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Damage Incorporated: Discovery of the Potent, Highly Selective, Orally Available ATR Inhibitor BAY 1895344 with Favorable Pharmacokinetic Properties and Promising Efficacy in Monotherapy and in Combination Treatments in Preclinical Tumor Models

Ulrich Lücking,* Lars Wortmann,* Antje M. Wengner, Julien Lefranc, Philip Lienau, Hans Briem, Gerhard Siemeister, Ulf Bömer, Karsten Denner, Martina Schäfer, Marcus Koppitz, Knut Eis, Florian Bartels, Benjamin Bader, Wilhelm Bone, Dieter Moosmayer, Simon J. Holton, Uwe Eberspächer, Joanna Grudzinska-Goebel, Christoph Schatz, Gesa Deeg, Dominik Mumberg, and Franz von Nussbaum

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ABSTRACT: The ATR kinase plays a key role in the DNA damage response by activating essential signaling pathways of DNA damage repair, especially in response to replication stress. Since DNA damage and replication stress are major sources of genomic instability, selective ATR inhibition has been recognized as a promising new approach in cancer therapy. We now report the identification and preclinical evaluation of the novel, clinical ATR inhibitor BAY 1895344. Starting from quinoline **2** with weak ATR inhibitory activity, lead optimization efforts focusing on potency, selectivity, and oral bioavailability led to the discovery of the potent, highly selective, orally available ATR inhibitor BAY 1895344, which exhibited strong monotherapy efficacy in cancer xenograft models that carry certain DNA damage repair deficiencies. Moreover, combination treatment of BAY 1895344 with certain DNA damage inducing chemotherapy resulted in synergistic antitumor activity. BAY 1895344 is currently under clinical investigation in patients with advanced solid tumors and lymphomas (NCT03188965).

INTRODUCTION

The DNA damage response (DDR) is a complex network of signaling pathways that coordinates the detection and repair of DNA damage to secure the integrity of the genome of eukaryotic cells.[1,2] DDR deficiencies are linked to human pathologies, including cancer.[3–5] Defects in or deregulation of certain DDR components can promote tumorigenesis but concurrently may increase the dependence of cancer cells on alternative repair pathways. The kinases ataxia telangiectasia and Rad3-related (ATR), ataxia telangiectasia mutated (ATM), and DNA-dependent protein kinase (DNA-PK) are key mediators of the DDR by responding to different DNA damage insults, which are primarily DNA double-strand breaks for ATM and DNA-PK, and replication stress for ATR.[6–9] These kinases belong to the phosphatidylinositol 3-kinase-related kinase (PIKK) family of serine/threonine protein kinases, which also includes the mammalian target of rapamycin (mTOR). The kinases of the PIKK family are characterized by a high degree of homology in the kinase domain.

DNA replication stress is a hallmark of cancer[10] and ATR plays a key role in the DNA replication stress response by stabilizing replication forks, regulating cell cycle progression, and activating DNA damage repair.[4,11–14] Since replication stress is a major source of genomic instability in cancer, ATR has recently emerged as a promising target in cancer therapy.[15,16] Selective ATR inhibition leads to increased replication stress and DNA damage, overall resulting in cellular lethality. Promising preclinical data provided a strong basis for the clinical evaluation of selective ATR inhibitors, both in combination with selected DNA damage inducing radio- or chemotherapy, and as monotherapy, following synthetic lethality approaches by treatment of tumors with certain DDR deficiencies.[8] Cancer cells with increased replication stress (e.g., oncogene-induced via Ras activation or MYC amplification) or a defective DDR (e.g., ATM loss of function) may provide the potential for single-agent activity of a selective ATR inhibitor, relying on the principle of synthetic lethality.[17,18] Therapeutic efficacy of standard-of-care cancer treatments, such as radio- and chemotherapy, relies on the induction of DNA damage, which is particularly cytotoxic for proliferating cells. DNA repair mechanisms may limit their effectiveness, especially in cancers that have activated the DDR to resist the lethal effects.[19] Selective ATR inhibition

may enhance sensitivity to certain DNA damage inducing therapies, as well as to certain DNA damage repair compromising therapies, providing a strong potential for ATR inhibitors in anticancer therapy.[13]

However, the discovery of highly selective ATR inhibitors is challenging.[20] The high degree of homology of the kinases of the PIKK family and related lipid kinases like phosphatidylinositol 3-kinases (PI3Ks) increases the risk for the inhibition of additional kinases, which may increase toxicity or counteract the therapeutic effects of ATR inhibition. So far, four ATR inhibitors are reported to have entered clinical trials as single agents and in combination therapy for the treatment of cancer.[13] The structures and preclinical properties of berzosertib (M6620/VX-970),[21] discovered by Vertex and licensed to Merck KGaA, and AstraZeneca's ceralasertib (AZD6738),[22] originating from tool compound AZ20,[23] were recently disclosed (Figure 1). Moreover, clinical evaluation of M4344/VX-803, whose structure has been disclosed recently[24] has been initiated (NCT02278250). In this report, we now describe the discovery and preclinical evaluation of the potent, highly selective, orally available ATR inhibitor BAY 1895344,[25] which is currently under clinical investigation in patients with advanced solid tumors and lymphomas (NCT03188965).[26]

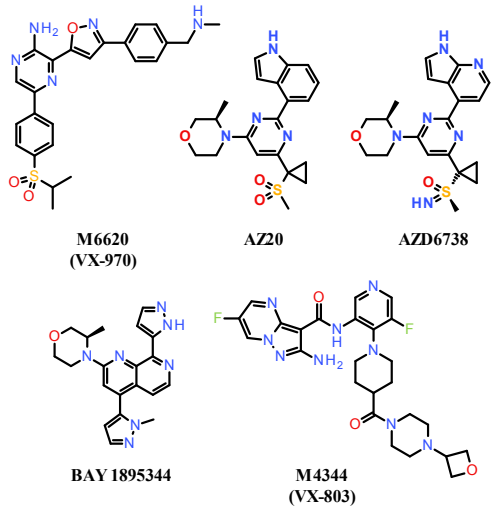


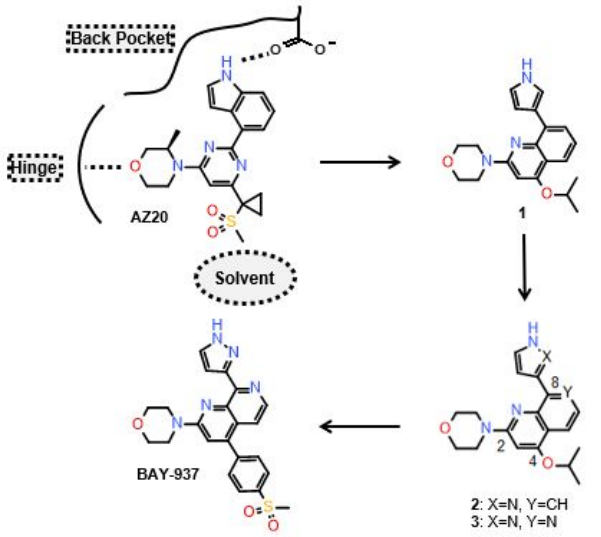
Figure 1. Structures of ATR inhibitors M6620 (VX-970), AZ20, AZD6738, BAY 1895344 and M4344 (VX-803).

RESULTS AND DISCUSSION

Search for a New ATR Lead Structure. To identify a new ATR lead structure, we initiated a high-throughput screen (HTS) and also evaluated known ATR inhibitors in an in-house homology model based on a published PI3Kγ crystal structure.[27]

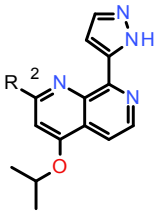
Tool compound AZ20, for instance, revealed two main interactions with the ATP binding pocket of ATR in this homology model (Scheme 1): one hydrogen bond via the morpholine oxygen to the hinge region and an additional hydrogen bond via the indole NH. Based on the overall findings from these modeling studies, including evaluation of the most interesting hits from the HTS, quinoline derivative **1** was suggested as a new ATR inhibitor. However, the electron-rich pyrrole moiety of the proposed structure **1** raised concerns about potential DMPK issues, and therefore we elected to synthesize the corresponding pyrazole analogue **2** instead. Due to the unprecedented 2,4,8-substitution pattern at the quinolone scaffold, synthesis of target molecule **2** turned out to be challenging and multiple synthetic routes were evaluated before the synthesis was finally accomplished (see Scheme 2). Despite all the effort, quinoline **2** revealed very low inhibitory activity against ATR in the biochemical in vitro assay compared to AZ20 [IC_{50}: 5050 nM (**2**) vs 3 nM (AZ20)]. However, due to the difficulties encountered in the synthesis of quinoline **2**, we had also initiated the synthesis of the corresponding naphthyridine analogue **3**, before the preparation of quinoline **2** was achieved. The additional nitrogen at the 7-position of the naphthyridine core was intended to simplify the synthesis with respect to the desired 2,4,8-regiochemistry at the scaffold, since it was envisaged to allow the introduction of the substituent at the 8-position (next to the 'added' nitrogen) by various methods. This time our efforts paid off and the new naphthyridine **3** surprisingly revealed promising ATR inhibitory activity in vitro (IC_{50}: 125 nM). Initial lead optimization efforts then led to the identification of the new lead compound BAY-937 which demonstrated further improved in vitro activity, both in the biochemical (IC_{50}: 59 nM, see Table 4) and in the cellular mechanistic assay in HT-29 cells (IC_{50}: 380 nM). The new lead compound also revealed good selectivity against the structurally related kinase ATM, based on the IC_{50} values from the corresponding biochemical in vitro assays (ratio of IC_{50} values ATM/ATR ~332). However, selectivity against mTOR, based on the IC_{50} values from the corresponding cellular mechanistic assays, was low (ratio of IC_{50} values mTOR/ATR ~13), which raised concerns about potential toxic effects resulting from this off-target inhibition. BAY-937 also demonstrated promising antiproliferative activities in vitro against various cancer cell lines, for example in the colorectal cancer (CRC) cell lines HT-29 (IC_{50}: 704 nM) and LoVo (IC_{50}: 315 nM). However, BAY-937 was characterized by a very low aqueous solubility of <1 mg/L and this impacts the oral bioavailability of only 14% that we recorded in in vivo pharmacokinetic studies in rats, even though BAY-937 has a high permeability coefficient (P_{app} A–B) of 102 nm/s and a low efflux ratio of 2.1 in Caco-2 cells (see Table 4). Moreover, the compound also raised safety concerns due to its activity in the hERG patch clamp assay in vitro (IC_{50}: 5.8 μM). However, in in vivo efficacy studies in the HT-29 xenograft model in mice, BAY-937 demonstrated moderate antitumor efficacy after twice daily (b.i.d.) oral administration (po), and the compound was finally selected as the new ATR lead structure.

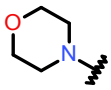
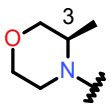
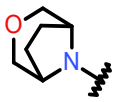
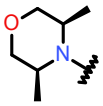
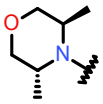
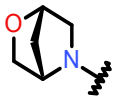
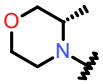
Scheme 1. Key Structural Modifications Leading to the New ATR Lead Structure BAY-937

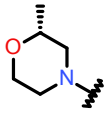
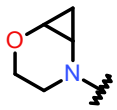
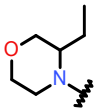
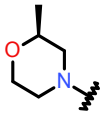
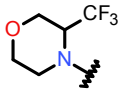
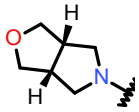
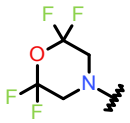
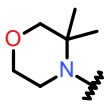
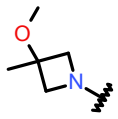


Lead Optimization Efforts Culminating in Clinical Candidate BAY 1895344. Lead optimization efforts initially focused on the hinge-binding morpholine moiety of BAY-937, by investigating the structure–activity relationship (SAR) of ATR inhibitory activity in the biochemical and in the cellular mechanistic assay in HT-29 cells. Since the low selectivity of BAY-937 against the structurally related PIKK mTOR was considered a potential toxicity issue, increasing selectivity against mTOR was another important optimization parameter. Due to the shorter syntheses, the structural variation of the hinge-binding moiety was initially evaluated in the related 4-isopropoxy series, using compound **3** as a point of reference. Introduction of a methyl group at the 3-position of the morpholine, with (*R*)-stereochemistry,[23] gave compound **4** with significantly improved inhibitory activity against ATR in vitro in the biochemical (IC_{50}: 38 nM, Table 1) and in the cellular assay (IC_{50}: 154 nM). Moreover, selectivity against the structurally related kinase mTOR, based on the ratio of the IC_{50} values from the corresponding cellular in vitro assays, was significantly improved to 25. In contrast, (*S*)-enantiomer **9** revealed significantly reduced in vitro activity against ATR (IC_{50}: 1000 nM). However, additional lead optimization efforts at the hinge-binding moiety did not result in any further improvement of ATR inhibitory activity in vitro, even though a broad variety of substituted morpholines (**5**–**14**, **16**, **17**) and potential morpholine bioisosteres (**15**, **18**) were evaluated at this position. Table 1 lists a selection of structurally diverse analogues that were tested.[28]

Table 1. Selected Examples of Lead Optimization Efforts at the Hinge-Binding 2-Position and Key In Vitro Properties[a]



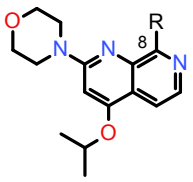
Compd	R	ATR, biochemical IC_{50} [nM]	ATR, cellular IC_{50} [nM]	Selectivity vs mTOR, ratio of cellular IC_{50} values
3		125	424[b]	5
4		38	154[b]	25
5		310	ND	ND
6		413	ND	ND
7		730	ND	ND
8		922	ND	ND
9		1000	ND	ND

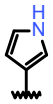
10		1500	ND	ND
11		1510	ND	ND
12		1860	ND	ND
13		1970	ND	ND
14		8610	ND	ND
15		9440	ND	ND
16		>20000	ND	ND
17		>20000	ND	ND
18		>20000[b]	ND	ND

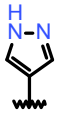
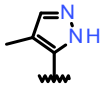
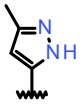
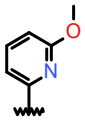
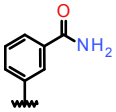
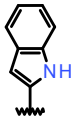
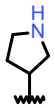
[a]IC_{50} values are reported as arithmetic means of multiple measurements except where stated otherwise (see SI for full details); ND: not determined. [b]Single measurement.

To our surprise, we also recorded a very steep SAR with respect to in vitro activity against ATR at the 8-position, exemplified by the selected compounds of Table 2.[28] The presence and the position of the two nitrogen atoms of the five-membered aromatic substituent at the 8-position are important for very high ATR inhibitory activity. Pyrrole analogues **19** (IC_{50}: 339 nM, Table 2) and **20** (IC_{50}: 589 nM), for instance, revealed reduced activity against ATR compared to inhibitor **3**. Shifting of the nitrogen atoms within the five-membered aromatic ring (**22**, IC_{50}: 3400 nM; **23**, IC_{50}: 3690 nM) or the introduction of additional nitrogen atoms into the five-membered ring (**31**, IC_{50}: >20000 nM) was also not tolerated with respect to in vitro activity. N-Methylation of the pyrazole NH reduced ATR inhibitory activity in vitro significantly [IC_{50}: 12500 nM (**25**) vs 125 nM (**3**)]. Introduction of additional substituents at the pyrazole ring reduced activity against ATR (**24**, IC_{50}: 5640 nM; **26**, IC_{50}: 12700 nM), too. Moreover, no suitable structural alternative for the pyrazole ring was identified (**21**, **27**–**29**, **32**–**35**). For instance, attempts to mimic the NH donor function of the pyrazole at the 8-position of compound **3** failed (**21**, **32**–**35**). The 8-unsubstituted analogue **36** was also inactive against ATR in vitro.

Table 2. Selected Examples of Lead Optimization Efforts at the 8-Position and Key In Vitro Properties[a]



Compd	R	ATR, biochemical IC_{50} [nM]	ATR, cellular IC_{50} [nM]
3		125	424[b]
19		339	>3000[b]
20		589	ND

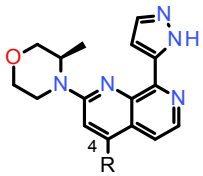
21		3300	ND
22		3400	ND
23		3690	ND
24		5640	ND
25		12500	ND
26		12700	ND
27		13200	ND
28		17200	ND
29		18100	ND
30		>20000[b]	ND
31		>20000	ND
32		>20000[b]	ND
33		>20000	ND
34		>20000	ND
35		>20000	ND
36		>20000	ND

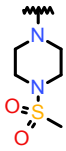
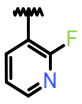
[a]IC_{50} values are reported as arithmetic means of multiple measurements except where stated otherwise (see SI for full details); ND: not determined. [b]Single measurement.

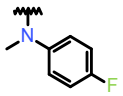
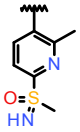
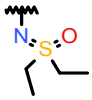
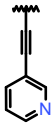
In contrast to the results of the structural variation at the 2- and 8-positions of the naphthyridine scaffold, we recorded a broad SAR with respect to biochemical and cellular activity against ATR at the 4-position. Our synthetic approach offered the late-stage introduction of C–C, C–N, C–O, and C–P bonds at the 4-position and many of the resulting, structurally diverse compounds demonstrated high ATR inhibitory activity in vitro. Table 3 shows a selection of examples from the more than 600 analogues that were synthesized in the course of the optimization efforts at this position.[28] Many of these compounds also demonstrated potent antiproliferative activity against selected cancer cell lines in vitro (Table 3). However, with respect to the desired kinase selectivity profile, a much tighter, unpredictable SAR was recorded. Lead compound BAY-937 revealed significant in vitro activity against mTOR, but many new compounds resulting from the structural variation at the 4-position demonstrated significantly improved selectivity against this off-target, for instance sulfoximine[29–31] **45** and imidazole **47**. Especially ortho-substituted (hetero)aromatic substituents at the 4-position of the naphthyridine scaffold, such as in compounds **39**, **41**, **42** or **47**, which were expected to induce a certain twist of

the neighboring aromatic groups, attracted our interest since such a twist was expected to disrupt crystal packing and thereby increase aqueous solubility.[32] However, many of the corresponding compounds also revealed significantly improved selectivity against mTOR. Unfortunately, numerous mTOR-selective compounds demonstrated insufficient selectivity against other kinases of the PIKK family or related lipid kinases like ATM, DNA-PK, or PI3K. Attempts to predict the selectivity properties of planned analogues based on computational chemistry approaches were unsuccessful. In a thorough preclinical evaluation, BAY 1895344 revealed the best preclinical overall profile in vitro and in vivo, and was ultimately selected as our clinical candidate.

Table 3. Selected Examples of Lead Optimization Efforts at the 4-Position and Key In Vitro Properties[a]



Compd	R	ATR, biochemical IC_{50} [nM]	ATR, cellular IC_{50} [nM]	Selectivity vs mTOR, ratio of cellular IC_{50} values	HT-29 antiproliferation IC_{50} [nM]
4		38	154[b]	25	929[b]
37		2	34[b]	18	93[b]
38		5	59[b]	44	233[b]
BAY 1895344		7	36	61	160
39		8	44[b]	50	175[b]

40		9	70[b]	42	381[b]
41		10	266[b]	16	323[b]
42		10	82[b]	59	125[b]
43		14	66[b]	41	292[b]
44		15	86[b]	71	268[b]
45		22	30[b]	217	168[b]
46		22	227[b]	8	405[b]
47		25	76[b]	263	192[b]
48		28[b]	289[b]	61	498[b]

[a]IC_{50} values are reported as arithmetic means of multiple measurements except where stated otherwise (see SI for full details); ND: not determined. [b]Single measurement.

Preclinical Profile of BAY 1895344. BAY 1895344 demonstrated very potent inhibition of ATR in vitro, both in the biochemical (IC_{50}: 7 nM, Table 4) and in the cellular mechanistic assay in HT-29 cells (IC_{50}: 36 nM). Evaluation of BAY 1895344 in an in-house kinase selectivity panel, as well as by KINOME*scan* profiling performed by DiscoverX

(San Diego, CA, USA), revealed a very promising kinase selectivity profile. Of the 31 kinases in the in-house panel, only the IC_{50} value from the biochemical mTOR assay (IC_{50}: 35 nM) was of the same order of magnitude as for ATR (IC_{50}: 7 nM). Although biochemical kinase profiling is a useful and tractable first step in investigating kinase activity profiles, it remains important to test compounds in relevant cellular assays to determine activity and selectivity. Based on the IC_{50} values from the corresponding cellular mechanistic assays, which we consider to be more relevant, BAY 1895344 showed good selectivity against mTOR (ratio of IC_{50} values: mTOR/ATR 61). Moreover, BAY 1895344 revealed high selectivity against other related kinases, such as DNA-PK (IC_{50}: 332 nM), ATM (IC_{50}: 1420 nM), and PI3Kβ (IC_{50}: 3270 nM). In the KINOME*scan* panel, 1 μM BAY 1895344 exhibited activity against only six of a total of 468 kinases (403 of these were nonmutant kinases; for more details, see the Supporting Information), for only three of these kinases K_d values below 1 μM were recorded [mTOR, cyclin G-associated kinase (GAK), and right open reading frame kinase 2 (RIOK2); K_d = 24, 580, and 660 nM, respectively].[25] BAY 1895344 also demonstrated very potent antiproliferative activity against various cancer cell lines in vitro,[25] for example in the CRC cell lines HT-29 (IC_{50}: 160 nM) and LoVo (IC_{50}: 71 nM), and in the B-cell lymphoma cell line SU-DHL-8 (IC_{50}: 9 nM). Lead compound BAY-937 and clinical candidate BAY 1895344 have an identical logD value of 2.1, determined at pH 7.5, but BAY 1895344 demonstrated significantly improved aqueous solubility at pH 6.5 (BAY 1895344: 34 mg/L vs BAY-937: <1 mg/L).[33] In line with our rationale to increase aqueous solubility by disrupting the crystal packing, the small molecule X-ray structure of BAY 1895344 shows a twist of the naphthyridine scaffold relative to the ortho-methyl-substituted pyrazole moiety at the 4-position (torsion angle: 54.8°) (Figure 2). Additionally, the (3*R*)-methyl substituent of the morpholine points out of the plane of the naphthyridine scaffold.

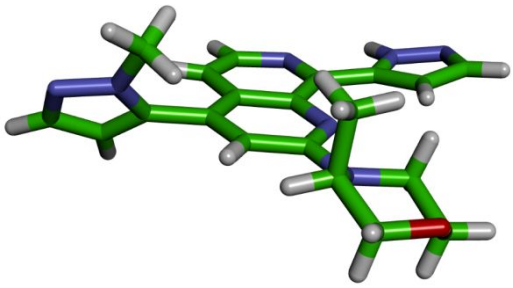


Figure 2. X-ray structure of BAY 1895344.

In vitro pharmacokinetic studies with BAY 1895344 revealed high metabolic stability in liver microsome preparations of human and rat origin tested at 1 μM compound concentration, resulting in a predicted maximal oral bioavailability (F_{max}) of the parent compound after incubation for 60 minutes of 86% and 88%, respectively. In liver microsome preparations of mouse and dog origin, a moderate metabolic stability was recorded (F_{max} of 44% and 54%, respectively). Stabilities in hepatocytes were very similar to those reported for liver microsomes. The inhibitory potency of BAY 1895344 towards human CYP isoforms was investigated in human liver microsomes using a standard set of CYP isoform selective substrates. Up to the highest test concentration (20 μM), no relevant change in CYP1A2- and CYP2D6-mediated metabolite formation was observed; thus, IC_{50} values could not be determined. BAY 1895344 showed competitive inhibitory potency of CYP2C8 (IC_{50}: 9.8 μM) and CYP2C9 (IC_{50}: 11 μM). The inhibitory potential of BAY 1895344 towards CYP3A4-dependent pathways was tested in the presence of the model substrates midazolam and testosterone in co-incubation (IC_{50}: 7.0 μM and >20 μM, respectively) as well as in pre-incubation assays in human liver microsomes. Time-dependent inhibition of CYP3A4 was not observed. In the Caco-2 model, a permeability coefficient (P_{app} A–B) of 211 nm/s and an efflux ratio of 0.7 was determined, suggesting a good intestinal permeability of the compound. The plasma protein binding of BAY 1895344 was investigated in vitro in plasma from mouse, rat, dog, and human and the unbound fraction (f_u) was found to be between 3.0% (human) and 17% (rat).

In vivo pharmacokinetic investigations revealed that BAY 1895344 has a low blood clearance in rat and dog (1.2 L/h/kg and 0.79 L/h/kg) and a moderate blood clearance in mouse (3.5 L/h/kg). The volume of distribution is high for mouse and rat (8.8 L/kg and 1.7 L/kg) and intermediate to high for dog (1.0 L/kg). The apparent half-life is intermediate (1.3 h, rat) to short (0.17 h, mouse; 1.0 h, dog). After oral administration, bioavailability is moderate in dog (51%) to high in rat (87%). In the hERG patch clamp assay in vitro, BAY 1895344 revealed no activity (IC_{50}: >10 μM).

Table 4. Properties of Lead Compound BAY-937 and Clinical Candidate BAY 1895344

	BAY-937	BAY 1895344
ATR, biochemical, IC_{50} [nM]	59	7
Selectivity vs ATM, ratio of biochemical IC_{50} values	332	202
ATR, cellular mechanistic assay in HT-29, IC_{50} [nM]	380	36
Selectivity vs mTOR, ratio of cellular mechanistic IC_{50} values	13	61
HT-29, IC_{50} [nM][a]	704	160
LoVo, IC_{50} [nM][a]	315	71
SU-DHL-8, IC_{50} [nM][a]	ND[b]	9
logD, pH 7.5	2.1	2.1
S_w, pH 6.5 [mg/L][c]	<1	34
P_{app} A–B [nm/s]	102	211
Efflux ratio	2.1	0.7
CL_b, human hepatocytes [L/h/kg]	ND[b]	0.41
CL_b, rat hepatocytes [L/h/kg]	0.64	0.74

CL_b, rat liver microsomes [L/h/kg]	0.69	0.52
CL_b, rat, in vivo, iv [L/h/kg]	1.5	1.2
V_{ss}, rat, in vivo, iv [L/kg]	2.0	1.7
$t_{1/2}$, rat, in vivo, iv [h]	0.93	1.3
F, rat, in vivo, po [%]	14	87
hERG patch clamp, IC_{50} [μM]	5.8	>10

[a]Cells were treated with test compounds for 96 h. [b]ND: not determined. [c]The solid state of the test compounds was not characterized.

BAY 1895344 exhibited strong monotherapy efficacy in various cancer xenograft models of different human cancer types that carry certain defects in DNA damage repair.[25]

For example, BAY 1895344 exhibited strong antitumor efficacy in the ATM-mutated SU-DHL-8 (*ATM*^{K1964E}) human germinal center B-cell-like diffuse large B-cell lymphoma (GCB-DLBCL) cell line derived xenograft model in female C.B-17 SCID mice, with a treatment-to-control (T/C) ratio of –0.03 ($p < 0.001$ vs vehicle) at acceptable tolerability, after oral (po) application of 50 mg/kg twice daily (b.i.d.) in an intermittent 3 days on and 4 days off dosing schedule (Figure 3).

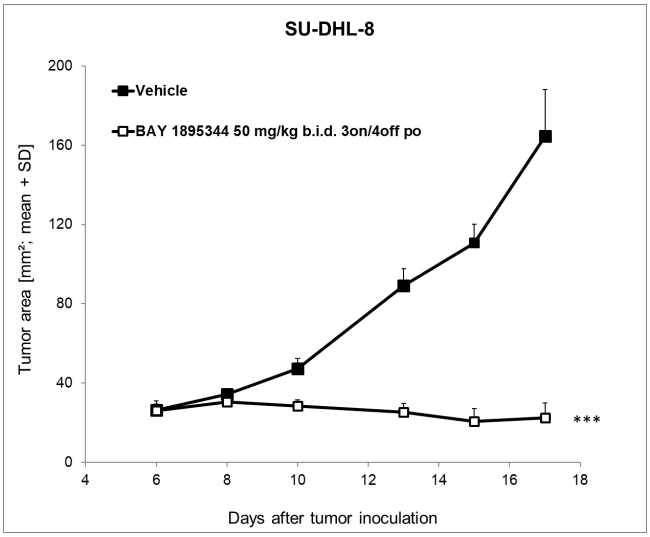


Figure 3. Antitumor efficacy of BAY 1895344 as monotherapy in the SU-DHL-8 GCB-DLBCL xenograft model in female C.B-17 SCID mice (n = 9/group). The optimal dose and treatment schedule for BAY 1895344 was 50 mg/kg (b.i.d. 3 days on/4 days off, po), based on best efficacy and tolerability in previously tested human xenograft models in mice. Treatments were well-tolerated, with a maximum body weight loss <10%. ***$p < 0.001$ vs vehicle.

The association between in vivo antitumor efficacy and ATR inhibition mediated by BAY 1895344 treatment has been demonstrated via two different approaches, ATR phosphorylation (pATR) as a direct measure of ATR kinase activity, and H2AX phosphorylation (pH2AX) as an indirect measure of ATR-mediated DNA repair. In tumor xenografts, BAY 1895344 treatment led to a reduction of pATR and in parallel to an increase of pH2AX indicating that blockade of ATR activity correlated with increased DNA damage. These findings are well in line with the unbound plasma levels of BAY 1895344 covering in vitro cellular IC_{50} values for the full dosing interval.[25]

To assess the activity of ATR inhibition with DNA damage inducing cancer therapy, we investigated the in vivo antitumor efficacy of BAY 1895344 in combination with the standard-of-care chemotherapy drug carboplatin in the platinum-resistant ATM protein low expressing CR5038 human CRC patient-derived xenograft (PDX) model in NOD/SCID mice. Oral (po) once daily (q.d.) application of BAY 1895344 at 20 mg/kg (reduced to 10 mg/kg from day 14) in an intermittent 2 days on and 5 days off dosing schedule in combination with 40 mg/kg carboplatin applied intraperitoneally (ip) once every week (1 day on/6 days off) resulted in synergistic antitumor activity, with a T/C ratio of 0.34 ($p < 0.05$ vs vehicle and respective monotherapies) at acceptable tolerability, with a transient body weight loss in the combination treatment group of 11–15% at days 11 to 14 and 28 to 31, whereby the BAY 1895344 dose was reduced at day 14. All other treatments were well-tolerated, with body weight loss <10% (Figure 4).

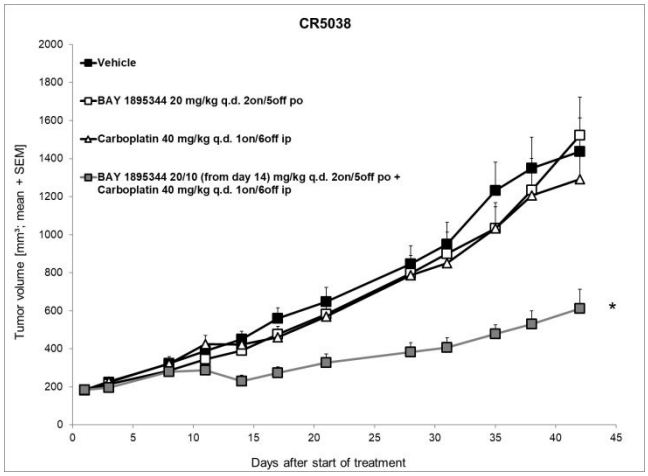


Figure 4. Antitumor efficacy of BAY 1895344 in combination with carboplatin in the CR5038 human CRC PDX model in NOD/SCID mice (n = 10/group). BAY 1895344 (20 mg/kg, and 10 mg/kg from day 14, q.d., 2 days on/5 days off, po) in combination with carboplatin (40 mg/kg, q.d., 1 day on/6 days off, ip). Acceptable tolerability of treatments with a transient body weight loss in the combination group at days 11 to 14 and 28 to 31 of 11–15%, otherwise <10%. *$p < 0.05$ vs vehicle and respective monotherapies.

