Supporting information

Biological and pharmacological characterization of Benzothiazole based CK-1δ inhibitors in models of Parkinson's disease

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KEYWORDS: Parkinson's disease, protein kinase CK-1δ inhibitors, drug discovery, neurodegeneration.

Biology

Safety studies (Zebrafish embryos and Ames test) of CK-1δ inhibitor 4

In Vivo activity on Zebrafish Embryos. All animal experiments were conducted and documented according to the federal and local regulation. Wild type zebrafish was used in this study. The embryos were collected and placed into 24-well plates, 10 embryos per well, and maintained in E2 medium at ~28 °C. Compounds were added 5 hours post fertilization (50% epiboly) and the embryos allowed growing in chemical compound solution up to 4 days. The phenotypes were compared using the Axio Scope. A1 microscope system from Carl Zeiss at 1, 2, 3, 4 days post fertilization. All embryo testing were stopped at day 5 of embryonic development.

Ames test. Suspensions of bacterial cells (*S. typhimurium* TA98 and *S. typhimurium* TA100) were exposed to 3 different concentrations of test compound **4** in 96-well flat-bottomed plates, as well as a positive and negative control for revertant growth and scoring. After 5 days of incubation, revertant colonies were detected by the change of

color from blue to yellow on solvent control plates. Results were also taken through days 3-7 for better interpretation.

All yellow, partially yellow or turbid wells were scored as positive, while all purple wells were scored as negative. The number of positive wells for each plate was recorded, and their number was counted and compared to that of spontaneous revertant colonies. The "Background" (i.e. no test material added) plate was used as reference for the level of spontaneous or background mutation of the assay organism. The statistical difference was determined as described by Gilbert.[1] The bacteria strains were maintained frozen and stored in total darkness until used. Both bacteria were inoculated in nutrient broth and incubated at 37 ºC for 20 h prior to the test.

Figure 1S.- Protein kinase CK-1δ expression in human dopaminergic SH-SY5Y cell line. Cultures were exposed for 24 h to 6-OHDA (35 μM), as described previously. (**A**) Immunocytochemical analysis of CK-1δ expression (green). Nuclei were counterstained with DAPI (blue). Scale bar, 10 μm. (**B**) Representative Western blot and quantification analysis showing expression levels of CK-1δ in SH-SY5Y cultures.

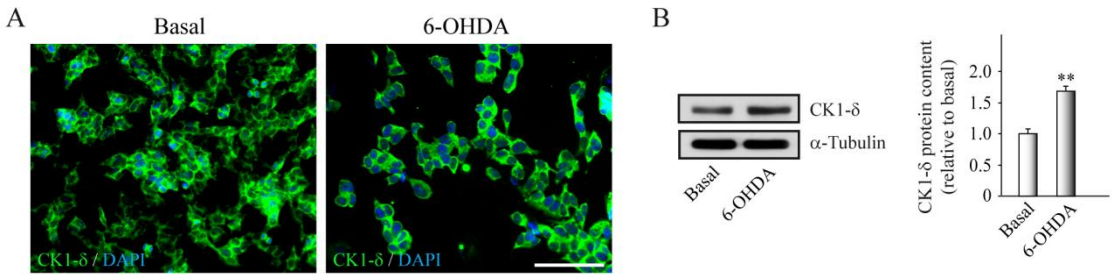


Figure 2S. Effect of protein kinase CK-1δ inhibitors on 6-hydroxydopamine (6-OHDA)-induced SH-SY5Y cell death. SH-SY5Y cells were exposed for 24h to 6-OHDA (35 μM) in the presence or absence of the CK-1δ inhibitors (0.1, 0.5, 1, 10 and 20 μM). The number of viable cells was measured by MTT assay. Each data point represents the mean ± SD of six replications in three different experiments. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 statistically significant differences between CK-1δ inhibitors and 6-OHDA treated cultures.

