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Synthesis and Antibacterial activity of Methyl Paraben derivatives

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Abstract : Parabens are class of chemicals widely used as preservatives in the cosmetic and pharmaceutical industries. They are effective preservatives in many types of formulae. These compounds and their salts are used primarily for their bacterial and fungicidal properties. They are also used as food additives. Their analogues *viz.* ethers and hybrid / fused molecules also possess various biological activities which prompted us to synthesize analogues for their future application as bioactive molecules. All the synthesized compounds were characterized by ^{1}HNMR and mass spectral data and screened for their potential antibacterial activity against Gram + ve and Gram – ve cultures. A Few of them are showing promising antibacterial activity.

Keywords : Methyl paraben, antibacterial, fungicidal, ^{1}HNMR, TOF MS ES, hybrid molecules, Gram + ve, Gram – ve.

INTRODUCTION :

Phenolic phytochemicals are known to exhibit anti-inflammatory, antioxidant, anticarcinogenic, antidiabetic, antiatherosclerosis and immunomodulatory activities in animals[1,2]. These are mostly polyphenols known as secondary plant metabolites[3] present in plant and trees. One such compound is 4-hydroxy benzoic acid which is used as antifungal, antimutagenic, antisickling, esterogenic[4] and antimicrobial[5] agent. It is primarily known as the basis for the preparation of its esters, known as parabens, which are used as preservatives in cosmetics. Parabens are used for their bactericidal and fungicidal properties. They can be found in shampoos, commercial moisturizers, shaving gels, personal lubricants, topical / parenteral pharmaceuticals, spray tanning solution, makeup and toothpaste. They are also used as food additives. Since methyl paraben is naturally occurring active compounds having antioxidant and antimicrobial properties and in continuation to our earlier work[6-8], we decided to make a library of compounds using various permutations and combinations to come up with novel ether and hybrid derivatives of methyl paraben. The objective of this study is to condense two molecules of the same disease domain to produce more potent candidate in the same disease domain or to condense two molecules of different disease domain to produce mixed variety of those disease domain or to have drug candidate with entirely different biological activity. In the present work, we are converting 4-hydroxy benzoic acid to methyl paraben which in turn further converted to ether and hybrid derivatives respectively using conventional / green chemistry methods and evaluated for their potential antimicrobial activity.

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MATERIALS AND METHODS

Materials : Chemicals used were of a laboratory grade. The reactions were monitored by TLC on aluminium-backed silica plate visualized by UV-light.

EXPERIMENTAL :-

Melting points were determined on a Thomas Hoover capillary melting point apparatus using digital thermometer. IR spectra were recorded on a Shimadzu FTIR Prestige model as KBr pellet. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer in $CDCl_3$. Chemical shifts were recorded in parts per million down field from tetramethyl silane. Mass spectra were recorded on a TOF MS ES mass spectrometer. Elemental analysis were carried out as a percentage on a Thermo finnigan, Flash EA 1112 series, Italy.

CHROMATOGRAPHIC SYSTEM

Column chromatography : For column chromatography 100 – 200 mesh Acme grade silica gel is used. The crude reaction mixture is concentrated under reduced pressure to yield crude mass which is preadsorbed on silica gel and purified by column chromatography with increase in concentration of Ethyl acetate in Petroleum ether. The fractions having similar 'R_f" values were pooled together, concentrated and subjected for characterization using various spectroscopic techniques.

Thin layer chromatography : TLC plates were prepared using silica gel G (ACME, BOMBAY). Pet. ether: EtOAc (85 : 15) was used as the solvent system.

Radial chromatography : The circular glass plates of thickness 1 mm, were prepared by using silica gel (PF254, E. MERCK, 50 g) in cold distilled water (105 ml). For elution, gradually increasing concentrations of EtOAc in pet ether were employed.

Preparation of Methyl paraben :- It was prepared by refluxing 4–hydroxy benzoic acid with methanol using sulphuric acid as a catalyst for 8 hrs. The progress of the reaction was monitored by TLC for the completion of reaction.

Work up :- The reaction mixture concentrated under reduced pressure to minimum and to that 200 ml of dichloromethane + 200 ml of water was added. The aqueous layer was extracted successively with dichloromethane (2 x 100 ml). The total organic layer was washed with water (200 ml), brine (100 ml) and concentrated to yield methyl paraben quantitatively. The general yield was 95 – 98 %.