We further tested the in vivo antitumor efficacy of BAY 1895344 in combination with another DNA damage repair compromising cancer drug, the PARPi olaparib, in the PARPi-insensitive SUM149 (*FANCD2*^{K50N}, *FANCI*^{D515H}, *BRCA1*^{N723X}, *TP53*^{M237I}) human triple-negative breast cancer (TNBC) xenograft model. Combination treatment with BAY 1895344 at sub-MTD and olaparib at MTD showed potent antitumor efficacy, with a T/C ratio of –0.06 ($p <$ 0.001 vs vehicle and respective monotherapies), pointing towards a synergistic combination effect. All treatments were well-tolerated, with a maximum body weight loss <10% (Figure 5).

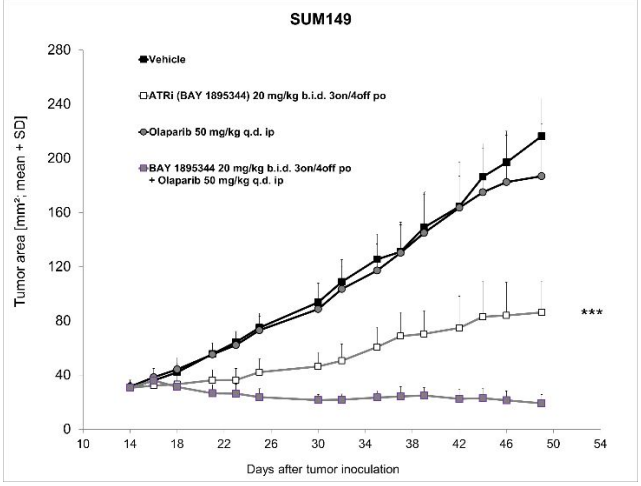
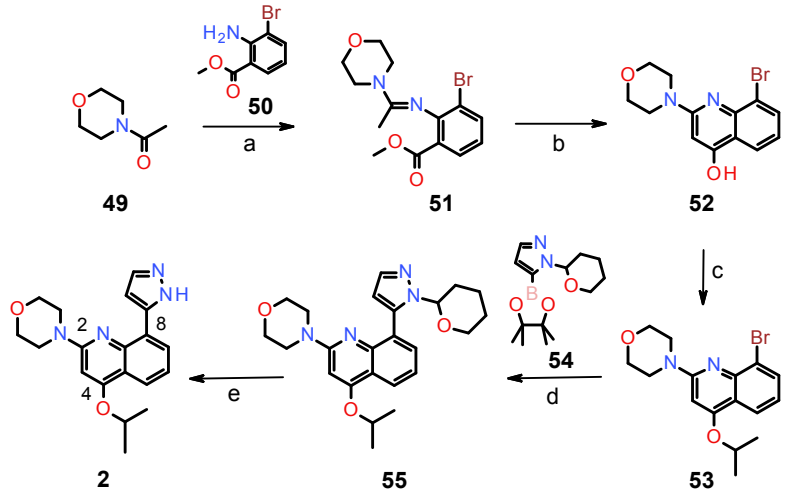


Figure 5. Antitumor efficacy of BAY 1895344 in combination with the PARPi olaparib in the SUM149 human TNBC xenograft model in nude mice (n = 10/group). BAY 1895344 (20 mg/kg, b.i.d., 3 days on/4 days off, po) in combination with olaparib (50 mg/kg, q.d., ip). Treatments were well-tolerated, with a maximum body weight loss <10%. ***p < 0.001 vs vehicle and respective monotherapies.

The data demonstrate and confirm that combination treatment of BAY 1895344 with certain DNA damage inducing therapies or certain DNA damage repair compromising cancer therapies results in synergistic activity and significant improvement of antitumor efficacy compared to the respective single-agent treatments, as we have demonstrated previously.[25] However, the tolerability of combination therapies inducing systemic DNA damage, directly or by inhibition of parallel DDR pathways, needs careful evaluation of dose and treatment schedule to ensure safety. Limitations caused by co-treatment induced systemic DNA damage could potentially be avoided in combination with treatments that specifically target the tumor only, such as local external beam radiation therapy (EBRT) or certain targeted alpha therapies .[25,34,35]

Synthesis. The successful synthesis of quinoline derivative **2** is depicted in Scheme 2. Condensation of commercially available *N*-acetylmorpholine (**49**) and anthranilic acid derivative **50** with phosphoryl chloride gave intermediate **51** in very good yield, which was then cyclized under basic conditions to give the quinoline scaffold **52** with the desired 2,4,8-regiochemistry in place.[36] Alkylation of the hydroxyl group, followed by a Suzuki reaction with boronic acid derivative **54** and final deprotection of the tetrahydropyranyl (THP) group under acidic conditions, gave rise to compound **2**.

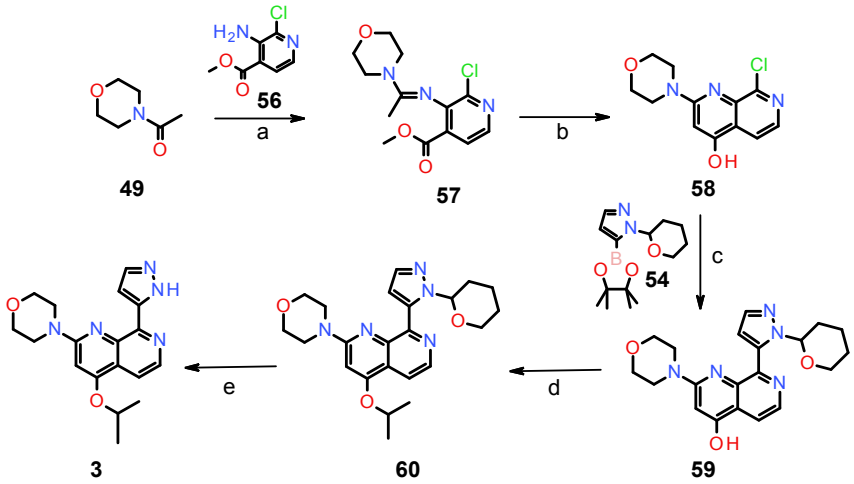
Scheme 2. Synthesis of Quinoline 2[a]



[a]Reagents and conditions: (a) $POCl_3$, DCE, rt to 80 °C, 93%; (b) LiHMDS, DMF, 0 °C to rt, 48%; (c) 2-iodopropane, K_2CO_3, MeCN, rt, 85%; (d) $Pd(dppf)Cl_2$, Cs_2CO_3, 1,4-dioxane, 85 °C, microwave, 54%; (e) 2 M aq HCl, 97%.

1,7-Naphthyridine derivative **3** was prepared using a similar synthetic approach (Scheme 3), which enabled the rapid structural variation of all three substituents of the central scaffold. Condensation of amide **49** and methyl 3-amino-2-chloroisonicotinate (**56**) yielded intermediate **57**, which was followed by cyclization under basic conditions to give central building block **58**. Suzuki coupling (**59**), ether formation (**60**), and final deprotection gave the desired 2,4,8-trisubstituted naphthyridine **3**.

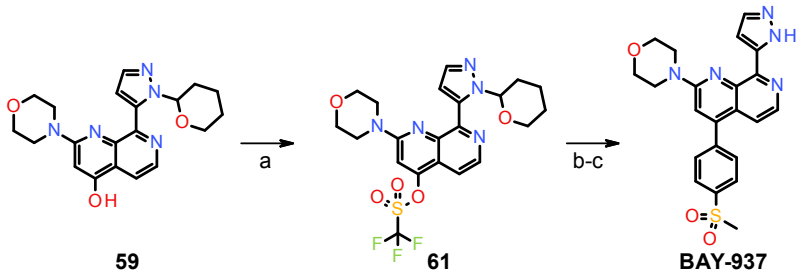
Scheme 3. Synthesis of 1,7-Naphthyridine 3[a]



[a]Reagents and conditions: (a) $POCl_3$, DCE, rt to 80 °C, 89%; (b) LiHMDS, DMF, rt, 65%; (c) $Pd(dppf)Cl_2$, Cs_2CO_3, 1,4-dioxane, 80 °C, 54%; (d) 2-iodopropane, MeCN, 85 °C, 81%; (e) TFA, H_2O, 59%.

4-Hydroxynaphthyridine derivatives like intermediate **59** were also utilized for rapid variation at the 4-position (Scheme 4). For instance, in the synthesis of lead compound BAY-937, intermediate **59** was converted into triflate **61**. A Suzuki coupling reaction of **61** with 4-(methylsulfonyl)phenylboronic acid pinacol ester and deprotection of the pyrazole THP group under acidic conditions provided BAY-937 in 51% overall yield from **59**.

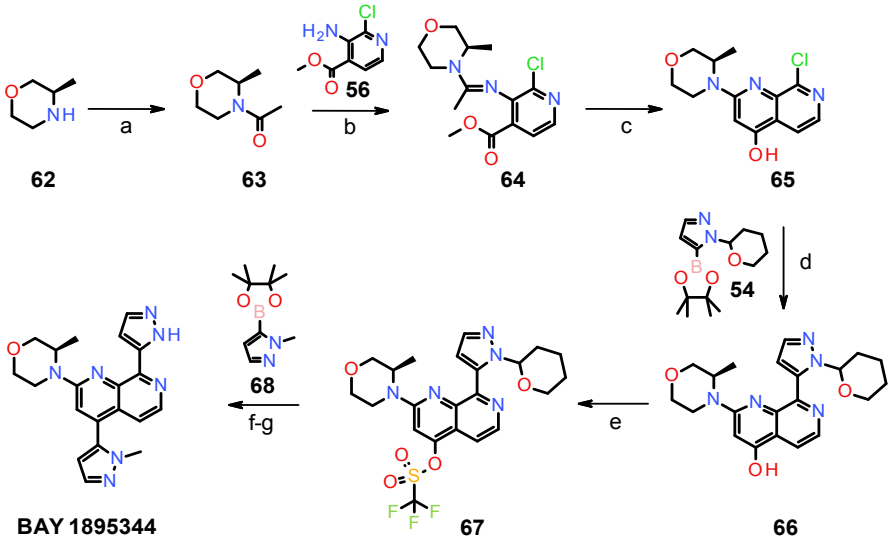
Scheme 4. Synthesis of BAY-937[a]



[a]Reagents and conditions: (a) *N*-phenyl-bis(trifluoromethanesulfonimide), DIPEA, DCM, rt, 88%; (b) 4-(methylsulfonyl)phenylboronic acid pinacol ester, $Pd(dppf)Cl_2$, Cs_2CO_3, 1,4-dioxane, 90 °C, 71%; (c) TFA, H_2O, 81%.

The research synthesis of BAY 1895344 is outlined in Scheme 5. Acetylation of commercial (3*R*)-3-methylmorpholine (**62**) gave amide **63**, which was condensed with **56** and then cyclized to yield central intermediate **65**. In analogy to the synthesis of BAY-937, a first Suzuki reaction introduced the protected pyrazole substituent at the 8-position (**66**). A second Suzuki reaction was used to introduce the methylpyrazole substituent at the 4-position, which was followed by acidic deprotection of the THP group to give BAY 1895344 in an overall yield of 28%.

Scheme 5. Synthesis of BAY 1895344[a]



[a]Reagents and conditions: (a) AcCl, K_2CO_3, DCM, rt, 95%; (b) $POCl_3$, DCE, rt to 80 °C, quant.; (c) LiHMDS, THF, 0 °C to rt, 64%; (d) $Pd(dppf)Cl_2$, Cs_2CO_3, 1,4-dioxane, 90 °C, 72%; (e) *N*-phenyl-bis(trifluoromethanesulfonimide), DIPEA, DCM, rt, 86%; (f) $Pd(PPh_3)_2Cl_2$, aq K_2CO_3, DME, 130 °C, MW; (g) H_2SO_4, 78%.

CONCLUSION

The integrity of the genome of eukaryotic cells is secured by complex signaling pathways, known as the DNA damage response (DDR). The serine/threonine protein kinase ATR has been recognized as a promising new target in cancer therapy since it plays a key role in the DNA replication stress response pathway. Based on promising preclinical data, four ATR inhibitors, from Merck KGaA (M6620, M4344), AstraZeneca (AZD6738), and Bayer AG (BAY 1895344), have entered clinical studies. BAY 1895344 resulted from extensive lead optimization efforts starting from quinoline **2**, which had been predicted as a promising ATR inhibitor in modeling studies, but revealed low in vitro activity against ATR. In addition to increased biochemical and cellular ATR inhibitory activity, high kinase selectivity against structurally related kinases of the PIKK family as well as promising physicochemical and DMPK properties were main optimization parameters. Thorough variation of all substituents of lead structure BAY-937 finally led to the discovery of the potent and highly selective ATR inhibitor BAY 1895344, which potently inhibits the proliferation of a broad spectrum of human tumor cell lines characterized by certain mutations affecting DDR pathways. In comparison to lead structure BAY-937, clinical candidate BAY 1895344 shows significantly improved aqueous solubility and oral bioavailability across species. Moreover, BAY 1895344 revealed no activity in the hERG patch clamp assay. Preclinical in vivo studies with BAY 1895344 demonstrated strong monotherapy antitumor efficacy with good tolerability in a variety of xenograft models of different human cancer types carrying certain defects in DNA repair. In addition, BAY 1895344 is likely to be particularly efficacious in combination treatment with certain DNA damage inducing therapies or certain DNA damage repair compromising therapies.

Taken together, the potent, highly selective, orally available ATR inhibitor BAY 1895344 shows great promise for the treatment of cancers with selected DDR deficiencies in monotherapy as well as in selected combinations by improving the therapeutic efficacy of standard-of-care therapies addressing DNA damage or DNA repair. BAY 1895344 is currently under clinical investigation in patients with advanced solid tumors and lymphomas (NCT03188965).[26]

EXPERIMENTAL SECTION

Synthetic Procedures

General Methods and Materials. Commercially available reagents and anhydrous solvents were used as supplied, without further purification. All air- and moisture-sensitive reactions were carried out in oven-dried (at 120 °C) glassware under an inert atmosphere of argon. A Biotage Initiator Classic microwave reactor was used for reactions conducted in a microwave oven. Reactions were monitored by TLC and UPLC analysis with a Waters Acquity UPLC MS Single Quad system; column: Acquity UPLC BEH C18 1.7 µm, 50 × 2.1 mm; basic conditions: eluent A: H_2O + 0.2 vol% aq NH_3 (32%), eluent B: MeCN; gradient: 0–1.6 min 1–99% B, 1.6–2.0 min 99% B; flow: 0.8 mL/min; acidic conditions: eluent A: H_2O + 0.1 vol% formic acid (99%), eluent B: MeCN; gradient: 0–1.6 min 1–99% B, 1.6–2.0 min 99% B; flow: 0.8 mL/min; temperature: 60 °C; DAD scan: 210–400 nm. Analytical TLC was carried out on aluminum-backed plates coated with Merck Kieselgel 60 F_{254}, with visualization under UV light at 254 nm. Flash chromatography was carried out using a Biotage Isolera One system with 200–400 nm variable detector. Preparative HPLC was carried out with a Waters AutoPurification MS Single Quad system; column: Waters XBridge C18 5 µm, 100 × 30 mm; basic conditions: eluent A: H_2O + 0.2 vol% aq NH_3 (32%), eluent B: MeCN; gradient: 0–0.5 min 5% B, flow: 25 mL/min; 0.51–5.50 min 10–100% B, flow: 70 mL/min; 5.51–6.5 min 100% B, flow: 70 mL/min; acidic conditions: eluent A: H_2O + 0.1 vol% formic acid (99%), eluent B: MeCN; gradient: 0–0.5 min 5% B, flow: 25

mL/min; 0.51–5.50 min 10–100% B, flow: 70 mL/min; 5.51–6.5 min 100% B, flow: 70 mL/min; temperature: 25 °C; DAD scan: 210–400 nm. NMR spectra were recorded at rt (22 ± 1 °C), unless otherwise noted, on Bruker Avance III HD spectrometers. ^{1}H NMR spectra were obtained at 300, 400, 500, or 600 MHz, and referenced to the residual solvent signal (7.26 ppm for $CDCl_3$, 2.50 ppm for DMSO-d_6).^{13}C NMR spectra were obtained at 125 or 150 MHz and also referenced to the residual solvent signal (39.52 ppm for DMSO-d_6). ^{1}H NMR data are reported as follows: chemical shift (δ) in ppm, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sept = septet, br = broad, m = multiplet), integration, and assignment. High-resolution mass spectra were recorded on a Xevo G2-XS Tof (Waters) instrument. Low-resolution mass spectra (electrospray ionization, ESI) were obtained via HPLC-MS (ESI) using a Waters Acquity UPLC system equipped with an SQ 3100 Mass Detector; column: Acquity UPLC BEH C18 1.7 µm, 50 × 2.1 mm; eluent A: H_2O + 0.05% formic acid (99%), eluent B: MeCN + 0.05% formic acid (99%); gradient: 0–0.5 min 5% B, 0.5–2.5 min 5–100% B, 2.5–4.5 min 100% B; total run time: 5 min; flow: 0.5 mL/min. Melting points were determined with a Büchi B-540 melting point apparatus. Optical rotations were recorded on a JASCO P-2000 polarimeter. The purity of all target compounds was at least 95%, as determined by LC-MS. A detailed description of all LC-MS methods is given in the SI. Compound names were generated using ICS software.

Methyl 3-Bromo-2-{[1-(morpholin-4-yl)ethylidene]-amino}benzoate (51). Under argon and at 0 °C, phosphorus oxychloride (0.57 mL, 6.09 mmol) was added to a solution of *N*-acetylmorpholine (**49**) (561 mg, 4.35 mmol) in DCE (3 mL) and the mixture was stirred at rt for 30 min. Methyl 2-amino-3-bromobenzoate (**50**) (500 mg, 2.17 mmol) was added and the reaction mixture was stirred at 80 °C for 3 h. After cooling, the mixture was added to aq $NaHCO_3$ solution and extracted with DCM (3 ×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, EtOAc/MeOH gradient 0–100%) to give **51** (700 mg, 2.05 mmol, 93%). MS (ESI+): m/z = 341.1 $[M+H]^+$.

8-Bromo-2-(morpholin-4-yl)quinolin-4-ol (52). 1.0 M Lithium bis(trimethylsilyl)amide in hexane (6.16 mL, 6.16 mmol) was added dropwise to solution of **51** (700 mg, 2.05 mmol) in DMF (10 mL) under argon and at 0 °C. The mixture was stirred for 3 h at rt. H_2O (2 mL) was added and the mixture was concentrated under reduced pressure to dryness. The residue was purified by flash chromatography (silica gel, EtOAc/MeOH gradient 0–100%) to give **52** (310 mg, 0.98 mmol, 48%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.89 (dd, J = 1.52, 8.08 Hz, 1H, quinoline), 7.84 (dd, J = 1.39, 7.45 Hz, 1H, quinoline), 7.04 (t, J = 7.71 Hz, 1H, quinoline), 6.44 (s, 1H, quinoline), 3.66–3.78 (m, 4H, morpholine), 3.42–3.63 (m, 4H, morpholine). MS (ESI+): m/z = 309.0 $[M+H]^+$.

8-Bromo-4-isopropoxy-2-(morpholin-4-yl)quinoline (53). K_2CO_3 (643 mg, 4.66 mmol) and 2-iodopropane (1.32 g, 7.76 mmol) were added to a solution of **52** (1.20 g, 3.88 mmol) in MeCN (50 mL) and the mixture was stirred for 8 h at rt. Then, the mixture was diluted with H_2O. The precipitate was collected by filtration and dried under reduced pressure to give **53** (1.18 g, 3.30 mmol, 85%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.85–7.89 (m, 2H, quinoline), 7.06 (t, J = 7.83 Hz, 1H, quinoline), 6.64 (s, 1H, quinoline), 5.02 (sept, J = 6.00 Hz, 1H, OCH), 3.73 (m, 8H, morpholine), 1.37 (d, J = 6.06 Hz, 6H, iPr). MS (ESI+): m/z = 351.1 $[M+H]^+$.

4-Isopropoxy-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]quinoline (55). A mixture of **53** (170 mg, 0.48 mmol), 1-(tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**54**) (202 mg, 0.73 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) $[Pd(dppf)Cl_2]$ (1:1 complex with DCM; 40 mg, 0.048 mmol), and Cs_2CO_3 (630 mg, 1.94 mmol) in 1,4-dioxane (5 mL) was stirred under argon for 3 h at 85 °C in a microwave reactor. After cooling, the mixture was diluted with H_2O and extracted with DCM (3 ×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by preparative HPLC (basic conditions) to give **55** (111 mg, 0.26 mmol, 54%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.97 (dd, J = 1.52, 8.34 Hz, 1H, quinoline), 7.55 (m, 1H, pyrazole), 7.53 (d, J = 6.88 Hz, 1H, quinoline), 7.24 (dd, J = 7.33, 8.08 Hz, 1H, quinoline), 6.60 (s, 1H, quinoline), 6.35 (d, J = 1.77 Hz, 1H, pyrazole), 5.13 (dd, J = 2.40, 9.47 Hz, 1H, NCHO), 5.01 (td, J = 6.06, 12.13 Hz, 1H, OCH), 3.76 (br d, J = 11.12 Hz, 1H, OCH_2), 3.59–3.68 (m, 4H, morpholine), 3.48–3.56 (m, 4H, morpholine), 3.12–3.21 (m, 1H, OCH_2), 2.29–2.38 (m, 1H, alkylene-CH_2), 1.95 (m, 1H, alkylene-CH_2), 1.79 (m, 1H, alkylene-CH_2), 1.34–1.53 (m, 9H, iPr, alkylene-CH_2). MS (ESI+): m/z = 423.0 $[M+H]^+$.

4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-pyrazol-5-yl)quinoline (2). 2 M HCl (10 mL) was added to a solution of **55** (104 mg, 0.25 mmol) in MeOH (30 mL) and the mixture was stirred for 30 min at rt. Then, the mixure was diluted with 1 N aq $NaHCO_3$ solution, and the precipitate was collected by filtration and dried under reduced pressure to give **2** (82 mg, 0.24 mmol, 97%). LC-MS [Method 1]: R_t = 0.97 min. MS (ESI+): m/z = 339.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.79–13.27 (m, 1H, pyrazole-NH), 7.88–8.13 (m, 2H, quinoline), 7.45–7.83 (m, 1H, pyrazole), 6.82–7.40 (m, 2H, quinoline, pyrazole), 6.55–6.75 (m, 1H, quinoline), 5.03 (m, 1H, OCH), 3.72–3.86 (m, 4H, morpholine), 3.63–3.70 (m, 4H, morpholine), 1.40 (d, J = 5.81 Hz, 6H, iPr); mixture of pyrazole tautomers. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 160.9, 159.2, 144.4, 140.9, 139.0, 128.5, 123.8, 121.7, 121.4, 118.4, 104.5, 90.3, 70.1, 66.1, 66.1, 45.6, 45.6, 21.6, 21.6. IR (ATR): ν = 3230–3340, 2970, 2865–2935, 2780–2835, 1614, 1407, 1122, 775 cm^{-1}. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{19}H_{23}N_4O_2$: 339.1821, found: 339.1817.

Methyl 2-Chloro-3-{[1-(morpholin-4-yl)ethylidene]-amino}isonicotinate (57). Under argon and at 0 °C, phosphorus oxychloride (2.44 mL, 25.4 mmol) was added to a solution of *N*-acetylmorpholine (**49**) (2.17 mL, 18.8 mmol) in anhydrous DCE (12 mL). The yellow solution was stirred at rt for 30 min. Methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (1.75 g, 9.4 mmol) was added and the mixture was stirred at 80 °C for 3 h. The DCE was distilled

off and the residue was purified by column chromatography (silica gel, EtOAc/MeOH 1:1) to give **57** (2.50 g, 8.4 mmol, 89%) as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.02 (d, *J* = 4.90 Hz, 1H, pyridine), 7.56 (d, *J* = 4.90 Hz, 1H, pyridine), 3.77 (s, 3H, methyl), 3.57–3.68 (m, 4H, morpholine), 3.47–3.57 (m, 4H, morpholine), 1.74 (s, 3H, methyl). MS (ESI+): *m*/*z* = 298.2 $[M+H]^+$.

8-Chloro-2-(morpholin-4-yl)-1,7-naphthyridin-4-ol (58). Under argon and at 0 °C, 1.0 M lithium bis(trimethylsilyl)amide in THF (20.1 mL, 20.1 mmol) was added dropwise to a solution of **57** (2.00 g, 6.7 mmol) in anhydrous DMF (20 mL). The mixture was stirred at rt for 3 h, then H_2O (2 mL) was added and the mixture was concentrated. The residue was purified by column chromatography (silica gel, EtOAc/MeOH 1:1) to give **58** (1.16 g, 4.4 mmol, 65%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.62 (s, 1H, OH), 7.98 (d, *J* = 5.2 Hz, 1H, naphthyridine), 7.73 (d, *J* = 5.2 Hz, 1H, naphthyridine), 6.62 (s, 1H, naphthyridine), 3.72–3.74 (m, 4H, morpholine), 3.63–3.65 (m, 4H, morpholine). MS (ESI+): *m*/*z* = 266.1 $[M+H]^+$.

2-(Morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-ol (59). Under argon, Pd(dppf)Cl_2 (1:1 complex with DCM; 244 mg, 0.30 mmol) and Cs_2CO_3 (650 mg, 2.00 mmol) were added to a suspension of 1-(tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**54**) (556 mg, 2.00 mmol) and **58** (266 mg, 1.00 mmol) in anhydrous 1,4-dioxane (4 mL). The reaction mixture was stirred at 80 °C for 16 h. The brown reaction solution was purified by column chromatography (silica gel, EtOAc) to give **59** (206 mg, 0.54 mmol, 54%) as a yellow oil. MS (ESI+): *m*/*z* = 382.3 $[M+H]^+$.

4-Isopropoxy-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine (60). K_2CO_3 (44 mg, 0.31 mmol) was added to a solution of **59** (100 mg, 0.26 mmol) and 2-iodopropane (45 mg, 0.26 mmol) in anhydrous MeCN (6 mL). The suspension was stirred at 85 °C for 7 h. The solvent was removed and the residue that remained was purified by column chromatography (silica gel, EtOAc) to give **60** (90 mg, 0.21 mmol, 81%) as a yellow oil. MS (ESI+): *m*/*z* = 424.3 $[M+H]^+$.

4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (3). A drop of H_2O and TFA (1 mL, 13 mmol) were added to **60** (80 mg, 0.19 mmol). After 10 min, the TFA was removed under reduced pressure and the residue that remained was adjusted to pH 7 using aq $NaHCO_3$ solution. The aqueous phase was extracted with DCM (3 ×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by column chromatography (silica gel, EtOAc) to give **3** (40 mg, 0.12 mmol, 59%) as a yellow foam; mp 73–74 °C. LC-MS [Method 3]: R_t = 2.80 min. MS (ESI+): *m*/*z* = 340.3 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 13.35 (br s, 1H, pyrazole-NH), 8.32 (d, *J* = 5.27 Hz, 1H, naphthyridine), 7.70 (d, *J* = 5.27 Hz, 1H, naphthyridine), 7.61 (s, 1H, pyrazole), 7.36 (s, 1H, pyrazole), 6.87 (s, 1H, naphthyridine), 5.01–5.10 (m, 1H, iPr), 3.78–3.81 (m, 4H, morpholine), 3.71–3.74 (m, 4H, morpholine), 1.41 (d, *J* = 6.02 Hz, 6H, iPr). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 159.8, 159.7, 140.3, 139.9, 139.6, 139.4, 138.8, 122.6, 114.1, 107.6, 93.2, 70.6, 66.0, 66.0, 45.5, 45.5, 21.5, 21.5. IR (ATR): ν = 3030–3650, 2976, 2852, 1612, 1431, 1228, 896–1080 cm^{-1}. ESI-HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{18}H_{22}N_5O_2$: 340.1773, found: 340.1774.

2-(Morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl Trifluoromethanesulfonate (61). Under argon, *N*,*N*-diisopropylethylamine (25 µL, 0.15 mmol) was added to a solution of **59** (28 mg, 0.073 mmol) and *N*-phenyl-bis(trifluoromethanesulfonimide) (39 mg, 0.11 mmol) in anhydrous DCM (3 mL). The reaction mixture was stirred at rt for 16 h. The brown reaction solution was purified by column chromatography (silica gel, EtOAc) to give **61** (34 mg, 0.066 mmol, 88%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.55 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.77 (s, 1H, naphthyridine), 7.62–7.66 (m, 2H, naphthyridine, pyrazole), 6.99 (d, *J* = 1.77 Hz, 1H, pyrazole), 6.09 (dd, *J* = 2.15, 9.51 Hz, 1H, NCHO), 3.65–3.81 (m, 9H, OCH_2, morpholine), 3.25–3.34 (m, 1H, OCH_2), 2.32–2.43 (m, 1H, alkylene-CH_2), 1.94–2.03 (m, 2H, alkylene-CH_2), 1.42–1.67 (m, 3H, alkylene-CH_2). MS (ESI+): *m*/*z* = 514.2 $[M+H]^+$.

4-[4-(Methylsulfonyl)phenyl]-2-(morpholin-4-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (BAY-937). Step a: 4-[4-(Methylsulfonyl)phenyl]-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine. Under argon, Pd(dppf)Cl_2 (1:1 complex with DCM; 25 mg, 0.03 mmol) and Cs_2CO_3 (391 mg, 1.2 mmol) were added to a suspension of **61** (154 mg, 0.30 mmol) and 4-(methylsulfonyl)phenylboronic acid pinacol ester (169 mg, 0.60 mmol) in anhydrous 1,4-dioxane (3 mL). The reaction mixture was stirred at 90 °C for 2 h. The mixture was chromatographed directly without workup (silica gel, EtOAc) to give the title compound (110 mg, 0.21 mmol, 71%) as a yellow foam. MS (ESI+): *m*/*z* = 520.3 $[M+H]^+$.

Step b: 4-[4-(Methylsulfonyl)phenyl]-2-(morpholin-4-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (BAY-937). A drop of H_2O and TFA (2 mL, 26 mmol) were added to 4-[4-(methylsulfonyl)phenyl]-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine (110 mg, 0.21 mmol) and the mixture was stirred for 3 h at rt. The TFA was removed using a rotary evaporator and the residue that remained was adjusted to pH 7 using aq $NaHCO_3$ solution. The aqueous phase was extracted with DCM (3 ×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated to dryness. MeOH (5 mL) was added to the residue. The resulting precipitated solid was collected by filtration and then dried to give BAY-937 (75 mg, 0.17 mmol, 81%). LC-MS [Method 4]: R_t = 0.65 min. MS (ESI+): *m*/*z* = 436.3 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 13.41 (br s, 1H, pyrazole-NH), 8.31–8.36 (m, 1H, naphthyridine), 8.11–8.16 (m, *J* = 8.28 Hz, 2H, phenyl), 7.84–7.88 (m, *J* = 8.28 Hz, 2H, phenyl), 7.64 (br s, 1H, pyrazole), 7.56 (s, 1H, naphthyridine), 7.43 (br s, 1H, pyrazole), 7.36 (d, *J* = 5.65 Hz, 1H, naphthyridine), 3.76-3.85 (m, 8H, morpholine), 3.33 (s, 3H, methyl). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 157.3, 146.7, 141.8, 141.0, 139.7, 139.6, 139.6, 130.5, 130.5, 127.4, 127.4, 125.4, 117.2, 114.6, 108.0, 66.0, 66.0, 45.2, 45.2, 43.5. IR (ATR): ν = 3100–3650, 2800–

3005, 2150, 1608, 1303, 1153, 765 cm^{-1}. ESI-HRMS: *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}N_5O_3S$: 436.1443, found: 436.1445.

(3*R*)-*N*-Acetyl-3-methylmorpholine (63). (3*R*)-3-Methylmorpholine (**62**) (12.8 g, 127 mmol) and K_2CO_3 (52.7 g, 381 mmol) were suspended in DCM (300 mL). The mixture was stirred at rt for 30 min, then acetyl chloride (19.9 g, 254 mmol) was added and the mixture was stirred at rt for 18 h. The precipitated solid was suction filtered and washed with DCM (200 mL). The mother liquor was concentrated to dryness to give **63** (17.19 g, 120.0 mmol, 95%) as a yellow oil. The crude product was used without further purification in the next step.

