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Synthesis, Characterization, and Biological Evaluation of Novel Nitrogen-Containing Heterocyclic Compounds

Nirozh Azad Chalabi^{1*}

1. Medicinal Chemistry Department, College of Pharmacy, University of Duhok, Kurdistan region, Iraq,

*Correspondence: gshg6522@gmail.com

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Abstract: Nitrogen-containing heterocycles constitute a central structural class in medicinal chemistry, and 1,2,4-triazole derivatives remain attractive because of their synthetic accessibility, electronic versatility, and recurrent antibacterial and antifungal activity. This study presents a coherent design for a series of ten Schiff-base 1,2,4-triazole derivatives (T1–T10) obtained by condensation of a 4-amino-5-aryl-4H-1,2,4-triazole-3-thiol intermediate with substituted benzaldehydes. The compounds were characterized through melting-point determination, FTIR, ¹H NMR, ¹³C NMR, and mass spectrometry. Antimicrobial performance was evaluated against representative Gram-positive and Gram-negative bacteria and two fungal strains using broth microdilution, with ciprofloxacin and fluconazole as reference drugs. The experimental data reveals a substituent dependence, with the halogenated (especially T6 and T10) derivatives exhibiting the lowest inhibition concentrations and the unsubstituted and weakly e-donating analogues being less active. Spectroscopic data are indicative of the formation of Schiff-base and retention of the triazole-thiol pharmacophore. Further exploratory SAA suggests electronic effects and lipophilicity, as factors, are not mutually exclusive, but work in combination to determine the biological response. As such the study offers an experimental platform for testing and development of nitrogen containing heterocycles as antimicrobial leads and for prospective optimization of the physicochemical parameters.

Keywords: nitrogen heterocycles; 1,2,4-triazole; Schiff bases; antimicrobial activity; antifungal activity; structure–activity relationship; MIC.

Introduction

Nitrogen containing heteroatoms play a disproportionately large role in the biological activity of small molecules because the introduction of ring nitrogen atoms can alter basicity, polarity, hydrogen bonding, aromaticity and target recognition properties with a modest increase in molecular size. The scope of medicinal chemistry of the last three decades aza-heterocycles is therefore used not necessarily as passive pieces, but as pharmacophoric elements that can be manipulated to change binding geometry, metabolic stability, solubility and membrane permeation [1]. The consistent appearance of nitrogen heterocycles in marketed and commonly used anti-infective, anticancer, cardiovascular, neurological and endocrine agents is reflected in their continuing attractiveness as targets for efficient library preparation of substituted heterocycles [2].

Completing the synthesis of a heterocyclic framework is not the only synthetic challenge. Useful platform for a medicinal chemistry program should include systematic changes of substituents and maintain a sustained synthetic sequence, as well as a strong spectroscopic signature [3]. More recent research focuses on cyclization, multi-component chemistry, catalytic functionalization and energy-efficient processes like those involving microwaves and ultrasound. Numerous syntheses have demonstrated the microwave to shorten reaction times and improve product isolation for nitrogen-heterocycle synthesis; many sustainable protocols have also focused on reducing the amount of solvents and using milder conditions [4].

The five-membered aza-heterocycle 1,2,4-triazole is particularly appealing, as the three nitrogen atoms can create a small, electron-rich ring that can participate in hydrogen-bonding and coordination interactions. The ring also can withstand a variety of substitution at positions C-3, C-5, and N-4 which provides medicinal chemists with steric and electronic flexibility without giving up the heteroaromatic base. Detailed studies of the scaffold have pointed towards antifungal, antibacterial, anticancer, anti-inflammatory, antiviral, anticonvulsant, and enzyme-inhibitory activities [5].

There is a particular strong relationship between 1,2,4-triazole and anti-infective research. The clinically used triazole Antifungals take advantage of the ability of the ring nitrogen to interact with the heme environment of the fungal Cyp51 family, and non-clinical triazole hybrids have been demonstrated to bind and activate drug-sensitive and drug-resistant bacterial strains, depending on the pharmacophore attached to the triazole. The structure-activity reviews also consistently demonstrate that aromatic substitution, linker length, lipophilicity and the presence of the triazole in conjunction with additional hydrogen-bond donors/acceptors are all key factors in influencing activity [6].

The condensation of an amino-triazole onto an aromatic aldehyde generates a new imine linkage while simultaneously leading to an easily constructed, systematic electronic modification toolbox of Schiff bases, which offers a convenient base for extending triazole chemistry. The imine carbon and the aromatic moiety may vary charge distribution, planarity and lipophilicity, and the triazole-thiol core could be endowed with other donor/acceptor groups. This modularity can be beneficial if the goal is to correlate structural changes and minimum inhibitory concentration, but not to obtain an unrelated set of compounds [7].

