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A diaminocyclohexyl analog of SNS-032 with improved permeability and bioavailability properties

Ingrid C. Choong *, Iana Serafimova, Junfa Fan, David Stockett, Erica Chan, Sravanthi Cheeti, Yafan Lu, Bruce Fahr, Phuongly Pham, Michelle R. Arkin, Duncan H. Walker, Ute Hoch*

Medicinal Chemistry, Sunesis Pharmaceuticals, 341 Oyster Point Boulevard, South San Francisco, CA, 94080, USA

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ABSTRACT

The identification of a selective CDK2, 7, 9 inhibitor **4** with improved permeability is described. Compound **4** exhibits comparable CDK selectivity profile to SNS-032, but shows improved permeability and higher bioavailability in mice.

The CDKs (cyclin-dependent kinases) are a family of serine/threonine protein kinases that, in conjunction with their cyclin (cyc) partners, play key roles in cell-cycle progression and transcriptional regulation.[1–3] Cell-cycle regulatory CDKs include cycD/CDK4 and cycD/CDK6, as well as cycE/CDK2, which sequentially phosphorylate the retinoblastoma protein to facilitate G1 → S transition. CDK2 and CDK1 paired with cycA, and cycB/CDK1 are required for orderly progression through S-phase and the G2 → M transition, respectively. Transcriptional CDKs include cycH/CDK7 and cycT/CDK9. The CDK7 subunit of transcription factor II H (TFIIH) phosphorylates the carboxy-terminal domain (CTD) of RNA polymerase II (pol II) on serine 5 at early stages of the transcriptional cycle.[4] CDK9, a member of the elongation factor P-TEFb, phosphorylates serine 2 on the CTD to transition RNA pol II into productive elongation.[5] Inhibition of these kinases is predicted to have the greatest effect on the expression of proteins with short half-lives, many of which are encoded by antiapoptotic and growth regulatory genes. Some of the CDKs are involved in both processes. For example, CDK7 is a component of the CDK-activating kinase (CAK). Full activation of CDKs 1, 2, 4, 5 and 6 requires phosphorylation by CAK; therefore, CDK7 serves as a 'master regulator' of the cell cycle. Inappropriate activation of both cell-cycle and transcriptional-regulatory CDKs can lead to unregulated proliferation, avoidance of apoptosis, and the presence of genetic instability in cancer cells. These attributes, which are among the hallmarks of cancer, suggest that CDKs may be important targets for cancer therapeutics. SNS-032 (Fig. 1, formerly BMS-387032) is such a dual-acting CDK inhibitor, with potency and selectivity against CDK2, 7 and 9 (Table 3). SNS-032 is currently in a phase 1 clinical trial for multiple myeloma and chronic lymphocytic leukemia as an intravenous agent.

Early reports showed that SNS-032 has oral bioavailability of about 31% in rats. Bioavailability was limited by absorption rather than extensive first-pass metabolism.[6] Since SNS-032 is a substrate of P-glycoprotein, this efflux transporter may be responsible for limiting its absorption. With an interest in a potential oral CDK program, we sought to develop a backup inhibitor to SNS-032 with comparable CDK2, 7, and 9 inhibitory activities, but with improved permeability and lack of transporter-mediated efflux. We hypothesized that improving the permeability of SNS-032 would possibly provide an inhibitor with improved oral bioavailability. In order to conserve the specificity and potency of SNS-032, we chose to maintain the general scaffold of SNS-032, but to replace the isonipecotic amide fragment and explore *N*-alkyl instead of *N*-acyl moieties.

Inhibitors **2**–**10** (Tables 1 and 2) were synthesized as summarized in Scheme 1. Intermediate **11** was prepared as reported pre-

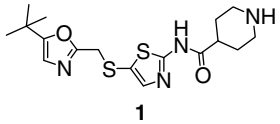

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Figure 1. SNS-032.

* Corresponding authors. Tel.: +1 650 619 6115.
E-mail addresses: ichoong2@gmail.com (I.C. Choong), uhoch@nektar.com (U. Hoch).

