Selection of a Water-Soluble Salt Form of a Preclinical Candidate, IIIM-290: Multiwell-Plate Salt Screening and Characterization

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Supporting Information

ABSTRACT: IIIM-290, a semisynthetic derivative of natural product rohitukine, is an orally bioavailable Cdk inhibitor, efficacious in the xenograft models of colon, pancreatic, and leukemia cancers. Its low aqueous solubility (~8.6 μg/mL) could be one of the reasons for achieving optimal in vivo efficacy relatively at a higher dose. Being a nitrogenous compound, salt formation was envisaged as one of the ideal approaches to enhance its solubility and dissolution profile. Thus, herein, a solubility-guided miniaturized 96-well plate salt screening protocol was devised for identification of the suitable salt form of this preclinical candidate. The solubility-guided strategy has resulted in the identification of hydrochloride as the most favorable counterion, resulting in 45-fold improvement in aqueous solubility. The HCl salt was then scaled up at a gram size and characterized using 1H and 13C NMR, scanning electron microscopy, powder X-ray diffraction, Fourier-transform infrared, and differential scanning calorimetry studies. The HCl salt displayed enhancement in the in vitro dissolution profile as well as improved plasma exposure in the pharmacokinetic study. The oral administration of the IIIM-290-HCl salt in BALB/c mice resulted in >1.5-fold improvement in areas under the curve, Cmax, and half-life. The prepared salt also did not alter its cyclin-dependent kinase (Cdk)-2 and Cdk-9 inhibition activity. This biopharmaceutically improved lead has a potential to investigate further in preclinical studies. The solubility-guided salt screening strategy implemented herein could be utilized for other preclinical leads.

INTRODUCTION

The solubility of a drug molecule containing an ionized functional group is frequently enhanced by preparing its suitable salt form. Usually, the salt form of a drug is more soluble in an aqueous medium than its nonionized form and is an effective method for increasing the dissolution rate of drugs. A suitable salt form can modulate physicochemical properties and thus in vivo performance of many drug candidates. Selection of an optimized or appropriate salt form is a crucial step in the drug discovery and development process as it has a profound impact on biopharmaceutical and pharmaceutical properties. The selection of a right salt form during the discovery stage also reduces the development timeline of a new drug candidate. The salt screening and solid form selection in early drug discovery has a major impact on both preclinical and clinical developments of the lead candidate. In a typical organic chemistry laboratory, the preparation of salt of any organic compound requires at least 50 mg quantity of the compound. Thus, optimization of a suitable counterion using such a strategy in a chemistry lab would require a larger amount of compound, may be up to 1–2 g. However, gram-sized quantities are only available when the molecule enters in the developmental stages. To address this need, researchers have attempted to miniaturize screening procedures such as high-throughput methods to identify potential salts.

Rohitukine is a chromone alkaloid isolated from Indian medicinal plant Dysoxylum binectariferum Hook. (Meliaceae). This natural product has inspired the discovery of two clinical candidates, viz. flavopiridol and riviciclib, among which the former has also received orphan drug status from U.S. Food and Drug Administration (FDA) for treatment of chronic lymphocytic leukemia and acute myeloid leukemia. Recently, our group has discovered an orally bioavailable preclinical candidate, IIIM-290, from this scaffold. IIIM-290, (1′R,2′S)-2-(2,6-dichlorostyryl)-5,7-dihydroxy-8-(3-hydroxy-1-methylpiperidin-4-yl)-4'H-chromen-4-one (Figure 1), is a potent inhibitor of cyclin-dependent kinase-9 (Cdk-9) exhibiting in vivo efficacy in colon, pancreatic, and leukemia xenograft models. It inhibits Cdk-9 with IC50 of 1.9 nM and exhibits cellular antiproliferative activity with the GI50 of 1.0 μM in leukemia and pancreatic cancer cell lines. In spite of its potent in vitro activity profile, the optimal in vivo efficacy was obtained at 50 mg/kg dose.

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which is relatively higher than the theoretical requirement. It is a basic compound \( pK_a = 5.4 \) with moderate solubility in water and in biorelevant media \( \text{pH} 1.2 - 7.4 \). The experimental octanol/water partition coefficient \( \log P \) and distribution coefficient \( \log D \) of IIIM-290 are 3.09 and 1.65, respectively. The inadequate aqueous solubility could be one of the reasons for its poor in vitro-in vivo correlation. To address the low solubility concern of this compound, the identification of an appropriate salt form of IIIM-290 was planned. Hence, we aimed at developing a screening procedure to identify the optimum salt form/s to improve its solubility and dissolution, which in turn is expected to modulate the in vivo exposure.

The present study describes miniaturized salt screening with various counterions to identify a suitable salt of IIIM-290. The solubility-guided screening was performed for the selection of appropriate counterions. With this screening approach, we focused on maximizing the hit rate in terms of solubility, its selection for scale-up, and subsequent characterization. The impact of optimum salt form/s on the solubility and dissolution of IIIM-290 was studied. The identified salt form was prepared in gram quantity and was completely characterized for its physicochemical parameters, in vitro dissolution profile, in vitro efficacy, and oral pharmacokinetics in mice.

### RESULTS AND DISCUSSION

**Identification of Appropriate Counterion/s via Solubility-Guided Salt Screening.** The developed solubility-based miniaturized protocol for the identification of a suitable counterion utilized a very small amount of compound \(~100 mg\), which can be manageable at the drug discovery stage. Therefore, it can be applied to early drug discovery and development where efforts on the preclinical candidate are required to make it druggable. The counterions for the salt screening experiment were chosen based on the criteria of \( pK_a \) difference of \( \geq 2 \) between the counterion and IIIM-290. On the basis of the \( pK_a \) values of counterions, phosphoric acid (PA) \( (2.15, 7.21, 12.32) \), maleic acid (MA) \( (1.9, 6.07) \), malonic acid (MlnA) \( (2.83, 5.69) \), oxalic acid (OA) \( (1.2, 4.2) \), HCl \( (−7) \), 4-aminobenzoic acid (4-ABA) \( (2.38) \), succinic acid (SA) \( (4.2, 5.6) \), fumaric acid (FA) \( (3.5, 4.5) \), formic acid (FoA) \( (3.77) \), hippuric acid (HA) \( (3.59) \), toluene sulfonic acid (TSA) \( (−2.8) \), and citric acid (CA) \( (3.73, 4.76, 6.4) \) were selected for primary salt screening with the possibility of formation of salt with IIIM-290 \( (pK_a = 5.4) \). Upon addition of counterion solutions and reaction solvents to the wells preloaded with IIIM-290, it was observed that the orange color of IIIM-290 turned to yellow in all wells except for 4-ABA and SA, which provided an indication of the chemical reaction between the respective counterion and the compound. The product formed in the wells was solid except for those wherein acetone/isopropyl alcohol (IPA) \((50:50 \text{ v/v}) \) and EtOH/acetonitrile (ACN) \((50:50 \text{ v/v}) \) were used as reaction solvents. These solvents yielded a solvated mass at the bottom of the well. Therefore, these two solvent combinations were omitted during secondary screening experiments. Furthermore, the in situ determination of thermodynamic equilibrium solubility data showed improved solubility of IIIM-290 in the wells loaded with HCl, FoA, and HA as counterions (Figure 2a). The average water solubility values of IIIM-290 in the presence of equimolar concentrations of HCl, FoA, and HA were found to be 483.87, 496.96, and 506.1 \( \mu g/mL \), respectively. The data

