RESEARCH PAPER

Synthesis, Characterization and Antioxidant Evaluation of Novel Ethyl 4-[2-cyano-3-(substituted phenyl) acrylamido]-2-hydroxybenzoate Derivatives

Doli Sherisha Bhavani[1*] and Kuchana Madhavi[2]

[1]*Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati -517 502, India*
[2]*Department of Pharmaceutical Chemistry, Bhaskar Pharmacy College, Hyderabad-500075, India*
[*]***Corresponding author:*** *Doli Sherisha Bhavani*
sherisha0110@gmail.com

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ABSTRACT
A novel series of ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoates, prepared using a Knoevenagel condensation method. It was reacted by the activation of the methylene group of ethyl 4 (2-cyanoacetamido)-2-hydroxybenzoate and condensed with different substituted benzaldehydes in toluene. High yields were achieved by the use of a catalytic amount of piperidine and acetic acid. In the current study, 1-cyanoacetyl-3,5-dimethylpyrazole was identified to be a cheap and effective starting material to use in the condensation reaction. In general, fifteen compounds could be obtained, purified through recrystallization with ethanol and completely characterized with physical and spectral analysis. The antioxidant potential of the individual compounds (5a-o) was compared, and it was discovered that the compounds with a 3, 4-dihydroxy (5h) substituent on the phenyl ring had the highest antioxidant potential at a concentration of 100 µM compared to others.

Keywords: Ethyl 4-Aminosalicylate, 1-Cyanoacetyl-3, 5-dimethylpyrazole, Knoevenagel condensation
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INTRODUCTION

Prodrugs are a superior approach in drug development, where biotransformation of inactive drug to active compounds takes place after the biochemical agents perform internal or external stimuli. The idea enhances various drug parameters, such as pharmacodynamics, pharmacokinetics, physicochemical behavior, and patient compliance (4P properties). Although they have these benefits, few drugs (10%) that are available in the market today use a prodrug approach [1]. Almost half of them are ester-based, allowing them to be used due to their tunable lipophilicity or to conceal ionizable groups. Nevertheless, they have poor predictability in their pharmacokinetics as a result of interspecies carboxylesterase activity variations as shown in the case of nalbuphine diester prodrug [2], as well as their oral bioavailability is often not more than 40–60% as a result of few functional classes of ester commonly being utilized [3].

The major research gap is the formation of better analogs of 4-aminosalicylic acid (4-ASA) which is an FDA-approved orphan drug that treats ulcerative colitis and has anti-inflammatory and anti-tubercular effects and can be used as an alternative medicine in patients with inflammatory bowel disease (IBD) [4, 5]. Previous work has investigated 4-ASA-based prodrugs as an improved colonic delivery system [6, 7], but there are still many difficulties with the system (mainly stability, high systemic excretion rate, and gastrointestinal intolerance), and so called structural analogue para-amino salicylic acid (PAS), which is characterized by moderate bioavailability (approximately 60%), has been previously shown to have severe gastrointestinal side effects at higher dosages [8]. The impact of these limitations is the necessity of new 4-ASA derivatives with enhanced pharmacokinetic properties and lowered gut-associated toxicity. Both aromatic rings substituted with either phenolic or hydroxyl groups have also widely been reported with cyanoacrylamide derivatives showing anti-inflammatory, antioxidant, and enzyme-modulating properties. Therefore, the cyanoacrylamide scaffold itself does not bring global structural innovation. This study introduces a new concept by incorporating a cyanoacetamide moiety that has never been integrated into a 4-ASA-derived salicylate framework in a unique fashion

Literature underlines the effectiveness of 1-cyanoacetyl-3,5-dimethylpyrazole as an efficient N-cyanoacetylating reagent for aromatic amines [9], and N-substituted cyanoacetamides have a variety of biological activities, which include tofacitinib and cephacetrile [10]. Butamben and benzocaknown asre also known established

**Author for Correspondence: sherisha0110@gmail.com*

therapeutic agents [11] which are alkyl 4-aminobenzoates. As a follow up to these precedents, the current work synthesizes ethyl 4-(2-cyanoacetamido)-2-hydroxybenzoate via cyanoacetylation of ethyl 4-aminosalicylate, which is then Knoevenagel condensed to form a sequence of ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoate analogues.

The intermediate (**compound 3**) is denoted as novel, but the reactions that are used are N-cyanoacetylation and condensation, which are routine and common to substituted anilides and aromatic amines. It is this novelty of structure of this cyanoacetamido-salicylate intermediate, a key precursor to a new type of 4-ASA-based cyanoacrylamide not merely the synthetic methodology itself. Also, developed structure–activity correlations (SAR) for cyanoacrylamide systems show that electron-donating substituents (–OH, –OCH_3, –NH_2) augment biological activity by adding electron density to the conjugated acrylamide backbone. Hydrogen bonding and stabilization of reactive intermediates of phenolic hydroxyl groups, and the cyano group enhances electrophilicity at the β-carbon, which are important in biological interactions.

