European Journal of Chemistry

Journal webpage: www.eurjchem.com

Synthesis and antimicrobial activity of some novel *N*-substituted benzimidazoles

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ARTICLE INFORMATION

DOI: 10.5155/eurjchem.8.2.149-154.1563

Received: 08 March 2017
Received in revised form: 08 April 2017
Accepted: 09 April 2017
Published online: 30 June 2017
Printed: 30 June 2017

KEYWORDS

Alkylation
Benzoylation
Benzimidazole
Antifungal activity
Antibacterial activity
Condensation reaction

ABSTRACT

Synthesis of a series of new substituted benzimidazole derivatives by the condensation of *o*-phenylenediamine with urea to give 1,3-dihydro-benzimidazol-2-one which reacted with phosphoryl chloride to give 2-chloro-1*H*-benzimidazole is reported. The product was then alkylated at the benzimidazole NH with different electrophilic reagents leading to functionalized derivatives. Structures of the newly synthesized products have been deduced on the basis of spectral and analytical data. The synthesized compounds were screened for their antimicrobial activity. This exhibited some promising results towards testing organism *in-vitro*.

Cite this: *Eur. J. Chem.* **2017**, *8(2)*, 149-154

1. Introduction

Benzimidazoles and their analogs are well known biologically active N-containing heterocycles [1], widely used as drugs such as proton pump inhibitor omeprazole [2,3], anti-helmentic albendazole [4,5], anti-dopaminergic domperidone [6,7], anti-psychotic pimozide [8,9], etc. Some of their analogs are the constitutional parts of the marine alkaloids, such as kealiiquinone and antitumor agents such as pyrrolo[1,2-a] benzimidazole quninone (APBI-A) [10,11]. Specifically, the 2-substituted analogues of benzimidazoles are known to be potent biologically active compounds [12-14]. Some of the important benzimidazole derivatives have been reported as thyroid receptor agonists [15], gonadotropin releasing hormone receptor antagonists [16], non-nucleoside HIV-1 reverse transcriptase inhibitors [17] and interestingly alkynyl benzimidazoles as modulators of metabotropic glutamate receptors [18] etc. We have also published some series of biologically active benzimidazoles [19-21]. Owing to the immense biological importance of benzimidazole derivatives, we now synthesized some novel class of benzimidazole derivatives and their biological activity screening studies.

2. Experimental

2.1. Chemicals

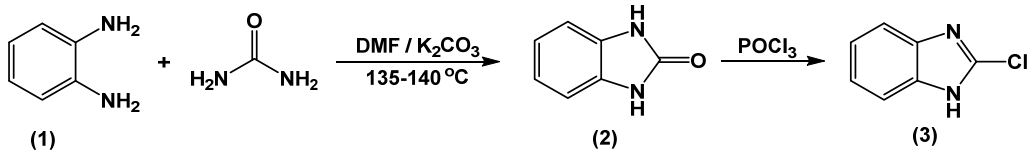
o-Phenylenediamine acid, urea, phosphoryl chloride obtained from Aldrich. Hydrochloric acid, sodium hydroxide, potassium carbonate, alkylating agent and solvents used were of commercial grade only.

2.2. Instrumentations

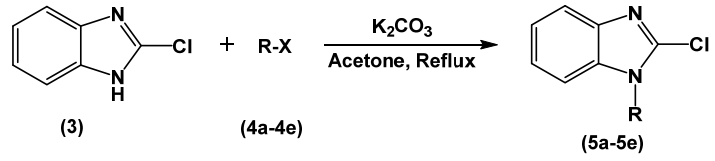
Melting points recorded on a MRVIS Series, Lab. India Instrument. TLC analysis was done using pre-coated silica gel plates and visualization was done using iodine/UV lamp. Infrared spectra were recorded on Perkin Elmer model FT-IR using the KBr disc. ^{1}H NMR spectra of the compounds were recorded on BRUKER Avance II 400 MHz NMR spectrometer with $CDCl_3$ as solvent unless otherwise mentioned. Elemental analysis was carried out on a Perkin Elmer Series II Elemental Analyzer 2400.