![Figure 1. Chemical structure of IIIM-290 and its physicochemical and efficacy data.](image)  
![Figure 2. Water solubility of IIIM-290 in the presence of counterions (a). Average water solubility of IIIM-290 in the presence of counterions in primary screening experiments. Statistical analysis; ns, \( P > 0.05 \); **, 0.01 < \( P < 0.02 \); *, 0.04 < \( P < 0.03 \); ****, \( P < 0.0001 \) (b). Effect of different ratios of IIIM-290 to counterions on water solubility.](image)
was statistically analyzed using Dunnett’s multiple comparisons test ($P < 0.05$) using GraphPad Prism 6.01.

On the basis of the observation from primary screening, IIIM-290 in combination with HCl, FoA, and HA was studied for improvement in the solubility of IIIM-290, if any, using different molar ratios, viz., 1:1, 1:2, and 1:4. Reaction solvents acetone/IPA (50:50 v/v) and EtOH/ACN (50:50 v/v) were exempted during secondary screening as they yielded solvated mass during primary screening. The average solubility of all of the experiments performed during secondary screening is depicted in Figure 2b. There was no proportionate increase in the aqueous solubility of IIIM-290 with counterions HA, HCl, and FoA in 1:2 and 1:4 ratios. The reason behind this observation was attributed to the fact that IIIM-290 contains only one site required for salt formation (piperidine ring containing single N). Formic acid was also eliminated from

Figure 3. (a) $^1$H NMR overlay of IIIM-290 and its salt forms; (b) $^1$H chemical shift perturbations of the IIIM-290-HCl salt.
further scale-up studies because it does not fall under the category of salt forms that were approved by the FDA. Hence, the scale-up of hydrochloride and hippurate salts of IIIM-290 was considered for further studies.

**Scale-up Synthesis of Selected IIIM-290 Salts.** The solubility of IIIM-290 in methanol was only $\sim 0.5$ mg/mL and is the limiting factor for scale-up synthesis. Hence, a combination of MeOH with water and CHCl$_3$ was tried to find out the optimum reaction solvent mixture that is suitable for scale-up synthesis. The process was optimized from 25 to 2000 mg scale. In the experiments involving hippuric acid as the counterion, various combinations of reaction solvents were used viz. MeOH/H$_2$O (80:20 v/v), MeOH/CHCl$_3$ (25:75 v/v), MeOH/CHCl$_3$/H$_2$O (70:20:10 v/v), and MeOH/CHCl$_3$ (75:25 v/v). Solubility of IIIM-290 was >10 mg/mL in the MeOH/CHCl$_3$ (75:25 v/v) mixture. However, the product obtained using all solvent combinations as mentioned above showed amorphous melting between 120 and 140 °C. Furthermore, the product obtained by reacting IIIM-290 with hydrochloric acid in the presence of MeOH/H$_2$O (80:20 v/v), MeOH/CHCl$_3$ (25:75 v/v), and MeOH/CHCl$_3$ (75:25 v/v) as reaction solvents showed the melting point of 312–315 °C. The obtained products were characterized and confirmed by NMR spectroscopy. Briefly, MeOH/CHCl$_3$ (75:25 v/v) was identified as the optimum reaction solvent for scale-up of IIIM-290 salt/s.

**1H NMR and 13C NMR.** The quaternization of the tertiary nitrogen atom in any organic compound gets reflected in the 1H NMR spectrum as a downfield shift in $\delta$ ppm values of hydrogen present on adjacent carbons. As expected, the 1H NMR spectroscopy of the IIIM-290-HCl salt showed a downfield shift in $\delta$ ppm values of the hydrogens of the piperidine ring. The difference in the chemical shifts of protons near the nitrogen atom in the 1H NMR spectrum of the free base and HCl salt was observed (Figure 3a). In general, a trend of downfield shift of chemical shift values of protons present near the tertiary nitrogen was observed in the HCl salt in comparison to that in the free base. The notable differences include the N−CH$_3$ group and the −CH$_2$ groups connected with a tertiary amine. The chemical shift value of the N−Me group in the free base was 2.56 ppm, which was downfield-shifted by 0.18 ppm in the HCl salt (to 2.74 ppm). One proton of the 6′-CH$_2$ group appears at 1.50 ppm in the free base; however, it has been downfield-shifted by 0.18 ppm in the HCl salt (to 1.87 ppm). The region of 2.79−3.43 ppm for other methylene protons from the piperidine ring (H-3″, H-5″, H-1″, H-6″) has also been shifted downfield (to 3.15−3.53 ppm) in the case of the HCl salt. The −CH proton (H-2″) of the piperidine ring has been slightly downfield-shifted by 0.05 ppm (shifted from 4.04 to 4.09 ppm) in the HCl salt. Interestingly, the H-3 and H-6 protons of the chromone ring were significantly downfield-shifted in the HCl salt. The H-3 proton appearing at 5.81 ppm in free base has been downfield-shifted by 0.59 ppm (shifted from 4.04 to 4.09 ppm) in the HCl salt. Similarly, the H-3 and H-6 protons of the chlorone ring were significantly downfield-shifted in the HCl salt. The H-3 proton appearing at 5.81 ppm in free base has been downfield-shifted by 0.59 ppm (shifted to 6.40 ppm in the HCl salt). Similarly, the H-6 proton appearing at 6.33 ppm in free base has been shifted by 0.22 ppm (shifted to 6.55 ppm in the HCl salt). There was no change in the chemical shift values of the styryl ring. 1H chemical shift perturbations observed for the IIIM-

![Figure 4. DSC curves of IIIM-290 and its salt forms.](image-url)
The 1H NMR spectrum of IIIM-290 hippurate did not show any change in chemical shift values of piperidine ring protons, indicating no quaternarization on the tertiary nitrogen. We also compared the 13C NMR spectra of the free base and HCl salt (Figure S10, Supporting Information). There was around 2 ppm shift in chemical shift values of all six carbons (region of δ 22.62–66.39 ppm) present in the piperidine ring (including N−Me). However, the aromatic region (δ 99.81–182.71 ppm) of the 13C NMR spectrum was not affected by salt formation. Thus, the NMR study confirmed the successful formation of the HCl salt; however, no such observation was noted in the case of the hippurate form, which indicated no any chemical interaction and thus no salt formation. It led to the formation of only an amorphous mixture.

