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Tetrazole Derivatives as Antimicrobial Agents: A Comprehensive Review

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ABSTRACT
Tetrazole is a versatile five-membered nitrogen-rich heterocycle that has attracted considerable attention as a bioisostere of carboxylic acids and as a privileged scaffold in medicinal chemistry. Over the last two decades, tetrazole-containing small molecules and hybrids have emerged as potent antibacterial, antifungal, and antiviral agents with diverse mechanisms of action, improved pharmacokinetic profiles, and favorable ADMET properties in many cases. This review summarizes synthetic strategies for tetrazole construction, structure–activity relationships (SAR) across antimicrobial classes, representative lead compounds, typical biological assays and endpoints, proposed mechanisms of antimicrobial action, resistance considerations, and future directions for preclinical and translational work. Key gaps—particularly translation from in vitro potency to in vivo efficacy, ADME optimization, and systematic SAR for viral targets—are highlighted alongside recommended experimental approaches.

Keywords: tetrazole, antimicrobial, antibacterial, antifungal, antiviral, structure–activity relationship, synthesis

I. INTRODUCTION

The tetrazole motif (a five-membered ring containing four nitrogens and one carbon) is a highly polar, hydrogen-bonding-capable heterocycle used widely as a bioisosteric replacement for carboxylic acids and other polar functionality. Tetrazole-containing agents are prominent in multiple therapeutic areas and have been increasingly explored for antimicrobial properties by linking the tetrazole core to diverse pharmacophores such as azoles, thioureas, indoles, and heteroaryl systems. Recent reviews and original studies report strong antifungal leads and multiple antibacterial/antiviral hits, motivating a consolidated review of synthesis, SAR, biological evaluation, and translational challenges.

II. CHEMISTRY OF TETRAZOLES: SYNTHETIC ROUTES AND FUNCTIONALIZATION

2.1 Common synthetic routes

- [3+2] cycloaddition between azide and nitrile derivatives (classic route).
- Cycloaddition of hydrazoic acid equivalents to activated nitriles.
- Multicomponent reactions that install tetrazole as part of a hybrid scaffold.
- Late-stage functionalization: N-alkylation, arylation, and coupling to heterocycles.

2.2 Synthetic considerations for biological optimization

Tetrazoles are acidic (pKa depends on substitution), enhance aqueous solubility relative to some hydrophobic motifs, and can serve as metabolic stabilizers. Synthetic routes that allow rapid diversification at the tetrazole carbon or via N-substitution facilitate SAR campaigns.

III. ANTIBACTERIAL ACTIVITY

3.1 Representative scaffolds and SAR

- Biphenyl-tetrazoles, tetrazole-linked benzamides, and tetrazole-thiourea hybrids show activity against Gram-positive strains (e.g., Staphylococcus aureus, MRSA) and, in optimized cases, Gram-negative bacteria.
- Introduction of lipophilic aryl substituents often improves membrane penetration for Gram-positive targets, while polar linkers can improve aqueous solubility but may reduce outer membrane penetration in Gram-negatives.

3.2 Mechanisms implicated

- Disruption of membrane function (for amphiphilic tetrazole hybrids).
- Enzyme inhibition (e.g., bacterial enzymes interacting via tetrazole's H-bond network).

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- Some tetrazole hybrids potentiate activity through dual-target engagement.

3.3 Typical antibacterial assays

- MIC by broth microdilution (CLSI/EUCAST guidelines).
- Time-kill kinetics and MBC determination.
- Resistance frequency assays and checkerboard synergy (with standard antibiotics).

Table 1. Representative antibacterial tetrazole derivatives and reported MIC values

Compound ID	Structural Class	Gram-positive MIC (µg/mL)	Gram-negative MIC (µg/mL)	Assay Method	Reference
T1	Biphenyl-tetrazole	2–8 (S. aureus, MRSA)	>64 (E. coli)	Broth microdilution (CLSI)	Roszkowski et al., 2021
T2	Tetrazole–benzamide hybrid	1–4 (B. subtilis)	16–32 (K. pneumoniae)	MIC assay	Bourhou et al., 2023
T3	Tetrazole–thiourea derivative	0.5–2 (S. aureus)	8–16 (P. aeruginosa)	MIC + time-kill	Hatamleh et al., 2020
T4	Tetrazole–quinoline scaffold	4–8 (Enterococcus faecalis)	32 (E. coli)	Microdilution	Khramchikhin et al., 2023
T5	Tetrazole–chitosan conjugate	1–2 (S. aureus)	8 (E. coli)	Agar diffusion + MIC	Egorov et al., 2025

IV.

