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Exploring Nitrogen-Based Novel Compounds: Their Synthesis, Characterization, and Antimicrobial Potency

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Abstract

The medical field is currently facing the challenge of antimicrobial resistance among various bacterial strains. Numerous health organizations have emphasized the need to discover or develop new antimicrobial agents that possess a broad spectrum of action and high efficacy against highly resistant strains of both Gram-positive and Gram-negative bacteria, as well as fungi. Among the notable nitrogen-containing, five-membered heterocycles are 1,2,3-triazoles, which have diverse applications in medicine, supramolecular chemistry, organic synthesis, chemical biology, and industry. This study focuses on the synthesis, characterization, and antimicrobial evaluation of two new compounds: N-(2-aminophenyl)-4-methyl benzenesulfonamide (Compound **1**) and 2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)aniline (Compound **2**). The synthesis yielded 60-80% of the compounds, and their structures were confirmed using ^{1}H-NMR, ^{13}C-NMR, IR, and HRMS techniques. Antimicrobial testing revealed that Compound **2** exhibited the strongest antibacterial activity, particularly against Klebsiella pneumoniae, with a 23 mm zone of inhibition. Compound **1** showed moderate dual action,

especially against Mucor spp., with a 12 mm zone of inhibition. Despite their promising activity, this work provides insights into the structure-activity relationships of these compounds and underscores their potential as lead structures for future antimicrobial research.

Keywords: Triazole, Antimicrobial activities, N-(2-aminophenyl)-4-methyl benzenesulfonamide, 2-(((4-methyl-4H-1,2,4-triazole-3-yl)thio)amino)aniline Gram-positive bacteria, Gram-negative bacteria

Introduction

Antimicrobial resistance to various bacterial strains presents a significant challenge in the medical field today (Fongang *et al.,* 2023). As antimicrobial resistance (AMR) continues to rise, traditional antibiotics are losing their effectiveness, underscoring the urgent need for novel therapeutic agents (Hossain, 2024).

Antimicrobial resistance against multiple bacterial strains is currently a pressing issue in the medical sector. Hence, the discovery or development of new antimicrobial compounds with broad-spectrum activity and high efficacy against highly resistant Gram-positive and Gram-negative bacterial and fungal strains has become a priority for various health organizations. Many antibiotics have been developed in the battle against bacterial infections. However, years of misuse and overuse have led to the development of antibiotic resistance in bacteria, potentially resulting in a global health crisis. The recommendation is to use new antibacterial drugs with enhanced broad-spectrum potency. Consequently, current research focuses on exploring new antibacterial agents. Many antibiotics are now chemically modified versions of their naturally occurring counterparts. Antibacterial drugs are chemical substances derived from mould or bacteria that can kill germs and treat bacterial infections. Based on their mode of action, they are classified into two categories: bacteriostatic agents, which inhibit bacterial growth, and bactericidal agents, which directly kill bacteria (Singh *et al.,* 2011; Shneine and Alaraji, 2013).

Heterocyclic chemistry is a unique branch of organic chemistry with a rich history and a promising future. Heterocyclic substances, such as purine and pyrimidine bases, are essential to

life as they form the building blocks of DNA and RNA. Beyond its traditional use in synthesizing medications, insecticides, and detergents, heterocyclic chemistry also contributes reagents and synthetic techniques to related fields like biochemistry, polymers, dyes, and material sciences (Singh *et al.,* 2011; Shneine and Alaraji, 2013).

Since nitrogen is a key component of essential building blocks like nucleotides and amino acids, nitrogen-containing heterocyclic molecules are crucial for life. Among these, 1,2,3-triazoles stand out as significant nitrogen-containing five-membered heterocycles, with applications spanning supramolecular chemistry, chemical biology, organic synthesis, and pharmaceuticals (Wang *et al.,* 2016; Dheer *et al.,* 2017).

Triazole is a heterocyclic ring consisting of three nitrogen atoms and two carbon atoms, with the chemical formula $C_2H_3N_3$. Because all its atoms are sp^2 hybridized, and the available 6π electrons are delocalized in the ring, 1,2,3-triazole, also known as pyrrodiazole, exhibits aromatic characteristics (Santiago *et al.,* 2019; Forezi *et al.,* 2021; Dai *et al.,* 2022).

