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Article

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Synthesis and structure activity relationships of DCLK1 kinase inhibitors based on a 5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one scaffold

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KEYWORDS: DCLK1, DCLK2, doublecortin-like kinase, kinase inhibitor, pancreatic cancer.

ABSTRACT: Doublecortin-like kinase 1 (DCLK1) is a serine/threonine kinase that is overexpressed in gastrointestinal cancers, including esophageal, gastric, colorectal and pancreatic cancers. DCLK1 is also used as a marker of tuft cells, which regulate Type II immunity in the gut. However, the substrates and functions of DCLK1 are understudied. We recently described the first selective DCLK1/ 2 inhibitor, DCLK1-IN-1, developed to aid the functional characterization of this important kinase. Here we describe the synthesis and structure activity relationships of 5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one DCLK1 inhibitors, resulting in the identification of DCLK1-IN-1.

INTRODUCTION

Doublecortin-like kinase 1 (DCLK1) was originally identified as a regulator of microtubule polymerization in migrating neurons and for these functions, the kinase domain has been shown to be largely dispensable. However, DCLK1 is also expressed in gastrointestinal cancers, including pancreatic cancer. In models of pancreatic ductal adenocarcinomas, DCLK1 has been reported to be expressed downstream of K-Ras activation, and as a putative K-Ras^{G12D} interacting protein.[1] The 5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one scaffold has been utilized to develop inhibitors for several kinases[2], including TNK2[3], PI3K-δ/γ[4], Aurora A/B/C[5] [6], LRRK2[7] and ERK5[8] [9]. The LRRK2 inhibitor LRRK2-IN-1[7] and the ERK5 inhibitors XMD8-92 and XMD8-85[10] display off-target activity against DCLK1 in profiling experiments (Figure 1). Confirmation of this DCLK1 affinity in follow-up kinase assays has been reported for LRRK2-IN-1 and XMD8-92[11] [12]. Recently, these molecules have also been shown to inhibit the bromodomains of BRD4, an important transcriptional regulator and cancer target.[9] [13]

LRRK2-IN-1 and XMD8-92 have been used to study the role of DCLK1 kinase activity in colorectal and pancreatic cancer[11,12,1]. However, the multi-targeted nature of these compounds makes them unsuitable for use as chemical probes for investigating the role of DCLK1 in cancer. We sought to develop 5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-ones with improved selectivity for DCLK1, by reducing affinity for ERK5, LRRK2, and the bromodomains of BRD4.

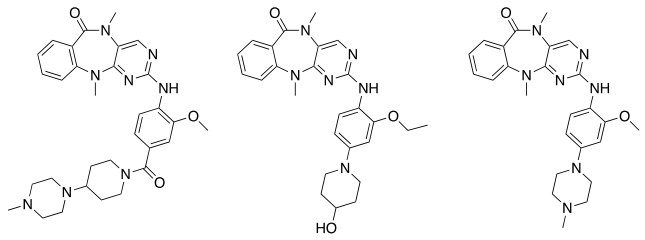
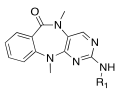


Figure 1. 5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-ones that inhibit DCLK1 kinase. Discovrx KINOME*scan* assay results for DCLK1 listed below. % control = percent of the control sample signal remaining upon treatment with 10 μM of compound.



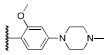
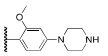
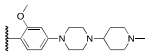
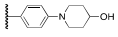
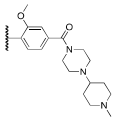
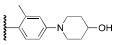
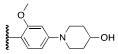
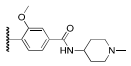
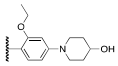
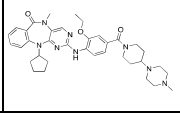
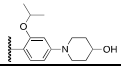
Compound ID	R_1	IC_{50} (nM) DCLK1	ATP conc (μM)	ERK5 % inhibition	IC_{50} (nM) LRRK2	Compound ID	R_1	IC_{50} (nM) DCLK1	ATP conc (μM)	ERK5 % inhibition	IC_{50} (nM) LRRK2
XMD8-85		11 ± 3	15	96	29 ± 3	XMD17-86		2 ± 3	15	98	ND
XMD13-43		11 ± 13	15	97	31 ± 2	FMF-03-047-1		16 ± 4	15	ND	6 ± 0.4
XMD13-44		52 ± 5	15	98	23 ± 2	XMD12-70-2		187 ± 34	15	98	7 ± 1
XMD8-90		104 ± 3	15	97	16 ± 0.8	XMD17-78		111 ± 2	15	95	6.1 ± 0.2
XMD12-1		109 ± 19	15	97	30 ± 1	XMD11-50 (LRRK2-IN-1)		58 ± 15	15	97	5 ± 0.4
XMD12-68		37 ± 7	15	96	35 ± 5						
XMD9-19		97 ± 27	15	98	14.1 ± 1	XMD11-52		1 ± 8	15	91	18 ± 3
XMD8-92		716 ± 57	15	96	46 ± 2	XMD17-109 (ERK5-IN-1)		22 ± 5	15	94	171 ± 30
XMD8-86		510 ± 36	15	68	160 ±7						

Table 1. Exploration of the R_1 position. DCLK1 IC_{50} values were determined using a MSA kinase assay and are reported as the average of three technical replicates ± the standard error of the mean (SEM). ERK5 % inhibition was measured using the KiNativ platform in cell lysates, at a concentration of 10 μM. LRRK2 IC_{50} values were determined using an ADAPTA kinase assay, with the ATP concentration set at the K_M, and are reported as the average of two technical replicates ± SEM.

We recently described the selective kinase inhibitor DCLK1-IN-1 as a chemical tool to examine the role of DCLK1 kinase activity. Here we present the structure activity relationships leading to the development of this molecule and report its anti-proliferative activity in K-Ras mutant patient derived organoid cultures.

RESULTS AND DISCUSSION

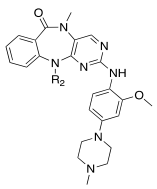
We developed a kinase peptide substrate mobility shift assay (MSA) to determine the IC_{50} of the reported compounds for DCLK1. The assay utilizes purified components including the DCLK1 kinase domain (residues 364- 689) and measures the conversion of peptide substrates, in this case a derivative of PRAK (p38 Regulated Activated Protein Kinase), to a phosphorylated form using microcapillary electrophoresis[14]. Of the reported inhibitors, XMD8-85 is the most potent (11 nM), and also display potent inhibition of ERK5 and LRRK2. LRRK2-IN-1 inhibits DCLK1 to a lesser extent (186 nM), and is significantly more potent against LRRK2 and ERK5. XMD8-92 was found to be a weakly active DCLK1 inhibitor (716 nM) (Table 1). We began by examining how variations in the R_1 substituent affected the activity of these three molecules, along with sulfonamide XMD17-86 identified in a screening effort (Table 1). The N-methyl piperazine on XMD8-85 could be substituted for oxygen without detriment to the activity. However, removal of the N-methyl group, or extension with a piperidine ring, led to reduced potency.

