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Der Pharmacia Lettre, 2013, 5 (5):60-64  
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## Synthesis and antibacterial studies of some N-(*p*-substituted benzylidene)-5-methyl-1,3,4-thiadiazole-2-amines

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### ABSTRACT

A series of Schiff's bases i.e., N-(*p*-substituted benzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amines were synthesized from 2-amino-5-methyl-1, 3, 4-thiadiazole **1** and studied for their *in vitro* antibacterial activity. Reaction of thiosemicarbazide with glacial acetic acid in presence of concentrated sulphuric acid led to the formation **1** which on further reaction with different *p*-substituted benzaldehydes yielded the title compounds **2**. These compounds were characterized by spectral analysis. All the synthesized compounds were evaluated for their *in vitro* for their antibacterial activity against two Gram positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and their minimum inhibitory concentration (MIC) were determined.

**Keywords:** 1, 3, 4-Thiadiazole, Schiff's base, antibacterial, minimum inhibitory concentration (MIC).

### INTRODUCTION

The wide-spread exploitation of chemotherapeutic agents for the treatment of infectious diseases leads to the development of microbial resistance to existing drugs. The emergence of resistance to the major classes of antibacterial drugs is recognized as a serious health concern. The hunt for novel antibacterial agents with different mode of actions will always remain an important and challenging task [1]. Compounds containing heterocyclic ring systems continue to attract considerable interest due to their wide range of biological activities. Amongst them five membered heterocyclic compounds occupy a unique place in the realm of natural and synthetic organic chemistry. Five membered heterocycles like 1, 3, 4-thiadiazole and their derivatives contain interesting biological activities. When various functional groups are attached to 1, 3, 4-thiadiazole nucleus, the resulting compounds interact with biological receptors and exhibit outstanding properties. Compounds containing 1, 3, 4-thiadiazole nucleus have been reported as antitumor agent [2], potent inhibitors of 5-lipoxygenase and cyclooxygenase [3], antimicrobials [4], anti-tuberculosis [5, 6], anti-inflammatory [7], antidepressant and anxiolytics [8], anticancer [9, 10], antihelminthic [11] etc. These reports including our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important compounds [12-15] inspired us to undertake the synthesis of some N-(*p*-substituted benzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amines. The synthesized compounds were characterized on the basis of IR and <sup>1</sup>H NMR spectral data. All the compounds were screened for their *in vitro* antibacterial activity against two Gram positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) respectively and their minimum inhibitory concentration (MIC) were also determined.

## MATERIALS AND METHODS

**Chemistry**

All the chemical and reagents used were of analytical grade and all the reaction were monitored by thin layer chromatography (TLC) using silica gel G as stationary phase, different solvent systems as mobile phase and iodine vapors as detecting agent. Melting points of the compounds were determined in open capillary tube by Decible Melting Point Apparatus and were uncorrected. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer using tetra-methyl silane as internal standard. Infrared Spectra were recorded by Perkins Elmer IR spectrophotometer using KBr pellets.

**Synthesis of 2-amino-5-methyl-1, 3, 4-thiadiazole (1)**

Synthesis of compound **1** was carried out according to the procedure reported in the literature [16]. Glacial acetic acid (0.15 M) and thiosemecarbazide (0.125 M.) in concentrated sulfuric acid (25 mL) were heated at 80-90 °C on thermostatically controlled water bath for about 7 hr. After cooling, the content was poured on crushed ice. The acid was neutralized with dilute ammonia solution. The crude product was filtered and washed with several time with cold distilled water and then recrystallized from hot distilled water.

**General procedure for the synthesis of N-(*p*-substituted benzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amines (2a-h)**

2-Amino-5-methyl-1, 3, 4-thiadiazole (0.01 M) and different *p*-substituted benzaldehydes (0.011 M) were refluxed in methanol in presence of few drops of glacial acetic acid for about 4 hr. After completion of reaction excess of methanol was distilled off under reduced pressure. The crude product so obtained was recrystallized from methanol. Physicochemical data of the title compounds are presented in Table 1.

