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A novel Parkinson's disease drug candidate with potent anti-neuroinflammatory effects through the Src signaling pathway

Yadan Wang,[‡] Xiuqi Bao,[‡] Song Xu, Wenwen Yu, Shengnan Cao, Jinping Hu, Yan Li, Xiaoliang Wang, Dan Zhang and Shishan Yu**

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China.

ABSTRACT: Numerous drug treatments are available for Parkinson's disease (PD), an age-related neurodegenerative disease, but most cause serious side effects. Therefore, novel therapeutic strategies that halt disease progression and allow for long-term administration are urgently needed. Neuroinflammation critically contributes to the pathogenesis of PD. Here, we report the discovery and optimization of phloroglucinol derivatives, a novel class of anti-neuroinflammatory compounds. Structural modifications of the hit compound 3-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one produced 43 derivatives, including a preclinical candidate (compound **21**) that exhibited potent *in vitro* anti-neuroinflammatory effects, good blood-brain barrier penetration, and desirable safety margins in mice at a median lethal dose (LD_{50}) > 5000 mg/kg. Its *in vivo* efficacy was demonstrated in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- and MPTP/probenecid (prob)-induced sub-acute and chronic PD models, respectively, and α-synuclein transgenic mice. Mechanistic studies revealed neuroinflammation inhibition by targeting Src/phosphatase and tensin homolog deleted on chromosome 10 (PTEN)/Akt signaling. We highlighted the potential usefulness of phloroglucinol derivatives in PD treatment.

INTRODUCTION

Parkinson's disease (PD) is an age-related, progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, which decreases the interstitial dopamine level and leads to bradykinesia, rigidity, and tremor.[1] Therefore, current treatment focuses on dopamine replacement, the inhibition of dopamine degradation using catechol-*O*-methyl-transferase (COMT) or monoamine oxidase type B (MAO-B) inhibitors, dopamine reuptake, and direct dopamine receptor agonists.[2-5] The available PD medications offer valuable symptomatic relief, but they are often associated with significant and intolerable side effects. Furthermore, importantly, these drugs cannot slow PD progression. Therefore, the development of broader and more fundamental therapeutic approaches to PD is critical.[6] The "neuroprotective therapy" concept has emerged as a potential treatment for PD and may effectively slow down or stop the disease progression.[7,8]

Neuroinflammation, which is well recognized as a key pathophysiological event contributing to progressive nigral dopaminergic neuron loss in PD,[9,10] may be another target for neuroprotective therapy. Microglia and astrocytes are major mediators of neuroinflammation in PD. Several reports have demonstrated the activation of microglial and astroglial cells near damaged or dying dopaminergic neurons in the substantia nigra.[11–13] Positron emission tomography (PET) imaging of PD patients shows increased microglial activation, and elevated levels of proinflammatory mediators are evident in the substantia nigra during postmortem examinations.[14,15] Epidemiological studies have demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs) confer a decreased risk of PD.[16] Therefore, preventative and curative treatments for PD have focused on discovering active substances that attenuate

excessive glial activation and protect dopaminergic neurons. Several agents that target inflammatory pathways are currently under investigation.[17]

In this article, we describe the discovery and optimization of a novel anti-PD series of phloroglucinol compounds with significant potential anti-neuroinflammatory effects and, more importantly, the drugability investigation that enabled the selection of a preclinical drug candidate with potent *in vitro* and *in vivo* anti-neuroinflammatory activities, desirable pharmacokinetic properties, and extremely low toxicity. Its underlying mechanisms were also investigated, and the results support investigating this novel agent as a potential PD therapy further.

RESULTS AND DISCUSSION

Discovery of the hit compound. 3-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**1**), a known phloroglucinol derivative, was previously isolated by our research group from *Lysidice rhodostegia*roots. It was identified as the initial hit compound by screening for biological activities, which revealed a moderate inhibitory effect on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced BV2 cells, with a half-maximal inhibitory concentration (IC_{50}) of 50.6 μM. Moreover, this compound showed certain "lead-like" properties, including low molecular weight, and a combination of polar and nonpolar moieties. Furthermore, it provided multiple diversification points for robust optimization.

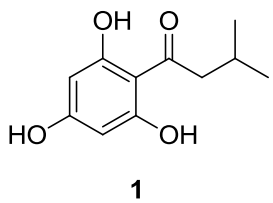

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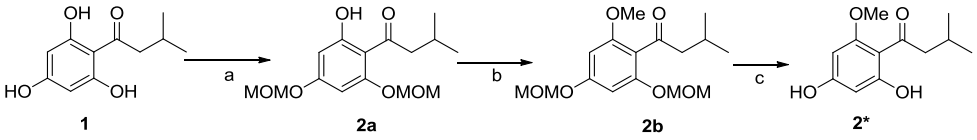
Figure 1. The structure of the hit compound.

Hit-to-lead medicinal chemistry optimization. With careful analysis, it was supposed that the three phenolic hydroxyl group contained in the hit compound might bring some negative effects as follows: 1) The polar of the molecule is strong, which may affect the absorption; 2) Glucuronidation of multi-hydroxyl groups may shorten the acting time of the compound within the body; 3) The stability of the compound is poor since the phenolic hydroxyl group is sensitive to chemical environment. Besides, the hit compound was not potent enough and its activity should be improved markedly. Therefore, structural modifications were performed to increase the anti-neuroinflammatory activities of the hit compound as well as improve its physicochemical properties. According to the basic principles of medicinal chemistry, the following strategies were used to achieve these purposes: 1) The structural skeleton of the hit compound was retained, and minimal modifications were made to adjust the lipid-water partition coefficients and/or improve its stability, such as changing the number of hydroxyl groups, methylating the hydroxyl groups at different positions, and altering the length of the sidechain. 2) Substantial changes were made by introducing various common nitrogen-containing activity-modifying pharmacophores into the molecule, such as amide, aminoketone, amino, propanolamine and furoxan units, to enhance the pharmacological effects. In the latter case, the number and methylation of hydroxyl groups, as well as the length of the sidechain can be modified simultaneously. As the hit compound was simple, slight change might lead to wide variation in the structure. Consequently, 43 phloroglucinol derivatives belonging six series (**2**–**8**, **9**–**10**, **11**–**17**, **18**–**25**, **26**–**43**, and **44**) were synthesized, 30 of which were new (indicated by *).

Compound **2** was synthesized from **1** by methylating the hydroxyl group at the ortho position of the carbonyl with Me_2SO_4 after protecting the other two with methoxymethyl (MOM) groups

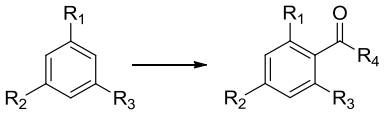
(Scheme 1), and **3**–**8** were prepared via Friedel–Crafts reactions, with BF_3–Et_2O as a catalyst (Scheme 2).[18]

Scheme 1. Synthesis of compound **2**.[a]



[a]Reagents and conditions: (a) CH_3OCH_2Cl, DIPEA, CH_2Cl_2, 0 ℃, 30 min; (b) Me_2SO_4, K_2CO_3, acetone, reflux, 5 h; (c) 2N HCl, reflux, 1 h.

Scheme 2. Synthesis of compounds **3**–**8**.[a]

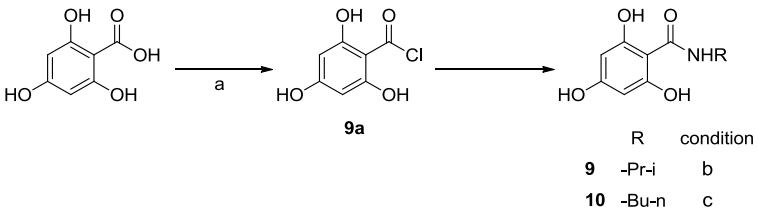


	R_1	R_2	R_3	R_4	condition
3	-OH	-OMe	-OMe	-Bu-i	a
4	-OMe	-OMe	-OMe	-Bu-i	a
5	-OH	-OMe	-H	-Bu-i	a
6	-H	-OH	-H	-Bu-i	a
7	-OH	-OH	-OH	-Bu-n	b
8	-OH	-OH	-OH	-Me	c

[a]Reagents and conditions: (a) isovaleryl chloride, BF_3–Et_2O, 80 ℃, 2 h; (b) valeryl chloride, BF_3–Et_2O, 80 ℃, 2 h; (c) acetyl chloride, BF_3–Et_2O, 80 ℃, 2 h.

Amides **9** and **10** were synthesized as follows: 2,4,6-trihydroxybenzoic acid was reacted with oxalyl chloride to give acyl chloride, which reacted with amines to yield the corresponding amides (Scheme 3).

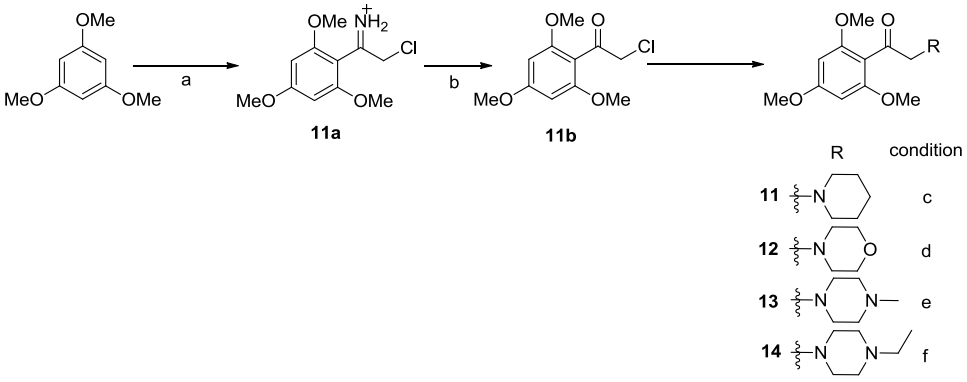
Scheme 3. Synthesis of compounds **9** and **10**.[a]



[a]Reagents and conditions: (a) oxalyl chloride, DMSO, CH_2Cl_2, rt, 1 h; (b) isopropylamine, THF, rt, 1 h; (c) butylamine, THF, rt, 1 h.

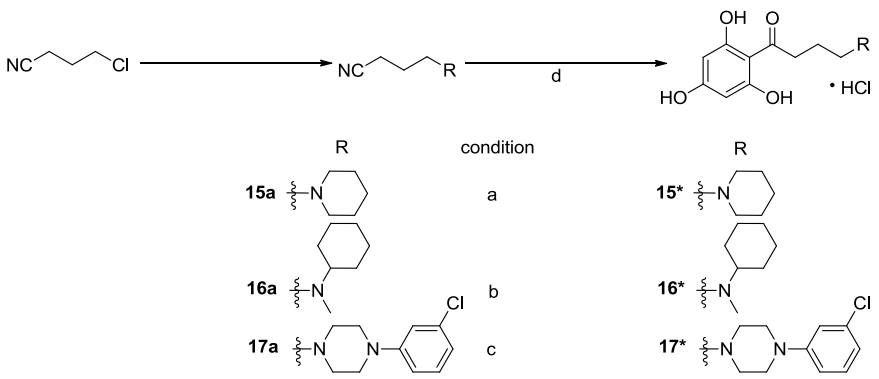
The aminoketone analogs **11**−**17** were prepared in two steps: acylation of 1,3,5-trimethoxybenzene or phloroglucinol with an omega-chloro alkyl nitril through Houben−Hoesch reactions catalyzed by HCl gas and introduction of amino groups by replacing the chloro group with various amines in the presence of K_2CO_3 (Schemes 4 and 5).[19] The order of the two steps could be reversed for the synthesis of **11**−**14**; however, the amines must first react with 4-chlorobutanenitrile for the synthesis of **15**−**17** because the hydroxyl groups of phloroglucinol were unstable in the alkaline environment created by K_2CO_3.

Scheme 4. Synthesis of compounds **11**−**14**.[a]



[a]Reagents and conditions: (a) chloroacetonitrile, HCl gas, PhCl, rt, 6 h; (b) H_2O, reflux, 1 h; (c) piperidine, K_2CO_3, acetone, reflux, 24 h; (d) morpholine, K_2CO_3, acetone, reflux, 24 h; (e) 1-methylpiperazine, K_2CO_3, acetone, reflux, 24 h; (f) 1-ethylpiperazine, K_2CO_3, acetone, reflux, 24 h.

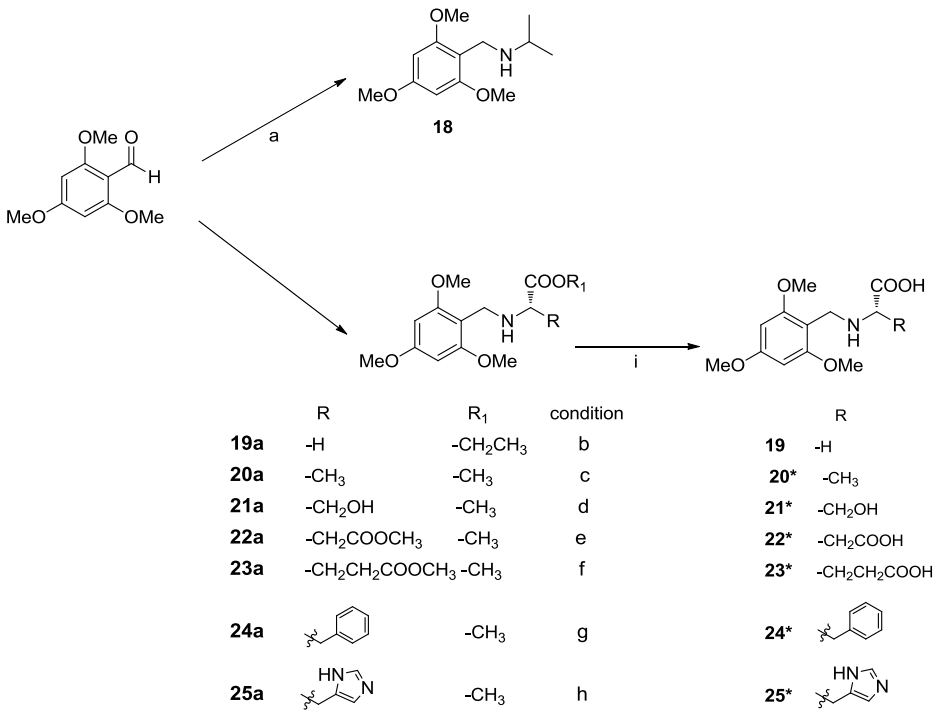
Scheme 5. Synthesis of compounds **15**−**17**.[a]



[a]Reagents and conditions: (a) piperidine, K_2CO_3, NaI, acetonitrile, rt, 20 h; (b) *N*-methylcyclohexanamine, K_2CO_3, NaI, acetonitrile, rt, 20 h; (c) 1-(3-chlorophenyl)piperazine, K_2CO_3, NaI, acetonitrile, rt, 20 h; (d) (i) phloroglucinol, HCl gas, $PhNO_2$, rt, 12 h; (ii) H_2O, reflux, 1 h.

Compounds **18**−**25** were obtained from 2,4,6-trimethoxybenzaldehyde by adding amines or alkyl (methyl or ethyl) ester of the amino acid hydrochloride salts, followed by treatment with $NaBH_3CN$ in anhydrous MeOH (pH ～ 6, Scheme 6) and, then, hydrolysis of the ester, if necessary.[20]

Scheme 6. Synthesis of compounds **18**−**25**.[a]

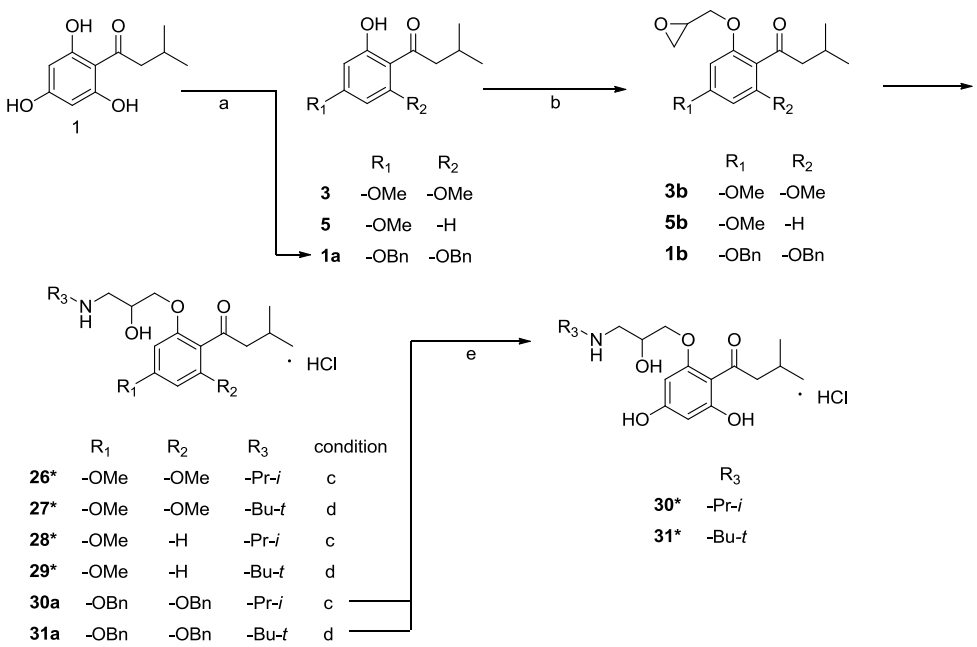


	R	R_1	condition		R
19a	-H	$-CH_2CH_3$	b	**19**	-H
20a	$-CH_3$	$-CH_3$	c	**20***	$-CH_3$
21a	$-CH_2OH$	$-CH_3$	d	**21***	$-CH_2OH$
22a	$-CH_2COOCH_3$	$-CH_3$	e	**22***	$-CH_2COOH$
23a	$-CH_2CH_2COOCH_3$	$-CH_3$	f	**23***	$-CH_2CH_2COOH$
24a		$-CH_3$	g	**24***	
25a		$-CH_3$	h	**25***	

[a]Reagents and conditions: (a) isopropylamine, $NaBH_3CN$, MeOH, HOAc, argon, rt, 3 h; (b) L-GlyOEt-HCl, $NaBH_3CN$, MeOH, argon, rt, 3 h; (c) L-AlaOMe-HCl, $NaBH_3CN$, MeOH, argon, rt, 3 h; (d) L-SerOMe-HCl, $NaBH_3CN$, MeOH, argon, rt, 3 h; (e) L-$Asp(OMe)_2$-HCl, $NaBH_3CN$, MeOH, argon, rt, 3 h; (f) L-$Glu(OMe)_2$-HCl, $NaBH_3CN$, MeOH, argon, rt, 3 h; (g) L-PheOMe-HCl, $NaBH_3CN$, MeOH, argon, rt, 3 h; (h) L-HisOMe-HCl, $NaBH_3CN$, MeOH, argon, rt, 3 h; (i) KOH, EtOH-H_2O (1:1), rt, 2 h, Amberlite 120 H^+ Resin.

