Scientific Research Publishing

Journal of Biophysical Chemistry, 2026, 17(3), 29-49
https://www.scirp.org/journal/jbpc
ISSN Online: 2153-0378
ISSN Print: 2153-036X

Hyperforin from *Hypericum perforatum* L.: Correct Stereochemistry, Structural Evidence, and Biological Activity

Larysa Karaliova*, Ilia Brondz#

Norwegian Drug Control and Drug Discovery Institute (NDCDDI) AS, Ski, Norway
Email: #ilia.brondz@gmail.com

How to cite this paper: Karaliova, L. and Brondz, I. (2026) Hyperforin from *Hypericum perforatum* L.: Correct Stereochemistry, Structural Evidence, and Biological Activity. *Journal of Biophysical Chemistry*, **17**, 29-49.
https://doi.org/10.4236/jbpc.2026.173003

Received: July 26, 2026
Accepted: August 28, 2026
Published: August 31, 2026

Abstract

Hyperforin is a major polyprenylated acylphloroglucinol from *Hypericum perforatum* L. whose complex stereochemistry is central to its chemical identity, biological activity, and biosynthetic reproducibility. This review critically reassesses the structural and stereochemical evidence for hyperforin and discusses its biological activity in relation to phytochemical characterization, stability, and analytical methodology. Hyperforin represents a rare example of a natural product whose biological significance can only be understood through the combined interpretation of analytical chemistry, stereochemistry, total synthesis, and pharmacology. The antimicrobial properties of *Hypericum perforatum* extracts, particularly against antibiotic-resistant cocci, were intensively investigated at the University of Oslo during 1978-1979. Subsequent investigations between 1979 and 1985 focused on the chemical composition of the plant, the isolation of hyperforin, and its structural characterization. These studies led to a revision of earlier structural representations of hyperforin. The first mass spectrometric characterization of hyperforin was followed by X-ray crystallographic analysis of its 3,5-dinitrobenzoate ester, which established the relative stereochemistry. Subsequent crystallographic analysis of the *p*-bromobenzoate ester demonstrated that earlier stereochemical assignments required correction, although erroneous structural representations continue to be cited in the literature. Accurate stereochemical determination is essential for understanding the biological activity, synthetic reproducibility, and structure-function relationships of hyperforin. Owing to its lipophilicity, chemical instability, membrane interactions, antimicrobial and immunomodulatory activity, and antitumor-related effects, hyperforin merits further investigation as a

*Deceased. This review is based in part on an unpublished manuscript chapter prepared by Larysa Karaliova. The corresponding author takes responsibility for the final submitted version.

#Corresponding author.

structurally complex and multifunctional bioactive natural product.

Keywords

Hyperforin, *Hypericum perforatum*, Polyprenylated Acylphloroglucinol, Stereochemistry, X-Ray Crystallography, GC-MS with SMB, Biosynthesis, Biological Activity

1. Introduction

This review is based on a chapter from an unpublished manuscript by Larysa Karaliova and was further developed by the co-author in her memory (Brondz [1]). Earlier reviews have not sufficiently distinguished between the early provisional stereochemical assignments, and the later configuration established by X-ray crystallography and supported by total synthesis.

During 1978-1979, intensive investigations were initiated at the University of Oslo to study the composition of *Hypericum perforatum* L. (Brondz, Greibrokk, and Aasen [2] [3]). The rapid global emergence of antimicrobial resistance (AMR), including resistance among clinically important species of cocci and methicillin-resistant *Staphylococcus aureus* (MRSA), represents one of the most serious challenges to modern medicine (Zapun, Vernet, and Pinho [4]). The effectiveness of conventional antibiotics, which target specific microbial structures such as the cell wall, ribosomes, or DNA replication machinery, is increasingly compromised by resistance mechanisms including enzymatic degradation, target modification, reduced membrane permeability, and active efflux systems.

This situation highlights a fundamental limitation of traditional antimicrobial strategies: the more specific the molecular target, the greater the selective pressure for the development of resistance. As a result, there is growing interest in alternative approaches that extend beyond direct pathogen elimination.

One such approach is host-directed therapy (HDT), which aims to enhance host innate and adaptive immune responses rather than directly targeting the pathogen (Brondz [5] [6], Kaufmann *et al.* [7]). By modulating host defense mechanisms, HDT strategies may reduce selective pressure on microorganisms and provide broader-spectrum protection, including against resistant strains.

Natural products have historically played a central role in drug discovery and remain a rich source of biologically active compounds (Gurevich *et al.* [8], and Newman, and Cragg [9]). *Hypericum perforatum* L. (St. John's wort) has been widely used in traditional medicine for centuries, particularly in the treatment of wounds, infections, and inflammatory conditions (Greeson, Sanford and Monti [10]). Its pharmacological properties have been extensively investigated, especially in relation to antidepressant activity (Butterweck, and Schmidt [11]).

Among its constituents, hyperforin has attracted considerable attention due to its broad pharmacological profile. In addition to antimicrobial activity (Schempp,

Pelz, Wittmer, Schöpf, and Simon [12]) and antiprotozoal properties, hyperforin exhibits anti-inflammatory, anticancer (Schempp *et al.* [13]) and immunomodulatory activities (Brondz [14] [15], Brondz, and Brondz [16], Cardile *et al.* [17], Gartner *et al.* [18], and Kacerovská *et al.* [19]). Importantly, hyperforin is capable of crossing biological barriers, including the blood-brain barrier (BBB) and blood-testis barrier (BTB), allowing distribution throughout various tissues (Brondz [5] [6]).

Despite these findings, hyperforin has not yet been fully integrated into contemporary antimicrobial research frameworks. Furthermore, discrepancies in its reported stereochemistry remain unresolved in parts of the literature, with earlier incorrect structural assignments still being cited.

The present review aims to clarify the correct relative and absolute stereochemistry of hyperforin, present its mass spectral characteristics, and evaluate its potential for analysis using advanced techniques such as gas chromatography-mass spectrometry with supersonic molecular beams (GC-MS with SMB). In addition, the review re-evaluates hyperforin within the context of modern antimicrobial challenges and proposes that it should be considered not merely as an antibiotic-like compound, but as a host-directed antimicrobial agent with a multitarget mechanism of action.

2. Literature Search and Scope of the Review

This article is a narrative review rather than a systematic review or meta-analysis. Its primary purpose is to critically reassess the structural and stereochemical evidence concerning hyperforin and to summarize the current knowledge regarding its biological activity. The literature considered includes the original publications describing the isolation and structural elucidation of hyperforin, later crystallographic investigations, total synthesis studies, analytical chemistry reports, and more recent publications addressing pharmacological, microbiological, antiviral, immunological, and anticancer properties. References were selected according to their scientific relevance to the historical development and current understanding of hyperforin rather than by predefined statistical inclusion criteria. Particular emphasis was placed on original experimental studies that established the stereochemistry of hyperforin and on publications that significantly advanced understanding of its biological properties. Because this review addresses the historical development of structural knowledge over approximately five decades, early publications of continuing scientific importance were retained together with recent investigations.

3. Ethnopharmacological Background

The ethnopharmacological use of *Hypericum perforatum* L. spans a wide range of geographical regions and historical periods, reflecting its long-standing importance as a medicinal plant. In European traditional medicine, the plant has been widely used for the treatment of wounds, burns, and inflammatory conditions (Osborn

[20], Brondz [14], European Medicines Agency [21]). Its application as a topical antiseptic indicates early empirical recognition of antimicrobial properties (Volosovets [22]).

