Derivatives of 8-Hydroxy-2-methylquinoline Are Powerful Prototypes for Zinc Sensors in Biological Systems

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The recent emphasis on understanding the myriad roles of zinc in both normal and diseased cells and tissues[1] has placed an ever increasing demand on methods for sensitive and selective methods for real-time monitoring of free Zn^{2+} in complex biological samples. Chelation-enhanced fluorescent sensors for zinc, based on fluorophores such as quinoline,[2] dansyl,[3] fluorescein,[4] and anthracene,[5] have been reported. While each of these agents has unique advantages, there remain issues with sensitivity, selectivity, and specificity that may be addressable with an alternate chromophore that is readily amenable to synthetic manipulation. Herein we report the systematic chemical modification of the 8-hydroxy-2-methylquinoline (Oxn) unit as a building block for the development of new sensors employing chelation-enhanced fluorescence. In particular, improvements in quantum yield from 0.004 to 0.70 and stepwise blue shifts in fluorescence emission wavelengths (to a total of over 70 nm) are reported.

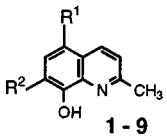
A selection of substituted quinoline derivatives bearing the electron-withdrawing nitro, sulfonic acid, and sulfonamide substituents (**1**−**9**) were prepared and screened for fluorescence response to Zn^{2+} under pseudobiological conditions.[6] Derivatives **1**−**6** were prepared by previously described methods.[7] The new sulfonamide derivatives, **7**−**9**, were prepared by simple two-step procedures involving the preparation of the corresponding sulfonyl chloride derivatives using chlorosulfonic acid,[8] followed by addition of these intermediates to excess amine in THF.

Table 1. Relevant Spectroscopic Data for Oxn Derivatives that Form Fluorescent Complexes with Zn^{2+}

derivative	λ_{ex}, nm	λ_{em}, nm	ϵ,[a] cm^{-1} M^{-1}	$\phi^{a,b}$
1	353	533	2286	0.004
2	425	460	14200	<0.001
4	355	515	3796	0.058
5	356	493	3433	0.33
7	354	499	5563	0.24
8	353	464	4564	0.70
9	369	465	4826	0.44
10	369	535	6061	0.10

[a] Spectra acquired in 150 mM NaCl, 50 mM HEPES, pH 7.01, 25 °C, with 1.00 μM **1**−**10** and 0.5−1.0 mM $ZnCl_2$. [b] Excitation of all species provided at λ_{max} (355−425 nm), 5 nm slit widths. Quantum yields were calculated with reference to a quinine sulfate standard.[10]

Deriv.	R^1	R^2
1	H	H
2	NO_2	H
3	NO_2	NO_2
4	SO_2OH	H
5	SO_2OH	SO_2OH
6	SO_2OH	NO_2
7	$SO_2N(CH_3)_2$	H
8	$SO_2N(CH_3)_2$	$SO_2N(CH_3)_2$
9	$SO_2N(n\text{-butyl})_2$	$SO_2N(n\text{-butyl})_2$



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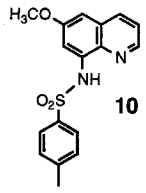

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Chemical substitution dramatically influenced the efficiency of both the absorption and emission properties of the Oxn derivatives. Table 1 lists relevant extinction coefficients and quantum yields of the fluorescent Zn^{2+}-bound derivatives. Substitution of nitro groups on the Oxn core resulted in significantly less fluorescent complexes with Zn^{2+}; complexes of **2** are weakly fluorescent, and those of **3** and **6** exhibited no measurable fluorescence in neutral solution. By contrast, derivatization with sulfonic acid or sulfonamide groups resulted in dramatically enhanced fluorescence properties. The improvement in quantum yield of the sulfonamide derivatives is particularly striking; that of **8** is 175 times greater than that of **1**. Although the excitation maxima for the compounds remained essentially unchanged, the emission wavelengths of the Zn^{2+}-bound derivatives were blue shifted compared to that from Zn^{2+}-bound **1**. The extent of blue shift was dependent on both the type (sulfonamide derivatives were more blue shifted than sulfonic acid derivatives) and degree (5,7-disubstituted derivatives were more blue shifted than 5-subsituted derivatives) of substitution (Figure 1a).[9]

