Supplementary Material (ESI) for Chemical Communications

ATP selective acridone based fluorescent probes for monitoring of metabolic events

Jatinder Kaur and Palwinder Singh*

Department of Chemistry, Guru Nanak Dev University, Amritsar-143005. India. Fax: 91 183 2258819; Tel: 91 183 2258802 x 3495; E-mail: palwinder_singh_2000@yahoo.com

Supporting Information

1. General Information

^{1}H and ^{13}C NMR spectra were recorded on JEOL 300 MHz using $CDCl_3$ as solvent. ^{1}H NMR titrations were carried out using DMSO-d_6 and D_2O as solvent. Chemical shifts are given in ppm with TMS as an internal reference. *J* values are given in Hertz. The reactions corresponding to epoxy ring opening with amines were performed in domestic microwave oven (INALSA model 1MW17EG) with microwave power 700W and operating frequency 2450 MHz. Reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel GF-254. Column chromatography was performed with 100-200 mesh silica. IR and UV spectral data were recorded on FTIR 8400S Shimadzu and BioTek PowerWave XS instruments respectively. The fluorescence spectra were run on Varian (Cary Eclipse) spectrofluorophotometer using 5 nm slit width (excitation at 253 nm). Pyruvate kinase, phosphoenolpyruvate were purchased from SPI-BIO bertin pharma. Hexokinase was purchased from HIMEDIA.

2. Experimental

2.1 Procedure for the synthesis of compounds 1a (Procedure A)

A mixture of 2-chlorobenzoic acid (1.56 g, 10 mmol), aniline (10 mmol), powdered CuO (25 mg) and K_2CO_3 (1.5 g, ~11 mmol) in isoamyl alcohol (10 ml) was heated at 160 ^{0}C for 10 h. After cooling, the alcohol was evaporated under vacuum and the residue was dissolved in hot water (120 ml) and acidified with 10N HCl. The precipitates were filtered and washed with hot water. The solid product was dissolved in ethyl acetate, the solution was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography over silica gel using a mixture of ethyl acetate and hexane (1:1) as the eluent. This product was taken in conc. H_2SO_4 and heated on water bath for 1.5 h. Reaction mixture was added to hot water and the resulting precipitates were filtered to get acridone **1a**.

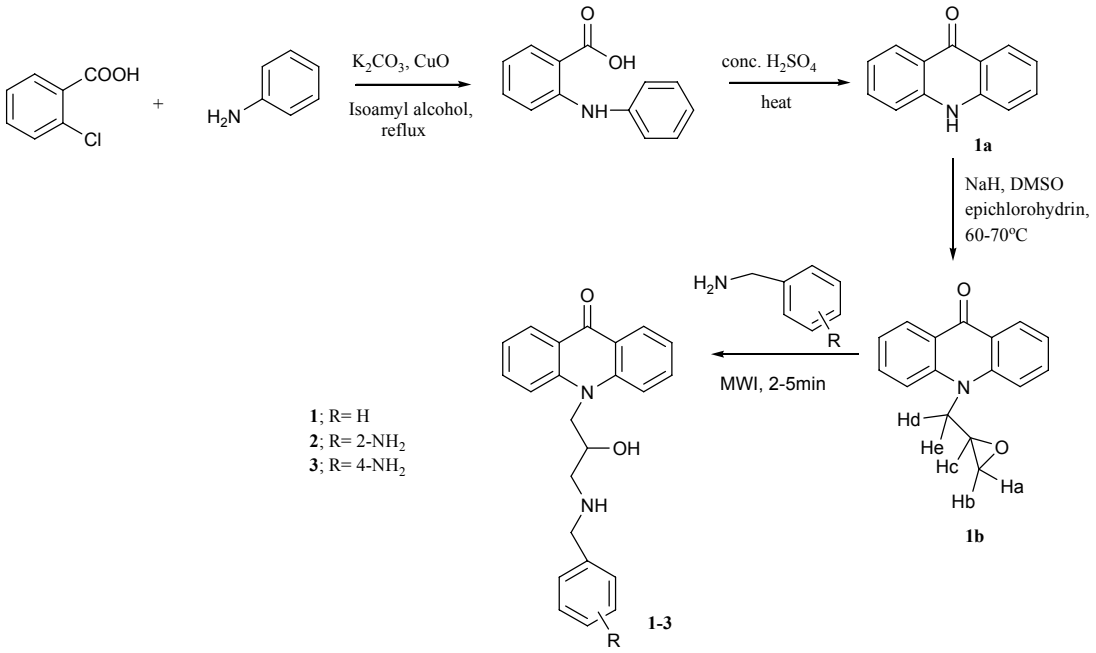
2.2 Procedure for the synthesis of compounds 1c (Procedure B)

Sodium hydride (3.0 mmol) was washed with dry hexane and taken in 15 ml of dimethyl sulphoxide. To this solution, acridones **1a** (2.5 mmol) and epichlorohydrin (3.0 mmol) were added and the reaction mixture was stirred for 17-18 h at 70-80 ^{0}C (TLC monitoring). The reaction mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4. The solvent was distilled off and the residue was column chromatographed using ethyl acetate and hexane (7:1) as eluents to isolate pure compound **1b**.

