Design, Synthesis, and Antimicrobial Evaluation of Novel Pyrazoles and Pyrazolyl 1,3,4-Thiadiazine Derivatives

Ibrahim Ali M. Radini

Chemistry Department, Faculty of Science, Jazan University, Jazan 2097, Saudi Arabia; iradini44@gmail.com; Tel.: +966-566-644-4196

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Abstract: A novel series of pyrazolyl 1,3,4-thiadiazines 5a–c, 8a–c, 12, 15a–c, 17a–c, and 20 was prepared from the reaction of pyrazole-1-carbothiohydrazide 1a, b with 2-oxo-N′-arylpropanehydrazonoyl chloride, 2-chloro-2-(2-arylhydrazono)acetate, and 3-bromoacetylcoumarin. Moreover, the regioselective reaction of 5-pyrazolone-1-carbothiohydrazide 1a with 4-substituted diazonium salts and 4-(dimethylamino)benzaldehyde gave the corresponding hydrazones 21a–c and 22. The newly prepared compounds were characterized by spectroscopy and elemental analysis. Many new synthesized compounds showed considerable antimicrobial activity against tested microorganisms. Hydrazones 21a–c and 22 showed remarkable antibacterial activity against tested microorganisms. 4-(2-(p-tolyl)hydrazineylidene)-pyrazole-1-carbothiohydrazide 21a displayed the highest antibacterial and antifungal activities with minimum inhibitory concentration (MIC) values lower than standard drugs chloramphenicol and clotrimazole, in the range of 62.5–125 and 2.9–7.8 µg/mL, respectively.

Keywords: pyrazole-1-carbothiohydrazide; 1,3,4-thiadiazines; hydrazonyl chlorides; antimicrobial activity; MIC

1. Introduction

Recently, the incidence of microbial infections has increased dramatically because of the misuse of antibiotics has caused the pathogens to become resistant to them and which has led to serious health hazards [1]. The rate of bacterial resistance to antibiotics is higher than the rate of development of new classes of antibiotics [2] so the design and synthesis of new compounds have potential antimicrobial activity are very important issue. Pyrazole derivatives have great attention due to their interesting biological and pharmaceutical activities such as antidepressant [3], antioxidant [4], anti-inflammatory [5], anticancer [6], antimicrobial [7–9], antiviral [10,11], anticonvulsant [12], and insecticidal activities [13]. In addition, the natural pyrazole C-glycoside, pyrazofurin (4-hydroxy-3β-D-ribofuranosyl-1H-pyrazole-5-carboxamide) has a broad spectrum of antimicrobial, antiviral, and antitumor activities [14]. It is well known that pyrazoles possess significant antibacterial activity. There are many antibiotic drugs containing pyrazole moiety such as Sulaphenazo-5 and PNU172576 (Figure 1).

Heterocycles containing the thiadiazine moiety have biological and pharmaceutical importance [15–18]. Recently, Khidre et al. [19] reported that 1,3,4-thiadiazine derivatives have a good antimicrobial activity. Motivated by the preceding information and continuation of my research program on the synthesis of novel bioactive heterocycles [7,20–23] I designed and synthesized a novel series of pyrazole and pyrazolyl 1,3,4-thiadiazine derivatives, for antimicrobial evaluation, starting from pyrazole-1-carbothiohydrazide 1a,b.
The molecular structure of compounds 1a,b was confirmed by elemental analyses and spectroscopic methods. The infrared spectrum of 1a showed characteristic bands at 3292, 3250, 3182, and 1685 cm\(^{-1}\) due to NH, NH\(_2\), and C=O functions, respectively. \(^1\)H-NMR revealed two singlet peaks at 2.02 and 3.28 ppm due to the CH\(_3\) and CH\(_2\), respectively. Also, molecular weight determination (MS) of 1a showed the molecular ion peaks at \(m/z\) 172.

The carbothiohydrazide moiety in 1a,b was reacted with selected electrophiles to prepare pyrazolyl 1,3,4-thiadiazine derivatives. Pyrazole-1-carbothiohydrazide 1a was reacted with 2-oxo-N\(^\prime\)-arylprenethydradononyl chlorides 2a-c in hot ethanol in the presence of Et\(_3\)N to yield 5-methyl-2-(5-methyl-6-(aryldiazeny)-4H-1,3,4-thiadiazin-2-yl)-2,4-dihydro-3H-pyrazol-3-ones 5a-c, in good yields, via intermediates 3 and 4 (Scheme 2). Similarly, 2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-6-(phenylidyazenyl)-4H-1,3,4-thiadizin-5(6H)-ones 8a-c were synthesized in high yields, from the reaction of 1a with ethyl 2-chloro-2-(2-arylhydrazono)acetate derivatives 6a-c under the same reaction conditions described for the preparation of 5a-c. 5-methyl-2-
(5-(2-oxo-2H-chromen-3-yl)-4H-1,3,4-thiadiazin-2-yl)-2,4-dihydro-3H-pyrazol-3-one 12 is furnished in a good yield when 1a was refluxed with 3-bromoacetyl coumarin 9 in ethanol (Scheme 2).

