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# Characterisation of three alpha-helical antimicrobial peptides from the venom of *Scorpio maurus palmatus*

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Keywords: Pore forming peptides; Infection; Scorpion venom; Venomics; Therapeutics

## Abstract

Scorpion venoms provide a rich source of anti-microbial peptides. Here we characterise three from the venom of *Scorpion maurus palmatus*. Smp13 is biologically inactive, despite sharing homology with other antimicrobial peptides, probably because it lacks a typically charged structure. Both Smp-24 and Smp-43 have broad spectrum antimicrobial activity, disrupting bacterial membranes. In addition, there is evidence that Smp24 may inhibit DNA synthesis in *Bacillus subtilis*. Smp24 haemolysed red blood cells but in contrast, Smp43 was non-haemolytic. The introduction of a flexible Gly-Val-Gly hinge into the middle of Smp24 did not alter the haemolytic activity of Smp24 (as might have been predicted from earlier studies with Pandinin2 (Pin2), although C-terminal truncation of Smp-24 reduced its haemolytic activity, in agreement with earlier Pin 2 studies. Smp24 and its derivatives, as well as Smp-43, were all cytotoxic (ATP release assay) toward mammalian HepG2 liver cells. Our results highlight the beneficial effect of helical-hinge-helical conformation on promoting prokaryotic selectivity of long chain scorpion AMPs, as well as the importance of examining a wide range of mammalian cell types in cytotoxicity testing.

## Introduction

Over the last few decades an increasing number of pathogenic microorganisms have developed resistance to conventional antibiotics posing problems in the management of infection. Although treatment options for some Gram positive pathogens have undoubtedly improved, our ability to successfully combat many Gram negative bacteria is becoming a serious issue (Livermore., 2009). The problems in modern antibiotic drug development of being able to design agents that are not easily susceptible to resistance (Jenssen *et al*., 2006) has meant that there has been a dramatic decline in the development of new antibiotics during the same period.

Antimicrobial peptides (AMPs) represent an ancient host defence mechanism of the innate immune system that transverses the evolutionary spectrum and to this day remains an effective strategy against invading pathogens in the animal kingdom. AMPs are found in all multicellular organisms, from molluscs to humans. They are present in vertebrate neutrophils and leukocytes, invertebrate haemolymph, amphibian skin secretions and nearly all types of plant cells (Bahar and Ren., 2013). The predominant mechanism of action of AMPs is to disrupt the structure and function of microbial cell membranes. Because of their selectivity for prokaryotes and their membrane-disruptive mechanisms for which microbes have little natural resistance, the spotlight in recent years has turned towards the development of novel antibiotics from these peptides (Zasloff., 2002, Hancock and Sahl., 2006)

AMP's are positively charged peptides of variable length (typically 12-70 amino acids) which can be broadly divided into four structural classes: (i) amphipathic linear α-helix, (ii) amphipathic β-sheet constrained and stabilised by disulphide bridges, (iii) extended linear peptides, lacking secondary structure and enriched with one or more amino acids (e.g. histidine, glycine, proline/ arginine) and (iv) loop peptides, which adopt a helical type-II

circular structure because of a high abundance of proline residues (Powers., 2003). Yeaman and Yount (2003) have reviewed various structural parameters- amphipathicity, charge, hydrophobicity, polar angle and conformation -which the authors suggest are crucial molecular determinants for identifying a peptide that has selective toxicity for prokaryotic membranes.

The key factor to membrane disruption is the initial electrostatic attraction of the peptide to the negatively charged bacterial membrane surface (Huang., 2000, Shin *et al*., 2001, Glukhov *et al*., 2005). After initial electrostatic attraction, a threshold concentration is often required before membrane disruption can occur (Huang., 2000, Melo *et al*., 2009). Following this initial step, a number of mechanisms have been proposed to account for membrane disruption: (i) Barrel stave mechanism - peptide oligomerization creates a central pore lumen characterised by peptide-peptide interactions (Baumann and Mueller., 1974). (ii) Toroidal mechanism - the pore lumen is lined by interactions between peptide and phospholipid head groups (Matsuzaki *et al*., 1996; Ludtke *et al*., 1996). (iii) Carpet model peptides cover ("carpet") the membrane surface and at a critical concentration, transient pores are formed which allow the peptide access to the inner leaflet (Shai *et al*., 1999 & Yamaguchi *et al*., 2001). In this last model, it is proposed that peptides can span the transmembrane bilayer on each membrane surface, causing curvature of the membrane to protect the acyl chains, which then leads to the disintegration of the bilayer, due to micelle formation (Teixeria *et al*., 2012).

AMPs have been found in the venoms of all venomous species examined. The physical process of envenomation can variously lead to damage of fangs or telsons, resulting in microbial infection. It is therefore logical to suggest that there has been evolutionary pressure on venoms to produce AMPs as a protective measure to sterilize any infection (de Lima *et al*., 2009). The AMP content of venoms varies widely between with a significant intra-species variation, dependent on such factors as age, sex, geography and climate (Calvete., 2013).

