Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 350 (2008) 35–42
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Solid form selection of zwitterionic 5-HT4 receptor agonist

Takashi Kojima *, Kiyohiko Sugano, Satomi Onoue [1], Noriaki Murase, Morimichi Sato, Yohei Kawabata [2], Takashi Mano

Science and Technology, Pfizer Global Research and Development, Nagoya Laboratories, Pfizer Japan Inc., 5-2 Taketoyo, Aichi 470-2393, Japan

Received 16 May 2007; received in revised form 11 July 2007; accepted 11 August 2007
Available online 24 August 2007

Abstract

From discovery synthesis of a zwitterionic pharmaceutical compound, 4-{[4-({[(3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid (compound A), two anhydrous ZW-I and ZW-II and two hydrate forms ZW-III and ZW-IV were identified. Although stable form ZW-I was chemically stable at 70 °C/75% RH for 10 days, it was transformed to hydrate form ZW-IV under ambient conditions within a few days, taking up water from atmospheric moisture. In order to select a solid form for further investigation, solid-state characterization, salt screening on 96-well plate, stable polymorph and hydrate screening and physical stability were performed. Based on the results of the salt screening, besylate, camsylate, hemi-edisylate, hemifumarate, monosuccinate salts of compound A were prepared, and their polymorphism and chemical and physical stability were evaluated. From the viewpoint of stability and manufacturability, a stable form of besylate salt (BSA-I), which had two anhydrous forms BSA-I and BSA-II and hydrate form BSA-III, was selected as a solid form. BSA-I was quite stable at high relative humidity and provided significant improvement of physical stability compared with ZW-I.

Keywords: Solid form selection; Salt screening; Raman spectroscopy; Hydrate; Polymorph; Physical stability

1. Introduction

From the viewpoint of the improvement of physicochemical properties and the protection of intellectual properties (Knapman, 2000), solid form selection (Gould, 1986; Morris et al., 1994; Tong and Whitesell, 1998) including salt, cocrystal (Vishweshwar et al., 2006) and polymorph screenings (Morissette et al., 2003) is recognized as an essential process in the drug discovery. Even though physicochemical properties, such as solubility and stability, of a drug candidate were not suitable for the development; appropriate solid form selection could realize the launch of a pharmaceutical product. Especially the solid-state stability of drug substances should be researched and improved during the preformulation study, since severe instability of drug substances might not be improved by the use of technologies of formulation and packaging.

The solid-state stability of crystalline drugs is generally classified into two categories; physical stability and chemical stability. Physical stability represents the stability of the solid form such as transformation of polymorphs and pseudopolymorphs and hydration (Bergren et al., 1996; Gandhi et al., 2000; Kojima et al., 2007a; Kushida and Ashizawa, 2002). Chemical stability represents resistance against chemical reactions under some storage conditions relating to humidity, temperature and photoirradiation (Byrn et al., 2001; Glass et al., 2004; Kojima et al., 2007b). Recently, improvements of chemical and physical stability of crystalline drugs in the solid-state by solid form selection were reported (Badawy, 2001; Bastin et al., 2000; Engel et al., 2000; Trask et al., 2005). Caffeine converted to hydrate with respect to humidity, whereas cocrystal of caffeine with oxalic acid exhibited high-stability without conversion to hydrate under

* Corresponding author. Fax: +81 569 74 4748.
E-mail address: tak_kojimajpn@yahoo.co.jp (T. Kojima).
[1] Present address: Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Surugaku, Shizuoka 422-8526, Japan.
[2] Present address: Pharmaceutical Research & Development, PreClinical Development, Banyu Pharmaceutical Co., Ltd., 3 Okubo, Tsukuba, Ibaraki 300-2611, Japan.

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.08.026

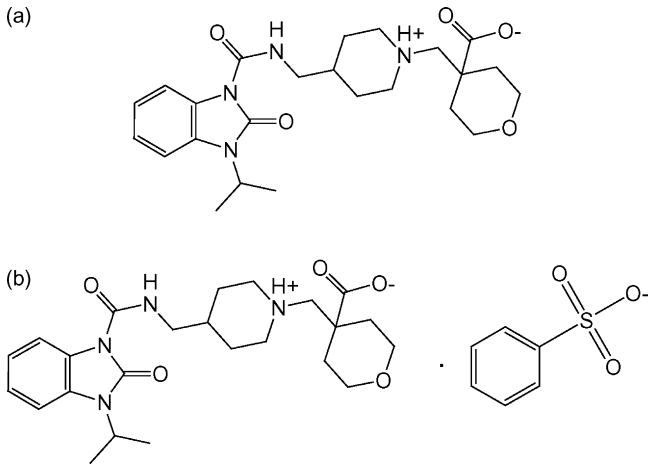


Fig. 1. Chemical structures of zwitterions (molecular weight, 458.6) (a) and besylate salt (molecular weight, 616.7) (b) of compound A.

exaggerated humidity (Trask et al., 2005). Mesylate salt of an ester prodrug of a IIb/IIIa receptor antagonist was more stable than acetate salt against ester and amidine hydrolysis under humidity (Badawy, 2001).

4-{[4-({[(3-Isopropyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid (compound A) is a crystalline zwitterionic compound with 5-HT4 receptor agonistic activity, which is though to be effective for the treatment of gastroesophageal reflux disease, non-ulcer dyspepsia, functional dyspepsia and irritable bowel syndrome (Fig. 1). From discovery synthesis, two polymorphs, ZW-I and ZW-II of zwitterionic pharmaceutical compound (zwitterion) has been identified. Compound A has had issues to develop as zwitterion, since stable form ZW-I was transformed to hydrate form ZW-IV including 21% water content under the ambient condition and an unexpected transformation causes the low potency of compound A during weighing of drug substance as zwitterion.

