Synthesis, Characterization and Antimicrobial Evaluation of Schiff Bases of 4-Amino-5-(4-*Tert*-butylphenyl)-4*H*-1,2,4-Triazole-3-Thiol

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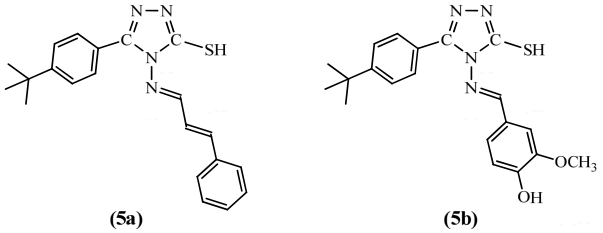
Abstract

*Triazoles are the five membered heterocyclic compounds having aromatic ring similar to that of pyrazole and imidazole but with an additional nitrogen atom in ring structure. Triazoles possess broad spectrum of biological activities which include antimicrobial, antifungal, antibacterial, antitubercular, anticancer, anti-oxidant, anti-inflammatory, antiviral and anticonvulsant activities. 1,2,4-triazole derivatives have received much attention due to their versatile biological properties as well as wide-ranging agrochemical and chemical properties. Hence, efforts have been made to synthesize 4-amino-5-(4-tert-butylphenyl)-4H-1,2,4-triazole-3-thiol and its Schiff bases using p-tert-butylbenzoic acid as precursor. The structures of newly synthesized compounds (**5a** and **5b**) were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectral analyses. These compounds were evaluated for their antimicrobial activity against bacterial strains - S. aureus, E. coli and S. typhi as well as the fungal strain, C. albicans. The results reveal that **5a** and **5b** displayed moderate activities against the tested microorganisms.*



(5a) **(5b)**

Keywords: *1,2,4-triazole, Schiff base, Thiol derivatives, Synthesis, Antimicrobial activities*

Introduction

Heterocyclic chemistry is the most challenging and more adequately rewarding field of organic chemistry with long history and future prospects[1]. The heterocyclic compounds containing nitrogen, oxygen and sulphur in the ring positions have significant interest in agrochemical[2] and medicinal chemistry due to their wide ranging biological targets[3]. Among heterocyclic compounds, the nitrogen containing heterocycles are one of the key intermediate and these are abundantly found in most of the medicinal compounds[4].

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Triazoles are the five membered aromatic heterocyclic compounds containing similar ring to that of azole, pyrazole and imidazole but with an additional nitrogen atom in the ring structure. Triazoles are said to be the isosters of imidazole, which is a part of the azole group of antifungal drugs such as Fluconazole, Itraconazole, Voriconazole, Ravuconazole and Posaconazole[5].Triazole and its derivatives represent one of the most biologically active classes of organic compounds that have received a great deal of attention since their discovery[6]. 1,2.4-triazole nucleus, being an important pharmacophore has a wide range of pharmacological properties such as anticancer[7-10], antifungal[11,12] antiviral[13-15], anti-inflammatory[16-18], anticonvulsant[19,20], antimalarial[21], antitubercular[22], antidepressant[23], antihypertensive[24,25], analgesic[26-28], and antioxidant[17,29] activities. In recent years, the synthesis of novel heterocycles from 1,2,4-triazole moiety has been attracting increasing interest for heterocyclic chemists due to their synthetic and effective medicinal importance. 1,2,4-Triazole ring has been incorporated into a wide variety of interesting drugs including H1/H2 histamine receptor blockers, CNS stimulants, anti-anxiety agents, and sedatives[30], some of these are Alprazolam[31], Furacyclin[32], Etizolam[33], Ribavirin[34] and Triazolam[35].

Schiff basses are the compounds containing azomethine functional group (-C=N-) that are typically formed by condensation of a primary amine and an aromatic aldehyde. Schiff bases have gained importance in medicinal and pharmaceutical fields due to their structural similarities with natural biological substances and relatively simple, flexible synthetic procedures that enable to design compounds with suitable structural properties [36-38].

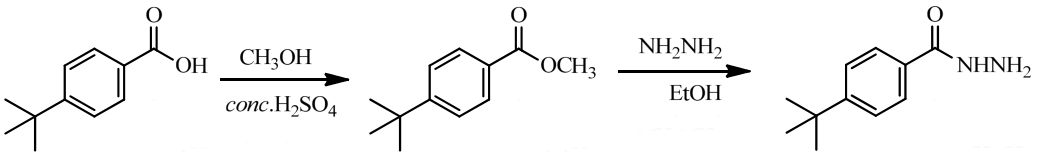
In present work we have synthesized 1,2,4-triazole-3-thiols incorporating 4-*tert*-butylphenyl moiety and its Schiff bases with vanillin and cinnamaldehyde.

