

Selective Toxicity of the Anthelmintic Emodepside Revealed by Heterologous  
Expression of Human KCNMA1 in *Caenorhabditis elegans*

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Molecular Pharmacology

**Supplementary Information S3.**

**Figure S3. Alignment of the amino acid sequences of *C. elegans* SLO-1a and human KCNMA1 (isoform b) using BLAST (NCBI; method compositional matrix adjust).**

The alignment shows 55% identity and 69% similarity/positives between the two sequences. Identical amino acids are indicated by their letter symbols and amino acids where side chains have similar physicochemical properties are indicated by "+". Gaps are indicated by "---" symbols and were introduced by the program algorithm (7% gaps) to enable analysis of the most conserved parts of the sequences. The sequences have been annotated with the following features; 'S0' to 'S6' are the transmembrane domains; 'voltage sensor'; 'P loop' is the pore domain; 'RCK1' and 'RCK2' are two domains that regulate the conductance of the potassium channel; 'Linker' is the domain between 'S6' and 'RCK1' and the length of this sequence alters the gating of the channel (Jiang Y, Lee A, Chen J, 2002: Crystal structure and mechanism of a calcium-gated potassium channel. Nature 417, 515-522); 'Calcium bowl' harbours calcium binding sites. The *slo-1* sequence has been annotated with the

location of the mutations which have been described to confer emodepside resistance (*js379*, *pd17*, *pd19*, *pd23*, *pd24*; Guest M, Bull K, Walker RJ, Amliwala K, O'Connor V, Harder A, Holden-Dye L, Hopper NA. 2007. The calcium-activated potassium channel, SLO-1, is required for the action of the novel cyclo-octadepsipeptide anthelmintic, emodepside, in *Caenorhabditis elegans*. Int J Parasitol 37:1577-88).

SLO-1	6	SPSQSKGFNQPYGYPMNCNLSRVFMEMTEEDRKCLEERKYWCFLSSITTFCASMILVVI	65
		S S S ++P M+ + V ME+ + R +R +W FL SS+ TF + +++++	
KCNMA1	54	SSSSSSSVHEIPVNT-terminalTMEVPCDSRG---QRMWWAFLASSMVTSS0GGLFIILL	107
SLO-1	66	WRVVTHL---CCQRREKEFVEEPIPAPEAVQINMNGSKHAPSETDPFLKQOE--KHLGWM	120
		WR + +L CC K EA +IN NGS A P +++E +GWM	
KCNMA1	108	WRTLKYLWTVCCHCYGGK-----TKEAQKIN-NGSSQADGTLKPVDEKEEAVAEVGWM	159
		Ethanol response	
		<b>S1</b>	
SLO-1	121	TEAKDWAGELISGQSLTGRFVLVLFVLSIGSLIIYFYDASFQNFQVETCIPWQDSPSQ	180
		T KDWAG +IS Q+LTGR LV+LVF LSIG+L+IYF D+S +E+C + + Q	
KCNMA1	160	TSVKDWAGVMISAQTLTGRVVLVLFVLSIGALVIYFIDSSNP---IESCQNFYKDFTLQ	216
SLO-1	181	IDLGFNIFFLVYFFIRFIAASDKVWFLLEMYSWIDFFTIPPSFVAIYLQRNWLGFRLRA	240
		ID+ FN+FFL+YF +RFIAA+DK+WF LE+ S +DFFT+PP FV++YL R+WLG RFLRA	
KCNMA1	217	IDMAFNVFLLYFGLRFIAANDKLWFVLEVNSVVDFFTVPVVFVSVYLNRSWLGLRFLRA	276
		<b>Voltage sensor</b>	
SLO-1	241	LRLMTVPDILQYLNILKTSSSIRLTQLVTVIFVAVCLTGAGLVHLENSGDFFKGFINPHR	300
		LRL+ +ILO+LNILKTS+SI+L L++IF++ LT AG +HL+ENSGD ++ F N	
KCNMA1	277	LRLIQFSEILQFLNILKTSNSIKLVNLLSIFISTWLTAAAGFIHLVENSGDPWENFQNNQA	336
		js379/STOP	
SLO-1	301	ITYADSVYFVLVTMSTVGYGDIYCTTLCGRLFMIFFILFGLAMFASYVPEIADLIGNRQK	360
		+TY + VY ++VTMSTVGYGD+Y T GRLFM+FFIL GLAMFASYVPEI +LIGNR+K	
KCNMA1	337	LTYWECVYLLMVTMSTVGYGDVYAKTTLGRLFMVFILGGLAMFASYVPEIIEELIGNRKK	396
		<b>Linker</b>	
SLO-1	361	YGGYKGEHGKHHIVVCGHITYDSVSHFLQDFLHEDRDDVDVEVVFHLRVVPDLELEGLF	420
		YGG Y G+KHIVVCGHIT +SVS+FL+DFLH+DRDDV+VE+VFLH + P+LELE LF	
KCNMA1	397	YGGYSYAVSGRKHIVVCGHITLESVSNFLKDFLHKDRDDVNVEIVFLHNISPNELEALE	456
		Ca <sup>2+</sup> binding sites (high affinity)	
		pd19/A	
SLO-1	421	KRHFTKVEFFTGTVMDSLDSRVKIGDADACLVLANKYSTNPDAEDAANIMRVISIKNY	480
		KRHFT+VEF+ G+V++ DL+RVKI ADAACL+LANKY +PDAEDA+NIMRVISIKNY	
KCNMA1	457	KRHFTQVEFYQGSVLNPHDLARVKIESADACLILANKYCADPDAEDASNIMRVISIKNYH	516
SLO-1	481	SDIRVIVQLMQYHNKAYLLNIPSWDWKRGGDVICLAEKLGFIQQSCLAPGFSTMMANLF	540
		IR+I Q++QYHNKA+LLNIPSW+WK GDD ICLAEKLGFIQQSCLA G STM+ANLF	
KCNMA1	517	PKIRIITQMLQYHNKAHLLNIPSWNWKEGDDAICLAEKLGFIQQSCLAQGLSTMLANLF	576
SLO-1	541	AMRSF-KTSPHTPLWLDYLRGAGMEMYTELSPSFANMSFPEAANLLFNRLGLLLLAIE	599
		+MRSF K T W YL G EMYTE LS +F +SFP L F +L LL++AIE	
KCNMA1	577	SMRSFIKIEEDT--WQKYYLEGVSNEMYTEYLSSAFVGLSFPTVCELFCVKKLLMIAIE	634
		Ca <sup>2+</sup> binding site (low affinity)	
		pd24/STOP	

