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Synthesis and Antibacterial Activity of New Chiral Aminoalcohol and Benzimidazole Hybrids

İlknur Çiçek,[a] Dr.Turgay Tunç,[b] Hatice Ogutcu,[c] Suzan Abdurrahmanoglu,[d] Aslıhan Günel,[a] and Nadir Demirel*[a]

New chiral aminoalcohol-benzimidazole hybrids have been synthesized from commercially available aminoalcohols [*S*(+)-Phenylglycinol, *S*(−)- Phenylalaninol and *S*(+)- Leucinol] and 2-(chloromethyl)-*N*-tosyl-1-*H*-benzimidazole. The synthesized compound were characterized by IR, NMR, and LC-MS analysis. The antibacterial properties of aminoalcohol-benzimidazole hybrid molecules **4a**, **4b**, and **4c** were investigated against both gram (+ve) and gram (-ve) bacterial pathogens by the well-diffusion method using several standarts. The cell-based biological experiment was consistent with *in silico* studies. Furthermore, *in silico* studies revealed that all synthesized compounds could be suggested as potential drugs for inhibition of both peptide deformylase for bacteria and sterol 14α-demethylase for yeast.

1. Introduction

Benzimidazole, first synthesized by Hobrecker in 1872, is an important heterocyclic compound found in many natural products.[1–5] In medicinal chemistry, benzimidazole derivatives are one of the highest significant and powerful structures.[6–7] Since benzimidazoles have considerable bioactivities, such as anti-protozoal, anti-microbial, anti-inflammatory, analgesic, antioxidant, anthelmintic, anti-hypertensive, and anticancer activity, they have an essential role in drug discovery.[8–9] Furthermore, compounds that bear benzimidazole ring also show important activity against several viruses such as human cytomegalo virus, HIV and influenza.[10–11] In recent years, some benzimidazole derivatives have found to have considerable antibacterial activity.[12–16] The chiral aspects of benzimidazoles derivatives have not attracted much attention due to the numerous articles on benzimidazoles in the category of numerous therapeutic agents in the medical field. Especially after the emergence of bifunctional benzimidazoles in recent years, strong researches have started in the chiral applications of benzimidazole derivatives. Benzimidazoles have acquired an important role in chiral processes because of their rigid structure, the capability to form hydrogen bonding, basic characteristics, (pKa = 5.4) high stability, nucleophilic properties, easy assembly of a chiral unit and having pyrrole and pyridine type nitrogen atoms fused to the benzene ring.[17–19] The biological and pharmacological properties of a chemical are influenced by the chiral nature of the compound. In biological processes, asymmetric centres have considerable importance.

The response of an organism to these molecules depends on how these molecules fit into biological receptors. The use of chiral chemicals as drugs such as anti-fungal, insecticides, anti-arrhythmic, antihistaminic and anti-cancer and anti-microbial reduces undesirable toxic and ecological effects as well as unnecessary excess drug use.[20–21]

A number of review articles have been published that provide a comprehensive overview of the antimicrobial activity of benzimidazole derivatives.[22–25]

Peptide deformylase's (PDF), which are essential metalloenzymes for cell growth, co-translationally remove the formyl group carried by the initiator methionine, which requires protein synthesis in bacteria.[26] They have been considered as a potential target for a new type of antibiotics for bacteria.[27–28] Besides the many types of PDF inhibitors,[29] Actinonin was the first one that synthesized for this aim.[30] Due to the lack of structural stability, actinonin showed weak or moderate activity against the bacteria. Johnson and coworkers have been examined the enzyme inhibition of some potential PDF inhibitors against community-acquired pneumonia experimentally in their studies.[31] On the other hand, inhibition of sterol 14α-demethylase, which is an essential enzyme for biosynthesis of sterols, is considered as the primary target for potential antifungal drug candidates.[32] The role of this enzyme activity presence of anti-fungal drugs has been extensively studied by researchers both experimentally and theoretically.[33–34] In order to examine the mechanism of antimicrobial effect of the novel compounds (**4a**, **4b**, and **4c**), an *in silico* study was planned

