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# Synthesis of 2-N-Salicylidene-5-(substituted)-1,3,4-thiadiazole as Potential Antimicrobial Agents

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Several new 2,5-disubstituted derivatives of 1,3,4-thiadiazoles containing imine moiety were obtained from cyclization of thiosemicarbazide and corresponding *p*-substituted carboxylic acid in phosphorus oxychloride then the resulted products were reacted with salicylaldehyde to give the final products **3a-h**. All the newly synthesized compounds were charac-terized using FTIR, $^{1}$H NMR, $^{13}$C NMR and elemental analysis. All compounds have been screened for their antimicrobial activity and showed that the introduction of 1,3,4-thiadiazole ring significantly increase the antibacterial activity.

**Key Words: 1,3,4-Thiadiazole ring, Antimicrobial activity, Imine moiety.**

## INTRODUCTION

The synthesis of compounds incorporating both 1,3,4-thiadiazole ring and imine moiety have attracted widespread attention due to their diverse pharmacological properties such as antimicrobial, antiinfammatory, analgesic and antitumoral activities[1-7]. Although there are a number of antibiotics which are commercially used in medicine. The synthesis of new compounds is of vital importance due to increasing drug resistance. Moreover, it is important to obtain therapeutically compounds having less toxic effects. 1,3,4-Thiadiazoles attracted the great attention after the discovery of sulfa drugs and their potent representative bearing this heterocyclic ring. Several 1,3,4-thiadiazoles have been found biologically active, *e.g*., they showed the anticancer and antiviral activity[8,9], antiinflammatory[10], carbonic-anhydrase inhibitory[11], anticonvulsant[12], H-2-antagonist[13], antibacterial[14] and fungicidal[15] activity.

Schiff bases-bimolecular condensation products of primary amines with aldehydes-represent valuable intermediates in organic synthesis and, at the same time, compounds with various applications. Schiff bases resulted from aromatic aldehydes *ortho*-substituted with a hydroxyl group have initially arouse the researchers' interest because of their wide biological activity range. Later, in studies[16,17] concerning quantitative structure-antitumor activity relationship of a series of Schiff bases derived from variously substituted aromatic amines and aldehydes, it has been shown that

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azomethines from salicylaldehydes gave the best correlation. Schiff bases of salicylaldehydes have also been reported as plant growth regulators[18] and antimicrobial[19] or antimycotic[20] activity. This paper presents a series of new Schiff bases with a potential biological activity resulted from condensation of salicylaldehyde with 2-amino-5-substituted-1,3,4-thiadiazole. These novel compounds could also act as valuable ligands whose biological activity has been shown to increase on complexation[21].

## EXPERIMENTAL

The chemicals used in this work were obtained from Aldrich Chemicals Company and they were all pure grade reagents.

**General procedure for the synthesis of 2-amino-5-substituted-1,3,4-thiadiazole (2a-h):** Mixture of appropriate carboxylic acid (0.01 mol), thiosemicarbazide (0.01 mol),phosphorus oxychloride (5 mL) was refluxed gently for 3 h. After cooling, water was added (50 mL). The mixture was refluxed for 4 h and filtered. The filtrate was neutralized with potassium hydroxide. The precipitate was filtered and washed with distilled water and crystallized from (ethanol-water) to give the desired products.

