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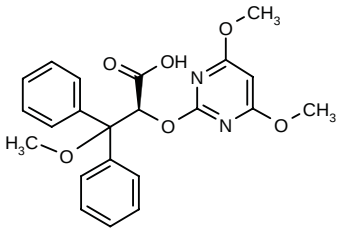
CCC: 0377-8282/99

LU-135252

Treatment of Heart Failure
Endothelin ET_A Antagonist

HMR-4005
LU-127043 (as racemic)

2(*S*)-(4,6-Dimethoxypyrimidin-2-yloxy)-3-methoxy-3,3-diphenylpropionic acid



$C_{22}H_{22}N_2O_6$ Mol wt: 410.4238

CAS: 171714-84-4

EN: 231818

Synthesis

The condensation of benzophenone (I) with chloroacetic acid methyl ester (II) by means of sodium methoxide in THF gives the epoxide (III), which is treated with methanol/BF_3.Et_2O, yielding 2-hydroxy-3-methoxy-3,3-diphenylpropionic acid methyl ester (IV). The condensation of (IV) with 4,6-dimethoxy-2-(methylsulfonyl)pyrimidine (V) by means of K_2CO_3 in DMF affords 2-(4,6-dimethoxypyrimidin-2-yloxy)-3-methoxy-3,3-diphenylpropionic acid methyl ester (VI), which is hydrolyzed with NaOH in hot dioxane/water to give the corresponding free acid (VII). Finally, this compound is submitted to optical resolution, affording LU-135252 (1, 2). Scheme 1.

Optical resolution of (VIII), the free acid derivative of (IV), with (*R*)-phenylethylamine, L-proline methyl ester or (*S*)-1-(4-nitrophenyl)ethylamine leads to the enantiomerically pure (*S*)-enatiomer (IX), which by reaction with 4,6-dimethoxy-2-(methylsulfonyl)pyrimidine (V) directly affords LU-135252 (1, 2). Scheme 1.

Description

Racemic, m.p. 165-8 °C.

Introduction

Endothelins are a family of endothelium-derived vasoactive and mitogenic peptides that include at least three isoforms, termed endothelin-1, endothelin-2 and endothelin-3 (ET-1, ET-2 and ET-3). They play a role in a variety of cardiovascular, renal and other disorders, and exert their actions via specific binding to at least two different receptor subtypes: ET_A and ET_B. ET_A is selective in that it binds only ET-1 and ET-2, whereas affinity to ET-3 is very low. ET_B is nonselective and has similar affinity to all three isoforms. Endothelin-induced vasoconstriction is mediated predominantly by vascular ET_A receptors, whereas activation of vascular ET_B receptors leads to vasorelaxation through the release of nitric oxide or prostacyclin. The physiological activity of endothelins and their role in the pathogenesis of cardiovascular disease is well documented (3-31).

Several selective endothelin ET_A antagonists, as well as a few mixed ET_A/ET_B antagonists, are currently undergoing clinical evaluation, as shown in Table I. The results of *in vitro* studies conducted with various endothelin ET_A antagonists are summarized in Tables II and III.

LU-135252 was recently discovered from a new class of nonpeptide selective ET_A receptor antagonists (1) with a 130-fold higher affinity for the human ET_A than for the ET_B receptor.

Pharmacology and Biochemistry

LU-135252 is the active enantiomer of racemic LU-127043 (30, 31). In its racemic formulation the compound has been shown be highly selective for the ET_A receptor subtype with K_i values for the displacement of [^{125}I]-ET-1 of 6 and 371 nM, respectively, for human ET_A and ET_B receptors expressed in CHO cells (30). Table II shows ET_A and ET_B receptor affinities for selected compounds acting preferentially as selective ET_A receptor antagonists. Functional antagonism of the compound was

