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Synthesis and Antibacterial Activity of Aromatic and Heteroaromatic Amino Alcohols

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Two series of aromatic and heteroaromatic amino alcohols were synthesized from alcohols and aldehydes and evaluated for their antibacterial activities. All the octylated compounds displayed a better activity against the four bacteria tested when evaluated by the agar diffusion method and were selected for the evaluation of minimal inhibitory concentration. The best results were obtained for *p*-octyloxybenzyl derivatives against *Staphylococcus epidermidis* (minimal inhibitory concentrations = 32 μM).

Key words: amino alcohols, antibacterial, aromatic, *E. coli*, lipophilicity

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Although approximately a third of them are potentially pathogenic, most of the bacteria are harmless to their host. The resident microbiota is associated with host protection, where it plays important roles against invading pathogens, in the detoxification and elimination of toxins or in the immune system modulation. These microscopic organisms display a quick cell cycle, dividing every 20–30 min, with only the most adapted bacteria persisting in the environment. This evolutive adaptation includes mutations and horizontal gene transfer, spreading antibiotic resistance. This phenomenon has become a major problem, particularly in hospitals (1–3), because of the appearance of multi-resistant bacterial strains, which makes the management of infectious diseases difficult. In this regard, there is an urgent need for prospective studies focused on the development of new active compounds with potential use in infection and microbial control.

Since the discovery of penicillin in 1928, a large number of antibiotics, natural or synthetic, have been developed. These compounds belong to several groups: cephalosporins, β-lactam, quinolones or aminoglycosides (4). The amino alcohol group is present in several of them, as in ethambutol, used in the treatment of tuberculosis, and new amino alcohols are being studied for their antimicrobial activity (5–7).

In previous studies, we were able to establish relationships between lipophilicity and biological activity (antibacterial or immunosuppressive) of glycosylated amino alcohol and *N*-acylated diamine derivatives (8,9). Expecting a synergistic effect between lipophilicity and the presence of an amino alcohol group, we describe in the present work the synthesis and antibacterial evaluation of a number of aromatic amino alcohols and amines, varying the aromatic cycle (phenyl, furyl, thienyl) and its substituents (none, bromo, octyloxy); the amino alcohol portion (ethanolamino, 2,2-dimethyl ethanolamino, diethanolamino); and/or the lipophilicity, introducing an octyl chain on the molecule. Antibacterial activity was first evaluated using agar diffusion method (10). Compounds displaying a diameter of zone of inhibition above 10 mm were selected for the evaluation of the minimal inhibitory concentrations (MIC).

Material and Methods

Chemistry

Synthesis of the glycidyl ethers

Epichlorohydrin (120 mmol) was slowly added to a cold mixture of 40% w/w aqueous sodium hydroxide (50 mL), aromatic alcohol (30 mmol) and tetrabutylammonium bromide (1.5 mmol). The progress of the reaction was monitored by thin layer chromatography (TLC) in dichloromethane. After completion of the reaction, the mixture was extracted twice with diethyl ether. The combined organic phase was dried with magnesium sulphate, filtered and evaporated to dryness (11). The ethers were purified by column chromatography on silica. Experimental data can be found in Appendix S1.

Epoxide ring opening

Method A: amine or diethanolamine (1.7 mmol) was slowly added to a solution of the epoxide (1.7 mmol) in methanol and the mixture was stirred at 50 °C for 24 h. The progress of the reaction was monitored by TLC in dichloromethane. After the completion of the reaction, the mixture was evaporated to dryness and purified by column chromatography on silica.

Method B: a mixture of 2,2-dimethyl ethanolamine or diethanolamine (1.7 mmol) in methanol was submitted to microwave irradiation (P 30w) for 10–15 min (12). After the completion of the reaction, the mixture was evaporated to dryness and purified by column chromatography on silica.

