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Article

# Design, Synthesis, Molecular Docking, and In Vitro Antibacterial Evaluation of Benzotriazole-Based β-Amino Alcohols and Their Corresponding 1,3-Oxazolidines

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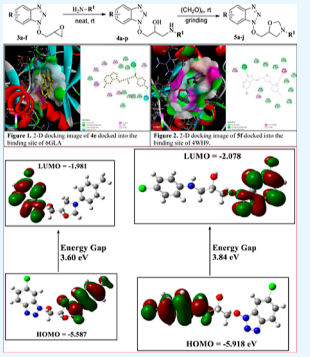
**ABSTRACT:** In the present study, a series of benzotriazole-based β-amino alcohols were efficiently synthesized in excellent yields via aminolysis of benzotriazolated epoxides under catalyst- and solvent-free conditions. Further these β-amino alcohols were successfully utilized to synthesize the corresponding benzotriazole-based oxazolidine heterocyclic derivatives. All the synthesized compounds were characterized by various spectroscopic techniques such as $^{1}$H NMR, $^{13}$C NMR, and mass spectroscopy for structure elucidation. The compounds were subjected to a microtiter plate-based antimicrobial assay. The antimicrobial activity results reveal that the compounds **4a**, **4e**, and **5f** were found to be active against *Staphylococcus aureus* (ATCC-25923) with minimum inhibitory concentrations (MICs) of 32, 8, and 64 μM, respectively. Also, the compounds **4a**, **4e**, **4k**, **4i**, **4m**, **4n**, **4o**, **5d**, **5e**, **5f**, **5g**, and **5h** showed effective activity against *Bacillus subtilis* (ATCC 6633) with MICs of 64, 16, 16, 16, 64, 16, 64, 64, 32, 64, 8, and 16 μM, respectively. A biological investigation was conducted, including molecular docking of two compounds with several receptors to identify and confirm the best ligand−protein interactions. Hence, this study found a significant strategy to diversify the chemical molecules. The synthesized compounds play a potential role as an antibacterial intensifier against some pathogenic bacteria for the development of antibacterial substances.




## 1. INTRODUCTION

Azoles, particularly the benzotriazole nucleus has a key role in both heterocyclic chemistry and medicinal chemistry.[1−3] Examples include the extensive use of benzotriazole-based compounds in the treatment of a wide range of diseases, including as anticarcinogenic,[4,5] antibacterial and antifungal,[6−8] and antimicrobial, antiprotozoal, antiviral, antimycobacterial, and antitubulin agents.[3] Furthermore, it should be noted that benzotriazole scaffolds have stacking interactions because of the conjugated benzene ring structure, which forms hydrogen bonds with a number of enzymes and receptors.[9] For instance, alizapride[4] and vorozole[5] both include a benzotriazole moiety, and these formulations exhibit outstanding inhibitory effects against a variety of proteins (Figure 1). Additionally, benzotriazole esters have been demonstrated to be effective mechanism-based inactivators of the (SARS) 3CL protease.[10]

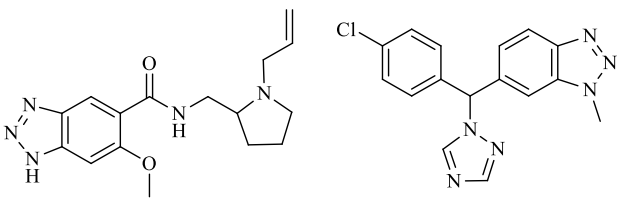


**Figure 1.** Benzotriazole moiety agonists.

Because of their improved solubility due to effective hydrogen bonding with biomolecular targets, benzotriazole derivatives were found to have a wide range of biological activities, including antitubercular, antibacterial, antiallergic, anti-HIV, antifungal, antiviral, and anti-inflammatory properties.

In light of the aforementioned biological applications, the benzotriazole moiety has been frequently used to construct biologically potent molecules which not only imparts significant properties like hydrophilicity, lipophilicity, and minimal side effects but also enhances their bioactivities.[11−14] Additionally, this will help to curb the drug-resistant pathogens, which will boost the prospects of developing superior drug alternatives.[15,16] On the other hand, β-amino alcohols are an elite class of compounds that have a wide range of medicinal applications.[17−19] Additionally, they were extensively used in the synthesis of unnatural amino acids[20,21] as well as in asymmetric synthesis such as chiral ligands and auxiliaries.[22,23] Therefore, it is anticipated that the

