Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Synthesis and Cellular Uptake of Cell delivering 2,6-pyridinediylbisalkanamide Submicron-sized sheets in HeLa Cells

J. S. Yadav*, Manoj K. Gupta, I. Prathap, Manika Pal Bhadra,* Parsi K. Mohan and Bulusu Jagannadh*

Email: *yadavpub@iict.res.in*; Fax: +91-40-7160387; Tel: +91-40-27193030

INDEX **Page No.**

Synthesis of of *N*,*N*'-2,6-Pyridinediylbisalkanamides (A-E)

Compounds used in this study were synthesized by following procedure and purified by column chromatography.

Typical Procedure for A, **C and E**: 2,6-Diaminopyridine (1.0 eq.) dissolved in dry tetrahydrofuran (THF) under nitrogen atmosphere and cooled to 0 ^{o}C. The coupling reaction was carried out by the dropwise addition of corresponding acid chloride (2.2 eq.) in dry THF in an ice bath using triethylamine (2.5 eq). The reaction was monitored by TLC, after the completion of the reaction, the excess base was neutralized by the addition of water and worked up to extract the organic layer containing the required product. The isolated crude product was further purified by column chromatography and the purity was checked by spectroscopic and high-resolution mass spectroscopy.

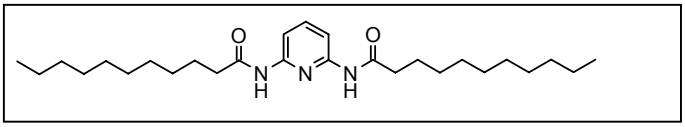
Typical Procedure for B and D: 2,6-Diaminopyridine (1.0 eq.) dissolved in dry tetrahydrofuran (THF) under nitrogen atmosphere and cooled to 0 ^{o}C. The coupling reaction was carried out by the dropwise addition of corresponding acid chloride (0.95 eq.) in dry THF in an ice bath using triethylamine (1.0 eq) of triethylamine. The monosubstituted DAP was coupled to another acid chloride to afford the desired compounds which were purified by column chromatography.

Experimental Section:

General Remarks: Melting points were recorded on Buchi R-535 apparatus and are uncorrected. IR spectra were recorded on a Thermo Nicolet Nexus 670 Spectrometer using KBr optics. Mass spectra (ESI/MS) were recorded on a LC-MSD-Trap-SL mass spectrometer. High Resolution Mass Spectrometry (ESI/HRMS) were recorded on QSTAR XL Mass Spectrometer. NMR spectra were recorded on Gemini and Avance–200 MHz and 300 MHz at 303 K with 7-10 mM solutions in $CDCl_3$ solvents using tetramethylsilane as internal standard or the solvent signals as secondary standards, and the chemical shifts are shown in scales. Multiplicity's of NMR signals are designated as s (singlet), d (doublet), t (triplet), br (broad), m (multiplet, for unresolved lines).

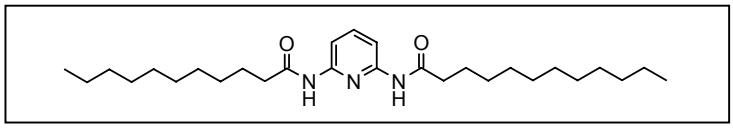
Spectroscopic data for compounds A-E:

(**A**) White Solid. m. p. = 93-96 ºC. IR (KBr): ν Amide A (3306), Amide I (1669), Amide II (1525), As (2919), S (2848).



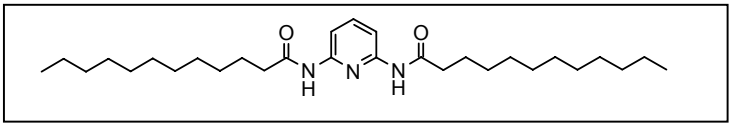
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (d, *J* = 8.3 Hz, 2H), 7.67 (t, *J* = 8.3 Hz, 1H), 7.43 (br. s, 2H, NH), 2.34 (t, *J* = 7.5 Hz, 4H), 1.75-1.65 (m, 4H), 1.43-1.26 (m, 28H), 0.88 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, Proton decoupled): δ 171.4 (NH**C**=O, 2C), 149.4 (**Ar**, 2C), 140.7 (**Ar**, 1C), 109.3 (**Ar**, 2C), 37.8 (CO**C**H, 2C), 31.8, 29.6, 29.3, 22.6 (peaks for **16C**), 13.7 (**C**H_3, 2C). ESI/MS: *m/z*: 446.4 $(M+H)^+$. HRMS calcd. for $C_{27}H_{48}N_3O_2$: 446.3755 $(M+H)^+$. Found: 446.3746.