Experimental Methods

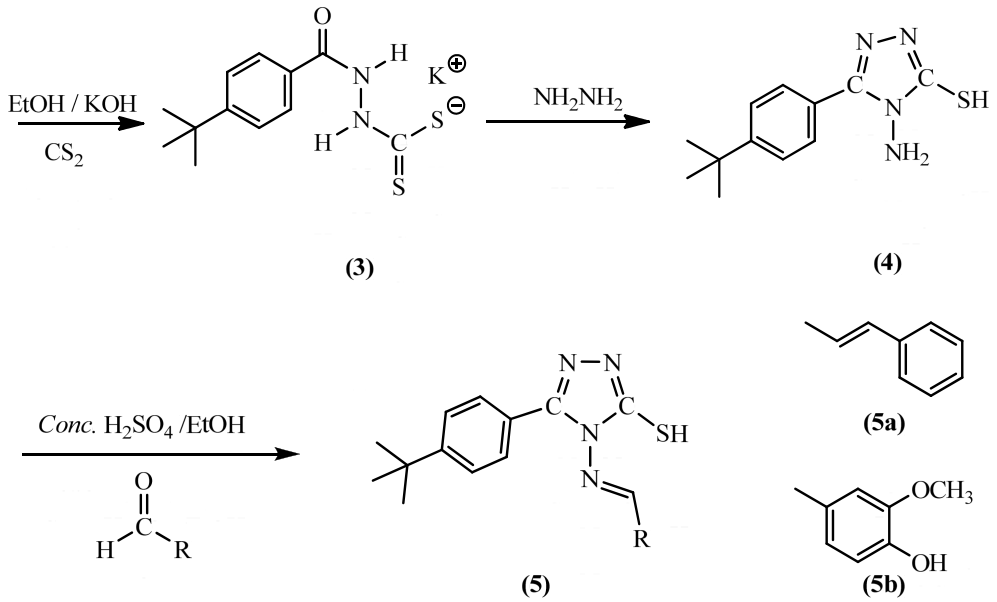
The progress of reaction was monitored by TLC using silica gel coated aluminum plates and spots were visualized in iodine chamber. Melting point was determined on Optics Technology electro-thermal apparatus by open capillary tube and are uncorrected; IR spectra were recorded on BRUKER 375-FTIR spectrometer.^{1}H-NMR and ^{13}C-NMR spectra were obtained from BRUKER Bio spin Advance III 400 MHz FT-NMR spectrometer using DMSO-d_6 as the solvent with TMS as internal standard. Chemical shifts are reported in parts per million (ppm) and signals are described as singlet (*s*), doublet (*d*), triplet (*t*) and multiplet (*m*).

Synthesis

The syntheses of the compounds were carried out as illustrated in Scheme 1.



(1) **(2)**



Scheme.1: Synthesis of Schiff base

Synthesis of methyl-4-tert-butylbenzoate **(1)**

A mixture of 4-*tert*-buytylbenzoic acid (17.82 g, 0.1 mol), anhydrous methanol (22 mL) and *conc.* sulphuric acid (1 mL) was refluxed for 5 h. The volume of solution was reduced to half by evaporating. After cooling it was diluted with 50 mL water and the ester was extracted with anhydrous diethyl ether. It was then washed with 20% (w/v) solution of sodium bicarbonate and then dried over anhydrous magnesium sulphate.

Yield 78%, (14.976 g), Colourless liquid.

Synthesis of 4-tert-butylbenzohydrazide **(2)**

99% hydrazine hydrate (0.750 g, 0.015 mol) was added drop wise, with constant stirring, to a solution of **1** (1.92 g, 0.01 mol) in absolute ethanol (20 mL) and refluxed for 6 h. The solution was then concentrated and cooled. The solid mass separated out was washed with ethanol, dried and recrystallized from ethanol.

Yield 64 %, (1.612 g), yellowish white, rhombus shaped crystal, m.p. 120 °C.

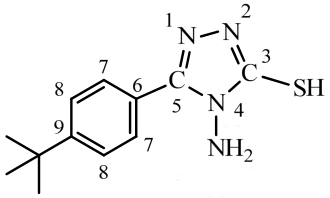
Synthesis of potassium 2-(4-tert-butylbenzoyl) hydrazinecarbodithioate **(3)**

Carbon disulphide (1.14 g) was added drop wise to ice cold mixture of potassium hydroxide (0.84 g, 0.015 mol), ethanol (20 mL) and acid hydrazide (1.92 g, 0.01 mol) and then refluxed for 16 h. Anhydrous diethyl ether (20 mL) was added and the solid separated out was washed several times with diethyl ether and recrystallized with ethanol.

