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β-adrenoreceptor blocking and antihypertensive activity of PP-24, a newly synthesized aryloxypropanolamine derivative

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Abstract

PP-24 is a newly synthesized putative β-adrenoceptor antagonist. The objective of the study was to the evaluate β-adrenoceptor blocking activity of PP-24 on isolated rat preparations: right atria, uterus and colon. Effects on the rat ECG and renal hypertension (induced by left renal artery ligation) were also investigated. Treatment with PP-24 (3 and 10 mg kg^{-1}) for 7 days in rats with renal hypertension significantly reduced the mean atrial blood pressure. Single i.v. injections of isoprenaline (0.3, 1 and 3 μg kg^{-1}) alone in normal anaesthetized rat caused hypotension and tachycardia, while PP-24 alone produced dose-dependent falls in mean aterial pressure and bradycardia. Pretreatment of anaesthetized rats with test compounds significantly blocked the hypotension response but not the tachycardia induced by isoprenaline (0.3, 1 and 3 μg kg^{-1}). The pA_2 of PP-24 to β_1-, β_2- and β_3-adrenoceptors was 7.72 ± 0.082, 7.40 ± 0.082 and 6.39 ± 0.16, respectively. The β_1/β_2 selectivity ratio was 2.08, compared with 1.27 for propranolol and 39.17 for atenolol. It is concluded that PP-24 possesses β-adrenoceptor blockade activity but with non-specific affinity for β_1- and β_2-adrenoceptor subtypes. The rank order of potency of the antagonists for β_1-adrenoceptors was atenolol > PP-24 > propranolol. The antihypertensive activity of PP-24 in rats with renal hypertension appears to be due to blockade of β-adrenoceptors.

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Introduction

An increase in sympathetic drive or stimulation increases the incidence of cardiovascular diseases. The roles of the three β-adrenoceptor subtypes β_1, β_2 and β_3 have been clearly established (Nisoli & Carruba 1997). β_1-adrenoceptors mediate the effects of sympathetic nerve stimulation and circulatory catecholamines mostly on the heart and renin release, whereas β_2-adrenoreceptors mediate bronchial and vascular dilation. Blockade of sympathetic stimulation by β-blockers is beneficial for the treatment of cardiovascular diseases such as angina pectoris, acute and post myocardial infarction, tachyarrhythmias, chronic heart failure, left ventricular diseases and hypertension (Hjalmarson 2000).

Hypertension is the most common cardiovascular disease and is a major public health issue in developed as well as developing countries. Although it is common and readily detectable, it can often lead to fatal complications if left untreated. Because of its high incidence and morbidity, various classes of drugs have been advocated for the control of hypertension (Badyal et al 2003). Despite the large armamentarium of drugs available for the treatment of hypertension, the last two decades have witnessed the introduction of a number of new antihypertensive drugs. Recent research during this period has also added considerably to our knowledge of the mechanisms involved in the pathogenesis of hypertension (Badyal et al 2003).

Well controlled and designed clinical trials have now demonstrated that cardioselective β_1-blockers do not produce any clinical adverse respiratory effects in patients with mild-to-moderate reversible airway diseases (Salpeter et al 2002). Catecholamine-induced myocardial injury appears to arise from stimulation of the β_1-receptor via a cAMP-dependent process, whereas stimulation of the β_2-receptor inhibits apoptosis via a Gi-coupled pathway (Communal et al 1999).

The usefulness of β-adrenoceptor antagonists in the treatment of various cardiovascular and non-cardiovascular complications has led us to search for new aryloxypropanolamine β-adrenoceptor antagonists (Jindal et al 2002a, b; Nandakumar et al 2005a, b, c; Bhatt et al 2007). Recently we described the antihypertensive and cardioselective activity of a compound having an ortho methoxy group with meta substitution to the phenyl ring (Bhatt et al 2007). The objective of the current study was to evaluate the in-vitro β-antagonistic activity and antihypertensive activity of 1-(isopropylamino)-3-(5-((isopropylamino)methyl)-2-methoxyphenoxy)propan-2-ol (PP-24), an aryloxypropanolamine derivative, in-vivo in rats with renal hypertension.

