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Synthesis and Antibacterial Evaluation of Novel Cyanoacrylamide Derivatives of 4-Aminosalicylic Acid

D. SHERISHA BHAVANI[1,*], K. MADHAVI[2] and K. SWATHI[2]

[1]Department of Pharmaceutical Chemistry, Bhaskar Pharmacy College, Hyderabad-500075, India
[2]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati-571502, India

*Corresponding author: E-mail: sherisha0110@gmail.com

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In this study, novel 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoic acid derivatives were synthesized starting from 4-(2-cyanoacetamido)-2-hydroxybenzoic acid. The latter was obtained through the cyanoacetylation of 4-aminosalicylic acid, leading to the formation of 4-(2-cyanoacetamido)-2-hydroxybenzoic acid as a key intermediate. The Knoevenagel condensation of the active methylene group of 4-(2-cyanoacetamido)-2-hydroxybenzoic acid was done with substituted benzaldehydes in toluene by using piperidine and glacial acetic acid as catalyzing agents. The procedure mentioned above was used to synthesize fifteen final compounds (**5a-o**). All the synthesized compounds were characterized by physico-chemical and spectral data. The synthesized compounds were tested for *in vitro* antibacterial activity by the cup-plate technique, using 0.1 mL of test solution at a concentration of 1000 µg/mL, against two Gram-positive organisms (*Bacillus subtilis*, *Staphylococcus aureus*) and two Gram-negative organisms (*Escherichia coli*, *Proteus vulgaris*). The outcomes of antibacterial activity were then compared to those obtained with the standard drug streptomycin and 4-aminosalicylic acid. Among all, the compounds **5l** and **5h** exhibited good antibacterial activity when compared with the standard drug.

Keywords: 4-Aminosalicylic acid, Cyanoacetylation, Knoevenagel condensation, Antibacterial activity.

INTRODUCTION

4-Aminosalicylic acid (PAS) is a well-established anti-tuberculosis drug whose inhibitory effect on *M. tuberculosis* was demonstrated by Lehmann, following Bernheim's earlier observations on the role of *para*-amino substitution [1,2]. PAS was later used for treating Monaldi fistulas and abscesses and became a standard therapy for pulmonary tuberculosis [3]. It remains an important second-line agent for multidrug-resistant tuberculosis due to its bacteriostatic mechanism [4,5] and recent studies also highlight its potential in cancer, inflammatory bowel disease and antiviral applications [6].

Activated nitriles have been widely employed in synthesizing heterocyclic systems [7,8] and 2-cyano-(3-substituted phenyl)acrylamide derivatives [9-11]. Many nitrile-containing pharmaceuticals are clinically prescribed and several nitrile derivatives have emerged as promising lead molecules [12]. Teriflunomide, the active metabolite of leflunomide, is a notable nitrile-based drug used in rheumatoid arthritis [13], with additional evaluations in multiple sclerosis and COVID-19 [14,15], as well as documented antitumor activity across several human cancers [16].

Cyanoacetylation of aromatic amines using reagents such as cyanoacetyl chloride, N-(cyanoacetyl)imidazole or 1-cyanoacetyl-3,5-dimethylpyrazole affords key cyanoacetamide intermediates, which readily undergo Knoevenagel condensation with aromatic aldehydes [17-20]. Cyanoacrylamide derivatives bearing phenolic or aromatic groups are also widely reported for diverse biological properties [21]. However, no studies have described N-cyanoacetylated PAS or its corresponding 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoic acid (**5a-o**), indicating an unexplored chemical space. SAR trends show that electron-withdrawing substituents (–NO_2, –Cl, –CN) often enhance antibacterial or anticancer activity, while the electron-donating groups (–OH, –OCH_3) may improve antioxidant or anti-inflammatory potential [22].

Therefore, the present work focuses on synthesizing novel PAS-based cyanoacrylamides (**5a-o**) *via* Knoevenagel condensation and evaluating their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus*

vulgaris to understand their biological potential and structure-activity relationships.

EXPERIMENTAL

All the chemicals and solvents used in this study were purchased from S.D. Fine and Sigma-Aldrich Chemicals of AR-grade. Pre-coated silica gel 60 F_{254} TLC sheets were used to track the reaction's development and Meswox UV cabinet was used to visualize the TLC spots. The melting points of synthesized compounds were determined by utilizing the Stuart melting point instrument. The IR spectra were obtained using a Bruker Infrared spectrophotometer using KBr pressed pellet technique. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrophotometer (500 MHz and 100 MHz), at the University of Hyderabad. The mass spectra of the compounds were recorded on Shimadzu 8040 single quadrupole LC-MS with ESI.

