Transforming Otilonium Bromide For Resilient Antimicrobial Combat

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Otilonium bromide is a cationic surfactant with established medical applications, revealing a promising dimension in its versatile nature. Beyond its conventional use, the compound demonstrates notable antifungal properties, signaling a substantial extension of its application potential. Due to the spreading drug resistance of many microorganisms to active substances and antibiotics, it is necessary to search for and create new compounds that will exhibit more aggressive mechanisms of action. Based on the methods of synthesis of otilonium bromide, new derivatives of the compound were created to broaden its applicability beyond its traditional pharmaceutical utility. Delving into the intricate synthesis processes employed, this study provides a detailed exploration of the newly created derivatives of otilonium bromide. The broadening of the applicability of otilonium bromide, especially in response to emerging challenges posed by microbial resistance, is intended to make a significant contribution to the evolving landscape of antimicrobial therapeutics. The findings not only unveil the expanded capabilities of otilonium bromide but also reveal new avenues for the development of compounds with enhanced efficacy and resilience in combating microbial threats.

Keywords: otilonium bromide; cationic surfactants; drugs; antifungal properties; quaternary compounds

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1. Introduction

Otilonium bromide (OB) [diethylmethyl (2-(4-(2-octyloxybenzamido)benzoyloxy)-ethyl) ammonium bromide] with the molecular formula $C_{29}H_{43}BrNO_4$ is classified as a quaternary ammonium compound. The solubility of OB in dimethylformamide (DMF) is 20 mg/mL, dimethyl sulfoxide (DMSO): 25 mg/mL, water: 0.0000295 mg/mL, ethanol: 10 mg/mL. Otilonium bromide was first discovered over thirty years ago by Menarini Research SpA. Currently it is known under several names as Doralin, Spasen, Spasomomen, in more than 40 countries around the world. The drug is mainly used to treat gastrointestinal diseases such as irritable bowel syndrome (IBS) through its ability to relieve smooth muscle spasms in the gastrointestinal tract [1–3]. OB is characterized as an anti-muscarinic, locally acting L-type calcium channel blocker, and tachykinin NK2 receptor antagonist, exhibiting minimal absorption from the gastrointestinal tract [4]. Through a number of clinical studies [5–10], its positive effects improving the quality of life of people with IBS have been confirmed.

Building on this rich history, the synthesis of Otilonium bromide has undergone significant advancements. The first documentation of OB chemical synthesis was presented by

Ghelardoni et al. [11] in their published work from 1973. In 2015, Chunxiang et al. [12] documented a patent no. CN105037193A. The document presents a number of methods for the preparation of OB. One approach is to start the process by synthesizing 4-(alkyloxy)benzoic acid and then reacting it with thionyl chloride. The next step is to add an amino acid. The resulting N -substituted amide undergoes a Fischer-Speier esterification reaction. The final step in the synthesis is quaternization of the nitrogen atom using bromomethane [12].

Recent studies by Zhen et al. [13], Cunningham et al. [14], and Gong and Kim [15] have collectively broadened the scope of OB's application. Zhen et al. [13] conducted a study on OB drug for antifungal activity against *Candida albicans* (SC5314) and *Cryptococcus neoformans* (H99) - susceptible or resistant to commonly used antifungal drugs. Concurrently, Cunningham et al. [14] investigated antimicrobial activity of OB against a range of Gram-negative (e.g. *Escherichia coli* B7A, Salmonella enterica serovar Typhimurium LT2, *Campylobacter jejuni* CG8421, with minimum inhibitory concentrations (MICs) between 1 and 64 μg/mL) and Gram-positive bacteria (e.g. *Staphylococcus aureus* USA100 635, *S. aureus* USA300 AH1263, *E. faecium* 1230933a, with MICs around 4 μg/mL). The authors suggested a membrane lytic mechanism of action, which is also supported by the difference in MIC and killing results over time between *E. coli* (shorter) and *S. aureus* (longer), which may be related to the accumulation of OB in the cytoplasmic membrane required to induce cell death. Gong and Kim [15] extended this exploration to investigate activity on *Vibrio vulnificus*. According to their research, OB has a broad spectrum of antifungal activity due to the cell division defects. OB is more potent against Gram-positive bacteria [13, 14]. OB exhibited low MIC value of 0.88 μg/mL also against *Candida albicans*, indicating significant antifungal activity. Additionally, OB demonstrated minimal cytotoxicity against human intestinal epithelial Caco-2 cells, with a half maximal inhibitory concentration IC50 value of 22.5 μg/mL, which surpasses the MIC value against *C. albicans*, suggesting its potential safety for therapeutic use in candidiasis [16]. Moreover, as was presented in Xu et al. [17] OB has shown significant potential in the fight against antimicrobial resistance (AMR). OB synergistically restores the activity of colistin against both resistant and susceptible Gram-negative bacteria, reducing the required dosage and, consequently, the drug's associated toxicity. Otilonium bromide demonstrated a 32 -fold reduction in colistin MIC (from 8 μg/ml to 0.28 μg/ml) when 20 μg/ml of OB was used in susceptibility tests against *E. coli* J53 strain carrying the *mcr-l*-bearing plasmid and from 1 μg/ml to $\leq$ 0.016 μg/ml in colistin-susceptible *E. coli* J53 strain when 10 μg/ml of OB was applied [18]. The various antifungal applications of otilonium bromide highlight the strategic interaction between molecular design and pharmacological functionality, demonstrating its potential not only in gastrointestinal therapies, but also as a versatile antifungal agent.

