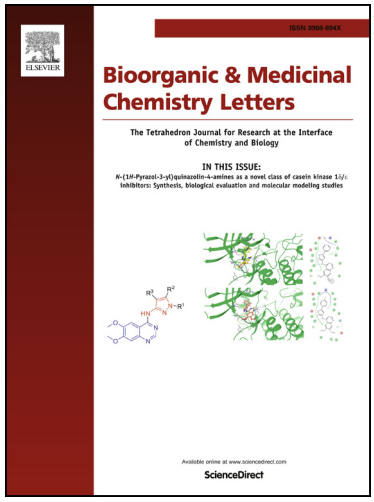


Discovery of Potent and Selective CDK8 Inhibitors through FBDD Approach

Xingchun Han, Min Jiang, Chengang Zhou, Zheng Zhou, Zhiheng Xu, Lisha Wang, Alexander V. Mayweg, Rui Niu, Tai-Guang Jin, Song Yang

PII: S0960-894X(17)30787-4
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.07.080
Reference: BMCL 25193

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 15 June 2017
Revised Date: 28 July 2017
Accepted Date: 29 July 2017

Please cite this article as: Han, X., Jiang, M., Zhou, C., Zhou, Z., Xu, Z., Wang, L., Mayweg, A.V., Niu, R., Jin, T-G., Yang, S., Discovery of Potent and Selective CDK8 Inhibitors through FBDD Approach, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.07.080

Discovery of Potent and Selective CDK8 Inhibitors through FBDD Approach

Xingchun Han[a,*], Min Jiang[a], Chengang Zhou[a], Zheng Zhou[b], Zhiheng Xu[b], Lisha Wang[b], Alexander V. Mayweg[a], Rui Niu[c], Tai-Guang Jin[c], and Song Yang [a,*]

[a] *Medicinal Chemistry, Roche Innovation Center Shanghai, Bldg 5, 720 Cailun Road, Shanghai 201203, China*
[b] *Chemical Biology, Roche Innovation Center Shanghai, Bldg 5, 720 Cailun Road, Shanghai 201203, China*
[c] *Discovery Oncology, Roche Innovation Center Shanghai, Bldg 5, 720 Cailun Road, Shanghai 201203, China*

* Corresponding author.
E-mail address: cyrus.han@roche.com (Xingchun Han)
simon.yang@roche.com (Song Yang)

Abstract: A fragment library screen was carried out to identify starting points for novel CDK8 inhibitors. Optimization of a fragment hit guided by co-crystal structures led to identification of a novel series of potent CDK8 inhibitors which are highly ligand efficient, kinase selective and cellular active. Compound **16** was progressed to a mouse pharmacokinetic study and showed good oral bioavailability.

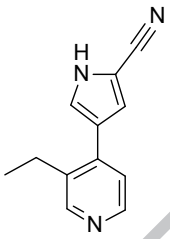
Keywords:
CDK8; inhibitor; fragment-based drug discovery (FBDD)

CDK8 is a Cyclin C-dependent serine-threonine kinase and a subunit of the Mediator complex that functions as a transcriptional regulator. In the past several years, multiple substrates of CDK8 have been identified, including histone H3, the RNA polymerase II (RNAPII) C-terminal domain (CTD), subunits of general transcription factors (GTFs) and certain transactivators.[1,2] CDK8 plays important roles in oncogenic signalling pathways, including the Wnt-β-catenin pathway,[3,4] the TGFβ signalling pathway,[5] the p53 pathway, [6,7] the serum and hypoxia response network,[8, 9] the Notch and STAT1 signaling,[10,11] and those governed by SMADs and the thyroid hormone receptor.[5, 12] Furthermore, CDK8 was reported to be frequently dysregulated in breast cancer,[13] colon cancer,[3] gastric cancer[14] and melanoma[15]. Inhibition of CDK8 by short hairpin RNA (shRNA) surpresses proliferation in cancer cells, and induces cell cycle arrest and apoptosis in *in vitro* and *in vivo* models.[3] Thus,

targeting gene transcription through CDK8 is a potential approach for cancer therapy. Recently, there has been increased interest in targeting CDK8 and a number of CDK8 inhibitors have been reported by pharmaceutical companies and academic groups.[16] Here, we disclose the identification of a novel class of potent and selective CDK8 inhibitors through fragment-based drug discovery (FBDD) approach.

