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Microwave Assisted Synthesis and Antimicrobial Activity of Novel Pyrrolidine Derivatives

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Abstract—Microwave assisted synthesis of 1-acetyl-2-benzylpyrrolidine-2-carboxylic acid and its derivatives has been developed with highly encouraging yields. 2-Benzyl-*tert*-butylpyrrolidine-1,2-dicarboxylate is used as an initial compound in the four steps synthesis of the target compounds. Structures of the products **5a**–**5h** have been confirmed by spectroscopic methods. According to antimicrobial tests 1-acetyl-2-benzylpyrrolidine-2-carboxamide **5c** is determined to be the most potent product.

Keywords: novel pyrrolidine derivatives, characterization, antimicrobial activity

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INTRODUCTION

The pyrrolidine ring system is a structural component of a variety of compounds with such pharmacological activities as antimicrobial [1, 2], antitumor [3], anti-HIV [4], and many more. For example, they are incorporated in the molecules of antibacterial agents Tosufloxacin, Clinafloxacin and Ceftobiprole.

In view of the above, we would like to report here an efficient MW assisted synthesis of new pyrrolidine derivatives as potential antimicrobial agents.

RESULTS AND DISCUSSION

The target pyrrolidine derivatives **5a**–**5h** were synthesized from 1-acetyl-2-benzylpyrrolidine-2-carboxylic acid according to the conventional and MW assisted methods (Schemes 1, 2). The latter approach turned out to be much more efficient than the conventional approach (Table 1). Structures of the synthesised products were characterized by ^{1}H and ^{13}C NMR spectral data.

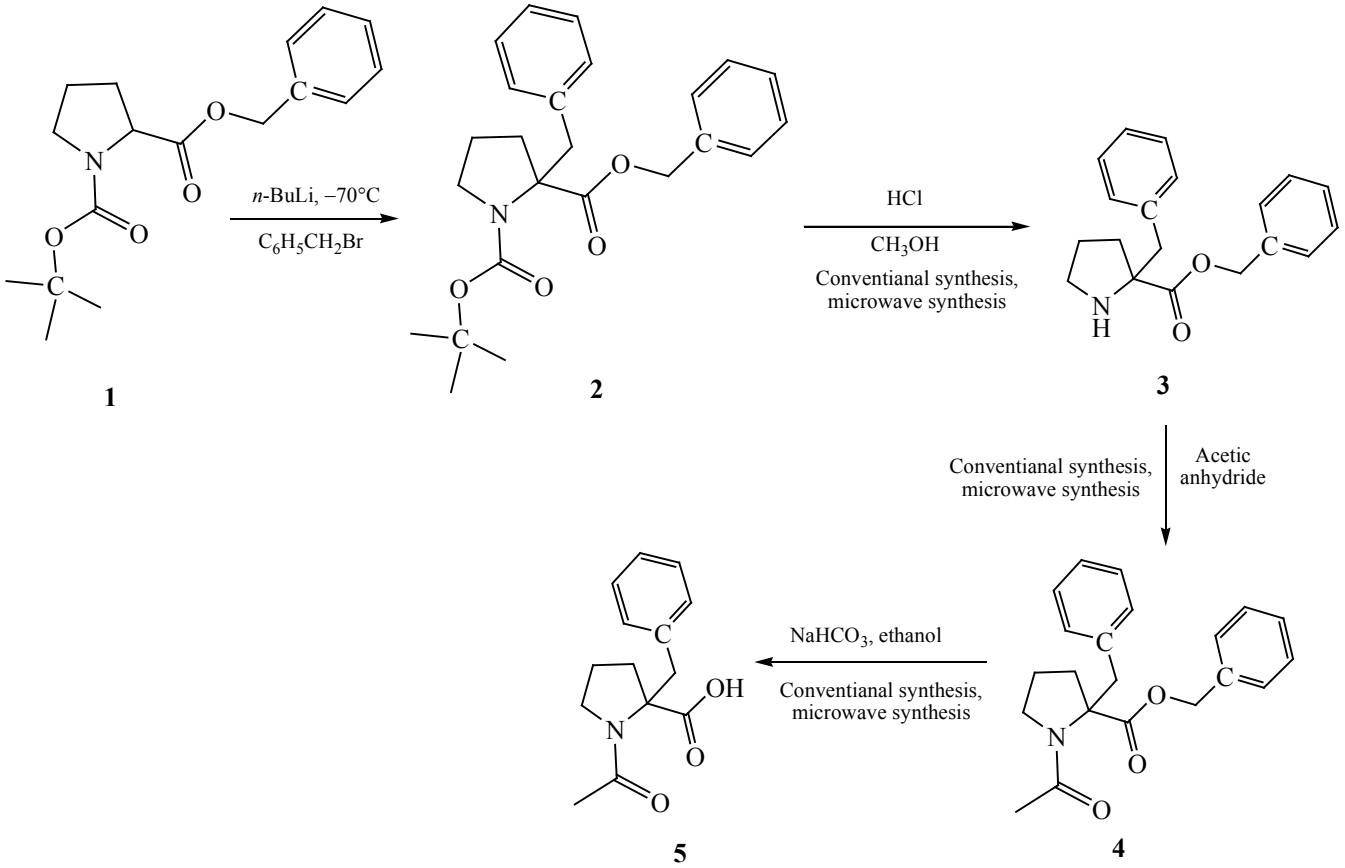
Antimicrobial activity. Serial dilutions of the test compounds and reference drugs were prepared in Müller–Hinton broth. Drugs (10 mg) were dissolved in DMSO (1 mL). Further progressive dilutions with melted Müller–Hinton agar were performed to obtain the required concentrations. In primary screening 1024 and 512 μg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 256, 128, 64, 32, 16, and 8 μg/mL against all microorganisms.

