Tetrakis(2-quinolinylmethyl)ethylenediamine (TQEN) as a new fluorescent sensor for zinc

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A new fluorescent sensor for zinc that binds 1 equivalent of zinc ion, N,N,N',N'-tetrakis(2-quinolylmethyl)-ethylenediamine (TQEN), has been prepared and characterized. Zinc-bound TQEN exhibits fluorescence around 383 nm upon excitation at 317 nm, while free TQEN emits very weak fluorescence. UV-Vis and ^{1}H NMR spectral changes also detected the binding of TQEN with zinc ion. The crystal structure of zinc complex with TQEN was determined by X-ray crystallography and compared with that of the TPEN–Zn complex (TPEN = N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine). The binding affinity of TQEN with zinc ion is very high ($K_d < 1$ µM in aqueous dmf solution). Competition experiments reveal that the zinc-binding affinity of TQEN is lower than the parent, strong metal ion chelator TPEN, and comparable to EGTA (EGTA = ethylene glycol-bis(2-aminomethyl)-N,N,N',N'-tetraacetic acid).

Introduction

Development of fluorescent zinc sensors is an attractive subject for bioinorganic chemists because zinc plays an important role in cellular events including enzyme catalysis,[1] expression of genes, apoptosis,[2] and neurotransmission.[3] In recent years, fluorescent zinc sensors based on a photoinduced electron-transfer (PET) mechanism of fluorescence have been studied extensively.[2,4–13] In these systems, zinc binding to a heteroatom closely located to a chromophore prevents PET, turning on the fluorescence of the sensor molecule.[14,15] The background fluorescence of the unbound sensor molecule is the important issue for molecular design of these PET-based zinc probes.

On the other hand, coordination-enhanced fluorescence (CEF) is another mechanism to create a fluorescent signal.[14,16] The mechanism of CEF relates to the character of the lowest energy orbital of the singlet excited state of the molecule. Emission from the quinoline molecule in the presence of Lewis acids such as BCl_3 is best described by this mechanism.[17] Upon complexation with zinc ion, the $^1n\pi^*$ state of the quinoline molecule moves to higher energies, which exhibits the $\pi\pi^*$-ground S_0 state emission.[14,17] Several quinoline-based zinc-sensors, including TSQ[18,19] and Zinquin,[20–23] have been developed (Chart 1).[24–26]
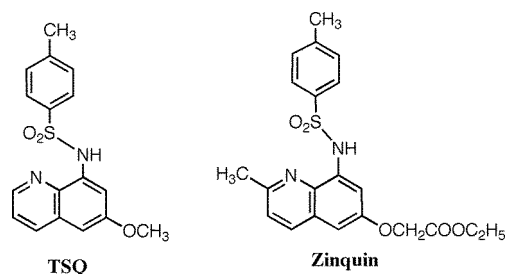

Chart 1

In this article, we wish to introduce N,N,N',N'-tetrakis(2-quinolylmethyl)ethylenediamine (TQEN), the first member of a future family of fluorescent zinc-sensing molecules derived from a well-known heavy metal chelator, N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (Chart 2). Replacing the pyridine ring with the quinoline group affords a sensor that utilizes both PET and CEF mechanisms. Zinc-binding affinity is adequately controlled by reduced donor ability of the ring
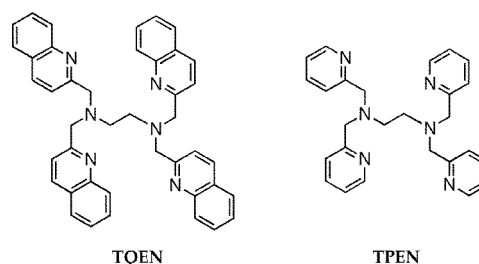

Chart 2

nitrogen atom and steric hindrance derived from the quinoline ring.

Experimental

General information

All reagents and solvents used for ligand synthesis were from commercial sources and used as received. N,N-Dimethylformamide (dmf, Dojin) was distilled over CaH_2 and passed through an alumina column. All aqueous solutions were prepared using deionized and redistilled water. Zinc perchlorate (Strem Chemicals) and the other metal salts were purchased from commercial sources: $Zn(CH_3COO)_2 \cdot 2H_2O$, $MgSO_4$, $CaCl_2$ and $AgNO_3$ (Nacalai); CH_3COONa, $CuCl_2 \cdot 2H_2O$, $Mn(CH_3COO)_2 \cdot 4H_2O$, $Fe(ClO_4)_3$, $Ni(NO_3)_2 \cdot 6H_2O$ and $CdCl_2 \cdot 2.5H_2O$ (Wako Pure Chemical, Co. Ltd); KBr (Ishizu Seiyaku). ^{1}H NMR (300.07 Hz) and ^{13}C NMR (75.00 Hz) spectra were recorded on a Varian GEMINI 2000 spectrometer and referenced to internal TMS or solvent signals. UV-Vis and fluorescence spectra were measured on a Jasco V-700 spectrophotometer and Jasco FP-720 spectrofluorometer, respectively. *CAUTION: Perchlorate salts of metal complexes with organic ligands are potentially explosive. All due precautions should be taken.*

