Synthesis and Antimicrobial Activity of Polyfunctionally Substituted Heterocyclic Compounds Derived from 5-Cinnamoylamino-2-Cyanomethyl-1,3,4-Thiadiazole

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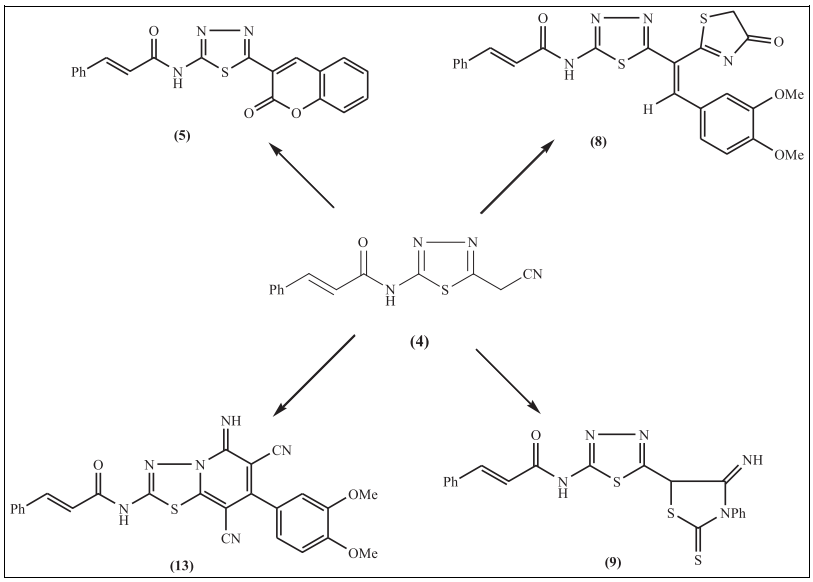

Cinnamoyl isothiocyanate **1** was reacted with 2-cyanoethanoic acid hydrazide **2** to afford 1-cyanoacetyl-4-substituted thiosemicarbazide **3,** which on treatment with a mixture of glacial acetic acid and acetic anhydride gave the desired 5-cinnamoylamino-2-cyanomethyl-1,3,4-thiadiazole **4**. Compound 4 was subjected to react with aromatic aldehydes, phenylisothiocyanate, carbon disulphide, and arylidene malononitrile to give coumarin **5**, thiazolidines **8**,**9,** and 1,3,4-thiadiazolo[3,2-a]pyridine **13** derivatives. The structures of all synthesized compounds were ascertained by spectral and analytical data. Antimicrobial activity of some of prepared compounds was investigated, and compounds **7, 8** were found to exhibit the highest strength.

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INTRODUCTION

Aroyl and acyl isothiocyanates are important reagents, which can be transformed to a variety of heterocyclic derivatives on reacting with polyfunctional molecules, either *via* addition followed by cyclization or *via* cycloaddition [1]. Treatment of aroyl and/or acyl isothiocyanates with acid hydrazids yields substituted thiosemicarbazides, which undergo cyclization to different heterocyclic compounds depending on the reaction conditions. Under basic conditions, substituted thiosemicarbazides undergo cyclization to 1,2,4-triazole-3-thione derivatives [2–10]. On the other hand, under acidic conditions, substituted thiosemicarbazides undergo dehydrative cyclization to 1,3,4-thiadiazole derivatives [4,7–13].

The 1,3,4-thiadiazole moiety is one of those compounds at the apex of chemists attention owing to its biological and pharmaceutical importance. A literature survey shows that 1,3,4-thiadiazole derivatives exhibit antimicrobial [14–18], anti-inflammatory [18,19], antitumor [20–23], antihyperlipidemic [24], anticonvulsant [25,26], antiviral [27], antioxidant [28], antifungal [29,30], antitubercular [31,32], and antidepressant activities [33]. In addition, 1,3,4-thiadiazole has important technological uses as corrosion and oxidation inhibitors [34], metal complexation agents [35,36], and in analytical fields [37]. In view of these fascinating and encouraging results and in continuation of our work on biologically active nitrogen and sulfur heterocycles [38–40], we have synthesized some 2,5-disubstituted 1,3,4-thiadiazoles by adopting a different methodology.

