Kingston University London

FACULTY OF HEALTH, SCIENCE, SOCIAL CARE AND EDUCATION

School of Life Sciences, Pharmacy and Chemistry

MSc Degree in

Medicinal Chemistry

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K2265116

Synthesis of novel analogues of Hipposudoric Acid for therapeutic use

September 2023

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ACKNOWLEDGMENTS

Completing this research project has been a transformative journey, and I am profoundly grateful for the individuals and institutions that have made it possible.

First and foremost, I extend my heartfelt gratitude to Dr. Stephen Wren, my supervisor and mentor. Your guidance, support, and expertise have been invaluable throughout this process. Your dedication to my academic growth has left an indelible mark on this work.

I am also thankful for Kingston University's exceptional staff, facilities and resources, which have provided the ideal environment for research and learning. The academic atmosphere here has truly fuelled my intellectual curiosity.

To my cherished family, your unwavering support has been my anchor. Your love and belief in me have been constant sources of inspiration, propelling me forward.
The successful completion of this thesis is a shared achievement. Thank you, Dr. Wren, Kingston University, and my family, for your instrumental roles in this important chapter of my academic journey.

LIST OF ABBREVIATION

DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
HCl	Hydrochloric acid
NMR	Nuclear magnetic resonance
TLC	Thin layer chromatography
UV	Ultraviolet
MIC	Minimum inhibitory concentration
IR	Infrared spectroscopy
MS	Mass spectroscopy
D6-DMSO	dimethyl sulfoxide-d6
FTIR	Fourier-Transfer Infrared
PCO	pyridinium chlorochromate
NBS	N-bromosuccinimide
BPO	benzoyl peroxide
TBAF	tetrabutylammonium fluoride
TMS	Trimethylsilyl.

CHAPTER I: INTRODUCTION

1.1 Exploring Hippopotamus Sweat

In a breakthrough study led by Kimiko Hashimoto and their team of Japanese researchers, a significant stride was taken in unravelling the mystery behind the enigmatic phenomenon of Hippopotamus skin colouration. By meticulously collecting sweat samples from these magnificent creatures, Hashimoto and their team have successfully isolated and characterised the pivotal compounds responsible for the fascinating colour transition observed on the hippopotamus's hairless skin.[14] This newfound understanding shattered the long-held, captivating myth that the hippopotamus sweats blood. Instead, the study revealed that these compounds, which undergo a striking metamorphosis from colourless to red and eventually brown, are produced by specialised skin glands.[14] Notably, these compounds, featuring a distinctive fluorenoid nucleus, exhibited exceptional biochemical properties. Remarkably potent and surpassing even the acidic strength of vinegar, this amalgamation of highly acidic compounds serves a dual purpose. Primarily, it functions as a formidable sunscreen, safeguarding the hippopotamus from the harsh grasp of solar radiation while ensuring its thermal comfort. Secondly, it serves as a potent antibiotic, endowing the pachyderm with a shield against pathogens, vital for a creature often predisposed to confrontations and injuries, thereby expediting the recovery process.[1,11]

1.2 Discovery of Hipposudoric Acid

The discovery of Hipposudoric acid marked an advancement in the study of natural compounds. Over recent years, Hipposudoric acid, originally isolated from the secretions of hippopotamus skin, has garnered attention for its remarkable biological and chemical properties.[1] In the late twentieth century, scientists investigated the antimicrobial properties of hippopotamus skin secretions. They stumbled upon a unique chemical compound that displayed antibacterial activity during their research. This marked the isolation and identification of the primary antimicrobial component: Hipposudoric Acid. This previously unknown compound was characterised Through isolation, extraction, purification, and spectroscopic analysis.

