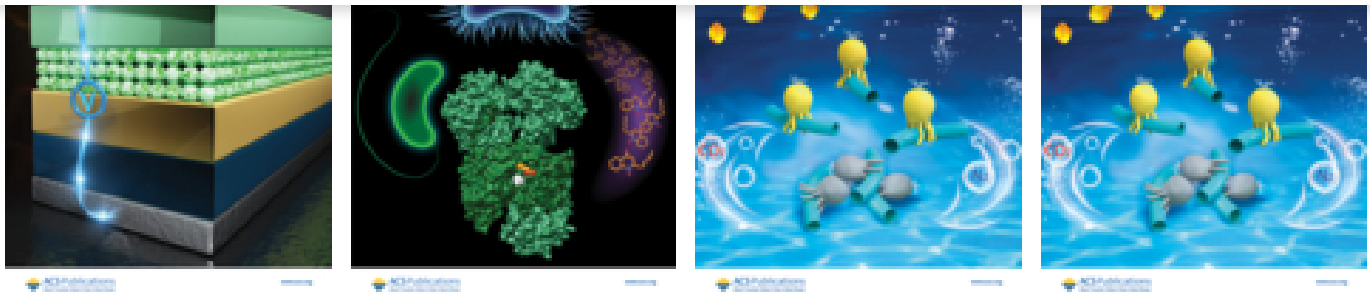

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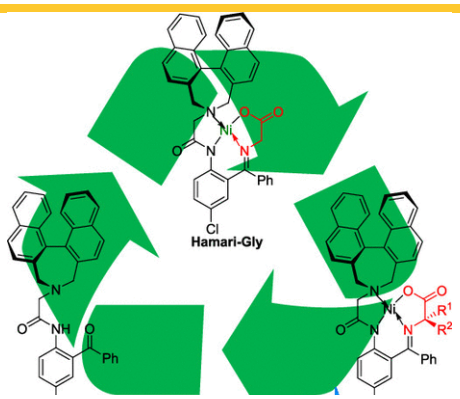

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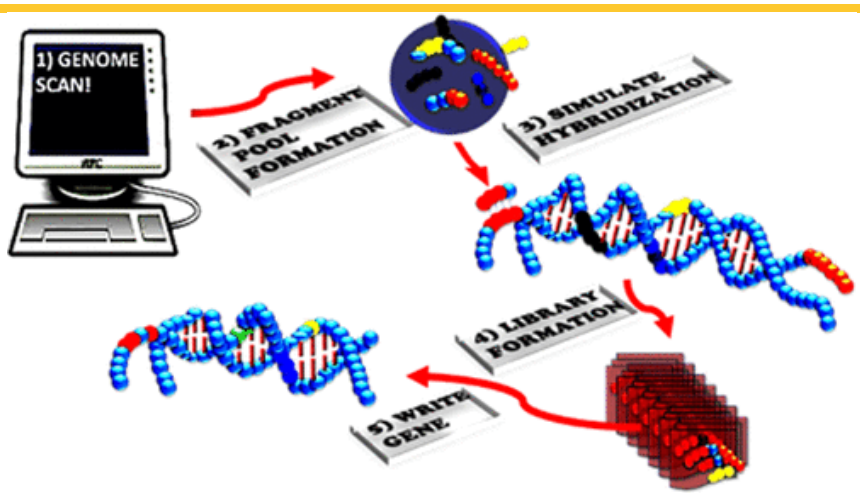

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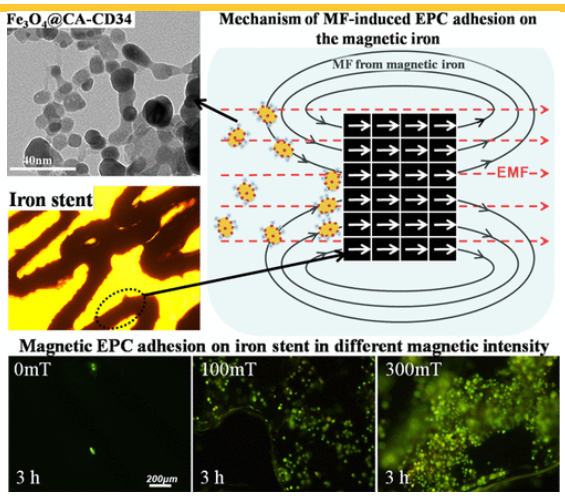