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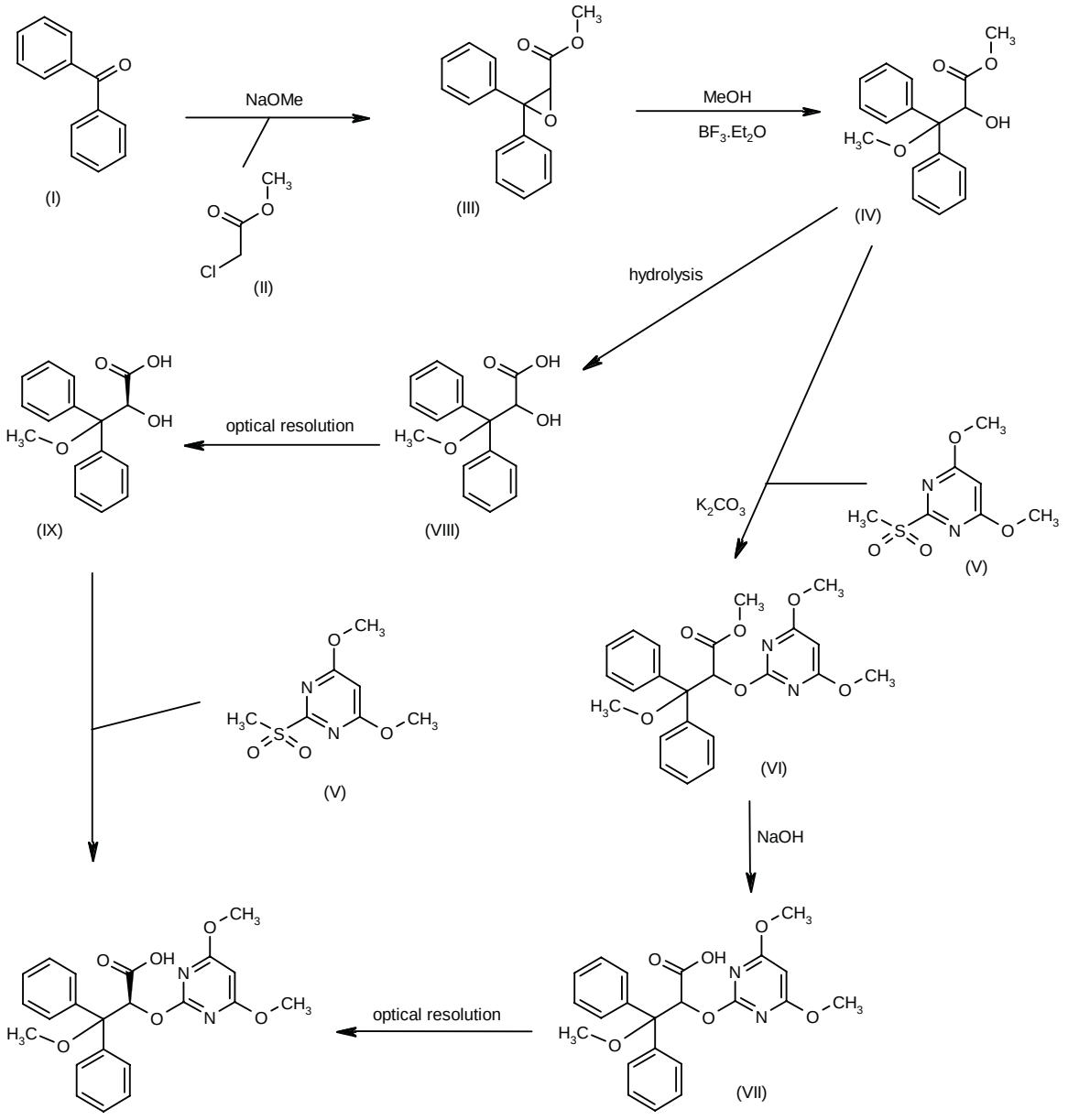
Scheme 1: Synthesis of LU-135252



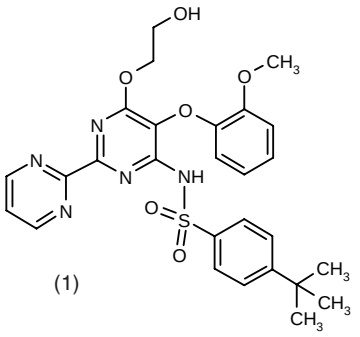
demonstrated since *in vitro* racemic LU-127043 was able to antagonize ET-1-induced vasoconstriction (pA_2 = 7.34) in isolated rabbit aortic rings, and *in vivo* it was shown to be orally active in preventing ET-1-induced lethality in rats (ED_{100} = 0 mg/kg p.o.) (1).

