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Imidazo[4,5-*a*]quinindolines as Highly Effective Antibacterial Agents[1]

Mehdi Pordel[a, 2], Shirin Ramezani[a], Maryam Jajarmi[b], and Mina Sokhanvar[a]

[a] *Department of Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran*

[b] *Khorasan Razavi Rural Water and Wastewater Co*

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Abstract—Resistance to antimicrobial agents is a concern that exists globally and has a considerable impact on human and animal health, so that the discovery of new antibacterial compounds has become increasingly more important in combating infectious disease. In this paper, imidazo[4,5-*a*]quinindolines are introduced as new antibacterial agents against Gram-positive and Gram-negative bacteria. These pentacyclic compounds are synthesized by the reaction of *N*-alkyl-5-nitrobenzimidazoles with 2-(1-alkyl-1*H*-3-indolyl)acetonitrile under basic conditions in excellent yields. The structures of newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. The antibacterial activities of the synthesized compounds were screened against standard strains of two Gram-positive and two Gram-negative bacteria using the broth microdilution method. Most of the compounds studied showed promising activities against both types of bacteria.

Keywords: 5-nitrobenzimidazoles, imidazo[4,5-a]quinindoline, Gram-positive and Gram-negative bacteria, antibacterial agents, MIC

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INTRODUCTION

Increasing global prevalence of antimicrobial resistance by enteric pathogens like *Escherichia coli*, *Campylobacter*, and *Salmonella* species against easily available and usually prescribed drugs has become one of the most important concerns throughout the world. Therefore, development of antibacterial compounds with novel structural features and activity against resistant pathogens is necessary. In these days, a number of drugs containing simple heterocycles or a combination of different moieties have been in use.

Indoloquinoline alkaloids have attracted obvious consideration due to their important biological properties and their function as pharmacophores. It has been revealed that indolo[2,3-*b*]quinolines, usually known as linear quinindoline, were utilized as antimicrobial, antimuscarinic, and antiviral agents [1–3]. They intercalate the DNA double helix causing remarkable changes in DNA conformation leading to inhibition of DNA replication and transcription [4].

On the other hand, imidazole and its derivatives have been reported to be bioactive molecules in many important biological systems with a wide range of pharmacological activity. In most cases, they are recognized as proton donors and/or acceptors in enzymatic systems, coordination system ligands, and as the basis of charge-transfer processes [5], in addition to antibacterial [6], antiepileptic [7], anti-inflammatory, and anticancer agents [8–10]. A combination of the imidazole nucleus with the quinindoline moiety may improve the mentioned biological properties.

Recently, we have reported a facile and efficient method for the synthesis of new fluorescent heterocyclic system imidazo[4,5-*a*]quinindoline (imidazo[4,5-*f*]-indolo[2,3-*b*]quinoline) from the reaction of *N*-alkyl-5-nitrobenzimidazoles with 2-(1-alkyl-1*H*-3-indolyl)acetonitrile under basic conditions [11]. In continuation with our research works on the synthesis and the activity of new heterocyclic compounds against bacteria [12–17], in this paper, in addition to the synthesis of some further new substituted imidazo[4,5-*a*]quinindolines, antibacterial activity of these compounds was also studied.

RESULTS AND DISCUSSION

Initially, the commercially available 5-nitro-1*H*-benzimidazole was alkylated with different alkyl halides in KOH and DMF to give 1-alkyl-5-nitro-1*H*-benzimidazoles (**Ia–d**) in very good yields [18]. Other precursors, 2-(1-alkyl-1*H*-3-indolyl)acetonitriles (**IIa**, **b**), were prepared in four steps explicated in the following manners. Reaction of alkylated indoles [19] with Mannich reagent led to the formation of *N*,*N*-dimethyl(1-alkyl-

