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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL VANILLIC ACID HYBRID DERIVATIVES

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ABSTRACT

Vanillic acid (4-hydroxy-3-methoxy benzoic acid) is a dihydroxybenzoic acid derivative used as a flavoring agent. It is used in the synthesis of various active pharmaceutical ingredients such as Etamivan, Modecainide, Brovanexine, Vanitiolide, Vanyldisulfamide etc. In this paper, novel ester / hybrid derivatives of vanillic acid were synthesized and tested for potential antibacterial activity. This combinatorial synthesis of novel vanillic ester / hybrid derivatives can be a useful approach to generate potent chemotherapeutic agents in developing new drug candidates.

Keywords: Vanillic acid, IR, ^{1}HNMR, TOF MS, DCC, DMAP, antibacterial, ditch-plate method.

INTRODUCTION

Phenolic phytochemicals are known to exhibit anti-inflammatory, antioxidant, anticarcinogenic, antidiabetic, anti-atherosclerosis and immunomodulatory activities in animals[1,2]. These are mostly polyphenols known as secondary plant metabolites, present in plants and trees. Polyphenols are commonly divided into flavonoids and the hydroxyl cinnamic acids[3-5]. Vanillic acid is a naturally occurring active compound having antimicrobial, anti-inflammatory and antioxidant / anticancer properties, we thought of synthesizing compounds with a novel ether, ester and hybrid derivatives of vanillic acid wherein vanillic acid would be etherified, esterified and hybridized with various other compounds and to check whether these compounds possess above biological activities[6-18]. 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 a mixed variety of those disease domain or to have drug candidate with entirely different biological activity.

EXPERIMENTAL

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

Melting points were determined on a Thomas Hoover capillary melting point apparatus using a digital thermometer. IR spectra were recorded on a Shimadzu FTIR Prestige model as KBr pellet. ^{1}H NMR spectra were recorded on a Varian 200 MHz spectrometer in $CDCl_3$. Chemical shifts were recorded in parts per million downfield from tetramethyl silane. Mass spectra were recorded on a TOF MS ES mass spectrometer. Elemental analysis was carried out as a percentage on a Thermo finnigan, Flash EA 1112 series, Italy.

RESULTS AND DISCUSSION

Preparation of 3,4 - dialkoxy benzoic acids :- Vanillic acid was subjected to esterification (MeOH / Conc. H_2SO_4) followed by etherification (K_2CO_3 / Acetone / Alkyl halide) to yield crude ether derivatives

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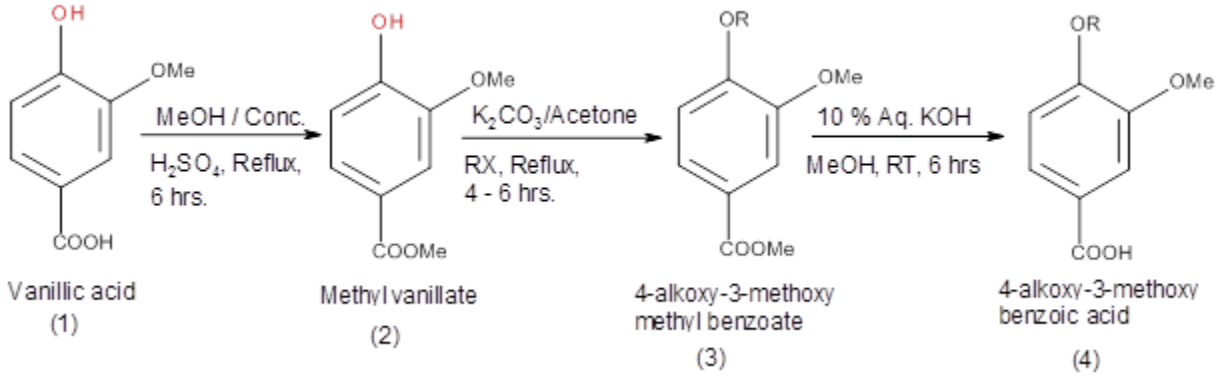
which were purified by column chromatography. These purified ether derivatives were subjected to hydrolysis (Aq. KOH / MeOH and then Conc. HCl) to yield 4-alkoxy vanillic acids respectively.