RESULTS AND DISCUSSION

In this investigation, the nucleophilic addition of 2-cyanoacetohydrazide **2** to cinnamoyl isothiocyanate **1** in refluxing dioxane afforded a mixture of two products, which were easily separated by fractional crystallization. The soluble fraction in methanol was identified as 5-cinnamoylamino-2-cyanomethyl-1,3,4-thiadiazole (63%) **4** and the insoluble fraction recrystallized from dioxane and was identified as 4-cinnamoyl-1-(2-cyanoacetyl) thiosemicarbazide (17%) **3**. Compound **3** readily cyclized to the corresponding 1,3,4-thiadiazole derivative **4** using a mixture of acetic anhydride and glacial acetic acid (Scheme 1)**.**

The structure of the acyclic product **3** was deduced from appropriate analytical and spectroscopic data. Thus, ^{1}H–NMR spectrum (DMSO-d_6) disclosed downfield three singlets each integrating for 1H (NH) at δ 12.5, 11.7, and 11.21 ppm, aromatic protons (5H) as a multiplet at δ 7.78–7.46 ppm together with two doublets each integrating for one hydrogen at δ 7.03 and 6.98 ppm with coupling constant J = 15 Hz characteristic of trans olefinic protons. The cyanomethylene protons (2H) of the cyanoacetamide group appeared upfield at δ 3.86 ppm. Complete evidence for the acyclic structure **3** was forthcoming from the mass spectrum, which showed the correct molecular ion peak at m/z = 288 (74.2%). In addition, the base peak at m/z = 131 attributable to the cinammoyl cation $PhCH = CHCO^+$ is in harmony with the assigned structure **3**. Cyclization of the thiosemicarbazide derivative **3** using a mixture of glacial acetic acid and acetic anhydride afforded the 1,3,4-thiadiazole derivative **4** as the sole product (Scheme 1). The structure of compound **4** was deduced from its spectroscopic and analytical data.

The proclivity of compound **4** towards electrophilic reagents such as aromatic aldehydes, carbon disulfide, phenyl isothiocyanate, and nucleophilic reagents such as hydrazine hydrate and hydrazides was investigated. Thus, the reaction of **4** with salicylaldehyde in refluxing dioxane in the presence of a catalytic amount of piperidine afforded the coumarin derivative **5**. On the other hand, treatment of the acyclic product **3** with salicyaldehyde under the same conditions affords **6,** which on treatment with a mixture of freshly distilled acetic anhydride and glacial acetic acid yielded coumarin derivative **5** (Scheme 2).

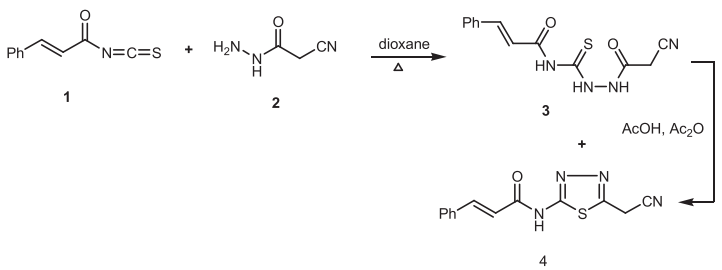
The structure of coumarin derivative **5** was verified with microanalytical data and was confirmed by spectroscopic data. Therefore, the IR spectrum of **5** revealed the absence of a stretching band for the nitrile group and the presence of an oxo-coumarin band at 1712cm^{-1}. Moreover, the ^{1}H–NMR spectrum of compound **5** displayed signals characteristic of the NH proton at δ 10.92 ppm as a broad singlet that disappeared with D_2O, a singlet for the C_4-H coumarin proton at δ 8.50 ppm, a multiplet for aromatic protons (9H) at δ 7.87–7.37 ppm, and two doublets for the trans-olefinic protons at δ 7.0 and 6.90 ppm, which are in accord with the proposed structure **5**. Furthermore, the highest recorded peak in the mass spectrum of **5** at m/z = 375 (9.7%) represents the molecular ion peak, which upon loss of cinnamoyl radical yielded the base peak at m/z = 245 (100%).

On the other hand, treatment of 1,3,4-thiadiazole derivative **4** with 3,4-dimethoxybenzaldehyde in dioxane and in the presence of a catalytic amount of piperidine afforded the corresponding arylidene derivative **7** (Scheme 3). The structure of **7** was confirmed by the analytical and spectroscopic data. Thus, the IR spectrum of **7** showed one weak absorption band of NH at 3198cm^{-1}, $\nu_{C\equiv N}$ (conjugated) at 2201cm^{-1} and $\nu_{C=O}$ at 1670cm^{-1}. The strong clue for the structure **7** was forthcoming from the study of its mass and ^{1}H–NMR spectra, which is compatible with the proposed structure.

