Self-assembly and photophysical studies of an unusual red colored dye which show green fluorescence in cell imaging

VivekshinhKshtriya[a], Bharti Koshti[a], Ashadul Haque[b], Ankit Gangrade[b], Ramesh Singh, Khashti BallabhJoshi [c], Sujoy Bandyopadhyay,[a] Dhiraj Bhatia, [b]* Nidhi Gour[a] *

[a] Department of Chemistry, Indrashil University, Kadi, Mehsana, Gujarat, India; E-mail: nidhi.gour@indrashiluniversity.edu.in; gournidhi@gmail.com

[b] Biological Engineering Discipline, Indian Institute of Technology Gandhinagar, Palaj, , Gandhinagar-382355, India; Email: dhiraj.bhatia@iitgn.ac.in

[c] Department of Chemistry, Dr. Hari Singh Gour University, Sagar, Madhya Pradesh, India

Abstract:

We report for the very first time self-assembly of a red color dye 7-Amino-6h-anthra[9,1-cd] [1,2]thiazol-6-one (**AAT**),its photophysical properties and its applications in cell imaging**.**Interestingly, **AAT** show intense red colour in visible light while it shows the orange colour fluorescence under UV light @312nm.Surprisingly, when this dye was used as cell imaging agent it revealed only green fluorescence inside cells and not red. Hence, the photophysical properties of this dye was very intriguing. Further, when self-assembling properties of this dye was examined it revealed formation of tree like branched structures which appeared red both under green and red filter which was again an unexpected

result..Interestingly **AAT** self-assembly also show morphological transition and the branched tree like structures changes to straight fibres as the solvent is changed from DMSO to THF. Hence, the results of self-assembly and cell imaging were contrary to each other and the photophysical properties of this dye is very unusual as compared to conventional dyes. Our future endeavours willaim to understand this anomalous behaviour in greater details in future through various biophysical assays.

Introduction:

Dyes are very important chemical compounds due to their wide range of applications such as textile,[1] plastic industry,[2] paper industries,[3] cell imaging application,[4] as antiseptic[5] and also as pharmaceutical drugs.[6] At present various scaffold-based dyes are available in the market which is based on some specific chromophore that imparts good photophysical properties to the dye, for instance, diazo based chromophore and others like Naphthalene,[7] Anthraquinone, Indigoid,[8] coumarin[9] etc. After azo dyes, anthraquinone based dyes are getting tremendous attention due to its very good electronic conjugation because of the presence of three rings that are fused together. Such chemical structure provide extra planarity and support for the preparation of various dyes. Anthraquinone-based dyes are very important due to their, good lightfastness,[10] brilliance[11] and chromophore stability under both acidic and basic conditions. [12] Hence, for these reasons, anthraquinone-based dyes are second most commonly synthesized dyes after the azo dyes.

The 9,10-anthraquinone is the main chromophore unit in anthraquinone based dyes. Substitution with different functional groups lead to changes in photophysical propertiesThe most bathochromic shift is observed when the electron releasing groups are introduced at the alpha position of anthraquinone.Functional groups like -NH2 and -OH are the most

favourable electron-donating group since they facilitate the donor-acceptor mechanisms operating in chromophore.[13]

Our group has been interested in assessing the self-assembling properties of amino acids,[14, 15] peptides, [14, 15] biopolymers, [16-19] and heterocyclic compounds.[20-22] Recently, we also reported self-assembly of pyridothiazole-based, aggregation-induced emission-enhancement (AIEE) luminogen 4-(5-methoxy-thiazolo[4,5-b]pyridin-2-yl)benzoic acid (PTC1) and its application for the sensitive detection and monitoring of amyloid fibrillation.[20] Hence, motivated by our previous studies we were motivated to synthesizenewanthraquinonescaffold which t can be effectively used for cell imaging application. Further since our research is based on correlating aggregation properties with the photophysicalphenomenon we also wanted to assess the self-assembling behaver of these dyes to assess the molecular mechanism behind its photophysical characteristics.

Herein this manuscript, we report for the very first time self-assembly and photophysical properties of unusual red dye7-Amino-6h-anthra[9,1-cd][1,2]thiazol-6-one (**AAT**)and its application in cell imaging. The photophysical properties of **AAT** were very unique as it exists in solid form as red color dye while in the solution it forms orange colour and show orange fluorescence under UV, while in cell imaging studiesit reveals green fluorescence only. The self-assembling properties of **AAT**were also exceptional since they reveal branched tree like red fluorescent structures both under green and red filter and do not revealed any green fluorescence as observed by cell imaging.