Reductive amination

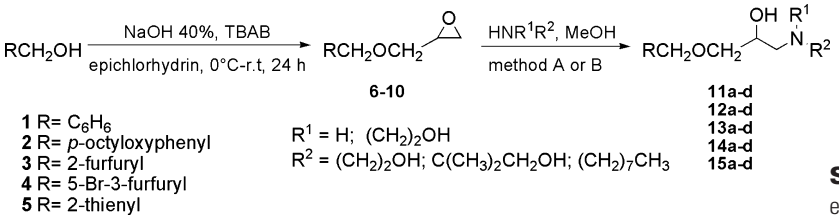
The aromatic aldehyde (2 mmol) and the octylamine or amino alcohol (2 mmol) were added to a cold stirred mixture of anhydrous methanol (8 mL) and dry sodium sulphate (1 g). The reaction was monitored by TLC in dichloromethane. After completion of the reaction, sodium borohydride (4 mmol) was added in portions with stirring at room temperature until completion of the reaction (TLC dichloromethane: methanol, 9:1 v/v). The sodium sulphate was removed by filtration and the resulting solution was concentrated and then extracted with dichloromethane and ammonium chloride (2:1 v/v). The organic phase was evaporated to dryness and purified by column chromatography on silica or by recrystallization.

Biology

Antibacterial activity

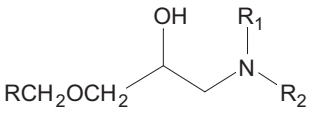
The antibacterial activity was assessed using the agar well diffusion and the broth dilution methods, against representative Gram-negative (*Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 2785) and Gram-positive (*Staphylococcus aureus* ATCC 3591, *Staphylococcus epidermidis* ATCC12228) strains obtained from the culture collection at the Laboratory of Bacterial Physiology and Molecular Genetics, Federal University of Juiz de Fora, Brazil. Nitrofurazone was used as a standard antibiotic for comparison.

Soft-agar was inoculated with a culture of the bacteria, poured into a Petri dish and allowed to set for 30 min at room temperature.



Scheme 1: Synthesis of glycidyl ethers and epoxide ring opening.

Table 1: Epoxide ring opening and reductive amination



R	R^1	R^2	Compound	Yield %	$RCH_2NR^1R^2$ Compound	Yield %
C_6H_6	H	$(CH_2)_2OH$	**11a**	30[a]	**22a** (19,20)	46
C_6H_6	H	$C(CH_3)_2CH_2OH$	**11b**	84[a]	**22b** (20)	71
C_6H_6	$(CH_2)_2OH$	$(CH_2)_2OH$	**11c**	60[a]	ns	–
C_6H_6	H	$(CH_2)_7CH_3$	**11d**	60	**22c** (21)	92
p-Octyloxy-C_6H_5	H	$(CH_2)_2OH$	**12a**	77[a]	**23a** (22)	49
p-Octyloxy-C_6H_5	H	$C(CH_3)_2CH_2OH$	**12b**	46[a]	**23b**	58
p-Octyloxy-C_6H_5	$(CH_2)_2OH$	$(CH_2)_2OH$	**12c**	40[a]	ns	–
p-Octyloxy-C_6H_5	H	$(CH_2)_7CH_3$	**12d**	79[a]	**23c**	50
2-Furfuryl	H	$(CH_2)_2OH$	**13a**	54[a]	**24a** (23)	56
2-Furfuryl	H	$C(CH_3)_2CH_2OH$	**13b**	40[b]	**24b**	80
2-Furfuryl	$(CH_2)_2OH$	$(CH_2)_2OH$	**13c**	59[a]	ns	–
2-Furfuryl	H	$(CH_2)_7CH_3$	**13d**	67[b]	**24c** (24)	60
5-Br-furfuryl	H	$(CH_2)_2OH$	**14a**	59[a]	**25a** (23)	60
5-Br-furfuryl	H	$C(CH_3)_2CH_2OH$	**14b**	45[b]	**25b**	80
5-Br-furfuryl	$(CH_2)_2OH$	$(CH_2)_2OH$	**14c**	59[a]	ns	–
5-Br-furfuryl	H	$(CH_2)_7CH_3$	**14d**	54[b]	**25c**	64
2-Thienyl	H	$(CH_2)_2OH$	**15a**	40[a]	**26a** (20)	54
2-Thienyl	H	$C(CH_3)_2CH_2OH$	**15b**	40[b]	**26b** (25)	62
2-Thienyl	$(CH_2)_2OH$	$(CH_2)_2OH$	**15c**	62[a]	ns	–
2-Thienyl	H	$(CH_2)_7CH_3$	**15d**	58[b]	**26c**	66

