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Syntheses and computational analyses of selected macrolide derivatives derived from clarithromycin A

BILJANA B. ARSIC[1,2]*, GARETH A. MORRIS[3], ABDOLREZA HASSANZADEH[2,4], OLGA P. JOVANOVIC[1], JILL BARBER[2] AND DJORDJE GLISIN[1]

[1]*University of Nis, Faculty of Sciences and Mathematics, Department of Chemistry, Visegradska 33, Nis, Republic of Serbia,* [2]*Division of Pharmacy and Optometry, School of Health Sciences, University of Manchester, Manchester, United Kingdom,* [3]*Department of Chemistry, University of Manchester, Manchester M13 9PL, United Kingdom,* [4]*Pharmaceutics Research Center, Institute of Pharmaceutical Sciences, Kerman University of Medical Sciences, Kerman, Iran.*

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Abstract: This study presents the synthesis and experimental and computational analysis of novel macrolide derivatives obtained from clarithromycin A, with the aim of exploring their potential to address the growing problem of antimicrobial resistance. The compounds synthesized include 2′-*O*-acetyl-clarithromycin A, its phosphoramidite derivative, and the corresponding phosphonyl derivative. Special attention was paid to the optimization of phosphitylation conditions due to the inherent instability of phosphoramidite compounds. The purity of the phosphoramidite derivative was successfully confirmed using diffusion-ordered NMR spectroscopy (DOSY). Comprehensive conformational analyses were carried out using molecular modeling techniques, followed by molecular docking and MM-GBSA calculations with a target protein from *Escherichia coli* to evaluate the relative binding affinities of clarithromycin A and its derivatives. The results indicate that the phosphoramidite and phosphonyl derivatives exhibit comparable binding affinities relative to the parent antibiotic. In addition, complex mass spectrometric fragmentation patterns of the phosphorus-containing derivatives were analyzed and rationalized using the MS Fragmenter computational tool.

Keywords: macrolide antibiotics; DOSY; synthesis; phosphoramidites; molecular docking.

INTRODUCTION

Rising antimicrobial resistance is a huge threat to human health.[1] A recent example is the hospital-acquired and ventilator-associated life-threatening pneumonia caused by *Acinetobacter*.[2] There are numerous initiatives to overcome

* Corresponding author. E-mail: Biljana.Arsic@pmf.edu.rs
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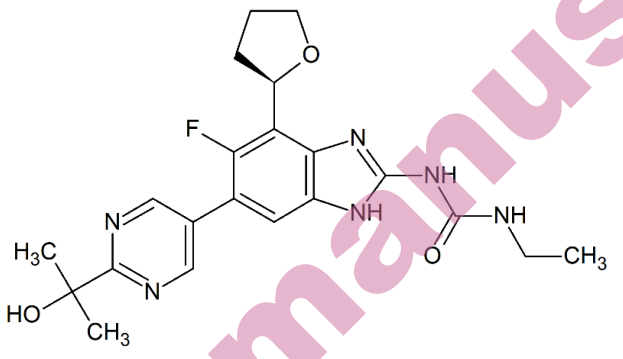
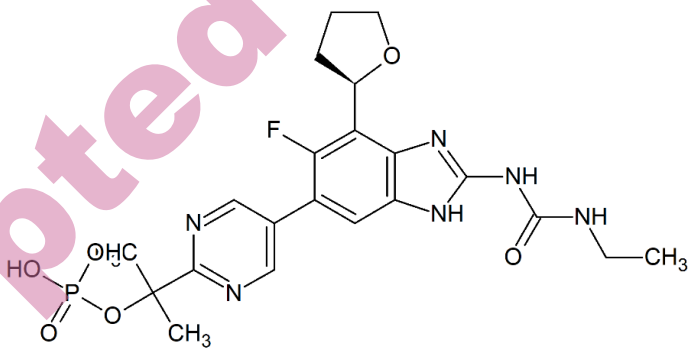
the resistance problem, and one of them is a new antibiotic class (representatives SPR719 and its prodrug SPR720, gyrase B inhibitors) with a novel mechanism of action.[3] The prodrug SPR720 (fobrepodacin) is a phosphate derivative (Fig 1), which encourages the synthesis of other phosphate derivatives. We are here interested in the synthesis of their precursors - phosphoramidites.



SPR719



SPR720

Fig 1. Structures of SPR719 and its prodrug, SPR720.

Phosphoramidite compounds are made in one of two ways, using the reagents 2- cyanoethyl diisopropylchlorophosphoramidite or 2-cyanoethyl *N*, *N*, *N*′, *N*′-tetra-isopropylphosphorodiamidite. Successful phosphitylation of modified nucleosides at the 3′ position with 2-cyanoethyl *N*,*N*,*N*,*N*-tetraisopropylphosphorodiamidite in the presence of *N*,*N*-diisopropylamine tetrazolide in acetonitrile gave synthetic blocks in significant yields (52-86%).[4] In the case of 2- cyanoethyl diisopropylchlorophosphoramidite, a mild base must be used, and the reaction time varied between 1-5 h.[5] Therefore, triethylamine has also been used as a base giving high yield of the synthesized compound (95%).[6]

DMAP has also been used to catalyse phosphorylations.[7] Cyanoethyl protection can be easily removed by piperidine in anhydrous acetonitrile, followed by addition of potassium carbonate solution in methanol,[8,9] or by concentrated ammonia.[10]

The aim of the current work was to synthesize acetyl and phosphoramidite derivatives of clarithromycin A (usually known as clarithromycin) (Fig 2).