The structural complexity of Hipposudoric acid might account for its intriguing attributes. Its intricate arrangement of atoms, functional groups, and stereochemistry immediately stood out, capturing the interest of chemists and biochemists who delved into its production and applications.[1] The separation of Hipposudoric acid raised questions and theories regarding its origin. It was postulated that the remarkable ability of hippos to heal wounds and protect themselves from infections might be attributed to the potent antimicrobial properties of this chemical.[2] This notion led to the proposal of an evolutionary adaptation that could potentially inspire the development of innovative medicinal substances.[1]

1.3 Chemical Properties of Hipposudoric Acid

Scientists and chemists are intrigued by the complex chemical structure of Hipposudoric acid, as characterised by its tricyclic fluorene framework. This framework encompasses essential elements, including a 5-hydroxy motif, a 1, 4, 8-trioxo unit, and a 3-alkyl substituent terminating in a carboxylic acid group as seen in figure 1.[3] Hipposudoric acid, a component of the family of phenolic acids found in natural products, has certain properties that give validity to the idea that it may have potential use in medicine. Its chemical structure is based on a polyphenolic scaffold with aromatic rings as major building blocks.[14] Because of the potential health advantages that these structural components may bring, their antioxidant properties have attracted much study. Because aromatic rings include conjugated double bonds, the molecule is better able to scavenge free radicals and reduce oxidative stress, both leading to an increase in the molecule's antioxidative action.[2,3] The acidity of its hydroxyl groups also influences Hipposudoric Acid's chemical behaviour. Its solubility, stability, and interactions with biological molecules may all be impacted by these hydroxyl groups' participation in hydrogen bonding interactions. When considering its possible medicinal uses, this element of its chemical characteristics is crucial as it determines how effectively the compound can be absorbed, transported, and interact with target molecules in the body, ultimately affecting its therapeutic effectiveness.[4]

Hipposudoric Acid's chemical reactivity also provides opportunities for analogue synthesis and structural modification. Chemists are investigating these possibilities to create new compounds with better pharmacokinetic or biological features.[1] To produce novel molecules for particular therapeutic uses, these initiatives seek to take advantage of the compound's intrinsic chemical features.

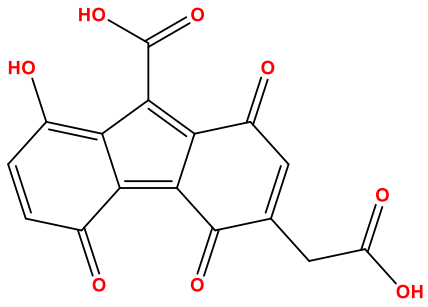


Figure 1. Structure of Hipposudoric acid.

1.4 Pharmacology of Hipposudoric Acid

Due to the possible therapeutic uses of various biological actions, Hipposudoric acid's pharmacology has been a research focus. The pharmacology of Hipposudoric acid remains a subject of scientific inquiry. While still in the nascent stages of understanding, preliminary research indicates potential antimicrobial, antioxidant, and photoprotective properties associated with this compound. As researchers continue to unravel the intricate mechanisms underlying these pharmacological attributes, the exploration of Hipposudoric acid's therapeutic potential presents an opportunity to uncover nature-inspired solutions to various health-related challenges, including antimicrobial resistance.[2,3] The compound's interactions have also identified potential implications for cancer research with cellular and molecular targets as DHFR inhibitors.[3]

1.5 Hipposudoric Acid as Antioxidants

One of the mainstays of Hipposudoric acid's biological relevance is its function as an antioxidant. Antioxidants like Hipposudoric Acid, powerful free radical scavengers, are essential in combating oxidative stress, a cellular imbalance associated with several illnesses and ageing. By facilitating electron transport and neutralising dangerous free

