

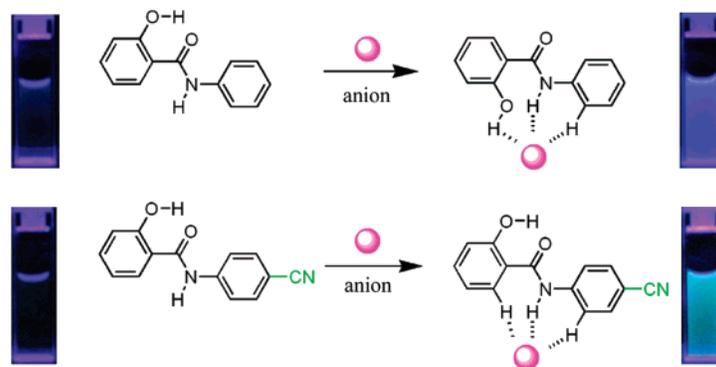
## Anion-Triggered Substituent-Dependent Conformational Switching of Salicylanilides. New Hints for Understanding the Inhibitory Mechanism of Salicylanilides

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A series of salicylanilides (**1a–h**) bearing varied substituents at the 3'- or 4'-position of the anilino moiety (substituent = *p*-OCH<sub>3</sub>, *p*-CH<sub>3</sub>, *m*-CH<sub>3</sub>, H, *p*-Cl, *m*-Cl, *p*-CO<sub>2</sub>CH<sub>3</sub>, and *p*-CN) were synthesized. In acetonitrile all of the substituted salicylanilides **1a–h** predominantly adopt the “closed-ring” conformation facilitated by a strong intramolecular OH $\cdots$ O=C hydrogen bond. In the presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, the conformation of **1a–h** was found to be modulated by the substituent. With our proposed proton-transfer fluorescence probing method, we were able to show that the conformation of **1a–f** bearing a not highly electron-withdrawing substituent was switched to the “open-ring” form by H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, whereas **1h** bearing a highly electron-withdrawing substituent, *p*-CN, remained in the “closed-ring” conformation. The significance of these findings for understanding, from a molecular structural point of view, the mechanism of salicylanilide-based inhibitors for inhibiting the protein tyrosine kinase epidermal growth factor receptor was discussed.

### Introduction

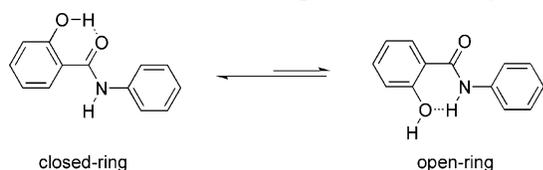
Salicylanilides have been the subject of intensive interest in medicinal chemistry, due to their ability to serve as inhibitors of the protein tyrosine kinase epidermal growth factor receptor (EGFR PTK) relating to cancer, psoriasis, and restenosis.<sup>1</sup> They are generally suggested to compete with ATP for binding at the catalytic domain of tyrosine kinase and stop the growth of tumors.<sup>1a,b</sup> Salicylanilides exist in a conformational equilibrium between the “closed-ring” and “open-ring” conformers, Scheme 1. The closed-ring conformation involving a strong intramolecular OH $\cdots$ O=C hydrogen bond has been proven to be

predominant and closely related to the inhibitory activities of salicylanilides.<sup>1</sup> Meanwhile, an electron-withdrawing substituent at the anilino moiety appears to be required to promote the inhibitory activity of salicylanilides.<sup>1</sup> Recent calculations, however, suggested that this would shift the conformational equilibrium of salicylanilides to the open-ring side,<sup>2</sup> not favor-

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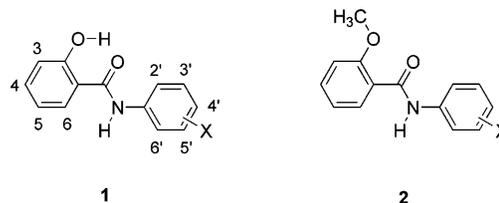
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## SCHEME 1. Conformational Equilibrium in Salicylanilide



able for their inhibitory functions. Although the exact equilibrium status is not yet clear, the role of the electron-withdrawing substituent at the anilino moiety in the inhibitory activity of salicylanilides remains to be clarified. Another issue untouched is the possible interaction of salicylanilides with ATP, in addition to their competition for binding sites in tyrosine kinase. In fact, salicylanilides, containing conventional hydrogen-bonding donor groups, phenolic OH and amido NH, are potent anion receptors.<sup>3,4</sup> Interaction of them with ATP is indeed expected, and whether this interaction, if any, influences the conformation of salicylanilides is unclear.

