Med Chem Res (2012) 21:3897–3901
DOI 10.1007/s00044-011-9933-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

11-Chloro-3-methyl-3*H*-imidazo[4,5-a]acridine (CMIA) as a potent and selective antimicrobial agent against clinical isolates of highly antibiotic-resistant *Acinetobacter baumannii*

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Received: 11 May 2011 / Accepted: 1 December 2011 / Published online: 11 December 2011

Abstract *Acinetobacter baumannii* is an increasingly nosocomial pathogen throughout the world, and the occurrence of multidrug-resistant (MDR) species is increasing. The aim of this study is to present the antimicrobial effects of a newly synthesized imidazoacridine, 11-chloro-3-methyl-3*H*-imidazo(4,5-a)acridine (CMIA), against MDR clinical isolates of *A. baumannii*. Standard dilution tube-test assay was performed to determine the MBC of CMIA for 91 clinical isolates of highly antibiotic-resistant bacteria with 28 of *A. baumannii* in them. The MBCs of CMIA ranged from 2.0 to 10.9 mg/l for Acinetobacter isolates while it was more than 47.9 mg/l for other clinical strains. The findings demonstrate that CMIA is a potent and selective antimicrobial agent against clinical strains of antibiotic-resistant *A. baumannii*.

Keywords *Acinetobacter baumannii* · Antimicrobial activity · Acridine · MBC

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Introduction

Acinetobacter is a common type of bacteria found in many places, including water, soil, and sewage. There are at least 25 different types of *Acinetobacter*. *Acinetobacter baumannii* is the particular type that is often associated with hospital infections.

The multidrug-resistant (MDR) types of *Acinetobacter* spp. are a series of the most important pathogens causing the nosocomial infections in communities (Abbo *et al*., 2005; Jain and Danziger, 2004). The Centers for Disease Control and Prevention recently highlighted the enormity and gravity of MDR *A. baumannii* infections in military medical facilities treating civilians and service personnel injured in Iraq, Kuwait and Afghanistan (Hujer *et al*., 2006). Antimicrobial evaluation of the mentioned species showed that more than half of them were resistant to three or more classes of antibiotics.

Another significant problem is the infection of large number of military personnel by MDR *Acinetobacter* spp. and finally widely disseminated types of bacteria over the world (Hujer *et al*., 2006). With the appearance of MDR strains of *A. baumannii* in ICUs, and burn units, as well as in soldiers returning from overseas, new treatments for such infections are necessary (Osterburg *et al*., 2009; Insa *et al*., 2007).

In this study, we present a recently synthesized analog of imidazoacridine as a selective growth inhibitor of MDR *A. baumannii* in comparison with common pathogenic strains of bacteria.

Results and discussions

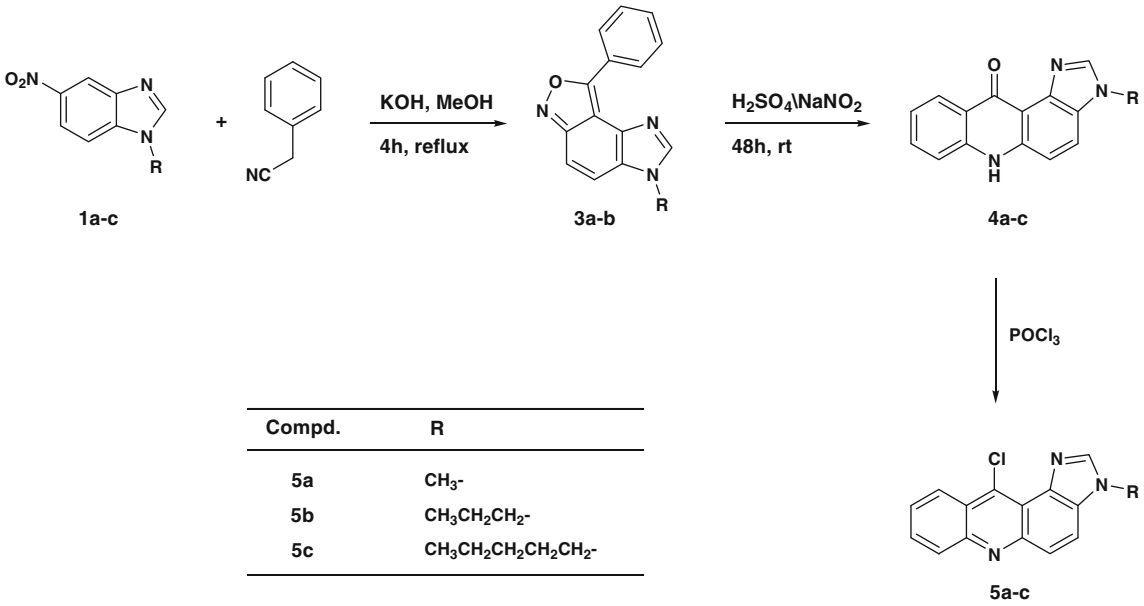
Our interest in imidazoacridine derivatives as bactericide agents emerges from the early research down by