The structure of the compounds 5, 8, and 12 was confirmed by elemental analyses and spectroscopic methods. The IR spectrum of thiadiazinyl pyrazolone 5c, as a representative example, revealed the lack of an NH2 absorption peak at 3292 and 3250 cm⁻¹ and appearance of an absorption peak at 3149 cm⁻¹ owing to the NH group. The 1H-NMR spectrum of 5c exhibited new signals at δ 1.41, 7.33, and 7.37 ppm assigned to methyl and aromatic protons, in addition, the D2O exchangeable signal at δ 11.57 ppm due to cyclic NH. Its 13C-NMR spectrum did not reveal the lack of C=S signal at 180 ppm and appearance of 12 carbon signals. Moreover, the mass spectra of compounds 5a–c gave molecular ion peaks at m/z 314, 328, and 348, respectively. This clearly indicates the carbothiohydrazide moiety was involved in cyclization reaction with hydrazonyl chlorides 2a–c to give thiadiazine.

![Scheme 2. Synthesis of compounds 5a–c, 8a–c, and 12.](image)

In a similar way, 6-(aryl diazenyl)-4H-1,3,4-thiadiazines 15a–c, 6-(aryl diazenyl)-4H-1,3,4-thiadiazin-5(6H)-ones 17a–c, and 5-aryl-4H-1,3,4-thiadiazines 20 were synthesized in very good yields from the reaction of pyrazole-1-carbothiohydrazide 1b with hydrazonyl chlorides 2a–c, 6a–c, and α-haloketone 9, respectively, under similar reaction condition as described before (Scheme 3). Compound 20 was previously synthesized from a one pot reaction of 3-(2-bromoacetyl)-2H-chromen-2-ones, thiocarbohydrazide, and pentane-2,4-dione [25]. The IR spectrum of 17b revealed the lack of NH2 band present in the IR spectra of starting pyrazole 1b and the appearance of new absorption bands at 3176 and 1680 cm⁻¹ corresponding to NH and CO functional groups, respectively. Likewise, the 1H-NMR
spectra showed a new singlet signal at δ 3.11 ppm due to H-6 of thiadiazine, two doublet signals at δ 7.13, 7.25 ppm integrated for four protons of 4-disubstitued benzene ring, and D$_2$O-exchangeable signals at 11.23 ppm due to NH. Its $^{13}$C-NMR spectrum did not exhibit the C=S signal at 180 ppm which observed in the starting material, but instead displayed 13 carbon signals. The mass spectra of 17a–c showed molecular ion peaks at $m/z$ 314, 328, and 348, respectively, which were in an accord with the calculated masses (c.f. experimental section).

Scheme 3. Synthesis of compounds 15a–c, 17a–c and 20.

The coupling reaction of 1a with 4-substituted arenediazonium chloride was performed in ethanol containing sodium acetate at 0–5 °C to give the corresponding hydrazones 21a–c. Also, the reaction of 1a with 4-(dimethylamino)benzaldehyde in ethanol containing few drops of HCl gave $N'$-(4-(dimethylamino)benzylidene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide 22 (Scheme 4). The $^1$H-NMR spectra of 21a–c showed lack the singlet signal due to CH$_2$ (C-4 of pyrazolone) and showed an aromatic multiplets in the region 7.22–7.84 ppm. In addition, new D$_2$O-exchangeable signals appeared in the region 11.74–12.24 ppm. These data support the successful coupling C-4 of pyrazolone with 4-substituted arenediazonium chloride. The IR spectrum of hydrazone 22 did not show an NH$_2$ band. The $^1$H-NMR spectrum of 22 showed a new singlet signal at 9.65 ppm due to the azamethine proton (N=CH) and an aromatic multiplets at 6.80 and 7.91 ppm. Also, the mass spectra of 21a–c and 22 are in an agreement with the calculated masses.
2.2. Antimicrobial Activity

In vitro antimicrobial screening of the newly synthesized compounds was carried out by the agar diffusion method using cultures of two fungal strains (Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 16404), as well as four bacteria strains, two Gram positive bacteria (Staphylococcus aureus (ATCC 29213), Bacillus subtilis (ATCC 6051), and two Gram negative bacteria (Klebsiella pneumoniae (ATCC 700603) and Escherichia coli (ATCC 25922). The standard antibiotic Chloramphenicol and Antifungal Clotrimazole was used as controls to evaluate the potency of the compounds being studied under the same conditions.

As shown in Table 1 compounds 5b, 8a, 12, and 17a were found to be inactive against all microorganisms while compounds 5a, 5c, 8c, 15a, 15b, 15c, 17b, 17c, and 20 exhibited low activity against some microorganisms only and inactive against others. Compounds 8b showed good activities against fungi and Gram positive bacteria. Compound 22 displayed good activities against all microorganisms except Candida albicans did not show any activity. Compounds 21a–c showed a broad spectrum activity against all microorganisms. Compound 21c showed the highest activity against Candida albicans with inhibition zones of 25 mm while compound 21a showed the highest activity against other strains, e.g., Aspergillus niger, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, and Escherichia coli with inhibition zones 35, 22, 30, 20, and 27 mm, respectively. The variation in the effectiveness of different compounds against microorganism depends on either the impermeability of the cells of the microbes or on differences in the ribosomes of microbial cells [26]. It may be concluded that the antimicrobial activity of the compounds is related to the cell wall structure of the bacterium as well as the structure of the pyrazole derivatives itself. It is possible because the cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids, but in contrast, Gram negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surround by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram-positive bacteria and are ineffective against Gram-negative pathogens [27].

