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Discovery of Riociguat (BAY 63-2521): A Potent, Oral Stimulator of Soluble Guanylate Cyclase for the Treatment of Pulmonary Hypertension

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Soluble guanylate cyclase (sGC) is a key signal-transduction enzyme activated by nitric oxide (NO). Impairments of the NO–sGC signaling pathway have been implicated in the pathogenesis of cardiovascular and other diseases. Direct stimulation of sGC represents a promising therapeutic strategy particularly for the treatment of pulmonary hypertension (PH), a disabling disease associated with a poor prognosis. Previous sGC stimulators such as the pyrazolopyridines BAY 41-2272 and BAY 41-8543 demonstrated beneficial effects in experimental models of PH, but were associated with unfavorable drug metabolism and pharmacokinetic (DMPK) properties. Herein we disclose an extended SAR exploration of this compound class to address these issues. Our efforts led to the identification of the potent sGC stimulator riociguat, which exhibits an improved DMPK profile and exerts strong effects on pulmonary hemodynamics and exercise capacity in patients with PH. Riociguat is currently being investigated in phase III clinical trials for the oral treatment of PH.

Introduction

Soluble guanylate cyclase (sGC) is a key signal-transduction enzyme. It is activated by the ubiquitous messenger nitric oxide (NO) and catalyzes the conversion of guanosine-5′-triphosphate (GTP) into the second messenger cyclic guanosine-3′,5′-monophosphate (cGMP). The increased level of cGMP modulates the activity of downstream effectors including protein kinases, phosphodiesterases (PDE), and ion channels, ultimately regulating a multitude of fundamental cellular processes including vasodilatation, vascular smooth-muscle cell growth, platelet aggregation, and neurotransmission. Impairments of the NO–sGC–cGMP signaling pathway have been implicated in the pathogenesis of cardiovascular, pulmonary, endothelial, renal, and hepatic diseases.[1] Among these, pulmonary hypertension (PH) is a devastating disease in which increased pulmonary vascular resistance causes right heart hypertrophy, eventually leading to right heart failure and death.[2] Although there have been significant advances in the treatment of PH including the clinical introduction of prostacyclin analogues, endothelin receptor antagonists, phosphodiesterase 5 (PDE5) inhibitors, and their combinations, there remains a major unmet need for additional therapeutic interventions.[3]

In PH patients a markedly impaired bioactivity of NO contributes to excessive pulmonary vasoconstriction.[4] Treatments that elevate NO levels (inhaled NO and NO-donor drugs) are unsuitable as long-term therapies for PH due to their short-lived effects, the development of tolerance, and nonspecific interactions of NO with various biomolecules. Precise regulation of NO levels is required in the pulmonary vasculature to direct blood flow preferentially to well-ventilated regions of the lung (ventilation/perfusion matching), thus ensuring optimal uptake of oxygen into the blood (Figure 1).[5] Therefore, therapies that act in synergy with endogenous NO to maintain ventilation/perfusion matching are highly desirable.[6]

The PDE5 inhibitor sildenafil augments the effects of endogenous NO, increasing cGMP levels by preventing its degradation. However, a significant number of patients with PH do not respond to sildenafil treatment,[7] indicating that endogenous NO in these patients is decreased to such an extent that sildenafil can no longer increase cGMP levels to a sufficient degree. Direct stimulation of sGC represents a promising alternative therapeutic strategy for such patients.

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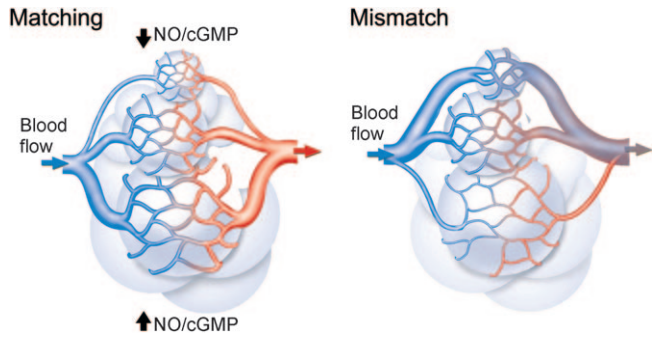


Figure 1. Regulation of ventilation/perfusion distribution in the lung. The adaptation of blood flow to ventilation is a critical function of the healthy lung (left). NO is a key regulator in the pulmonary vasculature, directing cGMP formation and thus blood flow preferentially to well-ventilated regions of the lung. In patients with PH, ventilation/perfusion mismatch limits blood oxygenation despite sufficient pulmonary blood flow (right). Unspecific vasodilation by traditional anti-hypertensives leads to a mismatch exacerbation. In contrast, sGC stimulators improve the ventilation/perfusion ratio by synergistically augmenting selective intrapulmonary vasodilation of NO.

Direct NO-independent sGC stimulation was first demonstrated in 1994 when Ko and colleagues reported cGMP-stimulating properties for benzylindazole YC-1 (**1**, Figure 2).[8] Our initial chemical optimization program based on YC-1 as a lead structure resulted in the identification of the sGC stimulators BAY 41-2272 (**2**) and BAY 41-8543 (**3**) with a pyrazolopyridinyl pyrimidine core.[9] The mode of action of these two compounds is similar to that of YC-1, but they demonstrate greatly increased potency and specificity for sGC. Their vasodilatory potency is approximately two to three orders of magnitude higher than that of YC-1.[1,9] They act synergistically to enhance the sensitivity of sGC to low levels of bioavailable NO and show a loss of stimulation after oxidation or removal of the prosthetic heme moiety of sGC. Recent studies have proposed that sGC stimulators bind to an allosteric nucleotide-binding site in the catalytic domains of sGC. The exact molecular mechanism of sGC stimulation, however, remains a matter of debate.[10]

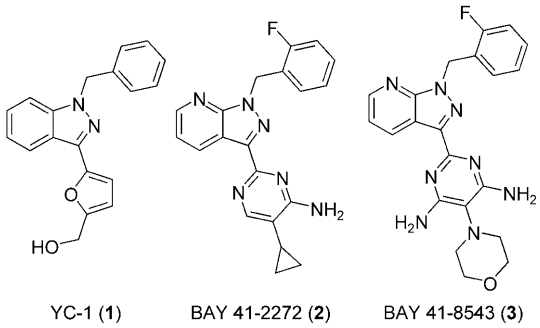


Figure 2. sGC stimulators YC-1, BAY 41-2272, and BAY 41-8543.

In various experimental models of PH, BAY 41-2272 (**2**) demonstrated beneficial effects, including a significant decrease in pulmonary arterial pressure, reversal in right ventricular hypertrophy, and structural remodeling of the lung vasculature.[1,11] Strong inhibition as well as induction of cytochrome P450 (CYP) isoenzymes, however, prevented BAY 41-2272 (**2**) from further advancement into preclinical development. Although the follow-up lead BAY 41-8543 (**3**) displayed no relevant CYP interaction, it was also not further advanced due to an unfavorable PK profile characterized mainly by high clearance and an undefined dose nonlinearity of plasma concentrations in all tested species.

In our earlier studies[9] we observed a steep SAR for stimulating sGC in the 1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine part of this lead series. In contrast, the pyrimidine moiety was tolerant against broad variations. Particularly, pyrimidine C5 turned out to be the most fruitful position to address the issues of pharmacokinetics and CYP interaction.

Herein we describe our efforts to optimize the drug metabolism and pharmacokinetic (DMPK) profile of our sGC stimulators by an extended SAR exploration of the pyrimidine region, leading to the identification of riociguat (**20**), which is currently in advanced clinical development for the treatment of PH.

Results and Discussion

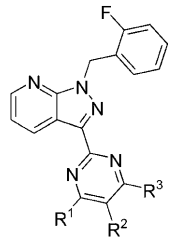
In the course of our optimization we investigated more than 800 pyrimidine derivatives differing mainly at C5. Representative examples are shown in Tables 1 and 2. The initial goal of our studies was to identify derivatives for a more detailed PK characterization that have no relevant CYP interaction potential, and at the same time retain the favorable in vitro and in vivo potency of BAY 41-2272 (**2**) and BAY 41-8543 (**3**, Table 1).

As primary in vitro assays to evaluate sGC-stimulating potency we monitored cGMP formation in a sGC-overexpressing Chinese hamster ovarian (CHO) cell line[12] and the inhibition of phenylephrine-induced contractions of rabbit aortic rings.[9c] In the former case we used the minimum effective concentration (MEC) for cGMP formation rather than the EC_{50} values for our SAR studies, as this provides a better correlation with the relaxation of isolated vessels and effective plasma concentrations in vivo. Apart from biological variability, deviations between the primary assays may be explained by differences in cell and tissue penetration. Blood pressure lowering effects were evaluated in conscious, spontaneously hypertensive rats equipped with a radiotelemetric device for continuous recording of hemodynamic parameters. Oral in vivo potency, efficacy, and duration of action in this model also provided initial hints on the PK profile.[9d]

Our lead compounds BAY 41-2272 (**2**) and BAY 41-8543 (**3**) stimulated the sGC-overexpressing cell line starting at 0.03 μM and inhibited the phenylephrine-induced contractions of rabbit aorta with IC_{50} values of 0.30 and 0.10 μM, respectively (Table 1). This translated into a potent, dose-dependent decrease of mean arterial blood pressure in spontaneously hypertensive (SH) rats with minimum effective doses (MED) of 1 and 0.3 mg kg^{-1} p.o., respectively.[9]

Throughout the project we synthesized numerous pairs of pyrimidine 4-amines and 4,6-diamines as exemplified by the pairs shown in Table 1 and Table 2 (**2**, **4**; **3**, **7**; **12**, **13**; **19**, **20**).

Table 1. Close analogues of the leads BAY 41-2272 (**2**) and BAY 41-8543 (**3**): effect on sGC stimulation and CYP inhibition.



Compd	R^1	R^2	R^3	cGMP Formation MEC [μM][a]	Rabbit Aorta IC_{50} [μM][b]	Conscious SH Rats MED [mg kg^{-1}][c]	CYP1A2 Co-incubation IC_{50} [μM][d]	CYP3A4 Co-incubation/Pre-incubation IC_{50} [μM][e]
2	H		NH_2	0.03	0.30	1	1.6	>20/4.3
3	NH_2		NH_2	0.03	0.10	0.3	>20	>20/>20
4	NH_2		NH_2	0.1	0.23	n.d.	1.4	14.9/2.4
5	NH_2	Et	NH_2	0.03	0.11	n.d.	2.4	20/14.8
6	NH_2	NMe_2	NH_2	0.01	0.79	n.d.	0.61	>20/>20
7	H		NH_2	0.03	0.26	3	>20	>20/>20
8	H		Me	0.08	0.67	n.d.	>20	>20/>20
9	H		H	0.5	0.93	>3	>20	>20/>20

[a] MEC: minimal effective concentration to achieve threefold stimulation of cGMP formation in a recombinant sGC-overexpressing cell line.[12] [b] Relaxing effect on pre-contracted rabbit aortic rings.[9c] [c] MED: minimal effective dose to induce a mean arterial blood pressure decrease of 10 mm Hg following oral administration to conscious, radiotelemetrically instrumented, spontaneously hypertensive (SH) rats (n.d. = not determined).[9d] [d] CYP1A2 inhibition following incubation of phenacetin in the presence of the test compound with human liver microsomes; LC–MS–MS analysis. [e] CYP3A4 inhibition following incubation of midazolam in the presence of the test compound with human liver microsomes; pre-incubation was conducted for 30 min; LC–MS–MS analysis of formed metabolites (paracetamol, 1′-hydroxymidazolam).