Beyond Western herbal traditions, Hypericum species have been used in various folk medical systems to prevent infection and promote wound healing (Greeson, Sanford, and Monti [10]). During the twentieth century, particularly in the Soviet Union, Eastern Europe, and Norway, more systematic pharmacological investigations were undertaken (Brondz [14]).

Standardized medicinal preparations, such as Novoimanin, were developed and applied clinically in the treatment of infected wounds and skin infections (Volosovets [23]). These preparations demonstrated pronounced antibacterial activity, particularly against Gram-positive microorganisms (Gurevich *et al.* [8], Brondz [14] Greeson, Sanford, and Monti [10]).

The continuity between ethnopharmacological knowledge and modern experimental findings supports the hypothesis that hyperforin is a principal contributor to the biological activity of *Hypericum perforatum*.

4. Chemistry of Hyperforin

Hyperforin is a polyprenylated acylphloroglucinol characterized by a highly lipophilic and structurally complex framework (Bystrov, Chernov, Dobrynin and Kolosov [24]; Bystrov, Dobrynin, Kolosov, Chernov, Chervin and Yakovlev [25]; Bystrov, Dobrynin, Kolosov, Popravko, and Chernov [26]-[28]; Bystrov *et al.* [29]). Its chemical instability, particularly under exposure to light and oxygen, presents significant analytical challenges and may affect reproducibility in pharmacological studies.

The stereochemical assignment of hyperforin has historically been controversial. Early structural interpretations proposed by Bystrov and co-workers were later revised using more direct structural approaches.

For comparison with the early structural studies of Bystrov *et al.* (1975-1978), an authentic sample of hyperforin obtained from the Academy of Sciences of the USSR was used. Both the relative structure and the absolute configuration determined in the later crystallographic studies were therefore compared with the same authentic material investigated in the earlier Soviet work.

Discrepancies between the structures proposed by Bystrov *et al.* and the later experimental data became apparent. The corrected relative configuration of hyperforin was established by X-ray crystallography of the 3,5-dinitrobenzoate ester (Brondz, Greibrokk, Groth, and Aasen [30]). Subsequently, the absolute configuration of hyperforin was determined by X-ray crystallographic analysis of its *p*-bromobenzoate ester (Brondz, Greibrokk, Groth, and Aasen [31]). These studies demonstrated that the stereochemical assignments proposed by Bystrov and coworkers required revision. The corrected relative and absolute configuration of hyperforin was consistent with later synthetic studies (Shimizu, Shi, Usuda, Kanai and Shibasaki [32]; Sparling, Moebius, and Shair [33]).

Hyperforin contains multiple stereogenic elements, and accurate stereochemical assignment is essential for understanding its biological activity, structure-function relationships, and synthetic reproduction. Analytical techniques such as GC-MS with SMB have proven useful for distinguishing structural isomers and elucidating stereochemical features (Brondz [34]).

5. Critical Review of Relative and Absolute Stereochemistry: X-Ray Crystallography, Mass Spectrometry, and Synthetic Confirmation

Stereochemical variations are known to significantly influence membrane interactions, pharmacodynamic behavior, and the biological activity of complex natural products (Zanoli [35]). Therefore, accurate determination of both relative and absolute stereochemistry is essential not only for correct synthetic reproduction, but also for understanding interactions with microbial cells, cancer cells, enzymes, cofactors, and host immune systems.

Hyperforin is a structurally complex polyprenylated acylphloroglucinol containing multiple stereogenic elements. Its spatial configuration is directly relevant to its biological activity, membrane interactions, and pharmacological interpretation. For such a molecule, no single indirect analytical method is sufficient to establish stereochemistry with full confidence. A reliable structural assignment requires convergent evidence from complementary approaches, including mass spectrometry, chromatographic separation, X-ray crystallography, biosynthetic studies, and total synthesis.

The first mass spectrum of hyperforin was reported in 1979 using chemical ionization (Brondz [14]), providing important molecular-characterization data. The observed molecular ion at m/z 537 was consistent with a molecular weight of 536 g/mol, reflecting protonation under the ionization conditions. The first chemical-ionization mass spectrum of hyperforin was later reproduced in Brondz (2017 [34]).

The relative stereochemistry of hyperforin was subsequently investigated by X-ray crystallography of its 3,5-dinitrobenzoate ester (Brondz, Greibrokk, Groth, and Aasen [30]). This represented a decisive advance over earlier spectroscopic interpretations, as the crystallographic approach allowed direct visualization of the three-dimensional arrangement of the molecular framework.

The absolute configuration was later established by X-ray crystallographic analysis of the *p*-bromobenzoate ester of hyperforin (Brondz, Greibrokk, Groth, and Aasen [31]). The use of the brominated derivative was particularly important because anomalous scattering by bromine provided an experimental basis for assigning the absolute configuration.

The configuration established by X-ray crystallography was also supported by later independent synthetic studies (Shimizu, Shi, Usuda, Kanai, and Shibasaki [32]; Sparling, Moebius, and Shair [33]). The agreement between X-ray crystallographic evidence and total synthesis is of particular significance because total synthesis provides an independent chemical validation of the stereochemical model.

Together, crystallography, GC-MS with SMB (Brondz, Fialkov, and Amirav [36]), and synthesis form a convergent body of evidence supporting the corrected relative and absolute stereochemistry of hyperforin.

Hyperforin may exist in tautomeric equilibrium, as discussed by Brondz (2017 [34] [37]). The major commonly used stereochemical form is (1R,5R,7S,8R)-4-hydroxy-1-isobutyryl-8-methyl-3,5,7-tris(3-methylbut-2-en-1-yl)-8-(4-methylpent-3-en-1-yl)bicyclo[3.3.1]non-3-ene-2,9-dione (**Figure 1**).

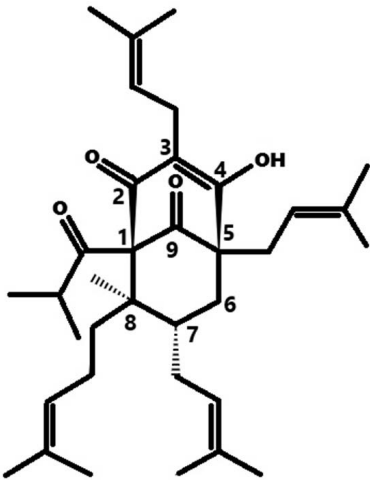

Figure 1. Corrected structure and atom numbering of hyperforin. Hyperforin (1R,5R,7S,8R)-4-hydroxy-1-isobutyryl-8-methyl-3,5,7-tris(3-methylbut-2-en-1-yl)-8-(4-methylpent-3-en-1-yl)bicyclo[3.3.1]non-3-ene-2,9-dione. The numbering of the bicyclic ring system follows IUPAC nomenclature.

The numbering and classification of this two-dimensional structure were discussed by Brondz (2017 [37]). NMR-based biosynthetic studies of hyperforin formation in *Hypericum perforatum* were also consistent with the stereochemical framework proposed by Brondz *et al.* (Adam, Arigoni, Bacher, and Eisenreich [38]). Earlier structural assignments proposed by Bystrov and co-workers (1975-1978) differ from the later configuration validated by X-ray crystallographic evidence and from evidence obtained through total synthesis, and NMR-based biosynthetic studies.

These discrepancies are best understood in the context of the analytical limitations, as well as inconsistencies in stereochemical interpretation and atom-numbering conventions by Bystrov and co-workers (1975-1978).

