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Original article

Enaminonitrile in heterocyclic synthesis: Synthesis and antimicrobial evaluation of some new pyrazole, isoxazole and pyrimidine derivatives incorporating a benzothiazole moiety

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ABSTRACT

Enaminonitrile **2** was used as key intermediate for the synthesis of polyfunctionally substituted heterocycles (e.g. pyrazoles, isoxazole, pyrimidines, thiazolo[3,2-*a*]pyrimidine, tetrazolo[1,5-*a*]pyrimidine, pyrido[1,2-*a*]pyrimidine, 1,5-benzodiazepine, and pyrazolo[1,5-*a*]pyrimidine) incorporating benzothiazole moiety *via* its reactions with some *N*-nucleophiles. The newly synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral studies. Representative compounds of the synthesized products were tested and evaluated as antimicrobial agents.

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1. Introduction

Enaminonitriles are versatile reagents and their chemistry has recently received a considerable attention as precursors to, otherwise not readily obtainable heteroaromatics [1–6]. Elnagdi et al. have reported several novel syntheses of azoles, azines, and azoloazines utilizing enaminonitriles as starting components [7–9]. On the other hand, the considerable biological and medicinal activities of benzothiazoles initiated considerable recent interest in the development of syntheses of these molecules [10–18]. In continuation of our interest in the synthesis of heterocycles containing a benzothiazole moiety [19,20], to identify new candidates that may be value in designing new, potent, selective and less toxic antimicrobial agents, we reported here a facial synthesis of some new pyrazole, isoxazole and pyrimidine derivatives pendant to a benzothiazole *via* the reactions of enaminonitrile with some nitrogen nucleophiles.

2. Results and discussion

2.1. Chemistry

The synthetic strategies adopted to obtain the target compounds are depicted in Schemes 1 and 2. The starting compound, *N*-(benzothiazol-2-yl)-2-cyanoacetamide (**1**) was prepared according to the previously reported procedure [21]. Thus, treatment of compound **1** with dimethylformamide dimethylacetal (DMF-DMA) in dry dioxane under reflux afforded a bright red crystalline product that was identified as *N*-(benzothiazol-2-yl)-2-cyano-3-(dimethylamino)acrylamide (**2**) in high yield. The structure of the latter product was established on the basis of its elemental analysis and spectral data. For example, its ^{1}H NMR spectrum displayed four singlet signals at δ 3.25, 3.32, 8.10, and 11.60 ppm due to magnetically nonequivalent $N(CH_3)_2$ group, methine, and NH protons, respectively, in addition to an aromatic multiplet in the region δ 7.26–7.90 ppm. To study the structure–activity relationship with respect to antimicrobial properties, the reactivity of enaminonitrile **2** towards some nitrogen nucleophiles was investigated. Thus, when enaminonitrile **2** was treated with ammonia, the transamination adduct **3** was formed in good yield. Cyclization of 3-amino-*N*-(benzothiazol-2-yl)-2-cyanoacrylamide (**3**) with triethyl orthoformate and acetic anhydride afforded a yellow crystalline product that was identified as 1-(benzothiazol-2-yl)-1,6-dihydro-6-oxopyrimidine-5-carbonitrile (**4**). The structure of compound **4** was established on the basis of its elemental analysis and spectral data. For example, its ^{1}H NMR spectrum showed two singlet signals at δ 8.63 and 9.86 ppm characteristic for H-4 and H-2 of the pyrimidine moiety. The mass spectrum of compound **4** showed the molecular ion peak at *m*/*z* 254 (M^+). Treatment of enaminonitrile **2** with hydrazine hydrate and

