JOURNAL OF NATURAL PRODUCTS

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Review

A Comprehensive Review of Patented Antimicrobial Peptides from Amphibian Anurans

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Cite This: *J. Nat. Prod.* 2024, 87, 600−616

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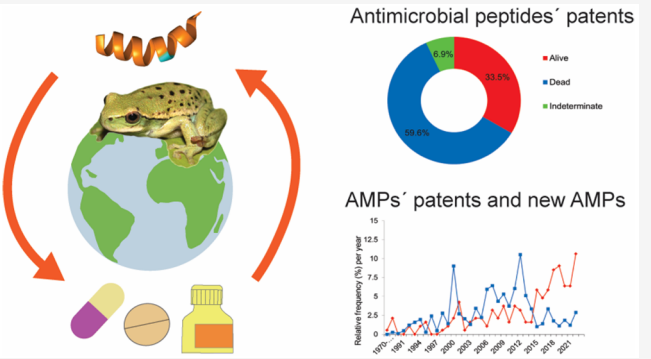
ABSTRACT: Since the 1980s, studies of antimicrobial peptides (AMPs) derived from anuran skin secretions have unveiled remarkable structural diversity and a wide range of activities. This study explores the potential of these peptides for drug development by examining granted patents, amino acid modifications related to patented peptides, and recent amphibians' taxonomic updates influencing AMP names. A total of 188 granted patents related to different anuran peptides were found, with Asia and North America being the predominant regions, contributing 65.4% and 15.4%, respectively. Conversely, although the Neotropical region is the world's most diversified region for amphibians, it holds only 3.7% of the identified patents. The antimicrobial activities of the peptides are claimed in 118 of these 188 patents. Additionally, for 160 of these peptides, 66 patents were registered for the natural sequence, 69 for both natural and derivative sequences, and 20 exclusively for sequence derivatives. Notably, common modifications include alterations in the side chains of amino acids and modifications to the peptides' N- and C-termini. This review underscores the biomedical potential of anuran-derived AMPs, emphasizing the need to bridge the gap between AMP description and practical drug development while highlighting the urgency of biodiversity conservation to facilitate biomedical discoveries.



INTRODUCTION

Despite all efforts to promote the sustainable use of natural resources, biodiversity loss remains one of humanity's "perverse" problems. The 2030 Agenda for Sustainable Development introduced the 17 Sustainable Development Goals (https://sdgs.un.org/goals), where biodiversity plays a key role. Within this reflection framework, current research aims to understand patterns of chemical diversity in broad ecological contexts, build a solid knowledge base to support sustainable use, and evaluate the valuation and conservation of biodiversity in terms of its pharmacological potential. Our analyses may contribute as information baselines for discussing environmental policies by informing the community about the economic value it can represent in the preserved form through ecosystem services or the generation of new products for people's health or well-being. Well-known examples are the studies from Newman and Cragg,[1] who have regularly published updates on pharmaceuticals that benefited from biological biodiversity. This broad view and its impact on the market have been critical in several political discussions. Most current drugs described in these reviews come from plants, marine organisms, and microorganisms, with a smaller portion being contributions from terrestrial animals.[1] To circumvent some information gaps, we have written this review focusing on antimicrobial peptides (AMPs) derived from the skin secretions of amphibian anurans.

To first understand the pharmaceutical significance of AMPs, it needs to be stressed that the use of peptide-based drugs has numerous advantages, such as high potency, selectivity, and low toxicity.[2−5] Peptides, compared to small-molecule drugs such as bestatin, bacitracin, vinblastine, and others, can be more specific for their target, so they have fewer side effects, and because of their short half-life are less likely to accumulate in tissues.[5−7] However, the development of peptide-based drugs also presents numerous limitations, such as metabolic instability due to cleavage by proteolytic enzymes and high sensitivity to physicochemical changes such as temperature and pH, poor absorption, and limited membrane permeability as a result of the presence of charge and hydrophilic or polar amino acids. Chemical modifications are the most common solutions to overcome these limitations at

Special Issue: Special Issue in Honor of Vanderlan da Silva Bolzani

Received: October 31, 2023
Revised: February 10, 2024
Accepted: February 14, 2024
Published: February 27, 2024

Table 1. Examples of AMPs under Clinical Trials (Modified from Ref 16)

Name	Natural Source	Source	Target Organism	Class	Clinical Trial	Ref
Surotomycin[a]	Microorganisms	Daptomycin analogue	Gram-positive bacteria	Synthetic cyclic lipopeptide	phase III	18
Bacteriocin OR-7	Microorganisms	*Lactobacillus salivarius*	Gram-negative bacteria	Bacteriocin	preclinical	19
Colicin E1	Microorganisms	*Escherichia coli H22H22*	Antibacterial	Bacteriocin	preclinical	20
HB-107	Insects	Cecropin B	Wound healing	Cecropin B	preclinical	21
Demegel CA(1−7)M(2−9)[a]	Insects	Cecropin analogue	Antifungal, antibacterial	Synthetic peptide	preclinical	22
Ruminococcin C	Microorganisms	*Ruminococcus gnavus*	Anticlostridial	Bacteriocin	preclinical	23
IDR-1002[a]	Bovine	Bactenecin derivative	*Staphylococcus aureus*	SyntheticPeptide	preclinical	24
Temporin10a	Anurans	*Rana ornativentris*	Gram-positive bacteria	Temporin	preclinical	25
Temporin A	Anurans	*Rana temporaria*	anti diabetic	Temporin	preclinical	26
Buforin II	Anurans	*Bufo gargarizans*	Broad spectrum antibacterial	Bofurin I	preclinical	27
Syphaxin	Anurans	*Leptodactylus syphax*	Broad spectrum antibacterial	Ocellatin-S1	preclinical	28
Pexiganan (MSI-78)[a]	Anurans	Magainin-derived peptide	Broad spectrumantibacterial	SyntheticPeptide	phase III	29

[a]Synthetic peptide or natural peptide analogue isolated from that source.

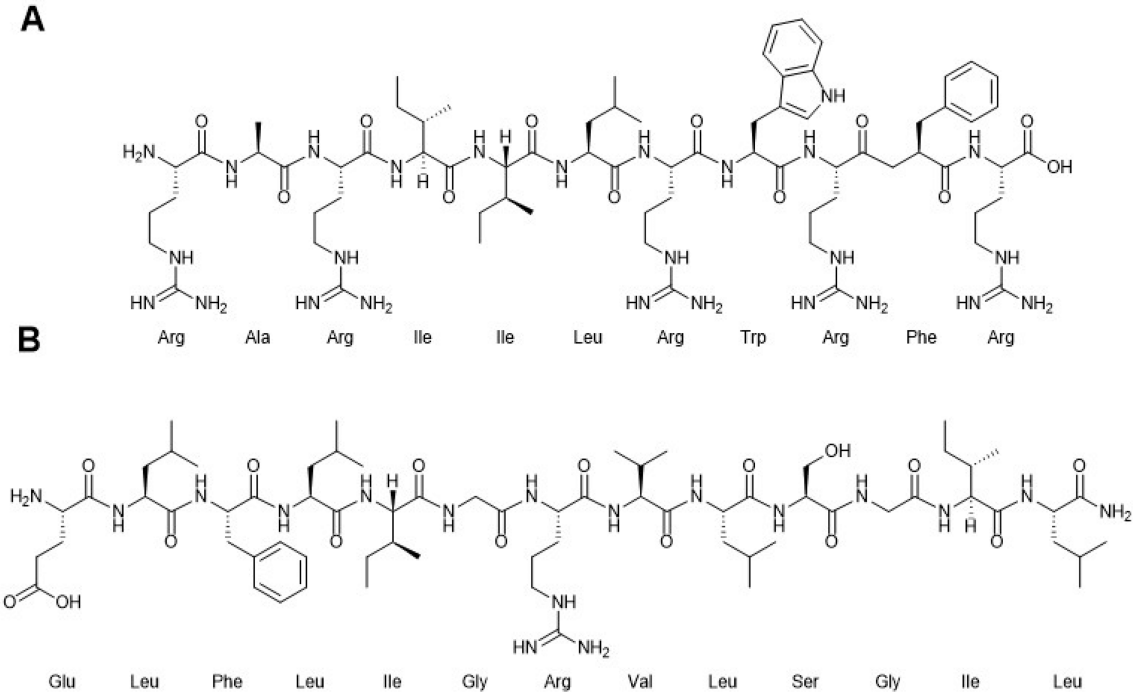


Figure 1. Examples of AMP in preclinical trials: (A) peptide W8 short peptide variant of anuran peptide kunitzin-RE; (B) temporin A peptide NH_2 modified at the C-terminus.

the expense of increasing production costs.[4,5] In addition, approving new drugs, in which peptides are not an exception, is a long process, usually requiring a minimum of 10 to 15 years and compliance with demanding regulatory standards.[8−13] In this sense, and despite the marked increase in clinical trials using peptides during the last two decades,[3,14] the number of studies at this stage is deficient, especially when considering the exponential ratio in which new AMPs are described yearly.[5,8,15] To make the matter more complicated, about 40% of the AMP-based drugs in clinical trials are of human origin, often leading to the development of endogenous resistance mechanisms.[3,16] Thus, AMPs from other natural sources, such as microorganisms, insects, marine organisms, and amphibians, represent promising avenues of pharmaceutical investigation (Table 1, Figure 1).[16,17]

As of February 2024, the Antimicrobial Peptide Database (APD3) (https://aps.unmc.edu/) contains 3940 antimicrobial peptides and proteins from six life kingdoms, of which 1079 active peptides are found in amphibians (1006 from frogs, 68 from toads, and 5 from others) with a great diversity of biological activity such as antibacterial, cytotoxic, antimutagenic, antifungal, antiviral, vermicidal, novel antiendotoxic, antidiabetic, antioxidant, among others.[14] AMPs are also used as pharmacological modulating agents to improve the immune response, wound healing processes, and the prevention of postsurgical adhesions.[3,14,30−43] The use of AMPs as new therapeutic antibiotics is today one of the most promising strategies to overcome the current multidrug-resistant *Staphylococcus aureus* (MRSA), which has become one of the significant health problems in recent years.[5,9−11] According to the first Global Research Agenda on Antimicrobial Resistance in Human Health of the World Health Organization (WHO), recently published, developing new drugs for treating MRSA

https://doi.org/10.1021/acs.jnatprod.3c01040
J. Nat. Prod. 2024, 87, 600−616

microorganisms is a priority due to the high incidence and mortality rate they are causing in the world.[44]