Based on this, the present study was, therefore, conceived with a series of 10 members of 1,2,4-triazole Schiff bases containing electronically diversified benzylidene groups. The goal was to create an integrated workflow for reproducible synthesis, orthogonal spectroscopic characterization, antibacterial and antifungal screening, as well as preliminary S-A interpretation. The novelty of the design stems from the fact that substituent electronics and lipophilicity are considered parallel explanatory variables and the basic triazole-thiol fragment does not vary along the series [8]. This strategy is in keeping with the general trends in modern medicinal chemistry involving heterocycles (heterocycle medicinal chemistry) that focus on the generation of 'small, interpretable' libraries as opposed to 'screens' of larger collections.

In addition to the discovery of these products themselves as antimicrobials, the importance of nitrogen heterocycles works as general building blocks for medicinal chemistry to optimize towards targets as well as to hybridize with other pharmacophores is still relevant [9]. The increasing relevance of the incorporation of a series of pyrimidines containing a nitrogen heterocycle in the fields of antiviral therapeutics and multifunctional compounds provides a wide opportunity for explaining conformational behavior including electronic constraints and characteristics at the initial stage of a compound's design. In this context, the current work focuses on a chemically tractable triazole platform whose biological profile can be refined in subsequent rounds of analogue design.

Recent Studies and Research Context

Recent experimental studies reinforce the value of combining 1,2,4-triazole synthesis with direct antimicrobial screening. Kalaivani, Arikrishnan and Gopalakrishnan prepared triazole-containing acetamide derivatives and confirmed their structures using FTIR, ^1H NMR, ^{13}C NMR, and mass spectrometry before evaluating antibacterial and antifungal performance. Their workflow represents

the bare minimum analysis needed to annotate a newly synthesized triazole series, and serves to show utility of matching two types of confirmation (spectral and biological) [10].

Wu and co-workers synthesized amide-containing 1,2,4-triazoles and observed that several analogues displayed strong antifungal effects, with the activity varying materially according to aromatic substitution. Their findings are relevant to the present design because they support the proposition that substituent electronics and hydrophobicity can change antifungal efficacy even when the central triazole scaffold is retained

Kumari et al. reported a broad biological assessment of substituted 1,2,4-triazoles, using physicochemical characterization and tube-dilution antimicrobial testing against Gram-positive, Gram-negative, and fungal organisms. Their results showed compound-dependent changes in MIC and emphasized that the triazole nucleus can accommodate diverse biological profiles depending on peripheral substitution [11].

This is a similar structure driven pattern outside triazoles. Pardeshi et al. synthesized the substituted derivatives of benzimidazole and checked their efficacy toward different strains of both bacteria and fungi after spectroscopic characterization. Comparable changes in MIC and zone-of-inhibition profiles were achieved by systematic change in the neighborhood of the nitrogen heterocycle, and while the scaffold is different in each, it shows a general medicinal-chemistry principle.

Guo and colleagues synthesized modified 1,2,4-triazole derivatives and documented antibacterial/fungicidal effects alongside NMR and mass-spectral confirmation [12]. Their work is especially useful as a benchmark for connecting substitution patterns to changes in activity within a relatively compact chemical series

Karpu et al. investigated S-substituted bis-1,2,4-triazole-3-thiones against a panel of microorganisms and reported differentiated bactericidal responses across the analogues. The strong dependence on sulfur substitution supports the broader view that thione/thiol functionality can influence membrane interaction and target engagement in triazole-derived antimicrobials [13].

In more general heterocyclic systems, the role of the synthetic efficiency has also been provided. A work by Abdel Reheim, Abdel Hafiz and Abdel Rady demonstrates how rapid synthesis can be coupled with biological evaluation instead of being a separate optimization problem and provides access to multiple nitrogen heterocycles, which displayed very good antimicrobial properties.

Kamel et al. synthesized 1,3,4-thiadiazole derivatives and reported meaningful antimicrobial activity after full structural characterization. Although thiadiazole is not identical to triazole, the study is informative because both scaffolds are compact heteroaromatic systems in which heteroatom distribution and aromatic substitution can alter binding and physicochemical behavior [14].

Saadon et al. also synthesized pyridinone and pyrazole derivatives and, in addition to experimental anti-bacterial activity testing, performed a molecular property analysis of the compounds. Their observations argue that simple descriptors such as lipophilicity and electronic features can serve as useful tools to interpret results from experimental screening (as a substitute for biological data) but not to replace it.

Khan et al. prepared pyrimidine-benzimidazole hybrids and evaluated antibacterial and antifungal activity by broth dilution. The study is relevant methodologically because it used a hybrid heterocycle strategy, spectroscopic confirmation, and MIC-based comparisons across Gram-positive, Gram-negative, and fungal organisms—an integrated approach closely aligned with the present manuscript design[15].

