Synthesis, Photophysical Properties, and Antimicrobial Activity of Novel Styryl Colorants Derived from 7-Methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline-6-carbaldehyde

K. J. Jarag,[a] A. R. Jagtap,[a] B. N. Borse,[b] S. R. Shukla,[b] and G. S. Shankarling[a]*

[a]Department of Dyestuff Technology, Institute of Chemical Technology, Matunga, Mumbai 400 019, India
[b]Department of Fibres and Textile Processing Technology, Institute of Chemical Technology, Matunga, Mumbai 400 019, India
*E-mail: gsshankarling@gmail.com or gs.shankarling@ictmumbai.edu.in

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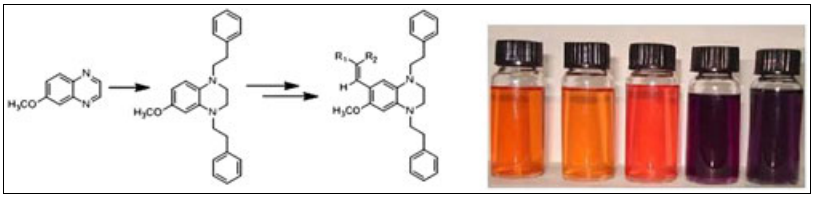

The novel 1,4-diphenethyl-1,2,3,4-tetrahydro-7-methoxyquinoxalin-6-carbaldehyde was synthesized by reductive alkylation of 6-methoxy quinoxaline with phenyl acetic acid and was further subjected to Knoevenagel condensation with various active methylene compounds to synthesize novel styryl colorants. Photophysical properties of styryl colorants were studied using UV–visible and fluorescence spectroscopy. These colorants displayed orange to violet hue and showed fluorescence emission maxima in the region of 560–640 nm, and displayed a large Stokes shift (85–104 nm). Compounds were subjected to thermogravimetric analysis which showed excellent stability up to 310°C. These styryl compounds were evaluated for their antimicrobial study as antifungal against *Candida albicans C. albicans* and *Aspergillus niger* and antibacterial against *Escherichia coli* and *Staphylococcus aureus*. The results revealed good antimicrobial activity against tested organisms. The synthesized chromophores were characterized using elemental analysis, FTIR, ^{13}C-NMR and ^{1}H-NMR spectroscopy and mass spectrometry.

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INTRODUCTION

Styryl colorants are of commercial importance, due to their application in high value-added products, such as sensitizers in photography, fluorescent probes, optical recording materials and dye lasers [1–5]. They are also used for DNA sequencing on gels [6]. Styryls are chemically stable and can be tailor-made for a broad range of applications by introducing different functional groups. Fluorescent styryl chromophores are generally known to have planar and rigid π-conjugation systems, such as stilbene, coumarin, naphthalimide, perylene, and quinoxaline [7].

