J Fluoresc
DOI 10.1007/s10895-013-1239-1

ORIGINAL PAPER

Synthesis, Physical Properties and Cytotoxicity of Stilbene-Triazine Derivatives Containing Amino Acid Groups as Fluorescent Whitening Agents

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Received: 4 March 2013 / Accepted: 14 May 2013

Abstract A series of novel stilbene-triazine derivatives containing amino acid groups were synthesized and screened to evaluate their cytotoxicity. The UV absorptions of the derivatives were in the range of 240–450 nm. The absorption peaks of the cis-isomers and trans-isomers were in 281–291 nm and 353–361 nm, respectively. Their fluorescence emission peaks of the derivatives were located in the range of 400–650 nm. The whiteness data indicated that all the stilbene-triazine-amino acid derivatives showed excellent whitening effect on cotton fiber compared to untreated cotton. The preliminary cytotoxicity of these derivatives on a mouse fibroblast cell line (L-929 cells) was also investigated. The results showed that the compounds (7a–h) were nontoxic to L-929 cells as fluorescent whitening agents.

Keywords Synthesis · Stilbene-triazine derivatives · Amino acid · Whiteness · Cytotoxicity

Introduction

Fluorescent whitening agents (FWA) are dyes that absorb light in the ultraviolet and violet region (usually 300–380 nm) of the electromagnetic spectrum, and re-emit light in the blue region (typically 400–450 nm) [1–3]. Fluorescent activity is a short term or rapid emission response, unlike phosphorescence, which is a delayed emission. These dyes are often used to enhance the appearance of color of fabric and paper, causing a "whitening" effect, making materials look less yellow by increasing the overall amount of blue light reflected. As a result, FWA are often widely used for whitening paper and textile [4–8]. Moreover, FWA have also been used as dyes in biology [9], light emitting diodes [10], and chemosensors [11]. However, FWA have long been well known for their toxicities to human health and environment [12]. With higher demands on healthy living conditions, it is very necessary to develop the alternatives with low toxicity or better biocompatibility. At present, many functional compounds with low-toxicity profile have been obtained by introducing amino acid groups [13–15]. However, a search of the literature revealed that stilbene-triazine derivatives (FWA) with amino acid group have not been described. Therefore their synthesis and properties study seemed to be an attractive task. Herein, we would like to report the synthesis, structures, photo-physical properties and cytotoxicity of the novel stilbene-triazine-amino acid derivatives (7a–h).

Results and Discussion

Synthesis of Derivatives

Reagents and conditions: (i) H_2O, NaOH (aq), 0–5 °C, 2 h; (ii) H_2O, compound 4, NaOH (aq), 40–45 °C, 5 h, then HCl (aq); (iii) H_2O, R_2H, NaOH (aq), 100 °C, 6 h, then HCl (aq) at room temperature.

The novel stilbene-triazine-amino acid derivatives (7a–h) were synthesized in a three-step synthetic route as summarized in Scheme 1. In brief, compound 3 was prepared from

