Electronic Supplementary Information

A novel pyrazole based single molecular probe for multi-analyte (Zn^{2+} and Mg^{2+}) detection in Human gastric adenocarcinoma cells

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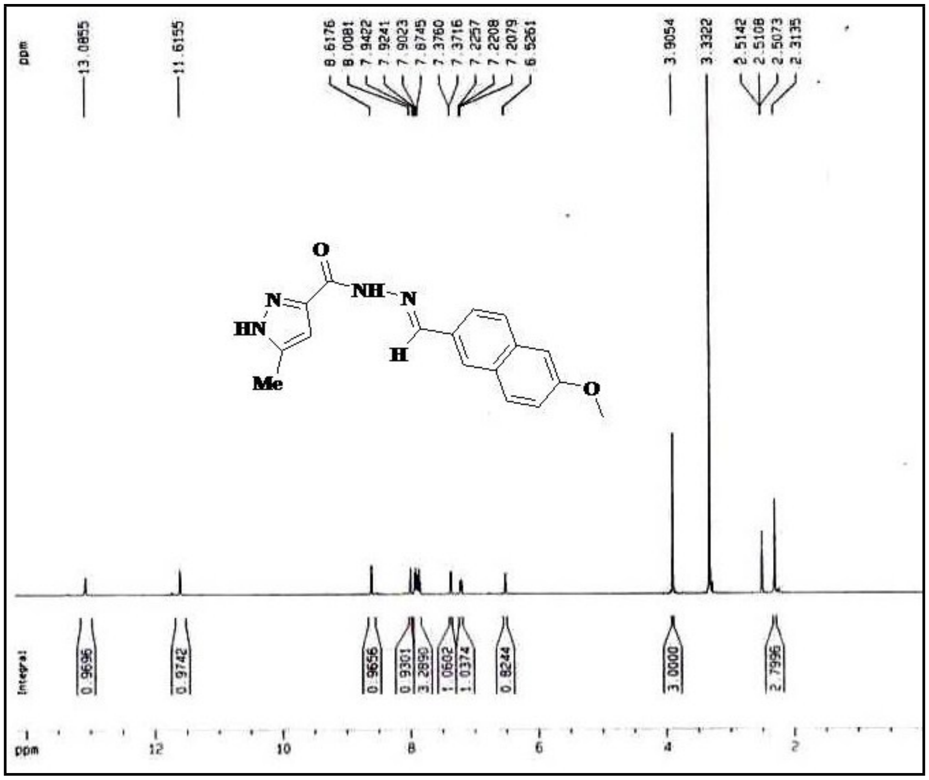

Fig. S1 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of the probe **PYN**.

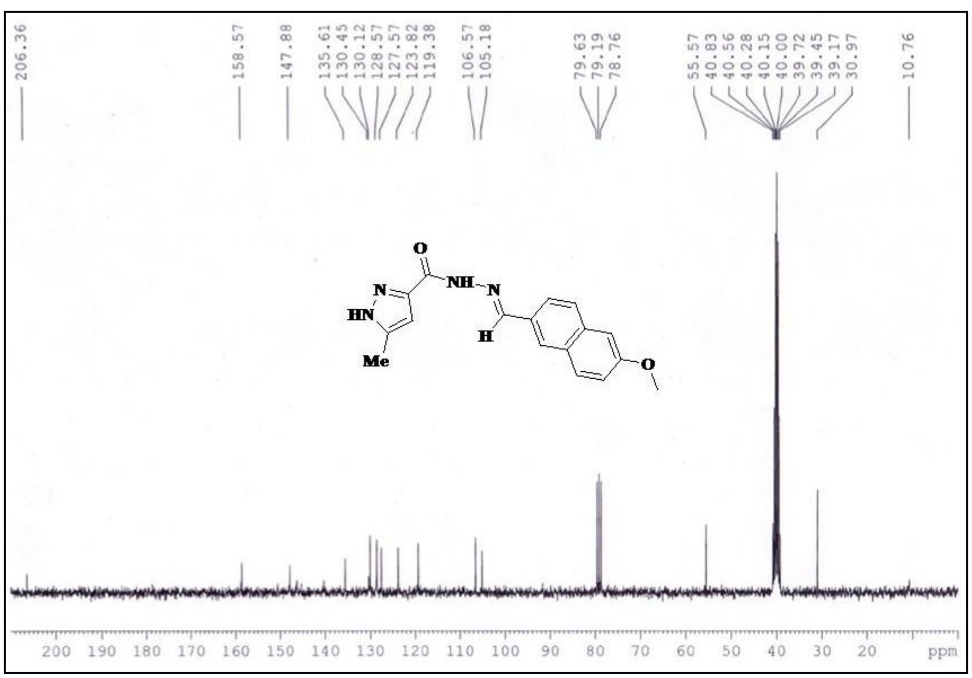

Fig. S2 ^{13}C NMR (DMSO-d_6, 300 MHz) spectrum of the probe **PYN**.