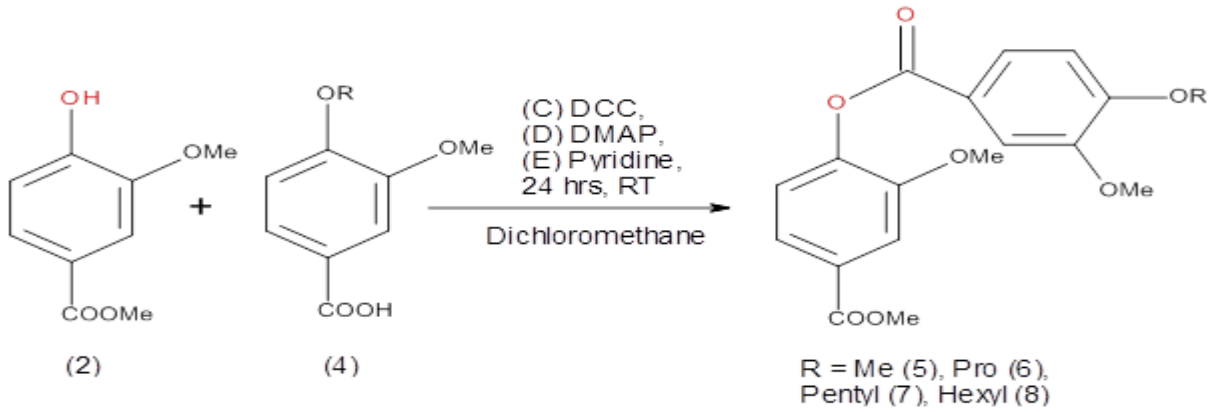


Scheme-1

Above 4-alkoxy-3-methoxy benzoic acids were then condensed with methyl vanillate under DCC/DMAP/Pyridine condition to yield series of hybrid derivatives whose structures were unambiguously confirmed by IR, ^{1}H NMR, Mass spectroscopy and elemental analysis and tested for their potential antimicrobial activity.

Synthesis of Fused Molecules Using Compounds (2) and (4)

These were prepared by following the general method. To a stirred solution of methyl vanillate [2] (1 eq.) in 30 ml, dichloromethane was added DCC [C] (1.3 eq.), DMAP [D] (0.05 eq.), pyridine [E] (0.5 eq.) and the reaction mixture stirred at room temperature for 5 min. A clear solution of the reaction mixture was obtained. To this, compound [4] aromatic / substituted aromatic acid (1.3 eq.) was added and stirring continued at room temperature for the next 24 hrs. As the reaction proceeds, urea derivative precipitates out as a by-product.

