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Pyridyl-urea Derivatives as Blockers of Aβ-induced mPTP Opening for Alzheimer's Disease

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Alzheimer's disease is a progressive neurodegenerative disorder in aged people and the pathogen is known as amyloid beta (Aβ) with different slicing.[1] There was a recent report that amyloid plaques have long been known as highly cytotoxic but many recent studies show that the real cytotoxic materials are small soluble Aβ oligomers.[2] Aβ oligomers localize to mitochondria and interfere with normal mitochondrial functions resulting in overproduction of reactive oxygen species, inhibition of respiration and ATP production and damage of mitochondrial structures.[3] In addition, Aβ oligomers induce mitochondrial calcium overload and the opening of mitochondrial permeability transition pores (mPTPs). This allows the influx of cytosolic molecules into the mitochondrial matrix and increase of the matrix volume, resulting in disruption of the mitochondrial outer membrane, subsequent destruction of mitochondrial functions, and cell death.[4] mPTP is composed of three major proteins: voltage-dependent anion channel (VDAC) in the outer membrane, adenine nucleotide translocator (ANT) and cyclophilin D (CypD) in inner membrane.[5] Cyclophilin D (CypD) is thought to regulate the opening of the mPTP because cyclospirin A (CsA), an inhibitor of CypD, inhibits the pore opening.[6] CypD inhibitors may block mPTP opening, resulting in protecting mitochondrial functions and structures and then possibly retarding Alzheimer's disease progress. In this paper, we designed and synthesized new cyclophilin D inhibitors and biologically evaluated whether those compounds recover Aβ-induced mitochondrial dysfunction through JC-1 assay, ATP production assay, and cell viability assay.[7]

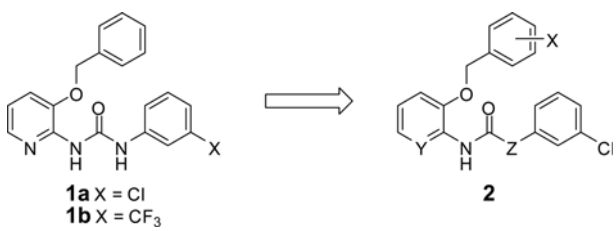
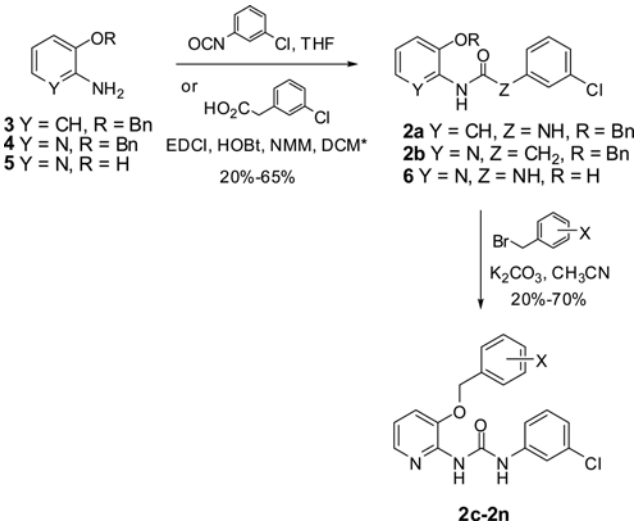


Figure 1. Designed cyclophilin D inhibitors **2**.

New cyclophilin D inhibitors were designed based on cyclophilin A inhibitors **1a** and **1b** because the sequence identity between cyclophilin A and cyclophilin D is 63% and the sequence similarity of positives is 81% (Figure 1).[8]

The designed target compounds **2** were prepared in two steps starting from compound **3**, **4**, or **5** (Scheme 1). The compound **3** reacted with *m*-chlorophenylisocyanate to afford a diaryl-urea compound **2a** in 20% yield, while the compound **4** underwent amide coupling reaction with *m*-chlorophenylacetic acid to give an amide compound **2b** in 30% yield. The compound **5** reacted with *m*-chlorophenylisocyanate to give a pyridyl-urea compound **6** in 65% yield, which was converted to the compounds **2c-2n** with various substituted benzyl groups in 20-70% yields.