The synthesis of racemic aryloxypropanolamine hydrochloride salts **26**–**31** is outlined in Scheme 7. Condensations of 2-(chloromethyl)oxirane with **3**, **5**, and **1a**, the *O*-benzyl-protected product of **1**,[21] which was achieved through treatment with NaH in anhydrous DMF, provided the epoxides **3b**, **5b** and **1b**, respectively. These subsequently reacted with various amines, resulting in the corresponding aryloxypropanolamines. The hydrochloride salts were obtained through treatment with ethereal HCl, followed by a deprotection step, if necessary.

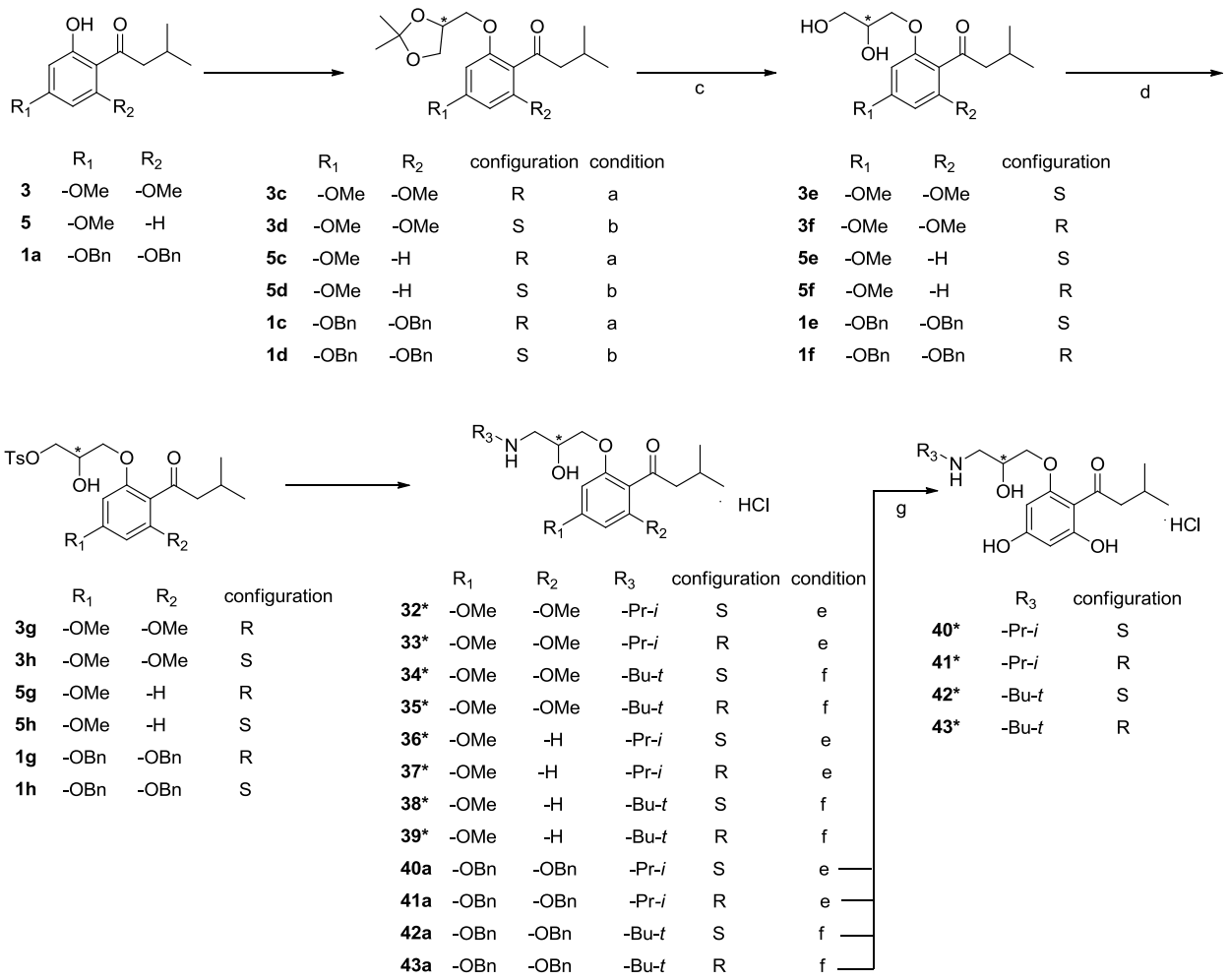
Scheme 7. Synthesis of compounds **26**–**31**.[a]



[a]Reagents and conditions: (a) benzyl *p*-toluenesulfonate, K_2CO_3, acetone, reflux, 3 h; (b) 2-(chloromethyl)oxirane, NaH, DMF, N_2, 90 ℃, 2 h; (c) (i) isopropylamine, 70 ℃, 3 h; (ii) ethereal HCl, 10 min; (d) (i) *tert*-butylamine, 70 ℃, 3 h; (ii) ethereal HCl, 10 min; (e) H_2, 3 atm, Pd–C, MeOH, HCl, rt, 12 h.

The synthesis of chiral aryloxypropanolamine hydrochloride salts **32**−**43** is shown in Scheme 8. Condensation of (*R*)-or (*S*)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane with **3**, **5**, and **1a**, followed by hydrolysis of 1,3-dioxolane to diols, *p*-toluenesulfonylation of the primary alcohol-OH and amination with various amines resulted in the corresponding chiral aryloxypropanolamine, which had the following configuration.

Scheme 8. Synthesis of compounds **32**−**43**.[a]



	R_1	R_2
3	-OMe	-OMe
5	-OMe	-H
1a	-OBn	-OBn

	R_1	R_2	configuration	condition
3c	-OMe	-OMe	R	a
3d	-OMe	-OMe	S	b
5c	-OMe	-H	R	a
5d	-OMe	-H	S	b
1c	-OBn	-OBn	R	a
1d	-OBn	-OBn	S	b

	R_1	R_2	configuration
3e	-OMe	-OMe	S
3f	-OMe	-OMe	R
5e	-OMe	-H	S
5f	-OMe	-H	R
1e	-OBn	-OBn	S
1f	-OBn	-OBn	R

	R_1	R_2	configuration
3g	-OMe	-OMe	R
3h	-OMe	-OMe	S
5g	-OMe	-H	R
5h	-OMe	-H	S
1g	-OBn	-OBn	R
1h	-OBn	-OBn	S

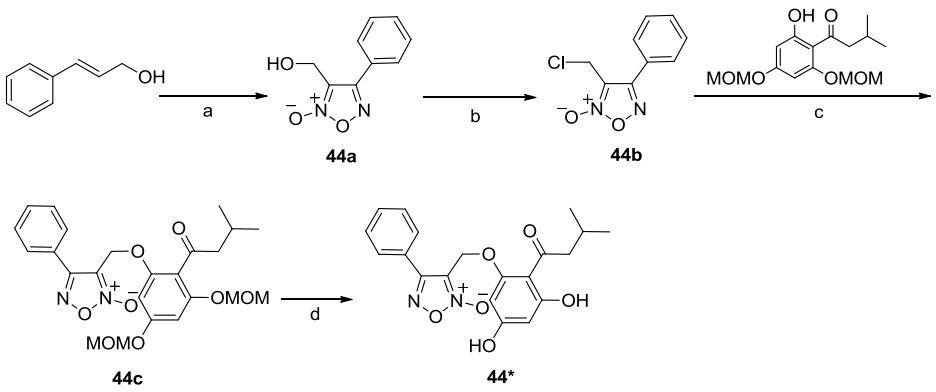
	R_1	R_2	R_3	configuration	condition
32*	-OMe	-OMe	-Pr-*i*	S	e
33*	-OMe	-OMe	-Pr-*i*	R	e
34*	-OMe	-OMe	-Bu-*t*	S	f
35*	-OMe	-OMe	-Bu-*t*	R	f
36*	-OMe	-H	-Pr-*i*	S	e
37*	-OMe	-H	-Pr-*i*	R	e
38*	-OMe	-H	-Bu-*t*	S	f
39*	-OMe	-H	-Bu-*t*	R	f
40a	-OBn	-OBn	-Pr-*i*	S	e
41a	-OBn	-OBn	-Pr-*i*	R	e
42a	-OBn	-OBn	-Bu-*t*	S	f
43a	-OBn	-OBn	-Bu-*t*	R	f

	R_3	configuration
40*	-Pr-*i*	S
41*	-Pr-*i*	R
42*	-Bu-*t*	S
43*	-Bu-*t*	R

[a]Reagents and conditions: (a) (*S*)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane, NaH, DMF, N_2, 90 ℃, 24 h; (b) (*R*)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane, NaH, DMF, N_2, 90 ℃, 24 h; (c) 4% H_2SO_4, THF, rt, 8 h; (d) TsCl, pyridine, CH_2Cl_2, rt, 48 h; (e) (i) isopropylamine, 70 ℃, 3 h; (ii) ethereal HCl, 10 min; (f) (i) *tert*-butylamine, 70 ℃, 3 h; (ii) ethereal HCl, 10 min; (g) H_2, 3 atm, Pd–C, MeOH, HCl, rt, 12 h.

Compound **44** was synthesized according to the sequence shown in Scheme 9. The reaction of cinnamyl alcoholand $NaNO_2$ in HOAc resulted in 4-hydroxymethyl-3-phenyl furoxan (**44a**),[22] which was converted into 4-chloromethyl-3-phenyl furoxan (**44b**) through treatment with $SOCl_2$. Condensation of the resulting chloride with **2a** in the presence of K_2CO_3, followed by deprotection using 2N HCl, provided the desired product **44**.

Scheme 9. Synthesis of compound **44**.[a]



[a]Reagents and conditions: (a) $NaNO_2$, HOAc, H_2O, rt, 24 h; (b) $SOCl_2$, pyridine, CH_2Cl_2, rt, 3 h; (c) K_2CO_3, KI, acetone, reflux, 5 h; (d) 2N HCl, reflux, 1 h.

All synthesized phloroglucinol derivatives were tested for anti-inflammatory activity using the same method as for the hit compound screen (Table 1). The activity profile exhibited varied results for each analogue, and their structure-activity relationships were discussed by structural series. Series 1: Compared with the hit compound **1**, the results of **2**−**6** showed that the numbers and positions of the phenolic hydroxyl groups and their methyl ethers greatly influenced the inhibitory activity against NO production in the LPS-induced BV2 cells. It seemed that reducing the number of hydroxyl groups to two (**5**) or one (**6**) might cause negative impact on the anti-inflammatory activity. While methylation of one (**2**) or all the three (**4**) rather than two (**3**) hydroxyl groups markedly increase the activity. Moreover, the results of **7** and **8** suggested that the length of the sidechain were also important for the activity, and valeryl would be more appropriate than isovaleryl and isobutyryl groups. Series 2 and 3: Introducing an amide (**9** and **10**) or an aminoketone (**11**−**17**) group seemed to make little sense to improve the activity. However, among the aminoketone analogues, **11**−**14** with substitution of methoxyl in place of hydroxyl groups and shorter length of the sidechain were slightly more effective. Series 4: Compounds **19**−**25**, which incorporated amino acid substituents, exhibited significantly different activities.

Weak inhibition of NO release was observed for compound **19**, which had a Gly substituent, whereas **21**−**23**, contained Ser, Asp, and Glu substituents, respectively, showed high inhibition levels, suggesting that sidechain substituents with a certain polarity and electrical properties might be necessary for activity. Furthermore, **24** and **25**, with Phe and His substituents, respectively, are mostly inactive, indicating that aromatic functional groups in the sidechain are not desirable. Series 5: Compounds **26**−**43** differed from the hit compound by an additional propanolamine unit at 2'-hydroxyl position, most of which showed moderate or marginal anti-neuroinflammatory activity except for compound **34** (IC_{50} 11.2 μM). The absolute configuration of the chiral carbon in propanolamine unit had certain influence on the activity, but no clear regularity could be concluded. Series 6: Introduction a furoxan substituent by formation of ether resulted in significant improvement in the activity, which may provide an effective way to get more potent anti-neuroinflammatory product. But unfortunately, only one compound was synthesized for a variety of reasons.

In a word, the structure activity relationships of the phloroglucinol derivatives are very complicated. Both minimal and substantial modification may influence the anti-neuroinflammatory effect. Finally, compounds **2**, **21**, **23**, and **44**, which had IC_{50} values of 5.8, 6.6, 8.4, and 8.5 μM, respectively, were selected as target compounds for further investigations. It is noteworthy that except for **2**, the other three proposed compounds are remarkably different from the hit compound in their structures, which may lead to a different mode of action.

Table 1. Inhibitory activities of compounds **1**−**44** against LPS-induced NO release in BV2 cells.

Compound	$IC_{50}{}^{a}$ (μM)	Compound	IC_{50} (μM)
1	50.7	**24**	—
2	5.8	**25**	—

3	51.5	**26**	84.8
4	11.1	**27**	75.0
5	113.4	**28**	—
6	90.7	**29**	137.5
7	11.2	**30**	74.0
8	194.7	**31**	21.7
9	92.6	**32**	138.4
10	37.1	**33**	41.3
11	24.9	**34**	11.2
12	32.4	**35**	—
13	95.3	**36**	25.6
14	22.7	**37**	40.9
15	182.2	**38**	53.8
16	54.8	**39**	28.2
17	—[b]	**40**	—
18	—	**41**	—
19	—	**42**	24.1
20	39.1	**43**	37.3
21	6.6	**44**	8.5
22	14.4	Curcumin[c]	2.2
23	8.4		

[a] half-maximal inhibitory concentration. [b] inhibition rate was less than 50% at the maximal concentration of 0.01 M. [c] positive control.

***In vitro* efficacy of lead compounds in primary cultures.** The four promising compounds were further examined for anti-neuroinflammatory effects in primary mixed mesencephalic neuronal/glial cultures. The microglia were activated by LPS stimulation, as indicated by the over-expression of membrane Iba-1, a marker of microglia activation, and they showed increased production of IL-1β and TNF-α. The neuroinflammatory response was greatly inhibited by

compounds **2**, **21**, **23**, and **44** at 10 μM (Figure 2A–C). The LPS-induced neuroinflammation also induced dopaminergic neurotoxicity, as reflected by the reduced expression of tyrosine hydroxylase (TH), which was rescued by these compounds (Figure 2D). These data suggest that the four compounds potentially suppressed neuroinflammation and protected dopaminergic neurons.

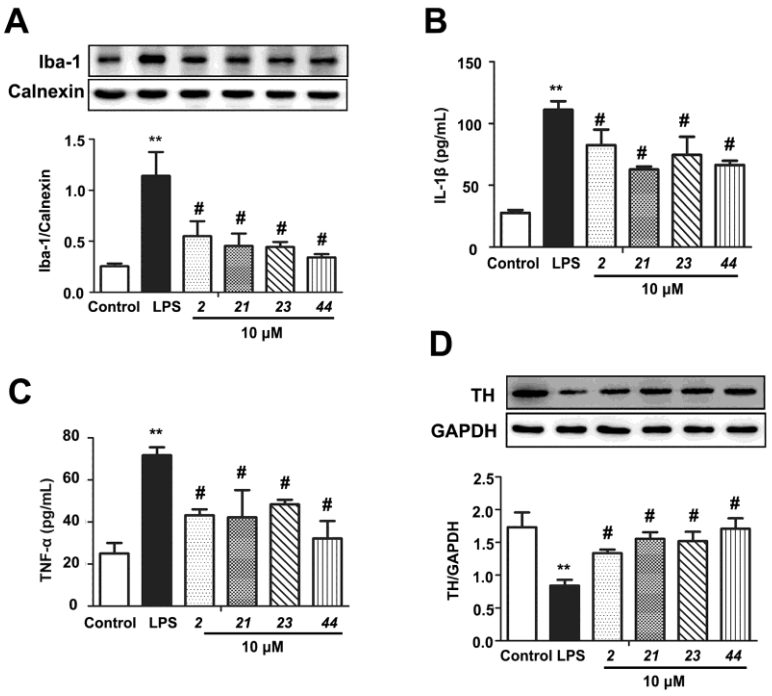


Figure 2. The effects of compounds **2**, **21**, **23**, and **44** on neuroinflammation and TH expression in primary mixed midbrain neuronal/glial cultures stimulated with LPS. Control group: primary mixed mesencephalic neuronal/glial cultures with no treatment; LPS group: primary mixed neuronal/glial cultures stimulated with LPS (100 ng/mL); Other groups: 10 μM of **2**, **21**, **23** and **44** incubated with cells for 1 hour before LPS challenge, and then incubated for 5 h to detect cytokines, 24 h to measure Iba-1 expression and 7 days to measure TH expression. (**A**) Effects of the indicated compounds on Iba-1 expression in microglia (n = 4). A representative image is shown. Inhibitory effects of the indicated compounds on (**B**) IL-1β and (**C**) TNF-α production in medium measured by ELISA assay (n = 6). (**D**) Effects of the

indicated compounds on TH expression measured by Western blot (n = 4). A representative immunoblot image is shown. Data are the mean ± standard deviation (SD),*P < 0.05 and **P < 0.01 *versus* control cells; $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01 *versus* LPS-treated cells.

