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Synthesis and Evaluation of the Anti-Microbial Activity of New Heterocycles Containing the 1,3,4-Thiadiazole Moiety

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Abstract: A new series of thiadiazole-enaminones **4** were synthesized via reactions of 5-acetyl-1,3,4-thiadiazoles **3** with dimethylformamide-dimethylacetal (DMF-DMA). The simple phenyl substituted thiadiazole-enaminone **4f** was used as a synthetic precursor for the preparation of a wide variety of new heterocyclic compounds, including the 5-substituted-1,3,4-thiadiazole derivatives **5**, **6**, **11**, **12** and **13**, which were obtained via reactions of **4f** with nitrogen nucleophiles. Also, reactions of enaminone **4f** with carbon nucleophiles afforded the respective 1,3,4-thiadiazoles **8a–d**. In addition, the results of the antimicrobial activities of thiadiazole-enaminones **4** and their precursors **2** and **3** indicate that some members of this series display promising activities against all tested microorganisms.

Keywords: antimicrobial activity; enaminone; 1,3,4-thiadiazole; nitrogen nucleophiles; carbon nucleophiles

1. Introduction

Substituted 1,3,4-thiadiazoles have attracted considerable interest owing to their wide spectrum biological activity, including antimicrobial, antituberculosis, anesthetic, antithrombotic, anticonvulsant, antihypertensive, anti-inflammatory and antiulcer properties [1-5]. Enaminones are polydentate reagents that have been utilized extensively in this decade as building blocks in organic synthesis [6-12]. In continuation of our previous reports on synthesis of bioactive heterocyclic compounds [10-16] in this investigation we have prepared a new series of enaminone-linked 1,3,4-thiadiazoles and

investigated their chemical reactivity with a variety of nucleophilic reagents. In addition, we tested the biological activity of the resulting thiadiazole derivatives against select microorganisms.

2. Results and Discussion

The new enaminone linked 1,3,4-thiadiazoles **4a–h** were prepared by reaction of the corresponding 5-acetyl-2-benzoylimino-3-aryl-1,3,4-thiadiazoles **3a–h** with dimethylformamide-dimethylacetal (DMF-DMA) under reflux in dry toluene (Scheme 1). The structures of the products were established based on their elemental and spectral data. For example, the ^{1}H-NMR spectra of these products contained two singlet signals at δ 5.8 and 8.1 ppm ($J = 12$ Hz) which correspond to the two trans-olefinic protons in the *E*-enaminone moieties [16,17].

Scheme 1. Synthesis of enaminones **4a–h**.

X: a, OCH$_3$; b, 4-CH$_3$; c, 4-Br; d, 4-Cl; e, 4-NO$_2$; f, H; g, 3-CH$_3$; h, 3-Cl
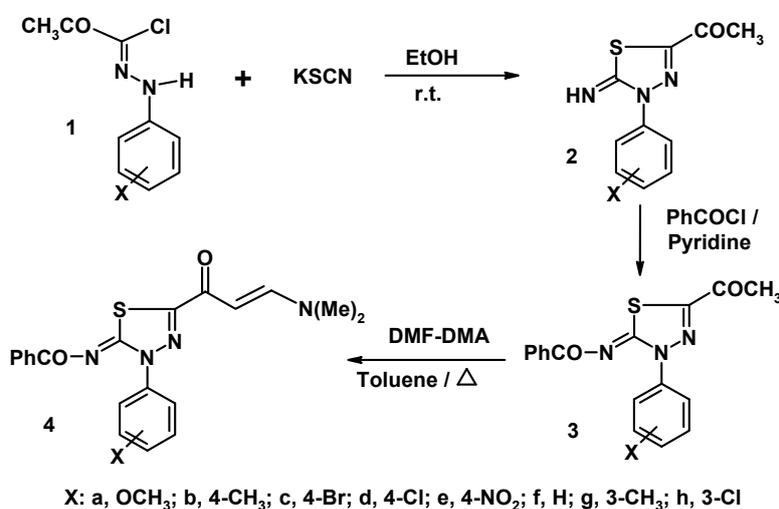
Reaction of enaminone **4f** with hydrazine hydrate in ethanol under reflux led to formation of the thiadiazole-pyrazole linked product **5** (Scheme 2) whose structure was assigned using spectroscopic and elemental analysis methods. For example, the IR spectrum of this compound contains a carbonyl band at 1,607 cm^{-1} attributed to the benzamide group and it does not contain an enaminone group-associated carbonyl band. Also, no olefinic or methyl proton resonances were observed in the ^{1}H-NMR spectrum of **5**, which did contain a singlet at δ 9.21 ppm due to the pyrazolyl-NH proton. In a related manner, reaction of enaminone **4f** with hydroxylamine hydrochloride in ethanol in the presence of potassium carbonate led to formation of the thiadiazole-isoxazole **6**. The structure of the latter compounds was also established based on both elemental and spectral data (see Experimental). The reactivity of enaminone **4f** towards several C- nucleophiles was explored next. Compound **4f** reacts with active methylene compounds in acetic acid in the presence of ammonium acetate under reflux to afford products that could have either of the regioisomeric linked thiadiazole-pyridine structures represented by either **8** or **10**. Two pathways are outlined in Scheme 3 for this reaction. The reaction may proceed by initial Michael addition (route A) of the active methylene compound to the activated double bond of **4f** to give the Michael adduct **7** followed by tandem elimination of dimethylamine and condensation with ammonia to give product **8** or the other suggested pathway (route B) may proceed by initial condensation of active methylene compound with the carbonyl group

