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A New Aspect of View in Synthesizing New Type β-adrenoceptor Blockers with Ancillary Antioxidant Activities

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Abstract—A series of vanilloid-type β-adrenoceptor blockers derived from antioxidant traditional Chinese herbal medicines were synthesized and tested for their antioxidant and adrenoceptor antagonistic activities. They all possessed significant β-adrenoceptor blocking activities under in vitro experiments and radioligand binding assays. In addition, some compounds were further examined in in vivo tests and produced antagonist effects matching that of propranolol and labetalol by measurements of antagonism toward (−)isoproterenol-induced tachycardia and (−)phenylephrine-induced pressor responses in anesthetized rats. Furthermore, all of the compounds had antioxidant effects inherited from their original structures. In conclusion, compound **11** had the most potent β-adrenoceptors blocking activity, **12** and **13** possessed high cardioselectivity, whereas **14**, **15** and **16** possessed additional α-adrenoceptor blocking activity and **15** is the most effective antioxidant of all. The antioxidant activity may be due to their α and β unsaturated side chain at position 1 and *ortho*-substituted methoxy moiety on 4-phenoxyethylamine.

Introduction

The use of β-adrenoceptor blockers is well established in the treatment of various cardiovascular disorders.[1] Their benefits have been demonstrated in many clinical investigations.[2,3] Recently, some β-adrenoceptor blockers have been found to inhibit lipid peroxidation in canine and swine models,[4,5] and it has been suggested that the inhibition of lipid peroxidation may provide additional cardioprotective effects for β-adrenoceptor blockers.[6,7] More recently, the third-generation β-adrenoceptor blockers, which possess ancillary cardiovascular actions other than β-adrenoceptor blockade, or cardioselective β-adrenoceptor blockers, have been shown to improve left ventricular function and decrease the risk of chronic heart failure.[8–10] The mechanism underlying the dramatic cardioprotective effects has been suggested to be reduction in myocardial workload and oxygen consumption.[11–12] Additional benefit is provided by vasodilation resulted from particular α_1-adrenoceptor blockade at peripheral vessels, which decreases preload, afterload and α_1-adrenoceptor mediated cardiac remodeling associated hypertrophy.[13,14]

From the history of developing β-adrenoceptor blockers, it has been generally accepted that the structure of aryloxypropanolamines led to β-adrenoceptor blocking activity[15,16] and the effect was also confirmed in our previous report.[17] Numerous natural products, including dehydrozingerone (**1**), zingerone (**2**), vanillin (**3**), eugenol (**4**), isoeugenol (**5**), and ferulic acid (**6**) (Scheme 1), used in traditional Chinese medicines have been shown to produce novel antioxidant activities.[18,19] All of them contain vanilloid (3-methoxy-4-hydroxy benzene) moiety and the phenolic hydroxyl groups provide the possibility to introduce a 4-ether-linked propanolamine side chain. Additionally, it is also well known that most of the initial β-adrenoceptor blockers contained an isopropyl- or *tert*-butylamine structure,[20] however, the substitution with aiacoxyethylamine was found to produce the α-adrenoceptor blocking activity.[21] In this article, we describe the synthesis of a series of 10 new β-adrenoceptor blockers derived from vanilloid nucleus (Table 1) and discuss the results from the examination

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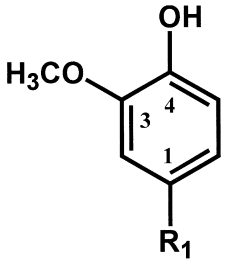
of them under in vivo, in vitro and receptor binding assays.

Results

Chemistry

The synthesis of compounds **7**–**16**, as shown in Scheme 2, represents a typical example of the general synthesis of vanilloid derivatives of **1**–**6**. Propanolamine derivatives were obtained by reacting compounds **1**–**6** with epichlorohydrin, and the obtained epoxide compounds were then reacted with isopropylamine, *tert*-butylamine or guaiacoxyethylamine, respectively, to yield **7**–**16**.[17]



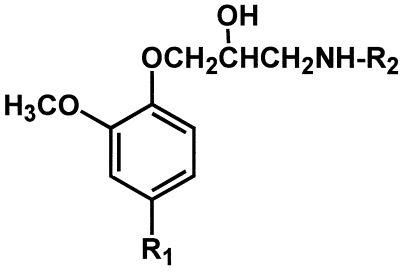
Dehydrozingerone (1): R_1 = CH=CHCOCH$_3$
Zingerone (2): R_1 = $CH_2CH_2COCH_3$
Vanillin (3): R_1 = CHO
Eugenol (4): R_1 = $CH_2CH=CH_2$
Isoeugenol (5): R_1 = CH=$CHCH_3$
Ferulic acid (6): R_1 = CH=CHCOOH

Scheme 1.

