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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 2,5-DISUBSTITUTED 1,3,4-THIADIAZOLES FOR THEIR POTENTIAL ANTIMICROBIAL ACTIVITY

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A series of novel N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-(4-substitutedbenzaldehyde)-semicarbazone **1-6**, N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-substitutedphenyl)ethanone]-semicarbazone **7-10** and N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-substitutedphenyl) (phenyl) methanone]-semicarbazone **11-14** were designed and synthesized for their potential antimicrobial activity. All the synthesized compounds were in good agreement with elemental and IR, ^{1}H-NMR and ^{13}C-NMR spectral data. The synthesized compounds were tested for their *in vitro* antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, the Gram negative bacteria *Proteus mirabilis* and *Pseudomonas aeruginosa*, the fungal strain *Aspergillus niger* and the yeast like pathogenic fungus *Candida albicans*, by disk diffusion method. The majority of the compounds were found active in the biological screening. The efforts were also made to establish structure activity relationships among synthesized compounds.

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Keywords: 1,3,4-thiadiazoles, Semicarbazones, Antimicrobial activity.

1. Introduction

Drug resistance is a steadily increasing process that is reaching alarming level in the treatment of infectious diseases caused by pathogenic bacteria, fungi, parasites and viruses. Over the past few decades, steadily increasing drug resistance in the treatment of infectious disease pose a serious problem in antimicrobial therapy and necessitates continuing research into novel classes of antimicrobials [1]. A number of researchers have reported antimicrobial activities in 2,5-disubstituted-1,3,4-thiadiazoles [2-4]. Keeping the above facts in view, we considered it of interest to synthesize some novel 2,5-disubstituted-1,3,4-thiadiazole derivatives for their antimicrobial properties.

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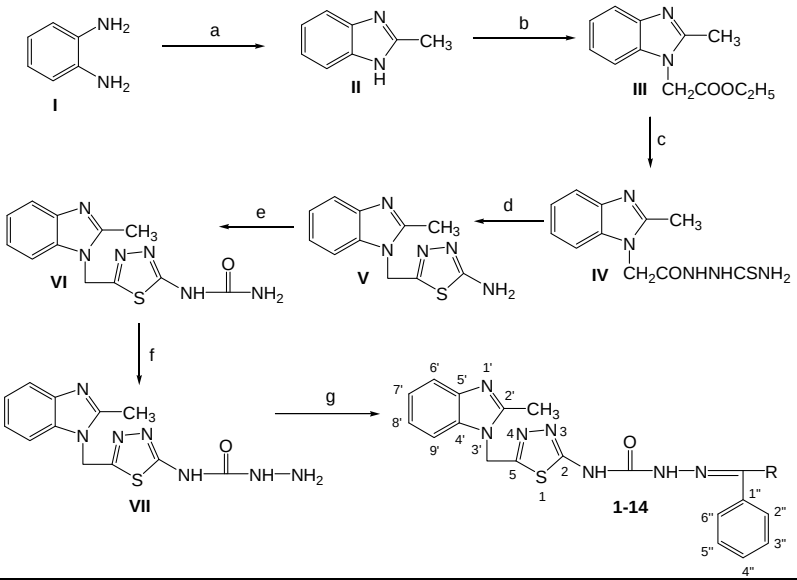
2. Experimental

2.1 Chemistry

The title compounds were prepared using the synthetic strategy described in figure 1. 2-Methyl-1*H*-benzimidazole **II** was prepared according to the reported method [5]. Compound **II** on N-ethoxylation with ethylchloroacetate in the presence of anhydrous K_2CO_3 in dry acetone gave ethyl (2-methyl-1*H*-benzimidazol-1-yl)acetate **III** which on treatment with thiosemicarbazide resulted in the formation of 2-[(2-methyl-1*H*-benzimidazol-1-yl)acetyl]-hydrazinecarbothioamide **IV**. Dehydrated annulation of compound **IV** with conc. H_2SO_4 followed by NH_3 treatment yielded 5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine **V** [6]. Compound **V** was treated with sodium cyanate in the presence of glacial acetic acid, to yield 1-[5-{(2-methyl-1*H*-benzimidazol-1-yl)methyl}-1,3,4-thiadiazol-2-yl]-urea **VI**. N-[5-{(2-Methyl-1*H*-benzimidazol-1-yl)methyl}-1,3,4-thiadiazol-2-yl]-hydrazine carboxamide **VII** was prepared by reaction of **VI** with hydrazine hydrate in the presence of sodium hydroxide. Title compounds **1-14** were prepared by reaction of the appropriate aldehyde or ketone with compound **VII.**

All the chemicals and solvents used in this study were purchased from Aldrich (Germany), Himedia (India) and Spectrochem Pvt Ltd (India). Melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on a Perkin Elmer IR spectrophotometer (KBr disc) (Perkin Elmer, Beaconsfield, UK), NMR spectra on a Bruker DRX-300 NMR spectrometer (DMSO-d_6, TMS) (Bruker Bioscience, Billerica, MA, USA) and the electrospray mass spectra on a Micromass Quattro II triple-quadrupole mass spectrometer (Methanol) (Micromass, Manchester, UK).



