Interaction of Triapine and related thiosemicarbazones with iron(III)/(II) and gallium(III): a comparative solution equilibrium study

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SUPPORTING INFORMATION

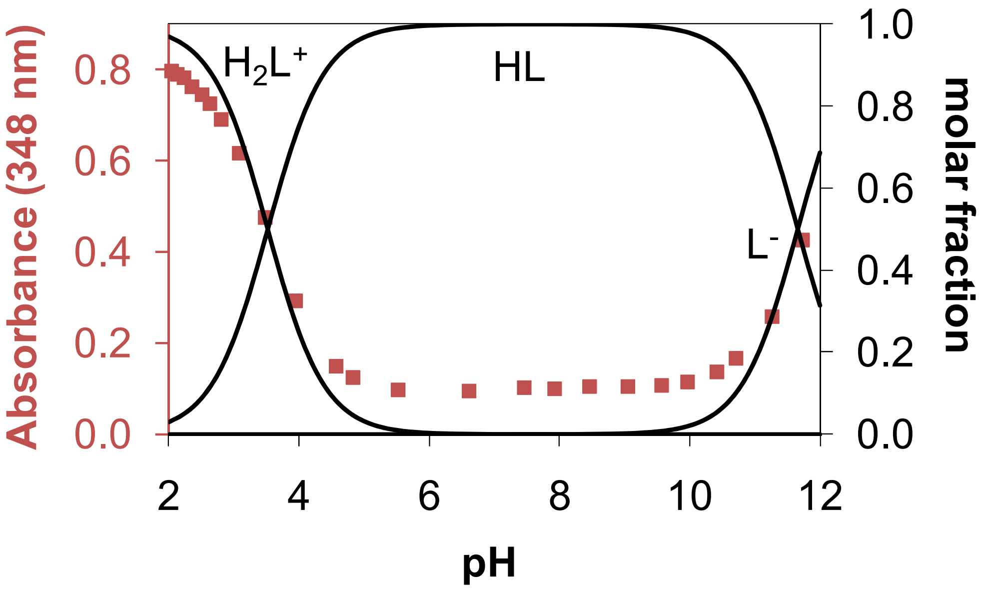

Fig. S1 Concentration distribution curves for species of AcTSC calculated with the constants obtained by UV–vis spectrophotometry depicted together with the absorbance values plotted against pH at 348 nm (■) {$c_{AcTSC} = 5 \times 10^{-5}$ mol dm^{-3}; $t = 25.0$°C, $I = 0.10$ mol dm^{-3} (KCl) in 30% (w/w) DMSO/H_2O}.

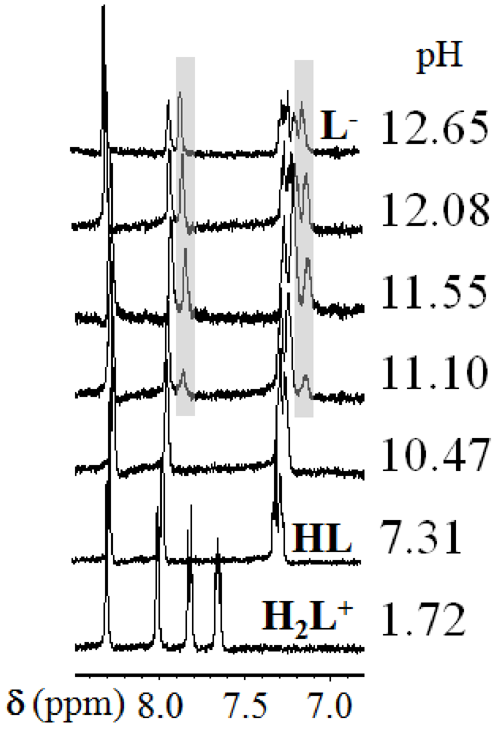

Fig. S2 ^{1}H NMR spectra of Triapine recorded at different pH values {$c_{ligand} = 4.0 \times 10^{-3}$ mol dm^{-3} in 30% (w/w) [D_6]DMSO/D_2O}. For the proton assignments see Table S1 in ref. 14.