Pharmacology

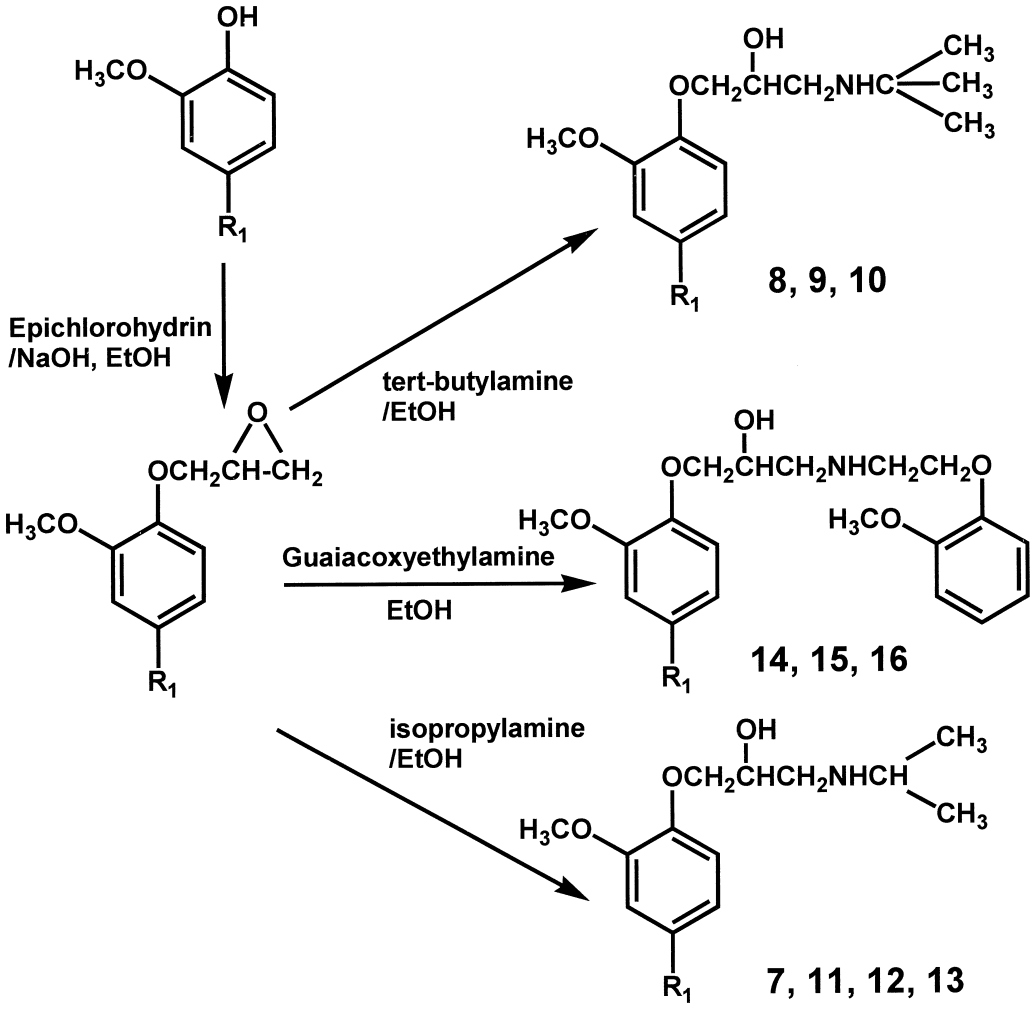
The adrenoceptor blocking activities of compounds **7**–**16** were evaluated by in vitro experiments and receptor binding assays. From the in vitro studies, all of the newly synthesized vanilloid derivatives concentration-dependently inhibited (−)isoproterenol-induced positive inotropic and chronotropic effects of the atria and tracheal relaxant responses in isolated guinea pig tissues. In addition, compounds **14**, **15** and **16** also produced significantly competitive antagonism of the norepinephrine-induced contraction in isolated rat thoracic aorta. The antagonistic potency of compounds **7**–**16** and the reference compounds were evaluated from the measurement of pA_2 values shown in Table 2. Furthermore, all of the compounds were then investigated in receptor binding assays using [^{3}H]CGP12177 as a ligand for β-adrenoceptors, and compounds **14**, **15** and **16** were further tested using [^{3}H]prazosin for α-adrenoceptors. Scatchard analysis[22] of the data was used to estimate the affinity and number of binding sites. The [^{3}H]CGP12177 was bound to β_1- and β_2-adrenoceptors in rat ventricle and lung membranes both in concentration-dependent and saturable manners with K_d values of 0.18 ± 0.04 and 1.25 ± 0.09 nM, respectively, and the B_{max} values were 45.2 ± 2.1 and 296.1 ± 18.4 fmol per mg protein at 25 °C, respectively. Compounds **7**–**16** competitively antagonized [^{3}H]CGP12177 bound to rat ventricle and lung membranes with K_i values shown in Table 3. [^{3}H]prazosin also concentration-dependently bound to α-adrenoceptors in rat brain membranes. The K_d value was 0.25 ± 0.01 nM and the B_{max} value was 71.3 ± 1.7 fmol per mg protein at 25 °C. The inhibitory constant K_i value of labetalol, an α/β-adrenoceptor blocker was 51.9 nM, and those of compounds **14**, **15** and **16** were 38.7, 38.9 and 32.5 nM, respectively.

Table 1. Physicochemical data of newly synthesized β-adrenoceptor blockers



Compounds	R_1	R_2	mp, °C	Recrystn solv	Yield (%)	Formula[a]
7	$CH=CHCOCH_3$	$CH(CH_3)_2$	109–111	CH_2Cl_2	55	$C_{17}H_{25}NO_4$
8	$CH=CHCOCH_3$	$C(CH_3)_3$	96–98	CH_2Cl_2	35	$C_{18}H_{27}NO_4$
9	$CH_2CH_2COCH_3$	$C(CH_3)_3$	75–77	EtOH	30	$C_{17}H_{27}NO_4$
10	CHO	$C(CH_3)_3$	135–137	EtOH	50	$C_{15}H_{23}NO_4$
11	$CH_2CH=CH_2$	$CH(CH_3)_2$	42–44	*n*-Hexane	68	$C_{16}H_{25}NO_3$
12	$CH=CHCH_3$	$CH(CH_3)_2$	120–122	*n*-Hexane	31	$C_{16}H_{25}NO_3$
13	CH=CHCOOEt	$C(CH_3)_3$	149–151	MeOH/Et_2O	45	$C_{19}H_{29}NO_5$
14	$CH_2CH=CH_2$	$CH_2CH_2O(C_6H_4)$-2-OCH_3	46–48	*n*-Hexane	25	$C_{22}H_{29}NO_5$
15	$CH=CHCH_3$	$CH_2CH_2O(C_6H_4)$-2-OCH_3	125–127	*n*-Hexane	52	$C_{22}H_{29}NO_5$
16	CH=CHCOOEt	$CH_2CH_2O(C_6H_4)$-2-OCH_3	167–169	*n*-Hexane	37	$C_{24}H_{31}NO_7$

[a]C, H, N were analyzed: the values are at ±0.4% of the theoretical values.



Scheme 2.