On the other hand, it is obvious that compounds 21a > 21b > 21c exhibited potent inhibition activity owing to its characteristic skeleton that containing free carbothiohydrazide moiety that confer its softness, six donating N atoms, and planar 4-substituted phenyl group compared with the other pyrazolyl 1,3,4-thiadiazine derivatives.
The compounds which showed greater antibacterial and antifungal activities were further assayed for minimum inhibitory concentration (MIC), and the values are listed in Table 2. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. Compounds 21a displayed low MIC value on *Aspergillus niger*, *Staphylococcus aureus*, *B. subtilis*, and *Klebsiella pneumoniae* than standard drug Clotrimazole and Chloramphenicol and showed MIC value on *Candida albicans* and *Escherichia coli* equal to standard drugs. The MIC value of compound 21b against *Aspergillus niger*, *B. subtilis*, and *Klebsiella pneumoniae* was equal to standard drugs Clotrimazole and Chloramphenicol. Moreover, Compound 21c showed a MIC value on *Aspergillus niger* *Staphylococcus aureus*, *B. subtilis*, *Klebsiella pneumoniae*, and *Escherichia coli* equal to the standard drugs.

The structure–activity relationship revealed that compounds with pyrazole-1-carbothiohydrazide unit displayed low MIC value on *Aspergillus niger*, *Staphylococcus aureus*, *B. subtilis*, and *Klebsiella pneumoniae* than standard drug Clotrimazole and Chloramphenicol and showed MIC value on *Candida albicans* and *Escherichia coli* equal to standard drugs. The presence of free carbothiohydrazide moiety increases the activity of 21a and the presence of electron donating substituents at the aromatic ring increased the activity of 21a.

### Table 1. In vitro antimicrobial activity of the synthesized compounds a,b.

| Comp. No. | Fungi | Gram Positive Bacteria | Gram Negative Bacteria |
|-----------|-------|------------------------|-----------------------|
|           | *Candida albicans* | *Aspergillus niger* | *Staphylococcus aureus* | *Bacillus subtilis* | *Klebsiella pneumoniae* | *Escherichia coli* |
| 5a        | 15 ± 1 | 20 ± 1.53             | na                    | na                   | na                    | 17 ± 1.00            |
| 5b        | na     | na                     | na                    | na                   | na                    | na                   |
| 5c        | 12 ± 1.53 | na                   | na                    | na                   | na                    | 11 ± 0.58            |
| 8a        | na     | na                     | na                    | na                   | na                    | na                   |
| 8b        | 20 ± 1.53 | 15 ± 2.65             | 13 ± 0.58             | 18 ± 0.58            | 11 ± 0.58             | na                   |
| 8c        | 9 ± 0.58 | na                    | na                    | 15 ± 2.08            | na                    | na                   |
| 12        | na     | na                     | na                    | na                   | na                    | na                   |
| 15a       | na     | 12 ± 0.00              | na                    | na                   | na                    | na                   |
| 15b       | na     | 12 ± 0.58              | na                    | na                   | na                    | na                   |
| 15c       | na     | na                     | 15 ± 0.58             | 29 ± 1.00            | 15 ± 1.15             | 23 ± 1.53            |
| 17a       | na     | na                     | na                    | na                   | na                    | na                   |
| 17b       | na     | na                     | na                    | 15 ± 0.58            | na                    | 14 ± 1.00            |
| 17c       | na     | na                     | 17 ± 1.15             | 16 ± 0.58            | na                    | 16 ± 0.58            |
| 20        | na     | na                     | na                    | na                   | na                    | 12 ± 0.58            |
| 21a       | 13 ± 0.00 | 35 ± 3.00             | 22 ± 1.15             | 30 ± 1.53            | 20 ± 0.58             | 27 ± 1.15            |
| 21b       | 18 ± 3.06 | 32 ± 0.58             | 21 ± 0.58             | 25 ± 0.58            | 18 ± 2.08             | 25 ± 0.58            |
| 21c       | 25 ± 3.00 | 29 ± 1.00             | 12 ± 1.53             | 23 ± 1.73            | 13 ± 0.58             | 25 ± 0.58            |
| 22        | na     | 26 ± 0.58              | 15 ± 0.00             | 20 ± 0.58            | 17 ± 0.58             | 20 ± 1.53            |
| Chloramphenicol | -  | 25 ± 0.58             | 30 ± 1.73             | 24 ± 1.15             | 24 ± 1.00             | -                    |
| Clotrimazole | 24 ± 4.51 | 20 ± 0.58             | -                     | -                   | -                     | -                    |

a Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of synthesized compounds against the pathological strains based on well diffusion assay. b The experiment was carried out in triplicate and the average zone of inhibition was calculated. c na No activity.