Amphibians are the earliest divergent clade of tetrapods and the first to venture into a terrestrial environment.[45] According to the Amphibian Species of the World database, as of October 2023, there are 8681 species reported, from which anurans (frogs and toads) are by far the most diversified order (7640 species, 88.0%), with these numbers increasing yearly with the descriptions of new species.[46] However, amphibian populations worldwide are declining mainly due to multiple environmental and anthropogenic stressors such as habitat fragmentation, environmental pollution, invasive alien species, climate change impacts, and the emergence of pathogenic fungi.[47−52] Significantly, and in contrast to the case of other extant terrestrial clades (i.e., squamates, birds, and mammals), amphibians' skin is highly dynamic, contributing to essential functions such as respiration and the body's osmotic balance.[53] In addition, it is considered a natural laboratory of organic chemistry because of the enormous diversity of compounds produced by the dermal glands,[54] which act against predators but also are essential elements of the innate immune system against pathogens[34,36,55] and as regulatory mechanisms of the microbiome.[56,57]

During the last 30 years, the study of anuran skin secretion has been a topic of great interest due to its unique chemical properties and the associated biosynthetic pathways, which make it a powerful source of natural compounds with biological activities.[30−32,39,54,58−60] The vast diversity of molecules found in anurans' dermal glands can be classified into five main groups: (1) peptides and proteins, (2) alkaloids, (3) other biogenic amines, (4) steroid bufadienolides, and (5) volatile organic compounds.[30−33,35,37−39,41,42,60−65] In recent years, our group has been working on the analysis of amphibian skins' secretions using spectrometry for the study of toxins,[60] volatile organic compounds,[66] pigmentation,[67] and AMPs' diversity,[68−71] analyzing chemical diversity in terms of their ecological relevance. More specifically, we now have a more comprehensive understanding, suggesting that many defense substances can also be interpreted regarding host-symbiotic relationships.[56] In this context, AMPs play a fundamental role in the interspecific interactions occurring in the hosts' dermosphere and thus represent complex models increasing the levels of biodiversity.

The high diversity of AMPs reported from amphibians in the last five decades has been summarized in several reviews focusing on their structure, physicochemical properties, and biological activities and also describing their biomedical potential.[30,31,33−35,37−42,61,63,64,67,72] This information has also been compiled in publicly accessible databases such as the Data Repository of Antimicrobial Peptides (DRAMP 3.0)[17] and the Collection of Antimicrobial Peptides ($CAMP_{R3}$).[73] One limitation of DRAMP 3.0 and $CAMP_{R3}$ is that they contain many predicted peptide sequences not linked to any biological activity and, thus, cannot be referred to as AMPs.[17,73−75] The APD3 is currently one of the most cited databases due to its inclusion criteria: (i) natural AMPs with (ii) a known amino acid sequence, (iii) biological activity, and (iv) less than 100 residues in size.[74,76]

Despite the large amount of scientific evidence supporting anuran AMPs as promising candidates for drug development,[30,31,33−35,37−42,61,63,64,67,72] the current status of the potential advancements is, with a few exceptions, unknown.[77−79] In-depth knowledge of patent information enables researchers to use existing patented technologies, compounds, or methodologies to advance drug development and supports interinstitutional research collaborations. Some databases compile patent information; the most prominent are the Derwent World Patents Index (DWPI) database, Google patent, Espacenet patent search, and others.[80−82] DWPI offers comprehensive global patent information, encompassing over 14.3 million primary innovations from nearly 60 patent-issuing authorities worldwide. It creates distinct patent families for each invention, facilitating a rapid study. Due to these attributes, it is one of the most comprehensive patent databases available. This database contains more than 81.1 million registered patents, compiling information from 59 patent authorities worldwide; this information is grouped into approximately 39.4 million DWPI patent families.[83] Google Patents and Espacenet are freely accessible online databases for searching for patent information. Google Patents contains data from 55 patent entities worldwide, with 12,770,061 granted patents and 7,437,605 patent applications. The Google Patents Public Data table features over 90 million bibliographic records of patent publications.[84] Espacenet is collaboratively developed by the European Patent Office (EPO) and the European Patent Organization member states. Espacenet boasts a database with records of over 140 million patent documents.[85]

In summary, the study of patents and the knowledge of clinical trials allow scientists to be aware of the state of the art in developing a new drug with biomedical potential. In this context, the present work intends to shed light on the impacts of amphibian secretions on the market as inspiration for the synthesis of new actives, detailing most of the patent documentation and chemistry of anurans' AMPs and updating the taxonomic classification of species that have mainly contributed to the AMPs' discovery.

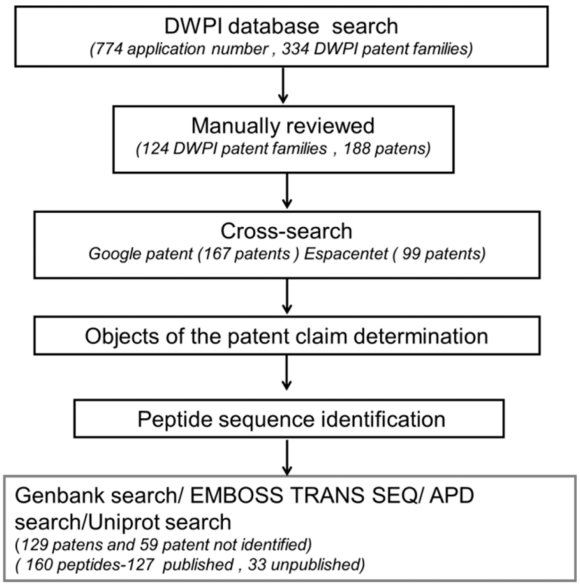
RESULTS AND DISCUSSION

Patented AMPs from Anuran Amphibians. A search in the DWPI database using the keywords "peptides", "antimicrobials", "frogs", and "anurans" was conducted to assess the status of treatments based on peptide molecules isolated from anurans (Scheme 1 and Search Methodology). Initially, 774 patent applications grouped into 334 DWPI families were found. After careful examination, 188 patents of AMPs were identified with anurans as the biological source (Scheme 1, Search Methodology, and Supporting Information 1.1). This number appears relatively low considering the abundance of anuran AMPs reported in the literature and their substantial clinical potential, 1079 amphibian AMPs (1006 from frogs, 68 from toads, and 5 from others) contained in the APD3 database as of February 2024.[14] Additionally, we cross-referenced patents from the DWIP database with Google Patent and Espacenet databases using publication number identifiers for each patent. The results showed that 21 patents (11.2%) were exclusively identified in DWPI, 68 patents (36.2%) were identified in both DWPI and Google Patents but not in Espacenet, and 99 patents (52.7%) were identified in the three databases. This high consistency in patent identification across databases, with 167 (88.9%) of the patents identified in two or more patent databases, supports our search strategy (Figure 2 and Supporting Information 1.1).

Current Status of Patented Anuran AMPs. The analysis of the validity status of the 188 patents related to anurans showed that there is a predominance of "dead" patents, accounting for 112 (59.6%) patents of the total, in contrast to

https://doi.org/10.1021/acs.jnatprod.3c01040
J. Nat. Prod. 2024, 87, 600−616

Scheme 1. Methodology for Identifying Patents Related to Anurans' Antimicrobial Peptides (AMPs)[a]



[a]First, a search was performed in Derwent Innovation, after which the results were manually refined by reviewing each of the patents followed by their categorization into DWPI families. Next, a cross-check was performed to validate the identified patents using Google Patents and the Espacenet database. Following this, an in-depth analysis of the patent information was conducted. Finally, peptide sequences were identified (Supporting Information 1.1−1.5).

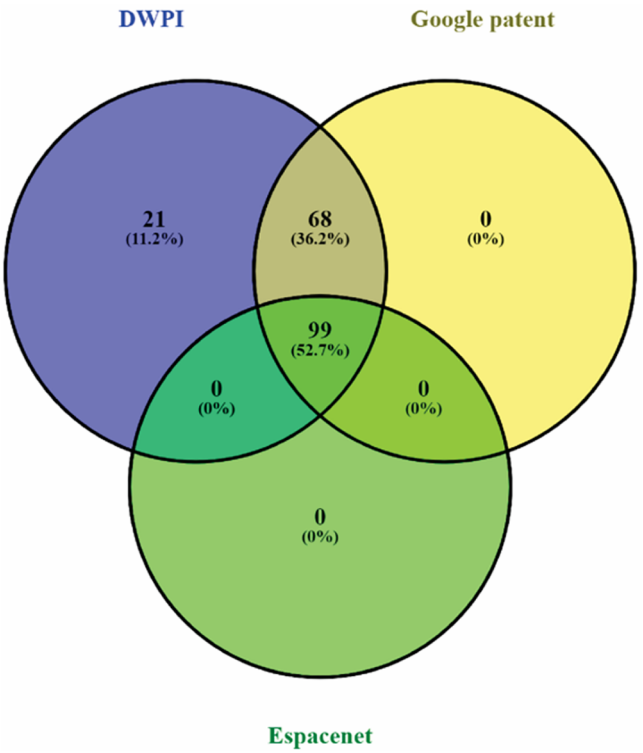


Figure 2. Venn diagram showing anuran-related patents identified by the DWPI, Espacent, and Google Patents databases. The areas delimited in these Venn diagrams show the number of patents and the percentage they represent (written in bold) (Supporting Information 1.1).