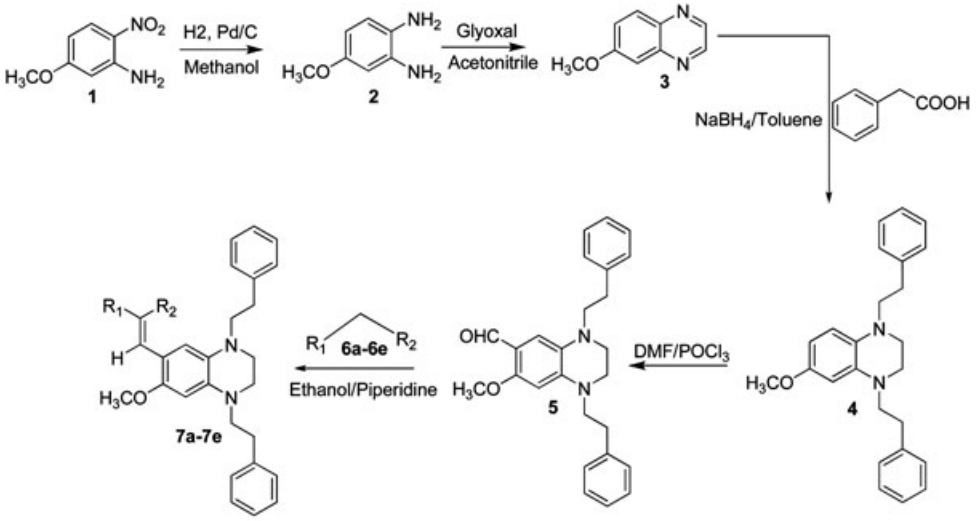
Quinoxaline derivatives, an important group of heterocyclic compounds, have been the subject of extensive study in the last two decades. Many quinoxaline derivatives showed excellent photoluminescence and electroluminescence properties. Compounds with fused quinoxaline aromatic rings are used in making organic light-emitting devices as well as other optoelectronic devices [8, 9]. Quinoxaline derivatives are well known in the pharmaceutical industry and have been shown to possess a broad spectrum of biological activities. They are known to possess antifungal and anticancer activities. Some quinoxaline derivatives have also been explored as antidiabetic and antiallergic agents [10–12]. Pyrazolo-[3,4-b]quinoxaline derivatives have been reported as photoinitiators [13, 14]. Our research group has been working in synthesis of various heterocyclic fluorescent colorants. Recently, we have reported the synthesis of fluorescent styryl colorants based on diphenylamine nucleus [15–17].

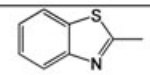
In this study, 6-methoxyquinoxaline was subjected to alkylative reduction using phenyl acetic acid to afford 7-methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline. This key intermediate was then used to prepare novel red to violet styryl chromophores by Knoevenagel condensation. All these chromophores showed absorption and emission properties depending on electron acceptors.

RESULTS AND DISCUSSION

Synthesis and characterization of styryl colorants. The synthetic procedure adopted to obtain the target compounds is depicted in Scheme 1. The novel quinoxaline colorants **7a–7e** were synthesized by classical Knoevenagel condensation of 1,4-diphenethyl-7-hydroxy-1,2,3,4-tetrahydroquinoxaline-6-carboxaldehyde

Scheme 1. Synthetic pathway of colorants **7a–7e**.



Active methylene	R_1	R_2	Chromophore
6a	-CN	-CN	**7a**
6b	-COOEt	-CN	**7b**
6c	-COOMe	-CN	**7c**
6d		-CN	**7d**
6e		-CN	**7e**

(5) with various active methylene compounds **6a–6e**. 4-Methoxy-1,2-phenylenediamine (2) was obtained by catalytic reduction of 4-methoxy-2-nitro aniline (1) over palladium on charcoal bed. Condensation of 4-methoxy-1,2-phenylenediamine with glyoxal in acetonitrile afforded 6-methoxy quinoxaline (3). The reaction of 6-methoxyquinoxaline with phenyl acetic acid and sodium borohydride in dry toluene gave 1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline (4). Vilsmeier–Haack formylation on 1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline yielded 1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline-6-carbaldehyde (5).

The structure (5) was established based on elemental and spectral data. The FTIR spectrum showed bands at 3025, 2829, and 1668 cm^{-1} accounting for aromatic C–H stretch, aldehydic C–H stretch and C=O stretch, respectively. Its ^{1}H-NMR spectrum displayed singlet signal at 10.2 ppm for aldehydic proton. Moreover, the mass spectrum of (5) exhibited the molecular ion peak at *m/z* 401, which is in agreement with its molecular formula $C_{26}H_{28}N_2O_2$. Knoevenagel condensation involving (5) with active methylenes (**6a–6e**) using piperidine as catalyst occurred smoothly giving colorants **7a–7e**. The structures of the new chromophores **7a–7e** were established by ^{1}H-NMR, ^{13}C-NMR, FTIR, mass spectrometry, and elemental analysis. All the colorants **7a–7e** as well as compounds **4** and **5** show typical pattern of methylenic protons in the region of 2.90–3.8 ppm. The two protons present on the phenyl ring attached to styryl linkage displayed prominent singlets, proton ortho to $-OCH_3$ always appeared at or around 5.90–6.10 ppm, while proton ortho to styryl linkages appeared at 7.80–8.10 ppm.

