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An Efficient Ultrasound Promoted One-Pot Three-Component Synthesis and Antibacterial Activities of Novel Pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-dione Derivatives

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Abstract: A series of novel pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-dione derivatives was synthesized by one-pot three-component reaction of the appropriate aldehyde, dimedone or cyclohexanedione and 6-aminothiouracil in ethanol under ultrasonic irradiation at 50 ^{o}C in short reaction times (1.5-3 min) and excellent yields (94-99%). The antibacterial activities of the synthesized compounds were evaluated on *M. leuteus, B. subtilis, E. coli* and *Ps. aeruginosa*. All the synthesized products showed good antibacterial activities.

Keywords: 6-aminothiouracil, antibacterial, MCR, one-pot, pyrimido[4,5-b]quinolone, pyridopyrimidine, pyrazole, ultrasound.

INTRODUCTION

Due to their significant roles in drug discovery, research on the synthesis of polyfunctinalized heterocyclic compounds, in particular compounds incorporating nitrogen heterocycles, has received much interest in recent years [1-5].

Fused heterocyclic systems containing pyrimidine structural units, such as pyrimidopyrimidines, pyrazoloquinolines, pyrazolopyridopyrimidines, and pyridopyrimidines have been reported to display a wide range of pharmacological activities [6-10]. Indeed, structures containing the pyrido[2,3-d]primidine moiety have shown strong biological activities such as, analgesic, anti-inflammatory [11], anticonvulsive [12,13], antipyretic [14], cardiotonic [15,16], antitumoral [17], bactericidal [18], antihistaminic [19], diuretic [20] and bronchiodilator [21] activities. Consequently, preparation of these compounds has attracted considerable attention in recent years and numerous methods have been reported for their synthesis. For example, a three-component reaction, catalyzed by KF-Al_2O_3 [22], a solvent-free methodology employing barbituric acids [23], one-pot reaction of isatin, barbituric acids and 1*H*-pyrazole-5-amines [10], a two-step convergent strategy [24], reaction of isoxazoles with 2,6-diaminopyridinone [25], a double annulation strategy using α-alkenoyl-α-carbamoylketene-(*S*,*S*)-acetals [26], ring transformation of isoxazolo[3,4-*d*]pyrimidine [27], reaction between 6-aminouracils with cyano olefins [28], intramolecular hetero Diels–Alder reactions involving 1-oxa-1,3-butadienes [29] and reaction of 3-(6-amino-1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxopyrimidin-5-yl)-3-oxo-propanenitrile with aromatic aldehydes [30]. However, some of these methods are limited because of slow reaction rate, poor yields, side products, tedious workup, and the use of toxic solvents or expensive catalysts. Therefore, there still remain many challenges in the synthesis of these biologically important compounds.

On the other hand, functionally substituted 1,3-diarylpyrazole derivatives have received considerable attention due to their wide range of useful biological properties, which include antimicrobial, anti-inflammatory (COX-2 inhibitor and ulcerogenic activity), antitubercular, antitumor, antiangiogenesis, anti-parasitic and antiviral activities [31-36]. Therefore, it would be beneficial to design a system which combines bio-labile nuclei such as pyrazole, pyridine and pyrimidine in a molecular framework and to evaluate their additive microbial effects. Thus, we report herein the synthesis of some new heteroaryl substituted pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-dione derivatives *via* a fast and multi-component reaction under ultrasonic irradiation, as a clean source of energy.

The resistance of common pathogens to standard drugs plays an important role in treatment failure [37]. Therefore, searching for new antimicrobial agents with specific activity is a prime objective of medicinal and synthetic chemists.