"alive" patents at 63 (33.5%), with "undetermined" documents totaling 13 (6.9%) (Figure 3A and Supporting Information 1.1). Interestingly, when considering DWPI families, more than 97 patents (51.6%) were classified as alive, 81 dead, and eight indeterminate. This difference could be because some patents within DWPI families are still active, even if other related documents in the same family have expired or were abandoned (Figure 3B and Supporting Information 1.1).

Figure 3C illustrates the behavior in relative frequencies of patents (RFP, red line) related to AMPs and relative frequencies of AMPs reported (RFAMP, blue line) by year. A notable discrepancy exists in the scientific literature regarding the first AMP's establishment. Some authors attribute it to magainin 2 (AP00144) isolated from the African frog *Xenopus laevis* in 1987, initiating amphibian AMP research.[43,86] In contrast, others suggest that the bombinin peptide (AP00049) from the European species *Bombina variegata* in 1970 was the first AMP described, though it was initially reported as a hemolytic peptide.[38,87,88] Between 1990 and 2014, RFP remained lower than RFAMP reported in the literature except for 2001, which saw a peak. This behavior reversed from 2015 to 2022, with RFP surpassing that of AMPs reported in the literature. RFAMP's behavior aligns with the absolute frequencies of frog AMPs in the APD3 database, with peak frequencies in 2000 and 2012 (Figure 3C and Supporting Information 1.1−1.3).[43]

Geographic and Taxonomic Distribution of Patented AMPs from Anuran Amphibians. Anuran AMP-related patents show a notable distribution across different regions, with Asia leading the records (65.4%), followed by North America (15.4%), Europe (8.5%), and finally South America and Australia (3.7% and 2.7%, respectively). Interestingly, scientific collaboration between Asia and North America, involving shared authorship, accounts for 4.3%. No patents were registered in Africa or Central America (Figure 4A and Supporting Information 1.1).

Analyzing the countries where patent applications have been submitted, China stands out with the highest number of applications (91), followed by the United States (26), Japan (12), and Australia (14). Examining the country of the patent priority, China maintains the lead (96), followed by the United States (45), Korea (10), Japan (7), and Brazil (7) (Figure 4C and Supporting Information 1.1). These results correspond with the inventors of patents by region. It is worth noting that the percentage of patents (23.9%) in which the priority is in the United States exceeds the percent of patents granted for the North American region (15.4%), indicating the importance authors attach to the recognition of their authorship by United States authorities. This distribution highlights the global recognition and influence associated with U.S. patents (Figures 4C and Supporting Information 1.1).

The distribution of anuran AMP-related patents granted in the Asian, North American, and European regions aligns with our geographical analysis of AMPs in the literature. Specifically, the Asian and North American regions consistently accounted for the highest percentages of patents granted related to anuran AMPs (65.4% and 15.4%, respectively) and AMPs found in the literature (47.2% and 16.2%, respectively) (Figure 4A,B and Supporting Information 1.1−1.3). This geographic pattern can be attributed to the focus on Ranidae and Hylidae, the primary sources of anuran AMPs studied (Figure 5A,B, Table 2, and Supporting Information 1.2). Ranidae has a worldwide distribution, except for southern South America, South Africa,

https://doi.org/10.1021/acs.jnatprod.3c01040
J. Nat. Prod. 2024, 87, 600−616

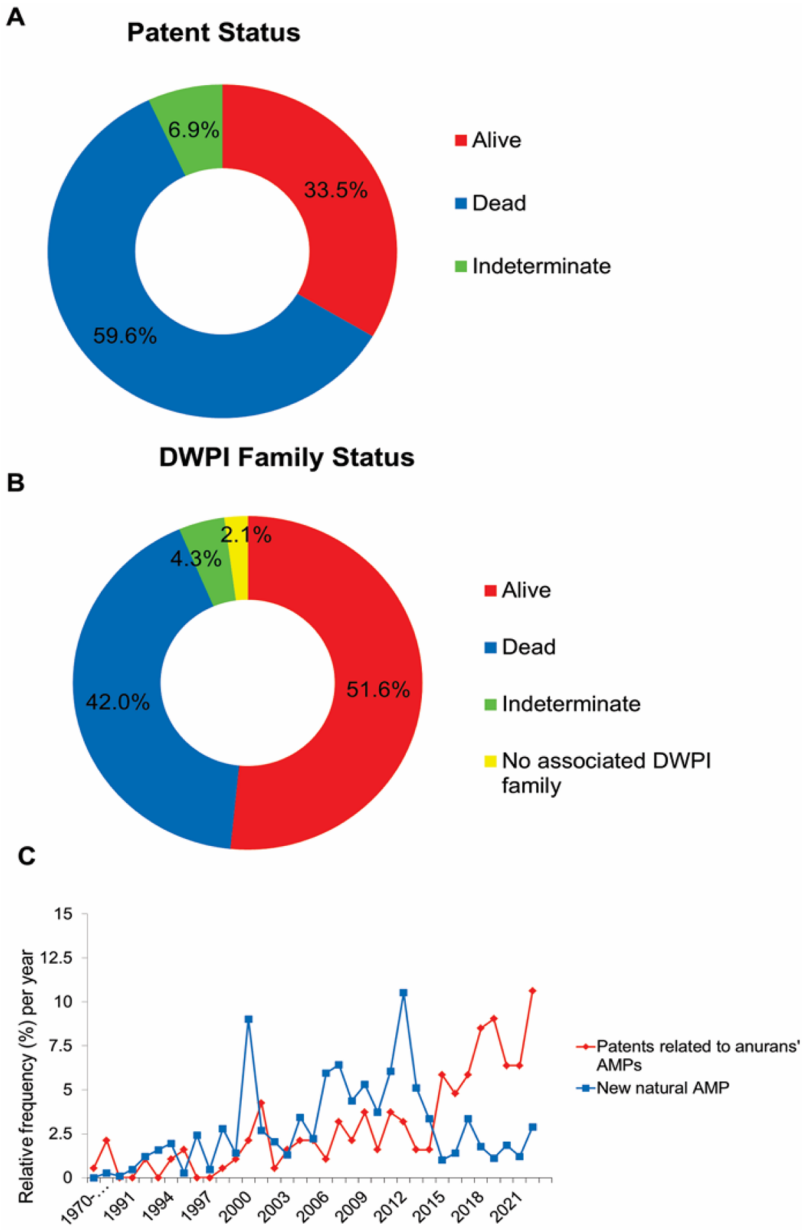


Figure 3. (A) Percentage distribution (%) of patent status according to current status granted by the Patent Coverage Authority. (B) Percentage distribution (%) of patent status according to current status DWPI families (Supporting Information 1.1). (C) Graph plot representing the relative frequencies in % (*y*-axis) per year (*x*-axis); the blue line represents the new natural peptides published, and the red line the number of patents related to anuran antimicrobial peptides (Supporting Information 1.1−1.3).

Madagascar, and most of Australia,[46] whereas Hylidae is well represented in the New World, but also in the Australo-Papuan Region, and temperate Eurasia, including extreme northern Africa and the Japanese Archipelago.[46]

Additionally, our analysis identified other anuran families in Asia and Europe as sources of patented and published AMPs. These families include Dicroglossidae, Microhylidae, and Rhacophoridae in Asia, as well as Bombinatoridae in Euro-Asia. In North America, AMPs were also isolated from the Ascaphidae family,[89] which is endemic to the region;[46] however, no patents related to peptides isolated from species of this family were found (Figure 5A,B, Table 2, and Supporting Information 1.2−1.4).

Furthermore, the granted patents related to anuran AMPs in the Australian region (4.3%) are lower than the half percentage (9%) of AMPs isolated from anurans reported in the literature for this region. This discrepancy can be attributed to the characteristics of Australia's amphibian populations, which, despite their endemism, are not among the world's most diverse, currently comprising approximately 247 recognized frog species.[46] Australia lacks amphibian clades like newts, salamanders, and cecilians found in other parts of the world.[46,90] The identified AMPs from this region were primarily isolated from anurans belonging to the families Hylidae, Limnodynastidae, and Myobatrachidae (Figures 4A,B and 5A,B, Table 2, and Supporting Information 1.1−1.3).[46]

Despite the high diversity of amphibians found in South America, Central America, and Africa,[91−96] we found that the percentage of patents related to AMPs granted in these regions is low (South America, 3.7%) or nonexistent. The fact that

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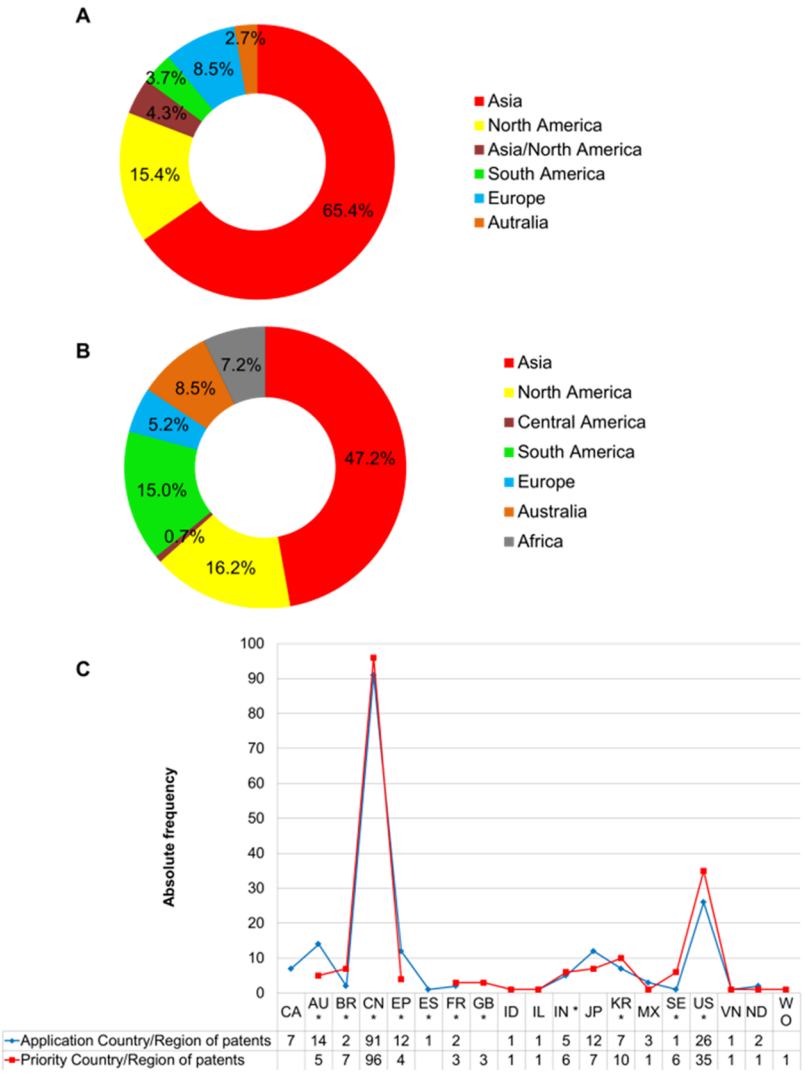

