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Original article

Synthesis and Effects of Novel Dihydropyridines as Dual Calcium Channel Blocker and Angiotensin Antagonist on Isolated Rat Aorta

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Abstract

Objective(s)

Four novel losartan analogues 5a-d were synthesized by connecting a dihydropyridine nucleus to imidazole ring. The effects of 5a and 5b on angiotensin receptors (AT_1) and L-type calcium channels were investigated on isolated rat aorta.

Materials and Methods

Aortic rings were pre-contracted with 1 μM Angiotensin II or 80 mM KCl and relaxant effects of losartan, nifedipine, 5a and 5b were evaluated by cumulative addition of these drugs to the bath solution.

Results

The results showed that compounds 5a and 5b have both L-type calcium channel and AT_1 receptor blocking activity. Their effects on AT_1 receptors are 1000 and 100,000 times more than losartan respectively. The activity of compound 5b on L-type calcium channel is significantly less than nifedipine but compound 5a has comparable effect with nifedipine.

Conclusion

Finally we concluded that these two new Compounds can be potential candidates to be used as effective antihypertensive agents.

Keywords: Angiotensin, Aorta, Dihydropyridine

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Introduction

Angiotensin II (ANG II) is an important regulator of the renal microcirculation and exerts major actions on afferent and efferent arterioles (1-3). Although it has been clearly demonstrated that ANG II increases intracellular calcium ($[Ca^{2+}]_i$) in vascular smooth muscle cells (RVSMC), the sequence of events following activation of ANG II type 1 receptors (AT_1) remains unclear (2, 4-6). Typically, the calcium response is characterized by a sharp transient rise in $[Ca^{2+}]_i$ followed by a fall toward a sustained plateau above baseline (2). Although the sustained increases in $[Ca^{2+}]_i$ are thought to be mediated through Ca^{2+} influx, the early peak response is generally considered to be due to mobilization of intracellular stores (6-9). AT_1 receptor-coupled G proteins activate PLC, which, in turn, activates IP_3 and DAG, leading to release of calcium from the sarcoplasmic reticulum (6, 10). It has been suggested that the increased $[Ca^{2+}]_i$ activates chloride channels (Cl_{Ca}) causing an efflux of chloride and subsequent depolarization of the cell membrane leading to opening of voltage-gated calcium channels (11-16). L-type calcium channel blockers prevent constriction of afferent arterioles and reduce sustained $[Ca^{2+}]_i$ increases as well (5, 10, 16-18).

Losartan (Dup-753) (Figure 1) is a nonpeptide angiotensin II receptor (type AT_1) antagonist discovered by Duncia *et al* in 1990 and its potassium salt (cozaar) has been marketed as an antihypertensive since 1995 (19).

To date, many orally available sartans have been developed and are used in the treatment of both hypertension and damage associated with diseases like atherosclerosis and diabetes. In particular, the good properties of new non peptide ANG II antagonists, such as losartan, have stimulated the design of many different congeners. All these drugs contain some common structural features represented by a biphenyl fragment bearing an acidic moiety (i.e. tetrazole, carboxylic- or sulphonamidocarboxyl group), linked to a heteroaromatic or acyclic system by means of a methylene group. Almost all of the chemical manipulations within the fundamental skeleton of sartans, concerned the substitution of the imidazole ring of losartan with several variously substituted heteroaromatic groups or acyclic structures (20).

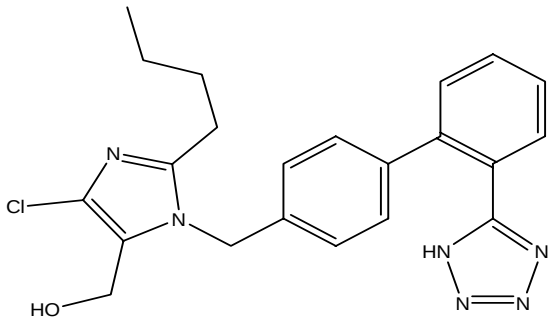


Figure 1. Losartan structure.

In this project some novel analogues of losartan were synthesized in which biphenyl fragment was retained and imidazole nucleus was connected to a dihydropyridine moiety. We proposed dual calcium channel blocking and AT_1 antagonist activity for the synthesized compounds.