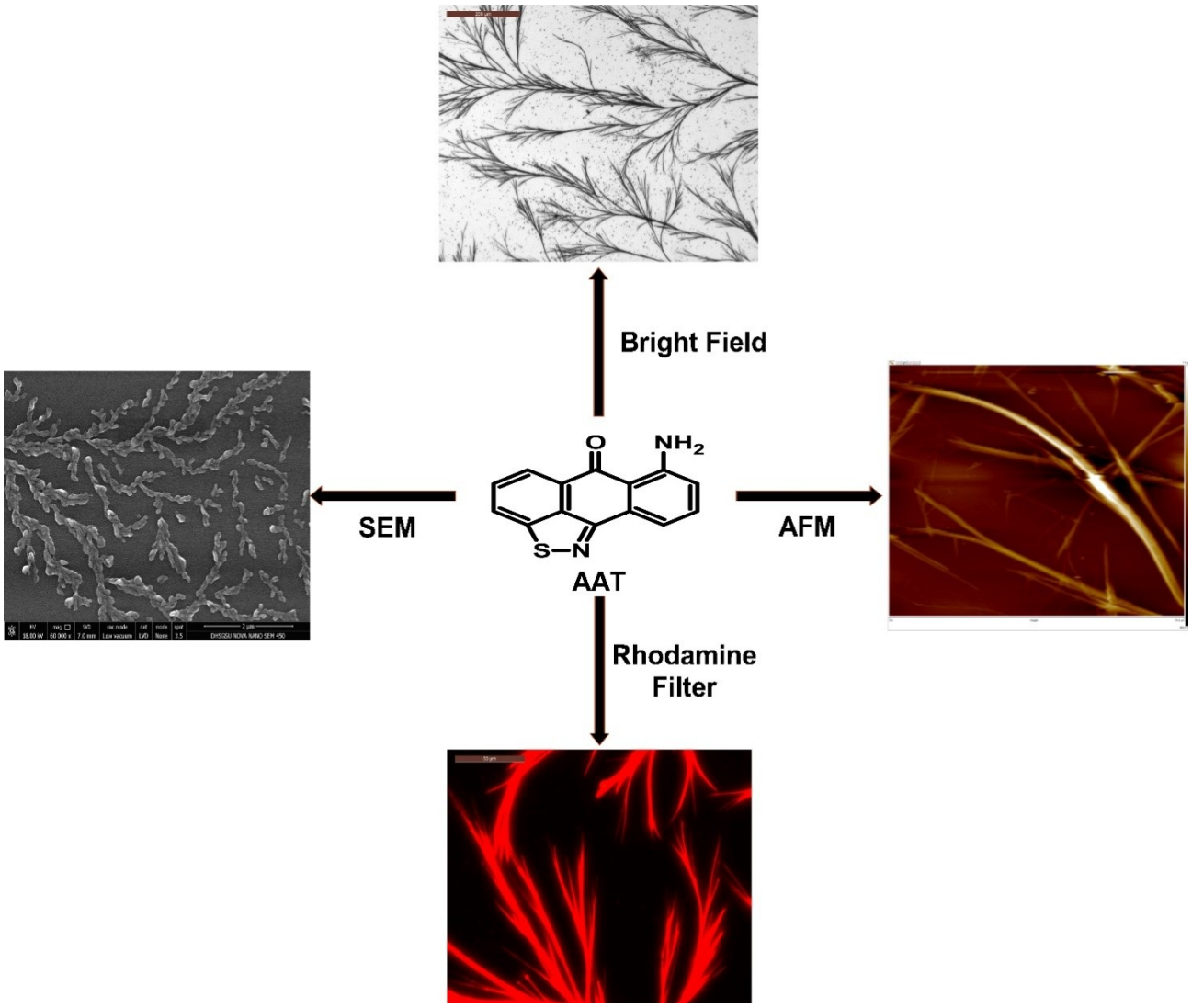
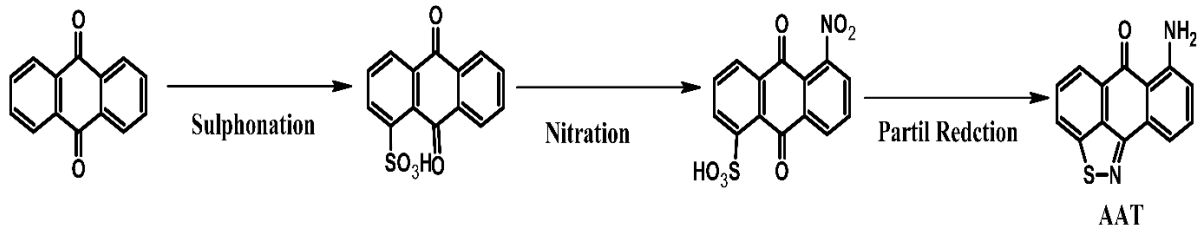


Figure 1. Self-assembled structures formed by **AAT** and its application in cell imaging.



