH groups. The ^1H nmr spectrum of **8** revealed two broad signals (D_2O–exchangeable) at δ 8.67 and δ 12.00 ppm due to the two NH protons in addition to the methyl protons at 2.29 ppm and aromatic protons resonating at 6.97-8.16 ppm.

Treatment of compound **1** with dimethylformamide dimethylacetal (DMF DMA) in dry xylene at reflux afforded the enaminone **9** [14] in good yield. Treatment of the latter compound with malononitrile dimer **10** gave the 1,8-naphthyridine-3-carbonitrile derivative **11** [8] in good yield. The structure of the latter product was established on the basis of elemental analysis and spectral data. Thus, the ir spectrum of the reaction product **11** showed absorption bands at 3430 - 3330 cm^{-1} due to NH and NH_2 groups in addition to the two strong absorption bands at 2209 and 1656 cm^{-1}, which can be assigned to the nitrile and amide carbonyl absorptions, respectively. The ^1H nmr spectrum of the product did not show signals for the dimethylamino group but showed singlets at δ_H 1.97 and δ_H 8.31 ppm for the methyl protons and H-7 of the naphthyridine respectively. Also revealed two broad signals (D_2O exchangeable) at δ_H 8.99 and δ_H 10.06 ppm due to NH_2 and NH protons and multiplets at δ_H 7.47-8.17 ppm corresponding to aromatic protons (Scheme 1).

Scheme 1



Compound **5b** exhibited high reactivity leading to a wide range of polyfunctionally substituted heterocycles. The amino group in compound **5b** was more nucleophilic than the methyl group.

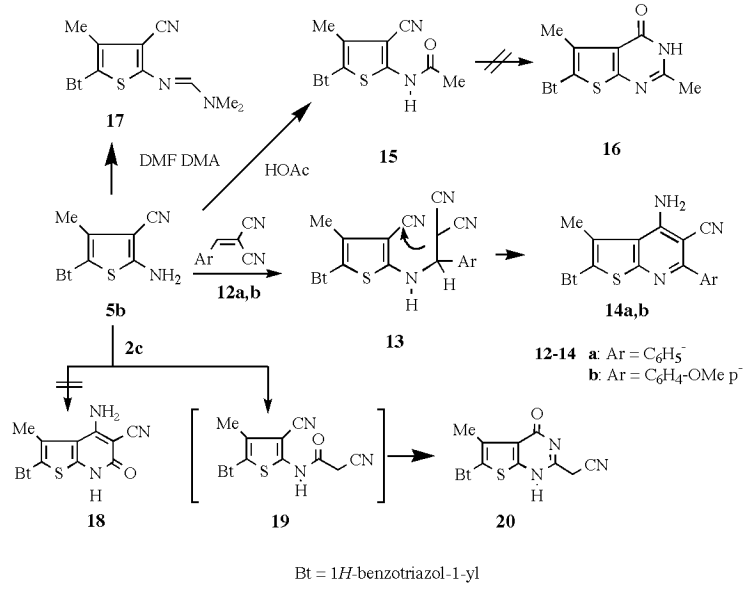
Thus, **5b** reacted with arylidenemalononitrile **12a,b** to afford thieno[2,3-*b*]-pyridine derivatives **14a,b**. The structure of **14** was established on the basis of its elemental analysis and spectral data. Thus, the ^{1}H nmr spectrum revealed the presence of a methyl singlet at δ 1.91 ppm. The formation of the product is assumed to proceed *via* addition of the amino group in **5b** to the double bond in **12** followed by cyclization and aromatization by loss of hydrogen cyanide to give **14** (Scheme 2). Compound **5b** was acylated with acetic acid under reflux to furnish the corresponding *N*-acylamino derivative **15**. An attempt to obtain the thieno[2,3-*d*]pyrimidinone **16** by increasing the reaction time, failed.

On the other hand, treatment of **5b** with dimethylformamide dimethylacetal (DMF DMA) at reflux temperature gave directly the amidine derivative **17** in high yield.

Similarly, compound **5b** reacted with ethyl cyanoacetate (**2c**) to give a single product of molecular formula $C_{15}H_{10}N_6OS$ corresponding to the isomeric structures **18** or **20**. Structure **18** was ruled out on the basis of the ^{1}H nmr spectrum of the reaction product which revealed the presence of CH_2- protons as a singlet at δ 4.13. Moreover, the ^{13}C nmr spectrum showed a carbon resonating at δ_C 165.17, assigned to the carbonyl carbon of structure **20**. The formation of **20** is assumed to take place *via* an intermediate **19**, which cannot be isolated and cyclizes into **20** under the reaction condition (Scheme 2).

