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Convenient Synthesis Of New Indeno[1,2-b]Pyridine Derivatives For Antimicrobial And Antioxidant Evaluation

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Abstract

A new set of 1H-indenopyrdine-based derivatives were synthesized using the compound 4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1H-indeno[1,2-b]pyridine-3-carbonitrile (**1**) as the key starting compound. The molecular structures of the new derivatives were identified using various spectroscopic techniques and elemental analysis. All the new analogues were screened as antimicrobial against different strains of Gram +ve, Gram -ve bacteria and the opportunistic pathogenic yeast C. albicans. Some of the new indenopyrdines exhibited moderate antimicrobial activity comparing to gentamicin as a standard drug. On the reverse, the free radical scavenging activity of the new compounds using DPPH assay protocol revealed that most of the compounds were potent antioxidant agents while comparing to ascorbic acid as a positive antioxidant control.

Keywords: 1H- indenopyrdine, Schiff's bases, Thiazolidinone ring, Antimicrobial, Antioxidant activities.

1. Introduction

Infectious diseases represent one of the main causes of a great number of deaths in both developing and developed countries [1,2]. The predominance of infectious diseases in recent decades refers to many different factors such as; hygiene literacy, climate change in addition to the problem of bacterial resistance to a large number of antimicrobial agents [3]. World Health Organization has classified antimicrobial resistance as one of the three most important public health threats of the 21st century. The misuse and overuse of various antibiotics are the main cause of genetic mutations in microbes leading to a change in their response to the antimicrobial drugs [4,5]. *E. coli*, *S. aureus* and *K. pneumonia* show resistance towards multiple drugs as they can highly adapt themselves to the hosts and healthcare conditions [6]. This challenging problem makes the antimicrobial treatment become ineffective leading to increased treatment costs and higher disease morbidity and mortality particularly in immunocompromised patients [7]. Therefore, novel antibacterial compounds with novel targets and selective toxicity need to be developed to overcome this problem [6,7]. DNA gyrase and topoisomerase IV are type II topoisomerases play a vital role DNA replication and repair thus, they are crucial for cell viability and offer the opportunity to develop novel antibacterial candidates which can overcome the bacterial resistance obstacle [8]. Also, it has been documented that the rigid 5*H*-indeno[1,2-*b*]pyridine ring has a planar configuration which facilitates the ring to intercalate into the topoisomerase I and II-DNA complex. In addition, the rigidity of structures leads to little conformational entropy, resulting in efficient fitting in the enzyme active site which inhibits the enzyme activity leading to cell growth arrest and even cell death [8].

Oxidative stress is a dynamic mechanism in the biological systems that is distinguished by an improper balance between the production of free radicals (FR) and the capacity of the body to expel these reactive species via using endogenous and exogenous antioxidants. Throughout various biochemical pathways, multiple reactions take place in which the boosters are the reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and the superoxide radical anion ($O2•−$), among others. Increased quantities of FR may result in damages of biomolecules, leading to

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severe pathological diseases such as; atherosclerosis, cancer, diabetes, cardiovascular, and chronic inflammation [9]. The biological systems contain endogenous antioxidant mechanisms to inactivate the excess ROS which are either enzymatic, like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, or non-enzymatic compounds, such as bilirubin and albumin. Thus, the consumption of antioxidants is the most efficient method to avoid many diseases related to the production of high levels (ROS) [10-12]. The clinical administration of drugs and the chemoprophylaxis of different diseases that happened via oxidizing agents need the development of the new antioxidants having the predicted antioxidant activity, as well as desired pharmacological properties [10,11]. The indeno-pyridine scaffold is a privileged heterocyclic ring system. It constitutes the main core of the 4-azafluorenone group of naturally occurring alkaloids. 4-Azafluorenone (5*H*-indeno [1,2-*b*]pyridin-5-one) derivatives have displayed a fascinating array of various bioactivities such as antioxidant, insecticidal, phosphodiesterase inhibition, antifungal, anti-spermatogenic, antifertility, antidepressant and antiarrhythmic activity [12,13]. In addition, due to their characteristic antimicrobial and antimalarial activities, different 4-azafluorenone analogues play a central role in drug discovery of new antimicrobial and antioxidant agents. **Fig. 1** exhibits representative members of this class of compounds.

Based on the aforementioned findings, and in continuation of our previous efforts to find out new potent antimicrobial and antioxidant agents aiming to combat the microbial resistance problem [14-17], this study deals with synthesis of two series of novel rigid analogs bearing 4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,3-dihydro-1H-indeno[1,2-b]pyridine-3-carbo-nitrile scaffold which is either conjugated with different heterocyclic rings at pyridine-2-position via an oxyacetamide linker or alkylated with different substituted alkyl chains at pyridine-N1- position. The structural formulae of the new derivatives were confirmed using microanalytical and spectral data.

The new *5H*-indeno[1,2-*b*]pyridine compounds were evaluated as antibacterial and antifungal agents against a panel of gram-positive and gram-negative bacteria and fungal strains. Determination of the minimum inhibitory concentration (MIC) was performed for the most active derivatives. Also, the antioxidant activity of the new derivatives was evaluated.

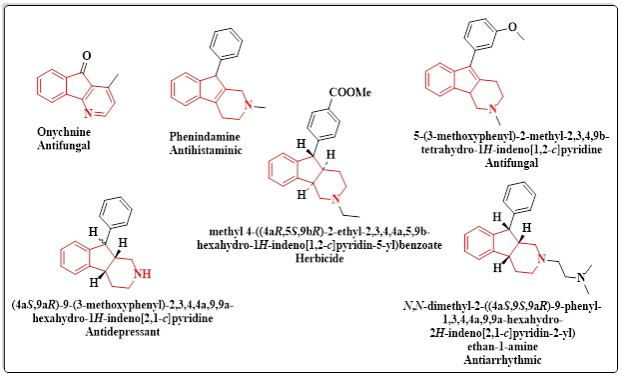


Fig. 1. Different indenopyridine derivatives of various bioactivities

2. Experimental

2.1. Chemistry

The TLC was performed using aluminum plates pre-coated with silica 60 F254 (Merck) and visualized by UV light (254 nm). Melting points are uncorrected and were determined on a Böetius PHMK (Veb Analytik Dresden) apparatus. The NMR spectra were recorded on a Varian Gemini 300 and Bruker DRX 400 spectrometer at 25 ºC, unless otherwise stated. ^{1}H- and ^{13}C-NMR signals were referenced to TMS and the solvent shift ($(CD_3)_2SO$ δ H 2.50 and δ C 39.5). Coupling constants are given in Hz and without sign. The IR-spectra were recorded (KBr) on a Jasco FT/IR-410 instrument. Mass spectrometry was carried out on a Varian FINNIGAN MAT 212 instrument and the elemental analysis on the Perkin Elmer 240 instrument.

4-(4-Hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1H-indeno[1,2-*b*]pyridine-3-carbonitrile (1)

A mixture of 4-hydroxy-3-methoxybezaldehyde (1.36 g, 10 mmol), 1-indanone (1.33g, 10mmol), ethylcyanoacetate (1.11g, 10mmol) and ammonium acetate (80 mmol) in ethanol (40 ml) was refluxed for 4 h, after cooling the formed precipitate was filtered, dried and recrystallized from glacial acetic acid to give the pure product 1. Yield: 80%; m.p. 292-294 °C. IR, ν, cm^{-1}: 3525–3245 (br, OH, NH),3048 (aromatic H), 2212 (CN), 1636 (C=O), ^{1}H NMR (DMSO-d_6) δ ppm: 3.42 (s, 2H, indene CH_2), 3.55 (s, 3H, -OCH_3), 6.90-7.80 (m, 7H, Ar-H), 9.99 (s, 1H, OH, D_2O exchangeable) 10.50 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR, δ ppm: 32.95 (CH_2), 56.00 (OCH_3), 110.90 (CN), 112.50, 113.50, 115.50, 116.20, 122.80, 125.10, 126.21. 127.90, 129.00, 130.20, 139.30, 143.90, 146.90, 147.40, 148.00, 149.70 (Ar-C), 170.22 (C=O). MS [m/z]: 332 [M]+ (100%), Analysis calcd. for $C_{20}H_{14}N_2O_3$ (332.36) %: C, 72.72; H, 4.27; N, 8.48; Found: C, 72.82; H, 4.17; N, 8.28.