In this report, we have demonstrated the solid form selection of compound A, using solid-state characterization, salt screening on 96-well plate based on our previous report (Kojima et al., 2006) and stable polymorph and hydrate screening. In addition, physical stabilities of the zwitterion and the selected form at different relative humidities were compared to assess their improvement.

2. Materials and methods

2.1. Materials

4-{[4-({[(3-Isopropyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid (compound A) was synthesized at Nagoya Laboratories, Pfizer Japan Inc. Methanesulfonic acid was obtained from Nacalai Tesque (Kyoto, Japan) and the other organic acids were obtained from Wako Pure Chemical Industries (Osaka, Japan). All solvents were purchased from Wako Pure Chemical Industries.

2.2. Powder X-ray diffractometory

Powder X-ray diffraction (PXRD) patterns were collected using a RINT-TTR (Rigaku, Tokyo, Japan) with Cu Kα radiation generated at 300 mA and 50 kV. Samples were placed on aluminum rotation-plates and rotated at 60 rpm at room temperature. Data were collected from 3 to 35° (2θ) at a step size of 0.02° and scanning speed of 4°/min.

2.3. Raman microscopy

Raman spectra were recorded on a LabRam HR-800/HTS-Multiwell (Jobin Yvon Horiba, Edison, NJ, USA) at room temperature, equipped with a back scattering light path system of a light-emitting diode laser (785 nm, 300 mW) as an excitation source and an air-cooled CCD detector. A 20-fold long working distance objective lens was used to collect the backscattered light. The spectra were acquired with 5.84 cm^{-1} spectral width and at least 30 s of exposure.

2.4. Thermal analysis

DSC was performed using a DSC 6200 system (Seiko Instruments, Chiba, Japan). A DSC thermogram was obtained in an aluminum pan system with a pin hole using a sample weight of ca. 3 mg and a heating rate of 5 °C/min under a nitrogen flow. TGA was performed using a TG/DTA 6200 system (Seiko Instruments, Chiba, Japan). A TGA thermogram was obtained in an open aluminum pan system under the same conditions as those for DSC.

2.5. Elemental analysis

The elemental analysis was performed on an Elementar Vario EL (Elementar Instrument, Germany) with helium (200 mL/min) as the carrier gas.

2.6. NMR spectroscopy

1H NMR data were collected on JNM-LA300 FT-NMR system (JEOL Ltd., Tokyo, Japan) operating at 300 MHz. Samples were dissolved in DMSO-d_6.

2.7. High pressure liquid chromatography (HPLC)

Chemical stability was evaluated by an HPLC system (Alliance 2695, Waters, Milford, MA, USA) at 215 nm. The packaged column was Zorbax Extend C18 (3.5 μm, 4.6 mm × 250 mm, Agilent Technologies, Inc., Santa Clara, CA, USA) operated at 40 °C at a flow rate of 1.0 mL/min. The mobile phase consisted of acetonitrile: 10 mM ammonium acetate buffer (27:73).

2.8. Moisture sorption analysis

Dynamic vapour sorption was recorded using an Automated Water Sorption Analyser (DVS-1, Surface Measurement Sys-

tems Ltd., London). Sample of ca. 10 mg was stored under humidity which varied between 0 and 95% RH at 25 °C.

2.9. Physical and chemical stability

The physical and chemical stabilities were evaluated by storing ca. 10 and 1 mg samples, respectively, at 75% RH at 70 °C for 10 days in desiccators. The long term physical stability was also evaluated by storing each sample of ca. 10 mg at 60, 69, 75, 81 and 84% RH at 25 °C in desiccators and samples were removed after 10, 20, 30 and 60 days. Samples after storage were subjected to an HPLC analysis for chemical stability, and PXRD and TG/DTA analyses for physical stability. Storage conditions were adjusted in the stability chamber (60% RH at 25 °C and 75% RH at 70 °C) and with saturated aqueous solutions of KI (69% RH), NaCl (75% RH), KBr (81% RH) and KCl (84% RH) at 25 °C.

2.10. Salt screening on 96-well plate

Salt formation of compound A on a 96-well plate was attempted using a combination of twelve kinds of crystallization solvents and 15 different acids according to the methods reported (Kojima et al., 2006). Methanol solutions of compound A and the acids, which were hydrochloric acid, sulfonic acid, 1,2-ethanedisulfonic acid, methanesulfonic acid, (1S)-(+)-10-camphorsulfonic acid, benzensulfonic acid, phosphoric acid, L-tartaric acid, fumaric acid, citric acid, gluconic acid, L-malic acid, L-lactic acid, succinic acid, and acetic acid were prepared in the concentration of 20 mM prior to use. Compound A solution (50 μL) was placed in all wells of the 96-well quartz plate (Hellma, Müllheim, Germany). Each methanol solution of acid (25 or 50 μL) was added into each row of the plate to give the binary mixture of compound A and acid in a molar ratio of 2:1 or 1:1. The plate was sealed and shaken at room temperature for 4 h. Solvent was evaporated off under reduced pressure at 40 °C overnight. Each crystallization solvent, 200 μL of methanol, ethanol, isopropyl alcohol, acetonitrile, acetone, ethyl acetate, isopropyl ether, tetrahydrofuran, toluene, dichloromethane, cyclohexane and mixture of isopropyl alcohol and water (v/v = 1/1), was added to each column of the plate. The plate was sealed again and shaken at 40 °C for 4 h and allowed to stand at room temperature overnight. All solvents in the plate were evaporated slowly in a nitrogen atmosphere to attempt crystallization. Solids on the 96-well plate were checked for crystallinity with a polarizing light microscope (PLM) and analyzed with a Raman microscope ($n = 2$ in each well).

