One Pot Syntheses and Biological Screening of New Vanillic Acid Esters

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Summary: High yielding syntheses of new esters (**1a-1l)** have been developed by way of facile one-pot reaction of vanillic acid (**1**) with a variety of 2-bromoacetophenone derivatives. Their structures were elucidated through spectroscopic data. Vanillic acid and its newly synthesized esters have been screened for antioxidant activity along with inhibition studies against the enzymes lipoxygenase and urease, respectively. The compound **1c** exhibited potent antioxidant activity with an IC_{50} value of 44.5±0.72 µ M, while **1e** showed significant inhibitory potential against lipoxygenase with an IC_{50} value of 11.4±0.22 µM. On the other hand, **1a**, **1d, 1e**, **1f** and **1k** revealed promising inhibitory activity against urease with IC_{50} values being 23.4±0.22, 22.2±0.42, 29.8±0.06, 24.6± 0.17 and 26.7±0.13µM, respectively.

Keywords: Vanillic acid esters, Syntheses, Antioxidant activity, Lipoxygenase and Urease inhibition.

Introduction

Polyphenols form a major component of human diet and their consumption is related to various beneficial effects on health related issues. Exploration of beneficial activity of polyphenols has been a matter of interest due to popularization of herbal medicines with minimum side effects. Polyphenols have two major classes, comprising of flavonoids and phenolic acids, respectively [1]. Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids These are widely distributed in plants and are known for their antioxidant activity which is the main cause of many biological activities including anti-inflammatory, antiviral, antiatherogenic, antibacterial, and anticancer [2]. Vanillic acid (4-hydroxy-3-methoxy benzoic acid), an oxidized form of vanillin, has been used as an intermediate in the production of pharmaceuticals such as Rhizoma, Picrorhizae, Ginseng, Propolis and BaiHao [3]. It has several medicinal properties including antifilarial, antimicrobial, free radical scavengers, chemopreventive, hepatoprotective, antihypertensive [4] and neuroprotective [5]. A number of vanillic acid derivatives have been reported to possess immunosuppressive properties [3], antibacterial [6], neuroprotective [7], anticancer[8] and antimicrobial [9] activities. These findings prompted us to synthesize further new derivatives of vanillic acid.

Herein we report the syntheses of a series of esters (**1a-1l**) by way of a facile one-pot synthesis involving vanillic acid (**1**) and a variety of 2-bromoacetophenone derivatives as alkylating agents. All the synthesized esters were subjected to biological screening to ascertain their possible therapeutic utility.

Experimental

Chemical

Vanillic acid was obtained in excellent yield from *Alstonia scholaris* (a member of the family Apocynaceae) while its additional quantity was procured from E-Merck (Darmstadt, Germany). All alkylating agents including 2-bromo-4'-chloroacetophenone, 2-bromo-2',5'-dimethoxyacetophenone, 2-bromo-4'-flouroacetophenone, 2-bromo-4'-phenylacetophenone, 2-bromo-3'-nitroacetophenone, 2-bromo-3',4'-dichloroacetophenone, 2-bromoacetophenone, 2-bromo-4'-nitroacetophenone, 2-bromo-4'-methylacetophenone and 2,4'-dibromoacetophenone were acquired from Sigma-Aldrich (Saint Louis, USA) and E. Merck, respectively.

Instruments

Melting points of the compounds were determined using a Buchi B 540 melting point apparatus and are uncorrected. IR spectra were recorded in the range of 4000-400 cm^{-1}. on a JASCO IRA instrument. Low and high resolution mass spectra were recorded on Varian-MAT 112S and Finnigan MAT -112312, double focusing mass spectrometers connected to DEC PDP 11/34 and IBM-AT compatible PC-based system, respectively. NMR spectra were recorded on Bruker AM 400 NMR spectrometer operating at 400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR. Chemical shifts (δ) are reported in ppm.

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Syntheses of vanillic acid esters

To a solution of vanillic acid **1** (1 mmol) in acetonitrile (35 ml), was added 1 ml of saturated solution of Na_2CO_3. After warming for a period of 30 min., added the appropriate 2-bromoacetophenone (2 mmol) was added. The solution was stirred at 70°C for 6h. The solvent was removed and the residue was partitioned between water and dichloromethane. The organic phase was successively washed with dilute sodium bicarbonate and water. Removal of solvent provided the crude products which were finally purified by column chromatography leading to pure esters **1a-1d**. Raising the temperature to 120°C and replacing the base with potassium carbonate furnished the esters **1e-1l**, respectively.

