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STEREOSTRUCTURE, ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF CYCLO-HEXENE, CYCLOHEXANOL AND PYRIDINE DERIVATIVES SYNTHESIZED FROM CHALCONES

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ABSTRACT: The main objective of this work is to study the stereostructures and biological activity of some highly substituted cyclohexenes, cyclohexanols and the intermediates there in. In order to get the desired cyclohexenes, the synthesis of cyclohexanols 2,4-bis(4-chlorobenzoyl)-1-(4-chlorophenyl)-3,5-bis(4-methoxyphenyl)-and 2,4-bis(4-chlorobenzoyl)-1,3,5-tris(4-chlorophenyl)-cyclohexan-1-ols (1b and 2b) from 4'- chloro-4-methoxy- and 4,4'-dichlorochalcones(1 and 2) via 1,5-bis(4-chlorophenyl)-3-(4-methoxyphenyl)-and 1,3,5-tris(4-chlorophenyl)-pentan-1,5-diones(1a and 2a) using p-chloroacetophenone in the presence of sodium hydroxide(molar ratio, 2:1:10) is first described. These are then dehydrated with p-TsOH which afford typical cyclohexenes, 4,6-bis(4-chlorobenzoyl) -1- (4-chlorophenyl) – 3,5 –bis (4-methoxyphenyl) and 4,6 –bis(4-chlorobenzoyl)-1,3,5 –tris (4-chlorophenyl) – cyclohex-1-enes (1c) and (2c) in quantitative yields containing β,γ-unconjugated double bond following stereoselective syn elimination. Also, in an attempt to synthesize dithiazolidin-4-one (3), the reaction of 1, 5-dione (1a) with mercapto acetic acid and ammonium carbonate is adopted. However, it fails to produce (3) and only a pyridine derivative, 2, 6-bis (4-chlorophenyl)-4-(4-methoxyphenyl) pyridine (4) is obtained.in 87% yield. Structural assignment, stereochemistry and biological assays are discussed. The characterization is done by modern spectroscopic techniques.

INTRODUCTION: Chalcones (1, 3-diaryl propenones) which are one of the plant secondary metabolite belonging to flavonoids family, have been widely explored[1] for various pharmacological activity, cosmetics, antioxidants and are easy to prepare simply by treating aryl methyl ketones with aryl aldehydes in the presence of a base.

Some cyclohexanol [2], cyclohexene [3] and pyridine [4] derivatives are present in essential oils [2], natural products [2-4] with many different kinds of applications, especially in the field of medicines[2-4] and cosmetics [2].

Several cyclohexenes isolated from plants have been reported to exhibit biological activity including antimicrobial, antitumor and antiproliferative activity against human cancer cells [3]. Cave and Raston [5, 6] have reported the synthesis of terpyridine by carrying out a reaction between 2-acetylpyridine and para-substituted benzaldehyde in the presence of sodium hydroxide in the solid

state and treating aftermath with ammonium acetate- acetic acid. However, when they repeated the same reaction in solution of sodium hydroxide, a different product, cyclohexanol was obtained. The importance of above findings and this reaction prompted us to undertake this problem. To obtain the target cyclohexenes, the corresponding cyclohexanols were first synthesized from chalcones and then dehydrated.

The cyclohexanols, 2,4-bis(4-chlorobenzoyl)-1-(4-chlorophenyl)-3,5-bis(4-methoxyphenyl)- and 2,4-bis(4-chlorobenzoyl)-1,3,5-tris(4-chlorophenyl) cyclohexan-1-ols (1b) and(2b) were synthesized from 4'-chloro-4-methoxy- and 4',4- dichloro-chalcones (1) and(2) via 1,5-bis(4-chlorophenyl)-3-(4-methoxyphenyl)-　　and　　1,3,5-tris(4-chlorophenyl) pentan-1,5-diones(1a) and(2a) by reaction with p-chloroacetophenone in the presence of sodium hydroxide (molar ratio, 2:1:10) involving double Michael addition followed by 1,6-intramolecular aldol condensation (**Scheme 1**).

The 1, 5-dione (1a) was also separately reacted with chalcone (1) in the presence of sodium hydroxide (molar ratio, 1:1.25:10) which yielded cyclohexanol(1b) as the sole product.　The cyclohexanols (1b) and(2b) were then dehydrated using a catalytic amount of p-TsOH in dry benzene which resulted in the formation of cyclohexenes, 4,6-bis(4-chlorobenzoyl) -1-(4-chlorophenyl) -3,5-(4-methoxyphenyl) and 4,6-bis(4-chlorobenzoyl) -1,3,5-trs(4-chlorophenyl) cyclohex-1-enes (1c) and(2c) (quantitative yields) with β,γ-unconjugated carbon-carbon double bond instead of α,β-conjugated carbon-carbon double bond containing cyclohexenes(1c') and (2c').

The reaction of dione (1a) with mercaptoaceticacid in the presence of ammonium carbonate(molar ratio, 1:3:10) yielded only a pyridine derivative, 2,6-bis(4-chlorophenyl)-4-(4-methoxyphenyl) pyridine[7,8] (3) in 87% yield.The synthesized compounds, cyclohexanols (1b), (2b) and cyclohexenes (1c), (2c) were evaluated for antimicrobial potentiality against two" Gram negative" bacteria (*Escherichia coli and Pseudomonas aeruginosa),* two " Gram positive" bacteria (Staphylococcus aureus and Bacillus subtilis) and one fungus (*Candida albicans).*

The cyclohexanol(1b) and the pyridine derivative (3) were also tested for cytotoxic activity against 3-cell lines of three types of human cancers: CNS, Breast and Lung.

MATERIALS AND METHODS:

Chemistry: Reagents and solvents were of commercial grade and were used without further purification. Thin layer chromatography and column chromatography were performed on 0.25 mm thick layer plates with silica gelG(Merck) and on silica gel(60-120 mesh LR,194013) respectively. Spots were visualized on exposure to iodine vapours. Melting points were determined on a Koffler hot-plate apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 621 spectrophotometer using the KBr disc technique.

^{1}H-and[13]C-NMR (δ-ppm) spectra were recorded on a Varian Unity 400 spectrometer and a Varian Unity 400spectrometer at 100 MHz respectively in acetone-d6 with TMS as the internal standard. The splitting patterns of[1]H-NMR were designated as follows:s: singlet; d: doublet; t: triplet; p: pentet; m: multiplet; dd: double doublet; ddd: triple doublet; dt: double triplet; dq: double quartet; br: broad; J: coupling constant in Hz. DCI-and APcI-mass spectra were recorded in a Ribermag R10-10B mass quadrupole mass spectrometer using ammonia as reagent gas (DCI-MS).

4'-Chloro-4-methoxychalcone (1) and 4, 4'-dichlorochalcone (2): were prepared with some modifications of the method given in the reference [11].

