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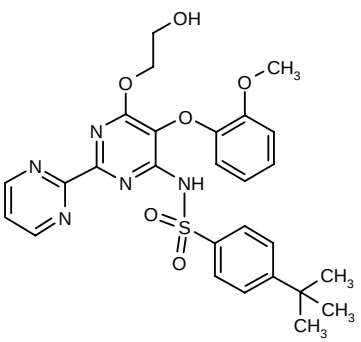
Bosentan

Rec INN; USAN

Treatment of Pulmonary Hypertension
Treatment of Heart Failure
Antihypertensive
Endothelin ET_A/ET_B Antagonist

Ro-47-0203
Tracleer®

4-*tert*-Butyl-*N*-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(2-pyrimidinyl)pyrimidin-4-yl]benzenesulfonamide



$C_{27}H_{29}N_5O_6S$ Mol wt: 551.6260

CAS: 147536-97-8
CAS: 157212-55-0 (as monohydrate)

EN: 203927

Synthesis

The condensation of diethyl (2-methoxyphenoxy)malonate (I) with pyrimidine-2-carboxamidine (II) by means of NaOMe (Na in MeOH), followed by treatment with NaOH, provides the dihydroxy pyrimidine derivative (III), which is converted into the dichloro derivative (IV) by treatment with refluxing PCl_5 and DIEA (1). Compound (IV) can also be obtained from the pyrimidinedione (V) by reaction with phosphorus oxychloride at 90 °C (2, 3). Reaction of compound (IV) with 4-*tert*-butylbenzenesulfonamide (VI), performed either directly in DMSO (1) or by means of benzyltriethylammonium chloride (BTEAC) or tetrabutylammonium bromide (TBAB) and K_2CO_3 in refluxing toluene (2, 3), gives compound (VII). Finally, this compound is converted into bosentan by reaction with sodium and ethylene glycol (VIII) (1-3).

Alternatively, the conversion of compound (VII) into bosentan can also be performed by reaction of compound (VII) with ethylene glycol mono *tert*-butyl ether (IX) by means of NaOH in toluene, yielding the *tert*-butyl ether derivative (X), which is then treated with formic acid at 85-90 °C in toluene to give the 2-(formyloxy)ethoxy derivative (XI). Finally, the formyl group is removed by treatment of (XI) with NaOH in H_2O (1-3). Scheme 1.

Introduction

Pulmonary hypertension is a rare and serious, progressive lung disorder characterized by blood pressure above the normal range within the pulmonary arterial system. There are two types of pulmonary hypertension: primary pulmonary hypertension which occurs in the absence of a known cause, and secondary pulmonary hypertension, which occurs as a result of another condition, usually scleroderma, chronic obstructive pulmonary disease (COPD) or systemic lupus erythematosus. Symptoms of the disorder include tiredness, shortness of breath after minimal exertion, edema, dyspnea, dizzy spells and fainting.

Although the pathogenesis of pulmonary hypertension is poorly understood, it is thought to begin with lung endothelial cell injury which leads to increased smooth muscle contraction and vessel narrowing, and eventually to the deposition of tissue in the pulmonary artery walls and scarring (fibrosis). The vessel walls then become stiff and thickened, or even completely blocked. Blood clots tend to form within the smaller arteries. The resulting increased resistance to blood flowing through the vessels places a strain on the right ventricle, which becomes enlarged. The overworked and enlarged right ventricle