Scorpion venoms provide an especially rich and diverse source of AMPs, with over 40 peptides having been characterised to date (see Harrison *et al.,* 2014 for a recent review); the applications of proteomic and genomic based approaches are identifying new peptides at an ever increasing rate. In one such study Abdel-Rahman *et al*., (2013) examined the venom of the North African scorpion *Scorpio maurus palmatus;* the study identified four potentially novel AMPs, namely a 76-residue cysteine-containing peptide (Smp76), and three alpha-helical peptides (Smp13, Smp24 and Smp43). Smp24 shares homology with Pandinin 2 (Pin 2) (54 %) an AMP from *Pandinus imperator*. Using Pin2 as a model AMP, Possani and colleagues (Rodriguez *et al*., 2011) have demonstrated the importance of a structural proline "kink" in the middle of an otherwise un-interrupted alpha-helix that confers potent non-specific pore-forming activity toward both eukaryotic and prokaryotic membranes; substitution of this Pro residue for a more hydrophobic Gly-Val-Gly "hinge", dramatically reduces the haemolytic activity of Pin2 and makes the modified peptide more selective for prokaryotic membranes. The present study describes the biological characterisation of Smp 13, Smp24 and Smp43 and in particular examines the effects of disrupting the central Pro residue of Smp24 with respect to its antimicrobial selectivity, as distinct from non-specific cytotoxic properties.

# Materials and Methods

## Materials

All peptides were synthesised using FMOC solid-phase chemistry and were purchased from Think Peptides (Oxford, UK) (Purity analysis see Supplementary data). All peptide sequences are listed in Table 1. All other solvents, chemicals and tissue culture reagents were of the highest grade available and were obtained from Sigma (Gillingham, UK).

**Bacterial strains**

Microbes used throughout this study were as follows: *Escherichia coli* JM109, *Pseudomonas aeruginosa* NCIMB 8295, *Bacilus subtilis* NCIMB 8024, *Staphylococcus aureus* SH1000, *Klebsiella pneumoniae* NCTC 13439, *Staphylococcus epidermidis* ATCC12228 & *Candida albicans* ATCC10231. Antibiotic resistant strains of *S. aureus* used were as follows: methicillin-resistant *S. aureus* (MRSA) ATCC 33591, epidemic methicillin-resistant *S. aureus* (EMRSA) 15 and EMRSA-16, clinical isolate MRSA mecA mupA positive, along with the vancomycin intermediate resistant *S. aureus* (VISA) Mu50, and a clinical VISA isolate KM126. All bacteria were from a culture collection held at Sheffield Hallam University.

**Antimicrobial assay**

All antimicrobial assays were carried out by the microplate dilution method (Andrews., 2001) using an automated plate reader (CENios Plus, Tecan, Switzerland). Synthetic AMPs were examined at a concentration range of 0-512 μg/ml with Muller-Hinton growth medium.

**Haemolysis assay**

Haemolytic activity was determined on sheep erythrocytes as described (Corzo *et al*., 2001). Whilst differences in lipid composition are observed between sheep and human erythrocytes they share significant similarities in terms of cholesterol content and neutrally charged lipid composition (Nelson 1967) and therefore provide a reliable first screen for toxicity.

Synthetic AMPs were examined at a concentration range of 0-512μg/ml using a plate reader (570nm, Infinite M200, Tecan, Switzerland) Triton X-100 (10%) was used as a positive control and deionised water as the negative control. All samples were tested in triplicate.

**ATP release assay**

An ATP release assay (Sigma, UK) was used to determine the cytotoxic potential of peptides against HepG2 liver cells in accordance with the manufacturer's protocol. HepG2 cells were grown in DMEM (glutamate max medium) containing 1 g/l D-glucose, 10% foetal calf serum and 1% penicillin-streptomycin. Cells were grown (37˚C, 5% $CO_2$ ) to 80% confluence. Cells were trypsinised, washed PBS and resuspended at the desired concentration to and seeded at a final density of 1.1 x $10^5$ cells/well. Cells were incubated for 24 hrs, washed (PBS) and peptides (0.5-512 μg/ml) added (15 mins 37˚C). ATP release was quantitated using luciferin/luciferase in a bioluminescent assay. All assays were performed in triplicate. Triton X-100 (10%) was used as a positive control and deionised water as the negative control. Luminescence was determined on a Wallac Victor2 1420 multi-label counter (Perkin Elmer, Llantrisant, UK).

**Membrane integrity assay**

Bacterial membrane integrity following antimicrobial peptide attack was determined by the BacLight method (adapted from Hillard *et al*., 1999). Membrane integrity was determined for both Gram positive (*S. aureus* SH100) and Gram negative (*E. coli* JM109) organisms using the live/dead BacLight kit (Invitrogen UK). Overnight cultures were diluted with Muller-Hinton broth to an optical density of between 0.5-0.6 at 600 nm. For each sample to be tested, 500 μl of culture was pelleted (10,000 x g, 15 mins) and washed with PBS. The pellet was resuspended and each peptide was added at 4x MIC. Peptides were incubated (10 mins, room temp) on a rocking platform. Each sample was then centrifuged (10,000 x g, 10 mins) and re-suspended in 500 μl of deionised water. The optical density was adjusted to 0.5 OD600nm and then 1.2 μl of BacLight reagent was added according to the manufacturer's instructions and incubated (15 mins, room temp) in the dark. The fluorescence excitation wavelength was set at 485nm and fluorescence emission measured at 645 nm and 530 nm to determine intact (green) and damaged (red) cells respectively. 100% loss of membrane

integrity was determined by the addition of Triton X-100 (10%) and deionised water was added as a negative control. All assays were performed in triplicate.