Figure 4. (A) Distribution of patents (%) related to peptide isolates from anuran antimicrobial peptides by geographic region. (B) Distribution of natural (%) anuran antimicrobial peptides reported by geographic region (Supporting Information 1.2 and 1.3). (C) Graph plot represents the absolute frequencies (*y*-axis) versus the distribution by regions/countries (*x*-axis). The blue line represents the patent applications by region/country, and the red line represents the priorities of the applied patents by region/country. (AU) Australia, (BR) Brazil, (CA) Canada, (CN) China, (EP) Europe Patent office, (ES) Spain, (FR) France, (ID) Indonesia, (IL) Israel, (IN) India, (JP) Japan, (KR) Korea, (MX) Mexico, (SE) Sweden, (US) United States, (VN) Vietnam, (ND) Not determined. * represents the regions/countries where part of their priority is also included in (WO) the World Intellectual Property Organization (Supporting Information 1.1).

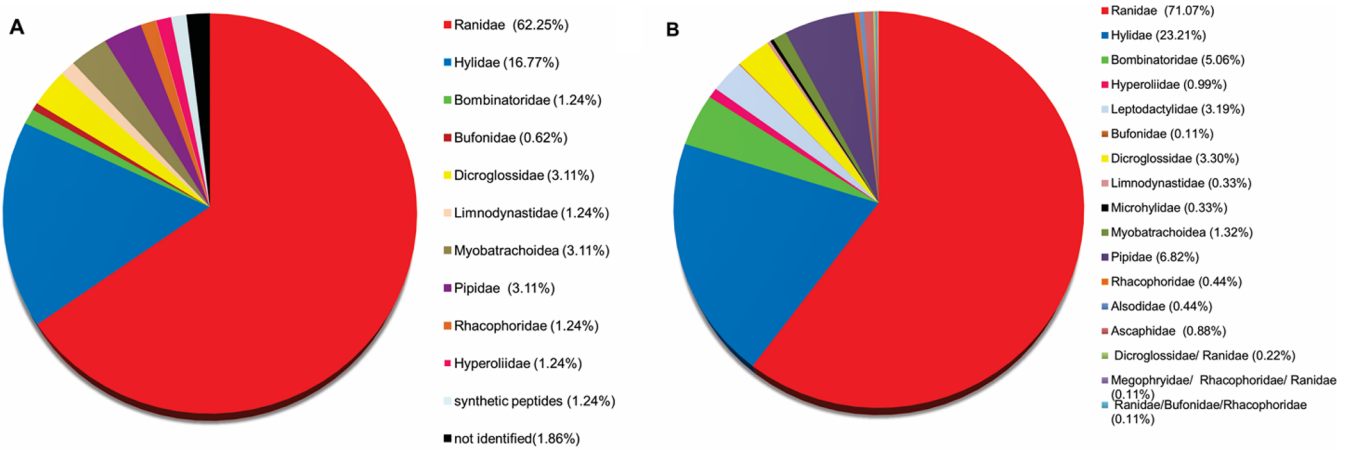

Figure 5. (A) Pie chart showing the distribution by anuran family of AMPs reported in the literature (Supporting Information 1.2 and 1.3). (B) Pie chart showing the distribution by anuran family according to patented AMPs (Supporting Information 1.2−1.4).

https://doi.org/10.1021/acs.jnatprod.3c01040
J. Nat. Prod. 2024, 87, 600−616

Table 2. Summary of the AMP Families Found in Anuran Species[a]

Family	Genus	Species	Peptide Family
Ranidae	*Amerana*	*Amerana aurora* (Baird and Girard, 1852)	Brevinin, Ranacyclin, Ranatuerin
		Amerana boylii (Baird, 1854)	Brevinin, Ranatuerin, Temporin
		Amerana cascadae (Slater, 1939))	Brevinin, Ranatuerin, Temporinn
		Amerana draytonii (Baird and Girard, 1852)	Temporin
		Amerana sierrae (Camp, 1917)	Brevinin
		Amerana pretiosa (Baird and Girard, 1853)	Brevinin, Esculentin, Ranatuerin, Temporin
		Amerana muscosa (Camp, 1917)	Ranatuerin, Temporin
	Aquarana	*Aquarana catesbeiana* (Shaw, 1802)	Cathelicidin, Ranauterin, Palustrin, Ranalexin, Ranatuerin, Temporin
		Aquarana grylio (Stejneger, 1901)	Ranalexin, Ranatuerin, Temporin
		Aquarana virgatipes (Cope, 1891)	Temporin
		Aquarana heckscheri (Wright, 1924)	Temporin
	Amolops	*Amolops afghanus* (Günther, 1858)	Brevinin, Esculentin, Nigrocin
		Amolops chunganensis (Pope, 1929)	Brevinin, Esculentin, Palustrin, Temporin
		Amolops daiyunensis (Liu and Hu, 1975)	Palustrin
		Amolops daiyunensis (Liu and Hu, 1975)	Brevinin, Palustrin
		Amolops hainanensis (Boulenger, 1900)	Brevinin, Hainanenin
		Amolops jinjiangensis Su, Yang, and Li, 1986	Temporin
		Amolops loloensis (Liu, 1950)	Brevinin, Cathelicidin, Esculentin, Temporin
		Amolops mantzorum (David, 1872)	Palustrin
		Amolops ricketti (Boulenger, 1899)	Brevinin
	Clinotarsus	*Clinotarsus curtipes* (Jerdon, 1853)	Brevinin
	Glandirana	*Glandirana rugosa* (Temminck and Schlegel, 1838)	Brevinin, Esculentin, Rugosin
		Glandirana susurra (Sekiya, Miura, and Ogata, 2012)	Brevinin, Granuliberin, Ranatuerin
	Hydrophylax	*Hydrophylax bahuvistara* Padhye, Jadhav, Modak, Nameer & Dahanukar, 2015	Esculentin, Brevinin
	Hylarana	*Hylarana aurantiaca* (Boulenger, 1904)	Brevinin
		Hylarana erythraea (Schlegel, 1837)	Brevinin
		Hylarana latouchii (Boulenger, 1899)	Brevinin, Esculentin, Palustrin, Temporin
		Hylarana maosonensis Bourret, 1937	Nigroain
		Hylarana picturata (Boulenger, 1920)	Brevinin, Temporin
		Hylarana nigrovittata (Blyth, 1856)	Gaegurin, Nigroain, Rugosin
		Hylarana taipehensis (Van Denburgh, 1909)	Brevinin, Esculentin, Palustrin, Temporin
		Hylarana guentheri (Boulenger, 1882)	Brevinin, Esculentin, Temporin
		Hylarana maosonensis Bourret, 1937	Brevinin, Brevinin, Temporin
		Hylarana nigrovittata (Blyth, 1856)	Brevinin, Ranacyclin, Temporin
		Hylarana spinulosa (Smith, 1923)	Brevinin, Esculentin, Temporin
	Indosylvirana	*Hylarana aurantiaca (Boulenger, 1904)*	Temporin
	Lithobates	*Lithobates areolatus* (Baird and Girard, 1852)	Temporin
		Lithobates areolatus (Baird and Girard, 1852)	Esculentin, Palustrin, Ranatuerin
		Lithobates berlandieri (Baird, 1859)	Brevinin, Esculentin, Ranatuerin
		Lithobates blairi (Mecham, Littlejohn, Oldham, Brown, and Brown, 1973)	Brevinin
		Lithobates capito (LeConte, 1855)	Brevinin, Esculentin, Ranatuerin, Temporin
		Lithobates chiricahuensis Platz and Mecham, 1979)	Brevinin, Esculentin, Ranatuerin
		Aquarana clamitans (Latreille, 1801)	Ranalexin, Ranatuerin
		Aquarana okaloosae (Moler, 1985)	Temporin
		Lithobates onca (Cope, 1875)	Ranatuerin
		Lithobates palmipes (Spix, 1824)	Brevinin, Temporin
		Lithobates palustris (LeConte, 1825)	Brevinin, Esculentin, Palustrin, Ranatuerin
		Lithobates pipiens (Schreber, 1782)	Brevinin, Esculentin, Ranatuerin
		Lithobates septentrionalis (Baird, 1854)	Brevinin, Ranatuerin, Temporin
		Lithobates sevosus (Goin and Netting, 1940)	Esculentin, Ranatuerin
		Lithobates sphenocephalus (Cope, 1886)	Brevinin, Temporin
		Lithobates tarahumarae (Boulenger, 1917)	Brevinin, Ranatuerin
		Lithobates yavapaiensis (Platz and Frost, 1984)	Brevinin, Ranatuerin
	Boreorana	*Boreorana sylvatica* (LeConte, 1825)	Brevinin
	Nidirana	*Nidirana adenopleura* (Boulenger, 1909)	Brevinin
		Nidirana daunchina (Chang, 1933)	Brevinin, Ranatuerin
		Nidirana pleuraden (Boulenger, 1904)	Pleurain

https://doi.org/10.1021/acs.jnatprod.3c01040
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Table 2. continued