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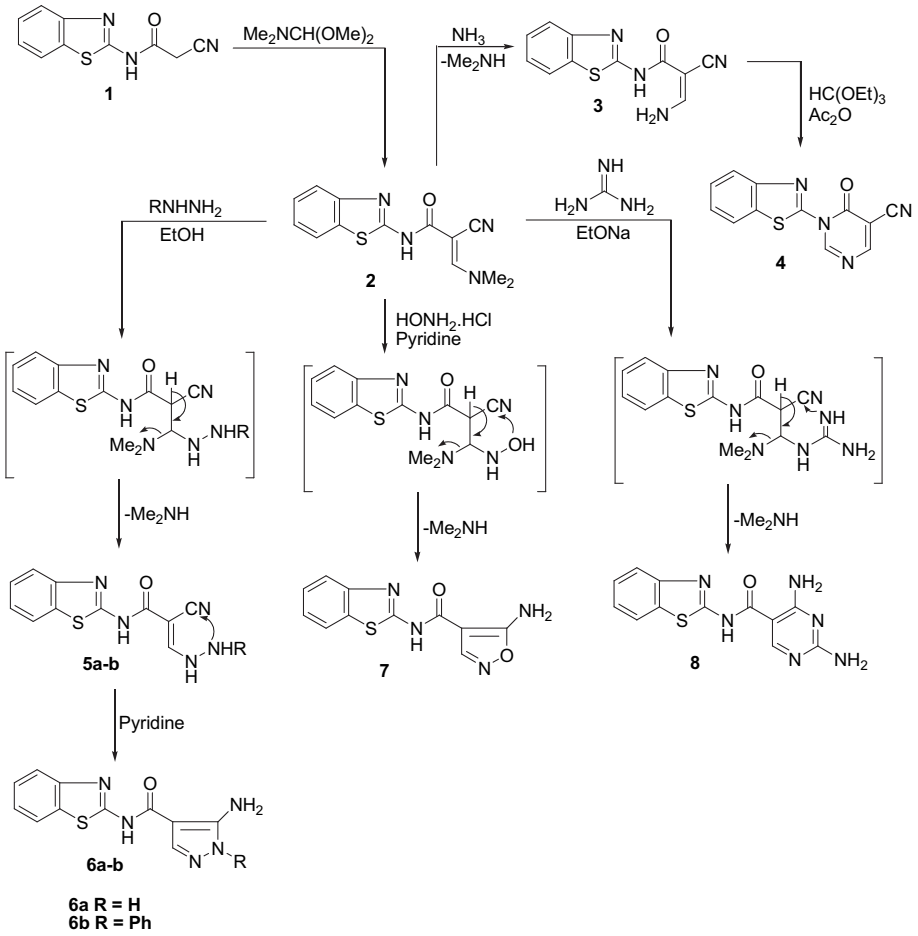
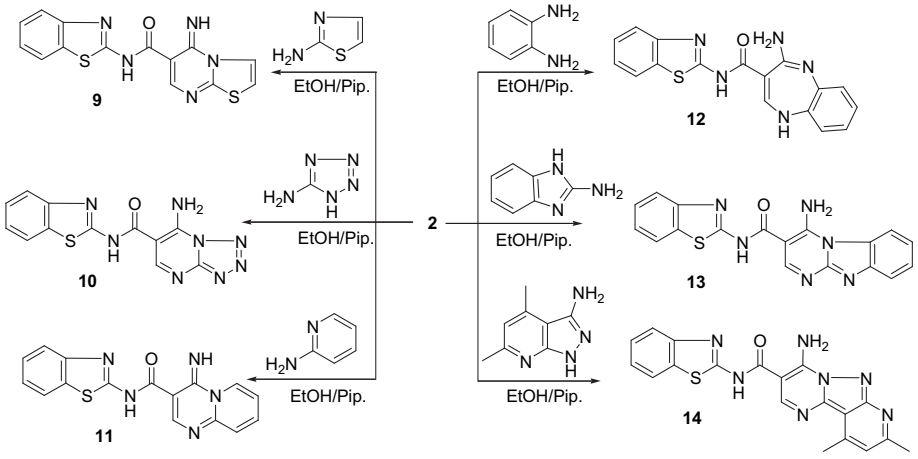


Scheme 1.

phenylhydrazine in refluxing ethanol furnished the isolated intermediates **5a** and **b** which could be cyclized to pyrazole derivatives **6a** and **b** upon boiling in pyridine. Structures of compounds **6a** and **b** were established on the basis of elemental analysis and spectral data. For example, the IR spectra of pyrazoles **6a** and **b** were free of nitrile function and showed absorption bands for NH_2 in the region 3450–3325 cm^{-1}, pyrazole **6a** showed also an absorption band due to NH at 3125 cm^{-1}.

Similarly, the enaminonitrile **2** reacts with hydroxylamine hydrochloride in refluxing pyridine to afford one isolable product identified as 5-amino-*N*-(benzothiazol-2-yl)isoxazole-4-carboxamide (**7**). The IR spectrum of isoxazole **7** showed absorption bands at 3379, 3271, 3138, 1648 cm^{-1} due to the amino, amide NH and carbonyl groups, respectively. We have also investigated the reactivity of enaminonitrile **2** towards guanidine. Thus, when compound **2** was treated with guanidine nitrate in the presence of



Scheme 2.

sodium ethoxide, it afforded an excellent yield of a product which was identified as 2,4-diamino-*N*-(benzothiazol-2-yl)pyrimidine-5-carboxamide (**8**) on the basis of its spectral data. Compounds **3**–**8** are assumed to be formed *via* addition of *N*-nucleophiles to the activated ethylenic double bond of enaminonitrile **2** followed by cyclization and elimination of a dimethylamine molecule as depicted in Scheme 1.