The central question concerns whether the relative and absolute stereochemistry proposed in the early studies of Bystrov *et al.* is consistent with later crystallographic and synthetic evidence. Later structural representations and renumbering introduced in the Bystrov series 1976-1978 complicate direct comparison with the original 1975 model introduced by Bystrov and co-workers. When translated into systematic stereochemical terminology, the Bystrov representation differs from the structure established by X-ray crystallography in both relative and absolute configuration.

The structure described in the Bystrov series of 1975-1978 may be interpreted as (1S,5R,6R,7S)-4-hydroxy-5-isobutyryl-6-methyl-3,5,7-tris(3-methylbut-2-en-1-yl)-6-(4-methylpent-3-en-1-yl)bicyclo[3.3.1]non-3-ene-2,9-dione, or may require further reinterpretation depending on the numbering convention applied.

Despite similarities in substituent positions, this representation is not identical to the crystallographically established structure. The carbon numbering presented in the 1975-1978 publications is not fully consistent with systematic IUPAC numbering principles, further complicating direct comparison of stereogenic centers.

Although the early Soviet studies were important in establishing the general molecular framework of hyperforin, they did not provide a definitive determination of its relative and absolute configuration. The persistence of earlier structural representations in the literature highlights the importance of critical evaluation of historical structural assignments and the need for consistent adoption of experimentally validated stereochemical models in ongoing research. Accurate relative and absolute stereochemical characterization remains essential for advancing the pharmacological understanding of hyperforin and for ensuring reproducibility in both experimental and applied studies.

6. Methodological Limitations of the Early Bystrov Assignments and the Evidentiary Strength of X-Ray Crystallography and Total Synthesis

The structural studies of Bystrov *et al.* (1975-1978) represent an important historical stage in the elucidation of hyperforin. These investigations contributed significantly to the understanding of the general molecular skeleton and the tetracarbonyl system of the molecule. However, the methods available at that time were not sufficient for an unambiguous determination of the relative and absolute stereochemistry of such a complex, highly substituted, and conformationally constrained natural product.

The early assignments relied primarily on spectroscopic and chemical interpretation, including nuclear magnetic resonance data, infrared spectroscopy, and chemical degradation or derivatization approaches. These methods were valuable for establishing functional groups and the general carbon framework, but they were indirect with respect to relative and absolute stereochemical determination. In a molecule such as hyperforin, with multiple stereogenic centers and closely related substituent environments, indirect spectroscopic interpretation can lead to ambiguous or erroneous stereochemical conclusions.

A further difficulty concerns atom-numbering conventions. The numbering system used in the early publications of Bystrov *et al.* (1975-1978) was chemically motivated, but it was not consistently maintained across later structural representations and was not consistent with IUPAC nomenclature for relative and absolute stereochemistry (Brondz [34] [37]). Changes in the numbering system used by Bystrov *et al.* (1975-1978) can obscure the correspondence between stereogenic centers and may lead to apparent or real discrepancies in stereochemical assign-

ment. This issue is particularly important when comparing historical structural proposals with crystallographic atom numbering, which necessarily assigns numbers to all atoms in the crystal structure.

By contrast, X-ray crystallography provides direct three-dimensional structural information. In the case of the *p*-bromobenzoate ester of hyperforin, the presence of bromine enabled the use of anomalous scattering to assign the absolute configuration experimentally (Brondz, Greibrokk, Groth, and Aasen [31]). This makes the crystallographic determination fundamentally stronger than an assignment based solely on indirect spectroscopic reasoning.

Total synthesis provides an external chemical test of the proposed configuration: if the synthesized molecule corresponds to the natural product in structure and properties, the assigned stereochemical model receives independent confirmation (Shimizu, Shi, Usuda, Kanai, and Shibasaki [32]; Sparling, Moebius, and Shair [33]). Biosynthetic NMR studies provide additional consistency with this structural framework (Adam, Arigoni, Bacher, and Eisenreich [38]). Thus, the convergence of X-ray crystallography, biosynthetic evidence, and synthesis provides a substantially stronger basis for the relative and absolute stereochemistry of hyperforin than the earlier indirect methodology.

Accordingly, the structures proposed by Bystrov *et al.* (1975-1978) should be regarded as historically important but methodologically limited. For current chemical, pharmacological, and synthetic work on hyperforin, the configuration established by X-ray crystallography and supported by total synthesis should therefore be used as the reference model.

7. Biosynthesis and Occurrence of Hyperforin in *Hypericum perforatum* L.

Hyperforin is one of the characteristic secondary metabolites of *Hypericum perforatum* L. and belongs to the group of polyprenylated acylphloroglucinols. This structural class is of particular phytochemical interest because it combines an acylphloroglucinol core with multiple prenyl-derived side chains, giving rise to a highly lipophilic and conformationally complex natural product. The prenylated architecture of hyperforin is also directly relevant to its chemical instability, chromatographic behavior, interaction with biological membranes, and biological activity.

The biosynthesis of hyperforin in *Hypericum perforatum* L. was investigated by Adam, Arigoni, Bacher, and Eisenreich (2002), who provided important experimental evidence for the origin of the carbon skeleton and prenyl substituents of this natural product. Their work established hyperforin as a biosynthetically complex plant metabolite in which acylphloroglucinol formation and subsequent prenylation steps are essential for generation of the final molecule. This biosynthetic background is important for understanding why correct structural and stereochemical assignment is not only a matter of nomenclature, but also a prerequisite for interpreting the natural origin, reproducibility, and biological properties

of hyperforin.

The extensive prenylation of hyperforin contributes to its distribution in lipophilic compartments of plant material and helps explain the practical difficulties associated with its isolation, storage, and analysis. Hyperforin is known to be chemically labile, particularly under conditions that may promote oxidation, isomerization, or degradation. Therefore, phytochemical studies of *Hypericum perforatum* L. must take into account not only the presence of hyperforin, but also the stability of the compound during extraction, chromatographic separation, and spectrometric analysis. These features make hyperforin a useful example of a natural product for which biosynthesis, stereochemistry, analytical methodology, and biological activity are closely interconnected.

8. Antimicrobial Activity

Hyperforin exhibits significant antibacterial activity, primarily against Gram-positive organisms, including Staphylococcus aureus and methicillin-resistant strains (MRSA) (Brondz [14]; Schempp, Pelz, Wittmer, Schöpf, Simon [12]; Volosovets [23]). Activity has also been reported against clinically important antibiotic-resistant cocci, including staphylococci, and against selected Gram-negative organisms such as Neisseria (Brondz [14]; Brondz [5]; Schempp, Pelz, Wittmer, Schöpf, Simon [12]).

In addition, hyperforin has been shown to enhance phagocytic activity, leading to indirect antimicrobial effects, including activity against Escherichia coli under opsonizing and non-opsonizing conditions (Brondz, Lingaas, Olssen, and Midtvedt [39]; Brondz [40]).

The sensitivity of Gram-positive bacteria is likely related to their cell-wall structure, which facilitates interaction between hyperforin and the cytoplasmic membrane. This interaction may disrupt membrane integrity and ion gradients.

Gram-negative bacteria are generally less susceptible because of the presence of an outer membrane barrier. However, selective activity against certain Gram-negative species has been reported, suggesting that permeability barriers alone do not fully explain the antimicrobial spectrum (Brondz [14]; Brondz, Lingaas, Olssen, and Midtvedt [39]; Brondz [40]).