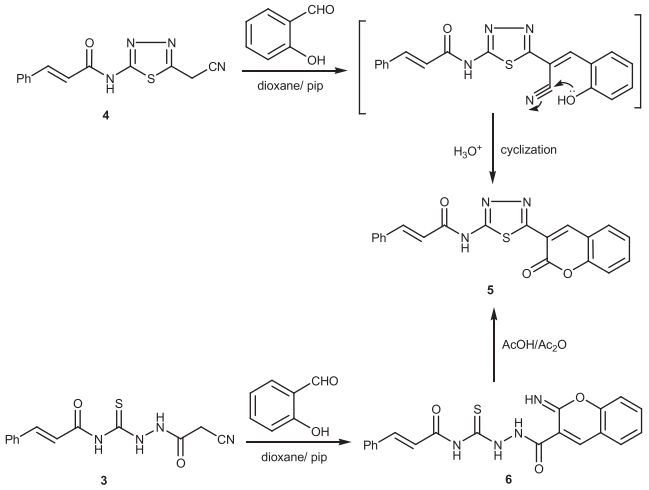
The reaction of the arylidene derivative **7** with mercaptoacetic acid in refluxing pyridine yielded the thiazolidinone derivative **8** (Scheme 3). The IR spectrum of **8** revealed the absence of stretching band of the nitrile group and retained carbonyl stretching band at 1695cm^{-1}. Location of a new $\nu_{C=N}$ at 1630cm^{-1} is in harmony with the assigned structure. The structure of **8** was further supported by mass spectroscopy as the molecular weight inferred the incorporation of one mercaptoacetic acid molecule in the reaction product.

Treatment of compound **4** with phenylisothiocyanate and elemental sulfur in presence of a catalytic amount of triethylamine in ethanol yielded the thiazole-2-thione

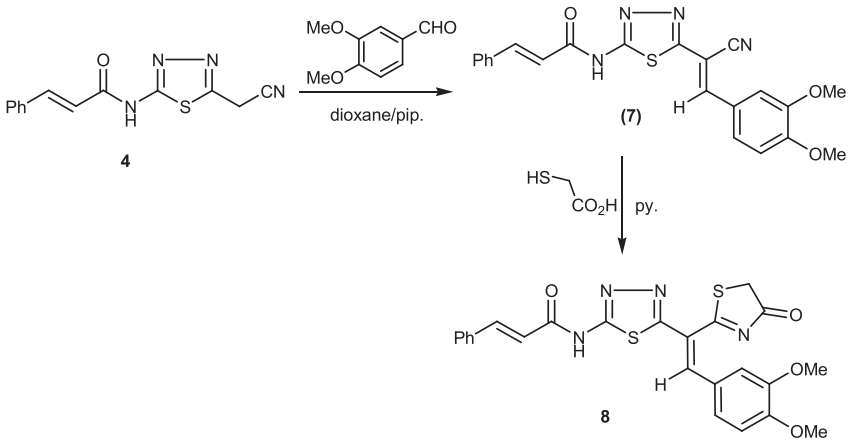
Scheme 1. Synthesis of 5-cinnamoylamino-2-cyanomethyl-1,3,4-thiadiazole.



Scheme 2. Synthesis of 2-(2-Oxo-2*H*-chromen-3-yl)-5- cinnamoylamino-1,3,4-thiadiazole.



Scheme 3. Synthesis of the 1,3,4-thiadiazole derivatives **7** and **8.**

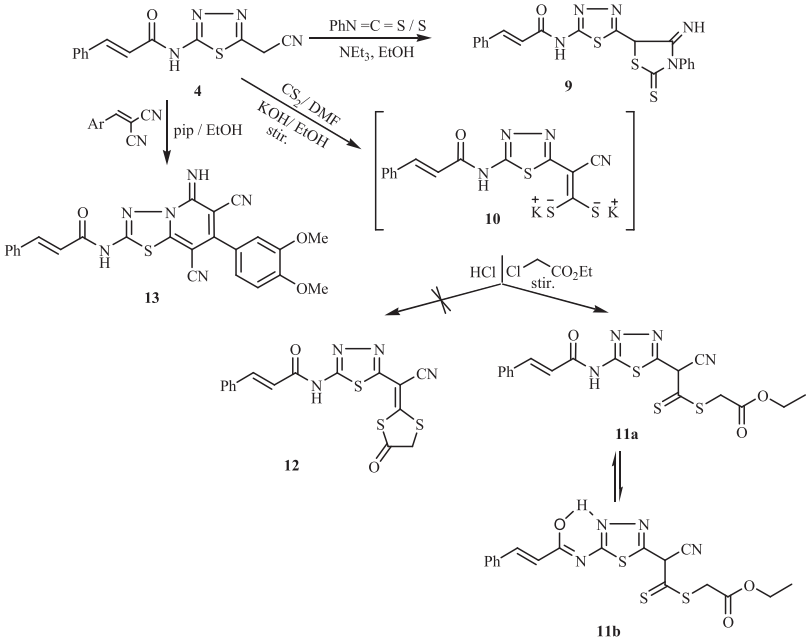


derivative **9** (Scheme 4). The IR spectrum of compound **9** revealed the absence of the stretching band of the nitrile group and retained three weak bands of NH at 3453, 3277, 3142cm^{-1} together with carbonyl stretching band at 1674cm^{-1}. Moreover, the ^{1}H–NMR spectrum of compound **9** revealed signals characteristic for three types of protons, which are consistent with the proposed structure **9**.