[a] *İ. Çiçek, Dr. Aslıhan Günel, Prof. Nadir Demirel*
Department of Chemistry, Faculty of Arts and Sciences
Ahi Evran University, 40100, Kırşehir, Turkey
Homepage: https://akademik.ahievran.edu.tr/site/nadirdemirel
E-mail: demireln@ahievran.edu.tr
[b] *Dr.Turgay Tunç*
Department of Chemistry Engineering and Process, Faculty of Engineering, University of Ahi Evran, Kırsehir, 40100, Turkey
[c] *Prof. Hatice Ogutcu*
Department of Field Crops, Faculty of Agriculture, University Ahi Evran, Kırsehir, 40100,Turkey
[d] *Dr. Suzan Abdurrahmanoglu*
Department of Chemistry, Faculty of Arts and Science, Marmara University, Istanbul, 34722, Turkey

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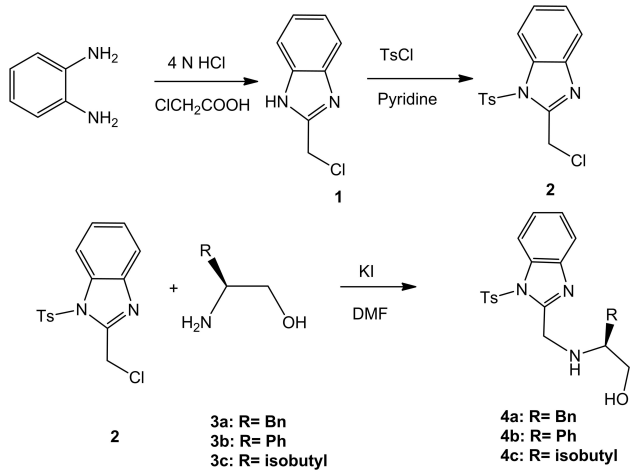
based on the binding interaction between the ligands (**4a**, **4b**, and **4c**) and peptide deformylase in both gram (−) and gram (+) bacteria and sterol 14 α-demethylase in *Candida albicans*.

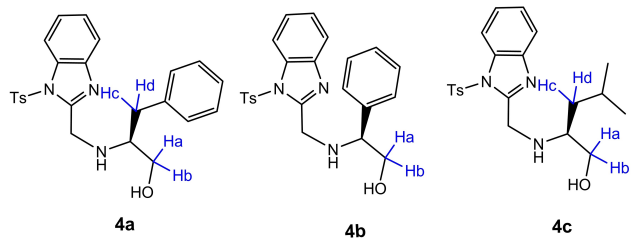
2. Results and Discussion

2.1. Chemistry

The synthesis of chiral amino alcohols-benzimidazole hybrid starts with the reaction of monochloro acetic acid and 1,2-diaminobenzene in 4 N HCl and then *N*-tosylation of 2-(chloromethyl)-1-*H*-benzimidazole **1** with *p*-toluensulfonyl chloride in pyridine gave 2-(Chloromethyl)-1-[(4-methylphenyl) sulfonyl]-1*H*-benzimidazole **2**. All spectroscopic and physical data of synthesized compound **1** and **2** are consistent with the literature.[35–36] (Scheme 1) The chiral amino alcohols-benzimidazole hybrid compounds were synthesized the reaction of commercially available chiral amino alcohols with the 2-(chloromethyl)-*N*-tosyl-1-*H*-benzimidazole **2**. (Scheme 1) The nucleophilic substitution was performed in the presence of KI in dry DMF using excess amino alcohols as a base. The reactions resulted in high yield. (84–90 %)



Scheme 1. The synthesis of chiral amino alcohols benzimidazole hybrids.



Scheme 2. The diastereotopic proton of methylene groups in chiral amino alcohol-benzimidazole hybrids.

NMR spectra of the synthesized compounds appear as expected. When the methylene protons are adjacent to an asymmetric centre, the methylene protons are not equivalent protons; as a result, they gave an AB system in the NMR spectrum. The NMR spectra revealed that the AB system was observed clearly in the case of Ha and Hb protons of **4a**, **4b, and 4c**. (**S4–S10**) **4a** and **4c** have two methylene groups adjacent to an asymmetric centre. (Scheme -**2**) But AB system was not observed in the case of Hc and Hd protons of **4a** and **4c**. Hc and Hd protons of **4a** seems as equivalent protons; as a result, they gave a doublet. (**S4**) In compound **4c**, the methylene protons (Hc and Hd) adjacent to the asymmetric centre has also another adjacent proton (methine), so the expected AB system became a more complicated one.(**S10**)The methylene proton of the benzimidazole unit gave two doublets. This system was observed in a similar

structure.[37–38] All the other spectral data were consistent with the structures.

