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Graphical Abstract

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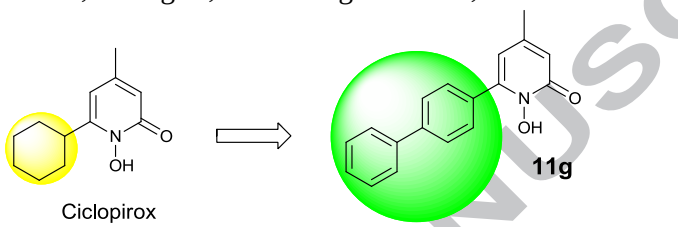

Mitochondrial metabolic activity in astrocytes exposed to H_2O_2

IC_{50} (μM) = 0.33 ± 0.05 IC_{50} (μM) = 0.21 ± 0.06

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Discovery of a novel series of *N*-hydroxypyridone derivatives protecting astrocytes against hydrogen peroxide-induced toxicity *via* improved mitochondrial functionality

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ABSTRACT

Astrocytes play a key role in brain homeostasis, protecting neurons against neurotoxic stimuli such as oxidative stress. Therefore, the neuroprotective therapeutics that enhance astrocytic functionality has been regarded as a promising strategy to reduce brain damage. We previously reported that ciclopirox, a well-known antifungal *N*-hydroxypyridone compound, protects astrocytes from oxidative stress by enhancing mitochondrial function. Using the *N*-hydroxypyridone scaffold, we have synthesized a series of cytoprotective derivatives. Mitochondrial activity assay showed that *N*-hydroxypyridone derivatives with biphenyl group have comparable to better protective effects than ciclopirox in astrocytes exposed to H_2O_2. *N*-hydroxypyridone derivatives, especially **11g**, inhibited H_2O_2-induced detrioration of mitochondrial membrane potential and oxygen consumption rate, and significantly improved cell viability of astrocytes. The results indicate that the *N*-hydoxypyridone motif can provide a novel cytoprotective scaffold for astrocytes via enhancing mitochondrial functionality.

1. Introduction

Astrocytes play a key role in the maintenance of homeostasis and function of the central nervous system. One of major homeostatic function of astrocytes provides defence mechanism against oxidative stress to neurons. In various neurological disorders associated with oxidative stress, astrocyte dysfunction has been implicated in the onset and progression of neurodegenerative process.[1] Furthermore, recent evidence suggests that enhancing or recovering astrocytic functions could represent a valuable strategy for neuroprotection.[2] For example, selective upregulation of astrocyte-derived factors such as Nrf2, lactate, or heme oxygenase-1 in astrocytes rescues activity or viability of nearby neurons under neurotoxic stimuli.[3–6] Therefore, astrocytes has been emerging as an attractive target for novel drug discovery, especially in neurodegenerative diseases such as Alzheimer's disease or Parkinson's diseases, as well as ischemic or traumatic brain injury.[7]

N-hydroxypyridone motif is included in the structure of a variety of bioactive compounds. Naturally occurring *N*-hydroxypyridone derivatives are often synthesized in a wide range for fungi as secondary metabolites and exhibit an extensive diversity of biological functions including anti-oxidant,[8] anti-bacterial,[9,10] anti-fungal,[11] and anti-malarial activities.[12]

Among synthetic *N*-hydroxypyridone derivatives, the anti-fungal agent, ciclopirox [6-cyclohexyl-1-hydroxy-4-methyl-2(1*H*)-pyridone] is one of the most well-known compounds. Although it has been used as a topical treatment for cutaneous fungal infections, recent phase I clinical study in patients with refractory hematologic malignancies demonstrated the oral systemic regimen was well tolerated in humans.[13] Furthermore, recent studies supported an appealing possibility to repurpose ciclopirox as an antibiotic against drug-resistant infections,[14] anti-tumor agent,[15] anti-human immunodeficiency virus drug,[16] or an agent to enhance angiogenesis and wound healing in diabetic environment.[17]

Another attractive possibility of repurposing ciclopirox is as a cytoprotective drug against oxidative stress, especially through protection of mitochondrial functionality. Mitochondria are the source of free radicals and in turn, the major target of oxidative stress. While impaired mitochondrial functionality in astrocytes contributes to neuronal injury,[18–20] protection of mitochondria in astrocytes enhances neuronal survival.[21] For example, the

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overexpression of antioxidant molecules, such as superoxide dismutase 2 (SOD2), glutathione, or chaperones, such as HSP70 or GRP 78 has proven to effectively reduce neuronal death in experimental models of brain diseases.[21] In line with this view, we previously found that ciclopirox protects astrocytes from peroxynitrite-induced cytotoxicity by attenuating mitochondrial dysfunction.[22] Ciclopirox inhibited peroxynitrite-induced depolarization of mitochondrial transmembrane potential ($\Delta\psi m$) and resulting ATP depletion. Ciclopirox also blocks H_2O_2-induced mitochondrial injury in SK-HEP-1 cells by maintaining $\Delta\psi m$ and preventing mitochondrial permeability transition pore (MPTP) opening.[23] Ciclopirox acted directly on isolated mitochondria and efficiently blocked H_2O_2-induced depolarization of $\Delta\psi m$.

In this context, we synthesized a series of various *N*-hydroxypyridone derivatives and explore the efficacy of ciclopriox and its derivatives against oxidative stress-evoked cytotoxicity in astrocytes. Here we demonstrate that ciclopirox and selected derivatives effectively inhibited H_2O_2-induced mitochondrial dysfunction and thus, resulting cytotoxicity along with structure-activity relationship (SAR) study of the derivatives.

2. Results and discussion

2.1. Chemistry

For the synthesis of *N*-hydroxypyridone derivatives, we focused on the modification of cyclohexane moiety of ciclopirox with diverse aromatic rings since multi-aromatic ring containing compounds such as flavonoids are well known for their anti-oxidant properties. For the purpose, retrosynthetic analysis was carried out as shown in figure 1. Figure 1A represents the classical approach of synthesis of ciclopirox where the source of cyclohexyl group was a substituent on the corresponding acid chloride.[24-25]

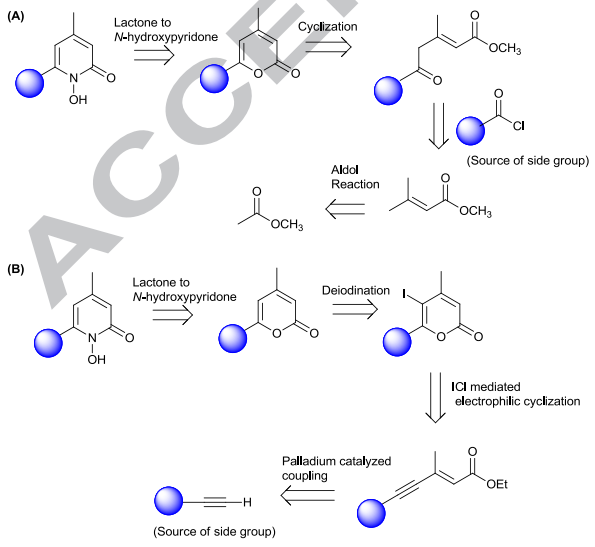
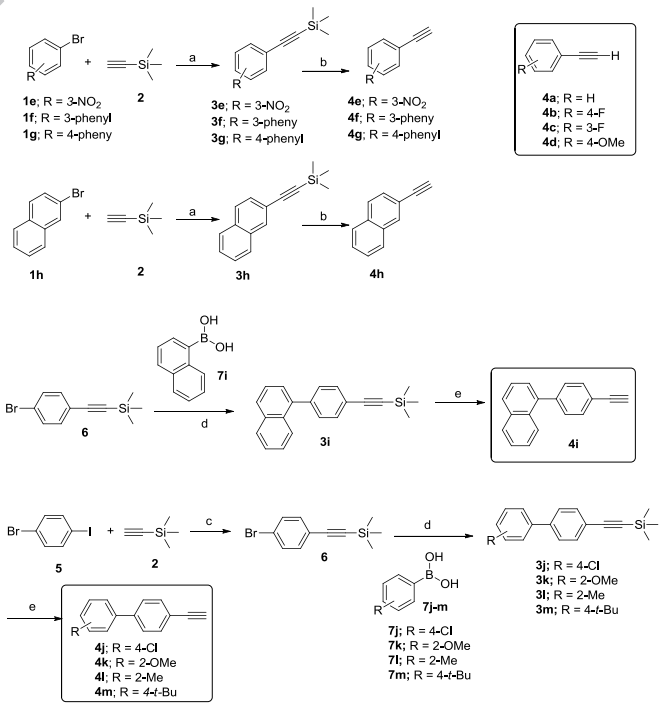


Fig. 1. Retrosynthetic plan for the synthesis of *N*-hydroxypyridone derivatives; A) Classical approach; B) Our approach.