We next examined the SAR around XMD8-92. To optimize the ortho-ethoxy substituent we explored the effects of reducing or increasing its' bulkiness. Potency improved as the ortho substituent size was reduced, with the ortho methyl-substituted analog XMD12-1 displaying the most potent inhibition in the series. However, complete removal or ortho substitution resulted in a 3-fold loss of activity. As previous work has demonstrated that an ortho methoxy substituent in this series confers desirable selectivity against the targets Aurora A/B/C and PI3K, relative to ortho-methyl analogs, ortho-methoxy was considered to afford the optimal balance of potency and selectivity.[4, 5] Overall, XMD9-19 is 20 fold less potent than XMD8-85, indicating that 4-N-methyl piperazine is preferred to 4-morpholine as the aromatic substituent (Table 1).

N-linked phenyl 4-sulfonamide R_1 substituents were found to be highly potent (XMD17-86), and ortho-methoxy substitution well tolerated (FMF-03-047-1). However, S-linked phenyl 4-sulfonamide R_1 substituents demonstrated significantly reduced affinity (XMD12-70-2).

Finally, when examining LRRK2-IN-1 analogs, shortened 2-ring systems such as XMD11-52 at R_1 displayed dramatically improved potency for DCLK1, similar to the trend observed in the XMD-8-85 analog series (Table 1).

Optimization of R_1 resulted in identification of potent DCLK1 inhibitors, however all lead compounds were also highly active against off targets LRRK2 and ERK5. Therefore we examined the effects of substitution of the R_2 position (Table 2), which had successfully been exploited to improve selectivity of ERK5-IN-1 against LRRK2.[10] As many of the analogs identified in Table 1 displayed high biochemical potency for DCLK1, the concentration of ATP was increased from 15 μM to 100 μM for testing future molecules, in order to increase the stringency of the assay and allow discrimination between potent analogs.



Compound ID	R_2	IC_{50} (nM) DCLK1	ATP conc (μM)	ERK5 % inhibition	IC_{50} (nM) ERK5	IC_{50} (nM) LRRK2
XMD8-85	Me	16 ± 7	100	96	87 ± 7	29 ± 3
XMD10-39	Et	154 ± 13	100	97	66 ± 7	57 ± 7
XMD10-78	iPr	71 ± 7	100	99	98 ± 12	289 ± 41
JWG-070	sec-butyl	32 ± 4	100	-	222 ± 21	743 ± 154
JWG-068	cyclobutyl	14 ± 2	100	-	79 ± 12	97 ± 6
XMD10-76	cyclopentyl	26 ± 4	100	98	146 ± 18	421 ± 84
XMD11-32	benzyl	4 ± 1	100	94	-	141 ± 5

Table 2. Exploration of the R_2 position. DCLK1 IC_{50} values were determined using a MSA kinase assay and are reported as the average of three technical replicates ± SEM. ERK5 % inhibition was measured using the KiNativ platform on cell lysates, at a concentration of 10 μM. LRRK2 IC_{50} values were determined using an ADAPTA kinase assay, with the ATP concentration set at the K_M, and are reported as the average of two technical replicates ± SEM. ERK5 IC_{50} values were assayed in a ^{32}P kinase assay, as described previously[8], and are reported as the average of two technical replicates ± SEM.

XMD8-85 was used as a reference compound, and displayed a 1.5-fold decrease in affinity as a result of increasing the ATP concentration (11 nM to 16 nM).

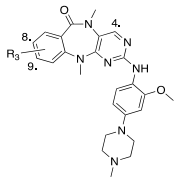
Increasing the bulk of the R_2 substituent improved the selectivity of the compounds against LRRK2, but not against ERK5, consistent with reported SAR[10]. No significant improvement in DCLK1 potency or selectivity was achieved by variation of R_2 (Table 2).

We next analyzed the effects of substitution of R_3 (Table 3). Substitution at the 4-position greatly reduced the potency for DCLK1, ERK5 and LRRK2, likely due to steric interference with the hinge binding interaction of the aminopyrimidine[10]. Previous work targeting PI3K-δ/γ[4] and Aurora kinases A/B/C[5] with this scaffold had shown that substitution at the 8-position confers significant selectivity.[4, 5] However, this modification was also not well tolerated by DCLK1, and compounds bearing an 8-methyl (XMD11-34) or 8-chloro (XMD10-126) suffered more than a 100-fold loss in biochemical activity. Finally, substitution at the 9-position was also not well tolerated by DCLK1, ERK5 or LRRK2 (Table 3).

Having failed to improve the selectivity via substitution of R_2 or R_3, we turned to exploration of the R_4 group. DCLK1 and LRRK2 possess a methionine gatekeeper residue (Met465 and Met1947 respectively), unlike ERK5, which has a leucine gatekeeper (Leu137). Examination of the ERK5-XMD8-92[15] and the DCLK1-XMD8-85 co-crystal structures revealed that the R_4 methyl group is oriented towards the gatekeeper residue (Supporting Figure 1). Therefore, we hypothesized that varying the R_4 group may lead to improved selectivity against ERK5.

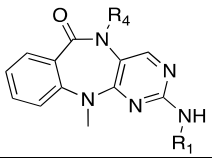
Kinase inhibitors bearing a 5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one scaffold have recently been reported to possess off-target activity for the bromodomains of BRD4.[9] BRD4 is a prominent anti-cancer target, and inhibition of BRD4 has pleiotropic effects[16]. Therefore, we sought to engineer out the BRD4 activity which would confound elucidation of DCLK1 dependent pharmacology. The N-methyl amide of XMD8-85 has been shown to bind in the acetyl lysine binding site of BRD4, and act as a substrate mimetic, with the core of the scaffold engaging in hydrophobic contacts.[13] We hypothesized that changes to this position would alter BRD4 binding and tested all R_4 substituted analogs using a BRD4 AlphaScreen assay, to assess the impact of varying the N-methyl amide.

We sought to both improve kinase selectivity and to remove BRD4 activity from our DCLK1 inhibitors by varying the R_4 position. We generated R_4 variants incorporating the three most potent R_1 substituents, and kept R_2 as methyl and R_3 as hydrogen (Table 4, and Ref 21 entries 1-5).



Compound ID	R_3	IC_{50} (nM) DCLK1	ATP conc (μM)	ERK5 % inhibition	IC_{50} (nM) ERK5	IC_{50} (nM) LRRK2
XMD10-47	4-Me	> 2000	100	58	> 10,000	501 ± 59
XMD11-34	8-Me	> 2000	100	77	3100 ± 620	928 ± 227
XMD10-126	8-Cl	> 2000	100	80	1240 ± 240	1330 ± 232
XMD10-147	9-Cl	> 2000	100	34	1138 ± 2040	3740 ± 3156

Table 3. Exploration of the R_3 substituent. DCLK1 IC_{50} values were determined using a MSA kinase assay and are reported as the average of three technical replicates ± SEM. ERK5 % inhibition was measured using the KiNativ platform in cell lysates, at a concentration of 10 μM. LRRK2 IC_{50} values were determined using an ADAPTA kinase assay, with the ATP concentration set at the K_M, and are reported as the average of two technical replicates ± SEM. ERK5 IC_{50} values were assayed in a ^{32}P kinase assay, as described previously[8], and are reported as the average of two technical replicates ± SEM.



