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Multifunctional host-defense peptides isolated from skin secretions of the banana tree dwelling frog *Boana platanera* (Hylidae; Hylinae)

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ABSTRACT

Five host-defense peptides (figainin 2PL, hylin PL, raniseptin PL, plasticin PL, and peptide YL) were isolated from norepinephrine-stimulated skin secretions of the banana tree dwelling frog *Boana platanera* (Hylidae; Hylinae) collected in Trinidad.

Raniseptin PL (GVFDTVKKIGKAVGKFALGVAKNYLNS.NH_2) and figainin 2PL (FLGTVLKLGKAIAKTVVPMLTNAMQPKQ. NH_2) showed potent and rapid bactericidal activity against a range of clinically relevant Gram-positive and Gram-negative ESKAPE $^+$ pathogens and *Clostridioides difficile*. The peptides also showed potent cytotoxic activity (LC_{50} values < 30 μM) against A549, MDA-MB-231 and HT29 human tumor-derived cell lines but appreciably lower hemolytic activity against mouse erythrocytes (LC_{50} = 262 ± 14 μM for raniseptin PL and 157 ± 16 μM for figainin 2PL). Hylin PL (FLGLIPALAGAIGNLIK.NH_2) showed relatively weak activity against microorganisms but was more hemolytic. The glycine-leucine-rich peptide with structural similarity to the plasticins (GLLSTVGGLVGGLLNNLGL.NH_2) and the non-cytotoxic peptide YL (YVPGVIESLL.NH_2) lacked antimicrobial and cytotoxic activities. Hylin PL, raniseptinPL and peptide YL stimulated the rate of release of insulin from BRIN-BD11 clonal β-cells at concentrations ≥100 nM. Peptide YL was the most effective (2.3-fold increase compared with basal rate at 1 μM concentration) and may represent a template for the design of a new class of incretin-based anti-diabetic drugs.

1. Introduction

Along with frogs belonging to the Ranidae [1,2], Pipidae [3] and Leptodactylidae [4] families, skin secretions from frogs from the extensive family Hylidae, currently 1051 species divided into three sub-families: Hylinae, Pelodryadinae and Phyllomedusinae [5], have proved to be a rich source of biologically active peptides with therapeutic potential. Although cytotoxicity against bacteria, fungi and viruses has been the property most extensively studied [6], it is now appreciated that such antimicrobial peptides are multifunctional with many compounds exhibiting clinically relevant immunomodulatory [7], wound healing [8], anti-tumor [9] and insulinotropic [10,11] activities. Consequently, they are more informatively described as host-defense peptides (HDPs).

Among frogs from the sub-family Hylinae, the genus *Boana* (currently 99 species [5]) are of particular interest and HDPs have been isolated from skin secretions of *B. albopunctata*, *B. boans, B. lundii*, *B. prasina*, *B. pulchella, B. raniceps* and *B. picturata* (reviewed in Ref. [12]). The taxonomy of the banana tree dwelling frog *Boana platanera* La Marca, Escalona, Castellanos, Rojas-Runjaic, Crawford, Señaris, Fouquet, Giaretta, and Castroviejo-Fisher, 2021 is particularly complex and incompletely resolved. Formerly classified as

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Abbreviations

HDP	Host-Defense Peptide
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time of Flight
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
T2D	Type 2 Diabetes

Hyla crepitans and subsequently as *Hypsiboas crepitans*, *B. platanera* is a natural resident of Colombia, Panama, Trinidad, Tobago, and Venezuela and inhabits humid and dry tropical forests and savannas but is also found in urban areas [5,13,14]. Its population is stable and it is described as a species of least concern [15]. *B. platanera* was formerly regarded as conspecific with the emerald-eyed treefrog *Boana xerophylla* (Duméril and Bibron, 1841). However, *B. xerophylla* (classified until 2017 as *Hypsiboas crepitans*) is a smaller species (average snout-vent length (SVL) 50 mm) and a more restricted geographical distribution, being found in eastern Venezuela south of the Orinoco and the Guianas highlands including adjacent northern Brazil [5,16].