We thus carried out a systematic investigation of the conformation in acetonitrile (CH<sub>3</sub>CN) of a series of salicylanilides bearing substituent X at the 3'- or 4'-position of the anilino moiety ranging from electron-donating *p*-OCH<sub>3</sub> to highly electron-withdrawing *p*-CN (**1a-h**, Figure 1) in the absence and presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, an alternative taken for ATP. Although infrared (IR) and NMR have been widely employed useful techniques for clarifying the conformation of amides and the involved hydrogen bonds,<sup>5</sup> we proposed a fluorescence method for the case of salicylanilides, not only because of the higher spectroscopy sensitivity and enlarged solvent availability, but more specifically due to the intriguing photophysical properties of salicylanilides. In the closed-ring conformer of salicylanilides, the phenolic OH is intramolecularly hydrogen bonded to the



X's: (a) 4'-OCH<sub>3</sub>, (b) 4'-CH<sub>3</sub>, (c) 3'-CH<sub>3</sub>, (d) H, (e) 4'-Cl, (f) 3'-Cl, (g) 4'-CO<sub>2</sub>CH<sub>3</sub>, (h) 4'-CN

**FIGURE 1.** Chemical structures of salicylanilides **1a-h** and methoxy counterparts **2a-g**.<sup>8a</sup>

carbonyl O atom, which could be signaled by the observation of excited-state intramolecular proton transfer (ESI<sub>intra</sub>PT) fluorescence. Phenolic OH in the open-ring conformer, on the other hand, is not intramolecularly hydrogen-bonded, and salicylanilides are hence prone to emit excited-state intermolecular proton transfer (ESI<sub>inter</sub>PT) fluorescence, especially when a proton acceptor such as an anion is present. As a consequence, the conformation of salicylanilides could be probed by the character of the proton-transfer fluorescence. Our investigations showed fluorescence spectroscopy indeed worked well in this regard and uncovered that H<sub>2</sub>PO<sub>4</sub><sup>-</sup> could switch the conformation of salicylanilides from the closed-ring form to the open-ring form, and this switching was subject to the electron-withdrawing ability of substituent X.

## Results and Discussion

Suezawa et al.<sup>5c</sup> in 2000 determined by NMR the conformation of unsubstituted salicylanilide **1d** in CCl<sub>4</sub> and suggested that **1d** adopted mainly the closed-ring conformation. It was thus wondered whether the closed-ring conformation was still predominant in more polar CH<sub>3</sub>CN, the solvent used here. <sup>1</sup>H NMR and NOESY spectra of **1d** in CD<sub>3</sub>CN were recorded. The phenolic OH signal was observed at 12.01 ppm, which is close to 11.98 ppm in CCl<sub>4</sub> but is shifted to far downfield compared to that of non-hydrogen-bonded phenolic OH normally observed at 4–6 ppm,<sup>5b</sup> indicating the OH...O=C intramolecular hydrogen bonding in **1d**. The NOESY spectrum shows obvious NOE correlations of the amido NH proton with the aryl CH proton at the 6- and 6'-positions (Figure 2), providing further evidence for the preference of the closed-ring to open-ring conformation in the equilibrium (Scheme 1). Correlation with the Hammett constant of substituent X of the NMR signals of phenolic OH and amido NH protons of **1a-h** in CD<sub>3</sub>CN and DMSO-*d*<sub>6</sub> (Figure 3) confirmed that indeed the phenolic OH proton was involved in an intramolecular hydrogen bond while the amido NH proton was exposed to solvent molecules to a much higher extent, and all of them took the closed-ring conformation in CH<sub>3</sub>CN and even in highly polar and hydrogen-bonding DMSO. It was also found in Figure 3 that, with increasing electron-withdrawing ability of X, the signal of the phenolic OH proton shifted to high field, suggesting a weakening OH...O=C hydrogen bond. This is in agreement with our molecular mechanic calculations which showed increasing OH...O=C hydrogen bond length (B3LYP/6-31G\* level)<sup>6</sup> and decreasing hydrogen-bonding energy<sup>7</sup> (Table 1). This means that the conformational equilibrium of **1** (Scheme 1) is shifted more toward the open-ring side when X becomes more electron-withdrawing.<sup>2</sup>

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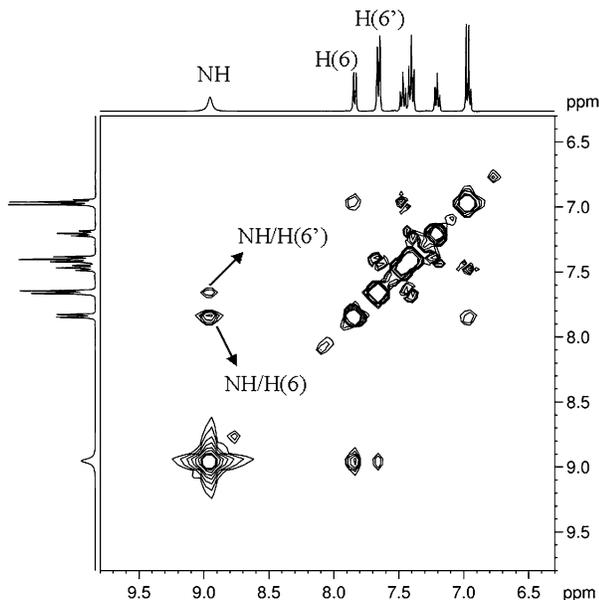


FIGURE 2. NOESY spectrum of **1d** in CD<sub>3</sub>CN.

