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Salt form selection and characterization of LY333531 mesylate monohydrate

Gary L. Engel [a], Nagy A. Farid [b], Margaret M. Faul [c,*], Lori A. Richardson [b], Leonard L. Winneroski [c]

[a] *Biopharmaceutics Department, Lilly Research Laboratories, A Division of Eli Lilly and Company, Indianapolis, IN 46285, USA*

[b] *Division of Drug Metabolism and Disposition, Lilly Research Laboratories, A Division of Eli Lilly and Company, Indianapolis, IN 46285, USA*

[c] *Chemical Process Research and Development Division, Lilly Research Laboratories, A Division of Eli Lilly and Company, Indianapolis, IN 46285, USA*

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Abstract

LY333531 is a potent protein kinase C_β (PKC_β) inhibitor currently under development for the treatment of diabetic complications. Seven salts of LY333531 (hydrochloride, sulfate, mesylate, succinate, tartrate, acetate and phosphate) were evaluated during the early phase of development. Physical property screening techniques including microscopy, DSC, TGA, XRPD, hygroscopicity and solubility were utilized to narrow the selection to two salts: the mesylate and hydrochloride. Identification of the optimal salt form was based upon solubility, bioavailability, physical stability and purity. During the evaluation process three hydrated forms (anhydrate, monohydrate, and tetrahydrate) of the hydrochloride salt were identified. The mesylate salt was found to give only one, a monohydrate. Processing parameters (e.g. filtration rate, crystal form stability) demonstrated that the anhydrate was the preferred form of the hydrochloride salt. Bioavailability studies in dogs indicated that the C_{max} and area under the plasma concentration vs. time curve (AUC) for LY333531 and its active metabolite, LY338522, following administration of the mesylate salt were approximately 2.6 times those obtained after the LY333531 HCl dose. This difference was presumed to be due primarily to the fact that the mesylate was five times more soluble than the hydrochloride salt in water. These factors led to selection and development of LY333531 mesylate monohydrate as the active pharmaceutical ingredient for clinical evaluation.

Keywords: Pharmaceutical salt form; Protein kinase C_β inhibitor; LY333531; Solubility; Bioavailability; Mesylate salt; Hydrochloride salt; Monohydrate; Anhydrate

1. Introduction

There has been a strong historical trend in the pharmaceutical industry to market the hydrochloride salt of amines. From an early literature re-

* Corresponding author.
E-mail address: faul_margaret_m@lilly.com (M.M. Faul)

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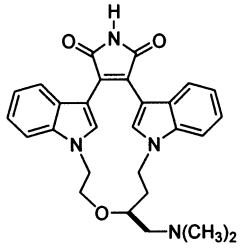
view by Berge et al. (1977) nearly 43% of the FDA commercially marketed salts were hydrochlorides. Only about 2% have been marketed as mesylate salts. More recently Bighley et al. (1996) compiled data from drug monographs listed in the 1993 edition of Martindale, the Extra Pharmacopeia, that showed a similar percentage of hydrochloride and mesylate salts (44 vs. 3.2%). Although the hydrochloride salts of pharmaceutical bases have advantages over other salt forming moieties because of their low molecular weight and low toxicity, they can have potential issues. The primary concern is from a decrease in solubility as a result of chloride anion common ion effects in the stomach. This was described by Miyazaki et al. (1981) and more recently by Thomas et al. (1996). Recent trends have indicated that mesylate salts are becoming more common as the marketed active pharmaceutical ingredient. Looking at the new chemical entities approved by the FDA over the last five years that had associated anionic salts, nearly 20% were reported to be mesylate salts (compiled from information found using the FDA website (www.fda.gov)). Explanations for the current increase in the number of mesylate salts have not been disclosed in the literature.

In addition, studies comparing the salt forms of basic drugs have been limited, and none have reported significant differences in bioavailability between different salt forms. Lin et al. (1972) reported no enhancement in bioavailability when salts of a basic antihypertensive agent, having significantly different intrinsic dissolution rates, were compared. Walmsley et al. (1986) also indicated that they did not observe a difference in the extent of bioavailability between oxalate and citrate salts of naftidrofuryl and Jamuludin et al. (1988) saw no significant differences in C_{max}, T_{max}, or AUC of the hydrochloride, sulfate, and ethyl carbonate salts of quinine.



Scheme 1. Structure of LY333531.