This study describes the application of peptidomic analysis (reversed-phase HPLC combined with MALDI-TOF mass spectrometry and automated Edman degradation) to purify and characterize structurally the HDPs present in major abundance in norepinephrine-stimulated skin secretions from *B. platanera* frogs collected in Trinidad. The therapeutic potential of synthetic replicates of the peptides was assessed by determining their antimicrobial activities against aerobic ESKAPE $^{+}$ pathogens and the anaerobe *Clostridioides difficile*, their cytotoxicities against a range of human tumor-derived cell lines, and their abilities to stimulate insulin release from BRIN-BD11 clonal β-cells [17]. The term ESKAPE^{+} refers to a group of virulent pathogenic bacteria that readily develop resistance to antibiotics in common usage [18].

2. Materials and methods

2.1. Collection of skin secretions

The banana tree dwelling frog *B. platanera* (n = 4, sex not determined, average SVL 60.3 mm, average weight 10.8 g) was collected in May 2022 at Brasso Seco, Trinidad (GPS coordinates 10°43′49.6″N 61°15′25.9″W). The animals were sampled in the field and subsequently released unharmed at the site of capture. Each frog was injected with norepinephrine hydrochloride (40 nmol/g body weight) as previously described [12] and placed in distilled water (100 mL) for 15 min. The collection solution was acidified by addition of concentrated hydrochloric acid (0.5 mL) and immediately frozen.

2.2. Purification of the peptides

Partial purification of the peptides in the pooled skin secretions on Sep-Pak C-18 cartridges (Waters Associates, Milford, MA, USA) and chromatography on a semipreparative Vydac 218TP510 (C-18) reversed-phase HPLC column (Grace, Deerfield, IL, USA) was carried out as previously described [12]. The peptides within the peaks designated 1–5 in Fig. 1 that were present in major abundance were purified to near homogeneity, as determined by peak symmetry and mass spectrometry, by successive chromatographies on semi-preparativeVydac 214TP510 (C-4) and Vydac 208TP510 (C-8) columns. Full details of the elution conditions are provided in Supplementary Material.

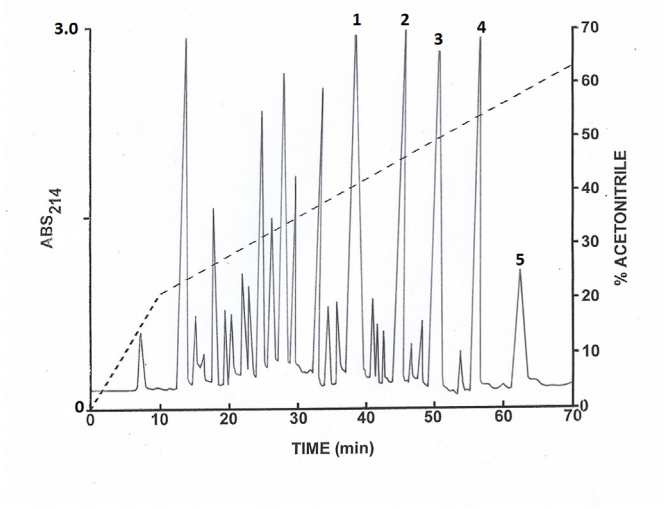

Fig. 1. Reversed-phase HPLC on a semipreparative Vydac C-18 column of pooled skin secretions from *B. platanera* after partial purification on Sep-Pak C-18 cartridges. The dashed line shows the concentration of acetonitrile in the eluting solvent. The peaks denoted 1–5 contained biologically active peptides that were purified further.

2.3. Structural characterization

MALDI-TOF mass spectrometry was carried out using an UltrafleXtreme instrument (Bruker Daltonik, Bremen, Germany). Full details of the procedure have been provided previously [19]. The primary structures of the purified peptides were determined by automated Edman degradation using an Applied Biosystems model 494 Procise sequenator (Applied Biosystems, Courtaboeuf, France).

2.4. Peptide synthesis

Figainin 2PL, hylin PL, peptide YL, plasticin PL and raniseptin PL were supplied in crude form by Synpeptide Co. Ltd. (Shanghai, China) and were purified to >98% purity by reversed-phase HPLC on a preparative Vydac 218TP1022 (C-18) column under conditions previously described [12]. The identities of the peptides were confirmed by electrospray mass spectrometry.