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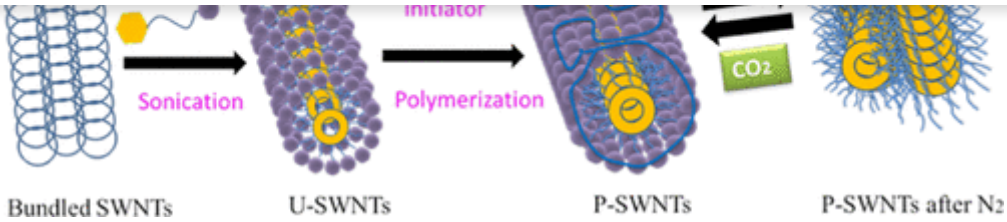

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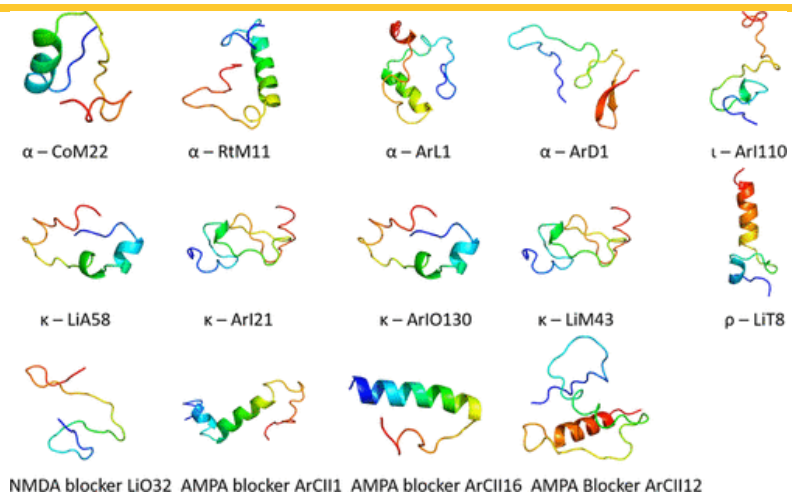

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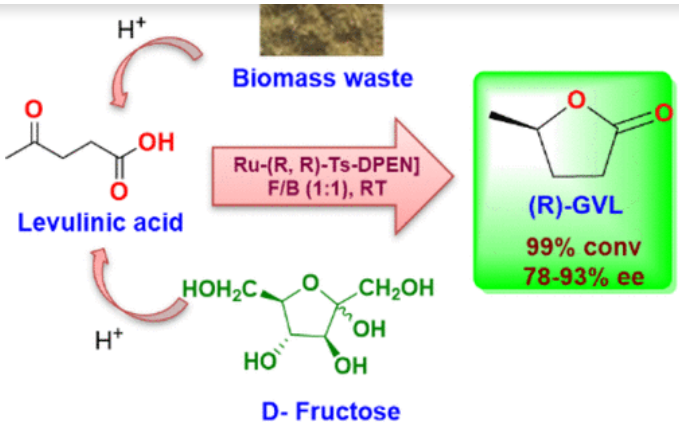

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Selecting Potential Neuronal Drug Leads from Conotoxins of Various Venomous Marine Cone Snails in Bali, Indonesia

Anak A. R. Sudewi,[†,¶] Ni M. Susilawathi,[†] Bayu K. Mahardika,[‡] Agung N. Mahendra,[§] Made Pharmawati,[∥] Mark A. Phuong,[⊥] and Gusti N. Mahardika*[,‡,#]

[†]Neurology Department of the Faculty of Medicine and [§]Pharmacology Department of the Faculty of Medicine, Udayana University, Jl. Sudirman, Denpasar 80226, Bali, Indonesia

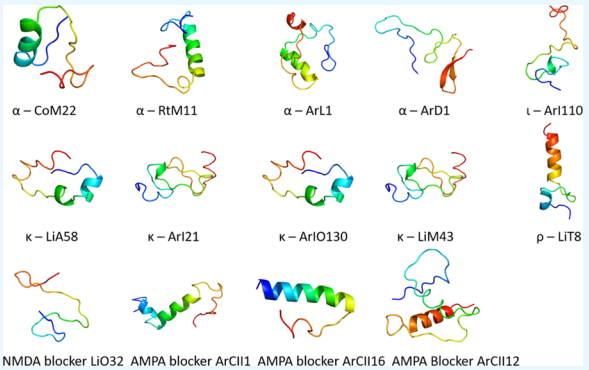
[‡]The Animal Biomedical and Molecular Biology Laboratory, Udayana University of Bali, Jl. Sesetan-Markisa 6, Denpasar 80223, Bali, Indonesia

[∥]Faculty of Mathematic and Natural Sciences, Udayana University of Bali, Kampus Bukit Jimbaran, Badung 80361, Bali, Indonesia

[⊥]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, Los Angeles 90095, California, United States

[#]The Indonesian Biodiversity Research Center, Jl. Sudirman, Denpasar 80225, Bali, Indonesia

ABSTRACT: Many conotoxins, natural peptides of marine cone snails, have been identified to target neurons. Here, we provide data on pharmacological families of the conotoxins of 11 species of cone snails collected in Bali. The identified definitive pharmacological families possibly targeting neuronal tissues were α (alpha), ι (iota), κ (kappa), and ρ (rho). These classes shall target nicotinic acetylcholine receptors, voltage-gated Na channels, voltage-gated K channels, and α1-adrenoceptors, respectively. The VI/VII-O3 conotoxins might be prospected as an inhibitor of *N*-methyl-D-aspartate. Con-ikot-ikot could be applied as an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor blocker medicine. The definitive pharmacology classes of conotoxins as well as those yet to be elucidated need to be further established and verified.