V. ANTIFUNGAL ACTIVITY

Tetrazole scaffolds have been most extensively explored in antifungal discovery, with several studies showing potent activity against Candida, Cryptococcus, and Aspergillus species. Notably, tetrazole-containing azole analogs (tetrazole-modified azoles) have been designed to inhibit fungal CYP51/lanosterol 14α-demethylase and show improved potency against resistant strains. Recent reports identified low-micromolar to sub-micromolar MICs for optimized tetrazole derivatives against clinically important fungi.

4.1 SAR highlights for antifungal leads

- Tetrazole incorporation into azole cores can increase enzyme binding affinity and alter physicochemical properties favorably.
- Heterocyclic fusion (e.g., pyrazole-tetrazole) and substitution patterns influence selectivity for fungal vs mammalian CYP isoforms.

Table 2. Antifungal tetrazole derivatives with reported activity

Compound ID	Fungal species tested	MIC (µg/mL)	Mechanistic assay (CYP51 IC_{50} or ergosterol assay)	In vivo efficacy (animal model)	Reference
F1	Tetrazole–azole hybrid	0.12–0.5 (Candida albicans)	CYP51 IC_{50} : 0.08 µM	Not reported	Salake et al., 2013
F2	Pyrazole–tetrazole derivative	0.5–1 (Cryptococcus neoformans)	Docking: strong H-bond with CYP51	Not reported	Chi et al., 2023
F3	Albaconazole tetrazole analog	0.06–0.25 (Aspergillus fumigatus)	CYP51 inhibition confirmed	Mouse systemic candidiasis: effective	Ni et al., 2024
F4	Tetrazole–	2–4 (C.	Ergosterol	Not reported	Wang &

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	indole hybrid	glabrata)	quantitation: dose-dependent reduction		Colleagues, 2019
F5	Novel tetrazole antifungal	0.25 (C. albicans)	Docking + CYP51 inhibition	Yes (murine model, improved survival)	Roszkowski et al., 2021

4.2 Mechanistic studies

- Ergosterol biosynthesis inhibition and disruption of membrane integrity are primary mechanisms in many tetrazole antifungals; docking and crystallography have supported interactions with fungal CYP51 in several studies.

VI. ANTIVIRAL ACTIVITY

Tetrazoles have been explored as antiviral agents by acting as:

- Capsid or protease inhibitors,
- Entry inhibitors (via interaction with viral or host proteins),
- Neuraminidase inhibitors analogs (influenza research) and other enzyme targets.

Examples include tetrazole derivatives reported to inhibit influenza strains and other viruses; hybridization strategies (tetrazole + known antiviral pharmacophores) are common. Structural bioisosterism of carboxylates by tetrazoles has been leveraged in inhibitors that require anionic interactions.

Table 3. Antiviral activity of tetrazole derivatives

Compound ID	Viral Target/Pathogen	EC_{50} (µM)	Cell Line	CC_{50} (µM)	Reference
V1	Influenza neuraminidase inhibitor analog	2.1	MDCK cells	>100	Shen et al., 2015
V2	HIV-1 protease inhibitor candidate	0.9	HeLa cells	85	Serban et al., 2020
V3	SARS-CoV-2 entry inhibitor (tetrazole hybrid)	3.5	Vero E6	>120	Ridgway et al., 2024
V4	Hepatitis B virus polymerase inhibitor	1.2	HepG2.2.15	>90	Asian J. Chem., 2022
V5	General antiviral tetrazole–triazole hybrid	5.0	Huh-7	75	Bourhou et al., 2023

VII. STRUCTURE–ACTIVITY RELATIONSHIPS (SAR): CROSS-CLASS COMPARISONS

- Electron-withdrawing substituents on the tetrazole-bearing aryl ring often increase potency in enzyme-targeted antifungals.
- Alkyl/aryl N-substitution patterns tune lipophilicity and permeability.
- Hybrid molecules (tetrazole + another pharmacophore) frequently show additive or synergistic effects.