Interestingly, the existence of an atypical endothelin receptor has been proposed from *in vitro* studies in primary cultures of rat astrocytes using LU-135252 as a specific ET_A receptor antagonist. In such a cell culture, LU-135252 and the selective ET_B receptor antagonist BQ-788 act synergistically both to compete with [^{125}I]-ET-1 and to increase extracellular ET-1 levels. However, these parameters were not significantly affected by ET_A and ET_B antagonists alone (24, 25). The results suggest that inhibition of the endothelin eliminatory activity of astrocytes by blocking the atypical endothelin astrocytic receptor – apparently involving ET_A and ET_B sites – could lead to increased extracellular endothelin levels in the brain (24, 25).

Cardioprotective effects of LU-135252 have been suggested from a range of animal models using either isolated tissues or in animals with experimental cardiovascular alterations. Thus, the cardioprotective actions of LU-135252 were demonstrated in studies using isolated

Table I: Endothelin antagonists under development (from Prous Science Ensemble database).

1. Bosentan
   Actelion (Phase III)[1]
2. BQ-123
   Banyu (Phase I)[2]
3. FR-139317
   Fujisawa (Phase II)
4. J-104132
   Banyu; Merck & Co. (Phase II)
5. LU-135252
   Knoll; Hoechst Marion Roussel (Phase II)
6. Ro-61-1790
   Roche; Vanguard Medica (Phase I)
7. S-0139
   Shionogi (Phase I)
8. SB-209670
   SmithKline Beecham (Phase II)
9. SB-217242
   SmithKline Beecham (Phase II)
10. TAK-044
    Takeda (Phase II)
11. TBC-11251
    Texas Biotechnology (Phase II)
12. ZD-1611
    Zeneca (Phase I)



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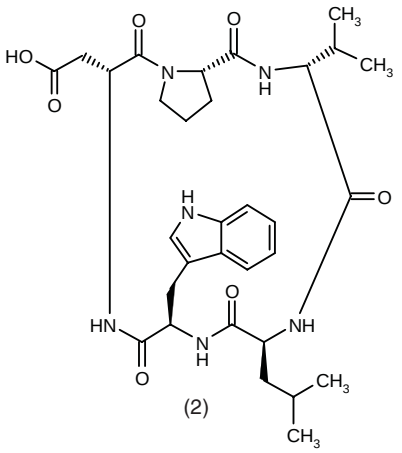

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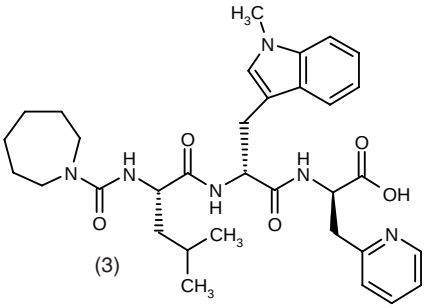

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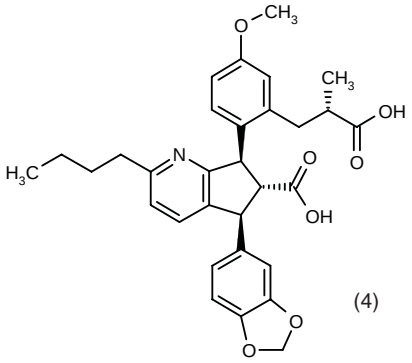

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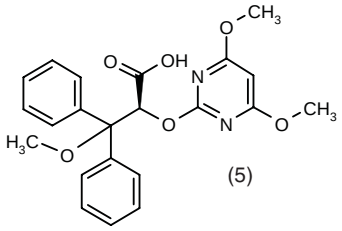

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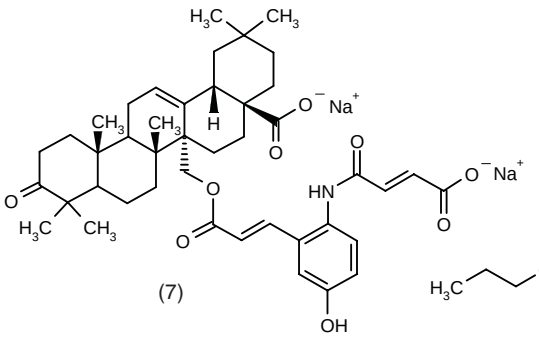

