***N*-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine (F2MPA): A Potential Cognitive Enhancer with MAO Inhibitor Properties**

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SUMMARY

Background: A considerable body of human and animal experimental evidence links monoaminergic systems and cognition. Monoamine oxidase inhibitors (MAOIs), being able to enhance monoaminergic transmission and having neuroprotective properties, might represent a promising therapeutic strategy in cognitive impairment in Alzheimer's disease (AD) and other dementias. **Methods:** The MAO-A and MAO-B inhibition profile of *N*-(furan-2-ylmethyl)-*N*-prop-2-yn-1-amine derivatives (compounds 1–3) were evaluated by fluorimetric method and their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties estimated. The effects of the selected compound 1, *N*-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine (F2MPA), were evaluated on the basic synaptic transmission, long-term potentiation (LTP), and excitability in the dentate gyrus (DG) of the hippocampus of anesthetized rats. **Results:** F2MPA is a partially reversible inhibitor of hMAO-B, with moderate to good ADMET properties and drug-likeness. Intraperitoneal administration of 1 mg/kg F2MPA greatly enhanced basic synaptic transmission, induced LTP, and potentiated electrically induced LTP in the dentate gyrus. Moreover, F2MPA did not modify seizure threshold of pilocarpine-induced convulsion in CD1 mice. **Conclusion:** Our findings suggest that, the MAO-B inhibitor, F2MPA improves DG synaptic transmission without triggering pathological hyperexcitability. Therefore, F2MPA shows promise as a potential cognition-enhancing therapeutic drug.

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Introduction

The dopamine (DA), norepinephrine (NE), and serotonin (5-HT) monoaminergic systems are deeply involved in cognitive processes via their influence on cortical and subcortical regions [1]. Imbalance or dysfunction in these systems has been implicated in numerous brain diseases, including dementia and the neuropsychiatric symptoms of Alzheimer's disease (AD) [2–5]. Indeed, monoaminergic systems are involved in cellular plasticity and memory processes in the hippocampus and cortex and in depression, psychosis, and agitation. Monoaminergic systems, including those located in the locus coeruleus, the raphe nuclei, and the tuberomammillary nucleus, undergo significant degeneration in AD, thereby depriving the hippocampal and cortical neurons from their critical modulatory influence [3]. Moreover, genome-wide association studies have linked polymorphisms in key genes involved in the function of monoaminergic systems and particular behavioral abnormalities in AD [6]. Consistently, increased monoaminergic function has been proven to restore cognitive function and to reduce AD-related pathology in animal models of neurodegeneration and its behavioral abnormalities in AD [7,8].

Therefore, besides acetylcholine (ACh) and acetylcholinesterase, monoamines and monoamine oxidase (MAO; EC 1.4.3.4) might represent a valuable therapeutic target for the treatment of AD [4,5,9]. MAO catalyzes the oxidative deamination of a variety of biogenic and xenobiotic amines, with the concomitant production of hydrogen peroxide [10]. MAO exists as two isozymes: MAO-A and MAO-B, both showing different substrate specificities, sensitivity to inhibitors, and amino acid sequences. MAO-A preferentially oxidizes NE and 5-HT and is selectively inhibited by clorgyline, while MAO-B preferentially deaminates β -phenylethylamine and is irreversibly inhibited by *l*-deprenyl [11].

Along with an enhancing effect on monoaminergic transmission [5], MAO-B inhibitors (MAO-BI) have shown neuroprotective properties [12]. For these reasons, MAO inhibition has been used in the design of multitarget-directed ligands (MTDLs) [9].

Based on our previous work on the synthesis and biological evaluation of novel MAO-BIs [13], we have decided to first investigate the MAO inhibition activity of simple propargylamines such as the *N*-(furan-2-ylmethyl)-*N*-prop-2-yn-1-amines 1–3 (Figure 1). The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of compounds 1–3 were investigated (Supplementary Material) to establish their suitability for *in vivo* studies. We then investigated the effect of intraperitoneal (i.p.) administration of compound 1, *N*-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine (F2MPA), on the excitability of the hippocampus of anesthetized rats *in vivo*. Long-term potentiation

(LTP) in the hippocampus is thought to be the major neurophysiological basis for the development of learning and memory [14] and its impairment has been seen in different animal models of AD as well as in patients with AD [15]. LTP in the dentate gyrus (DG) of the hippocampal formation has been implicated in associative learning [16]. For these reasons, we studied the effect of F2MPA on basal DG granular cell electrophysiological reactivity on an LTP paradigm to screen this candidate as a cognition-enhancing drug. The proconvulsant potential of F2MPA was investigated in naïve CD1 mice to test for potential side effects.