Compound Code	R	R′	Compound Code	R	R′	Compound Code	R	R′
1	H	H	6	H	4-Cl	11	C_6H_5	H
2	H	4-NO_2	7	CH_3	4-OH	12	C_6H_5	4-OH
3	H	4-OH	8	CH_3	4-OCH_3	13	C_6H_5	4-NO_2
4	H	4-CH_3	9	CH_3	4-NO_2	14	C_6H_5	4-OCH_3
5	H	4-OCH_3	10	CH_3	4-Cl			

Reaction conditions (a) CH_3COOH; (b) $ClCH_2COOC_2H_5$; (c) $NH_2NHCSNH_2$; (d) H_2SO_4, NH_3; (e) NaCNO, CH_3COOH; (f) $NH_2NH_2.H_2O$, NaOH; (g) Aldehydes or ketone, CH_3COONa.

Fig. 1.Scheme for synthesis of 2,5-disubstituted-1,3,4-thiadiazole derivatives 1–14.

2.1.1 General procedure for synthesis of 1-[5-{(2-methyl-1H-benzimidazol-1-yl)methyl}-1,3,4-thiadiazol-2-yl]-urea VI:

The compound **V** (0.01 mol) was dissolved in 10–30 ml of glacial acetic acid diluted to 50 ml with distilled water. To this equimolar (0.01 mol) quantity of sodium cyanate in 20–30 ml of warm water was added with stirring. The reaction mixture was allowed to stand several h followed by cooling on an ice bath. The precipitates obtained were collected by filtration, washed with cold water and recrystallized from 90% aqueous ethanol. MP (ºC) 178–179; Yield 66%; IR (KBr), 3052.9 (Aromatic C-H str), 1614.9 (C=N of benzimidazole ring), 738.3 (C-S of thiadiazole nucleus), 1647.5 (C=N of thiadiazole), 3345.1 (NH str of amide), 1688.5 (C=O str of amide), 3427.1 (NH str of NH_2); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 4.9 (s, 2H, NCH_2), 2.4 (s, 3H, CH_3), 7.2-7.8 (m, 4H, ArH), 6.2 (s, 2H, NH_2), 5.9 (s, 1H, NH); ESMS (Methanol) *m/z* 288.3 (M^+).

2.1.2 General procedure for synthesis of N-[5-(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl]-hydrazinecarboxamide VII:

Required quantity of the compound **VI** (0.01 mol) was dissolved in 30–40 ml of ethanol. To this was added equimolar solution of hydrazine hydrate in 5 ml of water. The reaction mixture was made alkaline by adding 4 g of sodium hydroxide pellets. The contents were then heated to reflux for 2–8 h, followed by cooling on an ice bath. The product was filtered and recrystallized from 90% aqueous ethanol. MP (ºC) 195–196; Yield 68%; IR (KBr), 3048.2 (Aromatic C-H str), 1609.4 (C=N of benzimidazole ring), 740.4 (C-S of thiadiazole nucleus), 1643.7 (C=N of thiadiazole), 3336.4 (NH str of amide), 1679.5 (C=O str of amide), 3438.5 (NH str of NH_2); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 4.9 (s, 2H, NCH_2), 2.6 (d, 2H, NH_2), 6.2 (t, 1H, N<u>H</u>NH_2), 6.1 (s, 1H, N<u>H</u>CO), 2.4 (s, 3H, CH_3), 7.2-7.8 (m, 4H, ArH); ESMS (Methanol) *m/z* 303.3 (M^+).

2.1.3 General procedure for synthesis of title compounds 1-14:

Equimolar quantities of compound **VII** (0.01 mol) and carbonyl compound (0.01 mol) were dissolved in 20–30 ml of ethanol. To this 5 ml of water was added. The turbidity if appeared was removed by adding ethanol with adequate stirring of the reaction mixture. The pH of the reaction mixture was adjusted between 4 and 5, by adding glacial acetic acid. The reaction mixture was refluxed for a period of time ranging from 2–3 h. Thereafter reaction mixture was cooled on an ice bath and the crystallized product so obtained was filtered under vacuum. The crude product was recrystallized from 90% aqueous ethanol.