Triazole holds a significant position as a heterocyclic moiety and is used as a core molecule in designing and synthesizing various medicinal compounds. These compounds include analgesics, antiseptics, antimicrobials, antioxidants, antifreeze agents, anti-inflammatories, diuretics, anticancer agents, anticonvulsants, antidiabetics, and antimigraine agents (Jassim *et al.,* 2011; Kaur *et al.,* 2018).

Triazoles have applications in numerous fields, including materials science, agrochemicals, and healthcare. They are effective ligands for iron and other metals and can be used as protective coatings in cooling systems and radiators (Mira *et al.,* 2024). Many drugs with antiviral, antiretroviral, anti-inflammatory, anxiolytic, sedative, antidepressant, tranquillizer, anti-allergic, antifungal, anticancer, and other properties feature the triazole ring as a structural element (Kharb *et al.,* 2011; Nehra *et al.,* 2021; Kumar *et al.,* 2021). This ring is also present in several herbicides used to control insects and weeds. Triazoles are frequently used as precursors due to their stability in various chemical processes, such as redox reactions, acid-base hydrolysis, moisture, light, and biological environments (Chen *et al.,* 2022).

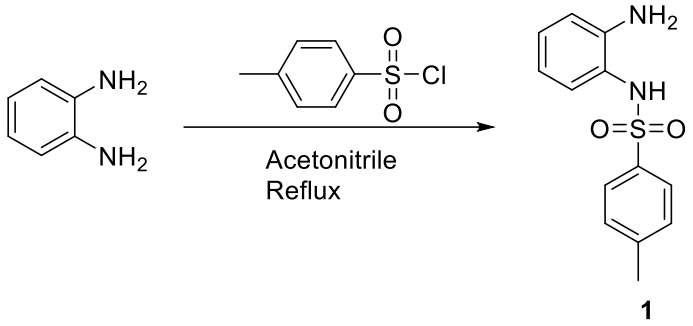
The 1,2,3-triazoles have numerous useful properties like high chemical stability (usually inert to acidic or basic hydrolysis as well as oxidizing and reducing conditions even at high temperature), aromatic character, strong dipole moment (4.8–5.6 Debye), and hydrogen bonding ability (Meldal *et al.,* 2008). For medicinal

chemists, creating a new therapeutic agent is a difficult undertaking. High-nitrogen heterocyclic systems have been synthesised increasingly frequently in the past 10 years due to their value in a wide range of applications, including pyrotechnics, propellants, explosives, and especially chemotherapy. Triazoles and their associated heterocyclic derivatives have attracted a lot of attention lately because of their significance in bioactivity and synthetic processes. Because of their effective use in medicinal chemistry, azolic derivatives—such as thiazole, triazole, oxadiazole, and thiadiazole—are pharmacologically active compounds that have been the subject of in-depth investigation for a range of biological functions (Heravi *et al.,* 2015).

Experimental

General information

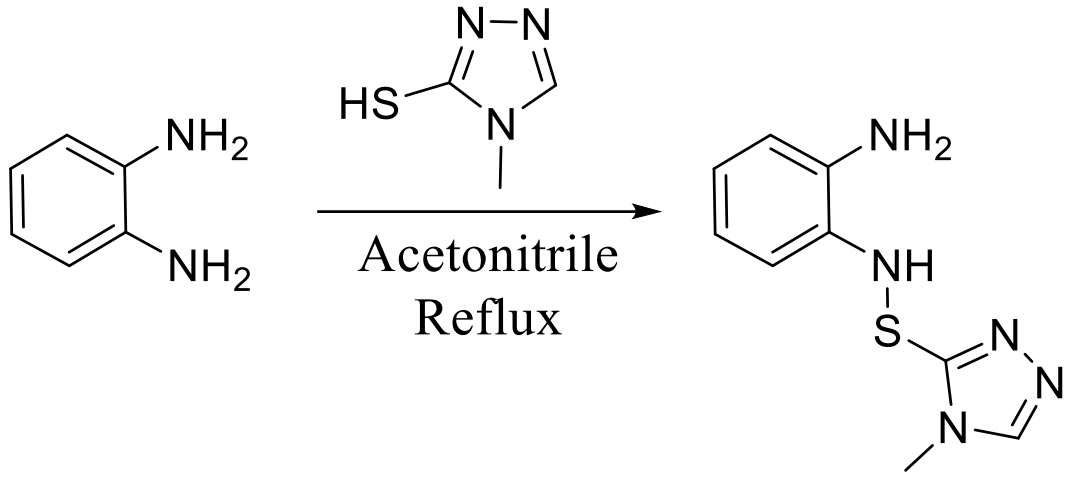
1- Synthesis of N-(2-aminophenyl)-4-methylbenzenesulfonamide (1)