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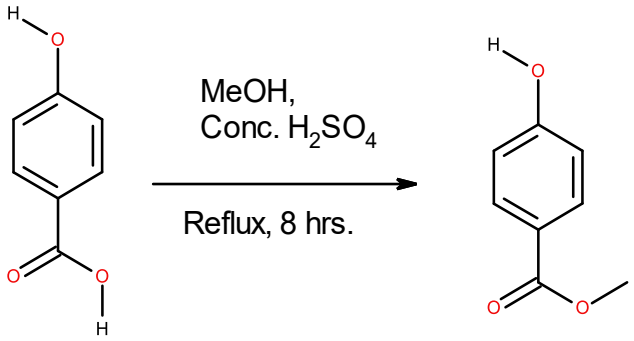
Reaction Scheme :



(1)
4-hydroxybenzoic acid

(2)
methyl 4-hydroxybenzoate

The above procedure can be scaled up to get more quantities of methyl paraben.

Methyl-4-hydroxybenzoate (2)

^{1}H NMR (400 MHz, $CDCl_3$) δppm : 3.9 (s, 3H, -**OCH_3** from –**$COOCH_3$** group), 6.41 (brs, 1H, -OH), 6.9 (d, *J* = 8.8 Hz, 2H, ArH), 7.96 (d, *J* = 8.8 Hz, 2H, ArH). IR (KBr) cm^{-1}: 2950 – 2800 (-CH stretching), 1725 (ester >C=O), 1588 (aromatic); TOF MS ES : 153 (M + H), 175 (M + Na). Molecular formula $C_8H_8O_3$; White solid (26.4 gms, 96.0 %). Melting range 125 – 128^{0}C; Anal. Calcd. for $C_8H_8O_3$: C 63.15, H 5.30, O 31.55 . Found C 63.18, H 5.33, O 31.52;

The methyl paraben was then subsequently converted to its ether and hybrid derivatives as mentioned below.

Diversification of Methyl paraben to its ether derivative (3):- It was prepared by following general method as depicted below.

To a stirred solution of [A] (1 eq.) in 30 ml acetone was added [B] (2.5 eq.) and stirring continued at 40^0 C for the next 30 min. for complete formation of K-salt. To this, compound [C] (2 eq.) was added drop wise and stirring continued at 45-50° C for the next 8 hrs. The progress of the reaction was monitored by TLC for the completion of the reaction.

Work Up:- The reaction mixture filtered through Buchner funnel, wash the cake with 15 ml acetone. The total organic layer was concentrated to minimum, preadsorbed on silica gel and purified by silica gel (100 - 200 mesh) column chromatography with increase in concentration of ethyl acetate in petroleum ether. The general yields ranges between 70 - 80 %.

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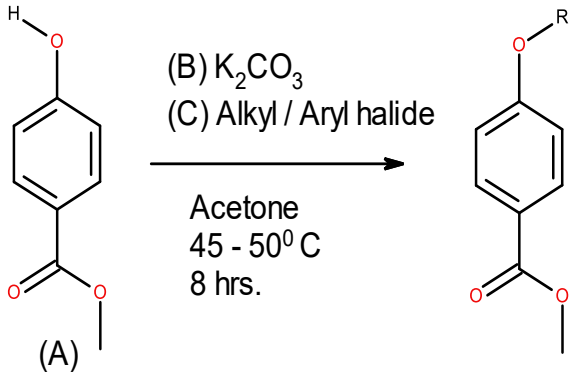
Reaction Scheme :



Methyl 4-hydroxybenzoate Methyl paraben ether derivative

Compound No.	R
3	Decyl

Methyl 4-decoxybenzoate (3)

^{1}H NMR($CDCl_3$, 400 MHz) δ ppm- 0.94 (t, *J* = 7.0 Hz, 3H, -CH_3 from decyl bromide moiety), 1.2 – 1.6 (m, 14H, 7 x –CH_2 from decyl bromide moiety), 1.7 – 1.9 (m, 2H, 1 x –CH_2 from decyl bromide moiety), 3.89 (s, 3H, -OCH_3 from –$COOCH_3$ group), 4.0 (t, *J* = 6.6Hz, 2H, 1 x -**OCH_2** from decyl bromide moiety), 6.9 (d, *J* = 8.8 Hz, 2H, ArH from methyl paraben moiety), 8.0 (d, *J* = 8.8 Hz, 2H, ArH from methyl paraben moiety); IR (KBr) cm^{-1}: 2980 – 2780 (-CH stretching), 1728 (ester >C=O), 1588 (aromatic); TOF MS ES: 293 (M + H); Molecular Formula $C_{18}H_{28}O_3$; Pure viscous mass (0.829 gms); Elemental Analysis, Calcd : C 73.93 %, H 9.65 %, O 16.41 % Found C 73.96 %, H 9.68 %, O 16.38 %;

Diversification of methyl paraben to its hybrid derivatives (4 - 5) :- These were prepared by following general method as depicted below.