The field of chemical and medical sciences should be constantly evolving, which requires a constant search for new compounds with improved therapeutic properties. For this reason, work is being carried out on the synthesis of OB derivatives, their characterization and the study of antimicrobial properties. The synthesis of OB derivatives may offer advantages, such as improved antimicrobial properties, solubility, and selectivity, which may expand the potential applications of OB derivatives.

In this article, a comprehensive investigation into various strategies of synthesis of OB derivatives is presented. Furthermore, a systematic evaluation is conducted to evaluate their structural, physicochemical, and antimicrobial properties. The objective is to understand the relationship between structural modifications and the resulting properties of these derivatives. This quest aims to contribute to a deeper scientific understanding of the structure-function relationships inherent in OB derivatives.

2. Results and discussion

2.1. Synthesis Optimization and Initial Compound Selection

Initially, efforts were focused on meticulously reproducing the OB synthesis reaction. After successfully obtaining a suitable compound, all steps of the synthesis were comprehensively modified. A series of experiments were methodically performed to optimize the method of preparation, including the selection of a suitable solvent among proton solvents and aprotic solvents. To refine and simplify the synthesis methodology, a systematic approach was adopted. The synthesis process was methodically divided into three steps, each developed in succession. This involved strategic alterations in the choice of solvents used, temperature, and reaction pressure [19]. This step-wise refinement not only enhanced the efficiency of the synthesis but also facilitated a deeper understanding of the influence of solvent dynamics, temperature variations, and reaction pressure on the overall synthetic process.

2.2. Step 1: Formation of 4-(Alkyloxy)benzoic Acid Derivatives

In the first step 4-n-Octyloxybenzoic acid, 4-n-Decyloxybenzoic acid, and 4-n-Dodecyloxybenzoic

acid (Fig. 1) were selected as the basis of reaction. This deliberate selection allowed to obtain compounds with three different chain lengths. The OB synthesis procedure will start with the formation of 4-(alkylloxy)benzoic acid by reacting with ethyl chloroformate. Once the acid is obtained, an N -substituted amide acid is added. The reaction to form the amino acid is long, taking more than 24 hours. Notably, an extended reaction duration was found to be conducive to enhancing the reaction yield. During the formation of 4-(alkyloxy)benzoic acid, the process was meticulously controlled maintaining a temperature under 5°C to prevent overheating of the reaction mixture and mitigate decomposition. Numerous solvents were systematically tested in the synthesis process, including dichloromethane, chloroform, diethyl ether, toluene. The reagent with the highest yield-dichloromethane was chosen for the synthesis procedure, optimizing the overall efficiency of the reaction.

