Summary of Professional Accomplishments

Study of the structures of selected natural products and the impact of their modification on antibacterial and antifungal activity

dr Krystian Pyta

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Spis treści

1. Name

Krystian Pyta

ORCiD ID: 0000-0001-5250-9990

Web of Science Researcher ID: D-7340-2011

2. Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhDdissertation

2012 doctor of chemical sciences; title of doctoral thesis: "Synthesis and structure of new amine analogues of the ansamycin antibiotic rifampicin"; Department of Biochemistry, Faculty of Chemistry, University of Adam Mickiewicz; Ph.D. thesis supervisor: prof. Ph.D. Piotr Przybylski; **doctoral thesis awarded by the Council of the Faculty of Chemistry of Adam Mickiewicz University**

2008 Master of Science in Chemistry; the title of master's thesis: "Synthesis, structure and complexing properties of new aza-derivatives of gossypol"; Department of Biochemistry, Faculty of Chemistry, University of Adam Mickiewicz; master's thesis supervisor: prof. Ph.D. Bogumił Brzezinski

3. Information on employment in research institutes or faculties/departments or school of arts

10.2012–now lecturer; Department of Natural Products Chemistry, Faculty of Chemistry, University of Adam Mickiewicz

10.2008-06.2012 doctoral studies; Department of Biochemistry, Faculty of Chemistry, University of Adam Mickiewicz

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

4.1 Title of scientific achievement

Study of the structures of selected natural products and the impact of their modification on antibacterial and antifungal activity.

4.2 List of scientific articles included in the habilitation thesis

The habilitation procedure is based on a scientific achievement which consists of a series of 8 publications (**H1**-**H8**). H1's work was funded by the "IUVENTUS PLUS" grant 0366/IP3/2013/72, which I managed. The order of the publications in this series is based on the discussion in the paper, not their year of publication. Attachments 5 and 6 contain copies of works **H1**-**H8** and statements from co-authors regarding their individual and substantive contributions to the creation of these works.

nr	publication	Impact Factor (IF) according to JCR database		MNiSW points**
		publication date	5-years***	
H1	**Pyta K.***, Blecha M., Janas A., Klich K., Pecyna P., Gajecka M., Przybylski P. „Synthesis, structure and antimicrobial evaluation of a new gossypol triazole conjugates functionalized with aliphatic chains and benzyloxy groups” *Bioorg. Med. Chem. Lett*, 26 (17), (**2016**), 4322-4326 DOI: 10.1016/j.bmcl.2016.07.033, link My participation consisted in designing and developing methods for the synthesis of new gossypol derivatives, spectroscopic characterization of the obtained compounds, developing and participating in the performance of tests checking the level of ergosterol in mushrooms, I participated in writing the manuscript and the supplement, and I was responsible for the review process.	2.454	2.6	70
H2	Przybylski P.*, **Pyta K.**, Klich K., Schilf W., Kamieński B. „^{13}C and ^{15}N CP/MAS, ^{1}H-^{15}N SCT CP/MAS and FTIR spectroscopy as tools for ualitative detection of the presence of zwitterionic and nonionic forms of ansa-macrolide 3-formylrifamycin SV and its derivatives in solid state” *Magn. Reson. Chem*. 52 (1-2), (**2014**), 10-21 DOI: 10.1002/mrc.4028, link I participated in developing the concept of the work and writing the manuscript, prepared the supplement, participated in the spectroscopic characterization of compounds and discussion of the results.	1.179	1.8	40
H3	**Pyta K.**, Klich K., Domagalska J., Przybylski, P.* „Structure and evaluation of antibacterial and antitubercular properties of new basic and heterocyclic 3-formylrifamycin SV derivatives obtained via 'click chemistry' approach” *Eur. J. Med. Chem.* 84, (**2014**), 651-676 DOI: 10.1016/j.ejmech.2014.07.066, link I participated in the development of the concept of the work, writing the manuscript and in the review process, I developed a method for the synthesis of derivatives and participated in the preparation of	3.447	6.5	200

	triazole and heterocyclic derivatives, I performed Huisgen cycloaddition reaction tests, I participated in the spectroscopic characterization of compounds and the discussion of the results including SAR analysis.			
H4	Czerwonka D., Domagalska J., **Pyta K.,** Kubicka M.M., Pecyna P., Gajecka M., Przybylski P.* „Structure-activity relationship studies of new rifamycins containing l-amino acid esters as inhibitors of bacterial RNA polymerases” *Eur. J. Med. Chem.*, 116, (**2016**), 216-221 DOI: 10.1016/j.ejmech.2016.03.061, link I participated in developing the concept of the work, writing the manuscript and in the review process, I participated in the spectroscopic characterization of compounds and discussion of the results.	4.519	6.5	200
H5	**Pyta K.**, Janas A., Szukowska M., Pecyna P., Jaworska M., Gajecka M., Bartl F., Przybylski P.* „Synthesis, docking and antibacterial studies of more potent amine and hydrazone rifamycin congeners than rifampicin” *Eur. J. Med. Chem.*, 167, (**2019**), 96-104 DOI: 10.1016/j.ejmech.2019.02.009, link I participated in the development of the concept of the work, writing the manuscript and in the review process, I participated in the preparation and purification of derivatives, I participated in the spectroscopic characterization of compounds and the discussion of results, including SAR analysis. I was responsible for preparing the supplement.	5.573	6.5	200
H6	**Pyta K.**, Przybylski P.*, Bartl, F. „Regioselective long-range proton transfer in new rifamycin antibiotics: A process in which crown ethers act as stronger brønsted bases than amines” *ChemPhysChem*, 16 (5), (**2015**), 938-942 DOI: 10.1002/cphc.201402892, link I participated in the development of the concept of the work, writing the manuscript and in the review process, I participated in the preparation and purification of derivatives, I participated in the spectroscopic characterization of compounds and discussion of the results, I prepared compounds for analysis and performed FT-IR spectra. I was responsible for preparing the supplement.	3.138	3.0	100