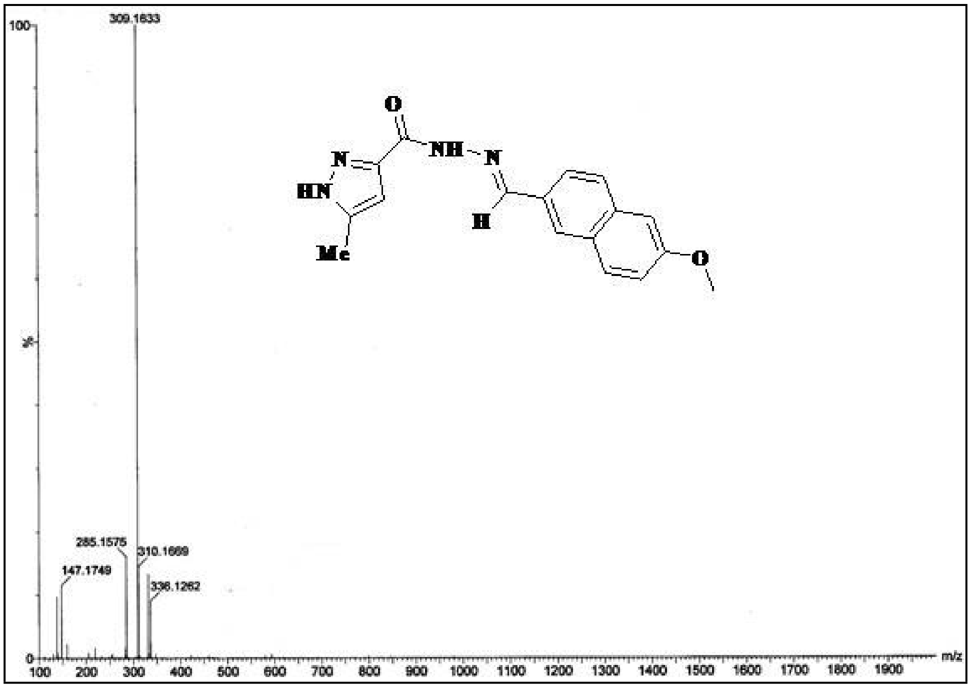

Fig. S3 MS spectra of the probe **PYN**.

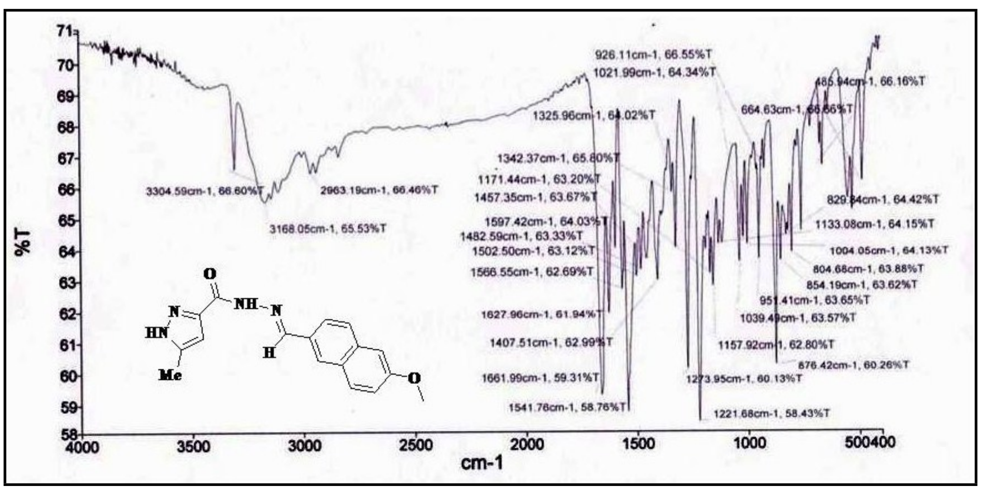

Fig. S4 FT- IR spectra of the probe **PYN**.

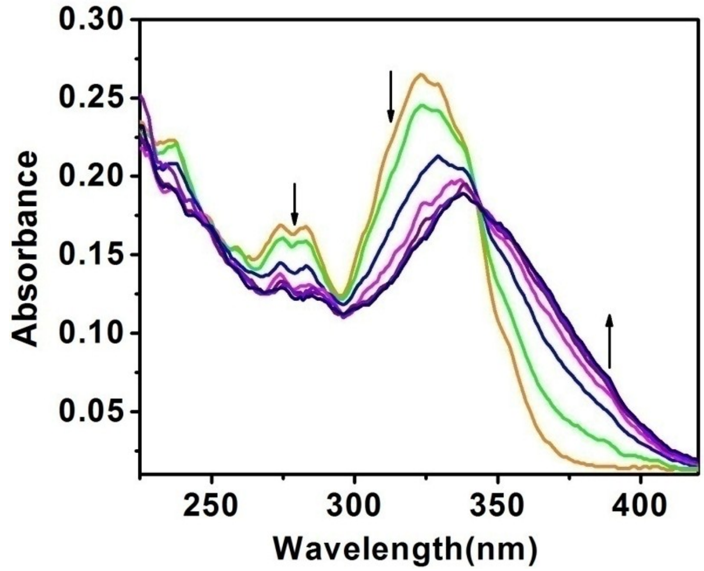

Fig. S5 Uv-vis spectra of the probe **PYN** (5 μM) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C), in the presence of different amount Zn^{2+}.

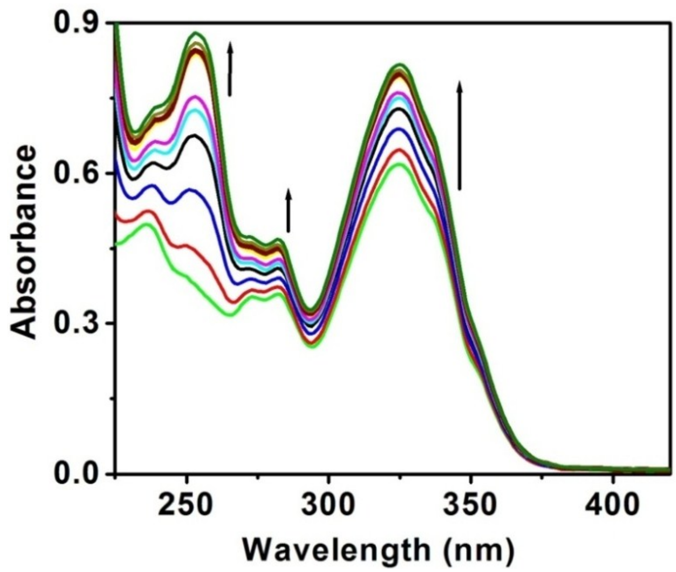

Fig. S6 UV-vis spectra of the probe **PYN** (5 μM) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C), in the presence of different amount Mg^{2+}.