The foregoing results prompted us to investigate the behavior of enaminonitrile **2** towards some bis-*N*-nucleophiles as well as heterocyclic amines as potential precursors for fused heterocyclic systems. Therefore, it was of interest to explore the scope, limitations and generality of enaminonitrile **2** as a precursor for the synthesis of some intricate to access polyfunctionally substituted fused pyrimidine derivatives for which we might expect, a wide spectrum of bioresponses. Thus, reaction of enaminonitrile **2** with equimolar amounts of heterocyclic amines namely 2-aminothiazole, 5-aminotetrazole and 2-aminopyridine upon reflux in ethanol containing a catalytic amount of piperidine, afforded the corresponding thiazolo[3,2-*a*]pyrimidine (**9**), tetrazolo[1,5-*a*]pyrimidine (**10**) and pyrido[1,2-*a*]pyrimidine (**11**) derivatives, respectively, in reasonable yields. The identity of the products was established on the basis of elemental analyses and spectral background in each case. *o*-Phenylene diamine has been reported as useful precursors for the synthesis of diazepine which possesses tranquilizers activity. Thus, treatment of enaminonitrile **2** with *o*-phenylene diamine under the same experimental condition afforded a yellow product identified as 4-amino-*N*-(benzothiazol-2-yl)-1*H*-benzo[*b*][1,4]diazepine-3-carboxamide (**12**). The IR spectrum of compound **12** showed two characteristic absorption bands at 3466, 3350, 3725, 3150 cm^{-1} due to amino group and two NH groups and no band due to the nitrile function. The mass spectrum of compound **12** showed a molecular ion peak at *m*/*z* 335 (M^+).

In the same manner, the enaminonitrile **2** reacts regioselectively with 2-aminobenzimidazole in refluxing ethanolic piperidine solution to give pyrimido[1,2-*a*]benzimidazole derivative (**13**). The structure of compound **13** was established on the basis of elemental analysis and spectral data of the isolated reaction product. The presence of an amino group in structure **13** was evidenced by the appearance of two absorption bands at 3452 and 3340 cm^{-1} in the IR spectrum of the reaction product. Similarly, the reaction of enaminonitrile **2** with 3-amino-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine [22] under the same experimental condition furnished only one isolable product, which was identified as 4-amino-*N*-(benzothiazol-2-yl)-8,10-dimethylpyrido[2′,3′:3,4]pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**14**). The spectral data of the isolated product were in complete agreement with the assigned structure. For example, the IR spectrum of the reaction product **14** revealed no bands due to nitrile function and showed characteristic absorption bands at 3430, 3301, 3179, 1678 cm^{-1} due to the amino, amidic-NH, and amidic carbonyl groups, respectively. The mass spectrum of compound **14** showed a molecular ion peak at *m*/*z* 389 (M^+) corresponding to a molecular formula $C_{19}H_{15}N_7OS$.

Formation of 2-heterarylbenzothiazoles **9**–**14** from enaminonitrile **2** and *N*,*N*-binucleophiles proceeds by initial substitution of the dimethylamino group followed by cyclization. It is worthwhile to mention that the intermediate substitution products **9**–**14** could not be isolated. The regioselectivity for the formation of compounds **9**–**14** is in line with the reported results of the reaction of enaminonitriles with heterocyclic amines [23].

3. Pharmacology

3.1. Antimicrobial evaluation

Thirteen of the newly synthesized target compounds were evaluated for their *in vitro* antibacterial activity against *Bacillus subtilis* and *Bacillus thuringiensis* as examples of Gram positive bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* as examples of Gram negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Botrytis fabae* and *Fusarium oxysporum* fungal strains.

Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activities. Streptomycin, Chloroamphenicol and Treflucan were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds showed significant growth inhibition zones (>10 mm) using twofold serial dilution method [24]. The MIC (μg/mL) and inhibition zone diameter values are recorded in Table 1.

Table 1
Minimal inhibitory concentrations (MIC, μg/mL) and inhibition zone (mm) of some new synthesized compounds.

