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Comparative Study of Procaine and Novobiocin: From Chemical Synthesis and Natural Extraction to *In Silico* Pharmacokinetic Profiling

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Abstract:

This review report presents a comparative study of two pharmacologically significant molecules usually used as local anesthetics and antibiotics. The two molecules, namely, Procaine (synthetic local anesthetic) and Novobiocin (natural aminocoumarin antibiotic), have been known for decades for their anesthetic and antibiotic properties. Procaine represents a milestone in medicinal chemistry, serving as the establishment point for a generation of less harmful anesthetics like lidocaine and articaine by simplifying the toxic structure of cocaine. Novobiocin, isolated from *Streptomyces niveus*, illustrates the structural intricacy and potent biological activity of microbial secondary metabolites. The study considers their expedition from historical discovery to modern algorithmic analysis. The aim of this work is to analyze and compare the chemical, pharmacological, and computational profiles of these two molecules using in silico prognostic tools such as SwissADME and ProTox-3 to evaluate their physicochemical properties, pharmacokinetics, and toxicological profiles. The findings show that while Procaine strictly adheres to Lipinski's Rule of five with high gastrointestinal absorption and oral bioavailability, Novobiocin's greater molecular weight (612.62g/mol) and high polarity (200.01Å²) result in several drug-likeness violations, which is typical of complex natural products. Toxicological modeling classified both molecules as Class 3/5 acute toxins (LD_{50} values between 175 and 5000 mg/kg) and highlighted a high probability of immunotoxicity (0.99), consistent with known clinical hypersensitivity risks. Ultimately, this study accentuates the necessity of combining computational screening with empirical testing and demonstrates the structural optimization of natural lead molecules to promote innovation in the pharmaceutical industry.

Keywords: *Procaine, Novobiocin, Medicinal Chemistry, Pharmacokinetics and Toxicology.*

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1. Introduction

The health issue is a universal matter, to save mankind by proposing solutions to quire diseases, by using medicinal chemistry (Waring *et al.*, 2002; Bhat *et al.*, 2021; Diass *et al.*, 2021; Abbaoui *et al.*, 2024). The field of medicinal chemistry is built upon a subtle balance between natural product discovery and

synthetic innovation (Ahmed & Jayaram, 2024). Throughout the history of pharmacology, scientists have continuously sought to either isolate potent bioactive compounds from natural sources or synthesize novel analogs to increase efficiency and reduce toxicity (Aniulis *et al.*, 2003). This twofold approach is key to addressing the changing issues in modern medicine, from the emergence of multidrug-resistant pathogens to the need for safer, targeted anesthetics (Nsele *et al.*, 2024; Sinclair *et al.*, 2025; Conboy-Stephenson *et al.*, 2025; Cheng *et al.*, 2023). This report focuses on two distinct and highly substantial molecules: Novobiocin and *Procaine* (Longombe *et al.*, 2022;Mair *et al.*, 2026). While both serve a very important medical purpose, they represent two different pathways in their pharmaceutical discovery. Procaine, a synthetic amino ester derivative, was developed in 1905 as a safer, non-addictive alternative to naturally occurring cocaine. *Novobiocin*, by contrast, is a classic natural product isolated from *Streptomyces niveus*, which played a vital role in the "Golden Age of Antibiotics" as a potent antibacterial agent against resistant strains (Hristova *et al.*, 2024; Attar *et al.*, 2025; Chopra *et al.*, 2025). This report focuses on two distinct and highly significant molecules: Procaine and Novobiocin. Although both molecules fulfill essential medical purposes, they represent different pathways in their pharmaceutical discovery (Berry *et al.*, 1997; Brunton *et al.*, 2012).

Procaine is a synthetic amino ester derivative of tropaine alkaloid that was developed in 1905 as a safer, non-additive alternative to naturally occurring cocaine (Hristova *et al*., 2024), and on the other hand, Novobiocin is a natural product compound isolated from *Streptomyces niveus*, which plays a vital role in the Golden Age of antibiotics by acting as a strong antibacterial agent against microbial resistant strains (Hristova *et al*., 2024; Attar *et al*, 2025). This mini review is following other works concerning medicine drugs containing pyrazole moieties (Chkaif, *et al.*, 2026; Lazrek, *et al.*, 2026, Akabli, *et al.*, 2026; Foufa, *et al.*, 2025, Snoussi, *et al.*, 2025). The aim of this study is to analyze and compare the physicochemical, pharmacological, and computational profiles of these two molecules. The specific goals include:

1. Review the historical progression and pharmacological origin of both molecules.
2. Examine their molecular structures and methods of extraction and synthesis.
3. Examine the biological activities and applications of the two molecules.
4. Evaluate their pharmacokinetic properties and toxicity profiles using computational tools (in *silico* methods such as SwissADME and Protox3).
5. Identifies research gaps and opportunities and provides guidance for future studies and potential drug discovery efforts using the two molecules.

Through this study, the review aims to contribute to the discussion of sustainability by bridging the gap among organic chemistry, pharmacology, and computational toxicology, delivering a comprehensive understanding of how these molecules function and interact within biological systems (Eddy *et al.*, 2023; Epstein *et al.*, 2023; Ismail *et al.*, 2018; Karunakar *et al.*, 2026).

2. Materials and methods

2.1 Material

This study used computational tools, scientific databases, and chemical structures of the selected compounds, Procaine and Novobiocin, to investigate their physicochemical, pharmacokinetic, and toxicological properties.

a. Computational Platforms and Software

SwissADME (Swiss Institute of Bioinformatics): An online platform used to calculate physicochemical descriptors and predict pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME). ProTox-3: A web-based toxicity prediction tool used to estimate acute toxicity (LD_{50}), toxicity class, and potential toxicological endpoints. Public Chemical Databases such as PubChem and ChemSpider were used to identify and verify the chemical structures of Procaine and Novobiocin.