**Table 1: Physicochemical data of N-(*p*-substituted benzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amines**

Compound	R	Molecular Formula	M.P. (°C)	% Yield
<b>2a</b>	H	C <sub>10</sub> H <sub>6</sub> N <sub>3</sub> S	232-234	69.3
<b>2b</b>	Cl	C <sub>10</sub> H <sub>8</sub> ClN <sub>3</sub> S	125-128	70.4
<b>2c</b>	Br	C <sub>10</sub> H <sub>8</sub> BrN <sub>3</sub> S	143-145	69.7
<b>2d</b>	F	C <sub>10</sub> H <sub>8</sub> FN <sub>3</sub> S	168-170	65.6
<b>2e</b>	NO <sub>2</sub>	C <sub>10</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub> S	135-137	71.7
<b>2f</b>	OCH <sub>3</sub>	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> OS	140-142	60.5
<b>2g</b>	CH <sub>3</sub>	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> S	203-205	61.4
<b>2h</b>	OH	C <sub>10</sub> H <sub>9</sub> N <sub>3</sub> OS	188-192	70.1

**Spectral data of the title compounds.****N-(benzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2a)**

IR (KBr, cm<sup>-1</sup>): 644 (C–S–C), 1078 (Ar), 1029 (N–N), 1688 (C=O), 1569 (C=N), 800 (*p*-di-substituted benzene); <sup>1</sup>H NMR (DMSO, *δ*, ppm): 7.01–7.14 (m, 4H, ArH), 8.12 (s, 1H, CH), 2.20 (s, 3H, CH<sub>3</sub>).

**N-(4-chlorobenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2b)**

IR (KBr, cm<sup>-1</sup>): 649 (C–S–C), 1088 (Ar–Cl), 039(N–N), 1575 (C=N), 817 (*p*- di-substituted benzene); <sup>1</sup>H NMR (DMSO, *δ*, ppm): 7.04-7.06 (m, 4H, ArH), 8.09 (s, 1 H, CH), 2.41 (s, 3H, CH<sub>3</sub>).

**N-(4-bromobenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2c)**

IR (KBr, cm<sup>-1</sup>): 642 (C–S–C), 1072 (Ar–Br), 1019 (N–N), 1571 (C=N), 803 (*p*-di-substituted benzene); <sup>1</sup>H NMR (DMSO, *δ*, ppm): 7.07-7.17 (m, 4H, ArH), 8.09 (s, 1H, CH), 2.25 (s, 3H, CH<sub>3</sub>).

**N-(4-fluorobenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2d)**

IR (KBr, cm<sup>-1</sup>): 640 (C–S–C), 1323 (Ar–F), 1030 (N–N), 1579 (C=N), 817 (*p*-di-substituted benzene); <sup>1</sup>H NMR (DMSO, *δ*, ppm): 7.07-7.19 (m, 4H, ArH), 8.10 (s, 1H, CH), 2.31 (s, 3H, CH<sub>3</sub>).

**N-(4-nitrobenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2e)**

IR (KBr, cm<sup>-1</sup>): 649 (C–S–C), 1327 (Ar–NO<sub>2</sub>), 1035 (N–N), 1577 (C=N), 814 (*p*-di-substituted benzene); <sup>1</sup>H NMR (DMSO, *δ*, ppm): 7.03-7.18 (m, 4H, ArH), 8.06 (s, 1H, CH), 2.31 (s, 3H, CH<sub>3</sub>).

**N-(4-methoxybenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2f)**

IR (KBr,  $\text{cm}^{-1}$ ): 648 (C–S–C), 1329 (Ar–OCH<sub>3</sub>), 1020 (N–N), 1570 (C=N), 809 (*p*-di-substituted benzene); <sup>1</sup>H NMR (DMSO,  $\delta$ , ppm): 7.02-7.19 (m, 4H, ArH), 8.09 (s, 1H, CH), 2.28 (s, 3H, CH<sub>3</sub>).

**N-(4-methylbenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2g)**

IR (KBr,  $\text{cm}^{-1}$ ): 646 (C–S–C), 1323 (Ar–CH<sub>3</sub>), 1032 (N–N), 1572 (C=N), 811 (*p*-di-substituted benzene); <sup>1</sup>H NMR (DMSO,  $\delta$ , ppm): 7.05-7.16 (m, 4H, ArH), 8.10 (s, 1H, CH), 2.37 (s, 3H, CH<sub>3</sub>).