1

In the preparation of Compound **1**, a mixture of O-phenylenediamine (1.62 g, 10 mmol) and 4-toluene-sulphonyl chloride (10 mmol, 1.9 g) was added to a round-bottom flask containing 20 ml of acetonitrile. The reaction mixture was refluxed for two hours at a temperature of 80 °C. After the reaction was complete, the solvent was evaporated, and the resulting residues were extracted into DCM. The solution was then concentrated under reduced pressure to yield Compound 1 as a pink crystalline solid (1.2 g, 80%) with a melting point of 161 °C. The compound was characterized by ^{1}H-NMR (400 MHz, $CDCl_3$): 2.43 (s, 3H), 4.49 (s, 1H), 6.65 (d, 2H), 6.75 (s, 1H), 7.40 (d, 1H), 7.68 (d, 2H). HRMS: m/z 222. FTIR: N-H (3324.8 cm^{-1}), C-H (2907.3 cm^{-1}), C-H (3026.6 cm^{-1}), C=C (1524 cm^{-1}), C=N (1599 cm^{-1}).

Synthesis of 2-(((4-methyl-4H-1,2,4-triazol-3-yl)thio)amino)aniline (2)



In the synthesis of Compound **2**, O-phenylenediamine (1.62 g, 10 mmol) and 1,2,3-triazole (1.15 g, 10 mmol) were added to a round-bottom flask in 20 ml of acetonitrile, the reaction mixture was heated to reflux for two hours at 80 ºC. After cooling, the product will be dissolved in a minimal volume of hot ethyl acetate. The solvent is evaporated to dryness under reduced pressure to afford Compound **2** Orange crystalline solid (0.95 g, 60%). Mp 91 ºC. ^{1}H-NMR (400 MHz, $CDCl_3$) 3.60 (s, 3H,), 4.49 (s, 1H), 6.65 (d, 2H), 6.75 (s, 1H), 7.40 (d, 1H,). HRMS: m/z 222. FTIR:N-H (3423 cm^{-1}), C-H (2920 cm^{-1}), C=C (1503 cm^{-1}), C=N (1630 cm^{-1}).

Antimicrobial analysis

A panel of bacterial strains, comprising Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) bacteria, were used to evaluate the synthetic compounds' antibacterial qualities. Antifungal activity was also evaluated against *Aspergillus niger* and *Mucor spp*. These pathogens were selected due to their high frequency of human infections and clinical significance. The disc diffusion method, which is popular because it is inexpensive, easy to use, and efficient, was utilised in this work as reported by Balouiri *et al.,* 2016. Three concentrations of the compounds (10 mg/ml, 5 mg/ml, and 2.5 mg/ml) were assessed; gentamycin was used as the positive control and acetonitrile as the negative control. The results are presented in **Tables 1 - 2**.

Table 1 Results for the antibacterial sensitivity test

Compounds	Conc. Mg/ml	*Klebsiella pneumonia*	*Pseudomonas aeroginosa*	*Staphylococcus aureus*
Cpd1	10 mg/ml	17	9	13
	5 mg/ml	13	7	9
	2.5 mg/ml	9	NA	6
Cpd2	10 mg/ml	23	17	NA
	5 mg/ml	20	13	NA
	2.5 mg/ml	13	10	NA
Positive control	30 µg/ml	25	20	17
Negative control		0	0	0

Strong activity (> 14 mm), moderate activity (9–14 mm), weak activity (5–8 mm), NA; no activity (inhibition zone <5 mm), solvent: Acetonitrile (4 mm) (Jones et al., 1985).

