

## Synthesis of Water Soluble Analogs of Arylsulfonylimidazolidinone (JSH-2282)

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To improve the water solubility of arylsulfonylimidazolidinone (JSH-2282), a potent anti-cancer agent, two urea derivatives, sodium (*S*)-2-(3-(4-((*S*)-2-oxo-4-phenylimidazolidin-1-ylsulfonyl)indoline-1-carbonyl)-phenyl)ureido)succinate (**2a**) and sodium (*S*)-2-(3-(4-((*S*)-2-oxo-4-phenylimidazolidin-1-ylsulfonyl)indoline-1-carbonyl)phenyl)ureido)pentanedioate (**2b**), were synthesized and studied for solubility and anti-cancer activity.

**Key Words :** Arylsulfonylimidazolidinone, Solubility, Cytotoxicity, Anti-cancer

### Introduction

Ever since the discovery of anti-cancer drugs, antimitotic agents such as vinca alkaloids, vinblastine and vincristine, as well as the taxanes (taxol and taxotere) have been recognized as effective medicine for the treatment of variety of cancers.<sup>1,2</sup> However, the mechanism of action and clinical use of all these antimitotic agents are associated with the problems of drug resistance, toxicity and poor bioavailability.<sup>3</sup> Therefore, nontoxic anti-cancer drug with increased bioavailability and selectivity is required to make safer medicine.

Previously, we have discovered a novel and promising anti-cancer candidate, JSH-2282 ((4*S*)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenylimidazolidin-2-one, Fig. 1),<sup>4</sup> which not only showed the potent antitumor *in vitro* activity but also proved its worth in the *in vivo* test by exhibiting approximately 45%, 42%, and 94% suppression of tumor growth against murine colon adenocarcinoma (colon 26), Lewis lung carcinoma (3LL), and human colon cancer (SW620) at a dose of 65 mg/kg (*p.o.*, q2d × 5), respectively. Unexpectedly, in the preclinical trials on beagle dog the drug was accumulated in the intestinal wall causing toxicity despite of the good therapeutic potential. This could be due to the poor solubility (0.0235 mg/mL) of JSH-2282 in water. Thus, its solubility has been identified as a critical parameter in preclinical trials. Therefore, we shifted our

focus to improve the water solubility of JSH-2282. It has been reported that introduction of an ionizable moiety into the structure of the compound is one of the common approaches to improve the water solubility.<sup>5-9</sup> In that context we observed that the structure of JSH-2282 has two plausible sites which can be connected with a linker, one is a nucleophilic amide (Site 1 in Fig. 1) of heterocyclic imidazolidin-2-one, and the other is a basic aromatic amine (Site 2 in Fig. 1) attached at 1*H*-indoline. The preliminary studies revealed that Site 1 was more restricted to the structure manipulations compared to Site 2 in terms of the activity (not published). Thus we decided to investigate modifications at Site 2. Therefore, we synthesized two newly designed analogs **2a** and **2b** of JSH-2282 with amino acid moieties as illustrated in Figure 1, which would allow ionization or salt formation.

### Results and Discussion

Compounds **2a** and **2b** were designed by linking with urea function of **1** with L-aspartic acid and L-glutamic acid moieties, respectively, to improve the water solubility (Fig. 1). In case of L-glutamic acid, we anticipated the facilitation of drug bioavailability *in vivo*, because L-glutamic acid is a key intermediate in the biosynthesis of other amino acids by a process known as transamination.<sup>10</sup>