of **4f** which leads to formation of intermediate **9** that cyclizes in the presence of ammonium acetate to give **10**. The latter product **10** was discarded however based on its ^{1}H-NMR spectral data. For example, the ^{1}H-NMR spectrum of compound **8a** revealed two singlet signals at δ 2.49 and 2.63 ppm assigned to the methyl and acetyl protons, in addition to two doublets at δ 8.04 and 8.39 ppm ($J = 7$–8 Hz) assigned to pyridine H-3 and H-4. Such value of coupling constant J is characteristic for pyridine H-3 and H-4 and much higher than that for H-2 and H-3 ($J = 4$–6 Hz) [18,19] in structure **10** (Scheme 3).

**Scheme 2.** Reactions of enaminone **4f** with hydrazine hydrate and hydroxylamine.
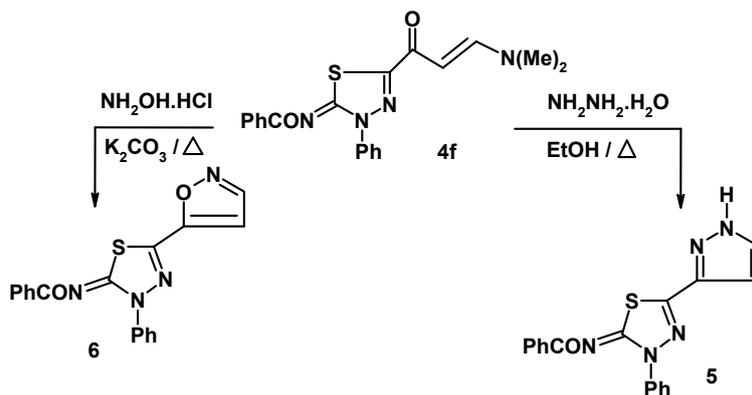
Scheme 3. Reaction of enaminone **4f** with active methylene compounds.

	R/R'
a	Me/ COMe
b	Me/COOEt
c	Ph/COOEt
d	Ph/COPh
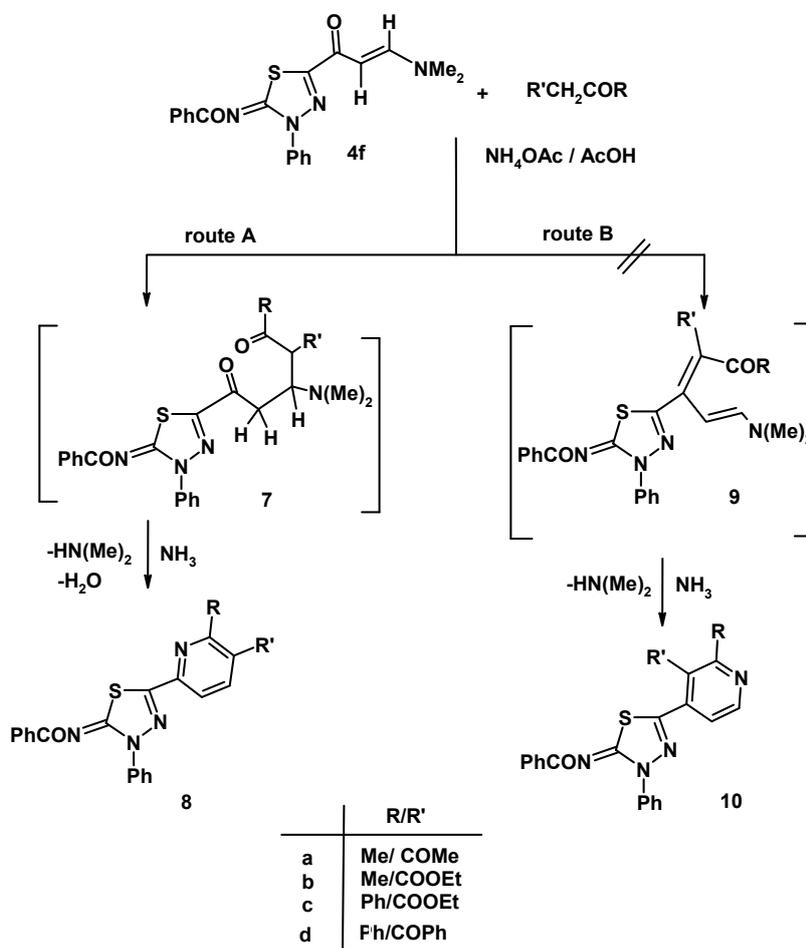
Also, the reactivity of the enaminone **4f** towards some heterocyclic amines was examined. For example, 5-amino-1,2,4-triazole was found to react with **4f** in acetic acid to yield the 1,2,4-triazolo[1,5-*a*]pyrimidine derivative **11** (Scheme 4). Similarly, treatment of **4f** with each of 2-aminobenzimidazole and 5-amino-3-phenylpyrazole under the same reaction conditions afforded the respective benzimidazo[1,2-*a*]pyrimidine **12** and pyrazolo[1,5-*a*]pyrimidine derivatives **13** (Scheme 4). The ^{1}H-NMR spectrum of each of the products **11**, **12** and **13** contains two doublets in the regions 7.93–8.11 ppm and 8.08–9.04 ppm with *J* values of 4.5 Hz that are assignable to the two vicinal protons in the pyrimidine moieties [20,21].

