In vitro Antimicrobial Activity of Some Synthetic Indolium Chloride Derivatives

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(Received: September 22, 2020; Accepted: November 15, 2020; Published (web): December 10, 2020)

ABSTRACT: A total of eight compounds including four starting materials and four indolium chloride derivatives were tested against Gram positive and Gram negative bacterial and fungal strains. Among them, *N*-acetyl-2-acetylindoliumchloride (**8**) exhibited significant antimicrobial activity at a concentration of 200 µg/disc, while the *N*-acetyl-2-benzoylindoliumchloride (**9**) showed moderate activity at 400 µg/disc. At the same time, *N*-acetyl-2-anisoylindoliumchloride (**10**) and *N*-acetyl-2-tolylindoliumchloride (**11**) demonstrated weak antimicrobial activity.

Key words: *N*-acetyl-2-acetylindoliumchloride, antimicrobial assay, disc diffusion.

INTRODUCTION

In many developed countries, most antibiotics are expensive and inaccessible for a significant percentage of the patients. Antibiotic resistance further compromises efficacy of the treatment. Many bacterial strains, including those that cause common infections of the skin, throat, urinary tract and lungs, are becoming immune to the widely available antibiotics leading to increased difficulty for treatment, misery and death.[1-7] Therefore, the production of new synthetic antimicrobial agents is a demand for time.

One of the biggest useful resources of novel compounds with diverse biological activities is heterocyclic chemistry, primarily due to the unusual ability of the resulting compounds to imitate the peptide structure and to bind reversibly to proteins.[8-11] In the drug discovery studies, indole derivatives are very useful heterocyclic compounds. They represent an influential class of molecules which play major roles in the biology of cells and are potential products that occur naturally. The use of indole derivatives as bioactive molecules against bacteria, cancer cells and various types of disorders in the human body has been becoming increasingly significant.[12]

In the current investigation, some derivatives of indolium chloride (**8**-**11**) (Figure 1) were screened for antimicrobial activity against some pathogenic microorganisms. The objective of the current study was both to investigate their impact on the pathogens tested and to identify the lead compounds with strong antimicrobial activity.

MATERIALS AND METHODS

General experimental procedures. Melting points were measured with a Gallenkamp (England) melting point apparatus in open capillary tubes. FT-IR spectra were recorded on a Shimadzu FTIR spectrophotometer. The UV spectra were obtained with a Shimadzu visible spectrophotometer in dry ethanol. The ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were acquired on a Bruker DPX-400 spectrophotometer and the chemical shifts are reported with respect to the signal of the internal standard, tetramethylsilane (TMS). Column chromatography and TLC were conducted over silica gel (60-120 mesh) and pre-coated silica gel 60F_{254} (E.

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Dhaka Univ. J. Pharm. Sci. **19**(2): 133-137, 2020 (December)

DOI: https://doi.org/10.3329/dujps.v19i2.50628

Merck), respectively and the spots on TLC plates were visualized with UV light. On a Perkin-Elmer 240C Analyser, elemental analyses (C, H, N) were performed. Acrylic esters, $(Ph_3P)_2PdCl_2$, and other reagents were procured from Fluka (Switzerland) and E. Merck (Germany), respectively.

Synthesis of indolium chloride derivatives. The compounds used in the current study were synthesized as stated by the methods shown in Scheme 1.