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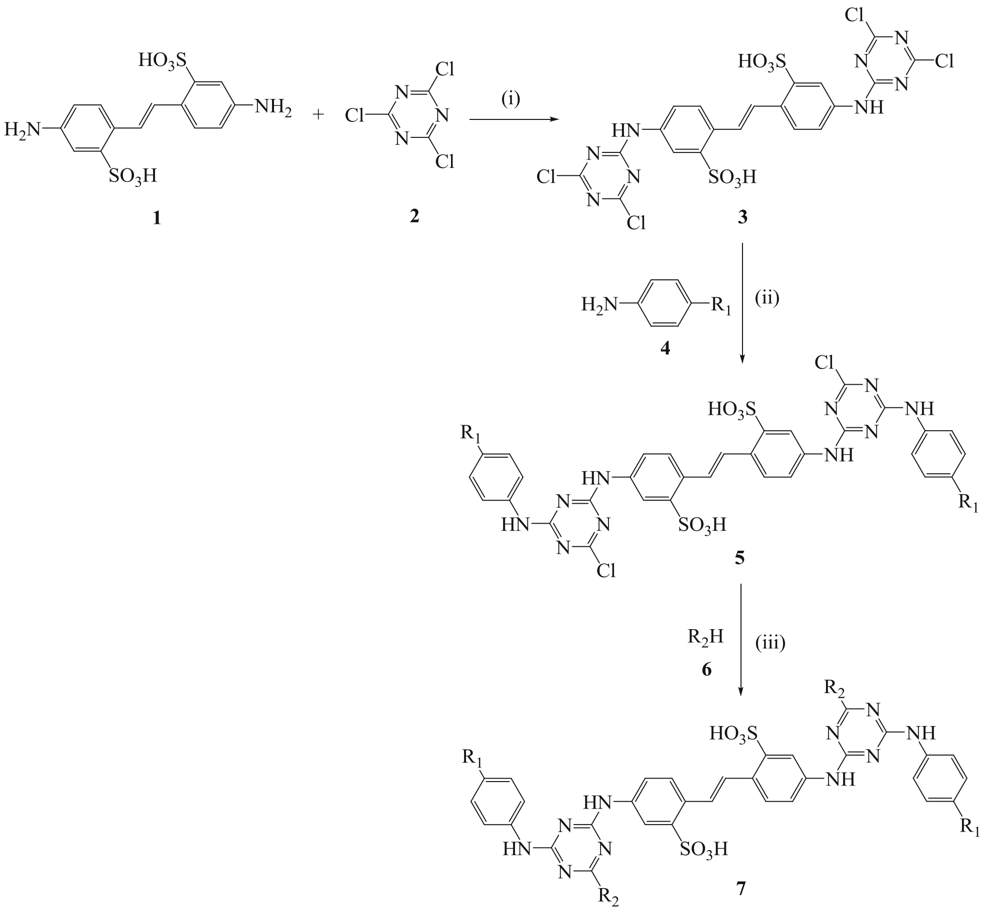

Scheme 1 Synthesis of the compounds (7a–h)

the reaction of 4,4′-diaminostilbene-2,2′-disulfonic acid (1) with 2,4,6-trichloro-1,3,5-triazine (2). This intermediate was further condensed with aromatic amine (4) via nucleophilic substitution reaction. Compounds 5 were isolated by acidifying to pH 3–4 with 4 mol/L HCl solution and then collected through vacuum filtration, and dried under vacuum overnight at 40 °C. Compounds 5 were further reacted with different amino acids (6), and the product of this reaction was precipitated by acidifying to pH 3–4 with 4 mol/L HCl solution, and the reaction mixture was filtered by vacuum to isolate the precipitate. The precipitate was washed with distilled water to remove sodium chloride and dried overnight under vacuum at 40 °C. The product was kept in a desiccator. The compounds (7a–h) were isolated and characterized. The substituents and yields of the compounds are presented in Table 1.

UV–Vis Spectra

The UV–vis spectra of the compounds (7a–h) are shown in Fig. 1. Their absorption bands ranged from 240 nm to 450 nm. The cis- and trans-isomers absorption peaks were in 281–291 nm and 351–361 nm, respectively. Triazine-stilbene compounds are well known for cis-trans isomerization from the strongly fluorescent trans-isomer to the non-fluorescent cis-isomer when they were exposed to light. As can be seen from Fig. 1, the cis-isomer absorption peaks of compounds 7c and 7g are much stronger compared to others. It indicates that 7c and 7g are very easy to light-induced to the cis-isomer because of the smaller substituents. The differences of UV absorption were due to the different substituents.