Scheme 1. Scheme for thesynthesis of**AAT.**

7-Amino-6h-anthra[9,1-cd][1,2]thiazol-6-one**(AAT)**was synthesised by reported methodology, In the first step the mono sulphonation wasdoneby using concentrated sulphuric acid which gives the 48% yield, remaining stating material recover which was further use for step-1 preparation. Followed by, in the second step selectively mono nitration has been carried out with use of fuming nitric acid and concentrated sulphuric acid and the final step followed the partial reduction of stage-2 material by using sodium sulfideNa_2S and liquid ammonia under pressure gives the **AAT.**

The as-synthesized **AAT** was characterized by ^{1}H and ^{13}C NMR spectroscopy and its purity ascertained through analytical HPLC. After complete structural characterization, the self-assembling properties of **AAT** were studied in solution to know the structure formation and to understand its morphology at the supramolecular level. The self-assembling properties of **AAT** molecules were studied at 1mM concentration in DMSO. Notably, **AAT** show branched tree like self-assembled structure in DMSO . Figure 2 illustrated branched tree like self-assembled structures formed by **AAT** in AFM. The SEM image of AAT (1mM) at higher magnification reveals beautiful branched tree like morphology. Since the size of assemblies were big, they could also be observed under optical and fluorescence microscope and the tree like assembly appear red under both red and green filter.

