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RIOCIGUAT

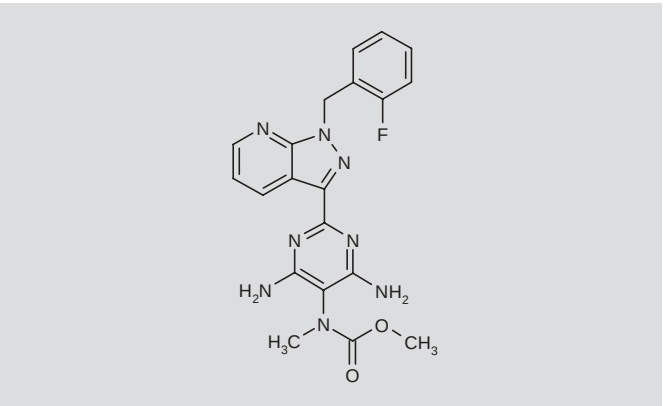
Rec INN

Soluble Guanylate Cyclase Stimulator
Treatment of Pulmonary Hypertension

BAY 63-2521

[4,6-Diamino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl]methylcarbamic acid methyl ester

InChI=1/C20H19FN8O2/c1-28(20(30)31-2)15-16(22)25-18(26-17(15)23)14-12-7-5-9-24-19(12)29(27-14)10-11-6-3-4-8-13(11)21/h3-9H,10H2,1-2H3,(H4,22,23,25,26)



$C_{20}H_{19}FN_8O_2$
Mol wt: 422.4157
CAS: 625115-55-1
EN: 355544

ABSTRACT

Pulmonary hypertension (PH) is a debilitating, ultimately fatal disease in which elevated pulmonary arterial pressure causes vascular remodeling, right heart hypertrophy and heart failure. Therapies have only been approved for one subcategory of PH (pulmonary arterial hypertension, or PAH), and survival rates remain low. Riociguat (BAY 63-2521) is a first-in-class drug developed by Bayer Schering Pharma as an oral PH therapy. It stimulates soluble guanylate cyclase (sGC), the receptor of the endogenous vasodilator nitric oxide (NO), by a dual mode of action: increasing sGC sensitivity to NO and directly stimulating sGC when NO is absent or low. Preclinical studies demonstrated vasorelaxant and antiremodeling properties. Phase I and proof-of-concept clinical trials supported further development. An open-label, uncontrolled phase II trial of riociguat in chronic thromboembolic PH and PAH has been completed, and phase III trials have begun in these indications. Further studies will investigate riociguat in PH associated with lung diseases.

SYNTHESIS*

Riociguat can be synthesized as follows:

Treatment of 2-fluorobenzyl bromide (I) with hydrazine hydrate in refluxing EtOH gives 2-fluorobenzyl hydrazine (II) (1), which by cyclocondensation with the sodium salt of ethyl cyanopyruvate (III) — prepared by reaction of diethyl oxalate (IV) with acetonitrile mediated by NaOEt (2) — by means of TFA in refluxing dioxane yields the 5-aminopyrazole derivative (V). Condensation of intermediate (V) with 3-dimethylaminoacrolein (VI) in the presence of TFA in refluxing dioxane provides the pyrazolo[3,4-*b*]pyridine derivative (VII), which is amidated with methanolic ammonia, affording amide (VIII). Treatment of amide (VIII) with pyridine and trifluoroacetic anhydride in THF followed by methanolysis of the resulting nitrile (IX) by means of NaOMe in MeOH generates the methyl imidoate (X), which, without isolation, undergoes amination with NH_4Cl in the presence of glacial acetic acid in refluxing MeOH to give the carboxamidine (XI). Coupling of amidine (XI) with phenylazomalononitrile (XII) by means of NaOMe in DMF at 110 °C affords diamine (XIII), which upon reduction with H_2 over Raney-Ni in H_2O/DMF at 62 °C provides triamine (XIV). Acylation of amine (XIV) with methyl chloroformate (XV) in pyridine affords carbamate (XVI), which is finally *N*-methylated by means of MeI and NaH in DMF (3) or LiHMDS in THF (4). Scheme 1.