Antibacterial activity. The bacterial activity of compounds **5a**–**5h** were evaluated and compared with Chloramphenicol as standard drug. Compounds **5c** and **5f** showed good antibacterial properties against four pathogenic bacterial strains compared with other compounds. Compound **5c** exhibited inhibition about 16 μg/mL against *B. subtilis* and *S. aureus.* Compound

Table 1. Comparison of the conventional and microwave assisted methods of synthesis of compounds **3, 4, 5a**–**5h**

Comp. no.	Reaction time		Yield, %	
	microwave synthesis, min	conventional synthesis, h	microwave synthesis, min	conventional synthesis, h
3	10	2	88	81
4	25	4	86	78
5	10	3	89	72
5a	25	5	79	66
5b	5	1	82	72
5c	20	6	91	79
5d	No active	2	No active	86
5e	15	3	79	71
5f	15	4	92	85
5g	10	2	90	76
5h	15	4	86	71

Scheme 1. Synthetic route to 1-acetyl-2-benzylpyrrolidine-2-carboxylic acid (**5**).



5f exhibited inhibition at ca 16 μg/mL against *B. subtilis*. Products **5d** and **5e** exhibited moderate antibacterial activity against all bacterial strains. Compounds **5a** and **5b** were determined to be of weak antibacterial activity against *S. aureus* and *B. subtilis*.

Antifungal activity. All the synthesized compounds **5a**–**5h** were tested against *C. Albicans*. Compounds **5c** and **5f** showed good antifungal properties than other compounds in the series against the tested strain. Compound **5c** showed inhibition at 32 μg/mL. The products **5d** and **5e** were moderately active.

In the present study, different groups attaching to the pyrrolidine ring influenced upon antimicrobial activity of the products. Antibacterial and antifungal activities of the tested compounds varied in the ranges 16–256 and 32–256 μg/mL, accordingly. Presence of the amino group in the moiety resulted in the enhanced activity of the corresponding derivatives. The electron donating hydroxyl and methoxy groups of pyrrolidine ring led to moderate activity of the respective products.

