Asian Journal of Chemistry; Vol. 30, No. 5 (2018), 1102-1108

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21202

Synthesis, Characterization, Stereochemistry and Biological Evaluation of Novel Cyclohexanol Derivatives

SAYEED MUKHTAR*, MESHARI A. ALSHARIF, MOHAMMED I. ALAHMDI and HUMAIRA PARVEEN

Department of Chemistry, Faculty of Science, University of Tabuk, Tabuk-71491, Kingdom of Saudi Arabia

*Corresponding author: E-mail: sayeed_mukhtar@hotmail.com

Received: 26 December 2017; *Accepted*: 18 February 2018; *Published online*: 29 March 2018; AJC-18849

This research includes the synthesis of 1,5-diketone from chalcone by Michael addition of *p*-chloroacetophenone in the presence of NaOH (molar ratio, 1:1:10), which led to the formation of a novel cyclohexanol derivative as a side product. The above reaction was repeated with different molar ratio of chalcone-acetophenone-sodium hydroxide to set the optimum condition for maximum yield of the novel cyclohexanol derivative. The dehydration of cyclohexanol using catalytic amount of *p*-TsOH produced quantitative yield of corresponding cyclohexene with β,γ-unsaturation instead of apparently more stable α,β-unsaturation. The compounds were well characterized by spectroscopic techniques and elemental analysis. Their stereochemistry is discussed and newly synthesized compounds are screened for anticancer and antimicrobial activities.

Keywords: Chalcones, Cyclohexanol derivatives, Stereochemistry, *in vitro* Anticancer activity, *in vitro* Antimicrobial activity.

INTRODUCTION

Recently, a considerable amount of research on biologically active cyclohexanol derivatives has been reported [1-6] and has attracted growing interest of medicinal chemists. An elaborative literature survey on cyclohexanol derivatives has revealed that they exhibited diverse biological activities such as acetylcholine-storage-blocking activities [7], analgesic [8], antidepressant [9], cardio-inhibitory effects [10] and also have been reported as potent microbial agents [11].

In view of these observations and as part of our program in search of biologically active compounds with sulphur and nitrogen containing heterocycles [12-18], we report here the synthesis, characterization, stereochemistry, anticancer and antimicrobial studies of cyclohexanol derivatives.

EXPERIMENTAL

Reagents and solvents were of commercial grade and used without further purification. Melting points were determined on a Koffler hot-plate apparatus and are uncorrected. Elemental analysis (C, H, N) were undertaken using an Elemental analyzer and within ± 0.4 % of the calculated values. IR spectra were recorded on a Perkin-Elmer 621 spectrophotometer while ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 MHz and 100 MHz, respectively in acetone-d_6 with TMS as the internal standard. DCI-mass spectra were recorded in a Ribermag R10-10B quadrupole mass spectrometer. Column chromatography was performed on silica gel (60-120 mesh).

4'-Chloro-4-methoxychalcone (**1a**) and 4,4'-dichlorochalcone (**1b**) were synthesized according to the published method [19].

General procedure for the synthesis of 1,5-diketones and cyclohexanol derivatives: A mixture of chalcone (**1a**) (500 mg, 1.83 mmol), *p*-chloroacetophenone (0.24 mL, 283 mg, 1.83 mmol) in an aqueous ethanolic solution of NaOH (7 mL, H_2O-EtOH, 1:1) (734 mg, 18.3 mmol) was heated on a water bath for 5 h monitoring the progress of the reaction by TLC at every 15 min. After completion, the reaction mixture was extracted with ethyl acetate, washed with water and the organic phase dried over Na_2SO_4. The solvent was distilled off under reduced pressure to a semi-solid residue which on column chromatography (silica gel, petroleum ether (60-80 ºC)-diethyl ether, 6:4 v/v) followed by crystallization from C_6H_6-EtOAc yielded compounds **2a** and **3a** as white crystalline needles.

1,5-*Bis*(4-chlorophenyl)-3-(4-methoxyphenyl)pentan-1,5-dione (2a): Yield: 68%; m.p 80 ºC, R_f 0.67 (benzene-diethyl ether, 9.5:0.5 v/v). IR (KBr, cm^{-1}): 1675 ν(C=O), 1610, 1580 ν(phenyl), 1510, 1400, 1350, 1250, 1170, 1075, 980, 810; ^{1}H NMR (400 MHz, acetone-d_6): δ 3.41 (dd, 2H, H-2up/4up, J = 16.94 Hz, J = 7.48 Hz); 3.51 (dd, 2H, H-2dn/4dn, J = 16.94 Hz, J = 6.56 Hz); 3.98 (br p, 1H, H-3, $J \approx 7$ Hz); 3.70 (s, 3H, H-OCH_3); 7.25 (d, 2H, H-Ar′-2,6, J = 8.85 Hz); 6.78 (d, 2H,

H-Ar′-3,5, *J* = 8.85 Hz); 7.98 (d, 4H, H-Ar-2, 6, *J* = 8.85 Hz); 7.49 (d, 4H, H-Ar-3,5, *J* = 8.85 Hz); ^{13}C NMR (100 MHz, acetone-d_6): δ 45.70 (C-2/4), 37.25(C-3), 55.42 (C-OCH_3) 129.51 (C-Ar′-2,6), 114.57 (C-Ar′-3,5), 130.62 (C-Ar-2,6), 129.57 (C-Ar-3,5), 136.96 (C-Ar′-1), 159.27 (C-Ar′-4), 136.86 (C-Ar-1), 139.45 (C-Ar-4), 198.06(C-CO), MS (DCI): 431/433/435 (M^+ + 1); Anal. Calcd for $C_{24}H_{20}O_3Cl_2$: C, 67.46; H, 4.72 %; Found: C, 67.39; H, 4.70 %.