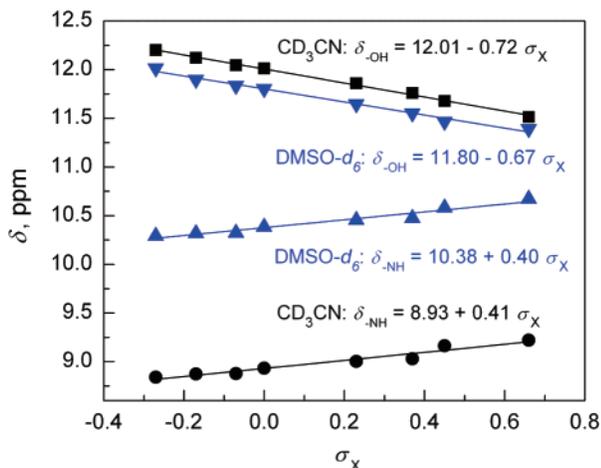


FIGURE 3. Linear relationship against the Hammett constant of substituent X in **1a–h** of the chemical shifts of the OH and NH protons in CD<sub>3</sub>CN and DMSO-*d*<sub>6</sub>. With **2**, the methoxy counterpart of **1**, in DMSO-*d*<sub>6</sub>,  $\delta(\text{NH}) = 10.12 + 0.61\sigma_X$ .<sup>8a</sup>

Due to intramolecular OH⋯O=C hydrogen bonding, salicylanilide **1d**, similar to salicylamide, exhibits an absorption band at 310 nm and an ESI<sub>intra</sub>PT fluorescence at 470 nm in CH<sub>3</sub>CN (Figure 4).<sup>8</sup> With introduction of a substituent at the 3'- or 4'-position of the anilino moiety, the characteristic absorption and fluorescence of salicylanilide at 310 and 470 nm were not much affected (Figures 4 and 5), indicating that

the predominant closed-ring conformation in **1a–h** remains despite varying 3'- or 4'-substitution.

Note that the emission maximum of **1** slightly shifts to the red with increasing electron-withdrawing ability of X (Figures 4b and 5), which is opposite to the intramolecular charge transfer (ICT) emission of its methoxy counterpart **2** that shifts to the blue.<sup>8a</sup> This provides additional support for the intramolecular proton-transfer nature of the emissive state of **1a–h** and hence their closed-ring conformation.

In the presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, interaction of **1** with H<sub>2</sub>PO<sub>4</sub><sup>-</sup> was expected that may influence the conformation of **1**. The phenolic OH proton of **1** in the closed-ring conformation is hydrogen-bonded to carbonyl O, and therefore, only the amido NH and aryl CH protons are available for binding H<sub>2</sub>PO<sub>4</sub><sup>-</sup>. In contrast, **1** in the open-ring form seems to be more favorable for binding H<sub>2</sub>PO<sub>4</sub><sup>-</sup> because the phenolic OH is also available (Scheme 2).<sup>3b</sup> To clarify the conformation **1a–h** adopt in the presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, we first carried out <sup>1</sup>H NMR titrations of **1d** by H<sub>2</sub>PO<sub>4</sub><sup>-</sup> in CD<sub>3</sub>CN.

Figure 6 shows that, upon addition of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, the signal of the amido NH proton is broadened and shifted downfield from 8.93 to 11.83 ppm whereas that of the phenolic OH proton originally at 12.01 ppm rapidly disappears. Meanwhile, downfield shifts in the NMR signals of CH(6) and CH(6') protons are also observed. These facts confirm hydrogen-bonding interaction of **1d** with H<sub>2</sub>PO<sub>4</sub><sup>-</sup>.<sup>11</sup> <sup>1</sup>H NMR dilution experiments on **1d** in CD<sub>3</sub>CN carried out over a **1d** concentration range of 0.1–20 mmol L<sup>-1</sup> indicated hardly any change in the chemical shifts of phenolic OH, amido NH, and aryl CH protons (Figures S1 and S2, Supporting Information), excluding the dimerization of **1d** at 10 mmol L<sup>-1</sup>, the concentration employed for NMR titration. Since the pK<sub>a</sub> of H<sub>3</sub>PO<sub>4</sub> (2.16 in water<sup>9</sup>) is much lower than that of **1d** (7.68 in water<sup>10</sup>), deprotonation of **1d** by H<sub>2</sub>PO<sub>4</sub><sup>-</sup> was ruled out; otherwise, a signal of the amido NH proton at ca. 16 ppm would have been observed<sup>5b</sup> and the signal of the C–H(6) proton shifted upfield due to an increase in the electron density in the salicyloyl ring via a through-bond mechanism.<sup>11</sup> These, however, are still not enough to make a clear assignment of the conformation of **1d**, because similar spectral changes are likely to be observed in both the closed-ring and open-ring conformations upon their interactions with H<sub>2</sub>PO<sub>4</sub><sup>-</sup>. In principle, binding H<sub>2</sub>PO<sub>4</sub><sup>-</sup> by **1d** in the closed-ring conformation (Scheme 2) will result in not only downfield shifts of the amido NH and aryl CH (6 and 6') signals but also an increase in the electron density at the carbonyl O atom and hence an enhancement in the intramolecular OH⋯O=C hydrogen bond. The latter would bring about a downfield shift and even the disappearance of the phenolic OH proton signal. Similarly, signals of phenolic OH, amido NH, aryl CH(6') and CH(6) protons of **1d** in the open-ring conformation could also be shifted downfield due to hydrogen bonding of **1d** with H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and polarization of the C–H(6) bond by a through-space effect.<sup>11</sup>

Absorption and fluorescence spectroscopies were then applied. Spectral traces given in Figure 7 for the case of **1d** indeed

