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SYNTHESIS, ANTIBACTERIAL ACTIVITY AND DOCKING STUDIES OF BENZYL ALCOHOL DERIVATIVES

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Abstract: Benzyl alcohol derivatives were synthesized, and characterized using NMR and FTIR spectroscopic techniques. For the first time, the antibacterial activities of the synthesized compounds were examined using disc diffusion method by measuring the diameter of the zones of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results demonstrated that the activity was concentration dependant, and that the compounds were generally potent against *P. aeruginosa*. Only two of the compounds were active against *S. aureus.* In terms of broad spectrum activity, compound **2d** (35 mm) was found to exhibit a promising efficacy which surpassed that of the standard drug (amoxicillin).The binding of compounds **2a-e** to the glucosamine-6-phosphate synthase (GlcN-6-P) active-site revealed that all the synthesized compounds fitted into the GlcN-6-P active-site receptor cavity, exhibited potential hydrogen-bonding interactions with the proximal amino acid residues and aligned similar to amoxicillin. Interestingly, it has been found that the most active compound, **2d** also appeared to have a relatively low binding energy (-52.8901 kcal/mol).

Keywords: Benzyl alcohol derivatives, antibacterial activity, docking studies , glucosamine-6-phosphate synthase.

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INTRODUCTION

The continuous increase in the antibiotics resistance and the attendant scarcity of new antimicrobial agents is arguably one of the major challenges in public health today, especially in the developing countries where up to one-half of deaths are attributed to infectious diseases (1-4). This could particularly be seen in the level of antibiotics resistance associated with a number of staphylococcus species which causes respiratory and skin infections, as well as pseudomonas species which are also responsible for the gastrointestinal and urogenital diseases (5). For instance, the clinical isolates of staphylococcus aureus — the leading cause of nosocomial infections — are becoming resistant to a range of popular antimicrobial agents such as vancomycin, trimethoprim-sulfamethoxazole, chloramphenicol, erythromycin, clindamycin, ciprofloxacin, penicillin, amikacin, tobramycin, and gentamicin (6).

In an attempt to address the aforementioned concerns, a number of workers have reported various bioactive molecules built around some promising scaffolds and assessed their antibacterial or antimicrobial potentials. These includes nitroimidazole derived oxazolidinones (7),

ciprofloxacin derivatives (8-11), icariin derivatives (12), 1-monolaurin (13), (*E*)-stilbene derivatives (14), benzimidazole-incorporated sulfonamide analogues (15), chalcone derivatives (16), 3-phenyl-1-methylquinolin-2-one derivatives (17), oxysterols (18), 2-thiazolylimino-5-arylidene-4-thiazolidinones (19), sulfonamide and carbamate derivatives of 5-Nitro-1H-indazole (20), polyfluorinated 4-thiazolidinone and α-aminophosphonic acid derivatives (21). Interestingly, we have noted a known bioactive scaffold that aparently escaped the attention of workers — benzyl alcohol.

Benzyl alcohol (Figure 1) is an aromatic alcohol commonly found in various essential oils such as jasmine, ylang-ylang, and hyacinth (22). Due to its antibacterial and antifungal activities, it is widely used as an ingredient in the manufacture of soaps, topical creams, skin lotions, shampoos, and facial cleansers. Unfortunately however, benzyl alcohol has been reported to be a contact allergen (23). It thus prompted us in this work to prepare benzyl alcohol derivatives in order to evaluate their structure activity relationships with the aim of developing lead compounds that could offer safe and efficacious oral or topical antibacterial agents.

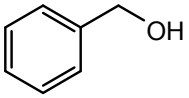


Figure 1. Structure of benzyl alcohol.

EXPERIMENTAL SECTION

Materials

All reagents and solvents were purchased from Aldrich and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. IR spectra were recorded on a Perkin–Elmer FT-IR Spectrum BX spectrophotometer. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected.