When we attempted to synthesize different aromatic *N*-hydroxypyridone derivatives with this approach; we observed no cyclization or very poor yield if any. To address this issue we designed another retrosynthetic plan as shown in figure 1B. In this synthetic plan, the substituents on the acetylene derivatives determines the side chain group on final *N*-hydroxypyridone derivatives, which would be employed to palladium catalyzed coupling with but-2-ynoate to afford enyne esters. The enyne esters would be subjected to electrophilic cyclization to allow the desired pyridones.

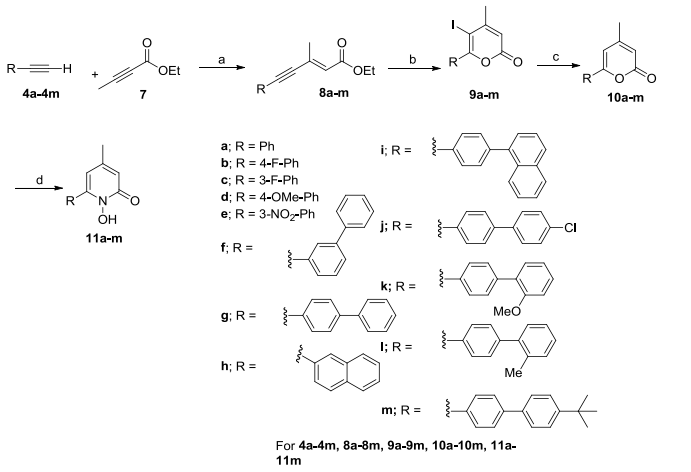
For this purpose, we first synthesized a variety of acetylene derivatives as shown in scheme 1. Acetylenes **4a—d** were commercially available whereas acetylenes **4e—h** were synthesized by Sonogashira coupling of trimethylsilyl acetylene (**2**) with different bromobenzene derivatives (**1e—h**) employing catalytic amount of $Pd(Ph_3)_2Cl_2$ and CuI in Et_3N at 60°C, followed by deprotection of trimethylsilyl group of corresponding compounds **3e—h** by K_2CO_3 in THF:MeOH (1:1) (scheme 1). Acetylene derivatives **4i—m** were synthesized by Sonogashira coupling of 1-bromo-4-iodobenzene (**5**) with ethynyltrimethylsilane (**2**) using $Pd(PPh_3)_2Cl_2$/CuI in Et_3N to afford ((4-bromophenyl)ethynyl)trimethylsilane (**6**), followed by coupling of later with different boronic acids (**7i—m**) via Suzuki reaction to afford products **3i—m**. The phenylacetylene derivatives **4i—m** were then obtained in quantitative yields by deprotection of corresponding compounds **3i—m** by K_2CO_3 in MeOH:THF (1:1).



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 60°C (79-90%); (b) K_2CO_3, THF:MeOH (1:1), r.t (90-98%); (c) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, r.t (98%); (d) $Pd(PPh_3)_2Cl_2$, aq. Na_2CO_3, DME, 120°C (69-91%); (e) K_2CO_3, THF:MeOH (1:1), r.t (69-98%).

Synthesis of compounds **11a—m** is shown in scheme 2. First, different acetylene derivatives (**4a—m**) were coupled with ethylbut-2-ynoate (**7**) using catalytic amount of $Pd(OAc)_2$ and TDMPP in benzene to afford corresponding products **8a—m** in moderate to high yields. Then the electrophilic cyclization of products **8a—m** was performed

with ICl in DCM to afford iodo-substituted cyclized products **9a—m** in quantitative yields, followed by their de-iodination with $Pd(OAc)_2/PPh_3$ in Et_3N/HCOOH to give products **10a—m**. Finally, compounds **11a—m** were synthesized by refluxing the corresponding compounds **10a—m** with $NH_2OH{\cdot}HCl$ in pyridine at 100°C (Scheme 2).



Scheme 2. Reagents and conditions: (a) $Pd(OAc)_2$, TDMPP, benzene (44-87%); (b) ICl, DCM (dry), r.t; (c) $Pd(OAc)_2$, PPh_3, Et_3N, HCOOH, DMF, 62°C (21-60%); (d) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 50°C (36-93%); (e) $NH_2OH.HCl$, pyridine, 100°C, 14 hr (50-71%).

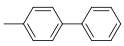
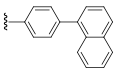
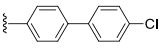
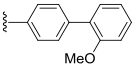
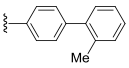
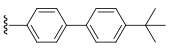
2.2. Biology

Mitochondrial metabolic activity assay The protective effects of ciclopirox and all the synthesized *N*-hydroxypyridone derivatives (**11a—m**) on oxidative stress was initially evaluated in primary astrocyte cultures exposed to H_2O_2. H_2O_2-induced mitochondrial dysfunction was measured by resazurin-based PrestoBlue® assay, where the non-fluorescent, blue resazurin reduces to the fluorescent resorufin depending on mitochondrial activity.

All the *N*-hydroxypyridone derivatives were tested in a series of dose escalation more than 8 points ranging from 1 nM to 10 µM. From the non-linear regression analysis of dose-response curves, the concentration of drug at which H_2O_2-induced deterioration of mitochondrial activity is half inhibited ($IC_{50,}$ a measure of potency) and the maximum inhibition (% as a measure of efficacy) that a drug can achieve were calculated. As shown in Table 1, ciclopirox protected mitochondrial metabolic activity against H_2O_2 in astrocytes, having an IC_{50} value of 330 nM with a 43.72% inhibition at maximum. To investigate the influence of the cyclohexyl moiety at the *N*-hydroxypyridone nucleus, systematic variations were carried out in this region. The replacement of the cyclohexyl to the phenyl ring markedly reduced the potency by 5 to 10 folds (compounds **11a—e**). Particularly, the addition of fluorine atom at position 3 of the phenyl ring resulted in complete loss of activity.

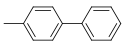
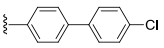
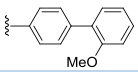
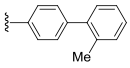
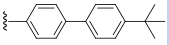
Further structure-activity relationship around the *N*-hydroxy-2-pyridone nucleus revealed that the addition of a biphenyl ring was optimal for potency and efficacy (compounds **11f**, **11g**, **11j—m**). The most active example in this series was the 4-biphenyl derivative **11g**, which showed an IC_{50} of 210 nM with a maximum inhibition value of

Table 1. The effects of *N*-hydroxypyridone derivatives on mitochondrial metabolic activity in astrocytes exposed to H_2O_2.

compds	Structure/R	PrestoBlue® assay IC_{50}[a] (µM)	PrestoBlue® assay Max inhibition[b] (%)
Ciclo-pirox		0.33 ± 0.05	43.72 (32.38–78.81)
11a		4.07 ± 1.46	57.92 (48.06–94.14)
11b		2.74 ± 0.35	26.83 (21.47–53.57)
11c		-	0.75 (0.61–0.79)
11d		1.99 ± 0.29	72.58 (38.35–85.07)
11e		4.74 ± 1.16	41.10 (37.54–70.68)
11f		0.45 ± 0.08	58.67 (35.02–74.27)
11g		0.21 ± 0.06	57.43 (42.26–71.14)
11h		7.10 ± 2.71	16.16 (5.24–54.21)
11i		1.86 ± 1.61	48.70 (42.39–67.34)
11j		0.61 ± 0.14	44.63 (31.98–58.52)
11k		0.34 ± 0.01	58.60 (34.09–66.38)
11l		0.43 ± 0.17	37.43 (34.35–66.45)
11m		0.30 ± 0.03	33.61 (24.56–79.29)

[a]Mitochondrial metabolic activity was determined by using Resazurin-based presto blue® assay following manufacturer's instructions. Cells were loaded with presto blue® reagent at 37°C for 15 minutes and the corresponding fluorescence change was measured (Ex = 540 nm, Em = 590 nm). Analysis of dose-response curve was performed using the non-linear regression fit analysis (log of dose vs. inhibitory response) to calculate the IC_{50} value of the drug that inhibited 50% of H_2O_2-induced mitochondrial metabolic dysfunction. IC_{50}: the dose of derivative required to inhibit H_2O_2-induced dysfunction of mitochondrial metabolic activity by 50%. Data are expressed as the mean ± SEM from the dose-response curve of 3 to 7 independent experiments; [b]Max inhibition (%): the maximal inhibitory response of the derivatives estimated from the non-linear regression curve fit. Data are expressed as a median (interquartile range) of inhibition percentage, N = 3 – 7.