In this manuscript we will disclose our results from a salt selection study preformed on LY333531 (Scheme 1), a free base of very low aqueous solubility, in which a salt form for the drug product was required in order to enhance solubility and increase bioavailability. LY333531 has recently been identified as a competitive reversible inhibitor of protein kinsae C_β and is being evaluated for the treatment of diabetic complications (e.g. retinopathy, erectile dysfunction) Ishii et al. (1996), Jirousek et al. (1996). Small lots (≤ 1 g) of seven salts (hydrochloride, sulfate, mesylate, succinate, tartrate, acetate, and phosphate) were initially crystallized and evaluated. Physical property tests including polarizing microscopy, hygroscopicity, differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), X-ray powder diffraction (XRPD), and aqueous solubility were performed on each of these salt forms to consider the feasibility of further development. Similar tiered approaches have been described in the literature by Morris et al. (1994). Five salts (sulfate, succinate, tartrate, acetate and phosphate) were then eliminated due to poor crystallinity, low solubility and difficulty in improving the chemical purity during preparation. A comprehensive analysis of the remaining two salts mesylate and hydrochloride, were finally assessed evaluating additional parameters including polymorphism/hydrate formation, stability, purification, filterability and the relative bioavailability in dogs. The results obtained from this analysis, and outlined in this manuscript, have led us to develop the mesylate salt as the optimal salt form of LY333531.

2. Materials and methods

2.1. Preparation of salts

2.1.1. Preparation of LY333531·mesylate monohydrate

The mesylate monohydrate salt of LY333531 was initially crystallized from methanol/water.

However, the potential to generate methyl methanesulfonate, a known mutagen, in methanol caused us to examine alternative solvent systems. It was determined that acetone/water (9/1 v/v) was a more appropriate solvent combination for processing and all of the subsequent development lots were prepared using these conditions.

2.1.2. Preparation of LY333531 anhydrate, hydrated hydrochloride

The anhydrate and hydrated forms of the hydrochloride salt of LY333531 were crystallized from methanol/water systems with the resultant crystal form being determined by the amount of water in the methanol. Acetone/water systems were found to increase the level of impurities relative to starting material.

2.2. Analytical assays

2.2.1. Solubility determination

Several different suspensions concentrations of LY333531 salts were stirred for 16 h, filtered, and assayed by HPLC using a Zorbax® SB-CN column (4.6 × 250 mm; 5 μm) and 0.1% trifluoroacetic acid (TFA)/acetonitrile (65/35, v/v) as the mobile phase. The flow rate was 1.0 ml/min, and ultraviolet detection was achieved at 233 nm. The sample solvent was acetonitrile/water (1/1, v/v).

2.2.2. Microscopy

Samples dispersed in immersion oil were observed using a Leitz Laborlux 12 Pol S polarizing microscope under crossed polarizers.

2.2.3. Hygroscopicity measurements

Vapor pressure isotherm data was obtained using a microbalance flow system manufactured by the VTI Corporation. A drying step at 60°C preceded the runs which were set up to cover a 10–95% relative humidity (RH) range for absorption and 95–5% for desorption, both in 5% RH steps.

2.2.4. X-ray powder diffraction (XRPD)

Samples were run on a Siemens Model D5000 Diffractometer, using Cu Kα radiation 1.5406 Å, 50 kV, 40 mA, and scanned from 4° to 35° 2θ at 0.03° stepwise with 2.0 s/step.

2.2.5. Thermal gravimetric analysis (TGA)

Measurements were performed on a TA Instruments Model 2950 thermogravimetric analyzer. Samples (2–5 mg) were placed in an open platinum pan and heated at a rate of 5°C/min.

2.2.6. Differential scanning calorimeter (DSC)

Measurements were performed using a TA Instruments Model 2910 differential scanning calorimeter. Samples (2–5 mg) were run using crimped aluminum pans heated at a rate of 5°C/min.

2.2.7. Total related substances (TRS)

The total related substances of samples of the hydrochloride and mesylate salts of LY333531 were determined using a Zorbax® SB-CN column (4.6 × 250 mm; 5μm) and 0.1% TFA/tetrahydrofuran (THF) as the mobile phase. A gradient system was employed using 40% THF for 10 min, 60% THF for 20 min, and reaching 70% THF in 30 min. The flow rate was 1.0 ml/min, and ultraviolet detection was achieved at 233 nm. The column temperature was 40°C.

