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Research Papers

Solubility of ionization behavior of the antifungal α-(2,4-difluorophenyl)-α-[(1-(2-(2-pyridyl)phenylethenyl)]-1H-1,2,4-triazole-1-ethanol bismesylate (XD405)

Michael B. Maurin, Rodney D. Vickery, Christine A. Gerard and Munir Hussain

Pharmacy R&D, The Du Pont Merck Pharmaceutical Company, P.O. Box 80336, Wilmington, DE 19880-0336 (USA)

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Summary

α-(2,4-Difluorophenyl)-α-[(1-(2-(2-pyridyl)phenylethenyl)]-1H-1,2,4-triazole-1-ethanol bismesylate (XD405) exhibits exceptional solubility for a triazole antifungal. The solubility of XD405 in water is high (790 mg/ml at pH 1.51). Changes in pH have a dramatic effect on the solubility with values of 3 μg/ml at pH 6. The high solubility is due to self-association. Surface tension studies show a critical micelle concentration of 10.9 mg/ml.

Introduction

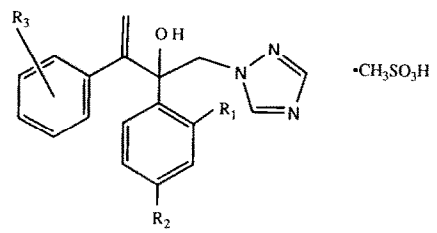
XD405, α-(2,4-difluorophenyl)-α-[(1-(2-(2-pyridyl)phenylethenyl)]-1H-1,2,4-triazole-1-ethanol bismesylate, is a novel triazole antifungal agent (Cuomo et al., 1990). The chemical structure is presented in Fig. 1. It is orally active antifungal agent that has demonstrated efficacy against *Aspergillus* and *Candida* in vitro and in vivo. As part of the physicochemical evaluation of XD405, the solubility and ionization behavior were characterized. The purpose of the studies was to provide a thorough understanding of the solution behavior of XD405 to facilitate formulation development and to gain insight into the reason(s) for the high aqueous solubility. The following data will describe the solution behavior of the compound and will provide a physicochemical rationale for the high aqueous solubility.

Correspondence to: M.B. Maurin, Pharmacy R&D, Du Pont Merck Pharmaceutical Company, P.O. Box 80336, Wilmington, DE 19880-0336, U.S.A.

Materials and Methods

Materials

XD405 was prepared in house. Methanol was HPLC grade (EM Science). The water was house distilled water that was passed through a Nanopure II ion exchange cartridge system (Barnstead) to obtain a conductivity of 18 MΩ cm. Sodium hydroxide (J.T. Baker, Inc.), hydrochloric acid



	R_1	R_2	R_3
DuP 860	H	Cl	2-Cl
DuP 991	F	F	2-Cl
XD405	F	F	4-

•CH_3SO_3H

Fig. 1. Chemical structure of XD405, DuP 860, and DuP 991.

(Anderson Laboratories) and methanesulfonic acid (Pennwalt) were of analytical grade.

Solubility determination

Solubility studies were carried out by placing excess XD405 into a suitable container with deionized water, adding varying amounts of either hydrochloric acid or sodium hydroxide to adjust the pH and rotating end-to-end for 24 h at room temperature (22°C). Two to three replicates were performed in water and in basic solutions, where highly reproducible pH control was permitted. In the mid pH range single replicates were performed. Preliminary experiments indicated that 24 h provided sufficient time to reach equilibrium. The suspension was passed through a 0.45 μm filter (Acrodisc® LC13 PVDF, Gelman Sciences) with the first portion discarded to ensure saturation of the filter. An aliquot of the filtrate was diluted and analyzed by HPLC and the remainder of the filtrate was employed for pH determination.

Surface tensiometry

Surface tension measurements were completed in triplicate with the DuNüoy ring method (Model 70545 Cenco®-DuNüoy® Interfacial Tensiometer, Cenco Instruments) at room temperature (22°C) with XD405 concentrations of 300–0.02 mg/ml. The stock solution of XD405 was prepared with deionized water at 300 mg/ml at pH 0.85. Serial dilutions were completed with 0.17 M methanesulfonic acid (pH 0.85).

