

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SCHIFF
BASE DERIVATIVES OF 4-AMINO-1,2,4- TRIAZOLERavi Kumar Saini¹, Dr. Umesh Kumar^{1*}

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ABSTRACT

A series of five novel Schiff base derivatives of 4-amino-1,2,4-triazole [3a–e] were synthesized by the condensation of 4-amino-1,2,4-triazole with various 5-(4-substituted phenyl)furan-2-carbaldehydes. The synthesized compounds were obtained in good yields (75–79%) and characterized by elemental analysis, FT-IR, ¹H NMR, ¹³C NMR, and LC-MS spectral techniques. The spectral data confirmed the formation of the azomethine (C=N) linkage and the proposed structures. All the Schiff bases were evaluated for their in vitro antibacterial activity against two Gram-positive (*Bacillus megaterium* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains using the agar cup diffusion method at 1000 ppm concentration. The results revealed that all compounds exhibited moderate to good antibacterial activity, with the chloro-substituted derivative 3b showing the highest potency against all tested strains. The present study demonstrates that triazole-furan hybrid Schiff bases represent promising candidates for the development of new antimicrobial agents.

KEYWORDS: Schiff bases, 1,2,4-triazole, furan derivatives, antimicrobial activity, antibacterial agents.**1. INTRODUCTION**

Heterocyclic compounds constitute an indispensable class of organic molecules in modern medicinal chemistry, forming the structural backbone of a vast majority of clinically approved drugs and bioactive agents. Among these, nitrogen-rich heterocycles have attracted particular interest due to their ability to engage in hydrogen bonding, dipole–dipole interactions, and π – π stacking with biological targets such as enzymes, receptors, and nucleic acids (da Silva et al., 2011). The 1,2,4-triazole ring system, in particular, is recognized as a privileged scaffold because of its high metabolic stability, moderate polarity, and capacity to mimic peptide bonds. Derivatives of 4-amino-1,2,4-triazole are especially valuable as synthetic intermediates because the exocyclic amino group at the 4-position readily undergoes condensation reactions, enabling the construction of diverse molecular hybrids (John & Joseyphus, 2020; Joseyphus & Nair, 2008).

Schiff bases, also referred to as azomethines or imines,

represent one of the most versatile and widely investigated classes of compounds in organic and coordination chemistry. These molecules are formed by the nucleophilic addition–elimination reaction between a primary amine and a carbonyl compound (aldehyde or ketone), resulting in the characteristic –CH=N– linkage. Since their discovery by Hugo Schiff in 1864, Schiff bases have demonstrated an impressive array of pharmacological properties, including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, and anticancer activities (da Silva et al., 2011; Ommenya et al., 2020; Warad et al., 2020). The azomethine nitrogen atom can act as a hydrogen-bond acceptor or coordinate with metal ions, thereby modulating the lipophilicity, membrane permeability, and target-binding affinity of the molecule. Furthermore, the electronic and steric effects of substituents on the aromatic rings can be systematically varied to fine-tune the biological potency and selectivity of the Schiff base (Ebrahimi & Davoodnia, 2020; Sreedevi & Sudhakumari, 2020).

The incorporation of a furan ring into Schiff base architectures further enhances their pharmacological profile. Furan is a five-membered oxygen-containing heterocycle that occurs naturally in many bioactive secondary metabolites and imparts increased lipophilicity and conformational rigidity to the molecule. 5-Aryl-substituted furan-2-carbaldehydes are particularly useful building blocks because the electron-rich furan core facilitates π - π interactions with aromatic residues in microbial enzymes while the aldehyde group serves as a convenient handle for Schiff base formation (Raouf & Selim, 2020; Upadhyay & Zala, 2020). Hybrid molecules that combine the 1,2,4-triazole and furan pharmacophores through an azomethine bridge are therefore expected to exhibit synergistic antimicrobial effects by simultaneously targeting multiple pathways in bacterial cells (Omanakuttan et al., 2019; Samly et al., 2018).