Yield 60 %, (2.754 g), white shining, needle shaped crystal, m.p. 310 °C

***Synthesis of 4-amino-5-(4-tert-butylphenyl)-4H-1,2,4-triazole-3-thiol* (4)**

A suspension of potassium dithiocarbazinate (3.06 g, 0.01 mol) in water (10 mL) and hydrazine hydrate (1 g, 0.02 mole) was refluxed till the evolution of hydrogen sulphide gas was ceased. The reaction mixture was cooled, diluted with water (100 mL) containing some crushed ice and acidified with *dil.* hydrochloric acid. The solid mass separated was filtered, washed with cold water and recrystallized with ethanol.



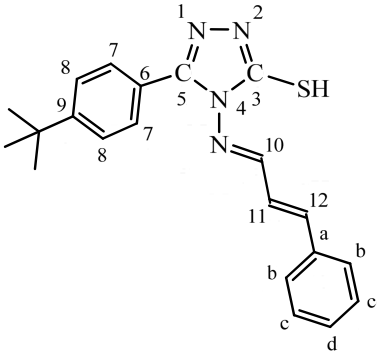
Yield 65%, (1.612 g, 0.0065 mol), light yellow, amorphous solid, m.p. 156 °C, IR (KBr, ν cm$^{-1)}$: 3220(m), 3199(m), 3109(m), 2950(s), 2849(s), 2589(w), 1655(m), 1521(s), 1437(s), 1161(s), 684(s); ^{1}H NMR (400MHz, DMSO-d_6) δ = 13.92 (1H, br. s., HS-), 7.78 (2H, d, J = 5.9 Hz, H-7), 7.58 (2H, d, J = 6.1 Hz, H-8), 1.28 (9H, br. s., C$\underline{H}_3$) ^{13}C NMR (100MHz, DMSO-d_6) δ = 177.7 (C-3), 161.0 (C-9), 155.7 (C-5), 126.7 (C-6), 126.4 (C-7), 120.2 (C-8), 35.3(*t*-Bu $\underline{C}$), 31.4 ($\underline{C}H_3$)

***Synthesis of Schiff base* (5)**

To the hot ethanolic solution of aromatic aldehyde containing 4-5 drops of *conc*. sulphuric acid, a hot ethanolic solution of **4** was added and refluxed for 5 h. The solid formed was filtered, washed with cold ethanol and recrystallized from ethanol.

***Synthesis of 5-(4-(tert-butylphenyl)-4-(3-phenylallylideneamino)-4H-1,2,4-triazole-3-thiol* (5a)**

To a solution of triazole (0.496 g, 0.002 mol) in anhydrous ethanol (5 mL), cinnamaldehyde (0.264 g, 0.002 mol), was added with constant stirring. After adding *conc.* sulphuric acid (5 mL), the mixture was refluxed for 5 h and cooled. The solid separated was filtered, washed with cold ethanol and recrystallized with ethanol.

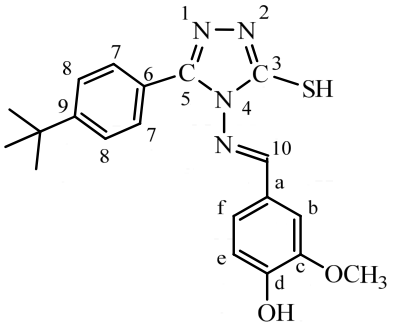


Yield 63%, (0.456 g, 0.00126 mol) pale yellow, amorphous solid, m.p. 182-184 °C, IR (KBr, ν cm$^{-1)}$: 3120(m), 3011(m), 2952(m), 2576(w), 1650(m), 1583(m), 1512(s), 1437(s), 1121(s), 675(s); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 14.07 (1H, br. s., HS-), 9.34 (1H, d, J = 9.5 Hz, H-10), 7.96 (2H, d, J = 8.8 Hz,

