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PARABEN DERIVATIVES AS FUTURE POTENTIAL DRUG

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ABSTRACT

Parabens are class of chemicals widely used as preservatives in the cosmetic and pharmaceutical industries. They are attractive preservatives in many types of formulas. These compounds and their salts are used primarily for their bacterial and fungicidal properties. They are also used as food additives. Their analogues also possess various biological activities which prompted us to synthesize few more 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 antibacterial activity against Gram + ve and Gram – ve cultures. Few of them are showing promising antibacterial activity.

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 of 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. In the present study, we are converting 4-hydroxy benzoic acid to methyl and ethyl paraben using conventional method and their further diversification to ester derivatives. Since methyl and ethyl paraben are naturally occurring active compounds having antioxidant and antimicrobial properties, we decided to make a library of compounds using various permutation and combinations to come up with novel ether and ester derivatives of methyl and ethyl paraben using conventional methods. 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 disease domain. In the present work, we are converting 4-hydroxy benzoic acid to methyl and ethyl paraben which in turn furher converted to ether and hybrid derivatives respectively using conventional methods.

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.

RESULTS AND DISCUSSION :

Preparation of methyl and ethyl paraben :- They were prepared by refluxing 4–hydroxy benzoic acid with methanol / ethanol using sulphuric acid as a catalyst for 8 hrs. The progress of the reaction is monitored by TLC for the completion of reaction.

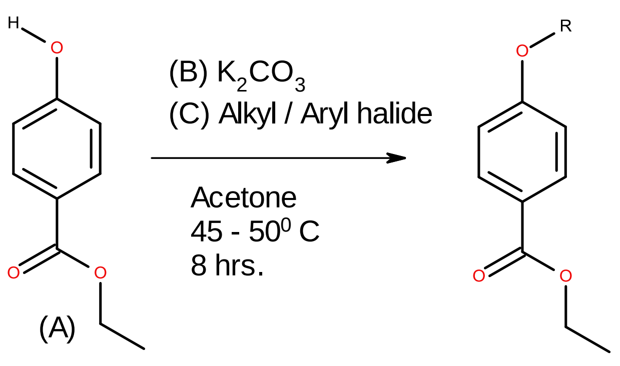
Work up :- The reaction mixture concentrated under reduced pressure to minimum and to that 200 ml of dichloromethane + 200 ml of water was added. The aqueous layer was extracted successively with dichloromethane (2 x 100 ml). The total organic layer was washed with water (200 ml), brine (100 ml) and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

General method for the preparation of compounds (I And II) :- These were 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 and stirring continued at 45-50° C for the next 8 hrs. The progress of this reaction is monitored by TLC for the completion of this reaction.

Work Up:- The reaction mixture filtered through Buchner funnel, wash the cake with 25 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 60-70 %.

Reaction Scheme 1 :



