An Insight into the Anticancer and Antibacterial Properties of New 4-(Benzylamino)benzoic Acid Derivatives: Synthesis and X-ray Crystallographic Analysis

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Article

An Insight into the Anticancer and Antibacterial Properties of New 4-(Benzylamino)benzoic Acid Derivatives: Synthesis and X-ray Crystallographic Analysis

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Abstract: A prominent class of reactions, reductive aminations are used extensively in research labs and industry to synthesize amines; which are applied in a variety of medicines, agrochemicals, and biomolecules. Amine compounds are essential in many aspects of life; amino groups exist in many natural and agricultural products. New 4-(benzylamino)benzoic acid derivatives with general formula of $C_{14}H_{12}NO_2R$, where [R= (H), (4-Cl), (4-NMe), (4-Br), (3-NO_2), (4-NO_2), (2-OMe), (3-OMe), (4-OMe), (2,3-OMe), (3,4-OMe), (2-OH, 3-OMe), (3-OMe, 4-OH), (3,5-OMe, 4-OH), (2-OH, 5-Br), (3-OH), (4-SMe), (2,3-OH), (3-CF_3)] (compounds **1-19**) were obtained by reductive amination reaction via reacting *p*-amino benzoic acid with benzaldehyde derivatives and sodium borohydride. All prepared compounds has been characterized by a variety of spectroscopic techniques (HRMS, FT-IR, ^{1}H, and ^{13}C{^{1}H} NMR) and elemental analysis. Furthermore, the structure of some of the synthesized derivatives were confirmed by single crystal X-ray diffraction (R = 4-Cl, 4-Br, 2-OMe, 3-OMe, 4-OMe, 2,3-OMe), compounds **2**, **4**, **7-9** and **11**, respectively. Moreover, some of these new compounds have shown somehow moderate antibacterial activities against different strains of bacteria (gram-positive and gram-negative) with MIC values ranging from 64-256 μg/ mL. The anticancer activities of all compounds (IC_{50} values, μM) were evaluated against different cell lines, HGF (Fibroblasts; normal cell line), A549 (Non-Small Cell Lung Cancer cell line) and H69 (Small Cell Lung Cancer cell line). Compound **18** showed a considerable IC_{50} value of 90.69 against A549 and an excellent value of 32.22 and H69, respectively.

Keywords: amines; reductive amination; x-ray structure; anticancer; antibacterial

1. Introduction

One of the main objectives of contemporary science is to promote the quality of life for all creatures. This primarily entails the creation of novel drugs and improving the methods for synthesizing already-existing pharmaceuticals in a medicinal context. Amine compounds are

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significantly important because the amino group is often discovered in many natural and agricultural products [1–6], they are also involved in pharmaceutical, chemical, and biological production [7–11]. Many different ways to produce amines include: (I) reduction of imine [12], nitriles [13], amides [14], nitro groups [15]. (II) nucleophilic substitution reactions: Gabriel Synthesis [16], Hofmann rearrangement [17], Curtius Rearrangement [18].

Reductive aminations (RA) constitute a significant class of reactions widely applied in research laboratories and industries for the synthesis of amines as well as pharmaceuticals, agrochemicals, and biomolecules [19–23]. According to researchers [24] 25% of C-N bond-forming reactions in drug industry take place via reductive amination reactions between carbonyl compounds (aldehyde/ ketones) with amines in the presence of a reducing agent, which represent a highly efficient and facile pathway to amine synthesis because of the readily available starting materials [25], suitable one-pot synthetic procedure, selective production of unsymmetrically substituted secondary, tertiary amines, and broad substance scope [26].

Para-aminobenzoic acid (4-aminobenzoic acid, PABA) is a well-known cyclic amino acid compound that belongs to the vitamins B group (vitamin B10) [27]. Furthermore, PABA well-known in biochemistry, as it synthesized by: yeasts [28], plants [29], and some bacteria [30]. As known, it is necessary for the synthesis of folic acid. In mammals and humans, PABA is not synthesized but formed by some bacteria, such as (*Escherichia coli*) in the human intestinal tract. Moreover, in medicinal chemistry, PABA was one of the main biologically active constituents to be utilized in sunscreen, as Patented in 1943 [31]. The initial in vivo experiment on mice demonstrated that PABA lessened UV damage and, in addition, ensured against skin cancer in rodents [32,33]. In industrial applications, *p*-aminobenzoic acid is used to synthesize Azo dyes that are used primarily to color textiles, leather, and paints [34].

Herein, we have done some modifications on earlier reported procedures that synthesized some 4-(benzylamino)benzoic acid derivatives [35–37]. To the best of our knowledge, there is no report on this modified procedure in the literature to prepare these compounds and some of our prepared derivatives are new and the rest were prepared with different methods as mentioned in the literature [38–40].

This work aimed at structurally modifying *para*-aminobenzoic acid via RA with different aldehydes to produce 4-(benzylamino)benzoic acid derivatives via milder reaction conditions, scalable procedures, and higher yields. Hence it is worth investigating their potential anticancer and antibacterial activities.

2. Experimental

2.1. General

All solvents and commercial reagents (*p*-amino benzoic acid, benzaldehyde, *p*-chlorobenzaldehyde, *p*-bromobenzaldehyde, *p*-dimethylaminobenzaldehyde, *m*-nitrobenzaldehyde, *p*-nitrobenzaldehyde, 5-bromo-2-hydroxybenzaldehyde, and *m*-hydroxybenzaldehyde, *o*-methoxy-benzaldehyde, *m*-methoxy-benzaldehyde, *p*-methoxy-benzaldehyde, 2,3-dimethoxy-benzaldehyde, 3,4-dimethoxy-benzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 2-hydroxy-3-methoxy-benzaldehyde, 4-hydroxy-3,5-dimethoxy-benzaldehyde, 4-methylthio-benzaldehyde, 2,3-dihydroxy-benzaldehyde, and 3-trifloromethyl-benzaldehyde) were purchased from Sigma Aldrich Chemical Company and used as received without purification unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254. Visualization of TLC was accomplished with UV light (254 nm). NMR spectra were recorded on a Bruker-Avance 400 MHz spectrometer. The residual solvent protons (^{1}H) or the solvent carbon (^{13}C) were used as internal standards. ^{1}H-NMR data are presented as follows: chemical shift in ppm (δ). The following abbreviations are used in reporting NMR data: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, mutiplet. ATR-FTIR spectra of the resulting compounds were recorded on a Bruker Vertex 70-FT-IR spectrometer at room temperature coupled with a Vertex Pt-ATR accessory, in the range of 4000 – 400 cm^{-1} at room temperature with 4 cm^{-1} resolutions. A UV-1800 UV-visible spectrophotometer (Shimadzu Corporation) was used to obtain UV-visible spectra at 25 °C in DMSO solution with a concentration

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of 5×10^{-5}M. High-Resolution Mass Spectra were recorded on SHIMADZU LC MSMS 8050With UHPLC 2060C.

2.2. General Procedure for Reductive Amination Reaction between 4-Aminobenzoic Acid and Benzaldehyde Derivatives to Produce 4-(benzylamino) Benzoic Acid Derivatives

The 4-(benzylamino) benzoic acid derivatives were synthesized in high yields by the reductive amination reaction as shown in Scheme 1. *P*-Aminobenzoic acid (1g, 7.29 mmol) was reacted with benzaldehyde, (0.77g, 7.29 mmol) in 5-10 mL solution of methanol. After a few minutes, the imine precipitated as a colored powder. Thereafter, sodium borohydride (0.41g, 10.9 mmol) was added in small portions to the reaction mixture, the precipitate dissolved, and the color faded. After that, a solution of 10% HCl (ca. 20 mL) was added until 4-(benzylamino)benzoic acid precipitated as powder. The product was filtered off, washed several times with distilled water and cold ethanol, and dried in the lab atmosphere. All other derivatives were prepared in the same manner, scheme 1.

2.2.1. Synthesis of 4-(benzylamino)benzoic Acid (1)

The title compound was synthesized using the general procedure and isolated 98 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2536-3100 (COOH), 3422 (NH), 1655 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.98 (s, 1H, COOH), 7.64 (d, J = 8.51 Hz, 2H, Ar-H), 7.34 (m, 4H, Ar-H), 7.24 (m, 1H, Ar-H), 7.03 (t, J = 5.98 Hz, 1H, NHCH$_2$), 6.60 (m, 2H, Ar-H), 4.33 (d, J= 5.99 Hz, 2H, HNCH$_2$). ^{13}C NMR: (DMSO-d_6,101 MHz) δ = 167.42 (1C, COOH), 152.39 (1C, Ar-C), 139.43 (1C, Ar-C), 131.04 (2C, Ar-CH), 128.34 (2C, Ar-CH), 127.14 (2C, Ar-CH), 126.78 (1C, Ar-CH), 117.21 (1C, Ar-C), 111.15 (2C, Ar-CH), 45.89 (1C, CH$_2$NH). Anal. Calcd. for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 73.84; H, 5.84; N, 6.20. λ_{max}: 305 nm, ε_{max}: 21325. HR-MS: $[M+H]^+$ at 228.00 m/z ($C_{14}H_{13}NO_2$ requires 227.09).