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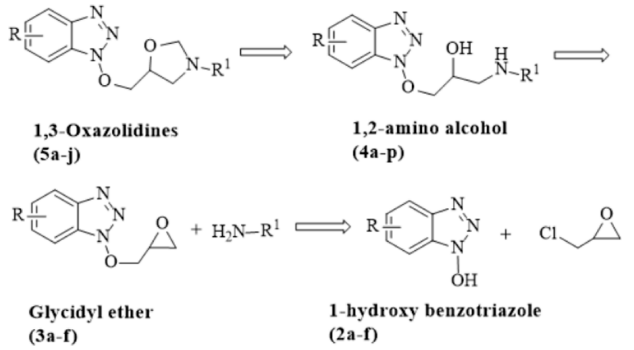
addition of a biologically active β-amino alcohol moiety to a benzotriazole scaffold will complement their action, perhaps improving the total pharmacological activity. It is important to note that screening of powerful and hybrid heterocycles has developed into a modern trend in biomedical development programs.[24−39]

## 2. RESULTS AND DISCUSSION

**2.1. Chemistry.** *2.1.1. Synthesis of Benzotriazole-Based β-Amino Alcohol and Corresponding 1,3-Oxazolidines.* The title compounds benzotriazole-based β-amino alcohols and the corresponding 1,3- oxazolidines were manufactured by utilizing the retrosynthetic method shown in Scheme 1. The major

**Scheme 1. Retrosynthetic Strategy for Benzotriazole Scaffolds**

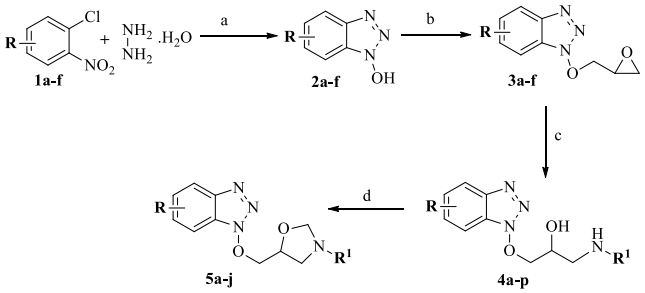



objective of this work was to improve benzotriazole chemistry[30,31] in order to create various types of β-amino alcohol analogues with 1,2,3-benzotriazole as the main heterocyclic component (for antibacterial screening, etc.). There are few reports in the literature that demonstrates the antibacterial activity of β-amino alcohols.[32−37] Therefore, to create medicinally advantageous antibacterial benzotriazole β-blocker analogues **4** and their equivalent oxazolidines **5**, a well-planned retrosynthetic technique was used to introduce a powerful β-amino alcohol unit into the benzotriazole nucleus (Scheme 1).

The main components needed to achieve the target compounds are 1-hydroxybenzotriazoles **2a−f** (HOBt) and benzotriazole epoxides **3a−f** (Scheme 2). The new significant protocol is the result of our ongoing efforts to synthesize benzotriazole-based motifs as possible pharmacophores.[38,39]

The fabricated HOBt **2a−f** and associated benzotriazolated glycidyl ethers **3a−f** served as starting materials for the synthetic methods (see the Experimental Section). Only a few reports on the synthesis of HOBt have been published in the literature,[40−50] and most of these are associated with one or more limitations, such as a long reaction time, a low yield, and vigorous reaction conditions. As a result, it was thought to be beneficial to devise a rapid and efficient procedure for producing HOBt from easily available raw materials for the synthesis of target compounds (Scheme 2, step a). We used the microwave (MW) irradiation condition as a nonconventional energy source to rapidly accomplish our goal. In this instance, MW reactor radiation was applied to an equimolar mixture of appropriate 2-nitrochlorobenzene and hydrazine hydrate in the presence of alcoholic sodium carbonate (Biotage, model: Initiator EXP EU 355301, 012180). Only

**Scheme 2. Preparation of Benzotriazole-Based β-Amino Alcohol and Corresponding 1,3-Oxazolidines**[a]




[a](a) Alcoholic $Na_2CO_3$, MW, 70/120 °C, 10/25 min; (b) (i) DMSO/$K_2CO_3$ (1 equiv), rt., (ii) (±)-epichlorohydrin, rt., 2−3 h, 80−90%; (c) neat, rt, few drops of dichloroform in case both reactants are solids; (d) $(CH_2O)_n$, neat grinding, rt.

10−25 min was required for the formation of products **2a−f** in an excellent yield of 80−90%.