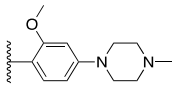
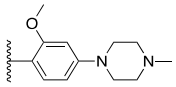
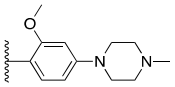
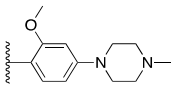
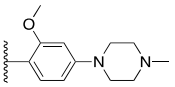
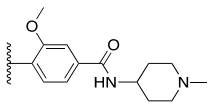
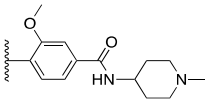
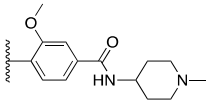
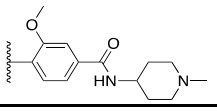
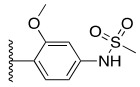
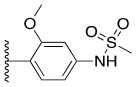
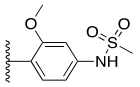
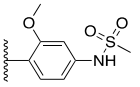
Compound ID	R_4	R_1	IC_{50} (nM) DCLK1	ATP conc (μM)	IC_{50} (nM) LRRK2	IC_{50} (nM) BRD4_1	IC_{50} (nM) ERK5
XMD8-87	H		> 1000	100	70 ± 6	> 10,000	1200 ± 220
XMD8-85	Me		16 ± 7	100	29 ± 3	888	87 ± 7
FMF-03-055-1	Et		3 ± 1	100	62 ± 9	830	1200 ± 130
FMF-03-146-1 (DCLK1-IN-1)	CH_2CF_3		17 ± 3	100	7000 ± 6490	> 10,000	3350 ± 650
FMF-03-149-1	iPr		411 ± 30	100	1900 ± 447	1200	-
XMD11-52	Me		4 ± 4	100	8.2 ± 3	4170	320 ± 50
FMF-03-059-1	Et		2 ± 0.5	100	14 ± 2	520	482 ± 75
FMF-03-148-1	CH_2CF_3		10 ± 3	100	378 ± 31	1570	-
FMF-03-151-1	iPr		126 ± 10	100	331 ± 26	2380	-
FMF-03-047-1	Me		3 ± 1	100	5.9 ± 0.4	125	276 ± 30
FMF-03-087-1	Et		36 ± 4	100	23 ± 3	4600	-
FMF-03-147-1 P2	CH_2CF_3		80 ± 7	100	8894 ± 8153	> 10000	-
FMF-03-150-2	iPr		> 1000	100	683 ± 131	7490	-

Table 4. Exploration of the R_4 position. DCLK1 IC_{50} values were determined using a MSA kinase assay and are reported as the average of three technical replicates ± SEM. LRRK2 IC_{50} values were determined using an ADAPTA kinase assay, with the ATP concentration set at the K_M, and are reported as the average of two technical replicates ± SEM. ERK5 IC_{50} values were assayed in a ^{32}P kinase assay, as described previously[8], and are reported as the average of two technical replicates ± SEM. BRD4 IC_{50}s were measured using an AlphaScreen displacement assay, and are reported as the average of three technical replicates ± SEM.

Removal of the R_4 methyl of XMD8-85 (XMD8-87) was not tolerated by DCLK1, ERK5 or BRD4, and led to a modest reduction in potency for LRRK2. Substitution of the R_4 methyl with an ethyl group was well tolerated by DCLK1 and LRRK2 in all cases (FMF-03-055-1, FMF-03-059-1, FMF-03-087-1). However, these molecules displayed significant reduction in affinity for ERK5 and BRD4. Based on this positive selectivity data, R_4 was further increased to an isopropyl group. This resulted in greater than 100-fold losses in activity for DCLK1 compared to the R_4 = methyl analogs.

Altering the polarity of the R_4 group to reduce its acetyl-lysine like properties, from a hydrophobic ethyl to an electronegative [1,1,1]-trifluoroethyl group led to a modest reduction in DCLK1 activity (5 – 25-fold), but a dramatic increase in selectivity against ERK5, LRRK2 and BRD4. FMF-03-146-1 displayed a superior selectivity profile against ERK5, LRRK2 and BRD4, and was profiled against the kinome. KINOME*scan* profiling at 1 μM compound concentration against a panel of 468 kinases revealed that this molecule displays exquisite selectivity for DCLK1 and DCLK2.[17]

As the function of DCLK1 is poorly characterized, we sought to develop a close structural analog of FMF-03-146-1 that could be used as a negative control compound, in order to increase the confidence that phenotypes observed in experiments with FMF-03-146-1 are DCLK1-kinase dependent. Therefore, we modified FMF-03-146-1 with R_1 or R_3 substituents that reduced DCLK1 activity in this series (Table 4), resulting in the identification of FMF-03-112-1.

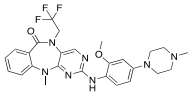
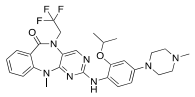
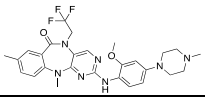
Compound ID	Structure	IC_{50} (nM) DCLK1	ATP conc (μM)	Selectivity vs FMF-03-146-1
FMF-03-146-1 (DCLK1-IN-1)		17 ± 3	100	1
FMF-04-077-1		214 ± 38	100	13
FMF-04-112-1		> 5000	100	303

Table 5. Generation of a DCLK1 negative control molecule. DCLK1 IC_{50} values were determined using a MSA kinase assay and are reported as the average of three technical replicates ± SEM.

In order to ensure additional kinase targets were not picked up by the negative control, FMF-03-112-1 was also profiled by KINOME*scan* at 1 μM and found to be inactive against the kinome.[17] With selective and well characterized molecules in hand, we sought to evaluate DCLK1 as a therapeutic target in PDAC. We previously reported that DCLK1$^+$ PDAC organoids derived from a patient at various points throughout chemotherapy and harboring a BRAF insertion mutation were sensitive to DCLK1 inhibition.[17]

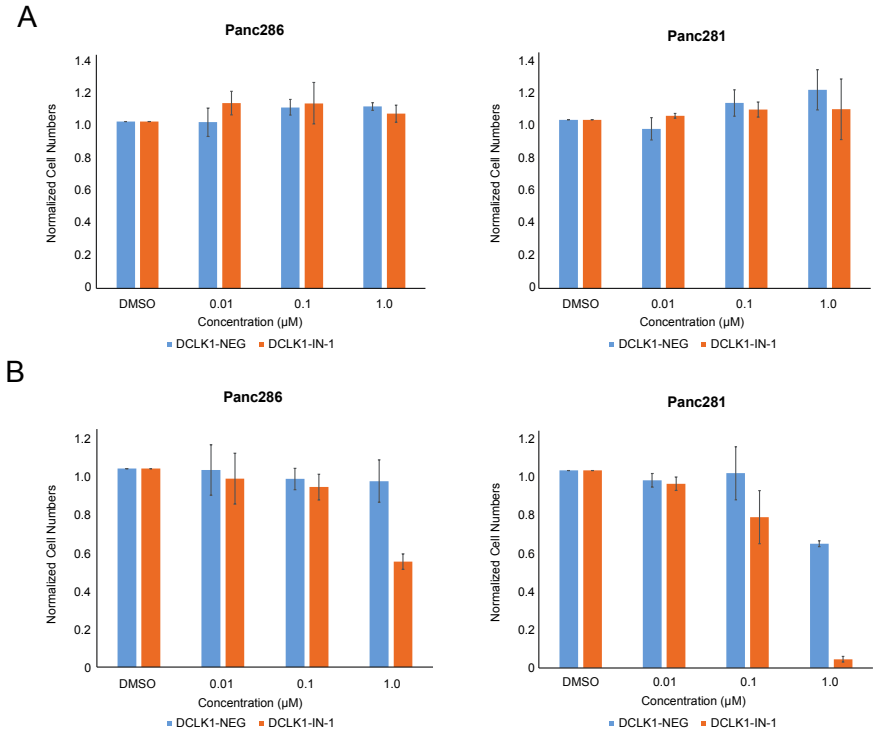


Figure 2. Biological characterization of DCLK1-IN-1 and DCLK1-NEG. A. Viability effects following organoid treatment with compounds at the indicated concentrations for 4 d. B. Viability effects following organoid treatment with compounds at the indicated concentrations for 12 d. Data in A, B, shown as the mean ± SEM (n = 3).

