

Supplementary Materials

Article

Efficiency of a Tetracycline-Adjuvant Combination Against Multidrug Resistant *Pseudomonas aeruginosa* Tunisian Clinical Isolates

Azza Troudi ^{1,2}, Meha Fethi ², Mohamed Selim El Asli ³, Jean Michel Bolla ¹, Naouel Klibi ² and Jean Michel Brunel ^{1,*}

¹ Aix Marseille Univ, INSERM, SSA, MCT, 13385 Marseille, France; azzatroudi.92@gmail.com (A.T.); jean-michel.bolla@univ-amu.fr (J.M.B.)

² Laboratory of Microorganisms and Active Biomolecules, Department of Biology, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia; meha.fethi@yahoo.fr (M.F.); n_klibi@yahoo.fr (N.K.)

³ Service of Microbiology, Military Hospital of Tunis HMPIT, Tunis, Tunisia; elasliselim@yahoo.fr

* Correspondence: jean-michel.brunel@inserm.fr

General procedure for the synthesis of polyaminoisoprenyl derivatives 3

To a solution of spermine (450 mg, 2.27 mmol) and triethylamine (450 μ L, 4.5 mmol) in distilled tetrahydrofuran (THF) (10 mL) is added dropwise farnesyl chloride **1** (mixture of isomers) (480 mg, 2 mmol) in distilled THF (15 mL). The reaction mixture is stirred at room temperature for 24 h. and evaporated to dryness. The crude residue is purified by column chromatography (eluant CH₂Cl₂/MeOH/conc.NH₄OH, 7:3:1) to afford the pure desired compound as a yellow solid in 64% yield (mixture of isomers).

Compound 3. 64% yield; Yellow solid; ¹H NMR (MeOD, 250 MHz): δ = 5.22-5.25 (m, 1H), 5.03-5.14 (m, 2H), 3.11-3.25 (m, 3H), 2.50-2.73 (m, 9H), 2.43-2.49 (m, 3H), 1.96-2.11 (m, 10H), 1.69-1.74 (m, 12H), 1.50-1.67 (m, 10H). ¹³C (MeOD): δ = 140.06, 139.44, 136.28, 136.25, 136.16, 136.13, 132.37, 132.13, 132.10, 125.94, 125.42, 125.38, 125.21, 123.15, 121.80, 54.58, 52.48, 52.25, 50.66, 50.52, 48.77, 48.24, 48.01, 47.63, 41.28, 41.18, 41.12, 40.94, 40.90, 40.80, 40.62, 33.26, 32.99, 30.35, 30.11, 28.53, 28.21, 27.84, 27.66, 27.43, 26.09, 26.04, 25.41, 23.84, 23.79, 17.90, 17.86, 16.61, 16.48, 16.25, 16.21. C₂₅H₅₀N₄ MS (ESI+) m/z 407.41 (100%, [M + H]⁺).