Pharmacokinetic properties and safety profiles. The *in vivo* preliminary pharmacokinetic properties of these four lead compounds were investigated in mice following the intragastric administration of each compound at a dose of 50 mg/kg (Table 2). All compounds were detectable in the plasma 5 min after administration. However, only **2** and **21** efficiently passed through the blood-brain barrier, whereas the concentrations of **23** and **44** in the brain were low (just above the limit of detection) and undetected, respectively. Therefore, compounds **2** and **21** were selected for further safety studies.

The results of *in vitro* toxicity study indicated that neither of the two compounds had selectivity for hERG. In the *in vivo* toxicity study, both showed satisfactory maximum tolerated doses after being orally administered to mice, with median lethal dose (LD_{50}) values of 2000 and >5000 mg/kg for compounds **2** and **21**, respectively. Nevertheless, the 14-day toxicity study showed that compound **2** exhibited hepatotoxicity and nephrotoxicity at a higher dose (1000 mg/kg), whereas compound **21** had no detectable adverse effects. Considering the long-term administration requirements for PD medications, compound **21** was chosen instead of compound **2** for further *in vivo* efficacy studies.

Table 2. Pharmacokinetic properties of the lead compounds.

Compound	Blood			Brain		
	$AUC_{0-\infty}$ (μg·[L·h])[a]	$t_{1/2}$ (h)[b]	C_{max} (μg/L)[c]	$AUC_{0-\infty}$ (μg·[L·h])	$t_{1/2}$ (h)	C_{max} (μg/L)
2	610.8 ± 42.2[d]	6.4 ± 0.7	1361.6 ± 561.1	1981.2 ± 346.7	3.4 ± 0.6	4668.0 ± 1624.4
21	5733.0 ±	1.1 ± 0.3	2590.0 ±	497.1 ±	1.5 ± 0.4	192.0 ±

	635.7		420.2	72.2		27.2
23	3195.1 ± 891.1	1.1 ± 0.2	1645.0 ± 233.4	≈ LOD[e]	–	≈ LOD
44	37.6 ± 18.0	1.2 ± 0.7	26.5 ± 8.4	ND	–	ND

[a] The area under the curve from 0 h to infinity. [b] Half-life. [c] Maximum concentration. [d] The values are the mean ± SD (n = 6). [e] Limit of detection

***In vivo* efficacy of compound 21 in PD mouse models.** Compound **21** was successively evaluated in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- and MPTP/probenecid (prob)-induced sub-acute and chronic PD mouse models, respectively, and in α-synuclein (syn) transgenic mice. Obvious dopaminergic neuron dysfunction and neuroinflammatory responses were observed in the three different PD models. Compound **21** greatly improved the motor behavior dysfunction of the mice by increasing their staying time on the rod in the rotarod tests and their performance scores in the pole tests. Furthermore, it increased the dopamine levels in the striatum, reversed the decline in TH-positive neurons in the substantia nigra, and decreased the productions of proinflammatory molecules, such as IL-1β and TNF-α, at doses of 10 and 20 mg/kg after short- (MPTP, 10-day) or long-term (MPTP/prob, 7-week and α-syn transgenic mice, 10-week) administration (Figures 3–5). The *in vivo* study demonstrated the potent neuroprotective effects of compound **21** on dopaminergic neurons, which were closely associated with its suppression of neuroinflammation in the brain.

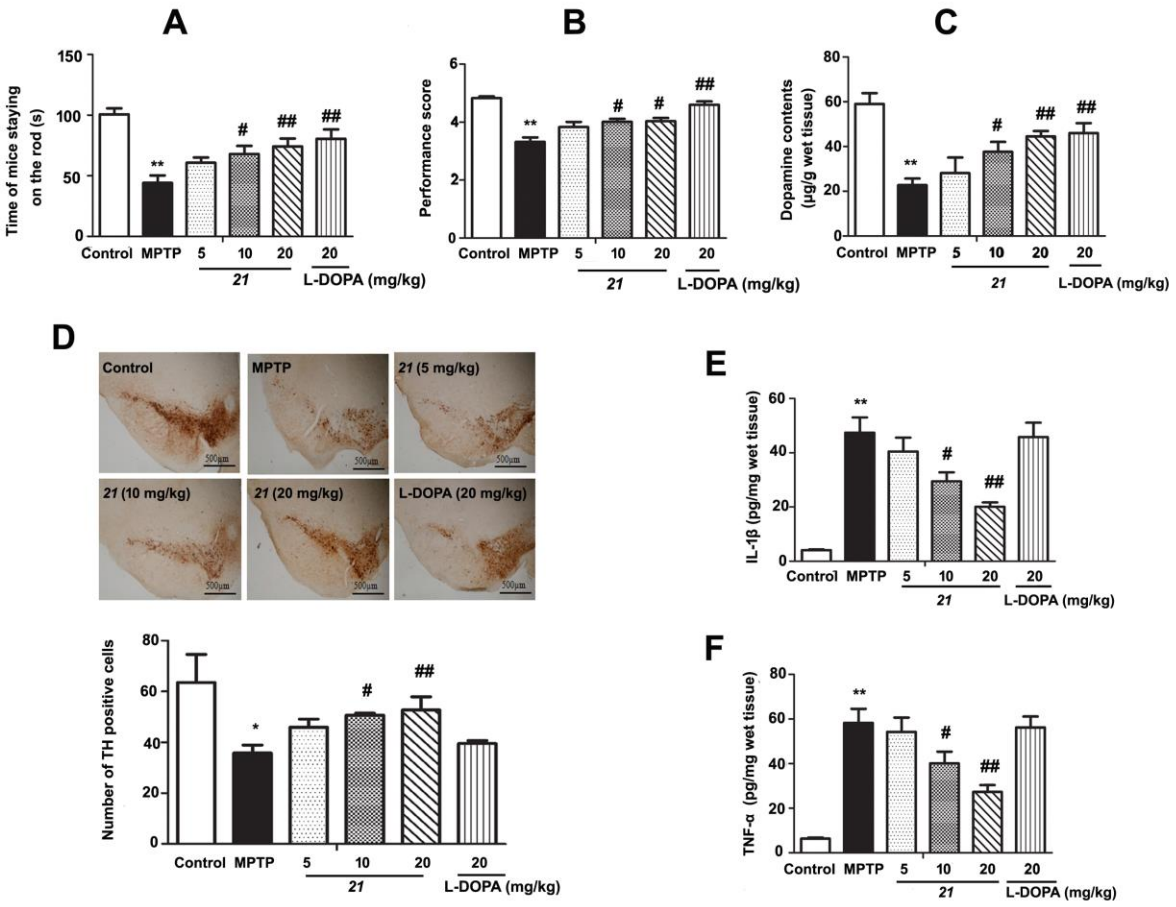


Figure 3. The effect of compound **21** on the MPTP-induced sub-acute PD mouse model. Control group: mice were treated with 0.5% sodium carboxymethylcellulose (CMC-Na); MPTP group: mice were injected with MPTP hydrochloride (30 mg/kg, i.p.) for 5 consecutive days; Other groups: mice were administered different doses of compound **21** (5, 10, 15 mg/kg) and L-DOPA (20 mg/kg) 30 min before each MPTP injection, and continued administered for the next 7 days. Compound **21** improved motor behavior of mice, as measured by (**A**) rotarod test and (**B**) pole test (n = 15). **(C**) The effect of compound **21** on dopamine level (n = 8). (**D**) The effect of compound **21** on the TH-positive staining neurons. Representative sections of substantia nigra from 5 mice are shown. Inhibitory effect of compound **21** on (**E**) IL-1β and (**F**) TNF-α production in the mouse midbrain measured by ELISA assay on the 12th day after the

administration of compound **21** (n = 6). Data are the mean ± SD; *P < 0.05 and **P < 0.01 *versus* control mice; #P < 0.05 and ##P < 0.01 *versus* MPTP injected mice.

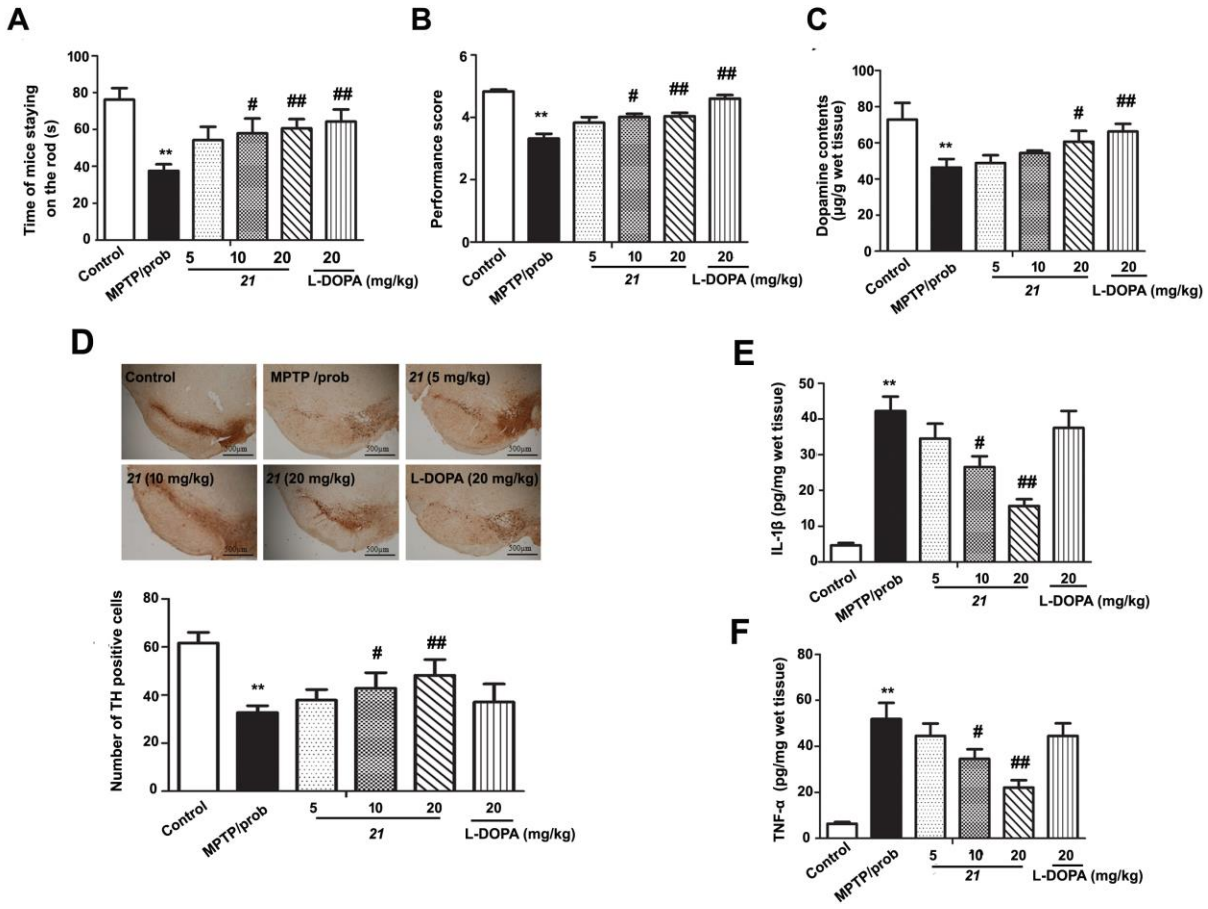


Figure 4. The effect of compound **21** on the MPTP/prob-induced chronic PD mouse model. Control group: mice were treated with 0.5% CMC-Na; MPTP/prob group: mice were administered 10 doses of MPTP hydrochloride (25 mg/kg, subcutaneously, s.c.) in combination with probenecid (250 mg/kg, i.p.) on a 5-week schedule; Other groups: mice showing behavioural dysfunction after the last MPTP/prob injection were administered different doses of compound **21** (5, 10, 15 mg/kg) and L-DOPA (20 mg/kg) once a day for 7 weeks. Compound **21** improved motor behavior of mice, as measured by (**A**) rotarod test and (**B**) pole test (n = 15). **(C**)

The effect of compound **21** on dopamine level (n = 8). (**D**) The effect of compound **21** on the TH-positive staining neurons. Representative sections of substantia nigra from 5 mice are shown. Inhibitory effect of compound **21** on (**E**) IL-1β and (**F**) TNF-α production in the mouse midbrain measured by ELISA assay (n = 6). Data are the mean ± SD; *P < 0.05 and **P < 0.01 *versus* control mice; $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01 *versus* MPTP/prob-treated mice.

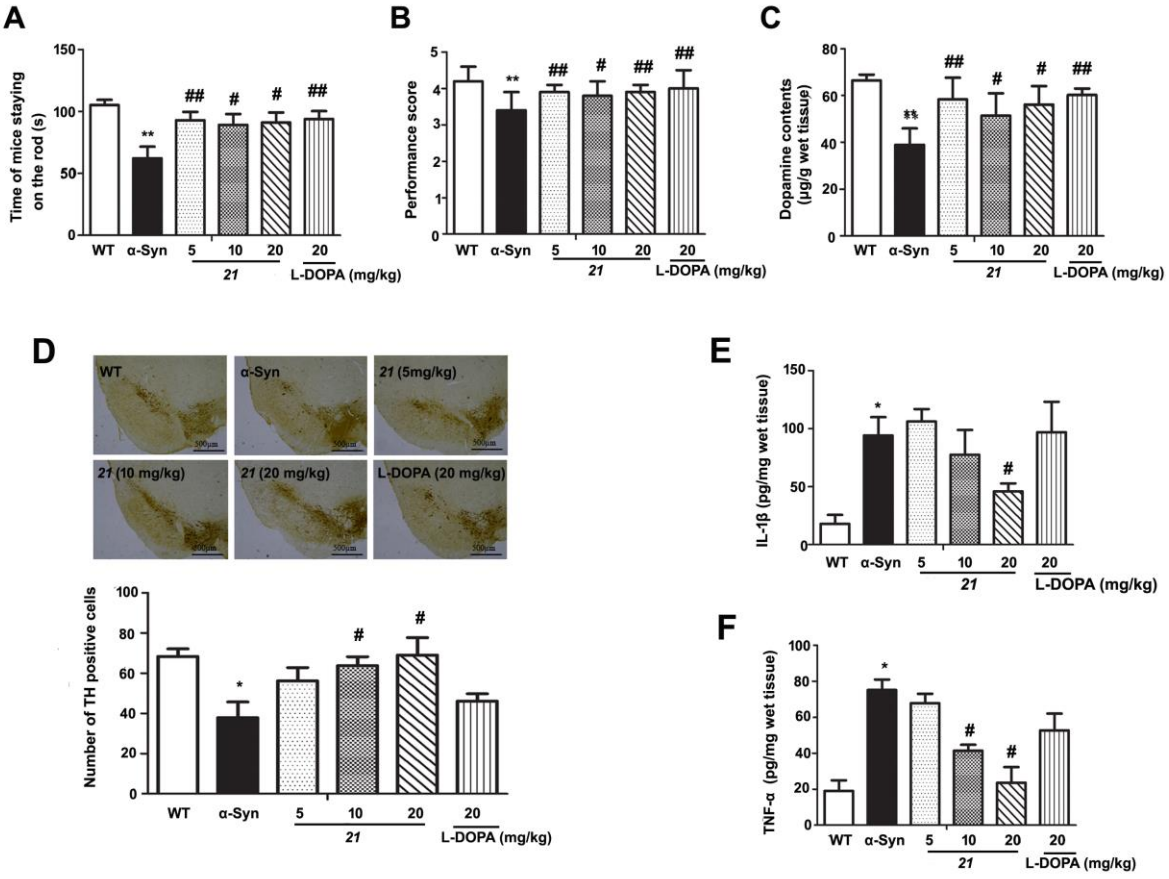


Figure 5. The effect of compound **21** on α-syn transgenic mice. WT group: mice were treated with 0.5% CMC-Na; α-Syn group: α-Syn (A53T) transgenic mice; Other groups: mice were administered different doses of compound **21** (5, 10, 15 mg/kg) and L-DOPA (20 mg/kg) once a day for 12 weeks. Compound **21** improved motor behavior of mice, as measured by (**A**) rotarod test and (**B**) pole test (n = 15). **(C**) The effect of compound **21** on dopamine level (n = 8).

(**D**) The effect of compound **21** on the TH-positive staining neurons. Representative sections of substantia nigra from 5 mice are shown. Inhibitory effect of compound **21** on (**E**) IL-1β and (**F**) TNF-α production in the mouse midbrain measured by ELISA assay (n = 6). Data are the mean ± SD; *P < 0.05 and **P < 0.01 *versus* control mice; $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01 *versus* α-syn transgenic mice.

