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Synthesis and evaluation of *N*-acyl sulfonamides as potential prodrugs of cyclin-dependent kinase inhibitor JNJ-7706621

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Abstract—A novel prodrug strategy for cyclin-dependent kinase inhibitor JNJ-7706621 has been explored. Through N-acylation of a sulfonamide substituent, tails containing different solubilizing groups (amino, carboxyl, alkoxyl, and hydroxyl) were attached to JNJ-7706621. Most of the prodrugs exhibited good aqueous solubility and the *N*-acyl groups on the sulfonamide were metabolically cleaved to generate active drug in rat PK study.

The cyclin-dependent kinases (CDKs) play a key role in regulating the cell cycle and cell proliferation.[1] For example, complexes of CDK4 and cyclin D1 control the early G1 phase of the cell cycle, while the activity of the CDK2/cyclin E complex is rate limiting for the G1 to S-phase transition. Similarly, the CDK2/cyclin A kinase complex is required for the progression through S-phase and the CDK1/cyclin B complex controls the entry into M-phase. A key regulator for these cell cycle transitions is CDK1, the primary activity required for a cell to enter mitosis in all eukaryotic cells. Uncontrolled proliferation is a hallmark of cancer cells, and CDKs are overexpressed with high frequency in many important solid tumors.[2] This evidence has led to an intense search for small molecular inhibitors targeted CDK family as an approach for cancer chemotherapy. As shown in Figure 1, several candidates have been advanced into clinical trials, including the 2-aminothiazole derivative BMS-387032 (SNS-032),[3] the nonselective CDK inhibitor flavopiridol,[4] and the purine analogue (*R*)-roscovitine (CYC-202).[5]

Our program to develop small ATP-competitive CDK inhibitors as cancer therapeutics has resulted in the discovery of JNJ-7706621, a potent inhibitor of CDK1 and CDK2 (IC_{50} = 6 and 2 nM, respectively).[6] In cell based assays, JNJ-7706621 displays antiproliferative activity against various human tumor cells (IC_{50} = 284, 254, and 447 nM for HeLa, HCT116, and A375, respectively). With low oral bioavailabilities in rodents (2% in nude mice and 8% in rats), possibly due to poor aqueous solubility (0.006 mg/mL at pH 2.0 and 0.017 mg/mL at pH 7.4), the compound was administered via the intraperitoneal (ip) route to nude mice for in vivo efficacy studies. Various formulation vehicles were evaluated in order to maximize the antitumor effect. JNJ-7706621 showed 95% tumor growth inhibition in A375 (human melanoma) tumor xenograft model study when formulated as a nanocrystal suspension and administered ip at a dose of 100 mg/kg. However, the suspension formulation is not suitable for intravenous (iv) injection in clinical studies where a clear solution is required. Though many clinically feasible drug delivery

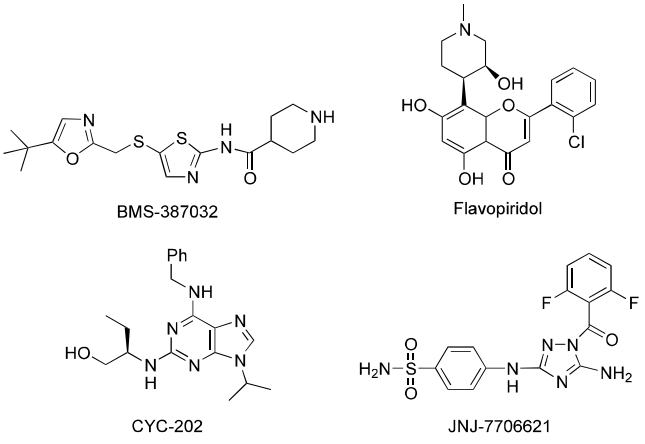

Figure 1.

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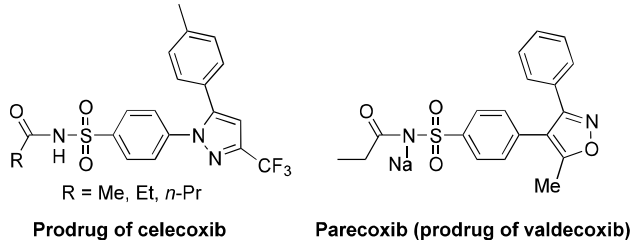
technologies were studied for JNJ-7706621, none was amenable to iv administration. Therefore, developing water-soluble prodrugs for JNJ-7706621 appears to be an attractive alternative strategy.