MATERIALS AND METHODS

The reagents required for this research possessed analytical reagent quality and were obtained at Sd-Fine and Sigma-Aldrich. TLC on silica gel 60 F_{254} coated chromatographic media enabled to monitor the progress of each reaction, and as well to pre-assess the purity of the product through visualization under a Meswox UV cabinet, and exposure to iodine vapors. The Stuart equipment was utilized to assess melting points of the obtained derivatives in open capillaries. Thermo Nicolet Nexus 670 and Bruker spectrophotometers were used to record infrared spectra by using KBr pellet methods. The Agilent and Varian equipment were used to acquire proton and carbon NMR spectra (^{1}H and ^{13}C) at 400 MHz with chemical shifts (delta) being reported against tetramethylsilane (TMS) and DMSO as the solvent at the Central University, Hyderabad. LC-MS analysis of the synthesized products was performed on an Agilent 1200 Infinity Series mass spectrometer to determine the molecular masses of the products synthesized.

Synthesis of ethyl 4-(2-cyanoacetamido)-2-hydroxybenzoate (Compound 3)

A solution of ethyl 4-aminosalicylate 11mM and 1-cyanoacetyl-3, 5 -dimethylpyrazole 10 mM in 30 ml of toluene was subjected to heated under reflux at 110^{o}C approximately one hour. TLC analysis was employed to monitor reaction progress. Upon completion, hot mixture permitted to cooled until it reached ambient conditions, and a solidified product formed [12, 13]. The precipitate obtained was filtrated and the crude solid was then dried and purified with the help of recrystallization using ethanol as described in (**Scheme-1**).

Ethyl 4-(2-cyanoacetamido)-2-hydroxybenzoate (Compound 3)

M.F: $C_{12}H_{12}N_2O_4$; M.wt: 248; M.P: 165-168; Yield: 86%

IR: 3509.75 (OH), 3331.05 (NH), 2215.07 (CN), 1736.96 (C=O, ester), 1685.61 (C=O, amide), 1603.90 (NH, def), 1465.61 (CH_2) cm^{-1}; ^{1}H-NMR: δ 10.69 (s, 1H, -NH), 10.34 (s, 1H, -OH), 7.99-7.96 (d, 1H, Ar), 7.84-7.82 (dd, 1H, Ar), 7.63-7.62 (d, 1H, Ar), 4.33-4.27 (q, 2H, -CH_2CH_3), 3.42 (s, 2H, -CH_2) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.18, 21.13, 60.54, 107.09, 116.01, 117.81, 125.23, 130.11, 142.62, 158.32, 160.97, 165.22 ppm; Mass m/z 249.2(M+1).

Synthesis of ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoate (5a-o)

Ethyl 4-(2-cyanoacetamido)-2-hydroxybenzoate 10 mM with substituted benzaldehyde 10 mM was introduced into 30 ml of toluene, followed by the addition of piperidine 0.39 ml and acetic acid 1.4 ml. The solution obtained was heated under reflux between 5 to 6 hours and the reaction process was followed through TLC [14-18]. The reaction mixture cooled until it reached ambient conditions, leading to the formation of a solid. The material produced under this condition was isolated through filtration. Crude product was subsequently purified through recrystallization from ethanol as depicted in (**Scheme 1**). Under this method, fifteen new compounds were prepared and identified in respect to their physical and spectral data.

Ethyl 4-(2-cyano-3-phenylacrylamido)-2-hydroxybenzoate (Compound 5a)

IR: 3523.23 (OH), 3280.03 (NH), 2235.27 (CN), 1733.27 (C=O, ester), 1702.30 (C=O, amide) cm^{-1}; ^{1}H-NMR: δ 10.73 (s, 1H, -NH), 10.33 (s, 1H, -OH), 8.32 (s, 1H, =CH), 8.01-7.96 (m, 3H, Ar), 7.85-7.82 (d, 2H, Ar), 7.56-7.53 (dd, 1H, Ar), 7.37-7.34 (m, 2H, Ar), 4.33-4.28 (q, 2H, -CH_2CH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.18, 60.52, 105.72, 108.08, 116.22, 117.79, 125.16, 129.07, 129.94, 130.11, 130.28, 142.69, 143.39, 151.23, 158.28, 161.14, 165.22 ppm; Mass m/z 337.1(M+1).

4-[2-cyano-3-(4-chlorophenyl)acrylamido]-2-hydroxybenzoate (Compound 5b)

IR: 3601.77 (OH), 3275.53 (NH), 2245.22 (CN), 1742.84 (C=O, ester), 1706.87 (C=O, amide), 1613.12 (NH) cm^{-1}; ^{1}H-NMR: δ 10.74 (s, 1H, -NH), 10.37 (s, 1H, -OH), 8.32 (s, 1H, =CH), 8.02-7.97 (m, 3H, Ar), 7.84-7.82 (d, 2H, Ar), 7.71-7.69 (dd, 1H, Ar), 7.43-7.42 (d, 1H, Ar), 4.33-4.27 (q, 2H, -CH_2CH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.22, 60.55, 106.32, 115.03, 117.45, 119.12, 124.32, 124.97, 130.29, 130.54, 133.53, 142.10, 142.42, 153.58, 155.75, 160.22, 165.21 ppm; Mass m/z 371.1(M+1).