2.2.2. Synthesis of 4-((4-chlorobenzyl)amino)benzoic Acid (2)

The title compound was synthesized using the general procedure and isolated 95 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2514-3000 (COOH), 3416 (NH), 1651 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.82 (s, 1H, COOH), 7.64 (d, J = 8.55 Hz, 2H, Ar-H), 7.36 (m, 4H, Ar-H), 7.04 (t, J = 5.80 Hz, 1H, NHCH$_2$), 6.57 (d, J = 8.60 Hz, 2H, Ar-H), 4.32 (d, J = 5.90 Hz, 2H, HNCH$_2$). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.47(1C, COOH), 152.21 (1C, Ar-C), 138.61 (1C, Ar-C), 131.34 (1C, Ar-C-Cl), 131.12 (2C, Ar-CH), 128.99 (2C, Ar-CH), 128.34 (2C, Ar-CH), 117.47 (1C, Ar-C), 111.27 (2C, Ar-CH), 45.19 (1C, CH$_2$NH). Anal. Calcd. for $C_{14}H_{12}ClNO_2$: C, 64.25; H, 4.62; N, 5.35. Found: C, 64.46; H, 4.83; N, 5.36. λ_{max}: 304 nm, ε_{max}: 22200. HR-MS: $[M+H]^+$ at 262.00 m/z ($C_{14}H_{12}ClNO_2$ requires 261.06).

2.2.3. Synthesis of 4-((4-(dimethylamino)benzyl)amino)benzoic Acid (3)

The title compound was synthesized using the general procedure and isolated 88 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2479-3111 (COOH), 3400 (NH), 1693 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.99 (s, 1H, COOH), 7.63 (d, J = 7.62 Hz, 2H, Ar-H), 7.27 (d, J = 7.26 Hz, 2H, Ar-H), 6.95 (t, J = 6.93 Hz, 1H, NHCH$_2$), 6.90 (d, J = 6.89 Hz, 2H, Ar-H), 6.59 (d, J = 6.58 Hz, 2H, Ar-H), 4.25 (d, J = 4.24 Hz, 2H, HNCH$_2$), 2.86 (s, 6H, N(CH3)$_2$). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.81(1C, COOH), 152.32 (1C, Ar-C), 149.60 (1C, Ar-C), 130.95 (2C, Ar-CH), 128.14 (2C, Ar-CH), 127.47 (1C, Ar-C), 126.64 (1C, Ar-C), 112.47 (2C, Ar-CH), 111.08 (2C, Ar-CH), 45.57 (1C, CH$_2$NH), 40.28 (2C, N(CH3)$_2$). Anal. Calcd. for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 69.9; H, 6.66; N, 10.30. λ_{max}: 307 nm, ε_{max}: 10200. HR-MS: $[M+H]^+$ at 271.25 m/z ($C_{16}H_{18}N_2O_2$ requires 270.14).

2.2.4. Synthesis of 4-((4-bromobenzyl)amino)benzoic Acid (4)

The title compound was synthesized using the general procedure and isolated 91 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2515-3100 (COOH), 3422 (NH), 1653 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 12.01 (s, 1H, COOH), 7.63 (d, J= 8.72 Hz, 2H, Ar-H), 7.50 (d, J = 8.35 Hz, 2H, Ar-H), 7.28 (d, J= 8.33 Hz, 2H, Ar-H), 7.05 (t, J= 6.05 Hz, 1H, NHCH$_2$), 6.56 (d, J= 8.76 Hz, 2H, Ar-H), 4.30 (d, J= 5.86 Hz, 2H, HNCH$_2$). ^{13}C NMR: (101 MHz, DMSO-d_6) δ = 167.37 (1C, COOH), 152.15 (1C, Ar-C),

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139.00(1C, Ar-C), 131.19 (2C, Ar-CH), 131.05 (2C, Ar-CH), 129.31 (2C, Ar-CH), 119.72 (1C, Ar-C-Br), 117.40 (1C, Ar-C), 111.20 (2C, Ar-CH), 45.18 (1C, CH_2NH). Anal. Calcd. for $C_{14}H_{12}BrNO_2$: C, 54.92; H, 3.95; N, 4.58. Found: C, 54.75; H, 4.02; N, 4.65. λ_{max}: 304 nm, ε_{max}: 10700. HR-MS: $[M+H]^+$ at 306.20 m/z ($C_{14}H_{12}BrNO_2$requires 305.01).

2.2.5. Synthesis of 4-((3-nitrobenzyl)amino)benzoic Acid (5)

The title compound was synthesized using the general procedure and isolated 96 % Yield as yellow powder. FTIR (ATR, ν, cm^{-1}): 2600-3150 (COOH), 3330 (NH), 1605 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 12.11 (s, 1H, COOH), 8.19 (s, 1H, Ar-H), 8.09 (dd, J = 8.13 Hz, 1H, Ar-H), 7.80 (dd, J= 7.69 Hz, 1H, Ar-H), 7.64 (m, 3H, Ar-H), 7.18 (t, J = 6.20 Hz, 1H, NHCH_2), 6.60 (d, J = 8.71 Hz, 2H, Ar-H), 4.49 (d, J = 6.17 Hz, 2H, HNCH_2). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.38 (1C, COOH), 151.96 (1C, Ar-C), 147.94 (1C, Ar-C-NO_2), 142.31 (1C, Ar-C), 133.85 (1C, Ar-CH), 131.14 (2C, Ar-CH), 129.92 (1C, Ar-CH), 121.85 (1C, Ar-CH), 121.59 (1C, Ar-CH), 117.72 (1C, Ar-C), 111.32 (2C, Ar-CH), 45.01 (1C, CH_2NH). Anal. Calcd. $C_{14}H_{12}N_2O_4$ for: C, 61.67; H, 4.44; N, 10.29. Found: C, 61.76; H, 4.41; N, 10.35. λ_{max}: 300 nm, ε_{max}: 20825. HR-MS: $[M+H]^+$ at 273.00 m/z ($C_{14}H_{12}N_2O_4$ requires 272.08).

2.2.6. Synthesis of 4-((4-nitrobenzyl)amino)benzoic Acid (6)

The title compound was synthesized using the general procedure and isolated 91 % Yield as yellow powder. FTIR (ATR, ν, cm^{-1}): 2500-3310 (COOH), 3410 (NH), 1604 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 12.06 (s, 1H, COOH), 8.20 (d, J = 8.31 Hz, 2H, Ar-H), 7.66 (d, J = 8.37 Hz, 2H, Ar-H), 7.60 (d, J = 8.42 Hz, 2H, Ar-H), 7.20 (t, J = 6.10 Hz, 1H, NHCH_2), 6.59 (d, J = 8.48 Hz, 2H, Ar-H), 4.51 (d, J = 5.93 Hz, 2H, HNCH_2). ^{13}C NMR: (101 MHz, DMSO-d_6) δ = 167.33 (1C, COOH), 151.95 (1C, Ar-C), 148.02 (1C, Ar-C), 146.49 (1C, Ar-C-NO_2), 131.09 (2C, Ar-CH), 128.03 (2C, Ar-CH), 123.52 (2C, Ar-CH), 117.72 (1C, Ar-C), 111.27 (2C, Ar-CH), 45.30 (1C, CH_2NH). Anal. Calcd. for $C_{14}H_{12}N_2O_4$: C, 61.67; H, 4.44; N, 10.29. Found: C, 61.70; H, 4.40; N, 10.31. λ_{max}: 302 nm, ε_{max}: 30700. HR-MS: $[M+H]^+$ at 273.10 m/z ($C_{14}H_{12}N_2O_4$ requires 272.08).

2.2.7. Synthesis of 4-((2-methoxybenzyl)amino)benzoic Acid (7)

The title compound was synthesized using the general procedure and isolated 96% Yield as white powder. FTIR (ν, cm^{-1}): 2515-3100 (COOH), 3422 (NH), 1653 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.97 (s, 1H, COOH), 7.67 (d, J = 8.54 Hz, 2H, Ar-H), 7.27 (m, 2H, Ar-H), 7.02 (d, J = 8.11 Hz, 1H, Ar-H), 6.92 (t, J = 7.39 Hz, 1H, NHCH_2), 6.88 (dd, J = 6.86 Hz, 1H, Ar-H), 6.59 (d, J = 8.59 Hz, 2H, Ar-H), 4.30 (d, J = 5.56 Hz, 2H, HNCH_2), 3.85 (s, 3H, OCH_3). ^{13}C NMR: (DMSO-d_6,101 MHz) δ = 167.40 (1C, COOH), 156.87 (1C, Ar-C-OCH_3), 152.48 (1C, Ar-C), 131.04 (2C, Ar-CH), 128.03 (1C, Ar-CH), 127.74 (1C, Ar-C), 126.49 (1C, Ar-CH), 120.11 (1C, Ar-CH), 116.99 (1C, Ar-C), 110.92 (2C, Ar-CH), 110.58 (2C, Ar-CH), 55.29 (1C, OCH_3), 40.79 (1C, CH_2NH). Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.05; H, 5.91; N, 5.50. λ_{max}: 305 nm, ε_{max}: 19725. HR-MS: $[M+H]^+$ at 258.10 m/z ($C_{15}H_{15}NO_3$ requires 257.11).

