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Synthesis, molecular docking, and biological evaluation of Schiff base hybrids of 1,2,4-triazole-pyridine as dihydrofolate reductase inhibitors

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ABSTRACT

In this study novel derivatives of 1,2,4-triazole pyridine coupled with Schiff base were obtained in altered aromatic aldehyde and 4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine reactions. Thin layer chromatography and melting point determination were employed to verify the purity of hybrid derivatives. The structures of the hybrid derivatives were interpreted using methods comprising infrared, nuclear magnetic resonance, and mass spectroscopy. The *in vitro* anti-microbial properties and minimum inhibitory concentration were determined with Gram-positive and Gram-negative bacteria. Among the derivatives produced, two derivatives comprising *(Z)-2-((4-((5-(pyridine*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methyl)phenol*and *(Z)-2-methoxy-5-((4-((5-(pyridine*-3-yl*)-4H-1,2,4-triazol-3- ylthio)methyl)phenylimino)methyl)phenol* obtained promising results as antibacterial agents. After synthesizing different derivatives, docking studies were performed and the scores range from −10.3154 to −12.962 kcal/mol.

1. Introduction

The preparation of 1,2,4-triazole and its biotic evaluation have facilitated the development of novel potent triazole derivatives (Chen et al., 2008; Bayrak et al., 2010; Agarwal et al., 2011). The established analogs of 1,2,4-triazole with diverse pharmacological properties, including analgesic, anti-inflammatory, anticancer, antihypertensive, anticonvulsant, and antiviral activities, have attracted much attention (Tozkoparan et al., 2007; Mhasalkar et al., 1970; Przegalinski and Lewandowska, 1979; Langley and Clissold, 1988; Kelley et al., 1995; Kumar et al., 2010; El-Nassan, 2011; El Sayed Aly et al., 2015; Hassan et al., 2020; Pagniez et al., 2020; Aly et al., 2020). Hybrids were obtained with a substituted benzyl group where, 5-mercapto-3-pyridyl-1,2,4-triazole was reacted to link the 1,2,4-triazole moiety with a pyridine ring. These hybrids of 1,2,4-triazole pyridine were shown to be active against Gram-negative and Gram-positive-bacteria. In particular, good activities against Gram-negative and Gram-positive bacteria were determined for the derivatives *3-(5-(2-bromobenzylthio)-4H-1,2,4-triazol*-3-yl*)pyridine* and *3-(5-(2,4-dibromobenzylthio)-4H-1,2,4-triazol*-3-yl*)pyridine*; in our previous study (Ahirwar et al., 2018).

Previous studies have also shown that Schiff bases have a broad range of biotic properties, including anticancer, antioxidant, and anti-inflammatory, activities (Nadia et al., 2017; Yasemin et al., 2016). Therefore, we hypothesized that including Schiff bases in hybrids with 1,2,4-triazole pyridine might allow the synthesis of derivatives with improved biological activities. Thus, the main aims of the present study were to obtain a novel bioactive series of 1,2,4-triazole Schiff bases with hybrids of pyridine and to assess their potential biotic activities.

As part of our ongoing research into hybrids derivatives, we synthesized a series of novel 1,2,4-triazole, and pyridine hybrids combined together with Schiff bases by reacting 4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine with different aromatic aldehydes to produce potent antimicrobial derivatives. *In-silico* investigations against dihydrofolate reductase(DHFR) were also performed to verify the antimicrobial activities. The residual interaction of the ligand with the receptor was visualized using DiscoveryStudiosoftware.

1.1. Experimental

Melting point determination was performed using an open capillary procedure followed by thin layer chromatography to check the purity of the compounds obtained (Dewangan et al., 2010, 2011).Fourier

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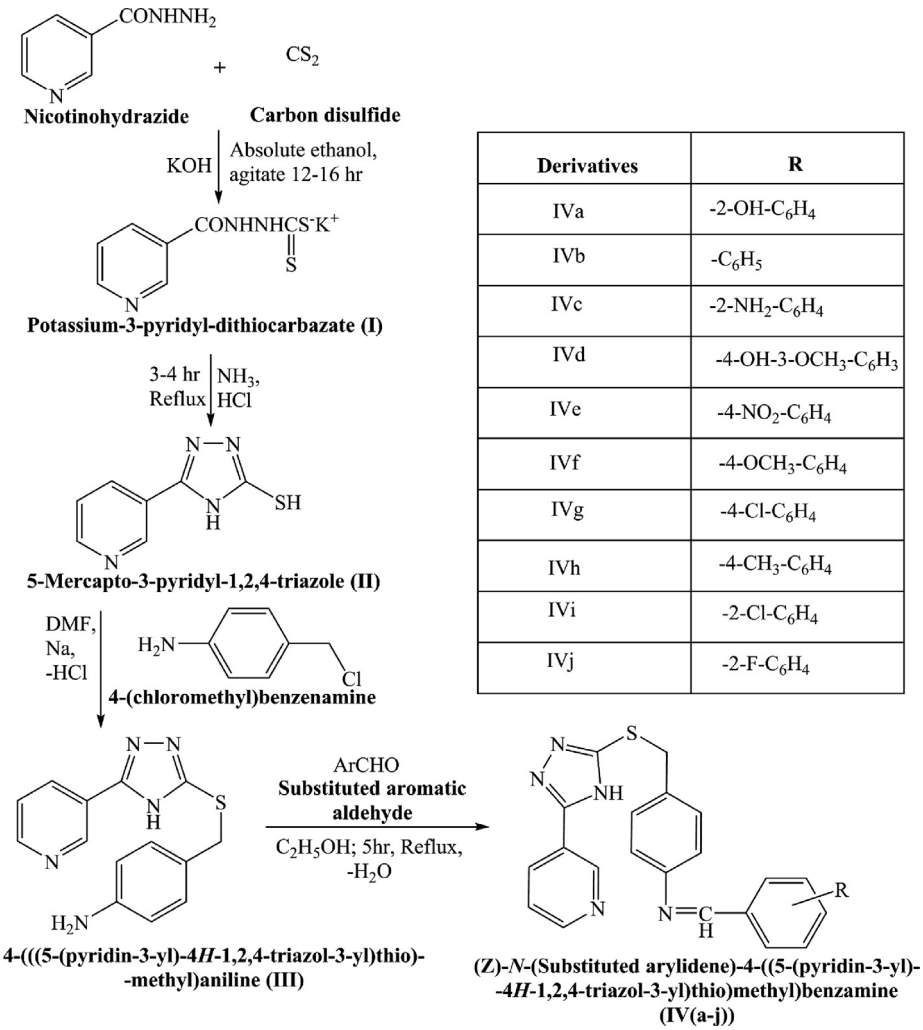

