Article

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 $\delta$ 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.

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 $\delta$ 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 $\delta$ 2.49 and 2.63 ppm assigned to the methyl and acetyl protons, in addition to two doublets at $\delta$ 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.
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 1H-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 Syncphalastrum 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.

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                  |
| GC                             | 12.5| 19.7| 18.4| 18.4| 18.4| 12.9| 27.1                  |
| CA                             | 11.0| 19.4| 15.2| 15.4| 15.4| 10.7| 26.1                  |
| SR                             | NA  | 18.4| NA  | 8.2 | NA  | NA  | 22.3                  |
| **Gram Positive Bacteria**     |     |     |     |     |     |     |                       |
| SA                             | 13.4| 22.04| 18.2| 20.4| 12.4| 11.2| 29.4                  |
| BS                             | 15.3| 24.08| 19.3| 21.1| 13.5| 12.3| 32.5                  |
| **Gram negative Bacteria**     |     |     |     |     |     |     |                       |
| PA                             | NA  | 22.9| NA  | 16.2| NA  | NA  | 28.3                  |
| EC                             | 10.4| 21.9| 9.4 | 18.9| 8.6 | 9.8 | 33.5                  |

Scheme 5. The mechanism of reaction of enaminone 4f with heterocyclic amine.
### Table 2. Anti-microbial activities.

| Microorganism | 2g | 2h | 2i | 3a | 3b | 3c | ST (30 µg/mL) |
|---------------|----|----|----|----|----|----|--------------|
| **Fungi**     |    |    |    |    |    |    |              |
| 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** |    |    |    |    |    |    |              |
| 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** |    |    |    |    |    |    |              |
| 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**     |    |    |    |    |    |              |
| 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** |    |    |    |    |    |              |
| 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** |    |    |    |    |    |              |
| 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**     |    |    |    |    |    |    |              |
| 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** |    |    |    |    |    |    |              |
| 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** |    |    |    |    |    |    |              |
| 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) |
|---------------|----|----|----------------|
|               | Itraconazole | Clotrimazole |
| **Fungi**     |    |    |                |
| 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 using an Electrotherm 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 C₁₇H₁₂BrN₃O₂S (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-dimethylacetel (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; ¹H-NMR (DMSO-d₆) 2.94 (s, 3H, CH₃), 3.21 (s, 3H, CH₃), 3.82 (s, 3H, OCH₃), 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 C₂₁H₂₀N₄O₃S (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; ¹H-NMR (DMSO-d₆) 2.43 (s, 3H, CH₃), 2.94 (s, 3H, CH₃), 3.21 (s, 3H, CH₃), 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 C₂₁H₂₀N₄O₂S (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; ¹H-NMR (DMSO-d₆) 2.89 (s, 3H, CH₃), 3.09 (s, 3H, CH₃), 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 C₂₀H₁₇BrN₄O₂S (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; ¹H-NMR (DMSO-d₆) 2.68 (s, 3H, CH₃), 2.97 (s, 3H, CH₃), 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; ^1H-NMR (DMSO-d_6) 2.98 (s, 3H, CH₃), 3.23 (s, 3H, CH₃), 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; ^1H-NMR (DMSO-d_6): 5.83 (d, J = 12 Hz, 1H, =CH), 2.95 (s, 3H, CH₃), 3.22 (s, 3H, CH₃), 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; ^1H-NMR (DMSO-d_6): 2.97 (s, 3H, CH₃), 3.23 (s, 3H, CH₃), 5.81 (d, J = 12 Hz, 1H, =CH), 7.48–7.72 (m, 9H, Ar-H), 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]-thiadiazol-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; ^1H-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}O_{2}S (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 benzoylacetaete 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, Ar-H), 8.39 (d, $J$ = 8 Hz, 1H, Ar-H); 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, Ar-H); 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, Ar-H), 8.32 (d, $J$ = 7 Hz, 1H, Ar-H); 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\(_4\)O\(_2\)S (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\(_7\)OS (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\(_6\)OS (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\(_6\)OS (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.

References and Notes

1. Li, Z.; Wang, X.; Da, Y. Synthesis of 2-(5-(2-chlorophenyl)-2-furoylamido)-5-aryloxymethyl-1,3,4-thiadiazoles under microwave irradiation. *Syn. Commun.* **2001**, *31*, 1829-1836.

2. Supuran, C.T.; Brigantl, F.; Tilli, S.; Chegwidden, W.R.; Scozzafava, A. Carbonic anhydrase inhibitors: Sulfonamides as antitumor agents? *Bioorg. Med. Chem.* **2001**, *9*, 703-714.

3. Liu, X.; Shi, Y.; Ma, Y.; Zhang, C.; Dong, W.; Pan, L.; Wang, B.; Li, B.; Li, Z. Synthesis, antifungal activities and 3D-QSAR study of N-(5-substituted-1,3,4-thiadiazol-2-yl)cyclopropanecarboxamides. *Eur. J. Med. Chem.* **2009**, *44*, 2782-2786.

4. Demirbas, N.; Karaoglu, S.A.; Demirbas, A.; Sancak, K. Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1,3,4]thiadiazol-2-yl)methyl-5-oxo-[1,2,4]triazole and 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-5-oxo-[1,2,4]triazole derivatives. *Eur. J. Med. Chem.* **2004**, *39*, 793-804.

