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Synthesis and evaluation of substituted benzimidazole motifs for preliminary antimicrobial drug target

Olayinka O. Ajani[1*], Damilola V. Aderohunmu[1], Shade J. Olorunshola[2], Bamidele M. Durodola[1] and Olatunde M. Ogunleye[1]

[1]Department of Chemistry, School of Natural and Applied Sciences, Covenant University, Canaan land, Km 10 Idiroko Road, P. M .B. 1023, Ota, Ogun State, Nigeria.
[2]Department of Biological Sciences, School of Natural and Applied Sciences, Covenant University, Canaan Land, Km 10 Idiroko Road, P. M. B. 1023, Ota, Ogun State, Nigeria.

Benzimidazole scaffolds are structural isosteres of naturally occurring nucleotides which allows them to interact with the biopolymers and enzymatic sites of living systems. The aim of this present work is to synthesize selected 2-alkanamino-benzimidazole derivatives in order to investigate their antimicrobial efficiency for possible future drug development. The series of targeted compounds were conventionally synthesized in good to excellent yields via [4+1]-cyclo-addition of o-phenylenediamine with some L-amino acid and purified by recrystallization or column chromatography where necessary. The chemical structures were confirmed by physico-chemical and spectral data which include UV, IR, 1H- and 13C-NMR. In addition, the antimicrobial properties of the synthesized benzimidazole derivatives were determined on six bacteria isolates alongside with gentamycin standard drug using agar diffusion method. The synthesized compounds showed broader activities spectrum than gentamycin and (1H-benzo[d]imidazol-2-yl)methanamine, 10a emerged as the most potent. Based on the versatility of the synthetic pathway and improved antibacterial activity, these compounds are recommended as good candidates for further studies in terms of MIC test, toxicity profile as some of them might pave way in the pharmaceutical research for future drug design.

Key words: Benzimidazole, cyclo-addition, zone of inhibition, spectroscopy, drug design.

INTRODUCTION

Benzimidazole is nitrogen containing heterocyclic scaffold with pKa of 12.75 while its conjugate acid has a pKa of 5.68, which is less basic than imidazole (Eicher et al., 2004). Among the large variety of heterocyclic systems developed, the ortho-fused bicyclic moiety benzimidazole appears to be a particularly effective heterocyclic system considering its interaction with the AT1 receptor (K-Vyas and Ghate, 2010) and important enzyme sites. Numerous compounds containing benzimidazole moieties have been reported to exhibit diverse biological and pharmacological properties including analgesic (Datar and Limaye, 2015),

*Corresponding author. E-mail: ola.ajani@covenantuniversity.edu.ng. Tel: +2348061670254.

antimicrobial (Meshram an. Vala, (2015), anticancer (Singla et al., 2015), anti-HCV (Zhao et al., 2015), anti-HIV (Pan et al., 2015), anthelmintic (Munguía et al., 2015), antitumor (Chu et al., 2015), antitubercular (Siddiki et al., 2015), antimalarial (Ndakala et al., (2011), antihypertensive (Zhang et al., 2015), antioxidant (Usta et al., 2015), and anti-inflammatory (Kumar et al., 2015) among others.

Conventionally, a drug is designated by its dominant or by its first recognized function; hence, benzimidazole nucleus is the core structure of commercially available drugs such as anthelmintics (albendazole, 2; thiabendazole, 3), antifungal agents (benomyl, 4), anticancer (bendamustine 5), anti-ulcer drug (rabeprazole, 6), receptor antagonist drug (astemizole, 7), antihypertensive (telmisartan 8) and anti-inflammatory and anti-ulcer drug (omeprazole 9) (Biswas et al., 2003). We have herein investigated the efficient synthesis of 2-substituted benzimidazole derivatives in order to evaluate their antimicrobial potential for future drug development.

MATERIALS AND METHODS

Physico-chemical and spectra study

Solvents used were of analytical grade and were used directly without further purification. Melting points were determined in open capillary tubes on Stuart melting point apparatus and were uncorrected. The IR spectra were run in solid state using the Bruker FT-IR while UV analyses of all the samples were run in ethanol, using UV-Genesys. The ^{1}H-NMR and ^{13}C-NMR of the compounds were run on Bruker NMR machine at 400 MHz and 100 MHz respectively using DMSO-d_6.

Antimicrobial activity

All the synthesized benzimidazole templates 10a-i and gentamycin standard drug were screened for antimicrobial activity on the targeted six organisms using agar well diffusion method as described by Russell and Furr (1977) method.

