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Synthesis, β-adrenergic blocking activity and β-receptor binding affinities of 1-substituted-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol oxalates

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Abstract

The compounds 1-isopropylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol oxalate (**5**) and 1-*tert*-butylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol oxalate (**6**) were synthesized from thymol (**1**), a naturally occurring agent in *Thymus vulgaris* L. Pharmacological evaluation of **5** and **6** were carried out using mouse ECG and isolated rat uterus models. Pretreatment of **5** (100 μg/kg, i.v.) and **6** (50 μg/kg, i.v.) antagonized isoprenaline (2 μg/kg, i.v.) induced tachycardia, similar to that of atenolol (CAS 29122-68-7, 20 μg/kg, i.v.) pretreatment in mouse ECG experiments as measured by R-R interval. Pretreatment of **5** and **6** blocked isoprenaline and adrenaline induced relaxation of isolated rat uterus (unprimed). Also the compounds **5** and **6** were subjected to in vitro β_1- and β_2-adrenergic receptor binding assay using turkey erythrocyte membrane (β_1) and lung homogenate of rats (β_2). Both **5** and **6** showed β-adrenergic receptor affinity comparable with that of propranolol (propranolol hydrochloride, CAS 318-98-9) with out selectivity to any one β-adrenergic receptor. These results suggest that both the compounds possess non-selective β-adrenergic blocking activity, with the *tert*-butyl derivative **6** being more active than the isopropyl derivative **5**.

Keywords: β-Adrenergic blocking activity; β-Adrenergic receptor binding affinity; Aryloxypropanolamines; Atenolol; Propranolol; Thymol

1. Introduction

Cardiovascular diseases such as angina pectoris, myocardial infarction, cardiac arrhythmias and congestive heart failure are the major cause of deaths worldwide, even surpassing deaths due to cancer. Of the various cardiovascular diseases, hypertension is of particular concern, since elevated blood pressure for prolonged periods of time increase the risks of developing angina pectoris, myocardial infarction, cerebral hemorrhage, stroke, kidney failure [1,2]. Implication of hypertension in the pathogenesis of coronary heart disease and stroke had made the health professionals to study and treat hypertension. Worldwide many drug classes including diuretics, β-adrenergic blocking agents, calcium antagonist, angiotensin converting enzyme inhibitors, angiotensin II antagonists and α-adrenergic blockers are being used for the control of blood pressure [3,4].

β-Adrenergic blocking agents are safe, cheap and effective for use as monotherapy or in combination with diuretics, calcium antagonists and α-adrenergic blockers [4]. Also β-adrenergic blocking agents are proven effective in reducing the symptoms of angina pectoris and in reducing morbidity and mortality after myocardial infarction [5]. Moreover, recent clinical trial results support the beneficiary action of β-adrenergic blocking agents in patients with mild to moderate congestive heart failure [6,7]. In addition to their usefulness in

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various cardiovascular diseases they are also used in the treatment of various non-cardiovascular disorders such as migraine, hyperthyroidism, anxiety, tremor and glaucoma [5]. The usefulness of β-adrenergic blocking agents in various cardiovascular and non-cardiovascular disorders prompted the present investigators to undertaken a research programme to search compounds with β-adrenergic blocking activity.

Recently many β-adrenergic blocking agents were reported which were synthesized from natural products [8–15], such as capsaicin [10], vanillin [12], gingerone [14] and eugenol [15]. Keeping this in view, we had planned to use other naturally occurring compounds as starting materials to synthesize β-adrenergic blocking agents. The aim of this study was to develop β-adrenergic blocking agents starting from thymol (**1**), which is a natural product obtained from *Thymus vulgaris* L., *Monarda punctata* L. or *Carum copticum* Benth. & Hook. f., and is mainly used for its antiseptic action [16]. Herein we report the synthesis, preliminary pharmacological screening and in vitro β_1-, β_2-adrenergic receptor binding assay of the aryloxypropanolamine derivatives of thymol.

2. Chemistry

Thymol (**1**) was condensed with epichlorohydrin in the presence of potassium carbonate to give the oxirane (**2**). ^{1}H NMR spectrum showed a one proton double doublet (dd) at δ 2.75 and a one proton triplet at δ 2.87 for $-CH_2$ of oxirane ring. Reaction of **2** with isopropylamine gave **3**, which in ^{1}H NMR spectrum showed a six proton doublet at δ 1.09 for $-NHCH(CH_3)_2$ and another six proton doublet at δ 1.20 for $ArCH(CH_3)_2$. While reaction of **2** with *tert*-butylamine gave **4**, which in ^{1}H NMR spectrum showed a nine proton singlet at δ 1.13 for $-NHC(CH_3)_3$ and a six proton doublet at δ 1.20 for $ArCH(CH_3)_2$.