The synthesized compounds **2** were biologically evaluated against Aβ-induced mitochondrial depolarization using JC-1 dye,[9] where the opening of mPTPs by Aβ makes mitochondrial membrane depolarized through influx of cytosolic



Scheme 1. Synthesis of designed pyridyl-urea derivatives **2** (*EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt: hydroxybenzotriazole, NMM: *N*-methylmorpholine, DCM: dichloromethane).

Table 1. Inhibitory activities of pyridyl-urea derivatives **2** against Aβ-induced mitochondrial depolarization (JC-1 assay), recovery of Aβ-suppressed mitochondrial ATP production, and cell viabilities

en.	Copd no.	X	Y	Z	JC-1 assay		ATP production recovery[c]	Cell viability[d]
					%remaining depolarization[a]	%inhibition[b]		
1	**1a**	–[e]	–[e]	–[e]	41%	59%	–[f]	–[f]
2	**1b**	–[e]	–[e]	–[e]	70%	30%	–[f]	–[f]
3	**2a**	H	CH	NH	150%	-50%	–[f]	–[f]
4	**2b**	H	N	CH_2	118%	-18%	–[f]	–[f]
5	**2c**	*o*-NO_2	N	NH	77%	23%	–[f]	–[f]
6	**2d**	*m*-NO_2	N	NH	121%	-21%	–[f]	–[f]
7	**2e**	*p*-NO_2	N	NH	109%	-9%	–[f]	–[f]
8	**2f**	*o*-CH_3	N	NH	42%	58%	–[f]	–[f]
9	**2g**	*m*-CH_3	N	NH	32%	68%	15%	93%
10	**2h**	*p*-CH_3	N	NH	115%	-15%	–[f]	–[f]
11	**2i**	*m*-OMe	N	NH	9%	91%	-28%	85%
12	**2j**	*p*-OMe	N	NH	43%	57%	-83%	75%
13	**2k**	*o*-Cl	N	NH	28%	72%	26%	107%
14	**2l**	*p*-Cl	N	NH	32%	68%	-15%	96%
15	**2m**	*o*-F	N	NH	30%	70%	-59%	83%
16	**2n**	*m*-F	N	NH	57%	43%	103%	130%

[a]%Remaining depolarization at 5 μM of each tested compound against Aβ-induced mitochondrial depolarization. [b]%Inhibition = 100% – %remaining depolarization. [c]%Recovery of ATP production at 5 μM of each tested compound against Aβ-suppressed mitochondrial ATP production. [d]Calcein viability assay at 5 μM of each tested compound. [e]No need. [f]Not determined

molecules and the membrane potential was measured by JC-1 dye (Table 1). All tests were performed using 5 μM of each compound. The reference compounds **1a** and **1b** showed good and marginal inhibitory activities against Aβ-induced mitochondrial depolarization with 59% and 30% inhibition, respectively. The compounds **2a** and **2b** without the pyridine moiety and the urea moiety, respectively, showed no inhibitory activities and further depolarized the mitochondrial membrane. Compounds **2c**, **2d** and **2e** with nitro substituents did not block Aβ-induced mitochondrial depolarization. The compounds with methyl (**2f** and **2g**), methoxy (**2i** and **2j**), chloro (**2k** and **2l**), and fluoro (**2m** and **2n**) substituents, with the exception of the compound **2h** with *p*-methyl substitutent, showed good inhibitory activities with 9%-57% remaining depolarization.

The compounds with good inhibitory activities were further biologically evaluated against recovery of Aβ-suppressed mitochondrial ATP production, as well as cell viabilities by calcein viability assay.[9] The compound **2n** fully recovered ATP production capability of mitochondria suppressed by Aβ. The compounds **2g** and **2k** partially recovered ATP production capability, while the compounds **2i**, **2j**, **2l**, and **2m** could not recover but further suppressed mitochondrial ATP production capability. The cell viability results were good for all the compounds with 75%-130% survival. Taken together, these three assay results indicate that the compound **2n** with *m*-fluoro substituent is the most promising agent to block Aβ-induced mPTP opening and recover ATP production capability with good cell viability.

In summary, new cyclophilin D inhibitors were designed and synthesized in a very convenient method. Among the synthesized compounds, the compound **2n** showed good inhibitory activity against Aβ-induced mitochondrial depolarization, full recovery of Aβ-suppressed mitochondrial ATP production, and good cell viability. Based on this study, extensive SAR (structure-activity relationship) study of compound **2n** to obtain better pharmacological profiles with *in vivo* activity in Alzheimer's disease animal model is warranted.

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