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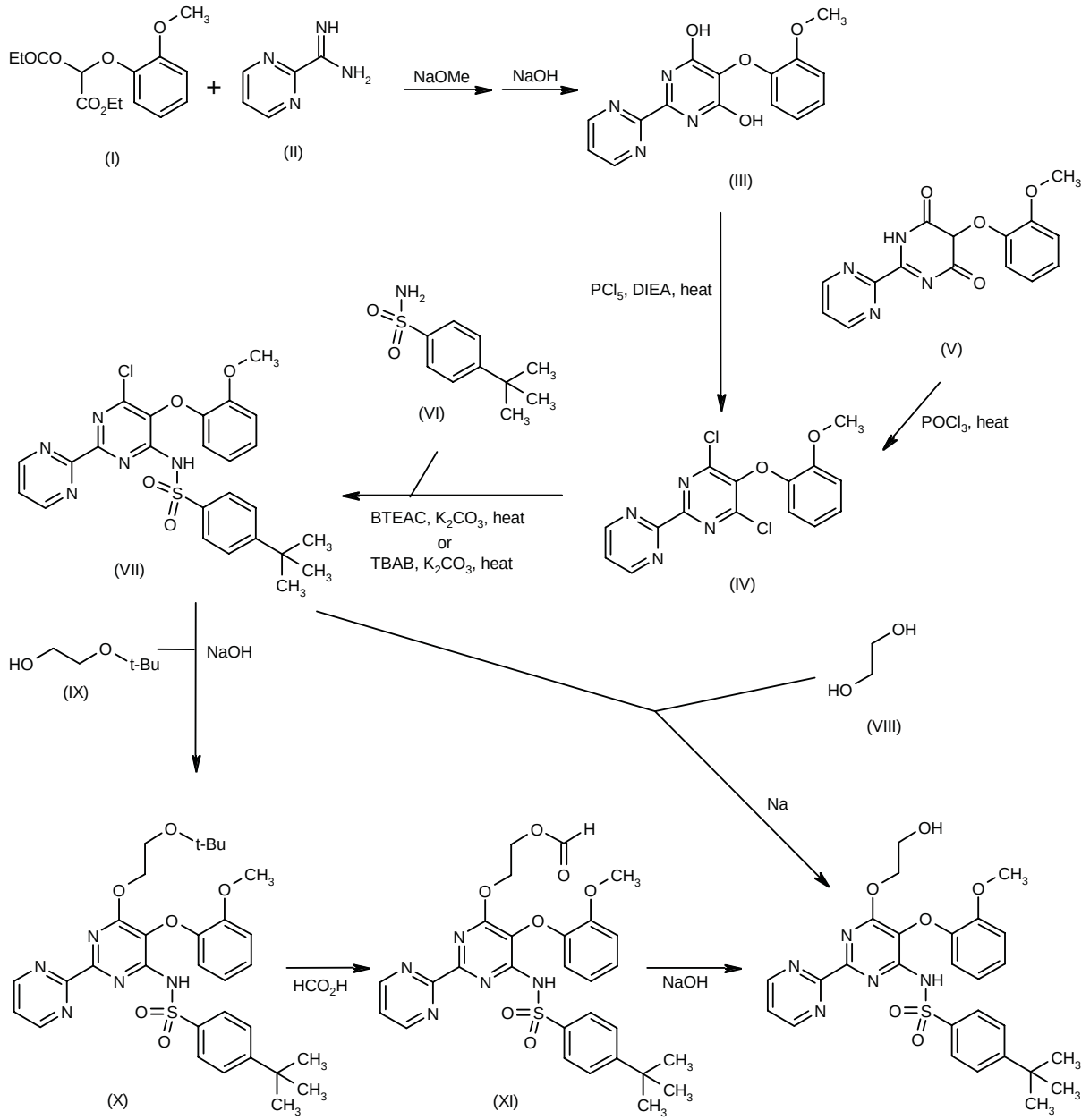
Scheme 1: Synthesis of Bosentan



gradually becomes weak and loses its ability to pump enough blood through the lungs. The right side of the heart may eventually fail altogether, resulting in death.

The disease is rare, occurring in an estimated 35,000-70,000 patients worldwide. The only known proven risk factors for primary pulmonary hypertension, according to the WHO, are female gender, HIV infection, the drugs aminorex, fenfluramine and dexfenfluramine, and toxic rapeseed oil. However, the long-term prognosis is poor, with a 5-year survival rate of approximately 50%.

Until recently, there were no treatments for patients diagnosed with pulmonary hypertension, but in recent years the availability of pulmonary vasodilators has significantly improved the treatment outcome. Treatment regimens include drugs designed to reduce or facilitate the work of the right ventricle. Anticoagulants prevent blood clots and allow blood to flow more freely; diuretics decrease the amount of fluid in the body, thereby reducing the workload of the heart; calcium channel blockers relax smooth muscles in blood vessels and the cardiac

wall, improving the heart's ability to pump blood; and prostacyclin, a vasodilator, helps the blood vessels to dilate and prevents blood clots from forming. Until now, only two drugs had been specifically approved for the indication of pulmonary hypertension: GlaxoSmithKline's epoprostenol sodium (Flolan®) and INO Therapeutics' nitric oxide for inhalation (INOmax®).