2.11. Salt preparation

Crystalline salts of compound A found by salt screen on 96-well plate were prepared on a 300-mg scale. Based on the results of salt screening on 96-well plate, besylate, camsylate, monosulfate, hemi-edisylate, hemifumarate and monosuccinate salts were crystallized from ethyl acetate, isopropyl ether, tetrahydrofuran, tetrahydrofuran, ethyl acetate and acetonitrile, respectively. The resultant products were filtrated and dried under reduced pressure.

2.12. Polymorph and pseudopolymorph screening

Zwitterion and salts of compound A (20 mg) were crystallized from each solvent (5 mL) used in the salt screen on the 96-well plate. Compounds which were not dissolved in crystallization solvent, were slurried overnight. The resultant products were filtrated and dried under a nitrogen atmosphere. Obtained solids were characterized by PXRD and TG/DTA.

2.13. Stable polymorph and hydrate screening

Stable polymorph screening of besylate salt was performed in sixteen solvents with a wide range of dielectric constants and eight solvents mixed with water (5% v/v) in order to increase the solubility. The 16 solvents were methanol, ethanol, 2-propanol, 1-buthanol, *tert*-amyl alcohol, acetonitrile, tetrahydrofuran, 2-methyltetrafuran, acetone, 2-butanone, methyl *tert*-butyl ether, ethyl acetate, dichloromethane, toluene, heptane and water. Eight solvents mixed with water (5% v/v) were methanol, ethanol, 2-propanol, acetone, acetonitrile, 1-buthanol, tetrahydrofuran and 2-butanone. Suspension of besylate salt in these solvents were prepared at the concentration of 40–200 mg/mL depending on the solubility. After 1-day slurry equilibration at 40 °C and then 3-day slurry equilibration at 25 °C, each suspension was filtrated and the obtained solids were characterized by PXRD and TG/DTA.

Hydrate screening of besylate salt was performed in the same eight solvents mixed with water (5% v/v) as stable polymorph screening, with a wide rage of water activity, methanol, ethanol, 2-propanol, acetone, acetonitrile, 1-buthanol, tetrahydrofuran, 2-butanone. Suspension was prepared and evaluated in the same manner as stable polymorph screening.

3. Results and discussion

3.1. Solid-state characterization of zwitterion

From the discovery synthesis, two anhydrous forms, stable form ZW-I and metastable form ZW-II, and two hydrate forms ZW-III and ZW-IV of zwitterion crystallized from water have been identified (Fig. 2 (1)). DSC curves of ZW-I and ZW-II showed melting endothermic peaks at 176.9 and 171.9 °C, respectively (Fig. 3 (1)). The desolvation of water occurred with 4.2 and 21% loss of the weight, respectively (Fig. 3 (2)). The results of DSC, TGA and elemental analysis suggested that ZW-

Table 1
Physicochemical properties of ZW-I

Melting point	176.9 °C
Chemical stability	Stable at 70 °C/75% RH for 10 days
Physical stability	Transformed to hydrate ZW-IV at 25 °C/75% RH within a few days
Polymorphism	2 Polymorphs (ZW-I and ZW-II) 2 Hydrates (ZW-III and ZW-IV)

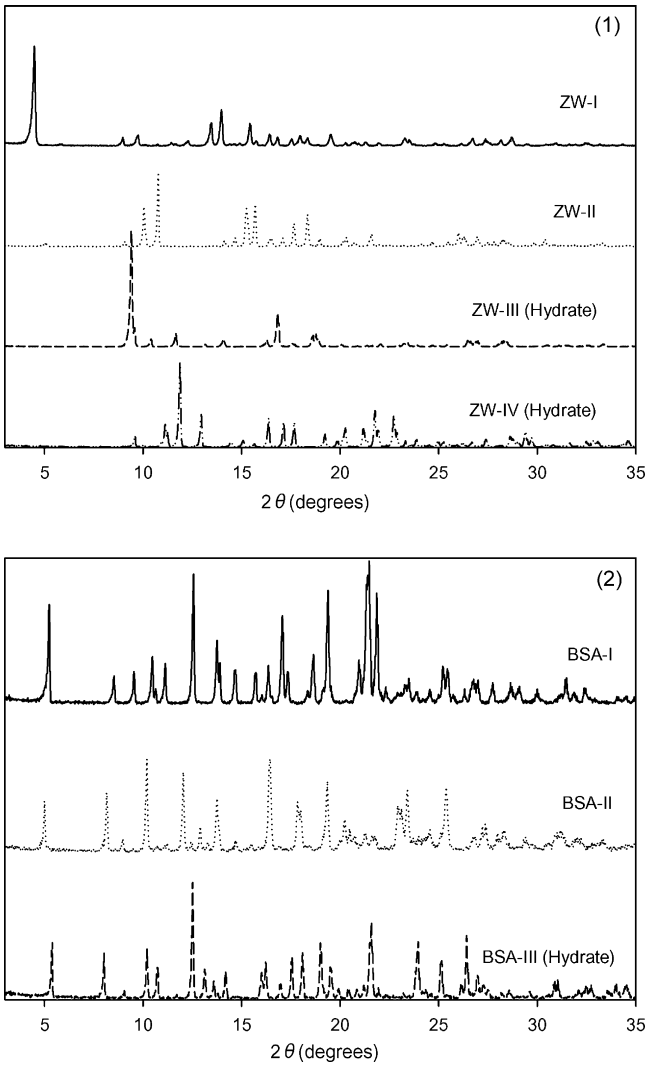


Fig. 2. (1) PXRD patterns of modifications of zwitterion. (2) PXRD patterns of modifications of besylate salt.