Methods

Chemistry

General Procedure for the preparation of benzyl alcohol derivatives (2a-e)

The aldehydes (13.2 mmol) were placed in a 250 mL round bottom flask containing ethanol (4 mL) and stirred at room temperature to obtain homogenous solutions. The resulting solutions were cooled on an ice bath prior to the addition of the reducing agent. Subsequently $NaBH_4$ (13.2 mmol) dissolved in 1M NaOH (3.8 mL) was slowly added over a period of 10 minutes. The resulting mixture was stirred at room temperature for 10 minutes after which it was cooled and then treated with 6M HCl dropwise until the evolution of H_2 gas stopped. The pH was checked to make sure the solution was acidic, and further stirred for another 10 minutes to allow the appropriate product to precipitate. The products were collected by filtration, washed twice with ice-cold water and then transferred to a dry piece of filter paper and air dried. But in the case of compound **2d**, the crude mixture was extracted with chloroform which after evaporation afforded the product as a film-like solid.

4-hydroxy-3-methoxybenzyl alcohol, 2a
White powder, 93% yield, mp 112-114°C. ^{1}HNMR (400MHz, $DMSO_4$-d_6) δ ppm 3.71 (3H, s, OCH_3), 4.37 (2H, s, CH_2), 5.14 (1H, s, OH), 6.70 (2H, d, *J* = 4, Ar-H), 6.88 (1H, s, Ar-H), 8.88 (1H, s, Ar-OH); ^{13}CNMR (100MHz, $DMSO_4$-d_6) δ ppm 39.52, 55.80, 63.36, 111.32, 1115.35, 119.51, 133.72, 145.54, 147.67; IR 3503, 1509, 1259cm^{-1}.

4-hydroxybenzyl alcohol, 2b
White powder, 87% yield, mp 260-265°C; ^{1}HNMR (400MHz, $DMSO_4$-d_6) δ ppm 4.37 (2H, s, CH_2), 5.07 (1H, s, OH), 6.71 (2H, d, *J* = 8, Ar), 7.11 (2H, d, *J* = 8, Ar), 9.36 (1H, s, Ar-OH); ^{13}CNMR (100MHz, $DMSO_4$-d_6) δ ppm 39.52, 63.09, 63.12, 115.10, 115.12, 128.43, 128.46, 132.94, 156.41, 156.42; IR 3377, 1513, 1233 cm^{-1}

4-nitrobenzyl alcohol, 2c
Yellow powder, 93% yield, mp 131-134°C. ^{1}HNMR (400MHz, $DMSO_4$-d_6) δ ppm 3.56 (2H, s, CH_2), 5.61 (1H, s, OH), 7.57 (2H, d, *J* = 8, Ar), 8.17 (2H, d, *J* = 4, Ar); ^{13}CNMR (100MHz, $DMSO_4$-d_6) δ ppm 39.52, 62.21, 123.49, 127.25, 148.50, 150.89 IR 3503, 1505, 1230 cm^{-1}

4-methoxybenzyl alcohol, 2d
Film-like white solid; 94% yield; mp 212-215°C; ^{1}HNMR (400MHz, $DMSO_4$-d_6) δ ppm 3.71 (3H, s, OCH_3), 4.40 (2H, s, CH_2), 5.15 (1H, s, OH), 6.87 (2H, d, *J* = 8, Ar), 7.22 (2H, d, *J* = 8, Ar); ^{13}CNMR (100MHz, $DMSO_4$-d_6) δ ppm 39.52, 55.22, 62.79, 113.67, 128.21, 128.82, 131.14, 134.60, 158.36, IR 3326, 1513, 1244 cm^{-1}.

4-bromobenzyl alcohol, 2e
Yellow powder; 95% yield; mp 212-215°C; ^{1}HNMR (400MHz, $DMSO_4$-d_6) δ ppm 4.46 (2H, s, CH_2), 5.38 (1H, s, OH), 7.27 (2H, d, *J* = 8, Ar), 7.48 (2H, d, *J* = 8, Ar); ^{13}C-NMR (100MHz, $DMSO_4$-d_6) δ ppm 39.52, 62.40, 62.43, 119.81, 119.83, 128.79, 131.12, 142.06; IR 3272, 1483, 1203 cm^{-1}.

