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Synthesis and antibacterial activity of benzyl-[3-(benzylamino-methyl)-cyclohexylmethyl]-amine derivatives

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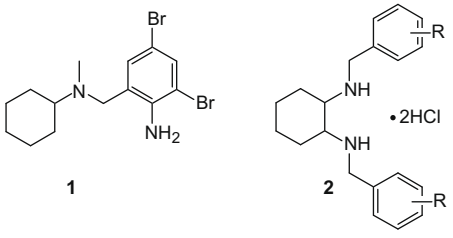
ABSTRACT

A series of benzyl-[3-(benzylamino-methyl)-cyclohexylmethyl]-amine derivatives with different substitution pattern on the aromatic ring have been prepared and evaluated for their antibacterial activity against Gram-positive and Gram-negative bacterial strains. Most of the compounds exhibit potent activity against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* while compounds **6l** and **6m** showed antibacterial activity against all the four bacterial strains with MIC values ranging from 0.002 to 0.016 μg/mL and no hemolytic activity up to 512 μg/mL in mammalian erythrocytes was observed.

Most of the antibacterial drugs in the clinical use have been discovered by the middle of last century and no new class of antibacterial was introduced in the market after 1962 until the discovery of linezolid in 2000.[1–3] Interestingly bacteria have developed resistance against most of these drugs soon after or even before their introduction in the market. For example linezolid was introduced in the market in 2000 and resistance of this compound was reported in 1999.[4–6] The multi-drug resistant has reached to the alarming stage[7–13] and situation has worsen due to the increasing incidences of methicillin resistant *Staphylococcus aureus* (MRSA), *Vancomycin-resistant enterococci* (VRE) and other antibiotic resistant human pathogenic microorganisms.[14–17]

To overcome the antibiotic resistant problem, there is an urgent need for the development of new antibacterial class that are not affected by resistance mechanisms already present in the bacterial population.[18,19] As a part of our ongoing efforts towards the synthesis of novel antibacterial agents,[20–22] we became interested to modify the bromhexine molecule (**1**). During the course of this study, we observed that simple structural manipulation led to the discovery of potent antibacterial agents (**2**) that exhibit antimicrobial activity at very low concentration without any hemolytic activity at very high concentration.[23,24]



Encouraged by this observation, we decided to synthesize structurally diverse compounds using 3-(aminomethyl-cyclohexyl)-methylamine as a starting material and to study their antibacterial activity.

Simple two step synthetic protocol was used for the preparation of title compounds (**6a–6u**). The first step involves treatment of diamine (**3**) with different substituted benzaldehydes (**4**) in dry methanol to give Schiff bases (**5a–5u**) in quantitative yield in the form of viscous liquid or solid. In the next step Schiff bases were reduced with sodium borohydride in dry methanol to give the substituted diamines (**6a–6u**) in quantitative yields. In most of the cases Schiff bases were soluble in methanol but in some cases dry THF was used as a co-solvent. All the synthesized amines were converted into their HCl salt by passing dry HCl gas in the solution of diamines in chloroform (Scheme 1). The formation of HCl salt of these diamines was confirmed by conductivity experiments. All these compounds were characterized spectroscopically (Table 1).[25]

In vitro antibacterial activity: Antimicrobial susceptibility testing was carried out using National Committee for Clinical Laboratory

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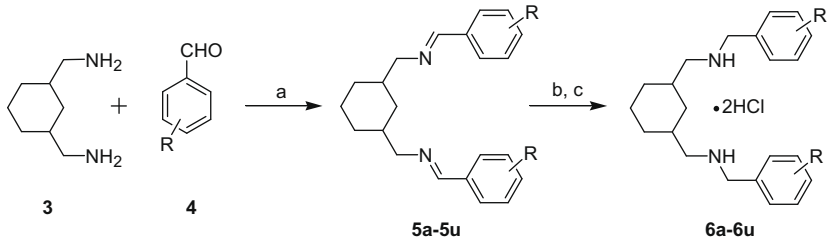

Scheme 1. Reagents and conditions: (a) MeOH, molecular sieves, 3–4 h, rt; (b) $NaBH_4$, MeOH, 2–3 h, rt; (c) $CHCl_3$, HCl gas.

