Published in final edited form as:

Bioorg Med Chem Lett. 2016 November 01; 26(21): 5350–5353. doi:10.1016/j.bmcl.2016.09.009.

Identification of small molecules that bind to the mitochondrial protein mitoNEET

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Abstract

MitoNEET (CISD1) is a 2Fe-2S iron-sulfur cluster protein belonging to the zinc-finger protein family. Recently mitoNEET has been shown to be a major role player in the mitochondrial function associated with metabolic type diseases such as obesity and cancers. The anti-diabetic drug pioglitazone and rosiglitazone were the first identified ligands to mitoNEET. Since little is known about structural requirements for ligand binding to mitoNEET, we screened a small set of compounds to gain insight into these requirements. We found that the thiazolidinedione (TZD) warhead as seen in rosiglitazone was not an absolutely necessity for binding to mitoNEET. These results will aid in the development of novel compounds that can be used to treat mitochondrial dysfunction seen in several diseases.

Keywords

Mitochondria; Bioenergetics; Glitazones; Type II diabetes

MitoNEET (CISD1) is a recently discovered protein found on the outer surface of mitochondria discovered by investigations of the off target pharmacological effects of the anti-diabetic drug pioglitazone.^{1,2} This protein belongs to a novel family of NEET proteins that contain 2Fe-2S clusters and are evolutionary conserved in several organisms including *Caenorhabditis elegans*, bacteria, and plants. MitoNEET belongs to the CDGSH-type zinc finger protein family, although it contains iron instead of the expected zinc, and has the characteristic amino acid sequence Asn-Glu-Glu-Thr.^{3–5} This family of proteins includes the genes CISD1 (mitoNEET), CISD2 (NAF-1, miner1) and CISD3 (miner2). In mitoNEET the

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iron-sulfur clusters are found to be coordinated in a 3Cys:1His configuration, which several groups have suggested act as a redox sensor for mitochondria to respond to oxidative changes in the cellular environment. The iron-sulfur cluster has been shown to be labile at $\text{pH} < 8$, leading to a loss of the cluster and transfer to other proteins in the cell such as anamorsin. The biological role of mitoNEET remains to be fully elucidated but it has been indicated to play an important role in diabetes and obesity, as well as in breast cancer cell proliferation. In obesity, for example, it was found that when mitoNEET is overexpressed in the adipose tissue of *ob/ob* genetic mice, systemic inflammation markers are significantly decreased in concert with a reduction in oxidative stress markers such as reactive oxygen species (ROS).⁶ These studies suggest that mitoNEET may constitute a novel drug target against mitochondrial dysfunction possibly impacting several disease states.²

The structure and function of mitoNEET has garnered a lot of attention in the past few years, but lacking is a comprehensive study showing the classes of compounds that can bind to mitoNEET. Currently known ligands that bind with mitoNEET belong to the thiazolidinedione (TZD or glitazones) family with NL-1 **1**, pioglitazone **23**, rosiglitazone **24** and (Fig. 1) as the best described.^{1,7} The current study builds on our earlier work that described the structure–activity relationships (SAR) for a small set of compounds and their binding to mitoNEET⁸ and expands our knowledge space of chemical interaction with mitoNEET.

MitoNEET was first discovered from a photo-affinity labeling via a derivative of pioglitazone. Pioglitazone belongs to the glitazone chemical class due to its thiazolidinedione (TZD) warhead moiety.¹ Although several studies have been published in recent years regarding the physiologic role of mitoNEET, few papers have appeared that focused on ligand binding characteristics of mitoNEET-associated compounds. This study was therefore initiated to fill that gap in knowledge. Utilizing a HTS screening assay, that we described previously,⁸ we expanded the compound set to explore both effects of the TZD warhead as well as other compounds to identify new scaffolds that might be able to bind to mitoNEET.⁸ Data on compound binding to human recombinant mitoNEET is given in Table 1. The K_i values were derived from the Cheng–Prusoff equation where the K_D for rosiglitazone was found to be 44 nM (Fig. 2).

Figure 3 shows properties of the compounds tested in this study. The majority of the compounds had a molecular weight less than 450 Da. The $\log D$, $\log P$ and total polar surface area (TPSA) were also determined and the majority of compounds were found to adhere to the rules of 5.⁹ No apparent correlation was seen between each chemical property and the binding affinity (K_i).