Derivatives	R
IVa	-2-OH-C_6H_4
IVb	-C_6H_5
IVc	-2-NH_2-C_6H_4
IVd	-4-OH-3-OCH_3-C_6H_3
IVe	-4-NO_2-C_6H_4
IVf	-4-OCH_3-C_6H_4
IVg	-4-Cl-C_6H_4
IVh	-4-CH_3-C_6H_4
IVi	-2-Cl-C_6H_4
IVj	-2-F-C_6H_4

Fig. 1. Scheme for the preparation of various 1, 2, 4-triazole pyridine hybrids Schiff base.

transform-infrared (IR) spectra were obtained using KBR pellets, with a PerkinElmer IR instrument (specific at ion number 283).^{1}H nuclear magnetic resonance (NMR) spectral peaks were recorded using a Bruker spectrometer that operated at300MHz.Mass spectra were obtained with an API 3000LC-MS system. The *in vitro* antimicrobial properties of the compounds (IVa–IVj) were determined using the disk diffusion method. The test procedure employed six specific bacterial strains, with three Gram-positive bacteria and three Gram-negative bacteria. The codes for the Gram-positive bacterial strains comprising *Staphylococcusaureus, Streptococcuspyogenes*, and *Enterococcusfaecalis* were MTCC96,MTCC442, andMTCC439,respectively.The codes for the Gram-negative bacterial strains comprising *Escherichiacoli, Pseudomonasaeruginosa*, and *Acinetobacterbaumannii* were MTCC443,MTCC424, and MTCC1425,respectively (Badwaik et al., 2011; Rajput et al., 2011).The standard drug methotrexate was used as a reference drug to assess the inhibitory effect based on the zone of inhibition. Micro-dilution susceptibility was employed to determine the minimum inhibitory concentrations (MICs) for the established compounds (Dewangan et al., 2019).

1.2. Method for synthesizing potassium-3-pyridyl-dithiocarbazate(I):

Potassium hydroxide solution at 0.15M (8.4g), absolute ethanol (200 mL), and pyridyl-2-carbohydrazide 0.10M (13.7g) were mixed and reacted by adding carbondisulfide at 0.15M(11.4g). Next, 150 mL of ethanol was added to the mixture, before diluting. After dilution, agitation was applied for 12–16h. After16h, 200 mL of dry ether was added to the resulting solution, before drying at 65 °C. The final product was used without further purification in the next step.

1.3. Method for synthesizing 5-mercapto-3-pyridyl-1,2,4-triazole(II):

First, 24g of mixture **I** (0.096M), 20 mL of 95% ammonia (0.864M), and 40 mL of distilled water were mixed and refluxed for 3–4 h, followed by stirring.After 3–4h, the mixture obtained was a yellow-colored solution. A white solid precipitate was obtained when the mixture was added to ice-cold water (100 mL) and hydrochloric acid (concentrated).The white solid was then filtered through a filter paper and dried. Recrystallization was conducted when the precipitate was completely dry.

1.4. Method for synthesizing 4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine(III):

First, compound **II** at 0.006M, anhydrous N,N-dimethylformamide at 6M(0.69g), and sodium solution at 6M (0.14g) were mixed in 2 mL of anhydrous methanol. The resulting mixture was stirred at room temperature for 10min and 4-(chloromethyl) benzenamine at 6M were then added. The resulting suspension was again stirred for 1–2h at room temperature with a $CaCl_2$ guard tube. Thin layer chromatography confirmed that there action was completed.

Table 1
Characterization data of synthesized Schiff bases of 1,2,4-triazole derivatives.