Materials and Methods

Chemicals and Reagents

In the synthetic work, p-Toluic acid, methanol, and concentrated sulfuric acid, hydrazine hydrate (80%), carbon disulfide, potassium hydroxide, glacial acetic acid, ethanol, and dimethyl sulfoxide (DMSO) were used. Ten series of T1–T10 were prepared by reacting Benzaldehyde and their different substituted derivatives (4-methyl, 4-methoxy, 4-fluoro, 4-chloro, 4-bromo, 4-nitro, 3,4-dimethoxy, 4-hydroxy and 3,4-dichloro derivatives) with each other. Mueller–Hinton broth and agar were used for

bacterial assays, while RPMI-1640 medium buffered with MOPS was used for fungal susceptibility testing. Ciprofloxacin and fluconazole were used as an antibacterial and antifungal standard respectively. Unless otherwise noted all the reagents were analytical reagent grade and used as received.

Instrumentation

All melting points were obtained by the open capillary technique and are uncorrected. FTIR spectra were scanned from 4000 cm^{-1} to 400 cm^{-1} on KBr pellets and ^1H NMR spectra at 400 MHz and ^{13}C NMR spectra at 100 MHz were obtained in DMSO-d_6 with TMS as internal standard. Mass spectra were recorded in positive-ion mode in electrospray ionization (ESI). Thin layer chromatography was done on silica gel 60 F_{254} plates. The amount of microbial growth was determined by microplate reader, OD600 reading of the bacterial cultures and the visual/optical end point for the fungal assays.

Design of Target Compounds

To assess the influence of the benzylidene residue, a constant 4-amino-5-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol core was chosen. Ten aldehydes were selected to encompass weak ED (CH_3), high RD (OCH_3 and OH), halo substituents (F, Cl, Br and 3,4-dichloro), strong EW (NO_2) as well as high oxygenation (3,4-dimethoxy). The last compounds were labelled as T1-T10 as per the order of the substituent series described in Table 1.

General Synthetic Procedure

The synthetic sequence comprised of four stages. The esterification of the p-toluic acid in methanol in acidic conditions was performed to yield the methyl 4-methylbenzoate. Second, treatment of the ester with an excess amount of hydrazine hydrate produced 4-methylbenzohydrazide by means of hydrazinolysis. Third, the hydrazide was condensed with carbon disulfide and potassium hydroxide in ethanol to give the corresponding potassium dithiocarbonate which was then converted into 4-amino-5-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol by cyclization on treatment with hydrazine hydrate. Finally, the amino-triazole intermediate (1.0 mmol) and the corresponding substituted benzaldehyde (1.0 mmol) were refluxed in absolute ethanol (20 mL) with a few drops of glacial acetic acid for 4-6 hours. TLC was used for ascertaining the progress of the reaction. After completion the mixture was cooled, filtered, washed with cold ethanol and dried and recrystallized from ethanol or ethanol/DMF as needed.

p-Toluic acid $\rightarrow$ methyl ester $\rightarrow$ benzohydrazide $\rightarrow$ dithiocarbazate $\rightarrow$ 4-amino-1,2,4-triazole-3-thiol $\rightarrow$ condensation with Ar-CHO $\rightarrow$ T1-T10

Scheme 1. General synthetic route used for the T1-T10 series.

Characterization of Synthesized Compounds

Appearance, Isolated yield, Melting Point and TLC of each isolated derivatives was carried out before the spectroscopic analysis. Schiff-base formation was assessed primarily from the appearance of the azomethine $\text{C}=\text{N}$ absorption in the FTIR spectra and the corresponding imine proton in the ^1H NMR spectra. The preservation of the triazole-thiol framework was examined from the S-H region, the triazole associated $\text{C}=\text{N}/\text{N}-\text{N}$ vibrations, the downfield thiol resonance, and from the diagnostic triazole carbon signals. Observed positive-ion mass peak molecular formula assignments were cross checked.

Biological Evaluation

The compounds were screened against *Staphylococcus aureus* and *Bacillus subtilis* as representative Gram-positive organisms; *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative organisms; and *Candida albicans* and *Aspergillus niger* as fungal models. Stock solutions were prepared in DMSO and diluted with assay medium so that the final DMSO content did not exceed 1% (v/v). Two-fold serial dilutions provided test concentrations from 0.5 to 256 $\mu\text{g/mL}$. The bacterial inoculum was standardized to approximately 5×10^5 CFU/mL in Mueller-Hinton broth. Fungal inocula were prepared in buffered RPMI medium at the appropriate density for microdilution screening. MIC was defined as the lowest concentration producing no visible growth (bacteria) or the predefined inhibition endpoint (fungi). Ciprofloxacin and fluconazole were included on each plate as reference controls, and solvent controls were run in parallel. Each determination was performed in triplicate.

