(1*R*,2*R*,6*S*)-2-(1*H*-1,2,4-Triazol-3-ylthio)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-enol) as promising molecule able to support the survival of primary cultured dopamine neurons

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Introduction:

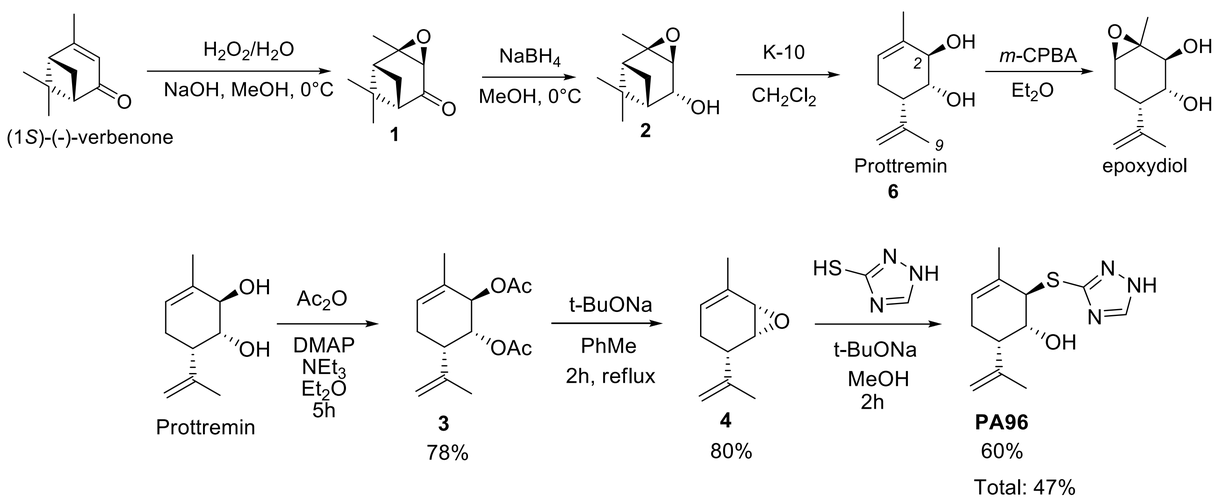
•Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with motor symptoms (tremor, rigidity, etc.) as well as cognitive and behavioral problems. [**1**]

• Levodopa, the main drug for Parkinson's disease treatment, has serious side effects including nausea and appetite loss, involuntary movements, twisting motions and abnormal postures (dystonia), etc. Prolonged use of Levodopa also gives rise to "on / off" episodes, resulting in additional complications. [**2**]

•Monoterpenoid (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (**Prottremin**) demonstrated high anti-PD activity *in vivo* on animal models in mice and rats. [**3**]. Likewise, Prottremin's monoepoxide estimated its anti-PD activity in *in vitro* and *in vivo*. Our data indicate that epoxydiol supports cultured naïve and MPTP -treated dopamine (DA) neurons, increases DA content in the brain, and alleviates motor symptoms of PD. [**4**]

•We present stereoselective synthesis of (1*R*,2*R*,6*S*)-2-(1*H*-1,2,4-triazol-3-ylthio)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-enol (PA96) with the same stereochemistry of all asymmetric centers as in parent compound (Prottremin). Derivative PA96 demonstrates high anti-PD in mice MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and haloperidol models of PD as well as restoration of dopamine concentration in midbrain and survival of cultured dopamine neurons.

Synthesis of **5**



MPTP-induced model of PD in mice

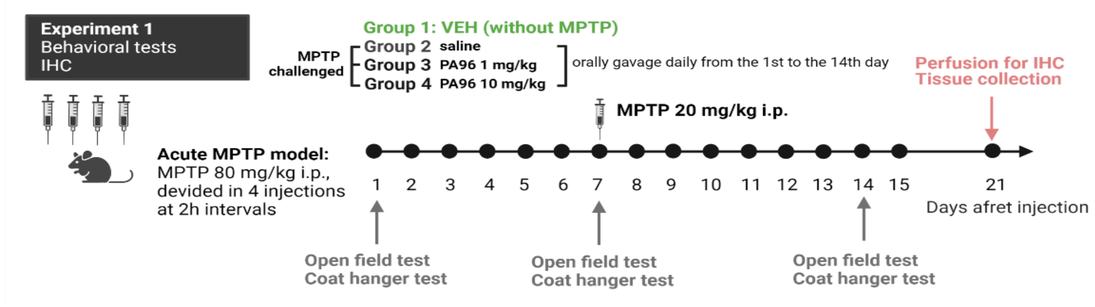


Fig.1. Study timeline of chronic experiment and the timing of assays. MPTP was injected intraperitoneally to C57Bl/6 male mice every 2 h in 8 h period in day 0 in a dose of 20 mg/kg for a total of four doses, and one additional dose of MPTP was injected on the 7th day. The timeline shows the treatment period of 14 days with PA96.