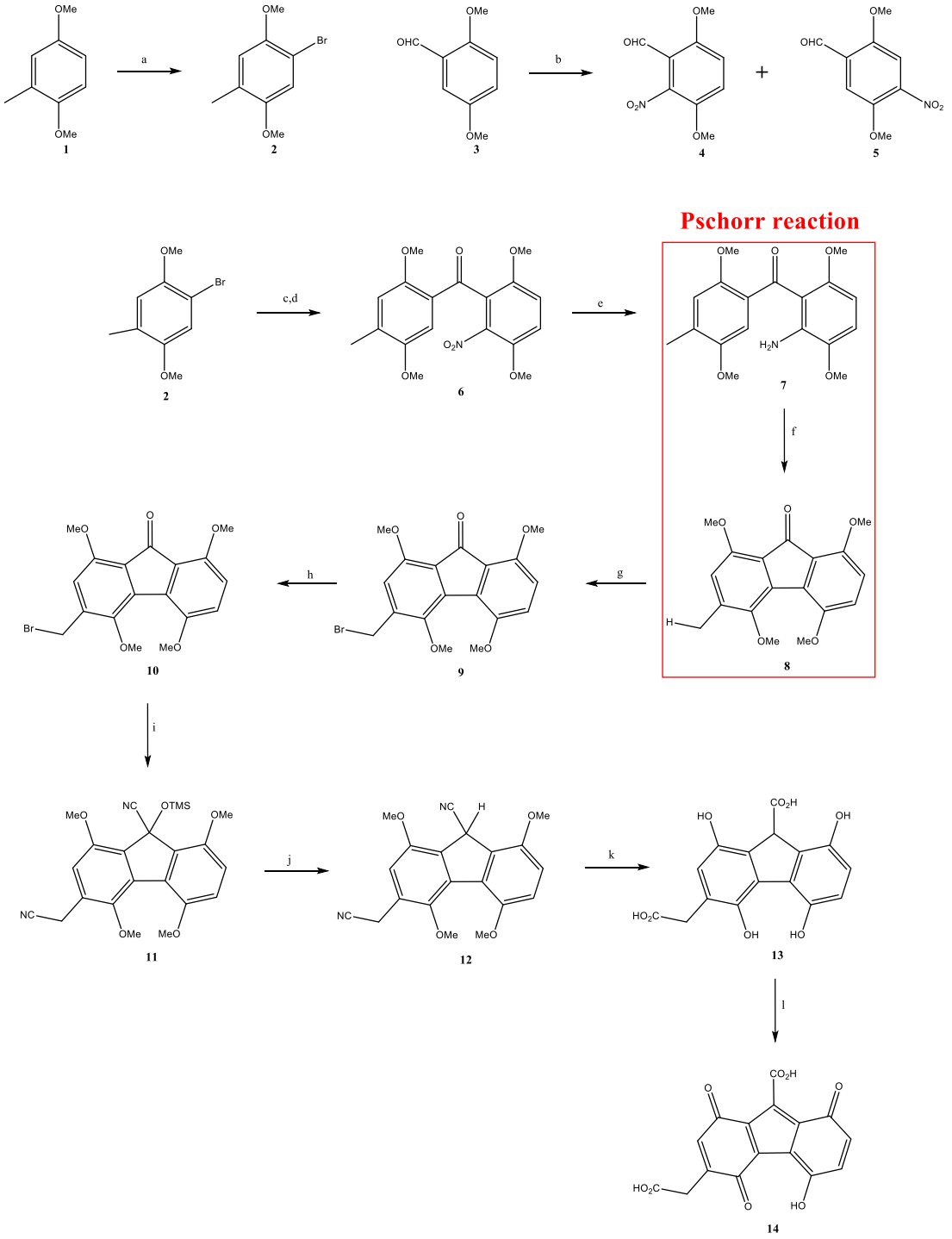
radicals, its polyphenolic structure, which comprises aromatic rings and hydroxyl groups, protects biomolecules, including proteins, lipids, and DNA, from oxidative damage.[5] It may alter important cellular signalling pathways involved in inflammation, the response to oxidative stress, and cell survival. This multimodal strategy may increase its promise to treat oxidative stress-related illnesses, including cardiovascular disease, neurological disorders, and metabolic syndromes.[5]