BACKGROUND

Pulmonary hypertension (PH) is a debilitating and ultimately fatal disease in which pulmonary vasoconstriction and pulmonary vascular remodeling progressively increase strain on the right side of the heart, eventually causing heart failure (5). According to the Venice classification, PH can be classified into five groups that differ in terms of etiology and treatment: 1) pulmonary arterial hypertension (PAH); 2) PH with left heart disease; 3) PH associated with lung diseases and/or hypoxemia; 4) chronic thromboembolic PH (CTEPH);

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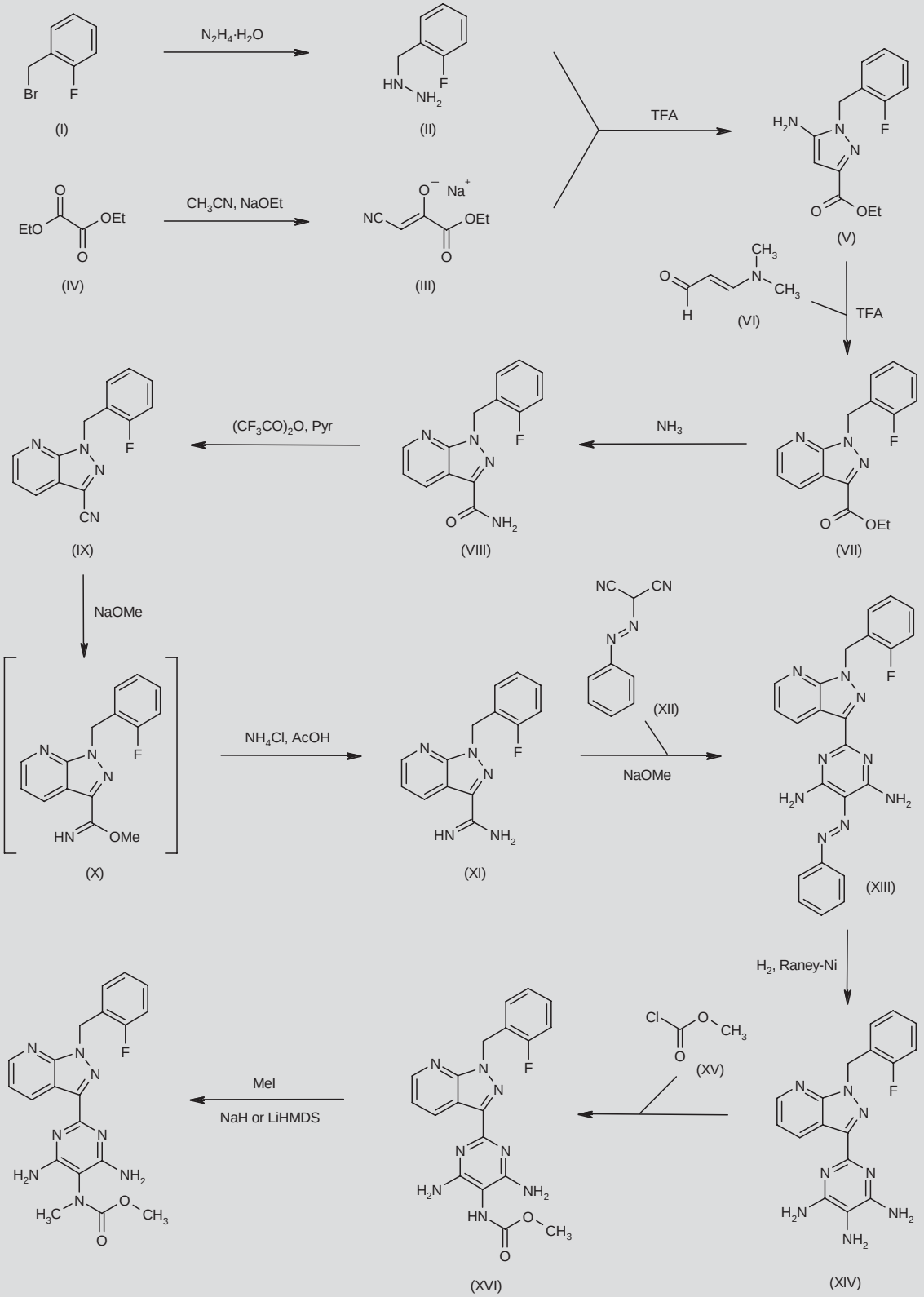
*Synthesis prepared by M. Vicente, R. Castañer.
Prous Science, Provenza 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Riociguat



and 5) miscellaneous (sarcoidosis, histiocytosis X, lymphangiomatosis and compression of pulmonary vessels) (6).