2.3. Bioavailability study

Each of four male beagle dogs, divided into two treatment groups, received both the hydrochloride and mesylate salts of LY333531 in a crossover design with a one week washout period between doses. A single 20 mg/kg oral dose of LY333531 (as the salt suspended by homogenization in 10% aqueous acacia) was administered in each treatment period. The dosing was based on the weight of the free base. Dogs in treatment group 1 received single doses of LY333531·HCl on Day 1 and LY333531·mesylate on Day 8. Dogs in treatment group 2 received single doses of LY333531·mesylate on Day 1 and LY333531·HCl on Day 8. Blood samples were collected (two dogs/time point/treatment group) at pre-determined timepoints in heparinized containers and stored on ice until centrifugation. The resulting plasma samples were stored at approximately

−70°C until the time of analysis. The concentrations of LY333531 and its active *N*-desmethyl metabolite, LY338522, in plasma samples were determined by the following procedure. Plasma was diluted with water containing an internal standard. Each sample was loaded onto a conditioned 1 ml C8 solid phase extraction cartridge, and washed with water, followed by 10% methanol in water. The analytes and internal standard were eluted with a solution of 95% methanol in water containing 0.1% trifluoroacetic acid (TFA). The eluent was dried under a gentle stream of N_2 at approximately 50°C, the residue was reconstituted in the mobile phase, and injected into an HPLC system. Chromatographic separation of LY333531, LY338522, and the internal standard was preformed using a Zorbax® RX-C8 (4.6 × 250 mm; 5 μm) column and 0.1% TFA/acetonitrile (33/67, v/v) as the mobile phase. The flow rate was 1.0 ml/min, and detection was achieved by visible absorbance at 460 nm. Concentrations below 10 ng/ml (the lowest point on the standard curve) were assigned a value of zero for calculation purposes.

Note: All chemicals in the above sections were commercial products of reagent grade and were used as received.

3. Results and discussion

3.1. Physicochemical tests/results

Both salts displayed birefringence under the polarizing microscope and were observed as thin plates with varying degrees of agglomeration. Visually the salts were red or red-orange in color.

Aqueous solubility of mesylate and the hydrochloride were 0.5 and 0.1 mg/ml (as LY333531), respectively. The solubility of the free base in water was below the HPLC limits of detection (< 1 μg/ml). Recent results from CaCO-2 screening and solubility testing in simulated gastric and intestinal fluids (M. Kuhfeld, B. Riebesehl; internal communications) have demonstrated that the LY333531 was both permeable and insoluble, and thus would be categorized as a Class II compound per the system described by Amidon et al. (1995).

During process scale-up it was discovered that the hydrochloride salt of LY333531 could be crystallized as different hydrated forms: an anhydrate and two different hydrated forms that were reversible and dependent on the relative humidity. Conversion between the two hydrated forms is demonstrated in the vapour pressure isotherms (Fig. 1). The XRPD of the two hydrated forms is shown (Fig. 2). Based on the plateau regions of the vapor pressure isotherm the forms were assumed to be a monohydrate and tetrahydrate, however, more definitive methods to better define the stoichiometery of the hydrates (e.g. single crystal X-ray diffraction) were not pursued. Since this crystal transition occurred in the 10–30% relative humidity range, it was thought that bulk manufacture and subsequent formulation would not be adequately controlled as a single form. The anhydrate form was found to be stable with changes in relative humidity (Fig. 3) and was therefore used for subsequent stability and bioavailability studies.

A vapor pressure study for the mesylate salt showed that the monohydrate form was stable up to 90% humidity after which it absorbed only about 0.1% water (Fig. 4). A small amount of hysteresis was seen on desorption, but no change in XRPD was observed.

Thermal investigations using DSC (Fig. 5) indicated that the mesylate salt had an initial small broad endotherm due to water loss and then a larger endotherm at 260–265°C representing the melt. The hydrochloride anhydrate, however, demonstrated only a very small endotherm near 250°C. As seen from the TGA data (Fig. 6), it was apparent that decomposition occurred for both salts by 270°C. Discoloration of solids at the end of the runs (300°C) also confirmed decomposition.