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-(benzaldehyde)-semicarbazone 1: MP (ºC) 161-163; Yield 54%; IR (cm^{-1}) (KBr) 3048.2 (Aromatic C-H str), 1602.5 & 1504.7 (Aromatic C-C str), 743.1 (C-S of 1,3,4-thiadiazole nucleus), 1644.8 (C=N of 1,3,4-thiadiazole nucleus), 1680.5 (C=O str of amide), 3435.8 (N-H str of amide), 1614.9 (C=N group); ^{13}C-NMR (75 MHz, DMSO-*d_6*, TMS, δ ppm): 141.3 (C-2'), 137.6 (C-4' & C-5'), 115.3 (C-6' & C-9'), 122.7 (C-7' & C-8'), 164.4 (C-2), 156.5 (C-5), 157.3 (NH<u>C</u>ONHNCH), 154.8 (NHCONHN<u>C</u>H), 129.1 (C-2" & C-6"), 128.7 (C-3" & C-5"), 130.8 (C-4"), 131.3 (C-1"), 9.3 (<u>C</u>H_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-*d_6*, TMS, δ ppm): 6.8-7.6 (m, 9H, ArH), 6.1 (s, 1H, N<u>H</u>CONH), 9.5 (s, 1H, NHCON<u>H</u>), 6.9 (s, 1H, imine H), 5.1 (s, 2H, CH_2), 2.5 (s, 3H, CH_3); ESMS (Methanol) m/z 391.4 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-(4-nitrobenzaldehyde)-semicarbazone 2: MP (ºC) 173-175; Yield 62%; IR (cm^{-1}) (KBr) 3044.3 (Aromatic C-H str), 1603.6 & 1505.8 (Aromatic C-C str), 745.2 (C-S of 1,3,4-thiadiazole nucleus), 1644.6 (C=N of 1,3,4-thiadiazole nucleus), 1683.8 (C=O str of amide), 3427.2 (N-H str of amide), 1613.7 (C=N group), 1522.5 & 1353.6 (N=O str of Ar-NO_2 group); ^{13}C-NMR (75 MHz, DMSO-*d_6*, TMS, δ ppm): 141.5 (C-2'), 137.8 (C-4' & C-5'), 115.4 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.6 (C-2), 156.4 (C-5), 157.5 (NH<u>C</u>ONHNCH), 154.7 (NHCONHN<u>C</u>H), 129.8 (C-2" & C-6"), 123.6 (C-3" & C-5"), 150.8 (C-4"), 137.4 (C-1"), 9.4 (<u>C</u>H_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-*d_6*, TMS, δ ppm): 7.2-8.2 (m, 8H, ArH), 6.2 (s, 1H, N<u>H</u>CONH), 9.4 (s, 1H,