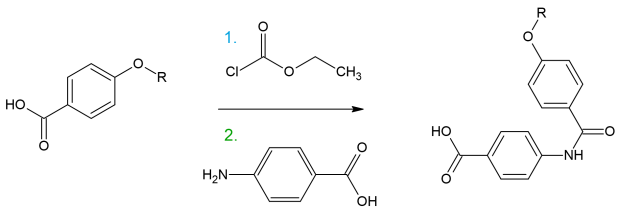


Fig. 1. Formation of 4-n-Octyloxybenzoic acid, 4-n-Decyloxybenzoic acid, 4-n-Dodecyloxybenzoic acid

2.3. Step 2: Synthesis of N-Substituted Amidoesters

In the second step, reactions of the products of stage 1 were carried out with 2-chloroethanediethylamine (Fig. 2). The implementation of Fischer-Speier esterification in this step facilitated the synthesis of an N -substituted amidoester compounds. The selection of appropriate solvents played a pivotal role in this stage, with acetone, ethyl acetate, acetonitrile being subjected to thorough testing. Comparable yields were obtained for acetone and ethyl acetate. This observation is consistent with findings from Bolchi et al. [20], which emphasizes the importance of solvent choice in esterification reactions for preparation methionine, arginine, tryptophan, and proline benzyl esters by Fischer-Speier reaction. In this study, green ethers such as Me-THF were preferred over more hazardous solvents and those that could induce racemization. The reaction yield was improved by intensively heating the reaction mixture with simultaneous stirring. It has been observed that too low a temperature (below 45°C) results in incomplete dissolution of the reaction substrates, leading to diminished yields.

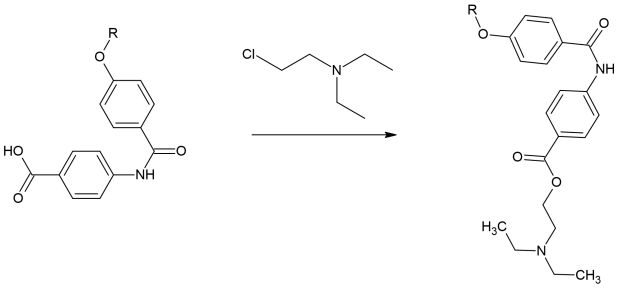


Fig. 2. Formation of N -substituted amidoester

2.4. Step 3: Quaternization and Final Product Formation

The final phase of the synthetic sequence is closely related to the quaternization of the nitrogen atom to obtain the final quaternary ammonium compounds. In this step, reactions of the products of stage 2 were carried out with the quaternary agent, as presented in Fig. 3. The solvent used for the synthesis depended on the quaternizing reagent. If the quaternary agent was hydrochloric acid and tetrafluoroboric acid, then water was used due to its limited solubility and form of occurrence. Conversely, for quaternization with 1,4-butanosultone, an extensive evaluation of solvents was conducted, such as acetone, ethyl acetate, ethanol, methanol. Because of the poor solubility of the obtained substrate in methanol and ethanol, the use of these solvents proved impossible. Consequently, acetone and ethyl acetate emerged as the optimal solvents for further studies. This meticulous selection of solvents aligns with the stringent criteria essential for the successful execution of the quaternization process, thereby ensuring the reproducibility and robustness of the synthetic outcomes [21]. For example as presented in Wang and Iou [22] the solvent effect on the quaternization of tertiary amines indicated that rate constant in aprotic solvent is larger than that in protic solvent, because the amine would form hydrogen bond with a protic solvent.

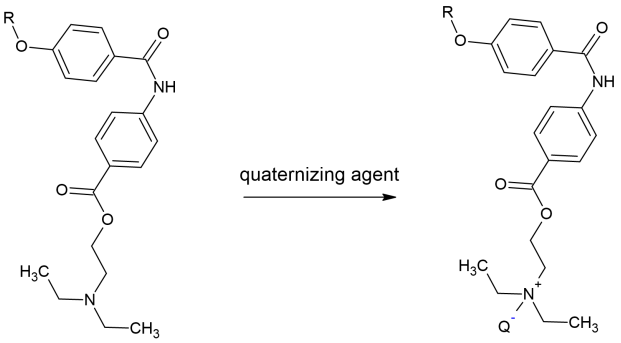
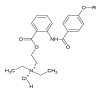


Fig. 3. Quaternization of the obtained compounds

Table 1. Synthesis of quaternary compounds with hydrochloric acid

No.	Abbreviation	Structural Formula	R	Yield %	Colour	Tm °C
1	C8PCl		C8	85.3	brown	86.2
2	C10PCl		C10	80.2	brown	107.5
3	C12PCl		C12	60.1	brown	102.9
4	C8A3Cl		C8	95.1	brown	60.2
5	C10A3Cl		C10	93.2	light brown	70.5
6	C12A3Cl		C12	64.3	light brown	90.5
7	C8A2Cl		C8	63.1	dark brown	55.0
8	C10A2Cl		C10	59.3	light brown	75.5
9	C12A2Cl		C12	51.2	light brown	105.2