CHAPTER II: REVIEW OF EXISTING LITERATURE

2.1 Previous Synthesis Strategies for Hipposudoric Acid and Analogues

The limited progress in the synthesis of Hipposudoric acid and its derivatives underscores the challenges faced by chemical research. While some synthetic compounds have been attempted, it's important to acknowledge that only the synthetic recreation of Hipposudoric acid itself has been achieved successfully. The primary focus of scientists has been on achieving a comprehensive understanding of Hipposudoric acid's properties and characteristics through synthetic methods. This foundational knowledge serves as a critical step towards enabling future researchers to potentially create new compounds. By utilising the insights gained from the synthetic recreation of Hipposudoric acid, scientists may eventually explore the development of innovative chemicals with modified structures while retaining essential pharmacophores. [1] Hipposudoric acid necessitates multi-step synthesis procedures that imitate its complicated structure. Previous attempts at synthesising Hipposudoric acid have employed a series of strategic reactions. The synthesis involved the utilisation of the Pschorr ring-closing reaction **8** as seen in scheme 1, which was followed by a cyanation process of compound **10** to introduce the carboxylic acid functionality **13**. Additionally, a meticulous oxidation procedure was employed to convert bis-hydroquinone **13** into bis-quinone **14**. Notably, these synthetic pathways yielded satisfactory yields of the target compound **14**. These strategies have paved the way for ongoing chemical and biological investigations involving both Hipposudoric acid and its analogues. [2]

Virtual screening and molecular docking investigations made possible the predicted potential binding affinities of analogues to certain biomolecular targets. [3] When successfully implemented, these computational insights could inform decisions regarding synthetic modifications and aid in the rational design of derivatives with potentially enhanced bioactivity. Researchers are also considering more environmentally friendly synthetic techniques to address concerns related to scalability and sustainability. [9]



Scheme 1. Synthesis of Hipposudoric acid **13**. Reagents: **(a)** Br2 (1.05 mol amt), CH2Cl2 **(b)** conc. HNO3 **(c)** (1.0 mol amt), t-BuLi (1.5 mol amt)/pentane, THF **(d)** PCC (1.6 mol amt), NaOAc, CH2Cl2 **(e)** Fe (3.6 mol amt), AcOH–H2O (9:1) **(f)** isoamyl nitrite (2.0 mol amt), AcOH, then hydroquinone (1.2 mol amt)/acetone **(g)** NBS (1.0 mol amt), BPO (0.06 mol amt), benzene **(h)** 1 M TBAF in THF (1.6 mol amt), TMSCN (solvent, 18 mol amt) **(i)** Et3SiH (5.0 mol amt), BF3ÆEt2O (5.0 mol amt), CH2Cl2 **(j)** aq HBr–AcOH **(k)** aq HBr–AcOH (3:1) **(l)** CuSO4 (1.0 mol amt), 0.5 M aq NaHCO3. [10]

2.2 Synthesis of Phenolic Compounds and Analogues

Due to their wide range of biological functions, synthesising phenolic compounds is essential to current organic chemistry and drug development. Phenolic compounds have a variety of pharmacological characteristics, including anti-inflammatory and antioxidant actions, as well as possible anti-cancer and anti-microbial activity. [13] Phenolic compounds are defined by a hydroxyl group connected to an aromatic ring. These compounds are synthesised using various techniques, each specifically catered to the necessary functional groups and structural complexity. The electrophilic aromatic substitution process is typical for producing phenolic compounds [12.] Through the interaction of an aromatic molecule with an electrophilic reagent, substituents are added to an aromatic ring using this technique. Oxidative coupling procedures, such as the biomimetic oxidative coupling of phenols, provide a productive way to create intricate phenolic frameworks and carbon-carbon bonds for more complicated structures. [12] Additionally, cross-coupling reactions like the Suzuki-Miyaura or Heck reactions are made possible by transition-metal catalysed reactions, which have grown in popularity [13]

