ORGANIC LETTERS

2013
Vol. 15, No. 2
235–237

# Development of a Fluorescent Chemosensor for the Detection of Kynurenine

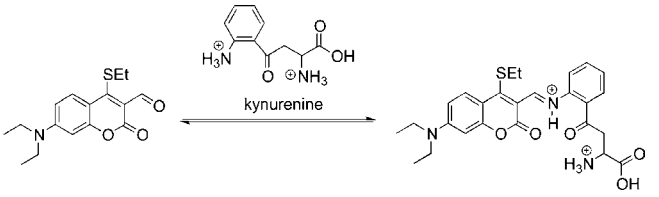
**Jessica L. Klockow and Timothy E. Glass***

*Department of Chemistry, University of Missouri, Columbia, Missouri 65211, United States*

*glasst@missouri.edu*

**Received November 24, 2012**

## ABSTRACT




**Kynurenine, a metabolite of tryptophan, is known to contribute to cancer progression when overproduced. A method for facile fluorescent sensing of kynurenine using sensor 1 has been developed. When bound at low pH, sensor 1 undergoes a very large bathochromic shift because kynurenine extends the conjugation of the fluorophore. This unusual mechanism of activation provides a 390-fold fluorescence enhancement that is very specific to kynurenine and a wavelength of fluorescence that extends into the red.**

Kynurenine is a metabolite of tryptophan in the synthetic pathway toward the production of picolinic acid and NAD.[1] Abnormally high concentrations of kynurenine are associated with coronary artery disease, invasive tumor growth, as well as Huntington's, Alzheimer's, and other neurological diseases.[2−4] Kynurenine acts as an endogenous ligand for the aryl hydrocarbon receptor (AHR) that inhibits antitumor immunological responses by suppressing allogeneic T-cell proliferation. As a result, elevated levels of kynurenine allow for unhindered invasive tumor growth.[5,6]

Enzymatic activation of indoleamine 2,3-dioxygenase (IDO) or tryptophan dioxygenase (TDO) initiates kynurenine production, and the ratio of kynurenine to tryptophan is often used as an indirect measure of IDO and TDO activity.[6] Increased IDO/TDO activity and high AHR levels correlate with poor prognosis in cancer patients and further tie the presence of kynurenine to cancer progression.[5,6]

The selective detection of kynurenine is material in the ongoing study of cancer progression. Current detection methods include HPLC, GC−MS, biosensors, and various electrochemical techniques that entail costly or time-consuming preparations.[7−12] In contrast, fluorescent chemosensors can provide a convenient and sensitive method for analyte detection in solution. Herein, we present a water-soluble fluorescent sensor based on the coumarin aldehyde scaffold that is selective for kynurenine over other biologically relevant aliphatic and aromatic primary amines. Moreover, the sensor demonstrates a profound bathochromic