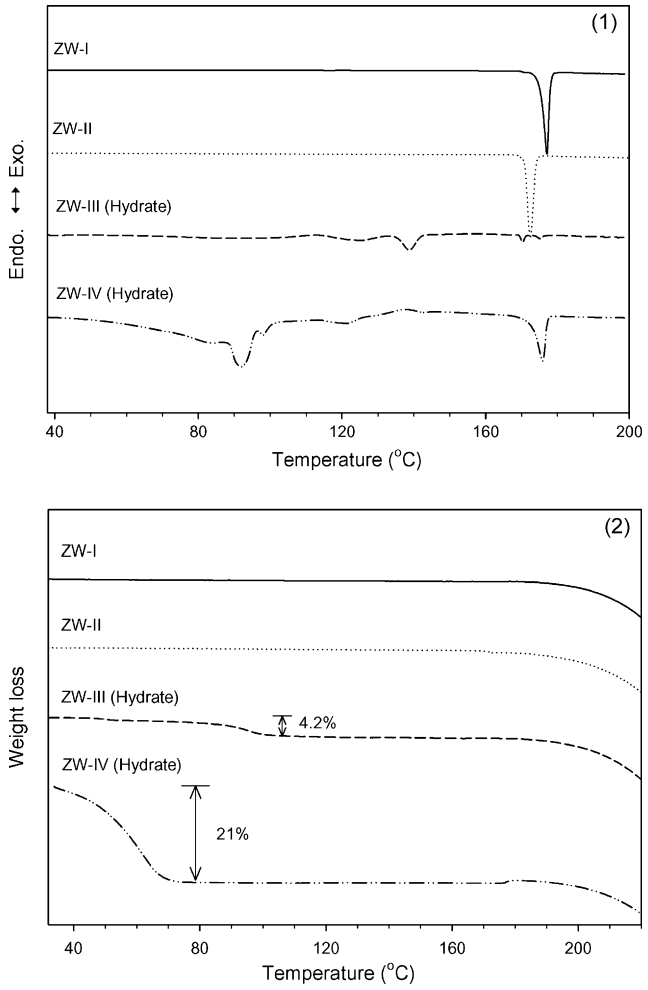


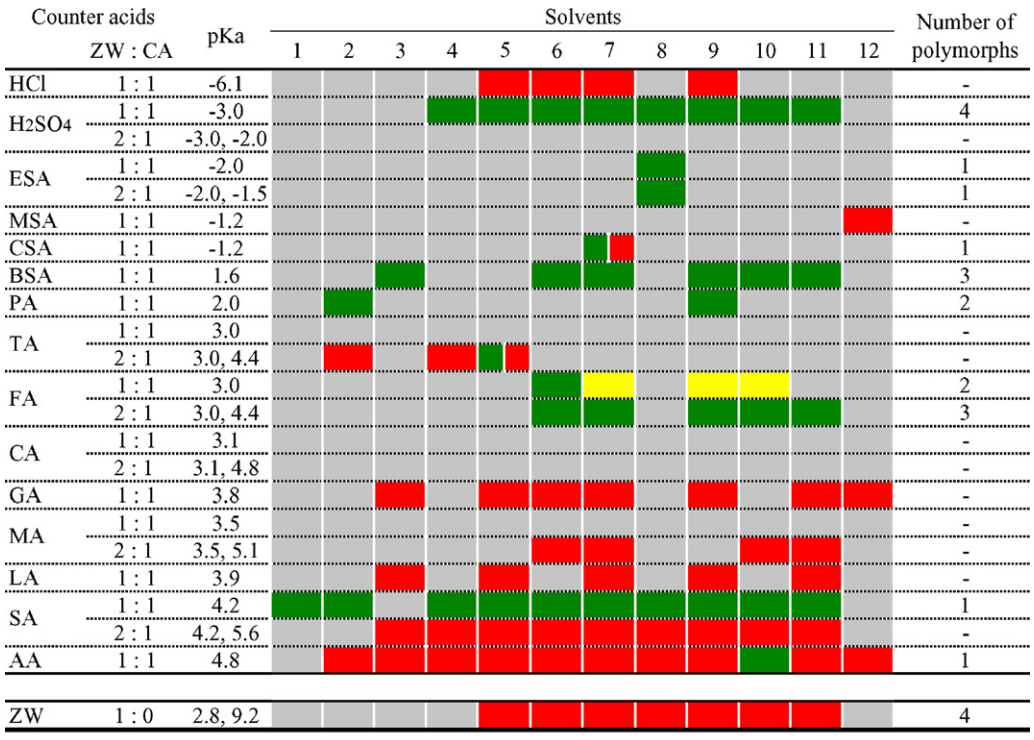
Fig. 3. (1) DSC thermograms of modifications of zwitterion. (2) TGA thermograms of modifications of zwitterion.

III and ZW-IV might be hydrates containing xH$_2$O with $x = 1$ and 6–7, respectively. ZW-I was chemically stable at exaggerated conditions such as 70 °C/75% RH for 10 days. However, ZW-I was not physically stable, since it took up water from atmospheric moisture and transformed to hydrate form ZW-IV at 70 °C/75% RH within a few days (Table 1). ZW-IV was also physically unstable with desolvation occurring at room temperature during TGA measurement (Fig. 3 (2)). These results suggested that ZW-I was transformed to ZW-IV including 21% water content under the ambient condition and an unexpected transformation caused the low content of compound A during the weighing of drug substance such as zwitterion. From the viewpoint of stability during the shelf life, alternative solid forms to zwitterion should be selected for the pharmaceutical development.

