Bioorganic & Medicinal Chemistry 18 (2010) 6708–6714

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design and synthesis of biphenyl derivatives as mushroom tyrosinase inhibitors

Kai Bao [a,b,†], Yi Dai [a,†], Zhi-Bin Zhu [b], Feng-Juan Tu [c], Wei-Ge Zhang [b,*], Xin-Sheng Yao [a,c,*]

[a] Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou 510632, PR China
[b] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[c] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, PR China

ARTICLE INFO

Article history:
Received 17 June 2010
Revised 26 July 2010
Accepted 27 July 2010
Available online 1 August 2010

Keywords:
Fortuneanoside E
Biphenyls
Tyrosinase inhibitors
Synthesis

ABSTRACT

Two new series of biphenyls, analogs of aglycone of natural product fortuneanoside E, were prepared using Suzuki–Miyaura cross-coupling and selective magnesium iodide demethylation/debenzylation, and their mushroom tyrosinase inhibitory activity was evaluated. Most of the 4-hydroxy-3,5-dimethoxyphenyl biphenyl compounds (series **II**, 20–36) were in general more active than 3,4,5-trimethoxyphenyl biphenyl compounds (series **I**, 1–19). Structure–activity relationships study showed that monosaccharide substituents, such as glucose, were not necessary and the presence of 4-hydroxy-3,5-dimethoxyphenyl moiety was crucial for inhibitory activity. Among the compounds synthesised, compound **21** (IC_{50} = 0.02 mM) was found to be the most active one, which exhibited an activity that was 7 times higher than that of fortuneanoside E (IC_{50} = 0.14 mM) and 10 times higher than that of arbutin (IC_{50} = 0.21 mM), known as potent tyrosinase inhibitors. The inhibition kinetics analyzed by Lineweaver–Burk plots revealed that compound **21** was a competitive inhibitor (K_i = 0.015 mM).

1. Introduction

Tyrosinase (EC 1.14.18.1), is a multifunctional copper-containing enzyme, that is, key in melanin biosynthesis, melanisation in animals, and browning in plants. Tyrosinase inhibitors can therefore be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. In addition, these inhibitors are also known to be useful in cosmetics for whitening and depigmentation after sunburn.[1,2] In the past few decades, a number of polyphenol tyrosinase inhibitors from both natural and synthetic sources, including flavonoids, stilbenes, and terpenoids, have been intensively investigated.[3–7] To our knowledge, however, only a few biphenyl inhibitors have been reported to date.[8,9]

Fortuneanoside E is a new biphenyl glycoside isolated from the fruit of Pyracantha fortuneana that has shown tyrosinase inhibitory activity. We previously reported the first total synthesis of this compound,[10,11] and it was noted that a synthetic intermediate of fortuneanoside E, compound **I**, was also found to show potent activities in the tyrosinase inhibition assay (Fig. 1).

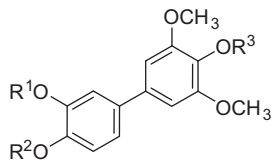
From examining the structural characteristics of fortuneanoside E and compound **I**, we designed and synthesized two series of biphenyls (series **I**: 3,4,5-trimethoxyphenyl series, 1–19 and series **II**: 4-hydroxy-3,5-dimethoxyphenyl series, 20–36) with different substitution patterns. We then examined the inhibitory effects of these compounds on mushroom tyrosinase to study the structure–activity relationships with these potential inhibitors.

2. Results and discussion

2.1. Chemistry

The synthetic procedures employed to obtain the target compounds **1**–**19** and **20**–**36** are depicted in Scheme 1.

Generally, there are several methods for the construction of biphenyls, including Stille coupling, the Gomberg–Bachmann reaction, the Ullmann reaction, and Suzuki–Miyaura cross-coupling. Considered the particularities of these methods and the characteristics of the target compounds, Suzuki–Miyaura cross-coupling

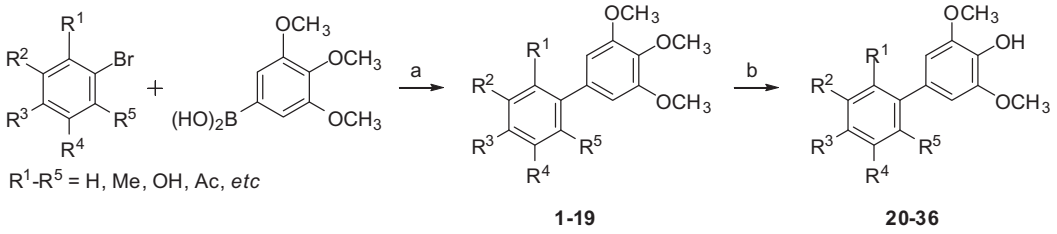


Fortuneanoside E R^1=H, R^2=Glc, R^3=H
Compound I R^1=Bn, R^2=H, R^3=CH_3

Figure 1. Chemical structures of fortuneanoside E and compound **I**.

* Corresponding authors. Tel.: +86 24 23986422; fax: +86 24 23986393 (W.-G.Z.); tel.: +86 20 85225849; fax: +86 20 85221559 (X.-S.Y.).
E-mail addresses: zhangweige2000@sina.com (W.-G. Zhang), yaoxinsheng@vip.tom.com (X.-S. Yao).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.07.062



Scheme 1. Synthesis of biphenyls **1**–**19** and **20**–**36**. Reagents and conditions: (a) K_2CO_3 (2.0 equiv), $Pd(OAc)_2$ (0.01 equiv), DMF, 110 °C; (b) MgI_2 (3.0 or 6.0 equiv), 80 °C.

was applied in the first step. To find the optimal conditions, a model reaction was performed using 2-(benzyloxy)-4-bromobenzaldehyde and 3,4,5-trimethoxy-phenylboronic acid under different conditions. As presented in Table 1, this reaction was found to be most efficient in the presence of K_2CO_3 and $Pd(OAc)_2$ in DMF at 110 °C and using these conditions, compound **1** could be prepared in 72% yield (Table 1, entry 9). With these optimized condition, biphenyls **2**–**19** were prepared from various bromobenzenes (commercially available or prepared by conventional methods) by Suzuki–Miyaura cross-coupling with 3,4,5-trimethoxy-phenylboronic acid, resulting in yields ranging from 68% to 92%.

Methyl and benzyl ethers have been widely used as stable protecting groups for hydroxyl groups in organic synthesis. A number of methods have been reported to perform cleavage of the methyl and benzyl groups in these ethers.[12,13] In our previous report,[11] we described a highly selective demethylation/debenzylation method that was effective in different kinds of pyrogallol trimethyl ethers using MgI_2 under solvent-free conditions. In these reactions, we found that a variety of functional groups were tolerated under our reaction conditions. Consequently, we applied this method in the synthesis of biphenyls **20**–**36**. Treatment of compounds **1**–**19** with MgI_2 at 80 °C under solvent-free conditions gave compounds **20**–**36** in good to excellent yields. If necessary, the amount of MgI_2 and the reaction temperature could be adjusted to improve the reaction yield. Analytical data of the previously reported compounds corresponded to the expected values and all new compounds were analyzed by ^1H, ^{13}C NMR, and MS.