Mechanistic studies of neuroprotective effects. To elucidate the molecular target of compound **21** in neuroinflammation, we performed isobaric tags for relative and absolute quantification (iTRAQ)-labelling proteomic studies in a MPTP/prob-induced chronic PD mouse model. We quantified 3508 proteins and applied a threshold of >1.5-fold to identify proteins that were differentially expressed, and 132 met the criteria (Supporting Information, Proteomics Data). Among these proteins, we selected those that were reportedly associated with neuroinflammation to validate the proteomic quantization. Finally, twenty proteins were analyzed, and eight were consistent with the proteomic analysis, as described in Figure 6. The Western blot analysis confirmed phosphatase and tensin homolog deleted on chromosome 10 (PTEN); Akt and NO synthase (NOS) were the most affected by compound **21**. Akt is reportedly the downstream effector of PTEN, and its phosphorylation may induce NOS activation.[23,24] PTEN counteracts the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway and controls inflammatory responses.[23,25] The present results indicated that the PTEN/Akt pathway and downstream NOS might be involved in the anti-inflammatory activity of compound **21**.

PTEN function is primarily regulated by phosphorylation through specific kinases, such as Src-tyrosine kinase, glycogen synthase kinase-3 (GSK3)-β, casein kinase 2 (CK2), liver kinase B1 (LKB1), Rho guanosine triphosphatases (GTPases) activating protein 21 (ARHGAP21), and Rho-

associated protein kinase 1 (ROCK1), which is frequently associated with functionally inactive PTEN.[26,27] Among these kinases, the expression levels of GSK3β, ARHGAP21, ROCK1 and Src were affected by compound **21** in the proteomic quantization. The four proteins were then validated, and Src-tyrosine kinase was the most obviously altered by compound **21** (Supporting Information, Figures S1 and S2). Src-tyrosine kinase is a major modulator in the proximal intracellular signaling pathways in innate cells and activates monocytes and macrophages.[28,29] Inhibition of Src reportedly suppresses neuroinflammation and protects mice from ischemia-induced brain damage.[30] Our present results revealed that in addition to PTEN and Akt phosphorylation, Src activation was also inhibited by compound **21** (Figures 6A–C). Src primarily phosphorylates PTEN at Tyr240, inactivating it and phosphorylating Akt.[31] We knocked down the Src gene in primary microglia and midbrain mixed neuronal/glial cultures and stimulated the cells with LPS, 1-methyl-4-phenylpyridinium ion (MPP^+), or α-synuclein. We found that the phosphorylation of PTEN and Akt decreased (Figure 6D), the inflammatory response was reduced, and the injury of dopaminergic neurons was alleviated (Figure 6E). Most importantly, we found that the suppression of neuroinflammation (Figures 6E and F) and the neuroprotective activity of compound **21** were mostly abrogated when Src was silenced (Figures 6G–I). The above data prove that Src tyrosine kinase was the main target of compound **21** in its neuroinflammation regulation and associated neuroprotection.

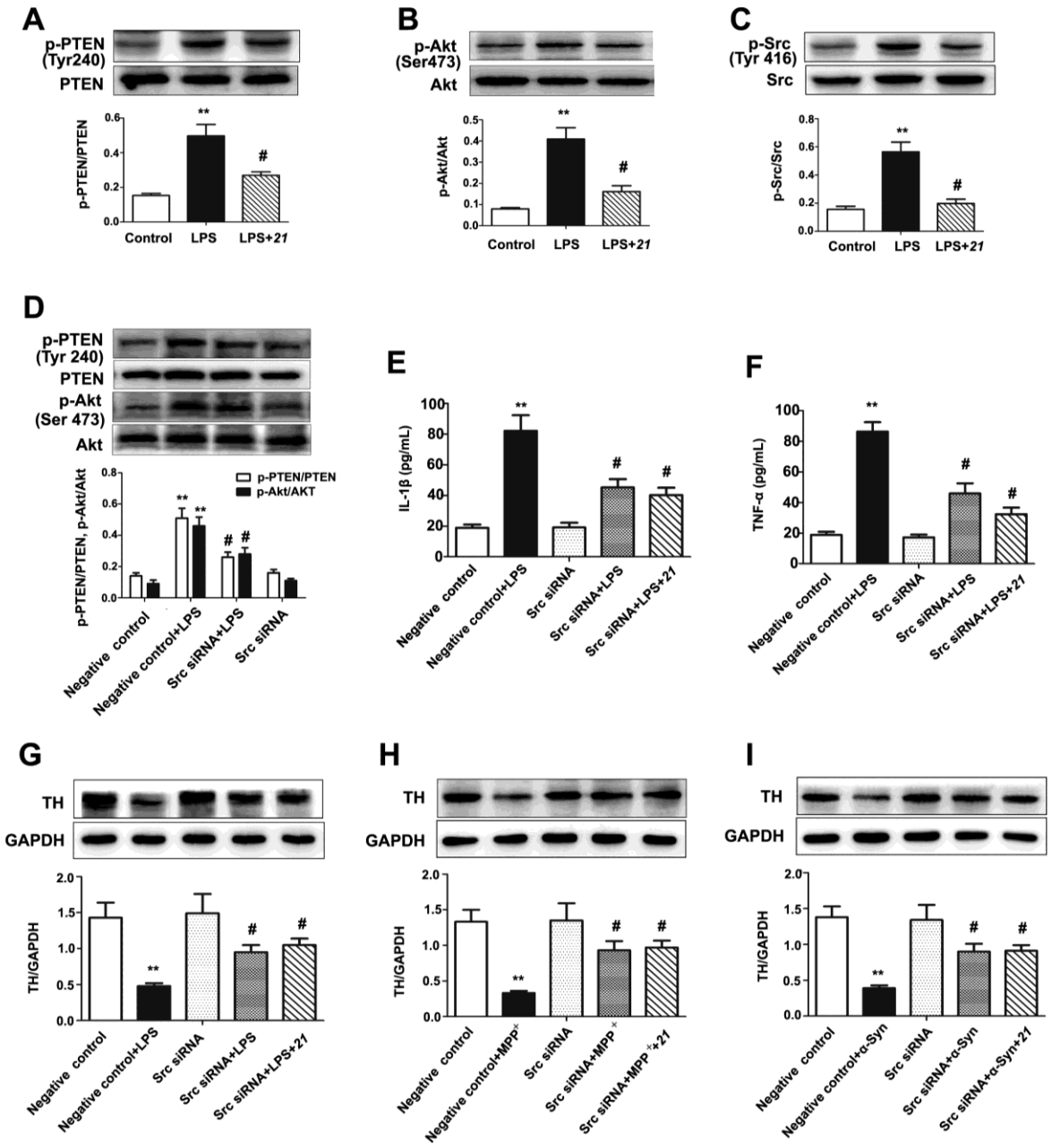


Figure 6. Compound **21** suppressed neuroinflammation by inhibiting Src signaling. Control group: primary microglia with no treatment; LPS group: primary microglia stimulated with LPS (100 ng/mL); LPS + **21** group: the microglia was incubated with compound **21** (10 μM) for 1 hour followed by LPS challenge; Negative control group: primary microglia or mixed mesencephalic neuronal/glial cultures were tranfected with empty vector;

Negative control + LPS group: primary microglia or mixed mesencephalic neuronal/glial cultures were tranfected with empty vector followed by LPS challenge; Src siRNA group: microglia or mixed mesencephalic neuronal/glial cultures were tranfected with Src siRNA; Src siRNA + LPS/MPP^+/α-Syn group: primary microglia or mixed mesencephalic neuronal/glial cultures were tranfected with Src siRNA, LPS (100 ng/mL), MPP^+ (30 μM), or α-syn (250 nM) was added 4 h later; Src siRNA + LPS/MPP^+/α-Syn +**21** group: primary microglia or mixed neuronal/glial cultures were tranfected with Src siRNA, compound **21** (10 μM) was added 1 h before LPS, MPP^+ or α-Syn challenge. The effects of compound **21** on (**A**) p-PTEN, (**B**) p-Akt and (**C**) p-Src expression in primary microglia cultures. (**D**) The regulation of Src on PTEN and Akt in primary microglia cultures (n = 4). The effects of compound **21** on (**E**) IL-1β and (**F**) TNF-α production in LPS-stimulated primary microglia cultures (n = 6). The effects of compound **21** on TH expression in (**G**) LPS-, (**H**) MPP^+- and (**I**) α-syn-stimulated primary mixed mesencephalic neuronal/glial cultures (n = 4). A representative immunoblot images are shown. The results are shown as the mean ± SD; $^{**}P < 0.01$ *versus* control cells; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *versus* LPS-, MPP^+- or α-syn- treated cells; $\Delta P < 0.05$ *versus* Src siRNA-treated cells.

CONCLUSIONS

In this paper, we reported the discovery and optimization of a novel class of anti-neuroinflammatory compounds: phloroglucinol derivatives. The structure-activity relationships of this series were complex, and changes in either the phenolic hydroxyl groups or the sidechain of the hit compound significantly influenced the activities. Note that the introduction of various nitrogen-containing pharmacophores into the hit

compound enhanced the potency more efficiently than minor modifications, such as changing the number of hydroxyl groups, methylating the hydroxyl groups at different positions, and altering the length of the sidechain. These results might provide a new strategy for the structural modification of other similar compounds.

Our study provided evidence supporting compound **21** as a potential candidate for PD treatment because of its potent *in vitro* and *in vivo* anti-neuroinflammatory activities, novel mechanism, impressive penetration of the blood-brain barrier, and low toxicity. Moreover, our results yielded further proof that neuroinflammation suppression might become an effective disease-modifying therapy that could delay or slow the progression of PD and thereby open up a new strategy for the future treatment of PD patients. We believe that further structural modification based on compound **21** and its exact mechanisms would enable the development of more potent agents used for PD. And these studies are currently underway in our laboratory.

EXPERIMENTAL SECTION

Chemistry. Reagents and solvents were of commercial quality and used without further purification. ^{1}H and ^{13}C NMR spectra were acquired on an INOVA-500, Mecury-400 or Mecury-300 spectrometer. HRESIMS data were obtained on an Agilent 6520 Accurate-Mass-Q-TOF LC/MS spectrometer. ESIMS data were recorded on an Agilent 1100 Series LC/MSD Trap/SL spectrometer. Optical rotations were measured on a JASCO P-2000 automatic polarimeter. TLC was conducted with glass precoated with silica gel GF254 (Qingdao Marine Chemical Inc., China) to monitor the progression of the reactions. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) were used for column chromatography (CC). The purity of the test compounds was determined by HPLC analysis using an Agilent 1100 series instrument equipped with a DAD detector and a YMC-Pack ODS column (100 mm × 4.6 mm, 5 μm) at 210 nm, and

was generally ≥ 95%, if not denoted. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with a SPD-20A detector using a YMC-Pack ODS-A column (250 × 50 mm, 5 μm).

*1-(2,4-Dihydroxy-6-methoxyphenyl)-3-methylbutan-1-one (**2**).* To a stirred suspension of **1** (2.10 g, 10.0 mmol) in anhydrous CH_2Cl_2 (50 mL) was added DIPEA (2.71 g, 21.0 mmol) and the reaction mixture was stirred for 15 min at 0 ℃, and then CH_3OCH_2Cl (2.00 g, 25.0 mmol) in CH_2Cl_2 (20 mL) was added dropwise. The mixture was stirred for 1 h at room temperature and subsequently poured into cold water (20 mL), and extracted with chloroform (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue obtained on column purification (silica, petroleum ether/EtOAc, 15:1 as eluent) afforded **2a** as a colorless oil (1.40 g, 47%).

To a solution of **2a** (0.30 g, 1.0 mmol) in anhydrous acetone (20 mL) was added anhydrous K_2CO_3 (0.27 g, 2.0 mmol), and the reaction mixture was refluxed for 15 min, followed by addition of Me_2SO_4 (100 μL, 2.0 mmol). After refluxing for an additional 5 h, the solvent was removed in vacuo. Water (20 mL) was then added to the residue, and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue obtained on column purification (silica, petroleum ether/EtOAc, 30:1 as eluent) afforded **2b** (0.25 g, 60%) as a yellow oil.

To a solution of **2b** (0.25g, 0.8 mmol) in MeOH (10 mL) was added 2N HCl (2 mL), and the reaction mixture was refluxed for 1 h. Then the solvent was removed in vacuo, and the resulting residue was dissolved in EtOAc (50 mL) and washed with water. The organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue obtained on column purification (silica, petroleum ether/EtOAc, 5:1 as eluent) afforded **2** as a white solid (0.18 g, 83%, mp 122–123 ℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.59 (br s, OH-2′, OH-4′), 5.94 (1H, s, H-5′), 5.85 (1H, s, H-

3′), 3.80 (3H, s, OCH_3), 2.77 (2H, d, *J* = 6.5 Hz, H-2), 2.07 (1H, m, H-3), 0.89 (6H, d, *J* = 6.5 Hz, CH_3-4, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.0, 165.7, 164.4, 162.4, 104.2, 95.3, 90.9, 55.3, 51.9, 24.5, 22.2 (2C); HRESIMS *m/z* = 225.1126 $[M + H]^+$ (calcd for $C_{12}H_{17}O_4$, 225.1121).

*General Procedure for Acylation by Friedel–Crafts Reaction for Compounds **3**–**8** (GP1).* The phenolic precursor (10.0 mmol) was dissolved in BF_3–Et_2O (20 mL), and an amount of 1 equiv of acyl chloride was added, and the reaction mixture was stirred at 80 ℃ for 2 h. After cooling, the reaction mixture was poured into ice-cooled 5% KOAc solution (50 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution and dried over Na_2SO_4 and concentrated in vacuo. The resulting product was obtained by column chromatography.

*1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-methylbutan-1-one (**3**).* This compound was prepared from 3,5-dimethoxyphenol and isovaleryl chloride by means of GP1 as a white solid (1.70 g, 71%, mp 54–55 ℃). Eluent for column chromatography: petroleum ether/EtOAc (30:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.59 (1H, s, OH-2′), 6.09 (1H, s, H-5′), 6.07 (1H, s, H-3′), 3.84 (3H, s, OCH_3-4′), 3.79 (3H, s, OCH_3-6′), 2.78 (2H, d, *J* = 6.5 Hz, H-2), 2.08 (1H, m, H-3), 0.91 (6H, d, *J* = 6.5 Hz, CH_3-4, CH_3-5); ESIMS *m/z* = 237 $[M + H]^+$.

*1-(2,4,6-Trimethoxyphenyl)-3-methylbutan-1-one (**4**).* This compound was prepared from 1,3,5-trimethoxybenzene and isovaleryl chloride by means of GP1 as a colorless oil (1.23 g, 49%). Eluent for column chromatography: petroleum ether/EtOAc (30:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.24 (2H, s, H-3′, H-5′), 3.79 (3H, s, OCH_3-4′), 3.72 (6H, s, OCH_3-2′, OCH_3-6′), 2.48 (2H, d, *J* = 7.0 Hz, H-2), 2.03 (1H, m, H-3), 0.87 (6H, d, *J* = 7.0 Hz, CH_3-4, CH_3-5); ESIMS *m/z* = 275 $[M + Na]^+$.

*1-(2-Hydroxy-4-methoxyphenyl)-3-methylbutan-1-one (**5**).* This compound was prepared from 3-methoxyphenol and isovaleryl chloride by means of GP1 as a colorless oil (1.95 g, 94%). Eluent for column chromatography: petroleum ether/EtOAc (25:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.81 (br s, OH- 2′), 7.82 (1H, d, J = 8.5 Hz, H-6′), 6.47 (1H, dd, J = 8.5, 2.0 Hz, H-5′), 6.44 (1H, d, J = 2.0 Hz, H-3′), 3.79 (3H, s, OCH_3), 2.79 (2H, d, J = 6.5 Hz, H-2), 2.12 (1H, m, H-3), 0.91 (6H, d, J = 6.5 Hz, CH_3-4, CH_3-5); ESIMS m/z = 209 $[M + H]^+$.

*1-(4-Hydroxyphenyl)-3-methylbutan-1-one (**6**).* This compound was prepared from phenol and isovaleryl chloride by means of GP1 as a white solid (1.00 g, 56%, mp 90–92 ℃). Eluent for column chromatography: petroleum ether/EtOAc (20:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (2H, d, J = 8.0 Hz, H-2′, 6′), 6.81 (2H, d, J = 8.0 Hz, H-3′, 5′), 2.73 (2H, d, J = 6.5 Hz, H-2), 2.10 (1H, m, H-3), 0.90 (6H, d, J = 6.5 Hz, CH_3-4, CH_3-5); ESIMS m/z = 179 $[M + H]^+$.

*1-(2,4,6-Trihydroxyphenyl)pentan-1-one (**7**).* This compound was prepared from phloroglucinol and valeryl chloride by means of GP1 as a white solid (1.19 g, 57%, mp 130–131 ℃). Eluent for column chromatography: CH_2Cl_2 /MeOH (30:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.21 (2H, s, OH-2′, OH-6′), 10.30 (1H, s, OH-4′), 5.78 (2H, s, H-3′, H-5′), 2.96 (2H, t, J = 7.5 Hz, H-2), 1.55 (2H, m, H-3), 1.31 (2H, m, H-4), 0.87 (3H, t, J = 7.5 Hz, CH_3-5); ESIMS m/z = 211 $[M + H]^+$.

*1-(2,4,6-Trihydroxyphenyl)ethanone (**8**).* This compound was prepared from phloroglucinol and acetyl chloride by means of GP1 as a white solid (0.70 g, 42%, mp 224 ℃). Eluent for column chromatography: CH_2Cl_2 /MeOH (30:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.21 (br s, OH-2′, OH-6′), 10.30 (br s, OH-4′), 5.78 (2H, s, H-3′, H-5′), 2.53 (3H, s, CH_3-2); ESIMS m/z = 169 $[M + H]^+$.

