



Synthesis, characterization and biological evaluation of novel pyrimidine linked 1,3,4-thiadiazole mannich-base derivatives for their antimicrobial activities

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ABSTRACT

To synthesize a variety of Pyrimidine linked with 1,3,4-thiadiazole mannich bases and their Biological activity was determined. Using 3-phenylpropionaldehyde and Acetamide hydro chloride, new compounds were synthesized. The structure of all the new compounds are established on the basis of FT-IR, ¹H NMR, ¹³C NMR and Mass spectral data. For antibacterial studies were done by *Staphylococcus aureus* ATCC 9144, *Bacillus Cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853. For antifungal, *Aspergillus Niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645 model. All the compounds were synthesized in good yield. Among the new compounds 8a, 8b are found to most biological activity. The results obtained justify the usage of these compounds from their antifungal and anti bacterial activity. Therefore the nature of groups is very important for antifungal and antibacterial activity.

Key words: 1, 3, 4-Thiadiazole, Synthesis, Antibacterial, Antifungal, NMR, Mannich bases, Pyrimidine

INTRODUCTION

In the last few decades, the chemistry of Heterocycles bearing a 1,3,4-thiadiazole moiety are reported to show a wide spectrum of biological activity [1-8] such as antibacterial [9], anti aggregatory agent [10], antiviral [11] and anti-inflammatory [12] activities. 1, 3, 4-Thiadiazoles exhibit broad spectrum of biological activities possibly due to the presence of toxophoric N-C-S moiety [13]. They found applications as antibacterial, antitumor, anti inflammatory agents, pesticides, herbicides, dyes, lubricants and analytical reagents. [14]

The 4-carbonyl derivative of thiazolidine is known as 4-thiazolidinone. 4-thiazolidinones have been well studied and a variety of biological activities have been reported for a large number of their derivatives. Such as antibacterial [15], antimicrobial [16], anti fungal [17], anti anthelmintic [18], anti inflammatory [19], anti tubercular [20] and diuretic agents [21], anti thyroid [22] and as a local anaesthetic.

