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Electronic and Resonance Effects on the Ionization of Structural Analogues of Efavirenz

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ABSTRACT The solubility of 4 analogues of efavirenz was studied as a function of pH. The study evaluated the ionization behavior and determined the relative contribution of electronegative substituents versus resonance effects on the pK_a value of the cyclic carbamate. The most profound lowering effect on the pK_a was due to the presence of multiple electronegative substituents and in particular the trifluoromethyl and acetylene groups. The presence of chlorine on the benzoxazinone ring was found to have a slight impact on the pK_a , although to a lesser extent. In the absence of any functional groups on the benzoxazinone ring system, the pK_a shifted to a value of 13.2, which is 3 pH units above that of efavirenz and more closely correlates with typical literature values for cyclic carbamates.

KEYWORDS: pKa, Ionization, Benzoxazinone, Carbamate, Solubility, Efavirenz Analogs

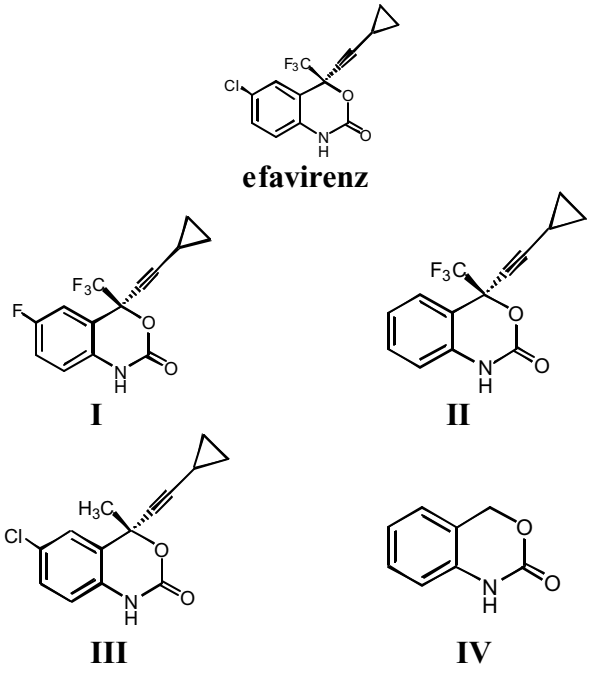

Figure 1. Chemical structure of efavirenz and 4 efavirenz analogues.

INTRODUCTION

Efavirenz (**Figure 1**) is a non-nucleoside reverse transcriptase inhibitor that has been approved by the Food and Drug Administration for the treatment of human immunodeficiency virus. The pK_a of efavirenz was determined previously by spectrophotometric and pH-solubility studies and found to be 10.2, which is anomalous with respect to typical ionization behavior of cyclic carbamates[1] . Although the basis for the lower-than-anticipated pK_a value was not understood completely, it was postulated that electronic withdrawing effects of the electronegative substituents and/or the potential for delocalization of the charge on the benzoxazinone ring system could be responsible for the lowering of the pK_a .

The objective of the current work was to address the contributions of electronegative and resonance effect through the study of 4 efavirenz analogs (**Figure 1**) to more definitively assess the relative influence of these factors on the ionization behavior of the carbamate of the benzoxazinone.

MATERIALS AND METHODS

Materials

All 4 structural analogues of efavirenz were synthesized by DuPont Pharmaceuticals Chemical and Physical Sciences Department (Wilmington, DE). Water was purified by a MilliQ-Plus™ ultra-pure water system (Millipore, Bedford, MA) with a specific resistance of >18 MO. All solvents were of high-performance liquid chromatography (HPLC) grade (EM Science, Gibbstown, NJ). Standard solutions of HCl and NaOH were purchased from VWR Scientific (West Chester, PA).

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Chromatographic Method

Chromatographic analysis of efavirenz analogues was done using an isocratic reversed-phase HPLC method and UV detection at 250 nm. Separations were performed on a Zorbax^{TM} R_X -C_8 column (4.6 x 250 mm) (Mac-Mod Analytical, Chadds Ford, PA) maintained at 35°C. The mobile phase consisted of 60% acetonitrile and 0.1% trifluoroacetic acid in water (vol/vol) at a flow rate of 0.6 mL/min. A vax-based data system, Multichrom2 (Fisons Instruments, Danvers, MA), was used to acquire data. Quantitation was based on the area response curve from standards prepared just prior to analysis.