Fluorescence Spectra

Figure 2 presents the emission spectra of the compounds (7a–h). The emitting fluorescence of the compounds (7a–h) located in the range of 400–650 nm although their intensity of

Table 1 Substituents and yields of the compounds (7a–h)

No	R_1	R_2	Yield (%)
7a	HO_3S-	$(H_3C)_2CHCH_2(HO_2C)CHNH-$	86
7b	HO_3S-	$HO_2C(CH_2)_2(HO_2C)CHNH-$	83
7c	HO_3S-	HO_2CCH_2NH-	86
7d	HO_3S-	(p)$OHPhCH_2CH(CO_2H)NH-$	81
7e	HO_2C-	$(H_3C)_2CHCH_2(HO_2C)CHNH-$	87
7f	HO_2C-	$HO_2CH(CH_2)_2(HO_2C)CHNH-$	85
7g	HO_2C-	HO_2CCH_2NH-	90
7h	HO_2C-	(p) $HOPhCH_2CH(CO_2H)NH-$	83

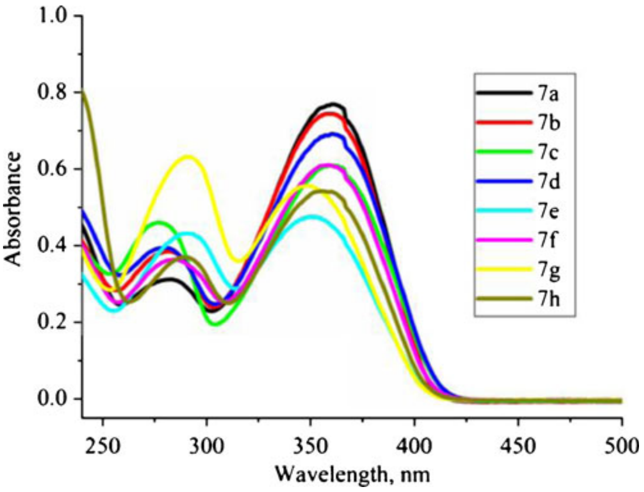

Fig. 1 UV absorption spectra of the compounds (7a–h)

fluorescence differed from each other. UV/visible absorption maxima and fluorescence emission maxima of these compounds were shown in Table 2. Because of the common parent structure, the compounds (7a–h) had the similar shapes of fluorescence emission spectra curve and UV absorption spectra curve.

Whiteness Assessment

The compounds (7a–h) were applied to pure cotton fiber at a concentration of 0.05 %, 0.1 %, 0.15 %, 0.20 %, and 0.25 % (o.w.f). From Table 3, we learned that all of the compounds had a significant whitening effect on cotton at different concentration. It is apparent that most of the compounds impart a high degree of whiteness at the optimum dye concentration (0.15 %). Among these compounds, 7 g was the most effective molecular and the whiteness value of treated cotton was highest (the whiteness=120.7 at the optimum dye concentration). The data obtained are given in Table 3.

Cytotoxicity Evaluation

FWA should be safe to living organisms. Therefore, after synthesizing the stilbene-triazine-amino acid derivatives and studying their physical properties, we evaluated the cytotoxicity of these derivatives to L-929 murine fibroblast cells. Cells were treated with the compounds (7a–h) for 24 and 48 h at concentrations of 5, 10, 15, 20, 25 and 50 ug/mL. Cell proliferation was evaluated by using MTT assay. The data demonstrated that all the stilbene-triazine-amino acid derivatives did not significantly inhibit the proliferation of L-929 cells (cell viability >95 %, Table 4) even if the concentration increased up to 50 ug/mL (the concentration > the optimum dye concentration) for 48 h. This result indicated that the newly synthesized stilbene-triazine-amino acid derivatives could not show considerable cytotoxicity to mouse fibroblast cells and would be green as FWA.

Conclusion

A series of novel stilbene-triazine compounds containing amino acid groups were synthesized through a three-step synthetic route. The obtained compounds were characterized by the analysis of the ^{1}H-NMR, ^{13}C-NMR and IR spectra. The photo-physical characteristics of the molecules were also investigated by measuring the UV spectra and the fluorescence spectra in aqueous solution. All compounds were applied to pure cotton fiber as fluorescent whitening agents and their performance were evaluated by measuring the degree of whiteness. The results showed that the compounds (7a–h) had a significant whitening effect on cotton. The maximum whiteness value of treated cotton with the compound 7 g at a concentration of 0.15 % (o.w.f) was found to be 120.7. The cytotoxicity of all synthesized compounds was evaluated using the MTT assay. Results demonstrated that the obtained stilbene-triazine-amino acid derivatives showed excellent biocompatibility with mouse fibroblast cells. It is encouraging that our compounds were not toxic to mouse fibroblast cells and may serve as green light-emitting materials. Further experiments are under way in our laboratory to confirm their biocompatibility to human cells in vivo.

