Supporting Information for

Ametoctradin is a Potent Q_o Site Inhibitor of the Mitochondrial Respiration Complex III

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CLUSTAL 2.1 Multiple Sequence Alignments

Sequence type explicitly set to Protein

Sequence format is Pearson

Sequence 1: 3CX5_N|PDBID|CHAIN|SEQUENCE 385 aa

Sequence 2: 1PPJ_P|PDBID|CHAIN|SEQUENCE 379 aa

Sequence 3: 1BCC_C|PDBID|CHAIN|SEQUENCE 380 aa

Sequence 4: 1ZRT_P|PDBID|CHAIN|SEQUENCE 437 aa

Sequence 5: 2YIU_D|PDBID|CHAIN|SEQUENCE 440 aa

Start of Pairwise alignments

Aligning...

Sequences (1:2) Aligned. Score: 50.92

Sequences (1:3) Aligned. Score: 51.32

Sequences (1:4) Aligned. Score: 47.01

Sequences (1:5) Aligned. Score: 48.05

Sequences (2:3) Aligned. Score: 74.67

Sequences (2:4) Aligned. Score: 49.08

Sequences (2:5) Aligned. Score: 48.02

Sequences (3:4) Aligned. Score: 46.32

Sequences (3:5) Aligned. Score: 46.58

Sequences (4:5) Aligned. Score: 82.84

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CLUSTAL 2.1 multiple sequence alignment
1PPJ_P|PDBID|CHAIN|SEQUENCE      ---------------MTNIRKSHPLMKIVNNAFIDLPAPSNISSWWNFGS 35
1BCC_C|PDBID|CHAIN|SEQUENCE      --------------MAPNIRKSHPLLKMINNSLIDLPAPSNISAWWNFGS 36
3CX5_N|PDBID|CHAIN|SEQUENCE      ----------------MAFRKSNVYLSLVNSYIIDSPQPSSINYWWNMGS 34
1ZRT_P|PDBID|CHAIN|SEQUENCE      MSGIPHDHYEPKTGIEKWLHDRLPIVGLVYDTIM-IPTPKNLNWWWIWGI 49
2YIU_D|PDBID|CHAIN|SEQUENCE      MAGIPHDHYEPKTGFERWLHRRLPIVSLVYDTLM-IPTPKNLNWWWIWGI 49
                                                  ::     : :: . ::  * *.:. **  *
1PPJ_P|PDBID|CHAIN|SEQUENCE      LLGICLILQILTGLFLAMHYTSDTTTAFSSVTHICRDVNYGWIIRYMHAN 85
1BCC_C|PDBID|CHAIN|SEQUENCE      LLAVCLMTQILTGLLLAMHYTADTSLAFSSVAHTCRNVQYGWLIRNLHAN 86
3CX5_N|PDBID|CHAIN|SEQUENCE      LLGLCLVIQIVTGIFMAMHYSSNIELAFSSVEHIMRDVHNGYILRYLHAN 84
1ZRT_P|PDBID|CHAIN|SEQUENCE      VLAFTLVLQIVTGIVLAMHYTPHVDLAFASVEHIMRDVNGGWAMRYIHAN 99
2YIU_D|PDBID|CHAIN|SEQUENCE      VLAFCLVLQIATGIVLVMHYTPHVDLAFASVEHIMRDVNGGYMLRYLHAN 99
                                 :*.. *: ** **:.:.***:..   **:** *  *:*: *: :* :***
1PPJ_P|PDBID|CHAIN|SEQUENCE      GASMFFICLYMHVGRGLYYGSYTFLET--WNIGVILLLTVMATAFMGYVL 133
1BCC_C|PDBID|CHAIN|SEQUENCE      GASFFFICIFLHIGRGLYYGSYLYKET--WNTGVILLLTLMATAFVGYVL 134
3CX5_N|PDBID|CHAIN|SEQUENCE      GASFFFMVMFMHMAKGLYYGSYRSPRVTLWNVGVIIFILTIATAFLGYCC 134
1ZRT_P|PDBID|CHAIN|SEQUENCE      GASLFFLAVYIHIFRGLYYGSYKAPREITWIVGMVIYLLMMGTAFMGYVL 149
2YIU_D|PDBID|CHAIN|SEQUENCE      GASLFFLAVYIHIFRGLYYGSYKAPREVTWIVGMLIYLMMMGTAFMGYVL 149
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1PPJ_P|PDBID|CHAIN|SEQUENCE      PWGQMSFWGATVITNLLSAIPYIGTNLVEWIWGGFSVDKATLTRFFAFHF 183
1BCC_C|PDBID|CHAIN|SEQUENCE      PWGQMSFWGATVITNLFSAIPYIGHTLVEWAWGGFSVDNPTLTRFFALHF 184
3CX5_N|PDBID|CHAIN|SEQUENCE      VYGQMSHWGATVITNLFSAIPFVGNDIVSWLWGGFSVSNPTIQRFFALHY 184
1ZRT_P|PDBID|CHAIN|SEQUENCE      PWGQMSFWGATVITGLFGAIPGIGPSIQAWLLGGPAVDNATLNRFFSLHY 199