2.3. Synthesis

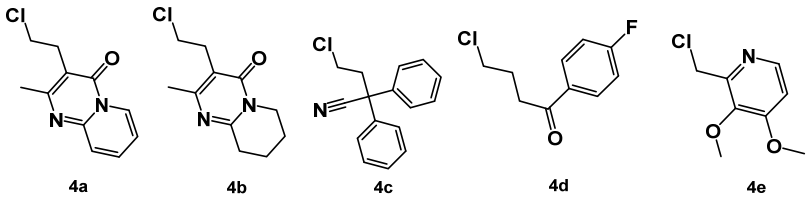
ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.8.2.149-154.1563



Scheme 1



Where, R-X=



Scheme 2

2.3.1. Synthesis of 1,3-dihydro-benzimidazol-2-one (2)

To a solution of *o*-phenylene diamine (**1**) (5 g, 0.046 mol) in DMF was added urea (5.52 g, 0.092 mol) and mixture heated to 135-140 °C for 12 h. When the reaction was complete, DMF was removed in vacuum; separated solid was washed with water and then dissolved in aq. 10% NaOH solution. The aqueous alkaline solution was filtered and neutralised with aq. HCl (35%). The separated product was filtered, washed and dried to obtain pure compound **2** (1,3-dihydro-benzimidazol-2-one) (Scheme 1). Yield: 5.8 g, 94%. M.p.: 99-101 °C (Lit. [22]: 100-102 °C).

2.3.2. Synthesis of 2-chloro-1H-benzimidazole (3)

A mixture of 1,3-dihydro-benzimidazol-2-one (**2**) (10 g, 0.07 mol), phosphoryl chloride (22.88 g, 0.14 mol) and catalytic amount of phenol was heated 103-107 °C for 12 h. After completion of the reaction, the mixture was cooled and sodium hydroxide (40%, 25 mL) solution was added. The reaction mixture was stirred until it solidify. The crude material was recrystallized to obtain pure product **3** (2-chloro-1*H*-benzimidazole) (Scheme 1). Yield: 11 g, 97 %. M.p.: 208-209 °C (Lit. [22]: 207-211 °C).

2.3.3. General procedure for the synthesis of N-alkylated derivatives of 2-chloro-1*H*-benzimidazole (5a-e)

To a solution of 2-chloro-1*H*-benzimidazole (**3**) (13.10 mmol) and potassium carbonate (19.65 mmol) in water (10 mL) was added compound **4a-e** (15.75 mmol) at room temperature. The reaction mixture was then heated to 70-75 °C for 3-4 h by TLC monitoring. After completion of reaction, added 20 mL ethyl acetate, stirred for 15 min layers were separated. Ethyl acetate layer was washed with 20 mL water, dried over sodium sulfate. After concentration of solvent under vacuum and recrystalization with aq. ethanol yielded corresponding *N*-substituted derivative (**5a-e**) as a white solid (Scheme 2) [23].

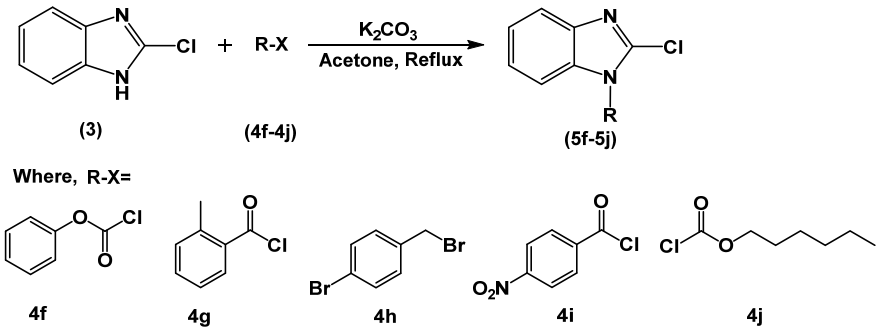
2.3.4. General procedure for the synthesis of N-alkylated derivatives of 2-chloro-1*H*-benzimidazole (5f-j)

To a solution of 2-chloro-1*H*-benzimidazole (**3**) (13.10 mmol) and potassium carbonate (19.65 mmol) in acetone (12 mL) was added compound **4f-j** (15.75 mmol) at room temperature. The reaction mixture was then heated to 50-55 °C for 4-6 h by TLC monitoring. After completion of reaction, solvent was evaporated and added 20 mL water and 25 mL ethyl acetate, stirred for 15 min layers were separated. Ethyl acetate layer was washed with 20 mL water, dried over sodium sulfate. After concentration of solvent under vacuum and recrystalization with aq. ethanol yielded corresponding *N*-substituted derivative (**5f-j**) as a white solid. (Scheme 3) [23].

