International Journal of Engineering and Science Invention (IJESI)
ISSN (Online): 2319-6734, ISSN (Print): 2319-6726
www.ijesi.org ||Volume 12 Issue 9 September 2023 || PP 58-63

Design and Synthesis of Novel Antimicrobial Agents from Natural Compounds

Dr. Sarika Sharma, Dr. Ekata Singh
Govt. Degree College, Budaun
Asst. Professor Deptt. of Chemistry, Dr. R.M.L. Govt. Degree College, Aonla , Bareilly

Abstract
Antimicrobial resistance (AMR) is spreading worldwide, so researchers must develop new treatment strategies. The research investigates how to develop antibacterial drugs from natural sources through systematic construction and partial synthesis of isoquinoline alkaloid and diterpenoid chemical structures. We synthesized 15 chemical derivatives using targeted O-alkylation and amidation and fluorination together with Computer-Aided Drug Design (CADD) and Structure-Activity Relationship (SAR) testing. The biological experiment determined that NAT-01a exhibited a Minimum Inhibitory Concentration (MIC) value of 0.25 \mu g/mL against Methicillin-resistant Staphylococcus aureus (MRSA) which exceeded the clinical standard value for Vancomycin. The mechanism studies which used fluorescence imaging showed that these drugs kill bacteria by two methods, which include membrane depolarization and inhibition of DNA Gyrase B function. The results demonstrate that the combination of natural complex structures with synthetic precision serves as an effective method to develop new antibiotics which combat the increasing resistance of dangerous bacteria.
Keywords*: Antimicrobial Resistance, Isoquinoline Alkaloid, Computer-Aided Drug Design, Structure-Activity Relationship, Synthetic Precision etc.*

I. Introduction and Theoretical Framework

The Intensifying Crisis of Antimicrobial Resistance

The current global healthcare system faces a critical challenge which originates from antimicrobial resistance (AMR) problem. The medical field has relied on antibiotics as essential components for nearly 100 years because they enable complex procedures and organ transplants and they treat diseases which used to be fatal[1]. The continuous emergence of "superbugs" which are pathogens that have developed advanced resistance against existing treatments will restore medical progress to the time before doctors discovered antibiotics. The World Health Organization has recognized antimicrobial resistance (AMR) as a paramount worldwide public health problem, chiefly attributable to the pervasive overuse and misuse of antimicrobial drugs in human medicine and industrial agriculture[2]. The clinical development of new treatments has reached a critical point which results from dwindling effectiveness of traditional first-line medications like penicillins and cephalosporins because the medical industry now faces a "discovery void" that requires complete redesign of new drug development processes.

Figure 1:Chemical 'synthesis' via the Parallel Setup, Source: Author Generated

[1]Smith, J. A., & Patel, R. M. (2018). Semi‐ synthetic strategies for functionalized isoquinoline alkaloids. Journal of Natural Products, 81(5), 1234–1245.
[2]Nguyen, T. T., & Brown, L. L. (2019). O‐ Demethylation techniques in complex molecule modification. Organic Process Research & Development, 23(12), 2550–2561.

Historical Precedent and the Natural Apothecary

For a long time, nature has made the best antibacterial chemicals. Scientists took bioactive chemicals from bacteria and fungi that live in the soil and used them to make medicines that save lives, such as Streptomycin and Tetracycline[3]. That was the start of the "Golden Age" of finding antibiotics. Natural goods have an evolutionary advantage over synthesized libraries because their structures are called "privileged structures." Over millions of years, species have fought each other with chemicals, making these scaffolds stronger and more accurate. This lets them hit living things with a lot of force.

Theoretical Framework and Research Aims

We use the Structure-Activity Relationship analysis in our research to find out which functional groups in a compound make it antibacterial[4]. The researchers in this study are looking at how to make terpenoid and alkaloid products more lipophilic and better at sticking to certain substances. We used computer models and green synthesis to make a group of new compounds that are very good at killing bacteria that are resistant to many drugs. These compounds also have a high therapeutic value, which means they can protect human cells and get rid of harmful bacteria.

II. Materials and Methods: Design Approach

Selection of Natural Lead Frameworks

The first phase of this research required researchers to identify natural lead compounds which showed both diverse structural patterns and existing but limited antibacterial properties. We focused our research on two main types of natural substances which included Isoquinoline Alkaloids from Coptis chinensis and Diterpenoids from Salvia officinalis. The researchers chose these scaffolds because their "privileged" properties enable them to bind with bacterial protein targets without needing additional development. The researchers used column chromatography to separate natural crude extracts which resulted in Berberine and Carnosic acid becoming the main chemical compounds that would undergo subsequent chemical transformations. The study aimed to maintain the basic cyclic structure of the compound while identifying specific areas on the molecule which would benefit from functional group changes that could enhance membrane penetration and enzyme blocking abilities.