Table 2. pA_2 and β_1/β_2-selectivity values from in vitro experiments

	β_1		β_2	α_1	
	pA_2 value[a]		pA_2 value[a]	pA_2 value[a]	β_1/β_2 ratio[b]
Compounds	Right atrium	Left atrium	Trachea	Aorta	ratio
7	7.57±0.09	7.42±0.08	6.93±0.04	< 5.00	4.4
8	7.68±0.06	7.53±0.02	6.76±0.05	< 5.00	8.3
9	7.50±0.07	7.62±0.09	6.77±0.05	< 5.00	5.4
10	7.67±0.03	7.89±0.11	7.66±0.15	< 5.00	1.0
11	8.23±0.04	8.36±0.13	8.18±0.12	< 5.00	1.1
12	7.63±0.08	7.89±0.12	6.12±0.05	< 5.00	32.4
13	7.62±0.05	7.54±0.07	6.28±0.11	< 5.00	21.9
14	7.88±0.12	7.52±0.05	7.33±0.15	7.05±0.03	3.6
15	7.83±0.12	7.80±0.09	7.76±0.11	7.47±0.45	1.2
16	8.04±0.09	8.03±0.15	7.51±0.06	7.05±0.03	3.4
Propranolol	8.24±0.06	8.07±0.12	8.07±0.12	< 5.00	1.5
Labetalol	7.91±0.09	7.54±0.16	7.54±0.16	6.87±0.08	2.3
Atenolol	7.34±0.03	5.70±0.06	5.81±0.06	< 5.00	34.7

[a]pA_2 values were obtained from the formula pA_2 = [log(DR−1)-log molar concentration antagonist] and calculated from individual Schild plot by regression. Each value was the mean±SEM of six to eight experimental results.
[b]The β_1/β_2-selectivity ratios were obtained from the antilogarithm of the differences between the mean pA_2 values obtained from right atrium and trachea.

Furthermore, compounds **14**, **15** and **16** were investigated under in vivo experiments. The results indicated that in ganglion-blocked anesthetized rats, injection of compounds **14**, **15, 16** and propranolol (0.5 mg/kg, iv) all significantly decreased the (−)isoproterenol-induced tachycardia responses (Fig. 1A). Likewise, in reserpine-pretreated rats, intravenous administration of (−)phenylephrine (1.0 μg/kg) increased the blood pressure 63.1±3.5 mmHg (mean±SEM; $n=8$), which was markedly inhibited by the iv injection of labetalol or compounds **14**, **15** and **16** (1.0 mg/kg, iv) but was not significantly affected by propranolol (1.0 mg/kg) (Fig. 1B).

Table 3. K_i values from receptor binding assays

Compounds	β_1 (Ventricle) K_i[a] (nM)	β_2 (Lung) K_i (nM)	α_1 (Brain) K_i (nM)	β_1/β_2 ratio
7	63.1±2.4	498.5±22.4	ND[b]	7.9
8	93.2±3.1	1248.6±55.3	ND	13.4
9	56.8±1.9	511.1±19.9	ND	9.0
10	8.0±0.2	9.8±0.3	ND	1.1
11	0.2±0.01	0.7±0.02	ND	3.5
12	209.0±0.7	6859.0±231.5	ND	32.2
13	103.0±4.6	2412.0±87.9	ND	23.4
14	9.7±0.5	48.3±0.9	38.7±0.9	5.0
15	43.4±2.0	53.5±2.5	38.9±1.8	1.2
16	3.4±0.1	13.8±0.4	32.5±1.3	4.1
Propranolol	0.2±0.01	0.6±0.02	ND	2.4
Labetalol	4.1±0.2	14.9±0.5	51.9±2.1	3.7
Atenolol	262.8±9.8	8478.6±387.2	ND	32.3

[a]K_i values were calculated from the equation $K_i = IC_{50}/(1+[^3H]ligand/K_d)$. K_d and [^{3}H]ligand denote the apparent dissociation constant and the free concentration of the radiolabel, respectively.
[b]ND, not determined.

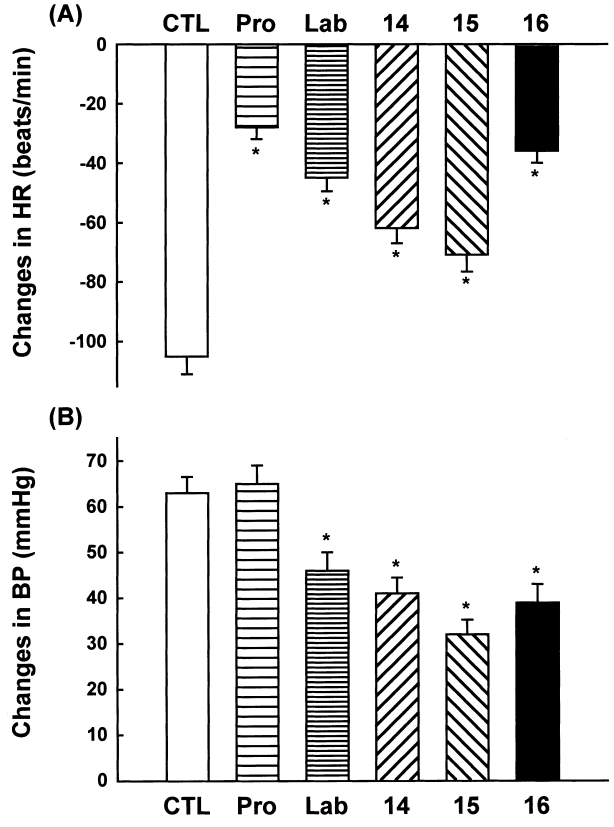


Figure 1. Effects of intravenous injection of (−)isoproterenol (0.5 μg/kg, panel **A**) in causing a tachycardia and (−)phenylephrine (10 μg/kg, panel **B**) in causing a pressor responses before □, CTL) and after (▤, propranolol; ▤ labetalol; ▨ **14**; ▧ **15**; ■ **16**, 0.5 mg/kg for β-adrenoceptor blockade and 1.0 mg/kg for α-adrenoceptor blockade) in ganglion-blocked anesthetized rats. Vertical bars, SEM changes from the baseline value, which was 269±18 beats/min for heart rate and 108±12 mmHg for blood pressure. Each value represents the average of six to eight rats. *$p<0.05$ (paired Student's t-test).