Antibacterial Assay

Sourcing of Bacteria for Assay

The bacteria used for the assay were obtained from the Department of Microbiology, Umaru Musa Yar'adua University, Katsina, Nigeria. The Gram-positive bacterium, *Staphylococcus aureus* was cultured in the medium prepared from mannitol salt agar. While the Gram-negative *Pseudomonas aeruginosa* was cultured in the medium prepared from nutrient agar.

Preparation of culture media

50 g of nutrient agar was dissolved in 1800 mL distilled water, autoclaved at 121°C for 15 minutes, spread into eighty (80) petri dishes and allowed to solidify. The Gram positive and negative bacteria were each inoculated into forty (40) prepared petri dishes (24).

Preparation of the test concentrations

Twenty milligrams (20 mg) of the compounds were dissolved in 1 mL of DMSO to afford the stock solution. Using serial double dilution, six different concentrations (10^{-1} mg/mL, 10^{-2} mg/mL, 10^{-3} mg/mL, 10^{-4} mg/mL, 10^{-5} mg/mL, 10^{-6} mg/mL) of each compound were prepared (24).

In vitro assay of the activity of the synthesized compounds on Staphylococcus aureus and Pseudomonas aeruginosa culture

Discs were made from filter paper and dipped into the different concentrations prepared from the five synthesized compounds. The discs were then transferred into the six (6) prepared petri dishes for the Gram-positive and negative bacteria which gave a total of thirty (30) petri dishes for each bacterium. The activity for each compound was determined by measuring the zones of inhibition (mm) of each bacterium after 24 hours of incubation (24).

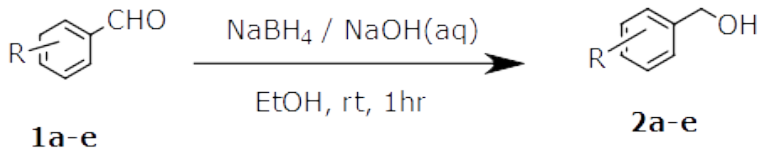
Molecular Docking studies

Discovery studio 2018 software was used to investigate the possible orientation and interactions of **2a-e** with glucosamine-6-phosphate GlcN-6-P synthase (25). The model structure of GlcN-6-P synthase, ID: 1XFF, was downloaded from the protein data bank. The target protein for molecular coupling was prepared based on the CHARMm force field with help of DS 2018. Structural and energy optimization of **2a-e** as ligands were prepared in DFT/B3LYP/6-31G base set using Gaussian 09 software. The docking studies estimate the binding site in the target model as Glcn-6-p synthase and the affinity of **2a-e** as ligands. CDOCKER was performed to place **2a-e** at the selected target binding sites. Docking results were evaluated with three-dimensional amino acid interactions and Binding energy values. Five possible structures of the ligands were generated and the best exposure was defined according to the lowest energy values for each complex.

RESULTS AND DISCUSSION

Chemistry

Benzyl alcohol derivatives, **2a-e** were synthesized (Scheme 1) by reacting the appropriate aldehyde with the basified solution of sodium borohydride, $NaBH_4$/NaOH(aq).



1a-e **2a-e**

	R
a	4-OH, 3-OCH_3
b	4-OH
c	4-NO_2
d	4-OCH_3
e	4-Br

Scheme 1: Synthesis of benzyl alcohol derivatives.