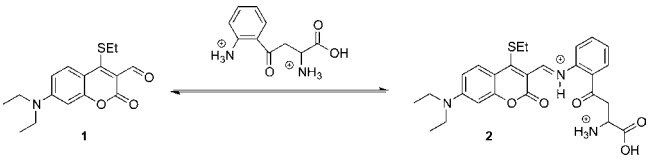
(1) Chen, Y.; Guillemin, G. *Int. J. Tryptophan Res.* **2009**, *2*, 1–19.
(2) Stone, T. W.; Forrest, C. M.; Darlington, L. G. *FEBS J.* **2012**, *279*, 1386–1397.
(3) Mezrich, J. D.; Fechner, J. H.; Zhang, X.; Johnson, B. P.; Burlingham, W. J.; Bradfield, C. A. *J. Immunol.* **2010**, *185*, 3190–3198.
(4) Chen, Y.; Stankovic, R.; Cullen, K. M.; Meininger, V.; Garner, B.; Coggan, S.; Grant, R.; Brew, B. J.; Guillemin, G. J. *Neurotox. Res.* **2010**, *18*, 132–142.
(5) Opitz, C. A.; Litzenburger, U. M.; Sahm, F.; Ott, M.; Tritschler, I.; Trump, S.; Schumacher, T.; Jestaedt, L.; Schrenk, D.; Weller, M.; Jugold, M.; Guillemin, G. J.; Miller, C. L.; Lutz, C.; Radlwimmer, B.; Lehmann, I.; von Deimling, A.; Wick, W.; Platten, M. *Nature* **2011**, *478*, 192–194.
(6) Prendergast, G. C. *Nature* **2011**, *478*, 192–194.
(7) Varghese, N.; Mogera, U.; Govindaraj, A.; Das, A.; Maiti, P.; Sood, A. K.; Rao, C. N. *Chem. Phys. Chem.* **2009**, *10*, 206–210.
(8) Li, H.; Tian, J.; Wang, L.; Zhang, Y.; Sun, X. *J. Mater. Chem.* **2011**, *21*, 824–828.
(9) Krcmova, L.; Solichova, D.; Melichar, B.; Kasparova, M.; Plisek, J.; Sobotka, L.; Solich, P. *Talanta* **2011**, *85*, 1466–1471.
(10) Guillemin, G. J.; Cullen, K. M.; Lim, C. K.; Smythe, G. A.; Garner, B.; Kapoor, V.; Takikawa, O.; Brew, B. J. *J. Neurosci.* **2007**, *27*, 12884–12892.
(11) Vaarmann, A.; Kask, A.; Maeorg, U. *J. Chromatogr. B* **2002**, *769*, 145–153.
(12) Notarangelo, F. M.; Wu, H.-Q.; Macherone, A.; Graham, D. R.; Schwarcz, R. *Anal. Biochem.* **2012**, *421*, 573–581.

10.1021/ol303025m 
**Published on Web 12/24/2012**

shift and fluorescence turn-on response upon binding to kynurenine.

Our group has developed a number of fluorescent sensors for the detection of biological amines using the coumarin aldehyde scaffold.[13−15] We utilize a similar model for our kynurenine sensor design (Scheme 1). The sensor functions through an internal charge transfer (ICT) mechanism whereby the tertiary nitrogen serves as the electron donor and the aldehyde acts as the electron acceptor.

**Scheme 1.** Proposed Binding of Sensor **1** to Kynurenine




The aldehyde of the sensor reversibly binds to primary amines to form a positively charged iminium ion that enhances the ICT and modulates both the absorbance and fluorescence. The red shift in absorption upon analyte binding permits excitation and monitoring of the sensor in both its unbound and bound states. Kynurenine is unusual in that it possesses both an aliphatic and an aromatic primary amine, and until now we had not examined the interaction of aromatic amines with our sensor system. Therefore, we explored the interaction of kynurenine with sensor **1**.

Initial efforts to test sensor binding to kynurenine were hampered by its low solubility in neutral aqueous media. Therefore, the solution pH was lowered to 1.0 to enhance kynurenine solubility and the absorbance and fluorescence spectra were monitored (Figure 1). Sensor **1** has an absorption band at 468 nm. This wavelength of absorption is typical for the coumarin aldehydes and indicates that the electron-poor aniline is not protonated even at this low pH. Upon addition of kynurenine, two new absorption bands were observed at 525 and 555 nm at pH 1. This represents an unprecedented bathochromic shift of 87 nm compared to the 35 nm shift that is typical for the coumarin aldehyde class of sensors.[14,15] The fluorescence emission spectrum mirrors the absorbance spectrum revealing two bands at 586 and 632 nm upon excitation at 555 nm. Because sensor **1** does not absorb above 530 nm in its unbound form, using exitation at 555 nm, binding to kynurenine provides a pronounced 390-fold fluorescence enhancement. Furthermore, the binding constant with kynurenine was 1880 $M^{-1}$, which is nearly 3 orders of magnitude higher than the binding of an aliphatic primary amine to the coumarin aldehyde sensors.[14]

We surmised that the aromatic amine must be involved in extending the conjugation of the chromophore. To test