Rahimizadeh *et al*., in which the synthesis and antibacterial activity of some imidazo(4,5-a)acridines was reported (Rahimizadeh *et al.*, 2009). It was shown that these series of compounds have growth-inhibitory activity against four standard strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. Herein, the minimum bactericidal concentrations (MBCs) of three 11-chloro-3-alkyl-3*H*-imidazo[4,5-a]acridines possessing methyl, *n*-propyl and *n*-pentyl substituents (**5a**–**5c**) (Scheme 1), were evaluated against *S. aureus* PTCC 1074, *P. aeruginosa* PTCC 1431, *Klebsiella pneumonia* PTCC 1053, and *E. coli* PTCC 1338. The MBCs were determined by using the dilution tube-test method, introduced by the National Committee for Clinical Laboratory Standards (Finegold and Garrod, 1995).

The results showed that the compounds with more lipophilic character exert less antibacterial activity. The octanol–water distribution coefficient (Log *D*) of **5a**–**5c** was measured by using shake flask method (Table 1) (Berthod and Carda-broch, 2004). Among the synthetic compounds, **5a** (11-chloro-3-methyl-3*H*-imidazo[4,5-a]acridine: CMIA), which exhibited the best bactericidal activity, was evaluated against some antibiotic-resistant gram-negative and gram-positive bacteria. 247 strains were isolated from different organs of patients at the Microbiological Laboratory of Ghaem Hospital (Medical University of Mashhad, Iran) and tested for antibiotic resistancy by disc diffusion method (Prescott *et al*., 2002). Among the isolates, 91 cases with highest antibiotic resistancy: *A. baumannii* (28 isolates), *E. coli* (13 isolates), *S. aureus* (15 isolates), *S. epidermidis* (4 isolates), *S. saprophyticus* (5 isolates), *K. pneumonia*, and *P. aeruginosa* (13 isolates) were evaluated. The experiments were also done for four groups of standard antibiotics:

Beta-lactam: Amoxicillin, Ceftazidime, Cefixime, and Cefotaxime; Fluoroquinolone: Nalidixic acid and Norfloxacine; aminoglycoside: Tobramycin, Kanamycin, and Tetracycline.

Table 2 shows the in vitro activities of CMIA in comparison with the reference agents against clinical and standard strains. These results are expressed as MBC values. Among the clinical isolates, CMIA showed potent activity against *A. baumannii* isolates. Among all *A. baumannii* cases, the MBCs of CMIA for bronchi and wound isolates were most potent (2.0–4.7 mg/l). The *A. baumannii*



Compd.	R
5a	CH_3-
5b	$CH_3CH_2CH_2$-
5c	$CH_3CH_2CH_2CH_2CH_2$-

Scheme 1 General procedure for the synthesis of compounds **5a**–**5c**

Table 1 Log *D* (octanol–water distribution coefficient) and MBC values of compounds **5a**–**5c**

Compounds	Log *D* ± SD	MBC (mg/l)			
		Staphylococcus aureus (1,074)	*Pseudomonas aeruginosa* (1,431)	*Escherichia coli* (1,338)	*Klebsiella pneumonia* (1,053)
5a	0.52 ± 0.07	3.10	75.0	6.25	12.5
5b	1.09 ± 0.05	6.25	>100	25.0	25.0
5c	1.48 ± 0.06	12.5	>100	50.0	25.0