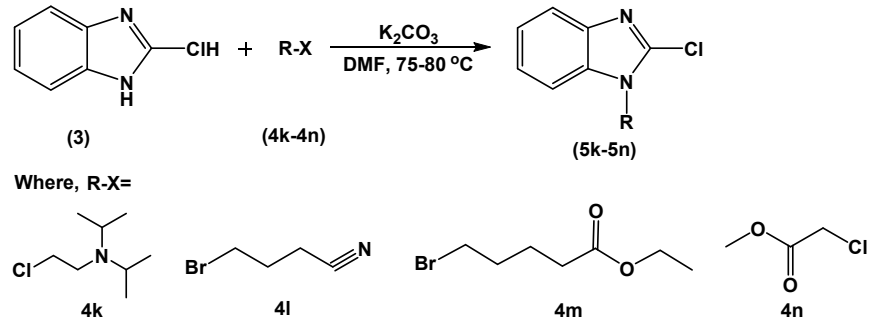
2.3.5. General procedure for the synthesis of N-alkylated derivatives of 2-chloro-1*H*-benzimidazole (5k-n)

To a solution of 2-chloro-1*H*-benzimidazole (**3**) (13.10 mmol) and potassium carbonate (19.65 mmol) in DMF (12 mL) was added compound **4k-n** (15.75 mmol) at 75-80 °C. The reaction mixture was then heated for 4-5 h and monitored by TLC. After the completion of reaction, it was quenched with water (25 mL) added 25 mL ethyl acetate, stirred for 15 min layers were separated. Ethyl acetate layer was washed with 25 mL water, dried over sodium sulfate. After concentration of solvent under vacuum and recrystallization with aq. ethanol yielded corresponding *N*-substituted derivative (**5k-n**) as a white solid. (Scheme 4) [23].

3-[2-(2-Chloro-benzimidazole-1-yl) -ethyl]-2-methyl-pyrido [1,2-a]pyrimidine-4-one (**5a**): Color: White. Yield: 80%. M.p.: 175-178 °C. FT-IR (KBr, ν, cm^{-1}): 1730 (C=O), 1664 (C=N), 1097 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.25 (s, 3H, CH_3-Ph), 3.22 (t, 2H, *J* = 7.2 Hz, -CH_2-CH_2-N), 4.49 (t, 2H, *J* = 7.2 Hz, -CH_2-CH_2-N), 7.15 (t, 1H, *J* = 6.72 Hz, Ar-H), 7.22-7.26 (m, 3H, Ar-H), 7.43-7.45 (m, 1H, Ar-H), 7.57-7.59 (m, 1H, Ar-H), 7.65-7.73 (m, 2H, Ar-H). MS (EI, *m/z* (%): 339.1 (M+1). Anal. calcd. for $C_{18}H_{15}ClN_4O$: C, 63.81; H, 4.46; N, 16.54. Found: C, 63.90; H, 4.41; N, 16.61%.



Scheme 3



Scheme 4

3-[2-(2-Chloro-benzimidazole-1-yl)-ethyl]-2-methyl-6, 7,8,9-tetrahydro-pyrido[1,2-a]pyrimidine-4-one (**5b**): Color: White. Yield: 74%. M.p.: 168-171 °C. FT-IR (KBr, ν, cm^{-1}): 1722 (C=O), 1650 (C=N), 1104 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.84-1.90 (m, 4H, CH_2-CH_2-CH_2), 2.00 (s, 3H, CH_3-Ph), 2.84 (t, 2H, *J* = 6.64 Hz, Aliphatic ring), 3.01 (t, 2H, *J* = 7.2 Hz, CH_2-CH_2-N), 3.95 (t, 2H, *J* = 6.2 Hz, Aliphatic ring), 4.40 (t, 2H, *J* = 7.2 Hz, CH_2-CH_2-Ph), 7.22-7.28 (m, 2H, Ar-H), 7.42 (dd, 1H, *J* = 6.64 Hz, Ar-H), 7.65-7.67 (m, 1H, Ar-H). MS (EI, *m/z* (%): 343.2 (M+1). Anal. calcd. for $C_{18}H_{19}ClN_4O$: C, 63.09; H, 5.59; N, 16.34. Found: C, 63.15; H, 5.60; N, 16.28 %.