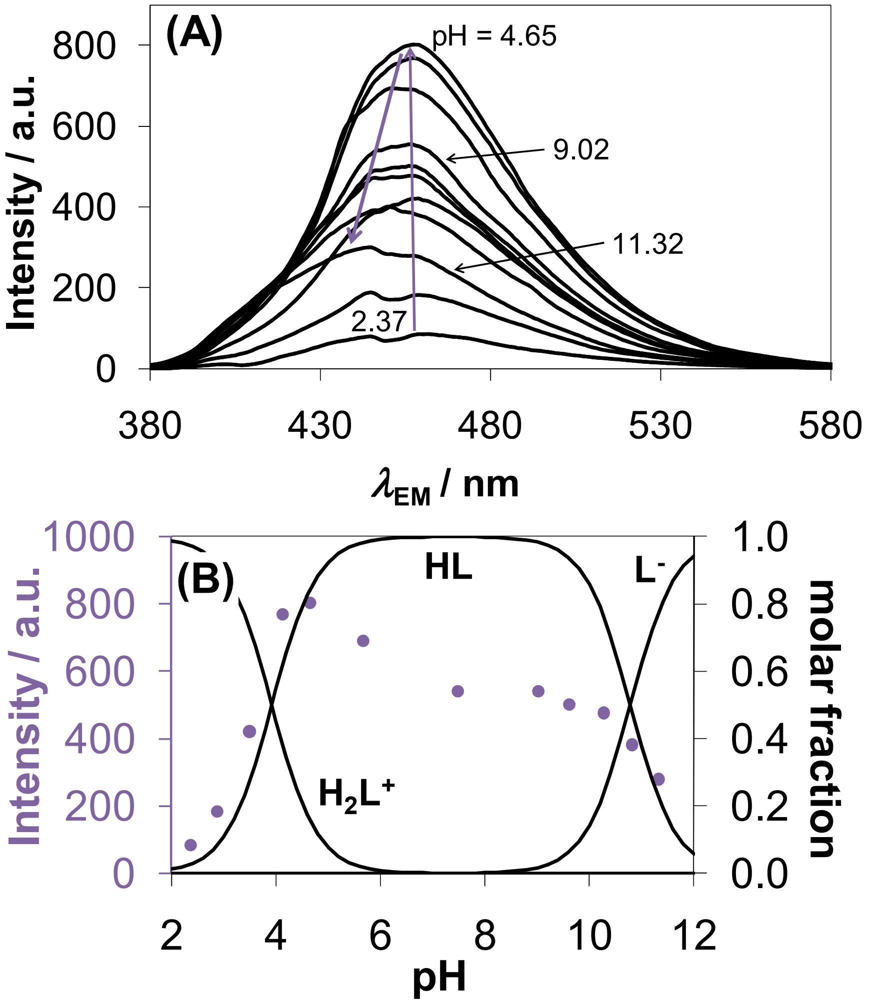

Fig. S3 Fluorescence emission spectra of Triapine recorded at different pH values (**A**) and concentration distribution curves for Triapine with the intensity of emission recorded at 458 nm (**B**) {λ_{EX} = 360 nm; $c_{ligand} = 1.02 \times 10^{-5}$ mol dm^{-3}; t = 25.0 °C, I = 0.10 mol dm^{-3} (KCl) in 30% (w/w) DMSO/H_2O}

Definition of the stability constants of the metal complexes collected in Table 4

General formula:

M = metal ion; L= deprotonated form of the ligand; H = H^+

Charges are omitted for simplicity.

$\beta(M_pL_qH_r) = [M_pL_qH_r]/[M]^p[L]^q[H]^r$	$pM + qL + rH \rightleftharpoons M_pL_qH_r$
constant:	equilibrium:
$\beta(\mathbf{MLH}) = [MLH]/[M][L][H]$	$M + L + H \rightleftharpoons MLH$
$\beta(\mathbf{ML}) = [ML]/[M][L]$	$M + L \rightleftharpoons ML$
$\beta(\mathbf{MLH_{-1}}) = [MLH_{-1}]/[M][L][H]^{-1}$	$M + L - H \rightleftharpoons MLH_{-1}$

