The delivery of arbidol by salt engineering: synthesis, physicochemical properties and pharmacokinetics

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ABSTRACT

The aim of the present study was to evaluate the feasibility of using the methanesulfonic salt of arbidol in order to improve its aqueous solubility and thus oral bioavailability. Arbidol mesylate (AM) was synthesized and then characterized using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM), and its apparent solubility and octanol–water partition coefficient were also studied. The results of NMR, IR, PXRD, SEM and DSC tests confirmed the salt formation. The apparent solubility of AM in water was 32-fold higher than that of the commercial product. A superior pH-dependent profile and an improved dissolution rate of AM were obtained in a variety of solutions with different pH values. In addition, AM exhibited a relatively higher peak plasma concentration (1460 versus 1297 ng/mL) and an increased AUC_{0-24} (2475 versus 1277 ng/mL × h) when comparing with the commercial product, indicating the improved bioavailability of the drug. This study suggests that AM may be able to improve the therapeutic efficacy of arbidol, which rendering it to be a promising candidate for further development.

Introduction

Arbidol (umifenovir), (1-methyl-2-phenylthiomethyl-3-carboethoxy-4-dimethylaminomethyl-5-hydroxy-6-bromoindoile) (Figure 1) is a small indole-derivative produced in Russia and used for prophylaxis and treatment of influenza A, influenza B and some other kinds of acute respiratory infections. Recently, it was found that arbidol has inhibitory activity in vitro against hepatitis C, Hantaan and chikungunya virus. In addition to its broad antiviral activity, it has also been reported to increase immunomodulatory activity and reduce the occurrence of influenza complications. Compared with other antiviral drugs, arbidol exhibits a relatively high efficacy, with only minor adverse effects and a low toxicity, but no studies have addressed the toxicity of arbidol during long-term use, such as a treatment for chronic diseases.

Arbidol (AS), as a free base, has poor aqueous solubility, which is the main drawback in association with its low bioavailability. As a consequence, arbidol has been developed as the hydrochloride monohydrate (AH) salt for commercial use. However, AH is practically insoluble in water and has a low bioavailability because approximately 40% of the drug is excreted within 48 h in the feces. For hydrochloride salts, a common-ion effect on the dissolution, have been evaluated and compared with those of AH. Finally, the pharmacokinetics of AM has been studied using male Sprague–Dawley (SD) rats with AH as the reference.
The infrared spectra of AS, AH and AM were recorded by the KBr FTIR twice with pure water and dried at 60°C for another 4 h. The precipitate was collected by filtration, washed until the pH of the solution was above 10 and stirring was kept reaction vessel during the process of adding sodium hydroxide under stirring. A light purple precipitate started to appear in the plant-shaped flask attached to a condenser at 55°C. AH (20 g) was dissolved in 100 mL ethanol and placed in an egg-plant-shaped flask attached to a condenser at 55°C and refluxed under stirring. A light purple precipitate started to appear in the reaction vessel during the process of adding sodium hydroxide (0.5 mol/L). Sodium hydroxide (0.5 mol/L) was continually added until the pH of the solution was above 10 and stirring was kept for another 4 h. The precipitate was collected by filtration, washed twice with pure water and dried at 60°C to give pure AS.

Precisely weighed AS (47.7 g, 0.1 M) and methanesulfonic acid were dissolved in 150 mL ethanol (molar ratio = 1:1) and the mixture was stirred at room temperature until a clear solution was obtained. The solution was cooled and allowed to crystallize slowly in fridge at 4°C, and then, the crystals were collected by filtration, washed twice with ethanol and dried at 60°C. Dried salt was crushed, passed through a 80 mesh ASTM sieve and kept in a closed container until physicochemical evaluation.

Characterization of AM

NMR

1H-NMR spectra of AS, AH and AM were recorded in deuterated dimethyl sulfoxide using a 400 MHz Bruker FT-NMR (Bruker India Scientific Pvt. Ltd., New Delhi, India) spectrometer. MestReNova software was used to interpret the results.

MS

Mass spectrograph (MS) of these compounds were established by AB SCIEX Instruments 4000 Q-Trap (Applied Biosystems, Foster City, CA) in electrospray ionization (ESI)-positive mode with a source temperature of 180°C. The compounds were dissolved in methanol to give a suitable concentration and analyzed in the range of 50–3000 m/z.