**Intracellular bio-reporter gene assay**

To determine if any of the peptides had intracellular targets, a *Bacillus subtilis* reporter gene assay (Urban *et al*., 2007) was performed by analysing the effects of stress on specific intracellular bacterial targets, using a bioluminescent luciferase gene reporter Bacteria ~~s~~were exposed to 1/2 x MIC of each peptide for 1, 1.5, 3 & 4 hours at 37˚C with luminescence determined using a Wallac Victor2 1420 multi-label counter (Perkin Elmer, Llantrisant, UK). All assays were performed in triplicate and responses were determined by calculating the % increase from the negative control (Phosphate buffered saline solution).

**CD spectra**

CD spectra were recorded on a JASCO J-700 spectropolarimeter at 22 ˚C using a 0.1 cm path-length cell. The spectral range was 190–250 nm and three scans were accumulated. Peptides (0.2 mg/ml) were dissolved in either water or 60% (v/v) aqueous 2,2,2-trifluoroethanol (TFE).

## Results

Using a microbroth dilution assay, no antimicrobial activity was detected (up to 512 μg/ml) with Smp13. In contrast, Smp24 and Smp43 displayed highest activity against Gram positive organisms (Table 2). Removing the last four C-terminal residues of Smp24 (truncated Smp24, Smp24T) made a negligible difference to antimicrobial activity. Similarly, increasing

the conformational flexibility of Smp24 by inserting a "hinge" region in the middle of the peptide (replacing Pro-14 with Gly-Val-Gly, Smp24GVG) had limited effect. Because of the strong activity observed with Smp24 towards *S. aureus* SH1000, this peptide was assayed against a wider range of *S. aureus* strains, including methicillin-resistant strains and strains with intermediate resistance towards vancomycin (Table 3). A wide range of MIC values were observed between strains, particularly between EMRSA-15 (MIC = 16 μg/ml) and EMRSA-16 (MIC = 512 μg/ml), highlighting the possibility of cross-resistance being acquired either through plasmid acquisition, mutation or phenotypic routes. Both Smp24 and Smp43 showed limited activity toward the yeast, *C.albicans*.

The effects of the biologically active peptides upon bacterial membrane integrity determined that all peptides examined were membrane active with maximum disruption against both Gram-positive and Gram-negative species at a concentration of 4 x the MIC value observed (Table 4).

Further evidence of membrane disruption was observed in *B.subtilis*, with all four active peptides inducing an almost x15-fold increases in the cell envelope stress response, as evidenced by the increased expression of the *ypuA* promoter after a 1 hour incubation. A significant stress response was still evident after 4 hours exposure to all peptides (Smp24 176% ± 35.6, Smp24T 166 % ± 28.1, Smp24GVG 183% ± 21.9 & Smp43 131 ± 26.2) (Fig.1). Interestingly, with the exception of Smp43, all peptides induced an up regulation of the gene associated with DNA synthesis stress; Smp24GVG showed the greatest (288.4% ± 14.2) increase in the expression of the *yorB* promoter after 3 hours whilst Smp24 and Smp24T increased expression by 228.1 % (± 12.1) and 163.3% (±6.4) respectively at the same time point. A comparison of cell envelope and DNA stress responses would suggest that the cell envelope has entered a recovery phase when the peptide is exerting maximum damage (either directly or indirectly) on bacterial DNA synthesis. In contrast to the induction

of DNA stress responses, the genes associated with RNA or protein synthesis related stress responses were not significantly induced by any of the peptides (Fig. 1).

In haemolysis assays Smp43 showed very low toxicity at the maximum concentration tested (1.2 ± 0.5 % lysis at 512 µg/ml), in comparison, Smp24 caused significant erythrocyte disruption (89.6 ±5.6 %) at the same concentration (Fig.2). The effects of modifications to Smp24 had contrasting outcomes (Fig 2). Truncated Smp24 showed a decrease in activity, with haemolysis reduced by 43% at the highest concentration compared with the parent peptide. However, inclusion of the flexible Gly-Val-Gly hinge region had little effect with no significant difference in haemolytic activity. Further cytotoxic testing was carried out on HepG2 cells by measuring the release of ATP (Fig 3) with cell damage occurring in a concentration-dependent fashion in response to all four peptides above a concentration of 32µg/ml. The consequence of Smp24 modification was similar to those observed in haemolysis assays with a decrease in cytotoxicity observed with the truncated derivative (11.8 % (±4.0) at 32 µg/ml compared with 15.9 % (±1.9)) whist Smp24GVG showed no significant difference compared with the parent peptide (Fig 3). However, there was a remarkable difference in the cytotoxicity of Smp43 when comparing erythrocyte haemolysis and the release of ATP from HepG2 cells (94.8% (±7.5) at 512 µg/ml), clearly highlighting differences in eukaryotic cell membrane architecture and the need for a range of diverse mammalian cell types to be assayed during cytotoxic testing (Fig. 3).

CD spectral analysis for both Smp24 and Smp43 revealed that both peptides are in an unordered structure in aqueous solution. However, in the presence of 60 % TFE both peptides adopt significant alpha helical regions characterised by two minima at 208 nm and 222 nm (Fig.4). Analysis of both peptides (DichroWeb server, Whitmore and Wallace, 2008) revealed Smp24 to have two regions of approximately 59 % and 22 % helical content whilst Smp43 showed two regions of approximately 70 % and 25 % helical content. The remaining

regions of both peptides were assigned as unordered. The percentage helical content was estimated using the SELCON method of analysis which determines helical content using statistical algorithms that were created by analysis of 29 reference proteins. This methodology was validated by analysing 4 unknown proteins where the structure was also confirmed by X-ray crystallography (Sreerama *et al.*, 1999).