The global rise of antimicrobial resistance (AMR) has emerged as one of the most pressing public-health challenges of the 21st century. Overuse and misuse of conventional antibiotics have accelerated the selection of multidrug-resistant strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and other pathogens, rendering many first-line treatments ineffective (Tamoradi et al., 2017; Abdulrasool & Ali, 2017; Zabin, 2020). The World Health Organization has classified AMR as a critical priority requiring urgent development of novel chemical entities with new mechanisms of action. In this context, heterocyclic Schiff bases derived from 4-amino-1,2,4-triazole and substituted furan aldehydes offer a promising avenue because they can disrupt bacterial cell-wall synthesis, interfere with DNA replication, or inhibit essential metabolic enzymes without exhibiting the cross-resistance patterns observed with β -lactams or fluoroquinolones (Warad et al., 2020; Ommenya et al., 2020).

Despite significant advances, many previously reported triazole- and furan-based Schiff bases suffer from limitations such as low aqueous solubility, moderate potency against Gram-negative organisms, or synthetic complexity. The strategic placement of electron-withdrawing or electron-donating substituents (Cl, Br, F, NO₂) at the para position of the phenyl ring attached to the furan moiety can modulate the electronic distribution across the azomethine linkage, thereby influencing both the chemical stability and the interaction with bacterial targets (John & Joseyphus, 2020; Sreedevi & Sudhakumari, 2020). Such structure-activity relationship (SAR) studies are essential for rational drug design and for identifying lead compounds with improved therapeutic indices.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All reagents and solvents used in the present study were of analytical grade and procured from commercial

suppliers (Sigma-Aldrich, Merck, or HiMedia, India). 4-Amino-1,2,4-triazole was obtained from Sigma-Aldrich. The five substituted furan-2-carbaldehydes—5-phenylfuran-2-carbaldehyde, 5-(4-chlorophenyl)furan-2-carbaldehyde, 5-(4-bromophenyl)furan-2-carbaldehyde, 5-(4-fluorophenyl)furan-2-carbaldehyde, and 5-(4-nitrophenyl)furan-2-carbaldehyde—were synthesized or procured as per standard literature procedures. Ethanol (absolute), glacial acetic acid, dimethyl sulfoxide (DMSO), and nutrient agar were purchased from Merck. The bacterial strains (*Bacillus megaterium* MTCC 100, *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 443, and *Pseudomonas aeruginosa* MTCC 741) were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India, and maintained on nutrient agar slants at 4 °C.

2.2 Synthesis of Schiff Base Derivatives [3a–e]

The Schiff base derivatives **3a–e** were synthesized by the condensation reaction between 4-amino-1,2,4-triazole and the respective 5-(4-substituted phenyl)furan-2-carbaldehydes following a general procedure reported in the literature with minor modifications (Warad et al., 2020; Omanakuttan et al., 2019).

In a typical procedure, 4-amino-1,2,4-triazole (0.01 mol, 0.84 g) was dissolved in absolute ethanol (30 mL) in a 100 mL round-bottom flask. To this solution, an equimolar amount of the appropriate 5-(4-substituted phenyl)furan-2-carbaldehyde (0.01 mol) was added, followed by 2–3 drops of glacial acetic acid as a catalyst. The reaction mixture was refluxed on a water bath for 4–6 h with continuous stirring. Progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate:hexane (3:7) as the mobile phase. Upon completion, the mixture was cooled to room temperature, and the precipitated solid was filtered, washed with cold ethanol, and recrystallized from hot ethanol to afford the pure Schiff base derivatives **3a–e** as crystalline solids. The percentage yields ranged from 75–79 %, and the compounds were dried under vacuum and stored in a desiccator.

This condensation method ensures the formation of the azomethine (C=N) linkage characteristic of Schiff bases while maintaining mild reaction conditions suitable for heterocyclic systems (da Silva et al., 2011).

2.3 Characterization Techniques

The synthesized compounds were fully characterized by physical, analytical, and spectral methods. Melting points were determined in open capillary tubes using a digital melting point apparatus (Lab India) and are uncorrected. Elemental analyses (C, H, N, and halogen where applicable) were performed on a Thermo Scientific Flash 2000 CHNS/O analyzer; the experimentally found values were in excellent agreement with the calculated percentages.