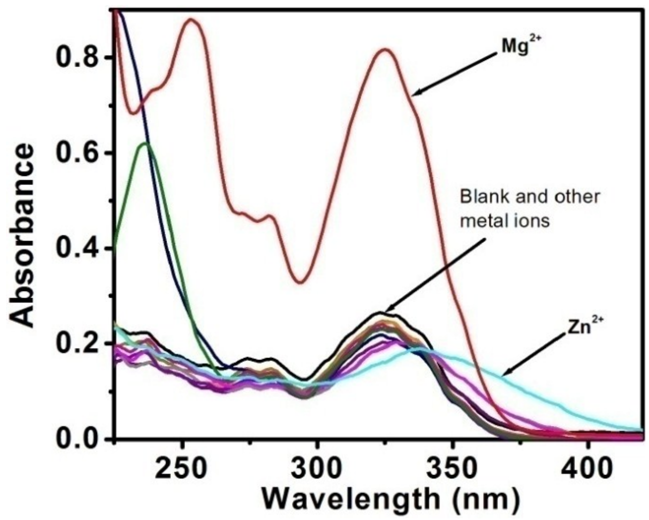

Fig. S7 Changes in the UV-Vis spectra of **PYN** (5 μM) after addition of 1.2 equivalents of various cations in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C).

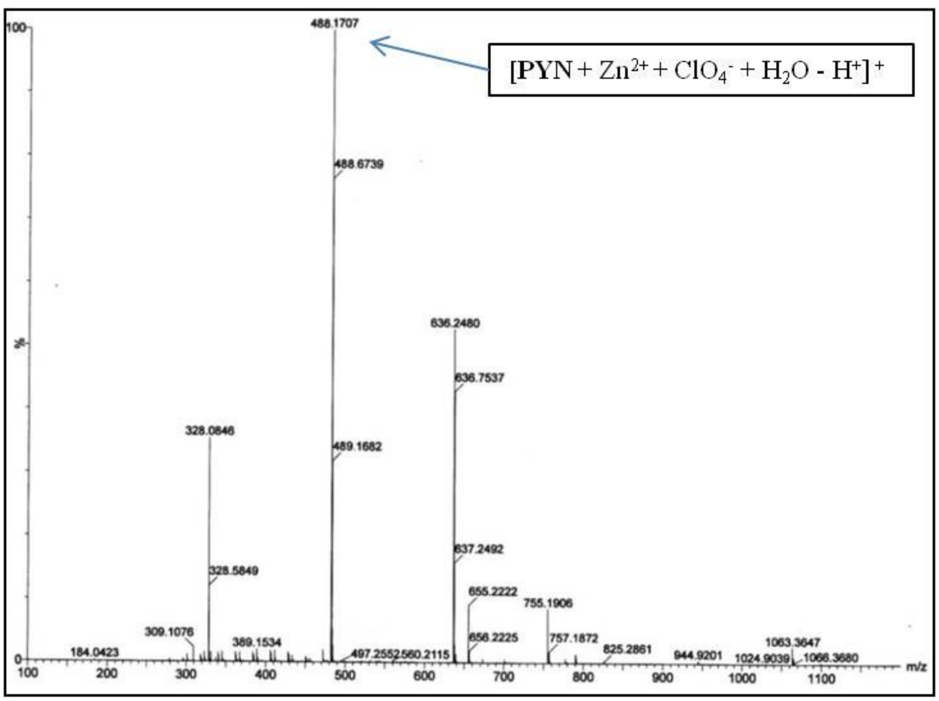

Fig. S8 TOF MS ES^{+} spectra of **PYN**-Zn^{2+} complex.

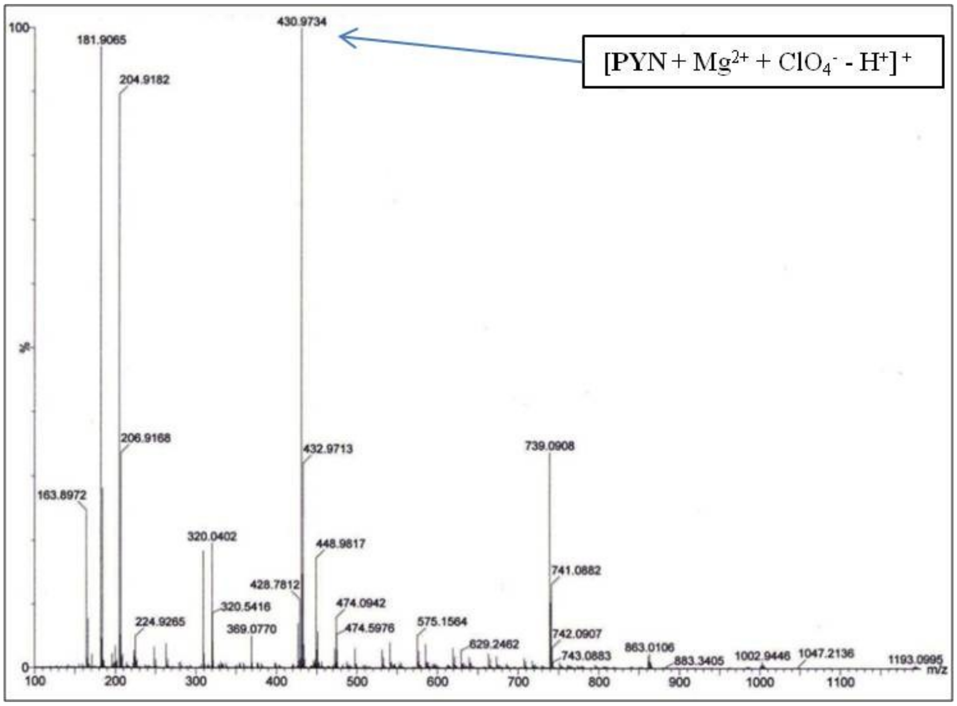

Fig. S9 TOF MS ES$^+$ spectra of **PYN**-Mg^{2+} complex.