Both the 3- and 5-amino groups on the triazole ring have low reactivity due to their conjugation with the heteroaryl group. On the other hand, the NH_2 of the sulfonamide shows greater flexibility and is more suitable for prodrug synthesis. This residue could be selectively acylated under appropriate conditions (vide infra). Two prodrugs derived from N-acylation of sulfonamide have been reported. As shown in Scheme 1, the prodrug of celecoxib demonstrated the proof-of-concept that an N-acylated sulfonamide could be metabolized to $–SO_2NH_2$ in rat tissue preparations as well as in whole animals after oral administration.[7] Parecoxib, the sodium salt of the propanoyl prodrug of valdecoxib that is marketed for the hospital treatment of postoperative pain, also exemplifies this principle.[8] Therefore, we hypothesized that iv-deliverable prodrugs for JNJ-7706621 could be generated via N-acylation of the sulfonamide moiety.[9]

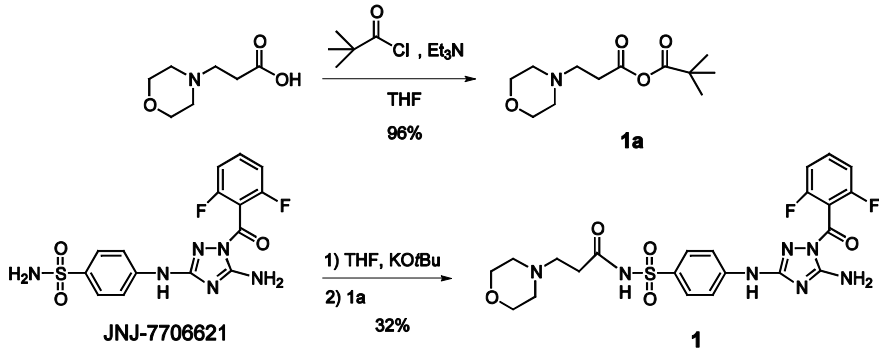
The chemistry used for prodrug synthesis is shown in Scheme 2. Mixed anhydride **1a** was formed by reacting 3-morpholin-4-ylpropionic acid with pivaloyl chloride in the presence of Et_3N. Compound **1a** was then added to a THF solution of the potassium salt of JNJ-7706621, prepared by treating the drug with potassium *tert*-butoxide to selectively deprotonate the $–SO_2NH_2$, generating prodrug **1** in 32% yield. The regio-selectivity for this N-acylation was confirmed by ^1H NMR (in DMSO), in which a singlet NH peak for the acylated sulfonamide appeared at 9.95 ppm, while the broad singlet peak at 7.09 ppm for the unacylated $–SO_2NH_2$ disappeared. Though a higher yield for the N-acylation step could not be achieved despite numerous optimization attempts, this methodology was still useful for generating many other prodrugs because only one step was needed.



Scheme 1. Prodrugs based on N-acylation of $–SO_2NH_2$.

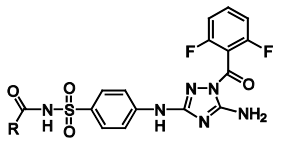
The prodrugs were evaluated for their ability to inhibit CDKs and tumor cell proliferation. The data are shown in Table 1. Compared to JNJ-7706621, the prodrugs were much less potent against CDK1 and CDK2, and were also less potent in cells. In our previous SAR study on the $–SO_2NH_2$, it was also observed that an unsubstituted NH_2 is critical for CDK1 and CDK2 inhibition; any modification of this NH_2 group was detrimental to kinase potency and cell activity.[6] Despite the significant loss in potency, most of the prodrugs have improved aqueous solubility and could be formulated as clear solutions for iv injection. With amino groups added, compounds **3**, **4**, and **7** were significantly more soluble than the parent compound in aqueous solution. Interestingly, the solubilities of acidic compound **2** and neutral compounds **5** and **6** were higher at pH 7.4 than at pH 2.0. Special attention was paid to formulate these prodrugs since their solubilities were pH dependent.

A standard rat pharmacokinetics assay was used to determine whether the *N*-acyl group on the sulfonamide could be metabolically cleaved in vivo to $–SO_2NH_2$. In the experiment, prodrugs were formulated as solutions in 10% w/v Solutol in 5% dextrose in sterile water vehicle and administered iv into rats. Blood samples (0.5 mL) were collected via orbital sinus puncture at 5, 15, and 30 min, 1, 2, 4, 6, 8, and 24 h after dosing and plasma levels for both prodrug and JNJ-7706621 were measured. The results, summarized in Table 2, demonstrate that the *N*-acyl group could be cleaved from the sulfonamide to produce active drug JNJ-7706621 in rats. However, the conversion ratio varied substantially with the *N*-acyl group. With prodrug **2** or **6**, high plasma ratios of JNJ-7706621 were achieved. On the other hand, only a small proportion of active drug was generated from prodrugs **1**, **3**, **4**, **5**, or **7** in vivo. For all prodrugs, T_{max} values for both prodrug and JNJ-7706621 were short, ranging from 0.08 to 0.12 h; $T_{1/2}$ values were also relatively short, ranging from 0.16 to 1 h. With good solubility



Scheme 2. Example procedure for prodrug synthesis.