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2YIU_D|PDBID|CHAIN|SEQUENCE      PWGQMSFWGATVITGLFGAIPGVGEAIQTWLLGGPAVDNPTLNRFFSLHY 199
                                  :****.*******.*:.*** :*  :  *  ** :*.:.*: ***::*:
1PPJ_P|PDBID|CHAIN|SEQUENCE      ILPFIIMAIAMVHLLFLHETGSNNPTGISS--------DVDKIPFHPYYT 225
1BCC_C|PDBID|CHAIN|SEQUENCE      LLPFAIAGITIIHLTFLHESGSNNPLGISS--------DSDKIPFHPYYS 226
3CX5_N|PDBID|CHAIN|SEQUENCE      LVPFIIAAMVIMHLMALHIHGSSNPLGITG--------NLDRIPMHSYFI 226
1ZRT_P|PDBID|CHAIN|SEQUENCE      LLPFVIAALVAIHIWAFHTTGNNNPTGVEVRRTSKADAEKDTLPFWPYFV 249
2YIU_D|PDBID|CHAIN|SEQUENCE      LLPFVIAALVVVHIWAFHTTGNNNPTGVEVRRGSKEEAKKDTLPFWPYFV 249
                                 ::** * .:. :*:  :*  *..** *:          . * :*: .*:
1PPJ_P|PDBID|CHAIN|SEQUENCE      IKDILGALLLILALMLLVLFAPDLLGDPDNYTPANPLNTPPHIKPEWYFL 275
1BCC_C|PDBID|CHAIN|SEQUENCE      FKDILGLTLMLTPFLTLALFSPNLLGDPENFTPANPLVTPPHIKPEWYFL 276
3CX5_N|PDBID|CHAIN|SEQUENCE      FKDLVTVFLFMLILALFVFYSPNTLGHPDNYIPGNPLVTPASIVPEWYLL 276
1ZRT_P|PDBID|CHAIN|SEQUENCE      IKDLFALALVLLGFFAVVAYMPNYLGHPDNYVQANPLSTPAHIVPEWYFL 299
2YIU_D|PDBID|CHAIN|SEQUENCE      IKDLFALAVVLVVFFAIVGFMPNYLGHPDNYIEANPLVTPAHIVPEWYFL 299
                                 :**:.   :.:  :  .. : *: **.*:*:  .*** **. * ****:*
1PPJ_P|PDBID|CHAIN|SEQUENCE      FAYAILRS------------------IPNKLGGVLALAFSILILALIPLL 307
1BCC_C|PDBID|CHAIN|SEQUENCE      FAYAILRS------------------IPNKLGGVLALAASVLILFLIPFL 308
3CX5_N|PDBID|CHAIN|SEQUENCE      PFYAILRS------------------IPDKLLGVITMFAAILVLLVLPFT 308
1ZRT_P|PDBID|CHAIN|SEQUENCE      PFYAILRAFAADVWVVILVDGLTFGIVDAKFFGVIAMFGAIAVMALAPWL 349
2YIU_D|PDBID|CHAIN|SEQUENCE      PFYAILRAFTADVWVVMLVNWLSFGIIDAKFFGVIAMFGAILVMALVPWL 349
                                   *****:                  :  *: **:::  :: :: : *
1PPJ_P|PDBID|CHAIN|SEQUENCE      HTSKQRSMMFRPLSQCLFWALVADLLTLTWIGGQPVEHPYITIGQLASVL 357
1BCC_C|PDBID|CHAIN|SEQUENCE      HKSKQRTMTFRPLSQTLFWLLVANLLILTWIGSQPVEHPFIIIGQMASLS 358
3CX5_N|PDBID|CHAIN|SEQUENCE      DRSVVRGNTFKVLSKFFFFIFVFNFVLLGQIGACHVEVPYVLMGQIATFI 358
1ZRT_P|PDBID|CHAIN|SEQUENCE      DTSKVRSGAYRPKFRMWFWFLVLDFVVLTWVGAMPTEYPYDWISLIASTY 399
2YIU_D|PDBID|CHAIN|SEQUENCE      DTSRVRSGQYRPLFKWWFWLLAVDFVVLMWVGAMPAEGIYPYIALAGSAY 399
                                 . *  *   ::   :  *: :. ::: *  :*.  .*  :  :.  .:
1PPJ_P|PDBID|CHAIN|SEQUENCE      YFLLILVLMPTAGTIENKLLKW------------------- 379
1BCC_C|PDBID|CHAIN|SEQUENCE      YFTILLILFPTIGTLENKMLNY------------------- 380
3CX5_N|PDBID|CHAIN|SEQUENCE      YFAYFLIIVPVISTIENVLFYIGRVNK-------------- 385
1ZRT_P|PDBID|CHAIN|SEQUENCE      WFAYFLVILPLLGATEKPEPIPASIEEDFNSHYG---NPAE 437
2YIU_D|PDBID|CHAIN|SEQUENCE      WFAYFLIILPLLGIIEKPDAMPQTIEEDFNAHYGPETHPAE 440
                                 :*  :*::.*  .  *:
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Note: the Q_o site residues were colored by red.