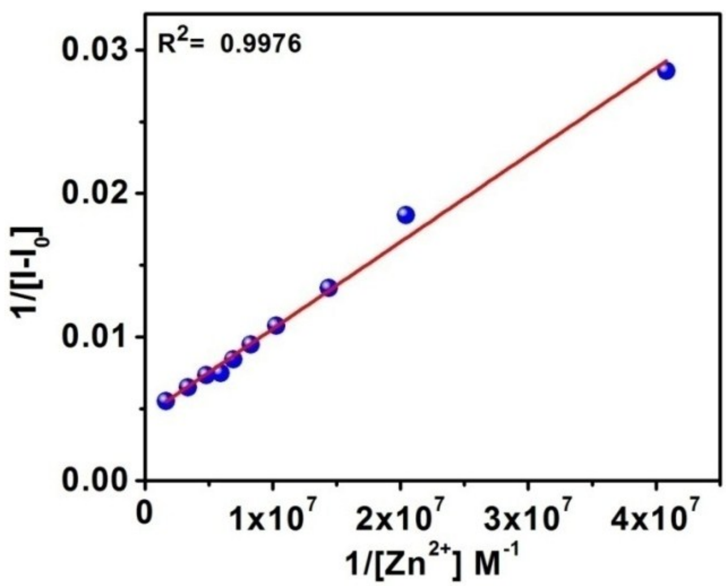

Fig. S10 Benesi-Hildebrand plot of the probe **PYN** (5 μM) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^oC), emission intensity at 456 nm assuming 1:1 stoichiometry between **PYN** and Zn^{2+}.

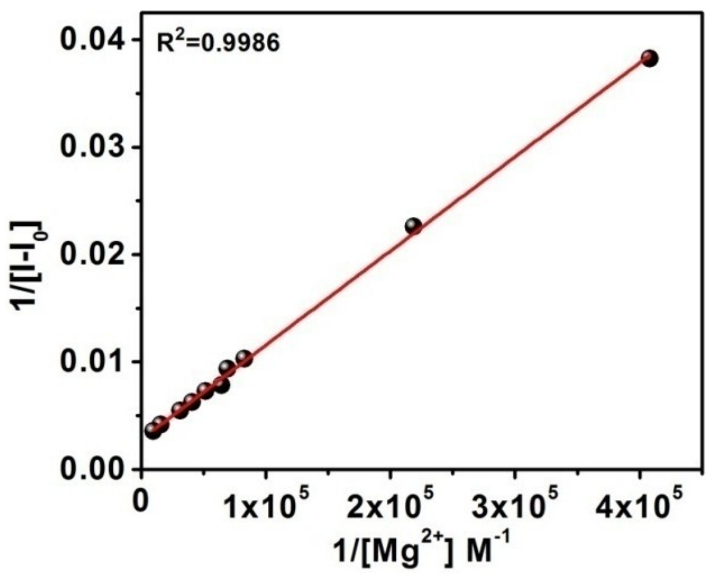

Fig. S11 Benesi-Hildebrand plot of the probe **PYN** (5 μM) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 °C), emission intensity at 472 nm assuming 1:1 stoichiometry between **PYN** and Mg^{2+}.

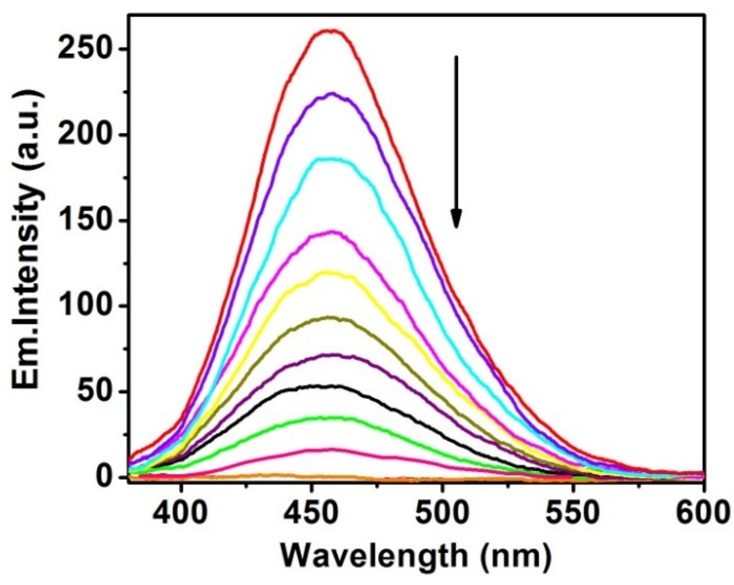

Fig. S12 Fluorescence titration of **PYN**-Zn^{2+} complex with EDTA in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 °C), (λ_{ex} = 370 nm). Intensity gradually decreases upon gradual addition of EDTA solution.