A previous study done by Bieganski et al. showed that mitoNEET may bind to polyphenolic compounds from natural product sources.¹⁰ We also found that the active metabolite of resveratrol, resveratrol-3-sulfate **22**, can bind to mitoNEET with a K_i of 144 nM; although the parent compound resveratrol did not bind to mitoNEET in our studies.¹¹ Other polyphenolic compounds found to have mitochondrial functional effects were tested to see whether they can bind to mitoNEET as well. We tested the natural compound curcumin **26**, which is isolated from the Indian spice turmeric, and found it to bind to mitoNEET at a K_i of

101 nM. The flavonoid kaempferol **27**, shown to have mitochondrial protective effects, was found to bind to mitoNEET at a K_i of 132 nM¹² (Figs. 4–6).

Based on the work previously done by us on the flavonoid from the plant *Rhus verniciflua* sulfuretin¹³ was also found that the benzofuran aurones bind to mitoNEET with varied affinity. For instance, compound **34** and **35** were non-binders while **36** bound to mitoNEET at a K_i of 52 nM and **37** with a K_i of 6 nM. This indicates that for aurones there are specific requirements for binding to mitoNEET to achieve optimum binding potency.

Since TZD warheads are the most well characterized mitoNEET ligand chemotype, we wanted to explore if opening of the TZD ring would perturb mitoNEET binding. A range of affinities can be seen with these TZD-type compounds, as the K_i values varied dramatically between the nanomolar to high micromolar range. Since the TZD warhead is constant in these compounds it appears the ability to bind to mitoNEET is largely dependent on the aromatic substituted moieties in the compounds. We tested a series of compounds and found that **42**, α -hydroxycinnamic acid, with a K_i of 1.7 μ M was only slightly less potent than a similar TZD compound, e.g., compound **3**, suggesting that the phenol group, present in both **3** and **42**, can have significant impact on potency. Similarly, nitrile containing compounds such as compound **56**, CCCP with a K_i of 1.49; compound **38**, AG104 with a K_i of 77 nM; and compound **62** with a K_i of 78 nM are all able to bind to mitoNEET without the TZD warhead as previously suggested but that phenols also seem to contribute to binding; **38** and **62** are substantially more potent than **56**, which lacks a phenol. Additionally, compound 3-AP **51** displayed high affinity with a K_i of 388, which supports our hypothesis that the TZD warhead could be replaced with other moieties. Also of interest was that nitrofurantoin **54**, an antibiotic used for urinary tract infections, binds to mitoNEET with a K_i of 816 nM while structurally related compounds such as furazolidone **65** and furaltadone **66** and dorzolamide **68**, are non-binders. The structurally similar muscle relaxant dantrolene **55** has a K_i of 2.06 μ M, suggesting the additional aromatic ring compared to nitrofurantoin positively influences binding to mitoNEET.

Several other FDA approved drugs were tested for mitoNEET binding, although most were found to be non-binders. We did however find the diuretic compound furosemide **41** was able to bind to mitoNEET with a K_i of 2.28 μ M. The non-steroidal anti-inflammatory drugs diclofenac **70** and naproxen **72** were found to be non-binders. Other type II anti-diabetic drugs were found to bind with mitoNEET such as glibenclamide (**43**; K_i of 1.67 mM) whereas metformin **71** did not bind to mitoNEET. Considering the different functional effects these compounds have shown on mitochondria,^{14,15} they may be useful as pharmacological tools to fully elucidate mitoNEET's mitochondrial activity.

In conclusion we show here a set of compounds that have been screened for binding to mitoNEET. We found the TZD warhead of the glitazones can be substituted for different structural groups, which may have the benefit of reducing side effect profiles seen before with TZDs.^{1,2} MitoNEET represents a novel protein drug target for the treatment of metabolic diseases where mitochondrial dysfunction is thought to play a critical role in the pathology, ranging from obesity to Parkinson's disease thereby with the promise of yielding novel drugs.

Acknowledgments

This work was funded in part by the Michael J. Fox Foundation for Parkinson's Research to WJG. The project described was supported by the National Institute of General Medical Sciences, U54GM104942. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Figure 1.

TZD ligands which bind to mitoNEET. Binding affinity is shown in microM (K_i).

Abbreviation: non-binding (NB).

Figure 2.

Determination of affinity for rosiglitazone to human recombinant mitoNEET.

[³H]rosiglitazone was used as a label. Human recombinant mitoNEET was attached via a His-tag to SPA beads, and binding determined from displacement of [³H]rosiglitazone by unlabeled rosiglitazone.

Figure 3.