To a stirred solution of [A] (1 eq.) in 30 ml dichloromethane was added [C] (1.3 eq.), [D] (0.05 eq.), [E] (0.5 eq.) and the reaction mixture stirred at room temperature for 5 min. Clear solution of reaction mixture was obtained. To this, compound [B] (1.3 eq.) was added and stirring continued at room temperature for next 24 hrs. As the reaction proceeds, urea derivative

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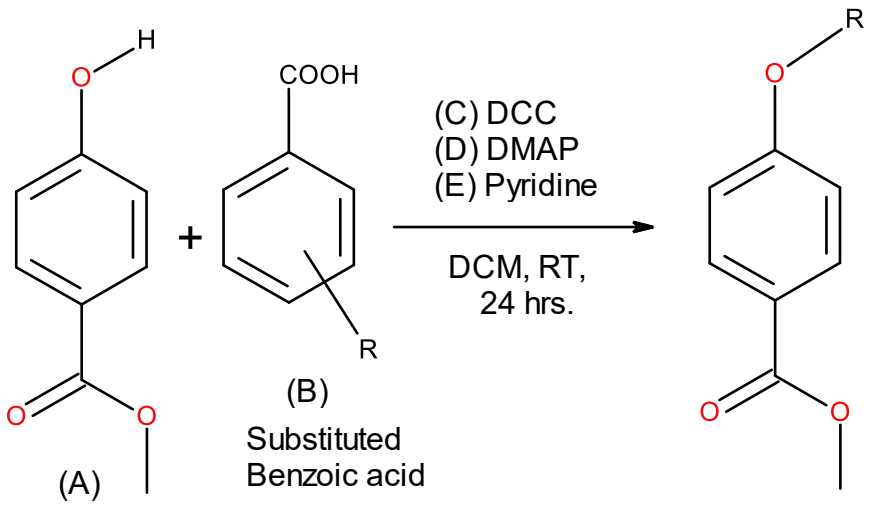
precipitates out as by product. The progress of the reaction is monitored by TLC for the completion of reaction.

Work up :-The reaction mixture filtered through celite bed which get rids of by product urea derivative. The filtrate was concentrated to minimum, preadsorbed on silica gel (100 – 200 mesh) and purified by column chromatography with increase in concentration of ethyl acetate in petroleum ether. The general yields of these reactions ranges between 70 – 80 %.

This is another method of preparing esters and follows green chemistry parameters. During the reaction by-product urea derivative precipitates out. The reaction mixture filtered through celite bed which gets rid of by product and the mother liquor on concentration yield the pure final product in a quantitative yield.

Reaction Scheme :



methyl 4-hydroxybenzoate

Hybrid derivatives

Compound No.	R
4	4-Nitro benzoyl
5	3-Nitro benzoyl

The general mechanism for this reaction can be given as follows.

