Journal of Molecular Graphics and Modelling 29 (2011) 965–973

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Novel ligands that target the mitochondrial membrane protein mitoNEET

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ARTICLE INFO

Article history:
Received 28 October 2010
Received in revised form 31 March 2011
Accepted 3 April 2011
Available online 13 April 2011

Keywords:
Mitoneet
Autodock
Docking
Fluorescence
Iron–sulfur
Thiazolidinedione

ABSTRACT

Ligands of the thiazolidinedione (TZD) class of compounds, pioglitazone (Actos™) and rosiglitazone (Avandia™) are currently approved for treatment of type 2 diabetes and are known to bind to the PPAR-γ nuclear receptor subtype. Recent evidence suggesting PPAR-γ independent action of the TZDs led to the discovery of a novel integral outer mitochondrial membrane protein, mitoNEET. In spite of the several reported X-ray crystal structures of the unbound form of mitoNEET, the location and nature of the mitoNEET ligand binding sites (LBS) remain unknown. In this study, a molecular blind docking (BD) method was used to discover potential mitoNEET LBS and novel ligands, utilizing the program AutoDock Vina (v 1.0.2). Validation of BD was performed on the PPAR-γ receptor (PDB ID: 1ZGY) with the test compound rosiglitazone, demonstrating that the binding conformation of rosiglitazone determined by AutoDock Vina matches well with that of the cocrystallized ligand (root mean square deviation of the heavy atoms 1.45 Å). The locations and a general ligand binding interaction model for the LBS were determined, leading to the discovery of novel mitoNEET ligands. An *in vitro* fluorescence binding assay utilizing purified recombinant mitoNEET protein was used to determine the binding affinity of a predicted mitoNEET ligand, and the data obtained is in good agreement with AutoDock Vina results. The discovery of potential mitoNEET ligand binding sites and novel ligands, opens up the possibility for detailed structural studies of mitoNEET–ligand complexes, as well as rational design of novel ligands specifically targeted for mitoNEET.

MitoNEET is a recently discovered iron–sulfur (2Fe–2S) outer mitochondrial integral membrane protein that binds the same ligands, specifically pioglitazone [1,2] and rosiglitazone [3], as the peroxisome proliferator-activated receptor gamma (PPAR-γ) nuclear receptor, and with similar binding affinity. Pioglitazone (**1**) and rosiglitazone (**2**) are members of the thiazolidinedione (TZD) class of compounds (Fig. 1), currently approved for treatment of type 2 diabetes. Recent evidence suggesting PPAR-γ independent action of the TZDs led to the discovery of mitoNEET through a cross-linking study with a photoactive form of pioglitazone [1]. Moreover, accumulating evidence indicates that many of the clinical effects of TZDs may originate by binding to mitoNEET. A recent report [4] suggests that mitoNEET plays a key role in regulating electron transport and oxidative phosphorylation, as there is a decrease in the complex I-dependent oxygen consumption in the mitochondria isolated from mitoNEET-deficient mice heart.

Pioglitazone was also reported to alter the oxidative capacity of mitochondria and exhibit negative regulatory effect on complex I activity in liver, muscle, and astroglioma cells [5,6], with positive regulatory effect in neuronal cells [7]. These correlations together with the mitochondrial localization of mitoNEET, strongly suggest that pioglitazone (and other TZDs) may exert their actions on mitochondria by regulating activities of the respiratory complexes via interaction with mitoNEET through yet unknown mechanism(s).

In other studies, pioglitazone has been shown as a potential treatment for several neurodegenerative diseases, particularly Alzheimer disease (AD) [8], amyotrophic lateral sclerosis (ALS) [9,10], multiple sclerosis (MS) [11–13], and Parkinson's disease (PD) [14]. Moreover, pioglitazone was shown to prevent LPS-induced activation of microglia, attenuate oxidative stress, and restore mitochondrial function, all of which are specific in dopaminergic neurodegeneration [15,16], implicated in PD [14]. Published evidence indicates that increased oxidative stress [17] is implicated in PD, suggesting that pioglitazone may have anti-inflammatory and anti-oxidative properties.

