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SUPPLEMENTARY MATERIAL TO

The design, synthesis and antimicrobial activity of new biquinoline derivatives

NIRAV K. SHAH, NIMESH M. SHAH, MANISH P. PATEL and RANJAN G. PATEL*

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India

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TABLE S-I. Physical data for compounds **2**(**a**–**d**)

Compound	R_1	M.p., °C	M.W., g mol^{-1}	Molecular formula	Yield, %
2a	H	145	191.61	$C_{10}H_6ClNO$	71
2b	Me	125–26	205.64	$C_{11}H_8ClNO$	73
2c	OMe	148–49	221.64	$C_{11}H_8ClNO_2$	68
2d	Cl	165–66	226.06	$C_{10}H_5Cl_2NO$	42

*Analytical and spectroscopic characterization data of 3-[(4-arylthiazole-2-yl)amino]cyclohex-2-en-1-ones **3**(**a**–**c**)*

*3-[(4-Phenylthiazol-2-yl)amino]cyclohex-2-en-1-one (**3a**)*. Yield: 74 %; m.p. 188–189 °C; Anal. Calcd. for $C_{15}H_{14}N_2OS$: C, 66.64; H, 5.22; N, 10.36 %. Found: C, 66.87; H, 4.98; N, 10.51 %; IR (KBr, cm^{-1}): 3395 (N–H str.), 1665 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.89–2.27 (6H, *m*, 3×CH_2), 5.23 (1H, *s*, CH), 6.72–7.85 (6H, *m*, Ar–H), 10.48 (1H, *s*, N–H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 20.19, 26.98 (CH_2), 37.02 (**C**H_2–CO), 106.41, 106.89, 112.47, 128.06, 132.79, 147.78, 153.56, 159.05, 161.67 (Ar-C), 197.28 (C=O).

*3-{[4-(4-Chlorophenyl)thiazol-2-yl]amino}cyclohex-2-en-1-one (**3b**)*. Yield: 70 %; m.p. 199–201 °C; Anal. Calcd. for $C_{15}H_{13}ClN_2OS$: C, 59.11; H, 4.30; N, 9.19 %. Found: C, 58.95; H, 4.44; N, 9.27 %; IR (KBr, cm^{-1}): 3430 (N–H str.), 1655 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.87–2.26 (6H, *m*, 3×CH_2), 5.27 (1H, *s*, CH), 6.87–7.94 (5H, *m*, Ar–H), 10.55 (1H, *s*, N–H); ^{13}C--NMR (100 MHz, DMSO-d_6, δ / ppm): 20.37, 27.60 (CH_2), 36.79 (**C**H_2–CO), 105.35, 106.78, 127.93, 129.25, 131.82, 148.63, 154.41, 159.30, 162.11 (Ar-C), 197.49 (C=O).

* Corresponding author. E-mail: patelranjanben@yahoo.com

*3-{[4-(4-Hydroxyphenyl)thiazol-2-yl]amino}cyclohex-2-en-1-one (**3c**).* Yield: 75 %; m.p. 210–212 °C; Anal. Calcd. for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 9.78 %. Found: C, 63.07; H, 4.75; N, 9.66 %; IR (KBr, cm^{-1}): 3415 (N–H str.), 1680 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.83–2.29 (6H, *m*, 3×CH_2), 5.25 (1H, *s*, CH), 6.81–7.71 (5H, *m*, Ar–H), 9.62 (1H, *s*, O–H), 10.34 (1H, *s*, N–H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 20.27, 27.33 (CH_2), 37.00 (**C**H_2–CO), 105.21, 106.62, 118.21, 127.10, 129.53, 148.87, 157.65, 159.48, 162.02 (Ar-C), 197.66 (C=O).