1,5-Bis(4-chlorophenyl)-3-(4-methoxyphenyl) pentan-1,5dione (1a) and 2,4-bis(4-chloro benzoyl)-1-(4-chlorophenyl)-3,5- bis(4-methoxy phenyl)cyclohexan-1-ol(1b)[Michael addition of p-chloroacetophenone to chalcone(1)]: A mixture of chalcone(1)(1.0g, 3.66mmol) and p-chloroacetophenone (293mg, 1.83mmol) in an aqueous-ethanolic solution (1:1) of sodium hydroxide (734mg,18.3 mmol) was heated on a water bath for 5h monitoring the progress of the reaction(TLC). After completion, the reaction mixture was extracted with ethyl acetate, washed with water and organic phase dried over Na_2SO_4.

The solvent was distilled off under diminished pressure to a semi-solid residue.It was then chromatographed over a silica gel column using pet. ether (40-60ºC)-diethyl ether (6:4 v/v) as an eluent. Elution of the column first yielded a white solid compound which on recrystallization from C_6H_6-EtOAc afforded (1a) as white crystalline needles, 66.5 mg (8.5%), m.p.= 80ºC, Rf= 0.67 (benzene : diethyl ether, 9.5:0.5 v/v).

IR(KBr) ν_{max}: 1675 (C=O), 1610,1580 (phenyl), 1510,1400, 1350, 1250, 1170, 1075,980, 810 cm^{-1}; ^{1}H-NMR(400MHz,acetone-d6) δ 3.41(2H, dd, J=16.94, 7.48 Hz, H-2up/4up), 3.51(2H, dd, J=16.94, 6.56 Hz, H-2dn/4dn), 3.98(1H, brp, J≈7.0 Hz, H-3), 3.70(3H,s, OCH$_3$; NOE: H-Ar'-3,5 ; H-3; H-2up/4up > H- 2dn/4dn),7.25(2H,d, J=8.85Hz, H-Ar'-2,6), 6.78(2H,d,J=8.85Hz, H-Ar'-3,5), 7.98 (4H,d, J=8.85Hz, 2× H-Ar-2,6), 7.49(4H,d, J=8.85Hz, 2×H-Ar-3,5); ^{13}C-NMR (100MHz, acetone-d6) δ 45.70(C-2/4, HETCOR correlation with H-2up/4up,H-2dn/4dn) 37.25(C-3, HETCOR correlation with H-3), 55.42(C-OCH$_3$, HETCOR correlation with H-OCH$_3$), 136.96^a(C-Ar'-1), 159.27(C-Ar'-4) 129.51(C-Ar'-2,6 HETCOR correlation with H-Ar'-2,6) 114.57(C-Ar'-3,5 HETCOR correlation with H-Ar'-3,5) 136.86^a(C-Ar-1) 139.45(C-Ar-4) 130.62(C-Ar-2,6 HETCOR correlation with H-Ar-2,6) 129.57(C-Ar-3,5 HETCOR correlation with H-Ar-3,5) 198.06(C=O) ; DCI-MS (NH$_3$) m/z (%): 427/429/431 [(M+1)$^+$ (33.5/26.2/6.7)], 273/275 [M-C$_8$H$_6$OCl]$^+$ (100.0/41.2), 139/141(68.3/25.3), 111/113 (25.67/7.5).

a. Assignments with the same superscript may be interchanged.

Further elution of the column yielded a dull white solid compound which on recrystallization from C_6H_6-EtOAc afforded (1b) as white crystalline globules, 815mg (64%), m.p. =232ºC, Rf=0.42(benzene-diethyl ether, 9.5:0.5 v/v).

IR(KBr) vmax:3480(OH),1665,1640(C=O),1610,1585(phenyl),1510,1400, 1250,1175,1085,1025,1005,820 cm^{-1};The ^{1}HNMR and ^{13}C-NMR values are given in **Table1**; APcI- MS, m/z%:699/701/703/705 [(M+1)$^+$,C$_{40}$H$_{33}$Cl$_3$O$_5$],681/683/685/687 [(M+ 1-

H$_2$O)$^+$, base peak],427/429 (11.11/7.02), 411/413 (26.90/11.70).

2,4-Bis(4-chlorobenzoyl)-l-(4-chlorophenyl)-3,5-bis(4-methoxyphenyl)cyclohexan-1-ol (1b)[Michael addition of 1,5-dione(1a) to chalcone(1)]:

A mixture of chalcone(1)(400mg,1.46mmol) and 1,5-dione (1a) (500mg, 1.17mmol) in an aqueous-ethanolic solution(1:1) of sodium hydroxide (470mg, 11.7mmol) was heated on a water bath for 5h.The reaction mixture was worked up as usual to a dull white solid product which on TLC examination showed the presence of only one compound.This on crystallization from C_6H_6-EtOAc afforded (1b) as white crystalline globules, 570mg(69.6%), m.p.=232ºC, Rf= 0.42(benzene-diethyl ether,9.5:0.5 v/v).

1,3,5-Tris(4-chlorophenyl)pentan-1,5-dione(2a) and 2,4-bis(4-chlorobenzoyl)-1,3,5-tris(4-chlorophenyl)cyclohexan-1-ol(2b)[Michael addition of p-chloroacetophenone to chalcone(2)]: A mixture of of chalcone(2) (1.0g, 3.62mmol) and p-chloroaceto phenone (278mg, 1.81mmol) in an aqueous-ethanolic(1:1) solution of sodium hydroxide (7.0ml, 725mg, 18.1mmol) was heated on a water bath for 5h as mentioned above and worked up as usual. The residue on purification by column chromatography (silica gel, pet. Ether (40-60)-diethyl ether,6:4v/v as eluent) followed by crystallization first yielded(2a) as white crystalline needles, 56.1mg(7.2%), m.p.=110ºC, R$_f$=0.85 (benzene-diethyl ether,9.5:0.5 v/v).

IR(KBr) ν_{max}: 1680 (C=O),1610,1585 (phenyl), 1520, 1400, 1350 1260, 1165, 1060, 980,810 cm^{-1} ; ^{1}H-NMR (400MHz, acetone-d6)δ 4.90 (2H, dd, J=17.24,7.63Hz, H-2$_{up}$/4$_{up}$), 3.59 (2H,dd, J=17.24, 6.41Hz ,H-2$_{dn}$/4$_{dn}$), 4.05 (1H,br p, J≈7Hz, H-3), 7.26 (2H,d, J=8.54Hz, H-Ar'-2,6,),7.40 (2H, d,J=8.54Hz, H- Ar'-3,5),8.00 (4H,d,J=8.70Hz, 2×H-Ar-2,6),7.52 (4H,d, J=8.70Hz, 2×H-Ar-3,5); ^{13}C-NMR(100 MHz, acetone-d6) δ 45.29 (C-2/4),37.23 (C-3), 132.44(C-Ar'-1), 144.20 (C-Ar'-4),130.48 (C-Ar'-2,6) ,129.11 (C-Ar'- 3,5), 136.74 (C-Ar/Ar-1),139.60 (C-Ar/Ar-4), 130.64 (C-Ar/Ar-2,6), 129.64 (C-Ar/Ar- 3,5),197.77 (C=O -1/5).