Typically, the $NaBH_4$ solution in aqueous sodium hydroxide was slowly added to the appropriate

solution of aldehyde in ethanol. The resulting mixture was stirred at room temperature for a few minutes. This was followed with the addition of aqueous HCl which resulted in the evolution of hydrogen gas signifying the quenching of the excess $NaBH_4$. The corresponding alcohol product precipitated and collected by filtration. After additional washings, the product was air-dried and analyzed. The Nuclear magnetic resonance (NMR) spectroscopic data of compounds **2a-e** revealed the positions as well as the type of the proton and carbon in each compound. While the IR data gave the functional group present, particularly the hydroxyl (OH) functionality. Typically, the ^{1}H NMR spectrum of **2a** revealed a signal at 4.37 ppm assigned to the two protons of the methylene group, ($-CH_2$). Further upfield, the signal at 6.70 ppm appeared in the aromatic region of the spectrum and was integrated and assigned to the two aromatic protons closer to the methylene group. The ^{1}H NMR spectrum revealed that the signal at 3.71 ppm was for the three protons of the methoxy group, ($-OCH_3$). Also the -OH proton attached to carbon-1 resonated at 5.14 ppm. The ^{13}C NMR spectrum showed a distinct signal at 55.80 ppm which corresponded to the carbon atom of the methoxy group. The signal at 66.36 ppm was assigned to the methylene carbon, while the signals at 111.32 ppm, 115.35 ppm, 119.51 ppm, 133.72 ppm, 145.54 ppm and 147.67 ppm were assigned to the carbons of the benzene ring. From the IR spectrum of compound **2a**, it was deduced that the band at 3503 cm^{-1} was due to the -OH stretching, while the C-O stretch (alcohol) was found at 1259 cm^{-1}.

Antibacterial Activity

The synthesized compounds were screened for *in vitro* antibacterial activity against Gram-positive and negative bacteria species, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively using disc diffusion method. The result for the activity was expressed as the length of diameter of the zones of inhibition as given in Table 1.

Table 1: Antibacterial activity of benzyl alcohol derivatives[a]

Compound	Organism	Zone of Inhibition (mm) Conc (mg/mL)					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
2a	*S. aureus*	08	00	00	00	00	00
	P. aeruginosa	27	11	11	09	08	08
2b	*S. aureus*	10	00	00	00	00	00
	P. aeruginosa	11	09	08	08	08	07
2c	*S. aureus*	08	08	07	00	00	00
	P. aeruginosa	07	00	00	00	00	00
2d	*S. aureus*	12	11	11	09	00	00
	P. aeruginosa	35	26	23	22	00	00
2e	*S. aureus*	00	00	00	00	00	00
	P. aeruginosa	12	11	07	00	00	00

[a]Amoxicillin disc (30µg/mL): *S. aureus* = 24 mm; *P. aeruginosa* = 13 mm

Generally, the antibacterial activities of all the compounds appeared to be concentration dependant. In the case of Gram-positive bacterium (*S. aureus*), it was found that at the highest concentration (10^{-1}), compound **2d** showed a moderate activity (12 mm). While all other compounds relatively exhibited weak activities. Specifically, compounds **2b**, **2a** and **2c** have 10 mm, 08 mm and 08 mm zones of inhibition respectively. Compound **2e** did not show any activity at all concentrations. Regarding the Gram-negative bacterium (*P. aeruginosa*), all of the compounds except **2c** exhibited some degree of activities at more than one concentration. It was interestingly found that compound **2d** demonstrated an excellent zone of inhibition (35 mm), and nearly matched the control antibiotic, amoxicillin even at a low concentration of 10^{-3} (23 mm). Compound **2a** has also showed a better activity at 10^{-1} concentration (27 mm) than the control drug. Overall, it could be commented that compound **2d** appeared quite promising as it demonstrated the ability to inhibit the growth of both Gram-positive and negative bacteria.

Molecular Docking Studies

The enzyme glucosamine-6-phosphate (GlcN-6-P) synthase is responsible for the catalysis of first and rate-limiting step in hexosamine biosynthesis which give rise to uridine 5'-diphospho N-

acelglucosamine (UDP-GlcNAc). Considering that UDP-GlcNAc is an essential building block in the fungal and bacterial cell walls, it thus becomes an interesting target for antifungal and antibacterial drug discovery (25).