Photophysical properties. To investigate photophysical properties, the absorption spectra of **7a–7e** in dichloromethane solution (1×10^{-4} *M*) were recorded. The electronic absorption spectra of the colorants **7a–7e** in dichloromethane displayed absorption maxima in the visible region from 508 to 536 nm.(Fig. 1) The colorants underwent bathochromic shift depending on the extent of conjugation as well as electron withdrawing nature of the acceptor group. The values of molar extinction coefficients were in the range from 16,723 to 67,251 L $(mol\ cm)^{-1}$. Insertion of conjugating aromatic electron

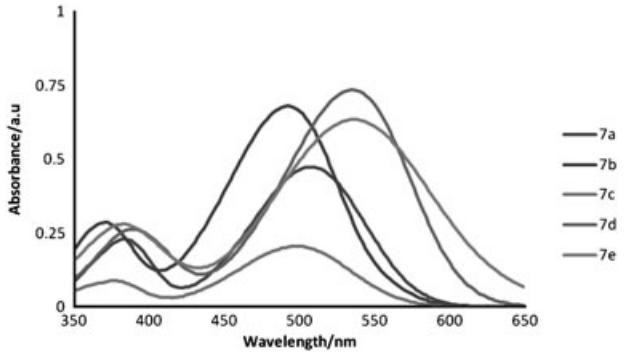


Figure 1. UV–vis spectra of dyes **7a–7e** (10^{-4} *M*) in dichloromethane.

acceptor in quinoxaline unit, due to extended overlap of orbitals, leads to higher molar extinction coefficients for the absorption. The colorants **7a** and **7b** having $(CN)_2CH_2$ and $CNCH_2COOEt$ as an acceptor group showed absorption and emission maximum equal to 504, 492 nm and 580, 586 nm, respectively. Colorants **7d** and **7e** showed bathochromic shift in absorption maximum as compared to colorants **7a**, **7b**, and **7c**. This may be due to the electron withdrawing nature of the substituent in colorants. Introduction of heterocyclic electron acceptor moiety in the parent quinoxaline structure leads to red shifted absorption. Colorant **7d** having cyanomethyl benzthiazole as an electron acceptor showed absorption and emission maximum equal to 528 and 616 nm, respectively. Colorant **7e** having 4-nitrophenyl acetonitrile as electron acceptor moiety showed absorbance maximum at 540 nm; however, it did not exhibit fluorescence. Introduction of nitro group increases the bathochromicity whereas it also quenches the fluorescence, thus colorant **7e** displayed bathochromic shift but is nonfluorescent. The absorption and emission spectra of colorants in dichloromethane are shown in Figures 1 and 2, respectively.

Solvatochromic properties of these colorants were studied using nonpolar (toluene and dichloromethane) and polar solvents (THF, acetone, methanol, and DMF).

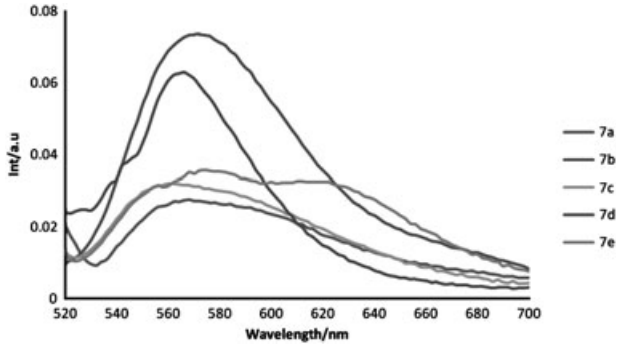


Figure 2. Fluorescence spectra of **7a–7e** (10^{-4} *M*) in dichloromethane.