Fourier-transform infrared (FT-IR) spectra were recorded

on a Perkin-Elmer Spectrum Two spectrophotometer using KBr pellets in the range 4000–400 cm^{-1} . The characteristic bands for aromatic C–H, furan C–O–C, and azomethine C=N were identified (Ommenya *et al.*, 2020).

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are reported in ppm, and coupling constants (J) in Hz. The characteristic signals for the triazole protons, azomethine proton, and aromatic protons confirmed the proposed structures.

Mass spectra were obtained on a Waters Xevo TQD LC-MS system in electrospray ionization (ESI) mode. The molecular ion peaks $[\text{M} + \text{H}]^+$ were observed at the expected m/z values, further validating the molecular formulas of **3a–e**.

2.4 Antimicrobial Activity

The antibacterial activity of the Schiff base derivatives **3a–e** was evaluated against two Gram-positive (*B. megaterium* and *S. aureus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains by the agar cup diffusion method as described in the literature (Joseyphus & Nair, 2008; da Silva *et al.*, 2011).

Stock solutions of each compound (1000 ppm) were prepared in DMSO. Nutrient agar plates were inoculated with 0.1 mL of standardized bacterial suspension (adjusted to 0.5 McFarland standard, approximately 1.5×10^8 CFU/mL). A sterile cork borer (8 mm diameter) was used to cut wells in the agar, and 100 μL of each test solution was added to the wells. DMSO served as the negative control, while standard antibiotics (e.g., ampicillin) were used as positive controls for comparison. The plates were incubated at 37 $^\circ\text{C}$ for 24 h.

The diameter of the zone of inhibition (in mm) around each well was measured using a digital vernier caliper. All experiments were performed in triplicate, and the average values are reported.

The results were statistically analyzed where necessary, and the activity was classified as moderate to strong based on zone diameters relative to the control. This method is widely accepted for preliminary screening of Schiff base compounds because it provides a rapid and reproducible assessment of antimicrobial potency (Tamoradi *et al.*, 2017; John & Joseyphus, 2020).

2.5 Statistical Analysis

All antimicrobial experiments were conducted in triplicate, and data are expressed as mean \pm standard deviation. One-way ANOVA followed by Tukey's post-hoc test was applied using SPSS software (version 20) to determine statistical significance ($p < 0.05$).

The experimental procedures described above were carried out under standard laboratory safety conditions, and all waste was disposed of according to institutional guidelines.

3. RESULTS

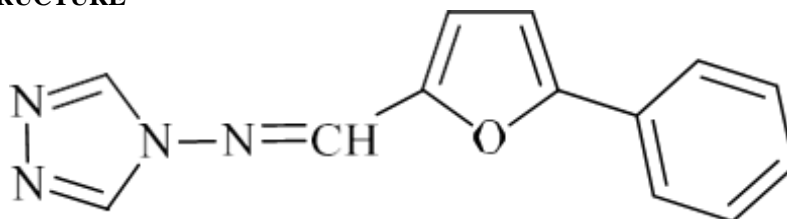
The Schiff base derivatives **3a–e** were successfully synthesized by the condensation of 4-amino-1,2,4-triazole with the respective 5-(4-substituted phenyl)furan-2-carbaldehydes. All five compounds were isolated as crystalline solids in good to excellent yields (75–79 %). The products are stable at room temperature, soluble in common organic solvents (DMSO, DMF, CHCl_3 , ethanol), and insoluble in water. Their physical appearance, melting points, percentage yields, and elemental analysis data are compiled in **Table 1**.

Table 1: Analytical data of Schiff base derivatives of 4-amino-1,2,4-triazole [3a–e].