## Discussion

A large number of scorpion AMPs have been characterised. They have a wide range of biological activities and their characterisation has been facilitated because their small size (<5kDa). Abdel Rahman *et al.*, (2013) identified four AMPs in the venom of *Scorpio maurus palmatus* and this study characterises the biological activity of three of them.

Smp43 shares high homology (75-86%) with opistoporins and pandinin 1 (Pin 1). Opistoporin 1 & Pin 1 both have been shown to have helix-hinge-helix structures (Moerman *et al*., 2002, Corzo *et al*., 2001). CD spectral analysis of Smp43 suggested the peptide to be unordered in aqueous solution whilst in the presence of 60 % TFE it adopted a di-helical structure, linked by a random coil region. Smp43 showed striking similarity to Pin 1 (helical regions between 3-18 and 26-37 linked by a random coil region) (Nomura *et al.*, 2005).

Smp24 showed a lower identity (54%) with pandinin-2 (Pin 2), although it shares evolutionarily conserved motifs with the shorter (17-20 residue) chain scorpion AMPs. The most notable among these is the LIPS motif which is found within the core of Pin 2 as well as BmKb1, ctriporin, imcroporin, meucin-18 and mucroporin. CD spectral analysis of Smp24 revealed, like Smp43, an unordered structure in aqueous solution that adopted a di-helical structure in 60% TFE. The structure of Smp24 however is in contrast to that of Pin 2 which

on the basis of NMR analysis, has only a single helical region, extending from the N-terminus to residue 18 (Nomura *et al*., 2004).

Despite the differences in structure the two peptides share a number of key features including length (24 residues), homology (13 identical residues) and biological activity (bactericidal & cytotoxic) which is highly suggestive of Smp24 belonging to the same family of mid-chain helical scorpion AMPs. The shorter Smp13 belongs to the family of cytotoxic peptides such as IsCT peptides (Dai *et al*., 2002). However, unlike other peptides within this family, Smp13 carries no charge and therefore it is not surprising that it has no antimicrobial activity. Its role within the venom remains to be elucidated.

The helix-hinge-helix topology of Smp43 is present in a number of longer chain AMPs, all of which have high therapeutic indices, for example cecroprin A **(**Holak *et al*., 1988), dermaseptin B2 (Galanth *et al*., 2009), Hadrurin (Torres-Larios *et al*., 2000), the opistoporins (Moerman *et al*., 2002), Pin1 (Corzo *et al*., 2001) and BmKbpp (Zeng *et al*., 2000). This suggests that this class of peptides has considerable potential for the future development of anti-infective agents.

Suggestions that substitution of a proline residue with a more flexible Gly-Val-Gly (GVG) "hinge" would reduce the haemolytic effects of AMPs has been proposed by Rodriguez and colleagues and, in the case of Pin2, has proved successful (Rodriguez et al., 2011). However there was no difference in the haemolytic activities of Smp24 and Smp24GVG.

It should be noted that the effect of proline within AMPs is ambiguous. On the one hand, many proline-deficient peptides (e.g. magainin-2) show low haemolytic/cytotoxic activity (Zasloff., 1987). In contrast, peptides such as melittin and pardaxin contain a proline in the

central region of their sequence and both have high antimicrobial and haemolytic potential. Removal of this proline residue further increases toxicity (Dempsey *et al*., 1991). Bodone *et al*. (2013) have suggested that a proline "kink" is required for prokaryotic membrane selectivity, with removal only increasing toxicity due to increased helical structure. This turn increases the hydrophobic face of the peptide and its propensity to bind to neutrally charged bilayers. In contrast, more compact proline-containing peptides will be less attracted to neutral surfaces and retain electrostatic attraction to negatively charged bilayers.

Reducing the length of the C-terminal of Smp24 by the removal of the last four residues increased the therapeutic index. This modification was chosen as it had been successfully performed with the evolutionary related Pin 2 where the shortening of the random coil region increased its therapeutic index despite a reduction in charge (Rodriguez *et al*., 2014) although this electrostatic attraction is less important towards eukaryotic membranes due to their zwitterionic nature (Jiang *et al*., 2008). The reduction in haemolysis seen in Pin 2 after truncation to 14 and 17 residue peptides is of interest as CD spectra showed a change in structure from a predominantly helical structure to a β-hairpin structure similar to Indolicidin (Rodriquez *et al*., 2014).

Although Smp43 exhibited low haemolysis it still caused significant ATP release in HepG2 cells suggesting disruption of eukaryotic membranes. However, it is worth noting cancer derived cells have a more negatively charged membrane than normal eukaryotic cells due to an increase in membrane phosphatidylserine content, differential branching and sialic acid content of N-linked glycans associated with transmembrane proteins and the increase in O-glycoslylated mucins (Gajski and Garaj-Vrhovac., 2013). On this basis it would be useful to assess any toxic effects on primary cells which exhibit normal membrane compositions both to redefine peptide toxicity and determine any beneficial anticancer properties.