In the antioxidant experiments, in order to eliminate the possibility of test compounds interfering with the assay, the test agents added directly to malondialdehyde (MDA) standards before the TBA agents were added. The results indicated that the test compounds nearly had no direct effects on the TBARS assay (data not shown). The abilities of test compounds and other reference agents on inhibiting lipid peroxidation in rat brain homogenates are compared in Table 4. Furthermore, compounds **14**, **15** and **16** were further investigated the scavenging activity of hydroxyl free radicals by EPR experiments. The formation of the spin adducts DMPO–OH, after the addition of DHF/Fe^{2+}–ADP to DMPO (90 mM in 0.9% saline), was evidenced by the appearance of the characteristic 1:2:2:1 EPR hyperfine splitting pattern (Fig. 2). Compounds **14**, **15** and **16** (10 μM) all significantly inhibited the height of DMPO–OH signal.

Discussion

In the present studies, compounds **7**–**16** were evaluated for their adrenoceptor blocking activities in vitro. Pharmacological and radioligand binding techniques were used to investigate the blocking activities at α- and β-adrenoceptors. In isolated guinea pig tissues, compounds **7**–**16** antagonized the (−)isoproterenol-induced positive inotropic and chronotropic responses of the atria and relaxant effects of the trachea, indicating that these newly synthesized compounds all possessed β-adrenoceptor blocking activities. The dramatic blocking activities of compounds **7**–**16** may be contributed to the basic propanolamine configuration. Most compounds

Table 4. Fifty concentration (IC_{50}) required inhibiting lipid peroxidation initiated by Fe^{2+}-ascorbic acid in rat brain homogenates[a]

Compounds	IC_{50} (μM)
1	35.7±1.5*
2	28.2±0.8*
3	6.9±0.3**
4	12.8±0.4**
5	1.5±0.1**
6	6.3±0.3**
7	83.4±4.0*
8	31.1±1.1*
9	103.0±4.9
10	15.7±0.9**
11	67.4±3.5*
12	35.8±1.4*
13	31.8±1.2*
14	55.1±2.7*
15	0.7±0.1**
16	7.1±0.4**
α-Tocopherol	1.8±0.1**
Trolox	3.0±0.2**
Ascorbic acid	4.1±0.2**

[a]Each value represents the mean. SEM of six to eight results (* $p<0.05$, ** $p<0.01$).

were less potent than propranolol, but more potent than atenolol in comparison with the estimated pA_2 values at β-adrenoceptors (Table 2). Particularly, compound **11** possesses the highest potency of all and compounds **12** and **13** are cardioselective β-adrenoceptor blockers with β_1/β_2 ratios of 32.4 and 21.9, respectively. These new findings encouraged us to modify vanilloid compounds **4**, **5** and **6** to obtain compounds **11**, **12** and **13** (Scheme 1), respectively, and to synthesize third generation β-adrenoceptor blockers **14**, **15** and **16** by substituting the epoxide compounds with guaiacoxyethylamine. Further examinations of α-adrenoceptor blocking activities were performed in rat aorta, precontracted with norepinephrine. The resulting pA_2 values indicated that **14**, **15** and **16** possess significant α-adrenoceptor blocking activities, however, the other compounds had only minor antagonistic actions and all their values were less than 5. These results further confirmed the previous report of Augstein et al.,[21] which suggested that a high degree molecule, requires 2-(2-methoxyphenoxy)ethylamine for optimal α-adrenoceptor blocking activity. In the α-adrenoceptor blocking experiments, the pA_2 values of **14**, **15** and **16** were greater than those of labetalol, a α/β-adrenoceptor blocker. However, **15** and **16** did not display the cardioselectivity that found in **12** and **13**. The β_1/β_2 ratios of **15** and **16** are 1.2 and 3.4, respectively, denoting the lower cardioselectivity than that of atenolol, which is a highly selective β_1-adrenoceptor blocker with the selective ratio of 34.7.

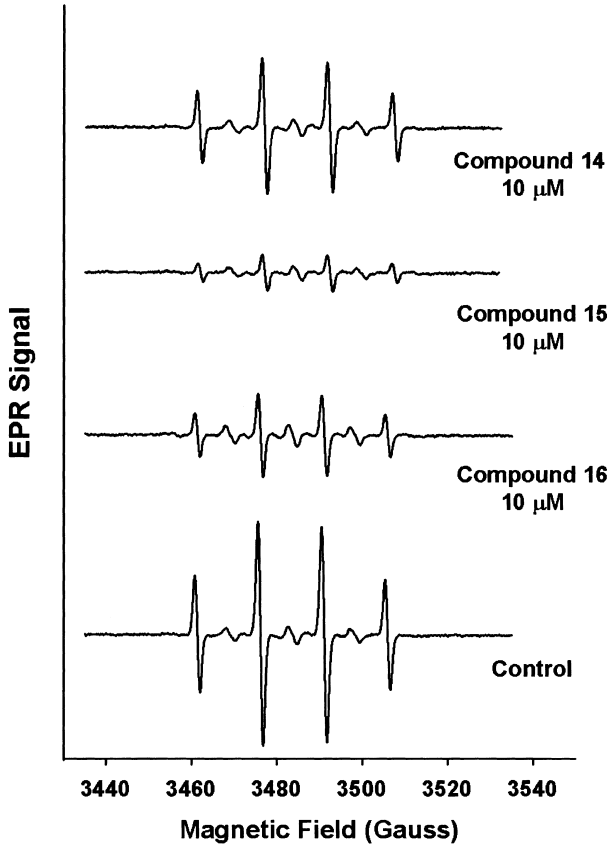


Figure 2. EPR spectra of DMPO–OH adduct formation in the absence (control) or presence of test compounds. The panel shows the representative tracings. The EPR signals were initiated by adding DHF/Fe^{2+}–ADP into 0.9% saline solutions containing 90 mM DMPO, with or without test compounds. These tracings were recorded at 10 min after the addition of DHF/Fe^{2+}–ADP.

The characterizations of the adrenoceptor blocking activities of **7**–**16** were determined from the receptor binding assays and compared with those of propranolol, labetalol and atenolol (Table 3). In the β-adrenoceptor binding study, all of the compounds showed various affinities at both β_1- and β_2-adrenoceptors. Above all, the binding affinity of compound **11** was similar to that of propranolol. All of the compounds showed the higher binding affinity than that of atenolol. In addition, **14**, **15** and **16** were further tested for their binding affinities on α-adrenoceptors and the obtained K_i values indicated that these agents displayed the higher binding affinity than that of labetalol. These results were then confirmed by in vivo experiments (Fig. 1). Compounds **14**, **15** and **16**, as well as propranolol and labetalol, all blocked (−)isoproterenol-induced tachycardia effects and indicated that they had β-adrenoceptor blocking activities. Additionally, **14**, **15**, **16** and labetalol (a dual α- and β-adrenoceptor antagonist) showed significant inhibition of pressor responses to (−)phenylephrine, suggesting that these agents also possess α-adrenoceptor blocking activities. However, propranolol did not show any blockade on α-adrenoceptors.