Table 2. The effects of *N*-hydroxypyridone derivatives on H_2O_2-induced astrocytic cell death.

compounds	Structure/R	LDH assay IC_{50}[a] (nM)	LDH assay Max inhibition[b] (%)
			
Ciclopirox		249.9 ± 65.7	50.3 ± 9.1
11f		104.5 ± 1.5	32.6 ± 4.3
11g		71.6 ± 30.7	53.5 ± 10.9
11j		167.0 ± 13.7	29.6 ± 1.4
11k		156.1 ± 14.5	58.8 ± 15.3
11l		106.5 ± 5.6	55.1 ± 9.4
11m		117.7 ± 63.7	59.1 ± 13.6

[a]Cell death was assessed by measuring the amount of lactate dehydrogenase (LDH) released into the culture supernatant using a diagnostic kit. The data were expressed as the percentage of total LDH release measured in repeatedly frozen and thawed parallel cell cultures. IC_{50}: the dose of derivative required to inhibit H_2O_2-induced astrocytic cell death by 50%. Data are expressed as the mean ± SEM from the dose-response curve of 3 independent experiments; [b]Max inhibition (%): the maximal inhibitory response of the derivatives estimated from the non-linear regression curve fit. Data are expressed as the mean ± SEM from the dose-response curve of 3 independent experiments.

57.43 % against H_2O_2-evoked mitochondrial metabolic dysfunction. The replacement of 4-biphenyl to 3-biphenyl ring as a substituent (**11f**) afforded two fold decrease in potency with comparable efficacy. The modification of the biphenyl ring by adding chlorine atom, or methoxy, methyl, or *tert*-butyl group (compounds **11j**, **11k**, **11l**, **11m**, respectively) was well-tolerated and slightly modulated the potency. While the addition of the electron-withdrawing chlorine atom (**11j**) at 4-position resulted in reduction of activity, while the effects of the electron-donating methoxy, methyl or *tert*-butyl groups (compounds **11k—m**) were not dramatic, showing comparable potencies. In contrast, replacement of one of phenyl ring of compound **11g** with the naphtyl ring (compound **11h**) caused noticeable deterioration of the activity. Substitution of the phenyl to 2-naphthyl ring (compound **11i**) also led to marked decrease of the activity.

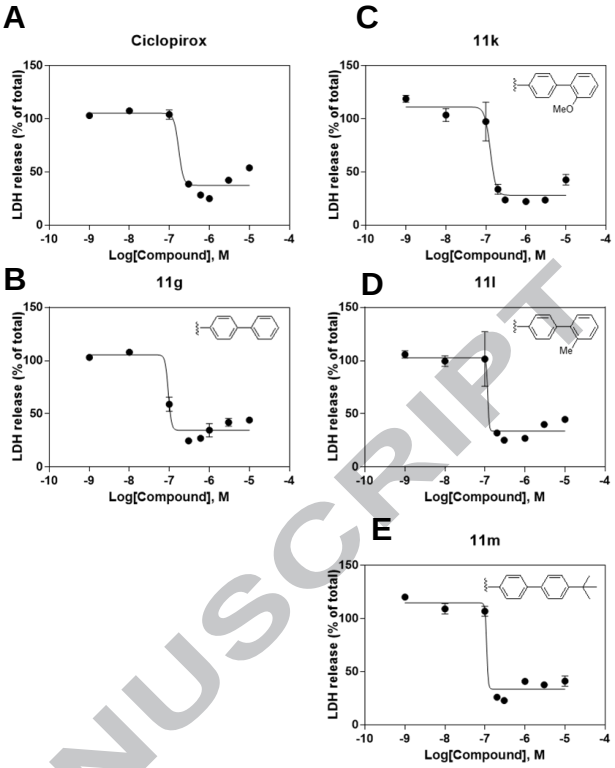


Fig. 2. Dose-response curves regarding protective effects of *N*-hydroxypyridone derivatives against H_2O_2-induced astrocytic cell death measured by the LDH assay. (A—E) Astrocytes were exposed to 500 μM H_2O_2 in the presence or absence of ciclopirox or selected biphenyl N-hydroxypyridone derivatives (**11g**, **11k—m**) ranging 1 nM to 10 μM. Data are expressed as the mean ± SEM (N=3) and the representative curves from 3 independent experiments were shown.

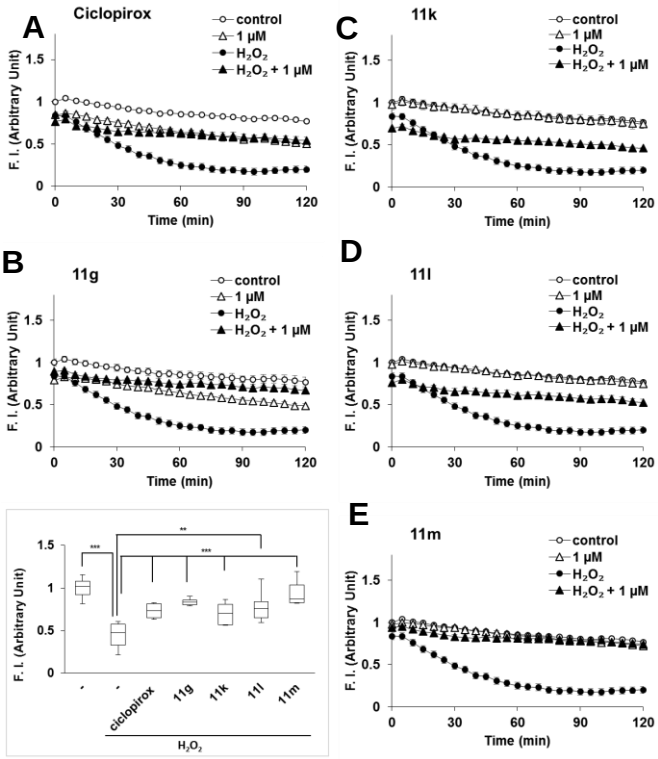


Fig. 3. Protective effects of *N*-hydroxypyridone derivatives in astrocytes against H_2O_2-evoked Δψm depolarization. (A—E) After loading with 500 nM TMRM at 37°C for 10 min, astrocytes were exposed to 500 μM

H_2O_2 in the presence or absence of ciclopirox or a selected biphenyl *N*-hydroxypyridone derivative (**11g, 11k—m**) at the concentration of 1 μM. Fluorescence intensities (F. I.) of TMRM were measured every 5 min for 2 h. Data are expressed as the mean ± SEM (N=4). The endpoint Δψm at 2 hours after treatment were determined based on F. I values of TMRM. (Inlet) Data are expressed as the median (horizontal bar), interquartile ranges (Q1 to Q3, vertical box), and min/max (whiskers). Data were analysed by Kruskal-Wallis test followed by Mann-Whitney test for multiple comparison (N=8). $^{**}P < 0.01$ or $^{***}P < 0.001$ vs H_2O_2 control.

After the initial round of screening, the biphenyl *N*-hydroxypyridone derivatives with high potency and efficacy (compounds **11f**, **11g**, **11j—m**) were selected for further biological characterization. The protective effect of derivatives against H_2O_2-induced cell death was assessed by measuring the cell-injury-evoked release of lactate dehydrogenase (LDH) from astrocytes (Table 2).

