ISSN 1070-4280, Russian Journal of Organic Chemistry, 2019, Vol. 55, No. 6, pp. 860–865. © Pleiades Publishing, Ltd., 2019.

Synthesis, Characterization, and *in vitro* Antibacterial Evaluation of Barbituric Acid Derivatives

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Received December 25, 2018; revised December 29, 2018; accepted December 30, 2018

Abstract—A series of 5,5′-(arylmethylene)bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-diones] have been synthesized by 2:1 condensation of 1,3-dimethylbarbituric acid with armatic aldehydes in the presence of methylamine. The synthesized compounds have been characterized by ^{1}H and ^{13}C NMR, IR, and mass spectra and tested for their *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Some of the compounds have shown antibacterial activity exceeding that of ampicillin used as reference drug.

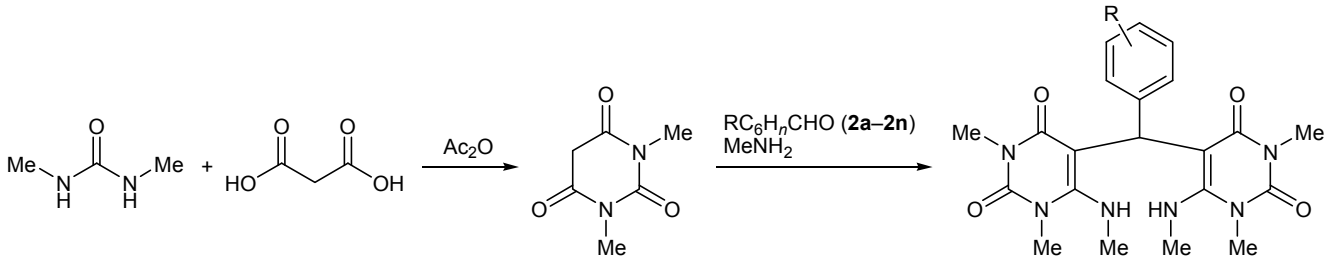
Keywords: barbituric acid derivatives, 5,5′-(arylmethylene)bis[1,3-dimethyl-6-(methylamino)pyrimidin-2,4(1*H*,3*H*)-diones, antibacterial activity.

DOI: 10.1134/S1070428019060174

Increased resistance to antimicrobial agents is nowadays a worldwide cause of concern for scientists, which has stimulated the synthesis of novel scaffolds and their structural modifications. In this context, pyrimidine moieties due to their ubiquity encourage chemists for further research in this area. It is well known that compounds having a pyrimidine nucleus display profound biological activities; examples are the antiviral drug idoxuridine [1–3], anti-HIV agent stavudine [4], antibacterial drug trimethoprim [5], and antimalarial and antibacterial drug sulfadoxine [6]. The therapeutic importance of pyrimidine derivatives such as barbituric and thiobarbituric acids is determined by their antineoplastic [7, 8], antiviral [9], antibiotic [10], and anti-inflammatory activities. Many synthetic drugs and chemotherapeutic agents derived from barbituric and thiobarbituric acids are well known [11]. Barbiturates constitute another class of nitrogen-containing heterocyclic moieties having a wide range of pharmacological applications as anaesthetics [12], anxiolytic, antifungal [13], antiviral [14, 15], urease inhibitor [16], anticancer [17], antitubercular [18], antiproliferative [19], and radiosensitizing agents [20]. With due consideration of all these facts, herein we report the synthesis of some barbituric acid derivatives, followed by accounting their biological activity against various bacterial strains.

Scheme 1 shows the reaction sequence involved in the synthesis of various barbituric acid derivatives **3a**–**3n**. Compounds **3a**–**3n** were synthesized by condensation of 1,3-dimethylbarbituric acid (**1**) with various substituted aromatic aldehydes **2a**–**2n** in the presence of methylamine according to the procedure described by us previously. Various spectroscopic techniques were used to characterize the newly synthesized compounds. The ^{1}H NMR spectrum of **3g** obtained by the reaction of **1** with 4-chlorobenzaldehyde in the presence of methylamine showed aromatic proton signals as one-proton doublets at δ 8.03, 7.53, 7.19, and 7.06 ppm. The singlets observed at δ 8.32 (1H) and 10 ppm were assigned to protons attached to the nitrogen atoms, and the singlet at δ 6.15 ppm (1H) is due to proton attached to the bridging carbon atom. The singlets at δ 3.28 (6H) and 3.17 ppm (6H) belong to methyl groups attached to the pyrimidine nitrogen atoms, and one more 6H-singlet at δ 3.28 ppm (6H) is due to the NHC**H**$_3$ groups. The IR spectrum of **3g** showed absorption bands at 3001 (=C–H), 2930

Scheme 1.