1-(4-Chlorophenyl)-1-oxoethyl-4-hydroxy-3-methoxybenzoate **(1a)**

Prepared from 2-bromo-4'-chloroacetophenone. Yield 74% **;** m.p. 148-150 °C; IR (KBr): 3512 (OH), 1680 (C=O), 1590 (aromatic C=C) cm^{-1}; HRMS (EI): *m/z* 320.0450 (calcd. 320.0452 for $C_{16}H_{13}ClO_5$), 322.0423 (calcd. 322.0266 for $C_{16}H_{13}{}^{37}ClO_5$); ^{1}H NMR (CD_3OD): δ 3.90 (3H, s, OMe), 5.58 (2H, s, H-2'), 6.86 (1H, d, *J*=8.0 Hz, H-5), 7.55 (2H, d, *J*=8.0 Hz, H-3''), 7.61 (1H, d, *J*=2.0 Hz, H-2), 7.62 (1H, dd, *J*=8.0, 2.0 Hz, H-6), 8.02 (2H, d, *J*= 8.0 Hz, H-2'').

1-(4-Nitrophenyl)-1-oxoethyl-4-hydroxy-3-methoxybenzoate **(1b)**

Obtained from 2-bromo-4'-nitroacetophenone. Yield 70% **;** m.p. 173-177 °C; IR (KBr): 3469 (OH), 1680 (C=O), 1595 (aromatic C=C) cm^{-1}; HRMS (EI): *m/z* 331.0792 (calcd. for [$C_{16}H_{13}NO_7]^+$: 331.0749); ^{1}H NMR ($CDCl_3$): δ 3.96 (3H, s, OMe), 5.53 (2H, s, H-2'), 6.98 (1H, d, *J*=8.3 Hz, H-5), 7.60 (1H, d, *J*=1.7 Hz, H-2), 7.75 (1H, dd, *J*=8.3 Hz, 1.7 Hz, H-6), 8.13 (2H, d, *J*=8.7 Hz, H-3'', 5''), 8.37 (2H, d, *J* = 8.7 Hz, H-2'', 6'').

1-(3-Nitrophenyl)-1-oxoethyl-4-hydroxy-3-methoxybenzoate **(1c)**

Prepared from 2-bromo-3'-nitroacetophenone. Yield 72%; m.p. 171-173°C; IR (KBr): 3467 (OH), 1685 (C=O), 1590 (aromatic C=C) cm^{-1} ; HRMS (EI): *m/z* 331.0745 (calcd. for $[C_{16}H_{13}NO]^+$: 331.0749); ^{1}H NMR ($CDCl_3$): δ 3.88 (3H, s, OMe). 5.49 (2H, s, H-2'), 6.88 (1H, d, *J*=8.2 Hz, H-5), 7.53 (1H, d, *J*=1.8 Hz, H-2), 7.64 (1H, dd, *J*=8.2, 1.8 Hz, H-6), 7.69 (1H, t, *J*=8.0 Hz, H-5''), 8.24 (1H, br.d, *J*= 7.7 Hz, H-6''), 8.41 (1H, br.d, *J*= 8.0 Hz, H-4''), 8.74 (1H, br.s, H-2'').

1-(4-Flurophenyl)- 1-oxoethyl-4-hydroxy-3-methoxybenzoate **(1d)**

Reaction of **1** with 2-bromo-3'-flouroacetophenone led to the compound **1d**. Yield 79% **;** m.p. 163-165°C; IR (KBr): 3469 (OH), 1686 (C=O), 1592 (aromatic C = C) cm^{-1} ; HRMS (EI): *m/z* 304.0746 (calcd. for $[C_{16}H_{13}FO_5]^+$: 394.0739); ^{1}H NMR ($CDCl_3$): δ 3.94 (3H, s, OMe), 5.48 (2H, s, H-2'), 6.98 (1H, d, *J* = 8.3 Hz, H-5), 7.16 (2H, t, *J* = 8.5 Hz, H-3'', H-5''), 7.60 (1H, d, *J*=2.0 Hz, H-2), 7.73 (1H, dd, *J*=8.3, 2.0 Hz, H-6), 7.99 (2H, dd, *J*= 8.5,1.5 Hz, H-2'', H-6'').