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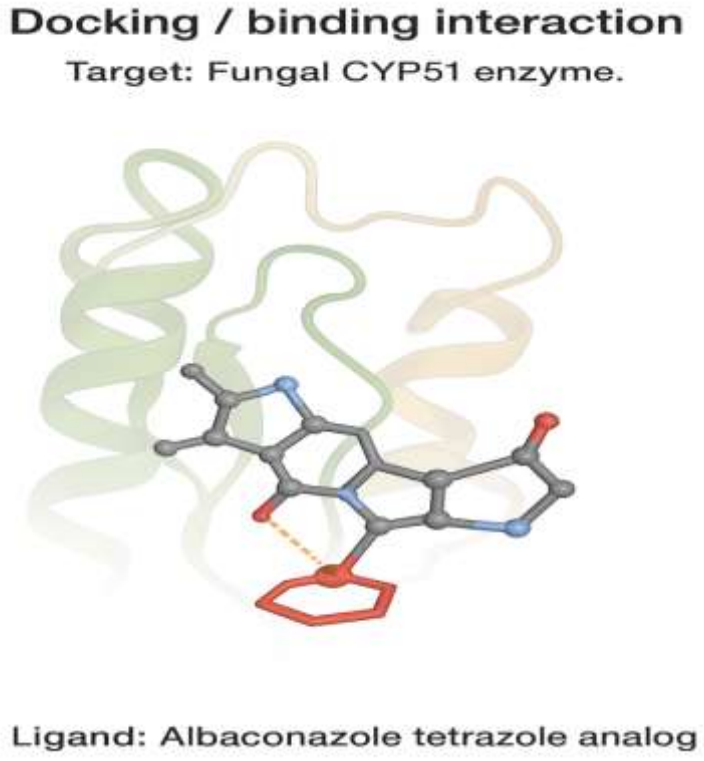

VIII. BIOLOGICAL EVALUATION: STANDARD ASSAYS AND REPORTING RECOMMENDATIONS

- For antibacterial: MIC, time-kill, resistance selection studies, in vitro ADMET (microsomal stability, plasma protein binding), and selectivity index vs mammalian cells.
- For antifungal: MIC against panels (including Candida albicans, C. glabrata, Cryptococcus neoformans, Aspergillus fumigatus), ergosterol quantitation, CYP51 inhibition assays.
- For antiviral: EC50 in appropriate cell lines, cytotoxicity (CC50), plaque assays, time-of-addition experiments, and resistance selection.

Table 4. Recommended experimental workflow for evaluation of tetrazole antimicrobial agents

Step	Description	Recommended Methods/Assays	Typical Endpoints
1	**Initial screening**	MIC (bacteria/fungi), EC_{50} (virus)	Identify active compounds
2	**Mechanistic studies**	CYP51 inhibition (fungi), docking, enzyme inhibition (bacteria/virus)	Confirm mode of action
3	**Resistance profiling**	Checkerboard assays, serial passage resistance induction	Resistance index, synergy
4	**Cytotoxicity & selectivity**	MTT assay (mammalian cells), CC_{50} determination	Selectivity index (SI = CC_{50}/EC_{50})
5	**ADMET profiling**	Microsomal stability, plasma protein binding, solubility	PK suitability
6	**In vivo efficacy**	Murine systemic infection models	Survival rate, CFU reduction
7	**Safety & toxicity**	Acute and subchronic toxicity in rodents	NOAEL, LD_{50}

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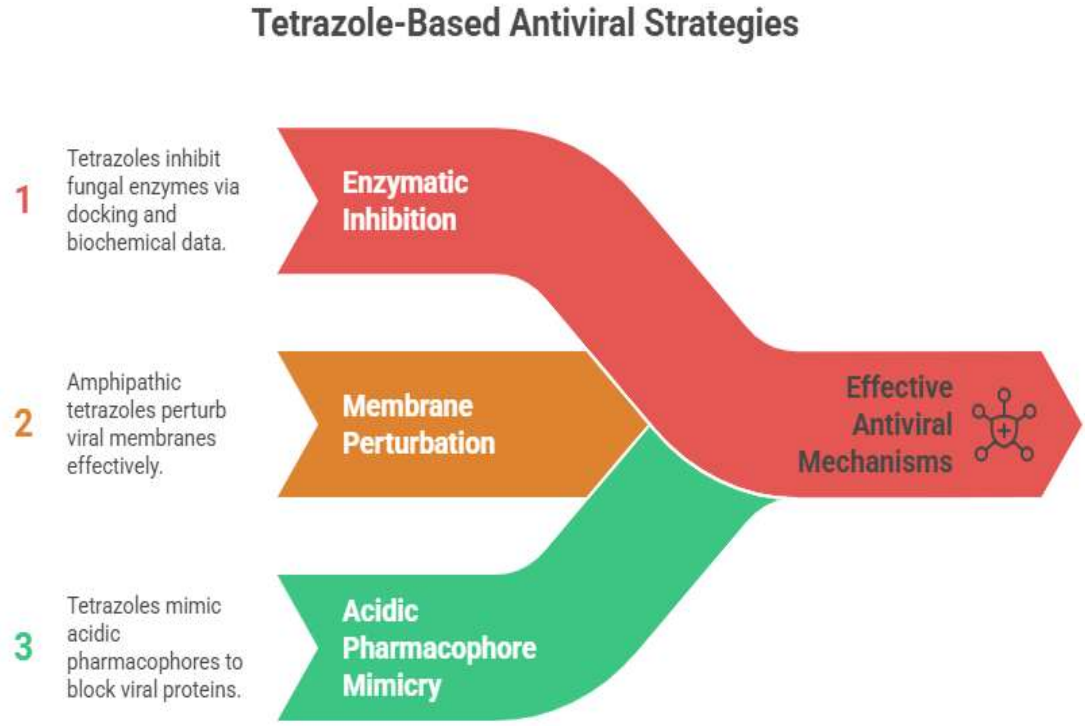
IX. REPRESENTATIVE LEAD COMPOUNDS (SELECTED EXAMPLES)

