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RESEARCH ARTICLE

Synthesis and Evaluation of Schiff Bases of 4-Amino-5-(chlorine substituted phenyl)-4*H*-1,2,4-triazole-3-thione as Antimicrobial Agents

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Abstract

Triazole ring system has attracted a continuously growing interest of synthetic organic chemists and those dealing with the medicinal compounds due to its versatile potential to interact with biological systems. Schiff bases are also considered as one of the most biologically active compounds. The aim of the present study was to synthesize new Schiff bases bearing triazole nucleus and to assess their antimicrobial activities. Four new Schiff base derivatives of 1,2,4-triazole-3-thione were synthesized by combining two different pharmacophores *viz*. triazole nucleus and Schiff base moiety and were characterized by spectral techniques (UV, FT-IR, and NMR). The Schiff bases were evaluated for antibacterial (*Staphylococcus aureus*, *Escherichia coli,* and *Klebsiella pneumoniae*) and antifungal (*Candida albicans*) activities. The synthesized compounds exhibited good to moderate activities against different strains of bacteria and fungi tested.

Keywords: 1,2,4-triazole-3-thione, chlorophenyl, Schiff base, antibacterial activity, fungal inhibition

Introduction

Heterocyclic chemistry is a distinct field of chemistry with a long history and prospects due to its versatile biological activities. Heterocyclic compounds were the earliest compound known to mankind. Among the heterocycles, triazole ring systems have received considerable attention due to their applications in the fields of medicine, industry, and agriculture as agrochemicals [1-3]. All triazoles are of synthetic origin and there is no triazole ring system detected as yet in nature.

Triazoles are heterocyclic organic compounds having a five-member aromatic ring with three nitrogen atoms and two carbon atoms. It is one of a pair of isomeric chemical compounds with chemical formula $C_2H_3N_3$. The triazole exists in two isomeric forms *viz.* 1,2,3-isomer and 1,2,4-isomer, with respect to the location of nitrogen in the ring [4]. Triazoles and their derivatives are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities such as antiviral [5], antibacterial [6-8], antitubercular, antimalarial [9], antifungal [10-12], anticancer [13-15], anti-inflammatory [16], antidepressant [17], antioxidant [18], local anesthetic, antiobesity, antidiabetic, anti-Parkinson's[19], analgesic [20], anticonvulsant [21], antianxiety, antihistaminic, antiepileptic, antihypertensive [22], etc. Various drugs of medicinal uses that contains the 1,2,4-triazole nucleus have already been synthesized. Some of the drugs are anastrozole (anticancer) [23], Ribavirin (antiviral) [24], voriconazole (antifungal) [5], posaconazole (antifungal) [6], trazodone (antidepressant) [25], estazolam (anticonvulsant) [26], rizatriptan (antimigraine) [27], alprazolam (tranquilizer) [28], rilmazafone (sedative-hypnotic) [29], diniconazole (fungicidal in agriculture) [6], Bitertanol (fungicidal), triazomol (pesticidal) and paclobutrazol (plant growth regulator) [29].

One of the most investigated reactions of triazoles is the formation of Schiff bases by condensing 4-amino-1,2,4-triazole-3-thiones with aldehydes [30-

33]. Schiff bases are the compounds carrying imine (>C=N–) or azomethine (–CH=N–) functional group [34]. Schiff bases possess structural similarities with natural biological substances and are also found in different enzymes such as tryptophan synthases, transaminases, transketolases etc. [35,36]. They are an important class of compounds in many fields such as analytical, biological, and inorganic chemistry and are a versatile pharmacophore [37].

Structural modifications of the triazole ring system by using different functionalities and aromatic rings are expected to result in potential candidates for antibacterial and antifungal agents. So, in the present work, some new compounds are synthesized by combining chemically different but pharmacologically compatible 1,2,4-triazole nucleus and Schiff base moiety in one frame to evaluate their antimicrobial activities.

Materials and Methods

Starting materials

4-chlorobenzoic acid, 2,4-dichlorobenzoic acid, and vanillin were purchased from Himedia, furfuraldehyde, methanol and potassium hydroxide from Fisher scientific, carbon disulphide from Merck and ethanol from Changshu Honsgsheng Fine Chemical. All chemicals and solvents used for the experiment were of synthetic grade and were used without further purification.