H-7), 7.55 - 7.80 (2H, m, H-b), 7.23 - 7.48 (6H, m, H-c, H-8, H-d, H-12), 7.13 (1H, dd, J = 16.1 Hz, J = 9.5 Hz, H-11), 1.37 (9H, br. s., C<u>H</u>$_3$); ^{13}C NMR (100MHz, DMSO-d_6) δ = 168.3 (C-3), 162.2 (C-10), 149.9 (C-9), 148.7 (C-5), 147.0 (C-a), 135.4 (C-12), 130.7 (C-c), 129.4 (C-b), 129.0 (C-d), 128.5 (C-6), 126.1 (C-8), 125.8 (c-7), 119.4 (C-11), 34.3 (*t*-Bu <u>C</u>) , 31.4 (<u>C</u>H$_3$)

***Synthesis of 5-(4-tert-butylphenyl)-4-(4-hydroxy-3-methoxybenzylidene- amino)-4H-1,2,4-triazole-3-thiol* (5b)**

To a solution of triazole (0.496 g, 0.002 mol) in anhydrous ethanol (5 mL), vanillin (0.304 g, 0.002 mol) was added with constant stirring. After adding *conc.* sulphuric acid (5 mL) the mixture was refluxed for 5 h and cooled. Thus formed solid was filtered, washed with cold ethanol and recrystallized with ethanol.



Yield 67%, (0.511 g, 0.00134 mol) light green, amorphous solid, m.p. 196-198 °C, IR (KBr, v cm$^{-1)}$: 3340(s), 2962(m), 2896(m), 2550(w), 1655(s), 1614(s), 1594(m), 1502(s), 1313(m), 1114(s), 676(s); ^{1}H NMR (400MHz, DMSO-d_6) δ = 14.04 (1H, br. s., HS-), 10.05 (1H, br. s., HO-), 9.98 (1H, br. s., H-10), 7.96 (2H, d, J = 8.8 Hz, H-7), 7.30 - 7.55 (3H, m, H-b & H-8), 7.15 - 7.30 (1H, m, H-f), 6.81 - 7.06 (1H, m, H-e), 3.77 (3H, br. s., C<u>H</u>$_3$O-), 1.37 (9H, br. s., C<u>H</u>$_3$); ^{13}C NMR (101MHz, DMSO-d_6) δ = 167.1 (C-3), 162.3 (C-10), 151.8 (C-9), 149.9 (C-5), 148.6 (C-d), 148.5 (C-c), 129.1(C-6) , 126.1 (C-a), 125.9 (C-8), 124.6 (C-7), 123.9 (C-f), 116.1 (C-e), 111.3 (C-b), 56.1 (<u>C</u>H$_3$O), 34.3 (*t*-Bu <u>C</u>) , 31.4 (<u>C</u>H$_3$)

Antimicrobial activity

The solution of newly synthesized compounds **5a** and **5b** prepared in DMSO were screened at a concentration of 3 mg.mL^{-1} for their antimicrobial activity against three bacterial species [one Gram positive, *Staphylococcus aureus* (ATCC: 25923) and two Gram negative, *Escherichia coli* (ATCC: 25922), *Salmonella typhi*] and one species of yeast, *Candida albicans* by well-diffusion method. The overnight culture of bacteria from nutrient agar medium and 48 hour culture of yeast from potato dextrose agar were inoculated in Muller-Hinton broth and incubated for 3-4 h at 37 °C till the turbidity matched with McFarland Standard 0.5. After standardization of the suspension, a sterile cotton swab was immersed in it and excess of suspension was pressed along the side of the tube. A lawn culture of organism was made on Mueller Hinton agar. Wells were made on these seeded agar plates by using a sterile metallic borer and the prepared solution (50 μL) of the test compounds and control (Ofloxacin, 3 mg.mL^{-1}) were loaded into each labeled well with the help of sterile micropipette. The Petri dishes were

incubated at 37 °C for 24 h and antimicrobial activity was determined by measuring the diameter of inhibition zone of each compound and compared with Ofloxacin as standard.

Results and Discussion

4-amino-5-(4-*tert*-butylphenyl)-4*H*-1,2,4-triazole-3-thiol (**4**) was synthesized by cyclization of potassium 2-(4-tert-butylbenzoyl) hydrazinecarbodithioate with hydrazine hydrate. Condensation of **4** with the aldehydes gave the Schiff bases 5-(4-(tert-butylphenyl)-4-(3-phenylallylideneamino)-4*H*-1,2,4-triazole-3-thiol (**5a**) and 5-(4-tert-butylphenyl)-4-(4-hydroxy-3-methoxybenzylidene- amino)-4*H*-1,2,4-triazole-3-thiol (**5b**). The structures of the newly synthesized compounds were confirmed by IR, ^{1}H and ^{13}C-NMR spectroscopy

