J. Biol. Chem. Chron. **2019,** 5*(1),* 85-90
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 – 7476
www.eresearchco.com/jbcc/

Synthesis, Characterization and Biological Evaluation of Novel N-alkylated 2-(4-bromophenyl)-1*H*-benzimidazole Derivatives

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(Received 16 Feb, 2018; Accepted 09 Apr, 2019; Published 16 Apr, 2019)

ABSTRACT: In present study *O*-pheylenediamine and 4–bromo benzoic acid were used as starting material and treated with poly phosphoric acid to obtained 2-(4-bromophenyl)-1*H*-benzimidazole.Using this compound, alkylation done at the benzimidazole –NH position with different benzyl halide reagent leading to the functionalized derivatives. Newly synthesized derivatives (5a to 5g) have been evaluated on the basis of spectral and analytical data like melting point, IR, ^{1}H-NMR and Mass spectroscopy. All the synthesized compounds were screened for their antimicrobial activity. This displayed some promising results towards testing organism in-vitro.

Keywords: Benzimidazole; Cyclisation; Condensation; Anti-bacterial activity; Anti-fungal activity.

INTRODUCTION: All the heterocyclic compounds are of great interest in pharmaceutical chemistry. Out of these heterocyclic compounds the benzofused heterocyclic compound, i.e. Benzimidazole and its derivatives are the building blocks of bioactive and clinical operations.[1 & 2] Benzimidazole derivatives are naturally occurring isostere of nucleotides[3], which shows a large number of biological activities towards antioxidant[4], Antimicrobial activity[5], antiflammatory - analgestic[6], anticancer[7], CNS depressant[8], androgen receptor antagonist[9], antitubercular[10], antihelmintic[11] and diabetic drugs[12], anti-ulcer[13], anticonvulsant[14], antiviral-antifungal[15] and antiprotozoal[16]. In addition the benzimidazole have played a very important role in the development of theory in heterocyclic chemistry and also extensively in organic synthesis.

Benzimidazole nucleus is present in vitamin-B12 (Merck index 2001). In this present study some novel derivatives of benzimidazole derivatives have been synthesized and their antibacterial and antifungal activity has been studied.

MATERIALS AND METHODS: O- phenylenediamine, 4-Bromo benzoic acid, polyphosphoric acid, Potassium carbonate, potassium iodide and alkylating agent. All the chemical reagents and solvents are commercial grade which were procured locally and are used in the study. Reaction monitoring thin layer chromatography (TLC) is used. TLC viewed under UV lamp and Iodine chamber. Melting points were determined in an open capillary using melting point apparatus. Infrared spectra of compounds were recorded in KBr on Jasco, FT/IR-4100 type-A.The proton magnetic resonance (^{1}H -NMR) spectraof the compounds were recorded on JEOL 500 MHz NMR spectrometer

RESULTS AND DISCUSSION: 2-(4-bromophenyl)-1*H*-benzimidazole[17](**3)**was synthesized by known method using o-phenylenediamine, 4–bromo benzoic acid in polyphosphoric acid at temperature 180°C with 90 % yield having m.p.296-298°C. Based on the spectral and analytical data the compound was assigned to be 2-(4-bromophenyl)-1*H*-benzimidazole (**3)**(Scheme-1). The alkylation of **3** with various electrophilic reagents in presence of potassium carbonate as base yielded the N-alkylated derivatives obtained Compounds **5a-5e** (Scheme-2) and **5f-5g** (Scheme-3)**.**

General Procedure of synthesis:

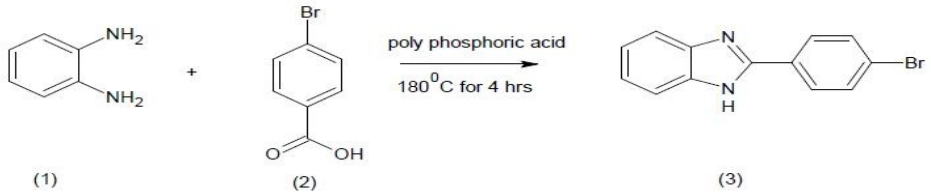
Synthesis of 2-(4-bromophenyl)-1*H*-benzimidazole *(3):* A mixture of *O*–phenylenediamine (10 gm, 0.07 moles), 4- bromo benzoic acid (22.88 gm, 0.14 moles) in presence polyphosphoric acid was heated 180^{0}C for 4 hrs. After confirmation of completion of the reaction, the mixture was chilled in ice and neutralized with 40% aqueous sodium hydroxide solution to pH-10. The obtained crude material which was recrystallized in ethanol water to obtain pure product **(3)(2-(4-**

bromophenyl)-1*H*-benzimidazole)[17] with yield 22.73 gm. 90%, Melting point 296-298°C (Scheme-1).