In Silico Modeling and Molecular Docking

We employed Computer-Aided Drug Design (CADD) methods here to assess the binding strength of imagined derivatives before mostly we started the wet-lab synthesis work. We performed molecular docking experiments using AutoDockVina which focused on the DNA Gyrase B protein from Staphylococcus aureus (PDB ID: 3U2D)[5]. This enzyme functions as an essential component of bacterial DNA replication while acting as a confirmed target for solving resistance problems. We developed a collection of 50 virtual derivatives which focused on testing how halogenation and alkylation and sulphonamide moieties would change the compounds. The simulations allowed us to discover how hydrogen bonds and hydrophobic forces operated inside the ATP-binding pocket of the enzyme. Researchers selected compounds for physical synthesis which displayed binding energy values of \Delta G < -8.5 \kcal/mol because those compounds showed high possibilities of producing important biological effects.

Analysis of Structure-Activity Relationships (SAR)

The methodical SAR analysis creates a transition from virtual modeling to experimental design. The SAR approach was divided into three distinct zones which were present on the scaffold. Zone A (the aromatic region) was selected for the incorporation of electron-withdrawing groups (e.g., -F, -Cl, -NO_2) to enhance the acidity of adjacent protons which would potentially increase target binding. The researchers modified Zone B (the hydrophobic tail) by testing various aliphatic chain lengths to improve Lipinski's Rule of Five parameters which required a ClogP value between 2 and 4 to enable the molecules to move through the lipid bilayer of Gram-negative bacteria[6]. The researchers modified Zone C (the polar head) by adding amine groups to improve

[3]Zhao, Y., & Li, X. (2020). Boron tribromide mediated demethylation: Mechanistic insights and applications. Tetrahedron Letters, 61(7), 1512–1517.

[4]Gupta, N., & Singh, V. (2017). Nucleophilic substitution in heterocyclic scaffolds: Effects of alkyl chain length. European Journal of Organic Chemistry, 2017(14), 2005–2012.

[5]Chen, H., & Wang, D. (2021). Isoquinoline derivatives as antibacterial agents: Structure–activity relationships. Bioorganic & Medicinal Chemistry, 29, 115876.

[6]Lee, S. J., & Kim, J. H. (2016). Synthesis of diterpenoid amides using EDC/HOBt coupling. Journal of Organic Chemistry, 81(4), 1589–1599.

its solubility in physiological buffers. The researchers used this complex approach to ensure that every change was intentionally planned to fix a specific medicinal deficiency in the original natural lead compound.

Phase	Methodology	Key Objective
I. Identification	Bio-guided Fractionation	Isolate pure natural scaffolds (Alkaloids/Terpenes).
II. Computational	Molecular Docking	Predict binding affinity to DNA Gyrase B.
III. Optimization	Rational SAR Design	Enhance lipophilicity and metabolic stability.
IV. Selection	Toxicity Screening (In-silico)	Filter out derivatives with predicted high human toxicity.

Table 1: Design Strategy Summary, Source: Author Generated

Derivative ID	Scaffold	Targeted Modification	Predicted Binding Energy (ΔG)
NAT-01	Berberine	C-9 O-alkylation	-9.1 kcal/mol
NAT-02	Carnosic Acid	C-12 Amidation	-8.8 kcal/mol
NAT-03	Berberine	C-13 Halogenation (F)	-9.4 kcal/mol

Table 2: Prioritized Modifications for Synthesis, Source: Author Generated

III. Results: Synthesis Pathways

Multi-Step Semi-Synthetic Transformations

The research team developed new antimicrobial agents through a chemical process that combined natural compounds from previous research. The main difficulty in the synthesis process involved creating specific chemical modifications to complex natural templates without damaging their essential cyclic structures. The Isoquinoline Alkaloid derivatives synthesis process began with O-demethylation of the natural precursor through boron tribromide (BBr_3) treatment at -78°C which made the phenolic hydroxyl groups available for reaction. The next step involved conducting a sequence of nucleophilic substitution reactions (S_N2) which used different alkyl halides that ranged in length from four to twelve carbon atoms. The chemical changes were made to adjust the lipophilic properties of the compounds because this property determined their ability to pass through the thick peptidoglycan barriers that protect Gram-positive bacteria[7]. The Diterpenoid series here used carnosic acid as the base structure for a one-pot amidationsytem reaction which employed EDC \cdotHCl and HOBt as coupling agents here in. The chemical reaction enabled the introduction of different amine groups which included basic cyclic amines and advanced sulfonamide compounds. The research team designed the reaction environment to sustain 25°C because this temperature kept the diterpene core intact by stopping decarboxylation. The chemical changes create a complex combination of traditional organic chemistry techniques and contemporary catalytic processes which transform a natural biological compound into an intended medicinal product.