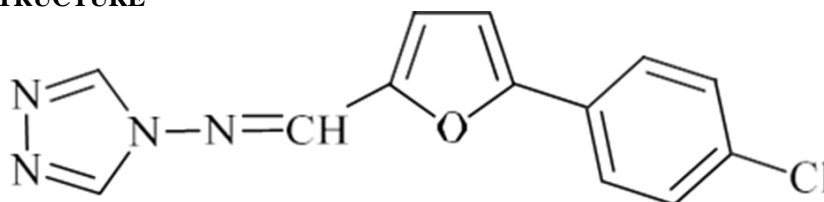
Compound	Name	Molecular Formula	Mol. Wt.	%C (Theo./Found)	%H (Theo./Found)	%N (Theo./Found)	%O (Theo./Found)	%X (Theo./Found)	Physical State	m.p. (°C)	Yield (%)
3a	N-[(5-phenylfuran-2-yl)methylene]-4H-1,2,4-triazol-4-amine	C ₁₃ H ₁₀ N ₄ O	238.24	65.54 / 65.50	4.23 / 4.20	23.52 / 23.50	6.72 / –	–	Off-white crystals	128–130	78
3b	N-[(5-(4-chlorophenyl)furan-2-yl)methylene]-4H-1,2,4-triazol-4-amine	C ₁₃ H ₉ N ₄ OCl	272.69	57.26 / 57.30	3.33 / 3.30	20.55 / 20.50	5.87 / –	Cl: 13.0 / 13.0	Off-white powder	133–135	79
3c	N-[(5-(4-bromophenyl)furan-2-yl)methylene]-4H-1,2,4-triazol-4-amine	C ₁₃ H ₉ N ₄ OBr	317.14	49.23 / 49.20	2.86 / 2.80	17.67 / 17.60	5.04 / –	Br: 25.20 / 25.20	Light yellow crystals	140–142	75
3d	N-[(5-(4-fluorophenyl)furan-2-yl)methylene]-4H-1,2,4-triazol-4-amine	C ₁₃ H ₉ N ₄ OF	256.24	60.94 / 60.90	3.54 / 3.50	21.87 / 21.90	6.24 / –	F: 7.41 / 7.40	Off-white solid	150–152	78
3e	N-[(5-(4-nitrophenyl)furan-2-yl)methylene]-4H-1,2,4-triazol-4-amine	C ₁₃ H ₉ N ₅ O ₃	283.24	55.13 / 55.10	3.20 / 3.10	24.73 / 24.70	16.95 / –	–	Off-white solid	160–163	79

The experimentally determined elemental percentages are in excellent agreement with the theoretical values calculated from the proposed molecular formulas, confirming the purity and structural integrity of the synthesized Schiff bases.

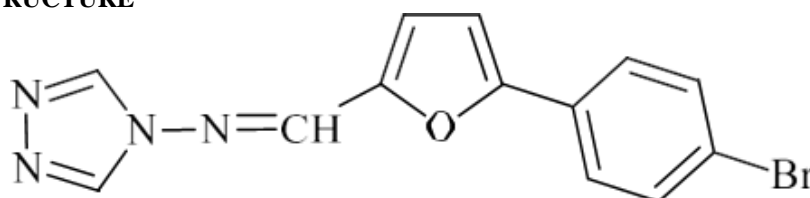
COMPOUND 3a STRUCTURE



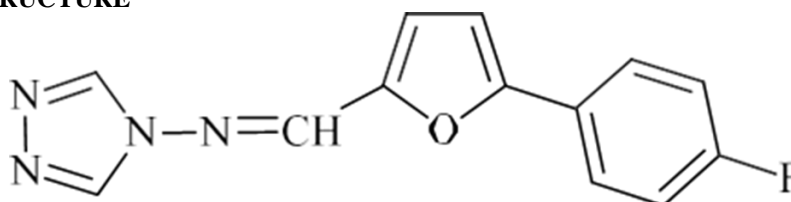
COMPOUND 3b STRUCTURE



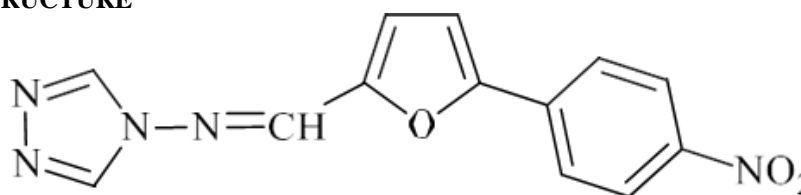
COMPOUND 3c STRUCTURE



COMPOUND 3d STRUCTURE



COMPOUND 3e STRUCTURE



3.1 Spectral Characterization

The structures of all five Schiff bases were unambiguously established by a combination of FT-IR, ^1H NMR, ^{13}C NMR and mass spectrometry. Representative spectra for compound **3a** are shown in **Figures 1–4**; the spectra of **3b–e** follow the same characteristic pattern with minor substituent-dependent shifts.