2.3 Procedure for the synthesis of compounds 1-3 (Procedure C).

A mixture of acridone **1c** (1 mmol) and appropriate amine (benzylamine/ o-amino benzylamine/ p-amino benzylamine (1 mmol) was irradiated in microwave oven for 2-5 minutes. On completion of the reaction (TLC), it was washed with diethyl ether to isolate pure products **1-3**.

2.4 Syntheses of compounds 1-3



Scheme S1. Syntheses of compounds **1**-**3**.

2.5 10-(3-Benzylamino-2-hydroxy-propyl)-10*H*-acridin-9-one (**1**): Compound **1** (0.035 g, 68%) was synthesized using procedure C as yellow solid, mp 157-160 ^{o}C; ν_{max}(KBr)/cm^{-1} 1580 (C=O), 3345 (NH), 3420 (OH); δ_H (300 MHz, $CDCl_3$) 2.76 (1H, dd, J^2 11.8 Hz, J^3 7.5 Hz, $NHCH_2$), 2.89 (1H, dd, J^2 12.0 Hz, J^3 3.9 Hz, $NHCH_2$), 3.77 (2H, s, Benzyl CH_2), 4.24 (1H, dd, J^2 15.1 Hz, J^3 3.1 Hz, NCH_2), 4.33-4.37 (1H, m, CH), 4.57 (1H, dd, J^2 15.1 Hz, J^3 8.2 Hz, NCH_2), 6.89-6.94 (2H, m, ArH), 7.19-7.25 (5H, m, ArH), 7.43-7.53 (4H, m, ArH), 8.0-8.03 (2H, m, ArH); δ_C (normal/DEPT-135) 50.2, 52.4, 53.9, 68.8, 115.4, 121.3, 121.6, 127.2, 128.1, 128.5, 133.6, 139.7, 142.3, 177.6; MS (FAB) 359 (M^++1); Anal. Calcd. for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82%. Found: C, 77.09; H, 6.16; N, 7.84%.

2.6 10-[3-(2-Amino-benzylamino)-2-hydroxy-propyl]-10*H*-acridin-9-one (**2**): Compound **2** (0.050 g, 67%) was synthesized using procedure C as yellow solid, mp 170-172 ^{o}C; ν_{max}(KBr)/cm^{-1} 1575 (C=O), 3395 (NH), 3410 (OH); δ_H (300 MHz, $CDCl_3$) 2.81 (1H, dd, J^2 11.8 Hz, J^3 6.7 Hz, $NHCH_2$), 2.90 (1H, dd, J^2 14.8 Hz, J^3 6.7 Hz, $NHCH_2$), 3.83 (2H, s, Benzyl

CH_2), 4.12 (1H, dd, J^2 15.9 Hz, J^3 2.1 Hz, NCH_2), 4.40-4.52 (2H, m, CH/1H of NCH_2), 6.57-6.64 (2H, m, ArH), 6.82-6.87 (2H, m, ArH), 6.98-7.04 (2H, m, ArH), 7.43-7.45 (4H, m, ArH), 7.85-7.88 (2H, m, ArH) ; δ_C (normal/DEPT-135) 50.3, 52.5, 52.6, 68.9, 115.8, 118.1, 121.3, 121.6, 123.5, 127.2, 128.7, 130.2, 133.7, 142.3, 146.4, 177.6; MS (FAB) 374 (M^++1); Anal. Calcd. for $C_{23}H_{23}N_3O_2$: C, 73.97; H, 6.21; N, 11.25. Found: C, 73.93; H, 6.19; N, 11.20.

2.7 10-[3-(4-Amino-benzylamino)-2-hydroxy-propyl]-10*H*-acridin-9-one (**3**): Compound **3** (0.055 g, 74%) was synthesized using procedure C as yellow solid, mp 165-166 ^oC; ν_{max}(KBr)/cm^{-1} 1585 (C=O), 3390 (NH), 3415 (OH); δ_H (300 MHz, $CDCl_3$) 2.53 (1H, dd, J^2 11.7 Hz, J^3 4.5 Hz, $NHCH_2$), 2.65 (1H, dd, J^2 12.4 Hz, J^3 4.3 Hz, $NHCH_2$), 3.45 (2H, s, Benzyl CH_2), 4.03-4.06 (1H, m, CH), 4.31-4.33 (2H, m, NCH_2), 6.39-6.40 (2H, m, ArH), 6.42-6.88 (2H, m, ArH), 6.99-7.04 (2H, m, ArH), 7.43-7.47 (2H, m, ArH), 7.62-7.64 (2H, m, ArH), 8.22-8.24 (2H, m, ArH); δ_C (normal/DEPT-135) 50.1, 52.4, 52.5, 68.7, 115.2, 118.3, 121.5, 121.7, 123.3, 127.5, 128.8, 130.1, 133.5, 142.2, 146.5, 177.3; MS (FAB) 374 (M^++1); Anal. Calcd. for $C_{23}H_{23}N_3O_2$: C 73.97; H, 6.21; N, 11.25. Found: C, 73.98; H, 6.23; N, 11.22.