Chemical properties of the compounds evaluated in this study. Shown are: (A) $\log D$, (B) $\log P$, (C) total polar surface area (TPSA) and (D) molecular weight. Below the count graphs, a linear regression plot between K_i (microM) and the chemical attribute.

Figure 4.

Natural products which bind to mitoNEET. Binding affinity is shown in microM (K_i).

Abbreviation: non-binding (NB).

Figure 5.

Benzofuran aurones which bind to mitoNEET. Binding affinity is shown in microM (K_i).

Abbreviation: non-binding (NB).

Figure 6.

FDA approved compounds and their affinity for mitoNEET. Binding affinity is shown in microM (K_i). Abbreviation: non-binding (NB).

Table 1
Binding affinity of compounds to human recombinant mitoNEET. [³H]rosiglitazone was used as radio-label *

#	Name/ID	IC ₅₀ (μM)	K _i (μM)	#	Name/ID	IC ₅₀ (μM)	K _i (μM)
1	NL-1	7.36	0.315	45	Pomalidomide	NB	NB
2	NL-2	9.56	0.409	46	7917584	3.3	0.141
3	NL-3	14	0.598	47	Acyclovir	NB	NB
4	NL-4	13.34	0.57	48	6209863	0.518	0.022
5	NL-5	3.26	0.139	49	Stiripentol	NB	NB
6	NL-6	5.23	0.224	50	4-Amino-1,8-naphthalimide	264	11.282
7	NL-7	15.12	0.646	51	Triapine	9.078	0.388
8	NL-8	4.98	0.213	52	Suramin	NB	NB
9	NL-9	2.93	0.125	53	Bosutinib	NB	NB
10	NL-10	0.86	0.037	54	Nitrofurantoin	19.1	0.816
11	NL-11	4.47	0.191	55	Dantrolene	48.33	2.065
12	NL-12	1.73	0.074	56	CCCP	35.04	1.497
13	NL-13	NB	NB	57	6636424	0.6861	0.029
14	NL-14	82.32	3.518	58	6373721	0.8389	0.036
15	NL-15	19.44	0.831	59	7320244	0.9454	0.04
16	NL-16	9.63	0.412	60	5472855	0.7701	0.033
17	NL-17	37320	1594.88	61	6634507	1.399	0.06
18	NL-18	22.27	0.952	62	5119666	1.829	0.078
19	NL-19	5.97	0.255	63	7138125	7.983	0.341
20	NL-20	11.05	0.472	64	Nitrofurazone	NB	NB
21	NL-21	13.65	0.583	65	Furazolidone	NB	NB
22	Resveratrol-3-sulfate	3.36	0.144	66	Furaladone	NB	NB
23	Pioglitazone	8.65	0.37	67	C10d	NB	NB
24	Rosiglitazone	0.731	0.031	68	Dorzolamide	NB	NB
25	NL-22	NB	NB	69	Chloro-imidazole	NB	NB
26	Curcumin	2.36	0.101	70	Diclofenac	NB	NB
27	Kaempferol	3.1	0.132	71	Metformin	NB	NB
28	NL-23	1.067	0.046	72	Naproxen	NB	NB

#	Name/ID	IC ₅₀ (μM)	K _i (μM)	#	Name/ID	IC ₅₀ (μM)	K _i (μM)
29	NL-24	1.964	0.084	73	5-Azacytidine	NB	NB
30	NL-25	3.18	0.136	74	L-732,138	NB	NB
31	NL-26	3.289	0.141	75	Homotaurine	NB	NB
32	NL-27	3.858	0.165	76	7722368	9.657	0.413
33	NL-28	0.91	0.039	77	5472855	1.485	0.063
34	NL-29	NB	NB	78	GSK-LSD1	3.454	0.148
35	NL-30	NB	NB	79	Tryprostatin-A	3.387	0.145
36	NL-31	1.22	0.052	80	Doxorubicin	80.76	3.451
37	NL-32	0.148	0.006	81	Chloroquine	NB	NB
38	AG104	1.8	0.077	82	Ursodiol	26.54	1.134
39	NL-33	6.94	0.297	83	ATP	NB	NB
40	NL-34	3.307	0.141	84	TC1	NB	NB
41	Furosemide	53.46	2.285	85	Methamphetamine	NB	NB
42	α-Hydroxy-cinnamic acid	40	1.709	86	Retigabine	NB	NB
43	Glibenclamide	39.1	1.671	87	Gemfibrozil	170.6	7.291
44	Dichloroacetate	NB	NB				

* Non-binding (NB).