### Table 2. Minimum inhibitory concentration (MIC) in (µg/mL) for compounds 8b, 21a–c, and 22.

| Comp. No. | *Candida albicans* | *Aspergillus niger* | *Staphylococcus aureus* | *Bacillus subtilis* | *Klebsiella pneumoniae* | *Escherichia coli* |
|-----------|--------------------|--------------------|------------------------|---------------------|-------------------------|-------------------|
| 8b        | 125 ± 2.52         | 187.5 ± 0.50       | 375 ± 3.00             | 500 ± 3.51          | 500 ± 4.51              | na                |
| 21a       | 7.8 ± 0.17         | 2.9 ± 0.06         | 125 ± 0.58             | 62.5 ± 0.50         | 62.5 ± 2.00             | 125 ± 2.52        |
| 21b       | 15.6 ± 0.76        | 5.8 ± 0.26         | 250 ± 8.08             | 125 ± 0.00          | 125 ± 2.65              | 187.5 ± 8.23      |
| 21c       | 11.6 ± 0.30        | 5.8 ± 0.65         | 187.5 ± 8.23           | 125 ± 1.00          | 125 ± 1.53              | 125 ± 0.00        |
| 22        | 93.7 ± 0.95        | 46.4 ± 0.84        | 250 ± 4.36             | 250 ± 4.58          | 250 ± 3.21              | 500 ± 8.00        |
| Chloramphenicol | —  | —                  | 187.5 ± 0.06           | 125 ± 0.58          | 125 ± 3.51              | 125 ± 1.73        |
| Clotrimazole | 7.8 ± 0.06       | 5.8 ± 0.06         | —                      | —                   | —                       | —                 |

na: No activity.
3. Experimental Section

3.1. General Information

All melting points were determined on a digital Gallen-Kamp MFB-595 instrument (Gallenkamp, London, UK) using open capillary tubes and are uncorrected. IR spectra were recorded on a Schimadzu FTIR 440 spectrometer (Shimadzu, Tokyo, Japan) using KBr pellets. Mass spectra were performed at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer (Shimadzu, Tokyo, Japan) provided with a data system. 1H-NMR and 13C-NMR spectra were recorded on a Bruker model (500 MHz) Ultra Shield NMR spectrometer (Bruker, Coventry, UK) in CDCl₃ or DMSO-d₆ using tetramethylsilane (TMS) as an internal standard; chemical shifts are reported as δ ppm units. Solvents were dried by standard techniques. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds was carried out and was run using thin layer chromatography (TLC) aluminum sheets silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany). Compound 1b was prepared previously by Alekseev et al. [24].

3.2. Synthesis

3-Methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (1a). Thiocarbohydrazide (10.6 g, 0.1 mol) was dissolved in a mixture of ethanol (20 mL) and HCl (1 mL) and ethyl acetoacetate was added (13 mL, 0.1 mol). The mixture was refluxed for 1 h. After cooling, the white precipitate was filtered off, washed with ethanol, and dried under reduced pressure. White crystals, yield (92%), m.p. 135–136 °C. IR (KBr) ν (cm⁻¹): 3292, 3250, 3182 (NH₂ & NH), 1685 (C=O), 1647 (C=N), 1H-NMR (500 MHz, CDCl₃) δ (ppm): 2.02 (s, 3H, CH₃), 3.28 (s, 2H, pyrazole-H₄), 8.7 (d, D₂O exchangeable, 2H, NH₂), 10.18 (s, D₂O exchangeable, 1H, NH); 13C-NMR (125 MHz, CDCl₃) δc (ppm): 15.5 (CH₃), 43.0 (CH₂, pyrazole-C₄), 160.5 (pyrazole-C₃), 166 (C=O), 179 (C=S); MS m/z (%): 172 [M⁺] (11%), 130 (100); Anal. Calcd. for C₅H₅N₂O (172.21): C, 34.87; H, 4.68; N, 32.54, Found: C, 34.57; H, 4.50; N, 32.41%.

3.2.1. General procedure for synthesis compounds 5, 8, 12, 15, 17, and 20

Equimolar amounts of 1a or 1b (1 mmol) and 2-oxo-N-arylpropaneydrozonoyl chloride 2a–c; ethyl 2-chloro-2-(2-arylhydrazineylidene)acetate 6a–c or 3-(2-bromoacetyl)-2H-chromen-2-one 8 (1 mmol) in absolute ethanol (30 mL) (few drops of triethylamine was added in case of 2a–c and 6a–c) was heated under reflux for 3–6 h (TLC), then left to cool. The solid was isolated by filtration, washed with ethanol, dried, and recrystallized from (EtOH).

5-Methyl-2-((5-methyl-6-(phenylidiazonyl)-4H-1,3,4-thiadiazin-2-yl)-2,4-dihydro-3H-pyrazol-3-one (5a). Red crystals, yield (85%), m.p. 193–194 °C (EtOH); IR (νmax, cm⁻¹): 3242 (NH), 1690 (C=O), 1600 (C=N), 1600–1440 (C=C); 1H-NMR (500 MHz, CDCl₃) δH (ppm): 1.25 (s, 3H, CH₃), 1.44 (s, 3H, CH₃), 3.10 (s, 2H, pyrazole-H₄), 7.33 (t, 2H, Ar-H), 7.44 (t, 2H, Ar-H), 8.04 (d, 1H, J = 8.5 Hz, Ar-H), 11.52 (s, D₂O exchangeable, 1H, NH); 13C-NMR (125 MHz, CDCl₃) δc (ppm): 12.2 (CH₃), 16.0 (CH₃), 44.0 (CH₂, pyrazole-C₄), 95.4, 121.7, 129.7, 148.2, 151.8, 154.6, 160.5, 166.3 (C=O); MS m/z (%): 314 [M⁺] (20%), 245(100); Anal. Calcd. for C₁₄H₁₄N₆O₆ (314.37): C, 53.49; H, 4.49; N, 26.73, Found: C, 53.69; H, 4.19; N, 26.67%.