Table 1
Synthesis of benzyl-[3-(benzylamino-methyl)-cyclohexylmethyl]-amine derivatives

Compd No.	R	Yield (%)	Mp (°C)
6a	H	70	110–112
6b	4-Me	62	114–116
6c	3-Me	70	198–200
6d	3,5-Me	68	152
6e	3,4-Me	71	240
6f	2-Me	65	196–198
6g	2,5-Me	70	150
6h	2,4-Me	80	198
6i	4-Et	55	218
6j	4-*n*-Pr	60	228
6k	4-*iso*-Pr	65	264
6l	4-*n*-Bu	80	202
6m	4-*t*-Bu	90	278
6n	4-Cl	85	252
6o	3-Cl	85	168
6p	2,6-Cl	90	120–122
6q	4-Br	75	202
6r	3-Br	65	182
6s	4-F	67	110–113
6t	3-F	75	150–152
6u	3-NO_2	78	210

Standards (NCCLS) microdilution assay. Briefly, the bacterial strains were grown in standard media until exponential growth was achieved. Tests were performed in a 96-well microtiter plate in a final volume of 100 μL. Test compounds were dissolved in 5% DMSO at an initial concentration of 500 μg and serially diluted in plate. Each well was then inoculated with ~2–5 × 10^5 bacterial cells and incubated at 37 °C for 24 h with shaking at 200 rpm. One well containing cells and 5% DMSO without any test compound (growth control), and one well containing only growth medium (sterility control) were used as controls. Growth of bacteria was determined using Power wave200 microplate scanning spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). Percent survival was calculated using growth without any compound as 100% survival. The MIC values are calculated using Grafit 4.0 software (Erithacus Software Ltd., Horley, Surrey, UK).

The compounds were evaluated against Gram-positive and Gram-negative bacterial strains. The minimum inhibitory concentration (MIC) values of each compound are shown in Table 2.

Compound **6a** in which both of the benzene rings are unsubstituted, showed no activity against any of the bacterial strains. Compounds with methyl group in the aromatic ring at different positions exhibit potent antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* with MIC values ranging from 0.125 to 1.00 μg/mL, with exception of compound **6f** in which methyl group is at *ortho*-position. Compound **6f** was found to be totally inactive against all the strains. Introduction of ethyl, *n*-propyl, *iso*-propyl, *n*-butyl or *t*-butyl groups at *para*-position of the phenyl ring have marked effect on the antibacterial activity of these compounds (entry **6i–6m**).

For example compounds having *n*-butyl or *tert*-butyl (entry **6l**, **6m**) exhibit antibacterial activity against all the four strains

Table 2
Antibacterial activity of benzyl-[3 (benzylamino-methyl)-cyclohexylmethyl]-amine derivatives

Compd No.	MIC (μg/mL)			
	E. coli	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
6a	*	*	*	*
6b	*	0.5	*	1.00
6c	*	1.00	*	1.00
6d	*	1.00	*	0.25
6e	*	0.125	*	*
6f	*	*	*	*
6g	*	*	*	*
6h	*	*	*	*
6i	*	0.125	*	0.25
6j	0.016	0.016	*	0.016
6k	*	0.125	*	0.125
6l	0.016	0.002	0.004	0.004
6m	0.002	0.004	0.016	0.008
6n	*	0.032	0.025	0.065
6o	*	0.125	*	0.25
6p	*	*	*	*
6q	*	0.032	*	0.125
6r	*	0.125	*	0.125
6s	*	*	*	*
6t	*	*	*	*
6u	*	*	0.02	*
Tet	0.001	0.01	0.01	0.02

[Tet] Tetracycline was used as a reference compound.
[*] MIC >250 μg/mL.