Fluorescence emission properties may be compared using a sensitivity index based on the product of the quantum yield and the extinction coefficient at λ_{ex}. A complete survey of the zinc complexes (Figure 1b) reveals the additive effect of substitution

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(1) (a) Kimura, E.; Kikuta, E. *J. Bioinorg. Chem.* **2000**, *5*, 139−155. (b) Cox, E. H.; McLendon, G. L. *Curr. Opin. Chem. Biol.* **2000**, *4*, 162−165. (c) Ramboarina, S.; Moreller, N.; Fournie-Zaluski, M.-C.; Roques, B. P. *Biochemistry* **1999**, *38*, 9600−9607.

(2) (a) Savage, D. D.; Montano, C. Y.; Kasarskis, E. J. *Brain Res.* **1989**, *496*, 257−267. (b) Budde, T.; Minta, A.; White, J. A.; Kay, A. R. *Neuroscience* **1997**, *79*, 347−358. (c) Nasir, M. S.; Fahrni, C. J.; Suhy, D. A.; Kolodsick, K. J.; Singer, C. P.; O'Halloran, T. V. *J. Biol. Inorg. Chem.* **1999**, *4*, 775−783. (d) Zalewski, P. D.; Forbes, I. J.; Seamark, R. F.; Borlinghaus, R.; Betts, W. H.; Lincoln, S. F.; Ward, A. D. *Chem. Biol.* **1994**, *1*, 153−161.

(3) (a) Koike, T.; Watanabe, T.; Aoki, S.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 12696−12703. (b) Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Eur. J. Inorg. Chem.* **1999**, 455−460. (c) Thompson, R. B.; Maliwal, B. P.; Feliccia, V. L.; Fierke, C. A.; McCall, K. *Anal. Chem.* **1998**, *70*, 4717−4723.

(4) (a) Hirano, T.; Kikuchi, K.; Urano, Y.; Higushi, T.; Nagano, T. *J. Am. Chem. Soc.* **2000**, *122*, 12399−12400. (b) Walkup, G. K.; Burdette, S. C.; Lippard, S. J.; Tsien, R. Y. *J. Am. Chem. Soc.* **2000**, *122*, 5644−5645.

(5) Akkaya, E. U.; Huston, M E.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 3590−3593.

(6) (a) The fluorescence properties of metal complexes of a related species have been used for detection of metal ions such as zinc following chromatographic separation. Soroka, K.; Vithanage, R. S.; Phillips, D. A.; Walker, B.; Dasgupta, P. K. *Anal. Chem.* **1987**, *59*, 629−636. Dickens, J. E.; Sepaniak, M. J. *J. Microcolumn Sep.* **1999**, *11*, 45−51. (b) Nitro-substituted quinoline units have been reported as potential chromophoric or fluorophoric sensors. Prodi, L.; Bargossi, C.; Montalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769−6770 and references therein.

(7) Oxn derivatives prepared and screened in this study: Derivative **1**: Aldrich. **2**, **3**: Petrow, V.; Sturgeon, B. *J. Chem. Soc.* **1954**, 570−574. **4**: prepared by hydrolysis of 5-sulfonyl chloride of **1**, characterization consistent with literature: Gershon, H.; McNeil, M. E.; Grefig, A. T. *J. Org. Chem.* **1969**, *34*, 3268−3270. **5**, **6**: Gershon, H.; McNeil, M. W. *J. Heterocycl. Chem.* **1972**, *9*, 659−667. **7**−**9**: prepared by addition of corresponding sulfonyl chloride to excess amine in THF. **10**: Molecular Probes.

(8) Clarke, H. T.; Babcock, G. S.; Murray, T. F. Benzenesulfonyl Chloride. In *Organic Syntheses*, 2nd ed.; Blatt, A. H., Gilman, H., Eds.; John Wiley and Sons: New York, 1961; Vol. 1, pp 85−87.