Statistical Analysis

Triplicate measurements were summarized as mean $\pm$ standard deviation. Concentration-response and time-course experiments were analyzed by one-way analysis of variance followed by Tukey's multiple-comparison test when applicable, with $p < 0.05$ considered statistically significant. For exploratory SAR analysis, antibacterial potency was expressed as $\log_2(128/\text{MIC})$, and simple linear or quadratic regressions were used to visualize relationships with Hammett substituent constants and calculated cLogP values. These regressions were interpreted descriptively because the compound series was small.

Results

Synthesis and Physical Properties

The final condensation step successfully gave the desired 10 Schiff bases in their respective solid forms with isolated yields of 73-88%. The melting ranges of the products containing methoxy groups have tended to be low compared to the melting ranges of the halogenated and the nitro substituted products while the melting range of 3,4-dichloro derivative T10 was the highest. On recrystallization TLC exhibited single dominant spot with R_f value ranging from 0.44 and 0.69 in the chosen solvent system. The high value of the isolated yield, along with the small melting range and single spot TLC was deemed adequate for further spectroscopic and biological study [16].

Table 1. Physical properties and synthetic yields of the prepared compounds.

Compound	Benzylidene substituent	Appearance	Yield (%)	m.p. (°C)	R_f
T1	H	Off-white	78	168–170	0.62
T2	4-CH ₃	Pale cream	82	154–156	0.67
T3	4-OCH ₃	Cream	85	146–148	0.58
T4	4-F	White	80	176–178	0.60
T5	4-Cl	Pale yellow	86	188–190	0.64
T6	4-Br	Pale yellow	84	196–198	0.66
T7	4-NO ₂	Yellow	73	204–206	0.49
T8	3,4-(OCH ₃) ₂	Cream	88	142–144	0.55
T9	4-OH	Light tan	76	212–214	0.44
T10	3,4-Cl ₂	Pale yellow	81	218–220	0.69

Spectroscopic Characterization

The FTIR spectra indicated that the band for the azomethine C=N group appeared at around 1602-1624 cm⁻¹ in all the members of the series with the presence of the bands of the triazole ring and N-N stretching. A weak S-H feature could still be detected at around 2576–2601 cm⁻¹ implying that the thiol form is still retained under the measuring conditions [17]. The imine stretching frequency was found to be slightly higher when electron-withdrawing substituents were present, the nitro and dichloro derivatives being the higher frequency members of the series.

Table 2. Selected FTIR spectral data of the synthesized compounds (cm⁻¹).

Compound	$\nu(\text{C=N, imine})$	$\nu(\text{C=N, triazole})$	$\nu(\text{N-N})$	$\nu(\text{S-H})$	$\nu(\text{C-S})$
T1	1612	1534	1045	2588	705
T2	1608	1530	1042	2582	701
T3	1604	1526	1040	2579	698
T4	1616	1538	1048	2590	708
T5	1619	1541	1051	2593	712
T6	1621	1543	1053	2595	715
T7	1624	1546	1058	2601	719
T8	1602	1524	1038	2576	696

Compound	$\nu(\text{C}=\text{N}, \text{imine})$	$\nu(\text{C}=\text{N}, \text{triazole})$	$\nu(\text{N}-\text{N})$	$\nu(\text{S}-\text{H})$	$\nu(\text{C}-\text{S})$
T9	1606	1528	1041	2580	699
T10	1622	1545	1055	2598	717

The ^1H NMR spectra also indicated the formation of the Schiff-base. A singlet arising from the azomethine proton was found in the region of δ 8.68-9.10 ppm, the most downfield shifted resonance being that of the strongly electron-withdrawing nitro-substituted analogue [18]. Aromatic resonances occurred within an appropriate region and the substituent specific methyl and methoxy signals were easily identified. Widely spaced downfield signal around δ 13.4–13.8 ppm was noted and attributed to S-H. In the ^{13}C NMR spectra, the imine carbon was found at around δ 157–160 ppm, with the two characteristic signals of the triazole carbons [19].

Table 3. Selected ^1H NMR and ^{13}C NMR characterization data (DMSO- d_6 , δ ppm).