2,4-*Bis*(4-chlorobenzoyl)-1-(4-chlorophenyl)-3,5-*bis*(4-methoxyphenyl)cyclohexan-1-ol (3a): Yield: 6.6 %, m.p. 232 ºC, R_f 0.42 (benzene-diethyl ether, 9.5:0.5 v/v). IR (KBr, cm^{-1}): 3480 ν(OH); 1665, 1640 ν(C=O); 1610, 1585 ν(phenyl), 1510, 1400, 1250, 1175, 1085, 1025, 1005, 820. The δ_H and δ_C values are given in Tables 1 and 2. MS (*ApcI*): *m/z* 681/683/685/687 $[M^+ + 1-H_2O]^+$.

The above reaction was also carried out with different molar ratios of chalcone **1a** and acetophenone in the presence of 10 equiv. NaOH (10 %) as described above which furnished compounds **2a** and **3a** in varying yields.

Further, Michael reaction of chalcone **1a** with the isolated 1,5-diketone **2a** was also conducted as follows: A mixture of chalcone **1a** (400 mg, 1.46 mmol) and 1,5-diketone **2a** (500 mg, 1.17 mmol) in NaOH solution (5.0 mL, H_2O-EtOH, 1:1) (470 mg, 11.7 mmol) was heated on a water bath for 5 h. The products on usual workup and crystallization afforded compound **3a** as white crystalline globules, 570 mg (69.6 %), m.p. 232 ºC, R_f 0.42 (benzene-diethyl ether, 9.5:0.5 v/v).

1,3,5-*Tris*(4-chlorophenyl)pentan-1,5-dione (2b): Yield: 72 %; m.p 110 ºC, R_f 0.85 (benzene-diethyl ether 9.5:0.5 v/v); IR (KBr, cm^{-1}): 1680 ν(C=O), 1610, 1585 ν(phenyl), 1520, 1400, 1350, 1260, 1165, 1060, 980, 810. ^{1}H NMR (400 MHz, acetone-d_6): 3.49 (dd, 2H, H-2up/4up, *J* = 17.24 Hz, *J* = 7.63 Hz); 3.59 (dd, 2H, H-2dn/4dn, *J* = 17.24 Hz, *J* = 6.41 Hz); 4.05 (br p, 1H, H-3, J ≈ 7 Hz); 7.26 (d, 2H, H-Ar′-2,6, *J* = 8.54 Hz); 7.40 (d, 2H, H-Ar′-3,5, *J* = 8.54 Hz); 8.00 (d, 4H, H-Ar-2,6, *J* = 8.70 Hz); 7.52 (d, 4H, H-Ar-3,5, *J* = 8.70 Hz). ^{13}C NMR (100 MHz, acetone-d_6): 45.29 (C-2/4), 37.23 (C-3), 130.48 (C-Ar′-2,6) 129.11 (C-Ar′-3,5), 130.64 (C-Ar-2,6), 129.64 (C-Ar-3,5), 132.44 (C-Ar′-1), 144.20 (C-Ar′-4), 136.74 (C-Ar-1), 139.60 (C-Ar-4), 197.77 (C-CO); MS (DCI): *m/z* 427/429/431/433 (M^+ +1); Anal. Calcd for $C_{23}H_{17}O_2Cl_3$: C, 63.98; H, 3.97 %. Found: C, 63.89; H, 3.96 %.

2,4-*Bis*(4-chlorobenzoyl)-1,3,5-tris(4-chlorophenyl)-cyclohexan-1-ol (3b): Yield: 7.3 %; m.p. 242 ºC, R_f 0.67 (benzene-diethyl ether 9.5:0.5 v/v); IR (KBr, cm^{-1}): 3475 ν(OH), 1665, 1645 ν(C=O), 1610, 1580 ν(phenyl), 1510, 1395, 1260, 1175, 1085, 1015, 820 cm. ^{1}H NMR (400 MHz, acetone-d_6): 2.00 (dd, 1H, H-6_{eq}, *J* = 3.66 Hz, *J* = 13.74 Hz), 2.86 (br dt, 1H, H-6_{ax}, *J* = 2.29 Hz, *J* = 11-12 Hz, *J* = 13.74 Hz), 3.92 (br dt, 1H, H-5_{ax}, *J* ≈ 11.10 Hz, *J* = 11-12 Hz, *J* = 3.66 Hz), 4.12 (br t, 1H, H-3_{ax}, *J* ≈ 11.45); 4.82 (br t, 1H, H-4_{ax}, *J* ≈ 11.14), 5.16 (d, 1H, H-2_{ax}, *J* = 11.60 Hz); 5.12 (d, H, H-OH, *J* = 2.29 Hz); 6.91-7.73 (br m, H-5 × Ar-rings). ^{13}C NMR (100 MHz, acetone-d_6): 46.51 (C-6), 44.22 (C-5), 48.47 (C-3), 55.50a (C-4), 56.08 (C-2), 76.11 (C-1), 202.34 (C-CO), 205.33 (C-CO), 127.84-146.28 (C-5 × Ar-rings). MS (*ApcI*): *m/z* 689/691/693/695 $[M^+ +1-H_2O]^+$.