Absorption maxima λ_{max}, emission maxima λ_{max} (em) and the associated Stokes shifts are shown in Table 1. Solvatochromic properties are dependent on structure of colorant as well as nature of chromophore and solvent [18]. These colorants showed a certain solvatochromism in the absorption and emission spectrum. For example for colorant **7a**, a change of solvent from least polar solvent toluene to polar DMF causes a positive bathochromic shift in absorption spectrum from 498 to 512 nm (Table 1). In case of emission spectrum of **7a**, we observed substantial quenching in the polar solvents.

The colorants exhibited orange to reddish pink fluorescence in most of the solvents. These colorants with donor-π-acceptor skeleton contains 1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline as electron donor unit conjugated to various electron withdrawing groups. The relatively low fluorescence intensities were observed for all colorants. It clearly indicates that excited state of colorant may be quenched by several nonradiative physical processes such as photoinduced electron transfer, resonance energy transfer to quencher, dipole–dipole interaction, *etc*.

Thermal stability of the colorants. Thermal properties of colorants **7a–7e** were examined by both thermogravimetric analysis and differential scanning calorimetry. Thermal stability data of compounds **7a–7e** is tabulated in Table 2. Stepwise isothermal ramping up to 600°C at 10°C min^{-1} was performed in a nitrogen atmosphere. Thermal stability is defined as the temperature up to which ~95% of the composition of the compound remains stable. The change in weight of the compound was measured as a function of temperature. Thermogravimetric analysis of compound **7b** showed the highest decomposition temperature, 97% of the weight composition was stable up to 307°C and underwent rapid thermal decomposition thereafter. Phase transition of the colorants **7a–7e** measured by differential scanning calorimetry showed sharp transitions. All of the colorants showed melting endotherms and crystallization exotherms with the exception of **7c**. Colorant **7d** showed highest phase transition temperature (166°C), and compound **7c** showed lowest phase transition temperature (124°C). It is clear from the presented data that all colorants have good thermal stability. The high thermal stability for the present colorants may be attributed to planar arrangement of 1,4-diphenethyl moiety which provides additional rigidity to molecule.

Antimicrobial activity.. All the synthesized compounds were tested *in vitro* for their antibacterial activity against microorganisms such as *Staphylococcus aureus* and *Escherichia coli*, using streptomycin as standard antibacterial. Minimum inhibitory concentrations (MIC) were determined by means of standard serial dilution method and are given in Table 3. It has been observed

Table 1

1UV–vis absorption and fluorescence emission maxima of colorants **7a–7e** in various solvents.

		7a	7b	7c	7d	7e
Toluene	λ_{max} (nm)	498	486	486	520	530
	λ_{em} (nm)	590	578	578	604	640
	Stokes shift	92	92	92	84	110
	ε [L (mol cm)$^{-1}$]	14,623	27,890	10,113	25,497	33,471
Acetone	λ_{max} (nm)	506	494	494	532	540
	λ_{em} (nm)	594	598	576	626	–
	Stokes shift	88	104	82	94	–
	ε [L (mol cm)$^{-1}$]	27,048	51,840	10,498	53,389	44,247
Dichloromethane	λ_{max} (nm)	504	492	494	528	540
	λ_{em} (nm)	580	586	570	616	–
	Stokes shift	76	94	76	88	–
	ε [L (mol cm)$^{-1}$]	53,602	53,129	16,723	67,251	67,160
THF	λ_{max} (nm)	502	490	490	528	540
	λ_{em} (nm)	600	592	588	618	–
	Stokes shift	98	102	98	90	–
	ε [L (mol cm)$^{-1}$]	23,818	42,275	12,954	39,248	34,940
Methanol	λ_{max} (nm)	508	492	498	534	536
	λ_{em} (nm)	568	572	560		566
	Stokes shift	60	80	62		30
	ε [L (mol cm)$^{-1}$]	21,261	33,701	9872	40,918	34,505
DMF	λ_{max} (nm)	512	502	502	542	552
	λ_{em} (nm)	–	604	562	630	–
	Stokes shift	–	102	60	88	–
	ε [L (mol cm)$^{-1}$]	20,230	23,392	6790	40,696	29,226

Table 2

Thermal properties of **7a–7e**.