Ethyl benzotriazol-1-yl acetate (**21**) [15] was allowed to react with hydrazine hydrate in ethanol to give 1*H*-benzotriazolacetic acid hydrazide **22** [2]. Condensation of the latter compound with benzoyl chloride (**23a**) or 2-thienoyl chloride (**23b**) in refluxing 1,4-diaxan as solvent and in the presence of ammonium acetate yielded the corresponding

Scheme 2



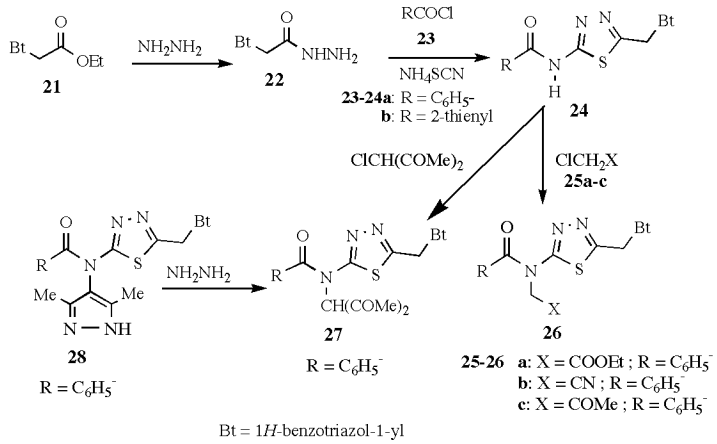
1,3,4-thiadiazole derivatives **24a,b**. The structures of **24a,b** were established *via* their elemental analysis and spectroscopic data. The mass spectrum of **24a** revealed a molecular ion peak at *m/z* (EI) = 336 (M^+). The ^1H nmr spectrum showed in addition to the aromatic signals, singlet signal at δ 6.46 for methylene protons and a signal at δ 10.50 was assigned to the NH proton. This signal underwent a facile hydrogen deuterium exchange upon addition of deuterium oxide.

The NH group of compound **24a** is highly reactive, and is more nucleophilic than the methylene carbon. Thus, **24a** reacted with ethyl chloroacetate (**25a**), chloroacetonitrile (**25b**) and chloroacetone (**25c**) in refluxing dimethylformamide and catalytic amounts of triethylamine to afford **26a-c**. Both elemental analysis and spectral data are compatible with the assigned structures. The NH band was not observed, in the ir spectra of compounds **26a-c** Moreover, treatment of compound **24a** with α-chloroacetylacetone in dimethylformamide and few drops of triethylamine at reflux gave a pale brown product which was identified as *N*-(1-Acetyl-2-oxopropyl)-*N*-(5'-benzotriazol-1-ylmethyl-[1',3',4']thiadiazol-2-yl)benzamide **27** (Scheme 3). Treatment of compound **27** with hydrazine hydrate in refluxing ethanol gave, *N*-(5-benzotriazol-1-ylmethyl[1,3,4]thiadiazol-2-yl)-*N*-(3,5-dimethyl-1*H*-pyrazol-4-yl)-benzamide **28** (Scheme 3).

Treatment of ethyl 4-chloroacetoacetate **29** with 1-*H*-benzotraizole to afford ethyl 4-benzotriazol-1-yl acetoacetate **30** which was allowed to react with phenylhydrazine in refluxing ethanol gave the hydrazone derivative **31**. On the other hand, the reaction of **30** with hydrazine hydrate under similar conditions afforded the pyrazolone **33** in good yield. The structure of the isolated products was confirmed on the basis of elemental analysis and spectral data.

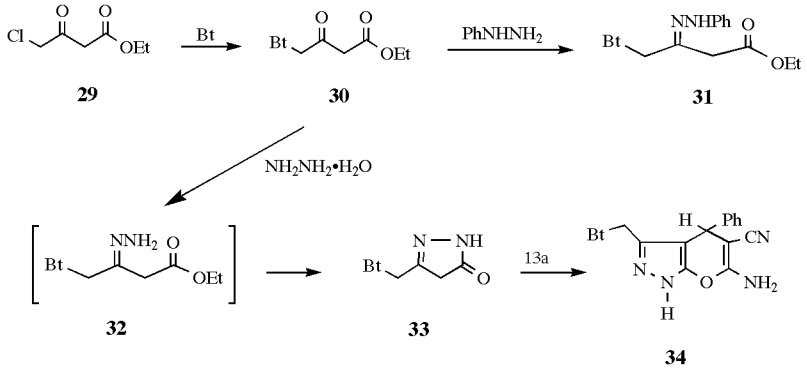
Treatment of 5-benzotriazol-1-ylmethyl-2,4-dihydropyrazol-3-one (**33**) with benzylidinemalononitrile (**13a**) in refluxing ethanol afforded the 4*H*-pyrano[2,3-*c*]pyrazole-5-carbonitrile derivative **34** [16,17] in good yield (Scheme 4). The ^1H nmr spectrum of compound **34** revealed a singlet at δ 4.58 corresponding to the pyran C-H proton and two doublets at δ 5.20 and 5.78 for the methylene protons, which indicate that they are not equivalent (Scheme 4).

Scheme 3



Bt = 1*H*-benzotriazol-1-yl

Scheme 4



Bt = 1*H*-benzotriazol-1-yl

Biological Activity.

The biological activities of some of the newly synthesized compounds were screened for their antifungal activity against *Aspergillus niger* and *Fusarium oxysporium*, while the antibacterial activity was tested against *Escherchia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. Most of the tested sample showed bactericidal and fungicidal activity (Table 1).