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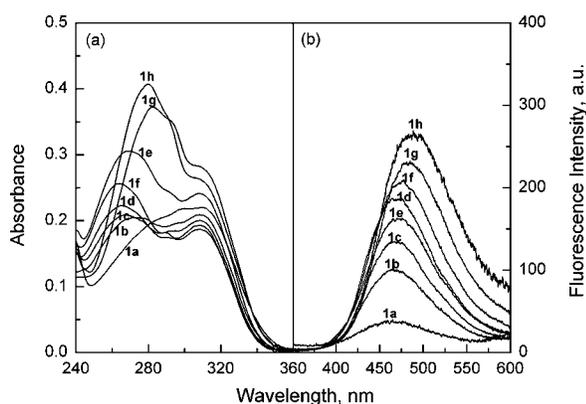
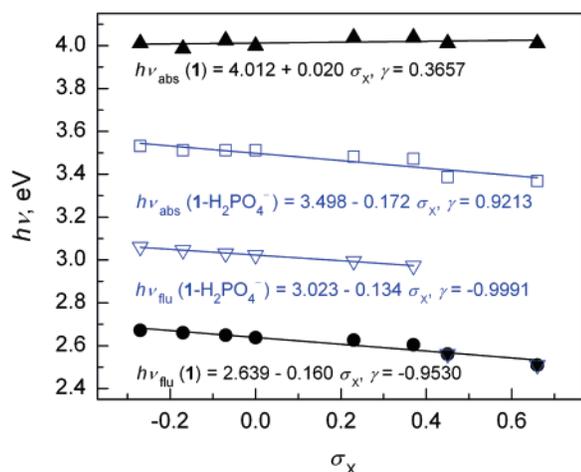
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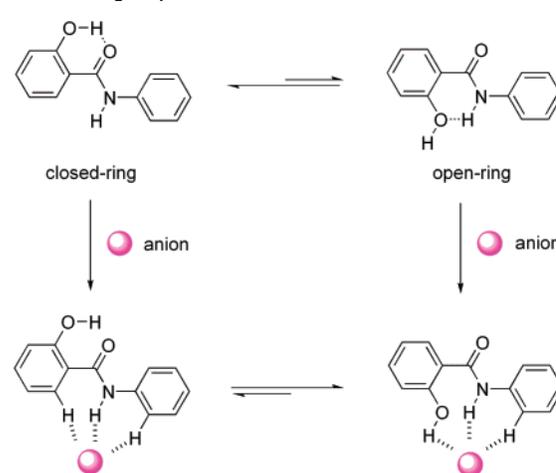
TABLE 1. Structural and Spectral Parameters of **1a–h** and **1·H<sub>2</sub>PO<sub>4</sub><sup>-</sup>** Complexes and Binding Constants in CH<sub>3</sub>CN

	OH...O, <sup>a</sup> Å	$E_{\text{HB}},^b$ kJ mol <sup>-1</sup>		absorption			fluorescence		
		CD <sub>3</sub> CN	DMSO- <i>d</i> <sub>6</sub>	$\lambda$ , nm		log $K$ , <sup>c</sup> mol <sup>-1</sup> L	$\lambda$ , nm		log $K$ , <sup>c</sup> mol <sup>-1</sup> L
				<b>1</b>	<b>1·H<sub>2</sub>PO<sub>4</sub><sup>-</sup></b>		<b>1</b>	<b>1·H<sub>2</sub>PO<sub>4</sub><sup>-</sup></b>	
<b>1a</b>	1.6776	23.72	12.96	284/309	351	3.81 ± 0.01	464	405	3.77 ± 0.01
<b>1b</b>	1.6807	23.38	12.46	273/311	353	3.81 ± 0.01	466	407	3.91 ± 0.02
<b>1c</b>	1.6804	23.06	12.21	267/308	353	3.87 ± 0.01	468	409	4.08 ± 0.01
<b>1d</b>	1.6832	22.93	12.04	266/310	353	3.85 ± 0.01	470	410	4.15 ± 0.01
<b>1e</b>	1.6885	22.29	11.41	269/307	356	3.90 ± 0.02	472	414	4.17 ± 0.02
<b>1f</b>	1.6867	21.87	10.99	265/307	357	4.13 ± 0.01	476	417	4.41 ± 0.02
<b>1g</b>	1.6911	21.53	10.66	282/309	366	4.01 ± 0.01	484	484	4.34 ± 0.02
<b>1h</b>	1.6963	20.84	10.33	280/309	368	4.24 ± 0.02	494	494	4.58 ± 0.03