General procedure for the synthesis of 4,6-bis(4-chlorobenzoyl)-1-(4-chlorophenyl)-3,5-*bis*(4-methoxyphenyl)-cyclohexene (4a): To a solution of compound **3a** (100mg, 0.142 mmol) in dry benzene (5 mL) was added *p*-toluene sulphonic acid (5 mg) and refluxed with stirring over an oil bath at 80 ºC for 3 h. The reaction mixture was cooled, washed with water and the organic phase dried over Na_2SO_4. The solvent was distilled off under reduced pressure and the residue left on usual workup and crystallization from C_6H_6-EtOAc afforded compound **4a** as white crystalline globules, 91 mg (93.4 %), m. p 218 ºC, R_f 0.60 (benzene:diethyl ether, 9.5:0.5 v/v). IR (KBr, cm^{-1}): 2835 ν(C-H), 1668 ν(C=O), 1613, 1587, 1569 ν(phenyl), 1512,1488, 1462, 1440, 1399, 1305, 1287, 1254 1214, 1179, 1092, 1031, 1011, 971, 862, 830, 751, 729, 697. The δ_H and δ_C values of compound **4a** are given in Table-3. MS (*ApcI*): *m/z* 681/683/685/687 (M^+ +1).

4-*Bis*(4-chlorobenzoyl)-1,3,5-tris(4-chlorophenyl)-cyclohexene (4b): Yield: 94.6%; m.p. 254 ºC, R_f 0.90 (benzene-diethyl ether 9.5:0.5 v/v); IR (KBr, cm^{-1}): 2858 ν(C-H), 1668

TABLE-1
^{1}H NMR DATA OF **3a** IN ACETONE-d_6

H-nr	δ(ppm)	Integration	Multiplicity	*J* (Hz) + COSY
6_{eq}	1.97	1H	Dd	$J_{5ax,6eq}$ 3.66; $J_{6ax,6eq.}$ 13.73
6_{ax}	2.78	1H	br dt	$J_{6ax,OH}$ 2.29; $J_{5ax,6ax}$ 11-12; $J_{6ax,6eq}$ 13.73
5_{ax}	3.86	1H	br dt	$J_{4ax,5ax}$ ≈ 11.14; $J_{5ax,6ax}$ 11-12; $J_{5ax,6eq}$ 3.66
3_{ax}	4.02	1H	br t	$J_{2ax,3ax} \approx J_{3ax,4ax} \approx$ 11.45
4_{ax}	4.71	1H	br t	$J_{3ax,4ax} \approx J_{4ax,5ax} \approx$ 11.14
2_{ax}	5.08	1H	D	$J_{2ax,\ 3ax}$ 11.75
1-OH	5.14	1H	D	$J_{OH,6ax}$ 2.29
3-[Ar'-2,6]	7.19	2H	d	$J_{Ar'-2,6,Ar'-3,5}$ 8.85
3-[Ar'-3,5]	6.42	2H	d	$J_{Ar'-2,6,Ar'-3,5}$ 8.85
3-[Ar'-OCH_3]	3.60	3H	s	
5-[Ar'-2,6]	7.26	2H	d	$J_{Ar'-2,6,Ar'-3,5}$ 8.70
5-[Ar'-3,5]	6.66	2H	d	$J_{Ar'-2,6,\ Ar'-3,5}$ 8.70
5-[Ar'-OCH_3]	3.43	3H	s	
4-Ar-2,6	7.42	2H	d	$J_{Ar-2,6,Ar-3,5}$ 8.69
4-Ar-3,5	7.15	2H	d	$J_{Ar-2,6,Ar-3,5}$ 8.69
2-Ar-2,6	7.52	2H	d	$J_{Ar-2,6,Ar-3,5}$ 8.85
2-Ar-3,5	7.19	2H	d	$J_{Ar-2,6,Ar-3,5}$ 8.85
1-Ar-2,6	7.71	2H	d	$J_{Ar-2,6,Ar-3,5}$ 8.69
1-Ar-3,5	7.19	2H	d	$J_{Ar-2,6,Ar-3,5}$ 8.69

TABLE-2
^{13}C NMR DATA OF COMPOUND **3a** IN ACETONE-d_6

C-no.	δ (ppm)	HETCOR correlation with	Long-range HETCOR correlation with
6	47.03	H-6_{ax}; H-6_{eq}	1-OH
5	43.94	H-5_{ax}	
3	48.38	H-3_{ax}	H-2_{ax}, H-4_{ax}
4	56.45[a]	H-4_{ax}	
2	56.36[a]	H-2_{ax}	
1	76.25		
CO	203.03		
CO	205.95		
3-[Ar-2,6]	128.84[b]	H-{3-[Ar-2,6]}	
3-[Ar-3,5]	114.28	H-{3-[Ar-3,5]}	
3-[Ar-4]	159.42		H-{3-[Ar'-OCH_3]}
3-[Ar-1]	132.16		
3-[Ar-OCH_3]	55.39[c]	H-{3-[Ar-OCH_3]}	
5-[Ar-2,6]	129.83	H-{5-[Ar-2,6]}	H-5_{ax}
5-[Ar-3,5]	114.62	H-{5-[Ar-3,5]}	
5-[Ar-4]	159.42		H-{5-[Ar'-OCH_3]}, H-{5-[Ar'-2,6]}
5-[Ar-1]	133.04		
5-[Ar-OCH_3]	55.26[c]	H-{5-[Ar-OCH_3]}	
4-Ar-2,6	130.17	H-{4-[Ar-2,6]}]	
4-Ar-3,5	128.68	H-{4-[Ar-3,5]}	
4-Ar-1	135.53[d]		
4-Ar-4	137.41[d]		
2-Ar-2,6	130.64	H-{2-[Ar-2,6]}	
2-Ar-3,5	128.94[b]	H-{2-[Ar-3,5]}	
2-Ar-1	138.34[d]		
2-Ar-4	138.47[d]		
1-Ar-2,6	128.84[b]	H-{1-[Ar-2,6]}	
1-Ar-3,5	127.81[b]	H-{1-[Ar-3,5]}	
1-Ar-1	139.69[d]		
1-Ar-4	146.73		