Differential Scanning Calorimetry (DSC), Fourier-Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), and Powder X-ray Diffraction (p-XRD) Analyses. The increase in the melting point of the salt form is usually accompanied by enhanced thermodynamic stability, easier processing conditions, and improved relative compatibility with formulation excipients.24 The thermal features of salts were revealed from DSC thermograms, as shown in Figure 4. DSC data of both IIIM-290 and IIIM-290·HCl showed a sharp melting endotherm at 247 and 324 °C, respectively. The IIIM-290·HCl salt showed a much higher melting point (~77 °C) than that of its free base. The physical
mixture of IIIM-290 and hippuric acid showed no amorphous transformation, which was evident due to their melting endotherm at 255 and 192 °C, respectively. Furthermore, the absence of melting endotherm in IIIM-290 hippurate indicated conversion of IIIM-290 to an amorphous state.

The moisture content in salts is one of the important developability parameters and has direct correlation with its chemical stability.5, 25 The moisture contents in IIIM-290, IIIM-290-HCl, and IIIM-290 hippurate form, at the end of 6 months, were determined by the loss on drying method. The % moisture content of IIIM-290-HCl and IIIM-290 hippurate form were found to be 3.5, 5, and 8.2% w/w, respectively. The FTIR spectra of IIIM-290-HCl (Figure S12) and IIIM-290 hippurate forms (Figure S13) showed broad peaks at 3418 and 3400 cm⁻¹, respectively, accounting for the moisture associated with it. IIIM-290 as a free base also showed the peak at 3416 cm⁻¹, for the −OH functionality present in its structure; however, the enhanced intensity of this peak in the HCl salt and hippurate form indicated moisture in the samples. The endotherm at 100 °C in the DSC spectrum of IIIM-290-HCl further confirmed the presence of moisture.

FTIR measurements of IIIM-290 and its salt forms were compared (Figures S11–S14). The assignments of some characteristic absorption bands are summarized in Table S1. The peaks in “region 1” due to the O−H stretching were absent in IIIM-290 hippurate, indicating amorphous transformation. The C−H and C−N stretching absorptions for IIIM-290 and its HCl salt, in region 2 and 4, indicated distinguished vibrations.

The morphology of IIIM-290, IIIM-290-HCl, IIIM-290 hippurate form and the physical mixture of IIIM-290 with hippuric acid was assessed by SEM. Intense morphological difference was observed between IIIM-290 (Figure S5a,b) and IIIM-290-HCl salt (Figure S5c,d) in SEM images. SEM micrographs revealed a rod-shaped (10−20 μm) morphology of IIIM-290, elongated needles (30–70 μm) of IIIM-290-HCl, and flake-like irregular aggregates (2–10 μm; Figure S5e,f) of the IIIM-290 hippurate form, indicating mixture of crystalline and amorphous particles. The physical mixture of hippuric acid and IIIM-290 showed a mixture of rods and flakes.

Their physical state was also evaluated by powder XRD (Figure 6). The p-XRD patterns of IIIM-290 and its HCl salt were distinctive, with the appearance of new, sharp, and characteristic peaks indicating the formation of a pure crystalline solid, and this was in agreement with ¹H and ¹³C NMR data. The physical mixture of IIIM-290 and hippuric acid retained crystallinity in comparison to that of the IIIM-290 hippurate form, wherein the p-XRD pattern indicated reduced crystallinity of IIIM-290.

Solubility, Partition Coefficient, in Vitro Dissolution, Kinetic Solubility, and pH-Solubility Profile. Solubility and partition coefficient (log P) are important preformulation parameters that have a direct impact on the absorption of orally administered drugs. Salt formation is one approach to improving the solubility of the poorly soluble drug candidate. IIIM-290-HCl and IIIM-290 hippurate demonstrated high water solubility as compared to that of free base (8.61 ± 1.8 μg/mL). The thermodynamic equilibrium solubility values of IIIM-290-HCl and hippurate were 362.23 ± 38.39 and 360.02 ± 13.19 μg/mL respectively. There was ~40-fold improvement in water solubility of both forms in comparison to that of the free base. The solubility data was in agreement with log P value, which is a measure of lipophilicity. Log P of IIIM-290-HCl was 1.82 ± 0.14, indicating the hydrophilic nature of salt over the free drug (log P = 3.1 ± 0.22). Interestingly, the IIIM-290 hippurate form showed enhanced solubility; however, no change in log P was observed with that of the free base. The plausible reason for significant improvement in solubility of IIIM-290 is the formation of a eutectic mixture between IIIM-290 and hippuric acid. Furthermore, ¹H NMR data confirmed no chemical interaction between IIIM-290 and hippuric acid. When ¹H NMR of the IIIM-290 hippurate form was compared with that of IIIM-290, no change in chemical shift was observed, which ruled out the possibility of formation of cocrystals of IIIM-290 with hippuric acid. If cocrystals have formed, due to the hydrogen bonding between IIIM-290 and hippuric acid, the chemical shift of participating atoms should have moved downfield because of increase in electron density.

For poorly soluble drugs, in traditional dissolution methods, sink conditions are recommended to discriminate the dissolution profiles of the poorly soluble compound/active pharmaceutical ingredient and its formulations. Dissolution testing of any solid oral dosage form involves either the basket or the paddle apparatus, which is based on the principle of operating under “sink conditions.”26 The standardized conditions are chosen to provide a gentle hydrodynamic regimen. “Physiological” media or solutions incorporating surfactants are preferred over water/organic solvent mixtures. According to pharmacopeial and regulatory considerations, the sink condition is defined as an excess solubilizing capacity of dissolution medium, wherein the concentration of the compound in dissolution media is 3−10 times higher than the

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saturation solubility of the compound. Particularly, in this case, the saturation solubility of IIIM-290 in water is ∼8.61 ± 1.8 μg/mL; thus, different concentrations of surfactants, namely, sodium lauryl sulfate (SLS), Tween 80, and Tween 40, were attempted to identify appropriate sink conditions. The saturation solubility of IIIM-290 and its sink index at different concentrations of surfactants is shown in Figure 7a,b, respectively. There was no linear increase observed in the solubility of IIIM-290 in Tween 80 and Tween 40. A linear increase in solubility of IIIM-290 was noted in 0.05, 0.1, 0.25, and 0.5% w/v SLS solutions. The sink index was calculated, and for dissolution experiments, 0.25% w/v SLS was added to physiological media to maintain sink conditions.

The in vitro dissolution profiles of IIIM-290, IIIM-290-HCl salt, and IIIM-290 hippurate form in physiological media: (a) water containing 0.25% SLS; (b) HCl buffer (pH 1.2) containing 0.25% SLS; (c) phosphate buffer (pH 6.8) containing 0.25% SLS, and (d) time-dependent solubility of IIIM-290 and its HCl salt at 37 °C.