3.2. Salt screening on 96-well plate

The salt screening of compound A on 96-well plates was conducted to facilitate possible salt formation. Except for amorphous or oily substances observed by PLM, crystals obtained were analyzed by Raman microscope. The results of Raman microscopy for salts of compound A on the 96-well plates are shown in Fig. 4. The Raman spectra of all crystals were sorted in comparison with each pattern in the shift region 600–1800 cm^{-1} and compared with those of zwitterion and counter acids measured separately, according to the method reported (Kojima et al., 2006). The Raman spectra of the crystals on the wells with combinations of compound A as zwitterion (ZW) and benzensulfonic acid (BSA) are shown in Fig. 5. The Raman spectra of binary mixture of ZW and BSA in molar ratio of 1:1 were identified as besylate salt with three polymorphs, because their spectra were different from the spectra of either ZW (ZW:BSA = 1:0) and BSA (ZW:BSA = 0:1).

Generally, the difference of pK_a in water between free base/acid and counter acid/base could provide an indication of salt formation and the tendency of salt formation was discussed in the field of high-throughput salt screening (Morissette et al., 2004). Fig. 4 also provides the information of tendency of salt formation even though pK_a values could be influenced by organic solvents. Raman spectra can indicate the formation of multi-component crystal such as



Abbreviations used in the figure; ZW, zwitterion; CA, counter acid; ESA, 1.2-ethanedisulfonic acid; MSA, methanesulfonic acid; CSA, Camphorsulfonic acid; BSA, benzenesulfonic acid; PA, phosphoric acid; TA, L-tartaric acid; FA, fumaric acid; CA, citric acid; GA, gluconic acid; MA, L-malic acid; LA, L-lactic acid; SA, succinic acid; AA, acetic acid.

Solvents; 1, methanol; 2, ethanol; 3, isoproplyl alcohol; 4, acetonitrile; 5, acetone; 6, ethyl acetate; 7, isopropyl ether; 8, tetrahydrofuran; 9, toluene; 10, dichloromethane; 11, cyclohexane; 12 mixed solvenet of isopropyl alcohol and water (v/v=1/1).

Raman spectra were classified in oil or amorphous, ; zwitterion, ; counter acid, ; and salt or cocrystal, .

Fig. 4. Raman spectra classification of salts on 96-well plate.

salt. It was found that compound A did not form salt with counter acid whose pK_a (Stahl and Wermuth, 2002a) was higher than 3.8 except for succinic acid (pK_a = 4.2, 5.6). Compound A could form six-membered intramolecular salt bridge between piperidine (pK_a = 9.2) and carboxylic acid (pK_a = 2.8). Counter acids whose pK_a were higher than 3.8 may not interfere with the intramolecular salt bridge and form salt with themselves.

In addition, crystals of fumaric acid and salt were detected in the wells in molar ratios of 1:1 and 2:1 (compound A: fumaric acid), respectively. Based on the methodology of our previous report (Kojima et al., 2006), compound A was supposed to form hemifumarate salt and the excess of fumaric acid was crystallized in the wells in molar ratio of 1:1. Raman spectra of crystalline form from each pair of binary mixtures of compound A and acid on the plate were also compared in order to predict the number of polymorphs (Fig. 4). Taken together the results of salt formation and the number of polymorphs, besylate, camsylate, monosulfate, hemi-edisylate, hemifumarate, monosuccinate salts were selected for further investigation.

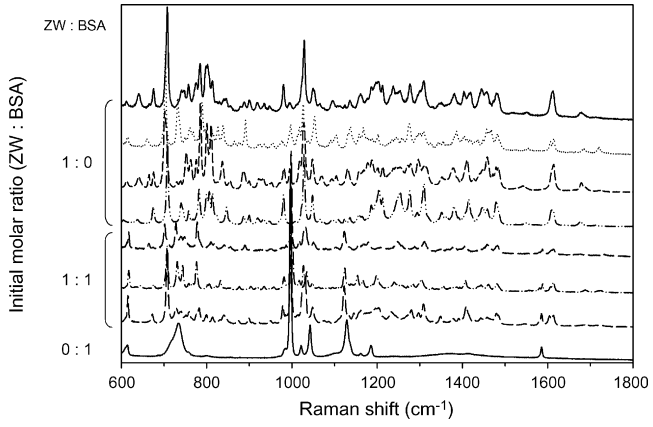


Fig. 5. Raman spectra of compound A as zwitterion (ZW:BSA = 1:0), besylate salt (ZW:BSA = 1:1) and benzensulfonic acid (ZW:BSA = 0:1) crystals on a 96-well plate.

3.3. Solid-state characterization of salts

Besylate, camsylate, monosulfate, hemi-edisylate, hemifumarate, monosuccinate salts of compound A, hit on 96-well plates, were prepared on a 300-mg scale. An HPLC analysis suggested that compound A was decomposed under preparation of monosulfate salt. The other salts obtained, besylate, camsylate, hemi-edisylate, hemifumarate, monosuccinate salts, were recrystallized or slurried in 12 solvents. Three forms of besylate,

Table 2
Physicochemical properties of salts

Salts	Besylate	Camsylate	Edisylate	Succinate	Fumarate
Stoichiometry (Compound A:Acid)	1:1	1:1	2:1	1:1	2:1
Polymorphism					
Modifications (melting point; °C)	BSA-I (220) BSA-II (200) BSA-III (hydrate)	CSA-I (188) CSA-II (hydrate)	6 solvates (MeOH, EtOH, THF, DCM, Acetone, MeCN)[a]	SA-I (111) SA-II (114)	FA-I (137) FA-II (145) FA-III (162)
Stability at 70 °C/75% RH for 10 days					
Crystalline form	BSA-I	CSA-I		SA-II	FA-III
Chemical stability	Potency >99%	Potency >99%	–[b]	Potency >99%	Potency >99%
Physical stability	Stable	Stable	–[b]	Unstable[c]	Unstable[c]