R = 2-ClC_6H_4 (**a**), 4-$Me_2NC_6H_4$ (**b**), 4-HOC_6H_4 (**c**), 2-HOC_6H_4 (**d**), Ph (**e**), 2-hydroxynaphthalen-1-yl (**f**), 4-ClC_6H_4 (**g**), 4-FC_6H_4 (**h**), 4-$O_2NC_6H_4$ (**i**), 3,4-$(HO)_2C_6H_3$ (**j**), 4-$MeOC_6H_4$ (**k**), 3-MeO-4-HOC_6H_3 (**l**), 3-HO-4-$MeOC_6H_3$ (**m**), naphthalen-1-yl (**n**).

(–C–H), 1671 (C=O), 3392 (N–H), 1217 (C–N), 1413 (C=C), and 670 cm^{-1} (C–Cl). In the ^{13}C NMR spectrum of **3g**, methyl carbons resonated at δ_C 27.99, 28.58, and 33.07 ppm, CH signal was located at δ 38.89 ppm, a sharp peak at δ_C 90.75 was assigned to C^5 ($C^{5'}$) of the pyrimidine moiety, aromatic carbon signals were observed at δ_C 127.38, 131.13, 136.65, and 142.84 ppm (C–Cl), and signals δ_C 151.02 and 162.93 ppm corresponded to the carbonyl carbons. The UV spectrum of **3g** displayed a strong band at λ_{max} 278 nm corresponding to the proposed structure. The molecular ion peak with *m*/*z* 459.23 was observed in the mass spectrum of **3g**.

The antibacterial activity of compounds **3a**–**3n** was screened against three bacterial strains, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*; ampicillin was taken as the standard drug. The results are summarized in Table 1. Compounds **3a**–**3f** showed high potency against all bacterial strains tested, and the antibacterial activities of **3a**–**3d** exceeded those of ampicillin. The antibacterial activity of **3a**–**3n** depended on the substituent in the phenyl ring, and compounds with electron-donating groups proved to be more active. Barbituric acid derivatives **3g**–**3n** displayed no activity.

In summary, barbituric acid derivative **3a**–**3n** have been synthesized and evaluated for their *in vitro* antibacterial activity against three bacterial strains, including gram positive and gram negative bacteria. Compounds **3a**–**3d** exhibited profound antimicrobial potency against the selected strains as compared to the reference drug ampicillin, while **3e** and **3f** were moderately active, and the others displayed no activity. Electron-donating substituents in the phenyl ring considerably enhanced the activity, and the activity against gram negative *P. aeruginosa* was higher than the activity against gram positive *B. subtilis* and *S. aureus*. Compounds **3a**–**3d** are thus promising as potent biologically active compounds with profound antimicrobial action.

Table 1. Antibacterial activity of compounds **3a**–**3f**[a]

Compound	*Staphylococcus aureus*		*Bacillus subtilis*		*Pseudomonas aeruginosa*	
	MIC, μg/mL	IZD, mm	MIC, μg/mL	IZD, mm	MIC, μg/mL	IZD, mm
3a	0.50	18.0	0.031	20.0	0.063	23.5
3b	1.0	21.0	1.0	18.0	1.0	25.0
3c	1.0	21.5	1.0	19.0	0.25	26.0
3d	1.0	24.0	0.50	16.0	1.0	22.5
3e	ND	12.0	ND	10.0	ND	14.5
3f	ND	13.5	ND	11.5	ND	13.0
Ampicillin	50	14.0		13.5	3.75	19.5

[a] MIC is minimum inhibitory concemtration, IZD is inibition zone diameter, and "ND" stands for not determined.

