Synthesis and antibacterial activity of new spiro[thiadiazoline-quinoxaline] derivatives

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Abstract

The 1,3-dipolar cycloaddition of 3-methylquinoxaline-2-thione and their *N*-alkylated derivatives to diphenylnitrile imine is presented. Using this method, spiro[thiadiazoline-quinoxaline] derivatives as biologically interesting compounds were produced in high to excellent yields. These compounds have been characterized on the basis of their spectroscopic et spectrometric data (^{1}H and ^{13}C NMR, IR, MS and X-ray). Antibacterial activity of the synthesized products has been studied by employing five bacterial strains. The compounds with ethyl group showed the best activity with MIC value of 32 µg/mL against *Streptococcus fasciens*.

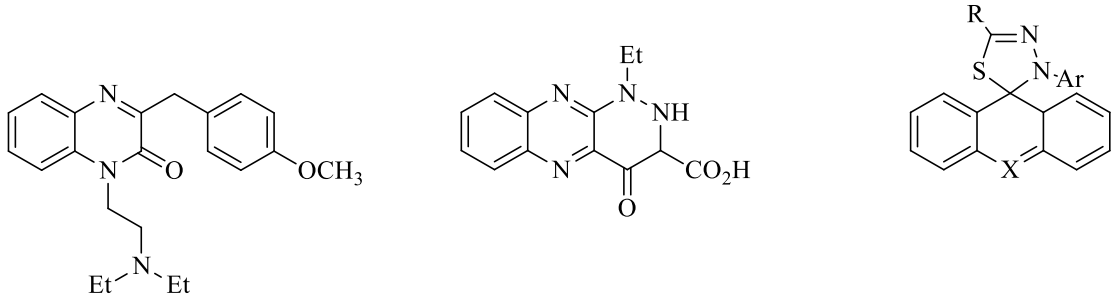
Keywords: 3-Methylquinoxaline-2-thione, 1,3-dipolar cycloaddition, spiro[thiadiazoline-quinoxaline], antibacterial activity

Introduction

The chemistry of quinoxalines has attracted considerable attention in the last decade for their chemical reactivity[1,2] biological properties,[3,4] and materials applications.[5,6] They exhibit a wide spectrum of biological activities such as antibacterial,[7] antifungal,[8] and anticancer.[9] Moreover, quinoxaline ring is a part of various antibiotics, such as Echinomycin, Levomycin and

Actinoleutin that are known to inhibit growth of gram positive bacteria and to be active against various tumors. Also, thiadiazoline moieties are present in the structure of various bioactive molecules found to act as anti-inflammatory,[10] analgesic[11] and allosteric modulator.[12]

The 1,3-dipolar cycloaddition has been the subject of intense research over the last decade, due to its great synthetic value. The cycloaddition is a process of synthesis of five-membered heterocycles, difficult to be prepared with other ways. It gives access to several substances with pronounced biological activities.[13-15]



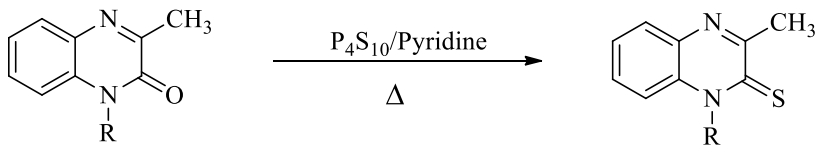
I **II** **III**

Figure 1. Examples of bioactive molecules derived from quinoxaline and thiadiazole.

In continuation of our work on the synthesis of quinoxaline derivatives,[16,17] we report the preparation and investigation of novel spiro[thiadiazoline-quinoxaline] as potential antibacterial compounds.

Results and Discussion

Initially, we have shown that the thionation of *N*-alkylquinoxaline **1a-b** with phosphorus pentasulfide in refluxing pyridine results in rapid formation of the corresponding alkyquinoxaline derivatives **1a-b**. The products were easily obtained by evaporation of pyridine and addition of warm water to the reaction mixture and the results were excellent in terms of yields and purity.