H7	Przybylski P.*, **Pyta K.**, Czerwonka D., Kubicka M.M., Gajecka, M. „The effect of complexation of 3-formylrifamycin SV macrocyclic ether derivatives with metal cations and small nitrogen-containing organic molecules on antibacterial activity against S. aureus and S. epidermidis” *Bioorg. Med. Chem. Lett.* 25 (18), (2015), 3903-3909 DOI: 10.1016/j.bmcl.2015.07.043, link I participated in the development of the concept of the work, writing the manuscript and in the review process, I participated in the preparation and purification of derivatives, I participated in the spectroscopic characterization of compounds and discussion of the results, including SAR analysis, I prepared compounds for analysis and performed FT-IR spectra. I was responsible for preparing the supplement.	2.486	2.6	70
H8	**Pyta K.**, Janas A., Skrzypczak N., Schilf W., Wicher B., Gdaniec M., Bartl F., Przybylski P.* „Specific Interactions between Rifamycin Antibiotics and Water Influencing Ability To Overcome Natural Cell Barriers and the Range of Antibacterial Potency” *ACS Infectious Diseases Article* 5(10), (**2019**), 1754-1763 DOI: 10.1021/acsinfecdis.9b00176, link I participated in the development of the concept of the work, writing the manuscript and in the review process, I participated in the spectroscopic characterization of compounds and discussion of the results, I prepared compounds for analysis and performed FT-IR spectra. I was responsible for preparing the supplement.	4.614	5.1	100
	Average value/publication	**3.426**	**4.325**	**122.5**

Scientometric informations – in September 2023

Total impact factor (IF) for journals in which works **H1**-**H8** were published	**27.41**
Total impact factor (IF) for journals according to the JCR database (for 2022)	**34.6**
Total number of points in which works **H1**-**H8** were published based on the list of journals scored by the Ministry of Science and Higher Education (for 2023)	**980**
The total number of scientific papers in which I am a co-author from the JCR list	**40**
h-index according to Web of Science database	**13**
Total number of citations according to the Web of Science database	**758**
Number of citations without self-citations according to the web of science database	**673**

4.3 Discussion of the most important achievements contained in the publications constituting the basis for the habilitation procedure

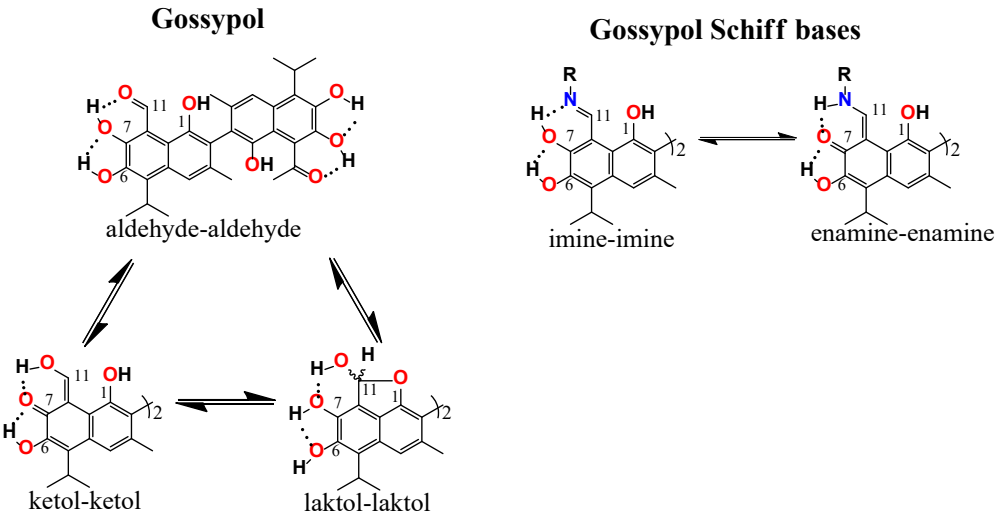
The habilitation thesis presented is titled "Study of the structures of selected natural products and the impact of their modification on antibacterial and antifungal activity". It contains 8 works (**H1**-**H8**) that were published after the doctoral degree was awarded. **H1** explores the effect of modifying the natural bisesquiterpene - gossypol on the antifungal activity of this group of compounds and their mechanism of action on fungi. **H2** presents results that show the possibility of rifampicin (**Rif**) existing in solid form with various structures, which may affect the process of determining these antibiotics quantitatively and qualitatively. **H3**-**H7** present the results of experimental research, including the synthesis of new rifampicin analogues and the correlation of their antibacterial activity with the structures of the obtained derivatives. Work **H8** describes the conformational equilibria in the group of ansamycin compounds and demonstrates the key role of water molecules in the stabilization of these structures.

This study is not a comprehensive discussion of all the obtained results. It provides a summary and presentation of the experimental research area and focuses on the significant advancements that meet the criteria of scientific innovation. All methodological details, numerical data (including crystallographic, biological, and theoretical studies), tables, drawings, spectra, etc. are available in the attached publications and supplementary materials, which are part of Annex 7. The publications included in the habilitation thesis are marked with the symbols [**H1**-**H8**], while the remaining cited literature is numbered based on their

appearance and listed as "List of cited literature" later in the self-report. The list of abbreviations used and their meanings can be found in subsection 4.3.4.