Without treatment, the median life expectancy following a diagnosis of PAH was 2.8 years in a patient registry study conducted in the 1980s (7). The development of PH also substantially worsens the prognosis for patients with left heart disease or lung diseases (8-10). The mean duration of survival following a diagnosis of CTEPH was reported to be 6.8 years without treatment (11), and decreased with increasing pulmonary arterial pressure (12).

Since the launch of intravenous epoprostenol for the treatment of PAH in 1996 (13), several vasodilator therapies have become available, targeting the prostacyclin, endothelin and nitric oxide (NO) signaling pathways (prostanoids, endothelin receptor antagonists and phosphodiesterase [PDE5] inhibitors, respectively; see Figure 1). Although these developments have clearly improved the lives of patients with PAH, survival rates and health-related quality of life remain relatively poor (14).

Furthermore, these therapies have only been approved for the treatment of patients with PAH, a small subset of the total population of patients with PH. Treatment of patients in other PH groups poses many challenges. In PH with left heart disease, reduction of pulmonary vascular resistance by vasodilatation may worsen pulmonary edema (10). In PH associated with chronic obstructive pulmonary disease (COPD), nonselective pulmonary vasodilatation may worsen ventilation/perfusion mismatch by causing vasodilatation in poorly ventilated areas of the lung, directing blood flow towards regions with low oxygen availability and thus reducing the efficiency of gas exchange (15, 16). While PAH therapies may be beneficial in patients with CTEPH (17), randomized, controlled trials of PAH therapies (bosentan, sildenafil and iloprost) in patients with CTEPH have failed to demonstrate any improvement in exercise capacity (18-20). There is, therefore, a substantial unmet need for effective pharmacological therapies to treat patients with PAH or other forms of PH. Since no pharmacological therapies are currently approved for types of PH other than PAH, even symptomatic relief would be a considerable step forward for such patients.

The full potential of the NO signaling pathway in PH pharmacotherapy has not yet been exploited. NO is an endogenous signaling molecule that is released from vascular endothelial cells and binds to its receptor, soluble guanylate cyclase (sGC), in nearby vascular smooth muscle cells. This interaction causes sGC to increase synthesis of the secondary messenger cyclic guanosine monophosphate (cGMP), which in turn promotes vasodilatation and inhibits cell proliferation (Fig. 1) (21, 22). In the lung, NO synthesis is increased in response to alveolar distension and oxygenation. Therefore, NO levels are