Compounds	TGA[a] (°C)	DSC[b] (°C)
7a	296	143
7b	307	137
7c	298	124
7d	288	166
7e	257	147

[a]Thermogravimetric analysis (TGA). Decomposition temperature (~95% stable) for the respective compounds.
[b]Differential scanning calorimeter (DSC). Transition temperature for the respective compounds.

Table 3

3MIC (ug mL^{-1}) determination using the modified resazurin assay.

Compd No.	Bacterial strains		Fungal strains	
	E. coli	*S. aureus*	*C. albicans*	*A. niger*
7a	5×10^3	–	2.5×10^3	2.5×10^3
7b	–	–	6.25×10^2	2.5×10^3
7c	2.5×10^3	5×10^3	1.25×10^3	2.5×10^3
7d	2.5×10^3	2.5×10^3	1.25×10^3	2.5×10^3
7e	2.5×10^3	1.25×10^3	2.5×10^3	2.5×10^3

that the compounds **7c–7e** show good activity, whereas **7b** does not show any antibacterial activity against *E. coli*. The compounds **7d–7e** showed moderate antibacterial activity against *S. aureus*.

The antifungal activity was tested against strain such as *Aspergillus niger* and *Candida albicans* using fluconazole as a standard. All the compounds showed good antifungal activity. The compounds **7a–7e** showed moderate activity against both strains. The good activity of compounds may be attributed to presence of heterocyclic ring.

CONCLUSIONS

We have synthesized novel styryl colorants (**7a–7e**) based on 1,4-diphenethyl-1,2,3,4-tetrahydro-7-methoxy-quinoxalin-6-carbaldehyde in good yields (62–86%). The colorants were thoroughly analyzed by spectral methods and subjected to photophysical and thermal studies. The absorption properties of the colorants are significantly influenced by nature of electron acceptor moiety attached to quinoxaline nucleus. The hues of these molecules were tuned from red to violet by changing the nature of electron acceptor. The low fluorescence intensities for these colorants highlight the dominance of nonradiating pathways leading to the quenching of the excited state. This is the first report of reductive alkylation reaction of 6-methoxy quinoxaline using substituted aryl carboxylic acid. These colorants showed promising thermal stability. The results indicate potential use of these molecules for the fabrication of amorphous thin films. These compounds showed antibacterial and antifungal activity against tested organisms.

EXPERIMENTAL

Materials and equipment. All solvents and chemicals were procured from SD fine chemicals (India) and were used without further purification. The reactions were monitored by TLC using 0.25-mm E-Merck silica gel 60 F254 precoated plates, which were visualized with UV light (254 and 366 nm). Melting points were measured on a standard melting point apparatus from Sunder Industrial Products, Mumbai. UV–vis absorption spectra were recorded on a Spectronic GENEYSIS 2 spectrophotometer instrument from dye solutions in chloroform. The fluorescence maxima were recorded on a Jasco FP- 1520 fluorimeter from dye solutions in chloroform. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, on a Varian Mercury Plus spectrometer Chemical shifts are expressed in δ (ppm) using TMS as an internal standard. Mass spectral data were obtained with a Micromass Q-Tof (YA105) spectrometer. Elemental analysis was done on a Harieus rapid analyzer. Thermogravimetric analysis was carried out on an SDT 226 Q600 v8.2 Build 100 model of TA instruments.

Synthesis and characterization. ***Synthesis of 6-methoxyquinoxaline (3).*** 6-Methoxyquinoxaline (**3**) was prepared using reported method [19]. m.p. 58–60°C (Lit: 60°C).