Solubility Determinations

The solubility as a function of pH was determined for each compound by placing excess solid in a suitable container and adding deionized water that had previously been adjusted to the desired pH value by the addition of HCl or NaOH. The samples were rotated end to end for a minimum of 1 hour; previous experiments had demonstrated this was adequate time for equilibration. The use of short equilibration times minimized the hydrolysis of the carbamate at the extreme pH conditions. The equilibrated suspensions were passed through 0.45-µm Whatman autovial (Teflon) syringeless filter devices (VWR Scientific). The first portion of the filtrate was discarded to ensure saturation of the filter, and the remaining filtrate was used for pH determination and diluted as necessary for HPLC analysis. The intrinsic solubility was determined by taking the average solubility over the pH range where only the un-ionized species was present.

RESULTS

The solubility profiles for the 4 efavirenz analogues are shown in **Figures 2 to 5**. All profiles are consistent with that of efavirenz, in which the carbamate undergoes deprotonation at high pH values to form a negatively charged species with increased solubility due to the ionization of the weak acid. Employing the same ionic equilibria previously used to determine the pK_a of efavirenz[1], the data may be plotted according to equation 1:

$$\left(\frac{S_T}{[AH]}\right) - 1 = \frac{K_a}{[H^+]} \qquad (1)$$

where S_T represents the total solubility measured at any given pH, $[AH]$ represents the solubility of the neutral species (intrinsic solubility), K_a is the ionization constant and is equal to the slope of the line, and $[H^+]$ is the hydrogen ion concentration at each pH value. The data for each analogue were plotted according to equation 1. **Table 1** summarizes the results of the linear regression analysis that was performed on all 4 analogues. Theoretical solubility curves based on the intrinsic solubility values and experimentally determined pK_a of each compound are shown in **Figures 2 to 5** and were in excellent agreement with experimental results.

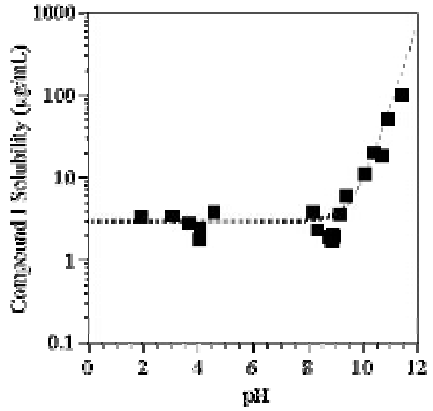

Figure 2. pH-Solubility profile of Compound I. The data points (squares) represent experimentally determined values and the theoretical line (dotted line) represents the theoretical solubility profile generated using an ionization constant of 2.43 x 10^{-10} and an intrinsic solubility of 2.8 µg/mL.

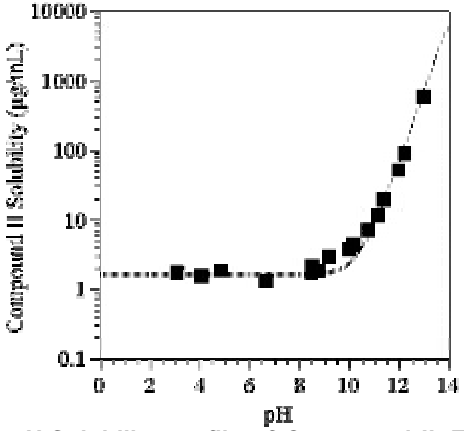

Figure 3.pH-Solubility profile of Compound II. The data points (squares) represent experimentally determined values, and the theoretical line (dotted line) represents the theoretical solubility profile generated using an ionization constant of 4.34 x 10^{-11} and an intrinsic solubility of 1.6 µg/mL.

Table 1. Summary of the Linear Regression and Ionization Constants from Equation 1

Compound	*Intrinsic Solubility (μg/mL)*	*Equation of the Line (R^2)*	*pK_a*
I	2.8	$y = 2.43 \times 10^{-10} x - 1.25 \times 10^{-1}$ ($R^2 = 1.000$)	9.6
II	1.6	$y = 4.34 \times 10^{-11} x + 8.05 \times 10^{-1}$ ($R^2 = 0.990$)	10.4
III	1.3	$y = 3.16 \times 10^{-12} x + 6.48 \times 10^{-3}$ ($R^2 = 0.999$)	11.5
IV	4400	$y = 6.71 \times 10^{-14} x + 1.07 \times 10^{-1}$ ($R^2 = 0.996$)	13.2