Physical measurements

Melting points of the synthesized compounds were determined on the Optics Technology electro-thermal apparatus by an open capillary tube. UV-Visible absorption spectra were monitored on a UV-Visible double beam spectrophotometer from Labtronics (Model LT-2802) using DMSO as a solvent. FT-IR spectra were recorded on spectrum GX Fourier transform infrared (FT-IR) spectrophotometer using the KBr pellet method. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at ambient temperature on Varian VNMRS 400 MHz NMR spectrometer using DMSO as the solvent with TMS as an internal standard.

Synthesis and analyses

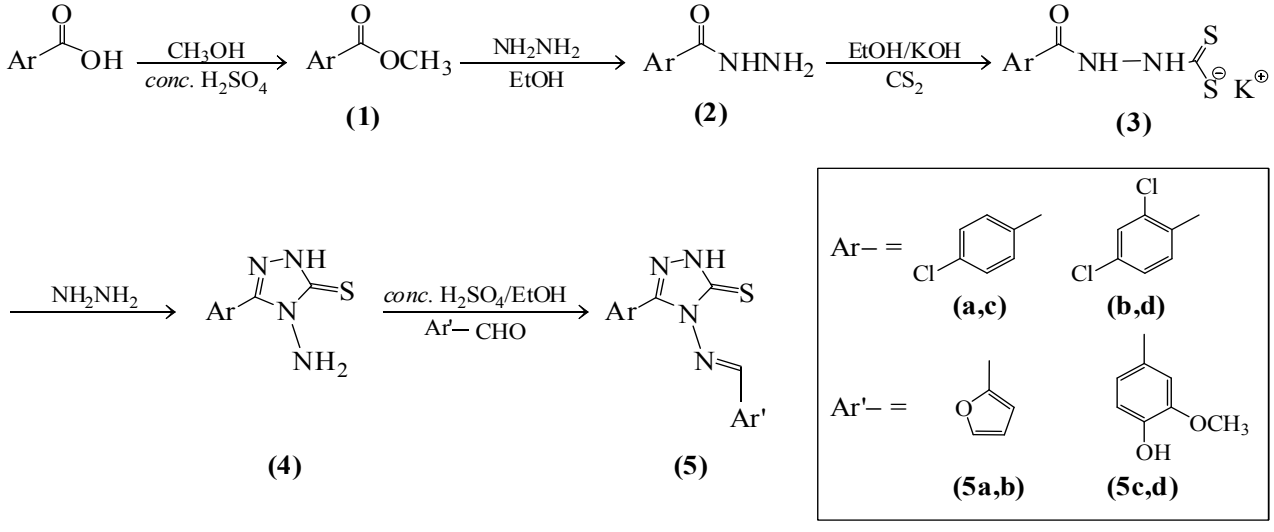
The compounds were synthesized from the starting materials according to Scheme 1.

Synthesis of methyl ester (1a,b) [38]

The respective substituted benzoic acid (0.1 mol) was dissolved in anhydrous methanol (50 mL) in a 250 mL round bottom flask fitted with a reflux condenser. Concentrated sulphuric acid (0.02 mol) was added and the reaction mixture subjected to reflux for 8-10 h. After the completion of the reaction, the excess methanol was removed and the contents were poured into water, neutralized with 20% (w/v) solution of sodium bicarbonate, and then dried over anhydrous magnesium sulphate.

Synthesis of acid hydrazide (2a,b) [38]

The ester (**1**) (0.10 mol) was dissolved in absolute ethanol (20 mL), 99% hydrazine hydrate (0.15



Scheme 1: *Synthetic routes of Schiff bases of 1,2,4-triazole derivatives*

mol) was added slowly with constant stirring and the reaction mixture was heated under reflux for 4 h. The mixture was concentrated and then cooled. The crude solid was filtered, washed with water and recrystallized from absolute ethanol to give aryl acid hydrazide.