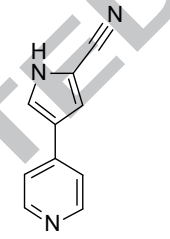
Our approach began with a fragment screening of ~6500 compounds (heavy atom count less than 19 atoms) against CDK8 in a biochemical assay at a concentration of 100 μM to identify 403 primary hits showing > 70% inhibition. Evaluation of 227 selected primary hits by dose response narrowed down the list to 48 interesting fragments showing IC_{50} < 50 μM with ligand efficiency (LE) > 0.3.

In this manuscript we outline our efforts to optimize one fragment hit (**1**) which had an IC_{50} of 4.63 μM against CDK8 with an LE of 0.49.[17] Initial exploration was conducted in an "SAR by catalog" approach utilizing similar compounds in Roche collection. This effort led to the identification of several compounds with improved potency, and accordingly higher ligand efficiency. As shown in Figure 1, removal of the ethyl group (compound **2**) resulted in a 16-fold increase in potency. Replacement of the cyano in compound **2** with a primary amide led to compound **3** showing slightly improved potency. *N*-monomethylation of the carbamoyl group (compound **4**) resulted in a ~2-fold loss in potency.



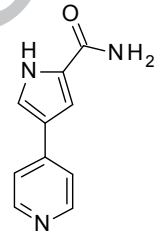
1

IC50=4.63μM
LE=0.49



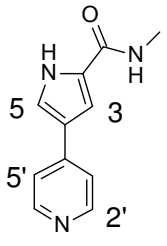
2

IC50=0.28μM
LE=0.69



3

IC50=0.17μM
LE=0.66



4

IC50=0.41μM
LE=0.58

Figure 1. Potency and ligand efficiency of compounds **1~4**

To get a better understanding of the binding mode to assist compound design, we tried to co-crystalize the fragments with CDK8/cyclin C complex, and a co-crystal structure of compound **4** was obtained (Figure 2). The X-ray structure revealed that compound **4** occupied the ATP binding region of the CDK8/cyclin C complex which adopted an active conformation. In the hinge region, the pyridine nitrogen atom establishes a hydrogen-bonding interaction with the residue of Ala100, and the C-H at position 2′ of pyridine (H-2′, Figure 1) forms C-H···O interaction with Asp98. Another direct hydrogen bond is formed between the amide carbonyl and the side chain of Lys52. A water molecule forms a hydrogen bond with the pyrrole nitrogen and thus indirectly links the compound **4** to the protein. The structure suggested that a small hydrophobic group at position 5 of the pyrrole or position 5′ of the pyridine would potentially fill a hydrophobic site in the protein, and a small group would be tolerated at position 2′ of the pyridine.

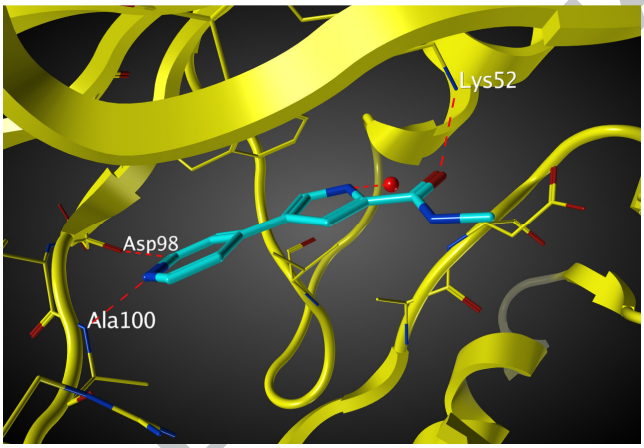
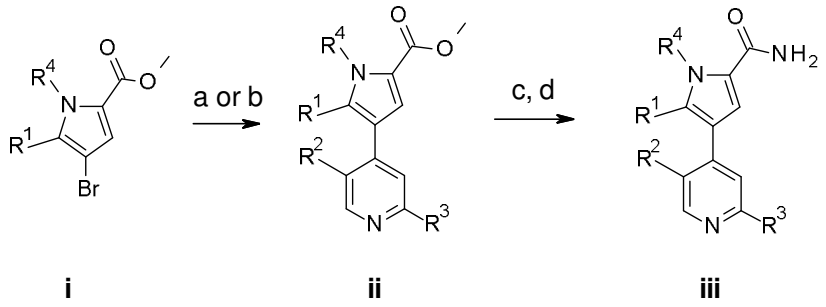


Figure 2. Co-crystal structure of compound **4** in CDK8/cyclin C (PDB code: 5XQX). Compound **4** is highlighted in cyan sticks. Red dash lines represent the key hydrogen bonds and C-H···O interaction.