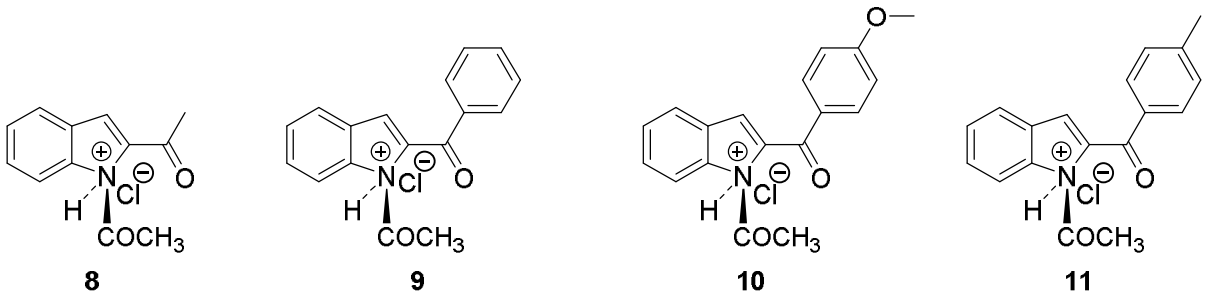
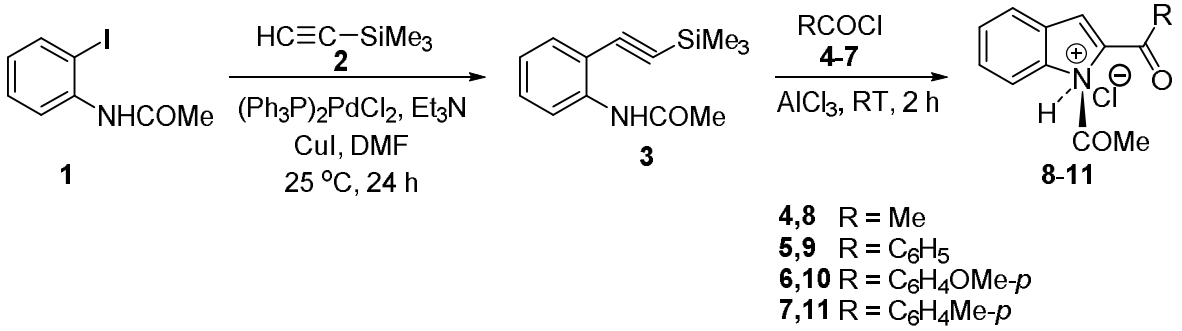


Figure 1. The synthesised indolium chloride derivatives.



Scheme 1. Synthesis of indolium chloride derivatives.

Synthesis of *N*-acetyl-2-substituted indolium chloride (8-11). N-acetyl-2-substituted indolium chloride (**8-11**) were prepared from 2-iodoacetanilide through palladium-catalysed reaction using trimethylsilyl acetylene followed by Friedel-Craft acylation of the resulting 2-(trimethylsilyl)-ethnylacetanilide with acyl chloride or acetic anhydride as shown in Scheme 1.

2-Iodoacetanilide (**1**) was easily reacted with trimethylsilyl acetylene (**2**) in *N,N*-dimethylform-amide (DMF) in presence of *bis*(triphenylphosphine) palladium (II) chloride (3.5 mol percent), trimethylamine (4 equiv), and copper(I) iodide (8 mol percent) at room temperature for 24 hours under the nitrogen atmosphere, giving 2-(trimethylsilyl)ethyn-ylacetanilide (**3**). The latter acetanilide was then acylated at 0-25 ^oC for 2 hours by Friedel-Craft reaction, stirring a mixture of **3**, anhydrous $AlCl_3$ (3 equiv) and acyl chloride (1 quiv) in tetrachloroethane to yield 2-acyl indole derivatives **8-11**.

2-Iodoacetanilide (**1**) was synthesised from 2-iodoanilines by acetylation reaction for 3 hours with $(CH_3CO)_2O/CH_3COOH/Zn$ at 80 ^oC and 2-iodoanilines was prepared from aniline using $KI/KIO_3/H^+$ in methanol by following a published method.[13]

2-Iodoacetanilide (1). White crystalline solid, mp 108-109 ^oC; R_f value 0.36 (n-hexane); IR (KBr): ν_{max} 3273.0 (NH), 1660.6 (C=O), 1573.8, 1529.4, 1463.9, 14433.0, 1411.8, 1292.2, 1253.6, 1014.5, 750.3, 663.5 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H, Ar-H), 7.75 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.42 (br. s, 1H, NH), 7.31 (t, 1H, *J* = 8 Hz, Ar-H), 6.82 (t, 1H, *J* = 8.0 Hz, Ar-H), 2.20 (s, 3H, $COCH_3$).

2-(Trimethylsilyl)ethynylacetanilides(3). Crystalline solid; mp 94-95 ^{o}C; UV (EtOH): λ_{max} = 296.2, 250.8 nm; IR (KBr): ν_{max} 3327 (NH), 2158 (C≡C), 1695 (C=O), 1672,1576,1516, 1444 cm^{-1}; ^{1}H NMR (400 MHz $CDCl_3$): δ 8.39 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.99 (br.s, 1H, NH), 7.41 (dd, *J* = 8.0, 8.3 Hz, 1H, Ar-H), 7.32 (dd, *J* = 8.0, 8.3 Hz, 1H, Ar-H), 7.01 (d, *J* = 8.0 Hz, 1H, Ar-H), 2.21 (s, 3H, $COCH_3$), 0.30 (s, 9H, $SiMe_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.0 (CO), 139.5, 131.4, 129.9, 123.10, 118.9, 111.5, 102.2, 100.2, 24.7 (CH_3), 0.10 ($SiMe_3$); Anal. calcd. for $C_{13}H_{17}SiNO$: C, 67.48; H, 7.40; N, 6.05. Found: C, 67.09; H, 7.43; N, 6.03.