4-(2-Chloro-benzimidazole-1-yl)-2, 2-diphenyl-butyronitrile (**5c**): Color: White. Yield: 76%. M.p.: 140-143 °C. FT-IR (KBr, ν, cm^{-1}): 2329 (C≡N), 1650 (C=N), 1137(C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.78 (t, 2H, *J* = 8.0 Hz, CH_2), 4.20 (t, 2H, *J* = 8.0 Hz, CH_2-N), 7.05-7.08 (m, 1H, Ar-H), 7.17-7.22 (m, 2H, Ar-H), 7.26-7.30 (m, 2H, Ar-H), 7.32-7.38 (m, 8H, Ar-H), 7.59-7.61 (m, 1H, Ar-H). MS (EI, *m/z* (%): 372.2 (M+1). Anal. calcd. for $C_{23}H_{18}ClN_3$: C, 74.29; H, 4.88; N, 9.53. Found: C, 74.35; H, 4.81; N, 9.60.

4-(2-Chloro-benzimidazole-1-yl)-1-(4-fluoro-phenyl) -butan-1-one (**5d**): Color: White. Yield: 68%. M.p.: 111-114 °C. FT-IR (KBr, ν, cm^{-1}): 1701 (C=O), 1658 (C=N), 1135 (C-F), 1097 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.18-2.25 (m, 2H, CH_2-N), 3.15 (t, 2H, *J* = 8 Hz, CH_2-N), 3.67 (t, 2H, *J* = 8 Hz, CH_2-C=O), 7.11-7.15 (m, 4H, Ar-H), 7.98-8.02 (m, 4H, Ar-H). MS (EI, *m/z* (%): 317.2 (M+1). Anal. calcd. for $C_{17}H_{14}ClFN_2O$: C, 64.46; H, 4.45; N, 8.84. Found: C, 64.50; H, 4.41; N, 8.91 %.

2-Chloro-1-(3,4-dimethoxy-pyridine-2-ylmethyl)-1H-benzimidazole (**5e**): Color: White. Yield: 81%. M.p.: 172-174 °C. FT-IR (KBr, ν, cm^{-1}): 2940 (OCH_3), 1652 (C=N), 1137 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.14 (s, 3H, OCH_3), 4.32 (s, 3H, OCH_3), 5.92 (s, 2H, CH_2-N), 7.34-7.41 (m, 2H, Ar-H), 7.75-7.79 (m, 2H, Ar-H), 8.04 (d, 1H, *J* = 6.68 Hz, Ar-H), 9.55 (d, 1H, *J* = 8 Hz, Ar-H). MS (EI, *m/z* (%): 304.2 (M+1). Anal. calcd. for $C_{15}H_{14}ClN_3O_2$: C, 59.31; H, 4.65; N, 13.83. Found: C, 59.42; H, 4.55; N, 13.91%.

2-Chloro-benzimidazole-1-carboxylic acid phenyl ester (**5f**): Color: White. Yield: 62%. M.p.: 138-141 °C. FT-IR (KBr, ν, cm^{-1}): 1752 (C=O), 1592 (C=N), 1068 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.05-7.09 (m, 1H, Ar-H), 7.10-7.15 (m, 1H, Ar-H), 7.16-7.18 (m, 1H, Ar-H), 7.32-7.36 (m, 3H, Ar-H), 7.44-7.48 (m, 2H, Ar-H), 7.75 (d, 1H, *J* = 7.92 Hz, Ar-H). MS (EI, *m/z* (%): 273.2 (M+1). Anal. calcd. for $C_{14}H_9ClN_2O_2$: C, 61.66; H, 3.33; N, 10.27. Found: C, 61.60; H, 3.39; N, 10.34%.

(2-Chloro-benzimidazole-1-yl)-o-tolyl-methanone (**5g**): Color: White. Yield: 65%. M.p.: 115-118 °C. FT-IR (KBr, ν, cm^{-1}): 1722 (C=O), 1607 (C=N), 1102 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.61 (s, 3H, CH_3), 7.15 (t, 1H, *J* = 6.73 Hz, Ar-H), 7.23-7.26 (m, 3H, Ar-H), 7.43-7.45 (m, 1H, Ar-H), 7.57-7.59 (m, 1H, Ar-H), 7.66-7.73 (m, 2H, Ar-H). MS (EI, *m/z* (%): 271.2 (M+1). Anal. calcd. for $C_{15}H_{11}ClN_2O$: C, 66.55; H, 4.10; N, 10.35. Found: C, 66.51; H, 4.16; N, 10.29 %.