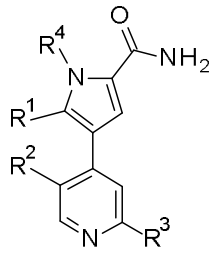
Based on these observations, we first synthesized compounds **5~11** to explore the SAR of these positions with the primary amide group fixed at position 2 of the pyrrole. The general synthetic strategy is depicted in Scheme 1. The key intermediate **ii** was synthesized by Suzuki coupling of substituted 4-Br pyrroles with appropriate 4-pyridylboronic acids, or converting the 4-Br pyrroles into the corresponding boronate ester followed by coupling with appropriate 4-Br pyridines.



Scheme 1. General synthetic approach for compounds **5~13**: (a) 4-pyridylboronic acids, Cs_2CO_3, $Pd(dppf)Cl_2$, EtOH/water, reflux; (b) $Pd_2(dba)_3$, bis(pinacolato)diboron, KOAc, butyldi-1-adamantylphosphine, DME, 100 °C 1 h, then add 4-bromo-pyridines, K_2CO_3, dioxane/ water, microwave 110 °C 1.5 h; (c) NaOH or LiOH, ethanol/water; (d) HATU or EDCI/HOBt, NH_3.

Table 1

Potency and Ligand efficiency for compounds **5~13**



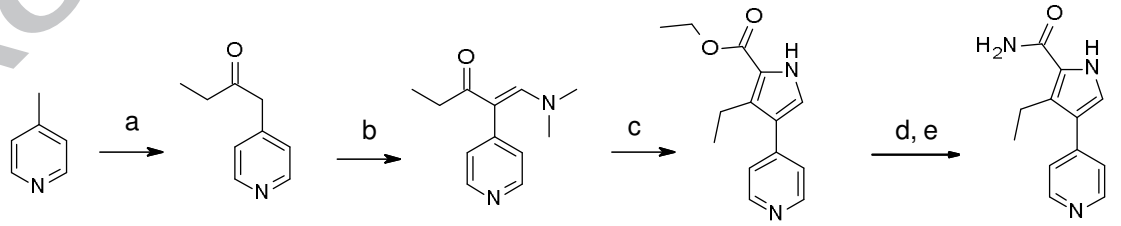
Compd	R^1	R^2	R^3	R^4	CDK8 IC_{50} (μM)	LE
5	Me	H	H	H	0.24	0.60
6	Cl	H	H	H	0.16	0.62
7	H	Cl	H	H	0.019	0.70
8	H	Me	H	H	0.087	0.64
9	H	Br	H	H	0.037	0.68
10	H	H	Me	H	0.48	0.58
11	H	H	Cl	H	0.35	0.59
12	H	H	H	Me	4.95	0.48
13	H	H	H	Bn	0.17	0.44

As shown in Table 1, the addition of a small hydrophobic group at the R^2 position generated compounds **7~9** with improved potency. Specifically, compound **7** showed the

best potency with an IC_{50} of 0.019 μM and LE up to 0.70. Methyl and chloro substitutions were well tolerated at the R^1 position (compounds **5** and **6**), but they led to diminished potency at the R^3 position (compounds **10** and **11**).