Endothelin has recently been shown to play a pivotal role in the development of pulmonary hypertension and elevated endothelin concentrations have been found to be strongly correlated with disease severity. Endothelin antagonists are therefore considered to represent an especially promising new approach to the treatment of pulmonary hypertension. The selective mixed endothelin ET_A/ET_B receptor antagonist bosentan (Tracleer®) has just become the first endothelin antagonist to reach the market for pulmonary hypertension.

Pharmacological Actions

Bosentan (Ro-47-0203) is a nonpeptide mixed endothelin ET_A and ET_B receptor antagonist obtained by structural modification of Ro-46-2005, the first orally active ET receptor antagonist. Binding assays have been performed using recombinant and native receptors. The compound displayed similar affinity for ET_A receptors in human smooth muscle cells and rat mesangial cells (K_i = 4.7 nM and 4.1 nM, respectively) and recombinant ET_A receptors expressed in CHO cells (K_i = 6.5 nM), while it had 10-fold lower affinity for the human receptor expressed in membranes of baculovirus-infected insect cells (K_i = 43.0 nM). Its affinity for ET_B receptors was lower than for ET_A receptors, as demonstrated using human placenta (K_i = 95 nM), porcine cerebellum membranes (K_i = 152 nM) and porcine tracheal membranes (K_i = 38-69 nM), and recombinant human receptors expressed in CHO cells (K_i = 343 nM) and Sf9 cells (K_i = 730 nM). Saturation binding experiments demonstrated competitive binding to both ET_A and ET_B receptor subtypes. Its selectivity for ET receptors was confirmed by its lack of effect on the binding of a range of neurotransmitters, peptides, eicosanoids and ion channels at concentrations up to 10 μM. In functional assays, bosentan competitively inhibited ET-1-induced contractions in isolated rat aorta (ET_A) and sarafotoxin S6C-induced contractions in isolated rat trachea (ET_B) with respective pA_2 values of 7.2 and 6.0, as well as sarafotoxin S6C-induced endothelium-dependent relaxation of rabbit superior mesenteric artery (ET_B; pA_2 = 6.7) (4).

In vivo, bosentan itself had no effect on blood pressure in rats, but it was able to significantly attenuate the pressor effect of big ET-1 in pithed rats at doses of 1-100 mg/kg p.o., and it also attenuated both the pressor (3-30 mg/kg i.v.) and depressor (10-30 mg/kg i.v.) effects of ET-1 in these animals. At a dose of 10 mg/kg i.v., it completely inhibited the depressor effect of the ET_B agonist sarafotoxin S6C, whereas a higher dose of 30 mg/kg i.v. was required for significant antagonism of the pressor response to sarafotoxin S6C. Bosentan demonstrated a long duration of action in these studies. The effect of big ET-1 was inhibited by 61% and 65%, respectively, at 45 min and 6 h after a dose of bosentan of 30 mg/kg p.o. Although at this dose only 16% inhibition was seen at 24 h after administration, the higher dose of 100 mg/kg p.o. inhibited the response to big ET-1 by 37% at 24 h (4).

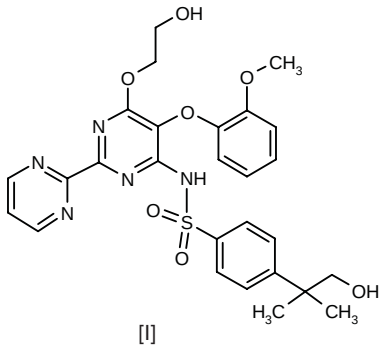
Bosentan has undergone extensive evaluation in experimental animal models, particularly models of hypertension, heart failure and pulmonary hypertension. In DOCA-salt hypertensive rats, chronically administered bosentan blunted the elevation in blood pressure, and suppressed cardiac hypertrophy, perivascular and subendocardial fibrosis, and renal, coronary and mesenteric arterial hypertrophy (5-8).

Rats with chronic heart failure following myocardial infarction showed a significant and sustained decrease in mean arterial pressure (MAP) following acute administration of bosentan, in the absence of an increase in heart rate, and the effect was additive in combination with the angiotensin-converting enzyme (ACE) inhibitor cilazapril (5, 9). In a similar study, chronic administration of bosentan suppressed cardiac remodeling and reduced mortality to a similar extent as ACE inhibitors. Treatment with bosentan was also associated with an increase in cardiac index and decreases in heart rate and left ventricular fibrosis and hypertrophy (5, 10). Studies in rats with congestive heart failure induced by aorto-caval fistula demonstrated its ability to significantly improve renal blood flow (5, 11). Bosentan also reduced pulmonary artery pressure, prevented left ventricular dysfunction and attenuated left ventricular remodeling following acute or chronic administration in dogs with heart failure induced by coronary microembolizations (5, 12-14).