And then (2b) as white crystalline globules 870mg (69%), m.p.=242ºC,R_f =0.67 (benzene- diethyl ether,9.5:0.5 v/v).

IR(KBr)ν_{max}:3475(OH),1665,1645(C=O),1610,1580(Phenyl),1510,1395,1260,1175,1085,1015,   820 cm$^{-1;}$ ^{1}HNMR(400 MHz, acetone-d6) δ 2.00 (1H,dd,J=3.66,13.74Hz,H-6$_{eq}$), 2.86 (1H,br dt, ,J=2.29,11.12,13.74Hz ,H-6$_{ax}$) ;3.92 (1H,br dt,J≈11.10,11.12,3.66Hz, H-5$_{ax}$),4,12 (1H,br t,J≈11.45Hz, H- 3ax),4.82(1H,br t ,J≈11.14Hz, H-4$_{ax}$),   5.16(1H,d,J=11.60Hz,H-2$_{ax}$),5.12 (1H,d,J=2.29Hz, OH-1),6.91-7.73 (20H,br m ,5×H-Ar-rings); ^{13}C-NMR(100 MHz,acetone-d6) δ 76.11 (C-1), 56.08^a (C-2), 48.47 (C-3),55.50^a (C-4),44.22 (C-5),46.51 (C-6), 202.34 (C=O),205.33 (C=O) 127.84-146.28 (5×C-Ar-rings).

   a. Assignments with the same superscript may be interchanged.

4, 6-Bis (4-chlorobenzoyl)-1, 3, 5-tris (4-chlorophenyl) cyclohex-1-ene (2c) [Dehydration of cyclohexanol(2b) using p-TsOH as a catalyst]: To a solution of the cyclohexan-1-ol (2b) (100 mg, 0.142 mmol) in anhydrous benzene (7.0 ml) was added *p*-toluene sulphonic acid (2.0 mg) and refluxed with stiring for 2.5h.The reaction mixture was then cooled, treated with NaHCO$_3$ (5%), washed with water and the organic phase dried over Na$_2$SO$_4$. The solvent was evaporated under diminished pressure to a white solid product which showed the presence of a single component on TLC (silica gel'G', benzene- diethyl ether, 9.5:0.5v/v) .It was then recrystallised from benzene –ethyl acetate to give (2c) as white crystalline globules, 91.0 mg (93.4%), m.p.= 218º C,$R_{f\ =}$ 0.60 (benzene-diethyl ether,9.5:0.5 v/v)

 IR(KBr)$\nu_{max:}$2858   (C-H),   1668   (>C=O), 1615,1589,1569 (C=C/Phenyl), 1488, 1402, 1360, 1317,1289,1215,1175,1094,1014,981,965,910,870, 842,805,761,738,720,677   cm^{-1}; ^{1}H-NMR(400MHz,acetone-d6)   and   13C-NMR(100MHz, acetone-d6) values are given in **Table 2**; APcI-MS;m/z (%) :688/690/692/694/696/ 698 [M$^+$, base peak].

4,6-Bis(4-chlorobenzoyl)-1-(4-chlorophenyl)-3,5-bis(4-methoxyphenyl)cyclohex-1-ene(1c)

[Dehydration of cyclohexanol (1b) using p-TsOH as a catalyst]: The cyclohexanol (1b) (100.0 mg, 0.141 mmol) was dehydrated using p-TsOH (2.0mg) in anhydrous benzene (7.0ml) as described for compound (2b) and the reaction mixture was worked up as usual. The white solid product obtained was recrystallized from benzene-ethyl acetate to afford (1c) as white crystalline globules, 92mg (94.4%), m.p.=254ºC, Rf=0.90 (benzene-diethyl ether,9.5:0.5v/v).

IR(KBr) ν_{max}:   2835(C-H),   1668(C=O), 1613,1587,1569, (C=C/Phenyl), 1512, 1488, 1462, 1440, 1399, 1305, 287, 1254, 1214, 1179, 1092, 1031, 1011, 971, 862, 830, 751, 729,697cm $^{-1}$; ^{1}H-NMR (400MHz, acetone-d6) δ 6.15 (1H, t, J$_{2,6pseud.ax}$, J$_{2,3pseud.ax}$=2.14Hz,H-2), 4.16 (1H,brdq, J$_{3pseud.ax,4ax}$≈11.1; J$_{2,3pseud.ax}$=2.14; J$_{3pseud.ax,6pseud.ax}$ ≈3.97Hz, H-3$_{pseud.ax}$),5.49 (1H, brdq, J$_{5ax,6pseud.ax}$ ≈11.1; J$_{3pseud.ax,6pseud.ax}$≈3.97; J$_{2,6pseud.ax}$= 2.14Hz,H-6$_{pseud.ax}$),   4.57(1H,brt,J$_{4ax,5ax}$≈J$_{3pseud.ax}$≈11.1Hz,H-4$_{ax}$), 3.74(1H,brt, J$_{5ax,6pseud.ax}$≈J$_{4ax,5ax}$≈11.1Hz,H-5$_{ax}$),3.54 (3H,s,H-3-Ar'-p-OCH$_3$),3.66 (3H,s,H-5-Ar'-p-OCH$_3$),6.47-7.74   (20H,   brm,five   p-substituted Ar- rings).

2, 6-Bis (4-chlorophenyl)-4-(4-methoxyphenyl) pyridine (3): A mixture of 1,5-diketone (1a) (1.5 g, 3.5m mol), mercaptoacetic acid (0.966 g, 10.5m mol) and ammonium carbonate (3.36 g, 35.0 m mol) in anhydrous benzene (30 ml) was refluxed for 25h and the reaction mixture was workedup as usual.The white solid product thus obtained was recrystallized from acetone-benzene to furnish (3) as white crystalline needles, 1.026 g (87%), m.p.=208º C ,$R_{f\ =}$ 0.63 (pet.ether-diethyl ether, 7:3 v/v).