5-Methyl-2-((5-methyl-6-(p-tolyldiazenyl)-4H-1,3,4-thiadiazin-2-yl)-2,4-dihydro-3H-pyrazol-3-one (5b). Brown powder, yield (86%), m.p. 219–220 °C (EtOH); IR (νmax, cm⁻¹), 3244 (NH), 1687 (C=O), 1591 (C=N), 1558–1440 (C=C); 1H-NMR (500 MHz, CDCl₃) δH (ppm): 1.27 (s, 3H, CH₃), 1.48 (s, 3H, CH₃), 1.54 (s, 3H, CH₃), 3.11 (s, 2H, pyrazole-H₄), 7.37 (dd, 2H, J = 8.5, 2.5 Hz, Ar-H), 7.82 (dd, 2H, J = 7.6, 2.5 Hz, Ar-H), 11.54 (s, D₂O exchangeable, 1H, NH); 13C-NMR (125 MHz, CDCl₃) δc (ppm): 12.9 (CH₃), 16.5 (CH₃), 21.5 (CH₃), 42.9 (CH₂- pyrazole-C₄), 94.9, 128.7, 129.9, 138.6, 145.1, 152.4, 155.4, 160.7, 165.7 (C=O); MS m/z (%): 328 [M⁺] (31%), 245 (100); Anal. Calcd. for C₁₅H₁₆N₆O₆ (328.39): C, 54.86; H, 4.91; N, 25.59, Found: C, 55.01; H, 4.67; N, 25.51%.
2-\((6-((4\text{-Chlorophenyl})\text{diazenyl})-5\text{-methyl-4H-1,3,4-thiadiazin-2-yl})\text{-5-methyl-2,4-dihydro-3H-pyrazol-3-one}\) (5e). Brown powder, yield (87%), m.p. 230–231 °C (EtOH); IR (\(v_{\text{max}}\) cm\(^{-1}\): 3149 (NH), 1692 (C=O), 1654 (C=N), 1593–1462 (C=C); \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta_H\) (ppm): 1.22 (s, 3H, CH\(_3\)), 1.41 (s, 3H, CH\(_3\)), 2.53 (s, 2H, pyrazole-H\(_4\)), 7.33 (dd, 2H, J = 9, 2.5 Hz, Ar-H), 7.37 (dd, 2H, J = 7, 2.5 Hz, Ar-H), 11.57 (s, D\(_2\)O exchangeable, 1H, NH); \(^13\)C-NMR (125 MHz, CDCl\(_3\)) \(\delta_C\) (ppm): 12.2 (CH\(_3\)), 15.8 (CH\(_3\)), 43.4 (CH\(_2\), pyrazole-C\(_4\)), 96.0, 129.7, 131.7, 134.8, 147.0, 151.6, 154.0, 161.4, 165.6 (C=O); MS m/z (%): 348 [M\(^+\) (35%)], 350 [M + 2\(^+\) (10)], 245(100); Anal. Calcd. for C\(_{14}\)H\(_{13}\)ClN\(_6\)O\(_3\) (348.81): C, 48.21; H, 3.76; N, 24.09; Found: C, 48.62; H, 3.46; N, 24.14%.

2-(3-Methyl-5-\text{o xo-4,5-dihydro-1H-pyrazol-1-yl})- 6-(\text{phenyl diazenyl})- 4H-1,3,4-thiadiazin-5(6H)-one (8a). Yellow crystals, yield (87%), m.p. 220–221 °C (EtOH); IR (\(v_{\text{max}}\) cm\(^{-1}\): 3180 (NH), 1695 (C=O), 1681 (C=O), 1633 (C=N), 1598–1496 (C=C); \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta_H\) (ppm): 2.14 (s, 3H, CH\(_3\)), 2.27 (s, 2H, pyrazole-H\(_4\)), 3.52 (s, 1H, thiaidine-H\(_4\)), 7.31 (t, 2H, Ar-H), 7.45 (t, 2H, Ar-H), 7.64 (d, 1H, J = 8.5 Hz, Ar-H), 10.6 (s, D\(_2\)O exchangeable, 1H, NH); \(^13\)C-NMR (125 MHz, CDCl\(_3\)) \(\delta_C\) (ppm): 14.9 (CH\(_3\)), 42.8, 81.5, 121.4, 126.5, 129.5, 151.4, 152.9, 159.7, 165 (C=O), 169 (C=O); MS m/z (%): 316 [M\(^+\) (20%)], 350 (8), 98 (100); Anal. Calcd. for C\(_{13}\)H\(_{12}\)N\(_6\)O\(_2\)S (316.34): C, 49.36; H, 3.82; N, 26.57; Found: C, 49.06; H, 3.79; N, 26.35%.

