

Published in final edited form as:

J Org Chem. 2012 May 4; 77(9): 4450–4453. doi:10.1021/jo300386u.

Proton-in-Flight Mechanism for the Spontaneous Hydrolysis of *N*-Methyl *O*-Phenyl Sulfamate: Implications for the Design of Steroid Sulfatase Inhibitors

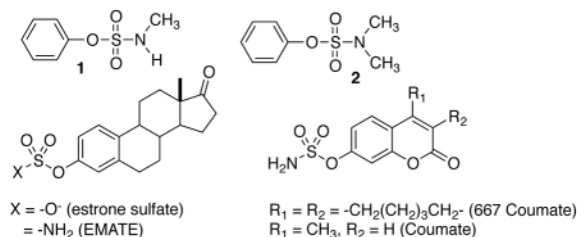
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Abstract

The hydrolysis of *N*-methyl *O*-phenyl sulfamate (**1**) has been studied as a model for steroid sulfatase inhibitors such as Coumate, 667 Coumate and EMATE. At neutral pH, simulating physiological conditions, hydrolysis of **1** involves an intramolecular proton transfer from nitrogen to the bridging oxygen atom of the leaving group. Remarkably, this proton transfer is estimated to accelerate the decomposition of **1** by a factor of 10^{11} . Examination of existing kinetic data reveals that the sulfatase *PaAstA* catalyzes the hydrolysis of sulfamate esters with moderate efficiencies of $\sim 10^4$; whereas, the catalytic rate acceleration generated by the enzyme for its cognate substrate is on the order of $\sim 10^{15}$. Rate constants for hydrolysis of a wide range of sulfuryl esters, ArOSO_2X^- , are shown to be correlated by a two parameter equation based on $\text{p}K_a^{\text{ArOH}}$ and $\text{p}K_a^{\text{ArOSO}_2\text{XH}}$.

Sulfamate esters such as Coumate, 667 Coumate and EMATE have been investigated as time dependent and irreversible inhibitors of human steroid sulfatase for the treatment of hormone dependent breast cancer and other ailments.¹ Sulfatase inhibition appears to involve decomposition of the parent sulfamate ester to generate a reactive electrophile $\{\text{HN}=\text{S}(=\text{O})_2\}$ that undergoes nucleophilic capture by groups positioned in and around the enzyme's active site.² Here, we describe a mechanistic study on the hydrolysis of **1**, which serves as a simulant for the sulfamate esters being developed as enzyme inhibitors. The *N*-methyl substituent of **1** precludes formation of the dianion $\text{PhOSO}_2\text{N}^{2-}$ simplifying the interpretation of kinetic effects at high pH.³ Based on the accumulated kinetic data, we propose a novel mechanism for the spontaneous hydrolysis of sulfamate esters under physiological conditions. We also present a physical organic description of sulfuryl transfer that should prove useful in predicting the hydrolytic stability of the next generation of sulfatase inhibitors.



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Supporting Information

A plot of absorbance versus pH for the UV-visible titration of **1**. Eyring plots for the hydrolysis of **1** and **2**. A plot of observed rate constant versus percent deuterium content used to calculate the solvent kinetic isotope effect on **1** (Figure S4). A table of kinetic constants for the hydrolysis of ArOSO_2X^- (Table S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

Pseudo-first order rate constants for the hydrolysis of **1** were determined by UV-visible spectrophotometry monitoring phenol or phenoxide production at 60 °C from 4.8 < pH < 13.6 ($I = 550$ mM, NaCl). A non-linear least squares fit of the rate data to eq. 1 indicates rate constants of $k^{1-} = (1.09 \pm 0.07) \times 10^{-5}$ and $k^1 = (2.0 \pm 0.1) \times 10^{-5} \text{ s}^{-1}$ corresponding to reaction along the high and low pH plateaus, respectively (Figure 1). The kinetic pK_a of 9.1 ± 0.3 is somewhat lower than that determined by spectrophotometric titration at 25 °C where $pK_a = 9.7 \pm 0.1$ (Figure S1, Supp. Info). The activation parameters for the hydrolysis of **1** at pH 5.9 were measured from $25 < T < 80$ °C and determined to be $\Delta H^\ddagger = 18.7 \pm 0.5$ kcal/mol and $\Delta S^\ddagger = -24 \pm 1$ cal/mol·K (Table 1).