Family	Genus	Species	Peptide Family
	Odorrana	*Odorrana andersonii* (Boulenger, 1882)	Brevinin, Esculentin, Nigrocin, Odorranain
		Odorrana chloronota (Günther, 1876)	Esculentin
		Odorrana grahami (Boulenger, 1917)	Brevinin, Esculentin, Nigrocin, Odorranain, Palustrin, Ranacyclin
		Odorrana hainanensis Fei, Ye, and Li, 2001	Brevinin, Temporin
		Odorrana hosii (Boulenger, 1891)	Brevinin, Esculentin, Nigrocin
		Odorrana ishikawae (Stejneger, 1901)	Brevinin, Esculentin, Nigrocin, Palustrin
		Odorrana jingdongensis Fei, Ye, and Li, 2001	Brevinin, Esculentin, Nigrocin, Odorranain
		Odorrana livida (Blyth, 1856)	Brevinin, Cathelicidin, Esculentin, Lividin, Nigrocin, Ranacyclin
		Odorrana margaretae (Liu, 1950)	Odorranain
		Odorrana chloronota (Günther, 1876)	Brevinin, Nigrocin
		Odorrana schmackeri	Brevinin, Nigrocin, Odorranain
		Odorrana tormota (Wu, 1977)	Nigrocin
		Odorrana versabilis (Liu and Hu, 1962)	Esculentin, Nigrocin, Ranatuerin, Temporin
		Odorrana wuchuanensis (Xu, 1983)	Brevinin, Nigrocin, Palustrin
	Pelophylax	*Pelophylax fukienensis* (Pope, 1929)	Pelophylaxin, Brevinin, Cathelicidin, Nigrocin, Temporin
		Pelophylax hubeiensis (Fei and Ye, 1982)	Brevinin, Pelophylaxin, Ranacyclin, Temporin
		Pelophylax lessonae (Camerano, 1882)	Brevinin, Esculentin, Kunitzin-RE, Ranacyclin
		Pelophylax nigromaculatus (Hallowell, *1861)*	Pelophylaxin, Brevinin, Cathelicidin, Nigrocin, Temporin
		Pelophylax plancyi (Lataste, 1880)	Pelophylaxin
		Pelophylax porosus (Cope, 1868)	Brevinin
		Pelophylax ridibundus (Pallas, 1771)	Brevinin, Temporin
		Pelophylax saharicus (Boulenger, 1913)	Brevinin, Temporin
	Rana	*Rana omeimontis* Ye and Fei, 1993	Japonicin, Palustrin, Nigrocin
		Rana chensinensis David, 1875	Brevinin, Palustrin, Temporin
		Rana chensinensis David, 1875	Japonicin
		Rana dalmatina Fitzinger, 1838	Brevinin
		Rana dybowskii Günther, 1876	Brevinin, Dybowskin, Temporin
		Rana italic Dubois, 1987	Brevinin, Temporin
		Rana japonica Boulenger, 1879	Brevinin, Japonicin, Temporin
		Rana luteiventris (Thompson, 1913)	Brevinin, Esculentin, Ranatuerin, Temporin
		Rana ornativentris Werner, 1903	Brevinin, Temporin
		Rana pirica Matsui, 1991	Brevinin
		Rana sakuraii Matsui and Matsui, 1990	Brevinin, Ranatuerin, Temporin
		Rana tagoi Okada, 1928	Temporin
		Rana temporaria Linnaeus, 1758	Brevinin, Ranatuerin, Temporin
		Rana tsushimensis Stejneger, 1907	Brevinin
		Rana tsushimensis stejneger Stejneger, 1907	Brevinin, Temporin
		Rana ulma Matsui, 2011	Brevinin
Hylidae	*Agalychnis*	*Agalychnis annae* (Duellman, 1963)	Dermaseptin, Dermatoxin
		Agalychnis callidryas (Cope, 1862)	Caerin, Dermaseptin, Phylloseptin
		Agalychnis dacnicolor (Cope, 1864)	Dermaseptin
		Agalychnis lemur (Boulenger, 1882)	Dermaseptin
		Agalychnis spurrelli Boulenger, 1913	Dermaseptin
	Boana	*Boana pulchella* (Duméril and Bibron, 1841)	Figainin, p2-hp-1935 AP02386, p3-hp-1891 AP02387
		Boana raniceps (Cope, 1862)	Raniseptin
		Boana semilineata (Spix, 1824)	hs-1 AP02527
	Callimedusa	*Callimedusa baltea* (Duellman and Toft, 1979)	Phylloseptin
		Callimedusa duellmani (Cannatella, 1982)	Dermaseptin, Phylloseptin
	Dendropsophus	*Dendropsophus columbianus* (Boettger, 1892)	Dendropsophin 1 ap02891
	Litoria	*Litoria ewingii* (Duméril and Bibron, 1841)	Uperin
		Litoria peronii (Tschudi, 1838)	Caerin
		Litoria rothii (De Vis, 1884)	Caerin
		Litoria fallax (Peters, 1880)	Fallaxidin
	Nyctimystes	*Nyctimystes infrafrenatus* (Günther, 1867)	Frenatin

https://doi.org/10.1021/acs.jnatprod.3c01040
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Table 2. continued

Family	Genus	Species	Peptide Family
	Phyllomedusa	*Phyllomedusa bicolor* (Boddaert, 1772)	Dermaseptin, Dermatoxin, Phylloseptin
		Phyllomedusa burmeisteri Boulenger, 1882	Phylloseptin
		Phyllomedusa camba De la Riva, 1999	Dermaseptin
		Phyllomedusa coelestis (Cope, 1874)	Phylloseptin
		Phyllomedusa distincta Lutz, 1950	Dermaseptin, Distinctin ap00493
		Phyllomedusa sauvagii Boulenger, 1882	Dermaseptin, Dermatoxin, Phylloseptin, Phylloxin
		Phyllomedusa tarsius (Cope, 1868)	Dermaseptin, Phylloseptin
		Phyllomedusa trinitatis Mertens, 1926	Phylloseptin
	Pithecopus	*Pithecopus azureus* (Cope, 1862)	Dermaseptin, Phylloseptin
		Pithecopus hypochondrialis (Daudin, 1800)	Phylloseptin, Hyposin
		Pithecopus-nordestinus (Caramaschi, 2006)	Phylloseptin
		Pithecopus-oreades (Brandão, 2002)	Dermaseptin
	Pseudis	*Pseudis paradoxa* (Linnaeus, 1758)	Pseudin
	Ranoidea	*Ranoidea aurea* (Lesson, 1829)	Aurein
		Ranoidea caerulea (White, 1790)	Caerin
		Ranoidea splendida (Tyler, Davies, and Martin, 1977)	Caerin, Caeridin
		Ranoidea raniformis (Keferstein, 1867)	Aurein
		Ranoidea chloris (Boulenger, 1892)	Caerin
		Ranoidea citropa (Péron, 1807)	Citropin
		Ranoidea dahlii (Boulenger, 1896)	Dahlein
		Ranoidea eucnemis (Lönnberg, 1900)	Caerin, Maculatin
		Ranoidea genimaculata (Horst, 1883)	Maculatin
		Ranoidea gilleni (Spencer, 1896)	Caerin
		Ranoidea xanthomera (Davies, McDonald, and Adams, 1986)	Caerin
		Ranoidea gracilenta (Peters, 1869)	Caerin
	Sphaenorhynchus	*Sphaenorhynchus lacteus* (Daudin, 1800)	Frenatin
Bombinatoridae	*Bombina*	*Bombina maxima* (Boulenger, 1905)	Maximin
		Bombina orientalis (Boulenger, 1890)	Bombinin
		Bombina variegata (Linnaeus, 1758)	Bombinin
Hyperoliidae	*Kassina*	*Kassina senegalensis* (Duméril and Bibron, 1841)	Kassinatuerin
	Hylambates	*Hylambates maculatus* Duméril, 1853	Kassinatuerin, Kassorin, Kassporin
Leptodactylidae	*Leptodactylus*	*Leptodactylus fallax* Müller, 1926	Ocellatin
		Leptodactylus insularum Barbour, 1906	Ocellatin
		Leptodactylus labyrinthicus (Spix, 1824)	Ocellatin
		Leptodactylus latrans (Steffen, 1815)	p1-ll-1577 AP03028, p2-ll-1298 AP03029, p3-ll-2085 AP03030
		Leptodactylus nesiotus Heyer, 1994	Ocellatin
		Leptodactylus paranaru Magalhães, Lyra, Carvalho, Baldo, Brusquetti, Burella, Colli, Gehara, Giaretta, Haddad, Langone, López, Napoli, Santana, de Sá, and Garda, 2020	Ocellatin
		Leptodactylus pentadactylus (Laurenti, 1768)	Ocellatin
		Leptodactylus pustulatus (Peters, 1870)	Ocellatin
		Leptodactylus syphax Bokermann, 1969	Ocellatin
		Leptodactylus validus Garman, 1888	Ocellatin
		Leptodatylus laticeps Boulenger, 1918	Ocellatin
	Pleurodema	*Pleurodema somuncurense* (Cei, 1969)	Somuncurin
Bufonidae	*Bufo*	*Bufo gargarizans* Cantor, 1842	Buforin
Dicroglossidae	*Euphlyctis*	*Euphlyctis cyanophlyctis* (Schneider, 1799)	Buforin
		Euphlyctis cyanophlyctis (Schneider, 1799)	Temporin
	Fejervarya	*Fejervarya limnocharis* (Gravenhorst, 1829)	Brevinin, Esculentin, Temporin, limnochariin-1 AP02606
	Hoplobatrachus	*Quasipaa spinosa (David, 1875)*	Cathelicidin
		Hoplobatrachus tigerinus (Daudin, 1802)	Tigerinin
	Limnonectes	*Limnonectes fragilis* (Liu and Hu, 1973)	Cathelicidin
		Limnonectes fujianensis Ye and Fei, 1994	Japonicin
		Limnonectes kuhlii (Tschudi, 1838)	Gaegurin, Rugosin, Temporin, Ranacyclin
	Nanorana	*Nanorana parkeri* (Stejneger, 1927)	Japonicin
		Nanorana ventripunctata Fei and Huang, 1985	Cathelicidin
		Nanorana yunnanensis (Anderson, 1879)	Cathelicidin
	Quasipaa	*Quasipaa exilispinosa* (Liu and Hu, 1975)	Brevinin, Esculentin
		Quasipaa robertingeri (Wu and Zhao, 1995)	Cathelicidin
		Quasipaa spinosa (David, 1875)	Defensin

https://doi.org/10.1021/acs.jnatprod.3c01040
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Table 2. continued