Overall, no major differences in in vitro potency were observed. In most cases, however, the diamino analogues displayed a slightly more potent relaxation of rabbit aorta than their monoamino counterparts. A seven- to tenfold loss in potency for relaxing rabbit aorta was observed upon replacement of the amino group of BAY 41-8543 (**3**) by methyl (compound **8**) or hydrogen (compound **9**). The weak blood pressure lowering effect of compound **9** suggests that the potency loss was even more dramatic in vivo, indicating a positive impact of the amino group on oral exposure.

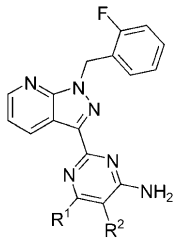
Regarding CYP inhibition, we found that in most cases a critical inhibition of isoform 1A2 could be attributed to small lipophilic substituents at C5 for both mono- and diaminopyrimidines, as evidenced by compounds **2**, **4**, **5**, and **6** (Table 1). In cases of small branched and unbranched alkyl side chains (compounds **2**, **4**, and **5**) we additionally observed a significant shift of CYP3A4 IC_{50} values following pre-incubation with human liver microsomes relative to those with co-incubation, indicating a time-dependent inhibition of this isoenzyme. Other CYP isoforms were not affected. BAY 41-8543 (**3**), and other derivatives (**7**–**9**) with the more polar morpholine substituent showed no relevant CYP inhibition.

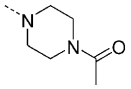
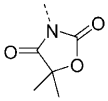
BAY 41-2272 (**2**) and various derivatives of this compound class were associated with the induction of CYP1A2 and/or 3A4 in cultured human hepatocytes. However, no clear structure–property relationships could be demonstrated. Consequently, the CYP1A2- and 3A4-inducing potential became an integral part of our screening cascade and was assessed for many advanced compounds.

In extension to our previous work,[9] we found that the pyrimidine C5 tolerates not only small lipophilic groups, but also a wide range of polar substituents for potent sGC stimulation, as exemplified by compounds **10** and **12**–**23** (Table 2). This helped us to overcome the CYP inhibition issue, as all compounds listed in Table 2 are devoid of relevant inhibition of all major CYP isoforms.

The introduction of various piperazines provided compounds such as **10** with equal or slightly lower in vitro potency. However, a greater than tenfold decrease in the potency of compound **10** to lower blood pressure in SH rats relative to

Table 2. Introduction of polar pyrimidine C5 substituents.



Compd	R^1	R^2	cGMP formation MEC [μM][a]	Rabbit Aorta IC_{50} [μM][b]	Conscious SH Rats MED [mg kg^{-1}][c]
10	NH_2		0.07	0.18	>3
11	H		>10	>10	n.d.
12	H		0.03	0.44	1
13	NH_2		0.02	0.37	0.3
14	H		0.05	1.3	3
15	NH_2		0.10	1.4	3
16	NH_2		0.03	0.77	3
17	NH_2		0.02	0.33	3
18	H		0.2	1.0	3
19	H		0.03	0.67	n.d.
20	NH_2		0.03	0.12	0.1
21	H		0.05	0.96	1
22	H		0.01	0.47	3

[a] MEC: minimal effective concentration to achieve threefold stimulation of cGMP formation in a recombinant sGC-overexpressing cell line.[12] [b] Ref. [9c]. [c] MED: minimal effective dose to induce a mean arterial blood pressure decrease of 10 mm Hg following oral administration to conscious, radiotelemetrically instrumented SH rats (n.d. = not determined).[9d]

BAY 41-8543 (**3**) indicated that no improvement of oral bioavailability was achieved.

The primary amide **11** turned out to be virtually inactive with only very weak inhibition of rabbit aortic ring contraction (22% at 28 μM). More promising oral in vivo potency could be achieved with pyridines **12** and **13**, which displayed MED values for rat blood pressure reduction of 1 and 0.3 mg kg^{-1}.

Sulfonyl ester **14** and lactam **18** exhibited an in vitro potency roughly tenfold lower than that of **3**, with IC_{50} values of 1.3 and 1.0 μM, respectively, for the relaxation of rabbit aortic rings. This correlates with the modest activity observed in SH rats with oral MED values of 3 mg kg^{-1} for both compounds.

The moderate potency of sulfonamides such as **15** on rabbit aortic rings could be improved by N-methylation (compound **16**) or cyclization (compound **17**). However, no positive effect on oral in vivo potency was observed. Furthermore, this subclass suffered from CYP3A4 induction and was not further pursued.

The introduction of carbamates provided highly potent analogues, as evidenced by *N*-methylcarbamate **20**,[13] which inhibited the contraction of rabbit aorta with an IC_{50} value of 120 nM and lowered blood pressure in SH rats starting at an oral threshold dose of 0.1 mg kg^{-1}.

The corresponding cyclic carbamate **21** exhibited lower in vitro and in vivo potency. The oxazolidine-2,4-dione **22** showed a promising IC_{50} value for the relaxation of isolated rabbit aorta. However, **22** demonstrated only short blood pressure lowering effects in rats at a threshold dose of 3 mg kg^{-1}, which may be related to a limited stability observed in rat plasma. In addition, CYP1A2 induction along with only low and non-dose-linear exposure in dog PK studies was observed for this compound.

On the basis of their favorable profile regarding sGC-stimulating potency in vitro, oral efficacy in rats, and CYP-inducing potential, we selected compounds **12** and **20** for a more detailed PK characterization in comparison with BAY 41-8543 (**3**).

Table 3. Pharmacokinetic profile of selected analogues in female Beagle dogs.[a]

Compd	CL_p [L h^{-1} kg^{-1}][b]	V_{dss} [L kg^{-1}][c]	$t_{1/2}$ [h]	F [%][d]
3	5.3	2.2	0.65	<1
12	2.0	1.9	1.4	29
20	0.25	0.73	2.4	79

[a] Mean values derived by intravenous (1–2 h infusion) and oral (gavage) administration of 0.3 mg kg^{-1} in EtOH/PEG400/H_2O vehicles. [b] Total plasma clearance. [c] Apparent volume of distribution at steady state. [d] Oral bioavailability.

A considerable improvement in the main parameters is represented by the PK profile in dogs (Table 3).

In comparison with BAY 41-8543 (**3**), the pyridine derivative **12** exhibited an improved oral bioavailability of 29%. The clearance, however, was still unacceptably high (2.0 L kg^{-1} h^{-1}). A superior PK profile in dogs was observed for the *N*-methylcarbamate **20**, which is characterized by low clearance (0.25 L kg^{-1} h^{-1}), a moderate volume of distribution (0.73 L kg^{-1}), an estimated plasma elimination half-life of 2.4 h, and good oral bioavailability (79%). Furthermore, and in contrast to BAY 41-8543 (**3**), the oral exposure of **20** in terms of AUC increased largely dose-proportionally in the dose range of 0.03–0.6 mg kg^{-1}.

Carbamate **20** stimulated purified recombinant sGC in a concentration-dependent manner up to 73-fold, from 0.1 to 100 μM, and showed the typical profile of sGC stimulators: strong synergistic enzyme activation when combined with NO-releasing agents and crucial dependence on the presence of the reduced prosthetic heme moiety.[14] In conscious SH rats, oral administration of **20** resulted in a long-lasting and dose-dependent blood pressure decrease (Figure 3). No development of tachyphylaxis was observed upon prolonged administration in this animal model. In two well-accepted rodent models of pulmonary arterial hypertension, chronic treatment with **20** significantly decreased hemodynamic changes, right heart hypertrophy, and structural remodeling of the lung vasculature.[13]

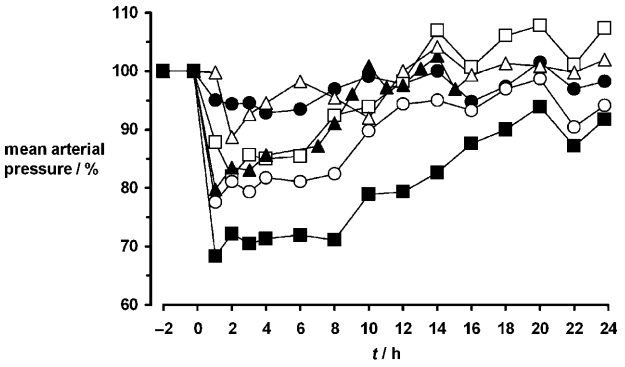


Figure 3. 24-hour profile of mean arterial blood pressure in conscious spontaneously hypertensive rats (SHR) after a single oral dose of riociguat (**20**). Controls were treated with vehicle. The substance doses were administered orally by gavage at 0 h. Shown are mean values of 6–12 animals as a percentage of initial values (131–142 mm Hg). An initial reflex increase in heart rate was observed starting at a dose of 0.03 mg kg^{-1}. [●: control, $n=12$; △: 0.03 mg kg^{-1}, $n=6$; □: 0.1 mg kg^{-1}, $n=6$; ▲: 0.3 mg kg^{-1}, $n=12$; ○: 1.0 mg kg^{-1}, $n=6$; ■: 3.0 mg kg^{-1}, $n=6$.]

The specificity of **20** was explored in 69 different enzyme and radioligand binding assays generated by MDS Pharma Services. No significant effects were observed at the highest tested concentration of 10 μM. In addition, **20** was tested in various PDE assays, applying human full-length PDE11A, 10A, 9A, 8A, 7B, 5, 4B, 3B, 2A, or purified bovine PDE6 and PDE1 enzymes. The IC_{50} value for PDE7B was 2.9 μM; IC_{50} values for all other tested PDEs were >10 μM.