Table 1

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

Compound	E- coli	B-subtilis	S-aureus	A-niger	*F-oxysporium*
5a	-	++	+	-	+++
5b	-	-	+++	-	++++
5c	-	-	+++	++	+++
8	-	+++	++++	++++	+++
11	-	-	++++	++++	-
14	-	++++	+++++	+++	-
15	-	++++	++++	++	++++
17	-	-	-	+++	++
20	-	+	++	-	++
24a	-	++	++++	-	+++
24b	-	+++	++++	+++	+++
26b	-	-	-	-	+++
26c	-	-	-	+++	++++
27	-	+	++++	-	+++
28	--	++++	+++++	--	**--**

No effect = -; Slight effect = +; Moderate effect = ++; Strong effect = +++, ++++ & +++++

Acknowledgement.

This work was financed by the University of Kuwait research grant SC 100. We are grateful to the Faculty of Science, Chemistry Department, SAF facility for the spectral and analytical data (Project G 01/01 & G 03/01). We are also grateful to Dr. I. H. Abbas for biological activity tests.

EXPERIMENTAL

All melting points are uncorrected. The ir spectra were recorded on a Shimadzu 2000 FTIR spectrometer. ^{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. Compounds **1**, **9**, **21** and **22** were prepared following literature procedure [14], [14], [15] and [2], respectively.

4-Benzotriazol-1-yl-2-benzoyl-3-methyl-2-butenenitrile (**4**).

A mixture of benzotriazol-1-yl acetone (1.75 g,10 mmol) and benzoylacetonitrile (1.45 g, 10 mmol) in ethanol (20 mL) and few drops of piperidine was refluxed for 4 hours and then left to cool at room temperature. The product, so formed, was collected by filtration and recrystallized from ethanol as yellow crystals 92% yield, mp 152-154 °C; ir: ν 2225 (CN) and 1697 cm^{-1} (CO); ^{1}H nmr (dimethyl-d_6 sulfoxide): δ_H 2.13 (s, 3H, Me), 5.70 (s, 2H, CH_2); 7.11-8.13 ppm (m, 9H, Ar-H).

Anal. Calcd. for $C_{18}H_{14}N_4O$: C, 71.51; H, 4.67; N, 18.53. Found: C, 71.17; H, 4.96; N, 18.52.

2-Amino-5-benzotriazol-1-yl-3-benzoyl-4-methylthiophene (**5a**).

Method A.

To a solution of **4** (3.34 g, 10 mmol) in ethanol (20 mL) were added elemental sulfur (0.32 g, 10 mmol) and a few drops of trimethylamine. The mixture was refluxed for 7 hours and then left to cool at room temperature. The product, so formed, was collected by filtration and recrystallized from ethanol/dimethylformamide (2:1).

Method B.

A mixture of benzotraizol-1-yl acetone (1.75 g, 10 mmol) benzoylacetonitrile (1.45 g, 10 mmol), elemental sulfur (0.32 g, 10 mmol) in ethanol (20 mL) and a few drops of piperidine was refluxed for 5 hours and then poured into ice cold water. The solid product, so formed, was collected by filtration and recrystallized from ethanol/dimethylformamide (2:1) as brown crystals in 71% yield; mp. 129-131 °C; ir: ν 3352, 3244 (NH_2) and 1661 cm^{-1} (CO); ^{1}H nmr (dimethyl-d_6 sulfoxide): δ_H 1.30 (s, 3H, Me), 7.46-8.16 (m, 9H, Ar-H) and 8.36 ppm (br, 2H, NH_2).

Anal. Calcd. for $C_{18}H_{14}N_4OS$: C, 64.66; H, 4.22; N, 16.76. Found: C, 64.85; H, 4.22; N, 17.14.

General Procedure for the Synthesis of **5b,c**.

A reaction carried out as described for the preparation of **5a**, by the method B, but with malononitrile (**2b**) (0.66 g, 10 mmol) or ethyl cyanoacetate (**2c**) (1.13 g, 10 mmol) in place of benzoylacetonitrile to give **5b** and **5c**, respectively.

2-Amino-5-benzotriazol-1-yl-4-methylthiophene-3-carbonitrile (**5b**).

This compound was recrystallized from ethanol as deep brown crystals (81%), mp 190-192 °C; ir, ν 3313, 3150 (NH_2) and 2209 cm^{-1} (CN); ^{1}H nmr (dimethyl-d_6 sulfoxide); δ_H 1.89 (s, 3H, Me), 7.47-8.16 (m, 4H, Ar-H) and 8.18 ppm (br, 2H, NH_2); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 145.64, 135.06, 134.36, 130.25, 128.49, 125.99, 125.12, 120.50, 119.73, 116.25, 111.43 (aromatic carbons & CN) and 13.06 ppm (Me).

Anal. Calcd. for $C_{12}H_9N_5S$: C, 56.47; H, 3.55; N, 27.44. Found: C, 56.21; H, 3.88; N, 27.38.

Ethyl 2-Amino-5-benzotriazol-1-yl-4-methylthiophene-3-carboxylate (**5c**).