General procedure for synthesis of 2-alkanaminobenzimidazole derivatives, 10a-i

o-Phenylenediamine (1.08 g, 10 mmol) and corresponding amino acids a-h or synthon i (10 mmol) was added sequentially to 10 mL of toluene in a quickfit flask. The reacting mixture was heated under reflux for 9 h under the influence of a magnetic stirrer to obtain coloured solution which was allowed to cool overnight. The crystals formed were filtered and air-dried to afford the 2-alkanaminobenzimidazoles, 10a-i in excellent yields.

Synthesis of (1*H*-benzo[*d*]imidazol-2-yl) methanamine, 10a

When **a =** glycine, yield 97.3%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å) $_{max}$: 236 (1.7782), 290 (1.324), 407 (0.8541). í in σm^{-1} (functionality responsible): 3384, 3363 (N-H of NH_2, 2 bands), 3245 (N-H), 3021 (C-H aromatic), 2930 (C-H aliphatic), 1605 (C=C), 1580 (C=N), 741 (Ar-H).

1-(1*H*-benzo[d]imidazol-2-yl)-2-methylpropan-1-amine, 10b

When b = L-valine, yield 85.1%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 209 (3.3653), 236 (2.893), 293 (2.5185). í in σm^{-1} (functionality): 3384, 3363 (N-H of NH_2, 2bands), 3117 (N-H), 2962 (C-H aliph. of CH_3), 1603 (C=C arom.), 1591 (C=N), 775 (Ar-H).

4-amino-4-(1*H*-benzo[d]imidazol-2-yl)butanamide, 10c

When **c =** L-glutamine, yield 84.3%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 209 (3.2842), 236 (3.1562), 293 (2.3054). í in σm^{-1} (functionality responsible): 3384, 3364 (N-H of NH_2, two bands), 3235 (N-H), 3200 (N-H), 2927 (C-H aliphatic of CH_2), 2803 (C-H aliphatic), 1685 (C=O of amide), 1605 (C=C aromatic), 1575 (C=N), 748 (Ar-H).

2-(pyrrolidin-2-yl)-1*H*-benzo[*d*]imidazole, 5d

When **d** = L-proline, yield 89.0%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 209 (3.3520), 239 (2.6522), 293 (2.2810). í in σm^{-1} (functionality): 3322 (N-H), 3124 (N-H aliph.), 3027 (C-H arom.), 2928 (C-H aliphatic of CH_2), 2857 (C-H aliph.), 1600 (C=C arom.), 1577 (C=N), 744.

4-(2-amino-2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)phenol, 5e

When e = L-tyrosine, yield 83.3%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 209 (3.3677), 236 (2.796), 293 (2.4698). í in σm^{-1} (functionality responsible): 3384, 3363 (N- H of NH_2, two bands), 3200 (N-H), 3117 (O-H, broad), 2929 (C-H aliphatic of CH_2), 2876, 2827 (C-H aliphatic), 1600 (C=C aromatic), 1584(C=N), 739 (Ar-H).

1-(1*H*-benzo[*d*]imidazol-2-yl)-2-phenylethanamine, 5f

When **f** = L-phenylalanine, yield 84.0%, m.p. > 200C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 212 (3.4050), 236 (2.9974), 293 (2.6857). í in σm^{-1} (functionality responsible): 3384, 3363 (N-H of NH_2, two bands), 3209 (N-H), 3022 (C=H aromatic), 2889 (C-H aliphatic), 1615 (C=C aromatic), 1575 (C=N), 749 (Ar-H).