Stirring compound **4** with carbon disulphide in ethanolic potassium hydroxide (10%) and dimethyl formamide for 3 h yielded the dipotassium disulphide salt **10,** which *in situ* added to ethyl chloroacetate followed by acidification with cold dilute hydrochloric acid gave the uncyclized product **11** (Scheme 4). No evidence was detected for the cyclized product **12** because the IR spectrum of the product obtained exhibited broad band at 3439cm^{-1} (bonded OH, NH), $\nu_{C\equiv N}$ at 2202cm^{-1}, $\nu_{C=O}$ (ester) at 1727cm^{-1}, $\nu_{C=O}$ (α,β-unsaturated amide) at 1677cm^{-1} and $\nu_{C=N}$ at 1624cm^{-1}.

Furthermore, when 1,3,4-thiadiazole derivative **4** was subjected to react with 3,4-dimethoxybenzylidene malononitrile in refluxing ethanol in presence of a catalytic amount of piperidine, the 1,3,4-thiadiazolo[3,2-a]pyridine derivative **13** was obtained (Scheme 4). Structure **13** was substantiated from the microanalytical and spectroscopic data. Thus, the IR spectrum of **13** displayed ν_{NH} at 3245 (w) and 3224 (w), 3170cm^{-1}, $\nu_{C\equiv N}$ (conjugated) at 2216cm^{-1}, $\nu_{C=O}$ at 1687cm^{-1}, and $\nu_{C=N}$ at 1628cm^{-1}, which agree well with the assigned structure. The ^{1}H–NMR spectrum of **13** revealed the presence of a singlet downfield integrating for one proton (NH) at δ 11.01 ppm, which is exchangeable with D_2O, a broad singlet for 1H (=NH) at 8.18 ppm, a multiplet involved the aromatic and olefinic protons at δ 7.68–6.98 ppm

Scheme 4. Synthetic routes of the 1,3,4-thiadiazole derivatives **9**, **11** and **13.**



integrating for 10H, and two singlets each integrating for 3H corresponding to the two methoxyl protons at δ 3.87 and 3.84 ppm. The formation of **13** could be formulated as depicted in Scheme 5.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of some of prepared compounds was investigated using the diffusion agar technique. The bacteria strains used were *Streptococcus pneumoniae* (RCMB 010010) and *Staphlococcus aureus* (RCMB 010028) for Gram-positive and *Pseudomonas aeruginosa* (RCMB 0010043) and *Escherichia coli* (RCMB 010052) for Gram-negative, and results were summarized at Table 1. *Aspergillus fumigatus* (RCMB 02568) and *Candida albicans* (RCMB 05036) were used for Fungi, and results were summarized at Table 2.

The outcomes listed in Table 1 showed that most of screened compounds displayed good inhibition in comparison with the standard drugs. Compounds **7, 8** show the highest strength against *Streptococcus pneumonia* and *Staphlococcus aureus* while compound **4** shows the lowest strength. On the other hand, compounds **7, 8** show the highest power against *Escherichia coli*, and compound **4** shows the lowest power, while all tested

Scheme 5. The suggested mechanism for the formation of 13

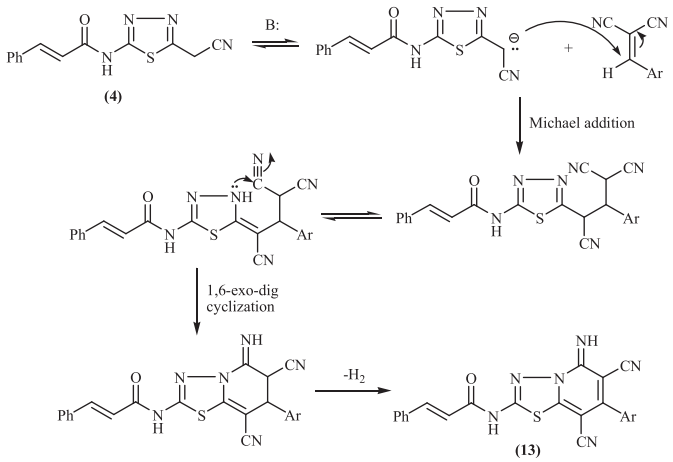


Table 1

Antibacterial activities of some synthesized compounds.