N,N,N',N'-tetrakis(2-quinolylmethyl)ethylenediamine (TQEN). A mixture of 2-chloromethylquinoline hydrochloride (1.07 g, 5 mmol), ethylenediamine (75.0 mg, 1.25 mmol) and potassium carbonate (2.07 g, 15 mmol) in acetonitrile (20 mL) was refluxed for 48 h. After the solvent was removed under reduced pressure, the residue was separated by chloroform/water and the organic phase was dried, evaporated, and the residue was washed with

Table 1 Crystallographic data for TQEN, [Zn(TQEN)](ClO$_4$)$_2$·2.5CH$_3$CN and [Zn(TPEN)](ClO$_4$)$_2$·0.67CH$_3$OH^a

Compounds	TQEN·4CHCl$_3$	[Zn(TQEN)](ClO$_4$)$_2$·2.5CH$_3$CN	[Zn(TPEN)](ClO$_4$)$_2$·0.67CH$_3$OH
Formula	C$_{46}$H$_{40}$Cl$_{12}$N$_6$	C$_{47}$H$_{43.5}$Cl$_2$N$_{8.5}$O$_8$Zn	C$_{26.67}$H$_{30.67}$Cl$_2$N$_6$O$_{8.67}$Zn
FW	1102.30	991.67	710.27
Space group	P-1	$C2/c$	$C2/c$
a/Å	10.271(6)	36.5983(14)	41.337(3)
b/Å	11.240(7)	12.5859(2)	9.4307(4)
c/Å	13.251(7)	24.8617(9)	24.1542(17)
α/°	71.05(2)	90	90
β/°	64.21(2)	127.1400(2)	108.421(3)
γ/°	74.27(3)	90	90
V/Å^3	1287.6(13)	9129.0(5)	8933.7(10)
Z	1	8	12
D_{calc}/g cm^{-3}	1.421	1.443	1.584
μ/cm^{-1}	6.83	7.19	10.67
$2\theta_{max}$/°	55.0	55.0	55.0
Temp./K	173	173	173
No. reflns collected	9893	44143	43100
No. reflns used	4492	9318	9086
No. of params	309	638	778
Final $R1$ ($I > 2\theta(I)$)	0.066	0.041	0.055
$wR2$ (all data)	0.172	0.124	0.185
GOF	1.07	1.09	1.44

^{a} $R1 = \Sigma \|F_o| - |F_c\|/\Sigma |F_o|$. $wR2 = [\Sigma w[(F_o{}^2 - F_c{}^2)^2]/\Sigma [w(F_o{}^2)^2]]^{1/2}$.

acetone to yield TQEN as a white powder (0.70 g, 89%). mp 196–198 °C. ^{1}H NMR (CDCl$_3$) δ (ppm): 2.89 (s, 4H), 3.96 (s, 8H), 7.49–7.54 (m, 8H), 7.66–7.71 (m, 8H), 7.87 (d, J = 8.4 Hz, 4H), 7.99 (d, J = 9.0 Hz, 4H). ^{13}C NMR (CDCl$_3$) δ (ppm): 52.58, 61.56, 120.85, 125.95, 127.16, 127.34, 128.88, 129.23, 136.08, 147.40, 160.29. ESI-MS: 625.3070. C$_{42}$H$_{37}$N$_6$ ([M + H]$^+$) requires 625.3074. Anal. Found: C, 80.69; H, 5.92; N, 13.41. C$_{42}$H$_{36}$N$_6$ requires C, 80.61; H, 5.96; N, 13.43.