Compound no.	MIC in μg/mL, and zone of inhibition (mm)					
	Bacteria				Fungi	
	Gram positive bacteria		Gram negative bacteria			
	B. subtilis	*B. thuringiensis*	*E. coli*	*P. aeruginosa*	*B. fabae*	*F. oxysporum*
2	100 (13–18)	25 (25–30)	100 (13–18)	50 (19–24)	25 (25–30)	25 (25–30)
3	100 (13–18)	50 (19–24)	25 (25–30)	100 (13–18)	6.25 (37–42)	12.5 (31–36)
4	12.5 (31–36)	50 (19–24)	50 (19–24)	50 (19–24)	12.5 (31–36)	50 (19–24)
5a	100 (13–18)	50 (19–24)	25 (25–30)	25 (25–30)	6.25 (37–42)	50 (19–24)
5b	100 (13–18)	50 (19–24)	12.5 (31–36)	25 (25–30)	6.25 (37–42)	12.5 (31–36)
6a	12.5 (31–36)	50 (19–24)	12.5 (31–36)	12.5 (31–36)	3.125 (43–48)	6.25 (37–42)
6b	12.5 (31–36)	50 (19–24)	50 (19–24)	50 (19–24)	12.5 (31–36)	50 (19–24)
7	50 (19–24)	25 (25–30)	50 (19–24)	50 (19–24)	6.25 (37–42)	25 (25–30)
8	12.5 (31–36)	25 (25–30)	25 (25–30)	50 (19–24)	3.125 (43–48)	6.25 (37–42)
9	12.5 (31–36)	25 (25–30)	25 (25–30)	6.25 (37–42)	12.5 (31–36)	25 (25–30)
10	3.125 (43–48)	12.5 (31–36)	50 (19–24)	50 (19–24)	3.125 (43–48)	6.25 (37–42)
11	6.25 (37–42)	12.5 (31–36)	12.5 (31–36)	50 (19–24)	6.25 (37–42)	25 (25–30)
12	6.25 (37–42)	12.5 (31–36)	25 (25–30)	6.25 (37–42)	12.5 (31–36)	25 (25–30)
Reference drugs						
Streptomycin	3.125 (43–48)	6.25 (37–42)	6.25 (37–42)	6.25 (37–42)	–	–
Chloramphenicol	6.25 (37–42)	6.25 (37–42)	6.25 (37–42)	6.25 (37–42)	–	–
Treflucan	–	–	–	–	3.125 (43–48)	3.125 (43–48)

The results depicted in Table 1 revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains, and also against antifungal strains.

In general, most of the tested compounds revealed better activity against the Gram positive rather than the Gram negative bacteria. It would be also noticed that compounds belonging to the cyclized pyrazole series exhibited better antibacterial potentials than the transamination adduct.

Regarding the activity of the pyrimidines against Gram positive bacteria, the results revealed that compounds **4**, **8**–**11** exhibited broad spectrum antibacterial profile against the tested organisms. In this view, compound **10** was equipotent to streptomycin in inhibiting the growth of *B. subtilis* (MIC 3.125 μg/mL), while its activity was 50% lower than streptomycin against *B. thuringiensis*. Compounds **11** and **12** showed 50% of the activity of streptomycin (MIC 6.25 μg/mL) but they were equipotent to Chloroamphenicol in inhibiting the growth of *B. subtilis* (MIC 6.25 μg/mL).

On the other hand, compounds **4**, **5a**, **b**, **6a**, **b** and **7** exhibited weak to moderate growth inhibitory activity against Gram positive bacteria as revealed from their MIC values (25–100 μg/mL). Among these compounds **6a** and **b** showed relatively good growth inhibitory profiles against *B. subtilis* (MIC 12.5 μg/mL) which were about 25% of the activity of streptomycin and 50% of Chloroamphenicol against the same organism.

Concerning the antibacterial activity of the compounds **4**, **6b**, **7**, and **10** revealed weak growth inhibitory against the tested Gram negative bacteria (MIC 50 μg/mL).

Regarding the activity of pyrazoles, pyrimidines and azolopyrimidines incorporating benzothiazole moiety, against antifungal strains, the results revealed that compounds **6b**, **8**, and **10** were equipotent to Treflucan in inhibiting the growth of *B. fabae* (MIC 3.125 μg/mL), while their reactivity was 50% lower than Treflucan against *F. oxysporum* (MIC 6.25 μg/mL).

It is worth mentioning that incorporation of benzothiazole to pyrimidine nucleus at position 5 *via* a carboxamide linker produced a high antimicrobial activity. Conversion of **5a** and **b** to pyrazoles **6a** and **b** also enhanced the antimicrobial activity. On the other hand, incorporation of benzothiazole nucleus to isoxazole at position 4 in compound **7** unfortunately produced weak antimicrobial activity.

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some new heterocycles incorporating benzothiazole moiety with the hope of discovering new structure leads serving as potent antimicrobial agents. Our aim has been verified by the synthesis of two different groups of structure hybrids comprising basically the benzothiazole moiety attached to either polysubstituted pyrazole or pyrimidine counter parts through various linkages of synergistic purpose. The obtained results clearly revealed that compounds derived from pyrimidines exhibited better antimicrobial activity than their pyrazole structure variants.