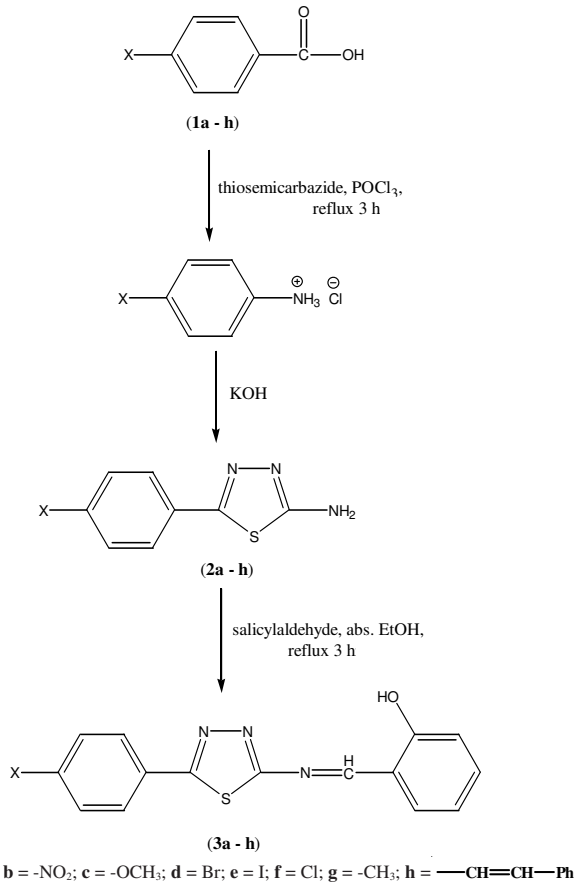
**General procedure for the synthesis of 2-N-salicylidene-5-(substituted)-1,3,4-thiadiazole (3a-h):** The resulted amino thiadiazole (0.01 mol) **2a-h** was refluxed for 3 h with (0.01 mol) salicyladehyde, the yellow precipitate which formed was filtered and crystallized from ethanol to give the final Schiff base products.

**Detection methods:** The percentage composition of the elements (CHN) for the compounds was 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.

## RESULTS AND DISCUSSION

**Synthesis and physical properties:** 2-Amino-5-substituted-1,3,4-thiadiazole **2a-h** being the starting materials were prepared from the reaction of different substituted aromatic carboxylic acids **1a-h** with thiosemicarbazide in phosphorus oxychloride then neutralization with potassium hydroxide. The 2-N-salicylidene-5-(substituted)-1,3,4-thiadiazole **3a-h** were formed through the reaction of compounds **2a-h** with salicylaldehyde in absolute ethanol. The structures of all the compounds were supported by spectral and analytical data. The strategy employed in the synthesis of the titled compounds is as follows (**Scheme-I**).

The purity of the synthesized compounds were checked by TLC using silica gel-G as adsorbent. Further evidence for the characterization of the synthesized compounds was obtained from C, H, N and S analysis, which are in agreement with the calculated values (Table-1).




**Scheme-I:** Protocol for synthesis of the title compounds (**3a-h**)

**Infrared spectroscopy:** Solid state infrared spectra of the synthesized compounds are recorded in the range 4000-700 $cm^{-1}$ and the most important bands are presented in Table-2. In the IR spectra of the compounds **2a-h**, the aromatic C-H stretching absorptions appeared in the region 3100-3010 $cm^{-1}$. On the other hand, the strong absorption band at 1254-1240 $cm^{-1}$ due to the stretching vibration of thiosemicarbazide C=S group was disappeared in the IR spectra of these compounds. However, a strong absorption band at about 3350-3300 $cm^{-1}$ was observed in each case due to the $-NH_2$ stretching vibrations, this band was disappeared in the IR spectra of compounds **3a-h**. Furthermore, the band at 3450-3400 $cm^{-1}$ together with the bands at 1620-1600 $cm^{-1}$ due to O-H and C=N stretching vibrations, respectively, are another important characteristic evidences for the proposed structures of the compounds **3a-h**.

**NMR spectroscopy:** The $^1H$ NMR spectra for all compounds were recorded in DMSO-$d_6$ using TMS as the internal standard (Table-3). The conclusions drawn from the data give a further support to suggested structures for the synthesized

