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Synthesis, characterization and antimicrobial activity of some carbamothioyl-1,3,4-thiadiazole derivatives

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Abstract : A series of 5-substituted-2-phenyl-1, 3, 4-thiadiazole were prepared by the reaction of different substituted benzoylisothiocyanate with 5-amino -2-phenyl -1, 3, 4-thiadiazole. The target molecules were characterized by CHNS analysis, IR, NMR and mass spectra. All these new compounds were screened for antibacterial and antifungal activity. Some of those compounds had promising antimicrobial activity.
Keywords: 1,3,4-Thiadiazole; Antimicrobial activity.

Introduction

The treatment of mycobacterial infections has become an important and challenging problem because the emergence of multiple-drug-resistance organisms and because of the acquired immunodeficiency syndrome (AIDS) pandemic [1]. The high rates of drug-resistant tuberculosis currently reported in many countries are alarming, since this phenomenon rapid drug susceptibility tests are needed and effective chemotherapy regiments with newly developed drugs are urgently being sought [2, 3]. Thidiazoles have been the subject of chemical and biological studies due to their interesting pharmacological properties, such as antimicrobial, anti-inflammatory, analgesic, and antitumoral activities [4, 5, 6]. Although there are a number of antibiotics, which are clinically used, the synthesis of new compounds is of vital importance due to increasing drug resistance. Moreover, it is important to obtain therapeutic compounds having less toxic effects. For this a number of synthesis methods for substituted heterocyclic derivatives have been developed [7]. In view of the above mentioned facts, we described herein the synthesis of some thiadiazole derivatives and evaluation of their antimicrobial activity. The reaction sequence leading to the formation of desired heterocyclic compounds are outlined in Scheme 1. The structures of the compounds were assigned on the basis of CHNS analysis, IR, ^{1}H, ^{13}C NMR and mass spectral data.

Experimental

Reagents and Techniques

All chemicals and solvents were purchased from Aldrich or Merck Chemical Companies and were used as received without further purification. The percentage compositions of the elements (CHNS) for the compounds were determined using an elemental analyzer CHNS Model Fison EA 1108. The infrared spectra were recorded as potassium bromide discs using a Perkin-Elmer spectrophotometer GX. The ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded using the JEOL JNM-ECP 400 spectrometer. Mass spectra were recorded by use of a Varian MTA CH-5 spectrometer (70 eV). The purity of the synthesized compounds was checked by TLC (aluminium foil-backed, 0.25mm silica gel 60 F_{254}; Merck)

General procedure for the preparation of substituted benzoylisothiocyanate 1-9

A mixture of different substituted benzoyl chloride (0.01mol) and ammonium thiocyanate (0.01 mol) in 25 mL acetone was refluxed with stirring for 1 h, and then filtered and the filtrate was used for further reaction [8].

General procedure for the preparation of 5-substituted-2-phenyl-1,3,4-thiadiazole 10-18

0.01 Mol of 2-phenyl-5-amino-1, 3, 4-thiadiazole in 20 mL acetone were added rapidly as possible on the above solution to maintain vigorous reflux. After refluxing for 6 h, the resulting solid was collected, washed with water and then with acetone and recrystalized with ethanol to afford the target products.

4-Fluoro-N-(methylcarbamothioylbenzamide-2-phenyl-1,3,4-thiadiazole 10

Yield 84%, m.p. 143-144 ̊C ; IR (KBr): ύ = 3138 (N-H), 3080 (C-H, aromatic), 1684 (C=O), 1604 (C=N), 1509 (C=C), 1318 (C=S), 627 (C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 7.41-8.12 (m, 9H, Ar-H), 10.30 (s, 1H, NH, D_2O exchangeable), 11.44 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 130.93-137.05 (10C, aromatic carbons), 150.24 and 152.16 (2C, thiadiazole carbons), 165.42 (1C, C=O), 187.08 (1C, C=S) ppm; Elemental analysis ($C_{16}H_{11}FN_4OS_2$); Calcd. C, 53.62; H, 3.09; N, 15.63; S, 17.89; Found C, 53.61; H, 3.08; N, 15.64; S, 17.90 %; M/S: *m/z* (%) = 374.07 (100.0%).