In our ongoing research for new synthetic methods for the development of efficient and environmentally friendly protocols for the synthesis of biologically important heterocyclic products [38], several novel derivatives of pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-dione (**6a-i**) have been synthesized by one-pot three-component cyclocondensation reaction of dimedone or cyclohexanedione (**1**),

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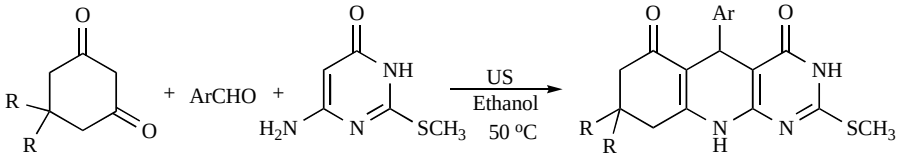


1 **2** **3** **4a-i**

R = H, CH_3

Scheme 1. An Efficient Synthesis of Pyrimido [4,5-b] quinoline-4, 6(3*H*,5*H*,7*H*,10*H*)-diones Under Ultrasonic Irradiations.

an appropriate aldehyde (**2**) and 6-aminothiouracil (**3**) at 50 °C, under ultrasonic irradiation (40 kHz) (Scheme **1**). This simple protocol furnished the desired products in short reaction times (1.5-3 min) and excellent yields (94–99%) (Table **1**). The structure of all the synthesized compounds was established by spectroscopic (IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analyses [39]. ^{1}H-NMR spectroscopy of the products (**6a-i**) clearly showed the dihydropyridine ring protons H-5 and H-10 as singlet at the range of 4.93-5.61 and 12.07-13.36 ppm respectively. The effect of different solvents was also examined on the efficiency of the reaction using preparation of **4a** as a model reaction (Table **2**). Of the solvents tested in the screening, ethanol was the solvent of choice. All the reactions described in this report were therefore carried out under optimized conditions (EtOH, 50 °C).

All the newly synthesized compounds were also screened for their antibacterial activities (Table **3**).

Table 1. Synthesis of Pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-dione (4a-i).

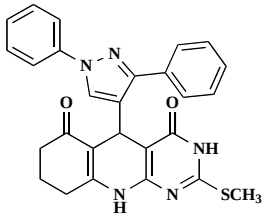
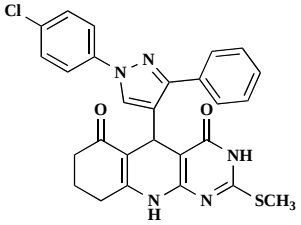
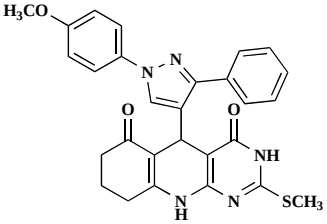
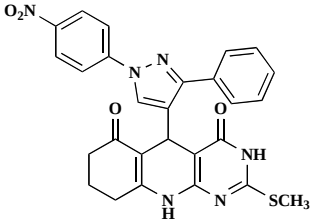
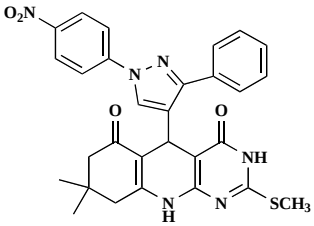
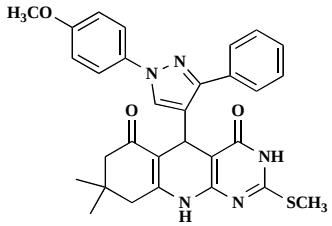
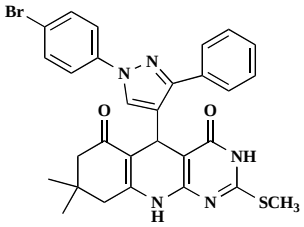
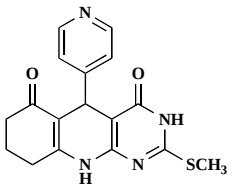
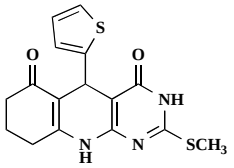
Entry	Product	Time (min)	Yield (%)[a,b]
4a		3 (240)[c]	96 (83)[c]
4b		2	97
4c		3	94
4d		2	97
4e		2	98

Table 1. contd…

Entry	Product	Time (min)	Yield (%)[a,b]
4f		3	96
4g		2	96
4h		1.5	99
4i		1.5	99

[a]Isolated yield. [b]Identified by spectroscopic (IR, ^{1}H NMR, ^{13}C NMR) and elemental analyses. [C]Classical reaction was carried out using cyclohexa-1,3-dione (1 mmol), arylaldehyde (**2a**) (1 mmol) and 6-amino-2-(methylthio)pyrimidin-4(3*H*)-one (1mmol) in ethanol (10 mL) under reflux condition which provided **4a** in 83% yield after 240 min.