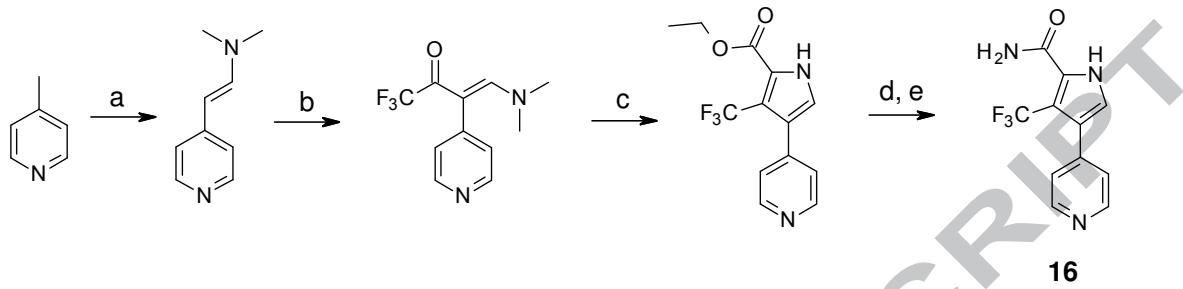
Compounds **12** and **13** (Table 1) were also synthesized using the route in Scheme 1 to explore the position 1 of the pyrrole. Methylation of the pyrrole nitrogen resulted in compound **12** with a ~29-fold loss in potency and a concomitant reduction in LE from 0.66 to 0.48, possibly due to the blockage of the hydrogen bonding interaction mediated by water. Unexpectedly, benzylation of the pyrrole nitrogen as in compound **13** did not diminish the potency. As the benzyl group also blocked the water mediated hydrogen bond, and might not be tolerated due to its relatively large size based on the co-crystal structure of compound **4** in CDK8, it was hypothesized that compound **13** and some other analogues might adopt a flip-over binding mode, in which the pyrrole nitrogen pointed outward while the hinge interactions were kept. The proposed flip-over binding mode also suggested that a small hydrophobic group at the position 3 of the pyrrole (R^5, Table 2) would potentially enhance the hydrophobic interaction.

To test the hypothesis we synthesized compounds **14~19**. Synthesis of compounds **14** and **15** started from 4-methylpyridine. Scheme 2 depicts the synthesis of compound **15** as a representative procedure. Deprotonation of the 4-methylpyridine by LDA, followed by treatment with acyl chloride afforded ketone **iv**. The ketone was heated with DMF-DMA to give intermediate **v**, which was then heated with diethyl aminomalonate to give pyrrole **vi** with an ester group. Saponification, followed by coupling with ammonia afforded the desired compound **15**. The trifluoromethyl analogue **16** was prepared starting from enamine formation of 4-methylpyridine with 1-*tert*-butoxy-*N*,*N*,*N*',*N*'- tetramethylmethanediamine. The route is shown in Scheme 3. A similar approach as the one shown in Scheme 1 was used to synthesize compounds **17**, **18** and **19**.



Scheme 2. Synthesis of compound 15: (a) LDA, THF, -78 °C, then propanoyl chloride, 21%; (b) DMF-DMA, dioxane, reflux, 8 h; (c) diethyl aminomalonate, acetic acid, reflux, 14 h, 20

% for 2 steps; (d) NaOH, ethanol/water, reflux, 4 h, 69%; (e) EDCI/HOBt, NH_3, DMF, rt, 5 h, 98%.

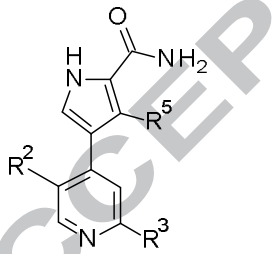


Scheme 3: (a) 1-*tert*-butoxy-*N*,*N*,*N*',*N*'- tetramethylmethanediamine, DMF, 130 ^{o}C, 16 h, 65%; (b) $(CF_3CO)O$, Et_3N, DCM, rt, overnight, 41%; (c) diethyl aminomalonate, acetic acid, 140 ^{o}C, 1 h, 11%; (d) conc. HCl, 80 ^{o}C, 4 h, 6%; (e) EDCI/HOBt, NH_3, DMF 1.5 h, 43%.

As can be seen from Table 2, the compounds with introduction of R^5 achieved very good potency, in which compound **16** was the most potent one (IC_{50} = 0.003 μM) and compound **17** achieved the highest ligand efficiency (LE = 0.76). The compounds were then tested in *in vitro* cell proliferation assay on AGS human gastric adenocarcinoma cells[18] (CDK8 wild-type) and showed sub-micromolar to low micromolar activity.

Table 2

Potency, Ligand efficiency, and cellular activity for compounds **14~19**



Compd	R^2	R^3	R^5	CDK8 IC_{50} (μM)	LE	AGS IC_{50} (μM)
14	H	H	Me	0.008	0.74	0.80
15	H	H	Et	0.013	0.67	1.12
16	H	H	CF_3	0.003	0.65	0.27
17	H	H	Cl	0.005	0.76	0.37
18	H	Me	Me	0.019	0.66	NA

19	Cl	H	Me	0.010	0.68	1.17

A co-crystal structure of compound **17** bound to CDK8 was subsequently generated and it confirmed our proposed flip-over binding mode (Figure 3). As predicted, the pyrrole nitrogen points outward and thus the chloro fills in the hydrophobic site. The pyridine nitrogen atom forms a hydrogen bond with Ala100 in the hinge region, while the primary amide forms a hydrogen bond with Asp173. A water molecule forms hydrogen networks linking the compound to Asn156 and Ala155.