Ethyl 4-[2-cyano-3-(p-tolyl)acrylamido]-2-hydroxybenzoate (Compound 5c)

IR: 3522.30 (OH), 3227.35 (NH), 2215.32 (CN), 1736.97 (C=O, ester), 1693.83 (C=O, amide), 1615.93 (-NH) cm^{-1}; ^{1}H-NMR: δ 10.69 (s, 1H, -NH), 10.26 (s, 1H, -OH), 8.27 (s, 1H, =CH), 7.99-7.96 (d, 1H, Ar), 7.93-7.91 (d, 2H, Ar),

7.84-7.82 (d, 2H, Ar), 7.60-7.59 (dd, 1H, Ar), 7.44-7.42 (d, 1H, Ar), 4.33-4.27 (q, 2H, -CH_2CH_3), 2.41 (s, 3H, -CH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.20, 21.29, 60.54, 105.72, 112.21, 116.25, 117.79, 125.15, 129.08, 129.96, 130.13, 130.30, 142.71, 143.41, 151.25, 156.11, 161.16, 165.24 ppm; Mass m/z 351.4 (M+1).

Ethyl 4-[2-cyano-3-(4-isopropylphenyl)acrylamido]-2-hydroxybenzoate (Compound 5d)

IR: 3523.25 (OH), 3285.27 (NH), 2252.83 (CN), 1745.27 (C=O, ester), 1701.87 (C=O, amide), 1631.76 (NH) cm^{-1}; ^{1}H-NMR: δ 10.70 (s, 1H, -NH), 10.17 (s, 1H, -OH), 8.28 (s, 1H, =CH), 7.99-7.94 (m, 3H, Ar), 7.84-7.82 (d, 2H, Ar), 7.66-7.64 (dd, 1H, Ar), 7.50-7.48 (d, 1H, Ar), 4.33-4.27 (q, 2H, -CH_2CH_3), 3.02-2.95 (m, 1H, -CH), 1.34-1.30 (t, 3H, -CH_2CH_3) and 1.25-1.23 (d, 6H, (-CH_3)$_2$) ppm; ^{13}C-NMR:14.18, 23.39, 33.57, 60.53, 105.81, 115.08, 116.24, 117.83, 125.18, 127.35, 129.86, 130.11, 130.45, 140.11, 142.67, 151.25, 158.88, 161.10, 165.23 ppm; Mass m/z 379.1(M+1).

Ethyl 4-[2-cyano-3-(4-dimethylaminophenyl)acrylamido]-2-hydroxybenzoate (Compound 5e)

IR: 3523.58 (OH), 3263.03 (NH), 2211.62 (CN), 1744.15 (C=O, ester), 1709.75 (C=O, amide), 1549.40 (NH) cm^{-1}; ^{1}H-NMR: δ 10.36 (s, 1H, -NH), 10.09 (s, 1H, -OH), 8.10 (s, 1H, =CH), 7.96-7.92 (m, 3H, Ar), 7.83-7.81 (d, 2H, Ar), 7.43-7.41 (dd, 1H, Ar), 6.87-6.85 (d, 1H, Ar), 4.32-4.27 (q, 2H, -CH_2CH_3), 3.08 (s, 6H, (-CH_3)$_2$) and 1.33-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.21, 44.02, 60.49, 111.72, 117.89, 118.50, 119.66, 124.68, 129.70, 130.05, 133.05, 142.15, 143.18, 151.17, 158.22, 162.25, 165.30 ppm; Mass m/z 380.1 (M+1).

Ethyl 4-[2-cyano-3-(4-methoxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5f)

IR: 3592.58 (OH), 3285.36 (NH), 2224.34 (CN), 1746.28 (C=O, ester), 1708.98 (C=O, amide), 1586.28 (NH) cm^{-1}; ^{1}H-NMR: δ 10.60 (s, 1H, -NH), 10.09 (s, 1H, -OH), 8.24 (s, 1H, =CH), 8.04-8.02 (d, 1H, Ar), 7.98-7.96 (d, 2H, Ar), 7.84-7.82 (d, 2H, Ar), 7.56-7.54 (dd, 1H, Ar), 7.19-7.17 (d, 1H, Ar), 4.33-4.27 (q, 2H, -CH_2CH_3), 3.87 (s, 3H, -OCH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.20, 55.68, 60.54, 103.32, 111.91, 114.94, 116.66, 117.77, 124.26, 125.06, 130.12, 132.71, 140.32, 142.81, 150.93, 161.43, 162.89, 165.26 ppm; Mass m/z 367.2 (M+1).

Ethyl 4-[2-cyano-3-(3, 4-dimethoxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5g)

IR: 3575.15 (OH), 3298.14 (NH), 2216.81 (CN), 1747.23 (C=O, ester), 1703.21 (C=O, amide), 1620.20 (NH) cm^{-1}; ^{1}H-NMR: δ 10.61 (s, 1H, -NH), 10.19 (s, 1H, -OH), 8.23 (s, 1H, =CH), 7.98-7.96 (d, 1H, Ar), 7.84-7.82 (d, 1H, Ar), 7.67-7.64 (dd, 1H, Ar), 7.50-7.49 (d, 1H, Ar), 7.33-7.27 (dd, 1H, Ar), 6.97-6.96 (d, 1H, Ar), 4.33-4.27 (q, 2H, -CH_2CH_3), 3.88 (s, 3H, -OCH_3), 3.82 (s, 3H, -OCH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.20, 55.87, 60.55, 103.29, 111.94, 112.39, 116.81, 117.80, 124.31, 125.09, 125.83, 130.12, 142.81, 148.73, 151.29, 152.84, 156.04, 158.30, 161.43, 165.28; Mass m/z 397.3 (M+1).