3. Biological activity

3.1. Antibacterial activity

The summary of inhibition zones (mm) was given in Table 1. It has been well known that *S.aureus,* a versatile pathogen, is multifarious in nature and varies in intensity of infection, affecting the skin, soft tissue, respiratory system, bone joints, and endovascular tissues.[39] After the breakage of the epithelium, this extracellular pathogen bacteremia may cause vital diseases such as pneumonia, osteomyelitis, endocarditis, and

Table 1. Inhibition zone (mm) of the synthesized compounds **4a**, **4b** and **4c** towards different Gram (+ve), Gram (−ve) and yeast.

Compds	*Gram (+)* *Micro-coccus luteus*	*Staphylo-coccus epidermis*	*Staphylo-coccus aureus*	*Bacillus cereus*	*Gram (−)* *Pseudo-monas putida*	*Klebsiella pneumonia*	*Entero-bacter aerogenes*	*Salmonella typhi H*	*Escherichia coli*	*Proteus-vulgaris*	*Yeast Candida albicans*
4a	20	23	21	17	19	20	18	17	20	20	19
4b	21	20	24	16	20	15	15	16	20	16	20
4c	20	22	26	20	23	22	20	20	22	21	20
AMP 10[a]	22	26	30	23	8	21	21	11	10	17	NT
SXT 25[a]	21	25	24	25	18	20	19	17	18	19	NT
AMC 30[a]	25	27	30	20	15	21	20	19	14	20	NT
K 30[a]	23	25	25	28	14	23	24	20	25	21	NT
NYS 100[a]	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	20

[a] SXT25, sulfamethoxazole 25 μg; AMP10, Ampicillin 10 μg; NYS100, Nystatin 100 μg; K30, Kanamycin 30 μg; AMC30, Amoxycillin 30 μg; NT: not tested; Diameter of zone inhibition (mm).

septic shock.[40] Although compound **4b** exhibited the same level of activity as STX25 (24 mm) against *S. aureus*, compound **4c** (26 mm) showed higher inhibition effect than standard antibiotics SXT25 (24 mm) and K30 (25 mm) against *S.aureus*. (Table 1, **S13**) Furthermore, compound **4c** showed good inhibition activity as a standard antibiotic AMC30 (20 mm) against well-known an opportunist pathogen *B. cereus* that is associated with food-borne illness.[41] In recent years, *P. putida* has become a significant human pathogen. *P. putida* creates nosocomial infections, especially in immunocompromised patients and in patients with medical devices or catheters, because they may colonize moist and lifeless hospital surfaces.[42–43] All compounds **4a**, (19 mm) **4b** (20 mm), and **4c** (23 mm) showed higher inhibition activity against *P. putida* than all standard antibiotics tested. (Table 1, **S13**) In the case of *K. pneumonia*, **4c** (22 mm) showed better activity than the standard antibiotics except for K30. (Table 1, **S13**) In addition, **4c** showed the same activity as K30 (20 mm) against *E. enterogenes*. (Table 1, **S13**) *Salmonella serovars* cause various clinical symptoms, from asymptomatic infection to acute typhoid-like syndromes in infants or certain highly susceptible animals.[44] Compounds **4a** and **4c** showed the same level of inhibition activity as standard antibiotics STX25 (17 mm) and K30 (20 mm) respectively against this important pathogen *Salmonella typhi H* (Table 1, **S13**) In the case of *E. coli*, **4a** (20 mm), **4b** (20 mm) and **4c** (22 mm) showed better activity than the standard antibiotics except for K30. (Table 1, **S13**) *P. vulgaris* is another crucial pathogen. Compounds **4a** and **4c** showed the same inhibition activity as standard antibiotics AMC30 (20 mm) and K30 (21 mm) against *P. vulgaris*. (Table 1, **S13**) Compound **4b** was found to have the same degree of inhibition activity as a standard antibiotic SXT 25 having a 21 mm zone against *M. luteus*. (Table 1, **S13**) Systemic fungal infections are vital for the immune compromised patient (organ or bond transplantation, aids, cancer chemotherapy). *Candida albicans* have revealed as significant causes of mortality and morbidity in this type of patient. **4a**, and **4c** showed similar inhibition activity as a standard antifungal against *C. albicans*. (Table 1, **S13**) In general, compound **4c** was found to be a higher level of inhibition activity than **4a** and **4b** in the case of gram (−) bacteria. The result also clearly showed that all compounds have approximately the same level of inhibition activity against gram (+) bacteria and yeast. Benzimidazole units are common in all three compounds, and the structural difference is due to the amino alcohol moiety.