2.5. Antimicrobial assays

Antimicrobial activities (MIC and MBC) of synthetic replicates of the *B. platanera* peptides were determined against the following bacterial strains: *Pseudomonas aeruginosa* PA01 DSM 50071, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Escherichia coli* DSM 787, vancomycin-resistant *Enterococcus faecalis* (VRE) MF06036, *Acinetobacter baumannii* DSM 30008, carbapenem-resistant *Klebsiella pneumoniae* (CRE) ATCC BAA 1705, *Staphylococcus epidermidis* DSM 28319, vancomycin-resistant *Enterococcus faecium* (VRE) NCTC 12201, *Clostridioides difficile* strains R20291 DSM 27147 and 630 ATCC BAA 1382 as previously described [20]. Full details are provided in Supplementary Material.

The rates at which figainin 2PL and raniseptin PL caused cell death of *S. aureus* MRSA (ATCC 43300), *E. coli* (DSM 787) and *C. difficile* (630 BAA1382) were determined as previously described [20]. Full details are provided in Supplementary Material.

2.6. Cytotoxicity assays

Effects of the peptides (1–100 μM) on the viability of A549 human non-small cell lung adenocarcinoma cells (RRID:CVCL_0023), MDA-MB-231 human breast adenocarcinoma cells (RRID:CVCL_0062), HT-29 human colorectal adenocarcinoma cells (RRID:CVCL_0320) and human umbilical vein endothelial cells (HUVEC) (RRID:CVCL_2959) following a 24 h incubation were determined as previously described [12]. Hemolytic activity of the peptides (15–500 μM) against freshly prepared erythrocytes from male NIH male Swiss mice (Harlan Ltd, Bicester, U.K.) was determined as previously described [21]. The LC_{50} value was taken as the mean concentration of peptide producing 50% hemolysis in three independent experiments.

2.7. Determination of insulin-releasing activity

BRIN-BD11 clonal β-cells were seeded into 24-well plates and allowed to attach during overnight incubation at 37 °C. Incubations with the synthetic *B. platanera* peptides (10^{-11} - 10^{-6} M; n = 8) were carried out for 20 min at 37 °C in Krebs-Ringer bicarbonate (KRB) buffer supplemented with 5.6 mM glucose as previously described [19]. After incubation, aliquots of cell supernatant were removed for insulin radioimmunoassay [22]. Control incubations were carried out in parallel with the well-established insulin stimulatory agents, alanine (10 mM) and KCl (30 mM).

2.8. Statistical analysis

Data were compared using unpaired Student's *t*-test (non-parametric with two-tailed P values and 95% confidence interval) and one-way ANOVA with Bonferroni post-hoc test wherever applicable. Values are presented as mean ± standard deviation (SD). Results are considered to be significantly different if $P < 0.05$.

3. Results

3.1. Purification of the peptides

The elution profile on a semipreparative Vydac C-18 reversed-phase column of the *B. platanera* skin secretions, after partial purification on Sep-Pak cartridges, is shown in Fig. 1. The peptides present in the peaks of major abundance designated 1–5 were selected for further purification to near homogeneity on Vydac C-4 and Vydac C-8 columns. Subsequent structural characterization showed that peak 1 contained peptide YL + hylin PL (1–10) fragment, peak 2 raniseptin PL, peak 3 figainin 2PL, peak 4 hylin PL, and peak 5 plasticin PL. The methodology is illustrated by the separation of peptide-YL from hylin PL-(1–10)-peptide present in peak 1 (Fig. 1) on a semipreparative Vydac C-4 column and purification to near homogeneity of peptide YL on a semipreparative Vydac C-8 column (Fig. 2).

3.2. Structural characterization of the peptides

The primary structures of the peptides were established without ambiguity by automated Edman degradation (Table 1), Agreement between the proposed amino acid sequence and the molecular masses determined by MALDI-TOF mass spectrometry was good and demonstrated that all the HDPs contained a C-terminally α-amidated residue. The peptides were assigned to recognized peptide families on the basis of structural similarity to peptides previously isolated from other frogs belonging to the Hylidae: raniseptin [23], figainin 2 [24], hylin [25] and plasticin [26]. A HDP with structural similarity to peptide YL has not been described previously.