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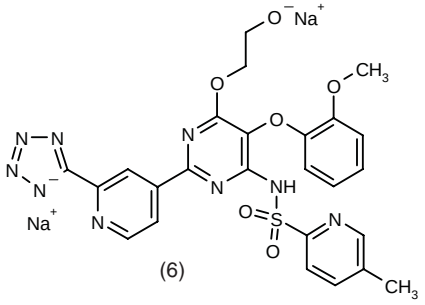

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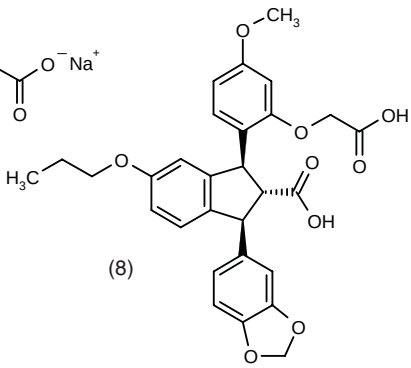

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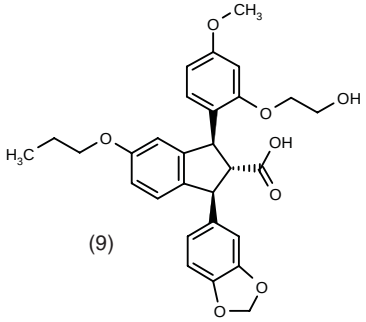

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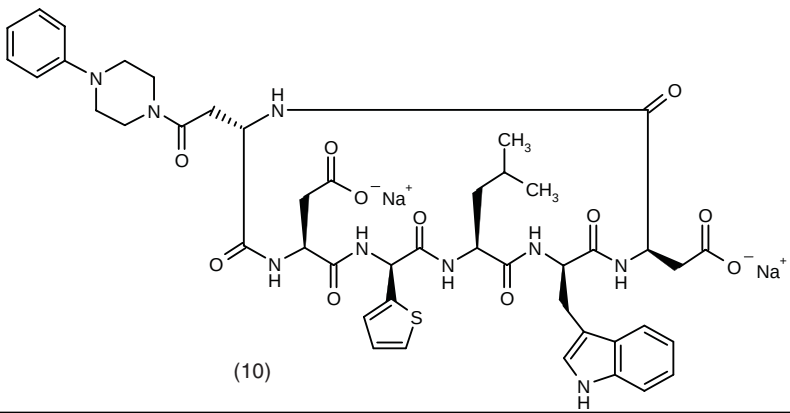

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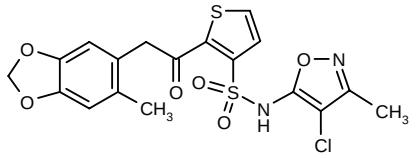
Table I: Continued.



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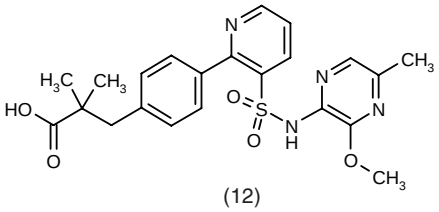

(12)

[1]Licensed from Roche. [2]Primarily a tool compound.

Table II: Binding affinity (displacement of [^{125}I]-endothelin-1 except where otherwise indicated) of selected compounds (data from Prous Science MFLine database).