EXPERIMENTAL

All chemicals were purchased from Merck, Sdfine, and Qualigens. Solvents and reagents were used without further purification, unless otherwise specified. The melting points were determined in open capillary in an electrically heated block and are uncorrected. The progress of reactions was monitored by TLC using TLC grade silica gel (G); spots were visualized by treatment with iodine vapor. The IR spectra were recorded on a Perkin Elmer 1430 spectrometer from samples prepared as KBr pellets. The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker 300 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane as internal standard. The mass spectra of compounds were recorded on a Jeol JMS-T100LC Accu TOF mass spectrometer.

1,3-Dimethylbarbituric acid (**1**) was synthesised as described in [21]. Yield 85%, mp 121°C, R_f 0.42 ($CHCl_3$–MeOH, 8:2). IR spectrum, ν, cm^{-1}: 2902 (C–H), 1710 (C=O), 1210 (C–N). ^{1}H NMR spectrum, δ, ppm: 3.02 s (6H), 3.07 (2H). ^{13}C NMR spectrum, δ_C, ppm: 29.06, 29.07, 38.01, 150.71, 165.78, 168.79. Mass spectrum: *m*/z 156.01.

5,5′-[(2-Chlorophenyl)methylene]bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3a). A solution of 0.312 g (2 mmol) of 1,3-dimethylbarbituric acid (**1**), 0.05 mL (1 mmol) of methylamine, and 0.141 g (1 mmol) of 4-chlorobenzaldehyde (**a**) was refluxed for ~3 h [24] (TLC). The solution was cooled, and the precipitate was filtered off, washed with water, and recrystallized from ethanol. Yield 80%, light yellow solid, mp 114°C, R_f 0.483 (hereinafter, $CHCl_3$–MeOH, 8:2). IR spectrum, ν, cm^{-1}: 3011 (=C–H), 2901 (–C–H), 1651 (C=O), 3352 (N–H), 1207 (C–N), 1420 (C=C), 688 (C–Cl). ^{1}H NMR spectrum. δ, ppm: 7.84–8.04 d (1H), 7.34–7.20 m (1H), 7.16–7.12 m (1H), 7.04–7.03 d (1H), 6.12 s (1H), 3.18 s (6H), 3.62 s (6H), 3.12 s (6H), 8.31 s (1H), 10.0 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 26.89, 28.34, 34.07, 39.89, 89.94, 128.16, 130.51, 136.75, 142.87, 151.06, 161.95. Mass spectrum: *m*/*z* 459.09.

Compounds **3b**–**3n** were synthesized in a similar way. The spectra of **3a** and **3g** are available from the authors.

5,5′-{[4-(Dimethyamino)phenyl]methylene}-bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3b). Yield 78%, light orange solid, mp 225°C, R_f 0.44. IR spectrum, ν, cm^{-1}: 3341 (N–H), 3021 (=C–H), 2921 (–C–H), 1641 (C=O), 1204 (C–N), 1422 (C=C), 1110 (C–N). ^{1}H NMR spectrum, δ, ppm 6.55–6.66 d (1H), 6.68–6.76 d (1H), 6.16–6.26 d (1H), 6.26–6.34 d (1H), 6.14 s (1H), 3.14 s (6H), 3.17 s (6H), 3.52 s (6H), 3.22 s (6H), 8.20 s (1H), 8.41 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 26.59, 28.54, 29.54, 29.77, 34.17, 88.62, 128.23, 130.41, 136.85, 142.89, 151.46, 160.95. Mass spectrum: *m*/*z* 469.24.

5,5′-[(4-Hydroxyphenyl)methylene]bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3c). Yield 75%, light orange solid, mp >250°C, R_f 0.44. IR spectrum, ν, cm^{-1}: 3510 (O–H), 3322 (N–H), 3115 (=C–H), 2912 (–C–H), 1630 (C=O), 1227 (C–N), 1431 (C=C), 1060 (C–O). ^{1}H NMR spectrum, δ, ppm: 6.60–6.66 d (1H), 6.67–6.75 d (1H), 7.16–7.19 d (1H), 7.20–7.29 d (1H), 6.25 s (1H), 3.15 s (6H), 3.42 s (6H), 3.36 s (6H), 8.63 s (1H), 8.77 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 27.19, 29.36, 36.17, 39.19, 127.16, 129.26, 135.91, 143.98, 151.16, 162.63. Mass spectrum: *m*/*z* 442.20.

