IN VITRO ACTIVITY OF
CEM-101 COMPARED TO CLARITHROMYCIN
AGAINST NOCARDIA SPECIES

S. Flores-Penalba[1], C. Shoen[2], M. DeStefano[2], M. Cynamon[2]

[1] Universitat de València. Facultat de Farmàcia, Dto. Química Orgànica, Vicent Andrés Estellés, Burjassot,46100, València, Spain.

[2] Veterans Affairs Medical Center, 800 Irving Avenue, Syracuse, 13210, NY, USA.

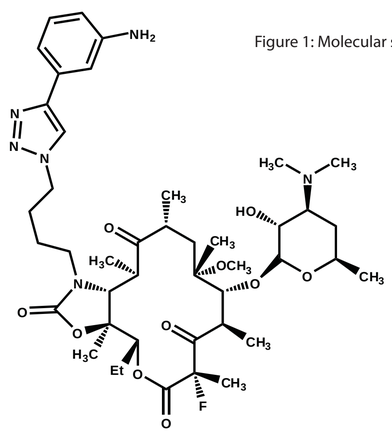
Contact: Sonia.Flores@uv.es

Introduction:

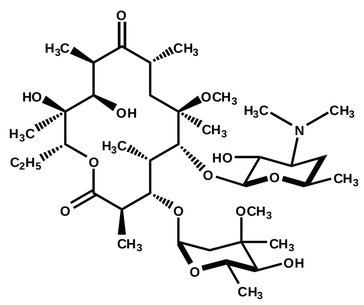
Sulfonamides are usually the treatment of choice for *Nocardia* infections. Central nervous system and disseminated nocardiosis, however, continue to be difficult to manage. Drug resistant species of *Nocardia* such as *N. farcinica* or *N. otitidiscaviarum*, and sulfonamide intolerance, especially in patients with AIDS, require the use of alternative drugs, alone or in combination. Although clarithromycin (CLA) is not the treatment of choice in nocardiosis, promising reports of its effectiveness have been published in cases of bacteremia and pneumonia. However, clinical experience is still limited.

In this study, the activity of CEM-101, a new ketolide (Figure 1A), was evaluated *in vitro* compared to CLA, a structurally related macrolide (Figure 1B), against *Nocardia spp*.

Figure 1: Molecular structures of CEM-101 and clarithromycin



1A. CEM-101



1B. Clarithromycin

Methods:

CLA, obtained from Abbott Laboratories (Abbott Park, IL), was dissolved in DMSO at a concentration of 1 mg/mL. CEM-101, provided by Cempra Pharmaceuticals Inc. (Chapel Hill, NC), was dissolved in double distilled water with 3% glacial acetic acid at a concentration of 1 mg/mL. Thirty one isolates of *Nocardia*, belonging to seven species, were obtained from the American Type Culture Collection (Manassas, VA), Barbara Body, and Betty Ann Forbes. Isolates were grown in cation supplemented Mueller-Hinton (MHcs) broth and diluted in MH broth to yield a standard turbidity of 100 Klett units per mL ($1.2x10^6$-$6.0x10^7$ CFU/mL). An *in vitro* broth microdilution method similar to that recommended by the Clinical and Laboratory Standards Institute (CLSI) was utilized for susceptibility testing.

Polystyrene 96-well round bottom plates (Corning Inc., Corning, NY) were prepared with 50 µl of MHcs broth per well. The drugs prepared at 4 X the maximal concentration tested (512 µg/ml) were added to the first well and then serially 2-fold diluted. The frozen bacterial cultures were thawed and diluted to a final concentration of about 1 x 10^5 CFU/ml (working stock) in MHcs broth. The actual inoculum was measured by titration and plating on MHcs agar. To each well 50 µl of the working stock was added. The microtiter plates were covered with SealPlate adhesive sealing film (Excel Scientific, Wrightwood, CA) and incubated at 37ºC in ambient air for 4-5 days prior to reading. The MIC was defined as the lowest concentration of antimicrobial agent yielding no visible turbidity. Each isolate was tested in duplicate and *Nocardia farcinica* 1 was used as a quality control in every experiment.

Results:

The results obtained for the 31 isolates tested are shown in Table 1. The end-points were generally sharp, with no more than one-dilution difference between duplicates. When this difference was observed, the higher MIC was reported. The MIC_{50} and MIC_{90} for CEM-101 were 0.062 mcg/mL and 32 mcg/mL, respectively, compared to 0.125 mcg/mL and 128 mcg/mL for CLA.

