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Modifications of the isonipecotic acid fragment of SNS-032: Analogs with improved permeability and lower efflux ratio

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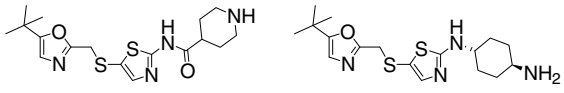
ABSTRACT

Modifications of the isonipecotic acid fragment of SNS-032 results in analogs which are more permeable and lower effluxed than SNS-032. The enantiomerically pure synthesis and the in vivo profile of analog **20** is described.

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 (CDK2, 4, 6 and 7) and transcriptional regulation (CDK7 and 9).[1–3] 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.[4–6]

SNS-032 (**1**, formerly BMS-387032, Fig. 1) is a dual-acting CDK inhibitor, with potency and selectivity against CDKs 2, 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. The incomplete oral bioavailability has been shown to be the result of poor absorption rather than extensive first-pass metabolism.[7] 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 CDK 2, 7, and 9 activities, but with improved permeability and lack of transporter-mediated efflux. Previous identification of **2** demonstrated that improving permeability resulted in significant higher bioavailabilities in mice.[8] Described here is a concurrent effort to identify a series which had both good permeability and lowered efflux.



1, SNS-032 **2**

Figure 1. SNS-032 and **2**.

In early efforts, we demonstrated that we could improve permeability and eliminate efflux by masking the isonipecotic secondary amine of **1** as a tertiary amine, sulfonamide or amide (data not shown). However, these compounds proved to be very unstable to liver microsomes, so we turned our attention to blocking potential P-glycoprotein binding with the secondary amine function through substitutions around the isonipecotic or nipecotic ring system.

Inhibitors **3–9** (Table 1) were synthesized as summarized in Scheme 1. Intermediate **10** was prepared as previously reported.[9] Under standard coupling conditions, treatment of **10** with different acids provided resulting amides in good yields. For *N*-Boc-protected analogs, deprotection with 4.0 N HCl in dioxane at 50 °C afforded the final products. The acids used above were commercially available or readily prepared according to the literature.[9,10] Specifically, **7–9** were synthesized through platinum reduction of the corresponding nicotinic acids to provided the saturated nipecotic acids as mixtures of *cis*-enantiomers. [11]

All the compounds were evaluated for inhibition of CDK2/cycA[12] enzymatic activity, inhibition of CDK9 phosphorylation of ser2 on RNA pol II[13] and permeability in MDCK cells.[14]

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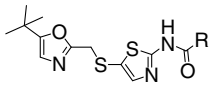
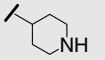
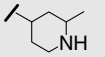
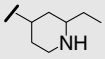
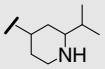
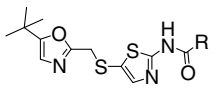
Table 1
In vitro profile of analogs **3**–**9**



Compound	R	IC_{50} (μM)		MDCK		% Parent Remaining @ 30/60 min	
		CDK2/cycA[a]	HCS CDK9[b]	P_{app} (A–B)[c]	Efflux ratio[d]	HLM	MLM
1		0.046	0.55	1.0	15	94/87	96/94
3		0.015	1.5	1.3	37	ND[e]	ND[e]
4		0.0030	0.68	4.5	13	60/41	ND[e]
5		0.017	0.81	3.2	16	33/7	24/1
6		0.020	0.55	7.5	6.0	83/74	68/44
7		0.095	1.6	25	3.0	12/4	7/2
8		0.014	0.37	24	2.0	71/49	26/8
9		0.14	0.27	14	2.5	60/39	49/27

[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.
[c] Units = 10^{-6} cm/min.
[d] Efflux ratio = P_{app} (B–A)/P_{app} (A–B).
[e] No data.

Table 2
In vitro profile of four isomers of **9**



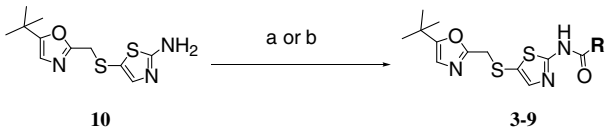
Compound	R	IC_{50} (μM)		MDCK		% remaining @ 30/60 min	
		CDK2/A[a]	HCS CDK9[b]	P_{app} (A–B)[c]	Efflux ratio[d]	HLM	MLM
9		0.14	0.27	14	2.5	60/39	49/27
18		0.036	3.2	10	6.3	57/29	50/33
19		0.0050	0.77	26	2.1	61/35	32/8
20		0.020	0.12	4.8	6.0	70/45	35/13
21		0.20	4.1	15	2.0	54/25	5/0

[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.
[c] Units = 10^{-6} cm/min.
[d] Efflux Ratio = P_{app} (B–A)/P_{app} (A–B).