Table 2. Synthesis of 4a in Different Solvents in Ultrasonic Condition.

Entry	Solvent	Time (min)	Yield (%)[a]
1	Ethanol	3	96
2	H_2O	5	65
3	THF	8	88
4	Ethanol/H_2O[b]	5	85
5	Methanol	3	92
6	Acetonitrile	5	88
7	Chloroform	10	66

[a]Isolated yield. [b]A 1:1 mixture.

ANTIBACTERIAL SCREENING

The purified products were screened for their antibacterial activity by using spectrophotometric (turbidimetric) analysis. Absorbance values were measured at 600 nm and converted to percentage antibacterial activity (AA %). It was calculated using the following formula: % Reduction in Absorbance = 100-[{(Abs_{sample}-Abs_{blank})/$Abs_{control}$} ×100]. Quantitative screening of antibacterial activity is given in Table **3**. Although all the compounds exhibited some antibacterial activity, it seems those pyrazole substituted products that carried a more polar group or were capable of forming hydrogen bonds (except **4c**) were more active; specifically, compounds **4b**, **4d**, **4e**, **4f** and thienyl substituted **4i** showed higher levels of antibacterial activity. Compounds **4a**, **4c**, **4g** and **4h**, overall, seem to have been least effective.

Table 3. Quantitative Screening of Antibacterial Activity.

Z	Compound	Absorbance	Absorbance at 600 nm			
			Bacillus subtilis	***Micrococcus leuteus***	***Pseudomonas aeruginosa***	***Escherichia coli***
1	Control	($Abs_{control}$)	0.220	0.529	0.338	0.374
2	**4a**	($Abs_{sample} - Abs_{blank}$)	0.189	0.340	0.239	0.297
		% Reduction in Absorbance (AA %)	14.1	35.72	29.28	20.58
3	**4b**	($Abs_{sample} - Abs_{blank}$)	0.043	0.101	0.105	0.053
		% Reduction in Absorbance (AA %)	80.45	80.90	68.93	85.82
4	**4c**	($Abs_{sample} - Abs_{blank}$)	0.116	0.385	0.220	0.350
		% Reduction in Absorbance (AA %)	47.27	27.22	34.91	6.41
5	**4d**	($Abs_{sample} - Abs_{blank}$)	0.076	0.124	0.159	0.108
		% Reduction in Absorbance (AA %)	75	76.55	52.95	71.12
6	**4e**	($Abs_{sample} - Abs_{blank}$)	0.050	0.047	0.074	0.120
		% Reduction in Absorbance (AA %)	77.27	91.11	78.1	67.91
7	**4f**	($Abs_{sample} - Abs_{blank}$)	0.043	0.101	0.124	0.141
		% Reduction in Absorbance (AA %)	80.45	80.9	63.31	62.29
8	**4g**	($Abs_{sample} - Abs_{blank}$)	0.217	0.430	0.104	0.166
		% Reduction in Absorbance (AA %)	1.36	18.71	69.23	55.61
9	**4h**	($Abs_{sample} - Abs_{blank}$)	0.203	0.260	0.225	0.304
		% Reduction in Absorbance (AA %)	7.72	50.85	33.43	18.71
10	**4i**	($Abs_{sample} - Abs_{blank}$)	0.099	0.088	0.109	0.194
		% Reduction in Absorbance (AA %)	55	83.36	67.75	48.12

In conclusion, a new series of substituted pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-dione derivatives **4a-i** has been synthesized *via* an MCR approach and was characterized by spectral and elemental analyses. This synthetic strategy allows the construction of relatively complicated nitrogen containing fused heterocyclic systems in short reaction times and excellent yields using a green protocol. All the derivatives of newly synthesized compounds, pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-dione derivatives (**4a-4i**), were screened for their antibacterial activities. Antibacterial activities of the synthesized compounds are given in Table **3**. Compounds **4b**, **4d**, **4e**, **4f**, **4i** showed good antibacterial activities in the case of all microorganism. Other products showed weakness to average antibacterial activities. In general introducing a more polar pyrazole moiety, except in the case of **4c**, increases the antibacterial activities of the synthesized pyrimido[4,5-b]quinoline-4,6-dione derivatives. More verification of the structure activity relation of these hetrocycles is underway.