[Zn(TQEN)](ClO$_4$)$_2$. A mixture containing TQEN (0.31 g, 0.50 mmol) and Zn(ClO$_4$)$_2$ (0.18 g, 0.49 mmol) in CH$_3$CN was stirred at room temperature overnight. All the material was dissolved after 5 min. After a small amount of insoluble material was filtered off, the solvent was removed under reduced pressure and the product was recrystallized from CH$_3$CN–ether to give suitable crystals for X-ray measurement. ^{1}H NMR (CD$_3$CN) δ (ppm): 3.18 (s, 4H), 4.1 (br., 4H), 4.43 (d, J = 17.4 Hz, 4H), 7.3 (br., 4H), 7.46 (d, J = 8.4 Hz, 4H), 7.5–7.7 (m, 4H), 8.07 (d, J = 7.8 Hz, 4H), 8.56 (d, J = 8.4 Hz, 4H). ^{13}C NMR (CD$_3$CN) δ (ppm): 57.00, 63.18, 123.17, 126.45, 129.19, 130.45, 130.79, 132.83, 142.94, 145.72, 160.78. ESI-MS: 787.1766. C$_{42}$H$_{36}$N$_6$ZnClO$_4$ ([Zn(TQEN)]ClO$_4$) requires 787.1773. Anal. Found: C, 55.85; H, 4.18; N, 9.35. C$_{42}$H$_{38}$N$_6$ZnCl$_2$O$_9$ ([Zn(TQEN)](ClO$_4$)$_2$·H$_2$O) requires C, 55.61; H, 4.22; N, 9.26.

[Zn(TPEN)](ClO$_4$)$_2$. [Zn(TPEN)](ClO$_4$)$_2$ was obtained by a similar procedure to that described for the preparation of [Zn(TQEN)](ClO$_4$)$_2$. The product was recrystallized from methanol to yield suitable crystals for X-ray measurement. ^{1}H NMR (CD$_3$CN) δ (ppm): 3.07 (s, 4H), 4.03 (d, J = 17.4 Hz, 4H), 4.31 (d, J = 17.4 Hz, 4H), 7.42–7.48 (m, 8H), 7.98 (dt, J = 1.5, 7.8 Hz, 4H), 8.17 (br., 4H). ^{13}C NMR (CD$_3$CN) δ (ppm): 58.09, 62.21, 125.89, 126.41, 142.08, 148.83, 156.53. ESI-MS: 587.1139. C$_{26}$H$_{28}$N$_6$ZnClO$_4$ ([Zn(TPEN)]ClO$_4$) requires 587.1147. Anal. Found: C, 44.53; H, 4.16; N, 12.01. C$_{26}$H$_{30}$N$_6$ZnCl$_2$O$_9$ ([Zn(TPEN)](ClO$_4$)$_2$·H$_2$O) requires C, 44.18; H, 4.28; N, 11.89.

X-Ray crystallography. Single crystals of TQEN·4CHCl$_3$, [Zn(TQEN)](ClO$_4$)$_2$·2.5CH$_3$CN and [Zn(TPEN)](ClO$_4$)$_2$·0.67-CH$_3$OH were covered by Paraton oil and mounted on a glass fiber. All the diffractions were collected at 173 K on a Rigaku Mercury CCD detector, with monochromated Mo-Kα radiation, operating at 50 kV/40 mA. Data were processed on a PC using CrystalClear Software (Rigaku). Structures were solved

by direct methods (SIR-97 or SHELXS-97) and refined by full-matrix least-squares methods on F^2. Hydrogen atoms were calculated (TQEN·4CHCl$_3$ and [Zn(TQEN)](ClO$_4$)$_2$·2.5CH$_3$CN) or found ([Zn(TPEN)](ClO$_4$)$_2$·0.67CH$_3$OH) and treated as a riding model. Hydrogen atoms in disordered solvent molecules for [Zn(TQEN)](ClO$_4$)$_2$·2.5CH$_3$CN were not included. The crystal data are summarized in Table 1. Selected bond lengths and angles for [Zn(TQEN)](ClO$_4$)$_2$·2.5CH$_3$CN and [Zn(TPEN)](ClO$_4$)$_2$·0.67CH$_3$OH are presented in Table 2.

CCDC reference numbers 246544–246546.

See http://www.rsc.org/suppdata/dt/b4/b411924j/ for crystallographic data in CIF or other electronic format.

Results and discussion

Synthesis and characterization of TQEN.

