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PREFORMULATION SELECTION OF A PROPER SALT FOR A WEAK ACID-BASE (RS-82856) - A NEW POSITIVE INOTROPIC AGENT

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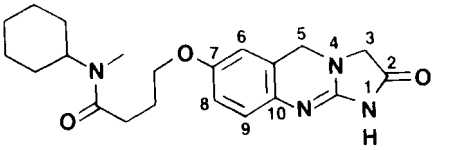
ABSTRACT

The solubility, hygroscopicity and intrinsic dissolution rate of the various salts of a weak acid-base (RS-82856) were evaluated. The phosphate salt was a mixture of monobasic and dibasic salts and was not physically stable. The hydrogen sulfate, and chloride salts were less hygroscopic and more soluble in water than the cation salts tested (sodium and potassium). All salts studied showed only slightly better intrinsic dissolution rates (~two-fold) than the parent drug at pH 3.1 and 7.0; only the hydrogen sulfate salt and the potassium salt had better dissolution rates at pH 1.2. This unusual dissolution behavior of the salts can be explained by the extremely low buffering effect of the salts in the dissolution medium. The hydrogen sulfate salt was recommended for development and was supported by the ~two-fold increase in bioavailability in a dog study when compared to the parent drug.

INTRODUCTION

The advantages of using the salt form of an ionizable drug have been well recognized in the literature.[1] However, the selection of an optimal salt could be a major task depending on the desired area(s) of improvement. Preformulation salt-screening studies for acidic or basic drugs are well known[2,3] and usually focus on the evaluation of the physio-chemical properties of the various salts. However, there are few examples on studies of weak acid-bases. In this paper, the preformulation salt selection of a new positive inotropic agent (RS-82856, 1), N-cyclohexyl-N-methyl-4-(7-oxo-1,2,3,5-tetrahydroimidazo[2,1,-b]quinazoline-2-one)butyramide, to be used in treating congestive heart failure[4,5] is described. The generality of this study to weak acid-base compounds is discussed.



1, RS-82856

EXPERIMENTAL

Materials: RS-82856 and the sodium, potassium, hydrogen sulfate, phosphate and chloride salts were used as received from Institute of Organic Chemistry, Syntex Research. All other chemicals and reagents were USP or HPLC grade and were used without further purification.

Instrumentation: High performance liquid chromatography (HPLC) was performed on an HP-1090 system equipped with an auto-injector, an HP-1040 diode-array detector, and an HP-3990A integrator-recorder. Differential Scanning Calorimetry (DSC) thermograms were recorded on a Perkin-Elmer DSC-2 Calorimeter. Microscopic observations were made using a Leitz Ortholux II

PDL-BK polarized light microscope. The determination of intrinsic dissolution rates was conducted using a Hansen dissolution apparatus (Model 72R115) and a compression die holder as described by Wood.[6]

HPLC Method: An Ultrasphere I.P. (Altex) 5-μ column and a mobile phase of 0.01M phosphate buffer (pH6.5)/CH_3CN/THF(70/25/5) were used. The flow rate was controlled at 1 mL/min. The eluent was monitored at 280 nm with a sensitivity of 0.04 AUFs.

Hygroscopicity Measurements: Samples of the solid drug (10 mg to 15 mg) were accurately weighed into small weighing bottles fitted with ground glass tops. The uncapped samples, were dried overnight under house vacuum (15-20 torr) at 80°C in the presence of phosphorous pentoxide, and the sample weight after drying was recorded. The dried samples were then stored at room temperature in desiccators maintained at constant relative humidities (RH) with various saturated salt solutions.[7] At predetermined time intervals, samples were weighed and the difference in the weight from the dry weight was recorded.

Solubility Measurements: To a 10 mL pyrex culture tube was added 5 mL of the appropriate buffer solution and an excess of the drug. The ionic strength of the buffer solutions was adjusted to 0.15 with KCl. The culture tube was sealed with a teflon-lined screw cap and the suspension was equilibrated in a 25°C water bath. At various time intervals, an aliquot of the solution was removed and filtered through a 0.45 μm Milipore® filter. The pH of the filtrate was measured, and the filtrate was then diluted quantitatively with mobile phase and assayed by HPLC.