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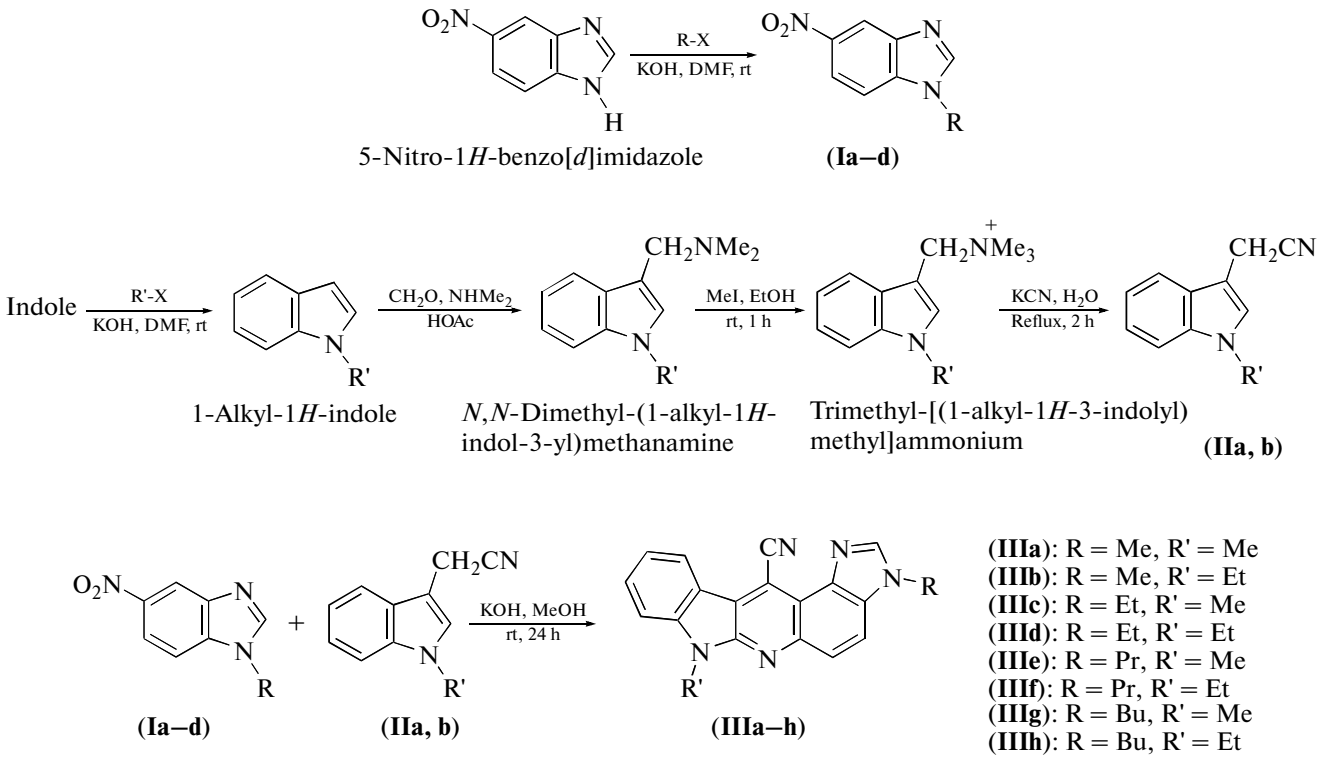
[2] Corresponding author: phone: +98 0513 8414182; fax: +98 0513 8424020; e-mail: mehdipordel58@mshdiau.ac.ir.

1*H*-indol-3-yl)methanamines. They were converted to the related salts by the reaction with MeI in excellent yields. Compounds (**IIa**, **b**) were finally obtained from the reaction of trimethyl[(1-methyl-1*H*-3-indolyl)methyl]ammonium salts with KCN [20].

Finally, 3,7-dialkyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitriles (**IIIa–h**) were synthesized from the one-pot reaction of 1-alkyl-5-nitro-1*H*-benzimidazole (**Ia–d**) with 2-(1-alkyl-1*H*-3-indolyl)acetonitriles (**IIa**, **b**) in basic MeOH solution via the nucleophilic substitution of hydrogen [11, 21] (Scheme 1). The structure of new target products (**IIIc**), (**IIId**), (**IIIg**), and (**IIIh**) was confirmed by NMR techniques, FT-IR spectroscopy, mass spectral, and microanalytical data. The spectral details of all these compounds are given in the Experimental section.



Scheme 1. Synthesis of compounds (**IIIa–h**).

All the synthesized compounds were screened for their antibacterial activity against standard strains of two Gram-positive (*Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633) and two Gram-negative bacteria (*Escherichia coli* ATCC 10538 and *Salmonella typhimurium* ATCC 14028) using broth microdilution method as described in our earlier studies [12–17]. We used amoxicillin and ciprofloxacin as reference compounds in the evaluation of antibacterial activity. The lowest concentration of the antibacterial agent that prevents growth of the test organism, as detected by lack of visual turbidity (matching the negative growth control), is assigned the minimum inhibitory concentration (MIC).