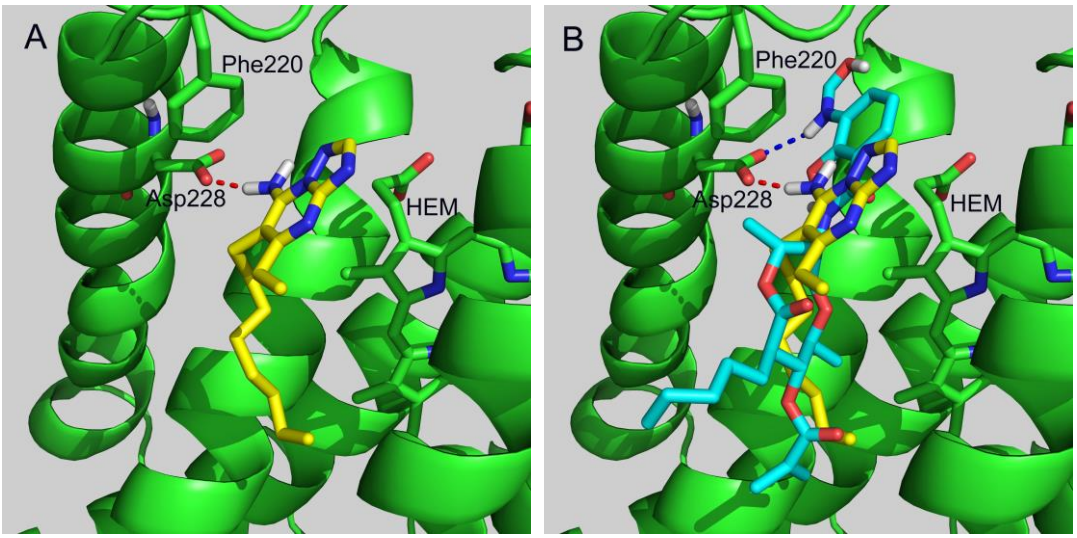

Figure 1S. (A) The binding mode of ametoctradin in the Q_i site. The red line represents the Hbond of ametoctradin with residue. (B) The overlay of ametoctradin and antimycin in the Q_i site. For clarity, only the key residues were shown. The blue and red line represents the Hbond of Asp228 with antimycin and ametoctradin, respectively.