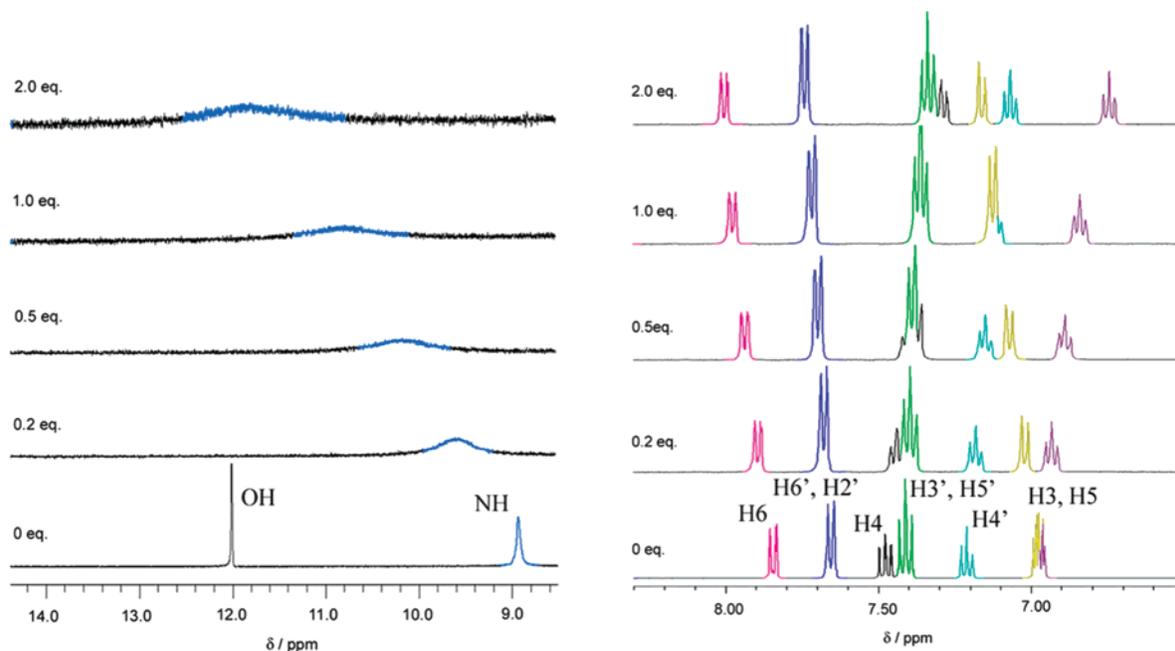
<sup>a</sup> OH...O=C intramolecular hydrogen bond length calculated at the B3LYP/6-31G\* level.<sup>6</sup> <sup>b</sup> Hydrogen-bonding energy calculated in CD<sub>3</sub>CN and DMSO-*d*<sub>6</sub> from the difference in the chemical shifts of the phenolic OH proton in **1a–h** and phenol (6.928 ppm in CD<sub>3</sub>CN and 9.32 ppm in DMSO-*d*<sub>6</sub>).<sup>7</sup> <sup>c</sup> Binding constant  $K$  obtained by nonlinear fitting assuming a 1:1 stoichiometry.<sup>12</sup> Nice fittings support this stoichiometry, which is also confirmed by importing absorption spectral data, for example, those in Figure 7a, into the Specfit/32 software (SPECFIT/32 Global Analysis System, v. 3.0, Spectrum Software Associates, Malborough, MA), which only allows for a perfect fitting under 1:1 stoichiometry.

FIGURE 4. Absorption (a) and fluorescence (b) spectra of **1a–h** in CH<sub>3</sub>CN.FIGURE 5. Linear correlation against the Hammett constant of substituent X of absorption and emission energies of **1** and its complex with H<sub>2</sub>PO<sub>4</sub><sup>-</sup> in CH<sub>3</sub>CN. With **2**, the methoxy counterpart of **1**,  $h\nu_{\text{flu}} = 2.42 + 0.378\sigma_X$ .<sup>8a</sup>

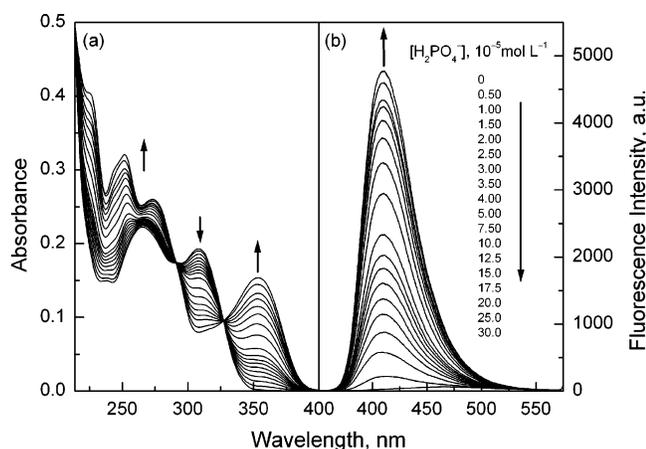
point to the interaction of it with H<sub>2</sub>PO<sub>4</sub><sup>-</sup>. The absorption spectrum of **1d** originally peaking at 266 nm splits, and a new band at 353 nm appears. During this course two clear isosbestic points at 290 and 327 nm are observed, establishing the formation of a well-defined ground-state hydrogen-bonding complex, as also suggested by the <sup>1</sup>H NMR titrations. Meanwhile, the fluorescence of **1d** originally peaking at 470 nm is