2.2.8. Synthesis of 4-((3-methoxybenzyl)amino)benzoic Acid (8)

The title compound was synthesized using the general procedure and isolated 96% Yield as white powder. FTIR (ν, cm^{-1}): 2479-3111 (COOH), 3400 (NH), 1693 (C=O).^{1}HNMR: (DMSO-d_6, 400 MHz) δ = 12.00 (s, 1H, COOH), 7.65 (d, J= 8.74 Hz, 2H, Ar-H), 7.24 (t, J= 8.09 Hz, 1H, NHCH_2), 7.02 (dd, J= 5.99 Hz, 1H, Ar-H), 6.92 (m, 3H, Ar-H), 6.59 (d, J= 8.77 Hz, Ar-H), 4.30 (d, J= 5.90 Hz, 2H, HNCH_2), 3.72 (s, 3H, OCH_3). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.39 (1C, COOH), 159.37 (1C, Ar-C-OCH_3), 152.37 (1C, Ar-C), 141.12 (1C, Ar-C), 131.01 (2C, Ar-CH), 129.39 (1C, Ar-CH), 119.27 (1C, Ar-CH), 117.21 (1C, Ar-C), 112.84 (2C, Ar-CH), 112.04 (1C, Ar-CH), 111.16 (1C, Ar-CH), 54.92 (1C, OCH_3), 45.82 (1C, CH_2NH). Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 69.99; H, 5.90; N, 5.47. λ_{max}: 305 nm, ε_{max}: 17475. HR-MS: $[M+H]^+$ at 258.10 m/z ($C_{15}H_{15}NO_3$ requires 257.11).

2.2.9. Synthesis of 4-((4-methoxybenzyl)amino)benzoic Acid (9)

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The title compound was synthesized using the general procedure and isolated in 86% Yield as white powder. FTIR (ν, cm^{-1}): 2500-3310 (COOH), 3410 (NH), 1604 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.99 (s, 1H, COOH), 7.64 (d, J= 8.70 Hz, 2H, Ar-H), 7.26 (d, J = 8.58 Hz, 2H, Ar-H), 6.95 (t, J=5.86 1H, NHCH$_2$), 6.89 (d, J = 8.67 Hz, 2H, Ar-H), 6.58 (d, J = 8.77 Hz, 2H, Ar-H), 4.25 (d, J = 5.76 Hz, 2H, HNCH$_2$), 3.72 (s, 3H, OCH$_3$). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.43 (1C, COOH), 158.21 (1C, Ar-C-OCH$_3$), 152.37 (1C, Ar-C), 131.15 (1C, Ar-C), 130.99 (2C, Ar-CH), 128.41 (2C, Ar-CH), 117.10 (1C, Ar-C), 113.76 (2C, Ar-CH), 111.13 (2C, Ar-CH), 55.00 (1C, OCH$_3$), 45.34 (1C, CH$_2$NH). Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.07; H, 5.83; N, 5.50. λ_{max}: 305 nm, ε_{max}: 18375. HR-MS: $[M+H]^+$ at 258.10 m/z ($C_{15}H_{15}NO_3$ requires 257.11).

2.2.10. Synthesis of 4-((2,3-dimethoxybenzyl)amino)benzoic Acid (10)

The title compound was synthesized using the general procedure and isolated 96% Yield as white powder. FTIR (ν, cm^{-1}): 2536-3100 (COOH), 3422 (NH), 1655 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.98 (s, 1H, COOH), 7.65 (d, J = 8.76 Hz 2H, Ar-H), 7.02 (m, 2H, Ar-H), 6.89 (m, 2H, Ar-H, and NHCH$_2$), 6.59 (d, J = 8.80 Hz, 2H, Ar-H), 4.31 (d, J = 5.86 Hz, 2H, HNCH$_2$), 3.80 (s, 3H, OCH$_3$), 3.77 (s, 3H, OCH$_3$). ^{13}C NMR: (101 MHz, DMSO-d_6) δ = 167.41 (1C, COOH), 152.39 (1C, Ar-C-OCH$_3$), 152.31 (1C, Ar-C-OCH$_3$), 146.46 (1C, Ar-C), 132.42 (1C, Ar-C), 131.05 (2C, Ar-CH), 123.76 (1C, Ar-CH), 120.00 (1C, Ar-CH), 117.08 (1C, Ar-C), 111.75 (1C, Ar-CH), 110.96 (2C, Ar-CH), 60.13 (1C, OCH$_3$), 55.63 (1C, OCH$_3$), 40.74 (1C, CH$_2$NH). Anal. Calcd. for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88. Found: C, 66.87; H, 5.97; N, 4.91. λ_{max}: 305 nm, ε_{max}: 31250. HR-MS: $[M+H]^+$ at 288.20 m/z ($C_{16}H_{17}NO_4$ requires 287.12).

2.2.11. Synthesis of 4-((3,4-dimethoxybenzyl)amino)benzoic Acid (11)

The title compound was synthesized using the general procedure and isolated 91% Yield as white powder. FTIR (ν, cm^{-1}): 2600-3150 (COOH), 3330 (NH), 1605 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.98 (s, 1H, COOH), 7.64 (d, J = 8.62 Hz, 2H, Ar-H), 6.96 (m, 4H, Ar-H), 6.60 (d, J = 8.64 Hz, 2H, Ar-H, and NHCH$_2$), 4.24 (d, J = 5.74 Hz, 2H, HNCH$_2$), 3.73 (s, 3H, OCH$_3$), 3.71 (s, 3H, OCH$_3$). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.49 (1C, COOH), 152.46 (1C, Ar-C-OCH$_3$), 148.77 (1C, Ar-C-OCH$_3$), 147.77 (1C, Ar-C), 131.68 (1C, Ar-C), 131.03 (2C, Ar-CH) 119.26 (1C, Ar-CH), 117.11 (1C, Ar-CH), 111.77 (1C, Ar-CH), 111.26 (2C, Ar-CH), 111.21 (1C, Ar-CH), 55.53 (1C, OCH$_3$), 55.43 (1C, OCH$_3$), 45.78 (1C, CH$_2$NH). Anal. Calcd. $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88. Found: C, 66.91; H, 5.92; N, 4.90. λ_{max}: 305 nm, ε_{max}: 18675. HR-MS: $[M+H]^+$ at 288.20 m/z ($C_{16}H_{17}NO_4$ requires 287.12).

2.2.12. Synthesis of 4-((2-hydroxy-3-methoxybenzyl)amino)benzoic Acid (12)

The title compound was synthesized using the general procedure and isolated 89 % Yield as white powder. FTIR (ν, cm^{-1}): 2600-3200 (COOH), 3386 (NH), 3322 (OH), 1670 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.95 (s, 1H, COOH), 8.75 (s, 1H, Ar-C-OH), 7.63 (d, J = 8.68 Hz, 2H, Ar-H), 6.85 (m, 4H, Ar-H), 6.57 (d, J = 8.71 Hz, 2H, Ar-H, and NHCH$_2$), 4.25 (d, J = 5.84Hz, 2H, HNCH$_2$), 3.73 (s, 3H, OCH$_3$), 3.71 (s, 3H, OCH$_3$). ^{13}C NMR: (101 MHz, DMSO-d_6) δ = 167.50 (1C, COOH), 152.60 (1C, Ar-C-OCH$_3$), 147.32 (1C, Ar-C-OH), 143.87 (1C, Ar-C), 131.07 (2C, Ar-CH), 125.53(1C, Ar-C), 120.08 (1C, Ar-C), 118.63 (1C, Ar-C), 116.87 (1C, Ar-CH), 110.95 (1C, Ar-CH), 110.42 (2C, Ar-CH), 55.81 (1C, OCH$_3$), 40.73 (1C, CH$_2$NH). Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13. Found: C, 65.86; H, 5.58; N, 5.09. λ_{max}: 305 nm, ε_{max}: 11600. HR-MS: $[M+H]^+$ at 274.00 m/z ($C_{15}H_{15}NO_4$ requires 273.10).