(9) Similar blue shifts in emission spectra of zinc complexes of a related quinoline derivative in methylene chloride have been reported in the literature. Hopkins, T. A.; Meerholz, K.; Shaheen, S.; Anderson, M. L.; Schmidt, A.; Kippelen, B.; Padias, A. B.; Hall, H. K. J.; Peyghambarian, N.; Armstrong, N. R. *Chem. Mater.* **1996**, *8*, 344−351. These authors postulate that blue shifts in emission wavelengths are due to increases in the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), and are generated by introducing electron-withdrawing groups to the quinoline core. Crystals of the zinc complex of **8** exhibit an intense pale blue fluorescence when irradiated at 365 nm.

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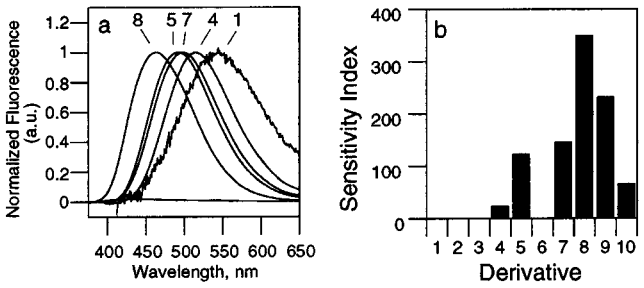

Figure 1. (a) Blue shift in normalized fluorescence emission spectra of **4**, **5**, **7**, and **8** compared to that of **1**. The baseline spectrum is that of **8** in the absence of zinc. (b) Sensitivity index of Zn^{2+}-bound Oxn derivatives, compared to Zn^{2+}-bound TSQ. Data are normalized relative to the parent Oxn, **1** (1.00). Experimental conditions are described in Table 1.

around the Oxn core on the fluorescence signal from the resulting zinc complexes. The sensitivity of the compounds presented in this study is compared with that of 6-methoxy-8-(*p*-toluenesulfonamido)quinoline (TSQ), **10**, a well characterized intracellular, biochemical, and analytical fluorescence indicator for zinc that exhibits spectroscopic properties representative of a class of 8-(*p*-toluenesulfonamido)quinoline indicators, including Zinquin.[2,11] The sensitivity indices of derivatives **5**, **7**, and **8** were superior to those of **10**, indicating that sensors prepared using these species might provide a useful fluorescence signal on binding Zn^{2+} in biological or near-neutral environmental samples.[12] The quantum yield of the fluorophores is the result of contributions from a number of sources, including molecular dipole, resonance structures, and solvent-fluorophore interactions, and further study will be required to describe the cumulative nature of these contributions.

To examine whether sulfonamide derivatives such as **7** and **8** would, in fact, prove useful components of zinc chemosensors, the more hydrophobic derivative **9** was prepared for intracellular experiments.[13] Fluorescent labeling of mouse fibroblasts (NIH/3T3) occurred after incubating the cells with **9** (Figure 2a). The fluorescence within the cell is nonuniform; some regions appear considerably brighter than the surrounding cellular material (Figure 2b). Recent reports have described similar "punctate" staining with other fluorescent staining agents for intracellular Zn^{2+} in fibroblast cell lines and attributed this feature to the existence of intracellular vesicles containing elevated concentrations of Zn^{2+}.[2c,d] It is likely that the nonuniform staining of cells by **9** results from a similar intracellular distribution of Zn^{2+}. The punctate fluorescence staining was reversed on incubation with 20 μM (*N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), a membrane permeable metal chelating agent,[2d] and not reversed by incubation with 20 μM EDTA (which does not transport through the cell membrane).[2c] Consequently, the observed fluorescence from **9** can be said to result from metal-bound probe located within the cell.