NHCONH), 6.8 (s, 1H, imine H), 5.0 (s, 2H, CH_2), 2.4 (s, 3H, CH_3); ESMS (Methanol) m/z 436.5 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)1,3,4-thiadiazol-2-yl}-N^4-(4-hydroxybenzaldehyde)-semicarbazone 3: MP (ºC) 193-194; Yield 55%; IR (cm^{-1}) (KBr) 3035.8 (Aromatic C-H str), 1603.6 & 1502.1 (Aromatic C-C str), 743.5 (C-S of 1,3,4-thiadiazole nucleus), 1640.4 (C=N of 1,3,4-thiadiazole nucleus), 1674.7 (C=O str of amide), 3425.1 (N-H str of amide), 1615.0 (C=N group), 821.7 (C-H def disubstituted benzene ring), 3461.9 (O-H str of alcoholic group), 1164.6 (C-O str of alcoholic group); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 141.4 (C-2'), 137.8 (C-4' & C-5'), 115.5 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.3 (C-2), 156.4 (C-5), 157.5 (NHCONHNCH), 154.7 (NHCONHNCH), 130.4 (C-2" & C-6"), 115.9 (C-3" & C-5"), 159.8 (C-4"), 123.8 (C-1"), 9.2 (CH_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8-7.7 (m, 8H, ArH), 6.2 (s, 1H, NHCONH), 9.5 (s, 1H, NHCONH), 6.8 (s, 1H, imine H), 5.0 (s, 2H, CH_2), 2.5 (s, 3H, CH_3), 5.3 (ArOH); ESMS (Methanol) m/z 407.4 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-(4-methylbenzaldehyde)-semicarbazone 4: MP (ºC) 168-170; Yield 57%; IR (cm^{-1}) (KBr) 3042.8 (Aromatic C-H str), 1605.8 & 1502.5 (Aromatic C-C str), 743.7 (C-S of 1,3,4-thiadiazole nucleus), 1646.4 (C=N of 1,3,4-thiadiazole nucleus), 1672.7 (C=O str of amide), 3429.4 (N-H str of amide), 2909.2 (aliphatic C-H str), 1445.1 (aliphatic C-H def), 1619.3 (C=N group), 826.4 (C-H def disubstituted benzene ring); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 141.5 (C-2'), 137.8 (C-4' & C-5'), 115.3 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.5 (C-2), 156.5 (C-5), 157.2 (NHCONHNCH), 154.7 (NHCONHNCH), 128.9 (C-2" & C-6"), 129.4 (C-3" & C-5"), 140.1 (C-4"), 128.3 (C-1"), 21.0 ($CH_3C_6H_5$), 9.5 (CH_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.9-7.7 (m, 8H, ArH), 6.0 (s, 1H, NHCONH), 9.6 (s, 1H, NHCONH), 6.8 (s, 1H, imine H), 5.1 (s, 2H, CH_2), 2.4 (s, 3H, CH_3), 2.3 ($ArCH_3$); ESMS (Methanol) m/z 405.4 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)1,3,4-thiadiazol-2-yl}-N^4-(4-methoxybenzaldehyde)-semicarbazone 5: MP (ºC) 197-199; Yield 58%; IR (cm^{-1}) (KBr) 3040.8 (Aromatic C-H str), 1606.9 & 1502.1 (Aromatic C-C str), 744.6 (C-S of 1,3,4-thiadiazole nucleus), 1645.0 (C=N of 1,3,4-thiadiazole nucleus), 1683.7 (C=O str of amide), 3424.2 (N-H str of amide), 1616.5 (C=N group), 825.3 (C-H def disubstituted benzene ring), 1266.4 (C-O of OCH_3 group); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 141.3 (C-2'), 137.7 (C-4' & C-5'), 115.3 (C-6' & C-9'), 122.9 (C-7' & C-8'), 164.4 (C-2), 156.7 (C-5), 157.4 (NHCONHNCH), 154.6 (NHCONHNCH), 129.9 (C-2" & C-6"), 114.5 (C-3" & C-5"), 164.3 (C-4"), 123.4 (C-1"), 56.1 ($OCH_3C_6H_5$), 9.3 (CH_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8-7.8 (m, 8H, ArH), 6.2 (s, 1H, NHCONH), 9.6 (s, 1H, NHCONH), 6.8 (s, 1H, imine H), 5.1 (s, 2H, CH_2), 2.4 (s, 3H, CH_3), 3.8 ($ArOCH_3$); ESMS (Methanol) m/z 421.5 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-(4-chlorobenzaldehyde)-semicarbazone 6: MP (ºC) 212-213; Yield 62%; IR (cm^{-1}) (KBr) 3036.3 (Aromatic C-H str), 1605.3 & 1505.6 (Aromatic C-C str), 750.5 (C-S of 1,3,4-thiadiazole nucleus), 1639.9 (C=N of 1,3,4-thiadiazole nucleus), 1693.6 (C=O str of amide), 3423.2 (N-H str of amide), 2911.7 (aliphatic C-H str), 1443.7 (aliphatic C-H def), 1604.4 (C=N group), 826.1 (C-H def disubstituted benzene ring), 719.4 (C-Cl str); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 141.4 (C-2'), 137.8 (C-4' & C-5'), 115.3 (C-6' & C-9'), 122.9 (C-7' & C-8'), 164.5 (C-2), 156.3 (C-5), 157.5 (NHCONHNCH), 154.9 (NHCONHNCH), 130.3 (C-2" & C-6"), 128.9 (C-3" & C-5"), 136.2 (C-4"), 129.2 (C-1"), 9.4 (CH_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.9-7.7 (m, 8H, ArH), 6.0 (s, 1H, NHCONH), 9.5 (s, 1H, NHCONH), 6.8 (s, 1H, imine H), 5.0 (s, 2H, CH_2), 2.5 (s, 3H, CH_3); ESMS (Methanol) m/z 425.9 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-hydroxyphenyl) ethanone]-semicarbazone 7: MP (ºC) 204-206; Yield 60%; IR (cm^{-1}) (KBr) 3040.7 (Aromatic C-H str), 1606.1 & 1506.0 (Aromatic C-C str), 745.8 (C-S of 1,3,4-thiadiazole