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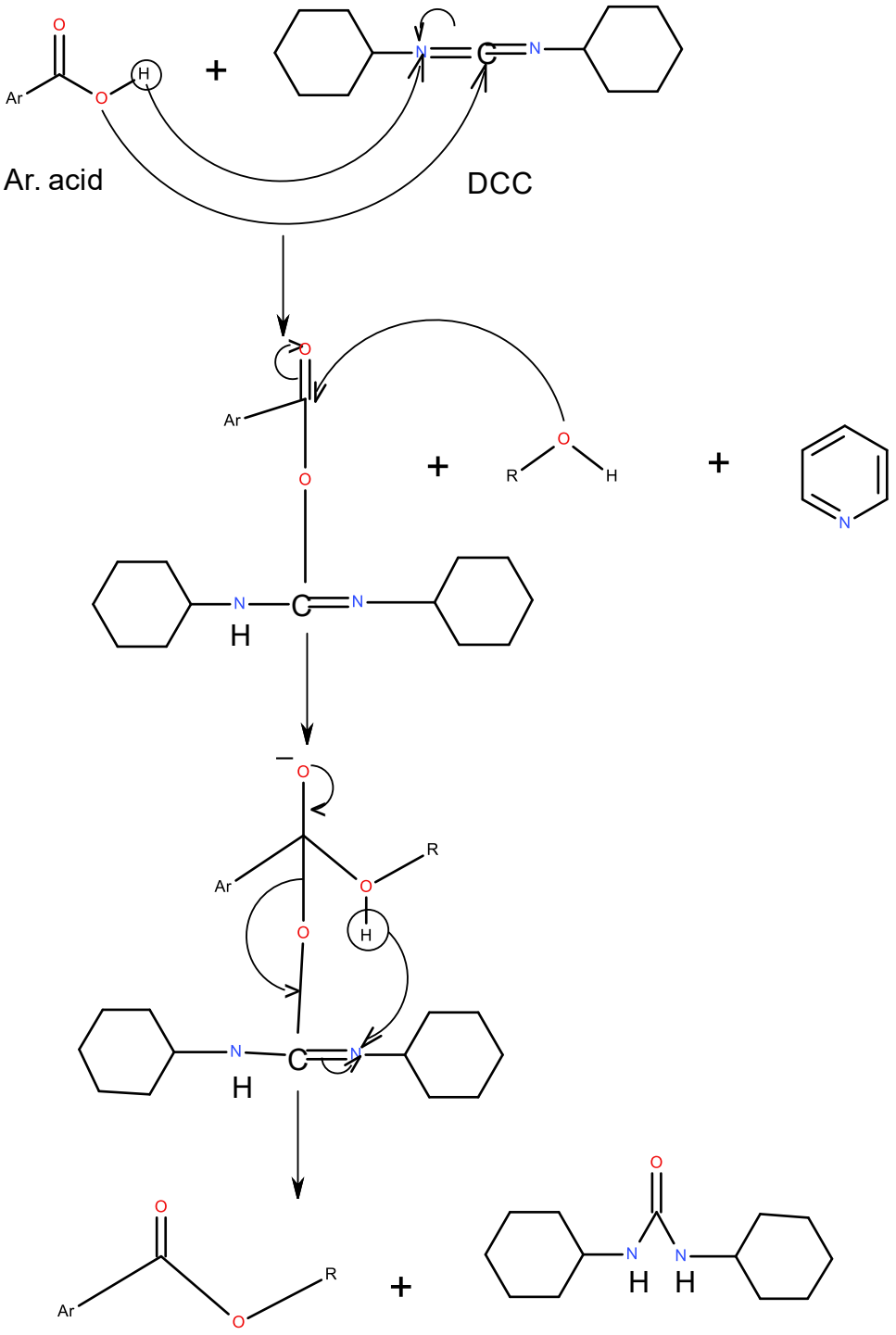
Probable mechanism for fused / hybrid molecules :



(4-methoxycarbonylphenyl) 4-nitrobenzoate (4)

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm :- 3.92 (s, 3H, -OCH_3 from –$COOCH_3$ group from methyl paraben moiety), 6.84 (d, *J* = 8.8 Hz, 2H, ArH from methyl paraben moiety), 7.3 (d, *J* = 8.6 Hz, 2H, ArH from 4-Nitro benzoic acid moiety), 7.92 (d, *J* = 8.8 Hz, 2H, ArH from methyl paraben moiety), 8.16 (d, *J* = 8.6 Hz, 2H, ArH from from 4-Nitro benzoic acid moiety); IR (KBr) cm^{-1}: 2950 – 2800 (-CH stretching), 1734 (ester >C=O), 1588 (aromatic); TOF MS ES : 302 (M + H); Molecular Formula $C_{15}H_{11}NO_6$; Pale yellow solid (0.96 gms). Melting range 128 – 132^{0}C; Elemental Analysis, Calcd : C 59.80 %, H 3.68 %, N 4.65 %, O 31.87 % Found C 59.82 %, H 3.70 %, O 31.85 %;

(4-methoxycarbonylphenyl) 3-nitrobenzoate (5)

^{1}H NMR($CDCl_3$, 400 MHz) δ ppm :- 3.86 (s, 3H, -OCH_3 from –$COOCH_3$ group from methyl paraben moiety), 6.80 (d, *J* = 8.8 Hz, 2H, ArH from methyl paraben moiety), 7.26 – 8.20 (m, 4H, ArH from 3-Nitro benzoic acid moiety), 7.92 (d, *J* = 8.8 Hz, 2H, ArH from methyl paraben moiety); IR (KBr) cm^{-1}: 2950 – 2800 (-CH stretching), 1736 (ester >C=O), 1590 (aromatic); TOF MS ES : 302 (M + H); Molecular Formula $C_{15}H_{11}NO_6$; Yellow solid (0.92 gms). Melting range 122 – 124^{0}C; Elemental Analysis, Calcd : C 59.80 %, H 3.68 %, N 4.65 %, O 31.87 % Found C 59.83 %, H 3.71 %, O 31.84 %;