Methods

Determination of hMAO Isoform Activity

The effects of the test compounds 1–3, as free bases, on the enzymatic activity of the hMAO isoform were evaluated by a fluorimetric method that follows the experimental protocol previously described by us [17]. Briefly, appropriate concentrations of the test drugs or reference inhibitors were diluted in 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing recombinant hMAO-A or hMAO-B. Enzyme concentration was adjusted to obtain the same reaction velocity for both MAO-A and MAO-B in our experimental conditions; that is, to oxidize 165 pmol of *p*-tyramine/min/mL in the control assay. Samples were incubated for 15 min at 37°C in a flat-black-bottom 96-well microtest™ plate (BD Biosciences, Franklin Lakes, NJ, USA), then placed in a dark fluorimetric chamber with the addition of 200 μ M Amplex® Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine to start the reaction. The production of H₂O₂ and, consequently, of resorufin was quantified at 37°C in a multidetection microplate fluorescence reader (FLX800TM; Bio-Tek® Instruments, Inc., Winooski, VT, USA) based on the fluorescence generated (excitation, 545 nm, emission, 590 nm) over a 15-min period. Control experiments were carried out, and the potential ability of the above test drugs to modify the fluorescence generated in the reaction mixture was also checked. Fluorescence emission was calculated after subtraction of background activity.

For reversibility assays, a 100 $\times$ concentration of the enzyme used in the experiments described above was incubated at room temperature with a concentration of inhibitor equivalent to 10-fold its IC₅₀. After 30 min, the mixture was diluted 100-fold into a reaction buffer containing Amplex® Red reagent, horseradish peroxidase, and *p*-tyramine and the reaction was then monitored for 15 min. Control tests were carried out by preincubating and diluting in the absence of an inhibitor [18].

Enzyme kinetic constants were determined by testing three different concentrations of the compound in the presence of four independent amounts of substrate *p*-tyramine, added after a 15-min preincubation. Slopes achieved in each experiment were registered and data were then analyzed by global nonlinear regression (3-parameter equation).

The chemicals used in the experiments were the new compounds, moclobemide (F. Hoffmann-La Roche Ltd., Basel, Switzerland), dimethylsulfoxide (DMSO, the vehicle), *l*-deprenyl hydrochloride, iproniazid phosphate, resorufin sodium salt, clorgyline hydrochloride, *p*-tyramine hydrochloride, sodium phosphate, horseradish peroxidase (supplied in the Amplex® Red

Figure 1 Structure of *N*-(furan-2-ylmethyl)-*N*-methylpropyl-2-yn-1-amine derivatives 1 (F2MPA), 2 and 3.

MAO assay kit from Molecular Probes), and membranous MAO isoforms prepared from insect cells (BTI-TN-5B1-4) and infected with recombinant baculovirus containing cDNA inserts for hMAO-A or hMAO-B (Sigma-Aldrich Química S.A., Alcobendas, Spain).

Animal Study

Surgical Procedures

The care and treatment of all animals conformed to Council Directive 86/609/EEC, the Animals Scientific Procedures Act 1986, and local regulations for the care and use of animals in research. All efforts were made to minimize the animals' pain and suffering, and to reduce the number of animals used. Experiments were conducted on male Sprague-Dawley rats and CD1 mice from Charles River Laboratories, Italy.

For the plasticity study, rats were anesthetized by i.p. urethane (1.2 g/kg) administration and positioned in a David Kopf stereotaxic frame. Body temperature was maintained by a heating pad and a temperature controller unit. Field potentials were evoked by stimulating the perforant path (PP) (AP: -8.3 L: 4.8 V: 3.4) with a bipolar stimulating electrode, then recorded by a bipolar electrode placed in the granule cell layer of the DG (AP: -4.8 L: 2.2 V: 3.6) [19] as previously described [20]. Location of the recording electrode was verified histologically. The hydrochloride salt of F2MPA was used for the *in vivo* experiments.