IR(KBr)ν_{max}:1610,1590,1570 (C=N/Phenyl), 1540, 1505, 1475, 1420, 1375, 1285, 1245, 1165, 1075, 1000,820 cm^{-1}; ^{1}H-NMR(400MHz, acetone-d6) δ 3.88(3H,s, Py-4-Ar'-p-OCH$_3$), 8.15(2H,s, Py-3,5), 7.94(2H, d, J+COSY=8.85Hz,   Py-4-Ar'-2,6) ,7.10(2H, d, J+CO SY=8.85Hz ,Py-4-Ar'-3,5) ,8.35(4H, d, J+COSY=8.69Hz, Py-2/6-Ar/Ar-2,6), 7.55(4H, d, J+COSY=8.69 Hz , Py-2/6-Ar/Ar-3,5); ^{13}C-NMR(100 MHz, acetone-d6) δ 55.82(Py-4-C-Ar'-p-OCH$_3$), 156.86(Py-C-2,6, HETCOR & Long-range HETCOR correlation with Ar-H-2,6) , 117.24 (Py-C- 3,5, HETCOR & Long-range

HETCOR correlation with Py-H-3,5 and Ar-H-3,5), 151.05(Py-C-4 , Long-range HETCOR correlation with Ar'-H-2,6; Ar'-H-3,5), 131.31(Py-4-Ar'-C-1), 161.96(Py-4-Ar'-C-4, Long-range HETCOR correlation with Ar'-H-OCH$_3$), 129.48(Py-4-Ar'-C-2,6 HETCOR correlation with Ar'-H-2,6) , 115.45(Py-4-Ar'-C-3,5 HETCOR correlation with Ar'-H-3,5) , 129.65(Py-2/6-Ar/Ar-C-2,6 HETCOR correlation with Ar-H-2,6), 129.59(Py-2/6-Ar/Ar-C-3,5 HETCOR correlation with Ar-H-3,5, Long-range HETCOR correlation with Ar-H-2,6) , 139.13(Py-2/6-Ar/Ar-C-1, Long- range HETCOR correlation with Ar-H-3,5), 135.60(Py-2/6-Ar/Ar-C-4, Longe –range HETCOR correlation with Ar-H-2,6 ; Ar-H-3,5); DCI-MS, m/z (%):406/408 /410 (100.0/80.52/20.0,M+H)$^+$.

In vitro antimicrobial activity: The synthesized compounds, cyclohexanols(1b), (2b) and cyclohexenes(1c),(2c) were screened *in vitro* for antibacterial activity by agar well diffusion method against four organisms. The antimicrobial activity of these test compounds , two cyclohexanols (1b),(2b) and two cyclohexenes (1c),(2c) was assayed on nutrient agar medium (Hi-Media Lab. Pvt. Mumbai, India). The antifungal activity was tested using Sabouraud dextrose agar medium (Hi-Media,Lab. India) by agar well diffusion method[12-14].

Briefly 0 .1 ml of the diluted inoculum (10^{6}CFUml^{-1}) of test organism was spread on NA/SDA (Nutrient Agar/Sabouraud dextrose Agar) plates. Wells of 8 mm diameter were punctured into the agar medium and filled separately with 100 µl of compound (250 µg ml^{-1} solvent blank and an antibiotic, chloramphenicol, 100 µg ml^{-1}) to which the test bacteria were sensitive. Fluconazole at the concentration of 100 µg ml^{-1} 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.

Cytotoxic activity: The compounds, cyclohexanol (1b) and 2, 4, 6-triaryl pyridine (3) were evaluated in a three cell lines panel of three types of human cancers: breast (MCF7), Lung (NCI-H460) and CNS (SF-268) in one dose primary anticancer assay at a single concentration, 1×10^{-4} M.

The results **Table 4** showed that these are inactive against cancers at this single concentration.

RESULTS AND DISCUSSION:

**Chemistry**: Chalcones (1) and (2) were prepared by condensing p-chloacetophenone

  (i)   With benzaldehydes

  (ii)   In the presence of sodium hydroxide [11].

The reactions of chalcones (1) and (2) with p-chloroacetophenone in the presence of aqueous-ethanolic sodium hydroxide (molar ratio, 2:1: 10) were then carried out refluxing the reaction mixture in anhydrous benzene for 5 hours. The products on chromatography over a silica gel column using pet. ether-diethyl ether (6:4) as eluent followed by crystallization yielded cyclohexanols, (1b) (64%) and (2b) (69%) alongwith 1,5-diones (1a) and (2a) respectively.

The dione (1a) was also separately reacted with chalcone (1) (molar ratio, 1.25:1:10) for 5h which yielded (1b) as a single product. The cyclohexanols (1b) and (2b) were then dehydrated by refluxing with a catalytic amount of *p*-TsOH in anhydrous benzene for 2.5h which furnished corresponding cyclohexenes (1c) and (2c) with β,γ –unconjugated C=C double bond in excellent yields (94.4% and 93.4%) %) **Scheme1**.

The reaction of dione (1a) with mercaptoacetic acid and ammonium carbonate (molar ratio, 1:3:10) in anhydrous benzene refluxing the reaction mixture for 25h, yielded a white solid product. This on crystallization from acetone-benzene afforded only a pyridine derivative 2, 6-bis (4-chlorophenyl)-4-(4-methoxyphenyl) pyridine (3) as white crystalline solid in 87% yield.