3-Nitro-N-(methylcarbamothioylbenzamide-2-phenyl-1,3,4-thiadiazole 11

Yield 86%, m.p. 191-192 ̊C ; IR (KBr): ύ = 3256 (N-H), 3100 (C-H, aromatic), 1683 (C=O), 1601 (C=N), 1527 and 1348 (NO_2), 1318 (C=S), 613 (C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 7.41-8.72 (m, 9H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable), 10.33 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 129.23-133.54 (10C, aromatic carbons), 152.15 and 153.72 (2C, thiadiazole carbons), 165.79 (1C, C=O), 187.81 (1C, C=S) ppm; Elemental analysis ($C_{16}H_{11}N_5O_3S_2$); Calcd. C, 49.86; H, 2.88; N, 18.17; S, 16.64; Found C, 49.85; H, 2.87; N, 18.16; S, 16.65%; M/S: *m/z* (%) = 401.06 (100.0%).

4-Cyano-N-(methylcarbamothioyl)benzamide-2-phenyl-1,3,4-thiadiazole 12

Yield 83%, m.p. 289-290 ̊C; IR (KBr): ύ = 3292 (N-H), 3032 (C-H, aromatic), 2231 (C≡N), 1674 (C=O), 1633 (C=N), 1536 (C=C), 1320 (C=S), 655 (C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ= 7.41-8.21 (m, 9H, Ar-H), 10.02 (s, 1H, NH, D_2O exchangeable), 11.33 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 118.65 (1C, C≡N), 129.23-133.57 (10C, aromatic carbons), 152.16 and 153.76 (2C, thiadiazole carbons), 165.41 (1C, C=O), 187.05 (1C, C=S) ppm; Elemental analysis ($C_{17}H_{11}N_5OS_2$); Calcd. C, 55.87; H, 3.03; N, 19.16; S, 17.55; Found C, 55.88; H, 3.04; N, 19.17; S, 17.54%; M/S: *m/z* (%) = 381.07 (100.0%).

2-Iodo-N-(methylcarbamothioyl)benzamide-2-phenyl-1,3,4-thiadiazole 13

Yield 70%, m.p. 122-123 ̊C; IR (KBr): ύ = 3239 (N-H), 3055 (C-H, aromatic), 1705 (C=O), 1630 (C=N), 1520 (C=C), 1335 (C=S), 667(C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 7.40-8.33 (m, 9H, Ar-H), 9.21 (s, 1H, NH, D_2O exchangeable), 11.44 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 132.29-140.41 (10C, aromatic carbons), 150.14 and 152.36 (2C, thiadiazole carbons), 167.43 (1C, C=O), 187.07 (1C, C=S) ppm; Elemental analysis ($C_{16}H_{11}IN_4OS_2$); Calcd. C, 41.21; H, 2.38; N, 12.01; S, 13.75; Found C, 41.22; H, 2.39; N, 12.02; S, 13.76%; M/S: *m/z* (%) = 481.97 (100.0%).

2-(4-Methoxyphenoxy)-N-(methylcarbamothioyl) acetamide-2-phenyl-1,3,4-thiadiazole 14

Yield 89%, m.p. 62-63 ̊C; IR (KBr): ύ = 3250 (N-H), 3070 (C-H, aromatic), 2988 (C-H, aliphatic), 1705 (C=O), 1627 (C=N), 1517 (C=C), 1318 (C=S), 1176 (C-O), 616(C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 3.83 (s, 3H, -OCH_3), 4.63 (s, 2H, -CH_2-), 6.88-8.03 (m, 9H, Ar-H), 9.23 (s, 1H, NH, D_2O exchangeable), 10.88 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 55.80 (1C, -OCH_3-),

66.71 (1C, -CH_2-), 133.43-139.01 (10C, aromatic carbons), 152.10 and 153.67 (2C, thiadiazole carbons), 172.63 (1C, C=O), 187.02 (1C, C=S) ppm; Elemental analysis ($C_{18}H_{16}N_4O_3S_2$); Calcd. C, 53.98; H, 4.03; N, 13.99; S, 16.01; Found C, 53.97; H, 4.04; N, 13.98; S, 16.02%; M/S: *m/z* (%) = 476.10 (100.0%).