**N-(4-hydroxybenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine (2h)**

IR (KBr,  $\text{cm}^{-1}$ ): 650 (C–S–C), 3300 (Ar–OH), 1039 (N–N), 1575 (C=N), 815 (*p*-di-substituted benzene); <sup>1</sup>H NMR (DMSO,  $\delta$ , ppm): 7.06-7.20 (m, 4H, ArH), 8.10 (s, 1H, CH), 2.51 (s, 3H, CH<sub>3</sub>), 4.9 (s, 1H, OH).

**Antibacterial activity**

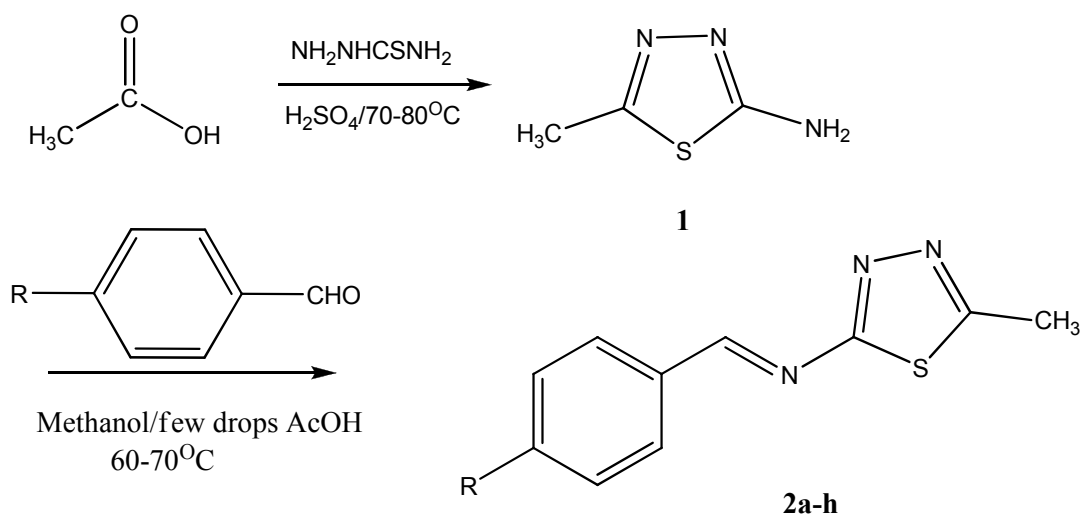
All the title compounds were screened for their *in vitro* antibacterial activity against two Gram positive strains, *i.e.*, *Bacillus subtilis* (MTCC 16) and *Staphylococcus aureus* (MTCC 3160) and two Gram negative strains, *i.e.*, *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 424) respectively. Ciprofloxacin was used as the standard drug for the present study. Serial two fold dilution technique was used for the study of antibacterial activity [17]. A stock solution (10  $\mu\text{g/mL}$ ) of all the title compounds and standard drug was prepared in dimethyl sulfoxide. Sterilized double strength nutrient broth (DSNB) was used as a growth media. The stock solution was serially diluted by DSNB aseptically to give concentrations of 5.0–0.01  $\mu\text{g/mL}$  into a series of sterilized culture tubes. All the tubes were inoculated by bacterial strain. The inoculum's size was approximately  $10^6$  colony forming units (CFU/mL). The inoculated tubes were incubated for 24 hr at  $37(\pm 1)$  °C. After 24 h, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The MIC for the title compounds and the standard drug, *i.e.*, ciprofloxacin are presented in Table 2.

Table 2: Antibacterial activity of N-(*p*-substituted benzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amines

Compound	Minimum inhibitory concentration (MIC $\mu\text{g/mL}$ )			
	<i>S. aureus</i> MTCC 3160	<i>B. subtilis</i> MTCC 16	<i>E. coli</i> MTCC 40	<i>P. aeruginosa</i> MTCC 424
2a	0.80	0.80	0.75	0.75
2b	0.75	0.70	0.60	0.65
2c	0.75	0.70	0.60	0.65
2d	0.55	0.55	0.50	0.50
2e	0.50	0.50	0.50	0.50
2f	0.95	0.95	0.90	0.90
2g	0.90	0.90	0.80	0.85
2h	0.65	0.65	0.55	0.60
Ciprofloxacin	0.15	0.12	0.01	0.25