1-(1*H*-benzo[d]imidazol-2-yl)-3-(methylthio)propan-1-amine, 5g

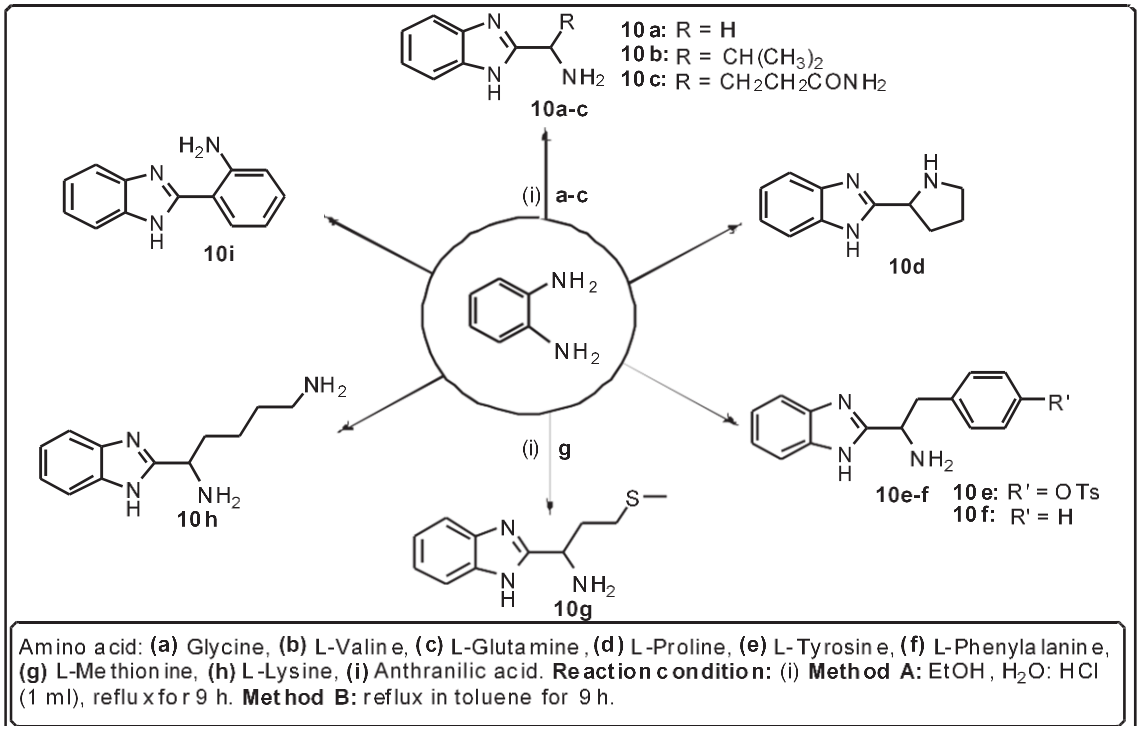
When **g** = L-methi- onine, yield 97.1%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 209 (3.3181), 236 (2.6064), 293 (2.2553). í in σm^{-1} (functionality responsible): 3384, 3363 (N-H of NH_2, two bands), 3276 (N-H), 3173 (N-H), 3029 (C-H aromatic), 2921 (C-H aliphatic of CH_2), 2885 (C-H aliphatic), 1600 (C=C), 1575 (C=N), 745 (Ar-H).

1-(1*H*-benzo[d]imidazol-2-yl)pentane-1,5-diamine, 5h

When **h** = L-lysine, yield 88.2%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 206 (2.6222), 236 (1.5911), 296 (1.6021). í in σm^{-1} (functionality responsible): 3383, 3365 (N-H of NH_2, two bands), 3276 (N-H), 3173 (N-H), 3026 (C-H aromatic), 2929 (C-H aliphatic of CH_2), 2845 (C-H aliphatic), 1603 (C=C aromatic), 1583 (C=N), 742 (Ar-H).

2-(1*H*-benzo[*d*]imidazol-2-yl)aniline, 5i

When **i**: anthranilic acid, yield 83.4%, m.p. > 200°C. $\ddot{e}_{max}$ in nm (log å)$_{max}$: 209 (3.1553), 242 (2.4914), 335 (2.0899). í in σm^{-1} (functionality responsible): 3384, 3366 (N-H of NH, two bands), 3201 (N-H), 3031



Scheme 1. Synthetic pathways for the preparation of targeted benzimidazole motifs 10a-I.

(C-H aromatic), 1576 (C=N), 748 (Ar-H).