b. Chemical Entities

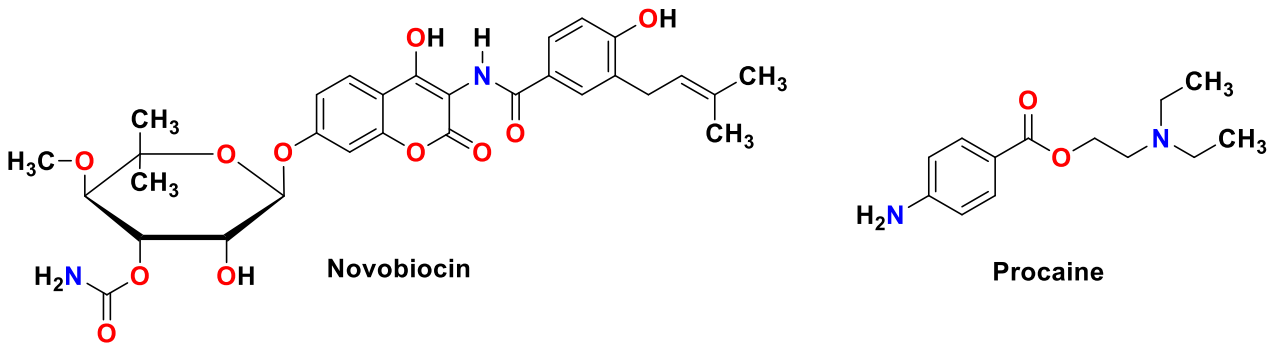


Figure 1. *Novobiocin and procaine structure*

3.0 Historical Progression and Pharmacological Origin of Procaine and Novocaine

3.1 The Procaine

Figure 2 image of coca plant

Procaine chemistry started in the 19th century; this is an era during which an alkaloid compound known as cocaine was extracted from the leaves of the coca plant. The compound was used as a primary local anesthetic in medical procedures (Carsetti *et al.*, 2023). Although cocaine was found to be effective at numbing localized areas. However, it presented severe drawbacks, including high toxicity to the central nervous system, severe vasoconstriction, and a strong potential for addiction and patient abuse (Sneader, 2005).

Figure 3 image of Alfred Einhorn

To tackle these limitations, German chemist Alfred Einhorn sought in 1905 to develop a synthetic molecule that retained the anesthetic properties of cocaine but lacked its addictive liability and toxicity. Einhorn successfully synthesized procaine. The synthesis was done by simplifying the complex tropane ring of cocaine alkaloid into a simpler, well-defined amino ester structure. This innovation resulted in an anesthetic agent that was much safer for broad clinical use, marketed under the trade name Novocain (derived from the Latin Novus, meaning new, combined with the suffix-Caine) (Fadah and Abolbashari, 2024).

3.2 The Novobiocin

Unlike Procaine, Novobiocin is a true natural product. It was isolated in the mid-1950s from the actinomycete bacterium *Streptomyces niveus* by multiple independent research teams, leading to its initial distribution under various names such as Albamycin and Cathomycin. The discovery occurred during the "**Golden Age of Antibiotics"**, a period defined by the rapid identification of microbial metabolites capable of treating severe infections (Hooper, 2001).

Figure 4: microscopic image of streptomyces niveus

Novobiocin appeared as a potent antibacterial agent during the rise of penicillin-resistant Staphylococcus aureus strains. By targeting the bacterial enzyme *DNA* gyrase, Novobiocin prevents *DNA* supercoiling, effectively halting replication and transcription in targeted bacteria and blocking microbial protein synthesis. This mechanism provided the medical community with a vital alternative during initial crises involving drug-resistant pathogens.

4.0 Extraction and Synthesis of the Molecules

4.1 Extraction and Purification Methods of Novobiocin

The production and isolation of novobiocin from natural sources follows a sequential workflow from microbial cultivation to complete purification (Altemimi *et al.*, 2017). Unlike the multi-step chemical

synthesis of *Procaine*, *Novobiocin* is a 100% natural product. It is not constructed atom-by-atom in a laboratory flask via organic synthesis; instead, it is biosynthesized by the bacterium *Streptomyces niveus*. The production in a lab setting involves large-scale fermentation, in which these microorganisms act as natural chemical factories. Because it is a product of biological fermentation, the efficiency of the process is not evaluated by a standard chemical yield calculation, which compares the theoretical mass of the product to the mass of the reactants. Instead, the yield for Novobiocin is determined by: Fermentation Titer: The concentration of the molecule produced per liter of culture medium (e.g., mg/L) and the extraction efficiency, presenting the percentage of the molecule successfully recovered from the fermentation broth during the purification stages. The extraction process includes:

a. The Fermentation and Acidification Process

While Procaine is synthesized by a chemist using glass equipment, Novobiocin is synthesized by the bacteria *Streptomyces niveus* inside a fermentation tank. This is a biological synthesis (biosynthesis) where the bacteria follow a specific 4-steps reaction pathway to assemble the molecule from basic biological building blocks. Novobiocin is produced by culturing the action my cete *Streptomyces niveus* in large-scale industrial bioreactors. The fermentation medium is optimized to maximize secondary metabolite yield (Waites *et al.*, 2001). Once fermentation is complete, acidification follows immediately, involving the entire broth, which is separated from the biomass and acidified. This step protonates the molecule, shifting its partition coefficient to favor extraction into organic solvents (Waites *et al.*, 2001).

Liquid-Liquid Extraction

The acidified broth is processed via liquid-liquid extraction using an appropriate organic solvent (such as ethyl acetate or dichloromethane). This step isolates novobiocin from water-soluble impurities and cellular debris. The organic phase is subsequently evaporated to yield a concentrated crude extract.

b. Purification by Chromatography

The purification involves dissolving the crude extract and fractionating it using column chromatography on silica gel or ion-exchange resins, and the purified fractions are concentrated and recrystallized to yield a high-purity, stable crystalline powder (Waites *et al.*, 2001).