	Microorganisms			
	G^+ bacteria		G^- bacteria	
Code no.	*Streptococcus pneumoniae*	*Staphlococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
4	15.1 ± 0.67	16.3 ± 0.56	NA	14.3 ± 0.72
5	16.3 ± 0.63	17.1 ± 0.36	NA	15.2 ± 0.36
7	21.3 ± 0.53	19.3 ± 0.44	NA	21.3 ± 0.23
8	24.2 ± 0.44	20.3 ± 0.63	NA	23.2 ± 1.50
9	17.1 ± 0.25	18.3 ± 0.44	NA	15.3 ± 0.23
11	16.4 ± 1.50	18.2 ± 0.63	NA	15.8 ± 1.50
13	17.3 ± 0.68	17.9 ± 0.36	NA	15.1 ± 0.58
Standard antimicrobial drug				
Ampicillin	23.8 ± 1.2	27.4 ± 0.72	—	—
Ciprofloxacin	—	—	20.6 ± 1.2	23 ± 0.63

*NA: no activity, data are expressed in the form of mean ± **SD.**

Table 2

Antifungal activities of some synthesized compounds.

	Fungi	
Code no.	*Aspergillus fumigatus*	*Candida albicans*
4	15.3 ± 0.63	14.2 ± 0.36
5	15.6 ± 0.63	15.2 ± 0.36
7	19.3 ± 0.53	17.4 ± 0.44
8	21.3 ± 0.58	18.6 ± 1.20
9	16.9 ± 0.25	14.5 ± 0.44
11	16.3 ± 1.20	15.3 ± 0.58
13	16.2 ± 0.25	15.9 ± 0.44
Amphotericin B	23.7 ± 1.20	21.9 ± 0.58

compounds showed no power against *Pseudomonas aeruginosa*. Moreover, all test compounds were found to have high toxicity for gram-positive than gram-negative bacteria, which may be attributed to the differences in cell-wall structural, where the walls of Gram-negative strains are more complex than those of Gram-positive strains. Again, compounds **7, 8** exhibit the highest strength against *Aspergillus fumigatus* and *Candida albicans* while compound **4** shows the lowest strength as shown in Table 2.

Minimum inhibition concentration for compounds **4, 7, 8** was determined and given in Table 3.

CONCLUSION

5-Cinnamoylamino-2-cyanomethyl-1,3,4-thiadiazole was used to synthesize different heterocyclic derivatives, and their structures were confirmed by analytical and spectroscopic analysis. Some of the newly synthesized compounds were tested for *in vitro* antibacterial and antifungal activities, and the interpretation of the obtained results shows that some of tested compounds have exhibit obvious antibacterial and antifungal activities.

EXPERIMENTAL

The melting points were measured on a Gallenkamp melting point apparatus. The FTIR spectra were recorded on a Pye Unicam SP-3-300 spectrometer. ^{1}H–NMR spectra were run on a Varian Mercury VX-300 NMR 300 MHz spectrometer. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at

Table 3

Minimum inhibition concentration (MIC) for compounds 4,7,8.

org.	4	7	8	Ampicillin	Ciprofloxacin	Amphotericin B
Streptococcus pneumoniae	31.25	1.95	0.98	0.98	—	—
Staphlococcus aureus	31.25	3.90	3.90	0.49	—	—
Pseudomonas aeruginosa	NA	NA	NA	—	1.95	—
Escherichia coli	62.50	1.95	0.98	—	0.98	—
Aspergillus fumigates	31.25	3.90	1.95	—	—	0.98
Candida albicans	62.50	15.63	3.90	—	—	0.49

*NA: no activity, MIC values were determined in μg/ml.

70e.V. Elemental analysis was performed by Vario EL-III elemental analysis.

Synthesis of (3) and (4). Ammonium thiocyanate (1 g, 0.01 mol) was added to a solution of cinnamoyl chloride (1.66 g, 0.01 mol) in dioxane (10 mL), and then the reaction mixture was stirred for 10 min. The reaction mixture was filtrated off, and the filtrate was added to a solution of cyanoacetohydrazide (1 g, 0.01 mol) in dioxane (10 mL), and then the reaction mixture was heated under reflux for 15 min. The formed solid (two spots on TLC) was collected by filtration, dried, and fractionally crystallized from methanol to give **4** (63%), while the residue was crystallized from dioxane to afford the **3** (17%).

