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Water-soluble prodrugs of an Aurora kinase inhibitor

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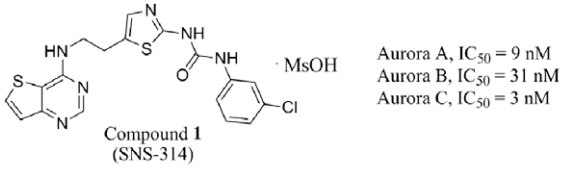
ABSTRACT

Compound **1** (SNS-314) is a potent and selective Aurora kinase inhibitor that is currently in clinical trials in patients with advanced solid tumors. This communication describes the synthesis of prodrug derivatives of **1** with improved aqueous solubility profiles. In particular, phosphonooxymethyl-derived prodrug **2g** has significantly enhanced solubility and is converted to the biologically active parent (**1**) following iv as well as po administration to rodents.

The Aurora serine/threonine kinases have emerged as promising anti-cancer targets for small-molecule intervention.[1–6] As a result, a number of Aurora kinase inhibitors have been disclosed and some have progressed into clinical trials.[1–10] Recently, we reported the discovery of a potent and selective Aurora kinase inhibitor, compound **1** (SNS-314, Fig. 1).[11] This compound has a compelling pre-clinical profile and is currently in clinical trials in patients with advanced solid tumors.[12] An issue commonly encountered when developing kinase inhibitors is low aqueous solubility. While we found that Captisol® can successfully be used to provide more than adequate aqueous solubility of **1** (Fig. 1) for parenteral administration, we sought alternative means in a parallel effort to identify Aurora kinase inhibitors with improved aqueous solubility profiles.[13] Initially, analogues of compound **1** containing solubilizing functionalities were explored. Unfortunately, efforts to improve solubility often compromised other pharmaceutical properties. For example, while amine-containing derivatives of **1** had significantly improved aqueous solubility at lower pH, they also tended to be heavily effluxed and have less favorable in vivo pharmacokinetic profiles.[11] Furthermore, of the analogues evaluated in vivo, compound **1** was found to display a superior activity profile. We therefore next considered a prodrug approach[14,15] where compound **1** would be directly derivatized with a bio-labile moiety designed to improve solubility. In addition to conferring improved solubility, the prodrug moiety would also have to be chemically stable in aqueous buffer yet labile in vivo to liberate the biologically active parent drug (**1**). In this letter, we describe our efforts directed towards these goals.

The synthesis of acyl-oxymethylene derived prodrugs of **1** is outlined in Schemes 1 and 2. Heating a mixture of isobutyryl chloride (**3**) and paraformaldehyde in the presence of zinc chloride yielded chloromethyl ester **4** in 56% yield (Scheme 1). Alkylation was achieved by treating the free base of compound **1** with sodium hydride followed by addition of **4** to afford prodrug **2b** in low yield (13%).[16] Similarly, carboxylic acid **5** was converted to the corresponding iodomethyl derivative **6** (48%) followed by alkylation of



Thermodynamic Solubilities (mg/mL)

Compound	Aqueous	Organic (PEG400)	Aqueous (20% Captisol®)
1	0.014 (pH 3.2)	35 (Free base)	14.3 (pH ~3.6)

Figure 1. Thermodynamic solubility profile of compound **1**.

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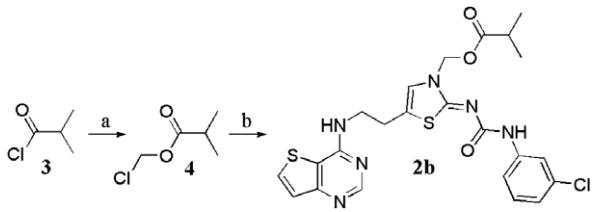
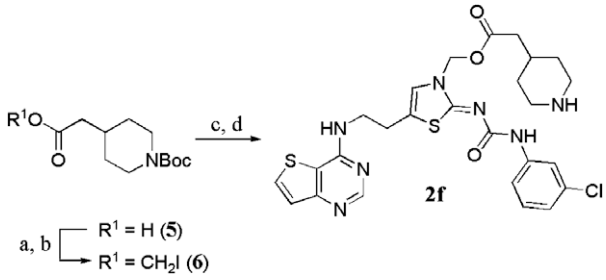