Typical Procedure for Preparation of pyrimido[4,5-b]quinoline-4,6(3*H*,5*H*,7*H*,10*H*)-diones (4a-j)

A mixture of 1,3- cyclohexanedione or dimedone (**1**) (1 mmol), appropriate aldehyde (**2**) (1mmol), and 6-amino-2-(methylthio)pyrimidin-4(3*H*)-one (**3**) (1mmol) in 10 mL absolute ethanol was irradiated in an ultrasonic cleaning unit (Elmasonic S 40H) at 50 ^{o}C for 1.5-3 minutes. The resultant precipitate was filtered and recrystallized from ethanol to furnish the desired pure products (**4a-j**) in 94-99% yields.

Product Characterization Data

4a: White solid; M.p. 306–309 °C; Found: C, 67.21; H, 4.68; N, 14.33 $C_{27}H_{23}N_5O_2S$ requires C, 67.34; H, 4.81; N, 14.54%; FT-IR (KBr): 3220, 3180, 3080, 2950, 2880, 1640, 1620, 1590, 1520, 1483, 1360, 1265, 1230, 1180, 1140, 950, 760, 690 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 1.82, 1.90 (m, 2H), 2.08-2.16 (m, 2H), 2.43-2.49 (m, 2H), 2.50 (s, 3H), 5.10 (s, 1H), 7.26 (t, 1H, J = 7.2 Hz), 7.34-7.47 (m, 5H), 7.82 (d, 2H, J = 8.0 Hz), 7.89 (d, 2H, J = 6.8 Hz,), 8.15 (s, 1H), 9.70 (s, NH), 12.1 (s, NH) ppm. ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 13.2, 21.1, 27.0, 29.2, 37.2, 98.8, 111.6, 118.4, 126.2, 127.6, 128.0, 128.1, 129, 129.2, 129.8, 135.1, 140.0, 151.5, 152.7, 158.2, 160.1, 162.2, 194.7.

4b: White solid; M.p. 220-221 °C; Found: C, 62.98; H, 4.05; N, 13.43 $C_{27}H_{22}ClN_5O_2S$ requires C, 62.84; H, 4.30; N, 13.57%; FT-IR (KBr) 3400, 3220, 3180, 3080, 2925, 2870, 1645, 1620, 1590, 1540, 1493, 1442, 1358, 1265, 1224, 1180, 1138, 1082, 1058, 1020, 958, 883, 835, 750, 722, 685 cm^{-1}; ^{1}H- NMR (400 MHz, DMSO-d_6): δ_H 1.79-1.86 (m,

2H), 2.11-2.16 (m, 2H), 2.50 (s, 2H), 2.51 (s, 3H), 5.06 (s, 1H), 7.26 (t, 1H, J = 7.2 Hz), 7.42-7.49 (m, 4H), 8.09 (d, 8.4 Hz), 8.16 (s, 1H), 9.76 (s, 1H), 12.20 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 13.2, 21.2, 25.1, 27.0, 37.2, 98.9, 111.8, 118.5, 126.3, 128.2, 129.5, 129.8, 130.9, 132.5, 134.1, 139.9, 150.2, 151.8, 152.7, 160.5, 162.0, 194.9.

4c: White solid; M.p. 260- 262 °C; Found: C, 65.87; H, 4.99; N, 13.53 $C_{28}H_{25}N_5O_3S$ requires C, 65.74; H, 4.93; N, 13.69%; FT-IR (KBr) 3400, 3320, 3220, 3180, 3050, 3000, 2920, 2800, 2780, 1650, 1640, 1558, 1520, 1500, 1445, 1361, 1280, 1240, 1185, 1052, 1018, 1000, 950, 900, 830, 790, 750, 690 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.72-1.83 (m, 2H), 2.09-2.20 (m, 2H), 2.43 (m, 2H), 2.51 (s, 3H), 3.82 (s, 3H), 5.07 (s, 1H), 6.96 (d, 2H, J = 8.0 Hz), 7.25 (t, 1H, J = 6.4 Hz), 7.44 (t, 2H, J = 7.0 Hz), 7.80 (t, 4H, J = 6.6 Hz), 8.10 (s, 1H), 9.69 (s, 1H), 12.08 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.2, 21.1, 25.1, 27.0, 37.2, 55.5, 81.6, 98.8, 111.7, 113.5, 118.3, 126.1, 127.6, 127.8, 128.9, 129.8, 130.4, 140.0, 151.3, 152.6, 159.1, 161.0, 163.9, 194.7.