[a]Assignment with the same superscript may be interchanged.

TABLE-3
^{1}H NMR SPECTRAL DATA OF COMPOUND **4a** IN ACETONE-d_6

H-no	δ (ppm)	Integration	Multiplicity	*J* (Hz) + COSY	C-no	δ (ppm)	HETCOR
2	6.15	1H	T	$J_{6ax,2,}$ $J_{2,3ax}$ 2.14	2	131.32	H-2
3_{ax}	4.16	1H	br dq	$J_{3ax,\ 4ax} \approx 11.10$; $J_{2,3ax}$ 2.14; $J_{3ax,\ 6pseud.ax} \approx 3.97$	3	47.16	H-$3_{pseud.ax}$
6_{ax}	5.49	1H	br dq	$J_{5ax,6ax} \approx 11.10$; $J_{3ax,6ax} \approx 3.97$; $J_{2,6}$ 2.14	6	55.15	H-$6_{pseud.ax}$
4_{ax}	4.57	1H	br t	$J_{5ax,4ax} \approx J_{3ax,4ax} \approx 11.10$	4	53.20	H-4_{ax}
5_{ax}	3.74	1H	br t	$J_{5ax,6} \approx J_{4ax,5ax} \approx 11.10$	5	50.42	H-5_{ax}
5 × Ar	6.47-7.74	20H	br m	–	CO	204.10	
H-OCH_3	3.54	3H	s	–	CO	206.83	
					5 × Ar	128.32-159.70	

(C=O), 1589, 1569 ν(phenyl), 1488, 1402, 1360, 1317, 1289, 1215, 1175, 1094, 1014, 981, 965, 910, 870, 842, 805, 761, 738, 720, 677. ^{1}H NMR (400 MHz, acetone-d_6): 6.17 (t, 1H, H-2_{eq}, J = 2.14 Hz); 4.23 (br dq, 1H, H-3_{ax}, $J \approx 11.14$ Hz, $J \approx 2.14$ Hz, $J \approx 3.66$ Hz); 3.82 (br t, 1H, H-5_{ax}, $J \approx 11.14$ Hz); 4.69 (br t, 1H, H-4_{ax}, $J \approx 11.14$ Hz); 5.56 (br dq, 1H, H-6_{ax}, J ≈ 11.14 Hz, $J \approx 2.14$ Hz, $J \approx 3.66$ Hz); 3.54 (s, 3H, H-OCH_3); 3.66 (s, 3H, H-OCH_3); 6.98-7.70 (br m, 20 H, 2 × H-Ar′ + 3 × H-Ar). ^{13}C NMR (100 MHz, acetone-d_6): 132.49 (C-2), 48.72 (C-3), 54.25 (C-4), 50.54 (C-5), 55.50 (C-6), 200.70 (C-CO), 202.43 (C-CO), 127.80-159.42 (C-5 × Ar); MS (*ApcI*): *m/z* 689/691/693/695 (M^+ +1).

General procedure for the synthesis of 2,6-bis(4-chlorophenyl)-4-(4-methoxyphenyl)pyridine (7a): A mixture of 1,5-diketone **2a** (1.5 g, 0.0035 mol), thioglycollic acid (0.966 g, 0.0105 mol) and ammonium carbonate (3.36 g, 0.350 mol) in dry benzene (30 mL) was refluxed for 28 h, collecting the generated water in an azeotropic collector. The reaction mixture was concentrated, extracted with diethyl ether, washed with water until filtrate was neutral and dried over sodium sulphate overnight. Evaporation of the solvent afforded a white solid mass which was recrystallized from acetone-benzene to furnish compound **7a** as white crystals, 1.026 g (87 %), m.p. 207-208 ºC, R_f 0.63 (petroleum ether-diethyl ether, 7:3 v/v); IR (KBr, cm^{-1}): 1610 ν(C=N and phenyl), 1590, 1570 ν(phenyl), 1540, 1505, 1475, 1420, 1375, 1285, 1245, 1165, 1075, 1000, 820. ^{1}H NMR (400 MHz, acetone-d_6): 3.88 (s, 3H, H-OCH_3); 8.15 (s, 2H, H-3,5); 7.94 (d, 2H, H-Ar′-2,6, J = 8.85 Hz); 7.10 (d, 2H, H-Ar′-3,5, J = 8.85 Hz); 8.35 (d, 4H, H-2, Ar-2,6, J = 8.69); 7.55 (d, 4H, H-2, Ar-3,5, J = 8.69). ^{13}C NMR (100 MHz, acetone-

d_6): 55.82 (C-OCH_3), 156.86 (C-2,6), 117.24 (C-3,5); 129.48 (C-Ar′-2,6); 115.45 (C-Ar′-3,5), 151.05 (C-4), 131.31 (C-Ar′-1), 161.96 (C-Ar′-4), 129.65 (C-2 Ar-2,6), 129.59 (C-2 Ar-3,5), 139.13 (C-2 Ar-1), 135.60 (C-2 Ar-4); MS (DCI): 406/408/410 (M^+ +1); Anal. Calcd for $C_{24}H_{17}NOCl_2$: C, 70.95; H, 4.22; N, 3.45 %. Found: C, 70.88; H, 4.21, N, 3.47 %.