The compounds **3** and **4** were converted to their oxalates **5** and **6**, respectively, by treatment with oxalic acid in refluxing methanol (Fig. 1). Their structures were confirmed by ^{1}H NMR, IR spectroscopy and by elemental analyses. Nomenclature of the compounds were done through internet at the website www.chemweb.com/autonom/.

3. Experimental

3.1. Chemistry

Melting points (melting point apparatus MP I, Veego, Mumbai, India) reported are uncorrected. ^{1}H NMR spectra were recorded on Bruker AC-300F, 300 MHz NMR instrument (Brucker AG, Fällanden, Switzerland) using tetramethylsilane (TMS) as the internal standard (chemical shifts in δ, ppm). IR spectra were recorded on Perkin–Elmer 882 spectrophotometer model (Perkin–Elmer Ltd., Beaconsfield, Buckinghamshire, England). IR spectra were obtained with potassium bromide pellets (γ max in cm^{-1}). The purity of the compounds were established by thin layer chromatography (TLC) and by elemental analysis (C, H, N). Elemental analyses were carried out on a Perkin–Elmer-2400 (Perkin–Elmer corporation, Norwalk, CT). Plates for TLC were prepared with silica gel G using ethyl acetate. Iodine vapors were used to develop the plates. Silica gel (100–200 mesh) for column chromatography was purchased from Acme synthetic chemicals, Mumbai, India.

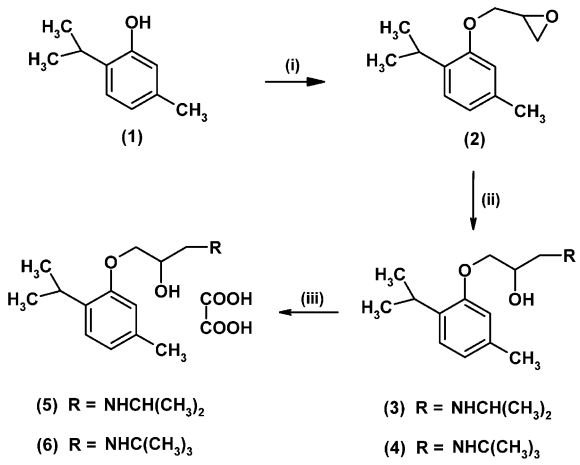

Fig. 1. Synthetic procedure to compounds **3** and **5**. Reagents and conditions: (i) epichlorohydrin/K_2CO_3, reflux; (ii) isopropylamine or *tert*-butylamine, reflux; and (iii) oxalic acid/methanol, reflux.

3.1.1. 2-(2-Isopropyl-5-methyl-phenoxymethyl)-oxirane (2)

Thymol (**1**) (5.0 g, 33.3 mmol), epichlorohydrin (50 ml) and potassium carbonate (5.0 g) were refluxed for 12 h while stirring. TLC confirmed completion of reaction. Filtered the residue and distilled off the excess epichlorohydrin under reduced pressure to give an oily residue, which was chromatographed over silica gel, using chloroform as the eluent to give an oily residue **2** (4.0 g, 58%). ^{1}H NMR ($CDCl_3$): δ 1.20 (d, 6H, $-CH(CH_3)_2$), 2.30 (s, 3H, $-CH_3$), 2.75 (dd, 1H, $-CH_2$ of oxirane), 2.87 (t,1H, $-CH_2$ of oxirane), 3.31 (m, 2H, $-CH$ of oxirane and $-CH(CH_3)_2$), 3.95 (dd, 1H, $-OCH_2-$), 4.20 (dd, 1H, $-OCH_2-$), 6.64 (s, 1H, Ar), 6.75 (d,1H, $J = 7.7$ Hz, Ar) and 7.09 (d, 1H, $J = 7.7$ Hz, Ar). M/S: m/z: 206 [M^+].