2.5. Structural and Thermal Properties of OB Derivatives

The obtained OB derivatives, their structures, yield and characteristics (colour and melting temperature, Tm) are shown in Tables 1 to 3. The presented table outlines the synthesis outcomes of various derivatives, each characterized by distinct abbreviations and structural formulas. For example, as shown in Table 1, the melting temperature (Tm) values, ranging from 55.0°C to 107.5°C, reflect the thermal characteristics of the synthesized compounds. The variations in Tm values suggest differences in the structural arrangements and molecular interactions within the synthesized derivatives. Similar relationship between melting temperature was observed by other authors, i.e. for benzoxazole derivatives [23]. It is evident that the choice of alkyl chain length and additional substituents influences both the yield and thermal properties of the synthesized compounds. This effect can be explained by steric hindrance effect. Longer alkyl chains introduce greater steric hindrance [24]. The bulkiness of longer chains can disrupt the efficient packing of molecules in the crystal lattice, leading to a less ordered structure. This results in a weaker and less stable arrangement, requiring less thermal energy to transition from a solid to a liquid state, hence lowering the melting temperature. Moreover, longer alkyl chains can exhibit reduced van der Waals forces and London dispersion forces per unit volume compared to shorter chains. While the overall intermolecular forces might increase due to a larger surface area, the forces per molecule can be less, contributing to a lower melting temperature [25]. The relationship between melting temperature and chain length is not so evident when analyzing the results in Tables 2 and 3. The choice of quaternizing agent (hydrochloric acid, N -butylsulfonate, tetrafluoroboric acid) influences the characteristics of the synthesized compounds. Longer alkyl chains generally correlate with lower melting temperatures, but this trend is not uniform across all compounds. Moreover, the choice of quaternizing agent (hydrochloric acid, N-butylsulfonate, tetrafluoroboric acid) introduces different counterions and influences the overall charge distribution in the molecules. This can affect intermolecular interactions and the response to temperature.

The comprehensive analysis of these synthesized derivatives reveals a nuanced interplay between structural modifications and their consequential effects on yield, colour, and melting temperature. These findings provide valuable insights for further exploration and optimization of the synthesis strategies for diverse applications.

2.6. Antifungal and Antibacterial Activity of OB Derivatives

Susceptibility of *Candida* spp. isolates to OB derivatives was assessed through the determination of the minimal inhibitory concentration (MIC), as outlined in Table 4. The compounds with the highest antifungal properties are: C10PS, C8A3S, C10A3S, C8A3BF4, C10A3BF4, C12A3BF4. This aligns with studies on quaternary ammonium compounds (QACs) where structural modifications, such as altering the alkyl chain length or introducing functional groups like aminobenzoic acid, enhance the biological activity. Research has demonstrated that quaternary ammonium salts exhibit significant antifungal activity against various fungal strains, including *Candida* spp., through mechanisms that involve disruption of fungal cell membranes [26, 27]. In particular, findings from Xu et al. [26] show that quaternisation with specific functional groups, such as butylsulfonate, can improve efficacy. For instance, longer alkyl chains often increase lipophilicity, enhancing the compound's interaction with fungal cell membranes and improving antifungal activity, as is observed in compounds with similar structures to C10A3BF4.

Based on the results presented in Table 4, it can be concluded that quaternisation with N-butylsulfonate improves the antifungal properties of the compounds. Furthermore, the incorporation of a 3-Aminobenzoic acid moiety is observed to have a favourable impact on the compounds,

Table 2. Synthesis of quaternary compounds with N -butylsulfonate

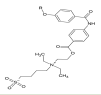
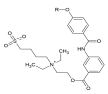
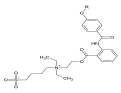
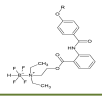
No.	Abbreviation	Structural Formula	R	Yield %	Colour	Tm °C
1	C8PS		C8	85.3	beige	50.1
2	C10PS		C10	85.4	beige	55.4
3	C12PS		C12	82.3	beige	70.0
4	C8A3S		C8	75.6	brown	50.4
5	C10A3S		C10	80.2	dark brown	57.4
6	C12A3S		C12	79.8	dark brown	65.2
7	C8A23S		C8	90.4	beige	53.9
8	C10A2S		C10	89.2	beige	68.2
9	C12A2S		C12	90.3	beige	90.4