INTRODUCTION

Conotoxins are natural peptide components of the venoms of marine cone snails of the *Conus* genus, which are remarkably diverse in terms of structure and function.[1] Many conotoxins have been identified to have neuronal targets. The snails capture prey using a diverse array of toxins, mainly neurotoxins,[2] although a few can be cardioactive.[3] Unique potency and selectivity profiles for a range of neuronal targets have made several conotoxins valuable as drug leads of analgesics, neuropsychiatric, and other neuropharmacologicals.[1,4,5] Neurologic application for pain reduction is the most common ongoing approved, preclinical, or clinical trial of conotoxins or their derivates. The ω-MVIIA conotoxin, marketed as ziconitide, was approved by the U.S. Food and Drug Administration in 2004 to treat chronic pain.[2,6] The χ-MrIA, ω-CVID, contulakin-G, α-Vc1.1, and μO-MrVIB conotoxins were in preclinical and clinical trials to cure neuropathic pain or neuroprotection.[7−12] Only one conotoxin, namely, κ-PVIIA, was in the preclinical phase to treat non-neurological complaints. This conotoxin was on trial to cure myocardial infarction.[13]

The classical organization of a conopeptide precursor is ER signal sequence, N-proregion, mature peptide region, and C-terminal proregions.[1,14] The precursor protein is then cleaved by proteases, generating active conotoxins that form key constituents of the venom.[15] The conotoxins are classified according to gene superfamily, cysteine framework, and pharmacological class.[14,16,17] The gene superfamily is based on the signal sequences, the cysteine framework is determined from the number of cysteine residues with estimated disulfide bonds of the mature peptide, and the pharmacological class is based on established pharmacological proof of certain conotoxins.[1] The pharmacological families are annotated with the Greek letters of α (alpha), γ (gamma), δ (delta), ε (epsilon), ι (iota), κ (kappa), μ (mu), ρ (rho), σ (sigma), τ (tau), χ (chi), and ω (omega).[18−29] Statistics of gene superfamily, cysteine framework, and pharmacological family[16,17] provide an estimate for the pharmacological family of newly discovered conotoxins. However, some may occur in an unknown gene superfamily and cysteine framework. The discovery of divergent and unassigned gene superfamilies is challenging in the determination of pharmacological families.

The transcriptomes of various species of marine cone snails found in Bali, Indonesia, have been described.[30] The evolution

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and systematic biology points of view of the findings have been published.[30−32] However, the pharmacological actions of discovered conotoxins have not been reported. Here, we provide data on the pharmacological families of the species published previously to provide insights into which to select and further study for bioprospecting potential drug leads from Indonesian marine snails.

MATERIALS AND METHODS

Published prosequences of conotoxins from 11 species of snails found in Bali[30] were analyzed. The species were *Conus arenatus, Conus coronatus, Conus ebraeus, Conus imperialis, Conus lividus, Conus marmoreus, Conus quercinus, Conus rattus, Conus sponsalis, Conus varius,* and *Conus virgo*.[30] The ER signal sequence, N-proregion, mature peptide region, and C-terminal proregions, as well as cysteine framework and gene superfamily, were identified using ConoServer (http://www.conoserver.org/).[1,16,17] The pharmacological families were predicted using statistics on pharmacological families available on the server based on previously published cysteine frameworks and gene superfamilies.[1] The data were further clustered as definitive pharmacological family (DPF), definitive combined pharmacological family (DCPF), nonalphabetical pharmacological family (NAPF), divergent gene family (DGF), newly proposed gene family,[30] novel gene family and cysteine framework combination (NGFCFC), unassigned gene family (UGF) SF,[1] and unknown conotoxin. The DCPF cluster was further assembled based on the cysteine framework and gene family. Protein modeling, prediction, and analysis of the representative mature toxin sequences were conducted using the Phyre2 server (http://www.sbg.bio.ic.ac.uk).[33]