Intrinsic Dissolution Rate Measurements: About 100 mg of the solid drug was finely ground and compressed at 2000 psi into a flat disk (surface area = 0.49 cm^2). The compression die was mounted onto the dissolution apparatus with a holder so that the die became the rotating member.[6] At zero time, the die containing the sample was lowered into a vessel containing 500 mL of

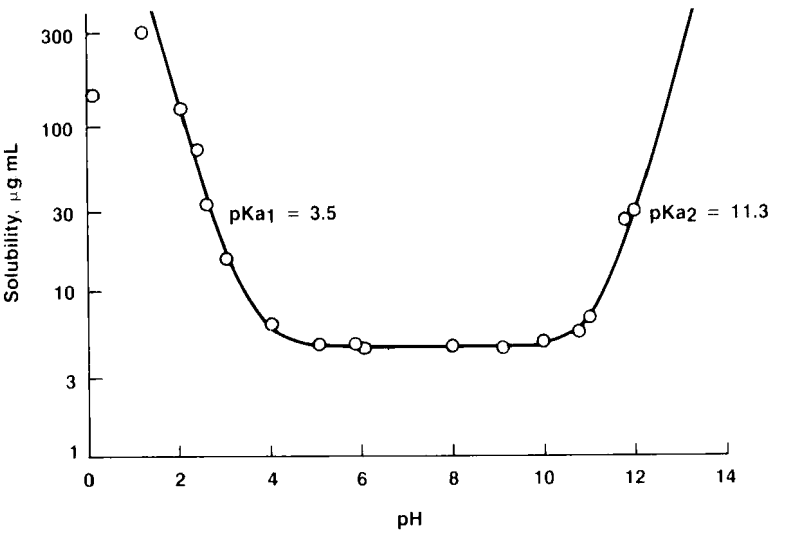


Figure 1. Aqueous solubility of RS-82856 as a function of pH at 25°C. The solid line is the theoretical curve defined by the relationship $S_{obs}=S_i(1+ [H^+]/Ka_1 + Ka_2/[H^+])$, where $S_i=4.6$ μg/mL, $Ka_1=3.2 \times 10^{-4}$ and $Ka_2=5.6 \times 10^{-12}$. The data points were obtained experimentally.

the dissolution medium equilibrated at 37° ± 0.5°C. The surface of the drug was maintained approximately 4 cm below the surface of the solution and the die was rotated at 100 rpm. At various time intervals, 1 mL aliquots of the solution were withdrawn and assayed by HPLC.

Effect of Excess Drug on Dissolution Medium pH: The pH of a medium in the presence of excess drug was determined by adding the medium gradually to 50 mg of material, mixing for 1 min with a vortex mixer after each addition, and recording the pH values.

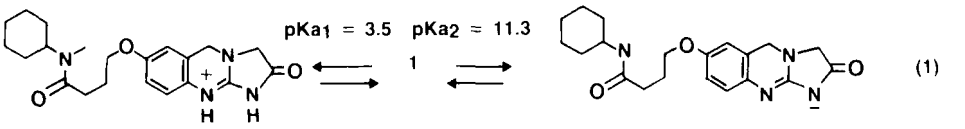
Bioavailability Studies: Three beagles each received, at one-week intervals, single doses of either 15 mg RS-82856 parent drug or 19.5 mg RS-82856 hydrogen sulfate salt in capsules. The dogs were kept in individual cages and fasted from 15 hours before to 5 hours after dosing, but water was allowed Ad libitum. A small volume of water (20 mL) followed each dosing. Blood samples were drawn from the vein at predetermined time intervals. The blood

was centrifuged to obtain plasma and the concentrations of the drug in plasma were determined by HPLC after extraction.