2.2.13. Synthesis of 4-((4-hydroxy-3-methoxybenzyl)amino)benzoic Acid (13)

The title compound was synthesized using the general procedure and isolated 95 % Yield as white powder. FTIR (ν, cm^{-1}): 2600-3200 (COOH), 3386 (NH), 3322 (OH), 1670 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.96 (s, 1H, COOH), 8.75 (s, 1H, Ar-C-OH), 7.64 (d, J = 8.68 Hz, 2H, Ar-H), 6.86 (m, 3H, Ar-H), 6.77 (t, J = 6.73 Hz, 1H, HNCH$_2$), 6.57 (d, J = 8.71 Hz, 2H, Ar-H), 4.26 (d, J = 5.84 Hz, 2H, HNCH$_2$), 3.80 (s, 3H, OCH$_3$). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.95 (1C, COOH), 153.05 (1C, Ar-C-OCH$_3$), 147.78 (1C, Ar-C), 144.32 (1C, Ar-C-OH), 131.52 (2C, Ar-CH), 125.98 (1C, Ar-C),

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120.54 (1C, Ar-CH), 119.08 (1C, Ar-CH), 117.33 (1C, Ar-C), 111.40 (2C, Ar-CH), 110.88 (1C, Ar-CH), 56.87 (1C, OCH_3), 40.73 (1C, CH_2NH). Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13. Found: C, 65.88; H, 5.49; N, 5.13. λ_{max}: 305 nm, ε_{max}: 4825. HR-MS: $[M+H]^+$ at 274.00 m/z ($C_{15}H_{15}NO_4$ requires 273.10).

2.2.14. Synthesis of 4-((4-hydroxy-3,5-dimethoxybenzyl)amino)benzoic Acid (14)

The title compound was synthesized using the general procedure and isolated 82 % Yield as white powder. FTIR (ν, cm^{-1}): 2516-3016 (COOH), 3353 (NH), 3152 (OH), 1679 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 12.03 (s, 1H, COOH), 8.20 (s, 1H, Ar-C-OH), 7.65 (d, J = 8.66 Hz, 2H, Ar-H), 6.86 (t, J = 5.66 Hz, 1H, HNCH), 6.63 (d, J = 6.61 Hz, 4H, Ar-H), 4.19 (d, J = 5.63 Hz, 2H, $HNCH_2$), 3.72 (s, 6H, OCH_3). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.50 (1C, COOH), 152.52 (1C, Ar-C), 147.99 (2C, Ar-C-OCH_3), 134.45 (1C, Ar-C-OH), 131.03 (2C, Ar-CH), 129.17 (1C, Ar-C), 117.10 (1C, Ar-C), 111.21 (2C, Ar-CH), 104.97 (2C, Ar-CH), 55.97 (2C, OCH_3), 46.32 (1C, CH_2NH). Anal. Calcd. for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.40; H, 5.62; N, 4.64. λ_{max}: 306 nm, ε_{max}: 14600. HR-MS: $[M+H]^+$ at 304.10 m/z ($C_{16}H_{17}NO_5$ requires 303.11).

2.2.15. Synthesis of 4-((5-bromo-2-hydroxybenzyl)amino)benzoic Acid (15)

The title compound was synthesized using the general procedure and isolated 85 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2600-3200 (COOH), 3386 (NH), 3322 (OH), 1670 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 10.02 (s, 1H, OH), 7.66 (d, J = 8.73 Hz, 2H, Ar-H), 7.21 (m, 3H, Ar-H and NH), 6.81 (d, J = 8.46 Hz, 1H, Ar-H), 6.57 (d, J = 8.73 Hz, 2H, Ar-H), 4.23 (d, 2H, $HNCH_2$). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.52 (1C, COOH), 154.47 (1C, Ar-C-OH), 152.28 (1C, Ar-C), 131.24 (2C, Ar-CH), 130.30 (1C, Ar-C), 130.28 (1C, Ar-CH), 128.02 (1C, Ar-CH), 117.40 (1C, Ar-CH), 117.17 (1C, Ar-CH), 111.15 (2C, Ar-CH), 110.12 (1C, Ar-C-Br), 40.48 (1C, CH_2NH). Anal. Calcd. for $C_{14}H_{12}BrNO_3$: C, 69.75; H, 5.85; N, 4.40. Found: C, 69.68; H, 5.80; N, 4.37. λ_{max}: 305 nm, ε_{max}: 12500. HR-MS: $[M+H]^+$ at 322.00 m/z ($C_{14}H_{12}BrNO_3$ requires 321.00).

2.2.16. Synthesis of 4-((3-hydroxybenzyl)amino)benzoic Acid (16)

The title compound was synthesized using the general procedure and isolated 80 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2516-3016 (COOH), 3353 (NH), 3152 (OH), 1679 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.98 (s, 1H, COOH), 9.33 (s, 1H, OH), 7.64 (d, J = 8.65 Hz, 2H, Ar-H), 7.11 (dd, J = 7.75 Hz, 1H, Ar-H), 7.00 (t, J = 5.89 Hz, 1H, $NHCH_2$), 6.57 (m, 5H, Ar-H), 4.25 (d, J = 5.98 Hz, 2H, $HNCH_2$). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.49 (1C, COOH), 157.49 (1C, Ar-C-OH), 152.49 (1C, Ar-C), 140.99 (1C, Ar-C), 131.07 (2C, Ar-CH), 129.36 (1C, Ar-CH), 117.68 (1C, Ar-C), 117.08 (1C, Ar-CH), 113.79 (2C, Ar-CH), 113.76 (1C, Ar-CH), 111.13 (1C, Ar-CH), 46.83 (1C, CH_2NH). Anal. Calcd. for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.22 1; H, 5.41; N, 5.74. λ_{max}: 305 nm, ε_{max}: 14975. HR-MS: $[M+H]^+$ at 244.10 m/z ($C_{14}H_{13}NO_3$ requires 243.09).

2.2.17. Synthesis of 4-((4-(methylthio)benzyl)amino) benzoic Acid (17)

The title compound was synthesized using the general procedure and isolated 80 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2516-3016 (COOH), 3333 (NH), 1665 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 12.06 (s, 1H, COOH), 7.64 (d, J= 8.47 Hz, 2H, Ar-H), 7.27 (d, J = 8.00 Hz, 2H, Ar-H), 7.20 (d, J = 8.00 Hz, 2H, Ar-H) 6.97 (t, J=5.86 1H, $NHCH_2$), 6.57 (d, J = 8.49 Hz, 2H, Ar-H), 4.27 (d, J = 5.04 Hz, $HNCH_2$), 2.42 (s, 3H, SCH_3). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.79 (1C, COOH), 152.26 (1C, Ar-C-SCH_3), 136.37 (1C, Ar-C), 136.33 (1C, Ar-C), 131.14 (2C, Ar-CH), 127.94 (2C, Ar-CH), 126.25 (2C, Ar-CH), 117.84 (1C, Ar-C), 111.29 (2C, Ar-CH), 48.71 (1C, SCH_3), 45.52 (1C, CH_2NH). Anal. Calcd. for $C_{15}H_{15}NO_2S$: C, 65.91; H, 5.53; N, 5.12. Found: C, 65.88; H, 5.50; N, 5.09. λ_{max}: 307 nm, ε_{max}: 11200. HR-MS: $[M+H]^+$ at 274.08 m/z ($C_{15}H_{15}NO_2S$ requires 273.08).