*Analytical and spectroscopic data for compounds **4(a–l)***

*Ethyl 2-amino-4-(2-chloro-3-quinolyl)-5-oxo-1-(4-phenylthiazol-2-yl)--1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4a**).* Yield: 87 %; m.p. 245–247 °C; Anal. Calcd. for $C_{30}H_{25}ClN_4O_3S$: C, 64.68; H, 4.52; N, 10.06 %. Found: C, 64.82; H, 4.48; N, 9.90 %; IR (KBr, cm^{-1}): 3425 & 3280 (asym. & sym. str. of –NH_2), 1678 (C=O str.), 1640 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / / ppm): 1.11 (3H, *t*, *J* = 7.16 Hz, CH_3), 1.71–2.20 (6H, *m*, 3×CH_2), 3.90 (2H, *q*, *J* = 7.16 Hz, OCH_2), 5.36 (1H, *s*, CH), 7.31–8.28 (11H, *m*, Ar–H), 8.42 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.45 (CH_3), 21.30, 26.82 (2C, CH_2), 35.40 (C4), 36.12 (**C**H_2–CO), 57.32 (OCH_2), 78.25 (**C**–COOEt), 113.31, 117.78, 119.89, 126.65, 126.96, 128.54, 129.12, 129.41, 131.23, 131.30, 133.30, 140.28, 144.93, 150.41, 150.60, 152.53, 152.73, 152.82, 156.91 (19C, Ar-C), 168.75 (**C**OOEt), 195.88 (C=O).

*Ethyl 2-amino-4-(2-chloro-6-methyl-3-quinolyl)-5-oxo-1-(4-phenylthiazol-2--yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4b**).* Yield: 89 %; m.p. 169–171 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_3S$: C, 65.20; H, 4.77; N, 9.81 %. Found: C, 65.28; H, 4.72; N, 9.93 %; IR (KBr, cm^{-1}): 3437 and 3333 (asym. and sym. str. of –NH_2), 1672 (C=O str.), 1620 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.09 (3H, *t*, *J* = 7.4 Hz, CH_3), 2.23 (3H, *s*, CH_3), 1.71–2.20 (6H, *m*, 3×CH_2), 3.93 (2H, *q*, *J* = 7.2 Hz, OCH_2), 5.31 (1H, *s*, CH), 7.29–8.25 (10H, *m*, Ar–H), 8.38 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.23 (CH_3), 20.14 (CH_3), 21.41, 26.02 (2C, CH_2), 35.10 (C4), 36.32 (**C**H_2–CO), 57.10 (OCH_2), 78.74 (**C**–COOEt), 113.14, 117.14, 119.41, 126.41, 126.47, 128.25, 129.45, 129.74, 131.13, 131.15, 133.25, 140.47, 144.78, 150.41, 150.45, 152.13, 152.71, 152.92, 156.45 (19C, Ar-C), 168.79 (**C**OOEt), 195.45 (C=O).

*Ethyl 2-amino-4-(2-chloro-6-methoxy-3-quinolyl)-5-oxo-1-(4-phenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4c**).* Yield: 83 %; m.p. 221––223 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_3S$: C, 63.42; H, 4.64; N, 9.54 %. Found: C, 63.58; H, 4.72; N, 9.73 %; IR (KBr, cm^{-1}): 3446 and 3240 (asym. and sym. str. of –NH_2), 1668 (C=O str.), 1635 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.01 (3H, *t*, *J* = 7.4 Hz, CH_3), 1.71–2.25 (6H, *m*, 3×CH_2), 3.89 (3H, *s*, OCH_3), 3.90 (2H, *q*, *J* = 7.1 Hz, OCH_2), 5.35 (1H, *s*, CH), 7.29–8.22 (10H, *m*,

Ar-H), 8.32 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.64 (CH_3), 21.23, 27.12 (2C, CH_2), 35.14 (C4), 36.00 (**C**H_2–CO), 57.23 (OCH_3), 59.10 (OCH_2), 77.74 (**C**–COOEt), 105.27, 114.41, 119.32, 126.40, 126.84, 128.23, 129.18, 129.78, 131.41, 131.97, 133.41, 140.40, 144.68, 150.74, 150.92, 152.44, 152.64, 152.90, 156.01 (19C, Ar-C), 168.14 (**C**OOEt), 195.38 (C=O).