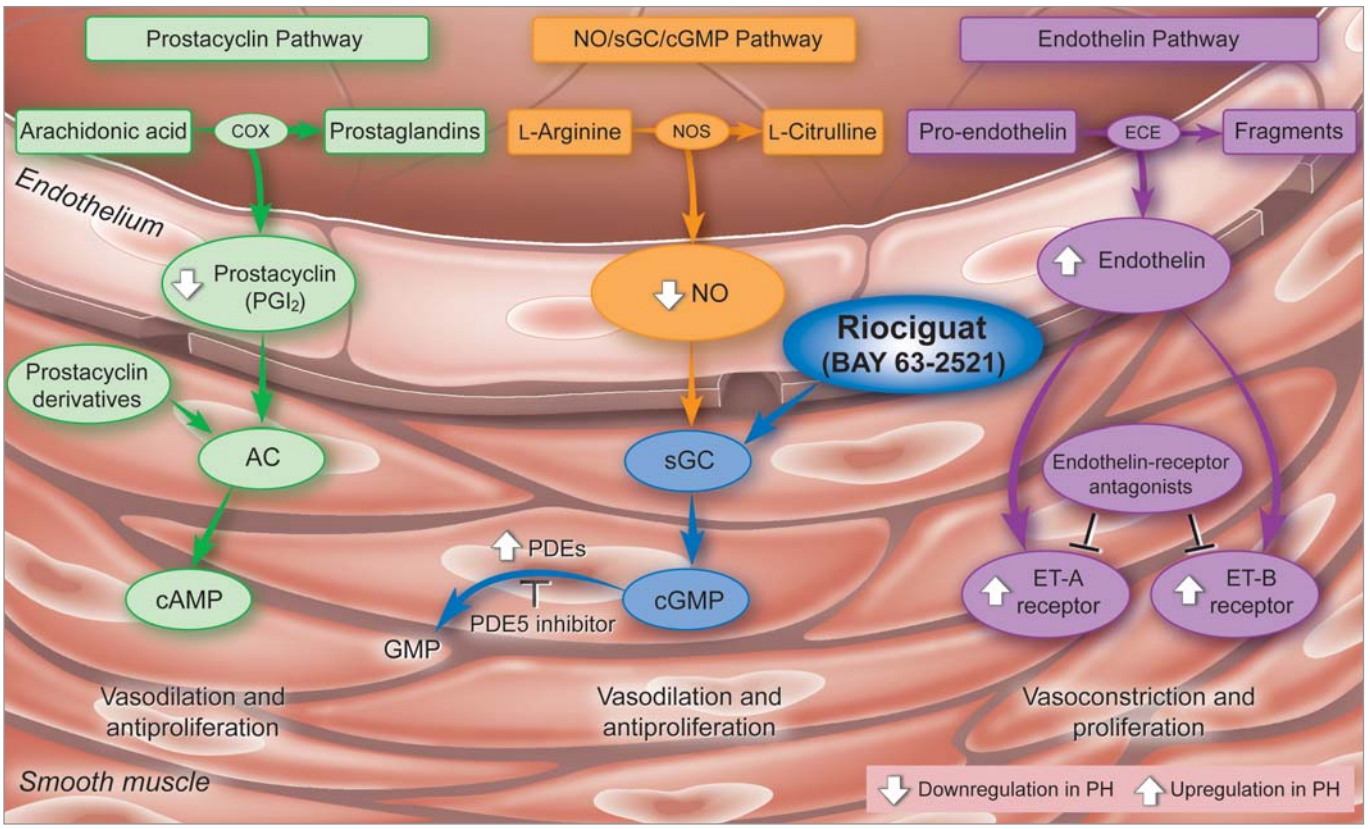


Figure 1. Three major therapeutic target pathways in pulmonary hypertension (PH). AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; (c)GMP, (cyclic) guanosine monophosphate; COX, cyclooxygenase; ECE, endothelin-converting enzyme; ET, endothelin; NO, nitric oxide; NOS, nitric oxide synthase; PDE, phosphodiesterase; sGC, soluble guanylate cyclase. Figure 6 reproduced from Ghofrani, A., Grimminger, F. *Modulating cGMP to treat lung diseases.* In: cGMP: Generators, Effectors and Therapeutic Implications. Schmidt, H.H.H.W., Hofmann, F., Stasch, J.P. (Eds.). Springer-Verlag: Berlin Heidelberg, 2009, 469-83, with permission from Springer-Verlag.

increased preferentially in well-ventilated areas of the lung, promoting local vasodilatation and thus directing blood flow to regions of the lung with the greatest supply of oxygen (23, 24). As such, therapies that act in synergy with endogenous NO would be expected to promote vasodilatation while maintaining ventilation/perfusion matching.

Phosphodiesterase PDE5 inhibitors such as sildenafil prevent cGMP degradation, and thereby increase cGMP levels (Fig. 1) (23, 24). However, these drugs depend on input from NO at the start of the pathway, and their efficacy may thus be limited in the presence of low NO levels. This limitation is relevant to PAH. Expression of endothelial NO synthase (eNOS) was found to be reduced in the lungs of patients with PAH compared with healthy individuals (25), and a small clinical study performed by Chockalingam and colleagues suggested that a substantial proportion of patients with PAH may not benefit from sildenafil treatment (26). In addition, studies of prolonged pulmonary artery obstruction in pigs found reduced pulmonary eNOS expression compared with controls, suggesting that NO production may also be compromised in CTEPH (27).

The discovery of the benzylindazole compound YC-1 as a direct stimulator of sGC provided a much-needed alternative means by which to increase cGMP levels. YC-1 has a dual mode of action, stimulating sGC directly in the absence of NO, and also acting in synergy with NO (28-31). YC-1 provided the lead structure for a chemical optimization program of 2,000 newly synthesized compounds that yielded 2 pyrazolopyridine derivatives, BAY 41-2272 and BAY 41-8543, with improved specificity and potency compared with YC-1 (32-34).