(*-H = deprotonation of a coordinated water molecule)*

$\beta(\mathbf{ML_2H}) = [ML_2H]/[M][L]^2[H]$	$M + 2\ L + H \rightleftharpoons ML_2H$
$\beta(\mathbf{ML_2}) = [ML_2]/[M][L]^2$	$M + 2\ L \rightleftharpoons ML_2$
$\beta(\mathbf{ML_2H_{-1}}) = [ML_2H_{-1}]/[M][L]^2[H]^{-1}$	$M + 2\ L - H \rightleftharpoons ML_2H_{-1}$

(-H = deprotonation of a coordinated water molecule or coordination of OH^-*)*

Derived stability constants:

$\log\beta([ML_2]) - 2\times\log\beta(H_2L) = \log K'$

$K' = [ML_2]\ [H]^4\ /[M][H_2L]^2$ $\qquad M + 2\ H_2L \rightleftharpoons ML_2 + 4\ H$

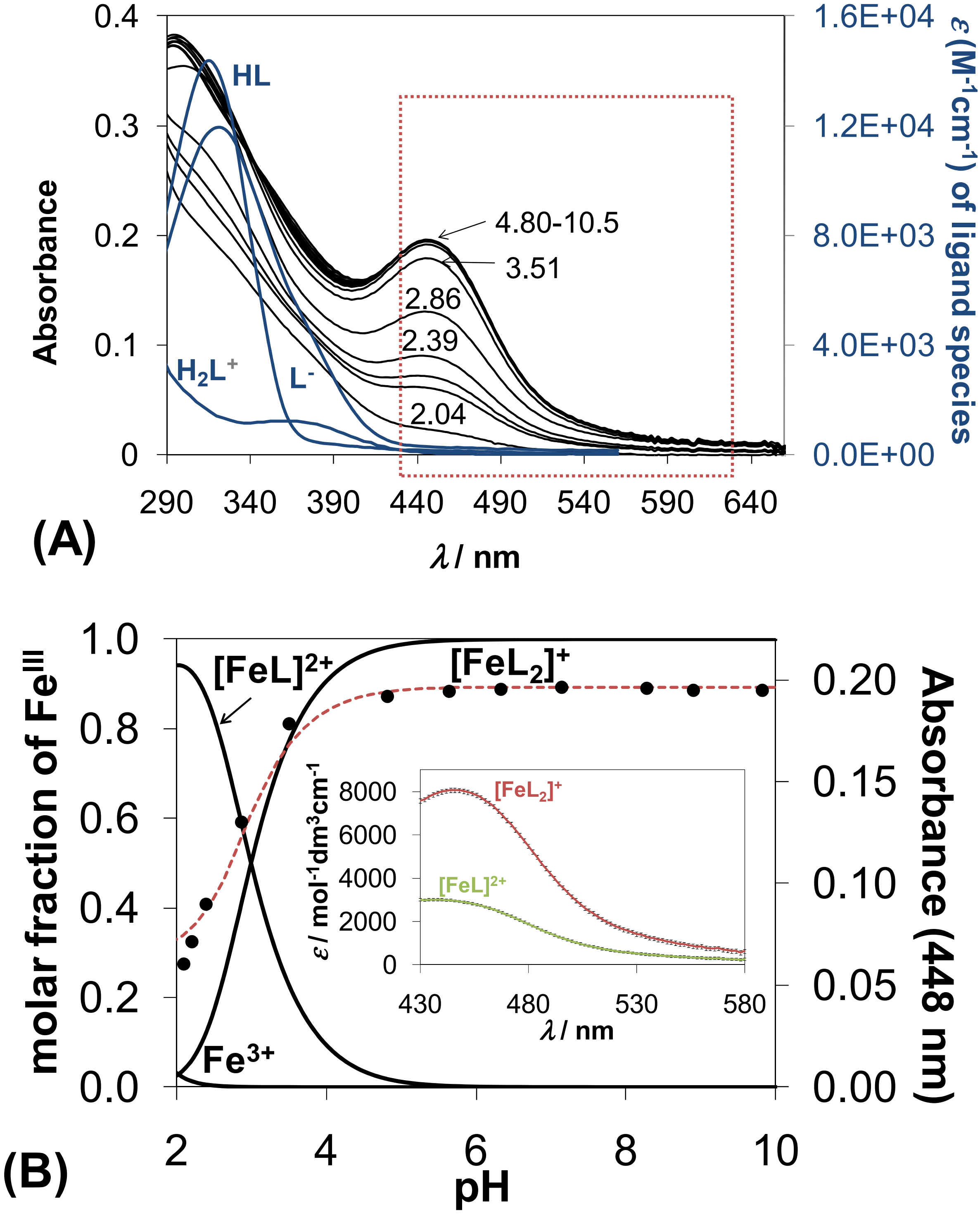

Fig. S4 UV–vis absorption spectra of Fe^{III} – FaTSC system recorded at different pH values together with the molar absorptivity values of the individual