4-Cinnamoyl-1-(2-cyanoacetyl)thiosemicarbazide (3). White crystals, Yield: 17%, mp: 235–236°C; ^{1}H–NMR (DMSO-d_6) δ (ppm): 12.50 (s, 1H, NH, exchangeable with D_2O), 11.71 (s, 1H, NH, exchangeable with D_2O), 11.21 (s, 1H, NH, exchangeable with D_2O), 7.78–7.46 (m, $5H_{arom.}$), 7.03 (d, H, PhCH=, J = 15.4 Hz), 6.98 (d, 1H, PhCH = CH, J = 15.4 Hz), 3.86 (s, 2H, $\underline{CH_2}$CN); IR (KBr) ν(cm^{-1}): 3297, 3221 (NH), 2267 (CN), 1680, 1666 (C = O); MS m/z (%): 288 ($M^{\cdot+}$; 74), 270 (21), 204 (24), 131 (100), 103 (90), 77 (89); *Anal*. Calcd. for $C_{13}H_{12}N_4O_2S$ (288.315): C, 54.16; H, 4.19; N, 19.43; S, 11.12. Found: C, 54.10; H, 4.08; N, 19.39; S, 11.08.

5-Cinnamoylamino-2-cyanomethyl-1,3,4-thiadiazole (4). White crystals, Yield: 63%, mp: 218–220°C; ^{1}H–NMR (DMSO-d_6) δ (ppm): 11.72 (s, 1H, NH, exchangeable with D_2O), 7.78–7.45 (m, $5H_{arom.}$), 7.03(d, 1H, PhCH=, J = 15.7 Hz), 6.98 (d, 1H, PhCH = CH, J = 15.7 Hz), 3.86 (s, 2H, $\underline{CH_2}$CN); IR (KBr) ν(cm^{-1}): 3158 (NH), 2252 (CN), 1691 (C = O), 1634 (C = N); MS m/z (%): 270 ($M^{\cdot+}$; 21), 202 (20), 131 (6), 76 (46), 69 (100). *Anal*. Calcd. for $C_{13}H_{10}N_4OS$ (270.302): C, 57.77; H, 3.73; N, 20.73; S, 11.86. Found: C, 57.80; H, 3.70; N, 20.68; S, 11.80.

Cyclization of (3). Thiosemicarbazide derivative **3** (1 g), freshly distilled acetic anhydride (10 mL) and glacial acetic acid (10 mL) was heated under reflux for 2 h. The reaction mixture was concentrated and then poured into ice/cold water. The yielded solid was separated by filtration, washed with water, dried, and recrystallized from methanol to give **4** (identity M.P., mixed M.P., IR, and TLC comparison).

2-(2-Oxo-2H-chromen-3-yl)-5-cinnamoylamino-1,3,4-thiadiazole (5). A mixture of thiadiazole **4** (2.7 g, 0.01 mol), salicylaldehyde (1.23 mL, 0.01 mol), and piperidine (0.5 mL) in dioxane (20 mL) was refluxed for 2 h. The reaction mixture was concentrated and then poured into ice/cold water and acidified with concentrated hydrochloric acid. The yielded solid was separated by filtration, washed with water, dried, and recrystallized from ethanol/dioxane mixture (7:3) to give **5** as yellow crystals, Yield: 51%, mp: 228–230°C; ^{1}H–NMR (DMSO-d_6) δ (ppm): 10.92 (s, 1H, NH, exchangeable with D_2O), 8.50 (s, 1H, C_4-H coumarin), 7.87–7.37 (m, $9H_{arom.}$), 6.92 (d, 1H, PhCH=, J = 15.4 Hz), 6.54 (d, 1H,PhCH = CH, J = 15.4 Hz). IR (KBr) ν(cm^{-1}): 3190 (NH), 1712, 1689 (C=O), 1636 (C=N) MS m/z (%): 375 ($M^{\cdot+}$; 10), 245 (100), 172 (79), 146 (71), 131 (90), 103 (98), 77 (78). *Anal*. Calcd. for $C_{20}H_{13}N_3O_3S$ (375.392): C, 63.99; H, 3.49; N, 11.19; S, 8.54. Found: C, 63.92; H, 3.52; N, 11.10; S, 8.58.

4-Cinnamoyl-1-(2-imino-2H-chromene-3-carbonyl)thiosemicarbazide (6). To a solution of compound **3** (2.88 g, 0.01 mol) in dioxane (20 mL), salicylaldehyde (1.23 mL, 0.01 mol) was added with piperidine (0.2 mL), and then the reaction mixture was refluxed for 3 h. After evaporation of excess solvent and acidification with dilute cold hydrochloric acid, the solid separated was collected by filtration, washed with water, dried, and then recrystallized from dioxane to give **6**, yellowish-white crystals, Yield: 43%, mp: 208–210°C; IR (KBr) ν(cm^{-1}): 3254, 3158 (NH), 1691 (C = O), 1634 (C = N); MS m/z (%): 392 ($M^{\cdot+}$; 14), 245 (79), 206 (40), 173 (41), 146 (92), 131 (100), 103 (89), 77 (99). *Anal*. Calcd. for $C_{20}H_{16}N_4O_3S$ (392.419): C, 61.22; H, 4.11; N, 14.28; S, 8.17. Found: 61.18; H, 4.17; N, 14.32; S, 8.10.