N-Acetyl-2-acetyl indolium chloride (8). Crystalline solid, mp 84-86 ^{o}C; UV (EtOH): λ_{max} 334, 305.4, 259.8, 238.4 nm; IR (KBr): ν_{max} 3221(NH), 1684 (C=O), 1652, 1608, 1576, 1560, 1502, 1130 cm^{-1}; ^{1}H NMR (400 MHz $CDCl_3$): δ 11.06 (br.s, 1H, NH), 8.59 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.65 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.46 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.05 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.10 (s, 1H, H-3), 2.18 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.31 (C=O), 185.31 (C=O), 168.89, 140.152, 135.85, 133.83, 129.06, 122.41, 121.02, 98.02, 25.40 ($CO\underline{C}H_3$), 23.15 ($CO\underline{C}H_3$).

***N*-Acetyl-2-benzoyl-1H-indolium chloride (9).** White crystalline solid, mp 63-64 ^{o}C;; IR (KBr): ν_{max} 3274.9 (NH), 3076.2 (C-H), 1703.0 (C=O), 1575.7, 1529.7 (C=C) and 1463.9, 1380.9, 1276.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.08 (br. s, 1H, NH), 8.64 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.94 (d, 2H, *J* = 7.2 Hz, Ar-H)), 7.82 (d,1H, *J* = 8.0 Hz, Ar-H) 7.52 (m, 4H, Ar-H), 7.13 (t, 1H, Ar-H), 6.79 (s, 1H, H-3), 2.23 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.70 (C=O), 179.53 (C=O), 168.98, 140.11, 133.99, 133.84, 132.58, 129.15, 128.82, 126.91, 123.12, 122.79, 121.51, 94.99, 25.49 ($COCH_3$).

***N*-Acetyl-2-anisoyl indolium chloride (10).** Light yellow crystal, mp. 112-113 ^{o}C; UV (EtOH): λ_{max} 380.80, 360.6, 238.0 nm; IR (KBr): ν_{max} 3327.9 (NH), 1683.7 (C=O), 1610, 1576.7, 1500, 1413, 1360, 1175 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.04 (br. s, 1H, NH), 8.62 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.91 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.77 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.51 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.12 (d, 1H, *J* = 7.2 Hz, Ar-H), 6.97 (d, 2H, *J* = 8 Hz, Ar-H), 6.70 (s, 1H, H-3), 3.88 (s, 3H, Ar-OCH_3), 2.22 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.46 (C=O), 180 (C=O), 168.93, 163.41, 139.83, 133.60, 131.17, 128.99, 126.14, 123.38, 122.75, 121.46, 114.09, 94.00, 55.53 (OCH_3) 25.48 ($COCH_3$). Anal. calcd for $C_{18}H_{16}ClNO_3$: C, 65.56; H, 4.89; N, 4.25. Found: C, 65.87; H, 4.75; N, 4.52.

***N*-Acetyl-2-toluoyl indolium chloride (11).** Amorphous solid, mp. 64-65 ^{o}C. UV (EtOH): λ_{max} 361.4, 256.6 nm; IR (KBr): ν_{max} 3325 (NH), 1687(C=O), 1569, 1508, 1446, 1423, 1365, 1190, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.07 (br. s, 1H, NH), 8.63 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.83 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.78 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.49 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.27 (d, 2H, *J* = 7.2, Ar-H), 7.11 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.74 (s, 1H, H-3), 2.41 (s, 3H, $COCH_3$), 2.21 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.92 (C=O), 179.85 (C=O), 168.88, 143.45, 139.97, 133.74, 131.71, 128.83, 126.16, 123.16, 122.68, 121.12, 120.81, 94.48, 25.48 ($COCH_3$), 21.62 (Ar-CH_3). Anal. calcd for $C_{18}H_{16}ClNO_2$: C, 68.90; H, 5.14; N, 4.46. Found: C, 69.20; H, 5.40; N, 4.77.

Antimicrobial screening. Disc diffusion method[14] was used to assess the antimicrobial activity of the test compounds, and the results observed are listed in Table 1. The samples were dissolved in chloroform/methanol at a ratio of 1:1 separately and applied at the concentration of 200- and 400-μg/disc to sterile filter paper disks. For antibacterial and antifungal assay kanamycin (30 μg/disc) and griseofulvin (25 μg/disc), respectively were used as positive controls. The standard test microorganisms were collected from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh.