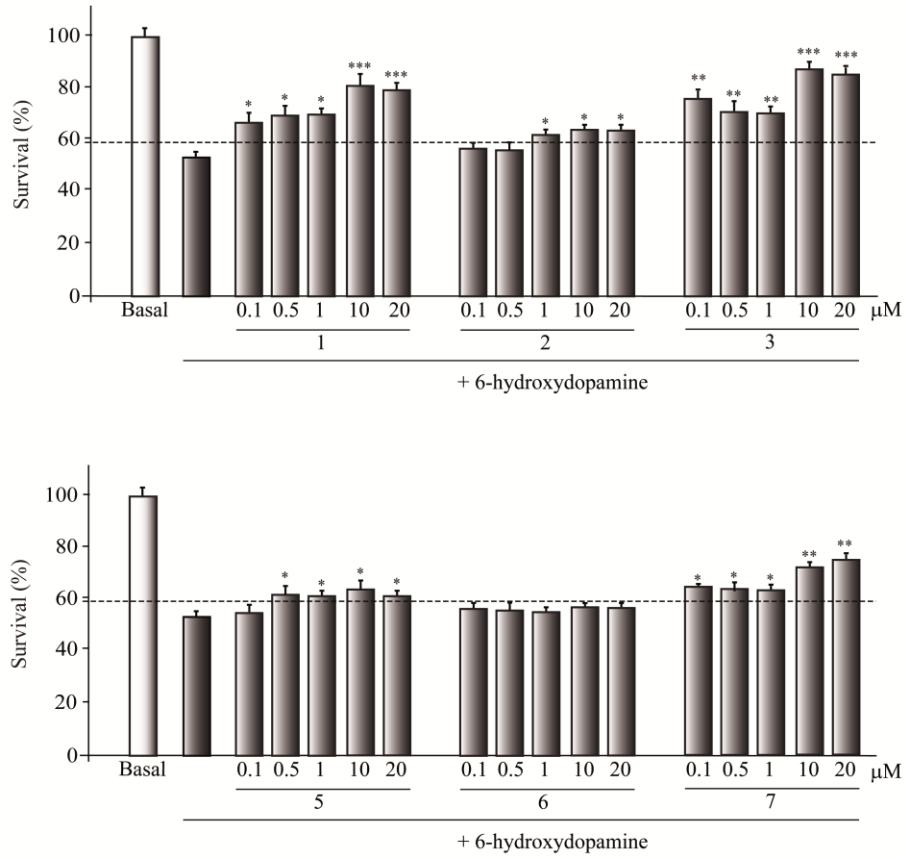
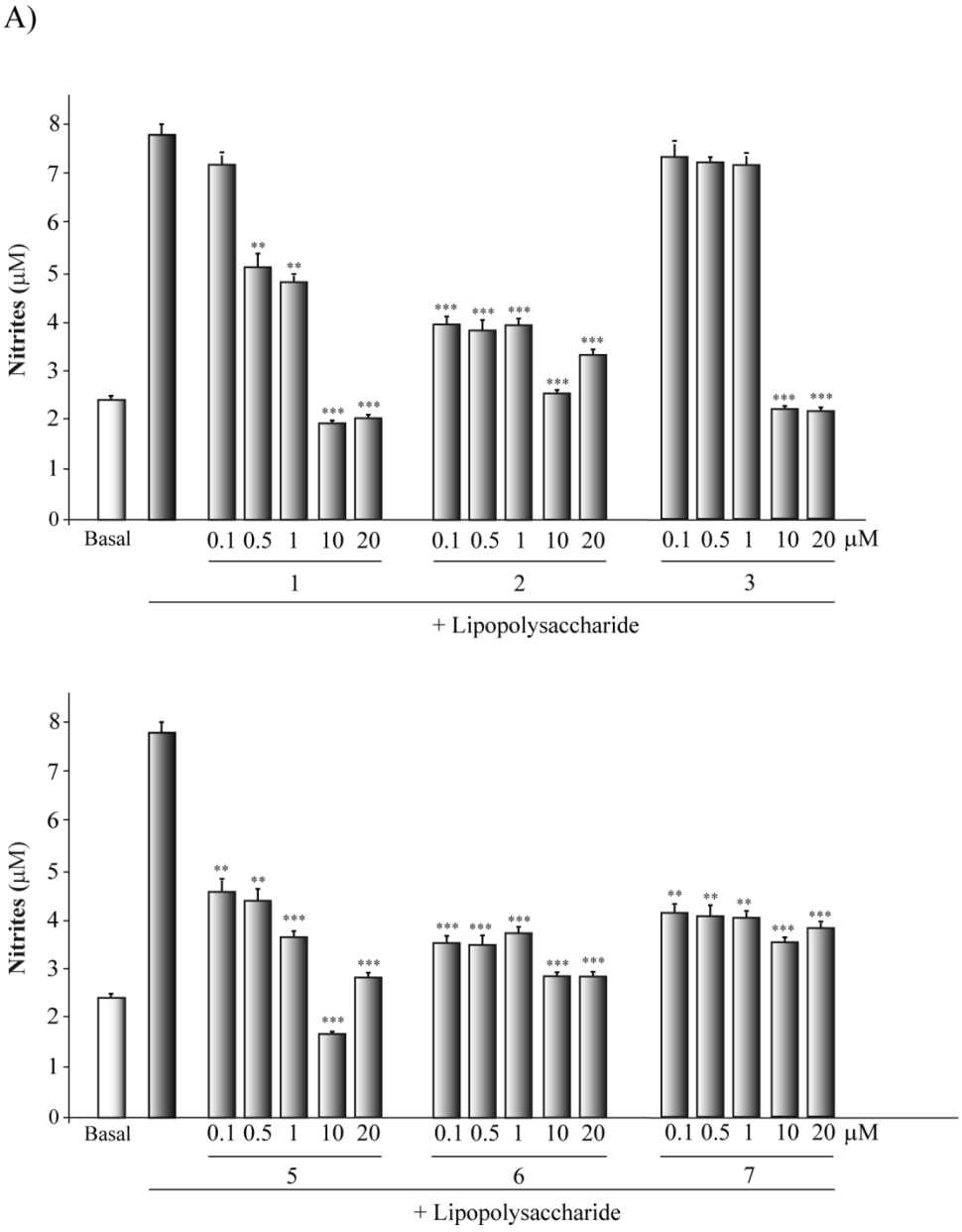