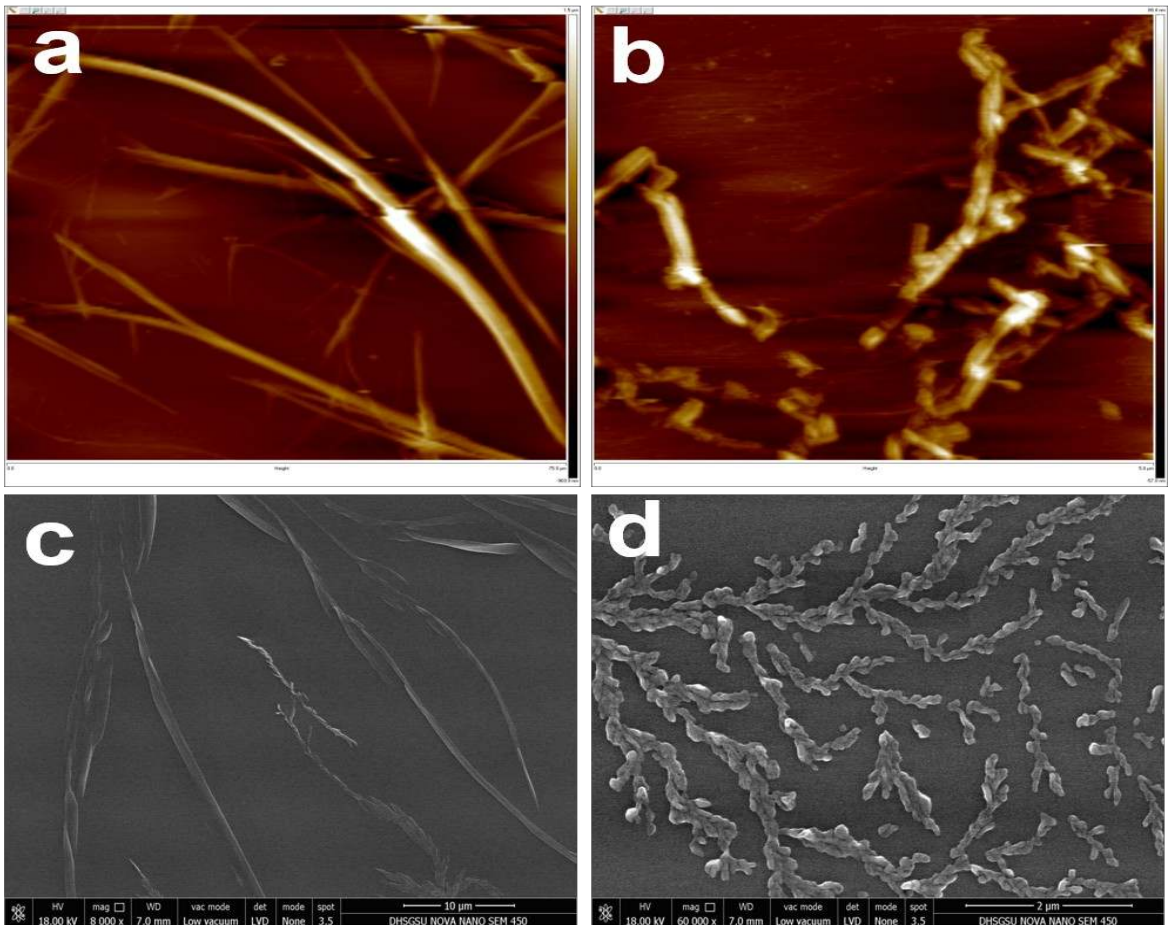


Figure 2: SEM images and AFM images of **AAT**at 1mM in

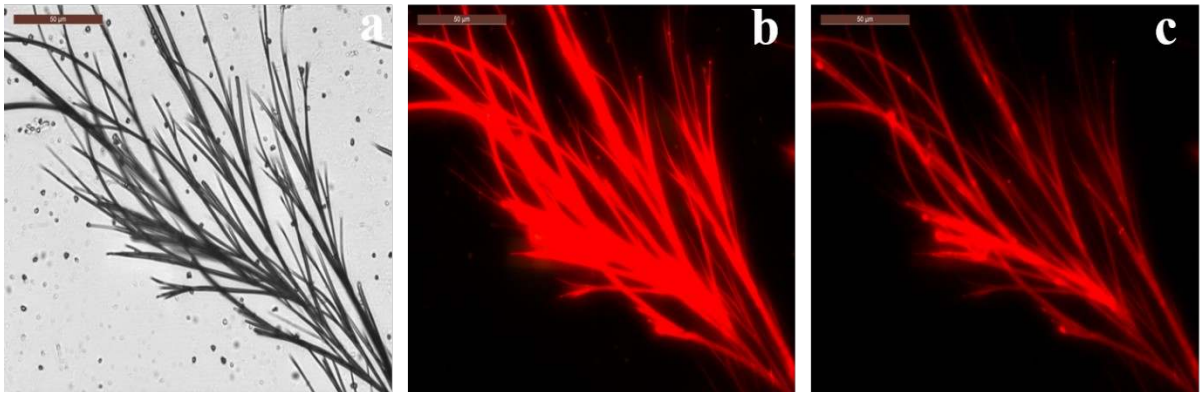


Figure 3. Optical Microscopic images of **AAT** under bright field, green filter and red filter in DMSO a-c) 1 mM concentration under 40X and scale bar 50µm

Interestingly, when AAT was incubated in non polar solvent like THF the branched tree like assemblies changed to fiber and small fractal like structures. Hence a morphological transition could be observed which suggest crucial role of polarity in the self-assembly.

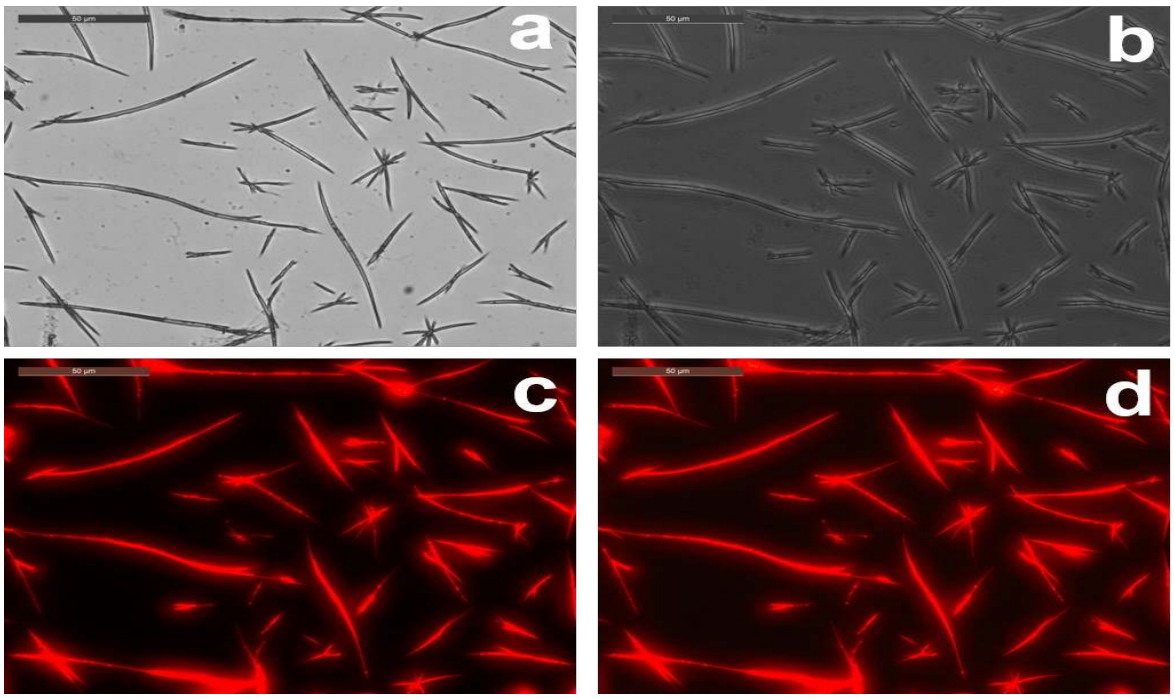


Figure 4. Optical and fluorescence microscopic images of **AAT** in THF. (a) in bright field (b) under phase contrast , (c) under green filter and d) under red filter at 1 mM concentration under40X, scale bar 50 µm.