The new fluorescent zinc-chelator, N,N,N',N'-tetrakis(2-quinolylmethyl)ethylenediamine (TQEN) was synthesized by refluxing an acetonitrile solution of ethylenediamine and four equivalents

Table 2 Selected bond distances (Å) and angles (°) for independent molecules of [Zn(TQEN)](ClO$_4$)$_2$ and [Zn(TPEN)](ClO$_4$)$_2$

|	[Zn(TQEN)](ClO$_4$)$_2$	[Zn(TPEN)](ClO$_4$)$_2$
Zn–N(1)	2.1497(14)	2.180(3), 2.201(2)
Zn–N(2)	2.1681(14)	2.188(3), 2.201(2)
Zn–N(3)	2.1543(14)	2.092(2), 2.094(2)
Zn–N(4)	2.4007(15)	2.203(3), 2.182(2)
Zn–N(5)	2.1271(14)	2.111(2), 2.094(2)
Zn–N(6)	2.3711(15)	2.233(3), 2.182(2)
N(1)–Zn–N(2)	82.17(6)	82.26(10), 82.39(14)
N(1)–Zn–N(3)	76.21(5)	81.00(10), 79.05(10)
N(1)–Zn–N(4)	78.00(5)	79.19(9), 78.81(9)
N(1)–Zn–N(5)	159.40(6)	156.10(10), 157.83(10)
N(1)–Zn–N(6)	92.18(5)	102.76(10), 95.12(9)
N(2)–Zn–N(3)	157.73(6)	156.30(10), 157.83(10)
N(2)–Zn–N(4)	90.63(5)	106.49(9), 95.12(9)
N(2)–Zn–N(5)	77.65(6)	79.82(10), 79.05(10)
N(2)–Zn–N(6)	77.96(5)	78.65(9), 78.81(9)
N(3)–Zn–N(4)	90.06(5)	86.69(10), 93.08(9)
N(3)–Zn–N(5)	124.25(5)	120.41(10), 121.44(14)
N(3)–Zn–N(6)	97.41(5)	88.82(10), 90.82(9)
N(4)–Zn–N(5)	98.01(5)	90.87(9), 90.82(9)
N(4)–Zn–N(6)	165.94(5)	174.77(9), 172.01(14)
N(5)–Zn–N(6)	87.63(5)	89.09(9), 93.08(9)
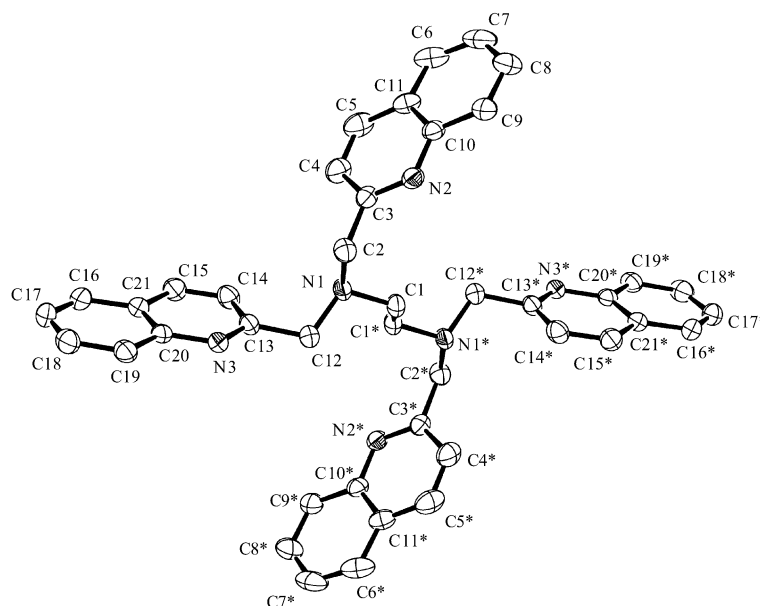
Fig. 1 ORTEP plot for TQEN. The symmetry operation needed to generate the atoms marked with an asterisk.

of 2-chloromethylquinoline hydrochloride in the presence of potassium carbonate. The product was obtained as a white powder in 89% yield and was characterized by ^{1}H and ^{13}C NMR, HRMS and elemental analysis. Single crystals suitable for X-ray analysis were obtained from chloroform solution of TQEN by slow evaporation at room temperature, affording the crystal structure of TQEN·4CHCl$_3$ (Table 1, Fig. 1). TQEN molecules are on the inversion centre in the crystal lattice. The chloroform molecules take part in well-defined CH···N hydrogen bonds to quinoline nitrogen atoms (C$_{chloroform}$ − N$_{quinoline}$ = 3.219(5) and 3.228(5) Å) in the crystal structure. TQEN is soluble in chloroform, dichloromethane and dmf; slightly soluble in acetonitrile and dmso; and almost insoluble in water, acetone, methanol and ethanol.

Fluorescent response of TQEN toward zinc and other metal ions.