Material and Methods

Animals

Wistar rats (175–250 g) were purchased from the National Toxicology Centre (Pune, India) and were housed in a clean environment at 25 ± 1°C, relative humidity 45–55% and with a 12 h light–dark cycle. Free access was allowed to normal rat chow and filtered water. The research protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy (Pune, India). The experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Enviroment, Government of India.

Drugs

PP-24 (Figure 1) was synthesized by Dr Poonam Piplani and Jyotika Bansal as part of development of new β-blockers with an aryloxypropanolamine group. The pharmacological agents used were obtained from the following sources: urethane and sodium chloride from Himedia Laboratories (Mumbai, India), atenolol hydrochloride from Khandewal Laboratory Ltd (Mumbai, India), propranolol hydrochloride from Sigma Chemical Co. (St Louis, MO, USA). The drugs were dissolved and diluted to appropriate concentrations with physiological saline. Drug solutions were prepared fresh on the day of experiment.

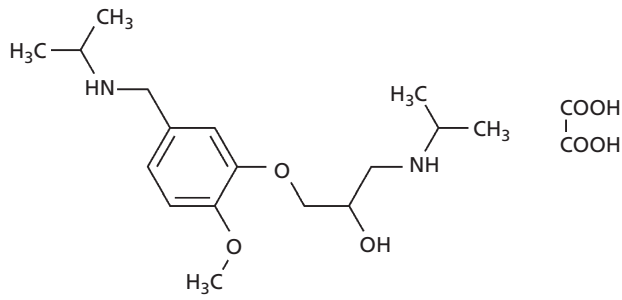

Figure 1 Structure of [1-(isopropylamino)-3-(5-((isopropylamino)-methyl)-2-methoxyphenoxy)propan-2-ol], (PP-24).

In-vitro β-adrenoceptor blocking activity

The specific β-adrenoceptor antagonistic activity was determined by constructing cumulative concentration–response curves (CRCs) for isoprenaline in the presence of the antagonists. β_1-Adrenoceptor blocking activity was determined in isolated rat right atria using the method described by Bhatt et al (2007). Control CRCs for the positive chronotropic effects of isoprenaline alone were constructed. The atria were allowed to restabilize for 30 minutes, after which various concentrations of antagonists were incubated with the atria for 30 min before cumulative concentrations of isoprenaline were added. β_2-Adrenoceptor blocking activity was determined in uterine horns from oestrus female rats according to the method of Nandakumar et al (2005b, c). β_3-Adrenoceptor blocking activity was evaluated using the isolated distal part of rat colon as described by Bhatt et al (2007).

Schild plots

For in-vitro studies agonist concentration ratios were determined from EC50 values with and without antagonists. The plot of the log agonist concentration ratio – 1.0 vs the log antagonist concentration (Arunlakshana & Schild 1959) was analysed by linear regression. Antagonism was considered competitive if the slope of the regression line was not significantly different from unity. In these cases mean pA_2 was obtained using the equation: $pA_2 = \log$ (agonist concentration ratio – 1.0) – log antagonist concentration.

In the case of experiments where the slope of the regression line was found to differ significantly from unity, the value obtained from the above equation was a pK_B rather than a pA_2 value.

In-vivo assessment of β-adrenoceptor blocking activity

Measurement of mean arterial pressure and heart rate

Male Wistar rats were anaesthetized with urethane (1.25 g kg^{-1} i.p.) and the trachea cannulated to assist spontaneous respiration. Heart rate was derived from the ECG recorded by subcutaneously placed electrodes. Mean arterial pressure (MAP) was measured by a pressure transducer in the left carotid artery. Output from the ECG and pressure transducer were recorded using a four-channel physiological data acquisition system.

The left jugular vein was cannulated for i.v. administration of drugs. Drugs were administered after 15–30 min' equilibration to allow stabilization of the cardiovascular parameters, and changes in heart rate and MAP were recorded. The dose of isoprenaline was optimized from the dose–response effect in preliminary studies. Next, a single dose of test drug was administered i.v. Isoprenaline (0.3, 1 and 3 μg kg^{-1} i.v.) was injected 15–20 min later and heart rate and MAP were recorded.