BAY 41-2272 and BAY 41-8543 both demonstrated efficacy in animal models of PH. BAY 41-2272 infusion caused strong pulmonary vasodilatation in sheep models of acute PH and persistent PH of the newborn (35, 36), and inhalation of BAY 41-2272 or BAY 41-8543 (both encapsulated in dry-powder, lipid/protein/sugar-based microparticles) caused pulmonary vasodilatation in a sheep model of acute PH without affecting mean arterial pressure (37). BAY 41-2272 also attenuated PH induced by heparin–protamine interaction in anesthetized dogs (38). Furthermore, BAY 41-2272 demonstrated antiremodeling properties in rats with hypoxia-induced PH, reducing cardiac hypertrophy and attenuating pulmonary arterial wall thickening (39).

Although BAY 41-2272 and BAY 41-8543 showed promise in preclinical studies, further pharmacokinetic optimization was required; profiling of approximately 1,000 additional compounds finally yielded the optimized sGC stimulator riociguat (BAY 63-2521) (4, 40).

PRECLINICAL PHARMACOLOGY

Riociguat (0.01-100 μM) stimulated the activity of purified sGC in a concentration-dependent manner up to 73-fold in vitro and acted in synergy with the NO donor diethylamine (DEA)/NO (0.1 μM) to stimulate sGC activity up to 112-fold (41). Riociguat also exhibited similar properties in endothelial cells, increasing cGMP levels in a concentration-dependent manner and in synergy with NO (42). Biochemical studies revealed that riociguat has the ability to act in an NO-independent but heme-dependent manner. At concentrations of up to 3 μM, riociguat did not inhibit the cGMP-metabolizing phosphodiesterase enzymes PDE1-11. Therefore, the effect of riociguat on cGMP levels is mediated by an increase in the synthesis of cGMP rather than by inhibition of its degradation (41 and Stasch, J.P., personal communication).

At concentrations of 0.01-1 μM riociguat inhibited the contraction of isolated blood vessels (rabbit aorta and saphenous artery, porcine coronary artery and canine femoral vein), and promoted vasorelaxation in arteries isolated from nitrate-tolerant rabbits, suggesting that it is not limited by the tachyphylaxis associated with repeated nitrate administration (4, 42). Riociguat (0.01 μM) decreased acute pulmonary vasoconstriction in isolated mouse lungs. Moreover, in mouse and rat models of PH (induced by hypoxia and monocrotaline, respectively) treatment with riociguat caused a decrease in right ventricular systolic pressure, and reduced right heart hypertrophy (Fig. 2) and vascular remodeling compared with untreated controls (41).

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics, safety and hemodynamic effects of riociguat have been investigated in two clinical trials: 1) a randomized, placebo-controlled, single-blind, parallel-group phase I study examined the effects of oral riociguat at doses of 0.25-5 mg in 45 healthy men aged 18-45 years (43); and 2) an open-label, uncontrolled, proof-of-concept study examined the effects of a single oral dose of riociguat (1 or 2.5 mg) in 15 patients with mild to moderate PH (mean pulmonary vascular resistance > 300 dyn.s/cm^5 and a diagnosis of PAH [n = 10] or distal CTEPH [n = 5]) (44).

Riociguat is orally active and readily absorbed; the mean time to reach peak plasma concentration (C_{max}) following administration of