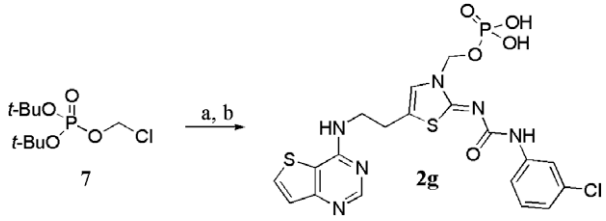
Scheme 1. Reagents and conditions: (a) CH_2O, $ZnCl_2$, 90 °C, 56%; (b) **1** (free base), NaH, DMF, rt, 13%.



Scheme 2. Reagents and conditions: (a) i—aqueous $NaHCO_3$, Bu_4NHSO_4, DCM, rt; ii—$ClCH_2OSO_2Cl$, DCM, 0–25 °C, 56%; (b) NaI, acetone, rt, 86%; (c) **1** (free base), NaH, DMF, rt, 36%; (d) 2 M HCl/dioxane, 91%.

compound **1** (36%, Scheme 2). Removal of the Boc-group under acidic conditions was achieved to give prodrug **2f** in 91% yield. Phosphonooxymethyl-derived prodrug **2g** was prepared according to Scheme 3. Reaction of compound **1** with di-*tert*-butyl chloromethyl phosphate (**7**)[17] under basic conditions followed by de-protection employing a mixture of water and acetic acid afforded phosphoric acid **2g** in 16% overall yield.

According to the SAR reported earlier, the 2-aminothiazole-urea portion in **1** is critical for good biological activity.[11] However, bi-aryl urea motifs of this type are notorious for conferring poor solubility profiles.[18] We therefore hypothesized that we could improve solubility by masking the urea in **1** with a prodrug moiety that would remove its bi-dentate hydrogen bonding capability



Scheme 3. Reagents and conditions: (a) **1** (free base), NaH, DMA, rt, 22%; (b) AcOH/H_2O (4:1), 65 °C, 72%.

(Fig. 2). Thus, the use of acyl-oxymethylene derived prodrugs **2a–c** was initially considered a potential approach to improve solubility. Further it was anticipated that the esters would undergo enzymatic hydrolysis in vivo to generate the biologically active parent (compound **1**) along with formaldehyde (cf. Fig. 2).[14,15] Although this first set of prodrugs was shown to be stable in aqueous buffer and DMSO (Table 1) they only showed modest improvements in their solubility profiles relative to the parent drug (**2a–c** vs **1**, Table 1). Our earlier studies indicated that incorporation of amines could significantly improve aqueous solubility of compounds in this series.[11] Therefore, we next focused on amine-containing acyl-oxymethylene derived prodrugs. Our initial attempts to explore these derivatives were unsuccessful due to instability of the prodrug. For example, although we were able to isolate prodrugs **2d** and **2e**, they were readily converted back to the parent in aqueous buffer and organic media (Table 1). However, we noticed that amine-containing prodrugs where the basic nitrogen was extended further from the ester-moiety appeared to be more stable (e.g., **2e** is more stable than **2d**, Table 1). Consistently, extending the basic amine in **2e** one atom further from the ester functionality did provide an amine-containing prodrug (**2f**) that showed no signs of decomposition over an eight hour time period (Table 1). As expected, this derivative was also found to have significantly enhanced aqueous solubility over the parent compound at a low pH (**2f** vs **1**, Table 1). Having achieved noteworthy improvements in solubility with a charged prodrug moiety, we next examined a phosphate-derived prodrug, compound **2g**. The prodrug moiety in **2g** is expected to be doubly charged at near-neutral pH and has successfully been used to improve the solubility of clinically used iv drugs, including Fosphenytoin[19–21] and Fospropofol.[22–24] Presumably, the phosphate moiety is cleaved in vivo by alkaline phosphatases to release the parent and formaldehyde (cf. Fig. 2).[14,15] In addition, phosphate-derived prodrugs have also been used to enhance the oral bioavailability (oral %*F*) of marketed drugs (e.g., the HIV-1 protease inhibitor Lexiva®).[15,25,26] Not surprisingly,