3.3. Antimicrobial activities

The antimicrobial activities (MIC and MBC) of synthetic replicates of hylin PL, raniseptin PL and figainin 2PL against ESKAPE $^{+}$ microbial pathogens and two strains of *C. difficile* are shown in Table 2. Of particular note is the high potency of raniseptin PL (MIC = 3 μg/mL; approx. 1 μM) against the Gram-negative bacteria *E. coli*, carbapenem-resistant *K. pneumoniae* and multidrug-resistant *A. baumannii* and against Gram positive vancomycin-resistant *E. faecium* and the anaerobe *C. difficile*. Synthetic replicates of peptide YL and plasticin PL at concentrations up to and including 100 μg/mL showed no detectable growth-inhibitory activity against S. *aureus* and *E. coli* and so were not investigated further. At concentrations equivalent to 1 *x* MIC, raniseptin PL and figainin 2PL produced 100% cell death of *E. coli*, *S. aureus* and *C. difficile* after a 1 h incubation (Fig. 3).

3.4. Cytotoxic activities

As shown in Table 3, figainin 2PL was the most potently cytotoxic ($LC_{50} < 20$ μM) against human-derived lung adenocarcinoma, breast adenocarcinoma and colorectal adenocarcinoma cells. At concentrations ≥30 μM, cell death was rapid occurring within <5 min. In contrast, the hemolytic activity against mouse erythrocytes was relatively low ($LC_{50} = 157 \pm 16$ μM during a 1 h incubation). Raniseptin PL was also potently and rapidly cytotoxic against the human tumor-derived cells ($LC_{50} < 30$ μM) while displaying weak hemolytic activity ($LC_{50} = 262 \pm 14$ μM). Both peptides were also potently cytotoxic against human umbilical vein endothelial cells (HUVECs) derived from non-neoplastic tissue ($LC_{50} < 20$ μM). Hylin PL was significantly less cytotoxic against tumor-derived cells and HUVECs compared with figainin 2PL and raniseptin PL but displayed greater hemolytic activity. Peptide YL and plasticin PL showed no detectable cytotoxicity against lung adenocarcinoma A549 cells following a 24 h incubation at 100 μM concentration and so were not investigated further. Plasticin PL was weakly hemolytic ($LC_{50} = 272 \pm 13$ μM) while peptide YL showed no hemolytic activity at concentrations up to 500 μM.

3.5. Insulinotropic activities

The rate of release of insulin from BRIN-BD11 clonal β-cells in the presence of 5.6 mM glucose was 0.96 ± 0.10 ng/10^6 cells/20 min. As shown in Fig. 4, incubation with 5.6 mM glucose containing 10 mM alanine resulted in a 2.6-fold increase in rate and incubation with 5.6 mM glucose containing 30 mM KCl resulted in a 3.4-fold increase in rate. Incubation with peptide YL, hylin PL and raniseptin PL at concentrations of 10^{-7} and 10^{-6} M significantly ($P < 0.05$) stimulated insulin release. The greatest effect was produced by peptide YL (1.9-fold increase at 10^{-7} M and 2.3-fold increase at 10^{-6} M). Plasticin PL also stimulated insulin release at 10^{-7} and 10^{-6} M but the increase in rate was not significant because of the variability in response. Figainin 2PL was without effect on insulin release at concentrations up to 10^{-6} M.

4. Discussion

This study is a component of an on-going program of investigation designed to characterize, both structurally and biologically, the HDPs in skin secretions of frogs from Trinidad with a view to identifying components with therapeutic potential. The present study has identified raniseptin PL and figainin 2PL as templates for development into agents for treatment of infections caused by