*General Procedure for Amidation for Compounds **9** and **10** (GP2).* To a stirred suspension of 2,4,6-trihydroxybenzoic acid (0.34 g, 2.0 mmol) and DMF (0.1 mL) in anhydrous CH_2Cl_2 (30 mL) was added dropwise oxalyl chloride (0.51 g, 4.0 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 1h and then evaporated in vacuo to give a clear oil. This crude oil was dissolved in anhydrous THF (5 mL), and amine (5.0 mmol) in THF (5 mL) was added dropwise in an ice-bath. After stirring at room temperature for 1h, the mixture was filtered and the filtrate was concentrated in vacuo to dryness. The resulting product was obtained by column chromatography.

*N-Isopropyl-2,4,6-trihydroxybenzamide (**9**).* This compound was prepared using isopropylamine by means of GP2 as a white solid (0.31 g, 80%, mp 100–102 ℃). Eluent for column chromatography: CH_2Cl_2 /MeOH (25:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.42 (1H, d, J = 7.5 Hz, NH), 5.77 (2H, s, H-3′, H- 5′), 4.03 (1H, m, H-3), 1.15 (6H, d, J = 6.0 Hz, CH_3-4, CH_3-5); ESIMS m/z = 212 $[M + H]^+$.

*N-Butyl-2,4,6-trihydroxybenzamide (**10**).* This compound was prepared using butylamine by means of GP2 as a white solid (0.32 g, 73%, mp 73–75 ℃). Eluent for column chromatography: CH_2Cl_2 /MeOH (25:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.62 (2H, s, OH-2′, OH-6′), 9.87 (1H, s, OH-4′), 8.60 (1H, t, J = 5.5 Hz, NH), 5.77 (2H, s, H-3′, H-5′), 3.26 (2H, dd, J = 13.0, 6.5 Hz, H-3), 1.47 (1H, m, H-4), 1.30 (1H, m, H-5), 0.89 (3H, t, J = 7.0 Hz, CH_3-6); LC-ESI-MS (m/z): ESIMS m/z = 226 $[M + H]^+$.

*General Procedure for Introduction of Aminoketone Unit for Compounds **11**−**14** (GP3).* To a stirred solution of 1,3,5-trimethoxybenzene (2.00 g, 12.0 mmol) in chlorobenzene (50 mL) was added chloroacetonitrile (0.90 g, 12.0 mmol), and the reaction mixture was stirred at room temperature with HCl gas bubbled in for 6 h, followed by filtration. A solution of the precipitate

in water (20 mL) was refluxed for 1h, and then cooled to 0 ℃. The key intermediate **11b** was obtained by filtration as a white soild (2.75g, 94%).

To a suspension of **11b** (0.50 g, 2.0 mmol) and anhydrous K_2CO_3 (0.34 g, 4.0 mmol) in anhydrous acetone (20 mL) was added an amount of 1 equiv of amines (2.0 mmol), and the mixture was refluxed for 24h, and then filtered. The filtrate was concentrated in vacuo, and the residue was recrystallized in EtOH/H_2O (4:1) to provide the resulting product.

*2-(Piperidin-1-yl)-1-(2,4,6-trimethoxyphenyl)ethanone (**11**).* This compound was prepared from **11b** and piperidine by means of GP3 as a white solid (0.48 g, 84%, mp 66–67 ℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.23 (2H, s, H-3′, H-5′), 3.78 (3H, s, OCH_3-4′), 3.71 (6H, s, OCH_3-2′, OCH_3-6′), 3.31 (2H, s, H-2), 2.37 (4H, t, J = 4.5 Hz, H-2″, H-6″), 1.43 (4H, m, H-3″, H-5″), 1.33 (2H, m, H-4″); ESIMS *m/z* = 294 $[M + H]^+$.

*2-Morpholino-1-(2,4,6-trimethoxyphenyl)ethanone (**12**).* This compound was prepared from **11b** and morpholine by means of GP3 as a white solid (0.41 g, 69%, mp 94–96 ℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.24 (2H, s, H-3′, H-5′), 3.79 (3H, s, OCH_3-4′), 3.72 (6H, s, OCH_3-2′, OCH_3-6′), 3.53 (4H, t, J = 4.5 Hz, H-3″, H-5″), 3.38 (2H, s, H-2), 2.43 (4H, br s, H-2″, H-6″); ESIMS *m/z* = 296 $[M + H]^+$.

*2-(4-Methylpiperazin-1-yl)-1-(2,4,6-trimethoxyphenyl)ethanone (**13**).* This compound was prepared from **11b** and N-methylpiperazine by means of GP3 as a white solid (0.41 g, 57%, mp 130–131 ℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.23 (2H, s, H-3′, H-5′), 3.79 (3H, s, OCH_3-4′), 3.72 (6H, s, OCH_3-2′, OCH_3-6′), 3.36 (2H, s, H-2), 2.43 (4H, br s, H-2″, H-6″), 2.27 (4H, br s, H-3″, H-5″), 2.13 (3H, s, CH_3-1‴); ESIMS *m/z* = 309 $[M + H]^+$.

*2-(4-Ethylpiperazin-1-yl)-1-(2,4,6-trimethoxyphenyl)ethanone (**14**).* This compound was prepared from **11b** and N-ethylpiperazine by means of GP3 as a white solid (0.47 g, 70%, mp

95–97 ℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.23 (2H, s, H-3′, H-5′), 3.78 (3H, s, OCH_3-4′), 3.71 (6H, s, OCH_3-2′, OCH_3-6′), 3.35 (2H, s, H-2), 2.43 (4H, br s, H-2″, H-6″), 2.30 (4H, br s, H-3″, H-5″), 2.26 (2H, q, J = 7.0 Hz, H-1‴), 0.95 (3H, t, J = 7.0 Hz, H-2‴); ESIMS m/z = 323 $[M + H]^+$.

*General Procedure for Introduction of Aminoketone Unit for Compounds **15**−**17** (GP4).* To a suspension of 4-chlorobutanenitrile (1.03 g, 10.0 mmol), anhydrous K_2CO_3 (1.40 g, 25.0 mmol) in anhydrous acetonitrile (20 mL) was added an amount of 1 equiv of amines (10.0 mmol), and the mixture was stirred at room temperature for 20 h, and then concentrated in vacuo. To the residue was added water (25 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to give a clear oil.

To a solution of the crude oil in nitrobenzene (20 mL) was added phloroglucinol (1.26 g, 10.0 mmol), and the reaction mixture was stirred at room temperature with HCl gas bubbled in for 12 h. Then water (15 mL) was added to the mixture until the precipitate was dissolved. The organic layer was decanted and washed with water (20 mL), and the washing added to the aqueous layer, which was refluxed for 1 h subsequently. The mixture was cooled to 0 ℃ and a precipitate appeared. The precipitate was filtered off and recrystallized in EtOH/H_2O to afford the resulting product.

*4-(Piperidin-1-yl)-1-(2,4,6-trihydroxyphenyl)butan-1-one hydrochlorate (**15**).* This compound was prepared from piperidine by means of GP4 as a white solid (0.67 g, 21%, mp 204–205 ℃). Solvent for recrystallization: EtOH/H_2O (5:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.23 (2H, s, OH-2′, OH-6′), 10.45 (1H, s, OH-4′), 5.81 (2H, s, H-3′, H-5′), 3.43 (2H, br d, J = 11.5 Hz, H-2″a, H-6″a), 3.08 (2H, t, J = 6.5 Hz, H-2), 3.05 (2H, overlap, H-4), 2.86 (2H, m, H-2″b, H-6″b), 1.94 (2H, m, H-3), 1.79 (2H, br d, J = 14.0 Hz, H-3″a, H-5″a), 1.65 (2H, overlap, H-3″b, H-5″b), 1.60

(1H, m, H-4″a,), 1.38 (1H, m, H-4″b); ^{13}C NMR (125 MHz, DMSO-d_6) δ 202.8, 164.5, 163.9, 163.9, 103.4, 94.3, 94.3, 55.2, 51.8, 51.8, 38.7, 22.3, 22.3, 21.0, 17.9; HRESIMS *m/z* = 280.1534 $[M + H]^+$ (calcd for $C_{15}H_{22}NO_4$, 225.1543).

*4-(Cyclohexyl(methyl)amino)-1-(2,4,6-trihydroxyphenyl)butan-1-one hydrochlorate (**16**).* This compound was prepared from N-methylcyclohexanamine by means of GP4 as a white solid (0.38 g, 11%, mp 160–162 ℃). Solvent for recrystallization: EtOH/H_2O (10:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.73 (2H, s, H-3′, H-5′), 2.98 (2H, t, *J* = 7.0 Hz, H-2), 2.44 (2H, t, *J* = 7.0 Hz, H-4), 2.35 (1H, m, H-1″), 2.18 (3H, s, NCH_3), 1.74 (2H, overlap, H-3), 1.73 (2H, overlap, H-2″a, H-6″a), 1.71 (2H, m, H-2″b, H-6″b), 1.54 (1H, br d, *J* = 12.5 Hz, H-4″a), 1.16 (2H, m, H-3″a, H-5″a), 1.12 (2H, m, H-3″b, H-5″b), 1.04 (1H, m, H-4″b); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.9, 164.8, 164.8, 164.7, 104.4, 94.7, 94.7, 62.1, 52.3, 40.3, 37.0, 27.8, 27.8, 25.9, 25.4, 25.4, 22.6; HRESIMS *m/z* = 308.1858 $[M + H]^+$ (calcd for $C_{17}H_{26}NO_4$, 308.1856).

*4-(4-(3-Chlorophenyl)piperazin-1-yl)-1-(2,4,6-trihydroxyphenyl)butan-1-one hydrochlorate (**17**).* This compound was prepared from 1-(3-chlorophenyl)piperazine by means of GP4 as a white solid (0.60 g, 13%, mp 169–171 ℃). Solvent for recrystallization: EtOH/H_2O (10:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.21 (2H, s, OH-2′, OH-6′), 10.43 (1H, s, OH-4′), 7.26 (1H, t, *J* = 8.0 Hz, H-5‴), 7.05 (1H, br s, H-2‴), 6.96 (1H, br d, *J* = 8.0 Hz, H- H-4‴), 6.87 (1H, br d, *J* = 8.0 Hz, H-6‴), 5.82 (2H, s, H-3′, H-5′), 3.90 (2H, br d, *J* = 12.5 Hz, H-2″a, H-6″a), 3.59 (2H, br d, *J* = 11.5 Hz, H-3″a, H-5″a), 3.19 (2H, overlap, H-2″b, H-6″b), 3.16 (2H, overlap, H-3″b, H-5″b), 3.10 (2H, t, *J* = 6.5 Hz, H-2), 3.03 (2H, t, *J* = 11.5 Hz, H-4), 1.99 (2H, m, H-3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 203.0, 164.8, 164.1, 164.1,150.7, 133.9, 130.6, 119.2, 115.2, 114.1, 103.6, 94.6, 94.6, 55.1, 50.5, 50.5, 44.9, 44.9, 38.9, 18.2; HRESIMS *m/z* = 391.1420 $[M + H]^+$ (calcd for $C_{20}H_{25}N_2O_4Cl$, 391.1419).

*N-(2,4,6-trimethoxybenzyl)propan-2-amine (**18**).* To a stirred solution of 2,4,6-trimethoxybenzaldehyde (196 mg, 1.0 mmol) in anhydrous MeOH (5 mL) was added isopropylamine (120 mg, 2.0 mmol) and $NaBH_3CN$ (76 mg, 1.2 mmol), followed by adjustment of the pH to ~6.0 with HOAc. The reaction mixture was stirred at room temperature for 3 h under an argon atmosphere and quenched with H_2O (20 mL). The solution was extracted with EtOAc (3 × 15 mL), and the combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue obtained on column purification (silica, petroleum ether/EtOAc, 5:1 as eluent) afforded **18** as a white solid (110 mg, 47%, mp 177 ℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.28 (2H, s, H-3′, H-5′), 3.93 (2H, s, H-1), 3.80 (6H, s, OCH_3-2′, OCH_3-6′), 3.79 (3H, s, OCH_3-4′), 3.32 (1H, m, H-3), 3.18 (1H, br s, NH), 1.25 (6H, d, J = 6.5 Hz, CH_3-4, CH_3-5); ESIMS m/z = 240 $[M + H]^+$.

*General Procedure for Reductive Amination for Compounds **19**−**25** (GP5).* To a stirred solution of 2,4,6-trimethoxybenzaldehyde (196 mg, 1.0 mmol) in anhydrous MeOH (5 mL) was added an amount of 2 equiv of L-amino acid methyl (ethyl) ester hydrochloride (2.0 mmol), followed by the addition of $NaBH_3CN$ (76 mg, 1.2 mmol). The reaction mixture was stirred at room temperature for 3 h under an argon atmosphere and quenched with H_2O (20 mL). The solution was extracted with EtOAc (3 × 15 mL), and the combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue obtained on column purification afforded the corresponding intermediate. A solution of the intermediate in 5% KOH in EtOH/H_2O (1:1, 10 mL) was stirred at room temperature for 1 h. The reaction mixture was then neutralized with ion-exchange resin (Amberlite IR-120 H^+), and the filtrate was concentrated in vacuo to yield a residue, which was subjected to prepared HPLC to afford the resulting product.

*2-((2,4,6-Trimethoxybenzyl)amino)acetic acid (**19**).* This compound was prepared from Glycine ethyl ester hydrochloride by means of GP5 as a white solid (110 mg, 43%, mp 160–161 °C). Eluent for column chromatography: petroleum ether/EtOAc (5:1); Conditions for prepared HPLC: CH_3CN/H_2O/TFA (18:82:0.03, 5 mL/min, t_R = 32 min); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.27 (2H, s, H-3′, H-5′), 4.05 (2H, br s, H-1), 3.79 (6H, s, OCH_3-2′, OCH_3-6′), 3.78 (3H, s, OCH_3-4′), 3.57 (2H, s, H-3); ESIMS *m/z* = 278 $[M + H]^+$.

*(S)-2-((2,4,6-Trimethoxybenzyl)amino)propanoic acid (**20**).* This compound was prepared from L-alanine methyl ester hydrochloride by means of GP5 as a white solid (102 mg, 38%, mp 174–175 °C). Eluent for column chromatography: petroleum ether/EtOAc (4:1); Conditions for prepared HPLC: CH_3CN/H_2O/TFA (20:80:0.03, 5 mL/min, t_R = 28 min); $[\alpha]_D^{20} = -10.1°$ (*c* = 0.333, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.27 (2H, s, H-3′, H-5′), 4.07 (2H, br s, H-1), 3.80 (6H, s, OCH_3-2′, OCH_3-6′), 3.79 (3H, s, OCH_3-4′), 3.36 (1H, m, H-3), 1.44 (3H, d, *J* = 6.0 Hz, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.8, 162.5, 159.7, 159.7, 99.3, 90.7, 90.7, 55.9, 55.9, 55.6, 54.0, 37.7, 15.2; HRESIMS *m/z* = 292.1155 $[M + Na]^+$ (calcd for $C_{13}H_{19}NO_5Na$, 292.1155).

*(S)-3-Hydroxy-2-((2,4,6-trimethoxybenzyl)amino)propanoic acid (**21**).* This compound was prepared from L-serine methyl ester hydrochloride by means of GP5 as a white solid (108 mg, 39%, mp 220–221 °C). Eluent for column chromatography: petroleum ether/EtOAc (1:1); Conditions for prepared HPLC: CH_3CN/H_2O/TFA (13:87:0.03, 5 mL/min, t_R = 35 min); $[\alpha]_D^{20} = -6.5°$ (*c* = 0.333, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.26 (2H, s, H-3′, H-5′), 4.10 (2H, br s, H-1), 3.85 (1H, overlap, H-5a), 3.75 (1H, overlap, H-5b), 3.79 (6H, s, OCH_3-2′, OCH_3-6′), 3.78 (3H, s, OCH_3-4′), 3.48 (1H, br s, H-3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.5, 162.4,

159.7, 159.7, 99.5, 90.9, 90.9, 60.9, 59.3, 56.0, 56.0, 55.6, 38.7; HRESIMS *m/z* = 284.1136 [M − H]$^-$ (calcd for $C_{13}H_{18}NO_6$, 284.1140).

*(S)-2-((2,4,6-Trimethoxybenzyl)amino)succinic acid (**22**).* This compound was prepared from L-aspartic acid dimethyl ester hydrochloride by means of GP5 as a white solid (138 mg, 44%, mp 138–139 ℃). Eluent for column chromatography: petroleum ether/EtOAc (5:1); Conditions for prepared HPLC: CH_3CN/H_2O/TFA (10:90:0.03, 5 mL/min, t_R = 41 min); $[\alpha]_D^{20}$ = – 7.3° (*c* = 0.333, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.28 (2H, s, H-3′, H-5′), 4.17 (1H, d, *J* = 13.0 Hz, H-1a), 4.13 (1H, d, *J* = 13.0 Hz, H-1b), 3.99 (1H, t, *J* = 6.0 Hz, H-3), 3.80 (3H, s, OCH_3-4′), 3.79 (6H, s, OCH_3 -2′, OCH_3 -6′), 2.84 (2H, br s, H-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.1, 169.2, 162.5, 159.6, 159.6, 99.2, 90.8, 90.8, 56.0, 56.0, 55.5, 54.7, 39.0, 34.0; HRESIMS *m/z* = 312.1092 [M − H]$^-$ (calcd for $C_{14}H_{18}NO_7$, 312.1089).