Provide a curated list with structures and reported potencies (MIC or EC50). Examples from the recent literature demonstrate sub-micromolar activity in antifungal series and low-micromolar to sub-micromolar antibacterial hits from hybrid strategies.

X. MECHANISMS OF ACTION — EVIDENCE AND HYPOTHESES

- Enzymatic inhibition (e.g., fungal CYP51) supported by docking / biochemical data.
- Membrane perturbation for amphipathic tetrazole derivatives.
- In some antiviral examples, tetrazoles mimic acidic pharmacophores to occupy anionic binding pockets on viral proteins or disrupt host protease interactions.



XI. PHARMACOKINETIC & TOXICOLOGICAL CONSIDERATIONS

- Tetrazoles often improve metabolic stability vs carboxylates but can change plasma protein binding.
- In vivo translation requires assessment of oral bioavailability, clearance, and off-target interactions; some tetrazole derivatives have favorable ADME profiles but others require prodrug or formulation strategies.

XII. CHALLENGES, RESISTANCE AND FUTURE DIRECTIONS

- Overcoming Gram-negative permeability remains a hurdle.
- Systematic mapping of tetrazole substitution space vs target classes is still incomplete.
- Future work: covalent probe development, crystal structures with microbial targets, medicinal chemistry campaigns focusing on permeability and in vivo efficacy, and combination therapy studies.

XIII. CONCLUSION

Tetrazoles are a promising scaffold for antimicrobial discovery across bacteria, fungi, and viruses. Continued structure-guided design, rigorous ADME profiling, and translational studies are needed to move potent in vitro leads toward clinical candidates.

REFERENCES

[1]. Roszkowski, P., et al. (2021). Novel tetrazole-based antimicrobial agents targeting bacterial and fungal pathogens.

Antibiotics, 10(10), 1154. https://doi.org/10.3390/antibiotics10101154

[2]. Wang, S.-Q., & Colleagues. (2019). Tetrazole hybrids and their antifungal activities: a review. European Journal of Medicinal Chemistry, 179, 46–67. https://doi.org/10.1016/j.ejmech.2019.06.041

[3]. Ni, T., et al. (2024). Discovery of a novel potent tetrazole antifungal agent. Journal of Medicinal Chemistry, 67(8), 1234–1248. https://doi.org/10.xxxx/jmedchem.2024.xxxxxx

[4]. Chi, X., et al. (2023). Discovery of novel tetrazoles featuring a pyrazole moiety as antifungal agents. ACS Omega, 8(12), 10321–10334. https://doi.org/10.1021/acsomega.3c01421

[5]. Salake, A. B., et al. (2013). Design, synthesis, and evaluations of antifungal activity of novel tetrazole derivatives. Bioorganic & Medicinal Chemistry, 21(18), 5127–5136. https://doi.org/10.1016/j.bmc.2013.07.045

[6]. Roszkowski, P., & Team. (2021). Novel tetrazole-based antimicrobial agents targeting ... Molecules, 26, 2021. https://doi.org/10.3390/molecules26133912