The beneficial effects of bosentan in pulmonary hypertension appear to result from a combination of vasodilating, antifibrotic and antiinflammatory effects in the lung. In a rat model of pulmonary hypertension induced by chronic hypoxia, bosentan given prophylactically or therapeutically reduced right ventricular hypertrophy, prevented pulmonary arterial remodeling and significantly reduced the development of pulmonary hypertension (5, 15, 16). The compound reduced pulmonary arterial pressure and right ventricular hypertrophy in a rat model of monocrotaline-induced chronic pulmonary hypertension (5, 17). Antiinflammatory and antifibrotic effects in the lung have also been reported (18, 19). Dogs with pulmonary hypertension induced by chronic pulmonary embolism showed almost complete attenuation of the pulmonary vascular remodeling seen in untreated controls, which was correlated with suppression of the increase in ET_A and ET_B receptor expression seen in the untreated animals (20).

Pharmacokinetics and Metabolism

Pharmacokinetic studies in animals demonstrated large interspecies differences in the plasma clearance of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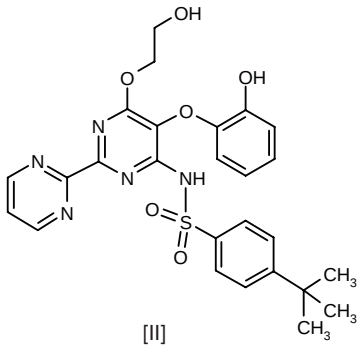
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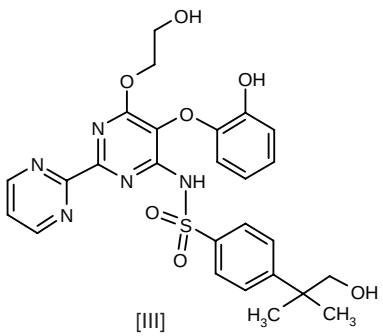
[III]

bosentan of about 60-fold, from approximately 1.5 ml/min/kg in dogs to 72 ml/min/kg in rabbits. As the compound has been reported to be almost exclusively eliminated in the bile following hepatic metabolism via the cytochrome P-450 system, its metabolism was investigated *in vitro* in liver microsomes and hepatocytes. A similar pattern of clearance (metabolism) was seen. The metabolism of bosentan by human liver microsomes and hepatocytes was slow and comparable to that for dog preparations. Based on these findings, a systemic plasma clearance of 1-2 ml/min/kg was predicted for man and was subsequently confirmed in healthy volunteers (~2 ml/min/kg) (21).

The metabolism of bosentan occurs mainly in the liver via cytochrome P-450 3A4 and 2C9, producing three metabolites: the hydroxylated compound [I], the demethylated metabolite [II] and the hydroxylated and demethylated metabolite [III] (22).

The pharmacokinetics of single rising oral and i.v. doses of bosentan were assessed in two double-blind, placebo-controlled studies in healthy volunteers. In one study, the subjects were administered oral doses of 3-2400 mg and i.v. doses of 10-750 mg, or placebo, and in the other study 6 subjects received a single oral dose of 650 mg and a single i.v. dose of 250 mg in a randomized fashion. The systemic plasma clearance and volume of distribution of the drug both decreased with increasing doses. Following i.v. dosing, clearance decreased from a mean of 11-12 l/h at doses of 10 and 50 mg to 6-7 l/h at doses of 500 and 750 mg, and steady-state volume of distribution decreased from a mean of 0.5-0.7 l/kg at the lower dose levels to about 0.2 l/kg at higher doses. The terminal elimination half-life was about 3-5 h after i.v. and oral dosing, except at higher oral doses (7-8 h after 1200 and 2400 mg p.o.). Peak plasma levels were reached within 2-3 h after oral dosing. Peak plasma levels and AUC increased proportionally with doses up to 600 mg, but at higher doses the increase was much less than proportional to dose. Oral bioavailability was about 50% at the dose of 600 mg, but appeared to decrease at higher doses. Urinary excretion of unchanged drug was very low, accounting for < 1% of dose after i.v. or oral administration. The increase in plasma ET-1 was directly related to the plasma levels of bosentan. Bosentan showed a tendency to decrease blood pressure and increase heart rate and was well tolerated. Nausea and vomiting were seen at higher i.v. doses, and mild headache was reported at oral doses of 300 mg or more and i.v. doses of 250 mg or above (23).