4d: Pale yellow solid; M.p. 276-277 °C; Found: C, 61.34; H, 4.11; N, 15.80 $C_{27}H_{22}N_6O_4S$ requires C, 61.59; H, 4.21; N, 15.96%; FT-IR (KBr) 3320, 3200, 3050, 3005, 2910, 2800, 2750, 1645, 1620, 1598, 1540, 1500, 1450, 1332, 1280, 1223, 1180, 1100, 1060, 1000, 960, 900, 860, 820, 750, 710, 690 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.86-2.16 (m, 4H), 2.43, (s, 2H), 2.50 (s, 3H), 5.08 (s, 1H), 7.30 (t, 1H, J = 7.0 Hz), 7.47 (t, 2H, J = 7.6 Hz), 7.86 (d, 2H, J = 8.0 Hz), 8.22 (s, 1H), 8.31 (s, 4H), 9.79 (s, 1H), 12.14 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.2, 21.2, 25.3, 26.9, 37.1, 81.6, 111.7, 118.7, 123.5, 126.7, 128.8, 129.9, 130.1, 130.3, 139.7, 142.2, 147.0, 149.3, 152.8, 156.4, 160.8, 162.2, 195.0.

4e: Yellow solid; M.p. 262-264 °C; Found: C, 62.65; H, 4.91; N, 15.02 $C_{29}H_{26}N_6O_4S$ requires C, 62.80; H, 4.73; N, 15.15%; FT-IR (KBr) 3500, 3460, 3300, 3200, 3000, 2920, 2750, 1660, 1620, 1580, 1540, 1442, 1295, 1240, 1222, 1180, 1145, 1050, 975, 900, 860, 795, 740, 690 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.92 (s, 3H), 0.99 (s, 3H), 2.15-2.24 (m, 3H), 2.36 (s, 3H), 2.44 (d, 1H, J = 16.8 Hz), 5.62 (s, 1H), 7.33 (t, 1H, J = 6.8 Hz), 7.52 (t, 1H, J = 7.0 Hz), 7.60 (d, 2H, J = 8.0 Hz), 7.84 (d, 2H, J = 7.2 Hz), 7.97 (s, 1H), 8.1 (d, 2H, J = 8.0 Hz), 11.99 (s, 1H), 13.32 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.0, 25.6, 28.1, 28.5, 31.7, 44.3, 50.0, 95.8, 114.6, 118.5, 121.5, 122.8, 124.1, 126.7, 127.6, 129.1, 129.3, 130.1, 139.8, 141.1, 146.6, 149.7, 159.4, 161.7, 163.9, 198.8.

4f: Milky solid; M.p. 266- 268 °C; Found: C, 66.53; H, 5.35; N, 16.12 $C_{30}H_{29}N_5O_3S$ requires C, 66.77; H, 5.42; N, 12.98%; FT-IR (KBr) 3500, 3480, 3300, 3200, 3000, 2920, 2730, 1640, 1615, 1580, 1445, 1295, 1242, 1220, 1015, 980, 900, 795 cm^{-1}; ^{1}H-NMR (400 MHz,DMSO-d_6): δ 0.91 (s, 3H), 0.96 (s, 3H), 2.16 (m, 3H), 2.34 (d, 1H, J = 17.2 Hz) 2.42 (s, 3H), 3.75 (s, 3H), 5.52 (s, 1H), 6.81 (d, 2H, J = 8.4 Hz), 7.24 (d, 2H, J = 8.4 Hz), 7.28 (t, 1H, J = 7.2), 7.49 (t, 2H, J = 7.8 Hz), 7.78 (d, 2H, J = 8.0 Hz), 7.89 (s, 1H), 12.07 (s, 1H), 13.36 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.0, 25.6, 28.1, 28.5, 44.2, 50.0, 55.6, 56.5, 95.8, 113.3, 114.8, 118.1, 120.1, 126.0, 126.6, 127.0, 129.3, 130.0, 140.0, 151.4, 158.9, 159.1, 161.7, 164.2, 198.7.