This compound was recrystallized from ethanol as pale green crystals (73%), mp 120-121 °C; ir, ν 3407, 3271 (NH_2) and 1696 cm^{-1} (ester CO); ^{1}H nmr (dimethyl-d_6 sulfoxide); δ_H 1.31 (t, 3H, J=7Hz, Me), 1.95 (s, 3H, Me), 4.24 (q, 2H, J=7Hz, OCH_2), 7.50-7.78 (m, 4H, Ar-H) and 8.18 ppm (br, 2H, NH_2 D_2O-exchangable); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 165.80 (ester CO), 145.71, 135.40, 134.39, 130.07, 128.38, 125.81, 125.48, 124.95, 120.51, 111.49 (aromatic carbons) 60.41 (OCH_2), 15.15 and 14.61 ppm (2Me).

Anal. Calcd for $C_{14}H_{14}N_4O_2S$: C, 55.62; H, 4.67; N, 18.54. Found: C, 55.94; H, 4.59; N, 18.32.

6-Anilino-5-benzotriazol-1-yl-1,2-dihydro-4-methyl-2-thioxopyridine-3-carbonitrile (**8**).

A mixture of **1** (1.75 g, 10 mmol) phenyl isothiocyanate (1.35 g, 10 mmol) and KOH (0.56, 10 mmol) in ethanol (20 mL) was stirred at room temperature for 3 hours. To the reaction mixture malononitrile (0.66 g, 10 mmol) was added and stirring was continued overnight. The product, so formed, was collected by filtration and recrystallized from ethanol as brown crystals 72% yield, mp 130-132 °C; ir: ν 3329 (2NH) and 2201 cm^{-1} (CN); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 2.29 (s, 3H, Me), 6.97-8.16 (m, 9H, Ar-H), 8.67 (br, 1H, NH) and 12.00 ppm (br, 1H, NH).

Anal. Calcd. for $C_{19}H_{14}N_6S$: C, 63.68; H, 3.94; N, 23.45. Found: C, 63.39; H, 4.17; N, 23.21.

4-Amino-6-benzotriazol-1-yl-1,2-dihydro-5-methyl-2-oxo-1,8-naphthyridine-3-carbonitrile (**11**).

A mixture of **9** (2.30 g, 10 mmol) and malononitrile dimer (**10**) (1.32 g, 10 mmol) was refluxed in sodium ethoxide (prepared from 0.6 g sodium metal and 60 mL ethanol) for 4 hours, then poured into ice-cold water and neutralized with HCl (10%). The product, so formed, was collected by filtration and recrystallized from ethanol as red crystals 75% yield, mp 230-232 °C; ir: ν 3430, 3330 (NH and NH_2) 2209 (CN) and 1656 cm^{-1} (CO); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 1.97 (s, 3H, Me), 7.47-8.17 (m, 4H, Ar-H), 8.31 (s, 1H, H-7), 8.99 (br, 2H, NH_2) and 10.06 ppm (br, 1H, NH).

Anal. Calcd. for $C_{16}H_{11}N_7O$: C, 60.56; H, 3.49; N, 30.90. Found: C, 60.32; H, 3.64; N, 30.63.

General Procedure for the Synthesis of Substituted Thieno[2,3-*b*]-pyridines **14a,b**.

To a suspension of **5b** (2.50 g, 10 mmol) in pyridine (20 mL) was added benzylidinemalononitrile (**12a**) (1.54g, 10 mmol) or *p*-methoxybenzylidinemalononitrile (**12b**) (1.84 g, 10 mmol). The mixture was stirred for 8 hours, neutralized with HCl (10%), then poured into ice-cold water. The solid product, so formed, was collected by filtration and recrystallized from ethanol.

4-Amino-2-benzotriazol-1-yl-3-methyl-6-phenylthieno[2,3-*b*]-pyridine-5-carbonitrile (**14a**).

This compound was obtained as deep brown crystals in 82% yield; mp. 160-162 °C; ir: ν 3336, 3197 (NH_2) and 2208 cm^{-1} (CN); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 1.91 (s, 3H, Me), 7.12-8.39 (m, 9H, Ar-H) and 8.69 ppm (br, 2H, NH_2).

Anal. Calcd. for $C_{21}H_{14}N_6S$: C, 65.96; H, 3.69; N, 21.98. Found: C, 66.03; H, 3.98; N, 22.02.

4-Amino-2-benzotriazol-1-yl-6-*p*-methoxyphenyl-3-methylthieno[2,3-*b*]pyridine-5-carbonitrile (**14b**).

This compound was obtained as deep green crystals in 73% yield; mp. 106-108 °C; ir: ν 3338, 3195 (NH_2) and 2210 cm^{-1} (CN); ^1H nmr (deuteriochloroform): δ_H 1.90 (s, 3H, Me), 3.82 (s, 3H, OMe); 7.17-8.71 (m, 8H, Ar-H) and 8.78 ppm (br, 2H, NH_2); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 164.23, 161.20, 153.79, 149.89, 145.23, 143.08, 135.07, 134.50, 130.18, 128.82, 126.75, 125.35, 124.78, 120.53, 120.06, 118.90, 115.90, 111.23 (aromatic carbons & CN), 56.70 (OMe) and 21.74 ppm (Me).