Phenyl(methylcarbamothioyl)carbamate-2-phenyl-1,3,4-thiadiazole 15

Yield 85%, m.p. 262-263 ◌̊C; IR (KBr): ύ = 3244 (N-H), 3020 (C-H, aromatic), 1700 (C=O), 1630 (C=N), 1518 (C=C), 1326 (C=S), 1176 (C-O), 613 (C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 7.41-8.68 (m, 10H, Ar-H), 9.29 (s, 1H, NH, D_2O exchangeable), 10.88 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 130.92-137.54 (10C, aromatic carbons), 150.63 and 151.12 (2C, thiadiazole carbons), 167.16 (1C, C=O), 188.24 (1C, C=S) ppm; Elemental analysis ($C_{16}H_{12}N_4O_2S_2$); Calcd. C, 53.92; H, 3.39; N, 15.72; S, 17.99; Found C, 53.91; H, 3.40; N, 15.71; S, 17.98%; M/S: *m/z* (%) = 432.07 (100.0%).

2-Bromo-N-(methylcarbamothioyl) benzamide-2-phenyl-1,3,4-thiadiazole 16

Yield 75%, m.p. 130-132 ◌̊C; IR (KBr): ύ = 3245 (N-H), 3075 (C-H, aromatic), 1702 (C=O), 1630 (C=N), 1520 (C=C), 1335 (C=S), 622(C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 7.41-8.03 (m, 9H, Ar-H), 9.20 (s, 1H, NH, D_2O exchangeable), 11.44 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 130.90-137.73 (10C, aromatic carbons), 150.11 and 153.06 (2C, thiadiazole carbons), 169.22 (1C, C=O), 187.35 (1C, C=S) ppm; Elemental analysis ($C_{16}H_{11}BrN_4OS_2$); Calcd. C, 45.83; H, 2.64; N, 13.36; S, 15.29; Found C, 45.82; H, 2.65; N, 13.37; S, 15.30%; M/S: *m/z* (%)= 419.95 (100.0%).

3-Methoxy-N-(methylcarbamothioyl) benzamide-2-phenyl-1,3,4-thiadiazole 17

Yield 75%, m.p. 154-155 ◌̊C; IR (KBr): ύ = 3266 (N-H), 3100 (C-H, aromatic), 2920 (C-H, aliphatic), 1705 (C=O), 1615 (C=N), 1521 (C=C), 1368 (C=S), 1185 (C-O), 616(C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 3.43 (s, 3H, -OCH_3), 6.90-8.03 (m, 9H, Ar-H), 9.20 (s, 1H, NH, D_2O exchangeable), 11.54 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 55.75 (1C, -OCH_3-), 129.20-133.51 (10C, aromatic carbons), 152.13 and 154.67 (2C, thiadiazole carbons), 170.15 (1C, C=O), 187.46 (1C, C=S) ppm; Elemental analysis ($C_{17}H_{14}N_4O_2S_2$); Calcd. C, 55.12; H, 3.81; N, 15.12; S, 17.31; Found C, 55.13; H, 3.80; N, 15.11; S, 17.32%; M/S: *m/z* (%) = 370.06 (100.0%).