$$\log(k_{\text{obs}}) = \log \left[k^1 \times \frac{10^{-\text{pH}}}{10^{-\text{pH}} + 10^{-\text{p}K_a}} + k^{1-} \times \frac{10^{-\text{p}K_a}}{10^{-\text{pH}} + 10^{-\text{p}K_a}} \right] \quad (\text{eq. 1})$$

The appearance of the pH rate profile (Figure 1) is consistent with an equilibrium mixture of **1** and **1**⁻ undergoing product formation by the two pathways k^1 and k^{1-} shown in Scheme 1. Williams previously studied a series of *N*-methyl *O*-aryl sulfamates hydrolysing at elevated pH via k^{1-} and found these to undergo spontaneous unimolecular decomposition to ⁻OAr and MeN=SO₂.⁴ Subsequent fast nucleophilic capture of MeN=SO₂ by solvent (or amine buffer) completes the transformation. The focus of the current study is on the k^1 process, which for **1** predominates below pH ~9 and provides an appropriate model for hydrolysis under physiological conditions of the *O*-aryl sulfamates being investigated as steroid sulfatase inhibitors.

At 60 °C the ratio of k^1/k^{1-} at ~2 is small and yet, there is a strong indication that the *N-H* group has a profound effect on the reactivity of **1**. Consider for example the hydrolysis of *N,N*-dimethyl *O*-phenyl sulfamate (**2**) which does not possess *N-H* groups. Spontaneous hydrolysis of **2** is too slow to monitor at 60 °C ($t^{1/2} = 4 \times 10^6$ years, *vide infra*) and kinetic experiments were carried out at pH 5.9 from $240 < T < 279$ °C. Observed first order rate constants for the hydrolysis of **2** were determined by ¹H NMR comparing the normalized signal intensities for unreacted starting material and phenol.⁵ Fitting the rate data for hydrolysis of **2** to the Eyring equation yielded the activation parameters $\Delta H^\ddagger = 38 \pm 3$ kcal/mol and $\Delta S^\ddagger = -14 \pm 6$ cal/mol·K (Table 1). The thermodynamic data allow us to estimate a rate constant of $k^2 = 7 \times 10^{-19} \text{ s}^{-1}$ for the spontaneous hydrolysis of **2** at 25 °C (Table 1). Remarkably, comparing this value to k^1 indicates that substitution of one of the *N*-methyl substituents in **2** with a hydrogen atom results in a greater than 10¹¹-fold rate acceleration at 25 °C. A rate constant of $7 \times 10^{-7} \text{ s}^{-1}$ for the hydrolysis of PhOSO₂NH₂ in 50% aqueous acetonitrile at 25 °C can be calculated from the reported activation parameters.⁶ This value is very similar to that for **1** indicating that a single *N-H* group is sufficient to achieve the full catalytic effect.

A solvent deuterium kinetic isotope effect of $k^H/k^D = 1.8 \pm 0.1$ was determined on k^1 for hydrolysis at 60 °C (Figure S4, Supp. Info.). Solvent isotope effects of 2.6 and 3.9 have previously been reported for the hydrolysis of *O*-4-nitrophenyl sulfamate {4-NO₂C₆H₄SO₂NL₂} and EMATE for reaction along the low pH plateau at 60 °C.^{7,8} These solvent isotope effects were used to support the proposal of an associative and bimolecular S_N2 pathway. Notably, any contribution of the *N-L* hydron to the observed kinetic isotope effects was specifically ruled out as it was deemed not to be involved in the slow step of sulfamate ester hydrolysis.⁸ However, the greater than 10¹¹-fold difference in reactivity between **1** and **2** seems to contradict this conclusion and suggests that the sulfamate *N-H* group is actually engaged in bonding interactions at the transition state that substantially reduce the free energy for hydrolysis ($\Delta\Delta G^\ddagger = 15.6$ kcal/mol).