*(S)-2-((2,4,6-Trimethoxybenzyl)amino)pentanedioic acid (**23**).* This compound was prepared from L-glutamic acid dimethyl esters hydrochloride by means of GP5 as a white solid (155 mg, 48%, mp 121–123 ℃). Eluent for column chromatography: petroleum ether/EtOAc (5:1); Conditions for prepared HPLC: CH_3CN/H_2O/TFA (13:87:0.03, 5 mL/min, t_R = 29 min); $[\alpha]_D^{20}$ = + 10.8° (*c* = 0.333, CH_3OH), ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.26 (2H, s, H-3′, H-5′), 4.03 (1H, d, *J* = 13.0 Hz, H-1a), 3.97 (1H, d, *J* = 13.0Hz, H-1b), 3.79 (6H, s, OCH_3 -2′, OCH_3 -6′), 3.78 (3H, s, OCH_3-4′), 3.44 (1H, t, *J* = 6.5Hz, H-3), 2.41 (1H, m, H-5a), 2.32(1H, m, H-5b), 2.01 (1H, m, H-6a), 1.94 (1H, m, H-6b); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.7, 169.9, 162.3, 159.6, 159.6, 99.9, 90.7, 90.7, 58.8, 55.9, 55.9, 55.5, 38.5, 30.3, 24.9; HRESIMS *m/z* = 326.1247 [M − H]$^-$ (calcd for $C_{15}H_{20}NO_7$, 326.1245).

*(S)-3-Phenyl-2-((2,4,6-trimethoxybenzyl)amino)propanoic acid (**24**).* This compound was prepared from L-phenylalanine methyl ester hydrochloride by means of GP5 as a white solid

(115 mg, 33%, mp 102 ℃). Eluent for column chromatography: petroleum ether/EtOAc (10:1); Conditions for prepared HPLC: CH_3CN/H_2O/TFA (30:70:0.03, 5 mL/min, t_R = 47 min); $[\alpha]_D^{20}$ = + 6.5° (*c* = 0.333, CH_3OH), ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.32 (2H, t, *J* = 7.5 Hz, H-3″, H-5″), 7.26 (1H, t, *J* = 7.5 Hz, H-4″), 7.22 (2H, d, *J* = 7.5 Hz, H-2″, H-6″), 6.27 (2H, s, H-3′, H-5′), 4.07 (2H, br s, H-1), 3.91 (1H, br s, H-3), 3.80 (3H, s, OCH_3-4′), 3.78 (6H, s, OCH_3 -2′, OCH_3 -6′), 3.32 (1H, dd, *J* = 13.5, 4.5 Hz, H-5a), 3.09 (1H, dd, *J* = 13.5, 8.0 Hz, H-5b); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.2, 162.4, 159.6, 159.6, 134.8, 129.3, 129.3, 128.4, 128.4, 127.1, 98.8, 90.6, 90.6, 59.3, 55.8, 55.8, 55.4, 38.3, 34.9; HRESIMS *m/z* = 346.1653 $[M + H]^+$ (calcd for $C_{19}H_{24}NO_5$, 346.1649).

*(S)-3-(1H-Imidazol-5-yl)-2-((2,4,6-trimethoxybenzyl)amino)propanoic acid (**25**).* This compound was prepared from L-histidine methyl ester hydrochloride by means of GP5 as a white solid (70 mg, 21%, mp 110–112 ℃). Eluent for column chromatography: petroleum ether/EtOAc (5:1); Conditions for prepared HPLC: CH_3CN/H_2O/TFA (10:90:0.03, 5 mL/min, t_R = 54 min); $[\alpha]_D^{20}$ = + 7.5° (*c* = 0.333, CH_3OH), ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (1H, s, H-3″), 7.47 (1H, s, H-5″), 6.26 (2H, s, H-3′, H-5′), 4.13 (1H, d, *J* = 13.5 Hz, H-1a), 4.07 (1H, d, *J* = 13.5 Hz, H-1b), 4.07 (1H, br s, H-3), 3.79 (3H, s, OCH_3-4′), 3.77 (6H, s, OCH_3 -2′, OCH_3 -6′), 3.34 (1H, dd, *J* = 15.5, 6.0 Hz, H-5a), 3.24 (1H, dd, *J* = 15.5, 8.0 Hz, H-5b); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.9, 162.6, 159.8, 159.8, 134.5, 127.6, 117.8, 99.1, 90.8, 90.8, 57.4, 55.9, 55.9, 55.6, 38.5, 24.8; HRESIMS *m/z* = 336.1556 $[M + H]^+$ (calcd for $C_{16}H_{22}N_3O_5$, 335.1554).

*General Procedure for Introduction of Propanolamine Unit for Compounds **26**−**31** (GP6).* To a solution of the phenolic precursor (**3**, **5**, or **1a**, 5.0 mmol) in anhydrous DMF (60 mL) was added NaH (0.12 g, 5.0 mmol), and the reaction mixture was stirred at room temperature for 20 min under an argon atmosphere, followed by the addition of (±)2-(chloromethyl)oxirane (2.31 g,

25.0 mmol), and then stirred at 90 ℃ for 2 h. After cooling, the mixture was poured into ice-cooled water (100 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water, and dried over Na_2SO_4, and concentrated in vacuo. The residue obtained on column purification using petroleum ether/EtOAc (30:1～10:1) as eluent afforded the corresponding epoxide.

The epoxide (1.0 mmol) was dissolved in amine (3 mL), and the solution was allowed to stand at 80 ℃ for 3 h in a sealed tube, and then evaporated in vacuo. To a sirred solution of the residue in absolute diethyl ether (15 mL) was added dropwise a saturated HCl-diethyl ether solution (1 mL), and a precipitate appeared, which was filtered off to afford the resulting hydrochlorate.

*1-(2-(2-Hydroxy-3-(isopropylamino)propoxy)-4,6-dimethoxyphenyl)-3-methylbutan-1-one hydrochlorate (**26**).* This compound was prepared using **3** and isopropylamine by means of GP6 as a white solid (110 mg, 19%, mp 128–130 ℃). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.27 (1H, br s, H-5′), 6.26 (1H, d, J = 1.5 Hz, H-3′), 4.14 (1H, m, H-2″), 4.01 (1H, dd, J = 10.4, 4.8 Hz, H-1″a), 3.96 (1H, dd, J = 10.4, 5.6 Hz, H-1″b), 3.79 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.29 (1H, m, H-5″), 3.02 (1H, m, H-3″a), 2.88 (1H, m, H-3″b), 2.54 (2H, br d, J = 5.6 Hz, H-2), 2.02 (1H, m, H-3), 1.25 (3H, d, J = 6.0 Hz, CH_3-6″), 1.17 (3H, d, J = 6.0 Hz, CH_3-7″), 0.88 (6H, d, J = 6.4 Hz, CH_3-4, CH_3-5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.7, 161.8, 157.7, 156.5, 113.4, 91.9, 91.5, 70.4, 65.0, 55.9, 55.6, 53.5, 49.9, 46.7, 24.3, 22.6, 22.6, 18.6, 18.2; HRESIMS m/z = 354.2277 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_5$, 354.2275).

*1-(2-(2-Hydroxy-3-(tert-butylamino)propoxy)-4,6-dimethoxyphenyl)-3-methylbutan-1-one hydrochlorate (**27**).* This compound was prepared using **3** and tertbutylamine by means of GP6 as a white solid (200 mg, 34%, mp 114–115 ℃). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.27 (1H, br s, H-5′), 6.25 (1H, br s, H-3′), 4.14 (1H, m, H-2″), 4.04 (1H, dd, J = 10.4, 4.8 Hz, H-1″a), 3.98 (1H,

dd, *J* = 10.0, 5.6 Hz, H-1″b), 3.92 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.04 (1H, m, H-3″a), 2.84 (1H, m, H-3″b), 2.54 (2H, d, *J* = 6.8 Hz, H-2), 2.02 (1H, m, H-3), 1.29 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.87 (6H, d, *J* = 6.4 Hz, CH_3-4, CH_3-5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.7, 161.8, 157.7, 156.4, 113.4, 91.9, 91.5, 70.3, 65.4, 56.4, 55.9, 55.7, 53.5, 44.3, 24.9, 24.9, 24.9, 24.4, 22.6, 22.6; HRESIMS *m/z* = 368.2437 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_5$, 368.2431).

*1-(2-(2-Hydroxy-3-(isopropylamino)propoxy)-4-methoxyphenyl)-3-methylbutan-1-one hydrochlorate (**28**).* This compound was prepared using **5** and isopropylamine by means of GP6 as a white solid (70 mg, 12%, mp 94–95 ℃). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (1H, d, *J* = 8.4 Hz, H-6′), 6.64 (1H, br s, H-3′), 6.59 (1H, dd, *J* = 8.4, 1.6 Hz, H-5′) , 4.31 (1H, m, H-2″), 4.14 (2H, br s, H-1″), 3.82 (3H, s, OCH_3), 3.32 (1H, m, H-5″), 3.15 (1H, m, H-3″a), 3.01 (1H, m, H-3″b), 2.85 (1H, dd, *J* = 16.0, 6.8 Hz, H-2a), 2.79 (1H, dd, *J* = 16.0, 6.8 Hz, H-2b), 2.08 (1H, m, H-3), 1.27 (3H, d, *J* = 6.0 Hz, CH_3-6″), 1.25 (3H, d, *J* = 6.0 Hz, CH_3-7″), 0.87 (6H, d, *J* = 6.0 Hz, CH_3-4, CH_3-5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.7, 163.8, 159.2, 131.8, 121.1, 106.3, 99.3, 70.7, 65.1, 55.7, 52.0, 49.9, 47.0, 24.6, 22.6, 22.6, 18.6, 18.3; HRESIMS *m/z* = 324.2173 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_4$, 324.2169).

*1-(2-(2-Hydroxy-3-(tert-butylamino)propoxy)-4-methoxyphenyl)-3-methylbutan-1-one hydrochlorate (**29**).* This compound was prepared from **5** and tertbutylamine by means of GP6 as a white solid (82 mg, 13%, mp 135–137 ℃). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (1H, d, *J* = 8.4 Hz, H-6′), 6.64 (1H, d, *J* = 2.0 Hz, H-3′), 6.60 (1H, dd, *J* = 8.4, 2.0 Hz, H-5′), 4.29 (1H, m, H-2″), 4.15 (2H, br s, H-1″), 3.82 (3H, s, OCH_3), 3.17 (1H, m, H-3″a), 2.95 (1H, m, H-3″b), 2.85 (1H, dd, *J* = 16.0, 6.8 Hz, H-2a), 2.80 (1H, dd, *J* = 16.0, 6.8 Hz, H-2b), 2.09 (1H, m, H-3), 1.32 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.87 (6H, d, *J* = 6.4 Hz, CH_3-4, CH_3-5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.7, 163.8, 159.2, 131.8, 121.2, 106.3, 99.3, 70.6, 65.4, 56.4, 55.7, 51.9, 44.5,

25.0, 25.0, 25.0, 24.6, 22.6, 22.6; HRESIMS *m/z* = 338.2331 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_4$, 338.2326).

*1-(2,4-Dihydroxy-6-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)-3-methylbutan-1-one hydrochlorate (**30**).* To a solution of **1** (3.00 g, 14.3 mmol) in acetone (200 mL) were added anhydrous K_2CO_3 (25.00 g, 181.0 mmol) and benzyl *p*-toluenesulphonate (7.86 g, 30.0 mmol), which was synthesized according to the method by Dewick, and the reaction mixture was refluxed for 3 h. After cooling, the solvent was removed in vacuo. The residue was dissolved in water (150 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue obtained on column purification (silica, petroleum ether/EtOAc, 30:1 as eluent) afforded **1a** as a white solid (2.98 g, 53%).

30a was prepared from **1a** and isopropylamine by means of GP6 as a white solid (400 mg, 60%).

To a solution of **30a** (170 mg, 0.3 mmol) in MeOH (30 mL) was added a catalytic amount of Pd-C, and the reaction mixture was stirred at room temperature for 12 h under H_2 at 3 atm. After filtration, the filtrate was concentrated in vacuo, and the residue obtained on prepared HPLC purification afforded **30** as a white solid (50 mg, 49%, mp 186–187 ℃). Conditions for prepared HPLC: CH_3CN/H_2O/TFA (22:78:0.03, 5 mL/min, t_R = 35 min); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.90 (1H, s, H-3′), 5.89 (1H, s, H-5′), 4.00 (1H, dd, *J* = 8.0, 3.5 Hz, H-1″a), 3.92 (1H, overlap, H-1″b), 3.88 (1H, m, H-2″), 2.93 (1H, dd, *J* = 16.0, 7.0 Hz, H-3″a), 2.84 (1H, dd, *J* = 16.0, 6.5 Hz, H-3″b), 2.77 (1H, m, H-5″), 2.72 (1H, dd, *J* = 13.5, 6.5 Hz, H-2a), 2.60 (1H, dd, *J* = 13.5, 7.0 Hz, H-2b), 2.13 (1H, m, H-3), 1.00 (3H, d, *J* = 6.0 Hz, CH_3-6″), 0.99 (3H, d, *J* = 6.0 Hz, CH_3-7″), 0.90 (3H, d, *J* = 6.5 Hz, CH_3-4), 0.88 (3H, d, *J* = 6.5 Hz, CH_3-5); ^{13}C NMR (125 MHz,

DMSO-d_6) δ 204.7, 166.2, 165.5, 162.3, 104.7, 95.9, 92.2, 71.6, 68.2, 52.3, 49.9, 48.5, 24.5, 22.7, 22.7, 22.6, 22.6; HRESIMS *m/z* = 326.1968 $[M + H]^+$ (calcd for $C_{17}H_{28}NO_5$, 326.1962).

*1-(2,4-Dihydroxy-6-(2-hydroxy-3-(tert-butylamino)propoxy)phenyl)-3-methylbutan-1-one hydrochlorate (**31**).* The intermediate **31a** was prepared from **1a** and tertbutylamine by means of GP6 as a white solid (450 mg, 66%). And the end product **31** was obtained from **31a** (150 mg, 0.3 mmol) as a white solid (59 mg, 64%, mp 205–206 ℃) by catalytic hydrogenation as described in synthesis of **30**. Conditions for prepared HPLC: CH_3CN/H_2O/TFA (22:78:0.03, 5 mL/min, t_R = 39 min); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.90 (1H, br s, H-3′), 5.81 (1H, br s, H-5′), 4.01 (1H, dd, *J* = 9.5, 4.0 Hz, H-1″a), 3.89 (1H, overlap, H-1″b), 3.84 (1H, m, H-2″), 2.94 (1H, dd, *J* = 16.0, 7.0 Hz, H-3″a), 2.85 (1H, dd, *J* = 16.0, 6.5 Hz, H-3″b), 2.64 (1H, dd, *J* = 12.0, 5.0 Hz, H-2a), 2.62 (1H, dd, *J* = 12.0, 6.0 Hz, H-2b), 2.13 (1H, m, H-3), 1.03 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.90 (3H, d, *J* = 7.0 Hz, CH_3-4), 0.89 (3H, d, *J* = 7.0 Hz, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.4, 166.3, 166.2, 162.3, 104.6, 95.9, 92.3, 71.5, 68.7, 52.3, 50.2, 45.4, 28.6, 28.6, 28.6, 24.6, 22.7, 22.6; HRESIMS *m/z* = 340.2123 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_5$, 340.2118).

*General Procedure for Introduction of Chiral Propanolamine Unit for Compounds **32**−**43** (GP7).* To a solution of the phenolic precursor (**3**, **5**, or **1a**, 20.0 mmol) in anhydrous DMF (60 mL) was added NaH (0.48 g, 20.0 mmol), and the mixture was stirred at room temperature for 20 min under an argon atmosphere, followed by the addition of an amount of 1.25 equiv of (*R*)- or (*S*)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane (25.0 mmol), and then stirred at 90 ℃ for 12 h. After cooling, the reaction mixture was poured into ice-cooled water (100 mL), and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated

in vacuo. The residue obtained on column purification using petroleum ether/EtOAc (30:1～20:1) as eluent afforded the corresponding intermediate with a 2,2-dimethyl-1,3-dioxolane moiety.

To a stirred solution of the intermediate (4.0 mmol) in THF (30 mL) was added dropwise 4% H_2SO_4 (30 mL), and the mixture was stirred at room temperature for 8 h. Then the organic solvent was removed in vacuo, and the residue was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution, and dried over Na_2SO_4, and concentrated in vacuo to provide the corresponding 1,2-diols.

The diols (3.0 mmol) was dissolved in anhydrous CH_2Cl_2 (40 mL), and a catalytic amount of pyridine was added, followed by the addition of TsCl (573 mg, 3.0 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature for 48 h, and concentrated in vacuo. The residue obtained on column purification using petroleum ether/EtOAc (25:1～15:1) as eluent afforded the ester of the primary hydroxyl group .

The ester (0.50 mmol) was dissolved in amine (3 mL), and the solution was allowed to stand at 80 ℃ for 3 h in a sealed tube, and then evaporated in vacuo. The residue was dissolved in absolute ether, and a saturated HCl-diethyl ether solution was added. The precipitate was filtered off to afford the resulting hydrochlorate.

*(S)-1-(2-(2-Hydroxy-3-(isopropylamino)propoxy)-4,6-dimethoxyphenyl)-3-methylbutan-1-one hydrochlorate (**32**).* This compound was prepared using **3**, (*S*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and isopropylamine by means of GP7 as a white solid (55 mg, 2%, mp 130–132 ℃). $[\alpha]_D^{20} = -22.5°$ (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.27 (1H, br s, H-5′), 6.25 (1H, br s, H-3′), 4.16 (1H, m, H-2″), 4.01 (1H, m, H-1″a), 3.96 (1H, m, H-1″b), 3.79 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.29 (1H, m, H-5″), 3.02 (1H, br d, *J* = 11.1 Hz, H-3″a), 2.90 (1H, m, H-3″b), 2.54 (2H, br d, *J* = 6.9 Hz, H-2), 2.02 (1H, m, H-3), 1.25 (3H, d, *J* = 6.0 Hz,

CH_3-6″), 1.23 (3H, d, *J* = 6.0 Hz, CH_3-7″), 0.88 (6H, d, *J* = 6.6 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 202.7, 161.8, 157.7, 156.5, 113.4, 91.9, 91.5, 70.4, 65.0, 55.9, 55.6, 53.5, 49.9, 46.7, 24.3, 22.6, 22.6, 18.6, 18.2; HRESIMS *m/z* = 354.2282 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_5$, 354.2275).