4.3.1 Synthesis and anticancer activity of new gossypol derivatives - introduction, aim of the work and discussion of the most important achievements (H1)

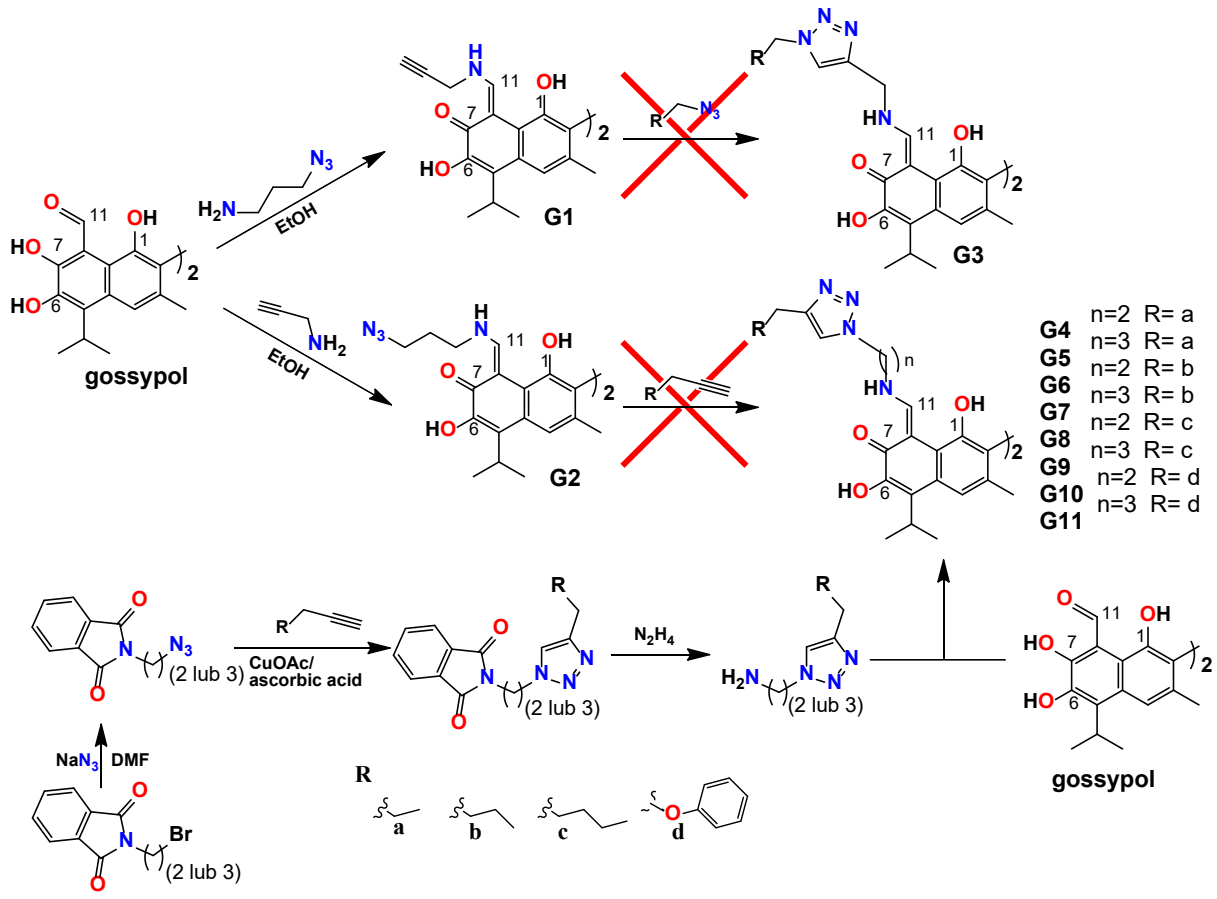
Gossypol (shown in Scheme 1) is a bisesquiterpene that possesses intriguing physico-chemical properties and displays promising biological activity.[1] This molecule contains an aldehyde group that is conjugated to a polyphenol system, which allows it to exist in three different tautomeric forms: aldehyde-aldehyde, lactol-lactol, and ketol-ketol (Scheme 1).[1] Additionally, due to the C(2)-C(2') bond's inhibited rotation, the gossypol molecule can exist as two enantiomers with different biological activity, known as atropisomerism.[1,2] The potential of gossypol's pharmacological applications is evident from over two thousand scientific publications reporting on the possibility of using this compound and its derivatives, such as Schiff bases, in the fields of pharmacy and medicine. Furthermore, gossypol shows promise as a temporary male contraceptive.[3] This substance has been found to possess powerful anti-cancer[4] and anti-malarial[5] properties. It is also being researched as a potential treatment for HIV, with its mechanism of action being linked to the inhibition of HIV reverse transcriptase.[6,7] My participation in a study on the synthesis and application of Schiff bases and gossypol hydrazones in pharmacy and agriculture revealed that these derivatives have both antibacterial[8] and antifungal[9] properties. The most promising results were obtained in antifungal tests against fungi from the *Fusarium* family.[9] Farmers and fruit growers face a major challenge with fungi, which often destroy crops. However, Gossypol derivatives have proven to be effective in inhibiting the growth of these fungi, with higher activity compared to gossypol itself.[9]



Scheme 1 Structure and tautomeric forms of gossypol and its Schiff bases.

Additionally, heteroaromatic substituents in the derivatives' structure have been found to have higher antibacterial activity than gossypol.[8] Unfortunately, despite its wide range of biological activity, gossypol cannot be used as a drug due to its high toxicity. This toxicity is attributed to the presence of two aldehyde groups in the molecule that can bind to proteins and enzymes (Scheme 1).[10]

Before the release of work **H1**, many studies had been conducted on Schiff bases, hydrazones, and oximes of gossypol. However, there was a lack of research on derivatives containing conjugates of gossypol derivatives with triazole systems in this particular library of compounds.[1] Triazole systems are known for their antifungal properties,[11,12] which could greatly enhance the antifungal properties of new gossypol derivatives if combined with the gossypol molecule. To create new enamine conjugates of gossypol with triazole systems substituted with aliphatic and aromatic groups, I planned to first obtain Schiff bases of gossypol with selected amines containing a propargyl or azide moiety (Scheme 2). Then, these groups would be used to perform the 1,4-dipolar Huisgen cycloaddition reaction, leading to the desired compounds (**G3**-**G11**). However, there was a problem during the attempt to obtain triazole systems, even after increasing the amount of catalyst and extending the reaction time. Compounds **G1** and **G2** have an amino nitrogen atom and oxygen atoms in the C(1) and C(1') positions. This promotes the formation of a complex with Cu^{+} copper cations and prevents the formation of a transition state in the cycloaddition reaction. To obtain triazole conjugates of gossypol, the strategy had to be changed. The dipolar Huisgen cycloaddition reaction was performed before combining the entire amine-triazole skeleton with gossypol (Scheme 2). Phthalimide bromides were used for this purpose and transformed into the appropriate azides, then the introduced functional group was transformed into triazole groups containing a selected substituent. The amino group was deblocked and a condensation reaction was conducted with a gossypol molecule. The amine derivatives of gossypol **G4**-**G11** obtained in this way were spectroscopically examined to confirm their structure and tested for antibacterial and antifungal activity.