(*Escherichia coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*) with MIC values ranging from 0.002 to 0.016 μg/mL, while compound with *n*-propyl group (entry **6j**) exhibit activity against *E. coli*, *P. aeruginosa* and *S. epidermidis* with MIC values 0.016 μg/mL. Interestingly compounds with ethyl and *iso*-propyl groups at *para*-position of the phenyl ring (entry **6i** and **6k**) showed good activity against *P. aeruginosa* and *S. epidermidis* only. Compounds with Cl, Br or NO_2

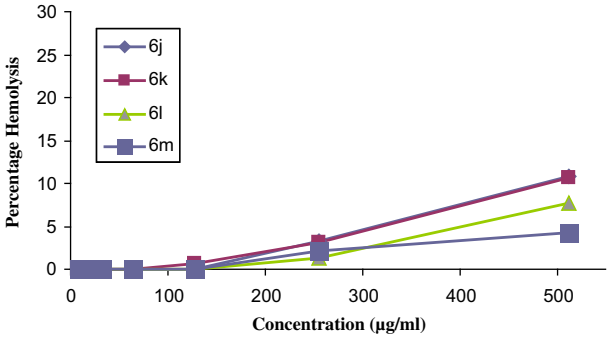

Figure 1. Hemolytic activity of the compounds. Fresh hRBC suspension (4 % v/v in 35 mM phosphate buffer with 150 mM NaCl) was used for the assay. After incubation of the test sample in the erythrocyte solution for 1 h at 37 °C, the solution was centrifuged and the supernatant absorbance was determined at 414 nm. Hemolysis affected by 0.1% Triton X-100 was considered as 100%.

at *meta*- or *para*-position exhibit good activity against *P. aeruginosa* and *S. epidermidis* (entry **6n**, **6o**, **6q**, **6r**, **6u**), while other compounds with fluoro group exhibit no activity against any of the bacterial strain (entry **6s**, **6t**). Other compounds having substitution at *ortho*-position of the phenyl ring were devoid of antibacterial activity (entry **6f**, **6g**, **6h**, and **6p**).

Toxicity of these compounds were investigated using human red blood cells (hRBC) the results showed that the concentration up to 512 μg/mL of compounds **6b–6f**, **6n–6r** did not show any toxicity while compounds **6j–6m** showed hemolysis between 4% and 10% at same concentration (Fig. 1). Compound **6i** lysed only 0.5% of the mammalian erythrocytes at 512 μg/mL concentration.

Out of 21 compounds, 14 compounds showed impressive antibacterial activity against *P. aeruginosa* and *S. epidermidis*, while two compounds exhibit potent antibacterial activity against all the bacterial strains with no hemolysis up to 512 μg/mL. Further chemical modification of selected compounds is under progress and results will be published in due course of time.