Synthesis of dithiocarbazinate (3a,b) [39]

The acid hydrazide (**2**) (0.05 mol) was added to a solution of potassium hydroxide (0.075 mol) in absolute ethanol (50 mL) at room temperature. Carbon disulphide (0.15 mol) was added and the mixture was stirred at room temperature for 18 h. The mixture was diluted with anhydrous diethyl ether (20 mL). The crude solid was filtered, washed several times with anhydrous diethyl ether, and dried in a desiccator.

Synthesis of 4-Amino-5-substituted-1,2,4-triazole-3-thione (4a,b) [40]

99% hydrazine hydrate (0.04 mol) was gradually added to the above potassium salt (**3**) (0.02 mol) dissolved in water (20 mL) with stirring and the mixture was refluxed gently till the evolution of hydrogen sulphide ceased. The mixture was then cooled to room temperature and diluted with 100 mL cold water containing some crushed ice and was acidified with concentrated hydrochloric acid. A yellow solid separated was filtered, washed with water, and recrystallized from ethanol.

Synthesis of Schiff bases of 4-Amino-5-substituted-1,2,4-triazole-3-thione (5a-5d) [41]

To the hot ethanolic solution of aldehyde (0.01 mol) containing 5 drops of concentrated sulphuric acid, a hot ethanolic solution of the triazole (**4**) (0.01 mol) was added in small portions over a period of 1 h and refluxed for 3 h. The reaction mixture was cooled, filtered, and recrystallized from ethanol to give (**5a-5d**).

5-(2-chlorophenyl)-4-(furan-2-ylmethyleneamino)-1,2,4-triazole-3(2H)-thione (5a)

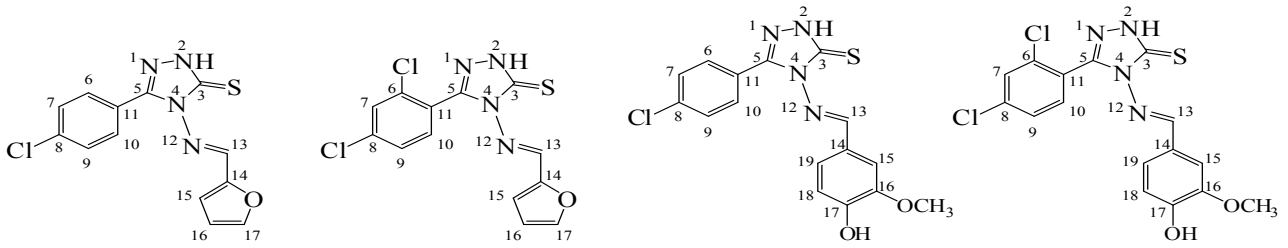
Pale yellow solid, yield 65% (1.976 g); mp 197 °C; UV-Visible spectrum (λ_{max}) nm = 302, 308, 344; IR ν_{max} in KBr (selected bands) cm^{-1} = 3095 (s), 1615 (s), 1573 (m), 1495 (s), 1476 (s), 1290 (s), 1045 (m), 840 (m), 760 (s); ^{1}H NMR (400MHz, DMSO-d_6) δ = 9.689 (1H, s, H-13), 7.972 (1H, br s, H-8), 7.677 - 7.59 (4H, m, H-17, H-11, H-9 and HN-), 7.156 (1H, td, *J* = 7.6, 2.0 Hz, H-10), 7.305 (1H, d., H-15), 6.728 (1H, dd. *J* = 3.2, 1.6 Hz, H-16); ^{13}C NMR (100MHz, DMSO-d_6) δ = 179.9 (C-3), 149.0 (C-14), 148.3 (C-5), 145.3 (C-17), 134.2 (C-13), 132.6 (C-11), 132.3 (C-7), 131.9 (C-9), 130.1 (C-8), 128.0 (C-10), 122.6 (C-6), 117.4 (C-15), 111.5 (C-16).