IR Spectra

The IR spectral analysis supports the presence of characteristic groups in the synthesized compounds. A couple of bands with medium absorption due to stretching vibrations of NH_2 are observed at the region 3220 and 3199 cm^{-1} in the spectra of compound **4**. The disappearance of these bands in the spectra of 5**a** and **5b** confirmed the formation of Schiff bases. The medium absorption at 3120 – 3109 cm^{-1} and strong absorptions at 3011– 2849 cm^{-1} are attributable to (C=CH) and (C–H) respectively. The strong absorptions in the region 1650 cm^{-1} – 1437 $cm^{-1}$are due to the stretching vibrations of azomethine (C=N) and ring breathing (C=C). Although triazole thiols can exist in thione-thiol tautomeric forms, the presence of weak absorption in the region 2589 cm^{-1} – 2550 cm^{-1} corresponding to stretching vibration of S-H suggest that synthesized compounds exist in thiol form in the solid state. The strong intensity band at 1161– 1114 cm^{-1} and 684 – 675 cm^{-1} are characteristic of N-N and Ar-H respectively. In addition, in the spectra of **5a** strong absorption at 3340 cm^{-1} and medium absorption at 1313 cm^{-1} also exist due to stretching and bending respectively of Ar-OH.

^{1}H-NMR Spectra

The ^{1}H-NMR spectra of synthesized compounds are consistent with the analogous triazole thiol Schiff bases. The ^{1}H-NMR spectra of the compounds display broad singlet at 13.92–14.07 ppm corresponding to the thiol proton (–SH) which is much deshielded due to adjacent strongly electron withdrawing triazole nucleus. The signals due to the protons of aromatic ring appear at 7.96 – 7.23 ppm. The low frequency broad singlet in the aliphatic regions is observed at 1.28 –1.37 ppm for the *t*-butyl protons. The formation of Schiff bases is also confirmed by absence of N–H band in IR spectra and the presence of signal due to imine proton (–N=CH–) at 9.34 and 9.98 ppm in ^{1}H NMR of **5a** and **5b** respectively. A doublet in spectra of **5a** at 9.34 ppm with *J* value of 9.5 HZ is attributed to imine proton while a doublet of doublet with *J* values at 16.1 and 9.5 ppm is due to the proton on the carbon adjacent to this. Whereas, in the spectra of **5b** there are additional signals at 10.05 ppm and 3.77 ppm due to the –OH and $–OCH_3$ protons.

^{13}C-NMR Spectral analysis

The appearance of a peaks at δ = 167.1 – 177.7 ppm indicate the C–SH and C=N carbons of triazole nucleus. The peak at 149.9 – 161.0 is attributed to the aromatic carbon adjacent to the *tert*-butyl group. The peak for other aromatic carbons appear in the region 120.2 – 148.6 ppm. The signals in the aliphatic region i.e. at 34.3 – 35.3 and 31.4 correspond to the carbons of *t*-butyl group. The signal at 56.1 ppm in the spectra of **5b** was attributable to the methoxy carbon.

Antimicrobial evaluation

The antimicrobial activity of Schiff bases **5a** and **5b** were examined against all tested microorganisms at a concentration of 3 $mg.mL^{-1}$ using Ofloxacin as standard and the results are summarized in table 1. Among these **5a** exhibited strong biological activity against *S. aureus* more than other Gram negatives. The compound **5b** displayed better activity against *S. typhi*, while showed moderate activity against *S. aureus* and *E. coli.* The Schiff bases **5a** and **5b** exhibited significant antifungal activity towards *Candida albicans* (yeast).

Table 1: Antimicrobial activities of Schiff bases

Compound Code	Diameter of Zone of Inhibition (mm)			
	Bacterial Strains			Fungal strain
	S. aureus	*E. coli*	*S.* Typhi	*C. albicans*
5a	29.5	18.5	20	15
5b	26	23	28	18
Ofloxacin	40	39	38	22

Conclusions

The Schiff bases (**5a** and **5b)** were successfully synthesized which are thermally stable and soluble in DMSO. The bonding of *N*-atom of amino group of thiol **(4)** with the carbonyl carbon of aromatic aldehydes as a result of condensation leading to the formation of Schiff bases were confirmed by analytical methods such as IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The spectroscopic evidence supports the proposed structures of synthesized compounds. The antimicrobial screening suggests that, the synthesized compounds exhibited moderate activities against all the tested microorganisms. **5a** exhibited stronger biological activity against *S. aureus* than Gram negatives, while it is reverse in case of **5b**. Further modification of this class of compounds may lead to safer antimicrobial agents.

Acknowledgements

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