Acknowledgments

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25. (*4-Ethyl-benzyl*)-{*3*-[(*4-ethyl-benzylamino*)-*methyl*]-*cyclohexylmethyl*}-*amine* (**6i**): Yield: 55%; mp: 218 °C; IR (Nujol, cm^{-1}): 2923, 2852, 2723, 2403, 1461, 1377, 1021, 828, 722; ^1H NMR (300 MHz, DMSO-d_6): 0.57–0.65 (m, 1H), 0.76–0.83 (m, 2H), 1.13–1.18 (t, 6H), 1.32–1.36 (m, 1H), 1.74–1.96 (m, 6H), 2.55–2.77 (m, 8H), 4.03 (s, 4H, $2CH_2Ph$), 7.21–7.24 (d, J = 9 Hz, 4H, Ar), 7.46–7.49 (d, J = 9 Hz, 4H, Ar), 9.23 (br s, 4H, $2NH_2^+$); ^{13}C NMR (75.5 MHz, DMSO-d_6): 15.53, 24.31, 27.87, 29.60, 30.67, 33.86 50.01, 51.83, 127.85, 129.23, 130.28, 144.43; ESI-HRMS (m/z) calculated for $C_{26}H_{38}N_2$: 378.3035, found: 378.3487 (M^+). (*4-n-Propyl-benzyl*)-{*3*-[(*4-n-propyl-benzylamino*)-*methyl*]-*cyclohexyl methyl*}-*amine* (**6j**): Yield: 60%; mp: 228 °C; IR (Nujol, cm^{-1}): 2924, 2845, 2751, 2599, 1584, 1462, 1027, 802; ^1H NMR (300 MHz, DMSO-d_6): 0.57–0.65 (m, 1H), 0.76–0.83 (m, 2H), 0.84–0.89 (t, 6H), 1.15–1.26 (m, 1H), 1.52–1.56 (m, 4H), 1.69–1.83 (m, 6H), 2.52–2.56 (t, 4H), 2.63–2.66 (d, 4H), 4.04 (s, 4H, $2CH_2Ph$), 7.20–7.23 (d, J = 9 Hz, 4H, Ar), 7.44–7.47 (d, J = 9 Hz, 4H, Ar), 9.18 (br s, 4H, $2NH_2^+$); ESI-HRMS (m/z) calculated for $C_{28}H_{42}N_2$: 406.3348, found: 406.3150 (M^+). (*4-Isopropyl-benzyl*)-{*3*-[(*4-isopropyl-benzyl amino*)-*methyl*]-*cyclohexylmethyl*}-*amine* (**6k**): Yield: 65%; mp: 264 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2754, 2379, 1584, 1459, 1022, 833; ^1H NMR (300 MHz, DMSO-d_6): 0.57–0.65 (m, 1H), 0.76–0.83 (m, 2H), 1.17–1.19 (d, 13H), 1.70–1.83 (m, 6H), 2.66–2.68 (t, 4H), 2.86–2.91 (m, 2H), 4.05 (s, 4H, $2CH_2Ph$), 7.26–7.29 (d, J = 9 Hz, 4H, Ar), 7.45–7.48 (d, J = 9 Hz, 4H, Ar), 9.11 (br s, 4H, $2NH_2^+$); ^{13}C NMR (75.5 MHz, DMSO-d_6): 23.76, 26.43, 29.57, 30.68, 33.17, 33.80, 50.01, 51.89, 126.38, 129.28, 130.34, 151.23; ESI-HRMS (m/z) calculated for $C_{28}H_{42}N_2$: 406.3348, found: 406.3291 (M^+). (*4-n-Butyl-benzyl*)-{*3*-[(*4-n-butylbenzyl-amino*)-*methyl*]-*cyclohexylmethyl*}-*amine* (**6l**): Yield: 80%; mp: 202 °C; IR (Nujol, cm^{-1}): 2923, 2854, 2739, 1585, 1461, 1377, 1026, 833; ^1H NMR (300 MHz, DMSO-d_6): 0.57–0.67 (m, 1H), 0.76–0.80 (m, 2H), 0.84–0.89 (t, 6H), 1.24–1.31 (m, 5H), 1.47–1.57 (m, 4H), 1.73–1.82 (m, 6H), 2.54–2.59 (t, 4H), 2.64–2.68 (d, 4H), 4.04 (s, 4H, $2CH_2Ph$), 7.22–7.23 (d, J = 9 Hz, 4H, Ar), 7.44–7.47 (d, J = 9 Hz, 4H, Ar), 9.12 (br s, 4H, $2NH_2^+$); ^{13}C NMR (75.5 MHz, DMSO-d_6): 13.65, 21.60, 24.21, 29.49, 32.95, 33.73, 34.14, 34.40, 49.91, 51.74, 128.31, 129.04, 130.15, 142.96; ESI-HRMS (m/z) calculated for: $C_{30}H_{46}N_2$: 434.3661, found: 434.69 (M^+). (*4-t-Butyl-benzyl*)-{*3*-[(*4-t-butyl-benzylamino*)-*methyl*]-*cyclohexylmethyl*}-*amine* (**6m**): Yield: 90%; mp: 278 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2475, 1586, 1458, 1377, 1023, 834; ^1H NMR (300 MHz, DMSO-d_6): 0.56–0.68 (m, 1H), 0.76–0.81 (m, 2H), 1.15–1.2 (m, 1H), 1.26 (s, 18H), 1.74–1.85 (m, 6H), 2.63–2.80 (d, 2H), 4.04 (s, 4H, CH_2Ph), 7.39–7.42 (d, J = 9 Hz, 4H, Ar), 7.50–7.53 (d, J = 9 Hz, 4H, Ar), 9.34 (br s, 4H, NH_2^+); ^{13}C NMR (75.5 MHz, DMSO-d_6): 24.49, 29.40, 31.51, 30.05, 34.09, 34.51, 50.85, 51.50, 125.86, 127.05, 130.22, 152.20; ESI-HRMS (m/z) calculated for: $C_{30}H_{46}N_2$: 434.3661, found: 434.3556 (M^+).