1-(4-Bromo-benzyl)-2-chloro-1H-benzimidazole (**5h**): Color: White. Yield: 88%. M.p.: 119-121 °C. FT-IR (KBr, ν, cm^{-1}): 1650 (C=N), 1137 (C-N), 1021 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.26 (s, 2H, CH_2-Ph), 6.97 (d, 2H, Ar-H), 7.12 (dd, 1H, *J* = 8 Hz, Ar-H), 7.14-7.23 (m, 2H, Ar-H), 7.36-7.41 (m, 2H, Ar-H), 7.63-7.65 (m, 1H, Ar-H). MS (EI, *m/z* (%): 322.2 (M+1). Anal. calcd. for $C_{14}H_{10}BrClN_2$: C, 52.29; H, 3.13; N, 8.71. Found: C, 52.36; H, 3.18; N, 8.65%.

(*2-Chloro-benzimidazole-1-yl)-(4-nitro-phenyl) -methanone* (**5i**): Color: White. Yield: 85%. M.p.: 155-158 °C. FT-IR (KBr, ν, cm^{-1}): 1722 (C=O), 1475 (NO_2), 1095 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.32-7.34 (m, 1H, Ar-H), 7.36-7.42 (m, 1H, Ar-H), 7.45 (m, 1H, Ar-H), 7.74 (dd, 2H, *J* = 8 Hz, Ar-H), 7.95-7.98 (m, 1H, Ar-H), 8.32-8.41 (m, 2H, Ar-H). MS (EI, *m/z* (%): 302.2 (M+1). Anal. calcd. for $C_{14}H_8ClN_3O_3$: C, 55.74; H, 2.67; N, 13.93. Found: C, 55.88; H, 2.61; N, 13.87%.

2-Chloro-benzimidazole-1-carboxylic acid hexyl ester (**5j**): Color: White. Yield: 87%. M.p.: 111-113 °C. FT-IR (KBr, ν, cm^{-1}): 1752 (C=O), 1592 (C=N), 1068 (C-N).

Table 1. Antibacterial activity (minimal inhibition concentration; MIC) of compound **5a-n**.

Compound	Antibacterial activity (MIC, µg/mL)			
	E. coli (Gram negative)	*P. aeruginosa* (Gram negative)	*S. aureus* (Gram positive)	*S. pyogenus* (Gram positive)
5a	100	62.5	125	62.5
5b	100	100	150	100
5c	62.5	100	125	125
5d	62.5	125	100	62.5
5e	50	125	62.5	100
5f	125	250	200	125
5g	100	100	200	250
5h	50	62.5	125	100
5i	50	100	62.5	150
5j	62.5	125	100	250
5k	125	100	100	500
5l	250	250	200	100
5m	200	250	200	125
5n	125	200	125	150
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	-	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.84 (t, 3H, *J* = 6.88 Hz, CH_3), 1.27-1.33 (m, 4H, 2×CH_2), 1.38-1.45 (m, 2H, CH_2), 1.75-1.82 (m, 2H, CH_2), 4.40 (t, 2H, *J* = 6.8 Hz, CH_2), 7.03-7.13 (m, 3H, Ar-H), 7.72-7.74 (d, 1H, *J* = 7.76 Hz, Ar-H). MS (EI, *m/z* (%): 281.2 (M+1). Anal. calcd. for $C_{14}H_{17}ClN_2O_2$: C, 59.89; H, 6.10; N, 9.98. Found: C, 9.95; H, 6.05; N, 10.04%.

[2-(2-Chloro-benzimidazole-1-yl)-ethyl] -diisopropyl-amine (**5k**): Color: White. Yield: 74%. M.p.: 123-126 °C. FT-IR (KBr, ν, cm^{-1}): 1582 (C=N), 1213 (C-N aliphatic), 1136 (C-N aromatic). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.97 (d, 12H, *J* = 6.56 Hz, 4×CH_3), 2.77 (t, 2H, *J* = 7.36 Hz, CH_2-N), 3.00-3.05 (m, 2H, CH-N), 4.12 (t, 2H, *J* = 7.2 Hz, CH_2-N), 7.23-7.32 (m, 3H, Ar-H), 7.66-7.69 (m, 1H, Ar-H). MS (EI, *m/z* (%): 280.2 (M+1). Anal. calcd. for $C_{15}H_{22}ClN_3$: C, 64.39; H, 7.92; N, 15.02. Found: C, 64.32; H, 7.99; N, 15.11%.