3.2. Stability

The hydrochloride anhydrate and mesylate monohydrate salts exhibited good thermal stability when stressed both at 50 and 40°C/75% RH for one month. Similar stability results were observed when the salts were mixed with three of the

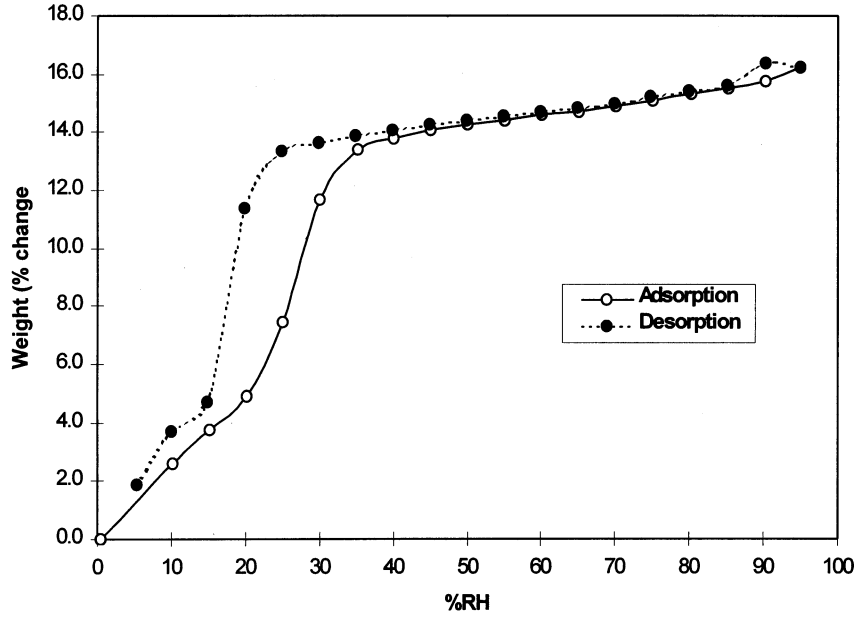


Fig. 1. Adsorption/desorption isotherm for LY333531 hydrochloride hydrated form.

more common pharmaceutical excipients: starch, lactose, and microcrystalline cellulose in excipient to drug ratios of 2/1 and 200/1. No significant decrease in potency, as determined by HPLC, was observed for any of the mixtures.

3.3. Purification and processing

Although difficult to assess at an early stage of development, there were some distinct processing differences between the mesylate monohydrate

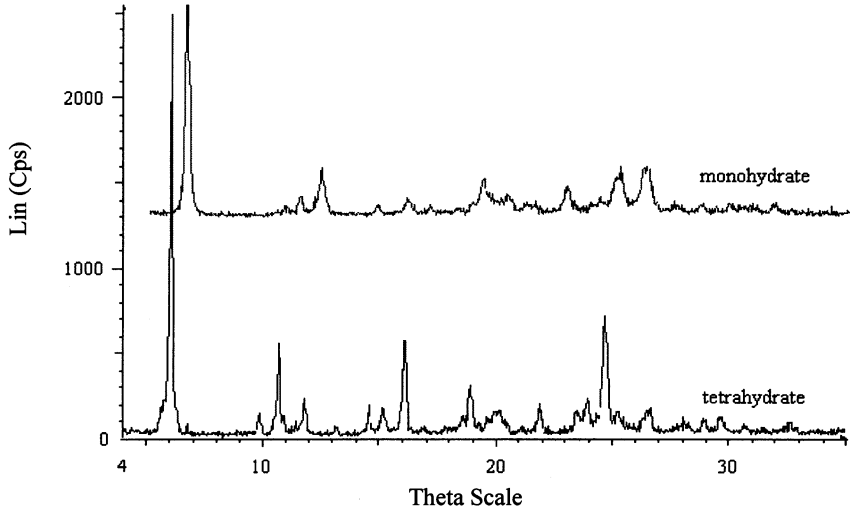


Fig. 2. LY333531 XRPD patterns for the hydrochloride monohydrate and tetrahydrate forms.