Scheme 1:
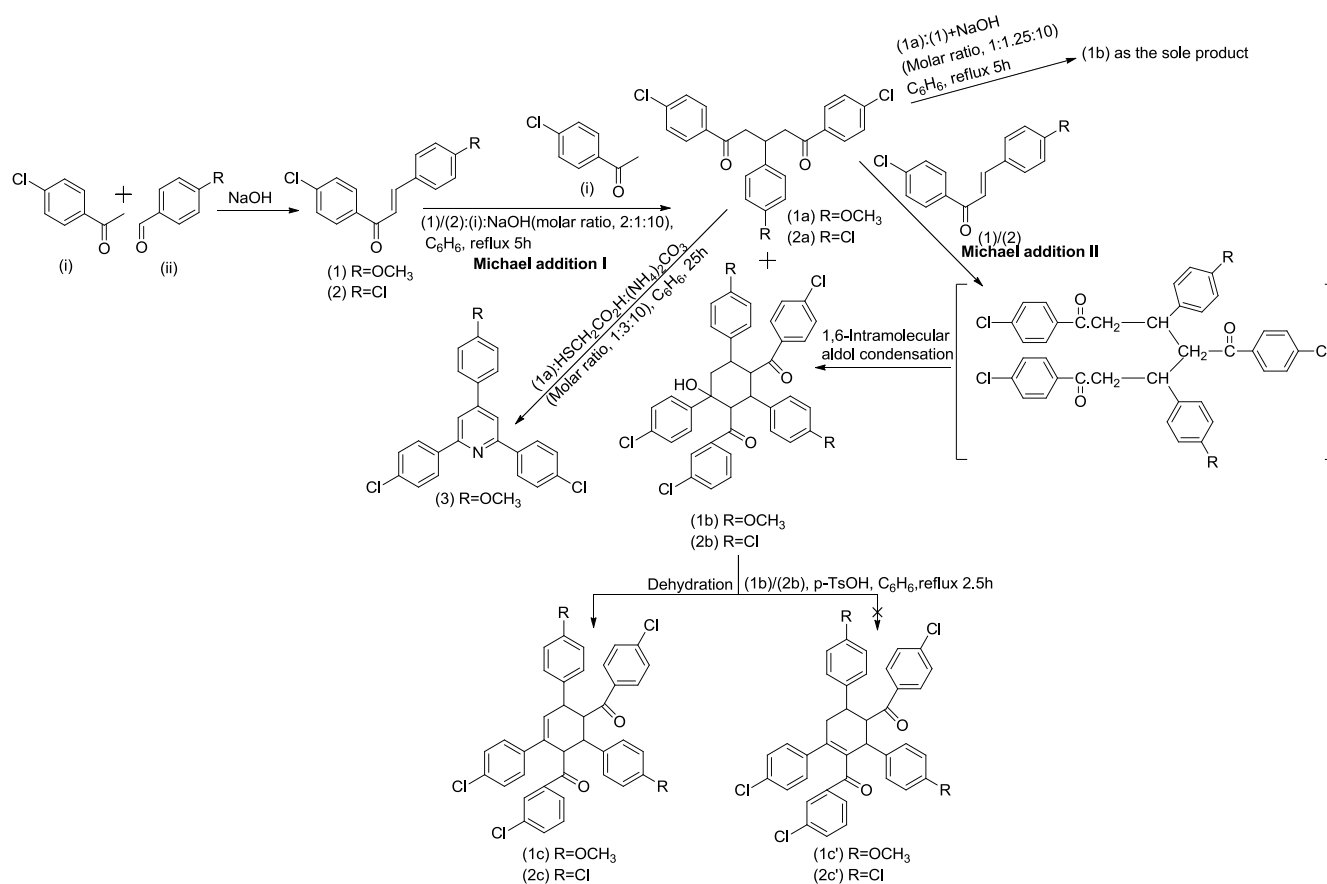
SCHEME 1

The stereostructures of (1a) and (2a) have been deduced as shown in **Fig. 1** by IR, DCI-MS, ^{1}H-NMR, NOE, ^{13}C-NMR, and HETCOR spectra (spectral data are shown in materials and methods). The assignments of all the signals to individual H- and C- atoms have been made on the basis of typical chemical shift values, coupling constants, relative integrations, NOE and HETCOR spectral data. The coupling constants of C-2up/4up (J=16.94, 7.48 Hz), C-2dn/4dn (J=16.94, 6.56 Hz) and C-3(J=7.0Hz) protons revealed that the two diastereotopic hydrogens at C-2/C-4 are anti- and syn-periplanar with C-3 hydrogen.

These assignments and couplings were further confirmed by HETCOR spectra which showed cross correlations of the signals of H-2up/4up& H-2dn/4dn; H-3 ; H-OCH$_3$; Ar'-H-2,6; Ar'-H-3,5; Ar-H-2,6 and Ar-H-3,5 with that of C-2/4, C-3, C-OCH$_3$, Ar'-C-2,6,Ar'-C-3,5,Ar-C-2,6 and Ar-C-3,5 respectively.

The relationships between the protons of H-2up/4up, H-2dn/4dn, H-3, H-OCH$_3$, Ar'-H-2, 6 and Ar'-H-3, 5 were further established by an additional NOE experiment. Irradiation of OCH$_3$ protons results in a NOE-enhancement of H-3 proton and also activates H-2up/4up & H-2dn/4dn protons but H-2up/4up proton more than H-2dn/4dn proton.

This means that these protons are near to each other and in a synclinal position of H-3 relative to H-2up/4up, H-2dn/4dn. Irradiation of Ar'-H-2,6 showed NOE-enhancement of Ar'-H-3,5 and also H-3 indicating that Ar'-H-2,6 and H-3 protons are very near to each other. All the signals of (2a) were found identical with that of (1a) except the absence of methoxyl signals .Based on above spectral evidence, structures are formulated as 1,5 –bis (4-chlorophenyl)-3-(4-methoxyphenyl)pentan-1,5 -dione (1a)and 1,3,5-tris(4-chlorophenyl)pentan-1,5-dione (2a) **(Fig. 1)**.
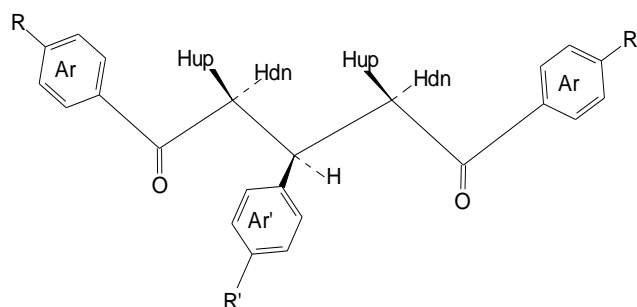
FIG-1: (1a) R=Cl, R'=OCH$_3$; (2a) R=R'=Cl

The structure and stereochemistry of (1b) have been confirmed by IR, APcI-MS,^{1}H-NMR, ^{13}C-NMR, COESY, HETCOR and Long-range HETCOR spectra and that of (2b) by a comparison with the spectra of (1b) (spectral data of (1b) are shown in **Table1** and that of (2b) in section MATERIALS AND METHODS). The IR spectra showed characteristic absorption peaks corresponding to OH, C=O and phenyl groups. The APcI-MS spectrum of (1b) showed a set of very low intensity [M +1]$^+$ peaks at m/z 699/701/703/705 confirming its mol.wt. as 698 which is consistent with the molecular formula C$_{40}$H$_{33}$Cl$_3$O$_5$.

The base peak appeared as a set of peaks at m/z 681/683/685/687 corresponding to the loss of a water molecule [M+1- H$_2$O] $^+$, indicating the presence of a hydroxyl group in it. The ^{1}H-NMR spectrum of (1b) showed a double doublet at δ1.97 with J=3.66&13.73 Hz and a broad double triplet at δ2.78 with J=2.29, 11.50 & 13.66 Hz , were assigned to 6eq and 6ax protons. A broad double triplet at δ3.86 with J=11.14, 11-12 & 3.66 Hz was assigned to5ax proton. The two broad triplets,one at δ4.02 with J=11.45 Hz and the other at δ4.71 with J=11.14 Hz were attributed to3ax and 4ax protons.

The signal for 2ax proton appeared as a doublet at δ5.08 with J=11.75 Hz.Thus,the large coupling constants of these protons indicated that the hydrogens at these positions are in axial dispositions and are mutually anti periplanar except the two diastereotopic hydrogens at C-6 which are syn and anti- periplanar with adjacent protons. A doublet at δ5.14 with J=2.29 Hz was assigned to the hydroxyl proton.