RESULTS AND DISCUSSION

The salt selection process began with the pKa determination of RS-82856. To achieve this, the pH solubility profile of the parent drug was established as shown in Figure 1. No data was obtained at pH's higher than 12 because the degradation of RS-82856 was too rapid[8] to allow an accurate determination of the solubility.

The solubility of the drug at intermediate pHs (pH 4 to 10) was found to be relatively constant at ~5 μg/mL; the increase in solubility in more acidic or basic solutions is due to ionization process shown in equation 1.



(1)

The effect of pH on the solubility obeys equation 2

$$S_{obs} = S_i\,(1+[H^+]/Ka_1 + Ka_2/[H^+]) \qquad (2)$$

where S_{obs} is the observed solubility, S_i is the intrinsic solubility, and Ka_1 and Ka_2 are the acid dissociation constants of RS-82856. The pKa_1 and pKa_2 derived from equation 2 were found to be 3.5 and 11.2 respectively. It is noted in figure 1 that the solubility of the drug at pH $\leq$ 1.12 deviated from the theoretically calculated values suggesting that a common ion effect with chloride ion may be significant at these pHs.

RS-82856 displays both weak acid and weak base characteristics, and so three anion salts in the form of chloride, hydrogen sulfate and phosphate and

Table 1. Solid State Physical Properties of RS-82856 and Its Salts

Compound	Melting Point[a] °C	Stoichiometry[b]	% Weight Gain[c] 47% RH[d]	68% RH	93% RH
Parent drug	245-247	---	1.6	1.9	2.9
Chloride salt	199-201	1:1	9.3	10.4	9.7
Hydrogen sulfate salt	204-205	1:1	4.0	4.6	8.2
Phosphate salt	199-201	1.75:1	1.8	2.1	15.7
Sodium salt	184-185	1:1	4.7	13.8	86.9
Potassium salt	189-192	1:1	1.8	19.7	93.4

[a]Determined by DSC.

[b]Determined by elemental analysis and based on a molar ratio.

[c]Samples were first dried under vacuum; weight gain was recorded after 16 hours in the designated humidities.

[d]RH=Relative humidity.

two cation salts in the form of potassium and sodium were prepared for evaluation. These salts were chosen because they enjoy common use in the pharmaceuticals.[1] Table 1 summarizes the various physical properties of these five salts and the parent drug.

All five salts exhibited sharp but lower melting points than that of the parent drug and appeared to be crystalline materials under microscopic examination. The elemental analysis revealed that a 1:1 drug:salt moiety molar ratio was obtained for the chloride, hydrogen sulfate, sodium and potassium salts. The phosphate salt had a 1.75:1 drug:salt moiety ratio indicating a mixture of mono- and dibasic salts. Attempts to prepare the dibasic sulfate salt also resulted in a mixture with its mono-basic (or hydrogen sulfate) salt. This behavior could be a result of the small difference in dissociation constants between the drug as a base (pKa = 3.5) and phosphoric acid (pKa = 2.12)[9] or bisulfuric acid (pKa = 1.92)[9] thus