**RESULTS AND DISCUSSION****Chemistry**

The synthesis of N-(*p*-substituted benzylidene)-5-methyl-1, 3, 4 -thiadiazole-2-amines were achieved following the steps outlined in the **Scheme 1**. Cyclization of thiosemicarbazide with acetic acid in presence of sulfuric acid gave 5-methyl-2-amino-1, 3, 4- thiadiazole **1**. Reaction of compound **1** with different *p*-substituted benzaldehydes in presence of few drops of glacial acetic acid furnished the Schiff's bases *i.e.*, N-(*p*-substituted benzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amines **2**. All the compounds were obtained in good yield. All the compounds were characterized by spectral analysis. The IR spectra of each compounds show a band for (C–S–C) stretching vibrations near  $644\text{ cm}^{-1}$  and (N–N) stretching vibrations were observed near  $1023\text{ cm}^{-1}$ . The bending vibrations for *p*-di-substituted benzene were appeared in the range of  $800\text{--}820\text{ cm}^{-1}$ . In case of <sup>1</sup>H NMR, the chemical shift value for methyl and methine protons of Schiff's base were appeared as singlet and observed at 2.20-2.41  $\delta$  (ppm) and 8.06-8.10  $\delta$  (ppm) respectively. The chemical shift value for aromatic protons was observed in the range of 7.01-7.14  $\delta$  (ppm) and appeared as multiplet.

**Compound R**

<b>2a</b>	<b>H</b>
<b>2b</b>	<b>Cl</b>
<b>2c</b>	<b>Br</b>
<b>2d</b>	<b>F</b>
<b>2e</b>	<b>NO<sub>2</sub></b>
<b>2f</b>	<b>OCH<sub>3</sub></b>
<b>2g</b>	<b>CH<sub>3</sub></b>
<b>2h</b>	<b>OH</b>

**Scheme 1:** Synthesis of N-(*p*-substituted benzylidene)-5-methyl-1,3,4-thiadiazole-2-amines**Antibacterial activity**

All the synthesized title compounds were screened for their *in vitro* antibacterial activity against two Gram positive bacterial strains *i.e.*, *Bacillus subtilis* (MTCC 16) and *Staphylococcus aureus* (MTCC 3160) and two Gram negative bacterial strains *i.e.*, *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 424) respectively and their minimum inhibitory concentration (MIC) were determined. A perusal of the **table 2** shows that all the title compounds were found to be active against all the bacterial strains used in this study. However, they showed more activity against the Gram negative than the Gram positive bacterial strains. The minimum inhibitory concentration (MIC) of the title compounds **2a-h** were found to be 0.95-0.50 µg/mL and 0.90-0.50 µg/mL against Gram positive and Gram negative bacterial strains respectively. The MICs of the title compounds containing electron withdrawing groups like fluoro, chloro, bromo or nitro were found somewhat less than the compounds containing electron releasing groups like methyl and methoxy. The reference standard ciprofloxacin inhibited Gram negative bacteria *viz.*, *E. coli* and *P. aeruginosa* at a MIC of 0.01 µg/mL and 0.25 µg/mL respectively whereas against Gram positive bacteria *viz.*, *S. aureus* and *B. subtilis* MIC was found to be 0.15 µg/mL and 0.12 µg/mL respectively. The results of the MIC for the standard drug, ciprofloxacin, against the bacterial strains used were found to be within the range as reported in the literature [18-20].

**CONCLUSION**

Present study describes the synthesis of a series of Schiff's bases of 5-methyl-1, 3, 4-thiadiazole-2-amine. The compounds were characterized by spectral techniques such as IR and proton NMR spectra. All the title compounds

were screened for their *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive) and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative) and their minimum inhibitory concentration (MIC) were determined. The results of antibacterial activity showed that compounds containing electron withdrawing groups e.g., chloro, bromo, fluoro or nitro were found to be more active than the compounds containing electron releasing groups such as methyl and methoxy. These results suggest that some more compounds using different aliphatic acids and hetero-aromatic aldehydes or ketones should be synthesized and screened for their antibacterial activity to explore the possibility of Schiff's bases of 5-alkyl-1, 3, 4-thiadiazole-2-amine as a new series of antibacterials.

#### Acknowledgements

The authors are thankful to Chairman, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar (Haryana) India for providing necessary facilities to carry out this work. Our sincere thanks are due to the Co-ordinator, Central Instrument Laboratory, Guru Jambheshwar University of Science and Technology, Hisar (Haryana), India for elemental and spectral analysis. The Director, IMTECH, Chandigarh is also duly acknowledged for providing bacterial strains.

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