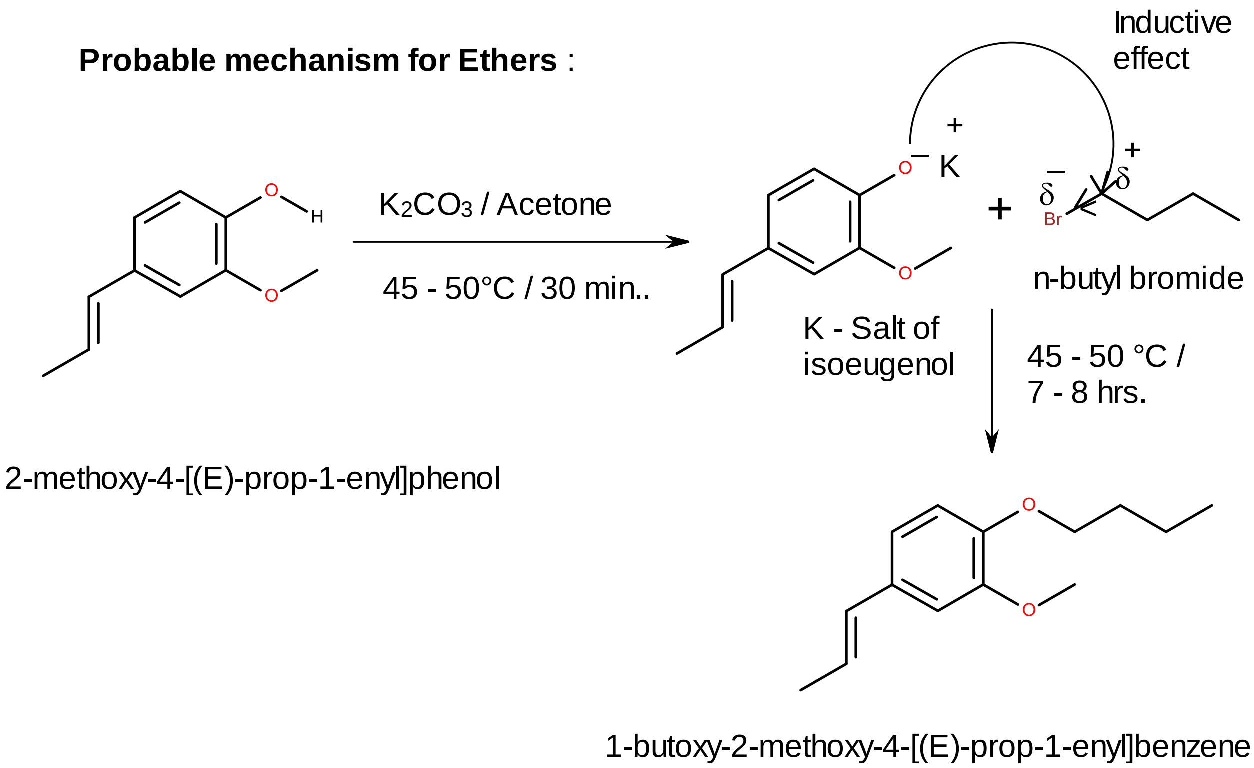
Ethyl 4-hydroxybenzoate Ethyl paraben ether derivatives

Table 1 : Ether derivatives of ethyl paraben.

Compound No.	R
I	Heptyl
II	3-Methyl butyl

Taking Isoeugenol as general example, the probable mechanism for ethers can be given as follows.

Probable mechanism for Ethers :



Compound 1 :- Ethyl 4 – heptoxybenzoate.

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm : 0.88 (t, J = 4.7 Hz, 3H, terminal $-CH_3$ from heptoxy moiety), 1.2 – 1.5 (m, 8H, 4 x $-CH_2$, methylenes from n- heptyl moiety), 1.37 (t, J = 6.9 H, 3H, from $-OCH_2$**CH_3** group) , 1.7 – 2.0 (m, 2H, 1x $-CH_2$ from n-heptyl moiety), 3.99 (t, J = 6.6 Hz, 2H, 1 x $-OCH_2$ from n-heptyl moiety), 4.34 (q, J = 7.7 Hz, 2H, $-OCH_2$ from -O**CH_2**CH_3 group), 6.89 (d, J = 8.7 Hz, 2H, ArH), 7.97 (d, J = 8.7 Hz, 2H, ArH); Molecular Formula $C_{16}H_{24}O_3$; TOF MS ES : 287 (M + Na); Pure viscous mass (0.954 gms, 60 %); Anal. Calcd. .for $C_{16}H_{24}O_3$: C 72.69 %, H 9.15 %, O 18.16 % Found C 72.66 %, H 9.11 %, O18.20 %.

Compound 2 :- Ethyl 4- isopentyloxybenzoate

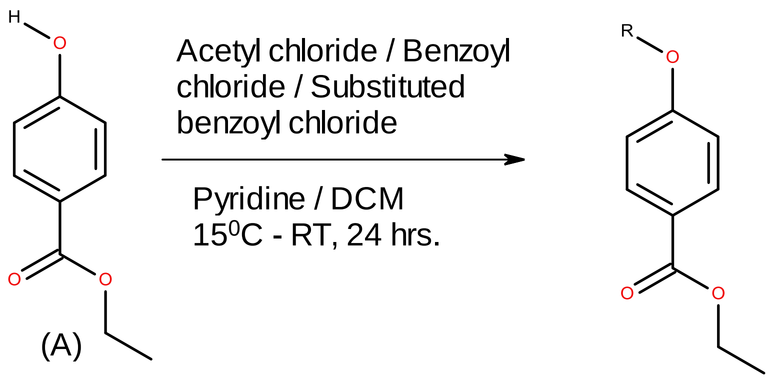
^{1}H NMR($CDCl_3$, 400 MHz) δ ppm- 0.98 (d, J = 6.6 Hz, 2 x $-CH_3$, 6H), 1.37 (t, J = 6.9Hz, 3H from $-OCH_2$**CH_3** group), 1.7 (m, 2H, $-CH_2$ from 3-Methyl butane moiety), 1.85(m, 1H, **CH**$(CH_3)_2$), 4.0 (t, J= 6.6Hz, 2H, 1 x -O**CH_2** from 3-Methyl butane moiety), 4.34 (q, J = 7.7 Hz, 2H, **-OCH_2** from ethyl paraben moiety), 6.89 (d, J = 8.7 Hz, 2H, ArH), 7.07 (d, J = 8.7 Hz, 2H, ArH); Molecular Formula $C_{14}H_{20}O_3$; TOF MS ES : 259 (M + Na); Pure viscous mass