The required benzotriazole-based glycidyl ethers **3a−f** were then produced in high percentage yields of 80−90% within 2−3 h via reactions between **2a−f** and (±)-epichlorohydrin at room temperature (Scheme 2 step b). The products **3a−f** were extracted by using chloroform, which were then purified by using flash chromatography with an ethyl acetate and *n*-hexane system and examined through spectral techniques such as $^1$H nuclear magnetic resonance (NMR), $^{13}$C NMR, and mass spectrometry (MS). Then, utilizing the optimal conditions stated in Scheme 2 (step c), benzotriazole-based glycidyl ethers **3a−f** were exposed to aminolysis to create a novel series of benzotriazole-based β-amino alcohols. Although there are a number of approaches for the regioselective synthesis of β-amino alcohols by opening the rings of epoxides,[51,52] the approach that uses aqueous conditions and catalyst-free conditions has caught our attention, particularly in light of green chemistry principles.[53−57] As a result, we planned to carry out aminolysis of **3a** using isopropylamine under aqueous conditions. As anticipated, an obvious reaction took place within 1 h, producing the necessary product **4m** with an excellent yield (90%). The reaction was also performed in other polar solvents, such as $CH_3CN$ (methyl cyanide), tetrahydrofuran, $CCl_4$ (carbon tetrachloride), and DCM (dichloromethane); however, water turned out to be most effective of the examined solvents. Interestingly, the reaction was investigated under neat conditions to produce the highest yield of the target chemical **4m** (Table 1, entry 9), indicating that catalyst- and solvent-free conditions were the most favorable for the anticipated reaction. However, in situations where both reactants are in the solid state, just a few drops of dichloroform were added to homogenize the reaction contents.

The process was then applied to various benzotriazole glycidyl ethers **3b−f** containing fluoro, chloro, methoxy, and nitro groups. The reaction outcome is summarized in Table 2. The synthetic benzotriazole glycidyl ethers **3** have presumably demonstrated more reactivity than regularly used and available epoxides (such as styrene epoxide, cyclohexene epoxide, and cyclopentene epoxide). It is significant to note that regioselectivity was also observed similar to the previous report.[38] Fourteen newer product molecules **4a−p** were generated without using a catalyst under neat reaction conditions, as indicated in Table 3.


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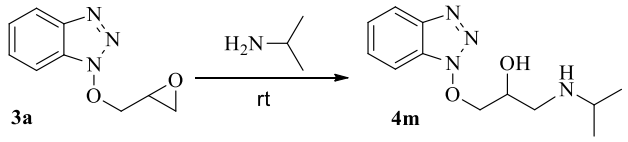
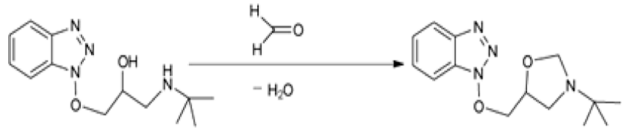
**Table 1. Optimization of Reaction Conditions**[a]




| entry | solvent | time (min)[b] | yield (%)[c] |
|---|---|---|---|
| 1 | $CH_3CN$ | 120 | 84 |
| 2 | $CHCl_3$ | 60 | 83 |
| 3 | $CH_2Cl_2$ | 60 | 82 |
| 4 | toluene | 180 | 69 |
| 5 | DMF | 130 | 73 |
| 6 | DMSO | 150 | 72 |
| 7 | water | 60 | 90 |
| 8 | hexane | 60 | 65 |
| **9** | **neat** | **25** | **95** |

[a]Stirring at room temperature. [b]Time required for completion of the reaction. [c]Yield of the isolated and purified product **4m**.

**Table 2. Optimization of Reaction Conditions**[a]




| entry | vatalyst (mol %/mg) | solvent | time (h/min)[b] | % yield (**2a**)[c] |
|---|---|---|---|---|
| 1 | catalyst (0) | formaldehyde[d] | 24 h | 20 |
| 2 | NaOH (10) | formaldehyde | 24 h | 40 |
| 3 | NaOH (10) | EtOH[e] | 10 h | 90 |
| 4 | NaOH (10) | MeOH | 10 h | 85 |
| **5** | **NaOH (10)** | neat | **05 min** | **98** |
| 6 | catalyst (0) | neat | 15 min | 30 |
| 7 | NaOH (5) | neat | 05 min | 80 |
| 8 | NaOH (15) | neat | 05 min | 98 |
| 9 | KOH (10) | neat | 05 min | 97 |
| 10 | $LiCO_3$ (10) | neat | 10 min | 70 |
| 11 | $K_2CO_3$ (10) | neat | 10 min | 68 |
| 12 | $NaHCO_3$ (10) | neat | 15 min | 65 |
| 13 | $Na_2CO_3$ (10) | neat | 10 min | 69 |
| 14 | basic alumina (50 mg) | neat | 15 min | 66 |

[a]1,2-Amino alcohol **4n** (0.5 mmol), paraformaldehyde (200 mg), and $Na_2SO_4$ (100 mg) were used. [b]Time taken for completion of the reaction (TLC). [c]Isolated yield of the purified product **5a**. [d]37−41% solution of formaldehyde in water was used (formalin). [e]2 mL of super dry alcohol (EtOH and MeOH) was used under reflux conditions.