When cells were treated with 500 μM H_2O_2 in the presence or absence of biphenyl *N*-hydroxypyridone derivatives, all tested compounds (**11f, 11g**, **11j**—**m**) showed substantial protective effects on cell viability in astrocytes. As shown in Table 1, the 4-biphenyl derivative **11g** showed the highest potency in these series. Interestingly, the replacement to 3-biphenyl ring (compound **11f**) or the addition of chlorine atom at the para position of the phenyl ring (compound **11j**) led to marked reduction in efficacy (39.1% or 44.6% reduction compared with compound **11g**, respectively). The representative dose-response curves of ciclopirox and other four biphenyl *N*-hydroxypyridone derivatives (**11g**, **11k**—**m**)

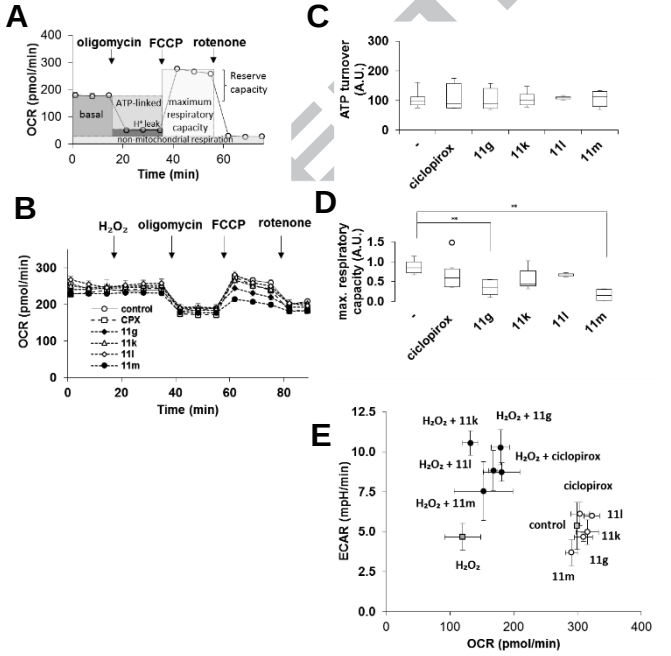


Fig. 4. Modulation of mitochondrial bioenergetics by *N*-hydroxypyridone derivatives in astrocytes. (A) Real-time mitochondrial respiration was monitored using the Seahorse XF24 analyzer after each addition of oligomycin, FCCP, and rotenone. The schematic curve demonstrates resulting changes in oxygen consumption rate (OCR) in response to each modulator and the relative contribution of each bioenergetic parameters. (B) Astrocytic OCR was measured in the presence of ciclopirox or selected biphenyl *N*-hydroxypyridone derivatives (**11g, 11k—m**) at the concentration 1 μM. Data are expressed as the mean ± SEM (N=8). (C, D) ATP turnover-linked OCR and maximum respiration capacity were calculated based on OCR curve. Data are expressed as the median (horizontal bar), interquartile ranges (Q1 to Q3, vertical box), and min/max (whiskers). Data were analysed by Kruskal-Wallis test followed by Mann-Whitney test for multiple comparison (N=8). $^{**}P < 0.01$ vs control. (E) Astrocytes were exposed to 500 μM H_2O_2 in the presence or absence of ciclopirox or selected biphenyl *N*-hydroxypyridone derivatives (**11g, 11k—m**). The effects on the mitochondrial bioenergetic profile were determined by plotting OCR vs. ECAR for each treatment. Data shown are the mean ± SEM (N = 8).

with both high potency and efficacy were shown in Fig. 2. The curves of all derivatives exhibited a steep slope,[26–28] with IC_{50} value ranging tens to several hundreds of nM and 50 to 60 % of maximum cell death inhibition. No apparent cytotoxicity to astrocytes was observed at the tested concentrations of all derivatives by themselves (up to 10 μM, data not shown).

Since the protective effects of ciclopirox on peroxynitrite-induced astrocytic cell death was closely related with its inhibitory effects on mitochondrial Δψ*m* depolarization,[22] we next investigated whether the selected biphenyl *N*-hydroxypyridone derivatives (**11g**, **11k—m**) affect H_2O_2-evoked Δψ*m* depolarization in astrocytes (Fig. 3).

The change of Δψ*m* was monitored by the fluorescence change of the indicator, tetramethylrhodamine methyl ester (TMRM). As shown in Fig. 3, H_2O_2 induced the dissipation of Δψ*m* in astrocytes prior to release of LDH. Ciclopirox and all four derivatives significantly inhibited H_2O_2-induced depolarization of Δψ*m* at 1 μM concentration. Derivatives did not induce Δψ*m* depolarization alone (Data not shown).

We next determined the effects of *N*-hydroxypyridone derivatives on mitochondrial respiration and glycolysis using extracellular flux analysis. This non-invasive technique allows the real-time measurements of O_2 consumption rate (OCR, an indicator of oxidative phosphorylation) and extracellular acidification rate (ECAR, an indicator of glycolysis) simultaneously in intact cells. As shown in a schematic diagram (Fig. 4A), the various parameters of mitochondrial function were determined by measuring OCR after sequential addition of pharmacological inhibitors, oligomycin (a ATP synthase inhibitor to block ATP synthesis), carbonyl cyanide p-[trifluoromethoxy]-phenyl-hydrazone (FCCP, a proton ionophore to uncouple ATP synthesis from the electron transport chain and allow for maximum electron flux), and rotenone (a complex I inhibitor to block the electron transport chain).

As shown in Fig. 4B, ciclopirox and biphenyl *N*-hydroxypyridone derivatives (**11g, 11k—m**) did not significantly change ATP turnover-linked OCR (Fig. 4C) and basal OCR (data not shown). Compound **11g** and **11m** suppressed maximal respiratory capacity (Fig. 4D) and the reserve capacity (data not shown) in astrocytes, without affecting cell survival. As a bioenergetic profile was

assessed by plotting OCR vs ECAR for each treatment, the control cells under basal conditions utilize both oxidative phosphorylation and glycolysis (Fig. 4E, a grey square). Ciclopirox and biphenyl *N*-hydroxypyridone derivatives did not significantly change this metabolic profile under basal conditions (Fig. 4E, white circles). In contrast, H_2O_2 caused a marked decrease in OCR, suggesting mitochondrial dysfunction consistent with loss of membrane potential in astrocytes (Fig. 4E, a dark grey square). The treatment of ciclopirox and biphenyl *N*-hydroxypyridone derivatives (**11g, 11k—m**) decreased the response of OCR to H_2O_2, and stimulated ECAR (Fig. 4E, dark circles), suggesting the restoration of mitochondrial bioenergetic function and an acute stimulation of glycolytic metabolism against H_2O_2. Glycolysis was known to supply glucose-6-phosphate, which can shunt into the pentose phosphate pathway (PPP). PPP drives regeneration of NADPH and Glutathione (GSH) in response to oxidative stress, thus potentially contributing to antioxidant redox status and survival of astrocytes, as previously shown in neurons.[29]

Taken together, these data strongly suggest the protective effects of ciclopirox and biphenyl *N*-hydroxypyridone derivatives (**11g**, **11k—m**) derivatives against H_2O_2-induced cytotoxicity in astrocytes *via* inhibition of mitochondrial dysfunction, by especially preserving mitochondrial $\Delta\psi m$ and bioenergetic profiles.

3. Conclusion

It was newly found that a novel class of *N*-hydroxy-2-pyridone-based derivatives efficiently protected astrocytes from reactive oxygen species like H_2O_2. On the basis SAR studies, we discovered that the addition of biphenyl ring to *N*-hydroxy-2-pyridone resulted in better potency and efficacy. The protective effects of ciclopirox and the biphenyl derivatives might be mainly due to their inhibitory actions against mitochondrial dysfunction. Among all compounds screened, compound **11g** was found to be the most potent showing IC_{50} values in the range of a few hundred nM, which equal or higher than that of ciclopriox. Considering the importance of astrocytes in neuronal bioenergetics and survival in various neurological disorders, the present study suggests that this new class of highly potent and efficient *N*-hydroxy-2-pyridone derivatives open an innovative therapeutic strategy for the treatment of neurodegenerative diseases.

Experimental

Chemistry

All melting points were determined on a Yamato melting point apparatus (model MP-J3). The NMR spectra were recorded on a VARIAN-500 MHz spectrometer. The chemical shifts were reported in parts per million (d) relative to TMS (0.0 ppm) as the internal standard, and the signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). The values of the coupling constant *J* are given in hertz. The mass spectra were recorded using high-resolution mass spectrometry (HRMS) (electron ionization MS) obtained on a JMS-700 mass spectrometer (Jeol, Japan) or using HRMS (electrospray ionization MS) obtained on a G2 QTOF mass spectrometer. TLC was performed on Merck silica gel 60 F254 plates. Column chromatography was conducted using silica gel 60 N (Kanto, 100e210 mm) or silica gel 60 (Kanto, 40e50 mm). HPLC spectra were noted on Water-2695 by using acetonitrile:water/60:40 as eluting system at flow rate of 1 mL/min at 310 nM wavelength.