Scheme 2 Synthetic strategies aimed at synthesizing new gossypol Schiff bases containing a triazole system.

The tests conducted on various bacterial strains revealed that only gossypol displayed activity against almost all Gram-positive bacteria at a level of 16 µg/ml. Out of the derivatives obtained, only the one containing the shortest alkyl substituent (ethyl) on the **G4** triazole system showed activity against bacterial strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pneumoniae* at a level of 256 µg/ml. In contrast, the results obtained from antifungal activity tests showed slightly greater variability in comparison to antibacterial activity tests (Table 1). For three strains of fungi: *Aspergillus brasiliensis*, *Aspergillus fumigatus*, and *Trichophyton mentagrophytes*, the obtained MIC values (mostly 256 µg/ml) indicated that these fungi are partially sensitive to the action of the derived gossypol (**G4**, **G6-G10**). However, it should be noted that only in two cases did their activity reach MIC = 128 µg/ml. The situation was somewhat different for the *Rhizopus stolonifer* strain because its sensitivity to the **G10** compound was higher (MIC = 64 µg/ml) (Table 1). The most intriguing results, however, were obtained for fungi of the genus *Fusarium oxysporum*, which were isolated from cabbage and tomatoes. Compounds **G10** and **G11** (with benzyloxy systems) were particularly noteworthy because they had MIC values of 16 µg/ml, which were equal to those obtained for the antifungal agent micoconazole (Table 1).

Table 1 Summary of antifungal activity tests and logP values for gossypol and its derivatives **G4**-**G11**. MIC values are given in µg/mL.

Fungi strain	*Aspergillus brasiliensis* ATCC 16404	*Aspergillus fumigatus* ATCC 204305	*Trichophyton mentagrophytes* ATCC 9533	*Rhizopus stolonifer* ATCC 56227b	*Fusarium acuminatum* ATCC 46651	*Fusarium oxysporum f.sp. betae*	*Fusarium oxysporum f. sp. lycopersici*	$logP_{calc}$*
compound								
gossypol	256	256	128	128	128	32	32	6.68
G4	256	256	256	128	256	64	64	7.30
G5	---	---	---	---	---	64	64	7.84
G6	256	256	256	128	256	64	64	8.35
G7	256	256	256	128	256	64	64	8.67
G8	256	256	256	128	256	64	64	8.89
G9	256	256	256	128	256	64	64	9.09
G10	128	256	256	64	128	16	16	8.25
G11	---	---	---	---	---	16	16	8.60
miconazole	---	---	---	---	---	16	16	5.72

*$logP_{calc}$ – values calculated in *Molinspiration property engine v2013.0,* http://www.molinspiration.com/.

During the **H1** work, it was crucial to identify an enzyme or process that could be targeted by certain compounds to disrupt the development of fungi. Previous studies have shown that gossypol can hinder the conversion of cortisone to cortisol by inhibiting 11-beta-hydroxysteroid dehydrogenase.[13,14] Additionally, many antifungal agents, such as fluconazole, interfere with sterol metabolism.[15,16] Thus, it was hypothesized that the antifungal activity of gossypol and its triazole derivatives could be linked to the inhibition of ergosterol biosynthesis. To check this hypothesis, tests were conducted to determine the effect of selected compounds on the level of ergosterol in mushroom cells. Analysis of the experiments conducted on *Rhizopus stolonifer* and *Fusarium acuminatum* strains exposed to gossypol and a **G10** derivative revealed a decrease in ergosterol levels over time. This suggests that the antifungal activity of gossypol and its derivatives is connected to the disruption of ergosterol biosynthesis in fungal cells (see Figure 1).

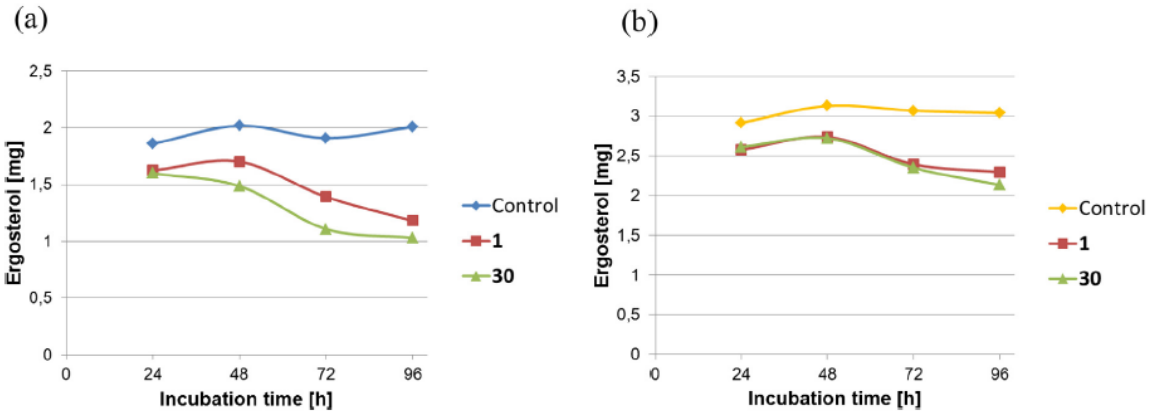
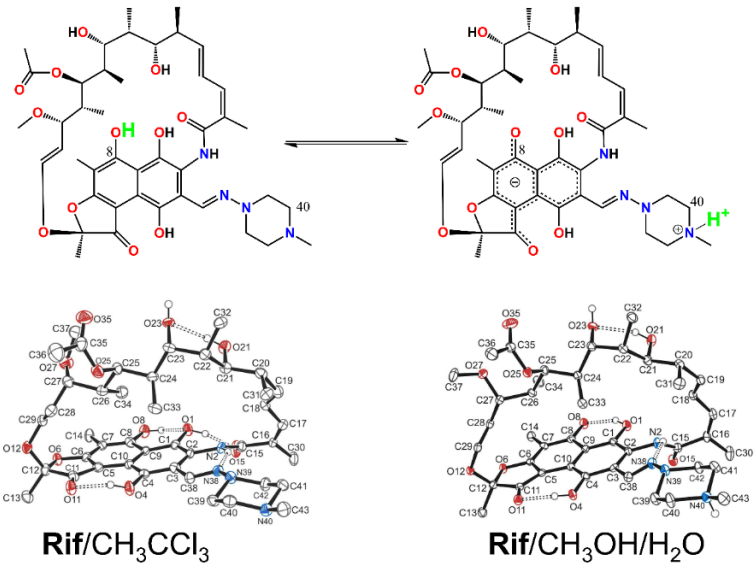


