© 2018 IJSRST | Volume 4 | Issue 2 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Synthesis and Antimicrobial Activity of Isoeugenol Hybrid Derivatives

Vijay D. Gangan*[1], S. S. Sankhe[2]

[1]Department of Chemistry, Thakur College of Science & Commerce, Thakur Village, Kandivali (E), Mumbai, Maharashtra, India

[2]Department of Chemistry, Patkar -Varde College, Goregaon (W), Mumbai, Maharashtra, India

ABSTRACT

Isoeugenol has wide medical applications. It is used in manufacturing perfumeries, flavorings, essential oils (odor description : Clove, spicy, sweet, woody) and in medicine (local antiseptic and analgesic). Its analogues also show various biological activities which prompted us to synthesize few more analogues (fused / hybrid molecules) for their future applications as bioactive molecules and are characterized by 1H NMR and Mass spectral data. Few of them are showing promising antibacterial activity.

Keywords : Isoeugenol Hybrid Derivatives, Synthesis And Antimicrobial Activity , Biological Activities , Perfumeries, Flavorings, Clove, Spicy, Sweet, Woody, DDC, DMAP

I. INTRODUCTION

Phenolic phytochemicals are known to exhibit anti-inflammatory, antioxidant, anticarcinogenic, antidiabetic, antiatherosclerosis and immunomodulatory activities in animals1,2. These are mostly polyphenols known as secondary plant metabolites3 present in plant and trees. One of such compound is isoeugenol which is a phenylpropene, a propenyl substituted guaiacol. It occurs in the essential oils of plants such as ylang–ylang. It can be synthesized from eugenol and had been used in the manufacture of vanillin. It may occur as either the cis (Z) or trans (E) isomer. Trans (E) isoeugenol is crystalline while cis (Z) isoeugenol is a liquid4. Since it is a naturally occurring active compound having antioxidant and antimicrobial properties, we decided to make a library of compounds using various permutation and combinations to come up with novel ester derivatives of isoeugenol using conventional method. 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.

II. RESULTS AND DISCUSSION

Isoeugenol dissolved in dichloromethane and treated with DDC, DMAP, pyridine and aromatic acids at room temperature for 24 hrs to yield respective hybrid molecules. The crude reaction mixture obtained in each stages were purified by column, radial and preparative thin layer chromatographic techniques and unambiguously characterized by 1H NMR and Mass spectroscopy techniques.

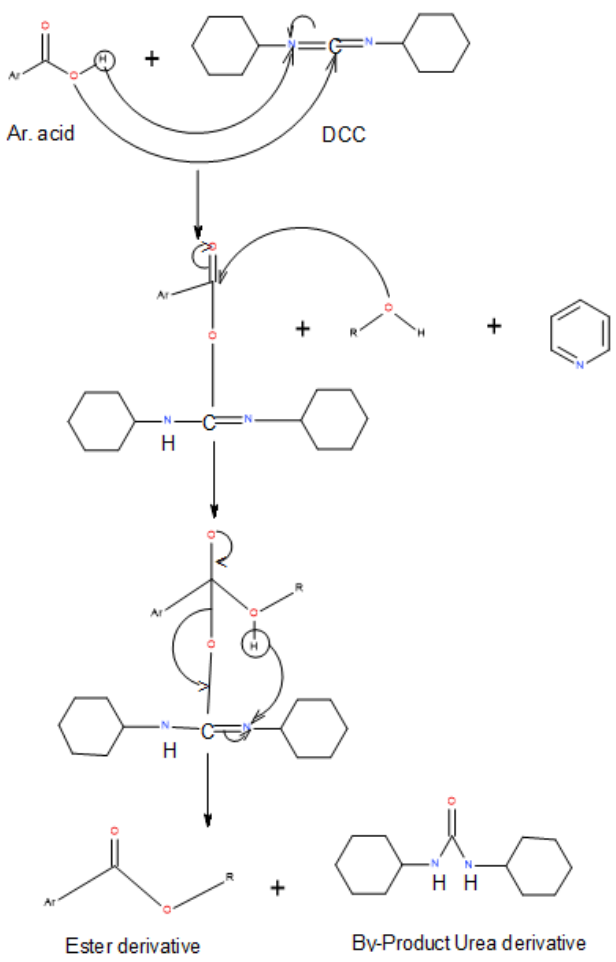
General method for the preparation of compounds (1 - 9) :- These were prepared by following general method as depicted below.