5-(2,4-dichlorophenyl)-4-(furan-2-ylmethyleneamino)-1,2,4-triazole-3(2H)-thione (5b)

Black solid, yield 61% (2.054 g); mp 204 °C; UV-Visible spectrum (λ_{max}) nm = 302, 309, 344; IR ν_{max} in KBr (selected bands) cm^{-1} = 3089 (m), 1614 (m), 1543 (m), 1501 (s), 1475 (s), 1280 (m), 1020 (m), 765 (s), 725 (m); ^{1}H NMR (400MHz, DMSO-d_6) δ = 9.741 (1H, br s, H-13), 7.989 (1H, br s, H-8), 7.875 (1H, d, *J* =2.0 Hz, H-17), 7.726 (1H, d, *J* = 8.0 Hz, H-11), 7.654 (1H, dd, *J* = 8.0, 2.0 Hz, H-10), 7.320 (1H, d, *J* = 3.6 Hz, H-15), 6.741 (1H, t, *J* = 2.0 Hz, H-16); ^{13}C NMR (100MHz, DMSO-d_6) δ =178.9 (C-3), 148.0 (C-14), 145.2 (C-5), 143.7 (C-17), 138.8(C-13), 137.9 (C-7), 131.2 (C-8), 130.5 (C-11), 129.9 (C-9), 126.7 (C-10),121.1 (C-6), 118.8 (C-15),113.4 (C-16).

5-(2-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylideneamino)-1,2,4-triazole-3(2H)-thione (5c)

Light green solid, yield 60% (2.148 g); mp 185 °C; UV-Visible spectrum (λ_{max}) nm = 302, 308, 344; IR



(5a) **(5b)** **(5c)** **(5d)**

Figure 1: *Structures of synthesized Schiff bases*

ν_{max} in KBr (selected bands) cm^{-1} = 3363 (m), 3062 (m), 1666 (m), 1597 (s), 1512 (s), 1463 (s), 1265(s), 1296 (s), 1261 (s), 1261 (s), 1026 (s), 817 (m), 717 (m); ^{1}H NMR (400MHz, DMSO-d_6) δ = 10.145 (1H, br s, HO-), 9.731 (1H, br s, H-13), 7.828 (1H, dd, *J* = 8.4, 2.0 Hz, H-8), 7.786 (1H, d, *J* = 8.4, Hz, H-15),7.746 (1H, dd, *J* = 8.8, 2.0 Hz, H-11), 7.626 (1H, m, H-9), 7.530 (1H, m, H-10), 7.438 (1H, dd, *J* = 8.4, 2.0 Hz, H-19), 6.918 (1H, d, *J* = 11.6, H-18), 3.833 (3H, br s, C$\underline{H}_3$); ^{13}C NMR (100MHz, DMSO-d_6) δ = 178.9 (C-3), 155.0 (C-13), 152.2 (C-17), 150.7 (C-16), 148.0 (C-5), 137.3 (C-11), 134.0 (C-7), 132.4 (C-9), 131.1 (C-14), 130.9 (C-8), 125.4 (C-10), 123.5 (C-6), 122.9 (C-19), 117.6 (C-18), 113.4 (C-15), 46.0 (C$\underline{H}_3$).

5-(2,4-dichlorophenyl)-4-(4-hydroxy-3-methoxybenzylideneamino)-1,2,4-triazole-3(2H)-thione (5d)