To explore the relationship between DCLK1 inhibition and viability in PDAC organoids harboring K-Ras mutations, we evaluated the effects of DCLK1-IN-1 and DCLK1-NEG on organoids derived from samples taken from two unrelated

PDAC patients using a previously reported method.[18] The organoids were treated for 4 days or 12 days of (Figure 2 A, B). These lines have multiple oncogenic mutations, including K-Ras^{G12D} (Panc030) and K-Ras^{G12V} (Panc281).[19] Here minimal effects were observed after 4 d treatment, but both organoids were sensitive over long term treatments, with between 50% and > 90% loss of viability observed by 12 d at 1 μM compound concentration. Minimal loss of viability with DCLK1-NEG was observed, suggesting these effects are on target.

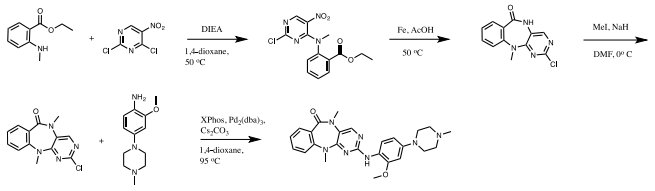
CONCLUSIONS

Here we present comprehensive structure activity relationships for a series of DCLK1 inhibitors based on a 5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one scaffold. This work culminated in the identification of DCLK1-IN-1, a chemical probe for the DCLK1 kinase domain. As previously reported, DCLK1-IN-1 has suitable pharmacokinetic properties for use in mice ($T_{1/2}$: 2.09 h, AUC: 1269.5 hr*ng mL^{-1}, Cl: 26.26 mL min^{-1} kg^{-1}) Using information garnered from the SAR study, we also designed structurally related negative control compound DCLK1-NEG, to be used in concert. Our previous studies demonstrated that DCLK1 inhibition could reduce viability in patient-derived PDAC organoids harboring an activating BRAF insertion mutation. In this work, we further the characterization of the role of DCLK1 in PDAC, this time examining effects in patient-derived organoids harboring K-Ras mutations, which were also sensitive to prolonged DCLK1 inhibition. We envisage these molecules will provide useful leads for the investigation of the effects of DCLK1 in PDAC and other DCLK1 expressing cancers.

EXPERIMENTAL SECTION

General Procedures. Unless otherwise noted, reagents and solvents were obtained from commercial suppliers and were used without further purification. ^{1}H NMR spectra were recorded on 500 MHz Bruker Avance III spectrometer, and chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Coupling constants (J) are reported in Hz. Spin multiplicities are described as s (singlet), br (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were obtained on a Waters Acquity UPLC. Preparative HPLC was performed on a Waters Sunfire C18 column (19 mm × 50 mm, 5 μM) using a gradient of 15−95% methanol in water containing 0.05% trifluoroacetic acid (TFA) over 22 min (28 min run time) at a flow rate of 20 mL/min. Assayed compounds were isolated and tested as TFA salts. Purities of assayed compounds were in all cases greater than 95%, as determined by reverse-phase HPLC analysis.

Scheme 1



ethyl 2-((2-chloro-5-nitropyrimidin-4-yl)(methyl)amino)benzoate (2) A mixture of ethyl 2-(methylamino)benzoate (1.44 g, 8.0 mmol), diisopropylethylamine (DIEA) (2.8 mL, 16.0 mmol) and 2,4-dichloro-5-nitropyrimidine (2.30g, 12.0 mmol) in dioxane (40 mL) was heated at 50˚C for 6 hours. After the reaction was complete as monitored by thin layer chromatography (TLC), the reaction solution was concentrated and the residue was purified by silica-gel column chromatography with ethyl acetate and hexane (1/20, v/v) to give the title compound (2.51 g, 93%). 1H NMR (600 MHz, CDCl3) δ 8.44 (s, 1H), 8.02 (d, J = 7.2 Hz, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.22 (d, J = 7.8 Hz, 1H), 4.28-4.18 (m, 2H), 3.58 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H). 13C NMR (150 MHz, CDCl3) δ 164.4, 160.8, 157.0, 155.2, 142.8, 134.1, 132.5, 128.9, 127.7, 61.6, 42.0, 14.0. MS (ESI) m/z 337 (M+H)+

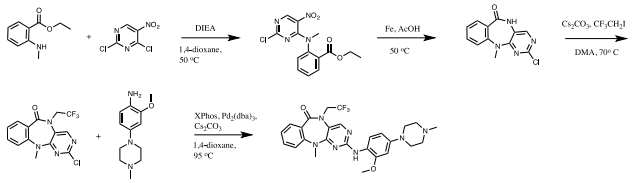
2-chloro-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (3). A mixture of compound 2 (2.35 g, 6.98 mmol) and iron power (3.9 g, 69.8 mmol) in acetic acid (100 mL) was heated at 50 ˚C for 9 hours. After the reaction was complete as monitored by reverse phase analytical liquid-chromatography electrospray mass spectrometry (LC-MS), the excess of iron was removed and the mixture was concentrated in vacuo. The resulting residue was poured into ice-water which resulted in a solid precipitate that was collected by filtration, washed with water and air dried to give the title compound (1.55 g, 85%). 1H NMR (600 MHz, DMSO-d6) δ 10.44 (s, 1H), 8.14 (s, 1H), 7.72 (d, J = 4.8 Hz, 1H), 7.58 (s, 1H), 7.27 (d, J = 6.0 Hz, 1H), 7.21 (s, 1H), 3.33 (s, 3H). 13C NMR (150 MHz, DMSO-d6) δ 167.6, 161.4, 153.4, 149.7, 147.9, 134.2, 132.0, 125.9, 124.6, 124.3, 120.1, 37.2. MS (ESI) m/z 261 (M+H)+

2-chloro-5,11-dimethyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (4). To a stirred suspension of compound 3 (688 mg, 2.64 mmol) and MeI (0.25 mL, 4.0 mmol) in dimethyl acetamide (DMA, 40.0 mL) was added NaH (360 mg, 60 % suspension in mineral oil) at -10 ˚C and the reaction was gradually warmed to 0 ˚C. After the reaction was complete as monitored by LC-MS, the solution was poured into ice-water which resulted in a solid precipitate. The precipitate was collected by filtration, washed with water and air dried to give the title compound (563 mg, 77%). 1H NMR (600 MHz, DMSO-d6) δ 8.57 (s, 1H), 7.68 (dd, J = 1.2, 7.2 Hz, 1H), 7.54-7.51 (m, 1H), 7.25 (d, J = 7.8 Hz, 1H), 7.20-7.18 (m, 1H), 3.41 (s, 3H), 3.32 (s, 3H). 13C NMR (150 MHz, DMSO-d6) δ 167.1, 163.8, 153.7, 153.4, 148.6, 133.5, 132.4, 128.7, 126.0, 124.6, 118.9, 38.1, 36.4. MS (ESI) m/z 275 (M+H)+.