4g: White solid; M.p. 276-278 °C; Found: C, 59.33; H, 4.36; N, 11.82 $C_{29}H_{26}BrN_5O_2S$ requires C, 59.18; H, 4.45; N, 11.90%; FT-IR (KBr) 3250, 3180, 3100, 3070, 2950, 1640, 1600, 1540, 1500, 1450, 1360, 1270, 1222, 1168, 1070, 1005, 960, 840, 830, 755, 735, 690 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.93 (s, 3H), 1.0 (s, 3H), 1.97-2.3 (m, 4H), 2.42 (s, 3H), 5.01 (s, 1H), 7.26 (t, 1H, J = 7.4 Hz), 7.45 (t, 2H, J = 7.8), 7.61 (d, 2H, J = 8.4), 7.79 (d, 2H, J = 7.6), 8.01 (d, 2H, J = 8.4), 8.13 (s, 1H), 9.72 (s, 1H), 12.08 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.2, 25.2, 27.7, 29.0, 31.2, 32.6, 50.7, 99.0, 110.6, 118.4, 121.2, 126.4, 128.1, 129.6, 129.9, 131.1, 131.2, 134.4, 139.8, 150.2, 150.7, 156.4, 160.0, 162.9, 194.6.

4h: White solid; M.p. 230-232 °C; Found: C, 59.70; H, 4.95; N, 16.21 $C_{17}H_{16}N_4O_2S$ requires C, 59.98; H, 4.74; N, 16.46 %; FT-IR (KBr) 3400, 3280, 3050, 2920, 2850, 2800, 2620, 1718, 1680, 1640, 1605, 1558, 1486, 1460, 1420, 1365, 1320, 1305, 1280, 1258, 1230, 1180, 1138, 1110, 1058, 1020, 1005, 990, 960, 935, 890, 880, 865, 820, 760, 750, 720, 690 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.75-1.95 (m, 2H), 2.15-2.35 (m, 2H), 2.51 (s, 3H), 2.52-2.66 (m, 2H), 4.94 (s, 1H), 7.20 (m, 2H), 8.40 (m, 2H), 9.96 (s, 1H), 12.43 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d6): δ 13.2, 21.2, 26.9, 33.8, 37.1, 97.0, 109.7, 123.3, 149.0, 150.6, 152.7, 154.3, 161.3, 162.0, 194.8.

4i: White solid; M.p. 308-310 °C; Found: C, 55.84; H, 4.49; N, 12.28 $C_{16}H_{15}N_3O_2S_2$ requires C, 55.65; H, 4.38; N, 12.16 %; FT-IR (KBr) 3220, 3120, 3080, 3020, 2950, 2860, 1655, 1610, 1545, 1478, 1445, 1360, 1262, 1222, 1180, 1140, 1078, 1000, 960, 880, 845, 785, 700 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.86-1.95 (m, 2H), 2.29 (m, 2H), 2.51 (s, 3H), 2.59 (m, 2H), 5.25 (s, 1H), 6.72 (s, br., 1H), 6.82 (s, br., 1H), 7.18 (s, br., 1H), 9.97 (s, 1H), 12.49 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.2, 21.3, 26.9, 28.6, 37.2, 97.9, 110.6, 123.4, 123.9, 127.0, 151.1, 152.4, 153.5, 161.0, 162.0, 194.7.

Determination of Antimicrobial Activity

Antibacterial activity was determined against *Eschrechia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Micrococcus leuteus*. The synthesized molecule, dissolved in DMSO, was added to 3 mL of nutrient broth (Merck) to obtain a final concentration of 125 μg/mL. The bacteria were inoculated into this mixture and allowed to grow for 18 h at 37 ^{o}C. Control samples were prepared by adding only the bacteria and DMSO, the DMSO alone, and the synthesized molecule alone. Degree of growth was determined spectrophotometerically by measuring absorbance at 600 nm, and corrections made using the controls.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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