3.1.2. 1-Isopropylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol (3)

2-(2-Isopropyl-5-methyl-phenoxymethyl)-oxirane (**2**) (2.0 g, 9.7 mmol) and isopropylamine (10 ml), were refluxed for 12 h. Completion of reaction was confirmed

by TLC. Removal of excess isopropylamine under reduced pressure gave an only residue **3** (2.5 g, 97%). ^{1}H NMR ($CDCl_3$): δ 1.09 (d, 6H, –NHCH(CH_3)$_2$), 1.20 (d, 6H, ArCH(CH_3)$_2$), 2.30 (s, 3H, ArCH_3), 2.7–3.02 (m, 5H, –CH(O*H*)CH_2N*H*C*H*<, two protons exchanged in D_2O), 3.27 (m, 1H, ArC*H*(CH_3)$_2$), 3.92 (m, 1H, –OCH_2–), 4.00 (m, 1H, –OCH_2–), 4.08 (bm, 1H, –C*H*(OH)–), 6.67 (s, 1H, Ar), 6.75 (d, 1H, $J = 7.7$ Hz, Ar) and 7.09 (d, 1H, $J = 7.7$ Hz, Ar). M/S: *m*/*z*: 265 [M^+].

3.1.3. 1-Isopropylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol oxalate (5) [DPJ-576]

1-Isopropylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol (**3**) (2.0 g, 7.5 mmol) was dissolved in acetone (50 ml) by warming, added a hot solution of oxalic acid (1.25 g, 9.9 mmol) in acetone (10 ml) and refluxed for 0.5 h. The reaction mixture was concentrated and left for crystallization to afford oxalate **5** (1.72 g, 64%), m.p. 143–146 °C. IR (KBr): 3396 (O–H), 3246 (N–H), 2963 (aliphatic C–H) and 811 (aromatic C–H) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.17 (q, 6H, –NHCH(CH_3)$_2$) 1.39 (d, 6H, ArCH(CH_3)$_2$), 2.28 (s, 3H, –CH_3), 3.21(m, 2H, 2 × –C*H*(CH_3)$_2$), 3.39 (m, 1H, –CH_2NH–), 3.51 (m, 1H, –CH_2NH–), 3.90 (m, 1H, –OCH_2–), 4.03 (m, 1H, –OCH_2–), 6.58 (s, 1H, Ar), 6.75 (d, 1H, $J = 7.7$ Hz, Ar), 7.08 (d, 1H, $J = 7.7$ Hz, Ar). *Anal Calc.* for $C_{18}H_{29}NO_6$: C, 60.82; H, 8.22; N, 3.94. Found: C, 60.29; H, 8.36; N, 4.06%.

3.1.4. 1-tert-Butylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol (4)

2-(2-Isopropyl-5-methyl-phenoxymethyl)-oxirane (**2**) (2.0 g, 9.7 mmol) and *tert*-butylamine (10 ml), were refluxed for 12 h. Completion of reaction was confirmed by TLC. Removal of excess *tert*-butylamine under reduced pressure gave an oily residue **4** (2.5 g, 92%). ^{1}H NMR ($CDCl_3$): δ 1.13 (s, 9H, –C(CH_3)$_3$), 1.20 (d, 6H, ArCH(CH_3)$_2$), 2.71–2.92 (m, 4H, –CH(O*H*)CH_2N*H*–, two protons exchanged in D_2O), 3.26 (m, 1H, –C*H*(CH_3)$_2$), 3.97 (m, 3H, –OCH_2C*H*(OH)–), 6.68 (s, 1H, Ar), 6.75 (d, 1H, $J = 7.7$ Hz, Ar), 7.09 (d, 1H, $J =$ 7.7 Hz, Ar). M/S: *m*/*z*: 279 [M^+].

3.1.5. 1-tert-Butylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol oxalate (6) [DPJ-577]

1-*tert*-Butylamino-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol (**4**) (1.5 g, 5.4 mmol) was dissolved in acetone (50 ml) by warming, added a hot solution of oxalic acid (1.0 g, 7.3 mmol) in acetone (10 ml) and refluxed the mixture for 0.5 h. Removal of excess solvent under reduced pressure gave oily residue, which was given washing with dry ether. Crystallized the residue from acetone to afford oxalate **6** (0.91 g, 46%). m.p. 193–194°C. IR (KBr): 3289 (N–H), 2981 (aliphatic C–H) and 845 (aromatic C–H) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.19 (d, 6H, ArCH(CH_3)$_2$), 1.40, (s. 9H, –C(CH_3)$_3$), 2.31 (s, 3H, ArCH_3), 3.01 (t, 1H, –CH_2NH–), 3.24 (m, 2H, –CH_2NHC*H*<), 3.91 (m, 1H, –OCH_2–), 4.10 (m, 1H, –OCH_2–), 4.41 (m, 1H, –C*H*(OH)–), 6.66 (s, 1H, Ar), 6.75 (d, 1H, $J = 7.7$ Hz, Ar), 7.07 (d, 1H, $J = 7.7$ Hz, Ar). *Anal Calc.* for $C_{19}H_{31}NO_6$: C, 61.76; H, 8.46; N, 3.79. Found: C, 61.95; H, 8.60; N, 3.89%.