Synthesis of trimethyl((3-nitrophenyl)ethynyl)silane (3e)

In a two neck rbf containing TEA (90 ml) was added 1-bromo-3-nitrobenzene **1e** (9.00 g, 44.55 mmol) at rt under nitrogen. The mixture was stirred for 5 minutes followed by addition of ethynyltrimethylsilane **2** (6.93 ml, 49.01 mmol), $Pd(PPh_3)_2Cl_2$ (1.57 g, 2.23 mmol) and CuI (849 mg, 4.46 mmol). The resulting mixture was stirred for 17 hours at 60°C and then cooled down to rt. The reaction mixture was evaporated in vacuum to remove excess of TEA followed by addition of 1N HCl until the pH reached to 1. The mixture was then extracted with EtOAc/water. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuum and the residue was purified by column chromatography (Hexane : EtOAc = 20 : 1) to give the desired compound **3e** (7.68 g) as a light brown solid; Yield = 79%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.02-7.19 (4H, m, Ar-H), 0.00 (9H, s, -Si(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 148.0, 137.4, 129.2, 126.7, 124.9, 123.0, 102.1, 97.6, -0.3.

Synthesis of acetylene derivatives 4e-h

Acetylene derivatives **4a-4d** were purchased from Sigma-Aldrich and were used as such without any further purification.

Synthesis of 1-ethynyl-3-nitrobenzene (4e)

In a two neck rbf containing THF (100 ml) and MeOH (100 ml) was added compound **3e** (7.55 g, 34.43 mmol) at rt. The mixture was stirred for 5 minutes followed by addition of K_2CO_3 (28.55 g, 206.58 mmol). The resulting mixture was stirred for 1 hour at the same temperature. The reaction mixture was filtered to remove K_2CO_3 and then extracted with EtOAc/water. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and dried in vacuum to give desired compound **4e** as a brown solid; Yield = 98 %; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.34-7.52 (4H, m, Ar-H), 3.24 (1H, s, -CH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 148.1, 137.7, 129.4, 126.9, 123.9, 123.5, 81.1, 79.9.

3-Ethynylbiphenyl (4f)

According to the procedure described for the synthesis of **4e**, Yield = 90%; Yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.73-7.23 (9H, m, Ar-H), 3.09 (1H, s, -CH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 140.6, 139.3, 130.0, 128.0, 126.8, 126.3, 121.7, 82.8.

4-ethynylbiphenyl (4g)

According to the procedure described for the synthesis of **4e**, Yield = 90%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.58-7.34 (9H, m, Ar-H), 3.11 (1H, s, -CH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 141.6, 140.3, 132.6, 128.9, 127.7, 127.1, 121.0, 83.6, 77.7.

2-Ethynylnaphthalene (4h)

According to the procedure described for the synthesis of **4e**, Yield = 91 %; Light brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.00-7.44 (7H, m, Ar-H), 3.12 (1H, s, -CH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 132.8, 132.6, 132.0, 128.3, 127.8, 127.5, 126.6, 126.3, 119.2, 83.8, 77.2.

((4-Bromophenyl)ethynyl)trimethylsilane (6)

In a two neck rbf containing TEA (200 ml) was added 1-Bromo-4-iodobenzene **5** (24.00 g, 84.83 mmol) at rt under nitrogen. The mixture was stirred for 5 minutes followed by addition of ethynyltrimethylsilane **2** (13.19 ml, 93.31 mmol), $Pd(PPh_3)_2Cl_2$ (2.98 g, 4.24 mmol) and CuI (1.62 g, 8.48 mmol). The reaction mixture was stirred for 30 minutes at the same temperature. The reaction mixture was evaporated in vaccum to remove excess of TEA followed by addition of 1N HCl until the pH reached to 1. The mixture was then extracted with EtOAc/water. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was purified by column chromatography (Hexane) to give the desired compound **6** (21.00 g) as a white solid; Yield = 98%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.19-7.06 (4H, m, Ar-H), 0.00 (9H, s, -$Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 133.5, 131.6, 122.8, 122.3, 104.0, 95.7, 0.00.

Synthesis of trimethyl((4-(naphthalen-1-yl)phenyl)ethynyl)silane (3i)

In a sealed tube was taken compound **6** (7.36 g, 29.08 mmol), 1-naphthaleneboronic acid **7i** (6.00 g, 34.89 mmol), $Pd(PPh3)_2Cl_2$ (1.02 g, 1.45 mmol), Na_2CO_3 (15.41 g, 145.40 mmol), water (18 ml) and DME (60 ml). The reaction mixture was stirred for 3 hours at 120^{o}C and then cooled down to rt. The mixture was then acidified with 1N HCl to bring pH ~1. The mixture was then extracted with EtOAc/water. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuum and the residue was purified by column chromatography (Hexane : EtOAc = 100 : 1) to give the desired compound **3i** (7.64 g) as a colourless oil; Yield = 87%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.58-7.06 (11H, m, Ar-H), 0.00 (9H, s, -$Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 140.9, 139.3, 133.7, 132.7, 131.7, 129.8, 128.2, 128.0, 127.6, 126.7, 126.0, 125.8, 125.2, 122.0, 105.0, 94.6, 60.1, -0.07.

Synthesis of acetylene derivatives 4i-4m

Synthesis of 1-(4-ethynylphenyl)naphthalene (4i)

According to the procedure described for the synthesis of **4e**; Yield 98%, Yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.89-7.37 (11H, m, Ar-H), 3.13 (1H, s, -CH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 141.1, 139.1, 133.6, 131.8, 131.1, 129.8, 128.1, 127.9, 127.8, 127.6, 126.62, 126.0, 125.5, 125.1, 120.8, 83.3, 77.3.

4-Chloro-4'-ethynylbiphenyl (4j)

According to the procedure described for the synthesis of **4i**; Yield = 72%; Yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.56-7.39 (8H, m, Ar-H), 3.13 (1H, s, -CH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 140.2, 138.6, 133.8, 132.6, 129.0, 128.2, 126.8, 121.3, 83.3, 78.0.

4'-Ethynyl-2-methoxybiphenyl (4k)

According to the procedure described for the synthesis of **4i**; Yield = 84%; Yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.69-7.10 (8H, m, Ar-H), 3.92 (3H, s, -OCH_3), 3.23 (1H, s, -CH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 155.3, 138.1, 130.7, 129.8, 129.6, 128.6, 128.1, 127.6, 127.0, 120.0, 119.5, 110.2, 82.9, 54.3

4'-Ethynyl-2-methylbiphenyl (4l)

According to the procedure described for the synthesis of **4i**; Yield = 91%; Yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.53-7.18 (8H, m, Ar-H), 3.08 (1H, s, -CH), 2.25 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 142.3, 140.9, 134.9, 131.6, 130.2, 129.6, 129.4, 129.1, 127.4, 125.6, 120.4, 83.4, 77.1, 20.2.

4-*tert*-Butyl-4'-ethynylbiphenyl (4m)

According to the procedure described for the synthesis of **4i**; Yield = 69%; Light yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.54-7.45 (8H, m, Ar-H), 3.10 (1H, s, -CH), 1.35 (9H, s, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 150.8, 141.3, 137.2, 132.4, 126.7, 126.59, 125.7, 120.6, 83.6, 77.5, 34.5, 31.2.

(*E*)-ethyl 3-methyl-5-phenylpent-2-en-4-ynoate (8a)

In a two neck rbf was stirred a mixture of $Pd(OAc)_2$ (275 mg, 1.22 mmol) and TDMPP (540 mg, 1.22 mmol) in benzene (30 ml) for 15 minutes at rt under nitrogen. To this mixture was added ethyl-2-butynoate **7** (2.84 ml, 24.48 mmol) and the resulting mixture was further stirred for 10 minutes at rt. Next, ethynylbenzene **4a** (2.50 g, 24.48 mmol) was added to this solution and the mixture was stirred for 5 to 10 minutes until reaction was completed. The workup of reaction was performed by evaporating the solvent in vacuum followed by column chromatography (Hexane : EtOAc = 100 : 1) to give the desired compound **8a** (2.62 g); Yield = 50%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.47-7.45 (2H, m, Ar-H), 7.36-7.33 (3H, m, Ar-H), 6.16 (1H, s, -CH), 4.21-4.17 (2H, q, J = 7.5 Hz, -$\underline{CH_2}CH_3$), 2.39 (3H, s, -CH_3), 1.31-1.28 (3H, t, J = 7.5 Hz, -$CH_2\underline{CH_3}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.9, 136.6, 130.7, 128.9, 127.8, 127.2, 122.9, 121.2, 92.4, 90.0, 58.9, 18.7, 13.1.