Ethyl 2-((3-cyano-4-(4-hydroxy-3-methoxy-phenyl)-5H-indeno[1,2-*b*]pyridin-2yl)-oxy)acetate (2)

A mixture of a compound 1 (10 mmol) with ethyl bromoacetate (10 mmol) and anhydrous potassium carbonate (40 mmol) in dry acetone (30 mL) was refluxed for 3 h. The excess solvent was evaporated under reduced pressure and the precipitated solid was recrystallized from ethanol to produce an ester 2. Yield 65%, m.p. 210–212 °C. IR spectrum, ν, cm^{-1}: 3445 (br, OH), 3054 (aromatic C–H), 2981, 2960 (aliphatic C–H), 2224 (CN), 1730 (C=O). ^{1}H NMR spectrum, δ, ppm: 1.22 (t, 3H, J = 5.2 Hz, CH_3CH_2), 3.45 (s, 2H, CH_2), 3.55 (s, 3H, OCH_3), 4.20 (q, 2H, J = 5.2 Hz, CH_2- CH_3), 4.60 (s, 2H, CH_2), 7.22- 7.89 (m, 7H, Ar-H), 10.00 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ, ppm: 14.35 (CH_3), 32.55 (CH_2), 56.00 (OCH_3), 62.25 (CH_2), 64.55 (CH_2), 110.90 (CN), 112.50, 113.50, 115.50, 116.20, 122.80, 126.10, 127.21. 128.90, 130.00, 131.20, 139.30, 145.90, 148.90, 149.40, 151.00, 159.20,160,20 (Ar-C), 166.92 (C=O). MS [m/z]: 416 $[M]^+$ (60%). Analysis calculated for $C_{24}H_{20}N_2O_5$, (416.43) %: C, 69.22; H, 4.84; N, 6.73. Found, %: C, 69.57; H, 4.90; N, 6.66.

2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]pyridin-2-yl)-oxy)acetohydrazide (3)

To a solution of a compound 2 (10 mmol) in dioxane (50 mL) was added hydrazine hydrate (20 mmol, 99%), and the mixture was stirred at 45°C for 3 h. Upon cooling down, the precipitated solid was recrystallized from ethanol to produce a hydrazide 3 . Yield 60%, m.p 227–225°C. IR spectrum, ν, cm^{-1}: 3445 (br, OH), 3338, 3261 (NH, NH_2), 3082 (aromatic C–H), 2855 (aliphatic C–H), 2220 (CN) and 1644 (C=O). 1H NMR spectrum, δ, ppm: 3.45 (s, 2H, indene CH_2), 3.55 (s, 3H, OCH_3), 4.40–4.42 (br.s, 2H, NH_2), 4.64 (s, 2H, CH_2), 7.24-7.90 (m, 7H, Ar-H), 8.90 (s, 1H, NH, D_2O exchangeable) 10.00 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ, ppm: 32.55 (CH_2), 56.00 (OCH_3), 64.55 (CH_2), 110.30 (CN), 112.50, 113.50, 115.50, 116.20, 122.80, 126.10, 127.21. 128.90, 130.00, 131.20, 139.30, 145.90, 148.90, 149.40, 151.00, 159.20,160.20 (Ar-C), 170.00 (C=O). MS [m/z]: 402 $[M]^+$ 60%. Analysis calculated for $C_{22}H_{18}N_4O_4$ (402.13) %: C, 65.66; H, 4.51; N, 13.92. Found %: C, 65.69; H, 4.55; N, 13.72.

General procedure for synthesis of compounds 4-6

To a solution of compound **3** (10 mmol) in glacial acetic acid (30 mL) phthalic anhydride, maleic anhydride and/or isatoic anhydride (10 mmol) was added. The mixture was refluxed for 8 h. then poured into ice water, the formed precipitate was filtered, washed with water and recrystallized from dioxane to produce the pure products.

2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]pyridin-2-yl)oxy)-N-(1,3-dioxo-isoindolin-2-yl)acetamide (4)

Yield 60%, m.p. 303-305°C. IR spectrum, ν, cm^{-1}: 3475 (br, OH), 3320 (NH), 3080 (aromatic C–H), 2850 (aliphatic C–H), 2220 (CN) and 1699 (2C=O), 1645 (C=O). **[1]**H NMR spectrum, δ, ppm: 3.42 (s, 2H, indene CH_2), 3.53 (s, 3H, OCH_3), 4.60 (2H, CH_2), 7.10-8.20 (m, 11H, Ar-H), 10.20 (s, 1H, OH, D_2O exchangeable), 10.50 (s, 1H, NH, D_2O exchangeable). MS [m/z]: 532 $[M]^+$ 65%. Analysis calculated for $C_{30}H_{20}N_4O_6$ (532.51)%: C, 67.67; H, 3.79; N, 20.52, Found %: C, 67.59; H, 3.55; N, 20.32.

2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]pyridin-2-yl)oxy)-N-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetamide (5)

Yield 70%, m.p. 190-192 °C. IR spectrum, ν, cm^{-1}: 3475 (br, OH), 3322 (NH), 3082 (aromatic C–H), 2870 (aliphatic C–H), 2218 (CN) and 1701 (C=O), 1645 (C=O). 1H-NMR spectrum, δ ppm: 3.45 (s, 2H, indene CH_2), 3.50 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2), 6.86(d, J=5.2, 2H, vinylic-H), 7.20-8.00 (m, 7H, ArH), 10.10 (s, 1H, OH, D2O exchangeable), 10.55 (s, 1H, NH, D2O exchangeable). MS [m/z]: 482 [M]+ 75%. Analysis calculated for $C_{26}H_{18}N_4O_6$ (482.45) %: C, 64.73; H, 3.76; N, 11.61, Found %: C, 64.83; H, 3.86; N, 11.51.

2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5*H*-indeno[1,2-*b*]pyridin-2-yl)oxy)-N-(2,4-dioxo-1,4-dihydro-quinazolin-3(2*H*)-yl)acetamide (6)

Yield 60%, m.p. 198-200 °C. IR spectrum, ν, cm^{-1}: 3465 (br, OH), 3333 (NH), 3082 (aromatic C–H), 2870 (aliphatic C–H), 2215 (CN) and 1698, 1695, 1645 (3C=O). ^{1}H NMR spectrum, δ, ppm: 3.45 (s, 2H, indene CH_2), 3.55 (s, 3H, OCH_3), 4.62 (s, 2H, CH_2), 7.20-8.20 (m, 11H, ArH), 10.30 (s, 1H, OH, D_2O exchangeable), 10.65,11 (2s, 2H, 2NH, D_2O exchangeable). MS [m/z]: 547 [M]+ 45%. Analysis calculated for $C_{30}H_{21}N_5O6$ (547.53)%: C, 65.81; H, 3.87; N, 12.79, Found %: C, 65.91; H, 3.97; N, 12.59.

General procedure for synthesis of hydrazone derivatives 7-11

A mixture of hydrazide **3** (10 mmol) and the appropriate aromatic aldehydes namely: 4-bromobenaldehyde, 4-fluorobenzaldehyde and 4-methoxybenzaldehyde and/or aldoses namely: D-glucose and D-xylose (10 mmol) in acetic acid (10 mL) was refluxed for 6-8 h. After the reaction completion the excess solvent was evaporated under reduced pressure and the residue was treated with ethanol (20

ml), the obtained solid was filtered, dried and recrystallized from ethanol to get the Schiff bases **7-11**.