Basal Dentate/Gyrus Cell Reactivity Recording

After 30 min following the surgery, a 15-min baseline of field potentials was recorded for each experiment. Five sweeps of each different interval were averaged. A 30 seconds gap was kept between sweeps. The average of these sweeps served as the control population spike (PS) and field excitatory postsynaptic potential (fEPSP) and their amplitude and slope were expressed as a percentage of these control values. Following the baseline recording, either saline or F2MPA was administered and the effect of this was recorded for 120 min.

Dentate Gyrus LTP Recordings

After postsurgical recovery, single pulses were applied. One pulse was applied every 60 seconds, and five responses were averaged. Pulse strength was set to evoke 40% of the maximum PS amplitude. After a 10-min baseline, either the saline or the drug was administered via i.p. The effect of the treatments on the baseline was observed for 30 min, then a high-frequency pulse train (HFS) was applied (same pulse parameters as in baseline, 1 second at 100 Hz) to evoke LTP. Following stimulation, the changes in amplitude of the population spike were measured for 1.5 h.

Systemic Pilocarpine-Induced Seizures

As an animal model of partial (focal) seizures with complex symptomatology and secondary generalization from the limbic focus [21], CD1 mice were treated with intravenous (i.v.) infusion of

pilocarpine (24 mg/mL) in the lateral tail vein at a constant rate of 150 μ L/min [22]. Methylscopolamine (1 mg/kg, i.p.) was injected 30 min before pilocarpine infusion to prevent peripheral cholinergic symptoms. During the experiment, the animal could freely move in a Plexiglas cage. Intravenous pilocarpine infusion induced head bobbing and bilateral forelimb clonus with rearing, followed by clonic convulsions with loss of righting reflexes (falling), tonic hindlimb extension, and death in all mice. Time was measured from the start of the infusion until the onset of these stages. Seizure thresholds were determined for each animal according to the following equation: dose (mg per kg) = (duration of infusion (s) $\times$ rate of infusion (mL per min) $\times$ drug concentration (mg per mL) $\times$ 1000)/(60 seconds $\times$ weight of mouse (g)). Either saline or F2MPA was administered 30 min before the pilocarpine i.v. infusion and the effect of this was evaluated on the different behaviors.

Statistical Analysis

Unless specified otherwise, results shown in the text and tables are expressed as mean $\pm$ standard error of the mean (SEM) from *n* experiments. Differences between means were determined by one-way analysis of variance (ANOVA) followed by the Dunnett's *post hoc* test for multiple comparisons. Graph Pad Prism software (GraphPad Software, San Diego, CA, USA) was used to perform statistical analysis and to calculate IC₅₀ values and kinetic parameters.

Evaluation of the electrically evoked potential data was conducted using Spike2 software (CED, Cambridge, UK). One-way analysis of variance (ANOVA) for repeated measures followed by Fisher's PLSD test for significance was used for the comparison of PS amplitude and fEPSP in vehicle and F2MPA-treated groups. The seizures threshold on the pilocarpine-induced stereotyped behavior data were analyzed using the two-tailed unpaired Student's *t*-Test. Differences were considered significant at $P < 0.05$.

Results

Chemistry

Starting with commercial and readily available 1-(furan-2-yl)-*N*-methylmethanamine (5) and furan-2-ylmethanamine (6), a reaction with propargyl bromide gave F2MPA and compounds 2, 3 in good yields (Scheme 1). Their analytical and NMR data are in very good agreement with those shown in the literature (Supplementary Material). Amine 3 has been reported here for the first time, but compounds F2MPA [23,24] and 2 [25] have been previously described.

Computer predictions were used to provide insight into the druggability of these compounds. Using ADMET Predictor 6.53 and ACD/Percepta 14.0.04 software packages, the predictions are satisfactory for these structures (Table S2). All of them show high intestinal absorption, and F2MPA is predicted to have lower plasma retention and better brain penetration than *l*-deprenyl. Although compounds 2 and 3 are predicted to show carcinogenicity, F2MPA lacks toxic effects, making F2MPA a promising compound for *in vivo* study.

Scheme 1 Synthesis of the target compounds 1–3.