*(R)-1-(2-(2-Hydroxy-3-(isopropylamino)propoxy)-4,6-dimethoxyphenyl)-3-methylbutan-1-one hydrochlorate (**33**).* This compound was prepared using **3**, (*R*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and isopropylamine by means of GP7 as a white solid (68 mg, 4%, mp 136–137 ℃). $[\alpha]_D^{20}$ = + 22.6° (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.27 (1H, br s, H-5′), 6.26 (1H, br s, H-3′), 4.10 (1H, m, H-2″), 4.01 (1H, m, H-1″a), 3.96 (1H, m, H-1″b), 3.79 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.29 (1H, m, H-5″), 3.02 (1H, br d, *J* = 11.1 Hz, H-3″a), 2.96 (1H, m, H-3″b), 2.54 (2H, br d, *J* = 6.9 Hz, H-2), 2.02 (1H, m, H-3), 1.25 (3H, d, *J* = 6.0 Hz, CH_3-6″), 1.23 (3H, d, *J* = 6.0 Hz, CH_3-7″), 0.88 (6H, d, *J* = 6.6 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 202.7, 161.8, 157.7, 156.5, 113.4, 91.9, 91.5, 70.4, 65.0, 55.9, 55.6, 53.5, 49.9, 46.7, 24.3, 22.6, 22.6, 18.6, 18.2; HRESIMS *m/z* = 354.2279 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_5$, 354.2275).

*(S)-1-(2-(2-Hydroxy-3-(tert-butylamino)propoxy)-4,6-dimethoxyphenyl)-3-methylbutan-1-one hydrochlorate (**34**).* This compound was prepared using **3**, (*S*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and tertbutylamine by means of GP7 as a white solid (86 mg, 3%, mp 116–117 ℃). $[\alpha]_D^{20}$ = − 23.9° (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.27 (1H, d, *J* = 1.5 Hz, H-5′), 6.25 (1H, d, *J* = 1.5 Hz, H-3′), 4.16 (1H, m, H-2″), 4.06 (1H, m, H-1″a), 3.97 (1H, m, H-1″b), 3.79 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.04 (1H, m, H-3″a), 2.86 (1H, m, H-3″b), 2.54 (2H, d, *J* = 6.9 Hz, H-2), 2.02 (1H, m, H-3), 1.29 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.87 (6H, d, *J* = 6.9 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 202.7, 161.8, 157.7, 156.5, 113.4,

91.9, 91.5, 70.3, 65.4, 56.4, 55.9, 55.6, 53.5, 44.3, 24.9, 24.9, 24.9, 24.4, 22.6, 22.6; HRESIMS *m/z* = 368.2437 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_5$, 368.2431).

*(R)-1-(2-(2-Hydroxy-3-(tert-butylamino)propoxy)-4,6-dimethoxyphenyl)-3-methylbutan-1-one hydrochlorate (**35**).* This compound was prepared using **3**, (*R*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and tertbutylamine by means of GP7 as a white solid (75 mg, 5%, mp 113–114 ℃). $[\alpha]_D^{20}$ = + 23.9° (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.27 (1H, d, *J* = 1.8 Hz, H-5′), 6.25 (1H, d, *J* = 1.8 Hz, H-3′), 4.15 (1H, m, H-2″), 4.06 (1H, m, H-1″a), 3.97 (1H, m, H-1″b), 3.79 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.04 (1H, m, H-3″a), 2.86 (1H, m, H-3″b), 2.54 (2H, d, *J* = 6.9 Hz, H-2), 2.02 (1H, m, H-3), 1.29 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.87 (6H, d, *J* = 6.6 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 202.7, 161.8, 157.7, 156.5, 113.4, 91.9, 91.5, 70.3, 65.4, 56.4, 55.9, 55.6, 53.5, 44.3, 24.9, 24.9, 24.9, 24.4, 22.6, 22.6; HRESIMS *m/z* = 368.2433 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_5$, 368.2431).

*(S)-1-(2-(2-Hydroxy-3-(isopropylamino)propoxy)-4-methoxyphenyl)-3-methylbutan-1-one hydrochlorate (**36**).* This compound was prepared using **5**, (*S*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and isopropylamine by means of GP7 as a white solid (93 mg, 7%, mp 98–99 ℃). $[\alpha]_D^{20}$ = − 1.4° (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.59 (1H, d, *J* = 8.7 Hz, H-6′), 6.64 (1H, d, *J* = 1.8 Hz, H-3′), 6.60 (1H, br d, *J* = 8.7 Hz, H-5′), 4.30 (1H, m, H-2″), 4.13 (2H, br s, H-1″), 3.82 (3H, s, OCH_3), 3.34 (1H, m, H-5″), 3.15 (1H, m, H-3″a), 3.00 (1H, m, H-3″b), 2.85 (1H, dd, *J* = 16.2, 6.9 Hz, H-2a), 2.79 (1H, dd, *J* = 16.2, 6.9 Hz, H-2b), 2.08 (1H, m, H-3), 1.27 (3H, d, *J* = 6.0 Hz, CH_3-6″), 1.26 (3H, d, *J* = 6.0 Hz, CH_3-7″), 0.87 (6H, d, *J* = 6.6 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 199.7, 163.8, 159.2, 131.8, 121.1, 106.3, 99.3, 70.7, 65.1, 55.7, 51.9, 49.9, 47.0, 24.6, 22.6, 22.6, 18.6, 18.3; HRESIMS *m/z* = 324.2178 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_4$, 324.2169).

*(R)-1-(2-(2-Hydroxy-3-(isopropylamino)propoxy)-4-methoxyphenyl)-3-methylbutan-1-one hydrochlorate (**37**).* This compound was prepared using **5**, (*R*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and isopropylamine by means of GP7 as a white solid (65 mg, 3%, mp 97–98 °C). $[\alpha]_D^{20}$ = + 1.4° (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.59 (1H, d, *J* = 8.7 Hz, H-6′), 6.64 (1H, d, *J* = 1.8 Hz, H-3′), 6.60 (1H, dd, *J* = 8.7, 1.8 Hz, H-5′), 4.31 (1H, m, H-2″), 4.14 (2H, br s, H-1″), 3.82 (3H, s, OCH_3), 3.32 (1H, m, H-5″), 3.15 (1H, m, H-3″a), 3.00 (1H, m, H-3″b), 2.85 (1H, dd, *J* = 16.2, 6.9 Hz, H-2a), 2.79 (1H, dd, *J* = 16.2, 6.9 Hz, H-2b), 2.08 (1H, m, H-3), 1.27 (3H, d, *J* = 6.0 Hz, CH_3-6″), 1.26 (3H, d, *J* = 6.0 Hz, CH_3-7″), 0.87 (6H, d, *J* = 6.6 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 199.7, 163.8, 159.2, 131.8, 121.1, 106.3, 99.3, 70.7, 65.1, 55.7, 51.9, 49.9, 47.0, 24.6, 22.6, 22.6, 18.6, 18.3; HRESIMS *m/z* = 324.2180 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_4$, 324.2169).

*(S)-1-(2-(2-Hydroxy-3-(tert-butylamino)propoxy)-4-methoxyphenyl)-3-methylbutan-1-one hydrochlorate (**38**).* This compound was prepared using **5**, (*S*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and tertbutylamine by means of GP7 as a white solid (37 mg, 3%, mp 148–149 °C). $[\alpha]_D^{20}$ = − 3.4° (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.59 (1H, d, *J* = 8.4 Hz, H-6′), 6.64 (1H, d, *J* = 2.1 Hz, H-3′), 6.60 (1H, dd, *J* = 8.4, 2.1 Hz, H-5′), 4.30 (1H, m, H-2″), 4.16 (2H, br s, H-1″), 3.82 (3H, s, OCH_3), 3.17 (1H, m, H-3″a), 2.97 (1H, m, H-3″b), 2.85 (1H, dd, *J* = 16.2, 6.6 Hz, H-2a), 2.80 (1H, dd, *J* = 16.2, 6.6 Hz, H-2b), 2.07 (1H, m, H-3), 1.32 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.87 (6H, d, *J* = 6.6 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 199.7, 163.8, 159.2, 131.8, 121.2, 106.3, 99.3, 70.6, 65.4, 56.4, 55.7, 51.9, 44.5, 25.0, 25.0, 25.0, 24.6, 22.6, 22.6; HRESIMS *m/z* = 324.2326 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_4$, 338.2334).

*(R)-1-(2-(2-Hydroxy-3-(tert-butylamino)propoxy)-4-methoxyphenyl)-3-methylbutan-1-one hydrochlorate (**39**).* This compound was prepared using **5**, (*R*)- 4-(chloromethyl)-2,2-dimethyl-

1,3-dioxolane and tertbutylamine by means of GP7 as a white solid (75 mg, 4%, mp 151–153 ℃). $[\alpha]_D^{20}$ = + 3.4° (*c* = 0.333, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.59 (1H, d, *J* = 8.4 Hz, H-6′), 6.64 (1H, d, *J* = 2.1 Hz, H-3′), 6.60 (1H, dd, *J* = 8.4, 2.1 Hz, H-5′), 4.29 (1H, m, H-2″), 4.17 (2H, br s, H-1″), 3.82 (3H, s, OCH_3), 3.17 (1H, m, H-3″a), 2.97 (1H, m, H-3″b), 2.85 (1H, dd, *J* = 16.2, 6.6 Hz, H-2a), 2.80 (1H, dd, *J* = 16.2, 6.6 Hz, H-2b), 2.09 (1H, m, H-3), 1.32 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.87 (6H, d, *J* = 6.9 Hz, CH_3-4, CH_3-5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 199.7, 163.8, 159.2, 131.8, 121.2, 106.3, 99.3, 70.6, 65.4, 56.4, 55.7, 51.9, 44.5, 25.0, 25.0, 25.0, 24.6, 22.6, 22.6; HRESIMS *m/z* = 324.2332 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_4$, 338.2334).

*(S)-1-(2,4-Dihydroxy-6-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)-3-methylbutan-1-one hydrochlorate (**40**)*. The intermediate **40a** was prepared using **1a**, (*S*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and isopropylamine by means of GP7 as a white solid (168 mg, 7%). The end product **40** was obtained from **40a** (168 mg, 0.3 mmol) as a white solid (58 mg, 52%, mp 207–209 ℃) by catalytic hydrogenation as described in synthesis of **30**. Conditions for prepared HPLC: CH_3CN/H_2O/TFA (22:78:0.03, 5 mL/min, t_R = 35 min); $[\alpha]_D^{20}$ = − 4.2° (*c* = 0.067, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.90 (1H, d, *J* = 1.5 Hz, H-3′), 5.81 (1H, d, *J* = 1.5 Hz, H-5′), 3.99 (1H, dd, *J* = 8.5, 3.0 Hz, H-1″a), 3.92 (1H, overlap, H-1″b), 3.88 (1H, m, H-2″), 2.93 (1H, dd, *J* = 16.0, 7.0 Hz, H-3″a), 2.84 (1H, dd, *J* = 16.0, 6.5 Hz, H-3″a), 2.77 (1H, m, H-5″), 2.72 (1H, m, H-2a), 2.62 (1H, dd, *J* = 11.5, 6.5 Hz, H-2b), 2.13 (1H, m, H-3), 1.00 (3H, d, *J* = 6.5 Hz, CH_3-6″), 0.99 (3H, d, *J* = 6.0 Hz, CH_3-7″), 0.90 (3H, d, *J* = 6.5 Hz, CH_3-4), 0.88 (3H, d, *J* = 6.5 Hz, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.7, 166.2, 165.5, 162.3, 104.6, 95.9, 92.3, 71.6, 68.1, 52.3, 49.9, 48.5, 24.5, 22.7, 22.7, 22.6, 22.6; HRESIMS *m/z* = 326.1972 $[M + H]^+$ (calcd for $C_{17}H_{28}NO_5$, 326.1962).

*(R)-1-(2,4-Dihydroxy-6-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)-3-methylbutan-1-one hydrochlorate (**41**)*. The intermediate **41a** was prepared using **1a**, (*R*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and isopropylamine by means of GP7 as a white solid (129 mg, 4%).

The end product **41** was obtained from **41a** (129 mg, 0.2 mmol) as a white solid (45 mg, 70%, mp 210–212 ℃) by catalytic hydrogenation as described in synthesis of **30**. Conditions for prepared HPLC were the same as those for **40**. $[\alpha]_D^{20}$ = + 4.2° (*c* = 0.067, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.92 (1H, d, *J* = 1.5 Hz, H-3′), 5.84 (1H, d, *J* = 1.5 Hz, H-5′), 3.99 (1H, br d, *J* = 5.5 Hz, H-1″a), 3.91 (1H, overlap, H-1″b), 3.89 (1H, m, H-2″), 2.94 (1H, dd, *J* = 16.0, 6.5 Hz, H-3″a), 2.84 (1H, dd, *J* = 16.0, 6.5 Hz, H-3″b), 2.76 (1H, m, H-5″), 2.72 (1H, m, H-2a), 2.63 (1H, dd, *J* = 11.0, 6.0 Hz, H-2b), 2.13 (1H, m, H-3), 1.00 (3H, d, *J* = 5.5 Hz, CH_3-6″), 0.99 (3H, d, *J* = 5.5 Hz, CH_3-7″), 0.90 (3H, d, *J* = 6.5 Hz, CH_3-4), 0.88 (3H, d, *J* = 6.5 Hz, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.6, 166.1, 165.0, 162.3, 104.7, 95.8, 1, 71.6, 68.1, 52.3, 49.9, 48.5, 24.5, 22.7, 22.7, 22.6, 22.6; HRESIMS *m/z* = 326.1967 $[M + H]^+$ (calcd for $C_{17}H_{28}NO_5$, 326.1962).

*(S)-1-(2,4-Dihydroxy-6-(2-hydroxy-3-(tert-butylamino)propoxy)phenyl)-3-methylbutan-1-one hydrochlorate (**42**)*. The intermediate **42a** was prepared using **1a**, (*S*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and tertbutylamine by means of GP7 as a white solid (95 mg, 4%).

The end product **42** was obtained from **42a** (95 mg, 0.2 mmol) as a white solid (34 mg, 54%, mp 218–219 ℃) by catalytic hydrogenation as described in synthesis of **30**. Conditions for prepared HPLC: CH_3CN/H_2O/TFA (22:78:0.03, 5 mL/min, t_R = 39 min); $[\alpha]_D^{20}$ = − 10.6° (*c* = 0.067, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.89 (1H, d, *J* = 2.0 Hz, H-3′), 5.81 (1H, d, *J* = 2.0 Hz, H-5′), 4.01 (1H, dd, *J* = 9.0, 3.5 Hz, H-1″a), 3.90 (1H, overlap, H-1″b), 3.84 (1H, m, H-2″), 2.93 (1H, dd, *J* = 16.0, 7.0 Hz, H-3″a), 2.85 (1H, dd, *J* = 16.0, 7.0 Hz, H-3″b), 2.67 (1H, dd, *J* =

11.5, 5.0 Hz, H-2a), 2.62 (1H, dd, *J* = 11.5, 6.5 Hz, H-2b), 2.13 (1H, m, H-3), 1.04 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.90 (3H, d, *J* = 6.5 Hz, CH_3-4), 0.89 (3H, d, *J* = 6.5 Hz, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.4, 166.3, 166.2, 162.3, 104.6, 95.9, 92.3, 71.5, 68.7, 52.3, 50.2, 45.4, 28.6, 28.6, 28.6, 24.6, 22.7, 22.6; HRESIMS *m/z* = 340.2117 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_5$, 326.2118).

*(R)-1-(2,4-Dihydroxy-6-(2-hydroxy-3-(tert-butylamino)propoxy)phenyl)-3-methylbutan-1-one hydrochlorate (**43**)*. The intermediate **43a** was prepared using **1a**, (*R*)- 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane and tertbutylamine by means of GP7 as a white solid (186 mg, 5%).

The end product **43** was obtained from **43a** (186 mg, 0.4 mmol) as a white solid (51 mg, 41%, mp 222–224 ℃) by catalytic hydrogenation as described in synthesis of **30**; Conditions for prepared HPLC were the same as those for **42**. $[\alpha]_D^{20}$ = + 10.6° (*c* = 0.067, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.92 (1H, d, *J* = 1.5 Hz, H-3′), 5.82 (1H, d, *J* = 1.5 Hz, H-5′), 4.01 (1H, dd, *J* = 9.0, 3.5 Hz, H-1″a), 3.90 (1H, overlap, H-1″b), 3.88 (1H, m, H-2″), 2.94 (1H, dd, *J* = 16.0, 7.0 Hz, H-3″a), 2.85 (1H, dd, *J* = 16.0, 7.0 Hz, H-3″b), 2.69 (1H, dd, *J* = 11.0, 6.0 Hz, H-2a), 2.62 (1H, dd, *J* = 11.5, 6.5 Hz, H-2b), 2.13 (1H, m, H-3), 1.04 (9H, s, CH_3-6″, CH_3-7″, CH_3-8″), 0.90 (3H, d, *J* = 6.5 Hz, CH_3-4), 0.89 (3H, d, *J* = 6.5 Hz, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.6, 166.2, 165.3, 162.3, 104.8, 95.9, 92.2, 71.6, 68.6, 52.3, 50.2, 45.3, 28.5, 28.5, 28.5, 24.6, 22.7, 22.6; HRESIMS *m/z* = 340.2115 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_5$, 326.2118).