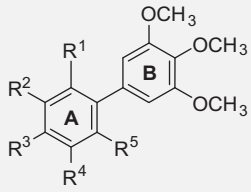
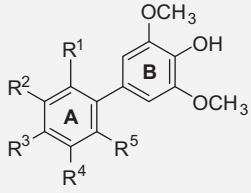
2.2. Inhibition of tyrosinase

The inhibition abilities of our synthetic biphenyls were initially investigated in vitro on mushroom tyrosinase using arbutin as the positive control (Table 2). From our preliminary investigation, we found that monosaccharide substituents, such as glucose, were not necessary for good inhibition activity. Some of the tested biphenyls, especially those containing the 4-hydroxy-3,5-dime-

Table 1
Suzuki–Miyaura cross-coupling of 2-(benzyloxy)-4-bromobenzaldehyde

Entry	Base	Catalyst	Solvent	Cond.	Time (h)	Yield (%)
1	KF	Pd/C	CH_3OH/H_2O = 1:1	rt (ultrasound)	3.0	—
2	KF	$Pd(PPh_3)_4$	CH_3OH/H_2O = 1:1	rt (ultrasound)	3.0	—
3	KF	$Pd(PPh_3)_4$	THF/H_2O = 4:1	rt (ultrasound)	3.0	—
4	K_2CO_3	$Pd(PPh_3)_4$	THF/H_2O = 4:1	Reflux	5.0	10
5	K_2CO_3	$Pd(PPh_3)_4$	Dioxane/H_2O = 6:1	90 °C	12.0	—
6	K_2CO_3	$Pd(OAc)_2$	THF/H_2O = 4:1	rt (ultrasound)	3.0	—
7	K_2CO_3	$Pd(OAc)_2$	THF/H_2O = 4:1	Reflux	5.0	32
8	K_2CO_3	$Pd(OAc)_2$	DMF	rt (ultrasound)	5.0	36
9	K_2CO_3	$Pd(OAc)_2$	DMF	110 °C	5.0	72

Table 2
Tyrosinase inhibitory activities of compounds **1**–**19** and **20**–**36**

Compound	R^1	R^2	R^3	R^4	R^5	IC_{50}[a] (mM)
						
1	H	OBn	CHO	H	H	>2.0
2	H	OH	CHO	H	H	>2.0
3	H	OBn	COOH	H	H	>2.0
4	H	OH	COOH	H	H	>2.0
5	H	OH	COOEt	H	H	>2.0
6	H	OBn	CONHBn	H	H	>2.0
7	H	OH	Me	H	H	>2.0
8	H	F	H	F	H	>2.0
9	F	H	F	H	H	>2.0
10	H	F	F	H	H	>2.0
11	F	OMe	H	H	H	>2.0
12	OMe	H	H	Ac	H	>2.0
13	OMe	H	H	$CHOHCH_3$	H	>2.0
14	H	OH	H	H	H	>2.0
15	H	OBn	H	H	H	0.55
16	Me	H	H	H	H	>2.0
17	H	H	COOH	H	H	>2.0
18	H	H	CONHBu	H	H	>2.0
19	H	H	CONHBn	H	H	>2.0
Compound **I**	H	OBn	OH	H	H	0.17
						
20	H	OH	CHO	H	H	0.06
21	H	OH	COOH	H	H	0.02
22	H	OH	COOEt	H	H	0.05
23	H	OH	CONHBn	H	H	0.03
24	H	OH	Me	H	H	0.09
25	H	F	H	F	H	0.08
26	F	H	F	H	H	0.06
27	H	F	F	H	H	0.07
28	F	OMe	H	H	H	0.06
29	F	OH	H	H	H	0.08
30	OMe	H	H	Ac	H	0.14
31	OMe	H	H	$CHOHCH_3$	H	0.19
32	H	OH	H	H	H	0.09
33	Me	H	H	H	H	0.17
34	H	H	COOH	H	H	0.05
35	H	H	CONHBu	H	H	0.07
36	H	H	CONHBn	H	H	0.12
Fortuneanoside E	H	OH	OGlc	H	H	0.14
Arbutin	0.21					

[a] The results are from the three concurrent readings, and each SD was usually within 2% of the mean.

thoxyphenyl moiety, were more potent inhibitors than fortuneanoside E and arbutin. With the exception of compound **I**, the 3,4,5-trimethoxyphenyl biphenyl compounds synthesized in series **I**, compounds **1**–**19**, showed very weak inhibitory activities against tyrosinase. However, when the methyl moiety at the C-4 position on ring B was cleaved, the activities sharply increased. From an examination of the structure–activity relationships of the two types of biphenyls in this study, it appears that the inhibitory activity is mainly dependent on the C-4 hydroxyl of ring B, whereas the presence of a hydroxy moiety on ring A does not contribute much to the inhibitory potency.

In series **II**, the most active compound was 3,4′-dihydroxy-3′,5′-dimethoxybiphenyl-4-carboxylic acid **21**, which exhibited an activity that was an order of magnitude higher than that of arbutin, 7 times higher than that of fortuneanoside E and 8.5 times higher than that of compound **I**. Comparing the IC_{50} of compound **21** (IC_{50} = 0.02) with those of compounds **32** and **34**, it is clear that when hydroxyl and carboxyl groups are both present on the A ring, the potency of the resulting compound **21** increases. In the case of the fluorine containing derivatives **25**–**29**, there was no remarkable difference in their activities. In addition, it was interesting to find that the activity of compound **34** (IC_{50} = 0.05), having 4-carboxyl substituents, substituents, was comparable to that of compound **21**. This finding also suggests that the hydroxy moiety on ring A did not have a great influence on the activity.

Among the tested biphenyls, compounds **21** showed the highest inhibitory activity, and hence, we carried out the kinetic analysis of **21** for tyrosinase inhibition with respect to L-tyrosine as a substrate. Lineweaver–Burk plots for the inhibition of tyrosinase by **21** were obtained with variable concentrations of **21** and the substrate (Fig. 2). The intersection of these lines on the vertical axis indicated that **21** was a competitive inhibitor of tyrosinase with respect to L-tyrosine as a substrate, with a K_i value of 0.015 mM. These data strongly suggested that **21** effectively inhibited the enzyme by binding to its active site. The inhibition kinetics of the selected compounds **23**, **34**, and **35** were also analyzed by Lineweaver–Burk plots and the K_i of these compounds binding were shown in Table 3. As shown in Table 3, the K_i of compound **21** was less than that of compounds **23**, **34**, and **35**, suggesting that compound **21** had most potent inhibitory effect.

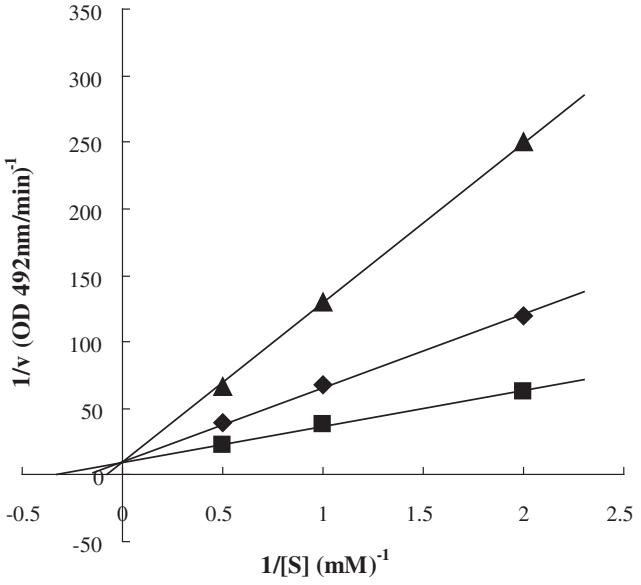


Figure 2. Lineweaver–Burk plots of mushroom tyrosinase and L-tyrosine without (■) and with (♦) 0.02 mM and (▲) 0.04 mM of compound **21**.

Table 3
Kinetics and inhibition constants of compounds **21**, **23**, **34**, and **35** on the activity of mushroom tyrosinase

Compound	Inhibition type	Inhibition constant (K_i) (mM)
21	Competitive	0.015
23	Competitive	0.060
34	Competitive	0.038
35	Competitive	0.031

3. Conclusions

In conclusion, two new series of biphenyls were prepared using Suzuki–Miyaura cross-coupling and selective magnesium iodide demethylation, and their activity as tyrosinase inhibitors was examined. Most of the compounds in series **II** possessed higher inhibitory activity compared to arbutin, fortuneanoside E and a synthetic intermediate that was known to be active in these assays, which suggested that the 4-hydroxy-3,5-dimethoxyl substituent on the B ring is crucial for tyrosinase inhibitory activity in this class of compounds. The present study revealed that activity could also be influenced by the type and position of substituents on the A ring—with electron withdrawing groups being somewhat advantageous—although explicit correlations would require further study. Among the compounds synthesized, compound **21** showed the strongest inhibitory activity. Kinetic study revealed that compound **21** acted as a competitive inhibitor of mushroom tyrosinase with K_i value of 0.015 mM. Further investigations into the structure–activity relationships of these compounds are currently in progress.