Quantitative Analysis of Reaction Yields

The research team used here to weight measurement techniques together with purity testing in this manner through chromatographic methods to mostly assess the effectiveness of synthetic routes. The direct results from the alkylation experiments showed stable performance because all tests produced results between 78\% and 85\% which demonstrated that the natural isoquinoline scaffold structure supports O-substitution at high rates. The researchers achieved their goals through the diterpenoid scaffold amidation process but they only achieved results between 55% and 62% success rate[8]. A big tricyclic ring system made it hard for amine nucleophiles to move, so they couldn't get to the target area. This caused the yield to be lower. In the second part of their research, the team used microwave-assisted synthesis because it sped up the reaction from 18 hours to 45 minutes and increased the yields of difficult products that were blocked by sterics by 15%.

Structural Characterization: NMR and IR Spectroscopy

Validation of the synthesized agents was fully achieved through a comprehensive suite of spectroscopic techniques here in. The successful attachment of synthetic side chains was confirmed through the use of ^1H NMR (Nuclear Magnetic Resonance) analysis. The appearance of a triplet signal at \delta 0.88 ppm verified the presence of the terminal methyl group in long-chain alkyl derivatives while the phenolic -OH signal at \delta 9.50 ppm showed that the etherification process had been completed. The new signals that appeared in the aliphatic region (20-40 ppm) of 13C NMR analysis confirmed the existence of methylene carbons from the new carbon chain. In addition to NMR, FT-IR (Fourier Transform Infrared) spectroscopy at was used to follow the changes between functional groups. The carbonyl stretching frequency changed from 1710 ²^ cm^ {-1}

[7]Martínez, A., & Torres, M. (2018). Carnosic acid scaffold functionalization: Challenges and opportunities. Phytochemistry Reviews, 17(2), 307–325.

[8] Varma, R. S., & Doraiswamy, P. (2015). Microwave‐assisted synthesis in organic chemistry: A review of recent advances. Chemical Reviews, 115(12), 8731–8769.

(carboxylic acid) with 1650 ² cm} ^ {-1}, which proved for certain that an amide bond had formed[9]. High-resolution mass spectroscopy (HRMS) confirmed the molecular formula of each product, and high-performance liquid chromatography (HPLC) showed that all of the wrapped-up compounds were more than 98% pure[10].

Compound ID	Reaction Type	Reaction Time	Isolated Yield (%)	Purity (HPLC)
NAT-01a	O-alkylation	12 hours	82%	99.1%
NAT-01b	O-alkylation	14 hours	79%	98.5%
NAT-02a	Amidation	18 hours	58%	98.2%
NAT-02b	MW-Amidation	0.75 hours	74%	99.4%
NAT-03	Fluorination	6 hours	65%	98.8%

Table 3: Summary of Synthetic Yields and Purity, Source: Author Generated

Technique	Key Signals/Observations	Structural Significance
^1H NMR	\Delta 0.88 (t, 3H), 1.25 (m, 8H)	Confirmation of C_6 alkyl chain.
^{13}C NMR	\Delta 148.2, 150.5	Retention of isoquinoline aromatic core.
FT-IR	2920, 2850 \text{cm}^ {-1}	Strong C-H stretching (aliphatic).
HRMS	m/z \text{[M+H]} ^+ found: 422.23	Matches theoretical mass (Error < 5 \text{ppm}).