4-(2-Chloro-benzimidazole-1yl)-butyronitrile (**5l**): Color: White. Yield: 88%. M.p.: 134-137 °C. FT-IR (KBr, ν, cm^{-1}): 2329 (C≡N), 1650 (C=N), 1137 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.18-2.24 (m, 2H, CH_2-CH_2), 2.39 (t, 2H, *J* = 7.12 Hz, CH_2-CN), 4.35 (t, 2H, *J* = 6.84 Hz, CH_2-N), 7.21-7.37 (m, 3H, Ar-H), 7.68-7.71 (m, 1H, Ar-H). MS (EI, *m/z* (%): 220.2 (M+1). Anal. calcd. for $C_{11}H_{10}ClN_3$: C, 60.14; H, 4.59; N, 19.13. Found: C, 60.19; H, 4.52; N, 19.09%.

5-(2-Chloro-benzimidazole-1yl)-pentanoic acid ethyl ester (**5m**): Color: White. Yield: 78%. M.p.: 118-121 °C. FT-IR (KBr, ν, cm^{-1}): 1752 (C=O), 1592 (C=N), 1174 (ester). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.23-1.33 (m, 5H, CH_2 & CH_3), 2.11-2.16 (m, 2H, CH_2), 2.37 (t, 2H, *J* = 7.04 Hz, CH_2), 4.11-4.17 (m, 2H, CH_2), 4.28 (t, 2H, *J* = 7.2 Hz, CH_2), 7.26-7.32 (m, 2H, Ar-H), 7.35-7.38 (m, 1H, Ar-H), 7.68-7.71 (m, 1H, Ar-H). MS (EI, *m/z* (%): 281.2 (M+1). Anal. calcd. for $C_{14}H_{17}ClN_2O_2$: C, 59.89; H, 6.10; N, 9.98. Found: C, 59.95; H, 6.06; N, 9.92%.

(2-Chloro-benzimidazole-1yl)-acetic acid methyl ester (**5n**): Color: White. Yield: 69%. M.p.: 131-134 °C. FT-IR (KBr, ν, cm^{-1}): 1752 (C=O), 1592 (C=N), 1068 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.67 (s, 3H, CH_3-O), 4.69 (s, 2H, CH_2-N), 7.25-7.30 (m, 2H, Ar-H), 7.43-7.47 (m, 1H, Ar-H), 8.07 (dd, 1H, *J* = 8.36 Hz, Ar-H). MS (EI, *m/z* (%): 225.2 (M+1). Anal. calcd. for $C_{10}H_9ClN_2O_3$: C, 53.47; H, 4.04; N, 12.47. Found: C, 53.39; H, 4.11; N, 12.51%.

2.4. Antimicrobial studies

2.4.1. Tested organisms

The newly synthesized compounds were screened for their antibacterial activity [24,25] against Gram-positive bacteria *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 443) and Gram negative *Escherichia coli* (MTCC-442), *Pseudomonas aeruginosa* (MTCC 441). The same compounds were screened for their antifungal activity [26] against *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282) and *Aspergillus clavatus* (MTCC 1323). Antibacterial activity was measured as per National Committee for Clinical Laboratory Standards (NCCLS) protocol by Mueller-Hinton Broth [27] (Becton-Dickinson, USA) Standard strains were procured by The Institute of Microbial Technology, Chandigarh. Compounds were primarily screened for their antibacterial activity in six sets against *E. coli*, *S. aureus*, *P. aeruginosa* and *S. pyogenes* at different concentrations of 1000, 500 and 250 µg/mL as shown in Table 1.

2.4.2. Antibacterial assay

The drugs found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 µg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inoculums size for test strain was adjusted to 1×10^6 CFU/mL (colony forming unit per milliliter) by comparing the turbidity (turbid metric method). Synthesized compounds were diluted to 2000 µg/mL concentration, as a stock solution. A control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of test organisms.