ligand species (blue lines) (**A**). Concentration distribution curves of complexes formed in the Fe^{III} – FaTSC system plotted against the pH with the measured (●) and calculated (red dashed line) absorbance values at 448 nm. *Inset shows the calculated individual spectrum of the* $[Fe^{III}L]^{2+}$ *and* $[Fe^{III}L_2]^{+}$ *species.* (**B**){$c_{ligand} = 5.0 \times 10^{-5}$ mol dm^{-3}; M:L = 1:2; t = 25.0°C, I = 0.10 mol dm^{-3} (KCl) in 30% (w/w) DMSO/H_2O}

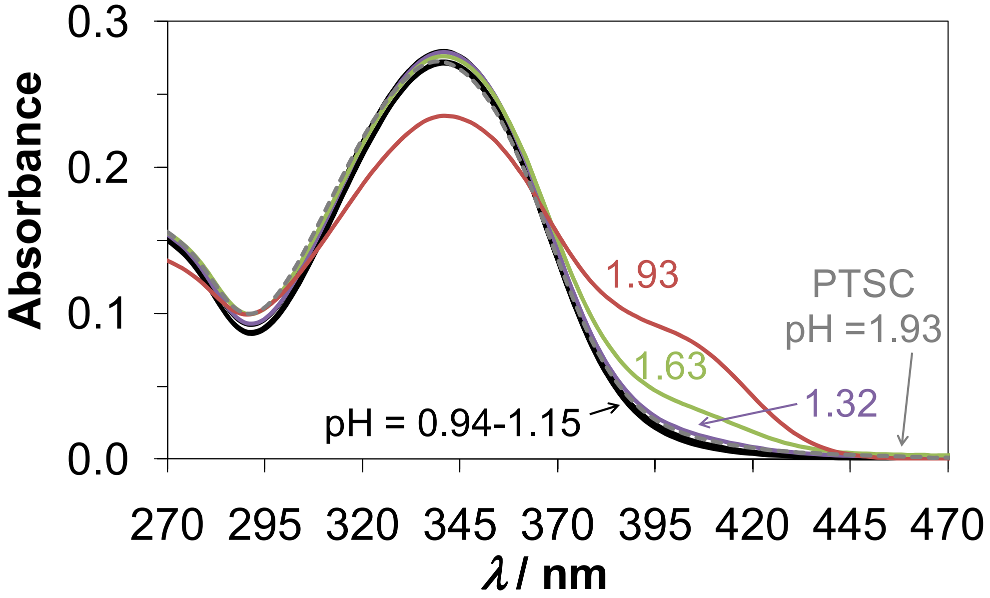

Fig. S5 UV□vis absorption spectra of Ga^{III} – PTSC system recorded at different pH values: pH < 2; $c_{ligand} = 2.0 \times 10^{-5}$ mol dm^{-3}; M:L = 1:1 (solid lines) and 0:1 (grey dashed line). {$t = 25.0$˚C, $I = 0.10$ mol dm^{-3} (KCl) in 30% (w/w) DMSO/H_2O}