Yellowish brown solid, yield 48% (1.879 g); mp 124 °C; UV-Visible spectrum (λ_{max}) nm = 302, 309, 322; IR ν_{max} in KBr (selected bands) cm^{-1} = 3280 (s), 3209 (m), 1654 (m), 1581 (s), 1482 (s), 1463 (s), 1261(m), 1291 (s), 1261 (s), 1031 (m), 814 (s), 734 (m); ^{1}H NMR (400MHz, DMSO-d_6) δ = 10.255, 10.029 (1H, br s, $\underline{H}$O-, *E* and *Z* geometrical isomers), 9.765, 9.508 (1H, br s, H-13, *E* and *Z* geometrical isomers), 7.929, 7.736 (1H, d, *J* = 8.4 Hz, H-11, *E* and *Z* geometrical isomers), 7.908, 7.859 (1H, d, *J* = 2.0 Hz, H-8, *E* and *Z* geometrical isomers), 7.663-7.608 (1H, m, H-15, *E* and *Z* geometrical isomers), 7.428, 7.228 (1H, dd, *J* = 8.4, 2.0 Hz, H-10, *E* and *Z* geometrical isomers), 7.383, 7.260 (1H, d, *J* = 1.6 Hz, H-19, *E* and *Z* geometrical isomers), 6.964, 6.881 (1H, d, *J*= 8.4 Hz, H-18, *E* and *Z* geometrical isomers), 3.835, 3.751 (3H, br s, C$\underline{H}_3$, *E* and *Z* geometrical isomers); ^{13}C NMR (100MHz, DMSO-d_6) δ = 178.8 (C-3), 159.5 (C-13), 150.0 (C-17), 149.7 (C-15), 148.0 (C-5), 134.2 (C-7), 133.4 (C-14), 129.1 (C-11), 128.9 (C-8), 127.4 (C-9), 127.0 (C-10), 122.9 (C-19), 121.1 (C-6), 117.0 (C-18), 112.1 (C-15), 46.0 (C$\underline{H}_3$).

Antimicrobial (antibacterial and antifungal) screening

The antimicrobial activity of the newly synthesized compounds (**5a-5d**) in two different concentrations (1% and 3%) were evaluated against various microorganisms, representing Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and fungus (*Candida albicans*) according to the cup-plate assay. The overnight culture of bacterial and fungal species from nutrient agar and potato dextrose agar respectively was adjusted to 0.5 McFarland standards and was spread on the surfaces of Muller-Hinton agar plates using a sterile cotton swab to prepare microbial lawns. The suitably spaced apart wells of 6 mm diameter were made on each agar plate and the labeled wells were loaded with 50 µL of each triazole solution. Ciprofloxacin (3%) and Ketoconazole (3%) were used as reference antibacterial and antifungal respectively. DMSO was used as a negative control. The Petri dishes were kept 30 minutes for diffusion and incubated at 37 °C for 24 h. After incubation, the diameters of the inhibition zones were measured in mm and the results of antimicrobial activities were interpreted.

Results and Discussion

Chemistry

Four new Schiff bases of 4-amino-1,2,4-triazole-3-thione (**5a-d**) were synthesized according to the synthetic route illustrated in scheme 1. The esters (**1a,b**) were prepared by heating the substituted benzoic acid with methanol and a catalytic amount of *conc.* H_2SO_4. The reaction of **1** with hydrazine hydrate in ethanol yielded corresponding aroyl hydrazides (**2a,b**). Treatment of **2** with alcoholic potassium hydroxide and carbon disulphide resulted in the formation of corresponding potassium 3-aroyl dithiocarbazate (**3a,b**). The ring closure of potassium 3-aroyl dithiocarbazate (**3a,b**) excess hydrazine hydrate in ethanol yielded 4-amino-5(substituted phenyl)-1,2,4-triazole-3-thione (**4a,b**). Condensation of 1,2,4-triazole (**4a,b**) with furfuraldehyde in refluxing ethanol in the presence of *conc.* H_2SO_4 as catalyst gave (**5a,b**), and while condensation with vanillin gave (**5c,d**).

Spectroscopic studies

The structures of the Schiff bases (**5a-d**) were confirmed by UV, ^{1}H-NMR, and ^{13}C-NMR spectral analysis.

UV-Visible analysis

The UV-Vis spectra of the compounds were studied in DMSO. The absorption spectra of the compounds

(**5a** – **5d**) exhibited three-bands around 302 nm, 309 nm, and 344 nm. The first two peaks are attributable to aromatic C=C and azomethine C=N ($\pi \rightarrow \pi^*$) transitions while the third band is attributable to the $n \rightarrow \pi^*$ transitions associated with the non-bonding electron pair of the azomethine nitrogen and sulphur atoms [42].