RESULTS AND DISCUSSION

For antimicrobial activity screening a total of four *N*-acetyl-2-substituted indolium chloride (**8**-**11**) have been tested against five Gram positive, eight Gram negative bacteria and three fungi. It is clear

from the antimicrobial screening (Table 1) that very prominent antimicrobial activity was revealed by *N*-acetyl-2-acetylindoliumchloride (**8**). *N*-acetyl-2-benzoylindoliumchloride (**9**), on the other hand, demonstrated mild to moderate activity, while *N*-acetyl-2-anisoylindoliumchloride (**10**) and N-acetyl-2-tolylindoliumchloride (**11**) displayed mild inhibition of microbial growth.

Table 1. Antimicrobial activity of compounds 8-11.

Test microorganisms	Diameter of zone of inhibition (mm)					
	8		**9**	**10**	**11**	Kanamycin (30 µg/disc)/ Griseofulvin (25 µg/disc)
	200 µg/disc	400 µg/disc	400 µg/disc	400 µg/disc	400 µg/disc	
Gram positive bacteria						
Bacillus cereus (BTCC-19)	16.4	18.2	10.5	9.4	8.6	35.1
B. megaterium (BTCC-18)	21.9	22.0	13.2	8.9	9.9	37.8
B. subtilis (QL 40)	14.4	14.5	9.9	8.4	8.3	33.4
Staphylococcus aureus (BTCC-43)	15.4	17.0	11.4	9.6	9.1	36.1
Sarcina lutea (ATCC-9341)	15.7	21.0	11.9	7.4	8.7	32.2
Gram negative bacteria						
Salmonella paratyphi (AM 16590)	16.1	14.5	12.4	9.9	10.2	32.9
S. typhi (AM 16406)	16.2	30.1	11.9	9.6	9.7	33.6
Vibrio parahemolyticus (AM 16362)	18.1	21.0	12.3	9.8	9.7	37.5
V. mimicus (N 1967)	14.5	17.0	12.5	9.5	10.2	36.9
Escherichia coli (BTCC-172)	14.8	20.0	9.9	8.7	9.4	34.7
Shigella dysenteriae (ATCC 26131)	17.4	16.5	11.8	9.2	9.8	36.3
S. boydii (ATCC 13147)	15.7	21.6	11.9	7.4	8.7	35.5
Pseudomonas aeruginosa	14.2	19.5	10.1	8.4	9.2	36.9
Fungi						
Saccharomyces cerevisiae	13.7	17.0	11.5	8.8	9.4	31.3
Candida albicans	17.7	17.0	11.7	9.6	10.1	37.2
Aspergillus niger	16.9	20.0	12.4	8.6	9.8	33.3

Diffusion time- 23 hours; Solvent used to dissolve the samples- $CHCl_3$/MeOH-1:1

In the screening, compound **8** exhibited maximum zone of inhibition. This compound was tested for twice at the concentration of 200- and 400-µg/disc and the activity in term of zone of inhibition was compared with the standard drugs, kanamycin and griseofulvin. Among the five Gram positive bacteria, the antimicrobial activity of compound **8** against *S. lutea* was highest with mean zone of inhibition of 21 mm and for Gram negative bacteria and the highest zone of inhibition was found for *S. typhi* (30 mm). In case of antifungal activity, *A. niger* was found to be most sensitive (20 mm) to the test compound **8** than the other fungal strains.

Compound **9** revealed the second-highest antimicrobial activity. It exhibited significant activity against *B. megaterium*, *V. parahemolyticus*, *V. mimicus* and *A. niger*. During antimicrobial screening against 16 bacteria and 3 fungi, compounds **10** and **11** displayed weak inhibitory zone ranging from 7.4 to 10.2 mm, where they showed maximum activity against *S. paratyphi* and *V. mimicus*, respectively. The antimicrobial activity of intermediate **3** has also been screened but it revealed no activity.

CONFLICT OF INTEREST

No conflict of interest is declared by the authors.

ACKNOWLEDGEMENTS

The research work was conducted by the financial support from Bangladesh University of Engineering and Technology (BUET), Dhaka, Bangladesh, the authors would like to thank the Department of Pharmaceutical Chemistry, Dhaka University, Bangladesh, for providing laboratory facility to conduct the antimicrobial screening of the tested compounds.

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