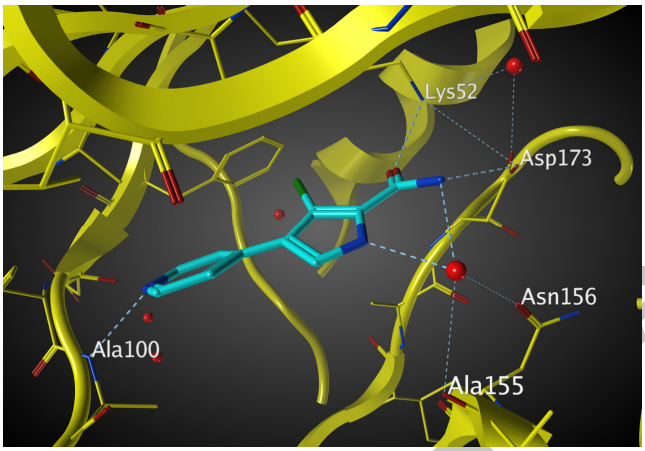


Figure 3. Co-crystal structure of compound **17** in CDK8/cyclin C (PDB code: 5XS2). Compound **17** is highlighted in cyan sticks. Cyan dash lines represent the key hydrogen bonds.

In order to investigate the kinase selectivity, compound **14** was measured against a panel of 43 kinases and this compound demonstrated an excellent kinase selectivity profile. At a concentration of 1 μM of the compound **14**, the inhibition against all kinases was less than 20% (Figure 4).

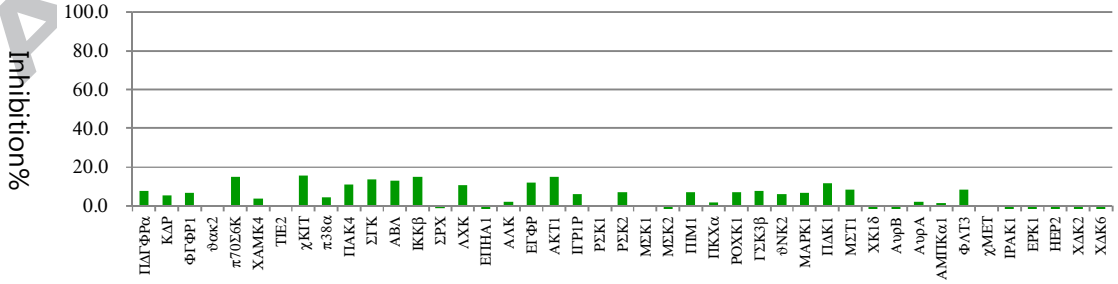


Figure 4. Kinase selectivity profile of compound **14**.

To understand the in vivo pharmacokinetic properties of this chemical series, compound **16** was selected for a mouse PK study. This compound displayed low systemic clearance, very good exposure and oral bioavailability (Table 3).

Table 3

Mouse pharmacokinetic profile of compound **16**.

Route	IV	PO
Dose level (mg/kg)	5	25
AUC	9378	25952
CL (mL/min/kg)	8.87	NA
Cmax (ug/L)	9940	12740
Tmax (h)	NA	0.25
T1/2 (h)	0.84	7.91
Vss (L/kg)	0.49	NA
Bioavailability (%)	NA	57.5

In the work presented herein, using fragment based screening followed by "SAR by catalog" approach, we have discovered low molecular weight compounds to inhibit CDK8. With the guidance of co-crystal structures, optimization of the compounds led to the identification of a group of potent and selective CDK8 inhibitors which are cellular active and highly ligand efficient. The potent compound **16** with an IC_{50} of 0.003 μM against CDK8 represents >1500-fold improvement in potency over the initial fragment hit. Compound **16** also showed good oral bioavailability in a mouse PK study. As the molecular weight is still low, this chemical series has ample space for further optimization of *in vitro* and *in vivo* properties.