Renal hypertension model

Wistar rats (175–200 g) were divided into seven groups of six animals per group. Group I served as normal animals and did not undergo renal ligation. Animals in the remaining

groups were anaesthetized with urethane (1.5 g kg^{-1} i.p.) and the left renal artery (LRA) ligated. After renal ligation animals were housed individually and provided with 1% sodium chloride solution instead of drinking water. After 6 weeks of LRA ligation, the following treatment protocol was used. Group II received vehicle (0.9% sodium chloride in water), 1 mL kg^{-1} orally), which served as control. Groups III, IV and V received oral PP-24 at doses of 1, 3 and 10 mg kg^{-1}, respectively. Group VI were given oral propranolol and Group VII oral atenolol (both 10 mg kg^{-1}). The treatment was continued for 7 days. At the end of the seventh week, the rats were anaesthetized 1 h after the administration of the last dose of saline or test drugs, and MAP and ECG were recorded as described above.

Statistical analysis

In the in-vitro studies, mean concentration curves for isoprenaline in the presence of antagonists were analysed using non-linear regression (Graph Pad Prism, Version 4.0, Graph Pad Inc., San Diego, CA, USA). The EC50 (negative logarithm of concentration at 50% inhibition of response) values of isoprenaline were obtained in the presence and absence of antagonist. Concentration ratios were determined from the EC50 values. Schild plots were analysed by linear regression as described above.

Data for the effect of PP-24 on MAP and heart rate induced by isoprenaline (Table 2) and in rats with renal hypertension (Table 3) were analysed by two-way analysis of variance, followed by Bonferroni's post-hoc test.

Results

In-vitro β-adrenoceptor blocking activity

PP-24 antagonized the positive chronotropic effect of isoprenaline in the isolated rat atria preparation. In addition, PP-24 caused a dose-dependent parallel shift to the right of the isoprenaline CRC (Figure 2). PP-24 (1, 3 and 10 μM) competitively antagonized the isoprenaline-induced responses in the atria preparation. PP-24 competitively antagonized isoprenaline-induced relaxation in isolated rat uterus and rat colon preconstricted with KCl. PP-24 caused a rightward shift of the CRC of isoprenaline, with a change in EC50 values