4. Experimental

All melting points were measured on a Gallenkamp electrothermal melting point apparatus. IR spectra were recorded for KBr disc on a Mattson 5000 FTIR spectrophotometer. ^{1}H NMR spectra were measured on a Bruker AC 300 (300 MHz) in $CDCl_3$ or DMSO-d_6 as solvent, using TMS as an internal standard, and chemical shifts are expressed as δ_{ppm}. Mass spectra were determined on Finnigan Incos 500 (70 eV). Elemental analyses were carried out in the Microanalytical Unit of the Faculty of Science, Cairo University. *N*-(Benzothiazol-2-yl)-2-cyanoacetamide (**1**) [21] and 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine [22] were prepared following literature procedure.

4.1. Synthesis of N-(benzothiazol-2-yl)-2-cyano-3-(dimethylamino)acrylamide (2)

A mixture of compound **1** (2.17 g, 10 mmol) and dimethylformamide dimethylacetal (1.2 g, 10 mmol) in dioxane (30 mL) was refluxed for 4 h. After cooling to room temperature, the solid precipitate that formed was filtered off, washed with light petroleum ether (bp 40–60 °C) and dried. Recrystallization from dioxane afforded compound **2**.

Bright red crystals; yield 81%; mp 231–232 °C (Lit. [9] 232 °C); IR (KBr): $\upsilon_{max}/cm^{-1} = 3400$ (NH), 2186 (CN), 1678 (CO). ^{1}H NMR ($CDCl_3$): $\delta_{ppm} = 3.25$ (s, 3H, CH_3), 3.32 (s, 3H, CH_3), 7.26 (t, $J = 8.1$ Hz, 1H, Ar–H), 7.40 (t, $J = 7.8$ Hz, 1H, Ar–H), 7.63 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.90 (d, $J = 7.8$ Hz, 1H, Ar–H), 8.10 (s, 1H, CH═), 11.6 (s, 1H, NH). MS: *m*/*z* (%) = 272 (M^+, 25), 177 (10), 149 (13), 123 (100), 122 (3.4), 80 (13). Anal. for $C_{13}H_{12}N_4OS$ (272.33): Calcd.: C 57.34; H 4.44; N 20.57%. Found: C 57.37; H 4.51; N 20.53%.

4.2. 3-Amino-N-(benzothiazol-2-yl)-2-cyanoacrylamide (3)

To a solution of the enaminonitrile **2** (0.217 g, 1 mmol) in ethanol (20 mL), concentrated aqueous ammonia (0.7 mL, sp. gr. 0.90) was added. The reaction mixture was refluxed for 6 h, then cooled. The solid product so formed was filtered off, washed with dry ether, dried and recrystallized from ethanol afforded compound **3**.

Yellow crystals; yield 73%; mp 226–227 °C; IR (KBr): $\upsilon_{max}/cm^{-1} = 3379$, 3274 ($NH_2$), 3166 (NH), 2195 (CN), 1655 (CO). ^{1}H NMR ($CDCl_3$): $\delta_{ppm} = 7.20$–7.94 (m, 4H, Ar–H), 8.21 (br s, 2H, NH_2), 8.32 (s, 1H, CH═), 9.86 (s, 1H, NH). Anal. for $C_{11}H_8N_4OS$ (244.27): Calcd.: C 54.09, H 3.30, N 22.94%; Found: C 54.12, H 3.26, N 22.89%.

4.2.1. 1-(Benzothiazol-2-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (4)

A solution of compound **3** (0.26 g, 1 mmol) in a mixture of triethyl orthoformate (2.5 mL) and acetic anhydride (2.5 mL) was heated under reflux for 3 h during which the product partially separated. The reaction mixture was allowed to cool to room temperature and the separated product was filtered off, washed with ethanol, dried, and recrystallized from acetic acid.

Yellow crystals; yield 53%; mp 282–283 °C; IR (KBr): $\upsilon_{max}/cm^{-1} = 2198$ (CN), 1695 (CO), 1625 (C═N). ^{1}H NMR (DMSO-d_6): $\delta_{ppm} = 7.22$–7.78 (m, 4H, Ar–H), 8.63 (s, 1H, pyrimidine–H_4), 9.25 (s, 1H, pyrimidine–H_2). MS *m*/*z* (%): 254 (M^+, 24), 227 (30), 203 (20), 150 (100), 149 (80), 122 (39), 95 (28), 68 (40), 51 (28). Anal. for $C_{12}H_6N_4OS$ (254.27): Calcd.: C 56.68, H 2.38, N 22.03%; Found: C 56.61, H 2.33, N 22.08%.

4.3. General procedure for the reaction of enaminonitrile 2 with hydrazines

To a solution of the enaminonitrile **2** (0.45 g, 2 mmol) in ethanol (20 mL), hydrazine hydrate (80%, 0.2 mL) or phenylhydrazine (0.2 mL, 2 mmol) was added. The reaction mixture was refluxed for 4 h, and then cooled. The solid product so formed was filtered off, washed with ethanol, dried and recrystallized from a mixture of DMF/EtOH (1:2) to give compounds **5a** and **b**.