Syntheses of compounds **2** were performed as outlined in

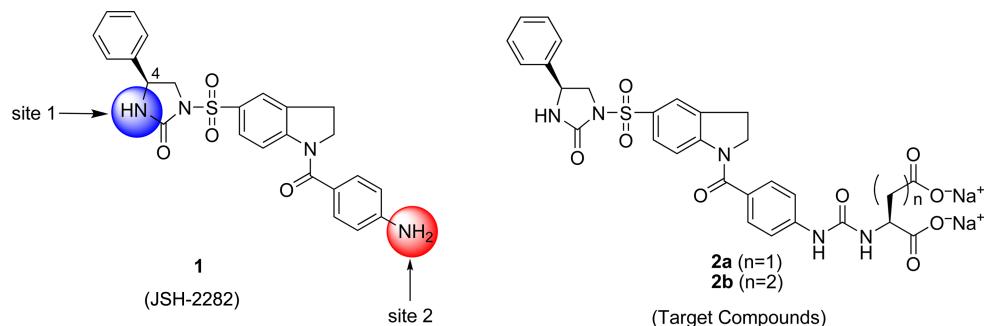
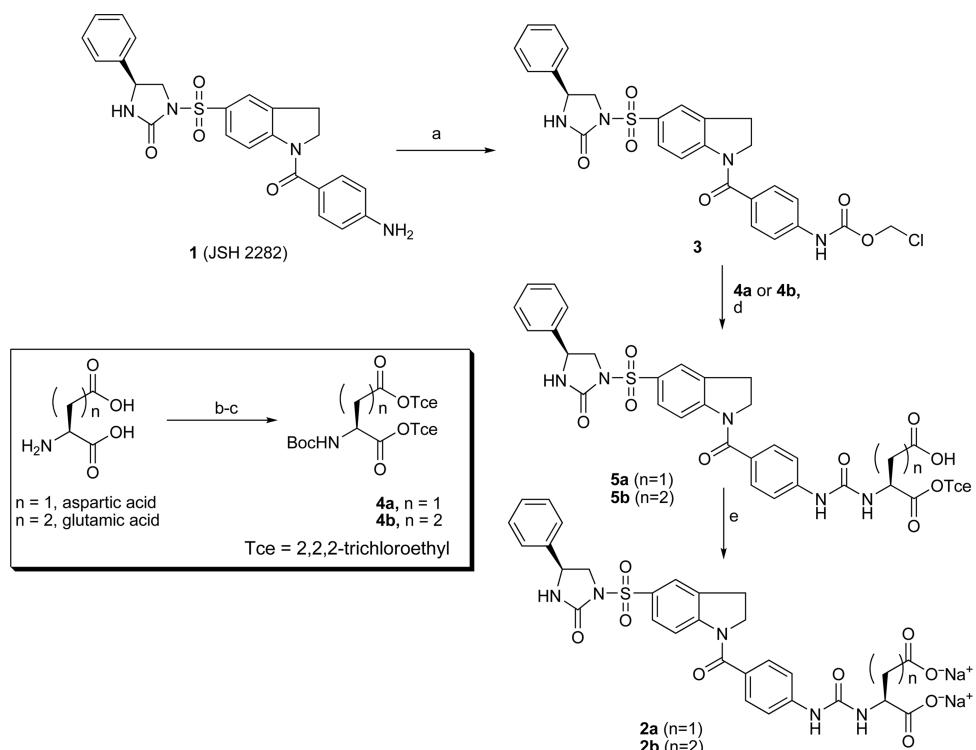


Figure 1. JSH-2282 (1) and Target compounds (2a and 2b).



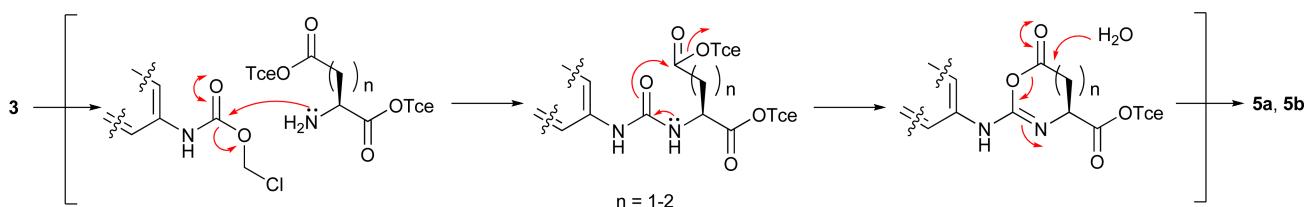
Reagents and conditions: (a) chloromethyl chloroformate, TEA, THF, 0 °C - rt, 12 h, 35–41%; (b) di-*tert*-butyl dicarbonate, NaOH, dioxane/H<sub>2</sub>O, rt, 18 h; (c) 2,2,2-trichloroethanol, DCC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C - rt, 24 h, 49% for **4a** and 69% for **4b** in two steps; (d) TFA/CH<sub>2</sub>Cl<sub>2</sub> (1:1), rt, 1 h, and then TEA, DMF, 0 °C - rt, 24 h, 58% for **5a** and 39% for **5b** in two steps; (e) NaHCO<sub>3</sub>, THF/H<sub>2</sub>O (2:1), 0 °C - rt, 24 h, 76% for **2a** and 61% for **2b**.