2,4,6-*Tris*(4-chlorophenyl)pyridine (7b): Yield 89 %, m.p. 220-222 ºC, R_f 0.78 (petroleum ether-diethyl ether, 7:3 v/v); IR (KBr, cm^{-1}): 1620 ν(C=N and phenyl), 1600, 1580 ν(phenyl), 1540, 1510, 1480, 1420, 1365, 1250, 1225, 1090, 985, 860, 810. ^{1}H NMR (400 MHz, acetone-d_6): 8.22 (s, 2H, H-3,5); 8.06 (d, 2H, H-Ar′-2,6, *J* = 8.70 Hz); 7.10 (d, 2H, H-Ar′-3,5, *J* = 8.70 Hz); 8.42 (d, 4H, H-2, Ar-2,6, *J* = 8.65); 7.60 (d, 4H, H-2, Ar-3,5, *J* = 8.65); ^{13}C NMR (100 MHz, acetone-d_6): 156.92 (C-2,6), 118.70 (C-3,5); 130.52 (C-Ar′-2,6); 116.55 (C-Ar′-3,5), 151.02 (C-4), 129.49 (C-Ar′-1), 147.35 (C-Ar′-4), 130.12 (C-2Ar-2,6), 129.89 (C-2Ar-3,5), 140.08 (C-2Ar-1), 136.22 (C-2Ar-4); MS (DCI): *m/z* 410/412/414 (M^+ +1); Anal. calcd for $C_{23}H_{14}NCl_3$: C, 67.26; H, 3.44; N, 3.41%. Found: C, 67.37; H, 3.42; N, 3.40%.

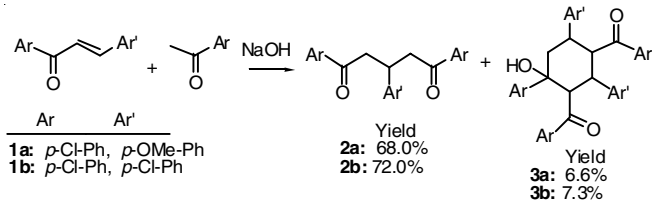
in vitro Anticancer activities: The compounds **3a**, **3b** and **7a** have been evaluated in the 3-cell line of three types of human cancers: breast (MCF7), Lung (NCI-H460) and CNS (SF-268) in the one dose primary anticancer assay at 10 μM concentration. In the current protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration and the culture incubated for 48 h. End point determinations were made with alamar blue [20]. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells.

in vitro Antimicrobial activities: A total of 4 compounds (**3a**, **3b**, **4a** and **4b**) were tested against two Gram +ve bacteria (*Staphylococcus aureus*, *Bacillus substilis*) and two Gram -ve bacteria (*Estcherichia coli*, *Pseudomonas aeruginosa*) and a yeast, *Candida albicans*. Sabour and dextrose (SD) and Nutrient broth agar media were obtained from Hi-Media Pvt. Ltd. (Bombay, India) were used for *Canadida albicans* and test bacteria, respectively. Freshly grown microbial cultures at 37 ºC were appropriately diluted in sterile normal saline solution to obtain a cell suspension of 10^6 CFU/mL.

Each synthetic compound was dissolved in DMSO to obtain a stock solution of different concentration ranging from 2500 μg/mL. The agar well diffusion method [21,22] was used. 0.1 mL of the diluted inoculum (10^6 CFU/mL) of test organism was spread on nutrient agar/sabour and dextrose agar plates. Wells of 8 mm diameter were punctured into the agar medium and filled with 100 μL of compound, solvent blank and an antibiotic (chloramphenicol, 100 μg/mL) to which the test bacteria were sensitive. Fluconozole at the concentration of 100 μg/mL was used as the control against *Candida albicans*. The plates were incubated for 18 h at 37 ºC. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

RESULTS AND DISCUSSION

The aim of the present work was to synthesize dithiazolidin-4-one derivatives from chalcones *via* the formation of 1,5-diketones by Michael addition of acetophenone, using thioglycollic acid in the presence of ammonium carbonate. Interestingly, during the preparation of 1,5-diketones (**2a** and **2b**) from chalcones (**1a** and **1b**) by the Michael addition of *p*-chloroacetophenone in the presence of NaOH (molar ratio, 1:1:10), a novel cyclohexanol derivative was obtained as a side product (**Scheme-I**).



Scheme-I: Synthesis of cyclohexanol derivatives (**3a** and **3b**)

It seems that the formation of cyclohexanol in the above reactions has occurred probably by double Michael addition [23] followed by intramolecular aldol condensation [24] of *bis*-adduct formed *in situ*. This was confirmed by carrying out Michael addition reaction of the isolated 1,5-diketone **2a** to chalcone **1a** under the same condition, which yielded cyclohexanol **4a** as the sole product. Keeping in view of the novelty of the side product, the same reaction was repeated with different molar ratios of chalcone-acetophenone-sodium hydroxide to set the optimum condition to obtain maximum yield of cyclohexanol and thus the ratio was fixed as 2:1:10 (Table-4).