TABLE-1
YIELD AND PHYSICAL DATA OF ALL COMPOUNDS PREPARED

| Comp. | Yield (%) | m.p. (ºC) | m.f. | Found (calcd.) (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | C | H | N | S |
| **2a** | 85 | 110-111 | $C_8H_7N_3S$ | 53.12 (54.22) | 4.32 (3.98) | 22.89 (23.71) | 19.42 (18.09) |
| **2b** | 83 | 78-79 | $C_8H_6N_4O_2S$ | 42.65 (43.24) | 3.01 (2.72) | 26.53 (25.21) | 13.76 (14.43) |
| **2c** | 73 | 135-136 | $C_9H_9N_3OS$ | 53.32 (52.16) | 3.84 (4.38) | 21.41 (20.27) | 16.28 (15.47) |
| **2d** | 75 | 156-157 | $C_8H_6N_3SBr$ | 36.87 (37.52) | 3.02 (2.36) | 15.63 (16.41) | 11.76 (12.52) |
| **2e** | 77 | 92-93 | $C_8H_6N_3SI$ | 30.98 (31.70) | 3.10 (2.00) | 14.03 (13.86) | 11.21 (10.58) |
| **2f** | 81 | 213-214 | $C_8H_6N_3SCl$ | 44.67 (45.39) | 2.42 (2.86) | 20.13 (19.85) | 16.42 (15.15) |
| **2g** | 79 | 187-188 | $C_9H_9N_3S$ | 55.76 (56.52) | 3.98 (4.74) | 22.39 (21.97) | 15.69 (16.77) |
| **2h** | 70 | 106-107 | $C_{16}H_{13}N_3S$ | 67.94 (68.79) | 5.10 (4.69) | 14.87 (15.04) | 10.85 (11.48) |
| **3a** | 72 | 152-153 | $C_{15}H_{11}N_3OS$ | 65.90 (64.04) | 2.85 (3.94) | 13.68 (14.94) | 10.97 (11.40) |
| **3b** | 79 | 115 d* | $C_{15}H_{10}N_4O_3S$ | 54.37 (55.21) | 2.73 (3.09) | 16.91 (17.17) | 10.02 (9.83) |
| **3c** | 75 | 182-183 | $C_{16}H_{13}N_3O_2S$ | 62.34 (61.72) | 3.98 (4.21) | 14.12 (13.50) | 11.12 (10.30) |
| **3d** | 73 | 144-145 | $C_{15}H_{10}N_3OSBr$ | 51.64 (50.01) | 3.03 (2.80) | 10.97 (11.66) | 9.21 (8.90) |
| **3e** | 85 | 167-168 | $C_{15}H_{10}N_3OSI$ | 43.56 (44.24) | 2.13 (2.48) | 11.15 (10.32) | 6.39 (7.87) |
| **3f** | 70 | 189-190 | $C_{15}H_{10}N_3OSCl$ | 57.89 (57.05) | 2.74 (3.19) | 12.58 (13.31) | 9.47 (10.15) |
| **3g** | 88 | 202-203 | $C_{16}H_{13}N_3OS$ | 64.53 (65.06) | 3.87 (4.44) | 13.45 (14.23) | 11.68 (10.86) |
| **3h** | 89 | 122-123 | $C_{23}H_{17}N_3OS$ | 73.15 (72.04) | 5.11 (4.47) | 11.32 (10.96) | 9.40 (8.36) |

*:d = decomposed.

compounds. The $^1$H NMR spectra of 2-amino-5-substituted-1,3,4-thiadiazole **2a-h** showed a multiple signals at 7.32-7.90 ppm and a singlet signal at about 9.14 ppm assignable for aromatic ring protons and -$NH_2$ group protons, respectively, the last one was further characterized by $D_2O$ exchange[22]. On the other hand, $^1$H NMR spectra for 2-N-salicylidene-5-(substituted)-1,3,4-thiadiazole **3a-h** showed singlet signal at about 7.35 ppm due to -N=CH- protons together with a multiple signals at 8.25-9.10 ppm attributable to aromatic protons[23]. Furthermore, the O-H proton signals appeared at 9.67-9.93 ppm and were further characterized by $D_2O$ exchange.