5,5′-[(2-Hydroxyphenyl)methylene]bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3d). Yield 75%, yellow solid, mp 215°C, R_f 0.45. IR spectrum, ν, cm^{-1}: 3565 (O–H), 3331 (N–H), 3115 (=C–H), 2912 (–C–H), 1630 (C=O), 1237 (C–N), 1433 (C=C), 1062 (C–O). ^{1}H NMR spectrum, δ, ppm: 6.90–6.96 d (1H), 6.97–6.99 m (1H), 7.29–7.31 d (1H), 7.32–7.36 m (1H), 5.45 s (1H), 6.01 s (1H), 3.25 s (6H), 3.32 s (6H), 3.39 s (6H), 8.14 s (1H), 8.17 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 28.59, 29.56, 36.37, 39.49, 89.25, 127.18, 129.36, 134.98, 143.88, 152.16, 162.64. Mass spectrum: *m*/*z* 442.20.

5,5′-(Phenylmethylene)bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3e). Yield 76%, pinkish orange solid, mp >250°C, R_f 0.59. IR spectrum, ν, cm^{-1}: 3322 (N–H), 3115 (=C–H), 2912 (–C–H), 1630 (C=O), 1227 (C–N), 1431 (C=C). ^{1}H NMR spectrum, δ, ppm: 7.14–7.19 d (1H), 7.20–7.25 d (1H), 7.26–7.29 d (1H), 7.30–7.33 d (1H), 7.34–7.39 m (1H) 6.05 s (1H), 3.35 s (6H), 3.44 s (6H), 3.46 s (6H), 8.13 s (1H), 8.27 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 29.23, 29.32, 36.57, 39.59, 89.81, 39.87, 127.56, 130.54, 135.81, 143.92, 150.06, 162.53. Mass spectrum: *m*/*z* 426.20.

5,5′-[2-Hydroxynaphthalen-1-yl)methylene]-bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3f). Yield 75%, brownish yellow solid, mp >250°C, R_f 0.58. IR spectrum, ν, cm^{-1}: 3550 (OH), 3350 (N–H), 3031 (=C–H), 2931 (–C–H), 1656

(C=O), 1217 (C–N), 1516, 1420 (C=C). ^{1}H NMR spectrum, δ, ppm: 5.67–5.70 s (1H), 6.87–6.89 d (1H), 7.53–7.58 d (1H), 8.16 s (1H), 7.45–7.48 m (1H), 7.53–7.55 m (1H), 8.20–8.33 d (1H), 6.22 s (1H), 3.17 s (6H), 3.22 s (6H), 3.35 s (6H), 9.83 s (1H), 8.44 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 28.85, 29.36, 34.18, 39.79, 89.92, 127.33, 128.26, 128.36, 128.37, 130.61, 136.75, 142.87, 151.38, 161.95. Mass spectrum: *m*/*z* 492.

5,5′-[(4-Chlorophenyl)methylene]bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3g). Yield 74%, light brown solid, mp 155°C, R_f 0.583. IR spectrum, ν, cm^{-1}: 3392 (N–H), 3001 (=C–H), 2930 (–C–H), 1671 (C=O), 1217 (C–N), 1413 (C=C), 670 (C–Cl). ^{1}H NMR spectrum, δ, ppm: 8.02–8.04 d (1H), 7.52–7.54 d (1H), 7.18–7.20 d (1H), 7.05–7.07 d (1H), 6.13 s (1H), 3.28 s (6H), 3.17 s (6H), 10 s (1H), 3.275 s (6H), 8.31 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 27.99, 28.58, 33.07, 38.89, 90.75, 127.38, 131.13, 136.65, 142.84, 151.02, 161.95. Mass spectrum: *m*/*z* 459.23.