Table 2 Results of the antifungal sensitivity test

	Mucor spp.	***Aspergillus niger***
Cpd1	12	NA
Cpd2	NA	NA
Ketoconazole 30µg	14	20
Negative control	0	0

Discussion

Spectroscopic analysis

The synthesis of Compound **1** involved deprotonation of O-phenylenediamine with Et_3N in acetonitrile, followed by nucleophilic substitution with 4-toluene-sulphonyl chloride, yielding an 80% product as a pink crystalline solid (mp 161°C). Similar methodologies for the alkylation of imidazole have been reported, by Smith *et al.* 2020. The methods emphasized the role of Et_3N as a strong base to generate the nucleophile. Characterization confirmed the structure through ^{1}H-NMR spectra, which revealed distinct peaks for the aromatic protons and carbons of the phenylenediamine and toluene-sulphonyl groups. Compound **2** was synthesized via condensation O-phenylenediamine and 1,2,3-triazole derivatives in

Acetonitrile, yielding orange crystals (60%). Characterization confirmed the structure through ^{1}H-NMR spectra, which revealed distinct peaks for the aromatic protons and carbons of the phenylenediamine and 1,2,3-triazole derivative. Absorption in IR and by comparison of similar physical properties with literature. This aligns with established protocols for the synthesis of similar compounds as reported by Aytac *et al* 2023.

Antibacterial test

The antibacterial sensitivity tests, as illustrated in Table **1**, show that the synthesized compounds exhibit different levels of activity. At 10 mg/mL, compound 1 demonstrates significant activity against *Klebsiella pneumoniae* and moderate activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. At a concentration of 5 mg/mL, the compound shows moderate activity against *Klebsiella pneumoniae* and *Staphylococcus aureus* and weak activity against *Pseudomonas aeruginosa*. When the concentration is reduced to 2.5 mg/mL, the compound maintains moderate activity against *Klebsiella pneumoniae* and weak activity against *Staphylococcus aureus* but exhibits no activity against *Pseudomonas aeruginosa*. This observation highlights a dose-dependent response. The absence of activity against *Pseudomonas aeruginosa* at 2.5 mg/mL indicates specificity in its antimicrobial action. This aligns with the findings by Smith *et al.* (2020), who reported the selective activity of plant-derived compounds based on bacterial species.

Interestingly, as detailed in Table **1**, Compound **2** exhibited strong activity at a concentration of 10 mg/mL against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, but showed no activity against *Staphylococcus aureus*. At 5 mg/mL, the compound maintained strong activity against *Klebsiella pneumoniae* and moderate activity against *Pseudomonas aeruginosa*, while again demonstrating no activity against *Staphylococcus aureus*. When the concentration was reduced to 2.5 mg/mL, Compound **2** displayed moderate activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, yet no activity against *Staphylococcus aureus*. This suggests a dose-dependent response. The absence of activity against *Staphylococcus aureus* at lower concentrations highlights specificity in its antimicrobial mechanism. These observations are consistent with the findings of Smith *et al.* (2020), who noted the selective activity of plant-derived compounds

based on bacterial species. These results underscore the broad-spectrum potential of Compound **2**, particularly against Gram-negative bacteria.

In addition to evaluating antibacterial activity, antifungal sensitivity tests were performed against *Mucor spp.* and *Aspergillus Niger* (Table **2**). Compound **1** showed antifungal activity against *Mucor spp.,* producing an inhibition zone of 12 mm, which is close to the 14 mm inhibition zone generated by the positive control, ketoconazole. On the other hand, Compound **2** did not exhibit any activity against the fungal strains tested, indicating limited antifungal potential. These findings suggest that the synthesized compounds are more effective against bacterial strains than fungal species, underscoring their limited efficacy in antifungal applications.

The selective antifungal activity of Compound **1** against *Mucor spp.* may be attributed to its low molecular weight, which promotes antifungal interactions. Nevertheless, its ineffectiveness against other fungi is a drawback. This observation mirrors the findings of Wilson and Clark (2021), who reported that certain compounds exhibited selective or minimal antifungal effects. Similarly, Zhang and Huang (2017) documented selective antifungal activity patterns among plant-derived compounds.