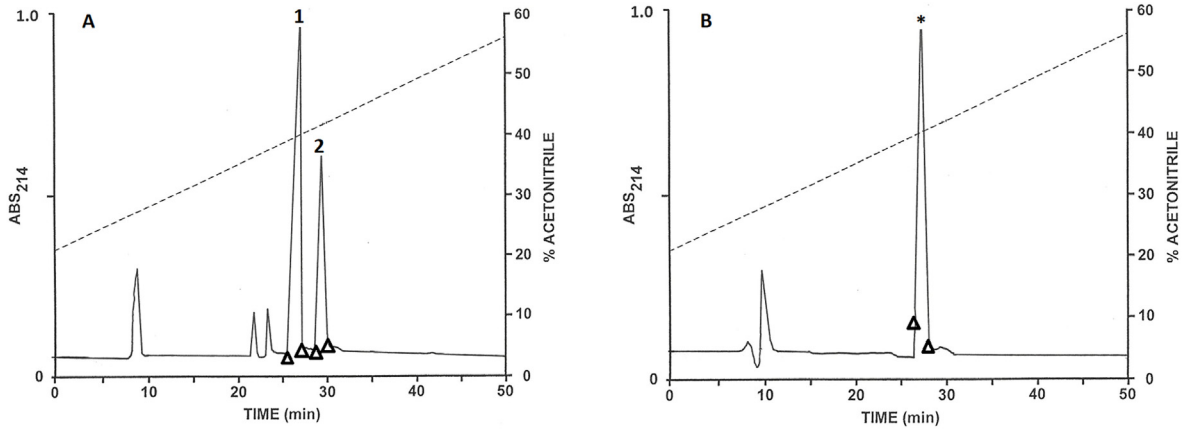

Fig. 2. (A) Separation of peptide YL (peak 1) from hylin PL (1–10) (peak 2) on a semipreparative Vydac C-4 column and (B) purification to near homogeneity of peptide-YL, denoted by *, on a semipreparative Vydac C-8 column. The dashed line shows the concentration of acetonitrile in the eluting solvent and the arrowheads show where peak collection began and ended.

Table 1
Primary structures and molecular masses of the peptides isolated from norepinephrine stimulated skin secretions from *B. platanera*.

Peak No.	Peptide	Primary structure	$[MH^+]_{obs}$	$MH^+]_{calc}$
1	Peptide YL	YVPGVIESLL.NH_2	1088.7	1088.6
1	Hylin PL(1-10)	FLGLIPALAG	971.6	971.6
2	Raniseptin PL	GVFDTVKKIGKAVGKFALGVAKNYLNS.NH_2	2823.9	2823.6
3	Figainin 2PL	FLGTVLKLGKAIAKTVVPMLTNAMQPKQ.NH_2	2996.9	2996.7
4	Hylin PL	FLGLIPALAGAIGNLIK.NH_2	1679.9	1680.0
5	Plasticin PL	GLLSTVGGLVGGLLNNLGL.NH_2	1765.9	1766.0

$[MH+]_{obs}$ denotes the experimentally determined molecular mass and $[MH+]_{calc}$ denotes the mass calculated from the proposed structures.

Table 2
Minimum Inhibitory Concentrations (μg/mL) and Minimum Bactericidal Concentrations (μg/mL) for peptides isolated from *B. platanera* skin secretions against a range of clinically relevant Gram-positive and Gram-negative aerobic pathogens and anaerobic *C. difficile*.

Pathogen	Hylin PL	Raniseptin PL	Figainin 2PL	Gen	Van
E. coli DSM 787	100 (>100)	3.13 (3.13)	6.25 (6.25)	0.98	NA
S. aureus ATCC 43300	100 (>100)	25 (25)	6.25 (12.5)	31.3	NA
S. epidermidis DSM 28319	>100 (>100)	6.25 (6.25)	12.5 (12.5)	0.49	NA
P. aeruginosa DSM 50071	>100 (>100)	50 (100)	100 (100)	3.91	NA
K. pneumoniae ATCC BAA 1705	>100 (>100)	3.13 (3.13)	25 (25)	0.98	NA
A. baumannii DSM 30008	100 (100)	3.13 (3.13)	6.25 (6.25)	0.98	NA
E. faecalis MF 06036	100 (100)	25 (25)	50 (100)	3.91	NA
E. faecium NCTC 12201	50 (50)	3.13 (3.13)	6.25 (6.25)	1.95	NA
C. difficile R20291	50 (>100)	3.13 (6.25)	6.25 (12.5)	NA	1.00
C. difficile 630 BAA1382	25 (50)	3.13 (3.13)	6.25 (12.5)	NA	1.00

Data show Minimum Inhibitory Concentration (MIC) and the values in parentheses show Minimum Bactericidal Concentration (MBC). NA: not applicable, GEN: gentamicin, VAN: vancomycin.

bacteria that are resistant to commonly used antibiotics. The peptides display potent bactericidal activity against a range of clinically relevant aerobic bacteria with a propensity to develop multi-drug resistance ($ESKAPE^+$ pathogens) and against the anaerobe *C. difficile*. Both peptides exhibited rapid ($\leq$1 h) killing of *E. coli*, a methicillin-resistant strain of *S. aureus* and *C. difficile*. Although neither MIC/MBC nor LC_{50} were determined under physiologically relevant conditions, the hemolytic activity of both peptides is appreciably less than the antimicrobial potency when tested *in vitro*.