Family	Genus	Species	Peptide Family
Limnodynastidae	*Limnodynastes*	*Limnodynastes interioris* Fry, 1913	Dynastin
		Limnodynastes dumerilii Peters, 1863	Dynastin
		Limnodynastes terraereginae Fry, 1915	Dynastin
Microhylidae	*Microhyla*	*Microhyla heymonsi* Vogt, 1911	Cathelicidin
		Microhyla pulchra (Hallowell, 1861)	Brevinin
	Kaloula	*Kaloula pulchra* Gray, 1831	Brevinin
Myobatrachoidea	*Uperoleia*	*Uperoleia crassa* Tyler, Davies, and Martin, 1981	Uperin
Pipidae	*Hymenochirus*	*Hymenochirus boettgeri* (Tornier, 1896)	Hymenochirin
		Pseudhymenochirus merlini Chabanaud, 1920	Hymenochirin
	Xenopus	*Xenopus amieti* Kobel, du Pasquier, Fischberg, and Gloor, 1980	XPF, Magainin, CPF
		Xenopus andrei Loumont, 1983	XPF, Magainin
		Xenopus borealis Parker, 1936	CPF
		Xenopus clivii Peracca, 1898	CPF
		Xenopus fraseri Boulenger, 1905	Magainin
		Xenopus mellotropicalis Evans, Carter, Greenbaum, Gvoždík, Kelley, McLaughlin, Pauwels, Portik, Stanley, Tinsley, Tobias, and Blackburn, 2015	XPF, CPF
		Xenopus epitropicalis Fischberg, Colombelli, and Picard, 1982	XPF, CPF
		Xenopus laevis (Daudin, 1802)	Magainin
		Xenopus lenduensis Evans, Greenbaum, Kusamba, Carter, Tobias, Mendel, and Kelley, 2011	CPF
		Xenopus muelleri (Peters, 1844)	CPF, Magainin
		Xenopus petersii Barboza du Bocage, 1895	CPF
		Xenopus tropicalis (Gray, 1864)	CPF, Magainin, Pxt
Rhacophoridae	*Theloderma*	*Theloderma corticale* (Boulenger, 1903)	Defensin, Pleurain
	Zhangixalus	*Zhangixalus dennysi* (Blanford, 1881)	Pelophylaxin
		Zhangixalus omeimontis (Stejneger, 1924)	Pelophylaxin
		Zhangixalus puerensis (He, 1999)	Cathelicidin, Defensin
Alsodidae	*Alsodes*	*Alsodes montanus* (Lataste, 1902)	Ascaphin
	Ascaphus	*Ascaphus truei* Stejneger, 1899	Ascaphin

[a]Note: We followed "Amphibians of the World" for anuran taxonomy" in October 2023.[46]

South America has 15.0% of the natural AMPs described in the literature (Figure 4A,B and Supporting Information 1.1−1.3) makes these numbers even more surprising. This discrepancy is likely related to the three basic scientific steps prior to patent registration while working with natural populations: to know the biological diversity at taxonomic and phylogenetic levels, to know the chemical diversity, and to conduct laboratory assays.[77,79,97] The significantly low number of patents granted in these regions can thus be related to the low investment in these steps for most countries. Thus, not surprisingly, Brazil, which has made continuous scientific investments since more than two decades ago, with a marked interruption between 2016 and 2022, is a notorious exception. Brazil has significantly increased the knowledge of its amphibian diversity,[94,98,99] with 1178 described species,[46] and it is the only country in the region with identified granted patents related to anuran AMPs (Figure 4C and Supporting Information 1.1 and 1.2).

The factors contributing to this research gap in Africa and Central and South America include challenging access to remote areas but mainly a lack of funding for conducting basic and applied research. Thus, as exemplified with Brazil, it is clear that the need for policies incentivizing such studies, including funds and resources, is crucial to unlocking the full potential of these highly biodiverse regions for novel drug discovery and conservation efforts.[91−96,100,101]

Primary Goals of Patented AMPs from Anuran Amphibians. Moreover, through the analysis of the information obtained, the primary purposes of the patents have been established into three groups: (1) structural targets (amino acid sequence of AMPs, amino acid sequence derivatives, and nucleotide sequence), (2) biological activities (antimicrobial activity, cytotoxic activity, antiviral activity, and other activities); and (3) applications (pharmaceutical composition, medical treatment, and production methods).

Principal Amino Acid Modification of Patented AMPs from Anuran Amphibians. Anuran AMPs are synthesized as precursors, encompassing a signal sequence, a propeptide, and the mature AMP (Figure 6).[68,102−104] It has been described

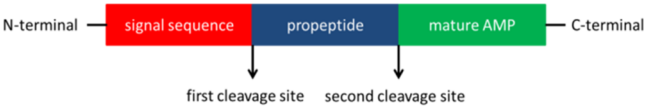

Figure 6. Structural composition of amphibian AMPs.

that the signal peptide (SP) is crucial for regulating the intracellular transport of the peptide,[102] and the propeptide region maintains the inactive form of the AMPs.[68,105] Both regions exhibit varying degrees of conservation, but they are much higher than the mature AMP, which has been widely described as the least conserved region within families of anuran AMPs.[102] The mature AMP carries out the biological activity of the peptide.[68,102−104] After removal of the signal sequence, the inactive precursor is transported to granular glands on the dorsal surface of anurans. Upon stimulation or injury, a second endopeptidase cleaves the inactive peptide, activating the mature peptides (Figure 6).[105,106] This transformation provides insights into the effectiveness of these peptides against pathogens.[68,104,106]

Among the modifications of the mature sequences of the peptide described in the patents, we can find the following: modification of the carboxylic acid of the C-terminal with an

https://doi.org/10.1021/acs.jnatprod.3c01040
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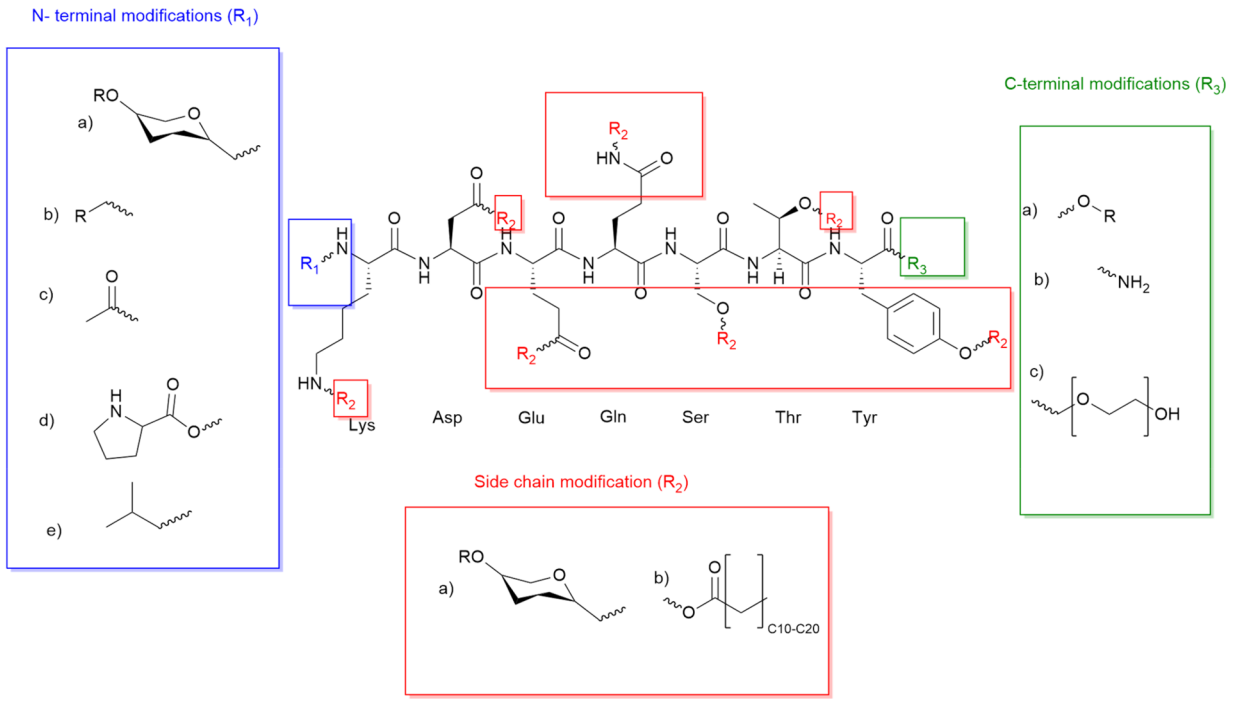

Figure 7. Examples of amino acid chemical modification strategies described for anuran antimicrobial peptides in granted patents. The blue boxes (R_1) represent modifications at the amino N-terminus: (a) glycation, (b) alkylation, (c) acetylation, (d) pyroglutamylation, and (e) isopropylation. The red boxes depict (R_2) modifications at the amino acid side chain: (a) glycation and (b) fatty acid addition (from C10−C20). The green boxes (R_3) depict modifications at the carboxyl C-terminus: (a) esterification, (b) amidation, and (c) addition of polyethylene glycol. It is worth noting that poly(ethylene glycol)s have also been attached to the amino acid side chains.