Mechanistically, hyperforin does not appear to act through a single defined molecular target. Proposed mechanisms include disruption of membrane potential, modulation of ion transport, and indirect antimicrobial effects mediated through host immune responses (Brondz [15]; Brondz, and Brondz [16]). These properties support the classification of hyperforin not only as a direct antimicrobial agent but also as a potential host-directed therapeutic compound.

9. Immunomodulatory Mechanism

In recent years, it has become increasingly evident that, despite the availability of numerous broad-spectrum antimicrobial agents, many infections remain difficult to treat, particularly in immunocompromised patients. In such individuals, im-

paired host defense mechanisms often result in severe and persistent microbial infections. A defining feature of hyperforin is its capacity to modulate the host immune response. Experimental studies have demonstrated that hyperforin enhances phagocytic activity (Brondz [40]) and supports innate immune functions (Brondz, and Brondz [16]). Phagocytosis is a fundamental component of innate immunity, involving the recognition, uptake, and destruction of pathogens by professional phagocytes such as macrophages and neutrophils. Hyperforin has been shown to significantly increase the uptake of bacteria by phagocytic cells, including under conditions where classical opsonin are absent. This observation suggests that hyperforin may exert an opsonin-like effect, facilitating interactions between pathogens and immune cells. *In vitro* studies demonstrated that hyperforin, at concentrations ranging from 1 to 100 μg/mL, stimulated the uptake of *Escherichia coli* by human polymorphonuclear neutrophils. The most pronounced effect was observed in the uptake of non-opsonized bacteria, where phagocytosis increased markedly at 100 μg/mL (Brondz [40]).

Unlike classical opsonins, which are typically proteins such as antibodies or complement factors, hyperforin is a small lipophilic molecule. Its physicochemical properties suggest that it may integrate into biological membranes and modulate membrane dynamics, thereby influencing cell-pathogen interactions.

In addition, hyperforin enhanced the uptake of IgG-opsonized bacteria, although to a lesser extent. These findings indicate that hyperforin does not merely augment classical immune pathways but may also facilitate phagocytic recognition and uptake independently of conventional opsonization mechanisms.

This dual effect suggests that hyperforin may function as a modulator of cell-pathogen interactions, potentially altering membrane properties or receptor-mediated processes involved in phagocytosis. Such activity is particularly relevant in immunocompromised conditions, where impaired opsonization and phagocytic function contribute to increased susceptibility to infection. The immunomodulatory properties of hyperforin support its classification as a host-directed antimicrobial agent. By enhancing host defense mechanisms rather than directly targeting microbial structures, hyperforin may reduce selective pressure for the development of antimicrobial resistance.

10. Antiviral Activity

Early Soviet investigations reported antiviral effects of Hypericum-derived preparations, including Imanin, against plant viruses (Gurevich *et al.* [8]). These historical observations are relevant to the broader pharmacological profile of Hypericum perforatum; however, they did not establish hyperforin as the sole constituent responsible for the reported activity.

More direct evidence has recently been provided for the antiviral activity of purified hyperforin against human coronaviruses. Raczkiewicz *et al.* (2024) demonstrated activity against four tested human coronaviruses, with IC_{50} values ranging from 0.24 to 2.55 μM. Time-of-addition experiments suggested that the inhibitory

effect occurred after viral entry, potentially during the replication stage. The antiviral effect was also demonstrated in primary human airway epithelial cells. These findings support the description of hyperforin as a compound with pan-coronavirus antiviral activity (Raczkiewicz *et al.* [41]).

The available evidence is nevertheless predominantly preclinical. The effective concentrations, mechanism of action, pharmacokinetic exposure, metabolic stability, and safety of hyperforin in the context of viral infection require further investigation. In particular, its known activation of the pregnane X receptor and induction of CYP3A4 may create clinically important drug-drug interactions and should be considered in any future antiviral development program (Sabarathinam, and Ganamurali [42]).

Oral administration and systemic distribution of hyperforin have been investigated in experimental animals and human volunteers (Biber *et al.* [43]; Brondz [15]; Brondz, and Brondz [16]). These studies are relevant to the feasibility of systemic delivery, although they do not themselves establish clinical antiviral efficacy. Further pharmacokinetic, toxicological, and *in vivo* antiviral studies are therefore required before therapeutic application can be considered.

11. Pharmacokinetics and Barrier Penetration

The high lipophilicity of hyperforin is a key physicochemical property that facilitates its interaction with biological membranes and contributes to its ability to cross physiological barriers (Friedemann [44]). Available evidence suggests that hyperforin is capable of penetrating the blood-brain barrier (BBB) (Brondz [15]; Brondz, and Brondz [16]; Pardridge [45]), which is consistent with its documented neuropharmacological effects (Butterweck, and Schmidt [11]). This characteristic is relatively uncommon among many conventional antibiotics and may have implications for infections involving the central nervous system.

Similarly, hyperforin appears to penetrate other biological barriers, including the blood-testis barrier (BTB) (Brondz [15]; Brondz, and Brondz [16]; Mruk, and Cheng [46]), suggesting potential distribution into immune-privileged tissues.

This property may be relevant in the context of infections associated with pathogens such as Neisseria gonorrhoeae and Neisseria meningitidis, which can involve tissues protected by physiological barriers. In such cases, therapeutic efficacy is often limited by restricted drug penetration. While inflammation can transiently increase barrier permeability, restoration of barrier integrity may subsequently reduce drug access, potentially contributing to incomplete pathogen clearance.

In this context, compounds capable of penetrating intact biological barriers may offer advantages over conventional antimicrobial agents. However, quantitative pharmacokinetic data for hyperforin remain limited, and further studies are required to establish its absorption, distribution, metabolism, and excretion (ADME) profile, as well as clinically relevant dosing parameters. These characteristics are important for interpreting the distribution, biological activity, and pharmacolog-

ical relevance of hyperforin, but quantitative ADME and tissue-exposure data remain necessary before any practical significance can be defined.

12. Antiprotozoal Activity and Analytical Context

Hyperforin has been reported to exhibit *in vitro* antimalarial activity against Plasmodium falciparum (Verotta, Appendino, Bombardelli, and Brun [47]). In the context of the present review, this finding should be interpreted not as evidence of clinical antimalarial efficacy, but as part of the broader biological activity profile of a polyprenylated acylphloroglucinol from H*ypericum perforatum* L. The antiprotozoal activity of hyperforin is therefore relevant primarily because it expands the range of biological systems in which this phytochemical has been tested and supports the need for correct structural and stereochemical assignment.

The comparison with established antimalarial drugs is useful mainly from an analytical and toxicological perspective. Earlier studies on primaquine diphosphate demonstrated that the biological and toxicological interpretation of antimalarial agents may be strongly influenced by chemical purity and by the presence of structurally related contaminants such as quinocide. HPLC, SFC/SFC-MS, GC-MS, and GC-MS with supersonic molecular beams were used to characterize such impurities and to distinguish primaquine from related contaminants (Brondz *et al.* [48]; Brondz *et al.* [49]; Brondz, Fialkov, and Amirav [36]). These studies illustrate a general principle that is also important for hyperforin: biological activity cannot be reliably interpreted unless the chemical identity, stereochemistry, purity, and stability of the investigated compound are adequately controlled.

Thus, the relevance of hyperforin to antimalarial research remains at the preclinical and phytochemical level. Its reported *in vitro* activity should be regarded as an additional indication of broad bioactivity, not as a basis for therapeutic claims. For Phytochemistry, the main significance lies in the relationship between natural-product structure, analytical characterization, stereochemistry, stability, and biological response.