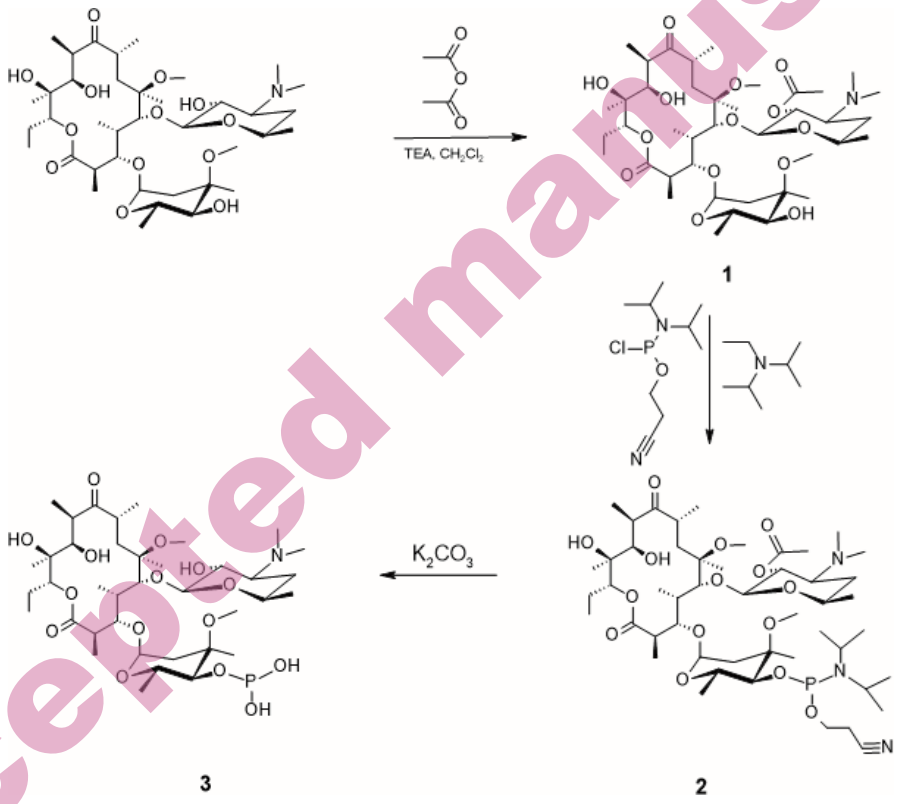


Fig 2. Scheme for the synthesis of compounds **1**-**3**.

DOSY NMR was used in the case of the phosphoramidite derivative of acetyl clarithromycin A to show the purity of the synthesized compound.[11] Conformational analysis for the macrolide antibiotics (Fig 3) gives insight into their possible biological conformations. Molecular docking was performed for clarithromycin A and compounds **1**-**3** with the target protein from *Escherichia coli*, and the relative affinity of the investigated compounds was further explored using Molecular Mechanics/Generalized Born Surface Area (MM-GBSA).

The complicated mass fragmentation patterns observed for 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**) and 4′′-*O*-phosphonyl-clarithromycin A (**3**) with ES^+ were analysed using the computational

tool MS Fragmenter 2023.1.1 (ACD/Labs, Canada) and are explained here in detail.