Figure 3S. Anti-inflammatory effect of protein kinase CK-1δ inhibitors on glial primary cultures. Astrocytes (A) and microglial cellS (B) cultures were isolated, plated and later on treated with lipopolysaccharide (LPS, 10 µg/mL) in the presence of the different CK-1δ inhibitors (0.1, 0.5, 1, 10 and 20 µM). Production of nitrite was measured by the Griess reaction. Each data point represents the mean±SD of six replications in three different experiments.***p < 0.001, statistically significant differences between CK-1δ inhibitors and LPS-treated cultures.



B)

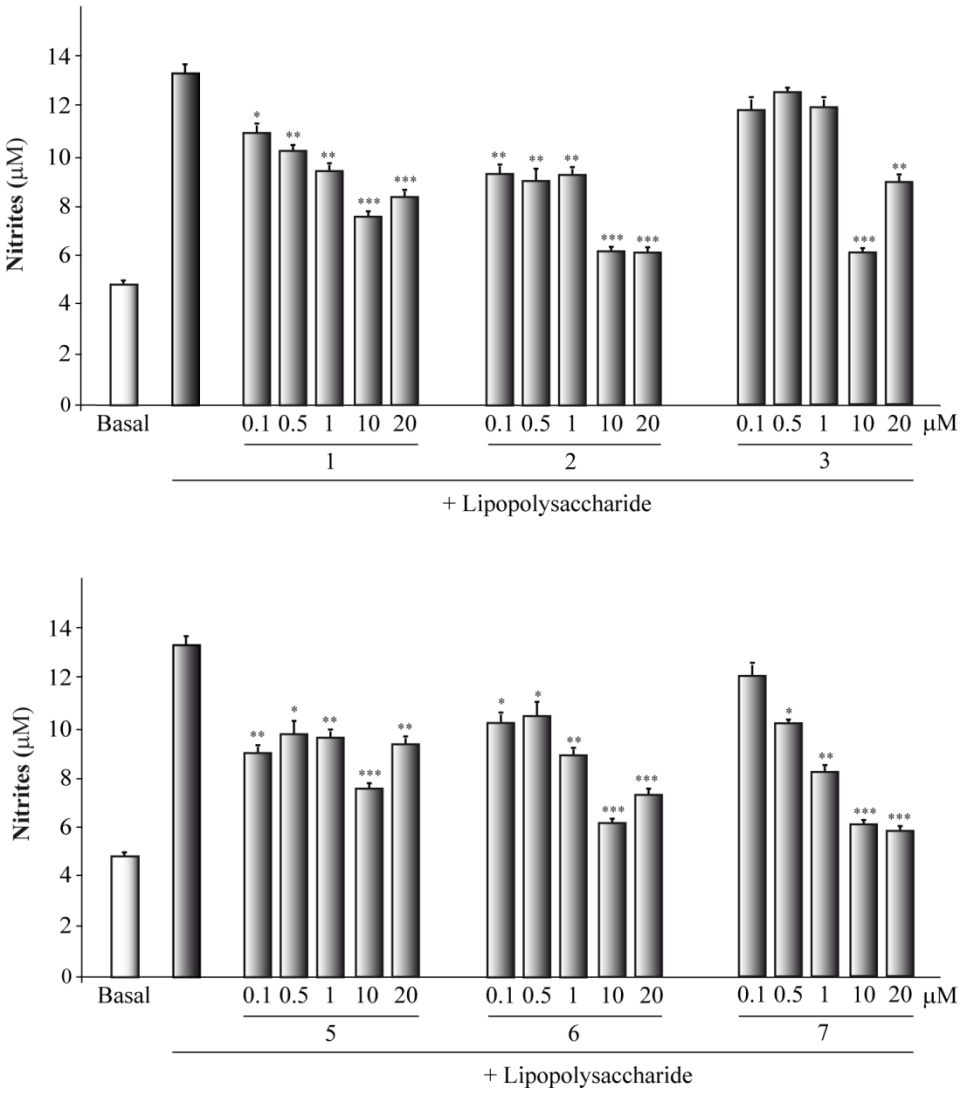