The tubes were then incubated at 37.8 °C for 24 h for bacteria. Ten µg/mL suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 h. The lowest concentration, which showed no growth after spot subculture was considered as MIC for each drug. The highest dilution (lowest concentration) preventing appearance of turbidity was taken as MIC i.e., the amount of growth from the control tube before incubation (which represents the original inoculums) was compared. The test mixture should contain 1×10^6 CFU/mL organisms. 2% DMSO and sterilized distilled water was used as negative control, while ampicillin antibiotic (1 U strength) was used as positive control. A set of tubes containing only seeded broth and solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The result of this was significantly affected by the size of inoculums. The standard drug used in the present study was ampicillin for evaluating antibacterial activity which showed 100, 100, 250 and 100 µg/mL MIC against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes*, respectively. For bacterial growth, in the present protocol, we have used Muller Hinton broth at 37.8 °C in aerobic condition for 24-48 h. Results of antimicrobial evaluation of derivatives **5a-n** are shown in Table 1.

Table 2. Antifungal activity (minimal inhibition concentration; MIC) of compound **5a-n**.

Compound	Antifungal activity (MIC, µg/mL)		
	C. albicans	*A. niger*	*A. clavatus*
5a	500	1000	>1000
5b	250	500	1000
5c	1000	500	500
5d	250	250	500
5e	500	250	>1000
5f	>1000	1000	>1000
5g	1000	500	500
5h	500	500	1000
5i	500	>1000	250
5j	1000	250	1000
5k	500	250	500
5l	>1000	500	500
5m	1000	1000	250
5n	500	>1000	1000
Nystatin	100	100	100
Griseofulvin	500	100	100

2.4.3. Antifungal assay

The same compounds were tested for antifungal activity as primary screening in six sets *against Candida albicans, Aspergillus niger* and *Aspergillus clavatus* at various concentrations of 1000, 500, 200 and 100 µg/mL as shown in Table 2. Results were recorded in the form of primary and secondary screening. Synthesized compounds were diluted to 1000 µg/mL concentration, as a stock solution. The synthesized compounds which were found to be active in this primary screening were further tested in a second set of dilution against all microorganisms. Griseofulvin was used as a standard drug for antifungal activity, which showed 500, 100 and 100 µg/mL MIC against *C. albicans*, *A. Niger* and *A. clavatus*, respectively. 2% DMSO and sterilized distilled water was used as negative control, while Griseofulvin (1 U strength) was used as positive control. For fungal growth, in the present protocol, we have used Sabourauds dextrose broth at 28.8 °C in aerobic condition for 48 h. Results of antifungal evaluation of derivatives **5a-n** are shown in Table 2.

3. Results and discussion

We have synthesized a series of *N*-substituted 2-chloro-1*H*-benzimidazole derivatives using a known procedure and obtained products with good yield (**5a-n**). 1,3-Dihydro-benzimidazol-2-one (**2**) was synthesized from the reaction of *o*-phenylenediamine with urea in dimethyl form amide (Scheme 1). Compound **2** was reacted with phosphoryl chloride in absent solvent to give 2-chloro-1*H*-benzimidazole **3** (Scheme 1). Compound **3** was combined with 3-(2-chloroethyl)-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one (**4a**) in presence of potassium carbonate in water to yield 3-[2-(2-chloro-benzimidazole-1-yl)-ethyl]-2-methyl-pyrido[1, 2-a]pyrimidine-4-one (**5a**) (Scheme 2). Its IR spectra showed the appearance of carbonyl absorption band at 1730 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 339.1 (M+1). Compound **3** was combined with 3-(2-chloro ethyl)-2-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrimidin-4-one (**4b**) in presence of potassium carbonate in water to yield 3-[2-(2-chloro-benzimidazole-1-yl)-ethyl]-2-methyl-6, 7, 8, 9-tetrahydro-pyrido[1,2-a]pyrimidine-4-one (**5b**) (Scheme 2). Its IR spectra showed the appearance of carbonyl absorption band at 1722 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 343.2 (M+1). Compound **3** was combined with 4-chloro-2,2-diphenylbutane nitrile (**4c**) in presence of potassium carbonate in water to yield 4-(2-chloro-benzimidazole-1-yl)-2,2-diphenyl-butyro nitrile (**5c**). Its IR spectra showed the appearance of nitrile absorption band at 2329 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 372.2 (M+1). Compound **3** was combined with 4-chloro-1-(4-fluorophenyl) butan-1-one (**4d**) in presence of potassium carbonate in water to yield 4-(2-chloro-benzimidazole-1-yl)-1-(4-fluoro-phenyl) -butan-1-one (**5d**). Its IR spectra showed the appearance of carbonyl absorption band at 1701 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 317.2 (M+1). Compound **3** was combined with 2-(chloromethyl)-4-methoxy pyridin-3-ol (**4e**) in presence of potassium carbonate in water to yield 2-chloro-1-(3,4-dimethoxy-pyridine-2-ylmethyl)-1*H*-benzimidazole (**5e**). Its IR spectra showed the appearance of methoxy absorption weak band at 2696 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 304.2 (M+1).