2.2.18. Synthesis of 4-((2,3-dihydroxybenzyl)amino) benzoic Acid (18)

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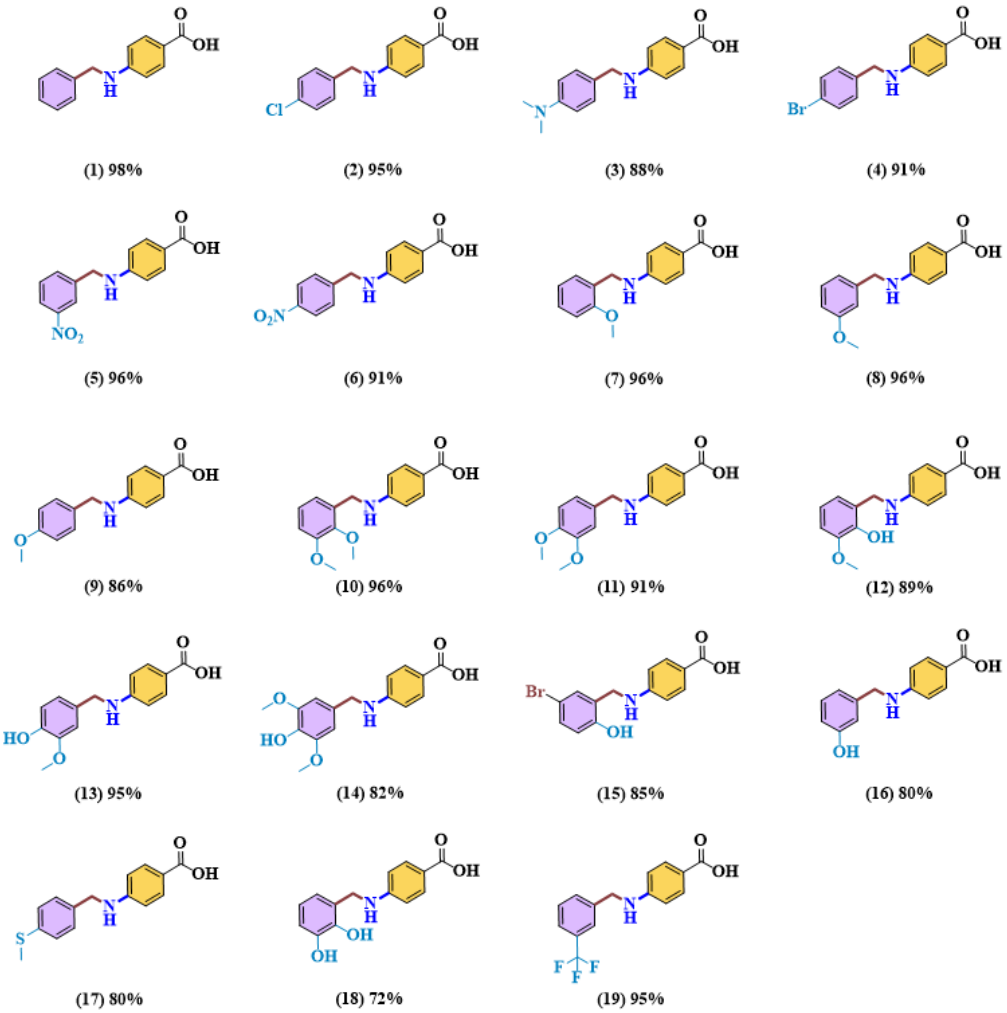
The title compound was synthesized using the general procedure and isolated 72 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2516-3020 (COOH), 3402 (NH), 3157 (OH), 1675 (C=O). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 11.96 (s, 1H, COOH), 9.31 (s, 1H, Ar-C-OH), 8.44 (s, 1H, Ar-C-OH), 7.63 (d, J = 8.52 Hz, 2H, Ar-H), 6.65 (m, 4H, Ar-H, and NHCH_2), 6.54 (d, J = 8.00 Hz, 2H, Ar-H), 4.29 (d, J = 4.00 Hz, 2H, HNCH_2). ^{13}C NMR: (101 MHz, DMSO-d_6) δ = 167.61 (1C, COOH), 152.73 (1C, Ar-C-OH), 145.02 (1C, Ar-C-OH), 143.10 (1C, Ar-C), 131.14 (2C, Ar-CH), 125.92 (1C, Ar-C), 118.78 (1C, Ar-C), 118.71 (1C, Ar-C), 116.88 (1C, Ar-CH), 114.04 (1C, Ar-CH), 111.05 (2C, Ar-CH), 41.01 (1C, CH_2NH). Anal. Calcd. for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.82; H, 5.08; N, 5.43. λ_{max}: 305 nm, ε_{max}: 30600. HR-MS: $[M+H]^+$ at 260.09 m/z ($C_{14}H_{13}NO_4$ requires 259.08).

2.2.19. Synthesis of 4-((3-(trifluoromethyl)benzyl)amino) benzoic Acid (19)

The title compound was synthesized using the general procedure and isolated 95 % Yield as white powder. FTIR (ATR, ν, cm^{-1}): 2500-3011 (COOH), 3424 (NH), 1671 (C=O). ^{1}HNMR: (DMSO-d_6, 400 MHz) δ = 12.06 (s, 1H, COOH), 7.63 (m, 6H, Ar-H, and, Ar-H), 7.13 (t, J= 8.09 Hz, 1H, NHCH_2), 6.61 (d, J= 8.00 Hz, 2H, Ar-H), 4.48 (d, J= 4.00 Hz, 2H, HNCH_2). ^{13}C NMR: (DMSO-d_6, 101 MHz) δ = 167.47 (1C, COOH), 152.17 (1C, Ar-C-CF_3), 141.24 (1C, Ar-C), 131.29 (1C, Ar-C), 131.17 (2C, Ar-CH), 129.45 (1C, Ar-CH), 129.02 (1C, CF_3), 125.67 (1C, Ar-C), 123.63 (1C, Ar-CH), 123.59 (1C, Ar-CH), 117.63 (1C, Ar-CH), 111.30 (2C, Ar-CH) 45.34 (1C, CH_2NH). Anal. Calcd. for $C_{15}H_{12}NO_2F_3$: C, 61.02; H, 4.10; N, 4.74. Found: C, 61.05; H, 4.07; N, 4.77. λ_{max}: 304 nm, ε_{max}: 16200. HR-MS: $[M+H]^+$ at 296.08 m/z ($C_{15}H_{12}NO_2F_3$ requires 295.08).



Scheme 1. Synthetic pathways of obtaining 4-(benzylamino) benzoic acid derivatives.

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2.3. Cell subculture and Growth Conditions

Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI)-1640 Medium (RPMI), and Dulbecco's Phosphate Buffered Saline (PBS) were acquired from Euroclone, Pero, Italy. Heat inactivated Fetal Bovine Serum (FBS) was acquired from Capricorn Scientific, Ebsdorfergrund, Germany. Primary Gingival Fibroblast cell line; Normal, Human, Adult (HGF) (ATCC PCS-201-018), Non-Small Cell Lung Cancer (NSCLC) cell line [A549 (ATCC CCL-185)], and Small Cell Lung Cancer (SCLC) cell line [NCI-H69 [H69] (ATCC HTB-119)] were acquired from ATCC®, Manassas, Virginia, USA. HGF cells were grown in 10% (v/v) FBS/DMEM (complete medium), while A549 and H69 cells were grown in 10% (v/v) FBS/RPMI (complete medium). Cells were incubated at 37 °C in humidified air atmosphere of 5% CO_2. HGF and A549 cells are adherent cells and were continuously washed with PBS and supplemented with fresh complete media to maintain their growth. H69 cells are suspended cells and complete media was continuously changed during splitting.

2.4. Cell Viability Assays Using Resazurin Dye Method

Resazurin sodium salt was acquired from Sigma-Aldrich, St. Louis, Missouri, USA. Compounds **1-19** were synthesized by our group. The cell viability assays were conducted using resazurin dye colorimetric method as previously described [41,42]. Briefly, HGF and A549 cells were seeded in 96-well plates at a seeding density of 2500 cells/200 µL/well in triplicates. They were allowed to adhere after 24 h incubation, then, media aspirated and fresh complete medium (180 µl) were added. H69 cells seeding density was 10000 cells/180 µL/well in triplicates and treatments were added at the same day. Compounds were dissolved in dimethyl sulfoxide (DMSO) (Fisher Chemicals, Hampton, New Hampshire, USA) to get a stock concentration of 10 mM and several diluted stock solutions ranging between 1000 µM to 0.1 µM were prepared using complete medium for the respective cell line used. Then, diluted stock solution of the compounds (20 µL) was added resulting in a final-well concentration ranging from 100 µM to 0.01 µM and plates incubated for 72 h. Untreated control samples were also prepared. Samples for background fluorescence for the resazurin dye were also prepared using only complete medium. After 72 h incubation, resazurin dye (20 µL; 125 g/L in PBS) was added into each well and plates incubated for 2 h (HGF and A549) or 4 h (H69). Then, fluorescence readings (excitation 540 nm / emission 620) were recorded using BioTeK SYNERGY HTX multi-mode plate reader. Assays were performed in triplicates in three independent trials. The readings were analyzed to calculate the percentage viability relative to controls and dose-response curves were generated using GraphPad Prism version 9.0 as discussed before [42].

2.5. Antibacterial Activity

The antibacterial activities were tested by evaluating the antibacterial behavior of the synthesized 4-(benzyl)amino) benzoic acid derivatives against six different pathogenic bacteria using two different methods: the agar diffusion method and the micro-broth dilution method to determine the Minimum Inhibitory Concentration (MIC, µg/mL) as reported previously [43,44]. The isolates include *Enterococcus faecalis (En), Staphylococcus aureus (Sa), Methicillin-Resistant Staphylococcus aureus (MRSA), Escherichia coli (Ec), Klebsiella pneumonia (Kp), and Salmonella enteritidis (Se),* which were obtained from the Ministry of Health-Jordan.