Materials and Methods

Chemistry

Melting points were determined on Electrothermal Capillary apparatus and were uncorrected. The ir spectra were obtained using a Perkin-Elmer Model 1000. 1H nmr was obtained on Bruker Ac-80 spectrophotometer and chemical shifts (δ) were in ppm relative to internal tetramethylsilane. C, H, and N analyses were within ±0.4% of theoretical values. Title compounds (5a-d) are sensitive to light; all chemical procedures involving these were shielded from light whenever present. Compounds 1, 3a and 3b were prepared as described previously (21).

2-(n-butyl)-1-[(2'-(1-trityltetrazol-5-yl) biphenyl-4-yl) methyl] imidazole-4 -carbaldehyde (3c) and 2-(n-butyl)-1-[(2'-(1-trityltetrazol-5-yl) biphenyl-4-yl) methyl] imidazole-5 –carbaldehyde (3d).

A solution of 1 (1 g, 3.3 mmoles), potassium carbonate (1.8 g, 13.04 mmoles) and 2b (4.0 g, 7.17 mmoles) in dried dimethylformamide (25 ml) was stirred at room temperature for 24 hr. The progress of reaction was controlled by thin layer chromatography on silica gel

(chloroform-methanol, 80:20). The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was diluted with water (20 ml) and extracted with chloroform (3×20 ml). The organic layer was collected and washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. A crude mixture of 3c and 3d (1.44 g) was separated as pale yellow oil. This crude mixture was used directly at the next step. Also, preparative thin layer chromatography of this mixture on silica gel (chloroform-methanol, 80:20 as eluent) afforded respectively 64% of 3c and 36% of 3d as pale yellow oils.

Compound 3c has ir (sodium chloride disk): ν 1675 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): δ 9.7 (s, 1H, CHO), 8.5-6.5 (m, 24H, arom, HC_4 imidazole), 4.5 (s, 2H, CH_2N), 2.5 (t, 3H, CH_2), 2.0-0.5 (m, 7H, CH_2, CH_3).

Anal. Calcld. for $C_{41}H_{36}N_6O$: C, 78.32 ; H, 5.77; N,13.37 .

Found: C, 78.11; H, 5.68; N, 13.41.

Compound 3d has ir (sodium chloride disk): ν 1675 cm^{-1} (C=O); 1H-nmr (deuteriochloroform): δ 9.6 (s, 1H, CHO), 8.5-6.5 (m, 9H, arom, HC_5 imidazole), 5.5 (s, 2H, CH_2N), 2.5 (t, 3H, CH_2), 2.0-0.5 (m, 7H, CH_2, CH_3).

Anal. Calcld. for $C_{41}H_{36}N_6O$: C, 78.32; H, 5.77; N,13.37.

Found C, 78.22; H, 5.68; N, 13.45.

Dimethyl 4-[2-butyl-1-(2'-carboxy biphenyl-4-yl) methylimidazol-4-yl]-1, 4-dihydro-2, 6-dimethylpyridine-3, 5-dicarboxylate (5a).

Ammonium solution (1.1 ml) was added to a stirring solution of crude mixture 3a, b (1.25 g, 3.32 mmol) and methyl acetoacetate (078 g, 6.68 mmol) in methanol (25 ml). The mixture was protected from light and refluxed overnight. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel (chloroform-methanol, 70:30) to give 1.03 g (56%) of 5a as a white solid, mp 216 ^oC (ethanol); ir (potassium bromide): ν 1687 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): 10.6 (s, 1H, COOH), 7.9-6.5 (m, 10H, arom, HC_4 imidazole, NH), 5.0 (m, 3H, HC_4 dihyropyridine, CH_2N) 3.59 (s, 6H, CH_3O), 2.0 (s, 6H, CH_3 dihydropyridine), 2.5 (m, 2H, CH_2), 1.8 - 0.4 ppm (m, 7H, CH_3, CH_2).

Anal. Calcld. for $C_{32}H_{35}N_3O_6$: C, 68.92; H, 6.33 ; N,7.54.

Found: C, 68.84; H, 6.19; N, 7.67.