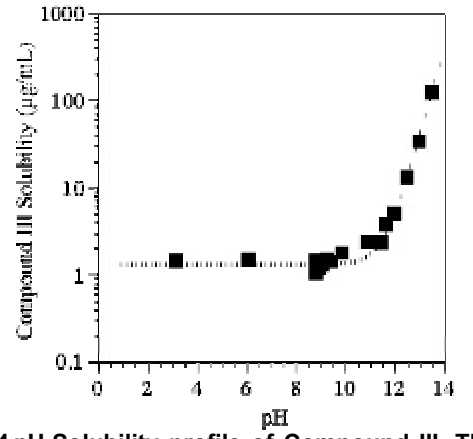

Figure 4.pH-Solubility profile of Compound III. The data points (squares) represent experimentally determined values, and the theoretical line (dotted line) represents the theoretical solubility profile generated using an ionization constant of 3.16 x 10^{-12} and an intrinsic solubility of 1.3 μg/mL.

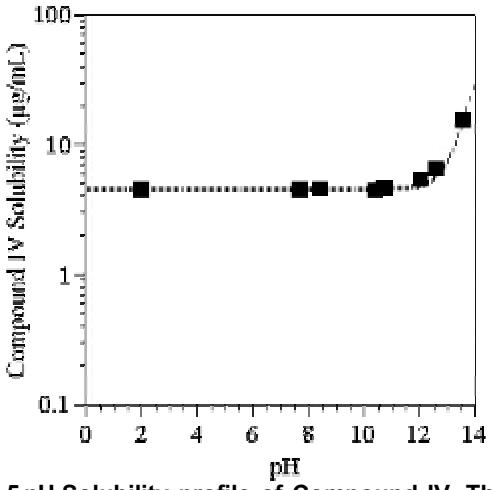

Figure 5.pH-Solubility profile of Compound IV. The data points (squares) represent experimentally determined values, and the theoretical line (dotted line) represents the theoretical solubility profile generated using an ionization constant of 6.71 x 10^{-14} and an intrinsic solubility of 4400 μg/mL.

Table 2. Electronegativities Associated with Atoms and Substituents on Efavirenz and Analogues of Efavirenz*

Atom or Substituent	*Electronegativity*
fluorine	3.95
chlorine	3.03
methyl	2.30
trifluoromethyl	3.35
acetylene	3.30

*Data in table are from reference (4).

DISCUSSION

Four analogues of efavirenz were studied to determine the relative contributions of electronegative substituents and that of delocalization effects through resonance on the ionization behavior of the carbamate functional group of the benzoxazinone. The electronegative atoms and functional groups present on efavirenz and the selected analogues are listed in **Table 2** along with their assigned electronegativity values. The impact of the electronegative chlorine atom positioned directly on the benzoxazinone ring was evaluated by substituting a fluorine atom (Compound I), which resulted in a decrease in the pK_a of 0.6 pH units. This result is consistent with an increase in the electronegativity of the fluorine atom compared to that of the chlorine and therefore increased stabilization of the negatively charged species. Conversely, the pK_a of the des-chloro analogue (Compound II) increased by only 0.2 pH units, suggesting that the chlorine alone has a minimal influence on the pK_a .

The trifluoromethyl substituent has an electronegativity value intermediate to that of chlorine and fluorine. Replacement of the trifluoromethyl with a methyl group (Compound III) resulted in an increase in the pK_a by 1.3 pH

units. The significant influence of this group may be due to its close proximity to the ionizable site as well as the larger difference in electronegativity values between the trifluoromethyl and methyl groups.

CONCLUSION

The influence of delocalization through resonance effects was evaluated in the absence of inductive effects by studying Compound IV, in which all substituents on the benzoxazinone ring were absent. The pK_a of Compound IV increased by 3 pH units over that of efavirenz, suggesting that the electron-withdrawing substituents as a whole contribute to the lowering of the pK_a to a much greater extent than resonance effects. The correlation between the ionization behavior and electronegativity is illustrated in **Figure 6**. The total electronegative contributions from all substituents on each compound on the benzoxazinone ring are plotted against the pK_a . In particular, the absence of the trifluoromethyl and acetylene groups with electronegativity values of 3.0 and 3.3, respectively, appear to have a profound impact on the ionization behavior of efavirenz. Delocalization of charge over a relatively small benzoxazinone ring system does not play a large role in the unusually low pK_a value of this particular series. Rather, the absence of the electronegative substituents results in a pK_a that bears closer resemblance to values reported in the literature (>13) for cyclic carbamates[2,3]. Efavirenz represents a unique case where the presence of multiple electronegative substituents near to the ionizable carbamate of the benzoxazinone significantly alters the ionization behavior of this compound.

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