The two singlets at δ3.60 and δ3.43 accounted for three protons each were attributed to two methoxyl groups. The aromatic signals at δ6.42-7.71 were accounted for 20 protons of A$_2$B$_2$ pattern of five benzene rings. The couplings of all these protons were confirmed by COESY spectra as shown in (Table 1). In the ^{13}C-NMR spectrum, the signals at δ203.03 and δ205.95 were assigned to two benzoyl carbonyl carbons and at δ55.39 and δ55.26 to the two methoxyl carbons.

The adjacent signals at δ47.03, δ48.38, δ56.45 and δ56.36 were attributed to C-6, C-3, C-4 and C-2 carbons respectively. The signals at δ43.94 and δ76.25 were assigned to C-5 and C-1 carbons. The signals at δ114.28-159.2 were assigned to aromatic carbons **Table 1**. The assignments and couplings of protons were further confirmed by extensive NMR analysis such as COESY, HETCOR and long range HETCOR spectra. In HETCOR spectra , the signal of methylene carbon at δ47.03 showed two cross correlation peaks with methylene protons (H-6ax and H-6eq) at δ1.97 as double doublet and at δ2.78 as broad double triplet.

The signal of methine carbons C-5,C-3,C-4 and C-2 at δ 43.94, 48.38, 56.45 and 56.36 showed cross correlation peaks with H-5ax, H-3ax, H-4-ax and H-2ax at δ3.86 as broad double triplet, at δ 4.02 as broad triplet, at δ 4.71 as broad triplet and δ 5.08 as doublet respectively. In the Long-range HETCOR spectra, the methylene carbon of C-6 at δ 47.03 showed cross correlation peaks with hydroxyl proton at δ 5.14 as doublet. The methine carbon of C-3 at δ 48.38 showed correlation peaks with its adjacent two methine protons, H-2ax and H-4ax at δ 5.08 and δ 4.71 as doublet and broad triplet respectively.

The signal at δ129.83 for aromatic carbons,[Ar'-C-2,6] at C-5 showed correlation with the methine proton H-5ax at δ 3.86 as broad double triplet.The signal at δ159.42 for aromatic carbon [Ar'-C-4] at C-3 showed correlation with the methoxyl protons [Ar'-p-OCH$_3$] at δ 3.60.Similar related correlation peaks in HETCOR and Long-range HETCOR were observed for the positions assigned **Table 1**. The ^{1}H-NMR and ^{13}C-NMR spectral data (in section MATERIALS AND METHODS) of (2b) were

found identical with that of (1b) except the absence of two methoxyl signals. On the basis of above spectral evidence, the conformational structure deduced is represented in **(Fig. 2)** and formulated as 2,4-bis(4-chlorobenzoyl)-1-(4-chlorophenyl)-3,5 - bis(4-methoxyphenyl) cyclohexan-1-ol (1b) and 2,4-bis(4-chlorobenzoyl)-1,3,5-tris(4-chlorophenyl) cyclohexan-1-ol (2b).
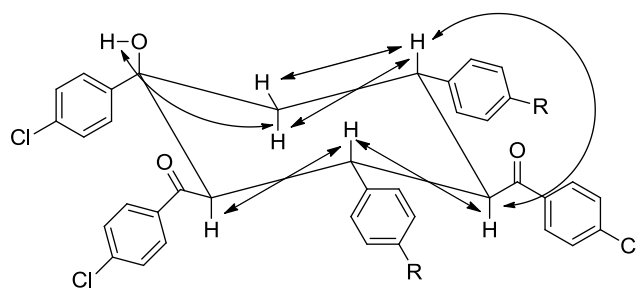

FIG. 2: (1b) R=OCH$_3$; (2b) R=Cl

TABLE 1: ^{1}H-NMR, 13C-NMR, COSY, HETCOR CORRELATION AND LONG-RANGE HETCOR CORRELATION DATA OF (1b)

^{1}H-NMR	δ(ppm)	Integration	Multiplicity	J(Hz)+COSY	^{13}C-NMR	δ (ppm)	HETCOR correlation with	Long-range HETCOR correlation with
6_{eq}	1.97	1H	dd	$J_{5ax,6eq}3.66; J_{6ax,6eq}13.73$	C-6	47.03	H-6_{ax};H-6_{eq}	1-OH
6_{ax}	2.78	1H	br dt	$J_{6ax,OH}2.29; J_{5ax,6ax}11.12; J_{6ax,6eq}13.73$	C-5	43.94	H-5_{ax}	
					C-3	48.38	H-3_{ax}	H-2_{ax},H-4_{ax}
5_{ax}	3.86	1H	br dt	$J_{4ax,5ax}≈11.14; J_{5ax,6ax}11.12; J_{5ax,6eq}3.66$	C-4	56.45^a	H-4_{ax}	
					C-2	56.36^a	H-2_{ax}	
3_{ax}	4.02	1H	br t	$J_{2ax,3ax}≈J_{3ax,4ax}≈11.45$	C-1	76.25		
4_{ax}	4.71	1H	br t	$J_{3ax,4ax}≈J_{4ax,5ax}≈11.14$	CO	203.03		
2_{ax}	5.08	1H	d	$J_{2ax,3ax}11.75$	CO	205.95		
1-OH	5.14	1H	d	$J_{OH,6ax}2.29$	3-[Ar'-C-2,6]	128.84^b	3-[Ar'-H-2,6]	
3-[Ar'-2,6]	7.19	2H	d	$J_{Ar'-2,6,Ar'-3,5}8.85$	3-[Ar'-C-3,5]	114.28	3-[Ar'-H-3,5]	
3-[Ar'-3,5]	6.42	2H	d	$J_{Ar'-2,6,Ar'-3,5}8.85$	3-[Ar'-C-4]	159.42		3-[Ar'-4-OCH$_3$]
					3-[Ar'-C-1]	132.16^d		
3-[Ar'-p-OCH$_3$]	3.60	3H	s	-------	3-[Ar'-4-C-OCH$_3$]	55.39^c	3-[Ar'-4-OCH$_3$]	
5-[Ar'-2,6]	7.26	2H	d	$J_{Ar'-2,6,Ar'-3,5}8.70$	5-[Ar'-C-2,6]	129.83	5-[Ar'-H-2,6]	
5-[Ar'-3,5]	6.66	2H	d	$J_{Ar'-2,6,Ar'-3,5}8.70$	5-[Ar'-C-3,5]	114.62	5-[Ar'-H-3,5]	
					5-[Ar'-C-4]	159.42		5-[Ar'-4-OCH$_3$], 5-[Ar'-H-2,6]
5-[Ar'-p-OCH$_3$]	3.43	3H	s	-------	5-[Ar'-C-1]	133.04^d		
					5-[Ar'-4-C-OCH$_3$]	55.26^c	5-[Ar'-4-OCH$_3$]	
4-[Ar''-2,6]	7.42	2H	d	$J_{Ar''-2,6,Ar''-3,5}8.69$	4-[Ar''-C-2,6]	130.17	4-[Ar''-H-2,6]	
4-[Ar''-3,5]	7.15	2H	d	$J_{Ar''-2,6,Ar''-3,5}8.69$	4-[Ar''-C-3,5]	128.68	4-[Ar''-H-3,5]	
2-[Ar''-2,6]	7.52	2H	d	$J_{Ar''-2,6,Ar''-3,5}8.85$	4-[Ar''-C-1]	135.53^d		
2-[Ar''-3,5]	7.19	2H	d	$J_{Ar''-2,6,Ar''-3,5}8.85$	4-[Ar''-C-4]	137.41^d		
1-[Ar-2,6]	7.71	2H	d	$J_{Ar-2,6,Ar-3,5}8.69$	2-[Ar''-C-2,6]	130.64	2-[Ar''-H-2,6]	
1-[Ar-3,5]	7.19	2H	d	$J_{Ar-2,6,Ar-3,5}8.69$	2-[Ar''-C-3,5]	128.94^d	2-[Ar''-H-3,5]	
					2-[Ar''-C-1]	138.34^d		
					2-[Ar''-C-4]	138.47^d		
					1-[Ar-C-2,6]	128.84^b	1-[Ar-H-2,6]	
					1-[Ar-C-3,5]	127.81^b	1-[Ar-H-3,5]	
					1-[Ar-C-1]	139.69^d		
					1-[Ar-C-4]	146.73^d		