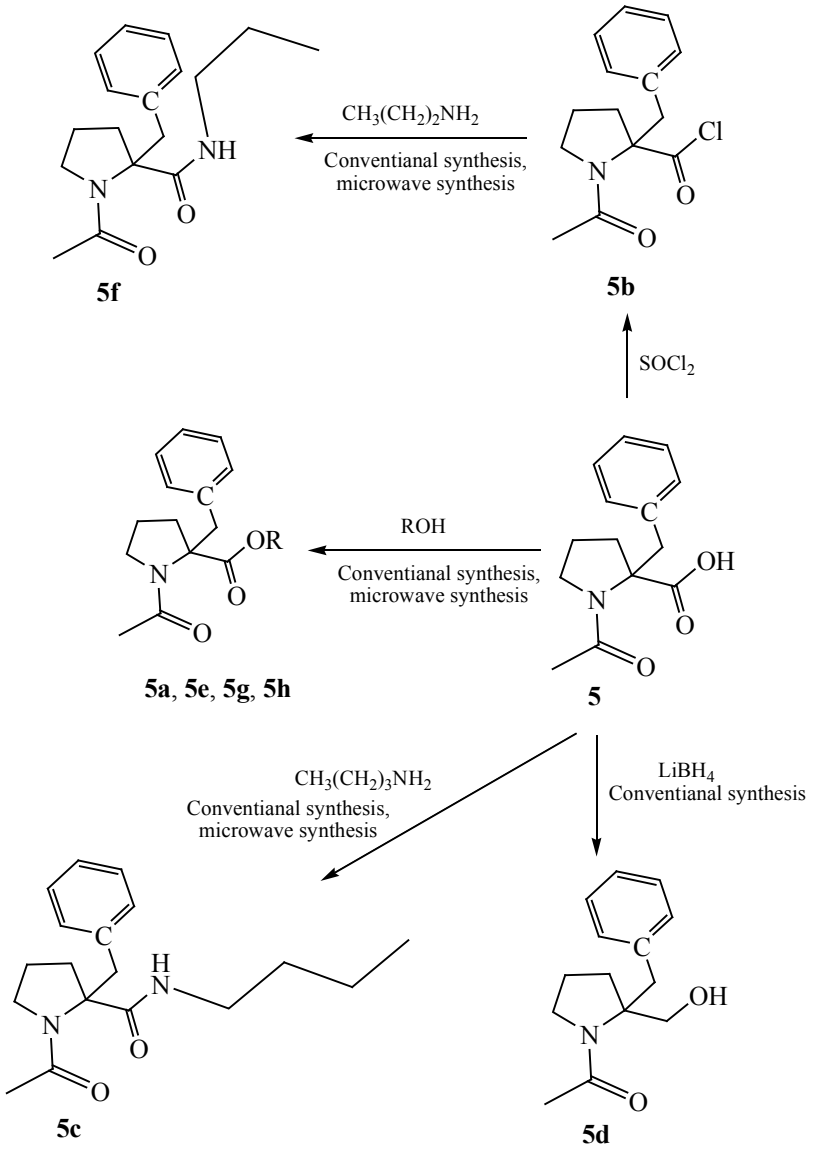
EXPERIMENTAL

Reagents were purchased from Across Ltd and used as it is. The solvents were of analytical grade and distilled before use. NMR spectra were measured on a Bruker 400 MHz spectrometer using DMSO-d_6 as a solvent and TMS as an internal reference. The microwave assisted syntheses were carried out using a Flexiwave–Milestone Microwave Synthesis Platform instrument.

Synthesis of 2-benzyl-1-*tert*-butyl-2-benzyl pyrrolidine-1,2-dicarboxylate (2). To a solution of compound **1** (1.6 mmol) in 20 mL of tetrahydrofuran were added *n*-butyl lithium (2.0 mmol) at –70°C upon stirring in nitrogen atmosphere, then benzyl bromide (1.5 mmol). The mixture was stirred for 1 h at room temperature. The resultant solution was quenched in dilute hydrochloric acid (4N), and the product was extracted by methylene chloride.

Benzyl-2-benzyl pyrrolidine-2-carboxylate (3). The compound **2** (2, 2.5 mmol) was dissolved in 15 mL of methanol, and hydrochloric acid (1.0 mmol) was added slowly. The reaction mixture was stirred for 10 min at

Scheme 2. Synthetic route to the compounds **5a**–**5h**.