(0.894 gms, 63 %); Anal. Calculated for $C_{14}H_{20}O_3$: C 71.16 %, H 8.53 %, O 20.31 % Found C 71.12 %, H 8.50 %, O20.35 %.

General method for the preparation of compounds (3 - 5) : These were prepared by following general method as depicted below.

To a stirred solution of Ethyl paraben (1 eq.) in dichloromethane (30 ml) was added pyridine (2.5 eq.) and cool the reaction in ice bath at 15° C. Clear solution of reaction mixture was obtained. To this, was added Acetyl chloride/ benzoyl chloride / substituted benzoyl chloride (2 eq.) at 15 - 20° C and stirred, allowed to attain the room temperature and stirring was continued for the next 24 hrs. (TLC). The organic layer was concentrated under reduced pressure to minimum, preadsorbed on the silica gel and purified by column chromatography (SiO_2, 100-200 mesh) with increase in concentration of Ethyl acetate in petroleum ether to yield pure compound. The purified compounds were unambiguously characterized by ^{1}HNMR, mass spectroscopy and elemental analysis. The general yields of these reactions were ranges between 60 - 80 %.

Reaction Scheme 2 :



Ethyl 4-hydroxybenzoate

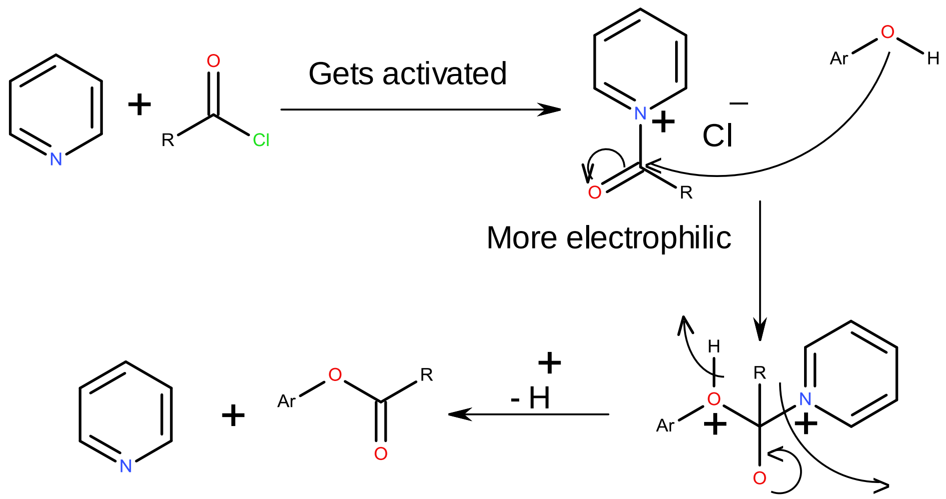
Ethyl paraben ester derivatives

Table 2 : Ester derivatives of ethyl paraben.

Compound No.	R
3	Acetyl
4	Benzoyl
5	4-Chloro benzoyl

Taking Isoeugenol as general example, the probable mechanism for esters can be given as follows.

Probable mechanism for Esters :



Compound 3 :- Ethyl 4 – acetoxybenzoate

[1]H NMR (CDCl$_3$, 400 MHz) δ ppm : 1.3 (t, J= 7 .7 Hz, 3H, from - OCH$_2$**CH$_3$**), 2.31 (S, 3H, from >CO**CH$_3$** group), 4.37 (q, J = 7, 14 Hz, 2H, from – OCH$_2$**CH$_3$**), 7.16 (d, J = 8.4 Hz, 2H, ArH), 8.07 (d, J = 8.4 Hz, ArH); Molecular Formula $C_{11}H_{12}O_4$; TOF MS ES : 231 (M + Na); Pure viscous mass (0.90 gms, 72 %); Anal. Calcd. for $C_{11}H_{12}O_4$: C 63.45 %, H 5.81 %, O 30.74 % Found C 63.41 %, H 5.78 %, O 30.78 %.