There are no consensus sequences that define the ability of frog skin HDPs to produce cell death but the vast majority of such peptides are cationic, contain a domain that includes several hydrophobic amino acids, and have the propensity to adopt an amphipathic α-helical conformation in a membrane-mimetic solvent such as 50% trifluoroethanol-water or in the presence of a (phospho)lipid vesicle [1,2]. The relative magnitude of potencies of such HDPs against bacteria and mammalian cells is determined by

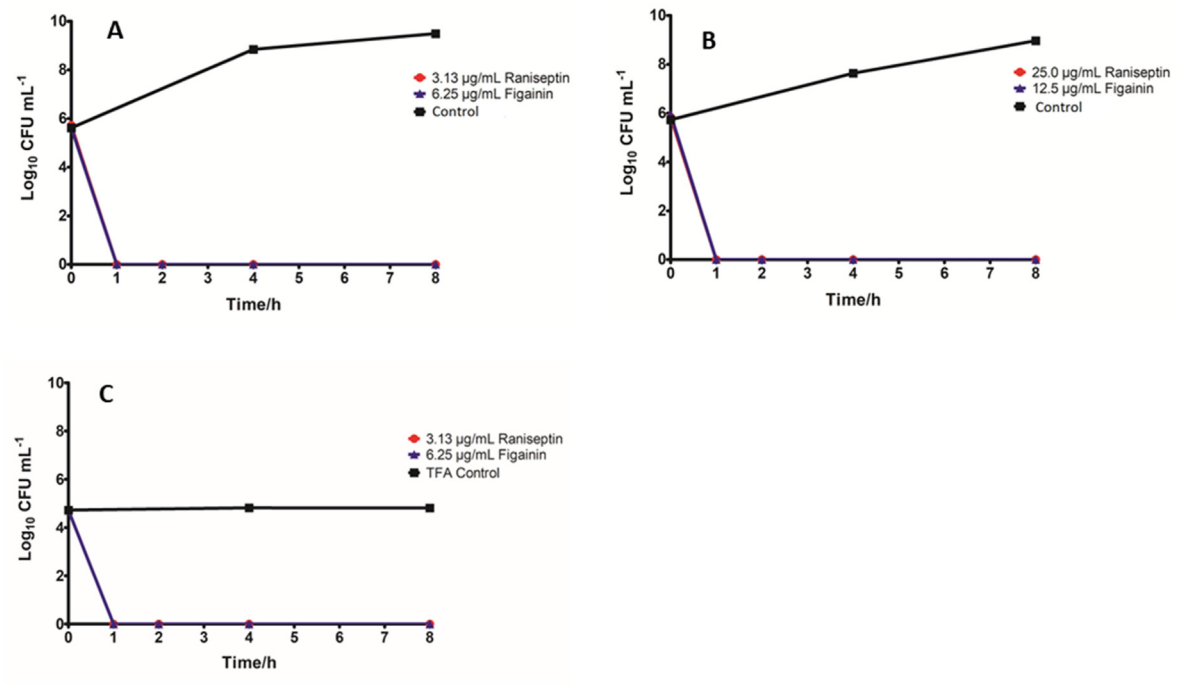

Fig. 3. Survival of (A) *E. coli* DSM 787, (B) *S. aureus* ATCC 43300 and (C) *C. difficile* 630 BAA1382 in liquid broth after addition of either 1 *x* MIC raniseptin PL or 1 *x* MIC figainin 2PL. Control incubations were conducted in the absence of peptide.

Table 3
Cytotoxicities of peptides from *B. platanera* against human lung adenocarcinoma A549 cells, breast adenocarcinoma MDA-MB-231 cells, colorectal adenocarcinoma HT-29 cells, human umbilical vein endothelial cells (HUVEC) and mouse red blood cells (RBC).

Cell type	Peptide YL	Plasticin PL	Hylin PL	Raniseptin PL	Figainin 2PL
A549	>100	>100	58 ± 2	5.8 ± 0.2	6.4 ± 0.3
MDA-MB-231	ND	ND	58 ± 2	10.5 ± 1.0	7.0 ± 0.5
HT-29	ND	ND	68 ± 2	29.1 ± 2.2	19.2 ± 0.8
HUVEC	ND	ND	58 ± 1	15.7 ± 0.7	16.6 ± 0.3
RBC	>500	272 ± 13	65 ± 5	262 ± 14	157 ± 16

Data show mean LC_{50} values (μM) ± S.D. ND: not determined.