(E)-N'-(4-Bromobenzylidene)-2-((3-cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]-pyridin-2-yl)oxy)acetohydrazide (7)

Yield 75%, m.p. 260-262 °C. IR spectrum, ν, cm^{-1}: 3430 (OH), 3261(NH), 3054 (aromatic C–H), 2218 (CN), 1643 (C=O). ^{1}H NMR spectrum, δ ppm: 3.40 (s, 2H, indene-CH_2), 3.50 (s, 3H, OCH_3), 4.62 (s, 2H, CH2), 7.00- 8.20 (m, 12H, ArH + N=CH), 9.90 (s, 1H, OH, D_2O exchangeable), 10.95 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ, ppm: 32.66 (CH_2), 56.22 (OCH_3), 62.25 (CH_2), 64.25 (CH_2), 110.20 (CN), 112.60, 114.00, 115.85, 122.80, 125.40, 126.00, 126.29. 127.20, 128.80, 129.2, 130.30, 131.30, 133.50, 139.20, 146.70, 148.90, 149.40, 151.40, 159.20, 160.20, 162.20 (Ar-C), 162.72(-C=N), 170.50 (C=O). MS [m/z]: 569.72 $[M]^+$ 60%. Analysis calculated for $C_{29}H_{21}$ BrN_4O_4 (569.7) %: C, 61.17; H, 3.72; N, 9.84. Found, %: C, 61.27; H, 3.75; N, 9.80.

(*E*)-2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]pyridin-2-yl)oxy)-N'-(4-fluoro-benzylidene)acetohydrazide (8)

Yield 70%, m.p.190-192°C. IR spectrum, ν, cm^{-1}: 3420 (OH), 3265 (NH), 3082 (aromatic C–H), 2220 (CN), 1648 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.42 (s, 2H, indene-CH_2), 3.55 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2), 7.20- 8.22 (m, 12H, ArH + N=CH), 9.92 (s, 1H, OH, D_2O exchangeable), 10.99 (s, 1H, NH, D_2O exchangeable). MS [m/z]: 508[M]+ 70%. Analysis calculated for $C_{29}H_{21}FN_4O_4$, (508.15)%: C, 68.50; H, 4.16; N, 11.02.Found, %: C, 68.60; H, 4.14; N, 11.00.

(E)-2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]pyridin-2-yl)oxy)-N'-(4-methoxy-benzylidene)acetohydrazide (9)

Yield 65%, m.p.180-182°C. IR spectrum, ν, cm^{-1}: 3440 (OH), 3268 (NH), 2220 (CN), 1651 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.42 (s, 2H, CH_2), 3.55 (s, 6H, $2OCH_3$), 4.65 (s, 2H, CH_2), 7.18- 8.20 (m, 12H, ArH + N=CH), 9.90 (s, 1H, OH, D_2O exchangeable), 11.01 (s, 1H, NH, D_2O exchangeable). MS [m/z]: 520[M]+ 65%. Analysis calculated for $C_{30}H_{24}$ N_4O_5 (520.55) %: C, 69.22; H, 4.65; N, 10.76. Found, %: C, 69.42; H, 4.55; N, 10.50.

(*E*)-2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]pyridin-2-yl)oxy)-N'-(2,3,4,5,6-penta hydroxyhexylidene)acetohydrazide (10)

Yield 58%, m.p.150-152 °C. IR spectrum, ν, cm^{-1}: 3440- 3268 (br, OH, NH), 2220 (CN), 1655 (C=O). ^{1}H-NMR spectrum, δ ppm: 2.30 (s, 5H, OH, D_2O exchangeable), 3.10 (m, 1H, H6'), 3.40, (s, 2H, indene-CH_2), 3.55 (s, 3H, OCH_3), 4.20 (t, J=7.1 Hz, 1H, H2'), 4.30 (t,1H, J=7.1 Hz, H4'), 4.60 (s, 2H, CH_2), 4.89 (m,1H, H5'), 5.16 (t,1H, J=7.1 Hz, H3'), 6.90 (d, J=6.9Hz, 1H, CH), 7.20- 8.08 (m, 7H, ArH + N=CH), 10.00 (s, 1H, OH, D_2O exchangeable), 11.01 (s, 1H, NH, D_2O exchangeable). MS [m/z]: 564[M]+ 45%. Analysis calculated for $C_{28}H_{28}F_4O_9$ (564.19) %: C, 59.57; H, 5.00; N, 9.92; Found, %: C, 59.77; H, 5.20; N, 9.62.

(*E*)-2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5*H*-indeno[1,2-b]pyridin-2-yl)oxy)-N'-(2,3,4,5-tetrahydroxypentylidene)acetohydrazide (11)

Yield 55%, m.p. 185-187 °C. IR spectrum, ν, cm^{-1}: 3442- 3300 (br, OH, NH), 2230(CN), 1649 (C=O). ^{1}H NMR spectrum, δ ppm: 2.49-2.53 (br, 4H, OH, D_2O exchangeable), 3.30, (t, J=7.1 Hz, 1H, H2'), 3.37 (t, J=7.1 Hz, 1H, H4'), 3.40 (s, 2H, indene-CH_2), 3.46 (t, J=7.3 Hz, 1H, H3'), 3.55 (s, 3H, OCH_3), 3.65 (m, 1H, H5''), 3.89 (m, 1H, H5'), 4.62 (s, 2H, CH2), 7.30-8.20 (m, 8H, ArH+ N=CH), 10.10 (s, 1H, OH, D_2O exchangeable), 11.31 (s, 1H. NH, D_2O exchangeable). MS [m/z]: 534 [M]+ 45%. Analysis calculated for $C_{27}H_{26}N_4O_8$ (534.53), %: C, 60.67; H, 4.90; N, 10.48.Found, %: C, 60.67; H, 4.90; N, 10.48.

***N*-(2-(4-Bromophenyl)-4-oxothiazolidin-3-yl)-2-((3-cyano-4-(4-hydroxy-3-methoxy-phenyl)-5H-indeno[1,2-*b*]-pyridin-2-yl)oxy)acetamide (12)**

A mixture of a Schiff base **7** (10 mmol) and thioglycolic acid (10 mmol) in dry benzene (20 mL) was refluxed for 5h. After the reaction completion the excess solvent was evaporated under reduced pressure and the residue was neutralized using Na_2CO_3, the obtained solid was filtered, dried and recrystallized from ethanol to get thiazolidinone 12, Yield 65%, m.p..225-227°C. IR spectrum, ν, cm^{-1}: 3410 (OH), 3265 (NH), 3054 (aromatic C–H), 2218 (CN), 1650, 1645 (2C=O). ^{1}H NMR spectrum, δ, ppm: 3.41 (s, 2H, indene-CH_2), 3.50 (s, 3H, OCH_3), 3.70 (s, 2H, CH_2 thiazolidinone ring), 4.62 (s, 2H, CH_2), 5.52 (s, 1H, CH thiazolidinone ring), 7.23-8.20 (m, 11H, ArH), 9.90 (s, 1H, OH, D_2O exchangeable), 10.96 (s, 1H, NH, D_2O exchangeable). $_{13}$C NMR spectrum, δ, ppm: 32.65 (CH_2), 35.20, 54.50 (CH_2, CH thiazolidinone ring), 56.20 (OCH_3), 64.25 (CH_2), 116.20 (CN), 112.60, 114.00, 115.85, 122.80, 125.40, 126.00, 126.29, 127.20, 128.80, 129.20, 130.30, 131.30, 133.50, 139.20, 146.70, 148.90, 149.4, 151.40, 159.20,160,20, 162,20 (Ar-C), 162.32(-C=N), 170.55, 172.20 (2C=O). MS [m/z]: 643 [M]+ 55%. Analysis calculated for $C_{31}H_{23}BrN_4O_5S$ (643.51), %: C, 57.86; H, 3.60; N, 8.71; Found, %: C, 57.66; H, 3.70; N, 8.61.

General procedure for the synthesis of compounds 13, 14

A mixture of hydrazide **3** (10 mmol) and different chloro derivatives namely: benzenesulfonylchloride and/or 4-acetamidobenzenesulfonyl chloride (10 mmol) in pyridine (10 mL) was refluxed for 6h. The reaction mixture was cooled and poured onto cold

water then acidified by diluted HCl, the obtained solid was filtered, dried and recrystallized from ethanol to get the compounds **13** and **14**.