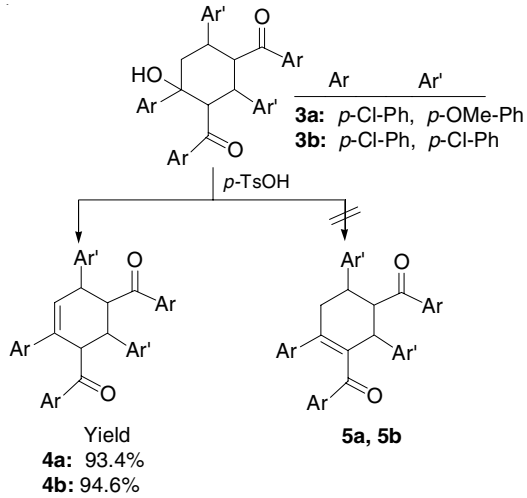
The dehydration of cyclohexanols **3a** and **3b** with catalytic amount of *p*-TsOH resulted in an unusual stereo selective synelimination producing quantitative yields of β,γ-unsaturated cyclohexenes **4a** (94.4 %) and **4b** (93.4 %) instead of apparently more stable α,β-unsaturated cyclohexenes **5a** and **5b** (**Scheme-II**).

This is probably due to the fact that the hydroxyl group in cyclohexanol being in axial disposition, the elimination went

TABLE-4
YIELDS OF COMPOUNDS **2a** AND **3a** WITH INCREASE IN MOLAR RATIOS OF CHALCONE-ACETOPHENONE

Substrate (chalcone)	Chalcone-acetophenone		Time (h)	Products* (% Yields-Isolated)	
	mmol	Molar ratio		**2a**	**3a**
1a	1.83:1.83	1:1	5	68	6.6
	2.29:1.83	1.25:1	5	42	30
	2.75:1.83	1.5:1	5	23	45
	3.21:1.83	1.75:1	5	8.4	58
	3.66:1.83	2:1	5	Minor amount	64

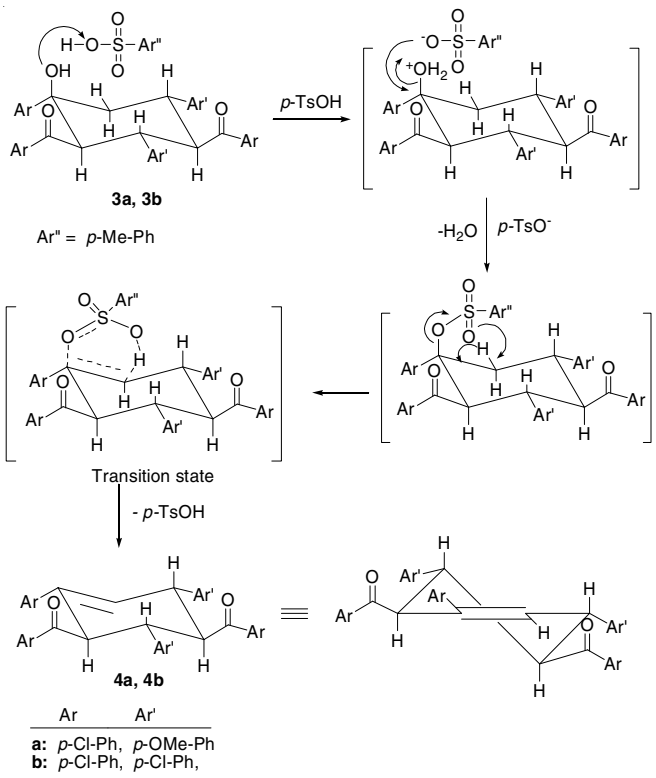
*The yields also vary with the concentration of NaOH. With 5 equivalents, the yield of compound **3a** was low while with 15 equivalents, an undesirable insoluble product was obtained. With 10 equivalents, the maximum yield was obtained.



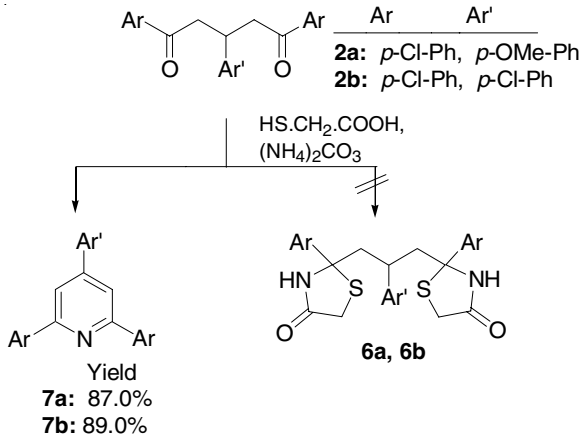
Scheme-II: Synthesis of β,γ-unsaturated cyclohexene derivatives

on that way where there is equatorial hydrogen (*cis* relationship) required for making up a six-membered transition state for elimination [25]. A plausible mechanism for this may be offered as shown in **Scheme-III**.