Methyl 2-Chloro-3-({1-[(3*R*)-3-methylmorpholin-4-yl]ethylidene}amino)isonicotinate (64). Under argon and at 0 °C, phosphorus oxychloride (17.1 mL, 188 mmol) was added to a solution of **63** (9.0 g, 62.8 mmol) in anhydrous DCE (78 mL) and the yellow solution was stirred at rt for 30 min. Methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (11.7 g, 62.8 mmol) was then added. The mixture was stirred at 80 °C for 1 h, at rt overnight, and on the next day at 80 °C for another 5 h. The DCE was distilled off. The remaining mixture was taken up in DCM (200 mL) and H_2O (100 mL), Na_2CO_3 as a solid was added slowly to adjust to pH 9, and the mixture was extracted with DCM (3 ×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated to dryness under reduced pressure to give **64** (19.5 g, 62.7 mmol, quant.) as a brown oil which was used without further purification in the next step. MS (ESI-APCI+): *m/z* = 312.2 $[M+H]^+$.

8-Chloro-2-[(3*R*)-3-methylmorpholin-4-yl]-1,7-naphthyridin-4-ol (65). Under argon and at 0 °C, a solution of lithium bis(trimethylsilyl)amide (31.4 g, 187 mmol) dissolved in anhydrous THF (250 mL) was added dropwise over 15 min to a solution of **64** (19.5 g, 62.8 mmol) in anhydrous THF (600 mL). The mixture was then stirred at rt for 3 h. H_2O (50 mL) was carefully added and the mixture was concentrated to dryness under reduced pressure. The residue was taken up in saturated aq NH_4Cl solution (600 mL) and extracted with DCM/iPrOH (4:1) (4 ×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to dryness. The residue was recrystallized (MeCN, 250 mL) to give pure product (7.6 g, 27.2 mmol). The mother liquor was concentrated and the residue was recrystallized (MeCN, 125 mL) to give a second batch of pure product (3.6 g, 13.2 mmol). Overall, 11.2 g of compound **65** (40.1 mmol, 64%) was obtained as a yellow-orange solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.12–12.07 (br s, 1H, OH), 7.97 (d, *J* = 5.27 Hz, 1H, naphthyridine), 7.72 (d, *J* = 5.27 Hz, 1H, naphthyridine), 6.58 (s, 1H, naphthyridine), 4.37–4.44 (m, 1H, morpholine), 4.05–4.17 (m, 1H, morpholine), 3.98 (m, 1H, morpholine), 3.77 (m, 1H, morpholine), 3.65 (m, 1H, morpholine), 3.49 (m, 1H, morpholine), 3.15–3.21 (m, 1H, morpholine), 1.22 (d, *J* = 6.78 Hz, 3H, methyl). MS (ESI-APCI+): *m/z* = 280.2 $[M+H]^+$.

2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-ol (66). Under argon, Pd(dppf)Cl_2 (1:1 complex with DCM; 146 mg, 0.18 mmol) and Cs_2CO_3 (2.33 g, 7.15 mmol) were added to a suspension of **65** (500 mg, 1.79 mmol) and 1-(tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**54**) (746 mg, 2.68 mmol) in anhydrous 1,4-dioxane (7.5 mL). The reaction mixture was stirred at 90 °C for 16 h. The brown reaction solution was purified by column chromatography (silica gel, EtOAc) to give **66** (506 mg, 1.28 mmol, 72%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.50 (br s, 1H, OH), 8.33 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.78 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.60 (d, *J* = 1.52 Hz, 1H, pyrazole), 6.89–6.95 (m, 1H, pyrazole), 6.59 (d, *J* = 4.31 Hz, 1H, naphthyridine), 6.10 (ddd, *J* = 2.03, 9.63, 16.48 Hz, 1H, NCHO), 4.20–4.41 (m, 1H), 3.90–4.10 (m, 2H), 3.58–3.79 (m, 3H), 3.37–3.51 (m, 1H), 3.09–3.30 (m, 2H), 2.32–2.43 (m, 1H), 1.91–2.03 (m, 2H), 1.40–1.64 (m, 3H), 1.15–1.23 (m, 3H). MS (ESI+): *m/z* = 396.3 $[M+H]^+$.

2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl Trifluoromethanesulfonate (67). Under argon, a solution of **66** (1.20 g, 3.03 mmol), *N*-phenyl-bis(trifluoromethanesulfonimide) (1.30 g, 3.64 mmol) and *N*,*N*-diisopropylethylamine (1.37 mL, 7.89 mmol) in anhydrous DCM (20 mL) was stirred for 3 d at rt. The solvent was distilled off under reduced pressure and the residue was chromatographed twice (silica gel, DCM/MeOH 98:2, EtOAc) to give **67** (1.42 g, 2.62 mmol, 86%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.54 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.73 (d, *J* = 2.79 Hz, 1H, naphthyridine), 7.61–7.67 (m, 2H, naphthyridine, pyrazole), 6.96–7.03 (m, 1H, pyrazole), 6.07–6.14 (m, 1H, NCHO), 4.44–4.51 (m, 1H), 4.08–4.18 (m, 1H), 3.94–4.05 (m, 1H), 3.60–3.84 (m, 3H), 3.39–3.55 (m, 1H), 3.21–3.32 (m, 2H), 2.31–2.46 (m, 1H), 1.92–2.03 (m, 2H), 1.50–1.65 (m, 1H), 1.45 (br s, 2H), 1.19–1.28 (m, 3H). MS (ESI+): *m/z* = 528.2 $[M+H]^+$.

2-[(3*R*)-3-Methylmorpholin-4-yl]-4-(1-methyl-1*H*-pyrazol-5-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (BAY 1895344). Sulfonate **67** (500 mg, 0.95 mmol), 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**68**) (415 mg, 1.90 mmol), 2 M aq K_2CO_3 solution (1.4 mL), and Pd$(PPh_3)_2Cl_2$ (67 mg, 0.094 mmol) were solubilized in DME (60 mL). The reaction mixture was stirred for 20 min at 130 °C under microwave irradiation. After cooling to rt, the mixture was filtered through a silicon filter and concentrated under reduced pressure. The crude material was purified by flash column chromatography (silica gel, hexane/EtOAc gradient 0–100%, followed by EtOAc/EtOH 9:1). The desired fractions were concentrated under reduced pressure and solubilized in concd H_2SO_4 (5 mL). The mixture was stirred for 3 h at rt. The mixture was then poured into ice and basified using solid $NaHCO_3$. The suspension was filtered and the solid was stirred with EtOH at 40 °C, filtered, and dried under reduced pressure to give BAY 1895344 (280 mg, 0.75 mmol, 78%). LC-MS [Method 2]: R_t = 0.99 min. MS (ESI+): *m/z* = 376.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.44 (br s, 1H, pyrazole-NH), 8.35 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.56–7.68 (m, 3H, pyrazole, naphthyridine), 7.42 (br s, 1H, pyrazole), 7.27 (d, *J* = 5.58 Hz, 1H, naphthyridine), 6.59 (d, *J* = 2.03 Hz, 1H, pyrazole), 4.60–4.69 (m, 1H, morpholine), 4.23 (br d, *J* = 11.66 Hz, 1H, morpholine), 4.00–4.09 (m, 1H, morpholine), 3.78–3.85 (m, 1H, morpholine), 3.75 (m, 4H, methyl, morpholine), 3.69–

3.74 (m, 1H, morpholine), 3.57 (m, 1H, morpholine), 1.30 (d, J = 6.59 Hz, 3H, methyl). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 156.5, 145.2, 140.0, 139.6, 139.5, 138.2, 137.4, 137.4, 125.7, 117.1, 115.5, 108.2, 107.7, 70.3, 66.1, 47.3, 39.7, 37.2, 13.3. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{20}H_{22}N_7O$: 376.1886, found: 376.1879. $[\alpha]_D$ –80.8 ± 1.04 (1.0000 g/ 100 mL $CHCl_3$).

X-ray Structure Determination of BAY 1895344

Data for BAY 1895344 were collected at 100 K on a Bruker system equipped with an APEX II CCD area detector and Cu X-ray radiation (Cu Kα, λ = 1.54178 Å). X-ray data collection and processing of data were performed using the APEX II v2014.11.0 package.[37] SHELXS was used for structure solution and SHELXL was used for full-matrix least-squares refinement on F^2.[38] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in geometrically ideal positions using the riding model. The isotropic temperature factors of all hydrogen atoms were 1.2 and 1.5 times the size of the temperature factors of the corresponding heavy atoms. The program XP from SHELXTL version 6.14 was used for all molecular representations.[39] Light yellow prisms of BAY 1895344 were obtained by slow evaporation of an isopropyl acetate solution of the compound at rt. A single crystal with dimensions 0.07 × 0.06 × 0.04 mm^3 was mounted on a CryoLoop using a protective oil. Two independent molecules of BAY 1895344 crystallized in the triclinic space group *P1* (Z = 2) with cell constants of a = 7.8579(3) Å, b = 11.3749(4) Å, c = 11.4596(4) Å, α = 68.330(2)°, β = 89.439(3)°, and γ = 88.361(1)°. The molecular formula is $C_{20}H_{21}N_7O_1$ with a molecular weight of 375.44 g/mol. A total of 23351 reflections of which 6531 are unique (R_{int} = 0.0189) were collected. The final R values were R_1 = 0.0430, I > 2σ(I), and wR_2 = 0.1280 for all data. The goodness-of-fit of the data was 1.054. The absolute structure was determined with a Flack parameter of –0.066(110).[40] The crystallographic data for BAY 1895344 have been deposited with the Cambridge Crystallographic Data Centre (CCDC) with deposition code CCDC 1983653.

4-Isopropoxy-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (4). Step a: 4-Isopropoxy-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine. K_2CO_3 (84 mg, 0.61 mmol) was added to a solution of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-ol (**66**; 200 mg, 0.51 mmol) and 2-iodopropane (101 µL, 1.01 mmol) in anhydrous MeCN (4 mL). The suspension was stirred at 85 °C for 3 h. The solvent was removed and the residue that remained was chromatographed (silica gel, EtOAc) to give 4-isopropoxy-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine as a yellow oil. Yield: 60 mg (27%). MS (ESI): m/z = 438.4 $[M + H]^+$. **Step b: 4-Isopropoxy-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (4).** 4-Isopropoxy-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine (80 mg, 0.18 mmol) was dissolved in MeOH (2 mL), 2 N aqueous HCl (2 mL, 4 mmol) was added, and the mixture was stirred for 1 h. MeOH was distilled off under reduced pressure and the residue that remained was adjusted to pH 7 using $NaHCO_3$ solution. The aqueous phase was extracted with DCM (3 × 20 mL). The combined organic phases were dried over Na_2SO_4 and then concentrated to dryness. The residue was chromatographed (silica gel, EtOAc) to give **4** as a yellow solid. Yield: 45 mg (70%). LC-MS [Method 3]: R_t = 2.92 min. MS (ESI+): m/z = 354.4 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.38 (br s, 1H, pyrazole), 8.31 (d, J = 5.32 Hz, 1H, naphthyridine), 7.69 (d, J = 5.07 Hz, 1H, naphthyridine), 7.60 (br s, 1H, pyrazole), 7.37 (br s, 1H, pyrazole), 6.82 (s, 1H, naphthyridine), 5.07 (m, 1H, CHO), 4.56–4.67 (m, 1H, morpholine), 4.15 (br d, J = 11.41 Hz, 1H, morpholine), 4.05 (br dd, J = 2.91, 11.28 Hz, 1H, morpholine), 3.83 (br d, J = 11.41 Hz, 1H, morpholine), 3.71 (dd, J = 2.53, 11.41 Hz, 1H, morpholine), 3.56 (dt, J = 2.66, 11.85 Hz, 1H, morpholine), 3.24–3.32 (m, 1H, morpholine), 1.41 (d, J = 6.08 Hz, 6H, iPr), 1.26 (d, J = 6.84 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.9, 158.9, 144.3, 140.4, 140.1, 139.5, 138.6, 122.4, 114.2, 107.4, 92.9, 70.6, 66.3, 47.4, 25.0, 21.5, 21.4, 13.2. HRMS (ESI, $[M + H]^+$): calcd for C_{19} H_{24} N_5 O_2, 354.1930; found, 354.1934.

4-Isopropoxy-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (5). Step a: 1-(3-Oxa-8-azabicyclo[3.2.1]octan-8-yl)ethanone. 3-Oxa-8-azabicyclo[3.2.1]octane hydrochloride (1:1) (CAS 904316-92-3, 457 mg, 3.05 mmol) was dissolved in pyridine (6.2 mL). The solution was flushed with argon. Then, acetic anhydride (2.9 mL, 31 mmol) was added and the reaction mixture was stirred for 3 h at rt. The mixture was diluted with EtOAc. The resulting suspension was filtered, and the filtrate was concentrated under reduced pressure, diluted with EtOAc, and filtered again. The filtrate was concentrated under reduced pressure to give 1-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)ethanone. Yield: 578 mg (quant.). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.35 (d, J = 6.82 Hz, 1H), 4.11 (br s, 1H), 3.49–3.57 (m, 3H), 1.97 (s, 3H), 1.68–1.94 (m, 5H). **Step b: Methyl 2-chloro-3-{(*E*)-[1-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)ethylidene]amino}isonicotinate**. An analogous method as described for **57** was used, with methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 257 mg, 1.38 mmol) and 1-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)ethanone (578 mg, 3.05 mmol) as the starting materials and stirring overnight at rt and for 2 h at 85 °C. Yield: 247 mg (41% with 74 % purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.03 (d, J = 4.94 Hz, 1H), 7.57 (d, J = 4.82 Hz, 1H), 4.11–4.58 (m, 2H), 3.81–3.95 (m, 1H), 3.75–3.81 (m, 3H), 3.40–3.73 (m, 3H), 2.54–2.63 (m, 1H), 1.86–1.97 (m, 3H), 1.71–1.85 (m, 3H). MS (ESI): m/z = 324.2 $[M + H]^+$. **Step c: 8-Chloro-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-1,7-naphthyridin-4-ol.** An analogous method as described for **58** was used, with methyl 2-chloro-3-{(*E*)-[1-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)ethylidene]amino}isonicotinate (125 mg, 386 µmol) as the starting material and stirring for 30 min at rt. Yield: 72.9 mg (61%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.17–12.35 (m, 1H), 7.96 (d, J = 5.32 Hz, 1H), 7.71 (d, J = 5.32 Hz, 1H), 6.50 (s, 1H), 4.56 (br s, 2H), 3.63–3.68 (m, 2H), 3.56–3.61 (m, 2H), 1.95–2.00 (m, 2H), 1.89–1.95 (m, 2H). MS (ESI+): m/z = 292.1 $[M+H]^+$. **Step d: 8-Chloro-4-isopropoxy-2-(3-**

oxa-8-azabicyclo[3.2.1]octan-8-yl)-1,7-naphthyridine. An analogous method as described for **60** was used, with 8-chloro-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-1,7-naphthyridin-4-ol (150 mg, 514 µmol) as the starting material and stirring overnight at 85 °C. Yield: 105 mg (61%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.98 (d, *J* = 5.31 Hz, 1H), 7.68 (d, *J* = 5.31 Hz, 1H), 6.78 (s, 1H), 5.03 (td, *J* = 5.97, 12.06 Hz, 1H), 4.78 (br s, 2H), 3.58–3.71 (m, 4H), 1.89–2.04 (m, 4H), 1.38 (d, *J* = 6.06 Hz, 6H). MS (ESI+): *m/z* = 334.1 [M+H]$^+$. **Step e: 4-Isopropoxy-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine.** 8-Chloro-4-isopropoxy-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-1,7-naphthyridine (104 mg, 312 µmol) was dissolved in DME (2.2 mL), and K_2CO_3 (95 mg, 685 µmol) and H_2O (0.6 mL) were added. The mixture was flushed with argon. 1-(Tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**54**) (CAS 903550-26-5, 130 mg, 467 µmol) and bis(triphenylphosphine)palladium(II) dichloride (17.5 mg, 25 µmol) were added and the mixture was stirred for 10 min at 130 °C in a microwave reactor. DCM was added and the reaction mixture was filtered through a silicon filter. The organic phase was separated and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, gradient: hexane/EtOAc 0–100%) to give 4-isopropoxy-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine. Yield: 118 mg (84%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.33 (d, *J* = 5.36 Hz, 1H), 7.73 (d, *J* = 5.04 Hz, 1H), 7.58 (d, *J* = 1.89 Hz, 1H), 6.85 (d, *J* = 1.89 Hz, 1H), 6.77 (s, 1H), 6.09 (dd, *J* = 2.36, 9.62 Hz, 1H), 5.02–5.08 (m, 1H), 4.62–4.68 (m, 2H), 3.55–3.72 (m, 5H), 3.24–3.29 (m, 1H), 2.35–2.41 (m, 1H), 1.85–2.02 (m, 6H), 1.53–1.62 (m, 1H), 1.43–1.51 (m, 2H), 1.40 (dd, *J* = 4.26, 5.83 Hz, 6H). MS (ESI+): *m/z* = 450.3 [M+H]$^+$. **Step f: 4-Isopropoxy-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (5)**. An analogous method as described for **3** was used, with 4-isopropoxy-2-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (116 mg, 258 µmol) as the starting material. Yield: 45.7 mg (48%). LC-MS [Method 4]: R_t = 0.64 min. MS (ESI+): *m/z* = 366.2 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.21–13.71 (m, 1H, pyrazole-NH), 8.32 (d, *J* = 5.31 Hz, 1H, naphthyridine), 7.70 (d, *J* = 5.31 Hz, 1H, naphthyridine), 7.54–7.66 (m, 1H, pyrazole), 7.31 (d, *J* = 1.77 Hz, 1H, pyrazole), 6.81 (s, 1H, naphthyridine), 5.07 (td, *J* = 6.13, 12.00 Hz, 1H, CHO), 4.77 (br s, 2H, CHN), 3.76 (br d, *J* = 10.86 Hz, 2H, CH_2O), 3.64–3.67 (m, 2H, CH_2O), 1.95–2.11 (m, 4H, CH_2-CH_2), 1.41 (d, *J* = 6.06 Hz, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.8, 157.3, 144.1, 140.6, 139.4, 138.7, 138.6, 122.5, 114.2, 107.5, 107.4, 70.7, 70.6, 70.2, 55.8, 26.6, 21.5, 21.4. HRMS (ESI, [M+H]$^+$): calcd for C_{20} H_{24} N_5 O_2, 366.1930; found, 366.1938.

2-[(3*R*,5*S*)-3,5-Dimethylmorpholin-4-yl]-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (6). Step a: 1-[(3*R*,5*S*)-3,5-Dimethylmorpholin-4-yl]ethanone. (3*R*,5*S*)-3,5-Dimethylmorpholine (CAS 45597-00-0, 0.50 g, 4.3 mmol) was dissolved in pyridine (8.6 mL, 0.11 mol) and acetic anhydride (4.0 mL, 42 mmol) was added. The reaction mixture was stirred for 16 h at rt, then concentrated under reduced pressure to give 1-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]ethanone. Yield: 0.64 g (95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.00 (br s, 2H), 3.65 (d, *J* = 11.41 Hz, 2H), 3.44 (dd, *J* = 11.28, 2.91 Hz, 2H), 2.00 (s, 3H), 1.22 (br s, 6H). MS (ESI+): *m/z* = 158.0. **Step b: Methyl 2-chloro-3-[(*E*)-{1-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]ethylidene}amino]isonicotinate.** 1-[(3*R*,5*S*)-3,5-Dimethylmorpholin-4-yl]ethanone (0.54 g, 3.4 mmol) was dissolved in DME (2.7 mL) and the reaction mixture was cooled to 0 °C. Phosphorus oxychloride (0.46 mL, 4.3 mmol) was added slowly and the reaction mixture was warmed to rt. After 30 min, methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 0.28 g, 1.5 mmol) was added in one portion and the mixture was stirred at 80 °C. After 6 h, the reaction mixture was cooled to rt and the solvent was removed under reduced pressure. The crude mixture was diluted with DCM and washed with saturated $NaHCO_3$ solution (3 ×). The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (gradient: 100% hexane to 100% EtOAc) to give methyl 2-chloro-3-[(*E*)-{1-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]ethylidene}amino]isonicotinate. Yield: 0.28 g (58%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.01 (d, *J* = 4.90 Hz, 1H), 7.56 (d, *J* = 4.90 Hz, 1H), 3.96–4.30 (m, 2H), 3.76–3.80 (m, 3H), 3.67–3.75 (m, 2H), 3.51–3.63 (m, 2H), 1.71–1.84 (m, 3H), 1.25–1.37 (m, 3H), 1.30 (br d, *J* = 6.59 Hz, 3H). MS (ESI+): *m/z* = 326.1. **Step c: 8-Chloro-2-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]-4-isopropoxy-1,7-naphthyridine.** Methyl 2-chloro-3-[(*E*)-{1-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]ethylidene}amino]isonicotinate (0.28 g, 0.86 mmol) was dissolved in anhydrous THF (6 mL) under inert atmosphere (argon). The reaction mixture was cooled to 0 °C and a solution of lithium 1,1,1,3,3,3-hexamethyldisilazan-2-ide (1.0 M in THF, 2.5 mL, 2.5 mmol) was added slowly. The reaction mixture was stirred for 16 h at rt. The reaction was quenched with H_2O and the mixture was concentrated under reduced pressure. The crude 8-chloro-2-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]-1,7-naphthyridin-4-ol (0.36 g) was used in the next step without further purification. MeCN (10 mL) was added to 8-chloro-2-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]-1,7-naphthyridin-4-ol (0.20 g, ca. 0.68 mmol). 2-Iodopropane (0.13 mL, 1.4 mmol) and K_2CO_3 (0.14 g, 0.81 mmol) were sequentially added to the suspension. The reaction mixture was stirred at 85 °C for 16 h, then cooled to rt, diluted with EtOAc, and washed with H_2O (3 ×). The organic phase was dried over sodium sulfate and concentrated under reduced pressure to give 8-chloro-2-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]-4-isopropoxy-1,7-naphthyridine, which was used in the next step without further purification. Yield: 95 mg (42 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.98 (d, *J* = 5.32 Hz, 1H), 7.69 (d, *J* = 5.32 Hz, 1H), 6.67 (s, 1H), 5.06 (spt, *J* = 5.96 Hz, 1H), 4.55 (br dd, *J* = 3.55, 6.34 Hz, 2H), 3.84 (d, *J* = 11.41 Hz, 2H), 3.65 (dd, *J* = 3.68, 11.53 Hz, 2H), 1.40 (d, *J* = 5.83 Hz, 6H), 1.32 (d, *J* = 6.84 Hz, 6H). MS (ESI+): *m/z* = 336.2. **Step d: 2-[(3*R*,5*S*)-3,5-Dimethylmorpholin-4-yl]-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (6).** 8-Chloro-2-[(3*R*,5*S*)-3,5-dimethylmorpholin-4-yl]-4-isopropoxy-1,7-

naphthyridine (0.10 g, 0.28 mmol) was dissolved in DME (3 mL). 1-(Tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**54**) (CAS 903550-26-5, 0.24 g, 0.84 mmol), K_2CO_3 (0.11 g, 0.84 mmol), bis(triphenylphosphine)palladium(II) dichloride (20 mg, 30 µmol), and H_2O (1.5 mL) were added sequentially. The reaction mixture was heated under microwave irradiation at 130 °C for 10 min. The crude reaction mixture was cooled to ambient temperature, filtered through a silicon filter and concentrated under reduced pressure. The crude mixture was purified by preparative HPLC (H_2O + HCOOH/MeCN 50:50 to 30:70). The purified product was concentrated under reduced pressure, dissolved in DCM, and washed twice with saturated $NaHCO_3$ solution. The organic phase was dried over sodium sulfate and concentrated under reduced pressure to give **6** as a solid. Yield: 56 mg (54%). LC-MS [Method 4]: R_t = 0.65 min. MS (ESI+): *m/z* = 368.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.37 (br s, 1H, pyrazole-NH), 8.29 (d, *J* = 5.07 Hz, 1H, naphthyridine), 7.69 (d, *J* = 5.07 Hz, 1H, naphthyridine), 7.59 (s, 1H, pyrazole), 7.36 (s, 1H, pyrazole), 6.69 (s, 1H, naphthyridine), 5.01–5.11 (m, 1H, CHO), 4.49 (br s, 2H, CH_2O), 3.88 (d, *J* = 11.41 Hz, 2H, CH_2O), 3.68 (br dd, *J* = 3.55, 11.41 Hz, 2H, CHN), 1.41 (d, *J* = 6.08 Hz, 6H, iPr), 1.36 (d, *J* = 6.84 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-*d6*) δ = 159.8, 157.5, 144.1, 140.6, 140.4, 139.5, 138.3, 122.1, 114.2, 107.1, 92.4, 70.8, 70.5, 45.9, 21.4, 18.2. HRMS (ESI, $[M+H]^+$): calcd for C_{20} H_{26} N_5 O_2, 368.2087; found, 368.2082.

2-[(3*R*,5*R*)-3,5-Dimethylmorpholin-4-yl]-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (7). Step a: 1-[(3*R*,5*R*)-3,5-Dimethylmorpholin-4-yl]ethanone. (3*R*,5*R*)-3,5-Dimethylmorpholine (CAS 591779-91-8, 0.50 g, 4.3 mmol) was dissolved in pyridine (8.6 mL, 0.11 mmol) and acetic anhydride (4.0 mL, 42 mmol) was added. The reaction mixture was stirred for 16 h at rt, then concentrated under reduced pressure to give 1-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]ethanone in quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.84–3.96 (m, 4H), 3.38–3.62 (m, 2H), 1.98–2.07 (m, 3H), 1.26 (br d, *J* = 6.32 Hz, 6H). MS (ESI+): *m/z* = 158.0. **Step b: Methyl 2-chloro-3-[(*E*)-{1-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]ethylidene}amino]isonicotinate.** 1-[(3*R*,5*R*)-3,5-Dimethylmorpholin-4-yl]ethanone (0.70 g, 4.4 mmol) was dissolved in DME (10 mL) and the reaction mixture was cooled to 0 °C. Phosphorus oxychloride (0.59 mL, 6.4 mmol) was added slowly and the reaction mixture was warmed to rt. After 30 min, methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 0.36 g, 1.9 mmol) was added in one portion and the mixture was stirred at rt. After 48 h, the reaction was quenched with saturated $NaHCO_3$ solution and the mixture was extracted with DCM (3 ×). The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (gradient: 100% hexane to 100% EtOAc) to give methyl 2-chloro-3-[(*E*)-{1-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]ethylidene}-amino]isonicotinate. Yield: 0.12 g (18%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.04 (t, *J* = 4.43 Hz, 1H), 7.56 (t, *J* = 4.89 Hz, 1H), 3.90–4.01 (m, 4H), 3.77 (d, *J* = 2.76 Hz, 3H), 3.48–3.59 (m, 2H), 1.79 (s, 3H), 1.33 (d, *J* = 6.53 Hz, 3H), 1.26 (d, *J* = 6.27 Hz, 3H). **Step c: 8-Chloro-2-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]-4-isopropoxy-1,7-naphthyridine.** Methyl 2-chloro-3-[(*E*)-{1-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]ethylidene)}amino]isonicotinate (0.12 g, 0.36 mmol) was dissolved in anhydrous THF (2.5 mL) under inert atmosphere (argon). The reaction mixture was cooled to 0 °C and a solution of lithium 1,1,1,3,3,3-hexamethyldisilazan-2-ide (1.0 M in THF, 1.1 mL, 1.1 mmol) was added slowly. The reaction mixture was stirred for 16 h at rt. The reaction was quenched with H_2O and the mixture was concentrated under reduced pressure. The crude 8-chloro-2-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]-1,7-naphthyridin-4-ol (0.13 g) was used in the next step without further purification. MeCN (6.8 mL) was added to 8-chloro-2-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]-1,7-naphthyridin-4-ol (0.14 g, ca. 0.46 mmol). 2-Iodopropane (0.10 mL, 0.90 mmol) and K_2CO_3 (74 mg, 0.55 mmol) were sequentially added to the suspension. The reaction mixture was stirred at 85 °C for 48 h, then cooled to rt, diluted with H_2O, extracted with DCM (3 ×), and the extract washed with saturated NaCl solution. The organic phase was dried (silicon filter) and concentrated under reduced pressure to give 8-chloro-2-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]-4-isopropoxy-1,7-naphthyridine, without further purification. Yield: 81 mg (52% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.06 (d, *J* = 5.32 Hz, 1H), 7.73 (d, *J* = 5.32 Hz, 1H), 6.85 (s, 1H), 5.02 (spt, *J* = 6.04 Hz, 1H), 4.17–4.25 (m, 2H), 4.02 (dd, *J* = 3.55, 11.15 Hz, 2H), 3.61 (dd, *J* = 4.56, 11.15 Hz, 2H), 1.39 (dd, *J* = 1.90, 5.96 Hz, 6H), 1.26 (d, *J* = 6.34 Hz, 6H). MS (ESI+): *m/z* = 336.2. **Step d: 2-[(3*R*,5*R*)-3,5-Dimethylmorpholin-4-yl]-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (7).** 8-Chloro-2-[(3*R*,5*R*)-3,5-dimethylmorpholin-4-yl]-4-isopropoxy-1,7-naphthyridine (40 mg, 0.12 mmol), 1-(tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**54**) (CAS 903550-26-5, 50 mg, 0.18 mmol), 2 M aqueous K_2CO_3 (0.18 mL, 0.36 mmol), and bis(triphenylphosphine)palladium(II) dichloride (8.5 mg, 11 µmol) were added sequentially to DME (1.1 mL). The reaction mixture was heated under microwave irradiation at 130 °C for 10 min. The reaction mixture was cooled to ambient temperature and filtered through a silicon filter and concentrated under reduced pressure. The crude mixture was purified by preparative HPLC (H_2O + HCOOH/MeCN 48:52 to 68:32) to give **7**. Yield: 9.8 mg (20%). LC-MS [Method 4]: R_t = 0.58 min. MS (ESI+): *m/z* = 368.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.41 (br s, 1H, pyrazole-NH), 8.36 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.73 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.59–7.64 (m, 1H, pyrazole), 7.44 (d, *J* = 1.77 Hz, 1H, pyrazole), 6.83 (s, 1H, naphthyridine), 5.04 (td, *J* = 6.05, 11.98 Hz, 1H, CHO), 4.20–4.33 (m, 2H, CH_2O), 4.02–4.20 (m, 2H, CHN), 3.67 (dd, *J* = 4.06, 11.15 Hz, 2H, CH_2O), 1.41 (dd, *J* = 2.41, 5.96 Hz, 6H, iPr), 1.26 (d, *J* = 6.34 Hz, 6H, CH_3).