nucleus), 1642.6 (C=N of 1,3,4-thiadiazole nucleus), 1683.1 (C=O str of amide), 3424.8 (N-H str of amide), 1607.2 (C=N group), 826.0 (C-H def disubstituted benzene ring), 3442.8 (O-H str of alcoholic group), 1144.9 (C-O str of alcoholic group); ^{13}C-NMR (75 MHz, DMSO-*d_6*, TMS, δ ppm): 141.4 (C-2'), 137.8 (C-4' & C-5'), 115.5 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.6 (C-2), 156.4 (C-5), 157.3 (NH<u>C</u>ONHNCCH$_3$), 155.7 (NHCONHN<u>C</u>CH$_3$), 11.4 (NHCONHNC<u>C</u>H$_3$), 130.5 (C-2" & C-6"), 115.7 (C-3" & C-5"), 159.8 (C-4"), 123.6 (C-1"); ^{1}H NMR (300 MHz, DMSO-*d_6*, TMS, δ ppm): 6.8-7.8 (m, 8H, ArH), 6.1 (s, 1H, N<u>H</u>CONH), 9.6 (s, 1H, NHCON<u>H</u>), 5.4 (ArOH), 1.1 (s, 3H, Carbimino CH$_3$), 5.1 (s, 2H, CH$_2$), 2.4 (s, 3H, CH$_3$); ESMS (Methanol) m/z 421.4 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-methoxyphenyl) ethanone]-semicarbazone 8: MP (ºC) 172-174; Yield 62%; IR (cm^{-1}) (KBr) 3043.0 (Aromatic C-H str), 1604.4 & 1501.6 (Aromatic C-C str), 739.2 (C-S of 1,3,4-thiadiazole nucleus), 1642.6 (C=N of 1,3,4-thiadiazole nucleus), 1683.5 (C=O str of amide), 3432.1 (N-H str of amide), 2913.7 (aliphatic C-H str), 1444.0 (aliphatic C-H def), 1618.3 (C=N group), 819.5 (C-H def disubstituted benzene ring), 1271.8 (C-O of OCH$_3$ group); ^{13}C-NMR (75 MHz, DMSO-*d_6*, TMS, δ ppm): 141.5 (C-2'), 137.9 (C-4' & C-5'), 115.4 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.5 (C-2), 156.4 (C-5), 157.4 (NH<u>C</u>ONHNCCH$_3$), 155.8 (NHCONHN<u>C</u>CH$_3$), 11.4 (NHCONHNC<u>C</u>H$_3$), 130.3 (C-2" & C-6"), 114.3 (C-3" & C-5"), 164.5 (C-4"), 123.6 (C-1"), 56.1 (O<u>C</u>H$_3$C$_6$H$_5$), 9.2 (<u>C</u>H$_3$ attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-*d_6*, TMS, δ ppm): 6.8-7.8 (m, 8H, ArH), 6.1 (s, 1H, N<u>H</u>CONH), 9.8 (s, 1H, NHCON<u>H</u>), 1.1 (s, 3H, Carbimino CH$_3$), 3.8 (ArOCH$_3$), 5.0 (s, 2H, CH$_2$), 2.5 (s, 3H, CH$_3$); ESMS (Methanol) m/z 435.5 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-nitrophenyl) ethanone]-semicarbazone 9: MP (ºC) 218-220; Yield 57%; IR (cm^{-1}) (KBr) 3044.6 (Aromatic C-H str), 1604.6 & 1503.7 (Aromatic C-C str), 740.8 (C-S of 1,3,4-thiadiazole nucleus), 1643.6 (C=N of 1,3,4-thiadiazole nucleus), 1685.9 (C=O str of amide), 3430.3 (N-H str of amide), 2911.7 (aliphatic C-H str), 1439.3 (aliphatic C-H def), 1617.6 (C=N group), 829.0 (C-H def disubstituted benzene ring), 1531.5 & 1359.1 (N=O str of Ar-NO$_2$ group); ^{13}C-NMR (75 MHz, DMSO-*d_6*, TMS, δ ppm): 141.6 (C-2'), 138.0 (C-4' & C-5'), 115.5 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.4 (C-2), 156.3 (C-5), 157.6 (NH<u>C</u>ONHNCCH$_3$), 155.8 (NHCONHN<u>C</u>CH$_3$), 11.3 (NHCONHNC<u>C</u>H$_3$), 130.0 (C-2" & C-6"), 123.8 (C-3" & C-5"), 150.6 (C-4"), 137.5 (C-1"), 9.4 (<u>C</u>H$_3$ attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-*d_6*, TMS, δ ppm): 7.2-8.2 (m, 8H, ArH), 6.2 (s, 1H, N<u>H</u>CONH), 9.7 (s, 1H, NHCON<u>H</u>), 1.1 (s, 3H, Carbimino CH$_3$), 5.0 (s, 2H, CH$_2$), 2.4 (s, 3H, CH$_3$); ESMS (Methanol) m/z 450.5 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-chlorophenyl) ethanone]-semicarbazone 10: MP (ºC) 187-189; Yield 60%; IR (cm^{-1}) (KBr) 3040.4 (Aromatic C-H str), 1606.2 & 1501.6 (Aromatic C-C str), 746.3 (C-S of 1,3,4-thiadiazole nucleus), 1649.7 (C=N of 1,3,4-thiadiazole nucleus), 1683.6 (C=O str of amide), 3426.4 (N-H str of amide), 2909.1 (aliphatic C-H str), 1442.0 (aliphatic C-H def), 1619.7 (C=N group), 825.9 (C-H def disubstituted benzene ring) 718.4 (C-Cl str); ^{13}C-NMR (75 MHz, DMSO-*d_6*, TMS, δ ppm): 141.7 (C-2'), 137.8 (C-4' & C-5'), 115.5 (C-6' & C-9'), 122.7 (C-7' & C-8'), 164.5 (C-2), 156.4 (C-5), 157.3 (NH<u>C</u>ONHNCCH$_3$), 155.7 (NHCONHN<u>C</u>CH$_3$), 11.4 (NHCONHNC<u>C</u>H$_3$), 130.4 (C-2" & C-6"), 129.2 (C-3" & C-5"), 136.2 (C-4"), 129.5 (C-1"), 9.3 (<u>C</u>H$_3$ attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-*d_6*, TMS, δ ppm): 7.2-7.9 (m, 8H, ArH), 6.3 (s, 1H, N<u>H</u>CONH), 9.8 (s, 1H, NHCON<u>H</u>), 1.2 (s, 3H, Carbimino CH$_3$), 5.1 (s, 2H, CH$_2$), 2.5 (s, 3H, CH$_3$); ESMS (Methanol) m/z 439.9 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(diphenylphenyl) methanone]-semicarbazone 11: MP (ºC) 240-242; Yield 55%; IR (cm^{-1}) (KBr) 3035.7 (Aromatic C-H str), 1605.2 & 1504.4 (Aromatic C-C str), 743.1 (C-S of 1,3,4-thiadiazole nucleus), 1645.2 (C=N of 1,3,4-thiadiazole nucleus), 1670.7 (C=O str of amide), 3427.0 (N-H str of amide), 1627.9 (C=N group), 709.1 & 765.5 (C-H def monosubstituted benzene ring); ^{13}C-NMR (75 MHz,