Diethyl 4-[2-butyl-1-(2'-carboxy biphenyl-4-yl) methylimidazol-4-yl]-1, 4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (5b).

This compound was prepared in a similar fashion to 5a affording 5b as a white solid in 57% yield; mp 189.5 (ethanol); ir (potassium bromide): ν 1687 cm^{-1} (C=O); 1H-nmr (deuteriochloroform): 10.8 (s, 1H, COOH), 8.4-6.7 (m, 10H, arom, HC_5 imidazole, NH), 5.0 (m, 3H, HC_4 dihyropyridine, CH_2N) 3.9 (q, 4H, CH_2O), 2.0 (s, 6H, CH_3 dihydropyridine), 2.2 (m, 2H, CH_2), 1.6 - 0.5 ppm(m, 13H, CH_3, CH_2).

Anal. Calcld. for $C_{34}H_{39}N_3O_6$: C, 69.72; H, 6.71; N,7.17.

Found: C, 69.68; H, 6.84; N, 7.05.

Dimethyl 4-[2-butyl-1-[2'-(1H-tetrazo-5-yl) biphenyl-4-yl] methylimidazol-4-yl]-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (5c).

This compound was prepared in a similar fashion to 5a affording 5c as an yellow oil in 30% yield; ir (sodium chloride): ν 1685cm^{-1} (C=O); ^{1}H-nmr (DMSO-d_6): 8.8 (s, 1H, NH tetrazole), 8.0-6.4 (m, 10H, arom, HC_5 imidazole, NH), 4.8-4.7 (m, 3H, HC_4 dihyropyridine, CH_2N), 3.35 (s, 6H, CH_3O), 2.3 (m, 2H, CH_2), 2.0 (6H, CH_3 dihydropyridine), 1.7 - 0.5 ppm (m, 7H, CH_3, CH_2).

Anal. Calcld. for $C_{32}H_{35}N_7O_4$: C, 66.08; H, 6.06; N,16.86.

Found: C, 66.17; H, 6.12; N, 16.95.

Diethyl 4-[2-butyl-1-[2'-(1H-tetrazo-5-yl) biphenyl-4-yl] methylimidazol-4-yl]-1, 4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (5d).

This compound was prepared in a similar fashion to 5a affording 5d as a yellow oil in 30% yield; ir (sodium chloride): ν 1685 cm^{-1}

(C=O); ^{1}H-nmr (DMSO-d_6): 8.9 (s, 1H, NH tetrazole), 8.0-6.4 (m, 10H, arom, HC_5 imidazole, NH), 5.3-4.5 (m, 3H, HC_4 dihyropyridine, CH_2N), 3.9 (q, 4H, CH_2O), 2.5 (t, 2H, CH_2), 2.1 (6H, CH_3 dihydropyridine), 1.9 - 0.4 ppm (m, 13H, CH_3, CH_2).

Anal. Calcld. for $C_{34}H_{39}N_7O_4$: C, 66.98; H, 6.45 ; N,16.08.

Found: C, 66.87; H, 6.56; N, 16.19.

Pharmacology

ANG II, Nifedipine, Losartan and KCl were supplied by Sigma. ANG II and KCl were dissolved in distilled water. Nifedipine, losartan and compounds 5a and 5b were dissolved in dimethylsulphoxide (DMSO). DMSO in organ baths did not affect smooth muscle relaxations induced by compounds. All drug solutions were prepared on a daily basis.