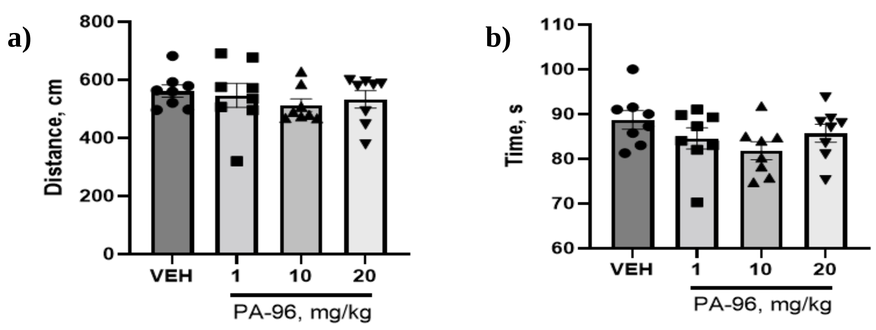


Fig.2. The influence of PA96 on locomotor activity of MPTP-treated mice: movement distance (**a**) and duration of locomotor activity (**b**) and. Mean ± SEM. N = 8 – 10 mice per group. * P < 0.05, ** P < 0.01, *** *P* < 0.001, **** *P* < 0.0001 compared to the VEH (vehicle) group. PA96 doesn't activate locomotor activity of MPTP-treated mice.

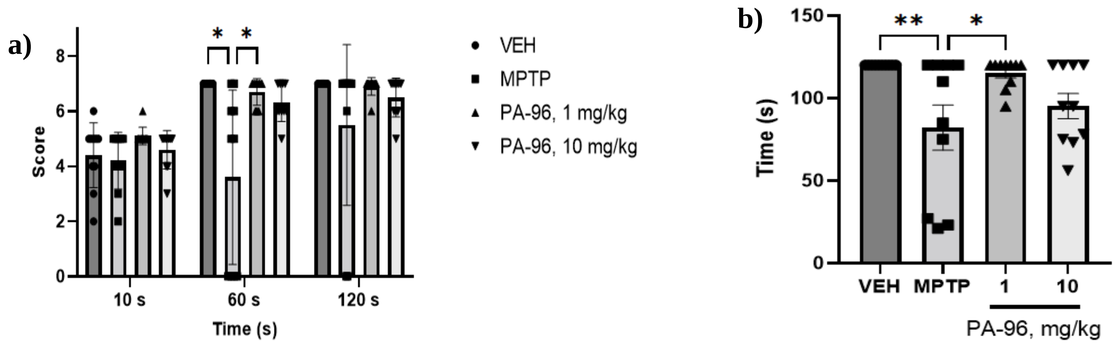


Fig.3. The influence of PA96 on motor coordination of MPTP-treated mice: appraised scores based on scoring criteria (**a**) and duration of movement activity (**b**). Mean ± SEM. N = 10 mice per group. * P < 0.05, ** P < 0.01 compared to the MPTP group.

Haloperidol-induced catalepsy in rats

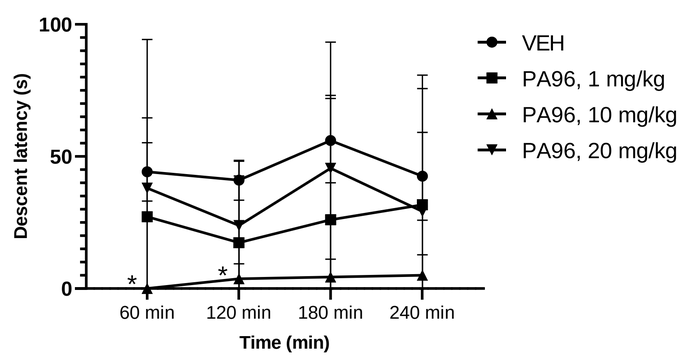


Fig.4. Time course of parallel bars test showing the effect of PA96 on haloperidol-induced catalepsy. One-way ANOVA with Dunnett's post-hoc test analysis was used to compare descent latency between VEH and other groups p<0.05* vs. VEH. Chi-square analysis was used to compare the percent of the cataleptic response to haloperidol between mice with VEH and PA96-treated ** P < 0.01, *** P < 0.001 vs. VEH.
Consequently, PA96 significantly decreased catalepsy time (Fig. 4), the percent of cataleptic animals.