FTIR

The infrared spectra of AS, AH and AM were recorded by the KBr disc method using an FTIR Spectrometer (Avatar-360, Nicolet, Madison, WI). Scanning was performed over the range 4000–400 cm⁻¹ at a resolution of 2 cm⁻¹.

DSC

The thermal behavior of these compounds was examined using differential scanning calorimeter (DSC-1, Mettler-Toledo, Switzerland). Samples were accurately weighed in aluminum spans and covered with an aluminum lid with a pinhole and calibrated with an empty aluminum pan. The DSC thermograms were recorded at a heating rate of 10°C/min from 30°C to 250°C in a nitrogen atmosphere.

PXRD

The powder X-ray diffraction spectra of AS, AH and AM were determined using an X-ray diffractometer (D/max-r A, Rigaku Denki, Japan) equipped with a Cu Kα radiation source of 45 kV using a current of 30 mA over the range 3° ≤ 2θ ≤ 45° at a scan rate of 1°/min.

SEM

Crystals were examined by scanning electron microscopy (SEM) (S-3400, Hitachi, Japan) at a working distance of 20 mm and an accelerated voltage of 5 kV after coating samples with gold before the examination.

Apparent solubility and octanol–water partition coefficient

Apparent solubility study

Apparent solubility investigations of AS, AH and AM were carried out involving the addition of an excess of solid bulk drug to 5 mL water, n-octanol and solutions at pH values of 1.0, 2.0 (HCl solutions), 4.5 (acetate buffer), 3.0, 6.0, 7.0 and 8.0 (phosphate buffers); these investigations were carried out in triplicate. The suspensions were shaken for 24 h at a constant temperature (37 ± 0.5°C), and then, excess insoluble drug was removed by centrifugation (3500 rpm, 10 min) at room temperature and the concentration of soluble drug in the diluted supernatant was determined by high-performance liquid chromatography (HPLC).

Octanol–water partition coefficient study

Octanol–water partition coefficient study of these compounds was conducted at 37°C using the shaking flask method. Initially, n-octanol and water were mutually saturated for 72 h by shaking at a constant temperature (37 ± 0.5°C) before use. Then, 3 mL saturated aqueous solution of the bulk drug along with 3 mL n-octanol (water saturation) were mixed and shaken for 24 h. Finally, the two phases were separated by centrifugation (3500 rpm, 10 min) at room temperature and the concentrations of the compounds in the aqueous phase and the n-octanol phase were determined by HPLC. Each determination was performed in triplicate.

Dissolution testing

Dissolution profiles were obtained with the USP Apparatus II setup using ZRS-8G (Tianjin Tianda Tianfa Technology Co., Ltd., Tianjin, China). The dissolution medium used in the present study include HCl solutions (pH 1.0, 1.2 and 2.0) and buffer solutions (pH 4.5, 5.0, 5.8, 6.0, 6.8 and 7.4). The buffer solutions were prepared as follows: (1) acetate buffer solution (pH 4.5): dissolve 7.7 g
ammonium acetate in 50 mL water, add 6 mL glacial acetic acid and dilute with water to 100 mL; (2) phosphate buffer solution (pH 5.0): sodium hydroxide solution was added to sodium dihydrogen phosphate solution (0.2 mol/L) to adjust pH value to 5.0; (3) phosphate buffer solution (pH 5.8) was prepared by dissolving 8.34 g potassium dihydrogen phosphate and 0.87 g dipotassium hydrogen phosphate in 1000 mL water; (4) phosphate buffer solution (pH 6.0): dissolve 8.34 g potassium dihydrogen phosphate and 0.87 g dipotassium hydrogen phosphate in 1000 mL water, and adjust pH value to 6.0 with sodium hydroxide solution; (5) phosphate buffer solution (pH 6.8): mix 250 mL potassium dihydrogen phosphate solution (0.2 mol/L) and 118 mL sodium hydroxide solution (0.2 mol/L), and dilute with water to 1000 mL; (6) phosphate buffer solution (pH 7.4): dissolve 1.36 g potassium dihydrogen phosphate in water, add 79 mL sodium hydroxide solution (0.1 mol/L), and dilute with water to 200 mL.

