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Synthesis and Biological Evaluation of Novel Isoxazolo[4,3-*e*]indoles as Antibacterial Agents[1]

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Abstract—The synthesis of a new series of 8-bromo-6-alkyl-1-aryl-6*H*-isoxazolo[4,3-e]indole derivatives is described. All the newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* HB101, *Staphylococcus aureus* pathogens (methicillin resistant *S. aureus* and methicillin susceptible *S. aureus), Pseudomonas aeruginosa*, and *Bacillus subtilis*; also MIC values of these compounds were determined.

Keywords: isoxazolo[4,3-e]indole, antibacterial agents, Staphylococcus aureus, MIC

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INTRODUCTION

Indoles are a pervasive class of compounds found in abundance in biologically active compounds such as pharmaceuticals [1, 2], agrochemicals [3] and alkaloids [4]. Indole myriad derivatives have, therefore, captured the attention of organic synthetic chemists. On the other hand, isoxazoles have found continuing application in medicinal chemistry, several examples of which have advanced to general medical practice [5]. As potential new chemical entities advance from in vitro screening through in vivo study towards clinical trials, pharmacokinetic properties collectively referred to as ADMET for Absorption, Distribution, Metabolism, Excretion and Toxicity [6, 7] become important considerations: half of drug attrition can be attributed to poor ADMET properties. An interesting facet of the biology of isoxazoles is that one significant route of metabolism and excretion is C-5 methyl hydroxylation [8, 9], mediated by the cytochrome P450 isoform 3A4 [10], often followed by conjugation to the glucuronide [11]. The existence of a safe route for drug metabolism is an important design feature for potential investigational new drugs containing the isoxazole. Combination of the isoxazole moiety with the indole nucleus may enhance these activities [12, 13].

Based on these facts and in continuation of research work on the synthesis of bioactive heterocycles [14–17] and evaluation of their biological activities, we decided to examine the transformation of 3-bromo-1-alkyl-5-nitro-1*H*-indoles to new heterocyclic system isoxazolo[4,3-*e*]indole by the reaction of nucleophilic hydrogen substitution, which may result in interesting biological activities.

RESULTS AND DISCUSSION

The target compounds 8-bromo-6-alkyl-1-aryl-6*H*-isoxazolo[4,3-*e*]indole derivatives (**3a**–**h**) were obtained as described in Scheme. The required starting materials 3-bromo-1-alkyl-5-nitro-1*H*-indoles (**1a**–**d**) were prepared by reaction of 5-nitro-1*H*-indole with bromine [18] and then different alkyl halides in DMF and KOH [19] using literature methods. The reaction of (**1a**–**d**) with arylacetonitriles (**2a,b**) led to the formation of the new 8-bromo-6-alkyl-1-aryl-6*H*-isoxazolo[4,3-*e*]indole (**3a**–**h**) *via* the nucleophilic substitution of hydrogen in basic EtOH solution in moderate yields (*cf.* [20–23]) (Scheme). The yield of the reaction was very low when instead of 3-bromo-1-alkyl-5-nitro-1*H*-indoles (**1a**–**d**), 1-alkyl-5-nitro-1*H*-indoles was used in this reaction. This can be due to the presence of bromine in the indole ring of compounds (**1a**–**d**) which are more electron-deficient than in 1-alkyl-5-nitro-1*H*-indoles.

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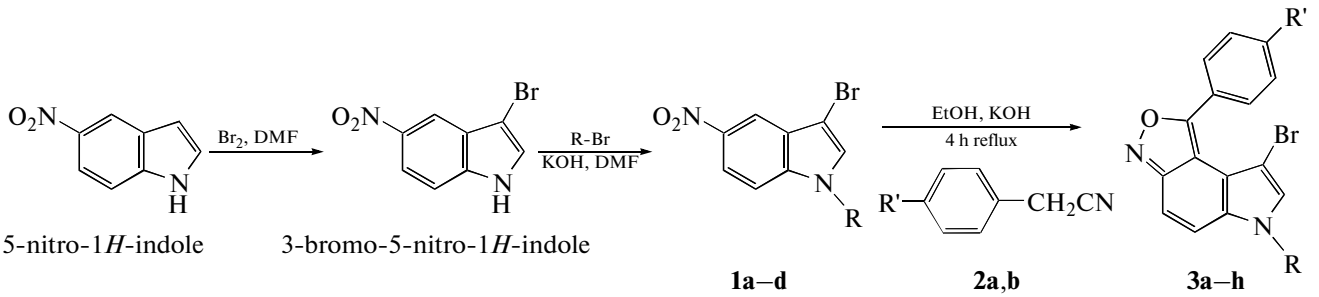
Antibacterial activity (MIC, μg mL^{-1}) of compounds **3a**–**h** against methicillin-resistant (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA)