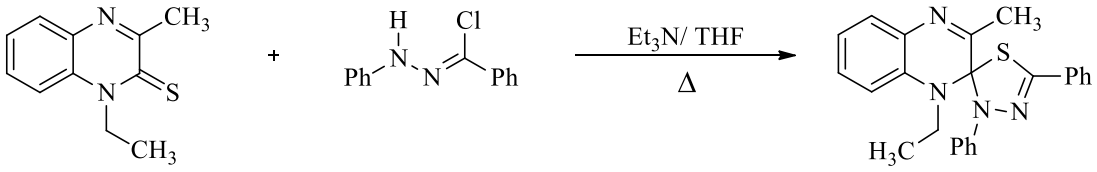


1 : R = H
1a : R = Ethyl
1b : R = Allyl

2 : R = H (90%)
2a : R = Ethyl (64%)
2b : R = Allyl (79%)

Scheme 1. Thionation of *N*-alkyl quinoxalin-2-one **1a-b**.

The structure of compounds **2a-b** was unambiguously characterized on the basis of their IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. The ^{1}H NMR spectra of these compounds showed in particular the presence of protons corresponding to the alkyl groups. After these, we investigated the reaction of 1,3-dipolar cycloaddition of diphenyl hydrazonoyl chloride with an equimolecular amount of 1-ethyl-3-methylquinoxaline-2-thione in dry tetrahydrofuran in the presence of the triethylamine (Scheme 2), one cycloadduct was obtained as a result of the 1,3-dipolar cycloaddition of diphenylnitrile imine ylide generated in situ from diphenyl hydrazonoyl chloride and triethylamine, on the dipolarophilic group C=S.



2a **3** **4a**

Scheme 2. 1,3-Dipolar cycloaddition of 1-ethylquinoxaline-2-thione **2a** and DPNI **3**.

The structural assignment of product **4a** was straight forward and relied upon the elemental analysis and structural data including IR, ^{1}H NMR and ^{13}C NMR spectra and single X-ray diffraction. The ^{1}H NMR spectrum of compound **4a** demonstrated a singlet at 2.16 ppm due to methyl protons at C3, the multiplets at 0.86 and 2.21 ppm were assigned to the ethyl protons appeared as ABX_3 patterns due to the presence of the chiral center at C2. The aromatic protons appeared as a multiplet at 4.89-7.49 ppm. The ^{13}C NMR spectrum exhibited the following signals : methyl carbons at 12.0 and 22.1 ppm, methylene carbon at 39.7 ppm, the spiro carbon at 102.6 ppm, the aromatic carbons at 113.4–132.6, the imine carbon at 156.6 and 142.7 ppm for (S–C=N). The mass spectrum shows a peak at *m/z* 399.1633 corresponding to [M+H]. An X-ray crystallographic study of a single crystal of **4a** (Figure 2, table 1) confirmed the structure deduced from NMR spectroscopic studies.

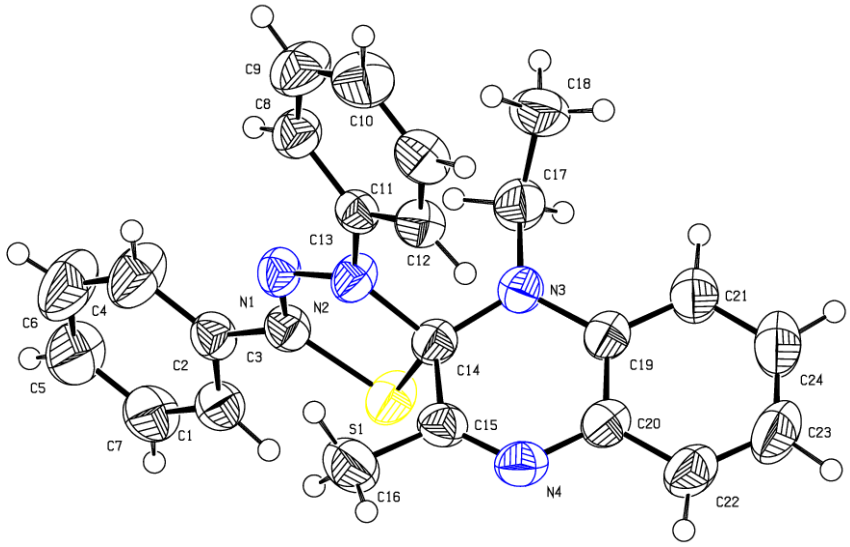
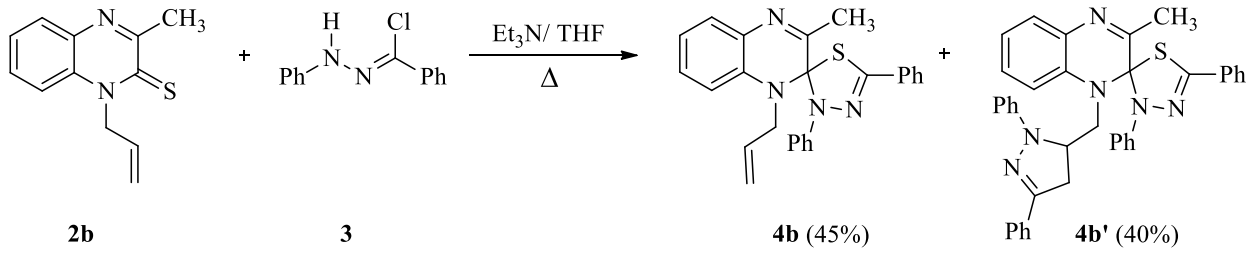