Synthesis of 6-methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline (4). 6-Methoxyquinoxaline (5.2 g, 0.032 mol) was dissolved in dry toluene (250 mL) and cooled to 5°C. To this cold solution, sodium borohydride (12.33g, 0.32mol) was added over a period of 30 min. Pale yellow slurry thus obtained was stirred for 15 min. Phenyl acetic acid (44.2 g, 0.32 mol) was dissolved in 100 mL of toluene and added to reaction mass dropwise over a period of 1 h maintaining the temperature 5–10°C. The brownish slurry that formed was stirred for another 1 h at 10°C and allowed to attain room temperature. It was then heated to gentle reflux for 8 h (reaction monitored on TLC). On cooling, thick reaction mass was poured in 250 mL of water. The toluene layer formed was separated, and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). Combined extracts and toluene layer were washed with 40% potassium hydroxide solution and brine, dried over anhydrous sodium sulfate, filtered, and vacuum evaporated to afford dark brown oil.

Yield: 76%. FTIR (KBr, cm^{-1}): ν∼ = 3027, 2203, 1610,1512,1338,1294 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.19–7.33 (m, 10H) ppm, 6.54 (s, 1H), 6.21–6.24 (dd, 2H, *J* = 8.43 Hz), 3.77 (s, 3H), 3.37–3.48 (m, 4H,), 3.28 (m, 2H), 3.19 (m, 2H), 2.90 (m, 4H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 152.99 ppm, 140.15, 139.83, 129.05, 128.84, 128.76, 128.52, 128.50, 126.58, 126.20, 126.12, 111.90, 100.18, 98.80, 55.54, 54.12, 53.52, 47.95, 47.16, 32.09, 31.92. $C_{25}H_{28}N_2O$: Calcd. C 80.61, H 7.58, N, 7.52; Found C 80.63, H 7.78, N, 7.65.

Synthesis of 7-methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline-6-carbaldehyde (5). In a 100 mL three neck round bottom flask (4.1 mL, 53 mmol) of anhydrous *N*,*N*-dimethylformamide was cooled (0–5°C) in an ice bath. To this solution, (3.39 mL, 36 mmol) of phosphorous oxychloride was added dropwise at 0–5°C. 6-Methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline (**4**) (7.65 g, 20 mmol) was added to the above solution and heated to 70° C for 5 h. This solution was then cooled to room temperature, poured in to ice water, and neutralized to pH 6–7 by dropwise addition of saturated aqueous sodium hydroxide solution. The mixture was extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulfate and then concentrated on rotary evaporator. The crude product on purification by column chromatography using silica gel 100–200 # and ethyl acetate: toluene (5:95) as eluent system afforded dark yellow solid.

Yield: 75%. FTIR (KBr, cm^{-1}): ν∼ = 2800–2900, 3000–3100, 1668, 1591, 1320. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 10.18 (s,1H) ppm, 7.16–7.32 (m, 10H), 7.08 (s, 1H), 6.05 (s, 1H), 3.82 (s, 3H), 3.57 (t, 2H), 3.45 (t, 2H,), 3.30 (t, 2H), 3.04 (t, 2H), 2.90 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 186.44, 158.23, 143.02, 139.58, 138.71, 128.86, 128.66, 128.60, 128.59, 128.42, 128.33, 128.05, 126.47, 126.23, 126.00, 125.13, 113.92, 108.46, 92.75, 55.75, 53.31, 53.22, 48.79, 45.78, 32.31, 31.45. ppm. Mass: *m/z* 401.33 (M + 1) $C_{26}H_{28}N_2O_{29}$(400.30): Calcd. C77.97, H 7.05, N 6.99; Found C 78.17, H 6.98, N 7.22.

Synthesis of 2-((7-methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxalin-6-yl)methylene)malononitrile (7a). 7-Methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxaline-6-carbaldehyde (1.5 g, 3 mmol) and malononitrile (0.247 g, 3.7 mmol) were dissolved in ethanol. Piperidine (0.1 mL) was added to it, and reaction mixture was refluxed for 4 h. Ethanol was removed by distillation under reduced pressure to afford dark red colored solid. The dye **7a** obtained was purified by column chromatography using silica gel 100–200 mesh and toluene as eluent system.