4. Experimental section

Unless otherwise noted, all the materials were obtained from commercially available sources and were used without purification. Thin-layer chromatography was performed on GF254 silica gel plates to monitor the reaction, and the plates were examined under UV light. The purification of the products was performed using column chromatography (60 Å, 200–300 mesh, Qingdao Ocean Chemicals) or silica gel plates (0.25 mm layer, Qingdao Ocean Chemicals) with the designated solvents. Melting points were measured on a hot-stage microscope (X-4, Beijing Taike Ltd) and are uncorrected. ESI-MS spectra were performed on a Finigan LCQ Advantage MAX or Agilent 1100 series MSD TRAP mass spectrometer. Elemental analyzes were conducted by the Analytical Centre of Jilin University, China. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ solution on Bruker ARX-300, Bruker AV-400, and Bruker AV-600 spectrometers with TMS as the internal reference. Chemical shifts were reported in ppm downfield from tetramethylsilane and proton–proton coupling constants (J) in Hz.

4.1. General procedure for the preparation of compounds 1–19

4.1.1. 3-(Benzyloxy)-3′,4′,5′-trimethoxybiphenyl-4-carbaldehyde (1)

To a solution of 2-(benzyloxy)-4-bromobenzaldehyde (0.87 g, 3.00 mmol) and 3,4,5-trimethoxyphenyl-boronic acid (0.42 g, 2.00 mmol) in DMF (20 mL), was added anhydrous K_2CO_3 (0.55 g, 4.00 mmol) and $Pd(OAc)_2$ (0.01 g, 0.06 mmol). The reaction mixture was stirred at 110 °C for 5 h, then cooled to room temperature and poured into water, filtered and recrystallized with ethyl acetate to afford **I** as a colorless solid (0.54 g, 72%). Mp 139–142 °C; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 10.57 (s, 1H, CHO), 7.90 (d, 1H, J = 8.0 Hz, 5-H), 7.48–7.36, 5.29 (OBn), 7.20 (dd, 1H, J = 8.0, 1.1 Hz, 6-H), 7.15 (d, 1H, J = 1.1 Hz, 2-H), 6.70 (s, 2H, 2′,6′-H), 3.91 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 4′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$,

TMS): δ 189.2 (CHO), 161.2 (C-3), 153.6 (C-3′/C-5′), 149.0 (C-1), 138.9 (C-4′), 136.2, 128.8, 128.3, 127.3, 70.8 (OBn), 135.9 (C-1′), 129.0 (C-5), 124.1 (C-4), 120.0 (C-6), 112.0 (C-2), 104.8 (C-2′/C-6′), 61.0 (4′-CH_3O), 56.3 (3′,5′-CH_3O); MS (ESI, *m/z*): 401.4 [M+Na^+], 779.1 [2M+Na^+]; Anal. Calcd for $C_{23}H_{22}O_5$: C, 73.00; H, 5.86. Found: C, 72.89; H, 5.90.

4.1.2. 3-Hydroxy-3′,4′,5′-trimethoxybiphenyl-4-carbaldehyde (2)

White solid, yield 83%. Mp 149–151 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 11.15 (s, 1H, CHO), 9.92 (s, 1H, OH), 7.62 (d, 1H, *J* = 8.1 Hz, 5-H), 7.23 (dd, 1H, *J* = 8.1, 1.6 Hz, 6-H), 7.18 (d, 1H, *J* = 1.6 Hz, 2-H), 6.82 (s, 2H, 2′,6′-H), 3.94 (s, 6H, 3′,5′-CH_3O), 3.91 (s, 3H, 4′-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 194.3 (CHO), 161.9 (C-3), 156.9 (C-1), 153.7 (C-3′/C-5′), 137.9 (C-4′), 136.8 (C-1′), 128.4 (C-5), 124.0 (C-6), 118.9 (C-4), 115.4 (C-2), 104.5 (C-2′/C-6′), 61.3 (4′-CH_3O), 56.5 (3′,5′-CH_3O); MS (ESI, *m/z*): 289.4 [M+H^+], 287.2 [M−H^-]; Anal. Calcd for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.57; H, 5.62.

4.1.3. 3-(Benzyloxy)-3′,4′,5′-trimethoxybiphenyl-4-carboxylic acid (3)

White solid, yield 76%. Mp 133–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 8.23 (d, 1H, *J* = 8.1 Hz, 5-H), 7.49–7.41, 5.39 (OBn), 7.30 (dd, 1H, *J* = 8.1, 1.2 Hz, 6-H), 7.22 (d, 1H, *J* = 1.2 Hz, 2-H), 6.70 (s, 2H, 2′,6′-H), 3.92 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 4′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 165.2 (COOH), 157.5 (C-3), 153.7 (C-3′/C-5′), 148.3 (C-1), 138.9 (C-4′), 135.2, 129.2, 127.9, 72.4 (OBn), 134.5 (C-1′), 134.3 (C-5), 121.1 (C-6), 116.7 (C-4), 112.0 (C-2), 104.7 (C-2′/C-6′), 61.0 (4′-CH_3O), 56.3 (3′,5′-CH_3O); MS (ESI, *m/z*): 811.9 [2M+Na^+], 827.7 [2M+K^+], 787.7 [2M−H^-]; Anal. Calcd for $C_{23}H_{22}O_6$: C, 70.04; H, 5.62. Found: C, 70.16; H, 5.51.

4.1.4. 3-Hydroxy-3′,4′,5′-trimethoxybiphenyl-4-carboxylic acid (4)

Light yellow solid, yield 84%. Mp 107–109 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.86 (d, 1H, *J* = 8.0 Hz, 5-H), 7.13–7.10 (m, 2H, 2,6-H), 6.86 (s, 2H, 2′,6′-H), 3.90 (s, 6H, 3′,5′-CH_3O), 3.78 (s, 3H, 4′-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 161.6 (COOH), 156.2 (C-3), 151.7 (C-3′/C-5′), 145.4 (C-1), 136.4 (C-4′), 133.8 (C-1′, C-5), 118.1 (C-6), 115.6 (C-4), 113.2 (C-2), 102.8 (C-2′/C-6′), 58.8 (4′-CH_3O), 54.5 (3′,5′-CH_3O); MS (ESI, *m/z*): 302.9 [M−H^-], 607.1 [2M−H^-]; Anal. Calcd for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 63.23; H, 5.27.

4.1.5. Ethyl 3-hydroxy-3′,4′,5′-trimethoxybiphenyl-4-carboxylate (5)

White solid, yield 92%. Mp 100–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 10.91 (s, 1H, OH), 7.89 (d, 1H, *J* = 8.3 Hz, 5-H), 7.18 (d, 1H, *J* = 1.5 Hz, 2-H), 7.08 (dd, 1H, *J* = 8.3, 1.5 Hz, 6-H), 6.81 (s, 2H, 2′,6′-H), 4.44 (q, 2H, *J* = 7.1 Hz, O*CH*$_2$CH$_3$), 3.94 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 4′-CH_3O), 1.44 (t, 3H, *J* = 7.1 Hz, OCH$_2$*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 170.1 (COOEt), 161.8 (C-3), 153.5 (C-3′/C-5′), 148.4 (C-1), 138.6 (C-4′), 135.5 (C-1′), 130.3 (C-5), 117.9 (C-6), 115.6 (C-4), 111.4 (C-2), 104.6 (C-2′/C-6′), 61.4 (O*CH*$_2$CH$_3$), 61.0 (4′-CH_3O), 56.3 (3′,5′-CH_3O), 14.2 (OCH$_2$*CH*$_3$); MS (ESI, *m/z*): 355.2 [M+Na^+], 687.9 [2M+Na^+], 331.2 [M−H^-]; Anal. Calcd for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.91; H, 6.14.