Based on its combined profile of excellent potency, specificity, efficacy, and safety, carbamate **20** was selected as a drug development candidate (riociguat, BAY 63-2521). Riociguat (**20**) demonstrated a favorable safety profile and was well tolerated in healthy volunteers[15] and in patients with PH.[16] Orally administered riociguat was efficacious in a proof-of-concept study of patients with PH, decreasing pulmonary vascular resistance and increasing cardiac output to a significantly greater extent than inhaled NO at doses of 1 and 2.5 mg.[15] Neither dose produced any deterioration in gas exchange, indicating that ventilation/perfusion matching was maintained. In a phase II study, riociguat (**20**) exerted strong effects on pulmonary hemodynamics and exercise capacity in subjects with the PH subforms of pulmonary arterial hypertension and chronic thromboembolic PH.[17]

Syntheses

Retrosynthetic analysis of scaffolds **A**, **B**, and **C** with none, one, or two amino groups as pyrimidine substituents, respectively, revealed amidine **23** as common precursor (Figure 4). Thus, condensation with a 1,3-dicarbonyl equivalent **a** should give access to symmetric pyrimidines **A**. Reagents of type **b** with a

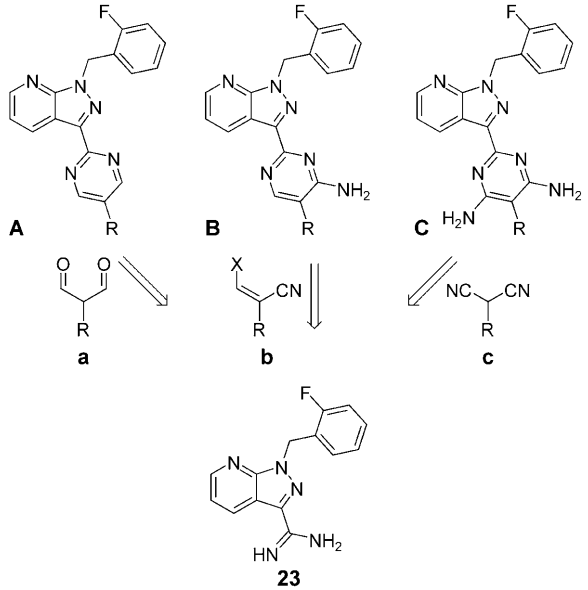
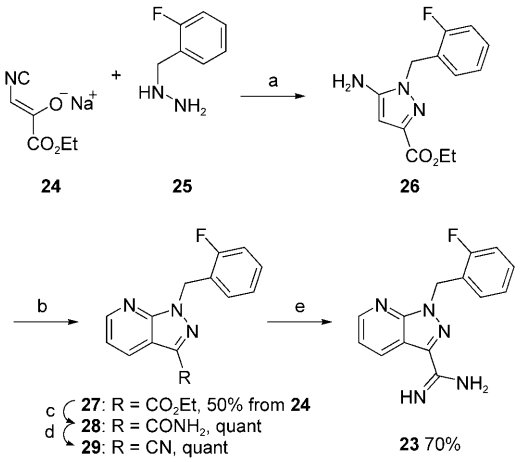


Figure 4. Retrosynthesis of des-, mono-, and diaminopyrimidines **A**, **B**, and **C**.

leaving group X were expected to form monoamino-substituted pyrimidines **B**, whilst malonic dinitrile derivatives **c** are known to undergo cyclization to diamino-substituted pyrimidines **C**.

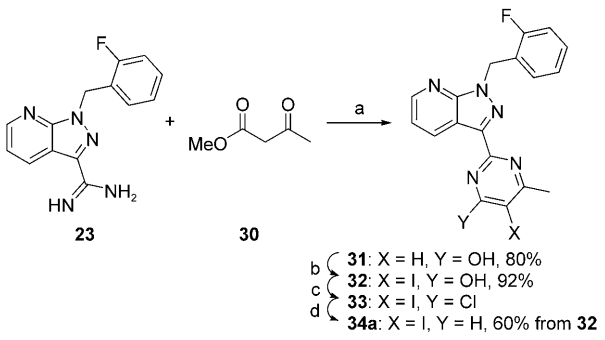
For the synthesis of amidine **23** we first generated aminopyrazole **26** from ethyl cyanopyruvate **24**[18] and 2-fluorobenzylhydrazine **25**[19] (Scheme 1). Subsequent cyclocondensation



Scheme 1. Synthesis of intermediate **23**. Reagents and conditions: a) TFA, dioxane, reflux, overnight; b) 3-dimethylaminoacrolein, TFA, reflux, 72 h; c) NH_3, MeOH, RT, 48 h; d) TFAA, Py, RT, overnight; e) 1. NaOMe, MeOH, RT, 2 h; 2. NH_4Cl, HOAc, reflux, overnight.

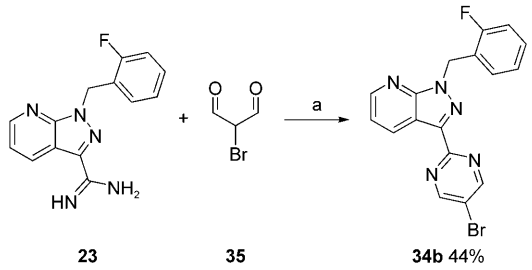
of the crude product with 3-dimethylaminoacrolein gave pyrazolopyridine **27** in 50% overall yield. In the following high-yielding steps the carboxylic ester moiety was transformed into the corresponding nitrile via classical amide formation and dehydration under standard conditions with trifluoroacetic acid anhydride,[20] providing compound **29**. Subsequent Pinner reaction of the cyano group with sodium methanolate and substitution with ammonia gave access to amidine **23** in a total 35% yield over five steps.

Condensation of amidine **23** with 3-oxobutanoic acid methyl ester **30** gave hydroxypyrimidine **31**, which was iodinated to the 5-iodo analogue **32** to allow later derivatization at this position (Scheme 2). The hydroxy group was then removed in a two-step sequence.[21] Chlorination afforded **33**, and the chlorine atom was selectively removed in the presence of the iodine atom by reduction with toluenesulfonyl hydrazide to give **34a**.



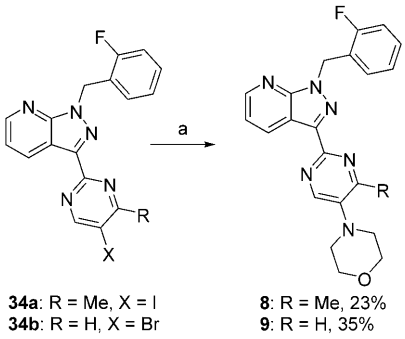
Scheme 2. Synthesis of intermediate **34a**. Reagents and conditions: a) toluene, reflux, overnight; b) I_2, NaOH, H_2O, reflux, overnight; c) $POCl_3$, 100 °C, 2 h; d) $TsNHNH_2$, $CHCl_3$, 48 h.

The 4,6-unsubstituted compound **34b** resulted from the condensation of amidine **23** with commercially available 2-bromomalondialdehyde **35** in acetic acid (Scheme 3).



Scheme 3. Synthesis of intermediate **34b**. Reagents and conditions: a) HOAc, 100 °C, 2 h.

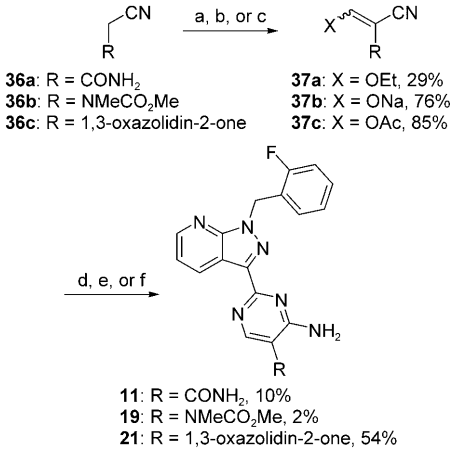
Compounds **8** and **9** were obtained in 23–35% yield by treating **34a** and **34b**, respectively, with morpholine under Buchwald's conditions[22] (Scheme 4).



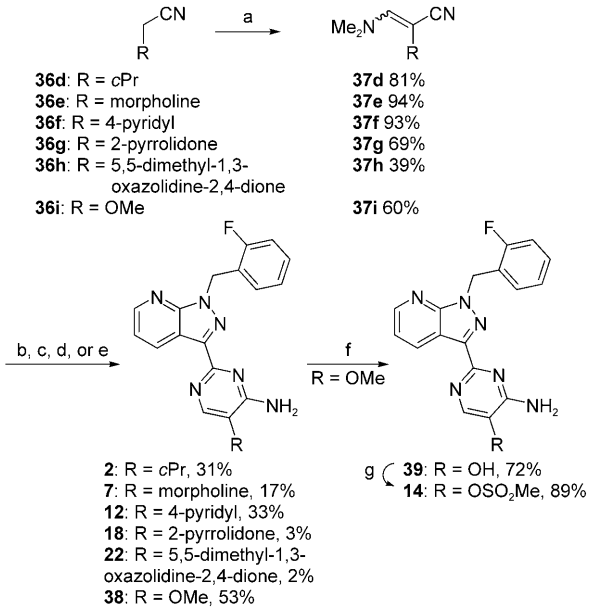
Scheme 4. Synthesis of compounds **8** and **9**. Reagents and conditions: a) morpholine, KO*t*Bu, [Pd_2dba_3] (cat), (*rac*)-BINAP (cat), toluene, 70 °C, overnight.

For the preparation of monoamino-substituted pyrimidines we investigated the condensation of amidine **23** with differently activated acrylonitrile building blocks (Schemes 5 and 6). Nitriles **36a–c** were converted into the corresponding enol ether **37a**, sodium enolate **37b**, or enol acetate **37c**. Condensation of amidine **23** with enol ether **37a** and sodium enolate **37b** provided aminopyridines **11** and **19**, respectively, in only low yields. Better results were obtained with enol acetate **37c** to afford oxazolidinone **21**.

In a more convenient approach we used enamines **37d–i** as reactants in the cyclocondensation, as these are readily accessible using Bredereck's reagent[23] (Scheme 6). During optimization of the heterocyclization step we found that prolonged heating in xylene resulted in only trace amounts of the aminopyrimidines (compounds **18** and **22**). Superior results were obtained by the addition of boron trifluoride (in the case of compound **12**) or running the reaction without solvent at reduced



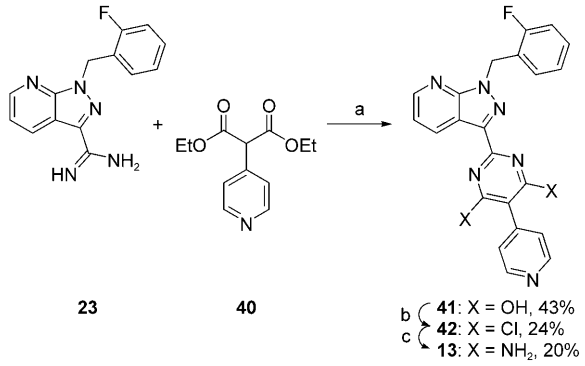
Scheme 5. Synthesis of compounds **11**, **19**, and **21**. Reagents and conditions: a) for **37a**: $HC(OEt)_3$, reflux, 2.5 h; b) for **37b**: HCO_2Et, NaOMe, THF, RT, overnight; c) for **37c**: HCO_2Et, KO*t*Bu, THF, RT, 1 h; then Ac_2O, HOAc, RT, 1 h; d) for **11**: **23**, piperidine, 3-methyl-1-butanol, 110 °C, overnight; e) for **19**: **23**, TEA, toluene, reflux, 9 h; f) for **21**: **23**, toluene, reflux, overnight.