ester or amide group,[107−115] or polyethylene glycol conjugation;[116−119] modification of the N-terminal by *N*-glycation, *N*-alkylation, *N*-acetylation, *N*-acylation, *N*-isopropylation, or *N*-pyroglutamylation of amino acids;[120,121] modification of side chains of specific amino acids (Figure 7). Lysine (K) has been coupled to fatty acids ranging from C10 to C20 or glycans at the amino group of the epsilon carbon. Similarly, at the gamma or delta carboxyl group of aspartic acid (D) or glutamic acid (E) residues, respectively, hydrolyzed glutamine (Q) residues have been coupled to fatty acids or glycans.[122] Additionally, the hydroxy group at the beta residue carbon of threonine (T) or serine (S) and the phenolic OH of tyrosine residues have been coupled to fatty acids or glycans.[122] These modifications are described as post-translational, and semisynthetic modifications typically improve pharmaceutical properties of peptides (Figure 7).[107−115]

Furthermore, conservative and nonconservative substitutions in the amino acid sequence were described.[107−110,116,117] Truncated peptides, lacking redundant segments of the original molecules,[122] and cyclization of peptides[123,124] were also described. The modification strategies identified through the analysis of patents align closely with those described in the scientific literature for the development of peptide-based drugs.[4,5]

Structural, Application, and Biological Targets of Patented AMPs from Anuran Amphibians. As can be seen in Figure 8A, in 156 of the patents identified in this study, the AMP amino acid sequence described was the target of their structural claims, of which 64 (40.3%) had only the amino acid sequence; 44 (27.7%) had the amino acid and DNA sequence; 37 (23.3%) had the amino acid sequence and any of its derivatives; 11 (6.9%) contained in its claims the amino acid sequence, amino acid sequence derivates, and nucleotide sequence; 2 (1.3%) patents included only derivatives of the sequence; and 1 (0.6%) had only the DNA sequence (Supporting Information 1.1).

The analysis of 129 of the 188 selected documents allowed the identification of 160 anuran AMP sequences with granted patents, of which 127 are published in the scientific literature (Supporting Information 1.2, 1.3, 1.5) and 33 are unpublished (Supporting Information 1.4). Of these 160 peptides, 66 were claimed for their natural sequence, 69 were patented for both the natural and their derivative sequences, 20 were exclusively for derivatives of the sequence, and five were only for their biological activity. Brevinin, temporin, and ranuterin had the highest number of patent-related structures, accounting for 30 (18.75%), 25 (15.62%), and 19 (11.87%) of the total peptides identified, respectively (Supporting Information 1.3−1.5).

Among the total number of identified patents, 165 concern the preparation of pharmaceutical compositions (Figure 8B). Within this category, 68 patents (37.6%) exclusively cover pharmaceutical compositions, 48 patents (26.5%) involve both pharmaceutical composition and biomedical treatment, 34 patents (18.8%) apply to a pharmaceutical composition and the production method, and 15 patents (8.3%) include pharmaceutical composition, biomedical treatment, and the production method. In contrast, only 15 patents (8.3%) are related to the production method. Additionally, one patent (0.6%) is solely associated with biomedical treatment (Supporting Information 1.1).

Claims concerning pharmaceutical compositions of peptides involve their formulation with a suitable carrier or diluent for patient administration.[111−113,122,125−127] Some of these compositions may also be combined with known antibiotics to produce a synergistic effect or combat antibiotic resistance.[118] Descriptions of systemic application routes

https://doi.org/10.1021/acs.jnatprod.3c01040
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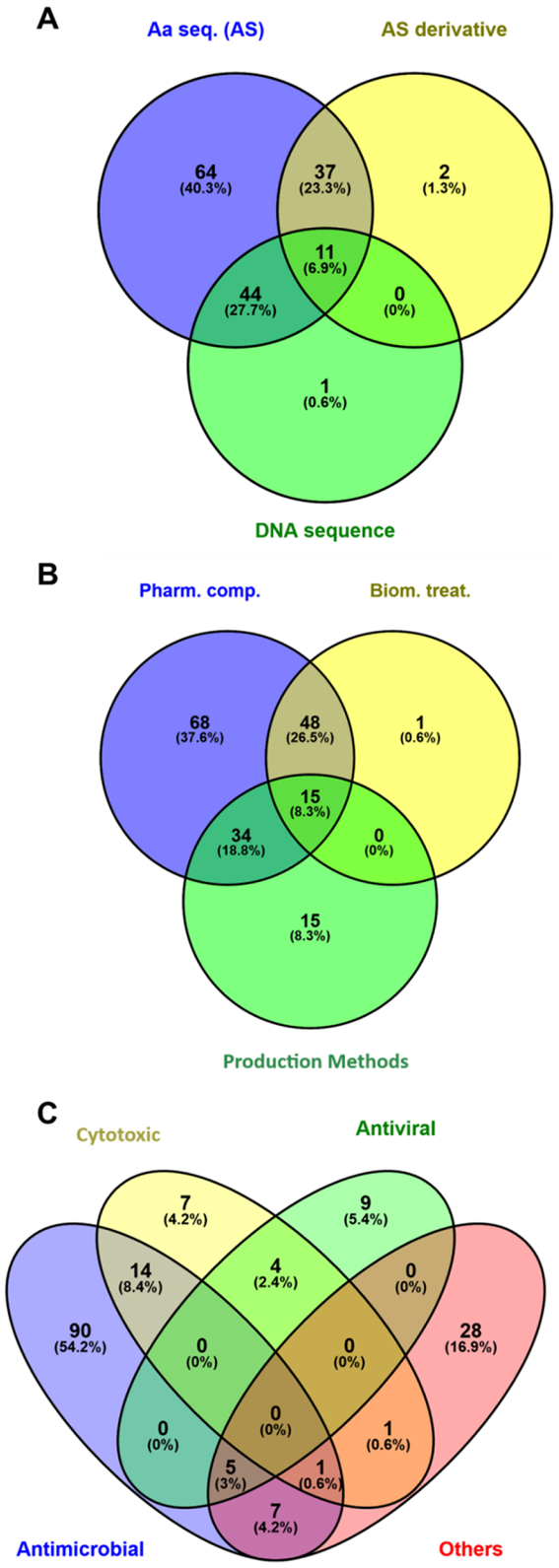

Figure 8. (A) Venn diagram showing structural targets of the patent claims: amino acid sequence, derived sequence, and nucleotide sequences. (B) Venn diagram showing applications targets of the patent claims: pharmaceutical composition, biomedical treatment, and production method of peptide. (C) Venn diagram showing biological activity targets of the patent claims (Supporting Information 1.1). The areas delimited in these Venn diagrams depict each category's number and percentage of peptides (written in bold).

varied, including vaccines, oral tablets, and external forms like gels, lotions, or sprays.[111−113,122,125−128] The medical treatments mentioned encompass human and veterinary patients for treating diseases.[122,129,130] Methods for obtaining natural peptides or RNA from anurans include ultrasonic extraction, microwave extraction, and electric milking of the skin. In turn, synthetic peptides are produced using mainly fundamentally solid-phase synthesis methods[131−133] and preparation of recombinant peptides in *Escherichia coli* or *Pichia pastoris* expression systems.[122,128]

There are FDA-approved peptide-based antimicrobial drugs described with similar modifications to those found in the patents.[5,134] For instance, dalbavancin, a lipoglycopeptide, and daptomycin, a lipopeptide, are employed to treat Gram-positive bacterial infections and administered intravenously, focusing on skin infections. Another example is colistin, a cyclic peptide administered intravenously, which targets multidrug-resistant infections caused by Gram-negative bacteria.[16] The applications of the patented inventions identified in the analysis of granted patents related to AMPs isolated from anurans contain a close overlap of chemical modifications with those recently published in a review of the status and recent advances in peptide-based drugs.[5]

The biological activities identified in the granted patents related to anurans' AMPs can be categorized into four main groups: antimicrobial, cytotoxic, antiviral, and other activities, with the latter including neuromodulatory, immune-modulatory, anti-inflammatory, inhibitory, wound healing, antioxidant, antidiabetic, antiparasitic, antithrombotic, and vasodepressor activities (Figure 8C). Among the total patents identified, 118 focused on antimicrobial activity, with 90 patents (54.2%) explicitly claiming only antimicrobial activity. Additionally, 14 patents (8.4%) claimed both antimicrobial and cytotoxic activities, five patents (3.0%) covered antimicrobial, antiviral, and other activities, seven patents (4.2%) included antimicrobial and other activities, and one patent (0.6%) encompassed antimicrobial, cytotoxic, and other activities. Furthermore, seven patents (4.2%) exclusively claimed cytotoxic activity, nine patents (5.4%) just claimed antiviral activity, and 28 patents solely pertained to other activities (Supporting Information 1.1).