Boana platanera	Plasticin-PL	GLL****STVGGLVGGLLNNLGL[a]
Agalychnis annae	Plasticin-A1	GLVSGLLNTAGGLLGDLLGSLGSLSG[a]
Agalychnis callidryas	Plasticin-C1	GLLSGILNTAGGLLGNLIGSLSN[a]
Agalychnis callidryas	Plasticin-C2	GLLSGILNSAGGLLGNLIGSLSN[a]
Pachymedusa danicolor	Plasticin-DA1	GVVTDLLNTAGGLLGNLVGSLSG[a]
Phyllomedusa trinitatis	Plasticin-TR	GLVSGLLNSVTGLLGNLAGGGL
Leptodactylus laticeps	Plasticin-L1	GLVNGLLSSVLGGGQGGGGLLGGIL
Leptodactylus fallax	Leptoglycin	GLLGGLLGPLLGGGGGGGGGLL

Fig. 4. Comparison of the primary structures of plasticin PL from *B. platanera* with non-cationic plasticins from *Agalychnis*, *Pachymedusa*, *Phyllomedusa* and *Leptodactylus*. Conserved residues are shown in red and the GXXXG motif is underlined. [a] denotes C-terminal α-amidation.

complex and incompletely understood interactions between molecular charge, hydrophobicity, hydrophobic moment (a measure of the degree of amphipathicity) and conformation (extent and stability of the helical domain) [27]. In broad terms, increasing peptide cationicity promotes interaction with the negatively charged cell membrane of bacteria leading to increased antimicrobial potency whereas increasing hydrophobicity promotes interaction with the zwitterionic membrane of mammalian erythrocytes leading to increased hemolytic activity [28]. As shown in Table 4, both raniseptin PL and figainin 2PL are strongly cationic (molecular

Table 4
Physicochemical properties of peptides with antimicrobial and cytotoxic activities isolated from *B. platanera* skin secretions.

Peptide	Charge at pH 7	Hydrophobicity	Hydrophobic moment	Predicted helical domain
Raniseptin PL	+5	0.328	0.433	6–26
Figainin 2PL	+5	0.524	0.360	5–14
Hylin PL	+2	0.826	0.601	7–14

Hydrophobicity was calculated using the hydrophobicity scale of Fauchere and Pliska [29] and hydrophobic moment [30]) using the HeliQuest webserver [31]. The extent of the helical domain was predicted using the PEP2D program [32].

charge +5 at pH 7) compared with hylin PL (molecular charge +2 at pH 7) which accounts, at least in part, for hylin PL's lower antimicrobial potency. In contrast, the greater hydrophobicity of hylin PL compared with raniseptin PL and figainin 2PL may account for its greater hemolytic activity (Table 3).

Naturally occurring cytotoxic peptides and their derivatives, including those from amphibia [33], are receiving increasing attention as potential anti-cancer agents particularly when tumors have developed resistance to commonly used drugs [34]. Figainin 2PL, raniseptin PL and hylin PL are cytotoxic to a range of human-derived tumors (Table 3) with figainin 2PL being the most potent. However, in common with other frog skin derived HDPs previously investigated (reviewed in Ref. [2]), their potential for development into therapeutically valuable anti-cancer agents is limited by the fact that they show no selectivity for tumor-derived cells. The LC_{50} values against non-neoplastic HUVECs are comparable to those determined for the tumor-derived cells. Bioinformatics algorithms have been used to develop a strategy for converting a peptide with antimicrobial properties into one with potential anticancer actions [35]. The strategy has been employed to design an analog of the 18 amino-acid-residue antimicrobial peptide AcrAP1 with potent anti-proliferative and apoptotic activities against a range of human tumor-derived cells and anti-angiogenic activity against HUVECs.