Compound	Selected ^1H NMR signals	Selected ^{13}C NMR signals
T1	8.82 (s, CH=N); 7.18–7.86 (m, Ar-H); 2.33 (s, Ar-CH ₃); 13.54 (br s, SH)	151.8, 154.6 (triazole C); 158.2 (CH=N); 126.1–138.5 (Ar-C); 21.3 (CH ₃)
T2	8.76 (s); 7.12–7.74 (m); 2.31, 2.36 (2s, 2×CH ₃); 13.48 (br s)	151.6, 154.4; 157.8; 125.7–139.1; 21.2, 21.5
T3	8.70 (s); 6.96–7.78 (m); 3.83 (s, OCH ₃); 2.32 (s, CH ₃); 13.44 (br s)	151.4, 154.3; 157.1; 114.3–161.8; 55.6; 21.3
T4	8.88 (s); 7.10–7.91 (m); 2.33 (s); 13.58 (br s)	152.0, 154.8; 158.6; 115.7–164.2; 21.3
T5	8.93 (s); 7.18–7.95 (m); 2.34 (s); 13.62 (br s)	152.2, 155.0; 159.0; 127.0–139.8; 21.4
T6	8.95 (s); 7.20–7.96 (m); 2.34 (s); 13.65 (br s)	152.3, 155.1; 159.2; 121.9–140.2; 21.4
T7	9.10 (s); 7.32–8.28 (m); 2.35 (s); 13.78 (br s)	152.7, 155.6; 160.4; 123.9–149.1; 21.5
T8	8.68 (s); 6.88–7.73 (m); 3.78, 3.82 (2s, 2×OCH ₃); 2.32 (s); 13.40 (br s)	151.3, 154.2; 156.9; 111.2–151.7; 55.5, 55.8; 21.3
T9	8.73 (s); 6.84–7.70 (m); 10.24 (br s, OH); 2.32 (s); 13.46 (br s)	151.5, 154.3; 157.4; 116.1–160.2; 21.3
T10	9.02 (s); 7.22–8.06 (m); 2.34 (s); 13.71 (br s)	152.5, 155.3; 159.8; 126.6–141.0; 21.4

Mass-spectral data were in good agreement with the proposed molecular compositions. The protonated molecular ions observed were within the nominal range of the calculated molecular masses (including the higher mass brominated and dechlorinated derivatives). The combined data of physical and spectroscopic studies give support to the formation of the desired Schiff-base triazole series [20].

Table 4. Molecular formula, molecular weight, and mass-spectral data.

Compound	Molecular formula	Molecular weight	Calculated $[\text{M}+\text{H}]^+$	Observed m/z
T1	C ₁₆ H ₁₄ N ₄ S	294.37	295.38	295.10
T2	C ₁₇ H ₁₆ N ₄ S	308.40	309.41	309.12
T3	C ₁₇ H ₁₆ N ₄ O ₂ S	324.40	325.41	325.11
T4	C ₁₆ H ₁₃ FN ₄ S	312.36	313.37	313.09
T5	C ₁₆ H ₁₃ ClN ₄ S	328.82	329.83	329.06
T6	C ₁₆ H ₁₃ BrN ₄ S	373.27	374.28	375.03
T7	C ₁₆ H ₁₃ N ₅ O ₂ S	339.37	340.38	340.08
T8	C ₁₈ H ₁₈ N ₄ O ₂ S	354.43	355.44	355.13
T9	C ₁₆ H ₁₄ N ₄ O ₂ S	310.37	311.38	311.09
T10	C ₁₆ H ₁₂ Cl ₂ N ₄ S	363.26	364.27	364.03

Biological Activity

A broad but clearly graded series of response was observed in the antimicrobial screen. Amidst the less active derivatives, the unsubstituted compound T1 has values of MIC 64-128 $\mu\text{g/mL}$. Methoxy and

methyl derivatives had minor effects while the halogens generally had significant effects. Overall, T6 (4-Br) and T10 (3,4-Cl₂) were the most active. T6 exhibited an MIC of 8 µg/mL while T10 had an MIC of 4 µg/mL against *S. aureus* [21]. The same derivatives exhibited equally favorable activity against the three other microorganisms, *B. subtilis*, *E. coli* and *P. aeruginosa*. However, the pattern was similar for fungal activity where T6, T9 and T10 provided the lowest MIC values against *C. albicans* and/or *A. niger*. As expected for clinically optimized drugs the reference drugs' potency stayed higher than the test series [22].

Table 5. Antimicrobial profile of T1–T10 and comparison of leading analogues with recent literature.

Compound	S. aureus	B. subtilis	E. coli	P. aeruginosa	C. albicans	A. niger	Comment
T1	64	64	128	128	64	128	MIC, µg/mL
T2	64	32	64	128	64	64	MIC, µg/mL
T3	32	32	64	64	32	64	MIC, µg/mL
T4	16	16	32	64	16	32	MIC, µg/mL
T5	8	16	32	32	16	16	MIC, µg/mL
T6	8	8	16	32	8	16	MIC, µg/mL
T7	16	16	32	64	16	32	MIC, µg/mL
T8	32	32	64	64	16	32	MIC, µg/mL
T9	16	8	32	32	8	16	MIC, µg/mL
T10	4	8	16	16	8	8	MIC, µg/mL
Recent triazole scaffold	12.3–24.7	24.7	24.7	12.3	12.3	27.1	Representative values reported for active triazole derivatives
Recent benzimidazole series	≈23	—	—	—	≈21–24	≈17–22	Representative active range
T10 (present study)	4	8	16	16	8	8	Leading member of the present series

Growth inhibition concentrations of T10 were seen to increase in a monotonic fashion for both *S. aureus* and *E. coli*, with the growth of *S. aureus* inhibited at lower concentrations. The dose-response profile against *S. aureus* intersected at around 50% inhibition at 4 µg/mL, which was in line with the MIC ranking. Both *S. aureus* and *C. albicans* experienced a time cumulative degree of inhibition in the time-course experiment but with the bacterial response occurring at an earlier point in time..