Chemistry

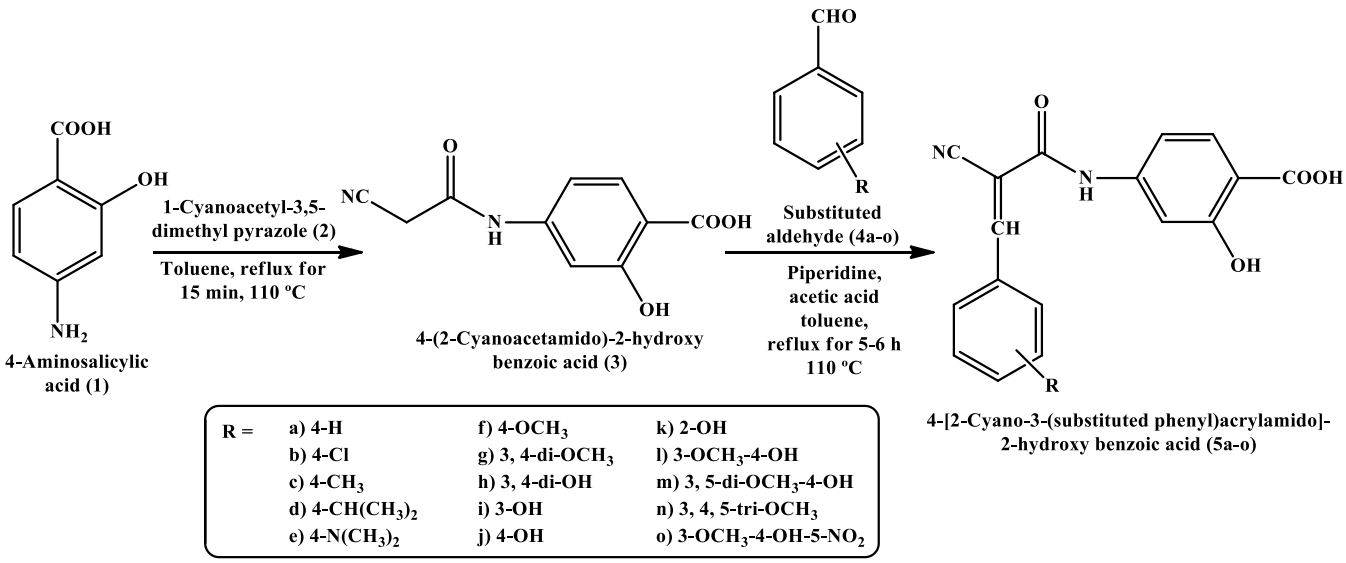
Synthesis of 4-(2-cyanoacetamido)-2-hydroxybenzoic acid (3): A reaction mixture of 1-cyanoacetyl-3,5-dimethyl-pyrazole (1.63 g, 10 mmol) and 4-aminosalicylic acid (1.53 g, 10 mmol) in toluene (20 mL) was allowed to reflux for 110 ºC for 60 min and reaction was monitored by TLC using benzene:ethyl acetate as mobile phase [23]. Once the reaction was complete, the solution was cooled to room temperature, filtered and finally recrystallized with ethanol to obtain compound **3** in pure form (**Scheme-I**).

4-(2-Cyanoacetamido)-2-hydroxybenzoic acid (3): m.f.: $C_{10}H_8N_2O_4$; m.w.: 220; R_f: 0.69; m.p.:198-201 ºC; yield: 82%. IR (KBr, ν_{max}, cm^{-1}): 3576 (-OH, *str.*), 3286 (-NH, *str.*), 3003 (Ar-CH, *str.*), 2976 (aliph-CH, *str.*), 2213 (-CN, *str.*), 1753 (C=O, acid, *str.*), 1691 (C=O, amide, *str.*). 1597 (-NH, *def.*). ^{1}H NMR (500 MHz, DMSO, δ ppm): 3.86 (s, 2H, -CH_2), 6.93-6.95 (d, 1H, Ar), 7.60-7.62 (dd, 1H, Ar), 8.05-8.06 (d, 1H, Ar), 10.26 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 26.98, 113.19, 116.37, 117.88, 121.23, 127.88, 130.49, 157.96, 161.23, 171.99; Mass *m/z* 218.65 (M-H).

General procedure for the synthesis of 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoic acid (5a-o): To a solution of 4-(2-cyanoacetamido)-2-hydroxy-benzoic acid (**3**, 10 mmol) in 25 mL of toluene, substituted benzaldehyde (10 mmol) was added. To this mixture 0.35 mL of piperidine and 1.3 mL of acetic acid were added and it was refluxed at 110 ºC along with continuous stirring for about 5-6 h [24]. By using TLC, the completion of reaction was observed. After cooling the reaction mixture to room temperature, the precipitate was filtered and washed thoroughly with toluene (2 × 5 mL) and recrystallized twice using ethanol to afford analytically pure final products (**Scheme-I**).