SCHEME 2. Conformational Equilibria of **1d** in the Presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>

shifted to 410 nm, which is continuously enhanced with increasing H<sub>2</sub>PO<sub>4</sub><sup>-</sup> concentration. This new emission is blue-shifted by 60 nm from the ESI<sub>intra</sub>PT fluorescence, excluding the closed-ring conformation of **1d**, since no enhancement was observed in the ESI<sub>intra</sub>PT fluorescence that would have been expected from binding of H<sub>2</sub>PO<sub>4</sub><sup>-</sup> with **1d** in the closed-ring conformation (Scheme 2), which shall increase the electron density of the carbonyl O atom and in turn facilitate ESI<sub>intra</sub>PT. The open-ring conformation was therefore assumed as the predominant conformation of **1d** in the presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>. **1d** in the open-ring conformation could form a hydrogen-bonding complex with H<sub>2</sub>PO<sub>4</sub><sup>-</sup> via its phenolic OH, amido NH, and aryl CH protons (Scheme 2). Upon photoexcitation, the acidity of phenolic OH proton is dramatically enhanced so that it is transferred to H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, leading to the observed ESI<sub>inter</sub>PT fluorescence of **1d** (Figure 7b). To support this assumption, a control experiment was carried out by using an organic base, triethylamine (TEA; p*K*<sub>b</sub> = 3.25 in water<sup>9</sup>), that is able to deprotonate phenolic OH (p*K*<sub>a</sub> = 7.68 in water<sup>10</sup>) and to result in an ESI<sub>inter</sub>PT fluorescence emission.<sup>3h</sup> Spectral responses of **1d** toward TEA in CH<sub>3</sub>CN (Figure S3, Supporting Information) show a profile similar to that observed toward H<sub>2</sub>PO<sub>4</sub><sup>-</sup>. This supports the occurrence of ESI<sub>inter</sub>PT of **1d** in the presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and the preference of the open-ring to closed-ring conformation of **1d** (Scheme 2). It is therefore made clear that the conformation of **1d** is switched from the closed-ring to the open-ring in the presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>.



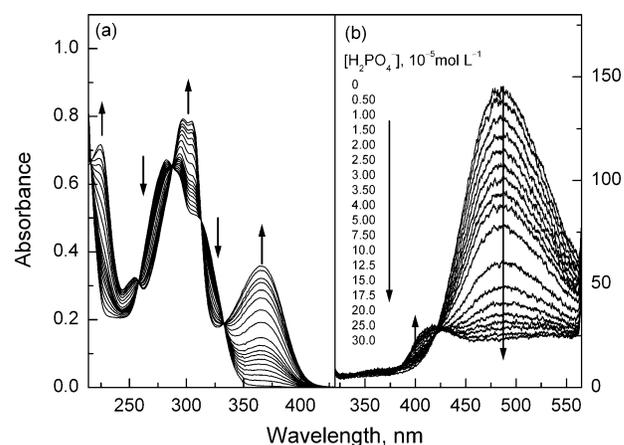
**FIGURE 6.** Portions of  $^1\text{H}$  NMR spectra of **1d** in  $\text{CD}_3\text{CN}$  in the presence of increasing equivalents of  $\text{H}_2\text{PO}_4^-$  as an  $n\text{-Bu}_4\text{N}^+$  salt.  $[\mathbf{1d}] = 10 \text{ mmol L}^{-1}$ . For the numbering of the protons in **1d** see Figure 1.



**FIGURE 7.** Absorption (a) and fluorescence (b) spectra of salicylanilide **1d** in  $\text{CH}_3\text{CN}$  in the presence of increasing concentrations of  $\text{H}_2\text{PO}_4^-$ .  $[\mathbf{1d}] = 2.0 \times 10^{-5}$  (a) and  $1.0 \times 10^{-5}$  (b)  $\text{mol L}^{-1}$ . The excitation wavelength employed to record the spectra in (b) was 290 nm, an isosbestic wavelength seen in (a).

As absorption and fluorescence spectral variation profiles of **1a–f** bearing a substituent from electron-donating  $p\text{-OCH}_3$  to electron-withdrawing  $m\text{-Cl}$  (Figures 5 and S4–S8, Supporting Information) are similar to those of **1d** (Figure 7), it is concluded that the conformation of **1a–f** is switched as is that of **1d** by  $\text{H}_2\text{PO}_4^-$ . Note in Figure 5 that the emission energy of **1a–f** in the presence of  $\text{H}_2\text{PO}_4^-$  has a dependence on the Hammett constant of X similar to that of the  $\text{ESI}_{\text{intraPT}}$  fluorescence of **1a–f**, again supporting the intermolecular proton-transfer character of the emission of **1a–f** in the presence of  $\text{H}_2\text{PO}_4^-$ .

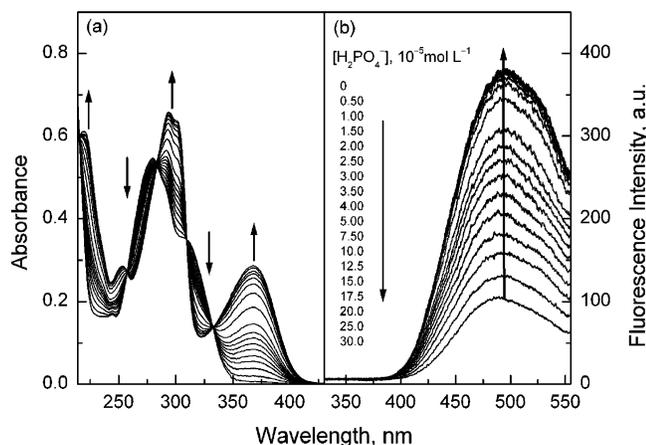
**1g** and **1h**, substituted by  $p\text{-CO}_2\text{CH}_3$  and  $p\text{-CN}$  of stronger electron-withdrawing ability, however, displayed differing spectral responses to  $\text{H}_2\text{PO}_4^-$  (Figures 8 and 9). With **1g** the  $\text{ESI}_{\text{interPT}}$  fluorescence at 410 nm increased very weakly upon adding  $\text{H}_2\text{PO}_4^-$ , at the expense of the  $\text{ESI}_{\text{intraPT}}$  fluorescence at 484 nm. In the case of **1h**,  $\text{H}_2\text{PO}_4^-$  induced an enhancement