Compound	Parameter (nM)	ET_A	ET_B	Material	Ref.
Bosentan	IC_{50}	7.13	475[a]	CHO cells, human receptor	65
	K_i	3.67	423[a]	CHO cells, human receptor	65
	K_i	3.40	29.6[a]	CHO cells, human receptor	66
BQ-123	K_i	40.5[b]	>2300[b]	CHO cells, human receptor	67
	K_d	0.73	24,300	Heart, human	68
	IC_{50}	22		Smooth muscle, pig	69
	IC_{50}		18,000	Cerebellum, pig	69
CI-1020	IC_{50}	0.23	2460[a]	CHO cells, human receptor	65
	K_i	0.17	134	CHO cells, human receptor	70
Endothelin-1	IC_{50}	0.3		CHO cells, human receptor	71
	IC_{50}		0.2	Placenta, human	71
	K_i		0.019[a]	Colon, rat	72
	K_i	0.10	0.07	CHO cells, human receptor	73
Endothelin-3	K_i	13	0.014[a]	Colon, rat	72
		20	0.07	CHO cells, human receptor	73
J-104132	K_i	0.034	0.104	CHO cells, human receptor	74
LU-127043[c]	K_i	6.0	371	CHO cells, human receptor	1
S-0139	K_i	1.0		Aortic smooth muscle, rat	75
	K_i		1000	Girardi heart cells, human	75
	IC_{50}	0.61		Aorta, pig	76
Ro-61-1790	K_i	0.6	1930	COS cells, human receptor	77
Sarafotoxin	K_i	>300	0.13	CHO cells, human receptor	73
SB-209670	K_i	0.38[d]	11.8[d]	CHO cells, human receptor	66, 74, 76, 78
SB-217242	K_i	1.23[d]	124[d]	CHO cells, human receptor	1, 74, 79
TAK-044	IC_{50}	0.24	130	CHO cells, human receptor	80
TBC-11251	IC_{50}	1.4		Human medulla blastocytes	81
	IC_{50}		9800	COS cells, human receptor	81
ZD-1611	IC_{50}	2.4	3162	MEL cells, human receptor	82

[a]Displacement of [^{125}I]-endothelin-3. [b]Displacement of [^{3}H]-SB-209670. [c]LU-127043 is the racemic form of the more active enantiomer LU-135252. [d]Mean value from different experiments using the same method.

rat hearts where the compound (1 and 10 μM) inhibited the polymorphonuclear leukocyte (PMN)- or neutrophil-mediated contractile dysfunction which follows myocardial ischemia. LU-135252 significantly enhanced the recovery of left ventricular developed pressure and the rate pressure product during reperfusion with human PMNs or neutrophils after 20 min of global ischemia (32, 33).

Further *in vivo* studies in rats have indicated that administration of LU-135252 (10 or 30 mg/kg/day) 7 days following myocardial infarction due to coronary artery ligation prevents left ventricular remodeling and improves cardiac function. In this model, pressor responses to ET-1 were significantly reduced and systolic blood pressure was decreased in LU-135252-treated rats as compared to controls. While only the high dose of LU-135252 was found to decrease heart rate, both doses concentration-dependently restored cardiac output and left ventricular dilatation and deterioration of the fractional shortening was decreased in a similar manner. Moreover, after

Table III: Inhibition of endothelin-1-induced vasoconstriction by selective ET_A receptor antagonists (data from Prous Science MFLine database).

Compound	Parameter	Value/range	Material	Ref.
Bosentan	pA_2	7.15[a]	Aortic artery, rat	83, 84
BQ-123	pA_2	7.4	Coronary artery, pig,	69
	pK_b	6.65	Pulmonary artery, human	85
CI-1020	pA_2	8.4	Mammary artery, human	86
	pA_2	8.1	Coronary artery, human	86
	pA_2	7.5	Femoral artery, rabbit	87
J-104132	pA_2	9.7	Iliac artery, rabbit	74
LU-127043[b]	pA_2	7.3	Aortic artery, rabbit	1
S-0139	pA_2	8.8	Aortic artery, rat	75
Ro-61-1790	pA_2	9.5	Aortic artery, rat	77
SB-209670	pA_2	9.8	Aortic artery, rabbit	1
	pK_b	9.2	Pulmonary artery, human	85
	pK_b	6.7	Pulmonary artery, rabbit	73
SB-217242	pA_2	8.10	Pulmonary artery, guinea pig	88
	pK_b	8.35	Aortic artery, rat	89
	pK_b	8.30[a]	Pulmonary artery, human	87, 89
TAK-044	pA_2	8.4	Coronary artery, pig	90

[a]Mean value from different experiments using the same method. [b]LU-127043 is the racemic form of the more active enantiomer of LU-135252.