In dmf or aqueous dmf solution (dmf : water = 1 : 1), TQEN exhibits very small fluorescence when excited with 317 nm light. Addition of an equimolar amount of zinc to the solution induces a 23 fold enhancement of fluorescence emission (λ_{em} = 383 nm, Fig. 2). A Job plot analysis reveals that TQEN binds one equivalent of zinc ion (Fig. 3). Significant fluorescence enhancement is achieved by the combination of PET and CEF mechanisms in TQEN.

Fig. 4 shows the fluorescent response of TQEN in the presence of other metal ions. Although TQEN emits in the presence
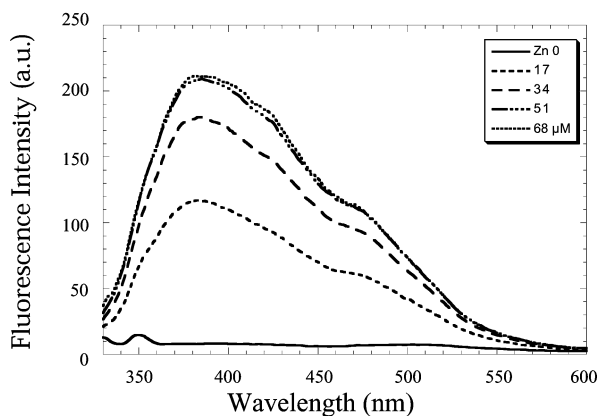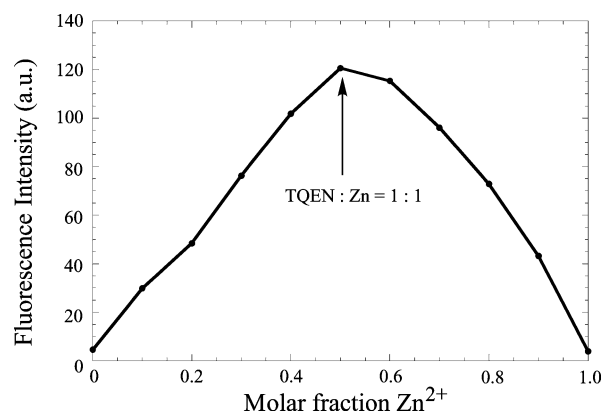

Fig. 3 Job's plot for fluorescent intensity of TQEN–zinc complex in dmf/H$_2$O (1 : 1). The sum of the concentrations of TQEN and zinc(II) is 34μM.

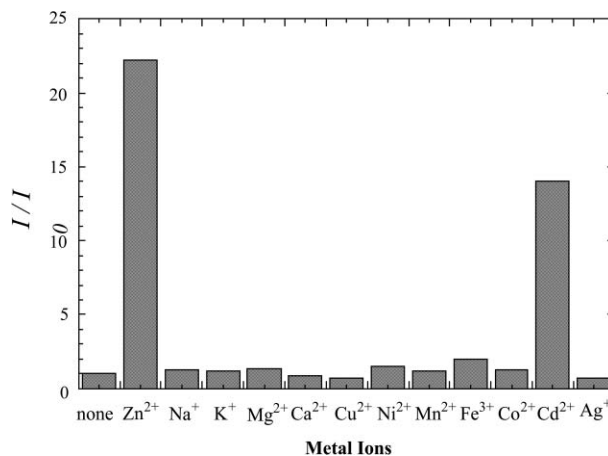**Fig. 4** The relative fluorescence intensity at 383 nm of TQEN (34 μM in dmf/H$_2$O (1 : 1)) responding to 1 equivalent of metal ions at 25 °C (λ_{ex} = 317 nm). I_0 is the emission intensity at 383 nm of TQEN (34 μM) in the absence of metal ions.

of cadmium ion with an intensity around 60% of the zinc–TQEN complex, the other metal ions induced little change in the emission of TQEN. Competition experiments revealed that Na$^+$, K$^+$, Mg^{2+}, Ca^{2+}, Ni^{2+} and Mn^{2+} did not quench the fluorescence of the TQEN–Zn complex (data not shown). On the other hand, zinc ion-induced emission of TQEN is prevented

Fig. 2 Fluorescence spectra (λ_{ex} = 317 nm) of 34 μM TQEN in dmf/H$_2$O (1 : 1) in the presence of various concentration of Zn^{2+} ranging from 0 to 68 μM.

in the presence of equimolar amounts of Cu^{2+}, Co^{2+}, Cd^{2+}, Ag^+ and excess Fe^{3+}, indicating the preferred complex formation with these metal ions (data not shown). The absence of fluorescence with these transition metals is probably due to the fluorescence quenching mechanism by the electron or energy transfer between fluorophore and metal cation.[6,8,14] The order in binding affinity (Fe < Cu > Zn) is in agreement with the Irving–Williams series of complex stability.[27]

pH Effect on fluorescent zinc ion sensing of TQEN.