1-(4-Fluorophenyl)-1-oxoethyl-4-O-[1-(4-fluorophenyl)-1-oxoethyl]-3-methoxybenzoate **(1e)**

The di-ester **1e** was prepared from 2-bromo-4'-floroacetophenone at 120°C. Yield 81% **;** m.p. 187-190 °C; IR (KBr): 2938 (CH), 1680 (C=O), 1590 (C = C) cm^{-1}; HRMS (EI): *m/z* 440.1071 (calcd. for [$C_{24}H_{18}F_2O_6]^+$: 440.1074) ; ^{1}H NMR ($CDCl_3$): δ 3.92 (3H, s, OMe), 5.35 (2H, s, H-2' '), 5.48 (2H, s, H-2'), 6.80 (1H, d, *J* = 8.4 Hz, H-5), 7.16 (4H, t, *J* = 8.5 Hz, H-3'', H-5''), 7.63 (1H, d , *J* = 1.8 Hz, H-2) , 7.69 (1H, dd, *J* = 8.4, 1.8 Hz, H-6), 7.98 (2H, dd, *J*= 8.6, 5.3 Hz, H-2'', H-6''), 8.04 (2H, dd, *J*= 8.6, 5.3 Hz, H-2''', H-6'''); ^{13}C-NMR ($CDCl_3$): δ 56.13 (OMe), 66.21 (C-2') ,71.51 (C-2'''), 112.91 (C-5) , 113.18 (C-2) , 116.03 (C-5'') , 116.06 (C-3''), 116.21 (C-5'''), 116.23 (C-3'''), 123.13 (C-1), 123.82 (C-6), 130.52 (C-6'') , 130.59 (C-2''), 130.94 (C-6'''),),131.03 (C-2'''), 149.22 (C-3) 151.65 (C-4),165.14 (C-4'''), 165.21 (C-4''), 165.54 (C-7), 167.17 (C-1''), 167.25 (C-1'''), 190.72 (C-1'), 192.30 (C-1''').

1-(2,5-Dimethoxyphenyl)-1-oxoethyl-4-O-[1-(2,5-dimethoxyphenyl)-1-oxoethyl]-3-methoxybenzoate **(1f)**

Prepared from 2-bromo-2',5'-dimethoxyacetophenone. Yield 78% **;** m.p. 187-190 °C; IR (KBr): 1685 (C=O), 1601 (C = C) cm^{-1}; HRMS (EI): *m/z* 524.1673 (calcd. for $[C_{28}H_{28}O_{10}]^+$: 524.1682); ^{1}H NMR ($CDCl_3$): δ 3.93 (3H, s, OMe), 5.38 (2H, s, H-2'''), 5.42 (2H, s, H-2'), 6.70 (1H, d, *J*=8.4, H-5), 6.92 (1H, d, *J*= 8.5 Hz, H-3''''), 6.94 (1H, d, J= 8.5 Hz, H-3''), 7.07 (1H, dd, *J*= 8.5, 3.2 Hz, H-4''''), 7.10 (1H, dd, *J*= 8.5, 3.2 Hz, H-4''), 7.44 (1H,d, 3.2 Hz, H-6''''),7.45 (1H,d, 3.2 Hz,H-6''), 7.64 (1H, d, *J*=1.9 Hz, H-2), 7.69 (1H, dd, *J*=8.4, 1.9 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 56.10, 56.10, 55.85, 55.81 (OMe), 70.38 (C-2'), 74.58 (C-2'''), 112.08 (C-5), 112.95 (C-2'

), 113.00 (C-3""), 113.03 (C-2), 122.09 (C-4", C-4""), 122.85 (C-1), 123.72 (C-6), 124.60 (C-1"), 124.70 (C-1'"), 148.87 (C-3), 151.95 (C-4),153.83 (C-5'""), 153.97 (C-2"), 154.10 (C-2""), 165.90(C-7), 192.95 (C-1'), 193.78 (C-1'").