R = CH_3CH_2 (**5a**), CH_3 (**5e**), $C_6H_5CH_2$ (**5g**), $CH(CH_3)_2$ (**5h**).

30–35°C in a MW oven then quenched in water, and extracted with diethyl ether.

Benzyl-1-acetyl-2-benzyl pyrrolidine-2-carboxylate (4). Compound **3** was mixed with acetic anhydride (2.0 mmol), 15 mL of methylene dichloride and sodium carbonate (3.0 mmol). The reaction mixture was stirred for 25 min in a MW oven, then quenched in water. The organic layer was separated and extracted with n-hexane.

1-Acetyl-2-benzylpyrrolidine-2-carboxylic acid (5). Solution of compound **4** (2.0 mmol) in 15 mL of ethanol was mixed with sodium bicarbonate. The reaction mixture was refluxed for 10 min in a MW oven, then quenched in water, extracted by dichloromethane, and concentrated to half of its volume. The remaining mixture was cooled down to 2–8°C upon stirring for 1 h. The product was filtered off and washed with diethyl ether.

Ethyl-1-acetyl-2-benzyl pyrrolidine-2-carboxylate (5a). To the product **5** dissolved in ethanol sulphuric acid was added slowly at 20–30°C. The reaction mixture was treated for 25 min in a MW oven, then quenched in ammonium bicarbonate solution, and the product was extracted by ethyl acetate and isolated. ^{1}H NMR, spectrum, δ, ppm: 1.1–1.5 t (3H, CH_2–CH_3), 4.2–4.6 m (2H, CH_2–CH_3), 7.50–8.40 m (5H, benzene), 2.0–2.3 m (pyr-

rolidine), 3.5–4.3 t (2H, pyrrolidine). ^{13}C NMR spectrum, δ, ppm: 14.2, 20.5, 24.2, 37.3, 46.7, 77.3, 124.7, 130.5, 132.3, 140.2, 170.6, 174.3. Found, %: C 69.71; H 7.72; N 5.11; O 17.41. $C_{16}H_{21}NO_3$. Calculated, %: C 69.79; H 7.69; N 5.09; O 17.43.

1-Acetyl-2-benzyl pyrrolidine-2-carbonyl chloride (5b). The mixture of compound **5** with toluene and thionyl chloride was heated to 40–50°C and maintained for 5 min in a MW oven. The pure product was distilled off. ^{1}H NMR spectrum, δ, ppm: 7.50–8.40 m (5H, benzene), 1.8–2.2 m (2H, pyrrolidine), 3.5–3.9 t (2H, pyrrolidine). ^{13}C NMR spectrum, δ, ppm: 20.5, 24.2, 36.2, 45.3, 70.8, 124.7, 127.4, 129.4, 139.4, 177.4, 172.9. Found, %: C 63.21; H 6.02; N 5.22; O 12.01. $C_{14}H_{16}ClNO_2$. Calculated, %: C 63.28; H 6.07; N 5.27; O 12.04.

1-Acetyl-2-benzyl-*n*-butylpyrrolidine-2-carboxamide (5c). To the mixture of compound **5**, methylene dichloride and 5-methoxy-2-iodophenylboronic was added butyl amine. The mixture was treated for 15 min in a MW oven, then quenched in water. The organic layer was separated to give the desired product. ^{1}H NMR spectrum, δ, ppm: 0.7–2.0 t (3H, C**H**$_3$–CH$_2$); 1.2–1.4 m (2H, CH$_2$–C**H**$_2$–CH$_3$); 1.4–1.8 m (2H, CH$_2$–C**H**$_2$–CH$_2$); 3.0–3.4 t (2H, **CH**$_2$–CH$_2$); 7.40–7.9 m (5H, benzene), 1.9–2.2 m (2H, pyrrolidine), 3.5–3.8 t (2H, pyrrolidine), 7.6–8.3 s (1H, NH). ^{13}C NMR spectrum, δ, ppm: 14.4, 21.6, 33.8, 41.4; 25.2, 38.3, 47.2, 78.5, 125.1, 129.2, 131.9, 136.4, 171.2, 174.5. Found, %: C 71.47; H 8.65; N 9.23; O 10.55. $C_{18}H_{26}N_2O_2$. Calculated, %: C 71.49; H 8.67; N 9.26; O 10.58.