AM and AH powders, equivalent to 100 mg arbidol, were weighed accurately and filled into hard gelatin capsules. The tests were conducted at 37 °C in 900 mL dissolution medium (water or solutions at different pH values (1.0, 1.2, 2.0, 4.5, 5.0, 5.8, 6.0, 6.8 and 7.4)) with a paddle stirring speed of 50 rpm. At predetermined intervals, 5.0 mL dissolution medium was withdrawn and replaced and 7.4)) with a paddle stirring speed of 50 rpm. At predetermined intervals, 5.0 mL dissolution medium was withdrawn and replaced and

High-performance liquid chromatography (HPLC) analysis

The HPLC analysis was conducted using a reverse phase HPLC system (Shimadzu, Japan) on a Thermo C18 column (200 × 4.6 mm, 5 μm) maintained at 25 °C. The mobile phase was methanol–water (containing 0.1 mol/L ammonium acetate) – glacial acetic acid (65:35:1, v/v/v) at a flow rate of 1.0 mL/min. The UV detection wavelength was set at 254 nm and the injection volume was 20 μL. The developed HPLC method was validated methodologically as per guideline of ICH21.

In vivo bioavailability assessment

Pharmacokinetic studies of AM and AH

Male SD rats (210–250 g), obtained from the Experimental Animal Center of Shenyang Pharmaceutical University, were fasted overnight with free access to water before drug administration. Commercially available AH was used as a reference and self-prepared AM was used for the tests. All tests involving animals were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People’s Republic of China.

Two groups of male SD rats, six animals in each group, were orally given AH (60 mg/kg) and AM (65 mg/kg), equivalent to a 54 mg/kg dose of arbidol. Blood samples (0.5 mL) were withdrawn from the eye veins before administration (0 h) and at different time intervals after administration. Afterwards, the blood samples were immediately centrifuged (13,000 rpm, 5 min) and stored at −20 °C until required for analysis.

Analysis of the arbidol concentration in rat plasma

The arbidol in rat plasma was extracted using liquid–liquid method and diazepam was used as the internal standard. Briefly, plasma samples (50 μL) were spiked with 50 μL internal standard solution (1 μg/mL), 50 μL methanol and 25 μL saturated solution of sodium hydrogencarbonate and vortex mixed for 1 min. Then, 1 mL tert-butyl methyl ether was added followed by vigorous vortexing for 3 min. After centrifugation at 13,000 rpm for 10 min, the upper organic layer was transferred to a centrifuge tube and dried at 37 °C under nitrogen. The residue was dissolved in 100 μL methanol, and 20 μL of this solution was injected into the HPLC system for analysis.

HPLC analysis was conducted using a Waters system (Waters Technologies, CA) consisting of a quaternary Waters e2695 pump, a Waters e2695 autosampler, and a Waters 2489 UV detector. Separation was carried out on a Thermo C18 column (200 × 4.6 mm, 5 μm) operated at 25 °C. The flow rate of the mobile phase (0.1 mol/L ammonium acetate buffer (pH 4.0)-methanol (35:65, v/v)) was 1.0 mL/min and the wavelength of the UV detector was set at 251 nm.

Pharmacokinetic data analysis

The maximum concentration (Cmax) and the time to reach this maximum concentration (Tmax) were obtained directly from the concentration-time data. The other pharmacokinetic parameters were calculated using DAS 2.1.1 software (Chinese Pharmacological Society). The relative bioavailability was the ratio of the AUC(0→t) of AM to AH.

Statistical analysis

The statistical analysis was performed by one-way analysis of variance (ANOVA) and unpaired t-test using the software of SPSS (version 17.0, Chicago, IL). Analyses were performed in apparent solubility, octanol–water partition coefficient studies and in vivo bioavailability assessment. All results were expressed as mean±SD, and the differences were deemed statistically significant at p < .05.