Experimental

Materials and Analysis

All reagents and materials were purchased from commercial suppliers. ^{1}H-NMR spectra were recorded in a Bruker

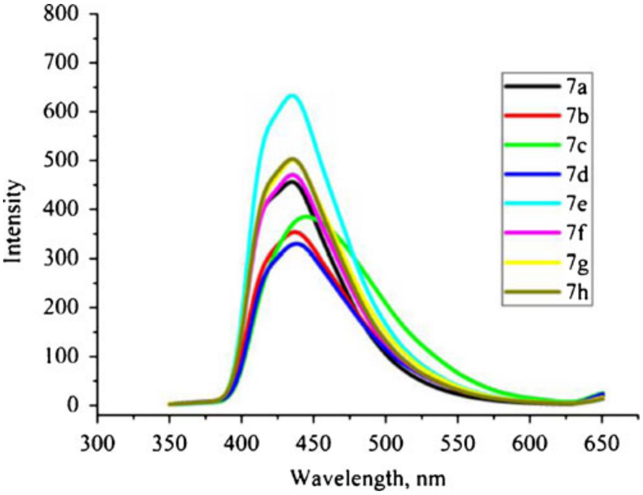

Fig. 2 Fluorescence emission spectra of the compounds (7a–h)

Table 2 UV absorbance and fluorescence characteristics of the compounds (7a–h)

No	UV absorbance		Fluorescence emission
	Cis λ_{max} (nm)	Trans λ_{max} (nm)	λ_{max} (nm)
7a	282	361	435
7b	281	361	437
7c	278	361	445
7d	281	360	437
7e	291	351	435
7f	285	359	434
7g	291	353	435
7h	290	356	435

Avance 400 (400 MHz) spectrometer, using TMS as internal standard, DMSO-d_6 or DMSO-d_6+D_2O as solvent and chemical shifts were expressed in ppm. ^{13}C-NMR spectra were recorded in a Bruker Avance 400 (100 MHz, DMSO-d_6 as solvent). IR spectra were recorded with an IR spectrophotometer Avtar 370 FT-IR. The UV spectra were obtained on a Shimadzu UV-2401PC. Fluorescence spectra were measured in aqueous solution in a quartz cell (1.0 cm). The progress of all reactions was monitored by the thin layer chromatography (TLC) technique, which was performed on 2.0×5.0 cm, using a mixture of CH_3OH, CH_2Cl_2 and NH_4OH as an eluent. The CIE Whiteness values were measured using a Datacolor 600 reflectance spectrophotometer.

General Method for the Synthesis of the Compounds 5

To a 1,000 mL round-bottom flask, equipped with a stirring bar, pH meter and thermometer, 400 mL distilled water and 100 g of ice were added. To this stirred mixture, 100 mmol 4,4′-diaminostilbene-2,2′-disulfonic acid (37.04 g) was added and the pH was maintained 4–5 by drop-wise addition of 1 mol/L NaOH solution. Then 200 mmol 2,4,6-trichloro-1,3,5-triazine (36.88 g) was gradually added to the mixture while maintaining temperature at 0–5 °C with external cooling. The reaction vessel was kept in an ice bath with stirring for 2 h. Aromatic amine 200 mmol (4-amino-benzoic acid and 4-amino-benzenesulfonic acid) was added to the reaction mixture. The mixture was heated to 40–45 °C and stirred for 5 h while maintaining pH 6–7 by drop-wise

Table 3 Degree of whiteness and color data for cotton treated with the compounds (7a–h)