General procedure for the synthesis of N- alkylated derivatives of 2-(4-bromophenyl)-1*H*-benzimidazole compounds (5a-5e): To a solution of 2-(4-bromophenyl)-1*H*benzimidazole(**3**)(10.0 mmoles) and potassium carbonate (15.0 mmoles) in acetone (70 ml) was added compound (4a-4e, 12.0 mmoles) at RT. The reaction mixture was heated to reflux for 3-4 hrs. Reaction progress was monitored on TLC. After reaction completion, Distilled out solvent under reduce pressure at 40-50^{0}C and added 30ml water and 30 ml ethyl acetate and stirred for 10 min. Ethyl acetate and aqueous layers were separated. Ethyl acetate layer was washed with 30 ml water, Distilled out ethyl acetate under reduced pressure to get degas mass and which was recrystallization in ethanol and water to obtained corresponding N-substituted derivative **(5a-5e**). (Scheme-2)

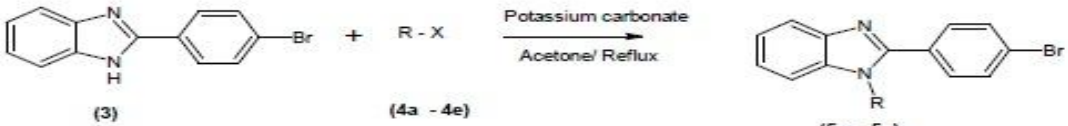
General procedure for the synthesis of N- alkylated derivatives of 2-(4-bromophenyl)-1*H*-benzimidazole compounds (5f-5g): To a solution of 2-(4-bromophenyl)-1*H*-benzimidazole(**3**)(10.0 mmoles) and potassium carbonate (15.0 mmoles),Potassium iodide (10.0 mmoles) in N,N –dimethyl formamide (30 ml) was added compound (**4f-4g,** 12.0 mmoles) at RT. The reaction mixture was then warmed 80 - 90^{0}C for 5-6 hrs. Reaction progress was monitored on TLC. After reaction completion, Reaction mass was quenched with 90 ml water and 90 ml ethyl acetate and stirred for 10 min. Ethyl acetate and aqueous layers were separated. Ethyl acetate layer was washed with 30 ml water, Distilled out ethyl acetate under reduced pressure to get degas mass and which was recrystallization in ethanol and water to obtained corresponding N-substituted derivative (**5f-5g**). (Scheme-3)

Scheme - 1



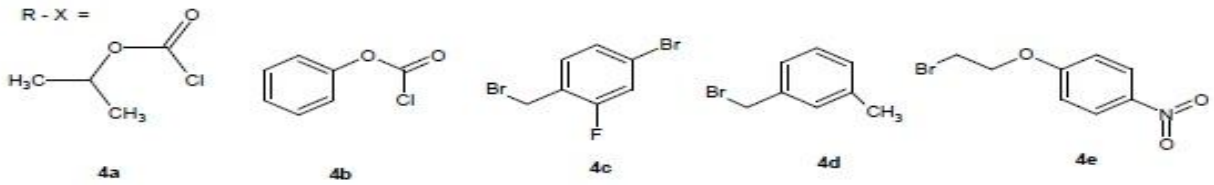
(1) (2) (3)