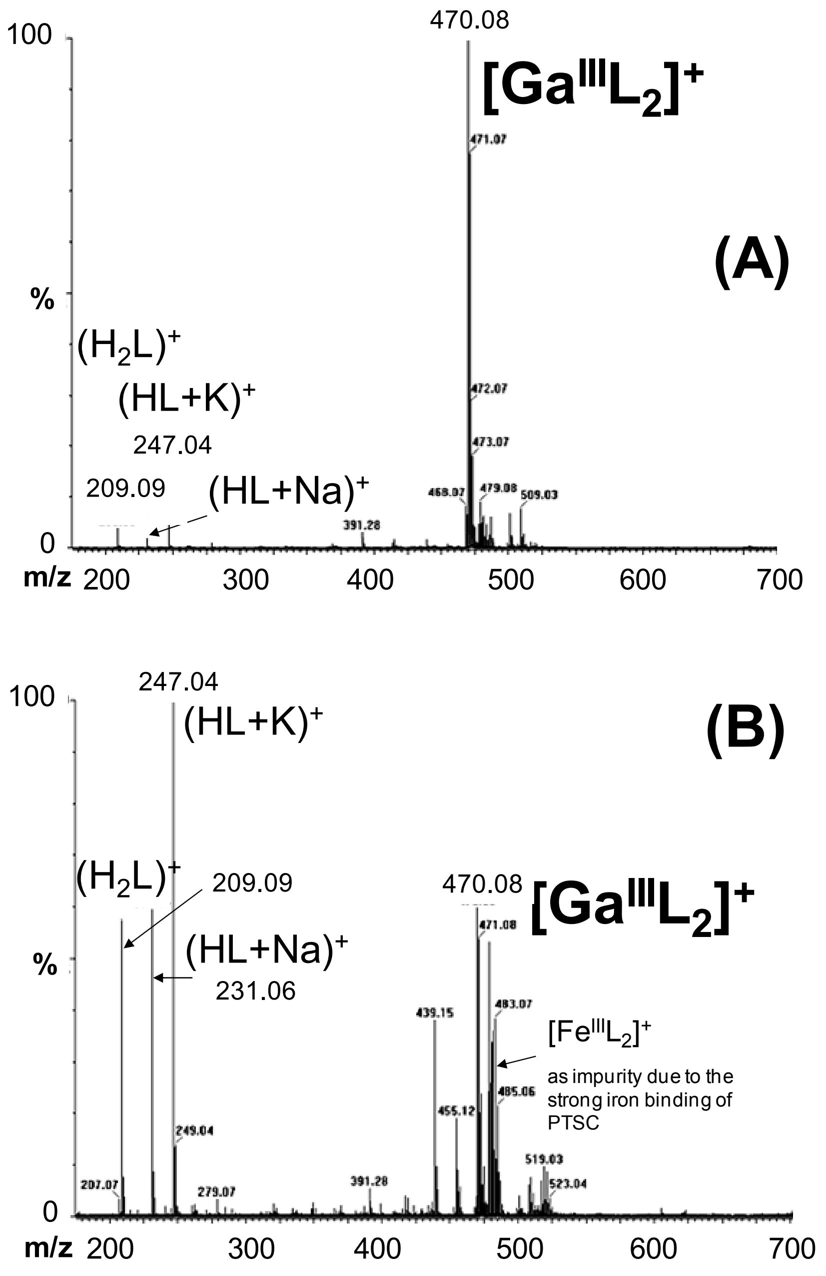

Fig. S6 ESI-MS spectra recorded in the Ga^{III} – PTSC system at pH 4.5 (**A**) and pH 9.5 (**B**) in positive mode at metal-ion-to-ligand ratio 1:2, $c_{ligand} = 1 \times 10^{-4}$ mol dm^{-3}.

(ESI-MS spectra were recorded on a Waters Q-TOF Premier instrument (Micromass MS Technologies, Manchester, UK) operating in positive ion mode. Samples were introduced into the ESI source by the syringe pump of the instrument. N_2 was used as nebulizer and cone gas, and the source temperature was set to 120 °C. The capillary voltage was set to 3.8 kV. Argon was employed as the collision gas and the collision energy was –25 eV. pH was set by the addition of aqueous NH_3 and HNO_3.)