Table 3. Synthesis of quaternary compounds with tetrafluoroboric acid

No.	Abbreviation	Structural Formula	R	Yield %	Colour	Tm °C
1	C8PBF4		C8	80.5	brown	95.5
2	C10PBF4		C10	79.7	dark brown	78.2
3	C12PBF4		C12	62.5	dark brown	136.3
4	C8A3PBF4		C8	77.5	light brown	80.5
5	C10A3PBF4		C10	72.5	light brown	96.7
6	C12A3PBF4		C12	68.9	brown	112.4
7	C8A2PBF4		C8	77.5	light brown	80.5
8	C10A2PBF4		C10	72.5	light brown	96.7
9	C12A2PBF4		C12	68.9	brown	112.4

further augmenting their antifungal efficacy. In analyzing the antifungal activity of the tested compounds, it is essential to connect their structural features with the observed biological effects. The compounds under investigation differ mainly in their carbon chain length, functional groups, and the presence of specific ions or moieties. These structural variations have a direct impact on their ability to inhibit the growth of different *Candida* species. C10PS, characterized by a 10 -carbon chain and a sulfonic group, demonstrated moderate antifungal activity, especially against *Candida tropicalis*, with an MIC of 32 μg/mL. However, it did not show any significant effect against *Candida albicans* or *Candida glabrata*. This result suggests that while the 10 -carbon chain may confer some lipophilic properties that aid in interacting with the fungal cell membrane, it is insufficient to impact all *Candida* species. The lack of activity against *C. albicans* and *C. glabrata* implies that additional functional groups or modifications might be necessary to enhance efficacy across different fungal strains. The introduction of an amino group alongside the sulfonic group, as seen in C8A3S and C10A3S, markedly improves antifungal properties. In particular, C10A3S exhibited the lowest MIC (32 μ/mL) against *C. albicans*, indicating its potential as a more potent antifungal agent. The presence of the amino group may increase the compound's ability to penetrate or interact with fungal cells more effectively, possibly by forming additional electrostatic interactions with membrane components or intracellular targets. The shorter-chain analog, C8A3S, exhibited slightly lower overall antifungal activity, suggesting that carbon chain length, alongside the functional groups, plays a significant role in determining efficacy. This observation highlights the delicate balance between hydrophobicity, determined by the carbon chain length, and hydrophilicity, contributed by the functional groups, which influences a compound's ability to permeate fungal cells. In comparison, compounds containing the tetrafluoroborate ion, such as C8A3BF4 and C10A3BF4, also exhibited antifungal activity, though their effectiveness was less pronounced than their sulfonic counterparts. C10A3BF4, for instance, demonstrated an MIC of 32 μg/mL against *C. glabrata*, while its activity against other Candida strains was limited. This selective efficacy could be attributed to the nature of the tetrafluoroborate ion, which might enhance interactions with certain fungal strains but not others [28]. The ion may interact differently with fungal cell membranes or intracellular proteins compared to sulfonic groups, leading to selective antifungal effects. Notably, C12A3BF4, with a longer carbon chain, only showed activity against *C. glabrata*, further supporting the hypothesis that longer chain lengths combined with

Table 4. Minimum inhibitory concentration of OB and its derivatives against human fungal and bacterial pathogens