4-(2-Cyano-3-phenylacrylamido)-2-hydroxybenzoic acid (5a): m.f.: $C_{17}H_{12}N_2O_4$; m.w.: 308; R_f: 0.74; Rf: 0.69; m.p.:226-228 ºC; yield: 78%. IR (KBr, ν_{max}, cm^{-1}): 3582 (-OH, *str.*), 3259 (-NH, *str.*), 3133 (Ar-CH, *str.*), 2938 (aliph-CH, *str.*), 2267 (-CN, *str.*), 1752 (C=O, acid, *str.*), 1696 (C=O, amide, *str.*), 1548 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 7.60-7.63 (m, 2H, Ar), 7.83-7.85 (m, 2H, Ar), 7.97-8.01 (m, 3H, Ar), 8.31 (s, 1H, Ar), 8.32 (s, 1H, C=CH), 10.73 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 107.66, 113.20, 116.61, 117.71, 122.73, 129.19, 129.77, 130.26, 130.51, 132.41, 132.92, 151.18, 158.34, 160.73, 171.99; Mass *m/z* 306.5 (M-H).

4-[2-Cyano-3-(4-chlorophenyl)acrylamido]-2-hydroxy-benzoic acid (5b): m.f.: $C_{17}H_{11}N_2O_4Cl$; m.w.: 342; R_f: 0.69; m.p.:214-217 ºC; yield: 71%. IR (KBr, ν_{max}, cm^{-1}): 3556 (-OH, *str.*), 3379 (-NH, *str.*), 3056 (Ar-CH, *str.*), 2982 (aliph-CH, *str.*), 2236 (-CN, *str.*), 1778 (C=O, acid, *str.*), 1705 (C=O, amide, *str.*), 1611 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 6.97-6.99 (d, 1H, Ar), 7.68-7.70 (d, 2H, Ar), 7.77-7.79 (dd, 1H, Ar), 7.99-8.01 (d, 2H, Ar), 8.14-8.15 (d, 1H, Ar), 8.28 (s, 1H, C=CH), 10.39 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 108.24, 113.31, 116.41, 117.71, 122.71, 129.09, 129.93, 130.16, 131.28, 132.18, 137.48, 149.81, 158.39, 160.53, 171.98; Mass *m/z* 340.95 (M-H).



Scheme-I: Systematic route of synthesizing target compounds **5a-o**

4-[2-Cyano-3-(*p*-tolyl)acrylamido]-2-hydroxybenzoic acid (5c): m.f.: $C_{18}H_{14}N_2O_4$; m.w.: 322; R_f: 0.82; m.p.:254-256 ºC; Yield: 84%. IR (KBr, ν_{max}, cm^{-1}): 3529 (-OH, *str.*), 3297 (-NH, *str.*), 3020 (Ar-CH, *str.*), 2990 (aliph-CH, *str.*), 2237 (-CN, *str.*), 1771 (C=O, acid, *str.*), 1705 (C=O, amide, *str.*), 1615 (-NH, def.); ^{1}H NMR (500 MHz, DMSO, δ ppm): 2.40 (s, 3H, CH_3), 7.41-7.43 (d, 1H, Ar), 7.82-7.84 (m, 2H, Ar), 7.91-7.92 (d, 1H, Ar), 7.96-7.99 (m, 2H, Ar), 8.27 (s, 1H, Ar), 8.31 (s, 1H, C=CH), 10.68 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 21.79, 106.22, 116.75, 117.20, 120.29, 125.66, 129.58, 130.46, 130.63, 130.80, 143.21, 143.91, 151.75, 158.26, 161.66, 165.74; Mass *m/z* 320.55 (M-H).

4-[2-Cyano-3-(4-isopropylphenyl)acrylamido]-2-hydroxybenzoic acid (5d): m.f.: $C_{20}H_{18}N_2O_4$; m.w.: 350; R_f: 0.89; m.p.:231-234 ºC; yield: 77%. IR (KBr, ν_{max}, cm^{-1}): 3537 (-OH, *str.*), 3253 (-NH, *str.*), 3027 (Ar-CH, *str.*), 2972 (aliph-CH, *str.*), 2830-2729 (unsaturated, saturated -CH, *str.*), 2240 (-CN, *str.*), 1782 (C=O, acid, *str.*), 1682 (C=O, amide, *str.*), 1619 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 1.24-1.25 (d, 6H, CH_3), 3.02-3.04 (m, 1H, -CH), 6.74-6.79 (d, 1H, Ar), 7.47-7.50 (d, 2H, Ar), 7.63-7.65 (dd, 1H, Ar), 7.91-7.94 (d, 2H, Ar), 8.020-8.024 (d, 1H, Ar), 8.24 (s, 1H, C=CH), 10.14 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 24.67, 31.03, 108.17, 116.29, 117.56, 120.31, 125.79, 129.98, 130.63, 131.16, 132.27, 137.68, 143.06, 143.35, 150.43, 158.01, 161.26, 165.71; Mass *m/z* 351.20 (M+H).