Compounds	R_1	Molecular formula	Molecular weight	Melting point (°C)	Appearance	Retention factor	Solubility	% yield (w/w)	λ max (nm)	Chemical Name
IVa	-2-OH-C_6H_4	$C_{21}H_{17}N_5OS$	387.46	215	Pale Yellow Solid	0.70	DMF	78.02	311	(Z)-2-((4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl) phenylimino)methyl)phenol
IVb	-C_6H_5	$C_{21}H_{17}N_5S$	371.46	206	Dark Brown	0.61	Ethanol	81.63	302	(Z)-N-benzylidene-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio) methyl)benzenamine
IVc	-2-NH_2-C_6H_4	$C_{21}H_{18}N_6S$	386.47	212	Yellow Solid	0.65	DMF	73.84	307	(Z)-2-((4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl) phenylimino)methyl)benzenamine
IVd	-4-OH-3-OCH_3-C_6H_3	$C_{22}H_{19}N_5O_2S$	417.48	237	Creamy White Solid	0.87	DMF	62.74	332	(Z)-2-methoxy-5-((4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio) methyl)phenylimino)methyl)phenol
IVe	-4-NO_2-C_6H_4	$C_{21}H_{16}N_6O_2S$	416.46	232	White Solid	0.82	DMF	81.26	330	(Z)-N-(4-nitrobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine
IVf	-4-OCH_3-C_6H_4	$C_{22}H_{19}N_5OS$	401.48	225	Creamy White	0.78	Ethanol	65.36	318	(Z)-N-(4-methoxybenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine
IVg	-4-Cl-C_6H_4	$C_{21}H_{16}ClN_5S$	405.9	230	Light Brown	0.81	Ethanol	73.58	328	(Z)-N-(4-chlorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine
IVh	-4-CH_3-C_6H_4	$C_{22}H_{19}N_5S$	385.48	211	Yellow Solid	0.62	DMF	72.84	305	(Z)-N-(4-methylbenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine
IVi	-2-Cl-C_6H_4	$C_{21}H_{16}ClN_5S$	405.9	228	Light Brown Solid	0.79	Ethanol	82.16	321	(Z)-N-(2-chlorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine
IVj	-2-F-C_6H_4	$C_{21}H_{16}FN_5S$	389.45	220	Creamy White Solid	072	Ethanol	63.74	315	(Z)-N-(2-fluorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine

1.5. Method for synthesizing (Z)-N-(substitutedarylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine(Schiffbases) (IV)

In a round-bottomed flask,4-*((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine***(III)** (0.01M) was diluted in 20 mL of ethanol. Next, aromatic aldehyde (0.1M) and 15 mL of ethanol were added to the solution, before refluxing for atleast 5–6h under reduced pressure. The volume of ethanol was reduced by half and the resulting solution was then placed over crushed ice. The precipitate obtained was then separated, dried, and recrystallized using ethanol.

1.6. In-silico/molecular docking approach:

Molecular docking studies conducted with Argus Lab version 4.0 software. Molecular docking was used to predict the interaction between the ligand and target protein. The protein targets in *Escherichiacoli* and *Lactobacillus* (4DFR) were obtained from the Protein Data Bank (PDB).The two-dimensional and three-dimensional structures of the molecules were generated using Chem Office version 10.0 software. Each of the lowest energy conformers of the new analogs were docked in the DHFR binding domain using the free available Discovery Studio software to determine the interaction between the protein and the ligand.

Table 2
Combustion analysis of synthesized Schiff bases of 1,2,4-triazole derivatives.

Compounds	Combustion Analysis	
	Theoretical Value (%)	Observed Values
IVa	C(65.10) H(4.42) N(18.08) O(4.13) S(8.28)	C(65.30) H(4.41) N(18.15) O(4.15) S(8.25)
IVb	C(67.90) H(4.61) N(18.85) S(8.63)	C(68.12) H(4.60) N(18.92) S(8.60)
IVc	C(65.26) H(4.69) N(21.75) S(8.30)	C(65.09) H(4.70) N(21.68) S(8.28)
IVd	C(63.29) H(4.59) N(16.78) O(7.66) S(7.68)	C(63.06) H(4.61) N(16.84) O(7.64) S(7.70)
IVe	C(60.56) H(3.87) N(20.18) O(7.68) S(7.70)	C(60.72) H(3.88) N(20.10) O(7.66) S(7.73)
IVf	C(65.81) H(4.77) N(17.44) O(3.99) S(7.99)	C(66.02) H(4.79) N(17.51) O(4.01) S(8.01)
IVg	C(62.14) H(3.97) Cl(8.73) N(17.25) S(7.90)	C(62.30) H(3.98) Cl(8.76) N(17.34) S(7.87)
IVh	C(62.14) H(3.97) Cl(8.73) N(17.25) S(7.90)	C(62.29) H(3.96) Cl(8.71) N(17.28) S(7.88)
IVi	C(62.14) H(3.97) Cl(8.73) N(17.25) S(7.90)	C(62.31) H(3.98) Cl(8.76) N(17.18) S(7.92)
IVj	C(64.76) H(4.14) F(4.88) N(17.98) S(8.23)	C(64.97) H(4.16) F(4.88) N(17.98) S(8.23)

2. Results and discussion

In this study, we determined the antimicrobial activities of 1,2,4-triazole pyridine Schiff base hybrids. The physical parameters of the established Schiff base derivatives were evaluated using techniques such as combustion analysis, thin layer chromatography, and IR, NMR, and mass spectroscopy.The *invitro* anti-microbial activities were assessed using Gram-positive and Gram-negative bacterial strains.