Table 1. CDK, antiproliferative, and solubility data for prodrugs



Compound	R	IC_{50} (μM)					Solubility (mg/ml)	
		CDK1	CDK2	HeLa	HCT116	A375	pH 2.0	pH 7.4
1	2-(Morpholin-4-yl)ethyl	1.55	0.118	20.91	50.8	>100	0.014	0.0047
2	$HOC(O)CH_2CH_2$	0.494	0.071	31.90	>100	20.25	0.0007	>1
3	2-(4-Methylpiperazin-1-yl)ethyl	1.34	0.266	>100	>10	>100	>1.0	0.82
4	H_2NCH_2	1.07	0.120	14.86	36.9	9.450	0.13	0.20
5	$CH_3OCH_2CH_2OCH_2$	1.66	0.247	>10	>10	>100	0.0054	>1.0
6	$CH_3CH_2OC(O)CH_2CH_2$	0.466	0.062	60.16	40.1	>10	0.0002	0.21
7	*R*-$HOCH_2CH(NH_2)$	1.41	0.140	4.024	71.2	>10	0.44	>1

Table 2. Standard rat PK results for prodrugs (iv dosing at 3 mg/kg)

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Prodrug AUC (μM-h)	20.5	47.0	23.2	11.8	42.9	72.2	8.15
JNJ-7706621 AUC (μM-h)	1.4	18.0	0.93	3.67	7.62	29.1	0.63
Conversion %[a]	7%	38%	4%	31%	18%	40%	8%

[a] =100 * AUC(JNJ-7706621)/AUC (prodrug).

at pH 7.4 and a high conversion percentage, compound **2** was scaled up for in vivo efficacy evaluation. Because of the technical difficulties associated with daily iv dosing of drugs in nude mice, the ip route was used as a surrogate. However, in an A375 (human melanoma) tumor xenograft model in nude mice, **2** failed to show significant antitumor effect when dosed IP at 100 mg/kg daily for 28 days. Possible explanations for this negative result include: lower conversion of prodrug in nude mice compared to rats, leading to lower plasma levels of JNJ-7706621; insufficient exposure to drug due to short plasma $T_{1/2}$ and rapid clearance of prodrug. Continuous iv infusion via an implantable pump would address the latter issue, but such a concept exceeded the scope of this work.

A novel prodrug strategy for JNJ-7706621 has been explored. Through N-acylation of a sulfonamide substituent, tails containing different solubilizing groups (amino, carboxyl, alkoxyl, and hydroxyl) were attached to JNJ-7706621. Most of the prodrugs exhibited good aqueous solubility and could be formulated as clear solutions for clinical iv injection. The *N*-acyl groups on the sulfonamide were metabolically cleaved to generate active drug in rat PK studies. The in vivo efficacy of these prodrugs will be further investigated.

Meanwhile, other prodrug strategies for JNJ-7706621 could be pursued.

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References and notes

1. (a) Morgan, D. O. *Nature* **1995**, *374*, 131; (b) Norbury, C.; Nurse, P. A. *Annu. Rev. Biochem* **1992**, *61*, 441.
2. Yasui, W.; Ayhan, A.; Kitadai, Y.; Nishimura, K.; Yokozaki, H.; Ito, H.; Tahara, E. *Int. J. Cancer* **1993**, *53*, 36.
3. 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.
4. Zhai, S.; Senderowicz, A. M.; Sausville, E. A.; Figg, W. D. *Ann. Pharmacother.* **2002**, *36*, 905.
5. McClue, S. J.; Blake, D.; Clarke, R.; Cowan, A.; Cummings, L.; Fisher, P. M.; MacKenzie, M.; Melville, J.; Stewart, K.; Wang, S.; Zhelev, N.; Zheleva, D.; Lane, D. P. *Int. J. Cancer* **2002**, *102*, 463.
6. (a) Lin, R.; Connolly, P. J.; Huang, S.; Wetter, S. K.; Lu, Y.; Murray, W. V.; Emanuel, S. L.; Gruninger, R. H.; Fuentes-Pesquera, A. R.; Rugg, C. A.; Middleton, S. A.; Jolliffe, L. K. *J. Med. Chem.* **2005**, *48*, 4208; (b) Emanuel, S.; Rugg, C. A.; Gruninger, R. H.; Lin, R.; Fuentes-Pesquera, A.; Connolly, P. J.; Wetter, S. K.; Hollister, B.; Kruger, W. W.; Napier, C.; Jolliffe, L.; Middleton, S. A. *Cancer Res.* **2005**, *65*, 9038.
7. Mamidi, R. N.; Mullangi, R.; Kota, J.; Bhamidipati, R.; Khan, A. A.; Katneni, K.; Datla, S.; Singh, S. K.; Rao, K. Y.; Rao, C. S.; Srinivas, N. R.; Rajagopalan, R. *Biopharm. Drug Dispos.* **2002**, *23*, 273.
8. Talley, J. J.; Bertershaw, S. R.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Kellogg, M. S.; Koboldt, C. M.; Yuan, J.; Zhang, Y. Y.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 1661.
9. Huang, S.; Lin, R.; Connolly, P. J.; Emanuel, S.; Middleton, S. A.; Wetter, S. K. *Abstracts of Papers*, 229th ACS National Meeting, San Diego, CA, United States, March 13–17, 2005.