1-(4-Bromophenyl)-1-oxoethyl-4-O-[1-(4-bromophenyl)-1-oxoethyl]-3-methoxybenzoate **(1g)**

Reaction of **1** with 2,4'-dibromoacetophenone furnished **1g**. Yield 80% **;** m.p. 169-174°C; IR (KBr): 1702 (C=O), 1587 (C=C) cm^{-1}; HRMS (EI): *m/z* 560.97 91 (calcd. for [$C_{24}H_{18}{}^{79}Br_2O_6]^+$: 560.9738) & 562.9648 (calcd. for $[C_{24}H_{18}{}^{81}BrBrO_6]^+$: 562.9698); ^{1}H NMR (C_3D_6O): δ 3.90 (3H, s, OMe), 5.64 (2H, s, H-2'"), 5.65 (2H, s, H-2'), 7.07 (1H, d, *J* = 8.0 Hz, H-5), 7.61 (1H, d, *J* = 1.8 Hz, H-2), 7.65 (1H, dd, *J* = 8.0, 1.8 Hz, H-6), 7.76 (2H, d, *J*=8.0 Hz, H-2", H-6"), 7.78 (2H, d, *J*= 8.0 Hz, H-2"", H-6""), 7.78 (2H, d, *J*= 8.0 Hz, H-2"", H-6""), 7.99 (2H, d, *J*= 8.0 Hz, H-2", -6"), 8.01 (2H, d, *J*= 8.0 Hz, H-2"", H-6""); ^{13}C-NMR ($CDCl_3$): δ 56.09 (OMe), 66.21 (C-2'), 71.48 (C-2'"), 112.91 (C-5),113.14 (C-2), 123.08 (C-1), 123.78 (C-6), 129.13 (C-1"), 129.28 (C-1'""), 122.29 (C- 2"),129.66 (C-2""), 132.23 (C-3"), 132.99 (C-3""), 133.02 (C-4""), 149.19 (C-3), 151.57 (C-4), 165.45 (C-7), 192.41 (C-1'), 192.94 (C-1'").

1-(4-Methylphenyl)-1-oxoethyl-4-O-[1-(4-methylphenyl)-1-oxoethyl]-3-methoxybenzoate **(1h)**

Prepared from 2-bromo-4'-methylacetophenone. Yield 81% **;** m.p. 148-152°C; IR (KBr): 1695 (C=O), 1603 (C = C) cm^{-1}; HRMS (EI): *m/z* 432.1579 (calcd. for $[C_{26}H_{24}O_6]^+$: 432.1573); ^{1}H NMR ($CDCl_3$): δ 2.43 (6H, s, CH_3), 3.94 (3H, s, OMe), 5.40 (2H, s, H-2'"), 5.51 (2H, s, H-2'), 6.80 (1H, d, *J* = 8.4 Hz, H-5), 7.30 (4H, d, *J* = 8.1 Hz, H-2", H-6", H-2"", H-6""), 7.66 (1H, d, *J* = 1.6 Hz, H-2), 7.72 (1H, dd, *J* = 8.4, 1.6 Hz, H-6), 7.87 (2H, d, *J* = 8.1 Hz, H-2", H-6"), 7.89 (2H, d, *J* = 8.1 Hz, H-2"", H-6""); ^{13}C-NMR ($CDCl_3$): δ 21.76 (6H, *C*H_3). 56.12 (OMe), 71.29 (C-2'"), 112.64 (C-5), 113.11 (C-2), 123.04 (C-1), 123.81 (C-6), 127.92 (C-2"), 128.16 (C-2""), 129.54 (C-3"), 129.57 (C-3""), 131.87 (C-1", C-1""), 144.80 (C-4"), 145.77 (C-4""), 149.12 (C-3), 151.77 (C-4), 165.65 (C-7), 191.87 (C-1'), 193.09 (C-1'").

1-([1,1'-Biphenyl]-4-yl)-1-oxoethyl 4-O-[1-{(1,1'-biphenyl)-4-yl}-1-oxoethyl]-3-methoxybenzoate **(1i)**