Figure 1 Amount of ergosterol [mg/1 g of dry weight of mushrooms], produced under the influence of gossypol and **G10**, a) for *R. stolonifer*; b) *F. acuminatum*.[H1]

4.3.2 Rifampicin and its amine analogues - introduction, aim of the work and discussion of the most important achievements

Rifampicin (**Rif** or **RMP**) is a type of antibiotic that belongs to the ansamycin group.[17] These antibiotics are named after the unique structure of their molecules, which consists of an aromatic core and a long aliphatic chain, known as the ansamycin bridge (Scheme 3). The bridge connects the core through non-adjacent carbon atoms, creating a "handle-like" appearance (Figure 2a). Due to its relatively inflexible structure, Rifampicin exhibits distinct chemical properties, such as the possibility of diastereoisomerism, which is influenced by the location of the ansamycin bridge around the aromatic ring. Its unique structure also contributes to its potent antibacterial activity.



Scheme 3 Ionic and non-ionic structure of **Rif** and its crystallographic structures obtained in a solution of CH_3Cl_3 and CH_3OH.[18]

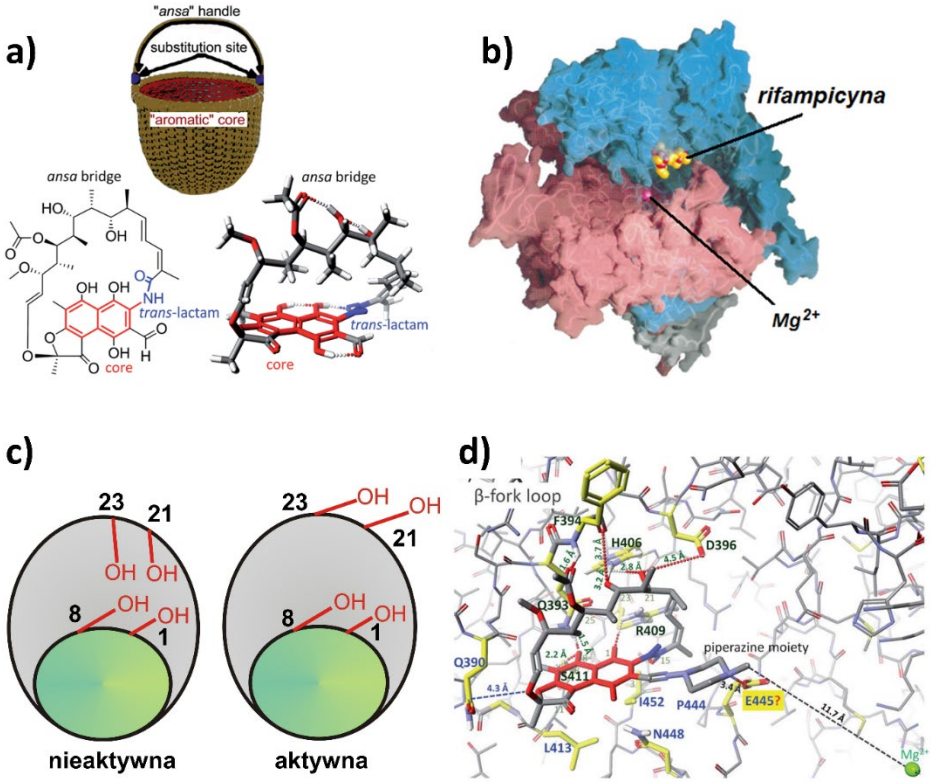


Figure 2 a) Example of the structure of ansamycin compounds based on the example of 3-formylrifamcin SV.[17]; b) Structure of the rifampicin-bacterial RNA polymerase complex.[19]; c) possible arrangements of hydroxyl and phenolic groups relative to each other; model of Rif binding to *Thermus aquaticus* RNAP[17]