4-[2-Cyano-3-(4-dimethylaminophenyl)acrylamido]-2-hydroxybenzoic acid (5e): m.f.: $C_{19}H_{17}N_3O_4$; m.w.: 351; R_f: 0.54; m.p.:212-214 ºC; yield: 86%. IR (KBr, ν_{max}, cm^{-1}): 3545 (-OH, *str.*), 3242 (-NH, *str.*), 3030 (Ar-CH, *str.*), 2928 (aliph-CH, *str.*), 2810-2742 (unsaturated, saturated -CH, *str.*), 2201 (-CN, *str.*), 1773 (C=O, acid, *str.*), 1676 (C=O, amide, *str.*), 1591 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 3.08 (s, 6H, CH_3), 6.85-6.87 (d, 1H, Ar), 7.81-7.83 (m, 3H, Ar), 7.92-7.95 (m, 3H, Ar), 8.09 (s, 1H, C=CH), 10.35 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 60.99, 97.79, 105.99, 112.20, 117.36, 118.98, 120.19, 125.23, 130.53, 133.55, 143.63, 143.95, 151.66, 153.74, 162.74, 165.82; Mass *m/z* 352.20 (M+H).

4-[2-Cyano-3-(4-methoxyphenyl)acrylamido]-2-hydroxybenzoic acid (5f): m.f.: $C_{18}H_{14}N_2O_5$; m.w.: 338; R_f: 0.88; m.p.:268-270 ºC; yield: 98%. IR (KBr, ν_{max}, cm^{-1}): 3512 (-OH, *str.*), 3299 (-NH, *str.*), 3079 (Ar-CH, *str.*), 2979 (aliph-CH, *str.*), 2229 (-CN, *str.*), 1760 (C=O, acid, *str.*), 1704 (C=O, amide, *str.*), 1619 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): δ 3.87 (s, 3H, -OCH_3), 6.90-6.91 (d, 1H, Ar), 7.16-7.18 (d, 2H, Ar), 7.71-7.74 (dd, 1H, Ar), 8.00-8.02 (d, 2H, Ar), 8.09 (d, 1H, Ar), 8.20 (s, 1H, C=CH), 10.21 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 56.18, 103.82, 108.58, 115.44, 117.16, 120.27, 124.76, 125.55, 130.62, 133.21, 143.32, 143.41, 151.42, 161.93, 163.38, 165.75; Mass *m/z* 336.50 (M-H).

4-[2-Cyano-3-(3,4-dimethoxyphenyl)acrylamido]-2-hydroxybenzoic acid (5g): m.f.: $C_{19}H_{16}N_2O_6$; m.w.: 368; R_f: 0.73; m.p.:218-220 ºC; yield: 91%. IR (KBr, ν_{max}, cm^{-1}): 3515 (-OH, *str.*), 3258 (-NH, *str.*), 3058 (Ar-CH, *str.*), 2975 (aliph-CH, *str.*), 2258 (-CN, *str.*), 1753 (C=O, acid, *str.*), 1701 (C=O, amide, *str.*), 1636 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO): δ 3.83-3.88 (2s, 6H, OCH_3), 6.91-6.93 (d, 1H, Ar), 7.18-7.20 (d, 1H, Ar), 7.62-7.64 (dd, 1H, Ar), 7.710-7.714 (d, 1H, Ar), 7.74-7.76 (dd, 1H, Ar), 8.10-8.11 (d, 1H, Ar), 8.19 (s, 1H, C=CH), 10.22 (s, 1H, -NH); ^{13}C NMR (500 MHz, DMSO, δ ppm): 55.96, 56.29, 103.94, 112.37, 112.79, 117.39, 122.81, 124.94, 126.04, 128.67, 129.37, 130.01, 149.17, 151.02, 153.08, 158.53, 161.11, 172.03; Mass *m/z* 366.55 (M-H).

4-[2-Cyano-3-(3,4-dihydroxyphenyl)acrylamido]-2-hydroxybenzoic acid (5h): m.f.: C_{17} $H_{12}N_2O_6$; m.w.: 340; R_f: 0.79; m.p.:204-207 ºC; Yield: 96%. IR (KBr, ν_{max}, cm^{-1}): 3597-3532 (-OH, *str.*), 3297 (-NH, *str.*), 3096 (Ar-CH, *str.*), 2930 (aliph-CH, *str.*), 2241 (-CN, *str.*), 1773 (C=O, acid, *str.*), 1701 (C=O, amide, *str.*), 1632 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 6.77-6.81 (m, 1H, Ar), 6.90-6.93 (m, 1H, Ar), 7.32-7.34 (dd, 1H, Ar), 7.57-7.65 (m, 2H, Ar), 7.97-8.03 (m, 2H, C=CH, Ar), 8.64 (s, 2H, -OH), 10.04 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 97.29, 101.83, 116.51, 116.71, 117.37, 120.24, 123.61, 125.42, 126.13, 130.58, 143.44, 146.29, 151.77, 151.95, 162.26, 165.77, 171.21; Mass *m/z* 338.55 (M-H).