We propose an alternative mechanism for the hydrolysis of **1** where the N-*H* proton is transferred, either directly or through a network of intervening water molecules⁹, to the phenoxy leaving-group as in transition structures **3** and **4** (Figure 2). This mechanism accounts for the small β^{LG} of -0.41 reported for the spontaneous hydrolysis of *O*-aryl sulfamates {ArOSO₂NH₂}.⁶ The magnitude of β^{LG} for these reactions indicates a modest degree of negative charge is accumulating on the aryloxy leaving-group as the sulfamate progresses from ground state to transition state.¹⁰ The transfer of the N-*H* proton to the bridging oxygen atom of the leaving group partially neutralizes the accumulating negative charge resulting from S-O bond fission and provides for the appearance of a shallow Brønsted coefficient or β^{LG} . Similar conclusions have been drawn regarding the hydrolysis of aryl phosphate monoester monoanions ($\beta^{\text{LG}} = -0.27$) and the acid catalysed hydrolysis of sulfate monoesters ($\beta^{\text{LG}} = -0.33$).^{11,12}

Impressive second order rate constants of $k_i/K_i \approx 10^6 \text{ M}^{-1}\text{s}^{-1}$ for irreversible inhibition of an aryl sulfatase from *P. aeruginosa* (*PaAstA*) have been reported for the inhibitors Coumate, 667 Coumate and EMATE.² The strong binding affinities of these compounds ($K_i < 1 \mu\text{M}$) might be expected to translate into high target selectivity; however, this does not appear to be the case. Active site titrations reveal that up to six equivalents of inhibitor are required for complete inactivation of *PaAstA*.² This suggests that off-target sulfamoylations are occurring with solvent and/or nucleophilic groups on the enzyme's surface acting as acceptors for the electrophile HN=S(O)₂. The high reactivity of the sulfamate esters likely contributes to their poor target selectivity; for example, Coumate has a half-time in dilute aqueous solution of only ~2 hours at pH 7 and 37 °C.

Shown in Figure 3 (■, dashed line) is a plot of $\log(k_i)$ versus $\text{p}K_a^{\text{LG}}$; where k_i is the first order rate constant for irreversible inhibition of *PaAstA* at pH 7, 37 °C under saturating concentrations of ArOSO₂NH₂.² Also included in Figure 3 is a plot of $\log(k_{\text{hydrolysis}})$ versus $\text{p}K_a^{\text{LG}}$ (●, solid line) for the uncatalyzed hydrolysis of ArOSO₂NH₂ at 37 °C.¹³ The vertical separation between the best-fit lines indicates that *PaAstA* provides a ~10⁴-fold rate acceleration for the cleavage of the S-OAr bond of simple sulfamate esters. This rate acceleration is small when compared with the level of catalysis achieved with sulfate monoesters, which are the enzyme's natural substrates. For example, a catalytic rate acceleration of $k_{\text{cat}}/k_{\text{uncat}} = 16.7/(2 \times 10^{-14}) = 8 \times 10^{14}$ can be calculated for the hydrolysis of 4-methoxyphenyl sulfate.^{2,12b} Steroid sulfatase inhibitors showing higher ratios of $k_i/k_{\text{hydrolysis}}$ are likely to result in fewer off-target sulfamoylations but it remains to be seen if this strategy will also result in a reduction in potency.

It would be desirable to predict the hydrolytic stability of sulfuryl esters before committing valuable resources towards synthesis of steroid sulfatase inhibitors. A plot of $\log(k)$ versus $\text{p}K_a^{\text{ROSO}_2\text{XH}}$ for the hydrolysis of a series of *anionic* sulfuryl esters with a common 4-nitrophenoxide leaving group at 25 °C adheres to the equation $\log(k) = (-0.70 \pm 0.05)\text{p}K_a^{\text{ROSO}_2\text{XH}} - (8.0 \pm 0.5)$ (Figure 4).¹⁴ The thirteen sulfuryl esters used to construct the plot are 4-NO₂C₆H₄OSO₃⁻, 4-NO₂C₆H₄OSO₂N⁻CH₂Ar, 4-NO₂C₆H₄OSO₂N⁻Me, 4-NO₂C₆H₄OSO₂C⁻(H)SO₂CH₃, 4-NO₂C₆H₄OSO₂C⁻(CH₃)SO₂CH₃, 4-NO₂C₆H₄OSO₂C⁻C₆H₅ (details are provided in the Supporting Information).^{3,4,6,12b,15,16} The inset in Figure 4 shows a series of parallel lines corresponding to the hydrolysis of sulfuryl esters ArOSO₂X⁻ with different aryloxy leaving groups (where ArO = PhO, 4-Cl-PhO, 3-NO₂-PhO and 4-NO₂-PhO). The best-fit lines indicate an average gradient of 0.71 ± 0.01 that is invariant with respect to leaving group and y-intercepts that increase with decreasing $\text{p}K_a^{\text{ArOH}}$.