Since Hipposudoric Acid has a complex polyphenolic structure and a variety of functional groups built into the molecule, synthesising analogues of it presents a unique difficulty. [2] An effective strategy for improving the antioxidant and anti-inflammatory properties of complex compounds is carefully modifying substituents on the aromatic rings. [2] The two main methods for achieving this tactical adjustment are complex multi-step synthetic sequences and transition-metal catalysed processes. [35,34] Controlled functional group insertion is made possible by transition-metal catalysis, allowing exact changes to the chemical characteristics of the analogues. Multi-step sequences, on the other hand, provide flexibility by permitting a gradual and focused alteration of the molecular framework. [15,34,35]

Amines play a crucial role as nucleophiles in diverse synthetic transformations, particularly in a notable technique involving the substitution of existing functional groups with amines. This technique leads to the creation of innovative analogues with distinct attributes.[16] Amidation, particularly using N,N'-Carbonyldiimidazole, emerges as a valuable approach for introducing amine moieties into compounds. This strategic incorporation of amine groups not only holds the potential to enhance solubility but also

facilitates interactions with specific enzymes or receptors, thereby enhancing the compound's potential interactions with biological targets. [16]

2.3 Bioactivity Studies of Hipposudoric Acid

Recent computational chemistry-driven studies have shed light on the potential of Hipposudoric acid analogues as inhibitors of DHFR. These analogues, structurally related to Hipposudoric acid, have been explored for their capacity to bind to the DHFR enzyme and potentially interfere with its essential function in nucleotide synthesis, as shown in **Figure 3**. [3] The inhibition of DHFR holds significance due to its involvement in critical cellular processes, making it a valuable target for therapeutic intervention. [17] However, it's important to approach these findings with caution, as computational studies provide a theoretical framework that requires validation through experimental assays. While these results offer a promising starting point for the development of novel inhibitors, further research, including biochemical and structural studies, is imperative to validate the inhibitory potential of Hipposudoric acid analogues against DHFR. This multidisciplinary approach will contribute to a comprehensive understanding of their mode of action and potential as therapeutic agents, thus advancing the field of drug discovery. In addition to its potential as a DHFR inhibitor through analogue studies, the inherent bioactivity of Hipposudoric acid itself presents a diverse range of possibilities for pharmaceutical and cosmetic applications. [1] Its antimicrobial properties make it a candidate for addressing microbial infections and related challenges. Furthermore, its photoprotective and antioxidant capabilities hold promise for skincare formulations and protection against the damaging effects of UV radiation and oxidative stress. [2] However, while the initial observations regarding these bioactivities are encouraging, it's vital to approach them with scrutiny. Rigorous experimentation is required to validate and fully understand the extent and mechanisms of Hipposudoric acid's antimicrobial, photoprotective, and antioxidant effects. Detailed studies involving cell culture models, animal testing, and clinical trials are necessary to assess their efficacy and safety in practical applications. As with any compound, the road from initial discovery to practical application is a complex one that demands comprehensive investigations across various scientific disciplines. [18] Collaborative efforts between medicinal and computational chemists, biologists, pharmacologists, and clinicians are essential to establish a solid foundation for the development of Hipposudoric acid and its analogues as potential therapeutic agents with

diverse bioactive properties. A crucial aspect to address is compound stability, and there is a need to identify novel, more stable versions of Hipposudoric acid, all the while preserving or augmenting its beneficial properties.

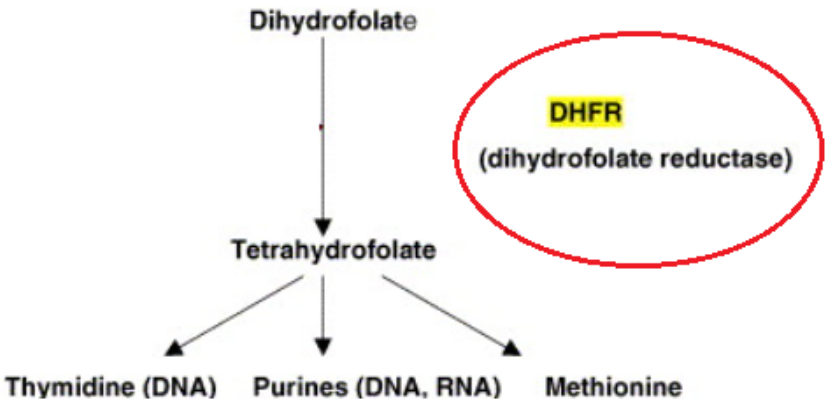


Figure 3. The reduction of dihydrofolate to tetrahydrofolate catalyzed by DHFR. [19]