DMSO-d_6, TMS, δ ppm): 141.5 (C-2'), 137.9 (C-4' & C-5'), 115.6 (C-6' & C-9'), 122.7 (C-7' & C-8'), 164.4 (C-2), 156.5 (C-5), 157.5 (NH<u>C</u>ONHNCC_6H_5), 155.6 (NHCONHN<u>C</u>C_6H_5), 129.1 (C-2" & C-6"), 128.6 (C-3" & C-5"), 130.9 (C-4"), 131.3 (C-1"), 129.1 (C-2"' & C-6"'), 128.6 (C-3"' & C-5"'), 130.9 (C-4"'), 131.3 (C-1"'), 9.3 (<u>C</u>H_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 7.2-7.9 (m, 14H, ArH), 6.2 (s, 1H, N<u>H</u>CONH), 9.7 (s, 1H, NHCON<u>H</u>), 5.0 (s, 2H, CH_2), 2.5 (s, 3H, CH_3); ESMS (Methanol) m/z 467.6 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-hydroxyphenyl) (phenyl) methanone]-semicarbazone 12: MP (ºC) 226-228; Yield 59%; IR (cm^{-1}) (KBr) 3040.6 (Aromatic C-H str), 1603.5 & 1507.0 (Aromatic C-C str), 746.9 (C-S of 1,3,4-thiadiazole nucleus), 1642.8 (C=N of 1,3,4-thiadiazole nucleus), 1680.2 (C=O str of amide), 3443.9 (N-H str of amide), 1619.5 (C=N group), 823.1 (C-H def disubstituted benzene ring), 3474.8 (O-H str of alcoholic group), 1159.7 (C-O str of alcoholic group), 708.6 & 762.2 (C-H def monosubstituted benzene ring); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 141.7 (C-2'), 137.9 (C-4' & C-5'), 115.2 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.3 (C-2), 156.4 (C-5), 157.2 (NH<u>C</u>ONHNCC_6H_5), 155.8 (NHCONHN<u>C</u>C_6H_5), 130.6 (C-2" & C-6"), 115.9 (C-3" & C-5"), 159.7 (C-4"), 123.6 (C-1"), 129.0 (C-2"' & C-6"'), 128.6 (C-3"' & C-5"'), 130.9 (C-4"'), 131.3 (C-1"'), 9.5 (<u>C</u>H_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8-7.9 (m, 13H, ArH), 6.2 (s, 1H, N<u>H</u>CONH), 9.8 (s, 1H, NHCON<u>H</u>), 5.3 (ArOH), 5.1 (s, 2H, CH_2), 2.4 (s, 3H, CH_3); ESMS (Methanol) m/z 483.5 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-nitrophenyl) (phenyl) methanone]-semicarbazone 13: MP (ºC) 186-187; Yield 60%; IR (cm^{-1}) (KBr) 3041.4 (Aromatic C-H str), 1604.6 & 1510.5 (Aromatic C-C str), 760.9 (C-S of 1,3,4-thiadiazole nucleus), 1648.2 (C=N of 1,3,4-thiadiazole nucleus), 1680.1 (C=O str of amide), 3425.8 (N-H str of amide), 1619.9 (C=N group), 824.7 (C-H def disubstituted benzene ring), 1520.8 & 1351.7 (N=O str of Ar-NO_2 group), 707.8 & 766.0 (C-H def monosubstituted benzene ring); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 141.8 (C-2'), 137.9 (C-4' & C-5'), 115.5 (C-6' & C-9'), 122.9 (C-7' & C-8'), 164.5 (C-2), 156.3 (C-5), 157.4 (NH<u>C</u>ONHNCC_6H_5), 155.7 (NHCONHN<u>C</u>C_6H_5), 130.1 (C-2" & C-6"), 123.9 (C-3" & C-5"), 150.9 (C-4"), 137.5 (C-1"), 129.0 (C-2"' & C-6"'), 128.6 (C-3"' & C-5"'), 130.7 (C-4"'), 131.3 (C-1"'), 9.3 (<u>C</u>H_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 7.2-8.2 (m, 13H, ArH), 6.1 (s, 1H, N<u>H</u>CONH), 9.7 (s, 1H, NHCON<u>H</u>), 5.0 (s, 2H, CH_2), 2.3 (s, 3H, CH_3); ESMS (Methanol) m/z 512.6 (M^+).