Figure 2. An ORTEP presentation of compound **4a**, showing 30% probability displacement ellipsoids.

In order to examine the *N*-substitution effect of alkyl group on the 1,3-dipolar cycloaddition, *N*-allyl-3-methylquinoxaline-2-thione was chosen to be employed to react with DPNI. So in this case it was found that both the double bond C=S and C=C of allyl group underwent the 1,3-dipolar cycloaddition reaction and formed regioisomeric mixture of novel spiro[thiadiazoline-quinoxaline] in excellent yield (Scheme 3). The two products obtained were separated by silica gel column chromatography.



Scheme 3. 1,3-dipolar cycloaddition of 1-ethylquinoxaline-2-thione **2b** and DPNI **3**.

The structure of these compounds was confirmed on the basis of their spectroscopic characteristics. In the ^{1}H NMR spectrum of **4b**, the allylic methylene group consists of two diastereotopic protons resonate as dd at 3.88 ppm. The other three consecutive signals correspond to the three vinylic protons resonate at 4.95, 5.11 and 5.51 ppm respectively. The aromatic protons resonate at 6.86-7.69 ppm. On the other hand the spiro carbon appeared at 102.4 ppm in the ^{13}C NMR, the allylic carbon showed resonance at 47.6, 118.4, 133.1 ppm. The

^{1}H NMR spectrum of **4b'** shows a multiplet in the region δ 2.70-3.00 for the CH_2 protons of pyrazoline ring. The N-CH_2 protons of the methylene group appeared as a multiplet in the region δ 3.47-3.69 while the CH proton of the pyrazoline ring appears as a multiplet at δ 4.39. The aromatic protons appeared at the region 6.56-7.77 ppm. The ^{13}C NMR shows signals at δ 36.1, 58.1 and 102.6 for CH, CH_2 pyrazolinic and spiro carbon, respectively, which confirms the structure of the products.

The structure of **4b'** was unequivocally evidenced by a single crystal X-ray data (Figure3; Table 1).