Compd.	MRSA	MSSA	Compd.	MRSA	MSSA
(**3a**)	22.0	15.2	(**3f**)	10.0	10.0
(**3b**)	15.7	10.0	(**3g**)	4.3	4.3
(**3c**)	10.0	10.0	(**3h**)	5.0	5.0
(**3d**)	17.5	10.6	Erythromycin	32.0	32.0
(**3e**)	15.3	15.3	Cephalexin	72.0	4.6



3a: R = Et, R' = Cl (49%), **3c**: R = Bu, R' = Cl (43%), **3e**: R = Et, R' = Me (51%), **3g**: R = Bu, R' = Me (55%), **3b**: R = Et, R' = Cl (45%), **3d**: R = iso-Bu, R' = Cl (55%), **3f**: R = Pr, R' = Me (50%), **3h**: R = iso-Bu, R' = Me (60%)

Scheme.

The structural assignments of compounds (**3a**–**h**) were based on the analytical and spectral data. For example, in the ^1H NMR spectrum of (**3a**), there are the doublet signals at δ 7.40 (J = 9.6 Hz, 1 H), δ 7.47 (J = 9.6 Hz, 1 H), δ 7.57 (J = 8.5 Hz, 2 H), δ 8.85 (J = 8.5 Hz, 2 H) ppm and singlet signal at δ 7.85 ppm attributed to seven protons of aromatic rings. Moreover, the ^{13}C NMR spectrum, molecular ion peak at m/z 379 (M^+ + 4) and microanalytical data strongly support the tricyclic structure of compound (**3a**).

ANTIMICROBIAL ACTIVITY

The test compounds listed in table were screened for the antibacterial activity against *Escherichia coli* HB101 (BA-7601C), *Staphylococcuse aureus* pathogens [methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA—ATCC 1112)], *Pseudomonas aeruginosa* (PTCC 1431), and *Bacillus subtilis* (PTCC 1365).

The Minimum inhibitory concentrations (MIC) of (**3a**–**h**) were determined in dilution test tube method, which had been introduced by NCCLS (National Committee for Clinical Laboratory Standards) [24]. For broth dilution methods, in which decreasing concentrations of the antimicrobial agents must be tested, usually prepared in serial two fold dilution of a broth medium is placed in tubes which will support the growth of the test microorganism (10^4 CFU mL^{-1}). After sufficient incubation (18 h), the tubes are examined for turbidity, indicating growth of the microorganism. The organism will grow in the tube that does not contain enough antimicrobial agents to inhibit growth. For further confidence, the samples were cultured onto Petri dishes containing Muller–Hinton agar (18 h at 37°C). 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 designated the minimum inhibitory concentration (MIC). A serial dilution of tested compounds (final concentration of 400 to 0.4 μg mL^{-1}), were added to the test bacteria in Mueller–Hinton broth and were incubated at 37°C for 18 h. Growth was presented in the medium control and was absent from the inoculum control [25].

The result indicates that these compounds are only effective against gram positive bacteria. It has been observed from the table, all compounds were found to exhibit considerable antibacterial activities against the mentioned organisms. These results are compared with MIC values of Cephalexin (72 and 4.6 μg mL^{-1} and Erythromycin (32 and 32 μg mL^{-1}). As the data in table display, compound **3g** shows the best inhibitory activity against methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA—ATCC 1112).

CONCLUSIONS

To summarize, we have synthesized some novel derivatives of isoxazolo[4,3-*e*]indole and shown them to be very effective *S. aureus* growth inhibitors. Such

compounds would appear to offer a suitable template for the design of more powerful antibacterial agents and further studies are under way to this end in our laboratory.

EXPERIMENTAL

Melting points were measured on an Electrothermaltype-9100 melting-point apparatus. ^{1}H and ^{13}C NMR spectra (δ, ppm, *J*, Hz) were recorded on a Bruker Avance DRX-400 FT spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C using $CDCl_3$ as solvent. The ^{1}H and ^{13}C NMR chemical shifts were referenced to tetramethylsilane (TMS) as internal standard. 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.

The microorganisms *S. aureus* ATCC 1112 were purchased from Pasteur Institute of Iran and *S. aureus* (methicillin resistant) was isolated from different specimens which were referred to the Microbiological Laboratory of Ghaem Hospital of Medical University of Mashhad-Iran and its methicillin resistance was tested according to the NCCLS guidelines [24].

*General Procedure for the Synthesis of (**3a**–**h**) from (**1a**–**d**) and (**2a**,**b**)*

Compounds (**1a**–**d**) (10 mmol) and (**2a**,**b**) (12 mmol) were added with stirring to a solution of KOH (20 g, 357 mmol) in ethanol (80 mL). The mixture was refluxed with stirring for 4 h, and then poured into water. The precipitate was collected by filtration, washed with water and air-dried to give crude (**3a**–**h**). More purification was achieved by crystallization from suitable solvent such as n-hexane-ethyl-acetate, MeOH or EtOH.