Ethyl 4-[2-cyano-3-(3, 4-dihydroxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5h)

IR: 3515.05-3503.24 (OH), 3213.77 (NH), 2217.70 (CN), 1733.71 (C=O, ester), 1704.37 (C=O, amide), 1609.33 (NH) cm^{-1}; ^{1}H-NMR: δ 10.50 (s, 1H, -NH), 9.90-9.32 (s, 3H, -OH), 8.16 (s, 1H, =CH), 7.99-7.96 (m, 2H, Ar), 7.83-7.81 (d, 1H, Ar), 7.60 (s, 1H, Ar), 7.37-7.34 (dd, 1H, Ar), 6.98-6.96 (d, 1H, Ar), 4.32-4.27 (q, 2H, -CH_2CH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.20, 60.52, 101.36, 116.01, 116.22, 116.88, 117.74, 123.13, 124.93, 125.63, 130.09, 142.93, 145.79, 151.23, 151.46, 154.32, 158.09, 161.76, 165.28 ppm; Mass m/z 369.4 (M+1).

Ethyl 4-[2-cyano-3-(3-hydroxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5i)

IR: 3501.35 (OH), 3290.79 (NH), 2233.28 (CN), 1745.75 (C=O, ester), 1704.37 (C=O, amide), 1609.33 (NH) cm^{-1}; ^{1}H-NMR: δ 10.79 (s, 1H, -NH), 10.27-8.58 (s, 2H, -OH), 8.21 (s, 1H, =CH), 7.96-7.93 (m, 2H, Ar), 7.84 (s, 1H, Ar), 7.70-7.69 (dd, 1H, Ar), 7.43-7.39 (m, 2H, Ar), 7.03-7.00 (m, 1H, Ar), 4.33-4.28 (q, 2H, -CH_2CH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.21, 60.54, 101.88, 108.87, 113.39, 116.03, 117.05, 119.77, 123.09, 125.00, 126.29, 130.11, 142.90, 147.80, 151.53, 155.11, 158.53, 161.63, 165.28; Mass m/z 353.2 (M+1).

Ethyl 4-[2-cyano-3-(4-hydroxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5j)

IR: 3585.28 (OH), 3293.10 (NH), 2202.10 (CN), 1747.14 (C=O, ester), 1703.18 (C=O, amide), 1605.83 (NH) cm^{-1}; ^{1}H-NMR: δ 10.55 (s, 1H, -NH), 10.19-9.59 (s, 2H, -OH), 8.18 (s, 1H, =CH), 7.98-7.97 (m, 3H, Ar), 7.83-7.81 (d, 2H, Ar), 7.56-7.53 (dd, 1H, Ar), 6.98-6.96 (d, 1H, Ar), 4.32-4.27 (q, 2H, -CH_2CH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.20, 60.55, 106.72, 115.98, 116.81, 117.96, 121.57, 125.19, 130.14, 130.38, 132.93, 142.68, 151.40, 154.13, 157.80, 161.10, 165.25 ppm; Mass m/z 353.2 (M+1).

Ethyl 4-2-cyano-3-(2-hydroxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5k)

IR: 3505.33 (OH), 3290.35 (NH), 2214.18 (CN), 1741.78 (C=O, ester), 1708.32 (C=O, amide), 1605.32 (NH) cm^{-1}; ^{1}H-NMR: δ 10.55 (s, 1H, -NH), 10.38-9.46 (s, 2H, -OH), 8.18 (s, 1H, =CH), 7.98-7.95 (d, 1H, Ar), 7.84-7.81 (d, 1H, Ar), 7.56-7.54 (dd, 1H, Ar), 7.34-7.23 (m, 2H, Ar), 6.99-6.97 (d, 2H, Ar), 4.32-4.27 (q, 2H, -CH_2CH_3) and 1.34-1.30 (t, 3H, -CH_2CH_3) ppm; ^{13}C-NMR: 14.18, 60.49, 101.86, 114.82, 116.32, 116.85, 117.74, 122.76, 124.98, 130.07, 133.13, 140.12, 142.87, 151.19, 161.59, 162.11, 165.25 ppm; Mass m/z 353.2 (M+1).

Ethyl 4-[2-cyano-3-(3-methoxy-4-hydroxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5l)

IR: 3533.28 (OH), 3232.19 (NH), 2217.12 (CN), 1743.41 (C=O, ester), 1708.11 (C=O, amide), 1601.13 (NH) cm^{-1}; ^{1}H-NMR: δ 10.92 (s, 1H, -NH), 10.26-9.32 (s, 2H, -OH),

8.58 (s, 1H, =CH), 8.00-7.95 (m, 1H, Ar), 7.88-7.80 (m, 2H, Ar), 7.63-7.59 (m, 1H, Ar), 7.32-7.26 (d, 2H, Ar), 4.32-4.27 (q, 2H, $-CH_2CH_3$), 3.91 (s, 3H, $-OCH_3$) and 1.34-1.30 (t, 3H, $-CH_2CH_3$) ppm; ^{13}C-NMR: 14.18, 56.18, 60.49, 101.86, 114.97, 116.32, 116.85, 117.74, 122.76, 124.98, 130.08, 133.13, 142.87, 143.88, 149.87, 151.20, 161.59, 162.11, 165.25 ppm; Mass m/z 383.1 (M+1).