4.2 Method of Synthesis of Procaine

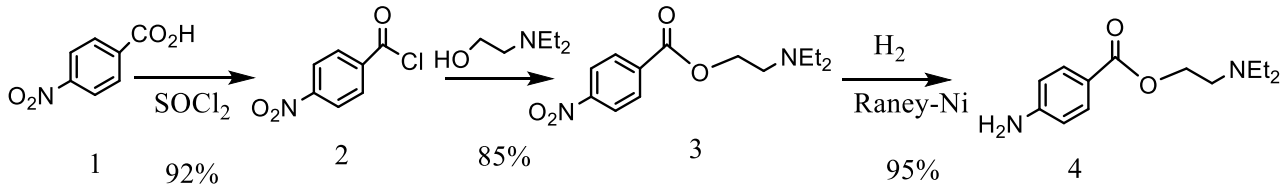
The synthesis of procaine from the tropane ring of cocaine alkaloid involves the following process: Reagents: 4-nitrobenzoic acid, thionyl chloride, 2-diethylaminoethanol, and dihydrogen (for the reduction step). Mechanism: The esterification proceeds via nucleophilic acyl substitution. The final reduction step converts the nitro group, NO_2, into an amine group, NH_2, which is essential for the

molecule's local anesthetic activity. The Three Steps of Chemical Synthesis**:** The synthesis of procaine involves esterifying the appropriate precursor to form the amino ester. The process is divided into three key steps:

Preparation of 4-nitrobenzoyl chloride: 4-nitrobenzoic acid is treated with thionyl chloride ($SOCl_2$) to obtain the corresponding acyl chloride (Vogel, 1989).

Esterification: 4-nitrobenzoylchloride reacts with 2-diethylaminoethanol in the presence of a base (such as pyridine) to form the intermediate compound, 2-(diethylamino) ethyl 4- nitrobenzoate.

Reduction of the Nitro Group: The nitro group is reduced to an aromatic amine via catalytic hydrogenation (using palladium on carbon) or by chemical reduction (for example, using iron and hydrochloric acid) to yield procaine (Einhorn & Uhlfelder, 1909).



Overall Synthesis Yield: 74.3%

Scheme1: Procaine synthetic reaction

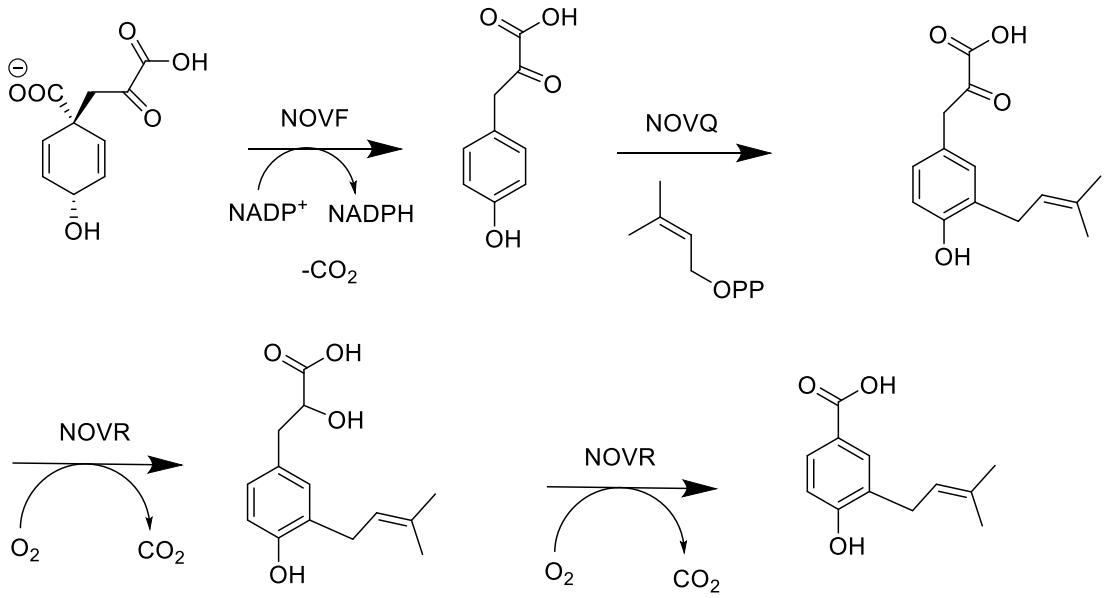
4.3 Natural Origin and Extraction yield of novobiocin

Unlike the multi-step chemical synthesis of Procaine, Novobiocin is a 100% natural product. It is not constructed atom-by-atom in a laboratory flask via organic synthesis; instead, it is biosynthesized by the bacterium *Streptomyces niveus.* The "production" in a lab setting involves large-scale fermentation, in which these microorganisms act as natural chemical factories (Berge *et al.,* 1977) . Because it is a product of biological fermentation, the efficiency of the process is not measured by a standard "chemical yield" calculation, which compares the theoretical mass of the product to the mass of the reactants. Instead, the yield of Novobiocin is determined by Fermentation Titer: the concentration of the molecule produced per liter of culture medium (e.g., mg/L). Extraction Efficiency: The percentage of the molecule successfully recovered from the fermentation broth during the purification stages (Ali *et al.,* 2026)**.**

a. The Fermentation Process:

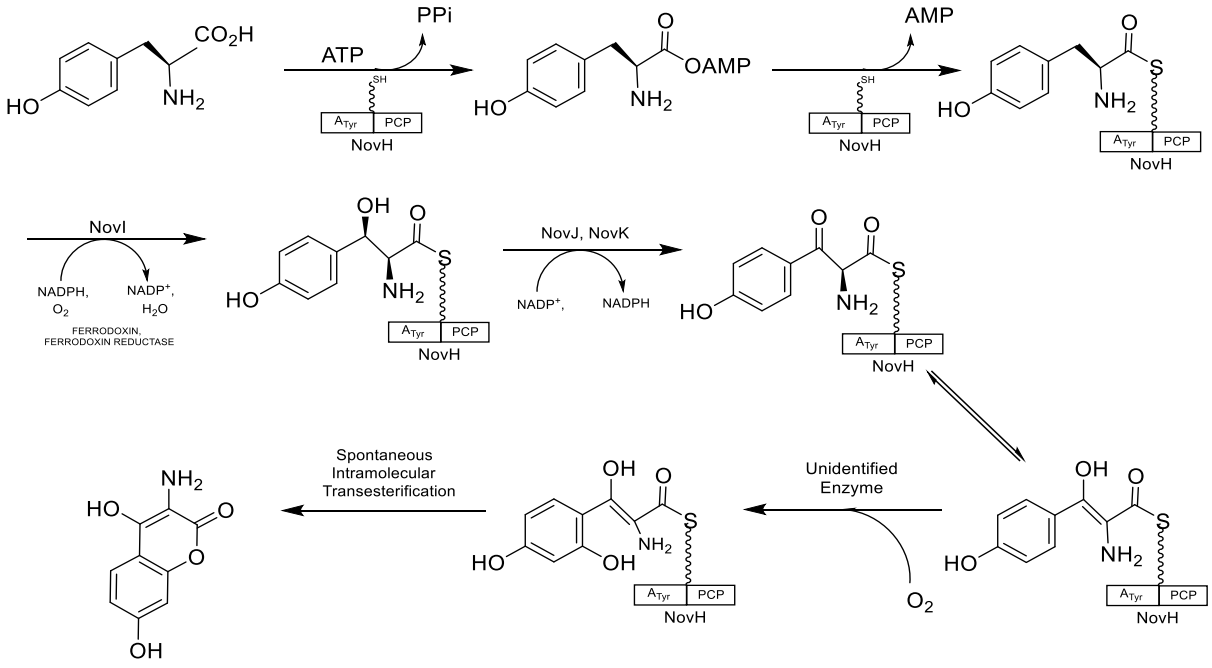
While Procaine is synthesized by a chemist using glass equipment, Novobiocin is synthesized by the bacteria *Streptomyces niveus* inside a fermentation tank (Mazzuchini & Canisso, 2026). This is a biological synthesis (biosynthesis) where the bacteria follow a specific 4-step reaction pathway to assemble the molecule from basic biological building blocks. Unlike a chemical reaction, where you calculate a molar yield, this biological process happens all at once as long as the agents (nutrients) are present in the tank. There are four steps involved in the biological process of synthesis, which include:

Step 1: Assembly of the Ring System (p-Hydroxybenzoic Acid). The reaction starts with the formation of the aromatic core. The bacteria use metabolic precursors to synthesize p-hydroxybenzoic acid, which serves as the foundation of the entire Novobiocin molecule.



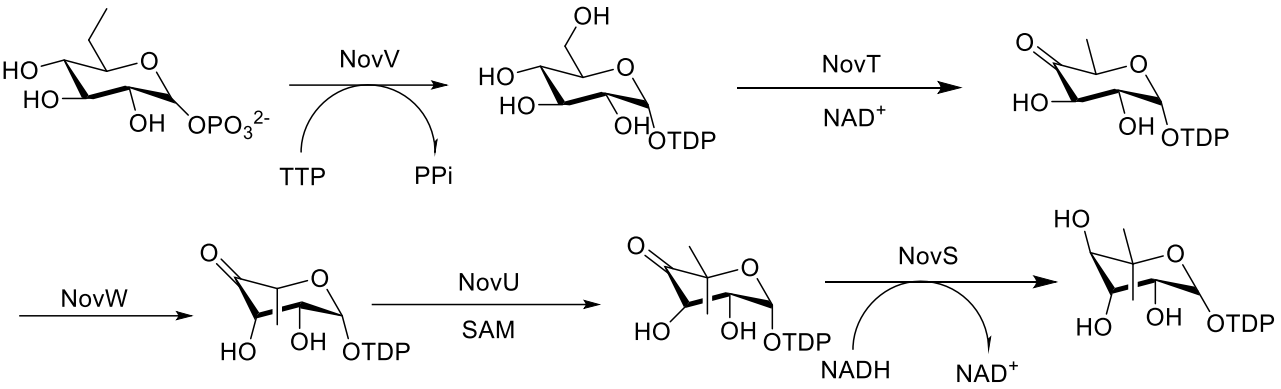
Scheme2: Enzymes reactions to prepare p-hydroxybenzoic acid

Step 2: Formation of the Coumarin Core (The Central Reaction) Inside the bacterial cell, specific enzymes catalyze the reaction between tyrosine (an amino acid) and other metabolites to form the Aminocoumarin ring. This is the "active" heart of the antibiotic that eventually binds to the DNA gyrase (Heide, 2009).



Scheme 3: Enzymes reaction to prepare Coumarin Core

Step 3: Sugar Synthesis (L-Noviose Formation). The bacterium produces a unique sugar called L-Noviose. In the fermentation tank, this reaction includes a series of enzymatic steps that transform glucose into this specialized sugar, which is essential for the molecule to penetrate bacterial cell walls. (Heide, 2009).