Equation 2 can be derived from these data to estimate rate constants for the decomposition of *anionic* sulfuryl esters based on $\text{p}K_a^{\text{ArOH}}$ and $\text{p}K_a^{\text{ArOSO}_2\text{XH}}$. The sulfuryl esters

$F_5C_6OSO_3^-$ and $4-CH_3C(=O)C_6H_4OSO_2C^-HC_6H_5$ are estimated to decompose at 25 °C with rate constants of 5×10^{-9} and $4 \times 10^6 \text{ s}^{-1}$, respectively, which do not differ greatly from the experimental values of 10^{-7} and $4 \times 10^7 \text{ s}^{-1}$.^{12b,16} The accuracy of the predictions, estimated to be within two orders of magnitude, is remarkable considering that the reactivities of the sulfonyl esters span a range of almost twenty orders of magnitude. At present there is insufficient kinetic data on the hydrolysis of *neutral* sulfonyl esters to construct an analogous correlation to that in Figure 4.

$$\log(k)=(0.71 \pm 0.01)pK_a^{ROSO_2XH}-(1.7 \pm 0.3)pK_a^{ArOH}+(4.7 \pm 2.5) \quad (\text{eq. 2})$$

In summary, the spontaneous hydrolysis of neutral sulfamates, containing one or more *N-H* groups, is shown to proceed through a novel proton-in-flight mechanism (Figure 2). The hydrolysis of **1** is accelerated by an impressive factor of 10^{11} relative to the hydrolysis of **2** and this effect is attributed to the simultaneous neutralization of charge on the bridging oxygen and non-bridging nitrogen atoms as a proton is transferred between these two atoms at the transition state. This mechanism suggests a rationale for the lack of irreversible inhibition observed with *N,N*-dialkyl *O*-aryl sulfamates such as **2**, which cannot react in a like manner.¹⁷ Moreover, the observed rates of sulfonyl transfer, at least for anionic esters with common leaving groups, can be satisfactorily correlated with their pK_a values. The data in Figure 4 suggests that a compound with a leaving group as activated as Coumate's ($pK_a^{ArOH} = 7.8$)¹⁸ would require a pK_a value of ~ 5 in order to attain a half-time in aqueous solution of 24 hours at 25 °C.

Experimental

N-methyl *O*-phenyl sulfamate (**1**) was prepared as described and determined to be 95% pure by comparison of the ^1H NMR integrated signal intensities of **1** to a known amount of pyrazine.⁴ The hydrolysis of **1** ($2.9 \times 10^{-4} \text{ M}$) was carried out in 1.5 ml polypropylene centerfuge vials immersed in a temperature controlled water bath. Reaction pH was maintained with 0.15 M acetate, phosphate, Tris or CAPS buffer at $I = 0.55 \text{ M}$ (NaCl). Variations in buffer concentration at constant pH did not show any observable effect on the rate constants. Periodically the reaction mixtures were transferred to 1 cm pathlength quartz cuvettes and the UV-visible spectra were obtained. Reaction progress was determined for reactions run below pH 9 by monitoring phenol production at 280 nm ($\epsilon^{280} = 1418 \text{ Abs/M/cm}$). Phenoxide production was monitored at 290 nm for higher pH reactions and an effective ϵ^{290} was determined under the exact experimental conditions in these cases. Observed first order rate constants were calculated by a non-linear least square fitting of the absorbance versus time data to a standard first order exponential equation. Good first order behavior was generally observed for greater than three half-times and a comparison of the UV spectra before and after complete hydrolysis demonstrated a 1:1 stoichiometry in all cases. A rate constant for the hydrolysis of **1** at pH 5.9 and 25 °C was determined by the method of initial rates. *N,N*-dimethyl *O*-phenyl sulfamate (**2**) was prepared as described and characterized by ^1H NMR.¹⁹ Hydrolysis of **2** (17 mM) in H_2O was carried out in vacuum sealed quartz tubes containing 0.2 M potassium phosphate buffer at pH 5.9. The sealed quartz tubes were inserted into stainless steel pipe bombs and placed in thermally equilibrated ovens as described.²⁰ Reaction progress was measured by diluting the reaction samples five-fold with D_2O and then obtaining a ^1H NMR spectrum on the reaction mixture and integrating the signals corresponding to $\text{PhOSO}_2\text{NMe}_2$ to PhOH . Control experiments reveal that hydrolysis of **2** at pH 5.9 is independent of hydronium ion concentration and that the spontaneous reaction extends up to at least pH 8.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health Grant GM-18325.