ns, not synthesized.
[a]Method A.
[b]Method B.

Holes (diameter of 5 mm) were then punched in the agar and filled with 100 μL of sample (5 mg/mL). The plates were then incubated overnight at 30 °C for growth of the bacteria, and the diameter of the inhibition zone was measured (13).

The MIC of the selected compounds was determined *in vitro* by the broth dilution method in Mueller Hinton medium (DIFCO Laboratories, Detroit, MI, USA), according to the CLSI guidelines (14). Each tested strain was previously grown in tryptic soy agar (DIFCO) at 35.5 °C for 24 h, and bacterial suspensions were adjusted to the turbidity of 0.5 McFarland (1.5 10^8 CFU/mL) with sterile saline solution (0.85% NaCl). Test compounds (5 mg) were dissolved in DMSO (2 mL) and the solutions were brought to 10 mL with distilled water. Further dilutions with Mueller Hinton broth were performed to obtain concentrations varying from 4 to 256 μg/mL, in a final volume of 3 mL. The test tubes, including a control without any inhibitory substance, were inoculated with 100 μL of the bacterial suspension and the systems were incubated at 35.5 °C for 24 h.

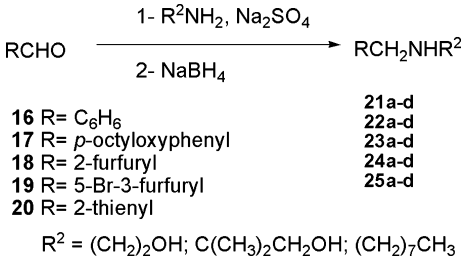
Bactericidal and bacteriostatic activities

To ensure the bactericidal concentrations, 0.1 mL from selected dilutions (two dilutions above MIC, dilution recorded as MIC, and two dilutions higher than MIC) was streaked out to a tryptic soy agar plate, which was incubated for 24 h (15). The bactericidal concentration of each chemical substance, for each bacterial strain, was considered as the lowest concentration of drug resulting in no bacterial growth (100% killing rate) (15).

Results and Discussion

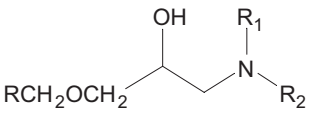
Reaction of aromatic alcohol with racemic epichlorohydrin under phase-transfer catalysis conditions led quantitatively to glycidyl ethers **6**–**10** (16,17) that were then used without further purification (Scheme 1). Benzyl alcohol derivative **2** was obtained from 4-hydroxybenzaldehyde, which was first alkylated with *n*-octyl bromide and then reduced with sodium borohydride, as described in the literature (75% over yield) (18).

Ring opening of the resulting epoxides was performed by treatment with amino alcohol (ethanolamine, diethanolamine, 2,2-dimethyl ethanolamine) or octylamine in methanol under conventional heating at 50 °C (method A) or under microwave irradiation (method B), leading to the corresponding aromatic amino alcohols in moderate yields after purification (Scheme 1, Table 1).



Scheme 2: Reductive amination.