Chromatographic method

The concentration of XD405 was determined with an isocratic HPLC method. Separation was performed on a 15 cm reversed-phase Nova-Pak C_{18} column (Waters Chromatography). The mobile phase was composed of methanol:water 65:35. A flow rate of 1.0 ml/min was employed (Model 510 Pump and Automated Gradient Controller, Waters Chromatography). Ultraviolet detection was utilized at 300 nm with AUFS 0.1 (Spectroflow Model 757, Applied Biosystems). Chromatograms were recorded on a Hewlett Packard 3390A integrator with programmed calculation of the peak area. Sample concentrations were determined from a standard curve based on the XD405 peak area. The standards were freshly prepared before each analysis.

Results

Solubility determinations

The solubility of XD405 in water (pH 1.51) was determined to be 790 mg/ml. The pH-solubility profile is provided in Fig. 2. The pH-solubil-

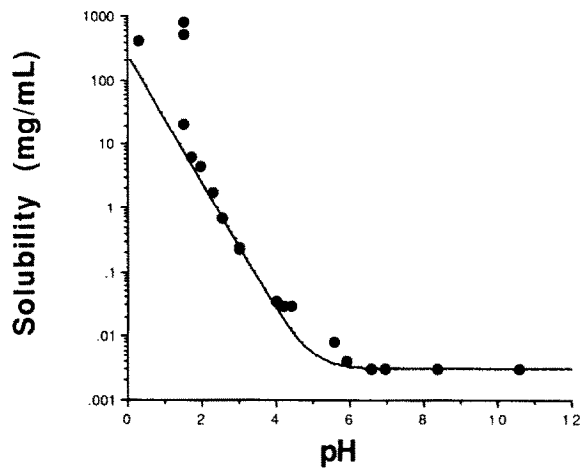


Fig. 2. Solubility of XD405 as a function of pH. Experimentally determined values (●) and theoretical line (———) derived with Eqn 4, with $pK_{a_1} = 1.68$ and $pK_{a_2} = 4.93$ and an intrinsic solubility of 3 μg/ml.

ity profile had two ionizable species, the triazole and pyridyl moieties.

The ionization behavior can be described with the following equilibria;

$$AH_2^{2+} \overset{K_{a_1}}{\rightleftharpoons} \underset{H^+}{AH^+} \overset{K_{a_2}}{\rightleftharpoons} A + H^+$$

where AH^{2+} is the diprotonated species, AH^+ denotes the monoprotonated species, A is the free base, and K_{a_1} and K_{a_2} represent the first and second ionization constants, respectively. The ionization constants are defined as;

$$K_{a_1} = \frac{[AH^+][H^+]}{[AH^{2+}]} \quad (1)$$

$$K_{a_2} = \frac{[A][H^+]}{[AH^+]} \quad (2)$$

The total solubility (S_T) is defined by:

$$S_T = AH^{2+}AH^+ + A \quad (3)$$

Rearranging Eqns 1 and 2 and substituting into Eqn 3 yields;

$$S_T = \frac{[A][H^+]^2}{(K_{a_1})(K_{a_2})} + \frac{[A][H^+]}{K_{a_2}} + [A] \quad (4)$$

Rearranging Eqn 4 produces;

$$(S_T/[A]) - 1 = \frac{[H^+]^2}{(K_{a_1})(K_{a_2})} + \frac{[H^+]}{K_{a_2}} \quad (5)$$

The data were analyzed by regression analysis with a zero intercept second order polynomial (JMP®, SAS Institute Inc.) that was based on Eqn 5 and employed an intrinsic solubility of 3 μg/ml. The resulting polynomial had a correlation coefficient of 0.997 and provided ionization constants ± standard error of $pK_{a_1} = 1.68 \pm 0.14$ and $pK_{a_2} = 4.93 \pm 0.096$.

Surface tensiometry

The surface tension was measured from 300 to 0.02 mg/ml. The data were analyzed by plotting the surface tension as a function of log XD405 concentration (Fig. 3) (Attwood and Florence, 1983). The surface tension decreased to a constant value of 57 dyne/cm at 25 mg/ml. The plot revealed a critical micelle concentration of 10.5 mg/ml with a 95% confidence interval (JMP®, SAS Institute Inc.) of 8.83–12.5 mg/ml.