*Ethyl 2-amino-4-(2,6-dichloro-3-quinolyl)-5-oxo-1-(4-phenylthiazol-2-yl)--1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4d**).* Yield: 76 %; m.p. 232–234 °C; Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_3S$: C, 60.92; H, 4.09; N, 9.47 %. Found: C, 60.86; H, 4.05; N, 9.60 %; IR (KBr, cm^{-1}): 3445 and 3345 (asym. and sym. str. of –NH_2), 1660 (C=O str.), 1640 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / / ppm): 1.01 (3H, *t*, *J* = 7.12 Hz, CH_3), 1.71–2.25 (6H, *m*, 3×CH_2), 3.92 (2H, *q*, *J* = 7.12 Hz, OCH_2), 5.30 (1H, *s*, CH), 7.37–8.24 (10H, *m*, Ar–H), 8.45 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.74 (CH_3), 21.19, 27.46 (2C, CH_2), 35.82 (C4), 36.58 (**C**H_2–CO), 59.24 (OCH_2), 77.93 (**C**–COOEt), 114.17, 117.70, 119.34, 126.61, 126.85, 128.22, 129.37, 129.87, 131.05, 131.81, 133.95, 140.70, 144.35, 150.66, 150.93, 152.55, 152.76, 152.87, 156.98 (19C, Ar-C), 168.99 (**C**OOEt), 195.87 (C=O); MS: 591.1 (M^++1).

*Ethyl 2-amino-1-[(4-chlorophenyl)thiazol-2-yl]-4-(2-chloro-3-quinolyl)-5--oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4e**).* Yield: 87 %; m.p. 195––197 °C; Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_3S$: C, 60.92; H, 4.09; N, 9.47 %. Found: C, 60.96; H, 4.00; N, 9.65 %; IR (KBr, cm^{-1}): 3441 and 3260 (asym. and sym. str. of –NH_2), 1668 (C=O str.), 1645 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.10 (3H, *t*, *J* = 7.16 Hz, CH_3), 1.70–2.21 (6H, *m*, 3×CH_2), 3.94 (2H, *q*, *J* = 7.12 Hz, OCH_2), 5.40 (1H, *s*, CH), 7.30–8.22 (10H, *m*, Ar–H), 8.40 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.98 (CH_3), 21.78, 26.54 (2C, CH_2), 35.45 (C4), 36.12 (**C**H_2–CO), 57.98 (OCH_2), 78.32 (**C**––COOEt), 113.87, 117.36, 119.74, 126.45, 126.65, 128.54, 129.56, 129.74, 131.36, 131.70, 133.12, 140.56, 144.56, 150.23, 150.45, 152.03, 152.65, 152.85, 156.19 (19C, Ar-C), 168.23 (**C**OOEt), 195.98 (C=O).

*Ethyl 2-amino-4-(2-chloro-6-methyl-3-quinolyl)-1-[(4-chlorophenyl)thiazol--2-yl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4f**).* Yield: 81 %; m.p. 208–210 °C; Anal. Calcd. for $C_{31}H_{26}Cl_2N_4O_3S$: C, 61.49; H, 4.33; N, 9.25 %. Found: C, 61.41; H, 4.23; N, 9.35 %; IR (KBr, cm^{-1}): 3442 and 3280 (asym. and sym. str. of –NH_2), 1678 (C=O str.), 1660 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.07 (3H, *t*, *J* = 7.2 Hz, CH_3), 1.72–2.45 (6H, *m*, 3×CH_2), 2.47 (3H, *s*, CH_3), 3.87 (2H, *q*, *J* = 7.2 Hz, OCH_2), 5.25 (1H, *s*, CH), 7.25–8.31 (9H, *m*, Ar–H), 8.48 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.12 (CH_3), 20.23 (CH_3), 21.39, 27.28 (2C, CH_2), 35.17 (C4), 36.36 (**C**H_2––CO), 59.65 (OCH_2), 78.54 (**C**–COOEt), 113.07, 114.78, 116.89, 122.54, 125.41, 126.65, 128.10, 128.32, 129.21, 140.14, 141.74, 141.90, 147.12, 149.23, 152.00, 152.36, 156.03, 158.36, 158.96 (19C, Ar-C), 169.19 (**C**OOEt), 195.37 (C=O).