***N'*-(2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5*H*-indeno[1,2-*b*]pyridin-2-yl)-oxy)-acetyl)benzenesulfonohydrazide (13)**

Yield 68%, m.p. 230-232 °C. IR spectrum, ν, cm^{-1}: 3425 (OH), 3300, 3260 (2NH), 3094 (aromatic C–H), 2218 (CN), 1650 (C=O), 1355 (SO_2). ^{1}H NMR spectrum, δ, ppm: 3.48 (s, 2H, indene-CH_2), 3.50 (s, 3H, OCH_3), 4.22(s, 2H, CH2), 7.23-8.20 (m, 12H, ArH), 9.99 (s, 1H, OH, D_2O exchangeable), 8.88, 11.30 (s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR spectrum, δ, ppm: 33.66 (CH_2), 56.22 (OCH_3), 66.25 (CH_2), 114.20 (CN), 112.68, 115.89, 116.56, 117.52, 122.60, 124.00, 125.85, 126.15, 128.89, 129.65, 132.80, 135.40, 139.00, 145.20, 146.70, 148.90, 149.40, 151.40, 159.20, 160,20, 162.20 (Ar-C), 168.50 (C=O). MS [m/z]: 542 [M]+ 45%. Analysis calculated for $C_{28}H_{22}N_4O_6S$ (542.13), %: C, 61.98; H, 4.09; N, 10.33; Found, %: C, 61.78; H, 4.19; N, 10.13.

***N*-(4-((2-(2-((3-Cyano-4-(4-hydroxy-3-methoxyphenyl)-5H-indeno[1,2-*b*]pyridin-2-yl)oxy)-acetyl)hydrazinyl)sulfonyl)phenyl)acetamide (14)**

Yield 58%, m.p.205-207 °C. IR spectrum, ν, cm^{-1}: 3425 (OH), 3300, 3265 (NH), 3094 (aromatic C–H), 2220 (CN), 1660, 1650 (2C=O), 1345 (S=O). ^{1}H NMR spectrum, δ, ppm: 2.13 (s, 3H, CH_3) 3.48 (s, 2H, indene-CH_2), 3.70 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 7.23- 8.20 (m, 11H, ArH), 9.89 (s, 1H, OH, D_2O exchangeable), 8.88, 10.20 11.30 (3s, 3H, 3NH, D_2O exchangeable). MS [m/z]: 599 [M]+ 38%. Analysis calculated for $C_{30}H_{25}N_5O_7S$ (599.15), %: C, 60.09; H, 4.20; N, 11.68; Found, %: C, 60.19; H, 4.40; N, 11.48.

General procedure for the synthesis of compounds 15-17

To a solution of compound **1** (10 mmol) in dry DMF (20 ml) sodium hydride (10 mmol) was added and the reaction mixture was stirred at room temperature for 1h. The appropriated alkyl halide (10 mmol) namely: 2-chloroethanol, bis-(2-chloroethylether) and/or 2-chloro-1,1-dimethoxyethane was added. The reaction mixture was stirred at 70 °C for 24h, after the reaction completion the solvent was evaporated under reduced pressure and the residue was washed with water several time the obtained solid was filtered, dried and recrystallized from ethanol.

4-(4-Hydroxy-3-methoxyphenyl)-1-(2-hydroxyethyl)-2-oxo-2,5-dihydro-1*H*-indeno[1,2-b]pyridine-3-carbonitrile (15)

Yield 70%, m.p. 265–267 °C. IR spectrum, ν, cm^{-1}: 3435-3400 (br, OH), 2210 (CN), 1643 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.42 (s, 2H, indene-CH_2), 3.55 (s, 3H, OCH_3), 3.72 (t, J = 4.5 Hz, 2H, N-CH_2), 4.50 (t, J = 4.5 Hz, 2H, CH_2-O), 5.10 (s, 1H, OH, D_2O exchangeable), 7.22- 7.89 (m, 7H, ArH), 10.00 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ, ppm: 33.35 (CH_2), 44.55 (CH_2), 53.00 (OCH_3), 59.25(CH_2), 110.90 (CN), 111.19, 112.50, 113.50, 115.50, 116.20, 122.80, 126.10, 127.21. 128.90, 130.00, 131.20, 133.90, 139.30, 145.90, 147.65, 148.90, 149.40, (Ar-C), 169.90 (C=O). MS [m/z]: 374 [M]+ 60%. Analysis calculated for $C_{22}H_{18}N_2O_4$ (374.40), %: C, 70.58; H, 4.85; N, 7.48. Found, %: C, 70.68; H, 4.65; N, 7.28.

1-(2-(2-Chloroethoxy)ethyl)-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1H-indeno[1,2-*b*]pyridine-3-carbonitrile (16)

Yield 62%, m.p. 258–260 °C. IR spectrum, ν, cm^{-1}: 3440 (br, OH), 2218 (CN), 1644 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.02 (t, J = 4.6 Hz, 2H, N-CH_2), 3.20 (t, J = 4.6 Hz, 2H, CH_2-O), 3.42 (s, 2H, indene CH_2), 3.55 (s, 3H, OCH_3), 3.65-3.80 (m, 4H, OCH_2-CH_2-Cl), 7.00-7.85 (m, 7H, Ar-H), 9.95 (s, 1H, OH, D_2O exchangeable). MS [m/z]: 436 [M]+ 85%. Analysis calculated for $C_{24}H_{21}ClN_2O^4$ (436.89), %: C, 65.98; H, 4.85; N, 6.41; Found, %: C, 65.86; H, 4.95; N, 6.21.

1-(2,2-Dimethoxyethyl)-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1*H*-indeno[1,2-*b*]-pyridine-3-carbonitrile (17)

Yield 70%, m.p. 260–262 °C. IR spectrum, ν, cm^{-1}: 3438 (br, OH), 2222 (CN), 1643 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.42 (s, 2H, indene CH_2), 3.53 (s, 3H, OCH_3), 3.75 (s, 6H, 2OCH_3), 4.22 (d, J = 4.6 Hz, 2H, N-CH_2), 4.65 (t, J =7.3 Hz, 1H, CH), 7.00- 7.15 (m, 7H, ArH), 10.00 (s, 1H, OH, D_2O exchangeable). MS [m/z]: 418 [M]+ 85%. Analysis calculated for $C_{24}H_{22}N_2O_5$ (418.48), %: C, 68.89; H, 5.30; N, 6.69; Found, %: C, 68.79; H, 5.40; N, 6.58.

General procedure for the synthesis of compounds 18-22.

Compounds **18-20** were prepared from compound **1** as described above for the preparation of compounds **13** and **14** using acid chloride derivatives namely: acetyl chloride, chloroacetyl chloride, benzoyl chloride, benzensulfonyl chloride and/or tosyl chloride respectively.

1-Acetyl-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1*H*-indeno[1,2-*b*]-pyridine-3-carbonitrile (18)

Yield 80%, m.p. >300°C. IR spectrum, ν, cm^{-1}: 3439 (br, OH), 2217 (CN), 1688 (C=O), 1637 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.65 (s, 3H, $COCH_3$) 3.43 (s, 2H, indene-CH_2), 3.52 (s, 3H, OCH_3), 7.00-7.80 (m, 7H, ArH), 9.90 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ, ppm: 23.25(CH_3, acetyl), 32.35

(CH_2), 56.00 (OCH_3), 110.90 (CN), 110.19, 113.50, 115.50, 116.20, 122.80, 126.10, 127.21, 131.20, 133.90, 139.30, 143.50, 145.90, 147.90, 149.40, 159.43, 160.53 (Ar-C), 169.90 (C=O), 172.90 (C=O). MS [m/z]: 372 [M]+ 60%. Analysis calculated for $C_{22}H_{16}N_2O_4$ (372.38), %: C, 70.96; H, 4.33; N, 7.52.Found, %: C, 70.76; H, 4.53; N, 7.32.