5,5′-[(4-Fluorophenyl)methylene]bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3h). Yield 88%, light brown solid, mp 145°C, R_f 0.56. IR spectrum, ν, cm^{-1}: 3321 (N–H), 3121 (=C–H), 2921 (–C–H), 1642 (C=O), 1237 (C–N), 1429 (C=C), 1213 (C–F). ^{1}H NMR spectrum, δ, ppm: 7.16–7.33 d (1H), 7.12–7.16 d (1H), 7.35–7.52 d (1H), 7.54–7.59 d (1H), 6.13 s (1H), 3.185 s (6H), 3.33 s (6H), 3.345 s (6H), 8.43 s (1H), 8.24 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 26.89, 29.34, 36.07, 39.39, 89.67, 127.47, 130.63, 136.91, 142.99, 150.07, 162.96. Mass spectrum: *m*/*z* 444.19.

5,5′-[(4-Nitrophenyl)methylene]bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3i). Yield 83%, light yellow solid, mp 210°C, R_f 0.423. IR spectrum, ν, cm^{-1}: 3341 (N–H), 3011 (=C–H), 2901 (–C–H), 1651 (C=O), 1543, 1303 (NO_2), 1247 (C–N), 1431 (C=C). ^{1}H NMR spectrum, δ, ppm: 8.16–8.32 d (1H), 8.27–8.29 d (1H), 7.26–7.32 d (1H), 7.19–7.28 d (1H), 6.22 s (1H), 3.28 s (6H), 3.32 s (6H), 3.42 s (6H), 8.23 s (1H), 8.14 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 24.89, 29.14, 35.07, 39.29, 90.23, 127.26, 129.53, 136.89, 142.97, 150.06, 162.95, 163.99. Mass spectrum: *m*/*z* 471.19.

5,5′-[(3,4-Dihydroxyphenyl)methylene]-bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3j). Yield 82%, orange yellow solid, mp 230°C, R_f 0.56. IR spectrum, ν, cm^{-1}: 3520, 3555 (O–H), 3101 (=C–H), 2911 (–C–H), 1660 (C=O), 3302 (N–H), 1237 (C–N), 1421 (C=C), 1055 (C–O). ^{1}H NMR spectrum, δ, ppm: 6.56–6.72 m (1H), 7.17–7.25 d (1H), 7.26–7.29 d (1H), 5.65 s (2H), 6.23 s (1H), 3.18 s (6H), 3.52 s (6H), 3.56 s (6H), 8.33 s (1H), 8.47 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 28.19, 29.84, 35.17, 39.39, 89.63, 127.46, 130.64, 136.91, 142.98, 152.11, 162.97. Mass spectrum: *m*/*z* 458.19.

5,5′-[(4-Methoxyphenyl)methylene]bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3k). Yield 86%, yellow solid, mp 120°C, R_f 0.58. IR spectrum, ν, cm^{-1}: 3321 (N–H), 3121 (=C–H), 2921, 2926 (–C–H), 1642 (C=O), 1237 (C–N), 1429 (C=C), 1140 (C–O). ^{1}H NMR spectrum, δ, ppm: 6.82–6.89 d (1H), 6.90–6.99 d (1H), 7.25–7.32 d (1H), 7.34–7.39 d (1H), 6.03 s (1H), 3.28 s (6H), 3.36 s (6H), 3.64 s (6H), 8.45 s (1H), 8.48 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 26.19, 28.34, 35.07, 39.19, 89.23, 127.70, 130.65, 136.81, 142.19, 150.37, 162.86. Mass spectrum: *m*/*z* 456.21.

5,5′-[(4-Hydroxy-3-methoxyphenyl)methylene]-bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3l). Yield 78%, creamish yellow solid, mp 190°C, R_f 0.58. IR spectrum, ν, cm^{-1}: 3516 (O–H), 3331 (N–H), 3115 (=C–H), 2912, 2926 (C–H), 1630 (C=O), 1237 (C–N), 1433 (C=C), 1062, 1140 (C–O). ^{1}H NMR spectrum, δ, ppm: 5.65 s (1H), 6.77 s (1H), 6.82–6.88 d (1H), 6.90–6.92 d (1H), 6.06 s (1H), 3.76 s (3H), 3.40 s (6H), 3.52 s (6H), 3.68 s (6H), 8.29 s (1H), 8.31 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 27.89, 29.76, 36.47, 39.39, 89.26, 127.48, 130.74, 135.81, 143.88, 152.76, 162.84. Mass spectrum: *m*/*z* 472.