4.1.6. *N*-Benzyl-3-(benzyloxy)-3′,4′,5′-trimethoxybiphenyl-4-carboxamide (6)

Colorless oil, yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 8.34 (d, 1H, *J* = 8.3 Hz, 5-H), 7.32–7.17 (m, 12H, Ar–H), 6.73 (s, 2H, 2′,6′-H), 5.21 (s, 2H, O*CH*$_2$), 4.59 (d, 2H, *J* = 5.5 Hz, NH*CH*$_2$), 3.92 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 4′-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 168.1 (CONH), 157.1 (C-3), 153.7 (C-3′/C-5′), 146.0 (C-1), 139.2 (C-4′), 138.5, 128.6, 127.7, 127.2, 44.0 (NHBn), 134.7, 128.9, 128.7, 127.8, 71.6 (OBn), 133.1 (C-1′), 127.2 (C-5), 126.0 (C-4), 118.7 (C-6), 111.5 (C-2), 104.8 (C-2′/C-6′), 61.0 (4′-CH_3O), 56.4 (3′,5′-CH_3O); MS (ESI, *m/z*): 484.1 [M+H^+], 506.4 [M+Na^+]; Anal. Calcd for $C_{30}H_{29}NO_5$: C, 74.52; H, 6.04; N, 2.90. Found: C, 74.46; H, 5.98; N, 2.93.

4.1.7. 3′,4′,5′-Trimethoxy-4-methylbiphenyl-3-ol (7)

White solid, yield 90%. Mp 138–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.16 (d, 1H, *J* = 7.8 Hz, 5-H), 7.03 (dd, 1H, *J* = 7.8, 1.8 Hz, 6-H), 6.96 (d, 1H, *J* = 1.8 Hz, 2-H), 6.72 (s, 2H, 2′,6′-H), 5.15 (s, 1H, OH), 3.88 (s, 9H, 3′,4′,5′-CH_3O), 2.29 (s, 3H, 4-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 154.1 (C-3), 153.4 (C-3′/C-5′), 140.5 (C-1), 137.5 (C-4′), 136.9 (C-1′), 131.2 (C-5), 122.9 (C-4), 119.2 (C-6), 113.6 (C-2), 104.3 (C-2′/C-6′), 61.0 (4′-CH_3O), 56.2 (3′,5′-CH_3O), 15.4 (4-CH_3); MS (ESI, *m/z*): 275.8 [M+H^+], 571.0 [2M+Na^+], 272.7 [M−H^-]; Anal. Calcd for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61. Found: C, 70.14; H, 6.55.

4.1.8. 3′,5′-Difluoro-3,4,5-trimethoxybiphenyl (8)

White solid, yield 75%. Mp 72–73 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.07 (d, 2H, *J* = 6.5 Hz, 2′,6′-H), 6.79 (m, 1H, 4′-H), 6.72 (s, 2H, 2,6-H), 3.94 (s, 6H, 3,5-CH_3O), 3.89 (s, 3H, 4-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 164.8, 161.6 (C-3′/C-5′), 153.6 (C-3/C-5), 144.6 (C-1′), 138.5 (C-4), 134.8 (C-1), 109.9 (C-2′/C-6′), 104.3 (C-2/C-6), 102.6 (C-4′), 61.0 (4-CH_3O), 56.3 (3,5-CH_3O); MS (ESI, *m/z*): 281.3 [M+H^+], 303.4 [M+Na^+]; Anal. Calcd for $C_{15}H_{14}F_2O_3$: C, 64.28; H, 5.03. Found: C, 64.15; H, 4.97.

4.1.9. 2,4-Difluoro-3′,4′,5′-trimethoxybiphenyl (9)

White solid, yield 69%. Mp 40–42 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.40 (m, 1H, 6-H), 6.98–6.87 (m, 2H, 3,5-H), 6.70 (s, 2H, 2′,6′-H), 3.90 (s, 9H, 3′,4′,5′-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 164.1 (C-4), 159.0 (C-2), 153.5 (C-3′/C-5′), 138.1 (C-4′), 131.6 (C-6), 130.8 (C-1′), 125.6 (C-1), 111.8 (C-5), 106.6 (C-2′/C-6′), 104.7 (C-3), 61.2 (4′-CH_3O), 56.5 (3′,5′-CH_3O); MS (ESI, *m/z*): 281.4 [M+H^+], 303.3 [M+Na^+], 319.3 [M+K^+]; Anal. Calcd for $C_{15}H_{14}F_2O_3$: C, 64.28; H, 5.03. Found: C, 64.21; H, 5.00.

4.1.10. 3′,4′-Difluoro-3,4,5-trimethoxybiphenyl (10)

White solid, yield 87%. Mp 69–72 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.38–7.31 (m, 1H, 2′-H), 7.28–7.16 (m, 2H, 5′,6′-H), 6.70 (s, 2H, 2,6-H), 3.92 (s, 6H, 3,5-CH_3O), 3.89 (s, 3H, 4-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 163.2 (C-3′), 156.4 (C-4′), 153.9 (C-3/C-5), 138.7 (C-1′), 138.3 (C-4), 135.4 (C-1), 123.2 (C-6′), 117.7 (C-2′), 116.2 (C-5′), 104.6 (C-2/C-6), 61.2 (4-CH_3O), 56.5 (3,5-CH_3O); MS (ESI, *m/z*): 281.4 [M+H^+], 303.3 [M+Na^+], 583.3 [2M+Na^+]; Anal. Calcd for $C_{15}H_{14}F_2O_3$: C, 64.28; H, 5.03. Found: C, 64.31; H, 4.94.

4.1.11. 2-Fluoro-3,3′,4′,5′-tetramethoxybiphenyl (11)

White solid, yield 83%. Mp 82–85 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.11 (m, 1H, 6-H), 7.00 (m, 1H, 5-H), 6.94 (m, 1H, 4-H), 6.76 (s, 2H, 2′,6′-H), 3.92 (s, 3H, 3-CH_3O), 3.90 (s, 3H, 4′-CH_3O), 3.89 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 153.1 (C-3′/C-5′), 150.7 (C-3), 148.3 (C-2), 137.9 (C-4′), 131.1 (C-1′), 129.8 (C-1), 123.8 (C-5), 121.8 (C-6), 112.2 (C-4), 106.5/106.4 (C-2′/C-6′), 60.8 (4′-CH_3O), 56.3 (3-CH_3O), 56.1 (3′,5′-CH_3O); MS (ESI, *m/z*): 315.7 [M+Na^+], 607.2 [2M+Na^+]; Anal. Calcd for $C_{16}H_{17}FO_4$: C, 65.74; H, 5.86. Found: C, 65.58; H, 5.99.

4.1.12. 1-(3′,4′,5′,6-Tetramethoxybiphenyl-3-yl)ethanone (12)

White solid, yield 91%. Mp 114–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.02 (d, 1H, *J* = 8.1 Hz, 5-H), 7.96 (dd, 1H, *J* = 8.1, 2.3 Hz, 4-H), 7.94 (d, 1H, *J* = 2.3 Hz, 2-H), 6.73 (s, 2H, 2′,6′-H), 3.90 (s, 6H, 3′,5′-CH_3O), 3.89 (s, 6H, 6,4′-CH_3O), 2.59 (s, 3H, 3-

$COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 196.8 (5-CO), 160.3 (C-6), 152.9 (C-3′/C-5′), 137.7 (C-4′), 133.0 (C-1′), 131.2 (C-2), 130.7 (C-3), 130.3 (C-4), 129.8 (C-1), 110.6 (C-5), 107.0 (C-2′/C-6′), 60.9 (4′-CH_3O), 56.3 (3′,5′-CH_3O), 55.9 (6-CH_3O), 26.4 (3-$COCH_3$); MS (ESI, *m/z*): 317.8 [M+H$^+$], 655.9 [2M+Na$^+$]; Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.44; H, 6.33.