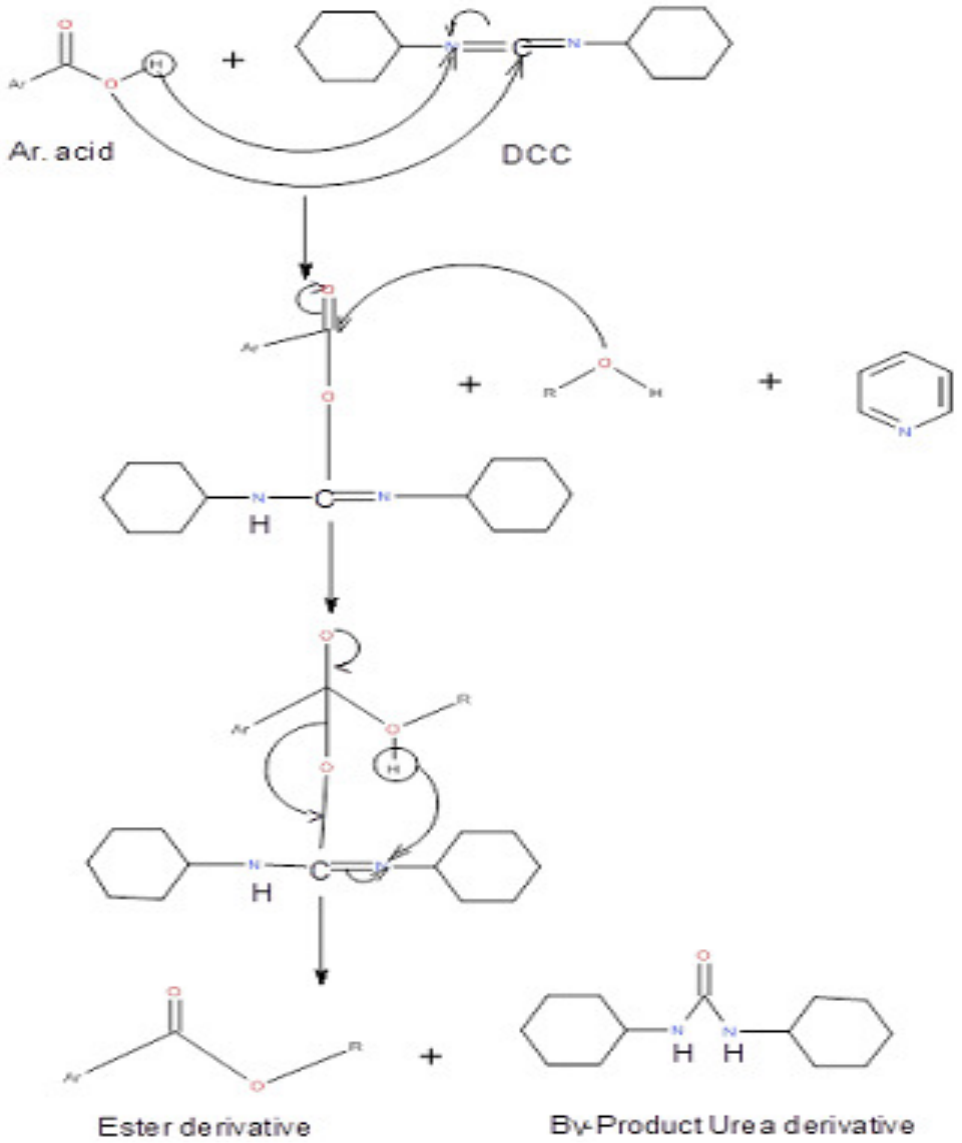


S. No.	2	4	Product (R)
1	Methyl vanillate	3,4-Dimethoxy benzoic acid	$-CH_3$ (5)
2	Methyl vanillate	3-methoxy-4-propoxy benzoic acid	$-C_3H_7$ (6)
3	Methyl vanillate	3-methoxy-4-pentoxy benzoic acid	$-C_5H_{11}$(7)
4	Methyl vanillate	3-methoxy-4-hexoxy benzoic acid	$-C_6H_{13}$ (8)

Scheme-2

The progress of the reaction is monitored by TLC for completion of the reaction. The reaction mixture filtered through celite bed which gets 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 an increase in the concentration of ethyl acetate in petroleum ether. The general yields of these reactions range between 70 – 90 %. This is another method of preparing esters and follows green chemistry parameters.



Scheme-3: Probable Mechanism For Fused/ Hybrid Molecules

Compound-1: (2-methoxy-4-methoxycarbonyl-phenyl) 3,4-dimethoxybenzoate (5)

^{1}H NMR ($CDCl_3$, 200 MHz)δ ppm: 3.87 (s, 6H, 2 x Ar–OCH_3), 3.89 (s, 3H, 1 x Ar–OCH_3), 3.92 (s, 3H, 1 x Ar–$COOCH_3$), 6.8 – 7.8 (m, 6H, ArH); TOF MS ES: 369 (M + Na); IR (KBr) cm^{-1}:- 2928, 2854 (Methyl, methines), 1715 (ester carbonyl), 1513 (aromatic); Molecular Formula $C_{18}H_{18}O_7$; Melting range 112 – 115^{0}C; Elemental Analysis, Calcd.: C 62.50 %, H 5.22 %, O 32.28 %. Found C 62.47 %, H 5.25 %, O 32.3.

Compound-2: (2-methoxy-4-methoxycarbonyl-phenyl) 3-methoxy-4-propoxy-benzoate (6)

^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm : 1.071 (t, J = 7.6 Hz, 3H, Terminal –CH_3 from propyl bromide moiety), 1.91 (m, 2H, - CH_2 from propyl bromide moiety), 3.88 (s, 3H, 1 x Ar–OCH_3), 3.93 (s, 3H, 1 x Ar–OCH_3), 3.94 (s, 3H, 1 x Ar–OCH_3), 4.07 (t, J = 6.0 Hz, 2H, -OCH_2 group), 6.6 – 8.0 (m, 6H, ArH); TOF MS ES: 397 (M + Na); IR (KBr) cm^{-1}:- 2968, 2947, 2876, 2849 (Methyl, methines), 1731

(ester carbonyl), 1508 (aromatic); Molecular Formula $C_{20}H_{22}O_7$; Melting range 110 – 112^{0}C; Elemental Analysis, Calcd.: C 64.21 %, H 5.89 %, O 29.90 %. Found C 64.18 %, H 5.92 %, O 29.91 %.

