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 (2Z)-2-((4-((5-(pyridine-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methyl)phenol and (2Z)-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 a 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).

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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 at 300MHz. 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 Staphylococcus aureus, Streptococcus pyogenes, and Enterococcus faecalis were MTCC96, MTCC442, and MTCC439, respectively. The codes for the Gram-negative bacterial strains comprising Escherichia coli, Pseudomonas aeruginosa, and Acinetobacter baumannii 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-carboxyhydrazide 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. After 16h, 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)benzeneamine(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) benzeneamine 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.
molecules were generated using Chem Of Lactobacillus (4DFR) were obtained from the Protein Data Bank. The protein targets in software. Molecular docking was used to predict the interaction between the ligand and target protein. The protein targets in DHFR binding domain using the free available Discovery Studio software to obtain the dockings cores, as shown in Table 1 (Supplementary Material). The parameters obtained for the derivatives were found to be: C6H4OCH3- C6H4C21H17N5S 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

Next, aromatic aldehyde (0.01M) was diluted in 20 mL of ethanol. The aldehyde was then placed over crushed ice. The precipitate obtained was then separated, dried, and recrystallized using ethanol.

In a round-bottomed flask, 4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine (II) (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 at least 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.

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

| Compounds | R₁ | Molecular formula | Molecular weight | Melting point (°C) | Appearance | Retention factor | Solubility | % yield (w/w) | λ max (nm) | Chemical Name |
|-----------|----|-------------------|------------------|-------------------|------------|-----------------|------------|--------------|-------------|---------------|
| IVa       | -2-OH- C₆H₄ | C_{21}H_{17}N₅OS | 387.46           | 215               | Pale Yellow Solid | 0.70          | DMF        | 78.02        | 311         | (Z)-2-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methylbenzenamine (Schiff bases) (IV) |
| IVb       | -C₂H₅ | C_{21}H_{18}N₅S | 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-NH₂- C₆H₄ | C_{21}H_{18}N₅S | 386.47           | 212               | Yellow Solid   | 0.65          | DMF        | 73.84        | 307         | (Z)-2-((4-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVd       | -4-OH-3-OCH₃- C₆H₄ | C_{22}H_{19}N₅O₂S | 417.48           | 237               | Creamy White Solid | 0.87          | DMF        | 62.74        | 332         | (Z)-N-(4-methoxybenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVe       | -4-NO₂- C₆H₄ | C_{21}H_{19}N₅O₂S | 416.46           | 232               | White Solid    | 0.82          | DMF        | 81.26        | 330         | (Z)-N-(4-(5-chlorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVf       | -4-OCH₃- C₆H₄ | C_{22}H_{19}N₅S | 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- C₆H₄ | C_{21}H_{19}CIN₅S | 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-CH₃- C₆H₄ | C_{21}H_{19}N₅S | 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- C₆H₄ | C_{21}H_{19}CIN₅S | 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- C₆H₄ | C_{21}H_{19}FN₅S | 389.45           | 220               | Creamy White Solid | 0.72          | Ethanol    | 63.74        | 315         | (Z)-N-(2-fluorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |

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 in vitro anti-microbial activities were assessed using Gram-positive and Gram-negative bacterial strains.

A scheme illustrating the synthesis of various 1,2,4-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 illustrated in 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, ¹H NMR, and MS spectral peaks were used.

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

| Compounds | R₁ | Chemical Name |
|-----------|----|---------------|
| IVa       | -2-OH- C₆H₄ | (Z)-2-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)phenylimino)methylbenzenamine (Schiff bases) (IV) |
| IVb       | -C₂H₅ | (Z)-N-benzylidene-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVc       | -2-NH₂- C₆H₄ | (Z)-2-((4-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVd       | -4-OH-3-OCH₃- C₆H₄ | (Z)-N-(4-methoxybenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVe       | -4-NO₂- C₆H₄ | (Z)-N-(4-(5-chlorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVf       | -4-OCH₃- C₆H₄ | (Z)-N-(4-methylbenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVg       | -4-Cl- C₆H₄ | (Z)-N-(4-chlorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVh       | -4-CH₃- C₆H₄ | (Z)-N-(4-methylbenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVi       | -2-Cl- C₆H₄ | (Z)-N-(2-chlorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
| IVj       | -2-F- C₆H₄ | (Z)-N-(2-fluorobenzylidene)-4-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-ylthio)methyl)benzenamine |
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 peak at m/z 416 \(^+\) and this agreed with the molecular formula for C\(_{33}\)H\(_{26}\)N\(_2\)O\(_3\)S. The spectral data obtained for the other derivatives are shown in Table 3.