$^{a, b, c, d}$ Assignments with the same superscript may be interchanged. Ar=p-chlorophenyl; Ar'=p-methoxyphenyl; Ar''=p-chlorobenzoyl

The structure and stereochemistry of (2c) have been established by a combined study of FT-IR, APcI-MS, ^{1}H-NMR, ^{13}C-NMR,COESY and HETCOR spectra **Table 2** and also by comparison with the spectra of the precursor (2b) and that of (1c) with the spectra of (2c).(spectral data of (2c) are shown in **Table 2** and that of (1c) in section MATERIALS AND METHODS).

The FT-IR spectra displayed characteristic absorption peaks corresponding to C=O, C =C and phenyl groups and no sign of OH peak. The APcI-MS of (2c) was compared with an Isotope model between m/z 680 and 720 and found to correspond with the molecular formula C$_{38}$H$_{25}$O$_2$Cl$_5$, confirming its molecular weight 688 which appeared as m/z 688 /690 /692 /694 /696 /698.

**TABLE 2: ^{1}H-NMR, ^{13}C-NMR AND HETCOR CORRELATION DATA OF (2c)**

^{1}H-NMR	δ(ppm)	Integration	Multiplicity	J(Hz)	^{13}C-NMR	δ(ppm)	HETCOR correlation with
2	6.17	1H	t	$J_{6pseud.ax,2}, J_{2,3pseud.ax}$ 2.14	2	132.49	H-2
3$_{pseud.ax}$	4.23	1H	br dq	$J_{3pseud.ax,4ax}≈11.14; J_{2,3pseud.ax}$ 2.14; $J_{3pseud.ax,6pseud.ax}≈3.66$	3	48.72	H-3$_{pseud.ax}$
6$_{pseud.ax}$	5.56	1H	br dq	$J_{5ax,6pseud.ax}≈11.14; J_{3pseu ax,6pseud.ax}≈3.66; J_{2,6pseud.ax}$2.14	6	55.15	H-6$_{pseud.ax}$
					4	54.25	H-4$_{ax}$
4$_{ax}$	4.69	1H	br t	$J_{5ax,4ax}≈J_{3pseud.ax,4ax}≈11.14$	5	50.54	H-5$_{ax}$
5ax	3.82	1H	br t	$J_{5ax,6pseud.ax}≈J_{4ax,5ax}≈11.14$	CO	200.70	
5×Ar	6.98-7.70	20H	br m	---	CO	202.43	
					5×Ar	127.84-159.42	

The disappearance of the hydroxyl signal, δ 5.12 of the respective cyclohexanol (2b) and appearance of a new low field signal at δ 6.17 in the spectrum of (2c) indicated that dehydration of cyclohexanol (2b) has occurred forming cyclohexene derivative. This low field signal at δ 6.17 as triplet with J=2.14 due to the couplings with the pseudo-ax protons at C-3 and C-6 was assigned to the olefinic proton (= CH-) at C-2.This showed that of the two adjacent protons C-6 and C-2 in cyclohexanol (2b), the C-6 proton has undergone elimination. The two broad double quartets at δ 4.23 and δ 5.56 each with J=11.14, 3.66 & 2.14 were assigned to H-3 pseud.ax and H-6 pseud.ax respectively.

The signals at δ3.82 and δ 4.69 as broad triplets were assigned to H-5ax and H-4ax. The aromatic protons signals as $A_2.B_2$ at δ6.98-7.70 accounted for twenty protons of five p-substituted phenyl rings. In ^{13}C-NMR spectrum, the downfield signals at δ 200.70 and δ 202.43 were assigned to two benzoyl carbonyl carbons. In the HETCOR spectrum, the C-2 signal at δ 132.49 correlates with H-2 signal at δ 6.17. The C-3 signal at δ 48.72 and C-5 signal at δ 50.54 correlate with H-3pseud.ax signal at δ 4.23 and H-5ax signal at δ3.82 respectively. The C-4 and C-6 signals at δ54.25 and δ55.15 correlate with H-4ax and H-6pseud.ax signals at δ4.69 and δ5.56 respectively.

The signals at δ127.84-159.42 were assigned to thirty carbons of five p-substituted phenyl rings. The structure of (1c) was confirmed by a comparison with the spectra of (2c). The signals of (1c) were found comparable with that of (2c) except the appearance of two methoxyl signals at δ3.54 and δ3.66 in two of the five p-substituted phenyl rings.

The conformational structure thus deduced is represented in (**Fig. 3**) and formulated as 4,6-bis(4-chlorobenzoyl)-1-(4-chlorophenyl)-3,5-bis(4-methoxyphenyl) cyclohex-1-ene (1c) and 4,6-bis (4-chlorobenzoyl)-1,3,5-tris(4-chlorophenyl) cyclohex-1-ene (2c).
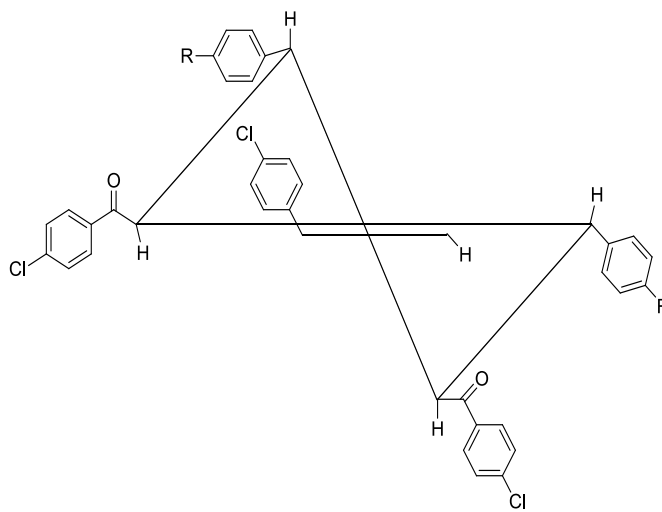
FIG. 3: (1c) R=OCH$_3$; (2c) R=Cl

The structure of (3) was confirmed on the basis of IR, DCI-MS, ^{1}H-NMR, ^{13}C-NMR, COSY, HETCOR and Long- range HETCOR spectra (Spectral data are shown in Section MATERIALS AND METHODS).