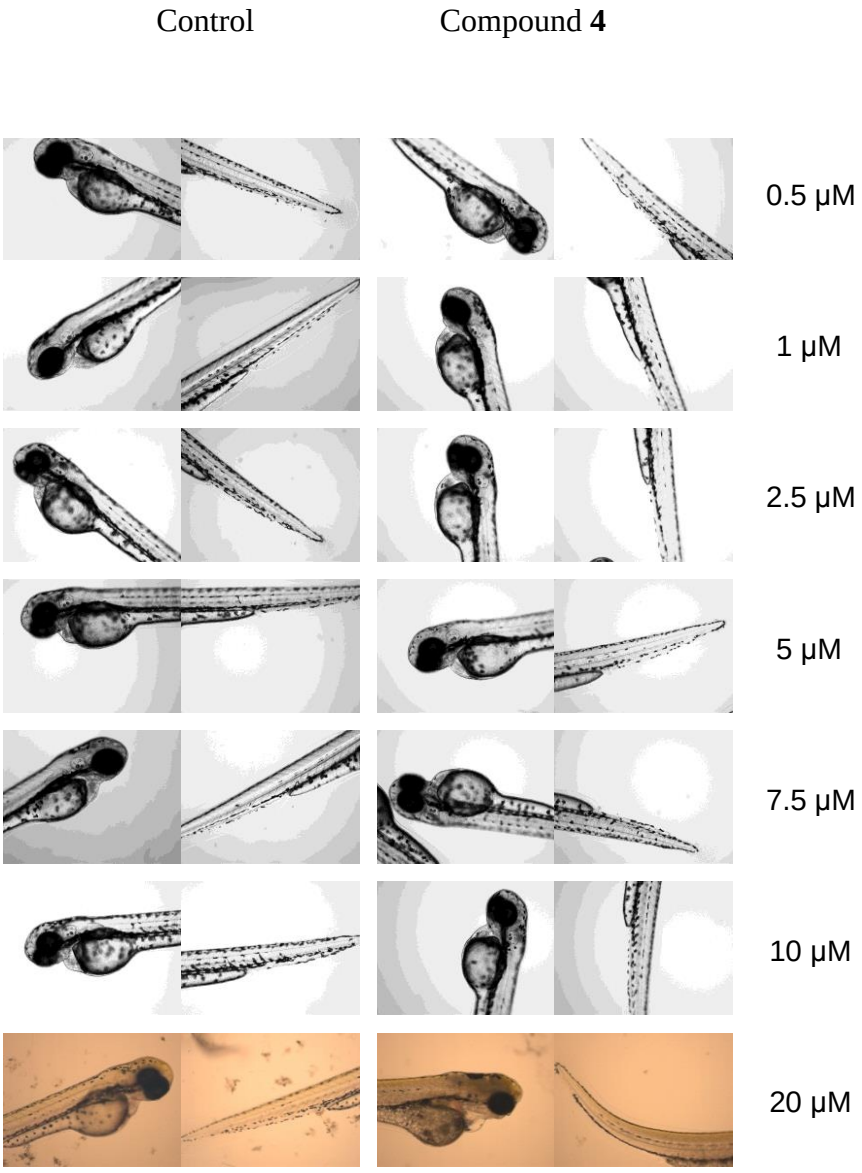

Nitrites (μM)
0
2
4
6
8
10
12
14
Basal
0.1 0.5 1 10 20 0.1 0.5 1 10 20 0.1 0.5 1 10 20 μM
1
2
3
+ Lipopolysaccharide
Nitrites (μM)
0
2
4
6
8
10
12
14
Basal
0.1 0.5 1 10 20 0.1 0.5 1 10 20 0.1 0.5 1 10 20 μM
5
6
7
+ Lipopolysaccharide

Figure 4S.- Control Embryos (left columns) and incubated embryos with compound **4** (right columns).



References

(1) Gilbert, A. L.; Giles, M. K.; Flachs, G. M.; Rogers, R. B.; Hsun, U. Y., A real-time video tracking system. *IEEE Trans. Pattern. Anal. Mach. Intell.* **1980,** *2* (1), 47-56.