Yield: 83%, Melting point: 142–145°C. FTIR (KBr): ν∼ = 3027, 2203, 1610, 1512, 1338, 1294 cm^{-1}. ^{1}H-NMR (400 MHz,$CDCl_3$): δ = 8.00 (s, 1H), 7.55 (s, 1H), 7.32–7.35 (m, 4H, *J* = 8.85 Hz), 7.28–7.30 (m, 4H *J* = 8.45 Hz), 7.18 (t, 2H) 5.94 (s, 1 H), 3.80 (s, 3 H), 3.62 (t, 2H), 3.46 (t, 2H), 3.28 (t, 2H), 3.04 (t, 2H), 2.92 (t, 4H) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 156.68, 150.04, 144.69, 139.80, 138.44, 129.14, 128.99, 128.83, 128.49, 127.02, 126.28, 118.81, 110.02, 107.48, 92.28, 56.07, 53.70, 53.12, 49.42, 46.24, 32.83, 31.90 ppm. Mass: *m/z* 449 (M + 1). $C_{29}H_{28}N_4O$ (448): calcd. C 77.65, H 6.29, N 12.49; found C 77.39, H 5.94, N 12.79.

The following compounds were synthesized by aforementioned procedure, using compound **5** and different active methylene compounds **6b–6e**.

Synthesis of (E)-ethyl 2-cyano-3-(7-methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxalin-6-yl)acrylate (7b). Dark red solid, Yield: 62%. Melting point: 137–139°C. IR (KBr): ν∼ = 3022, 2206, 1697, 1514, 1346, 1234 cm^{-1}, ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.63 (s, 1H), 7.81 (s, 1H), 7.20–7.31 (Ar-H, 10H), 6.00 (s, 1 H), 4.32 (q, 2H), 3.81 (s, 3H), 3.60 (t, 2H), 3.51 (t, 2H), 3.30 (t, 2H), 3.07 (t, 2H), 2.94 (m, 4H), 1.37 (t, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 156.68, 150.04, 144.69, 139.80, 138.44, 129.14, 128.99, 128.83, 128.49, 127.02, 126.28, 118.81, 110.02, 107.48, 92.28, 56.07, 53.70, 53.12, 49.42, 46.24, 32.83, 31.90 ppm. Mass: *m/z* = 496 (M + 1). $C_{31}H_{33}N_3O_3$(495): calcd. C 75.13, H 6.71, N 8.48; found C 75.45, H 6.40, N 8.84.

(E)-methyl 2-cyano-3-(7-methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxalin-6-yl)acrylate (7c). Yield: 80%. Melting point: 124–127°C. IR (KBr): ν∼ = 3021, 2203, 1696, 1514, 1347, 1234 cm^{-1}. 1H-NMR (400 MHz, $CDCl_3$): δ = 8.63 (s, 1H), 7.80 (s, 1H), 7.18–7.27 (Ar-H, 10H), 6.00 (s, 1 H), 3.80 (s, 3H), 3.60 (t, 2H), 3.51 (t, 2H), 3.30 (t, 2H), 3.07 (t, 2H), 2.94 (m, 4H), 1.37 (t, 3H) ppm. 13C-NMR (100 MHz, $CDCl_3$): δ = 156.80, 147.24, 143.37, 140.08, 138.74, 129.22, 128.92, 128.84, 128.44,

126.86, 126.13, 119.06, 109.65, 109.00, 92.88, 90.85 53.90, 53.63, 49.29, 46.46, 32.91, 31.80 ppm. Mass: *m/z* = 482 (M + 1). $C_{30}H_{31}N_3O_3$ (481): calcd. C 74.82, H 6.49, N 8.73; found C 75.11, H 6.40, N 8.94.