Ethyl 4-[2-cyano-3-(3,5-dimethoxy-4-hydroxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5m)

IR: 3593.75 (OH), 3250.23 (NH), 2232.57 (CN), 1740.31 (C=O, ester), 1701.17 (C=O, amide), 1603.18 (NH) cm^{-1}; ^{1}H-NMR: δ 10.56 (s, 1H, -NH), 9.79-9.33 (s, 2H, -OH), 8.19 (s, 1H, =CH), 7.98-7.96 (d, 1H, Ar), 7.84-7.82 (dd, 1H, Ar), 7.45 (s, 2H, Ar), 6.84-6.83 (d, 1H, Ar), 4.33-4.27 (q, 2H, $-CH_2CH_3$), 3.83 (s, 6H, $-OCH_3$) and 1.34-1.30 (t, 3H, $-CH_2CH_3$) ppm; ^{13}C-NMR: 14.18, 56.01, 60.50, 102.12, 108.63, 117.07, 119.74, 121.75, 124.99, 130.08, 141.08, 142.85, 147.91, 151.79, 156.01, 158.07, 161.53, 165.25 ppm; Mass m/z 413.1 (M+1).

Ethyl 4-[2-cyano-3(3, 4, 5-trimethoxyphenyl)acrylamido]-2-hydroxybenzoate (Compound 5n)

IR: 3583.25 (OH), 3268.96 (NH), 2215.28 (CN), 1746.37 (C=O, ester), 1707.96 (C=O, amide), 1637.66 (NH) cm^{-1}; ^{1}H-NMR: δ 10.68 (s, 1H, -NH), 10.37 (s, 1H, -OH), 8.25 (s, 1H, =CH), 7.99-7.97 (d, 1H, Ar), 7.84-7.82 (dd, 1H, Ar), 7.43 (s, 2H, Ar), 7.25-7.23 (d, 1H, Ar), 4.33-4.27 (q, 2H, $-CH_2CH_3$), 3.86-3.84 (s, 6H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$) and 1.34-1.30 (t, 3H, $-CH_2CH_3$) ppm; ^{13}C-NMR: 14.19, 55.17, 56.05, 60.55, 105.46, 108.05, 116.45, 117.82, 125.19, 126.92, 130.13, 141.33, 142.67, 151.38, 152.94, 158.92, 161.07, 165.24 ppm; Mass m/z 427.1 (M+1).

Ethyl 4-2-cyano-3-(3-methoxy-4-hydroxy-5-nitrophenyl)acrylamido]-2-hydroxybenzoate (Compound 5o)

IR: 3578.15 (OH), 3228.92 (NH), 2214.18 (CN), 1746.28 (C=O, ester), 1708.96 (C=O, amide), 1628.16 (NH) cm^{-1}; ^{1}H-NMR: δ 10.54 (s, 1H, -NH), 10.37-9.56 (s, 2H, -OH), 8.18 (s, 1H, =CH), 7.97-7.95 (d, 1H, Ar), 7.82 (s, 1H, Ar), 7.72 (s, 1H, Ar), 7.56-7.53 (dd, 1H, Ar), 6.98-6.96 (d, 1H, Ar), 4.32-4.27 (q, 2H, $-CH_2CH_3$), 3.83 (s, 3H, $-OCH_3$) and 1.34-1.30 (t, 3H, $-CH_2CH_3$) ppm; ^{13}C-NMR: 14.19, 55.56, 60.51, 101.36, 113.40, 116.02, 117.03, 119.76, 123.07, 124.98, 126.28, 130.08, 142.87, 147.79, 151.49, 151.88, 158.12, 161.60, 165.26 ppm; Mass m/z 428.1 (M+1).

Antioxidant Evaluation

Free Radical Scavenging Assay of DPPH

The evaluation of antioxidant potential of the synthesized analogues (**5a-o**) was determined by DPPH assessment which is a well-utilized approach in calculating free-radical scavenging activity. DPPH is a non-paired, nitrogen-centered radical in the form of a free radical, and its strong absorption at 517 nm enables it to have a deep violet color in ethanol. When reacts with an antioxidant, DPPH receives an electron or hydrogen atom to produce the stable, pale yellow diamagnetic 1,1-diphenyl-2-picrylhydrazine (DPPH–H). This coloration gives a convenient spectrophotometric system of evaluating antioxidant potential [19].

The test compounds were put into ethanolic solutions (100 μM). DPPH solutions (100 μM) were then made in 95% ethanol and mixed with the test compound. The mixtures were swirled and left at room temperature to enable the reaction to obtain equilibrium after 20 minutes. The absorbance of a solution at 517 nm was subsequently recorded with the help of UV-Visible spectrophotometer [20]. A solvent control was made in an identical condition, with ascorbic acid (100 μM) serving as the reference standard. All the experiments were repeated 3 times, and the mean of these three results was calculated to get the percentage DPPH radical scavenging as shown in the following equation:

Scavenging percentage of DPPH free radical = (Control - Test)/Control x 100

Free Radical Scavenging Assay of Nitric oxide

Nitric oxide scavenging of synthesized analogues (**5a-o**) was determined. At physiological pH in aqueous solution, sodium nitroprusside reacts spontaneously to produce nitric oxide which reacts with dissolved oxygen in the solution to yield nitrite ions. The quantification of these nitrite ions was done through the Griess reagent [21].