2.6. Single-Crystal X-ray Diffraction Measurements (SC-XRD)

Single-crystal X-ray diffraction (SC-XRD) data for compounds **2, 4, 7, 8, 9,** and **11** were collected using a Bruker D8 QUEST ECO diffractometer equipped with a sealed tube source (Mo-Kα, λ = 0.71073 Å) operating at 50 mV/20 mA and a Photon 50 detector. Suitable crystals were mounted using a dual-thickness MiTeGen Micro Loop. The crystals were maintained at 296 K during data collection. Cell parameters were determined and refined using all observed reflections. The structure was then solved by direct methods with the APEX 3 software suite, followed by further refinement using the Olex2 (V1.2.10) program [45], in conjunction with the SHELXL refinement package [46]. Hydrogen

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atoms were calculated and refined using the software. CCDC 2379919–2379924 contain the supplementary crystallographic data for this paper (Table 1). These data can be obtained free of charge from The Cambridge Crystallographic Data Centre (CCDC; https://www.ccdc.cam.ac. uk).

Table 1. Crystal data and details on structure refinement for the reported compounds.

Compound	2	4	7	8	9	11
CCDC deposition number	2379919	2379920	2379921	2379922	2379923	2379924
Empirical formula	$C_{14}H_{12}ClNO_2$	$C_{14}H_{12}BrNO_2$	$C_{15}H_{15}NO_3$	$C_{15}H_{15}NO_3$	$C_{15}H_{15}NO_3$	$C_{16}H_{17}NO_4$
Formula weight	261.70	306.16	257.28	257.28	257.28	287.30
Crystal system	triclinic	triclinic	monoclinic	monoclinic	triclinic	monoclinic
Space group	P-1	P-1	$P2_1/c$	$P2_1/c$	P-1	$P2_1/c$
Cell metric a/Å	5.9490(4)	5.9204(2)	12.0311(8)	8.2327(16)	8.082(2)	10.763(4)
b/Å	8.6353(5)	8.7920(3)	10.0807(6)	28.167(5)	10.565(3)	10.915(4)
c/Å	12.4159(7)	12.3226(5)	12.2667(9)	11.239(2)	16.078(4)	25.978(9)
α/°	97.416(3)	95.916(2)	90	90	93.781(10)	90
β/°	96.441(3)	97.411(2)	118.669(3)	93.263(7)	103.741(9)	101.871(7)
γ/°	103.954(3)	100.743(2)	90	90	96.597(10)	90
Cell volume/Å^3	606.98(6)	619.57(4)	1305.34(15)	2602.0(9)	1318.5(6)	2986.5(18)
Molecules per cell Z	2	2	4	8	4	4
ϱ_{calc}g/cm^3	1.432	1.641	1.309	1.314	1.296	0.639
μ/mm^{-1}	0.307	3.310	0.092	0.092	0.091	0.046
Electron per cell F(000)	272.0	308.0	544.0	1088.0	544.0	608.0
Crystal size/mm^3	0.853 × 0.341 × 0.28	0.758 × 0.694 × 0.414	? × ? × ?	0.993 × 0.364 × 0.362	0.73 × 0.531 × 0.524	0.767 × 0.572 × 0.258
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.926 to 41.582	4.756 to 46.896	5.588 to 43.176	4.642 to 38.322	5.244 to 46.684	4.062 to 34.272
Index ranges	-5 ≤ h ≤ 5, -8 ≤ k ≤ 8, -12 ≤ l ≤ 12	-6 ≤ h ≤ 6, -9 ≤ k ≤ 9, -13 ≤ l ≤ 13	-12 ≤ h ≤ 12, -10 ≤ k ≤ 10, -12 ≤ l ≤ 12	-7 ≤ h ≤ 7, -25 ≤ k ≤ 25, -10 ≤ l ≤ 10	-8 ≤ h ≤ 9, -11 ≤ k ≤ 11, -17 ≤ l ≤ 17	-8 ≤ h ≤ 8, -9 ≤ k ≤ 9, -21 ≤ l ≤ 20
Reflections collected	7350	12314	9379	29492	28182	9063
Independent reflections	1254 [R_{int} = 0.0267, R_{sigma} = 0.0185]	1809 [R_{int} = 0.0292, R_{sigma} = 0.0180]	1510 [R_{int} = 0.0413, R_{sigma} = 0.0266]	2127 [R_{int} = 0.0450, R_{sigma} = 0.0174]	3766 [R_{int} = 0.0427, R_{sigma} = 0.0247]	1742 [R_{int} = 0.0554, R_{sigma} = 0.0387]
Data/restraints/parameters	1254/0/168	1809/0/168	1510/0/178	2127/0/351	3766/0/354	1742/0/393
Goodness-of-fit on F^2	1.084	1.123	1.086	1.118	1.046	1.071
Final R indexes [I>=2σ (I)]	R_1 = 0.0281, wR_2 = 0.0669	R_1 = 0.0237, wR_2 = 0.0530	R_1 = 0.0346, wR_2 = 0.0849	R_1 = 0.0354, wR_2 = 0.0882	R_1 = 0.0384, wR_2 = 0.0896	R_1 = 0.0341, wR_2 = 0.0779
Final R indexes [all data]	R_1 = 0.0348, wR_2 = 0.0711	R_1 = 0.0295, wR_2 = 0.0565	R_1 = 0.0552, wR_2 = 0.0996	R_1 = 0.0459, wR_2 = 0.0991	R_1 = 0.0577, wR_2 = 0.1024	R_1 = 0.0564, wR_2 = 0.0896
Largest diff. peak/hole / e Å^{-3}	0.13/-0.13	0.27/-0.35	0.13/-0.14	0.13/-0.13	0.15/-0.14	0.11/-0.13

3. Result and Discussion:

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3.1. Elemental Analysis & Mass Spectra

The elemental analysis data for carbon, hydrogen and nitrogen of all prepared complexes (**1-19**) confirmed the purity of all of them. Furthermore, the mass spectra showed that all the prepared compounds have the expected m/z value. Figure 1 represents the mass spectra of compound **1** as a representative example. All other spectra of compounds (**2-19**) can be found in the supplementary information part.

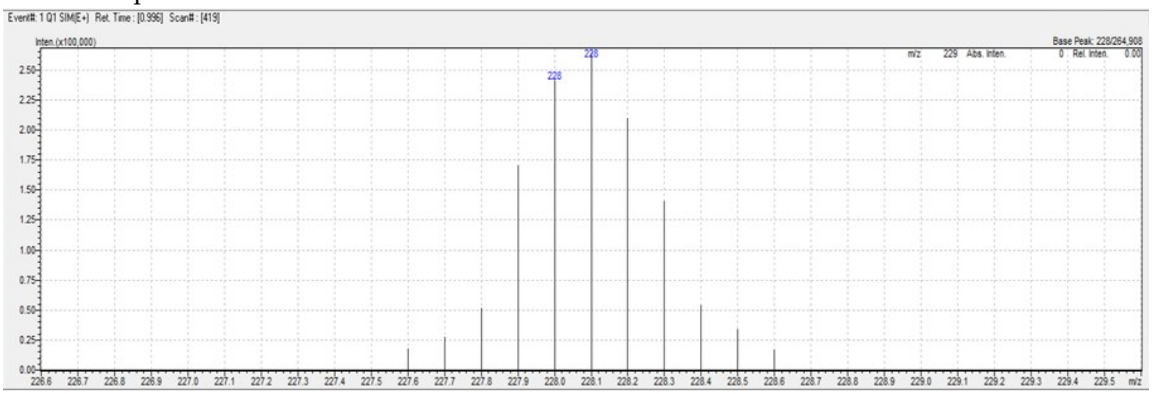

Figure 1. Mass spectra of compound **1**.

3.2. NMR Spectroscopy

^{1}H, and $^{13}C\{^{1}H\}$ NMR spectra were utilized as confirming tools to prove the structures of our synthesized compounds **1-19**. The protons of the carboxylic acid moiety had appeared as a singlet peak at a chemical shift between δ = 11.82 - 12.11 ppm. The protons of the aliphatic carbon (CH_2) had appeared as a doublet peak at a chemical shift between δ = 4.20 - 4.51 ppm, with entire number of 2 protons. The protons of the amino group neighbored to methylene carbon (CH_2) in all compounds were located as triplet peaks in the range of δ = 6.59 - 7.26 ppm.

Aromatic protons appeared with various kinds of coupling: singlet, doublet, doublet of doublet, triplet, and multiplet, depending on the type of the aromatic substitution whether ortho, meta or para, at chemical shifts δ = 6.54 - 8.20 ppm

The $^{13}C\{^{1}H\}$-NMR revealed various peaks at different chemical shifts depending on the type of carbon. The aliphatic carbon (CH_2) was located at chemical shift δ = 40.28-46.83 ppm with a total number of one carbon for each compound and this indicated the occurrence of the reduction step for the imine function group successfully. The aromatic carbons were shown at the aromatic region δ = 110.97 – 159.37 ppm, some peaks represented two carbons, and the other peaks represented one carbon. Also, there was a peak related to the COOH appeared in the range between δ = 167.33 – 167.81 ppm. The carbon of the carbonyl (C=O) group is more de-shielded than the aromatic carbons (C=C) and are located at higher ppm as shown in the spectra (**supplementary materials)**, because it's adjacent to the electronegative oxygen atom in contrast to the aliphatic carbons that appeared at lower ppm. Figure 2 shows the NMR spectra of compound **4** as a representative example.