A scheme illustrating the synthesis of the various1,2,4-triazole pyridine Schiff base hybrids is shown in Fig. 1. In total, 11 different Schiff bases were prepared by treating *4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine* with aromatic aldehyde.ArgusLab version 4 was used to conduct docking studies. Different docking parameters were set to obtain the dockings cores, as shown in Table 1 (Supplementary Material).The structural properties of the ligands are illustratedin Table 2 (Supplementary Material) and the chemical properties of the ligands are shown in Table 3 (Supplementary Material). The binding affinities of the standard drug and ligands with DHFR (4DFR) are shown in Table 4 (Supplementary Material). The parameters obtained for the derivatives comprising the melting points, chemical and physical structural properties, and combustion analysis results are presented in Table 1 and Table 2, respectively. The IR, ^{1}HNMR, and MS spectral peaks were used

Table 3
Spectral data of synthesized Schiff bases of 1,2,4-triazole derivatives.

Compounds	IR (KBr cm^{-1})	1H NMR δ (ppm) (DMSO-d_6)	MS
IVa	2978.85(Ar-C-H str), 1630.41(Ar-C=C str), 1154.87 (Ar–C–C str), 1595.76(C=Nstr), 1252.41(-C-N- str), 657.11(-C-S str), 735.83(C-Cl str)	6.76–7.45 (m 8H, Ar-H), 4.21 (s 2H, -CH_2), 7.44–8.55(m 4H, pyridine ring), 8.40(s 1H, imine)	386.74^+
IVb	3088.25(Ar-C-H str), 1610.41(Ar-C=C str), 1173.78 (Ar–C–C str), 1542.56(C=Nstr), 1200.41(-C-N- str), 647.12(-C-S str), 717.33(C-Cl str)	7.10–8.29 (m 9H, Ar-H), 4.20 (s 2H, -CH_2), 7.42–8.82(m 4H, pyridine ring), 8.42(s 1H, imine)	370.95^+
IVc	3108.64(Ar-C-H str), 1684.40(Ar-C=C str), 1112.08 (Ar–C–C str), 1521.24(C=Nstr), 1221.56(-C-N- str), 612.41.11(-C-S str), 712.98(C-Cl str)	6.48–7.39 (m 8H, Ar-H), 4.19 (s 2H, -CH_2), 7.44–8.81(m 4H, pyridine ring), 8.39(s 1H, imine), 4.1(s 2H, -NH_2)	385.23^+
IVd	2968.46(Ar-C-H str), 1598.35 (Ar-C=C str), 1175.47 (Ar–C–C str), 1500.36(C=Nstr), 1285.47(-C-N- str), 611.81(-C-S str), 765.79(C-Cl str)	6.65–7.12 (m 7H, Ar-H), 4.21 (s 2H, -CH_2), 7.44–8.81(m 4H, pyridine ring), 8.34(s 1H, imine), 5.1(s 1H, -OH), 3.73(s 3H, -OCH_3)	416.93^+
IVe	2912.56(Ar-C-H str), 1623.56(Ar-C=C str), 1121.67(Ar–C–C str), 1521.12(C=Nstr), 1213.87(-C-N- str), 641.78(-C-S str), 735.13(C-Cl str)	7.12–8.23 (m 8H, Ar-H), 4.10 (s 1H, -CH_2), 7.44–8.82 (m 4H, pyridine ring), 8.39(s 1H, imine)	415.28^+
IVf	2890.45(Ar-C-H str), 1611.76(Ar-C=C str), 1108.45(Ar–C–C str), 1541.10(C=Nstr), 1286.45(-C-N- str), 698.34(-C-S str), 812.12(C-Br str)	6.80–7.52 (m 8H, Ar-H), 4.16 (s 2H, -CH_2), 7.44–8.82 (m 4H, pyridine ring), 3.75(s 1H, -OCH_3), 8.35(s 1H, imine)	400.93^+
IVg	3134.78(Ar-C-H str), 1652.89(Ar-C=C str), 1146.89(Ar–C–C str), 1511.21(C=Nstr), 1264.76(-C-N- str), 698.98(-C-S str), 842.45(C-Br str)	7.12–7.56 (m 8H, Ar-H), 4.19 (s 2H, -CH_2), 7.42–8.81 (m 4H, pyridine ring), 8.38(s 1H, imine)	404.28^+
IVh	3078.45(Ar-C-H str), 1662.67(Ar-C=C str), 1109.78(Ar–C–C str), 1500.90(C=Nstr), 1210.43(-C-N- str), 690.56(-C-S str), 832.56(C-Br str)	7.09–7.51 (m 8H, Ar-H), 4.20 (s 2H, -CH_2), 7.42–8.84 (m 4H, pyridine ring), 8.35(s 1H, imine), 2.42(s 3H, -CH_3)	384.17^+
IVi	2910.56Ar-C-H str), 1623.56(Ar-C=C str), 1101.67(Ar–C–C str), 1561.12(C=Nstr), 1210.87(-C-N- str), 691.78(-C-S str), 842.12(C-Br str)	7.10–7.56 (m 8H, Ar-H), 4.16 (s 2H, -CH_2), 8.35(s 1H, imine), 7.44–8.58 (m 4H, pyridine ring)	404.37^+
IVj	2922.12(Ar-C-H str), 1615.45(Ar-C=C str), 1135.60(Ar–C–C str), 1515.10(C=Nstr), 1235.10(-C-N- str), 690.80(-C-S str), 820.90(C-Br str)	7.02–7.61 (m 8H, Ar-H), 4.24 (s 2H, -CH_2), 7.40–8.84 (m 4H, pyridine ring), 8.41(s 1H, imine)	388.26^+

to assess the structures formed in the different*3-(5-(substituted-benzylthio)-4H-1,2,4-triazol*-3-yl)*pyridine*derivatives.