1-[2-Benzyl-2-(hydroxymethyl)pyrrolidine-1-yl]-ethanone (5d). To the solution of compound **5** in THF at 2–8°C were added BuLi and lithium borohydride. The mixture was stirred for 2 h then quenched in water, the product was extracted by methylene dichloride and isolated upon evaporation of the solvent. ^{1}H NMR spectrum, δ, ppm: 17.30–7.80 m (5H, benzene), 1.8–2.1 m (2H, pyrrolidine), 3.5–3.8 m (2H, pyrrolidine), 3.6–3.9 s (2H, CH$_2$–OH), 3.2.3.6 s (1H, OH). ^{13}C NMR spectrum, δ, ppm: 23.2, 19.5, 36.4, 46.5, 71.5, 124.1, 124.4, 130.8, 138.3, 171.2. Found, %: C 72.01; H 8.16; N 5.97; O 13.65. $C_{14}H_{19}NO_2$. Calculated, %: C 72.07; H 8.21; N 6.00; O 13.72.

Methyl-1-acetyl-2-benzyl pyrrolidine-2-carboxylate (5e). To the solution of compound **5** in methanol was added sulphuric acid upon stirring at 20–30°C. Then the reaction mixture was treated for 15 min in a MW oven. Upon completion of the process the mixture was quenched in ammonium bicarbonate solution, and the product was extracted by ethyl acetate and isolated upon evaporation of the solvent. ^{1}H NMR spectrum, δ, ppm: 7.30–7.60 m (5H, benzene), 1.8–2.1 m (2H, pyrrolidine), 3.5–3.6 m (2H, pyrrolidine), 3.6–3.9 s (3H, CH$_3$–O). ^{13}C NMR spectrum, δ, ppm: 21.5, 24.6, 37.8, 45.2, 76.1, 126.2, 129.4, 130.1, 137.7, 171.5, 172.8. Found, %: C 68.91; H 7.29; N 5.31; O 18.32. $C_{15}H_{19}NO_3$. Calculated, %: C 68.94; H 7.33; N 5.36; O 18.37.

1-Acetyl-2-benzyl-*N*-propylpyrrolidine-2-carboxamide (5f). To the solution of compound **5** in methylene dichloride propyl amine was added slowly, and the mixture was treated for 15 min in a MW oven, then quenched in water. The organic layer was separated, and the product was isolated. ^{1}H NMR spectrum, δ, ppm: 0.8–1.1 t (3H, C**H**$_3$–CH$_2$),1.4–1.7 m (2H, CH$_2$–C**H**$_2$–CH$_3$), 7.30–7.60 m (5H, benzene), 1.8–2.2 m (2H, pyrrolidine), 3.5–3.7 m (2H, pyrrolidine), 7.1–7.5 t (2H, C**H**$_2$–CH$_2$–NH), 8.0–8.3 s (1H, NH group). ^{13}C NMR spectrum, δ, ppm: 11.7, 24.4, 43.5; 24.8, 42.6, 46.2, 76.3, 123.4, 127.5, 128.6, 137.5, 173.3, 175.9. Found, %: C 70.77; H 8.36; N 9.73; O 11.14. $C_{17}H_{24}N_2O_2$. Calculated, %: C 70.80; H 8.39; N 9.71; O 11.10.

Benzyl 1-acetyl-2-benzylpyrrolidine-2-carboxylate (5g). To the mixture of compound **5** with benzyl alcohol was added sulphuric acid upon stirring at 20–30°C. The reaction mixture was treated for 10 min in a MW oven, then quenched in ammonium bicarbonate solution. The product was extracted by ethyl acetate and isolated. ^{1}H NMR spectrum, δ, ppm: 7.20–7.90 m (10H, benzene), 1.8–2.2 m (2H, pyrrolidine), 3.5–3.9 m (2H, pyrrolidine), 5.2–5.6 s (2H, O–C**H**$_2$–Ph). ^{13}C NMR spectrum, δ, ppm: 25.6, 36.3, 47.2, 77.8, 67.5, 123.2, 128.3, 130.4, 139.9, 173.8.4, 171.2. Found, %: C 74.71; H 6.84; N 4.12; O 14.21. $C_{21}H_{23}NO_3$. Calculated, %: C 74.75; H 6.87; N 4.15; O 14.23.