Reaction of **1** with 2-bromo-4'-phenyloacetophenone. Furnished **1i.** Yield 75% **;** m.p. 131-133°C; IR (KBr): 1689 (C=O), 1601 (C = C) cm^{-1}; HRMS (EI): *m/z* 556.1909 (calcd. for [$C_{36}H_{28}O_6]^+$: 556.1944); ^{1}H NMR ($CDCl_3$): δ 3.91 (3H, s, OMe), 5.42 (2H, s, H-2'"), 5.53 (2H, s, H-2'), 6.81 (1H, d, *J* = 8.4 Hz, H-5), 7.36-7.59 (10H, m, H-2"", H-6"", H-2"", H-6""), 7.64 (1H, d, *J*= 1.6 Hz, H-2), 7.69 (4H, m, H-3", H-5", H-3"", H-5""), 7.70 (1H, m, H-6), 8.00 (2H, d, *J* = 8.3 Hz, H-2", H-6"), 8.04 (2H, d, *J*=8.3 Hz, H-2"", H-6""); ^{13}C-NMR ($CDCl_3$): δ 56.10 (OMe), 112.07 (C-5), 113.12 (C-2), 124.03 (C-6),127.50 (4C, C-3", C-5", C-3"", C-5""), 128.42 (C-2", C-6"), 128.80 (C-2"", C-6""), 132.05 (C-1", C-1'""), 146.00-128.12 (C-1"", C-6"", C-1", C-6""), 147.01 (C-4", C-4""), 149.21 (C-3), 153.01 (C-4), 166.00 (C-7), 192.02 (C-1'), 193.30 (C-1'").

1-(Phenyl)-1-oxoethyl-4-O-[1-(phenyl)-1-oxoethyl]-3-methoxybenzoate **(1j)**

Vanillic acid reacted with 2-bromoacetophenone leading to **1j**. Yield 82% ; m.p. 106-110°C; IR (KBr): 1693 (C=O), 1596 (C=C) cm^{-1}; HRMS (EI): *m/z* 440.1246 (calcd. for [$C_{24}H_{20}O_6]^+$: 440.126); ^{1}H NMR ($CDCl_3$): δ 3.90 (3H, s, OMe), 5.42 (2H, s, H-2'"), 5.52 (2H, s, H-2'), 6.81 (1H, d, *J*=8.3 Hz, H-5),7. 48 (2H, m, H-6", H-6""), 7.58 (4H, m, H-3", H-5", H-3"", H-5""), 7.63 (1H, d, *J*= 1.5 Hz, H-2), 7.70 (1H, d, *J* = 8.3, 1.5 Hz, H-6), 7.94 (2H, d, *J* = 8.6 Hz, H-2", H-6"), 7.99 (2H, d, *J* = 8.6 Hz, H-2"", H-6""); ^{13}C-NMR ($CDCl_3$): δ 55.95 (OMe), 66.30 (C-2'), 71.15 (C-2'"), 112.67 (C-5), 112.99 (C-2), 123.64 (C-6), 123.88 (C-1), 127.67-133.79 (C-2", C-6", C-2"", C-6""), 133.85 (C-1"), 134.17(C-1'""), 149.00 (C-3), 151.61 (C-4), 165.44 (C-7), 192.18 (C-1'), 193.34 (C-1'").

1-(3,4-Dichlorophenyl)-1-oxoethyl-4-O-[1-(3,4-dichlorophenyl)-1-oxoethyl]-3-methoxybenzoate **(1k)**

Prepared from 2-bromo-3',4'-dichloroacetophenone. Yield 75% **;** m.p. °C; IR (KBr): 1690 (C=O), 1592 (C = C) cm^{-1}; HRMS (EI): *m/z* 539.9704 (calcd. for $[C_{24}H_{16}{}^{35}Cl_4O_6]^+$: 539.9701), 541.9816 (calcd. for [$C_{24}H_{16}{}^{37}ClCl_3O_6]^+$: 541.9857), 543.9642 (calcd. 543.9641 for $[C_{24}H_{16}{}^{37}Cl_2Cl_2O_6]^+$: 543.9642), 545.9610 (calcd. for $[C_{24}H_{16}{}^{37}Cl_3ClO_6]^+$: 545.9612); ^{1}H NMR ($CDCl_3$): δ 3.94 (3H, s, OMe), 5.31 (2H, s, H-2'"), 5.46 (2H, s, H-2'), 6.83 (1H, d, *J*=8.4 Hz, H-5), 7.58 (1H,d, *J*= 8.3 Hz, H-5"), 7.60 (1H, d, *J*=8.3 Hz, H-5""), 7.64 (1H, d, *J*=1.2 Hz, H-2), 7.71 (1H, dd, *J*=8.4, 1.2 Hz, H-6), 7.78 (1H, dd, *J*=8.3, 1.3 Hz, H-6"), 7.87 (1H, dd, *J*=8.3,1.3 Hz, H-6""), 8.04 (1H, br.d, *J*=1.3 Hz, H-2), 8.16 (1H, br.d, *J*=1.3 Hz, H-2""); ^{13}C-NMR ($CDCl_3$): δ 56.13 (OMe), 66.19 (C-2'), 71.84 (C-2'"), 113.36 (C-2), 113.43 (C-5), 123.83 (C-6), 124.04 (C-1), 124.04 (C-1), 129.90 (C-2"), 130.47 (C-2""), 131.01 (C-5""), 131.09 (C-5""), 133.87 (C-3", C-3""), 134.02 (C-4", C-4""), 138.85 (C-1"), 138.96 (C-1""), 149.54 (C-3), 151.68 (C-4), 165.60 (C-7), 171.84 (C-1'"), 190.60 (C-1'), 192.38 (C-1'").