(E)-2-(benzo[d]thiazol-2-yl)-3-(7-methoxy-1,4-diphenethyl-1,2,3,4 tetrahydroquinoxalin-6-yl)acrylonitrile (7d). Yield (64%) Melting point: 165–167°C. IR (KBr): ν∼ = 2839, 2217, 1500 cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 8.57 (s, 1H), 8.02 (dd, 2H), 7.82 (d, 1H), 7.37–7.18 (Ar-H, 11H), 7.18 (t, 2H) 6.06 (s, 1 H), 3.86 (s, 3H), 3.22 (t, 2H), 3.1 (t, 2H), 2.96 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 166.32, 155.85, 154.17, 142.26, 140.49, 140.08, 138.87, 134.57, 129.24, 128.89, 128.85, 128.46, 126.81, 126.33, 126.14, 124.69, 122.80, 121.31, 119.26, 110.29, 108.63, 95.35, 93.24, 56.24, 54.01, 53.61, 49.17, 46.57, 32.80, 31.96 ppm. Mass: *m/z* = 557.07 (M + 1). $C_{35}H_{32}N_4OS$(556): calcd. C 75.51, H 5.79, N 10.06, S 5.76; found C 75.19, H 5.46, N 9.98, S 5.73.

(Z)-3-(7-methoxy-1,4-diphenethyl-1,2,3,4-tetrahydroquinoxalin-6-yl)-2-(4-nitrophenyl)acrylonitrile (7e). Yield (86%) Melting point: 147–149°C. IR (KBr): ν∼ = 2932, 2853, 2194, 1513, 1315, 841 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.22 (t, 2H), 8.14 (s, 1H), 7.77 (dd, 2H), 7.74 (s, 1H), 7.34–7.19 (m, 10H), 6.07 (s, 1H), 3.83 (s, 3 H), 3.4 (m, 4H), 3.33 (t, 2H), 3.13 (t, 2H), 2.98 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 154.97, 146.10, 143.30, 141.42, 140.01, 138.90, 138.60, 129.13, 128.82, 128.63, 128.41, 126.73, 126.13, 125.30, 124.14, 119.90, 110.59, 108.34, 98.56, 93.37, 56.19, 53.95, 53.51, 48.93, 46.57, 32.71, 31.91ppm. Mass: *m/z* 544.93 (M + 1). Anal. Calcd. for $C_{34}H_{32}N_4O_3$ (544.25): calcd. C 74.98, H 5.92, N 10.92; found C 74.98, H 5.75, N 11.21.

Determination of antimicrobial activity. ***General.*** Incubator at 35 and 37°C; pipettes of various sizes (Gilson); sterile tips, 100, 200, 500, and 1000 μL; sterile normal saline; sterile isosensitest agar (Southern Group Laboratory, SGL); antibiotic solutions (Sigma–Aldrich); sterile solution of 10% (v/v) DMSO in water (Sigma–Aldrich).

Medium. Isosensitest medium was used throughout this assay, as it is pH buffered. Although NCCLS recommends the use of Mueller Hinton medium for susceptibility testing [20], the isosensitest medium had comparable results for most of the tested bacterial strains [21].

Preparation of the plates. Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 μL of test material in 10% (v/v) DMSO (usually a stock concentration of 4 mg mL^{-1}) was pipetted into the first row of the plate. To all other wells, 50 μL of nutrient broth. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50 μL of the test material in serially descending concentrations. To each well, 10 μL of resazurin indicator solution was added. Using a pipette, 30 μL of 3.3 × strength isosensitized broth was added to each well to ensure that the final volume was single strength of the nutrient broth. Finally, 10 μL of bacterial suspension (5 × 106 cfu mL^{-1}) was added to each well to achieve a concentration of 5 × 105 cfu mL^{-1}. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control, a column with all solutions with the exception of the test compound, and a column with all solutions with the exception of the bacterial solution adding 10 μL of nutrient broth instead. The plates were prepared in triplicate, and placed in an incubator set at 37°C for 18–24 h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial or fungal strain [21].

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