2.8 Equation for calculation of binding constants

Binding constants of compound-anion complex were calculated using Benesi-Hildebrand Equation.

$$\frac{1}{(A_f - A_{obs})} = \frac{1}{(A_f - A_{fc})} + \frac{1}{K\,(A_f - A_{fc})\,[\text{Ligand}]}$$

Where A_f is absorbance of free host, A_{obs} is absorbance observed, A_{fc} is absorbance at saturation, K is the binding constant.

2.9 UV-vis and fluorescence studies with ATP

Stock solutions of compounds **1**–**3** were prepared at 10^{-3} M concentration in HEPES buffer (10^{-2} M, pH 7.2). 10^{-2} M stock solution of ATP was prepared by dissolving NaATP in HEPES buffer. Taking the ligand concentration constant (50 μM), incremental addition of ATP (up to 150 μM) to compounds **1**–**3** resulted in decrease in absorbance in the UV spectra of these

compounds. For recording the fluorescence spectra, 0−1.7 μM of ATP was added to 0.1 μM solutions of compounds **1**–**3**.

2.10 Selectivity for ATP

To check the selectivity of compounds **1**–**3** for ATP amongst its homologues ADP, AMP and adenosine, the stock solutions of these compounds were prepared in HEPES buffer with same concentration as above. Upon addition of even 10 μM of adenosine/ ADP/AMP/ GDP/ IDP/ CDP/ UDP/ GTP/ ITP/ CTP/ UTP to 0.1 μM solution of compounds **1**–**3**, no change in fluorescence intensity was observed.

2.11 Competitive binding of acridones with ATP

Stock solutions of the compounds and other reagents were prepared with same conc. as in the above experiments. Addition of 10 μM of adenosine/ADP/AMP/ GDP/ IDP/ CDP/ UDP/ GTP/ ITP/ CTP/ UTP to 0.1 μM solution of compounds **1**–**3** did not make change in their fluorescence spectra. Addition of 1.7 μM of ATP to the above solution resulted in quenching of fluorescence to the same extent as in experiment 2.9 indicating that ATP competitively binds to acridones in presence of its homologues.

2.12 Fluorescence monitoring of last step of payoff phase of glucose metabolism

An assay solution containing ADP (0-1.7 μM), PEP (100 μM), compound **1** (0.1 μM) was prepared in HEPES buffer (pH 7.2) and its fluorescence was recorded. When excited at 253 nm, the solution gave same fluorescence intensity as due to pure compound. Addition of PK (50 μM) and Mg^{2+} (50 μM) to the above solution and excitation at 253 nm resulted in decrease in the fluorescence intensity of the solution. The change in fluorescence intensity was converted into the initial velocity (v_0, μM min^{-1}) using a calibration curve obtained from the fluorescence

titration under the assay conditions. The data obtained was analyzed using Lineweaver-Burk Plot to obtain the Michaelis constant (K_m, μM)

2.13 Fluorescence monitoring of First step of preparatory phase of glucose metabolism

An assay solution containing ATP (17 μM), glucose (17 μM) and compound **1** (0.1 μM) was prepared in HEPES buffer (pH 7.2). After addition of HK (10-80 units) and Mg^{2+} (17 μM), fluorescence intensity (excitation at 253 nm) was recorded. An increase in fluorescence intensity up to 50% (w.r.t compound) was observed. The change in fluorescence intensity was converted into the initial velocity (v_0, μM min^{-1}) using a calibration curve obtained from the fluorescence titration under the assay conditions.