Scheme 4. Reaction of enaminone **4f** with heterocyclic amines.
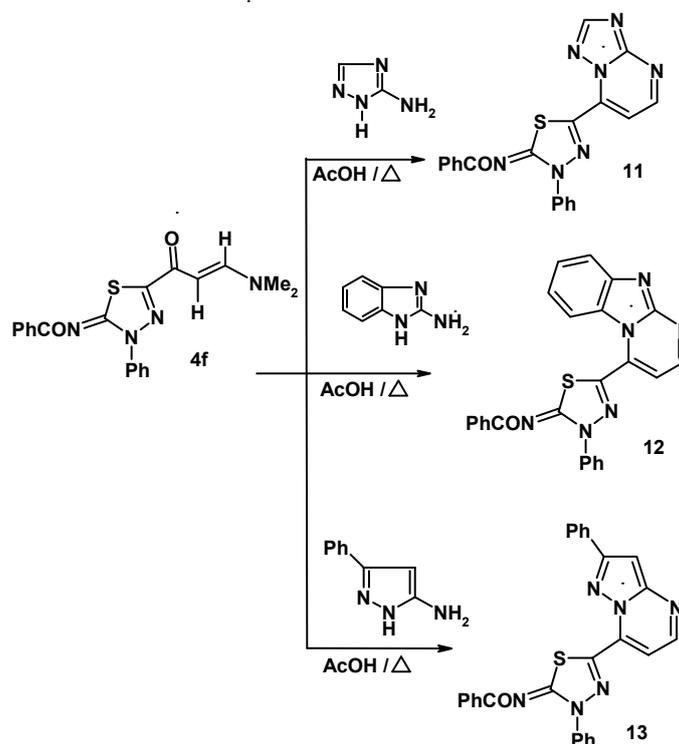
A plausible mechanistic pathway for formation of **11**, **12** and **13** involves Michael addition of the exocyclic amino group of the amines to the enaminone double bond of **4f** followed by *in situ* tandem elimination of dimethylamine and dehydrative cyclization (route A) (Scheme 5). Another route (B), producing regioisomer **16** via intermediate **15** does not operate in this process.

2.1. Biological Screening Anti-Microbial Activities

In vitro anti-microbial screening of the compounds **2**, **3** and **4** prepared in this study was carried out using four fungal strains, including *Aspergillus fumigatus* RCMB 002003 (AF), *Geotrichum candidum* RB 052006 (GC)*, Candida albicans* RCMB 005002 (CA) and *Syncephalastrum racemosum* RCMB 005003 (SR), and four bacterial species, including the Gram positive bacteria *Staphylococcus aureus* RCMB 000106 (SA) and *Bacillus subtilis* RCMB 000107 (BS), and the Gram negative bacteria *Pseudomonas aeruginosa* RCMB 000102 (PA) and *Escherichia coli* RCMB 000103 (EC). The results of the investigations with the thiadiazole derivatives **2a–h** (Tables 1 and 2) showed that **2b** displays

high activities against all the tested microorganisms. This finding suggests that the presence of an electron-donating C-4 methyl group in the phenyl ring linked to the 1,3,4-thiadiazole moiety promotes increased biological activity. In addition, compound **3c** showed high activities against all tested microorganisms, especially AF, when compared to the standard fungicides itraconazole and clotrimazole. In addition, **3c** showed high activity against all tested bacteria species, especially BS, when compared with the standard bactericides penicillin G and streptomycin. The data obtained by probing the antimicrobial activities of enaminones **4** are given in Tables 3, 4 and 5. The results indicate that **4c** is highly potent against all tested microorganisms. Based on these results, we can conclude that the presence of a bromine substituent at the C-4 of the phenyl group linked to the 1,3,4-thiadiazole moiety causes increased antimicrobial activity.

Scheme 5. The mechanism of reaction of enaminone **4f** with heterocyclic amine.
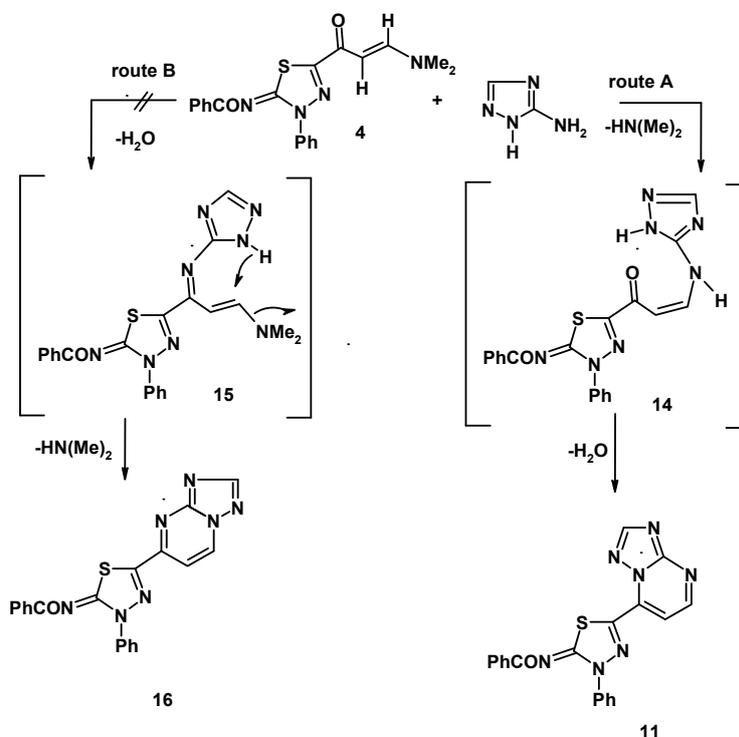

Table 1. Anti-microbial activities.

