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Original Article

# SYNTHESIS OF SOME NOVEL TRIAZOLE DERIVATIVES AS SCHIFF BASES AND THEIR ANTIMICROBIAL EVALUATION

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**ABSTRACT**

**Objective:** This work involves the synthesis of some novel schiff base derivatives synthesized from *p*-amino benzoic acid.

**Methods:** A series of 4-[4-(arylidene amino-5-mercapto-4H-[1, 2, 4] triazol-3-yl]-benzoic acid complexes were synthesized from 4-(4-amino-5-mercapto-4H-[1, 2, 4] triazol-3-yl)-benzoic acid by reaction with different aromatic aldehydes. All the synthesized schiff base derivatives were characterized by using analytical techniques (FT-IR, $^1$H NMR and Mass spectroscopy). The title compounds were evaluated for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae)* and Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa)* and anti-fungal activity against (*Candida albicans* and *Aspergillus niger).*

**Results:** Schiff base derivatives 5(a-h) were synthesized in good yields and showed antimicrobial activity, among them, compounds 5c, 5d, 5e and 5f were significantly active against gram positive, gram negative bacterial and fungal strains and rest of compounds showed moderate to weak activity.

**Conclusion:** The Schiff base obtained showed variation in the antimicrobial and antifungal activity, based on the structure of the substituted aromatic aldehydes.

**Keywords:** Anti-bacterial, Anti-fungal, Aldehyde, Schiff base


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## INTRODUCTION

Schiff bases are the product of primary amines and carbonyl compounds, named after Hugo Schiff, who discovered it in 1864. The common structural feature of these compounds is the azomethine group with a general formula RHC=N-$R_1$, where R and $R_1$ are alkyl, aryl, cycloalkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethines. Schiff bases are also synthesized by some researchers using solvent free and environmentally friendly methods [1]. The general reaction for the synthesis of Schiff bases is given as

$$RNH_2 + RCHO \longrightarrow R\text{-}N{=}CH\text{-}R + H_2O$$

The presence of a lone pair of electrons in a $sp^2$ hybridized orbital of the nitrogen atom of the azomethine group is of considerable chemical and biological importance. Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, Schiff bases are generally excellent chelating agents, especially when a functional group like–OH or–SH is present close to the azomethine group so as to form a five or six-membered rings with the metal ion [2]. Their metal complexes such as copper, nickel and zinc complexes have been widely studied and proved as competent anticancer agents [3]. Further some investigations are going on in the field on analytical, inorganic and industrial chemistry due to its versatile nature of the compounds.

Now a day, in the research field, dealing with Schiff base coordination chemistry has expanded enormously. Schiff bases resulted from aromatic aldehydes ortho-substituted with a hydroxyl group have initially aroused the researcher's interest because of their ability to act as bidentate ligands for transitional metal ions [4]. Schiff bases are characterized by the-N=CH-(imine) group which imports in elucidating the mechanism of transamination and racemization reaction in the biological system. Schiff bases have been reported for their biological properties, such as, anti-inflammatory agents, anti-cancer agents, various metal complexes as antimicrobial agents [5-8], anti-convulsant [9], analgesic, anti-inflammatory agents [10-11] and anti-tubercular agents [12]. They serve as models for biologically important species. In this research article, we have synthesized some novel Schiff base derivatives from p-amino benzoic acid, and all the newly synthesized compounds were tested against the anti-microbial action.

## MATERIALS AND METHODS

### General

All the chemicals and solvents used in synthesis were purchased from the Himedia, Nice and Merck. The progress of reaction and purity of the synthesized compounds were checked by thin layer chromatography (TLC) with silica gel plates.

The melting points were determined by a capillary method using Thermoscientific melting point apparatus and found to be uncorrected. The FT-IR spectra (nujol) were recorded on Shimadzu FT-IR spectrophotometer. The $^1$H NMR spectra were recorded (in DMSO) on a BRUKER AVANCE-400 (400 MHZ) spectrometer using TMS as an internal standard. Mass spectra were recorded on Water, Q-TOF Micro mass (LC-MS).