3. ^{1}H NMR spectra of compounds 1-3

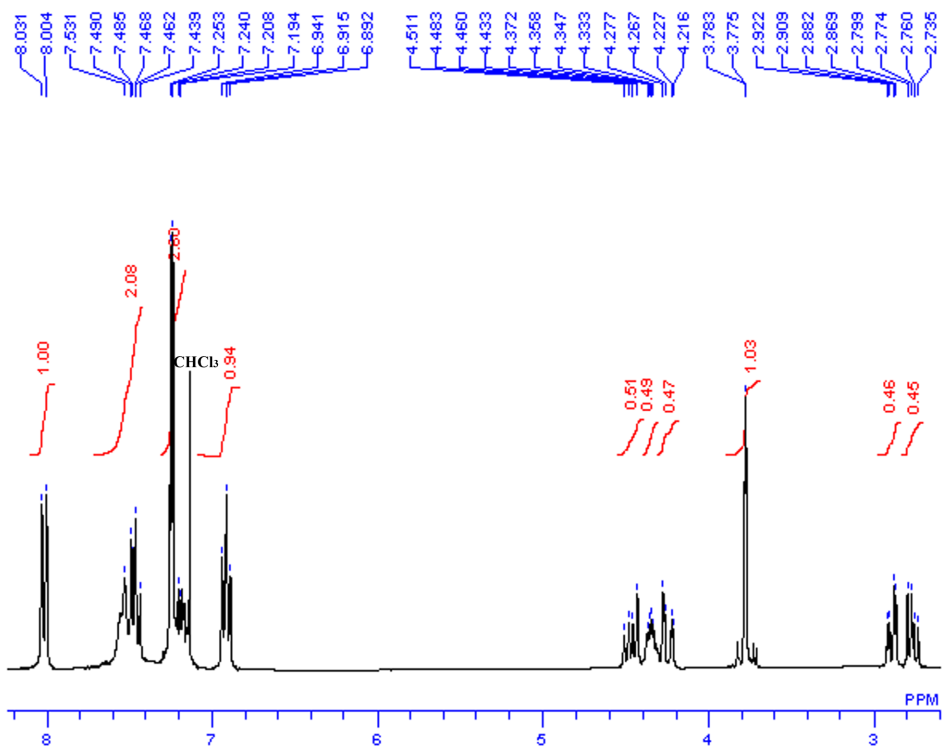


Fig. S1 ^{1}H NMR spectrum of compound **1**.

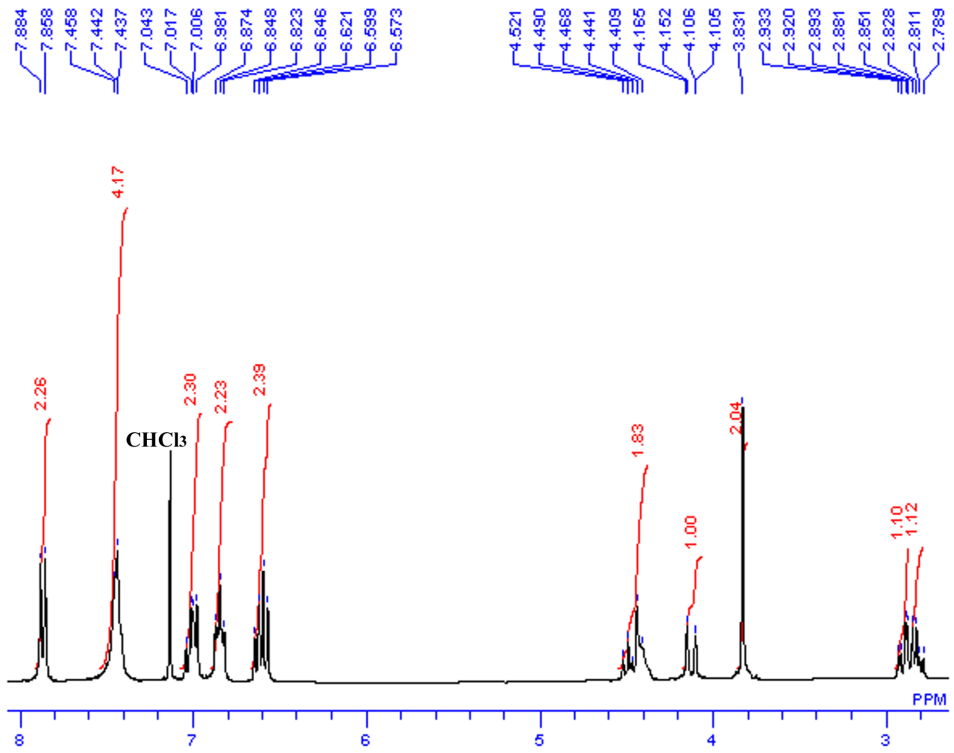


Fig. S2 ^{1}H NMR spectrum of compound **2**.