**Scheme 1.** Synthesis of **2a** and **2b**.

Scheme 1. Accordingly, amino acids are protected with 2,2,2-trichloroethyl (Tce) ester group in order to exclude the possibility of intramolecular cyclization in the reaction process<sup>11</sup> as well as in order to make the easy removal of the introduced protecting groups. The suitable fully-protected compound **4a** (BocNH-Asp(OTce)-OTce) and **4b** (BocNH-Glu(OTce)-OTce) were synthesized from L-aspartic acid and L-glutamic acid in two steps (49% and 69% yield, respectively). Treatment of compound **4a** (BocNH-Asp(OTce)-OTce) or **4b** (BocNH-Glu(OTce)-OTce) with trifluoroacetic acid gave de-Boc intermediate *in situ* and then chloromethoxycarbonyl compound **3** in the presence of TEA was added and stirred at 0 °C for 24 h to produce the urea intermediates **5a** or **5b**, which possesses one free carboxylic acid. In these reactions, it is also possible to form a S<sub>N</sub>2 substitution product. However <sup>1</sup>H-NMR spectra confirmed that the CH<sub>2</sub> peak of aminomethoxyl (-C(O)O-CH<sub>2</sub>-NH-) did not appear and only one of CH<sub>2</sub> peak (4.82 ppm, s, 2H

for **5a** and 4.79 ppm, s, 2H for **5b**, respectively) for 2,2,2-trichloroethyl appeared. MS data were matched with compound **5a** and **5b**. Thus formation of urea rather than substitution was confirmed in this reaction. The proposed mechanism for the formation of compounds **5a** or **5b** is illustrated in Figure 2 and we hypothesized that these compounds may be formed through heterocyclic intermediate 4,5-dihydro-1,3-oxazin-6-one after formation of urea. Finally, the target sodium salt compounds **2a** and **2b** were obtained *via* base hydrolysis from urea products **5a** and **5b**, respectively.

The obtained urea derivatives **2a** and **2b** were initially tested for their water solubility in distilled water.<sup>12</sup> The solubility of **2a** (9.714 mg/mL) and **2b** (14.880 mg/mL) was increased remarkably over 400 times compared to that of JSH-2282 (< 0.0235 mg/mL). These results indicate that the obtained urea derivatives are successful in improving the solubility. As the compounds **2a** and **2b** showed far better



**Figure 2.** A possible mechanism for formation of compound **5a** and **5b**.

solubility, their cytotoxicity was investigated against SW-620 cell lines. Surprisingly, the cytotoxicity of **2a** ( $IC_{50} = 5.46 \mu\text{M}$ ) and **2b** ( $IC_{50} = 10.89 \mu\text{M}$ ) against SW-620 cell line was far less as compared to that of JSH-2282 ( $IC_{50} < 0.39 \mu\text{M}$ ). The above results confirmed one of the previous finding that the ionized form has low lipid solubility but high water solubility (*i.e.* hydrophilicity) and thus cannot penetrate the cell membrane easily which may lead to lower cytotoxicity.<sup>13</sup>

Since urea linkage is possibly cleaved in plasma to generate JSH-2282, reconversion tests with these compounds in rat plasma under *in vitro* and *in vivo* conditions were performed but JSH-2282 was not formed until 8 h in both the conditions. Thus these tests confirmed that compounds **2a** and **2b** are very stable and the cytotoxicity obtained is purely due to **2a** and **2b** and not because of the parent compound JSH-2282.

### Conclusion

Two water soluble analogs **2a** and **2b** of arylsulfonylimidazolidinone (JSH-2282) were prepared by conjugating an amino acid (L-aspartic acid or L-glutamic acid) using urea linkage and studied for the solubility and anti-cancer activity. The newly discovered compounds showed significant stability both under *in vitro* as well as *in vivo* system and remarkably improved water solubility compared to the parent compound. However, relatively low cytotoxicity hampered the *in vivo* anti-cancer study. Therefore, further elaboration for finding the other analogs with better pharmacological activity is in progress.