10 weeks, left ventricular end-diastolic and central venous pressures were normalized. Although LU-135252 treatment did not prevent cardiac hypertrophy, left ventricular collagen density was decreased (34).

In contrast, another study has shown that early administration of LU-135252 (60 mg/kg b.i.d by gavage for 4 weeks) to rats 24 h following experimental acute myocardial infarction resulted in impairment in scar healing and in left ventricular dilatation and dysfunction. LU-135252 treatment reduced pulmonary ET-1 levels, although cardiac concentrations were unaffected. However, a decrease in right ventricular systolic pressure and right atrial pressure was observed (35).

The cardioprotective effects of LU-135252 have also been demonstrated in the pig. LU-135252 treatment (1 and 5 mg/kg i.v.) resulted in a significant reduction in mean arterial pressure, whilst heart rate, coronary blood flow and resistance were not affected. When LU-135252 was administered i.v. 20 min prior to myocardial ischemia (induced by 45 min coronary occlusion followed by 4 h reperfusion), the infarct size was significantly reduced from 81 ± 5% in untreated animals to 64 ± 35 and 35 ± 4% in animals receiving 1 or 5 mg/kg, respectively (36).

Studies have also been performed using a species of pig (German Landrace) with hearts lacking collateral circulation thus making them susceptible to ischemic rhythm disturbances and ventricular fibrillation. LU-135252 (1 or 3 mg/kg i.v.) was administered to pigs 15 min prior to occlusion of the left anterior descending coronary artery for up to 90 min to induce fibrillation. Although neither dose affected heart rate, blood pressure or ventricular contractility during the 15 min pretreatment period, within 20 min of ischemia, the 3 mg/kg dose prolonged regular sinus rhythms and significantly reduced the incidence of ventricular extrasystoles by 50 and 87%, respectively. The total incidence of ventricular fibrillation was reduced (80% *vs.* 50%, $p = 0.17$) and late ventricular fibrillation occurring after 20 min of ischemia was decreased (78% *vs.* 38%, $p = 0.12$) in LU-135252-treated animals (37).

LU-135252 was found to significantly reduce blood pressure in normotensive dogs (38) and to decrease the number of cyclic coronary flow reductions due to intraarterial heart thrombus formation and vasoconstriction following local coronary artery injury (39). In addition, the agent was effective in the canine model of experimental heart failure where dogs received the agent (50 mg/kg/day p.o.) beginning on day 3 and continued for 3 weeks during pacing-induced cardiomyopathy. A significant attenuation in the increase in mean pulmonary artery and left ventricular end-diastolic pressure was observed in treated dogs after 2 weeks of pacing. The increase in plasma norepinephrine levels was also significantly attenuated as compared to placebo-treated control animals (40). Other studies using canine models of ventricular pacing-induced congestive heart failure also demonstrated improvements in cardiovascular hemodynamics including cardiac output in animals subjected to severe (21 day pacing) congestive heart failure; no improvements were observed in animals receiving less severe (10 day) pacing (41). Interestingly, another study in dogs showing increased expression of preproET-1 mRNA in the left ventricle and lung following severe heart failure (3 weeks of rapid pacing), has reported that although expression was unaltered in the left ventricle of LU-135252-treated dogs, a significant reduction in preproET-1 mRNA was observed in the lung as compared to untreated animals (42).

As cited above, vasoconstrictor properties *in vitro* of the compound in its less active formulation, racemic LU-127043, were demonstrated (1). Table II further shows the activity of selected compounds for antagonizing ET-1-induced contractile response in a range of arterial tissues. Although LU-135252 may decrease blood pressure in normotensive animals (36, 38), antihypertensive properties of LU-135252 have mainly been shown *in vivo* by using a range of animal models such as those using the salt-related hypertension. Studies have demonstrated that ET-1 through activation of ET_A receptors causes vasoconstriction and proliferation resulting in alterations in vascular function and structure in salt-sensitive hypertension, a major risk factor in cardiovascular disease. Thus, blockade of this receptor can improve endothelial function and prevent structural changes.