N^1-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-methoxyphenyl) (phenyl) methanone]-semicarbazone 14: MP (ºC) 254-256; Yield 58%; IR (cm^{-1}) (KBr) 3045.9 (Aromatic C-H str), 1607.5 & 1502.6 (Aromatic C-C str), 745.1 (C-S of 1,3,4-thiadiazole nucleus), 1647.4 (C=N of 1,3,4-thiadiazole nucleus), 1688.3 (C=O str of amide), 3429.0 (N-H str of amide), 1619.2 (C=N group), 821.9 (C-H def disubstituted benzene ring), 1255.2 (C-O of OCH_3 group), 709.7 & 767.2 (C-H def monosubstituted benzene ring); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 141.5 (C-2'), 137.9 (C-4' & C-5'), 115.5 (C-6' & C-9'), 122.8 (C-7' & C-8'), 164.4 (C-2), 156.6 (C-5), 157.3 (NH<u>C</u>ONHNCC_6H_5), 155.9 (NHCONHN<u>C</u>C_6H_5), 130.3 (C-2" & C-6"), 114.5 (C-3" & C-5"), 164.6 (C-4"), 123.6 (C-1"), 129.0 (C-2"' & C-6"'), 128.6 (C-3"' & C-5"'), 130.8 (C-4"'), 131.4 (C-1"'), 56.2 (O<u>C</u>$H_3C_6H_5$), 9.4 (<u>C</u>H_3 attached to benzimidazole); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8-7.8 (m, 13H, ArH), 6.2 (s, 1H, N<u>H</u>CONH), 9.8 (s, 1H, NHCON<u>H</u>), 3.8 (ArOCH_3), 5.0 (s, 2H, CH_2), 2.4 (s, 3H, CH_3); ESMS (Methanol) m/z 497.6 (M^+).

2.2 Antimicrobial activity

The antimicrobial properties of the compounds were investigated against bacterial strains *i.e.*, *Proteus mirabilis* (MTCC-425), *Pseudomonas aeruginosa* (MTCC-424), *Bacillus subtilis* (MTCC-619), and *Staphylococcus aureus* (MTCC-96) and fungal strains *i.e.*, *Aspergillus niger* (MTCC-1344) and *Candida albicans* (MTCC-227) using disk diffusion method [7, 8]. (Table 1)

Norfloxacin and Clotrimazole were used as standard drug for antibacterial and antifungal studies respectively. Nutrient Agar [9] (beef extract 1g, Yeast extract 2g, peptone 5g, sodium chloride 5g, Agar 15g and distilled water q. s. to 1,000 ml) was employed as culture media for antibacterial studies. For antimycotic evaluation against *Aspergillus niger*, Czapek yeast extract agar [9] (Czapek concentrate 10 ml, K_2HPO_4 1g, yeast extract 5g, sucrose 30g, Agar 15g and distilled water q. s. to 1,000 ml, where czapek concentrate comprises of $NaNO_3$ 30g, KCl 5g, $MgSO_4.7H_2O$ 5g, $FeSO_4.7H_2O$ 0.1g and distilled water q. s. to 1,000 ml) was employed.

Table 1: Antimicrobial activity[#] of the synthesized compounds using disc-diffusion method (100 μg/ 8mm disc)

Compound Code	Zone of Inhibition (mm)					
	Antibacterial activity				Antifungal activity	
	Staphylococus aureus **(MTCC-96)**	***Bacillus subtilis*** **(MTCC-619)**	***Proteus mirabilis*** **(MTCC-425)**	***Pseudomonas aeruginosa*** **(MTCC-424)**	***A. niger*** **(MTCC-1344)**	***C. albicans*** **(MTCC-227)**
1.	12	12	11	-	12	11
2.	13	11	12	11	13	11
3.	15	12	13	13	13	12
4.	12	-	-	-	-	13
5.	14	12	14	15	13	13
6.	14	13	11	-	13	-
7.	15	14	13	15	13	13
8.	14	15	14	13	11	12
9.	14	15	13	12	13	13
10.	12	13	12	-	11	10
11.	14	13	15	16	13	14
12.	16	14	16	16	15	14
13.	17	14	16	17	15	17
14.	14	13	15	14	14	14
Norfloxacin	24	17	22	20	NT	NT
Clotrimazole	NT	NT	NT	NT	21	23

NT= Not tested.