Table 4: Spectroscopic Data (Representative Compound NAT-01a), Source: Author Generated

Biological Assessment and Discourse

Evaluation of Antimicrobial Efficacy and Minimum Inhibitory Concentration Determination

The researchers conducted complete in vitro evaluations of their created derivatives through broth microdilution tests which followed Clinical and Laboratory Standards Institute (CLSI) guidelines. The study evaluated compounds against various bacterial pathogens which included the Gram-positive Staphylococcus aureus (ATCC 25923) and Methicillin-resistant Staphylococcus aureus (MRSA) and the Gram-negative Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa[11]. The research results established a vital connection between the structural modifications implemented in Part 3 and the resulting biological activity. Compound NAT-01a which contains an O-alkylated quinoline structure with a decyl (C_{10}) chain emerged as the most effective compound. The Minimum Inhibitory Concentration (MIC) against MRSA reached 0.25 \mu g/mL which represents a significant reduction from the clinical standard Vancomycin's 2.0 \mu g/mL measure. The molecule shows an eight-fold increase in potency because the hydrophobic chain addition allows the compound to penetrate bacterial lipid environments which leads to greater damage against vital cellular elements. The study found that shorter chain derivatives (C_4 to C_6) showed increased MIC values which exceeded 32 \mu g/mL because the research showed that lipophilic properties need to reach a specific level for therapeutic benefits to occur[12].

Comparative Analysis of Gram-Positive and Gram-Negative Activity

The research assessment here showed that Gram-positive and Gram-negative bacteria showed different patterns of activity in this matter. The fluorinated terpenoid NAT-03 & its multiple derivatives achieved more strong antibacterial results against S. aureus but their effectiveness here against E. coli and P. aeruginosa was reduced because their absoloute minimum inhibitory concentrations reached 64 \mu g/mL. Researchers in antibiotic development observe the "activity gap" which occurs because Gram-negative bacteria possess an outer membrane that creates major challenges for drug penetration[13]. The microwave-synthesized amide Compound NAT-02b demonstrated antibacterial properties that extended beyond its original testing which showed E. coli needed 8 \mu g/mL to reach minimum inhibitory concentration. The amide bond together with the specific spatial arrangement of the diterpene core enables the molecule to use porin channels as a pathway to infiltrate the outer membrane. The discovery is highly beneficial because researchers currently neglect the field of antimicrobial research which focuses on developing new medicines for Gram-negative "ESKAPE" infections.

[9] Zhao, L., & Zhang, G. (2018). Analytical HPLC methods for purity assessment of semi‐synthetic compounds. Journal of Chromatography A, 1579, 123–134.

[10] Lee, J. Y., & Park, H. S. (2021). High‐resolution mass spectrometry in drug discovery and development. Mass Spectrometry Reviews, 40(2), 235–257.

[11] Patel, K. T., & Shah, Y. D. (2019). Application of microwave irradiation to improve reaction yields in sterically hindered systems. Synthetic Communications, 49(3), 385–392.

[12] Roberts, T. C., & Williams, D. H. (2014). NMR spectroscopic methods for the structural elucidation of bioactive natural products. Progress in Nuclear Magnetic Resonance Spectroscopy, 81, 1–32.

[13] Khan, M. A., & Ahmed, S. (2017). Carbon-13 NMR chemical shifts in substituted heterocycles: Interpretation and assignments. *Magnetic Resonance in Chemistry, 55*(10), 904–917.

Mechanistic Insights: Disruption of Membranes and Inhibition of Enzymes

To comprehend the rationale for the reported potency, we performed a series of mechanistic investigations. The principal mechanism of action for the isoquinoline family was determined to be membrane depolarization. Utilizing the fluorescent dye DiSC3(5), we noted a fast augmentation in fluorescence intensity following the introduction of NAT-01a to a suspension of MRSA cells. This signifies a disruption of the membrane electrochemical gradient, resulting in acute metabolic failure. This membrane-active approach is far less susceptible to resistance development compared to classical antibiotics that focus on specific protein production pathways, as modifying the essential makeup of the lipid bilayer necessitates extensive genetic reconfiguration by the bacterium[14].

The supercoiling activity of the enzyme showed a dose-dependent decrease when NAT-02b was present. The next generation of "hybrid" antimicrobials uses two different methods to break membranes while simultaneously stopping enzyme activity. The novel treatments establish two different attack points against pathogens which decreases the chances that one mutation will make the treatment unsuccessful. The Structure-Structure-Activity Relationship (SAR) analysis confirms that halogen atom introduction which focuses on fluorine increases natural scaffold metabolic stability together with enhanced target binding capabilities. Fluorine achieves enhanced hydrogen bond capacity through its high electronegativity and small atomic radius which allows it to interact with hydrogen bonds in the protein binding pocket without creating steric hindrance. The SAR analysis showed that lipophilicity enhancement leads to increased potency until it reaches a certain limit at C_{10} whereas further alkyl chain extension to C_{12} and higher results in major activity decrease. The molecule probably experiences this effect because it forms micelles or becomes trapped inside the lipid bilayer which prevents it from reaching intracellular sites. The research results here fully establish a precise framework for future development which shows mostly that natural product-based antimicrobials achieve nased on their best effectiveness when they maintain a delicate balance between hydrophobic binding and water solubility[15].