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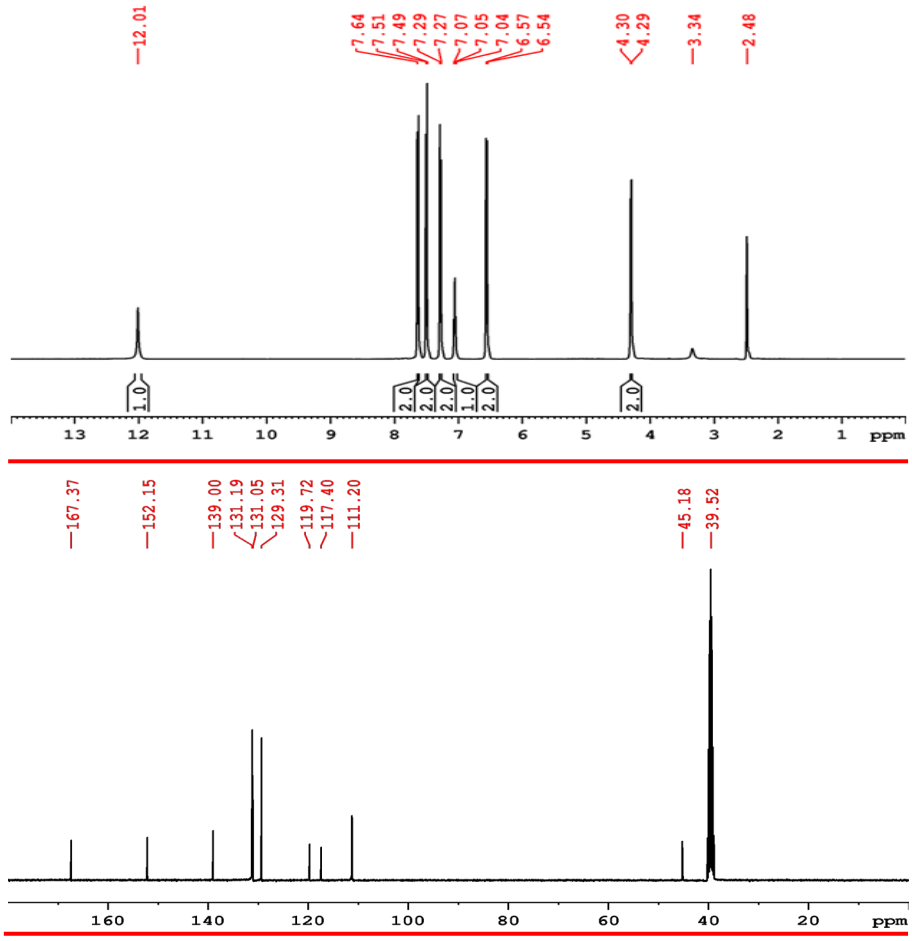

Figure 2. (a) ^{1}H-NMR spectrum and (b) ^{13}C{^{1}H}-NMR spectrum of compound **4** recorded in DMSO-d_6.

3.3. Infra-Red Spectroscopy

The infrared spectra of compounds **1-19** exhibit three major bands in the range of 2500-3150 cm^{-1}, 1604-1693 cm^{-1} and 3330-3422 cm^{-1} which are assigned for OH, CO in COOH, and NH functional groups, respectively. The appearance of the NH stretching as a sharp peak confirms the occurrence of the reduction step in the reductive amination reaction. Furthermore, stretching phenolic hydroxyl bands appear in the range of 3322-3152 cm^{-1}, for compounds **12-16** and **18** (Figure 3).

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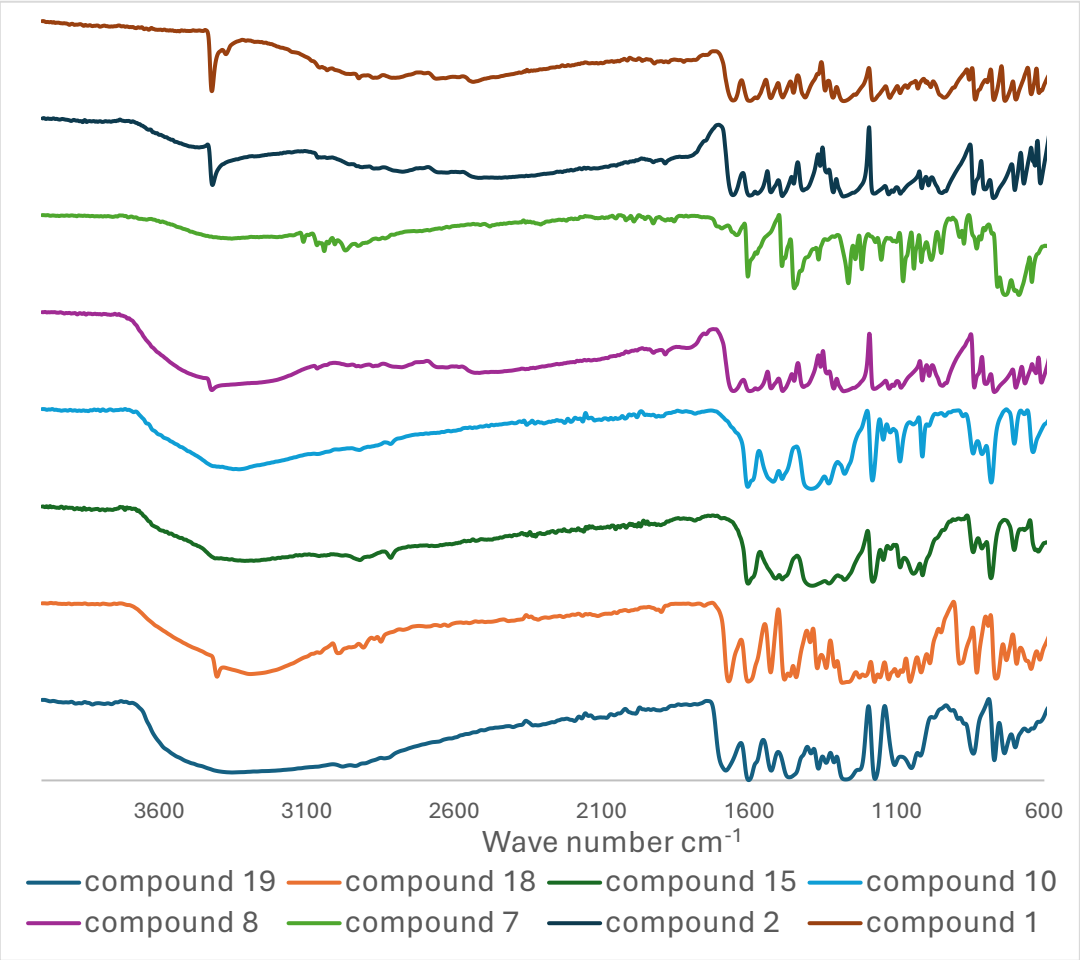

Figure 3. Representative FTIR spectra of some prepared compounds.

3.4. Molecular Structures

The structure of the 4-(benzylamino) benzoic acid derivatives is supported by X-ray diffraction methods for products, **2**, **4**, **7**-**9** and **11**, as shown in Figure 4. Suitable crystals for single-crystal X-ray analysis were approachable by slow evaporation of a saturated solution of compounds **2**, **4**, **7**-**9**, and **11** in THF at room temperature. Two crystallographically independent molecules in the unit cell of compounds **8**, **9**, and **11** were revealed; only one of them is shown in Figure 4. It is worth pointing out that the nature and the position of R substituent at the aryl ring have negligible effect on the bond lengths of these compounds.