**FIGURE 8.** Absorption (a) and fluorescence (b) spectra of **1g** ( $X = p\text{-CO}_2\text{CH}_3$ ) in  $\text{CH}_3\text{CN}$  in the presence of increasing concentrations of  $\text{H}_2\text{PO}_4^-$ .  $[\mathbf{1g}] = 3.5 \times 10^{-5}$  (a) and  $1.8 \times 10^{-5}$  (b)  $\text{mol L}^{-1}$ . The excitation wavelength employed to record the spectra in (b) was 287 nm, an isosbestic wavelength seen in (a).

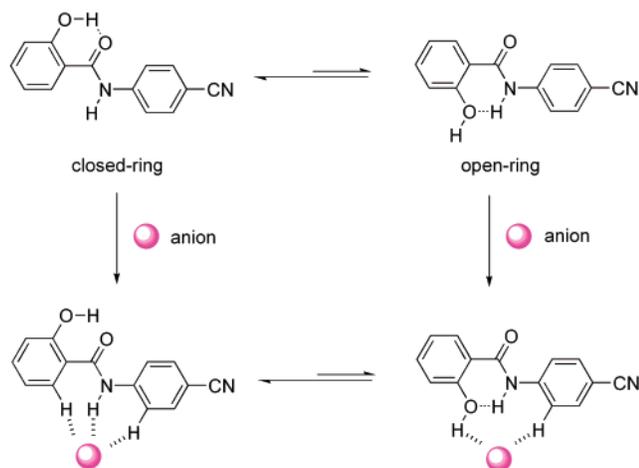
in the  $\text{ESI}_{\text{intraPT}}$  fluorescence at 494 nm. Following analysis previously given for **1d**, **1h** in the presence of  $\text{H}_2\text{PO}_4^-$  was concluded to remain predominantly in the closed-ring conformation (Scheme 3), opposite to **1d** which was switched by  $\text{H}_2\text{PO}_4^-$  to the open-ring conformation (Scheme 2).

Although the phenolic OH proton of **1a–f** is strongly bonded to the carbonyl O atom, they still take an open-ring conformation in the presence of  $\text{H}_2\text{PO}_4^-$ . It thus appears that the  $\text{NH}\cdots\text{O}$  hydrogen bond between the amido NH proton and the phenolic O atom in the open-ring conformation plays a more important role in controlling the conformation of substituted salicylanilides. In case the acidity of the NH proton is relatively low, this  $\text{NH}\cdots\text{O}$  hydrogen bond would be weak.<sup>5c</sup> The amido NH proton is relatively more accessible, which allows for a cooperation of phenolic OH, aryl CH, and NH protons in their hydrogen binding to  $\text{H}_2\text{PO}_4^-$ . The conformational equilibrium of **1a–f**



**FIGURE 9.** Absorption (a) and fluorescence (b) spectra of **1h** ( $X = p\text{-CN}$ ) in  $\text{CH}_3\text{CN}$  in the presence of increasing concentrations of  $\text{H}_2\text{PO}_4^-$ .  $[\mathbf{1h}] = 2.0 \times 10^{-5}$  (a) and  $1.0 \times 10^{-5}$  (b)  $\text{mol L}^{-1}$ . The excitation wavelength employed to record the spectra in (b) was 283 nm, an isosbestic wavelength seen in (a).

**SCHEME 3. Conformational Equilibria of Salicylanilide **1h** in the Presence of  $\text{H}_2\text{PO}_4^-$**



with a weakly acidic amido NH proton is thus shifted to the open-ring conformation (Scheme 2). With **1h**, however, the highly electron-withdrawing substituent  $p\text{-CN}$  at the anilino phenyl ring enhances the acidity of the amido NH proton. The  $\text{NH}\cdots\text{O}$  hydrogen bond in the open-ring conformation is relatively strong so that the amido NH proton is now less accessible for  $\text{H}_2\text{PO}_4^-$ .<sup>3b</sup> The closed-ring conformation of **1h** therefore remains preferable during its binding to  $\text{H}_2\text{PO}_4^-$  (Scheme 3). The acidity of the amido NH proton in **1g** is between those of **1f** and **1h**, and its conformation in the presence of  $\text{H}_2\text{PO}_4^-$  could be reasonably assumed as a mixture of opening and closed-ring conformations, as indeed suggested by its fluorescence titration traces.