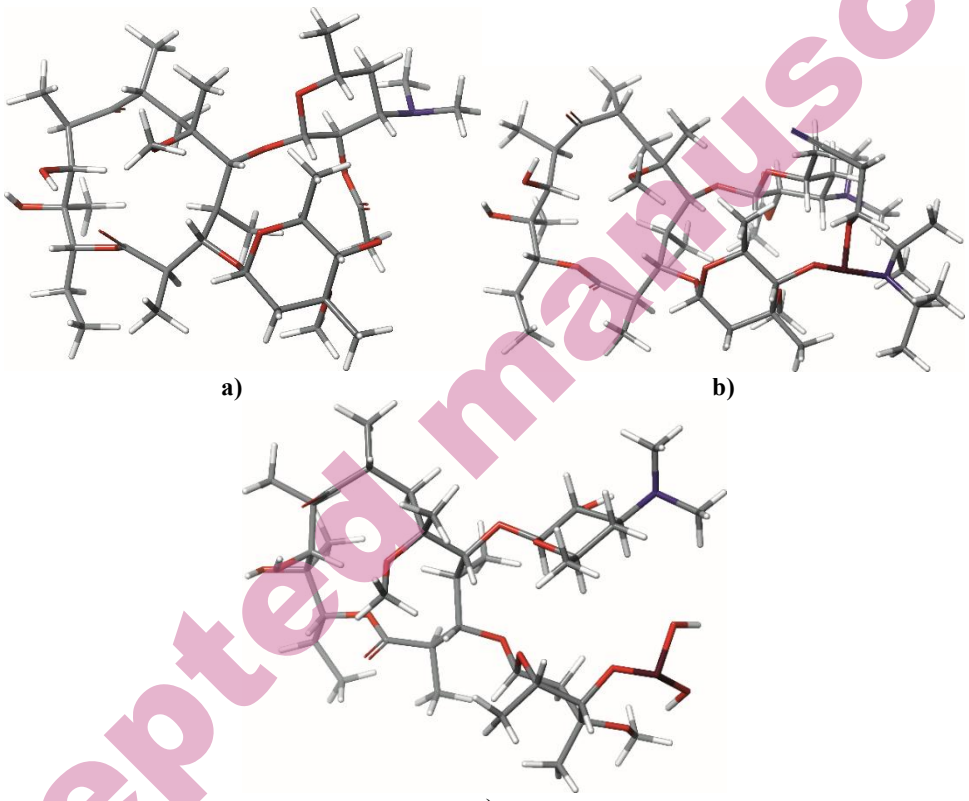


Fig 3. Structure of a) 2′-*O*-acetyl-clarithromycin A (**1**); b) 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**); c) 4′′-*O*-phosphoryl-clarithromycin A.

EXPERIMENTAL

Instrumentation

NMR spectrometers

Bruker Avance-300 spectrometer

NMR spectra were recorded using a Bruker Avance-300 spectrometer (Bruker, Billerica, MA, USA) (7.05 T) equipped with a 5 mm single-axis Z-gradient four-nucleus probe, operating at 300 MHz for ^{1}H, 75 MHz for ^{13}C and 121 MHz for ^{31}P. The spectrometer was operated using the XWIN NMR system software. Chemical shifts (δ) are reported in parts per million (ppm), with peak positions relative to Me_4Si (0 ppm) as internal reference for ^{1}H and ^{13}C.

Varian Unity 500 spectrometer

A Varian Unity 500 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) was operated with an 11.74 T Oxford Instruments magnet. In this field, the ^{1}H resonated at 500 MHz. A 5 mm PFG (Pulse Field Gradient) probe was used. The temperature was controlled using the Varian VT unit with a precision of ±0.1 ºC.

Mass spectrometer

Electrospray ionisation-mass spectra (ESI-MS) were acquired on a Micromass Platform mass spectrometer (Waters Corporation, Milford, MA, USA), and data were analysed using the program PLATFORM with a Masslynx data system. The sample (10 µL) was injected using a Hewlett Packard auto-sampler, and the machine was operated at a cone value of 30 eV, at 80 ºC.

Melting point apparatus

All melting points were determined using a Bibby SMP-10 (Stuart Scientific, Staffordshire, UK) melting point apparatus and were reported uncorrected. The samples were placed in a capillary tube and inserted in the melting point apparatus and were heated at a rate of 1 ºC min^{-1} until the compound melted.

Two-dimensional NMR spectroscopy

HMQC and HMBC

HMBC (Heteronuclear Multiple Bond Connectivity) and HMQC (Heteronuclear Multiple Quantum Coherence) spectra were acquired on the Varian Unity 500 spectrometer. An HMBC spectrum gives a two-dimensional spectrum, with ^{13}C chemical shifts on one axis and ^{1}H chemical shifts on the other, correlating spins coupled through multiple bonds; HMQC correlates ^{13}C and ^{1}H through one-bond couplings.

DOSY

High resolution DOSY (Diffusion-Ordered Spectroscopy) data were acquired using the ONESHOT DOSY pulse sequence. Twelve spectra were acquired, with gradient pulse durations of 2.9 ms and nominal gradient amplitudes ranging from 6 to 63 G cm^{-1} in equal steps of gradient squared. The FIDDLE (free induction decay deconvolution for line shape enhancement) algorithm[12] was used to correct line shapes using TSP as a reference standard. DOSY spectra were constructed after baseline correction by taking the first echo spectrum and distributing the intensities of the individual signals in the second dimension according to their respective diffusion coefficients.[13,14]

Synthetic procedures

*2′-O-acetyl-clarithromycin A (**1**).* The compound 2′-*O*-acetyl-clarithromycin A (**1**) was synthesized according to previously published procedure.[15] A solution of clarithromycin A (0.0972 g, 0.13 mmol) and triethylamine (0.16 mmol) in dichloromethane was cooled in an ice-water bath. Acetic anhydride (0.27 mmol) was added to the solution, the bath was removed, and the reaction was stirred for 5.5 h at room temperature. The reaction was monitored using TLC, with mobile phase dichloromethane: ethyl acetate: ethanol= 5:5:2, and the plate was sprayed with the mixture *p*-anisaldehyde: ethanol: conc. sulfuric acid= 1:9:1. A solution of 0.5 M NaH_2PO_4 was added to the reaction mixture and the aqueous layer was separated. The aqueous phase was extracted three times with chloroform. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give crude product which was recrystallized from acetonitrile, affording 0.0704 g (yield 68.6%) of 2′-*O*-acetyl-clarithromycin

A: mp 249-251 ºC; ^{1}H NMR ($CDCl_3$) δ 4.75 (dd, CHOAc), 3.37 (s, 3H, C-3´´OCH_3), 3.01 (s, 3H, C-6 OCH_3), 2.26 (s, 6H, $N(CH_3)_2$), 2.05 (s, 3H, $OCOCH_3$), 1.14 (s, 3H, C-12 CH_3); ^{13}C NMR ($CDCl_3$) δ 175.7 (C-1), 170 ($OCOCH_3$), 71.8 (C-2´); MS (ES^+) (*m/z*): $[M+H]^+$ 790. The melting point was in accordance with the previously published value.[15] NMR spectra (^{1}H and ^{13}C) of the compound are available in the Supplementary Material (Figure S-1).