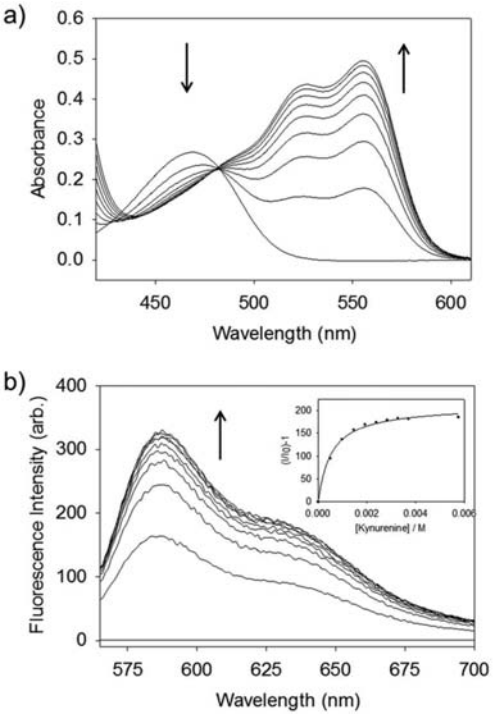



**Figure 1.** UV/vis (a) and fluorescence (b) spectra ($\lambda_{ex}$ = 555 nm) of sensor **1** (10 $\mu$M) in buffer (50 mM $H_3PO_4$, 120 mM NaCl, pH 1, 1% DMSO) adding aliquots of 50 mM kynurenine. Inset is the fit to a one-site binding isotherm.

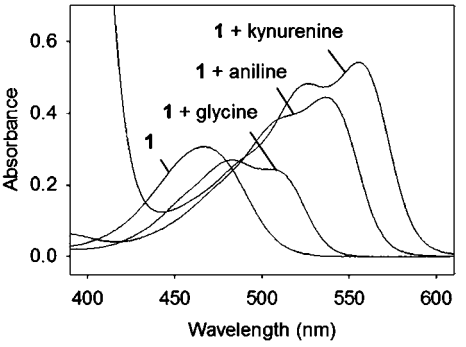


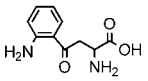
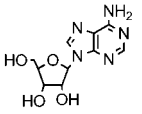
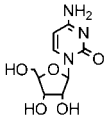

**Figure 2.** UV/vis spectrum of sensor **1** (10 $\mu$M) with various analytes (kynurenine 5 mM; aniline 16 mM; glycine 360 mM) in buffer (50 mM $H_3PO_4$, 120 mM NaCl, pH 1, 1% DMSO).

this hypothesis, we titrated sensor **1** with aniline which gave a similar, though smaller, bathochromic shift with two distinct bands (Figure 2). The larger shift and higher binding constant of kynurenine is attributed to the *o*-carbonyl group, which extends the conjugation of the chromophore with an additional electron-withdrawing group.

A pH titration of the sensor−kynurenine bound complex gave a p$K_a$ of 2.5 that is consistent with the p$K_a$ of an aniline Schiff base (Figure S1, Supporting Information).[16]

(13) Secor, K.; Plante, J.; Avetta, C.; Glass, T. *J. Mater. Chem.* **2005**, *15*, 4073–4077.
(14) Feuster, E. K.; Glass, T. E. *J. Am. Chem. Soc.* **2003**, *125*, 16174–16175.
(15) Secor, K. E.; Glass, T. E. *Org. Lett.* **2004**, *6*, 3727–3730.

**Table 1.** Association Constants and Spectroscopic Results from the Binding of Sensor **1** (10 $\mu$M in 50 mM $H_3PO_4$, 120 mM NaCl, pH 1, 1% DMSO) with Various Analytes

| analyte | association constant $K_a$ ($M^{-1}$)[a] | $\lambda_{max1}$ (nm) | $\lambda_{max2}$ (nm) | $I_{sat}/I_0$ ($\lambda_{ex}$ = 555 nm) |
|---|---|---|---|---|
|  kynurenine | 1880 | 525 | 555 | 390 |
|  glycine | 8.0 | 483 | 507 | n.d. |
|  adenosine | 5.3 | 470 | - | n.d. |
|  cytidine | 1.6 | 470 | - | n.d. |
|  aniline | 600 | 510 | 536 | 220 |

[a] Error in $K_a$ is ±5% based on triplicate titrations.