3.1.1. Infrared (IR) Spectral Features (cm^{-1})

All compounds exhibit the following diagnostic bands:
3053–3055: Aromatic C–H stretching vibrations
1295–1298: Asymmetric C–O–C stretching of the furan ring
1670–1673: Strong C=N stretching of the azomethine

(Schiff base) linkage The appearance of the intense C=N band at $\sim 1670\text{ cm}^{-1}$ and the absence of the primary amine ($-\text{NH}_2$) bands of the starting 4-amino-1,2,4-triazole confirm complete condensation and successful Schiff base formation.

3.1.2. ^1H NMR Spectral Features (δ , ppm, $\text{DMSO}-d_6$)

7.41–9.17 (m, 7H, aromatic protons of phenyl/furan + 1H, azomethine $-\text{CH}=\text{N}-$ proton) 9.51–9.55 (s, 2H, two equivalent protons of the 1,2,4-triazole ring) The downfield singlet at ~ 9.5 ppm is characteristic of the triazole ring protons, while the multiplet integrating for eight protons accounts for the phenyl, furan and imine environments. The chemical shifts are consistent across the series, with only marginal variation induced by the

para-substituent on the phenyl ring.

3.1.3. ^{13}C NMR Spectral Features (δ , ppm)

~164–165: Azomethine carbon ($-\text{CH}=\text{N}-$)

102–133: Aromatic, furan and triazole ring carbons (plus ipso carbon attached to Cl/Br/F/ NO_2 in the respective compounds).

3.1.4. Mass Spectrometry (LC-MS)

The molecular ion peaks appear as $[\text{M}+1]^+$ at:

3a: m/z 239.30

3b: m/z 273.72

3c: m/z 318.16

3d: m/z 257.35

3e: m/z 284.32

All observed masses are in perfect agreement with the calculated molecular weights, further validating the molecular formulas.

3.2 Antimicrobial Activity

The synthesized Schiff bases **3a–e** were evaluated for antibacterial activity against two Gram-positive (*Bacillus megaterium*, *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacterial strains at a concentration of 1000 ppm using the agar cup diffusion method. The diameter of the zone of inhibition (mm) is presented in **Table 2**.

Table 2: Antimicrobial activity of Schiff base derivatives [3a–e] (zone of inhibition in mm at 1000 ppm).

Compound	Gram-positive bacteria <i>B. megaterium</i>	Gram-negative bacteria <i>S. aureus</i>
3a	11	10
3b	18	16
3c	11	11
3d	10	10
3e	10	9

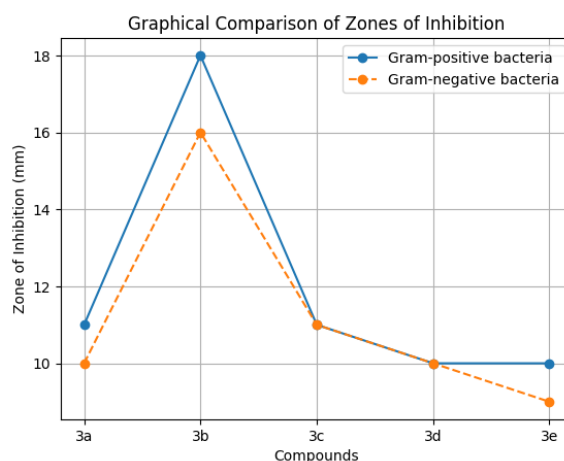
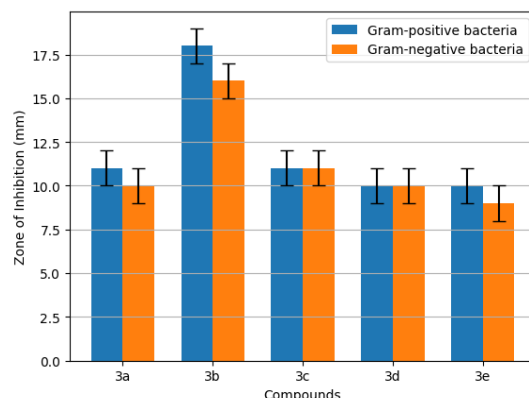