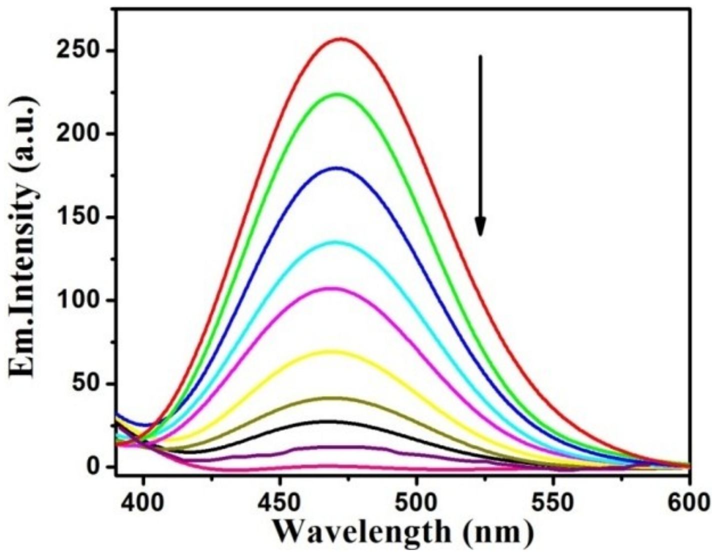

Fig. S13 Fluorescence titration of **PYN**-Mg^{2+} complex with EDTA in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C), (λ_{ex} = 370 nm). Intensity gradually decreases upon gradual addition of EDTA solution.

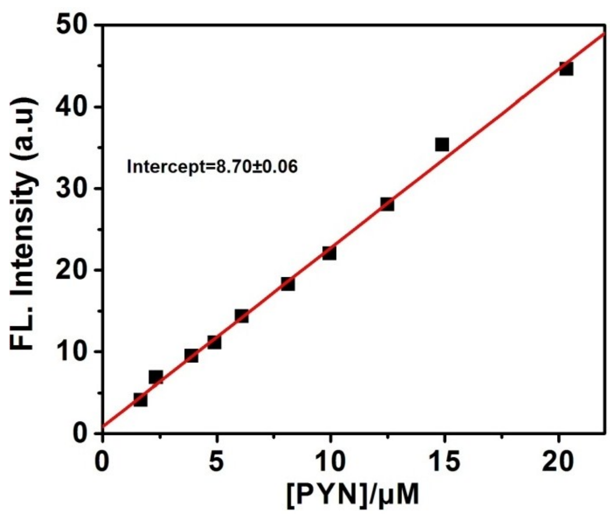

Fig. S14 Determination of Sb of the blank, **PYN** solution.

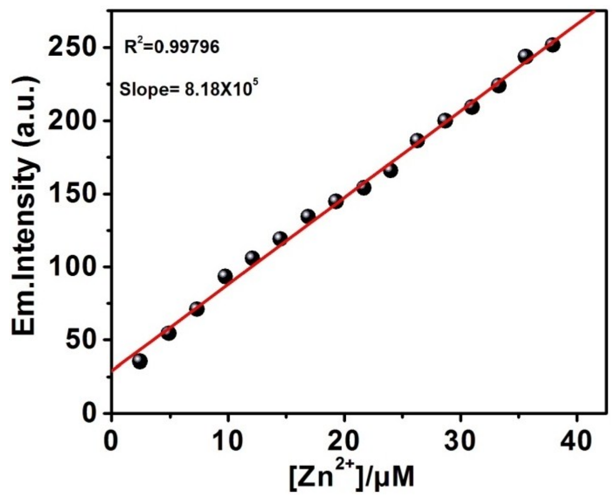

Fig S15 Determination of the detection limit of Zn^{2+} by **PYN** (5 μM) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C), (λ_{ex} = 370 nm, λ_{em} = 456 nm). LOD (Zn^{2+}) = (3x0.06)/8.18x10^5=2.2x10^{-7}

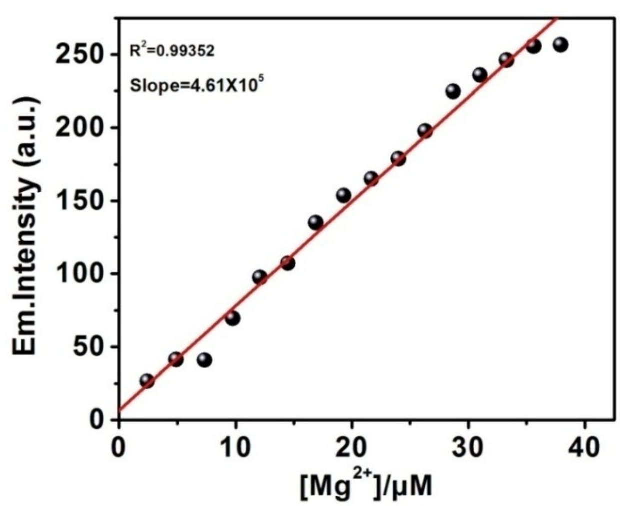

Fig. S16 Determination of the detection limit of Mg^{2+} by **PYN** (5 μM) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C), (λ_{ex} = 370 nm, λ_{em} = 472 nm). LOD (Mg^{2+}) = (3x0.06)/4.61x10^5=3.9x10^{-7}

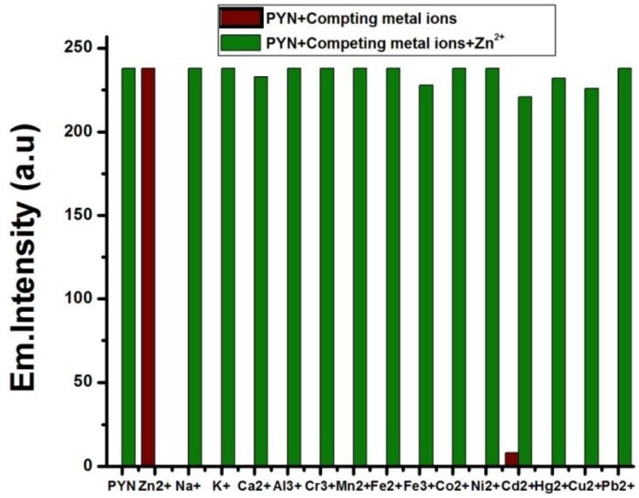

Fig. S17 Fluorescence intensity of **PYN** (5 μM) upon the addition of different metal ions (wine bar) and the addition of Zn^{2+} in the presence of background cations (olive bar) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C). The intensities were recorded at 456 nm.