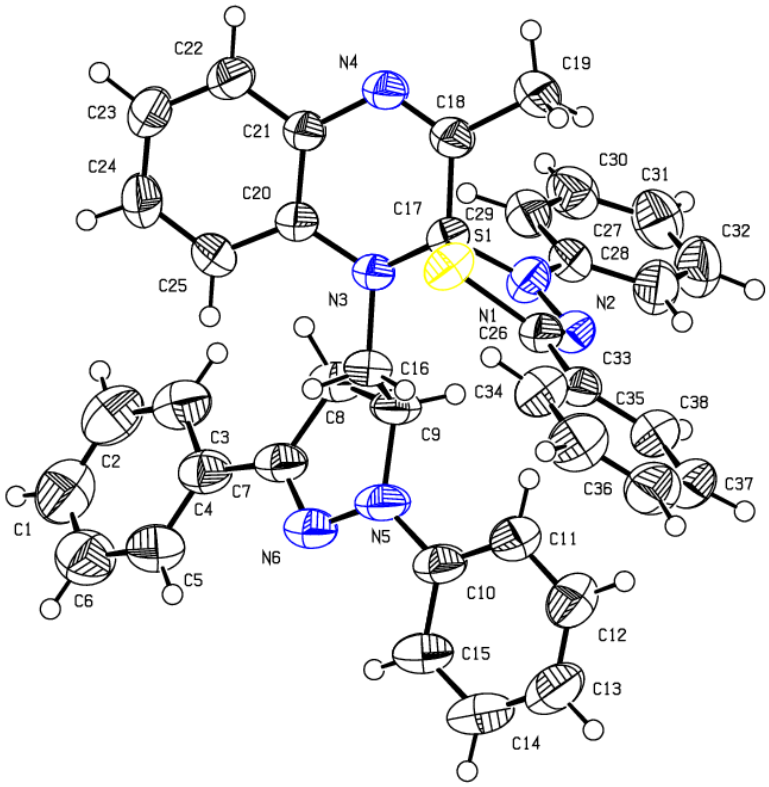


Figure 3. An ORTEP presentation of compound **4b'**, showing 30% probability displacement ellipsoids.

Table 1. Crystallographic data of compounds **4a** and **4b'**

Compounds	**4a**	**4b'**
Empirical formula	$C_{24}H_{22}N_4S$	$C_{38}H_{32}N_6S$
Formula weight	398.16	604.24
Temperature	293(2) K	293(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/n$	P1

Table 1. Continued

Compounds	**4a**	**4b'**
Unit cell dimensions	a = 9.9492(3) Å	a = 11.2893(5) Å
	α = 90°	α = 97.197(4)°.
	b = 14.9849(5) Å	b = 11.3662(5) Å
	β =108.791(2)°	β= 103.861(4)°.
	c = 14.7859(5) Å	c = 13.9207(10) Å
	γ = 90°	γ = 111.130(2)°
Volume	2086.90(12) $Å^3$	1572.84(15) $Å^3$
Z	4	2
Density (calculated)	1.613 Mg/m^3	1.585 Mg/m^3
Absorption coefficient	0.771 mm^{-1}	0.758 mm^{-1}
F(000)	1026	760
Crystal size	0.35x0.31x0.22 mm^3	0.27x0.18x0.17 mm^3
Theta range for data collection	1.99 to 24.43°	1.55 to 26.47°
Index ranges	-11<=h<=11, -17<=k<=17, -17<=l<=17	-14<=h<=14, -14<=k<=14, -17<=l<=17
Reflections collected	21000	39178
Independent reflections	3443 [R(int) = 0.035]	6431 [R(int) = 0.0442]
Refinement method	Full-matrix least-squares on F_2	
Data / restraints / parameters	3443 / 0 / 264	6431 / 0 / 407
Goodness-of-fit on F^2	1.201	0.977
Final R indices [I>2sigma(I)]	R_1 = 0.0369, wR_2 = 0.0916	R_1 = 0.0433, wR_2 = 0.1134
R indices (all data)	R_1 = 0.0553, wR_2 = 0.1196	R_1 = 0.0796, wR_2 = 0.1462
Largest diff. peak and hole	0.208 and -0.230 e.$Å^{-3}$	0.417 and -0.266 e.$Å^{-3}$
CCDC number	775780	775782

In vitro antibacterial activity

The compounds were evaluated for their in vitro antibacterial activity against *Escherichia coli* ATCC 4157, *Pseudomonas aeroginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Staphylococcus aureus* ATCC 25923 and *Streptococcus fasciens* ATCC 29212 using an Agar dilution method.[18] The microorganisms used were procured from the Department of Microbiology Medicinal, National Institute of health, Rabat, Morocco. All bacteria were grown on Mueller–Hinton Agar (Hi-media) plates (37 °C, 24 h). The Minimum Inhibitory Concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculum. The results of the screening are shown in Table 2.

Table 2. Antibacterial activity of the compounds: MIC's in mg/mL

Drugs	Microorganisms				
	E. coli	*S. aureus*	*S. fasciens*	*P. vulgaris*	*P. aeroginosa*
2	-	32	16	-	-
2a	-	64	256	-	-
2b	-	-	-	-	-
4a	-	128	-	-	-
4b	-	-	-	-	-
4b'	-	-	-	-	-
Tetracycline	-	30	30	-	-
Clotrimazole	-	-	30	30	-

MIC–Minimum inhibitory concentration

In antibacterial activity studies, an introduction of ethyl group reflected better activity 64 µg/mL against *Staphylococcus aureus* and 256 µg/mL against *Streptococcus fasciens* followed in decreasing order by 1-ethyl-spiro[thaidiazoline-quinoxaline] with 128 µg/mL against *S. aureus*. In contrast, surprisingly the other molecules were found to be totally inactive against all test bacteria species. By comparison of the inhibiting effects of the alkyl groups, we can deduce that the presence of ethyl group conferred the highest antibacterial action.[19,20] When compared to Clotrimazole and Tetracycline, compound **2a** was less active and compound **2** was more active against *Streptococcus fasciens* than both references.