- (dash) = No activity

[#]Microbial strains were procured from Institute of Microbial Technology (IMTECH) Chandigarh, INDIA.

Malt yeast Agar [9] (Malt extract 3g, yeast extract 3g, Peptone 5g, glucose 10g, Agar 20g and distilled water q. s. to 1,000 ml) with pH 7.0 was employed as culture media in antimicrobial studies against *Candida albicans*. The sterilization of the culture medias, petridishes and other glasswares was done by autoclaving at 15 1b/sq inch pressure for 30 min. For antibacterial studies, incubation was carried out at 37 $\pm$ 1ºC for 48 h except for *Bacillus subtilis* where incubation was carried out at 26 $\pm$ 1º C for similar time period. Incubation conditions for *Aspergillus niger* and *Candida albicans* was 25 $\pm$ 1ºC for 72 h.

The cell density of each inoculum was adjusted with hemocytometer in order to procure a final concentration of approximately 10^5 CFU ml^{-1}. During antimicrobial evalution the medium after sterilization was poured into sterile petridishes under aseptic conditions in a laminar flow chamber. When the medium in the plate solidified, 0.5 ml of (10^5 CFU ml^{-1}) culture of test organism was inoculated and uniformly spread over the agar surface using a sterile L-shaped glass rod. Solutions of the test compound (100 μg/ml) were prepared by dissolving the test compound in dimethyl formamide (DMF). The sterile filter paper disc (8mm diameter) were moistened with the test compounds solution in DMF of specific concentration (100μg/disc) placed on the agar culture plates that had been previously inoculated with specific microorganisms. Controls were maintained with DMF and standard drug chosen in the respective case. Inhibition zones were

measured and the diameter was calculated in millimeters. All the tests were performed in triplicate for determination of MIC's.

3. Results and discussion

The structures of the compounds were elucidated on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. C, H and N estimations were found within 0.4% of the calculated values. IR data of synthesized thiadiazole analogues clearly shows C=N stretching band around 1644 cm^{-1} and C–S absorption band around 740 cm^{-1} which indicates ring closure of 1,3,4-thiadiazole ring. All the final compounds have strong absorption around 3040 cm^{-1} which is evidence for the presence of aromatic C–H bonds. Presence of aromatic C–C bonds was confirmed by presence of absorption band around 1602 and 1504 cm^{-1}. IR data also confirms the presence of specific functional groups present in the final synthesized compounds. In ^{13}C-NMR spectra, C-2 and C-5 of the thiadiazole nucleus were seen around 164 and 156 ppm respectively. The chemical shift of all other carbons of final compounds was seen as expected. The ^{1}H-NMR and mass spectra of title compounds were in conformity with the assigned structure. The mass spectra of these compounds showed molecular ion peaks corresponding to their molecular formula.

Out of all the fourteen compounds evaluated for antimicrobial studies, compound no. 13 showed appreciable antibacterial activity against all six microbial strains used (zone of inhibition in disk diffusion method- 17 mm against *Staphylococcus aureus*, 14 mm against *Bacillus subtilis*, 16 mm against *Proteus mirabilis*, 17 mm against *Pseudomonas aeroginosa*, 15 mm against *Aspergillus niger* and 17 mm against *Candida albican*). On critical overview of synthesized compounds, it has been found that compounds bearing the groups like nitro, hydroxy on distant phenyl ring possess high potency in disk diffusion test. Whereas replacement of these groups with methoxy and methyl groups on the distant phenyl ring has resulted in compounds with decrease in antimicrobial activity. Replacement of the proton on the carbimino carbon atom by methyl group *i.e.*, **7** to **10** or phenyl ring *i.e.*, **11** to **14** has demonstrated variation in activity due to increase in the dimension of the group at this position of the molecule. Compounds with phenyl ring were found to possess considerable activity in comparison to methyl group. The increased antimicrobial activity of compounds **11-14** may be attributed to the presence of phenyl substitution which might be responsible for penetration of the compound inside the microbial strains used due to their increased lipophilic character.

4. Conclusions

A series of novel 2,5-disubstituted-1,3,4-thiadiazoles were synthesized and evaluated for their potential antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans* by disk diffusion method. The results obtained showed that the majority of the compounds exhibited antimicrobial activity. In the present studies, N^{1}-{5-[(2-methyl-1H-benzimidazol-1-yl)-1,3,4-thiadiazol-2-yl}-N^{4}-[1-(4-nitrophenyl) (phenyl) methanone]-semicarbazone **13** came out as the most active compound, showing a broad spectrum of activity. These new data might be valuable in the future development of 2,5-disubstituted-1,3,4-thiadiazoles as novel antimicrobial agent.

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