### Chemical synthesis

### General procedure

### Synthesis of Thiocarbohydrazide (3)

Schiff bases derivatives were prepared according to reported literature [13]. Hydrazine hydrate (1) (1.0 mol) was placed in a round bottom flask, 0.2 mol of carbon disulphide (2) ($CS_2$) (15.2 g, 12.1 ml) was added dropwise into the flask while maintaining the temperature below 15 °C and the temperature was gradually raised for 1.5 hr. The completion of the reaction was confirmed by TLC. The reaction mixture was cooled down; the precipitate was filtered and washed with water. The product was recrystallized with ethanol.

### Synthesis of 4-(4-Amino-5-mercapto-4H-[1, 2, 4] triazol-3-yl)-benzoic acid (4)

A mixture of *p*-amino benzoic acid (0.01 mol) and thiocarbohydrazide (3) (0.015 mol) were taken in a round bottomed flask.

The completion of the reaction was confirmed by TLC. The reaction mixture was cooled; the precipitate was filtered and washed with water. The product was recrystallized with ethanol.

**Synthesis of 4-[4-(arylidene amino-5-mercapto-4H-[1, 2, 4] triazol-3-yl]-benzoic acid 5(a-h)**

Suspension of 4-(4-amino-5-mercapto-4*H*-[1, 2, 4] triazol-3-yl)-benzoic acid (4) (0.2 mol) and equimolar amount of the corresponding substituted benzaldehyde with 3 to 4 drops of sulphuric acid was added. The reaction mixture was refluxed for 2-3h. The completion of reaction was confirmed by TLC. The reaction mixture was cooled; precipitate was filtered and washed with water. The product was recrystallized with ethanol. Various substituted aldehyde used in the reaction are mentioned in table 1.

**5a: Synthesis of: 4-[4-(Benzylidene-amino)-5-mercapto-4*H*-[1,2,4]triazol-3-yl]-benzoic acid**

Yield (80%); m. p. 188 °C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3022 (C-H, Ar str.), 2921(C-H str), 1701(C=O str in COOH), 1570 (CH=N), 1509 (C=C str), 1216 (C-O str), 949 (C-S bend), 732 (C-H bend); $^1$H NMR(DMSO, ppm): 4.3 (t, 1H, NH), 5.8 (s, 2H, N-$CH_2$-NH), 7.9-7.6 (m, 6H, Ar H), 8.4 (s, 1H, Ar-H); MS (m/z): 313.1 ($M^+$).

**5b: Synthesis of 4-{5-Mercapto-4-[(4-nitro-benzylidene)-amino]-4*H*-[1,2,4] triazol-3-yl}-benzoic acid**

Yield (75%); m. p. 217°C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3020(C-H Ar str), 2788(C-H str), 1700.32(C=O in COOH gp.), 1681(C=C in Ar str), 1585(CH=N), 1409(N-O), 937(C-S); $^1$H NMR: 11.62 (s, H, COOH), 10.07 (s,1H,C-SH), 8.41-7.53(m,9H, Ar-H); MS (m/z): 357.1 ($M^+$).

**5c: Synthesis of 4-{5-Mercapto-4-[(3-nitro-benzylidene)-amino] -4*H*-[1,2,4]triazol-3-yl}-benzoic acid**

Yield (75%); m. p. 214°C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3035(C-H Ar str), 2772(C-H str.), 1716(C=O str), 1572(CH=N), 1533(C=C), 1512(N-O ortho); $^1$H NMR: 10.70 (s,1H,COOH), 10.15 (s,1H,C-SH), 8.42-7.50 (m,9H, Ar-H); MS (m/z): 357.1 ($M^+$).