The brain tissue, rich in lipids, was a commonly used model[23] for the study of lipid peroxidation and therefore in the present study it was chosen to evaluate the inhibition of lipid peroxidation of compounds **1**–**16** (Table 4). MDA formation assayed by the TBA method was used as an index of membrane lipid peroxidation because of its sensitivity and simplicity. In this test, all of the compounds, including the vanilloid type natural products **1**–**6** and the newly synthesized β-adrenoceptor blockers **7**–**16**, showed remarkable inhibitory actions on Fe^{2+}-induced lipid peroxidation. The antioxidant effect of these compounds may be due to their α and β unsaturated side chain at the 1-position and *ortho*-substituted[24] methoxy group at 3-position of 4-phenoxyethylamine derived from vanilloid base (**7**–**16**). To ensure the scavenging activity, compounds **14**, **15** and **16** were further investigated by using EPR spectroscopy to determine whether or not these compounds inhibit OH radical generation in a Fenton-type reaction. Because EPR signal height is proportional to the amount of DMPO-OH adduct produced, the decrease of signal in the presence of compounds **14**, **15** and **16** indicates that these compounds effectively suppress the formation of DMPO-OH (Fig. 2). In addition, there are significantly positive correlations between the newly synthesized vanilloid compounds and their original structures, indicating that the antioxidant activities are inherited from their basic vanilloid moiety. For example, the rank of antioxidant potency was **5** > **6** > **4**, whereas the potency of compounds **15**, **16** and **14**, which were derived from **5**, **6** and **4**, respectively, was also **15** ≥ **16** ≥ **14**. The measured IC_{50} values of **14**, **15** and **16** were less than those of **11**, **12** and **13,** and indicated that the guaiacoxyethylamine series is more potent than the *tert*-butylamine series in their antioxidant activities.

In summary, all of the new vanilloid derivatives described in this article have potent β-adrenoceptor blocking activities, which match those of reference compounds in in vitro and receptor binding studies. Compound **12** and **13** have the high cardioselectivity equal to that of atenolol. Compounds **14**, **15** and **16**, which are substituted with guaiacoxyethylamine, all possess α-adrenoceptor blocking activities. Additionally, we have demonstrated that all of the compounds possessed potent antioxidant activities inherited from their original structures. These findings suggested that it is valuable to modify the natural products from traditional Chinese herbal medicines to synthesize new β-adrenoceptor blockers with the vasodilatoty α-adrenoceptor blocking activity by substituting the 4-hydroxyl group.

Experimental

General information

All melting points were measured with a Yanaco MP-J3 micromelting point apparatus and are uncorrected. Infrared spectra were recorded through a KBr disk (ν in cm^{-1}) on a Hitachi 270-30 IR spectrophotometer. ^1H nuclear magnetic resonance spectra were recorded on a Varian Gemini 400 FT-NMR spectrophotometer, using $CDCl_3$ as solvent and TMS as internal standard (chemical shift in δ, ppm). Mass spectra were recorded with a JEOL-D100 GC-mass spectrophotometer. Elemental analyses were performed on a Heraeus CHN-O-Rapid analyzer and were within 0.4% of the theoretical values unless otherwise indicated.

Vanillin, eugenol, isoeugenol, ferulic acid and guaiacol were purchased from Tokyo Chemical Industry Co. (TCI). Epichlorohydrin and $CDCl_3$ were obtained from Janssen. Guaiacoxyethylamine was synthesized via Mannich reactions[19] from guaiacol. All the other reagents used in this study were EP-grade products of E. Merck. Animals were obtained from the Experimental Animal Center, Cheng-Kung National University Medical College, Tainan, Taiwan.

Synthesis

1-{[4-(1-Buten-3-one)-2-methoxy]phenoxy}-3-(isopropylamino)-propanol (7). ^1H NMR ($CDCl_3$) δ 1.08–1.11 (d, 6H, CH_3×2), 2.37 (s, 3H, $COCH_3$), 2.57 (br s, 1H, exchangeable O*H*), 2.80–2.86 (s, 3H, -CH_2NHC*H*-), 3.89 (s, 3H, Ar–OCH_3), 4.07–4.20 (m, 3H, Ar–OCH_2-C*H*(OH)), 5.71 (s, 1H, -N*H*-), 6.56–6.64 (d, 1H, Ar–CH=C*H*), 6.89–7.12 (m, 3H, Ar–*H*), 7.42–7.50 (d, 1H, Ar–C*H*CH); IR (KBr) 3300, 1680, 1600 cm^{-1}; MS *m*/*z* 308 $(M+H)^+$.

1-{[4-(1-Buten-3-one)-2-methoxy]phenoxy}-3-(*tert*-butylamino)propanol (8). ^1H NMR ($CDCl_3$) δ 1.03 (s, 9H, CH_3×3), 2.31 (s, 3H, $COCH_3$), 2.45 (br s, 1H, exchangeable O*H*), 2.54–2.69 (s, 3H, -CH_2NHC-), 3.83 (s, 3H, Ar–OCH_3), 3.90–4.05 (m, 3H, Ar–OCH_2-C*H*(OH)), 5.78 (s, 1H, -N*H*-), 6.70–6.80 (d, 1H, Ar–CH=C*H*), 7.01–7.45 (m, 3H, Ar–*H*), 7.52–7.60 (d, 1H, Ar–C*H*=CH); IR (KBr) 3300, 1690, 1600 cm^{-1}; MS *m*/*z* 322 $(M+H)^+$.