Additionally, it has been confirmed through spectroscopy that exclusive formation of $\beta$-amino alcohols with a secondary alcohol functionality was observed in the present investigation. The formation of single regiomers has also been supported by the theoretical studies (see Supporting Information).

A series of matching benzotriazole-based 1,3-oxazolidines **5a**−**j** were produced in excellent yield by cyclizing the synthesized $\beta$-amino alcohol molecules utilizing paraformaldehyde in the presence of 10 mol % alkali, as shown in Table 3.[39]

As these scaffolds are known to exhibit a wider range of bioactivities,[58−65] we then carried out the synthesis of certain benzotriazolated 1,3-oxazolidines as medicinally privileged pharmacophores that may operate as a potential therapeutic molecule (Scheme 2, step d). The formation of target molecules, i.e., benzotriazolated $\beta$-amino alcohols **4a**−**p** and benzotriazolated 1,3-oxazolidines **5a**−**j**, were satisfactorily confirmed through $^1$H NMR, $^{13}$C NMR, and MS techniques.[38,39]

**2.2. Antimicrobial Activity of Compounds.** The 96-well microtiter plate assay results reveal that the compounds **4a**, **4e**, and **5f** were found active against *Staphylococcus aureus* [American Type Culture Collection (ATCC) 25923] with minimum inhibitory concentrations (MICs) of 32, 8, and 64 $\mu$M, respectively. Also, the compounds **4a**, **4e**, **4k**, **4i**, **4m**, **4n**, **4o**, **5d**, **5e**, **5f**, **5g**, and **5h** showed effective activity against *Bacillus subtilis* (ATCC 6633) with MICs of 64, 16, 16, 16, 64, 16, 64, 64, 32, 64, 8, and 16 $\mu$M, respectively.

**2.3. Biological Screening (Antimicrobial Screening of Compounds).** The compounds were evaluated for their antimicrobial activity using the microdilution-based method [as per Clinical and Standards Institute (CLSI) guidelines] against two Gram-positive bacterial strains (*S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633), two Gram-negative bacterial strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), one yeast (*Candida albicans* ATCC 24433) and one filamentous fungi (*Aspergillus niger* ATCC 16404). The microdilution assays were performed in accordance with the procedures outlined by the CLSI. Mueller−Hinton agar (MHA) and Mueller−Hinton broth (MHB) were prepared as per the manufacturer's instructions (HiMedia Laboratories, India) for the antibacterial assay, and for antifungal testing Sabouraud dextrose broth (SDB) was used. The precultures of the strains were prepared in MHB and SDB by fresh inoculums of the cultures incubated at 37 °C for 18−24 h (for bacteria) and at 28 °C for 48 h for the fungal cultures with 100 rpm shaking to obtain concentrations of approximately 5−6 log CFU/mL (evaluated and adjusted spectrophotometrically at 625 nm). The bacterial suspensions were further diluted with MHB to obtain a final inoculum of 5 × $10^5$ CFU/mL, and for fungal suspension 1 × $10^3$ CFU/mL inoculum was used in SDB. The antibacterial assays were performed in clear 96-well *U*-bottom microtiter plates. Initially, the test compounds were screened for antibacterial activity at a slightly higher concentration (128 $\mu$g/mL), and those that show inhibition in primary screening were further tested at different concentrations to obtain MIC and minimum bactericidal concentration (MBC) values. Each experiment was accompanied by a positive control containing broth, pathogen, and a known inhibitory compound amphotericin B or ciprofloxacin [16−0.03 $\mu$g/mL], i.e., standard antifungal and antibacterial agents, respectively, and a negative control containing broth and pathogen. The plates were incubated for 24 h at 37 °C for bacterial cultures and at 28 °C for 48 h for fungal cultures, and then observations were recorded visually. The well containing a minimum concentration of the compound in which there is no visual growth is considered as MIC. A loopy inoculum from the wells containing no visual growth was streaked on the MHA/SDB plate for MBC/MFC (minimum fungicidal concentration).