Table 1. Dissolution Results of IIIM-290, IIIM-290-HCl Salt and IIIM-290 Hippurate Form

|                | T_{max} (min) | C_{max} (μg/mL) (mean ± SEM) | AUC_{0−t} (μg/mL min) (mean ± SEM) | AUC ratio^a |
|----------------|---------------|------------------------------|-----------------------------------|-------------|
| IIIM-290       | 30            | 6.93 ± 0.91                  | 3557.23 ± 247.03                  | 1           |
| IIIM-290-HCl   | 60            | 15.42 ± 1.61                 | 5240.98 ± 350.59                  | 1.47        |
| IIIM-290 hippurate | 60        | 1.20 ± 0.13                  | 1048.09 ± 92.58                   | 0.29        |
| IIIM-290       | 15            | 16.06 ± 0.49                 | 2102.46 ± 80.09                   | 1           |
| IIIM-290-HCl   | 30            | 19.51 ± 0.09                 | 2223.07 ± 81.96                   | 1.1         |
| IIIM-290 hippurate | 30        | 14.11 ± 0.48                 | 1789.91 ± 78.19                   | 0.86        |
| IIIM-290       | 15            | 9.52 ± 1.26                  | 4898.69 ± 317.03                  | 1           |
| IIIM-290-HCl   | 15            | 13.58 ± 0.30                 | 5896.10 ± 366.69                  | 1.2         |
| IIIM-290 hippurate | 15       | 2.53 ± 0.32                  | 2395.56 ± 173.57                  | 0.49        |

^a[AUC_{0−t, sample}]/[AUC_{0−t, IIIM-290}]. SEM = standard error of the mean. Here, sample means IIIM-290-HCl or IIIM-290 hippurate form.
those of the free base was observed in water as well as in physiological media, namely, HCl (pH 1.2) and phosphate buffer (pH 6.8). However, the IIIM-290 hippurate form showed a lower dissolution rate and AUC from 0 to infinity than those of the free base. The comparatively low dissolution rate of the IIIM-290 hippurate form may be attributed to the coformer, hippuric acid. The dissolution values of IIIM-290, HCl salt, and hippurate form in water at the end of 120 min were 49, 78, and 12% w/v, respectively (Figure 8a). Similarly, as shown in Figure 8b, the % drug dissolved in HCl buffer (pH 1.2) at 30 min for the IIIM-290-HCl salt was 99% in comparison to that for its free base (83%) and hippurate form (71% w/v). The same observation was noted down (Figure 8c) when dissolution was performed in phosphate buffer (pH 6.8). At the end of 60 min, the HCl salt showed a higher dissolution (84%) compared to that of IIIM-290 and its hippurate form (63 vs 26% w/v). At acidic pH 1.2, which corresponds to a fasting state of the stomach, both components, viz., IIIM-290 and its HCl salt, were highly soluble, as might be expected (Figure 8b).

However, whether a drug is acidic or basic, most of its absorption occurs in the small intestine (pH 6–8) and hence the dissolution at pH 6.8 is more relevant. In summary, the dissolution enhancement is due to the salt formation as typically seen for pharmaceuticals. The time-dependent solubility of the free base and its HCl salt at 37 °C was determined to find out their extent of supersaturation state in water. The HCl salt of IIIM-290 allows it to remain in the solution state for a longer period in comparison with the parent compound (Figure 8d). The HCl salt form of IIIM-290 evidenced to inhibit its crystallization for a prolonged period of time (the “parachute” effect). The sustained parachute effect provides high apparent solubility, which is responsible for the improvement in its efficacy. The experimental and calculated \( \text{pH}_{\text{max}} \) values were determined from the pH-solubility profile of the IIIM-290-HCl salt. \( \text{pH}_{\text{max}} \) is the pH value above which the salt can potentially convert to its free base form. The \( \text{pH}_{\text{max}} \) was calculated using formula \( \text{pH}_{\text{max}} = pK_a + \log(\text{sol FA/sol HCl}) \). The calculated and experimental \( \text{pH}_{\text{max}} \) values for the IIIM-290-HCl salt were 3.59 and 3.0, respectively, which were in accordance with each other (Figure 9). The pH shift after solubility determination in water was also monitored, and it was 3.6. The \( \text{pH}_{\text{max}} \) is crucial to determine the instability issue of salt during developmental stages and thus excipients which will elevate microenvironmental pH above the \( \text{pH}_{\text{max}} \) can be avoided during formulation development of salts.

**In Vitro Inhibition of Cdk-2/A and Cdk-9/T1 and Pharmacokinetics of the IIIM-290-HCl Salt.** To test the potency of HCl salt of IIIM-290, the kinase inhibition of Cdk-2/A and Cdk-9/T1 was studied in comparison to the free base at a concentration of 500 nM. The % inhibition data is depicted in Table 2, which supported retained biological activity by the HCl salt form.

### Table 2. In Vitro Inhibition of Cdk-2/A and Cdk-9/T1 by IIIM-290 and Its HCl Salt at 500 nM

| test sample | % inhibition (±SD) |
|-------------|---------------------|
| IIIM-290    | 90 ± 0.2            |
| IIIM-290-HCl| 85 ± 0.5            |

The pharmacokinetics of IIIM-290 and its HCl salt was evaluated in BALB/c mice following a single 50 mg/kg dose administration by oral route. The exposure (\( \text{C}_{\text{max}} \) and AUC from 0 to infinity) of the IIIM-290-HCl salt was higher than that of the free base and was found to be 1030 versus 656 ng/mL and 3710 versus 2570 ng h/mL, respectively. The elimination half-life (\( T_{1/2} \)) values were found to be 1.92 and 5.06 h, respectively, for the free base and IIIM-290-HCl salt. This indicated decreased clearance of IIIM-290 when administered as a salt form. Overall, the pharmacokinetic exposure following oral administration of the IIIM-290-HCl salt was higher compared to for the free base. The data presented in Table 3 indicated improved ADME parameters of the HCl salt over IIIM-290 as a free base. Furthermore, the AUC from 0 to infinity ratio obtained from the in vitro dissolution result was in agreement with the pharmacokinetic studies of IIIM-290-HCl with respect to its free base (1.47 vs 1.44).