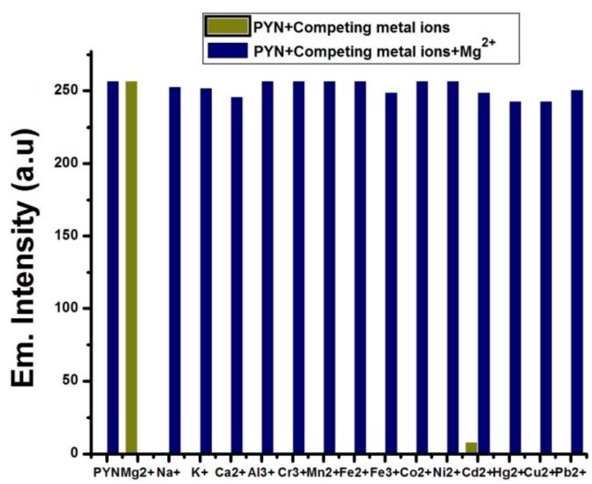

Fig. S18 Fluorescence intensity of **PYN** (5 μM) upon the addition of different metal ions (dark yellow bar) and the addition of Mg^{2+} in the presence of background cations (navy bar) in 7:3 (v/v) MeCN-water solution (10 mM Tris-HCl, pH 7.2 at 25 ^{o}C). The intensities were recorded at 472 nm.

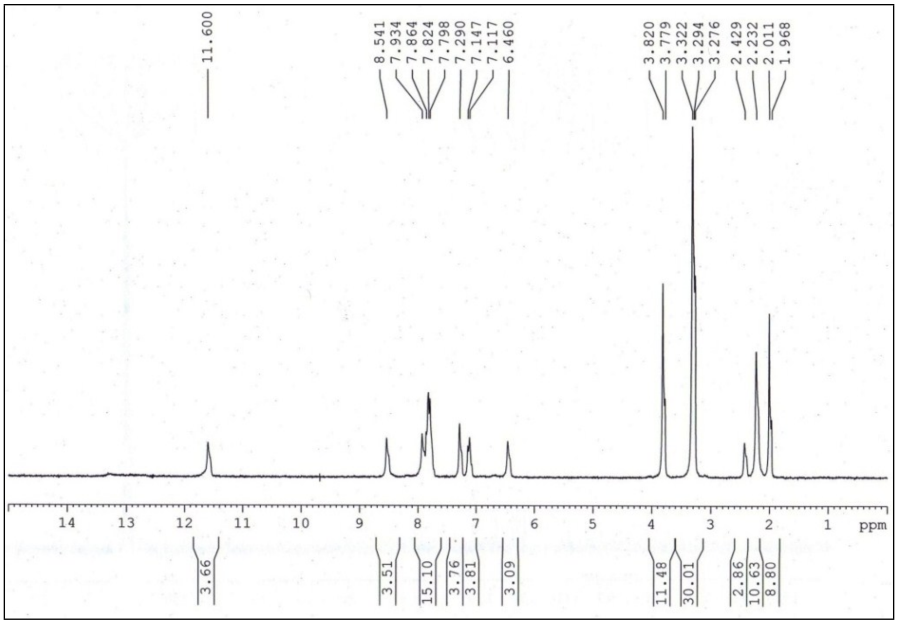

Fig. S19 ^{1}H NMR spectrum of **PYN-**Zn^{2+} (1:1), in DMSO-d_6 in Bruker 300 MHz instrument.

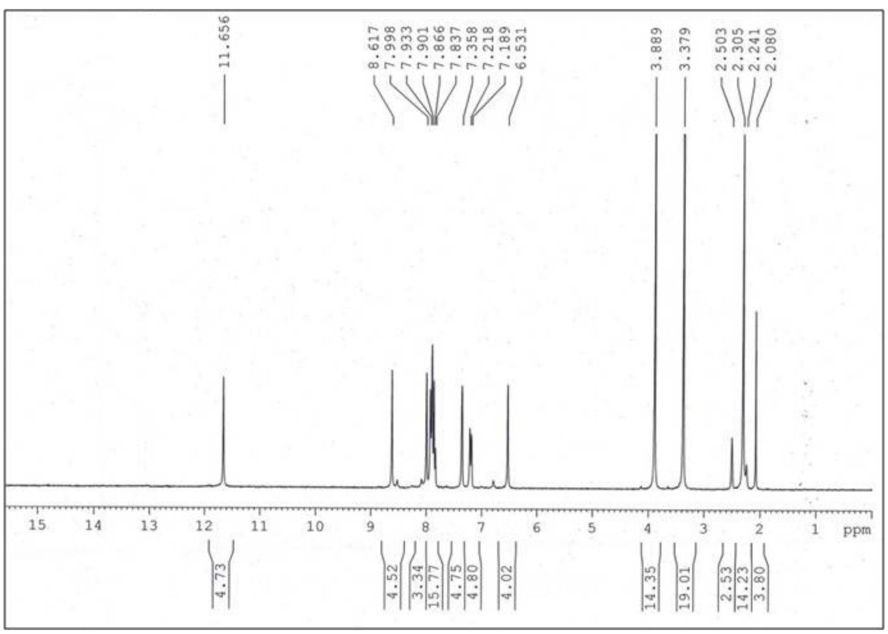

Fig. S20 ^{1}H NMR spectrum of **PYN-**Mg^{2+} (1:1), in DMSO-d_6 in Bruker 300 MHz instrument