**2.4. Molecular Docking.** Molecular docking studies help medicinal chemists to identify novel drugs at low cost and in a short amount of time. AutoDock Vina and Chimera software[66,67] were used to efficiently achieve molecular docking. The docking approach was used to discover the optimum match of ligands and proteins with the least amount of energy. The compound **4e** was docked with 6GLA protein


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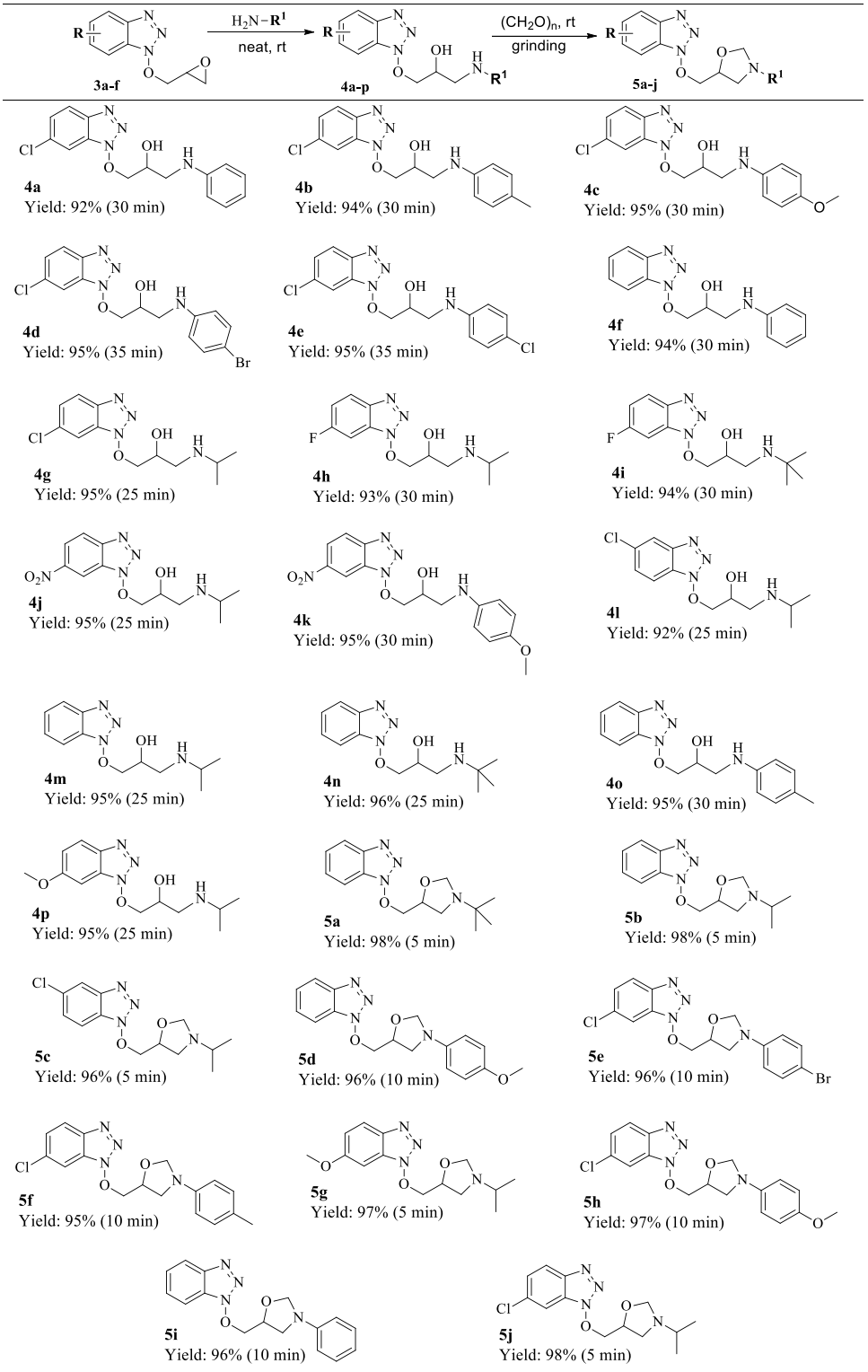
**Table 3. Substrate Scope for the Synthesis of 4 and 5 under Optimized Conditions**[a]




[a]All compounds gave satisfactory spectral ($^1$H NMR, $^{13}$C NMR, and ESI-MS) data and C, H, and N analysis within ±0.4%.