FT-IR analysis

In the IR spectra of compounds **5a-d,** no absorption bands were detected at about 1651–1707 cm^{-1} indicating the absence of C=O group of compound **3a-b** which is evidence for the conversion of dithiocarbazinate to triazoles [43]. The most characteristic absorptions due to the triazole nucleus are observed at 1666-1614 cm^{-1} (C=N), and 1290-1261 cm^{-1} (C=S) [35]. The absence of medium intensity bands in the region 3500-3200 cm^{-1} attributable to NH_2 protons of **4a-b** demonstrates the formation of Schiff bases [44]. Since the 1,2,4-triazole contains thioamide –NH-C=S functional group, the sulphur at 3-position of the ring can be incorporated as a thiol (Fig. 2a) [45] or thione (Fig. 2b) [46] function. There is no absorbance band in the region c.a. 2600 cm^{-1} attributable to stretching of S–H, but the presence of band *v*(N–H) stretching vibration at 3084-3092 cm^{-1} indicating that the triazole Schiff base remains in thione form [47]. The strong absorption at 814 cm^{-1} is due to the stretching vibration of the (C-Cl) group.

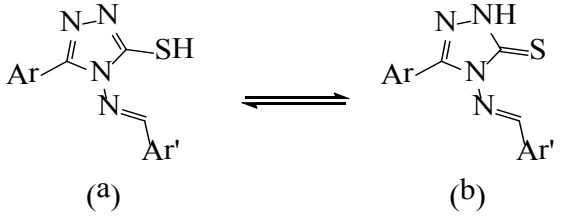


Figure 2: *Tautometric forms of Schiff base (a) thiol and (b) thione*

^{1}H-NMR analysis

In ^{1}H-NMR spectra of **5a-d**, the absence of signals approximately at δ_H 5.76 ppm (NH_2) and the presence of a sharp azomethine group (H–C=N) singlet at δ_H 9.741-9.508 ppm demonstrate the formation of Schiff bases. [48]. The lack of the exchangeable SH signal c.a. 4.0 ppm indicated the predominance of the thione tautomer in DMSO-d_6 [49]. A singlet appeared in the downfield region at δ_H 10.145 and 10.255 ppm in the ^{1}H-NMR spectra of **5c** and **5d** respectively corresponded to the phenolic OH, proton [50]. The signals for the aromatic ring protons appeared at their usual chemical shifts with δ_H 6.741–7.989 ppm [51]. Besides, -OCH_3 group of compound **5c** and **5d** resonated at 3.833 and 3.835 ppm respectively integrating three protons as a singlet. [52].

^{13}C-NMR analysis

The ^{13}C-NMR signals at δ_C 134.0 ppm (**5a**), 138.8 ppm (**5b**), 155.0 ppm (**5c**), and 159.5 ppm (**5d**) due to the azomethine carbon confirm the formation of Schiff bases [53]. Moreover, a downfield chemical shift at δ_C 179.9-178.8 ppm indicates the existence of the C=S group and thus the predominance of the thione tautomer in solution [54]. The aromatic carbons of the phenyl rings gave signals between δ_C 112.1–152.2 ppm while those of the furanyl ring gave signals between δ_C 111.5–149.0 ppm [55]. In **5b** and **5d** the signal at 46.0 ppm i.e. in the aliphatic region is attributable to the methoxy carbon (OCH_3) [52].

Antimicrobial screening

The antimicrobial screening revealed that the tested compounds showed moderate to good activity at the concentrations of 1% and 3% in DMSO.