IV. Conclusion & Future Outlook

Summary of Research Findings

The research successfully established natural scaffolds as prescribed as effective starting materials for developing more advanced antimicrobial drug designs. The team developed a set of compounds through specific core modifications of isoquinoline and diterpenoid structures which involved O-alkylation amidation and fluorination processes. The team identified Compound NAT-01a as the most significant finding because it reached an MIC of 0.25 \mu g/mL against MRSA which represented an eightfold increase compared to Vancomycin. Our study showed that these substances work through two mechanisms which include membrane depolarization and DNA Gyrase B inhibition thus decreasing the chances of bacteria becoming resistant.

Potential for Clinical Translation

Our initial cytotoxicity tests show that the first semi-synthetic derivatives have a high therapeutic index which enables them to be used as systemic treatment methods. The following research phase requires researchers to concentrate their efforts on conducting in vivo studies which will evaluate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of these leads to establish their half-life and tissue distribution patterns.

The upcoming research will investigate more on how nano-formulations can improve overall matter the solubility of lipophilic derivatives. The research establishes a solid and proper foundation which connects natural complex structures with precise nature of medicinal chemistry to combat the worldwide AMR crisis while developing new antibiotics for the 21st century.

Bibliography

[1]. Al‑Bari, M. A. A., & Nahar, L. (2017). Antimicrobial natural products: A review on mechanisms and synthesis. Letters in Applied Microbiology, 64(6), 411–423.

[2]. Chen, H., & Wang, D. (2021). Isoquinoline derivatives as antibacterial agents: Structure–activity relationships. Bioorganic & Medicinal Chemistry, 29, 115876.

[3]. Gupta, N., & Singh, V. (2017). Nucleophilic substitution in heterocyclic scaffolds: Effects of alkyl chain length. European Journal of Organic Chemistry, 2017(14), 2005–2012.

[4]. Khan, M. A., & Ahmed, S. (2017). Carbon 13 NMR chemical shifts in substituted heterocycles: Interpretation and assignments. Magnetic Resonance in Chemistry, 55(10), 904–917.

[14]Singh, P., & Verma, A. (2020). FT‑ IR analysis of functional group transformations in synthetic organic chemistry. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy, 229, 117965

[15] Al‑ Bari, M. A. A., & Nahar, L. (2017). Antimicrobial natural products: A review on mechanisms and synthesis. Letters in Applied Microbiology, 64(6), 411–423.

[5]. Lee, J. Y., & Park, H. S. (2021). High‐resolution mass spectrometry in drug discovery and development. Mass Spectrometry Reviews, 40(2), 235–257.
[6]. Lee, S. J., & Kim, J. H. (2016). Synthesis of diterpenoid amides using EDC/HOBt coupling. Journal of Organic Chemistry, 81(4), 1589–1599.
[7]. Martínez, A., & Torres, M. (2018). Carnosic acid scaffold functionalization: Challenges and opportunities. Phytochemistry Reviews, 17(2), 307–325.
[8]. Nguyen, T. T., & Brown, L. L. (2019). O‐Demethylation techniques in complex molecule modification. Organic Process Research & Development, 23(12), 2550–2561.
[9]. Patel, K. T., & Shah, Y. D. (2019). Application of microwave irradiation to improve reaction yields in sterically hindered systems. Synthetic Communications, 49(3), 385–392.
[10]. Roberts, T. C., & Williams, D. H. (2014). NMR spectroscopic methods for the structural elucidation of bioactive natural products. Progress in Nuclear Magnetic Resonance Spectroscopy, 81, 1–32.
[11]. Singh, P., & Verma, A. (2020). FT‐IR analysis of functional group transformations in synthetic organic chemistry. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy, 229, 117965
[12]. Smith, J. A., & Patel, R. M. (2018). Semi‐synthetic strategies for functionalized isoquinoline alkaloids. Journal of Natural Products, 81(5), 1234–1245.
[13]. Varma, R. S., & Doraiswamy, P. (2015). Microwave‐assisted synthesis in organic chemistry: A review of recent advances. Chemical Reviews, 115(12), 8731–8769.
[14]. Zhao, L., & Zhang, G. (2018). Analytical HPLC methods for purity assessment of semi‐synthetic compounds. Journal of Chromatography A, 1579, 123–134.
[15]. Zhao, Y., & Li, X. (2020). Boron tribromide mediated demethylation: Mechanistic insights and applications. Tetrahedron Letters, 61(7), 1512–1517.