To a stirred solution of Isoeugenol (1 eq.) in dichloromethane (30 mL) was added pyridine (0.5 eq.), DMAP (0.05 eq.), DCC (1.3 eq.) and aromatic /

substituted aromatic acid (1.3 eq.) respectively at room temperature and stir it for next 24 hrs. As the reaction proceeds the by-product urea derivative precipitates out of the reaction mixture and floats on the surface (TLC). The organic layer was concentrated under reduced pressure to minimum, preadsorbed on silica gel and purified by column chromatography (SiO2, 100 – 200 mesh) with increase in concentration of ethyl acetate in petroleum ether to yield pure compound. The purified compounds were unambiguously characterized by 1H NMR and Mass spectroscopy.

Compound No.	R
1	2-Methoxy benzoyl
2	2-Ethoxy benzoyl
3	Phenyl acetyl
4	4-Chloro phenyl acetyl
5	4-Ethoxy-3-methoxy benzoyl
6	3,4-Dimethoxy benzoyl
7	2,6-bis(trifluoro methyl) benzoyl
8	4-Allyloxy benzoyl
9	3-Amino benzoyl

Probable mechanism for fused / hybrid molecules :



Compound 1 : [2-methoxy-4-[(E)-prop-1-enyl]phenyl]-2- methoxybenzoate

^{1}H NMR (400 MHz, $CDCl_3$) δppm : 1.88 (d, J = 6.6 Hz, terminal methyl from isoeugenol moiety), 3.83 (s, 3H, Ar x –OCH_3), 3.97 (s, 2H, Benzylic –CH_2), 6.1 – 6.3 (m, 1H, olefinic proton 'a'), 6.39 (d, J = 15.8 Hz, 1H, olefinic proton "b'), 6.8 – 8.2 (m, 7H, ArH from isoeugenol and 2-methoxy benzoic acid moiety TOF MS ES : 299 (M + H), 321 (M + Na). Molecular formula $C_{18}H_{18}O_4$. Pure viscous mass (1.36 gms, 74.7 %). Anal. Calcd. for $C_{18}H_{18}O_4$: C 72.47, H 6.08, O 21.45 Found C 72.43, H 6.05, O 21.49;

Compound 2 : [2-methoxy-4-[(E)-prop-1-enyl]phenyl]-2-ethoxy benzoate

^{1}H NMR (400 MHz, $CDCl_3$) δppm : 1.45 (t, J = 7.0 Hz, -CH3 from –OCH_2CH_3 group), 1.88 (d, J = 6.5 Hz, 3H,

terminal methyl from isoeugenol moiety), 3.83 (s, 3H, Ar x –OCH_3), 4.14 (q, J = 7.0 Hz, 2H, –OCH_2 from –OCH_2CH_3 group), 6.1 – 6.3 (m, 1H, olefinic proton 'a'), 6.38 (d, J = 15.6 Hz, 1H, olefinic proton "b'), 6.8 – 8.1 (m, 7H, ArH from isoeugenol and 2-ethoxy benzoic acid moiety. ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 14.96 (-CH_3, terminal methyl from isoeugenol moiety), 18.67 (CH_3 from -OCH_2CH_3 group), 56.14 (–OCH_2), 64.84 (Ar x –OCH_3), 109.97 (=CH), 113.62 (=CH), 118.63 (ArC), 119.75 (ArC), 120.28 (ArC), 123.17 (ArC), 126.01 (ArC), 130.87 (q, >C<), 132.50 (ArC), 134.25 (q, >C<), 137.10 (ArC), 139.17 (q, ArC-O), 151.56 (q, ArC-O), 159.48 (q, ArC-O), 164.29 (q, >C=O). TOF MS ES : 313 (M + H), 335 (M + Na). Molecular formula $C_{19}H_{20}O_4$. Pure viscous mass (1.32 gms, 69.5 %). Anal. Calcd. for $C_{19}H_{20}O_4$: C 73.06, H 6.45, O 20.49 . Found C 73.10, H 6.42, O 20.52;