2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5,11-dimethyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (XMD8-85-1). A mixture of 4 (26 mg, 0.1 mmol), 2-methoxy-4-(4-methylpiperizin-1-yl)benzamine (22 mg, 0.1 mmol), X-Phos (4.3 mg), Pd2(dba)3 (5.5 mg) and K2CO3 (41.5 mg, 0.3 mmol) in 1.2 mL of *t*-BuOH was heated at 100 °C in a seal tube for 4 h. Then the reaction was filtered through celite and eluted with dichloromethane. The dichloromethane was removed in vacuo and the resulting crude product was purified by reverse-phase prep-HPLC using a water (0.05% TFA)/acetonitrile (0.05% TFA) gradient to afford the title compound 10 as TFA salt (20 mg, yield: 36%). 1H NMR (600 MHz, CD3OD) δ 7.83 (dd, *J* = 1.2, 7.8 Hz, 1H), 7.71 (s, 1H), 7.62-7.59 (m, 2H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.26 (t, *J* = 7.2 Hz, 1H), 6.75 (d, *J* = 1.8 Hz, 1H), 6.66 (d, *J* = 7.2 Hz, 1H), 3.92-3.87 (m, 5H), 3.66-3.60 (m, 2H), 3.49 (s, 3H), 3.30-3.24 (m, 2H), 3.14-3.08 (m, 2H), 2.97 (s, 3H). MS (ESI) *m/z* 446

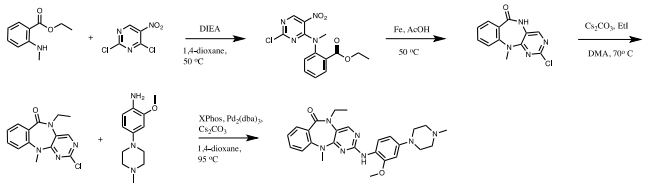
(M+H)+.

Scheme 2



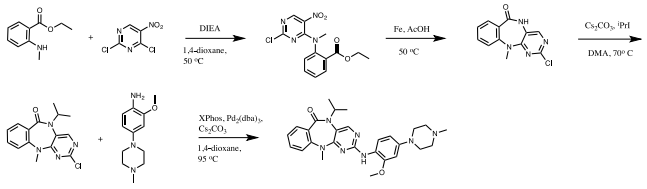
2-chloro-11-methyl-5-(2,2,2-trifluoroethyl)-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (5). Compound 3 (500mg, 1.9 mmol), Cs_2CO_3 (1.3 g, 4.0 mmol) and trifluoroethyl iodide (250 µL, 2.6 mmol) were dissolved in DMA (5 mL) and heated to 70 °C for 2 h. The solvent was evaporated and the residue purified by silica column chromatography (0 – 5% MeOH in DCM) to give the title compound (116 mg, 18 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.84 (s, 1H), 7.73 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.59 (ddd, *J* = 8.4, 7.3, 1.7 Hz, 1H), 7.34 (dd, *J* = 8.4, 0.9 Hz, 1H), 7.26 (ddd, *J* = 8.0, 7.3, 1.0 Hz, 1H), 5.04 (br s, 2H), 3.38 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.79, 165.40, 155.37, 149.08, 134.04, 132.42, 126.15, 125.90, 125.17, 124.92, 123.66, 119.15, 48.61 (q, *J* = 32.5 Hz), 36.30. MS (ESI) *m/z* 343 (M+H)+

Scheme 3



2-chloro-5-ethyl-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (6). Compound 3 (500mg, 1.9 mmol), Cs_2CO_3 (1.3 g, 4.0 mmol) and ethyl iodide (210 µL, 2.6 mmol) were dissolved in DMA (5 mL) and heated to 70 °C for 2 h. The solvent was evaporated and the residue purified by silica column chromatography (0 – 5% MeOH in DCM) to give the title compound (280 mg, 51 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.66 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.53 (ddd, *J* = 8.4, 7.3, 1.7 Hz, 1H), 7.27 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.22 (td, *J* = 7.5, 1.0 Hz, 1H), 4.10 (q, *J* = 5.2 Hz, 2H), 3.18 (s, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.06, 164.74, 153.97, 153.61, 148.71, 133.30, 132.08, 127.49, 126.71, 124.70, 118.82, 46.09, 36.31, 13.82. MS (ESI) *m/z* 290 (M+H)+

Scheme 4



2-chloro-5-isopropyl-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (7). Compound 3 (500mg, 1.9 mmol), Cs_2CO_3 (1.3 g, 4.0 mmol) and isopropyl iodide (250 µL, 2.2 mmol) were dissolved in DMA (5 mL) and heated to 70 °C for 2 h. The solvent was evaporated and the residue purified by silica column chromatography (0 – 5% MeOH in DCM) to give the title compound (167 mg, 29 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58 (s, 1H), 7.69 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.52 (ddd, *J* = 8.3, 7.3, 1.7 Hz, 1H), 7.27 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.21 (td, *J* = 7.5, 1.0 Hz, 1H), 4.52 (hept, *J* = 6.8 Hz, 1H), 3.35 (s, 3H), 1.80 – 0.85 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.49, 166.18, 154.39, 148.79, 133.11, 132.29, 126.90, 125.90, 124.56, 118.46, 54.22, 36.01. MS (ESI) *m/z* 304 (M+H)+

All other compounds were prepared from intermediates 4, 5, 6 or 7 in an analogous fashion to XMD8-85.

5-ethyl-2-((4-(4-hydroxypiperidin-1-yl)-2-methoxyphenyl)amino)-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (XMD9-22). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.94 (s, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.63 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.50 – 7.45 (m, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.16 (t, *J* = 7.4 Hz, 1H), 6.61 (d, *J* = 2.6 Hz, 1H), 6.49 (dd, *J* = 8.8, 2.6 Hz, 1H), 4.15 – 4.01 (m, 1H), 3.80 (s, 3H), 3.62 (tq, *J* = 8.5, 4.4 Hz, 1H), 3.55 – 3.46 (m, 2H), 3.35 (s, 2H), 3.27 (s, 3H), 1.83 (dq, *J* = 12.4, 4.0 Hz, 2H), 1.50 (dtd, *J* = 13.0, 9.5, 3.8 Hz, 2H), 1.15 (t, *J* = 7.1 Hz, 3H). MS (ESI) *m/z* 475 (M+H)$^+$

5-ethyl-2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-03-055-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.35 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.64 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.49 (ddd, *J* = 8.7, 7.2, 1.7 Hz, 1H), 7.24 – 7.13 (m, 2H), 6.72 (d, *J* = 2.6 Hz, 1H), 6.57 (dd, *J* = 8.8, 2.6 Hz, 1H), 4.20 – 3.92 (m, 1H), 3.89 (s, 2H), 3.83 (s, 3H), 3.54 (d, *J* = 12.1 Hz, 2H), 3.28 (s, 3H), 3.18 (q, *J* = 10.9 Hz, 2H), 2.94 (t, *J* = 12.6 Hz, 2H), 2.89 (d, *J* = 3.4 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 3H), .MS (ESI) *m/z* 474 (M+H)$^+$