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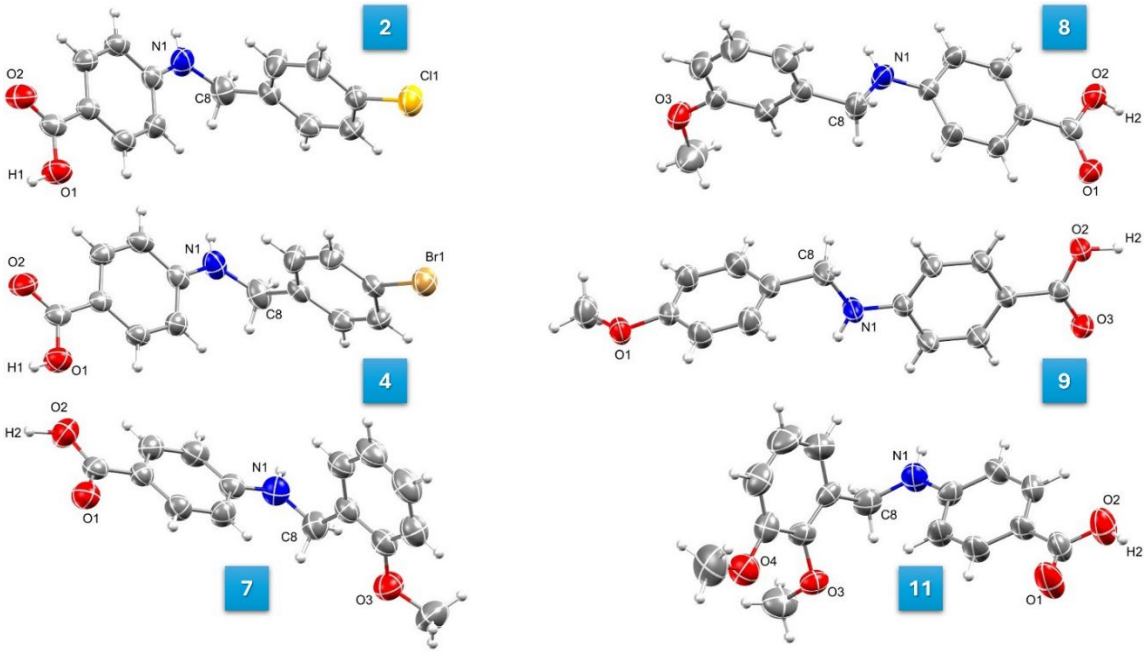

Figure 4. Molecular structures (50% probability) of compounds **2**, **4**, **7**-**9**, and **11**.

3.5. Anti-Cancer Properties

Cell viability assays were conducted using resazurin dye colorimetric method by treating HGF, A549, and H69 cell lines for 72 h with compounds **1** to **19**. Compounds were initially tested on HGF cell line to identify their toxicity on normal cell line and later tested on cancer cell lines. The results are summarized in **Table 2**.

Table 2. Cytotoxicity (IC_{50}) of compounds **1** to **19** on HGF, A549, and H69 cell lines after treatment for 72 h; n = 3.

Compound	IC_{50} (± SEM) μM; n=3		
	HGF (Fibroblasts; normal cell line)	**A549 Non-Small Cell Lung Cancer cell line**	**H69 Small Cell Lung Cancer cell line**
1			
2			
3			
4			
5			
6			
7			
8			
9		>100	>100
10	>100		
11			
12			
13			
14			
15			
16			
17			
18		**90.69 ± 10.23**	**32.22 ± 7.08**
19		>100	>100

*: ~ 50% cell viability at 100 μM.

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The results showed that all compounds were noncytotoxic on the normal HGF cell line. Hence, their investigation on cancer cell lines would be worth investigating. NSCLC (A549) and SCLC (H69) cell lines were used. However, only compound **18** showed a cytotoxic effect on A549 and H69 cell lines with IC_{50} values of 90.69 µM and 32.22 µM, respectively. The dose-response curves for compound **18** are presented in **Figures 5** and **6**. In addition, the selectivity index [41,42] for compound **18** on H69 cell line is greater than 3 and this presents a selective toxic agent on SCLC cell line. Although compound **18** did not show a marked cytotoxicity on A549 cell line, compound **18** may be further evaluated against other cancer cell lines as well as being optimized to increase its toxicity, especially on H69 cell line. In addition, all these compounds may be further investigated on other cancer cell lines and they may show a cytotoxic effect.

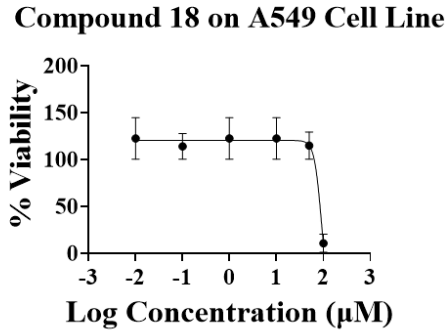

Figure 5. Dose-response curve for compound **18** on A549 cell line, generated by GraphPad Prism version 9.0, n=3.

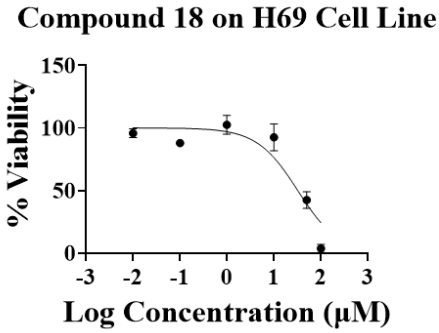

Figure 6. Dose-response curve for compound **18** on H69 cell line, generated by GraphPad Prism version 9.0, n=3.

3.6. Antibacterial Properties

The antibacterial activity of compounds **(1-19)** was tested in vitro by determining MIC values via the micro broth dilution method against several pathogenic bacteria (*En, Sa, MRSA, Ec, Kp,* and *Se*). The MIC was defined as the minimal inhibitor concentration that has an optical density less than that of amoxicillin (positive control). Amoxicillin was taken as a standard reference drug and evaluated under conditions similar to those used for all synthesized compounds. As a negative control, DMSO solvent does not affect the antibacterial activity of tested bacteria.

Table 3 shows that compounds **1, 4, 6, 9,** and **12** were inactive against neither gram negative nor gram positive bacteria strains. All other compounds showed differentiated MIC values against all tested bacterial strains. The MIC values showed that compounds **3, 5, 11, 13, 14, 16-19** were entirely inactive toward all types of gram-positive bacteria and against gram negative *Ec*. Besides, compounds **3, 5, 11, 13, 14** and **16** showed moderate activity against gram negative *Kp* and *Se* (MIC = 64 µg/mL). All other compounds showed low activities toward different strains of bacteria, (MIC = 128-256 µg/mL).

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Higher activity observed against gram-negative bacteria can be explained by considering the effect of lipopolysaccharide (LPS), a major component of the surface of such organisms.

The LPS is an important entity in determining the virulence and pathogenicity of gram-negative bacteria and the effectiveness of the outer membrane barrier function [47]. The Ln(III) complexes can penetrate the bacterial cell membrane by coordinating the metal ion through nitrogen or oxygen donor atoms to LPS, causing damage to the outer cell membrane, and inhibiting the nucleic acid synthesis and protein synthesis and therefore inhibiting bacterial growth [48].

Table 3. Antibacterial activity data of compounds **1-19**.

Compound	Minimum Inhibitory Concentration (MIC, µg/ mL)					
	Gram-Positive			Gram-Negative		
	En	*Sa*	*MRSA*	*Ec*	*Kp*	*Se*
1	N	N	N	N	N	N
2	128	N	N	N	64	64
3	N	N	N	N	64	64
4	N	N	N	N	N	N
5	N	N	N	N	64	64
6	N	N	N	N	N	N
7	128	N	N	N	64	64
8	256	N	N	N	64	64
9	N	N	N	N	N	N
10	256	N	N	N	64	64
11	N	N	N	N	64	64
12	N	N	N	N	N	N
13	N	N	N	N	64	64
14	N	N	N	N	64	64
15	64	N	N	N	64	128
16	N	N	N	N	64	64
17	N	N	N	N	256	N
18	N	N	N	N	256	N
19	N	N	N	N	256	N
DMSO (-ve control)	N	N	N	N	N	N
Amoxicillin (+ve control)	16	16	16	32	16	16

4. Conclusion

New 4-(benzylamino)benzoic acid derivatives, compounds **1-19**, with general formula of $C_{14}H_{12}NO_2R$, where [R= (H), (4-Cl), (4-NMe), (4-Br), (3-NO_2), (4-NO_2), (2-OMe), (3-OMe), (4-OMe), (2,3-OMe), (3,4-OMe), (2-OH, 3-OMe), (3-OMe, 4-OH), (3,5-OMe, 4-OH), (2-OH, 5-Br), (3-OH), (4-SMe), (2,3-OH), (3-CF_3)] were synthesized and characterized). Compound **18** showed a considerable IC_{50} value of 90.69 against A549 and an excellent value of 32.22 and H69, respectiv by a variety of spectroscopic techniques (HRMS, FT-IR, ^1H, and ^{13}C NMR) and elemental analysis. Furthermore, the structure of some of the synthesized derivatives were confirmed by single crystal X-ray diffraction (R = 4-Cl, 4-Br, 2-OMe, 3-OMe, 4-OMe, 2,3-OMe), compounds **2**, **4**, **7**, **8**, **9** and **11**, respectively. A number of these compounds showed low to moderate antibacterial activities toward different strains of bacteria, gram negative & gram positive. The anticancer activities of all compounds (IC_{50} values, µM) were evaluated against different cell lines, HGF (Fibroblasts; normal cell line), A549 (Non-Small Cell Lung Cancer cell line) and H69 (Small Cell Lung Cancer cell lineely.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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