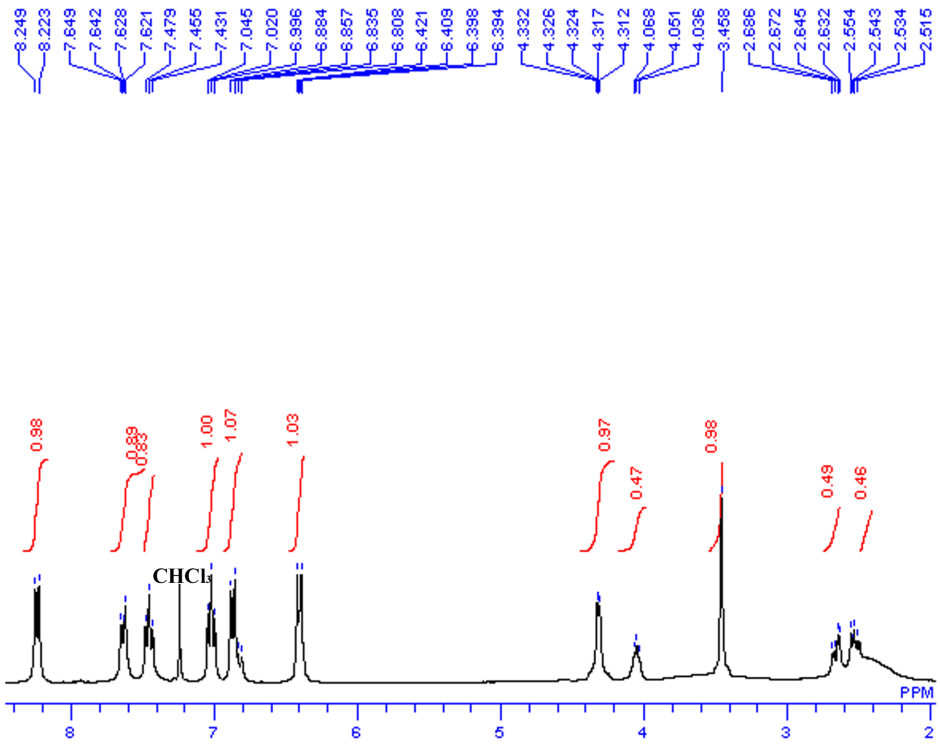


Fig. S3 ^{1}H NMR spectrum of compound **3**.

4. UV-visible spectra of compounds 1-3 with ATP

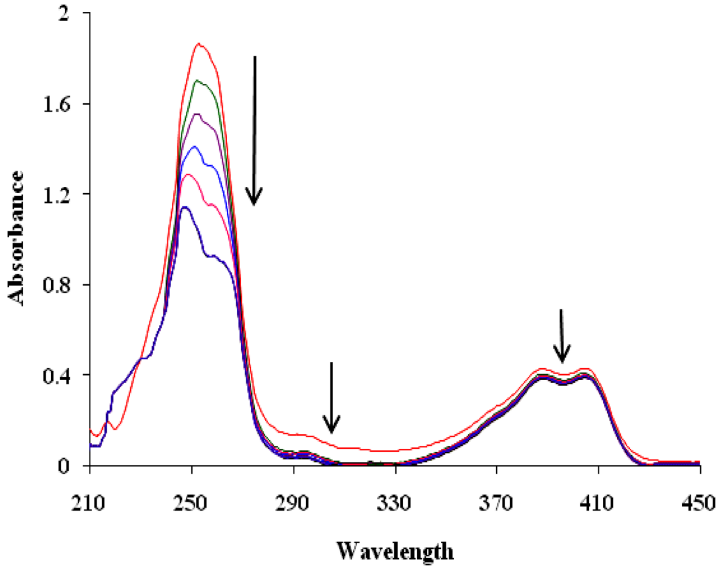


Fig. S4 Absorption spectrum of compound **1** (50 μM) on titration with 0-3 equiv. of ATP in HEPES buffer.

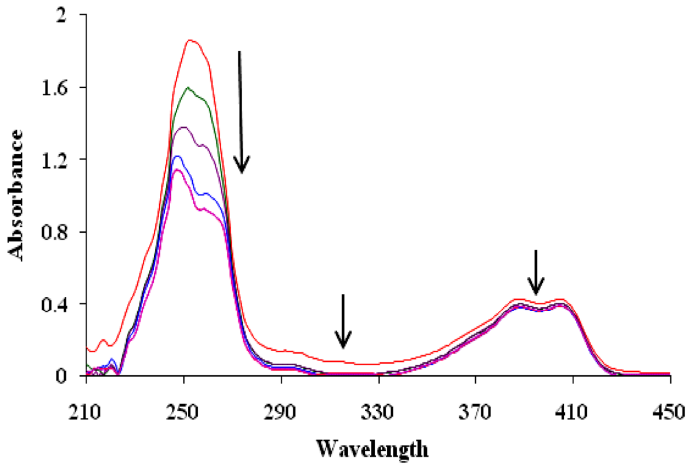


Fig. S5 Absorption spectrum of compound **2** (50 μM) on titration with 0-3 equiv. of ATP in HEPES buffer.

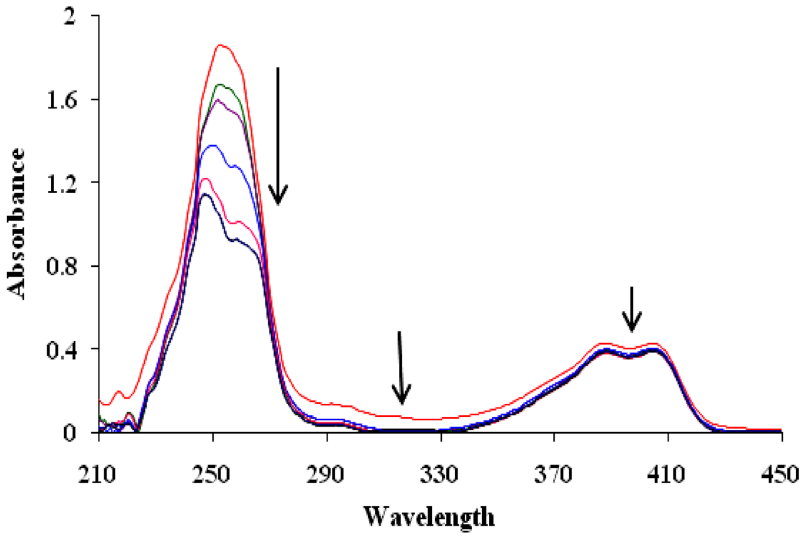


Fig. S6 Absorption spectrum of compound **3** (50 μM) on titration with 0-3 equiv. of ATP in HEPES buffer.