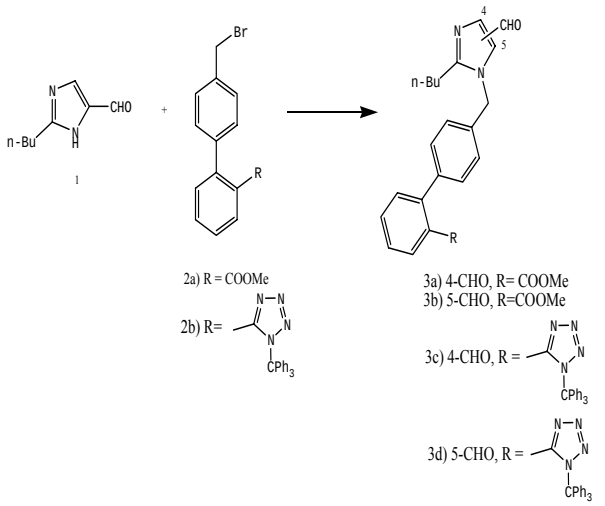
Male Wistar rats weighing 200–250 g were used. Animals were anesthetized with interaperitoneal injection of sodium thiopental (80 mg/kg) and their thoracic aorta was removed, cleaned of adhering fat and cut into rings of 3-4 mm long. All rings were mounted on stainless steel hooks in 20 ml organ baths. These organ chambers were filled with Krebs-Henseleit solution (KHS), with a composition (in mM) of: NaCl 118, KCl 4.7, $MgSO_4$ $2H_2O$ 1.2 KH_2PO_4, 2 H_2O 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11.1, aerated with a mixture of 95% O_2/5% CO_2 and kept at 37 °C. The pH of the saturated solution was 7.4. Changes in tension were recorded with an isometric transducer and displayed on a Washington recorder. After mounting, the preparations were allowed to equilibrate for 1 hr. During this time the resting tension was adjusted to 2 g and solution was renewed every 15 min.

Aortic rings were pre-contracted with 1 µM ANG II or 80 mM KCl and concentration-response curve for losartan, nifedipine and compounds 5a and 5b were obtained by cumulative addition of these drugs to the bath solution. The relaxant effects of the compounds were expressed as percentage of the pre-contraction. To evaluate the effects of the compounds, pD2 values (the negative logarithm of the concentration for the half-maximal response (EC_{50})) were calculated. All data are expressed as mean±standard error. Statistical comparisons between groups were performed using unpaired t-test and *P* values of less than 0.05 were considered to be statistically significant.

Results and Discussion

Chemistry

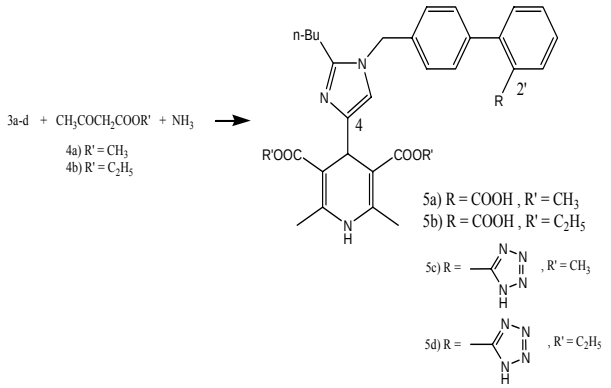
Compound 1 was synthesized as reported previously (21). Alkylation of 1 with bisphenylmethyl bromide 2a, b gave a 70:30 mixture of isomeric 4(5)-formylimidazoles 3a-d with 4 -isomers predominance. The proportion of isomers was determined by ^{1}H-NMR. Benzylic protons of 5-isomer (3b, d) were more deshielded than that of 4-isomer (3a, c) which appeared at 5.63 and 5.17 respectively (scheme 1).



Scheme 1. Synthesis of compounds 3a-d.

The cured isomeric mixture was reacted with acetoacetate 4a, b and ammonia in methanol according to classical Hanzsch condensation to afford dihydropyridines 5a-d (scheme 2). Mostly, the predominant 4 isomer 3a, c was condensed. This may be related to steric hinderance of 5 isomer dihydropyridines if formed. It was interesting that trityl protecting groups were removed *in situ* during reaction in ammonia solution. Usually trityl protecting group is removed at acidic media (22). Ester groups on 2' position of bisphenyl (R) were also hydrolyzed while dihydropyridine esters (R') remained intact. Carbonyl group of ester at 2' position is co-planar with phenyl ring and consequently is more prone to hydrolysis than

carbonyl group of esters at 3 and 5 position of dihydropyridine nucleus.



Scheme 2. . Synthesis of compounds 5a-d.