Scheme 4: Enzymes reaction to make L-Noviose

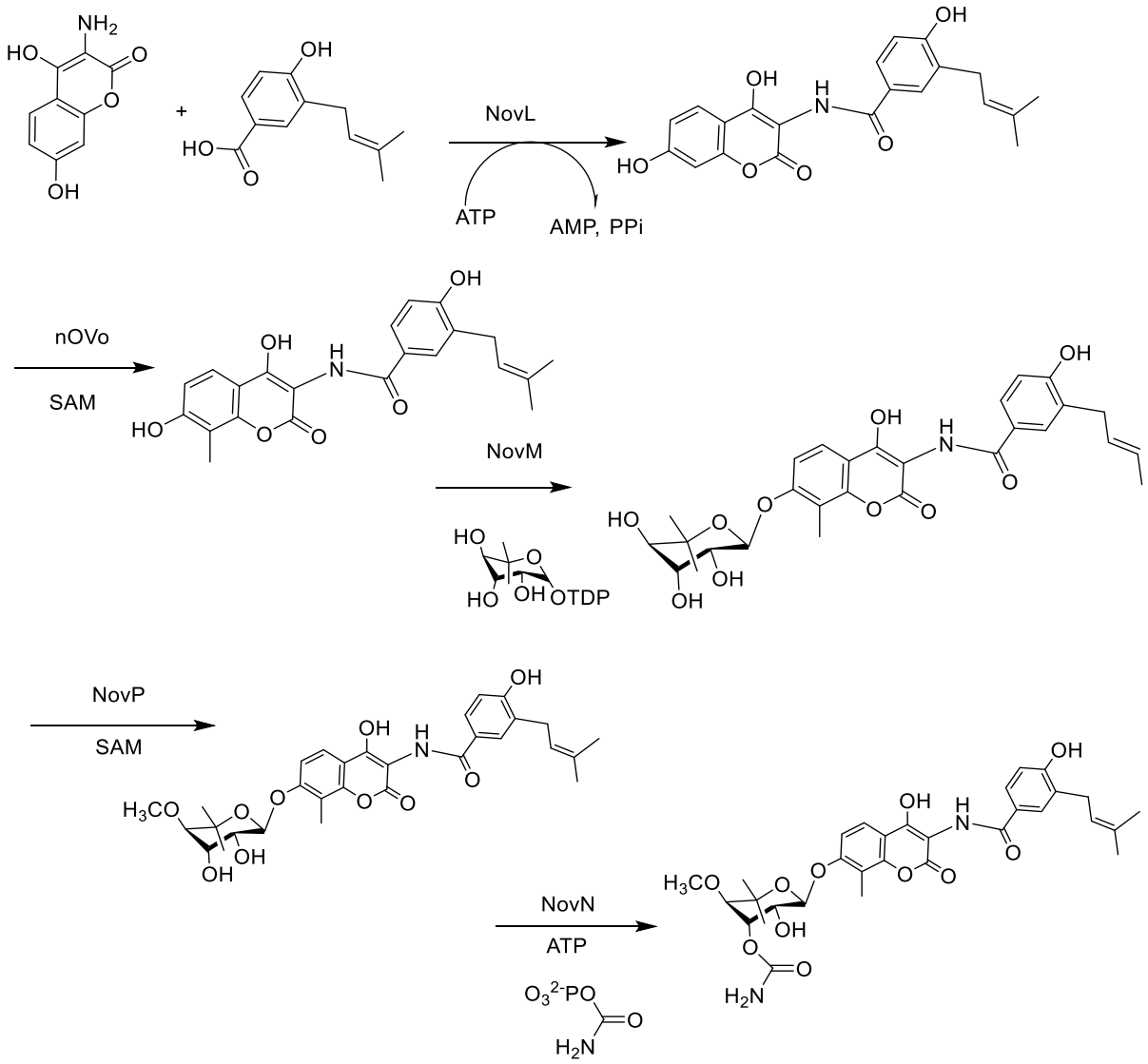
Step 4: Final Condensation (The "Closing" Reaction). The final step in the tank is the condensation reaction. An enzyme called an acyltransferase links the sugar, the coumarin ring, and the aromatic acid together. Once these three components are "snapped" together, the bacteria secrete the finished 100% natural Novobiocin into the surrounding broth (Heide, 2009).

Note on Yield: As discussed, because this is a 100% natural extraction from a lab-controlled biological process, the yield is not computed step by step as in a chemical synthesis. Instead, it is measured by the total concentration of Novobiocin found in the tank after Step 4 is complete (Kominek, 1972).

5.0 Biological Activities and Applications of Procaine and Novobiocin

5.1 Procaine as a Local Anesthetic

Procaine acts by reversibly blocking nerve conduction, through binding to voltage-gated sodium channels on the intracellular side of the cell membrane, it prevents sodium influx, thereby inhibiting depolarization and the propagation of the action potential (Levine & DeMaria, 2015). Figure 5, illustrates the possible biological activity pathways or mode of action of the procaine molecule in the biological system.



Scheme 5: Finale assembly to make novobiocin

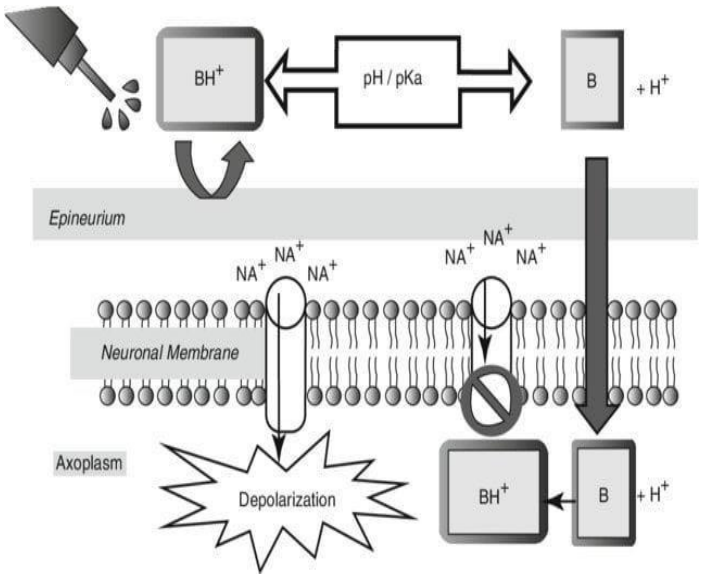


Figure 5: *Mechanism of Local Anesthesia* (Levine & DeMaria 2015)

5.2 Novobiocin as an Antibiotic and DNA Gyrase Inhibitor

Novobiocin is a potent inhibitor of bacterial DNA gyrase (specifically the GyrB subunit). Blocking the ATP-binding site prevents the enzyme from supercoiling DNA, thereby halting bacterial DNA replication and transcription (Zuzanna Pakosz-Stepien, 2021).