| Table 3 | Spectral data of synthesized Schiff bases of 1,2,4-triazole derivatives. |
|---------|-----------------------------------------------------------------------|
| Compounds | IR (KBr cm\(^{-1}\)) | 1H NMR \(\delta\) (ppm) | MS |
|----------|------------------------|---------------------|-----|
| IVa | 2976.85(Ar-C-H str), 1630.41(Ar-C-C str), 1154.87(Ar-C-C str), 1595.76(C=N-C str), 2522.41(C-N str), 657.11(C-S str), 735.83(C-O str) | 6.76-7.45 (m 8H, Ar-H), 386.74 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| Ivb | 3088.25(Ar-C-H str), 1610.41(Ar-C-C str), 1173.79(Ar-C-C str), 1542.56(C-N str), 1200.41(C-N str), 642.75(C-S str), 717.33(C-O str) | 7.10-8.29 (m 9H, Ar-H), 370.95 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVc | 3108.46(Ar-C-H str), 1684.40(Ar-C-C str), 1112.08(Ar-C-C str), 1521.24(Ar-C-N str), 1211.56(C-N str), 612.41(C-S str), 712.98(C-O str) | 6.48-7.39 (m 9H, Ar-H), 385.23 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVd | 2986.46(Ar-C-H str), 1598.35(Ar-C-C str), 1175.47(Ar-C-C str), 1500.36(C-N str), 1285.47(C-N str), 611.80(C-S str), 765.79(C-O str) | 6.76-7.15 (m 7H, Ar-H), 416.93 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVe | 2912.56(Ar-C-H str), 1623.56(Ar-C-C str), 1121.67(Ar-C-C str), 1512.12(Ar-C-N str), 1213.87(C-N str), 641.76(C-S str), 735.13(C-O str) | 7.12-8.27 (m 8H, Ar-H), 415.28 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVf | 2890.45(Ar-C-H str), 1611.76(Ar-C-C str), 1108.45(Ar-C-C str), 1541.10(C-N str), 1286.45(C-N str), 698.34(C-S str), 812.12(C-O str) | 6.80-7.52 (m 8H, Ar-H), 400.93 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVg | 3134.78(Ar-C-H str), 1652.89(Ar-C-C str), 1146.89(Ar-C-C str), 1551.21(Ar-C-N str), 1264.76(C-N str), 698.96(C-S str), 842.45(C-O str) | 7.12-7.56 (m 8H, Ar-H), 404.28 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVh | 3078.45(Ar-C-H str), 1662.67(Ar-C-C str), 1197.08(Ar-C-C str), 1500.90(Ar-C-N str), 1210.43(C-N str), 690.50(C-S str), 832.56(C-O str) | 7.09-7.51 (m 8H, Ar-H), 384.17 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVi | 2910.56(Ar-C-H str), 1623.56(Ar-C-C str), 1101.67(Ar-C-C str), 1561.12(Ar-C-N str), 1210.87(C-N str), 691.76(C-S str), 842.12(C-O str) | 7.10-7.56 (m 8H, Ar-H), 404.37 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |
| IVj | 2922.12(Ar-C-H str), 1615.45(Ar-C-C str), 1135.60(Ar-C-C str), 1515.10(C-N str), 1235.10(C-N str), 690.80(C-S str), 820.00(C-O str) | 7.02-7.61 (m 8H, Ar-H), 388.26 \(^+\) | 1H NMR \(\delta\) (ppm) | MS |

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-alkly, p-sigma, p-anion, and p-cation. The synthesized derivatives had binding energy values in the range from ~10.3154 to ~12.7796 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 derivatives.
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.

| 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            | 8              | 12         | 12         | 8          | 8          | 9          |
| IVc       | 11     | 10           | 12             | 12         | 13         | 7          | 7          | 8          |
| IVd       | 11     | 7            | 6.5            | 11         | 10         | 6.5        | –          | –          |
| IVe       | 10     | 10           | –              | 12         | 11.5       | 8          | –          | –          |
| IVf       | 12     | 9            | 5.5            | 12         | 9          | 7          | –          | –          |
| IVg       | 12     | 10           | 12             | 10         | 10         | 8          | 9          | –          |
| IVh       | 10     | 12           | 8              | 12         | 11         | 7          | –          | 8          |
| IVi       | 8      | 11           | 7              | 11         | 10         | 7.5        | –          | –          |
| DMSO      | –      | –            | –              | –          | –          | –          | –          | –          |
| Mithotrexate | 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      | –       | –            | –             | –          | –          | –          | –          | –          |
| Mithotrexate | 0.8   | 0.82         | 0.95          | 0.96       | 0.8         | –          | NT          | NT          |
| Fluconazole | NT     | NT           | NT            | NT         | NT         | NT         | 1.10        | 1.20        |

NT = Not Tested.
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.

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