Table 1
In vitro stabilities and aqueous solubilities of prodrugs **2a–g**

Compound	DMSO[a] (% remaining at 8 h)	Buffer, pH 7.4[a] (% remaining at 8 h)	Aqueous solubility[b] [mg/mL]
1			0.014 (pH 3.2)
2a	100	100	0.12 (pH 2.5)
2b	NT	NT	0.17 (pH 3.2)
2c	100	100	0.28 (pH 3.2)
2d	0	0	NT
2c	84	52	NT
2f	100	100	2.5 (pH 3.5)
2g	100	100	4.7 (pH 7.8)

[a] In vitro stabilities as determined by LC/MS after 8 h at room temperature.
[b] Thermodynamic solubilities. Values are typically means of two measurements, variation < 30%. See Ref. 13 for protocol.

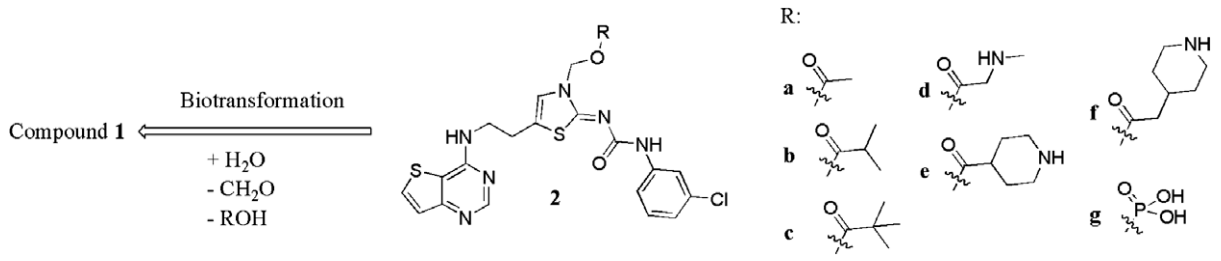


Figure 2. Prodrug strategy employed to improve the solubility profile of compound **1** involved derivatization of the bi-aryl urea portion of the molecule. Prodrugs **2a–g** were prepared and evaluated. In vivo cleavage of the prodrug moieties (*R*) was expected to liberate the biologically active parent (compound **1**) along with formaldehyde.

compound **2g** has significantly improved aqueous solubility at near-neutral pH and is also stable in organic media as well as in buffer (Table 1).

Model studies conducted in vitro indicated that the selected prodrug moieties are cleaved as hypothesized to release the biologically active parent compound upon exposure to plasma and S9 fractions (human and rat, data not shown). Thus, having identified a series of prodrugs with overall favorable in vitro profiles, we decided to determine their preliminary in vivo pharmacokinetic (PK) profiles. These studies, conducted in CD1 mice, indicated that only compound **2g** displayed satisfactory in vivo properties (data not shown) while the other prodrugs yielded low plasma concentrations of the parent. For example, the bulky pivaloyl ester derivative **2c** was only slowly converted to **1** following intravenous (iv) administration and also yielded low levels of the parent drug following oral delivery (po). Although cleaved somewhat faster, acetate-derived ester **2a** also resulted in disappointingly low exposure levels of the parent drug. These results combined with only modest improvements in solubility led us to discontinue examination of the acyl-oxymethylene derived prodrugs. While the amine-containing ester **2f** did show quite promising solubility properties (Table 1), the mouse PK study indicated that neither the prodrug nor the parent could be detected in systemic circulation following iv or oral administrations. Thus, only **2g** was found to have preliminary in vitro and in vivo properties warranting further studies. Therefore, it was decided to focus the ensuing efforts on this compound.