*Ethyl 2-amino-4-(2-chloro-6-methoxy-3-quinolyl)-1-[(4-chlorophenyl)thiazol-2-yl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4g**)*. Yield: 79 %; m.p. 225–227 °C; Anal. Calcd. for $C_{31}H_{26}Cl_2N_4O_4S$: C, 59.91; H, 4.22; N, 9.01. Found: C, 59.82; H, 4.42; N, 9.23 %; IR (KBr, cm^{-1}): 3445 and 3285 (asym. and sym. str. of $–NH_2$), 1670 (C=O str.), 1650 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.01 (3H, *t*, *J* = 7.2 Hz, CH_3), 1.75–2.41 (6H, *m*, 3×CH_2), 3.85 (2H, *q*, *J* = 7.1 Hz, OCH_2), 3.95 (3H, *s*, OCH_3), 5.32 (1H, *s*, CH), 7.29–8.24 (9H, *m*, Ar–H), 8.46 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.78 (CH_3), 21.89, 27.96 (2C, CH_2), 35.65 (C4), 36.54 (**C**H_2–CO), 56.41 (OCH_3), 59.12 (OCH_2), 78.23 (**C**–COOEt), 105.32, 114.21, 116.14, 116.45, 122.55, 125.65, 128.85, 128.96, 129.74, 140.17, 141.20, 141.39, 147.23, 149.11, 152.75, 152.85, 156.63, 158.74, 158.32 (19C, Ar-C), 169.32 (**C**OOEt), 195.97 (C=O).

*Ethyl 2-amino-1-[(4-chlorophenyl)thiazol-2-yl]-4-(2,6-dichloro-3-quinolyl)--5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4h**)*. Yield: 75 %; m.p. 198–200 °C; Anal. Calcd. for $C_{30}H_{23}Cl_3N_4O_4S$: C, 57.56; H, 3.70; N, 8.95. Found: C, 57.72; H, 3.85; N, 8.77 %; IR (KBr, cm^{-1}): 3439 and 3281 (asym. and sym. str. of $–NH_2$), 1670 (C=O str.), 1635 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.03 (3H, *t*, *J* = 7.4 Hz, CH_3), 1.72–2.41 (6H, *m*, 3×CH_2), 3.87 (2H, *q*, *J* = 7.16 Hz, OCH_2), 5.20 (1H, *s*, CH), 7.25–8.28 (9H, *m*, Ar–H), 8.40 (2H, *s*, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.98 (CH_3), 21.23, 27.45 (2C, CH_2), 35.32 (C4), 36.89 (**C**H_2–CO), 59.97 (OCH_2), 78.56 (**C**–COOEt), 112.71, 114.82, 116.10, 116.98, 122.38, 125.30, 128.56, 128.91, 129.13, 140.17, 141.88, 141.99, 147.06, 149.12, 152.58, 152.30, 156.85, 158.06, 158.12 (19C, Ar-C), 169.87 (**C**OOEt), 195.74 (C=O).

*Ethyl 2-amino-4-(2-chloro-3-quinolyl)-1-[(4-hydroxyphenyl)thiazol-2-yl]-5--oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4i**)*. Yield: 73 %; m.p. 198––200 °C; Anal. Calcd. for $C_{30}H_{25}ClN_4O_4S$: C, 62.88; H, 4.40; N, 9.78. Found: C, 63.07; H, 4.56; N, 9.67 %; IR (KBr, cm^{-1}): 3439 and 3295 (asym. and sym. str. of $–NH_2$), 1675 (C=O str.), 1640 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.05 (3H, *t*, *J* = 7.12 Hz, CH_3), 1.68–2.44 (6H, *m*, 3×CH_2), 3.90 (2H, *q*, *J* = 7.12 Hz, OCH_2), 5.21 (1H, *s*, CH), 7.20–8.26 (10H, *m*, Ar–H), 8.29 (2H, *s*, NH_2), 9.65 (1H, *s*, OH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.11 (CH_3), 20.92, 27.14 (2C, CH_2), 35.47 (C4), 36.85 (**C**H_2–CO), 59.52 (OCH_2), 78.36 (**C**––COOEt), 113.36, 114.69, 116.78, 116.92, 122.12, 125.98, 128.32, 128.87, 129.40, 140.71, 141.43, 141.61, 147.91, 149.73, 152.82, 152.49, 156.93, 158.71, 158.25 (19C, Ar-C), 169.61 (**C**OOEt), 195.40 (C=O).