Compound 3 : [2-methoxy-4-[(1E)-prop-1-en-1-yl]phenyl]2-phenylacetate

^1H NMR (400 MHz, $CDCl_3$) δppm : 1.86 (d, 3H, J = 8.5 Hz, terminal methyl from isoeugenol moiety), 3.76 (s, 3H, Ar x –OCH_3), 3.87 (s, 2H, Benzylic –CH_2), 6.0 – 6.2 (m, 1H, olefinic proton 'a'), 6.34 (d, J = 16.1 Hz, 1H, olefinic proton "b'), 6.6 – 7.0 (m, 3H, ArH from isoeugenol moiety), 7.2 – 7.6 (m, 5H, ArH from phenyl acetic acid moiety). ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 14.96 (-CH_3, Terminal methyl from isoeugenol moiety), 56.18 (Ar x –OCH_3), 110.0 (=CH), 118.65 (=CH), 122.97 (ArC), 126.49 (ArC), 126.92 (ArC), 129.51 (ArC), 130.74 (ArC), 131.47 (q, >C<), 132.31 (ArC), 133.23 (q, >C<), 134.67 (ArC), 137.52 (q, >C<), 138.74 (q, ArC-O), 151.31 (q, ArC-O), 163.77 (q, >C=O). TOF MS ES : 282 (M + H), 305 (M + Na). Molecular formula $C_{18}H_{18}O_3$. Pure viscous mass (1.38 gms, 80.23 %). Anal. Calcd. for $C_{18}H_{18}O_3$: C 76.57, H 6.43, O 17.0 Found C 76.53, H 6.45, O 17.04;

Compound 4 : . [2-methoxy-4-[(1E)-prop-1-en-1-yl]phenyl]-2-(4-chlorophenyl)acetate

^1H NMR (400 MHz, $CDCl_3$) δppm : 1.86 (d, 3H, J = 8.5 Hz, terminal methyl from isoeugenol moiety), 3.76 (s, 3H, Ar x –OCH_3), 3.84 (s, 2H, Benzylic –CH_2), 6.0 – 6.2 (m, 1H, olefinic proton 'a'), 6.34 (d, J = 15.8 Hz, 1H, olefinic proton "b'), 6.6 – 7.0 (m, 3H, ArH from isoeugenol moiety), 7.2 – 7.6 (m, 4H, ArH from 4-chloro phenyl acetic acid moiety). TOF MS ES : 317 (M + H), 339 (M + Na). Molecular formula $C_{18}H_{17}ClO_3$. Pure viscous mass (1.34 gms, 69.43 %). Anal. Calcd. For $C_{18}H_{17}ClO_3$: C 68.25, H 5.41, O 16.15, Cl 11.19 % Found C 68.21, H 5.38, O 16.19;

Compound 5 : [2-methoxy-4-[(E)-prop-1-enyl]phenyl]-4-ethoxy-3-methoxy benzoate

^1H NMR (400 MHz, $CDCl_3$) δppm : 1.51 (t, J = 7.0 Hz, -CH_3 from –OCH_2CH_3 group), 1.89 (d, J = 6.4 Hz, 3H, terminal methyl from isoeugenol moiety), 3.82 (s, 3H, Ar x –OCH_3), 3.95 (s, 3H, Ar x –OCH_3), 4.19 (q, J = 6.8 Hz, 2H, –OCH_2 from –OCH_2CH_3 group), 6.1 – 6.3 (m, 1H, olefinic proton 'a'), 6.38 (d, J = 15.6 Hz, 1H, olefinic proton 'b'), 6.8 – 8.0 (m, 6H, ArH from isoeugenol and 4-ethoxy-3-methoxy benzoic acid moiety). ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 14.88 (-CH_3, terminal methyl from isoeugenol moiety), 18.68 (-CH_3 from -OCH_2CH_3 group), 56.17 (–OCH_2), 56.26 (Ar x –OCH_3), 64.69 (Ar x –OCH_3), 110.0 (=CH), 111.53 (=CH), 112.9 (ArC), 118.66 (ArC), 121.81 (ArC), 123.13 (q, >C<), 124.74 (ArC), 126.17 (q, >C<), 130.77 (ArC), 137.21 (ArC), 139.16 (q, ArC-O), 149.1 (q, ArC-O), 151.55 (q, ArC-O), 153.06 (q, ArC-O), 164.9 (q, >C=O). TOF MS ES : 343 (M + H), 365 (M + Na). Molecular formula $C_{20}H_{22}O_5$. Pure viscous mass (1.42 gms, 67.94 %). Anal. Calcd. for $C_{20}H_{22}O_5$: C 70.16, H 6.48, O 23.36 Found C 70.12, H 6.52, O 23.39;

Compound 6 : [2-methoxy-4-[(E)-prop-1-enyl]phenyl]-3,4-dimethoxy benzoate

^1H NMR (400 MHz, $CDCl_3$) δppm : 1.89 (d, J = 6.4 Hz, 3H, terminal methyl from isoeugenol moiety), 3.82 (s, 3H, Ar x –OCH_3), 3.96 (s, 3H, Ar x –OCH_3), 3.97 (s, 3H, Ar x –OCH_3). 6.18 – 6.26 (m, 1H, olefinic proton