Further studies in healthy male volunteers indicated no clinically relevant effects of bosentan on the pharmacokinetics of digoxin (24). However, concomitant administration of bosentan increased the elimination of the (*R*)- and (*S*)-enantiomers of warfarin (38% and 29% decreases in mean AUC, resectively), resulting in reduced anticoagulant effects of the drug in the majority of subjects and suggesting the need for close monitoring of patients on warfarin upon initation or discontinuation of bosentan (25).

Clinical Studies

Although pharmacological and clinical studies of bosentan suggested potential in several cardiovascular disorders, including hypertension (26-28), coronary artery disease (29) and heart failure (30-35), its clinical development has been initially geared to the treatment of pulmonary hypertension due to the lack of therapeutic options for this disorder.

A double-blind, randomized, placebo-controlled study was conducted in 32 patients with primary pulmonary hypertension or pulmonary hypertension associated with scleroderma, administered bosentan as oral doses of 62.5 mg b.i.d. for 4 weeks followed by 125 mg b.i.d. for 12 weeks, or placebo. The primary endpoint of the trial was the change in exercise capacity, *i.e.*, 6-min walk distance. Bosentan-treated patients showed a significant improvement in exercise capacity at 12 weeks compared to placebo-treated patients, with an increase of about 70 m *versus* a decrease of about 6 m, an effect which was maintained for at least 20 weeks. Cardiac index increased on bosentan and pulmonary vascular resistance decreased on bosentan while it increased on placebo. Bosentan treatment was also associated with a reduction in the Borg dyspnea index and improved WHO functional class. Adverse events were minimal and mainly consisted of reversible elevations in hepatic enzymes (36, 37) (Table I).

Table I: Randomized, double-blind clinical trials of bosentan in patients with primary or secondary pulmonary hypertension.

Study drug	n	Cardiac index (l/min/m^2)	Pulmonary vascular resistance (dyne/s/cm^5)	Pulmonary artery pressure (mmHg)	Pulmonary capillary wedge pressure (mmHg)	6-min walk test (m)	WHO functional class	Conclusions	Ref.
Bosentan	32							Bosentan improved	36
62.5-125 mg po bid x 12 w		+0.5*	–223*	–1.6**	0.1**	+70**	↓**	exercise capacity	
Placebo		–0.5	+191	5.1	3.9	–6	=	and cardiopulmonary hemodynamics and improved WHO functional class, with good tolerability	
Bosentan	213							Bosentan improved	38
125 mg po bid x 16 w						+27*	↓**	exercise capacity	
Bosentan							=	and reduced the risk	
250 mg po bid x 16 w						+46*		of clinical worsening,	
Placebo						–8		with good tolerability	

*$p < 0.001$; **$p < 0.05$

The BEATHE-1 study was designed to extend the results from the above preliminary study to a larger patient population. A total of 213 patients with primary or secondary pulmonary hypertension were enrolled in this multinational, double-blind, randomized, placebo-controlled trial to receive either placebo or bosentan at doses of 125 mg b.i.d. or 250 mg b.i.d. for at least 16 weeks. As above, the primary endpoint was change in exercise capacity (6-min walk test). An improvement in walking distance was obtained on bosentan, as was improvement in the Borg dyspnea index and WHO functional class. These beneficial effects did not appear to be dose-dependent and both doses were well tolerated. The most frequent adverse event on bosentan was asymptomatic elevations in liver transaminases (38) (Table I).

An analysis of the safety database of bosentan clinical trials indicated that reversible liver injury, *i.e.*, asymptomatic transaminase elevations, occurred in 2-18% of patients and was dose-dependent. This side effect of the drug appeared to be mediated, at least in part, by inhibition of the hepatocanalicular bile salt export pump (Bsep in rodents; BSEP or ABCB11 in humans), leading to intracellular accumulation of cytotoxic bile salts (39).

Bosentan has just recently been introduced for the first time in the U.S. by Actelion for improving exercise capacity and decreasing the rate of worsening in patients with pulmonary arterial hypertension with significant limitation of physical activity (WHO class III and IV). It is available as tablets of 62.5 mg and 125 mg (40).

Manufacturers

Discovered by F. Hoffmann-La Roche AG (CH), licensed to Actelion Ltd. (CH) who is developing the product in collaboration with Genentech, Inc. (US).

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