4.1.13. 1-(3′,4′,5′,6-Tetramethoxybiphenyl-3-yl)ethanol (13)

Colorless oil, yield 92%. ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 7.34–7.32 (m, 2H, 2,4-H), 6.97 (d, 1H, *J* = 9.1 Hz, 5-H), 6.74 (s, 2H, 2′,6′-H), 4.91 (q, 1H, *J* = 6.4 Hz, 3-*CH*OHCH$_3$), 3.89 (s, 3H, 4′-CH_3O), 3.88 (s, 6H, 3′,5′-CH_3O), 3.82 (s, 3H, 6-CH_3O), 1.52 (d, 3H, *J* = 6.4 Hz, 3-CHOH*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 155.8 (C-6), 152.8 (C-3′/C-5′), 138.2 (C-4′), 134.0 (C-1′), 137.3 (C-3), 130.7 (C-2), 128.1 (C-4), 125.6 (C-1), 111.4 (C-5), 107.0 (C-2′/C-6′), 70.0 (3-*C*HOHCH$_3$), 60.9 (4′-CH_3O), 56.2 (3′,5′-CH_3O), 55.8 (6-CH_3O), 25.1 (3-CHOH*C*H$_3$); MS (ESI, *m/z*): 319.1 [M+H$^+$], 341.2 [M+Na$^+$], 659.8 [2M+Na$^+$]; Anal. Calcd for $C_{18}H_{22}O_5$: C, 67.91; H, 6.97. Found: C, 67.80; H, 6.93.

4.1.14. 3′,4′,5′-Trimethoxybiphenyl-3-ol (14)

White solid, yield 75%. Mp 98–101 °C; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 7.27 (m, 1H, 5-H), 7.09 (m, 1H, 6-H), 7.03 (m, 1H, 2-H), 6.83 (dd, 1H, *J* = 8.1, 2.0 Hz, 4-H), 6.75 (s, 2H, 2′,6′-H), 3.89 (s, 3H, 4′-CH_3O), 3.88 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 156.2 (C-3), 153.1 (C-3′/C-5′), 143.0 (C-1), 137.6 (C-4′), 137.0 (C-1′), 129.9 (C-5), 119.3 (C-6), 114.3 (C-2), 114.2 (C-4), 104.5 (C-2′/C-6′), 61.0 (4′-CH_3O), 56.2 (3′,5′-CH_3O); MS (ESI, *m/z*): 259.7 [M−H$^-$], 519.6 [2M−H$^-$]; Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 69.38; H, 6.05.

4.1.15. 3′-(Benzyloxy)-3,4,5-trimethoxybiphenyl (15)

White solid, yield 77%. Mp 87–89 °C; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 7.47–7.14 (m, 8H, Ar–H), 6.96 (m, 1H, 4′-H), 6.75 (s, 2H, 2,6-H), 5.13 (s, 2H, OBn), 3.90 (s, 6H, 3,5-CH_3O), 3.89 (s, 3H, 4-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 159.1 (C-3′), 153.4 (C-3/C-5), 142.9 (C-1′), 137.9 (C-4), 137.0, 128.6, 128.0, 127.5, 70.2 (OBn), 136.9 (C-1), 129.7 (C-5′), 119.8 (C-6′), 114.2 (C-2′), 113.3 (C-4′), 104.6 (C-2/C-6), 60.9 (4-CH_3O), 56.2 (3,5-CH_3O); MS (ESI, *m/z*): 373.1 [M+Na$^+$], 723.5 [2M+Na$^+$]; Anal. Calcd for $C_{22}H_{22}O_4$: C, 75.41; H, 6.33. Found: C, 75.56; H, 6.18.

4.1.16. 3′,4′,5′-Trimethoxy-2-methylbiphenyl (16)

Light yellow oil, yield 86%. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 7.27–7.24 (m, 4H, 3,4,5,6-H), 6.52 (s, 2H, 2′,6′-H), 3.90 (s, 3H, 4′-CH_3O), 3.86 (s, 6H, 3′,5′-CH_3O), 2.30 (s, 3H, 2-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 153.1 (C-3′/C-5′), 142.3 (C-4′), 137.9 (C-2), 137.1 (C-1′), 135.7 (C-1), 130.6 (C-3), 129.8 (C-4), 127.6 (C-5), 126.0 (C-6), 106.7 (C-2′/C-6′), 61.2 (4′-CH_3O), 56.4 (3′,5′-CH_3O), 20.8 (2-CH_3); MS (ESI, *m/z*): 259.4 [M+H$^+$], 281.4 [M+Na$^+$]; Anal. Calcd for $C_{16}H_{18}O_3$: C, 74.39; H, 7.02. Found: C, 74.32; H, 7.11.

4.1.17. 3′,4′,5′-Trimethoxybiphenyl-4-carboxylic acid (17)

Light yellow solid, yield 79%. Mp 154–156 °C; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 8.14 (d, 2H, *J* = 8.0 Hz, 3,5-H), 7.62 (d, 2H, *J* = 8.0 Hz, 2,6-H), 6.80 (s, 2H, 2′,6′-H), 3.93 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 4′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 173.0 (COOH), 153.6 (C-3′/C-5′), 146.2 (C-1), 138.5 (C-4′), 135.8 (C-1′), 130.7 (C-4), 127.0 (C-2, C-3, C-5, C-6), 104.7 (C-2′/C-6′), 60.9 (4′-CH_3O), 56.3 (3′,5′-CH_3O); MS (ESI, *m/z*): 287.4 [M−H$^-$]; Anal. Calcd for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.49; H, 5.50.

4.1.18. *N*-Butyl-3′,4′,5′-trimethoxybiphenyl-4-carboxamide (18)

White solid, yield 74%. Mp 116–118 °C; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 7.83 (d, 2H, *J* = 8.5 Hz, 3,5-H), 7.60 (d, 2H, *J* = 8.5 Hz, 2,6-H), 6.79 (s, 2H, 2′,6′-H), 3.49, 1.62, 1.43, 0.98 (NHBu), 3.93 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 4′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 167.1 (CONH), 153.6 (C-3′/C-5′), 144.2 (C-1), 138.3 (C-4′), 136.0 (C-1′), 133.5 (C-4), 127.3 (C-3, C-5), 127.1 (C-2, C-6), 104.6 (C-2′/C-6′), 61.0 (4′-CH_3O), 56.3 (3′,5′-CH_3O), 39.8, 31.8, 20.2, 13.8 (NHBu); MS (ESI, *m/z*): 344.2 [M+H$^+$], 709.3 [2M+Na$^+$]; Anal. Calcd for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 69.87; H, 7.46; N, 4.02.

4.1.19. *N*-Benzyl-3′,4′,5′-trimethoxybiphenyl-4-carboxamide (19)

White solid, yield 68%. Mp 119–121 °C; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 7.86 (d, 2H, *J* = 8.2 Hz, 3,5-H), 7.61 (d, 2H, *J* = 8.2 Hz, 2,6-H), 7.38–7.31(5H, Ar–H), 6.79 (s, 2H, 2′,6′-H), 4.69 (d, 2H, *J* = 5.7 Hz, NH*CH*$_2$), 3.93 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 4′-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 167.3 (CONH), 153.9 (C-3′/C-5′), 144.8 (C-1), 138.5 (C-4′), 136.2 (C-1′), 133.3 (C-4), 129.1 (C-3, C-5), 128.2 (C-2, C-6), 104.8 (C-2′/C-6′), 61.3 (4′-CH_3O), 56.6 (3′,5′-CH_3O), 138.4, 128.0, 127.8, 127.5, 44.5 (NHBn); MS (ESI, *m/z*): 378.4 [M+H$^+$], 400.4 [M+Na$^+$], 416.3 [M+K$^+$], 375.8 [M−H$^-$]; Anal. Calcd for $C_{23}H_{23}NO_4$: C, 73.19; H, 6.14; N, 3.71. Found: C, 73.07; H, 6.03; N, 3.86.