As planned for the synthesis of dithiazolidin-4-ones, the reaction of 1,5-diketone **2a** was carried out with thioglycollic acid in presence of ammonium carbonate (molar ratio 1:5:10) in anhydrous benzene, refluxing the reaction mixture for 30 h. The products on crystallization from acetone-benzene yielded an undesired compound, pyridine derivatives **7a** (87.0 %) and **7b** (89 %) instead of the target compound, dithiazolidin-4-ones (**6a** and **6b**) (**Scheme-IV**).



Scheme-III: A plausible mechanism for the formation of β,γ-unsaturated cyclohexene derivatives



Scheme-IV: Formation of pyridine derivatives

Since, the cyclization of 1,5-diketone with ammonia to form pyridine derivative is a well-known reaction [26,27], it is understood that ammonium carbonate being in excess underwent ring closure with 1,5-diketone prior to the attack with thioglycollic acid. The structures and stereochemistry of the synthesized compounds have been established by the combined study of FTIR, DCI-mass, APcI-mass, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H-COSY, HETCOR and long-range HETCOR spectra. The assignments of all the signals to individual H or C atoms (Tables 1-3) have been performed from their typical chemical shift values, coupling constants and relative integrations.

The coupling constants of compound **3a** (Table-1) indicated that all hydrogen atoms are in axial positions and mutually anti-periplanar except the diastereotopic CH_2 hydrogen atoms at C-6 position. From H and C values of compound **4a** (Table-3), it was evident that the double bond of cyclohexene ring is not in conjugation with carbonyl group on C-6 (α,β-unsaturated ketone) as expected, but dehydration went in the other direction affording β,γ-unsaturated ketone.

Anticancer activities: The results of anticancer activity of three compounds **3a**, **3b** and **7a** against 3-cell line of three types of human cancers: breast (MCF7), Lung (NCI-H460) and CNS (SF-268) in the one dose primary anticancer assay are given in Table-5. As results showed in Table-5, all these compounds have not shown any remarkable cytotoxic activity at 10 μM concentration.

Antimicrobial activities: The results for antimicrobial activities of the tested compounds detected against the test organisms *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus*

TABLE-5
RESULT OF ANTICANCER ACTIVITY OF THE COMPOUNDS AGAINST 3-CELL LINES OF HUMAN CANCERS

Test compound	Sample	Concentration	Units	Retardation of growth (%)		
				(Breast) MCF7	(Lung) NCI-H460	(CNS) SF-268
3a	1	1.000E-04	Molar	72	86	77
3b	1	1.000E-04	Molar	90	91	53
7a	1	1.000E-04	Molar	75	81	106

TABLE-6
ANTIMICROBIAL ACTIVITY OF COMPOUNDS BY AGAR WELL DIFFUSION METHOD

Test compound	Effective concentration (µg/well)	Antimicrobial activity in terms of zone of inhibition (mm)				
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
3a	250	–	–	19	13	–
3b	250	–	–	20	12	–
4a	250	–	–	12	10	12
4b	250	–	–	20	15	–
Chloramphenicol	100 µg/well	25	20	24	30	–
Fluconozole	100 µg/well	–	–	–	–	25

aureus, *Pseudomonas aeruginosa* and *Candida albicans* are presented in Table-6. Solvent control (DMSO) showed a non-significant inhibition to test microorganisms. Antimicrobial activity against Gram -ve bacteria was deduced in all compounds. However, such activity was not detected in all compounds against Gram +ve bacteria.

The compounds exhibited antimicrobial activity at the concentration of 250 µg/100 µL. One compound **4a** also demonstrated antifungal (anticandidal) activity. Collectively, it has been found that all compounds (**3a**, **3b**, **4a** and **4b**) showed significant antimicrobial activity against Gram -ve bacterial strains. These compounds may be directly explored in the preparation of topical anti-infective agents.

Conclusion

The aim of this work was to synthesize dithiazolidin-4-one derivatives from chalcones *via* the formation of 1,5-diketones. During the synthesiz of 1,5-diketones (**2a** and **2b**) from chalcones (**1a** and **1b**) by the Michael addition of *p*-chloroacetophenone in the presence of NaOH (molar ratio, 1:1:10), a novel cyclohexanol derivative was obtained as a side product. Keeping in view of the novelty of the side product, the same reaction was repeated with different molar ratios of chalcone-acetophenone-sodium hydroxide to set the optimum condition to obtain maximum yield of cyclohexanol and thus the ratio was fixed as 2:1:10. The dehydration of cyclohexanols **3a** and **3b** with catalytic amount of *p*-TsOH resulted in an unusual stereoselective *syn*-elimination producing quantitative yields of β,γ-unsaturated cyclohexenes **4a** (94.4%) and **4b** (93.4%) instead of apparently more stable α,β-unsaturated cyclohexenes. Compounds **3a**, **3b** and **7a** have not shown any significant anticancer activity against three types of human cancers in the one dose primary anticancer assay. The results of antimicrobial studies showed that all compounds demonstrated significant antimicrobial activity against Gram -ve bacterial strains at the concentration of 250 µg/100 µL.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support for this work from the Deanship of Scientific Research (DSR), University of Tabuk, Tabuk, Saudi Arabia under Grant No. (S-0116-1437). The authors are also thankful to National Cancer Institute (NCI), Bethesda, Maryland, USA for *in vitro* anticancer screening and Prof. Iqbal Ahmad, Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India for performing antimicrobial studies of synthesized compound.