The fluorescence of this type of zinc sensor is often pH-sensitive.[10,28] In the PET systems, the heteroatom (in many cases, nitrogen atom) responsible for zinc binding also binds proton enhancing the fluorescence of the chromophore in the low pH region. Using the aniline nitrogen instead of aliphatic amine as the zinc-binding PET heteroatom lowers the pK_a, shifting the pH-sensitive region towards the low pH region.[7,11] Fig. 5 shows the effect of pH for zinc-induced fluorescence of TQEN, indicating that TQEN is able to act as a fluorescent zinc sensor in a wide pH range. In this probe, protonation at the aliphatic nitrogen does not turn on the fluorescence of TQEN because PET quenching is not the sole mechanism for the fluorescent response of TQEN. Considering the fact that quinaldine (2-methylquinoline) exhibits little fluorescence at neutral pH under the present experimental conditions, the small fluorescent response around pH 4–5 in Fig. 5 is probably from 'PET-off' TQEN·2H$^+$, that has protonated aliphatic nitrogens and proton-free quinoline nitrogens. Subsequent protonation at the quinoline nitrogen in the lower pH region quenches the fluorescence by a secondary PET process between quinoline chromophores.[28]
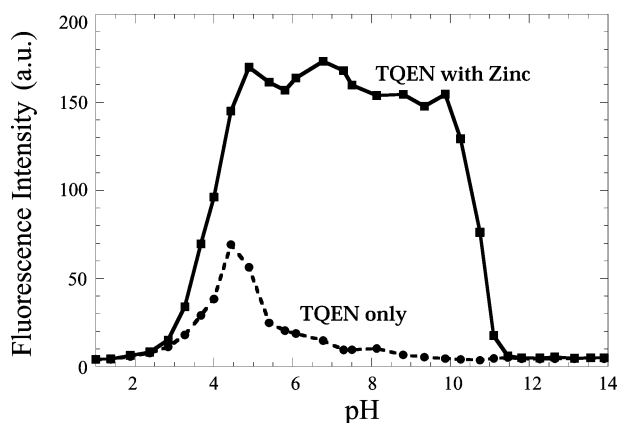

Fig. 5 Effect of pH on fluorescence intensity at 383 nm of TQEN [34 μM in dmf/H_2O (1 : 1)] in the absence (●) and presence (■) of Zn^{2+} ion (1 equiv.) at 25 °C (λ_{ex} = 317 nm).

Absorbance change of TQEN with zinc and other metal ions.

TQEN was titrated with zinc and monitored by UV-Vis spectrometry (Fig. 6). The change in absorption exhibits an isosbestic point at 284 nm. The metal ions in the former group in the competition experiment with zinc (Na^+, K^+, *etc.*, see previous section) did not induce any UV-Vis spectral changes for TQEN, however, the latter group of metals except for Fe^{3+} (Cu^{2+}, Co^{2+}, *etc.*) induced a similar spectral change with zinc ion as observed in Fig. 6.

NMR titration of TQEN with zinc ion.

NMR titration of TQEN by zinc ion in $CDCl_3$: CD_3CN (3 : 1 (v/v)) exhibited separated peaks for free TQEN and zinc-bound TQEN, indicating that the exchange rate for free TQEN and Zn-bound TQEN is slow compared to NMR timescales (Fig. 7). The protons in the benzylic position of zinc-bound species exhibited doublet and broad signals due to geminal
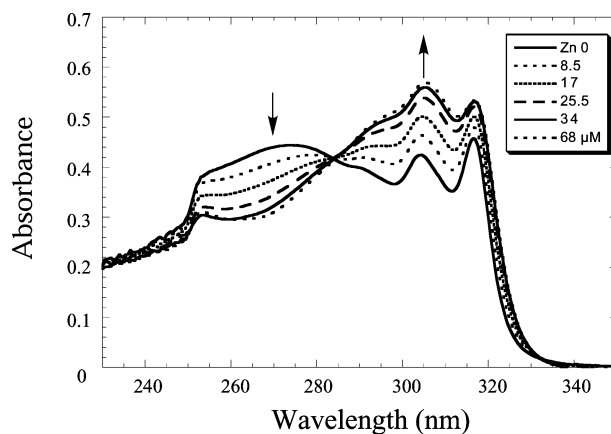

Fig. 6 UV-Vis spectra of 34 μM TQEN in dmf/H_2O (1 : 1) in the presence of various concentrations of Zn^{2+} ranging from 0 to 68 μM.