4.3.1. N-(Benzothiazol-2-yl)-2-cyano-3-hydrazinylacrylamide (5a)

Pale yellow crystals; yield 83%; mp 303–304 °C; IR (KBr): $\upsilon_{max}/cm^{-1} = 3436$, 3328 ($NH_2$), 3273, 3135 (2NH), 2194 (CN), 1667 (CO). ^{1}H NMR (DMSO-d_6): $\delta_{ppm} = 5.13$ (br s, 2H, NH_2), 7.26 (t, $J = 7.6$ Hz, 1H, Ar–H), 7.41 (t, $J = 7.6$ Hz, 1H, Ar–H), 7.60 (d, $J = 5.4$ Hz, 1H, Ar–H), 7.81 (d, $J = 6$ Hz, 1H, Ar–H), 7.86 (s, 1H, olefinic CH═), 8.30 (s, 1H, NH–hydrazine), 10.6 (s, 1H, NH). MS *m*/*z* (%): 259 (M^+, 36%), 226

(7.5), 185 (6.6), 177 (6.8), 150 (96), 110 (100), 83 (22), 69 (27.5). Anal. for $C_{11}H_9N_5OS$ (259.29): Calcd.: C 50.95, H 3.50, N 27.01%; Found: C 50.90, H 3.45, N 27.13%.

4.3.2. 3-(2-Phenylhydrazinyl)-N-(benzothiazol-2-yl)-2-cyanoacrylamide (**5b**)

Yellow crystals; yield 67%; mp 135–136 °C; IR (KBr): υ_{max}/cm^{-1} = 3429, 3325, 3175 (3NH), 2216 (CN), 1660 (C=O). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 7.26–7.90 (m, 4H, Ar–H), 8.11 (s, 1H, olefinic CH=), 8.52 (s, 1H, NH), 10.2 (s, 1H, Ph–NH), 10.6 (s, 1H, NH). MS *m*/*z* (%): 335 (M^+, 14.6), 272 (20), 186 (68), 123 (100), 77 (39). Anal. for $C_{17}H_{13}N_5OS$ (335.38): Calcd.: C 60.88, H 3.91, N 20.88%; Found: C 60.79, H 3.87, N 20.78%.

4.4. General procedure for synthesis of pyrazole derivatives (**6a** and **b**)

A solution of compounds **5a** and **b** (1 mmol) in pyridine (20 mL) was refluxed for 8 h. The solution was evaporated under vacuum and triturated with ethanol. The precipitated product was filtered off, washed with ethanol and recrystallized from a mixture of EtOH/DMF (1:1) to give pyrazoles **6a** and **b**.

4.4.1. 5-Amino-N-(benzothiazol-2-yl)-1H-pyrazole-4-carboxamide (**6a**)

Yellow powder; yield 88%; mp 284–285 °C (Lit. [9] 286 °C); IR (KBr): υ_{max}/cm^{-1} = 3450, 3369 (NH_2), 3275, 3125 (2NH), 1660 (CO), 1607 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 6.12 (br s, 2H, NH_2), 7.27 (t, *J* = 7.8 Hz, 1H, Ar–H), 7.40 (t, *J* = 8.1 Hz, 1H, Ar–H), 7.72 (d, *J* = 8.1 Hz, 1H, Ar–H), 7.93 (d, *J* = 7.8 Hz, 1H, Ar–H), 8.16 (s, 1H, pyrazole–H_5), 11.93 (br s, 2H, 2NH). Anal. for $C_{11}H_9N_5OS$ (259.29): Calcd.: C 50.95, H 3.50, N 27.01%; Found: C 50.87, H 3.42, N 27.12%.

4.4.2. 5-Amino-N-(benzothiazol-2-yl)-1-phenyl-1H-pyrazole-4-carboxamide (**6b**)

Yellow powder; yield 65%; mp 246–247 °C; IR (KBr): υ_{max}/cm^{-1} = 3441, 3328 (NH_2), 3219 (NH), 1666 (C=O), 1637 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 6.70 (s, 2H, NH_2), 7.29–7.94 (m, 9H, Ar–H), 8.41 (s, 1H, pyrazole–H_5), 12.15 (s, 1H, NH). Anal. for $C_{17}H_{13}N_5OS$ (335.38): Calcd.: C 60.88, H 3.91, N 20.88%; Found: C 60.69, H 3.85, N 20.77%.

4.5. Synthesis of 5-amino-N-(benzothiazol-2-yl)isoxazole-4-carboxamide (**7**)

A mixture of enaminonitrile **2** (0.45 g, 2 mmol) and hydroxylamine hydrochloride (2.3 mmol) in 30 mL pyridine was refluxed for 8 h and then allowed to cool to room temperature and diluted with ice-cold water (20 mL). The solid product so formed was collected by filtration, washed with water, dried, and recrystallized from ethanol to give compound **7**.