N-(Methylcarbamothioyl)benzamide-2-phenyl-1,3,4-thiadiazole 18

Yield 70%, m.p. 86-87 ◌̊C; IR (KBr): ύ = 3274 (N-H), 3080 (C-H, aromatic), 2842 (C-H, aliphatic), 1696 (C=O), 1620 (C=N), 1578 (C=C), 1320 (C=S), 635 (C-S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ= 7.51-8.43 (m, 10H, Ar-H), 9.45 (s, 1H, NH, D_2O exchangeable), 10.69 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 129.23-136.40 (10C, aromatic carbons), 149.62 and 152.03 (2C, thiadiazole carbons), 165.42 (1C, C=O), 187.53 (1C, C=S) ppm; Elemental analysis ($C_{16}H_{12}N_4OS_2$); Calcd. C, 56.45; H, 3.55; N, 16.46; S, 18.84; Found C, 56.44; H, 3.56; N, 16.45; S, 18.83%; M/S: *m/z* (%) = 340.05 (100.0%).

Antimicrobial tests

All the newly synthesized compounds were evaluated for their in *vitro* antibacterial activity against *Staphylococcus aureus*, *Streptcoccus viridans* and *Escherichia coli*. Disk diffusion method [9] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 ◌̊C for an hour. The test compounds were prepared with different concentrations using DMF. 1 mL containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 discs. Disks of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 ◌̊C for 24 h. Ofloxacin was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted.

On the other hand, the newly prepared compounds were screened for their in *vitro* antifungal activity against *Cibberela*, *Cercospora arachidicola* and *Physolospora piricola* in DMSO by the serial plate dilution method [10]. All the fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species. Agar media (20 mL) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 ◌̊C for 1 h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37 ◌̊C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Ketoconazole was

Table 1: Antibacterial activity of compounds 10-18

Comp. No.	*Staphylococcus aureus*		*Streptcoccus viridans*		*Escherichia coli*	
	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition
10	11	68.75	11	64.70	12	75.00
11	13	81.25	12	70.85	11	68.75
12	14	87.50	15	88.23	13	81.25
13	12	75.00	15	88.23	11	68.75
14	13	81.25	12	70.58	12	75.00
15	11	68.75	11	64.70	12	75.00
16	12	75.00	16	94.11	13	81.25
17	15	93.75	13	76.47	11	68.75
18	14	87.50	12	70.58	13	81.25
Ofloxacin	16	100.0	17	100	16	100

Table 2 : Antifungal activity of compounds 10-18

Comp. No.	*Cibberela*		*Cercospora arachidicola*		*Physolospora piricola*	
	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition
10	18	60.00	13	65.00	16	53.33
11	16	53.33	12	60.00	11	36.66
12	14	46.66	12	60.00	17	56.66
13	12	40.00	16	80.00	14	46.66
14	26	86.66	17	85.00	12	40.00
15	24	80.00	13	65.00	16	80.00
16	16	53.33	14	70.00	11	55.00
17	11	36.66	13	65.00	13	65.00
18	14	46.66	14	70.00	12	60.00
Ketoconazol	20	100.0	30	100	20	100

used as a standard drug. The zone of inhibition observed after respective incubation was measured and percent inhibition of the compounds was calculated. The results are presented in Table 1 and Table 2.

Results and Discussion

Chemistry

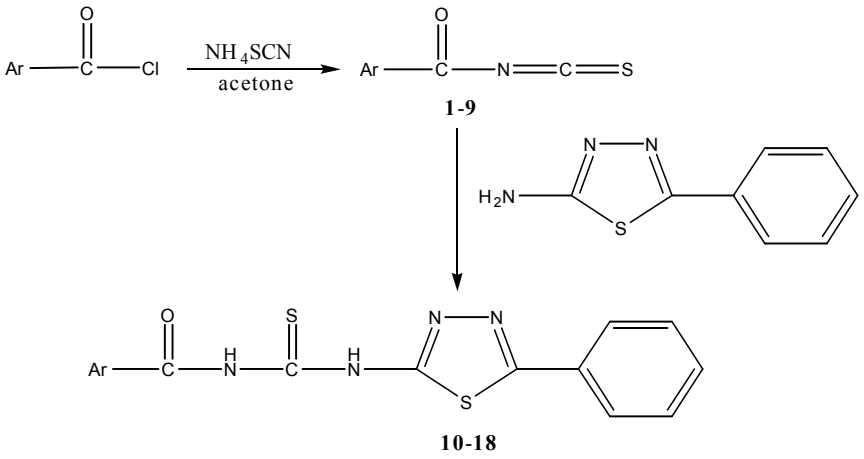
The starting materials, substituted benzoyl isothiocyanate (**1-9**), were synthesized by refluxing different substituted benzoyl chloride with ammonium thiocyanate in acetone [8]. Treatment of compounds (**1-9**) with 2-amino-5-phenyl-1, 3, 4-thiadiazole in acetone gave the corresponding 1,3,4-thiadiazole derivatives (**10-18**) in 70-89% (**Scheme 1**).