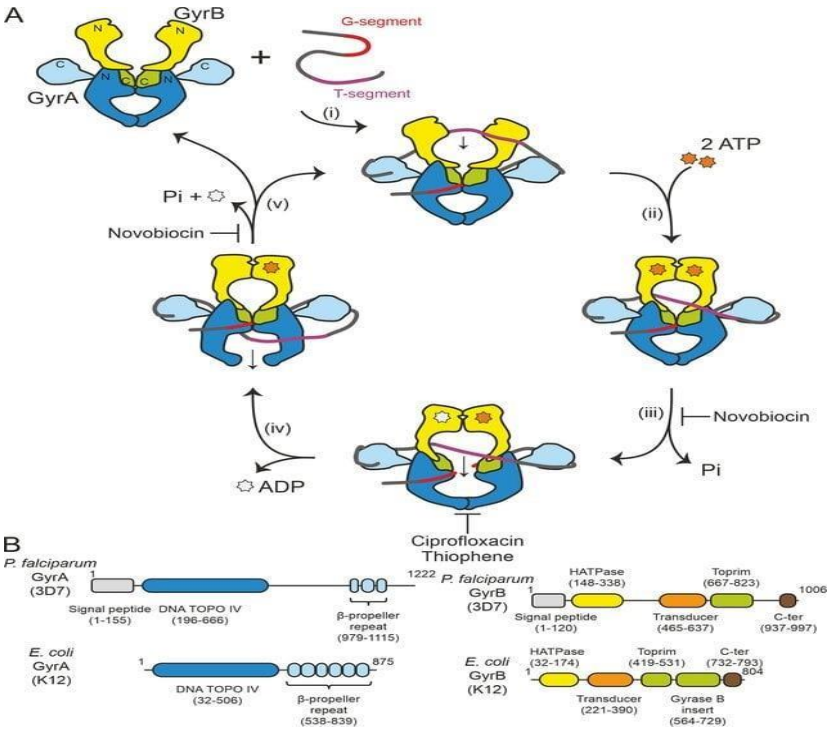


Figure 6 *: DNA Gyrase Inhibition*

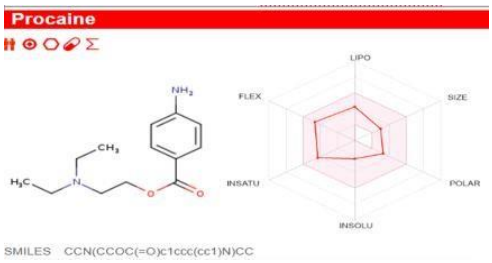
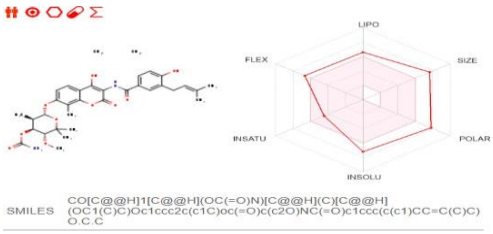
6.0 Pharmacokinetics and Toxicological Profiles of Procaine and Novobiocin

The use of *in silico* tools allows researchers to evaluate a molecule's behavior in the body and its possible toxicity before conducting in vitro and in vivo experiments (Giudicelli & Tillement, 1977; Merzouki *et al.*, 2025; Elsharkawy *et al.*, 2026). This approach accelerates drug discovery and lead optimization. This study uses in silico *t*ools such as SwissADME to evaluate the pharmacokinetic properties and Protox3 for toxicological analysis of the two molecules under study.

6.1 SwissADME Parameters of Procaine and Novobiocin:

SwissADME is a comprehensive web-based computational tool developed by the Swiss Institute of Bioinformatics (SIB) that predicts the ADME profile of small organic molecules. By inputting a chemical structure, the tool applies advanced mathematical models to estimate key ADME parameters: Absorption, Distribution, Metabolism, and Excretion, along with drug-likeness and medicinal chemistry friendliness (Kaddouri *et al.*, 2022; Er-rajy *et al.*, 2025; Merzouki *et al.*, 2025;).

6.1.1 SwissADME of Procaine and Novobiocin: demonstrated bellow

	Procaine	Novobiocin
The Bioavailability Radar Diagram		
Physicochemical Properties	Physicochemical Properties Formula C13H20N2O2 Molecular weight 236.31 g/mol Num. heavy atoms 17 Num. arom. heavy atoms 6 Fraction Csp3 0.46 Num. rotatable bonds 7 Num. H-bond acceptors 3 Num. H-bond donors 1 Molar Refractivity 69.06 TPSA 55.56 Å²	Physicochemical Properties Formula C34H46N2O10 Molecular weight 642.74 g/mol Num. heavy atoms 46 Num. arom. heavy atoms 16 Fraction Csp3 0.44 Num. rotatable bonds 10 Num. H-bond acceptors 10 Num. H-bond donors 4 Molar Refractivity 177.50 TPSA 179.78 Å²
Lipophilicity	Lipophilicity Log $P_{o/w}$ (iLOGP) 2.77 Log $P_{o/w}$ (XLOGP3) 1.92 Log $P_{o/w}$ (WLOGP) 1.78 Log $P_{o/w}$ (MLOGP) 1.93 Log $P_{o/w}$ (SILICOS-IT) 1.61 Consensus Log $P_{o/w}$ 2.00	Lipophilicity Log $P_{o/w}$ (iLOGP) 0.00 Log $P_{o/w}$ (XLOGP3) 6.03 Log $P_{o/w}$ (WLOGP) 5.98 Log $P_{o/w}$ (MLOGP) 2.22 Log $P_{o/w}$ (SILICOS-IT) 3.86 Consensus Log $P_{o/w}$ 3.62
Water Solubility	Water Solubility Log *S* (ESOL) -2.31 Solubility 1.15e+00 mg/ml ; 4.85e-03 mol/l Class Soluble Log *S* (Ali) -2.71 Solubility 4.60e-01 mg/ml ; 1.95e-03 mol/l Class Soluble Log *S* (SILICOS-IT) -3.46 Solubility 8.10e-02 mg/ml ; 3.43e-04 mol/l Class Soluble	Water Solubility Log *S* (ESOL) -7.22 Solubility 3.86e-05 mg/ml ; 6.01e-08 mol/l Class Poorly soluble Log *S* (Ali) -9.58 Solubility 1.68e-07 mg/ml ; 2.61e-10 mol/l Class Poorly soluble Log *S* (SILICOS-IT) -6.88 Solubility 8.57e-05 mg/ml ; 1.33e-07 mol/l Class Poorly soluble
Pharmacokinetics (Absorption & Distribution)	Pharmacokinetics GI absorption High BBB permeant Yes P-gp substrate No CYP1A2 inhibitor No CYP2C19 inhibitor No CYP2C9 inhibitor No CYP2D6 inhibitor No CYP3A4 inhibitor No Log K_p (skin permeation) -6.38 cm/s	Pharmacokinetics GI absorption Low BBB permeant No P-gp substrate Yes CYP1A2 inhibitor No CYP2C19 inhibitor No CYP2C9 inhibitor No CYP2D6 inhibitor No CYP3A4 inhibitor Yes Log K_p (skin permeation) -5.94 cm/s
Drug-Likeness & Medicinal Chemistry	Druglikeness Lipinski Yes; 0 violation Ghose Yes Veber Yes Egan Yes Muegge Yes Bioavailability Score 0.55 Medicinal Chemistry PAINS 0 alert Brenk 1 alert: aniline Leadlikeness No; 1 violation: MW<250 Synthetic accessibility 1.89	Druglikeness Lipinski No; 2 violations: MW>500, NorO>10 Ghose No; 4 violations: MW>480, WLOGP>5.6, MR>130, #atoms>70 Veber No; 1 violation: TPSA>140 Egan No; 2 violations: WLOGP>5.88, TPSA>131.6 Muegge No; 3 violations: MW>600, XLOGP3>5, TPSA>150 Bioavailability Score 0.17 Medicinal Chemistry PAINS 0 alert Brenk 2 alerts: cumarine, isolated_alkene Leadlikeness No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5 Synthetic accessibility 6.54