Biological Activity

The biological evaluation of the test drugs on hMAO activity was investigated by measuring their effects on the production of H₂O₂ from *p*-tyramine, a common substrate for both hMAO-A and hMAO-B, using the above method following the general procedure previously described by us [17]. Test drugs (new compounds and reference inhibitors) were unable to react directly with the Amplex[®] Red reagent, indicating that these drugs do not interfere with the measurements. The hMAO-A displayed a Michaelis constant ($K_m = 514 \pm 46.8 \mu\text{M}$) and a maximum reaction velocity ($V_{\text{max}} = 301.4 \pm 27.9 \text{ nmol/min/mg protein}$), whereas hMAO-B showed a $K_m = 104.7 \pm 16.3 \mu\text{M}$ and a $V_{\text{max}} = 28.9 \pm 6.3 \text{ nmol/min/mg protein}$ ($n = 5$).

The hMAO inhibitory activities of the novel compounds and reference inhibitors are reported in Table 1. Compounds 2 and 3 showed no inhibitory activity against hMAO-A and hMAO-B in the concentration range studied. F2MPA was able to inhibit hMAO-B, displaying an IC_{50} of $5.16 \pm 0.86 \mu\text{M}$ and a selectivity ratio >19 (no inhibition of MAO-A was observed). Comparing with the inactive compound 2, these results highlight the importance of a methyl group, as in F2MPA, but not a second propargyl

moiety, as in compound 3, for the hMAO inhibition and selectivity. Compared with other reference MAOIs used as standards, F2MPA is 12-fold more potent than clogyline (MAO-A selective), 1.4-fold less active than iproniazide, and 270-fold less active than *l*-deprenyl (MAO-B selective), but more potent than the reversible MAO-A selective inhibitor, moclobemide, for the inhibition of MAO-B.

Furthermore, we studied the reversibility of compound F2MPA by checking the enzyme activity restoration after dilution of the control. Figure 2 shows its partially reversible behavior in contrast to the irreversible inhibitor *l*-deprenyl that remains at 9% of activity after incubation and dilution.

Kinetic assays were also performed for F2MPA that showed uncompetitive behavior; if the inhibitor binding were fully reversible, the K'_i would be $0.27 \pm 0.05 \mu\text{M}$ (Figure 3).

Table 1 IC_{50} values and MAO-B selectivity ratios ($\text{IC}_{50} [\text{MAO-A}]/\text{IC}_{50} [\text{MAO-B}]$) for the inhibitory effects of *N*-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine (F2MPA) and reference inhibitors, on the enzymatic activity of human recombinant MAO isoforms expressed in baculovirus-infected BTI insect cells

DRUG	MAO-A (IC_{50})	MAO-B (IC_{50})	Ratio
F2MPA	^a	$5.16 \pm 0.86 \mu\text{M}$	$>19^c$
Clogyline	$4.5 \pm 0.3 \text{ nM}^{**}$	$61.4 \pm 1.1 \mu\text{M}$	0.00007
<i>l</i> -deprenyl	$67.3 \pm 1.0 \mu\text{M}^{**}$	$19.6 \pm 0.9 \text{ nM}$	3.4
Iproniazide	$6.6 \pm 0.8 \mu\text{M}$	$7.6 \pm 0.4 \mu\text{M}$	0.9
Moclobemide	$361 \pm 19.3 \mu\text{M}$	^b	$<0.4^c$

All IC_{50} values shown in this table are the mean $\pm$ SEM from five experiments. Level of statistical significance: $**P < 0.01$ versus the corresponding IC_{50} values obtained against MAO-B, as determined by ANOVA/Dunnett's *post hoc* test. ^aInactive at 100 μM (highest concentration tested). ^bInactive at 1 mM (highest concentration tested). ^cValues obtained under the assumption that the corresponding IC_{50} against MAO-A or MAO-B is the highest concentration tested.

Figure 2 Recovery of hMAO-B activity after the dilution of the incubation (30 min at room temperature) of the 100 $\times$ -enzyme concentration and 10-fold IC_{50} of F2MPA (filled circles) or *l*-deprenyl (filled triangles). Control tests were carried out by preincubating and diluting in the absence of an inhibitor (empty circles). Represented data are mean $\pm$ SEM of three independent assays.

Figure 3 Double reciprocal plots showing $1/v$ versus $1/[S]$ where v is expressed in nmol/min/mg protein and $[S]$ is the substrate concentration (μM). Data shown are mean $\pm$ SEM of three independent assays for control (empty circles) and $0.25 \mu\text{M}$ (empty squares), $0.83 \mu\text{M}$ (filled circles), and $2.5 \mu\text{M}$ (filled squares) of F2MPA.