5-Methyl-2-(2-(2-oxo-2-\text{H-chromen-3-yl})-6H-1,3,4-thiadiazin-2-yl)-2,4-dihydro-3H-pyrazol-3-one (12). Brown powder, yield (87%), m.p. 260–261 °C (EtOH); IR (\(v_{\text{max}}\) cm\(^{-1}\): 3184 (NH), 1693, 1689 (C=O), 1625 (C=N), 1583–1529 (C=C); \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta_H\) (ppm): 1.92 (s, 3H, CH\(_3\)), 2.13 (s, 2H, pyrazole-H\(_4\)), 3.74 (s, 1H, thiaidine-H\(_4\)), 7.24 (dd, 2H, J = 7, 2 Hz, Ar-H), 7.33 (dd, 2H, J = 6.5, 2 Hz, Ar-H), 10.98 (s, D\(_2\)O exchangeable, 1H, NH); \(^13\)C-NMR (125 MHz, CDCl\(_3\)) \(\delta_C\) (ppm): 13.7 (CH\(_3\)), 21 (CH\(_2\)), 79.5, 123.4, 129.5, 134.4, 144, 148, 151.4, 159.7, 165 (C=O), 169 (C=O); MS m/z (%): 330 [M\(^+\) (25%)], 350 (8), 98 (100); Anal. Calcd. for C\(_{14}\)H\(_{14}\)N\(_6\)O\(_2\)S (330.37): C, 50.90; H, 4.27; N, 25.44; Found: C, 50.43; H, 4.15; N, 25.27%.
2-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-methyl-6-(p-toluidazinyl)-4H-1,3,4-thiadiazine (15b). Brown powder, yield (86%), m.p. 205–206 °C (EtOH); IR (v_{max}, cm^{-1}): 3156 (NH), 1683 (C=N), 1591–1456 (C=C); ^{1}H-NMR (500 MHz, CDCl_{3}) \delta_{H} (ppm): 2.26 (s, 3H, CH_{3}), 2.32 (s, 3H, CH_{3}), 2.51 (s, 3H, CH_{3}), 2.57 (s, 3H, CH_{3}), 6.04 (s, 1H, pyrazole-H_{4}), 7.32 (dd, 2H, J = 6 Hz, Ar-H), 7.86 (dd, 2H, J = 8.5 Hz, Ar-H), 11.52 (s, D_{2}O exchangeable, 1H, NH); ^{13}C-NMR (125 MHz, CDCl_{3}) \delta_{C} (ppm): 8.54 (CH_{3}), 13.2 (CH_{3}), 18.6 (CH_{3}), 18.6 (CH_{2}), 103.3, 114.9, 121.0, 129.1, 129.3, 129.6, 148.9, 150.6, 159.2, 189.5; MS m/z (%): 326 [M]^+ (30%), 299 (100); Anal. Calcd. for C_{16}H_{18}N_{6}S (326.42): C, 58.87; H, 5.56; N, 25.75; Found: C, 58.49; H, 5.27; N, 25.41%.

6-((4-Chlorophenyl) diazenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-methyl-4H-1,3,4-thiadiazine (15c). Brown crystals, yield (84%), m.p. 187–188 °C (EtOH); IR (v_{max}, cm^{-1}): 3178 (NH), 1681 (C=O), 1650 (C=N), 1595–1487 (C=C); ^{1}H-NMR (500 MHz, CDCl_{3}) \delta_{H} (ppm): 2.12 (s, 3H, CH_{3}), 2.24 (s, 3H, CH_{3}), 3.2 (s, 1H, H6 thia diazinone), 6 (s, 1H, pyrazole-H_{4}), 7.33 (t, 2H, J = 9, Ar-H), 7.44 (t, 2H, Ar-H), 7.65 (d, 1H, J = 8.5 Hz, Ar-H), 11 (s, D_{2}O exchangeable, 1H, NH); ^{13}C-NMR (125 MHz, CDCl_{3}) \delta_{C} (ppm): 12.7 (CH_{3}), 14.9 (CH_{3}), 85.2, 114, 121.4, 129.5, 135.4, 142.9, 145.7, 148.6, 152.0, 169.1; MS m/z (%): 348.97 [M + 3]^+ (20%), 345.94 [M]^+ (31%), 317 (100); Anal. Calcd. for C_{15}H_{19}ClN_{6}S (346.84): C, 51.95; H, 4.36; N, 24.23; Found: C, 51.60; H, 4.15; N, 24.07%.

2-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(phenyl diazenyl)-4H-1,3,4-thiadiazin-5(6H)-one (17a). Green powder, yield (85%), m.p. 126–127 °C (EtOH); IR (v_{max}, cm^{-1}): 3169 (NH), 1640 (C=O), 1620 (C=N), 1575–1473 (C=C); ^{1}H-NMR (500 MHz, CDCl_{3}) \delta_{H} (ppm): 2.13 (s, 3H, CH_{3}), 2.23 (s, 3H, CH_{3}), 2.46 (s, 3H, CH_{3}), 3.11 (s, 1H, thia diazinone-H_{6}), 6.04 (s, 1H, pyrazole-H_{4}), 7.13 (d, 2H, J = 7 Hz, Ar-H), 7.25 (dd, 2H, J = 6.5, 2 Hz, Ar-H), 11.23 (s, D_{2}O exchangeable, 1H, NH); ^{13}C-NMR (125 MHz, CDCl_{3}) \delta_{C} (ppm): 12.4 (CH_{3}), 14.8 (CH_{3}), 21.7 (CH_{3}), 86.0, 113, 125.7, 130.7, 134.8, 144.0, 147.6, 149, 154.0, 169.6; MS m/z (%): 328 [M]^+ (3%), 321 (20), 148.8 (90), 85.9 (100); Anal. Calcd. for C_{15}H_{18}N_{6}OS (328.39): C, 54.86; H, 4.91; N, 25.59; Found: C, 54.39; H, 4.69; N, 25.41%.