No	o.w.f (%)	Whiteness	L*	a*	b*	No	o.w.f (%)	Whiteness	L*	a*	b*
Untreated		75.0	94.78	−0.55	2.66						
7a	0.05	108.9	95.58	0.95	−4.44	7e	0.05	104.7	95.73	0.74	−3.40
	0.1	110.6	95.69	1.29	−4.72		0.10	104.8	95.67	0.66	−3.47
	0.15	112.4	95.72	1.13	−4.73		0.15	104.2	95.63	0.62	−3.35
	0.2	110.5	95.58	1.13	−5.21		0.20	104.1	95.53	0.53	−3.37
	0.25	110.2	95.42	0.84	−4.71		0.25	102.8	95.57	0.68	−3.08
7b	0.05	103.1	95.11	0.83	−3.36	7f	0.05	98.3	95.61	0.47	−2.05
	0.1	108.7	95.72	1.08	−4.30		0.10	98.6	95.53	0.40	−2.16
	0.15	110.1	95.85	0.77	−4.55		0.15	101.1	95.46	0.41	−2.76
	0.2	109.2	95.53	0.81	−4.51		0.20	101.0	95.70	0.39	−2.61
	0.25	108.4	95.93	0.82	−4.14		0.25	95.8	95.6	0.23	−2.17
7c	0.05	103.6	95.51	0.85	−3.27	7g	0.05	116.2	95.90	1.55	−5.91
	0.10	105.2	95.61	0.84	−3.58		0.10	116.5	95.98	1.42	−5.93
	0.15	106.2	95.65	0.88	−3.78		0.15	120.7	96.10	1.51	−6.59
	0.20	105.8	95.76	0.82	−3.64		0.20	119.7	96.05	1.41	−6.85
	0.25	103.8	95.80	0.48	−3.17		0.25	117.8	95.92	1.44	−6.24
7d	0.05	111.4	95.49	1.17	−5.02	7h	0.05	93.9	95.36	−0.26	−1.21
	0.1	112.1	95.62	1.13	−5.11		0.1	94.9	95.69	−0.53	−1.24
	0.15	113.7	95.71	1.14	−5.44		0.15	96.6	95.66	−0.22	−1.64
	0.2	113.4	95.85	1.04	−5.30		0.2	92.6	95.38	−0.64	−0.91
	0.25	111.7	95.63	0.85	−5.02		0.25	85.4	95.66	−1.51	0.82

o.w.f, the weight ratio of the dye and the cotton fiber; L*, the lightness value; a*, the red/green value; b*, the yellow/blue value

Table 4 Effects of the compounds (7a–h) on mouse fibroblast L-929 cell viability

No	L-929 cell viability (%) for 24 h						L-929 cell viability (%) for 48 h					
	5 ug/mL	10 ug/mL	15 ug/mL	20 ug/mL	25 ug/mL	50 ug/mL	5 ug/mL	10 ug/mL	15 ug/mL	20 ug/mL	25 ug/mL	50 ug/mL
7a	107.1	103.6	106.3	102.3	99.1	98.7	100.3	104.2	107.4	100.8	95.5	98.5
7b	108.2	97.4	99.2	97.1	96.1	96.8	102.1	101.8	100.9	97.9	102.3	100.1
7c	97.1	95.4	96.3	103.2	100.1	104.2	104.3	95.9	96.9	99.8	99.3	105.7
7d	105.7	98.3	102.2	110.6	98.4	105.8	104.2	99.5	102.8	104.9	104.1	104.1
7e	103.7	97.2	100.7	108.5	107.6	105.6	106.3	100.5	98.1	98.4	107.2	104.1
7f	111.4	97.1	100.4	103.5	95.7	105.0	108.8	98.2	111.2	104.1	108.5	98.8
7g	105.3	102.1	99.4	98.1	104.1	103.0	102.5	100.3	97.1	95.2	96.9	97.6
7h	101.8	101.9	103.3	104.1	104.4	104.2	100.1	97.7	103.2	95.3	97.1	106.5

addition of 1 mol/L NaOH solution. Compounds 5 were isolated by aciding to pH 3–4 with 4 mol/L HCl solution and collecting through vacuum filtration, and drying under vacuum overnight at 40 °C, and the compounds 5 were obtained in 96 % and 93 % yield.