2´-O-acetyl-4´´-O-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (2). 2´-*O*-acetyl-clarithromycin A (**1**) (0.1074 g, 0.14 mmol) was dissolved in dry dichloromethane (dried over thermally treated Al_2O_3) in an atmosphere of dry nitrogen. Then 60.7 µL 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (ρ=1.061 g mL^{-1}) (0.28 mmol) was added, and 94.8 µL of *N*-ethyldiisopropylamine (ρ=0.742 g mL^{-1}, 0.56 mmol). Then 0.0024 g (0.02 mmol) 4-dimethylaminopyridine was added and the reaction mixture was stirred. The reaction was monitored using TLC every 15 min during 2 h, with mobile phase dichloromethane: ethyl acetate: ethanol= 5:5:2, and the plate was sprayed with the mixture *p*-anisaldehyde:ethanol:conc. sulfuric acid= 1:9:1. The reaction was left with stirring overnight at room temperature. Then a few drops of absolute ethanol were added, followed by aqueous saturated $NaHCO_3$, and the extraction was performed with dichloromethane. The organic layer was dried over anhydrous sodium sulfate. A crude product was obtained after vacuum evaporation and kept at -21 ºC. MS (ES^+) (*m/z*): $[M+K]^+$ 1028. Mass spectrum (ES^+) is available in the Supplementary Material (Figure S-2). NMR spectra (^{1}H, HMBC, HMQC) recorded at 500 MHz for the crude product are not shown.

*2´-O-acetyl-4´´-O-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**)*. 2´-*O*-acetyl-clarithromycin A (**1**) (0.1000 g, 0.13 mmol) was dissolved in dry dichloromethane in an atmosphere of dry nitrogen. Then, using an automated pipette, 141.4 µL 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (ρ=1.061 g mL^{-1}, 0.65 mmol) was added, and 176.6 µL of *N*-ethyldiisopropylamine (ρ=0.742 g mL^{-1}, 1.04 mmol). The reaction mixture was monitored using TLC every 15 min during 2 h, with mobile phase dichloromethane: ethyl acetate: ethanol= 5:5:2, and the plate was sprayed with the mixture *p*-anisaldehyde:ethanol:conc. sulfuric acid= 1:9:1. The reaction was left overnight stirring. MS (ES^+) (*m/z*): $M^{+\cdot}$ 989. Mass spectrum (ES^+) is available in the Supplementary Material (Figure S-3).

*4´´-O-phosphonyl-clarithromycin A (**3**)*. 2´-*O*-acetyl-4´´-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**) (0.5165 g, 0.52 mmol) was dissolved in 15 mL acetone, 10 mL methanol and 5 mL water. The mixture was left at room temperature overnight then a saturated solution of potassium carbonate was added. Afterwards, the reaction mixture was extracted with methylene chloride three times and then dried over anhydrous sodium sulfate. Filtration was performed, and evaporation with a vacuum evaporator, giving 0.1465 g 4´´-*O*-phosphonyl-clarithromycin A (**3**) (yield 34.7 %). MS (ES^+) (*m/z*): $[M-63]^+$ 748. Mass spectrum (ES^+) is available in the Supplementary Material (Figure S-4).

Conformational analysis

Unconstrained conformational search

SMILES string from PubChem was used for the generation of 3D structure of clarithromycin A. A 3D structure of 2´-*O*-acetyl derivative of clarithromycin A was constructed using 2D Sketcher (Beta) option. Their unconstrained conformational analyses were performed using MacroModel under the Schrodinger Suite 2025-4 and with Maestro 14.6 as the interface. Chloroform was used as the solvent. The minimizations were first performed with charges from the force field (AMBER*)[16], the cut-off was extended, the minimization method was TNCG (Truncated Newton Conjugate Gradient), and the maximum number of iterations was set to

10,000, with the gradient convergence, and its threshold of 0.05. AMBER* force field was used previously for this type of macrocycle as the results of the conformational analysis showed good agreement with NMR data.[17] Conformational search torsional sampling was MCMM (Monte Carlo multiple minimum) with automatic setup during the calculation, and torsion sampling options were set to intermediate. The maximum number of steps was 10,000, with 100 steps per rotatable bond. The number of structures to be saved for each search was 100, energy window for saving structures was 21 kJ/mol, and the maximum atom deviation cut-off was 0.5 Å. In the case of 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**), minimization and unconstrained conformational search were performed in Macromodel using MMFFs force field and chloroform as solvent, with the previously found parameters for the minimization and unconstrained conformational analysis of clarithromycin A and 2′-*O*-acetyl derivative of clarithromycin A. AMBER* force field does not contain the parameters for phosphorus containing compounds like from this study, so MMFFs was chosen as a force field as it provides good agreement with the experimental data on this type of compounds.[18] MMFFs force field was used in the minimization and the conformational analysis of 4′′-*O*-phosphoryl-clarithromycin A as well.