In vivo Activity

N-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine was chosen for *in vivo* tests in animal models, using rats and mice. The effects of the hydrochloride salt of F2MPA on basal and LTP-induced field potential recordings in the DG were considered. Moreover, the F2MPA proconvulsant properties were tested in the pilocarpine tail vein infusion model.

Time Course of the Effect Induced by F2MPA on the Population Spike Amplitude and Field Excitatory Postsynaptic Potential in the Dentate Gyrus

Systemic administration of F2MPA hydrochloride (1 mg/kg , i.p.; $n = 3$) produced a significant increase in the amplitude of the PS that attained a maximum of $166.6 \pm 41.3\%$ (F2MPA vs. saline; $F_{1,6} = 12.507$, $P < 0.01$) of baseline values (Figure 4), respectively, when compared with the vehicle group (saline, $n = 5$). As shown in Figure 4A, the effect became apparent after 10 min ($+21.4 \pm 10.6$ $P = 0.03$) and the PS amplitudes increased gradually until the end of the recording. The increase in the PS amplitude of more than 60% over the baseline (which lasted for more than 120 min) indicated a F2MPA-induced LTP in the hippocampal DG. Conversely, the slope of fEPSP was unaffected overall by F2MPA treatment (F2MPA vs. saline, $F_{1,6} = 4.607$, $P = 0.10$) with a trending increase over time (Figure 4B).

Time Course of Long-Term Potentiation Caused by F2MPA Registered in the Dentate Gyrus

During the pre-HSF period, peripheral administration of F2MPA hydrochloride (1 mg/kg , i.p.; $n = 3$) significantly increased the amplitude of the PS ($137.5 \pm 17.9\%$ after 40 min, F2MPA vs. saline, $F_{1,8} = 7.495$, $P < 0.05$; $n = 6$) without influencing the slope of the fEPSP ($104.1 \pm 2.3\%$ after 40 min, F2MPA vs. saline

Figure 4 Time-course plots of perforant path (PP)-evoked population spike (PS) responses of dentate granular cells of the control group and F2MPA groups. Empty circles, saline ($n = 5$); filled circles, F2MPA (1 mg/kg , i.p.; $n = 3$). Drugs were injected i.p. at the arrow (time = 0). Each point represents the mean $\pm$ SEM of the response, as a percentage of baseline values. In (A) the F2MPA sustained and significant increase effect on population spike (PS) amplitude and in (B) on fEPSP slope versus the vehicle group. *N*-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine (F2MPA). One-way ANOVA for repeated measures followed by Fisher's PLSD post hoc test; F2MPA versus saline group, $*P < 0.05$, $**P < 0.01$.

$F_{1,8} = 0.326$, $P = 0.5834$; $n = 6$) compared with the vehicle group (saline, $n = 4$) (Figure 5). As can be noticed in Figure 5A, these values were similar to the results that we obtained in the time-course experiments (time 40, PS amplitude $131.4 \pm 15.6\%$; fEPSP $103.1 \pm 4.1\%$). According to well-established findings [26], HFS induced LTP for PS amplitude and fEPSP slope and these changes were followed for up to 90 min for both vehicle and drug-treated groups (Figure 5A,B). The HSF produced a moderate potentiation of evoked potentials in the saline group. The PS amplitude was increased by 130% after HSF, which was then decreased to 122.6% at 30 min, 118.8% at 60 min, and 112.7% at 90 min. Field EPSP slope reached 122.4% of increase after HSF and returned to 116.8% at 90 min. Figure 5 shows the significant effects of F2MPA on the induction of LTP (F2MPA vs. saline; $F_{1,8} = 10.212$, $P < 0.05$). The PS amplitude was increased by 1 mg/kg F2MPA by 247% after HSF, which was then decreased to 211.4% at 30 min, 205.9% at 60 min, and 207.1% at 90 min. In