RESULTS AND DISCUSSION

Benzimidazole derivatives are crucial structural scaffolds found in diverse library of biologically active compounds which are therapeutically useful agents in drug discovery and medicinal chemistry research. Hence, in the continuation of our effort on the efficient synthesis of benzimidazole (Ajani et al., 2013) and investigation of their antimicrobial potential, we have herein synthesized some 2-substituted benzimidazole derivatives via [4+1]-cyclo-addition of o-phenylenediamine and nine COOH donors. The direct condensation of the –COOH functionality of eight amino acids a-h and anthranilic acid synthon i with the NH_2 groups of the *o*-phenylenediamine was employed in this category in order to get the alkanamino- bearing benzimidazole derivatives 10a-i (Scheme 1), because it was envisaged that the amino acid insertion in benzimidazole framework at 2-position might create a synergistic effect to boost the biological activity of the titled compounds. In detail, the reaction optimization study was conducted by comparing the synthesis in the presence of HCl: H_2O mixture (Method A) to that of simple reflux in catalyst-free medium using toluene solvent (Method B) as shown in Figure 1. The reaction of the equimolar amount of *o*-phenylenediamine with nine different amino acids in aqueous HCl, (hydrochloric acid) was successfully achieved by heating the mixture under reflux at a carefully controlled temperature for 9 h to afford alkanamino-bearing benzimidazole derivatives 10a-i. Method B involved the use of the same stoichiometry in toluene solvent without any catalyst. Method B was discovered to be eco-friendly, atom-economical, afforded the products via easier work-up and in higher yields (Figure 1). From the spectroscopic point of view, the uv transition with the lowest wavelengths for the compounds 10a-i observed at 206 to 212 nm, were as a result of the ð→ ð* transition of C=C which depicted the presence of benzene ring in all the compounds. Bathochromic shifts led to the presence of other peaks at higher wavelengths ranging from 236 to 406 nm. Some of these were as a result of ð→ n transition which may be ascribed to the auxochromic C=N group; characteristic of K bands of C=N functional group (Komurcu et al.,1995). The infrared spectra of the compounds 10a-i showed absorption bands due to the stretching vibrations of N-H, C-H aromatic, C-H aliphatic, C=C and C=N at 3384-3201, 3031-3021, 2962-2885, 1615-1600 and 1591-1575 cm^{-1} respectively. An additional band was noticed in 10c at 1685 cm^{-1} which depicted the presence of C=O of amide in 10c alone.

The result of antimicrobial activity *in vitro* was as shown in the Table 1. It was discovered that gentamycin was least active against *K. pneumonia* and *E. coli* whereas 10a-i exhibited high to excellent activities on these two organisms. Also, gentamycin was moderately active on *S. aureus, S. faecalis, P. vulgaris* and *P. aeruginosa* while all benzimidazoles possessed high to excellent activities on

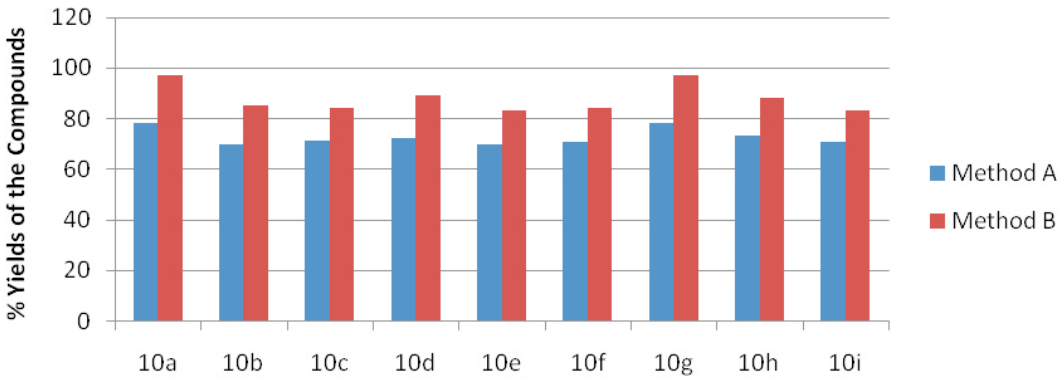


Figure 1. Comparative study of two methods for optimization study.

Table 1. Result of *in vitro* antimicrobial screening with zones of inhibition in (mm).

Comp No↓ / Organism→	*Staphylococcus aureus*	*Streptococcus faecalis*	*Klebsiella pneumonia*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Escherichia coli*
10a	++++	++++	++++	++++	+++	++++
10b	++++	++++	++++	+++	++	++++
10c	+++	+++	++++	+++	++++	++++
10d	++++	++++	++++	++	++++	++++
10e	+++	+++	+++	++	+++	+++
10f	+++	+++	+++	+++	+++	+++
10g	+++	+++	+++	++	+++	+++
10h	+++	+++	+++	+++	+++	+++
10i	+++	+++	+++	+++	+++	+++
Gentamycin	++	++	+	++	++	+

+ = Least activity (1 – 10 mm); ++ = Moderate activity (11 – 20 mm); +++ = High activity (21 – 30 mm), ++++ = Excellent activity (>30 mm).

these four organisms except in the case of 10e, 10d and 10g against *P. vulgaris* and 10b against *P. aeruginosa* where moderate activities were noticed (Table 1).