### Table 3. Pharmacokinetic Parameters of IIIM-290 and IIIM-290-HCl Salt in BALB/c Mice

| PK parameter | IIIM-290 | IIIM-290-HCl | PO, 50 mg/kg |
|--------------|----------|--------------|-------------|
| \( T_{1/2} \) (h) | 1.92     | 5.06         | 2570        |
| \( C_{\text{max}} \) (ng/mL) | 656     | 1030         | 3600        |
| \( T_{\text{max}} \) (h) | 1.0      | 2.0          | 3710        |
| AUC from 0 to infinity (ng h/mL) | 2570 | 3600 |           |
| AUC from 0 to infinity (ng h/mL) | 2570 | 3710 |           |

**CONCLUSIONS**

The oral route is the most preferred way of drug administration. However, solubility and dissolution of the drug candidate are the limiting factors for its oral absorption. Our investigation enabled successful development of the HCl salt of IIIM-290 with improved solubility and dissolution with better pharmacokinetic exposure. This research enabled successful development of solubility-based miniaturized 96-well plate salt screening methodology. The protocol was used for the identification of a suitable counterion required for the preparation of the salt of preclinical candidate, IIIM-290. The hydrochloride salt was identified and prepared up to 2 g scale. The characterization by p-XRD, \(^1\)H and \(^13\)C NMR, FTIR, SEM, and DSC analyses confirmed the formation of a new

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**Figure 9.** pH-solubility profile of the IIIM-290-HCl salt.
crystalline form. The HCl salt exhibited better physicochemical properties such as high water solubility and decreased log P value compared to those of the free base. It also led to dissolution enhancements in different physiological buffers (pH 1.2−6.8). Time-dependent solubility also proved that the HCl salt allows IIIM-290 to remain in the solution state for a longer period. The pharmacokinetic exposure of the IIIM-290·HCl salt by the oral route in BALB/c mice was higher and comparable with the free base.

## MATERIALS AND METHODS

### Materials

The preclinical candidate, IIIM-290, was synthesized in the laboratory up to 60 g scale with high-performance liquid chromatography (HPLC) purity of ~99.7% w/w. Counterions, viz., phosphoric acid (PA) and formic acid (FoA), were purchased from RFCL Limited (RANKEM), India. Maleic acid (MA), succinic acid (SA), fumaric acid (FA), hippuric acid (HA), 4-aminobenzoic acid (4-ABA), and toluene sulfonic acid (TSA) were procured from Alfa Aesar, India. Malonic acid (MInA) and hydrochloric acid (HCl) were purchased from S. D. Fine Chem. Ltd, India. Oxalic acid (OA) and citric acid (CA) were received from Merck Ltd., India, and Sigma-Aldrich, India, respectively. HPLC-grade methanol and water (Fisher Scientific, Mumbai) were used throughout the study. HPLC (Shimadzu, LC-6AD), Chromolith performance RP-18e (100−4.6 mm, Merck) HPLC column, vortex (IKA vortex Genius 3), microplate shaker (Eppendorf, Thermo-Mixer, Germany), microcentrifuge 5430R (Eppendorf), sonicator and micropipettes (Eppendorf) were used for the study.

$^1$H NMR spectrum was recorded on a Bruker-Avance DPX FT-NMR 400 MHz instrument. FTIR spectra were recorded on a PerkinElmer IR spectrophotometer. Melting points were recorded using a Buchi melting point M-560 apparatus. Differential scanning calorimetry was performed using TA Instruments Q-10 DSC. Powder X-ray diffraction measurements were carried out on a PANalytical’s X-ray diffractometer. Dissolution studies were performed using the paddle method on a dissolution apparatus (Labindia DS 8000) (Indian Pharmacopeia, 2007).

### Primary Screening for Selection of Counterion

Solutions of IIIM-290 and respective counterions were loaded in a 96-well plate in the equimolar ratio as shown in Figure 10. Into the 96-well plate, 50 μL of methanolic solution (25 mM) of IIIM-290 was loaded, and the plate was shaken at 50 °C and 300 rpm for evaporation of methanol. The plate was further subjected to overnight drying by keeping it in the desiccator. This exercise was performed to load accurately a very small quantity of IIIM-290 (~570 μg) into each well. In the next step, 50 μL of 25 mM methanolic solution of all counterions except for HCl, formic acid, and phosphoric acid. Aqueous solutions (10 μL) of 125 mM volatile acids, viz., HCl, formic acid, and phosphoric acid, were used for the study. Furthermore, 200 μL of reaction solvents, as depicted in Figure 10, was added to the corresponding wells. The 96-well plate was stored in a vacuum desiccator for 24 h for complete removal of solvent/s, if any. The physical characteristics like change in the color and mass distribution of the IIIM-290·HCl salt by the oral route in BALB/c mice was higher and comparable with the free base.

![Figure 10. Schematic diagram of the 96-well plate salt screening protocol for IIIM-290.](image-url)
pattern of the solid mass formed in the wells were noted down. The equilibrium saturation solubility of the product formed in each well was determined as per our previously published protocol. Briefly, 200 μL of water was added to each well and the plate was shaken at 300 rpm at 25 °C for 24 h. The obtained solution was transferred to microcentrifuge tubes and centrifuged at 16 000 rpm at 25 °C. The supernatant was diluted appropriately with methanol and analyzed by HPLC.

**Secondary Screening To Identify Optimum Counterion Ratio and Reaction Solvent/s.** On the basis of the solubility improvement of IIIM-290 in primary screening, the counterions, viz., hippuric acid, HCl, and formic acid, were selected further to identify the optimum ratio of counterions with the compound, IIIM-290. Three different molar ratios, viz., 1:1, 1:2, and 1:4, of the compound, IIIM-290, and counterions were chosen. The compound and respective counterions were loaded into the 96-well plate as mentioned in the above section. The visual observation and the obtained counterions were loaded into the 96-well plate as mentioned in the previous section. Reaction solvents such as the mixture acetone/IPA (50:50 v/v) and EtOH/ACN (50:50 v/v) were not considered during the selection of the optimum reaction solvents. The reason being they yielded solvated material. The plate was dried overnight in the desiccator, and visual observations of the product formed in each well were noted down. The saturation solubility of all samples was determined as mentioned in the earlier section.

**Scale-up Synthesis of HCl and Hippurate Salt of IIIM-290.** For scale-up of HCl and hippurate salt of IIIM-290, the reaction vehicle comprising methanol in mixture with another solvent was selected on the basis of the nature of the reaction mixture formed in the well. The limiting factor for scale-up experiments was the low solubility of IIIM-290 (0.5 mg/mL) in methanol. Hence, we tried CHCl3 in combination with MeOH to select the optimum reaction solvent for scale-up of IIIM-290. Three different molar ratios, viz., 1:1, 1:2, and 1:4, of the compound, IIIM-290, and counterions were chosen. The compound and respective counterions were loaded into the 96-well plate as mentioned in the above section. The visual observation and the obtained counterions were loaded into the 96-well plate as mentioned in the earlier section.