*Ethyl 2-amino-4-(2-chloro-6-methyl-3-quinolyl)-1-[(4-hydroxyphenyl)thiazol-2-yl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4j**)*. Yield: 64 %; m.p. 236–238 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_4S$: C, 63.42; H, 4.64; N, 9.54 %. Found: C, 63.22; H, 4.48; N, 9.74 %; IR (KBr, cm^{-1}): 3442 and 3280 (asym. and sym. str. of $–NH_2$), 1678 (C=O str.), 1630 (C=O str.); ^{1}H-NMR (400 MHz,

DMSO-d_6, δ / ppm): 1.03 (3H, *t*, *J* = 7.2 Hz, CH_3), 1.70–2.41 (6H, *m*, 3×CH_2), 2.45 (3H, *s*, CH_3), 3.89 (2H, *q*, *J* = 7.16 Hz, OCH_2), 5.24 (1H, *s*, CH), 7.25–8.23 (9H, *m*, Ar–H), 8.30 (2H, *s*, NH_2), 9.62 (1H, *s*, OH); ^{13}C-NMR (100 MHz, DMSO-d_6 / δ , ppm): 14.23 (CH_3), 20.98 (Ar–CH_3), 21.12, 27.78 (2C, CH_2), 35.45 (C4), 36.98 (**C**H_2–CO), 59.56 (OCH_2), 78.89 (**C**–COOEt), 112.54, 114.32, 116.36, 116.52, 122.00, 125.35, 128.34, 128.47, 129.10, 140.87, 141.71, 141.99, 147.40, 149.59, 152.81, 152.99, 156.63, 158.25, 158.86 (19C, Ar-C), 169.10 (**C**OOEt), 195.32 (C=O); MS: 586.6 (M^++1).

*Ethyl 2-amino-4-(2-chloro-6-methoxy-3-quinolyl)-1-[(4-hydroxyphenyl)thiazol-2-yl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4k**)*. Yield: 81 %; m.p. 244–246 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_5S$: C, 61.74; H, 4.51; N, 9.29 %. Found: C, 61.88; H, 4.70; N, 9.07 %; IR (KBr, cm^{-1}): 3445 and 3260 (asym. and sym. str. of –NH_2), 1680 (C=O str.), 1660 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.05 (3H, *t*, *J* = 7.4 Hz, CH_3), 1.70–2.41 (6H, *m*, 3×CH_2), 3.91 (2H, *q*, *J* = 7.2 Hz, OCH_2), 3.91 (3H, *s*, OCH_3), 5.28 (1H, *s*, CH), 6.87–8.17 (9H, *m*, Ar–H), 8.27 (2H, *s*, NH_2), 9.72 (1H, *s*, OH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.72 (CH_3), 21.26, 27.47 (2C, CH_2), 35.63 (C4), 36.60 (**C**H_2–CO), 56.08 (Ar–OCH_3), 59.13 (OCH_2), 78.35 (**C**–COOEt), 105.79, 114.48, 116.04, 116.25, 122.99, 125.28, 128.09, 128.59, 129.14, 140.27, 141.20, 141.90, 147.47, 149.01, 152.69, 152.91, 156.73, 158.10, 158.38 (19C, Ar-C), 169.06 (**C**OOEt), 195.81 (C=O).