General Method for the Synthesis of the Compounds (7a–h)

To a 250 mL reaction flask, equipped with a stirring bar and a reflux condenser, 100 mL distilled water was added. Then the flask was charged with 6 mmol compounds 5 and 12 mmol amino acid 6. The reaction mixture was stirred at reflux for 6 h while maintaining pH 8–9 by drop-wise addition of 1 mol/L NaOH solution. The disappearance of compounds 5 were confirmed by TLC analysis. After 6 h, the product was isolated by cooling and aciding to pH 3–4 with 4 mol/L HCl solution. The product was precipitated out and filtered by vacuum, and the precipitate was washed with distilled water (100 mL). The resulting compounds (7a–h) were dried under vacuum overnight at 40 °C, obtained in 81–90 % yield, and stored in a desiccator.

Synthesis of 2,2'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4,1-Phenylene))bis(Azanediyl)bis(4-(4-Sulfophenylamino)-1,3,5-Triazine-6,2-Diyl))bis(Azanediyl)bis(4-Methylpentanoic Acid) (7a)

^{1}H-NMR (400 MHz; DMSO-d_6, δ): 0.91 (d, 12H, 2×$(CH_3)_2$), 1.57–1.73 (m, 6H, 2×CH_2CH), 4.54 (t, 2H, 2×CH), 7.42 (d, 2H, ArH), 7.57–7.63 (d, 6H, ArH), 7.65 (s, 2H, ArH), 7.74 (s, 2H, CH=CH), 7.89–8.11(d, 4H, ArH), 10.15 (s, 2H, 2×NH), 10.49 (s, 4H, 4×NH); IR (KBr, cm^{-1}): 3445–3028, 2981, 1620, 1472, 1400; ^{13}C-NMR (100 MHz, DMSO-d_6): 22.6, 24.8, 41.1, 62.9, 121.9, 126.8, 135.1, 136.4, 145.9, 150.4, 171.8, 173.6.

Synthesis of 2,2'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4,1-Phenylene))bis(Azanediyl)bis(4-(4-Sulfophenylamino)-1,3,5-Triazine-6,2-Diyl))bis(Azanediyl)Dipentanedioic Acid (7b)

^{1}H-NMR (400 MHz; DMSO-d_6+D_2O, δ): 1.98 (q, 4H, 2×CH_2), 2.37 (q, 4H, 2×CH_2), 4.55 (t, 2H, 2×CH), 7.48 (d, 2H, ArH), 7.58 (d, 4H, ArH), 7.64 (d, 2H, ArH), 7.76 (s, 2H, CH=CH), 7.94 (s, 2H, ArH), 8.01 (d, 4H, ArH); IR (KBr, cm^{-1}): 3441–3028, 2970, 1621, 1472, 1400; ^{13}C-NMR (100 MHz, DMSO-d_6): 26.6, 30.4, 67.8, 120.5, 120.9, 122.6, 123.1, 127.1, 127.5, 136.4, 143.9, 145.5, 173.7, 174.1.

Synthesis of 2,2'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4,1-Phenylene))bis(Azanediyl)bis(4-(4-Sulfophenylamino)-1,3,5-Triazine-6,2-Diyl))bis(Azanediyl)Diacetic Acid (7c)

^{1}H-NMR (400 MHz; DMSO-d_6+D_2O, δ): 4.01 (s, 4H, 2×CH_2), 7.53 (d, 2H, ArH), 7.57 (d, 4H, ArH), 7.40 (s, 2H, ArH), 7.81 (s, 2H, CH=CH), 7.96 (d, 2H, ArH), 8.14 (d, 4H, ArH); IR (KBr, cm^{-1}): 3441–3028, 2981, 1621, 1472, 1400; ^{13}C-NMR (100 MHz, DMSO-d_6): 42.9, 119.1, 120.2, 121.4, 126.5, 129.4, 138.7, 141.1, 143.5, 164.1, 165.9, 172.4.