3.2. Pharmacological methods

Swiss albino mice of either sex (20–30 g) and female rats of Wistar strain (200–250 g) were purchased from National Toxicology Centre, Pune, India. All animals were housed in clean environment under 12 hours light and 12 hours dark cycle. The animals had free access to food pellets (Chakan oil mills, Pune, India) and water was made available ad libitum.

Isoprenaline (ISOLIN®, Samarth Pharma Pvt. Ltd., Mumbai, India), Adrenaline (Adrenaline inj. I.P., Walker's Pharmaceuticals Pvt. Ltd., Pune, India), Atenolol hydrochloride (Khandewall labs ltd, Mumbai, India) and Urethane (Wilson laboratories, Mumbai, India) were diluted/dissolved to appropriate concentrations with physiological saline. Fresh drug solutions were prepared for each day's work.

3.2.1. Mouse ECG experiments

Mice of either sex weighing 25–35 g were anesthetized with freshly prepared urethane (1.75 g/kg i.p.) and stainless steel needles of gauge 21 × 1/2 were inserted subcutaneously in the flexor aspect of limbs. The needles were clipped to SS2L electrodes of BIOPAC Student lab pro (Model MP 30, BIOPAC Systems, Inc., Santa Barba, CA).

The drugs in the appropriate doses were administered intravenously though the tail vein. ECG recordings (Lead I) were taken at 5, 15, 30, 45 min, 1 hour and then hourly response till the action of the drug ends. At the end of the experiment the needles were unclipped and detached from the limbs of the animals and the animals were observed intermittently till full recovery.

3.2.2. Isolated rat uterus preparation

Isolated rat uterus preparation was carried as described by Levy and Tozzi [17]. Spontaneous motility of the unprimed rat uterus was recorded by means of force transducer of Student's Physiograph (Bio-Devices, Ambala, India). The drugs were injected in to the isolated organ bath (INCO, Ambala, India) and the effects on the contraction of the uterus were recorded.

3.3. β-Adrenergic receptor binding assay

All the used solvents and powders were for analysis (J.T. Baker, Deventer, Holland). Propranolol hydrochloride and ICI 118551 were purchased from Sigma

Chemical Co., St. Louis, MO, USA. [^{3}H]Dihydroalprenolol ([^{3}H]DHA) (New England Nuclear, Boston, MA, USA), having a specific activity of 99.9 Ci/Mol and radiochemical purity >98.5%, was used as ligand.

3.3.1. β_1-Adrenergic receptor binding assay

Pellets containing β_1 type adrenergic receptors were obtained from turkey erythrocyte membranes as described in the literature [18].

β_1-Adrenergic receptor binding assay was determined as follows: 300 μl of membrane (~1.2 mg/ml of protein, dilution 1: 8 v/v) were incubated for 15 min at 37 °C with 100 μl of 4 nM [^{3}H]DHA and 100 μl of various concentrations of the test compounds (dissolved in water and diluted with saline buffer) and 12 M Tris–HCl, pH 7.5 (total vol. 1 ml). The incubations were stopped adding 4 ml of cold buffer (12 M Tris–HCl) followed by rapid filtration through glass fiber filter Whatman GF/B disks (Brandel Biomedical Research and Laboratories Inc., Gaithersburg, MD, USA). The samples were subsequently washed three times with 4.5 ml of the same buffer and placed into scintillation vials 10 ml of Filter-Count liquid scintillation cocktail (Packard BioScience s.r.l., Pero, Milan, Italy) was then added to each vial and counting was carried out by scintillation spectrometer (Packard TRI-CARB® 2000CA-Packard BioScience s.r.l., Pero, Milan, Italy).

Non-specific binding was defined as non-displaceable binding in the presence of 100 μl of 10 μM propranolol.

Competition experiments were analyzed by the 'Easy Fit' program (EasyFit 1.4, 1989–1991, Matteo Vaccari and Mario Negri Institute Milan, Italy) to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}), with six concentrations or displacers, each performed in triplicate.