*Ethyl 2-amino-4-(2,6-dichloro-3-quinolyl)-1-[(4-hydroxyphenyl)thiazol-2-yl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4l**)*. Yield: 79 %; m.p. 256–258 °C; Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_4S$: C, 59.31; H, 3.98; N, 9.22 %. Found: C, 59.50; H, 4.05; N, 9.47 %; IR (KBr, cm^{-1}): 3440 and 3275 (asym. and sym. str. of –NH_2), 1675 (C=O str.), 1640 (C=O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.02 (3H, *t*, *J* = 7.12 Hz, CH_3), 1.70–2.41 (6H, *m*, 3×CH_2), 3.91 (2H, *q*, *J* = 7.12 Hz, OCH_2), 5.25 (1H, *s*, CH), 7.25–8.20 (9H, *m*, Ar–H), 8.26 (2H, *s*, NH_2), 9.70 (1H, *s*, OH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.12 (CH_3), 21.23, 27.36 (2C, CH_2), 35.32 (C4), 36.21 (**C**H_2–CO), 59.45 (OCH_2), 78.56 (**C**–COOEt), 105.65, 114.23, 116.10, 116.98, 122.34, 125.30, 128.23, 128.87, 129.13, 140.17, 141.78, 141.99, 147.56, 149.32, 152.23, 152.30, 156.30, 158.96, 158.32 (19C, Ar-C), 169.87 (**C**OOEt), 195.96 (C=O).

DETERMINATION OF THE ANTIMICROBIAL ACTIVITY

Sample preparation

A 1000 ppm solution of newly synthesized compounds was prepared in DMF.

Culture media for the study of antibacterial activity

The media: Nutrient broth (Hi-Media, Mumbai, India) having following composition was used for the preparation of inoculums.

Ingredient	g L^{-1}
Peptic digest of animal tissue	5.00

Yeast extract	1.50
Beef extract	1.50
Sodium chloride	5.00

Weighed quantities of all the components were dissolved in freshly prepared hot distilled water.

Sterilization for the study of antibacterial activity

The sterilization of culture media, culture tubes, and other materials was realized by autoclaving them at 15 lbs/sq. inch pressure for 15 min. The Petri-dishes were sterilized by keeping them overnight in an electrically heated air oven at 140 °C.

Preparation of nutrient plates for the study of antibacterial activity

Sterilized media (20 mL) was poured into each sterilized Petri dish and allowed to solidify.

Preparation of inoculums for the study of antibacterial activity

The inoculums of the organisms were prepared by transferring a loop full of the corresponding organism from the stock culture into the sterile broth and incubated at 37 °C for 24 h under shaking conditions. The organisms were sub-cultured on the nutrient agar slants. The inoculums were prepared by dispensing colonies in sterile distilled water to prepare a suspension.

Antibacterial susceptibility testing

A test tube containing sterile melted soft agar (approximately 15 ml) was cooled to 45 °C and inoculated with 0.2 ml of a suspension of the test culture, mixed thoroughly and poured into a Petri dish containing sterile nutrient agar medium and allowed to solidify for five minutes. A cup-borer (8 mm) was sterilized by dipping into absolute alcohol and flaming and then left to cool. Cups were bored in the agar with the sterile cup-borer and marked. The cups were filled with 0.1 ml of the required test sample solution and the test sample was allowed to diffuse for 10 to 15 min in a refrigerator. The plate was incubated at 37 °C for 24 h and on the next day, the zone of inhibition of surrounding each cup was observed and measured in mm.

Sample preparation for the study of antifungal activity

A 1000 ppm solutions of freshly synthesized compounds were prepared in DMF.

Culture media for the study of the antifungal activity

The media: potato-dextrose agar (Hi-Media, Mumbai, India) having the following composition was used for the preparation of inoculums for the antifungal study:

Ingredient	g L^{-1}
Potatoes infusion	200.00
Dextrose	20.0
Agar	15.0

Weighed quantities of all the components were dissolved in freshly prepared hot distilled water.

All experimental conditions for the antifungal activity were the same as those for the antibacterial activity except for the culture media and the plate was incubated at 28 °C for 48 h before the zone of inhibition surrounding each cup was observed and measured in mm.