Compound 4 :- Ethyl 4 – benzoyloxybenzoate

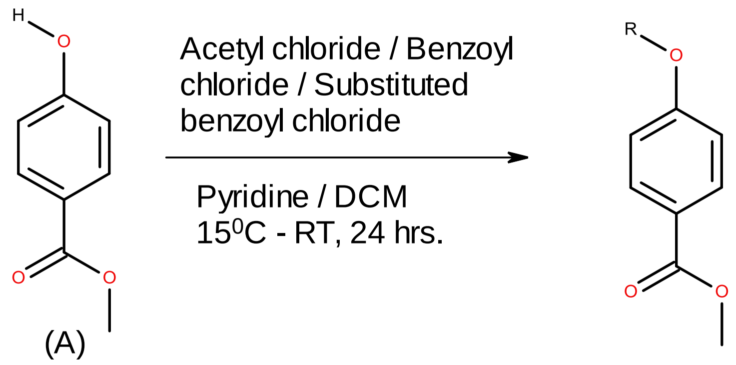
[1]HNMR (CDCl$_3$, 400 MHz) δppm : 1.4 (t, J = 4.7 Hz, 3H, -CH$_3$ from – OCH$_2$**CH$_3$** group), 4.34 (q, J=7.7 Hz, 2H,-**OCH$_2$** from Etyl paraben moiety), 7.2 - 8.4 (m, 9H, Ar-H); Molecular Formula $C_{16}H_{14}O_4$; TOF MS ES : 293 (M + Na); Off white solid (0.975 gms, 64 %); Melting range 65 – 68^{0}C; Anal. Calcd. for $C_{16}H_{14}O_4$: C 71.10 %, H 5.22 %, O 23.68 % Found C 71.06 %, H 5.19 %, O 23.71 %.

Compound 5 :- Ethyl 4-(4-chlorobenzoyl)oxybenzoate

[1]H NMR (CDCl$_3$, 400 MHz) δppm : 1.4 (t, J = 4.7 Hz, 3H, -CH$_3$ from – OCH$_2$**CH$_3$** group), 4.34 (q, J = 7.7 Hz, 2H, OCH$_2$ from ethyl paraben moiety), 7.0 - 8.7 (m, 8H, ArH); Molecular Formula $C_{16}H_{13}ClO_4$; TOF MS ES : 327 (M^+ + Na) and 329(M^+ + 2 + Na); Off white solid (1.34 gms, 74 %). Melting range 100 - 102^{0}C Anal. Calcd .for $C_{16}H_{13}ClO_4$: C 63.06 %, H 4.30 %, O 21.00 %, Cl 11.63 % Found C 63.10 %, H 4.26 %, O 21.04 %

General method for the preparation of compounds (6 - 9) :- These were prepared by the general method as mentioned for ethyl paraben.

Reaction Scheme 3 :



Methyl 4-hydroxybenzoate | Methyl paraben ester derivatives

Table 3 : Ester derivatives of methyl paraben.

Compound No.	R
6	Acetyl
7	Benzoyl
8	4-Chloro benzoyl
9	2-Chloro Benzoyl

The general mechanism for ester is same as depicted for ethyl paraben.

Compound VI :- Methyl 4-acetoxybenzoate

^{1}HNMR (CDCl$_3$, 400 MHz) δppm : 2.3 (s, 3H, >CO-**CH$_3$**), 3.9 (s, 3H, -COO**CH$_3$**), 7.16 (d, J = 8.4 Hz, 2H, ArH), 8.07 (d, J = 8.4 Hz, ArH); Molecular Formula $C_{10}H_{10}O_4$; TOF MS ES : 217 (M + Na); White solid (1.297 gms, 68 %). Melting range 70 - 75^0C. Anal.Calcd .for $C_{10}H_{10}O_4$: C 61.85 %, H 5.19 %, O 32.96 % Found C 61.81 %, H 5.16 %, O 32.99 %.