1-{[4-(3-Butanone)-2-methoxy]phenoxy}-3-(isopropylamino)propanol (9). ^1H NMR ($CDCl_3$) δ 1.10–1.13 (s, 6H, CH_3×2), 2.15 (s, 3H, $COCH_3$), 2.51 (br s, 1H, exchangeable O*H*), 2.74–2.79 (s, 4H, Ar–CH_2CH_2), 2.80–2.88 (s, 3H, CH_2NHC*H*-), 3.85 (s, 3H, Ar–OCH_3), 4.05–4.19 (m, 3H, Ar–OCH_2CH(OH)), 5.81 (s, 1H, -N*H*-), 6.68–6.86 (m, 3H, Ar–*H*); IR (KBr) 3300, 1710, 1590 cm^{-1}; MS *m*/*z* 310 $(M+H)^+$.

1-[(4-Aldehyde-2-methoxy)phenoxy]-3-(tert-butylamino)-propanol (10). ^1H NMR ($CDCl_3$) δ 1.25 (s, 9H, CH_3 × 3), 2.45 (br s, 1H, exchangeable O*H*), 3.17–3.39 (m, 2H, -CH_2NHC-), 3.90 (s, 3H, Ar–OCH_3), 4.16–4.24 (m, 3H, Ar–OCH_2CH(OH)), 5.71 (s, 1H, -N*H*-), 6.99–7.40 (m, 3H, Ar–*H*), 9.85 (s, 1H, C*H*O); IR (KBr) 3250, 2900, 1670 cm^{-1}; MS *m*/*z* 282 $(M+H)^+$.

1-[(4-Allyl-2-methoxy)phenoxy]-3-(isopropylamino)-propanol (11). ^1H NMR ($CDCl_3$) δ 1.06–1.09 (d, 6H, CH_3 × 2), 1.95 (d, 1H, exchangeable O*H*), 2.69–2.89 (m, 3H, -CH_2NHC*H*-), 3.32–3.35 (dd, 2H, Ar–CH_2), 3.85 (s, 3H, Ar–OCH_3), 3.91–4.07 (m, 3H, Ar–OCH_2CH(OH)), 5.04–5.10 (m, 2H, -CH=CH_2), 5.86–6.06 (m, 1H, -C*H*=CH_2), 6.69–6.88 (m, 3H, Ar–*H*); IR (KBr) 3300, 3150, 1600 cm^{-1}; MS *m*/*z* 280 $(M+H)^+$.

1-[(4-Propenyl-2-methoxy)phenoxy]-3-(isopropylamino)-propanol (12). ^1H NMR ($CDCl_3$) δ 1.18–1.22 (d, 6H, CH_3×2), 1.85–1.89 (d, 3H, Ar–CH=CHCH_3), 2.13 (br s, 1H, exchangeable O*H*), 2.83–3.08 (m, -CH_2NHC*H*-), 3.86 (s, 3H, OCH_3), 4.03–4.06 (m, 2H, Ar–OCH_2), 4.14–4.21 (m, 1H, -C*H*OH), 6.02–6.19 (m, 1H, Ar–CH=C*H*CH3), 6.29–6.38 (d, 1H, Ar–C*H*=CHCH$_3$), 6.84–6.88 (t, 3H, Ar–*H*); IR (KBr) 3600, 3300, 1600 cm^{-1}; MS *m*/*z* 280 $(M+H)^+$.

1-[(4-Propenoic acid ethyl easter-2-methoxy)phenoxy]-3-(*tert*-butyl amino)propanol (13). ^1H NMR ($CDCl_3$) δ 1.12 (s, 9H, CH_3×3), 1.34 (q, 3H, -$COOCH_2CH_3$), 2.60–2.91 (m, 2H, -CH_2NH-), 3.88 (s, 3H, ArOCH_3), 3.90-4.l0 (m, 2H, ArOCH_2-), 4.21–4.32 (m, 2H, -$COOCH_2CH_3$), 4.60–4.62 (m, 1H, -C*H*OH), 6.27–6.35 (d, 1H, Ar–CH=C*H*-), 6.88–7.10 (m, 3H, Ar–*H*), 7.57–7.67 (d, 1H, Ar–C*H*=CH-), IR (KBr) 3300, 1700 cm^{-1}, MS *m*/*z* 352 $(M+H)^+$.

1-[(4-Allyl-2-methoxy)phenoxy]-3-[(2-methoxyphenoxyethyl)amino]-propanol (14). ^1H NMR ($CDCl_3$) δ 2.41–2.79 (br s, 1H, -N*H*-), 2.93–3.16 (m, 4H, -CH_2NHCH_2-), 3.31–3.34 (t, 2H, Ar–CH_2), 3.80–3.83 (s, 6H, 2×Ar–OCH_3), 4.00–4.03 (m, 4H, 2×Ar–OCH_2-), 4.14–4.16 (m, 1H, -C*H*(OH)), 5.03–5.12 (m, 2H, -CH=CH_2), 5.88–6.01 (m, 1H, -C*H*=CH_2), 6.67–6.92 (m, 7H, Ar–*H*); IR (KBr) 3300, 3150, 1600 cm^{-1}; MS *m*/*z* 388 $(M+H)^+$.

1-[(4-Propenyl-2-methoxy)phenoxy]-3-[(2-methoxyphenoxyethyl)amino]-propanol (15). ^1H NMR ($CDCl_3$) δ 1.85–1.88 (d, 3H, Ar–CH=CH-CH_3), 2.15–2.22 (br s, 1H, -N*H*-), 2.83–3.10 (m, 4H, -CH_2NHCH_2-), 3.84–3.85 (s, 6H, 2×Ar–OCH_3), 4.03–4.04 (m, 2×4H, Ar–OCH_2-),

4.12–4.15 (m, 1H, -C*H*(OH)), 6.05–6.17 (m, 1H, Ar–CH=C*H*-CH_3), 6.30–6.36 (m, 1H, Ar–C*H*=CH-CH_3), 6.84–6.92 (m, 6H, Ar–*H*); IR (KBr) 3600, 3300, 1600 cm^{-1}; MS *m*/*z*: 388 $(M+H)^+$.

1-[(4-Propenoic acid ethyl easter-2-methoxy)phenoxy]-3-[(2-methoxyphenoxyethyl)amino]-propanol (16). ^1H NMR ($CDCl_3$) δ 1.34 (t, 3H, -$COOCH_2CH_3$), 2.93–3.18 (m, 4H, -CH_2NHCH_2-), 3.77–3.84 (s, 6H, 2×$ArOCH_3$), 4.06–4.14 (m, 4H, 2×$ArOCH_2$-), 4.17–4.20 (m, 3H, -$COOCH_2CH_3$), 4.24–4.31 (m, 1H, C*H*(OH)), 6.26–6.34 (d, 1H, Ar–CH=C*H*), 6.86–7.04 (m, 7H, Ar–*H*), 7.57–7.65 (d, 1H, Ar–C*H*=CH-), IR (KBr) 3300, 1700 cm^{-1}, MS *m*/*z* 446 $(M+H)^+$.