Abbreviation	MIC (μg/mL)				
	(*C. albicans* SC5314)	(*C. glabrata* CBS138)	(*C. tropicalis* MYA3404)	(*S. aureus* S01-10-0202)	(*P. aeruginosa* M06-06-0213)
OB	2	8	2	8	> 128
C8PS	> 128	> 128	> 128	> 128	> 128
C10PS	>128	>128	32	> 128	> 128
C12PS	> 128	128	128	> 128	> 128
C8PCl	> 128	> 128	> 128	> 128	> 128
C10PCl	>128	> 128	>128	> 128	> 128
C12PCl	>128	> 128	128	> 128	> 128
C8PBF4	>128	>128	>128	>128	>128
C10PBF4	> 128	> 128	> 128	> 128	> 128
C12PBF4	> 128	128	>128	>128	> 128
C8A3Cl	> 128	64	128	64	> 128
C10A3Cl	64	> 128	> 128	128	> 128
C12A3Cl	>128	>128	>128	>128	>128
C8A3S	128	32	64	128	>128
C10A3S	32	> 128	64	32	> 128
C12A3S	>128	>128	> 128	8	> 128
C8A3BF4	128	32	>128	32	>128
C10A3BF4	>128	32	> 128	> 128	> 128
C12A3BF4	>128	32	>128	>128	> 128
C8A2Cl	>128	> 128	> 128	128	>128
C10A2Cl	>128	> 128	> 128	128	> 128
C12A2Cl	128	> 128	> 128	> 128	> 128
C8A23S	64	64	> 128	>128	> 128
C10A2S	> 128	> 128	> 128	64	>128
C12A2S	>128	> 128	>128	>128	> 128
C8A2BF4	>128	> 128	> 128	128	> 128
C10A2BF4	> 128	> 128	> 128	128	> 128
C12A2BF4	>128	>128	>128	>128	>128

tetrafluoroborate ions might favor interactions with specific fungal species rather than providing broad-spectrum antifungal activity. This discernment underscores the potential of specific synthetic modifications to amplify the therapeutic attributes of OB derivatives, specifically in the context of combating *Candida* spp. infections. Moreover, the structure-activity relationship of these compounds shows that specific modifications, such as chain length adjustments and the introduction of functional groups like sulfonic or tetrafluoroborate ions, significantly impact their antifungal properties. The presence of an amino group, particularly in compounds like C10A3S, enhances activity against *Candida albicans*, while tetrafluoroborate-containing compounds exhibit selective efficacy against *Candida glabrata*. Meanwhile, a gram-positive *Staphylococcus aureus* S01-10-0202 and a gram-negative *Pseudomonas aeruginosa* M06-06-0213 were chosen in order to test the antibacterial activity of OB and its derivatives. The results found that OB and its derivatives have no antibacterial activity against *P. aeruginosa*, while exhibited variable antibacterial activity against *S. aureus*. OB and its derivative C12A3S showed antibacterial activity at 8 μg/mL, while C10A3S and C8A3BF4 at

32 μg/mL. These findings suggest that strategic structural modifications could be a promising approach to developing more potent and selective antimicrobial agents targeting specific *Candida* species and *S. aureus*.

3. Experimental section

3.1. Step 1: Synthesis of 4-(n-Octyloxy)benzoic Acid Derivatives

In the first step, 5 g of 4-n-Octyloxybenzoic acid was dissolved in 50 mL of dichloromethane. The reaction of adding triethylamine was carried out at a temperature below 5°C.2.3 g of ethyl chloromethane was added. After one hour, 2.86 g of N -substituted aminobenzoic acid was added. The reaction was pretreated at room temperature for another week. For purification, 100 mL of distilled water was added, the phases were separated and the organic phase was evaporated. The resulting product was dissolved in water and the pH was changed to acidic, then the product was filtered and dried.

3.2. Step 2: Synthesis of N-Substituted Amidoesters

In the second step, 4.5 g of the product obtained in step 1 was dissolved in acetone. The liquid was heated and 1.98 g of 2-chloroethyldiethylamine was added. The reaction was carried out for about a week, gradually adding NaOH to obtain pH about 7. The solution was filtered and then evaporated.

3.3. Step 3: Quaternization of N-Substituted Amidoesters

In the third step, the resulting sludge was divided into 3 parts and the compounds were quaternized in a 1 : 1 ratio.

3.4. Structural Characterization of Synthesized Derivatives

The structure of the final product was confirmed by elementary analysis, IR, and NMR. Examples of 13C NMR for group representatives:

C8PCl: 13C NMR: δ 9.3 (2C, s), 14.0 (1C, s), 22.6 (1C, s), 25.8 (1C, s), 29.3 (1C, s), 29.3-29.4 (2C, 29.4 (s), 29.4 (s)), 31.8 (1C, s), 55.5-55.5 (3C, 55.5 (s), 55.5 (s)), 59.2 (1C, s), 69.1 (1C, s), 114.3 (2C, s), 117.9 (2C, s), 129.4-129.7 (3C, 129.5 (s), 129.6 (s)), 130.9 (2C, s), 133.6 (1C, s), 137.4 (1C, s), 158.5 (1C, s), 164.8 (1C, s), 166.2 (1C, s).