### Experimental

**Chemistry.** All reagents and solvents were dried prior to use according to the standard methods. Commercial reagents were used without further purification unless otherwise stated. Melting points were measured on an Electrothermal melting point apparatus. IR spectra were obtained on KBr disks using a JASCO Report 100 spectrophotometer. <sup>1</sup>H-NMR spectra were recorded on JNM-AL 400 (Jeol) instrument operating at 400 MHz and the chemical shift ( $\delta$ ) were reported in parts per million downfield from tetramethylsilane (TMS). Electro spray mass spectra (ESIMS) were recorded on PE SCIEX API 2000 (triple quadrupole) LC-MS/MS system (Applied Biosystems, Foster City, CA, USA). Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040-0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063-0.200 mm; Merck). The reactions were followed by thin-layer chromatography (TLC) on Merck glass silica gel (Kiesel gel 60 F<sub>254</sub>) plates that were visualized under a UV lamp. HPLC was performed using a Shimadzu liquid chromatograph model Class-vp version 6.12, equipped with a SPD-10A UV-vis detector (Shimadzu). The HPLC analysis was performed using a Symetry TM® C-18 column (3.9 × 150 mm, Part No. WAT054205, Waters, Milford, MA). The elution condition was a linear gradient of

31-95% acetonitrile in 0.1% TFA for 30 min at a flow rate of 1 mL/min with UV detection at 254 nm. All solvents for HPLC were filtered through a 0.45  $\mu\text{m}$  membrane filter (Waters, USA).

### General Synthetic Procedure for Compounds **2a** and **2b** (Scheme 1).

**Synthesis of (S)-Chloromethyl 4-(5-(2-oxo-4-Phenylimidazolidin-1-yl-sulfonyl)indoline-1-carbonyl) Phenyl Carbamate (3):** Under N<sub>2</sub>, a solution of **1** (JSH-2282, 300 mg, 0.61 mmol) and TEA (260  $\mu\text{L}$ , 3 eq) in dry THF (10 mL) was stirred in ice bath for 0.5 h, and then chloromethyl chloroformate (82  $\mu\text{L}$ , 1.5 eq) in dry THF (5 mL) was added. After continuous stirring for another 12 h, the reaction mixture was quenched by 50 mL of diluted water. It was taken up with EtOAc (100 mL × 3) and its organic layer was sequentially washed with saturated aqueous NaHCO<sub>3</sub> solution (50 mL × 2), brine (50 mL × 2) and water (100 mL × 2). The organic layer was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and was then evaporated under reduced pressure to give a crude solid, which was purified by column chromatography using the Hexane-EtOAc (3:1 → 1:1) as eluent to give **3** (120 mg, 35.4%) as a pale yellow solid.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.91 (s, 1H), 7.78 (m, 2H), 7.60-7.53 (m, 4H), 7.37 (d,  $J = 6.4$  Hz, 3H), 7.25 (d,  $J = 3.2$  Hz, 2H), 5.84 (s, 2H), 4.78 (t,  $J = 7.6$  Hz, 1H), 4.31 (t,  $J = 9.2$  Hz, 1H), 4.18 (t,  $J = 8.4$  Hz, 2H), 3.69 (dd,  $J = 9.6, 7.2$  Hz, 1H), 3.19 (t,  $J = 8.4$  Hz, 2H); IR (cm<sup>-1</sup>, KBr) 3300, 1730, 1560; ESIMS: *m/z* = 556.0 [M+1]<sup>+</sup>.

**Synthesis of BocNH-Asp(OTce)-OTce (4a):** To a solution of L-aspartic acid (1.0 g, 7.51 mmol) in dioxane/H<sub>2</sub>O (1:1, 40 mL) NaOH was added (1.2 g, 4 eq). The reaction mixture was stirred at room temperature for 0.5 h, and then cooled to 0 °C (ice bath). To the cooled reaction mixture (Boc)<sub>2</sub>O (2.5 g, 1.5 eq) was added and allowed to warm up to room temperature and further stirred for 18 h. After completion of the reaction, the mixture was diluted with water (200 mL) and titrated to pH 3-4 with 1 N HCl. The aqueous solution was extracted with EtOAc (100 mL × 3) and the EtOAc layers were all combined. The organic layer was washed with brine (100 mL × 2), water (100 mL × 2), and then evaporated *in vacuo*. The given BocNH-Asp-OH as colorless syrup was directly used in the next step.