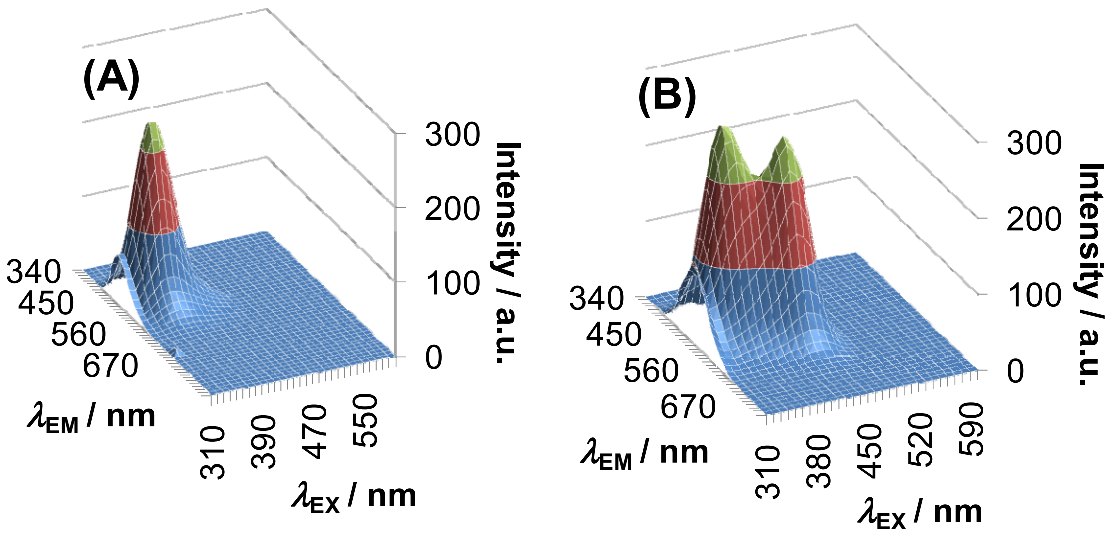

Fig. S7 3D fluorescence spectra of Triapine (A) and Ga^{III} – Triapine (B) systems {c$_{ligand}$ = 4.0 × 10^{-6} mol dm^{-3}; Ga^{III} : Triapine = 1:1; pH = 3.70; λ_{EX} = 300-600 nm; t = 25.0 ˚C, I = 0.10 mol dm^{-3} (KCl)}