Cyclization of (6). Thiosemicarbazide derivative **6** (1 g), acetic anhydride (10 mL), and glacial acetic acid (10 mL) were heated under reflux for 2 h. The reaction mixture was concentrated and then poured into ice/cold water. The yielded solid was collected by filtration, washed with water, dried, and recrystallized from dioxane to give **5** (identity M.p., mixed M.p., IR, and TLC comparison).

2-[1-Cyano-2-(3,4-dimethoxyphenyl)vinyl]-5-cinnamoylamino-1,3,4-thiadiazole (7). A mixture of thiadiazole **4** (2.7 g, 0.01 mol), 3,4-dimethoxybenzaldehyde (1.66 g, 0.01 mol) and piperidine (0.5 mL) in ethanol (20 mL) was refluxed for 1 h. The produced solid on hot was separated by filtration, dried, and recrystallized from dioxane to give **7** as yellow crystals, Yield: 48%, mp:160–162°C; ^{1}H–NMR (DMSO-d_6) δ (ppm): 11.68 (s, 1H, NH, exchangeable with D_2O), 8.20 (s, 1H, $\underline{CH}$=), 7.78–7.73 (m,$3H_{arom.}$), 7.71–7.47 (m,$5H_{arom.}$), 7.20 (d, 1H, PhCH=, J = 15.2 Hz), 7.04 (d, 1H, PhCH = CH, J = 15.2 Hz), 3.88(s, 3H,OCH_3), 3.83 (s, 3H,OCH_3); IR (KBr) ν(cm^{-1}): 3189 (NH), 2201 (C≡N), 1670 (C=O), 1618 (C=N); MS m/z (%): 418 ($M^{\cdot+}$; 23.9), 288 (23), 151 (54), 131 (100), 103 (99), 77 (55). *Anal*. Calcd. for $C_{22}H_{18}N_4O_3S$ (418.455): C, 63.15; H, 4.33; N, 13.39; S,7.66. Found: C, 63.20; H, 4.29; N, 13.35; S, 7.70.

2-[2-(3,4-Dimethoxyphenyl)-1-(4-oxo-4,5-dihydrothiazol-2-yl)vinyl]-5-cinnamoylamino-1,3,4-thiadiazole (8). A mixture of arylidene derivative **7** (2.1 g, 0.005 mol) and mercaptoacetic acid (0.46 g, 0.005 mol) and in pyridine (15 mL) was heated under reflux for 7 h. The reaction mixture was concentrated and then poured into ice/cold water and acidified with concentrated hydrochloric acid.

The yielded solid was separated by filtration, washed with water, dried, and recrystallized from methanol to give **8**, pale yellow crystals, Yield: 47%, mp:270–272°C; IR (KBr) ν(cm^{-1}): 3196 (NH), 1695 (C=O), 1630 (C=N); MS m/z (%): 492 ($M^{\cdot+}$; 1), 459 (2), 287 (11), 260 (11), 216 (21), 151 (100), 131 (64), 103 (36), 77 (55). *Anal*. Calcd. for $C_{24}H_{20}N_4O_4S_2$ (492.556): C, 58.52; H, 4.09; N, 11.37; S,13.02. Found: C, 58.48; H, 3.99; N, 11.42; S, 13.08.

2-(4-Imino-3-phenyl-2-thioxothiazolidin-5-yl)-5-cinnamoyl-amino-1,3,4-thiadiazole (9). A mixture of arylidene derivative **7** (2.1 g, 0.005 mol), phenylisothiocyanate (0.65 mL, 0.005 mol), elemental sulfur (0.16 g, 0.005 mol), and triethylamine (0.5 mL) in ethanol (20 mL) was heated under reflux for 4 h. The produced solid after cooling was separated by filtration, dried, and recrystallized from dioxane to give **9** as yellow crystals, Yield: 47%, mp: 270–272°C; ^{1}H–NMR (DMSO-d_6) δ (ppm): 11.28 (s, 1H, =NH, exchangeable with D_2O), 10.36 (s, 1H, NH, exchangeable with D_2O), 7.44–7.35 (m,12H, $10H_{arom}$ + 2H olefinic protons), 4.92 (s, 1H,C_5-H of thiazole ring); IR (KBr) ν(cm^{-1}): 3453, 3277, 3142 (NH), 1674 (C=O), 1280 (C=S); MS m/z (%): 437 ($M^{\cdot+}$; 9), 307 (10), 208 (70), 131 (91), 103 (92), 77 (90). *Anal*. Calcd. for $C_{20}H_{15}N_5OS_3$ (437.552): C, 54.90; H, 3.45; N, 16.00; S,21.99. Found: C, 54.95; H, 3.40; N, 16.05; S, 21.92.