Table 1: Minimum inhibitory concentrations (MICs, mcg/mL) of CEM-101

Entry	Isolate	CEM-101	Clarithromycin
1	*N asteroides 4*	32	128
2	*N. asteroides 9*	0.015	0.125
3	*N. asteroides 13*	16	8
4	*N. asteroides 22*	≤ 0.0075	≤ 0.0075
5	*N. asteroides 651*	≤ 0.0075	≤ 0.0075
6	*N. asteroides 720*	0.062	0.015
7	*N. asteroides 1170*	≤ 0.0075	≤ 0.0075
8	*N. asteroides 1260*	≤ 0.0075	0.015
9	*N. asteroides 2039*	≤ 0.0075	≤ 0.0075
10	*N. asteroides F71743*	16	32
11	*N. asteroides S3840*	≤ 0.0075	0.015
12	*N. asteroides W34408*	32	64
13	*N. brasiliensis 9*	16	32
14	*N. caviae 243*	16	128
15	*N. caviae 2497*	≤ 0.0075	0.25
16	*N. farcinica 1*	32	64
17	*N. farcinica 1253N6*	32	128
18	*N. farcinica 6*	16	64
19	*N. farcinica ATCC 3318*	16	128
20	*N. nova 2*	32	64
21	*N. nova 5*	≤ 0.0075	≤ 0.0075
22	*N. nova 7*	0.031	0.031
23	*N. nova 8*	0.015	0.015
24	*N. nova 10*	0.015	0.031
25	*N. nova 11*	0.015	0.015
26	*N. nova 12*	0.015	0.015
27	*N. nova 14*	0.062	0.015
28	*N. nova 1276*	≤ 0.125	≤ 0.125
29	*N. otitidiscaviarum 14629*	16	128
30	*N. otitidiscaviarum S49536*	32	128
31	*N. veterana 3*	32	128
	MIC_{50}	0.062	0.125
	MIC_{90}	32	128

Table 2: MIC50 and MIC90 of the different groups of isolates

Group (*n*)		CEM-101	Clarithromycin
Overall group (31)	MIC range	32-≤0.0075	128-≤0.0075
	MIC_{50}	0.062	0.125
	MIC_{90}	32	128
Resistant group (14)	MIC range-R	32-16	128-16
	MIC_{50}-R	16	64
	MIC_{90}-R	32	128
Susceptible group (17)	MIC range-S	0.062-≤0.0075	0.25-≤0.0075
	MIC_{50}-S	≤0.0075	0.015
	MIC_{90}-S	0.031	0.031

Two groups of isolates could be clearly distinguished in terms of susceptibility. A resistant group, with MICs >=8 mcg/mL (14 isolates) and a susceptible group, with MICs <=0.25 mcg/mL (17 isolates), as shown in Table 2. There was good concordance with the activity of CEM-101 (Figure 2A) and CLA (Figure 2B) in each of these groups. Some species e.g. *N. farcinica* and *N. otitidiscaviarum* appeared to be resistant to these drugs. Other species, such as *N. asteroides* or *N. nova*, showed variable susceptibility patterns. Similar results in terms of variable *Nocardia* susceptibilities against clarithromycin had been previously reported (Eur. J. Clin. Microbiol. Infect. Dis., 2004; 23:69-70).

Figure 2A: Distribution of *Nocardia* isolates for CEM-101

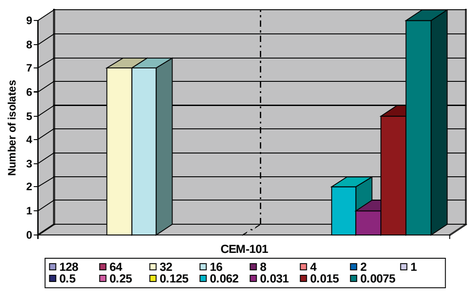


Figure 2B: Distribution of *Nocardia* isolates for CLA

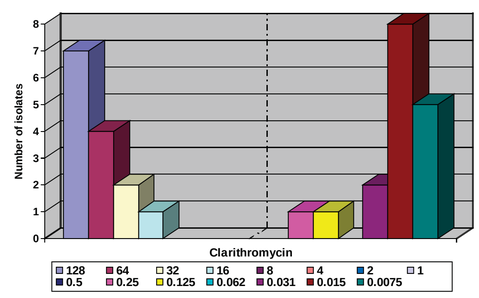


Figure 2: Distribution of Nocardia isolates in relation to the MIC (mcg/mL). Difference between resistant and susceptible groups for CEM-101 and clarithromycin.

Conclusions:

The new ketolide CEM-101 was demonstrated to be active *in vitro* against *Nocardia spp*, showing equal or better activity than CLA against most of the isolates tested. CEM-101 should be further evaluated against *Mycobacteria* and in animal models of nocardial and mycobacterial infection to define its spectrum of activity.

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