TABLE-2
INFRARED SPECTRAL ($cm^{-1}$) DATA OF ALL COMPOUNDS PREPARED

| Comp. | ν(O-H) | ν($NH_2$) | ν(C-H) aromatic | ν(C-H) aliphatic | ν(C=N) | ν(C=C) | ν($NO_2$) | ν(C-S) | γ(C-H) aromatic |
|---|---|---|---|---|---|---|---|---|---|
| **2a** | – | 3342, 3310 | 3022 | – | 1576 | 1518 | – | 657 | 745, 663 |
| **2b** | – | 3367, 3324 | 3087 | – | 1574 | 1512 | 1543, 1367 | 645 | 832 |
| **2c** | – | 3345, 3315 | 3025 | 2987, 2854 | 1556 | 1510 | – | 648 | 830 |
| **2d** | – | 3356, 3321 | 3040 | – | 1573 | 1522 | – | 650 | 833 |
| **2e** | – | 3346, 3332 | 3067 | – | 1565 | 1521 | – | 645 | 835 |
| **2f** | – | 3336, 3314 | 3010 | – | 1545 | 1520 | – | 634 | 838 |
| **2g** | – | 3337, 3312 | 3075 | 2978, 2865 | 1556 | 1518 | – | 637 | 840 |
| **2h** | – | 3358, 3234 | 3090 | – | 1585 | 1513 | – | 648 | 842 |
| **3a** | 3450 | – | 3059 | – | 1610, 1580 | 1517 | – | 650 | 845 |
| **3b** | 3478 | – | 3083 | – | 1612, 1576 | 1512 | – | 640 | 830 |
| **3c** | 3467 | – | 3042 | 2949, 2865 | 1610, 1558 | 1515 | – | 638 | 835 |
| **3d** | 3458 | – | 3067 | – | 1609, 1575 | 1520 | – | 637 | 834 |
| **3e** | 3493 | – | 3079 | – | 1613, 1570 | 1523 | – | 638 | 845 |
| **3f** | 3478 | – | 3037 | – | 1612, 1551 | 1516 | – | 635 | 844 |
| **3g** | 3456 | – | 3068 | 2958, 2873 | 1613, 1558 | 1518 | – | 643 | 836 |
| **3h** | 3468 | – | 3089 | – | 1610, 1588 | 1517 | – | 646 | 835 |

Table-4 shows the most relevant $^{13}C$ NMR data. Due to scant solubility of the synthesized compounds, their spectra were recorded in DMSO-$d_6$. The two thiadiazole carbons appeared at about 87.65 and 90.78 ppm while the signals attributed to aromatic carbons appeared at about 125.46-132.57 ppm for compounds **2a-h** and **3a-h**. The signal at 110.85-112.63 ppm was due to -N=CH- group of compounds **3a-h**[24].

**Antimicrobial activity:** All the synthesized compounds **2a-h** and **3a-h** were screened for their antibacterial activity against *S. aureus*, *S. typhi* and *E. coli* (Table-5) by the drug diffusion method[25]. The inhibition zone was measured in mm and was compared with standard drug. DMSO was used as control and Streptomycin

TABLE-3
$^{1}$H-NMR DATA (δ, ppm) OF COMPOUNDS (**2a-h**; **3a-h**) PREPARED

| Comp. | -CH$_3$ | -OCH$_3$ | -CH=CH- | -N=CH- | Aromaticprotons | -NH$_2$ | O-H |
|---|---|---|---|---|---|---|---|
| **2a** | – | – | – | – | 7.30-7.89 | 9.15 | – |
| **2b** | – | – | – | – | 7.33-7.90 | 9.14 | – |
| **2c** | – | 2.39 | – | – | 7.32-7.76 | 9.13 | – |
| **2d** | – | – | – | – | 7.30-7.86 | 9.12 | – |
| **2e** | – | – | – | – | 7.32-7.91 | 9.13 | – |
| **2f** | – | – | – | – | 7.33-7.90 | 9.14 | – |
| **2g** | 1.23 | – | – | – | 7.32-7.91 | 9.15 | – |
| **2h** | – | – | 5.43 | – | 7.31-7.87 | 9.13 | – |
| **3a** | – | – | – | 7.34 | 8.25-9.03 | – | 9.67 |
| **3b** | – | – | – | 7.38 | 8.23-9.05 | – | 9.90 |
| **3c** | – | 2.67 | – | 7.35 | 8.25-9.11 | – | 9.91 |
| **3d** | – | – | – | 7.37 | 8.24-9.10 | – | 9.92 |
| **3e** | – | – | – | 7.32 | 8.25-9.10 | – | 9.67 |
| **3f** | – | – | – | 7.36 | 8.23-9.11 | – | 9.68 |
| **3g** | 1.45 | – | – | 7.34 | 8.25-9.12 | – | 9.90 |
| **3h** | – | – | 5.26 | 7.35 | 8.24-9.10 | – | 9.93 |