The assignments and couplings of ^{1}H- and ^{13}C-NMR signals were established by correlations of C-signals with that of the corresponding H-signals in HETCOR, Long-range HETCOR and ^{1}H-^{1}H signals in COSY spectra. The structure thus assigned is formulated as 2, 6-bis (4-chlorophenyl)-4-(4-methoxyphenyl) pyridine (**Fig. 4**).
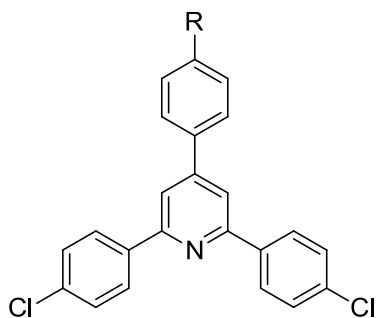
FIG. 4: R=OCH₃

Study for Biological activity: The newly synthesized compounds were evaluated for the antimicrobial (antibacterial and antifungal) activity and also for their cytotoxic activity against malignant human tumor cells.

In vitro **antimicrobial activity of cyclohexanols (1b),(2b) and cyclohexenes (1c),(2c):** The *in vitro* antimicrobial activity against four antibacterial organisms, *Staphylococcus aureus* (IOA-106), *Bacillus subtilis* (MTCC-121 laboratory isolate), *Escherichia coli* (U.P.-2566) and *Pseudomonas aeruginosa* (IOA-110) and one antifungal organism, *Candida albicans* (SC-5314 laboratory isolate) of the four compounds (1b), (2b),(1c) and

(2c) were evaluated by agar well diffusion method [12-14]. The results for the antimicrobial study of the tested compounds against the test organisms are depicted in **Table 3**.

The antibacterial activity against "Gram negative" bacteria (*E. coli* and *P. aeruginosa*) was demonstrated in all compounds. However, least activity/no activity could be detected in them against *"Gram positive"* bacteria *(S. aureus / B. subtilis).* It is interesting to note that the compounds having greater chlorine moiety (2b) and (2c) showed enhanced activity against *"Gram negative"* bacteria and is nearly comparable with that of the standard antibiotic drug, *chloramphenicol* against *E. coli*. These compounds also demonstrated little antifungal (anticandidal) activity. Effective concentration of these active compounds was 250 μg per well. Further exploration requires detailed study on exact mode of interaction of these peculiar compounds with "Gram negative" bacteria. In vivo protection and possible toxicity data on these compounds are to be generated further.

TABLE 3: ANTIMICROBIAL ACTIVITY OF THE COMPOUNDS (1b), (2b), (1c) AND (2c) BY AGARWELL DIFFUSION METHOD

Test Compounds	Effective concentration μg per well	Antimicrobial activity in terms of zone of inhibition in mm				
		SA	BS	EC	PA	CA
(1b)	250	09	-	19	13	-
(2b)	250	10	-	20	12	07
(1c)	250	09	-	12	10	-
(2c)	250	10	-	20	15	09
Chloramphenicol	100 μg per well	25	20	24	30	-
Fluconazole	100 μg per well	-	-	-	-	25

SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*; EC, *Escherichia coli*; PA, *Pseudomonas aeruginosa*; CA, *Candida albicans*

Cytotoxic activity against malignant human tumor cells: Two compounds, the cyclohexanol (1b) and 2, 4, 6-tri aryl-substituted pyridine (3) were submitted to the National Cancer Institute (NCI) developmental therapeutic program for the in vitro cell line screening to investigate their antitumor activity. The compounds were first evaluated as one dose primary anticancer assay in a three cell lines panel consisting of three types of human cancers: breast (MCF7), lung (NCI-H460) and CNS (SF-268) [9, 10]. In the screening protocol, each cell line was inoculated and preincubated for 24 - 48 h. on a microtiter plate.

Test agents were then added at a single concentration (1×10^{-4}M) and the culture incubated for further 48 h. End point determinations were made with alamar blue. Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells. Compounds that reduced the growth of any one of the cell lines to approximately 32% or less are considered to be active. The preliminary screening results are shown in **Table 4** according to which these compounds demonstrated to be inactive as the percentage growth of the treated cells are above 32%.

TABLE 4: CYTOTOXIC ACTIVITY OF THE COMPOUNDS AGAINST THREE CELL LINES OF HUMAN CANCERS

Test compounds	Concentration	Retardation of growth (%)			Activity
		MCF7 (Breast)	NCI-H460 (Lung)	SF-268 (CNS)	
(1b)	1×10^{-4}M	90	91	53	Inactive
(3)	1×10^{-4}M	75	81	106	Inactive

CONCLUSION: The dehydration of cyclo-hexanols(1b) and (2b) using a catalytic amount of p-TsOH afforded typical cyclohexenes (1c) and (2c) with β,γ-unconjugated C=C double bond in excellent yields, instead of getting apparently more stable conjugated α,β-C=C double bond containing cyclohexenes (1c') and (2c'). It is worthy to note that the dehydration has undergone by stereoselective syn elimination rather than as usual anti-elimination probably due to steric reason.

The conformational structures of (1a), (1b), (1c), (2a), (2b) and (2c) were established on the basis of extensive spectral studies such as ^{1}H-NMR, ^{13}C-NMR, COSY, HETCOR and Long range HETCOR spectra. The 1, 5-dione (1a) when reacted with mercaptoacetic acid in the presence of ammonium carbonate, a pyridine derivative (3) was obtained. The compounds (1b), (2b), (1c) and (2c) were evaluated for antimicrobial activity. These compounds demonstrated significant antibacterial activity against "Gram negative" bacteria (*E.coli* and *P. aeruginosa*).

It is noteworthy that the compounds having greater number of chlorine moiety [(2b) and (2c)] showed enhanced antibacterial activity. The cyclohexanol (1b) and 2, 4, 6-triaryl pyridine (3) were screened for cytotoxic activity in a three cell lines panel against three types of human cancers: CNS, Breast, and Lung at a single concentration 1×10^{-4} M. These compounds showed no any sign of cytotoxic activity in a three cell line panel at this single concentration. Further investigations for other biological assays are required to explore their potentialities in future.

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