Table 2: Diameter of the inhibition zone (cm)



$RCH_2NR_1R_2$

	E. coli	*P. aeruginosa*	*S. aureus*	*S. epidermidis*		*E. coli*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
11a	–	0.7	–	–	**22a**	–	–	–	–
11b	–	0.7	–	–	**22b**	–	–	–	–
11c	–	0.9	–	–	**22c**	1.9	1.3	2.1	1.7
11d	2.1	1.5	1.9	1.9	**23a**	2.1	1.9	2.5	2.7
12a	1.1	0.7	0.9	–	**23b**	2.9	0.9	2.3	1.1
12b	0.9	0.7	1.5	1.5	**23c**	0.9	–	1.5	–
12c	2.1	0.9	1.3	1.3	**24a**	–	0.7	–	–
12d	1.5	1.1	1.3	1.1	**24b**	1.5	–	–	–
13a	–	–	–	–	**24c**	2.5	4.5	1.7	4.5
13b	–	–	–	1.5	**25a**	–	0.9	–	–
13c	–	–	–	–	**25b**	–	–	–	–
13d	2.3	1.9	1.5	1.7	**25c**	1.7	–	–	1.3
14a	–	–	1.7	–	**26a**	1.7	1.5	–	–
14b	0.9	0.7	–	–	**26b**	1.7	1.7	0.9	–
14c	–	–	–	–	**26c**	–	1.3	–	1.9
14d	3.1	1.3	2.9	2.1					
15a	3.5	–	2.3	–					
15b	–	–	–	–					
15c	–	1.1	–	–					
15d	2.1	1.3	1.7	1.7					
Nitrofurazone	3.5	1.5	3.1	2.5					

Table 3: Minimal inhibitory concentrations (MIC)

	MIC (μg/mL)										
	11d	**12b**	**12c**	**12d**	**13d**	**14d**	**15d**	**22c**	**23a**	**23b**	**24c**
Escherichia coli	128	256	128	32	256	64	64	256	64	64	256
Pseudomonas aeruginosa	256	>256	16	>256	128	128	>256	256	128	256	256
Staphylococcus aureus	128	16	>256	64	128	64	32	128	64	32	64
Staphylococcus epidermidis	64	32	32	32	>256	128	128	256	64	64	128

Table 4: Bactericidal activities

	Minimal bactericidal concentrations (μg/mL)										
	11d	**12b**	**12c**	**12d**	**13d**	**14d**	**15d**	**22c**	**23a**	**23b**	**24c**
Escherichia coli	256	256	256	32	–[a]	64	64	–[a]	64	128	256
Pseudomonas aeruginosa	–[a]	–	128	–	256	256	–	–[a]	–[a]	–[a]	–[a]
Staphylococcus aureus	256	64	–	256	256	128	64	256	64	128	128
Staphylococcus epidermidis	128	64	32	32	–	128	128	–[a]	128	256	128

Bacteriostatic.

The reductive amination was performed at room temperature through a one-pot procedure. Intermediate imines, formed by the treatment of aldehydes **16**–**20** with ethanolamine, 2,2-dimethyl ethanolamine or octylamine in methanol in the presence of dry sodium sulphate, were reduced with sodium borohydride, leading to the desired aromatic amino alcohols and amines in moderate yields after purification (Scheme 2, Table 1).

Antibacterial activity

The antibacterial activity was first assessed using the agar well diffusion method, using *E. coli*, *S. aureus*, *S.* e*pidermidis* and *P. aeruginosa* strains. The results (Table 2) suggest that of all the compounds assayed, the octylated ones were the most effective, as they showed significant inhibition of the tested bacteria. This can be observed in the series **11**, **13**, **14**, **15**, **21**, **23** and **24**, in which only the octylated compounds were active. Compound **14d** was comparable with nitrofurazone used as standard. All the *O*-octylated compounds of series **12** inhibited the bacteria, although the introduction of a second octyl group did not seem to improve the antibacterial activity, as the activity of the dialkylated compound **12d** was similar to those of monoalkylated **12a**–**c**.