Scheme 6. Synthesis of compounds **2**, **7**, **12**, **14**, **18**, and **22**. Reagents and conditions: a) *t*BuOCH$(NMe_2)_2$, 80–100 °C, slight vacuum, 24–48 h; b) for **2** and **7**: **23**, neat, 100–120 °C, slight vacuum, overnight; c) for **12**: **23**, BF_3, xylene, 140 °C, 19 h; d) for **18** and **22**: xylene, 120 °C, overnight; e) for **38**: **23**, 3-methyl-1-butanol, 110 °C, 72 h; f) PhSH, K_2CO_3, NMP, 190 °C, 1 h; g) $MeSO_2Cl$, Py, RT, overnight.

pressure (for **2** and **7**). In the case of the methoxy-substituted pyrimidine **38** neopentyl alcohol turned out to be the preferred solvent; however, prolonged reaction times of three days were required. The methyl ether of **38** was cleaved with thiophenol[24] leading to phenol **39**, which was subsequently converted into sulfonic ester **14**.

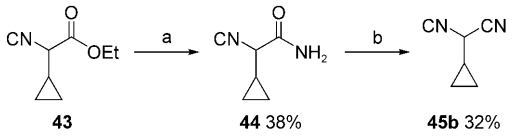
Our first route to synthesize diaminopyrimidines started with the reaction of amidine **23** with a malonic ester derivative **40**[25] leading to dihydroxypyrimidine **41** (Scheme 7). However, subsequent chlorination followed by chlorine–ammonia ex-



Scheme 7. Synthesis of compound **13**. Reagents and conditions: a) toluene, reflux, overnight; b) $POCl_3$, DMF (cat), reflux, 3 h; c) NH_3 (aq), 140 °C, autoclave, overnight.

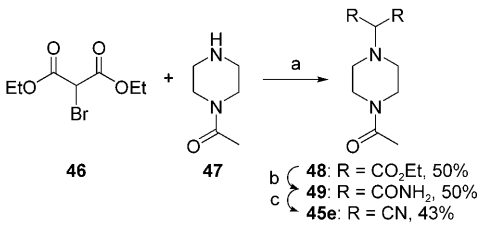
change gave only low yields. Condensation of substituted malonic dinitriles with amidines proved to be a better alternative for the construction of 4,6-diaminopyrimidines.[26]

To synthesize dinitrile **45b**, known ethyl cyano(cyclopropyl)-acetate **43**[27] was treated with methanolic ammonia to yield amide **44**, which was dehydrated with Burgess' reagent[28] to yield cyclopropylpropanedinitrile **45b** (Scheme 8).



Scheme 8. Synthesis of intermediate **45b**. Reagents and conditions: a) NH_3 (7 N in MeOH), RT, 96 h; b) Burgess' reagent, toluene, RT, 1.5 h.

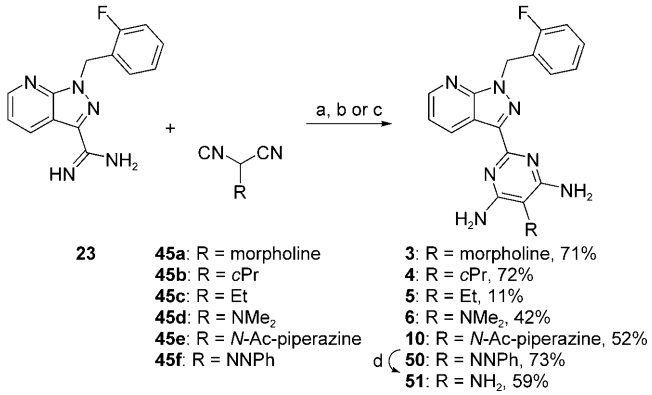
(4-Acetylpiperazin-1-yl)propanedinitrile (**45e**) was synthesized in a three-step sequence (Scheme 9). Diethyl bromopro-



Scheme 9. Synthesis of intermediate **45e**. Reagents and conditions: a) K_2CO_3, MeCN, 50 °C, 28 h; b) NH_3 (7 N in MeOH), RT, 90 h; c) Burgess' reagent, THF/CH_2Cl_2 (3:1), RT, 1.5 h.

panedioate (**46**) was substituted with *N*-acetyl piperidine (**47**) in MeCN with potassium carbonate as base.[29] Subsequently, the malonic diester was transformed into primary diamide **49** by aminolysis, followed by dehydration to dinitrile **45e** using Burgess' reagent.

The alkyl-substituted malonic dinitriles **45b** and **45c**[30] as well as the amino-substituted analogues **45a**,[31] **45d**,[31,32] and **45e** reacted neat with amidine **23** to afford the diaminopyrimidines in 40–70% yield (Scheme 10). In the case of ethyl-substi-



Scheme 10. Synthesis of compounds **3**–**6**, **10**, and intermediate **51**. Reagents and conditions: a) for **3**–**6** and **10**: neat, 105 °C, slight vacuum, 3–12 h; b) for **5**: NMP, 150 °C, overnight; c) for **50**: NaOMe, DMF, 110 °C, overnight; d) H_2 (65 bar), Raney-Ni (cat), DMF, 62 °C, 22 h.

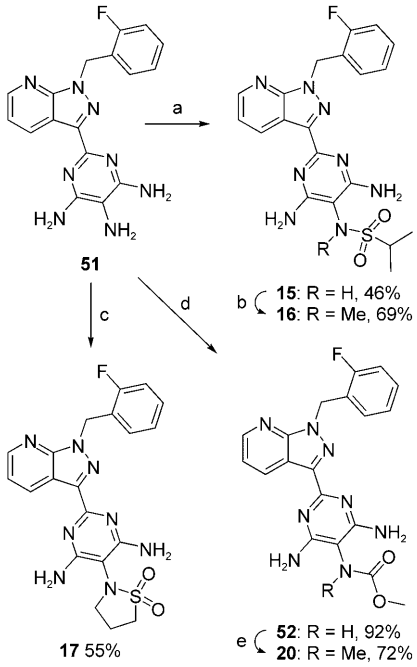
tuted compound **5**, we used *N*-methylpyrrolidone (NMP) as solvent, but even after heating at 150 °C overnight the reaction resulted in only 11% yield. The phenyldiazo-substituted malonic dinitrile **45 f** is a well-known compound[33] that has been reported several times to react with formamidine as well as aliphatic or aromatic amidines[34] to the corresponding phenyldiazopyrimidines, giving access to 4,5,6-triaminopyrimidines by reduction of the diazo group. The cycloaddition was performed in the presence of sodium methanolate in DMF and gave **50** in 73% yield. Subsequent reduction with Raney nickel as catalyst provided the triamine **51**.

During cyclocondensations with **23**, typical side reactions involve trimerization of **23** to the corresponding triazine derivative, which can be removed due to its extreme insolubility. Triazine formation can also occur from two molecules of **23** and one of DMF (from Bredereck's reagent). Traces of dimethylamine can lead to the dimethylamidine congener of **23**, and prolonged heating leads to the formation of nitrile **29**.

The 5-amino group is the most reactive amino function in **51** and reacted with a multitude of electrophilic reagents (Scheme 11). With sulfonic acid chlorides we obtained sulfonamides such as the isopropyl derivate **15**, which was further alkylated to the *N*-methyl analogue **16**. The cyclic isothiazolidine 1,1-dioxide **17** was generated in a two-step procedure using 3-chloropropane-1-sulfonyl chloride. The cycloalkylation step required elevated temperatures. With methyl chloroformate, triamine **51** reacted to give carbamate **52** in high yield. This compound was methylated with iodomethane after deprotonation with LiHMDS and yielded *N*-methyl carbamate **20**.

Conclusions

Our continuous efforts to optimize the unfavorable DMPK profile of previous sGC stimulators led to the identification of riociguat (**20**). Its pharmacodynamic and pharmacokinetic properties suggest that riociguat may offer a unique mode of action for the treatment of PH by inducing pulmonary vasodilation and thus decreasing the workload of the right heart. Its synergistic action with endogenous NO is thought to lead to vasodilation preferentially in well-ventilated regions of the lung, thus preventing ventilation/perfusion mismatch. Based on the positive findings in a phase IIb study in patients with PH, riociguat has recently entered phase III clinical trials.



Scheme 11. Synthesis of compounds **15**–**17** and **20**. Reagents and conditions: a) $iPrSO_2Cl$, Py, RT, overnight; b) MeI, K_2CO_3, acetone, RT, overnight; c) 1. $Cl(CH_2)_3SO_2Cl$, Py, RT, overnight; 2. K_2CO_3, DMF, 80 °C, overnight; d) $ClCO_2Me$, Py, 0 °C, 2 h, → RT, 12 h; e) LiHMDS, THF, 0 °C, 30 min; then MeI, 0 °C, 1 h.

Experimental Section

General methods and materials: ^1H NMR and ^{13}C NMR spectra were recorded in $[D_6]$DMSO at RT on Bruker Avance spectrometers operating at 300, 400, and 500 MHz for ^1H NMR, and at 125 MHz for ^{13}C NMR. Flash column chromatography was performed on silica gel 60 (0.063–0.200 mm) purchased from Merck KGaA (Germany). Preparative HPLC was performed on a 250×30 mm column packed with YMC gel ODS-AQ S-5/15 μM, with MeCN/H_2O as eluent and UV detection. Solvents for extraction and chromatography were reagent grade and used as received. Commercial reagents were used without purification.