Anal. Calcd. for $C_{22}H_{16}N_6OS$: C, 64.07; H, 3.89; N, 20.38. Found: C, 64.11; H, 4.12; N, 20.28.

2-Acetylamino-5-benzotriazol-1-yl-4-methylthiophene-3-carbonitrile (**15**).

A solution of **5b** (2.5 g, 10 mmol) in acetic acid (20 mL) was refluxed for 3 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 a mixture of ethanol/dimethylformamide (2:1) as brown crystals in 75% yield; mp. 167-169 °C; ir: ν 3198 (NH), 2217 (CN) and 1643 cm^{-1} (CO); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 1.91 (s, 3H, Me), 2.07 (s, 3H, Me), 7.43-8.31 (m, 4H, Ar-H) and 12.22 ppm (br, 1H, NH, D_2O-exchangable); ^{13}C nmr (dimethyl-d_6-sulfoxide): δ_C 172.87, 148.58, 134.93, 132.37, 130.08, 128.63, 125.80, 124.90, 121.63, 120.72, 114.63, 111.37 (aromatic carbons & CN), 21.38 and 23.23 ppm (2Me).

Anal. Calcd. for $C_{14}H_{11}N_5OS$: C, 56.56; H, 3.73; N, 23.56. Found: C, 56.78; H, 3.90; N, 23.81.

5-Benzotriazol-1-yl-2-(dimethylaminomethylenimino)-4-methylthiophene-3-carbonitrile (**17**).

A mixture of **5b** (2.5 g, 10 mmol) and dimethylformamide dimethylacetal (1.33 g, 10 mmol) in 20 mL dimethylformamide was refluxed for 3 hours. The solvent was evaporated under reduced pressure and the residue recrystallized from a mixture of ethanol/dimethylformamide (2:1) as pale green crystals in 85% yield; mp. 140-142 °C; ir: ν 2211 cm^{-1} (CN); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 2.01 (s, 3H, Me), 3.03 (s, 6H, NMe_2), 5.87 (s, 1H, methylene CH) and 7.52-8.21 (m, 4H, Ar-H).

Anal. Calcd. for $C_{15}H_{14}N_6S$: C, 58.05; H, 4.55; N, 27.09. Found: C, 58.46; H, 4.36; N, 26.92.

6-Benzotriazol-1-yl-5-methyl-4-oxo-1,4-dihydrothieno[2,3-*d*]pyrimidin-2-ylacetonitrile (**20**).

A mixture of compound **5b** (2.50 g, 10 mmol) and ethyl cyanoacetate **2c** (1.13 g, 10 mmol) was heated for 15 minutes at 150 °C in oil bath, then 20 mL of pyridine was added. The reaction mixture was refluxed for 3 hours, then cool and neutralized with 10% HCl. The solid product, so formed, was collected by filtration and recrystallized from ethanol/DMF (1:2) as brown crystals in 69% yield; mp. 202-204 °C, ir: ν 3339 (NH), 2213 (CN), 1637 cm^{-1} (CO); ^1H nmr (dimethyl-d_6 sulfoxide); 2.09 (s, 3H, Me), 4.13 (s, 2H, CH_2), 7.41-8.15 (m, 4H, Ar-H) and 8.79 (br, 1H, NH); ^{13}C (dimethyl-d_6 sulfoxide): 165.17, 154.01, 149.74, 142.76, 134.42, 133.91, 129.95, 128.64, 126.64, 125.69, 120.64, 118.14 and 116.33 (aromatic carbons & CN), 50.39 (CH_2) and 21.84 ppm (Me).

Anal. Calcd. for $C_{15}H_{10}N_6OS$: C, 55.90; H, 3.13; N, 26.08. Found: C, 55.63; H, 3.44; N, 25.96.

General Procedure for the Synthesis of **24a,b**.

A mixture of benzoyl chloride **23a** (1.40 g, 10 mmol) or 2-thiophene carbonylchloride **23b** (1.07 g, 10 mmol) and ammonium thiocyanate (0.76 g, 10 mmol) in 20 mL of 1,4-dioxan was heated under reflux for 20 minutes. To the reaction mixture, compound **22** (1.91 g, 10 mmol) was added. The reaction mixture was refluxed for 2 hours and then poured into ice-cold water. The solid product, so formed, was collected by filtration and recrystallized from ethanol.

N-(5-Benzotriazol-1-ylmethyl-[1,3,4]thiadiazol-2-yl)benzamide (**24a**).

This compound was obtained as pale yellow crystals in 78% yield, mp. 250-252 °C. ir: ν 3436 (NH) and 1664 cm^{-1} (CO); ^1H

nmr (dimethyl-d_6 sulfoxide); δ 6.46 (s, 2H, CH_2), 7.49-8.03 (m, 9H, Ar-H) and 10.50 ppm (br, 1H, NH, D_2O-exchangable); ms: (EI), *m/z* 336 $(M)^+$.

Anal. Calcd. for $C_{16}H_{12}N_6OS$:C, 57.13; H, 3.59; N, 24.98. Found: C, 57.30; H, 4.35; N, 24.56.