Figure 5 Changes in long-term potentiation (LTP) after F2MPA administration in the perforant path (PP)-dentate gyrus (DG) synapses. **(A)** Time-course changes of the amplitude of population spike (PS) before and after HSF-induced LTP (100 Hz, 1 second) in the F2MPA and vehicle group. **(B)** Time-course changes of the slope of fEPSP before and after HSF-induced LTP in the F2MPA and saline group. Drug and vehicle were injected i.p. at the arrow. *N*-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine (F2MPA). One-way ANOVA for repeated measures followed by Fisher's PLSD post hoc test; F2MPA versus saline group, * $P < 0.05$, ** $P < 0.01$.

the group of rats treated with F2MPA, the fEPSP slope reached 125.4% of increase after HSF. This increase was not statistically significant compared with control (F2MPA vs. saline; $F_{1,8} = 0.255$, $P = 0.62$) (Figure 5B). Thus, in our study, F2MPA increased PS amplitude in normal condition and after tetanic stimulation, while the fEPSP was not affected by either electrical paradigms.

Effect of F2MPA Treatment on Pilocarpine-Induced Limbic Seizures with Secondary Generalization

Infusion of pilocarpine into the lateral tail vein of mice produced an array of rapidly progressing behaviors (Figure 6). We did not reveal any significant effects of F2MPA on the threshold dose of pilocarpine for inducing shivering (F2MPA $n = 10$, 215.5 ± 20.84 ; saline $n = 10$, 251.6 ± 15.40 ; student's unpaired *t*-Test; $P = 0.1815$), tail twitching (F2MPA $n = 10$, 325.10 ± 12.81 ;

Figure 6 Effects of F2MPA treatment on seizure threshold of CD1 mice. Graphical representation of the threshold doses of pilocarpine ($n = 10$ for each group) needed to induce the typical consecutive behaviors in saline and F2MPA-treated groups, saline and F2MPA were administered 30 min prior pilocarpine infusion. No statistically significant changes were observed (unpaired Student's test). Tonic hindlimb extension (THE); *N*-(furan-2-ylmethyl)-*N*-methylprop-2-yn-1-amine (F2MPA).

saline $n = 10$, 372.3 ± 22.38 ; student's unpaired *t*-Test; $P = 0.0842$), rearing (F2MPA $n = 10$, 419 ± 38.30 ; saline $n = 10$, 445.1 ± 27.9 ; student's unpaired *t*-Test; $P = 0.5898$), clonic convulsions with loss of righting reflexes (F2MPA $n = 10$, 431.0 ± 28.25 ; saline $n = 10$, 460.5 ± 26.81 ; student's unpaired *t*-Test; $P = 0.4592$), tonic hindlimb extension (F2MPA, $n = 9$, 464.4 ± 30.91 ; saline, $n = 10$, 484.3 ± 33.63 ; student's unpaired *t*-Test; $P = 0.6710$), and death (F2MPA, $n = 10$, 468.7 ± 31.19 ; saline, 500.9 ± 33.29 ; student's unpaired *t*-Test; $P = 0.4900$).

Discussion

In this work, we have reinvestigated inhibition of MAO by F2MPA [23] and two related and readily synthesized propargylamines, *N*-(furan-2-ylmethyl)prop-2-yn-1-amine (2) and *N*-(furan-2-ylmethyl)-*N*-(prop-2-yn-1-yl)prop-2-yn-1-amine (3). Only F2MPA is a partially reversible and uncompetitive inhibitor of hMAO-B with a selectivity ratio over MAO-A > 19 . The IC_{50} is $5.16 \pm 0.86 \mu M$, and a K_i of $0.27 \pm 0.05 \mu M$ was determined. Compared with other reference MAOIs, F2MPA is 12-fold more potent than the MAO-A-selective clorgyline, but 270-fold less active than *l*-deprenyl for the inhibition of MAO-B. The ADMET properties of F2MPA (Supplementary Material) identify it as a suitable lead compound for the development of new therapeutic strategies for the cognitive decline of AD. The latter assumption is further confirmed by the *in vivo* electrophysiological results in urethane-anesthetized rats, obtained by i.p. injection of the hydrochloride salt of F2MPA. Here, we show the effect of F2MPA in the PP-DG synapse by means of electrophysiological recording of granular cell reactivity and electrical induction of LTP. We chose to study DG granular cells as they receive prominent monoaminergic input from the midbrain and brain stem nuclei [27]. We found that the PS was potentiated within 10 min following administration of F2MPA at a dose of 1 mg/kg. This effect was sustained over time, reaching its maximum value (about 60%) at the end of recording (2 h), resembling the chemically induced LTP.