First, the thermodynamic solubility profile of **2g** was studied in more detail by examining the effects of the counter-ion. Not surprisingly, the bis-salts were shown to be more soluble than the corresponding mono-salts (Table 2). We also found that the solubility is dependent on the identity of the counter-ion. In particular, the use of choline achieves an aqueous solubility of 9.4 mg/mL (Table 2). This is a significant (>500-fold) improvement over the parent drug and provides levels of solubility that could reduce or eliminate the need to use a solubilizing excipient such as Captisol® for iv-formulations.

Having narrowed the selection to one prodrug, we also decided to conduct additional in vivo pharmacokinetic studies, this time in rats. First, the iv PK profile of compound **1** derived in vivo from prodrug **2g** was compared to the profile of **2g** itself (Fig. 3). The anticipated[15] conversion of the prodrug ($t_{1/2}$ = 0.5 ± 0.1 h) to the parent was observed and resulted in significant levels of the liberated parent **1** ($t_{1/2}$ = 2.1 ± 0.3 h). In fact, the PK profile of compound **1** derived following iv administration of **2g** is similar to the profile from an equivalent iv dose of the parent itself (Fig. 4). The exposure to **1** generated from **2g** is 91% of that achieved from dosing with **1** (AUC_{INF} = 1910 ± 230 ng h/mL vs 2090 ± 430 ng h/mL).

We also studied the rat PK profile following oral administration of **2g** (Fig. 5). While modest levels of the parent (**1**) were detected in plasma following oral delivery of **2g** (dose equivalent to 10 mg/kg of **1**), the resulting AUC_{INF} value is somewhat lower than the value obtained when an equivalent dose of the parent (**1**) is administered (2200 ± 1200 ng h/mL vs 3650 ± 1700 ng h/mL). The corresponding oral bioavailabilities with respect to compound **1** are 44% and 72% following oral dosing of **2g** and **1**, respectively. In contrast to iv administration of **2g** (see above), following oral delivery, no prodrug could be detected in the systemic circulation consistent with a low permeability profile of **2g** as determined in the MDCK cell line (<1.0 × 10^{-6} cm/s). Presumably, alkaline phosphatases, present in the brush border of the gut, convert the prodrug to compound **1** prior to absorption.[27]

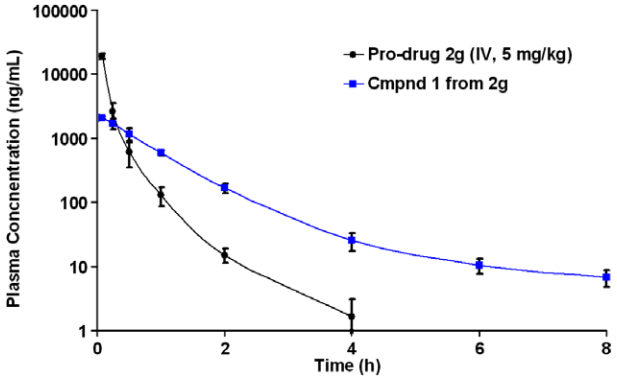


Figure 3. Rat iv pharmacokinetic profiles of prodrug **2g** and compound **1** formed in vivo from **2g**. The profiles were determined in male Sprauge–Dawley rats (n = 3) and the dose of prodrug **2g** was equivalent to 5 mg/kg of compound **1**.

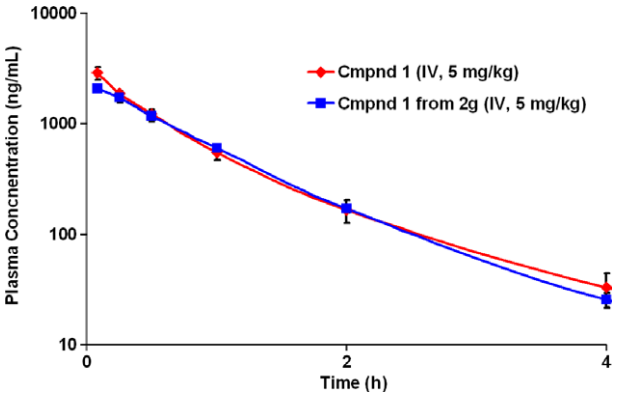


Figure 4. Comparison of the rat iv pharmacokinetic profile of compound **1** formed in vivo from **2g** to the profile of an equivalent iv dose of compound **1**. The profiles were determined in male Sprauge–Dawley rats (n = 3).