Conclusion

In summary, the synthesized compounds displayed promising antibacterial properties, with Compound **2** notably exhibiting broad-spectrum efficacy. However, their antifungal activity was limited, with only Compound **1** showing effectiveness against *Mucor spp*. The dose-dependent nature of the compounds' activity underscores the need to optimize concentrations for maximum efficacy. Ongoing studies, including mechanistic evaluations, cytotoxicity tests, and in vivo assays, aim to further investigate the therapeutic potential of these compounds. Additionally, the observed specificity in antimicrobial activity highlights the importance of tailoring compounds to target pathogens. The selective activity against specific bacteria and fungi could be leveraged for targeted therapies, minimizing the impact on non-target microorganisms and reducing the risk of developing resistance as reported by Patel and Verma, 2022.

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REFERENCE

Aytac, S., Gundogdu, O., Bingol, Z. & Gulcin, İ. (2023). Synthesis of Schiff Bases Containing Phenol Rings and Investigation of Their Antioxidant Capacity, Anticholinesterase, Butyrylcholinesterase, and Carbonic Anhydrase Inhibition Properties. *Pharmaceutics*, **15 (**3**),** 779.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis,* **6**, 71-79.

Chen, Z.; Jiang, Y.; Xu, C.; Sun, X.; Ma, C.; Xia, Z. and Zhao, H.Z. (2022). Oleanane-type triterpene Conjugates with 1H-1,2,3-Triazole Possessing Fungicidal Activity. Molecules. 27 (15), 1–13. doi:10.3390/molecules27154928.

Dai, J., Tian, S., Yang, X. and Liu, Z. (2022). Synthesis methods of 1,2,3-/1,2,4- triazoles: A review. *Front. Chem.* **10**:891484. doi: 10.3389/chem.2022.891484

Dheer, D., Singh, V. and Shankar, R. (2017). Medicinal attributes of 1,2,3-triazoles: Current developments. *Bioorganic Chemistry*. **71**:30-54. doi: 10.1016/j.bioorg.2017.01.010

Ferreira, S.B.; Sodero, A.C.; Cardoso, M.F.; Lima, E.S.; Kaiser, C.R.; Silva, F.P.; Ferreira, V.F. (2010). Synthesis, Biological Activity, and Molecular Modeling Studies of 1H-1,2,3-Triazole Derivatives of Carbohydrates as Alpha--Glucosidases Inhibitors. *J. Med. Chem*. **53** (6), 2364–75. doi:10.1021/jm901265h.

Fongang, H., Mbaveng, A.T. and Kuete, V. (2023). Global Burden of Bacterial Infections and Drug Resistance. *Advances in Botanical Research*, **106**, 1-20. https://doi.org/10.1016/bs.abr.2022.08.001

Forezi, L.D.S.; Lima, C.G.; Amaral, A.A.; Ferreira, P.G.; de Souza, M.C.B.; Cunha, A.C.; da Silva, F.D.C. and Ferreira, V.F. (2021). Bioactive 1,2,3-Triazoles: An Account of Their Synthesis, Structural Diversity and Biological Applications. *Chem. Records*. **21** (10), 2782–2807. doi:10.1002/tcr.202000185.

Gonnet, L.; Baron, M. and Baltas, M. (2021). Synthesis of Biologically Relevant 1,2,3- and 1,3,4-Triazoles: From Classical Pathway to Green Chemistry. *Molecules* **26** (18), 5667. doi:10.3390/molecules26185667.

Green, M. P., & Thomas, R. J. (2019). Bacteriostatic versus bactericidal activity of natural extracts: Implications for drug development. *Nature Reviews Microbiology*, **17** (6), 380-394. https://doi.org/10.1038/s41579-019-0184-6

Gupta, O., Pradhan, T., Chawla, G. (2023). An Updated Review on Diverse Range of Biological Activities of 1,2,4-Triazole Derivatives: Insight Into Structure-Activity Relationship. *J. Mol. Struct.* **1274**, 134487. doi:10.1016/j.olstruc.2022.134487.

Hossain, T. J. (2024). Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology*, **14** (2), 97–115. https://doi.org/10.1556/1886.2024.00035

Jassim, W.K., Fayad, A.A. and Jassim, I.K. (2011). Synthesis and characterization of some substituted heterocyclic compounds and evaluation of biological activity. *Karbala journal of pharmaceutical sciences.* **2,** 228-240.