13. Anti-Angiogenic and Antitumor Activity

In the human organism, continuous cellular turnover generates a substantial number of abnormal or potentially transformed cells. Under physiological conditions, the immune system identifies and eliminates many of these cells, thereby contributing to the prevention of malignant transformation. The immunomodulatory activity of hyperforin may contribute to this surveillance system (Brondz, Brondz [16]). In addition to its effects on innate immunity, hyperforin has been reported to influence key mechanisms involved in inhibition of tumor development and progression (Schempp *et al.* [13]). Tumor cells often exhibit impaired apoptotic pathways, which facilitate survival and proliferation. Hyperforin has been shown to induce apoptosis in malignant cells, thereby partially restoring this critical regulatory function (Hostanska, Reichling, Bommer, Weber, and Saller [50]; Schempp,

Simon-Haarhaus, and Simon [51]).

Beyond its pro-apoptotic effects, hyperforin demonstrates significant anti-angiogenic activity. Angiogenesis is a fundamental process in tumor growth and metastasis, as it provides the blood supply necessary for tumor expansion. Hyperforin has been reported to interfere with multiple steps of angiogenesis, including inhibition of matrix metalloproteinase-9 (MMP-9) activity, suppression of neutrophil activation, and reduction of cell motility and recruitment (Lorusso [52]; Schempp *et al.* [53]).

Furthermore, hyperforin appears to attenuate inflammation-driven angiogenesis and may exert protective effects against fibrosis, including in pulmonary tissues. Given the central role of inflammatory processes in tumor-associated angiogenesis, these findings contribute to the broader biological profile of hyperforin as a multifunctional natural product.

The inhibition of tumor-associated angiogenesis is relevant to cancer biology, because angiogenesis supports tumor growth, invasion, and metastasis (Albini, Tosetti, Benelli, and Noonan [54]). Hyperforin has also been reported to affect cancer-cell invasion and metastatic behavior (Donà *et al.* [55]). Taken together, the reported pro-apoptotic, anti-angiogenic, anti-inflammatory, and immunomodulatory effects support the classification of hyperforin as a multitarget bioactive phytochemical. However, these findings remain primarily preclinical and require further mechanistic and toxicological validation.

14. Stability, Standardization, and Formulation Challenges

Hyperforin remains technically challenging to study because of its chemical instability and pronounced lipophilicity. The compound is readily oxidized by atmospheric oxygen and is sensitive to light, which complicates extraction, purification, storage, standardization, and reproducible biological testing. Its low aqueous solubility also creates difficulties for preparation of experimental formulations. Commercial hyperforin standards are useful for analytical comparison, but they do not solve the broader problems of stability, purity, sterility, and formulation required for pharmacological or toxicological evaluation. These limitations underline the need for careful phytochemical standardization before biological results can be reliably compared across studies.

15. Recent Developments and Future Perspectives

Recent studies have increasingly focused on the pharmacological consequences of the established structure of hyperforin rather than on its structural elucidation. Current investigations emphasize the multiple pharmacological activities of hyperforin, including modulation of inflammatory pathways and TRPC6-mediated signaling, potential effects on antimicrobial resistance mechanisms, and possible applications in oncology and neuroprotection (Brahmachari [56]; Cardile *et al.* [17]; Li *et al.* [57]; Sabarathinam, and Ganamurali [42]).

16. Discussion

Hyperforin is a highly substituted polyprenylated acylphloroglucinol with a complex three-dimensional architecture. Even relatively small differences in stereochemical assignment may influence conformational behavior, membrane interactions, receptor binding, pharmacodynamic properties, synthetic reproducibility, and the interpretation of structure-activity relationships.

The revision of hyperforin stereochemistry is therefore not merely a historical or nomenclatural issue. The early assignments proposed by Bystrov and co-workers between 1975 and 1978 were important in establishing the general molecular framework, but they were based primarily on the indirect spectroscopic and chemical methods available at that time. These approaches were insufficient for an unambiguous determination of the absolute configuration of a molecule of such complexity.

Later X-ray crystallographic studies of hyperforin derivatives provided a more direct experimental basis. Analysis of the 3,5-dinitrobenzoate ester established the relative stereochemistry, whereas analysis of the *p*-bromobenzoate ester permitted determination of the absolute configuration. The bromine atom was particularly important because anomalous scattering enabled experimental assignment of absolute stereochemistry. These crystallographic results were subsequently supported by independent total syntheses, providing chemical confirmation of the corrected structural model.

GC-MS with supersonic molecular beams provided additional analytical support by improving the differentiation of structurally related compounds and contributing to the evaluation of isomeric and stereochemical features. Although this method cannot replace X-ray crystallography for determination of absolute configuration, it provides complementary evidence within a convergent analytical framework. The agreement among crystallographic analysis, advanced mass spectrometry, and total synthesis supports the corrected stereochemical model as the appropriate reference structure for future chemical, pharmacological, and synthetic investigations.

The physicochemical properties of hyperforin are also important to its pharmacological potential and for interpreting its biological activity. Its pronounced lipophilicity promotes interaction with biological membranes and may influence tissue distribution, permeability, extraction behaviour, chromatographic properties, and formulation challenges. These features link the phytochemical structure of hyperforin directly to its analytical handling and biological interpretation.

The present review integrates structural, phytochemical, analytical, microbiological, and pharmacological evidence to reassess hyperforin as a multifunctional bioactive natural product. The available data indicate that hyperforin should not be regarded solely as a conventional natural antimicrobial substance. Rather, its biological profile includes direct antimicrobial activity, modulation of host defense mechanisms, antiprotozoal effects, antiviral potential, inhibition of angiogenesis, and antitumor-related activity.

A particularly important feature of hyperforin is its ability to influence innate immune defense. Conventional antimicrobial agents act primarily by targeting microbial structures or metabolic pathways and may therefore generate selective pressure that contributes to the emergence of resistance. Hyperforin, by contrast, has been reported to enhance phagocytic activity, including the uptake of non-opsonized bacteria by human polymorphonuclear neutrophils. This finding suggests that hyperforin may facilitate pathogen clearance through mechanisms that are not restricted to classical opsonin-dependent pathways.

Such an immunomodulatory effect may be especially relevant in patients with impaired host defense, in whom defective phagocytic or innate immune functions contribute to severe, recurrent, or persistent infection. At the same time, hyperforin exhibits direct antimicrobial activity, particularly against Gram-positive organisms, including antibiotic-resistant strains. The combination of direct antimicrobial action and enhancement of host immunity represents a potentially valuable dual mechanism and supports consideration of hyperforin as a multitarget bioactive phytochemical whose activity may involve both direct antimicrobial effects and modulation of host-cell responses. Accurate determination of the relative and absolute stereochemistry of hyperforin is essential for interpreting these biological activities. Quantitative pharmacokinetic information remains limited, and systematic studies of absorption, distribution, metabolism, excretion, tissue exposure, and clinically relevant dosing are still required.

The antiviral findings add another dimension to the pharmacological profile of hyperforin. Recently *in vitro* studies demonstrated activity against several human coronaviruses and suggested an effect occurring after viral entry, possibly during replication. These results are promising but remain preclinical. The mechanism of action, achievable tissue concentrations, metabolic stability, and safety must therefore be clarified before any biological or pharmacological relevance can be defined.