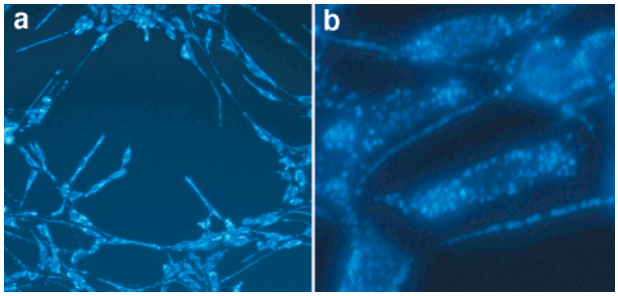

Figure 2. Fluorescence microscope images of NIH/3T3 mouse fibroblasts labeled with **9** in metal free Hank's balanced salt solution. (a) Wide field view of cells loaded with **9** (all cells take up the ligand). (b) Image acquired at higher magnification of cells showing nonuniform "punctate" patterning of intracellular fluorescence following incubation with **9** (10 μM, 45 min, 21 °C).

In summary, the quantum yields of new derivatives **7**−**9**, when complexed to Zn^{2+} in pseudobiological conditions, are superior to quinoline derived probes reported in the literature. The affinity of these simple species for zinc may make them useful probes of cellular events associated with elevated zinc levels.[2b,14] Moreover, these new derivatives are very good candidates for subsequent incorporation into sensor designs with an extended superstructure[15] for sensitive and selective detection of zinc in biological and environmental applications, studies of which are in progress.

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Supporting Information Available: Experimental details, including synthetic procedures, relevant spectra, pH profiles, and metal ion selectivity of **9**, determination of quantum yields, and imaging of probe-loaded cells (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) (a) Chen, R. F.; Kernohan, J. C. *J. Biol. Chem.* **1967**, *242*, 5813−5823. (b) Chen, R. F. *Anal. Biochem.* **1967**, *19*, 374−387.

(11) (a) Compano, R.; Ferrer, R.; Guiteras, J.; Prat, M. D. *Analyst* **1994**, *119*, 1225−1228. (b) Nowicki, J. L.; Johnson, K. S.; Coale, K. H.; Elrod, V. A.; Lieberman, S. H. *Anal. Chem.* **1994**, *66*, 2732−2738.

(12) Job's plot analyses indicate that at pH 7.0 a mixture of 1:1 and 2:1 complexes of Zn(II) is formed. Half-maximal intensity is reached at a $[Zn^{2+}]_{free}$ of 6.0 and 27 μM for **7** and **8**, respectively. This compares well with 8-(*p*-toluenesulfonamido)quinoline indicators such as TSQ and Zinquin (ligands with K_d 1−20 μM), ref 2, and references therein. Cell permeable indicators with higher affinity for Zn^{2+} have been reported in the literature, including refs 2c and 4.

(13) Of the biologically relevant metal ions, Cu^{2+}, Fe^{3+}, Na^+, and K^+ do not form fluorescent complexes with the ligands. Only Cu^{2+}, and to a lesser extent Fe^{3+}, displaced Zn^{2+} from the corresponding complexes. Levels of both species, particularly copper, are highly regulated in cells (Rae, T. D.; Schmidt, P. J.; Pufahl, R. A.; Culotta, V. C.; O'Halloran, T. V. *Science* **1999**, *284*, 805−808). Addition of Ca^{2+} and Mg^{2+} resulted in weak fluorescence responses. Fluorescence emission from **9**, either in the apo form or the Zn^{2+} complex, was essentially independent of pH in the pH 4−8 range. The affinity of **9** for zinc is similar to that of **8**; low solubility of complexes of **9** made it difficult to obtain a precise value for an apparent K_d. Imaging experiments were performed with either the presence or absence of **9** in the media. In the second instance, some loss of intracellular fluorescence intensity was observed after an extended period of observation (ca. 45 min).

(14) (a) Frederikson, C. J.; Suh, S. W.; Silva, D.; Frederickson, C. J.; Thompson, R. B. *J. Nutr.* **2000**, *130*, 1471S−1483S. (b) Cole, T. B.; Wenzel, H. J.; Kafer, K. E.; Schwartzkroin, P. A.; Palmiter, R. D. *Proc. Natl. Acal. Sci.* **1999**, *96*, 1716−1721.

(15) Walkup, G. K.; and Imperiali, B. *J. Org. Chem.* **1998**, *63*, 6727−6731.