Procaine:

The radar chart for Procaine shows the red line almost entirely within the pink shaded area. This indicates that Procaine has an optimal physicochemical profile for a drug. LIPO (Lipophilicity): It falls perfectly within the range (LogPo/wbetween0.7and 5.0). Size: At236.31g/mol, it is well within the ideal range (150–500g/mol). POLAR (Polarity): Its TPSA is within the acceptable 20–130 Å 2 range. FLEX (Flexibility): Ithas7rotatable bonds, meeting the criteria for flexibility ≤ 9, and the INSATU &INSOL: It meets the requirements for saturation and solubility.

Formula: $C_{13}H_{20}N_2O_2$, Molecular Weight: 236.31 g/mol. Num. heavy atoms: 17. Num of aroma indicating heavy atoms: 6 (the benzene ring), Fraction Csp3: 0.46 (balance of saturated and unsaturated carbons) and the TPSA: 52.32 $Å^2$ (This low value suggests the molecule can pass through cell membranes easily).

Log $P_{o/w}$: 2.14. This value is ideal. It means the molecule is lipophilic enough to cross the fatty nerve cell membranes to block sodium channels, but hydrophilic enough to remain stable in the bloodstream.

Log S (ESOL): -2.49 (Soluble). Class: Procaine is classified as Soluble, meaning it dissolves well in aqueous environments, which is essential for injectable anaesthetics.

GI Absorption: High. This indicates that the molecule is very well absorbed by the body. BBB Permeant: Yes. Procaine is capable of crossing the Blood-Brain Barrier. This explains why high doses can cause central nervous system side effects. P-gp Substrate: No. It is not pumped out of cells by P-glycoprotein, allowing it to stay at the target site effectively. CYP Inhibitor: It does not inhibit major cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4), suggesting a low risk for drug-drug interactions

Lipinski's Rule of Five: Yes; 0 violations. This confirms that Procaine has the characteristics of an ideal orally active drug. Veber & Ghose Rules: It passes all standard drug-likeness filters. Bioavailability Score: 0.55 (Standard high score for drugs passing Lipinski's rules). PAINS & Brenk Alerts: 0 alerts, meaning there are no "unstable" or problematic chemical groups in the structure. Synthetic Accessibility: 1.94 (On a scale of 1 to 10, where 1 is very easy). This indicates that Procaine is very simple to synthesize in a laboratory.

Novobiocin:

Unlike the previous small molecule, Novobiocin’s red plot stretches far outside the pink zone in three specific directions: SIZE: Significantly exceeds the limit (MW > 500). POLAR: Very high polarity (TPSA > 140). INSOLU: Hits the edge, indicating very poor solubility. This is a "non-drug-like" profile by classical standards, which is typical for large, potent antibiotics that act on specific bacterial targets rather than general human receptors.

Formula: C34H46N2O10, Molecular Weight: 642.74 g/mol, TPSA: 179.78 Å²: this very high polar surface area explains why it cannot pass through lipid membranes easily and Rotatable Bonds: 10 indicating is a highly flexible molecule.

Consensus Log P $_{o/w}$: 3.62. This shows that it is much greasier. This high lipophilicity is necessary because it must penetrate the complex cell walls of Gram-positive bacteria to reach the DNA machinery inside.

Class: Poorly soluble (Log S between -7.22 and -6.88). Because it is poorly soluble, Novobiocin is often administered as a salt (sodium novobiocin) to improve its ability to dissolve in the body.

GI Absorption: Low. This indicates that the drug is not well absorbed through the gut, which is why it was historically used primarily for skin infections or specific systemic treatments. BBB Permeant :No. It cannot cross the blood-brain barrier, protecting the central nervous system from the drug's effects. P-gp Substrate: Yes. The body recognizes this molecule as a "foreign invader" and uses P-glycoprotein pumps to actively expel it from cells. CYP3A4Inhibitor: Yes. This is a major finding; it means Novobiocin could slow down the metabolism of other drugs, which may lead to drug-drug interactions.

Lipinski Violations: 2 violations (MW>500 and NorO> 10). Bioavailability Score: 0.17. This is very low compared to Procaine (which was 0.55), confirming that this natural product is a "difficult" molecule to deliver as a standard pill. Synthetic Accessibility: 6.54. On a scale where 1 is easy and 10 is difficult, a score of 6.54 indicates that Novobiocin is difficult to synthesize in the laboratory. This is why it is produced via fermentation using *Streptomyces niveus* rather than total chemical synthesis.

7.0 Toxicity Analysis (Protox3) of Procaine and Novobiocin

7.1 Toxicity Analysis (Protox3) of Procaine

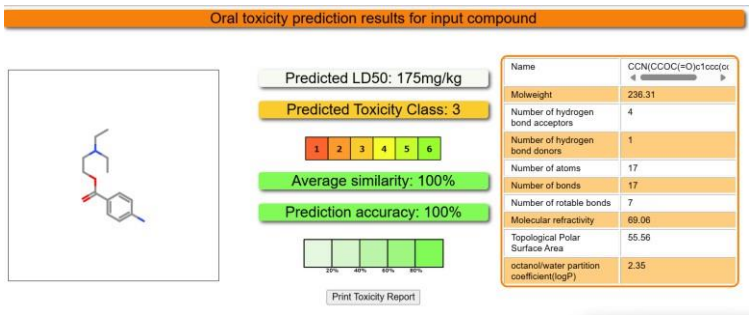


Figure 7: Toxicity Analysis of Procaine

According to the ProTox-3 analysis, Procaine is classified as a Class 3 acute toxin with an LD_{50} of 175mg/kg. While it shows a very safe profile regarding carcinogenicity, mutagenicity, and hormonal disruption (Nuclear Receptor Signaling), it shows a very high probability of immunotoxicity (0.99). This confirms its clinical tendency to cause hypersensitivity or allergic reactions in certain individuals.