**5d: Synthesis of 4-{4-[(4-Chloro-benzylidene)-amino]-5-mercapto-4*H*-[1,2,4] triazol-3-yl}-benzoic acid**

Yield (85%); m. p. 240°C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3502(O-H str), 3034(C-H Ar str), 1608(C=C), 1581(CH=N), 972(C-S), 601(C-Cl); $^1$H NMR: 11.25(s,1H,COOH), 9.90(s,1H,C-SH), 7.70-7.30(m,9H, Ar-H); MS (m/z): 346.5 ($M^+$).

**5e: Synthesis of 4-{4-[(4-Hydroxy-benzylidene)-amino]-5-mercapto-4*H*-[1,2,4]triazol-3-yl}-benzoic acid**

Yield (80%); m. p. 162°C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3253(O-H str), 1034 (C-S), 1588 (CH=N), 719 (C-H bend); $^1$H NMR: 14.04(s,1H,OH), 9.62 (s,1H,CH=N), 9.70 (s,1H, C-SH), 7.70-6.79 (m, 9H, Ar-H); MS (m/z): 328.1 ($M^+$).

**5f: Synthesis of 4-{ 4-[(2-Hydroxy-bezylidene)-amino]-5-mercapto-4*H*-[1,2,4]triazol-3-yl}-benzoic acid**

Yield (85%); m. p. 245°C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3217(O-H str), 3015 (C-H), 1578 (CH=N), 972 (C-S); $^1$H NMR: 14.05 (s,1H,OH), 10.79 (s,1H,COOH), 10.03 (s,1H, C-SH), 7.58-6.80 (m,9H, Ar-H); MS (m/z): 328.1 ($M^+$).

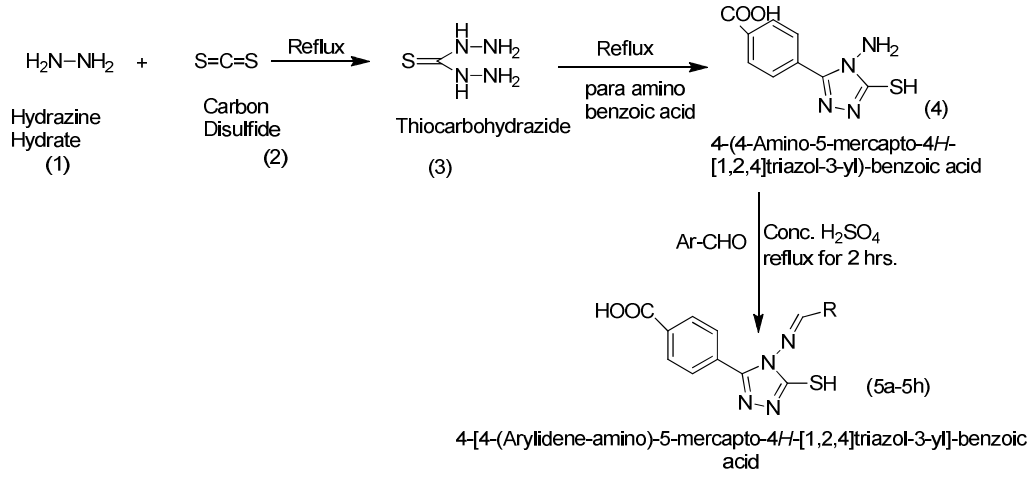
**5g: Synthesis of 4-{5-Mercapto-4-[(3,4,5-trimethoxy-bezylidene)-amino]-4*H*-[1,2,4]-triazol-3-yl-}-benzoic acid**

Yield (70%); m. p. 275°C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3022(C-H), 2832(O-$CH_3$), 1585(CH=N), 967 (C-S), 738 (C-H bend); $^1$H NMR: 9.95 (s, 1H, C-SH), 9.74 (s, 1H, CH=N), 7.70-6.87 (m, 6H, Ar-H), 3.81 (s, 3H, $OCH_3$); MS (m/z): 402.1 ($M^+$).