3.2. Molecular Docking

Results of the computational studies were evaluated by means of binding energies of all the novel compounds with the PDF of *E. coli, S. aureus,* and sterol 14α-demethylase of *C. albicans*. The binding affinities of **4a**, **4b**, and **4c**, which observed as a negative score with unit expressed kcal/mol, were presented in Table 2. As seen in Table 2, all the compounds were successfully docked with the targeted proteins. Average binding energies for all compounds were calculated as −8.0 ± 0.5 kcal/mol except the one between **4b** and *C. albicans* (5TZ1) as −9.4 kcal/mol, which is the highest score.

Table 2. Binding affinities of compounds (kcal/mol).

Compounds	*E. coli* (1LRU)	*S. aureus* (1LM4)	*C. albicans* (5TZ1)
4a	−8.3	−8.3	−8.4
4b	−8.0	−8.2	−9.4
4c	−7.6	−8.1	−8.4

Ligand **4a** has formed hydrogen bonds with the active sites such as Arg97, Gly89, Ile44, Val62 of 1LRU (*E.coli*) with bond length < 3 Å as seen in Figure 1. Besides, there are hydrophobic (pi-alkyl) interaction between ligand and Leu91, Cys129 residues, and also van der Waals interaction with Arg97 and Glu88. It was observed that the other two ligands showed similar interactions.

In Figure 2, docking pose and binding interaction between ligand **4a** and *S.auerus* (1LM4) were presented. In this case, the ligand has formed hydrogen bonds (length less than 3 Å) with the active residues Asp87, Met89 besides the hydrophobic interaction with Ile140 (alkyl), Met189 (pi-sigma), Try88 (pi-alkyl) residues and also van der Waals interaction with Asp134 residue.

For the discussion of antimicrobial properties of synthesized compounds, the binding pose and interaction with the sterol 14α-demethylase in *C. albicans* were also examined. As seen in Figure 3, all intermolecular interactions could be evidence of the inhibition effect of ligand **4b**, which showed the highest binding energy.[32] For instance, there are hydrogen bonds (bond length < 3 Å) formed between His377 and Ser378, between Phe380 and Try118, between His377 and ligand, etc., Hydrophobic interactions were also determined between Ala61 and Leu87 (alkyl), between Leu88 and ligand (pi-sigma), etc., All these interactions are inconsistent with obtained by using other antifungal drugs such as posaconazole.[32]

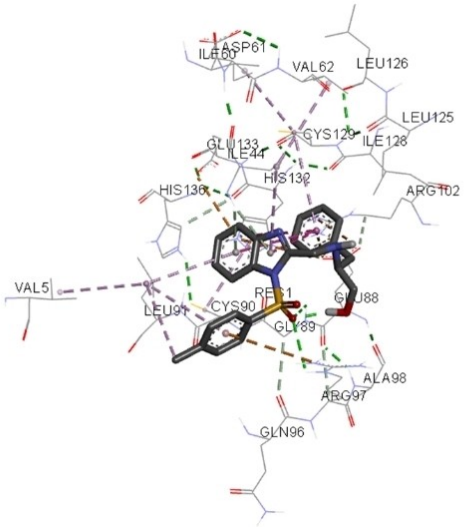


Figure 1. Interaction between compound **4a** and peptide deformylase (PDB: 1LRU) of *E. coli* (Discovery studio image).

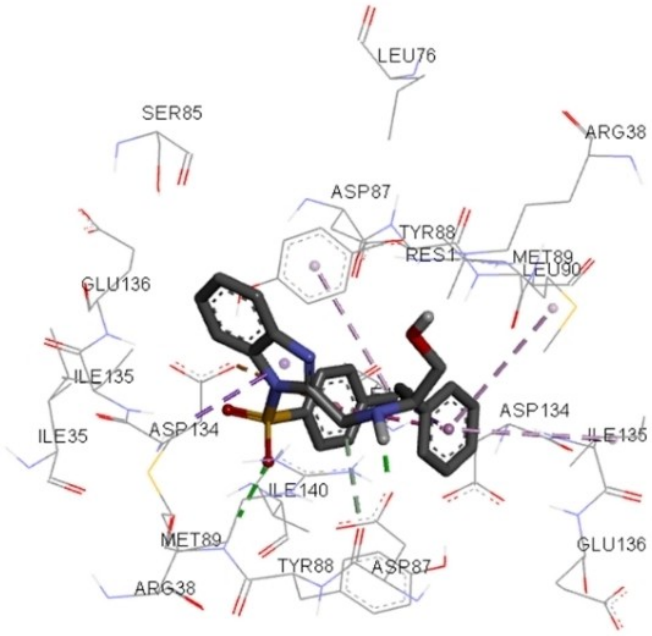


Figure 2. Interaction between compound **4a** and peptide deformylase (PDB:1LM4) of *S.auerus* (Discovery studio image).