5. Holla, B.S.; Poorjary, K.N.; Rao, B.S.; Shivananda, M.K. New bis-aminomercaptotriazoles and bis-triazolothiadiazoles as possible anticancer agents. *Eur. J. Med. Chem.* **2002**, *37*, 511-517.

6. Ferraz, H.M.C.; Goncalo, E.R.S.; Recent preparations and synthetic applications of enaminones. *Quim. Nova* **2007**, *30*, 957-964.

7. Lue, P.; Greenhill, J.V. Enamines in heterocyclic synthesis. *Adv. Heterocycl. Chem.* **1997**, *67*, 207-215.

8. Stanovnik, B.; Svete, J. Synthesis of heterocycles from alkyl 3-(dimethylamino) propenoates and related enamines. *Chem. Rev.* **2004**, *104*, 2433-2480.

9. Yermolayev, S.A.; Gorobets, N.Y.; Lukinova, E.V.; Shishkin, O.V.; Shishkina, S.V.; Desenko, V.M. An efficient synthesis of N1-substituted 2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide via enolate salts. *Tetrahedron* **2008**, *64*, 4649-4655.

10. Riyadh, S.M.; Farghaly, T.A.; Abdallah, M.A.; Abdalla, M.M.; Abdel-Aziz, M.R. New pyrazoles incorporating pyrazolopyrazole moiety: Synthesis, anti-HCV and antitumor activity. *Eur. J. Med. Chem.* **2010**, *45*, 1042-1050.

11. Farghaly, T.A.; Abdalla, M.M. Synthesis, tautomerism, and antimicrobial, anti-HCV, anti-SSPE, antioxidant, and antitumor activities of arylazobenzosuberones. *Bioorg. Med. Chem.* **2009**, *17*, 8012-8019.

12. Abbas, E.M.H.; Farghaly, T.A. Synthesis, reactions and biological activity of 1,4-benzothiazine derivatives. *Monatsh. Chem.* **2010**, *141*, 661-667.

13. Riyadh, S.M.; Farghaly, T.A.; Gomah, S.M.; Novel polyaza: heterocyclic systems: Synthesis, antitumor, and antimicrobial activities. *Arch. Pharm. Res.* **2010**, *33*, 1721-1728.
14. Shawali, A.S.; Zayed, M.M.; Farghaly, T.A. Synthesis and biological activity of new 1H-pyrazolo[3,4-b]quinoxalines (Flavazoles). *J. Heterocycl. Chem.* **2005**, *42*, 185-189.

15. Abdel Hafez, N.A.; Farghaly, T.A.; Al-Omar, M.A.; Abdalla, M.M. Synthesis of bioactive polyheterocyclic ring systems as 5α-reductase inhibitors. *Eur. J. Med. Chem.* **2010**, *45*, 4838-4844.

16. Farghaly, T.A.; Abdel Hafez, N.A.; Ragab, E.A.; Awad, H.M.; Abdalla, M.M. Synthesis, anti-HCV, antioxidant, and peroxynitrite inhibitory activity of fused benzosuberone derivatives. *Eur. J. Med. Chem.* **2010**, *45*, 492-500.

17. Shawali, A.S.; Farghaly, T.A.; Al-Dahshoury, A.R. Synthesis, reactions and antitumor activity of new β-aminovinyl 3-pyrazolyl ketones. *ARKIVOC* **2009**, *xiv*, 88-99.

18. Breitmaier, E. *Structure Elucidation by NMR in Organic Chemistry: A Practical Guide*; John Wiley: Chichester, UK, 1993; p. 27.

19. Shawali, A.S.; Farghaly, T.A.; Al-Dahshoury, A.R. An efficient synthesis of functionalized 3-(hetaryl)pyrazoles. *ARKIVOC* **2010**, *ix*, 19-30.

20. Ho, Y.W. 5-(1-Pyrrolyl)-2-phenylthieno[2,3-d]pyrimidine as building block in heterocyclic synthesis: Novel synthesis of some pyrazoles, pyrimidines, imidazo[1,2-a]pyrimidines, pyrazolo[1,5-a]pyrimidines, pyrido-(pyrimido)pyrazolo[1,5-a]pyrimidines, 1,2,4-Triazolo[1,5-a]pyrimidine and a 1,2,3,4-Tetrazolo[1,5-a]pyrimidine derivative. *J. Chin. Chem. Soc-Taipei* **2007**, *54*, 1075-1085.

21. Li-Rong, W.; Shu-Wen, W.; Ming, L.; Hua-Zheng, Y. Reaction of enaminones with aminopyrazoles: Synthesis, structures and bioactivities of 7-Aryl-3-cyano-2-substituted pyrazolo[1,5-a] pyrimidines. *Chin. J. Chem.* **2005**, *23*, 1231-1235.

22. Eweiss, N.F.; Osman, A.O. Synthesis of heterocycles. Part II. New routes to acetylthiadiazolines and alkylazothiazoles. *J. Heterocycl. Chem.* **1980**, *17*, 1713-1717.

23. Smania, J.A.; Monache, F.D.; Smania, E.F.A.; Cuneo, R.S. Antibacterial activity of steroidal compounds isolated from *Ganoderma applanatum* (Pers.) Pat. (Aphyllophoromycetideae) fruit body. *Int. J. Med. Mushrooms* **1999**, *1*, 325-330.

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

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