Scheme - 2

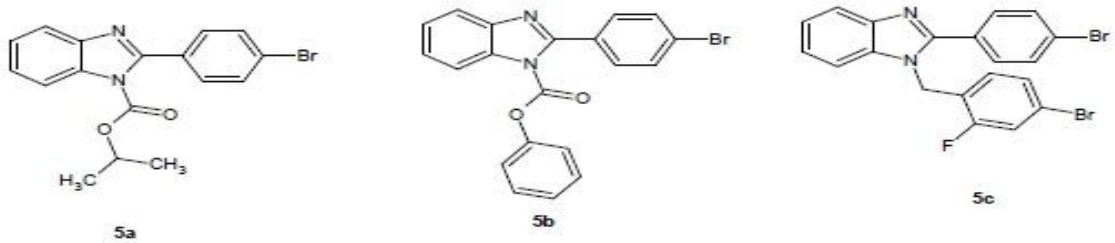


(3) (4a - 4e) (5a - 5e)

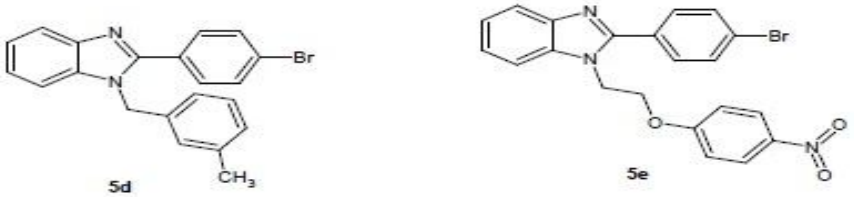
Where R - X =



4a 4b 4c 4d 4e

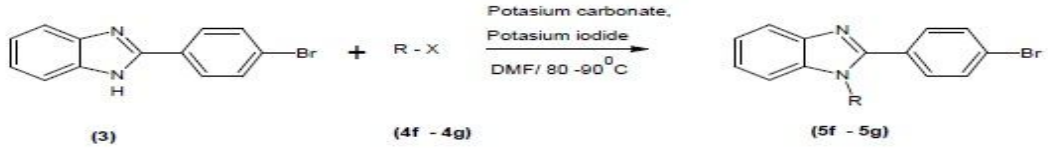


5a 5b 5c



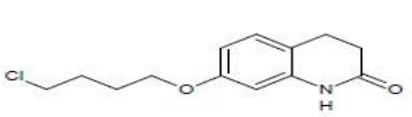
5d 5e

Scheme - 3

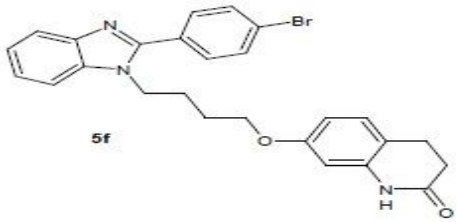


(3) (4f - 4g) (5f - 5g)

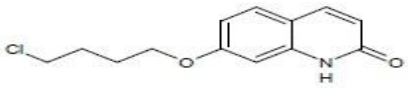
Where R - X =



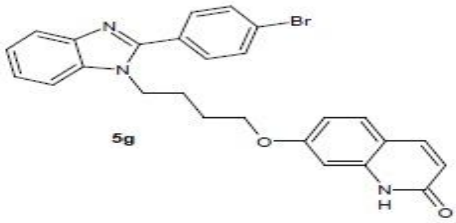
4f



5f



4g



5g

Analytical Characterization:

Propan-2-yl 2-(4-bromophenyl)-1*H*-benzimidazole-1-carboxylate *(5a):*
Yield=90%; M.P.-115-120^{0}C; IR(KBr):582(C-Br),1455(C-H),1537(C=C),1749(C=0),2353(-CH_2)cm^1;
^{1}H-NMR($CDCl_3$): δ1.29-1.30(d,J1=6.2*Hz*,6H),5.14-5.19(m,1H),7.39-7.41(m,2H,ArH),7.53-7.54(d, J1=8.4*Hz*,2H, ArH), 7.60-7.61(d,J1=8.3*Hz*,2H, ArH), 7.78-7.79(m, 1H, ArH), 8.02-8.04(m, 1H, ArH); MS (m/z): 359.17 (M^++1).

Phenyl 2-(4-bromophenyl)-1*H*-benzimidazole-1-carboxylate *(5b):*
Yield=80%; M.P.-165-168^{0}C; IR (KBr): 742(C-Cl),1328(C-F),1461(C=C),1617(C=N)2357(-CH_2) cm^{-1}, ^{1}H-NMR($CDCl_3$): 7.15-7.17 (m,5H, ArH), 7.27-7.30(m,2H,ArH), 7.60-7.64(d,d,J1=14.2*Hz*,J2=8.7*Hz*, 4H,ArH),7.84-7.85(m,1H,ArH), 8.10-8.12(t, J1=4.6*Hz*,1H, ArH).
MS (m/z): 394.23(M^++1).