Pharmacology

Measurements of isolated atria and trachea of the guinea pigs. Experiments were performed following the method described in a previous report from our laboratory.[25] Right atria preparations, which retained a spontaneous rhythm, were used for assessment of chronotropic effects, while inotropic effects were examined with left atrial preparations. The experiments were carried out at 37 °C containing Kreb's solution of the following composition (mM) NaCl 113, KCl 4.8, $CaC1_2$ 2.2, KH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, dextrose 11.0, bubbled with 95% O_2 and 5% CO_2. As β-agonist, (−)isoproterenol was administrated to the preparations in cumulative fashion after an equilibration period of 90 min in Kreb's solution and the concentration-response curve was established. The atria were then allowed a 30–60 min washout period to restablize, after which time various concentrations of the test compounds were incubated with the atrium 30 min before (−)isoproterenol. The activity of the test compound was expressed as pA_2 value, which was calculated from the parallel shifts of the cumulative concentration-response curves of (−)isoproterenol.[26]

Measurement of isolated aorta of the rats. The preparation of Wistar thoracic aorta rings was similar to that originally described by Honda et al.[27] In brief, the rat thoracic aorta preparations were set up in 10 mL organ baths containing Kreb's solution at 37 °C under a resting tension of 1.5 g. Cumulative concentration-response curves for norepinephrine were constructed by increasing the concentration of the agonist bath approximately 3-fold. Test compounds were added to the bath medium after a control concentration-response curve to norepinephrine had been obtained. The tissues were exposed to the test compounds for 30 min before rechallenging with norepinephrine. Contractions were expressed as a percentage of the maximum contraction obtained by the first challenge to the tissues.

Receptor binding assay procedures. Wistar rats (either sex, 250–300 g) were sacrificed and their ventricle and lung were removed quickly and chilled in ice-cold TE buffers (10 mM Tris–HCl, 1mM EDTA, 0.1 mM ascorbic acid, pH 7.4). The membrane preparations for β_1- and β_2-adrenoceptor binding assays were prepared according to the methods of Wu et al.[28] The ventricle or lung preparations were homogenized with a polytron (IKA-Labortechnik, Model T25-S1, Germany) in 20 volumes of buffer containing 10 mM Tris–HCl, 1 mM EDTA and 0.1 mM ascorbic acid (pH 7.4) under ice-cold conditions. The homogenate was centrifuged further at 1000 *g* for 10 min at 4 °C and then the supernatant was centrifuged again at 10,000 *g* for 12 min. The second supernatant was further centrifuged at 30,000 *g* for 15 min at 4 °C and the final pellet was resuspended in an assay buffer (75 mM Tris–HCl, 25 mM $MgCl_2$, pH 7.4) and stored at −80 °C. The membrane preparation for α_1-adrenoceptor binding assay was prepared according to the method of Miach et al.[29] The cortex and the whole brain minus the cerebellum were homogenized in 20 volumes of ice-cold buffer (0.25 mM sucrose, 1 mM $MgCl_2$, 5 mM Tris–HCl, 0.05% ascorbic acid, pH 7.4). The homogenate was centrifuged at 900 *g* for 10 min at 4 °C and then the supernatant was centrifuged at 12,000 *g* for 20 min at 4 °C. The resulting pellet was resuspended in the incubation buffer (mM: $MgCl_2$ 12.5, Tris–HCl 62.5, pH 7.5) and stored at −80 °C. Protein concentration was determined using the method of Lowry et al.,[30] using bovine serum albumin as standards.

Adrenoceptor binding assay, including [^3H]CGP-12177 binding to rat ventricular and lung membranes and [^3H]prazosin to rat brain membrane, were determined by the methods of Huang et al.[25] [^3H]CGP-12177 and ventricular or lung membranes (200–300 μg) were incubated for 60 min at 25 °C in 75 mM Tris–HCl buffer composed of $MgCl_2$ 25 mM, with or without the addition of 10 μM propranolol, to make a final volume of 250 μL. [^3H]prazosin and brain membrane (250–300 μg) were incubated for 60 min at 37 °C with or without 10 μM phentolamine, in 50 mM Tris–HCl buffer including $MgCl_2$ 10 mM, to make a final volume of 500 μL.

After incubation, the mixture was filtered rapidly through Whatman GF/C glass fiber filters supported on a 12-port filter manifold (Millipore). The filters were immediately washed three times with 5 mL of each of the ice-cold buffer (75 mM Tris–HCl and 25 mM $MgCl_2$ buffer for β-adrenoceptor binding and 50 mM Tris–HCl and 10 mM $MgCl_2$ buffer for α-adrenoceptor binding), and dried in an oven at 80 °C for 2 h. The radioactivity was counted in 4 mL of Triton-toluene based scintillation fluid with about 45% efficiency in a Beckman LS6500 scintillation system (Fullerton, CA, USA). Specific binding was defined as the excess over blank in the presence of 10 μM propranolol for β-adrenoceptors, 10 μM phentolamine for α-adrenoceptors.

Measurement of lipid peroxidation in rat brain homogenates. The rat brain homogenate was made in 0.9% saline containing 10 mg tissue/mL. The rates of membrane lipid peroxidation were measured by the formation of TBARS. Rat brain homogenates (1 mL) were incubated at 37 °C for 5 min with 10 μL of test compound or vehicle. Lipid peroxidation was initiated by the addition of 0.1 mL of 0.25 mM $FeCl_2$ and 1 mM ascorbic acid.[23] After 30 min of incubation, the reaction was arrested by adding 0.1 mL of 0.2% BHT. Thiobarbituric

acid reagent was then added and the mixture was heated for 30 min in a boiling water bath. The TBARS was extracted with *n*-butanol and measured at 532 nm. The amount of TBARS was quantified using the linear regression obtained from malondialdehyde standards.