RESULTS

The pharmacological classes of conotoxins discovered in various venomous marine cone snails in Bali, Indonesia, based on pharmacological family and cysteine framework, are listed in Table 1. Meanwhile, Table 2 shows the detailed list of conotoxin peptides clustered in DCPF and NAPF identified in various species. The result shows that the DPFs, listed from the most frequent, were α, κ, ι, and ρ with 66, 54, 37, and 4 conotoxin sequences, respectively. The total number of conotoxins annotatable to definitive families was 161. There were 400, 121, 119, 71, 63, 35, 12, and 3 conotoxins annotated to DCPF as follows, listed in the order of frequency: $\delta/\gamma/\kappa/\mu/\omega$, $\alpha/\iota/\kappa/\mu$, $\varepsilon/\mu/\tau$, γ/ω, α/ρ, α/κ, α/σ, and $\alpha/\kappa/\mu$, respectively. A total of 824 conotoxins were assigned to these clusters. The number of conotoxins in the clusters of NAPFs of con-ikot-ikot, conkunitzin, conodipin, conoporin, and conophysin was 101, 58, 30, 28, and 20, respectively. The other 90 conotoxins were clustered into DGF. NGFCFC consists of 315 sequences. UGFs of SF-04, mi1, and mi2 conotoxins were 50, while 319 were ungrouped conotoxins with certain gene families with poor cysteine residue. Each cluster was further subclustered based on the cysteine framework and gene family.

The conotoxins of NGFCFC are presented in Table 3. The most common combination was IX-P, followed by XV-V, XI-I3, XV-N, VI/VII-O3, and XXII-E. The combinations of conotoxins in the clusters of nonalphabetical families with a certain or novel cysteine framework (NCF) were V, XXI, and NCF—con-ikot-ikot; IX, XII, XIV, and NCF—conkunitzin; VIII and NCF—conodipin; NCF—conoporin; as well as

Table 1. Pharmacological Classes of Conotoxins Discovered in Various Venomous Marine Cone Snails in Bali, Indonesia, Based on Pharmacological Family and Cysteine Framework[a]

	DPF						divergent		NGPF						UGFSF		
species	A	I	K	R	DCPF	NAPF	Div1	Div2	NPGF1	NPGF2	NPGF3	NPGF4	NPGF5	NGFCFC	SF-04	SF-mi1	SF-mi2	UKC
C. arenatus	9	20	7	0	128	45	0	1	2	0	1	1	2	48	2	2	0	58
C. coronatus	11	2	9	0	158	44	4	0	0	0	0	13	1	30	1	10	0	48
C. ebraeus	10	0	0	0	13	11	4	0	2	1	0	0	0	12	0	2	2	18
C. imperialis	1	2	2	0	29	3	2	0	0	0	2	3	0	19	0	0	0	7
C. lividus	14	0	8	1	92	27	0	0	5	1	2	2	0	47	2	0	1	42
C. marmoreus	0	1	0	3	19	1	0	0	0	0	0	0	0	5	0	0	0	11
C. quercinus	2	0	5	0	42	7	0	0	2	2	1	0	0	22	1	0	4	9
C. rattus	11	0	3	0	20	34	1	1	4	0	0	2	2	10	0	2	3	9
C. sponsalis	5	10	2	0	199	31	5	0	3	2	1	0	0	60	2	7	3	71
C. varius	3	2	3	0	71	25	1	0	0	1	0	8	0	48	0	0	3	33
C. virgo	0	0	15	0	53	9	0	0	3	1	1	0	0	14	1	1	1	13
total	66	37	54	4	824	237	17	2	21	8	8	29	5	315	9	24	17	319

[a]DPF = definitive pharmacological family; DCPF = definitive combined pharmacological family; NAPF = nonalphabetical pharmacological family; DGF = divergent gene family; Div1 = DivMKFPLLFISL; Div2 = DivMKVAVVLLVS; NPGF = newly proposed gene family (NPGF); NPGF1 = MEFRR; NPGF2 = MKFLL; NPGF3 = MKISL; NPGF4 = MMLFM; NPGF5 = MRFYM;[30] NGFCFC = novel gene family and cysteine framework combination; UGF = unassigned gene family;[1] UKC = unknown conotoxin.