The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prussoff [19], from the following equation: $K_i = IC_{50}/(1+S/K_D)$ where S represents the concentration of the ligand used and K_D its receptor dissociation constant (K_D values, obtained by Scatchard analysis [20], for [^{3}H]DHA is 3.6×10^{-9} M).

3.3.2. β_2-Adrenergic receptor binding assay

Preparation of lung homogenate: male Sprague–Dawley rats (Harlan Italy s.r.l., Correzzana, Milan) were sacrificed by decapitation. The right lung was removed free of the major bronchi. Lungs were homogenized with Polytron® Apparatus-Kinematica GmbH, Littau, Switzerland (setting 5 for 15 s) in 50 volumes buffer, 75 M Tris–HCl (pH 7.65), 25 M $MgCl_2$ and then centrifuged at 30,000 × g for 10 min twice. The resulting pellets were resuspended in 100 volumes of buffer 75 M Tris–HCl (pH 7.65), 25 M $MgCl_2$, then were frozen at −80 °C before being assayed [21,22]. [^{3}H]dihydroalprenolol was used as ligand.

Three hundred microliters of membrane of lung (~13.05 mg of fresh tissue, dilution 1:10) were incubated for 30 min at 37 °C with 100 μl of 6 nM [^{3}H]DHA, 100 μl of ketanserine 10^{-7} M 5HT antagonist and 100 μl of various concentrations of the test compounds (dissolved in water and diluted with buffer) and 75 M Tris–HCl (pH 7.65), 25 M $MgCl_2$ (total vol. 1 ml). The samples were subsequently washed with 4.5 ml of the same buffer and placed into scintillation vials. 10 ml of Filter-Count liquid scintillation cocktail (Packard BioScience s.r.l., Pero, Milan, Italy) was then added to each vial and counting was carried out by scintillation spectrometer (Packard TRI-CARB® 2000CA-Packard BioScience s.r.l., Pero, Milan, Italy).

Non-specific binding was measured in the presence of 100 μl of 10 μM unlabelled ICI 118551, and specific binding as the difference between total and non-specific binding.

The concentration of the test compounds that inhibited [^{3}H]DHA binding by 50% (IC_{50}) was determined as above reported.

4. Results

Pharmacological evaluation of newly synthesized compounds **5** (DPJ-576) and **6** (DPJ-577) have been carried out using mouse E.C.G and isolated rat uterus. Table 1 shows the effect of isoprenaline, atenolol, **5** and **6** alone, and the effect of isoprenaline after pretreatment with atenolol, **5** and **6** on heart rate, on mouse ECG recordings. Both **5** and **6** antagonized the relaxant effect of isoprenaline and adrenaline on isolated unprimed rat uterus, the results are shown in Table 2. Also both the compounds were subjected to in vitro β_1- and β_2-adrenergic receptor binding assay using turkey erythrocyte membrane (β_1) and lung homogenate of rats (β_2) and the results are shown in Table 3. The binding affinities were compared with that of propranolol (propranolol hydrochloride, CAS 318-98-9), a non-selective β-adrenergic blocking agent and atenolol, a cardioselective β-adrenergic blocking agent.

5. Discussion

In mouse ECG experiment administration of isoprenaline (2 μg/kg i.v.) decreased the R-R interval representing increase in the heart rate. The onset of action was 5 min and the peak was observed between 15 and 30 min. Complete restoration of normal heart rate was observed at 60 minutes. Administration of atenolol, **5** and **6** produced a dose dependent decrease in mouse heart rate. Administration of isoprenaline (2 μg/kg i.v.) failed to produce tachycardia in atenolol (20 μg/kg i.v.) pretreated mice after a pretreatment time of 3 hours,

Table 1
Effect of isoprenaline, atenolol, **5** (DPJ-576) and **6** (DPJ-577) on mouse ECG parameters

Sr. no.	Drug treatment and dose		Time (min)	Heart Rate
	1	2		
1	isoprenaline (2 μg/kg)		30	617.46±39.68
2	atenolol (20 μg/kg)		180	321.56*±24.99
3	atenolol (20 μg/kg)	isoprenaline (2 μg/kg)	180	327.00*±20.40
4	**5** (DPJ-576) (100 μg/kg)		60	337.40*±65.00
5	**5** (DPJ-576) (100 μg/kg)	isoprenaline (2 μg/kg)	60	414.70*±20.76
6	**6** (DPJ-577) (50 μg/kg)		60	371.60*±36.73
7	**6** (DPJ-577) (50 μg/kg)	isoprenaline (2 μg/kg)	60	375.25*±10.49