**HPLC Method for Estimation of IIIM-290 Concentration.** The reversed-phase HPLC method for estimation of IIIM-290 involved reversed-phase C18 Chromolith performance RP-18e (100–4.6 mm) column using a photodiode detector (SPD-M20A, Prominence, Shimadzu). For gradient elution, CH3OH and 0.1% v/v formic acid in water as a mobile phase were pumped with the flow rate of 1 mL/min through pumps A and B (LC-6AD Shimadzu), respectively. The gradient comprised 70% B for 0–2 min, 70–30% B for 2–10 min, 30% B for 10–12 min, 30–70% B for 12–17 min, and 70% B for 17–20 min. The injection volume was 3.0 μL (SIL-20A HT Prominence auto-sampler). The column oven (CTO-10ASVP) temperature was 37 °C. The calibration curve was obtained by injecting methanolic solutions of IIIM-290 using different concentrations, viz., 5, 10, 20, 40, and 80 μg/mL (r² = 1.0, RSD < 6%). The retention time of the compound was 11.99 min. Each analysis was performed in triplicate.

**1H NMR and 13C NMR Analyses.** The chemical interaction, if any, among IIIM-290, counterions, and their respective salt forms was studied by 1H NMR. Briefly, 1H NMR spectra of IIIM-290 along with its HCl salt and hippurate form were recorded using a Bruker-Avance DPX FT-NMR 400 MHz instrument using deuterated dimethyl sulfoxide (DMSO-d6). 13C NMR spectra were recorded at 125 or 100 MHz.

\[ \text{2-((E)-2,6-Dichlorostyryl)-5,7-dihydroxy-8-((3S,4R)-3-hydroxy-1-methylpipеридin-4-yl)4H-chromen-4-one (IIIM-290).} \]

Yellow solid; mp 235–237 °C; HPLC tR = 12.9 min (99% purity); 1H NMR (400 MHz, DMSO-d6): δ 7.59 (d, J = 8 Hz, 2H), 7.57 (d, J = 16 Hz, 1H), 7.4 (t, J = 8 Hz, 1H), 7.07 (d, J = 16 Hz, 1H), 6.33 (s, 1H), 5.81 (s, 1H), 4.04 (brs, 1H), 3.43 (m, 1H), 3.24 (m, 2H), 2.94 (d, J = 12 Hz, 1H), 2.84 (m, 2H), 2.56 (s, 3H), 1.23 (d, J = 8 Hz, 1H). 13C NMR (125 MHz, DMSO-d6): δ 182.6, 163.99, 160.88, 160.08, 154.54, 134.99, 131.76, 129.83, 129.8, 129.7, 129.2, 128.32, 110.57, 106.92, 105.33, 101.69, 68.96, 62.13, 56.2, 45.77, 37.69, 24.9.

**FTIR (KBr):** νmax 3550, 3469, 3416, 3241, 3059, 2924, 2853, 1656, 1590, 1550, 1447, 1420, 1382, 249, 124.9.

**Differential Scanning Calorimetry.** Thermal behavior of IIIM-290 and its salt forms were recorded using a Q10 TA Instruments thermoanalyzer, which was equipped with Trios V4.1 software. The instrument was calibrated using indium for
temperature and enthalpy change. For thermal analysis, 3–5 mg of the sample was placed in a sealed aluminum pan and scanned from 40 to 400 °C at the heating rate of 10 °C/min. The onset temperature and enthalpy change for each thermal event were calculated.

**Fourier-Transform Infrared Spectroscopy.** FTIR analysis was performed using a PerkinElmer FTIR spectrophotometer equipped with spectrum software version 10.03.06. FTIR spectra were obtained from 16 scans over the range of 4000–700 cm⁻¹. KBr disks were prepared by grinding 2 mg of the sample with 200 mg of KBr and the pellet was directly compressed by applying pressure of 2–3 t for 2 min.

**Scanning Electron Microscopy (SEM).** Representative SEM images of IIIM-290 samples were taken using a scanning electron microscope, JEOL JSM-IT300, with gold coating. For the SEM observations, each sample was fixed on an aluminum sample holder using double-sided carbon tape.

**Powder X-ray Diffraction.** Powder X-ray diffraction measurements were carried out on a PANalytical’s X’Pert Pro X-ray diffractometer. For X-ray radiation source, a Cu Kα (λ = 45 kV, 40 mA) anode was used, ranging 2θ between 5 and 50° with 0.5 s/step scan rate with 0.017° increment.

**Solubility and Partition Coefficient Determination.** The thermodynamic equilibrium solubility in water and partition coefficient (log P) of HCl and hippurate salt of IIIM-290 were determined using our previously published protocols. For determining solubility in water, briefly, in a 1.5 mL microcentrifuge tube, an excess of the sample was added to 500 μL of water, which was mixed using a vortex mixer for 5 min. The samples were further shaken at 300 rpm at 25 °C for 24 h. Eppendorf tubes were centrifuged at 14 000 rpm for 10 min, and the supernatant was analyzed using the developed HPLC method to find out solubility of samples. Furthermore, the log P value was determined by our previously reported and validated miniaturized shake flask method. Briefly, in a 1.5 mL microcentrifuge tube, 200 μL of the stock solution of IIIM-290 and its salt forms (1000 μg/mL prepared in n-octanol) was added. The volume was made up to 1 mL with 300 μL of presaturated n-octanol and 500 μL of presaturated water. Eppendorf tubes were shaken overnight at 500 rpm and centrifuged at 16 000 RCF (G-force) for 20 min to separate aequous and organic layers. The concentration of the compound in both, organic and aequous, phases was determined by the HPLC method, as mentioned in the above section. The presaturated solutions were used wherein the organic phase consisted of n-octanol saturated with water and the aqueous phase consisted of water saturated with n-octanol.

**Determination of Solubility and Sink Conditions for Dissolution Studies.** Sink conditions were determined by measuring saturation solubility of IIIM-290 using different concentrations of surfactants (0.05, 0.1, 0.25, 0.5, 0.75, and 1% w/v) such as sodium lauryl sulfate (SLS), Tween 80, and Tween 40. The sink index was calculated for 10 mg of IIIM-290 in 250 mL of dissolution media using the following equation

\[
sink\ index = \frac{C_s}{C_q}
\]

where \(C_s\) and \(C_q\) are the saturation solubility and concentration of the compound in the dissolution medium, respectively.

**Dissolution Tests.** All dissolution tests were carried out at 37 ± 0.5 °C at 50 rpm using a USP dissolution apparatus (Labindia dissolution tester, model: DS 8000; type 2, paddle) with 250 mL of dissolution medium. Sampling for all dissolution tests was performed manually at 15, 30, 60, 120, 240, and 360 min. Dissolution of IIIM-290 and its salts was determined in water under sink conditions using 0.25% w/v SLS. Apart from this, to mimic conditions of stomach and intestine, in vitro dissolution was carried out under sink conditions, as mentioned above, in HCl buffer (pH 1.2) and phosphate buffer (pH 6.8). At each sampling time, 1 mL of medium was removed and filtered through a 0.45 μm poly(vinylidene difluoride) (PVDF) syringe filter (Millex-HV, Bedford, Massachusetts), substituting 1 mL of fresh, prewarmed medium to maintain the 250 mL volume. The obtained filtrate was diluted appropriately with methanol and analyzed by the HPLC method. All dissolution tests were performed in triplicate. The area under the curve (AUC) was determined using trapezoidal rule integration to the last time point (AUC₀→ₜ). The maximum concentration (\(C_{max}\)) and time to reach the maximum concentration (\(T_{max}\)) were also obtained. The extent of dissolution enhancement was quantified from the AUC ratio ([AUC₀→ₜ,sample]/[AUC₀→ₜ,IIIM-290]).