The results of assay of minimum inhibitory concentration, as well as the bacterial activity, were determined for the octylated compounds by broth dilution method, and are shown in Tables 3 and 4. All the tested compounds were active against *E. coli*, with MIC values ranging from 256 to 32 μg/mL. Compound **12d**, the most active compound, was also bactericidal in the same concentration (32 μM). Compounds **11d**, **14d**, **21c**, **22a**, **22b** and **23c** were active against the four tested bacteria, and compounds **13d** and **12c** did not inhibit *S. epidermidis* and *S. aureus*, respectively, at the maximum concentration tested (256 μM). The comparison of the MIC values obtained for compounds **11d**, **13d** and **15d**, where the aromatic cycles are respectively phenyl, 2-furyl and 2-thienyl, shows that the thiophene analogue is the most active against three of the tested organisms. The introduction of a bromine atom on the furan ring enhances the activity, as the MIC value determined for compound **14d** is quite similar to that of **15d**. *N*-Phenyloctylamine **21c**, the simplest compound synthesized in this work, was less active than all its aminoalcohol analogues, suggesting that a lipophilic chain and at least one hydroxyl group would be necessary for the activity. The role of a second hydroxyl group is not clear: the results of the determination of the antibacterial activity by the agar well diffusion method suggest that the compounds of the first series (derived from epichlorohydrin) are more active than their analogues obtained by reductive amination. In spite of the fact that octylated derivatives are most active than the other tested compounds, suggesting that lipophilicity is important for the biological activity, no relationship could be established between lipophilicity (logP) and MIC values.

Conclusion

The two series of amino alcohols synthesized in this work displayed activity against Gram-positive and Gram-negative bacteria. The best results were obtained for the compounds having an octyl chain in their structure and at least one amino alcohol group, suggesting that the lipophilicity is as important as the chemical group for the activity. More studies are needed to determinate what would be the ideal length of the alkyl chains, and how these compounds inhibit the bacterial growth.