Microorganism	2a	2b	2c	2d	2e	2f	ST (30 μg/mL)	
Fungi							Itraconazole	Clotrimazole
AF	12.5	22.3	15.9	20.1	15.2	11.5	28.5	26.1
GC	12.5	19.7	18.4	18.3	18.4	12.9	27.1	23.1
CA	11.0	19.4	15.2	15.4	15.4	10.7	26.1	18.3
SR	NA	18.4	NA	8.2	NA	NA	22.3	20.5
Gram Positive Bacteria							Penicillin G	Streptomycin
SA	13.4	22.04	18.2	20.4	12.4	11.2	29.4	25.1
BS	15.3	24.08	19.3	21.1	13.5	12.3	32.5	29.1
Gram negative Bacteria							Penicillin G	Streptomycin
PA	NA	22.9	NA	16.2	NA	NA	28.3	24.3
EC	10.4	21.9	9.4	18.9	8.6	9.8	33.5	25.6

Table 2. Anti-microbial activities.

Microorganism	2g	2h	2i	3a	3b	3c	ST (30 µg/mL)	
Fungi							Itraconazole	Clotrimazole
AF	19.2	19.2	18.3	13.3	11.2	25.3	28.5	26.1
GC	20.4	20.4	18.2	12.4	10.4	24.4	27.1	23.1
CA	17.4	18.2	17.1	11.2	9.4	17.2	26.1	18.3
SR	16.4	14.4	12.0	9.4	NA	19.9	22.3	20.5
Gram Positive Bacteria							Penicillin G	Streptomycin
SA	18.2	19.4	17.5	16.9	13.2	24.7	29.4	25.1
BS	20.3	21.4	19.8	18.2	14.3	28.2	32.5	29.1
Gram negative Bacteria							Penicillin G	Streptomycin
PA	16.4	11.4	14.9	12.8	NA	22.8	28.3	24.3
EC	19.5	16.8	18.9	11.9	9.4	24.4	33.5	25.6

Table 3. Anti-microbial activities.

Microorganism	3d	3e	3f	3g	3h	ST. (30 µg/mL)	
Fungi						Itraconazole	Clotrimazole
AF	10.8	23.1	23.9	12.3	18.4	28.5	26.1
GC	10.2	22.3	17.2	11.4	19.6	27.1	23.1
CA	8.9	17.0	14.5	10.2	16.2	26.1	18.3
SR	NA	19.2	16.2	8.0	14.2	22.3	20.5
Gram Positive Bacteria						Penicillin G	Streptomycin
SA	10.5	23.2	18.5	12.9	14.4	29.4	25.1
BS	12.7	27.3	15.8	13.2	18.4	32.5	29.1
Gram negative Bacteria						Penicillin G	Streptomycin
PA	NA	21.2	19.9	11.0	NA	28.3	24.3
EC	8.5	24.8	20.9	10.8	13.8	33.5	25.6

Table 4. Anti-microbial activities.

Microorganism	4a	4b	4c	4d	4e	4f	ST. (30 µg/mL)	
Fungi							Itraconazole	Clotrimazole
AF	17.3	14.8	24.3	18.5	16.1	15.3	28.5	26.1
GC	18.4	13.9	22.4	19.1	17.1	16.2	27.1	23.1
CA	16.2	12.8	17.8	16.1	11.3	14.7	26.1	18.3
SR	17.9	NA	18.9	12.3	10.5	NA	22.3	20.5
Gram Positive Bacteria							Penicillin G	Streptomycin
SA	17.9	15.7	22.7	16.9	12.4	20.04	29.4	25.1
BS	18.2	17.2	25.2	19.7	13.5	22.08	32.5	29.1
Gram negative Bacteria							Penicillin G	Streptomycin
PA	12.3	NA	21.8	15.4	NA	12.7	28.3	24.3
EC	15.8	12.4	22.4	17.8	8.6	18.6	33.5	25.6

Table 5. Anti-microbial activities.