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Table 2. Number of Conotoxin Peptides Clustered in DCPF and NAPF Identified in Various Species Marine Cone Snails Found in Bali, Indonesia[a]

	DCPF								NAPF				
species	$\alpha/\iota/\kappa/\mu$	$\alpha/\kappa/\mu$	α/κ	α/ρ	α/σ	$\delta/\gamma/\kappa/\mu/\omega$	$\varepsilon/\mu/\tau$	γ/ω	CII	CKNZ	CNDP	CNPR	CNPS
C. arenatus	2	11	3	14	4	80	7	7	26	13	4	0	2
C. coronatus	45	13	0	9	0	67	12	12	21	10	3	8	2
C. ebraeus	4	0	0	0	0	8	0	1	1	2	5	1	2
C. imperialism	6	0	0	1	1	9	7	5	0	0	0	2	1
C. lividus	20	2	0	16	0	31	16	7	14	11	0	2	0
C. marmoreus	6	0	0	0	1	1	9	2	0	1	0	0	0
C. quercinus	8	1	0	8	0	16	1	8	4	0	2	0	1
C. rattus	2	2	0	0	1	11	3	1	17	6	2	8	1
C. sponsalis	14	5	0	3	0	125	32	20	9	12	7	0	3
C. varius	12	1	0	7	5	24	19	3	8	2	4	7	4
C. virgo	2	0	0	5	0	28	13	5	1	1	3	0	4
total	121	35	3	63	12	400	119	71	101	58	30	28	20

[a]CII = con-ikot-ikot; CKNZ = conkunitzin; CNDP = conodipin; CNPR = conoporin; CNPS = conophysin.

Table 3. Number of Conotoxin Peptides of Novel Pharmacological Family with Definitive Cysteine Framework and Gene Superfamilies Identified in Each Species[a]

CF and GF combinations		species											
CF	GF	*C. arenatus*	*C. coronatus*	*C. ebraeus*	*C. imperialis*	*C. lividus*	*C. marmoreus*	*C. quercinus*	*C. rattus*	*C. sponsalis*	*C. varius*	*C. virgo*	total
IX	P	17	9	1	11	4	0	0	2	16	12	0	72
XV	V	4	3	0	0	19	0	6	0	2	0	3	37
XI	I3	3	2	2	0	0	0	0	0	1	16	0	24
XV	N	4	3	2	1	4	0	1	2	1	0	6	24
XXII	E	4	3	0	1	2	3	3	1	2	2	0	21
XIX	N	0	2	0	0	0	0	0	3	4	8	0	17
XII	I4	2	8	0	0	1	0	0	0	3	0	0	14
XV	O2	2	0	0	0	0	0	0	1	10	1	0	14
XVII	Y	2	0	1	0	2	0	1	0	5	0	2	13
XII	U	0	0	0	0	2	0	5	0	4	0	1	12
VI/VII	V	0	0	1	0	9	0	0	0	1	0	0	11
VI/VII	U	0	1	0	0	0	0	0	1	5	0	0	7
XVI	Q	0	0	0	0	4	0	3	0	0	0	0	7
XVI	T	7	0	0	0	0	0	0	0	0	0	0	7
IX	M	0	0	3	1	0	0	0	0	0	2	0	6
XVI	M	0	0	0	0	0	0	0	0	0	6	0	6
XXIII	K	0	0	0	5	0	0	0	0	0	1	0	6
XIV	T	0	0	0	0	0	0	0	0	4	0	0	4
XVIII	I2	0	0	0	0	0	0	1	0	0	0	1	2
XVIII	O1	2	0	0	0	0	0	0	0	0	0	0	2

[a]Listed from the most frequent; CF = cysteine framework; GF = gene superfamily; single peptide combinations were not shown. These were XX—D, IX—E, XII—I2, VI/VII—I4, IX—N, I—O1, XII—O1, XVIII—O1, XIV—O3, III—Q.

NCF—conophysin. The divergent DivMKFPLLFISL occurred with the cysteine framework VI/VIII, while DivMKVAVVLLVS occurred with XIV. The new proposed gene families and cysteine framework combinations were IX—MEFRR, VI/VII—MKFLL, IX and VI/VII—MKISL, VIII and XIV—MMLFM, as well as VI/VII—MRFYM. The UGFs are presented in combinations of XIII—SF-04, XIII—SF-mi1, and NCF—SF-mi2.

We clustered the mature toxins of the combined families $\alpha/\iota/\kappa/\mu$, α/κ, α/ρ, α/σ, $\delta/\gamma/\kappa/\mu/\omega$, $\varepsilon/\mu/\tau$, and γ/ω and conducted protein prediction with some representatives of each group. The PDB data for >50% identity and homology show that only γ/ω representative resulted in 78.8% homology and 60% identity in the established pharmacological class. Those with cysteine framework VI/VII and gene superfamily O2 are close to ω-conotoxin MVIIV. The other representatives could not be estimated in any established pharmacological class (not shown).