by using AutoDock software. The ligand was docked into the functional sites of the relevant protein one at a time, with the docking energy analyzed to find the lowest value. Figure 2 shows that **4e** is involved in the pi−sigma interaction with Leu956, many alkyl, as well as pi−alkyl interactions with Tyr904, Val836, Ala966, Met902, Val884, and Ala853. However, compound **4e** also bind firmly to the active site through one conventional hydrogen bond (Leu828). While Pro906 formed a halogen (Cl) interaction, besides other residues also found to be bound to control drugs through van der Waals interaction depicted in a two-dimensional (2D) plot (Figure 2). Compound **5f** binds through one conventional



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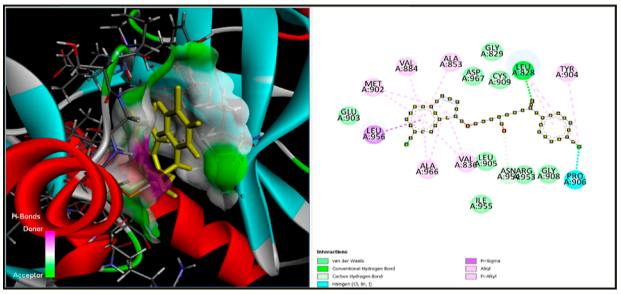

**Figure 2.** 2D docking image of **4e** docked into the binding site of 6GLA.

hydrogen bond (Arg485) and three pi−alkyl interactions with Leu398, Arg488, besides these, one pi−pi T-shaped with Phe386, other interactions are also found to bound through, carbon−hydrogen bonding and van der Waals interaction depicted in a 2D plot (Figure 3). The binding affinity energies of the control drugs are mentioned in Table 4.

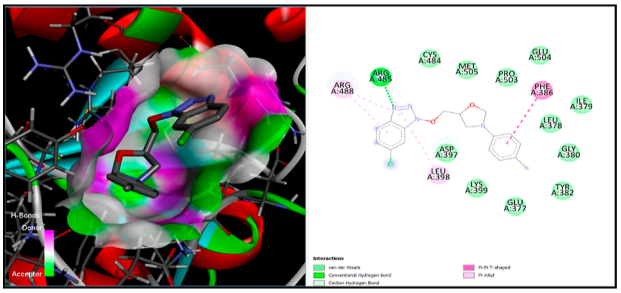

**Figure 3.** 2D docking image of **5f** docked into the binding site of 4WH9.

**Table 4. Binding Affinity Energy of Control Drugs**

| compound | protein ID | bond distance (Å) | inhibition constant (micromolar) | binding energy (k cal $mol^{-1}$) |
|---|---|---|---|---|
| **4e** | 6GLA | 2.326, 2.519 | 1.876 | −7.8 |
| **5f** | 4WH9 | 2.267, 2.322, 2.587 | 0.680 | −8.4 |

If we compare the binding affinity energy of control drugs, compounds **4e** and **5f** have the highest negative binding energy with −7.8 and −8.4 kcal/mol, respectively.

**2.5. HOMO−LUMO analysis.** The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) can be easily observed by using the GaussView program[68] and are given in Figure 4. The Frontier molecular orbitals make it possible to understand the local reactivity of molecules. According to Fukui,[69] to study a chemical reaction, only the two molecular orbitals HOMO and LUMO are of real interest. The HOMO provides information on the electron-donor character (nucleophilic site), whereas the LUMO indicates the electron-receptor character of the molecule (electrophilic site). The green color corresponds to the negative phase, and the red color indicates the positive phase. It can be seen from the plot of HOMO level of compound **5f**, all positive and negative regions are spread over the six-membered ring attached with the methyl group and partially over the five-membered rings. In the LUMO level of