6-((4-Chlorophenyl) diazenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,3,4-thiadiazin-5(6H)-one (17b). Green powder, yield (80%), m.p. 164–165 °C (EtOH); IR (v_{max}, cm^{-1}): 3169 (NH), 1680 (C=O), 1612 (C=N), 1599–1465 (C=C); ^{1}H-NMR (500 MHz, CDCl_{3}) \delta_{H} (ppm): 2.13 (s, 3H, CH_{3}), 2.23 (s, 3H, CH_{3}), 2.46 (s, 3H, CH_{3}), 3.11 (s, 1H, thia diazinone-H_{6}), 6.04 (s, 1H, pyrazole-H_{4}), 7.13 (d, 2H, J = 8 Hz, Ar-H), 7.25 (dd, 2H, J = 6.5, 2 Hz, Ar-H), 11.23 (s, D_{2}O exchangeable, 1H, NH); ^{13}C-NMR (125 MHz, CDCl_{3}) \delta_{C} (ppm): 12.4 (CH_{3}), 14.8 (CH_{3}), 81.0, 112, 124.2, 131.5, 135.6, 143.0, 146.7, 148.9, 153.0, 168.6; MS m/z (%): 348 [M]^+ (25%), 350 (8), 86 (100); Anal. Calcd. for C_{15}H_{16}ClN_{6}S (348.81): C, 48.21; H, 3.76; N, 24.09; Found: C, 47.99; H, 3.62; N, 23.89%.

### 3.2.2. General procedure for synthesis 21a-c

To a stirred solution of compound 1a (0.516 g, 3 mmol) in ethanol (30 mL) sodium acetate trihydrate (0.39 g, 3 mmol) was added. After stirring for 15 min, the mixture was chilled at 0 °C and treated with a cold solution of the respective aniline (4-chloroaniline (0.381 g, 3 mmol), p-toluidine (0.310 g, 3 mmol), or 4-aminobenzenesulfonamide (0.516 g, 3 mmol)) in 6 M hydrochloric acid (1.5 mL) with a sodium nitrite solution (0.21 g, 3 mmol, in 3 mL water). The addition of the diazonium salt was stirred for an additional 2 h at 0–5 °C and then left for 8 h in a refrigerator (4 °C). The resulting
solid was collected by filtration, washed thoroughly with water, and dried. The crude product was crystallized from ethanol to give hydrazones 21a–c.

3- Methyl- 5- oxo- 4-(2- (p-tolyl) hydrazineylidene)- 4,5- dihydro- 1H-pyrazole- 1-carbothiohydrazide (21a).
Brown powder, yield (80%), m.p. 120–121 °C (EtOH); IR (ν\text{max}, cm⁻¹): 3254–3142 (NH₂ & 2NH), 1698 (C=O), 1595 (C=N), 1567–1485 (C=C); ¹H-NMR (500 MHz, CDMSO-d₆) δH (ppm): 2.31 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 6.85 (d, D₂O exchangeable, 2H, NH₂), 7.46 (m, 4H, Ar-H), 11.31 (s, D₂O exchangeable, 1H, NH), 11.74 (s, D₂O exchangeable, 1H, NH); ¹³C-NMR (125 MHz, DMSO-d₆) δC (ppm): 11.9 (CH₃), 21.4 (CH₃), 116.4, 127.4, 128.4, 130, 142.1, 147.5, 163.8, 194.0; MS m/z (%): 290 [M⁺] (5%), 285.78 (30), 267.95 (90); Anal. Calcd. for C₁₁H₁₄N₄O₂S (310.76): C, 42.52; H, 3.57; N, 27.04; Found: C, 42.36; H, 3.29; N, 27.49.

4-(2- (4-Chlorophenyl)hydrazineylidene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (21b).
Red crystals, yield (85%), m.p. 117–118 °C (EtOH); IR (ν\text{max}, cm⁻¹): 3383–3172 (NH₂ & 2NH), 1674 (C=O), 1593 (C=N), 1544–1485 (C=C); ¹H-NMR (500 MHz, CDMSO-d₆) δH (ppm): 2.31 (s, 3H, CH₃), 6.99 (s, D₂O exchangeable, 2H, NH₂), 7.1 (s, D₂O exchangeable, 1H, NH), 7.48 (m, 4H, Ar-H), 11.76 (s, D₂O exchangeable, 1H, NH); ¹³C-NMR (125 MHz, DMSO-d₆) δC (ppm): 11.9 (CH₃), 116.4, 117.4, 118.4, 128, 129.5, 147.1, 163.8 (C=O), 194 (C=S); MS m/z (%): 310 [M⁺] (5%), 285.78 (30), 267.95 (90); Anal. Calcd. for C₁₁H₁₄ClN₄O₂S (303.38): C, 55.43; H, 5.65; N, 23.08; Found: C, 55.13; H, 5.30; N, 22.97%.