Acknowledgments

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References

1. Tally F.P., DeBruin M.F. (2000) Development of daptomycin for gram-positive infections. J Antimicrob Chemother;46:523–526.
2. Sader H.S., Gales A.C., Pfaller M.A., Mendes R.E., Zoccoli C., Barth A., Jones R.N. (2001) Pathogen frequency and resistance patterns in Brazilian hospitals: summary of results from three years of the SENTRY Antimicrobial Surveillance Program. Braz J Infect Dis;5:200–214.
3. Obritsch M.D., Fish D.N., MacLaren R., Jung R. (2005) Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. Pharmacotherapy;25:1353–1364.
4. Demain A.L., Sanchez S. (2009) Microbial drug discovery: 80 years of progress. J Antibiot;62:5–16.
5. Katiyar D., Tiwari K.V., Tewari N., Verma S.S., Sinha S., Gaikwad A., Srivastava A., Chaturvedi V., Srivastava R., Srivastava B.S., Tripathi R.P. (2005) Synthesis and antimycobacterial activities of glycosylated amino alcohols and amines. Eur J Med Chem;40:351–360.
6. Salmi C., Loncle C., Letourneux Y., Brune J.M. (2008) Efficient preparation of secondary aminoalcohols through a Ti(IV) reductive amination procedure. Application to the synthesis and antibacterial evaluation of new 3β-N-[hydroxyalkyl]aminosteroid derivatives. Tetrahedron;64:4453–4459.
7. Almeida M.V., Le Hyaric M., Amarante G.W., Lourenço M.C.S., Brandão M.L.L. (2007) Synthesis of amphiphilic galactopyranosyl diamines and amino alcohols as antitubercular agents. Eur J Med Chem;42:1076–1083.
8. Almeida C.G., Garbois G.D., Amaral L.M., Diniz C.G., Le Hyaric M. (2010) Relationship between structure and antibacterial activity of lipophilic *N*-acyldiamines. Biomed Pharmacother;64:287–290.
9. Correa T.A., Reis E.F.C., Alves L.L., Alves C.S., Castro S.B.R., Dias A.T., Taveira A.F., Le Hyaric M., Couri M.R.C., Ferreira A.P., Almeida M.V. (2010) Preparation of amino alcohols condensed with carbohydrates: evaluation of cytotoxicity and inhibitory effect on NO production. Chem Biol Drug Des;76:451–456.
10. Collins C.H., Lynes P.M., Grange J.M. (1995) Microbiological Methods, 7th edn. Britain: Butterwort-Heinemann Ltd.; p. 175–190.
11. Sun F., Xu G., Wu J., Yang L. (2006) Efficient lipase-catalyzed kinetic resolution of 4-arylmethoxy-3-hydroxybutanenitriles: application to an expedient synthesis of a statin intermediate. Tetrahedron Asymmetry;17:2907–2913.
12. Sabitha G., Subba Reddy B.V., Abraham S., Yadav J.S. (1999) Microwave promoted synthesis of β-aminoalcohols in dry media. Green Chem;1:251–252.
13. National Committee for Clinical Laboratory Standards (2001) Methods for Dilution and Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 5th edn, Vol. 20, no. 2. Approved Standard M 7.A5. Wayne, PA: NCCLS.
14. CLSI (2007) Performance Standards for Antimicrobial Susceptibility Testing, 17th Informational Supplement. CLSI Document M100-S16, 26. Wayne, PA: CLSI.
15. Alviano W.S., Alviano D.S., Diniz C.G., Antoniolli A.R., Alviano C.S., Farias L.M., Carvalho M.A.R., Souza M.M.G., Bolognese A.M. (2008) In vitro antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. Arch Oral Biol;53:545–552.
16. Gokel G.W., Dishong D.M., Diamond C.J.J. (1980) Lariat ethers. Synthesis and cation binding properties of macrocyclic polyethers possessing axially disposed secondary donor groups. J Chem Soc Chem Commun;5:1053–1054.
17. Hu Y., Qiao L., Qin Y., Zhao X., Chen X., Wang X., Wang F. (2009) Synthesis and Stabilization of Novel Aliphatic Polycarbonate from Renewable Resource. Macromolecules;42:9251–9254.
18. Hasegawa J., Hamada M., Miyamoto T., Nishide K., Kajimoto T., Uenishi J., Node M. (2005) The application of phenylmethanethiol and benzenethiol derivatives as odorless organo-sulfur reagents in the synthesis of thio-sugars and thio-glycosides. Carbohydr Res;340:2360–2368.
19. Chong H., Ma X., Lee H., Bui P., Song H.A., Birch N. (2008) Synthesis and evaluation of novel polyaminocarboxylate-based antitumor agent. J Med Chem;51:2208–2215.
20. Fujieda H., Usui S., Suzuki T., Nakagawa H., Ogura M., Makishima M., Miyata N. (2007) Phenylpropanoic acid derivatives bearing a benzothiazole ring as PPARδ-selective agonists. Bioorg Med Chem Lett;17:4351–4357.
21. Meyers A.I., Himmelsbach R.J., Reuman M. (1983) Reductive cleavage of aryl oxazolines to benzaldehydes and substituted toluenes. J Org Chem;48:4053–4058.
22. Cho T.K., Kang S.K. (2005) Direct and indirect reductive amination of aldehydes and ketones with solid acid-activated sodium borohydride under solvent-free conditions. Tetrahedron;61:5725–5734.
23. Schirok H., Li-Sommer Y., Brands M., Lobell M., Tersteegen A., Himmel H., Schlemmer K., Lang D., Petersen K., Renz M., Mumberg D., Hoffmann J., Siemeister G., Boemer U. (2009) Preparation of tricyclic nitrogen heterocyclic compounds and related heteroanalogs for the prevention or treatment of cancer. PCT Int. Appl.WO 2009033581.
24. Alonso F., Riente P., Yus M. (2008) Hydrogen-transfer reductive amination of aldehydes catalysed by nickel nanoparticles. Synlett;9:1289–1292.
25. Leskovsek V., Urleb U. (1994) A new approach to the synthesis of *N*-arylalkyl amino alcohols. Synth Com;24:1415–1424.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental data.

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