**5h: Synthesis of 4-{5-Mercapto-4-[(4-methyl-bezylidene)-amino]-4*H*-[1,2,4] triazol-3-yl}-benzoic acid**

Yield(80%); m. p.219°C; FT-IR (NaCl, $\lambda_{max}cm^{-1}$): 3031 (C-H str), 1583 (CH=N), 942 (C-S); $^1$H NMR: 10.58 (s, 1H, COOH), 9.98 (s,1H,CH=N), 7.73-7.46 (m, 8H, Ar-H), 2.42 (s, 1H, $CH_3$); MS (m/z): 326.1 ($M^+$).

**Scheme**



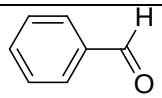
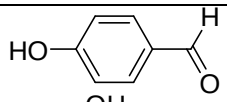
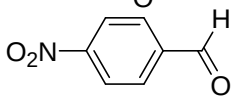
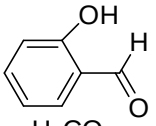
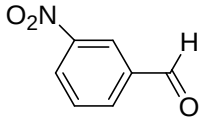
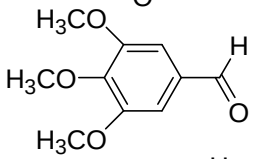
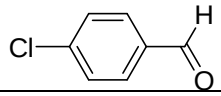
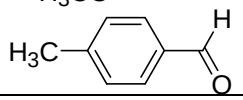

**Antimicrobial activity testing**

**General procedure**

The anti-microbial activities of the test compound 5(a-h) were evaluated by using cup plate method [14]. The synthesized compounds were screened for antibacterial activity against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative strains (*E. coli* and *P. aeruginosa*). Antifungal activity was done against *C. albicans* and *A. niger*. Muller Hinton agar medium was used for the evaluation of antibacterial activity. Screening of antifungal activity was done using Sabouraud's dextrose agar medium. Stock solutions of the compounds were first prepared by dissolving them in a solution of 30% DMF in water. 20 ml sterilized dehydrated nutrient agar medium was poured in each petri dish and was allowed to set. Eight cups were then made by punching the agar surface with a sterile cork bore (6 mm). The Petri plates were placed at 37 °C for 24 h.

At the end of the period the inhibition zones formed on media were measured with a zone reader in millimeters. 30% of Dimethyl formamide (DMF) was used as blank. Ciprofloxacin was used as a standard for anti-bacterial activity. Fluconazole used as a standard for anti-fungal activity. The experiment was conducted in triplicate (n=3), and the average values were calculated.

**Table 1: Various substituted aldehyde used are**

| Compound | R | Compound | R |
|---|---|---|---|
| 5a |  | 5e |  |
| 5b |  | 5f |  |
| 5c |  | 5g |  |
| 5d |  | 5h |  |