The IR spectrum obtained for *(Z)-2-methoxy-5-((4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methyl)phenol* contained a strong C=N stretching band at 1500.36 cm^{-1} and a C-N absorption band at1285.47 cm^{-1}, which indicated the closure of the 1,2,4-triazole ring. The absorption band at 2968.46 cm^{-1}, C=C stretching band at 1598.35 cm^{-1}, C-Cl stretching at 765.79 cm^{-1}, and C-S stretching band at 611.81 cm^{-1} demonstrated the existence of aromatic C-H stretching. The strong absorption around 3078.85 cm^{-1} and 620.47 cm^{-1} found in all of the final derivatives indicated the existence of aromatic C-Hand C=C bonds, respectively. The specific functional groups were confirmed by the ^{1}HNMR data obtained for the final synthesized derivatives.The^{1}HNMR spectrum obtained for *(Z)-2-methoxy-5-((4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3ylthio)methyl)phenylimino)methyl)phenol* contained peaks for seven aromatic protons in the region of 6.65–7.12, for four pyridine protons from 7.44 to 8.81, and for two methylene protons at 4.21.The shift value of 5.1 in the spectra for the synthesized derivatives confirmed the presence of an –OH group. The mass spectra obtained for *Z)-2-methoxy-5-((4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methyl)phenol* contained a peak at *m/z* 416^+ and this agreed with the molecular formula for $C_{22}H_{19}N_5O_2S$. The spectral data obtained for the other derivatives are shown in Table 3.

All of the hybrid derivatives of 1,2,4-triazole pyridine were tested to assess their antibacterial activities at a concentration of 10 mg/mL based on the zone of inhibition. The antibacterial activity assessments showed that the zones of inhibition obtained for the synthesized derivatives ranged from 5.5 to 13 mm against Gram-positive and Gram-negative bacteria, and the standard drug methotrexate produced a zone of inhibition that ranged from 10 to 14 mm.The results also showed that the synthesized derivatives had very weak antifungal activities as shown in Table 4. The MIC values obtained for all of the synthesized compounds are shown in Table 5.

Among the synthesized derivatives, the two derivatives comprising *(Z)-2-((4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methyl)phenol* and *(Z)-2-methoxy-5-((4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methyl)phenol* had the most potent antibacterial activities (Fig. 2).Compounds that contain a triazole nucleus have good pharmacological activities. Molecular modifications of the triazole nucleus at position-3 and position-5 have significant effects, and these sites are important for determining the pharmacologically significant activities of synthesized triazole derivatives.

In the present study, triazole derivatives were developed by modification at position 2 in the triazole ring and their potencies were compared with those of our previously synthesized 1,2,4-triazole derivatives (Hassan et al., 2020). We synthesized analogs of 1,2,4-triazole--pyridine hybrids modified with Schiff bases. Most Schiff bases are active against Gram-positive and Gram-negative bacteria and the zones of inhibition range from 12 to 13 mm at a concentration of 10 mg/mL. Our results suggested that the 1,2,4-triazole pyridine hybrids produced with Schiff bases had significantly higher antibacterial activities compared with 1,2,4-triazole-pyridine hybrids. Thus, the addition of Schiff bases enhanced the biotic activities of the 1,2,4-triazole-pyridine hybrids.

The 1,2,4-triazole pyridine Schiff base hybrids produced in this study were highly effective against Gram-positive and Gram-negative bacteria with 80–90% efficacy, and their antibacterial activities were comparable to that of the standard drug methotrexate.

2.1. Insilico/docking study

The potencies of the synthesized compounds were evaluated further in docking studies. The structure of 4DFR (in *Escherichia coli* and *Lactobacillus*) in the DHFR protein was obtained from PDB (RCSB) and the binding affinity was calculated in the docking study. The receptor complex and standard drug binding energies are shown in Table 2.

The inhibition of the DHFR(4DFR) enzyme was indicated by the higher affinity of the synthesized molecules for binding with the protein, as shown by the higher binding energies in Table 2. The interactions between the DHFR enzyme and all of the synthesized molecules were strong due to various bonds, *i.e.*, carbon-hydrogen bonds, hydrogen bonds, van Der Waals forces, p-sigma, p-sulfur, p-donor hydrogen, p-alkyl, p-sigma, p-anion, and p-cation. The synthesized derivatives had binding energy values in the range from −10.3154 to–12.7962 kcal/mol, which are greater than the binding energy of the standard drug methotrexate. The different amino acids in the receptor bound with the pyridine and nitrogen atoms in the triazole ring in the synthesized

Table 4
Anti –microbial activity of synthesized Schiff bases of 1,2,4-triazole derivatives using plate hole diffusion method(10 mg/mL).