1-(4-Chlorophenyl)-1-oxoethyl- 4-O-[1-(4-chlorophenyl)-1-oxoethyl]-3-methoxybenzoate **(1l)**

Synthesized from 2-bromo-4'-chloroacetophenone. Yield 77%**;** m.p. 161-163°C; IR (KBr): 2942 (CH), 1685 (C=O), 1600 (aromatic C=C) cm^{-1}; HRMS(EI): *m/z* 472.0465 (calcd. for $[C_{24}H_{18}Cl_2O_6]^+$: 472.0480), 474.0481(calcd. for $[C_{24}H_{18}{}^{37}ClClO_6]^+$: 474.0479), 476.0522 (calcd. for $[C_{24}H_{18}{}^{37}Cl_2O_6]^+$: 476.0518); ^{1}H NMR ($CDCl_3$+CD_3OD): δ 3.91 (3H, s, OMe), 5.34 (2H, s, H-2'''), 5.47 (2H, s, H-2'), 6.81 (1H, d, *J* = 8.4 Hz, H-5), 7.46 (4H, d, *J* = 8.3 Hz, H-3'', H-5'', H-3'''', H-5''''), 7.62 (1H, d, *J* =1.5 Hz, H-2), 7.68 (1H, dd, *J* = 8.4, 1.5 Hz, H-6), 7.80 (2H, d, *J* = 8.4 Hz, H-2'', H-6''), 7.94 (2H, d, *J* = 8.4 Hz, H-2'''', H-6''''); ^{13}C-NMR ($CDCl_3$+CD_3OD): δ 56.13 (OMe), 66.26 (C-2'), 77.21 (C-2'''), 112.97 (C-5), 113.19 (C-2), 123.82 (C-6), 129.25 (C-3'', C-5'', C-3'''', C-5''''), 129.28 (C-2'', -6''), 129.64 (C-2'''', C-6''''), 132.63 (C-1''), 132.65 (C-1''''), 140.44 (C-4''''), 140.58 (C-4''), 149.23 (C-3), 151.61 (C-4), 165.51 (C-7), 191.22 (C-1'), 192.77 (C-1''').

Procedures for Bioassays

Determination of DPPH Assay: DPPH radical-scavenging activity was achieved by the method defined by Gülçin *et al.* [10]. Lipoxygenase Inhibition Assay: The method used for lipoxygenase assay as reported previously by Wahab *et al.*, [11]. Urease Inhibition Assay: Urease activity was determined by measuring ammonia production using the method described by Weatherburn [12].

Results and Discussion

A variety of alkylating agents derived from acetophenone have been employed in the current studies including 2-bromo-4'-chloroacetophenone, 2-bromo-2',4'-dimethoxyacetophenone, 2-bromo-4'-flouroacetophenone, 2-bromo-4'-phenylacetophenone, 2-bromo-3'-nitroacetophenone, 2-bromo-3',4'-dichloroacetophenone, 2-bromoacetophenone, 2-bromo-4'-nitroacetophenone, 2-bromo-4'-methylacetophenone and 2-bromo-4'-bromoacetophenone. Their reactions with vanillic acid (**1**) were to be temperature dependent**.** The reactions at 70^o C in acetonitrile and sodium carbonate alkylated the more activated carboxylic group leading to esters **1a-1d**. Raising the temperature to 120^{o}C and replacing the base with potassium carbonate resulted in alkylation of both the carboxylic and phenolic functionalities to furnish the esters **1e-1l** (Figures 1 and 2). The structures of the synthesized derivatives were elucidated by spectroscopic techniques including IR, mass and NMR. The esters **1a-1d** invariably showed absorption bands for phenolic moiety between 3300-3400 $cm^{-1}$and oxymethylene protons in ^{1}H-NMR spectra between 5-6 ppm. On the other hand, the esters **1e-1l** did not show the absorption for phenolic moiety in IR spectra and the ^{1}H- NMR now showed a pair of oxymethylene protons between 5-6 ppm.