Table 3
In vitro profile of **20**

Compound	IC_{50} (μM)[a]					MDCK		% Parent remaining @ 30/60 min	
	CDK1/cycB	CDK2/cycA	CDK4/cycD	CDK7/H	CDK9/T	P_{app} (A-B)[b]	Efflux Ratio[c]	HLM	MLM
1	0.48	0.038	0.93	0.062	0.0040	1.0	15	94/87	96/94
20	0.12	0.020	0.25	0.11	0.0040	4.8	6.0	70/45	35/13

[a] Except for CDK2/A, all enzymatic data obtained from Upstate.
[B] Units = 10^{-6} cm/min.
[c] Efflux ratio = P_{app} (B–A)/P_{app} (A–B).



Scheme 1. Synthesis of analogs. Reagents and conditions: (a) acid, DMAP, EDCI, DCM, rt; (b) a, then 4.0 M HCl in dioxane, 50 °C, 2 h.

Disappointingly, flanking substituents around the isonipecotic amine did not improve permeability or decrease efflux (compounds **3**–**5**). However, since nipecotic amide **6** showed good permeability and only moderate efflux, we decided to explore incorporating substituents on the nipecotic ring. Indeed, compounds incorporating a methyl substituent at various positions around the ring demonstrated improved permeability and lowered efflux (**7**–**9**). Given that **9** demonstrated the highest stability in mouse liver microsomes while maintaining CDK2/cycA and CDK9 activity, we synthesized the discrete enantiomers as well as the two other isomers not represented in the enantiomeric mixture of **9** to identify which isomer(s) had the most promising in vitro profile.

Synthesis of the four single isomers of **9** is summarized in Scheme 2. Commercially available (*S*)-3-bromo-2-methyl-propanol was oxidized with Dess–Martin periodinane to give aldehyde **12**, which was condensed with secondary amine under standard reductive amination conditions to afford tertiary amine **13**. Intramolecular alkylation of **13** with LDA at low temperature provided diasteroisomers **14** and **15**. Exchanging protecting groups followed by hydrolysis provided *N*-Boc protected nipecotic acid intermediates **16** and **17**. Standard amide coupling conditions provided diasteroisomers **18** and **19**, which were easily separated by column chromatography. Isomers **20** and **21** were synthesized following the same route using (*R*)-3-bromo-2-methyl-propan-ol as the starting material.

The in vitro profile of the four isomers of **9** is summarized in Table 2. Isomers **19** and **20** retained both biochemical activity against CDK2 and cellular activity in the HCS assay. More significantly, isomer **19** showed good permeability and low efflux. On the basis of the permeability data and acceptable liver microsomal stability (Table 3),[14,15] **20** is a potential candidate for pharmacokinetic studies.

In summary, through modifications of the isonipecotic acid fragment of SNS-032, we identified compound **20** which exhibits comparable CDK selectivity profile to SNS-032, but shows improved permeability and lower efflux. Pharmacokinetic studies will determine bioavailability in mice.

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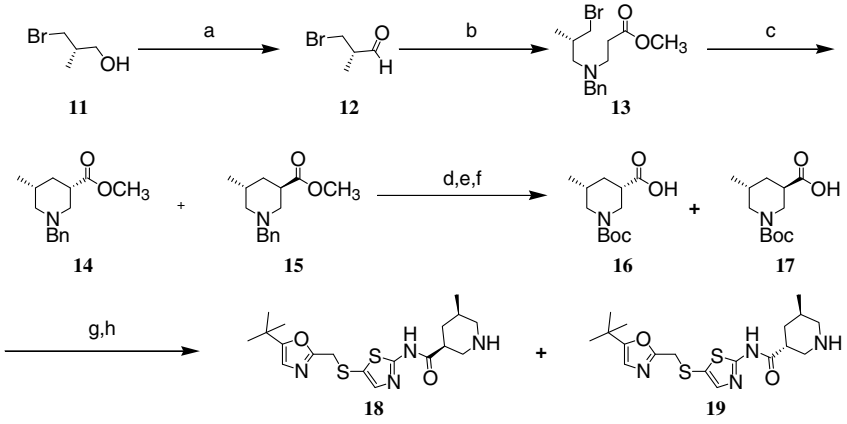
12. Inhibition of CDK2-cyclinA was assayed with the IMAP (Molecular Devices) fluorescence polarization assay and was used according to the manufacturer's guidelines. Briefly, 1 mM DTT and 100 μM sodium vanadate was added to the IMAP tween reaction buffer. The final ATP concentration



Scheme 2. Synthesis of single isomers of **9**. Reagents and conditions: (a) Dess–Martin periodinane, dichloromethane, 95%; (b) $BnNHCH_2CH_2CO_2CH_3$, $NaHB(OAc)_3$, dichloroethane, HOAc, 75%; (c) LDA, ether, HMPA, −78 °C, 80%; (d) Pd-C, H_2, 95%; (e) $(Boc)_2O$, 1.0 M NaOH, dioxane, 97%; (f) 1.0 M LiOH, CH_3OH/H_2O, 90%; (g) DMAP, EDCI, **10**, dichloromethane, 87%; (h) 4.0 N HCl in dioxane, 50 °C, 2 h, 90%.

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.

13. 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.
14. MDCK II cells were cultured in DMEM supplemented with 10% FBS and 100 units 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 five 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.
15. 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.