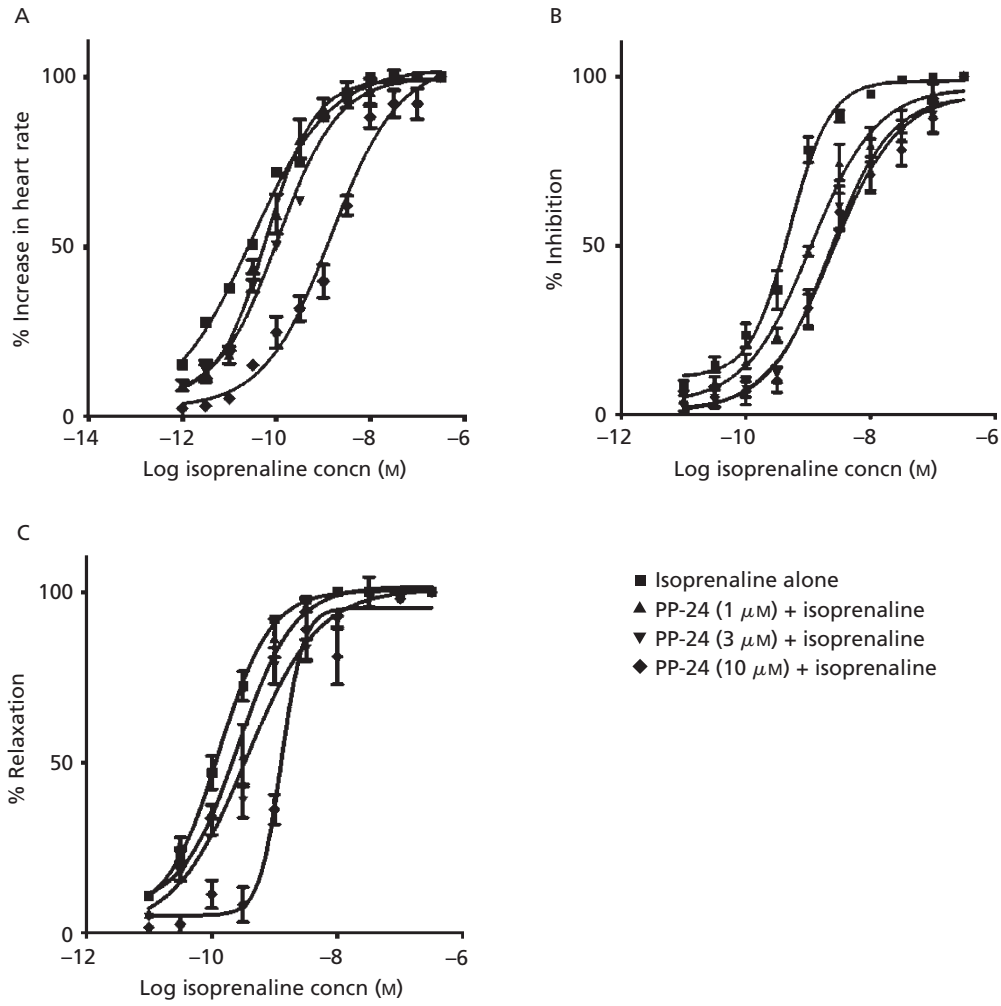

Figure 2 Antagonism of isoprenaline responses by PP-24 in isolated tissues: (A) rat right atria; (B) rat uterus; (C) rat colon.

Table 1 Potencies of PP-24 and reference adrenoreceptor antagonists on isolated preparations

	Rat right atria		Rat uterus		Rat distal colon		β_1/β_2 selectivity	β_1/β_3 selectivity
	pA_2/pK_B	Slope of Schild plot	pA_2/pK_B	Slope of Schild plot	pA_2/pK_B	Slope of Schild plot		
PP-24	7.72 ± 0.082	1.26 ± 0.48	7.40 ± 0.08	0.94 ± 0.27	6.39 ± 0.16	0.49 ± 0.22	2.08	21.37
Propranolol	8.27 ± 0.02	1.04 ± 0.07	8.18 ± 0.02	0.96 ± 0.01	6.64 ± 0.16	0.53 ± 0.17	1.27	44.16
Atenolol	7.16 ± 0.12	1.18 ± 0.09	5.57 ± 0.11	0.99 ± 0.11	4.62 ± 0.33	0.15 ± 0.05	39.17	346.37

The ratio values were obtained from the anti-logarithm of the difference between the mean pA_2 values from in-vitro studies. pA_2/pK_B = [log (Dose ratio −1) − log molar concentration of antagonist].

(Figure 2) in both these preparations. Atenolol and propranolol competitively antagonized the chronotropic effect of isoprenaline in isolated rat atria and its relaxant effect on the uterus preparation. However, the β-blocking activity produced in rat colon was blocked by PP-24, atenolol and propranolol and was found to be non-competitive in nature. Table 1 shows the pA_2 values for PP-24, atenolol and propranolol treatment in the different isolated preparations. The result indicated that PP-24 had antagonistic activity at the different β-adrenoceptor subtypes.

In-vivo assessment of β-adrenoceptor blocking activity

Isoprenaline (3 μg kg^{-1} i.v.) caused an increase in heart rate and reduction in MAP. The isoprenaline-induced hypotensive response was transient (5–10 min) whereas the isoprenaline-induced tachycardia lasted slightly longer (10–20 min). Intravenous injection of PP-24 (1 and 3 mg kg^{-1}), propranolol (0.5, 1 and 2 mg kg^{-1}) or atenolol (0.1, 0.3 and 1 mg kg^{-1}) alone reduced MAP and heart rate. Pretreatment with PP-24 (1, 3 and 10 mg kg^{-1} i.v.), propranolol (0.5, 1 and 2 mg kg^{-1} i.v.) or atenolol (0.1, 0.3 and 1 mg kg^{-1} i.v.) for 15–20 min significantly blocked the isoprenaline-induced hypotension in a dose-dependent manner (Table 1). Atenolol (1 mg kg^{-1} i.v.) was more effective in blocking isoprenaline-induced tachycardia compared with PP-24 (1, 3 and 10 mg kg^{-1}), propranolol (0.5, 1 and 2 mg kg^{-1}) and also lower doses of atenolol (0.1 and 0.3 mg kg^{-1}). These results suggested that PP-24, atenolol and propranolol possess β-adrenoceptor blocking activity.