As shown in the table, all of the synthesized compounds showed high antibacterial activity against both Gram-positive and Gram-negative strains and most of them showed greater inhibitory activity against *Staphylococcus aureus* and *Salmonella typhimurium* bacteria than the well known antibacterial agents amoxicillin and ciprofloxacin. The synthesized compounds exhibited a little stronger inhibitory activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhimurium* than against *Escherichia coli*. Analysis of the inhibitory activity of compounds (**IIIa–h**) against Gram-positive and Gram-negative bacteria showed that the length of the alkyl chains appeared to have a direct impact on the antibacterial activity of the 3,7-dialkyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile derivatives. As the length of alkyl chains increased in the compounds starting from (**IIIa**) onwards, the MIC gradually decreased (the change in R group was more sensible than that in R' group), with the compound (**IIIh**) (R = Bu and R' = Et) being the most active. It can be proposed that the chain lengths might change the binding characteristics of ligands to their respective receptors and thus increase the biological activity [16, 17].

Inhibitory activity (MIC, μg mL^{-1}) of compounds (**IIIa–h**) and controls against bacteria

Compound	R, R'	*Staphylococcus aureus* (ATCC 29213)	*Bacillus subtilis* (ATCC 6633)	*Escherichia coli* (ATCC 10538)	*Salmonella typhimurium* (ATCC 14028)
(IIIa)	R, R' = Me	15	15	25	25
(IIIb)	R = Me, R' = Et	10	10	25	15
(IIIc)	R = Et, R' = Me	10	10	25	10
(IIId)	R = Et, R' = Et	5	5	10	5
(IIIe)	R = Pr, R' = Me	3	3	10	5
(IIIf)	R = Pr, R' = Et	3	3	10	3
(IIIg)	R = Bu, R' = Me	1	0.5	5	1
(IIIh)	R = Bu, R' = Et	0.5	0.5	3	0.5
Ciprofloxacin		16	0.05	1	>128
Amoxicillin		25	0.06	150	>128

CONCLUSIONS

Imidazo[4,5-*a*]quinindoline compounds were synthesized through the simple methods. The structures of new synthesized compounds were confirmed by FT-IR, NMR, and mass spectra. These compounds were evaluated for in vitro antibacterial activities against four strains of bacteria. Compound (**IIIh**) showed the highest biological activity among the compounds studied, however, most of them showed significant activities for both Gram-positive and Gram-negative bacteria. The results of this study suggest that further development of such compounds may be of therapeutic interest.

EXPERIMENTAL

General

Methanol, *N*,*N*-dimethylformamide (DMF), methyl iodide, ethyl bromide, *n*-propyl bromide, *n*-butyl bromide, isobutyl bromide, dimethylamine, formaldehyde, potassium cyanide, 5-nitro-1*H*-benzimidazole, and indole were purchased from Merck. Amoxicillin, ciprofloxacin, and potassium hydroxide were purchased from Sigma-Aldrich. All solvents were dried according to standard procedures. Compounds (**Ia–d**) [18] and (**IIa, b**) [19, 20] were synthesized as described in the literature. The microorganisms *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 10538, and *Salmonella typhimurium* ATCC 14028 were purchased from Pasteur Institute of Iran.

Melting points were measured on an IA9100 (Electrothermal) melting-point apparatus. The IR spectra (ν, cm^{-1}) were obtained in KBr discs on a Tensor 27 spectrometer and only noteworthy absorptions are listed. ^{13}C NMR (100 MHz) and the ^{1}H NMR (400 MHz) spectra were recorded on a Bruker Avance DRX-400 FT spectrometer in $CDCl_3$. Chemical shifts are reported in ppm downfield from TMS as internal standard; coupling constant *J* is given in Hz. The mass spectra were recorded on a Varian Mat, CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer. All measurements were carried out at room temperature.

General Procedure for the Synthesis of Compounds (IIIa–h)

1-Alkyl-5-nitro-1*H*-benzimidazole (**Ia–d**) (20 mmol) and 2-(1-alkyl-1*H*-3-indolyl)acetonitrile (**IIa**, **b**) (25 mmol) were added with stirring to a solution of KOH (15 g, 268 mmol) in methanol (70 mL). The mixture was stirred at room temperature for 24 h. After concentrating the solution at reduced pressure, the precipitate was collected by filtration, washed with water, following with acetone, and then air dried to give practically pure compounds (**IIIa–h**). More purification was attained by crystallization from a proper solvent such as EtOH.