1-(4-bromo-2-fluorobenzyl)-2-(4-bromophenyl)-1*H*-benzimidazole *(5c):*
Yield=76% M.P.-148-152^{0}C; IR (KBr): 743(C-Br),1376(C-F)1472(C=C),1605(C=N),2353(-CH_2) cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ5.41(s, 2H), 6.63-6.66(t,1H, ArH)), 7.17-7.21(d,d,J1=11.7*Hz*,J2=8.5*Hz*,2H ,ArH), 7.28-7.34(m,3H,ArH),7.51-7.52(d,J1=8.4*Hz*,2H,ArH), 7.61-7.62(d,J1=8*Hz*,2H,ArH),7.86-7.87(d,J1=7.9*Hz*,1H,ArH), MS (m/z): 461.09 (M^++1).

2-(4-bromophenyl)-1-(3-methylbenzyl)-1*H*-benzimidazole *(5d):*
Yield=82%; M.P.-132-137^{0}C; IR (KBr): 739(C-Br),1482(C=C),1610(C=N),2353(-CH_2)cm^{-1};
^{1}H-NMR ($CDCl_3$): δ2.30(s,3H), 5.39(s,2H), 6.86-6.91(m, 2H,ArH), 7.10-7.12 (d, J1=7.4*Hz*, 1H,ArH), 7.20-7.24 (m, 3H,ArH), 7.31-7.33 (m, 1H,ArH), 7.53-7.59 (d,d, J1=13.9*Hz*, J2=8.6*Hz*,4H,ArH),7.85-7.87(d,J1=8.1*Hz*,1H,ArH), MS (m/z): 378.92 (M^++1).

2-(4-bromophenyl)-1-[2-(4nitrophenoxy)ethyl]-1*H*-benzimidazole *(5e):*
Yield=77%; M.P.-195-200^{0}C; IR(KBr):752(C-Br), 3083(CH),1454(NO2) 1110(C-N) cm-1,
^{1}H-NMR($CDCl_3$): δ4.35-4.37 (t,J1=5.3*Hz*2H),4.70-4.72 (t, J1=5.3*Hz*,2H), 6.74-6.76 (d, J1=9.1*Hz*, 2H,ArH),7.33-7.38(m,2H,ArH),7.49-7.51(d, J1=7.1*Hz*, 1H,ArH), 7.68 (d,J1=1.7*Hz*,4H,ArH), 7.83-7.84(d,J1=7.1*Hz*,1H,ArH),8.11-8.13(d,J1=9.1*Hz*,2H,ArH).MS(*m/z*):438.22(M+1).

7-(4-(2-Bromo-1*H*-benzo[d]imidazol-1-yl)butoxy)-3,4-dihydro quinolin-2(1H)-one *(5f):*
Yield=75%; M.P.-168-172^{0}C; IR(KBr):743(C-Br), 1471(C=C),1625(C=N),1680(C=O),2354(CH_2), 2948(CH),cm^{-1};^{1}H-NMR (DMSO-D_6): δ1.69-1.73 (d,dJ1=13.4*Hz*,J2=6.4*Hz*,2H),2.00-2.01 (t,J1=7.4*Hz*,2H),2.60-2.63 (m, 2H), 2.88-2.91 (t,

J1=7.5*Hz*, 2H), 3.81-3.83 (t, J1=5.9*Hz*, 2H), 4.30-4.33 (t, J1=7.7*Hz*, 2H), 6.20 (m, 1H,ArH),6.41-6.43(d,d, J1=8.2*Hz*,J2=2.2*Hz*,1H,ArH), 7.03-7.05 (d, J1=8.3*Hz*, 1H,ArH), 7.31-7.33 (m, 2H,ArH), 7.42-7.43 (m, 1H,ArH),7.58 (s, 4H,ArH), 7.81-7.83 (m,1H,ArH). MS (m/z): 490.30 (M^++1).