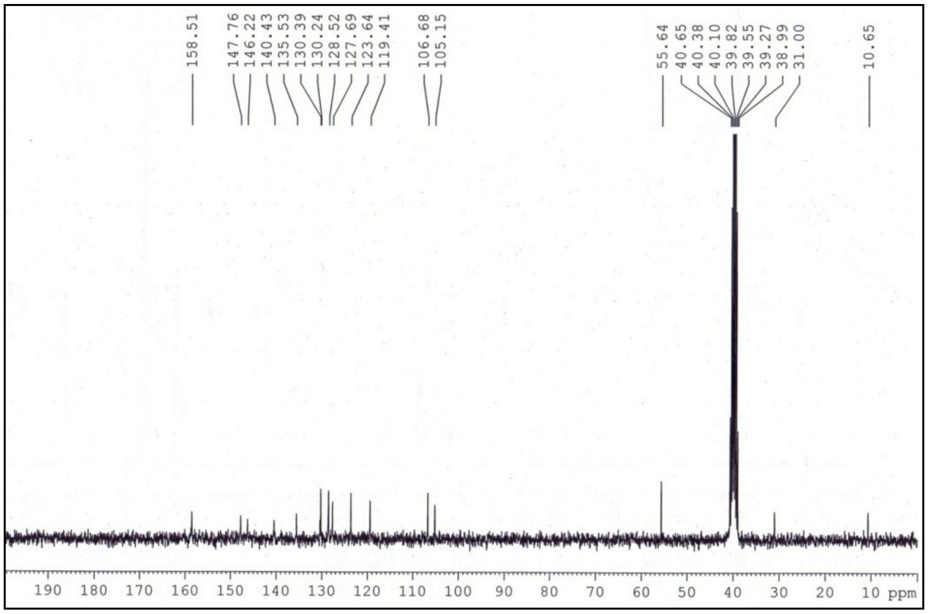

Fig. S21 ^{13}C NMR spectrum of **PYN-**Zn^{2+} (1:1), in DMSO-d_6 in Bruker 300 MHz instrument.

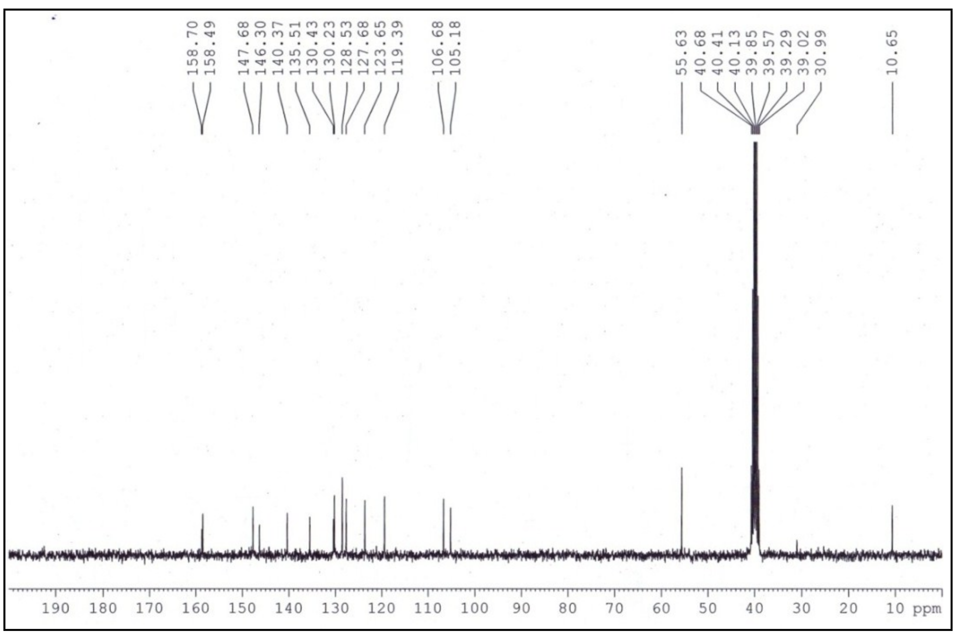

Fig. S22 ^{13}C NMR spectrum of **PYN-**Mg^{2+} (1:1), in DMSO-d_6 in Bruker 300 MHz instrument.

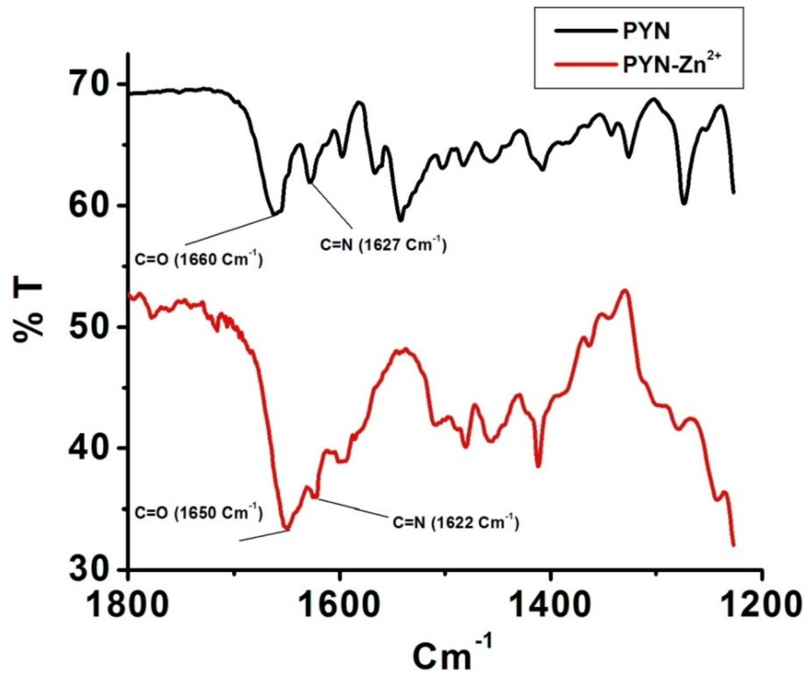

Fig. S23 FT-IR spectrum of **PYN**-Zn^{2+} in KBr pellet.