Compound VII :- Methyl 4-benzoyloxybenzoate

^{1}HNMR(CDCl$_3$, 400 MHz) δppm : 3.9 (s, 3H, from -COO**CH$_3$** group), 7.22 (d, J = 8.4 Hz, 2H, ArH), 8.07(d, J = 8.4 Hz, ArH from methyl paraben moiety), 7.2 - 8.3 (m, 5H, ArH from Benzoyl Moiety); Molecular Formula $C_{15}H_{12}O_4$; TOF MS ES : 279 (M + Na); White solid (1.09 gms,65 %); Melting range 106 - 110^0C Anal.Calcd .for $C_{15}H_{12}O_4$: C 70.31 %, H 4.72 %, O 24.97 %. Found C 70.34 %, H 4.69 %, O 24.95 %.

Compound VIII :- Methyl 4–(4–chlorobenzoyl)oxybezoate

^{1}H NMR ($CDCl_3$, 400 MHz) δppm : 3.9(s, 3H,-OCH_3 from –COO**CH_3** group), 7.22(d, J = 8.4 Hz, 2H, ArH), 8.1 (d, J = 8.4 Hz, 2H, ArH from Methyl paraben moiety), 7.2 - 8.2 (m, 4H, ArH from 4-Chloro benzoyl moiety); Molecular Formula $C_{15}H_{11}ClO_4$; TOF MS ES : 313 (M^+ + Na) and 315 (M^+ + 2 + Na); Off white solid (1.297 gms, 68 %). Melting range 126 - 129^{0}C; Anal. Calcd .for $C_{15}H_{11}ClO_4$: C 61.98 %, H 3.81 %, O 22.02 %, Cl 12.20 % Found C 61.95 %, H 3.78 %, O 22.05 %.

Compound IX :- (4- methoxycarbonyl phenyl)–2–chlorobenzoate

^{1}HNMR ($CDCl_3$, 400 MHz) δppm : 3.9 (s, 3H,-OCH_3 from –CO**OCH_3** group), 7.24 (d, J = 8.4 Hz, 2H, ArH from Methyl paraben moiety), 8.1 (d, J = 8.4 Hz, H, ArH from methyl paraben moiety), 7.2 - 8.2 (m, 4H, ArH from 2-Chloro benzoyl moiety); Molecular Formula $C_{15}H_{11}ClO_4$; TOF MS ES : 313 (M^+ + Na) and 315 (M^+ + 2 + Na); White solid (1.297 gms, 68 %). Melting range 67 - 69^{0}C Anal. Calcd .for $C_{15}H_1ClO_4$ C 61.98 %, H 3.81 %, O 22.02 %, Cl 12.20 % Found C 61.95 %, H 3.78 %, O 22.05 %.

EXPERIMENTAL

Mps. are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Varian spectrometer and Mass spectra on TOF MS ES mode. Elemental analysis was carried out as a percentage on a Thermo finnigan, Flash EA 1112 series, Italy.

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 'rf' 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.

BIOLOGICAL ACTIVITY:

Antibacterial activity using agar diffusion method[6] :- Concentration 100 µm

The synthesized molecules were screened for their antibacterial activity using agar diffusion method at 100 µm concentration against Gram positive (Staphylococcus aureus) and Gram negative (Escherichia coli) bacterial species qualitatively. The results of the antibacterial activities are summarized in Table 4.

Table 4 : Antibacterial Activity Results

Sr. No	Compound No.	Antibacterial Activity	
		Against Gram - ve bacteria species (Escherichia coli)	Against Gram +ve bacterial species (Staphylococcus aureus)
1	Methyl paraben	+	+
2	Ethyl paraben	-	-
3	1	-	-
4	2	+	-
5	3	-	-
6	4	-	-
7	5	-	+
8	6	-	-
9	7	-	-
10	8	-	+
11	9	-	-

The above results shows that the base molecule Methyl paraben has anti-bacterial activity against both the bacterial culture. Its derivative *viz.* 5 and 8 were active against *Staphylococcus aureus* (Gram + ve bacteria) and 2 was active against *Escherichia coli* (Gram - ve bacteria) respectively. Thus, 3-methyl butyl and 4-chloro benzoyl 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 paraben derivatives suggests that this class of compounds is 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 paraben derivatives develop new therapeutic molecules that might result in candidates having better activity.

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