Further, the utility of **AAT** for cell imaging applications were assessed by incubating **AAT** with fixed human. The**AAT**-treated cells were excited with three lasers, and corresponding emission was collected as follows. (excitation 633 nm; emission 643 to 796 nm, excitation 488 nm; emission 500 to 600 nm, excitation 405 nm; emission 415 to 500 nm). Approximately 30 cells were selected from three different images, and their fluorescence intensity was quantified (Figure 10). It can be observed that cells only revealed green fluorescence while minimal fluorescence could be observed in red and blue channel. This observation was very surprising as from self-assembly studies, AAT was expected to show red fluorescence.

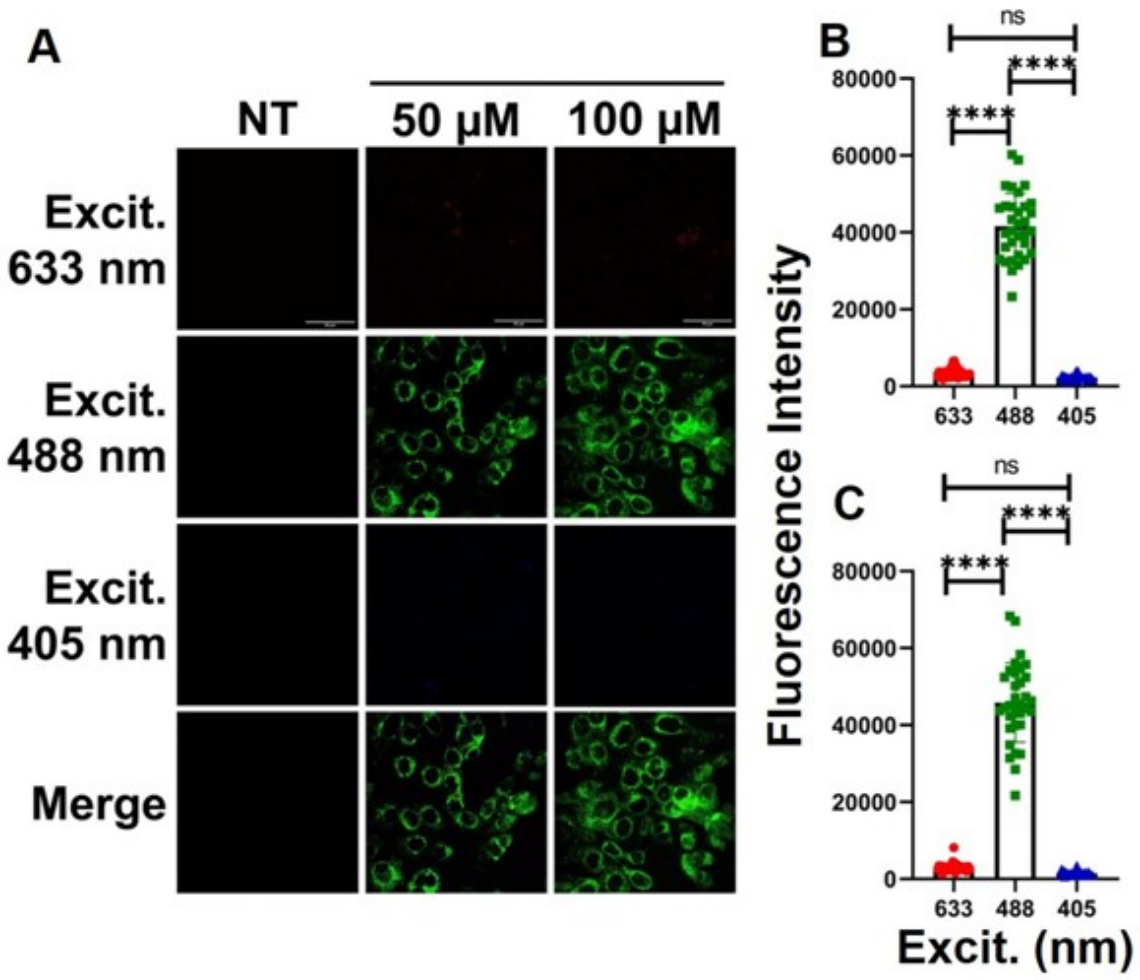


Figure 5. Representative **(A)** confocal microscopy images of human breast cancer cell line (MDA-MB-231) treated with 50 µM and 100µM synthesized **AAT** compound. Fluorescence quantification of **(B)**50 µM and **(C)**100uM concentration **PA** treated cells replotted all three excitation wavelengths. **** Indicates significance level at P < 0.0001, * indicates significance level at P < 0.0329, and ns indicates no significance between the fluorescence intensities.