4-[2-Cyano-3-(3-hydroxyphenyl)acrylamido]-2-hydroxybenzoic acid (5i): m.f.: $C_{17}H_{12}N_2O_5$; m.w.: 324; R_f: 0.63; m.p.:204-207 ºC; yield: 84%. IR (KBr, ν_{max}, cm^{-1}): 3593 (-OH, *str.*), 3294 (-NH, *str.*), 3093 (Ar-CH, *str.*), 2919 (aliph-CH, *str.*), 2236 (-CN, *str.*), 1753 (C=O, acid, *str.*), 1694 (C=O, amide, *str.*), 1582 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 6.84-6.86 (d, 1H, Ar), 6.87-6.89 (d, 1H, Ar), 6.99-7.02 (m, 1H, Ar), 7.54-7.56 (dd, 1H, Ar), 7.70-7.72 (dd, 1H, Ar), 7.98-7.99 (d, 1H, Ar), 8.08-8.09 (d, 1H, Ar), 8.16 (s, 1H, C=CH), 8.52 (s, 1H, -OH), 10.22 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 102.38, 108.19, 113.94, 116.53, 117.50, 120.26, 123.58, 125.51, 126.77, 130.56, 143.36, 148.30, 151.97, 152.38, 158.26, 162.08, 165.75; Mass *m/z* 323.05 (M-H).

4-[2-Cyano-3-(4-hydroxyphenyl)acrylamido]-2-hydroxybenzoic acid (5j): m.f.: $C_{17}H_{12}N_2O_5$; m.w.: 324; R_f: 0.86; m.p.:215-218 ºC; yield: 91%. IR (KBr, ν_{max}, cm^{-1}): 3521 (-OH, *str.*), 3253 (-NH, *str.*), 3050 (Ar-CH, *str.*), 2953 (aliph-CH, *str.*), 2251 (-CN, *str.*), 1733 (C=O, acid, *str.*), 1691 (C=O, amide, *str.*), 1553 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 6.81-6.83 (d, 1H, Ar), 6.96-6.98 (d, 2H, Ar), 7.66-7.68 (dd, 1H, Ar), 7.91-7.93 (d, 2H, Ar), 8.05 (s, 1H, Ar), 8.14 (s, 1H, C=CH), 8.74 (s, 1H, -OH), 10.12 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 107.40, 116.64, 117.19, 120.19, 121.88, 122.92, 126.82, 128.08, 129.56, 129.80, 130.79, 133.58, 151.01, 158.42, 158.82, 160.71, 171.96; Mass *m/z* 322.5 (M-H).

4-[2-Cyano-3-(2-hydroxyphenyl)acrylamido]-2-hydroxybenzoic acid (5k): m.f.: $C_{17}H_{12}N_2O_5$; m.w.: 324; R_f: 0.84; m.p.:223-226 ºC; yield: 73%. IR (KBr, ν_{max}, cm^{-1}): 3559 (-OH, *str.*), 3293 (-NH, *str.*), 3036 (Ar-CH, *str.*), 2912 (aliph-CH, *str.*), 2258 (-CN, *str.*), 1771 (C=O, acid, *str.*), 1709 (C=O, amide, *str.*), 1615 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 6.93-6.98 (m, 2H, Ar), 7.52-7.54 (m, 2H, Ar), 7.70-7.71 (m, 1H, Ar), 7.75-7.77 (m, 1H, Ar), 8.11-8.14 (m, 2H, C=CH, Ar), 8.74 (s, 1H, -OH), 10.30 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 97.84, 102.67, 115.75, 116.82, 117.56, 123.13, 123.38, 127.51, 129.39, 133.36, 143.64, 150.73, 158.87, 161.19, 162.38, 172.29; Mass *m/z* 322.5 (M-H).