Amoxicillin as the standard drug binds with GlcN-6-P synthase with binding affinity of -169.504 kcal/mol. The binding sites of amoxicillin on GlcN-6-P synthase target included twelve residues involved in the hydrogen bond, five residues involved in hydrophobic interaction and three residues involved in other interactions (Figure 2, left panel). There were two strongest hydrogen bonds that occurred with GLY99 with functional group 016 (bond length = 2.09381) and H27 (bond length = 2.72711). then with TRP24 through functional group H34 (bond length = 2.7042) and H41 (bond length = 2.9026). Similarly, another type of hydrogen bond designated as Carbon Hydrogen Bond has also occurred with GLY99 through functional group H43 (bond length = 2.10665).

The binding of compounds **2a-e** to the GlcN-6-P synthase active-site revealed that all the synthesized compounds fitted into the GlcN-6-N active-site receptor cavity, exhibited potential hydrogen-bonding as well as hydrophobic interactions with the proximal amino acid residues (Figure 2, right panel).

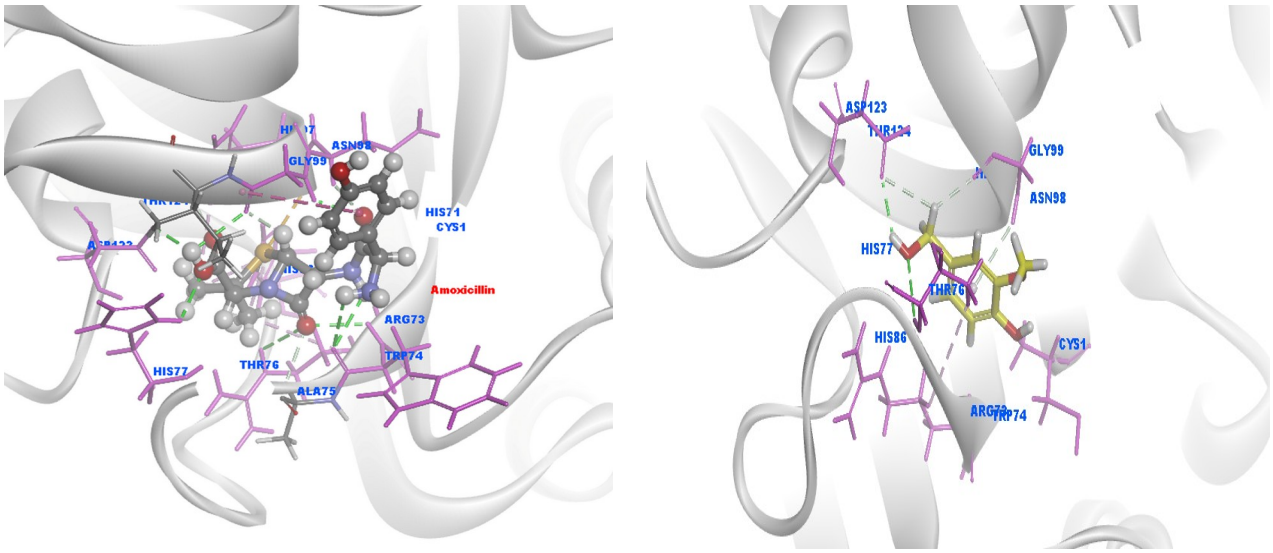


Figure 2. 3D interactions of amoxicillin as reference compound in the GlcN-6-P synthase binding region (left panel); Compound **2a** superimposed in the binding site of GlcN-6-P synthase (right panel).

Compound 2a was found to dock at seven sites (binding energy = -28.0758 kcal/mol). There were two hydrogen bond interactions with ASP123 through functional group H17 and H15 (bond length = 2.126 and 2.874 respectively). Another two hydrogen bonds were revealed with GLY99 through functional groups H15 and HN (bond length = 2.300 and 3.067 respectively). Other types of hydrogen bond as well as the hydrophobic interactions accounted for the remaining binding sites.