Constrained conformational search

Constrained conformational search was performed on clarithromycin A and macrolides **1**-**3** starting from the global minima of the unconstrained search of the investigated compounds and using the same parameters as for unconstrained conformational search with the distances constraints (H4-H11 was constrained to 2.5 ± 0.3 Å, H5-H18 to 2.5 ± 0.3 Å and H15-H16 to 2.5 ± 0.3 Å).

Molecular docking

The molecular docking studies with selected compounds (clarithromycin A (6-*O*-methyl erythromycin A), compounds **1**-**3**) were performed using protein chain from *Escherichia coli* as a target interacting with co-crystalized erythromycin A (entry 4V7U[19] from Protein Data Bank) in folded-out conformation. They were achieved at the binding site of erythromycin A in *E. coli*. Protein chain was prepared for docking using the Protein Preparation and Refinement tool of Schrodinger Suite 2025-4. The previously optimized structures of clarithromycin A and compounds **1**-**3** in MacroModel through constrained conformational search were ligands in the molecular docking studies. Molecular docking was performed with Glide under Schrodinger Suite 2025-4.

MM-GBSA studies

MM-GBSA studies were conducted on docking complexes of ligands (clarithromycin A, compounds **1**-**3**) and the target-protein chain from *E. coli* using Prime under Schrodinger Suite 2025-4. Solvation model was VSGB and used force field was OPLS 4.

Rules-based fragment prediction for mass spectrometry

MS Fragmenter[20] was used for the prediction of mass spectral fragmentation of 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**) and 4′′-*O*-phosphonyl-clarithromycin A (**3**). The compounds **2** and **3** were drawn, and then ionization polarity and fragmentation options were selected.

RESULTS AND DISCUSSION

Synthesis of the phosphoramidite derivative of 2′-O-acetyl clarithromycin A

There are many published syntheses[21] of acetyl derivatives of macrolide antibiotics (such as 2′-*O*-acetyl-(10*E*)-10,11-ene-11-deoxy-clarithromycin), but there are no available data on the synthesis of the phosphoramidite derivatives. Phosphoramidites are unstable, and preferably stored under an inert atmosphere at −20 °C to minimize oxidation and hydrolysis.[22] Although stored at -21 ºC prior to NMR analysis, the phosphoramidite derivative of 2′-*O*-acetyl clarithromycin A showed several signals in the ^{31}P NMR spectrum. A simple experiment with water, 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite and *N*-ethyldiisopropylamine showed that the reagent is not so easily degradable (^{31}P δ 15.4 ppm), and that there are numerous products with various chemical shifts (Figure S-5 in the Supplementary Material). The most intense can be observed at δ 3.4 ppm (^{31}P). 2′-*O*-Acetyl clarithromycin A did not show any reaction towards phosphoramidite derivative in 1 day when there was no catalyst in the system. After 1 day when 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite was in 5 fold excess, the desired product appeared (^{31}P δ 7 ppm) (Figure S-6 in the Supplementary Material). The same result was achieved with 1) DMAP (4-dimethylaminopyridine) as a catalyst (Figure S-7 in the Supplementary Material), 2) on heating on 40 ºC under reflux (Figure S-8 in the Supplementary Material), 3) in ratio of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite and the substrate of 0.95:1, and the ratio of base to the reagent of 2:1, and 4) iodine as a catalyst. *N*-ethyldiisopropylamine causes some slight changes in 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, particularly if the reaction requires longer time, so other bases are preferred. The synthesis of 4′′-*O*-phosphonyl-clarithromycin A (**3**) was easily achieved.

*DOSY NMR analysis of the purity of the synthesized compound: 2′-O-acetyl-4′′-O-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**)*

Diffusion-ordered NMR spectroscopy (DOSY) is a method for distinguishing between the NMR signals of (macro)molecules of different molecular weights present in solution at millimolar concentrations.[23] The number of components identifiable in solution is limited only by resolution.[24,25]

The purity of the synthesized compound, 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A using DMAP as a catalyst was checked using DOSY NMR at 500 MHz. The recorded spectrum revealed several compounds with different diffusion coefficients (Fig 4), however, most of the signals with different diffusion coefficients belong to solvent and reagents used. A line of signal at diffusion coefficient (~3-4 × 10^{-10} m^2 s^{-1}) shows chemical shifts characteristic of macrolides,[26] suggesting the presence of a single compound with, crucially, no degradation products, such as cladinose.