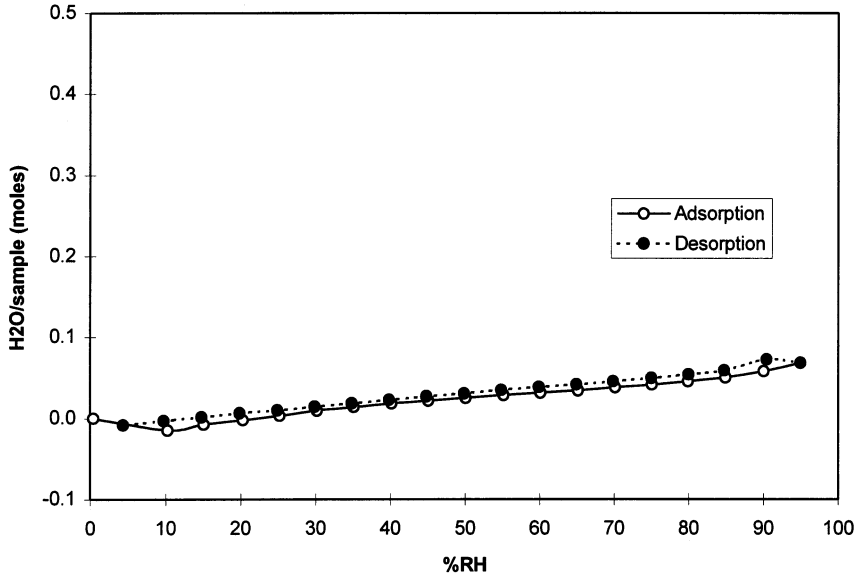


Fig. 3. Adsorption/desorption isotherm for LY333531 hydrochloride anhydrated.

and the hydrochloride salt forms. The mesylate monohydrate crystals were easier to filter and gave a significant purification of the starting free base material. The hydrochloride anhydrate also filtered well but offered no advantage in purification. In contrast, the hydrated form filtered poorly, but afforded a moderate purification. Table 1 summarizes the crystallization data.

3.4. Bioavailability

To eliminate, or minimize, the effect of inter-animal variability on the determination of the relative bioavailability of LY333531 salts, a crossover design was selected. LY338522 (*N*-desmethyl LY333531) was identified in preliminary metabolism studies as a metabolite of

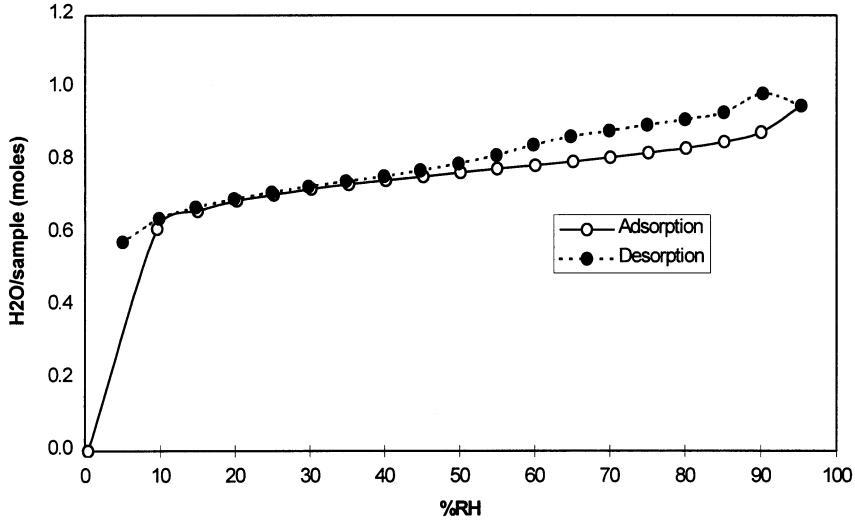


Fig. 4. Adsorption/desorption isotherm for LY333531 mesylate monohydrate.