Synthesis of 2,2'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4,1-Phenylene))bis(Azanediyl)bis(4-(4-Sulfophenylamino)-1,3,5-Triazine-6,2-Diyl))bis(Azanediyl)bis(3-(4-Hydroxyphenyl)Propanoic Acid) (7d)

^{1}H-NMR (400 MHz; DMSO-d_6, δ): 2.97 (d, 4H, 2×CH_2), 4.06 (t, 2H, 2×CH), 4.79 (s 2H, 2×OH), 6.70 (d, 2H, ArH), 7.02–7.56 (d, 12H, ArH), 7.57 (s, 2H, ArH), 7.67 (s, 2H, CH=CH), 7.78 (d, 2H, ArH), 8.14 (d, 4H, ArH), 9.60 (s, 2H, 2×NH), 10.39 (s, 4H, 4×NH); IR (KBr, cm^{-1}): 3441–3028, 2881, 1624, 1472, 1400; ^{13}C-NMR (100 MHz, DMSO-d$_6$): 35.4, 70.9, 115.6, 122.6, 125.1, 126.7, 127.1, 130.9, 138.2, 145.9, 150.4, 156.6, 157.0, 170.8, 172.5.

Synthesis of 4,4'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4, 1-Phenylene))bis(Azanediyl)bis(4-(1-Carboxy-3-Methylbutylamino)-1,3,5-Triazine-6,2-Diyl))bis(Azanediyl) Dibenzoic Acid (7e)

^{1}H-NMR (400 MHz; DMSO-d$_6$, δ): 0.94 (d, 12H, $2\times(CH_3)_2$), 1.64 (m, 2H, 2×CH), 1.76(t, 4H, $2\times CH_2$), 3.80 (t, 2H, 2×CH), 7.61 (d, 2H, ArH), 7.70 (d, 4H, ArH), 7.74 (s, 2H, CH=CH), 7.90 (s, 2H, ArH), 8.01 (d, 2H, ArH), 8.15 (d, 4H, ArH), 9.53 (s, 2H, 2×NH), 9.74 (s, 2H, 2×NH), 10.46 (s, 2H, 2×NH); IR (KBr, cm^{-1}): 3426–3028, 2960, 1623, 1489, 1405; ^{13}C-NMR (100 MHz, DMSO-d$_6$):35.4, 36.2, 53.9, 56.1, 115.7, 115.9, 120.2, 127.1, 127.5, 138.2, 143.6, 145.7, 157.0, 165.5, 170,6, 172.5.

Synthesis of 2,2'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4, 1-Phenylene))bis(Azanediyl)bis(4-(4-Carboxyphenylamino)-1, 3,5-Triazine-6,2-Diyl))bis(Azanediyl)Dipentanedioic Acid (7f)

^{1}H-NMR (400 MHz; DMSO-d$_6$+D$_2$O, δ): 1.82–2.38 (m, 8H, $2\times CH_2CH_2$), 3.31 (t, 2H, 2×CH), 7.59 (d, 2H, ArH), 7.70 (d, 4H, ArH), 7.81 (d, 2H, ArH), 7.86 (s, 2H, CH=CH), 7.94 (s, 2H, ArH), 8.10 (d, 4H, ArH); IR (KBr, cm^{-1}): 3442–3028, 2930, 1592, 1489, 1408; ^{13}C-NMR (100 MHz, DMSO-d$_6$): 26.8, 29.4, 57.3, 113.0, 117.6, 119.7, 126.5, 131.6, 143.2, 150.4, 153.5, 158.7, 168.2, 168.4, 172.6, 175.2.

Synthesis of 4,4'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4, 1-Phenylene))bis(Azanediyl)bis(4-(Carboxymethylamino)-1, 3,5-Triazine-6,2-Diyl))bis(Azanediyl)Dibenzoic Acid (7g)

^{1}H-NMR (400 MHz; DMSO-d$_6$, δ): 4.05 (d, 4H, $2\times CH_2$), 7.67 (d, 2H, ArH), 6.69–7.04 (d, 12H, ArH), 7.76 (s, 2H, CH=CH), 7.88–7.89 (d, 8H, ArH), 9.22 (s, 2H, 2×NH), 10.30 (s, 2H, 2×NH), 10.70 (s, 2H, 2×NH); IR (KBr, cm^{-1}): 3425–3028, 2910, 1621, 1490, 1407; ^{13}C-NMR (100 MHz, DMSO-d$_6$): 42.9, 120.1, 120.9, 121.7, 124.1, 126.8, 127.9, 133.8, 139.9, 142.5, 145.8, 167.2, 167.4, 169.3, 170.8.