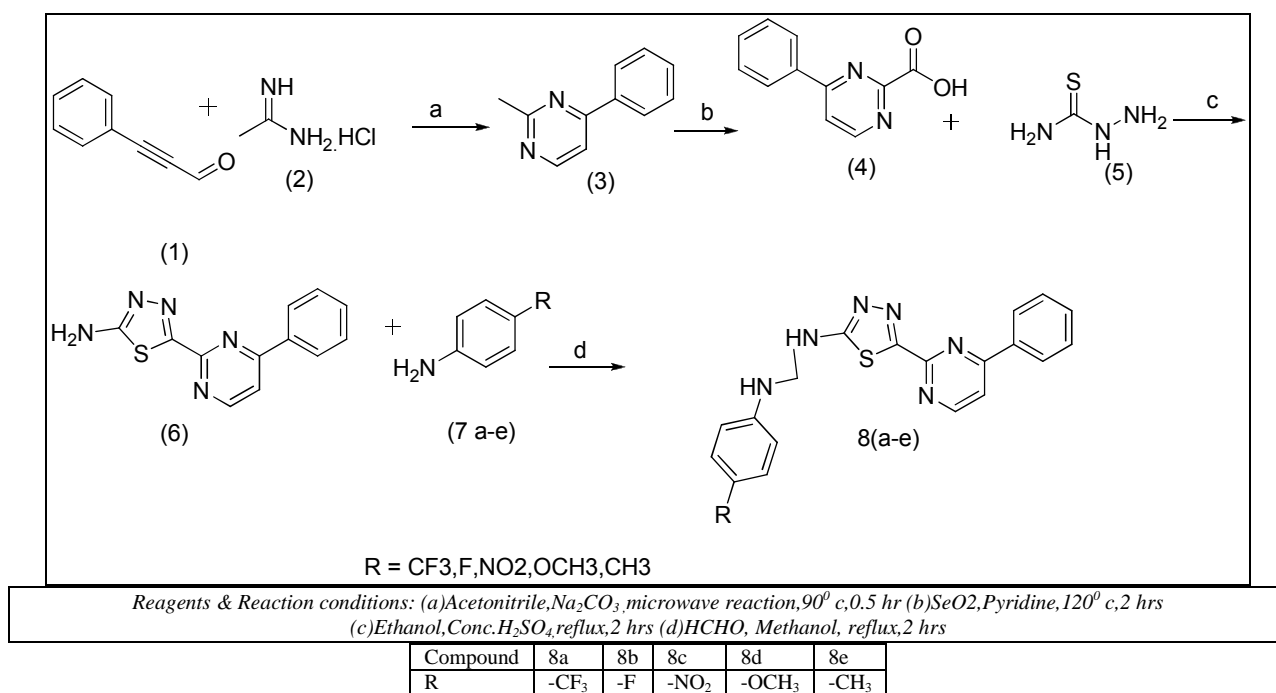
EXPERIMENTAL SECTION

Material and Methods:

Melting points were determined using an electro thermal digital apparatus and are uncorrected. Purity of the compound was checked by thin layer chromatography (TLC). IR spectra were prepared on a FT-IR spectrophotometer using KBr discs. ¹H PMR spectra were recorded on Bruker spectrophotometer (300 MHz) in DMSO-d₆ or CDCl₃ using TMS as an internal standard.

All the reagents and solvents were reagent grade and were used without further purification unless otherwise specified. Microwave reactions were conducted using a focused single mode microwave unit. The machine consists of a continuous focused microwave power delivery system with operator selectable power output. The reactions were performed either in a round-bottomed flask equipped with condenser, or in a glass tube sealed with a septum under the pressure set at 100 psi. The reported reaction temperature was monitored using a calibrated infrared temperature control mounted under the reaction vessel. The reaction mixture was magnetically stirred. Reactions were monitored by TLC using aluminum plates pre-coated with a 0.25 mm layer of silica gel containing a fluorescent indicator (Merck Art. 5544). Kieselgel 60 (40–63 μm) was used for column chromatography. Melting points are uncorrected. Chemical shifts (δ) are given in parts per million (ppm) relative to δH 7.24 / δC 77.0 (central line of t) for $\text{CHCl}_3/\text{CDCl}_3$, δH 3.31 / δC 49.0 $\text{CH}_3\text{OD}/\text{CD}_3\text{OD}$, and δH 2.49 (m) / δC 39.5 (m) for $(\text{CH}_3)_2\text{SO}/(\text{CD}_3)_2\text{SO}$. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Coupling constants (J) are given in Hz.

Scheme:



The title compounds 8(a-d) were synthesised in four sequential steps using different reagents and reaction conditions the 8(a-d) were obtained in moderate yields. The structures of 8(a-d) were established by spectral (IR, ¹H-NMR, ¹³C-NMR and mass) and analytical data.

EXPERIMENTAL SECTION

Synthesis of 2-methyl-4-phenylpyrimidine(3):

A mixture of 3-phenylpropionaldehyde (0.01 mol) and acetimidamide hydro chloride (0.01 mol) was stirred in Dry AcetoNitrile(10 ml) and Dry Na₂CO₃ (0.02mol) was added to it. The stirring was continued for 0.5 hr under Micro Wave conditions at 90^oc .Reaction progress was monitored by TLC.After completion of reaction cool to RT. Then concentrated under reduced pressure by using rota evaporator & Purified by column chromatography(100-200 mesh size silica) with elution of 10% Ethyl acetate to get pure yellow solid yield:47 % mp: 130^oc-132^oc.

Synthesis of 4-phenylpyrimidine-2-carboxylic acid(4):

A mixture of compound (3) (0.01 mol), selenium di oxide (0.01 mol), and pyridine (5 ml) was refluxed for 2 hours. Reaction progress was monitored by TLC. after completion of compound 3,concentrated under reduced pressure, then added water (10 ml),acidified with Conc.HCl,white solid was formed, filter off,dried,to get 75 % yield. M.P.: 187-189^oC.