Antihypertensive activity in rats with renal hypertension

LRA ligation increased MAP and heart rate in the rats after 7 weeks. The MAP and heart rate of control kidney-ligated rats were 192.33 mmHg and 354 beats min^{-1}, respectively. Significant reduction in MAP and heart rate was observed in hypertensive rats treated with PP-24 (3 and 10 mg kg^{-1}), propranolol (10 mg kg^{-1}) and atenolol (10 mg kg^{-1}). The fall in MAP produced by PP-24 (10 mg kg^{-1}) was less than that with atenolol (10 mg kg^{-1}) and propranolol (10 mg kg^{-1}), as shown in Table 3.

Discussion

In-vitro experiments

Tissue experiments were conducted to assess the antagonist potency of PP-24 towards different β-adrenoceptor subtypes and its selectivity for β_1-adrenoceptors. In accordance with

Table 2 Effect of isoprenaline alone (3 μg kg^{-1}) and in the presence of PP-24, propranolol and atenolol on rat heart rate and mean arterial pressure (MAP)

Antagonist (mg kg^{-1})	Decrease in MAP (mmHg)			Change in mean heart rate (beats min^{-1})		
	Isoprenaline alone	Antagonist	Antagonist + isoprenaline	Isoprenaline alone	Antagonist	Antagonist + isoprenaline
PP-24 (1)	35.00 ± 0.55	7.60 ± 0.75**	23.80 ± 3.47***	114.20 ± 4.02	-50.40 ± 3.80***	79.40 ± 3.66
PP-24 (3)	35.40 ± 4.41	9.80 ± 2.35**	26.80 ± 4.18*	48.00 ± 43.57	-28.80 ± 18.44***	68.40 ± 32.41
PP-24 (10)	30.60 ± 0.98	34.60 ± 1.96^{ns}	18.20 ± 1.39***	100.40 ± 11.48	-66.20 ± 7.57***	62.40 ± 22.58*
Propranolol (0.5)	42.40 ± 2.09	8.00 ± 0.32***	34.00 ± 1.14*	49.60 ± 5.27	-25.60 ± 2.25***	27.00 ± 1.61
Propranolol (1)	43.20 ± 2.08	12.60 ± 0.87***	30.60 ± 1.47***	54.00 ± 1.64	-30.60 ± 1.08***	22.20 ± 1.88
Propranolol (2)	45.60 ± 1.63	13.80 ± 0.49***	19.00 ± 0.63***	59.80 ± 1.11	-44.20 ± 0.97***	15.60 ± 1.47**
Atenolol (0.1)	46.20 ± 1.53	13.80 ± 1.59***	28.40 ± 1.03***	42.80 ± 1.80	-34.40 ± 0.81***	30.75 ± 1.60
Atenolol (0.3)	47.80 ± 1.69	13.00 ± 1.22***	21.40 ± 1.03***	56.40 ± 0.98	-39.00 ± 1.61***	14.00 ± 0.63*
Atenolol (1)	48.20 ± 2.37	16.20 ± 0.49***	14.20 ± 0.73***	59.60 ± 4.11	-65.20 ± 3.34***	11.80 ± 1.43**

Response to isoprenaline (3 μg kg^{-1}) was taken after 10 min of treatment. Values are mean ± s.em. (n = 5). Data were analysed separately for MAP and heart rate by two-way analysis of variance followed by Bonferroni's post-hoc test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs isoprenaline (3 μg kg^{-1}) group alone.

Table 3 Effect of PP-24, propranolol and atenolol in rats with renal hypertension (induced by left renal artery ligation)

	Mean arterial pressure (mmHg)	Mean heart rate (beats min^{-1})
Normal	98.16 ± 1.9	339.66 ± 4.0
Renal hypertensive control	192.33 ± 2.3###	354.00 ± 10.5
PP-24 (1 mg kg^{-1})	181.00 ± 2.3	329.00 ± 3.8*
PP-24 (3 mg kg^{-1})	171.83 ± 2.5***	304.66 ± 9.1***
PP-24 (10 mg kg^{-1})	154.00 ± 5.4***	290.00 ± 9.8***
Propranolol (10 mg kg^{-1})	152.80 ± 4.1***	298.83 ± 6.4***
Atenolol (10 mg kg^{-1})	147.5 ± 7.2***	303.50 ± 6.8***

Values are mean ± s.e.m. (n = 6). ###$P < 0.001$ vs normal group; *$P < 0.05$, ***$P < 0.001$ vs renal hypertensive control group (two-way analysis of variance followed by Bonferroni's post-hoc test).

previous reports, isoprenaline-induced chronotropic effects were competitively antagonized by atenolol (Tesfamariam & Allen 1994; Wu et al 2000). PP-24 also competitively antagonized the chronotropic effect of isoprenaline. As indicated by the pA_2 value in atrial preparation, PP-24 has antagonistic activity at β_1-adrenoceptors (Table 1, Figure 2).