(**B**) White Solid. m. p. = 99-102 ºC. IR (KBr): ν Amide A (3314), Amide I (1670), Amide II (1524), As (2919), S (2848).



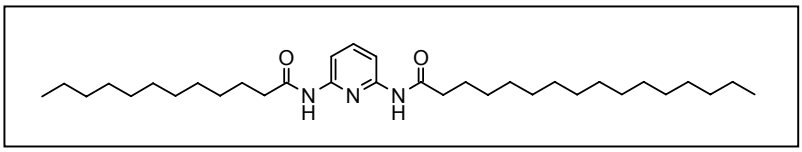
^{1}H NMR (200 MHz, $CDCl_3$): δ 7.87 (d, *J* = 8.5 Hz, 2H), 7.72-7.64 (m, 1H), 7.43 (br. s, 2H, NH), 2.35 (t, *J* = 7.6 Hz, 4H), 1.78-1.64 (m, 4H), 1.40-1.26 (m, 30H), 0.88 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, Proton decoupled): δ 171.4 (NH**C**=O, 2C), 149.5 (**Ar**, 2C), 140.7 (**Ar**, 1C), 109.3 (**Ar**, 2C), 37.7 (CO**C**H, 2C), 31.8, 29.5, 29.4, 29.1, 22.6 (peaks for **17C**), 13.9 (**C**H_3, 2C). ESI/MS: *m/z*: 482.4 $(M+Na)^+$. HRMS calcd. for $C_{28}H_{50}N_3O_2$: 460.3915 $(M+H)^+$. Found: 460.3903.

(**C**) White Solid. m. p. = 80-83 ºC. IR (KBr): ν Amide A (3313), Amide I (1670), Amide II (1524), As (2919), S (2848).



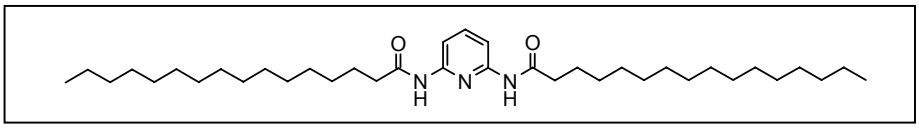
^{1}H NMR (200 MHz, $CDCl_3$): δ 7.88 (d, *J* = 7.7 Hz, 2H), 7.72-7.64 (m, 1H), 7.50 (br. s, 2H, NH), 2.35 (t, *J* = 7.6 Hz, 4H), 1.75-1.64 (m, 4H), 1.32-1.26 (m, 32H), 0.89 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, Proton decoupled): δ 171.5 (NH**C**=O, 2C), 149.5 (**Ar**, 2C), 140.8 (**Ar**, 1C), 109.4 (**Ar**, 2C), 37.8 (CO**C**H, 2C), 31.8, 29.5, 29.4, 29.3, 29.2, 29.1, 25.3, 22.6 (peaks for **18C**), 14.0 (**C**H_3, 2C). ESI/MS: *m/z*: 474.4 $(M+H)^+$. HRMS calcd. for $C_{29}H_{52}N_3O_2$: 474.4042 $(M+H)^+$. Found: 474.4059.

(**D**) White Solid. m. p. = 92-95 ºC. IR (KBr): ν Amide A (3314), Amide I (1670), Amide II (1526), As (2918), S (2848).



^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (d, *J* = 7.5 Hz , 2H), 7.72-7.66 (m, 1H), 7.56 (br. s, 2H, NH), 2.36 (t, *J* = 7.5 Hz, 4H), 1.76-1.67 (m, 4H), 1.30-1.25 (m, 40H), 0.88 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, Proton decoupled): δ 171.4 (NH**C**=O, 2C), 149.4 (**Ar**, 2C), 140.8 (**Ar**, 1C), 109.3 (**Ar**, 2C), 37.8 (CO**C**H, 2C), 31.8, 29.5, 29.4, 29.3, 29.2, 25.3, 22.6 (peaks for **22C**), 14.0 (**C**H_3, 2C). ESI/MS: *m/z*: 552.5 $(M+Na)^+$. HRMS calcd. for $C_{33}H_{60}N_3O_2$: 530.4665 $(M+H)^+$. Found: 530.4685.