The test compounds (100 μM) were incubated with sodium nitroprusside (10 μM) in an alcohol solution in the presence of phosphate buffer (pH 7.4) to a reaction mixture at 25°C and incubated over 150 minutes [22]. The reaction mixture (2 mL) was then incubated and then 2 mL mixture was added to 2 mL Griess reagent. The color change through diazotization of sulphanilamide and subsequent reaction with N-naphthylethylenediamine dihydrochloride was conducted. Absorbance of the solution obtained was obtained at 546 nm [23]. Alpha tocopherol (100 μM) was used as a reference standard to make a solvent control under the same condition. Averages of 3 findings obtained after each of the experiments were repeated to establish the percentage NO radical scavenging as below:

Scavenging % = (Control-Test)/Control x 100

RESULTS AND DISCUSSION

Chemistry

Research work is an advanced process of the preparation of ethyl 4-(2-cyanoacetamido)-2-hydroxybenzoate (**3**) through reacting ethyl 4-aminosalicylate (**2**) with 1-cyanoacetyl-3,5-dimethylpyrazole (**1**) as the cyanoacetylating agent. The resulting intermediate (**3**) possesses two nucleophilic sites, as at which the amide nitrogen and the methylene carbon are situated, which contain huge potential of additional structural modification. Knoevenagel condensation of the methylene

carbon, which is more reactive than the amide nitrogen. The activated methylene carbon is easily subjected to Knoevenagel condensation reaction with the substituted derivatives of benzaldehydes (**4a-o**) in toluene using catalytic quantities of piperidine and acetic acid. This conversion provided the respective ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoates (**5a–o**) with isolated yields of 65 to 91 percent. IR, (^{1}H and ^{13}C) NMR and along with mass spectrometric analyses were performed to structural confirm the synthesized analogues. Physical parameters that were synthesized were characterized and their structures were resolved by the spectrometry as shown by **Table 1**.

Synthesized analogues (**5a-o**) exhibited the IR spectra that indicated the presence of the OH stretching at the wavelength of 3601.77-3501.35. The amide group N–H stretches were found between 3331.05-3213.77 cm^{-1}. The CN characteristic bands were found at 2252.83-2202.10 cm^{-1}. The maximum absorption corresponding to the carbonyls of ester and amide groups was recorded at 1747.23-1733.27 cm^{-1} and 1709.75-1685.61 cm^{-1}, respectively. N–H deformation bands were found between 1637.66-1549.40 cm^{-1}, where compounds that had methoxy substituents were found with absorption bands at 1293.29-1187.38 cm^{-1}.

The ^{1}H NMR spectra recorded for analogues (**5a-o**) had typical signals, indicating the presence of ethyl ester fragment. A triplet with δ 1.34-1.30 ppm was attributed to (–CH_3), and quartet at δ 4.33-4.27 ppm was correspond to (–O–CH_2–). The region at δ 8.04-6.83 ppm was observed to have aromatic protons as doublets, double of doublets and multiplets, which correspond to a substituted benzene ring. A unique singlet at δ 8.58-8.10 ppm indicating the presence of benzylidene proton (–CH=) was indicative of successful Knoevenagel condensation in the system and is characteristic of α, β-unsaturated cyanoacrylamide systems. Exchangeable protons were also detected with singlets at δ 10.38-8.58 ppm being the phenolic –OH group and the signals at δ 10.92-10.36 ppm being the amide –NH proton. The presence of singlets at **5f**, **5g**, **5l**, **5m**, **5n**, and **5o** is due to the presence of methoxy substituents which were observed at δ 3.91-3.82 ppm which is the position of the protons at the -OCH_3. The isopropyl bearing analogue **5d** with δ 1.25-1.23 ppm had a doublet, related to methyl moiety, multiplet related to methine proton at δ 3.02-2.95 ppm. In the **5e** compound, the dimethylamino group formed a single point at 3.08 ppm in the delta-frequency, which indicated the existence of the -$N(CH_3)_2$ group. One similarity that was evident in all final products was the loss of the methylene proton signal at δ 3.42 ppm of compound (**3**) with previous detection, and the distinct resonance of CH= proton signal at δ 8.58-8.10 ppm range, which indicated successful formation of the acrylamide framework.

The ^{13}C NMR spectra of compounds (**5a-o**) were typical resonances that verified the structural framework of the synthesized molecules. The signals at δ 14.22-14.18 ppm indicated the presence of the methyl carbon (–CH_3) and the signals at δ 60.55-60.49 ppm showed the presence of methylene carbon (–O–CH_2–). The existence of the cyano group was evidently observed through nitrile carbon resonance at δ 117.96-117.03 ppm. The presence of aromatic carbons was found in the δ 148.73-107.09 ppm range, but with the presence of substituted phenyl rings. A characteristic signal at δ 153.58-150.93 ppm was associated with the carbon of the benzylidene (–CH=), which verified the successful Knoevenagel condensation. The carbon directly coupled to the phenolic –OH group was at δ 161.59-149.87 ppm, which was typical of oxygenated aromatic carbons. The carbonyl carbons were also evident: the amide C=O was found at δ 162.89-160.22 ppm, and the ester C=O appeared at δ 165.30-165.21 ppm, respectively, in all the derivatives, indicating the integrity of the cyanoacrylamide–ester framework.
The mass spectra of the products obtained were recorded in positive ion mode and the molecular ions were identified with their respective $[M+H]^+$ masses, and the molecular masses were found to be as expected. In combination with the typical IR absorptions and the diagnostic ^{1}H NMR and ^{13}C NMR signals, the mass spectral data made it possible to collectively validate the structures of all the produced analogues.