Isoprenaline-induced relaxation in spontaneously contracting unprimed uterus is mediated by β_2-adrenoceptors (Levy & Tozzi 1963). The presence of large numbers of β_2-adrenoceptors in rat uterus has been demonstrated by autoradiography (Tolszczuk & Pelletier 1988); β_2-adrenoceptor-mediated responses are influenced by gestation (Borda et al 1979). In the present investigation, administration of isoprenaline inhibited the spontaneous contraction of the uterus, with EC50 values of 0.12–0.16 nM, which is comparable with earlier reports (Bianchetti & Manara 1990). The pA_2 values for standard β-adrenoceptor antagonists like propranolol (8.19 ± 0.077) and atenolol (5.58 ± 0.29) were similar to those reported previously (Wilson et al 1984) (Table 1, Figure 2). The pA_2 values of PP-24 (7.40 ± 0.082) reveals that the compound has significant antagonistic effect at β_2-adrenoceptors. The potency of the antagonists for the β_2-adrenoceptors was in the order: propranolol > PP-24 > atenolol (Table 1).

Isoprenaline-induced relaxation in rat colon preconstricted with KCl is mediated through β_3 receptors, and is resistant to conventional β_1- and β_2-antagonists. In the present investigation, propranolol, atenolol and PP-24 noncompetitively antagonized the relaxant response of isoprenaline, as the slope of Schild's plot was not near to unity (Table 1, Figure 2).

In-vivo effect of PP-21 against isoprenaline

Intravenous infusion of isoprenaline lowers peripheral vascular resistance, primarily in skeletal muscles and also in renal and mesenteric vascular beds. Diastolic blood pressure falls but systolic blood pressure may remain unchanged or rise. Although MAP falls, cardiac output increases because of positive inotropic and chronotropic effects of isoprenaline. β-adrenoceptor antagonists prevent the hypotensive effects of isoprenaline, which is primarily due to stimulation of β_1-adrenoceptors. The tachycardia produced by isoprenaline is primarily due to β-adrenoceptor activity in the heart, and the hypotensive effect is primarily due to β_2-adrenoceptors in the blood vessels (Kannan et al 1999; Chiu et al 2000). Though β_1-, β_2- and β_3-adrenoceptors are present in mammalian heart, positive ionotropic and chronotropic effect of isoprenaline in-vivo are brought about predominantly through β_1-adrenoceptors (Piercy 1988; Wellstein et al 1988).

In the present investigation, PP-24 (10 mg kg^{-1}), propranolol (2 mg kg^{-1}) and atenolol (0.3 mg kg^{-1}) reduced isoprenaline-induced tachycardia (Table 2), and isoprenaline-induced hypertension was reduced by all doses of all three antagonists. These reductions in isoprenaline-induced tachycardia and hypotension indicate that PP-24 possesses β_1-adrenoceptor blocking activity.

Effects of PP-24 in rats with renal hypertension

The mechanism involved in the development of hypertension in the LRA ligation model appears to depend on the species examined. In the rat, constriction of one renal artery with an intact contralateral artery produces hypertension with elevated plasma renin activity and the animals are responsive to angiotensin blockade (Cangiano et al 1979). One of the mechanisms of the antihypertensive effects of β-blockers is reduction in renin release by juxtaglomerular cells. In the present investigation, PP-24 (3 and 10 mg kg^{-1}) showed a significant antihypertensive effect, similar to that of propranol (10 mg kg^{-1}) and atenolol (10 mg kg^{-1}).

Conclusion

PP-24 possessed non-specific β-adrenoceptor antagonistic activity in-vitro and in-vivo similar to that of propranolol. Effects in the renal hypertension model suggest the use of PP-24 as a potential antihypertensive agent.

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