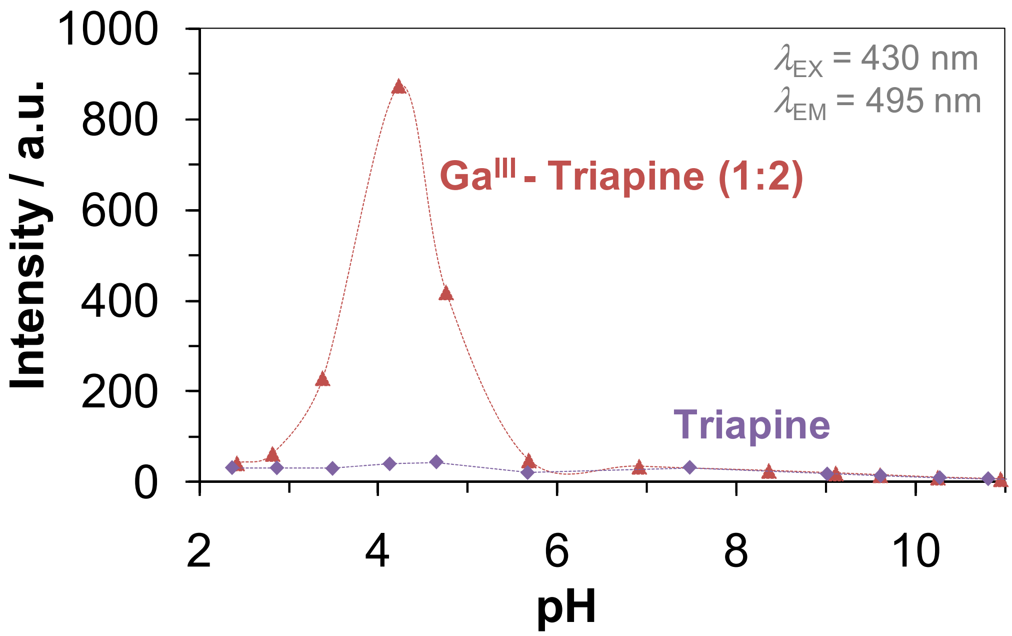

Fig. S8 Fluorescence emission intensity recorded at 495 nm for Triapine and for Ga^{III}-Triapine (1:2) system at different pH values {λ_{EX} = 430 nm; c$_{ligand}$ = 1.00 × 10^{-5} mol dm^{-3}; t = 25.0 ˚C, I = 0.10 mol dm^{-3} (KCl) in 30% (w/w) DMSO/H_2O}

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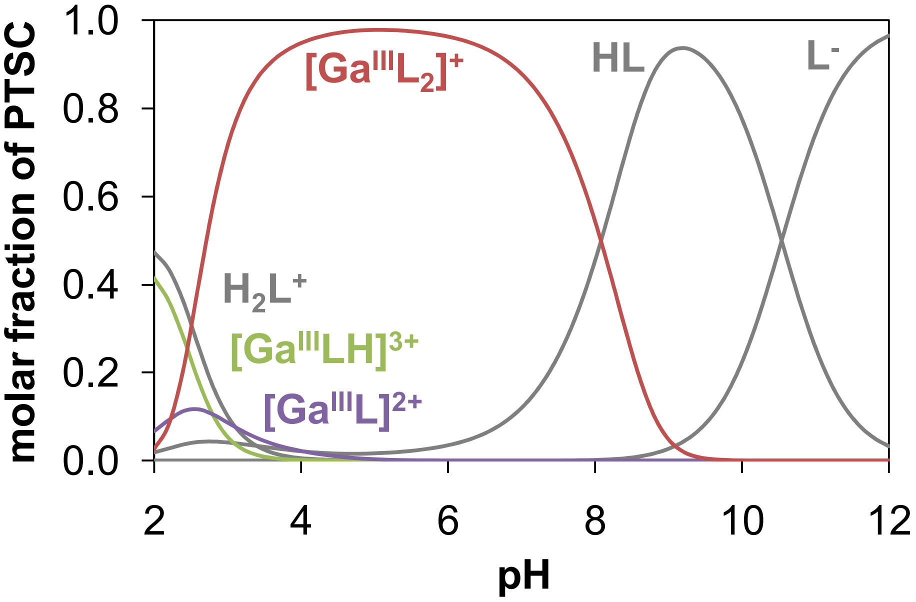

Fig. S9 Concentration distribution curves of species formed in the Ga^{III} – PTSC system plotted against the pH at metal-ion-to-ligand ratio 1:2 calculated on the basis of the pH-potentiometric results, $c_{ligand} = 1 \times 10^{-3}$ mol dm^{-3}. {ligand species (grey lines); Ga^{III} containing species (colored lines)}