5. Benesi-Hildebrand plot of 1.ATP.

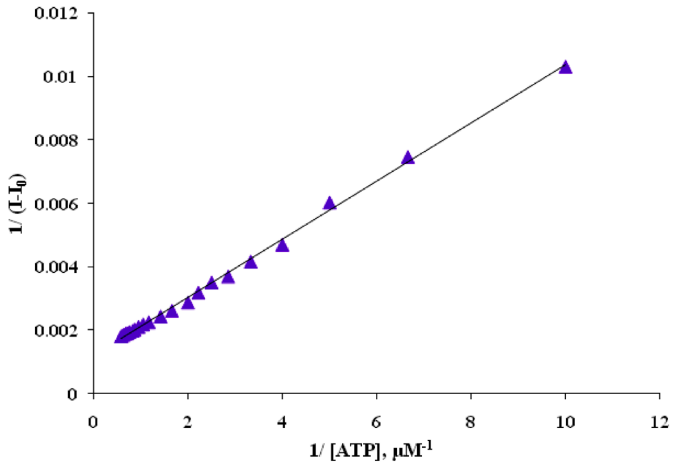


Fig. S7 Benesi-Hildebrand plot of **1.ATP** indicating 1:1 stoichiometry.

6. Fluorescence emission spectra of compounds 2 and 3 upon incremental addition of ATP

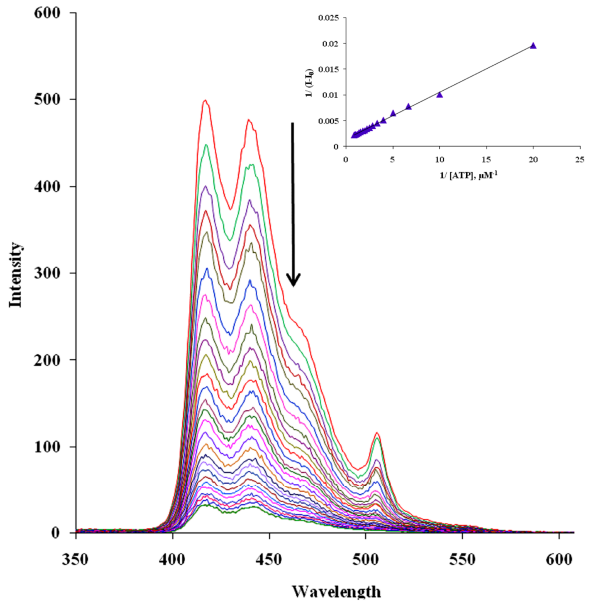


Fig. S8 Fluorescence spectrum of compound **2** (0.1 μM) upon incremental addition of ATP. Inset shows Benesi-Hildebrand plot indicating 1:1 stoichiometry of **2**.ATP complex. Excitation at 253 nm.

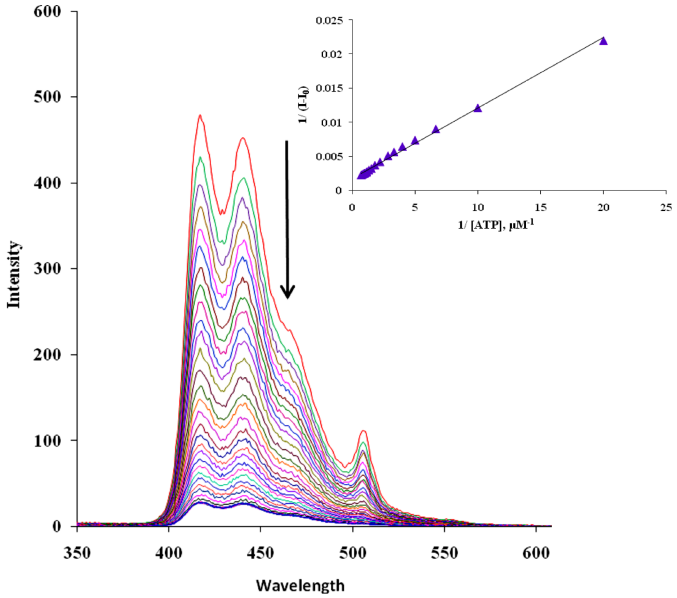


Fig. S9 Fluorescence spectrum of compound **3** (0.1 μM) upon incremental addition of ATP. Inset shows Benesi-Hildebrand plot indicating 1:1 stoichiometry of **3**.ATP complex. Excitation at 253 nm.