2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-11-methyl-5-(2,2,2-trifluoroethyl)-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-03-146-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.85 (s, 1H), 8.48 (s, 1H), 8.18 (s, 1H), 7.78 – 7.67 (m, 2H), 7.57 – 7.50 (m, 1H), 7.30 – 7.25 (m, 1H), 7.20 (td, *J* = 7.6, 1.0 Hz, 1H), 6.71 (d, *J* = 2.6 Hz, 1H), 6.61 – 6.53 (m, 1H), 3.86 (d, *J* = 4.4 Hz, 1H), 3.83 (s, 2H), 3.54 (d, *J* = 12.0 Hz, 2H), 3.29 (s, 2H), 3.23 – 3.12 (m, 2H), 2.95 (t, *J* = 12.5 Hz, 2H), 2.88 (s, 3H). MS (ESI) *m/z* 528 (M+H)$^+$

5-isopropyl-2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-03-149-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.71 (s, 1H), 8.25 (s, 1H), 8.11 (s, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.66 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.46 (ddd, *J* = 8.7, 7.2, 1.7 Hz, 1H), 7.27 – 7.12 (m, 2H), 6.71 (d, *J* = 2.6 Hz, 1H), 6.57 (dd, *J* = 8.8, 2.6 Hz, 1H), 4.60 (hept, *J* = 6.8 Hz, 1H), 3.82 (s, 6H), 3.54 (d, *J* = 12.2 Hz, 2H), 3.28 (s, 3H), 3.17 (dd, *J* = 12.8, 9.2 Hz, 2H), 2.94 (t, *J* = 12.7 Hz, 2H), 2.88 (d, *J* = 3.3 Hz, 3H), 1.49 – 1.36 (m, 3H), 1.08 (d, *J* = 6.8 Hz, 3H). MS (ESI) *m/z* 488 (M+H)$^+$

4-((5-ethyl-11-methyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxy-*N*-(1-methylpiperidin-4-yl)benzamide (FMF-03-059-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.38 (s, 1H), 8.49 (s,

1H), 8.36 (dd, *J* = 8.0, 5.3 Hz, 2H), 8.23 – 8.16 (m, 1H), 7.66 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.54 – 7.46 (m, 2H), 7.25 (dt, *J* = 8.4, 1.9 Hz, 1H), 7.19 (td, *J* = 7.5, 1.1 Hz, 1H), 4.61 (br s, 2H), 4.11 – 3.98 (m, 1H), 3.95 (d, *J* = 3.8 Hz, 3H), 3.49 (d, *J* = 12.2 Hz, 2H), 3.36 (s, 3H), 3.17 – 3.05 (m, 2H), 2.79 (d, *J* = 4.6 Hz, 3H), 2.08 – 1.98 (m, 2H), 1.82 – 1.69 (m, 2H), 1.18 (t, *J* = 7.0 Hz, 3H). MS (ESI) *m/z* 516 $(M+H)^+$

2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-11-methyl-5-(2,2,2-trifluoroethyl)-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-03-146-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.85 (s, 1H), 8.48 (s, 1H), 8.18 (s, 1H), 7.78 – 7.67 (m, 2H), 7.57 – 7.50 (m, 1H), 7.30 – 7.25 (m, 1H), 7.20 (td, *J* = 7.6, 1.0 Hz, 1H), 6.71 (d, *J* = 2.6 Hz, 1H), 6.61 – 6.53 (m, 1H), 3.86 (d, *J* = 4.4 Hz, 1H), 3.83 (s, 2H), 3.54 (d, *J* = 12.0 Hz, 2H), 3.29 (s, 2H), 3.23 – 3.12 (m, 2H), 2.95 (t, *J* = 12.5 Hz, 2H), 2.88 (s, 3H). MS (ESI) *m/z* 528 $(M+H)^+$

3-methoxy-4-((11-methyl-6-oxo-5-(2,2,2-trifluoroethyl)-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-*N*-(1-methylpiperidin-4-yl)benzamide (FMF-03-148-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.63 (s, 1H), 8.38 (d, *J* = 7.5 Hz, 1H), 8.34 (d, *J* = 10.0 Hz, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 7.71 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.56 (ddt, *J* = 10.1, 8.7, 3.3 Hz, 2H), 7.53 – 7.48 (m, 1H), 7.30 (dd, *J* = 8.2, 3.0 Hz, 1H), 7.25 – 7.20 (m, 1H), 5.27 (s, 1H), 4.62 (s, 1H), 4.17 – 3.97 (m, 1H), 3.94 (d, *J* = 3.7 Hz, 3H), 3.48 (s, 2H), 3.37 (d, *J* = 2.8 Hz, 3H), 3.18 – 3.05 (m, 2H), 2.79 (d, *J* = 4.6 Hz, 3H), 2.08 – 2.01 (m, 2H), 1.78 (qd, *J* = 13.7, 3.9 Hz, 2H). MS (ESI) *m/z* 570 $(M+H)^+$

4-((5-isopropyl-11-methyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxy-*N*-(1-methylpiperidin-4-yl)benzamide (FMF-03-151-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.44 (s, 1H), 8.39 (s, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.26 (s, 1H), 7.68 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.57 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.24 (dt, *J* = 8.7, 1.9 Hz, 1H), 7.18 (td, *J* = 7.5, 1.0 Hz, 1H), 4.59 (p, *J* = 6.8 Hz, 1H), 4.20 – 3.98 (m, 1H), 3.94 (d, *J* = 3.9 Hz, 3H), 3.49 (d, *J* = 12.2 Hz, 2H), 3.36 (s, 3H), 3.17 – 3.06 (m, 2H), 2.79 (d, *J* = 4.6 Hz, 3H), 2.09 – 1.99 (m, 2H), 1.78 (qd, *J* = 13.6, 3.9 Hz, 2H), 1.47 (s, 3H), 1.11 (s, 3H). MS (ESI) *m/z* 530 $(M+H)^+$

***N*-(4-((5,11-dimethyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxyphenyl)methanesulfonamide (FMF-03-047-1).** ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.36 (s, 1H), 8.16 (s, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.69 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.51 (ddd, *J* = 8.7, 7.2, 1.8 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 2.3 Hz, 1H), 6.84 (dd, *J* = 8.6, 2.3 Hz, 1H), 3.82 (s, 3H), 3.39 (s, 3H), 3.32 (s, 4H), 2.98 (s, 3H). MS (ESI) *m/z* 455 $(M+H)^+$

***N*-(4-((5-ethyl-11-methyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxyphenyl)methanesulfonamide (FMF-03-087-1).** ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.39 (s, 1H), 8.16 (s, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.64 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.53 – 7.44 (m, 1H), 7.24 (dd, *J* = 8.4, 0.9 Hz, 1H), 7.17 (td, *J* = 7.5, 1.0 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.84 (dd, *J* = 8.6, 2.3 Hz, 1H), 4.02 (s, 2H), 3.82 (s, 3H), 3.31 (s, 3H), 2.98 (s, 3H), 1.16 (t, *J* = 7.1 Hz, 3H).
MS (ESI) *m/z* 469 $(M+H)^+$

***N*-(3-methoxy-4-((11-methyl-6-oxo-5-(2,2,2-trifluoroethyl)-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)phenyl)methanesulfonamide (FMF-03-147-1).** ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.72 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.32 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.23 (td, *J* = 7.5, 0.9 Hz, 1H), 6.80 (s, 1H), 6.70 (d, *J* = 1.3 Hz, 2H), 3.75 (s, 3H), 3.65 (s, 2H), 3.29 (s, 3H), 3.17 (s, 3H). MS (ESI) *m/z* 523 $(M+H)^+$