Conclusions

In conclusion, new spiro[thiadiazoline-quinoxaline] were synthesized and their structure were determined and also assayed for their in vitro antibacterial activity. This study should extend on tests anti-inflammatory drug, antifungal and anti-cancer because the literature gives results enormously interesting on these subjects. Also of other bacteria should be also selected to widen the investigation.

Experimental Section

General. The melting points were taken on an Electrothermal capillary melting point apparatus. Infrared spectra (ν-cm^{-1}) were recorded on a Perkin Elmer 577, using KBr disks. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance 300 NMR Spectrometer in DMSO-d_6. Spectra were internally referenced to TMS. Peaks are reported in ppm downfield of TMS. Mass

spectra recorded in a SYNAPT G2 HDMS (Waters) spectrometer in Electrospray Ionization (ESI).

Thionation

Phosphorus pentasulfide (1.67 g, 3.75 mmol) was suspended in 40 mL of pyridine and 3-methylquinoxalin-2-one (0.5 g, 3.1 mmol) was added. The reaction mixture was refluxed for 5 h. Then 200 mL of warm water was added to the contents of the flask. The precipitate was filtered off and recrystallized in petroleum ether to afforded corresponding compounds.

3-Methylquinoxaline-2-thione (2). Rdt.90%; mp = 250 °C (250-251 lit)[21]; IR (KBr): $\nu_{C=S}$:1074 cm^{-1}; ν_{N-H}: 3000 cm^{-1}; ν_{CN} :1359 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (3H, s, CH_3); 3.38 (1H, s, NH); 7.35-7.74 (4H, m, CH); ^{13}C RMN (DMSO-d_6) δ ppm: 25.1 (CH_3); 116.1; 126.0; 128.4; 130.5 (CH_{Ar}); 132.1; 135.3; 161.8; 175.5 (Cq). HRMS (ESI) [M+H] : *m/z* = 177.0477. Anal. Calcd for $C_9H_8N_2S$: C, 61.34; H, 4.58; N, 15.90; Found : C, 61.20; H, 4.64; N, 15.83.

1-ethyl -3-Methylquinoxaline-2-thione (2a). Yield = 64%; mp: 124 °C. IR (KBr): $\nu_{C=S}$:1134 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1,33 (3H, t, *J* = 7.2 Hz, CH_3); 2.70 (3H, s , CH_3); 4.91 (2H, q, *J* = 7.2 Hz, CH_2); 7.48–7.80 (4H, m, H_{Ar}). ^{13}C NMR (DMSO-d_6) δ ppm : 10.8 (CH_3); 26.4 (CH_3); 44.6 (CH_2); 115.6-129.6 (CH_{Ar}); 130.3; 134.98; 160.9; 175.3 (Cq). HRMS (ESI) [M+H] : *m/z* =205.0786. Anal. Calcd for $C_{11}H_{12}N_2S$: C, 64.67; H, 5.92; N, 13.71; Found : C, 64.80; H, 6.01; N, 13.52.

1-allyl-3-Methylquinoxaline-2-thione (2b). Yield =.79%; mp : 38 °C. ^{1}H NMR (DMSO-d_6) δ ppm : 2.71 (3H, s, CH_3); 5.03 (1H, q, CH); 5.18 (1H, q, CH); 5.57 (2H, dd, *J* = 4.8 Hz, CH_2); 5.93 (1H, q, CH); 7.48 - 7.85 (4H, m, H_{Ar}). ^{13}C NMR (DMSO-d_6) δ ppm : 26.6 (CH_3); 51.5 (CH_2); 116.2 (CH); 117.8 (CH_2); 125.7-130.3 (CH_{Ar}); 132.2; 134.7; 160.9; 176.1 (Cq). HRMS (ESI) [M+H] : *m/z* = 217.0790. Anal. Calcd for $C_{12}H_{12}N_2S$: C, 66.63; H, 5.59; N, 12.95; Found : C, 66.72; H, 5.47; N, 13.01.