4- (2- (1- (Hydrazinocarbonothioyl)- 3- methyl- 5- oxo- 1,5- dihydro- 4H- pyrazol- 4- ylidene) hydrazineyl benzene sulphonamide (21c).
Brown powder, yield (80%), m.p. 140–141 °C (EtOH); IR (ν\text{max}, cm⁻¹): 3296–3221 (NH₂ & 2NH), 1693 (C=O), 1595 (C=N), 1543–1489 (C=C); ¹H-NMR (500 MHz, CDMSO-d₆) δH (ppm): 2.28 (s, 3H, CH₃), 6.98 (s, D₂O exchangeable, 2H, NH₂), 7.08 (s, D₂O exchangeable, 2H, NH₂), 7.18 (s, D₂O exchangeable, 2H, NH₂), 7.72 (d, 2H, J = 7.5 Hz, Ar-H), 7.84 (d, 2H, J = 8.5 Hz, Ar-H), 11.31 (s, D₂O exchangeable, 1H, NH), 12.24 (s, D₂O exchangeable, 1H, NH); ¹³C-NMR (125 MHz, DMSO-d₆) δC (ppm): 11.8 (CH₃), 116.6, 126.8, 140.9, 144, 153.8, 160.7, 169 (C=O), 180 (C=S); MS m/z (%): 354.93 [M⁺] (4%), 255.68 (100); Anal. Calcd. for C₁₁H₁₃N₈O₃S₂ (355.39): C, 37.18; H, 3.69; N, 27.59; Found: C, 36.98; H, 3.56; N, 27.47%.

Synthesis of N°- (4-(Dimethylamino) benzilidene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (22).
A few drops of HCl were added to a mixture of 1a (0.516 g, 3 mmol), and 4-(dimethylamino) benzaldehyde (0.447 g, 3 mmol) in EtOH (20 mL), and the reaction mixture was stirred 6 h. The precipitate formed was collected by filtration, dried, washed with EtOH, and recrystallized from EtOH. Red powder, yield (89%), m.p. 261–262 °C (EtOH); IR (ν\text{max}, cm⁻¹): 3269 (NH), 1689 (C=O), 1510 (C=N), 1504–1456 (C=C); ¹H-NMR (500 MHz, CDMSO-d₆) δH (ppm): 2.68 (s, 3H, CH₃), 3 (s, 6H, N(CH₃)₂), 3.04 (s, 2H, CH₂, pyrazole-N), 6.80 (m, 2H, Ar-H), 7.91 (m, 2H, Ar-H), 9.65 (s, 1H, CH=N), 11.97 (s, D₂O exchangeable, 1H, NH); ¹³C-NMR (125 MHz, DMSO-d₆) δC (ppm): 16.6 (CH₃), 40 (CH₃), 41 (CH₂), 111.6, 124, 129.5, 131.3, 154.4, 158, 163 (C=O), 190.5 (C=S); MS m/z (%): 290 [M⁺] (25%); Anal. Calcd. for C₁₄H₁₇N₅O₃S (303.38): C, 55.43; H, 5.65; N, 23.08; Found: C, 55.13; H, 5.30; N, 22.97%.

3.3. Antimicrobial Evaluation

The antibacterial activity of the synthesized compounds was tested against a panel of two gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative bacteria (Klebsiella pneumoniae and Escherichia coli). The antifungal activities of the compounds were tested against two fungi (Candida albicans and Aspergillus flavus). A solution of each compounds in DMSO with concentration 1 mg/mL was prepared separately, paper discs of Whatman filter paper were prepared with standard size (5 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the compound solution were placed aseptically in the petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with Staphylococcus aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Candida albicans, and Aspergillus flavus. The petri dishes were incubated at 36 °C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic
chloramphenicol and antifungal clotrimazole were also recorded using the same procedure as above at the same concentration and solvents [28].

The MIC was determined using the disc diffusion technique by preparing discs containing 1.9–1000 µg/mL of each compound against gram positive, gram negative, and fungi. Twofold dilutions of the solution were prepared. The microorganism suspensions at 10 CFU/mL (colony forming unit/mL) concentrations were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 h for the bacteria. The standard antibiotic chloramphenicol and antifungal clotrimazole was also recorded using the same procedure as above at the same concentration and solvents. At the end of the incubation period, the minimum inhibitory concentrations (MIC) values were recorded as the lowest concentration of the substance that had no visible turbidity. Control experiments with DMSO and uninoculated media were run parallel to the test compounds under the same condition [28].

4. Conclusions

A novel series of pyrazole and pyrazolone derivatives was synthesized, in good yields, starting from pyrazole-1-carbothiohydrazide 1a,b. A number of prepared compounds showed moderate to good antimicrobial activities. Hydrazones 21a–c showed significant antimicrobial activities with MIC values equal to or lower than standard drugs chloramphenicol and clotrimazole. It is clearly that the presence of free carbothiohydrazide moiety increases antimicrobial activity.

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Sample Availability: Samples of the compounds 1a, 1b, 5a, 8a, 12, 15c, 17b, 20, 21b, and 22 are available from the author. © 2018 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).