SLO-1 600 LKDEENKECNIAINPGPHIVI QPQTQGFFIAQSADEVKRAFFWCKQCHDDIKDVSLIKK 659  
 K N+E I INPG H+ IQ T GFFIA A EVKRAFF+CK CHDDI D IKK  
 KCNMA1 635 YK-SANRESRILINPGNHLKI QEGTLGFFIASDAKEVKRAFFYCKACHDDITDPKRIKK 693

SLO-1 660 KCKNLALFRRNTKHSTAARDYSDFDALFYQNDARATDVLQQFQPQAPAGPMGHLGQQVQL 719  
 CK L+ QP  
 KCNMA1 694 GCKR-----LEDEQPST----- 705

**RCK2**

SLO-1 720 RMINQQRPSGGRNSMSIPP-----DGRGVDFSKDFEQFQDM-KYDSTGMFHWCP SRN 773  
 + +++ +GG RNS + P D + + + ++ KYDSTGMFHW C +  
 KCNMA1 706 -LSPKKKQRNGGMRNSPNTSPKLMRHDFLLIPGNDQIDNMDSNVKKYDSTGMFHWCAPKE 764

SLO-1 774 LEDCVLERHQAAMTVLNGHVVVCLFADQDSPLIGLRNFIMPLRSSNFHYHELKHVVIVGD 833  
 +E +L R +AAMTVL+GHVVVC+F D S LIGLRN +MPLR+SNFHYHELKH+V VG  
 KCNMA1 765 IEKVILTRSEAAMTVLSGHVVVCIFGDVSSALIGLRNLVMPLRASNPFHYHELKHIVFVGS 824

pd17V

SLO-1 834 LEYLRKEWKTLYNLPKISILNGSPLSRADLRAVNINLCDCMVIISARVPNTEDTTLADKE 893  
 +EYL++EW+TL+N PK+SIL G+PLSRADLRAVNINLCDCMVI+SA N +DT+L DKE  
 KCNMA1 825 IEYLRKEWETLHNFPKVSILPGTPLSRADLRAVNINLCDCMVILSANQNNIDDTSLQDKE 884

SLO-1 894 AILASLNIAKMQFDDTLGFFPMRHQ-----TGDRSPLGSP I--SMQKKGAKFGTNVPMI 945  
 ILASLNIAK+MQFDD++G Q SP SP+ +++ G N+P+I  
 KCNMA1 885 CILASLNIAKMQFDDSIGVLQANSQGFTPPGMDRSSPDNSPVHGMLRQPSITTVGNIPII 944

**“Calcium bowl”**

SLO-1 946 TELVND<sup>SNVQFLDQ</sup><sup>DDDD</sup><sup>PDTELYLTQ</sup>PFACGTAFASVLDLSMSTTYFNDSALTLIRT 1005  
 TELVND+<sup>NVQFLDQ</sup><sup>DDDD</sup><sup>PDTELYLTQ</sup>PFACGTAFASVLDLSMSTTYFNDSALTLIRT  
 KCNMA1 945 TELVND<sup>SNVQFLDQ</sup><sup>DDDD</sup><sup>PDTELYLTQ</sup>PFACGTAFASVLDLSMSTTYFNDSALTLIRT 1004

Ca<sup>2+</sup> binding sites

SLO-1 1006 LVTGGATPELELILAEAGALRGGYSTPETLSNRDRCRQAQISLQDNPNYDGVVHNTTYGAM 1065  
 LVTGGATPELE ++AE LRGYSTP+TL+NRDRCR+AQ++L D P+ + YG +  
 KCNMA1 1005 LVTGGATPELEALIAEENALRGGYSTPQTLANRDRCRVAQLALLDGGPFADLGDGGCYGDL 1064

SLO-1 1066 FTIALRRYQQLCIGLYRLHD---QDNPDMSKRYVITNPPAELRIKNTDYVYVLEQFD 1119  
 F AL+ Y LC G+YRL D KRYVITNPP E + TD ++ L QFD  
 KCNMA1 1065 FCKALKTYNMLCFGIYRLRDAHLSTPSQCTKRYVITNPPYEFELVPTDLIFCLMQFD 1121