4-Isopropoxy-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (8). Step a: 1-[(1*S*,4*S*)-2-Oxa-5-azabicyclo[2.2.1]heptan-5-yl]ethanone was prepared using an analogous method as described for **63**, from (1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptane hydrochloride

(1:1) (CAS 31560-06-2, 100 mg, 737 µmol). Yield: 84.7 mg (81%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.59–4.72 (m, 1H), 4.56 (s, 1H), 3.70 (s, 1H), 3.61 (s, 1H), 3.32–3.44 (m, 1H), 3.12–3.23 (m, 1H), 1.84–2.02 (m, 3H), 1.77–1.84 (m, 1H), 1.73 (s, 1H). **Step b: Methyl 2-chloro-3-({1-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]ethylidene}amino)isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 74 mg, 0.397 mmol) and 1-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]ethanone (84.0 mg, 595 µmol). Yield: 111 mg (81 % with 90 % purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.97–8.09 (m, 1H), 7.51–7.65 (m, 1H), 4.64 (br s, 2H), 3.90 (s, 1H), 3.67–3.83 (m, 5H), 3.36–3.57 (m, 2H), 1.69 (br s, 4H). MS (ESI+): *m/z* = 310.1 [M+H]$^+$. **Step c: 8-Chloro-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-({1-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]ethylidene}amino)-isonicotinate (109 mg, 352 µmol). Yield: 59.2 mg (61%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.60 (br s, 1H), 7.93 (d, *J* = 5.32 Hz, 1H), 7.70 (d, *J* = 5.32 Hz, 1H), 6.30 (br s, 1H), 5.05 (br d, *J* = 1.52 Hz, 1H), 4.70 (s, 1H), 3.77–3.88 (m, 1H), 3.69 (d, *J* = 7.35 Hz, 1H), 3.34–3.54 (m, 2H), 1.84–1.96 (m, 2H). MS (ESI+): *m/z* = 278.1 [M+H]$^+$. **Step d: 8-Chloro-4-isopropoxy-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-1,7-naphthyridin-4-ol (57.2 mg, 206 µmol). Yield: 66.8 mg (91% with 90% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.88 (d, *J* = 5.32 Hz, 1H), 7.60 (d, *J* = 5.32 Hz, 1H), 6.34–6.78 (m, 1H), 5.11 (br s, 1H), 4.95 (spt, *J* = 6.00 Hz, 1H), 4.66 (s, 1H), 3.75–3.81 (m, 1H), 3.64 (d, *J* = 7.35 Hz, 1H), 3.49–3.54 (m, 1H), 3.37–3.47 (m, 1H), 1.80–1.90 (m, 2H), 1.29–1.35 (m, 6H). MS (ESI+): *m/z* = 320.1 [M+H]$^+$. **Step e: 4-Isopropoxy-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine** was prepared using an analogous method as described for **66**, from 8-chloro-4-isopropoxy-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-1,7-naphthyridine (65.0 mg, 203 µmol). Yield: 59 mg (60% with 90% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.30 (d, *J* = 5.32 Hz, 1H), 7.71 (dd, *J* = 2.53, 5.32 Hz, 1H), 7.55–7.65 (m, 3H), 6.08–6.18 (m, 1H), 4.98–5.09 (m, 2H), 4.70 (s, 1H), 3.81 (dd, *J* = 6.97, 15.08 Hz, 1H), 3.65–3.75 (m, 2H), 3.53 (br dd, *J* = 5.58, 9.89 Hz, 1H), 3.38–3.48 (m, 1H), 3.21–3.29 (m, 1H), 2.33–2.43 (m, 1H), 1.87–1.98 (m, 4H), 1.43–1.65 (m, 3H), 1.37–1.43 (m, 6H). MS (ESI+): *m/z* = 436.3 [M+H]$^+$. **Step f: 4-Isopropoxy-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (8)** was prepared using an analogous method as described for BAY 1895344, from 4-isopropoxy-2-[(1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (57.0 mg, 131 µmol). Yield: 12.5 mg (27%). LC-MS [Method 1]: R_t = 0.81 min. MS (ESI+): *m/z* = 352.3 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.40 (br s, 1H, pyrazole), 8.27 (d, *J* = 5.07 Hz, 1H, naphthyridine), 7.67 (d, *J* = 5.07 Hz, 1H, naphthyridine), 7.60 (d, *J* = 1.77 Hz, 1H, pyrazole), 7.37 (d, *J* = 1.52 Hz, 1H, pyrazole), 6.65 (br s, 1H, naphthyridine), 5.14 (s, 1H, CHO), 5.05 (td, *J* = 5.96, 11.91 Hz, 1H, iPr), 4.78 (s, 1H, CHN), 3.89 (br d, *J* = 7.35 Hz, 1H, CH_2O), 3.77 (br s, 1H, CH_2N), 3.71 (br d, *J* = 10.14 Hz, 1H, CH_2O), 3.45–3.60 (m, 1H, CH_2N), 1.95–2.04 (m, 2H, CH_2-C), 1.42 (d, *J* = 3.55 Hz, 3H, CH_3), 1.40 (d, *J* = 3.80 Hz, 3H, CH_3).

4-Isopropoxy-2-[(3*S*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (9). The title compound was prepared as described for its enantiomer **4**. The analytical data were identical to **4**. $[\alpha]^{20}_D$ +66.13 (*c* = 1, DMSO). LC-MS [Method 4]: R_t = 0.64 min. MS (ESI+): *m/z* = 354.2 [M+H]$^+$

4-Isopropoxy-2-[(2*R*)-2-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (10). Step a: 1-[(2*R*)-2-Methylmorpholin-4-yl]ethanone was prepared using an analogous method as described for **63**, from (2*R*)-2-methylmorpholine hydrochloride (1:1) (CAS 168038-14-0, 500 mg, 3.63 mmol). Yield: 413 mg (75% with 95% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.10–4.22 (m, 1H), 3.74–3.81 (m, 1H), 3.60–3.74 (m, 1H), 3.32–3.48 (m, 2H), 3.09 (ddd, *J* = 3.42, 11.72, 13.24 Hz, 0.5H), 2.77 (dd, *J* = 10.27, 13.05 Hz, 0.5H), 2.60 (dt, *J* = 3.68, 12.61 Hz, 0.5H), 2.21–2.31 (m, 0.5H), 1.94–2.04 (m, 3H), 1.08 (dd, *J* = 3.93, 6.21 Hz, 3H). **Step b: Methyl 2-chloro-3-[(*E*)-{1-[(2*R*)-2-methylmorpholin-4-yl]ethylidene}amino]isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 269 mg, 1.44 mmol) and 1-[(2*R*)-2-methylmorpholin-4-yl]ethanone (413 mg, 2.88 mmol). Yield: 294 mg (63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.02 (d, *J* = 4.82 Hz, 1H), 7.56 (d, *J* = 5.07 Hz, 1H), 3.99–4.17 (m, 2H), 3.80–3.90 (m, 1H), 3.42–3.59 (m, 2H), 3.32 (s, 3H), 2.85–3.08 (m, 1H), 2.62–2.74 (m, 1H), 1.75 (s, 3H), 1.12 (d, *J* = 6.34 Hz, 3H). MS (ESI+): *m/z* = 312.1 [M+H]$^+$. **Step c: 8-Chloro-2-[(2*R*)-2-methylmorpholin-4-yl]-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-[(*E*)-{1-[(2*R*)-2-methylmorpholin-4-yl]ethylidene}-amino]isonicotinate (294 mg, 944 µmol). Yield: 368 mg (quant.). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.68 (d, *J* = 5.05 Hz, 1H), 7.61 (d, *J* = 5.05 Hz, 1H), 5.69 (s, 1H), 4.16 (br t, *J* = 14.27 Hz, 2H), 3.87 (dd, *J* = 2.02, 11.37 Hz, 1H), 3.48–3.59 (m, 2H), 2.66–2.76 (m, 1H), 2.40 (dd, *J* = 10.23, 12.76 Hz, 1H), 1.15 (d, *J* = 6.32 Hz, 3H), OH not visible. MS (ESI+): *m/z* = 280.1. [M+H]$^+$. **Step d: 8-Chloro-4-isopropoxy-2-[(2*R*)-2-methylmorpholin-4-yl]-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-[(2*R*)-2-methylmorpholin-4-yl]-1,7-naphthyridin-4-ol (368 mg, 1.32 mmol). Yield: 330 mg (74% with a purity of 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.99 (d, *J* = 5.31 Hz, 1H), 7.68 (d, *J* = 5.56 Hz, 1H), 6.84 (s, 1H), 5.07 (td, *J* = 6.06, 12.13 Hz, 1H), 4.47 (br d, *J* = 12.88 Hz, 2H), 3.96 (dd, *J* = 2.27, 11.62 Hz, 1H), 3.52–3.62 (m, 2H), 2.95–3.03 (m, 1H), 2.65–2.72 (m, 1H), 1.39 (dd, *J* = 1.26, 6.06 Hz, 6H), 1.20 (d, *J* = 6.32 Hz, 3H). MS (ESI+): *m/z* = 322.1 [M+H]$^+$. **Step e: 4-Isopropoxy-2-[(2*R*)-2-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (10)** was prepared over two steps using analogous methods as described for **66** and

BAY 1895344, from 8-chloro-4-isopropoxy-2-[(2*R*)-2-methylmorpholin-4-yl]-1,7-naphthyridine (330 mg, 1.03 mmol). Yield: 255 mg (67% over two steps). LC-MS [Method 4]: R_t = 0.64 min. MS (ESI+): *m/z* = 354.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.37 (br s, 1H, pyrazole-NH), 8.31 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.55–7.77 (m, 2H, pyrazole, naphthyridine), 7.36 (s, 1H, pyrazole), 6.88 (s, 1H, naphthyridine), 5.09 (spt, *J* = 6.00 Hz, 1H, iPr), 4.37 (br t, *J* = 13.43 Hz, 2H, CH_2O), 4.01 (dd, *J* = 2.53, 11.41 Hz, 1H, CHO), 3.57–3.70 (m, 2H, CH_2N), 3.06 (dt, *J* = 3.55, 12.42 Hz, 1H, CH_2N), 2.74 (dd, *J* = 10.77, 12.55 Hz, 1H, CH_2N), 1.41 (d, *J* = 5.83 Hz, 6H, iPr), 1.23 (d, *J* = 6.08 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-*d6*) δ = 159.8, 159.4, 144.4, 140.4, 140.0, 139.4, 138.8, 122.5, 114.2, 107.5, 93.3, 71.1, 70.6, 65.7, 51.3, 44.8, 21.5, 18.9. HRMS (ESI, $[M+H]^+$): calcd for $C_{19}H_{24}N_5O_2$, 354.1930; found, 354.1936.

4-Isopropoxy-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (11). Step a: 1-(2-Oxa-5-azabicyclo[4.1.0]heptan-5-yl)ethanone was prepared using an analogous method as described for **63**, from 2-oxa-5-azabicyclo[4.1.0]heptane hydrochloride (1:1) (CAS 1354952-28-5, 500 mg, 3.69 mmol). Yield: 400 mg (77%). MS (ESI+): *m/z* = 142.0 $[M + H]^+$. **Step b: Methyl 2-chloro-3-{(*E*)-[1-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)ethylidene]amino}isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 264 mg, 1.42 mmol) and 1-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)ethanone (400 mg, 2.83 mmol). Yield: 155 mg (35%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.03 (d, *J* = 5.13 Hz, 1H), 7.57 (d, *J* = 4.82 Hz, 1H), 3.73–3.81 (m, 5H), 3.62–3.73 (m, 1H), 3.35–3.48 (m, 1H), 3.09–3.32 (m, 1H), 2.78–2.91 (m, 1H), 1.91 (br s, 3H), 0.96 (br s, 1H), 0.48–0.62 (m, 1H). MS (ESI+): *m/z* = 310.0 $[M+H]^+$. **Step c: 8-Chloro-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-{(*E*)-[1-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)ethylidene]amino}isonicotinate (155 mg, 500 µmol). Yield: 82 mg (59%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.73 (br s, 1H), 7.97 (d, *J* = 5.07 Hz, 1H), 7.75 (d, *J* = 5.32 Hz, 1H), 6.80 (s, 1H), 3.72–3.89 (m, 4H), 3.37 (br d, *J* = 3.80 Hz, 1H), 2.76 (br d, *J* = 4.56 Hz, 1H), 0.98 (q, *J* = 6.34 Hz, 1H), 0.58 (td, *J* = 4.06, 6.34 Hz, 1H). MS (ESI+): *m/z* = 278.0 $[M+H]^+$. **Step d: 8-Chloro-4-isopropoxy-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-1,7-naphthyridin-4-ol (82.0 mg, 295 µmol). Yield: 73 mg (77%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.01 (d, *J* = 5.31 Hz, 1H), 7.73 (d, *J* = 5.31 Hz, 1H), 6.86 (s, 1H), 5.02 (td, *J* = 5.97, 12.06 Hz, 1H), 3.73–3.92 (m, 4H), 3.41 (dd, *J* = 3.41, 9.47 Hz, 1H), 2.94 (br d, *J* = 4.04 Hz, 1H), 1.43 (dd, *J* = 4.42, 5.94 Hz, 6H), 1.04 (q, *J* = 6.57 Hz, 1H), 0.59 (td, *J* = 4.04, 6.32 Hz, 1H). MS (ESI+): *m/z* = 320.3 $[M+H]^+$. **Step e: 4-Isopropoxy-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine** was prepared using an analogous method as described for **66**, from 8-chloro-4-isopropoxy-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-1,7-naphthyridine (73.0 mg, 228 µmol). Yield: 85 mg (86%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.35 (dd, *J* = 1.27, 5.32 Hz, 1H), 7.77 (dd, *J* = 0.76, 5.32 Hz, 1H), 7.08 (s, 1H), 6.84 (br d, *J* = 5.58 Hz, 1H), 6.16–6.24 (m, 1H), 4.99–5.07 (m, 1H), 3.65–3.92 (m, 4H), 3.58–3.65 (m, 1H), 3.35–3.37 (m, 1H), 3.24–3.33 (m, 2H), 2.89–2.94 (m, 1H), 2.32–2.40 (m, 1H), 1.91–2.03 (m, 2H), 1.54–1.64 (m, 1H), 1.42–1.48 (m, 8H), 0.99–1.05 (m, 1H), 0.53–0.60 (m, 1H). MS (ESI+): *m/z* = 436.2 $[M+H]^+$. **Step f: 4-Isopropoxy-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (11)** was prepared using an analogous method as described for BAY 1895344, from 4-isopropoxy-2-(2-oxa-5-azabicyclo[4.1.0]heptan-5-yl)-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (85.0 mg, 195 µmol). Yield: 59 mg (82% with 95% purity). LC-MS [Method 1]: R_t = 0.79 min. MS (ESI+): *m/z* = 352.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.54 (br s, 1H, pyrazole), 8.33 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.73 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.59–7.62 (m, 1H, pyrazole), 7.39 (s, 1H, pyrazole), 6.86 (br s, 1H, naphthyridine), 5.04 (td, *J* = 5.99, 12.10 Hz, 1H, iPr), 3.92–4.03 (m, 2H, CH_2O), 3.84 (dt, *J* = 3.80, 11.03 Hz, 1H, CH_2N), 3.62 (br s, 1H, CH_2N), 3.50–3.58 (m, 1H, CHO), 2.92–3.00 (m, 1H, CHN), 1.44 (dd, *J* = 2.91, 5.96 Hz, 6H, iPr), 1.06–1.17 (m, 1H, cyclopropyl), 0.56–0.63 (m, 1H, cyclopropyl). HRMS (ESI, $[M+H]^+$): calcd for $C_{19}H_{22}N_5O_2$, 352.1773; found, 352.1772.

2-(3-Ethylmorpholin-4-yl)-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (12). Step a: 1-(3-Ethylmorpholin-4-yl)ethanone was prepared using an analogous method as described for **63**, from 3-ethylmorpholine (CAS 55265-24-2, 500 mg, 4.12 mmol). Yield: 789 mg (quant.). MS (ESI+): *m/z* = 158.0 $[M+H]^+$. **Step b: Methyl 2-chloro-3-{(*E*)-[1-(3-ethylmorpholin-4-yl)ethylidene]amino}isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 367 mg, 1.96 mmol) and 1-(3-ethylmorpholin-4-yl)ethanone (789 mg, 4.52 mmol, 90% purity). Yield: 255 mg (40%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.02 (d, *J* = 5.02 Hz, 1H), 7.56 (d, *J* = 5.02 Hz, 1H), 3.68–3.79 (m, 5H), 3.32–3.41 (m, 4H), 1.72 (s, 3H), 1.46 (s, 3H), 1.37 (s, 3H). MS (ESI+): *m/z* = 326.1 $[M+H]^+$. **Step c: 8-Chloro-2-(3-ethylmorpholin-4-yl)-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-{(*E*)-[1-(3-ethylmorpholin-4-yl)-ethylidene]amino}isonicotinate (255 mg, 782 µmol). Yield: 341 mg (quant.). The material was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.56–7.66 (m, 2H), 5.59 (s, 1H), 4.09 (br d, *J* = 14.95 Hz, 3H), 3.79–3.90 (m, 2H), 3.41–3.53 (m, 2H), 2.97 (dt, *J* = 3.68, 12.74 Hz, 1H), 1.67–1.81 (m, 1H), 1.42–1.53 (m, 1H), 0.89 (t, *J* = 7.48 Hz, 3H). MS (ESI+): *m/z* = 294.1 $[M+H]^+$. **Step d: 8-Chloro-2-(3-ethylmorpholin-4-yl)-4-isopropoxy-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-(3-ethylmorpholin-4-yl)-1,7-naphthyridin-4-ol (241 mg, 821 µmol). Yield: 165 mg (60%). The material was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.97 (d, *J* = 5.31 Hz, 1H), 7.66 (d, *J* = 5.31 Hz, 1H), 6.78 (s, 1H), 5.05 (td, *J* = 6.03, 11.94 Hz, 1H), 4.46 (br s, 1H), 4.39 (br d, *J* = 14.15 Hz, 1H), 3.83–3.99 (m, 2H), 3.43–3.60 (m, 2H), 3.14–3.24 (m, 1H), 1.63–1.84 (m, 2H), 1.38 (dd, *J* = 5.94, 8.72 Hz,

6H), 0.89 (t, *J* = 7.45 Hz, 3H). MS (ESI+): *m/z* = 336.1 $[M+H]^+$. **Step e: 2-(3-Ethylmorpholin-4-yl)-4-isopropoxy-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine** was prepared using an analogous method as described for **66**, from 8-chloro-2-(3-ethylmorpholin-4-yl)-4-isopropoxy-1,7-naphthyridine (145 mg, 433 µmol). Yield: 125 mg (52% with 74% purity). MS (ESI+): *m/z* = 452.4 $[M+H]^+$. **Step f: 2-(3-Ethylmorpholin-4-yl)-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (12)** was prepared using an analogous method as described for BAY 1895344, from 2-(3-ethylmorpholin-4-yl)-4-isopropoxy-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (125 mg, 277 µmol). Yield: 99 mg (92% with 95% purity). LC-MS [Method 4]: R_t = 0.65 min. MS (ESI+): *m/z* = 368.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.36 (br s, 1H, pyrazole-NH), 8.29 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.68 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.60 (br s, 1H, pyrazole), 7.34 (br s, 1H, pyrazole), 6.81 (s, 1H, naphthyridine), 5.07 (td, *J* = 5.96, 11.91 Hz, 1H, CH), 4.40 (br s, 1H, CH_2O), 4.23 (br d, *J* = 12.67 Hz, 1H, CH_2O), 3.88–4.09 (m, 2H, CH_2O), 3.50–3.65 (m, 2H, CH_2N), 3.21–3.29 (m, 1H, CHN), 1.76–1.98 (m, 1H, CH_2), 1.59–1.74 (m, 1H, CH_2), 1.40 (t, *J* = 6.46 Hz, 6H, iPr), 0.91 (t, *J* = 7.48 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.7, 159.0, 144.1, 140.5, 140.2, 139.4, 138.5, 122.3, 114.2, 107.4, 92.9, 70.5, 67.4, 66.2, 53.1, 21.6, 21.3, 20.3, 10.9. HRMS (ESI, $[M+H]^+$): calcd for $C_{20}H_{26}N_5O_2$, 368.2087; found, 368.2082.

4-Isopropoxy-2-[(2*S*)-2-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (13). The title compound was prepared as described for its enantiomer **10**. The analytical data were identical to **10**. LC-MS [Method 4]: R_t = 0.64 min. MS (ESI+): *m/z* = 354.2 $[M+H]^+$.

4-Isopropoxy-8-(1*H*-pyrazol-5-yl)-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridine (14). Step a: 1-[3-(Trifluoromethyl)morpholin-4-yl]ethanone was prepared using an analogous method as described for **63**, from 3-(trifluoromethyl)morpholine hydrochloride (1:1) (CAS 1196152-13-2, 908 mg, 4.74 mmol). Yield: 886 mg (85% with 90% purity). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.98 (br dd, *J* = 4.14, 9.61 Hz, 1H), 3.33–4.23 (m, 6H), 2.05–2.12 (m, 3H). MS (ESI+): *m/z* = 198.0 $[M + H]^+$. **Step b: Methyl 2-chloro-3-[(*E*)-{1-[3-(trifluoromethyl)morpholin-4-yl]ethylidene}amino]isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 377 mg, 2.02 mmol) and 1-[3-(trifluoromethyl)morpholin-4-yl]ethanone (886 mg, 4.04 mmol, 90% purity). Yield: 451 mg (58% with 95% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.09 (d, *J* = 4.80 Hz, 1H), 7.60 (d, *J* = 4.80 Hz, 1H), 3.89–4.22 (m, 3H), 3.75–3.82 (m, 3H), 3.41–3.53 (m, 1H), 3.30 (s, 3H), 1.81–1.87 (m, 3H). MS (ESI+): *m/z* = 366.2 $[M+H]^+$. **Step c: 8-Chloro-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-[(*E*)-{1-[3-(trifluoromethyl)morpholin-4-yl]ethylidene}amino]isonicotinate (447 mg, 1.22 mmol). Yield: 98 mg (20% with 82% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.85 (br s, 1H), 8.01–8.09 (m, 1H), 7.72–7.80 (m, 1H), 6.70 (s, 1H), 4.24 (br d, *J* = 12.88 Hz, 2H), 4.02 (dd, *J* = 3.54, 11.37 Hz, 1H), 3.64–3.92 (m, 2H), 3.52–3.63 (m, 1H), 3.33–3.48 (m, 1H). MS (ESI+): *m/z* = 334.1 $[M+H]^+$. **Step d: 8-Chloro-4-isopropoxy-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridin-4-ol (95.0 mg, 285 µmol). Yield: 70 mg (65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98–8.14 (m, 1H), 7.72 (d, *J* = 5.52 Hz, 1H), 6.89–7.02 (m, 1H), 5.72–5.82 (m, 1H), 5.46–5.70 (m, 1H), 4.99–5.16 (m, 1H), 4.38–4.61 (m, 1H), 4.19–4.34 (m, 1H), 3.97–4.13 (m, 1H), 3.73–3.85 (m, 1H), 3.51–3.64 (m, 1H), 1.39 (dd, *J* = 6.02, 11.80 Hz, 6H). MS (ESI+): *m/z* = 376.2 $[M+H]^+$. **Step e: 4-Isopropoxy-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridine** was prepared using an analogous method as described for **66**, from 8-chloro-4-isopropoxy-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridine (68.0 mg, 181 µmol). Yield: 77 mg (82%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.41 (d, *J* = 5.32 Hz, 1H), 7.78 (d, *J* = 5.32 Hz, 1H), 7.61 (d, *J* = 1.77 Hz, 1H), 6.73–6.97 (m, 2H), 5.98 (dd, *J* = 2.15, 9.51 Hz, 1H), 5.44 (br s, 1H), 5.04–5.13 (m, 1H), 4.14–4.35 (m, 2H), 3.97–4.06 (m, 2H), 3.65–3.84 (m, 2H), 3.46–3.57 (m, 1H), 3.20–3.29 (m, 2H), 2.30–2.35 (m, 1H), 1.88–1.96 (m, 1H), 1.38–1.60 (m, 9H). MS (ESI+): *m/z* = 492.4 $[M+H]^+$. **Step f: 4-Isopropoxy-8-(1*H*-pyrazol-5-yl)-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridine (14)** was prepared using an analogous method as described for BAY 1895344, from 4-isopropoxy-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-2-[3-(trifluoromethyl)morpholin-4-yl]-1,7-naphthyridine (75.0 mg, 153 µmol). Yield: 22 mg (36%). LC-MS [Method 3]: R_t = 0.94 min. MS (ESI+): *m/z* = 408.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.37 (br s, 1H, pyrazole-NH), 8.37 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.74 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.60 (s, 1H, pyrazole), 7.31 (br s, 1H, pyrazole), 6.99 (s, 1H, naphthyridine), 5.52 (br d, *J* = 6.34 Hz, 1H, iPr), 5.04–5.13 (m, 1H, CH), 4.22–4.43 (m, 2H, CH_2O), 4.10 (br d, *J* = 9.63 Hz, 1H, CH_2O), 3.84 (br d, *J* = 11.15 Hz, 1H, CH_2O), 3.52–3.71 (m, 1H, CH_2N), 3.33–3.49 (m, 1H, CH_2N), 1.41 (dd, *J* = 5.83, 10.90 Hz, 6H, iPr). ^{19}F NMR (377 MHz, DMSO-d_6): δ = –67.32. MS (ESI+): *m/z* = 408.3 $[M + H]^+$. ^{13}C NMR (101 MHz, DMSO-*d6*) δ = 160.1, 158.7, 144.9, 139.9, 139.4, 127.8, 124.9, 122.7, 114.2, 107.7, 93.1, 70.9, 65.8, 63.6, 51.1, 41.2, 21.6, 21.2. HRMS (ESI, $[M + H]^+$): calcd for $C_{19}H_{21}N_5O_2F_3$, 408.1647; found, 408.1636.

4-Isopropoxy-8-(1*H*-pyrazol-5-yl)-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-1,7-naphthyridine (15). Step a: 1-[(3a*R*,6a*S*)-Tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]ethanone was prepared using an analogous method as described for **63**, from (3a*R*,6a*S*)-hexahydro-1*H*-furo[3,4-*c*]pyrrole hydrochloride (1:1) (CAS 57710-36-8, 400 mg, 2.67 mmol). Yield: 369 mg (84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.89–3.96 (m, 2H), 3.62–3.74 (m, 4H), 3.48 (dd, *J* = 4.04, 12.63 Hz, 1H), 3.35 (dd, *J* = 4.80, 10.86 Hz, 1H), 2.90–3.07 (m, 2H), 2.05 (s, 3H). **Step b: Methyl 2-chloro-3-[(*E*)-{1-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]ethylidene}-amino]isonicotinate** was prepared using an analogous method as described **64**, from methyl 3-amino-2-

chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 293 mg, 1.57 mmol) and 1-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]ethanone (365 mg, 2.35 mmol). Yield: 247 mg (46%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.99 (d, *J* = 4.82 Hz, 1H), 7.53 (d, *J* = 5.07 Hz, 1H), 3.78–3.83 (m, 2H), 3.76 (s, 3H), 3.66 (br s, 2H), 3.53–3.62 (m, 2H), 3.35–3.42 (m, 2H), 2.98 (br s, 2H), 1.73 (s, 3H). MS (ESI+): *m/z* = 324.1 [M+H]$^+$. **Step c: 8-Chloro-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-[(*E*)-{1-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]ethylidene}amino]isonicotinate (240 mg, 741 µmol). Yield: 186 mg (86%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.06–12.23 (m, 1H), 7.93 (d, *J* = 5.32 Hz, 1H), 7.70 (d, *J* = 5.32 Hz, 1H), 6.34 (s, 1H), 3.83 (dd, *J* = 6.72, 8.74 Hz, 2H), 3.73 (br dd, *J* = 7.60, 10.90 Hz, 2H), 3.57–3.66 (m, 2H), 3.40–3.50 (m, 2H), 2.99–3.10 (m, 2H). MS (ESI+): *m/z* = 292.1 [M+H]$^+$. **Step d: 8-Chloro-4-isopropoxy-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-1,7-naphthyridin-4-ol (180 mg, 617 µmol). Yield: 170 mg (83%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.95 (d, *J* = 5.04 Hz, 1H), 7.67 (d, *J* = 5.36 Hz, 1H), 6.49 (s, 1H), 5.01 (td, *J* = 5.99, 11.98 Hz, 1H), 3.77–3.89 (m, 4H), 3.53–3.66 (m, 4H), 3.07 (br dd, *J* = 3.47, 6.62 Hz, 2H), 1.40 (d, *J* = 5.99 Hz, 6H). MS (ESI+): *m/z* = 334.2 [M+H]$^+$. **Step e: 4-Isopropoxy-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine** was prepared using an analogous method as described for **66**, from 8-chloro-4-isopropoxy-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-1,7-naphthyridine (170.0 mg, 509 µmol). Yield: 110 mg (48%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.30 (d, *J* = 5.32 Hz, 1H), 7.72 (d, *J* = 5.32 Hz, 1H), 7.10 (d, *J* = 1.77 Hz, 1H), 6.49 (s, 1H), 6.22 (dd, *J* = 2.28, 9.63 Hz, 1H), 5.02 (quin, *J* = 6.02 Hz, 1H), 3.81–3.93 (m, 2H), 3.67–3.76 (m, 3H), 3.47–3.62 (m, 4H), 3.22–3.31 (m, 2H), 3.00–3.09 (m, 2H), 2.32–2.40 (m, 1H), 1.91–2.04 (m, 2H), 1.43–1.65 (m, 3H), 1.42 (dd, *J* = 2.79, 6.08 Hz, 6H). MS (ESI+): *m/z* = 450.3 [M+H]$^+$. **Step f: 4-Isopropoxy-8-(1*H*-pyrazol-5-yl)-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-1,7-naphthyridine (15)** was prepared using an analogous method as described for BAY 1895344, from 4-isopropoxy-2-[(3a*R*,6a*S*)-tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (110.0 mg, 245 µmol). Yield: 36 mg (40%). LC-MS [Method 4]: R_t = 0.62 min. MS (ESI+): *m/z* = 366.2 [M+H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.70 (br s, 1H, pyrazole-NH), 8.39 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.74 (d, *J* = 5.35 Hz, 1H, naphthyridine), 7.71 (s, 1H, pyrazole), 7.24 (s, 1H, pyrazole), 6.17 (s, 1H, naphthyridine), 4.77–4.86 (m, 1H, CH), 3.94–4.15 (m, 4H, CH_2O), 3.84 (dd, *J* = 3.55, 9.12 Hz, 2H, CH), 3.63–3.73 (m, 2H, CH_2N), 3.18–3.27 (m, 2H, CH_2N), 1.52 (d, *J* = 5.83 Hz, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.2, 157.0, 143.5, 141.2, 140.5, 139.4, 138.2, 122.0, 114.2, 107.2, 93.6, 73.2, 70.7, 52.0, 42.8, 21.4. HRMS (ESI, [M+H]$^+$): calcd for C_{20} H_{24} N_5 O_2, 366.1930; found, 366.1933.

4-Isopropoxy-8-(1*H*-pyrazol-5-yl)-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-1,7-naphthyridine (16). Step a: 1-(2,2,6,6-Tetrafluoromorpholin-4-yl)ethanone was prepared using an analogous method as described for **63**, from 2,2,6,6-tetrafluoromorpholine (CAS 65502-95-6, 500 mg, 3.14 mmol). Yield: 208 mg (33%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.33 (t, *J* = 8.53 Hz, 2H), 4.19 (t, *J* = 8.91 Hz, 2H), 2.11 (s, 3H). **Step b: Methyl 2-chloro-3-{(*E*)-[1-(2,2,6,6-tetrafluoromorpholin-4-yl)ethylidene]amino}isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 129 mg, 689 µmol) and 1-(2,2,6,6-tetrafluoromorpholin-4-yl)ethanone (208 mg, 1.03 mmol). Yield: 140 mg (54%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.13 (d, *J* = 4.80 Hz, 1H), 7.61 (d, *J* = 4.80 Hz, 1H), 4.33–4.48 (m, 4H), 3.74 (s, 3H), 1.85 (s, 3H). MS (ESI+): *m/z* = 370.1 [M+H]$^+$. **Step c: 8-Chloro-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-{(*E*)-[1-(2,2,6,6-tetrafluoromorpholin-4-yl)ethylidene]amino}isonicotinate (139 mg, 375 µmol). Yield: 35 mg (26%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.09 (br s, 1H), 8.09 (d, *J* = 5.32 Hz, 1H), 7.82 (d, *J* = 5.32 Hz, 1H), 6.78 (s, 1H), 4.50–4.61 (m, 4H). MS (ESI+): *m/z* = 338.1 [M+H]$^+$. **Step d: 8-Chloro-4-isopropoxy-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-1,7-naphthyridin-4-ol (35 mg, 104 µmol). Yield: 50.7 mg (quant.). The material was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.12 (d, *J* = 5.32 Hz, 1H), 7.75–7.79 (m, 1H), 7.11 (s, 1H), 5.09–5.18 (m, 1H), 4.67 (br t, *J* = 8.87 Hz, 4H), 1.38–1.43 (m, 6H). MS (ESI+): *m/z* = 380.2 [M+H]$^+$. **Step e: 4-Isopropoxy-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-8-{1-[(2*R*)-tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine** was prepared using an analogous method as described for **66**, from 8-chloro-4-isopropoxy-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-1,7-naphthyridine (48 mg, 107 µmol with 85% purity). Yield: 94 mg crude (quant.). The material was used in the next step without further purification. MS (ESI+): *m/z* = 496.3 [M+H]$^+$. **Step f: 4-Isopropoxy-8-(1*H*-pyrazol-5-yl)-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-1,7-naphthyridine (16)** was prepared using an analogous method as described for BAY 1895344, from 4-isopropoxy-2-(2,2,6,6-tetrafluoromorpholin-4-yl)-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (94 mg, 190 µmol). Yield: 25 mg (29%). LC-MS [Method 3]: R_t = 0.96 min. MS (ESI+): *m/z* = 412.2 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.07 (br s, 1H, pyrazole-NH), 8.60 (br s, 1H, pyrazole), 8.33 (d, *J* = 5.32 Hz, 2H, naphthyridine, pyrazole), 7.61 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.06 (s, 1H, naphthyridine), 5.09–5.18 (m, 1H, CH), 4.57–4.66 (m, 4H, CH_2), 1.39–1.44 (m, 6H, iPr).