Microorganism	4g	4h	ST. (30 µg/mL)	
Fungi			Itraconazole	Clotrimazole
AF	14.8	20.2	26.1	28.5
GC	13.9	19.7	23.1	27.1
CA	12.4	16.8	18.3	26.1
SR	NA	11.3	20.5	22.3
Gram Positive Bacteria			Penicillin G	Streptomycin
SA	15.1	21.4	25.1	29.4
BS	16.4	26.1	29.1	32.5
Gram negative Bacteria			Penicillin G	Streptomycin
PA	NA	19.2	24.3	28.3
EC	10.4	20.9	25.6	33.5

3. Experimental

3.1. General

Melting points were determined uisng an Electrothermal Gallenkamp apparatus and are reported uncorrected. IR spectra were recorded in KBr using PyUnicam SP-1000 Spectrometer. ^{1}H-NMR spectra were recorded using CDCl$_3$ and DMSO-d_6 solutions using a Varian Em-300 MHz Spectrometer and chemical shifts are reported in ppm relative to that of TMS, which was used as an internal standard. Mass spectra were recorded using a AEI MS 30 mass spectrometer operating at 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Antimicrobial activities were carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

3.2. Synthesis of Compounds **2a–h** and **3a–h**

Compounds **2a–h** and **3a–h** were prepared using previously described methods [22]. Compounds **2c** and **3c** are newly prepared and their physical constants, together with spectral and elemental analysis are shown below:

3.2.1. *5-Acetyl-3-(4-bromophenyl)-2-imino-1,3,4-thiadiazole* (**2c**)

Gray solid, (80% yield), mp 160 °C (EtOH); IR (KBr) 3238 (NH), 1680 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.46 (s, 3H, CH$_3$), 7.37 (d, *J* = 9 Hz, 2H, ArH), 7.51 (d, *J* = 9 Hz, 2H, ArH), 10.75 (s, 1H, NH); MS *m/z* (%) 298 (M$^+$, 2), 278 (13), 276 (42), 198 (25), 171 (25), 90 (54), 76 (4). Anal. Calcd. for C$_{10}$H$_8$BrN$_3$OS (298.16): C, 40.28; H, 2.70; N, 14.09. Found: C, 40.08; H, 2.51; N, 14.00%.

3.2.2. *N-[5-Acetyl-3-(4-bromophenyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide* (**3c**)

Orange solid, (80% yield), mp 100 °C (EtOH); IR (KBr) 1686, 1608 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.66 (s, 3H, COCH$_3$), 7.48–7.63 (m, 5H, ArH), 7.75 (d, *J* = 9 Hz, 2H, ArH), 8.01 (d, *J* = 9 Hz,

2H, ArH); MS *m/z* (%) 402 (M$^+$, 1), 122 (50), 111 (13), 103 (23), 94 (35), 82 (41), 76 (100). Anal. Calcd. for C$_{17}$H$_{12}$BrN$_3$O$_2$S (402.27): C, 50.76; H, 3.01; N, 10.45. Found: C, 50.53; H, 3.11; N, 10.36%.

3.3. Synthesis of N-[3-aryl-5-(3-dimethylaminoacryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamides **4a–h**

A mixture of the appropriate 1,3,4-thiadiazole derivative **3** (10 mmol) and dimethylformamide-dimethylacetal (DMF-DMA) (2.4 g, 20 mmol) in dry toluene was stirred under reflux for 2 h. After cooling, methanol was added and the resulting solid was collected by filtration, washed with methanol, dried and crystallized from the appropriate solvent to afford the respective enaminones **4a–h**.

3.3.1. N-[3-(4-Methoxyphenyl)-5-(3-dimethylaminoacryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide (**4a**)

Orange solid, (90% yield), mp 260 °C (EtOH); IR (KBr), 1642, 1616 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.94 (s, 3H, CH$_3$), 3.21 (s, 3H, CH$_3$), 3.82 (s, 3H, OCH$_3$), 5.81 (d, *J* = 12 Hz, 1H, =CH), 7.17 (d, *J* = 9 Hz, 2H, ArH), 7.45–7.59 (m, 5H, ArH), 7.83 (d, *J* = 9 Hz, 2H, ArH), 7.91 (d, *J* = 12 Hz, 9H, =CH). Anal. Calcd. for C$_{21}$H$_{20}$N$_4$O$_3$S (408.47): C, 61.75; H, 4.94; N, 13.72. Found: C, 61.53; H, 4.79; N, 13.65%.

3.3.2. N-[3-(4-Methylphenyl)-5-(3-dimethylaminoacryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide (**4b**)

Yellow crystals, (90% yield), mp 250 °C (EtOH/dioxane); IR (KBr) 1644, 1625 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.43 (s, 3H, CH$_3$), 2.94 (s, 3H, CH$_3$), 3,21(s, 3H, CH$_3$), 5.82 (d, *J* = 12 Hz, 1H, =CH), 7.43–7.59 (m, 5H, ArH), 7.82 (d, *J* = 9 Hz, 2H, ArH), 7.91 (d, *J* = 12 Hz, 1H, CH=), 8.08 (d, *J* = 9 Hz, 2H, ArH). MS *m/z* (%) 392 (M$^+$, 12), 104 (24), 85 (14), 77 (17), 98 (100), 97 (16), 104 (24), Anal. Calcd. for C$_{21}$H$_{20}$N$_4$O$_2$S (392.48): C, 64.27; H, 5.14; N, 14.27. Found: C, 64.43; H, 5.07; N, 14.38%.