2.4 Spectroscopic Techniques for Compound Characterization

In the field of compound characterization, nuclear magnetic resonance (NMR) spectroscopy played a pivotal role as the primary method used for characterizing compounds. [24] It offered crucial insights into molecular dynamics and structural information. NMR spectroscopy relied on nuclear spin and resonance, revealing intra-molecular interactions that facilitated the identification of chemical structures. Carbon-13 and hydrogen (protons) nuclei, owing to their inherent nuclear spin, exhibited magnetic properties within the framework of NMR spectroscopy. The application of radiofrequency pulses within a magnetic field induced these nuclei to exhibit distinct resonance frequencies, thereby unveiling their local environments. NMR spectra encompassed essential parameters such as chemical shifts, coupling constants, and integrals, all of which played pivotal roles in detecting functional groups, molecular connectivity, and stereochemistry in compounds. This analytical technique proved invaluable as it discriminated between isomers and tautomers with precision.

Mass spectroscopy, another prominent method, emerged as a versatile tool for the chemical characterization of ions based on their mass-to-charge ratios. This approach streamlined the determination of the molecular composition of chemical substances. Key methodologies involved in mass spectrometry included atomic mass calculations,

analysis of fragmentation patterns, and examination of isotopic distributions. The process commenced with the ionization of a sample, followed by the categorization of ions according to their mass-to-charge ratio. These techniques were widely employed to uncover intricate details about the molecular composition of compounds and their structural elucidation. [25]

Infrared (IR) spectroscopy, on the other hand, played a pivotal role in the identification of functional groups within chemical compounds by inspecting the vibrational modes of molecular bonds. Specific vibrational transitions occurred when molecules absorbed infrared light, offering insights into the types of bonds and functional groups present. The characteristic peaks observed in IR spectra were directly linked to distinct functional groups. Bonds such as C-H, O-H, N-H, C=O, and C=C gave rise to stretching and bending vibrations that manifested as distinct peaks in the spectrum. The positions and intensities of these peaks served as valuable indicators for inferring the presence of specific functional groups and gaining a deeper understanding of the overall structure of the compound. This technique found extensive use in various applications, including the identification of unknown compounds and the analysis of complex mixtures. [26]

2.5 Novel Compounds: Pioneering a Therapeutic Frontier

The production of new compounds was a creative and revolutionary step in antibacterial research and medicinal development. Some of the synthesised analogues of Hipposudoric acid stood out as new substances with distinctive structural alterations and improved properties. By providing new approaches for battling microbial infections and tackling treatment difficulties, these innovative chemicals had the potential to push the limits of therapeutic possibilities. [3] These new compounds were significant because they underwent structural changes. These chemicals might have interacted differently with microbial targets due to structural deviations, making them possibly more effective, selective, and flexible. In addition to demonstrating the adaptability of the original Hipposudoric Acid molecule. [32,33]

Introducing new compounds injects enthusiasm and hope into the area, even if the road from synthesis to medicinal use may require rigorous testing and validation. These substances could be the solution to solving unmet medical needs, defeating resistant strains, and revolutionizing antimicrobial treatment. The promise of introducing a new class of therapeutic agents energises researchers and emphasises the dynamic

character of medicinal chemistry. These novel compounds will open a new chapter in antimicrobial research as they undergo characterisation, antimicrobial activity testing, and prospective clinical trials. Their introduction into the field of research serves as a reminder of how medical science is still developing and how difficult it is to understand the complexity of microbial illnesses fully.

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