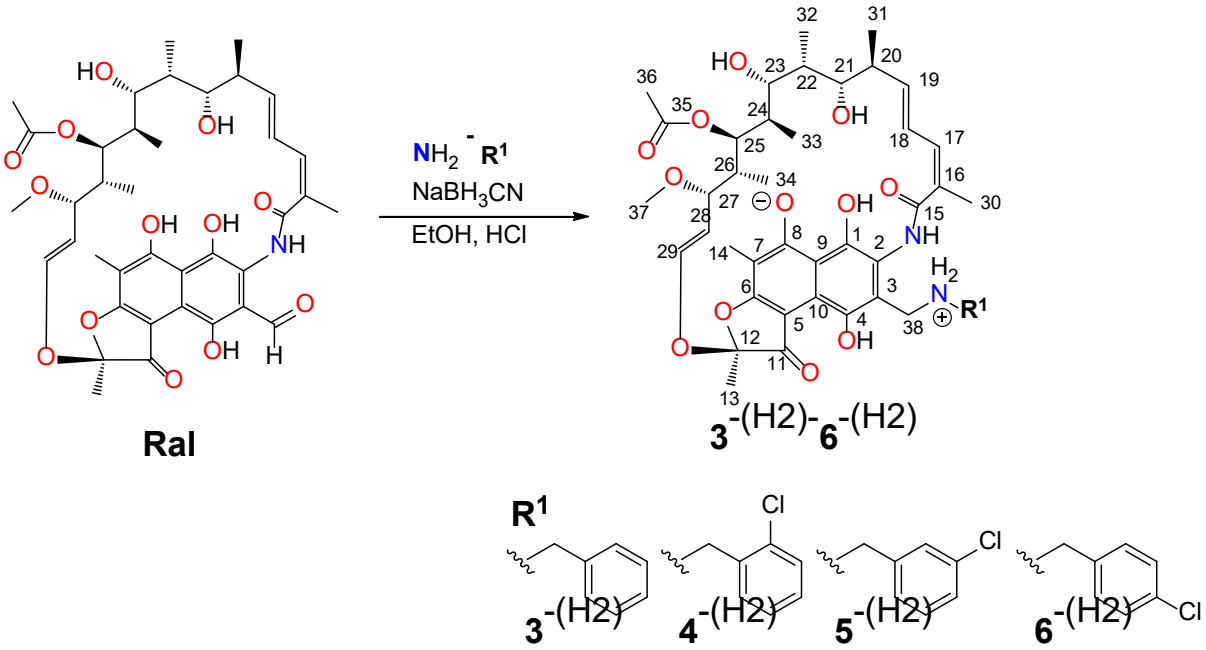
Rifampicin is a highly effective antibiotic with a broad range of biological activity. It is a semi-synthetic ansamycin that works by blocking bacterial DNA-dependent RNA polymerase, which is a key structural element responsible for the effective interaction of the antibiotic with RNAP (Fiqure 2b i d). The orientation of the hydroxyl groups O(21)H and O(23)H of the ansamycin bridge is crucial in this regard, and it must be directed "outwards" to realize the hydrogen bonds that stabilize **Rif** in the RNAP binding site (Figure 2c).[20] Rifampicin inhibits the transcription process in bacterial cells, thus preventing their proper development[21,22], and it is the drug of first choice for serious clinical cases caused by tuberculosis, atypical, and leprosy bacilli.[19] Additionally, it is used for septic conditions caused by various pathogens. However, the use of this antibiotic is limited due to its relatively high hepatotoxicity and the development of defense mechanisms by bacteria, which lead to drug resistance. Bacterial strains can become resistant to rifampicin by modifying the structure of RNA polymerase or through various chemical modifications of the antibiotic.[23] Consequently, new analogues that are resistant to the deactivation processes carried out by bacteria are being sought.

Bacteria become resistant to **Rif**, among others, by hydrolyzing the hydrazone bond, which indicates its important role in binding to RNAP. The aim of the discussed works was to obtain, by means of reductive amination reactions, new amine analogues of rifampicin containing various functional groups in their structure. Attaching a substituent to the C(3) carbon atom through an amino group resulted in lack of susceptibility to hydrolysis by bacteria. It was also important to determine the amine structures of **Rif** analogues in various solvents and compare them with the structure of **Rif**. The analysis of antibacterial activity test results combined with information on the structure of this group of derivatives was aimed at establishing the structure-activity relationship (SAR) for this type of ansamycins.

Although the structure of **Rif** has been known for many decades, until our research team published results in 2012 regarding the possibility of this antibiotic occurring in two forms in solution and in a solid,[18,24] there was no mention in the literature on this topic. These works showed that in aprotic solutions and during the crystallization of **Rif** from such solutions, this antibiotic occurs in a non-ionic form (Scheme 3). However, in protic solutions and when such solutions are used to crystallize **Rif**, a proton is transferred from the phenol group O(8)-H to the piperazine nitrogen atom N(43) of the hydrazone group, which causes **Rif** to exist in an ionic form (Scheme 3). This fact is crucial in stabilizing the antibiotic in the binding site of bacterial RNA polymerase. The presence of the protonated nitrogen atom N+(40)-H enables additional stabilization of **Rif** during complex formation with RNAP, thanks to the formation of an interaction with the negatively charged residue of the conserved glutamate E_{445} (Figure 2d).

4.3.2.1 Discussion of the work H2

Taking into account the possibility of **Rif** occurring in two forms in solution (nonionic and ionic), it was interesting to investigate whether these two forms of **Rif** in a solid would also be observed in CP/MAS/NMR studies. Until the publication of the results in **H2**, solid-state NMR studies of **Rif** were described in the literature, but the authors of these studies did not pay attention to the possibility of this compound occurring in two forms[25] or the quality of the solid-state NMR spectra was not sufficient to draw constructive conclusions[26]. Perfect for studying the structures of pharmaceuticals in solids modern CP/MAS/NMR spectroscopic technique is suitable.[27,28] In the work **H2**, ^{13}C and ^{15}N CP/ MAS and FT-IR. These results were then used to test their possible use in qualitative solid-state **Rif** analysis.



Scheme 4 Scheme for the synthesis of amine analogues of **Rif**.