2-(3,3-Dimethylmorpholin-4-yl)-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (17). Step a: 1-(3,3-Dimethylmorpholin-4-yl)ethanone was prepared using an analogous method as described for **63**, from 3,3-dimethylmorpholine hydrochloride (CAS 59229-64-0, 500

mg, 3.23 mmol). Yield: 588 mg (99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.63–3.69 (m, 2H), 3.31–3.39 (m, 2H), 3.27 (s, 2H), 1.95 (s, 3H), 1.29 (s, 6H). MS (ESI+): m/z = 158.0 $[M+H]^+$. **Step b: Methyl 2-chloro-3-{(*E*)-[1-(3,3-dimethylmorpholin-4-yl)ethylidene]amino}isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 273 mg, 1.46 mmol) and 1-(3,3-dimethylmorpholin-4-yl)ethanone (588 mg, 3.36 mmol with 90% purity). Yield: 162 mg (34%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.01 (dd, J = 1.13, 4.89 Hz, 1H), 7.55 (t, J = 5.09 Hz, 1H), 3.95 (br s, 1H), 3.84 (br d, J = 10.79 Hz, 1H), 3.73–3.81 (m, 5H), 3.44–3.54 (m, 1H), 3.35–3.44 (m, 1H), 1.62–1.84 (m, 6H), 0.87–0.97 (m, 3H). MS (ESI+): m/z = 326.1 $[M+H]^+$. **Step c: 8-Chloro-2-(3,3-dimethylmorpholin-4-yl)-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-{(*E*)-[1-(3,3-dimethylmorpholin-4-yl)ethylidene]amino}isonicotinate (162 mg, 496 µmol). Yield: 229 mg (quant.). The material was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.59–7.78 (m, 2H), 5.69–5.84 (m, 1H), 3.88–4.37 (m, 4H), 3.73 (br t, J = 5.07 Hz, 2H), 1.44–1.55 (m, 6H), OH not visible. MS (ESI+): m/z = 294.1 $[M+H]^+$. **Step d: 8-Chloro-2-(3,3-dimethylmorpholin-4-yl)-4-isopropoxy-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-(3,3-dimethylmorpholin-4-yl)-1,7-naphthyridin-4-ol (129 mg, 439 µmol). Yield: 72.8 mg (49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.06 (d, J = 5.30 Hz, 1H), 7.71 (d, J = 5.31 Hz, 1H), 6.93 (s, 1H), 5.01 (quin, J = 6.00 Hz, 1H), 3.83 (t, J = 5.05 Hz, 2H), 3.56–3.67 (m, 2H), 3.41 (s, 2H), 1.52–1.60 (m, 6H), 1.37 (d, J = 6.06 Hz, 6H). MS (ESI+): m/z = 336.1 $[M+H]^+$. **Step e: 2-(3,3-Dimethylmorpholin-4-yl)-4-isopropoxy-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine** was prepared using an analogous method as described for **66**, from 8-chloro-2-(3,3-dimethylmorpholin-4-yl)-4-isopropoxy-1,7-naphthyridine (72.8 mg, 217 µmol). Yield: 53 mg (56%). **Step f: 2-(3,3-Dimethylmorpholin-4-yl)-4-isopropoxy-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (17)** was prepared using an analogous method as described for BAY 1895344, from 2-(3,3-dimethylmorpholin-4-yl)-4-isopropoxy-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (53.2 mg, 118 µmol). Yield: 46 mg (quant.). LC-MS [Method 4]: R_t = 0.65 min. MS (ESI+): m/z = 368.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.39 (br s, 1H, pyrazole-NH), 8.40 (d, J = 5.07 Hz, 1H, naphthyridine), 7.75 (d, J = 5.07 Hz, 1H, naphthyridine), 7.62 (br s, 1H, pyrazole), 7.42 (br s, 1H, pyrazole), 6.91 (s, 1H, naphthyridine), 4.95–5.06 (m, 1H, CH), 3.81–3.92 (m, 2H, CH_2O), 3.64 (br s, 2H, CH_2O), 3.41–3.48 (m, 2H, CH_2N), 1.47 (s, 6H, CH_3), 1.41 (d, J = 6.08 Hz, 6H, iPr). HRMS (ESI, $[M+H]^+$): calcd for $C_{20}H_{26}N_5O_2$, 368.2087; found, 368.2082.

4-Isopropoxy-2-(3-methoxy-3-methylazetidin-1-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (18). Step a: 1-(3-Methoxy-3-methylazetidin-1-yl)ethanone was prepared using an analogous method as described for **63**, from 3-methoxy-3-methylazetidine (CAS 877665-31-1, 500 mg, 4.9 mmol). Yield: 589 mg (75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.01 (d, J = 8.87 Hz, 1H), 3.85 (dd, J = 1.01, 8.62 Hz, 1H), 3.71 (d, J = 9.89 Hz, 1H), 3.58 (d, J = 9.89 Hz, 1H), 3.15 (s, 3H), 1.76 (s, 3H), 1.38 (s, 3H). **Step b: Methyl 2-chloro-3-{(*E*)-[1-(3-methoxy-3-methylazetidin-1-yl)ethylidene]amino}isonicotinate** was prepared using an analogous method as described for **64**, from methyl 3-amino-2-chloropyridine-4-carboxylate (**56**) (CAS 173435-41-1, 273 mg, 1.46 mmol) and 1-(3-methoxy-3-methylazetidin-1-yl)ethanone (535 mg, 3.36 mmol with 90% purity). Yield: 378 mg (67% with 81% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.00–8.08 (m, 1H), 7.54 (d, J = 5.07 Hz, 1H), 4.03 (q, J = 7.10 Hz, 2H), 3.79 (s, 5H), 3.13–3.21 (m, 3H), 1.61 (s, 3H), 1.44 (s, 3H). MS (ESI+): m/z = 312.2 $[M + H]^+$. **Step c: 8-Chloro-2-(3-methoxy-3-methylazetidin-1-yl)-1,7-naphthyridin-4-ol** was prepared using an analogous method as described for **65**, from methyl 2-chloro-3-{(*E*)-[1-(3-methoxy-3-methylazetidin-1-yl)ethylidene]amino}isonicotinate (378 mg, 1.21 mmol). Yield: 344 mg (quant.). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.67 (br s, 1H), 7.96 (d, J = 5.32 Hz, 1H), 7.72 (d, J = 5.32 Hz, 1H), 6.16 (s, 1H), 3.97–4.11 (m, 2H), 3.89 (d, J = 9.12 Hz, 2H), 3.17–3.23 (m, 3H), 1.47 (s, 3H). MS (ESI+): m/z = 280.1 $[M+H]^+$. **Step d: 8-Chloro-4-isopropoxy-2-(3-methoxy-3-methylazetidin-1-yl)-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 8-chloro-2-(3-methoxy-3-methylazetidin-1-yl)-1,7-naphthyridin-4-ol (100 mg, 357 µmol). Yield: 77.5 mg (67%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.98 (d, J = 5.32 Hz, 1H), 7.69 (d, J = 5.32 Hz, 1H), 6.36 (s, 1H), 4.96 (spt, J = 6.04 Hz, 1H), 3.92–4.10 (m, 4H), 3.22–3.25 (m, 3H), 1.49 (s, 3H), 1.39 (d, J = 6.08 Hz, 6H). MS (ESI+): m/z = 322.2 $[M+H]^+$. **Step e: 4-Isopropoxy-2-(3-methoxy-3-methylazetidin-1-yl)-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (18)**. 8-Chloro-4-isopropoxy-2-(3-methoxy-3-methylazetidin-1-yl)-1,7-naphthyridine (69 mg, 214 µmol), 1-(tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**54**) (CAS 903550-26-5, 119 mg, 429 µmol), and bis(triphenylphosphine)palladium(II) dichloride (18 mg, 26 µmol) were dissolved in DME (17 mL). 2 M Aqueous K_2CO_3 (0.24 mL, 472 µmol) was added and the mixture was stirred for 10 min at 130 °C in a microwave reactor. The mixture was diluted with EtOAc and filtered through a silicon filter. The filtrate was concentrated under reduced pressure. The residue was purified by preparative HPLC (acidic conditions). The combined product fractions were concentrated under reduced pressure, dissolved in MeOH, and stirred with concd HCl at rt overnight. The reaction mixture was quenched with saturated $NaHCO_3$ solution and extracted with DCM. The combined organic layers were dried (silicon filter) and concentrated under reduced pressure to give **18**. Yield: 52 mg (68%). LC-MS [Method 4]: R_t = 0.64 min. MS (ESI+): m/z = 354.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.47 (br s, 1H, pyrazole-NH), 8.29 (d, J = 5.32 Hz, 1H, naphthyridine), 7.68 (d, J = 5.32 Hz, 1H, naphthyridine), 7.57–7.63 (m, 1H, pyrazole), 7.40 (s, 1H, pyrazole), 6.38 (s, 1H, naphthyridine), 4.98 (spt, J = 6.08 Hz, 1H, CH), 4.15 (d, J = 8.87 Hz, 2H, CH_2N), 4.03 (d, J = 9.13 Hz, 2H, CH_2N), 3.24–3.28 (m, 3H, OCH_3), 1.53 (s, 3H, CH_3), 1.41 (d, J = 5.83 Hz, 6H, iPr). HRMS (ESI, $[M+H]^+$): calcd for $C_{19}H_{24}N_5O_2$, 354.1930; found, 354.1952.

4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-pyrrol-2-yl)-1,7-naphthyridine (19). Step a: *tert*-Butyl 2-[4-

isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]-1*H*-pyrrole-1-carboxylate. Under argon, [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 20 mg, 24 µmol) was added to a mixture of 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (75 mg, 0.24 mmol) and [1-(*tert*-butoxycarbonyl)-1*H*-pyrrol-2-yl]boronic acid (CAS 135884-31-0, 57 mg, 0.27 mmol) in MeCN (2 mL) and 2 M aqueous K_2CO_3 (2 mL). The mixture was stirred in a microwave oven at 130 °C for 10 min. After cooling, DCM was added and the mixture was filtered using a Whatman filter paper. The organic phase was concentrated and the residue was purified by HPLC (basic conditions) to give *tert*-butyl 2-[4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]-1*H*-pyrrole-1-carboxylate. Yield: 35 mg (33%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.21 (d, *J* = 5.31 Hz, 1H), 7.64 (d, *J* = 5.56 Hz, 1H), 7.38 (dd, *J* = 1.77, 3.28 Hz, 1H), 6.77 (s, 1H), 6.40 (dd, *J* = 1.77, 3.28 Hz, 1H), 6.30 (t, *J* = 3.20 Hz, 1H), 5.06 (quin, *J* = 6.00 Hz, 1H), 3.59–3.71 (m, 4H), 3.48–3.56 (m, 4H), 1.38 (d, *J* = 6.06 Hz, 6H), 0.93 (s, 9H). **Step b: 4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-pyrrol-2-yl)-1,7-naphthyridine (19)**. TFA (7 µL, 0.096 mmol) was added to a solution of *tert*-butyl 2-[4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]-1*H*-pyrrole-1-carboxylate (9 mg, 0.020 mmol) in DCM (2 mL) and the reaction mixture was stirred at rt for 150 min. Additional TFA (7 µL, 0.096 mmol) was added and the reaction mixture was stirred overnight. Additional TFA (23 µL, 0.32 mmol) was added and the reaction mixture was stirred for 8 h. The mixture was basified by the addition of aqueous $NaHCO_3$ solution and extracted with DCM (2 ×). The combined organic phases were filtered using a Whatman filter paper and concentrated to give **19**. Yield: 9 mg (0.027 mmol, 79% with 95% purity). LC-MS [Method 2]: R_t = 1.25 min. MS (ESI+): *m*/*z* = 339.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.53 (br s, 1H, pyrrole-NH), 8.28–8.36 (m, 1H, naphthyridine), 7.60 (d, *J* = 5.31 Hz, 1H, naphthyridine), 7.48 (br s, 1H, pyrrole), 7.29 (s, 1H, pyrrole), 7.03 (br s, 1H, pyrrole), 6.38–6.45 (m, 1H, naphthyridine), 4.80 (td, *J* = 5.94, 11.87 Hz, 1H, CH), 3.89–4.03 (m, 4H, CH_2O), 3.64–3.81 (m, 4H, CH_2N), 1.44–1.59 (m, 6H, iPr).

4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-pyrrol-3-yl)-1,7-naphthyridine (20). Under argon, [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 13 mg, 16 µmol) was added to a mixture of 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (50 mg, 0.16 mmol) and 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrrole (CAS 214360-77-7, 34 mg, 0.18 mmol) in MeCN (1.5 mL) and 2 M aqueous K_2CO_3 (1.5 mL). The mixture was stirred at 130 °C in a microwave oven for 10 min. After cooling, DCM was added and the mixture was filtered using a Whatman filter paper. The organic phase was concentrated and the residue was purified by preparative HPLC (acidic conditions) to give **20**. Yield: 5 mg (9%). LC-MS [Method 4]: R_t = 0.62 min. MS (ESI+): *m*/*z* = 339.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.95 (br s, 1H, pyrrole-NH), 8.19 (d, *J* = 5.56 Hz, 1H, naphthyridine), 8.07–8.10 (m, 1H, pyrrole), 7.45 (d, *J* = 5.31 Hz, 1H, naphthyridine), 6.99–7.02 (m, 1H, pyrrole), 6.81 (s, 1H, pyrrole), 6.79 (s, 1H, naphthyridine), 5.03 (quin, *J* = 6.06 Hz, 1H, CH), 3.75–3.83 (m, 4H, CH_2O), 3.63–3.74 (m, 4H, CH_2N), 1.39 (d, *J* = 5.81 Hz, 6H, iPr). HRMS (ESI, $[M+H]^+$): calcd for $C_{19}H_{23}N_4O_2$, 339.1821; found, 339.1813.

4-Isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine-8-carboxamide (21). 8-Chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (150 mg, 487 µmol) was dissolved in DMSO (7 mL). [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 40 mg, 48.4 µmol), 0.4 M ammonia in THF (6 mL, 2.4 mmol), and triethylamine (410 µL, 2.9 mmol) were added. The mixture was stirred at 100 °C under carbon monoxide (13 bar) for 23 h. Then, the mixture was filtered through Celite and washed with EtOAc and MeOH. The filtrate was concentrated under reduced pressure. The residue was purified by preparative HPLC (acidic conditions) to give **21**. Yield: 24 mg (14%). LC-MS [Method 1]: R_t = 0.70 min. MS (ESI+): *m*/*z* = 317.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.48 (br s, 1H, NH), 8.22–8.32 (m, 1H, naphthyridine), 7.68–7.85 (m, 1H, naphthyridine), 7.60 (br s, 1H, NH), 6.84 (s, 1H, naphthyridine), 5.07 (quin d, *J* = 6.06, 12.02 Hz, 1H, CH), 3.58–3.81 (m, 8H, morpholine), 1.39 (m, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 167.9, 163.1, 159.8, 159.0, 140.5, 138.5, 122.3, 115.9, 93.0, 70.7, 66.0, 45.2, 21.4. HRMS (ESI, $[M+H]^+$): calcd for $C_{16}H_{21}N_4O_3$, 317.1614; found, 317.1610.

8-(1*H*-Imidazol-5-yl)-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (22). Step a: 4-Isopropoxy-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-imidazol-5-yl]-1,7-naphthyridine. 8-Chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (100 mg, 325 µmol), 1-(tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-imidazole (CAS 1029684-37-4, 207 mg, 746 µmol), bis(triphenylphosphine)palladium(II) dichloride (64 mg, 98 µmol), and K_2CO_3 (103 mg, 748 µmol) were stirred in DME (3 mL) and H_2O (1 mL) for 24 h at 90 °C. Then, the mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, DCM/MeOH 95:5) to give 4-isopropoxy-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-imidazol-5-yl]-1,7-naphthyridine. Yield: 137 mg (quant.). MS (ESI/APCI+): *m*/*z* = 424.3 $[M+H]^+$. **Step b: 8-(1*H*-Imidazol-5-yl)-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (22).** To a solution of 4-isopropoxy-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-imidazol-5-yl]-1,7-naphthyridine (135 mg, 319 µmol) in MeOH (10 mL) was added 2 M aqueous HCl (3 mL) and the mixture was stirred for 1 h at rt. The mixture was concentrated under reduced pressure, and to the residue was added saturated $NaHCO_3$ solution. The aqueous phase was extracted with DCM. The combined organic phases were dried over Na_2SO_4 and then concentrated to dryness. The residue was purified by flash chromatography (silica gel, DCM/MeOH 95:5) to give **22**. Yield: 29 mg (27%). LC-MS [Method 4]: R_t = 0.62 min. MS (ESI+): *m*/*z* = 340.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.28–8.29 (m, 2H, imidazole, naphthyridine), 7.76 (s, 1H, imidazole), 7.66 (d, *J* = 5.6 Hz, 1H, naphthyridine), 6.40 (s, 1H, naphthyridine), 4.78 (spt, *J* = 6.4 Hz, 1H, CH), 3.90–3.92 (m, 4H, morpholine), 3.68–3.70 (m, 4H, morpholine), 1.48 (d, *J* = 6.4 Hz, 6H, iPr), NH not visible. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.9, 159.6, 145.8, 139.7, 138.7, 136.6,

131.6, 130.1, 122.1, 112.6, 93.2, 70.5, 66.0, 45.6, 21.5. HRMS (ESI, $[M+H]^+$): calcd for $C_{18}H_{22}N_5O_2$, 340.1773; found, 340.1792.

4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-pyrazol-4-yl)-1,7-naphthyridine (23). Step a: 2-(Morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,7-naphthyridin-4-ol was prepared using an analogous method as described for **22** (step a), from 8-chloro-2-(morpholin-4-yl)-1,7-naphthyridin-4-ol (**58**) (2.0 g, 7.53 mmol) and 1-(tetrahydro-2*H*-pyran-2-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (CAS 1003846-21-6, 3.14 g, 11.3 mmol). Yield: 1.4 g (49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.38 (s, 1H), 8.79 (s, 1H), 8.37 (s, 1H), 8.23 (d, *J* = 5.09 Hz, 1H), 7.61 (d, *J* = 5.09 Hz, 1H), 6.64 (s, 1H), 5.50 (m, 1H), 3.95 (m, 1H), 3.79 (m, 4H), 3.67 (m, 1H), 3.60 (m, 4H), 2.03 (m, 3H), 1.70 (m, 1H), 1.56 (m, 2H). **Step b: 4-Isopropoxy-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,7-naphthyridine** was prepared using an analogous method as described for **60**, from 2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,7-naphthyridin-4-ol (100 mg, 262 µmol). Yield: 96 mg (86%). **Step c: 4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-pyrazol-4-yl)-1,7-naphthyridine (23)** was prepared using an analogous method as described for BAY 1895344, from 4-isopropoxy-2-(morpholin-4-yl)-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,7-naphthyridine (96 mg, 227 µmol). Yield: 58 mg (77%). LC-MS [Method 4]: R_t = 0.62 min. MS (ESI+): *m/z* = 340.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.95 (s, 1H, pyrazole-NH), 8.66 (s, 1H, pyrazole), 8.40 (s, 1H, pyrazole), 8.23 (d, *J* = 5.09 Hz, 1H, naphthyridine), 7.55 (d, *J* = 5.09 Hz, 1H, naphthyridine), 6.84 (s, 1H, naphthyridine), 5.04 (m, 1H, CH), 3.79 (m, 4H, morpholine), 3.69 (m, 4H, morpholine), 1.40 (m, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.9, 159.5, 149.3, 139.9, 139.1, 129.8, 122.0, 120.5, 112.1, 93.1, 70.4, 66.1, 45.7, 21.5. HRMS (ESI, $[M+H]^+$): calcd for $C_{18}H_{22}N_5O_2$, 340.1773; found, 340.1770.

4-Isopropoxy-8-(4-methyl-1*H*-pyrazol-5-yl)-2-(morpholin-4-yl)-1,7-naphthyridine (24). The title compound was prepared using an analogous method as described for **20**, from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (50 mg, 162 µmol) and (4-methyl-1*H*-pyrazol-5-yl)boronic acid (CAS 1562244-77-2, 31 mg, 244 µmol). Yield: 16 mg (28%). LC-MS [Method 4]: R_t = 0.67 min. MS (ESI+): *m/z* = 354.3 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.20 (br s, 1H, pyrazole-NH), 8.36 (br d, *J* = 5.09 Hz, 1H, naphthyridine), 7.67 (d, *J* = 5.09 Hz, 1H, naphthyridine), 7.42 (br s, 1H, pyrazole), 6.86 (br s, 1H, naphthyridine), 5.04–5.11 (m, 1H, CH), 3.77 (br s, 4H, morpholine), 3.68 (br s, 4H, morpholine), 2.23–2.40 (m, 3H, CH_3), 1.41 (d, *J* = 6.04 Hz, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 160.0, 159.4, 146.3, 140.8, 140.0, 139.2, 137.4, 122.2, 115.7, 113.5, 93.1, 70.7, 66.0, 45.3, 21.5, 10.8. HRMS (ESI, $[M+H]^+$): calcd for $C_{19}H_{24}N_5O_2$, 354.1930; found, 354.1925.

4-Isopropoxy-8-(1-methyl-1*H*-pyrazol-5-yl)-2-(morpholin-4-yl)-1,7-naphthyridine (25). The title compound was prepared using an analogous method as described for **20**, from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (75 mg, 244 µmol) and (1-methyl-1*H*-pyrazol-5-yl)boronic acid (CAS 720702-41-0, 34 mg, 268 µmol). Yield: 1 mg (1%), after preparative HPLC (acidic conditions). LC-MS [Method 1]: R_t = 1.06 min. MS (ESI+): *m/z* = 354.3 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.81 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.59 (d, *J* = 1.77 Hz, 1H, pyrazole), 6.92 (d, *J* = 1.77 Hz, 1H, pyrazole), 6.38 (s, 1H, naphthyridine), 4.81 (td, *J* = 6.08, 12.17 Hz, 1H, CH), 4.06 (s, 3H, CH_3), 3.78–3.84 (m, 4H, morpholine), 3.64–3.70 (m, 4H, morpholine), 1.51 (d, *J* = 6.08 Hz, 6H, iPr).

4-Isopropoxy-8-(5-methyl-1*H*-pyrazol-3-yl)-2-(morpholin-4-yl)-1,7-naphthyridine (26). The title compound was prepared using an analogous method as described for **20**, from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (75 mg, 244 µmol) and (5-methyl-1*H*-pyrazol-3-yl)boronic acid (CAS 1163248-54-1, 34 mg, 268 µmol). Yield: 7 mg (8%). LC-MS [Method 4]: R_t = 0.71 min. MS (ESI+): *m/z* = 354.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.22 (d, *J* = 5.30 Hz, 1H, naphthyridine), 7.85 (d, *J* = 5.31 Hz, 1H, naphthyridine), 7.54 (d, *J* = 1.52 Hz, 1H, pyrazole), 6.81 (s, 1H, naphthyridine), 6.22 (s, 1H, pyrazole-NH), 5.07 (td, *J* = 5.97, 12.06 Hz, 1H, CH), 3.59–3.70 (m, 4H, morpholine), 3.51–3.58 (m, 4H, morpholine), 2.08 (s, 3H, CH_3), 1.41 (d, *J* = 6.06 Hz, 6H, iPr).

4-Isopropoxy-8-(6-methoxypyridin-2-yl)-2-(morpholin-4-yl)-1,7-naphthyridine (27). 8-Chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (209 mg, 679 µmol), 2-(6-methoxypyridin-2-yl)-6-methyl-1,3,6,2-dioxazaborocane-4,8-dione (CAS 1227700-45-9, 897 mg, 3.4 mmol), K_2CO_3 (469 mg, 3.40 mmol), copper(II) acetate monohydrate (61.7 mg, 310 µmol), palladium(II) acetate (7.62 mg, 34.0 µmol), tris(dibenzylideneacetone)dipalladium(0) (62.2 mg, 67.9 µmol), and dicyclohexyl[2',4',6'-tri(propan-2-yl)biphenyl-2-yl]phosphane (XPhos, 129 mg, 272 µmol) were stirred in DMF (3.1 mL) and isopropyl alcohol (780 µL) for 20 h at 100 °C and for 1 h at 100 °C in a microwave reactor. The mixture was filtered, washed with EtOAc and MeOH, and the filtrate was concentrated under reduced pressure. 250 mg from the residue was purified by preparative HPLC (acidic conditions) to give **27**. Yield: 5 mg (2%). LC-MS [Method 4]: R_t = 0.66 min. MS (ESI+): *m/z* = 381.2 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_2Cl_2): δ = 8.35 (d, *J* = 5.32 Hz, 1H, pyridine), 7.78 (d, *J* = 5.32 Hz, 1H, pyridine), 7.63 (t, *J* = 7.69 Hz, 1H, pyridine), 7.45 (d, *J* = 7.03 Hz, 1H, pyridine), 6.72 (d, *J* = 8.11 Hz, 1H, pyridine), 6.34 (s, 1H, pyridine), 4.77 (spt, *J* = 6.04 Hz, 1H, CH), 3.90 (s, 3H, OCH_3), 3.68–3.74 (m, 4H, morpholine), 3.49–3.56 (m, 4H, morpholine), 1.43 (d, *J* = 6.08 Hz, 6H, iPr).

3-[4-Isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]benzamide (28). The title compound was prepared using an analogous method as described for **22** (step a), from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (200 mg, 650 µmol) and (3-carbamoylphenyl)boronic acid (CAS 351422-73-6, 161 mg, 975 µmol). Yield: 177 mg (66%). LC-MS [Method 4]: R_t = 0.68 min. MS (ESI+): *m/z* = 393.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.80 (s, 1H, phenyl), 8.37 (d, *J* = 5.34 Hz, 1H, naphthyridine), 8.27 (m, 1H, phenyl), 7.97 (s, 1H, NH), 7.90 (d, *J* = 5.38 Hz, 1H, naphthyridine), 7.72 (m, 1H, phenyl),

7.52 (m, 1H, phenyl), 7.31 (s, 1H, NH), 6.82 (s, 1H, naphthyridine), 5.06 (m, 1H, CH), 3.68 (m, 8H, morpholine), 1.41 (m, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.1, 160.0, 158.9, 153.7, 141.1, 139.1, 139.0, 133.5, 133.1, 130.3, 127.2, 127.0, 122.3, 114.3, 92.5, 70.5, 66.1, 45.3, 21.5. HRMS (ESI, [M+H]$^+$): calcd for $C_{22}H_{25}N_4O_3$, 393.1927; found, 393.1918.

4-Isopropoxy-2-(morpholin-4-yl)-8-(pyridin-3-yl)-1,7-naphthyridine (29). The title compound was prepared using an analogous method as described for **6** (step d), from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (100 mg, 325 µmol) and pyridin-3-ylboronic acid (CAS 1692-25-7, 60 mg, 487 µmol). Yield: 61.5 mg (54%). LC-MS [Method 4]: R_t = 0.69 min. MS (ESI+): *m/z* = 351.2 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.30 (dd, *J* = 0.76, 2.02 Hz, 1H, pyridine), 8.58 (dd, *J* = 1.77, 4.80 Hz, 1H, pyridine), 8.48 (td, *J* = 1.86, 8.15 Hz, 1H, pyridine), 8.39 (d, *J* = 5.31 Hz, 1H, pyridine), 7.75 (d, *J* = 5.31 Hz, 1H, pyridine), 7.49 (ddd, *J* = 0.76, 4.80, 8.08 Hz, 1H, pyrdidine), 6.84 (s, 1H, pyridine), 5.07 (td, *J* = 5.97, 12.06 Hz, 1H, CH), 3.62–3.74 (m, 8H, morpholine), 1.41 (d, *J* = 5.81 Hz, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 160.0, 159.2, 151.9, 150.9, 148.7, 141.2, 139.3, 137.6, 134.7, 122.7, 122.4, 114.7, 92.7, 70.6, 66.0, 45.2, 21.5. HRMS (ESI, [M+H]$^+$): calcd for $C_{20}H_{23}N_4O_2$, 351.1821; found, 351.1819.

8-(1*H*-Indol-2-yl)-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (30). 8-Chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (144 mg, 468 µmol), *tert*-butyl 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole-1-carboxylate (CAS 1072944-96-7, 241 mg, 702 µmol), and tetrakis(triphenylphosphine)palladium(0) (108 mg, 93.6 µmol) were stirred in DMF (4 mL) and 2 M aqueous Na_2CO_3 (580 µL, 1.2 mmol) for 20 h at 100 °C. The mixture was filtered, washed with EtOAc and MeOH, and the filtrate was concentrated under reduced pressure. The residue was dissolved in DCM, and TFA (1.8 mL, 23 mmol) was added. The mixture was stirred for 16 h at rt. Saturated $NaHCO_3$ solution was added, the mixture was extracted with DCM, and the collected organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by preparative HPLC (acidic conditions) to give **30**. Yield: 31.5 mg (17%). LC-MS [Method 4]: R_t = 0.86 min. MS (ESI+): *m/z* = 389.3 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.68 (s, 1H, indole-NH), 8.35 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.87 (d, *J* = 1.01 Hz, 1H, indole), 7.67 (d, *J* = 5.49 Hz, 1H, naphthyridine), 7.63 (d, *J* = 8.21 Hz, 1H, indole), 7.52 (dd, *J* = 0.76, 8.11 Hz, 1H, indole), 7.13 (ddd, *J* = 1.14, 6.97, 8.11 Hz, 1H, indole), 6.99 (t, *J* = 7.40 Hz, 1H, indole), 6.90 (s, 1H, naphthyridine), 5.09 (spt, *J* = 6.00 Hz, 1H, CH), 3.76–3.88 (m, 8H, morpholine), 1.42 (d, *J* = 6.08 Hz, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 160.0, 159.7, 146.1, 140.7, 138.8, 136.4, 136.1, 128.6, 122.5, 122.4, 121.1, 119.1, 113.4, 111.9, 106.0, 93.2, 70.6, 66.1, 45.7, 21.5. HRMS (ESI, [M+H]$^+$): calcd for $C_{23}H_{25}N_4O_2$, 389.1978; found, 389.1984.