3,7-Dimethyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIIa). mp 363–365°C (lit. [11] mp 360–362°C); ^{1}H NMR: 4.05 (3H, s, CH_3), 4.27 (3H, s, CH_3), 7.46 (1H, td, J = 8.0, 0.8, ArH), 7.57 (1H, d, J = 8.4, ArH), 7.71 (1H, td, J = 8.0, 1.2, ArH), 7.89 (1H, d, J = 9.2, ArH), 8.09 (1H, d, J = 9.2, ArH), 8.22 (1H, s, ArH), 8.96 (1H, d, J = 8.0, ArH).

7-Ethyl-3-methyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIIb). mp 342–344°C (lit. [11] mp 342–345°C); ^{1}H NMR: 1.51 (3H, t, J = 7.2, $CH_2\mathit{CH_3}$), 4.28 (3H, s, CH_3), 4.33 (2H, q, J = 7.2, $\mathit{CH_2}CH_3$), 7.45 (1H, td, J = 8.0, 0.8, ArH), 7.56 (1H, d, J = 8.4, ArH), 7.72 (1H, td, J = 8.0, 1.2 , ArH), 7.88 (1H, d, J = 9.2, ArH), 8.10 (1H, d, J = 9.2, ArH), 8.21 (1H, s, ArH), 8.96 (1H, d, J = 8.0, ArH).

3-Ethyl-7-methyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIIc) was obtained as shiny yellow needles (EtOH), yield 79%, mp 302–305°C;

IR: 2225 (CN); ^{1}H NMR: 1.66 (3H, t, J =7.2, CH_2CH_3), 4.07 (3H, s, CH_3), 4.42 (2H, q, J = 7.2, CH_2CH_3), 7.42 (1H, td, J = 8.0, 0.8, ArH), 7.51 (1H, d, J= 8.4, ArH), 7.72 (1H, td, J = 8.0, 1.2, ArH), 7.86 (1H, d, J = 9.2, ArH), 8.09 (1H, d, J = 9.2, ArH), 8.22 (1H, s, ArH), 8.90 (1H, d, J = 8.0, ArH); ^{13}C NMR: 14.7, 31.6, 37.7, 104.5, 109.3, 113.3, 116.2, 117.7, 118.8, 119.0, 120.8, 124.3, 125.1, 129.6, 129.6, 137.3, 141.0, 142.4, 144.9, 149.5; MS, *m/z*: 325 (M^+). Calcd. for $C_{20}H_{15}N_5$ (325.4): C, 73.83; H, 4.65; N, 21.52. Found: C, 74.06; H, 4.69; N, 21.28.

3,7-Diethyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIId) was obtained as shiny yellow needles (EtOH), yield 65%, mp 295–297°C; IR: 2225 (CN). ^{1}H NMR: 1.53 (3H, t, J = 7.2, CH_2CH_3), 1.66 (3H, t, J = 7.2, CH_2CH_3), 4.35 (2H, q, J = 7.2, CH_2CH_3), 4.43 (2H, q, J = 7.2, CH_2CH_3), 7.43 (1H, td, J = 8.0, 0.8, ArH), 7.50 (1H, d, J = 8.4, ArH), 7.75 (1H, td, J = 8.0, 1.2, ArH), 7.84 (1H, d, J = 9.2, ArH), 8.07 (1H, d, J = 9.2, ArH), 8.23 (1H, s, ArH), 8.91 (1H, d, J = 8.0, ArH); ^{13}C NMR: 13.9, 14.5, 32.5, 37.5, 104.5, 109.7, 113.1, 116.4, 117.8, 118.2, 119.0, 120.4, 124.4, 125.2, 129.7, 129.8, 137.1, 141.3, 142.6, 144.7, 149.5; MS, *m/z*: 339 (M^+). Calcd. for $C_{21}H_{17}N_5$ (339.4): C, 74.32; H, 5.05; N, 20.63. Found: C, 73.97; H, 5.01; N, 20.45.

7-Methyl-3-propyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIIe). mp 305–307°C (lit. [11] mp 303–305°C); ^{1}H NMR: 1.03 (3H, t, J = 7.2, $CH_2CH_2CH_3$), 1.98–2.08 (2H, m, $CH_2CH_2CH_3$), 4.09 (3H, s, CH_3), 4.35 (2H, t, J = 7.2, $CH_2CH_2CH_3$), 7.44 (1H, td, J = 8.0, 0.8, ArH), 7.53 (1H, d, J = 8.4, ArH), 7.73 (1H, td, J = 8.0, 1.2, ArH), 7.87 (1H, d, J = 9.2, ArH), 8.12 (1H, d, J = 9.2, ArH), 8.23 (1H, s, ArH), 8.92 (1H, d, J = 8.0, ArH).