The identified DPFs possibly targeting neuronal tissues were α (alpha), ι (iota), κ (kappa), and ρ (rho), while those of other groups were the VI/VII-O3 conotoxins as an inhibitor of *N*-methyl-D-aspartate (NMDA) and the con-ikot-ikot as an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor blocker. The representatives of the conotoxins possibly targeting neuronal tissues found in Bali,Indonesia, are listed in Table 4, which were selected based on cysteine framework and/or gene superfamily. PDB search of some conotoxins found no template, which has high percentage of

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Table 4. Representatives of the Conotoxins Possibly Targeting Neuronal Tissues Found in Bali, Indonesia, and the Result of PDB Search

conotoxin name[a]	species	cysteine framework	gene superfamily	pharmacological class	PDB search (% confidence/PID)[b]
CoM22	*C. coronatus*	I	M	α	none
RtM11	*C. rattus*	II	M	α	metallothionein mt_nc (65.3/67)
ArL1	*C. arenatus*	XIV	L	α	none
ArD1	*C. arenatus*	XX	D	α	αd-conotoxin gexxa (99.9/49)
ArI110	*C. arenatus*	XI	I1	ι	conotoxin g117 (85.9/41)
LiA58	*C. lividus*	VI/VII	A	κ	α-conotoxin vc1a (85.4/60)
ArI21	*C. arenatus*	XI	I2	κ	none
ArO130	*C. arenatus*	XI	O1	κ	aptotoxin vii (79.77/88)
LiM43	*C. lividus*	XXVII	M	κ	none
LiT8	*C. lividus*	I	T	ρ	none
LiO32	*C. lividus*	VI/VII	O3	NMDA blocker	none
ArCII1	*C. arenatus*	V	UK	CII/AMPA blocker	defensin, α (47.5/55)
ArCII16	*C. arenatus*	XXI	UK	CII/AMPA blocker	con-ikot-ikot (100/38)
ArCII12	*C. arenatus*	UK	UK	CII/AMPA blocker	none

[a]The first two characters are abbreviated species name, followed by gene superfamily and the number of the sequence in the database as previously published;[30] PID = percentage of identity; NMDA blocker = putative NMDA blocker; AMPA blocker = putative AMPA blocker; UK = unknown; CII = con-ikot-ikot. [b]Only search results of confidence level of >40% are shown; PDB search was conducted in website http://www.sbg.bio.ic.ac.uk/?phyre2/html/page.cgi?id=index.[33]

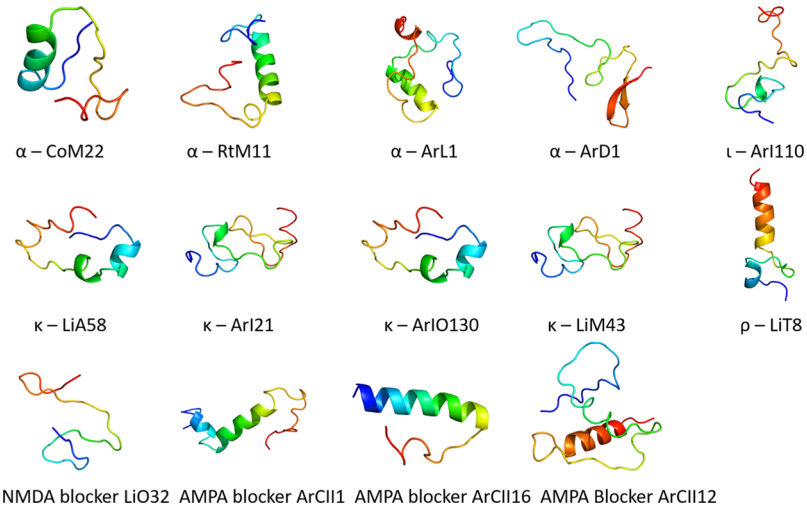

Figure 1. Final model of the mature peptide region of the representatives of the conotoxins possibly targeting neuronal tissues found in Bali,Indonesia. The peptide names are the same as described in Table 4. The N-proregion was included in the modeling for the mature peptides of less than 30 residues. Modeling was conducted at http://www.sbg.bio.ic.ac.uk/?phyre2/html/page.cgi?id=index.[33]

confidence, while others found template database with confidence levels of 47.5−100%, with percentages of identity of 38.5−88%. The results of protein modeling, prediction, and analysis of the representative of mature toxin sequences are presented in Figure 1. The figure explains that some peptides consist of random coil and α helix, while LiO32 is merely random coiled. Sequences of all species are available at dryad (doi:10.5061/dryad.1v5d3).[30] The cDNAs of the representatives of the conotoxins possibly targeting neuronal tissues described in this article are available in GenBank with Acc. no. MN580095-MN580108.

DISCUSSION

As expected, the total number of conotoxins identified in our study was large or 1996. Such an abundance is very common in marine cone snails. Each sea snail species typically possesses an average of 100−200 conotoxins,[34] which are employed to paralyze prey.[35] Of the total, only 161 (8.1%) can be assigned to an established pharmacological family. Another 824 (41.3%) are assigned to possible combinations of established families. The number of conotoxins in the clusters of nonalphabetical families of con-ikot-ikot, conkunitzin, conodipin, conoporin, and conophysin was 237 (11.9%). The other 90 conotoxins were clustered into divergent groups and 71 to new proposed families.