Indeed, the striking effect of F2MPA is similar to that of the long-lasting potentiation effect that NE has on the DG PS, termed as NE-LLP or NE-LTP (to distinguish it from tetanic LTP), which is mediated by activation of β -adrenoreceptors and increase of cAMP in granular cells [28,29]. Conversely, F2MPA did not significantly potentiate the fEPSP evoked by PP single pulse stimulation, suggesting a preferential postsynaptic locus of action, at the transition between the synapse and the spike-generating mechanism in the granular layer, without affecting release of glutamate by PP fibers. The present finding is consistent with the lack of effect of NE on fEPSP in DG [28], further suggesting a NE involvement in the effects of F2MPA. The most striking result to emerge from our data is that F2MPA pretreatment induced a strong potentiation of the induction of LTP by PP tetanic stimulation (100 Hz), seen as an increase in PS amplitude (about 200 times larger of that obtained in the vehicle group). Similar to the effects on basal synaptic responses, F2MPA did not modify the increase of the slope of the fEPSP induced by LTP, further confirming a preferential influence on the postsynaptic component observed in basal conditions. Other monoamines might also be involved in the effects of F2MPA. Indeed, 5-HT increases PS amplitude with no concomitant change to the fEPSP [30,31]. Considering that MAO-Is can also increase synaptic concentration of GABA [32] and reduce formation of oxygen radicals and nitric oxide [4] in different brain areas showing additional antioxidant properties [12], it is possible that the integration of multiple mechanisms underlies the changes produced in granular cell excitability following administration of F2MPA. Further work needs to be carried out to establish which mechanism is the most important for F2MPA to exhibit its effects in granular cell excitability.

This is the first electrophysiological study on the effect of a MAO-BI in the DG. The few available findings in literature are indeed limited to the Schaeffer collateral-commissural pathway of the rat hippocampus, are also *in vitro* [33–35] and show a different scenario in the CA3-CA1 synapses to our data in the PP-DG. For instance, *l*-deprenyl reduced the amplitude of EPSP and this attenuation was DA-dependent by decreasing glutamate release from presynaptic terminals [35], but it did not affect the induction and maintenance of LTP in CA1 [33]. Conversely, chronic administration of the MAO-A selective inhibitor, moclobemide, shifted hippocampal synaptic plasticity toward facilitation of long-term depression (LTD) and blockade of LTP in control mice, while attenuated impairment in LTP was seen in mice with impaired glucocorticoid receptor function [34]. In agreement with the latter

evidence, MAO-BIs antagonise scopolamine-induced impairments [36,37] and improved cognitive performance in middle-aged rats in the Morris water maze [38]. The study of the link between MAO-I and learning and memory by behavioral experiments in rodents has also produced opposite results. For instance, inhibition of MAO-A or MAO-B, alone or in combination, did not facilitate spatial learning in rats [39], and MAO-B knockout mice do not show protection from the age-dependent deficits in spatial learning [40]. It is also possible that some of the cognitive effects by MAO-BIs are not attributable to MAO-B inhibition *per se* [33,38].

Regardless of the mechanisms, MAO-BIs might be an effective treatment for AD, and the ongoing clinical trials [41] will give some answers about this therapeutic possibility.

Although preliminary, our *in vitro* and *in vivo* results demonstrated a potentially novel effect of [33–35] as a CNS drug. Indeed, [33–35] is a moderate, but selective, partially reversible, and uncompetitive MAO-B inhibitor with moderate to good ADMET properties and drug-likeness and also has high biological activity. It is effective when peripherally administered increasing the reactivity of DG granular cells and inducing/potentiating LTP in the PP-DG synapse. It is notable that [33–35] did not modify the threshold for the induction of pilocarpine-induced (limbic and generalized) epilepsy, ruling out potential risk for inducing convulsion. This finding is especially important in consideration of the fact that AD is a risk factor for epilepsy and seizures and can occur in some patients [42]. Further research is needed to clarify the link between MAO-I, AD [3–5,9] and learning and memory [4,39], but F2MPA could offer new hope to sufferers of this devastating disease. In conclusion, F2MPA shows potential for development as a therapeutic drug for cognition improvement in patients with AD and other dementias.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Data S1. Supplemental material.

Table S2. Physicochemical properties for structures.