**Kinetic Solubility Assessment.** The solubility of IIIM-290 and its HCl salt was also investigated over a period of 120 min. All experiments were carried out using the HPLC method as described earlier. The time-dependent solubility profile of IIIM-290 and the selected salt form was measured in water at 37 °C. An excess of the compound was added to 10 mL of water under shaking at 300 rpm. An aliquot of 1 mL was taken out at 5, 10, 20, 40, 60, and 120 min. The samples were filtered through a 0.45 μm PVDF syringe filter, diluted with methanol, and analyzed by HPLC to find out solubility.

**pH-Solubility Profile of the IIIM-290-HCl Salt.** The solubility of the HCl salt of IIIM-290 was determined, as given in the above section, at different pH values. Eight different USP buffers were used: hydrochloric acid buffers pH 1.2 and 2.0 and phosphate buffers pH 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0. The pH-solubility curve was plotted to determine the pHₘₚₚₜₜ of the IIIM-290-HCl salt.

**In Vitro Biological Activity and Oral Pharmacokinetic Studies.** The Cdk inhibition assay was carried out using a radioactive ([³²P]-ATP) filter-binding assay at the International Centre for Kinase Profiling, U.K. The assay for Cdk-2 and Cdk-9 was performed as per our published protocol. The % enzyme activity remaining in comparison to control was determined. The data is reported as % inhibition, which is calculated as (100 – % enzyme activity).

Oral pharmacokinetic studies of IIIM-290 and its HCl salt were carried out in BALB/c male mice of age 8–13 weeks, by administering compounds orally at a dose of 50 mg/kg in a selected vehicle (1% v/v Tween 80 + 0.5% w/v sodium CMC q.s.). Blood samples were collected (\(n = 3\) time point) at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h, postdose. At each time point, about 120 μL of blood was collected by retro-orbital sinus puncture into a labeled microfuge tube containing 2.4 μL of 200 mM K₂EDTA solution. The blood samples were processed to obtain the plasma samples within 30 min of scheduled sampling time. All plasma samples were stored below −60 °C until bioanalysis. The plasma samples were analyzed for IIIM-290 using a fit-for-purpose liquid chromatography−mass spectrometry (LC-MS/MS) method with a lower limit of
quantification of 5.05 ng/mL. The PK parameters of IIIM-290 were calculated using the noncompartmental analysis tool of validated Phoenix WinNonlin software (version 6.3). The PK studies were conducted at Eurofins Advinus Ltd., Bengaluru, on a commercial basis (Institutional Animal Ethics Committee number: ATL-43_PKM-035/Jun-2017).

**ASSOCIATED CONTENT**

* Supporting Information

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'H, 13C, distortionless enhancement by polarization transfer NMR, and FTIR scans of all compounds (PDF)

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**Notes**

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**REFERENCES**

(1) Gardner, C. R.; Walsh, C. T.; Almarsson, O. Drugs as materials: valuing physical form in drug discovery. Nat. Rev. Drug Discovery 2004, 3, 926–934.

(2) Lipinski, C. A. Poor aqueous solubility—An industry wide problem in drug discovery. Ant. Pharm. Rev. 2002, 5, 82–85.

(3) Serajuddin, A. T. Salt formation to improve drug solubility. Adv. Drug Delivery Rev. 2007, 59, 603–616.

(4) Hawley, M.; Morozowich, W. Modifying the diffusion layer of soluble salts of poorly soluble basic drugs to improve dissolution performance. Mol. Pharm. 2010, 7, 1441–1449.

(5) Paulekhuin, G. S.; Dressman, J. B.; Saal, C. Salt screening and characterization for poorly soluble, weak basic compounds: case study albendazole. Pharmazie 2013, 68, 555–564.

(6) Taniguchi, C.; Inoue, R.; Kato, M.; Yamashita, K.; Kawabata, Y.; Wada, K.; Yamada, S.; Onoue, S. New dipyrindamole salt with improved dissolution and oral bioavailability under hypochlorhydric conditions. Drug Metab. Pharmacokinet. 2013, 28, 383–390.

(7) Vioglio, P. C.; Chierotti, M. R.; Gobetto, R. Pharmaceutical aspects of salt and cocrystal forms of APIs and characterization challenges. Adv. Drug Delivery Rev. 2017, 117, 86–110.

(8) Gross, T. D.; Schaab, K.; Ouellette, M.; Zook, S.; Reddy, J. P.; Shurtleff, A.; Sacanä, A. I.; Alebic-Kolbah, T.; Bozijań, H. An approach to early-phase salt selection: Application to NBI-75043. Org. Process Res. Dev. 2007, 11, 365–377.

(9) Morissette, S. L.; Almarsson, O.; Peterson, M. L.; Remenar, J. F.; Read, M. J.; Lemmo, A. V.; Ellis, S.; Cima, M. J.; Gardner, C. R. High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids. Adv. Drug Delivery Rev. 2004, 56, 275–300.

(10) Morris, K. R.; Fakes, M. G.; Thakur, A. B.; Newman, A. W.; Singh, A. K.; Venöt, J. J.; Spagnuolo, C. J.; Serajuddin, A. T. M. An integrated approach to the selection of optimal salt form for a new drug candidate. Int. J. Pharm. 1994, 105, 209–217.

(11) Tong, W. Q.; Whitesell, G. In situ salt screening—a useful technique for discovery support and preformulation studies. Pharm. Dev. Technol. 1998, 3, 215–223.

(12) Remenar, J. F.; MacPhee, J. M.; Larson, B. K.; Tyagi, V. A.; Ho, J. H.; McIlroy, D. A.; Hickey, M. B.; Shaw, P. B.; Almarsson, O. Salt selection and simultaneous polymorphism assessment via high-throughput crystallization: The realization of sertraline. Org. Process Res. Dev. 2003, 7, 990–996.

(13) Collman, B. M.; Müller, J. M.; Seadeek, C.; Stambek, J. A.; Blackburn, A. C. Comparison of a rational vs. high throughput approach for rapid salt screening and selection. Drug Dev. Ind. Pharm. 2013, 39, 29–38.