Hence, to understand this anomalous photophysical properties of **AAT** in more detail, fluorescence spectra of **AAT** was recorded at 10 µM concentration**. It was observed that AAT** itself show orange color fluorescence in solution in broad daylight and reveal excitation maxima at 362 and emission maxima at 480 nm which is evince the mirror image spectra obtained in Figure 5. The emission spectra hence corresponded to green fluorescence unlike the orange colored fluorescence which could be seen through the naked eye.

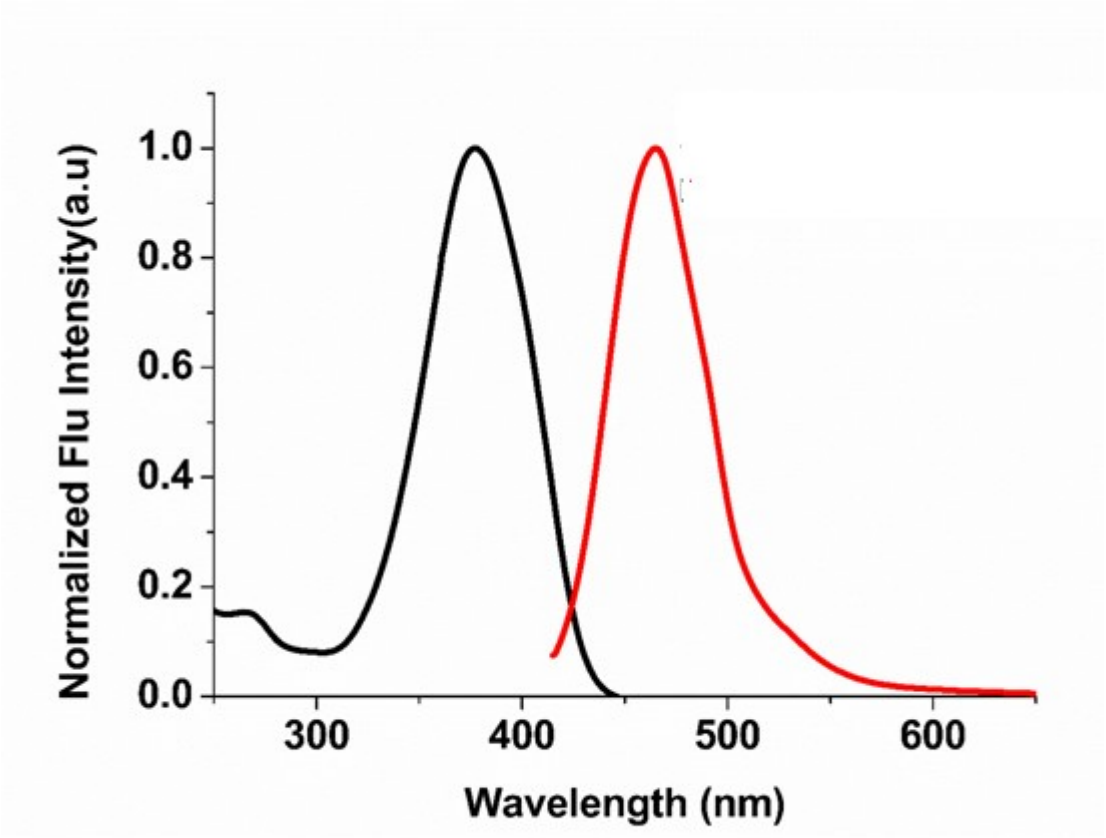


Figure 5. Excitation and Emission spectra of **AAT** in DMSO at 10uM concentration

Further, to correlate the studies of AAT self-assembly studies, we also recorded fluorescence spectra of AAT by giving excitation 470 which corresponds to green filter of microscope and excitation 538 nm which corresponds to red filter. Under both excitation wavelengths a very broad spectra was obtained which covered red region of the spectra and explains the red fluorescence obtained. (Figure 6)