Characterization of the compounds (10-18)

IR spectra of compounds (**10-18**) showed characteristic bands at about 3200, 1700, 1630 and 1300cm^{-1} corresponding to the N-H, C=O, C=N and C=S [11, 12]. Beside this, the most characteristic evidence for structure given to those compounds in ^{1}H NMR spectra is the signals around δ 7.15-8.3 and 8.78 ppm attributed to aromatic and N-H protons, respectively. The assignment of N-H group in these compounds was achieved by D_2O exchange. Furthermore, ^{13}C-NMR spectra showed signals at about δ 173.03 and 180.50 ppm due to C=O and C=S groups, respectively [13, 14].

Antimicrobial evaluation

Microorganisms have existed on the earth for more than 3.8 bilion years and exhibit the greatest genetic and metabolic diversities. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. It is believed that they compose about 50% of the living biomass. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. The disease-causing microorganisms have particularly been vulnerable to man's selfishness for survival who has sought to deprive them of their habitat using antimicrobial agents. These microorganisms have responded by developing resistance mechanisms to fight off this offensive. Currently antimicrobial resistance among bacteria, viruses, parasites, and other disease causing organisms is a serious threat to infectious disease management globally [15].



Compound No.	Ar
10	$4\text{-}FC_6H_4$
11	$3\text{-}NO_2C_6H_4$
12	$4\text{-}CNC_6H_4$
13	$2\text{-}IC_6H_4$
14	$CH_3O\text{-}C_6H_4\text{-}OCH_2$
15	$C_6H_5\text{-}O\text{-}$
16	$2\text{-}BrC_6H_4$
17	$3\text{-}CH_3OC_6H_4$
18	C_6H_5

Scheme 1

In order to appreciate the mechanisms of resistance, it is important to understand how antimicrobial agents act. Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents. These include generally the following:

- Inhibition of the cell wall synthesis
- Inhibition of ribosome function
- Inhibition of nucleic acid synthesis
- Inhibition of folate metabolism
- Inhibition of cell membrane function

Microorganisms were increasingly becoming resistant to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded. They achieved this through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted. The resistance mechanisms, therefore, depend on which specific pathways are inhibited by the drugs and the alternative ways available for those paths ways that the organisms can modify to get a way around in order to survive [16, 17]. Resistance can be described in two ways:

(a) intrinsic or natural whereby microorganisms naturally do not posses target sites for the drugs and, therefore, the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to effect their action or
(b) acquired resistance whereby a naturally susceptible microorganism acquires ways of not being affected by the drug.

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *Staphylococcus aureus*, *Streptococcus viridans* and *Escherichia coil*. For antifungal, *Gibberela*, *Cercospora arachidicola* and *Physolospora piricola*

were used as microorganisms. The data are summarized in Table 1 and Table 2, and show that all compounds display certain activity against the tested microorganisms. From SAR we can see that the antibacterial and antifungal activity of the synthesized compounds may be due the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules, which facilitate the crossing through the biological membrane of the microorganism and thereby inhibit their growth.

Conclusions

The preparation procedure follow in this work for synthesis of the title compounds offers reduction in the reaction time, operation simplicity, cleaner reaction, easy work-up and improved yields. All spectroscopic analysis confirmed the proposed structures of these compounds. Biological activity data have shown that the synthesized compounds have a significant biological activity against the tested microorganisms. From these observations, with slight modification in the structure one can plan for the drug design.

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