Salt-sensitive and salt-resistant Dahl rats were fed a high sodium diet with or without LU-135252 (60 mg/kg/day) for 2 months. Treatment with the antagonist was found to partly reduce the sodium-induced increases in systolic blood pressure caused by the diet. However, LU-135252 treatment significantly normalized the sodium-induced changes in vascular reactivity, tissue ET-1 content and vascular structure; no changes were observed in the salt-resistant animals (43). Similar results were obtained in rats in which deoxycorticosterone acetate (DOCA)-salt hypertension was induced by unilateral nephrectomy and implantation of DOCA (200 mg/rat). LU-135252 (50 mg/kg/day p.o. for 4 weeks) was also shown to lower systolic blood pressure and reduced development of small mesenteric and renal artery hypertrophy. Moreover, this reduction in blood pressure and inhibition of small artery morphological changes appeared to improve survival rates (44).

Since it is known that flow or shear stress-induced dilation is attenuated in hypertension, a study has compared the effects of LU-135252 (50 mg/kg/day) treatment for 2 weeks on the dilator response of isolated resistance arteries to flow in normotensive and spontaneously hypertensive (SHR) rats. Although systolic arterial pressure was not altered by LU-135252 in either strain, flow-induced dilation was significantly increased by 73% in SHR but not normotensive rats suggesting that flow-induced ETA stimulation was effectively suppressed. In addition, acetylcholine-induced dilation was also improved by chronic LU-135252 treatment; myogenic and phenylephrine-induced tone was not affected by LU-135252 treatment (45).

LU-135252 (50 mg/kg/day) not only normalized blood pressure in rats in which L-NAME was administered to induce hypertension (NO deficient hypertension model) (46), but it also has been shown to reduce neointimal thickening in several animal models. In rats in which angiotensin II (200 ng/kg/min) was administered to induce hypertrophy of small arteries, LU-135252 (50 mg/kg/day) partially reduced blood pressure and blocked the increase in media thickness, media/lumen ratio and cross-sectional area of basilar and small mesenteric arteries (47).

On the other hand, in a pharmacological model of pulmonary hypertension LU-135252 administration (50 mg/kg/day by gavage) inhibited that pulmonary hypertension in rats induced by monocrotaline – pulmonary hypertension is induced by monocrotaline because of reducing responsiveness of vascular smooth muscle to NO –, thus showing in this model its effectiveness against pulmonary hypertension (48). Title compound was also shown to have possible therapeutic value as a treatment for atherosclerosis. In apoE knock out mice fed a high fat diet and given the agent for 30 weeks, lesion formation was minimized throughout the aortic tree treatment although no effects were observed on plasma cholesterol levels (49). Furthermore, stenosis and neointimal formation were significantly reduced LU-135252 (20-100 mg/kg/day) treatment in several animals models including rabbits in which nonoccluding silastic collars were perivascularly implanted and in rats, dogs and pigs following percutaneous transluminal coronary angioplasty (PTCA) (50-52). In addition, in the diabetic rat, vascular hyalin deposition was dramatically reduced by LU-135252 treatment demonstrating that this agent may be of therapeutic importance in blocking diabetic angiopathy (53).

ET-1 has been shown to play a role in the pathogenesis of acute renal failure and a number of studies have demonstrated the protective effects of LU-135252 during this pathology. LU-135252 (100 mg/kg/day p.o.) was administered to rats 1 h following clamping of both renal arteries and continued for 14 days or rats were a given the agent (10 mg/kg i.v.) on days 0, 1, 3, 6, 9 and 14 following acute renal failure. With the former treatment, serum creatinine and fractional sodium excretion were significantly lower while creatinine clearance significantly increased in treated animals as compared to the vehicle group. In rats receiving the latter regimen, renal and cortex blood flow were increased for the first 9 days and medulla blood flow significantly increased on days 1 and 6 following acute renal failure (54). Similar improvements in renal function were obtained in other studies in which rats were subjected to two stage five-sixth nephrectomies and treated with LU-135252 (50 mg/kg/day) for 3 weeks (55).