4-[2-Cyano-3-(3-methoxy-4-hydroxyphenyl)acrylamido]-2-hydroxybenzoic acid (5l): m.f.: $C_{18}H_{14}N_2O_6$; m.w.: 354; R_f: 0.52; m.p.:210-212 ºC; yield: 98%. IR (KBr, ν_{max}, cm^{-1}): 3521 (-OH, *str.*), 3283 (-NH, *str.*), 3006 (Ar-CH, *str.*), 2964 (aliph-CH, *str.*), 2250 (-CN, *str.*), 1773 (C=O, acid, *str.*), 1705 (C=O, amide, *str.*), 1614 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 3.83 (s, 3H, OCH_3), 6.63-6.66 (m, 2H, Ar), 7.42 (s, 1H, Ar), 7.51-7.53 (m, 1H, Ar), 7.93 (d, 1H, Ar), 8.15-8.16 (m, 2H, C=CH, Ar), 9.02 (s, 1H, -OH), 10.02 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 56.52, 96.18, 103.37, 108.90, 115.96, 117.85, 122.54, 123.58, 125.65, 139.37, 141.09, 147.68, 148.38, 150.93, 158.82, 160.86, 172.48; Mass *m/z* 352.55 (M-H).

4-[2-Cyano-3-(3,5-dimethoxy-4-hydroxyphenyl)acrylamido]-2-hydroxybenzoic acid (5m): m.f.: C_{19} $H_{16}N_2O_7$; m.w.: 384; R_f: 0.65; m.p.:321-324 ºC; Yield: 94%. IR (KBr, ν_{max}, cm^{-1}): 3590 (-OH, *str.*), 3277 (-NH, *str.*), 3009 (Ar-CH, *str.*), 2931 (aliph-CH, *str.*), 2209 (-CN, *str.*), 1702 (C=O, acid, *str.*), 1690 (C=O, amide, *str.*), 1619 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 3.83 (s, 6H, OCH_3), 6.63-6.67 (m, 1H, Ar), 7.42 (s, 2H, Ar), 7.51-7.53 (m, 1H, Ar), 8.15-8.16 (m, 2H, C=CH, Ar), 9.05 (s, 1H, -OH), 10.02 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 56.53, 96.74, 103.39, 108.92, 115.98, 117.87, 122.56, 123.61, 125.69, 139.10, 141.12, 148.40, 150.95, 158.66, 162.89, 171.03; Mass *m/z* 382.55 (M-H).

4-[2-Cyano-3-(3,4,5-trimethoxyphenyl)acrylamido]-2-hydroxybenzoic acid (5n): m.f.: $C_{20}H_{18}N_2O_7$; m.w.: 398; R_f: 0.72; m.p.:284-287 ºC; Yield: 89%. IR (KBr, ν_{max}, cm^{-1}): 3594 (-OH, *str.*), 3204 (-NH, *str.*), 3037 (Ar-CH, *str.*), 2939 (aliph-CH, *str.*), 2236 (-CN, *str.*), 1765 (C=O, acid, *str.*), 1703 (C=O, amide, *str.*), 1624 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 3.78 (s, 3H, OCH_3), 3.85 (s, 6H, OCH_3), 6.97-6.99 (d, 1H, Ar), 7.41 (s, 2H, Ar), 7.79-7.81 (dd, 1H, Ar), 8.14-8.15 (d, 1H, Ar), 8.21 (s, 1H, C=CH), 10.34 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 56.51, 60.79, 105.99, 108.45, 113.08, 116.71, 117.04, 122.72, 127.53, 129.25, 130.33, 141.67, 151.27, 153.40, 158.29, 160.84, 172.02; Mass *m/z* 396.65 (M-H).

4-[2-Cyano-3-(3-methoxy-4-hydroxy-5-nitroyphenyl)-acrylamido]-2-hydroxybenzoic acid (5o): m.f.: $C_{18}H_{13}N_3O_8$; m.w.: 399; R_f: 0.68; m.p.:234-236 ºC; yield: 74%. IR (KBr, ν_{max}, cm^{-1}): 3592 (-OH, *str.*), 3278 (-NH, *str.*), 3123 (Ar-CH, *str.*), 2991 (aliph-CH, *str.*), 2239 (-CN, *str.*), 1773 (C=O, acid, *str.*), 1703 (C=O, amide, *str.*), 1616 (-NH, *def.*); ^{1}H NMR (500 MHz, DMSO, δ ppm): 3.81 (s, 3H, OCH_3), 7.52-7.55 (d, 1H, Ar), 7.65 (s, 1H, Ar), 7.71 (s, 1H, Ar), 7.76-7.78 (dd, 1H, Ar), 7.90-7.94 (m, 1H, Ar), 8.17 (s, 1H, C=CH), 10.49 (s, 1H, -OH), 10.66 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO, δ ppm): 55.55, 101.38, 115.81, 116.16, 117.26, 118.45, 119.59, 122.67, 126.52, 127.41, 130.15, 130.40, 141.87, 147.95, 151.36, 152.73, 161.56, 167.40; Mass *m/z* 400.10 (M+H).