There is only one N(2) nitrogen atom in the **Ral** structure, however, due to its chemical nature (lactam), it cannot participate in the intramolecular transfer of a proton from the phenolic O(8)H group, so this molecule was an ideal reference point for the non-ionic form of rifamycins in CP/MAS studies. In turn, amine analogues of **Rif** in the solution, regardless of the solvent used, existed in the zwitterionic form with the protonated nitrogen atom N+(38)-H and the phenolate system in the C(8) position of the naphthalene core, they were a very good model for observing the ionic form of rifamycins in the body constant. It is known from previous studies that crystallization of Rif from aprotic solvents allows obtaining crystals in which this antibiotic occurs only in a non-ionic form, while crystallization from protic systems leads to obtaining an ionic form in a solid.[18] It should be emphasized here that in the structures crystallographic tests, solvent molecules are present and their presence stabilizes the appropriate forms of **Rif**, what is more, it is known that both forms of **Rif** can undergo mutual exchange transformations into themselves, among others, by transferring the compound in question, the need to transform both types of crystals into powder (for CP/MAS measurements) will not result in a change in the ionic form in relation to the initial **Rif** crystals. he analysis of the results obtained in CP/MAS spectra proved that in the case of **Ral** and **Rif** powder obtained from crystals in CH_3Cl_3, the carbonyl group C(11)=O retains its unsaturated character because the resonance frequency for this carbon atom in both ^{13}C NMR spectra was above 195 ppm (Figure 3a and b). Also, other signals from carbonyl groups in the ^{13}C CP/MAS spectra were recorded with similar chemical shift values. The data obtained from solid-state NMR experiments coincide with those obtained

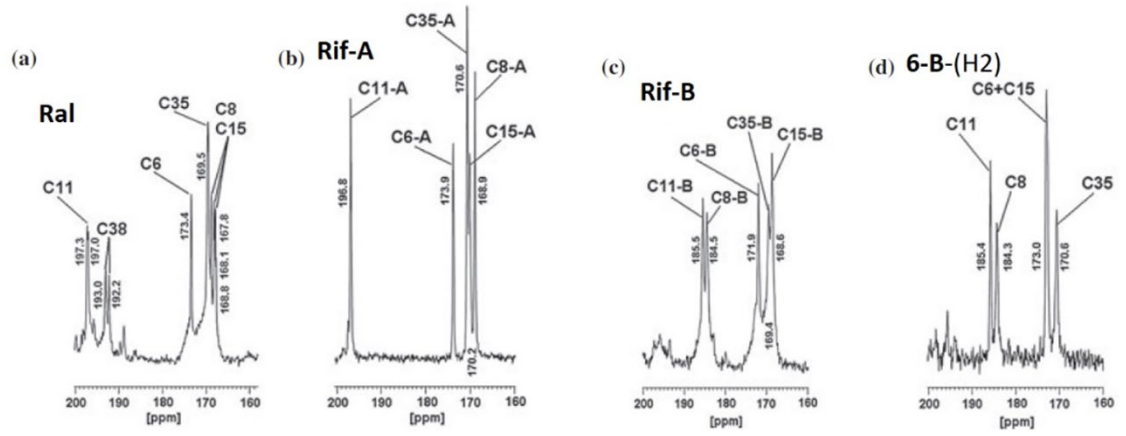


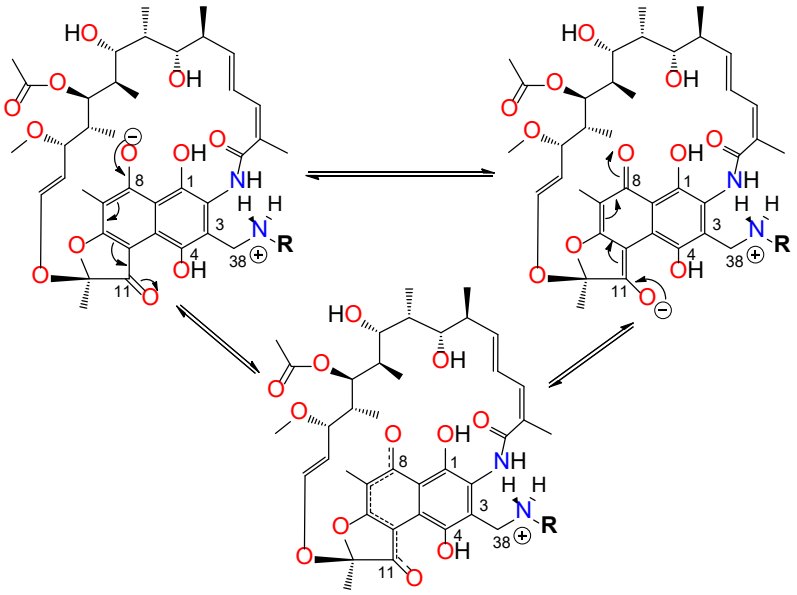
Figure 3 Comparison of fragments of 13C CP/MAS spectra for **Ral**, **Rif-A** (powder from crystals with CH_3Cl_3), **Rif-B** (powder from crystals with CH_3OH) and the **6-B** derivative.

Table 2 Chemical shift values in a solid state ^{15}N NMR for **Ral**, **Rif-A** (powder from crystals with CH_3Cl_3), **Rif-B** (powder from crystals with CH_3OH) and the derivative **6-B**.

Nitrogen atom	**Ral** CP/MAS (powder)	**Rif-A** CP/MAS (crystal **Rif** z CH_3Cl_3	**Rif-b** CP/MAS (crystal **Rif** z CH_3OH	**6**-(H2) CP/MAS (powder)	**6**-(H2) + KOH CP/MAS (powder)
N15	-250.7*	-241.4*	-248.3*	-252.2*	-257.4*
N38	---	-42.3	-32.3	-331.1*	-338.4*
N39	---	-253.4	-261.8	---	---
N43	---	-343.4	-336.1*	---	---
* Signals observed in the experiment ^{1}H-^{15}N SCT CP/MAS					

for the recorded ^{13}C NMR spectrum of **Rif** in a non-polar solvent - $CDCl_3$ (Table 1 in paper **H2**). The situation is different in the case of measurements of ^{13}C NMR spectra in a solid state for powder obtained from **Rif** crystals in methanol and amine Rif analogues and is analogous to the results observed in the ^{13}C NMR **Rif** spectrum obtained in CD_3OD solution (Table 1 in work **H2**). In these cases, there is no characteristic signal from the C(11) ketone carbon atom above 195 ppm (Figure 3c and d). In the discussed cases, this signal is more strongly shielded and visible below 190 ppm, while the resonance value for the C(8) carbon atom is higher and this is related to the revealing effect associated with the strong resonance phenomenon between the C(8)O^- phenolate group and the carbonyl C(11)O (Scheme 5). Similar differences are visible almost in the entire spectral range, which is also visible in the values of chemical shifts recorded for nitrogen atoms in the ^{15}N CP/MAS/NMR spectra (Table 2). Particularly important from the