N-(5'-Benzotriazol-1-ylmethyl[1',3',4']thiadiazol-2'-yl)thiophene-2-carboxamide (**24b**).

This compound was obtained as pale yellow crystals in 93% yield, mp. 277-279 °C, ir: ν 3425 (NH) and 1644 cm^{-1} (CO); ^1H nmr (dimethyl-d_6 sulfoxide); δ 6.47 (s, 2H, CH_2), 7.24-8.11 (m, 7H, Ar-H) and 8.27 ppm (bs, 1H, NH, D_2O-exchangable); ms: (EI), *m/z* 336 $(M)^+$.

Anal. Calcd. for $C_{14}H_{10}N_6S_2O$: C, 49.12; H, 2.92; N, 24.56. Found: C, 49.25; H, 3.07; N, 24.27.

General Procedure for the Synthesis of **26a-c**.

A mixture of **24a** (3.36 g, 10 mmol), ethyl cyanoacetate (**25a**) (1.13 g, 10 mmol) or chloroacetonitrile (**25b**) (0.73 g, 10 mmol) or chloroacetone (**25c**) (0.76 g, 10 mmol) in 20 mL of dimethylformamide and few drops of triethylamine was refluxed for 3 hours. The solid product, so formed, was collected by filtration and recrystallized from the proper solvent.

Ethyl *N*-[(5-Benzotriazol-1-ylmethyl[1,3,4]thiadiazol-2-yl)benzoylamino]acetate (**26a**).

This compound was recrystallized from ethanol as brown crystals (71%), mp 99-101 °C, ir, ν 1750 (ester CO); 1671 (amide CO); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 1.16 (t, 3H, J=7Hz, Me), 4.16 (q, 2H, J=7Hz, OCH_2); 5.30 (s, 2H, CH_2), 6.44 (s, 2H, -CH_2) and 7.47-8.16 ppm (m, 9H, Ar-H); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 173.55, 167.07, 166.07, 153.62, 145.74, 135.51, 133.29, 129.97, 128.83, 127.66, 124.96, 119.88, 118.45 and 110.94 (aromatic & other carbon atoms) 61.96 (OCH_2), 52.05 (CH_2), 46.63 (CH_2) and 22.48 ppm (Me).

Anal. Calcd for $C_{20}H_{18}N_6O_3S$: C, 56.87; H, 4.30; N, 19.90. Found: C, 56.83; H, 4.50; N, 19.60.

N-(5-Benzotriazol-1-ylmethyl[1,3,4]thiadiazol-2-yl)-*N*-(1-cyanomethyl)benzamide (**26b**).

This compound was crystallized from ethanol as pale brown crystals (69%), mp 140-142 °C; ir, ν 2197 (CN) and 1625 cm^{-1} (amide CO); ^1H nmr (dimethyl-d_6 sulfoxide); δ_H 5.71 (s, 2H, CH_2), 6.45 (s, 2H,CH_2), and 7.45-8.28 (m, 9H, Ar-H); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 174.02, 166.18, 155.18, 146.09, 133.75, 132.55, 130.37, 129.68, 128.96, 125.24, 120.30, 118.89, 115.52, 111.48 (aromatic & other carbon atoms) 47.00 (CH_2), and 28.93 ppm (CH_2).

Anal. Calcd. for $C_{18}H_{13}N_7OS$: C, 57.60; H, 3.49; N, 26.12. Found: C, 57.52; H, 3.76; N, 26.43.

N-(5-Benzotriazol-1-ylmethyl[1,3,4]thiadiazol-2-yl)-*N*-(2-oxopropyl)benzamide (**26c**).

This compound was crystallized from ethanol/dimethylformamide (2:1) as pale brown crystals in 89%, mp 93-95 °C; ir, ν 1737 (CO) and 1625 cm^{-1} (amide CO); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 2.31 (s, 3H, Me), 5.47 (s, 2H,CH_2), 6.42 (s, 2H, CH_2), and 7.46-8.17 (m, 9H, Ar-H); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ 201.52 (CO), 173.90, 166.47, 154.03, 145.00, 136.20, 133.69, 132.32, 130.42, 129.26, 125.40, 120.31, 118.90, and 111.42 (aromatic & other carbon atoms) 59.98 (CH_2), 46.99 (CH_2) and 28.21 ppm (Me).

Anal. Calcd. for $C_{19}H_{16}N_6O_2S$: C, 58.16; H, 4.11; N, 21.42. Found: C, 58.20; H, 4.25; N, 21.42.

N-(1-Acetyl-2-oxopropyl)-*N*-(5'-benzotriazol-1-ylmethyl-[1',3',4']thiadiazol-2-yl)benzamide (**27**).