5-Iodo-4-methyl-6-phenyl-2H-pyran-2-one (9a)

In a two neck rbf containing a solution of compound **8a** (2.62 g, 12.23 mmol) in CH_2Cl_2 (20 mL) was added 1 M solution of ICl (18.35 ml, 18.35 mmol) dropwise under nitrogen. The mixture was stirred at rt for 30 minutes followed by its quenching with sodium thiosulfate solution (20 ml) at same temperature. The reaction mixture was extracted with CH_2Cl_2/water. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuum and the residue was purified by

column chromatography (Hexane : EtOAc = 25 : 1) to give the desired compound **9a** (1.53 g); Yield = 40%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.65-7.63 (2H, m, Ar-H), 7.48-7.44 (3H, m, Ar-H), 6.26 (1H, s, Ar-H), 2.38 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.2, 160.8, 158.4, 134.5, 130.6, 129.5, 128.1, 112.1, 29.1.

Typical procedure for the synthesis of compounds 10a-10m

4-Methyl-6-phenyl-2H-pyran-2-one (10a)

In a two neck rbf was stirred a mixture of compound **9a** (1.53 g, 4.90 mmol), $Pd(OAc)_2$ (22 mg, 0.098 mmol) and Ph_3P (51 mg, 0.196 mmol) in DMF (40 ml) for 5 minutes at rt under nitrogen. TEA (2.05 ml, 14.70 mmol) was added to this solution and the mixture was further stirred for 5 minutes followed by addition of 98% formic acid (0.41 ml, 10.78 mmol). The reaction mixture was stirred for 5 hours at 62^{o}C. After completion of reaction, the mixture was cooled down to rt and then extracted with EtOAc/water. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuum and the residue was purified by column chromatography (Hexane : EtOAc = 20 : 1) to give the desired compound **10a** (730 mg); Yield = 80%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.83-7.81 (2H, m, Ar-H), 7.46-7.44 (3H, m, Ar-H), 6.52 (1H, s, Ar-H), 6.08 (1H, s, Ar-H), 2.23 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.0, 159.2, 155.7, 131.1, 130.4, 128.6, 125.3, 111.4, 103.8, 21.3; MS (TOF ES+): m/z calcd for $C_{12}H_{11}O_2$ $[M + 1]^+$, 187.0753; found, 187.0753.

6-(4-Fluorophenyl)-4-methyl-2H-pyran-2-one (10b)

According to the procedure described for the synthesis of **10a**; Yield 81%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.81-7.78 (2H, m, Ar-H), 7.14-7.10 (2H, m, Ar-H), 6.46 (1H, s, Ar-H), 6.05 (1H, s, Ar-H), 2.22 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.7, 162.7, 161.0, 159.7, 158.4, 131.8, 130.6, 115.4, 115.3, 112.2, 29.0; MS (TOF ES$^+$): m/z calcd for $C_{12}H_{10}FO_2$ $[M + 1]^+$, 205.0659; found, 205.0683.

6-(3-Fluorophenyl)-4-methyl-2H-pyran-2-one (10c)

According to the procedure described for the synthesis of **10a**; Yield = 81%; Light brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.59-7.11 (4H, m, Ar-H), 6.52 (1H, s, Ar-H), 6.09 (1H, s, Ar-H), 2.23 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.7, 161.8, 157.7, 155.6, 133.3, 130.3, 121.0, 117.4, 112.5, 112.1, 104.5, 21.3; MS (TOF ES+): m/z calcd for $C_{12}H_{10}FO_2$ $[M + 1]^+$, 205.0659; found, 205.0672.

6-(4-Methoxyphenyl)-4-methyl-2H-pyran-2-one (10d)

According to the procedure described for the synthesis of **10a**; Yield = 72%; Yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.77-7.75 (2H, d, *J* = 9.0 Hz, Ar-H), 6.95-6.93 (2H, d, *J* = 9.0 Hz, Ar-H), 6.41 (1H, s, Ar-H), 6.00 (1H, s, Ar-H), 3.85 (3H, s, -OCH_3), 2.21 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.6, 161.6, 159.5, 156.5, 127.2, 123.7, 114.2, 110.3, 102.7, 55.4, 21.6; MS (TOF ES$^+$): m/z calcd for $C_{13}H_{13}O_3$ $[M + 1]^+$, 217.0858; found, 217.0864.

4-Methyl-6-(3-nitrophenyl)-2H-pyran-2-one (10e)

According to the procedure described for the synthesis of **10a**; Yield = 36%; Yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.63-7.65 (4H, m, Ar-H), 6.66 (1H, s, Ar-H), 6.18 (1H, s, Ar-H), 2.29 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.2, 156.5, 155.5, 148.6, 133.0, 131.0, 130.0, 124.9, 120.3, 113.1, 105.4, 21.5; MS (TOF ES+): m/z calcd for $C_{12}H_{10}NO_4$ $[M + 1]^+$, 232.0604; found, 232.0612.

6-(Biphenyl-3-yl)-4-methyl-2H-pyran-2-one (10f)

According to the procedure described for the synthesis of **10a**; Yield = 81%; Brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.03-7.37 (9H, m, Ar-H), 6.57 (1H, s, Ar-H), 6.10 (1H, s, Ar-H), 2.24 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.3, 159.2, 156.3, 141.9, 140.0, 131.7, 129.3, 128.9, 127.8, 127.1, 124.3, 124.2, 111.7, 104.4, 21.6; MS (TOF ES$^+$): m/z calcd for $C_{18}H_{15}O_2$ $[M + 1]^+$, 263.1066; found, 263.1068.

6-(Biphenyl-4-yl)-4-methyl-2H-pyran-2-one (10g)

According to the procedure described for the synthesis of **10a**; Yield = 86%; Light-brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.89-7.37 (9H, m, Ar-H), 6.55 (1H, s, Ar-H), 6.08 (1H, s, Ar-H), 2.23 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.3, 159.2, 155.9, 143.4, 139.8, 130.1, 128.9, 127.9, 127.4, 127.0, 126.0, 111.6, 103.9, 21.6; MS (TOF ES$^+$): m/z calcd for $C_{18}H_{15}O_2$ $[M + 1]^+$, 263.1066; found, 263.1073.

4-Methyl-6-(naphthalen-2-yl)-2H-pyran-2-one (10h)

According to the procedure described for the synthesis of **10a**; Yield = 87%; Brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.37-7.51 (7H, m, Ar-H), 6.60 (1H, s, Ar-H), 6.08 (1H, s, Ar-H), 2.22 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.3, 159.3, 156.0, 134.1, 133.0, 128.8, 128.6, 128.3, 127.6, 127.5, 126.8, 126.0, 121.9, 111.6, 104.4, 21.56; MS (TOF ES$^+$): m/z calcd for $C_{16}H_{13}O_2$ $[M + 1]^+$, 237.0909; found, 237.0908.

4-Methyl-6-(4-(naphthalen-1-yl)phenyl)-2H-pyran-2-one (10i)

According to the procedure described for the synthesis of **10a**, Yield = 88%; Brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.95-7.42 (11H, m, Ar-H), 6.60 (1H, s, Ar-H), 6.11 (1H, s, Ar-H), 2.25 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.2, 159.2, 155.9, 143.3, 138.9, 133.7, 131.2, 130.5, 130.1, 128.3, 128.1, 126.8, 126.2, 125.8, 125.4, 125.2, 111.6, 104.0, 21.5; MS (TOF ES$^+$): m/z calcd for $C_{22}H_{17}O_2$ $[M + 1]^+$, 313.1222; found, 313.1226.

6-(4'-Chlorobiphenyl-4-yl)-4-methyl-2H-pyran-2-one (10j)

According to the procedure described for the synthesis of **10a**; Yield = 88%; Brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.86-7.40 (8H, m, Ar-H), 6.54 (1H, s, Ar-H), 6.07 (1H, s, Ar-H), 2.22 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.3, 159.0, 156.1, 142.1, 138.3, 134.2, 130.5, 129.2, 128.3, 127.3, 126.2, 111.8, 104.2, 21.7; MS (TOF ES$^+$): m/z calcd for $C_{18}H_{14}ClO_2$ $[M + 1]^+$, 297.0676; found, 297.0688.