Biological activity

***In vitro* antibacterial activity:** The antibacterial activity of synthesized compounds (**5a-o**) was conducted against two Gram-positive bacteria *viz.*, *Bacillus subtilis*, *Staphylococcus aureus* and two Gram-negative bacteria *viz.*, *Escherichia coli*, *Proteus vulgaris* by using cup plate approach. The zone of inhibition (ZOI) was utilized to assess the outcomes of the antibacterial screening. Streptomycin and 4-aminosalicylic acid were employed as a reference standard to compare the results.

The nutrient agar medium was sterilized by autoclaving at 121 ºC (15 lb/sq.inch) for 15 min. The petriplates, boiling tubes and flasks plugged with cotton were sterilized in a hot-air oven at 160 ºC, for 1 h. Into each sterilized petriplate (10 cm diameter), poured about 125 mL of molten nutrient agar medium which was already inoculated with the respective strain of bacteria (5 mL of inoculum to 250 mL of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow solidification. After solidification, the cups of each 6 mm diameter were made by scooping out medium with a sterilized cork borer from a petridish. Under sterile conditions, 0.1 mL of each test solution, containing 100 μg, was carefully added to the cups. At the same time, 0.1 mL of a standard solution with 100 μg of streptomycin and 4-aminosalicylic acid was added to separate petriplates. All plates were labelled accordingly. The labelled plates were then incubated for 24 h at 37 ± 1 ºC. After the incubation period, the diameters of the circular inhibition zones (mm) were measured [23].

RESULTS AND DISCUSSION

In present research, 4-[2-cyano-3-(substituted phenyl)-acrylamido]-2-hydroxybenzoic acids (**5a-o**) were synthesized by using a novel intermediate 4-(2-cyanoacetamido)-2-hydroxybenzoic acid (**3**) with substituted benzaldehydes (**4a-o**) through Knoevenagel condensation reaction. The reaction was performed using catalytic amounts of acetic acid and piperidine in toluene as illustrated in **Scheme-I**. All the synthesized compounds (**5a-o**), were effectively synthesized with good yields ranging from 71-98%. All the structures of synthesized compounds (**5a-o**) were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis.

The IR spectrum of intermediate 4-(2-cyanoacetamido)-2-hydroxybenzoic acid (**3**) revealed the absorption bands at 3286.78 cm^{-1} and 1465.70 cm^{-1}, indicating N-H stretching and an active methylene group. The absorption bands at 2213.78 cm^{-1} show C≡N stretching. In the IR spectrum of the target compounds **5a-o**, showed the absorption band between 3597.70-3512.81 cm^{-1} indicative of O-H stretching. The absorption bands corresponding to N-H stretching vibration range from 3379.96-3204.77 cm^{-1}. The absorption bands associated with C≡N stretching appeared in the region from 2267.70-2201.26 cm^{-1}. The cyano group is a strong electron withdrawing group, which increases electron deficiency on the adjacent carbonyl carbon. This increased electron deficiency results in a higher stretching frequency with a higher wave number. As a result, the carbonyl group of acid and amide emitted bands in the range from 1782.09-1702.33 and 1709.01-1676.06 cm^{-1}. The N-H deformation appeared in the region of 1636.78-1548.82 cm^{-1}.

The ^{1}H NMR spectrum of the intermediate, 4-(2-cyanoacetamido)-2-hydroxybenzoic acid (**3**), showed a characteristic singlet at δ 10.26 ppm, confirming the presence of the amide N–H proton. The aromatic region displayed two

doublets at δ 8.06-8.05 and δ 6.95-6.93 ppm, along with a double of doublet at δ 7.62-7.70 ppm, corresponding to the protons of the 2-hydroxybenzoic acid ring. A distinct singlet at δ 3.86 ppm was observed for the active methylene (–CH_2–) protons. In the final compounds **5a-o**, the complete disappearance of this methylene singlet at δ 3.86 ppm and the appearance of benzylidene (C=CH) protons as singlets in the region of δ 8.32-8.03 ppm provide clear evidence for the successful Knoevenagel condensation, thus confirming the formation of the target acrylamido derivatives. The ^{1}H NMR spectra of all synthesized compounds **5a-o** exhibited a characteristic amide N–H singlet in the region of δ 10.73-10.02 ppm, confirming the presence of the acrylamido moiety across the series. In addition, compounds **5h**, **5i**, **5j**, **5k**, **5l**, **5m** and **5o**, which contain phenolic or salicylic hydroxyl groups, showed distinct O–H singlets appearing between δ 10.49-8.52 ppm, supporting the presence of free hydroxyl functionalities. The aromatic region of all compounds **5a-o** was represented by multiplets, doublets and doublets of doublets within δ 8.31-6.63 ppm, corresponding to the protons of both the salicylic ring and the substituted phenyl ring attached to the acrylamide linkage. Substituent-specific signals were also clearly observed. A singlet at δ 3.88-3.81 ppm, attributed to methoxy (–OCH_3) protons, appeared in compounds **5f**, **5g**, **5l**, **5m**, **5n** and **5o**, consistent with their methoxy-substituted phenyl systems. For the dimethylamino derivative (**5e**), the $N(CH_3)_2$ protons resonated as a characteristic singlet at δ 3.08 ppm. The isopropyl-substituted compound (**5d**) showed a well-defined doublet at δ 1.25-1.24 ppm corresponding to the isopropyl CH_3 groups and a multiplet at δ 3.04-3.02 ppm assigned to the methine (CH) proton, confirming the presence of the isopropyl moiety.