Ethyl-5-amino-1-(2-fluorobenzyl)-1*H*-pyrazole-3-carboxylate (26): Trifluoroacetic acid (TFA; 75 mL, 980 mmol) was admixed to the sodium salt of ethyl cyanopyruvate[18] (100 g, 613 mmol) in dioxane (2.5 L) at RT with efficient stirring, and the mixture was stirred for 10 min, during which a large portion of the starting material dissolved. 2-Fluorobenzylhydrazine (85.9 g, 613 mmol) was added, and the mixture was heated at reflux overnight. After cooling, the precipitated crystals were filtered off with suction and washed with dioxane. The product was used in the next step without purification; ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 1.24$ (t, $J = 7.1$ Hz, 3H), 4.18 (q, $J = 7.1$ Hz, 2H), 5.27 (s, 2H), 5.59 (s, 2H), 5.77 (s, 1H), 6.83 (t, $J = 7.4$ Hz, 1H), 7.15 (t, $J = 7.5$ Hz, 1H), 7.19–7.25 (m, 1H), 7.31–7.37 ppm (m, 1H); ^{13}C NMR (125 MHz, $[D_6]$DMSO): $\delta = 14.2$, 44.6 (d, $^3J_{C,F} = 4.4$ Hz), 59.8, 90.0, 115.2 (d, $^2J_{C,F} = 20.8$ Hz), 124.1 (d, $^2J_{C,F} = 14.8$ Hz), 124.5 (d, $^4J_{C,F} = 3.5$ Hz), 128.9 (d, $^3J_{C,F} = 4.2$ Hz), 129.4 (d,

$^3J_{C,F}$ = 8.1 Hz), 141.7, 148.6, 159.6 (d, $^1J_{C,F}$ = 245 Hz), 162.1 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{13}H_{14}FN_3O_2$: 264.1143, found: 264.1145.

Ethyl-1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine-3-carboxylate (27): A mixture of aminopyrazole **26** (161 g, 613 mmol), 3-dimethylaminoacrolein (60.8 g, 613 mmol) and TFA (83.9 g, 736 mmol) in dioxane (2.5 L) was heated at reflux for three days. The solvent was subsequently removed under reduced pressure. The residue was added to H_2O (2 L), and the mixture was extracted with EtOAc (3×1 L). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash chromatography (toluene, then toluene/EtOAc 4:1) to afford the title compound (91.6 g, 50% yield over two steps); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.38 (t, *J* = 7.1 Hz, 3H), 4.41 (q, *J* = 7.1 Hz, 2H), 5.87 (s, 2H), 7.14–7.18 (m, 1H), 7.20–7.26 (m, 2H), 7.35–7.41 (m, 1H), 7.49 (dd, *J* = 8.1, 4.4 Hz, 1H), 8.50 (dd, *J* = 8.1, 1.3 Hz, 1H), 8.72 ppm (dd, *J* = 4.4, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 14.2, 44.6 (d, $^3J_{C,F}$ = 4.2 Hz), 60.8, 114.6, 115.5 (d, $^2J_{C,F}$ = 20.8 Hz), 119.8, 123.2 (d, $^2J_{C,F}$ = 14.8 Hz), 124.7 (d, $^4J_{C,F}$ = 3.5 Hz), 130.2 (d, $^3J_{C,F}$ = 8.3 Hz), 130.3 (d, $^3J_{C,F}$ = 3.7 Hz), 131.1, 133.9, 150.0, 150.3, 159.9 (d, $^1J_{C,F}$ = 246 Hz), 161.1 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{16}H_{14}FN_3O_2$: 300.1143, found: 300.1135.

1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine-3-carboxamide (28): Ester **27** (10.2 g, 34.0 mmol) was added to MeOH (150 mL) saturated with NH_3 at 0–10 °C, and the mixture was stirred at RT for 48 h. The mixture was then concentrated in vacuo to yield the title amide as a tan solid (9.19 g, quant), which was used without purification; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 5.82 (s, 2H), 7.11–7.18 (m, 2H), 7.23 (dd, *J* = 9.9, 8.5 Hz, 1H), 7.33–7.38 (m, 1H), 7.39 (dd, *J* = 8.0, 4.4 Hz, 1H), 7.51 (brs, 1H), 7.77 (brs, 1H), 8.57 (dd, *J* = 8.0, 1.4 Hz, 1H), 8.65 ppm (dd, *J* = 4.4, 1.4 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 44.0 (d, $^3J_{C,F}$ = 4.4 Hz), 114.0, 115.4 (d, $^2J_{C,F}$ = 21.0 Hz), 119.0, 123.5 (d, $^2J_{C,F}$ = 14.6 Hz), 124.6 (d, $^4J_{C,F}$ = 3.5 Hz), 129.8 (d, $^3J_{C,F}$ = 3.7 Hz), 129.9 (d, $^3J_{C,F}$ = 8.1 Hz), 131.6, 137.3, 149.6, 150.7, 159.7 (d, $^1J_{C,F}$ = 246 Hz), 163.0 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{14}H_{11}FN_4O$: 271.0990, found: 271.0989.

1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine-3-carbonitrile (29): Carboxamide **23** (36.1 g, 133 mmol) was dissolved in THF (330 mL), and pyridine (27 g, 341 mmol) was added. Over the course of 10 min, trifluoroacetic anhydride (TFAA; 71.7 g, 341 mmol) was added, during which the temperature rose to 40 °C. The mixture was stirred at RT overnight and was then added to H_2O (1 L). It was extracted with EtOAc (3×0.5 L), and the combined organic layers were washed with a saturated solution of $NaHCO_3$ and with 1 N HCl, dried ($MgSO_4$), and concentrated under reduced pressure to yield the title compound (33.7 g, 100%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 5.88 (s, 2H), 7.11–7.26 (m, 2H), 7.30–7.36 (m, 1H), 7.37–7.42 (m, 1H), 7.54 (dd, *J* = 8.2, 4.5 Hz, 1H), 8.49 (dd, *J* = 8.2, 1.3 Hz, 1H), 8.80 ppm (dd, *J* = 4.5, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 45.1 (d, $^3J_{C,F}$ = 3.9 Hz), 113.0, 115.5 (d, $^2J_{C,F}$ = 20.8 Hz), 116.2, 120.2, 122.5 (d, $^2J_{C,F}$ = 14.6 Hz), 124.6 (d, $^4J_{C,F}$ = 3.5 Hz), 129.4, 130.5 (d, $^3J_{C,F}$ = 8.1 Hz), 130.7 (d, $^3J_{C,F}$ = 3.5 Hz), 149.1, 151.1, 160.0 ppm (d, $^1J_{C,F}$ = 247 Hz), C3 of pyrazolopyridine not visible; HRMS: *m/z* $[M]^+$ calcd for $C_{14}H_9FN_4$: 252.0811, found: 252.0810.

1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine-3-carboximidamide (23): NaOMe (30.4 g, 562 mmol) was dissolved in MeOH (1.5 L), and nitrile **29** (36.5 g, 145 mmol) was added. The mixture was stirred at RT for 2 h. Subsequently, the solution was admixed with glacial HOAc (32.2 mL, 33.8 g, 562 mmol) and NH_4Cl (9.28 g, 173 mmol), and the mixture was heated at reflux overnight. Subsequently, the solvent was evaporated under reduced pressure, the residue was triturated with acetone, and the precipitated solid was filtered off with suction. The product was added to H_2O (2 L), and Na_2CO_3 (31.8 g, 300 mmol) was added with stirring. The solution was extracted with EtOAc (3×1 L), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated to yield the title compound (27.5 g, 70%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 5.88 (s, 2H), 7.12–7.17 (m, 1H), 7.20–7.29 (m, 2H), 7.34–7.41 (m, 1H), 7.49 (dd, *J* = 8.2, 4.4 Hz, 1H), 8.59 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.74 (dd, *J* = 4.4, 1.4 Hz, 1H), 9.49 ppm (brs, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 44.5 (d, $^3J_{C,F}$ = 4.2 Hz), 112.9, 115.5 (d, $^2J_{C,F}$ = 21.0 Hz), 119.2, 122.9 (d, $^2J_{C,F}$ = 14.6 Hz), 124.6 (d, $^4J_{C,F}$ = 3.7 Hz), 130.1 (d, $^3J_{C,F}$ = 3.5 Hz), 130.2 (d, $^3J_{C,F}$ = 8.1 Hz), 131.1, 133.3, 150.3, 150.4, 157.7, 159.8 ppm (d, $^1J_{C,F}$ = 246 Hz); HRMS: *m/z* $[M+H]^+$ calcd for $C_{14}H_{12}FN_5$: 270.1150, found: 270.1150.

3-{4-Amino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-5-yl}-1,3-oxazolidin-2-one (21): Compound **37c** (45.0 g, 229 mmol) was added to amidine **23** (47.5 g, 176 mmol) in toluene (200 mL), and the mixture was heated at reflux overnight. Subsequently, the solvent was removed in vacuo. The residue was triturated with EtOAc, and the precipitate was collected by suction filtration. The crude product was recrystallized from EtOH/H_2O (10:1), then twice triturated with EtOAc/MeOH (1:1) and collected by suction filtration to yield **21** as a tan solid (38.8 g, 54%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.84 (t, *J* = 7.9 Hz, 2H), 4.47 (t, *J* = 7.9 Hz, 2H), 5.84 (s, 2H), 7.11–7.27 (m, 3H), 7.33–7.39 (m, 2H), 7.39 (dd, *J* = 7.57, 4.41 Hz, 2H), 8.32 (s, 1H), 8.65 (dd, *J* = 4.4, 1.3 Hz, 1H), 8.95 ppm (dd, *J* = 8.2, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 44.0 (d, $^3J_{C,F}$ = 4.6 Hz), 45.8, 62.5, 114.5, 115.0, 115.4 (d, $^2J_{C,F}$ = 21.3 Hz), 118.3, 123.9 (d, $^2J_{C,F}$ = 14.8 Hz), 124.5 (d, $^4J_{C,F}$ = 3.7 Hz), 129.9 (d, $^3J_{C,F}$ = 8.3 Hz), 130.0 (d, $^3J_{C,F}$ = 4.6 Hz), 133.0, 140.9, 149.1, 150.8, 154.4, 156.6, 158.3, 159.8 (d, $^1J_{C,F}$ = 246 Hz), 160.4 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{20}H_{16}FN_7O_2$: 406.1422, found: 406.1416.

(2*E*/*Z*)-2-Cyclopropyl-3-(dimethylamino)prop-2-enenitrile (37d): Cyclopropylacetonitrile[35] (25.0 g, 293 mmol) and bis(dimethylamino)-*tert*-butyloxymethane (25.5 g, 146 mmol) were heated at 100 °C under a riser pipe for 46 h, while dimethylamine and *tert*-butanol were removed at reduced pressure. After evaporation of excess starting material and other volatile components, the residue was distilled (0.15 mbar, bp: 60–65 °C) to yield the title compound as a slightly yellow liquid (16.2 g, 81%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): mixture of two compounds (*E* and *Z*); major component: δ = 0.31–0.36 (m, 4H), 0.55–0.62 (m, 4H), 1.37–1.45 (m, 1H), 2.97 (s, 6H), 6.66 (s, 1H); minor component: δ = 0.41–0.46 (m, 4H), 0.73–0.78 (m, 4H), 1.57–1.65 (m, 1H), 3.04 (s, 6H), 6.69 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): mixture of two compounds (*E* and *Z*); major component: δ = 5.47, 12.5, 41.2, 72.7, 121.6, 150.6 ppm; minor component: δ = 7.60, 8.97, 41.9, 76.3, 124.1, 151.2 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_8H_{12}N_2$: 137.1073, found: 137.1072.