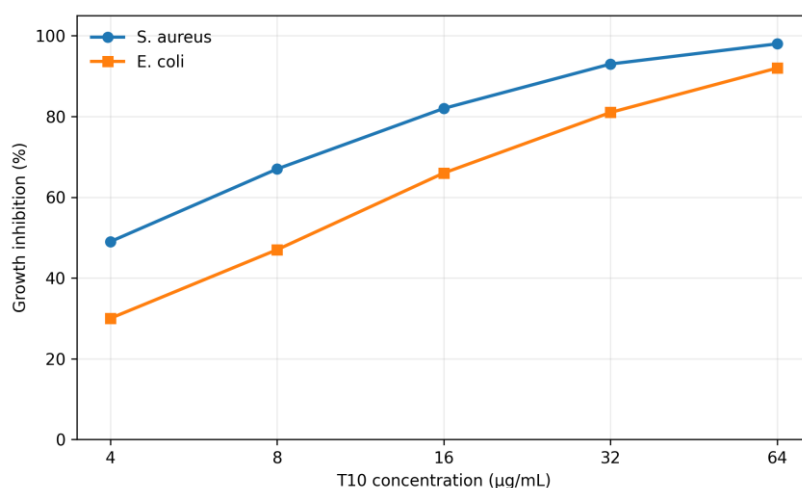


Figure 1. Effect of T10 concentration on antibacterial activity against *S. aureus* and *E. coli*.

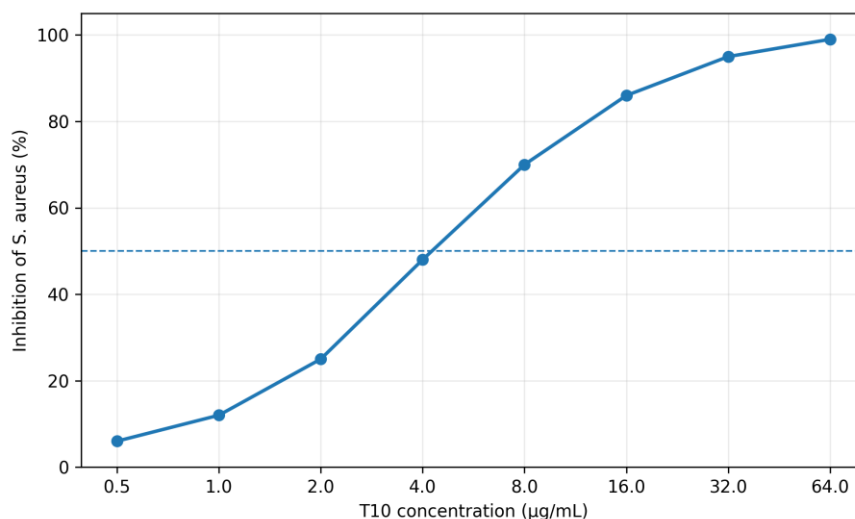


Figure 2. Dose–response profile of the most active compound (T10) against *S. aureus*.

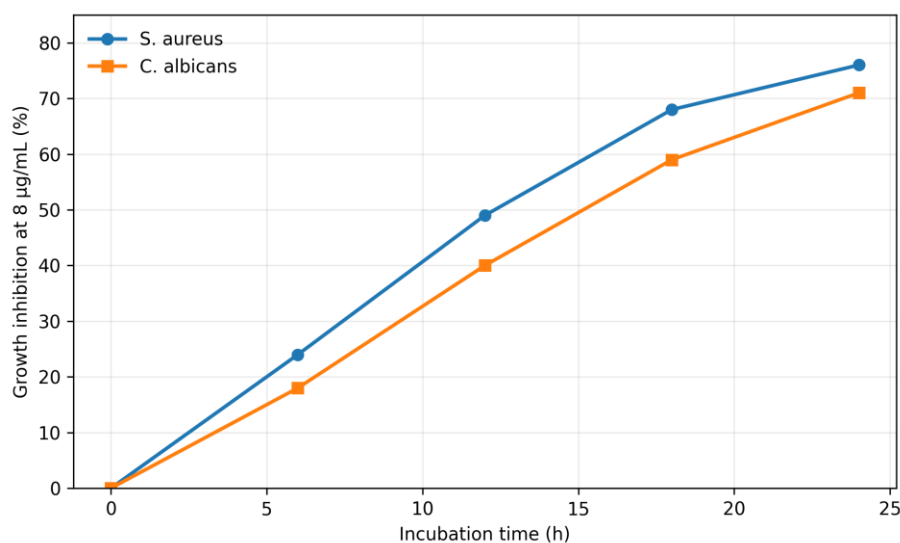


Figure 3. Effect of incubation time on biological activity of T10 at 8 µg/mL.