6-(2'-Methoxybiphenyl-4-yl)-4-methyl-2H-pyran-2-one (10k)

According to the procedure described for the synthesis of **10a**, Yield = 90%; Yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.84-6.98 (8H, m, Ar-H), 6.53 (1H, s, Ar-H), 6.05 (1H, s, Ar-H), 3.80 (3H, s, -OCH_3), 2.20 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.2, 159.3, 156.3, 155.9, 141.0, 130.4, 129.8, 129.5, 129.2, 129.1, 125.1, 120.8, 111.3, 103.7, 55.4, 21.4; MS (TOF ES$^+$): m/z calcd for $C_{19}H_{17}O_3$ [M + 1]$^+$, 293.1171; found, 293.1173.

4-Methyl-6-(2'-methylbiphenyl-4-yl)-2H-pyran-2-one (10l)

According to the procedure described for the synthesis of **10a**, Yield = 87%; Light brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.88-7.22 (8H, m, Ar-H), 6.56 (1H, s, Ar-H), 6.09 (1H, s, Ar-H), 2.28 (3H, s, -CH_3), 1.24 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.2, 159.3, 155.8, 144.4, 140.6, 135.0, 130.3, 129.7, 129.4, 127.6, 125.8, 125.3, 111.5, 103.8, 21.5, 20.2; MS (TOF ES$^+$): m/z calcd for $C_{19}H_{17}O_2$ [M + 1]$^+$, 277.1222; found, 277.1221.

6-(4'-*tert*-Butylbiphenyl-4-yl)-4-methyl-2H-pyran-2-one (10m)

According to the procedure described for the synthesis of **10a**, Yield = 93%; Brown solid; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.88-7.47 (8H, m, Ar-H), 6.54 (1H, s, Ar-H), 6.07 (1H, s, Ar-H), 2.23 (3H, s, -CH_3), 1.36 (9H, s, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.3, 159.3, 155.9, 151.2, 143.3, 136.8, 129.8, 127.2, 126.7, 126.0, 125.8, 111.5, 103.8, 34.6, 31.3, 21.6; MS (TOF ES$^+$): m/z calcd for $C_{12}H_{23}O_2$ [M + 1]$^+$, 319.1692; found, 319.1697.

Typical procedure for the synthesis of compounds 11a-11m

1-Hydroxy-4-methyl-6-phenylpyridin-2(1H)-one (11a)

In a two neck rbf was stirred a mixture of compound **10a** (730 mg, 3.92 mmol) and $NH_2OH.HCl$ (9.53 g, 137.20 mmol) in pyridine (20 mL) for 17 hours at 100^{o}C under nitrogen. After completion of reaction, the mixture was cooled down to rt and extracted with CH_2Cl_2/water. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuum and the residue was recrystallized with ethanol to give the desired compound **11a** (425 mg) as a light yellow solid; Yield = 54%; m.pt. 130-131^{o}C; HPLC purity: 99.4%, t_R = 3.1 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.66-7.64 (2H, m, Ar-H), 7.48-7.46 (3H, m, Ar-H), 6.53 (1H, s, Ar-H), 6.19 (1H, s, Ar-H), 2.28 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 158.3, 148.8, 143.6, 131.6, 129.7, 129.1, 128.3, 114.5, 109.9, 21.4; HRMS (ESI) [M-H]$^-$ Calcd for $C_{12}H_{10}NO_2$: 200.0717, Found: 200.0724.

6-(4-Fluorophenyl)-1-hydroxy-4-methylpyridin-2(1H)-one (11b)

According to the procedure described for the synthesis of **11a**; Yield = 52%; Pale yellow solid; m.pt. 146-147^{o}C; HPLC purity: 98.8%, t_R = 3.3 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.66-7.64 (2H, m, Ar-H), 7.17-7.14 (2H, m, Ar-H), 6.52 (1H, s, Ar-H), 6.17 (1H, s, Ar-H), 2.27 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.5, 162.6, 157.8, 148.8, 141.9, 131.1, 127.4, 115.6, 115.4, 114.1, 109.4, 21.5; HRMS (ESI) [M-H]$^-$ Calcd for $C_{12}H_9$ FNO_2: 218.0623, Found: 218.063.

6-(3-Fluorophenyl)-1-hydroxy-4-methylpyridin-2(1H)-one (11c)

According to the procedure described for the synthesis of **11a**; Yield = 57%; Yellow solid; m.pt. 149-150^{o}C; HPLC purity: 99.6%, t_R = 3.3 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44-7.16 (4H, m, Ar-H), 6.54 (1H, s, Ar-H), 6.20 (1H, s, Ar-H), 2.28 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.3, 161.3, 157.6, 148.7, 141.3, 133.2, 130.0, 124.7, 117.0, 114.4, 109.4, 21.4; HRMS (ESI) [M-H]$^-$ Calcd for $C_{12}H_9$ FNO_2: 218.0623, Found: 218.0616.

1-Hydroxy-6-(4-methoxyphenyl)-4-methylpyridin-2(1H)-one (11d)

According to the procedure described for the synthesis of **11a**; Yield = 50%; Light yellow solid; m. pt. 175-176^{o}C; HPLC purity: 98.7%, t_R = 3.3 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.61-7.59 (2H, d, *J* = 8.5 Hz, Ar-H), 6.97-6.95 (2H, d, *J* = 9.0 Hz, Ar-H), 6.46 (1H, s, Ar-H), 6.15 (1H, s, Ar-H), 3.84 (3H, s, -OCH_3), 2.23 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.8, 157.9, 148.6, 142.7, 130.4, 123.7, 113.8, 113.2, 109.0, 55.3, 21.4; HRMS (ESI) [M-H]$^-$ Calcd for $C_{13}H_{12}NO_3$: 230.0817, Found: 230.0823.

1-Hydroxy-4-methyl-6-(3-nitrophenyl)pyridin-2(1H)-one (11e)

According to the procedure described for the synthesis of **11a**; Yield = 67%; Light yellow solid; m. pt. 172-173^{o}C; HPLC purity: 97.8%, t_R = 3.0 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.52-7.66 (4H, m, Ar-H), 6.60 (1H, s, Ar-H), 6.27 (1H, s, Ar-H), 2.31 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.4, 148.7, 148.0, 140.0, 134.7, 132.6, 129.4, 124.3, 123.8, 115.1, 109.5, 21.2; HRMS (ESI) [M-H]$^-$ Calcd for $C_{12}H_9N_2O_4$: 245.0563, Found: 245.0570.

6-(Biphenyl-3-yl)-1-hydroxy-4-methylpyridin-2(1H)-one (11f)

According to the procedure described for the synthesis of **11a**; Yield = 60%; Yellow semisolid; HPLC purity: 98.9%, t_R = 6.4 min; ^{1}H NMR (MeOD, 500 MHz): δ 7.89-7.85 (1H, m, Ar-H), 7.71-7.33 (8H, m, Ar-H), 6.50 (1H, s, Ar-H), 6.39 (1H, s, Ar-H), 2.28 (3H, s, -CH3); ^{13}C NMR (MeOD, 125 MHz): δ 163.3, 159.0, 158.3, 141.9, 140.0, 131.7, 129.2, 129.1, 128.6, 127.5, 126.6, 124.0, 123.6, 110.7, 104.7, 20.2; HRMS (ESI) [M-H]$^-$ Calcd for $C_{18}H_{14}NO_2$: 276.1025, Found: 276.1041

6-(Biphenyl-4-yl)-1-hydroxy-4-methylpyridin-2(1H)-one (11g)

According to the procedure described for the synthesis of **11a**; Yield = 62%; Yellow solid; m. pt. 210-211^{o}C; HPLC purity: 95.3%, t_R = 8.2 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.75-7.25 (9H, m, Ar-H), 6.54 (1H, s, Ar-H), 6.24 (1H, s, Ar-H), 2.30 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.4, 148.5, 142.8, 141.8, 140.0, 130.0, 129.3, 128.8,

127.8, 127.1, 113.4, 109.0, 21.4; HRMS (ESI) $[M-H]^-$ Calcd for $C_{18}H_{14}NO_2$: 276.1025, Found: 276.1035.