C8PS: 13C NMR: δ 8.2 (2C, s), 14.0 (1C, s), 22.6-22.8 (2C, 22.6 (s), 22.7 (s)), 25.8 (1C, s), 29.1-29.3 (2C, 29.2 (s), 29.3 (s)), 29.3-29.4 (2C, 29.4 (s), 29.4 (s)), 31.8 (1C, s), 53.5 (2C, s), 58.5 (1C, s), 59.1 (1C, s), 64.0 (1C, s), 69.1 (1C, s), 71.7 (1C, s), 114.3 (2C, s), 117.9 (2C, s), 129.4-129.7 (3C, 129.5 (s), 129.6 (s)), 130.9 (2C, s), 133.6 (1C, s), 137.4 (1C, s), 158.5 (1C, s), 164.8 (1C, s), 166.2 (1C, s).

C8PBF4: 13C NMR: δ 8.6 (2C, s), 14.0 (1C, s), 22.6 (1C, s), 25.8 (1C, s), 29.3 (1C, s), 29.3-29.4 (2C, 29.4 (s), 29.4 (s)), 31.8 (1C, s), 49.1 (1C, s), 52.5 (2C, s), 66.5 (1C, s), 69.1 (1C, s), 114.3 (2C, s), 117.9 (2C, s), 129.4-129.7 (3C, 129.5 (s), 129.6 (s)), 130.9 (2C, s), 133.6 (1C, s), 137.4 (1C, s), 158.5 (1C, s), 164.8 (1C, s), 166.2 (1C, s).

3.5. Antimicrobial Activity of OB Derivatives

The minimal inhibitory concentrations (MICs) were determined following CLSI protocols M27-A3 for fungi and M07-A9 for bacteria. Yeast strain (*C. albicans*, *C. glabrata*, and *C. tropicalis*) were cultured in 3 mL YPD broth medium overnight at 30°C, while bacterial strain (*S. aureus* and *P. aeruginosa*) were cultured in 3 mL Cation-Adjusted Mueller-Hinton Broth (CAMHB) medium overnight at 37°C. After incubation, all microbial suspensions were washed twice and resuspended with sterile water. Yeast concentrations were determined using a hemocytometer, while bacterial concentrations were determined by colony count on inoculum suspensions.

The broth microdilution method was employed using 96-well microtiter plates. In each well, 100μ L of the diluted test compound was mixed with 100μ L of the inoculums prepared in CAMHB medium for bacteria or RPMI 1640 medium for fungi. The final concentrations of test compounds ranged from 0.125 to 128μ g/mL. Microbial suspensions were initially standardized to 0.5 McFarland standard and then diluted to achieve final inoculum concentrations of 1.25×10^3 cells /mL for fungi and 2.5×10^4CFU/mL for bacteria.

The plates were incubated at 35°C for 48 h for fungi and at 37°C for 24 h for bacteria. The MIC was defined as the lowest concentration of compound that completely inhibited visible microbial growth.

4. Conclusions

In summary, the investigation has yielded valuable insights into the synthesis of Otilonium Bromide (OB) derivatives through a systematic reaction method. This approach allows to obtain a series of homologous OB derivatives. Some of the obtained compounds show antifungal properties, which gives hope for obtaining further compounds with even better properties. These collective observations underscore the intricate interplay between molecular structure, intermolecular forces, and environmental factors in determining the diverse properties of OB derivatives. While some compounds exhibit promising antifungal attributes, the comprehensive understanding of structure-activity re-

lationships, as discussed earlier, provides a foundation for the rational design of derivatives with enhanced properties. Moving forward, the identified compounds with notable antifungal properties pave the way for further exploration and refinement of the synthetic strategies. The prospect of obtaining compounds with even superior antimicrobial characteristics is an encouraging avenue for future research, offering the potential for advancing the therapeutic applications of OB derivatives in combating fungal and bacterial infections. The integration of structural insights and antimicrobial assessments positions the synthesized derivatives as promising candidates for continued investigation and optimization in the realm of antimicrobial therapeutics.

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