290 Along with their membrane disruptive mechanism the stress responses with *B. subtilis* whole
291 cell reporter assays showed a possible interaction with DNA for Smp24 and its derivatives. A
292 number of peptides have been shown to interact with intracellular targets including DNA.
293 These include buforin II (BFII) as a prototypical example (Yonezawa *et al*., 1992, Park *et al*.,
294 1998, Zhang *et al*., 2014), which also contains a proline in the centre of the peptide (Park *et*
295 *al*., 1998). BFII penetrates the membrane without causing general membrane
296 permeabilisation and accumulates in the cytosol before binding to DNA. In contrast, proline
297 deficient magainin 2 (MAG 2), causes membrane permeabilisation but stays bound to the
298 phospholipid bilayer and has a low propensity for DNA binding (Kobayashi *et al*., 2004).
299 Both peptides form transient toroidal pores although BFII pores have a significantly shorter
300 half-life (Park *et al*., 2000). Pore collapse is electrostatically driven by repulsive forces
301 within the helical portion of the peptide (Kobayashi *et al*., 2004). Further evidence for the
302 destabilising effect of proline is found in alamethicin where substitution of alanine for proline
303 is known to destabilise the pore (Kaduk *et al*., 1998). Since Smp24 both disrupts membranes
304 and may have an intracellular target, we propose that the highly charged nature of the peptide
305 (+4) together with the presence of proline could create a "half-way house" between BFII and
306 MAG 2. Although Smp24 has a longer pore half-life than BFII, we suggest that, unlike MAG
307 2, Smp24 can still dissociate from the phospholipid bilayer and interact with DNA. In this
308 context however, the observation that Smp24GVG did not diminish the DNA stress response,
309 needs further investigation.

310 The fact that the intracellular stress responses of Smp43 were limited to the cell envelope is
311 perhaps not surprising, when considering that Pin1 causes complete disruption of the bilayer
312 (Nomura *et al*., 2005). Biophysical studies in our own laboratory (unpublished observations)
313 support a carpet-like mechanism for Smp43, similar to Pin 1.

Antimicrobial assays of the peptides from *S. maurus palmatus* studied in our experiments show wide differences in MIC values, especially between Gram-positive and Gram-negative bacteria, with preferential activity towards Gram-positive bacteria, in keeping with previously tested α-helical peptides from scorpion venom (Harrison *et al.,* 2014). This is perhaps not surprising when considering the failure to produce Gram-negative specific invectives in recent years and highlights the intrinsic difficulty in identifying suitable Gram-negative specific agents.

In summary, three scorpion AMPs have been characterised from the venom of *S. maurus* palmatus. Two of these peptides (Smp24 & Smp43) showed broad spectrum antimicrobial activity but differed significantly in their haemolytic effects whilst the shortest peptide (Smp13) had no biological activity. Different modifications of Smp24 led to both beneficial and adverse biological effects and highlighted the potential for further manipulation of these peptides as AMP scaffolds. Both Smp24 and Smp43 exhibited a membrane disruptive mechanism of action, however Smp24 and its derivatives showed evidence of interference with DNA synthesis. In accord with previous studies (Torres-Larios *et al*., 2000, Moerman *et al*., 2002, Corzo *et al*., 2001 and Zeng *et al*., 2000), our experiments further highlight the beneficial effect that a helical-hinge-helical conformation has on long chain scorpion AMPs in promoting prokaryotic selectivity.

## Acknowledgements

All work contained within this study was supported by a grant provided by the Biomolecular Sciences Research Centre, Sheffield Hallam University, UK.

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**Table 1.** Sequence information of all peptides used in this study

**Table 2.** Minimum inhibitory activity (MIC) of AMPs

**Table 3.** MIC of Smp24 against a range of antibiotic sensitive and antibiotic resistant *S. aureus* strains

**Table 4.** Membrane damage mediated by scorpion AMPs on Gram-positive and Gram-negative membranes

**Figure 1.** Stress response of *B. subtilis* reporter constructs following exposure to Smp24 (blue), Smp43 (red) Smp24GVG (green) and Smp24T (purple).

**Figure 2.** Haemolytic activity of *Scorpio maurus palmatus* AMPs and their derivatives.

**Figure 3.** Cytotoxic effects of *Scorpio maurus palmatus* AMPs and their derivatives against HepG2 cells measured as a function of ATP release.

**Figure 4.** Solvent dependent effects of Smp24 and Smp43 on peptide secondary structure.

**Table 1.**

| Peptide name | Sequence |
|---|---|
| ***Smp13*** | ILQDIWNGIKNLF-$NH_2$ |
| ***Smp24*** | IWSFLIKAATKLL**P--**SLFGGG-KKDS |
| ***Pandinin-2*** | FWGALAKGALKLIP--SLFSSFSKKD |
| ***Smp24GVG*** | IWSFLIKAATKLL**GVG**SLFGGG-KKDS |
| ***Smp24T*** | IWSFLIKAATKLL--PSLFGG |
| ***Smp43*** | G-VWDWIKKTAGKIWNSEPVKALKSQALNAAKNFVAEKIGATPS |
| ***Pandinin-1*** | GKVWDWIKSAAKKIWSSEPVSQLKGQVLNAAKNYVAEKIGATPT |

**Table 2.**

| Bacteria strain | Gram class | MIC (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | **Smp 13** | **Smp 24** | **Smp 24 GVG** | **Smp 24T** | **Smp 43** |
| *B. subtilis* NCIMB 8024 | + | No Activity | 4 | 8 | 8 | 4 |
| *S. epidermidis* sp. | + | No Activity | 8 | 16 | 16 | 64 |
| *S. aureus* SH100 | + | No Activity | 8 | 16 | 16 | 32 |
| *E. coli* JM109 | - | No Activity | 64 | 64 | 128 | 128 |
| *K. pneumoniae* NCTC 13439 | - | No Activity | 128 | 128 | 128 | 64 |
| *P. aeruginosa* | - | No Activity | 256 | 256 | 256 | 64 |
| *C. albicans* | fungi | No Activity | 32 | 128 | 128 | 128 |