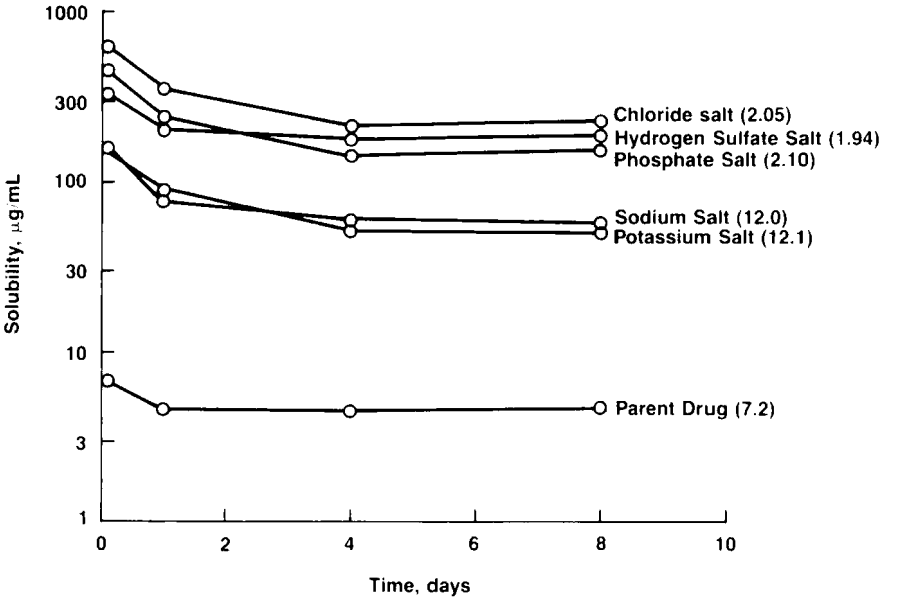


Figure 2. Apparent solubility of RS-82856 and various salts in water as a function of time. The values in the parenthesis indicate the pH of the final solution.

preventing the formation of a physically stable[10] monobasic phosphate salt or dibasic sulfate salt.

Screening the hygroscopicity properties of the parent drug and salt forms at 47%, 68% and 93% relative humidity (RH) conditions showed that the parent drug is relatively non-hygroscopic adsorbing less than 1 mole of water even at 93% RH. Among the salts, the order of increasing hygroscopicity was: hydrogen sulfate salt ~ phosphate salt < chloride salt << sodium ~ potassium. No conversion of the salts to the parent drug was observed after storage for up to 7 days at 93% RH.

The solubility of various drug forms in water was studied as a function time. Figure 2 summarizes the results. Since all of the salts are salts of a weak base or a weak acid, a large excess amount of drug was used in the solubility study to ensure that the true solubility of the salt was obtained.[11] This is supported by the observation that the final pHs of the

Table 2. Intrinsic Dissolution Rate Constants and Diffusion Layer pH of RS-82856 and Its Four Salts at 37°C.

Compound	Bulk pH					
	1.1		3.1		7.0	
	$pH_{h\to0}$[a]	k, mg/min/cm^2	$pH_{h\to0}$[a]	k, mg/min/cm^2	$pH_{h\to0}$[a]	k, mg/min/cm^2
Parent drug	1.12	0.11	3.12	0.0028	7.01	0.0012
Chloride salt	1.10	0.056	2.05	0.0068	6.90	0.0020
Hygroden sulfate salt	1.12	0.25	2.14	0.0052	6.75	0.0027
Sodium salt	1.12	0.082	3.35	0.0044	7.10	0.0026
Potassium salt	1.11	0.14	3.25	0.0055	7.08	0.0036

[a]Diffusion layer pH. See experimental for determination

salt solutions (Figure 2) are in good agreement with that predicted from the pH-solubility profile. In general, the anion salts have better solubilities than the cation salts which, in turn, are much better than the parent drug. Although the initial solubility for each compound appeared to be larger, it decreased as time proceeded and reached an apparent equilibrium value after four days. DSC analysis of the suspended solids showed that the phase transformation was responsible for the decrease in solubility for the free base, sulfate salt and chloride salt. Both the potassium salt and sodium salt resulted in a solution pH of ~12.0. Again, some apparent chemical degradation was observed by HPLC and the reason for the decrease in solubility was not investigated in detail. The phosphate salt was found to partially convert back to the free base, consistent with the unstable nature of this salt. The phosphate salt was therefore eliminated from further consideration.

The intrinsic dissolution rate of the remaining four salts and the parent drug was studied at three different pHs (Table 2). In agreement with the pH-solubility profile, the dissolution rate of each compound increases as the

pH of the medium decreases. At pH 3.1 and 7.0, these salts showed comparable dissolution rates which were about two-fold fuster than that of the parent drug. At pH 1.1 both the sodium salt and the potassium salt showed similar dissolution rate as that of the parent drug while the hydrogen sulfate salt exhibited the highest dissolution rate, but again, only about two-fold that of the parent drug. The chloride salt had the slowest dissolution rate at pH 1.1 due to the common ion effect of chloride ion, in agreement with the suppressed solubility at this pH (Figure 1).