'a'), 6.40 (d, J = 15.8 Hz, 1H, olefinic proton 'b'), 6.8 – 8.0 (m, 6H, ArH from isoeugenol and 3, 4-dimethoxy benzoic acid moiety). ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 18.67 (-CH_3, terminal methyl from isoeugenol moiety), 56.041 (Ar x –OCH_3), 56.23 (Ar x –OCH_3), 56.38 (Ar x –OCH_3), 109.99 (=CH-), 110.55 (=CH-), 112.70 (ArC), 118.67 (ArC), 122.1 (ArC), 123.13 (ArC), 124.79 (ArC), 126.20 (q, >C<), 130.69 (q, >C<), 137.24 (ArC), 139.13 (q, ArC-O), 149.93 (q, ArC-O), 151.53 (q, ArC-O), 153.65 (q, ArC-O), 164.85 (q, >C=O). TOF MS ES : 329 (M + H), 351 (M + Na). Molecular formula $C_{19}H_{20}O_5$. Pure viscous mass (1.36 gms, 68.0 %). Anal. Calcd. for $C_{19}H_{20}O_5$: C 69.50, H 6.14, O 24.36 . Found C 69.54, H 6.10, O 24.40;

Compound 7 :- [2-methoxy-4-[(E)-prop-1-enyl]phenyl]-2,6-bis(trifluoromethyl) benzoate

^{1}H NMR (400 MHz, $CDCl_3$) δppm : 1.91 (d, J = 6.4 Hz, 3H, terminal methyl from isoeugenol moiety), 3.84 (s, 3H, Ar x –OCH_3), 6.20 – 6.29 (m, 1H, olefinic proton 'a'), 6.41 (d, J = 15.8 Hz, 1H, olefinic proton 'b'), 6.8 – 7.2 (m, 3H, ArH from isoeugenol moiety), 8.13 (s, 1H, ArH from 2,6-bis(trifluoromethyl) benzoyl moiety), 8.66 (s, 2H, ArH from 2,6-bis(trifluoromethyl) benzoyl moiety due to symmetry) ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 18.66 (-CH_3, terminal methyl from isoeugenol moiety), 56.07 (Ar x –OCH_3), 109.98 (=CH-), 118.67 (=CH-), 121.74 (ArC), 122.63 (ArC), 124.45 (ArC), 127.0 (ArC), 130.59 (ArC), 131.95 (ArC), 132.40 (ArC), 132.74 (ArC), 133.08 (q, 2 x –CF_3), 137.98 (q, 2 x –CF_3), 138.32 (q, ArC-O), 151.08 (q, ArC-O), 162.43 (q, >C=O). TOF MS ES : 405 (M + H), 427 (M + Na). Molecular formula $C_{19}H_{14}F_6O_3$. Pure viscous mass (1.76 gms, 71.54 %). Anal. Calcd. for $C_{19}H_{14}F_6O_3$: C 56.44, H 3.49, O 11.87, F 28.29 Found C 56.40, H 3.52, O 11.90;

Compound 8 : [2-methoxy-4-[(E)-prop-1-enyl]phenyl]-4-allyloxy benzoate

^{1}H NMR (400 MHz, $CDCl_3$) δppm : 1.88 (d, J = 6.4 Hz, 3H, terminal methyl from isoeugenol moiety), 3.81 (s, 3H, Ar x –OCH_3), 4.61 (d, J = 6.1 Hz, 2H, benzylic –CH_2), 5.32 (d, J = 10.6 Hz, 1H, olefinic proton), 5.43 (d, J = 17.3 Hz, 1H, olefinic proton), 6.1 – 6.3 (m, 1H, olefinic proton 'a'), 6.38 (d, J = 15.6 Hz, 1H, olefinic proton "b'), 6.8 – 8.2 (m, 7H, ArH from isoeugenol and 4-allyloxy benzoic acid moiety). ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 18.5 (-CH_3, terminal methyl from isoeugenol moiety), 55.9 (–OCH_2), 68.9 (Ar x –OCH_3), 109.8 (=CH), 114.48 (=CH), 114.58 (=CH), 118.23 (=CH), 118.26 (ArC), 121.96 (ArC), 122.9 (q, >C<), 125.9 (q, >C<), 130.6 (q, >C<), 132.4 (2 x ArC), 137.0 (2 x ArC), 138.9 (ArC), 151.4 (q, ArC-O), 162.8 (q, ArC-O), 164.6 (q, >C=O). TOF MS ES : 325 (M + H), 347 (M + Na). Molecular formula $C_{20}H_{20}O4$. Pure viscous mass (1.32 gms, 67.0 %). Anal. Calcd. for $C_{20}H_{20}O_4$: C 74.06, H 6.21, O 19.73 Found C 74.02, H 6.18, O 19.77;