As mentioned above, the diversity of patented activities for anuran peptides confirms the potential of these compounds for the development of peptide-based drugs. It is common in the scientific literature to find anuran AMPs with more than one activity. For example, raniseptin-3 (AP03622), raniseptin-6 (AP03621),[135] palustrin-Ca (AP02142),[136] and dermaseptin-PS1 (AP03039) show antimicrobial and cytotoxic activity,[137] frenatin 2. 3S (AP03034) shows antimicrobial and antiviral activity,[138] maximin 1 (AP00832) presents the three previous activities,[139] figainin 2 (AP03190) in addition to presenting the three activities mentioned above is antiparasitic (Supporting Information 1.2).[140]

Based on a review of the scientific literature, patent-related anuran AMPs have a broad spectrum of antibacterial and antifungal activities. Remarkably, some peptides such as B1CTcu2, B1CTcu3, B1CTcu4,[78,141] brevinin-1JDc,[142] DMS-PS1, DMS-PS2,[143] esculentin-2 HYba1, esculentin-2 HYba2,[78,144] ranalexin,[145] temporin-1Ga,[146] and temporin-SHf[147] have shown activity against MRSA strains. It is essential to highlight that some of these peptides, such as buforin I and II[148] and dermaseptin-S4 showed activity against *Candida neoformans*,[149] whereas B1CTcu5 stands out for its anti-

https://doi.org/10.1021/acs.jnatprod.3c01040
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tuberculosis (anti-TB) activity (Supporting Information 1.5),[9,119] which are priority pathogens according to the WHO's agenda for antimicrobial resistance in human health.[44]

Some patents involving derived peptide molecules or naturally occurring amphibian AMPs, such as gaegurin-5[150−154] and dermaseptin-PP,[155,156] were also found to be aimed at cytotoxic activities. Evidence has also been published regarding other activities such as antiparasitic, antidiabetic, hemolytic, and antibiofilm properties. Patented peptide structures from the tryptophyllins and rubellidins families, known for their neuromodulatory and neurotramismic activity, were also found (Supporting Information 1.5).[39,157]

Interestingly, peptides classified within the cathelicidin family (cathelicidin-NV, cathelicidin-PP, cathelicidin-PR1, cathelicidin-PR2, and cathelicidin-RC1) have progressed through the preclinical phase. For instance, the peptide LL37 fragment of human cathelicidin is currently in a phase II (NCT04098562) clinical trial to treat diabetic foot ulcers (Supporting Information 1.3−1.5).[158] It is important to note that cathelicidin peptides are found in several vertebrate groups. A phylogenetic study observed that amphibian cathelicidin predates that of reptiles but aligns with the evolutionary timeline of fish. This suggests that these peptides may have played a role in the evolutionary transition that allowed amphibians to bridge the gap between aquatic and terrestrial vertebrates.[159]

Nomenclature and Evolutionary Comments on Anuran AMPs. Establishing a nomenclature system for peptides isolated from amphibians has been a challenge. Numerous authors have established a convention to name amphibian peptides based on the species from where they were first described, based on a two- or three-letter code.[160] Others suggested adding the family name of the species,[30] for example, the peptide esculentin-2-OA2 (AP01867) isolated from *Odorrana adenorsinii*.[161] The esculentin family was first identified from *Pelophylax lessonae*, previously known as *Rana esculenta*.[162,163] However, these systems have limitations due to the constant updating of the amphibian taxonomy and the lack of a link between peptide names and biological function or evolution.[46,160] In addition, as it was recently discussed, names are provided using only the mature peptide as reference.[68,105] Though a clear evolutionary understanding of AMPs is limited because of their short length and the hypervariability of the mature regions, some alternatives to overcome these problems include using the complete sequence of the peptides.[68,105]

As reviewed in the literature among the main AMP families isolated from anurans, we can find bombinin, magainin, XPF, dermaseptin, brevinin, caerin, esculentin, ranalexin, rugosin, temporin, uperin, ranatuerin, maculatin, citropin, aurein, phylloxin, palustrin, kassinatuerin, tigerinin, pseudin, distinctin, nigrocin, dahlein, japonicin, ranacyclin, ocellatin, ascaphin, phylloseptin, lividin, pelophylaxin, dybowskin, pleurain, odorranain, hyposin, fallaxidin, nigroain, andersonin, cathelicidin, hymenochirin, frenatin, defensin, and kassorins.[30,31,33−35,37−42,61,63,64,67,72] Additionally, this analysis includes other significant peptides isolated from anurans due to their biological significance (Table 2 and Supporting Information 1.2).

CONCLUSIONS

Despite the substantial diversity and potential clinical applications of anuran AMPs, the number of patents granted in this field is low. The prevalence of "dead" patents accounts for most identified patents. These facts highlight the challenges in translating AMP research into practical applications. Though our findings suggest that anuran AMPs can still be considered promising clinical candidates, closing the gap between scientific discovery and patentable inventions is essential. In this sense, our analyses have uncovered exciting patterns in the distribution of patents, with Asia leading the in-patent grants, followed by North America and Europe. These patterns are consistent with the regional distribution of published AMPs, which can be traced to the higher investments made on ranids occurring in Eurasia. However, the complex relationship between patent percentages and amphibian distribution in regions such as South America, Central America, and Africa presents a significant challenge. These biodiverse regions hold immense scientific discovery potential, but research and exploration are often hampered mainly because of their limited resources and socioeconomic problems. Our analysis of patent applications showed the main structural modifications of patented anuran AMPs, including C-terminal and N-terminal end alterations, polyethylene glycol conjugation, and side-chain modifications. These strategies closely align with those described in the scientific literature for peptide-based drug development, offering valuable insights for future therapeutic innovation. Our analysis of patent claims relating to anuran peptides emphasized the significance of property claims in incorporating these molecules into pharmaceutical compositions. These patents encompass formulations with suitable carriers and diluents, various administration routes, and applications in medical treatments for both human and veterinary use. The versatility of anuran AMPs in pharmaceutical applications underscores their potential to address a wide range of diseases. Furthermore, our analyses revealed the multifunctionality of anuran AMPs in the granted patents. Beyond their antimicrobial properties, these peptides exhibit cytotoxic, antiviral, and various other activities, including neuromodulation, immune modulation, and wound healing. This diversity in biological activities expands the potential therapeutic applications of anuran AMPs beyond antimicrobial functions.

SEARCH METHODOLOGY

The work methodology was divided into two parts. In the first part, the identification of the patents related to AMPs isolated from anurans and the title of the sequences of the natural AMPs was carried out. First, an advanced search was performed in the DWPI database with the keywords "frogs OR/AND anurans OR/AND antimicrobial peptides" to investigate anuran-related patents. The selection criteria were all published patents (up to the date of 2022) that had the above terms in the title, abstract, or claims. Next, a screening of the exported information was performed for each patent, eliminating duplicate information and grouping patents according to their DWPI family number. DWPI families are groups of related patents, often including multiple patents filed in different countries for the same invention.[83] After that, with the publication number identified for each patent, a search was performed in the public patent databases Google Patents and Espacenet to cross-check the identified patents; both databases were searched using the unique publication number of the patents identified in the DWPI patent database search.[84,85]

The next step was to determine the validity status of the patents by using the information obtained from the DWPI. A patent is considered "live" if it is still in force, "dead" if it has

https://doi.org/10.1021/acs.jnatprod.3c01040
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expired or been abandoned, and "undetermined" if there is uncertainty or a lack of information about its current status.[83]

Following that, a careful analysis of the patent claims, description, and abstract (not shown) was conducted to identify the objectives of the patent claims, which were grouped into three main categories with subgroups. The first group was designated as structural targets, including patents that, in their suits, present (a) the amino acid sequence described in the patent of the AMP; (b) derivatives of the amino acid sequence, including any of the modifications to the sequences described in patent (a); and (c) the nucleotide sequence for obtaining (a) or (b). The second group included the applications described in the patent claims: (a) pharmaceutical composition (preparations, vaccines, lyophilized products, gel), (b) medical treatment (application in humans or animals), (c) production methods (extraction of the natural peptide from anurans, recombinant obtaining, or synthetic obtaining), and (d) agricultural application. The third group of analyses aimed to determine the biological activities described in the patent claims and was subdivided as follows: (a) antimicrobial activity (anti-Gram +/−, antifungal, and anti-MRSA), (b) cytotoxic, (c) antiviral, and (d) other activities (antioxidant, antihealing, inhibitor, etc.).

The subsequent step involved the identification of the structures of natural antimicrobial peptides isolated from anurans related to or patented in patent complaints. For the identification of peptide structures, information obtained from patent abstracts, claims, and descriptions such as nucleotide sequences, partial peptide sequences, peptide masses, disulfide bridges, and biological activities were analyzed in combination. The combined analysis of this information was performed by searching the databases: (1) the APD3 for searching the AMP sequence and for the calculation of the physicochemical properties of peptides with the Antimicrobial Peptide Calculator and Predictor Tool,[2] (2) the Uniprot database for identified peptide or protein sequences using the Uniprot BLAST alignment tool,[164] and (3) the GenBank nucleotide database for searching nucleotide sequences.[50] In addition, the nucleotide sequence translation tool EMBOSS Transeq from EMBL's European Bioinformatics Institute was used to translate the nucleotide sequence,[165] and (4) Google Scholar, Web of Science, and Pubmed were used for manual screening in the scientific literature. The methodology presented above is summarized in Scheme 1 for reference.

Simultaneously, we searched APD3 for the main families of antimicrobial peptides isolated from anurans described in the scientific literature.[3,14,30−43,61,63,64,67,72] Additionally, the taxonomy of all anurans mentioned in this article was updated using data from the Amphibians of the World database as of October 2023.[46]

The statistical analysis and the graphs of this study were performed in Microsoft Excel 2010, while the Venn diagrams were constructed using Venny v 2.1.240 software, which is accessible at https://bioinfogp.cnb.csic.es/tools/venny/.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.3c01040.

Tables that compile the information on the data reviewed: Supporting Information 1.1: Summary table of patents involving antimicrobial peptides isolated from frogs and information obtained from the DWPI patent database Data on Derwent Innovation; Supporting Information 1.2: Summary table of 1078 antimicrobial peptides isolated/derivated from anurans found in the Antimicrobial Peptide Database (APD3) available at https://aps.unmc.edu/; Supporting Information 1.3: Summary table of some frog and toad antimicrobial peptides found in the literature (not found in the APD3); Supporting Information 1.4: Summary table of peptides identified only in the patents; Supporting Information 1.5: Summary table of biological activities of anuran AMPs related to patents published in the scientific literature (XLSX)

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The manuscript was written through the contributions of all authors. All authors approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by the thematic project number 2020/02207-5 titled "Inventorying secondary metabolism through metabolomics: Contribution to the valuation of Brazilian Biodiversity" of the São Paulo State Research Foundation (FAPESP) and F. Almeida García' Fellowships Granted as Search Auxiliary/BCO - Direct Doctorate - Continuous Flow with process number 2023/00837-0. A.E.

Brunetti also thanks Agencia Nacional de Promoción Científica y Tecnológica grant number PICT 2020 # 2939, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) grant number PIP 2021-2023, and co-funded scholarship CONICET-Fulbright.

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