The BocNH-Asp-OH was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and the reaction mixture was cooled to 0 °C, and then 2,2,2-trichloroethanol (1.1 mL, 2.4 eq), DCC (2.2 g, 2.4 eq), and DMAP (290 mg, 0.5 eq) was sequentially added and the mixture was stirred for 24 h under N<sub>2</sub>. The insoluble urea was removed by filtering through celite aid, and the filtrate was diluted with EtOAc 200 mL and washed with saturated NaHCO<sub>3</sub> (50 mL × 2), brine (50 mL × 2), and water (100 mL × 2). The organic layer was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and then evaporated *in vacuo*. The residue was purified by a Si gel column chromatography using the Hexane-EtOAc (5 : 1) as eluent to give compound **4a** (1.815 g, 49% for two steps) as a colorless syrup.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  4.81 (s, 2H), 4.76 (s, 2H), 4.13 (m, 1H), 3.25 (dd,  $J = 17.6, 4.8$  Hz, 1H), 3.10 (dd,  $J =$

17.4, 4.8 Hz, 1H), 1.46 (s, 9H).

**Synthesis of BocNH-Glu(OTce)-OTce (4b):** The procedure for the preparation of **4a** was applied for the synthesis of compound **4b** with L-glutamic acid (1.0 g, 6.8 mmol) as a starting material to give pure **4b** (810 mg, 68.8% for two steps) as a colorless syrup.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 4.76 (s, 2H), 4.75 (s, 2H), 4.53 (m, 1H), 2.68–2.62 (m, 2H), 2.83 (m, 1H), 2.08 (m, 1H), 1.45 (s, 9H).

**Synthesis of (S)-4-Oxo-3-(3-(4-(5-((S)-2-oxo-4-phenylimidazolidin-1-ylsulfonyl)indoline-1-carbonyl)phenyl)ureido)-4-(2,2,2-trichloroethoxy)butanoic acid (5a):** TFA (5 mL) was added to a stirred solution of BocNH-Asp(OTce)-OTce (**4a**, 53 mg, 0.11 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL). The reaction mixture was stirred at room temperature for 1 h. Volatile compounds in the reaction mixture were removed under reduced pressure. The residue was triturated and washed with anhydrous Et<sub>2</sub>O, and then the solid was isolated by decantation. The solid H-Asp(OTce)-OTce was dried under vacuum to remove the residual Et<sub>2</sub>O and was used in the next step without further purification.

Under N<sub>2</sub>, to a cooled (0 °C) stirred solution of compound H-Asp(OTce)-OTce in dry DMF (10 mL) was slowly added TEA (38 μL, 3 eq) and dropwise added a solution of compound **3** (50 mg, 0.09 mmol) in dry DMF (10 mL). The reaction mixture was stirred at 0 °C for 1 h and then at ambient temperature for 24 h. The reaction mixture was diluted with water (50 mL) and extracted EtOAc (50 mL × 3). The combined organic layer was washed successively with saturated NaHCO<sub>3</sub> (50 mL × 2), brine (50 mL × 2), and water (100 mL × 2). The organic layer was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo*. The residue was purified by a Si gel column chromatography using the cHx:EtOAc = 1:4 → 1:5 as eluent to give compound **5a** (40 mg, 58%) as a pale yellow solid.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 7.90 (s, 1H), 7.81 (m, 2H), 7.64 (dd, *J* = 27.2, 8.4 Hz, 4H), 7.36 (d, *J* = 6.8 Hz, 3H), 7.24 (d, *J* = 8.0 Hz, 2H), 4.82 (s, 2H), 4.77 (t, *J* = 7.6 Hz, 1H), 4.57 (m, 1H), 4.29 (t, *J* = 9.0 Hz, 1H), 4.17 (t, *J* = 8.8 Hz, 2H), 3.67 (dd, *J* = 8.0, 7.2 Hz, 1H), 3.29 (dd, *J* = 17.6, 3.2 Hz, 1H), 3.19 (t, *J* = 8.4 Hz, 2H), 3.04 (dd, *J* = 17.6, 8.4 Hz, 1H); IR (cm<sup>-1</sup>, KBr) 3300, 1720, 1650, 1170, 760; ESIMS: *m/z* = 754.0 [M+1]<sup>+</sup>.