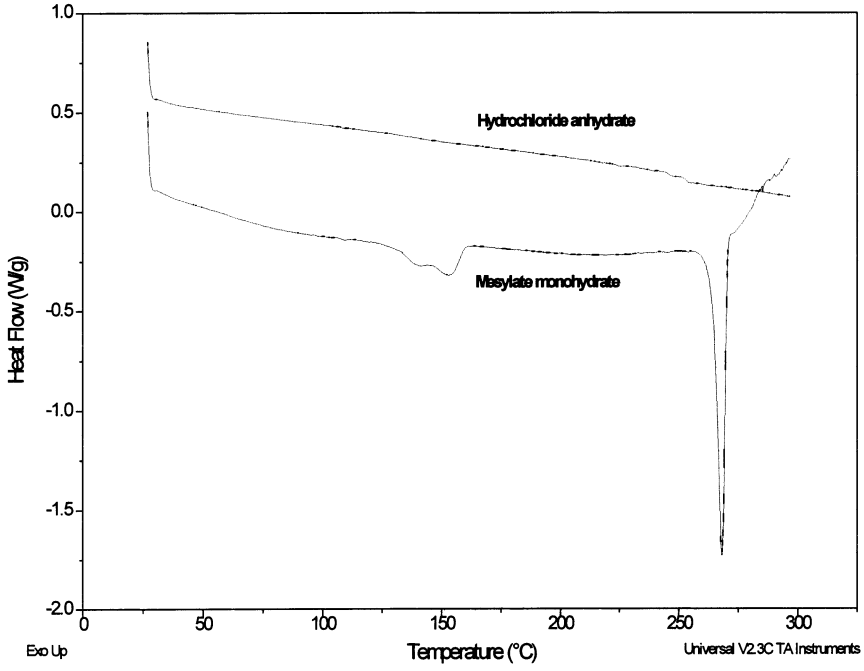


Fig. 5. LY333531 DSCs of the hydrochloride anhydrate and mesylate monohydrate salts.

LY333531, and was found to be equally active (Ishii et al. 1996; Jirousek et al. 1996). The structure of LY338522 is shown below (Scheme 2).

Higher plasma concentrations of both LY333531 and LY338522 were achieved in each dog following an oral dose of the mesylate salt, than from an equivalent dose of the hydrochloride salt (Fig. 7). The mean maximum plasma concentration ($C_{max} \pm$ std error) following the administration of the HCl salt was 400 ± 142 ng/ml for LY333531 and 862 ± 255 ng/ml for LY338522. The corresponding values following administration of the mesylate salt were 896 ± 243 ng/ml (LY333531) and 2455 ± 930 ng/ml (LY338522). This represented an approximately 250% increase in plasma concentration of the compound and the metabolite.

The ratio of the area under the plasma concentration versus time curve (AUC) represents a measure of the relative oral bioavailability of the compound. The AUC values for LY333531 and LY338522 in each dog were approximately 2.6 times higher following administration of LY333531 mesylate, compared to the values obtained from the same LY333531·HCl dose (Table 2).

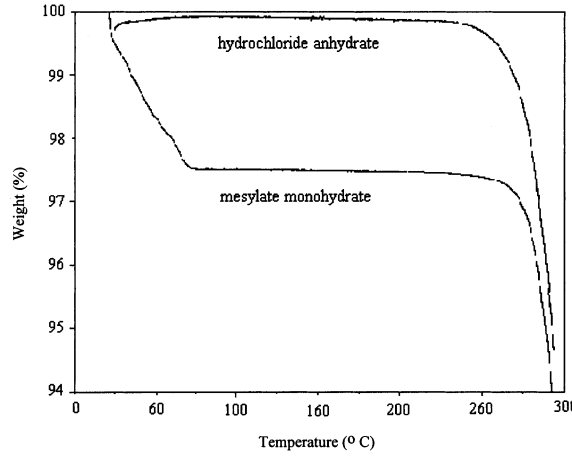
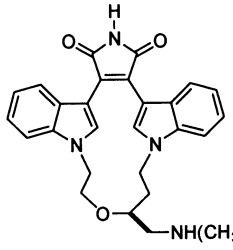


Fig. 6. LY333531 TGAs of the hydrochloride anhydrate and mesylate monohydrate salts.

Table 1
Total related substances in LY333531·mesylate monohydrate and hydrochloride crystals

Salt	Crystal form	TRS (%)[a]
HCl	Anhydrate	4.72
HCl	Mono/tetrahyrate	9.80
Mesylate	Monohydrate	2.00

[a] LY333531 free base with 9.98% TRS was employed as starting material.



Scheme 2. Structure of LY338522, a metabolite of LY333531.

4. Conclusion

A systematic two-tier approach has been applied to the selection of the optimum salt form of LY333531 in an expeditious manner. Initial evaluation of the physical properties of seven salt forms

Table 2
Ratios of AUC from a single oral 20 mg/kg dose of LY333531 administered as the mesylate monohydrate and HCl salts

Dog	LY333531	LY338522
1	3.89	2.77
2	1.62	2.97
3	2.14	2.02
4	2.56	2.74
Mean	2.55	2.62
SEM	0.49	0.21

of LY333531 (hydrochloride, sulfate, mesylate, succinate, tartrate, acetate, and phosphate) led us to select the mesylate and hydrochloride as potential salts for development. Comprehensive analysis of these two salt forms indicated that the mesylate monohydrate salt was more soluble (5X), more stable more bioavailable (2.6X) and easier to process than the hydrochloride salt. LY333531 mesylate monohydrate is currently being developed as the clinical candidate.