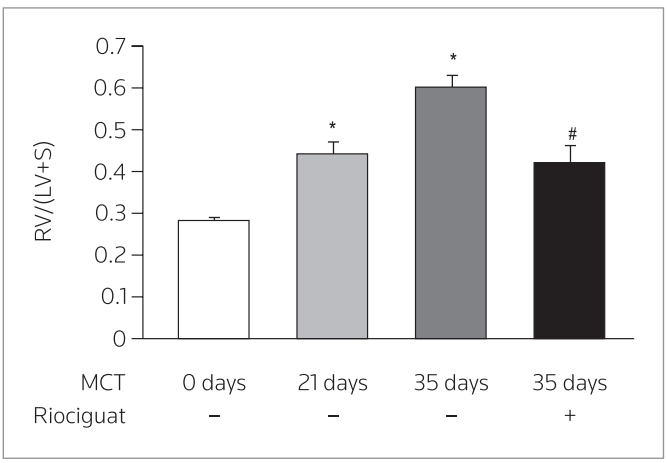


Figure 2. Effect of riociguat on right heart hypertrophy in rats with monocrotaline (MCT)-induced pulmonary hypertension. Rats were injected with MCT on day 0 and assessed on day 21 and day 35. Riociguat (10 mg/kg) was administered orally from day 21 to day 35 (N = 8). Right ventricular hypertrophy was assessed by the ratio of right ventricle weight to left ventricle and septum weight (RV/[LV+S]). *P < 0.05 versus control; #P < 0.05 versus MCT at day 35. Figure 11a reproduced from Schermuly, R.T., Stasch, J-P., Pullamsetti, S.S., Middendorff, R., Müller, D., Schlüter, K-D., Dingendorf, A., Hackemack, S., Kolosionek, E., Kaulen, C., Dumitrascu, R., Weissmann, N., Mittendorf, J., Klepetka, W., Seeger, W., Ghofrani, H.A., Grimminger, F. *Expression and function of soluble guanylate cyclase in pulmonary arterial hypertension*. Eur Respir J 2008, 32(4): 881-91, with permission from European Respiratory Society Journals Ltd.

Table I. *Riociguat pharmacokinetic parameters in patients with pulmonary hypertension following single-dose administration of 1 and 2.5 mg riociguat.*

Parameter	Unit	Riociguat 1 mg (n = 5)			Riociguat 2.5 mg (n = 10)		
		Geometric mean	CV (%)	Range	Geometric mean	CV (%)	Range
AUC	µg.h/L	602.3	14.9	456.5-749.6	1411	39.2	597.5-3121
C_{max}	µg/L	59.43	5.9	53.49-65.05	119.4	16.1	74.69-172.4
t_{max}	h	0.750*		0.500-1.500	0.500*		0.250-1.500
$t_{½}$	h	9.953	8.6	8.737-12.14	11.65	38.6	4.680-28.58
Vz/f	L/kg	0.354	7.3	0.307-0.393	0.378	20.7	0.005-0.609
CL/f	L/h	1.660	14.9	1.334-2.191	1.771	39.2	0.801-4.184

*Median; AUC, area under plasma concentration–time curve from zero to infinity after single dose; CL/f, total body clearance of drug from plasma calculated after oral administration; C_{max}, maximum drug concentration in plasma after single-dose administration; CV, coefficient of variance (geometric); $t_{½}$, terminal elimination half-life; t_{max}, time to reach maximum drug concentration in plasma; Vz/f, apparent volume of distribution during terminal phase after oral administration. Adapted from Table 4 from Grimminger F., Weimann, G., Frey, R., Voswinckel, R., Thamm, M., Bölkow, D., Weissmann, N., Mück, W., Unger, S., Wensing, G., Schermuly, R.T., Ghofrani, H-A. *First acute haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension*. Eur Respir J 2009, 33(4): 785-92, with permission from European Respiratory Society Journals Ltd.

a single oral dose was 0.5-0.75 h in the proof-of-concept study of riociguat in patients with PH (Table I) (44). Solution and tablet formulations of riociguat (2.5 mg) were tested in healthy men and demonstrated similar bioavailability (43). The mean terminal half-life ($t_{½}$) of riociguat was 10-12 h in patients with PH (44). Riociguat exhibited dose proportionality and pronounced interindividual variability in both studies. Individual dose titration of riociguat was recommended for future studies to address the variability in pharmacokinetics among patients.

SAFETY

In the phase I study of riociguat in healthy men, 50% of the total study population (29/58) and 53% of the group administered riociguat (24/45) reported at least one adverse event (all mild or moderate) (43). In the proof-of-concept study of riociguat in PH, 21% of the study population (4/19) reported at least one adverse event (all mild) (44). Neither study revealed any treatment-emergent abnormalities in electrocardiogram data or laboratory values, and the proof-of-concept study found no deterioration in ventilation/perfusion matching, consistent with the synergistic action of riociguat with endogenous NO.

Drug-related adverse events were consistent with the mode of action of riociguat as a vasodilator. The most common drug-related adverse events in the phase I study were headache (n = 11), flushing (n = 8), orthostatic hypotension (n = 7), nasal congestion (n = 7) and feeling hot (n = 7). In this study, the adverse event rate in the active treatment group was dose-dependent. However, no relevant differences were seen in the rate of adverse events between placebo and riociguat doses up to 1 mg. In the proof-of-concept study, three drug-related adverse events (hot flush, dizziness and nasal congestion) were each reported once by three patients (44).