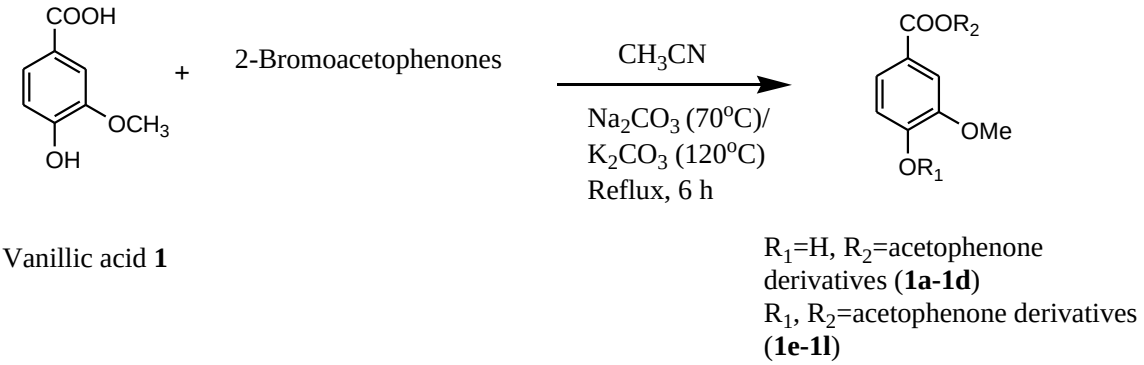

Fig. 1: Scheme for the synthesis of vanillic acid esters.