1-(2-Chloroacetyl)-4-(4-hydroxy-3-methoxy-phenyl)-2-oxo-2,5-dihydro-1H-indeno-[1,2-*b*]-pyridine-3-carbonitrile (19)

Yield 78%, m.p.>300 °C. IR spectrum, ν, cm^{-1}: 3429 (br, OH), 2218 (CN), 1706 (C=O), 1637 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.42 (s, 2H, indene CH_2), 3.53 (s, 3H, OCH_3), 4.36 (s, 2H, CO-CH_2Cl), 7.00-7.55 (m, 7H, ArH), 9.90 (s, 1H, OH, D_2O exchangeable). MS [m/z]: 406 [M]+ 55%. Analysis calculated for $C_{22}H_{15}ClN_2O_4$ (406.82), %: C, 64.95; H, 3.72; N, 6.89; Found, %: C, 64.85; H, 3.82; N, 6.69.

1-Benzoyl-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1*H*-indeno[1,2-*b*]-pyridine-3-carbonitrile (20)

Yield 75%, m.p. 275-277 °C. IR spectrum, ν, cm^{-1}: 3439 (br, OH), 2220 (CN), 1711 (C=O), 1644 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.42 (s, 2H, indene CH_2), 3.53 (s, 3H, OCH_3), 7.00- 8.00 (m, 12H, ArH), 9.95 (s, 1H, OH, D_2O exchangeable). MS [m/z]: 434 [M]+ 65%. Analysis calculated for $C_{27}H_{18}N_2O_4$ (434.45), %: C, 74.65; H, 4.18; N, 6.45; Found, %: C, 74.75; H, 4.28; N, 6.25.

4-(4-Hydroxy-3-methoxyphenyl)-2-oxo-1-(phenyl-sulfonyl)-2,5-dihydro-1*H*-indeno-[1,2-*b*]pyridine-3-carbonitrile (21)

Yield 70%, m.p.186-188 °C. IR spectrum, ν, cm^{-1}: 3421 (br, OH), 2215 (CN), 1630 (C=O), 1360 (S=O). ^{1}H NMR spectrum, δ, ppm: 3.42 (s, 2H, indene CH_2), 3.55 (s, 3H, OCH_3), 7.26- 8.10 (m, 12H, ArH), 9.95 (s, 1H, OH, D_2O exchangeable). MS [m/z]: 470 [M]+ 65%. Analysis calculated for $C_{26}H_{18}N_2O_5S$ (470.50), %: C, 66.37; H, 3.86; N, 5.95; Found, %: C, 66.57; H, 3.76; N, 5.75

.

4-(4-Hydroxy-3-methoxyphenyl)-2-oxo-1-tosyl-2,5-dihydro-1*H*-indeno[1,2-*b*]-pyridine-3-carbo-nitrile (22)

Yield 82%, m.p. 238-240 °C. IR spectrum, ν, cm^{-1}: 3420 (br, OH), 2213 (CN), 1644 (C=O), 1355 (SO_2). ^{1}H NMR spectrum, δ, ppm: 2.15 (s, 3H, CH_3), 3.45 (s, 2H, indene-CH_2), 3.60 (s, 3H, OCH_3), 7.20- 8.10 (m, 11H, ArH), 9.98 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ, ppm: 21.35 (CH3), 32.66 (CH_2), 56.00 (OCH_3), 110.20 (CN), 112.68, 115.89, 116.56, 117.52, 122.60, 124.00, 125.85, 129.65, 132.80, 135.40, 139.00, 145.20, 146.70, 148.9, 149.4, 151.40, 159.20,160,20, 165.30 (Ar-C), 171.50 (C=O). MS [m/z]: 484 [M]+ 65%. Analysis calculated for $C_{27}H_{20}N_2O_5S$ (484.53), %: C, 66.93; H, 4.16; N, 5.78; Found, %: C, 86.93; H, 4.06; N, 5.58.

2.2. Antimicrobial activity bioassay

The nineten compounds were screened for their antibacterial and antiyeast activities using the agar diffusion bioassay [18] against two Gram positive bacteria (Staphylococcus aureus ATCC 29213 and Bacillus subtilis ATCC6633), two Gram negative bacilli (Sarcina lutea and Escherichia coli ATCC 25922) and one yeast (Candida albicans NRRL Y–477). Mullar Hinton agar (for bacteria) and PDA (for yeast) were inoculated with 100 µl of 24h cultures of tested strains standardized to obtain a final OD600 of 1. Wells of 0.9 cm diameter were made with sterile cork porer and filled with 100 µl containing 5 mg of each sterilized compound dissolved in Dimethyl sulfoxide (DMSO). Plates were incubated aerobically at 37°C for 24h. A clear zone of inhibition of at least 20 mm in diameter after 24h incubation was considered as a positive result. Gentamycin (10 mg/ml) and clotrimazole (10 mg/mL) were used as standard antibacterial and anti-yeast references, respectively. The antimicrobial activity of DMSO was also tested to avoid any possible antimicrobial interference with the tested compounds. The minimum inhibitory concentration (MIC) was determined for potent compounds showing inhibition zone more than 20 mm in diameter using different concentrations of the compound (0.2, 0.5, 1, 1.5 and 2 mg/well). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of an antimicrobial compound that is bacteriostatic (prevents the visible growth of bacteria) after incubation at 37°C for 16–24 h.

2.3. Antioxidant activity assay

The antioxidant activity of the compounds was assessed by the DPPH assay method as described [19-22] with minor modification. Briefly, 500 µL of ethanolic DPPH solution (0.4 mmol) was vigorously mixed with 500 µL of compounds or water (as a control) and incubated at 37 °C in the dark for 1 h. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The percentage of scavenging activity was calculated as [1-(As - Ab)/Ac] × 100, whereas Ab, Ac and As is the absorbance of the blank (ethanol and sample), the control (DPPH and deionised water) and the sample (DPPH and sample), respectively. Ascorbic acid at the concentration of 0.1% was used as a positive control.

For determination of IC50, four different concentrations of ethanolic solutions of the compounds (4.2, 3.3, 2.5 and 0.83 gm) were prepared and assayed for antioxidant activity as mentioned before. IC50 is the maximal concentration of the compound to cause 50% scavenging activity of DPPH.