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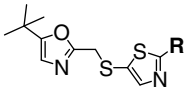
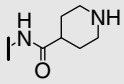
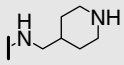
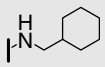
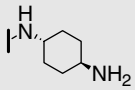
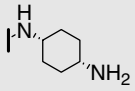
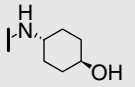
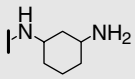
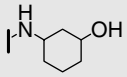
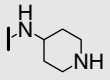
Table 1
Inhibition of enzymatic CDK2 and cellular CDK9



Compound	R	IC_{50} (μM)	
		CDK2/cycA[a]	HCS CDK9[b]
1 (SNS-032)		0.046	0.458
2		0.229	>10
3		0.168	>10
4		0.030	0.355
5		0.503	>10
6		0.007	3.5
7		0.158	>10
8		0.028	>10
9		0.009	>10
10		0.538	>10
11		0.690	>10

[a] IMAP enzymatic assay measuring inhibition of phosphorylation of fluorescein-labeled substrate.
[b] ArrayScan high-content cellular assay measuring phospho-ser2 of RNA pol II.

viously.[7] Treatment of **11** with $NaNO_2$ in CH_3CN provided 2-bromothiazole **12** in 40–60% yield. Exposure of **12** to various primary and secondary amines in dimethylacetamide at 110 °C afforded the final products, **2–10**.

All the compounds were screened in a CDK2/cycA[8] biochemical assay and a CDK9 high-content screen cellular assay[9] and compared against SNS-032 (Compound **1**). Inhibition of CDK2/cycA was readily achieved, consistent with CDK2/SNS-032 crystallography which shows that the piperidinyl ring is directed into solvent,[10] and therefore replaceable. However, sub-micromolar CDK9 cellular activity was only observed for the 1,4-diaminocyclohexyl substitution in **4** (Table 1).

Further exploration around **4** indicated a CDK9 preference for *trans*-1,4-diaminocyclohexyl fragment over the *cis*-isomer **5**, as well as the 1,4-regioisomer vs the 1,3-isomer **7** (Table 1). Similar SAR was not determined for SNS-032 amide series.

Compound **4** was further evaluated in in vitro studies, where it was shown to have comparable activities against CDKs 2, 7, and 9 (Table 2). More significantly, **4** exhibited over 5-fold improved permeability in MDCK[11] cells over **1** (Table 2). On the basis of the permeability data and acceptable liver microsomal stability,[12] **4** was selected for pharmacokinetic studies.

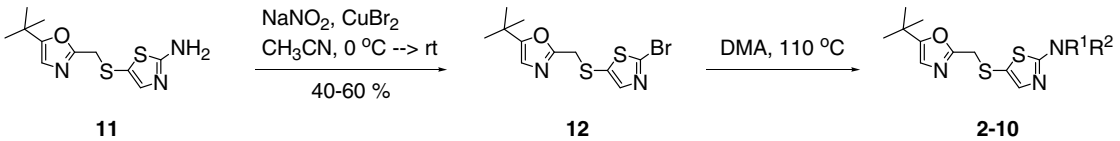
Plasma concentration–time profiles after IV and PO administration of 5 and 10 mg/kg, respectively, of compounds **1** and **4** are shown in Figure 2.[13] Pharmacokinetic parameters are summarized in Table 3. Pharmacokinetics after intravenous administration are similar for **1** and **4**. Compounds **1** and **4** show moderate to high clearance, a large volume of distribution, and terminal half-lives of 0.6 and 1.9 h, respectively. After oral administration of 10 mg/kg, bioavailability of **1** was 14%, whereas **4** showed bioavailability of 62%. Administration of 30 mg/kg compound **4**, led to a more than dose-linear increase in AUC (4.4-fold increase for a 3-fold increase in dose), resulting in a bioavailability of 92%. The high bioavailability was maintained at the next higher dose level of 45 mg/kg. Given the similar pharmacokinetics after intravenous administration, the increased bioavailability of **4** compared to **1** may be the result of the improved permeability.

In summary, the incomplete bioavailability of compound **1** (SNS-032) in mice that resulted from poor absorption may be remedied by improving the permeability of the compound. Through replacing the *N*-isonipecotic fragment with the *N*-1,4-*trans*-diaminocyclohexyl fragment, we identified compound **4** which exhibits comparable CDK selectivity profile to SNS-032, but shows improved permeability and higher bioavailability in mice. Further evaluation of compound **4** is required to determine the effects of improved permeability on in vivo efficacy.