References and Footnotes

1. a) Maltais R, Poirier D. *Steroids*. 2011; 76:929. [PubMed: 21458474] b) Day JM, Purohit A, Foster PA, Woo LW, Potter BV, Reed MJ. *Ann NY Acad Sci*. 2009; 1155:80. [PubMed: 19250195]
2. Bojarová P, Denehy E, Walker I, Loft K, DeSouza DP, Woo LWL, Potter BVL, McConville MJ, Williams SJ. *ChemBioChem*. 2008; 9:613. [PubMed: 18288656]
3. For an example of a complex pH rate profile for the hydrolysis of a sulfamate ester see: Thea S, Cevalco G, Guanti G, Williams A. *J Chem Soc Chem Commun*. 1986:1582.
4. Willams A, Douglas KT. *J Chem Soc Perkin Trans*. 1974; 2:1727.
5. We do not specify a mechanism for the spontaneous hydrolysis of 2 and consider that it could occur by S-O bond cleavage with direct displacement of phenol. An alternative process involving hydrolysis of 2 via S-N bond fission to initially afford phenyl sulfate and dimethylamine (or its ammonium ion) cannot be ruled out based on the kinetic data because the subsequent hydrolysis of phenyl sulfate [$\Delta H^\ddagger = 32.9$ kcal/mol $\Delta S^\ddagger = -15$ cal/mol/K, data from Ref. 12b] to form phenol and inorganic sulfate is predicted to occur faster than its formation.
6. Spillane WJ, Thea S, Cevalco G, Hynes MJ, McCaw CJA, Maguire NP. *Org Biomol Chem*. 2011; 9:523. [PubMed: 20976313]
7. Spillane WJ, McCaw CJA, Maguire NP. *Tetrahedron Lett*. 2008; 49:1049.
8. Spillane WJ, Malaubier J-B. *Tetrahedron Lett*. 2010; 51:2059.
9. Shi F-Q, Li X, Xia Y, Zhang L, Yu Z-X. *J Am Chem Soc*. 2007; 129:15503. [PubMed: 18027935]
10. An alternative and equally valid viewpoint would be that a small amount of positive charge is lost from the aryloxy leaving-group as the sulfamate progresses from ground state to transition state.
11. a) Kirby AJ, Vargolis AG. *J Am Chem Soc*. 1967; 89:415. b) Grzyska PK, Czyryca PG, Purcell J, Hengge AC. *J Am Chem Soc*. 2003; 125:13106. [PubMed: 14570483]
12. a) Hoff R, Larsen P, Hengge A. *J Am Chem Soc*. 2001; 123:9338. [PubMed: 11562216] b) Edwards DR, Lohman DC, Wolfenden R. *J Am Chem Soc*. 2012; 125:525. [PubMed: 22087808]
13. Rate constants for hydrolysis of $\text{ArOSO}_2\text{NH}_2$ at 37 °C were calculated from the activation parameters reported in reference 7.
14. A similar but less extensive plot appears in reference 16.
15. a) Guthrie JP. *J Am Chem Soc*. 1980; 102:5177. b) Thea S, Guanti G, Hopkins AR, Williams A. *J Org Chem*. 1985; 50:5592.
16. Davy MB, Douglas KT, Loran JS, Steltner A, Williams A. *J Am Chem Soc*. 1977; 99:1196.
17. a) Woo LWL, Ganeshapillai D, Thomas MP, Sutcliffe OB, Malini B, Mahon MF, Purohit A, Potter BVL. *ChemMedChem*. 2011; 6:2019. [PubMed: 21990014] b) Howarth NM, Purohit A, Reed MJ, Potter BVL. *J Med Chem*. 1994; 37:219. [PubMed: 8295207]
18. Wolfbeis OS, Fuerlinger E, Kroneis H, Marsoner H. *Fresenius Z Anal Chem*. 1983; 314:119.
19. Spillane WJ, Taheny AP, Kearns MM. *J Chem Soc Perkin I*. 1982:677.
20. Wolfenden R. *Chem Rev*. 2006; 106:3379. [PubMed: 16895333]