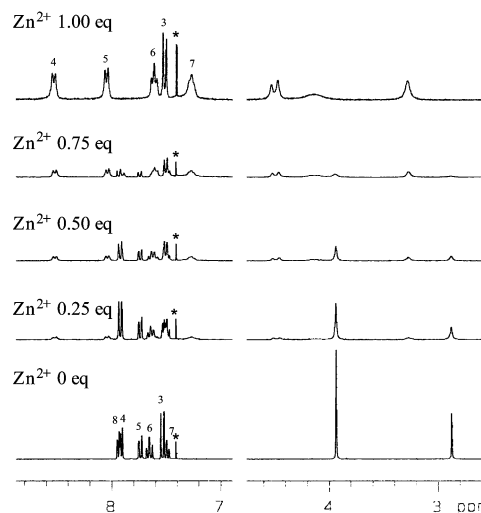**Fig. 7** Partial ^1H NMR spectra for TQEN in $CD_3CN/CDCl_3$ (1 : 3) in the presence of increasing amounts of zinc ion. The asterisk indicates the solvent peak.

coupling by the restriction of conformational freedom in the zinc complex. Careful investigation of the aromatic region of the TQEN–Zn complex reveals that the H-8 (peri position to the ring nitrogen) proton signal becomes very broad. This significant broadening should be as a result of the combination of metal contact shift and conformation effect upon complex formation (*vide infra*). Two signals in the lower field are assigned to H-4 and H-5, indicating that zinc coordination to the nitrogen atom reduces electron density of the quinoline ring.

### Crystal structure of Zn^{2+}-complexes of TQEN and TPEN.

The binding mode of TQEN and TPEN toward zinc ion was revealed by X-ray crystallography (Fig. 8 and 9). Suitable crystals for X-ray crystallography were obtained in CH_3CN–ether solution for the TQEN–Zn complex and MeOH solution for the TPEN–Zn complex. The details for the X-ray measurement and crystal data are summarized in Table 1. TPEN–Zn complex has two crystallographically independent molecules with slightly different structures, one of these structures is shown in Fig. 9. The selected bond distances and angles are listed in Table 2. Although several metal complexes of TPEN have been characterized by X-ray crystallography,[29–37] the structure determination for zinc-bound TPEN has been accomplished for the first time in this study.

TQEN and TPEN bind zinc ion by six nitrogen atoms, affording a distorted octahedral coordination environment and helical structure around the zinc center with two different
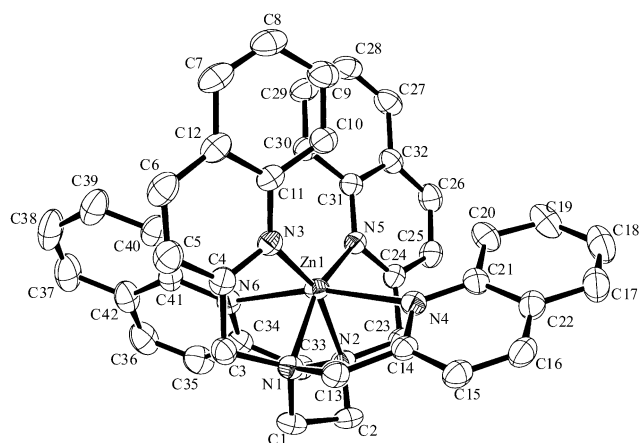
Fig. 8 ORTEP plot for the cation part of [Zn(TQEN)]·(ClO$_4$)$_2$· 2.5CH$_3$CN.
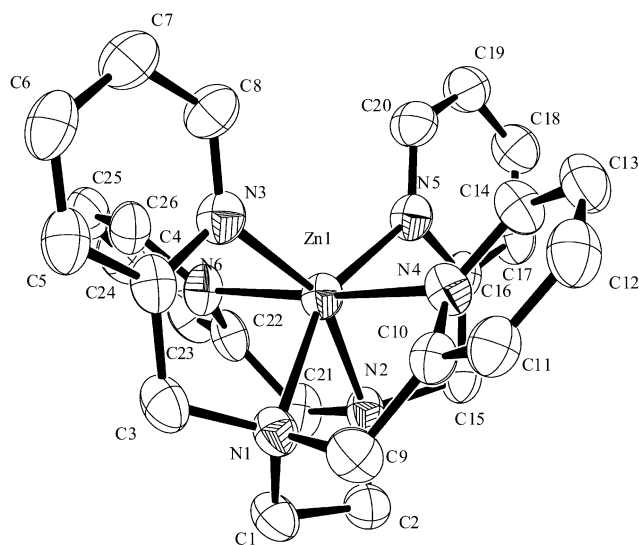
Fig. 9 ORTEP plot for cation part of one of two crystallographically independent molecules of [Zn(TPEN)]·(ClO$_4$)$_2$·0.67CH$_3$OH.