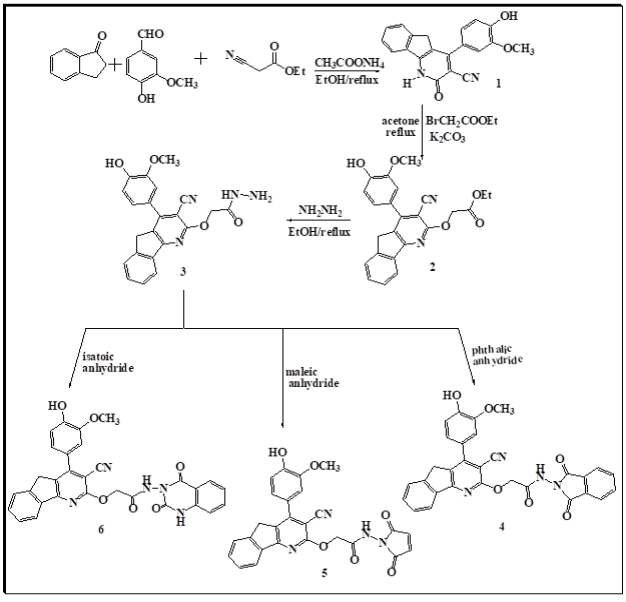
3. Results and discussion

3.1. Chemistry

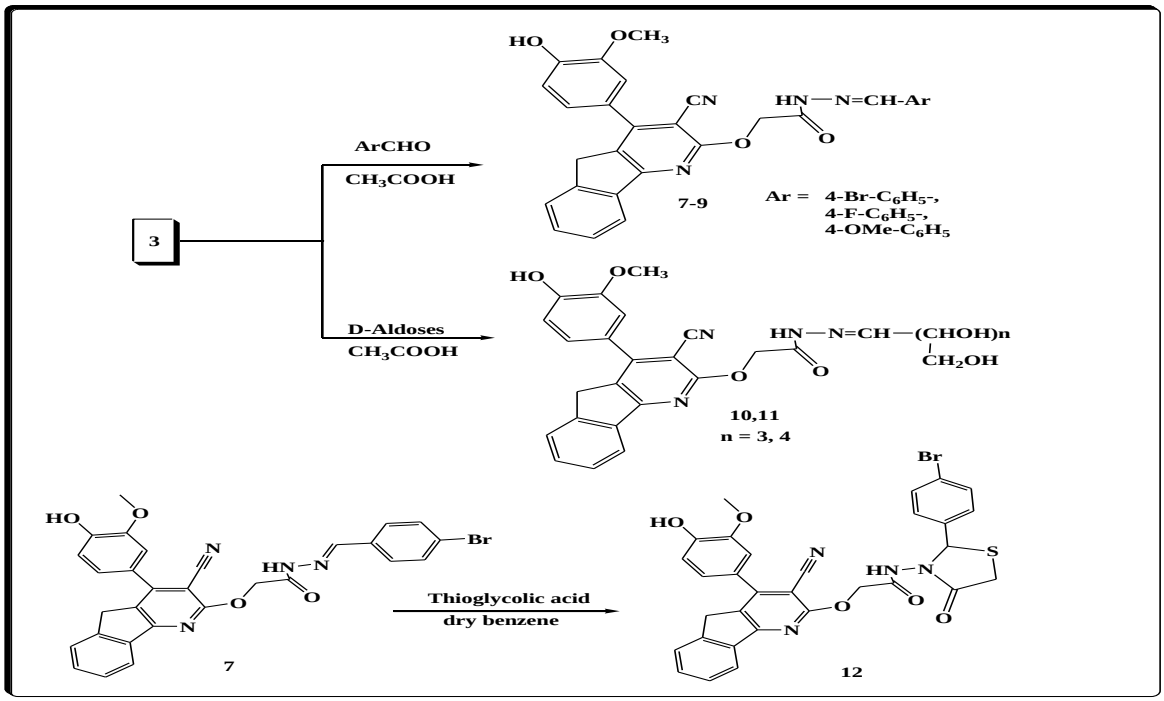
The new synthesis of 1*H*-indenopyrdine derivatives are depicted in **Schemes 1-5**. The identification of all structures of these compounds were carried out by spectroscopic techniques and elemental analysis. The starting compound 4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1H-indeno[1,2-b]pyridine-3-carbo-nitrile (**1**) was synthesized via one pot multicomponent condensation [23-27] of 1-indanon, 4-hydroxy-3-methoxybenzaldehyde, ethylcyanoacetate in ethanol in the presence of ammonium acetate. IR spectrum of compound **1** showed characteristic absorption bands at 3525-3225, 2212 and 1636 cm^{-1} due to OH, NH, CN and C=O, respectively. ^{1}H-NMR spectrum of compound **1** displayed signals at δ 10.50 and 9.99 ppm referring to NH and OH protons (D_2O exchangeable), 6.90-7.80 ppm representing the aromatic protons, in addition to two singlet signals at δ 3.55 and 3.42 ppm due to $–OCH_3$ and indene $-CH_2$ respectively. ^{13}C NMR spectrum of the same derivative **1** revealed various signals at δ (32.95, 56.00 and 170.22 ppm due to indene CH_2, OCH_3, and C=O groups, respectively. Upon the reaction of the key starting pyridone derivative **1** with ethyl bromoacetate in dry acetone in the presence of anhydrous potassium carbonate led to the formation of the corresponding O-ethyl ester derivative **2**. IR spectrum of the latter derivative **2** showed the absence of the absorption band of NH group and the appearance of a strong absorption band at 1730 cm^{-1} for C=O (ester). Furthermore, ^{1}H NMR spectrum of **2** revealed a new singlet signal at δ 4.60 ppm due to $-OCH_2$ as well as triplet and quartet signals at δ 1.22 and 4.20 ppm representing OCH_2-CH_3, respectively. The ^{13}C-NMR spectrum of **2** showed signals at δ 166.92 ppm (C=O), 64.55 ppm (O-CH_2), 62.25 ppm (-CH_2) and 14.35 ppm (-CH_3). The acid hydrazide 3 was obtained by hydrazinolysis of the ester **2** with hydrazine hydrate (**Scheme 1**). IR spectrum of **3** showed absorption bonds at 3338, 3261 cm^{-1} for NH, NH_2 and at 1644 cm-1 for C=O group. ^{1}H NMR spectrum of the latter derivative revealed singlet signals at δ 4.40-4.42 and 8.90 ppm for NH_2 and NH. The condensation of the hydrazide compound **3** with different anhydrides such as; phthalic anhydride, maleic anhydride and/or isatoic anhydride afforded the corresponding compounds **4**-**6**.

IR spectra of compounds **4**-**6** showed absorption bands at 3320-3333 and 2218-2220 cm^{-1} due to NH, CN functionalities, respectively and three characteristic bands at 1701-1695 and 1645 cm^{-1} due to 3C=O groups. Moreover, the mass spectra of 4-6 represented their molecular ion peaks at m/z 532 (65%), 482(75%) and 547 (55%) which were in agreement with their molecular formulae.

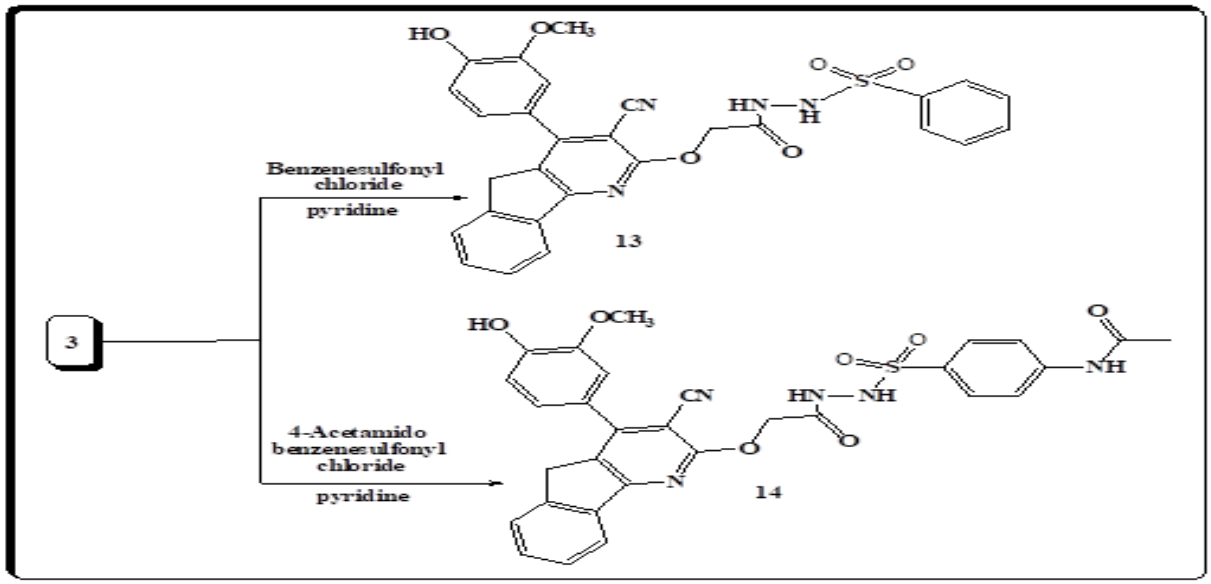
Furthermore, the Schiff bases **7-11** were synthesized by the reaction of the hydrazide **3** with different aromatic aldehydes such as: 4-bromobenzaldehyde, 4-fluorobenzaldehyde, 4-methoxybenzaldehyde and/or aldoses namely: D-glucose and D-xylose in acidic medium (**Scheme 2**). The formed hydrazones were confirmed by spectral data and elemental analysis. Their ^{1}HNMR spectra showed the presence of the azomethine proton (CH=N) and the sugar protons in the expected regions (cf.exp). Upon condensation of Schiff base **7** with thioglycolic acid in dry benzene, the thiazolidinone derivative **12** was formed. It's IR spectrum showed a characteristic absorption bond at 1645 cm^{-1} due to C=O groups. Also, ^{1}HNMR spectrum revealed singlet signals at δ 3.70 and 5.52 ppm assigned to the methylene protons (CH_2) and the methine proton (N-CH-S) of the formed thiazolidinone ring, respectively. ^{13}C NMR represented signals at δ 170.55 and 172.20 ppm referring to 2 C=O groups as well as at δ 54.50 and 35.20 for the methine and methylene carbons of thiazolidinone ring, respectively