Results

AM characterization

1H-NMR research

As shown in Figure 2, the 1H NMR (400 MHz, DMSO-d6) chemical shift (δ, parts per million [ppm]) data of AM were 9.37 (s, 1H), 8.74 (s, 1H), 8.04 (s, 1H), 7.43–7.26 (m, 5H), 4.84 (d, J = 5.3 Hz, 2H), 4.74 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 3.69 (s, 3H), 2.77 (d, J = 4.6 Hz, 6H), 2.30 (d, J = 3.3 Hz, 3H), and 1.25 (t, J = 7.1 Hz, 3H). For AH, the chemical shift [δ, parts per million (ppm)] data were 9.38 (s, 1H), 8.85 (s, 1H), 8.04 (s, 1H), 7.42–7.26 (m, 5H), 4.89–4.83 (m, 2H), 4.74 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 3.70 (s, 3H), 3.36 (s, 2H), 2.75 (s, 6H), 1.25 (t, J = 7.1 Hz, 3H). As for AS, data were 8.75 (s, 1H), 7.71 (s, 1H), 7.38–7.23 (m, 5H), 4.63 (s, 2H), 4.14 (t, J = 7.1 Hz, 2H), 4.02 (s, 2H), 3.66 (s, 3H), 2.26 (s, 6H) and 1.23 (t, J = 7.1 Hz, 3H). The proton was completely transformed from salt former to parent drug as demonstrated by the disappearance of peaks at δ 4.02 (s, 2H) and δ2.26 (s, 6H) together with the appearance of peaks at δ 4.74 (s, 2H) and δ 2.77 (d, J = 4.6 Hz, 6H). Further evidence towards formation of the desired salt was that all protons of AM were observed in the spectra and had different chemical shift values as compared to AS.

MS

In ESI mode of mass spectrometry, AM gave a molecular (M+ +1) ion peak at 479.0902 m/z, corresponding to molecular weight of arbidol and also being equal to those of AH and AS (data were
not shown), which demonstrated the integrity of the parent molecule after salt formation.

**FTIR spectra**

As shown in Figure 3, AH and AM showed a sharp signal at 3124 cm\(^{-1}\) and 3134.0 cm\(^{-1}\), respectively, and characteristic of the N–H bond stretching vibrations was absent in AS, suggesting that methanesulfonic acid was incorporated in the parent molecule. In addition, AM also exhibited a strong broad peak in the range 1300–1000 cm\(^{-1}\) with a maximum peak around 1189.5 cm\(^{-1}\), which was assigned to an asymmetric SO\(_3\)-stretching vibration.

**DSC curves**

The results obtained are shown in Table 1 and Figure 4. For AH, a relatively broad endothermal band (90–150 °C) was observed due to the loss of water and the melting point was found to be around 180 °C (ΔH: 16.70 J/g). AS was characterized by a sharp melting endothermal peak at 132 °C (19.22 J/g). During AM scanning, a single, new and sharp endothermal peak was shown at 174 °C (ΔH: 14.65 J/g). Generally, an increase in the enthalpy of fusion (ΔH) accompanies an increase in the crystal lattice energies\(^{22}\). Therefore, this suggested that the crystal structure of arbidol was destabilized by new salt formation.

**PXRD patterns**

In this study, the PXRD spectrum of AM was compared with AS and AH (Figure 5). Obvious differences in the PXRD patterns of these compounds were presented due to their different crystallinity.

**Morphological study**

The morphology of AS, AH and AM examined by SEM is presented in Figure 6. AS was in the form of irregular cubes and AH with an aggregated state had a rod-like shape. In contrast, AM was in the form of irregular-shaped particles with a wide particle size distribution. The surface morphology of arbidol both in salts and in free base forms were quite different since their crystalline phases vary from each other, which can be supported by the DSC and PXRD results.

**Apparent solubility and octanol–water partition coefficient**

Figure 7 shows the apparent solubility of AM, AS and AH in water, n-octanol and solutions with different pH values. AM (85.78 mg/mL) exhibited substantial increases in apparent solubility compared with AS (0.002 mg/mL) and AH (2.68 mg/mL) in water. While the apparent solubility of AM in n-octanol was 4.12 mg/mL, which was a little lower than that of AH (6.45 mg/mL) and higher than that of AS (3.14 mg/mL). In addition, arbidol is a week basic drug; therefore, it shows better apparent solubility in the acidic conditions (\(S > 0.13\) mg/mL) than the basic ones (\(S < 0.01\) mg/mL). The apparent solubility of AH in the relatively basic conditions (pH 4.5, 6.0, 7.0 and 8.0) were 13.06, 157.00, 760.00, 14.29 times greater (\(p < .001\)) than that of AS. However, at pH 1.0, 2.0 and 3.0, the apparent solubility of AH was similar to AS (\(p > .05\)). The conversion of the solid form from hydrochloride salt to mesylate contributed to a statistically significant (\(p < .001\)) enhancement in the apparent solubility of arbidol in all tested solutions. The apparent solubility of AM was 4.75-, 86.07-, 3.00-, 13.06-, 67.04-, 110.79- and 971.00-fold higher than that of AH in pH 1.0, 2.0, 3.0, 4.5, 6.0, 7.0 and 8.0 Solutions.