(2*E*/*Z*)-3-(Dimethylamino)-2-(morpholin-4-yl)prop-2-enenitrile (37e): Morpholinoacetonitrile[36] (8.13 g, 64.5 mmol) and *tert*-butoxy-bis(dimethylamino)methane (4.16 g, 64.5 mmol) were heated at 80 °C overnight. Subsequently, the mixture was concentrated in vacuo, and the residue was distilled (3.6 mbar, bp: 105 °C) to yield the title compound (11.0 g, 94%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 2.47–2.51 (m, 4H), 3.04 (s, 6H), 3.59–3.62 (m, 4H), 6.37 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 41.7, 52.5, 65.7, 91.6, 119.2, 144.4 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_9H_{15}N_3O$: 182.1288, found: 182.1286.

5-Cyclopropyl-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-4-amine (2): Amidine **23** (2.00 g, 7.43 mmol) and ami-

noacrylonitrile **37d** (4.00 g, 29.4 mmol) were mixed thoroughly with an ultrasonic bath until a homogeneous milk was formed. Water pump vacuum was applied, causing the mixture to foam. With shaking, the mixture was subsequently immersed into an oil bath at 106 °C and was heated overnight under reduced pressure. The resulting solid was triturated with toluene, filtered off with suction, and washed with Et_2O. The residue was taken up in boiling MeCN (50 mL) and filtered off with suction. The residue obtained was taken up in boiling DMF (25 mL) and filtered off with suction. Both filtrates were combined and concentrated to yield the title compound as a tan solid (850 mg, 31%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 0.57–0.67 (m, 2H), 0.85–0.97 (m, 2H), 1.62–1.70 (m, 1H), 5.82 (s, 2H), 7.02–7.19 (m, 4H), 7.19–7.26 (m, 1H), 7.31–7.40 (m, 2H), 8.63 (dd, J = 4.4, 1.3 Hz, 1H), 8.96 ppm (dd, J = 8.2, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 5.3, 8.3, 43.9 (d, $^3J_{C,F}$ = 4.6 Hz), 114.4, 115.4 (d, $^2J_{C,F}$ = 20.4 Hz), 116.8, 118.1, 123.9 (d, $^2J_{C,F}$ = 14.8 Hz), 124.5 (d, $^4J_{C,F}$ = 3.7 Hz), 129.8 (d, $^3J_{C,F}$ = 8.3 Hz), 130.0 (d, $^3J_{C,F}$ = 4.6 Hz), 133.1, 141.2, 149.0, 150.8, 151.0, 157.1, 159.8 (d, $^1J_{C,F}$ = 245 Hz), 163.4 ppm; HRMS: m/z [M+H]$^+$ calcd for $C_{20}H_{17}FN_6$: 361.1571, found: 361.1567.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-5-(morpholin-4-yl)pyrimidin-4-amine (7): A thorough mixture of amidine **23** (1.00 g, 3.72 mmol) and morpholinoacrylonitrile **37e** (2.00 g, 11.0 mmol) was treated with ultrasound for 5 min and subsequently stirred at 120 °C under reduced pressure (membrane pump) overnight. The mixture was cooled to RT and stirred with *tert*-butylmethyl ether. The resulting precipitate was collected by suction filtration and purified by flash chromatography (*c*Hex/EtOAc gradient 100:1→1:1) to yield the title compound as a tan solid (262 mg, 17%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 2.87–2.93 (m, 4H), 3.75–3.80 (m, 4H), 5.81 (s, 2H), 6.83 (br s, 2H), 7.10–7.19 (m, 2H), 7.20–7.26 (m, 1H), 7.32–7.36 (m, 1H), 7.36 (dd, J = 7.6, 3.8 Hz, 1H), 8.04 (s, 1H), 8.62 (d, J = 3.8 Hz, 1H), 8.95 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 43.8 (d, $^3J_{C,F}$ = 3.7 Hz), 50.3, 66.1, 114.3, 115.4 (d, $^2J_{C,F}$ = 20.3 Hz), 118.0, 124.0 (d, $^2J_{C,F}$ = 14.8 Hz), 124.5 (d, $^4J_{C,F}$ = 3.7 Hz), 129.5, 129.8 (d, $^3J_{C,F}$ = 8.3 Hz), 130.0 (d, $^3J_{C,F}$ = 3.7 Hz), 133.2, 141.5, 143.6, 149.0, 150.8, 158.5, 159.8 ppm (d, $^1J_{C,F}$ = 246 Hz); HRMS: m/z [M+H]$^+$ calcd for $C_{21}H_{20}FN_7O$: 406.1786, found: 406.1783.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-5-pyridin-4-ylpyrimidin-4-amine (12): Amidine **23** (0.50 g, 1.9 mmol) and 3-(dimethylamino)-2-(4-pyridyl)acrylonitrile[37] (0.32 g, 1.9 mmol) were suspended in xylene (5 mL), and BF_3 etherate (71 μL, 0.56 mmol) was added. The mixture was heated at 140 °C for 19 h. Subsequently, the solvent was evaporated, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 20:1). The compound was triturated with MeCN and collected by suction filtration to yield the title compound as a tan solid (0.24 g, 33%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 5.85 (s, 2H), 7.05–7.27 (m, 5H), 7.34–7.39 (m, 1H), 7.40 (dd, J = 8.0, 4.5 Hz, 1H), 7.53–7.56 (m, 2H), 8.28 (s, 1H), 8.64–8.69 (m, 3H), 9.04 ppm (dd, J = 8.0, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 44.1 (d, $^3J_{C,F}$ = 4.2 Hz), 113.9, 114.7, 115.5 (d, $^3J_{C,F}$ = 20.8 Hz), 118.4, 123.3, 123.9 (d, $^2J_{C,F}$ = 14.6 Hz), 124.6 (d, $^4J_{C,F}$ = 3.5 Hz), 130.0 (d, $^3J_{C,F}$ = 8.1 Hz), 130.1 (d, $^3J_{C,F}$ = 3.9 Hz), 133.3, 141.0, 142.6, 149.3, 150.2, 150.9, 155.3, 159.5, 159.9 (d, $^1J_{C,F}$ = 246 Hz), 160.5 ppm; HRMS: m/z [M+H]$^+$ calcd for $C_{22}H_{16}FN_7$: 398.1524, found: 398.1520.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-5-methoxypyrimidin-4-amine (38): Amidine **23** (46.8 g, 135 mmol) was dissolved in isoamyl alcohol, and 3,3-bis(dimethylamino)-2-methoxypropionitrile **37i** (24.7 g, 144 mmol) was added. The mixture was heated at 110 °C for three days, then cooled to 0 °C, and the precipitated product was collected by suction filtration. It was washed with cool Et_2O and dried in a vacuum oven at 50 °C to yield the title compound as a tan solid (25.4 g, 53%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 3.89 (s, 3H), 5.80 (s, 2H), 6.93 (br s, 2H), 7.11–7.19 (m, 2H), 7.20–7.26 (m, 1H), 7.33–7.38 (m, 1H), 7.35 (dd, J = 7.6, 4.4 Hz, 1H), 7.99 (s, 1H), 8.61 (d, J = 4.1 Hz, 1H), 8.92 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 43.7 (d, $^3J_{C,F}$ = 3.7 Hz), 55.7, 114.2, 115.4 (d, $^2J_{C,F}$ = 21.3 Hz), 117.9, 124.1 (d, $^2J_{C,F}$ = 14.8 Hz), 124.5 (d, $^4J_{C,F}$ = 3.7 Hz), 129.8 (d, $^3J_{C,F}$ = 8.3 Hz), 130.0 (d, $^3J_{C,F}$ = 3.7 Hz), 133.1, 133.5, 138.9, 141.5, 148.9, 150.8, 152.2, 155.2, 159.8 ppm (d, $^1J_{C,F}$ = 246 Hz); HRMS: m/z [M+H]$^+$ calcd for $C_{18}H_{15}FN_6O$: 351.1364, found: 351.1362.

4-Amino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-5-ol (39): Methyl ether **38** (25.3 g, 72.2 mmol) was dissolved in NMP (500 mL), and thiophenol (7.96 g, 72.2 mmol) and K_2CO_3 (2.50 g, 18.1 mmol) were added. The mixture was heated at 190 °C for 1 h. The solvent was then removed in vacuo, and the residue was mixed with a solution of NH_4Cl (half-concd, 1.0 L) and extracted with EtOAc (3×500 mL). The product precipitated and was collected by suction filtration and dried in a vacuum oven at 50 °C to yield the title compound as a gray solid (18.1 g, 72%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 5.79 (s, 2H), 6.70 (br s, 2H), 7.11–7.18 (m, 2H), 7.20–7.25 (m, 1H), 7.33 (dd, J = 7.6, 4.4 Hz, 1H), 7.33–7.38 (m, 1H), 7.83 (s, 1H), 8.60 (d, J = 4.4 Hz, 1H), 8.92 (d, J = 7.6 Hz, 1H), 9.85 ppm (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 43.7 (d, $^3J_{C,F}$ = 3.7 Hz), 114.1, 115.4 (d, $^2J_{C,F}$ = 20.3 Hz), 117.7, 124.1 (d, $^2J_{C,F}$ = 13.9 Hz), 124.5 (d, $^4J_{C,F}$ = 3.7 Hz), 129.7 (d, $^3J_{C,F}$ = 8.3 Hz), 130.0 (d, $^3J_{C,F}$ = 3.7 Hz), 133.2, 136.4, 136.6, 141.8, 148.9, 150.8, 151.3, 155.1, 159.8 ppm (d, $^1J_{C,F}$ = 246 Hz); HRMS: m/z [M+H]$^+$ calcd for $C_{17}H_{13}FN_6O$: 337.1208, found: 337.1209.