(**E**) White Solid. m. p. = 110-112 ºC. IR (KBr): ν Amide A (3396), Amide I (1670), Amide II (1595), As (2918), S (2848).



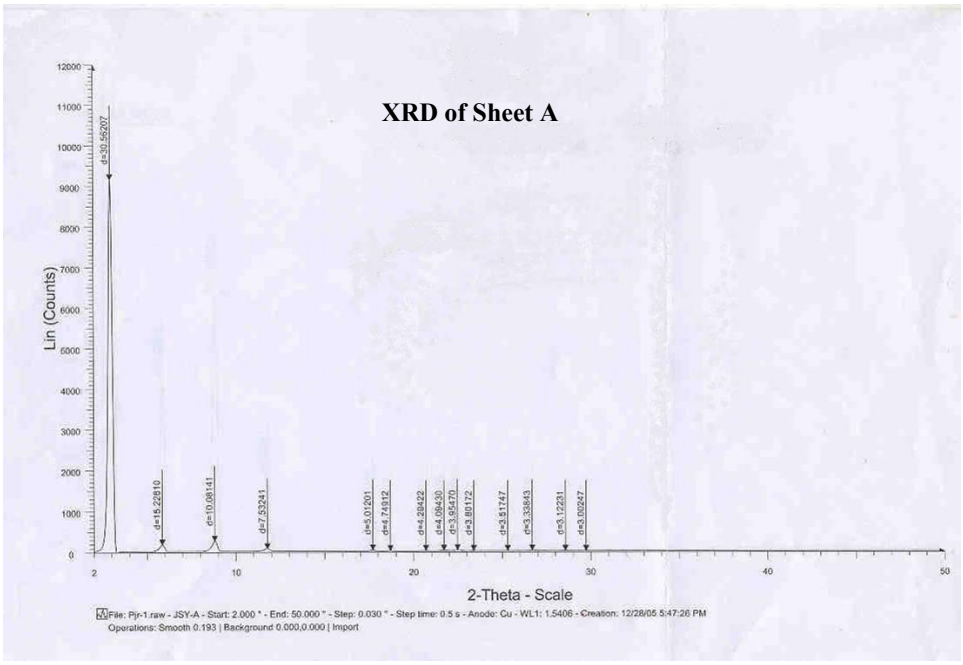
^{1}H NMR (200 MHz, $CDCl_3$): δ 7.87 (d, *J* = 8.1 Hz, 2H), 7.71-7.63 (m, 1H), 7.47 (br. s, 2H, NH), 2.34 (t, *J* = 7.3 Hz, 4H), 1.69-1.62 (m, 4H), 1.37-1.12 (m, 48H), 0.88 (t, *J* = 6.5 Hz, 6H). ESI/MS: *m/z*: 586.5 $(M+H)^+$. HRMS calcd. for $C_{37}H_{68}N_3O_2$: 586.5313 $(M+H)^+$. Found: 586.5311.

Powder X-ray Diffraction (XRD)

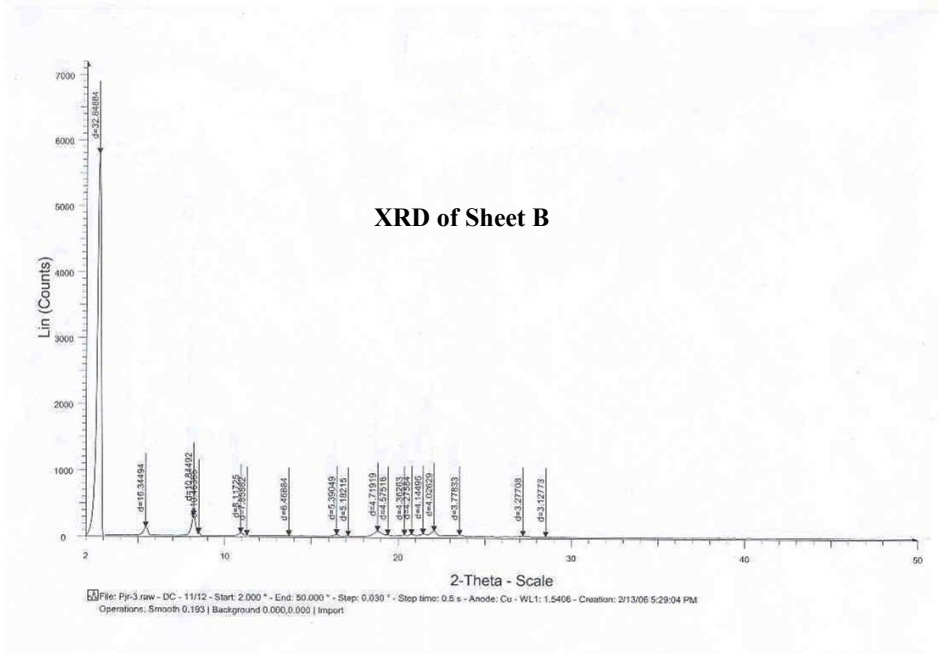
Powder X-ray Diffraction was recorded on Siemens D-5000 in sealed Cu tube (2.2 kw) using graphite crystals. The powdered submicron-sized sheets were filled in the sample tube and were analyzed to produce a single diffraction peak in the small angle region X-ray diffraction patterns were acquired for 2θ values ranging from 3 to 50 for **A-E** powders.