**Synthesis of (S)-5-Oxo-4-(3-(4-(5-((S)-2-oxo-4-phenylimidazolidin-1-ylsulfonyl)indoline-1-carbonyl)phenyl)ureido)-5-(2,2,2-trichloroethoxy)pentanoic acid (5b):** The procedure for the preparation of **5a** was applied for the synthesis of compound **5b** with BocNH-Glu(OTce)-OTce (**4b**, 383 mg, 0.75 mmol) as a starting material to give pure **5b** (153 mg, 40%) as a pale yellow solid.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 7.92 (s, 1H), 7.81 (d, br, *J* = 8.0 Hz, 2H), 7.64 (dd, *J* = 28.8, 8.4 Hz, 4H), 7.37 (d, *J* = 6.8 Hz, 3H), 7.25 (d, *J* = 8.4 Hz, 2H), 4.79 (s, 2H), 4.77 (t, *J* = 7.6 Hz, 1H), 4.36 (t, *J* = 6.4 Hz, 1H), 4.31 (t, *J* = 9.2 Hz, 1H), 4.16 (t, *J* = 8.0 Hz, 2H), 3.69 (dd, *J* = 9.4, 7.2 Hz, 1H), 3.20 (t, *J* = 8.4 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.46–2.36 (m, 1H), 2.31–2.24 (m, 1H); IR (cm<sup>-1</sup>, KBr) 3310, 1720,

1650, 1180, 1100, 760; ESIMS: *m/z* = 768.0 [M+1]<sup>+</sup>.

**Synthesis of Sodium (S)-2-(3-(4-(5-((S)-2-Oxo-4-phenylimidazolidin-1-ylsulfonyl)indoline-1-carbonyl)phenyl)ureido)succinate (2a):** To a solution of compound **5a** (186 mg, 0.247 mmol) in THF/H<sub>2</sub>O (2:1, 22.5 mL) was added NaHCO<sub>3</sub> (46 mg, 2.2 eq) and stirred at 0 °C for 24 h. The reaction mixture was concentrated at 40 °C under vacuum. The residue was triturated and washed with anhydrous Et<sub>2</sub>O (5 mL × 3), and then the solid was isolated by decantation. The solid was again recrystallized with a cooled solution of acetone and ether, to give the desired compound **2a** (144 mg, 76%) as a white amorphous solid.

<sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD) δ 7.84 (s, 1H), 7.76 (s, br, 2H), 7.54 (s, br, 4H), 7.34–7.30 (m, 3H), 7.24 (d, *J* = 8.0 Hz, 2H), 4.78 (dd, *J* = 8.8, 6.0 Hz, 1H), 4.53 (t, *J* = 6.0 Hz, 1H), 4.31 (t, *J* = 9.4 Hz, 1H), 4.21 (t, *J* = 8.0 Hz, 2H), 3.60 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.19 (m, 2H), 2.83 (dd, *J* = 11.2, 5.2 Hz, 2H); IR (cm<sup>-1</sup>, KBr) 3300, 1710, 1650, 760; ESIMS: *m/z* = 622.6 [M+1]<sup>+</sup>.

**Synthesis of Sodium (S)-2-(3-(4-(5-((S)-2-Oxo-4-phenylimidazolidin-1-ylsulfonyl)indoline-1-carbonyl)phenyl)ureido)pentanedioate (2b):** The procedure for the synthesis of compound **2b** from compound **5b** (63 mg, 0.08 mmol) was similar to that used for compound **2a**. The compound **2b** (33 mg, 61%) was obtained as a white solid.

<sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD) δ 7.86 (s, 1H), 7.82 (d, br, 2H), 7.67 (dd, *J* = 52.0, 8.4 Hz, 4H), 7.36–7.31 (m, 3H), 7.21 (dd, *J* = 8.0, 2.0 Hz, 2H), 4.79 (dd, *J* = 8.6, 6.4 Hz, 1H), 4.34–4.29 (m, 2H), 4.19 (t, *J* = 8.4 Hz, 2H), 3.60 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.24 (t, *J* = 9.4 Hz, 2H), 2.36–2.34 (m, 2H), 2.03 (m, br, 2H); IR (cm<sup>-1</sup>, KBr) 3310, 1725, 1650, 1180, 1120, 760; ESIMS: *m/z* = 636.6 [M+1]<sup>+</sup>.