Compound 9 : [2-methoxy-4-[(E)-prop-1-enyl]phenyl]-3-amino benzoate

^{1}H NMR (400 MHz, $CDCl_3$) δppm : 1.88 (d, J = 6.7 Hz, terminal methyl from isoeugenol moiety), 3.6 – 3.8 (brs, 2H, -NH_2), 3.81 (s, 3H, Ar x –OCH_3), 6.1 – 6.3 (m, 1H, olefinic proton 'a'), 6.39 (d, J = 15.8 Hz, 1H, olefinic proton 'b'), 6.8 – 7.7 (m, 7H, ArH from isoeugenol and 3-amino benzoic acid moiety). TOF MS ES : 284 (M + H), 306 (M + Na). Molecular formula $C_{17}H_{17}NO_3$. Pure viscous mass (1.24 gms, 72 %). Anal. Calcd. for $C_{17}H_{17}NO_3$: C 72.07, H 6.05, N 4.94, O 16.94 . Found C 72.03, H 6.08, O 16.98;

Advantages :-

1. Use of simple and inexpensive reactants.
2. High yields and purity of products.
3. Simplicity of the procedure.
4. Reaction carried out at room temperature.
5. Highly scalable.
6. The synthesis of Hybrid derivatives using DCC / DMAP is another approach to prepare ester derivatives.

III. EXPERIMENTAL

Mps. are uncorrected. ^{1}H and ^{13}CNMR spectrum 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.

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 '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 ditch plate method5 :- Conc 100 µg/ml

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,) bacterial species qualitatively. The results of the antibacterial activities are summarized in Table 1.

Theory : One of the many ways to test the anti-bacterial activity of compounds / drugs is ditch plate method. Ditch plate method is a preliminary method to screen the test compounds / drugs for their potential as anti-microbials . In this method , the compound to be tested for antimicrobial activity is seeded in the agar plate and the test organisms are streaked across.

Procedure : A ditch 10mm wide is cut into sterile MH agar plate. The test drug / compound is added to 5 ml molten MH agar butt at 40^{0}C 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 .

RESULTS : The following test samples showed anti-bacterial activity against the organisms mentioned in the following Table 1.

Table 1 : Antibacterial Activity Results

SAMPLE NO.	ACTIVITY AGAINST
Isoeugenol	*Staphylococcus aureus* [Gram positive] *Escherichia coli* [Gram negative]
4	*Staphylococcus aureus* [Gram positive] *Escherichia coli* [Gram negative]
7	*Staphylococcus aureus* [Gram positive] *Corynebacterium diphtheriae* [Gram positive] *Salmonella typhi* [Gram negative]

	Escherichia coli [Gram negative]
8	*Corynebacterium diphtheriae* [Gram positive] *Salmonella typhi* [Gram negative]

The above results shows that the base molecule, isoeugenol has antibacterial activity against both the bacterial cultures. Its derivatives viz. 4, 7 and 8 were also active against both the bacterial cultures. In depth analysis of these compounds through structure activity relationship studies would provide further insight and can be an interesting topic of future studies.

The structural diversity and the pronounced biological activities encountered in the isoeugenol hybrid 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 isoeugenol hybrid derivatives develop new therapeutic molecules that might result in candidates having better activity.

IV. REFERENCES

[1]. Wattenberg L. W., Coccia J. B.,Lam L. K. Inhibitory effects of phenolic compounds on benzo(a)pyrene-induced neoplasia. Cancer Res., 1980, 40, pp. 2820 - 2823.

[2]. Talalay P., De Long MU., Prochaska HJ. Identification of a common chemical signal regulating the induction of enzymes that protects against chemical carcinogenesis. Proc. Natl. Acad. Sci. USA.

[3]. Macheix JJ., Fleuriet A.;Billot J. Fruit phenolics. Boca Raton, FL; CTC; 1990.

[4]. The Merck Index, 12th edition, Merck & Co., Whitehouse Station, New Jersey, USA, 1996.

[5]. Finn R. K. Theory of Agar Diffussion Methods for Bioassay. Anal. Chem., 1959, 31 (6), pp 975 - 977.