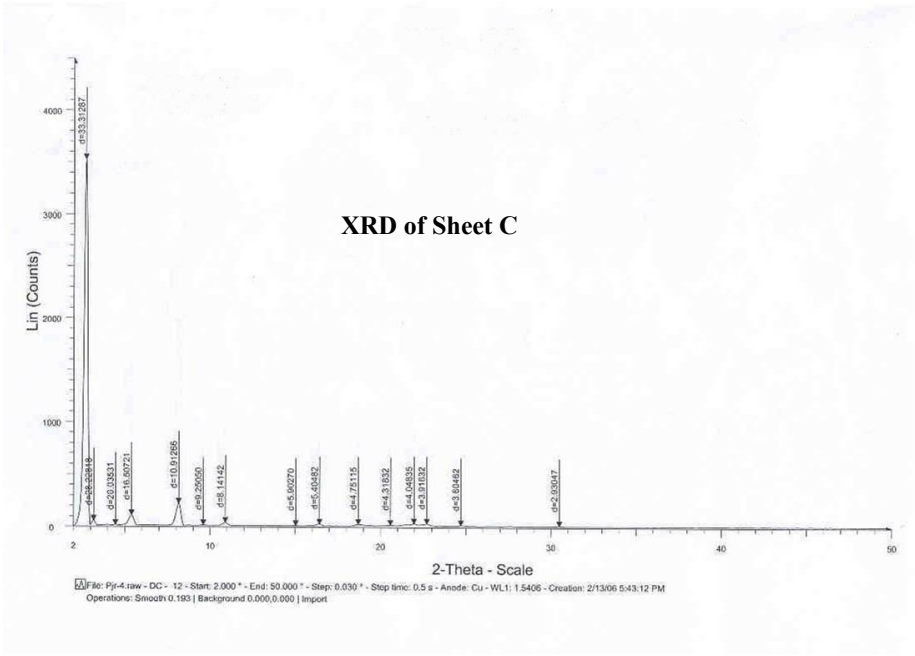
Sample	**d** (XRD) in nm	**l** (Calculated) in nm
A	3.06	2.92
B	3.28	3.34
C	3.33	3.45
D	3.79	3.96
E	4.55	4.46