3.3.3. N-[3-(4-Bromophenyl)-5-(3-dimethylaminoacryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide (**4c**)

Yellow solid, (80% yield), mp 125 °C (EtOH); IR (KBr) 1669, 1598 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.89 (s, 3H, CH$_3$), 3.09 (s, 3H, CH$_3$), 6.20 (d, *J* = 12 Hz, 1H, CH=), 7.30–7.41 (m, 9H, ArH), 7.60 (d, *J* = 12 Hz, 1H, CH=); MS *m/z* (%) 459 (M$^+$, 1), 252 (100), 186 (70), 176 (54), 155 (59), 138 (60), 128 (36), 233 (84), 201 (61), 77(10). Anal. Calcd. for C$_{20}$H$_{17}$BrN$_4$O$_2$S (457.34): C, 52.52; H, 3.75; N, 12.25. Found: C, 52.30; H, 3.54; N, 12.09%.

3.3.4. N-[3-(4-Chlorophenyl)-5-(3-dimethylaminoacryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide (**4d**)

Orange solid, (80% yield), mp 320 °C (EtOH/dioxane); IR (KBr) 1643, 1619 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.68 (s, 3H, CH$_3$), 2.97 (s, 3H, CH$_3$), 5.82 (d, *J* = 12 Hz, 1H, =CH), 7.48–7.60 (m, 5H, ArH), 7.72 (d, *J* = 8 Hz, 2H, ArH), 7.93 (d, *J* = 12 Hz, 1H, =CH), 8.04 (d, *J* = 8 Hz, 2H, ArH);

MS *m/z* (%) 415 (M$^+$+2, 3), 413 (M$^+$, 7), 111 (4), 98 (100), 77 (23). Anal. Calcd. for C$_{20}$H$_{17}$ClN$_4$O$_2$S (412.89): C, 58.18; H, 4.15; N, 13.57. Found: C, 58.02; H, 4.08; N, 13.46%.

3.3.5. *N-[3-(4-Nitrophenyl)-5-(3-dimethylaminoacryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide* (**4e**)

Yellow solid, (86% yield), mp 280 °C (EtOH/dioxane); IR (KBr) 1720, 1638 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-*d$_6$*) 2.98 (s, 3H, CH$_3$), 3.23 (s, 3H, CH$_3$), 5.87 (d, *J* = 12 Hz, 1H, =CH), 7.49–7.63 (m, 5H, Ar-H), 7.93 (d, *J* = 12 Hz, 1H, =CH), 8.39 (d, *J* = 8 Hz, 2H, ArH), 8.51 (d, *J* = 8 Hz, 2H, ArH); MS *m/z* (%) 423 (M$^+$, 5), 105 (40), 98 (100), 97 (11), 77 (31). Anal. Calcd. for C$_{20}$H$_{17}$N$_5$O$_4$S (423.45): C, 56.73; H, 4.05; N, 16.54. Found: C, 56.92; H, 4.08; N, 16.49%.

3.3.6. *N-[3-Phenyl-5-(3-dimethylamino-acryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide* (**4f**)

Orange Solid , (80% yield), mp 268 °C (EtOH); IR (KBr), 1641, 1624 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-*d$_6$*): 5.83 (d, *J* = 12 Hz, 1H, =CH), 2.95 (s, 3H, CH$_3$), 3.22 (s, 3H, CH$_3$), 8.08 (d, *J* = 12 Hz, 1H, =CH) 7.46–7.98 (m, 10H, Ar-H); MS *m/z* (%): 378 (M$^+$, 22), 361 (12), 331 (26), 105 (51), 98 (100), 77 (38). Anal. Calcd. for C$_{20}$H$_{18}$N$_4$O$_2$S (378.45): C, 63.47; H, 4.79; N, 14.80. Found: C, 63.50; H, 4.59; N, 14.67 %.

3.3.7. *N-[3-(3-Methylphenyl)-5-(3-dimethylamino-acryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide* (**4g**)

Orange solid, (82% yield), mp 200 °C (EtOH/Dioxane); IR (KBr) 1690, 1638 (2C=O) cm^{-1}; MS *m/z* (%) 392 (M$^+$, 11), 337 (24), 132 (35), 131 (22), 105 (100), 77 (69). Anal. Calcd. for C$_{21}$H$_{20}$N$_4$O$_2$S (392.48): C, 64.27; H, 5.14; N, 14.27. Found: C, 64.15; H, 5.01; N, 14.10%.

3.3.8. *N-[3-(3-Chlorophenyl)-5-(3-dimethylamino-acryloyl)-3H-[1,3,4]-thiadiazol-2-ylidene]-benzamide* (**4h**)

Orange solid, (90% yield), mp 250 °C (EtOH); IR (KBr) 1646, 1639 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-*d$_6$*) 2.97 (s, 3H, CH$_3$), 3.23 (s, 3H, CH$_3$), 5.81 (d, *J* = 12 Hz, 1H, =CH), 7.48–7.72 (m, 9H, ArH), 7.79 (d, *J* = 12 Hz, 1H, =CH); MS *m/z* (%) 415 (M$^+$+2, 17), 413 (M$^+$, 33), 395 (25), 329 (14), 98 (100), 105 (61), 77 (59). Anal. Calcd. for C$_{20}$H$_{17}$ClN$_4$O$_2$S (412.89): C, 58.18; H, 4.15; N, 13.57. Found: C, 58.20; H, 4.28; N, 13.64%.