4.2. General procedure for the preparation of compounds 20–36

4.2.1. 3,4′-Dihydroxy-3′,5′-dimethoxybiphenyl-4-carbaldehyde (20)

MgI_2 (0.88 g, 3.18 mmol) in dry ether (10 mL) was added to a solution of **1** (0.2 g, 0.53 mmol) in CH_2Cl_2 (5 mL). The solvent was removed under reduced pressure and the residuary solid mixture was heated at 80 °C for 4 h. Then H_2O (20 mL) and $Na_2S_2O_3$ was added and the mixture was poured into 5% hydrochloric acid (10 mL), extracted with EtOAc (2 × 15 mL). The combined organic phase was washed with saturated sodium bicarbonate solution (20 mL) and saturated brine (20 mL), dried over $MgSO_4$ and evaporation in vacuo. The crude product was purified by silica gel flash chromatography (*P*/*E*: 2/1) to give **20** as a white solid (0.12 g, 84%). Mp 60–62 °C; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 11.12 (s, 1H, CHO), 9.90 (s, 1H, 3-OH), 7.58 (d, 1H, *J* = 8.0 Hz, 5-H), 7.20 (dd, 1H, *J* = 8.0, 1.5 Hz, 6-H), 7.17 (d, 1H, *J* = 1.5 Hz, 2-H), 6.85 (s, 2H, 2′,6′-H), 5.64 (s, 1H, 4′-OH), 3.96 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 195.8 (CHO), 162.0 (C-3), 150.0 (C-1), 147.5 (C-3′/C-5′), 134.0 (C-4′), 130.8 (C-1′), 121.0 (C-4), 119.4 (C-5), 118.5 (C-6), 115.3 (C-2), 104.5 (C-2′/C-6′), 56.5 (3′,5′-CH_3O); MS (ESI, *m/z*): 275.8 [M+H$^+$], 273.3 [M−H$^-$]; Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.76; H, 5.04.

4.2.2. 3,4′-Dihydroxy-3′,5′-dimethoxybiphenyl-4-carboxylic acid (21)

Light yellow solid, yield 86%. Mp 77–79 °C; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 9.90 (s, 1H, 3-OH), 7.58 (d, 1H, *J* = 7.9 Hz, 5-H), 7.21 (dd, 1H, *J* = 7.9, 1.5 Hz, 6-H), 7.16 (d, 1H, *J* = 1.5 Hz, 2-H), 6.85 (s, 2H, 2′,6′-H), 5.69 (s, 1H, 4′-OH), 3.96 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 195.8 (COOH), 161.9 (C-3), 150.0 (C-3′/C-5′), 147.5 (C-1), 136.0 (C-4′), 134.0 (C-1′), 130.7 (C-5), 119.3 (C-6), 115.3 (C-4), 118.5 (C-2), 104.4 (C-2′/C-6′), 56.5 (3′,5′-CH_3O); MS (ESI, *m/z*): 290.9 [M+H$^+$], 312.9 [M+Na$^+$], 288.7 [M−H$^-$]; Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.07; H, 4.86. Found: C, 61.94; H, 4.77.

4.2.3. Ethyl 3,4′-dihydroxy-3′,5′-dimethoxybiphenyl-4-carboxylate (22)

White solid, yield 81%. Mp 127–129 °C; ^1H NMR (600 MHz, $CDCl_3$, TMS): δ 10.91 (s, 1H, 3-OH), 7.87 (d, 1H, *J* = 8.4 Hz, 5-H), 7.08 (dd, 1H, *J* = 8.4, 1.8 Hz, 6-H), 7.16 (d, 1H, *J* = 1.8 Hz, 2-H), 6.84 (s, 2H, 2′,6′-H), 5.62 (s, 1H, 4′-OH), 4.44 (q, 2H, *J* = 7.1 Hz, COO*CH*$_2$CH$_3$), 3.96 (s, 6H, 3′,5′-CH_3O), 1.44 (t, 3H, *J* = 7.1 Hz, COOCH$_2$*CH*$_3$); ^{13}C NMR (150 MHz, $CDCl_3$, TMS): δ 170.2 (COOEt),

161.9 (C-3), 148.5 (C-1), 147.4 (C-3′/C-5′), 138.5 (C-4′), 135.5 (C-1′), 130.3 (C-5), 117.8 (C-6), 115.3 (C-4), 111.1 (C-2), 104.2 (C-2′/C-6′), 61.4 (OCH_2CH_3), 56.4 (3′,5′-CH_3O), 14.2 (OCH_2CH_3); MS (ESI, *m/z*): 319.1 [M+H$^+$], 341.1 [M+Na$^+$], 316.9 [M−H$^-$]; Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 64.08; H, 5.87.

4.2.4. *N*-Benzyl-3,4′-dihydroxy-3′,5′-dimethoxybiphenyl-4-carboxamide (23)

Light yellow solid, yield 79%. Mp 179–181 °C; ^{1}H NMR (600 MHz, $CDCl_3$, TMS): δ 12.43 (s, 1H, 3-OH), 7.39–7.36 (m, 6H, Ar–H), 7.19 (d, 1H, *J* = 1.7 Hz, 2-H), 7.02 (dd, 1H, *J* = 8.3, 1.7 Hz, 6-H), 6.82 (s, 2H, 2′,6′-H), 5.61 (s, 1H, 4′-OH), 4.67 (d, 2H, *J* = 5.5 Hz, NH*CH*$_2$), 3.95 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (150 MHz, $CDCl_3$, TMS): δ 168.1 (CONH), 162.0 (C-3), 147.3 (C-3′/C-5′), 144.5 (C-1), 137.5 (C-4′), 135.4, 128.9, 128.8, 127.9, 43.7 (NHBn), 132.3 (C-1′), 130.9 (C-5), 117.2 (C-6), 116.4 (C-2), 111.6 (C-4), 104.1 (C-2′/C-6′), 56.4 (3′,5′-CH_3O); MS (ESI, *m/z*): 402.9 [M+Na$^+$], 378.7 [M−H$^-$]; Anal. Calcd for $C_{22}H_{21}NO_5$: C, 69.64; H, 5.58; N, 3.69. Found: C, 69.74; H, 5.43; N, 3.85.

4.2.5. 3′,5′-Dimethoxy-4-methylbiphenyl-3,4′-diol (24)

Colorless oil, yield 91%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.16 (d, 1H, *J* = 7.8 Hz, 5-H), 7.03 (dd, 1H, *J* = 7.8, 1.3 Hz, 6-H), 6.97 (d, 1H, *J* = 1.3 Hz, 2-H), 6.77 (s, 2H, 2′,6′-H), 5.50 (s, 1H, 3-OH), 4.72 (s, 1H, 4′-OH), 3.94 (s, 6H, 3′,5′-CH_3O), 2.28 (s, 3H, 4-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 154.1 (C-3), 147.3 (C-3′/C-5′), 141.0 (C-1), 134.5 (C-4′), 132.6 (C-1′), 131.3 (C-5), 122.5 (C-4), 119.3 (C-6), 113.5 (C-2), 104.0 (C-2′/C-6′), 56.4 (3′,5′-CH_3O), 15.4 (4-CH_3); MS (ESI, *m/z*): 261.4 [M+H$^+$], 259.2 [M−H$^-$]; Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 69.36; H, 6.29.