Table 2 In vitro activities of CMIA and nine reference antibiotics against standard and clinical isolated bacteria

Strains	Cases	MBC (mg/l)									
		CMIA	Amoxicillin	Ceftazidime	Cefixime	Cefotaxime	Nalidixic acid	Norfloxacine	Tobramycin	Kanamycin	Tetracyclin
Acinetobacter baumannii	Bronchi ($n = 8$)	**4. 7 ± 1.4**	>100	90.6 ± 12.9	87.5 ± 13.3	96.9 ± 24.7	>100	37.5 ± 13.7	68.0 ± 14.8	>100	>100
Acinetobacter baumannii	Wound ($n = 12$)	**2.0 ± 0.6**	>100	91.7 ± 12.3	82.8 ± 18.2	95.8 ± 20.8	>100	41.7 ± 12.3	83.3 ± 19.4	>100	>100
Acinetobacter baumannii	Urine ($n = 8$)	**10.9 ± 2.2**	>100	93.7 ± 11.5	75.0 ± 21.7	90.6 ± 12.9	>100	62.5 ± 23.1	78.1 ± 20.8	>100	87.5 ± 13.0
Escherichia coli	Urine ($n = 8$)	56.4 ± 12.4	76.2 ± 10.4	14.4 ± 3.2	12.1 ± 2.9	17.3 ± 3.9	24.0 ± 5.2	7.3 ± 2.4	64.3 ± 7.2	84.4 ± 12.5	20.4 ± 4.2
Escherichia coli	Bronchi ($n = 5$)	89.3 ± 20.2	>100	45.4 ± 10.2	23.1 ± 6.2	24.5 ± 5.2	22.7 ± 6.8	12.2 ± 4.6	74.0 ± 18.2	85.5 ± 13.0	27.1 ± 7.2
Staphylococcus aureus	Wound ($n = 9$)	72.3 ± 14.2	>100	63.5 ± 8.2	33.1 ± 6.0	12.7 ± 3.4	81.6 ± 16.2	9.8 ± 2.9	17.3 ± 4.7	17.9 ± 4.6	12.5 ± 3.5
Staphylococcus aureus	Urine ($n = 6$)	91.0 ± 22.8	>100	80.2 ± 18.2	45.2 ± 9.2	77.2 ± 12.2	>100	11.5 ± 4.0	11.8 ± 3.7	55.1 ± 9. 4	26.3 ± 5.5
Staphylococcus epidermidis	Eye ($n = 4$)	73.7 ± 24.2	>100	73.7 ± 24.2	28.7 ± 14.2	75.0 ± 20.1	>100	75.0 ± 20.1	50.0 ± 20.4	25.0 ± 17.6	81.2 ± 12.5
Staphylococcus saprophyticus	Bronchi ($n = 5$)	93.7 ± 12.5	>100	82.4 ± 13.5	24.5 ± 5.2	95.0 ± 11.2	>100	50.0 ± 17.7	25.0 ± 15.3	40.0 ± 13.7	45.0 ± 11.2
Pseudomonas aeruginosa	Urine ($n = 5$)	85.0 ± 13.7	>100	43.8 ± 10.5	20.0 ± 6.8	16.3 ± 5.6	>100	13.7 ± 2.8	13.7 ± 2.8	47.9 ± 16.6	21.2 ± 5.6
Pseudomonas aeruginosa	Wound ($n = 8$)	75.0 ± 16.6	>100	90.6 ± 12.9	96.9 ± 8.8	81.2 ± 11.6	>100	25.8 ± 8.4	26.5 ± 8.0	48.4 ± 14.0	54.7 ± 13.2
Klebsiella pneumonia	Urine ($n = 6$)	47.9 ± 5.1	>100	22.9 ± 5.1	20.8 ± 6.4	25.0 ± 7.9	29.1 ± 6.4	11.5 ± 4.7	28.1 ± 7.6	12.5 ± 3.9	26.0 ± 10.1
Klebsiella pneumonia	Wound ($n = 7$)	85.7 ± 13.3	>100	92.8 ± 12.2	44.6 ± 9.8	51.8 ± 11.2	25.9 ± 9.1	50 ± 12.5	24.1 ± 10.5	16.0 ± 6.1	30.3 ± 15.9
Staphylococcus aureus (1,074)	–	3.10	3.10	1.55	1.55	1.55	12.5	1.55	3.10	6.25	3.10
Pseudomonas aeruginosa (1,431)	–	75.0	25	1.55	1.55	3.10	12.5	3.10	6.25	12.5	12.5
Escherichia coli (1,338)	–	6.25	12.5	3.10	1.55	1.55	6.25	1.55	0.77	12.5	6.25