**RESULTS AND DISCUSSION**

A novel series of 4-[4-(Arylidene amino-5-mercapto-4*H*-[1, 2, 4] triazol-3-yl]-benzoic acid complexes from *p*-amino benzoic acid and thiocarbohydrazide was synthesized and characterized using analytical methods like TLC, IR, $^1$H-NMR and Mass spectroscopy. The structures of newly synthesized compounds were characterized on the basis of spectral data and elemental analysis. The physicochemical factors of the above-synthesized compounds were mentioned in table 2. The synthesized compounds were soluble in chloroform and methanol. All the synthesized Schiff bases were screened for *in vitro* antibacterial and antifungal activities. The antimicrobial activity of the synthesized compounds was evaluated by using Cup Plate method. The Schiff base complexes synthesized from p-amino benzoic acid were characterized by using FT-IR technique. The range of 3035-3020 cm$^{-1}$ indicates the presence of (C-H) aromatic group. The IR spectra of Schiff base complexes synthesized from p-amino benzoic acid mainly characterized by absorption width at the range 1588-1570 cm$^{-1}$which attribute the presence of CH=N group. Various functional groups of different aldehydes in compounds can be perceived by different ranges. 1512 cm$^{-1}$indicated the presence of nitro group at ortho position. The presence of chlorine deduced by observing the 601 cm$^{-1}$. 3253 cm$^{-1}$ showed the presence of hydroxyl group in 4-{4-[(4-Hydroxy-benzylidene)-amino]-5-mercapto-4*H*-[1, 2, 4] triazol-3-yl}-benzoic acid. The presence of (C-S) group illustrated by vibrations observed between 972-937 cm$^{-1}$. The $^1$H-NMR spectrum suggested the presence of a number of hydrogen atoms. A multiple peak was observed on  scale between 8.4-6.8 ppm which showed the presence of the aromatic ring in compound 5(a-h). Similarly, a single peak was observed on  scale between 9.98-9.62 ppm indicate the presence of CH=N group in derivatives. Observation of singlet peak on  scale between 10.07-9.7 ppm confirmed the presence of C-SH group in the compounds. The MS spectrum suggested that all the synthesized compounds are in given range.

**Anti-microbial activity**

The anti-microbial activities of the newly synthesized Schiff base 5(a-h) were assessed against Gram-positive (*Staphylococcus aureus, Streptococcus pneumonia)* and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa)* using the cup-plate method and antifungal activity against *Candida albicans* and *Aspergillus niger*. Ciprofloxacin and fluconazole were used as a standard.

It was found that Compounds 5(c), 5(d) and 5(e) showed significant activity against *S. aureus, S. pneumoniae*, *E. coli*, *P. aeruginosa,* whereas compounds 5(c), 5(d) and 5(f) showed significant antifungal activity against *C. albicans* and *A. niger*. Whilst, 5(a), 5(b) showed moderate activity against bacterial strains. Anti-microbial data of all the synthesized compounds were shown in table 3**.** The presence of chlorine atom at para position in 5(d) has increased the antimicrobial activity of the compound [15]. The similarly presence of nitro group at meta position increased the antibacterial activity of 5(c), whereas, the remarkable antifungal activity of 5(e) observed due to the presence of hydroxyl group at ortho position. Similarly, the good anti-bacterial activity of 5(d) was also reported due to the presence of hydroxyl group at para position [15]. Antimicrobial activity depends on the nature of bacterial strain, the solvent and chelating ability of the Schiff base. It is believed that Schiff bases act by forming a chelate with the bacterial strain.

This may involve hydrogen bonding through the azomethine group with the active centers of cell constituents thus resulting in an interference with normal cell process [16]. Hence, stronger the hydrogen bonding, more will be activity. Compounds 5(c), 5(d), 5(e) and 5(f) showed a wider zone of inhibition as compared to other synthesized compounds. The reason for the enhanced anti-microbial activity in these compounds was the–I effect of their substituents. Electron withdrawing ability of chlorine yielded a compound with significant anti-bacterial activity. Similarly, the activity of 5(e) and 5(f) was also increased due to the presence of hydroxyl group and its electron withdrawing effect [17]. In my present work some compounds like 5b (containing nitro group) and 5d (containing a hydroxyl group) showed better performance as an anti-microbial agent as compare to the compounds which were reported in previous literature containing same group [15].

**Table 2: Physicochemical data of 4-[4-(arylidene amino-5-mercapto-4*h*-[1, 2, 4] triazol-3-yl]-benzoic acid complexes**