491 **Table 3.**

| ***S.aureus* strain** | **MIC (µg/ml)** |
|---|---|
| SH1000 | 32 |
| S.*aureus* ATCC 25923 | 32 |
| MRSA ATCC 33591 | 512 (Partial) |
| MRSA mecA mupA +ve | 16 |
| EMRSA-15 | 16 |
| EMRSA-16 | 512 (Partial) |
| VISA MU50 | 32 |
| VISA KM126 | 64 |

492 **Table 4.**

| | **% Membrane damage** | | | |
|---|---|---|---|---|
| **Species/Strain** | **Smp24** | **Smp24GVG** | **Smp24T** | **Smp43** |
| ***E.coli* JM109** | 110.5 (±3.9) | 97.2 (±1.2) | 99.5 (±3.5) | 105.4 (±1.8) |
| ***S.aureus* SH1000** | 101.2 (±4.8) | 105.3 (±4.5) | 11.6 (±2.9) | 103.2 (±5.1) |

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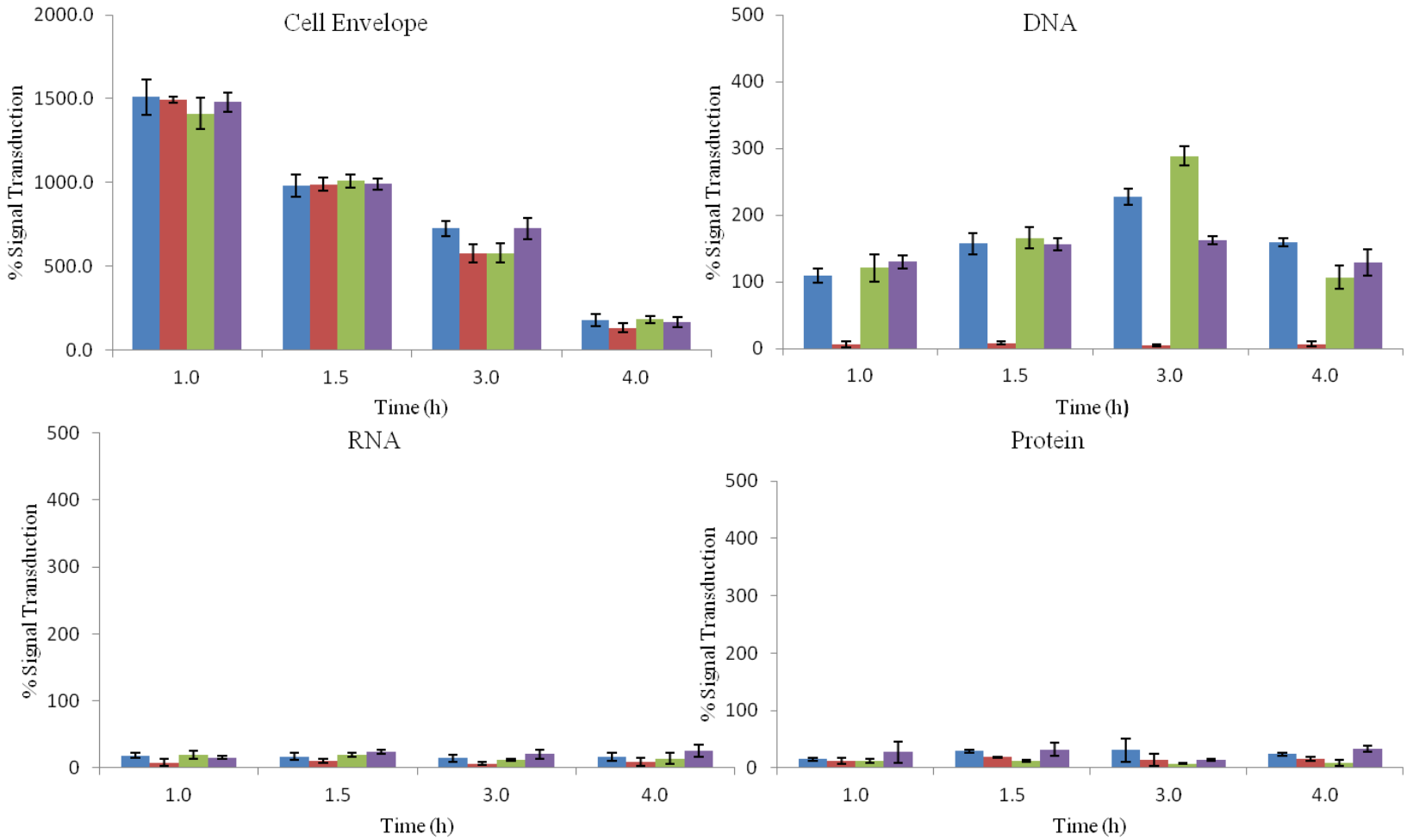
Cell Envelope
% Signal Transduction
2000.0
1500.0
1000.0
500.0
0.0
1.0
1.5
3.0
4.0
Time (h)
DNA
% Signal Transduction
500
400
300
200
100
0
1.0
1.5
3.0
4.0
Time (h)
RNA
% Signal Transduction
500
400
300
200
100
0
1.0
1.5
3.0
4.0
Time (h)
Protein
% Signal Transduction
500
400
300
200
100
0
1.0
1.5
3.0
4.0
Time (h)