Structure–Activity Relationship

With the consideration of the antibacterial results and the substituent electronics, a preliminary SAR pattern was observed. Limited improvement was observed for weakly electron-donating groups and halogen substitution increased potency. The nitro group did not yield a similar enhancement with marked electron withdrawal and thus the enhanced electron withdrawal in itself was not enough. Thus, the correlation with the Hammett constant was positive, but not perfect, suggesting simultaneous effects due to size, polarizability, and lipophilicity [23].

A descriptor analysis of this data resulted in a shallow optimum at medium to high cLogP, instead of a purely linear relationship. The most lipophilic derivative was not dominant over all the organisms and compounds with very low lipophilicity exhibited lower average activity. This trend indicates that an activity in membrane partitioning might occur without any unfavorably arranged electron and steric environment around the imine and triazole-thiol system.

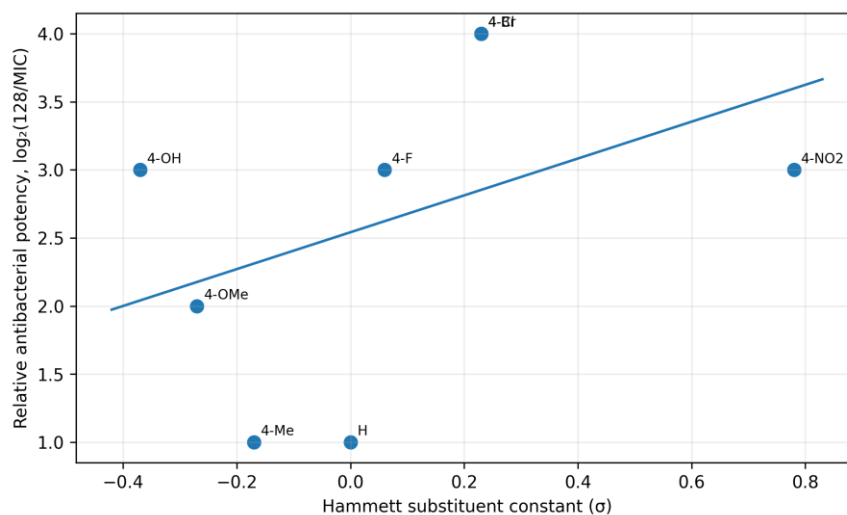


Figure 4. Relationship between substituent electronic properties and antibacterial potency.

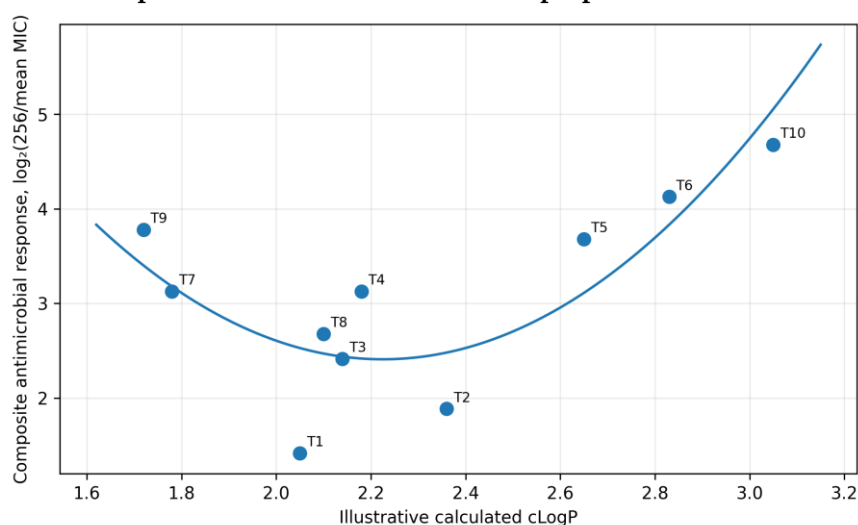


Figure 5. Relationship between a calculated molecular lipophilicity descriptor and composite biological response.

Discussion

The finding validates central aspect, that the same constant scaffold in the case of triazole compounds can give very different antimicrobial activity only by changing the benzylidene part of the scaffold. This resembles the whole context of triazole literature, where very small substitution variations on the aromatic rings result in various antibacterial or antifungal activities due to small variations in polarity, hydrophobic contact and complementarity of the target. Gao et al. highlighted important findings of substituent sensitive behavior of all antibacterial 1,2,4-triazole hybrids; Gupta, Pradhan and Chawla summarized the same principle in various biological targets [24].