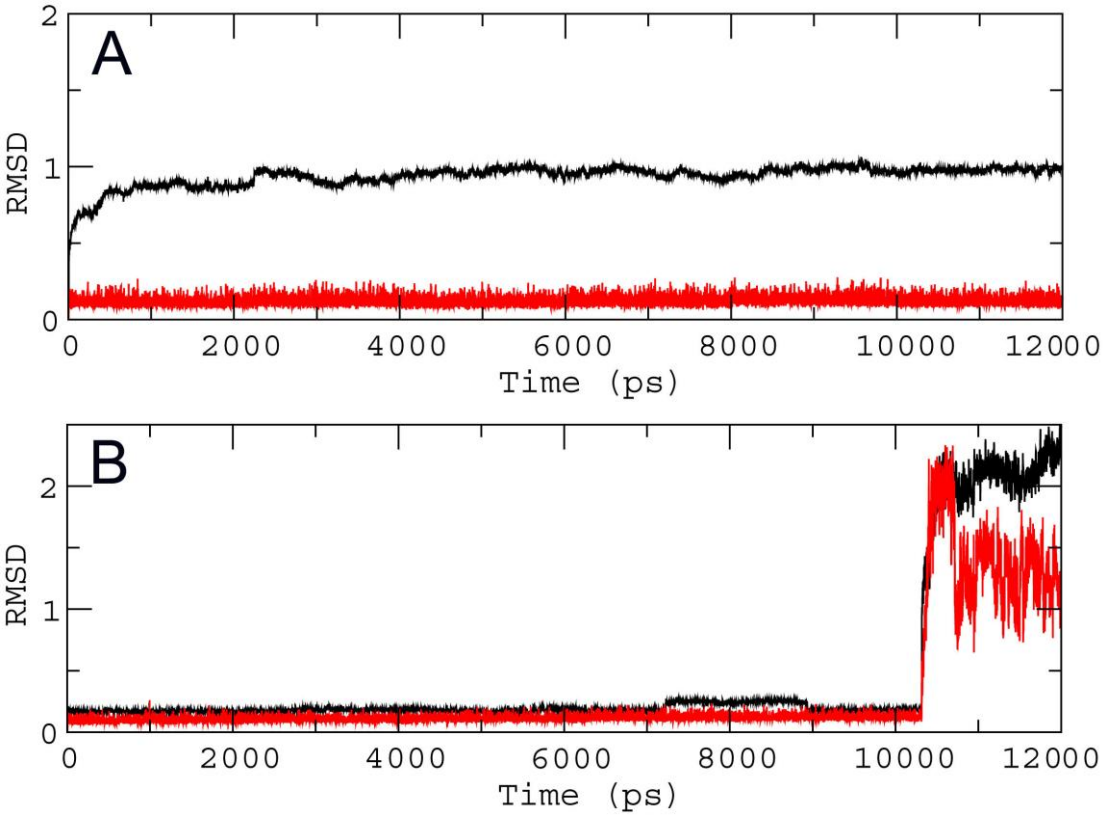

Figure 2S. Plots of the RMSDs of ametroctradin-Q_o(A) and ametroctradin-Q_i (B). The black line represents the RMSD of the backbone atoms, and the red line represents the RMSD of the heavy atom of ligand.

Table 1S. The individual energy terms of the calculated free energy of title compounds. (kcal/mol).

Q_o site								
No.	ΔE_{ele}	ΔE_{VDW}	ΔE_{np}	ΔE_{pol}	ΔH	$-T\Delta S$	ΔG_{cal}	ΔG_{exp} [a]
4a	-10.75	-47.30	-5.18	31.25	-31.98	8.59	-23.39	-9.41
4b	-7.63	-49.27	-5.21	33.87	-28.24	11.67	-16.57	-6.03
4c	-9.10	-47.74	-5.03	30.72	-31.15	9.10	-22.05	-9.27
4g	-23.83	-45.07	-4.66	41.62	-31.94	14.88	-17.06	-6.26
4i	-19.62	-46.79	-4.65	40.60	-30.45	13.87	-16.58	-6.21
4k	-25.14	-45.07	-4.64	45.10	-29.76	15.07	-14.69	-5.55
4m	-27.03	-46.03	-4.80	44.81	-33.06	14.37	-18.69	-6.74
4o	-28.45	-48.24	-5.06	46.30	-35.45	15.89	-19.58	-7.10
4p	-25.72	-43.83	-4.66	44.25	-29.97	14.06	-15.91	-5.94
4q	-23.31	-45.39	-4.81	42.84	-30.67	12.01	-18.66	-7.34
4x	-21.37	-44.26	-4.50	40.54	-29.59	13.36	-16.23	-6.09
4y	-23.30	-44.83	-4.55	42.33	-30.34	13.64	-16.70	-6.25
ametoctradin	-19.39	-49.17	-5.05	40.66	-32.94	11.26	-21.68	-8.86
(R^2) [b]	0.25	0.30	0.44	0.37	0.20	0.64	0.96	1.00

[a] $\Delta G_{exp} = -RTlnIC_{50}$.

[b] the correlation between ΔG_{exp} and the individual energy terms of the calculated free energy