**Figure 1.**

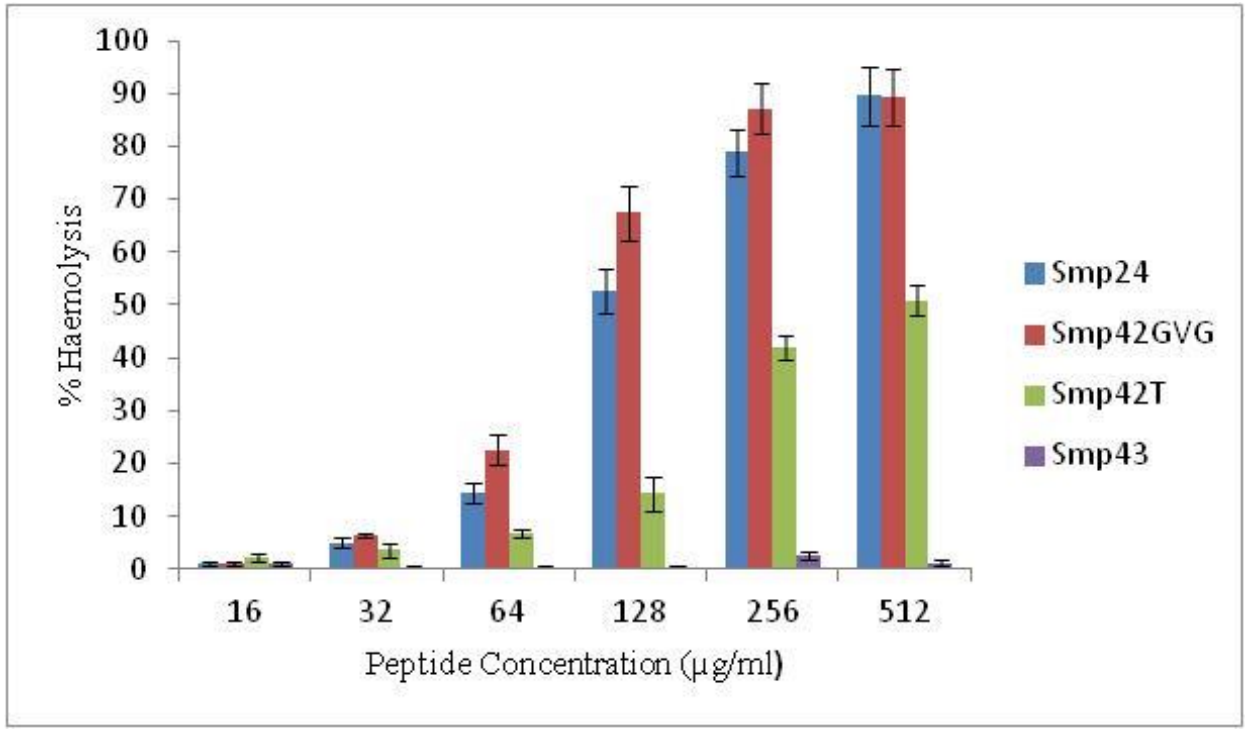
100
90
80
70
60
50
40
30
20
10
0
% Haemolysis
16
32
64
128
256
512
Peptide Concentration (µg/ml)
Smp24
Smp42GVG
Smp42T
Smp43

**Figure 2.**

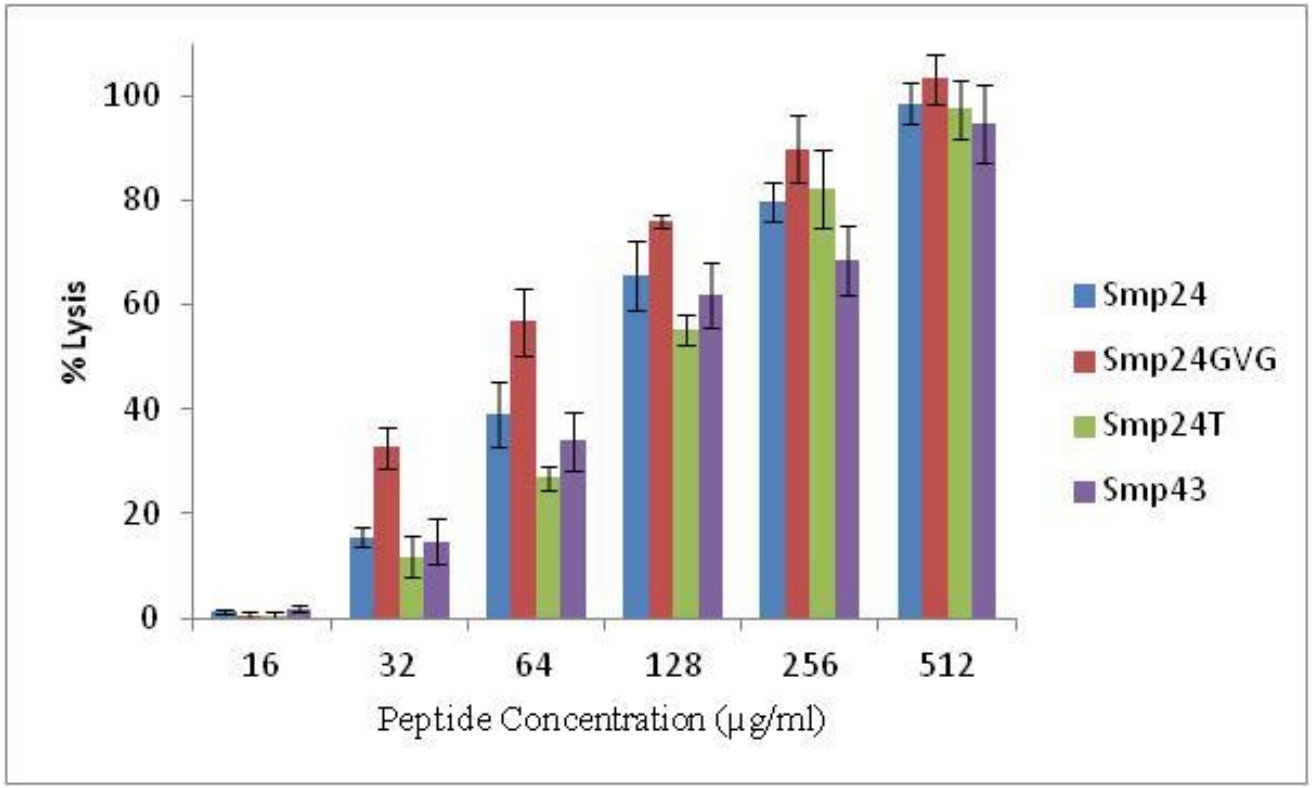
100
80
60
40
20
0
% Lysis
16
32
64
128
256
512
Peptide Concentration (µg/ml)
Smp24
Smp24GVG
Smp24T
Smp43