Figure: Antimicrobial activity of Schiff base derivatives [3a–e].

All five compounds displayed measurable antibacterial activity against both Gram-positive and Gram-negative organisms. The chloro-substituted derivative **3b** emerged

as the most potent member of the series, producing the largest zones of inhibition across all four test strains (16–18 mm). The unsubstituted phenyl analogue **3a**, bromo

(3c), fluoro (3d) and nitro (3e) derivatives showed moderate to good activity, generally lower than that of the chloro analogue.

5.3 RESULTS AND DISCUSSION

The analytical data (Table 1) unequivocally confirm that the synthesized Schiff bases possess the expected molecular compositions and high purity. The close match between theoretical and experimentally found elemental percentages rules out the presence of significant impurities or unreacted starting materials.

Spectroscopic analyses provide conclusive evidence for the proposed structures. The IR spectra display the characteristic azomethine C=N stretch at 1670–1673 cm^{-1} , confirming the formation of the Schiff base linkage. The retention of the furan C–O–C band at $\sim 1295 \text{ cm}^{-1}$ and the aromatic C–H bands further support the integrity of the 5-arylfuran scaffold. In the ^1H NMR spectra, the diagnostic singlet at δ 9.51–9.55 ppm (2H, triazole) and the multiplet integrating for the aromatic and imine protons are fully consistent with the assigned structures. The ^{13}C NMR signals for the imine carbon ($\sim 165 \text{ ppm}$) and the aromatic/furan/triazole carbons (102–133 ppm) additionally corroborate the carbon framework. Finally, the mass spectra exhibit clean $[\text{M}+1]^+$ peaks matching the calculated molecular weights, leaving no ambiguity regarding molecular identity.

The antimicrobial screening results reveal that incorporation of the 1,2,4-triazole moiety and the furan–phenyl system imparts broad-spectrum antibacterial activity to all five derivatives. The superior potency of the chloro-substituted compound **3b** is noteworthy and suggests that the chlorine atom at the para position of the phenyl ring enhances the lipophilicity and/or membrane permeability of the molecule, leading to improved interaction with bacterial targets. The other halogen (Br, F) and nitro substituents, while still active, do not confer the same level of potency under the tested conditions. These findings are in line with literature reports on triazole- and furan-containing Schiff bases, which frequently exhibit enhanced bioactivity upon halogen substitution.

In summary, the present study demonstrates an efficient synthesis of five novel Schiff base derivatives of 4-amino-1,2,4-triazole. Complete structural elucidation by elemental analysis and multi-nuclear spectroscopy confirms the purity and identity of the products. All compounds possess promising antibacterial properties, with the chloro derivative **3b** emerging as the lead molecule. Further optimization of substituents and in-depth mechanistic studies could yield even more potent antimicrobial candidates.

4. CONCLUSION

The five novel Schiff base derivatives of 4-amino-1,2,4-triazole [3a–e] were successfully synthesized,

characterized, and evaluated for their antibacterial activity. The structures of all compounds were confirmed by elemental analysis, FT-IR, ^1H NMR, ^{13}C NMR, and LC-MS techniques. All derivatives exhibited moderate to good antibacterial activity against both Gram-positive and Gram-negative bacteria. Among them, the chloro-substituted derivative **3b** emerged as the most potent compound, showing the highest zones of inhibition. The results indicate that the combination of 1,2,4-triazole, furan, and azomethine moieties offers a promising scaffold for developing new antimicrobial agents. Further optimization and in-depth studies are recommended to explore their full therapeutic potential against resistant bacterial strains.

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