Pharmacology

The relaxant effect of the test compounds 5a and 5b, nifedipine and losartan on isolated rings of rat aorta precontracted by potassium chloride (KCl) or angiotensin II (ANG II) were given in Tables 1 and 2. The results of the first part of the study indicate that compounds 5a and 5b both caused dilation against potassium chloride-induced contraction. The activity of compound 5b is significantly lower than nifedipine (P<0.05) but compound 5a has comparable effect with nifedipine. Losartan also causes dilation against potassium chloride-induced contraction which was significantly lower than nifedipine (P<0.05). Altogether, compounds 5a and 5b, nifedipine and losartan exerted concentration-dependent relaxation responses on aortic rings precontracted by KCl with potency order of: nifedipine ≥ 5a > losartan> 5b.

Regarding the mechanism of contraction induced by KCl, which activates L-type calcium channels of smooth muscles (23), our data indicates that the blocking effect of compound 5b on L-type calcium channels is less than nifedipine but for compound 5a is comparable with nifedipine. Our data also show that losartan has blocking effect on L-type calcium channels with lower potency compared to nifedipine. This finding is in accordance with the results of the work of Louch *et al* which showed that losartan by itself reduces incidence of transient inward current in ventricular myocytes (24).

To investigate the effect of compounds on Angiotensin receptor 1 (AT1), aortic rings were contracted by ANG II and then the relaxant effects of compounds were measured. The results showed that both compounds 5a and 5b cause dilation against ANG II-induced contraction. The activity of compounds 5a and 5b is significantly higher than losartan (P<0.05) as positive control. The EC_{50} of compound 5b and 5a are 100,000 and 1000 times more than losartan respectively. Comparing two compounds with each other we can say that compound 5b has higher potency than compound 5a on AT_1 receptor while we showed that on L-type calcium channels, the potency of compound 5a was higher than compound 5b. Our data show that nifedipine also has relaxant effect on ANG II-induced contraction in isolated aorta but its effect is significantly lower than losartan (P<0.05). Although nifedipine doesn't have antagonistic activity on AT_1 receptors, it should be considered that a part of contraction induced by ANG II is due to activation of L-type calcium channels. Therefore nifedipine can diminish contraction on ANG II but with a lesser potency than losartan. In another study it has been shown that a part of the ANG II-induced constriction of renal resistance vessels is mediated by voltage-dependent L-type calcium channels responsive to the dihydropyridine nifedipine (16).

Regarding these data, we should consider that for compounds 5a and 5b and also for losartan, relaxant effect on ANG II contracted aorta is due to both AT_1 receptor antagonism and calcium channel blocking activity.

Table 1. Relaxant effects of nifedipine, losartan and compounds 5b and 5a on KCl-induced contraction

Compound	Contractile stimulus	pD2	Statistics (Comparison whit nifedipine)
5b	KCl	-4.29±0.72	*
5a	KCl	-5.29±0.16	n.s.
Losartan	KCl	-4.58±0.1	*
Nifedipine	KCl	-5.58±0.19	_

Relaxation is expressed as pD2 of precontraction induced by KCl (80 mM). pD2 values represent mean value±SE for 6 experiments. ns, P> 0.05; *, P< 0.05 when compared with control (nifedipine). (unpaired t-test).

Table 2. Relaxant effects of nifedipine, losartan and compounds 5a and 5b on angiotensin-induced contraction.

Compound	Contractile stimulus	pD2	Statistics (Comparison with losartan)
5a	Angiotensin II	-8.67±0.18	*
5b	Angiotensin II	-10.52±0.18	*
Nifedipine	Angiotensin II	-4.50±0.1	*
Losartan	Angiotensin II	-5.66±0.16	_

Relaxation is expressed as pD2 of precontraction induced by Angiotensin II (1 μM). pD2 values represent mean value±SE for 6 muscle rings ; *, $P< 0.05$ when compared with control (losartan). (unpaired t-test).

Anyway, in comparison AT_1 receptor antagonistic activity of these compounds, we can at least say that compound 5b is more potent than losartan, because as we mentioned above the relaxant effect of compound 5b on ANG II contracted aorta is 100,000 times more than losartan; while compound 5b and losartan are semi potent on L-type calcium channels.

It can be concluded from these data that compounds 5b and 5a have both calcium channel and AT_1 receptor blocking activity.

Their effects on AT_1 receptors are 100,000 and 1000 times more than losartan respectively. Finally we can conclude that these two new compounds can be potential candidates to be used as effective antihypertensive agents.

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