LU-135252 treatment (50 mg/kg b.i.d. p.o.) was also effective in uninephrecotomized rats with induced passive Heymann nephritis. Results showed a decrease in proteinuria of 23% while combination therapy with the agent and the angiotensin converting enzyme (ACE) inhibitor, trandolapril (1 mg/kg) in the drinking water, reduced urinary proteins by 45%. These results suggest that blocking both angiotensin II and the ET_A receptor may have the therapeutic advantage in treatment of renal disease in those patients who respond only partially to ACE treatment alone (56). Similarly, although plasma ET-1 levels were unaffected, urinary concentrations levels were found to decrease more than 50% in rats with streptozotocin-induced diabetes mellitus receiving long term LU-135252 (100 mg/kg/day) for 160 days (57).

Further renoprotective effects were noted in a study demonstrating that the increased ET-1 levels in rat kidney

tissue accompanying angiotensin II (200 ng/kg/min) treatment were significantly reduced from 58 ± 10 to 17.6 ± 6.7 pg/mg tissue with combination LU-135252 (50 mg/kg/day) treatment (58). Renal protection as reflected by improved renal function (*i.e.*, reduced proteinuria) was also observed with LU-135252 in combination with the vasodilator hydralazine in stroke prone SHR (SHRSP) rats (59). Moreover, when salt-loaded SHRSP rats were subjected to uninephrectomy, LU-135252 not only abolished glomerulosclerosis, vascular damage and tubulointerstitial damage but prevented death associated with salt loading (60).

LU-135252 (30 mg/kg/day p.o. for 14 days) was suggested to improve recovery following posttransplant acute renal failure in bilaterally nephrectomized rats without immunosupressant treatment, although creatinine clearance, fractional sodium excretion and urinary ET-1 excretion were unaffected by compound treatment (61).

Although LU-135252 (80 mg/kg/day p.o. for 6 weeks) treatment following biliary duct occlusion in rats was shown to significantly reduce liver and spleen weights reflecting a 40-50% reduction in collagen accumulation and a down regulation of hepatic procollagen levels, mortality was significantly increased up to 50% due to intestinal hemorrhage (62).

Pharmacokinetics

As of yet, the pharmacokinetics of LU-135252 has not been clearly reported. However, development of a sensitive radioreceptor assay using porcine aortic smooth muscle membranes and [^{125}I]-ET-1 radioligand could allow future elucidation of the pharmacodynamics of this agent. ET-1 at pathophysiological and clinical (40 pg/ml) concentrations did not interfere with the assay. Recovery of 60-1000 nM LU-135252 ranged from 79-91%. The sensitivity of this assay for plasma or urine was determined to be 19 nM with constructed calibration curves ranging from 18.7 to 2400 nM. The assay was tested with urine and plasma from rats and dogs and found to practical and reproducible. Although a slight loss of signal was detected after 24 h at room temperature, stable storage at 4 °C of LU-135252 added to plasma was observed for up to 1 week (63).

Clinical Studies

LU-135252 is currently undergoing phase II trials for the treatment of congestive heart failure and hypertension. It is expected to block the deleterious effects of ET-1 and prevent structural damage in blood vessels and the heart, and may reverse remodeling in the heart (64).

Conclusions

ET-1 is an endothelium-derived peptide with potent vasoconstrictor and pressor effects and co-mitogenic properties, which acts through two identified subtype receptors, ET_A and ET_B. ET-1 in its mature form is derived from its immediate precursor “big ET-1” by means of ECE-1. ET-1 is known to play an important role in maintaining peripheral vascular tone and blood pressure, affecting functionality of heart, kidney and CNS. Thus, compounds acting on endothelin system have focussed great attention by which the hypotensive effects of ECE inhibitors and endothelin receptor antagonists should be useful in the treatment of hypertension and related diseases. ET-1 receptor acting agents emerge as one of the most interesting pharmacological goals for the treatment of such diseases and pre-clinical studies with either mixed ET_A/ET_B or selective ET_A receptor antagonists have provide exiting results. Indeed, with the promising results revealed in the preclinical studies involving animals models, the selective ET_A antagonist LU-135252 has the potential to be therapeutically useful in chronic heart failure and hypertension, two extremely common conditions. In addition, LU-135252 may also be effective against renal failure associated with several conditions. The results from the first clinical trials are awaited.

Manufacturer

Knoll AG (DE); codeveloped with Hoechst Marion Roussel AG (DE).

References

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