A mixture of compound **24a** (3.36 g, 10 mmol), α-chloroacetylacetone (1.35 g, 10 mmol) in dimethylformamide (20 mL) and few drops of triethylamine were refluxed for three hours then allowed to cool. To the reaction mixture 20 mL of ethanol and water were added. The solid product, so formed, was collected by filtration and recrystallized from ethanol as pale brown crystals in 88% yield; mp. 102-104 °C, ir: ν 1671 (2CO), 1625 (amide CO); ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 2.07 (s, 3H, Me), 2.17 (s, 3H, Me), 2.50 (s, 1H, CH), 6.44 (s, 2H, CH_2), 7.44-8.13 (m, 9H, Ar-H); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 189.00, 186.97 (2CO), 173.98, 167.30, 161.19, 158.83, 154.53, 145.77, 135.59, 131.81, 129.46, 124.87, 119.88, 118.44, 110.96 (aromatic & other carbon atoms), 46.94 (CH_2), 36.64 (CH), 31.62 ppm (2Me); ms: (EI), *m/z* 434.1 (M^+).

Anal. Calcd. for $C_{21}H_{18}N_6O_3S$: C, 58.06; H, 4.18; N, 19.35. Found: C, 58.44; H, 4.27; N, 19.48.

N-(5-Benzotriazol-1-ylmethyl[1,3,4]thiadiazol-2-yl)-*N*-(3,5-dimethyl-1*H*-pyrazol-4-yl)-benzamide (**28**).

A suspension of compound **27** (3.86 g, 10 mmol) in ethanol (20 mL) was treated with hydrazine hydrate (0.5 g, 10 mmol). The reaction mixture was refluxed for 3 hours then allowed to cool. The solid product, so formed, was collected by filtration and recrystallized from ethanol as pale brown crystals in 69% yield; mp 154-156 °C, ir: ν 3370 (NH), 1625 (amide CO). ^1H nmr (dimethyl-d_6 sulfoxide): δ_H 2.04 (s, 3H, Me), 2.07 (s, 3H, Me), 6.05 (bs, 1H, NH), 6.41 (s, 2H, CH_2), 7.45 - 8.12 ppm (m, 9H, Ar-H).

Anal. Calcd. For $C_{21}H_{18}N_8SO$: C, 58.60; H, 4.21; N, 26.03. Found: C, 58.60; H, 4.52; N, 25.84.

Ethyl 4-Benzotriazol-1-ylacetoacetate (**30**).

A mixture of 1-*H*-benzotriazole (1.19 g, 10 mmol) in toluene (20 mL) and sodium hydride (0.24 g, 10 mmol) was refluxed for 1 hour then allowed to cool at room temperature. To the mixture, compound **29** (1.65 g, 10 mmol) was added and the mixture refluxed for 6 hours. The solvent was evaporated under reduced pressure and the resulting solid product, so formed, was collected by filtration and recrystallized from ethanol as pale brown crystals in 67% yield; mp; 120-121 °C, ir: ν 1726 and 1743 cm^{-1} (2CO); ^1H nmr (deuteriochloroform): δ_H 1.28 (t, 3H, J=7 Hz, Me), 3.58 (s, 2H, CH_2), 4.18 (q, 2H, J=7 Hz, CH_2), 5.66 (s, 2H, CH_2) and 7.33-8.05 (m, 4H, Ar-H); ^{13}C nmr (deuteriochloroform): δ_C 200.41 (ketone CO), 172.38 (ester CO), 146.27, 139.10, 128.60, 124.68, 120.34, 118.55 (aromatic carbons), 62.39 (OCH_2), 58.63 (CH_2), 50.18 (CH_2) and 14.43 ppm (Me).

Anal. Calcd. For $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 58.00; H, 5.35; N, 16.69.

Ethyl 4-Benzotraiazol-1-yl-3-phenylhydrazonobutanoate (**31**).

A solution of compound **30** (2.47 g, 10 mmol) in ethanol (20 mL) was treated with phenylhydrazine (1.08 g, 10 mmol). The reaction mixture was refluxed for 3 hours, then allowed to cool. The solid product, so formed, was collected by filtration and recrystallized from ethanol as yellow crystals in 76% yield; mp. 140-142 °C, ir: ν 3443 (NH), 1728 (ester CO); ^1H nmr (deuterio-

chloroform): δ_H 1.28 (t, 3H, J=7 Hz, Me), 3.35 (s, 2H, CH_2), 3.75 (q, 2H, J=7 Hz, CH_2), 5.56 (s, 2H, CH_2) and 6.96-8.09 (m, 9H, Ar-H) and 8.73 ppm (br, 1H, NH).

Anal. Calcd. for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.63; N, 20.76. Found: C, 64.33; H, 5.53; N, 20.77.

5-(Benzotriazol-1-ylmethyl)-2,4-dihydropyrazol-3-one (**33**).

A suspension of compound **30** (2.47 g, 10 mmol) in ethanol (20 mL) was treated with hydrazine hydrate (0.5 g, 10 mmol). The reaction mixture was refluxed for 3 hours then allowed to cool. The solid product, so formed, was collected by filtration and recrystallized from ethanol as pale brown crystals in 61% yield; mp. 139-141 °C, ir: ν 1625 cm^{-1} (CO); ^{1}H nmr (dimethyl-d_6 sulfoxide): δ_H 5.20 (s, 2H, CH_2), 5.78 (s, 2H, CH_2), 7.37-8.04 (m, 4H, Ar-H) and 10.50 ppm (br, 1H, NH, D_2O exchangeable); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 158.95 (CO), 146.18, 141.90, 133.40, 128.14, 124.86, 119.69 and 111.33 (aromatic carbons), 45.36 (CH_2) and 56.95 ppm (CH_2).