Yellow crystals; yield 73%; mp 260–261 °C; IR (KBr): υ_{max}/cm^{-1} = 3379, 3271 (NH_2), 3138 (NH), 1648 (CO). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 7.20–7.92 (m, 4H, Ar–H), 8.02 (br s, 2H, NH_2), 8.31 (s, 1H, isoxazole–H_3), 9.86 (s, 1H, NH). MS *m*/*z* (%): 260 (M^+, 34%), 259 (30), 217 (7), 177 (29), 150 (77), 149 (81), 123 (19), 122 (24.5), 77 (24), 68 (100), 67 (70). Anal. for $C_{11}H_8N_4O_2S$ (260.27): Calcd.: C 50.76, H 3.10, N 21.53%; Found: C 50.66, H 3.05, N 21.43%.

4.6. Synthesis of 2,4-diamino-N-(benzothiazol-2-yl)pyrimidine-5-carboxamide (**8**)

A mixture of enaminonitrile **2** (0.45 g, 2 mmol) and guanidine nitrate (0.28 g, 2.3 mmol) in sodium ethoxide solution (prepared by dissolving 0.23 g of sodium in 20 mL absolute ethanol) was refluxed for 8 h. The reaction mixture was allowed to cool to room temperature and diluted with ice-cold water (30 mL) containing few drops with HCl. The solid product so formed was collected by filtration, washed with water, dried, and recrystallized from a mixture of EtOH/DMF (2:1) to give compound **8**.

Buff powder; yield 86%; mp 333–334 °C; IR (KBr): υ_{max}/cm^{-1} = 3360, 3256 (NH_2), 3144 (NH), 1660 (C=O). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 7.33 (t, *J* = 7.2 Hz, 1H, Ar–H), 7.46 (t, *J* = 7.2 Hz, 1H, Ar–H), 7.65 (br s, 2H, NH_2), 7.64 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.95 (d, *J* = 7.8 Hz, 1H, Ar–H), 8.72 (s, 1H, pyrimidine–H_6), 8.89 (br s, 2H, NH_2), 12.80 (s, 1H, NH). MS *m*/*z* (%): 286 (M^+, 13%), 137 (98), 136 (100), 95 (53), 94 (53), 68 (19), 67 (18). Anal. for $C_{12}H_{10}N_6OS$ (286.31): Calcd.: C 50.34, H 3.52, N 29.35%; Found: C 50.31, H 3.43, N 29.31%.

4.7. General procedure for the reaction of enaminonitrile **2** with heterocyclic amines and o-phenylene diamine

A mixture of the enaminonitrile **2** (0.45 g, 2 mmol) and an equimolar amount of the appropriate heterocyclic amines (2-aminothiazole, 5-aminotetrazole, 2-aminopyridine, 2-aminobenzimidazole or 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine) or *o*-phenylene diamine in 30 mL ethanol containing few drops of piperidine (3 drops) was refluxed for 8 h, then left to cool to room temperature. The solid deposited was collected by filtration and recrystallized from a mixture of EtOH/DMF (3:1) to give compounds **9**–**14**.

4.7.1. N-(Benzothiazol-2-yl)-5-imino-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxamide (**9**)

Yellow crystals; yield 74%; mp 283–284 °C; IR (KBr): υ_{max}/cm^{-1} = 3345, 3169, (2NH), 1665 (CO), 1625(C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 6.98, 7.22 (2d, *J* = 4.5 Hz, thiazole–H), 7.25–7.86 (m, 4H, Ar–H), 8.96 (s, 1H, pyrimidine–H_4), 9.21 (s, 1H, NH), 9.65 (s, 1H, NH). Anal. for $C_{14}H_9N_5OS_2$ (327.38): Calcd.: C 51.36, H 2.77, N 21.39%; Found: C 51.21, H 2.67, N 21.42%.

4.7.2. 7-Amino-N-(benzothiazol-2-yl)-tetrazolo[1,5-a]pyrimidine-6-carboxamide (**10**)

Yellow crystals; yield 54%; mp 272–273 °C; IR (KBr): υ_{max}/cm^{-1} = 3434, 3329 (NH_2), 3169 (NH), 1672 (CO). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 6.98 (s, 2H, NH_2), 7.23–7.98 (m, 4H, Ar–H), 8.23 (s, 1H, pyrimidine–H_4), 9.87 (s, 1H, NH). MS *m*/*z* (%): 312 (39), 225 (33), 258 (39), 177 (28), 135 (21), 123 (37), 77 (10). Anal. for $C_{12}H_8N_8OS$ (312.31): Calcd.: C 46.15, H 2.51, N 35.88%; Found: C 46.11, H 2.47, N 35.76%.