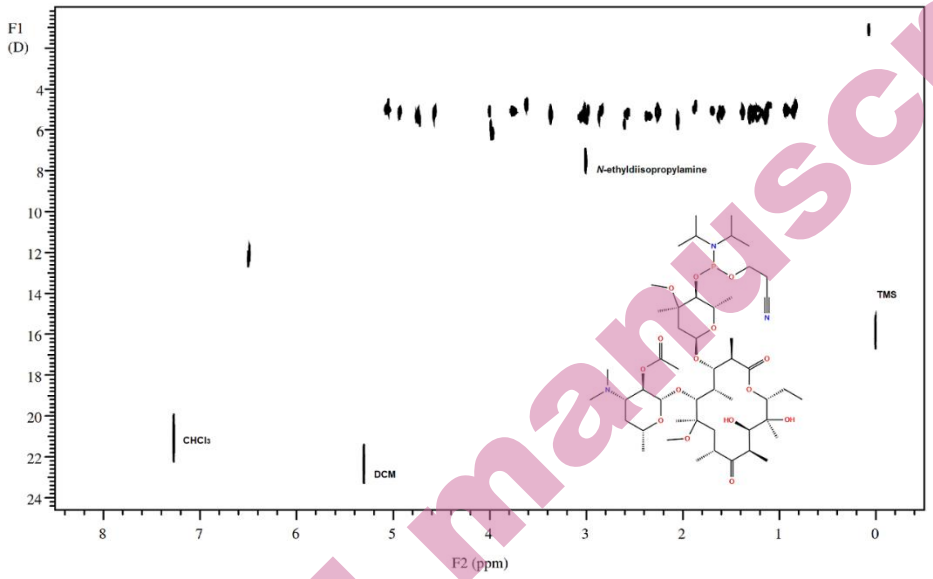


Fig 4. DOSY NMR spectrum at 500 MHz of 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**).

Conformational analysis

Unconstrained conformational analysis of clarithromycin A, 2′-*O*-acetyl-clarithromycin A (**1**) and 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**) gave as global minima folded-out[17,27] structures (H4-H11 (2.90 Å), H5-H18 (2.36 Å), H15-H16 (2.94 Å) in the case of clarithromycin A; H4-H11 (2.88 Å), H5-H18 (2.36 Å), H15-H16 (2.95 Å) in the case of 2′-*O*-acetyl-clarithromycin A (**1**); H4-H11 (3.03 Å), and H5-H18 (2.44 Å), H15-H16 (2.91 Å) in the case of 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**)). A longer distance H15-H16 than 2.8 Å in the case of clarithromycin A, 2′-*O*-acetyl-clarithromycin A (**1**) and 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**) is not so significant, owing to the possibility of free rotation of methyl groups, which obviously does not require a high amount of energy. Conformational analysis of 4′′-*O*-phosphoryl-clarithromycin A did not give a folded-out structure of the global minimum (H4-H11 (5.18 Å), H5-H18 (2.94 Å), H15-H16 (5.79 Å)). The analysis of the the conformational landscapes, low-energy conformers, and relative populations of clarithromycin A and macrolide antibiotics (**1**-**3**) reflects the known conformational diversity of macrolides (Table I).

Table I. Global minima energies and their number of times, and the population of folded-out in the first ten lowest energy conformations as results of the unconstrained conformational search, as well as global minima energies and their number of times as results of constrained conformational search

Compound	Unconstrained conformational search			Constrained conformational search	
	Global minimum		Population of folded-out in the first ten lowest energy conformations	Global minimum	
	Energy (kJ/mol)	Number of times		Energy (kJ/mol)	Number of times
Clarithromycin A	85.78	13	4/10	130.57	19
2'-*O*-acetyl-clarithromycin A (**1**)	26.17	23	6/10	76.26	10
2'-*O*-acetyl-4''-*O*-(2-cyanoethyldiisopropyl phosphoramidite)-clarithromycin A (**2**)	464.99	2	3/10	521.97	15
4''-*O*-phosphonyl-clarithromycin A (**3**)	493.64	8	0/10	516.28	29

Our previously published work on erythromycin A and clarithromycin A (6-*O*-methyl erythromycin A) shows that their active conformations when bound weakly to bacterial (*Escherichia coli*) ribosomes are folded-out conformations.[17] Obtained clarithromycin A conformations with the lowest energy-global minima in water both using Macromodel and SYBYL[17] showed folded-out structure. Unconstrained conformational search on clarithromycin A and compounds **1** and **2** in chloroform showed folded-out conformation with negligible longer H4-H11 distance from 2.8 Å. However, compound **3** showed completely different conformation. Since biologically active conformation of clarithromycin A is folded-out, all four compounds (clarithromycin A and compounds **1**-**3**) were put for constrained conformational search, and obtained global minima were used in further computational studies.

Molecular docking

Macrolide antibiotics inhibit bacterial protein synthesis by reversibly binding to the 50S subunit of the bacterial ribosome. This binding blocks the ribosomal exit tunnel near the peptidyl transferase center, thereby preventing elongation of the growing peptide chain.[28]

Due to the reason that there is no available crystal structure of clarithromycin A with the ribosomal bacterial proteins, the crystal structure of erythromycin A with *E. coli* ribosome was used for the molecular docking study (PDB ID: 4V7U)[19]. Clarithromycin A (6-*O*-methyl erythromycin A) is a semi-synthetic macrolide derived from erythromycin A.

Erythromycin A shows folded out conformation in the bound state in the crystal structure with ribosome of *E. coli:* H4-H11 2.42 Å, H5-H18 2.65 Å and H15-H16 3.12 Å. It established interactions with amino-acids residues Ala 89, Lys 90, Gly 91 and Arg 92. These data were used for the molecular studies with clarithromycin A and compounds **1-3**.

Molecular docking studies on clarithromycin A and compounds **1-3** show quite different Glide scores (Table II). Compounds **2** and **3** were shown better inhibition compared to clarithromycin A.