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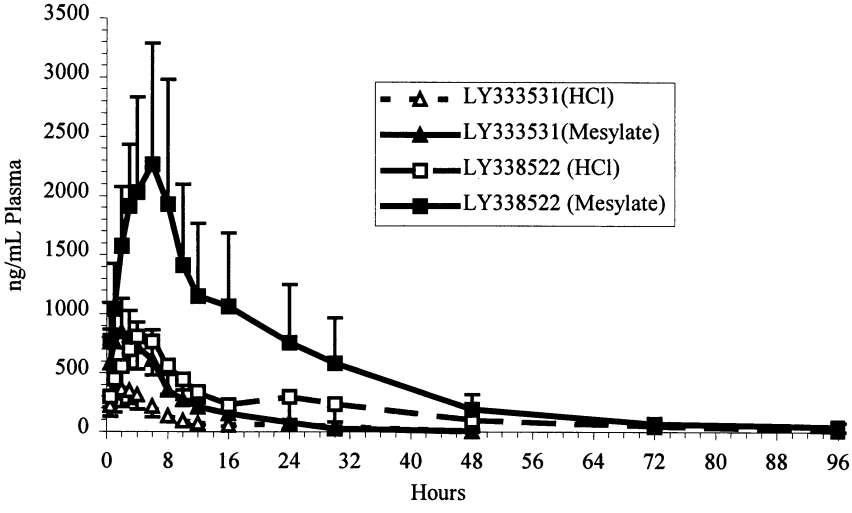

Fig. 7. Mean plasma concentrations of LY333531 and LY338522 in male beagle dogs orally administered LY333531 HCl LY333531 mesylate (20 mg LY333531/kg).

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References

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theroretical basis for a biopharmaceutical drug classifcation: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Berge, S.M., Bighley, L.D., Monkhouse, D.C., 1977. Pharmaceutical salts. J. Pharm. Sci. 66, 1–19.

Bighley, L.D., Berge, S.M., Monkhouse, D.C., 1996. Preservation of pharmaceutical products to salt forms of drugs and absorption. In: Swarbrick, J., Boylan, J.C. (Ed.), Encyclopedia of Pharmaceutical Technology 13. Marcel Dekker, New York, pp. 453–499.

Ishii, H., Jirousek, M.R., Koya, D., Takagi, C., Xia, P., Clermont, A., et al., 1996. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC_{β} inhibitor. Science 272, 728–731.

Jamuludin, A., Mohamad, M., Navartnam, V., Selliah, K., Tan, S.C., Wernsdorfer, W.H., 1988. Relative bioavailability of the hydrochloride, sulfate, and ethyl carbonate salts of quinine. Br. J. Clin. Pharm. 25, 261–263.

Jirousek, M.R., Gillig, J.R., Heath, W.F., Johnston, C.M., McDonald III, J.H., Neel, D.A., Rito, C.J., Singh, U., Stramm, L.E., Melikian-Badalian, A., Baevsky, M., Ballas, L.M., Hall, S. E., Faul, M.M., Winneroski, L.L., 1996. (*S*)-13-[(Monomethylamino)methyl]-10,11,14,15-tetrahydro-4,9:16,21-dimethen-1H,13H-dibenzo(E,K]pyrrolo-[3,4-H][1,4,13]oxadiaza-cyclohexadecine-1,3(2H)-dione (LY333531) and related analogues. Isozyme selective inhibitors of protein kinsae C_{β} (PKC_{β}). J. Med. Chem. 39, 2664–2671.

Lin, S.L., Lachman, L., Schwartz, C.J., Huebner, C.F., 1972. Preformulation investigation I: relation of salt forms and biological activity of an experimental antihypertensive. J. Pharm. Sci. 61, 1418–1422.

Miyazaki, S., Oshiba, M., Nadai, T., 1981. Precaution on use of hydrochloride salts in pharmaceutical formulations. J. Pharm. Sci. 70, 594–595.

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

Thomas, E., Rubino, J., 1996. Solubility, melting point and salting-out relationships in a group of secondary amine hydrochlorides. Int. J. Pharm. 130, 179–183.

Walmsley, L.M., Taylor, T., Wilkinson, P.A., Brodie, R.R., Chasseaud, L.F., Alun-Jones, V., 1986. Plasma concentrations and relative bioavailability of naftidrofuryl from different salt forms. Biopharm. Drug Dispos. 7, 327–334.