Conclusion

The alkanamino-benzimidazoles were successfully achieved in a higher yield, easier work-up and highly economical procedure using toluene solvent as compared with former conventional method in aqueous acid as catalyst. The compounds were screened in vitro against six bacterial isolates most of which were associated with the gastrointestinal tract damage in man and animal. All the compounds exhibited better activity than the gentamycin standard drug. Hence, they are good candidates for further study as the new replacement for gentamycin might emerge from this series.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Ajani OO, Ezeoke EK, Edobor-Oso A, Ajani OA (2013). Facile synthesis and characterization of new 2,3-disubstituted benzimidazole derivatives. International Research Journal of Pure and Applied Chemistry 3:10-21.

Biswas K, Bandyopadhyay, U, Chattopadhyay I, Varadaraj A, Ali E,. Baner- jee RK (2003). A novel antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of hydroxyl radical. Journal of Biological Chemistry 278:10993-11001.

Chu B, Liu F,Li L, Ding C, Chen K, Sun Q, Shen Z, Tan Y, Tan C, Jiang Y (2015). A benzimidazole derivative exhibiting antitumor activity blocks EGFR and HER2 activity and upregulates DR5 in breast cancer cells. Cell Death Disease 6(3):1686.

Datar PA, Limaye SA (2015). Design and synthesis of mannich bases as benzimidazole derivatives as analgesic agents. Antiinflamm. Antiallergy. Agents Medicinal Chemistry 14:35-46.

Eicher T, Hauptmann S, Speicher A (2004). The chemistry of heterocycles: Structure, reactions, syntheses, and applications.

Wiley-VCH GmbH and Co. KGaA, Germany.
Komurcu SG, Rollas S, Uglen M, Gorro JW (1995). Evaluation of some aryl hydrazones of p-aminobenzoic acid hydrazide as antimicrobial agents and their in-vitro hepatic microsomal metabolism. Bollettino Chimico Farmaceutico 134:375-379.
K-Vyas V, Ghate M (2010). Substituted benzimidazole derivatives as angiotensin II-AT1 receptor antagonist: A review. Mini Reviews in Medicinal Chemistry 10(14):1366-1384.
Kumar N Sharma CS, Ranawat MS, Singh HP, Chauhan LS, Dashora N (2015). Synthesis, analgesic and anti-inflammatory activities of novel mannich bases of benzimidazoles. Journal of Pharmaceutical Investigation 45:65-71.
Meshram GA, Vala VA (2015). Synthesis, characterization and antimicrobial activity of benzimidazole-derived chalcones containing 1,3,4-oxadiazole moiety. Chemistry of Heterocyclic Compounds, 51:44-50.
Munguía B, Michelena M, Melian E, Saldaña J, Ures X, Manta E, Domínguez L (2015). Development of novel valerolactam-benzimidazole hybrids anthelmintic derivatives: Diffusion and biotransformation studies in helminth parasites. Experimental Parasitology 153:75-80.
Ndakala AJ, Gessner RK, Gitari PW, October N, White KL, Hudson A, Fakorede F, Shackleford, DM, Kaiser M, Yeates C, Charman SA, Chibale K (2011). Antimalarial pyrido[1,2- a]benzimidazoles. Journal of Medicinal Chemistry 54:4581-4589.
Pan T, He X, Chen B, Chen H, Geng G, Luo H, Zhang H, Bai C (2015). Development of benzimidazole derivatives to inhibit HIV-1 replication through protecting APOBEC3G protein. European Journal of Medicinal Chemistry 95:500-513.
Russell AD, Furr JR (1977). The antibacterial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid. Journal of Applied Bacteriology 43(2):253-260.
Siddiki AA, Bairwa VK, Telvekar VN (2015). Synthesis and biological evaluation of novel N' (4-aryloxybenzylidene)-1H-benzimidazole-2 carbohydrazide derivatives as anti-tubercular Agents. Combinatorial Chemistry and High Throughput Screening 17:630-638.
Singla P, Luxami V, Paul K (2015). Triazine-benzimidazole hybrids: anticancer activity, DNA interaction and dihydrofolate reductase inhibitors. Bioorganic and Medicinal Chemistry 23:1691-1700.
Usta A, Yýlmaz F, Kapucu G, Baltaþ N, Menteþe E (2015), Synthesis of some new benzimidazole derivatives with their antioxidant activities Letters in Organic Chemistry 12:227-232.
Zhang Y, Xu J, Li Y, Yao H, Wu X (2015), Design, synthesis and pharmacological evaluation of novel no-releasing benzimidazole hybrids as potential antihypertensive candidate. Chemical Biology and Drug Design 85:541-548.
Zhao F, Liu N, Zhan P, Jiang X, Liu X (2015). Discovery of HCV NS5B thumb site I inhibitors: core-refining from benzimidazole to indole scaffold. European Journal of Medicinal Chemistry 94:218-228.