The octanol–water partition coefficients (Log P) of AS, AH and AM in the n-octanol/water system were measured and the results
are summarized in Table 2. These results show that AS had a higher octanol–water partition coefficient than the solid salt forms and the partition coefficient was reduced from 3.43 to 0.26 and 0.01 for AH and AM, respectively. The reduced Log \( p \) for AM was attributed to the formation of a salt between the parent molecule and methanesulfonic, which statistically significantly \((p < .001)\) increased its solubility in water and slightly reduced its solubility in \( n \)-octanol \((p > .05)\) compared with AH.

**In vitro dissolution profiles**

Purified water and solutions with different pH values (1.0, 1.2, 2.0, 4.5, 5.0, 5.8, 6.0, 6.8 and 7.4) were selected as dissolution medium and the dissolution profiles are shown in Figure 8 and Figure S1. At pH 1.0, only 2.07% of AH was released after 5 min, whereas the dissolution of AM was 33.77% under the same condition (Figure S1(a)). In addition, the percentage dissolution of AM in water (Figure 8(f)) at 5 min and 60 min were 72.81% and 93.81%, respectively, which were significantly faster than that of AH (5 min: 29.81%; 60 min: 79.24%). Similarly, such dissolution behavior was also observed under other conditions, showing that the newly designed solid form exhibited improved dissolution. However, in solutions of pH 6.0, 6.8 and 7.4, a sharp decline in dissolution profiles was observed, especially at pH 6.8 and 7.4. For example, at pH 6.0 (Figure S1(d)), the maximum dissolution of AH at 15 min was 32.61%, but it declined from 32.61% to 30.20% in 5 min, while dissolution of AM showed a dramatic drop (about 30%) from 15 min (106.12%) to 20 min (76.28%). Despite the drug being precipitated, the dissolution of AM was still significantly higher than that of AH in those medium.

**Pharmacokinetic study in SD rats**

The developed HPLC method used to determine the content of arbidol in rat plasma was also validated methodologically as per guideline of ICH24. A linear calibration curve was obtained at plasma concentration ranging from 40 to 10,000 ng/mL. In addition, the method was shown to be reliable and reproducible with intra-/inter-day precision below 5%, accuracy within ±3.0% and mean extraction recovery more than 72.9%.

The effect of the readily soluble salt on the oral exposure of arbidol was examined using male SD rats. The key pharmacokinetic parameters and the mean plasma concentration–time curves are shown in Table 3 and Figure 9, respectively. Following a single administration equivalent to a 54 mg/kg dose of arbidol, AM exhibited a relatively slower elimination (a prolonged \( t_{1/2} \)) and a higher (1.13-fold) peak plasma concentration (1460 ng/mL) in comparison with AH (1297 ng/mL), while the AUC0–t \((p < 0.05)\) of AM (2475 ng/mL \( \times \) h) was 1.94 times greater than that of AH (1277 ng/mL \( \times \) h). No statistically significant difference was observed in \( T_{max}, C_{max} \) and \( t_{1/2} \) among the solid salt forms.