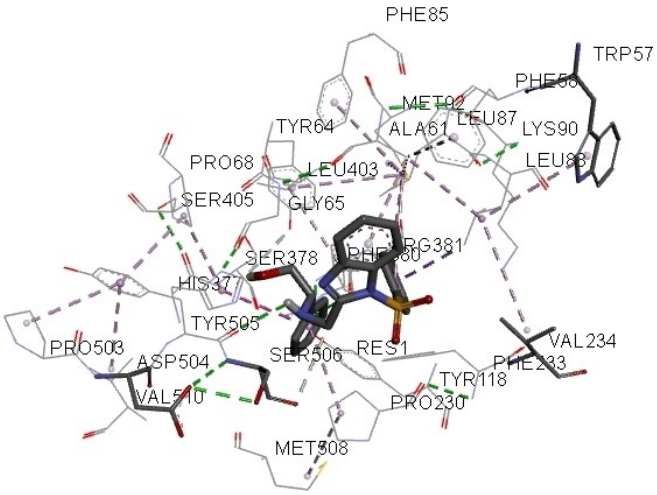


Figure 3. Interaction between compound **4a** and sterol 14α-demethylase (PDB:5TZ1) of *Candida albicans* (Discovery studio image).

The results of ADME (absorption, distribution, metabolism, and excretion) predictions of all compounds by SwissADME were given in Table 3. Lipinski's rules state that oral bioavailability of a drug is likely to exist if it possesses the following features: MW ≤ 500; H-bond donors ≤ 5; H-bond acceptors ≤ 10; c logP values ≤ 5. The results of all three compounds showed that they all obeyed the Lipinski's rules. (Table 3) The ADME results also showed that with high gastrointestinal absorption, no brain-blood barrier (BBB) permeation, and even negative lop K_p value, which refers to less skin permeation, all of them could be recognized as a drug like potential.

Table 3. The results of ADME analysis of compounds.

Sample	MW/ $gmol^{-1}$	HBD (≤ 5)	HBA (≤ 10)	cLogP (≤ 5)	Molar refractivity	Log K_p (cm/s)
4a	435.54	2	5	3,56	121.61	−6.28
4b	421.51	2	5	3,33	116.80	−6.54
4c	401.52	2	5	3,41	11.54	−6.26

MW: Molecular weight < 500, HBD: Hydrogen bond donor ≤ 5, HBA: Hydrogen bond acceptor ≤ 10, cLogP: High lipophilicity (expressed as consensus LogP) < 5, Molar refractivity should be between 40 and 130, Log K_p: skin permeability: The more negative log K_p, the less skin permeant is the compound

4. Conclusion

In conclusion, new chiral amino alcohol-benzimidazole hybrids **4a**, **4b**, and **4c** were synthesized and antibacterial properties were investigated against both Gram (+ve) and Gram (-ve) bacterial pathogens. All three chiral molecules showed good activity against tested pathogens. Molecular docking studies for the **4a**, **4b**, and **4c** indicated that all compounds interacted with the the PDF of *E. coli*, *S. aureus*, and sterol 14α-demethylase of *C. albicans* with high binding energies. The highest binding energy was observed between **4b** and *C. albicans* (5TZ1) as −9.4 kcal/mol. Furtheremore all three new chiral molecules interacted with the target protein PDF of *E. coli*, *S. aureus*, and sterol 14α-demethylase of *C. albicans* through H-bond, hydrophobic and van der Waals interaction with high binding energies.

The ADME (absorption, distribution, metabolism, and excretion) studies indicated that all three new chiral molecules **4a**, **4b**, and **4c** have high gastrointestinal absorption, no brain-blood barrier (BBB) permeation, and less skin permeation. In briefly, new chiral aminoalcohol-benzimidazole hybrid molecules could be recognized as a drug like potential towards PDF of *E. coli*, *S. aureus*, and sterol 14α-demethylase of *C. albicans.*

Supporting Information Summary

Experimental Section, General Method, Test Microorganisms, Molecular Docking, Detection of Antimicrobial Activity and Preparation and Analytical Data of **4a**, **4b**, and **4c** can be found in the Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Benzimidazole · Antibacterial Agent · Aminoalcohol · PDF inhibitor · Molecular docking

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