4-Amino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-5-yl methanesulfonate (14): Methanesulfonyl chloride (75 mg, 0.65 mmol) was added to a solution of phenol **39** (200 mg, 0.60 mmol) in pyridine (10 mL), and the mixture was stirred at RT overnight. Subsequently, H_2O (150 mL) was added. The precipitate was collected by suction filtration, washed with H_2O, and dried in vacuo to yield the title compound as a tan solid (220 mg, 89%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 3.51 (s, 3H), 5.84 (s, 2H), 7.11–7.26 (m, 3H), 7.32–7.38 (m, 1H), 7.40 (dd, J = 7.9, 4.7 Hz, 1H), 7.56 (br s, 2H), 8.29 (s, 1H), 8.63–8.66 (m, 1H), 8.92–8.96 ppm (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 37.7, 44.0 (d, $^3J_{C,F}$ = 4.6 Hz), 114.4, 115.4 (d, $^2J_{C,F}$ = 21.3 Hz), 118.4, 123.8 (d, $^2J_{C,F}$ = 14.8 Hz), 124.6 (d, $^4J_{C,F}$ = 3.7 Hz), 128.4, 129.9 (d, $^3J_{C,F}$ = 8.3 Hz), 130.0 (d, $^3J_{C,F}$ = 3.7 Hz), 133.0, 140.6, 148.2, 149.2, 150.8, 156.8, 157.6, 159.8 ppm (d, $^1J_{C,F}$ = 246 Hz); HRMS: m/z [M+H]$^+$ calcd for $C_{18}H_{15}FN_6O_3S$: 415.0983, found: 415.0977.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-5-pyridin-4-ylpyrimidine-4,6-diol (41): Amidine **23** (3.27 g, 12.1 mmol) and diethyl pyridin-4-ylpropanedioate[25] (2.88 g, 12.1 mmol) were suspended in toluene (40 mL) and heated at reflux overnight. The precipitate was collected by suction filtration to yield the title compound (2.43 g; 43%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 5.86 (s, 2H), 7.11–7.44 (m, 6H), 7.49 (dd, J = 8.1, 4.4 Hz, 1H), 8.25 (br s, 2H), 8.40–8.48 (m, 2H), 8.72 (dd, J = 4.4, 1.5 Hz, 1H), 8.92 ppm (dd, J = 8.1, 1.5 Hz, 1H).

3-(4,6-Dichloro-5-pyridin-4-ylpyrimidin-2-yl)-1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine (42): Dihydroxypyrimidine **41** (2.39 g, 5.77 mmol) was heated in $POCl_3$ (10 mL) with a catalytic amount of DMF (3 drops) for 3 h at reflux. Subsequently, the mixture was poured into an ice-cold Na_2CO_3 solution (150 mL), and the mixture was extracted with CH_2Cl_2 (150 mL). The solvent was removed in

vacuo to afford the title compound as a brownish solid (670 mg, 24%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=5.95 (s, 2H), 7.12–7.29 (m, 3H), 7.34–7.43 (m, 1H), 7.52–7.59 (m, 3H), 8.75 (dd, J=4.4, 1.6 Hz, 1H), 8.78–8.82 (m, 2H), 8.82 ppm (dd, J=8.1, 1.6 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=44.7 (d, $^3J_{C,F}$=4.2 Hz), 114.6, 115.6 (d, $^2J_{C,F}$=21.0 Hz), 119.8, 123.4 (d, $^2J_{C,F}$=14.6 Hz), 124.7 (d, $^4J_{C,F}$=3.5 Hz), 125.4, 128.6, 130.2 (d, $^3J_{C,F}$=3.5 Hz), 130.2 (d, $^3J_{C,F}$=8.6 Hz), 131.9, 138.0, 143.7, 148.1, 150.1, 150.9, 158.9, 159.8, 160.0 ppm (d, $^1J_{C,F}$=246 Hz); HRMS: m/z [M+H]$^+$ calcd for $C_{22}H_{13}Cl_2FN_6$: 451.0636, found: 451.0623.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-5-pyridin-4-ylpyrimidine-4,6-diamine (13): Dichloropyrimidine **42** (200 mg, 0.443 mmol) was suspended in 25% aqueous NH_3 (5.0 mL) and heated overnight at 140 °C in an autoclave. Subsequently, the mixture was extracted with CH_2Cl_2 (3×50 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 30:1). A further purification step by preparative HPLC afforded the title compound (45 mg, 20% yield); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=5.95 (s, 2H), 7.14–7.21 (m, 2H), 7.23–7.29 (m, 1H), 7.29–7.50 (m, 5H), 7.54 (dd, J=8.1, 4.4 Hz, 1H), 7.68 (d, J=5.0 Hz, 2H), 8.77 (dd, J=4.4, 1.3 Hz, 1H), 8.88 (d, J=5.0 Hz, 2H), 9.04 ppm (dd, J=8.1, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=44.7 (d, $^3J_{C,F}$=4.2 Hz), 90.9, 114.2, 115.6 (d, $^2J_{C,F}$=20.8 Hz), 119.8, 123.3 (d, $^2J_{C,F}$=14.6 Hz), 124.7 (d, $^4J_{C,F}$=3.5 Hz), 127.6, 129.8 (d, $^3J_{C,F}$=3.7 Hz), 130.2 (d, $^3J_{C,F}$=8.1 Hz), 132.6, 147.7, 150.6, 151.1, 158.0, 158.3, 158.6, 159.8 ppm (d, $^1J_{C,F}$=246 Hz); HRMS: m/z [M]$^+$ calcd for $C_{22}H_{17}FN_8$: 412.1560, found: 412.1553.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-5-(morpholin-4-yl)pyrimidine-4,6-diamine (3): Amidine **23** (200 mg, 0.74 mmol) and 2-*N*-morpholinomalonic dinitrile[31] (400 mg, 2.65 mmol) were heated at 105 °C under reduced pressure for 12 h. Subsequently, the solid residue was dissolved in DMF, silica gel (600 mg) was added, and the solvent was evaporated under reduced pressure. Flash chromatography (gradient: EtOAc, then MeOH) gave the title compound as a tan solid (222 mg, 71%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=2.87–3.01 (m, 4H), 3.64–3.83 (m, 4H), 5.78 (s, 2H), 6.13 (br s, 4H), 7.07–7.15 (m, 2H), 7.20–7.25 (m, 1H), 7.32 (dd, J=7.9, 4.7 Hz, 1H), 7.32–7.37 (m, 1H), 8.59 (dd, J=4.4, 1.3 Hz, 1H), 9.07 ppm (dd, J=8.2, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=43.8 (d, $^3J_{C,F}$=4.6 Hz), 47.9, 67.1, 106.8, 114.6, 115.4 (d, $^2J_{C,F}$=20.4 Hz), 117.8, 124.2 (d, $^2J_{C,F}$=14.8 Hz), 124.6 (d, $^4J_{C,F}$=3.7 Hz), 129.8 (d, $^3J_{C,F}$=8.3 Hz), 129.9 (d, $^3J_{C,F}$=4.0 Hz), 134.0, 141.8, 148.8, 150.8, 155.8, 160.1 (d, $^1J_{C,F}$=246 Hz), 160.6 ppm; HRMS: m/z [M+H]$^+$ calcd for $C_{21}H_{21}FN_8O$: 421.1901, found: 421.1887.

5-Ethyl-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidine-4,6-diamine (5): Amidine **23** (13.0 g, 42.5 mmol) and ethylmalononitrile[29] (4.00 g, 42.5 mmol) were heated in NMP (19 mL) at 150 °C overnight. The mixture was concentrated in vacuo, and the crude product was purified by flash chromatography (EtOAc/toluene 1:1, then EtOAc, then MeOH). The product fractions were concentrated, and the residue was purified by preparative HPLC to yield the title compound as a tan solid (1.69 g, 11%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=1.03 (t, J=7.3 Hz, 3H), 2.47 (t, J=7.3 Hz, 2H), 5.92 (s, 2H), 7.09–7.21 (m, 2H), 7.22–7.29 (m, 1H), 7.34–7.41 (m, 1H), 7.62 (br s, 4H), 7.52 (dd, J=8.2, 4.4 Hz, 1H), 8.76 (d, J=4.4 Hz, 1H), 8.99 ppm (dd, J=8.2 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=11.1, 15.5, 44.6 (d, $^3J_{C,F}$=4.6 Hz), 93.3, 113.8, 115.5 (d, $^2J_{C,F}$=21.3 Hz), 119.6, 123.2 (d, $^2J_{C,F}$=14.8 Hz), 124.7 (d, $^4J_{C,F}$=2.8 Hz), 129.6 (d, $^3J_{C,F}$=3.7 Hz), 130.1 (d, $^3J_{C,F}$=8.3 Hz), 132.5, 134.8, 148.0, 150.6, 151.0, 159.7 ppm (d, $^1J_{C,F}$=245 Hz); HRMS: m/z [M+H]$^+$ calcd for $C_{19}H_{18}FN_7$: 364.1687, found: 364.1674.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-*N*^{5},*N*^{5}-dimethylpyrimidine-4,5,6-triamine (6): Amidine **23** (1.54 g, 5.72 mmol) and (dimethylamino)propanedinitrile[32] (1.87 g, 17.2 mmol) were heated at 105 °C overnight. Subsequently, MeOH and silica gel (6 g) were added, and the mixture was concentrated in vacuo. The product was purified by flash chromatography (*c*Hex/EtOAc 1:1, then EtOAc/MeOH 4:1) to yield the title compound as a tan solid (901 mg, 42%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=2.67 (s, 6H), 5.78 (s, 2H), 6.05 (br s, 4H), 7.07–7.15 (m, 2H), 7.19–7.25 (m, 1H), 7.32 (dd, J=8.2, 4.4 Hz, 1H), 7.32–7.38 (m, 1H), 8.59 (d, J=4.3 Hz, 1H), 9.06 ppm (d, J=8.2 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=40.6, 43.7 (d, $^3J_{C,F}$=4.6 Hz), 108.0, 114.5, 115.4 (d, $^2J_{C,F}$=21.3 Hz), 117.7, 124.2 (d, $^2J_{C,F}$=14.8 Hz), 124.5 (d, $^4J_{C,F}$=3.7 Hz), 129.7 (d, $^3J_{C,F}$=8.3 Hz), 129.8 (d, $^3J_{C,F}$=3.7 Hz), 133.8, 141.9, 148.7, 150.7, 155.3, 159.8 (d, $^1J_{C,F}$=246 Hz), 160.3 ppm; HRMS: m/z [M+H]$^+$ calcd for $C_{19}H_{19}FN_8$: 379.1789, found: 379.1779.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-5-[(*E*)-phenyldiazenyl]pyrimidine-4,6-diamine (50): Sodium methanolate (3.87 g, 71.7 mmol) and then phenylazomalonitrile[33] (12.2 g, 71.7 mmol) were added to a stirred solution of amidine **23** (21.9 g, 71.1 mmol) in DMF (160 mL). The mixture was heated at 110 °C overnight and then allowed to cool to RT. The solid which precipitated thereby was filtered off with suction and washed with EtOH. Drying resulted in the desired compound (23.0 g, 73%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=5.85 (s, 2H), 7.13–7.26 (m, 3H), 7.34–7.42 (m, 3H), 7.49 (dd, J=8.0, 7.4 Hz, 2H), 7.84 (br s, 2H), 8.01 (d, J=7.4 Hz, 2H), 8.47 (br s, 2H), 8.65 (dd, J=4.4, 1.5 Hz, 1H), 9.20 ppm (dd, J=8.0, 1.5 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=44.0 (d, $^3J_{C,F}$=4.2 Hz), 112.1, 115.0, 115.4 (d, $^2J_{C,F}$=21.0 Hz, 118.3, 121.9, 123.9 (d, $^2J_{C,F}$=14.6 Hz), 124.6 (d, $^4J_{C,F}$=3.7 Hz), 129.0, 129.9 (d, $^3J_{C,F}$=8.1 Hz), 130.0 (d, $^3J_{C,F}$=3.9 Hz), 133.9, 141.1, 149.1, 150.8, 152.5, 158.3, 159.9 (d, $^1J_{C,F}$=246 Hz), 160.6 ppm; HRMS: m/z [M+H]$^+$ calcd for $C_{23}H_{18}FN_9$: 440.1742, found: 440.1734.