Anal. Calcd. for $C_{10}H_9N_5O$: C, 55.81; H, 4.22; N, 32.54. Found: C, 55.63; H, 4.37; N, 32.25.

6-Amino-3-benzotriazol-1-ylmethyl-4-phenyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (**34**).

To a solution of **33** (2.15 g, 10 mmol) in ethanol (20 mL) benzilidinemalononitrile (1.54 g, 10 mmol) was added. The reaction mixture was refluxed for 3-4 hours, then left to cool to room temperature. The solid products was collected by filtration and recrystallized from ethanol as brown crystals (72%); mp. 190-192 °C. ir: ν 3413, 3325 (NH_2 and NH) and 2189 cm^{-1} (CN); ^{1}H nmr (dimethyl-d_6 sulfoxide): δ_H 4.58 (s, H, CH), 5.43 (d, 1H, J=13Hz, CH),5.65 (d, 1H, J=13Hz, CH), 6.98-7.98 (m, 11H, Ar-H & NH_2) and 12.92 ppm (br,1H,NH); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ_C 161.40, 156.00, 145.89, 144.64, 133.74, 133.14, 131.35, 130.39, 129.25, 128.22, 127.97, 124.82, 121.34, 120.00, 110.89 and 100.50 (aromatic & other carbon atoms), 56.90 (CH_2) and 36.90 ppm (CH).

Anal. Calcd. for $C_{20}H_{15}N_7O$: C, 65.57; H, 4.09; N, 26.54. Found: C, 65.31; H, 4.49; N, 26.45.

Biological Testing.

The newly synthesized compounds were tested against the specified microorganisms, using 400 μg/ml (w/v) solutions in sterile dimethyl-d_6 sulfoxide. A solution of the tested compound (0.5 mol) was poured aseptically in a well of 6 mm diameter made by a borer in a seeded agar medium. After pipetting the same volume in wells of all tested microorganisms, bacteria test plates were incubated at 37 °C for 24 hours and fungal test plates were incubated at 25 °C for 48 hours. The activities were expressed as inhibition zones (mm diameter, as clear areas). The least concentration that showed inhibitory effect on any specific microorganism was considered as the minimum inhibitory concentration (MIC), which was determined using streptomycin and mycostatin as references.

REFERENCES AND NOTES

[1] A. K. Sengupta, M. P. Bajaj and U. Chand. *J. Indian. Chem. Soc*., **55**, 992 (1978).

[2] S. M. El-Khawass and N. S. Habib, *J. Heterocyclic Chem.* **26**, 177 (1989).

[3] L. L. Bannett and H. I. Baker, *J. Org. Chem*. **22**, 707 (1957).

[4] S. Rao and A. S. Mitra. *Indian J. Chem.* **15B**, 1060 (1977).

[5] S. D. Srivastava and T. R. Rawat. *Indian J. Chem*. **38B**, 623 (1999).

[6] P. G. Baraldi, A. N. Zaid, I. Lampronti, F. Fruttarolo, M. G. Pavani, M. A. Tabrizi, J. C. Shryock, E. Leung and R. Romagnoli, *Bioorganic & Medicinal Chem. Lett.* **10**, 1953 (2000).

[7] R. W. Sabnis, D. W. Rangnekar and N. D. Sonawane, *J. Heterocyclic Chem.* **36**, 333 (1999).

[8] V. P. Litvinov, S. V. Roman and V. D. Dyachenko, *Russian Chem. Review*, **69**, 201 (2000).

[9] S. Buttacharya and D. F. Ewing, *J. Chem. Perkin Trans. 1*, 689 (1994).

[10] S. El-Meligie. *Indian J. of Chem*. **36B**, 1126 (1997).

[11] A. Baba, A. Mori, T. Yasuma, S. Unno, H. Makino and T. Sohda. *Chem. Pharm. Bull.* **47**, 993 (1999).

[12] B. H. Lee, F. E. Dutton, M. F. Clothier, J. W. Bowman, J. P. Davis, S. S. Johnson, E. M. Thomas, M. R. Zantello, E. W. Zinser, J. C. McGuire, D. P. Thompson and T. G. Geary. *Bioorganic & Medicinal Chem. Lett*. **9**, 1727 (1999).

[13] K. Gewald, E. Schinke and H. Bottcher., *Chem. Ber*. **99**, 94 (1966).

[14] F. Al-Omran, *J. Heterocyclic Chem.* **37**, 1219 (2000).

[15] F. Al-Omran, N. Al-Awadi, O. Yousef and M. H. Elnagdi, *J. Heterocyclic Chem*. **37**, 167 (2000).

[16] Y. A. Mohamed, M. A. Zahran, M. M. Ali, A. M. El-Agrody and H. H. El-Said, *J. Chem. Res(s)*, 322 (1995).

[17] Yu. A. Sharanin, M. P. Goncharenko and V. P. Litvinov, *Russian Chem. Review*, **67**, 393 (1998).