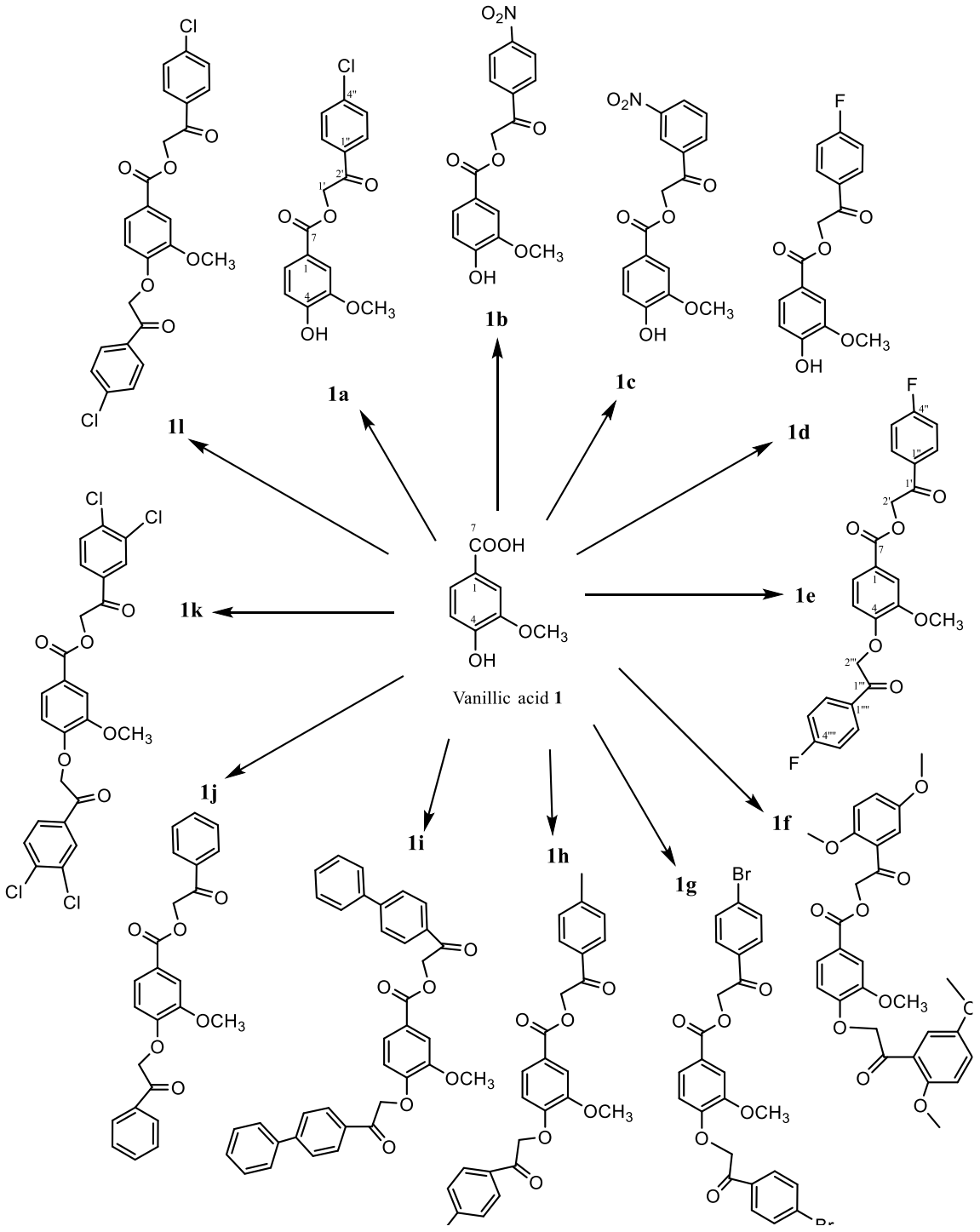

Fig. 2: Esters of vanillic acid **1a-1l**.

Vanillic acid (**1**) and its synthesized derivatives were screened for their DPPH radical scavenging assay and inhibitory studies against the enzymes urease and lipoxygenase (Table 1). Vanillic acid **1** and its derivative **1c** showed significant antioxidant activity as compared to the standard BHA, while **1h** and **1l** showed good activity (Table 1). It is interesting to note that, **1c** which has a nitro group at C-3 exhibited pronounced antioxidant activity as compared to **1b** which is a positional isomer of **1c** with the nitro group at C-4. The ester **1e** carrying fluorine at C-4 revealed potent inhibitory potential against the enzyme lipoxygenase while significant activity was exhibited by derivatives **1b**, **1c**, **1d** and **1i**. On the other hand, potent urease inhibitory activity was observed for **1a**, **1d**, **1e**, **1f** and **1k** (Table-1).

Conclusion

High yielding facile syntheses of esters of vanillic acid have been achieved by way of one pot alkylation reactions of the starting material with a variety of 2-bromoacetophenone derivatives used as alkylating agents. Biological screening of the target molecules revealed some of these to exhibit good to excellent antioxidant activity and inhibitory potential against the enzymes lipoxygenase and urease, respectively

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Table-1: Radical scavenging and enzyme inhibitory activities of **1** and its derivatives **1a-1l**.

Compounds	Antioxidant Activity IC_{50} value (µM)	Lipoxygenase Inhibition IC_{50} value (µM)	Urease Inhibition IC_{50} value (µM)
1	**46.7±0.21**	**>100± 0.35**	**35.7± 0.73**
1a	**60.9±0.23**	**60.5± 0.78**	**23.4± 0.22**
1b	**81.2±0.16**	**23.4± 0.53**	**63.2±0.03**
1c	**44.5±0.72**	**27.5± 0.77**	**65.5± 0.67**
1d	**70.3±0.31**	**21.07±0.10**	**22.2±0.42**
1e	**84.8±±0.92**	**11.4± 0.22**	**29.8± 0.06**
1f	**77.7±0.28**	**75.9± 0.29**	**24.6± 0.17**
1g	**77.7±0.78**	**42.3± 0.27**	**79.2±0.07**
1h	**56.7±0.54**	**46.3± 0.82**	**90.1±0.33**
1i	**65.7±0.63**	**29.8± 0.56**	**32.4± 0.48**
1j	**67.5±0.64**	**56.3± 0.72**	**82.2±0.08**
1k	**85.6±0.27**	**59.4± 0.62**	**26.7±0.13**
1l	**54.7±0.38**	**56.8± 0.81**	**76.4± 0.33**
BHA	**44.2 ± 0.51**	-	-
Thiourea	-	-	**21.2 ± 0.09**
Baicalein	-	**22.6 ± 0.08**	-

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