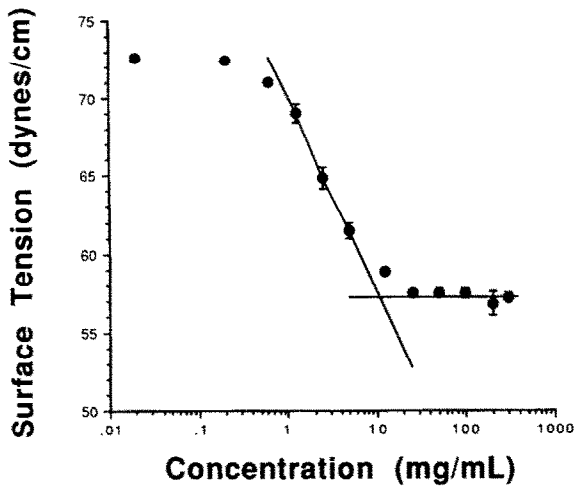


Fig. 3. Surface tension of XD405 as a function of concentration. The data points and error bars represent the mean ± S.D. of three replicates.

Discussion

XD405 contains a triazole and pyridyl function. The solubility studies indicate two distinct ionization constants of 1.68 and 4.93. The ionization constant of 1.68 can be assigned to the triazole. The value is consistent with literature pK_a values of 2.27 for unsubstituted triazole (Albert and Sarjent, 1984) as well as for the pK_a determined for DuP 860 and DuP 991 (Fig. 1), two structurally similar triazoles, of 1.79 and 2.15, respectively (Maurin et al., 1993). Pyridine is reported to have a pK_a of 5.23 (Albert and Sarjent, 1984). A phenyl substituent in the *ortho* position is expected to result in a pK_a decrease. The pK_a of 4.93 is consistent for what would be expected for the *ortho* phenyl pyridyl function of XD405.

The maximum solubility for two closely related analogs DuP 860 and DuP 991 has been reported as 40 and 90 μg/ml, respectively. However, DuP

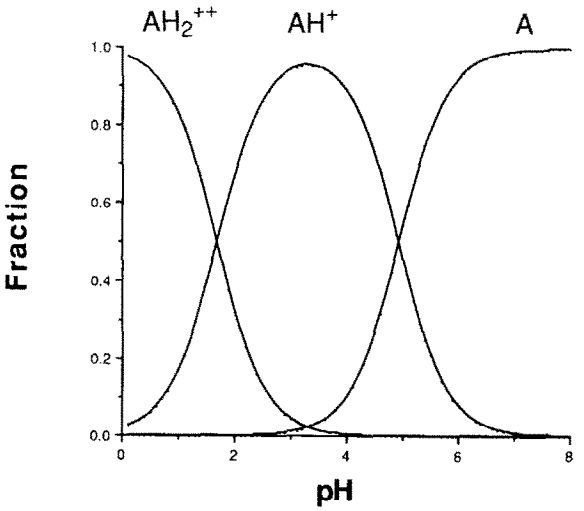


Fig. 4. Distribution diagram for XD405 where AH^{2+} represents the species with the triazole and pyridyl ionized, AH^+ denotes the species with the triazole unionized and the pyridyl ionized and A represents the species with both of the triazole and pyridyl unionized.

860 and DuP 991 lack a second ionizable site, such as the pyridyl. It is striking that the second ionization confers an increase in solubility of approx. 4 orders in magnitude and a positive deviation from the curve generated with Eqn 5 at pH values below 2. Positive deviations from pH-solubility profiles have been determined previously for prostaglandin $F_{2\alpha}$ tromethamine (Roseman and Yalkowsky, 1973), clindamycin 2-palmitate hydrochloride (Rowe, 1979), and brequinar sodium (King et al., 1989) and were attributed to micelle formation in the cases of prostaglandin $F_{2\alpha}$ tromethamine and clindamycin 2-palmitate hydrochloride and molecular aggregation via a stacking self-association in the case of brequinar sodium. The concentrated solutions of XD405 do foam, suggesting a self-association phenomenon. Further evaluation with surface tensiometry revealed a distinct break in a plot of surface tension vs log concentration consistent with the micellar hypothesis (Mukerjee, 1974). The surface tension decreased from 72.5 to 57.2 dyne/cm as the concentration of XD405 increased from 0.02 to 25 mg/ml, with concentrations above 25 mg/ml providing no further reduction in surface tension. The self-association resulted in the dramatic solubility increase in acidic pH. The distribution diagram for the three species is presented in Fig. 4.

The results indicated that the solubility of XD405 is highly pH dependent and required acidic environments for maximum solubility with the micelle formation playing a critical role in the enhanced aqueous solubility in acid.

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