To understand such unusually small dissolution rate differences among the salts and between the salts and the parent drug, the effect of the salts on the diffusion layer pH was determined. The approximation method which measures the pH of various dissolution media in equilibrium with excess drug was used.[12] The results are summarized in Table 2. Except for the small decrease (less than 1 pH unit) observed for the hydrogen sulfate salt and chloride salt at pH 3.1, the diffusion layer pHs of the salts and the parent drug are virtually the same as those of the dissolution medium. Thus, the extremely low self-buffering effects of the various salts of RS-82856 resulted in the $\leq$ two-fold differences in their dissolution rates.[12,13]

Based on the above physical properties, the hydrogen sulfate salt appears to be the best salt among the five salts studied. To further screen the suitability of this salt as a drug raw material, the hydrogen sulfate salt was subjected to 80°C and 81%RH for two weeks and was found to be physically (did not convert to the parent drug) and chemically stable (97% remaining). Under similar conditions, the parent drug was chemically stable (99%). Between the parent drug and the hydrogen sulfate salt, the former has a better hygroscopicity property and the latter provides two-fold increase in the dissolution rate. However, because a potential absorption problem might be expected for a poorly soluble drug like RS-82856 (intrinsic aqueous solubility equals ~5 μg/mL)[14], the hydrogen sulfate salt was recommended for development.

To substantiate the concern of possible absorption problems, the

Table 3. Pharmacokinetic Parameters of RS-82856 Parent Drug and Hydrogen Sulfate Salt in Dogs

Formulation	Dog Identification	AUC, 0-7 hr (ng/mL-hr)	C_{max} (ng/mL)	t_{max} (hr)
Parent drug	A	264	72	2.0
	B	315	113	2.0
	C	135	38	2.0
	Mean	238 ± 54	74 ± 21	2.0 ± 0.0
Hydrogen sulfate salt	A	1,274	318	1.0
	B	335	85	2.0
	C	487	184	2.0
	Mean	698 ± 291	195 ± 68	1.67 ± 0.58

bioavailability of the hydrogen sulfate salt and the parent drug was compared in dogs. The various pharmacokinetic data are summarized in Table 3. Large animal-to-animal variations were observed in the values of area under plasma RS-82856 concentration-time curves (AUC) and concentration maximum (Cmax), for either the parent drug (2-3 fold variation) or the hydrogen sulfate salt (3-4 fold variation). This is an indication of incomplete absorption of the drug.

Because of the large animal-to-animal variation observed and the small number (three) of test animals, no statistical evaluation of the bioavailability data was performed. The mean AUC values for the three dogs given the parent drug and the hydrogen sulfate salt were 238 and 698 ng/mL/hr, respectively, while the mean C_{max} values were 74 and 195 ng/mL respectively. Since the time to reach maximum concentration (t_{max}) was similar for both compounds, it was concluded that the hydrogen sulfate salt was absorbed approximately 2-3 times as efficiently as the parent drug. The bioavailability data therefore parallel the intrinsic dissolution rate and are thus supportive of the selection of the hydrogen sulfate salt of RS-82856 for further development.

CONCLUSION

RS-82856 has a very high pKa(11.2) as an acid and a very low pKa(3.5) as a base. This results in a solubility minimum (5μg/mL) between pH 4-10. The primary goal during the salt selection process was to maximum drug absorption while achieving good physical and chemical stability. Because of the weak acid-base nature of RS-82856, only very strong bases and acids could be used to make physically stable salts. The low buffering ability of the various salt forms of drug resulted in a small variation in the dissolution rates (~two-fold) in a given medium. Nonetheless, it was demonstrated from the bioavailability data that a two-fold difference in dissolution rate can be important, and that it is the dissolution rate rather than the aqueous solubility that correlates well with the absorption data. Since the buffering effect of the cation salts (e.g. sodium and potassium) of a weak acid-base would be expected to increase the pH of the medium and thus decrease the solubility, anion salts of weak acid-base drugs are preferred to be developed.

ACKNOWLEDGEMENTS

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