Compounds	Zone of inhibition (mm)							
	Antibacterial activity						**Antifungal activity**	
	Gram-ve bacteria			**Gram + ve bacteria**				
	E.coli	***P. aeruginosa***	***A. baumannii***	***S. aureus***	***S. pyrogenes***	***E. faecalis***	***A. clavatus***	***C. albicans***
IVa	12	12	10	12	12	9	9	8
IVb	12	9	7	11	11	–	–	5
IVc	11	10	12	12	12	7	–	–
IVd	13	12	11	12	13	8	8	7
IVe	11	7	6.5	11	10	6.5	–	–
IVf	10	10	–	12	11.5	8	–	6
IVg	12	9	5.5	12	9	7	–	–
IVh	12	10	12	10	10	8	9	–
IVi	10	12	8	12	11	7	–	8
IVj	8	11	7	11	10	7.5	–	–
DMSO	–	–	–	–	–	–	–	–
Mithotrixate	14	13	12	13	14	10	–	–
Fluconazole	–	–	–	–	–	–	12	12

#Diameter of zone of inhibition expressed in mm.

Table 5
Antimicrobial activity of the synthesized Schiff bases of 1,2,4- triazole derivatives expressed as MIC (mg/mL).

Compounds	MIC (mg/mL)							
	Antibacterial activity						Antifungal activity	
	Gram-ve bacteria			Gram + ve bacteria				
	E.coli	*P. aeruginosa*	*A. baumannii*	*S. aureus*	*S. pyrogenes*	*E. faecalis*	*A. clavatus*	*C. albicans*
IVa	0.99	0.98	1.78	1	0.99	1.25	1.45	1.50
IVb	1.65	1.80	2.96	1.65	1.55	15.7	7.5	3.2
IVc	1.23	1.53	2.02	1.95	1.75	2.9	18.1	20.4
IVd	0.90	0.97	1.26	0.98	0.92	3.1	1.9	2.1
IVe	1.31	2.89	3.46	1.45	1.95	3.5	18.0	14.5
IVf	1.88	1.93	10.03	1.75	1.45	2.75	14.1	18.9
IVg	1.34	1.58	4.09	1.75	1.45	2.45	13.0	14.8
IVh	1.65	1.82	2.45	1.87	1.88	2.12	2.10	17.5
IVi	1.55	2.21	2.45	1.45	1.30	2.5	14.1	1.65
IVj	2.23	1.48	2.80	1.45	1.90	2.8	20.5	21.5
DMSO	–	–	–	–	–	–	–	–
Mithotrixate	0.8	0.82	0.95	0.96	0.8	1.25	NT	NT
Fluconazole	NT	NT	NT	NT	NT	NT	1.10	1.20

NT = Not Tested.

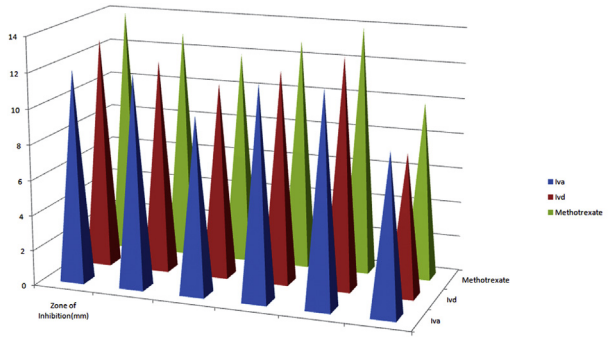

Fig. 2. Comparative antibacterial evaluation of compound IVa and IVd with Methotrexate.

compounds (Table 2). For compounds Iva and IVd, amino acid residues 4 and 5 in the DHFR enzyme were similar to the 15 amino acid residues responsible for the formation of bonds with methotrexate. The binding interactions between compounds Iva and IVd with DHFR (4DFR) are shown as two-dimensional and three-dimensional representations in Figs. 3 and 4. The differences in the biological activities of the test compounds could be explained based on the interactions with particular amino acid residues and the alignment with the receptor binding pocket despite their similar chemical structures according to the docking study results. The binding pocket alignments of compounds Iva and IVd were similar to that for the standard drug methotrexate, and their antibacterial activities were also similar to that of methotrexate.

3. Conclusion

In this study, we synthesized a novel series of Schiff bases by reacting *4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine* with an aromatic aldehyde. Chromatographic and spectroscopic techniques were used to determine the physical and chemical properties of the newly synthesized 1,2,4-triazole derivatives. Antimicrobial screening showed that two compounds comprising *(Z)-2-((4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methyl)phenol* and *(Z)-2-methoxy-5-((4-((5-(pyridin*-3-yl*)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino) methyl)phenol* had greater antibacterial activities compared with the other derivatives. Docking analysis also demonstrated that the activities of the synthesized compounds against bacteria were due to inhibition of the DHFR enzyme. The results obtained in this study may facilitate the development of novel antibacterial analogs in the future.