Isopropyl 1-acetyl-2-benzylpyrrolidine-2-carboxylate (5h). To the solution of compound **5** in isopropyl alcohol sulphuric acid was added upon stirring at 20–30°C. The reaction mixture was treated for 15 min in a MW oven then quenched in ammonium bicarbonate solution, the product was extracted by ethyl acetate and isolated. ^{1}H NMR spectrum, δ, ppm: 1.2–1.5 d [6H, (C**H**$_3$)$_2$CH]; 4.7–5.2 m [1H, (CH$_3$)$_2$C**H**]; 7.30–7.70 m (5H, benzene), 1.8–2.1 m (2H, pyrrolidine), 3.5–3.7 m (2H, pyrrolidine), 2.0–2.4 s (3H, O=CH$_3$). ^{13}C NMR spectrum, δ, ppm: 21.9; 69.4; 21.2, 24.6, 38.4, 47.5, 76.2, 124.6, 127.8, 128.3, 137.2, 171.8. Found, %: C 70.53; H,8.04; N,4.81;

Table 2. Antimicrobial activity (MIC values) of the synthesized compounds **5a**–**5h**

Compound	MIC, μg/mL				
	gram negative bacteria		gram positive bacteria		fungus
	E. Coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
5a	128	64	256	128	256
5b	256	128	512	256	128
5c	32	64	16	16	32
5d	32	64	128	128	64
5e	64	32	128	64	128
5f	32	32	16	32	64
5g	128	64	128	128	256
5h	64	256	32	64	128
Chloramphenicol	16	16	16	16	–
Ketoconazole	–	–	–	–	16

O,16.56. $C_{17}H_{23}NO_3$. Calculated, %: C 70.56; H 8.01; N 4.84; O 16.59.

Antimicrobial activity. The standard microbial strains were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. The antimicrobial activity of the synthesized compounds was determined by broth micro dilution method as recommended by the NCCLS [5, 6]. The compounds synthesized were evaluated against gram positive bacteria *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), gram negative bacteria *Pseudomonas aeruginosa* (NCIM 2036), *Escherichia coli* (NCIM 2065), and fungal species *Candida albicans* (NCIM 3102) (Table 2).

Antibacterial assay. The cultures were obtained in Müller–Hinton broth for all the bacteria after 24 h of incubation at 37±1°C. Testing was carried out at pH 7.4, and the 2-fold serial dilution technique was applied. The microorganisms were grown overnight and the final inoculum size was 10^5 CFU/mL. A set of tubes containing only inoculated broth was kept as control. After incubation for 24 h at 37±1°C, the last tube with no growth of microorganism was recorded to represent MIC expressed in μg/mL. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The antibiotic Chloramphenicol (16 μg/mL) and Ketoconazole (16 μg/mL) are used as reference antibacterial and antifungal agents, respectively for comparison.

Antimycotic assay. The yeasts were maintained in Sabouraud dextrose broth after incubation for 24 h at 25±1°C. Testing was carried out in Sabouraud dextrose broth at pH 7.4 and the 2-fold serial dilution technique was applied. The microorganisms were grown overnight and the final inoculum size was 10^4 CFU/mL. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25±1°C for the antifungal assay, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/mL. Each experiment for antimycotic activity and antibacterial assay was replicated twice in order to define the MIC values.

CONCLUSIONS

A series of novel 1-acetyl-2-benzylpyrrolidine-2-carboxylic acid derivatives has been synthesized by conventional and microwave technology. The structures of products have been confirmed by spectral analysis. The microwave assisted synthesis is the more efficient approach to the desired compounds. The results of preliminary bioassays indicate that a number of the products exhibit antibacterial activity against gram-positive and gram-negative bacteria. Modification of the pyrrolidine ring of the parent compounds allows to develop more active derivatives.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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