8-Bromo-1-(4-chlorophenyl)-6-ethyl-6*H*-isoxazolo[4,3-*e*]indole (3a) was obtained as pale yellow crystals (EtOH), yield (49%), mp: 151–153°C; ^{1}H NMR ($CDCl_3$) δ 1.57 (3 H, t, *J* = 7.3), 4.27 (2 H, q, *J* = 7.3), 7.40 (1 H, d, *J* = 9.6), 7.47 (1 H, d, *J* = 9.6), 7.57 (2 H, d, *J* = 8.5), 7.85 (1 H, s), 8.85 (2 H, d, *J* = 8.5); 13C NMR ($CDCl_3$): δ 15.25, 43.56, 93.89, 112.24, 124.33, 127.45, 131.02, 132.87, 132.95, 133.23, 145.87, 148.55, 158.83, 160.19, 164.17; MS (*m/z*) 379 (*M* + 4); Anal. calcd. for $C_{17}H_{12}BrClN_2O$ (375.6): C, 54.36; H, 3.22; N, 7.46. Found: C, 54.21; H, 3.18; N, 7.58.

8-Bromo-1-(4-chlorophenyl)-6-propyl-6*H*-isoxazolo[4,3-*e*]indole (3b) was obtained as pale yellow needles (MeOH), yield (45%), mp: 141–143°C; ^{1}H NMR ($CDCl_3$) δ 0.98 (3 H, t, *J* = 7.3), 1.95–1.87 (2 H, m), 4.21 (2 H, t, *J* = 7.3), 7.41 (1 H, d, *J* = 9.6), 7.46 (1 H, d, *J* = 9.6), 7.56 (2 H, d, *J* = 8.5), 7.85 (1 H, s), 8.85 (2 H, d, *J* = 8.5) ppm; ^{13}C NMR ($CDCl_3$): δ 11.23, 27.09, 44.59, 93.75, 112.23, 124.56, 127.56, 131.08, 132.90, 132.96, 133.23, 145.89, 148.63, 158.91, 160.23, 164.15; MS (*m/z*) 393 (*M* + 4); Anal. calcd. for $C_{18}H_{14}BrClN_2O$ (389.7): C, 55.48; H, 3.62; N, 7.19. Found: C, 55.65; H, 3.69; N, 7.02.

8-Bromo-6-butyl-1-(4-chlorophenyl)-6*H*-isoxazolo[4,3-*e*]indole (3c) was obtained as yellow needles (MeOH), yield (43%), mp: 154–156°C; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, *J* = 7.2), 1.33–1.42 (2 H, m), 1.84–1.91 (2 H, m), 4.21 (2 H, t, *J* = 7.2), 7.41 (1 H, d, *J* = 9.6), 7.46 (1 H, d, *J* = 9.6), 7.56 (2 H, d, *J* = 8.5), 7.85 (1 H, s), 8.85 (2 H, d, *J* = 8.5); ^{13}C NMR ($CDCl_3$): δ 13.23, 20.95, 34.45, 49.56, 94.00, 112.25, 124.57, 127.56, 131.09, 132.92, 132.95, 133.23, 145.87, 148.67, 158.95, 160.20, 164.09; MS (*m/z*) 407 (*M* + 4); Anal. calcd. for $C_{19}H_{16}BrClN_2O$ (403.7): C, 56.53; H, 3.99; N, 6.94. Found: C, 56.43; H, 3.90; N, 7.06.

8-Bromo-6-isobutyl-1-(4-chlorophenyl)-6*H*-isoxazolo[4,3-*e*]indole (3d) was obtained as pale yellow needles (n-hexane-ethyl-acetate), yield (55%), mp: 146–148°C; ^{1}H NMR ($CDCl_3$) δ 0.98 (6 H, d, *J* = 6.5), 2.23–2.16 (1 H, m), 4.01 (2 H, d, *J* = 7.2), 7.42 (1 H, d, *J* = 9.6), 7.47 (1 H, d, *J* = 9.6), 7.57 (2 H, d, *J* = 8.5), 7.85 (1 H, s), 8.85 (2 H, d, *J* = 8.5); ^{13}C NMR ($CDCl_3$): δ 20.65, 26.16, 51.89, 93.86, 112.21, 124.54, 127.55, 131.07, 132.90, 132.95, 133.25, 145.86, 148.45, 158.96, 160.21, 164.07; MS (*m/z*) 407 (*M* + 4); Anal. calcd. for $C_{19}H_{16}BrClN_2O$ (403.7): C, 56.53; H, 3.99; N, 6.94. Found: C, 56.41; H, 3.93; N, 7.05.