References and notes

[1] Bernecky C, Grob P, Ebmeier CC, Nogales E, Taatjes DJ. Molecular architecture of the human Mediator-RNA polymerase II-TFIIF assembly. *PLoS Biol*. 2011;9(3):e1000603.
[2] Galbraith MD, Donner AJ, Espinosa JM. CDK8: a positive regulator of transcription. *Transcription.* 2010;1(1):4-12.
[3] Firestein R, Bass AJ, Kim SY, Dunn IF, Silver SJ, Guney I, Freed E, Ligon AH, Vena N, Ogino S, Chheda MG, Tamayo P, Finn S, Shrestha Y, Boehm JS, Jain S, Bojarski E, Mermel C, Barretina J, Chan JA, Baselga J, Tabernero J, Root DE, Fuchs CS, Loda M, Shivdasani RA, Meyerson M, Hahn WC. CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. *Nature* 2008;455(7212):547–551.

[4] Morris EJ, Ji J-Y, Yang F, Di Stefano L, Herr A, Moon NS, Kwon EJ, Haigis KM, Näär AM, Dyson NJ. E2F1 represses beta-catenin transcription and is antagonized by both pRB and CDK8. *Nature* 2008;455(7212):552-556.

[5] Alarcón C1, Zaromytidou AI, Xi Q, Gao S, Yu J, Fujisawa S, Barlas A, Miller AN, Manova-Todorova K, Macias MJ, Sapkota G, Pan D, Massagué J. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. *Cell* 2009;139(4):757-769.

[6] Donner AJ, Szostek S, Hoover JM, Espinosa JM. CDK8 is a stimulus-specific positive coregulator of p53 target genes. *Mol Cell* 2007;27(1):121-133.

[7] Donner AJ, Hoover JM, Szostek SA, Espinosa JM. Stimulus-specific transcriptional regulation within the p53 network. *Cell Cycle* 2007;6(21):2594-2598.

[8] Donner AJ, Ebmeier CC, Taatjes DJ, Espinosa JM. CDK8 is a positive regulator of transcriptional elongation within the serum response network. *Nat Struct Mol Biol* 2010;17(2):194-201.

[9] Galbraith MD, Allen MA, Bensard CL, Wang X, Schwinn MK, Qin B, Long HW, Daniels DL, Hahn WC, Dowell RD, Espinosa JM., HIF1A employs CDK8-mediator to stimulate RNAPII elongation in response to hypoxia. *Cell* 2013;153(6):1327–1339.

[10] Fryer CJ, White JB, Jones KA. Mastermind recruits CycC:CDK8 to phosphorylate the Notch ICD and coordinate activation with turnover. *Mol Cell* 2004;16(4):509–520.

[11] Bancerek J, Poss ZC, Steinparzer I, Sedlyarov V, Pfaffenwimmer T, Mikulic I, Dölken L, Strobl B, Müller M, Taatjes DJ, Kovarik P. CDK8 kinase phosphorylates transcription factor STAT1 to selectively regulate the interferon response. *Immunity* 2013;38(2):250-262.

[12] Belakavadi M, Fondell JD. CDK8 positively cooperates with mediator to promote thyroid hormone receptordependent transcriptional activation. *Mol Cell Biol* 2010;30(10):2437-2448.

[13] Broude EV, Győrffy B, Chumanevich AA, Chen M, McDermott MS, Shtutman M, Catroppo JF, Roninson IB. Expression of CDK8 and CDK8-interacting Genes as Potential Biomarkers in Breast Cancer. *Curr Cancer Drug Targets*. 2015;15(8):739-749.

[14] Kim MY, Han SI, Lim SC. Roles of cyclin-dependent kinase 8 and β-catenin in the oncogenesis and progression of gastric adenocarcinoma. *Int J Oncol*. 2011;38(5):1375-1383.

[15] Kapoor A, Goldberg MS, Cumberland LK, Ratnakumar K, Segura MF, Emanuel PO, Menendez S, Vardabasso C, Leroy G, Vidal CI, Polsky D, Osman I, Garcia BA, Hernando E, Bernstein E. The Histone Variant macroH2A Suppresses Melanoma Progression Through Regulation of CDK8. *Nature* 2010;468(7327):1105-1109.