7.3 Novobiocin Toxicity Analysis (Protox3) of Novobiocin

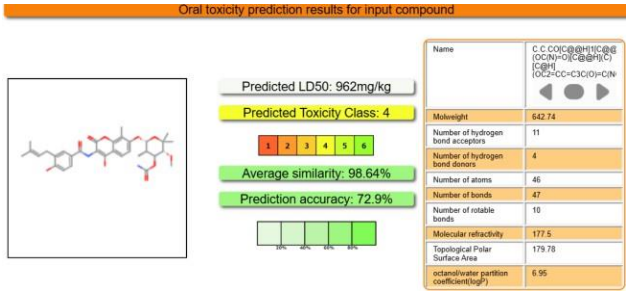


Figure 8: Toxicity Analysis of Novobiocin

The in-silico profile of Novobiocin illustrates the typical characteristics of a secondary metabolite. While it violates several 'Drug-Likeness' rules due to its size and polarity, its Class 4 toxicity rating and LD_{50} of 962 mg/kg demonstrate a high level of safety regarding acute overall toxicity. However, the high immunotoxicity (0.99) suggests that, like many antibiotics, it carries a risk of inducing allergic responses.

7.4 Comparative Study of the Pharmacokinetics and Toxicology Profile of the Molecules

The comparative study of Procaine and Novobiocin discloses two distinct philosophies in pharmaceutical development: the structural optimization of a natural lead (synthetic innovation) and the direct utilization of complex microbial metabolites (natural isolation). The SwissADME and ProTox-3 computational assessments provide a vital first look into the "life" of these molecules inside a biological system. For Procaine, the data confirm why it revolutionized surgery: its low molecular weight and ideal lipophilicity enable rapid diffusion. However, the high probability of immunotoxicity (0.99) predicted by ProTox-3 explains the allergic reactions often seen in clinical practice with ester-type anesthetics. For Novobiocin, the analysis shows the limitations of the "Rule of Five." While its high molecular weight and polarity lead to "violations" of traditional drug-likeness filters, its antibiotic efficacy is not diminished. This suggests that natural products commonly operate through specific active transport routes rather than simple passive diffusion. Predicting hepatotoxicity serves as a warning for dosage regulation in treatment environments. It is important to note that while SwissADME and ProTox-3 are powerful prognostic tools based on elaborate algorithms, they are not 100% accurate. These results are based on statistical probability and chemical similarity; they do not replace the necessity of in vitro assays and in vivo clinical trials. A prediction of "toxicity" in a computer model is a signal for caution, not definitive proof of danger, and a "safe" prediction does not guarantee the absence of side effects in a living organism.

8.0 Research Gap and Future Perspectives

Despite the long-standing clinical use of Procaine and Novobiocin and the important insights provided by computational tools such as SwissADME and ProTox-3, several gaps remain in appreciating their

pharmacological and toxicological characteristics, including the following;

1. Most available studies focus on the historical therapeutic applications of these compounds, while comparative investigations integrating synthetic and naturally derived drugs using modern in silico approaches are scarce. In particular, direct comparisons of their physicochemical properties, pharmacokinetic behavior, and toxicity profiles have received limited attention.

2. Although computational predictions show considerable immunotoxicity and possible hepatotoxicity, there is insufficient experimental evidence to validate these findings. Comprehensive in vitro and in vivo studies are needed to confirm the predicted toxicological endpoints and clarify the underlying molecular mechanisms responsible for adverse effects.

3. The poor drug-likeness characteristics and limited oral bioavailability predicted for Novobiocin point to the need for structural optimization and the synthesis of novel analogs with improved pharmacokinetic properties. However, relatively few studies have explored medicinal chemistry tactics to enhance the absorption, bioavailability, and safety of this natural product.

4. The inability of traditional drug-likeness rules, particularly Lipinski's Rule of Five, to adequately describe the behavior of structurally complex natural products demonstrates the need for alternative predictive systems and more comprehensive computational approaches. Investigation into active transport routes and carrier-mediated uptake pathways for natural compounds such as Novobiocin remains limited.

5. Finally, although SwissADME and ProTox-3 may provide rapid and cost-effective predictions, their outputs are based on statistical algorithms and chemical similarity models. There is still a need for integrated studies that combine computer modeling with molecular docking, molecular dynamics simulations, pharmacogenomic analyses, and laboratory verification to establish more reliable structure-activity and structure-toxicity relationships.

Therefore, upcoming research should focus on the rational design of novel Procaine and Novobiocin derivatives, validation of computational predictions via laboratory and clinical studies, and the development of refined predictive frameworks for complex natural products to facilitate safer and more effective drug discovery**.**

9.0 Conclusion

This comparative study shows the complementary roles of Procaine and Novobiocin in pharmaceutical development. Procaine exhibited favorable physicochemical properties, high gastrointestinal absorption, and full compliance with Lipinski's Rule of Five, whereas Novobiocin, despite several drug-likeness violations due to its bigger molecular size and higher polarity, retained the significant therapeutic

potential characteristic of complex natural products. In silico analyses using SwissADME and ProTox-3 revealed that both compounds belong to the moderate acute toxicity class and have a high probability of immunotoxicity, while Novobiocin also showed potential hepatotoxicity. These findings show that natural products do not always conform to conventional drug-likeness criteria and may depend on alternative biological mechanisms for their activity. Overall, this study emphasizes the value of computational tools for preliminary screening while stressing the need for practical validation through in vitro and in vivo studies. The integration of computational approaches with the structural optimization of natural lead compounds remains a promising strategy for advancing modern drug discovery and developing safer, more effective therapeutic agents.

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