Table 2
In vitro profiles of **1** and **4**

Compound	IC_{50} (μM)[a]					MDCK		% Parent remaining after 30/60 min	
	CDK1/cycB	CDK2/cycA	CDK6/cycD	CDK7/cycH	CDK9/cycT	P_{app} $(A-B)$[b]	Efflux ratio[c]	HLM	MLM
1 (SNS-032)	0.480	0.038	nd[d]	0.062	0.004	1	15	94/87	96/94
4	0.763	0.020	>2	0.105	0.097	5.8	9.4	64/53	76/77

[a] Except for CDK2/cycA, all enzymatic data obtained from Upstate.
[b] Units = 10^{-6} cm/min.
[c] Efflux ratio = P_{app} $(B-A)/P_{app}$ $(A-B)$.
[d] No data.



Scheme 1. Synthesis of *N*-alkyl analogs.

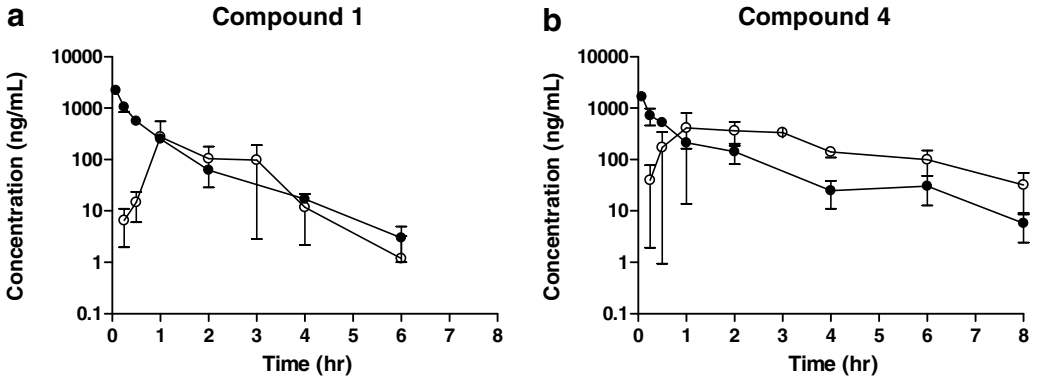


Figure 2. Concentration vs time profiles of compounds **1** and **4** in mice after 5 mg/kg IV (closed circles) and 10 mg/kg PO (open circles). Concentrations are the averages of three animals per timepoint.

Table 3
Pharmacokinetic parameters for **1** and **4**

Compound	Dose (mg/kg)	Route	C_{max} (ng/mL)	AUC (ng h/mL)	CL (mL/min/kg)	Vss (L/kg)	$t_{1/2}$ (h)	%F
1 (SNS-032)	5	iv	2230	1510	55	3	0.6	
	10	po	275	434			0.5	14
4	5	iv	1660	1160	72	6	1.9	
	10	po	411	1430			1.5	62
	30	po	2390	6390			1.2	92
	45	po	4600	10,700			1.2	100