Spin-trapping experiments. The procedure for spin-trapping experiments was described previously by Yue et al.[24] (1989). Briefly, oxygen-derived free radicals were generated from auto-oxidation of DHF (830 μM) in the presence of $FeCl_3$ (25 μM) chelated by ADP (250 μM). DMPO (90 mM) dissolved in 0.9% saline was exposed to the free radical generation system in the presence or absence of test compounds. The formation of the radical spin adduct, DMPO–OH, was monitored using a Bruker EMX-10 EPR spectrometer interfaced to an IBM PC/ATX computer.

Measurement of β- and α-adrenergic responses. Rats were pretreated with mecamylamine (5 mg/kg, iv), a ganglion-blocking agent, to ensure uniform initial heart rate. (−)Isoproterenol (0.5 mg/kg) was administered via a femoral vein and the resultant tachycardia recorded as the control. A single dose of test compound was then administered intravenously. After 10 min, a further injection of (−)isoproterenol was given. Additionally, rats were pretreated with reserpine (5 mg/kg, ip) 24 hr prior to the injection of (−)phenylephrine (10 μg/kg, iv), followed 15 min later by the intravenous injection of a single dose of test compound. After 10 min, a further injection of (−)phenylephrine was given.

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References and Notes

1. Frishman, W. H. *Med. Clin. North Am.* **1988**, *72*, 37.
2. Sponer, G.; Bartsch, W.; Strein, K.; Mulle-Beckmann, B.; Bohm, E. *J. Cardiovasc. Pharmacol.* **1987**, *9*, 317.
3. Ruffolo, R. R.; Boyle, D.; Venuit, R. P.; Lukas, M. *Drug of Today* **1991**, *27*, 465.
4. Mak, I. T.; Arroyo, C. M.; Weglicki, W. B. *Circ. Res.* **1989**, *65*, 1151.
5. Yue, T. L.; Cheng, H. Y.; Lysko, P. G.; McKenna, P. J.; Feuerstein, R.; Gu, J. L.; Lysko, K. A.; Davis, L. L.; Feuerstein, G. Z. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 92.
6. Weglicki, W. B.; Mak, I. T.; Simic, M. G. *J. Mol. Cell. Cardiol.* **1990**, *22*, 1190.
7. Feuerstein, G. Z.; Yue, T. L.; Ma, X. L.; Ruffolo, R. R. *Prog. Cardiovasc. Dis.* **1998**, *41* (Suppl. 1), 17.
8. Heesch, C. M.; Marcoux, L.; Hatfield, B.; Eichhorn, E. J. *Am. J. Cardiol* **1995**, *75*, 360.
9. Packer, M.; Bristow, M. R.; Cohn, J. N.; Colucci, W. S.; Fowler, M. B.; Gilbert, E. M.; Shusterman, N. H. *N. Engl. J. Med.* **1996**, *334*, 1349.
10. Packer, M. *Prog. Cardiovasc. Dis.* **1998**, *41* (Suppl. 1), 39.
11. Bril, A.; Slivjak, M.; DiMartino, M. J.; Feuerstein, G. Z.; Linee, P.; Poyser, R. H.; Ruffolo, R. R.; Smith, E. F. *Cardiovasc. Res.* **1992**, *26*, 518.
12. Feuerstein, G. Z. *J. Cardiovasc. Pharmacol.* **1992**, *19* (Suppl. 1), S138.
13. Bristow, M. R. *Am. J. Cardiol.* **1993**, *71*, 12 C.
14. Hamburger, S. A.; Barone, F. C.; Feuerstein, G. Z.; Ruffolo, R. R. *Pharmacol.* **1991**, *43*, 113.
15. Wu, E. S.; Cole, T. E.; Davidson, T. A.; Blosser, J. C.; Borrelli, A. R.; Kinsolving, C. R.; Milgate, T. E.; Parker, R. B. *J. Med. Chem.* **1987**, *30*, 788.
16. Hoffman, B. B.; Lefkowitz, R. J. In *The Pharmacological basis of Therapeutics*, 8th ed; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds. New York: Pergamon; 1991; pp 221–243.
17. Chen, I. J.; Yeh, J. L.; Liou, S. J.; Shen, A. Y. *J. Med. Chem.* **1994**, *37*, 938.
18. Rajakumar, D. A.; Rao, M. N. A. *Biochem. Pharmacol.* **1993**, *46*, 2067.
19. Uchida, M.; Nakajin, S.; Toyoshima, S.; Shinoda, M. *Biol. Pharm. Bull.* **1996**, *19*, 623.
20. Blanc, M.; Tamir, A.; Aubriot, S.; Michel, M. C.; Bouzoubaa, M.; Leclerc, G.; Demenge, P. *J. Med. Chem.* **1998**, *41*, 1613.
21. Augstein, J.; Austin, W. C.; Boscott, R. J.; Green, S. M.; Worthing, C. R. *J. Med. Chem.* **1965**, *8*, 356.
22. Scatchard, G. *Ann. N. Y. Acad. Sci.* **1949**, *51*, 660.
23. Braughler, J. M.; Pregenzer, J. F.; Chase, R. L.; Duncan, L. A.; Jacobsen, E. J.; Mccall, J. M. *J. Biol. Chem.* **1987**, *262*, 10438.
24. Yue, T. L.; Cheng, H. Y.; Lysko, P. G.; Mckenna, P. J.; Feuerstein, R.; Gu, J. L.; Lysko, K. A.; Davis, L. L.; Feruerstein, G. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 92.
25. Huang, Y. C.; Yeh, J. L.; Wu, B. N.; Lo, Y. C.; Liang, J. C.; Lin, Y. T.; Sheu, S. H.; Chen, I. J. *Drug Dev. Res.* **1999**, *47*, 77.
26. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
27. Honda, K.; Takenaka, T.; Miyata-osawa, A.; Teral, M. *J. Pharmacol. Exp. Ther.* **1986**, *236*, 776.
28. Wu, B. N.; Huang, Y. C.; Wu, H. M.; Hong, S. J.; Chiang, L. C.; Chen, I. J. *J. Cardiovasc. Pharmacol.* **1998**, *31*, 750.
29. Miach, P. J.; Dausse, J. P.; Cardot, A.; Meyer, P. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *312*, 23.
30. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.