XRD of Sheet A

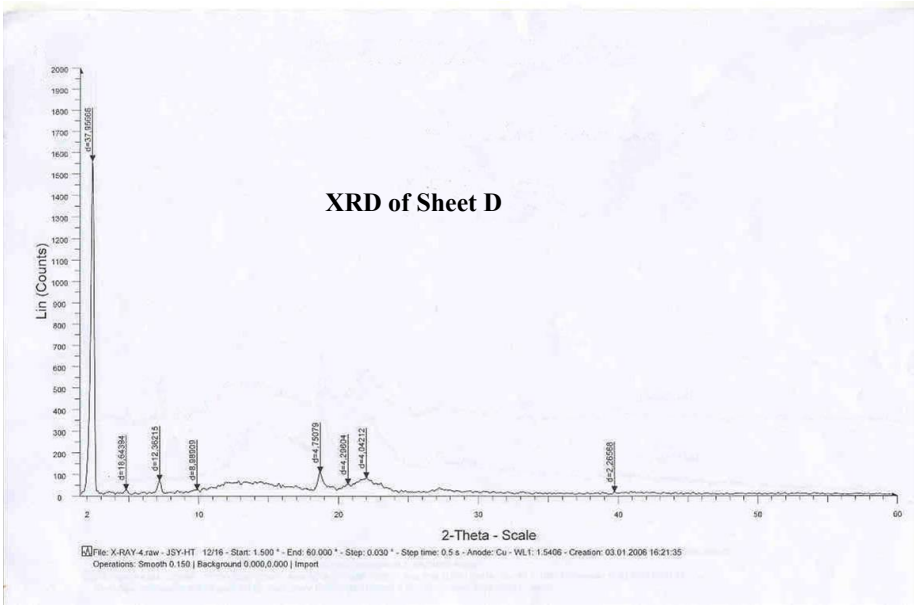


XRD of Sheet B

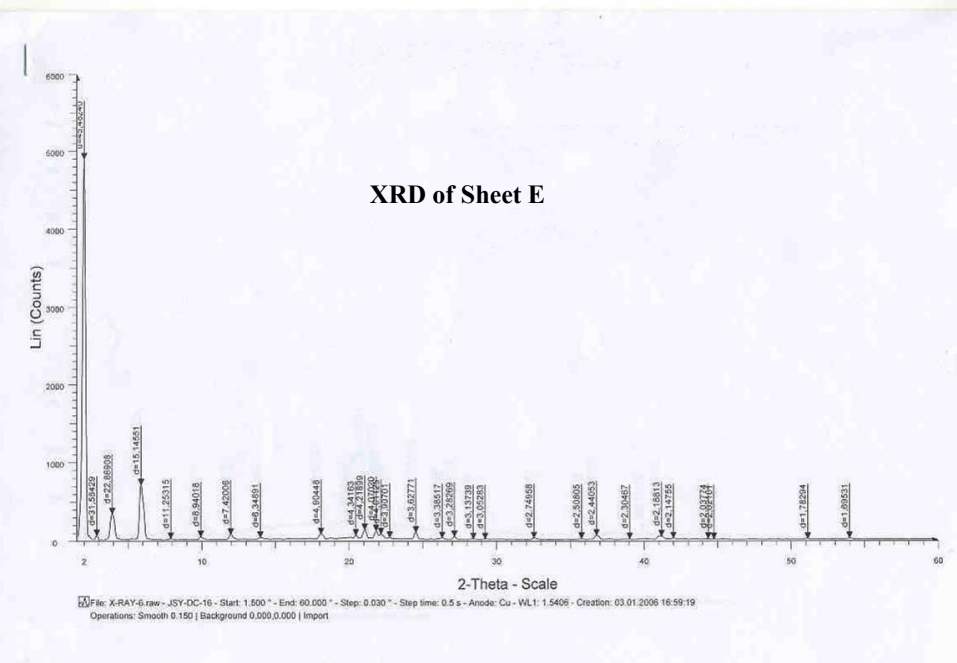




XRD of Sheet C



XRD of Sheet D


XRD of Sheet E
Lin (Counts)
2-Theta - Scale
File: X-RAY-6.raw - JSY-DC-16 - Start: 1.500 ° - End: 60.000 ° - Step: 0.030 ° - Step time: 0.5 s - Anode: Cu - WL1: 1.5406 - Creation: 03.01.2006 16:59:19
Operations: Smooth 0.150 | Background 0.000,0.000 | Import

Flourescence and recognition of self-assembles structures of 2,6-Pyridinediylbisalkanamides

Materials and Methods:

The sample is prepared in 70% methanol and heated up to 100^0 C for 5 min and on slow cooling it forms submicron-sized sheets that are stable for more than 30 min. These sheets formation can be read by checking the fluorescence using fluorometer (Hitachi, F-4500 model).

The nucleoside bases are used about series of concentrations ranging from 10 μM to 300 μM. The micro/nano assemblies are quenched completely at different concentrations and urea samples are not shown any change in the fluorescence.

Sample **A** is completely quenched by thymidine at 250 μM concentration and **C**, **E** at 150 μM, **B** and **D** (Fig 3: A, C, E, G and I) are at 150 μM, 90 μM and 100 μM, respectively. The similar experiment carried out with other nucleosides is almost completely quenched at 300 μM concentration. There is no change in the fluorescence of compound **A** – **E** with urea (Fig 3: B, D, F, H and J).