4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-tetrazol-5-yl)-1,7-naphthyridine (31). Step a: 4-Isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine-8-carbonitrile. 8-Chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (200 mg, 650 µmol) and zinc cyanide (91.6 mg, 780 µmol) were suspended in DMF (10 mL). Tetrakis(triphenylphosphine)palladium(0) (22.5 mg, 19.5 µmol) was added under argon and the mixture was stirred for 3 h at 130 °C. Saturated $NaHCO_3$ solution was added, the mixture was extracted with DCM, and the collected organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, $CHCl_3$/MeOH 50:1) to give 4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine-8-carbonitrile. Yield: 188 mg (97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.33 (d, *J* = 5.17 Hz, 1H), 7.89 (d, *J* = 5.22 Hz, 1H), 6.35 (s, 1H), 4.79 (m, 1H), 3.82 (m, 8H), 1.48 (m, 6H). **Step b: 4-Isopropoxy-2-(morpholin-4-yl)-8-(1*H*-tetrazol-5-yl)-1,7-naphthyridine (31)**. To a solution of 4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine-8-carbonitrile (184 mg, 617 µmol) in DMF (10 mL) were added sodium azide (180 mg, 2.8 mmol) and ammonium chloride (147 mg, 2.8 mmol). The mixture was stirred at 125 °C for 6 d. The suspension was filtered and the fitrate was concentrated under reduced pressure. Citric acid (10%) was added, the mixture was extracted with $CHCl_3$, and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, DCM/MeOH 9:1) to give **31**. Yield: 25 mg (12%). LC-MS [Method 4]: R_t = 0.62 min. MS (ESI+): *m/z* = 342.2 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.43 (d, *J* = 5.21 Hz, 1H, naphthyridine), 7.91 (d, *J* = 5.11 Hz, 1H, naphthyridine), 6.87 (s, 1H, naphthyridine), 5.71 (s, 1H, NH), 5.10 (m, 1H, CH), 3.72 (br s, 8H, morpholine), 1.41 (m, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.7, 159.4, 159.1, 141.6, 139.2, 122.7, 117.2, 93.2, 70.9, 66.1, 45.0, 36.0, 21.4. HRMS (ESI, [M+H]$^+$): calcd for $C_{16}H_{20}N_7O_2$, 342.1678; found, 342.1670.

3-[4-Isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]pyridin-2(1*H*)-one (32). Step a: 4-Isopropoxy-8-(2-methoxypyridin-3-yl)-2-(morpholin-4-yl)-1,7-naphthyridine was prepared using an analogous method as described for **30**, from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (210 mg, 682 µmol) and (2-methoxypyridin-3-yl)boronic acid (CAS 163105-90-6, 157 mg, 1.0 mmol). Yield: 7 mg (3%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.28 (d, *J* = 5.48 Hz, 1H), 8.23 (d, *J* = 4.93 Hz, 1H), 7.67–7.72 (m, 2H), 7.07 (dd, *J* = 4.94, 7.22 Hz, 1H), 6.76 (s, 1H), 5.04 (spt, *J* = 6.04 Hz, 1H), 3.73–3.80 (m, 3H), 3.59–3.65 (m, 4H), 3.44–3.51 (m, 4H), 1.40 (d, *J* = 6.08 Hz, 6H). MS (ESI+): *m/z* = 381.2 [M + H]$^+$. **Step b: 3-[4-Isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]pyridin-2(1*H*)-one (32).** A mixture of 4-isopropoxy-8-(2-methoxypyridin-3-yl)-2-(morpholin-4-yl)-1,7-naphthyridine (311 mg, 817 µmol), acetic acid (14 mL, 250 mmol), and hydrobromic acid (2 mL, 37 mmol) was stirred for 7 h at rt. Saturated $NaHCO_3$ solution was added, the mixture was extracted with DCM, and the collected organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by preparative HPLC (acidic conditions) to give **32**. Yield: 115 mg (38%). LC-MS [Method 4]: R_t = 0.61 min. MS (ESI+): *m/z* = 367.0 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.69 (br s, 1H, pyridone-NH), 8.24 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.67 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.49 (br s, 1H, pyridone), 7.43 (br d, *J* = 5.32 Hz, 1H, pyridone), 6.74–6.81 (m, 1H, naphthyridine), 6.26 (br t, *J* = 6.46 Hz, 1H, pyridone),

5.04 (spt, *J* = 6.00 Hz, 1H, CH), 3.50–3.73 (m, 8H, morpholine), 1.39 (d, *J* = 5.83 Hz, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 163.1, 161.2, 159.8, 158.5, 155.0, 141.8, 140.1, 138.6, 135.3, 121.3, 114.1, 104.6, 92.8, 70.4, 66.0, 45.1, 21.5. HRMS (ESI, [M+H]$^+$): calcd for $C_{20}H_{23}N_4O_3$, 367.1770; found, 367.1769.

6-[4-Isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]pyridin-2(1*H*)-one (33). The title compound was prepared using an analogous method as described for **32** (step b), from 4-isopropoxy-8-(6-methoxypyridin-2-yl)-2-(morpholin-4-yl)-1,7-naphthyridine (**27**) (1.2 g, 3.14 mmol). Yield: 10.8 mg (1%). LC-MS [Method 1]: R_t = 1.16 min. MS (ESI+): *m/z* = 367.3 [M+H]$^+$. ^{1}H NMR (400 MHz, CD_2Cl_2): δ = 13.81–14.00 (m, 1H, pyridone), 8.34 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.80 (d, *J* = 5.07 Hz, 1H, naphthyridine), 7.59 (d, *J* = 7.10 Hz, 1H, pyridone), 7.46 (dd, *J* = 7.22, 9.00 Hz, 1H, pyridone), 6.42 (d, *J* = 9.07 Hz, 1H, pyridone), 6.37 (s, 1H, naphthyridine), 4.76 (spt, *J* = 6.00 Hz, 1H, CH), 3.79–3.87 (m, 4H, morpholine), 3.63–3.70 (m, 4H, morpholine), 1.42 (d, *J* = 6.08 Hz, 6H, iPr). MS (ESI+): *m/z* = 367.3 [M + H]$^+$. HRMS (ESI, [M+H]$^+$): calcd for $C_{20}H_{23}N_4O_3$, 367.1770; found, 367.1768.

4-Isopropoxy-2-(morpholin-4-yl)-8-(pyrrolidin-3-yl)-1,7-naphthyridine (34). Step a: *tert*-Butyl 4-[4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]-2,3-dihydro-1*H*-pyrrole-1-carboxylate was prepared using an analogous method as described for **20**, from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (120 mg, 390 µmol) and *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydro-1*H*-pyrrole-1-carboxylate (CAS 1401165-14-7, 127 mg, 429 µmol). Yield: 35 mg (20%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.32 (s, 1H), 8.18 (d, *J* = 5.31 Hz, 1H), 7.48 (d, *J* = 5.05 Hz, 1H), 6.83 (s, 1H), 5.03 (quin, *J* = 6.00 Hz, 1H), 3.63–3.81 (m, 10H), 3.10–3.29 (m, 2H), 1.48 (s, 9H), 1.39 (d, *J* = 6.06 Hz, 6H). MS (ESI+): *m/z* = 441.5 [M+H]$^+$. **Step b: *tert*-Butyl 3-[4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]pyrrolidine-1-carboxylate.** *tert*-Butyl 4-[4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]-2,3-dihydro-1*H*-pyrrole-1-carboxylate (29 mg, 65.8 µmol) was dissolved in EtOH (30 mL), 10% palladium on carbon (7 mg, 6.58 µmol) was added, and the mixture was stirred under hydrogen atmosphere for 3.5 h at rt. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure to give *tert*-butyl 3-[4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]pyrrolidine-1-carboxylate. Yield: 37 mg (quant.). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.17 (d, *J* = 5.56 Hz, 1H), 7.58 (d, *J* = 5.56 Hz, 1H), 6.79 (s, 1H), 5.03 (td, *J* = 5.84, 11.81 Hz, 1H), 4.47 (br t, *J* = 7.71 Hz, 1H), 3.65–3.88 (m, 10H), 3.48 (br s, 2H), 2.23 (br d, *J* = 6.06 Hz, 2H), 1.37–1.42 (m, 15H). MS (ESI+): *m/z* = 443.4 [M+H]$^+$. **Step c: 4-Isopropoxy-2-(morpholin-4-yl)-8-(pyrrolidin-3-yl)-1,7-naphthyridine (34)**. To a solution of *tert*-butyl 3-[4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridin-8-yl]pyrrolidine-1-carboxylate (33.0 mg, 74.6 µmol) in DCM (2 mL) was added TFA (1.0 mL, 13 mmol) and the mixture was stirred for 2 h at rt. The mixture was diluted with H_2O and $NaHCO_3$ solution until pH >8. DCM was added and the mixture was filtered through phase separator paper. The filtrate was concentrated under reduced pressure to give **34**. Yield: 14 mg (52%). LC-MS [Method 4]: R_t = 0.62 min. MS (ESI+): *m/z* = 343.3 [M+H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (d, *J* = 5.56 Hz, 1H, naphthyridine), 7.69 (d, *J* = 5.56 Hz, 1H, naphthyridine), 6.37 (s, 1H, naphthyridine), 4.71–4.86 (m, 2H, pyrrolidine, CH), 3.85–3.90 (m, 4H, morpholine), 3.58–3.71 (m, 6H, morpholine, pyrrolidine), 3.40–3.56 (m, 2H, pyrrolidine), 2.38–2.59 (m, 1H, pyrrolidine), 2.27 (br d, *J* = 5.05 Hz, 1H, pyrrolidine), 1.48 (d, *J* = 6.06 Hz, 6H, iPr), NH not visible.

4-Isopropoxy-*N*-methyl-2-(morpholin-4-yl)-1,7-naphthyridine-8-carboxamide (35). The title compound was prepared using an analogous method as described for **21**, from 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (150 mg, 487 µmol) and 2 M methanamine (1.5 mL, 2.9 mmol) in DMSO (6.9 mL). Yield: 35.5 mg (21%). LC-MS [Method 1]: R_t = 0.52 min. MS (ESI+): *m/z* = 331.0 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.99 (br d, *J* = 4.82 Hz, 1H, NH), 8.26 (d, *J* = 5.07 Hz, 1H, naphthyridine), 7.76 (d, *J* = 5.32 Hz, 1H, naphthyridine), 6.83 (s, 1H, naphthyridine), 5.01–5.12 (m, 1H, CH), 3.70–3.77 (m, 4H, morpholine), 3.64–3.70 (m, 4H, morpholine), 2.86 (d, *J* = 4.56 Hz, 3H, CH_3), 1.39 (d, *J* = 6.08 Hz, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 166.6, 159.8, 159.0, 150.6, 140.7, 138.5, 122.3, 115.9, 93.0, 70.7, 66.0, 45.2, 25.9, 21.4. HRMS (ESI, [M+H]$^+$): calcd for $C_{17}H_{23}N_4O_3$, 331.1770; found, 331.1766.

4-Isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (36). Under argon, [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 53 mg, 65 µmol) and Cs_2CO_3 (847 mg, 2.60 mmol) were added to a suspension of [3-(hydroxymethyl)phenyl]boronic acid (CAS 87199-15-3, 148 mg, 975 µmol) and 8-chloro-4-isopropoxy-2-(morpholin-4-yl)-1,7-naphthyridine (200 mg, 650 µmol) in anhydrous 1,4-dioxane (5.0 mL). The reaction mixture was stirred at 130 °C for 6 h and overnight at 100 °C. The mixture was concentrated under reduced pressure. The residue was dissolved in MeOH, sodium borohydride was added, and the mixture was stirred overnight at rt. Saturated $NaHCO_3$ solution was added, the mixture was extracted with DCM, and the collected organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, $CHCl_3$/MeOH 95:5) and preparative HPLC, to give **36** as major byproduct. Yield: 100 mg (56%). LC-MS [Method 4]: R_t = 0.61 min. MS (ESI+): *m/z* = 274.2 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.84 (m, 1H, naphthyridine), 8.23–8.24 (m, 1H, naphthyridine), 7.65–7.67 (m, 1H, naphthyridine), 6.78 (s, 1H, naphthyridine), 5.02–5.05 (m, 1H, CH), 3.72–3.74 (m, 4H, morpholine), 3.68–3.72 (m, 4H, morpholine), 1.38–1.40 (m, 6H, iPr). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.7, 159.6, 149.5, 143.4, 139.6, 121.2, 114.4, 92.9, 70.5, 66.1, 45.2, 21.5. HRMS (ESI, [M+H]$^+$): calcd for $C_{15}H_{20}N_3O_2$, 274.1556; found, 274.1555.

3,3-Dimethyl-2-{2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridin-4-yl}butan-2-ol (37). Step a: Methyl 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxylate. In an autoclave, a mixture of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-

pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (2.527 g, 4.79 mmol), 1,3-bis(diphenylphosphino)propane (203 mg, 0.48 mmol), palladium(II) acetate (108 mg, 0.48 mmol), and triethylamine (1.3 mL, 9.6 mmol) in DMF (34 mL) and MeOH (18 mL) was purged with carbon monoxide at rt. The autoclave was pressured with carbon monoxide to 16.5 bar and the mixture was stirred at rt for 30 min. The autoclave was depressurized and then pressured with carbon monoxide to 20.9 bar. The mixture was stirred at 80 °C for 20 h. The autoclave was depressurized and, after cooling, the mixture was diluted with H_2O and extracted with EtOAc (2 ×). The combined organic phases were dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (gradient: 100% hexane to 100% EtOAc) to give methyl 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxylate. Yield: 1.537 g (73%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.50 (s, 1H), 8.33 (d, *J* = 5.32 Hz, 1H), 7.77 (d, *J* = 5.32 Hz, 1H), 7.60 (d, *J* = 1.77 Hz, 1H), 6.91 (dd, *J* = 1.77, 15.46 Hz, 1H), 6.59 (d, *J* = 4.31 Hz, 1H), 6.02–6.17 (m, 1H), 4.20–4.38 (m, 1H), 3.90–4.03 (m, 2H), 3.67–3.77 (m, 2H), 3.57–3.65 (m, 1H), 3.34 (s, 3H), 3.20–3.29 (m, 1H), 3.08–3.20 (m, 1H), 2.26–2.40 (m, 1H), 1.89–1.99 (m, 2H), 1.52–1.65 (m, 1H), 1.39–1.49 (m, 2H), 1.15–1.21 (m, 3H). MS (ESI+): *m/z* = 438.2 $[M+H]^+$. **Step b: 2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxylic acid.** Methyl 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxylate (1.10 g, 2.51 mmol) was dissolved in THF (11 mL) and MeOH (5 mL). 1.0 M Aqueous NaOH (2.8 mL, 2.8 mmol) was added and the mixture was stirred for 10 min at rt. The solvent was removed under reduced pressure and the aqueous phase was acidified to pH 5 using 1 M aqueous HCl. The aqueous solution was lyophilized to give 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxylic acid, without further purification. Yield: 1.10 g (quant.). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (dd, *J* = 2.15, 5.45 Hz, 1H), 8.30 (d, *J* = 4.82 Hz, 1H), 7.54–7.63 (m, 1H), 7.41 (d, *J* = 5.07 Hz, 1H), 6.83 (dd, *J* = 1.77, 10.65 Hz, 1H), 5.94–6.05 (m, 1H), 4.36–4.48 (m, 1H), 4.06 (br t, *J* = 14.32 Hz, 1H), 3.94 (td, *J* = 3.93, 11.15 Hz, 1H), 3.67–3.76 (m, 2H), 3.58–3.67 (m, 1H), 3.37–3.51 (m, 1H), 3.09–3.29 (m, 2H), 2.31–2.41 (m, 1H), 1.89–2.01 (m, 2H), 1.38–1.63 (m, 3H), 1.18 (dd, *J* = 6.59, 10.14 Hz, 3H), OH not visible. MS (ESI+): *m/z* = 424.0 $[M+H]^+$. **Step c: *N*-Methoxy-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxamide**. *N*-Methoxymethanamine hydrochloride (1:1) (861 mg, 8.83 mmol) was dissolved in DMF (20 mL). *N*,*N*-Diisopropylethylamine (3.1 mL, 18 mmol) and *N*-[(dimethylamino)(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methylidene]-*N*-methylmethanaminium hexafluorophosphate (HATU, 2.52 g, 6.62 mmol) were added and the mixture was stirred for 10 min at rt. 2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxylic acid (1.10 g, 2.21 mmol with 85% purity) was then added and the mixture was stirred for 16 h at rt. *N*,*N*-Diisopropylethylamine (3.1 mL, 18 mmol) and HATU (2.52 g, 6.62 mmol) were added again and the reaction mixture was stirred for 16 h at rt. H_2O was added and the mixture was stirred for 10 min. The aqueous phase was extracted with EtOAc and the combined organic layers were washed with half-saturated NaCl solution. The organic layer was dried (silicon filter) and concentrated under reduced pressure. The crude material was purified by flash column chromatograpy (from hexane/EtOAc gradient 0–100% to EtOAc/EtOH gradient 0–20%) to give *N*-methoxy-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxamide. Yield: 1.05 g (quant.). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.40 (d, *J* = 5.32 Hz, 1H), 7.63 (s, 1H), 7.60 (d, *J* = 3.88 Hz, 1H), 7.43 (d, *J* = 5.32 Hz, 1H), 6.96 (dd, *J* = 1.77, 15.46 Hz, 1H), 6.06–6.14 (m, 1H), 4.51 (br t, *J* = 6.59 Hz, 1H), 4.10–4.20 (m, 1H), 3.93–4.01 (m, 1H), 3.60–3.77 (m, 3H), 3.46–3.56 (m, 3H), 3.38–3.45 (m, 2H), 3.15–3.30 (m, 2H), 2.67–2.70 (m, 3H), 2.31–2.46 (m, 1H), 1.93–1.98 (m, 1H), 1.40–1.64 (m, 3H), 1.18–1.25 (m, 3H). MS (ESI+): *m/z* = 467.5 $[M+H]^+$. **Step d: 1-{2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}ethanone.** *N*-Methoxy-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxamide (710 mg, 1.52 mmol) was dissolved in THF (20 mL) and the mixture was cooled to 0 °C. 3.0 M MeMgBr in diethylether (1.5 mL, 4.6 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 30 min and at rt for 1.5 h. Additional 3.0 M MeMgBr in diethylether (1.5 mL, 4.6 mmol) was added and the reaction mixture was stirred for 16 h. The reaction was quenched with saturated ammonium chloride solution and the mixture was extracted with DCM. The organic phase was filtered and concentrated under reduced pressure to give 1-{2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}ethanone, which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.41 (d, *J* = 5.58 Hz, 1H), 7.95 (d, *J* = 5.58 Hz, 1H), 7.84 (d, *J* = 3.04 Hz, 1H), 7.62 (t, *J* = 1.52 Hz, 1H), 6.87 (dd, *J* = 1.77, 14.19 Hz, 1H), 5.96–6.06 (m, 1H), 4.50–4.62 (m, 1H), 4.17 (br d, *J* = 13.18 Hz, 1H), 3.94–4.02 (m, 1H), 3.57–3.80 (m, 5H), 3.41–3.55 (m, 1H), 3.19–3.32 (m, 2H), 1.89–2.03 (m, 2H), 1.73–1.80 (m, 2H), 1.39–1.62 (m, 3H), 1.22 (dd, *J* = 6.72, 14.32 Hz, 3H). MS (ESI+): *m/z* = 422.3 $[M+H]^+$. **Step e: 3,3-Dimethyl-2-{2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridin-4-yl}butan-2-ol (37).** 1-{2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}ethanone (100 mg, 237 µmol) was dissolved in THF (2.0 mL) and the mixture was cooled to 0 °C. 1.0 M *t*BuMgCl in THF (710 µL, 710 µmol) was added dropwise. The mixture was stirred at 0 °C for 0.5 h and at rt for 1.5 h. The reaction was quenched with H_2O and 3 M aqueous HCl (0.5 mL) was added. The mixture was stirred for 72 h at rt. The reaction was quenched with $NaHCO_3$ solution and the mixture was extracted with DCM. The organic phase was dried (silicon filter) and concentrated under reduced pressure. The crude material was purified by preparative HPLC (basic conditions) to give 3,3-dimethyl-2-{2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-

pyrazol-5-yl)-1,7-naphthyridin-4-yl}butan-2-ol as a mixture of diastereomers. Yield: 25 mg (24%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.31 (br s, 1H), 8.88–8.97 (m, 1H), 8.26 (d, *J* = 5.58 Hz, 1H), 7.59 (s, 1H), 7.32 (br s, 1H), 7.11 (br s, 1H), 5.59 (s, 1H), 4.53 (br s, 1H), 3.98–4.17 (m, 2H), 3.68–3.87 (m, 2H), 3.58 (br t, *J* = 11.28 Hz, 1H), 3.23–3.32 (m, 1H), 1.70 (s, 3H), 1.17–1.28 (m, 3H), 0.95 (s, 9H). MS (ESI+): *m/z* = 396.3 [M+H]$^+$. **Step f: (2*S*)-3,3-Dimethyl-2-{2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridin-4-yl}butan-2-ol (37).** The diastereomers of 3,3-dimethyl-2-{2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridin-4-yl}butan-2-ol (**37**) (210 mg) were separated by preparative chiral HPLC to give 3,3-dimethyl-2-{2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridin-4-yl}butan-2-ol (**37**, single diastereomer with unknown configuration of the alcohol stereogenic center; 26 mg). Preparative chiral HPLC method: instrument: Labomatic HD5000, Labocord-5000, Gilson GX-241, Labcol Vario 4000; column: Chiralpak AD-H 5µm, 250 × 30 mm; eluent A: hexane + 0.1 vol% diethylamine (99%), eluent B: EtOH; isocratic: 90% A + 10% B; flow: 40.0 mL/min; UV 280 nm. Analytical chiral HPLC method: instrument: Agilent HPLC 1260; column: Chiralpak AD-H 5µm, 150 × 4.6 mm; eluent A: hexane + 0.1 vol% diethylamine (99%), eluent B: EtOH; isocratic: 90% A + 10% B; flow: 1.4 mL/min; temperature: 25 °C; DAD 280 nm; ^{t}R = 3.97 min. LC-MS [Method 4]: R_t = 0.66 min. MS (ESI+): *m/z* = 396.3 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.30 (br s, 1H, pyrazole-NH), 8.92 (br d, *J* = 5.07 Hz, 1H, naphthyridine), 8.26 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.59 (s, 1H, pyrazole), 7.32 (s, 1H, pyrazole), 7.10 (br s, 1H, naphthyridine), 5.59 (br s, 1H, OH), 4.52 (br s, 1H, morpholine), 4.00–4.17 (m, 2H, morpholine), 3.82 (br d, *J* = 11.15 Hz, 1H, morpholine), 3.68–3.77 (m, 1H, morpholine), 3.49–3.66 (m, 1H, morpholine), 3.17–3.31 (m, 1H, morpholine), 1.71 (br s, 3H, CH_3), 1.24 (d, *J* = 6.59 Hz, 3H, CH_3), 0.95 (s, 9H, tBu). HRMS (ESI, [M+H]$^+$): calcd for $C_{22}H_{30}N_5O_2$, 396.2400; found, 396.2392.

2-[(3*R*)-3-Methylmorpholin-4-yl]-4-[4-(methylsulfonyl)piperazin-1-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (38). Step a: 2-[(3*R*)-3-Methylmorpholin-4-yl]-4-[4-(methylsulfonyl)-piperazin-1-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine. A mixture of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (150 mg, 0.28 mmol) and 1-(methylsulfonyl)piperazine (CAS 55276-43-2, 159 mg, 0.97 mmol) in MeCN (0.42 mL) was stirred at 70 °C for 8 h under argon. After cooling, the reaction mixture was diluted with EtOAc and THF, and washed with saturated aqueous NaCl solution. The organic phase was filtered using a Whatman filter paper and then concentrated to give 2-[(3*R*)-3-methylmorpholin-4-yl]-4-[4-(methylsulfonyl)piperazin-1-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine, which was used in the next step without further purification. MS (ESI+): *m/z* = 542.4 [M+H]$^+$. **Step b: 2-[(3*R*)-3-Methylmorpholin-4-yl]-4-[4-(methylsulfonyl)piperazin-1-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (38)**. A solution of crude 2-[(3*R*)-3-methylmorpholin-4-yl]-4-[4-(methylsulfonyl)piperazin-1-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine (267 mg, 0.49 mmol) in MeOH (2.3 mL) and 2 N aqueous HCl (0.57 mL) was stirred for 1 h at rt. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc/THF (1:1) (2 ×). The combined organic phases were filtered using a Whatman filter paper and then concentrated to dryness. The residue was purified by preparative HPLC (basic conditions) to give **38**. Yield: 55 mg (24%). LC-MS [Method 4]: R_t = 0.60 min. MS (ESI+): *m/z* = 458.3 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.21 (br s, 1H, pyrazole-NH), 8.32 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.63 (d, *J* = 5.22 Hz, 1H, naphthyridine), 7.61 (s, 1H, pyrazole), 7.34 (d, *J* = 1.77 Hz, 1H, pyrazole), 6.83 (s, 1H, naphthyridine), 4.59 (br d, *J* = 4.56 Hz, 1H, morpholine), 4.13 (br d, *J* = 11.66 Hz, 1H, morpholine), 4.04 (dd, *J* = 3.30, 11.41 Hz, 1H, morpholine), 3.82 (d, *J* = 11.41 Hz, 1H, morpholine), 3.69 (dd, *J* = 2.79, 11.41 Hz, 1H, morpholine), 3.54 (dt, *J* = 2.79, 11.79 Hz, 1H, morpholine), 3.37–3.45 (m, 4H, piperazine), 3.22–3.31 (m, 5H, piperazine, morpholine), 3.00 (s, 3H, CH_3), 1.27 (d, *J* = 6.84 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 158.0, 156.3, 145.5, 140.8, 140.7, 139.0, 138.5, 123.2, 115.9, 107.5, 101.1, 70.5, 66.3, 51.1, 47.3, 45.5, 34.1, 13.2, one carbon not visible (under solvent). HRMS (ESI, [M+H]$^+$): calcd for $C_{21}H_{28}N_7O_3S$, 458.1974; found, 458.1972. $[\alpha]^{20}_D$ –68.80 (*c* = 1, DMSO).

4-(2-Fluoropyridin-3-yl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (39). 2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (0.25 g, 0.47 mmol) was dissolved in 1,4-dioxane (2.5 mL). (2-Fluoropyridin-3-yl)boronic acid (CAS 174669-73-9, 0.20 g, 1.4 mmol) was added in one portion followed by the addition of Cs_2CO_3 (0.62 g, 1.90 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 77 mg, 94 µmol). The reaction mixture was heated for 4 h in a sealed tube at 110 °C. The mixture was then cooled to rt and filtered. The solid was washed with DCM and the filtrate was concentrated under reduced pressure. The crude material was purified by flash column chromatography (gradient of hexane/EtOAc 0-100% and EtOAc/EtOH 0-20%). The combined product fractions were concentrated under reduced pressure (~ 140 mg), dissolved in MeOH (3 mL), and 3 N aqueous HCl (10 mL) was added. The mixture was stirred for 2 h at rt and then basified with 3 M aqueous NaOH. The suspension was filtered and washed with H_2O. The solid was dried under reduced pressure at 60 °C to give **39**. Yield: 109 mg (90% with 95% purity). LC-MS [Method 4]: R_t = 0.66 min. MS (ESI+): *m/z* = 391.3 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.44 (br s, 1H, pyrazole-NH), 8.44–8.48 (m, 1H, pyridine), 8.32 (d, *J* = 5.32 Hz, 1H, pyridine), 8.18 (ddd, *J* = 1.90, 7.48, 9.63 Hz, 1H, pyridine), 7.59–7.67 (m, 3H, pyridine, pyrazole), 7.43 (br s, 1H, pyridine), 7.17 (dd, *J* = 1.77, 5.32 Hz, 1H, pyrazole), 4.63 (br d, *J* = 4.56 Hz, 1H, morpholine), 4.22 (br d, *J* = 12.17 Hz, 1H, morpholine), 3.98–4.10 (m, 1H, morpholine), 3.76–3.87 (m, 1H, morpholine), 3.67–3.76 (m, 1H, morpholine), 3.50–3.63 (m, 1H, morpholine), 3.38 (br s, 1H, morpholine), 1.30 (d, *J* = 6.84 Hz, 3H, CH_3). HRMS (ESI, [M + H]$^+$): calcd for

$C_{21}H_{20}N_6OF$, 391.1683; found, 391.1689. $[\alpha]^{20}_D$ –80.22 (*c* = 1, DMSO).

***N*-(4-Fluorophenyl)-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridin-4-amine (40). Step a: *N*-(4-Fluorophenyl)-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-amine.** A mixture of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (150 mg, 0.28 mmol) and 4-fluoro-*N*-methylaniline (CAS 459-59-6, 121 mg, 0.97 mmol) in MeCN (0.42 mL) was stirred at 70 °C for 3 h under argon. After cooling, the reaction mixture was diluted with EtOAc and THF, and washed with saturated aqueous NaCl solution. The organic phase was filtered using a Whatman filter paper and then concentrated to give *N*-(4-fluorophenyl)-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-amine, which was used in the next step without further purification. **Step b: *N*-(4-Fluorophenyl)-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridin-4-amine (40).** A solution of crude *N*-(4-fluorophenyl)-*N*-methyl-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-amine (273 mg) in MeOH (2.5 mL) and 2 N aqueous HCl (0.63 mL) was stirred for 1 h at rt. Then, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc/THF (1:1) (2 ×). The combined organic phases were filtered using a Whatman filter paper and then concentrated to dryness. The residue was purified by preparative HPLC (basic conditions) to give **40**. Yield: 54 mg (24%). LC-MS [Method 4]: R_t = 0.65 min. MS (ESI+): *m/z* = 419.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.26 (br s, 1H, pyrazole-NH), 8.08 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.61 (d, *J* = 1.52 Hz, 1H, pyrazole), 7.35 (d, *J* = 1.77 Hz, 1H, pyrazole), 6.95–7.14 (m, 6H, phenyl, naphthyridine), 4.55–4.63 (m, 1H, morpholine), 4.15 (br d, *J* = 11.41 Hz, 1H, morpholine), 4.05 (dd, *J* = 3.30, 11.15 Hz, 1H, morpholine), 3.83 (d, *J* = 11.41 Hz, 1H, morpholine), 3.72 (dd, *J* = 2.91, 11.53 Hz, 1H, morpholine), 3.57 (dt, *J* = 2.91, 11.72 Hz, 1H, morpholine), 3.44 (s, 3H, CH_3), 3.34–3.40 (m, 1H, morpholine), 1.29 (d, *J* = 6.84 Hz, 3H, CH_3). ^{19}F NMR (377 MHz, DMSO-*d6*) δ = -121.35 (m, 1F). HRMS (ESI, $[M+H]^+$): calcd for $C_{23}H_{24}N_6OF$, 419.1996; found, 419.1994. $[\alpha]^{20}_D$ –64.22 (*c* = 1, DMSO).