ANTIOXIDANT EVALUATION

Radical scavenging Activity of DPPH and Nitric Oxide

The antioxidant evaluation of the obtained analogues (**5a-o**) assessment was performed with two known assays, which consisted of: interaction with free radical DPPH, and free radical scavenging of NO, and the results are indicated in **Table 2**.

The compound with the highest antioxidant activity of the analyzed derivatives was compound **5h** which had a comparable or higher antioxidant activity than that of the standard. This compound is even more effective because phenolic hydroxyl groups at the 3rd and the 4th position on the phenyl ring create a catechol system that enables efficient donation and stabilization of the resultant phenoxyl radicals by resonance and intramolecular hydrogen bonding. Compound **5l** and **5m** were also found to be significant antioxidants. The higher activity may be because the methoxy groups are ortho to the phenolic hydroxyl, hence, creating a sterically hindered phenol, which is better at stabilizing free radicals than the phenolic moiety alone, which has been reported in the literature to be sterically hindrance to increase antioxidant activity.

Nevertheless, placing the hydroxyl group in any of the other positions para (4th) to the meta (3rd) or ortho (2nd) positions (as in compounds **5i** and **5k**), led to a significant reduction in activity, indicating that hydroxyl positioning is important in radical stabilization. In addition, the antioxidant activity of the methylated hydroxyl group, in compounds **5f**, **5g** and **5n**, influenced the antioxidant capacity considerably, which demonstrated that free phenolic -OH group is a major factor of radical scavenging. The outcome of antioxidant analysis based on the DPPH and nitric oxide scavenging models were not

dissimilar, which again validates the requirements of the phenolic moiety to the radical-scavenging activity of these derivatives.

CONCLUSION

This study is a facile, eco-friendly, and effective synthesis of a novel series of ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoate analogs (**5a-o**). The method had easy reaction conditions and a simple work-up with satisfactory to excellent purity and yields of compounds. Ethyl 4(2-cyanoacetamido)-2-hydroxybenzoate was synthesized via cyanoacetylation followed by Knoevenagel condensation with various aromatic aldehydes using piperidine and glacial acetic acid as catalysts. Each derivative was characterized and validated by IR, (^{1}H and ^{13}C) NMR, along with mass spectrometry. Antioxidant activity of all the compounds *in vitro* was evaluated with the help of DPPH and NO radical scavenging method at a concentration of 100 µM. Compound **5h** with a 3, 4-dihydroxy (catechol) moiety, was most active, and showed how the free phenolic hydroxyl groups are vital in the stabilization of the radicals. Ortho-methoxy derivatives (**5l**, **5m**) were highly active because of steric protection of the phenol, but activities were impaired by methylation or displacement of hydroxyl groups (**5f**, **5g**, **5i**, **5j**, **5n**). The trends of the structure–activity observed here demonstrate the significance of hydroxyl positioning and electronic/steric influence on potential of antioxidants. The findings indicate that the compounds that are produced should be further studied in relation to their antibacterial, antitubercular, and anticancer effects.