7. Table S1 Fluorescence quenching of compounds **1**–**3** in presence of ATP

Compound	Fluorescence quenching with ATP (%)
1	97.5
2	94.0
3	94.3

8. ^{1}H NMR Titrations of compounds 1-3 with ATP

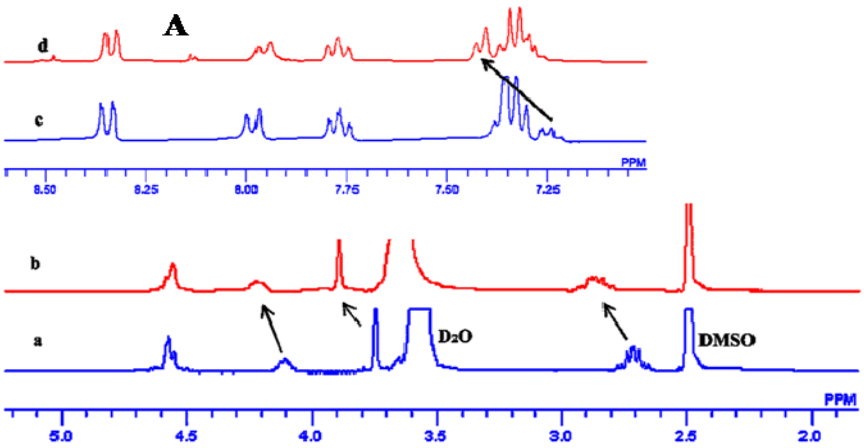


Fig. S10 Shifts in ^{1}H NMR signals of (a) **1**, on addition of (b) 3 equiv of ATP, (c) aromatic part of **1** (expanded), on addition of (d) 3 equiv of ATP.

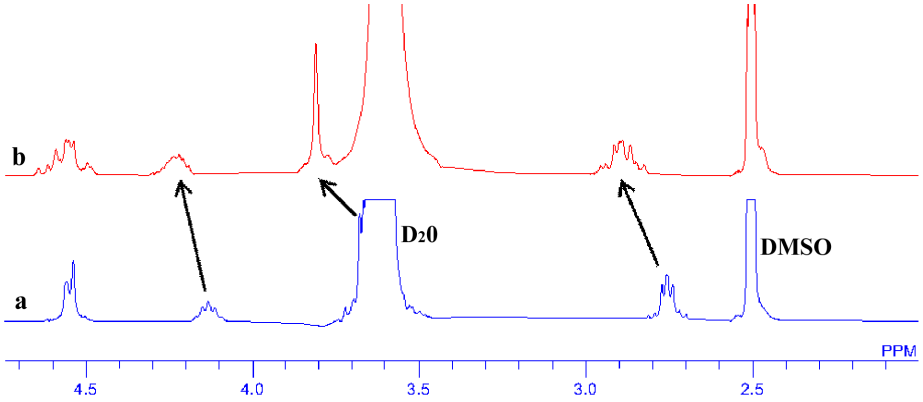


Fig. S11 Shifts in ^{1}H NMR signals of (a) compound **2,** on addition of (b) 3 equiv. of ATP.

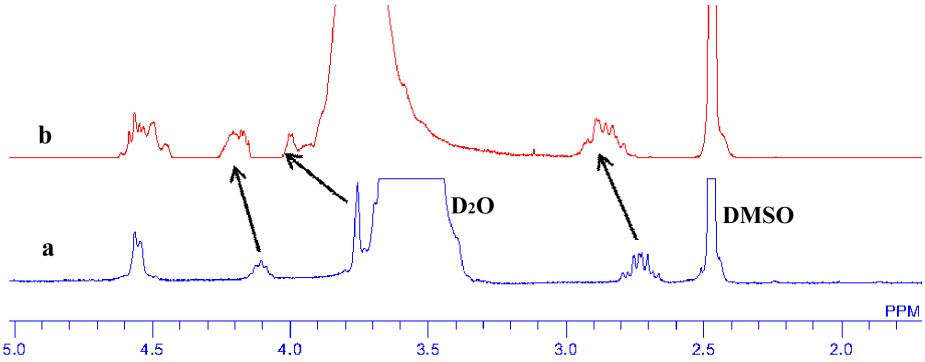
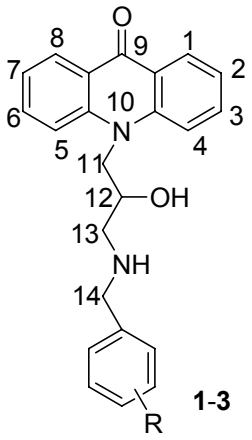


Fig. S12 Shifts in ^{1}H NMR signals of (a) compound **3,** on addition of (b) 3 equiv. of ATP.