Compound 2b exhibited a total of nine docked interactions (binding energy = -34.4725 kcal/mol). One hydrogen bond with the residue ARG73 through functional group O9 (bond length = 2.456) and hydrophobic interaction with the same residue (bond length = 5.05659) were found. But for the residue TRP74, it was found that the conventional hydrogen bonds have occurred through functional groups O8 and H16 (bond length = 2.141 and 2.55708 respectively). The rest of the interactions have occurred with other residues through various functional groups.

Compound 2c docked with the enzyme (binding energy = -66.1382 kcal/mol) with lower energy than 2a and 2b. This might be due to the fact that there were some residues that each interacted multiple times with the compound. For instance, CYS1 interacted three times using different bonding types through functional groups O17, O18 and O19 (bond length = 4.4659, 1.86553 and 3.06222 respectively). Also the residue ARG73 doubly interacted using hydrogen bond through functional group O8 (bond length = 1.87337) and a hydrophobic interaction. Similarly, the residue ASP123 interacted using hydrogen bond through functional groups H15 and H13 (bond length = 3.08947 and 2.27452 respectively). In total, there were ten various interactions observed with compound 2c.

Compound 2d (binding energy = -52.8901 kcal/mol) docked at a total of eight sites. It exhibited double interactions with ARG73, a hydrogen bonding through functional group O8 (bond length = 1.87521) and a hydrophobic interaction (bond length = 5.00408). The rest are single interactions with the remaining seven residues.

Compound 2e docked with the enzyme (binding energy = -74.6045 kcal/mol) at six sites which are fewer than all the compounds. It demonstrated double hydrophobic interactions with the residue HIS86 (bond length = 4.83674 and 4.91763). The remaining residues interacted singly through various bonding types.

Apparently, in terms of the binding energy, compound **2e** demonstrated the least value (-74.6045 kcal/mol) second to the standard drug, amoxicillin (-169.504 kcal/mol). This is in disagreement with the in vitro assay where compound **2d** exhibited the best zone of inhibition of the bacterial strains even better than the standard drug. Though it also possessed some significant low binding energy (-52.8901 kcal/mol). Mismatching of the results of biological assay with the docking studies had been observed in some works. For instance, a standard drug with the most potent in vitro bioactivity was found to exhibit a lesser docking score compared to the tested compounds and vice versa (26). Furthermore, some tested compounds exhibited similar *in vitro* bioactivities but appeared to differ in their docking results (27).

We opined that these discrepancies might be due to the fact that the tested compounds could be hitting targets other than the enzyme being studied. Other contributing factors might be due to the solubility issues, change in binding conformation of proteins, temperature and ionic strength of the buffer which influence entropy but often neglected in the docking software.

CONCLUSIONS

Benzyl alcohol derivatives were synthesized using simple $NaBH_4$ reduction. Although the compounds are known, but for the first time, their antibacterial activities have been investigated. And the results demonstrated that the activity was concentration dependant, and that the compounds were generally potent against *P. aeruginosa*. Only two of the compounds were active against *S. aureus.* In terms of broad spectrum activity, compound **2d** was found to exhibit a promising efficacy which surpassed that of the standard drug. In order to understand the mode of interaction of the compounds, an *in silico* studies were carried out by docking the compounds against glucosamine-6-phosphate synthase (GlcN-6-P) as potential target. Interestingly, all the compounds fitted into the cavity and demonstrated a close alignment of the ligands with the enzyme active site. Of note was the binding energy of the most biologically active compound, **2d** which appeared to have a relatively favorable binding energy.

This work recommends further studies on the pharmacokinetic properties of compound **2d.** Based on the information obtained, it may also be subjected to *in vivo* studies to fully explore its potential as a possible lead that could be optimized to clinical trials in order to have a new antibacterial agent.

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