Synthesis of 4,4'-(6,6'-(4,4'-(Ethene-1,2-Diyl)bis(3-Sulfo-4, 1-Phenylene))bis(Azanediyl)bis(4-(1-Carboxy-2-(4-Hydroxyphenyl)Ethylamino)-1,3,5-Triazine-6,2-Diyl)) bis(Azanediyl)Dibenzoic Acid (7h)

^{1}H-NMR (400 MHz; DMSO-d$_6$, δ): 2.85 (d, 4H, $2\times CH_2$), 3.62 (t, 2H, 2×CH), 6.68 (d, 2H, ArH), 7.06 (d, 2H, CH=CH), 7.60–8.05 (m, 20H, ArH), 9.29 (d, 2H, 2×NH), 10.51(s, 2H, 2×NH), 10.76 (s, 2H, 2×NH); IR (KBr, cm^{-1}): 3439–3139, 2929, 1590, 1513,1407; ^{13}C-NMR (100 MHz, DMSO-d$_6$): 35.4, 71.9, 115.9, 120.9, 125.1, 126.7, 127.2, 131.0, 136.5, 150.4, 156.7, 157.0, 171.0, 172.5.

NMR Spectra

NMR spectra were performed on a Bruker AVANCE 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz). Splitting patterns reported here are: s (singlet), d (doublet), t (triplet), and m (multiplet).

FT-IR Spectra

FT-IR spectra were recorded on a spectrophotometer Avtar 370 FT-IR. About 600 mg of KBr was ground in a mortar with a pestle, and sufficient amounts of synthesized compounds were ground with KBr to make a 0.5 wt% mixture. After each sample was loaded, the sample chamber was purged with nitrogen for a minimum of 10 min before data collection.

UV-Visible Absorption and Fluorescence Emission Spectra

Steady-state UV–vis is spectra of the monomers (10^{-5} M solutions in deionized water at pH 8 with 1 mol/L NaOH solution) were collected on a Shimadzu UV-2401PC Spectrophotometer. Fluorescence data for the monomers (10^{-7} M solutions in deionized water at pH 8 with 1 mol/L NaOH solution) were collected on a Shimadzu RF-5301 PC Spectrofluorophotometer.

Dyeing Procedure

Dyeing of pure cotton fiber that contained no FWA was carried out for 30 min using different concentration of a solution (pH 8 with 1 mol/L NaOH solution) of the compounds (7a–h) at 0.05 %, 0.10 %, 0.15 %, 0.20 % and 0.25 % (o.w.f). The liquor to pure cotton fiber ratio was adjusted to 40:1; temperature was increased from 20 °C to 50 °C at a rate of 2 °C/min within 15 min; Then the bath was maintained at 50 °C for 15 min. Treated samples were removed from the treatment bath, rinsed in cold water and dried at room temperature.

Whiteness Measurements

The compounds (7a–h) were applied at a concentration of 0.05 %, 0.10 %, 0.15 %, 0.20 % and 0.25 % to pure cotton fiber by the previously described dyeing procedure. The average whiteness value of three measurements from each sample at different locations was recorded and the L*a*b* coordinates were determined.

Cell Culture

L-929 cells (mouse fibroblast cell line) were maintained in RPMI-1640 medium supplemented with 10 % (v/v) bovine calf serum and 80 U/ml gentamicin. Cells were cultured in a 5 % CO_2, humidified incubator at 37 °C and detached by 0.05 % trypsin-EDTA.

Cell Viability Assay

Cells were grown on 96-well plates in culture media until approximately 80 % confluency was reached. The compounds (7a–h) in medium (5, 10, 15, 20, 25 and 50 ug/mL) were added. After incubation (for 24 and 48 h), cell viability was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] assay. The absorbance of all samples was measured at 499 nm using Spectra MAX 190 microplate spectrophotometer (GMI Co., USA).

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