Upon raising the pH of the solution, the long wavelength absorption is lost. The sensor response requires that the imine be protonated, thus the sensor only operates at low pH. It should be noted that a Schiff base from an aliphatic amine has p$K_a$ values of ~6–8.[17] This supports the notion that it is the aromatic amine of kynurenine that binds to the sensor.

To test for selectivity, sensor **1** was titrated with other biological amines and the fluorescence was monitored. The various analytes bound to sensor **1** weakly and provided only modest bathochromic shifts. In turn, no fluorescence response was observed when excited at 555 nm (Table 1). Even aromatic primary amines such as adenosine provided no response. Thus, sensor **1** is highly selective for kynurenine.

A competitive binding study was performed to investigate the effect of competing analytes on the binding of sensor **1** to kynurenine (Figure 3). Sensor **1**, kynurenine (10 mM), and various competing analytes (100 mM) were combined and the fluorescence intensities observed with an excitation of 555 nm.

(16) Reeves, R. L.; Smith, W. F. *J. Am. Chem. Soc.* **1963**, *85*, 724–728.
(17) Kayser, R. H.; Pollack, R. M. *J. Am. Chem. Soc.* **1977**, *99*, 3379–3387.

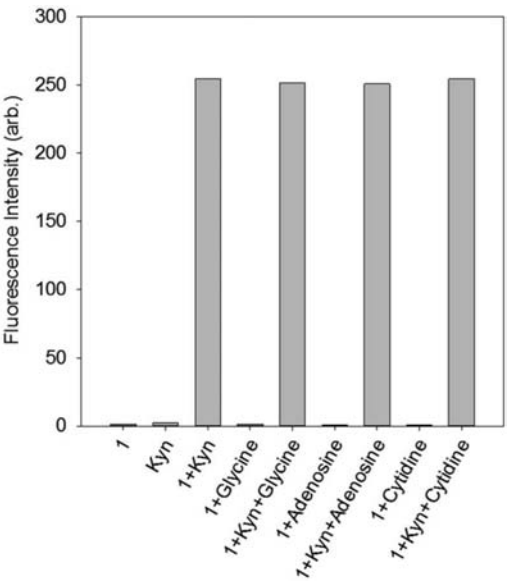



**Figure 3.** Fluorescence emissions of sensor **1** (10 $\mu$M) at $\lambda_{em}$ = 586 nm with kynurenine (10 mM) in the presence of various analytes (100 mM) in buffer (50 mM $H_3PO_4$, 120 mM NaCl, pH 1, 1% DMSO).

The relative fluorescence intensity did not change when competing analytes were added in 10-fold excess. These data indicate that sensor **1** can selectively sense kynurenine in complex mixtures containing other biological aromatic and aliphatic primary amines.

In conclusion, we have developed sensor **1** as a tool for the selective detection of kynurenine. The coumarin aldehyde confers a turn-on fluorescence response upon binding due to an enhanced charge transfer. This method is unique in that kynurenine is incorporated as part of the fluorophore, giving exquisite selectivity for the target analyte due to an unprecedented bathochromic shift. Other biological amines form bound complexes that do not absorb at such high wavelengths. Thus, sensor **1** could be used to detect kynurenine in serum. Although the sensor requires that the media be adjusted to acidic pH, the selectivity seen among similar analytes is compelling. The considerable fluorescence enhancement seen upon binding to kynurenine is unperturbed by the presence of excess competing analytes and further substantiates sensor **1** as a means to monitor kynurenine in order to facilitate studies of cancer initiation and progression.

**Acknowledgment.** This work was supported by the National Science Foundation (CHE-1112194).

**Supporting Information Available.** Experimental methods and synthesis of sensor **1**, UV/vis absorption, and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.