1-Hydroxy-4-methyl-6-(naphthalen-2-yl)pyridin-2(1H)-one (11h)

According to the procedure described for the synthesis of **11a**; Yield = 56%; Yellow solid; m. pt. 205-206°C; HPLC purity: 99.4%, t_R = 4.7 min; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.12 (1H, s, Ar-H), 7.98-7.95 (3H, m, Ar-H), 7.68-7.67 (1H, d, J = 8 Hz, Ar-H), 7.59-7.57 (2H, m, Ar-H), 6.41 (1H, s, Ar-H), 6.25 (1H, s, Ar-H), 2.19 (3H, s, -CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 158.6, 148.6, 145,5, 133.3, 132.8, 130.5, 128.8, 128.7, 128.0, 127,7, 127.6, 127.1, 126.9, 116.3, 108.9, 21.1; HRMS (ESI) $[M-H]^-$ Calcd for $C_{16}H_{12}NO_2$: 250.0873, Found: 250.0884.

1-Hydroxy-4-methyl-6-(4-(naphthalen-1-yl)phenyl)pyridin-2(1H)-one (11i)

According to the procedure described for the synthesis of **11a**; Yield = 61 %; Light yellow solid; m. pt. 242-243°C; HPLC purity: 98.0%, t_R = 11.4 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.93-7.44 (11H, m, Ar-H), 6.56 (1H, s, Ar-H), 6.30 (1H, s, Ar-H), 2.31 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.7, 148.6, 142.6, 142.2, 139.2, 133.8, 131.4, 130.2, 128.9, 128.4, 128.1, 127.0, 126.3, 125.9, 125.7, 125.4, 113.7, 109.3, 21.5; HRMS (ESI) $[M-H]^-$ Calcd for $C_{22}H_{16}NO_2$: 326.1186, Found: 326.1185.

6-(4'-Chlorobiphenyl-4-yl)-1-hydroxy-4-methylpyridin-2(1H)-one (11j)

According to the procedure described for the synthesis of **11a**; Yield = 56.6%; Light yellow solid; m. pt. 198-199°C; HPLC purity: 91.8%, t_R = 9.6 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.74-7.43 (8H, m, Ar-H), 6.54 (1H, s, Ar-H), 6.23 (1H, s, Ar-H), 2.29 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.3, 148.3, 141.6, 141.3, 138.3, 133.9, 130.2, 129.2, 128.9, 128.1, 126.6, 113.4, 108.9, 21.2; HRMS (ESI) $[M-H]^-$ Calcd for $C_{18}H_{13}ClNO_2$: 310.0640, Found: 310.0634.

1-Hydroxy-6-(2'-methoxybiphenyl-4-yl)-4-methylpyridin-2(1H)-one (11k)

According to the procedure described for the synthesis of **11a**; Yield = 71%; Yellow solid; m. pt. 242-243°C; HPLC purity: 97.8%, t_R = 8.3 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.71-7.00 (8H, m, Ar-H), 6.53 (1H, s, Ar-H), 6.24 (1H, s, Ar-H), 3.84 (3H, s, -OCH_3), 2.29 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.5, 156.4, 148.4, 142.1, 140.3, 130.7, 129.5, 129.4, 129.1, 128.4, 120.9, 113.2, 111.3, 109.1, 55.5, 21.4; HRMS (ESI) $[M-H]^-$ Calcd for $C_{19}H_{16}NO_3$: 306.1135, Found: 306.1139.

1-Hydroxy-4-methyl-6-(2'-methylbiphenyl-4-yl)pyridin-2(1H)-one (11l)

According to the procedure described for the synthesis of **11a**; Yield = 66%; Yellow solid; m. pt. 184-185°C; HPLC purity: 97.8%, t_R = 8.2 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.72-7.25 (8H, m, Ar-H), 6.54 (1H, s, Ar-H), 6.26 (1H, s, Ar-H), 2.31 (3H, s, -CH_3), 2.29 (3H, s, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.5, 148.5, 143.6, 142.1, 140.8, 135.1, 130.3, 129.6, 129.1, 128.6, 127.6, 125.8, 113.4, 109.2, 21.4, 20.3; HRMS (ESI) $[M-H]^-$ Calcd for $C_{19}H_{16}NO_2$: 290.1186, Found: 290.119.

6-(4'-*tert*-Butylbiphenyl-4-yl)-1-hydroxy-4-methylpyridin-2(1H)-one (11m)

According to the procedure described for the synthesis of **11a**; Yield = 67%; Light yellow solid; m. pt. 170-171°C; HPLC purity: 99.6%, t_R = 3.3 min; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.73-7.48 (8H, m, Ar-H), 6.53 (1H, s, Ar-H), 6.24 (1H, s, Ar-H), 2.28 (3H, s, -CH_3), 1.37 (9H, s, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.5, 150.9, 148.5, 142.6, 142.1, 137.1, 129.7, 129.2, 126.8, 126.7, 125.8, 113.4, 109.1, 34.5, 31.2, 21.4; HRMS (ESI) $[M-H]^-$ Calcd for $C_{22}H_{22}NO_2$: 332.1651, Found: 332.1664.

Biological methods

Cell culture

Mixed glial cells were prepared from the prefrontal cortices of 1 to 2-day-old Sprague-Dawley rat pups as previously described[30]. In brief, meninges-free cerebral hemispheres were dissociated by titurating through a Pasteur pipettes. Cells were then plated on poly-D-lysine (1 μg/ml)-coated 175-cm^2 flask and maintained in MEM supplemented with 10% FBS (Hyclone, Logan, UT) for a week. To prepare pure astrocytic cultures, the flasks were shaken at 260 rpm overnight at 37°C on day 8 of the mixed glial cultures. After removal of supernatant containing detached microglial cells and oligodendrocytes, the remaining astrocytes were trypsinized, washed, and replated at a density 3.0 × 10^2 cells/mm^2 in the growth medium on poly-D-lysine (10 μg/ml)-coated plates. All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by Korea University Institutional Animal Care & Use Committee (KUIACUC-2014-233). Cells were used for experiments 4 or 5 day after plating.

Presto Blue assay

Mitochondrial metabolic activity was determined by Resazurin-based presto blue assay (Invitrogen, Grand Island, NY), following manufacturer's instructions. In brief, cells were loaded for 15 minutes with presto blue reagent at 37°C. The fluorescence change was determined by using a microplate reader (Ex = 540 nm, Em = 590 nm; SpectraMax GeminiEM, Molecular Devices, Sunnyvale, CA). Analysis of dose-response curve was performed using the non-linear regression fit (log of dose vs. inhibitory response) function of GraphPad Prism® v. 6.07 to calculate the IC_{50} value of the drug that inhibited 50% of H_2O_2-induced mitochondrial metabolic dysfunction.

LDH assay

Cell death were assessed by measuring the amount of lactate dehydrogenase (LDH) released into the culture supernatant using a diagnostic kit (Sigma-Aldrich, St. Louis, MO), as described previously[31]. The data were expressed as the percentage of total LDH release, as measured with repeatedly frozen and thawed parallel cell cultures.

Measurement of mitochondrial membrane potential

Mitochondrial membrane potential was assessed by the mitochondrial uptake of Tetramethylrhodamine methyl ester

(TMRM, Molecular probes) as previously described[32]. In brief, cells were loaded with 500 nM TMRM at 37°C for 10 min and the changes in the mitochondrial accumulation of TMRM was analysed by using a microplate reader (SpectraMax GeminiEM, Molecular Devices).

Measurement of oxygen consumption rate

Mitochondrial oxygen consumption rate (OCR), an indicator for mitochondrial respiration functionality, was measured using the XF24 extracellular flux analyser (Seahorse Bioscience, Billerical, MA), as previously described[31]. After sequential addition of oligomycin (an ATP synthase inhibitor; Sigma-Aldrich), FCCP (a protonophore; Sigma-Aldrich), and rotenone (a complex I inhibitor; Sigma-Aldrich) in the presence or absence of each derivative and H_2O_2 to astrocytes, OCR was assessed according to the manufacturer's protocol. The extracellular acidification rate (ECAR), an indicator for cellular oxygen-independent glycolysis was also measured simultaneously. Bioenergetic parameters were normalized to baseline and calculated as ATP turnover (the difference between the starting basal OCR and oligomycin-repressed OCR) as well as maximal respiration capacity (the difference between FCCP and rotenone-induced OCR changes).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and analysed for statistical significance by using either non-parametric Kruskal-Wallis test followed by Mann-Whitney U test or an analysis of variance (ANOVA) followed by the post-hoc Bonferroni test for multiple comparisons, depending on the Levene test results (SPSS; release20.0.0.1, IBM Corp.).

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