**Water Solubility.** JSH-2282 and analogs **2a** and **2b** were saturated in distilled water and shaken vigorously. The saturated solutions were sonicated for 30 min at 25 °C and passed through a syringe filter (Cellulose acetate 0.45 μm filter unit, DISMIC-13cp, Advantec). The concentrations of the filtrates were analyzed using reverse phase HPLC (RP-HPLC).

**In Vitro Plasma Reconversion Kinetics Studies.** Plasma was obtained from the purchased Male Sprague-Dawley rats. The stock solution (1 mg/mL) of compound **2a** and **2b** in dimethylsulfoxide (DMSO) was again diluted 100 times with the plasma and the mixture was maintained at 37 ± 0.5 °C by a Precision shaking water bath. No plasma protein precipitation was observed at this concentration of DMSO. The plasma was sampled at selected times (0 h, 1 h, 2 h, 4 h, and 8 h), and then it was later subjected to the sample preparation method described below and then analyzed with the modified HPLC assay (Table 1).

**Sample Preparation.** Plasma samples (40 μL) were diluted with a sufficient volume (3 mL) of methylene chloride to precipitate the plasma proteins as well as to extract the compounds of interest. After vortex mixing for 5 min and centrifuging at 3000 g for 10 min, the organic layer was carefully taken out and dried. The plasma extracts were reconstituted in 160 μL of methanol with vortexing. After

**Table 1.** The water solubility and cytotoxicity of **2a** and **2b** against SW620 and results of reconversion test (*in vitro*, *in vivo*)

Compound	Solubility (mg/mL)	IC <sub>50</sub> (μM) (SW620 <sup>a</sup> )	Reconversion test (h) <sup>b,c</sup>				
			0	1	2	4	8
<b>2a</b>	9.7140	5.46	X	X	X	X	X
<b>2b</b>	14.880	10.89	X	X	X	X	X
<b>1</b> (JSH-2282)	0.0235	0.39					

<sup>a</sup>SW620 is human colorectal adenocarcinoma cell line. <sup>b,c</sup>Yes (O) or No (X) on the detection of JSH-2282 under the above described HPLC condition on assigned time (0 h-8 h) *in vitro* rat plasma as well as *in vivo*.

syringe filtration (0.45 μm, Waters), the filtrate was analyzed using RP-HPLC.

#### In Vivo Kinetics Reconversion Studies Using Rats.

**Animals:** Male Sprague-Dawley rats, about 7 weeks old, were obtained from Nippon SLC Co. Ltd. (Hamamatsu, Japan). The rats were housed under controlled environmental conditions and fed commercial feed pellets. All rats had free access to food and water.

**In Vivo Kinetics Reconversion:** Five male Sprague-Dawley rats weighing 240-300 g were fasted overnight with free access to water for at least 12 h. A jugular vein cannula was equipped to sample systemic blood. At 30 min before drug administration, 250 μL of the blank blood sample was removed *via* cannula. Doses of the compound **2a** and **2b** were freshly prepared as saline solution immediately before administration. The administered volume of drugs was 1 mg/kg/mL. Compound **2a** and **2b** were administered intravenously, and then systemic blood was sampled *via* a jugular vein cannula (250 μL) at selected times (0 min, 15 min, 30 min, 60 min, 120 min, 240 min, and 480 min) after dosing. Blood samples were kept on ice, heparinized, and centrifuged, then the plasma was immediately stored at -80 °C until analysis by RP-HPLC. The plasma samples were combined with 700 μL of ethyl acetate, vortexed vigorously for 5 min, and shaken for 1 h at room temperature followed by centrifugation at 2500 x g for 15 min. The organic layer (550 μL) was evaporated to dryness using a rotary evapo-

rator (SC100, Savant), and then samples were reconstituted in 60 μL of methanol with vortexing. After syringe filtration (0.45 μm, Waters), the filtrate was analyzed using RP-HPLC.

**Bioassay.** The cytotoxicity of JSH-2282, compounds **2a** and **2b** was measured using the SRB assay described previously against SW-620 cancer cell lines.<sup>14</sup> Doxorubicin was used as the positive control. The results are shown in Table 1.

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