4-[2-Methyl-6-(*S*-methylsulfonimidoyl)pyridin-3-yl]-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (41). Step a: 4-[2-Methyl-6-(methylsulfanyl)pyridin-3-yl]-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine. A suspension of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (250 mg, 0.47 mmol), [2-methyl-6-(methylsulfanyl)pyridin-3-yl]boronic acid (CAS 1451392-60-1, 87 mg, 0.47 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 38 mg, 47 µmol), and K_2CO_3 (164 mg, 1.19 mmol) in MeCN (10.0 mL) and H_2O (3.3 mL) was degassed with argon. Under argon, the reaction mixture was stirred at 130 °C for 10 min in a microwave reactor. After cooling, the reaction mixture was diluted with saturated aqueous NaCl solution and extracted with EtOAc (2 ×). The combined organic phases were filtered using a Whatman filter paper and then concentrated. The residue was purified by column chromatography (gradient: 100% hexane to 100% EtOAc) to give 4-[2-methyl-6-(methylsulfanyl)pyridin-3-yl]-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine. Yield: 170 mg (70%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.33 (d, *J* = 5.58 Hz, 1H), 7.57–7.66 (m, 2H), 7.40–7.45 (m, 1H), 7.32 (d, *J* = 8.11 Hz, 1H), 7.09 (ddd, *J* = 2.03, 3.49, 5.39 Hz, 1H), 6.95–7.04 (m, 1H), 6.09–6.18 (m, 1H), 4.52 (br s, 1H), 4.12–4.24 (m, 1H), 3.93–4.05 (m, 1H), 3.69–3.77 (m, 2H), 3.60–3.69 (m, 1H), 3.37–3.54 (m, 1H), 3.17–3.31 (m, 2H), 2.55–2.60 (m, 3H), 2.32–2.41 (m, 1H), 2.25 (t, *J* = 3.80 Hz, 3H), 1.92–2.03 (m, 2H), 1.50–1.65 (m, 1H), 1.46 (br s, 2H), 1.18–1.28 (m, 3H). MS (ESI+): *m/z* = 517.2 $[M+H]^+$. **Step b: 2,2,2-Trifluoro-*N*-{methyl[6-methyl-5-(2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridin-4-yl)pyridin-2-yl]-λ⁴-sulfanylidene}acetamide.** Under an atmosphere of argon, a solution of 2,2,2-trifluoroacetamide (43 mg, 0.38 mmol) in THF (0.20 mL) was added dropwise to a solution of sodium *tert*-butoxide (24 mg, 0.25 mmol) in THF (0.25 mL), such that the temperature of the mixture remained below 10 °C. Subsequently, a freshly prepared solution of 1,3-dibromo-5,5-dimethylhydantoin (47 mg, 0.16 mmol) in THF (0.25 mL) was added dropwise to the stirred mixture, such that the temperature of the mixture remained below 10 °C. Then, the mixture was stirred for 10 min at 10 °C. Finally, a solution of 4-[2-methyl-6-(methylsulfanyl)pyridin-3-yl]-2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine (130 mg, 0.25 mmol) in THF (0.8 mL) was added dropwise to the stirred mixture, such that the temperature of the mixture remained below 10 °C. The mixture was stirred for 3 h at 10 °C and then at rt overnight. The mixture was diluted with toluene (1.0 mL) under cooling and a solution of sodium sulfite (32 mg, 0.25 mmol) in H_2O (0.9 mL) was added such that the temperature of the mixture remained below 15 °C. The mixture was extracted with EtOAc (3 ×). The combined organic phases were washed with aqueous NaCl solution, filtered using a Whatman filter paper, and concentrated. The residue was purified by column chromatography (silica gel, EtOAc) to give 2,2,2-trifluoro-*N*-{methyl[6-methyl-5-(2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridin-4-yl)pyridin-2-yl]-λ⁴-sulfanylidene}acetamide containing slight impurities. Yield: 28 mg (18%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.32–8.41 (m, 1H), 8.04–8.18 (m, 1H), 7.87–7.98 (m, 1H), 7.65 (d, *J* = 1.52 Hz, 1H), 7.52–7.59 (m, 1H), 6.90–7.09 (m, 2H), 6.10–6.21 (m, 1H), 4.52 (br s, 1H), 4.08–4.26 (m, 1H), 3.97 (br d, *J* = 8.36 Hz, 1H), 3.68–3.77 (m, 2H), 3.43–3.68 (m, 2H), 3.16–3.27 (m, 4H), 2.52–2.59 (m, 1H), 2.34–2.46 (m, 4H), 1.98 (br d, *J* = 4.56 Hz, 2H), 1.50–1.65 (m, 1H), 1.46 (br s, 2H), 1.13–1.27 (m, 3H). MS (ESI+): *m/z* = 628.4 $[M + H]^+$. **Step c: 4-[2-Methyl-6-(*S*-methylsulfonimidoyl)pyridin-3-yl]-2-[(3*R*)-3-**

methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine. 2,2,2-Trifluoro-*N*-{methyl[6-methyl-5-(2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridin-4-yl)pyridin-2-yl]-λ⁴-sulfanylidene}acetamide (28 mg, 0.045 mmol) was dissolved in MeOH (0.87 mL). To this solution, H_2O (0.31 mL) was added. The pH was adjusted to 10.5 by the addition of 25% aqueous KOH solution. Oxone (23 mg, 0.038 mmol) was added and the mixture was stirred at rt for 5 h. Additional Oxone (23 mg, 0.038 mmol) was added. The pH was adjusted to 10.5 by the addition of 25% aqueous KOH solution. The mixture was stirred at rt for 3 h. The mixture was filtered and the filtrate was adjusted to pH 6–7 by the addition of 1 N aqueous HCl. The mixture was diluted with aqueous NaCl solution and extracted with DCM (2 ×). The combined organic phases were washed with 10% aqueous sodium sulfite solution, filtered using a Whatman filter paper, and concentrated to give crude 4-[2-methyl-6-(*S*-methylsulfonimidoyl)pyridin-3-yl]-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (10 mg), which was used without further purification. **Step d: 4-[2-Methyl-6-(*S*-methylsulfonimidoyl)pyridin-3-yl]-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (41).** A solution of crude 4-[2-methyl-6-(*S*-methylsulfonimidoyl)pyridin-3-yl]-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (10 mg) in MeOH (1.0 mL) and 2 N aqueous HCl (0.02 mL) was stirred for 2 h at rt. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (2 ×). The combined organic phases were dried over sodium sulfate, filtered, and concentrated to dryness. The residue was purified by preparative HPLC (acidic conditions) to give **41** as a mixture of diastereomers. Yield: 2 mg (9% over two steps). LC-MS [Method 2]: R_t = 0.68 min. MS (ESI+): *m/z* = 464.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.45 (br s, 1H, pyrazole-NH), 8.30 (dd, *J* = 2.79, 5.32 Hz, 1H, pyridine), 8.04–8.10 (m, 2H, pyridine), 7.65 (br s, 1H, pyrazole), 7.57 (br d, *J* = 3.04 Hz, 1H, pyridine), 7.45 (br s, 1H, pyrazole), 6.95–7.02 (m, 1H, pyridine), 4.61 (br s, 1H, morpholine), 4.52 (br d, *J* = 18.76 Hz, 1H, morpholine), 4.19–4.27 (m, 1H, morpholine), 4.05 (br d, *J* = 10.90 Hz, 1H, morpholine), 3.82 (br dd, *J* = 5.83, 11.66 Hz, 1H, morpholine), 3.72 (br d, *J* = 11.91 Hz, 1H, morpholine), 3.52–3.64 (m, 1H, morpholine), 3.22–3.26 (m, 3H, CH_3), 2.35 (d, *J* = 2.03 Hz, 3H, CH_3), 1.30 (t, *J* = 6.21 Hz, 3H), sulfoximine NH not visible.

4-(6,7-Dihydro-5*H*-pyrrolo[1,2-*a*]imidazol-3-yl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (42). Step a: 4-(6,7-Dihydro-5*H*-pyrrolo[1,2-*a*]imidazol-3-yl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine. A suspension of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (100 mg, 0.19 mmol), (6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazol-3-yl)boronic acid (CAS 1876473-42-5, 57 mg, 0.38 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 15 mg, 19 μmol), and K_2CO_3 (65 mg, 0.47 mmol) in MeCN (2.0 mL) and H_2O (1.0 mL) was degassed with argon. Under argon, the reaction mixture was stirred at 130 °C for 10 min in a microwave reactor. After cooling, the reaction mixture was diluted with saturated aqueous NaCl solution and extracted with EtOAc (2 ×). The combined organic phases were filtered using a Whatman filter paper and then concentrated to give 4-(6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazol-3-yl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine, which was used in the next step without further purification. MS (ESI+): *m/z* = 486.3 $[M+H]^+$. **Step b: 4-(6,7-Dihydro-5*H*-pyrrolo[1,2-*a*]imidazol-3-yl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (42).** A solution of crude 4-(6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazol-3-yl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (166 mg) in MeOH (3.2 mL) and 2 N aqueous HCl (0.34 mL) was stirred for 90 min at rt. The reaction mixture was diluted with saturated aqueous NaCl solution and extracted with EtOAc (2 ×). The combined organic phases were dried over sodium sulfate, filtered, and concentrated to dryness. The residue was purified by preparative HPLC (basic conditions) to give **42**. Yield: 1 mg (1 % over two steps). LC-MS [Method 2]: R_t = 0.92 min. MS (ESI+): *m/z* = 402.4 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.41 (br s, 1H, pyrazole-NH), 8.37 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.78 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.63 (s, 1H, naphthyridine), 7.34–7.46 (m, 3H, pyrazole, imidazole), 4.62 (br d, *J* = 4.82 Hz, 1H, morpholine), 4.20 (br d, *J* = 11.41 Hz, 1H, morpholine), 4.03–4.14 (m, 3H, pyrrolidine, morpholine), 3.83 (d, *J* = 11.41 Hz, 1H, morpholine), 3.72 (dd, *J* = 2.66, 11.53 Hz, 1H, morpholine), 3.58 (dt, *J* = 2.66, 11.72 Hz, 1H, morpholine), 2.85–2.92 (m, 2H, pyrrolidine), 2.52–2.68 (m, 3H, pyrrolidine, morpholine), 1.28 (d, *J* = 6.59 Hz, 3H, CH_3).

2-[(3*R*)-3-Methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-4-(tetrahydro-2*H*-pyran-4-yl)-1,7-naphthyridine (43). Step a: 4,8-Dichloro-2-[(3*R*)-3-methylmorpholin-4-yl]-1,7-naphthyridine. 8-Chloro-2-[(3*R*)-3-methylmorpholin-4-yl]-1,7-naphthyridin-4-ol (**65**) (0.50 g, 1.8 mmol) was suspended in phosphorus oxychloride (1.6 mL, 17 mmol) and the mixture was stirred at 95 °C for 3 h. The reaction mixture was cooled to rt and then placed in an ice bath. The reaction was carefully quenched by the dropwise addition of 3 N aqueous NaOH until pH 9. The aqueous phase was extracted with DCM (3 ×). The organic layer was dried (silicon filter) and concentrated under reduced pressure. The crude mixture was then stirred with MeOH and filtered. The solid was dried under reduced pressure at 40 °C to give 4,8-dichloro-2-[(3*R*)-3-methylmorpholin-4-yl]-1,7-naphthyridine, without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.14 (d, *J* = 5.32 Hz, 1H), 7.79 (d, *J* = 5.55 Hz, 2H), 4.62 (br d, *J* = 4.56 Hz, 1H), 4.29 (br d, *J* = 12.17 Hz, 1H), 3.95–4.03 (m, 1H), 3.77 (d, *J* = 11.41 Hz, 1H), 3.65 (dd, *J* = 3.04, 11.41 Hz, 1H), 3.35–3.54 (m, 1H), 3.17–3.32 (m, 1H), 1.25 (d, *J* = 6.84 Hz, 3H). **Step b: 4-Chloro-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine.** 4,8-Dichloro-2-[(3*R*)-3-methylmorpholin-4-yl]-1,7-naphthyridine (0.5 g, 1.7 mmol), 1-(tetrahydro-2*H*-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-

2-yl)-1*H*-pyrazole (CAS 903550-26-5, 0.47 g, 1.7 mmol), and bis(triphenylphosphine)palladium(II) dichloride (0.12 g, 0.17 mmol) were dissolved in DME (15 mL). 2 M Aqueous K_2CO_3 (2.5 mL, 5.0 mmol) was added and the reaction mixture was heated for 10 min under microwave irradiation at 130 °C. The mixture was cooled to ambient temperature, dried by filtration through a silicon filter and concentrated under reduced pressure. The crude material was purified by flash column chromatography (gradient from 100% hexane to 100% EtOAc) to give 4-chloro-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine. Yield: 0.5 g (45%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.49 (d, *J* = 5.31 Hz, 1H), 7.83 (d, *J* = 5.31 Hz, 1H), 7.76 (d, *J* = 3.54 Hz, 1H), 7.62 (s, 1H), 6.92 (dd, *J* = 1.77, 14.91 Hz, 1H), 6.00–6.09 (m, 1H), 4.49 (br t, *J* = 6.82 Hz, 1H), 4.11 (br t, *J* = 11.37 Hz, 1H), 3.92–4.00 (m, 1H), 3.57–3.82 (m, 3H), 3.40–3.53 (m, 1H), 3.16–3.29 (m, 2H), 2.31–2.47 (m, 1H), 1.90–2.03 (m, 2H), 1.39–1.64 (m, 3H), 1.15–1.24 (m, 3H). MS (ESI+): *m/z* = 414.3 [M+H]$^+$. **Step c: 2-[(3*R*)-3-Methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-4-(tetrahydro-2*H*-pyran-4-yl)-1,7-naphthyridine (43).** 4-Chloro-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (0.10 g, 0.22 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydro-2*H*-pyran (CAS 287944-16-5, 95 mg, 0.43 mmol) were dissolved in 1,4-dioxane (5 mL). Cs_2CO_3 (0.28 g, 0.87 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (1:1 complex with DCM, 18 mg, 21 µmol) were added sequentially. The reaction mixture was heated for 4 h in a sealed tube at 110 °C. 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydro-2*H*-pyran (53 mg, 0.22 mmol) was added and the mixture was stirred for 48 h at 110 °C. The reaction mixture was then cooled to rt, diluted with H_2O, and extracted with EtOAc. The organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude mixture was dissolved in DCM (6 mL) and 1 M aqueous HCl (1.2 mL) was added. The reaction mixture was stirred overnight and basified using saturated $NaHCO_3$ solution. Then, the mixture was diluted with H_2O and extracted with DCM. The organic phase was washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude material (42 mg) was dissolved in MeOH (2 mL) and was hydrogenated in an autoclave (10.5 bar) at rt for 18 h using 10% palladium on carbon (20 mg). The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The crude material was purified by flash chromatography (NH-cartridge, hexane/EtOAc 95:5) to give **43**. Yield: 11 mg (13%). LC-MS [Method 1]: R_t = 0.74 min. MS (ESI+): *m/z* = 380.4 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.36 (br s, 1H, pyrazole-NH), 8.37 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.89 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.52–7.55 (m, 1H, pyrazole), 7.37 (s, 1H, pyrazole), 7.31 (s, 1H, naphthyridine), 4.64 (br d, *J* = 6.08 Hz, 1H, morpholine), 4.19 (br d, *J* = 11.41 Hz, 1H, morpholine), 3.92–4.09 (m, 3H, tetrahydropyran, morpholine), 3.77–3.87 (m, 1H, morpholine), 3.49–3.75 (m, 5H, tetrahydropyran, morpholine), 3.23–3.31 (m, 1H, morpholine), 1.89 (dq, *J* = 4.06, 12.25 Hz, 2H, tetrahydropyran), 1.73–1.81 (m, 2H, tetrahydropyran), 1.23–1.29 (m, 3H, CH_3).

2-[(3*R*)-3-Methylmorpholin-4-yl]-4-[1-(methylsulfonyl)cyclopropyl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (44). Step a: {2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}methanol. To a solution of methyl 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine-4-carboxylate (**37**, step a) (190.5 mg, 0.435 mmol) in anhydrous THF (19 mL) was added 1 M diisobutylaluminum hydride in toluene (871 µL, 0.871 mmol) under argon at rt and the reaction mixture was stirred for 1.5 h at 80 °C. Then, the mixture was cooled with ice, saturated aqueous ammonium chloride solution (20 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified using a FlashMaster chromatography system (silica gel, $CHCl_3$/MeOH 98:2) to give {2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}methanol. Yield: 118 mg (66%). MS (ESI+): *m/z* = 410.3 [M+H]$^+$. **Step b: {2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}methyl methanesulfonate.** To a solution of {2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}methanol (118 mg, 0.288 mmol) and triethylamine (52 µL, 0.375 mmol) in anhydrous THF (5 mL) was added dropwise under argon at 0 °C methanesulfonyl chloride (25 µL, 0.317 mmol), and the reaction mixture was allowed to stir for 1 h at 0 °C. With intervals of 2 h, additional methanesulfonyl chloride (3 × 25 µL, 0.317 mmol) was added, and the reaction mixture was allowed to stir for another 16 h at rt. After the addition of another portion of methanesulfonyl chloride (25 µL, 0.317 mmol), the reaction mixture was stirred at 40 °C for 2 h. Then, the mixture was filtered and the filtrate was concentrated to give {2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}methyl methanesulfonate, which used in the next step without further purification. Yield: 219 mg (quant.). MS (ESI+): *m/z* = 488.2 [M+H]$^+$. **Step c: 2-[(3*R*)-3-Methylmorpholin-4-yl]-4-[(methylsulfonyl)methyl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine.** To a solution of {2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl}methyl methanesulfonate (219 mg, 0.45 mmol) in anhydrous DMSO (2 mL) was added sodium methanesulfinate (161 mg, 1.57 mmol), and the reaction mixture was allowed to stir at 120 °C for 20 min. Then, the reaction mixture was diluted with H_2O (10 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified using a PuriFlash chromatography system (silica gel, DCM/MeOH 95:5) to give 2-[(3*R*)-3-methylmorpholin-4-yl]-4-[(methylsulfonyl)methyl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine as a yellow solid. Yield: 84 mg (40%). MS

(ESI+): m/z = 472.3 $[M+H]^+$. **Step d: 2-[(3*R*)-3-Methylmorpholin-4-yl]-4-[1-(methylsulfonyl)cyclopropyl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine.** To a solution of 2-[(3*R*)-3-methylmorpholin-4-yl]-4-[(methylsulfonyl)methyl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine (84 mg, 0.178 mmol), 1,2-dibromoethane (15 µL, 0.178 mmol), and tetrabutylammonium bromide (6 mg, 0.018 mmol) in anhydrous THF (1.68 mL) was added 50% aqueous NaOH solution (185 µL), and the reaction mixture was stirred at rt for 1 h. The suspension changed color, to dark green/dark brown. Additional 1,2-dibromoethane (15 µL, 0.178 mmol), tetrabutylammonium bromide (6 mg, 0.018 mmol), and 50% aqueous NaOH solution (185 µL) were added, and the reaction mixture was stirred at 60 °C for 5 h. Then, the mixture was diluted with H_2O (10 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified using a FlashMaster chromatography system (silica gel, DCM/MeOH 95:5) to give 2-[(3*R*)-3-methylmorpholin-4-yl]-4-[1-(methylsulfonyl)cyclopropyl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine as a yellow solid. Yield: 25 mg (28%). The product was used in the next step without further purification. MS (ESI+): m/z = 498.3 $[M + H]^+$. **Step e: 2-[(3*R*)-3-Methylmorpholin-4-yl]-4-[1-(methylsulfonyl)cyclopropyl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (44).** To a solution of 2-[(3*R*)-3-methylmorpholin-4-yl]-4-[1-(methylsulfonyl)cyclopropyl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridine (25 mg, 0.05 mmol) in MeOH (2 mL) was added 2 M aqueous HCl. The reaction mixture was stirred for 18 h at 50 °C. MeOH was removed under reduced pressure and the pH of the residue was adjusted to 7 by the addition of aqueous $NaHCO_3$ solution. The aqueous layer was extracted with DCM (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to give **44** as a yellow solid. Yield: 16 mg (73%). LC-MS [Method 1]: R_t = 0.81 min. MS (ESI+): m/z = 414.2 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.48–8.49 (m, 1H, naphthyridine), 7.82–7.83 (m, 1H, naphthyridine), 7.71 (s, 1H, pyrazole), 7.45 (s, 1H, naphthyridine), 7.32 (s, 1H, pyrazole), 4.39–4.46 (m, 1H, morpholine), 4.16–4.20 (m, 1H, morpholine), 3.98–4.03 (m, 1H, morpholine), 3.91–3.95 (m, 1H, morpholine), 3.83–3.88 (m, 1H, morpholine), 3.67–3.75 (m, 1H, morpholine), 3.51–3.58 (m, 1H, morpholine), 2.86 (s, 3H, CH_3), 1.97–2.36 (m, 4H, cyclopropyl), 1.45 (m, 3H, CH_3), NH not visible.

4-{[Diethyl(oxido)-λ⁶-sulfanylidene]amino}-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (45). Step a: 4-{[Diethyl(oxido)-λ⁶-sulfanylidene]amino}-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine. Under argon, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (XantPhos, 8 mg, 0.014 mmol) and tris(dibenzylideneacetone)dipalladium(0) (7 mg, 7 µmol) were added to a mixture of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (75 mg, 0.142 mmol), (*S*-ethylsulfonimidoyl)ethane (CAS 92523-32-5, 22 mg, 0.19 mmol), and Cs_2CO_3 (69 mg, 0.21 mmol) in toluene (0.67 mL). The mixture was stirred at 110 °C for 3 h. After cooling, the reaction mixture was diluted with EtOAc and washed with aqueous NaCl solution. The organic phase was filtered using a Whatman filter paper and concentrated to give 4-{[diethyl(oxido)-λ⁶-sulfanylidene]amino}-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine, which was used without further purification. MS (ESI+): m/z = 499.2 $[M+H]^+$. **Step b: 4-{[Diethyl(oxido)-λ⁶-sulfanylidene]amino}-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (45).** 2 N Aqueous HCl (0.14 mL, 0.29 mmol) was added to a solution of crude 4-{[diethyl(oxido)-λ⁶-sulfanylidene]amino}-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (71 mg) in MeOH (3.2 mL), and the reaction mixture was stirred at rt for 1 h. The mixture was basified by the addition of aqueous $NaHCO_3$ solution and extracted with EtOAc (2 ×). The combined organic phases were filtered using a Whatman filter paper and concentrated. The residue was purified by preparative HPLC (basic conditions) to give **45**. Yield: 25 mg (42% over two steps). LC-MS [Method 4]: R_t = 0.61 min. MS (ESI+): m/z = 415.3 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ = 13.34 (br s, 1H, pyrazole-NH), 8.28 (d, *J* = 5.07 Hz, 1H, naphthyridine), 7.87 (d, *J* = 5.32 Hz, 1H, naphthyridine), 7.59 (s, 1H, pyrazole), 7.33 (s, 1H, pyrazole), 6.83 (s, 1H, naphthyridine), 4.38 (br d, *J* = 4.82 Hz, 1H, morpholine), 3.94–4.08 (m, 2H, morpholine), 3.82 (d, *J* = 11.41 Hz, 1H, morpholine), 3.71 (dd, *J* = 2.53, 11.41 Hz, 1H, morpholine), 3.51–3.62 (m, 5H, morpholine, CH_2), 3.22–3.31 (m, 1H, morpholine), 1.30–1.36 (m, 6H, CH_3), 1.24 (d, *J* = 6.59 Hz, 3H, CH_3). HRMS (ESI, $[M+H]^+$): calcd for $C_{20}H_{27}N_6O_2S$, 415.1916; found, 415.1906.

2-[(3*R*)-3-Methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-4-(pyridin-3-ylethynyl)-1,7-naphthyridine (46). 2-[(3*R*)-3-Methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (75.0 mg, 0.142 mmol), copper(I) iodide (2.71 mg, 14.2 µmol), and triethylamine (400 µL, 2.8 mmol) were dissolved in MeCN (2.0 mL), degassed, and placed under a blanket of argon. 3-Ethynylpyridine (CAS 2510-23-8, 44.0 mg, 0.427 mmol) and bis(triphenylphosphine)palladium(II) dichloride (5.32 mg, 7.58 µmol) were added and the reaction mixture was stirred at 45 °C overnight. The mixture was filtered and washed with EtOAc. The filtrate was concentrated to dryness. The residue was dissolved in DCM and H_2O, and the aqueous phase was extracted with DCM (3 ×). The combined organic layers were dried (silicon filter) and concentrated under reduced pressure. The filtrate was purified by preparative HPLC (acidic conditions). The combined product fractions were concentrated under reduced pressure, dissolved in MeOH, and stirred with concd HCl overnight at rt. The reaction mixture was quenched with saturated $NaHCO_3$ solution and extracted with DCM (3 ×). The combined organic layers were dried (silicon filter) and concentrated under reduced pressure. The solid was purified by preparative HPLC (basic conditions) to give **46**. Yield: 9 mg (16%). LC-MS [Method 2]: R_t = 1.11 min. MS (ESI+): m/z =

397.2 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ = 13.43 (br s, 1H, pyrazole-NH), 8.97 (dd, *J* = 0.76, 2.03 Hz, 1H, pyridine), 8.70 (dd, *J* = 1.77, 4.82 Hz, 1H, pyridine), 8.46 (d, *J* = 5.32 Hz, 1H, pyridine), 8.20 (td, *J* = 1.93, 8.05 Hz, 1H, pyridine), 7.99 (d, *J* = 5.07 Hz, 1H, pyridine), 7.85 (s, 1H, pyridine), 7.60 (s, 1H, pyrazole), 7.53–7.60 (m, 1H, pyridine), 7.37 (br s, 1H, pyrazole), 4.62 (br d, *J* = 6.84 Hz, 1H, morpholine), 4.20 (br d, *J* = 13.43 Hz, 1H, morpholine), 4.06 (br d, *J* = 8.11 Hz, 1H, morpholine), 3.83 (br d, *J* = 11.41 Hz, 1H, morpholine), 3.72 (br dd, *J* = 2.66, 11.28 Hz, 1H, morpholine), 3.51–3.64 (m, 1H, morpholine), 3.35–3.41 (m, 1H, morpholine), 1.29 (br d, *J* = 6.84 Hz, 3H, CH_3).

4-(2-Methyl-1*H*-imidazol-1-yl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (47). 2-Methyl-1*H*-imidazole (CAS 693-98-1, 22.3 mg, 272 µmol) was dissolved in anhydrous DMF (1.5 mL), purged with argon, and sodium hydride (8.70 mg, 362 µmol) was added. The mixture was stirred for 10 min and 4-chloro-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (**43**, step b) (75.0 mg, 181 µmol) in DMF (1.5 mL) was added. The mixture was stirred for 4 h at 100 °C. Concd HCl (0.3 mL) was added and the mixture was stirred for 1 h at rt. The mixture was basified with solid $NaHCO_3$ and filtered. The filtrate was purified by preparative HPLC (basic conditions) and preparative TLC (silica gel, DCM/MeOH 9:1) to give **47**. Yield: 16 mg (24%). LC-MS [Method 4]: R_t = 0.57 min. MS (ESI+): *m/z* = 376.1 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6): δ = 13.48 (br s, 1H, pyrazole-NH), 8.39 (d, *J* = 5.27 Hz, 1H, naphthyridine), 7.74 (s, 1H, imidazole), 7.61–7.71 (m, 1H, pyrazole), 7.44–7.49 (m, 2H, imidazole, pyrazole), 7.11 (d, *J* = 1.13 Hz, 1H, naphthyridine), 6.98 (br d, *J* = 4.89 Hz, 1H, naphthyridine), 4.66 (br s, 1H, morpholine), 4.26 (br d, *J* = 12.80 Hz, 1H, morpholine), 4.08 (br d, *J* = 8.66 Hz, 1H, morpholine), 3.85 (br d, *J* = 11.29 Hz, 1H, morpholine), 3.71–3.78 (m, 1H, morpholine), 3.56–3.65 (m, 1H, morpholine), 3.38–3.44 (m, 1H, morpholine), 2.20 (s, 3H, CH_3), 1.33 (br d, *J* = 6.78 Hz, 3H, CH_3). HRMS (ESI, $[M+H]^+$): calcd for $C_{20}H_{22}N_7O$, 376.1886; found, 376.1877.

4-(Diethylphosphoryl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (48). Step a: 4-(Diethylphosphoryl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine. A mixture of 2-[(3*R*)-3-methylmorpholin-4-yl]-8-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-5-yl]-1,7-naphthyridin-4-yl trifluoromethanesulfonate (**67**) (150 mg, 0.28 mmol), diethylphosphane oxide (CAS 7215-33-0, 32 mg, 0.28 mmol), palladium(II) acetate (1.3 mg, 6 µmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with DCM; 3.5 mg, 6 µmol), and *N*,*N*-diisopropylethylamine (0.06 mL, 0.37 mmol) in DMF (1.2 mL) and DME (0.14 mL) was degassed with argon. Under argon, the reaction mixture was stirred at rt for 10 min and overnight at 110 °C. After cooling, the reaction mixture was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$ solution and saturated aqueous NaCl solution. The aqueous phase was saturated with solid NaCl and extracted with THF/EtOAc (1:1). The combined organic phases were filtered using a Whatman filter paper and then concentrated to give 4-(diethylphosphoryl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine, which was used in the next step without further purification. **Step b: 4-(Diethylphosphoryl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-(1*H*-pyrazol-5-yl)-1,7-naphthyridine (48).** A solution of crude 4-(diethylphosphoryl)-2-[(3*R*)-3-methylmorpholin-4-yl]-8-{1-[tetrahydro-2*H*-pyran-2-yl]-1*H*-pyrazol-5-yl}-1,7-naphthyridine (190 mg) in MeOH (1.8 mL) and 2 N aqueous HCl (0.45 mL) was stirred for 60 min at rt. Then, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (2 ×). The combined organic phases were filtered using a Whatman filter paper and concentrated to dryness. The residue was purified by preparative HPLC (basic conditions) to give **48**. Yield: 25 mg (22% over two steps). LC-MS [Method 4]: R_t = 0.53 min. MS (ESI+): *m/z* = 400.3 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ = 13.39 (br s, 1H, pyrazole-NH), 8.49 (d, *J* = 5.58 Hz, 1H, naphthyridine), 8.38 (d, *J* = 5.58 Hz, 1H, naphthyridine), 7.54–7.75 (m, 2H, naphthyridine, pyrazole), 7.36 (br s, 1H, pyrazole), 4.62 (br d, *J* = 4.56 Hz, 1H, morpholine), 4.17 (br d, *J* = 11.15 Hz, 1H, morpholine), 4.06 (br dd, *J* = 3.42, 11.28 Hz, 1H, morpholine), 3.84 (br d, *J* = 11.41 Hz, 1H, morpholine), 3.73 (dd, *J* = 2.79, 11.41 Hz, 1H, morpholine), 3.53–3.63 (m, 1H, morpholine), 3.37–3.39 (m, 1H, morpholine), 2.14–2.27 (m, 4H, CH_2), 1.27 (d, *J* = 6.84 Hz, 3H, CH_3), 0.96–1.08 (m, 6H, CH_3).

Pharmacology

Vectors for Expression of ATR/ATRIP in HEK293-6E Cells. The cDNAs encoding the protein sequences of full-length human ATR sequence (Q13535) with an N-terminally fused Flag tag as well as of the full-length human ATRIP (Q8WXE1) were optimized for expression in eukaryotic cells and synthesized by GeneArt technology at Life Technologies. Both cDNAs also encoded att site sequences at the 5'- and 3'-ends for subcloning into the following destination vectors using Gateway technology: pD-MamA (an in-house derivate of the vector pEAK from Edge BioSystems but with a human CMV promoter) which provides an N-terminal fusion of a GST tag to the integrated gene of interest; pD-MamB (an in-house derivative of pTT5 from NRCC, Y. Durocher) which provides an N-terminal fusion of a STREP II tag to the integrated gene. The cDNAs of ATR and ATR-DN were cloned into pD-MamA and the ATRIP-FL into pD-MamB.

Co-expression of ATR/ATRIP by Transient Transfection in HEK293-6E Cells. For transient transfection of HEK293-6E cell suspension, a Biostat CultiBag bioreactor with 5 L culture volume (starting volume) in a 20 L culture bag was used. The cells were cultured in F17 medium (Gibco, Invitrogen, Cat 05-0092DK) with the following supplements: Pluronic F68 (10 mL of 10% solution/L, Gibco, 24040), GlutaMAX (20 mL of 100 × solution/L), L-alanylglutamine (200 mM, Invitrogen, 25030), G418 (final concentration 25 pg/mL, PAA, P02-012). The applied culture conditions were 37 °C, rocking rate 18 rpm, pH 7.0, pO_2 55%. At the day of transfection, the cell culture had reached a cell density of 1.6 × 10 cells/mL and a viability of 99%. For preparation of the transfection

solution, to 500 mL F17 medium (without the supplements), 4 mg of the ATR-encoding plasmid, 1 mg of the ATRIP-encoding plasmid, and 10 mg PEI (polyethylenimine, linear, Polysciences 23966, as 1 mg/mL stock solution) were sequentially added, carefully mixed, and incubated at rt for 15 min. This transfection solution was then added to the 5 L cell culture in the culture bag. This cell culture was incubated for 5 h and afterwards 5 L of F17 medium with the mentioned supplements was added and the rocking rate increased to 19 rpm. 48 h after transfection, the cells were harvested by centrifugation (30 min, 1000×g, 15 °C) and the cell pellets stored at –80 °C.