**Figure 3.**

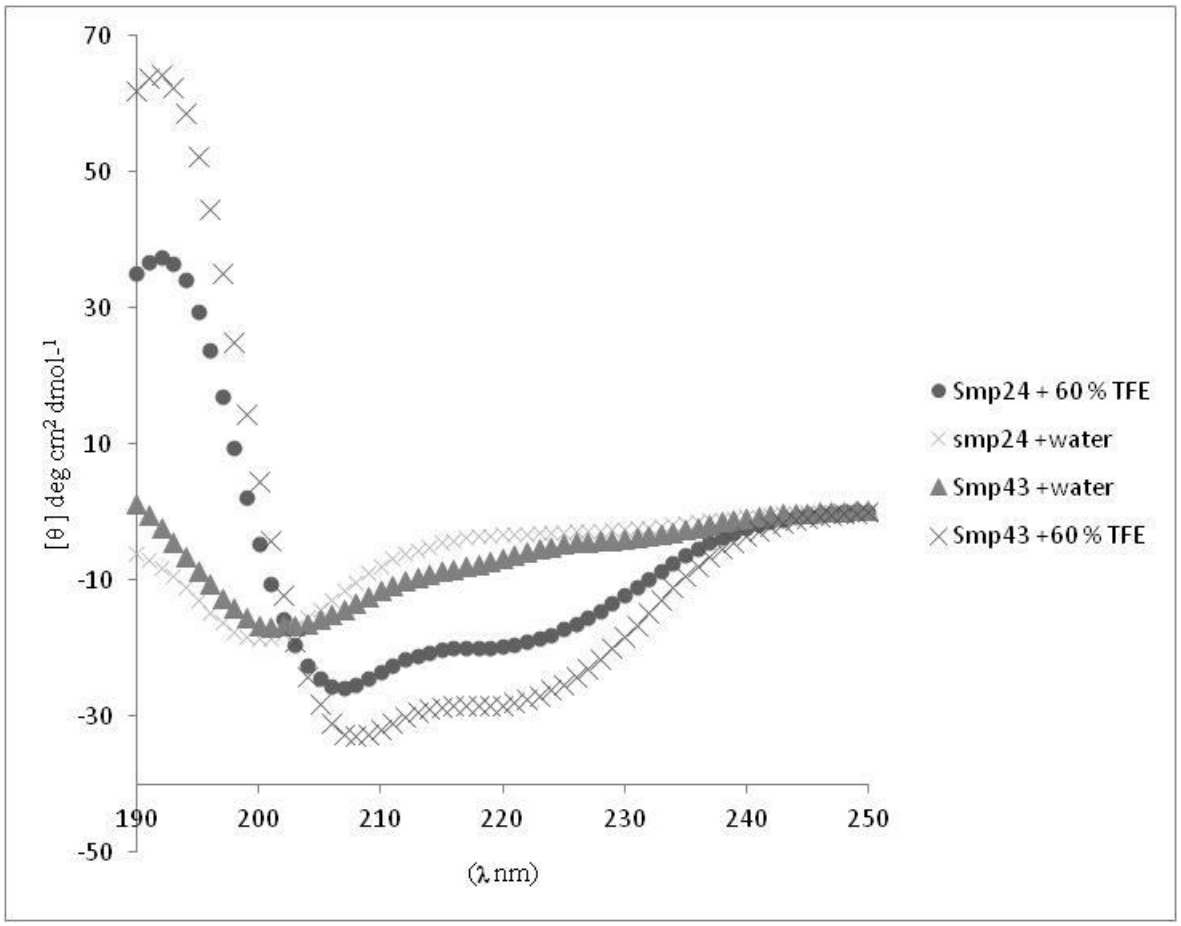
70
50
30
10
-10
-30
-50
190
200
210
220
230
240
250
[θ] deg cm² dmol-1
(λ nm)
Smp24 + 60 % TFE
smp24 +water
Smp43 +water
Smp43 +60 % TFE

**Figure 4**