2-[1-(2-Fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidine-4,5,6-triamine (51): The diazo compound **50** (5.00 g, 11.4 mmol) was hydrogenated with Raney nickel (800 mg, 50% in H_2O) in DMF under a pressure of 65 bar H_2 and at 62 °C for 22 h. The catalyst was filtered off with suction through Celite, and the solution was evaporated in vacuo. The residue was stirred with 5 N HCl. The yellow–brown precipitate was collected by suction filtration and dried to yield the desired compound as a trihydrochloride (3.10 g, 59%). The free base was obtained by shaking the salt with a dilute solution of $NaHCO_3$ and subsequent extraction with EtOAc. The solid which was virtually insoluble in both phases was filtered off with suction; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=4.02 (s, 2H), 5.75 (s, 2H), 5.78 (s, 4H), 7.10–7.14 (m, 2H), 7.20 (dd, J=10.5, 8.1 Hz, 1H), 7.28 (dd, J=8.0, 4.4 Hz, 1H), 7.31–7.37 (m, 1H), 8.56 (dd, J=4.4, 1.5 Hz, 1H), 9.03 ppm (dd, J=8.0, 1.5 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=43.6 (d, $^3J_{C,F}$=4.4 Hz), 106.4, 114.3, 115.4 (d, $^2J_{C,F}$=21.0 Hz), 117.3, 124.4 (d, $^2J_{C,F}$=14.8 Hz), 124.5 (d, $^4J_{C,F}$=3.7 Hz), 129.7 (d, $^3J_{C,F}$=8.1 Hz), 129.9 (d, $^3J_{C,F}$=3.9 Hz), 133.8, 142.5, 148.6, 149.0, 150.9, 151.2, 159.9 ppm (d, $^1J_{C,F}$=246 Hz); HRMS: m/z [M+H]$^+$ calcd for $C_{17}H_{15}FN_8$: 351.1477, found: 351.1483.

***N*-{4,6-Diamino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl}propane-2-sulfonamide (15)**: Triaminopyrimidine **51** (380 mg, 1.09 mmol) was dissolved in pyridine (25 mL). Isopropylsulfonyl chloride (232 mg, 1.63 mmol) was added, and the mixture was stirred at RT overnight. Subsequently, the solvent was removed in vacuo, and the residue was purified by flash chroma-

tography (CH_2Cl_2/MeOH 20:1) to afford the title compound (230 mg, 46% yield); ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 1.31 (d, J = 6.7 Hz, 6H), 3.41 (sept, J = 6.7 Hz, 1H), 5.80 (s, 2H), 6.29 (br s, 4H), 6.98–7.16 (m, 2H), 7.22 (dd, J = 10.5, 8.2 Hz, 1H), 7.30–7.42 (m, 1H), 7.35 (dd, J = 8.0, 4.5 Hz, 1H), 8.30 (s, 1H), 8.61 (dd, J = 4.5, 1.6 Hz, 1H), 9.06 ppm (dd, J = 8.0, 1.6 Hz, 1H).

***N*-{4,6-Diamino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl}-*N*-methylpropane-2-sulfonamide (16)**: Sulfonamide **15** (217 mg, 0.475 mmol) was dissolved in acetone (54 mL). K_2CO_3 (328 mg, 2.38 mmol) and CH_3I (67 mg, 0.48 mmol) were added, and the mixture was stirred at RT overnight. Subsequently, H_2O was added, and the mixture was extracted with CH_2Cl_2 (100 mL). The solvent was removed in vacuo, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 30:1) to yield the title compound (155 mg, 69% yield); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.31 (d, J = 6.8 Hz, 6H), 3.06 (s, 3H), 3.60 (sept, J = 6.8 Hz, 1H), 5.80 (s, 2H), 6.37 (br s, 4H), 7.07–7.16 (m, 2H), 7.23 (dd, J = 10.1, 8.6 Hz, 1H), 7.31–7.40 (m, 1H), 7.35 (dd, J = 8.1, 4.4 Hz, 1H), 8.61 (dd, J = 4.4, 1.6 Hz, 1H), 9.07 ppm (dd, J = 8.1, 1.6 Hz, 1H).

Methyl-{4,6-diamino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl}carbamate (52): Triamine **51** (30.5 g, 87.0 mmol) was dissolved in pyridine (470 mL) and cooled to 0 °C. Methyl chloroformate (8.22 g, 87.0 mmol) was added, and the mixture was stirred at 0 °C for 2 h. Subsequently, it was warmed to RT and stirred for a further 12 h. After concentration in vacuo, the residue was washed with H_2O and dried. Further purification was effected by stirring in boiling Et_2O (300 mL). The precipitated product was filtered off with suction and dried in vacuo to yield the title compound (32.6 g, 92%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 3.61 (s, 3H), 5.80 (s, 2H), 6.13 (br s, 4H), 7.10–7.15 (m, 2H), 7.21 (dd, J = 10.0, 8.5 Hz, 1H), 7.31–7.37 (d, 2H), 7.97 (br s, 1H), 8.60 (dd, J = 4.4, 1.5 Hz, 1H), 9.06 ppm (dd, J = 8.0, 1.5 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 43.8 (d, $^3J_{C,F}$ = 4.2 Hz), 51.7, 94.2, 114.6, 115.4 (d, $^2J_{C,F}$ = 21.0 Hz), 117.8, 124.1 (d, $^2J_{C,F}$ = 14.6 Hz), 124.5 (d, $^4J_{C,F}$ = 3.5 Hz), 129.7 (d, $^3J_{C,F}$ = 8.1 Hz), 129.8 (d, $^3J_{C,F}$ = 3.9 Hz), 133.8, 141.7, 148.8, 150.8, 155.0, 156.7, 159.8 (d, $^1J_{C,F}$ = 246 Hz), 160.2 ppm; HRMS: m/z $[M+H]^+$ calcd for $C_{19}H_{17}FN_8O_2$: 409.1532, found: 409.1526; Anal. calcd for $C_{19}H_{17}FN_8O_2$: C 55.88, H 4.20, N 27.44, F 4.65, found: C 55.75, H 4.20, N 27.35, F 4.70.

Methyl-{4,6-diamino-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl}methylcarbamate (20): Methyl carbamate **52** (20.0 g, 49.0 mmol) was dissolved in THF (257 mL) and cooled to 0 °C. LiHMDS was added as a 1 M solution in THF (53.9 mL, 53.9 mmol) within 15 min. After 20 min of stirring at 0 °C, CH_3I (6.95 g, 53.9 mmol) was added. The mixture was stirred for 1 h at 0 °C and then warmed to RT. An aqueous solution of NH_4Cl was added, and it was extracted with EtOAc and CH_2Cl_2. The combined organic layers were concentrated in vacuo, and the residue was triturated with THF/CH_2Cl_2 (1:1). The remaining crystals were collected by suction filtration, added to MeOH, and heated at reflux for 1 h. The precipitate was collected. Dioxane/CH_2Cl_2 (1:1, 100 mL) was added and heated at reflux. MeOH (~20 mL) was added until the material was completely dissolved. Charcoal was added. The mixture was heated at reflux and filtered through Celite. The solvent was evaporated, and the residue was stirred in MeOH for 1 h. The white crystals were collected by suction filtration to yield 14.9 g (72%) of the title compound; ^{1}H NMR (500 MHz, [D$_6$]DMSO): two rotamers; δ = 3.01 (s, 3H), 3.54 and (s, 3H), 5.80 (s, 2H), 6.33 and 3.31 (br s, 4H), 7.07–7.14 (m, 2H), 7.23 (dd, J = 10.0, 8.5 Hz, 1H), 7.30–7.37 (m, 2H), 8.59 (dd, J = 4.0, 1.1 Hz, 1H), 9.05 ppm (dd, J = 8.0, 1.1 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): two rotamers; δ = 34.2 (major component a) and 34.6 (minor component b), 43.8 (d, $^3J_{C,F}$ = 4.4 Hz), 52.3 (b) and 52.4 (a), 99.3 (a) and 100.3 (b), 114.6, 115.4 (d, $^2J_{C,F}$ = 21.0 Hz), 117.8, 124.1 (d, $^2J_{C,F}$ = 14.8 Hz), 124.5 (d, $^4J_{C,F}$ = 3.5 Hz), 129.7 (d, $^3J_{C,F}$ = 7.8 Hz), 129.8 (d, $^3J_{C,F}$ = 3.5 Hz), 133.8, 141.7, 148.8, 150.8, 155.0 (b) and 155.4 (a), 157.1, 159.4, 159.7, 159.8 ppm (d, $^1J_{C,F}$ = 245.5 Hz); HRMS: m/z $[M+H]^+$ calcd for $C_{20}H_{19}FN_8O_2$: 423.1688, found: 423.1692; Anal. calcd for $C_{19}H_{17}FN_8O_2$: C 56.9, H 4.5, N 26.6, F 4.5, found: C 57.1, H 4.7, N 26.7, F 4.3.

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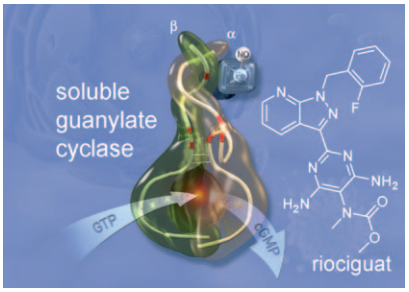
Discovery of Riociguat (BAY 63-2521): A Potent, Oral Stimulator of Soluble Guanylate Cyclase for the Treatment of Pulmonary Hypertension



Direct stimulation of soluble guanylate cyclase (sGC) represents a promising therapeutic strategy for the treatment of a range of diseases, including the severely disabling pulmonary hypertension (PH). Optimization of the unfavorable DMPK profile of previous sGC stimulators provided riociguat, which is currently being investigated in phase III clinical trials for the oral treatment of PH.