8-Bromo-6-ethyl-1-(4-methylphenyl)-6*H*-isoxazolo[4,3-*e*]indole (3e) was obtained as pale yellow needles (EtOH), yield (51%), mp: 151–153°C; ^{1}H NMR ($CDCl_3$) δ 1.56 (3 H, t, *J* = 7.3), 2.44 (3 H, s), 4.25 (2 H, q, *J* = 7.3), 7.41 (2 H, d, *J* = 8.0), 7.43 (1 H, d, *J* = 9.5), 7.47 (1 H, d, *J* = 9.5), 7.86 (1 H, s), 8.77 (2 H, d, *J* = 8.0); ^{13}C NMR ($CDCl_3$): δ 15.25, 21.33, 42.13, 93.95, 112.22, 124.89, 127.12, 127.76, 129.87, 129.95, 136.11, 145.45, 148.98, 158.33, 160.09, 165.19; MS (*m/z*) 357 (*M* + 2); Anal. calcd. for $C_{18}H_{15}BrN_2O$ (355.2): C, 60.86; H, 4.26; N, 7.89. Found: C, 60.71; H, 4.20; N, 8.00.

8-Bromo-1-(4-methylphenyl)-6-propyl-6*H*-isoxazolo[4,3-*e*]indole (3f) was obtained as pale yellow needles (MeOH), yield (50%), mp: 146–148°C; ^{1}H NMR ($CDCl_3$) δ 0.99 (3 H, t, *J* = 7.3), 1.89–1.98 (2 H, m), 2.45 (3 H, s), 4.19 (2 H, t, *J* = 7.3), 7.41 (2 H, d, *J* = 8.0), 7.44 (1 H, d, *J* = 9.5), 7.48 (1 H, d, *J* = 9.5), 7.87 (1 H, s), 8.76 (2 H, d, *J* = 8.0); ^{13}C NMR ($CDCl_3$): δ 11.22, 21.32, 27.07, 44.57, 94.12, 112.13, 124.91, 127.14, 127.76, 129.85, 129.96, 136.10, 145.47, 149.00, 158.31, 160.09, 165.17; MS (*m/z*) 371 (*M* + 2); Anal. calcd. for $C_{19}H_{17}BrN_2O$ (369.3): C, 61.80; H, 4.64; N, 7.59. Found: C, 61.94; H, 4.69; N, 7.44.

8-Bromo-6-butyl-1-(4-methylphenyl)-6*H*-isoxazolo[4,3-*e*]indole (3g) was obtained as yellow needles (n-hexane-ethyl-acetate), yield (55%), mp: 136–139°C; ^{1}H NMR ($CDCl_3$) δ 0.97 (3 H, t, *J* = 7.2), 1.35–1.45 (2 H, m), 1.84–1.91 (2 H, m), 2.45 (3 H, s), 4.21 (2 H, t, *J* = 7.2), 7.42 (2 H, d, *J* = 8.0), 7.45 (1 H, d, *J* = 9.5), 7.48 (1 H, d, *J* = 9.5), 7.86 (1 H, s), 8.77 (2 H, d, *J* =

8.0); ^{13}C NMR ($CDCl_3$): δ 13.19, 20.81, 21. 33, 34.48, 49.57, 94.11, 112.12, 124.91, 127.19, 127.75, 129.86, 129.97, 136.10, 145.49, 149.04, 158.39, 160.12, 165.16; MS (*m/z*) 385 (*M* + 2); Anal. calcd. for $C_{20}H_{19}BrN_2O$ (383.3): C, 62.67; H, 5.00; N, 7.31. Found: C, 62.49; H, 4.91; N, 7.50.

8-Bromo-6-isobutyl-1-(4-methylphenyl)-6*H*-isoxazolo[4,3-*e*]indole (3h) was obtained as pale yellow needles (MeOH), yield (60%), mp: 156–158°C; ^{1}H NMR ($CDCl_3$) δ .97 (6 H, d, *J* = 6.7), 2.21–2.14 (1 H, m), 2.43 (3 H, s), 4.00 (2 H, d, *J* = 7.2), 7.41 (2 H, d, *J* = 8.0), 7.45 (1 H, d, *J* = 9.5), 7.47 (1 H, d, *J* = 9.5), 7.87 (1 H, s), 8.76 (2 H, d, *J* = 8.0); ^{13}C NMR ($CDCl_3$): δ 20.65, 21.35, 26.16, 51.89, 94.08, 112.11, 124.90, 127.21, 127.80, 129.86, 129.99, 136.11, 145.51, 149.02, 158.39, 160.14, 165.14; MS (*m/z*) 385 (*M* + 2); Anal. calcd. for $C_{20}H_{19}BrN_2O$ (383.3): C, 62.67; H, 5.00; N, 7.31. Found: C, 62.43; H, 4.93; N, 7.59.

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