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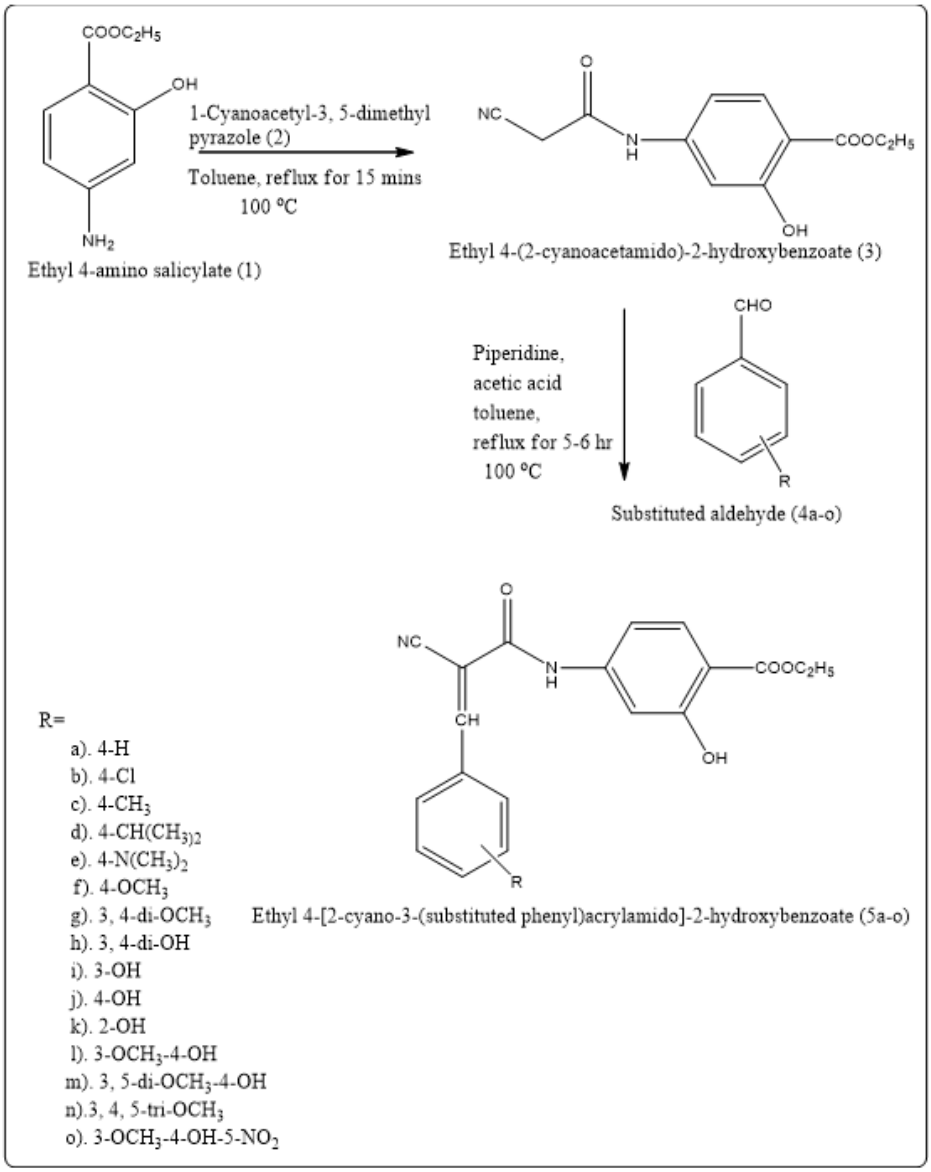
Figure legends

Scheme 1: Synthesis of ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxy benzoates (**5a-o**).

Table 1: Physical data of Ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2- hydroxybenzoates (**5a-o**).

Table 2: *In vitro* antioxidant activity of Ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]- 2-hydroxybenzoates (**5a-o**).



Scheme 1: Synthesis of ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxy benzoates **(5a-o)**.

Table 1: Physical data of Ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxy benzoates (5a-o)

Compound	R	M.F	M.Wt	M.P	R_f Value	% Yield
5a	H	$C_{19}H_{16}N_2O_4$	336.34	176-178	0.31	78
5b	4-Cl	$C_{19}H_{15}ClN_2O_4$	370.79	207-219	0.35	66
5c	4-CH_3	$C_{20}H_{18}N_2O_4$	350.37	219-222	0.53	82
5d	4-$CH(CH_3)_2$	$C_{22}H_{22}N_2O_4$	378.42	166-168	0.55	87
5e	4-$N(CH_3)_2$	$C_{21}H_{21}N_3O_4$	379.41	230-232	0.44	93

5f	4-OCH_3	$C_{20}H_{18}N_2O_5$	366.37	198-201	0.34	76
5g	3,4-di-OCH_3	$C_{21}H_{20}N_2O_6$	396.39	164-166	0.49	82
5h	3,4-di-OH	$C_{19}H_{16}N_2O_6$	368.34	188-190	0.4	91
5i	3-OH	$C_{19}H_{16}N_2O_5$	352.34	156-158	0.36	86
5j	4-OH	$C_{19}H_{16}N_2O_5$	352.34	178-180	0.31	75
5k	2-OH	$C_{19}H_{16}N_2O_5$	352.34	168-170	0.29	68
5l	3-OCH_3-4-OH	$C_{20}H_{18}N_2O_6$	382.37	197-180	0.5	83
5m	3,5-di-OCH_3-4-OH	$C_{21}H_{20}N_2O_7$	412.39	192-194	0.3	65
5n	3,4,5-tri-OCH_3	$C_{22}H_{22}N_2O_7$	426.42	217-219	0.6	83
5o	3-OCH_3-4-OH-5-NO_2	$C_{20}H_{17}N_3O_8$	427.36	208-210	0.48	74

***Solvent system:** Benzene: Ethyl acetate

*** Recrystallization solvent**: Ethanol

Table 2: *In vitro* antioxidant activity of Ethyl 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxy benzoates (5a-o)

Compound	R	% Reduction of DPPH	% Inhibition of Nitric oxide
5a	H	31.16	15.38
5b	4-Cl	46.02	36.26
5c	4-CH_3	48.05	13.18
5d	4-$CH(CH_3)_2$	35.06	25.27
5e	4-$N(CH_3)_2$	58.44	36.26
5f	4-OCH_3	28.57	24.17
5g	3,4-di-OCH_3	22.6	21.97
5h	3,4-di-OH	83.1	63.73
5i	3-OH	63.63	30.76
5j	4-OH	68.83	51.64
5k	2-OH	50.64	46.15
5l	3-OCH_3, 4-OH	76.62	61.53
5m	3,5-di-OCH_3-4-OH	74.02	53.84
5n	3,4,5-tri-OCH_3	29.87	15.16
5o	3-OCH_3-4-OH-5-NO_2	33.76	19.78
Ascorbic acid	-	86.01	-
α-Tocopherol	-	-	69.23