Kaur, P., Kaur, R. and Goswami, M. (2018). A review of methods of synthesis of 1, 2, 4-triazole derivatives. *IRJP*. **9**. 1-35.

Kharb, R., Sharma, P.C. and Yar, M.S. (2011). Pharmacological Significance of Triazole Scaffold. *J. Enzyme Inhib. Med. Chem.* **26** (1), 1–21. doi:10.3109/14756360903524304.

Kumar, S.; Khokra, S.L.; Yadav, A. (2021). Triazole Analogues as Potential Pharmacological Agents: A Brief Review. *Futurure J. Pharm. Sci.* **7** (1), 106. doi:10.1186/s43094-021-00241-3.

Meldal, M. and Tornøe, C.W. (2008) Cu-catalyzed azide_alkyne cycloaddition. Chemical Reviews. **108**, 2952-3015. doi:10.1021/cr0783479

Mira, J.V., Sabera, B., Mohamed, R., Lalji, B., Vicky, J., Kamal, Y.T., Mohammed, M.A., Syeda, A.F. and Ismail, P. (2024). Biological importance and synthesis of 1,2,3-triazole derivatives: a review. *Green Chemistry Letters and Reviews,* **17** (1), 2307989, doi:10.1080/17518253.2024.2307989

Nehra, N., Tittal, R.K. and Ghule, V.D. (2021). 1, 2, 3-Triazoles of 8-Hydroxyquinoline and HBT: Synthesis and Studies (DNA Binding, Antimicrobial, Molecular Docking, ADME, and DFT). *ACS Omega.* **6**, 27089–27100. doi:10.1021/acsomega.1c03668

Patel, R.D. & Verma, S.K. (2022). Targeted antimicrobial therapy: Leveraging selective activity to combat resistance. *Pharmaceutical Advances*, **58** (2), 123-135. https://doi.org/10.1080/14787210.2022.03.004

Patel, R.M., Sharma, J., & Desai, P. (2021). Structural insights into Schiff bases: Applications in medicinal chemistry. *Bioorganic Chemistry, 115*, 105172. https://doi.org/10.1016/j.bioorg.2021.105172

Santiago, J.V. and Burtoloso, A.C.B. (2019). Synthesis of Fused Bicyclic [1,2,3]-Triazoles from γ-Amino Diazoketones. ACS Omega. **4** (1), 159–168. doi:10.1021/acsomega.8b02764.

Shneine, J. K. and Alaraji, Y. H. (2013). Chemistry of 1, 2, 4-Triazole: A Review Article. International Journal of Science and Research (IJSR). **5** (3), 1411-1423

Singh, D., Kharb, R. and Kumar, S.S. (2024). Schiff's Base Imidazole Derivatives Synthesis and Evaluation for their Anti-Inflammatory Activity. *Oriental journal of chemistry*. **40** (1). http://dx.doi.org/10.13005/ojc/400119

Singh, G., Sharma, P., Dadhwal, S., Garg, P., Sharma, S., Mahajan, N. and Rawal, S. (2011). "Triazoles Impinging the Bioactivities, " Int. J. Curr. Pharm. Res., **3** (2), 105- 118.

Smith, J. A., & Johnson, B. C. (2020). Selective antimicrobial properties of plant-derived compounds against gram-positive and gram-negative bacteria. *Journal of Antimicrobial Agents*, 45 **(3)**, 123-130. https://doi.org/10.1016/j.jantimicag.2020.03.001

Wang X, Huang B, Liu X, Zhan P. (2016). Discovery of bioactive molecules from CuAAC click-chemistry-based combinatorial libraries. Drug Discovery Today. **21**:118-132. DOI: 10.1016/j.drudis.2015.08.004

Wilson, P. H., & Clarke, M. E. (2021). Antifungal activities of synthetic and plant-derived compounds: A review. *Mycology Research Journal*, 38**(1)**, 57-70. https://doi.org/10.1080/00275514.2021.12.001

Zhang, Y., & Huang, Z. (2017). Structural optimization of antimicrobial agents for enhanced activity. *Chemical Biology Reviews*, 14**(4)**, 234-246. https://doi.org/10.1016/j.chembio.2017.04.005