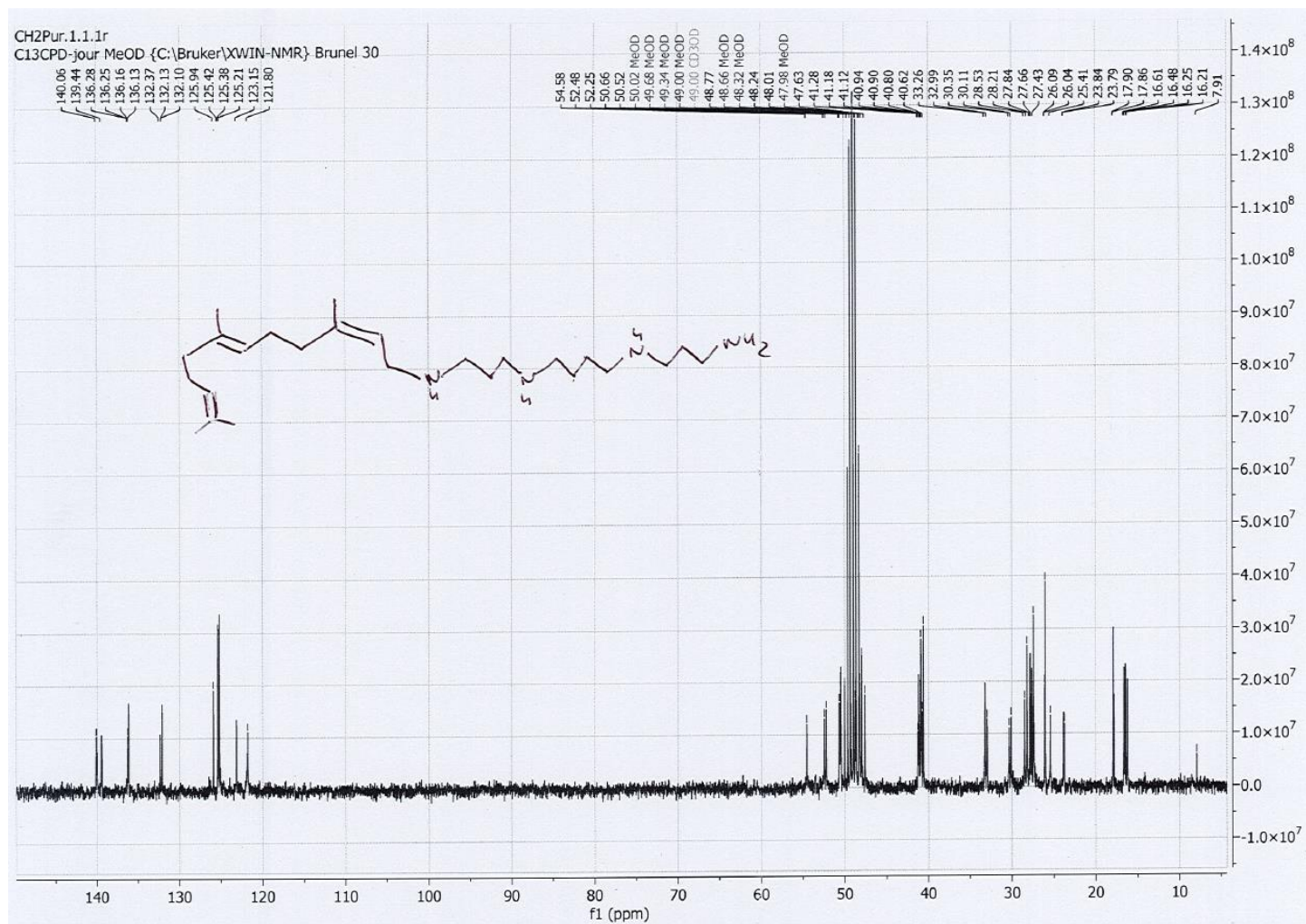


Figure S2. ^{13}C NMR spectrum of 3.

Table S1. Origins, antimicrobial resistance profile, and resistance genes of the 21 clinical *P. aeruginosa* strains.

Strain	Ward	Origin	Profil of resistance	Resistance genes	Efflux pump test
P1	ICU	blood	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	SHV	-
P2	ICU	tracheal aspirate fluid	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	VIM, GES	+
P3	ICU	catheter	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	SHV	+
P4	ICU	blood	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	VIM, GES	+
P6	orthopedic surgery	pus	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, GM, TM, CIP, LVX	SHV	-
P8	ICU	tracheal aspirate fluid	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT AN, GM, TM, CIP, LVX	GES	+
P11	ICU	expectoration	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	SHV	+
P12	ICU	tracheal aspirate fluid	IMP, MEM, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	GES	+
P26	vascular surgery	pus	IMP, MEM, FEP, TCC, PIP, PIT, TM, CIP, LVX	-	+
P28	ICU	tracheal aspirate fluid	IMP, MEM, FEP, TCC, PIP, PIT, AZT AN, GM, TM, CIP, LVX	GES	+
P29	ICU	catheter	IMP, MEM, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	GES	+
P34	ICU	tracheal aspirate fluid	IMP, MEM, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	GES	+
P45	ICU	blood	IMP, MEM, TCC, PIP, AZT		+
P50	ICU	tracheal aspirate fluid	IMP, MEM, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	GES	+
P51	ICU	blood	IMP, MEM, FEP, TCC, PIP, AZT, AN, GM, TM, CIP, LVX	-	+
P54	ICU	blood	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, GM, TM, CIP, LVX	VIM	+
P69	ICU	tracheal aspirate fluid	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	GES	+
P72	ICU	tracheal aspirate fluid	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	GES	+
P73	urology	urine	IMP, MEM, CAZ, FEP, TCC, PIP, AZT, GM, TM, CIP, LVX	GES	+
P74	bacteriology	tracheal aspirate fluid	IMP, MEM, CAZ, FEP, TCC, PIP, PIT, AZT, AN, GM, TM, CIP, LVX	SHV	+
P569	-	tracheal aspirate fluid	IMP, MEM, AZT, AN, GM, TM, LVX	GES	+

amikacin (AN), aztreonam (ATM), cefepime (FEP), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IPM), levofloxacin (LVX), meropenem (MEM), piperacillin (PIP), piperacillin-tazobactam (PIT), ticarcillin-clavulanic acid (TCC), tobramycin (TM), Intensive care Unit (ICU).