3.4. *Synthesis of N-[3-phenyl-5-(1H-pyrazol-3-yl)-3H-[1,3,4]thiadizol-2-ylidene]-benzamide* (**5**)

A mixture of enaminone **4f** (1.89 g, 5 mmol) and hydrazine hydrate (5 mL) in absolute ethanol was stirred at reflux for 10 h and cooled. The formed solid was separated by filtration and crystallized from ethanol to give **5** as white solid, (65%, yield), mp 220 °C; IR (KBr) 3303 (NH), 1607 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-*d$_6$*) 6.82–7.51 (m, 10H, Ar-H), 7.58 (d, *J* = 8 Hz, 1H, pyrazole-H), 8.02 (d, *J* = 8 Hz, 1H, pyrazole-H), 9.21 (s, 1H, NH); MS *m/z* (%), 347 (M$^+$,1), 257 (5), 235 (68), 132 (19), 103 (80), 90 (24), 76 (100). Anal. Calcd. for C$_{18}$H$_{13}$N$_5$OS (347.40): C, 62.23; H, 3.77; N, 20.16. Found: C, 62.09; H, 3.54; N, 20.07%.

3.5. Synthesis of N-(5-isoxazol-5-yl-3-phenyl-3H-[1,3,4]-thiadiazol-2-ylidene)-benzamide (**6**)

To a solution of **4f** (1.89 g, 5 mmol) in absolute ethanol (20 mL) was added hydroxylamine hydrochloride (0.35 g, 5 mmol) and anhydrous potassium carbonate (0.5 g, 5 mmol). The mixture was stirred at reflux for 5 h, cooled and the precipitate formed was separated by filtration and crystallized from ethanol to give **6** as yellow solid, (70%, yield), mp 160 °C; IR (KBr) 1606 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 7.36–7.72 (m, 10H, Ar-H), 7.95 (d, J = 8 Hz, 1H, isoxazole-H), 8.13 (d, J = 8 Hz, 1H, isoxazole-H); MS (*m/z*) (%) 348 (M$^+$, 2), 121 (12), 105 (100), 91 (24), 77 (76). Anal. Calcd. for C$_{18}$H$_{12}$N$_4$O$_2$S (348.39): C, 62.06; H, 3.47; N, 16.08. Found: C, 61.98; H, 3.55; N, 16.11%.

*3.6. Reaction of Enaminone **4f** with Active Methylene Compounds*

3.6.1. General Procedure

To a solution of **4f** (1.89 g, 5 mmol) in glacial acetic acid (20 mL) was added the corresponding active methylene compound (acetylacetone, ethyl acetoacetate, ethyl benzoylacetate or dibenzoyl methane) (5 mmol) and ammonium acetate (0.5 g, 6 mmol). The reaction mixture was stirred at reflux for 5–10 h (reaction progress monitored by using TLC) and poured into cold water. The formed solid was separated by filtration and crystallized from ethanol to give the respective products **8a–d**.

3.6.2. *N-[5-(5-Acetyl-6-methyl-pyridin-2-yl)-3-phenyl-3H-[1,3,4]thiadiazol-2-ylidene]-benzamide* (**8a**)

Yellow solid, (80%, yield), mp 290 °C; IR (KBr), 1679, 1605 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.49 (s, 3H, CH$_3$), 2.63 (s, 3H, COCH$_3$), 7.47–7.72 (m, 10H, Ar-H), 8.04 (d, J = 8 Hz, 1H, ArH), 8.39 (d, J = 8 Hz, 1H, ArH); MS *m/z* (%) 415 (M$^+$+1, 2), 414 (M$^+$, 5), 136 (6), 104 (77), 90 (68), 76 (100). Anal. Calcd. for C$_{23}$H$_{18}$N$_4$O$_2$S (414.49): C, 66.65; H, 4.38; N, 13.52. Found: C, 66.49; H, 4.26; N, 13.28%.

3.6.3. *6-(5-Benzoylimino-4-phenyl-4,5-dihydro-[1,3,4]-thiadiazol-2-yl)-2-methyl-nicotinic Acid Ethyl Ester* (**8b**)

Yellow solid, (80%, yield), mp 200 °C; IR (KBr) 1715, 1617 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 1.36 (t, J = 7 Hz, 3H, CH$_3$), 2.83 (s, 3H, CH$_3$), 4.35 (q, J = 7 Hz, 2H, CH$_2$), 7.50–7.71 (m, 10H, Ar-H), 8.03 (d, J = 8 Hz, 1H, ArH), 8.35 (d, J = 8 Hz, 1H, ArH). MS *m/z* (%) 444 (M$^+$, 5), 104 (82), 91 (51), 77 (100). Anal. Calcd.for C$_{24}$H$_{20}$N$_4$O$_3$S (444.52): C, 64.85; H, 4.54; N, 12.60. Found: C, 64.90; H, 4.35; N, 12.49 %.