The MICs of CMIA against *Acinetobacter baumannii* isolates were distinguished by bold format

MBC values of the reference antibiotics were more than 70 mg/l except for Norfloxacine. CMIA inhibited the rest of gram-negative and gram-positive strains at or above 47.9 mg/l. The data of Table 1 shows that the higher concentration of CMIA is needed for effective growth inhibition of *A. baumannii* urinary isolates. Unlike the *A. baumannii* isolates, many other strains were completely resistant to CMIA at the doses tested.

Data from this study indicates that CMIA is a potent and selective antimicrobial agent against *A. baumannii*.

Conclusion

It is well documented that the 11-chloro-3-alkyl-3*H*-imidazo[4,5-a]acridines are notable bactericidal agents and their efficacies are decreased by descending the N-alkyl length. Here, it was also found that the CMIA is the most potent and selective bactericidal analog against clinical isolates of highly antibiotic-resistant *A. baumannii*. The observed bactericidal activity of CMIA represents a potentially attractive alternative for topical treatment of *A. baumannii* infections.

Materials and methods

Determination of MBCs

The MBCs were determined by dilution tube-test method, introduced by National Committee for Clinical Laboratory Standards (Finegold and Garrod, 1995). A serial dilution of tested compounds (final concentration of 200–0.4 mg/l), were added to the test bacteria in Mueller–Hinton broth and were incubated at 37°C for 24 h (5×10^5 CFU/ml). After sufficient incubation (24 h), the tubes were examined for turbidity, indicating growth of the microorganism. For further confidence, the samples were cultured in Petri dishes containing Muller–Hinton agar (24 h at 37°C). The lowest drug concentration that prevents the test organism growth (<99.9%) is introduced as MBC (Pachon-Ibanez *et al*., 2004). Growth was observed in medium control but not in the inoculum control (Finegold and Garrod, 1995).

Determination of Log *D*

One milligram of solute **5a–5c** was independently deposited in a test tube. 5 ml of each saturated layer (octanol and aqueous phosphate buffer 50 mM, pH 7.4) was added to the sample, and the tube was capped and equilibrated for 6 h on a mechanical shaker. For each of the compounds, a blank solution was prepared. The test was repeated four times for each compound. The absorbance values of the equilibrated layers and the blanks were read at 402 nm, and the partition coefficient was calculated with the equation: $\text{Log } D = \text{Log } [(A_{oct} - A_{blank})/(A_{wat} - A_{blank})]$.

Chemistry

Compounds **5a–5c** were synthesized according to the procedure reported by Rahimizadeh et al. by starting from *N*-Alkyl-5-nitrobenzimidazoles and phenylacetonitrile (Rahimizadeh *et al*., 2009). The compounds **3a–3c** were obtained via the nucleophilic substitution of hydrogen of *N*-alkyl-5-nitrobenzimidazoles **1a–1c** with phenylacetonitriles in KOH/MeOH under reflux condition (4 h). Compounds **3a–3c** rearranged to their corresponding imidazo[4,5-a]acridones **4a–4c** in concentrated sulfuric acid containing nitrous acid after 24 h at room temperature. Treatment of imidazo[4,5-a]acridones **4a–4c** in boiling $POCl_3$ gave imidazo[4,5-a]acridines **5a–5c** (Scheme 1). The structure of the new synthetic compound, **5c**, was confirmed by ^{1}H NMR spectroscopy and CHN analysis.