**Discussion**

**Design of AM**

As mentioned earlier, AH is practically insoluble in water and has a low bioavailability since nearly half of the drug is excreted in the feces1. To address the issue, a new salt composition of arbidol was designed and methanesulfonic acid was chosen as the salt former based on the following considerations. Firstly, methanesulfonic acid has been a pharmaceutically acceptable acid widely used in the pharmaceutical industry as an orally administered salt due to its hypo-toxic and biocompatible23. Secondly, one attractive hypothesis in our study is that it is much easier for methanesulfonic acid than chloride to form hydrogen bonds with water, and
The octanol-water partition coefficient of AS, AH and AM (data are shown as mean ± SD).

| Solubility (µg/mL)       | Saturated water | Saturated n-octanol | Log p  |
|--------------------------|-----------------|---------------------|-------|
| AS                       | 0.03 ± 0.00     | 75.55 ± 5.93        | 3.43 ± 0.06 |
| AH                       | 20.36 ± 0.56a   | 37.40 ± 1.63a       | 0.26 ± 0.26a |
| AM                       | 31.31 ± 0.95b   | 31.77 ± 1.75        | 0.01 ± 18.6b |



**Characterization of AM**

In the area of salt and co-crystal development, the ΔpK_a (ΔpK_a = pK_a(base) – pK_a(acid)) rule is widely used to predict the solid form of components between the parent drugs and coformers. It is generally assumed that components with ΔpK_a > 3 mostly form salts, whereas if ΔpK_a < 0, it tends to be co-crystals, and complex compounds can be obtained if 0 < ΔpK_a < 3.24,25. The pK_a value of arbidol is 6.0,26, and ΔpK_a value for arbidol and methanesulfonic acid would be 7.2, and thus, the ΔpK_a rule suggests salt formation.

**Apparent solubility enhancement**

Up to now, arbidol salts with benzoic (BA), salicylic (SA), maleic (MA), glutaric (GL), gentisic acid (GE), fumaric acid (FU) and succinic (SU) had been investigated for solubilization13. The apparent solubility reported with MA (2.7 mg/mL), SA (0.15 mg/mL), GL (1.3 mg/mL) and GE (0.26 mg/mL) in water were significantly lower than AM (85.74 mg/mL). Herein, the apparent solubility of AS (0.15 mg/mL) and AH (0.13 mg/mL) were comparable with those measured for AS and AH, respectively, were 0.15 mg/mL and 0.12 mg/mL in HCl solution with pH value around 1.0. These data demonstrated that an increased aqueous solubility of arbidol could be achieved by salt formation, and the lowering of microenvironment-pH phenomenon would be a possible explanation for the significantly increased apparent solubility27,28. The salt forms are strong acids, which can create acidic microenvironment around the surface of arbidol molecule that forced its ionization and caused more drugs to dissolve. Here, the greater solubilization of AM could be explained by the following aspects. Firstly, AM could destabilize the crystal structure of arbidol and endow the drug with a lower crystal lattice energy, thus exhibiting an increased aqueous solubility. This phenomenon had been confirmed by DSC assay that ΔH of AM was lower than those of AH and AS. Secondly, it is much easier for methanesulfonic acid than chloride to form hydrogen bonds with water, resulting in the enhancement of dissolution. Stahl et al. reported that there is a good correlation between the aqueous solubility and hygroscopicity of salts.29 The moisture adsorption study reported here shown that the hygroscopicity of AM was 4.22-fold higher than that of AH under 80% relative humidity (RH) condition for one day (data were not shown), illustrating that AM had a greater affinity with water than AH.

**Dissolution rate measurement**

The dissolution profiles (Figures 8 and S) showed that AM has the potential ability to increase the dissolution of poorly soluble drug. To compare the dissolution rates of AM and AH, a monoequation (MEE) was used to evaluate with hopes to give a full and direct view. The MEE is shown as follows30.

\[
\log(y_\infty - y) = \log y_\infty - \frac{k}{2.303} t
\]

where \(y_\infty\) represents the maximum percentage dissolution of drug, \(y\) is the cumulative percentage dissolution of drug in time \(t\) and \(k\) is the dissolution rate constant.