The last group was further classified as unknown pharmacological class of conotoxins with definitive gene family and cysteine framework, nonalphabetical family, divergent, variant MEFR, variant MKFL, variant MMLFM, variant MRFYM, SF-04, and SF-mi1, as well as SF-mi2 gene families with definitive or NCFs. NGFCFC consists of 315 sequences. UGFs of SF-04, mi1, and mi2 conotoxins were 50, while 319 were ungrouped conotoxins with certain gene families but NCF.

The identified DPFs were α, κ, ι, and ρ, while one group is close to ω-conotoxin. Conotoxins assigned to these families can be explored further as drug leads for neurological use. Pharmacological families and the pharmacological actions of

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conotoxins assigned to them have been identified as stimulating or blocking receptors, ion channels, or transporters. The α family works at nicotinic acetylcholine receptors (nAChRs),[18] the ι family at voltage-gated Na channels,[22] the κ family at voltage-gated K channels,[23] and ρ family at α1-adrenoceptors.[26] The ω family is a voltage-gated Ca channel blocker,[29] which might be useful for cardiovascular disorder.[36]

To further predict the pharmacological action of uncertain or unknown conotoxins, we clustered the data based on gene superfamily and cysteine framework. The five most common combination of conotoxins with DCPF was IX—P, followed XV—V, XI—I3, XV—N, and XXII—E. The combination of IX—P has been described in TxIXA as a prototype of P-superfamily conotoxin,[37] which causes "spasmodic" symptoms on intracerebral injection in mice. The known O3-gene superfamily conotoxins have the VI/VII cysteine framework.[38] The only characterized O3 superfamily is "bromosleeper", which causes lethargy, drowsiness, and sleep in mice.[39] This conotoxin is thought to be similar to conantokin, an inhibitor of NMDA receptors.[1] The pharmacological actions of other combinations are yet to be described.

Assembling the conotoxins annotated as belonging to nonalphabetical gene superfamilies, we listed the combination of gene superfamilies with an established framework or NCF as conkunitzin combined with the frameworks IX, XII, XIV, and NCF; con-ikot-ikot with the frameworks V, XXI, and NCF; and SF-04 and SF-mi1 with the framework XIII. Meanwhile, SF-mi2, conopidin, conophysin, and conoporin have novel frameworks. Conkunitzin of conus snails displayed high sequence similarity to the kunitz domain of dendrotoxin peptides, which are K^+ channel blockers found in black mamba venom.[40] Conkunitzins in our data are associated with the frameworks IX, XII, XIV, and NCF. There has been no information published on the cysteine framework of con-kunitzin. Our con-ikot-ikot data show cysteine frameworks V, XXI, and NCF. The published con-ikot-ikot cysteine framework is XXI.[41−44] The combination with the framework V is also not new.[45] Con-ikot-ikot has an effect on AMPA receptors, inhibiting channel desensitization.[43] Conodipine is a unique conotoxin, originally found in *Conus magus*, with two polypeptide chains.[46] It has phospholipase-A2 activity like animal venoms, with potent neurotoxicity to mammalian tissues and bacteria.[1] Conophysin displayed a primary structure and cysteine framework typical of the neurophysin peptide family.[47] The role of this peptide in venom is not clear. Conophysins and conopressins are thought of as products of the same peptide precursor. Conopressin, which is not identified in our dataset, can produce a "scratching effect" following intracerebral injection in mice.[48] Conoporin belongs to conoprotein, the high-molecular-weight component of *Conus* spp. venom, which may be involved in conotoxin maturation.[1]

We identified peptides that belong to SF-04 and SF-mi1 gene superfamilies with the XIII framework. SF-mi1, mi2, and mi3 are temporarily annotated as undescribed or belonging to superfamilies with the frameworks VI/VII and XIII.[1] Our SF-mi1 sequence data show framework XIII; however, the SF-mi3 data show an undescribed cysteine framework. The pharmacological effect of these configurations has yet to be explored.

We also identified divergent and various newly proposed superfamilies combined with various cysteine frameworks. The divergent superfamily occurs in combination with the frameworks VI/VII and XIV. The various unassigned superfamilies occur in combination with IX—MEFR, NCF—MEFR, VI/VII—MKFL, IX—MMLFM, VIII—MMLFM, XIV—MMLFM, and VI/VII—MRFYM. MEFR, MKFL, MMLFM, and MRFYM were proposed to be reclassified or assigned as new superfamilies to "(1) novel groupings of conotoxin gene superfamilies and (2) groups of conopeptides with similarity to previously characterized conotoxins but were not given a formal classification" with a cutoff of 70% signal sequence identity in the established gene superfamilies.[30] The pharmacological actions of these conotoxins are yet to be elucidated.