*4-((3,5-Dihydroxy-2-(3-methylbutanoyl)phenoxy)methyl)-3-phenyl-furoxan (**44**)*. To a stirred solution of $NaNO_2$ (3.00g, 72.0 mmol) in water (50 mL) was added dropwise cinnamyl alcohol (2.00 g, 15.0 mmol) in HOAc (5 mL). The reaction mixture was stirred at room temperature for 4 h, and then neutralized with $NaHCO_3$, followed by the extraction with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue

obtained on column purification (silica, petroleum ether/EtOAc, 7:1 as eluent) afforded the furoxan alcohol **44a** (0.86 g, 30%).

To a stirred solution of **44a** (192 mg, 1.0 mmol) and pyridine (0.32 mL, 4 mmol) in anhydrous CH_2Cl_2 (15 mL) was added $SOCl_2$ (0.35 mL) in an ice-bath. The mixture was stirred at room temperature for 3 h, and then washed with ice water, saturated $NaHCO_3$ solution, and brine successively. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give a clear oil.

To a solution of the crude oil and **2a** (220 mg, 1.0 mmol) in acetone (20 mL) was added anhydrous K_2CO_3 (210 mg, 1.5 mmol). After refluxing for 5 h, the reaction mixture was concentrated in vacuo. Water (20 mL) was then added to the residue, and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue obtained on column purification (silica, petroleum ether/EtOAc, 15:1 as eluent) afforded **44b** as a yellow oil (200 mg, 42%).

To a solution of **44b** (200 mg, 0.42 mmol) in MeOH (10 mL) was added 2N HCl (2 mL) and the mixture was refluxed for 1 h, and then the solvent was removed in vacuo. The resulting residue was dissolved in EtOAc (50 mL) and was washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue obtained on column purification (silica, petroleum ether/EtOAc, 5:1 as eluent) afforded **44** as a white solid (150 mg, 90%, mp 125–127 ℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.43 (1H, s, OH-6′), 10.73 (1H, s, OH-4′), 7.79 (2H, d, J = 7.5 Hz, H-2‴, H-6‴), 7.63 (2H, t, J = 7.5 Hz, H-3‴, H-5‴), 7.59 (1H, t, J = 7.5 Hz, H-4‴), 6.08 (1H, d, J = 1.5 Hz, H-3′), 5.96 (1H, d, J = 1.5 Hz, H-5′), 5.24 (2H, s, H-1″), 2.44 (2H, d, J = 7.0 Hz, H-2), 1.91 (1H, m, H-3), 0.66 (6H, d, J = 6.5 Hz, CH_3-4, CH_3-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 204.1, 165.7, 164.5, 160.2, 156.9, 131.8, 129.7, 129.7, 127.6,

127.6, 125.6, 112.3, 105.2, 96.9, 92.6, 59.1, 52.2, 24.5, 22.3, 22.3; HRESIMS *m/z* = 385.1395 $[M + H]^+$ (calcd for $C_{20}H_{20}N_2O_6$, 385.1394).

BV-2 cell culture and treatment. BV-2 cells were cultured at 37 ℃ in an atmosphere of 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. For the compound screening experiments, the cells were plated in 96-well plates at a density of 5×10^3 cell/well and 24 h later were treated with compounds **1**−**44** at six different concentrations (100, 50, 10 5, 1, and 0.1 μM, respectively). Then, 1 h later the cells were treated with LPS (300 ng/mL), incubated for another 24 h, and the culture media were collected for the detection of the NO concentration.

Primary mixed mesencephalic neuronal/glial culture and treatment. Neuronal-glial cultures were prepared from the ventral mesencephalic tissue of embryonic day-14 Sprague-Dawley rats (Beijing Vital River Animal Center). Briefly, the whole brain was removed, mesencephalon was dissected, blood vessels and meninges were removed, and then pooled mesencephalic tissues were dissociated using mild mechanical trituration in ice-cold phosphate-buffered saline (PBS, pH 7.4). After pelleting and centrifugation, the cells were resuspended and plated (5×10^5/well) into 24-well cell culture plates pre-coated with 10 mg/mL poly-D-lysine. The cells were maintained at 37 ℃ in a humidified atmosphere of 95% air/5% CO_2 in minimal essential medium (MEM) containing 10% FBS, 10% horse serum, 1 μg/mL glucose, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 μM non-essential amino acids, 100 U/mL penicillin, and 100 μg/mL streptomycin. Seven-day-old cultures were used for the experiments, the cells were treated with LPS (100 ng/mL) in the presence or absence of the indicated compounds at different concentrations (added 1 h before LPS stimulation), and then incubated for 5 h, 24 h, and 7 days to determine cytokine, Iba-1, or TH expressions, respectively.

Primary microglia culture and treatment. Primary microglia-enriched cultures were prepared from the whole brains of 1-day-old Sprague-Dawley rats (Beijing Vital River Animal Center). The brain tissue was cleared of the meninges and blood vessels, dissociated using mechanical trituration, and then placed in ice-cold PBS. The tissue was subsequently digested with 0.25 mg/mL trypsin, triturated in DMEM by pipetting, and filtered to remove large debris. The isolated cells (5×10^7) were seeded into 150-cm^2 culture flasks coated with 10 mg/mL poly-D-lysine in DMEM/F12 supplemented with 10% FBS, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamic acid, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator with 95% air/5% CO_2 at 37 ℃. Following their attainment of confluence (12–14 days), the microglia were separated from the astroglia by shaking the flasks at 180 rpm for 1 h. The purity of the microglia-enriched cultures was > 98%, as determined by immunocytochemical staining. The isolated microglia were plated on six-well plates, the cells were treated with LPS (100 ng/mL) in the presence or absence of compound **21** at 10 μM (added 1 h before LPS challenge), and then incubated for 3 h followed by the measurement of phosphorylated (p)-PTEN, p-Akt, and p-Src expression.

RNAi. To silence the *Src* gene using RNAi, the primary microglia or mixed mesencephalic neuronal/glial cultures were transfected with optimized concentrations of Src siRNA (Invitrogen, CA, USA). The transfections were performed using the Lipofectamine RNAi MAX Reagent (Invitrogen, CA, USA) and 25 nM of the appropriate siRNA according to the manufacturer's instructions. The transfection medium was replaced with fresh DMEM/F-12 complete medium 4 h later. The microglia culture was incubated with compound **21**, followed by LPS (100 ng/mL), and then p-PTEN and p-Akt expression was measured 3 h later while IL-1β and TNF-α expressions were assayed 5 h later. The primary mixed midbrain neuronal/glial cultures were

incubated with compound **21** and then treated with LPS (100 ng/mL), MPP^+ (30 μM), or α-syn (250 nM) 4 h later. The TH expression was measured 7 days later.

***In vitro* NO production assay.** The NO accumulation in the medium was assayed by measuring the production of nitrite (NO^{2-}) using the Griess assay as described previously.[32]

Enzyme-linked immunosorbent assay (ELISA) of cytokines. Following the predetermined treatments, the cell culture media were collected, and the IL-1β and TNF-α levels were detected. For cytokine assay of the brain tissue, the midbrain samples were homogenised with non-denaturing lysis buffer containing a protease inhibitor tease. IL-1β and TNF-α levels were subsequently measured using commercially available ELISA kits (R&D System, MN, USA) following the manufacturer's instructions.

Western blot analysis. The cells and midbrain were lysed in non-denaturing lysis buffer and the immunoblotting was performed as described previously.[33] The blot was developed using the LAS3000 chemiluminescence system (Fujifilm, Tokyo, Japan), and the densities of the bands were determined using the Gel-Pro Analyzer 4.0 software.

Pharmacokinetic analysis. Male ICR mice weighing 22-25 g (Animal Center of the Chinese Academy of Medical Sciences) were intragastrically administered compounds **2**, **21**, **23,** and **44,** each at a single dose of 50 mg/kg after a 16-h fast. Then, blood samples and brain tissue were collected at 5, 15, and 30 min, and 1, 2, 4 and 8 h after dosing, following decapitation of the animal. All experimental procedures were performed in accordance with the guidelines of the Beijing Municipal Ethics Committee for the Care and Use of Laboratory Animals. The plasma was prepared by centrifuging the blood at 4000 rpm for 5 min. The brains were homogenised with saline, centrifuged at 12000 rpm for 25 min, and then a 5-μL aliquot of the plasma or brain extract was injected into the liquid chromatography/tandem mass spectrometry (LC/MS/MS)

system on an Agilent 6890 gas chromatography (GC) apparatus (Agilent Corp., CA, USA). For the detection of compounds **21** and **23**, an isocratic chromatographic procedure was used with a mobile phase of 70% acetonitrile (1% trifluoroacetic acid) and 30% water (H_2O) at a flow rate of 0.2 mL/min. The chromatographic procedure was performed using the CAPCELL PAK C18 column (2.1 mm × 100 mm, 5 μm) at 30ºC. Compounds **2** and **44** were detected using the Zorbax C18 column (2.1 mm × 50 mm, 3.5 μm) at 30ºC, and the mobile phase was 70% acetonitrile (1% trifluoroacetic acid) and 30% H_2O at a flow rate of 0.2 mL/min.

hERG safety assay. The whole-cell voltage clamp analysis was performed using an internal solution of potassium chloride (KCl) 130 mM, magnesium chloride ($MgCl_2$) 1 mM, ethylene glycol tetraacetic acid (EGTA) 5 mM, adenosine triphosphate (ATP)-Mg 5 mM, and HEPES 10 mM (pH 7.2). The external bath solution consisted of sodium chloride (NaCl) 137 mM, calcium chloride ($CaCl_2$) 1.8 mM, KCl 4 mM, $MgCl_2$ 1 mM, glucose 5 mM, and HEPES 10 mM (pH 7.4). The potassium current (Ik) was elicited from a holding potential of -70 mV using 2-s long depolarizing steps to 50 mV applied in 10 mV increments every 15 s while the tail current (Itail) was recorded using a step to -40 from +50 mV for 2 s using an EPC10 amplifier (Heka Instruments). The current signals were filtered at 2 kHz and digitized using an AD-DA converter for subsequent analysis with the PulseFit software ver. 8.74. The Ik and Itail were measured at the end of and the beginning of the depolarizing and repolarizing pulses, respectively.

***In vivo* toxicity assessment.** ICR mice (Animal Center of the Chinese Academy of Medical Sciences) weighing 22-25 g were used. The mice were treated with compounds **2** and **21** via oral gavage each at 250, 500, and 1000 mg/kg for 14 consecutive days. In addition to regular monitoring of the animal's general condition, the clinical chemistry of the liver and kidney were

assessed. The experimental procedures were performed in accordance with the guidelines of the Beijing Municipal Ethics Committee for the Care and Use of Laboratory Animals.

Establishment of PD mouse models and drug administrations. Male C57/BL mice (Animal Center of the Chinese Academy of Medical Sciences) weighing 22−25 g were used to establish the MPTP- and MPTP/prob-induced PD models. Male B6; C3H mice over-expressing mutant α-syn (A53T) were supplied by the Model Animal Research Center of Nanjing University and were maintained on a 12-h light/dark cycle at 24 ℃ in a room with a relative humidity of 60%. Furthermore, the animals were provided with food and water *ad libitum* and allowed to adapt to the conditions described above for 1 week, before the experimentation. In the sub-acute PD model, MPTP (30 mg/kg of MPTP hydrochloride, intraperitoneally, i.p.) was injected into the mice consecutively for 5 days. Compound **21** (5, 10, and 20 mg/kg) and L-DOPA (20 mg/kg) suspended in 0.5% CMC-Na were administered 30 min before each MPTP injection and after the last injection of MPTP, the compounds were continually administered to the mice for the next 7 days. In the chronic study, the mice were administered 10 doses of MPTP hydrochloride (25 mg/kg in saline, s.c.) in combination with probenecid (250 mg/kg in DMSO, i.p.). The 10 doses were administered on a 5-week schedule. After the last MPTP/prob injection, the mice were subjected to the rotarod behavioural test. Only those showing behavioural dysfunction were used in the subsequent experiments. Then, the mice were randomly divided into the MPTP/prob-, compound **21** (5, 10 and 20 mg/kg) and L-DOPA-treated groups. Compound **21** and L-DOPA were administered to mice once a day for 7 weeks. In the α-syn (A53T) transgenic mice, compound **21** and L-DOPA were administered once a day for 12 consecutive weeks, which commenced when they were 4-month-old. All experiments were performed in accordance with

the guidelines of the Beijing Municipal Ethics Committee for the Care and Use of Laboratory Animals.

Rotarod test. The rotarod test, which requires animals to balance and walk on a rotating cylinder, is used to measure coordinated motor skills. The mice were positioned on the rotarod, which was then set to revolve at 14 rpm for up to 120 s. The rotarod automatically recorded the time when the animals first fell off the rod, which was designated as the latency. The mice were tested thrice, and the latency was recorded each time. The animals were allowed to rest for 1 h between each trial.

Pole test. The pole test was originally used to evaluate bradykinesia in PD mice. The pole used in this study was constructed of wood with height and diameter of 50 and 3 cm, respectively, and wrapped in gauze to prevent slipping while the base was positioned in the home cage. A wooden ball was glued to the top of the pole to prevent animals from sitting there and to help position the animals on the pole. The performance of the mice while they descended the pole was then scored with 5 and 1 as the highest and lowest scores, respectively. If the mouse did not descend within 60 s, it was guided down. The mice were pre-trained before the experimentation, and they each performed two successive trials, with a 1-h interval between the trials.

Histochemical analysis. The TH immunohistochemical analysis was performed as previously described[7]. Briefly, the brains of the mice were fixed and cut into 40-μm sections using a freezing microtome and the coronal sections through the substantia nigra were processed. The sections were incubated with primary antibodies against TH (Abcam, MA, USA) and the labelled proteins were visualized using 0.04% hydrogen peroxidase and 0.05% 3,3′-diaminobenzidine. The sections were observed using a light microscope (NIKON E600, Japan),

and the number of positively stained cells in each group was recorded. All quantifications were performed blindly.

Striatal dopamine analysis. The mouse striatal dopamine was analysed using HPLC as described previously.[34] Briefly, the striatum was suspended in 0.6N perchloric acid, sonicated, and then centrifuged. Potassium dihydrogen phosphate solution consisting of potassium citrate 20 mM, potassium dihydrogen phosphate 300 mM, and ethylenediaminetetraacetic acid (EDTA)·Na_2 (2 mmol) was added to the supernatant and centrifuged. An aliquot of the supernatant was subsequently analysed using an HPLC system (Waters, MA, USA).

Statistical analysis. The data were expressed as mean ± SD and were analysed using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. $P < 0.05$ was considered statistically significant.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI:

NMR (^{1}H and ^{13}C) spectra of all target compounds (PDF)

HPLC analysis of compounds (PDF)

Proteomics studies (PDF)

Proteomics data (CSV)

AUTHOR INFORMATION

Corresponding Author

*For S. Y.: phone, +86-10-63165326; fax, 86-10-63017757; email, yushishan@imm.ac.cn.

*For D. Z.: phone, +86-10-63165203; fax, 86-10-63017757; e-mail, danzhang@imm.ac.cn.

Author Contributions

‡The authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; LD50, median lethal dose; COMT, catechol-O-methyl-transferase; MAO-B, monoamine oxidase type B; PET, positron emission tomography; NSAIDs, nonsteroidal anti-inflammatory drugs; NO, nitric oxide; LPS, lipopolysaccharide; IC50, half-maximal inhibitory concentration; MOM, methoxymethyl; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; DMF, dimethylformamide; DIPEA, N,N-diisopropylethylamine; Gly, glycine; Ala, alanine; Ser, serine; Asp, aspartic acid; Glu, glutamic acid; Phe, phenylalanine; His, histidine; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; TH, tyrosine hydroxylase; CMC-Na, sodium carboxymethylcellulose; prob, probenecid; syn, synuclein; iTRAQ, isobaric tags for relative and absolute quantification; PTEN, phosphatase and tensin homolog deleted on chromosome 10; NOS, NO synthase; PI3K, phosphoinositide 3-kinase; GSK3, glycogen synthase kinase-3; CK2, casein kinase 2; LKB1, liver kinase B1; GTPases, guanosine

triphosphatases; ROCK1, Rho-associated protein kinase 1; ARHGAP21, Rho guanosine triphosphatases activating protein 21; MPP+, 1-methyl-4-phenylpyridinium ion; CC, column chromatography; DMEM, Dulbecco's modified Eagle medium; FBS, foetal bovine serum; MEM, minimal essential medium; EGTA, ethylene glycol tetraacetic acid; ATP, adenosine triphosphate; EDTA, ethylenediaminetetraacetic acid.

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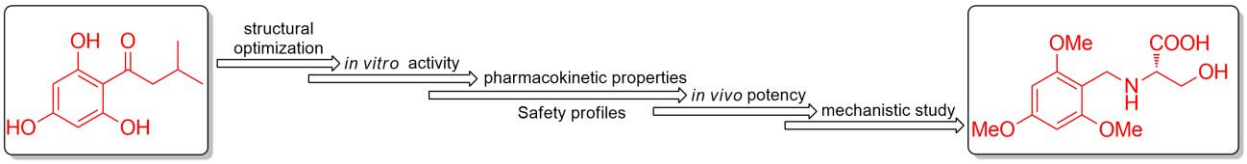
Table of Contents Graphic



Hit compound

Drug candidate for PD