A preliminary dose-finding study performed as part of the proof-of-concept study, in which four patients with PH received hourly incremental doses of riociguat up to a total of 2.5 mg (0.5 mg + 1 mg + 1 mg; two patients) or 5 mg (1 mg + 2 mg + 2 mg; two patients), revealed that riociguat was well tolerated at doses up to 2.5 mg, whereas the 5-mg dose caused asymptomatic hypotension in one patient (44).

CLINICAL STUDIES

The acute hemodynamic effects of riociguat were also investigated in the phase I and proof-of-concept studies (43, 44).

Heart rate is considered a very sensitive noninvasive parameter for indirect estimation of the effect of a vasodilating agent on the cardiovascular system in healthy young volunteers, and was therefore adopted as a measure of vasodilatation in the study of riociguat in healthy men. Doses of 1-5 mg riociguat significantly increased heart rate compared with placebo in a dose-dependent manner, thus confirming the vasodilating properties of riociguat. Diastolic blood pressure and mean arterial pressure both decreased slightly but significantly at riociguat doses of 1 and 5 mg; systolic blood pressure did not decrease significantly in this study (43).

Vasoactive hormones were measured in the phase I study to assess the effect of riociguat on blood pressure control mechanisms in healthy participants. Only the 5-mg dose caused a significant increase in norepinephrine levels. Doses from 1 mg upwards increased plasma renin activity compared with placebo, but no significant change was observed in plasma aldosterone and angiotensin II levels. Significant increases in plasma cGMP levels (overspill from intracellular cGMP) were detected with 2.5- and 5-mg riociguat doses compared with placebo (43).

The proof-of-concept study assessed the hemodynamic effects of a single oral dose of riociguat in 15 patients with mild to moderate PH. Based on the results of the preliminary dose-finding study in four patients, riociguat doses of 1 and 2.5 mg were tested. Riociguat was given in the morning after an overnight fast and following a washout period of at least 8 h for patients taking acute vasodilators, such as calcium channel blockers. Hemodynamic parameters were assessed before, during and after inhalation of NO (10-20 ppm for 10 min), and riociguat was given after hemodynamic parameters had returned to baseline values. The effects of riociguat were compared with peak values during NO inhalation and post-NO baseline values. Riociguat plasma concentrations correlated significantly with pulmonary arterial pressure, pulmonary vascular resistance, systolic blood pressure, systemic vascular resistance and cardiac index. Both doses of riociguat produced clinically relevant reductions from