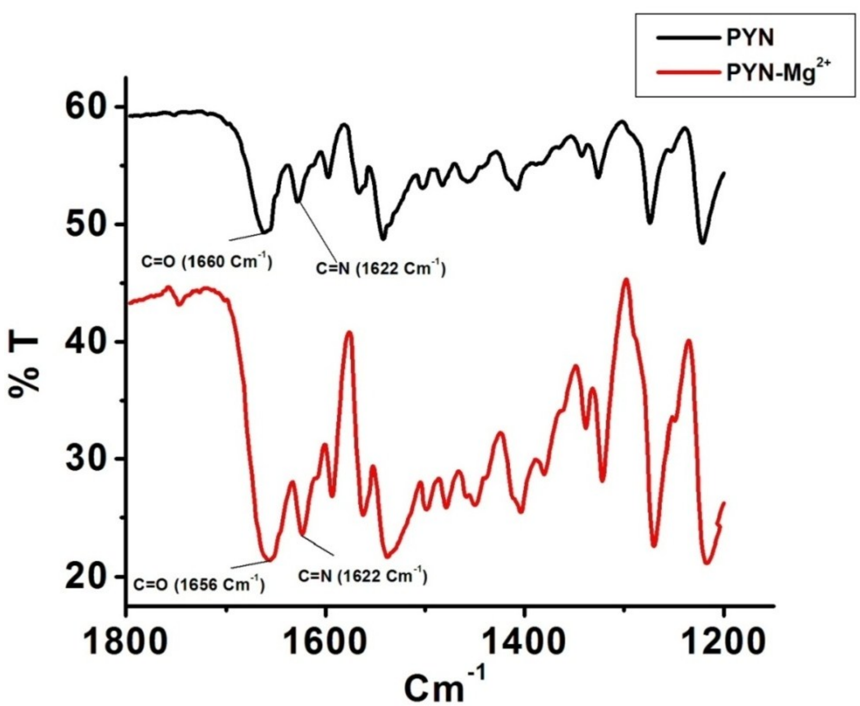

Fig. S24 FT-IR spectrum of **PYN**-Mg^{2+} in KBr pellet.

Table 1

Fluorescence lifetimes of **PYN** and **PYN**-Zn^{2+}/Mg^{2+} complex in ACN/H_2O solvent

ACN/ H_2O	**τ_1(ns)**	**τ_2(ns)**	**τ_3(ns)**	**α_1**	**α_2**	**α_3**	**χ^2**	**τ_{av}**	**Φ**
PYN	1.07	5.17	0.06	0.06	0.02	0.93	0.96	0.71	0.003
PYN+Zn^{2+}	1.73	7.48	0.17	0.20	0.06	0.74	1.09	4.42	0.15
PYN+Mg^{2+}	1.18	7.48	0.17	0.18	0.03	0.79	0.91	3.46	0.12

Table 2

Calculated selected bond angles (^{0}) and bond lengths (A^o) data of **PYN** and complex **PYN-Zn^{2+}/Mg^{2+}** (B3LYP/ LANL2DZ level of theory).

bond angles (^{0})	**PYN-Zn^{2+}**	bond angles (^{0})	**PYN-Mg^{2+}**	bond angles (^{0})	**PYN**
O_{22}-Zn_{39}-N_{26}	79.32	O_{22}-Mg_{39}-N_{26}	79.26	O_{22}-C_{21}-N_{20}	123.86
Zn_{39}-N_{26}-N_{27}	120.73	Mg_{39}-N_{26}-N_{27}	145.30	N_{20}-N_{19}-C_{17}	117.64
N_{26}-C_{23}-C_{21}	117.90	N_{26}-C_{23}-C_{21}	116.78	O_{22}-C_{21}-C_{24}	124.24
Zn_{39}-O_{22}-C_{21}	18.07	Mg_{39}-O_{22}-C_{21}	84.97	C_{21}-N_{20}-N_{19}	121.13
Zn_{39}-N_{26}-C_{23}	108.12	Mg_{39}-N_{20}-C_{23}	44.07	C_{21}-C_{24}-N_{27}	119.24
O_{22}-C_{21}-C_{23}	116.58	O_{22}-C_{21}-C_{23}	15.10	C_{24}-N_{27}-N_{28}	104.28
O_{22}-C_{21}-N_{20}	129.36	O_{22}-C_{21}-N_{20}	130.41		
C_{21}-N_{20}-N_{19}	114.31	C_{21}-N_{20}-N_{19}	114.12		

Bond lengths (Å)	**PYN-Zn^{2+}**	Bond lengths (Å)	**PYN**
Zn_{39}-O_{22}	2.181	C_{21}-O_{22}	1.206
Zn_{39}-N_{26}	1.991	C_{21}-N_{20}	1.394
O_{22}-C_{21}	1.296	N_{19}-N_{20}	1.352
C_{21}-C_{23}	1.486	N_{19}-C_{17}	1.280
N_{26}-C_{23}	1.338	C_{21}-C_{24}	1.494
N_{26}-N_{27}	1.349	C_{24}-N_{27}	1.330
C_{21}-N_{20}	1.304	N_{27}-N_{28}	1.391
N_{20}-N_{19}	1.379	C_{24}-C_{25}	1.421

Bond lengths (Å)	**PYN-Mg^{2+}**
Mg_{39}-O_{22}	2.091
Mg_{39}-N_{26}	2.372
O_{22}-C_{21}	1.295
C_{21}-C_{23}	1.487
N_{26}-C_{23}	1.341
N_{26}-N_{27}	1.350
C_{21}-N_{20}	1.302
N_{20}-N_{19}	1.380