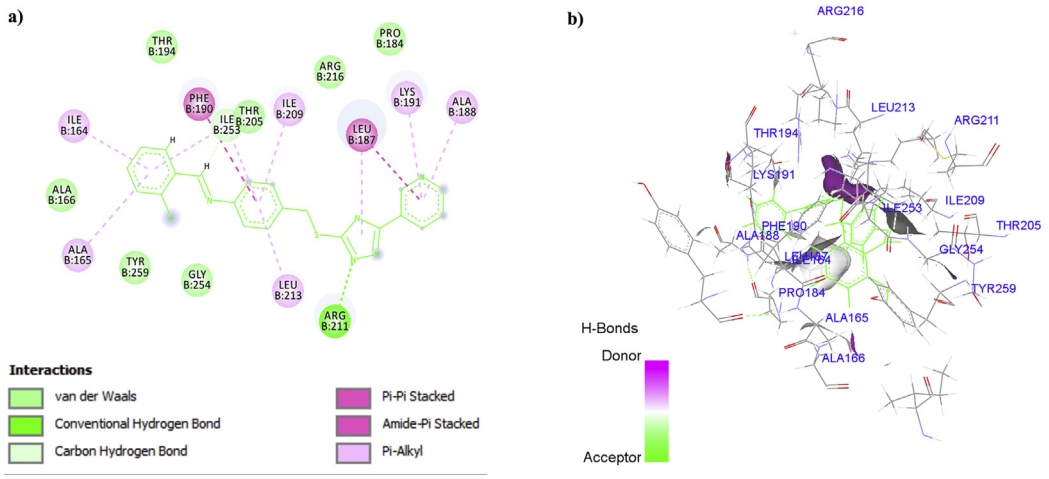

Fig. 3. Binding interaction of compound IVa with dihydrofolate reductase enzyme (4DFR): a) 2D representation, b) 3D representation.

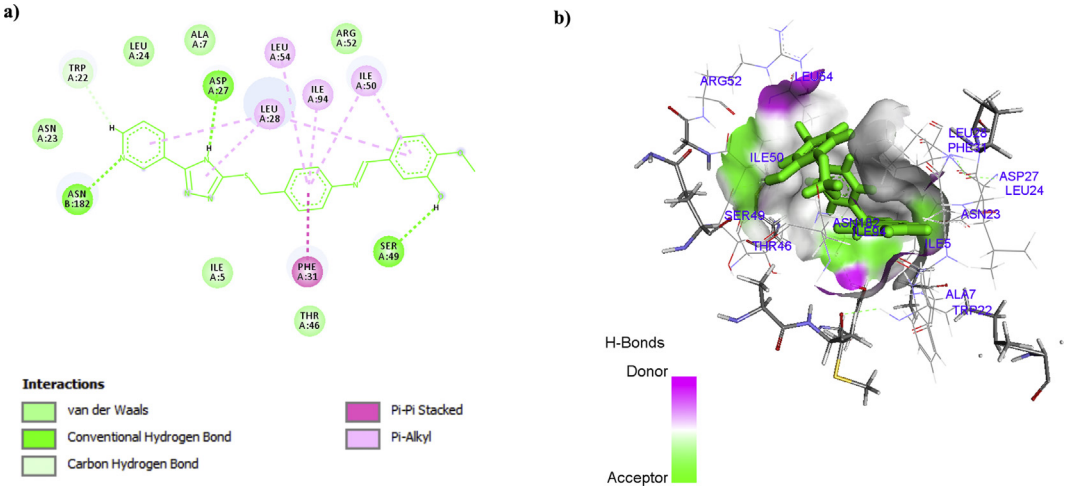

Fig. 4. Binding interaction of compound IVd with dihydrofolate reductase enzyme (4DFR): a)2D representation, b) 3D representation.

CRediT authorship contribution statement

D. Dewangan: Conceptualization, Methodology, Software, Validation, Writing – original draft. **Y. Vaishnav:** Formal analysis, Writing – review & editing. **A. Mishra:** Investigation, Supervision. **A.K. Jha:** Resources. **S. Verma:** Data curation. **H. Badwaik:** Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crphar.2021.100024.

References

Agarwal, N., Kumar, R., Dureja, P., Khurana, J.M., 2011. Synthesis, antimicrobial evaluation and QSAR analysis of novel nalidixic acid based 1,2,4-triazole derivatives. Eur. J. Med. Chem. 46 (9), 4089–4099. https://doi.org/10.1016/j.ejmech.2011.06.009.

Ahirwar, J., Ahirwar, D., Lanjhiyana, S., Jha, A.K., Dewangan, D., Badwaik, H., 2018. Synthesis, characterization, molecular modeling, and biological evaluation of 1,2,4-Triazole-pyridine hybrids as potential antimicrobial agents. J Het Chem 55, 2598–2608. https://doi.org/10.1002/jhet.3319.

Aly, A.A., Hassan, A.A., Makhlouf, M.M., Bräse, S., 2020. Chemistry and biological activities of 1,2,4-triazolethiones—antiviral and anti-infective drugs. Molecules 25 (13), 3036–3090. https://doi.org/10.3390/molecules25133036.

Badwaik, H., Vekatraman, S., Thakur, D., Vinod, M., Raamamurthy, J., Dshmukh, K., 2011. Antibacterial evaluation of novel 2-aryl-quinoline -3- carbaldehyde derivatives. Asian J. Res. Chem. 4 (6), 893–895.

Bayrak, H., Demirbas, A., Demirbas, N., Karaoglu, S.A.M., 2010. Cyclization of some carbothioamide derivatives containing antipyrine and triazole moieties and investigation of their antimicrobial activities. Eur. J. Med. Chem. 45 (11), 4726–4732. https://doi.org/10.1016/j.ejmech.2010.07.018.

Chen, Q., Zhu, X.L., Jiang, L.L., Liu, Z.M., Yang, G.F., 2008. Synthesis, antifungal activity and CoMFA analysis of novel 1,2,4-triazolo[1,5-a]pyrimidine derivatives. Eur. J. Med. Chem. 43 (3), 595–603. https://doi.org/10.1016/j.ejmech.2007.04.021.