9. Table S2 ^{1}H NMR shift of compounds **1-3** in presence of ATP.



1-3

Compd.	**^{1}H NMR Shift (ppm) on addition of 3 equiv of ATP (DMSO+D_2O)**			
	12-H	**13-H**	**14-H**	**ArH**
1	0.116	0.154	0.144	0.165
2	0.094	0.141	0.140	0.045
3	0.095	0.131	0.245	0.050

10. Energy minimized structure of 1.ATP

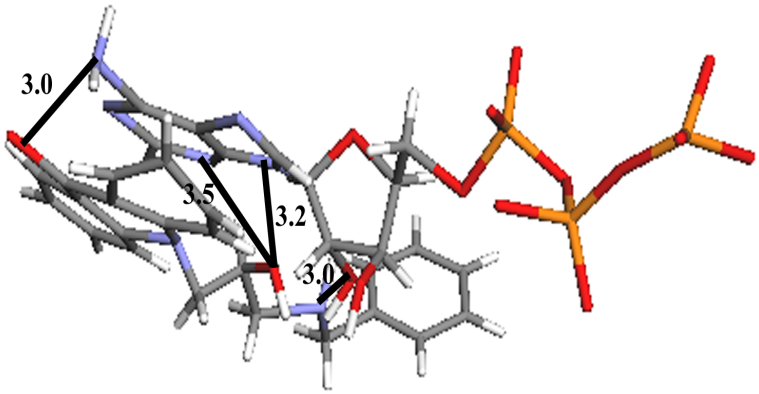


Fig. S13 Energy minimized structure of **1**.ATP complex (PM3) showing interactions of ATP with *O* of carbonyl group, *OH* and *NH* groups of compound **1**

11. Table S3. Binding constants of compounds **1**-**3** with ATP.

Compound.	Binding constant with ATP (M^{-1})
1	8.33×10^7
2	7.14×10^7
3	6.66×10^7

12. Competition experiments of compounds 2 and 3 with homologues/analogues of ATP

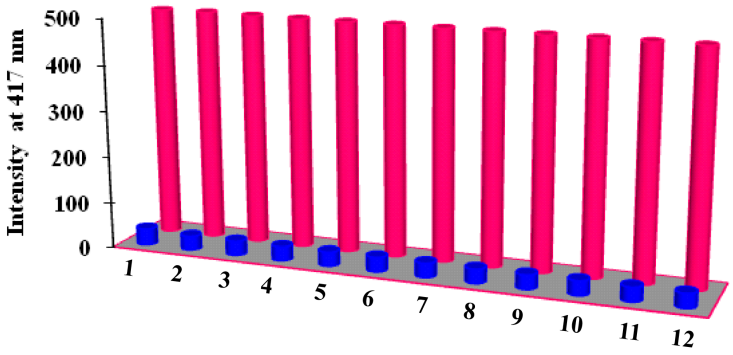


Fig. S14 Fluorescence response of compound **2** to homologues/analogues of ATP. Pink bars represent the addition of 100 equivalents of appropriate homologue/analogue to 0.1 μM solution of compound **2**. Blue bars represent subsequent addition of 14 equivalents of ATP to the solution. 1) **2**+ATP, 2) **2** +Adenosine +ATP, 3) **2** +AMP +ATP, 4) **2** +ADP +ATP, 5) **2** +GTP +ATP, 6) **2** +GDP +ATP, 7) **2** +ITP +ATP, 8) **2** +IDP +ATP, 9) **2** +CTP +ATP, 10) **2** +CDP +ATP, 11) **2** +UTP +ATP, 12) **2** +UDP +ATP.

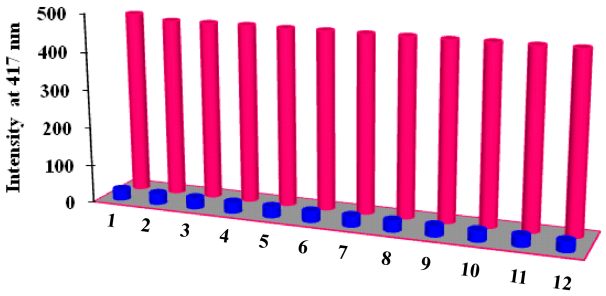


Fig. S15 Fluorescence response of compound **3** to homologues of ATP. Pink bars represent the addition of 100 equivalents of appropriate homologue/analogue to 0.1 μM solution of compound **3**. Blue bars represent subsequent addition of 15 equivalents of ATP to the solution. 1) **3**+ATP, 2) **3** +Adenosine +ATP, 3) **3** +AMP +ATP, 4) **3** +ADP +ATP, 5) **3** +GTP +ATP, 6) **3** +GDP +ATP, 7) **3** +ITP +ATP, 8) **3** +IDP +ATP, 9) **3** +CTP +ATP, 10) **3** +CDP +ATP, 11) **3** +UTP +ATP, 12) **3** +UDP +ATP.