7-(4-(2-Bromo-1*H*-benzo[d]imidazol-1-yl)butoxy)quinolin-2(1H)-one *(5g):*

Yield=70 %; M.P.-175-180°C;IR(KBr):743(C-Br), 1470(C=C),1622(C=N),1645(C=O),2340(CH_2), 2948(CH),^{1}H-NMR (DMSO-D_6):δ1.65 (m,2H),1.86 (m,2H),3.92 (t, 2H), 4.38-4.39 (d, J1=6.7*Hz*, 2H), 6.28-6.30 (d, J1=9.1*Hz*,1H,ArH), 6.69-6.71(d,J1=10.3*Hz*, 2H,ArH), 7.24-7.32(m,2H,ArH),7.53-7.54(d,J1=8.3*Hz*,1H,ArH), 7.69-7.73 (m, J1=8.3*Hz*,6H,ArH), 7.78-7.80 (d,J1=9.5*Hz*,1H,ArH), 11.59 (s, 1H), MS (*m/z*): 488.30 (M+1).

Antimicrobial activity: Novel N-alkylated 2-(4-bromophenyl)-1*H*-benzimidazole Derivatives has been synthesized and evaluate on different bacterial and fungal strains. The synthesized compounds **(5a-5g)** were evaluated for their antibacterial activity[18] against human pathogenic Gram negative bacteria such as *Escherichia coli* MTCC442, *Pseudomonasaeruginosa* MTCC441 and Gram positive bacteria

Some derivatives of benzimidazole **5a-5g** were synthesized by nucleophilic substitution of 2-(4-bromophenyl)-1*H*-benzimidazole (3) and were evaluated for antimicrobial activities[19-21] toward *Candida albicans* MTCC227, *Aspergillus Niger* MTCC282 and *Aspergillus clavatus* MTCC1323. Broth dilution method was used to evaluate the antibacterial activity. It is carried out in tubes. Mueller Hinton Broth[22]was used as nutrient medium. Serial dilutions were prepared in primary and secondary screening. Each synthesized drug was diluted obtaining 2000 µg/mL concentration, as a stock solution. In primary screening 1000, 500 and 250 µg/mL concentrations of the synthesized drugs were taken. The drugs found active in primary screening were similarly diluted to obtain 200, 100, 50, 25, 12.5, and 6.250 µg/mL, and concentrations. The highest dilution showing at least 99% inhibition zone was taken as MIC.

We have synthesized *N*-substituted 2-(4-bromophenyl)-1*H*-benzimidazole derivatives by using different condensation conditions and were thoroughly evaluated biological activity of compound 5a-5g such as antifungal(Table-1) and antibacterial (Table-2).Some of the compounds were found to have promising antibacterial activity against E. *coli* such as **5d**. Whereas **5a, 5b, 5c, 5d, 5e 5f** and **5g** the compounds were highly active against S. *aureus* when compared to the Ampicillin as a standard. These compounds were also screened against C. *albicans*, A. *niger* and A. *Clavatus* for antifungal activity. Unfortunately, not a single compound shows the prominent antifungal activity when compared to the Greseofulvin and Nystatin as standard.

Table 1: Antifungal activity of compound (5a-5g). (Minimal inhibition concentration; MIC µg/ml).

Compound	C. *albicans*	A. *niger*	A. *clavatus*
5a	500	1000	>1000
5b	500	500	1000
5c	1000	500	500
5d	500	500	500
5e	1000	500	500
5f	500	500	>1000
5g	>1000	1000	>1000
Nystatin	100	100	100
Greseofulvin	500	100	100

Table 2: Antibacterial activity of compound (5a-5g). (Minimal inhibition concentration; MIC µg/ml).

Antibacterial activity (MIC, µg/ml)				
Compound	**E. coli (Gram negative)**	**P. Aeruginosa (Gram negative)**	**S. Aureus (Gram positive)**	**S. Pyogenus (Gram positive)**
5a	100	100	75	125
5b	100	100	100	100
5c	100	100	125	125
5d	75	125	100	100
5e	100	100	200	250
5f	100	125	62.5	100
5g	125	250	75	100
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

CONCLUSION: The structures of all synthesized compounds were well supported by spectroscopy analysis data. In antibacterial study the derivatives were subjected for their antibacterial evaluation where some of the compounds found to have excellent antibacterial potential. However, antifungal activity of all synthesized compounds was unsatisfactory.

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