The ^{13}C NMR spectra exhibited a resonance peak between δ 172.48-165.71 ppm representing the acid carbonyl carbon signal and δ 165.77-160.53 ppm representing the amide carbonyl carbon signal. Resonance peak at δ 151.94-146.29 ppm representing the benzylidene carbon signal and δ 117.85-117.16 ppm representing the nitrile carbon signal. The resonance peak at δ 162.26-96.18 and δ 60.79-55.55 ppm representing the aromatic carbons signal and methoxy carbon signal. The mass spectra of all the synthesized compounds **5a-o** revealed [M+H] and [M-H] peak equivalent to their molecular weight.

Biological evaluation

***In vitro* antibacterial activity:** The antibacterial activities of the synthesized compounds **5a-o** were assessed against *B. subtilis*, *S. aureus*, *E. coli* and *P. vulgaris* using the cup-plate method (1000 µg/mL). The mean ZOI values are summarized in Table-1. Streptomycin and 4-aminosalicylic acid served as reference standards. Overall, the series showed better inhibition against Gram-negative strains, indicating favourable permeability through their cell membranes.

Among all derivatives, **5h** (3,4-di-OH) and **5l** (3-OCH_3, 4-OH) exhibited the highest antibacterial activity, with ZOI values comparable to streptomycin and 4-aminosalicylic acid. A clear SAR trend emerged based on the nature and position of substituents. Phenolic OH groups significantly enhanced activity (**5i**, **5j**, **5h**), attributed to increased polarity and stronger hydrogen-bonding interactions. Compounds containing both -OH and -OCH_3 groups (**5l**, **5m**) showed further improvement, suggesting a synergistic electron-donating effect that enhances target binding. Electron-rich substituents also contributed positively; the dimethylamino analogue **5e** showed higher activity than the sterically similar **5d**, indicating the role of lone-pair electron donation in improving antibacterial interaction. In contrast, steric hindrance and unfavourable substitution positions reduced activity. The *ortho*-substituted compound **5k** and bulky trimethoxy derivative **5n** showed diminished potency. Introduction of an electron-withdrawing nitro group (**5o**) slightly decreased activity, confirming that reduced electron density on the aromatic ring negatively impacts binding. Overall, electron-donating substituents, particularly *para*-hydroxyl and methoxy groups, were most favourable, while bulky or electron-withdrawing groups attenuated antibacterial potency.

TABLE-1
ANTIBACTERIAL ACTIVITY OF 4-[2-CYANO-3-(SUBSTITUTED PHENYL)ACRYLAMIDO]-2-HYDROXYBENZOIC ACIDS (**5a-o**)

Compounds	Zone of inhibition (mm)			
	B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*
5a	06	08	07	09
5b	10	11	08	10
5c	10	09	11	08
5d	08	09	09	10
5e	14	16	16	17
5f	10	08	08	08
5g	11	07	07	09
5h	15	16	18	18
5i	13	14	15	17
5j	10	10	10	18
5k	08	08	06	07
5l	17	18	21	19
5m	14	14	15	16
5n	09	07	10	07
5o	14	16	16	16
Streptomycin	19	23	25	21
4-Aminosalicylic acid	16	17	19	19

Conclusion

A novel series of 4-[2-cyano-3-(substituted phenyl)acrylamido]-2-hydroxybenzoic acid (**5a-o**) was successfully synthesized *via* a Knoevenagel condensation of intermediate **3** with substituted benzaldehydes. Antibacterial screening demonstrated that all derivatives possessed measurable activity, with compounds **5l** and **5h** exhibiting the highest inhibition against *B. subtilis*, *S. aureus*, *E. coli* and *P. vulgaris*. Electron-donating substituents, particularly 3-OCH_3, 3-OH and 4-OH groups, significantly enhanced activity, likely due to improved hydrogen bonding and increased electron density facilitating stronger bacterial target interactions. In contrast, sterically hindered *ortho*-substitution and electron-withdrawing groups resulted in reduced potency. Overall, the findings indicate that hydroxyl- and methoxy-substituted derivatives serve as promising lead molecules. Based on their encouraging antibacterial profiles, these compounds will be further evaluated for antitubercular, antioxidant and anticancer activities in subsequent studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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