Apparent binding constants are as follows:

Submicron-sized Sheets	**Thymidine**	**Adenosine**
A	~3.606 x 10^5 M^{-1} (250μM)	~2.017 x 10^5 M^{-1} (200μM)
B	~3.928 x 10^5 M^{-1} (130μM)	~2.825 x 10^5 M^{-1} (100μM)
C	~4.498 x 10^5 M^{-1} (150μM)	~2.179 x 10^5 M^{-1} (200μM)
D	~4.668x 10^5 M^{-1} (150μM)	~3.438 x 10^5 M^{-1} (100μM)
E	~2.507 x 10^5 M^{-1} (100μM)	~1.723 x 10^5 M^{-1} (60μM)

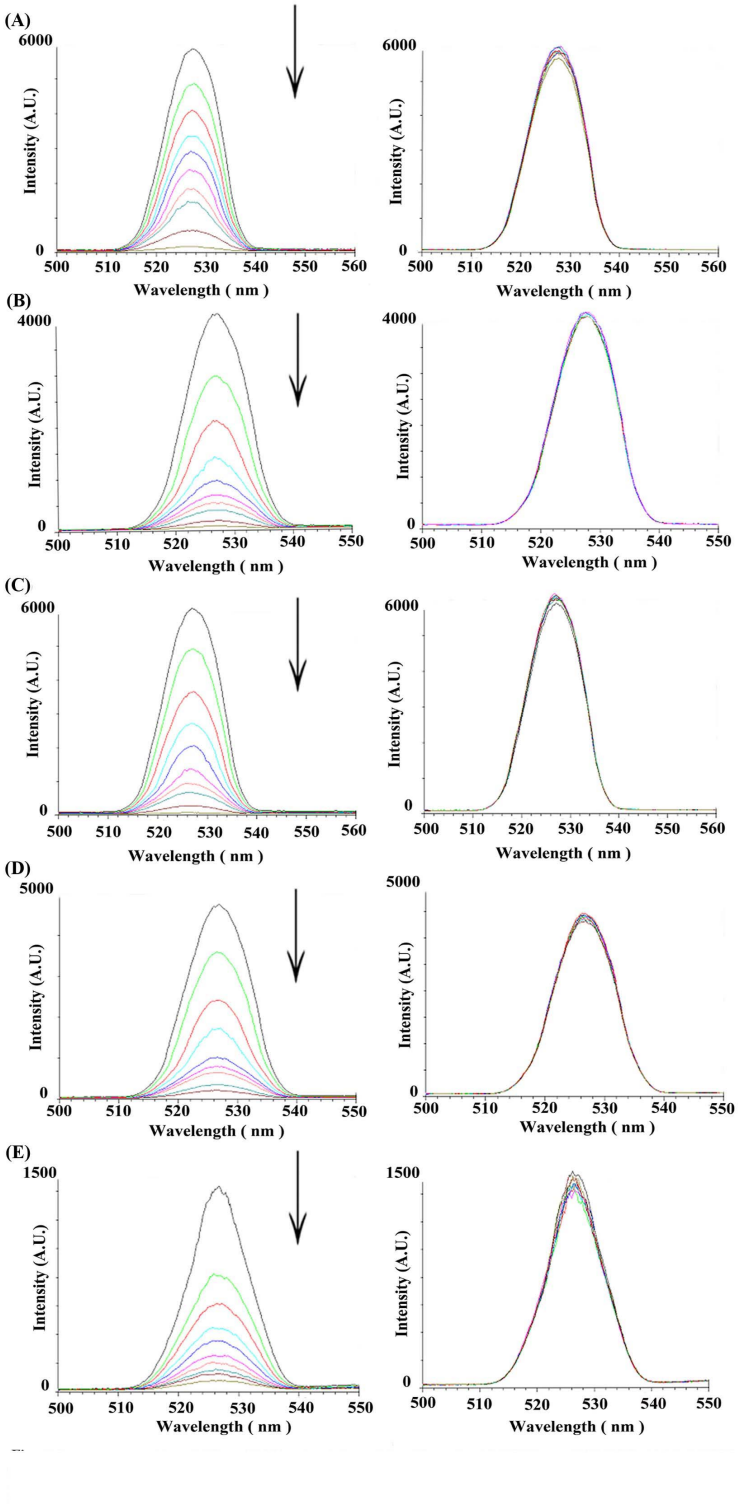


Figure S3 Fluoroscence quenching of different DAP based submicron-sized sheets using thymidine as a quencher. Thymidine concentartion in the graph (Top to bottom) ranged from 10uL to 250 uL and urea used as negative control for the experiment. Fluoroscence quenching by thymidine are shown left side and right side are corresponding urea graph.