2-[Ethyl 2-(2-cyanoethanethioylthio)acetate]-5-cinnamoy lamino-1,3,4-thiadiazole (11). The thiadiazole **4** (2.7 g, 0.01 mol) was added to a cold suspension of finally deviated potassium hydroxide (0.56 g, 0.01 mol) in dry dimethyl formamide (20 mL), and then the reaction mixture was stirred for 15 min. Carbon disulphide (0.76 mL, 0.01 mol) was added drop wise, and the reaction mixture was allowed to stand for 3 h. The reaction mixture was cooled to 0°C and stirred with ethyl chloroacetate (1.23 mL, 0.01 mol) at room temperature for 3 h and then was allowed to stand overnight. The reaction mixture was poured into ice/cold water and acidified with concentrated hydrochloric acid. The obtained solid was separated by filtration, washed with water, dried, and recrystallized from methanol to give **11** as pal yellow crystals, Yield: 75%, mp:180–182°C; ^{1}H–NMR (DMSO-d_6) δ (ppm): 10.28 (s, 1H, NH, exchangeable with D_2O), 7.64–7.48 (m,5H_{arom}), 7.06 (d, 1H, PhCH=, J = 15.4 Hz), 6.96 (d, 1H, PhCH = CH, J = 15.4 Hz), 4.23 (s, 1H, CHCN), 4.207 (q, 2H, CH_2CH_3, J = 7.2 Hz), 4.21 (s, 2H, S CH_2CO), 1.22 (t,3H, CH_2CH_3, J = 7.2 Hz); IR (KBr) ν(cm^{-1}): 3439 (NH), 2202 (C≡N), 1727, 1677 (C=O), 1624 (C=N); MS m/z (%): 432 ($M^{\cdot+}$, 11), 386 (11), 358 (2), 267 (4), 229 (6), 131 (17), 103 (8), 77 (11). *Anal*. Calcd. for $C_{18}H_{16}N_4O_3S_3$ (432.528): C, 49.98; H, 3.72; N, 12.95; S, 22.24. Found: 49.92; H, 3.68; N, 13.05; S, 22.30.

2- Cinnamoylamino -6,8-dicyano-7-(3,4-dimethoxyphenyl)-5-imino-5H-1,3,4-thiadiazolo[3,2-a]pyridine (13). A mixture of thiadiazole **4** (2.7 g, 0.01 mol), 3,4-dimethoxybenzylidene malononitrile (2.2 g, 0.01 mol) and piperidine (0.5 mL) in ethanol (20 mL) was refluxed for 6 h. The solid formed after concentration was separated by filtration, dried, and recrystallized from dioxane to give **13** as light brown crystals, Yield:48%, mp: 270–272°C; ^{1}H–NMR (DMSO-d_6) δ (ppm): 11.01 (s, 1H, NH, exchangeable with D_2O), 8.18 (s, 1H, =NH, exchangeable with D_2O), 7.68–6.98 (m,10H, $8H_{arom}$. + 2H olefinic proton), 3.87 (s, 3H,OCH_3), 3.84 (s, 3H,OCH_3); IR (KBr) ν(cm^{-1}): 3245, 3224, 3170 (NH), 2216 (C≡N), 1687 (C=O), 1628 (C=N); MS m/z (%): 456 ($M^{\cdot+}$-$CN^{\cdot}$; 28), 441 (98), 417 (50), 247 (20), 131 (100), 103 (89), 77 (54). *Anal*. Calcd. for $C_{25}H_{18}N_6O_3S$ (482.500): C, 62.23; H, 3.73; N, 17.42; S,6.65. Found: 62.23; H, 3.46; N, 17.46; S, 6.70.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity was evaluated using the agar diffusion methodolgy [41]. The used bacteria and fungi were preserved on nutrient agar medium and Sabouraud Dextrose agar medium, respectively. The tested compound was disolved in DMF (showed on inhibition zone) to concentration of 5 mg/mL. The diameter of inhibition zone (mm) was measured after 24 h of incubation at 30°C for bacteria and 48 h of incubation at 28°C for fungi. Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm). Ammpicillin and Ciprofloxacin were taken as reference for antibacterial activity and Amphotericin B as reference for antifungal activity, and the results were summarized in Tables 1 and 2. Minimum inhibition concentration values were determined in μg/mL, and the results were summarized in Table 3.

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