Binding constant analysis did show strong interaction of  $\text{H}_2\text{PO}_4^-$  with **1a–h** in  $\text{CH}_3\text{CN}$ . Data obtained from nonlinear fitting assuming a 1:1 stoichiometry<sup>12</sup> (Table 1) clearly indicate that **1h** exhibits a higher binding affinity toward  $\text{H}_2\text{PO}_4^-$  despite the participation in anion binding of the aryl CH instead of the more acidic phenolic OH proton as in, for example, **1d**. This is

in agreement with the recently reported calculations on  $\text{C}_6\text{H}_6^-$  anion complexes by Hay et al.<sup>13</sup> It was revealed that simple aryl CH protons form hydrogen bonds with anions that can be as strong as over 50% of those formed by acidic OH and NH protons. Moreover, when substituted by an electron-withdrawing substituent, the aryl CH protons could become more powerful hydrogen bond donors to form stronger hydrogen-bonding complexes even than those from conventional OH and NH protons, as observed here.

## Conclusions

We showed that in  $\text{CH}_3\text{CN}$  all of the substituted salicylanilides **1a–h** adopted predominantly the closed-ring conformation involving a strong intramolecular  $\text{OH}\cdots\text{O}=\text{C}$  hydrogen bond. In the presence of  $\text{H}_2\text{PO}_4^-$ , however, the conformation of salicylanilides **1a–h** was subject to the substituent. The observed switching of intramolecular to intermolecular proton-transfer fluorescence of **1a–f** by  $\text{H}_2\text{PO}_4^-$  directly pointed to the opening conformation of them in the presence of  $\text{H}_2\text{PO}_4^-$ . **1h** bearing a highly electron-withdrawing substituent,  $p\text{-CN}$ , however, was shown to remain in the closed-ring conformation in the presence of  $\text{H}_2\text{PO}_4^-$ . The observed enhancement in the excited-state intramolecular instead of the intermolecular proton-transfer fluorescence of **1h** in the presence of  $\text{H}_2\text{PO}_4^-$  served as a direct indication. The character of the proton-transfer fluorescence, *inter-* or *intramolecular*, clearly probes the conformation of salicylanilides, which also enables direct fluorescence imaging of the conformations by strongly contrasted colors.

These conclusions would be of considerable significance for understanding the inhibitory mechanism of salicylanilides. As more hydrophobic salicylanilide is preferred in its action as an inhibitor,<sup>1</sup> the binding site in the catalytic domain shall be less polar. One issue then that deserves attention is the occurrence of interaction with ATP of the salicylanilide-based inhibitors, in addition to their competition for binding to the catalytic domain. This also helps to understand the experimental observations that the inhibitory activity of salicylanilides is positively related to the electron-withdrawing ability of the substituent at the anilino moiety.<sup>1</sup> On the basis of our model investigations, this substituent of higher electron-withdrawing ability facilitates the closed-ring conformation of salicylanilides required to be functional. Introduction of a strongly electron-withdrawing substituent at the anilino ring is thus suggested, and it is even better if hydrophobicity can also be enhanced by doing so.

## Experimental Section

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were acquired on a 400 or 500 MHz NMR spectrometer in  $\text{DMSO}-d_6$  or  $\text{CD}_3\text{CN}$  using TMS as an internal standard. Absorption and fluorescence spectral titrations for anion binding were carried out by adding an aliquot of anion solution to a bulk salicylanilide solution at a given concentration.

Chemicals used for syntheses were commercially available, were of AR grade, and were used as received. Solvents used for spectral investigations were further purified by redistillations so that no fluorescent impurity could be detected at the employed excitation wavelength.

Salicylanilides **1a–h** are known compounds in the literature,<sup>1b,c</sup> but synthesis procedures and characterization data are provided as modified procedures were employed in this work.

(12) (a) Kato, R.; Nishizawa, S.; Hayashita, T.; Teramae, N. *Tetrahedron Lett.* **2001**, *42*, 5053–5056. (b) Madrid, J. M.; Mendicuti, F. *Appl. Spectrosc.* **1997**, *51*, 1621–1627.

(13) (a) Bryantsev, V. S.; Hay, B. P. *Org. Lett.* **2005**, *7*, 5031–5034. (b) Bryantsev, V. S.; Hay, B. P. *J. Am. Chem. Soc.* **2005**, *127*, 8282–8283.

**General Procedure for Preparation of Salicylanilides 1a–h.**

A mixture of salicylic acid (1.0 g, 7.0 mmol) and thionyl chloride (2.5 mL, 35 mmol) was heated at 50 °C for 2 h. The excess SOCl<sub>2</sub> was removed by distillation in vacuo at room temperature. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> and the solvent removed as before; this procedure was repeated twice. The freshly formed salicyloyl chloride dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> was added dropwise to a solution of the substituted aniline, pyridine, and DMAP in dry CH<sub>2</sub>Cl<sub>2</sub>. The resultant solution was stirred at room temperature for 2 h, and the solvent was removed. The residue was washed with 1 mol L<sup>-1</sup> HCl and dissolved in 2 mol L<sup>-1</sup> NaOH. The filtrate was neutralized with 3 mol L<sup>-1</sup> HCl, and a white solid was precipitated, which was collected by filtration and purified by recrystallization

from CH<sub>3</sub>CN/H<sub>2</sub>O (9:1, v/v). **1a–h** were fully characterized, and detailed spectral data are supplied in the Supporting Information.

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**Supporting Information Available:** NMR dilution experiment data of **1d** in CD<sub>3</sub>CN, fluorescence spectra of **1d** in CH<sub>3</sub>CN in the presence of triethylamine, absorption and fluorescence spectra of **1a–f** in CH<sub>3</sub>CN in the presence of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, characterization data, and <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of **1a–h**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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