3.6.4. *6-(5-Benzoylimino-4-phenyl-4,5-dihydro-[1,3,4]-thiadiazol-2-yl)-2-phenyl-nicotinic Acid Ethyl Ester* (**8c**)

Orange solid, (80 %, yield), mp 210 °C; IR (KBr) 1719, 1611 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 1.06 (t, J = 7 Hz, 3H, CH$_3$), 4.18 (q, J = 7 Hz, 2H, CH$_2$), 7.46–8.11 (m, 15H, Ar-H), 8.22 (d, J = 7 Hz, 1H, ArH), 8.32 (d, J = 7 Hz, 1H, ArH); MS *m/z* (%) 506 (M$^+$, 10), 117 (12), 105 (87), 77 (100). Anal. Calcd. for C$_{29}$H$_{22}$N$_4$O$_3$S (506.59): C, 68.76; H, 4.38; N, 11.06. Found: C, 68.53; H, 4.22; N, 11.20.%

3.6.5. *N-[5-(5-Benzoyl-6-phenyl-pyridin-2-yl)-3-phenyl-3H-[1,3,4]thiadiazol-2-ylidene]-benzamide* (**8d**)

Yellow solid, (75%, yield), mp 175 °C; IR (KBr) 1669, 1618 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 7.49–7.94 (m, 20H, Ar-H), 8.02 (d, J = 8 Hz, 1H, pyridine-H), 8.20 (d, J = 8 Hz, 1H, pyridine-H); MS m/z (%) 539 (M$^+$+1, 7), 538 (M$^+$, 10), 222 (15), 105 (100), 98 (82), 91 (27), 77 (76). Anal. Calcd. for $C_{33}H_{22}N_4O_2S$ (538.63): C, 73.59; H, 4.12; N, 10.40. Found: C, 73.44; H, 4.22; N, 10.23%.

3.7. Reaction of Enaminone **4f** *with Heterocyclic Amines*

3.7.1. General Procedure

To a solution of **4f** (1.89 g, 5 mmol) in acetic acid (20 mL) was added the appropriate heterocyclic amine (5 mmol). The mixture was stirred at reflux for 6 h then cooled. The formed solid was separated by filtration and crystallized from the appropriate solvent.

3.7.2. *5-(2-Benzoylimino-3-phenyl-1,3,4-thiadiazol-5-yl)-triazolo[1,5-a]pyrimidine* (**11**)

Yellow solid, (80%, yield), mp 300 °C (EtOH); IR (KBr) 1608 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 7.45–8.10 (m, 10H, Ar-H), 8.11 (d, J = 4.5 Hz, 1H, ArH), 8.97 (s, 1H, triazole-H), 9.04 (d, J = 4.5 Hz, 1H, ArH); MS m/z (%) 399 (M$^+$, 16), 229 (2.9), 173 (0.3), 105 (80), 77 (100); Anal. Calcd. for $C_{20}H_{13}N_7OS$ (399.44): C, 60.14; H, 3.28; N, 24.55. Found: C, 60.08; H, 3.21; N, 24.64%.

3.7.3. *4-(2-Benzoylimino-3-phenyl-1,3,4-thiadiazol-5-yl)-benzimidazo[1,2-a]pyrimidine* (**12**)

Yellow solid, (80%, yield), mp 230 °C (Dioxane); IR (KBr) 1635 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 7.46–7.69 (m, 14H, Ar-H), 7.93 (d, J = 4.5 Hz, 1H, pyrimidine-H), 8.08 (d, J = 4.5 Hz, 1H, pyrimidine-H); MS m/z (%), 448 (M$^+$, 1), 377 (7), 105 (22), 98 (100), 77 (19). Anal. Calcd .for $C_{25}H_{16}N_6OS$ (448.51): C, 66.95; H, 3.60; N, 18.74. Found: C, 66.68; H, 3.51; N, 18.59%.

3.7.4. *4-(2-Benzoylimino-3-phenyl-1,3,4-thiadiazol-5-yl)-7-phenyl-pyrazolo[1,5-a]pyrimidine* (**13**)

Yellow solid, (80%, yield), mp 280 °C (Dioxane); IR (KBr) 1606 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) 7.43–7.79 and 8.06–8.17 (m, 15H, Ar-H), 7.79 (d, J = 5 Hz, 1H, pyrimidine-H), 8.0 (s, 1H, pyrazole-H), 8.63 (d, J = 5 Hz, 1H, pyrimidine-H); MS m/z (%), 474 (M$^+$, 39), 397 (16), 104 (100), 91 (30), 77 (77); Anal. Calcd. For $C_{27}H_{18}N_6OS$ (474.55): C, 68.34; H, 3.82; N, 17.71. Found: C, 68.20; H, 3.64; N, 17.56%.

3.8. Agar Diffusion Well Method to Determine the Antimicrobial Activity

The microorganism inoculums were uniformly spread using sterile cotton swab on a sterile Petri dish containing malt extract agar (for fungi) and nutrient agar (for bacteria). Each sample (100 μL) was added to each well (6 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24–48 h at 37 °C (for bacteria) and at 28 °C (for fungi). After incubation, microorganism growth was observed. Inhibition of the bacterial and fungal growth were measured in mm. Tests were performed in triplicate [23].

4. Conclusions

In the investigation described above, a new series of 1,3,4-thiadiazole derivatives was prepared. In addition, 1,3,4-thiadiazole derivatives substituted at position-5 with heterocyclic rings were synthesized via reaction of enaminone **4f** with C- and N-nucleophiles. The antimicrobial properties of some of the prepared compounds were evaluated. The results demonstrate that selected members of this series, including **2b**, **3c** and **4c**, show excellent activities against all tested microorganisms compared with the standard fungicides itraconazole and clotrimazole and bactericides penicillin G and streptomycin.

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Sample Availability: Samples of the compounds **2–13** are available from the authors.