MM-GBSA studies

MM-GBSA estimates the binding free energy of a ligand–protein complex in a more physically realistic way than simple docking scores. It includes terms for molecular mechanics energies and solvent effects *via* an implicit solvation model. Because of this, MM-GBSA is often more accurate at ranking ligands by binding affinity, especially for lead optimization or for smaller sets of compounds selected after docking.[29,30]A common strategy is to use docking to generate possible binding poses quickly, then apply MM-GBSA to rescore these poses with a more realistic energy model. This helps refine rankings and improve discrimination between strong and weak binders.[31]

Obtained docking complexes of protein chain from *E. coli* and investigated compounds (clarithromycin A and **1-3**) were used in MM-GBSA studies. Clarithromycin A and compound 2 were showen to possess similar binding affinities, and less activity was shown by compound 3, ans particularly compound 1 (Table II).

Table II. GlideScores from molecular docking and ΔG_bind energies from MM-GBSA studies for the investigated compounds

Compounds	Glide score (kJ/mol)	ΔG_bind (kJ/mol)
Clarithromycin A		-83.30
2'-*O*-acetyl-clarithromycin A (**1**)		-34.52
2'-*O*-acetyl-4"-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**)	-2.13	-82.01
4"-*O*-phosphonyl-clarithromycin A (**3**)	-0.96	-74.81

Rules-based fragment prediction for mass spectrometry

Electrospray ionization of 2′-*O*-acetyl-4′′-*O*-(2-cyanoethyldiisopropylphosphoramidite)-clarithromycin A (**2**) was performed under two different sets of conditions. In the first case (electrospray), one additional peak was observed which has *m/z* equal to 200.2 (Fig 5a), and which was predicted with MS Fragmenter. In the second case (default conditions), MS Fragmenter successfully predicted the existence of peaks with following *m/z* values that were found in experimental ES^+ mass spectra of **2**: 102, 219, 614, 790, 877, 920, 921, 930, 947, 962 (Fig 5b).

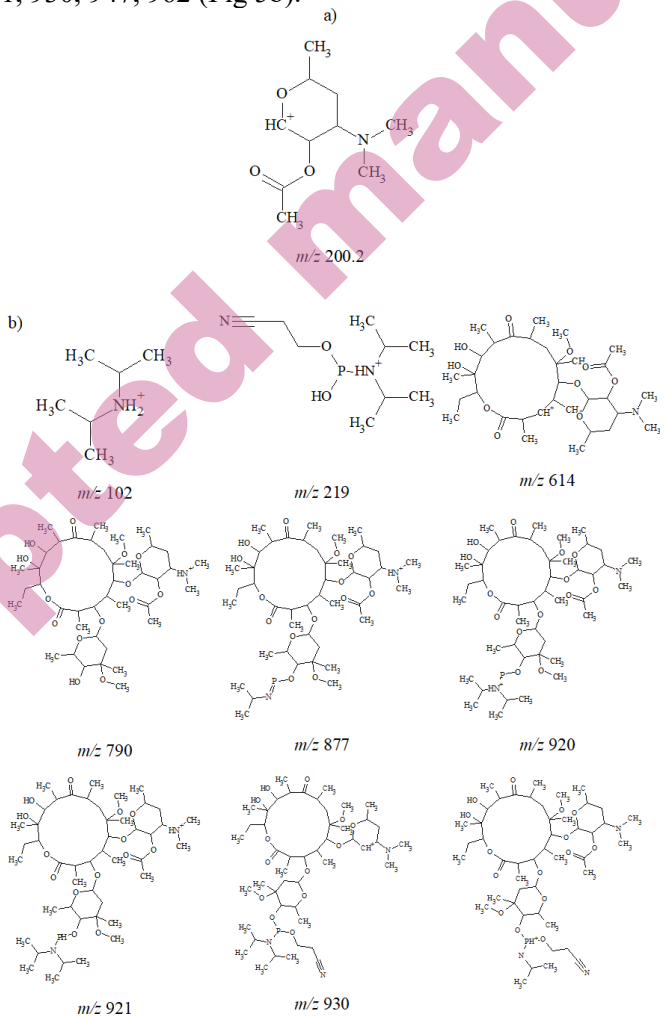


Fig 5. Fragments of compound **2** predicted using MS Fragmenter: a) a fragment only seen under milder conditions of electrospray ionization; b) fragments seen in mass spectra under default conditions.

The fragments at *m/z* equal to 776, 771, 750, 748, 734 and 198 in the ES^+ mass spectrum of 4′′-*O*-phosphonyl-clarithromycin A (**3**) were predicted using MS Fragmenter (Fig 6).