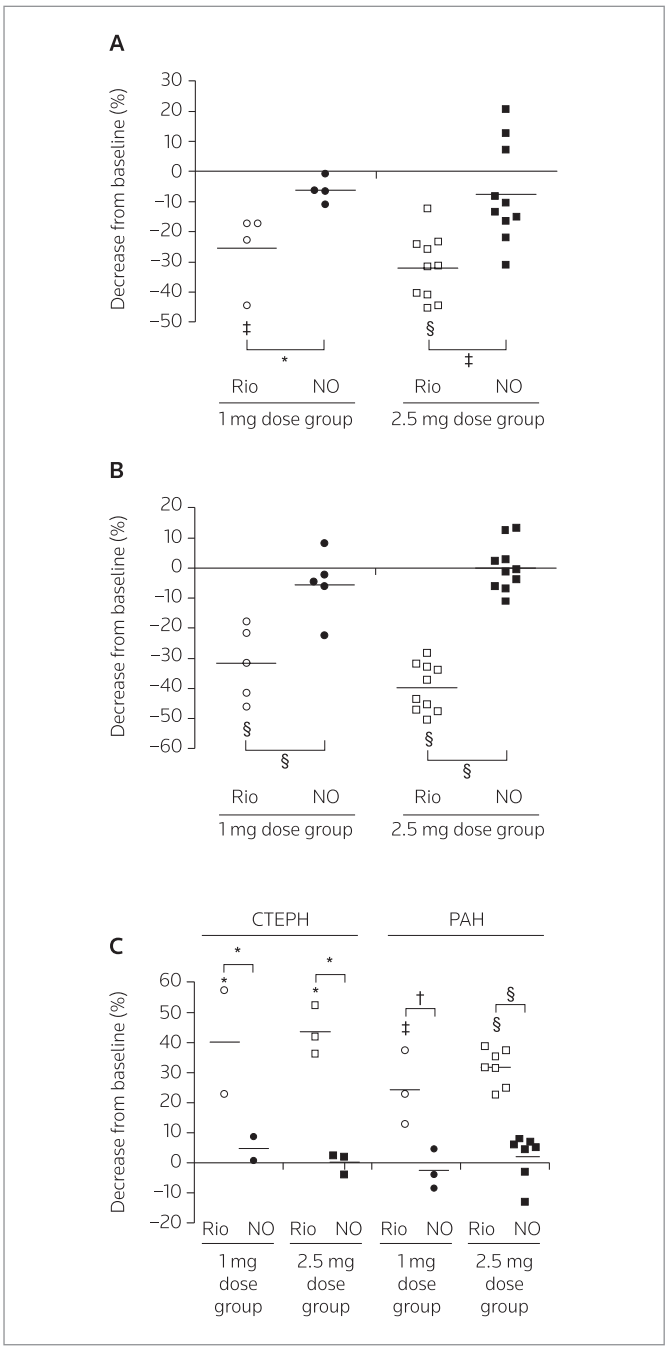


Figure 3. Changes in (**A**) pulmonary and (**B**) systemic vascular resistance and (**C**) cardiac index in patients with pulmonary hypertension following a single dose of riociguat compared with inhaled nitric oxide. Horizontal lines indicate point estimates (least-squares means) in each case. Statistical significance was measured by the F statistic (*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; §$P < 0.0001$). CTEPH, chronic thromboembolic pulmonary hypertension; PAH, pulmonary arterial hypertension; Rio, riociguat; NO, inhaled nitric oxide. Figure 2C-D and Figure 3 reproduced from Grimminger, F., Weimann, G., Frey, R., Voswinckel, R., Thamm, M., Bölkow, D., Weissmann, N., Mück, W., Unger, S., Wensing, G., Schermuly, R.T., Ghofrani, H-A. *First acute haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension*. Eur Respir J 2009, 33(4): 785-92, with permission from European Respiratory Society Journals Ltd.

baseline in pulmonary arterial pressure and pulmonary vascular resistance, and an increase in cardiac index. In comparison, inhaled NO had little effect (Fig. 3). Analysis of the CTEPH and PAH patient subgroups revealed that riociguat had a similar hemodynamic effect in both groups, increasing cardiac index to a greater extent than inhaled NO (44) (Fig. 3C).

Riociguat produced significant decreases in systolic blood pressure and systemic vascular resistance in the proof-of-concept study (Fig. 3B), but mean systolic blood pressure did not fall below 110 mmHg and the observed systemic effects were asymptomatic, possibly because of a marked elevation in cardiac output. However, patients remained supine throughout the study, and the long-term effects of riociguat in mobile patients must be examined in future studies (44).

Dose titration of riociguat, which has since been investigated in a phase II study, will help to promote pulmonary vasodilatation while maintaining adequate systemic blood pressure. The phase II trial (45, 46) is an open-label, uncontrolled, 12-week study investigating the feasibility of individual dose titration of oral riociguat according to systolic blood pressure in patients with CTEPH or PAH. This trial enrolled 75 patients, of whom 68 chose to continue treatment with riociguat in a long-term extension of the study. Interim analyses have been sufficiently encouraging to warrant the initiation of randomized, double-blind, placebo-controlled phase III clinical studies of riociguat in patients with PAH or CTEPH (47-49).

Participants with other forms of PH are being recruited for two non-randomized, uncontrolled trials of riociguat: a proof-of-concept study in PH associated with COPD (50) and a phase II study in PH associated with interstitial lung disease (ILD) (51). The proof-of-concept study in PH associated with COPD will recruit 20 patients who will routinely undergo invasive hemodynamic assessment. The study will investigate the effect of a single dose of riociguat (1 or 2.5 mg) on hemodynamic parameters, gas exchange and lung function in these patients, as well as assessing safety, tolerability and pharmacokinetics. The phase II study in PH associated with ILD will investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of riociguat in 20 patients. Riociguat dose will be titrated from 1 mg t.i.d. up to a maximum of 2.5 mg t.i.d. over the 12-week study period, and patients will have the option to continue into a long-term extension phase. Measures of efficacy will include 6-min walking distance, the modified Borg dyspnea scale, and assessments of quality of life and hemodynamic parameters.

SOURCE

Bayer Schering Pharma AG (DE).

DISCLOSURE

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