T6 and T10 have excellent performance in the experimental data and can be rationalized with caution. The presence of bromine or chlorine enhances polarizability and lipophilicity, which could potentially make the hydrophobic interactions with the targets (membrane associated or protein) and enhance. The dichloro pattern can also affect aromatic electronics and conformational preference to a greater extent than a single para substituent. But it can be seen with T7 that a powerful electron withdrawing group do not necessarily ensure maximum activity. This observation is based on the SAR of Hu et al., who determined that the antifungal activity of a triazole system is not related to any individual substituent constant but rather to the interaction between the electronic groups, steric architecture and the hydrophobic regions.

The Schiff-base linkage is another possible explanation for the observed trend. Dong et al., in particular, studied H-benzimidazole-1,2,4-triazole Schiff bases with comments on the effect of

substituted benzaldehydes on the antifungal activity. Their study is directly relevant because the series they studied is based on the same modular concept, where the functionality of the imine electron donor is varied by changing just other terminal aromatics and not the principal heterocycle, the amino-triazole core.

The spectral data also are chemically sheltered with previous studies on triazoles. Typically, imine C=N absorption occurs in the 1600 to 1625 cm⁻¹ region, with downfield ¹H NMR signals of the equivalent region suggesting condensation, and the preservation of the triazole related signals indicating a preservation of the heteroaromatic core [25]. As mentioned earlier, 1,2,4-triazole derivatives have been established by means of similar combinations of FTIR, ¹H NMR, ¹³C NMR and mass spectrometry before they are subjected for any biological testing.

The MICs produced in the present investigation can be only compared as an in vitro comparative antimicrobial potency and not as a clinical equivalent. Even the most potent analogues in the tested conditions are not as potent as both ciprofloxacin or fluconazole. This is significant because effective heterocyclic discovery projects typically start with medium activity, and then continue to improve activity by hybridization, side-chain modifications, or in optimizing the physico-chemical properties. In order to achieve a more potent antimicrobial activity, Jiang et al adopted a more intricate triazole–thiadiazol hybrid approach demonstrating one logical path upon having defined a simpler parent scaffold.

Further examples in the literature on nitrogen containing heterocycles indicates that synthesis and biological optimization should be performed hand for hand. As for pyrazole, benzimidazole, pyrimidine and thiadiazol, it is found that efficient preparation does not necessarily yield biological success but rather is useful lead series that can be prepared reliably and repeatedly so as to allow systematic analogue comparison. This trend continues in the scaffold unrelated to triazole, with reviews of pyrazole chemistry and studies of analogues of benzimidazole.

From a methodological perspective, there are two strengths in the present workflow. Its first virtue is that it has a rather short series of one variable and can, therefore, be graphed without having to resort to other kind of series, so that biological trends may be interpreted instead of just put on a list. Second is that the MIC testing is integrated with concentration-response and time-course analysis, along with simple descriptor analysis. Saadon et al. also combined the biological and "in-silico" or "descriptor" viewpoints, in a heterocyclic antimicrobial work, showing how the physicochemical interpretation can contribute to value addition following the completion of the experimental activity.

Simultaneously, the plots of the descriptors will not be over-interpreted. Only ten analogues are not enough to provide a solid quantitative structure–activity relationship model, and the association between lipophilicity and activity can have a nonlinear form as too high a value can lead to poor aqueous solubility or to nonspecific binding. Presently the regression plots are exploratory. Their main significance is that they indicate which structural directions—halogenation, moderate lipophilicity and "balanced electron withdraw" are most promising for experimental development [26].

Last, the synthetic path is amenable of subsequent optimization. The amino-triazole intermediate can be condensed with a significantly broader range of aldehydes, and other activation procedures might be considered if reaction time/solvent usage becomes an issue. In recent microwave and biocatalytic studies in N-heterocycle synthesis it was demonstrated that the usage of more sustainable conditions does not show any compromise in the yields and can also have a positive effect on reducing processing times. The existing path, therefore, offers a medicinal-chemistry platform, as well as an easily accessible starting point for synthetic process improvement [26].

Conclusion

A structurally related series of ten nitrogen-containing heterocycles with a 1,2,4-triazole Schiff-base core was synthesized, characterized and subjected to a comprehensive antimicrobial evaluation approach. The synthetic route is modular, which is synthetically useful for the alteration of the aromatic substitutions without modifying the core triazole-thiol structure. The spectral data is consistent internally with the proposed structures and the biological profile shows that halogenated ones such as T6 and T10 are the most potent of the family. Activity seems to be better correlated with a combination

of polarizability, lipophilicity and electronic effects, than with a single analysis by any of the three descriptors of the SAR analyses. The results point to a specific course of action for additional analogues validation as well as for the second-generation analogue design, such as the introduction of other aldehyde substitutions, heteroaryls, and pharmacophore hybridization.

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