Compound-3: (2-methoxy-4-methoxycarbonyl-phenyl) 3-methoxy-4-pentoxy-benzoate (7)
^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm : 0.88 (t, J = 7.4 Hz, 3H, terminal methyl from pentyl bromide moiety), 0.9 – 1.9 (m, 6H, 3 x –CH_2 from pentyl bromide moiety), 3.88 (s, 3H, 1 x –OCH_3 group), 3.91 (s, 3H, Ar–OCH_3), 3.91 (s, 3H, Ar x –OCH_3), 3.93(s, 3H, Ar x -OCH_3), 4.04 (t, J = 6.0 Hz, 2H, -OCH_2 group), 6.6 – 7.9 (m, 6H, ArH) moiety); TOF MS ES: 425 (M + Na); IR (KBr) cm^{-1}:- 2927, 2945, 2870, 2852 (Methyl, methines), 1732 (ester carbonyl), 1507 (aromatic); Molecular Formula $C_{22}H_{26}O_7$; Melting range 103 – 105^{0}C; Elemental Analysis, Calcd.: C 65.70 %, H 6.50 %, O 27.80 %. Found C 65.66 %, H 5.53 %, O 27.81 %.

Compound-4: (2-methoxy-4-methoxycarbonyl-phenyl) 4-hexoxy-3-methoxy-benzoate (8)
^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm : 0.95 (t, J = 6.8 Hz, 3H, terminal methyl from hexyl bromide moiety), 1.2 – 2.0 (m, 8H, 4 x –CH_2 from hexyl bromide moiety), 3.89 (s, 3H, Ar x –OCH_3), 3.94 (s, 3H, Ar x –OCH_3), 3.95 (s, 3H, 1 x –OCH_3 from Ar x –$COOCH_3$ group), 4.16 (t, J = 6.8 Hz, 2H, -OCH_2 group), 6.8 – 8.0 (m, 6H, ArH); TOF MS ES: 439 (M + Na); IR (KBr) cm^{-1}:- 2951, 2927, 2850 (Methyl, methines), 1731 (ester carbonyl), 1512 (aromatic); Molecular Formula $C_{23}H_{28}O_7$; Melting range 133 – 135^{0}C; Elemental Analysis, Calcd.: C 66.32 %, H 6.78 %, O 26.90 %. Found C 66.29 %, H 6.81 %, O 26.89 %.

Chromatographic Study
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 an increase in the 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 was employed.

Biological Activity
Antibacterial Activity using ditch plate method[19]
The synthesized molecules were screened for their antibacterial activity at 100 µg/ml concentration using ditch plate method against Gram + ve (*Staphylococcus aureus*, *Corynebacterium diphtheria*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*) bacterial species qualitatively. The results of the antibacterial activities are summarized in Table-1.

Theory
Ditch plate method is the method 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 the 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 a 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.

Table-1: Antibacterial Activity Results

Sample No.	Active Against	
Base molecule Methyl vanillate (2)	*Staphylococcus aureus*	[Gram positive]
	Salmonella typhi	[Gram negative]
	Klebsiella pneumoniae	[Gram negative]
	Corynebacterium diphtheriae	[Gram positive]
	Escherichia coli	[Gram negative]
(7)	*Staphylococcus aureus*	[Gram positive]
	Salmonella typhi	[Gram negative]
	Corynebacterium diphtheriae	[Gram positive]
	Escherichia coli	[Gram negative]
(8)	*Staphylococcus aureus*	[Gram positive]
	Klebsiella pneumoniae	[Gram negative]
	Corynebacterium diphtheriae	[Gram positive]
	Escherichia	[Gram negative]

CONCLUSION

The novel hybrid derivatives of vanillic 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 a dehydrating agent in a reasonably good yield. The probable mechanism for the formation of the hybrid derivative was also discussed.

The biological activity suggests that the base molecule methyl vanillate have anti-bacterial activity against both the bacterial cultures. Its derivatives *viz.* 7 and 8 were also active against certain Gram + ve and Gram-ve cultures. Thus, fused molecules of vanillic acid (7 & 8) having long alkyl side chain 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.

The structural diversity and the pronounced biological activities encountered in the vanillic acid 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 vanillic acid derivatives develops new therapeutic molecules that might result in candidates having better activity. All synthesized hybrid derivatives were chemically new and confirmed by SciFinder search.

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