[a] Anhydrous form of hemi-edisylate salt was not obtained.
[b] Not determined.
[c] Transformed to hydrate ZW-IV of zwitterions.

two forms of camsylate, six forms of edisylate, two forms of succinate and three forms of fumarate were detected by PXRD and TG/DTA (Table 2). Three forms of besylate salts were identified as two anhydrous forms BSA-I and BSA-II and one hydrate form BSA-III (Figs. 2 (2), 6 (1) and (2)). Two forms of camsylate salts were identified as an anhydrous form CSA-I and a hydrate form CSA-II. Two forms of succinate salts were identified as two anhydrous forms SA-I and SA-II. Three forms of fumarate salts were identified as anhydrous forms FA-I, FA-II and FA-III. Crystallization of fumarate and succinate salt from the mixture of isopropyl alcohol and water (v/v = 1/1) resulted in a transformation to hydrate form ZW-IV of zwitterion. It was speculated that the salt was dissociated and transformed to ZW-IV, since acidities of fumaric acid and succinic acid were lower than that of the carboxylic acid of compound A and could not maintain the salt form (Stahl and Nakano, 2002b). Six forms of edisylate salt were identified as methanol, ethanol, tetrahydrofuran, dichloromethane, acetone and acetonitrile solvates by TGA and NMR, and anhydrous form was not obtained. From the viewpoint of the manufacturing process of pharmaceutical substances and products including crystallization and wet granulation, it was concluded that fumarate, succinate and edisylate salts were not suitable for pharmaceutical development.

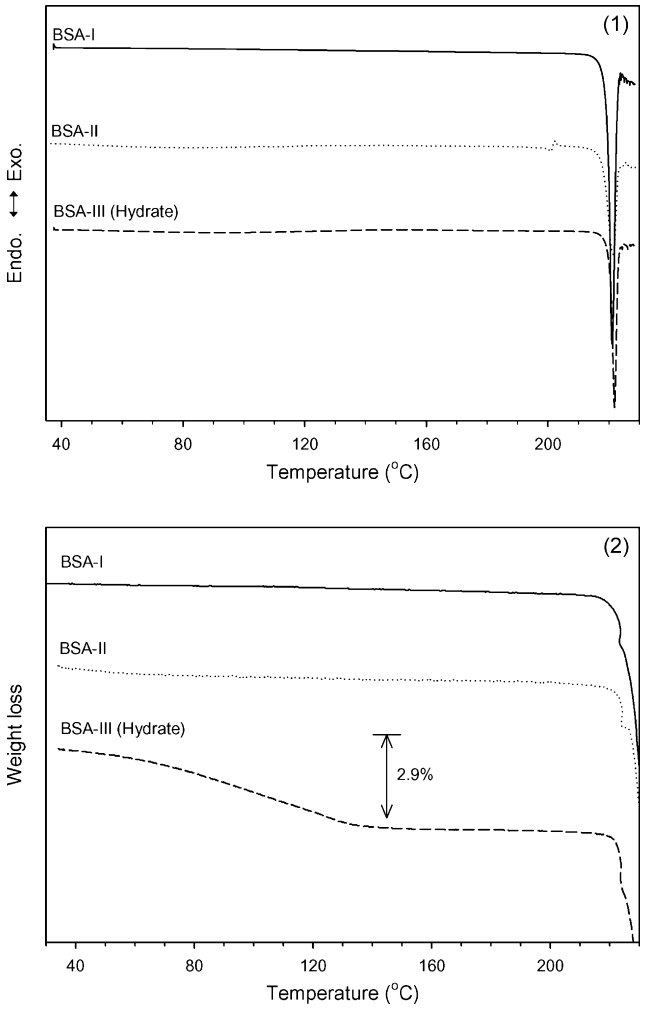


Fig. 6. (1) DSC thermograms of modifications of besylate salt. (2) TGA thermograms of modifications of besylate salt.

The chemical and physical stability of anhydrous forms of salts, BSA-I of besylate, CSA-I of camsylate, SA-II of succinate and FA-III of fumarate, was evaluated at 75% RH at 70 °C for 10 days. HPLC analyses did not show any significant decrease in the purity of each salt (data not shown). The results of PXRD and TG/DTA suggested that BSA-I and CSA-I were physically stable at these conditions, whereas SA-II and FA-III were transformed to hydrate form of zwitterion, ZW-IV. Moisture sorption analyses of BSA-I of besylate, CSA-I of camsylate, SA-II of succinate and FA-III of fumarate salts were also performed and compared with that of ZW-I (Fig. 7). CSA-I and FA-III salts were kinetically stable up to 90% RH, however these salts took up water drastically at 95% RH and would be transformed to hydrate form. SA-II gradually took up water from moisture as the relative humidity increased. Whereas, BSA-I was stable up to 95% RH. Therefore, BSA-I was the only crystalline form which showed less hygroscopicity than ZW-I. The results of stability at 75% RH at 70 °C and moisture sorption analyses suggested that BSA-I of besylate salt would be a preferable solid form for pharmaceutical development.