| S. No. | Molcular formula | Mol. Wt. | Melting point | Yield | Physical state | Solubility | $R_f$ |
|---|---|---|---|---|---|---|---|
| 5a | $C_{15}H_{12}O_2N_4S$ | 312.0 | 188 | 80 | Yellowish amorphous | $CHCl_3$,$CH_3OH$ | 0.45 |
| 5b | $C_{15}H_{11}O_4N_5S$ | 357.0 | 217 | 75 | Light orange amorphous | $CHCl_3$,$CH_3OH$ | 0.57 |
| 5c | $C_{15}H_{11}O_4N_5S$ | 357.0 | 214 | 75 | Dark orange | $CHCl_3$,$CH_3OH$ | 0.53 |
| 5d | $C_{15}H_{11}O_2ClN_4S$ | 346.5 | 240 | 85 | Yellow crystalline | $CHCl_3$,$CH_3OH$ | 0.70 |
| 5e | $C_{15}H_{12}N_4O_3S$ | 328.00 | 162 | 80 | Crystalline orange | $CHCl_3$,$CH_3OH$ | 0.63 |
| 5f | $C_{15}H_{12}N_4O_3S$ | 328.00 | 245 | 85 | Creamish, fluffy | $CHCl_3$,$CH_3OH$ | 0.61 |
| 5g | $C_{18}H_{18}N_4O_5S$ | 402.0 | 275 | 70 | Whitish Yellow | $CHCl_3$,$CH_3OH$ | 0.65 |
| 5h | $C_{16}H_{14}O_2N_4S$ | 326.00 | 219 | 80 | Creamish, Amorphous | $CHCl_3$,$CH_3OH$ | 0.53 |

**Table 3: Antimicrobial activity of synthesized schiff base derivatives**

| Compound | Zone of inhibition in (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* | *P. aeruginosa* | *S. aureus* | *S. pneumoniae* | *C. albicans* | *A. niger* |
| 5a | 14.76±0.45 | 16.22±0.23 | 13.23±0.23 | 14.05±0.12 | 12.91±0.21 | 14.16±0.16 |
| 5b | 15.97±0.82 | 15.35±0.32 | 13.24±0.26 | 12.12±0.11 | 10.30±0.28 | 8.03±0.13 |
| 5c | 17.47±0.47 | 17.98±0.15 | 17.26±0.23 | 17.15±0.17 | 17.23±0.23 | 16.22±0.21 |
| 5d | 19.16±0.17 | 18.99±0.16 | 18.01±0.21 | 16.16±0.18 | 17.21±0.20 | 16.21±0.21 |
| 5e | 19.05±0.25 | 20.96±0.20 | 18.08±0.11 | 16.18±0.17 | 14.17±0.26 | 12.33±0.28 |
| 5f | 10.2±0.24 | 13.91±0.48 | 12.05±0.16 | 11.28±0.25 | 18.01±0.13 | 17.42±0.21 |
| 5g | 11.07±0.39 | 13.89±0.58 | 9.99±0.16 | 11.08±0.38 | 12.18±0.21 | 11.31±0.16 |
| 5h | 13.80±0.22 | 13.41±0.37 | 15.13±0.13 | 16.16±0.18 | 14.15±0.16 | 13.32±0.17 |
| Cipro floxacin (Std.) | 19.67±0.58 | 21.75±0.22 | 20.12±0.11 | 20.18±0.16 | - | - |
| Fluconazole (Std.) | - | - | - | - | 20.33±0.58 | 20.31±0.13 |
| DMF | - | - | - | - | - | - |

Values are expressed as mean±SEM. (n=3). SEM (Standard error mean)

## CONCLUSION

The Schiff base compounds were synthesized by using thiocarbohydrazide, p-amino benzoic acid and aromatic aldehydes. Due to the presence of different substituted aldehydes, novel Schiff base derivatives were synthesized. All the compounds were subjected to antimicrobial activity, and mostly compounds showed significant to moderate activity. The purpose of this paper is to throw light on the fact that substitution on Schiff base compounds synthesized from the same precursor leads to the formation of novel Schiff base derivatives, which showed good antimicrobial activity. Compounds containing chlorine group and nitro exhibited significant antimicrobial activity. Whereas, other derivatives containing other groups like methoxy and methyl group showed moderate to weak anti-microbial activity.

## CONFLICT OF INTERESTS

Declared none

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