Hyperforin also exhibits biological effects relevant to oncology. Reported activities include induction of apoptosis, inhibition of angiogenesis, modulation of inflammatory signaling, and interference with matrix metalloproteinase activity and cellular recruitment. These mechanisms affect several processes involved in tumor progression. The combination of anti-angiogenic, pro-apoptotic, and immunomodulatory actions may therefore be relevant to the broader biological profile of hyperforin as a multitarget natural product.

The reported *in vitro* antimalarial activity of hyperforin further supports its classification as a broad-spectrum bioactive compound. In view of the toxicity and therapeutic limitations of several established antimalarial drugs, compounds with alternative or multitarget mechanisms remain of interest. Nevertheless, the antimalarial activity of hyperforin remains preclinical and requires further mechanistic, analytical, and biological validation.

Several limitations must be acknowledged. Much of the available evidence is derived from *in vitro* or other preclinical studies, while comprehensive pharmacokinetic, toxicological, and clinical data remain insufficient. The direct molecular

targets responsible for individual pharmacological effects have not been fully defined, and differences in extraction, purification, formulation, storage, and analytical methods may contribute to variation among published results. Because hyperforin is unstable in the presence of light and oxygen, strict analytical standardization is essential for reproducibility.

Drug-drug interactions represent an additional and clinically important limitation. Hyperforin is a potent non-steroidal agonist of the pregnane X receptor and may induce CYP3A4, thereby altering the metabolism of co-administered drugs. This interaction potential must be considered when interpreting biological activity and when evaluating hyperforin in pharmacological or toxicological models (Sabarathinam, and Ganamurali [42]).

Future research should integrate structural chemistry, stereochemical validation, phytochemical analysis, stability assessment, formulation science, pharmacokinetics, microbiology, immunology, and toxicology. Particular attention should be given to the relationship between the corrected stereochemical structure of hyperforin and its biological functions. The convergence of X-ray crystallography, GC-MS with supersonic molecular beams, biosynthetic evidence, and total synthesis provides a reliable structural foundation for such investigations. On this basis, hyperforin merits further study as a structurally complex and multifunctional bioactive natural product from *Hypericum perforatum* L. It should be emphasized that hyperforin is not currently an approved therapeutic drug. Most available evidence remains preclinical and originates from biochemical, microbiological, cellular, or animal studies. Consequently, the biological activities discussed in this review should not be interpreted as evidence of established clinical efficacy but rather as a scientific basis for continued investigation.

Limitations of the Review:

As a narrative review, this article summarizes and critically evaluates published experimental evidence but does not generate new experimental data. Consequently, the conclusions presented are limited by the quality and availability of the published literature. Although substantial progress has been made in understanding the stereochemistry and biological activity of hyperforin, many pharmacological observations remain preclinical and require further confirmation in animal models and well-designed clinical studies.

Future Perspectives:

Future investigations should focus on improving the chemical stability of hyperforin, reducing its metabolic liabilities, minimizing CYP-mediated drug interactions, and developing stable formulations suitable for *in vivo* and clinical evaluation. Such advances may determine whether hyperforin can eventually progress from an experimentally valuable natural product to a clinically useful therapeutic agent.

17. Conclusions

Hyperforin represents a structurally complex and biologically active natural prod-

uct whose biological significance extends beyond conventional antimicrobial activity.

The evidence reviewed in this article supports its consideration as a multifunctional bioactive natural product with antimicrobial, immunomodulatory, antiprotozoal, anti-angiogenic, and antitumor properties. Its ability to enhance phagocytic activity, interact with biological membranes, and penetrate physiological barriers such as the blood-brain barrier and blood-testis barrier further distinguishes it from many classical antimicrobial agents.

A central conclusion of this review is that the correct relative and absolute stereochemistry of hyperforin is essential for any meaningful interpretation of its biological activity, pharmacological potential, and synthetic reproducibility. Earlier structural assignments proposed by Bystrov and co-workers were historically important but methodologically limited. Later crystallographic studies, supported by advanced mass-spectrometric analysis, biosynthetic evidence, and total synthesis, provide a stronger and experimentally validated basis for the structural understanding of hyperforin.

At the same time, important limitations remain. Hyperforin is chemically unstable, highly lipophilic, difficult to purify, sensitive to oxygen and light, and challenging to formulate. These properties complicate standardization, stability assessment, analytical reproducibility, toxicological evaluation, and biological interpretation. Therefore, further work should integrate structural chemistry, phytochemical analysis, stability studies, formulation science, pharmacokinetics, toxicology, microbiology, immunology, and relevant biological models.

Author Contributions

Larysa Karaliova: Conceptualization; Investigation; Writing—original draft.

Ilia Brondz: Conceptualization; Investigation; Formal analysis; Writing—review and editing, Visualization; Project administration.

Acknowledgements

The authors sincerely thank the anonymous reviewers for their constructive comments and valuable suggestions, which substantially improved the scientific quality and clarity of this review. The authors also acknowledge the use of AI-assisted language editing during manuscript preparation. All scientific interpretations, critical analyses, conclusions, and the final version of the manuscript were reviewed, verified, and approved by the corresponding author, who accepts full responsibility for the accuracy and integrity of the work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Brondz, I. (2025) To Remembrance Larysa Karaliova. *Voice of the Publisher*, **11**, 1-3.

https://doi.org/10.4236/vp.2025.111001

[2] Brondz, I., Greibrokk, T. and Aasen, A.J. (1983) N-Alkanes of *Hypericum perforatum*: A Revision. *Phytochemistry*, **22**, 295-296. https://doi.org/10.1016/s0031-9422(00)80110-7

[3] Brondz, I., Greibrokk, T. and Aasen, A.J. (1983) N-1-Alkanols of *Hypericum perforatum*. *Journal of Natural Products*, **46**, 940-941. https://doi.org/10.1021/np50030a025

[4] Zapun, A., Vernet, T. and Pinho, M.G. (2008) The Different Shapes of Cocci. *FEMS Microbiology Reviews*, **32**, 345-360. https://doi.org/10.1111/j.1574-6976.2007.00098.x

[5] Brondz, I. (2016) Super Antibiotics, Part I. Hyperforin. *Voice of the Publisher*, **2**, 19-27. https://doi.org/10.4236/vp.2016.24004

[6] Brondz, I. (2016) Super Antibiotics, Part II. Hyperforin, Mass Spectroscopy (MS) and Gas Chromatography-Mass Spectrometry (GC-MS), Evidence of Permeability of the Blood-Testis Barrier (BTB) and the Blood-Brain Barrier (BBB) to Hyperforin. *International Journal of Analytical Mass Spectrometry and Chromatography*, **4**, 66-73. https://doi.org/10.4236/ijamsc.2016.44007

[7] Kaufmann, S.H.E., Dorhoi, A., Hotchkiss, R.S. and Bartenschlager, R. (2018) Host-directed Therapies for Bacterial and Viral Infections. *Nature Reviews Drug Discovery*, **17**, 35-56. https://doi.org/10.1038/nrd.2017.162

[8] Gurevich, A.I., Dobrynin, V.N., Kolosov, M.N., Popravko, S.A., Ryabova, I.D., Chernov, B.K., *et al.* (1971) Hyperforin, an Antibiotic from *Hypericum perforatum* L. *Antibiotiki Kimioterapi*, **16**, 510-513. (In Russian)

[9] Newman, D.J. and Cragg, G.M. (2020) Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of Natural Products*, **83**, 770-803. https://doi.org/10.1021/acs.jnatprod.9b01285