In addition, mitoNEET is likely to play a vital role in mitochondrial energy homeostasis and metabolism [4], transporting and sequestering iron, electron transfer, and oxidation of fatty acids [1]. Pioglitazone binding to mitoNEET was also found to stabilize the mitoNEET dimer formation, which correlates with decreased oxidative stress [18]. The fact that PPAR-γ ligands bind to mitoNEET suggests an alternate mode of action for these ligands, with the

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doi:10.1016/j.jmgm.2011.04.001

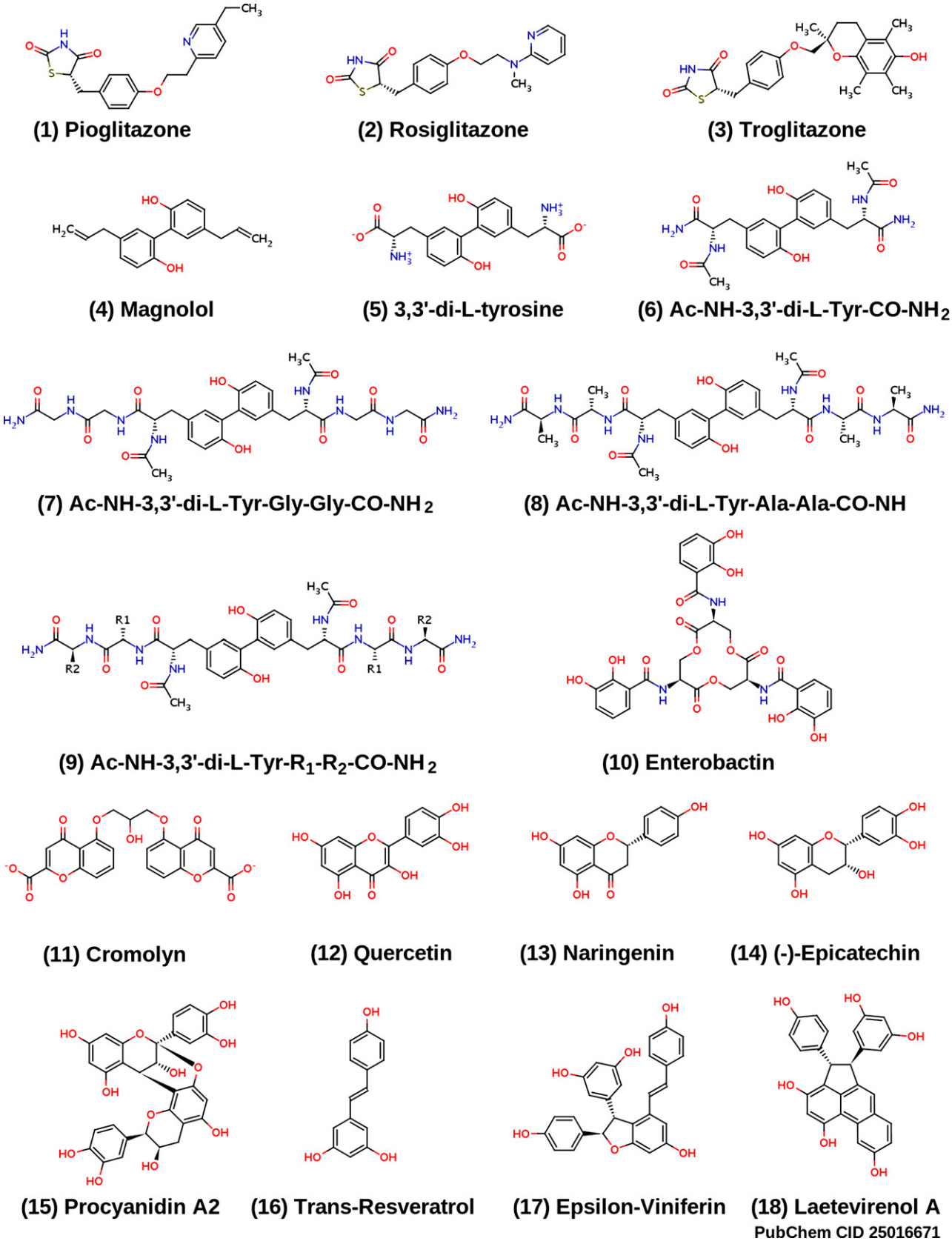

Fig. 1. Structures of 15 compounds predicted to bind to mitoNEET, as well as the structures of pioglitazone (**1**) and rosiglitazone (**2**).