Synthesis of 5-(4-phenylpyrimidin-2-yl)-1,3,4-thiadiazol-2-amine(6):

A mixture of thiosemicarbazide(NH₂-CS-NH-NH₂) (0.01 mol), compound(4) (0.01 mol), and conc. Sulphuric acid (5 ml) in 50 ml of ethanol was refluxed for 2 hour and poured onto crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to separate the first step product.

Yield: 85 % M.P.: 140-142^o C

Synthesis of N-(5-(4-phenylpyrimidin-2-yl)-1,3,4-thiadiazol-2-yl)-N-(4-(trifluoromethyl/Fluoro/nitro/methoxy/methyl)phenyl)methanediamine (8 a-e):

A methanolic solution of first Compound 6(0.001mole) was charged into a three neck flask equipped with a stirred and dropping funnel. The solution was stirred to dissolve it completely. To this methanolic solution, formaldehyde (7 ml, 37%) was added drop wise during 15-20 minutes. The resulting mixture was stirred during half an hour to complete reaction of formaldehyde and To this reaction mixture, the methanolic solution of Para substituted amine (0.001 mol) was added dropwise with stirring in about half an hour at 30 o C temperature and refluxed for two hour at 65-70 oC.it was allowed to cool and poured in ice water. The solid obtained was filtered off washed thoroughly with hot water and dried.

Table 1: Characterization data of synthesized compounds

Compound	Molecular formula	Molecular weight	Melting point	Yield
3	C ₁₁ H ₁₀ N ₂	170.21	130 ^o C-132 ^o C	47 %
4	C ₁₁ H ₈ N ₂ O ₂	200.19	187-189 ^o C.	75 %
6	C ₁₂ H ₉ N ₃ S	255.30	140-142 ^o C	85 %
8a	C ₂₀ H ₁₄ F ₃ N ₅ S	413.42	164-166 ^o C	70%
8b	C ₁₉ H ₁₄ FN ₅ S	363.41	140-142 ^o C	65%
8c	C ₁₉ H ₁₄ N ₆ O ₂ S	390.42	180-182 ^o C	68%
8d	C ₂₀ H ₁₇ N ₅ OS	375.45	145-147 ^o C	55%
8e	C ₂₀ H ₁₇ N ₅ S	359.45	85-87 ^o C	58%

Analytical data of synthesized compounds:**Compound 3:**

IR(KBr,cm⁻¹):3100 (Ar.C-H str.), 1628 (Ar.C-C str.), 1450 (C-N str.); ¹H NMR (DMSO-d₆, ppm):2.5(3H,S),8.4(1H,d,J=7HZ), 7.8(1H,d,j=7HZ),7.4-7.8(5H,m); ¹³C NMR (DMSO-d₆,ppm): 24(-CH₃ in Pyrimidine ring), 167,155,112,160,135,125,130,128(10 aromatic carbons)

Compound 4:

IR(KBr,cm⁻¹): 3100 (Ar.C-H str.), 1628 (Ar.C-C str.), 1450 (C-N str.),1720(C=O str.),3200(-OH Str.),1310(C-O str.); ¹H NMR (DMSO-d₆,ppm):9(1H,d,j=8 HZ),8.5(1H,d,j=8HZ),7.4-7.8(5H,m),11(-COOH); ¹³C NMR (DMSO-d₆,ppm) : 115-160(10 aromatic carbons),175(carbonyl carbon)

Compound 6:

IR(KBr,cm⁻¹): 3100 (Ar.C-H str.), 1628 (Ar.C-C str.), 1450 (C-N str.),3200-3400(Two bands indicates -NH₂),1055(C-S Str.)¹H NMR (DMSO-d₆,ppm):9(1H,d,j=8 HZ),8.5(1H,d,j=8HZ),7.4-7.8(5H,m),7(2H,bs); ¹³C NMR (DMSO-d₆,ppm) : 115-170(12 aromatic carbons)

Compound 8a:

IR(KBr,cm⁻¹): 3420 (N-H str.), 3150 (Ar.C-H str.), 1670 (Ar.C-C str.), 1465 (C-N str.), 1125 (C-S str.), 1550 (C=C str.), 1200(C-F Str.),1200(C-O str.)¹H NMR (DMSO-d₆,ppm):9(1H,d,j=8HZ),8.5(1H,d,j=8HZ),7.4-7.8(5H,m),4(1H,S,bs,-NH),4.4(2H,S),7.2(2H,d,j=8 HZ),7.5(2H,d,j=8HZ); ¹³C NMR (DMSO-d₆,ppm) : 115-170(17 aromatic carbons),174(C-2 of 1,3,4 thiadiazole ring),125(-CF₃), 50(NH-CH₂)Elemental Analysis: Calculated: C, 58.10; H, 3.41; N, 16.94; found: C, 58.10; H, 3.40; N, 16.92MS m/z: 413.09 (100.0%), 414.10 (21.8%)

Compound 8b:

IR(KBr,cm⁻¹): 3420 (N-H str.), 3150 (Ar.C-H str.), 1670 (Ar.C-C str.), 1465 (C-N str.), 1125 (C-S str.), 1550 (C=C str.), 1200(C-F Str.), 1200(C-O str.); ¹H NMR (DMSO-d₆,ppm):9(1H,d,j=8HZ),8.5(1H,d,j=8HZ),7.4-7.8(5H,m),4(1H,S,bs,-NH),4.4(2H,S),7.2(2H,d,j=8 HZ),7.42H,d,j=8HZ); ¹³C NMR (DMSO-d₆,ppm) :115-170(17 aromatic carbons),174(C-2 of 1,3,4 thiadiazole ring),50(NH-CH₂); Elemental Analysis: Calculated: C, 62.79; H, 3.88; N, 19.27found: C, 62.76; H, 3.86; N, 19.25; MS m/z: 363.10 (100.0%), 364.10 (20.7%)

Compound 8c:

IR(KBr,cm⁻¹): 3420 (N-H str.), 3150 (Ar.C-H str.), 1670 (Ar.C-C str.), 1465 (C-N str.), 1125 (C-S str.), 1550 (C=C str.), 1555 (N-O str.),1200(C-O str.)¹H NMR (DMSO-d₆,ppm):9(1H,d,j=8HZ),8.0(1H,d,j=8HZ),7.4-

7.8(5H,m),4(1H,S,bs,-NH),4.4(2H,S),7.6(2H,d,j=8 HZ),8.4(2H,d,j=8HZ)¹³C NMR (DMSO-d₆,ppm): 115-170(17 aromatic carbons),174(C-2 of 1,3,4 thiadiazole ring),50(NH-CH₂); Elemental Analysis: Calculated: C, 58.45; H, 3.61; N, 21.53Found: C, 58.43; H, 3.60; N, 21.52; MS m/z: 390.09 (100.0%), 391.09 (23.6%)

Compound 8d:

IR(KBr,cm⁻¹): 3420 (N-H str.), 3150 (Ar.C-H str.), 1670 (Ar.C-C str.), 1465 (C-N str.), 1125 (C-S str.), 1550 (C=C str.), 1555 (N-O str.), 1200(C-O str.); ¹H NMR (DMSO-d₆,ppm):9(1H,d,j=8HZ),8.0(1H,d,j=8HZ),7.4-7.8(5H,m),4(1H,S,bs,-NH),4.4(2H,S),7.3(2H,d,j=8 HZ),7.0(2H,d,j=8HZ),3.8(3H,S); ¹³C NMR (DMSO-d₆,ppm): 115-170(17 aromatic carbons),174(C-2 of 1,3,4 thiadiazole ring),50(NH-CH₂); 55(-OCH₃)Elemental Analysis: Calculated: C, 63.98; H, 4.56; N, 18.65Found: C, 63.96; H, 4.54; N, 18.63

Compound 8e:

IR(KBr,cm⁻¹): 3420 (N-H str.), 3150 (Ar.C-H str.), 1670 (Ar.C-C str.), 1465 (C-N str.), 1125 (C-S str.), 1550 (C=C str.)¹H NMR (DMSO-d₆,ppm):9(1H,d,j=8HZ),8.0(1H,d,j=8HZ),7.4-7.8(5H,m),4(1H,S,bs,-NH),4.4(2H,S,-N-CH₂),7.1(2H,d,j=8HZ),7.0(2H,d,j=8HZ),2.2 (3H,S); ¹³C NMR (DMSO-d₆,ppm):115-170(17 aromatic carbons),174(C-2 of 1,3,4 thiadiazole ring),50(NH-CH₂),23(aromatic methyl gp)MS m/z: 359.12 (100.0%), 360.12 (24.3%)Elemental Analysis: Calculated: C, 66.83; H, 4.77; N, 19.48Found: C, 66.81; H, 4.75; N, 19.47

Biological Activity:

Antibacterial activity:

All the newly synthesized 1, 3, 4-thiadiazole derivatives were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were Staphylococcus aureus ATCC 9144, Bacillus Cereus ATCC 11778, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 2853. For antifungal, Aspergillus Niger ATCC 9029 and Aspergillusfumigatus ATCC 46645 were used as organism. Both microbial studies were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method. For this, the compound whose MIC has to be determined is dissolved in serially diluted DMSO Then a standard drop of the culture prepared for the assay is added to each of the dilutions, and incubated for 16–18 hrs at 37^oc. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity. The results are shown in the table: 2.

compound	Antibacterial data in MIC(μg/ml)				Antifungal data in MIC (μg/ml)	
	Gram +ve Bacteria		Gram -ve Bacteria		<i>A.niger</i>	<i>A.fumigatus</i>
	<i>S. aureus</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>E.coli</i>		
8a	9	9	10	9	16	18
8b	8	8	9	9	16	16
8c	8	7	5	7	15	15
8d	7	6	4	6	13	14
8e	5	4	4	5	12	13

RESULTS AND DISCUSSION

Synthesis:

All the 1,3,4thiadiazole derivatives (8 a-e) were synthesized in multistep synthetic path way as shown in scheme.2-methyl-4-phenyl pyrimidine(3) was synthesized according to the reported procedure[23]. The reaction of 2-methyl-4-phenyl pyrimidine with selenium di oxide in pyridine to affordthe corresponding 4-phenyl pyrimidine-2-carboxylic acid(4) as per the reported procedure[24], which was reacted with Thiosemicarbazide in ethanol as per the reported procedure[25] to afford 5-(4-phenylpyrimidin-2-yl)-1,3,4-thiadiazol-2-amine(6), which was reacted with formaldehyde and Para substituted anilines in methanol as per the reported procedure[26] to afford 5-(4-phenylpyrimidin-2-yl)-N-(4-(trifluoromethyl/fluoto/Nitro/methoxy)benzyl)-1,3,4-thiadiazol-2-amine (8 a-e).

The structures of the synthesized compound were determined on the basis of their FTIR and ¹H NMR data. The spectral data for FTIR and ¹H NMR which confirms the structure of synthesized compounds. In vitro antibacterial activity data of 1,3,4-Thiadiazole derivatives against tested organisms displayed significant activity with a wide degree of variation. It is found that compound8a,8b,8c have shown significant antibacterial activity against gram positive bacteria. Rest of the compounds has exhibited significant to substantial activity against the same strain. Compound 8a,8b,and 8c have shown highestactivity against gram negative bacteria. Substantial activity is achieved in case of compounds 8d against S. aureus, B.cereus, E.coli, P. aeruginosa and the remaining compounds are significantly active against the same species. All the 1,3,4-Thiadiazole derivatives have exhibited significant to moderate activity against gram positive and gram negative bacteria.

CONCLUSION

A series of novel 1,3,4-Thiadiazole derivatives were synthesized and the structure of the entire compounds were confirmed by recording by their ¹H NMR, and IR spectra. In conclusion, we feel that the preliminary in vitro activity results of this class of compounds may possess potential for design of future molecules with modifications on the aryl substituent's as well as NH₂ side chain. All the synthesized compounds showed moderate activity against bacteria and fungi. The screening studies have demonstrated that the newly synthesized compounds exhibit promising antibacterial and antifungal properties. Therefore, it is concluded that there exists example scope for further study in this class of compounds.

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