[16] (a) Rzymski T, Mikula M, Wiklik K, Brzózka K. CDK8 kinase - An emerging target in targeted cancer therapy. *Biochim Biophys Acta*. 2015;1854(10 Pt B):1617-1629. (b) Pelish HE, Liau BB, Nitulescu II, Tangpeerachaikul A, Poss ZC, Da Silva DH, Caruso BT, Arefolov A, Fadeyi O, Christie AL, Du K, Banka D, Schneider EV, Jestel A, Zou G, Si C, Ebmeier CC, Bronson RT, Krivtsov AV, Myers AG, Kohl NE, Kung AL, Armstrong SA, Lemieux ME, Taatjes DJ, Shair MD. Mediator kinase inhibition further activates super-enhancer-associated genes in AML. *Nature*. 2015;526(7572):273-276. (c) Czodrowski P, Mallinger A, Wienke D, Esdar C, Pöschke O, Busch M, Rohdich F, Eccles SA, Ortiz-Ruiz MJ, Schneider R, Raynaud FI, Clarke PA, Musil D, Schwarz D, Dale T, Urbahns K, Blagg J, Schiemann K. Structure-Based Optimization of Potent, Selective, and Orally Bioavailable CDK8 Inhibitors Discovered by High-Throughput Screening. *J Med Chem*. 2016;59(20):9337-9349. (d) Koehler MF, Bergeron P, Blackwood EM, Bowman K, Clark KR, Firestein R, Kiefer JR, Maskos K, McCleland ML, Orren L, Salphati L, Schmidt S, Schneider EV, Wu J, Beresini MH. Development of a Potent, Specific CDK8 Kinase Inhibitor Which Phenocopies CDK8/19 Knockout Cells. *ACS Med Chem Lett* 2016;7(3):223–228. (e) Mallinger A, Schiemann K, Rink C, Stieber F, Calderini M, Crumpler S, Stubbs M, Adeniji-Popoola O, Poeschke O, Busch M, Czodrowski P, Musil D, Schwarz D, Ortiz-Ruiz MJ, Schneider R, Thai C, Valenti M, de Haven Brandon A, Burke R, Workman P, Dale T, Wienke D, Clarke PA, Esdar C, Raynaud FI, Eccles SA, Rohdich F, Blagg J. Discovery of Potent, Selective, and Orally Bioavailable Small-Molecule Modulators of the Mediator Complex-Associated Kinases CDK8 and CDK19. *J Med Chem*. 2016;59(3):1078-1101. (f) Bergeron P, Koehler MF, Blackwood EM, Bowman K, Clark K, Firestein R, Kiefer JR, Maskos K, McCleland ML, Orren L, Ramaswamy S, Salphati L, Schmidt S, Schneider EV, Wu J1, Beresini M. Design and Development of a Series of Potent and Selective Type II Inhibitors of CDK8. *ACS Med Chem Lett*. 2016;7(6):595-600. (g) Rzymski T, Mikula M, Żyłkiewicz E, Dreas A, Wiklik K, Gołas A, Wójcik K, Masiejczyk M, Wróbel A, Dolata I, Kitlińska A, Statkiewicz M, Kuklinska U, Goryca K, Sapała Ł, Grochowska A, Cabaj A, Szajewska-Skuta M, Gabor-Worwa E, Kucwaj K, Białas A, Radzimierski A, Combik M, Woyciechowski J, Mikulski M, Windak R, Ostrowski J, Brzózka K. SEL120-34A is a novel CDK8 inhibitor active in AML cells with high levels of serine phosphorylation of STAT1 and STAT5 transactivation domains. *Oncotarget*. 2017;8(20):33779-33795.

[17] Patent applications describing our CDK8 inhibitors have been published. Assays and full experimental procedures for analogues in this letter are contained in the patent WO2014154723A1.

[18] beta-catenin, K-ras and PIK3CA genes are mutated in AGS cell line: (a) Yoon YK, Kim HP, Han SW, Hur HS, Oh DY, Im SA, Bang YJ, Kim TY. Combination of EGFR and MEK1/2 inhibitor shows synergistic effects by suppressing EGFR/HER3-dependent AKT activation in human gastric cancer cells. *Mol Cancer Ther.* 2009;8(9):2526-2536. (b) Ikenoue T, Ijichi H, Kato N, Kanai F, Masaki T, Rengifo W, Okamoto M, Matsumura M, Kawabe T, Shiratori Y, Omata M. Analysis of the beta-catenin/T cell factor signaling pathway in 36 gastrointestinal and liver cancer cells. *Jpn J Cancer Res.* 2002;93(11):1213-20.

1

IC50=4.63µM
LE=0.49
Fragment hit

16

IC50=0.003µM
LE=0.65