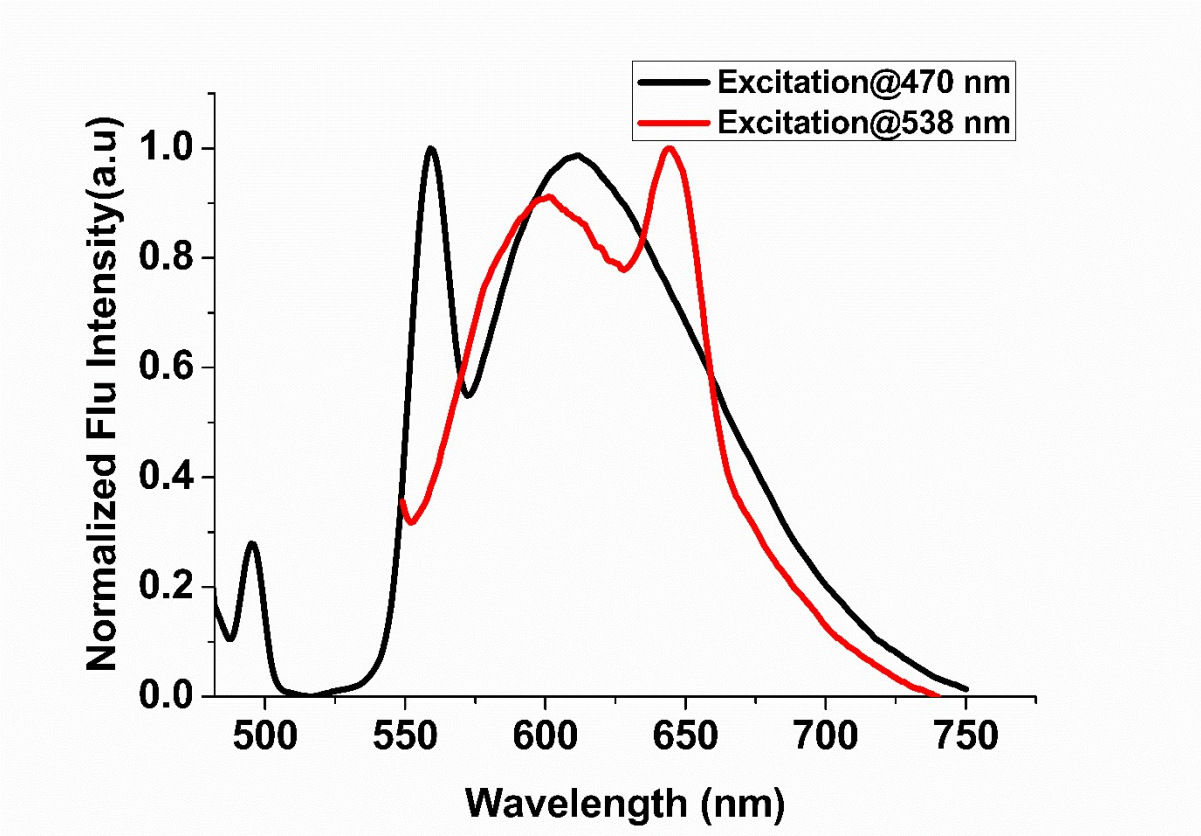


Figure 6: Emission spectra of **AAT** at 10 uM concentration correspond to the FITC filter and Rhodamine filter in Fluorescence microscopy,

Further, fluorescence spectra of AAT was also recorded by giving the excitation 405, 488 and 633 nm which was used in confocal microscopy for the cell imaging experiments,(Figure 7) The emission spectra in this case appears most prominent under green channel probably because of which the green flurescence is observed.