The most significant features of this methodology are (a) good accessibility of the reagent and its stability (b) a stoichiometric amount of reagent can be used by direct weighing, avoiding excess (c) no evolution of hazardous vapours during the reaction (d) the total elimination of the use of toxic organic solvents (e) a simple experimental procedure (g) good control over the outcome of the reaction by varying the amount of reagent (h) less expensive and (i) very simple reaction work up with avoidance of by-product. The aforesaid protocol thus provides an improved procedure for the synthesis of useful hybrid derivatives having important pharmaceutical, agricultural and other physicochemical properties.

BIOLOGICAL ACTIVITY

Antibacterial Activity using ditch plate method[9] :-

The synthesized molecules were screened for their antibacterial activity using ditch plate method at 100 µg / ml concentration against Gram positive (*Staphylococcus aureus*, *Corynebacterium diphtheriae*) and Gram negative (*Escherichia coli*, *Salmonella typhi, Proteus vulgaris*) bacterial species qualitatively.

Theory : Ditch plate method is the method of chosen to test the anti-bacterial activity of compounds. It is a preliminary method to screen the anti-microbial potential of compounds / drugs, which are insoluble or partially soluble in aqueous phase. In this method , the test

compound is seeded in an agar plate and the test organisms are streaked across to test the inhibition of the growth as a marker of anti-microbial activity.

PROCEDURE : A ditch (10 mm x 70 mm) is cut into sterile MH agar plate. The test drug / compound is added to 5 ml molten MH agar butt at 40^0C and this mixture is poured into the ditch and allowed to solidify. The ditch should be made in level with the rest of the agar by pouring the mixture . The different bacterial cultures are streaked perpendicular to the ditch using nichrome wire loop. The plate is then incubated at 37^0 C for 24 hours. The results are observed as inhibition of bacterial growth on the ditch as well as adjacent to the ditch. The following test samples showed antibacterial activity against the organisms mentioned in the following **Table 1**.

Table 1 : Antibacterial Activity Results

SAMPLE NO.	ACTIVE AGAINST
2 (Methyl Paraben)	*Staphylococcus aureus* [Gram positive] *Salmonella typhi* [Gram negative] *Klebsiella pneumonia* [Gram negative] *Corynebacterium diphtheriae* [Gram positive] *Escherichia coli* [Gram negative]
Ampicillin (Std. Drug)	*Staphylococcus aureus* [Gram Positive] *Corynebacterium diphtheriae* [Gram positive] *Escherichia coli* [Gram negative] *Proteus vulgaris* [Gram negative] *Salmonella typhi* [Gram negative]
4	*Staphylococcus aureus* [Gram positive] *Escherichia coli* [Gram negative]
5	*Staphylococcus aureus* [Gram positive] *Escherichia coli* [Gram negative]

RESULTS AND DISCUSSION

The novel ether and hybrid derivatives of 4-hydroxy benzoic acid were synthesized by cost effective industry viable process following the principle of green chemistry. The synthesis of hybrid derivatives is another way to prepare ester derivatives using DCC as dehydrating agent in a reasonably good yield. The probable mechanism for the formation ether and hybrid derivatives were also discussed.

The biological activity suggest that the base molecule methyl paraben has anti-bacterial activity against both the bacterial cultures. Its derivatives *viz.* 4 and 5 were active against both *Staphylococcus aureus* (Gram + ve bacteria) and *Escherichia coli* (Gram - ve bacteria)

respectively. Thus, 4 and 5 derivatives were potential antibacterial candidates. In depth analysis of these compounds through structure activity relationship studies would provide further insight and can be an interesting topic of future studies.

CONCLUSION

The structural diversity and the pronounced biological activities encountered in the 4-hydroxy benzoic acid derivatives suggests that these class of compounds were worthy for further studies that may lead to derivatives by using combinatorial chemistry approach is an alternative strategy to new therapeutic discovery. In other words the generation of diverse hydroxyl benzoic acid derivatives develop new therapeutic molecules that might result in candidates having better activity.

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