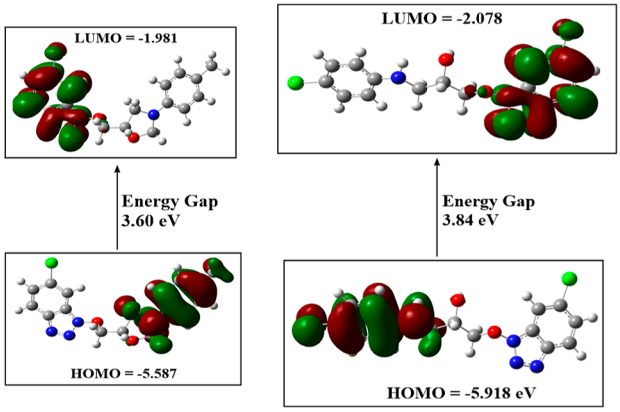


**Figure 4.** HOMO−LUMO with an energy gap.

compound **5f**, positive and negative regions are distributed over the six-membered ring attached with a chlorine atom. Whereas in the HOMO of compound **4e**, the positive and negative regions are spread over the phenyl ring and in the LUMO of compound **4e**, the positive and negative regions are spread on the phenyl ring as well as on five-membered rings. The difference between the energy of the HOMO and LUMO is defined as the energy gap $\Delta E$. This reflects the polarizability and chemical activity within a molecule. It promotes the transfer of charges in the molecule. This energy quantity allows characterizing the chemical reactivity and the kinetic stability of the molecule. When the HOMO−LUMO energy gap is high, the flow of electrons at the higher energy state is difficult, making the molecule hard and less reactive. Results reveal that the HOMO−LUMO energy gap ($E_{HOMO}-E_{LUMO}$) is equal to 3.60 eV in **5f** and 3.84 eV in **4e**, respectively.

## 3. EXPERIMENTAL SECTION

**3.1. Materials and Methods.** *o*-Nitrochlorobenzenes, hydrazine monohydrate hydrate (98%), substituted anilines, (±)- epichlorohydrin (Sigma-Aldrich), sodium carbonate, potassium carbonate, aliphatic amines such as tertiary butylamine (HiMedia), and isopropyl amine (SDFCL) were used as received. Melting points were recorded in open capillaries using the ANALAB melting point apparatus. $^{1}H$ NMR (400 MHz) and $^{13}C$ NMR (101/126 MHz) spectra were recorded in $CDCl_3/CD_3OD$ solutions with TMS as the internal standard on a Bruker AVANCE III HD spectrometer. Mass analysis was carried out using Nexera UHPLC @ 130 MPa with a SIL-30AC Nexera autosampler coupled to a liquid chromatography−mass spectrometry (LC−MS) 8030 tandem mass spectrometer manufactured by Shimadzu Corporation. Tokyo, Japan. The analysis of all these compounds was performed in a full scan mode with nitrogen as the interface gas.

**3.2. Microbial Strains.** *Bacterial strains S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853; the yeast *C. albicans* ATCC 24433; and the fungal strain *A. niger* ATCC 16404 used for antimicrobial activity screening were procured from ATCC. The strains were cultured in potato dextrose broth (PDB) and nutrient agar at 28 and 37 °C, respectively, in Petri dishes for 3−5 days.

**3.3. General Procedure for Synthesis of HOBt (2a−f).** A mixture[a] of *o*-nitro chlorobenzenes **1** (3 mmol) and hydrazine hydrate (9 mmol) in 5 mL of ethanol in the


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presence of powdered $Na_2CO_3$ (3 mmol) was subjected to irradiation at the temperature of 70 °C in a MW reactor (Biotage, model: Initiator EXP EU 355301, 012180) for 10 min (except for **2f** in which *n*-heptanol was used as the solvent and the MW vial was subjected to irradiation at 120 °C for 20−25 min). When the reaction got completed [according to thin-layer chromatography (TLC)], the mixture was poured in 20 mL of ice-cold water and its acidification was carried using 1 M HCl (pH, 3.2−3.5) to produce the crude product in the form of a white precipitate (**2c** was collected as a yellow precipitate). The precipitates were then subjected to filtration, washed using 5% NaCl (aqueous solution), and were subsequently subjected to purification by means of recrystallization either by utilizing EtOH or $CHCl_3$/MeOH solvent system to produce products devoid of impurities in a very high yield (80−90%)

**3.4. General Procedure for Synthesis of Derivatives of 1-(Oxirane-2-ylmethoxy)-1*H*-benzo[*d*][1,2,3] Triazoles (3a−f).** A mixture of 1-hydroxy benzotriazoles 2 (3 mmol) and (±)-epichlorohydrin (3.5 mmol) in 5 mL of dimethyl sulfoxide (DMSO) in the presence of powdered $K_2CO_3$ (3 mmol, activated at 40 °C) was stirred at room temperature for a duration of 2−3 h. Ten milliliters of distilled water was then added to the reaction mixture, and the product was extracted using chloroform (3 × 10 mL). The combined organic layers were washed using saturated $NaHCO_3$ aq. solution (2 × 3 mL), followed by distilled water (3 × 5 mL), then rinsed with 2 mL brine, and finally dried over anhydrous $Na_2SO_4$. Removal of excess solvent was carried out under reduced pressure conditions, and the product obtained in a crude form was subjected to purification using column chromatography to obtain respective benzotriazole glycidyl ethers **3a−f** in a high yield (80−90%).