It has been demonstrated that several frog skin HDPs that were first identified as a result of their ability to inhibit the growth of microorganisms have later been shown to stimulate the release of insulin from clonal β-cells and isolated mouse islets as well as lowering blood glucose in murine models of type 2 diabetes (T2D) (reviewed in Refs. [2,10]). Such incretin peptides may be divided into two groups (A) peptides with cytotoxic properties that nevertheless stimulate release of insulin at a concentration at least an order of magnitude less than the concentration that impairs the integrity of the mammalian plasma cell membrane and (B) peptides that lack antimicrobial and cytotoxic properties even at high concentrations. While raniseptin PL and hylin PL belong to the first group, peptide YL joins frenatin 2D (DLLGTLGNLPLPFI.NH_2) from the Tyrrhenian painted frog *Discoglossus sardus* (Alytidae) [36] and tigerinin-1R (RVCSAIPLPICH.NH_2) from the Vietnamese common lowland frog *Hoplobatrachus rugulosus* (Dicroglossidae) [37] in the second group of non-cytotoxic peptides. No single mechanism of action has been identified that mediates the insulin-releasing action of the frog skin HDPs. Incubation of BRIN-BD11 cells with most of the cationic incretin peptides produced membrane depolarization and an increase in intracellular Ca^{2+} concentrations but incubation of the cells with frenatin 2D [36] and the temporins [38] had no significant effect on membrane potential or intracellular Ca^{2+} concentrations suggesting that these peptides are acting via an alternative, as yet uncharacterized, K_{ATP} channel-independent mechanism.

The plasticins are conformationally flexible peptides containing one or more copies of the GXXXG motif that were first isolated from frogs of the Phyllomedusinae sub-family and later from frogs of the Leptodactylidae family (reviewed in Refs. [4,26]). The plasticins may be divided into two groups in terms of structural similarity with the strongly cationic peptides displaying varying degrees of antimicrobial and cytotoxic activities and the weakly cationic peptides lacking antimicrobial activity and having only low or absent hemolytic activities [26]. The primary structures of the weakly cationic plasticins, including plasticin PL, are compared in Fig. 4. As expected, plasticin PL (molecular charge = +1 at pH 7) lacks antimicrobial activity and only very weak hemolytic activity. Its role, if any, in host-defense is unclear. However, it is worthwhile to point out that the weakly cationic plasticin-L1 from *L. laticeps* displays immunomodulatory properties, stimulating production of the proinflammatory cytokines IL-1β, IL-12 IL-23 and TNF-α from mouse peritoneal macrophages [39]. This suggests the possibility that such peptides may act as an activation signal for the system of innate immunity that protects the frog against invasion by pathogenic microorganisms in the environment.

The potent insulin-releasing activity coupled with its ease of synthesis and its lack of hemolytic activity at concentrations up to 500 μM identify peptide YL as a template for the design of peptides with therapeutic potential for T2D therapy. Future studies will investigate the *in vivo* antidiabetic properties of the peptides in animal models of T2D (high-fat fed mice and *db/db* mice) and attempt to elucidate its mechanism of action.

5. Conclusions

Recent years have seen the emergence of multi-drug resistant strains of numerous pathogenic bacteria and fungi as well as marked increases in the world-wide incidence of T2D. The present study has identified two peptides in the skin secretions of *B. platanera*: raniseptin PL and figainin 2PL that show potential for development into therapeutically valuable antibacterial agents. Both peptides display potent broad-spectrum activity against ESKAPE pathogens and *C. difficile* concomitant with much lower cytotoxicity against erythrocytes. Future studies will address the synthesis of non-toxic analogs with increased potency against microorganisms and longer half-lives in the circulation [40].

The efficacy of long-acting GLP-IR agonists (liraglutide, semaglutide) and more recently the GLP-1R/GIPR dual agonist tirzepatide in normalizing circulating glucose concentrations in patients with T2D is well established [41] but these compounds are not without their limitations. The most common off-target effects are nausea, vomiting, diarrhea and loss of muscle mass, which are tolerated in most patients, but result in abandoning of treatment in a significant number [42,43]. Consequently, there is a need for new types of non-toxic, anti-diabetic drugs, particularly for patients for whom GLP-1/GIP-derived agents are inappropriate. Preliminary data in this study have identified peptide YL as an effective and non-cytotoxic *in vitro* insulin-releasing agent with therapeutic potential. T2D of long duration, in addition to impaired insulin release, is associated with reduced β-cell mass and so future studies will investigate the ability of peptide YL to promote β-cell regeneration and protect against cytokine-mediated β-cell destruction [38].

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Declaration of competing interest

Authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biochi.2024.03.012.

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