Table S2. MICs of doxycycline, minocycline, and compound **3** against PA01 and *P. aeruginosa* Tunisian clinical strains.

Strains	MIC of doxycycline ($\mu\text{g/mL}$)	MIC of minocycline ($\mu\text{g/mL}$)	MIC of compound 3 (μM) ($\mu\text{g/mL}$)
PA01	64	32	25 (10)
P1	64	32	25 (10)
P2	64	32	25 (10)
P3	64	32	25 (10)
P4	64	32	25 (10)
P6	64	32	12.5 (5)
P7	128	128	25 (10)
P8	64	32	25 (10)
P11	128	64	25 (10)
P12	64	32	25 (10)
P14	64	32	25 (10)
P26	128	128	25 (10)
P28	64	64	25 (10)
P29	64	64	25 (10)
P34	64	64	25 (10)
P45	128	64	25 (10)
P50	64	64	25 (10)
P51	64	32	25 (10)
P54	64	32	25 (10)
P69	64	32	25 (10)
P72	64	32	25 (10)
P73	128	128	25 (10)
P74	128	64	50 (20)
P569	64	64	50 (20)

Table S3. Dose-dependent effect of compound 3 to enhance doxycycline and minocycline activities against PA01 and *P. aeruginosa* Tunisian clinical strains.

Strains	MIC of doxycycline in the presence of compound 3 at different concentrations			MIC of minocycline in the presence of compound 3 at different concentrations		
	2.5 μ M	5 μ M	10 μ M	2.5 μ M	5 μ M	10 μ M
	(Gain factor)					
PA01	8 (8)	2 (64)	0.5 (128)	4 (8)	1 (64)	0.25 (128)
P1	2 (32)	1 (64)	<0.005 (>1024)	2 (16)	0.5 (64)	<0.005 (>1024)
P2	4 (16)	1 (64)	0.25 (256)	4 (8)	1 (32)	0.25 (128)
P3	4 (16)	2 (32)	0.25 (256)	4 (8)	2 (16)	0.25 (128)
P4	4 (16)	2 (32)	0.25 (256)	8 (4)	1 (32)	0.25 (128)
P6	1 (64)	0.5 (128)	<0.005 (>1024)	1 (32)	0.25 (128)	<0.005 (>1024)
P7	4 (16)	2 (64)	0.25 (256)	4 (16)	2 (64)	0.25 (256)
P8	8 (16)	2 (32)	0.5 (256)	8 (8)	2 (16)	0.5 (128)
P11	8 (16)	2 (64)	0.5 (256)	8 (8)	2 (32)	0.5 (128)
P12	4 (16)	2 (32)	0.5 (128)	4 (8)	2 (16)	0.5 (64)
P14	8 (8)	2 (32)	1 (64)	8 (4)	1 (32)	1 (32)
P26	8 (16)	2 (64)	1 (128)	8 (16)	1 (128)	0.5 (256)
P28	8 (8)	2 (32)	1 (64)	4 (16)	1 (64)	0.5 (128)
P29	4 (16)	1 (64)	0.125 (512)	4 (16)	1 (64)	0.125 (512)
P34	4 (16)	1 (64)	0.25 (256)	4 (16)	1 (64)	0.125 (512)
P45	8 (16)	2 (64)	1 (128)	4 (16)	2 (32)	0.5 (128)
P50	8 (8)	2 (32)	0.5 (128)	8 (8)	4 (16)	0.5 (128)
P51	4 (16)	1 (64)	0.25 (256)	8 (4)	2 (16)	0.25 (128)
P54	8 (8)	1 (64)	0.5 (128)	8 (4)	1 (32)	0.25 (128)
P69	8 (8)	1 (64)	0.125 (512)	8 (4)	1 (32)	0.125 (256)
P72	4 (16)	1 (64)	0.5 (128)	8 (4)	2 (16)	0,0031 (1024)
P73	64 (2)	16 (8)	8 (16)	32 (4)	8 (16)	2 (64)
P74	32 (4)	8 (16)	4 (32)	16 (4)	4 (16)	1 (64)
P569	16 (4)	2 (32)	0.5 (128)	16 (4)	8 (16)	0.5 (128)