***N*-(4-((5-isopropyl-11-methyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxyphenyl)methanesulfonamide (FMF-03-150-2)**
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.30 (s, 1H), 8.16 (s, 1H), 7.96 (d, *J* = 8.6 Hz, 1H), 7.66 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.50 – 7.43 (m, 1H), 7.25 – 7.21 (m, 2H), 7.16 (td, *J* = 7.5, 1.0 Hz, 1H), 6.91 (d, *J* = 2.3 Hz, 1H), 6.83 (dd, *J* = 8.6, 2.3 Hz, 1H), 4.55 (dq, *J* = 38.7, 6.8 Hz, 1H), 3.81 (s, 3H), 3.30 (s, 3H), 2.98 (s, 3H), 1.44 (d, *J* = 5.6 Hz, 4H), 1.09 (d, *J* = 6.6 Hz, 4H). MS (ESI) *m/z* 483 $(M+H)^+$

5-ethyl-2-((2-methoxy-4-(4-(pyrrolidin-1-yl)piperidine-1-carbonyl)phenyl)amino)-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-03-058-2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.47 (s, 1H), 8.29 (d, *J* = 8.2 Hz, 1H), 8.20 (s, 1H), 7.66 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.50 (ddd, *J* = 8.7, 7.3, 1.8 Hz, 1H), 7.25 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.19 (td, *J* = 7.5, 1.0 Hz, 1H), 7.09 – 7.00 (m, 2H), 4.05 (s, 2H), 3.91 (s, 3H), 3.53 (s, 2H), 3.47 – 3.37 (m, 1H), 3.35 (s, 3H), 3.16 – 3.05 (m, 2H), 2.96 (s, 1H), 2.55 (s, 2H), 2.09 (s, 2H), 2.06 – 1.92 (m, 2H), 1.93 – 1.78 (m, 2H), 1.63 – 1.49 (m, 1H), 1.17 (t, *J* = 7.1 Hz, 3H). MS (ESI) *m/z* 556 $(M+H)^+$

***N*-(4-((5-ethyl-11-methyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)phenyl)methanesulfonamide (FMF-03-083-1).** ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.66 (s, 1H), 9.44 (s, 1H), 8.43 (s, 1H), 7.74 – 7.68 (m, 2H), 7.64 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.48 (ddd, *J* = 8.8, 7.2, 1.7 Hz, 1H), 7.26 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.16 (d, *J* = 8.8 Hz, 3H), 4.15 – 3.59 (m, 2H), 3.35 (s, 3H), 2.93 (s, 3H), 1.17 (t, *J* = 7.0 Hz, 3H). MS (ESI) *m/z* 439 $(M+H)^+$

5-ethyl-2-((2-methoxy-4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)amino)-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-03-086-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.14 (s, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.64 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.48 (ddd, *J* = 8.7, 7.2, 1.8 Hz, 1H), 7.25 – 7.12 (m, 2H), 6.66 (d, *J* = 2.7 Hz, 1H), 6.58 (dd, *J* = 8.8, 2.7 Hz, 1H), 4.21 (t, *J* = 5.2 Hz, 2H), 4.05 (s, 2H), 3.81 (s, 3H), 3.41 (s, 4H), 3.27 (s, 3H), 3.17 (s, 4H), 2.82 (s, 3H), 1.16 (t, *J* = 7.1 Hz, 3H). MS (ESI) *m/z* 518 $(M+H)^+$

4-((5-ethyl-11-methyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)benzenesulfonamide (FMF-03-088-1/-2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.51 (s, 1H), 7.95 – 7.88 (m, 2H), 7.79 – 7.72 (m, 2H), 7.66 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.53 – 7.47 (m, 1H), 7.27 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.21 – 7.15

(m, 3H), 3.53 (s, 2H), 3.39 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 425 $(M+H)^+$

***N*-(2-(dimethylamino)ethyl)-4-((5-ethyl-11-methyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxybenzamide (FMF-03-061-1).** ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.64 (t, J = 5.7 Hz, 1H), 8.50 (s, 1H), 8.38 (d, J = 8.5 Hz, 1H), 8.23 (s, 1H), 7.66 (dd, J = 7.7, 1.7 Hz, 1H), 7.57 (dd, J = 8.5, 1.9 Hz, 1H), 7.53 (d, J = 2.0 Hz, 1H), 7.50 (ddd, J = 8.8, 7.2, 1.7 Hz, 1H), 7.25 (dd, J = 8.4, 1.0 Hz, 1H), 7.19 (td, J = 7.5, 1.0 Hz, 1H), 4.09 (s, 2H), 3.94 (s, 3H), 3.62 (q, J = 5.9 Hz, 2H), 3.36 (s, 3H), 3.28 (q, J = 5.9 Hz, 2H), 2.87 (d, J = 4.7 Hz, 6H), 1.18 (t, J = 7.0 Hz, 3H). MS (ESI) *m/z* 490 $(M+H)^+$

2-((4-(3-(dimethylamino)pyrrolidine-1-carbonyl)-2-methoxyphenyl)amino)-5-ethyl-11-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-03-067-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.48 (s, 1H), 8.32 (d, J = 8.2 Hz, 1H), 8.22 (s, 1H), 7.66 (dd, J = 7.7, 1.7 Hz, 1H), 7.50 (ddd, J = 8.7, 7.3, 1.7 Hz, 1H), 7.27 – 7.15 (m, 4H), 3.95 (s, 2H), 3.92 (s, 3H), 3.75 – 3.66 (m, 2H), 3.61 (s, 2H), 3.35 (s, 3H), 2.85 (s, 6H), 2.40 – 2.26 (m, 1H), 2.12 (t, J = 10.6 Hz, 1H), 1.17 (t, J = 7.0 Hz, 3H). MS (ESI) *m/z* 516 $(M+H)^+$

2-((2-isopropoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-11-methyl-5-(2,2,2-trifluoroethyl)-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-04-077-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.50 (s, 1H), 8.02 (s, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.70 (dd, J = 7.7, 1.7 Hz, 1H), 7.58 – 7.50 (m, 1H), 7.27 (dd, J = 8.5, 1.0 Hz, 1H), 7.20 (td, J = 7.5, 1.0 Hz, 1H), 6.73 (d, J = 2.6 Hz, 1H), 6.57 (dd, J = 8.8, 2.6 Hz, 1H), 5.29 (d, J = 37.9 Hz, 1H), 4.68 (h, J = 6.1 Hz, 1H), 4.55 (s, 1H), 3.81 (d, J = 13.1 Hz, 2H), 3.30 (s, 3H), 3.17 (t, J = 5.5 Hz, 2H), 2.94 (t, J = 12.5 Hz, 2H), 2.90 – 2.85 (m, 3H), 1.25 (d, J = 6.0 Hz, 7H). MS (ESI) *m/z* 556 $(M+H)^+$