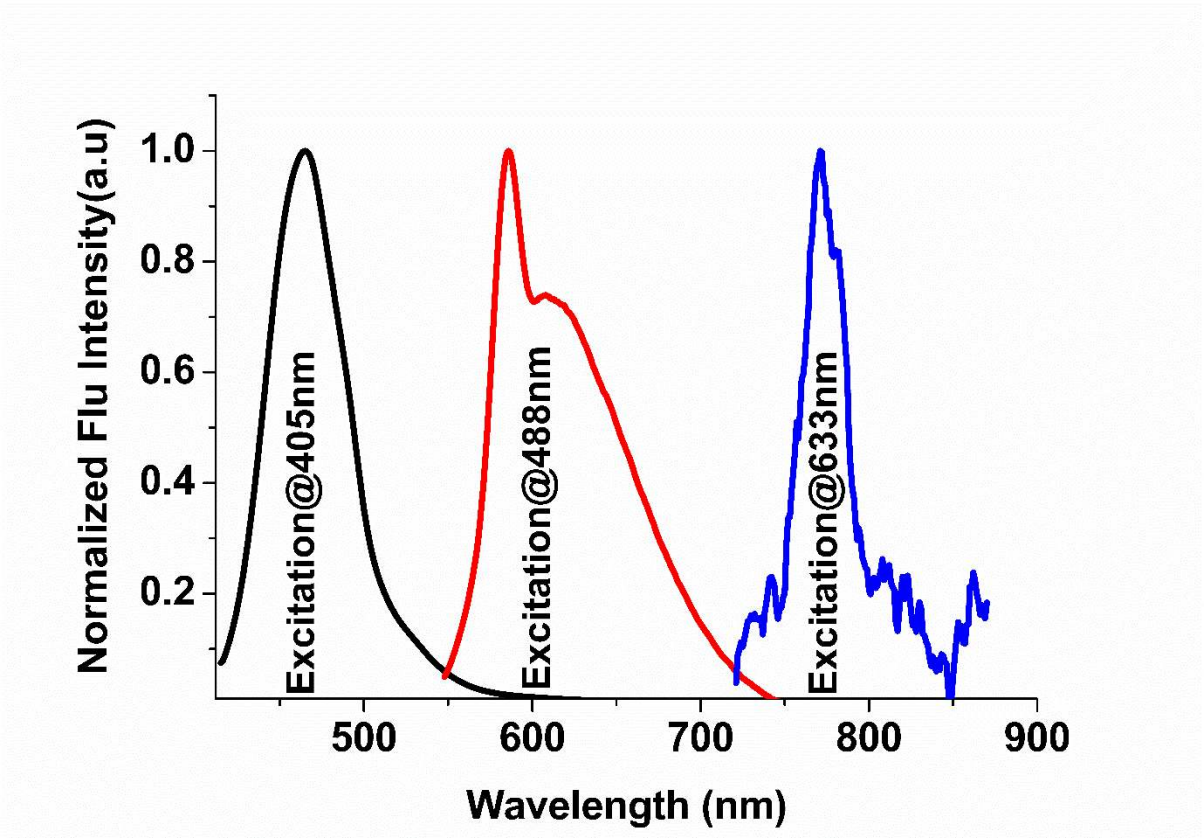


Figure 7: Emission spectra of **AAT** at 10 uM concentration correspond to the confocal microscopy excitation at 405, 488 and 633 nm.

Materials and method:

Self-assembling properties of AAT

The self-assembly property of **AAT**was assessed by scanning electron microscopy and optical microscopy, at 1 to 10 mM concentration.

Scanning electron microscopy (FE-SEM)

SEM images were taken using a JSM7600F (Jeol) FE-SEM 450 microscope (the accelerating voltage ranging from 1 to 15 kV). SEM samples were prepared on silicon wafers. A 10 µL**AAT** of a 1mM solution were dispensed and dried at room temperature. The samples were analyzed without gold coating under a low vacuum.

Atomic Force Microscopy (AFM)

Neat and co-incubated solutions of **AAT** were imaged under an atomic force microscope. The samples were placed on freshly cleaved muscovite mica surfaces followed by imaging with an AFM (INNOVA, ICON analytical equipment, Bruker, operating under the acoustic AC mode (AAC or tapping mode), with the aid of a cantilever (NSC 12(c) from MikroMasch, Silicon Nitride Tip) by NanoDrive version 8 software. The force constant was 2.0 N/m, while the resonant frequency was ~276 kHz. All of the images were taken in the air at rt, with the scan speed of 1.5 lines/sec. The data analysis was done using the nanoscope analysis software. The sample-loaded substrates were dried at dust-free space under a 40W lamp for 30 min followed by high-vacuum drying and subsequently examined under AFM.

Preparation of stock solution:

All the microscopic and spectroscopic studiesof **AAT**were done using10 mM stock solution of **AAT** in DMSO.

UV visible study

The UV-visible spectra havebeen recorded at 1 to 25 μMconcentration by using the 10 mM stock solution of **AAT**in different solvents.The spectra were recorded by usingShimadzu UV-1900 UV-vis spectrophotometer.

Fluorimetric analysis

The excitation and emission spectra of **AAT** were recorded at 10 μM concentration in DMSO. The emission spectra of **AAT** were recorded by giving an excitation wavelength as 375 nm and recorded from 385 to 800 nm. Similarly, the excitation spectra were recorded from 200 to 800 nm, where the maximum emission wavelength (λem) was found to be 462 nm. The experiment has been performed on Jasco spectrofluorometer FP 8300.

Solvent dependent study of AAT

The solvent dependent study has been done at 10 μM concentration by using 10 mM stock solution in DMSOthrough UV-visible and Spectrofluorometer instrument in DMSO, THF, Water,Hexane, Etoc, IPA, chloroform, ACN, MeOH, Ethanol, Acetone,

Cytotoxic assay:

Cell imaging:

Human breast cancer (MDA-MB-231) cells were grown in DMEM cell culture media supplemented with 10% FBS and 1% antibiotic in a 5% CO2 incubator. First, the cells were seeded onto a glass coverslip and fixed with 4% paraformaldehyde. Later the fixed cells were treated with 50 μM and 100 mM AAT for 15 min at room temperature. The coverslips were then mounted on a glass slide, and images were captured in Leica confocal microscope.

Conclusions.

In conclusion, we have reported self-assembly and photophysical properties of 7-Amino-6h-anthra[9,1-cd][1,2]thiazol-6-one (**AAT**). AAT self-assembles to branched tree like morphologies in DMSO and these assemblies show red fluorescence in both green and red filters. These morphologies changes to fiber like structuresin THF which also reveal red fluorescence. . However, when the utility of **AAT** is assessed as a cell imaging agent in human breast cancer cell lines (MDA-MB-231) the cells revealed green emission and no red fluorescence. These results were very surprising and show anomaolous behavior of this dye. We have tried to explain this unusual behavior through fluorescence spectroscopy of AAT in varying excitation wavelengths. However, more studies need to be pursued to find the exact reasoning for this anomaly.

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