The reaction of compound **3** with phenylchloroformate (**4f**) in presence of potassium carbonate in acetone to yield 2-chloro-benzimidazole-1-carboxylic acid phenyl ester (**5f**) (Scheme 3). Its IR spectra showed the appearance of carbonyl absorption band at 1752 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 273.2 (M+1). Compound **3** was reacted with 2-methylbenzoyl chloride (**4g**) in presence of potassium carbonate in acetone to yield (2-chloro-benzimidazole-1-yl)-*o*-tolyl-methanone (**5g**). Its IR spectra showed the appearance of carbonyl absorption band at 1722 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 271.2 (M+1). Compound **3** was reacted with 1-bromo-4-(bromomethyl)benzene (**4h**) in presence of potassium carbonate in acetone to yield 1-(4-bromo-benzyl)-2-Chloro-1*H*-benzimidazole (**5h**). Its IR spectra showed the appearance of C-Br absorption band at 1021 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 322.2 (M+1). Compound **3** was reacted with 4-nitrobenzoyl chloride (**4i**) in presence of potassium carbonate in acetone to yield (2-Chloro-benzimidazole-1-yl)-(4-nitro-phenyl)-methanone (**5i**). Its IR spectra showed the appearance of carbonyl absorption band at 1722 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 302.2 (M+1). Compound **3** was reacted with hexylchloro formate (**4j**) in presence of potassium carbonate in acetone to yield 2-chloro-benzimidazole-1-carboxylic acid hexyl ester (**5j**). Its IR spectra showed the appearance of carbonyl absorption band at 1752 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 281.2 (M+1).

The reaction of compound **3** with *N*-(2-chloroethyl)-*N*-isopropylpropan-2-amine (**4k**) in presence of potassium carbonate in DMF to yield [2-(2-chloro-benzimidazole-1-yl)-ethyl]-diisopropyl-amine (**5k**) (Scheme 4). Its IR spectra showed the appearance of C-N Stretch alkyl absorption band at 1136 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 280.2 (M+1). Compound **3** was reacted with 4-bromobutanenitrile (**4l**) in presence of potassium carbonate in DMF to yield 4-(2-chloro-benzimidazole-1yl)-butyronitrile (**5l**). Its IR spectra showed the appearance of nitrile absorption band at 2329 cm^{-1}. Its mass

spectrum showed a peak corresponding to its molecular ion at *m/z* 220.2 (M+1). Compound **3** was reacted with ethyl 5-bromopentanoate (**4m**) in presence of potassium carbonate in DMF to yield 5-(2-chloro-benzimidazole-1yl)-pentanoic acid ethyl ester (**5m**). Its IR spectra showed the appearance of carbonyl absorption band at 1752 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 281.2 (M+1). Compound **3** was reacted with methyl 2-chloroacetate (**4n**) in presence of potassium carbonate in DMF to yield (2-chloro-benzimidazole-1yl)-acetic acid methyl ester (**5n**). Its IR spectra showed the appearance of carbonyl absorption band at 1752 cm^{-1}. Its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 225.2 (M+1).

All the compounds prepared herein were screened for their antimicrobial activities. The structures of all the synthesized compounds were characterized by spectroscopic data, and allowed these molecules for study of antibacterial and antifungal activities (Table 1 and 2).

4. Conclusion

Newly synthesized *N*-substituted 2-chloro-1*H*-benzimidazole derivatives were thoroughly characterized and some of them (**5c**, **5d**, **5e**, **5h**, **5i** and **5j**) were found to possess high activity against *Escherichia coli* whereas compounds **5a**-**n** were highly active against *Staphylococcus aureus* and compound **5a** and **5d** have also exerted very good activity against *Streptococcus pyogenes* employed for screening when compared to the standard ampicillin. The compounds **5b** and **5d** exhibited antifungal activity. However, antifungal activity of the other synthesized compounds was unsatisfactory.

Acknowledgement

The authors express their thanks to Sophisticated Analytical Instrumentation Facility Chandigarh, India for NMR and Mass spectra and Micro-care laboratory, Surat, Gujarat, India for biological activity.

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