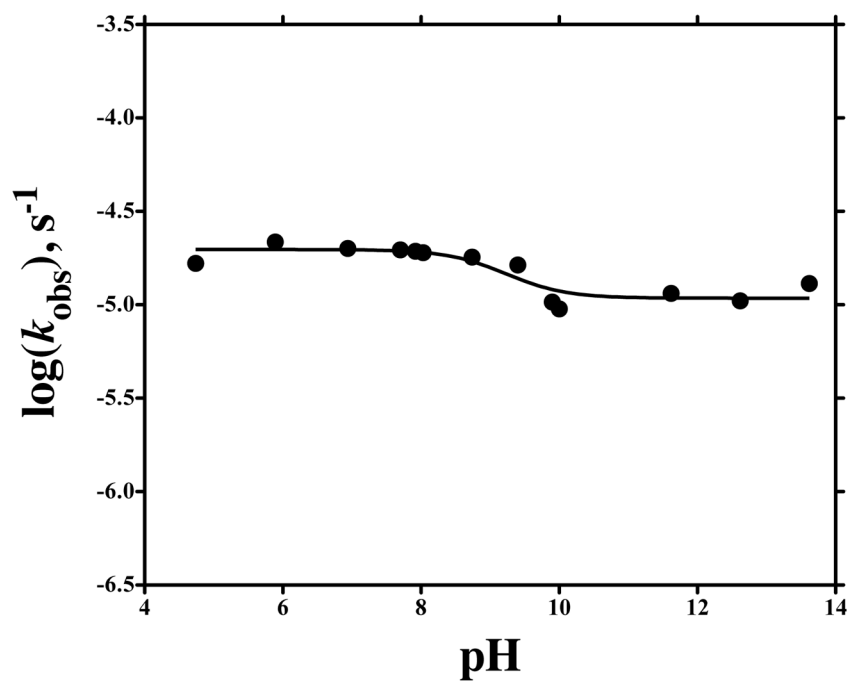


Figure 1.

A plot of $\log(k_{\text{obs}})$ versus pH for the hydrolysis of **1** at 60 °C, $I = 0.55 \text{ M}$ (NaCl). The fitted parameters $k^{1-} = (1.09 \pm 0.07) \times 10^{-5} \text{ s}^{-1}$, $k^1 = (2.0 \pm 0.1) \times 10^{-5} \text{ s}^{-1}$ and $\text{p}K_{\text{a}} = 9.1 \pm 0.3$ were obtained from a fit of the data to equation 1.

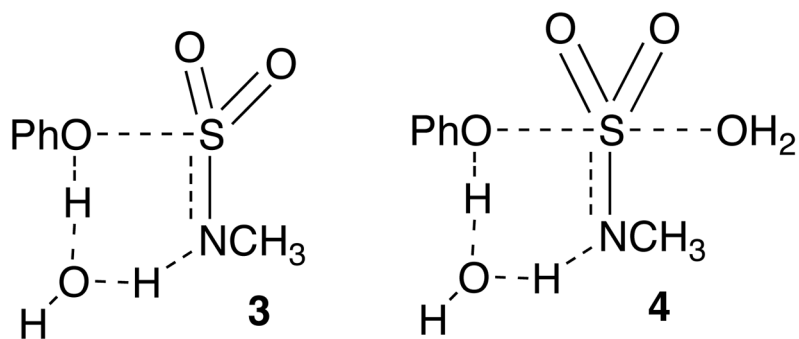


Figure 2.
Possible transition structures for the hydrolysis of **1**.