2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-8,11-dimethyl-5-(2,2,2-trifluoroethyl)-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (FMF-04-112-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.74 (s, 1H), 8.45 (s, 1H), 8.15 (s, 1H), 7.75 (d, J = 8.7 Hz, 1H), 7.49 (d, J = 2.2 Hz, 1H), 7.34 (dd, J = 8.6, 2.3 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 6.71 (d, J = 2.6 Hz, 1H), 6.57 (dd, J = 8.8, 2.6 Hz, 1H), 5.23 (s, 1H), 4.54 (s, 1H), 4.07 – 3.84 (m, 2H), 3.81 (s, 3H), 3.59 – 3.47 (m, 3H), 3.20 (d, J = 22.5 Hz, 2H), 2.95 (t, J = 12.8 Hz, 2H), 2.88 (s, 4H), 2.28 (s, 3H). MS (ESI) *m/z* 542 $(M+H)^+$

methyl 2-(4-(3-methoxy-4-((11-methyl-6-oxo-5-(2,2,2-trifluoroethyl)-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)phenyl)piperazin-1-yl)acetate (FMF-04-117-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.21 (s, 1H), 7.73 (d, J = 8.7 Hz, 1H), 7.70 (dd, J = 7.8, 1.7 Hz, 1H), 7.54 (ddd, J = 8.4, 7.3, 1.7 Hz, 1H), 7.27 (dd, J = 8.4, 1.1 Hz, 1H), 7.21 (td, J = 7.5, 1.0 Hz, 1H), 6.71 (d, J = 2.6 Hz, 1H), 6.57 (dd, J = 8.8, 2.6 Hz, 1H), 5.28 (s, 1H), 4.56 (s, 1H), 4.35 (s, 3H), 3.81 (s, 3H), 3.77 (d, J = 2.3 Hz, 2H), 3.73 – 3.61 (m, 4H), 3.29 (s, 3H), 3.12 (d, J = 15.9 Hz, 2H), 3.05 – 2.92 (m, 1H). MS (ESI) *m/z* 586 $(M+H)^+$

2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-4,5,11-trimethyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (XMD10-47). ^{1}H NMR (600 MHz, MeOD-*d4*) 7.88 (d, J = 9.0 Hz, 1H), 7.76 (dd, J = 8.4 Hz, 1.2 Hz, 1H), 7.53 (m, 1H), 7.26 (m, 2H), 6.76 (d, J = 2.4 Hz, 1H), 6.67 (dd, J = 9.0 Hz, 2.4 Hz, 1H), 3.90 (s, 3H), 3.86 (m, 2H), 3.63 (m, 2H), 3.46 (s, 3H), 3.31 (s, 3H), 3.29 (m, 2H), 3.09 (m, 2H), 2.98 (s, 3H), 2.42 (s, 3H). MS (ESI) *m/z* 474 $(M+H)^+$

11-benzyl-2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-methyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (XMD11-32). ^{1}H NMR (600 MHz, $CDCl_3$) 8.14 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.80 (dd, J = 7.2 Hz, 1.2 Hz, 1H), 7.39 (d, J = 7.2 Hz, 2H), 7.31 (m, 2H), 7.25 (m, 2H), 7.17 (m, 1H), 7.08 (m, 2H), 6.59 (dd, J = 8.4 Hz, 3.0 Hz, 1H), 6.54 (d, J = 2.4 Hz, 1H), 5.12 (br, 2H), 3.86 (s, 3H), 3.54 (s, 3H), 3.22 (m, 4H), 2.65 (m, 4H), 2.40 (s, 3H)**.** MS (ESI) *m/z* 536 $(M+H)^+$

2-((2-methoxy-5-(4-(4-methylpiperazin-1-yl)piperidine-1-carbonyl)phenyl)amino)-5,11-dimethyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (XMD11-56). ^{1}H NMR (600 MHz, MeOD-*d4*) 8.42 (d, J = 1.8 Hz, 1H), 8.27 (s, 1H), 7.77 (dd, J = 7.8 Hz, 1.8 Hz, 1H), 7.53 (m, 1H), 7.26 (d, J = 8.4 Hz, 1H), 7.21 (m, 2H), 7.12 (d, J = 8.4 Hz, 1H), 4.69 (s, 2H), 4.02 (s, 2H), 3.98 (s, 3H), 3.52 (s, 4H), 3.48 (s, 3H), 3.46 (s, 3H), 3.41 (s, 4H), 2.93 (s, 3H), 2.12 (s, 2H), 1.68 (s, 2H). MS (ESI) *m/z* 571 $(M+H)^+$

2-((4-(4-hydroxypiperidin-1-yl)-2-methylphenyl)amino)-5,11-dimethyl-5,11-dihydro-6*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6-one (XMD12-68). ^{1}H NMR (600 MHz, DMSO-d_6) 8.52 (s, 1H), 8.21 (s, 1H), 7.64 (dd, J = 7.8 Hz, 1.8 Hz, 1H), 7.46 (m, 1H), 7.18 (d, J = 8.4 Hz, 2.0 Hz, 2H), 7.13 (m, 1H), 6.76 (s, 1H), 6.71 (m, 1H), 4.63 (d, J = 3.0 Hz, 1H), 3.59 (m, 1H), 3.46 (m, 2H), 3.34 (s, 3H), 3.18 (s, 3H), 2.77 (m, 2H), 2.12 (s, 3H), 1.79 (m, 2H), 1.47 (m, 2H). MS (ESI) *m/z* 445 $(M+H)^+$

***N*-(4-((5,11-dimethyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)phenyl)methanesulfonamide (XMD17-86).** ^{1}H NMR (600 MHz, DMSO-d_6) 9.62 (s, 1H), 9.42 (s, 1H), 8.37 (s, 1H), 7.68 (m, 3H), 7.48 (m, 1H), 7.25 (d, J = 8.4 Hz, 1H), 7.14 (m, 3H), 3.80 (s, 3H), 3.34 (s, 3H), 2.91 (s, 3H). MS (ESI) *m/z* 425 $(M+H)^+$

ASSOCIATED CONTENT

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Author Contributions

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Conflict of Interest

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ABBREVIATIONS

DCLK1, doublecortin-like kinase 1. SEM, standard error of the mean. MSA, mobility shift assay.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Experimental protocols, compound characterization, molecular formula strings, supporting figures (PDF)

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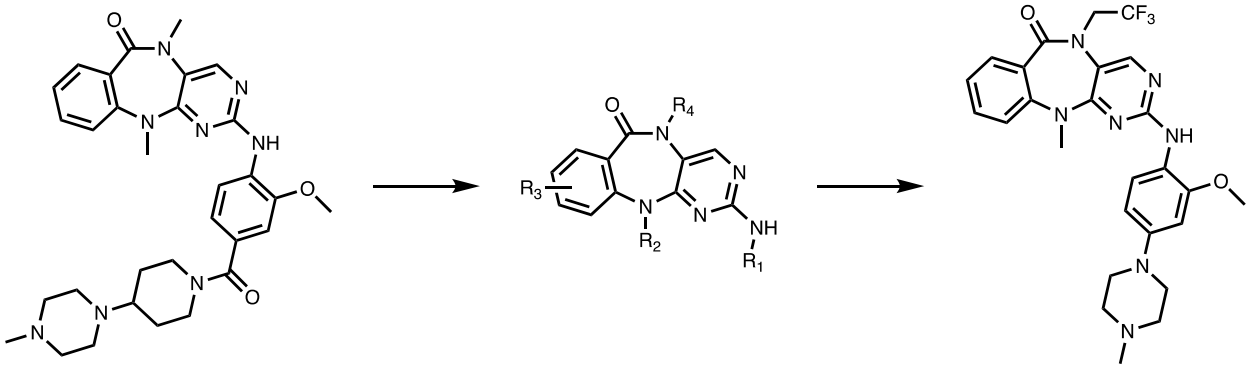

Kinase / bromodomain binding scaffold.

SAR

Highly selective DCLK1/2 inhibitor.