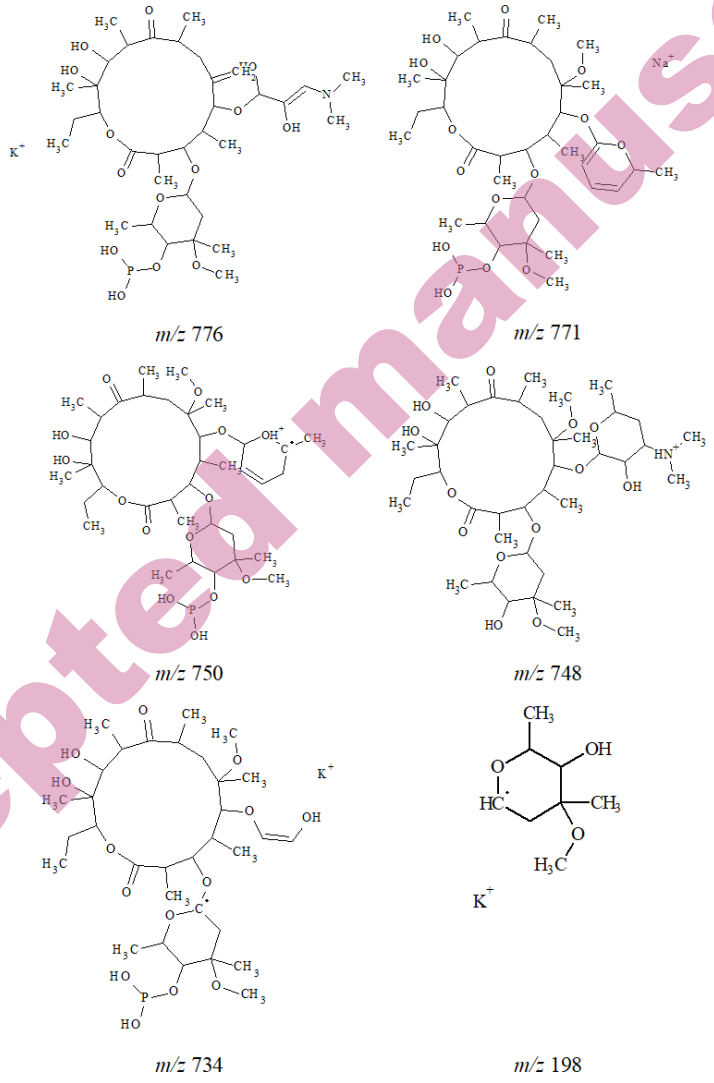


Fig 6. Fragments in the ES^+ mass spectrum of 4′′-*O*-phosphonyl-clarithromycin A (**3**) predicted using MS Fragmenter.

CONCLUSION

In this work, clarithromycin A was successfully modified through the introduction of acetyl, phosphoramidite, and phosphonyl functional groups, yielding structurally well-characterized macrolide derivatives. The synthesis of the

phosphoramidite derivative is particularly significant, given the scarcity of literature data and the challenges associated with its chemical instability. The application of DOSY NMR spectroscopy proved to be a powerful method for confirming the purity of this complex macrolide derivative. Computational conformational analyses revealed that clarithromycin A and its acetyl and phosphoramidite derivatives predominantly adopt folded-out conformations consistent with the biologically active form known to bind bacterial ribosomes, whereas the phosphonyl derivative displayed a markedly different conformational preference. Molecular docking and MM-GBSA studies further demonstrate that derivatives containing phosphorus-based substituents retain binding affinity toward the ribosomal target when compared to clarithromycin A. Overall, the results highlight chemical modification of macrolide antibiotics as a promising strategy for the development of new compounds with potentially improved pharmacological properties. This study provides a solid foundation for further biological evaluation and supports the future design of novel macrolide-based prodrugs and derivatives aimed at combating antimicrobial resistance.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13617, or from the corresponding author on request.

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И З В О Д

СИНТЕЗЕ И КОМПЈУТЕРСКЕ АНАЛИЗЕ ОДАБРАНИХ МАКРОЛИДНИХ ДЕРИВАТА ДОБИЈЕНИХ ИЗ КЛАРИТРОМИЦИНА А

БИЉАНА Б. АРСИЋ[1,2], GARETH A. MORRIS[3], ABDOLREZA HASSANZADEH[2,4], ОЛГА П. ЈОВАНОВИЋ[1], JILL BARBER[2] И ЂОРЂЕ ГЛИШИН[1]

[1]Универзитет у Нишу, Природно-математички факултет, Департман за хемију, Вишеградска 33, Ниш, Република Србија, [2]Division of Pharmacy and Optometry, School of Health Sciences, University of Manchester, Manchester, United Kingdom, [3]Department of Chemistry, University of Manchester, Manchester M13 9PL, United Kingdom, [4]Pharmaceutics Research Center, Institute of Pharmaceutical Sciences, Kerman University of Medical Sciences, Kerman, Iran.

У раду су приказане синтезе и експерименталне и компјутерске анализе нових макролидних деривата добијених из кларитромицина А, са циљем испитивања њиховог потенцијала у превазилажењу проблема антимикробне резистенције. Синтетисани су 2′-*O*-ацетил-кларитромицин А, његов фосфорамидитни дериват и одговарајући фосфонилни дериват. Посебна пажња посвећена је оптимизацији услова фосфорилације, с обзиром на нестабилност фосфорамидита. Чистоћа фосфорамидитног деривата потврђена је применом DOSY NMR спектроскопије. Изведене су детаљне конформационе анализе коришћењем молекулског моделирања, као и молекулско доковање и MM-GBSA прорачуни са циљним протеином из *Escherichia coli*, како би се проценио афинитет везивања синтетисаних једињења. Резултати показују да фосфорамидитни и фосфонилни деривати имају упоредив афинитет везивања у односу на кларитромицин А. Додатно, фрагментација у масеној спектрометрији анализирана је и рационализована применом MS Fragmenter софтвера.

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