The \(k\) values of AM were significantly higher than those of AH in dissolution medium at all pH values, apart from pH 4.5 (Table 4 and Figure 10). To a certain extent, these data demonstrated that the dissolution rate of arbidol was improved by AM in comparison with AH in those medium. However, several points need to be considered. Firstly, at pH 5.0, although the \(k\) value of AM (0.159) was higher than that of AH (0.099), the correlation coefficient (R) of AH (0.732) was significantly lower than that of other pH conditions, suggesting that the dissolution rate of drug did not remain constant during dissolution process. To verify this supposition, \(k\) values were obtained by fitting dissolution to the MEE from 0 min to 20 min. We found that the \(k\) of AM (0.216; R, 0.991) was lower than that of AH (0.251; R, 0.977). Thus, at pH 5.0, the dissolution rate of AM was lower than that of AH since the dissolution rate is determined by the first 2–3 dissolution points. Secondly, a sharp decline in the dissolution profiles was observed at pH 6.0, 6.8 and 7.4, and thus, the \(k\) values were calculated by fitting dissolution to the MEE from 0 min to the peak time (\(T_{\text{max}}\)). Thirdly, at pH 6.0, the MEE was also used to evaluate the settling rate, when we found that the settling rate of AM (\(k = -0.039\)) was slower than that of AH (\(k = -0.012\)). In conclusion, these results demonstrated that AM could be used to increase the dissolution rate of the poorly soluble drug. The lowering of microenvironment-pH phenomenon and its great apparent solubility might give a full explanation for the increased dissolution rate.

**Bioavailability of AM**

To reach the systemic circulation, drugs need to dissolve in GI fluid and then penetrate the membranes of the GI tract. As mentioned earlier, the main drawback of arbidol is its poor water solubility and low dissolution rate, resulting in limited...
absorption. In our study, a new salt was designed to address the above problem by replacing hydrochloride salt with mesylate. As expected, AM showed a $C_{\text{max}}$ value about 1.13 times and an AUC value nearly 1.94 times higher than that of AH. The relative oral bioavailability of AM compared with AH was 193.8%, and the reasons for this can be explained as follows. Firstly, AM significantly increases the aqueous solubility of arbidol, thus more drugs could reach the systemic circulation; secondly, AM is effective in improving drug dissolution, which caused an increased drug concentration throughout the whole GI tract. The apparent solubility and dissolution tests had given a detailed explanation in the mechanism, and it can be concluded that the desirable oral bioavailability of arbidol could be achieved by preparing soluble salt AM.

Conclusions
In our study, AM was successfully prepared and proton transformation between arbidol and the salt former was directly/indirectly characterized by $^1$H-NMR, FTIR, DSC, PXRD and SEM techniques. Based on our physicochemical evaluation, as well as the in vivo bioavailability study of male SD rats, it can conclude that AM is able to substantially increase the apparent solubility, as well as the rate and extent of the in vitro dissolution of arbidol, thus improving its oral bioavailability.

In conclusion, AM is a novel salt form of arbidol with much better bioavailability and physicochemical properties. Hence, AM is a promising candidate for further development and is an excellent practical alternative to the current commercially available bulk drug.

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Figure 9. The mean plasma concentration-time curves of arbidol after oral administration of AM (T: circular) and AH (R: square) (data were expressed as mean ± SD, n = 6).

Table 4. The monoequation (MEE), correlation coefficient (R) and dissolution rate constant (k) of AH and AM in water, as well as in dissolution medium at different pH values.

| pH   | MEE | R    | K    |
|------|-----|------|------|
| pH 1.0 | y = -0.026x + 1.818 | 0.991 | 0.060 |
| pH 1.2 | y = -0.030x + 1.884 | 0.991 | 0.069 |
| pH 2.0 | y = -0.052x + 1.912 | 0.978 | 0.120 |
| pH 4.5 | y = -0.064x + 1.848 | 0.975 | 0.147 |
| pH 5.0 | y = -0.043x + 1.293 | 0.732 | 0.099 |
| pH 5.8 | y = -0.053x + 1.693 | 0.972 | 0.122 |
| pH 6.0 | y = -0.094x + 1.557 | 0.987 | 0.216 |
| pH 6.8 | y = -0.022x + 1.142 | 0.992 | 0.051 |
| pH 7.4 | y = -0.032x + 1.000 | 0.974 | 0.074 |
| Water | y = -0.026x + 1.797 | 0.967 | 0.060 |

The results were obtained by fitting dissolution to the MEE from 0 min to 20 min.

The results were obtained by fitting dissolution to the MEE from 0 min to the peak time (T_{max}).

The results were obtained by fitting dissolution to the MEE from 20 min to 60 min.

Figure 10. The dissolution rate constant of AM and AH in all test dissolution medium.

Disclosure statement
The authors report no declarations of interest. The authors alone are responsible for the content and writing of this article.

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