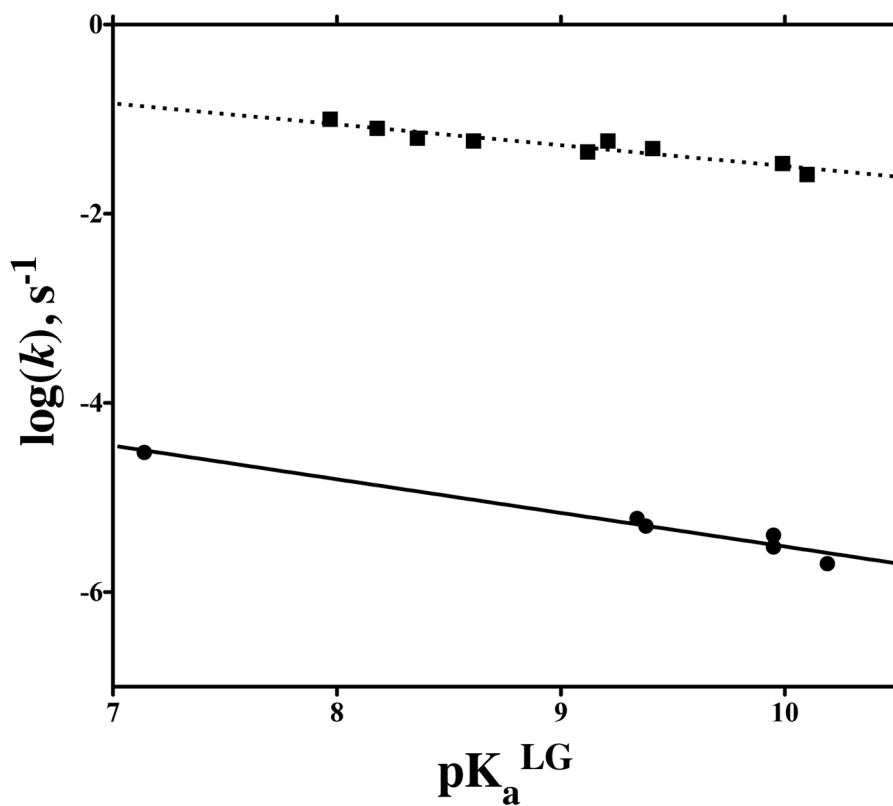


Figure 3. a) Plot of $\log(k_i)$ versus $\text{p}K_a^{\text{LG}}$ (■, dashed line) for the irreversible inhibition of *PaA*stA by $\text{ArOSO}_2\text{NH}_2$ (pH 7, 37 °C). The data are obtained from Ref. 2. b) Plot of $\log(k_{\text{hydrolysis}})$ versus $\text{p}K_a^{\text{LG}}$ (●, solid line) for the uncatalyzed hydrolysis of $\text{ArOSO}_2\text{NH}_2$ at 37 °C. The data for this series are obtained from Ref. 7. See Table S1 of the Supporting Information.