Scheme 1. Synthesis of new indenopyridine acetamide compounds



Scheme 2. Synthesis of new Schiff bases 7-11 and the thiazolidinone derivative 12

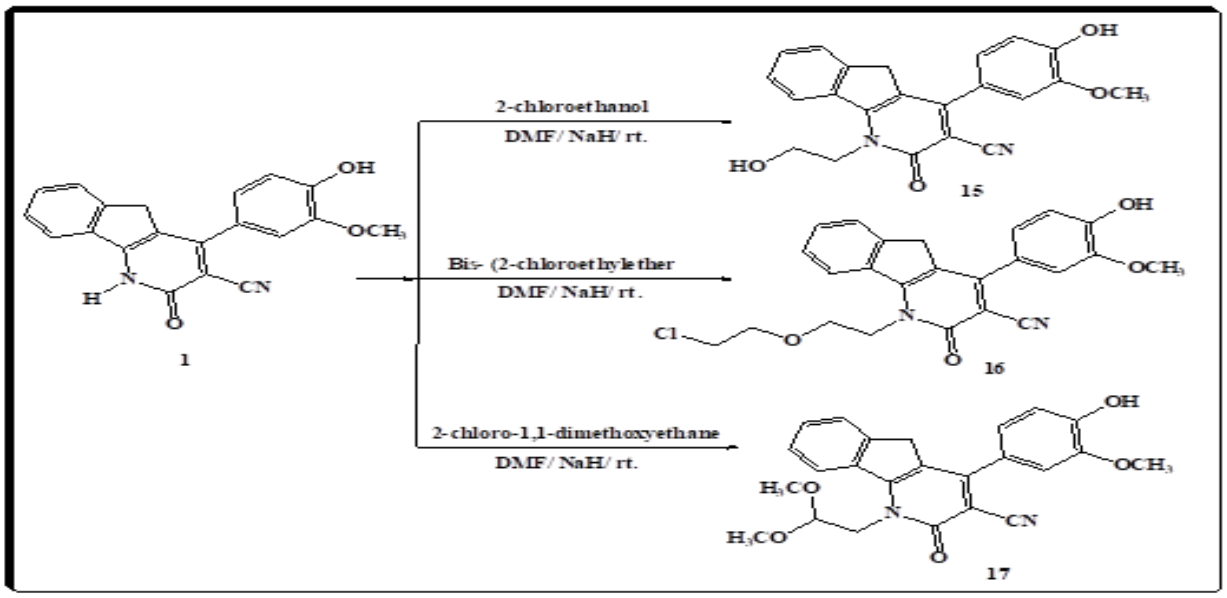


Scheme 3. Synthesis of new sulfonamide derivatives 13, 14

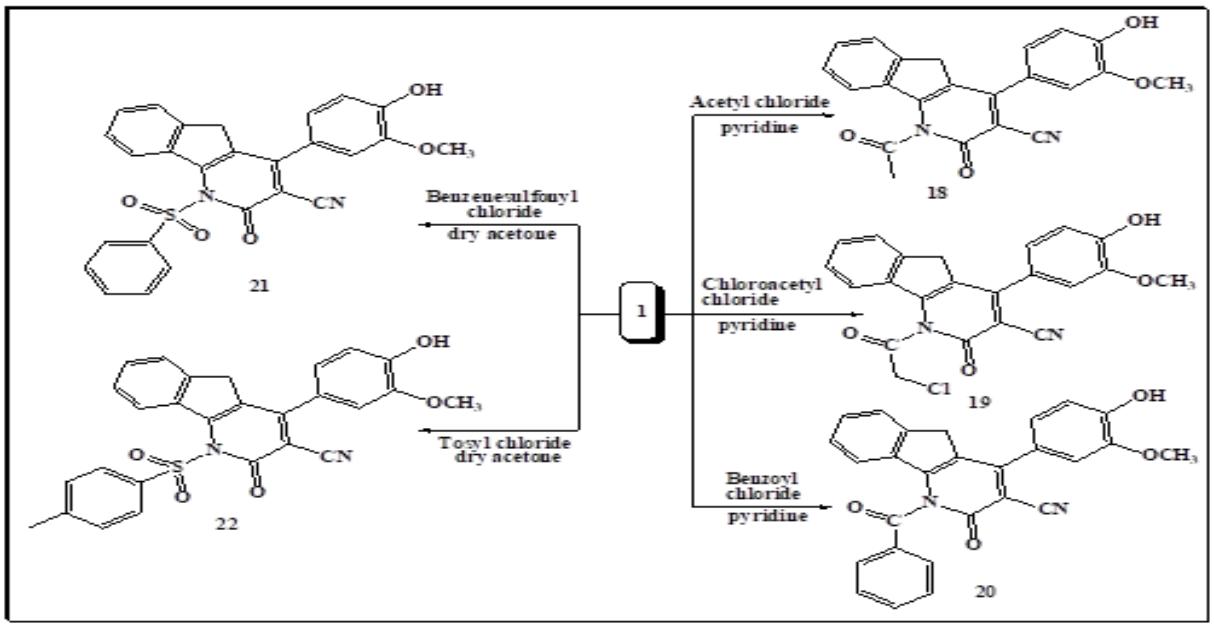
Moreover, the N-salfonylated products **13, 14** were synthesized by condensation of the hydrazide **3** with benzenesulfonylchloride and 4-acetamidobenzene-sulfonylchloride derivatives (**Scheme 3**). IR spectra of **13, 14** displayed absorption bands at 3300-3260 and 1355-1345 cm^{-1} due to 2NH and $2SO_2$ groups in addition to the main bands of the parent molecules (**cf.exp**). ^{1}HNMR spectrum of **14** showed a singlet at δ 2.13 ppm related to CH_3, in addition to the expected signals. The mass spectra of **13**, **14** revealed their ion peaks at 542 and 599.15 respectively, which were in agreement with their expected molecular formulae.

On the other hand, the acyclic N-glycoside products **15-17** were synthesized by the reaction of the starting indenopyridine **1** with 2-chloroethanol, bis- (2-

chloroethylether) and 2-chloro-1,1-dimethoxyethane. The structures of the compounds **15-17** were confirmed by their spectral data and elemental analysis. Their IR spectra showed the absence of NH absorption band. ^{1}H-NMR for compound **15** (for example) showed two triplet signals at δ 3.72, 4.50 ppm related to N-CH_2-CH_2-OH alongside with a new singlet at δ 5.10 ppm for the hydroxyl group (**Scheme 4**). The N-alkylated derivatives **18-20** were accomplished by refluxing the indenopyridine **1** with acid chloride derivatives (such as: acetyl chloride, chloroacetyl chloride and benzoyl chloride) in pyridine. Also, the N-salfonylated derivatives **21, 22** were achieved by reaction of the treatment of **1** with benzenesulfonyl chloride and tosyl chloride (**Scheme 5**). IR spectra of **18-22** showed the absence of NH absorption band and the appearance of new bands related to the carbonyl and alkyl or aryl side chain. ^{1}H-NMR showed a singlet signal at δ 2.65 ppm due to the acetyl protons of $COCH_3$ of compound **18** and at δ 4.36 ppm due to the methylene protons of compound **19**. IR spectra of compounds **21** and **22** showed absorption bands at 1360 and 1355 cm^{-1} related to SO_2 groups. Mass spectra of all derivatives **18-22** showed the expected molecular ion peaks (cf. exp).