References and notes

1. Sausville, E. A. *Trends Mol. Med.* **2002**, *8*, 32.
2. Shapiro, G. I. *J. Clin. Oncol.* **2006**, *24*, 1770.
3. Marshall, N. F. et al *J. Biol. Chem.* **1996**, *271*, 27176.
4. Akoulitchev, S.; Makela, T. P.; Weinber, R. A.; Reinberg, D. *Nature* **1995**, *377*, 557.
5. Marshall, N. F.; Price, D. H. *J. Biol. Chem.* **1995**, *270*, 12335.
6. Kamath, A. V.; Chong, S. *Cancer Chemother. Pharmacol.* **2005**, *55*, 110.
7. Kim, K. S.; Kimball, D. S.; Cai, Z.; Rawlins, D. B.; Misra, R. N.; Poss, M. A.; Webster, K. R.; Hunt, J. T.; Han, W. U.S. Patent 6,521,759, 2003.
8. Inhibition of CDK2-cyclinA was assayed with the IMAP (Molecular Devices) florescence polarization assay and was used according to the manufacturer's guidelines. Briefly, 1 mM DTT and 100 μM sodium vanadate was added to the IMAP tween reaction buffer. The final ATP concentration was 10 μM. CDK2/cyclinA was used at a final concentration of 2 nM (Cell Signaling, 7521) and the substrate, FAM-H1-peptide, was used at a final concentration of 100 μM (Molecular Devices, R7439). Compounds were serial diluted in an 11-point titration and IC_{50}s, the concentration required to inhibit enzyme activity by 50%, was determined for each compound under the assay conditions described.
9. HCT116 cell lines were obtained from ATCC. Array Scan: HCT116 cells were treated for 16 h with serial dilutions of compound and fixed and permeabilized with 100% MeOH. The cells were then stained with either anti-RNA polymerase II serine2 (Abcam #ab5095) antibody in combination with AlexaFluor 488 anti-rabbit IgG secondary antibody (Invitrogen #A11008). The cell nuclei were stained using Hoechst 33342 (Invitrogen #3570). Fluorescence levels in the cells were then analyzed by HCS using a Cellomics ArrayScan instrument.
10. Misra, R. N.; Xiao, H.; Kim, K. S.; Lu, S.; Han, W.; Barbosa, S. A.; Hunt, J. T.; Rawlins, D. B.; Shan, W.; Ahmed, S. Z.; Qian, L.; Chen, B.; Zhao, R.; Bednarz, M. S.; Kellar, K. A.; Mulheron, J. G.; Batorsky, R.; Roongta, U.; Kamath, A.; Marathe, P.; Ranadive, S. A.; Sack, J. S.; Tokarski, J. S.; Pavletich, N. P.; Lee, F. Y. F.; Webster, K. R.; Kimball, S. D. *J. Med. Chem.* **2004**, *47*, 1719.
11. Microsomal stability assays were performed in 100 mM sodium phosphate buffer, pH 7.4, containing 3.3 mM $MgCl_2$, 0.5 mg/mL liver microsomal protein, 1 μM test article, and 1 mM NADPH. The reaction mixture was allowed to equilibrate to 37 °C for 10 min before reactions were started by addition of NADPH. Aliquots were removed immediately, 30 min, and 60 min after addition of NADPH. Reactions were stopped by addition of acetonitrile containing internal standard (verapamil). Samples were placed on ice until centrifugation (4100*g*, 10 min) to remove protein content and analyzed by LC–MS/MS. Incubations with lidocaine and dextromethorphan served as positive controls and indicated that reactions functioned properly.
12. MDCK II cells were cultured in DMEM supplemented with 10% FBS and 100 U antibiotic solution (Cellgro) per milliliter. Cells were seeded into wells of 12-transwell plates at a seeding density of 50,000 cells/well and cultured for 5 days. Prior to addition of the dosing solutions, MDCK monolayers were washed with PBS. The bi-directional permeability studies were initiated by adding an appropriate volume of DMEM media containing 2 μM compound to either the apical (apical to basolateral transport) or basolateral (basolateral to apical transport) side of the monolayer. Aliquots were taken from both the apical and basolateral compartments after incubation for 3 h in a humidified CO_2 incubator at 37 °C. Samples were diluted with acetonitrile containing internal standard (verapamil) and SNS-032 concentrations were analyzed using a LC–MS/MS method. The permeability coefficient (P_{app}) was calculated as follows: $P_{app} = (1/A) \; C_0 \; (dQ/dt)$, with A representing the surface are of the membrane, C_0 the initial concentration, and dQ/dt the drug flux.
13. All animals used were handled according to Sunesis Pharmaceuticals, Inc. institutional guidelines. Compounds **1** and **4** were dissolved in 2.1 mM tartaric acid (pH 4.0) to obtain dosing solutions of 1, 3, or 4.5 mg/mL. Female, 6- to 8-week-old CD-1 (3 mice/timepoint) received a single IV dose of 5 mg/kg compound 1 or 2 via the tail vein at 5 mL/kg or single po doses of 10, 30, and 45 mg/kg via oral gavage at 10 mL/kg. After euthanasia, blood samples were collected via cardiac puncture at 0.083 (iv), 0.25, 0.5, 1, 2, 3 (po), 4, 6, and 8 h postdose into a K_2-EDTA tube and placed on ice until centrifugation to harvest plasma. Plasma samples were stored frozen until analyzed by LC–MS/MS. The lower limit of detection was 1 ng/mL. Back-calculated standards and quality control samples (15, 125, and 500 ng/mL) did not deviate by more than 20% from their nominal concentration. Concentrations per timepoint were averaged before estimating PK parameters.