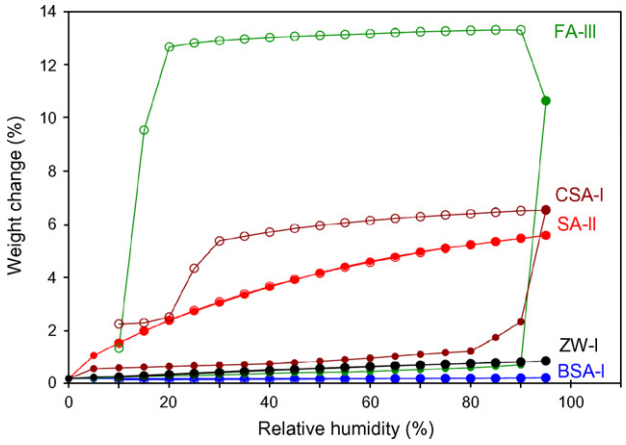


Fig. 7. Sorption/desorption step-isotherm for water vapor on various salts. Filled circle (●), sorption; open circle (○), desorption.

3.4. Stable polymorph and hydrate screening

Stable polymorph and hydrate screening of besylate salt was performed to ensure that BSA-I was the most preferable form for pharmaceutical development. In the solid-state characterization, two anhydrous forms BSA-I and BSA-II, and one hydrate form BSA-III of besylate salt were identified (Figs. 2 (2), 6 (1) and (2)). The DSC curves of BSA-I of besylate salt showed the melting endothermic peak at 220.5 °C. Whereas, the DSC curves of BSA-II showed endothermic and exothermic peaks corresponding with the transformation to form I at 200.1 °C and an endothermic peak at 221.9 °C corresponded with the melting of BSA-I.

BSA-I of besylate salt was slurried in 16 pure solvents and eight solvents mixed with water (5% v/v). PXRD patterns and TG/DTA thermograms of crystals obtained from pure organic solvents and methanol and ethanol mixed with water showed the same pattern as intact form BSA-I and indicated that BSA-I of besylate salt would be the most stable form. Recently, the effect of solubility on transformation to thermodynamically most stable form was discussed (Miller et al., 2005). In the report, solubility of 8 mM is required to ensure transformation. In our experiments, five solvents had enough solubility to allow for BSA-I.

In addition, hydrate screening was also conducted in water and eight solvents mixed with water (5% v/v) with a wide rage of water activity ranging from 0.2 to 1.0 at room temperature. Hydrate form BSA-III of besylate salt was obtained in water and organic solvent mixed with water and identified as the same form crystallized from the mixture of isopropyl alcohol and water (v/v = 1/1). In order to evaluate hydrate formation, water activity has been given attention recently (Sacchetti, 2004; Ticehurst et al., 2002; Zhu et al., 1996). Some reports have discussed the relationship between critical water activity and critical relative humidity. In this study, we have ranged the water activity from 0.2 to 1.0 at room temperature. Transformation to hydrate form BSA-III was detected in the solvent except for methanol and ethanol mixed with water which showed lower water activity than 0.4. These results suggested that critical water activity of besylate salt was 0.4 which is corresponded with ambient relative humidity according to the report (Ticehurst et al., 2002) and physical stability in the solid-state should be required.

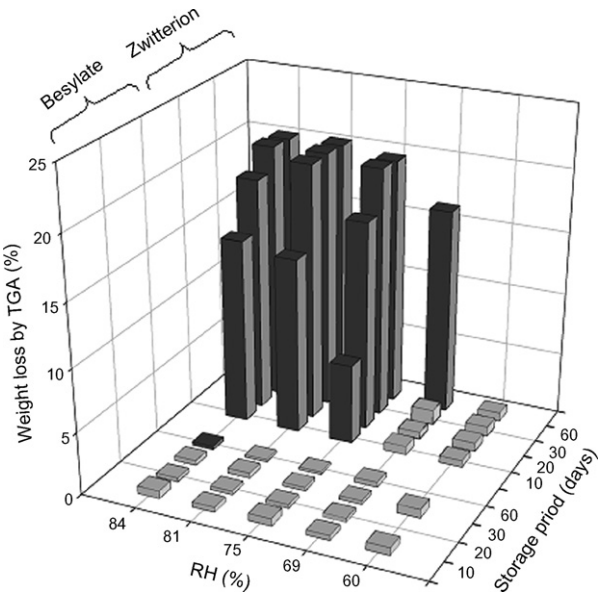


Fig. 8. Results of physical stability of ZW-I of zwitterion and BSA-I of besylate salt. Light gray column, anhydrous form; dark gray column, hydrate.

3.5. Physical stability of zwitterions and besylate salt at different relative humidities

Physical stability of ZW-I of zwitterions and BSA-I of besylate salt was evaluated at different humidities at 25 °C (Fig. 8). The results of PXRD patterns and TG/DTA thermograms suggested that zwitterion was transformed to hydrate form ZW-IV at 69% RH, whereas besylate salt was quite stable at 69% RH and transformed to hydrate form BSA-III at 84% RH. The results of the TGA suggested that physical stability under humid condition is significantly improved in besylate salt compared with zwitterion. Moisture content of ZW-IV and BSA-III was 21 and 2.9%, respectively (Figs. 3 (2) and 6 (2)). From the viewpoint of manufacturing a pharmaceutical product, unexpected transformation of ZW-I to ZW-IV causes the low potency of compound A during the weighing of a drug substance as zwitterion and BSA-I of besylate salt might solve this issue.

4. Conclusion

We have demonstrated the effective solid form selection of compound A as zwitterionic pharmaceutical compound and that stable form BSA-I of besylate salt was an appropriate solid form for further studies. Three forms of besylate salt, anhydrous forms BSA-I and BSA-II and hydrate form BSA-III, were also identified. The stable form of BSA-I provided significant improvement in physical stability compared with zwitterion developed in the early stage.