**3.5. General Procedure for Synthesis of Benzotriazole-Based *β*-Amino Alcohols (4a−p).** A mixture of **3** (1 mmol) and an appropriate aromatic amine (1 mmol) was stirred at room temperature under solvent-free conditions. After completion of the reaction, as indicated by TLC, the product was purified by flash column chromatography using ethyl acetate/hexane as the solvent system. However, in the case of compounds **4g−j** and **4l−n** where an excess of isopropyl/tertiary butyl amine (2.5 mmol) was used, it was removed under reduced pressure before further purification. The structure of products **4** was confirmed by their elemental and spectral data.[38]

**3.6. General Procedure for Synthesis of Benzotriazole-Containing 1,3-Oxazolidines from *β*-Blockers (5a−j).** A mixture of *β*-amino alcohol (0.5 mmol) and paraformaldehyde (200 mg) in the presence of 10 mol % NaOH and sodium sulfate (100 mg) were ground for 5−10 min using a mortar and pestle.[39] Reaction progress was checked through the traditional TLC method using *n*-hexane/EtOAc (1:1) as the solvent system until completion (TLC, Table 3) Afterward, 2−3 mL of DCM or chloroform was added, and the resulting product was purified through filtration. The residue on the filter paper was subsequently washed with additional 2−3 mL of the solvent. In the end, the so-collected filtrate was reduced under vacuum evaporation. The crude products so obtained were purified by chromatography utilizing 60−120 mesh size silica gel to get pure 1,3-oxazolidines **5a−j** in an excellent yield.

## 4. CONCLUSIONS

The envisaged methodology has the advantage of opening the epoxide ring without any catalyst and solvent to afford the target molecule in an excellent yield (Table 3). In conclusion, a revised and efficient protocol advocating a catalyst- and solvent-free route is demonstrated toward the synthesis of diverse benzotriazole-based *β*-amino alcohols **4a−p** in good to excellent yield. Subsequently, these *β*-amino alcohols were utilized to furnish the corresponding oxazolidines **5a−j**. The structure of these compounds was thoroughly analyzed by $^1$H NMR, $^{13}$C NMR, and MS. All of the synthesized compounds were screened for in vitro pharmacological activities. It was observed that the compounds **4a**, **4e**, and **5f** showed high potential antibacterial activity against *S. aureus* (ATCC 25923), while the compounds **4a**, **4e**, **4k**, **4i**, **4m**, **4n**, **4o**, **5d**, **5e**, **5f**, **5g**, and **5h** reveal potent activity against *B. subtilis* (ATCC 6633) and induced postantibiotic effects against both Gram-positive pathogens (Supporting Information). Molecular docking studies of compounds **4e** and **4f** were carried out on the proteins 6GLA and 4WH9. The resultant binding energy was −7.8 and −8.4 kcal/mol, indicating that the title compounds can be studied further for their medicinal application.

Our aim was to explore the bioactive antibacterial potential of the compounds that are synthesized through a novel methodology. It can be inferred that **4e**, **4k**, **4l**, **4n**, **5g**, and **5h** may act as potential chemical entities for the development of antibacterial substances. The molecular docking study confirms that these compounds are most suitable for further studies toward the development of antibacterial substances. Literature also has reported that these molecules show positive antibacterial activity.[32−37] Therefore, here, through successful chemical modification and synthesis of the corresponding molecule with a high yield, we tried to evaluate the bioactive properties of the aforementioned molecules, and the results revealed that these chemical molecules successfully enhanced their antibacterial properties (pictures are presented in the Supporting Information). It is anticipated that silent metabolic pathways might have been involved, which become active after the successful synthesis of the chemical molecules. Hence, further efforts should be made, and further research should be conducted utilizing in vivo models to determine their efficacy as antibiotics.

Additionally, it is pertinent to mention that the present investigation is a result of our continuous efforts toward the development of greener methodologies for the creation of targeted bioactive heterocyclic scaffolds,[38,39,70−74] quantification, and isolation cum purification techniques for biologically active components obtained from different sources.[75−79]

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c07315.

$^1$H NMR, $^{13}$C NMR, and MS data and spectra; molecular docking study; biological activity images; HOMO−LUMO structures; and DFT study (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ADDITIONAL NOTE

[a]Caution: Preliminary mixing of corresponding *o*-nitro chlorobenzenes and hydrazine hydrate in a MW vial should be performed under ice-cold conditions and continuous stirring (exothermic reaction). Subsequent to this, the vial may be subjected to MW irradiation.

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