4.2.6. 3′,5′-Difluoro-3,5-dimethoxybiphenyl-4-ol (25)

Light yellow solid, yield 88%. Mp 60–61 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.04 (m, 2H, 2′,6′-H), 6.80 (m, 1H, 4′-H), 6.75 (s, 2H, 2,6-H), 5.65 (s, 1H, 4-OH), 3.95 (s, 6H, 3,5-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 163.6 (C-3′/C-5′), 147.7(C-3/C-5), 145.0 (C-1′), 135.6 (C-4), 135.1 (C-1), 109.9 (C-2′/C-6′), 104.2 (C-2/C-6), 102.3 (C-4′), 56.7 (3, 5-CH_3O); MS (ESI, *m/z*): 267.6 [M+H$^+$], 289.6 [M+Na$^+$], 265.1 [M−H$^-$]; Anal. Calcd for $C_{14}H_{12}F_2O_3$: C, 63.16; H, 4.54. Found: C, 63.05; H, 4.39.

4.2.7. 2′,4′-Difluoro-3,5-dimethoxybiphenyl-4-ol (26)

Light yellow solid, yield 80%. Mp 54–57 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.38 (m, 1H, 6′-H), 6.95–6.84 (m, 2H, 3′,5′-H), 6.71 (d, 2H, 2,6-H), 5.67 (s, 1H, 4-OH), 3.91 (s, 9H, 3,5-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 162.3 (C-4′), 159.9 (C-2′), 147.3 (C-3/C-5), 135.0 (C-4), 131.5 (C-6′), 126.4 (C-1), 125.7 (C-1′), 111.7 (C-5′), 106.2 (C-2/C-6), 104.8 (C-3′), 56.7 (3,5-CH_3O); MS (ESI, *m/z*): 267.3 [M+H$^+$], 289.3 [M+Na$^+$], 555.3 [2M+Na$^+$]; Anal. Calcd for $C_{14}H_{12}F_2O_3$: C, 63.16; H, 4.54. Found: C, 63.01; H, 4.43.

4.2.8. 3′,4′-Difluoro-3,5-dimethoxybiphenyl-4-ol (27)

White solid, yield 85%. Mp 70–72 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.35–7.28 (m, 1H, 2′-H), 7.25–7.14 (m, 2H, 5′,6′-H), 6.71 (s, 2H, 2,6-H), 5.58 (s, 1H, 4-OH), 3.95 (s, 6H, 3,5-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 150.8 (C-3′), 149.8 (C-4′), 147.7 (C-3/C-5), 138.9 (C-1′), 135.2 (C-4), 131.1 (C-1), 123.0 (C-6′), 117.7 (C-2′), 116.0 (C-5′), 104.2 (C-2/C-6), 56.7 (3,5-CH_3O); MS (ESI, *m/z*): 267.3 [M+H$^+$], 289.3 [M+Na$^+$], 264.6 [M−H$^-$]; Anal. Calcd for $C_{14}H_{12}F_2O_3$: C, 63.16; H, 4.54. Found: C, 63.06; H, 4.36.

4.2.9. 2′-Fluoro-3,3′,5-trimethoxybiphenyl-4-ol (28)

White solid, yield 67%. Mp 100–102 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.10 (m, 1H, 5′-H), 6.99 (m, 1H, 6′-H), 6.93 (m, 1H, 4′-H), 6.78 (s, 2H, 2,6-H), 5.57 (s, 1H, 4-OH), 3.92 (s, 6H, 3,5-CH_3O), 3.93 (s, 3H, 3′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 150.8 (C-2′), 148.4 (C-3′), 147.0 (C-3/C-5), 134.8 (C-4), 130.1 (C-1′), 126.7 (C-1), 123.8 (C-5′), 121.9 (C-6′), 112.0 (C-4′), 106.2 (C-2/C-6), 56.4 (3,5-CH_3O); MS (ESI, *m/z*): 301.6 [M+Na$^+$], 277.4 [M−H$^-$]; Anal. Calcd for $C_{15}H_{15}FO_4$: C, 64.74; H, 5.43. Found: C, 64.88; H, 5.51.

4.2.10. 2-Fluoro-3′,5′-dimethoxybiphenyl-3,4′-diol (29)

Colorless oil, yield 58%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.07 (m, 1H, 6-H), 6.99 (m, 2H, 4,5-H), 6.77 (s, 2H, 2′,6′-H), 3.93 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 149.5 (C-2), 147.0 (C-3′/C-5′), 144.2 (C-3), 134.9 (C-4′), 129.7 (C-1), 126.5 (C-1′), 124.4 (C-5), 121.6 (C-6), 115.8 (C-4), 106.1 (C-2′/C-6′), 56.4 (3′,5′-CH_3O); MS (ESI, *m/z*): 287.5 [M+Na$^+$], 263.3 [M−H$^-$]; Anal. Calcd for $C_{14}H_{13}FO_4$: C, 63.63; H, 4.96. Found: C, 63.48; H, 4.82.

4.2.11. 1-(4′-Hydroxy-3′,5′,6-trimethoxybiphenyl-3-yl)ethanone (30)

White solid, yield 74%. Mp 144–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.01 (d, 1H, *J* = 7.0 Hz, 5-H), 7.96–7.94 (m, 2H, 2,4-H), 6.74 (s, 2H, 2′,6′-H), 5.58 (s, 1H, 4′-OH), 3.92 (s, 6H, 3′,5′-CH_3O), 3.90 (s, 3H, 6-CH_3O), 2.59 (s, 3H, 3-$COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 196.9 (5-CO), 160.4 (C-6), 146.8 (C-3′/C-5′), 134.5 (C-4′), 131.2 (C-1′), 130.9 (C-2), 130.4 (C-3), 129.5 (C-4), 128.6 (C-1), 110.6 (C-5), 106.7 (C-2′/C-6′), 56.5 (3′,5′-CH_3O), 55.9 (6-CH_3O), 26.4 (3-$COCH_3$); MS (ESI, *m/z*): 303.7 [M+H$^+$], 627.7 [2M+Na$^+$], 301.7 [M−H$^-$]; Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.59; H, 6.08.

4.2.12. 5′-(1-Hydroxyethyl)-2′,3,5-trimethoxybiphenyl-4-ol (31)

White solid, yield 71%. Mp 103–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 6.96 (d, 1H, *J* = 9.3 Hz, 3′-H), 7.32 (m, 2H, 4′,6′-H), 6.76 (s, 2H, 2,6-H), 4.92 (q, 1H, *J* = 6.5 Hz, 5′-*CH*OHCH$_3$), 3.91 (s, 6H, 3,5-CH_3O), 3.82 (s, 3H, 2′-CH_3O), 1.52 (d, 3H, *J* = 6.5 Hz, 5′-CHOH*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 155.8 (C-2′), 146.7 (C-3/C-5), 138.1 (C-4), 134.2 (C-5′), 130.8 (C-1), 129.5 (C-6′), 128.0 (C-4′), 125.3 (C-1′), 111.4 (C-3′), 106.6 (C-2/C-6), 70.0 (5′-*C*HOHCH$_3$), 56.4 (3,5-CH_3O), 55.8 (2′-CH_3O), 25.1 (5′-CHOH*C*H$_3$); MS (ESI, *m/z*): 327.9 [M+Na$^+$], 632.2 [2M+Na$^+$], 303.5 [M−H$^-$]; Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 66.93; H, 6.53.

4.2.13. 3′,5′-Dimethoxybiphenyl-3,4′-diol (32)

White solid, yield 76%. Mp 121–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.29 (m, 1H, 5-H), 7.12 (m, 1H, 2-H), 7.02 (m, 1H, 6-H), 6.79 (s, 2H, 2′,6′-H), 6.78 (m, 1H, 4-H), 3.95 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 156.7 (C-3), 147.3 (C-3′/C-5′), 143.3 (C-1), 134.7 (C-4′), 132.4 (C-1′), 129.9 (C-5), 119.6 (C-6), 114.0 (C-4), 113.8 (C-2), 104.2 (C-2′/C-6′), 56.4 (3′,5′-CH_3O); MS (ESI, *m/z*): 269.4 [M+Na$^+$], 245.1 [M−H$^-$]; Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.16; H, 5.57.