Purification. Purification of the ATR (Flag tag)/ATRIP (STREP tag) complex was achieved by affinity chromatography using anti-FLAG-resin (Sigma, A220). Cells were harvested by centrifugation (4000×g) and lysed in buffer A (50 mM Tris HCl pH 7.5, 150 mM NaCl, 5% glycerol, 1 mM Na_3VO_4, 1 mM NaF, 10 mM β-glycerophosphate, 1% Tween 20, 0.1% NP40, complete with EDTA) for 1 h at 4 °C. The supernatant (20000×g) was then bound to Flag-agarose and eluted after several washing steps using buffer B (50 mM Tris HCl pH 7.4, 150 mM NaCl, 10% glycerin, 200 pg/mL Flag peptide from Sigma, F3290). Elution fractions were aliquoted and shock-frozen using liquid nitrogen. The final concentration of ATR in the final preparation was 250 pg/mL calculated densitometrically using BSA as a standard in a Coomassie stained gel. The yield of co-purified ATRIP was far below a 1:1 ratio with ATR, but was essential for ATR activity.

Biochemical Assay for ATR. A time-resolved fluorescence resonance energy transfer (TR-FRET) based ATR binding competition assay was used to determine the affinity of compounds to ATR using 40 nM (= KD) of the fluorescent 5-TAMRA-labeled tracer 3',6'-bis(dimethylamino)-*N*-(4-{[2-(1*H*-indol-4-yl)-6-(morpholin-4-yl)pyrimidin-4-yl]amino}butyl)-3-oxo-3*H*-spiro[2-benzofuran-1,9'-xanthene]-5-carboxamide, an ATP-competitive ATR inhibitor (synthesized at Bayer AG, Germany; for further details see ref 28).

Biochemical Assay for ATM. ATM phosphorylates biotinylated peptide derived from Rad17. Detection of phosphorylated peptide is achieved by HTRF using Streptavidin-XL, anti-phospho Rad17 antibody and anti-rabbit-IgG-Terbium. Excitation of Europium with 337 nm light results in emission of fluorescent light with 620 nm. In case a tetrameric detection complex has formed, part of the energy will be transferred to the Streptavidin-XL fluorophore that itself emits light of 665 nm. Unphosphorylated peptide does not give rise to light emission at 665 nm, because no FRET-competent detection complex can be formed. N-terminal FLAG™-tagged recombinant ATM (full-length), expressed in HEK 293-6E cells and purified by anti-FLAG™ agarose affinity chromatography, was used as enzyme. As substrate for the kinase reaction biotinylated peptide biotin-PEG2-SVEPPLSQETFSD (C-terminus in amide form) was used which can be purchased e. g. from Biosyntan (Berlin-Buch, Germany). For the assay 50 nl of a 100-fold concentrated solution of the test compound in DMSO was pipetted into either a black low volume 384-well microtiter plate or a black 1536-well microtiter plate (both Greiner Bio-One, Frickenhausen, Germany), 2 µl of a solution ATM in aqueous assay buffer [50 mM HEPES pH 7.0, 10 mM magnesium chloride, 50 mM potassium chloride, 1 mM dithiothreitol, 0.1 % (w/v) bovine serum albumin, 0.01% (v/v) Igepal CA-630 (Sigma-Aldrich, #I3021)] were added and the mixture was incubated for 15 min at 22 °C to allow pre-binding of the test compounds to the enzyme before the start of the kinase reaction. Then the kinase reaction was started by the addition of 3 µl of a solution of adenosine-tri-phosphate (ATP, 16.7 µM => final conc. in the 5 µl assay volume is 10 µM) and substrate (1.67 µM => final conc. in the 5 µl assay volume is 1 µM) in assay buffer and the resulting mixture was incubated for a reaction time of 90 min at 22 °C. The concentration of ATM was adjusted depending of the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range, a typical concentration was 15 nM. The reaction was stopped by the addition of 3 µl of a solution of TR-FRET detection reagents (0.33 µM streptavidine-XL665 [Cisbio Bioassays, Codolet, France, # 610SAXLG] and 3.33 nM anti-phospho p53(Ser15)-K [Cisbio Bioassays, # 61P08KAY]) in an aqueous EDTA-solution (166.7 mM EDTA, 0.2 % (w/v) bovine serum albumin, 800 mM potassium fluoride in 100 mM HEPES pH 7.5). The resulting mixture was incubated 1 h at 22 °C to allow the formation of complex between the phosphorylated biotinylated peptide and the detection reagents. Subsequently the amount of phosphorylated substrate was evaluated by measurement of the resonance energy transfer from the Eu-cryptate to the streptavidine-XL. Therefore, the fluorescence emissions at 620 nm and 665 nm after excitation at 350 nm was measured in a TR-FRET reader, e.g. a Pherastar FS (BMG Labtechnologies, Offenburg, Germany) or a Viewlux (Perkin-Elmer). The ratio of the emissions at 665 nm and at 622 nm was taken as the measure for the amount of phosphorylated substrate. The data were normalised (enzyme reaction without inhibitor = 0 % inhibition, all other assay components but no enzyme = 100 % inhibition). Usually the test compounds were tested on the same microtiterplate in 11 different concentrations in the range of 20 µM to 0.07 nM (20 µM, 5.7 µM, 1.6 µM, 0.47 µM, 0.13 µM, 38 nM, 11 nM, 3.1 nM, 0.9 nM, 0.25 nM and 0.07 nM, the dilution series prepared separately before the assay on the level of the 100-fold concentrated solutions in DMSO by serial dilutions, exact concentrations may vary depending pipettors used) in duplicate values for each concentration and IC50 values were calculated using Genedata Screener™ software.

Phospho-H2AX Assay. Phospho-Ser139 histone H2AX (also known as γH2AX) represents a cellular early marker for the DDR. In particular, H2AX is phosphorylated by ATR upon DNA replication stress representing a suitable readout for activity against ATR if measured after short-term incubation.[41,42] HT-29 human colorectal tumor cells (ACC-299), originally obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig), were plated out at a density of 12000 cells/measurement point in a black-walled, clear-bottomed 96-well multititer plate in 100 µL of growth medium

[DMEM/Ham's F12, 2 mM L-glutamine, 10% fetal calf serum (FCS)] and cultured for 24 h at 37°C. Test compounds (final concentration 1 nM–10 μM respectively DMSO for controls, were added using a Tecan HP D300 digital dispenser (Tecan Group Ltd., Switzerland), followed by the addition of 100 μL of hydroxyurea (final concentration 2.5 mM) in growth medium. Samples were incubated for 30 min at 37 °C, then the growth medium was aspirated, and the cells were fixed by adding ice-cold MeOH for 15 min. After fixation, the cells were washed once with PBS, followed by incubation with Odyssey blocking buffer (Li-Cor Biosciences, USA) for 1 h at 22 °C. Cells were then labeled with mouse anti-phospho-histone H2A.X (Ser139) antibody (1:500, clone JBW301, Merck Millipore, Germany) in blocking buffer for 1 h at 22 °C or overnight at 4 °C. Cells were washed with PBS (3 ×) and the secondary antibody, Alexa Fluor 488 [1:500, Fluor 488-linked anti-mouse-IgG antibody (donkey), Thermo Fisher Scientific, USA], was applied for 1 h at 22 °C. Subsequently, cells were washed three times with PBS and fluorescence was measured with an acumen Explorer laser scanning cytometer (TTP Labtech, UK).

The change (%) in the level of γH2AX was calculated by normalizing the measured values to the fluorescence values of untreated control wells (= 0%) and the fluorescence of the induced, DMSO-treated controls without test compounds (= 100%). Half-maximal γH2AX inhibition values (IC_{50}) were determined using a four-parameter logistic fit.

Cellular mTOR Assay. PI3K/AKT/mTOR signaling pathway activity was investigated in MCF7 breast cancer cells (HTB-22, ATCC) by quantification of the phosphorylation of AKT at Ser473 as a direct mTOR phosphorylation site. MCF7 cells were thawed, washed, and resuspended in Opti-MEM (Gibco) at 1.0×10^6 cells/mL. First, 50 nL aliquots of test compounds (final concentration as given below, DMSO as control) were transferred to a 384-well microtiter plate using a Hummingbird liquid handler. Next, 3 μL of cell suspension (assay medium as control) was added to the wells using a Multidrop dispenser (Thermo Fisher Scientific). Samples were incubated for 30 min at 37 °C/5% CO_2. For the detection and quantification of phosphorylated AKT (Ser473), a commercial HTRF detection kit [phospho-AKT (Ser473) assay kit, Cisbio, France] was used, according to the manufacturer's instructions. Fluorescence (excitation 337 nm, emission 1 620 nm, emission 2 665 nm) was measured with a PHERAstar plate reader. The ratiometric data (665 nm/620 nm) were normalized to the vehicle (DMSO = 0% inhibition) and inhibitor (medium only = 100% inhibition) controls. Compounds were tested in duplicate at 11 concentrations (20 μM, 5.7 μM, 1.6 μM, 0.47 μM, 0.13 μM, 38 nM, 11 nM, 3.1 nM, 0.89 nM, 0.25 nM, and 0.073 nM). IC_{50} values were calculated using a four-parameter logistic fit (Screener software package, Genedata, Switzerland).

Proliferation Assays. HT-29 (ACC-299) and LoVo (ACC-350) human colorectal tumor cells and SU-DHL-8 (ACC-573) human B-cell lymphoma cells were originally obtained from the DSMZ. HT-29 and LoVo cells were plated out at a density of 2000 (HT-29) or 3000 (LoVo) cells/measurement point in a 96-well microtiter plate in 200 μL of growth medium (DMEM/Ham's F12, 2 mM L-glutamine, 10% FCS). Cells were allowed to adhere for 24 h, and then the test compounds were added at various concentrations (0 μM, and in the range 0.001–10 μM; final concentration of the solvent DMSO 0.1%) using a Tecan HP D300 digital dispenser. After continuous incubation for 4 d at 37°C the cells were fixed with glutaraldehyde, stained with crystal violet,[43] and the absorbance was recorded at 595 nm using Tecan Sunrise equipment (Tecan Group Ltd., Switzerland). All measurements were performed in quadruplicate. The values were normalized to the absorbance of solvent-treated cells (100%), and the absorbance of a reference plate which was fixed at the time point of compound application (0%). Half-maximal growth inhibition values (IC_{50}) were determined using a four-parameter logistic fit.

Viability (corresponding to cell number) of non-adherent SU-DHL-8 cells was determined using the CellTiter-Glo (CTG) cell viability assay (Promega, Madison, WI, USA), according to the manufacturer's instructions. Briefly, cells were plated out at 4000 cells/measurement point in a 96-well multititer plate in 100 μL growth medium (RMPI-1640, 2 mM L-glutamine, 10% FCS), cultured for 24 h at 37 °C, and then incubated with different concentrations of test compounds (0.001–10 μM) for 4 d. Then CTG solution was added and, after 10 min, luminescence was measured with a VICTOR V Multilabel plate reader (PerkinElmer). The change in cell viability was calculated by normalization with respect to the cell number at the beginning of the treatment and to the cell number of the untreated control group. Half-maximal growth inhibition values (IC_{50}) were determined using a four-parameter logistic fit.

Pharmacokinetic Studies

Caco-2 Permeability Assay. Caco-2 cells (purchased from the DSMZ) were seeded at a density of 2.5×10^5 cells/well on 24-well insert plates, 0.4 μm pore size, 0.3 cm^2 (Costar) and grown for 13–15 d in DMEM medium supplemented with 10% FCS, 1% GlutaMAX (100 ×, Gibco), 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco), and 1% non-essential amino acids (100 ×). Cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere. Medium was changed every 2–3 d.

The bidirectional transport assay for the evaluation of Caco-2 permeability was undertaken in 24-well insert plates using a robotic system (Tecan). Before the assay was run, the culture medium was replaced by transport medium (FCS-free HEPES carbonate transport buffer pH 7.2). For the assessment of monolayer integrity, the transepithelial electrical resistance (TEER) was measured. Only monolayers with a TEER of at least 400 Ω*cm^2 were used. Test compounds were predissolved in DMSO and added either to the apical or basolateral compartment at a final concentration of 2 μM. Evaluation was done in triplicate. Before and after incubation for 2 h at 37 °C, samples were taken from both compartments and analyzed, after precipitation with MeOH, by LC-MS/MS. The apparent permeability coefficient (P_{app}) was calculated both for the apical to basolateral (A→B) and the basolateral to apical (B→A) direction using following equation: $P_{app} = (V_r/P_0)(1/S)(P_2/t)$, where V_r is the volume of medium in the

receiver chamber, P_0 is the measured peak area of the test compound in the donor chamber at t = 0, S is the surface area of the monolayer, P_2 is the measured peak area of the test compound in the acceptor chamber after incubation for 2 h, and t is the incubation time. The efflux ratio basolateral (B) to apical (A) was calculated by dividing P_{app} B–A by P_{app} A–B.

In Vitro Metabolic Stability in Liver Microsomes. The in vitro metabolic stability of test compounds was determined by incubation at 1 μM in a suspension of liver microsomes in 100 mM phosphate buffer pH 7.4 ($NaH_2PO_4 \cdot H_2O$ + $Na_2HPO_4 \cdot 2H_2O$) and at a protein concentration of 0.5 mg/mL at 37 °C. The microsomes were activated by adding a cofactor mix containing 8 mM glucose-6-phosphate, 4 mM $MgCl_2$, 0.5 mM NADP, and 1 IU/mL glucose-6-phosphate dehydrogenase in phosphate buffer pH 7.4. The metabolic assay was started shortly afterwards by adding the test compound to the incubation at a final volume of 1 mL. Organic solvent in the incubations was limited to ≤0.01% DMSO and ≤1% MeCN. During incubation, the microsomal suspensions were continuously shaken at 580 rpm and aliquots were taken at 2, 8, 16, 30, 45, and 60 min, to which an equal volume of cold MeOH was immediately added. Samples were frozen at –20 °C overnight and after thawing subsequently centrifuged for 15 min at 3000 rpm. The supernatant was analyzed with an Agilent 1200 HPLC system with LC-MS/MS detection. The half-life of a test compound was determined from the concentration–time plot. From the half-life, the intrinsic clearances and the hepatic in vivo blood clearance (CL) and maximal oral bioavailability (F_{max}) were calculated using the 'well-stirred' liver model[44] together with the additional parameters liver blood flow, specific liver weight, and microsomal protein content. The following parameter values were used: liver blood flow: 5.4, 4.2, 2.1, and 1.32 L/h/kg for mouse, rat, dog, and human, respectively. Specific liver weight: 43, 32, 39, and 21 g/kg body weight for mouse, rat, dog, and human, respectively. Microsomal protein content: 40 mg/g for all species.

In Vitro Metabolic Stability in Hepatocytes. Hepatocytes from Han/Wistar rats were isolated via a two-step perfusion method. After perfusion, the liver was carefully removed from the rat: the liver capsule was opened and the hepatocytes were gently shaken out into a Petri dish with ice-cold Williams' medium E (WME). The resulting cell suspension was filtered through sterile gauze into 50 mL Falcon tubes and centrifuged at 50×g for 3 min at rt. The cell pellet was resuspended in WME (30 mL) and centrifuged twice through a Percoll gradient at 100×g. The hepatocytes were washed again with WME and resuspended in medium containing 5% FCS. Cell viability was determined by trypan blue exclusion. For the metabolic stability assay, liver cells were distributed in WME containing 5% FCS to glass vials at a density of 1.0×10^6 vital cells/mL. The test compound was added to a final concentration of 1 μM. During incubation at 37° C, the hepatocyte suspensions were continuously shaken at 580 rpm and aliquots were taken at 2, 8, 16, 30, 45, and 90 min, to which an equal volume of cold MeCN was immediately added. Samples were frozen at –20 °C overnight and after thawing subsequently centrifuged for 15 min at 3000 rpm. The supernatant was analyzed with an Agilent 1200 HPLC system with LC-MS/MS detection. The half-life of a test compound was determined from the concentration–time plot. From the half-life, the intrinsic clearances and the hepatic in vivo blood clearance (CL) and maximal oral bioavailability (F_{max}) were calculated using the 'well-stirred' liver model[44] together with the additional parameters liver blood flow, specific liver weight, and amount of liver cells in vivo and in vitro. The same parameters for liver blood flow and specific liver weight as given above were used; liver cells in vivo: 1.1×10^8 cells/g liver, liver cells in vitro: 1.0×10^6/mL.

Plasma Protein Binding. HT equilibrium dialysis was used to determine the plasma protein binding, as outlined by Banker et al.[45] In brief, a semipermeable membrane separated the plasma from the buffer compartment. The test compound was added to the plasma side at a concentration of 3 μM and incubated for 7 h at 37 °C and 5% CO_2 with 99% humidity and moderate shaking. 10 μL of the plasma side was transferred to a deep-well plate containing 90 μL of buffer and 90 μL of the buffer side was added to 10 μL of blank plasma. All samples were precipitated with 400 μL ice-cold MeOH and frozen overnight at –20 °C. After thawing and mixing, the samples were centrifuged for 10 min at 3000 rpm. The supernatant was transferred to a 96-well plate, and LC-MS/MS measurements were undertaken. From the quotient of buffer and plasma concentration, the unbound fraction (f_u) was calculated.

CYP Inhibition Assay. The inhibitory potency of test compounds towards cytochrome P450 dependent metabolic pathways was determined in human liver microsomes (pool) by applying individual CYP isoform selective standard probes (CYP1A2, phenacetin; CYP2A6, coumarin; CYP2B6, bupropion; CYP2C8, amodiaquine; CYP2C9, diclofenac; CYP2C19, (*S*)-mephenytoin; CYP2D6, dextromethorphan; CYP2E1, chlorzoxazone; CYP3A4, midazolam, testosterone). Reference inhibitors were included as positive controls. Incubation conditions (protein and substrate concentration, incubation time) were optimized with regard to linearity of metabolite formation. Assays were processed in 96-well plates at 37 °C using a Freedom EVO Workstation (Tecan, Crailsheim, Germany). Reactions were stopped by addition of 100 μL acetonitrile containing the respective stable labeled internal standard. Precipitated proteins were removed by centrifugation of the well plate (1800 rcf, 10 min, 10°C). Metabolite formation was quantified by LC-MS/MS analysis (QTRAP 6500 system, Sciex, Canada), followed by inhibition evaluation and IC_{50} calculation.

CYP Induction Assay. To evaluate the CYP induction potential in vitro, cultured human hepatocytes from three separate livers were treated once daily for three consecutive days with vehicle control, one of eight concentrations of test compound, or known human CYP inducers (e.g., omeprazole, phenobarbital, rifampin). After treatment, the cells were incubated in situ with the

appropriate marker substrates for the analysis of CYP3A4, CYP2B6, and CYP1A2 activity by LC-MS/MS. Following the in situ incubation, the same hepatocytes from the same treatment groups were harvested for RNA isolation and analyzed by qRT-PCR to assess the effect of test compound on CYP1A2, CYP2B6, and CYP3A4 mRNA expression levels.

In Vivo Pharmacokinetic Studies. All animal experiments were conducted in accordance with the German Animal Welfare Law and were approved by local authorities. For in vivo pharmacokinetic (PK) experiments, test compounds were administered to rodents (male Wistar rats or female NMRI mice) or female beagle dogs intravenously at doses of 0.3 to 0.5 mg/kg and intragastrically at doses of 0.6 to 1 mg/kg formulated as solutions using solubilizers such as PEG400 in well-tolerated amounts. For PK after intravenous (iv) administration, test compounds were given as iv bolus and blood samples were taken at 2 min, 8 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h after dosing. For PK after intragastral (ig) administration, test compounds were given ig to fasted rats and blood samples were taken at 5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h after dosing. Blood was collected into lithium heparin tubes (Monovette, Sarstedt) and centrifuged for 15 min at 3000 rpm. An aliquot of 100 µL from the supernatant (plasma) was taken and precipitated by the addition of cold MeCN (400 µL). Samples were frozen at −20 °C overnight, and subsequently thawed and centrifuged at 3000 rpm, 4 °C for 20 min. Aliquots of the supernatants were analyzed using an Agilent 1200 HPLC system with LC-MS/MS detection. PK parameters were based on the plasma concentration and time data and calculated (e.g., using the linear–log trapezoidal rule for AUC estimation) using an Excel-based program. PK parameters derived from concentration–time profiles after iv administration: CL_{plasma}: total plasma clearance of test compound (in L/h/kg), CL_{blood}: total blood clearance of test compound: $CL_{plasma}*C_p/C_b$ (in L/h/kg) with C_p/C_b being the ratio of concentrations in plasma and blood. PK parameters calculated from concentration–time profiles after ig administration: C_{max}: maximal plasma concentration (in mg/L), $C_{max,norm}$: C_{max} divided by the administered dose (in kg/L), T_{max}: time point at which C_{max} was observed (in h). Parameters calculated from both iv and ig concentration–time profiles: AUC_{norm}: area under the concentration–time curve from $t = 0$ to infinity (extrapolated) divided by the administered dose (in kg*h/L), $AUC_{(0-t\ last)norm}$: area under the concentration–time curve from $t = 0$ to the last time point for which plasma concentrations could be measured divided by the administered dose (in kg*h/L), $t_{1/2}$: apparent half-life (in h), F: oral bioavailability: AUC_{norm} after ig administration divided by AUC_{norm} after iv administration (in %).

Safety Assay

Automated hERG K^+ Current Voltage-Clamp Assay. The hERG K^+ current assay is based on a recombinant HEK293 cell line with stable expression of the *KCNH2 (HERG)* gene.[46,47] The cells were cultured using a humidified incubator (37 °C, 5% CO_2) and a standard culture medium [MEM with Earle's salts and L-glutamine, 10% non-inactivated FCS, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, penicillin/streptomycin (50 µg/mL each), 0.4 mg/mL Geneticin]. Approximately 0.5–8 h following cell dissociation, the cells were investigated by means of the 'whole-cell voltage-clamp' technique[48] in an automated 8-channel system (Patchliner; Nanion Technologies, Munich, Germany) with PatchControlHT software (Nanion) to control the Patchliner system and to handle data acquisition and analysis. Voltage-clamp control was provided by two EPC 10 Quadro amplifiers under control of PatchMasterPro software (both: HEKA Elektronik, Lambrecht, Germany) and with NPC-16 medium resistance (~2 MΩ) chips (Nanion) serving as planar substrate at rt (22–24 °C). NPC-16 chips were filled with intra- and extracellular solution [intracellular solution: 10 mM NaCl, 50 mM KCl, 60 mM KF, 20 mM EGTA, 10 mM HEPES pH 7.2 (KOH); extracellular solution: 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES pH 7.4 (NaOH)] and with cell suspension. After formation of a GΩ seal and entering whole-cell mode (including several automated quality control steps), the cell membrane was clamped to the holding potential (−80 mV). Following an activating clamp step (+20 mV, 1000 ms), exclusively hERG-mediated inward tail currents were elicited by hyperpolarizing voltage steps from +20 to −120 mV (duration 500 ms); this clamp protocol was repeated every 12 s.[49] After an initial stabilization phase (5–6 min), test compounds were added either as a single concentration (10 µM) or in ascending concentrations (0.1, 1, and 10 µM; 5–6 min per concentration), followed by several washout steps. Effects of test compounds were quantified by analyzing the amplitude of the hERG-mediated inward tail currents (in % of predrug control) as a function of test compound concentration (Igor Pro Software). Mean concentration–response data were fitted with a standard sigmoidal four-parameter logistic equation of the form: Y=Bottom+(Top−Bottom)/(1+10^((LogIC$_{50}$−X)*HillSlope)), where Y is the current inhibition (in % of predrug control), X is the logarithm of drug concentration, and IC_{50} is the drug concentration producing half-maximal current inhibition, and using the following constraints: Top = 100%, Bottom = 0%. No curve fitting was performed in cases with an obvious lack of a concentration-dependent current inhibition and/or a too small effect size (ca. ≤ 20%).

Physicochemical Assays

Aqueous Solubility of Compound–DMSO Solutions. Aqueous solubility at pH 6.5 was determined by an orientating HTS method.[50] Test compounds were applied as 1 mM DMSO solutions. After addition of buffer pH 6.5, solutions were shaken for 24 h at rt. Undissolved material was removed by filtration. The compound dissolved in the filtrate was quantified by HPLC-MS/MS.

LogD Measurement. LogD values at pH 7.5 were recorded using an indirect method for determining hydrophobicity constants by reversed-phase HPLC.[51] A homologous series of *n*-alkan-2-ones (C_3–C_{16}, 0.02 M in MeCN) was used for calibration. Test compounds were applied as 0.67 mM DMSO stock solutions in MeCN/H_2O (1:1). The lipophilicity of compounds was then assessed by comparison to the calibration curve.

In Vivo Pharmacology Studies

All animal experiments were conducted in accordance with the German Animal Welfare Law and were approved by local authorities. Experiments were initiated after an acclimatization period of at least 7 d. Mice were kept in a 12 h light/dark cycle, food and water were available *ad libitum*, and the housing temperature was 23 °C.

BAY 1895344 was formulated in PEG400/EtOH/H_2O (60:10:30), carboplatin in 0.9% NaCl, and olaparib in 10% HPβCD [(2-hydroxypropyl)-β-cyclodextrin] in 0.9% NaCl. The oral and intraperitoneal application volume was 10 mL/kg and the time interval between two applications per day was 6–7 h.

Assessment of antitumor Efficacy. Tumor response was assessed by measuring tumor area (length × width) using a caliper. Animal body weight was monitored as a measure of treatment-related toxicity. Tumor area and body weight were determined 2–3 times per week. Changes in body weight throughout the study compared to initial body weight at treatment start were considered a measure of treatment-related toxicity (>10% = critical, treatment on hold until recovery; >20% = toxic, termination). Mice were euthanized when showing signs of toxicity (>20% body weight loss), or when tumors reached a maximum size of 225 mm^2. Tumor growth inhibition is presented as the T/C (treatment-to-control) ratio, calculated with tumor areas or tumor volume at study end. Relative tumor growth inhibition based on tumor area ($T/C_{rel.\ area}$) was calculated using the formula [(tumor area of treatment group at day x) – (tumor area of treatment group at day before first treatment)] / [(tumor area of vehicle group at day x) – (tumor area of vehicle group at day before first treatment)]. Tumor volume was calculated using the equation (longest diameter x shortest diameter^2)/2). Relative tumor growth inhibition based on tumor volume (T/C_{volume}) was calculated using the formula [(tumor volume of treatment group at day x) – (tumor volume of treatment group at day before first treatment)] / [(tumor volume of vehicle group at day x) – (tumor volume of vehicle group at day before first treatment)].

In Vivo Efficacy of Monotherapy in SU-DHL-8 Tumor Xenografts. The antitumor efficacy and tolerability of BAY 1895344 were evaluated in the cell line derived human germinal center B-cell-like diffuse large B-cell lymphoma (GCB-DLBCL) model SU-DHL-8 (DSMZ, ACC-573; mutations: ATM^{K1964E}, $FANCD2^{R1165Q}$, $FEN1^{L190V}$, $REV3L^{V1004E}$, $PRKDC^{fs}$, $MYC^{p72S,Q10H}$, $BRAF^{T599TT}$, $CDC7^{K42N}$, $TOP2A^{G1197E}$, $TP53^{R249G,Y234N}$) growing as xenograft in female C.B-17 SCID mice (Taconic M&B). SU-DHL-8 cells from mid-log phase (70%) cultures were harvested and inoculated subcutaneously by injection of 5 × 10^6 cells in 100 µL of cell suspension (in 100% Matrigel) into the flank of mice. When tumors reached a size of 26 mm^2, mice were randomized into treatment and control groups (n = 9 mice/group), and treatment was started. For assessment of monotherapy efficacy, BAY 1895344 was applied orally (po) at 50 mg/kg twice daily (b.i.d.) for 3 days on and 4 days off.

In Vivo Efficacy of Combination Therapy with Carboplatin in CR5038 Tumor Xenografts. The antitumor efficacy and tolerability of BAY 1895344 were evaluated in the patient-derived human CRC model CR5038 (Crown Bioscience, San Diego) growing as xenograft in female NOD/SCID mice (Envigo Laboratories, The Netherlands). Frozen tumor material was thawed and washed with PBS. Cells were resuspended at a concentration of 98000 viable cells/100 µL in 50% PBS/50% Matrigel. 200 µL of cell suspension was injected subcutaneously into the flank of naïve mice. When tumors reached a size of 184 mm^3, mice were randomized into treatment and control groups (n = 10 mice/group), and treatment was started. For assessment of combination therapy efficacy, BAY 1895344 was applied orally (po) at 10 or 20 mg/kg once daily (q.d.) for 2 days on and 5 days off, and carboplatin was applied intraperitoneally (ip) at 40 mg/kg once (q.d.) every week (1 day on/6 days off), both as monotherapy or in combination treatment.

In Vivo Efficacy of Combination Therapy with Olaparib in SUM149 Tumor Xenografts. The antitumor efficacy and tolerability of BAY 1895344 were evaluated in the cell line derived human triple-negative breast cancer (TNBC) model SUM149 (ATCC; $FANCD2^{K50N}$, $FANCI^{D515H}$, $BRCA1^{N723X}$, $TP53^{M237I}$) growing as xenograft in female nude mice (Janvier Labs, France). SUM149 cells from mid-log phase (70%) cultures were harvested and inoculated subcutaneously by injection of 5 × 10^6 cells in 100 µL of cell suspension (in 100% Matrigel) into the flank of mice. When tumors reached a size of 30 mm^2, mice were randomized into treatment and control groups (n = 10 mice/group), and treatment was started. For assessment of combination therapy efficacy, BAY 1895344 was applied po at 20 mg/kg twice daily (b.i.d.) for 3 days on and 4 days off, and olaparib was applied at 50 mg/kg ip once every day (q.d.), both as monotherapy or in combination treatment.

Statistical Analyses. Data are expressed as means ± SEM. Statistical analysis was performed using SigmaStat software. A one-way analysis of variance was performed and differences to the control or single-agent treatment groups were compared by a pairwise comparison procedure (Dunn's method). To evaluate the cooperativity of the combination of compound A with compound B, expected additivity was calculated according to the Bliss model (C=A+B–A*B; where C is the expected T/C ratio of the combination of drug A and drug B if they act additively, A is the T/C ratio of drug A, and B is the T/C ratio of drug B). Excess >10% over the expected additive effect is assumed to indicate synergism of the two drugs; less than 10% of the expected additive effect is assumed to indicate antagonism.[52]

ASSOCIATED CONTENT

Supporting Information.
Kinase selectivity panel data for BAY 1895344
LC-MS methods for determination of purity
Molecular formula strings
This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written with contributions from all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): U.L., L.W., A.M.W., J.L., P.L., H.B., G.S., U.B., K.D., M.S., M.K., K.E., F.B., B.B., W.B., D.M., S.J.H, U.E., J.G.-G., C.S., G.D., D.M., and F.v.N. are or have been employees and stockholders of Bayer AG.

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ABBREVIATIONS

DDR, DNA damage response; ATR, ataxia telangiectasia and Rad3-related; ATRIP, ATR interacting protein; ATM, ataxia telangiectasia mutated; DNA-PK, DNA-dependent protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PIKK, phosphatidylinositol 3-kinase-related kinase; PARP, poly (ADP-ribose) polymerase; CRC, colorectal cancer; GCB-DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma.

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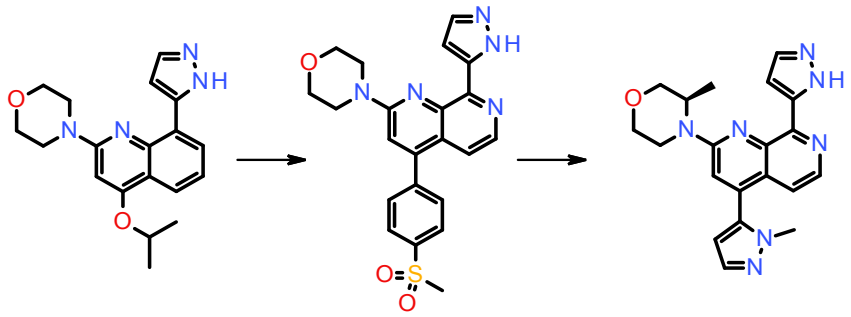

2
IC_{50} ATR: 5050 nM

BAY-937
IC_{50} ATR: 59 nM
vs mTOR: 13
F, rat, in vivo: 14%

BAY 1895344
IC_{50} ATR: 7 nM
vs. mTOR: >60
F, rat, in vivo: 87%