7-Ethyl-3-propyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIIf). mp 304–306°C (lit. [11] mp 303–306°C); ^{1}H NMR: 1.05 (3H, t, J = 7.2, $CH_2CH_2CH_3$), 1.57 (3H, t, J = 7.2, CH_2CH_3), 2.00–2.09 (2H, m, J = 7.2, $CH_2CH_2CH_3$), 4.35 (2H, t, J = 7.2, $CH_2CH_2CH_3$), 4.69 (2H, q, J = 7.2, CH_2CH_3), 7.43 (1H, td, J = 8.0, 0.8, ArH), 7.55 (1H, d, J = 8.4, ArH), 7.72 (1H, td, J = 8.0, 1.2, ArH), 7.87 (1H, d, J = 9.2, ArH), 8.11 (1H, d, J = 9.2, ArH), 8.21 (1H, s, ArH), 8.95 (1H, d, J = 8.0, ArH).

3-Butyl-7-methyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIIg) was obtained as shiny yellow needles (EtOH), yield 64%, mp 296–298°C; IR: 2225 (CN). ^{1}H NMR: 1.01 (3H, t, J = 7.0, $CH_2CH_2CH_2CH_3$), 1.45 (2H, m, $CH_2CH_2CH_2CH_3$), 1.99 (2H, m, $CH_2CH_2CH_2CH_3$), 4.09 (3H, s, CH_3), 4.37 (2H, t, J = 7.2, $CH_2CH_2CH_2CH_3$), 7.43 (1H, td, J = 8.0, 0.8, ArH), 7.52 (1H, d, J = 8.4, ArH), 7.72 (1H, td, J = 8.0, 1.2, ArH), 7.86 (1H, d, J = 9.2, ArH), 8.10 (1H, d, J = 9.2, ArH), 8.19 (1H, s, ArH), 8.91 (1H, d, J = 8.0, ArH); ^{13}C NMR: 13.5, 20.0, 33.5, 35.2, 42.7, 104.1, 109.4, 114.0, 115.6, 117.7, 118.7, 119.1, 120.4, 124.3, 125.3, 129.5, 129.7, 138.2, 141.5, 142.4, 144.2, 149.5; MS, *m/z*: 353 (M^+). Calcd. for $C_{22}H_{19}N_5$ (353.4): C, 74.77; H, 5.42; N, 19.82. Found: C, 74.37; H, 5.36; N, 19.50.

3-Butyl-7-ethyl-3,7-dihydroimidazo[4,5-*a*]quinindoline-12-carbonitrile (IIIh) was obtained as shiny yellow needles (EtOH), yield 65%, mp 286–289°C; IR: 2225 (CN). ^{1}H NMR: 1.01 (3H, t, J = 7.0, $CH_2CH_2CH_2CH_3$), 1.43 (2H, m, $CH_2CH_2CH_2CH_3$), 1.59 (3H, t, J = 7.2, CH_2CH_3), 1.98 (2H, m, $CH_2CH_2CH_2CH_3$), 4.37 (2H, t, J = 7.2, $CH_2CH_2CH_2CH_3$), 4.67 (2H, q, J = 7.2, CH_2CH_3), 7.44 (1H, td, J = 8.0, 0.8, ArH), 7.53 (1H, d, J = 8.4, ArH), 7.72 (1H, td, J = 8.0, 1.2, ArH), 7.85 (1H, d, J = 9.2, ArH), 8.11 (1H, d, J = 9.2, ArH), 8.18 (1H, s, ArH), 8.90 (1H, d, J = 8.0, ArH); ^{13}C NMR: 13.4, 13.7, 20.0, 31.2, 33.6, 42.5, 104.3, 109.6, 114.2, 115.2, 117.4, 118.7, 119.1, 120.5, 124.6, 125.7, 129.2, 129.7, 138.4, 141.6, 142.4, 144.2, 149.4; MS, *m/z*: 367 (M^+). Calcd. for $C_{23}H_{21}N_5$ (367.4): C, 75.18; H, 5.76; N, 19.06. Found: C, 74.89; H, 5.72; N, 18.84.

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