Dewangan, D., Pandey, A., Sivakumar, T., Rajavel, R., Dubey, R., 2010. Synthesis of some novel 2, 5- disubstituted 1, 3, 4-oxadiazole and its analgesic, AntiInflammatory, antibacterial and anti-tubercular activity. Int. J. Chem. Res. 2 (3), 1397–1412.

Dewangan, D., Tripathi, D.K., Verma, T., Nagori, K., Shah, V., Rajawel, R., 2011. Synthesis, characterization and anti-inflammatory, analgesic, and antimicrobial activities of substituted 4-(4-oxo-2-phenyl quinazolin-3 (4H)-yl) N-aryl-methylene benzene sulfonamide derivatives. Int. J. Chem. Res. 3 (1), 948–954.

Dewangan, D., Nakhate, K., Mishra, A., Thakur, A., Rajak, H., Dwivedi, J., Sharma, S., Paliwal, S.H., 2019. Design, synthesis, and characterization of quinoxaline

derivatives as a potent antimicrobial agent. J Het Chem 56 (2), 566–578. https://doi.org/10.1002/jhet.3431.

El Sayed Aly, M.R., Saad, H.A., Mohamed, M.A.M., 2015. Click reaction based synthesis, antimicrobial, and cytotoxic activities of new 1,2,3-triazoles. Bioorg. Med. Chem. Lett 25 (14), 2824–2830. https://doi.org/10.1016/j.ejmech.2011.02.055.

El-Nassan, H.B., 2011. Synthesis and antitumor activity of novel pyrido[2,3-d][1,2,4] triazolo[4,3-a]pyrimidin-5-one derivatives. Eur. J. Med. Chem. 46 (6), 2031–2036. https://doi.org/10.1016/j.ejmech.2011.02.055.

Hassan, A., Askar, A.A., M Naglah, A., A Almehizia, A., Ragab, A., 2020. Discovery of new Schiff bases tethered pyrazole moiety: design, synthesis, biological evaluation, and molecular docking study as dual targeting DHFR/DNA gyrase Inhibitors with immunomodulatory activity. Molecules 25 (11), 2593–2616. https://doi.org/10.3390/molecules25112593.

Kelley, J.L., Koble, C.S., Davis, R.G., McLean, E.W., Soroko, F.E., Cooper, B.R., 1995. 1-(Fluorobenzyl)-4-amino-1H-1,2,3-triazolo[4,5-c]pyridines: synthesis and anticonvulsant activity. J. Med. Chem. 38 (4), 4131–4134. https://doi.org/10.1021/jm00020a030.

Kumar, D., Kumar, N.M., Chang, K.H., Shah, K., 2010. Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4-thiadiazoles. Eur. J. Med. Chem. 45 (10), 4664–4674. https://doi.org/10.1016/j.ejmech.2010.07.023.

Langley, M.S., Clissold, S.P., 1988. Brotizolam. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy as an hypnotic. Drugs 35 (2), 104–122. https://doi.org/10.2165/00003495-198835020-00002.

Mhasalkar, M.Y., Shah, M.H., Nikam, S.T., Deliwala, K.G., Ananthanarayana, C.V., 1970. 4-alkyl-5-aryl-aryl-4H-1, 2, 4-triazole-3-thiols as hypoglycemic agents. J. Med. Chem. 13 (4), 672–674. https://doi.org/10.1021/jm00298a021.

Nadia, G.K., Mansoura, M., Hind, M.I., 2017. Synthesis of new Schiff bases bearing 1,2,4-triazole, thiazolidine and chlor-azetidine moieties and their pharmacological evaluation. J. Enzym. Inhib. Med. Chem. 31 (1), 119–129. https://doi.org/10.1080/14756366.2016.1238365.

Pagniez, F., Lebouvier, N., Min Na, Y., Ourliac-Garnier, I., Picot, C., Le Borgne, M., Le Pape, P., 2020. Biological exploration of a novel 1,2,4-triazole-indole hybrid molecule as antifungal agent. J. Enzym. Inhib. Med. Chem. 35 (1), 398–403. https://doi.org/10.1080/14756366.2019.1705292.

Przegalinski, E., Lewandowska, A., 1979. The effect of etoperidone, a new potential antidepressant drug, on the central serotonin system. J. Neural. Transm. 46 (4), 303–312. https://doi.org/10.1007/BF01259336.

Rajput, S., Sisodia, D., Badwaik, H., Thakur, D., Nagori, K., 2011. Synthesis, characterization and antimicrobial activity of a 5(4-(4-Substituted) Aminobenzylidine)Thiazolidine-2,4-dione derivatives. Asian J. Res. Chem. 4 (1), 40–43.

Tozkoparan, B., Kupeli, E., Yesilada, E., Ertan, M., 2007. Preparation of 5-aryl-3-alkylthio-l,2,4-triazoles and corresponding sulfones with antiinflammatory–analgesic activity. Bioorg. Med. Chem. Lett 15 (14), 1808–1814. https://doi.org/10.1016/j.bmc.2006.11.029.

Yasemin, U., Sadik, D., Fatih, C., Zeynep, A., Murat, K., Kemal, S., 2016. Synthesis of new 1,2,4-triazole compounds containing Schiff and Mannich bases (morpholine) with antioxidant and antimicrobial activities. J. Enzym. Inhib. Med. Chem. 31 (53), 89–95. https://doi.org/10.1080/14756366.2016.1206088.