(14) Naik, R. G.; Kattige, S. L.; Bhat, S. V.; Alreja, B.; de Souza, N. J.; Rupp, R. H. An antiinflammatory cum immunomodulatory piperidinylbenzopyranone from Dysoxylum bincaritiferum: Isolation, structure and total synthesis. Tetrahedron 1988, 44, 2081–2086.

(15) Mohanakumara, P.; Sreejayan, N.; Priti, V.; Ramesha, B. T.; Ravikant, G.; Ganeshiah, K. N.; Vasudeva, R.; Mohan, J.; Santoshkumar, T. R.; Mishra, P. D.; Ram, V.; Shaanker, R. U. Dysoxylum bincaritiferum Hook.f (Meliaceae), a rich source of rohitukine. Fitoterapia 2010, 81, 145–148.

(16) Kumar, V.; Guru, S. K.; Jain, S. K.; Joshi, P.; Gandhi, S. G.; Bharate, S. B.; Bhushan, S.; Bharate, S. S.; Vishwakarma, R. A. A chromatography-free isolation of rohitukine from leaves of Dysoxylum bincaritiferum: Evaluation for in vitro cytotoxicity, Cdk inhibition and physicochemical properties. Biogorg. Med. Chem. Lett. 2016, 26, 3457–3463.

(17) Blachly, J. S.; Byrd, J. C.; Grever, M. Cyclin-dependent kinase inhibitors for the treatment of chronic lymphocytic leukemia. Semin. Oncol. 2016, 43, 265–273.

(18) Wiernik, P. H. Alvocidib (flavopiridol) for the treatment of chronic lymphocytic leukemia. Expert Opin. Invest. Drugs 2016, 25, 729–734.

(19) Cassaday, R. D.; Goy, A.; Advani, S.; Chowla, P.; Nachankar, R.; Gandhi, M.; Gopal, A. K. A phase II, single-arm, open-label, multicenter study to evaluate the efficacy and safety of P276-00, a cyclin-dependent kinase inhibitor, in patients with relapsed or refractory mantle cell lymphoma. Clin. Lymphoma, Myeloma Leuk. 2015, 15, 392–397.

(20) Zeidner, J. F.; Karp, J. E. Clinical activity of alvocidib (flavopiridol) in acute myeloid leukemia. Leuk. Res. 2015, 39, 1312–1318.

(21) Bharate, S. B.; Kumar, V.; Jain, S. K.; Mintoo, M. J.; Guru, S. K.; Nuthakki, V. K.; Sharma, M.; Bharate, S. S.; Gandhi, S. G.; Mondhe, D. M.; Bhushan, S.; Vishwakarma, R. A. Discovery and preclinical development of I.IIM-290, an orally active potent cyclin-dependent kinase inhibitor. J. Med. Chem. 2018, 61, 1664–1687.

(22) Vishwakarma, R. A.; Bharate, S. B.; Bhushan, S.; Mondhe, D. M.; Jain, S. K.; Meena, S.; Guru, S. K.; Pathania, A. S.; Kumar, S.; Behl, A.; Mintoo, M. J.; Bharate, S. S.; Joshi, P. Rohitukine Analoges as Cyclin dependent Kinase Inhibitors and a Process for the Preparation Thereof. WO2014170914A1, US20160052915, EP2986605, CA2908084, IN2013DE01142, Oct 23, 2014.

(23) Berge, S. M.; Bighley, L. D.; Monkhouse, D. C. Pharmaceutical salts. J. Pharm. Sci. 1977, 66, 1–19.
(24) Mroso, P. V.; Po, A. L. W.; Irwin, W. J. Solid-state stability of aspirin in the presence of excipients: kinetic interpretation, modeling, and prediction. *J. Pharm. Sci.* 1982, 71, 1096–1101.

(25) Guerrieri, P.; Rumondor, A. C.; Li, T.; Taylor, L. S. Analysis of relationships between solid-state properties, counterion, and developability of pharmaceutical salts. *AAPS PharmSciTech* 2010, 11, 1212–1222.

(26) Anonymous. Appendix XII B. Dissolution. *British Pharmacopoeia*, Vol IV, 2010; [http://www.drugfuture.com/Pharmacopoeia/BP2010/data/896.html](http://www.drugfuture.com/Pharmacopoeia/BP2010/data/896.html) (retrieved on April, 11, 2018).

(27) Sun, D. D.; Wen, H.; Taylor, L. S. Non-sink dissolution conditions for predicting product quality and in vivo performance of supersaturating drug delivery systems. *J. Pharm. Sci.* 2016, 105, 2477–2488.

(28) Liu, P.; De Wulf, O.; Laru, J.; Heikkila, T.; van Veen, B.; Kiesvaara, J.; Hirvonen, J.; Peltonen, L.; Laaksonen, T. Dissolution studies of poorly soluble drug nanosuspensions in non-sink conditions. *AAPS PharmSciTech* 2013, 14, 748–756.

(29) Kulkarni, A. P.; Shahnawaz, M.; Zaheer, Z.; Dehghan, M. H. G. Development and validation of a dissolution method for pioglitazone tablets. *Dissolution Technol.* 2012, 36–46.

(30) Washington, N.; Washington, C.; Wilson, C. G. The Stomach. In *Physiological Pharmaceutics—Barriers to Drug Absorption*, 2nd ed.; Washington, N., Washington, C., Wilson, C. G., Eds.; Taylor and Francis Inc.: New York, 2001; pp 75–108.

(31) Babu, N. J.; Nangia, A. Solubility advantage of amorphous drugs and pharmaceutical cocrystals. *Cryst. Growth Des.* 2011, 11, 2662–2679.

(32) Bharate, S. S.; Vishwakarma, R. A. Thermodynamic equilibrium solubility measurements in simulated fluids by 96-well plate method in early drug discovery. *Bioorg. Med. Chem. Lett.* 2015, 25, 1561–1567.

(33) Kumar, V.; Bharate, S. S.; Vishwakarma, R. A. Modulating lipophilicity of rohitukine via prodrug approach: Preparation, characterization, and in vitro enzymatic hydrolysis in biorelevant media. *Eur. J. Pharm. Sci.* 2016, 92, 203–211.

(34) Garcia, C. V.; Paim, C. S.; Steppe, M. J.; Schapoval, E. E. Development and validation of a dissolution test for rabeprazole sodium in coated tablets. *J. Pharm. Biomed. Anal.* 2006, 41, 833–837.

(35) Fung, M.; Börzıp, K. R.; Suryanarayanan, R. Physical stability and dissolution behavior of ketoconazole-organic acid coamorphous systems. *Mol. Pharm.* 2018, 1628–1641.

(36) John, C. T.; Xu, W.; Lupton, L. K.; Harmon, P. A. Formulating weakly basic HCl salts: relative ability of common excipients to induce disproportionation and the unique deleterious effects of magnesium stearate. *Pharm. Res.* 2013, 30, 1628–1641.