TABLE-4
$^{13}$C-NMR DATA (δ, ppm) OF COMPOUNDS (**2a-h**; **3a-h**) PREPARED

| Comp. | -CH$_3$ | -OCH$_3$ | -CH=CH- | -N=CH- | Thiadiazole carbons | Aromatic carbons |
|---|---|---|---|---|---|---|
| **2a** | – | – | – | – | 87.74, 90.80 | 126.48-133.15 |
| **2b** | – | – | – | – | 87.63, 90.75 | 125.46-132.57 |
| **2c** | – | 16.18 | – | – | 87.64, 90.79 | 125.71-132.34 |
| **2d** | – | – | – | – | 87.65, 90.78 | 126.32-133.62 |
| **2e** | – | – | – | – | 87.70, 90.80 | 125.53-132.70 |
| **2f** | – | – | – | – | 87.63, 90.75 | 124.63-132.89 |
| **2g** | 13.24 | – | – | – | 87.64, 90.78 | 125.61-132.57 |
| **2h** | – | – | 66.32 | – | 87.66, 90.77 | 125.34-132.80 |
| **3a** | – | – | – | 110.86 | 87.67, 90.74 | 125.50-132.63 |
| **3b** | – | – | – | 111.12 | 87.70, 90.79 | 125.43-132.54 |
| **3c** | – | 16.83 | – | 112.67 | 87.67, 90.77 | 125.55-132.53 |
| **3d** | – | – | – | 110.85 | 87.68, 90.80 | 125.50-132.62 |
| **3e** | – | – | – | 111.49 | 87.64, 90.79 | 125.47-132.55 |
| **3f** | – | – | – | 112.34 | 87.63, 90.76 | 125.51-132.60 |
| **3g** | 13.56 | – | – | 110.56 | 87.66, 90.78 | 125.45-132.52 |
| **3h** | – | – | 67.41 | 111.63 | 87.64, 90. 80 | 125.43-132.55 |

was used as antibacterial standard. All the compounds were tested at 100 and 250 μg/mL concentration. From the antimicrobial screening of the compounds it could observed that the introduction of 1,3,4-thiadiazole show a significant increase in antibacterial activity, also compounds **3a-h** show good antibacterial activity.

TABLE-5
ANTIBACTERIAL ACTIVITY OF ALL COMPOUNDS PREPARED

| Comp. | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* | | *S. typhi* | | *E. coli* | |
| | 100 µg | 200 µg | 100 µg | 200 µg | 100 µg | 200 µg |
| **2a** | – | + | + | ++ | ++ | ++ |
| **2b** | – | ++ | + | ++ | ++ | ++ |
| **2c** | – | ++ | – | + | – | + |
| **2d** | – | + | + | ++ | ++ | ++ |
| **2e** | ++ | ++ | ++ | +++ | ++ | +++ |
| **2f** | +++ | +++ | +++ | +++ | +++ | +++ |
| **2g** | + | ++ | ++ | +++ | +++ | +++ |
| **2h** | ++ | +++ | ++ | +++ | +++ | +++ |
| **3a** | + | ++ | ++ | ++ | ++ | +++ |
| **3b** | +++ | +++ | +++ | +++ | +++ | +++ |
| **3c** | +++ | +++ | +++ | +++ | +++ | +++ |
| **3d** | ++ | ++ | ++ | +++ | ++ | +++ |
| **3e** | +++ | ++ | +++ | ++ | +++ | +++ |
| **3f** | +++ | +++ | +++ | +++ | +++ | +++ |
| **3g** | +++ | +++ | +++ | +++ | +++ | +++ |
| **3h** | +++ | ++ | + | ++ | + | ++ |

Disc size: 6.35 mm Standard: Streptomycin Control: DMSO
Duration: 24 h Resistant (11 mm/less) Intermediate (12-14 mm)
Sensitive (15 mm/more)

+++: high activity, ++: moderate activity, +: low activity, –: no activity.

## Conclusion

From the present study, it can be concluded that the newly synthesized compounds have a significant antimicrobial activity against the tested microorganisms. The results also indicate that chlorosubstituted compounds **2f** and **3f** in position 6 of the benzene ring was essential for the activity and they are exhibit a growth inhibitory activity. From these observations and with slight modification in the structure one can plan for the drug design.

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