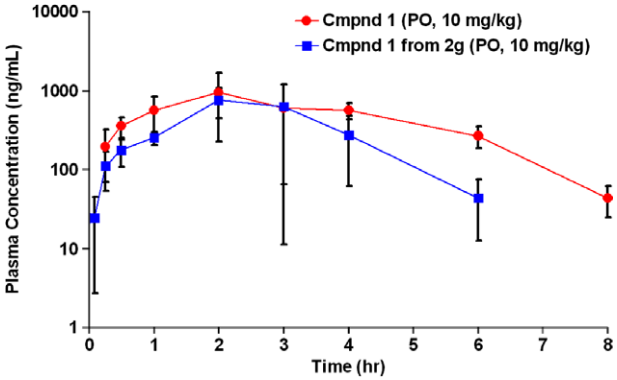


Figure 5. Comparison of the rat pharmacokinetic profile of compound **1** formed in vivo from **2g** following oral delivery to the profile of an equivalent oral dose of compound **1**. The profiles were determined in male Sprauge–Dawley rats (n = 3).

Table 2
Effect of counter-ion on solubility of prodrug **2g**

Counter-ion	Salt type	Aqueous solubility[a] [mg/mL]
Sodium	Mono	0.51
Sodium	Bis	4.7
Potassium	Mono	0.86
Potassium	Bis	6.0
Choline	Mono	2.4
Choline	Bis	9.4

[a] Thermodynamic solubilities. Values are typically means of two measurements, variation < 20%. See Ref. 13 for protocol.

In conclusion, we have shown that the aqueous solubility profile of our clinical stage Aurora kinase inhibitor SNS-314 (**1**) can be significantly improved by derivatizing the 2-aminothiazole-urea moiety with bio-labile prodrug appendages. In particular, phosphonooxymethyl-derived prodrug **2g** displays high aqueous solubility and is stable in buffer as well as organic media. In addition, it is converted to the biologically active parent (**1**) upon iv and oral deliveries to rodents. The identification of **2g** also suggests new avenues for further explorations. For example, substitutions on the oxymethylene unit of **2g** as well phosphate ester modifications may yield additional enhancements in pharmaceutical properties.[14,15]

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13. *General procedure for determination of thermodynamic solubilities:* The test compound was accurately weighed out in a suitable size screw cap glass vial followed by the addition of the appropriate media. The resulting slurry was sonicated for 20 min at room temperature, 10 min at 50 °C, and then another 10 min at room temperature. The slurry was then agitated for a minimum of two days at room temperature at the end of which they were inspected under polarized light microscope (Olympus BX51) for crystallinity of the residual solid. Additional time for equilibration was allowed if crystallinity was not observed. On the day of analysis, the slurry was aliquoted into 1.5 mL propylene centrifuge tubes and centrifuged at 15,000 rpm at 20 ± 2 °C for 20 min. The supernatant was analyzed for concentration by HPLC (Agilent 1100 binary pump and a Phenomenex Synergi Hydro-RP column (30 × 2 mm, 4 μm, 80 Å particle size)) and for pH by pH meter (where applicable). When mono-salts were prepared, sufficient volume of the aqueous solution of the base was added such that the molar ratio of the base to that of the drug is 1.05:1. When bis-salts were prepared, sufficient volume of the aqueous solution of the base was added such that the molar ratio of the base to that of the drug is 2.1:1.
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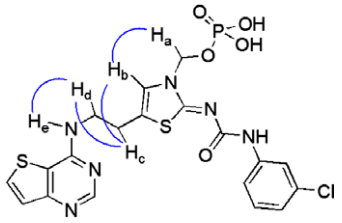
16. The regio-chemical identity of the prodrugs was verified by NOE studies. For example, key enhancements observed for prodrug **2g**:



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