Scheme 4. Synthesis of new acyclic N-glycoside compounds 15-17



Scheme 5. Synthesis of N-alkylated and N-salfonylated derivatives 18- 22

3.2. Biological activity

3.2.1. Antimicrobial activity

The agar diffusion bioassay was used to evaluate the antagonistic activity of the new nineteen compounds at concentration of 5 mg/well against some selected Gram +ve and Gram -ve bacteria and yeast. The spectra of inhibition were different among the tested compounds, the inhibition zones (IZ) varied from 10 to 30 mm for bacteria and from 14 to 16 mm for yeast. According to **table 1**, the thiazolidinone derivative **12**, the N-carbonyl **18-20** and N-sulfonyl derivative **21** showed moderate antibacterial activity against the tested Gram +ve and Gram –ve bacteria and the tested fungal strain (IZs ranges from 12-17 mm; ZOI Gentamycin, 25-35 mm, IZ Clotrimazole, 30 mm) except the chloro-compound **19** which appeared to be completely inactive agent. Although the tosyl compound **22** appeared to be inactive against the tested +gm bacteria and moderately active against the yeast strain *C. albicans* (IZ, 15 mm), it showed promising activity against *E. coli* (IZ; 30 mm) comparing to gentamicin of IZ; 35 mm. The 2,5-dioxo-pyrrole compound **5** exhibited moderate activity against the tested Gram -ve bacteria *S. lutea* and *E. coli* as well as the +gm bacteria B. subtilis (IZ; 10-12 mm) and inactivity against *S. aureus* and the yeast *C. albicans*, while the acetohydrazide **11**, and the acetamide **14** derivatives represented only moderate antifungal activity (IZ, 14, 16 mm, respectively) The rest of the compounds lost the activity as antibacterial and antifungal candidates.

Even though *E. coli* can be an innocent resident of the gastrointestinal tract of human, it has also the pathogenic ability to cause severe diarrhea and extra-intestinal diseases. Pathogenic *E. coli* causes much mortality worldwide [28]. Consequently, finding compounds with antimicrobial activity against this pathogen could be of a great interest. Thus, MIC test was carried out for compound **22** against *E. coli* ATCC 25922 and it was found to be 0.5 mg /mL.

3.2.2. Antioxidant activity

Scavenging of DPPH free radicals is routinely used as a determinant of antioxidant activity of a compound. In this study, in vitro antioxidant activity of the new nineteen compounds was tested. The results in **Table 2** indicated that sixteen out of the nineteen compounds exhibited potent antioxidant activity (ranging from 74.07 to 99.6%). The parent indeno-pyridine-3-carbonitrile compound **1** exhibited the highest DPPH scavenging activity of about 99.6% at a concentration of 4.2 mg, and compounds **8-12**, 14, 19 and 22 also exhibited excellent antioxidant activity exceeded 90% at the same concentration. On the other hand, compounds **2, 5** and **7** showed moderate antioxidant activity (37.6, 36 and 41.6 %, respectively).

Four varying concentrations (4.2, 3.3, 2.5 and 0.83 mg/mL) of the sixteen most potent compounds were tested to determine their IC50's. The tested compounds demonstrated different percentages of antioxidant activity in a concentration dependent manner. Interestingly, the IC50's of all the tested compounds were closely related to the reference ascorbic acid (IC50; 0.9 - 2.9 mg/mL, IC_{50} ascorbic acid; 0.8 mg/mL) (**Table 2, Fig. 2**). The obtained results indicated that the new indeno-pyridines could be considered as basic nuclei for further derivatization of new compounds as potent antioxidant agents.

Table 1: Antmicrobial activity of the new indeno-pyridine derivatives

Compd (5 mg/well)	Growth inhibition zone (mm)				
	S. aureus Gram +ve	*B. subtilis* Gram +ve	*S. lutea* Gram –ve	*E. Coli* Gram -ve	*C. albicans* Yeast
1	—	—	—	—	—
2	—	12	—	—	—
3	—	10	15	—	—
4	—	—	—	10	—
5	—	11	12	10	—
6	—	—	—	—	—
7	—	—	—	—	—
8	—	—	—	—	—
9	—	—	—	—	—
10	—	—	—	—	—
11	—	—	—	—	14
12	10	15	13	—	16
14	—	—	—	—	16
17	11	—	17	11	—
18	12	13	13	13	14
19	11	12	13	15	—
20	15	15	17	17	15
21	14	14	16	15	15
22	—	—	—	30	15
DMSO	—	—	—	—	—
Genta-mycin (10mg/ml)	25	40	40	35	—
Clotrimazole (10mg/ml)	—	—	—	—	30

Table 2: Antioxidant activity of the new indeno-pyridines and their IC50

Comd.	Antioxidant activity (%) ± SD				IC_{50} (mg/mL)
	Sample conc. (mg/mL)				
	4.2	3.3	2.5	0.83	
1	99.59±0.71	96.32±1.41	74.21±1.41	36.05±1.41	1.4
2	37.86±1.41	–	–	–	–
3	83.13±2.12	68.16±0.71	52.37±0.71	43.42±2.12	2.1
4	81.28±0.71	62.89±2.12	51.58±1.41	42.63±0.71	2.2
5	36.01±0.71	–	–	–	–
6	81.28±0.71	67.11±0.71	51.84±2.12	37.63±2.12	2.4
7	41.56±0.71	–	–	–	–
8	98.97±1.41	94.21±0.71	66.05±1.41	45.26±0.71	1.2
9	98.56±0.71	94.21±1.41	68.16±2.12	42.89±2.12	1.3
10	98.97±0.71	86.58±0.71	70.00±0.71	45.53±2.12	1.1
11	92.80±2.12	90.79±2.12	75.26±0.71	46.32±0.71	1.0
12	96.91±1.41	88.42±0.71	70.53±1.41	38.68±2.12	1.4
14	89.92±1.41	87.63±1.41	67.89±0.71	49.47±0.71	0.9
17	86.42±2.12	62.11±2.12	35.53±1.41	21.05±1.41	2.9
18	74.07±1.41	68.95±0.71	50.00±2.12	45.79±1.41	2.5
19	92.59±2.12	90.79±0.71	76.32±0.71	40.26±0.71	1.2
20	82.92±0.71	42.89±0.71	31.32±2.12	26.32±1.41	3.5
21	94.65±1.41	79.47±2.12	61.58±1.41	48.68±1.41	0.9
22	92.80±0.71	87.63±0.71	75.26±2.12	48.95±2.12	0.9
Ascorbic acid	100±0.00	100±0.00	91.35±2.12	49.88±1.41	0.8

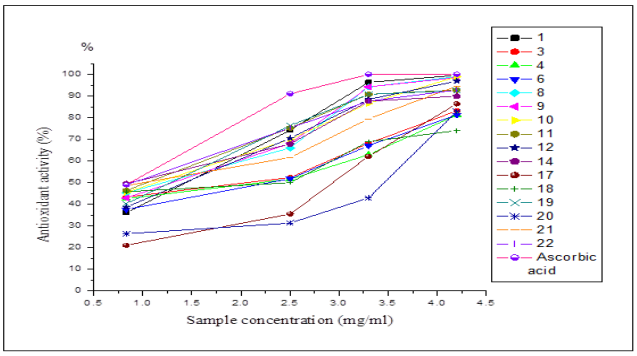


Fig. 2. Antioxidant activity of the new indeno-pyridines

4. CONCLUSION

This study represents the synthetic approaches of new 1*H*-indeno[1,2-*b*]pyridine derivatives using 4-(4-hydroxy-3-methoxyphenyl)-2-oxo-2,5-dihydro-1H-indeno[1,2-b]pyridine-3-carbo-nitrile (1) as the key starting compound. Antimicrobial evaluation revealed that most of the new compounds were moderately active against different Gram +ve, Gram-ve bacteria and the pathogenic yeast *C. albicans*. The tosyl compound **22** represented considerable activity against E. coli of IZ; 30 mm comparable to the reference antibiotic gentamicin of IZ; 35 mm with MIC 0.5 mg/mL. The new derivatives were further evaluated as antioxidant agents. They exhibited significant activity of IC50's ranging from 0.9 to 3.5 mg/mL in comparison with ascorbic acid as a positive control of IC50; 0.9 mg/mL. The new compounds bearing indenopyridine core could be considered as basic scaffolds for further design and synthesis of new potent antioxidant candidates.

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