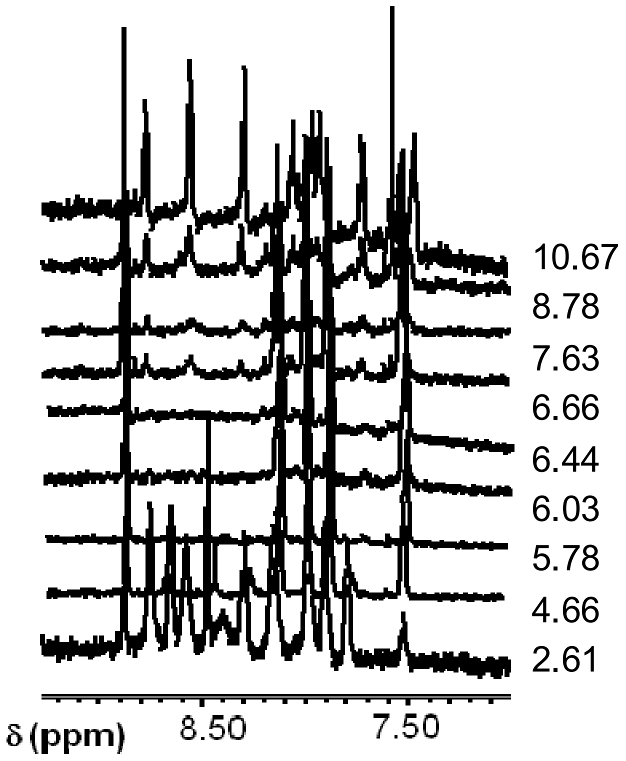

Fig. S10 ^{1}H NMR spectra of Ga^{III} – PTSC system recorded at different pH values at metal-ion-to-ligand ratio 1:2 {$c_{ligand} = 1.0 \times 10^{\square 3}$ mol dm$^{\square 3}$ in 30% (w/w) [D$_6$]DMSO/D$_2$O}.

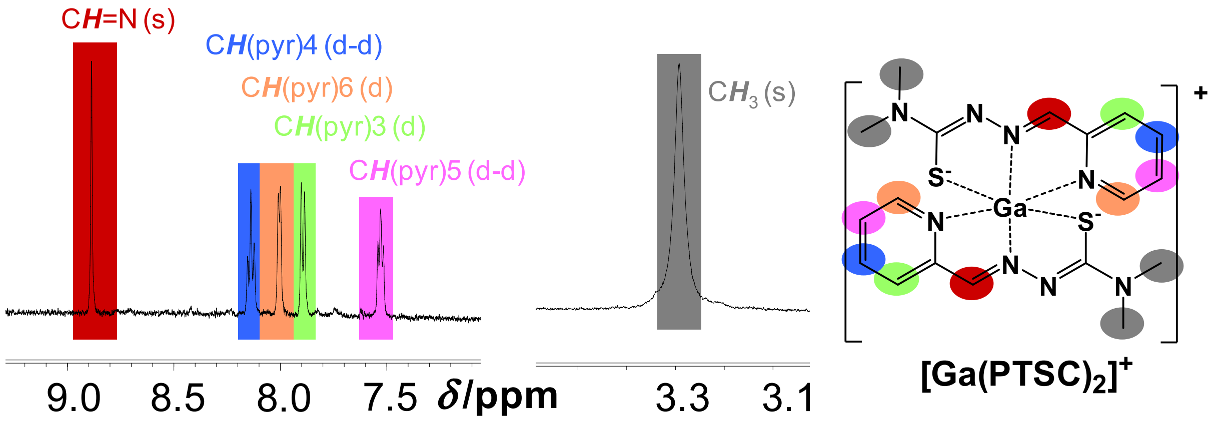

Fig. S11 ^{1}H NMR spectra of [GaL$_2$]$^+$ complex of PTSC {$c_{ligand} = 1.0 \times 10^{\square 3}$ mol dm$^{\square 3}$; M:L = 1:2; 30% (w/w) [D$_6$]DMSO/D$_2$O} and proton assignments.

Table S1 Cytotoxicity of R-N-Heterocyclic TSCs and their Ga^{III} and Fe^{III} *bis*-ligand complexes in two different human cancer cell lines (IC_{50} values in μmol dm^{-3})[a]

cell line	species tested	Triapine	FTSC	PTSC	APTSC	FaTSC	AcTSC
41M	HL	0.45	2.9	0.0040	0.210	4.9	2.5
	$[Ga^{III}L_2]^{+b}$	0.50	3.0	0.0058	0.148	6.8	2.4
	$[Fe^{III}L_2]^{+b}$	3.0	5.4	0.22	10.4	>200	56
	ratio[c]	*6.7*	*1.9*	*55*	*50*	*41*	*22*
SK-BR-3	HL	0.52	3.2	0.0098	0.290	5.6	3.6
	$[Ga^{III}L_2]^{+b}$	0.70	3.0	0.0100	0.162	9.2	2.0
	$[Fe^{III}L_2]^{+b}$	3.4	9.8	0.30	12.0	>200	>200
	ratio[c]	*6.5*	*3.1*	*31*	*41*	*35*	*56*

[a] Taken from ref. 8. [b] The IC_{50} values are doubled for the comparison as the *bis*-ligand complexes consist of two ligands. [c] IC_{50} of $[Fe^{III}L_2]^+$/ IC_{50} of HL.