5,5′-[(3-Hydroxy-4-methoxyphenyl)methylene]-bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3m). Yield 76%, yellow solid, mp 210°C, R_f 0.56. IR spectrum, ν, cm^{-1}: 3520, 3516 (O–H), 3112 (=C–H), 2910, 2924 (–C–H), 1630 (C=O), 3330 (N–H), 1237 (C–N), 1430 (C=C), 1060, 1130 (C–O). ^{1}H NMR spectrum, δ, ppm: 5.65 s (1H), 6.77–6.79 d (1H), 6.80–6.88 d (1H), 6.84 s (1H), 6.20 s (1H), 3.78 s (3H), 3.22 s (6H), 3.52 s (6H), 3.69 s (6H), 8.19 s (1H), 8.21 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 28.99, 29.96, 36.97, 39.42, 90.29, 127.28, 130.64, 135.91, 143.98, 152.96, 162.74. Mass spectrum: *m*/*z* 472.21.

5,5′-(Naphthalen-1-ylmethylene)bis[1,3-dimethyl-6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione] (3n). Yield 70%, brownish yellow solid, mp >250°C, R_f 0.53. IR spectrum, ν, cm^{-1}: 3352 (N–H), 3011 (=C–H), 2901 (–C–H), 1651 (C=O), 1207

(C–N), 1526, 1420 (C=C). ^{1}H NMR spectrum, δ, ppm: 7.09–7.15 d (1H), 7.44–7.46 m (1H), 7.94–7.98 d (1H), 8.16–8.20 d (1H), 7.56–7.60 m (1H), 7.62–7.66 m (1H), 8.20–8.24 d (1H), 6.02 s (1H), 3.28 s (6H), 3.32 s (6H), 3.45 s (6H), 9.87 s (1H), 8.21 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 25.89, 29.34, 34.17, 39.79, 89.27, 127.23, 130.51, 136.55, 142.97, 151.18, 161.85. Mass spectrum: *m*/*z* 476.22.

***In vitro* antimicrobial evaluation.** The antimicrobial activities of compounds **3a**–**3n** were evaluated by measuring the minimum inhibitory concentrations (MIC) and inhibition zone diameters (IZD). For the determination of MICs, bacterial strains *Bacillus subtilis* MTCC 121 (Bc), *Staphylococcus aureus* MTCC96 (Sa), and *Pseudomonas aeruginosa* MTCC 741 (Pa) were allowed to grow on LB liquid medium for 8 h at 37°C, and the growths of bacterial strains were measured by recording the optical density of the solution at λ 600 nm. Bacterial suspension, 100 μL, was then added to a conical tube containing compound **3a**–**3n** at a final concentration of 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98, 0.49, or 0.244 μg/mL in 10 mL of LB medium. In a control experiment, 100 μl of bacterial suspension was added to a conical tube containing 10 mL of LB medium alone. The growth of bacteria for each conical tube was measured by recording the optical density of the solution at λ 600 nm after incubation for 24 h at 37°C. The optical density versus compound concentration was plotted together, and the drop in the optical density at respective concentration was taken as the MIC value [22].

The inhibition zone diameters were measured by the agar diffusion method. Nutrient agar medium was prepared and poured into sterile petri plates. After solidification, the plates were inoculated with bacterial pathogens (*Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). Wells were prepared using standard method, and 100 μL of a solution of **3a**–**3n** was placed into each well of nutrient agar medium with the bacterial lawn. The plates were incubated overnight at 37°C, and the diameter of inhibition zone was measured [22, 23].

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Chemistry, University of Lucknow, Lucknow, for providing necessary laboratory facilities; to the Director, Central Drug Research Institute (CDRI), Lucknow, India, for providing spectral data; and to the Director, Faculty of Biotechnology, Institute of Biosciences and Technology, Shri Ramswaroop Memorial Unversity, for providing biological activity data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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