The number of potential neuronal drugs from conotoxins discovered in various venomous marine cone snails in Bali, Indonesia, is huge. The marine cone snails seem to be well equipped with mainly neurotoxic venoms, but also a very few cardiotoxic venoms, to immobilize the prey. A small portion of the conotoxins from Bali could be annotated to specific pharmacological classifications, which could be the first stepping stone to develop neurological drugs. A much larger portion has yet to be assigned, but the pharmacological action can be predicted based on the published literature. PDB searches of mature toxins with an undescribed cysteine framework or novel framework pattern combinations should give an insight into their possible pharmacological actions.

Conotoxins that work on nAChRs might be developed as antidepressants. nAChR modulation is an area with significant promise for future antidepressant drug development.[49] Furthermore, this group of cholinergic receptors has been recently known to be involved in the nicotine reward effect.[50] Because ACh is known as a dopamine release regulator, α-conotoxins may potentially exhibit a salutary effect in psychoses and Tourette's syndrome treatment.[51] We assigned 27 peptides from six species to the α pharmacological class, which acts upon this receptor. Another 14 peptides are annotated to ι (iota), with molecular targets of voltage-gated Na channels.[22] It is intriguing to discuss the therapeutic potentials of an ι-conotoxin found in our current study. This conotoxin has been revealed to possess agonistic activity against three sodium channels, namely, Nav1.2, Nav1.6, and Nav1.7.[52] Because these sodium channels are implicated in many diseases such as migraine, epilepsy, autism, ataxia, pain disorders, paroxysmal itch, and anosmia,[53] we can utilize this group as a chemical tool to support the research on developing novel drugs against these pathological conditions. The ι family could be developed into drugs targeting chronic pain, epilepsy, and cardiac arrhythmias.[54] The κ targets the voltage-gated K channels,[23] which might be beneficial to be developed as new drugs for cancer; autoimmune diseases; and metabolic, neurological, and cardiovascular disorders.[55] The ρ class was identified in three peptides from two species. This class specifically targets the α1-adrenoceptors,[26] which play a key role in the modulation of sympathetic nervous system activity, as well as being a site of action for many therapeutic agents.[56]

The NMDA blocker framework VI/VII—O3 was identified in this study. This could have the potential to treat some neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis.[57,58] Con-ikot-ikot was identified in 10 species (but not *C. imperialis*), and the number of peptides was 101. This class is reported to exhibit an effect on AMPA receptors, inhibiting channel desensitization.[43] This can be a potential antiepileptic drug.[59]

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PDB search of conotoxins shows that the representatives of the conotoxins possibly targeting neuronal tissues found in this study are novel. Some have no confidence template in the database, while others have percentages of identity of 38.5−88%. The variety of secondary structures of random coil and α helix might explain the mechanism of action and protein targets.

The pharmacological effect of the definitive as well as uncertain pharmacological classes of conotoxins should be determined and proven. Being peptides, the production and purification of conotoxins should be straightforward. For example, conotoxins can be produced using recombinant DNA technology[60−62] or synthetic peptides.[63−65] Simple clustering based on suspected pharmacological families or gene families and cysteine framework conducted in our study should be a simple approach to select conotoxin(s) of interest.

CONCLUSIONS

The identified DPFs possibly targeting neuronal tissues were α (alpha), ι (iota), κ (kappa), and ρ (rho) as well as NMDA and AMPA receptor blocker. The definitive pharmacology classes of conotoxins as well as those yet to be elucidated need to be further established and verified.

AUTHOR INFORMATION

Corresponding Author

*E-mail: gnmahardika@unud.ac.id.

ORCID

Gusti N. Mahardika: 0000-0001-5525-0793

Present Address

¶The Rector of Udayana University of Bali, Udayana University, Kampus Bukit Jimbaran, Badung, Bali, Indonesia.

Author Contributions

Conceptualization, A.A.R.S., M.A.P., and G.N.M.; data curation, N.M.S., B.K.M., A.N.M., and M.P.; original draft preparation, B.K.M.; writing—review & editing, A.A.R.S. and G.N.M.

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Notes

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ABBREVIATIONS

Ach, acetylcholine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; DCPF, definitive combined pharmacological family; DPF, definitive pharmacological family; nAChRs, nicotinic acetylcholine receptors; NAPF, non-alphabetical pharmacological family; NCF, novel cysteine framework; NGFCFC, novel gene family and cysteine framework combination; NMDA, *N*-methyl-D-aspartate; PDB, Protein Data Base; UGF, unassigned gene families

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