aromatic ring environments. The bond lengths for N1,N2–Zn are slightly shortened for the TQEN complex with slight elongation of the N3,N5–Zn bonds compared to the TPEN–Zn complex. Significant bond elongation for N4,N6–Zn bonds is observed for the TQEN–Zn complex. Two of four pyridine rings of the TPEN–Zn complex, which are in *trans* geometry against the aliphatic nitrogen, are located in almost the same plane with a twist angle of ~8°. For the TQEN–Zn complex, steric hindrance between two closely located quinoline rings (with a twist angle of ~22°) forces all four quinoline rings into the propeller-like configuration.

The NMR spectrum of the TQEN–Zn complex (Fig. 7) suggests that the helix inversion is faster than the NMR timescale at room temperature, exhibiting mean signals of the two proton environments corresponding to each conformer. The large chemical shift difference between the two H-8 protons in different quinoline environments, owing to metal contact shift and conformation effect upon complex formation, and possibly partial dissociation of the quinoline coordination due to steric hindrance cause the significant broadening of this signal (Fig. 6). These structural and coordination features should provide a lot of information for the molecular design of next generation TQEN derivatives.

Binding affinity of TQEN with zinc ion.

The estimation of the binding constant for TQEN to Zn has not yet been measured because the binding affinity is too high (K_d < 1 µM) to calculate directly from the titration data based on the fluorescent or absorption spectral change.[23] The determination of accurate binding constant requires a suitable competitor molecule whose binding constant with zinc ion is known in the experimental media. Such an experiment will be performed when water-soluble TQEN derivatives become available. As a preliminary assessment, competition experiments were performed (Fig. 10). In the presence of 1 equiv. of TPEN, complete quenching of the emission from the TQEN– Zn complex was achieved, suggesting that the zinc ion from the TQEN–Zn complex was extracted by the strong zinc chelator TPEN. However, the zinc was not completely removed from the TQEN–Zn complex using 1 equiv. of EGTA [ethylene glycol-bis(2-aminomethyl)-N,N,N′,N′-tetraacetic acid, K_{Zn} = ~10^9 M]. TQEN–Zn fluorescence was ~40% quenched by EGTA. These data indicate that TQEN binds to the zinc ion with a similar affinity to EGTA. Reduced donor ability of the ring nitrogen atom and steric hindrance derived by the quinoline ring plays an important role for lowering the zinc-binding affinity.
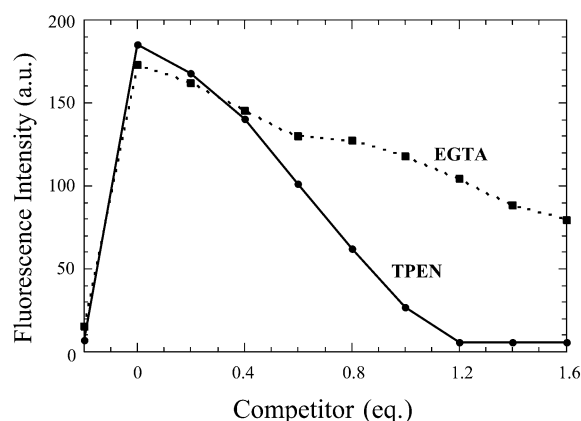
Fig. 10 Effect of competitor on fluorescence intensity (at 383 nm) of the TQEN–Zn complex. Spectra were recorded for 34 µM TQEN and Zn^{2+} in dmf/H$_2$O (1 : 1) with increasing amounts of TPEN or EGTA.

Conclusions

A new hexadentate nitrogen ligand, TQEN is the first member of a new family of fluorescent zinc sensor molecules based on TPEN-like structures. Replacement of the pyridine ring of TPEN with a quinoline ring provides fluorescent properties and moderate zinc-binding affinity. Among the fluorescent sensors for metal ions reported to date,[38–40] TQEN is easily prepared and the simple scaffold has several unique properties as a fluorescent sensor platform. Introduction of appropriate substituents into TQEN will improve the optical properties and water-solubility of TQEN, in addition modification of the skeleton of heteroaromatics should provide a wide variety of molecules of significant interest. Such studies are now in progress in our laboratory.

Acknowledgements

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