Supports the Survival of Naïve and MPP+ Treated Dopamine Neurons

PA96 screened on cultured primary DA neurons. PA96 was taken at a dose of 0.1 and 1 μM (based on effective doses for the epoxydiol [**3**]. PA96 promoted the survival of naïve tyrosine hydroxylase (TH)-positive neurons in a dose-dependent manner. In the wells treated with 1 nM PA96, the number of TH-positive neurons was 40% greater (P = 0.04, ANOVA with Dunnett's post-hoc test) than that in the VEH-treated wells. PA96 protected cultured dopamine neurons against MPP^+-induced degeneration. In the wells treated with MPP^+ and 1 nM PA96 the number of TH-positive neurons was 2.4 - fold greater than in VEH-treated wells (P =0.03, paired t-test)

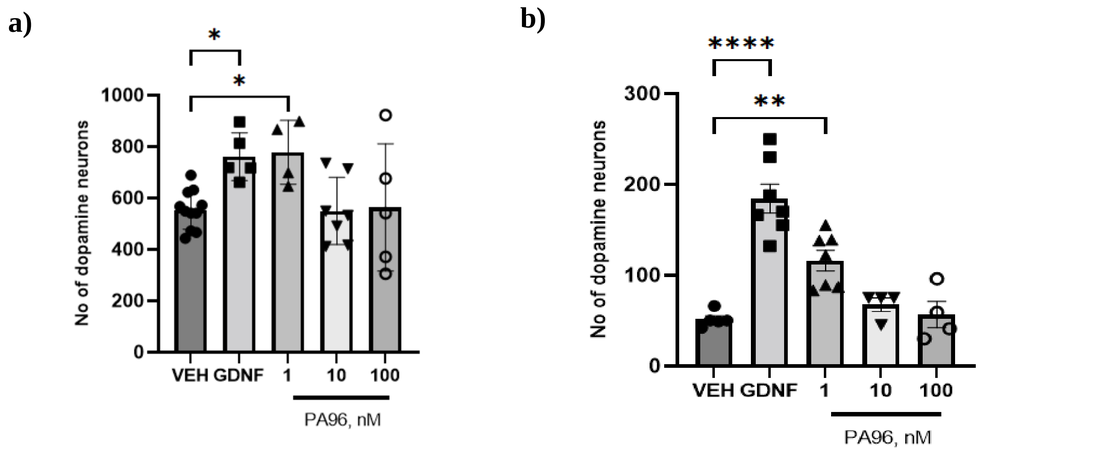


Fig.5. PA96 similarly to glial cell line-derived neurotrophic factor (GDNF) promotes the survival of naïve (**a**) and MPP^+-treated (**b**) primary midbrain dopamine neurons from wild-type mice *in vitro*. * P < 0.05, ** P < 0.01, **** *P* < 0.0001, ANOVA with Dunnett's post-hoc test (**a**) and paired t-test (**b**) compared to VEH. Number of independent experiments (N) = 4-10.

Evaluation of the neuroprotective properties of PA96 in the nigrostriatal dopamine system

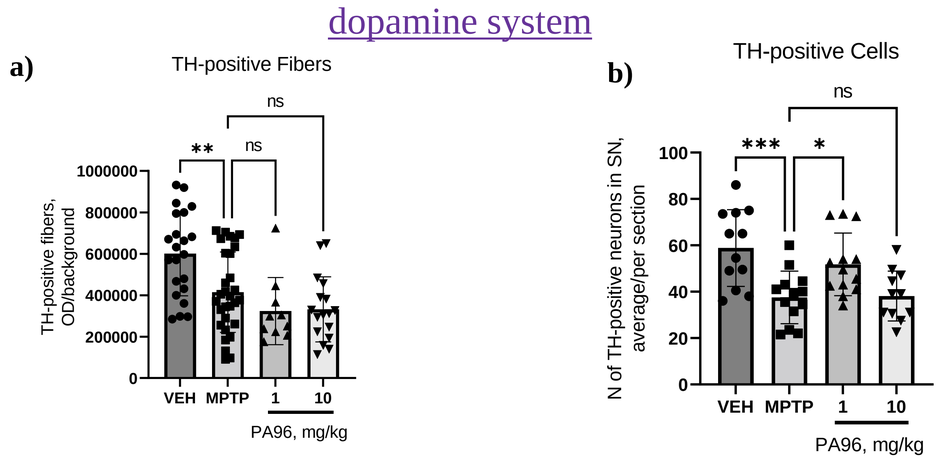


Fig. 6 The influence of PA96 (1 and 10 mg/kg) on the density of TH-positive fibers in the striatum (**a**) and the number of TH-positive cells in substantia nigra (SN), (**b**) of MPTP-treated male C57Bl/6 mice. The data are presented as Mean ± SEM, N = 3–6 per group. * *P* < 0.05, ** *P* < 0.01,*** *P* < 0.001 compared to the MPTP group. Administration of MPTP reduced the density of TH-positive fibers in the ST by 1.5 times as compared to VEH-treated mice. However, significant changes in the density of TH-positive fibers in the ST were observed in none of the treatment groups. We also studied the effect of PA96 on the number of TH-positive neurons in the SN. Treatment of MPTP mice with 1 mg/kg of PA96 increased by 37% the number of TH-positive neurons as compared to that in MPTP-treated mice.

Reference

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[3] Ardashov, O.V., et al. Highly Potent Activity of (1R,2R,6S)-3-Methyl-6-(prop-1-en-2-yl)cyclohex3-ene-1,2-diol in Animal Models of Parkinson's Disease. J. Med. Chem 2011;54: 3866- 3874.

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