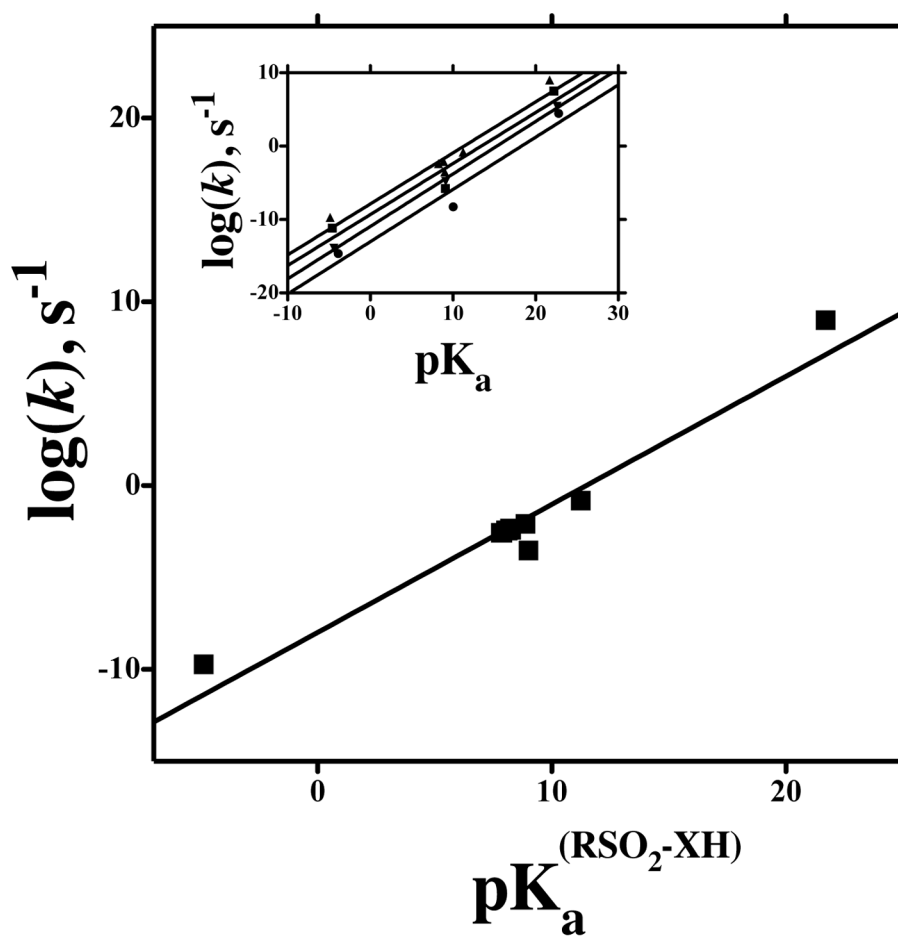
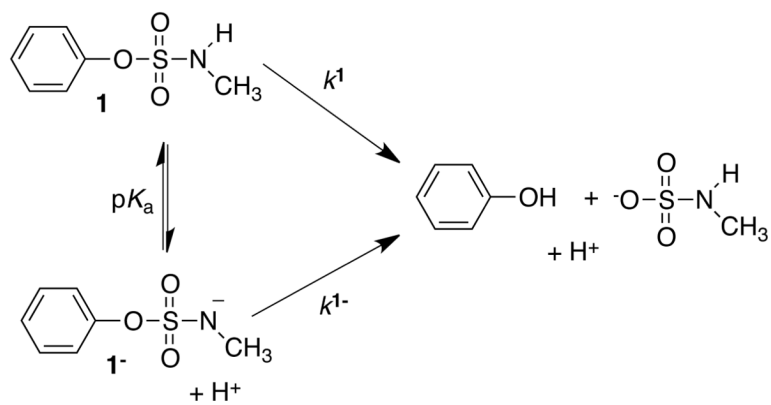


Figure 4.
 a) Plot of $\log(k, \text{s}^{-1})$ versus $\text{pK}_a^{\text{RSO}_2\text{XH}}$ for the hydrolysis of $4\text{-NO}_2\text{-C}_6\text{H}_4\text{O-SO}_2\text{X}^-$ at 25°C . The data are fit to $\log(k) = (-0.70 \pm 0.05)\text{pK}_a^{\text{RSO}_2\text{XH}} - (8.0 \pm 0.5)$ with $r^2 = 0.95$ (13 data). Full details are provided in the Supporting Information.



Scheme 1.
Hydrolysis of 1 and 1⁻.

Table 1Kinetic and thermodynamic data for the hydrolysis of sulfamate esters **1** and **2**.

	ΔH^\ddagger (kcal/mol)	ΔS^\ddagger (cal/mol • K)	k_{25} (s ⁻¹)	k_{60} (s ⁻¹)
1	18.7 ± 0.5	-24 ± 1	2 × 10 ⁻⁷	(2.0 ± 0.1) × 10 ⁻⁵
1⁻	---	---	---	(1.1 ± 0.1) × 10 ⁻⁵
2^a	38 ± 3	-14 ± 6	7 × 10 ⁻¹⁹	7 × 10 ⁻¹⁶

^aThe rate constants k_{25} and k_{60} for hydrolysis of **2** at 25 and 60 °C were calculated from the activation parameters using the Eyring equation.