[10] Greeson, J.M., Sanford, B. and Monti, D.A. (2001) St. John's Wort (*Hypericum perforatum*): A Review of the Current Pharmacological, Toxicological, and Clinical Literature. *Psychopharmacology*, **153**, 402-414. https://doi.org/10.1007/s002130000625

[11] Butterweck, V. and Schmidt, M. (2007) St. John's Wort: Role of Active Compounds for Its Mechanism of Action and Efficacy. *Wiener Medizinische Wochenschrift*, **157**, 356-361. https://doi.org/10.1007/s10354-007-0440-8

[12] Schempp, C.M., Pelz, K., Wittmer, A., Schöpf, E. and Simon, J.C. (1999) Antibacterial Activity of Hyperforin from St John's Wort, against Multiresistant *Staphylococcus aureus* and Gram-Positive Bacteria. *The Lancet*, **353**, 2129-2132. https://doi.org/10.1016/s0140-6736(99)00214-7

[13] Schempp, C.M., Kirkin, V., Simon-Haarhaus, B., Kersten, A., Kiss, J., Termeer, C.C., *et al.* (2002) Inhibition of Tumour Cell Growth by Hyperforin, a Novel Anticancer Drug from St. John's Wort That Acts by Induction of Apoptosis. *Oncogene*, **21**, 1242-1250. https://doi.org/10.1038/sj.onc.1205190

[14] Brondz, I. (1979) Antibiotikumet "Hyperforin" og andre innholdsstoffer i drogen *Hypericum perforatum* L. Master's Thesis, University of Oslo.

[15] Brondz, I. (2012) Enhancement of the Immunity in AIDS and Other Immunocompromised Patients by Hyperforin, an Antibiotic from *Hypericum perforatum* L. 2*nd ARIP European Conference on Antimicrobial Resistance & Infection Prevention*, Vilnius, 4-5 October 2012, p. 61.

[16] Brondz, I. and Brondz, A. (2012) Recent Enhancement of the Immunity in AIDS and Other Immunocompromised Patients by Hyperforin, an Antibiotic from *Hypericum*

perforatum L. (*In Vitro* Model). Part I. *Journal of Biophysical Chemistry*, **3**, 304-310. https://doi.org/10.4236/jbpc.2012.34037

[17] Cardile, A., Zanrè, V., Campagnari, R., Asson, F., Addo, S.S., Orlandi, E., *et al.* (2023) Hyperforin Elicits Cytostatic/Cytotoxic Activity in Human Melanoma Cell Lines, Inhibiting Pro-Survival NF-κB, STAT3, AP1 Transcription Factors and the Expression of Functional Proteins Involved in Mitochondrial and Cytosolic Metabolism. *International Journal of Molecular Sciences*, **24**, Article 1263. https://doi.org/10.3390/ijms24021263

[18] Gartner, M., Müller, T., Simon, J.C., Giannis, A. and Sleeman, J.P. (2005) Aristoforin, a Novel Stable Derivative of Hyperforin, Is a Potent Anticancer Agent. *ChemBioChem*, **6**, 171-177. https://doi.org/10.1002/cbic.200400195

[19] Kacerovská, D., Pizinger, K., Majer, F. and Šmíd, F. (2008) Photodynamic Therapy of Nonmelanoma Skin Cancer with Topical *Hypericum perforatum* Extract—A Pilot Study. *Photochemistry and Photobiology*, **84**, 779-785. https://doi.org/10.1111/j.1751-1097.2007.00260.x

[20] Osborn, E.M. (1943) On the Occurrence of Antibacterial Substances in Green Plants. *British Journal of Experimental Pathology*, **24**, 227-231.

[21] European Medicines Agency (2022) European Union Herbal Monograph on *Hypericum perforatum* L., Herba (Revision 1). European Medicines Agency. https://www.ema.europa.eu/documents/herbal-monograph/final-european-union-herbal-monograph-hypericum-perforatum-l-herba-revision-1_en.pdf

[22] Volosovets, P.S. (1964) Microflora of Suppurative Wounds and the Quantitative Changes of the Microflora due to the Effect of Treatment with Amanine and Novoimanine. *Mikrobiologichnyi Zhurnal*, **26**, 60-65.

[23] Volosovets, P.S. (1986) The Effect of Novoimanine on the Course of Experimental Staphy-Lococcal Sepsis. *Mikrobiologichnyi Zhurnal*, **48**, 60-64.

[24] Bystrov, N.S., Chernov, B.K., Dobrynin, V.N. and Kolosov, M.N. (1975) The Structure of Hyperforin. *Tetrahedron Letters*, **16**, 2791-2794. https://doi.org/10.1016/s0040-4039(00)75241-5

[25] Bystrov, N.S., Dobrynin, V.N., Kolosov, M.N., Chernov, B.K., Chervin, I.I. and Yakovlev, G.I. (1976) Stereokhimiya Antibiotika Hyperforina. *Doklady Akademii Nauk SSSR*, **226**, 338-341. (In Russian)

[26] Bystrov, N.S., Dobrynin, V.N., Kolosov, M.N., Popravko, S.A. and Chernov, B.K. (1978) Chemistry of Hyperforin. VI. General Chemical Characterization. *Bioorganicheskaya Khimiya*, **4**, 791-797. (In Russian)

[27] Bystrov, N.S., Dobrynin, V.N., Kolosov, M.N., Popravko, S.A. and Chernov, B.K. (1978) Chemistry of Hyperforin. VIII. Differentiation of the Side Chains. *Bioorganicheskaya Khimiya*, **4**, 943-947. (In Russian)

[28] Bystrov, N.S., Dobrynin, V.N., Kolosov, M.N., Popravko, S.A. and Chernov, B.K. (1978) Chemistry of Hyperforin. IX. The Structure of Hyperforin. *Bioorganicheskaya Khimiya*, **4**, 948-955. (In Russian)

[29] Bystrov, N.S., Gupta, S.R., Dobrynin, V.N., Kolosov, M.N., Chernov, B.K., Chervin, I.I., *et al.* (1976) Structure of the Antibiotic Hyperforin. *Doklady Akademii Nauk SSSR*, **226**, 88-90. (In Russian)

[30] Brondz, I., Greibrokk, T., Groth, P.A. and Aasen, A.J. (1982) The Relative Stereochemistry of Hyperforin—An Antibiotic from L. *Tetrahedron Letters*, **23**, 1299-1300. https://doi.org/10.1016/s0040-4039(00)87088-4

[31] Brondz, I., Greibrokk, T., Groth, P., Aasen, A.J., Seip, R. and Brunvoll, J. (1983) The

Absolute Configuration of Hyperforin, an Antibiotic from *Hypericum perforatum* L., Based on the Crystal Structure Determination of Its P-Bromobenzoate Ester. *Acta Chemica Scandinavica*, **37**, 263-265. https://doi.org/10.3891/acta.chem.scand.37a-0263

[32] Shimizu, Y., Shi, S.L., Usuda, H., Kanai, M. and Shibasaki, M. (2010) Catalytic Asymmetric Total Synthesis of *Ent*-Hyperforin. *Angewandte Chemie International Edition*, **49**, 1103-1106. https://doi.org/10.1002/anie.200906678

[33] Sparling, B.A., Moebius, D.C. and Shair, M.D. (2013) Enantioselective Total Synthesis of Hyperforin. *Journal of the American Chemical Society*, **135**, 644-647. https://doi.org/10.1021/ja312150d