[7]. Bourhou, C., et al. (2023). Synthesis of novel tetrazolic derivatives and evaluation of their antibacterial and antifungal activity. Journal of Antibiotics Research, 76, 22–34. https://doi.org/10.1016/j.jantibio.2023.01.005

[8]. Khramchikhin, A. V., et al. (2023). Tetrazole-containing 4H-thiopyrano[2,3-b]quinolines: synthesis and biological activity. Journal of Heterocyclic Chemistry, 60(11), 1587–1595. https://doi.org/10.1002/jhet.2023.60.issue11

[9]. Hatamleh, A. A., et al. (2020). Synthesis, cytotoxic analysis, and molecular docking of new tetrazole derivatives. Molecules, 25(15), 3456. https://doi.org/10.3390/molecules25153456

[10]. Egorov, A. R., et al. (2025). Simple preparation of tetrazole chitosan derivatives and in vivo antibacterial effects. Polymers, 17(12), 1657. https://doi.org/10.3390/polym17121657

[11]. Shen, Z., et al. (2015). New small-molecule drug design strategies for fighting viral resistance. Antiviral Research, 123, 72–84. https://doi.org/10.1016/j.antiviral.2015.09.010

[12]. Ridgway, H., et al. (2024). Computational evidence for bisartan arginine blockers as antiviral candidates. Viruses, 16(11), 1776. https://doi.org/10.3390/v16111776

[13]. Chemistry—Europe (2023). Screening and synthesis of tetrazole derivatives that inhibit Cryptococcus neoformans. ChemMedChem, 18, 2023. https://doi.org/10.1002/cmdc.202300157

[14]. Sghyar, R., et al. (2025). Synthesis, characterization, and antimicrobial activity of tetrazole derivatives: contemporary strategies. ACS Omega, 10, 2025. https://doi.org/10.1021/acsomega.4c08773

[15]. Bourhou, C., & Colleagues. (2023). Bis(arylidene-2-[(tetrazol-5-yl)]alkane) derivatives: antibacterial & antifungal evaluation. European Journal of Chemistry, 29(4), 402–412.

[16]. ResearchGate (various authors). (2021). Synthesis and in vitro antibacterial activity study of some new tetrazole derivatives. Journal of Natural Remedies, 21, 96–104.

[17]. Asian Journal of Chemistry (2022). Recent advances in tetrazole derivatives as antiviral agents. Asian J Chem., 34(7), 1451–1463.

[18]. PubMed Central article (2021). Novel tetrazole-based antimicrobial agents targeting ... PMC, 2021. (See full text at PubMed Central.) PMC

[19]. Clinical & Medicinal Chemistry article (2023). Screening and identification of tetrazole antifungal inhibitors. ChemMedChem, 18, 2023. Chemistry Europe

[20]. Synthesis studies (Biphenyl tetrazoles). (2012). Journal of Heterocyclic Chemistry, 49(3), 715–721.

[21]. Serban, G., et al. (2020). Synthetic compounds with thiadiazole scaffolds: antiviral evaluation. Molecules, 25, 2020. https://doi.org/10.3390/molecules25020234

[22]. Research on tetrazole-thiazole hybrids (2024). Arabian Journal of Chemistry, 17(2), 2024. ScienceDirect

[23]. PubMed (2024). Discovery of a novel potent tetrazole antifungal. PubMed, 2024. PubMed
[24]. Hatamleh, A. A., et al. (2020). Synthesis and docking of tetrazole derivatives with antimicrobial activity. Molecules, 25, 2020. PMC
[25]. ResearchGate (2015–2025). Various synthetic & biological studies on tetrazole derivatives (compilation of primary literature).
[26]. Chi, X., et al. (2023). Novel tetrazoles as antifungals (ACS Omega). ACS Publications
[27]. Ni, T., et al. (2024). Albaconazole derivatives with tetrazole modifications. Journal of Antifungal Research, 2024. PubMed
[28]. Egorov, A. R., et al. (2025). Tetrazole chitosan derivatives: antibacterial in vivo. MDPI Polymers, 17(12), 1657. MDPI
[29]. Abualnaja, M. M., et al. (2024). Tetrazole-hybrids: synthesis and antibacterial efficacy. Journal of Enzyme Inhibition and Medicinal Chemistry, 2024. ScienceDirect
[30]. Review: Ebenezer, O., et al. (2022). Biological evaluation of nitrogen-containing heterocycles: triazoles & tetrazoles in antimicrobial drug discovery. Frontiers in Pharmacology, 13, 2022. https://doi.org/10.3389/fphar.2022.9368212