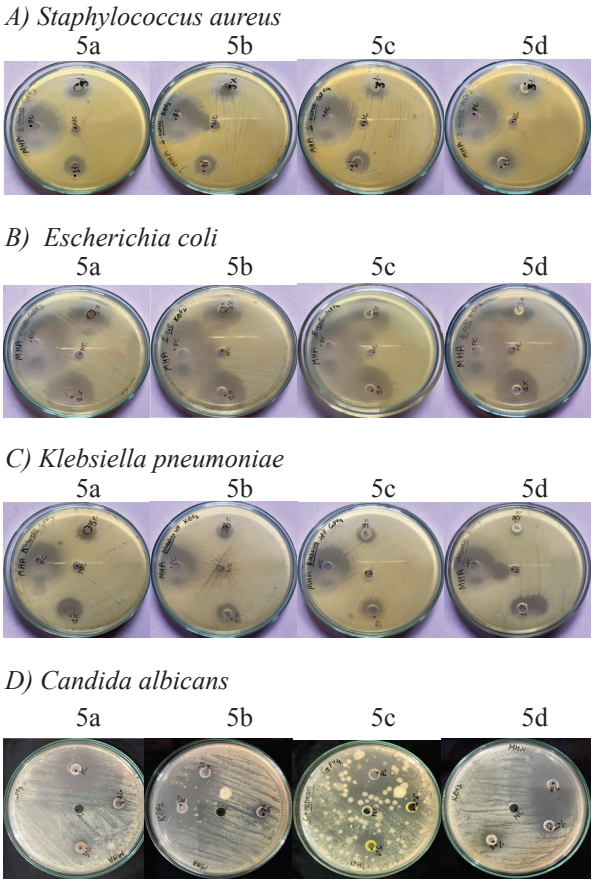


Figure 3: *Antimicrobial activities of synthesized compounds against (A) S. aureus (B) E. coli (C) K. pneumonia, and (D) C. albicans*

Table 1: *Antimicrobial activities of synthesized compounds and the reference antibiotics*

Sample Code	Concentration (%)	Diameter of Inhibition Zone (mm)			
		S. aureus	*E. coli*	*K. pneumoniae*	*C. albicans*
5a	1	9	17	10	4
	3	8	16	8	5
5b	1	7	16	9	6
	3	7	14	7	7
5c	1	8	14	8	6
	3	6	12	7	8
5d	1	6	12	8	7
	3	5	7	3	9
Ciprofloxacin	3	23	30	21	-
Ketoconazole	3	-	-	-	24

The compounds showed comparatively good activity against Gram-negative bacterial strains compared to the ciprofloxacin reference.

The results are summarized in Table 1.

All four Schiff bases showed promising antibacterial activity against *E. coli* than other bacterial strains. Based on the zone of inhibition produced against the tested bacterial species, compound **5b** showed was found to be more effective than other compounds (**5a**, **5c**, and **5d**).

In the case of antifungal activity, all the synthesized compounds exhibited moderate activity against a fungal strain *Candida albicans* (yeast) compared to the standard drug ketoconazol. The compound **5d** showed more potent activity than other compounds (**5a**, **5b**, and **5c**) against a fungal strain.

Compounds **5a** and **5c** with 2-chlorphenyl group at C-5 showed greater antibacterial activity than compounds **5b** and **5d** containing 2,4-dichlorophenyl group. The addition of electron-withdrawing Cl atom on the phenyl ring at C-5 therefore tends to decrease antibacterial activity [56]. On the other hand, Compounds **5a** and **5b** with a furanyl group on azomethine carbon (–CH=N–) showed more antibacterial activity compared to compounds **5c** and **5d** with a 4-hydroxy-3-methoxyphenyl group. The bulkiness of group on the azomethine carbon tends to decrease antibacterial activity. In the case of antifungal activity, the observations were just reverse. The results are in agreement with the observation that electron density of substituent at the C-5 position of the 1,2,4-triazole nucleus and on the azomethine carbon determine the antibacterial and antifungal activities [57]. The small difference in the antimicrobial activity of synthesized compounds suggests that the triazole nucleus may be playing an important role in the microbial inhibition.

Conclusion

The four new novel 1,2,4-triazole derivatives (**5a**, **5b, 5c** & **5d**) were prepared successfully in the laboratory. All the synthesized compounds were obtained in good yield. The spectroscopic techniques (UV, FT-IR, ^{1}H-NMR, and ^{13}C-NMR) confirmed the structure of the newly-synthesized derivatives. The synthesized compounds act as better antibacterial agents than as antifungal agents. The compound **5a** showed prominent bacterial inhibition whereas compound **5d** exhibited good fungal inhibition among the synthesized compounds. The increase in electron deficiency of the phenyl group at C-5 tends to decrease the antibacterial activity, while it tends to enhance the antifungal activity. Moreover, the bulkiness of substituent at azomethine carbon also tends to decrease the antibacterial activity, but increase the antifungal activity.

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