[34] Brondz, I. (2017) Super Antibiotics, Part IV. Hyperforin, Relative and Absolute Stereochemistry Elucidated by Gas-Chromatography Mass-Spectrometry with Supersonic Molecular Beams. *International Journal of Analytical Mass Spectrometry and Chromatography*, **5**, 70-85. https://doi.org/10.4236/ijamsc.2017.53005

[35] Zanoli, P. (2004) Role of Hyperforin in the Pharmacological Activities of St. John's Wort. *CNS Drug Reviews*, **10**, 203-218. https://doi.org/10.1111/j.1527-3458.2004.tb00022.x

[36] Brondz, I., Fialkov, A.B. and Amirav, A. (2009) Analysis of Quinocide in Unprocessed Primaquine Diphosphate and Primaquine Diphosphate Tablets Using Gas Chromatography-Mass Spectrometry with Supersonic Molecular Beams. *Journal of Chromatography A*, **1216**, 824-829. https://doi.org/10.1016/j.chroma.2008.11.043

[37] Brondz, I. (2017) Super Antibiotics: Part III. Hyperforin, Revision of the Relative and Absolute Stereochemistry Presented by Bystrov *et al*. *Voice of the Publisher*, **3**, 15-24. https://doi.org/10.4236/vp.2017.32002

[38] Adam, P., Arigoni, D., Bacher, A. and Eisenreich, W. (2002) Biosynthesis of Hyperforin in *Hypericum perforatum*. *Journal of Medicinal Chemistry*, **45**, 4786-4793. https://doi.org/10.1021/jm0209782

[39] Brondz, I., Lingaas, E., Olssen, I. and Midtvedt, T. (1984) The Influence of Hyperforin on Phago-Cytosis of *Escherichia coli* by Human Polymorphonuclear Neutrophils *in Vitro*. 14*th International Symposium on the Chemistry of Natural Products*, Poznań, 9-14 July 1984, 590.

[40] Brondz, I. (1987) Influence of Hyperforin Upon Phagocytic Functions in Human Polymorphonuclear Leucocytes. *Fourth International Conference on Chemistry and Biotechnology of Biologically Active Natural Products*, Budapest, 10-14 August 1987, p. 119.

[41] Raczkiewicz, I., Rivière, C., Bouquet, P., Desmarets, L., Tarricone, A., Camuzet, C., *et al*. (2024) Hyperforin, the Major Metabolite of St. John's Wort, Exhibits Pan-*Coronavirus* Antiviral Activity. *Frontiers in Microbiology*, **15**, Article ID: 1443183. https://doi.org/10.3389/fmicb.2024.1443183

[42] Sabarathinam, S. and Ganamurali, N. (2026) Hyperforin as a Non-Steroidal Modulator of the PXR-CYP3A4 Regulatory Axis: Mechanistic and Computational Insights into Xenobiotic Metabolism. *The Journal of Steroid Biochemistry and Molecular Biology*, **256**, Article 106886. https://doi.org/10.1016/j.jsbmb.2025.106886

[43] Biber, A., Fischer, H., Römer, A. and Chatterjee, S. (1998) Oral Bioavailability of Hyperforin from Hypericum Extracts in Rats and Human Volunteers. *Pharmacopsychiatry*, **31**, 36-43. https://doi.org/10.1055/s-2007-979344

[44] Friedemann, U. (1942) Blood-Brain Barrier. *Physiological Reviews*, **22**, 125-145. https://doi.org/10.1152/physrev.1942.22.2.125

[45] Pardridge, W.M. (2012) Drug Transport across the Blood-Brain Barrier. *Journal of Cerebral Blood Flow & Metabolism*, **32**, 1959-1972. https://doi.org/10.1038/jcbfm.2012.126

[46] Mruk, D.D. and Cheng, C.Y. (2015) The Mammalian Blood-Testis Barrier: Its Biology and Regulation. *Endocrine Reviews*, **36**, 564-591. https://doi.org/10.1210/er.2014-1101

[47] Verotta, L., Appendino, G., Bombardelli, E. and Brun, R. (2007) *In Vitro* Antimalarial Activity of Hyperforin, a Prenylated Acylphloroglucinol. A Structure-Activity Study. *Bioorganic & Medicinal Chemistry Letters*, **17**, 1544-1548. https://doi.org/10.1016/j.bmcl.2006.12.100

[48] Brondz, I., Klein, U., Mantzilas, D., Ekeberg, D., Hvattum, E., Schultz, H., *et al.* (2005) Nature of the Main Contaminant in the Drug Primaquine Diphosphate: GC-MS Analysis. *Asian Journal of Chemistry*, **17**, 1678-1688. https://asianpubs.org/index.php/ajchem/article/view/14090

[49] Brondz, I., Mantzilas, D., Klein, U., Ekeberg, D., Hvattum, E., Lebedeva, M.N., *et al.* (2004) Nature of the Main Contaminant in the Anti Malaria Drug Primaquine Diphosphate: A Qualitative Isomer Analysis. *Journal of Chromatography B*, **800**, 211-223. https://doi.org/10.1016/j.jchromb.2003.09.042

[50] Hostanska, K., Reichling, J., Bommer, S., Weber, M. and Saller, R. (2003) Hyperforin a Constituent of St John's Wort (*Hypericum perforatum* L.) Extract Induces Apoptosis by Triggering Activation of Caspases and with Hypericin Synergistically Exerts Cytotoxicity towards Human Malignant Cell Lines. *European Journal of Pharmaceutics and Biopharmaceutics*, **56**, 121-132. https://doi.org/10.1016/s0939-6411(03)00046-8

[51] Schempp, C.M., Simon-Haarhaus, B. and Simon, J.C. (2002) Phototoxic and Apoptosis-Inducing Capacity of Pseudohypericin. *Planta Medica*, **68**, 171-173. https://doi.org/10.1055/s-2002-20264

[52] Lorusso, G., Vannini, N., Sogno, I., Generoso, L., Garbisa, S., Noonan, D.M., *et al.* (2009) Mechanisms of Hyperforin as an Anti-Angiogenic Angioprevention Agent. *European Journal of Cancer*, **45**, 1474-1484. https://doi.org/10.1016/j.ejca.2009.01.014

[53] Schempp, C., Kiss, J., Kirkin, V., Averbeck, M., Simon-Haarhaus, B., Kremer, B., *et al.* (2005) Hyperforin Acts as an Angiogenesis Inhibitor *in Vitro* and *in Vivo*. *Planta Medica*, **71**, 999-1004. https://doi.org/10.1055/s-2005-871303

[54] Albini, A., Tosetti, F., Benelli, R. and Noonan, D.M. (2005) Tumor Inflammatory Angiogenesis and Its Chemoprevention. *Cancer Research*, **65**, 10637-10641. https://doi.org/10.1158/0008-5472.can-05-3473

[55] Donà, M., Dell'Aica, I., Pezzato, E., Sartor, L., Calabrese, F., Barbera, M.D., *et al.* (2004) Hyperforin Inhibits Cancer Invasion and Metastasis. *Cancer Research*, **64**, 6225-6232. https://doi.org/10.1158/0008-5472.can-04-0280

[56] Brahmachari, G. (2017) Discovery and Development of Neuroprotective Agents from Natural Products. Elsevier.

[57] Li, X.X., Yan, Y., Zhang, J., Ding, K., Xia, C.Y., Pan, X.G., *et al.* (2023) Hyperforin: A Natural Lead Compound with Multiple Pharmacological Activities. *Phytochemistry*, **206**, Article 113526. https://doi.org/10.1016/j.phytochem.2022.113526