* $P < 0.05$ level significance applying paired Student's t-test.

indicating blockade of β_1-adrenergic receptors. Similarly isoprenaline (2 μg/kg i.v.) injected after pretreatment of **5** (100 μg/kg i.v.) in mice failed to produce tachycardia. Also isoprenaline (2 μg/kg i.v.) injected after **6** (50 μg/kg i.v.) pretreatment in mice failed to produce tachycardia, indicating blockade of β_1-adrenergic receptors. In the present experiment atenolol (20 μg/kg i.v.) was more effective than **5** (100 μg/kg i.v.) or **6** (50 μg/kg i.v.) as a β_1-adrenergic blocking agent as it showed better inhibition of isoprenaline induced increase in heart rate. Out of the two compounds the *tert*-butyl derivative **6** was more active as a β_1-adrenergic blocker than the isopropyl derivative **5**.

Isolated rat uterus (unprimed) is an experimental model specific for adrenergic β_2-receptor. Both **5** and **6** blocked isoprenaline (0.02 μg/ml) and adrenaline (0.02 μg/ml) induced relaxation of isolated rat uterus The rank order of potency of inhibition of relaxation was, **6** (2.5 μg/ml) > **5** (5 μg/ml). This experiment shows that both **5** and **6** possess β_2-adrenergic blockade, with **6** being a better β_2-adrenergic blocker.

The β-adrenergic receptor binding assay of **5** and **6** (Table 3), show that both the compounds possess β-adrenergic receptor affinity comparable to that of propranolol without selectivity to any one receptor, while atenolol has a selective affinity to β_1-adrenergic receptor. Both the compounds **5** and **6** have similar binding profile with the isopropyl derivative (**5**) being slightly selective for β_1-adrenergic receptor, compared to the *tert*-butyl derivative (**6**). Thus the results of the functional studies are in accordance with the β-adrenergic receptor binding affinity for both **5** and **6**. They both show a lesser β_1-adrenergic blocking activity as measured by the ability to inhibit the tachycardia induced by isoprenaline in mouse ECG experiments when compared to atenolol, but the binding affinities of **5** and **6** are better than that of atenolol. This difference in functional and receptor binding studies may be due to pharmacokinetic factors of the newly synthesized compounds that may also be responsible for the activity of the drug in vivo.

Table 3
Inhibition of [^{3}H]DHA binding on β_1- and β_2-adrenergic receptor and selectivity ratio (β_1/β_2) of compounds **5** (DPJ-576)and **6** (DPJ-577)

Compound	$K_i\beta_1 \pm$SD (M)	$K_i\beta_2 \pm$SD (M)	Selectivity ratio (β_1/β_2)
5 (DPJ-576)	$1.39 \times 10^{-10} \pm$ 0.052	$1.21 \times 10^{-9} \pm$ 0.454	8.70
6 (DPJ-577)	$4.77 \times 10^{-10} \pm$ 1.312	$2.66 \times 10^{-10} \pm$ 1.141	0.56
propranolol	$1.60 \times 10^{-9} \pm$ 0.134	$2.50 \times 10^{-9} \pm$ 0.177	1.56
atenolol	$2.70 \times 10^{-8} \pm$ 0.401		

In conclusion the results of functional and receptor binding studies indicate that both the newly synthesized compounds **5** and **6** starting from thymol possessed non-selective β-adrenergic receptor blocking activity, with the *tert*-butyl derivative (**6**) being more active than the isopropyl derivative (**5**). Further modification of these compounds are underway to obtain more potent and cardioselective β-adrenergic blocking agents.

Table 2
Effect of adrenaline, isoprenaline alone and in presence of **5** (DPJ 576) and **6** (DPJ 577) on isolated rat uterus (unprimed)

Drug	Height (mm)					
	Normal	Adrenaline (0.02 μg/ml)	Drug+adrenaline	Normal	Isoprenaline (0.02 μg/ml)	Drug+isoprenaline
DPJ-576 (5 μg/ml)	22.00±1.73	0.00±0.00*	21.33±1.15	12.67±6.51	0.00±0.00*	12.00±5.29
DPJ-577 (2.5 μg/ml)	23.00±11.53	4.00±6.93*	24.33±11.24	22.33±6.08	3.67±3.52*	23.33±5.36

* $P < 0.05$ level significance applying paired Student's t-test.

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