4.7.3. N-(Benzothiazol-2-yl)-4-imino-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**11**)

Yellow crystals; yield 74%; mp 288–289 °C; IR (KBr): υ_{max}/cm^{-1} = 3320, 3195 (2NH), 1660 (CO), 1618 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 7.21–7.85 (m, 8H, Ar–H), 8.16 (s, 1H, pyrimidine–H_4), 8.98 (s, 1H, NH), 9.95 (s, 1H, NH). Anal. for $C_{16}H_{11}N_5OS$ (321.36): Calcd.: C 59.80, H 3.45, N 21.79%; Found: C 59.69, H 3.35, N 21.61%.

4.7.4. 4-Amino-N-(benzothiazol-2-yl)-1H-1,5-benzodiazepine-3-carboxamide (**12**)

Yellow powder; yield 68%; mp 206–207 °C; IR (KBr): υ_{max}/cm^{-1} = 3466, 3350 (NH_2), 3275, 3150 (2NH), 1678 (CO). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 6.82 (s, 2H, NH_2), 7.25–8.36 (m, 8H, Ar–H), 8.62 (s, 1H, CH=), 9.87 (s, 1H, NH), 12.22 (s, 1H, NH). MS *m*/*z* (%): 335 (M^+, 100), 267 (55), 178 (14), 150 (67), 135 (12), 133 (33), 123 (31), 108 (31), 77 (47). Anal. for $C_{17}H_{13}N_5OS$ (335.38): Calcd.: C 60.88, H 3.91, N 20.88%; Found: C 60.73, H 3.89, N 20.72%.

*4.7.5. 4-Amino-N-(benzothiazol-2-yl)-pyrimido[1,2-a]benzimidazole-3-carboxamide (**13**)*

Yellow powder; yield 49%; mp 285–286 °C; IR (KBr): υ_{max}/cm^{-1} = 3452, 3340 (NH_2), 3218 (NH), 1645 (CO). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 7.19–8.12 (m, 8H, Ar–H), 8.51 (s, 2H, NH_2), 8.89 (s, 1H, pyrimidine–H_6), 12.82 (s, 1H, NH). MS *m*/*z* (%): 360 (M^+, 8), 343 (100), 212 (36), 177 (2), 150 (16), 134 (7), 123 (5), 90 (15), 77 (9), 63 (17). Anal. for $C_{18}H_{12}N_6OS$ (360.39): Calcd.: C 59.99, H 3.36, N 23.32%; Found: C 59.83, H 3.32, N 23.24%.

*4.7.6. 4-Amino-N-(benzothiazol-2-yl)-8,10-dimethylpyrido[2′,3′:3,4]pyrazolo[1,5-a] pyrimidine-3-carboxamide (**14**)*

Deep red powder; yield 54%; mp 296–297 °C; IR (KBr): υ_{max}/cm^{-1} = 3430, 3301 (NH_2), 3179 (NH), 1678 (CO). ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.70 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 6.98 (s, 1H, pyridine–H_5), 7.36–7.68 (m, 4H, Ar–H), 7.88 (s, 2H, NH_2), 8.27 (s, 1H, pyrimidine–H_4), 10.24 (s, H, NH). MS *m*/*z* (%): 389 (M^+, 61), 356 (17), 240 (100), 173 (25), 150 (23), 77 (6.5). Anal. for $C_{19}H_{15}N_7OS$ (389.43): Calcd.: C 58.60, H 3.88, N 25.18%; Found: C 58.45, H 3.61, N 25.07%.

5. Antimicrobial evaluation

Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the test compound in DMF (1 mg/mL) were placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *B. subtilis* and *B. thuringiensis* as examples of Gram positive bacteria and *E. coli* and *P. aeruginosa* as examples of Gram negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *B. fabae* and *F. oxysporum* fungal strains. Streptomycin, Chloroamphenicol and Treflucan were used as standard antibacterial and antifungal agents, respectively. DMF alone was used as control at the same above-mentioned concentration. The plates were incubated at 37 °C for 24 h for bacteria and for 48 days for fungi. Compounds that showed significant growth inhibition zones (>10 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

5.1. Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activities, respectively. Stock solutions of the tested compounds, Streptomycin, Chloroamphenicol and Treflucan were prepared in DMF at concentration of 1000 μg/mL followed by twofold dilution at concentrations of (500, 250,…, 3.125 μg/mL). The microorganism suspensions at 10^6 CFU/mL (Colony Forming Unit/mL) concentration were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h and the minimal inhibitory concentrations (MICs) were determined. Control experiments were also done.

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