4.2.14. 3,5-Dimethoxy-2′-methylbiphenyl-4-ol (33)

Light yellow oil, yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.24–7.23 (m, 4H, 3′,4′,5′,6′-H), 6.53 (s, 2H, 2,6-H), 3.88 (s, 6H, 3,5-CH_3O), 2.28 (s, 3H, 2′-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 147.0 (C-3/C-5), 142.4 (C-4), 135.8 (C-2′), 134.0 (C-1), 133.5 (C-1′), 130.6 (C-3′), 130.0 (C-4′), 127.5 (C-5′), 126.0 (C-6′), 106.4 (C-2/C-6), 56.6 (3,5-CH_3O), 20.8 (2′-CH_3); MS (ESI, *m/z*): 245.3 [M+H$^+$], 267.3 [M+Na$^+$], 511.4 [2M+Na$^+$]; Anal. Calcd for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60. Found: C, 73.62; H, 6.76.

4.2.15. 4′-Hydroxy-3′,5′-dimethoxybiphenyl-4-carboxylic acid (34)

Light yellow solid, yield 88%. Mp 228–230 °C; ^{1}H NMR (600 MHz, CD_3SOCD_3, TMS): δ 8.05 (d, 2H, *J* = 8.2 Hz, 3,5-H), 7.63 (d, 2H, *J* = 8.2 Hz, 2,6-H), 6.86 (s, 2H, 2′,6′-H), 3.95 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (150 MHz, CD_3SOCD_3, TMS): δ 172.0 (COOH),

148.2 (C-3′/C-5′), 144.9 (C-1), 136.2 (C-4′), 131.7 (C-1′), 129.7 (C-2, C-6), 129.6 (C-4), 126.1 (C-3, C-5), 104.4 (C-2′/C-6′), 56.1 (3′,5′-CH_3O); MS (ESI, *m/z*): 274.9 [M+H$^+$], 296.9 [M+Na$^+$], 273.3 [M−H$^-$]; Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.81; H, 5.05.

4.2.16. *N*-Butyl-4′-hydroxy-3′,5′-dimethoxybiphenyl-4-carboxamide (35)

Light yellow solid, yield 79%. Mp 136–139 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.86 (d, 2H, J = 8.3 Hz, 3,5-H), 7.69 (d, 2H, J = 8.3 Hz, 2,6-H), 6.93 (s, 2H, 2′,6′-H), 3.92 (s, 6H, 3′,5′-CH_3O), 3.40, 1.63, 1.44, 0.99 (NHBu); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 167.5 (CONH), 147.7 (C-3′/C-5′), 144.6 (C-1), 135.4 (C-4′), 133.4 (C-1′), 131.9 (C-4), 127.6 (C-3, C-5), 127.2 (C-2, C-6), 104.4 (C-2′/C-6′), 56.7 (3′,5′-CH_3O), 40.1, 32.1, 20.5, 14.1 (NHBu); MS (ESI, *m/z*): 681.9 [2M+Na$^+$], 328.5 [M−H$^-$]; Anal. Calcd for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04; N, 4.25. Found: C, 69.12; H, 6.95; N, 4.33.

4.2.17. *N*-Benzyl-4′-hydroxy-3′,5′-dimethoxybiphenyl-4-carboxamide (36)

Light yellow oil, yield 86%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.85 (d, 2H, J = 8.5 Hz, 3,5-H), 7.60 (d, 2H, J = 8.5 Hz, 2,6-H), 7.39–7.37 (5H, Ar–H), 6.81 (s, 2H, 2′,6′-H), 4.69 (d, 2H, J = 5.7 Hz, NHCH_2), 3.97 (s, 6H, 3′,5′-CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 162.6 (CONH), 155.7 (C-3′/C-5′), 147.8 (C-1), 138.5 (C-4′), 137.7 (C-1′), 132.9 (C-4), 129.1 (C-3, C-5), 127.8 (C-2, C-6), 104.5 (C-2′/C-6′), 56.8 (3′,5′-CH_3O), 135.4, 128.3, 128.0, 127.2, 46.2 (NHBn); MS (ESI, *m/z*): 362.1 [M−H$^-$]; Anal. Calcd for $C_{22}H_{21}NO_4$: C, 72.71; H, 5.82; N, 3.85. Found: C, 72.64; H, 5.76; N, 3.68.

4.3. Tyrosinase assay

This assay was performed according to the procedure of Mason et al. with slight modifications, using L-tyrosine as a substrate. Forty microliters of mushroom tyrosinase solution (100 units/mL), 40 μL of 0.1 mg/mL L-tyrosine solution in phosphate-buffered saline (PBS) solution (25 mM, pH 6.8), 80 μL of phosphate-buffered saline (PBS) solution (25 mM, pH 6.8), and 40 μL of sample in 20% MeOH solution were added to a 96-well microplate. The assay mixture was incubated at 37 °C for 30 min. Instead of a sample in 20% MeOH solution, a 20% MeOH solution was added to a blank solution. Before and after incubation, the amount of dopachrome produced in the reaction mixture was measured at 492 nm in the microplate reader. The percentage of the inhibition of tyrosinase activity was calculated by the following equation: inhibition (%) = $[(A-B)-(C-D)]/(A-B)\times 100$, where A is absorbance of blank solution after incubation, B is absorbance of blank solution before incubation, C is absorbance of sample solution after incubation, and D is absorbance of sample solution before incubation.

The inhibitory kinetics of the selected compounds **21**, **23**, **34**, and **35** on mushroom tyrosinase for the oxidation of L-tyrosine was determined from Lineweaver–Burk double reciprocal plots. The velocity equation for the competitive inhibition in reciprocal form is: $1/V = K_m/V_{max}(1 + [I]/K_i)1/[S]+1/V_{max}$. The inhibition constants (K_i) of the competitive inhibitors were calculated by the following equation: $K_{mapp} = K_m[1 + ([I]/K_i)]$ where K_{mapp} is the apparent K_m, in the presence of an inhibitor.

Acknowledgment

This work was supported by the National Science Foundation for Post-doctoral Scientists of China (Grant No. 20080440774).

References and notes

1. Chang, T. S. *Int. J. Mol. Sci.* **2009**, *10*, 2440.
2. Kima, Y. J.; Uyamab, H. *Cell. Mol. Life Sci.* **2005**, *62*, 1707.
3. Okombi, S.; Rival, D.; Bonnet, S.; Mariotte, A.-M.; Perrier, E.; Boumendjel, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2252.
4. Karioti, A.; Protopappa, A.; Megoulas, N.; Skaltsa, H. *Bioorg. Med. Chem.* **2007**, *15*, 2708.
5. Khan, M. T. H.; Khan, S. B.; Ather, A. *Bioorg. Med. Chem.* **2006**, *14*, 938.
6. Shimizu, K.; Yasutake, S.; Kondo, R. *Chem. Pharm. Bull.* **2003**, *51*, 318.
7. Khan, K. M.; Mughal, U. R.; Khan, M. T. H.; Ullah, Z.; Peerven, S.; Choudhary, M. I. *Bioorg. Med. Chem.* **2006**, *14*, 6027.
8. Kim, Y. J.; No, J. K.; Lee, J. H.; Chung, H. Y. *Biol. Pharm. Bull.* **2005**, *28*, 323.
9. Nakamura, K.; Yoshida, M.; Uchiwa, H.; Kawa, Y.; Mizoguchi, M. *Pigment Cell Res.* **2003**, *16*, 494.
10. Dai, Y.; Zhou, G. X.; Kurihara, H.; Ye, W. C.; Yao, X. S. *J. Nat. Prod.* **2006**, *69*, 1022.
11. Bao, K.; Zhang, L.; Zhang, W. G.; Cheng, M. S.; Yao, X. S. *Org. Biomol. Chem.* **2009**, *7*, 5084.
12. Nishioka, H.; Nagasawa, M.; Yoshida, K. *Synthesis* **2000**, *2*, 243.
13. Punna, S.; Meunier, S.; Finn, M. G. *Org. Lett.* **2004**, *6*, 2777.