We have also demonstrated that besylate, hemi-edisylate, hemifumarate and monosuccinate salt could be prepared on a 300-mg scale, based on the results of the salt screening on 96-

well plate. We have previously reported that the stoichiometrical information obtained on the 96-well plate was useful for efficient salt selection for scale up (Kojima et al., 2006). In this study, the methodology of stoichiometrical prediction on the 96-well plate was firstly applied to a new pharmaceutical compound.

In the solid form selection, the multi-dimensional evaluation including solubility, stability and manufacturability should be performed. Especially, issues of physicochemical property of pharmaceutical compounds would be the motivation for solid form selection, which is positioned as essential evaluation item and has influenced the strategy and period of the solid form selection. The effective solid form selection was demonstrated based on the strategy in order to improve the physical stability of zwitteion as pharmaceutical compound and BSA-I of besylate salt could solve the issues for the development.

References

Badawy, S.I., 2001. Effect of salt form on chemical stability of an ester prodrug of a glycoprotein IIb/IIIa receptor antagonist in solid dosage forms. Int. J. Pharm. 223, 81–87.

Bastin, R.J., Bowker, M.J., Slater, B.J., 2000. Salt selection and optimization procedures for pharmaceutical new chemical entities. Org. Process Res. Dev. 4, 427–435.

Bergren, M.S., Chao, R.S., Meulman, P.A., Sarver, R.W., Lyster, M.A., Havens, J.L., Hawley, M., 1996. Solid phases of delavirdine mesylate. J. Pharm. Sci. 85, 834–841.

Byrn, S.R., Xu, W., Newman, A.W., 2001. Chemical reactivity in solid-state pharmaceuticals: formulation implications. Adv. Drug Deliv. Rev. 48, 115–136.

Engel, G.L., Farid, N.A., Faul, M.M., Richardson, L.A., Winneroski, L.L., 2000. Salt form selection and characterization of LY333531 mesylate monohydrate. Int. J. Pharm. 198, 239–247.

Gandhi, R.B., Bogardus, J.B., Bugay, D.E., Perrone, R.K., Kaplan, M.A., 2000. Pharmaceutical relationships of three solid state forms of stavudine. Int. J. Pharm. 201, 221–237.

Glass, B.D., Novák, C., Brown, M.E., 2004. The thermal and photostability of solid pharmaceuticals. J. Therm. Anal. Calorim. 77, 1013–1036.

Gould, P.L., 1986. Salt selection for basic drugs. Int. J. Pharm. 33, 201–217.

Knapman, K., 2000. Polymorphic predictions. Modern Drug Discovery, vol. 3. American Chemical Society, pp. 53–54, 57.

Kojima, T., Onoue, S., Murase, N., Katoh, F., Mano, T., Matsuda, Y., 2006. Crystalline form information from multi-well plate salt screening by use of Raman microscopy. Pharm. Res. 23, 806–812.

Kojima, T., Kato, F., Teraoka, R., Matsuda, Y., Kitagawa, S., Tsuhako, M., 2007a. Physicochemical characterization of tamoxifen citrate pseudopolymorphs, ethanolate and methanolate. Chem. Pharm. Bull. 55, 407–411.

Kojima, T., Onoue, S., Katoh, F., Teraoka, R., Matsuda, Y., Kitagawa, S., Tsuhako, M., 2007b. Effect of spectroscopic properties on photostability of tamoxifen citrate polymorphs. Int. J. Pharm. 336, 346–351.

Kushida, I., Ashizawa, K., 2002. Solid state characterization of E2101, a novel antispastic drug. J. Pharm. Sci. 91, 2193–2202.

Miller, J.M., Collman, B.M., Greene, L.R., Grant, D.J., Blackburn, A.C., 2005. Identifying the stable polymorph early in the drug discovery-development process. Pharm. Dev. Technol. 10, 291–297.

Morissette, S.L., Soukasene, S., Levinson, D., Cima, M.J., Almarsson, O., 2003. Elucidation of crystal form diversity of the HIV protease inhibitor ritonavir by high-throughput crystallization. Proc. Natl. Acad. Sci. USA 100, 2180–2184.

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., 2004. High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids. Adv. Drug Deliv. Rev. 56, 275–300.

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

Sacchetti, M., 2004. Determining the relative physical stability of anhydrous and hydrous crystal forms of GW2016. Int. J. Pharm. 273, 195–202.

Stahl, P.H., Wermuth, C.G., 2002a. Monographs on Acids and Bases. In: Stahl, P.H., Wermuth, C.G. (Eds.), Handbook of Pharmaceutical Salts: Properties, Selection, and Use. VHCA Verlag Helvetica Chimica Acta, WILEY-VCH, Zürich (Switzerland), Weinheim (Federal Republic of Germany), ISBN 3-906390-26-8, pp. 266–327.

Stahl, P.H., Nakano, M., 2002b. Pharmaceutical Aspects of the Drug Salt Form. In: Stahl, P.H., Wermuth, C.G., Eds.), Handbook of Pharmaceutical Salts: Properties, Selection, and Use, pp. 83–116.

Ticehurst, M.D., Storey, R.A., Watt, C., 2002. Application of slurry bridging experiments at controlled water activities to predict the solid-state conversion between anhydrous and hydrated forms using theophylline as model drug. Int. J. Pharm. 247, 1–10.

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

Trask, A.V., Motherwell, W.D., Jones, W., 2005. Pharmaceutical cocrystallization: engineering a remedy for caffeine hydration. Cryst. Growth Des. 5, 1013–1021.

Vishweshwar, P., McMahon, J.A., Bis, J.A., Zaworotko, M.J., 2006. Pharmaceutical co-crystals. J. Pharm. Sci. 95, 499–516.

Zhu, H., Yuen, C., Grant, D.J.W., 1996. Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 1. Theophylline. Int. J. Pharm. 135, 151–160.