REFERENCES

1. J. Barluenga, L. Alvarez-Rodrigo, F. Rodriguez, F. Fananas, T. Sordo and P. Campomanes, *Angew. Chem. Int. Ed.*, **46**, 2607 (2007); https://doi.org/10.1002/anie.200605172.
2. G. Lu and C. Cai, *J. Chem. Res.*, **35**, 147 (2011); https://doi.org/10.3184/174751911X12977065405044.
3. I. Mamedov, R. Abbasoglu, M. Bayramov and A. Maharramov, *Magn. Reson. Chem.*, **54**, 315 (2016); https://doi.org/10.1002/mrc.4377.
4. Y. Obata, C. Jie Li, M. Fujikawa, K. Takayama, H. Sato, K. Higashiyama, K. Isowa and T. Nagai, *Int. J. Pharm.*, **212**, 223 (2001); https://doi.org/10.1016/S0378-5173(00)00608-6.
5. S. Pandey, S. Singh, B. Patro and A. Ghosh, *Indian J. Chem.*, **43B**, 2705 (2004).
6. L. Rong, X. Wei, Y. Lu and Z. Zong, *Youji Huaxue*, **32**, 1999 (2012); https://doi.org/10.6023/cjoc201204004.
7. G. Rogers, S. Parsons, D. Anderson, L. Nilsson, B. Bahr, W. Kornreich, R. Kaufman, R. Jacobs and B. Kirtman, *J. Med. Chem.*, **32**, 1217 (1989); https://doi.org/10.1021/jm00126a013.
8. K. Anan, M. Masui, S. Hara, M. Ohara, M. Kume, S. Yamamoto, S. Shinohara, H. Tsuji, S. Shimada, S. Yagi, N. Hasebe and H. Kai, *Bioorg. Med. Chem. Lett.*, **27**, 4194 (2017); https://doi.org/10.1016/j.bmcl.2017.06.076.
9. A. Dhir, S. Malik, S.V. Kessar, K.N. Singh and S.K. Kulkarni, *Eur. Neuropsychopharmacol.*, **21**, 705 (2011); https://doi.org/10.1016/j.euroneuro.2010.12.003.
10. D.C. Kosegarten, J.J. Defeo and D.R. DeFanti, *J. Pharm. Sci.*, **56**, 1104 (1967); https://doi.org/10.1002/jps.2600560911.
11. V. Mahyavanshi, S.I. Marjadi and R. Yadav, *Arab. J. Chem.*, **10**, S804 (2017); https://doi.org/10.1016/j.arabjc.2012.12.009.
12. H. Parveen, F. Hayat, S. Mukhtar, A. Salahuddin, A. Khan, F. Islam and A. Azam, *Eur. J. Med. Chem.*, **46**, 4669 (2011); https://doi.org/10.1016/j.ejmech.2011.05.055.
13. H. Parveen, S. Mukhtar and A. Azam, *J. Heterocycl. Chem.*, **53**, 473 (2016); https://doi.org/10.1002/jhet.2427.
14. H. Parveen, R.A.S. Alatawi, N.H. El Sayed, S. Hasan, S. Mukhtar and A.U. Khan, *Arab. J. Chem.*, **10**, 1098 (2017); https://doi.org/10.1016/j.arabjc.2015.05.002.
15. S. Mukhtar, M.I. Al-Ahmdi and H. Parveen, *Asian J. Chem.*, **28**, 2589 (2016); https://doi.org/10.14233/ajchem.2016.19908.
16. K. Salat, A. Moniczewski and T. Librowski, *Mini Rev. Med. Chem.*, **13**, 335 (2013); https://doi.org/10.2174/1389557511313030003.
17. T. Torroba, *J. Prakt. Chem*., **341**, 99 (1999); https://doi.org/10.1002/(SICI)1521-3897(199902)341:2<99::AID-PRAC99>3.0.CO;2-Z.
18. V.P.M. Rahman, S. Mukhtar, W.H. Ansari and G. Lemiere, *Eur. J. Med. Chem.*, **40**, 173 (2005); https://doi.org/10.1016/j.ejmech.2004.10.003.
19. S. Mukhtar, M. Rahman, W. Ansari, G. Lemière, A. De Groot and R. Dommisse, *Molecules*, **4**, 232 (1999); https://doi.org/10.3390/40700232.
20. G.D. Gray and E. Wickstrom, *Biotechniques*, **21**, 780 (1996).

21. C. Perez, M. Pauli and P. Bazerque, *Acta Biol. Exp.*, **15**, 113 (1990).
22. A.Z. Beg and I. Ahmad, *World J. Microbiol. Biotechnol.*, **16**, 841 (2000); https://doi.org/10.1023/A:1008991724288.
23. A. Lubineau and J. Auge, *Tetrahedron Lett.*, **33**, 8073 (1992); https://doi.org/10.1016/S0040-4039(00)74720-4.
24. R. Antonioletti, F. Bonadies, E.S. Monteagudo and A. Scettri, *Tetrahedron Lett.*, **32**, 5373 (1991); https://doi.org/10.1016/S0040-4039(00)92389-X.
25. J. March, Advanced Organic Chemistry, John Wiley & Sons, edn 4, p. 1009 (1992).
26. M. Weiss, *J. Am. Chem. Soc.*, **74**, 200 (1952); https://doi.org/10.1021/ja01121a051.
27. F. Krohnke, *Synthesis*, 1 (1976); https://doi.org/10.1055/s-1976-23941.