1,3-Dipolar cycloaddition

To a solution of *N*-alkyl-3-methylquinoxaline-2-thione (10^{-2} mole) and diphenylnitrilimine ($1{,}3.10^{-2}$ mole) in THF (20 mL), was added triethylamine (2 mL). The mixture was refluxed for 24 hours. The precipitate was recovered by filtration and was separated by chromatography on silica gel (hexane/Ethyl Acetate: 9/1).

1-Ethyl-3-methyl-3',5'-diphenyl-1*H*,3'*H*-spiro[quinoxaline-2,2'-[1,3,4]thiadiazole (4a). Yield = 80%; mp : 151 °C. ^{1}H NMR (DMSO-d_6) δ ppm : 0.86 (3H, t, *J* = 7.2 Hz, CH_3); 2.16 (3H, s, CH_3); 3.30 (2H, q, *J* = 7.2 Hz, CH_2); 6.88-7.49 (14H, m, H_{Ar}). ^{13}C NMR (DMSO-d_6) δ ppm : 12.0 (CH_3); 22.1 (CH_3); 39.7 (CH_2); 113.4; 115.4; 120.8; 121.9; 126.6; 128.7; 129.5; 129.8; 130.2; 130.6 (CH_{Ar}); 102.6; 130.1; 131.9; 132.5; 140.9; 142.7; 156. 6 (Cq). HRMS (ESI) [M+H] : *m/z* = 399.1633. Anal. Calcd for $C_{24}H_{22}N_4S$: C, 72.33; H, 5.56; N, 14.06; Found : C, 72.41; H, 5.62; N, 13.98.

1-Allyl-3-methyl-3',5'-diphenyl-1*H*,3'*H*-spiro[quinoxaline-2,2'-[1,3,4]thidiazole] (4b). Yield = 45%. Mp : 169 °C. ^{1}H NMR (DMSO-d_6) δ ppm : 2.20 (3H, s, CH_3); 3.88(2H, dd, CH_2); 5.51

(1H, m, CH); 4.95 (1H, dd, *J* = 17.4 Hz, CH); 5.11 (1H, dd, *J* = 17.4 Hz, CH); 6.86-7.69 (14H, m, H_{Ar}). ^{13}C NMR (DMSO-d_6) δ ppm : 22.1 (CH_3); 47.6 (CH_2); 118.4 (=CH_2); 131.1 (CH); 114.1; 115.7; 121.1; 122.1;126.6; 128.6; 129.4; 129.7; 129.8; 130.5; 133.1 (CH_{Ar}); 102.4; 132.0; 133.0; 133.1; 141.0; 142.6; 157.0 (Cq). HRMS (ESI) [M+H] : *m/z* = 411.1640. Anal. Calcd for $C_{25}H_{22}N_4S$: C, 73.14; H, 5.40; N, 13.65; Found : C, 73.09; H, 5.51; N, 13.70.

1-((1,3-Diphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)methyl)-3-methyl-3',5'(diphenyl-1*H*,3'*H*-spiro[quinoxaline-2,2'-[1,3,4]thiadiazole (4b'). Yield = 40%; mp : 249 °C. ^{1}H NMR (DMSO-d_6) δ ppm : 2.17 (3H, s, CH_3); 2.72 (1H, m, CH); 3.00 (2H, m, CH_2); 3.53 (2H, m, CH_2); 6.56-7.77 (25H, m, CH). ^{13}C NMR (DMSO-d_6) δ ppm : 22.2 (CH_3); 36.1 (CH_2); 43.7 (CH_2); 58.6 (CH); 112.7; 113.4; 114.3; 115.3; 119.4; 121.5; 122.3; 126.2; 128.6; 128.8; 129.1; 129.3; 129.5; 129.8; 130.0; 130.2; 130.8 (CH_{Ar}); 102.6; 130.6; 130.8; 132.0; 132.5; 134.5; 142.9; 144.9; 148.5; 157.0 (Cq). HRMS (ESI) [M+H] : *m/z* = 605.2485. Anal. Calcd for $C_{38}H_{32}N_6S$: C, 75.47; H, 5.33; N, 13.90; Found : C, 75.38; H, 5.44; N, 13.99.

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