General procedure for the synthesis of 5a–5c

Compounds **1a–1c** (5 mmol) and phenyl acetonitrile (6 mmol) were added with stirring to a solution of 10 g KOH in 40 ml methanol. After the mixture was refluxed with stirring for 4 h, it was then poured into water. The precipitate was collected by filtration, washed with water, and air-dried to give **3a–3c**.

Sodium nitrite (2.5 g, 75 mmol) was slowly added with stirring to a solution of **3a–3f** (3.5 mmol) in 50 ml sulfuric acid 98% at −10°C. After the addition was completed, the mixture was allowed to warm to room temperature and left at room temperature (24 h). After pouring the mixture into 300 ml ice-water, precipitated solid was separated, washed with water, and dried to give **4a–4c**.

A mixture of **4a–4c** (2 mmol) and 3 ml $POCl_3$ was refluxed (3 h). After cooling to room temperature, the mixture was poured on to crushed ice and neutralized with NaOH (10%). The product was extracted with ethyl acetate (2 × 25 ml). The extract was dried and evaporated to give **5a–5c**.

*11-Chloro-3-methyl-3H-imidazo[4,5-a]acridine (**5a**)*

Yellow crystals (acetonitrile), yield 75%, mp: 240–241°C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.02$ (s, 3H), 7.61 (d, $J = 8.9$ Hz, 1H), 7.65–8.05 (m, 5H), 8.65 (dd, $J = 9.0$ Hz, $J = 2.0$ Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 157.9, 156.7, 150.3, 142.6, 134.6, 131.6, 131.3, 130.7, 127.4, 125.8, 122.0, 119.0, 111.7, 110.6, 33.2$ ppm; MS (70 eV): $m/z = 267$ (M^+); Anal. Calcd. for $C_{15}H_{10}ClN_3$: C, 67.30; H, 3.77; N, 15.70. Found: C, 67.49; H, 3.69; N, 15.81.

11-Chloro-3-*n*-propyl-3*H*-imidazo[4,5-a]acridine (**5b**)

Yellow crystals (acetonitrile), yield 79%, mp 185–187°C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.01$ (t, $J = 7.2$ Hz, 3H), 1.88–2.11 (m, 2H), 4.30 (t, $J = 7.2$ Hz, 2H), 7.68–8.32 (m, 6H), 8.67 (dd, $J = 9.0$ Hz, $J = 2.0$ Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 157.7$, 156.6, 148.3, 142.3, 134.6, 131.7, 130.8, 130.3, 127.0, 125.6, 121.5, 118.8, 111.5, 110.5, 52.3, 21.6, 10.1 ppm; MS (70 eV): $m/z = 295$ (M^+); Anal. Calcd. for $C_{17}H_{14}ClN_3$: C, 69.03; H, 4.77; N, 14.21. Found: C, 69.39; H, 4.65; N, 14.17.

11-Chloro-3-*n*-pentyl-3*H*-imidazo[4,5-a]acridine (**5c**)

Yellow crystals (acetonitrile), yield 75%, mp: 175–176°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, $J = 6.8$ Hz, 3H), 1.21–1.70 (m, 4H), 1.73–2.01 (m, 2H), 4.31 (t, $J = 6.8$ Hz, 2H), 7.63-8.31 (m, 6H), 8.66 (dd, $J = 9.0$ Hz, $J = 2.0$ Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 157.7$, 156.5, 147.7, 141.9, 134.5, 131.6, 130.8, 130.3, 126.9, 125.6, 121.4, 118.7, 111.2, 110.1, 49.7, 30.8, 20.4, 20.1, 11.1 ppm; MS (70 eV): $m/z = 321$ (M^+); Anal. Calcd. for $C_{19}H_{18}ClN_3$: C, 70.47; H, 5.60; N, 12.98. Found: C, 70.35; H, 5.65; N, 12.91.

Acknowledgments We express our sincere gratitude to Mohammad Naser Shafiee (Faculty of Health Sciences, Mashhad University of Medical Sciences) for reviewing the manuscript. We thank Mr. Mohammad Ramezanpour and Mr. Mohammad Jafar Arjmand (Microbiology Laboratory, Ghaem Hospital) for their assistance in MIC determination. We are also grateful to Mashhad University of Medical Sciences for financial support of this work.

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