point of view of the transfer process proton are the chemical shift values recorded for the N(43) nitrogen atom, for whose resonance value after protonation is higher by 7.3 ppm and confirms the location of a proton transferred from the phenol group O(8)H in the **Rif** ionic form on this N(43) nitrogen atom. The above statement is confirmed by an experiment performed for one of the **Ral** amine derivatives, for which, after the deprotonation of the N(38) nitrogen atom, the resonance frequency decreases by approximately 7 ppm. These differences are also perfectly visible in the FT-IR spectra, in which for ionic forms a shift of the ν(C=O) stretching band for the carbonyl group C(11)=O towards lower frequencies is observed, which is superimposed with the stretching vibration from groups C(8)=O (Fig. 3 in work **H2**). A characteristic band of δ(N+-H) deformation vibrations at approximately 1680 cm^{-1} is also visible. Therefore, it was shown that the form of **Rif** spectra recorded in a solid depends on the method of purification of this antibiotic (the method of crystallization, which is extremely important due to controlling the synthesis process of this drug and checking its purity). It is worth emphasizing the differences in the position of the absorption bands in the FT-IR spectra and the signals in the NMR spectra for both forms of **Rif**. The differences in question are so large that in the case of **Rif** and other antibiotics from this group of compounds, special attention should be paid to the possibility of these compounds occurring in many structural forms. Omission of this fact may result in incorrect analysis, for example of a preparation containing this compound, or rejection of some portion of the antibiotic due to spectroscopic differences in the solid.



Scheme 5 The phenomenon of resonance in the ionic structures of rifamycins - an example amine derivatives of **Ral**.

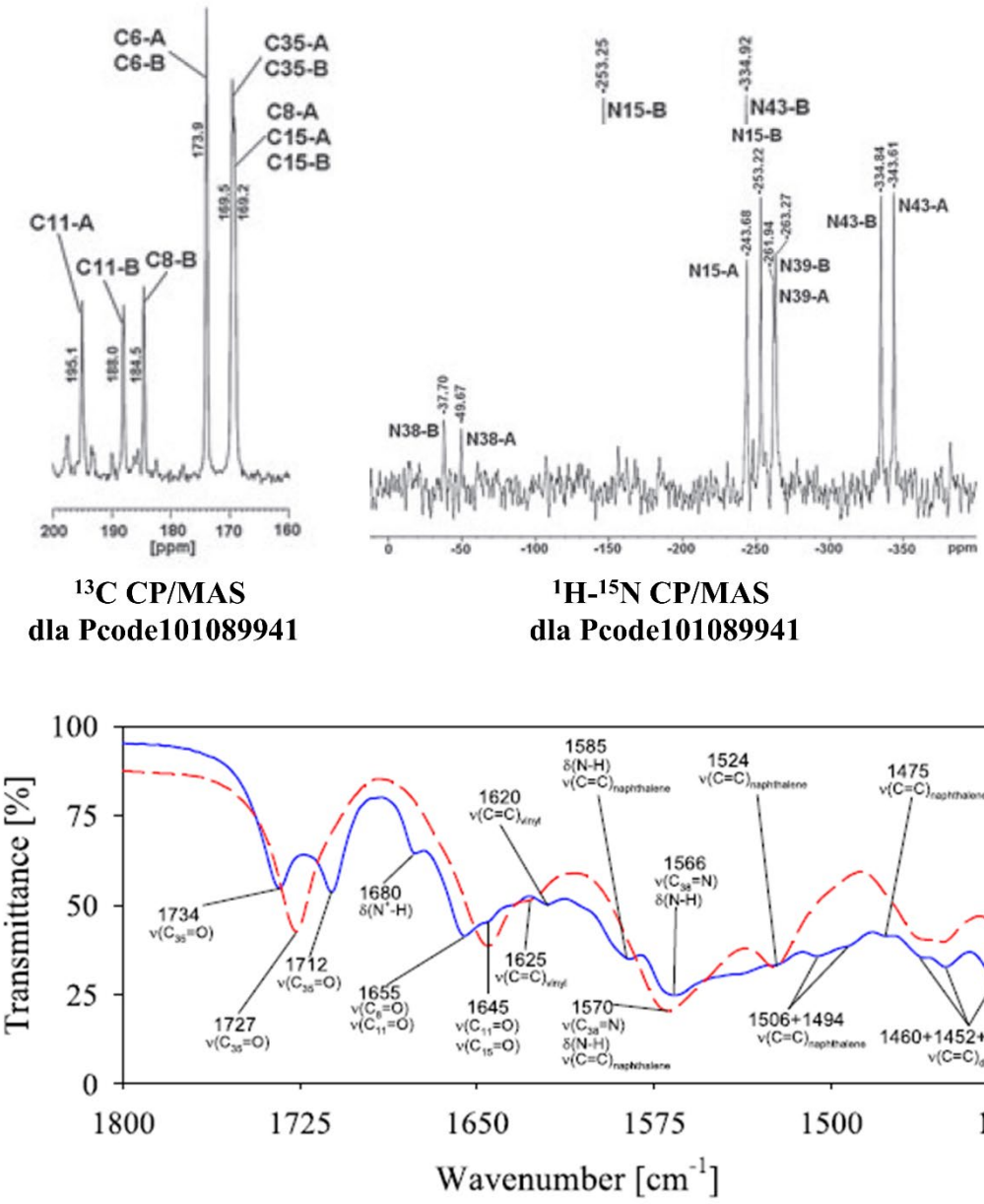


Figure 4 A fragment of the ^{13}C NMR spectrum in a solid state, ^{13}N NMR spectrum in a solid state and the FTIR spectrum recorded in KBr pellets in the range of 1400 - 1800 cm^{-1}.

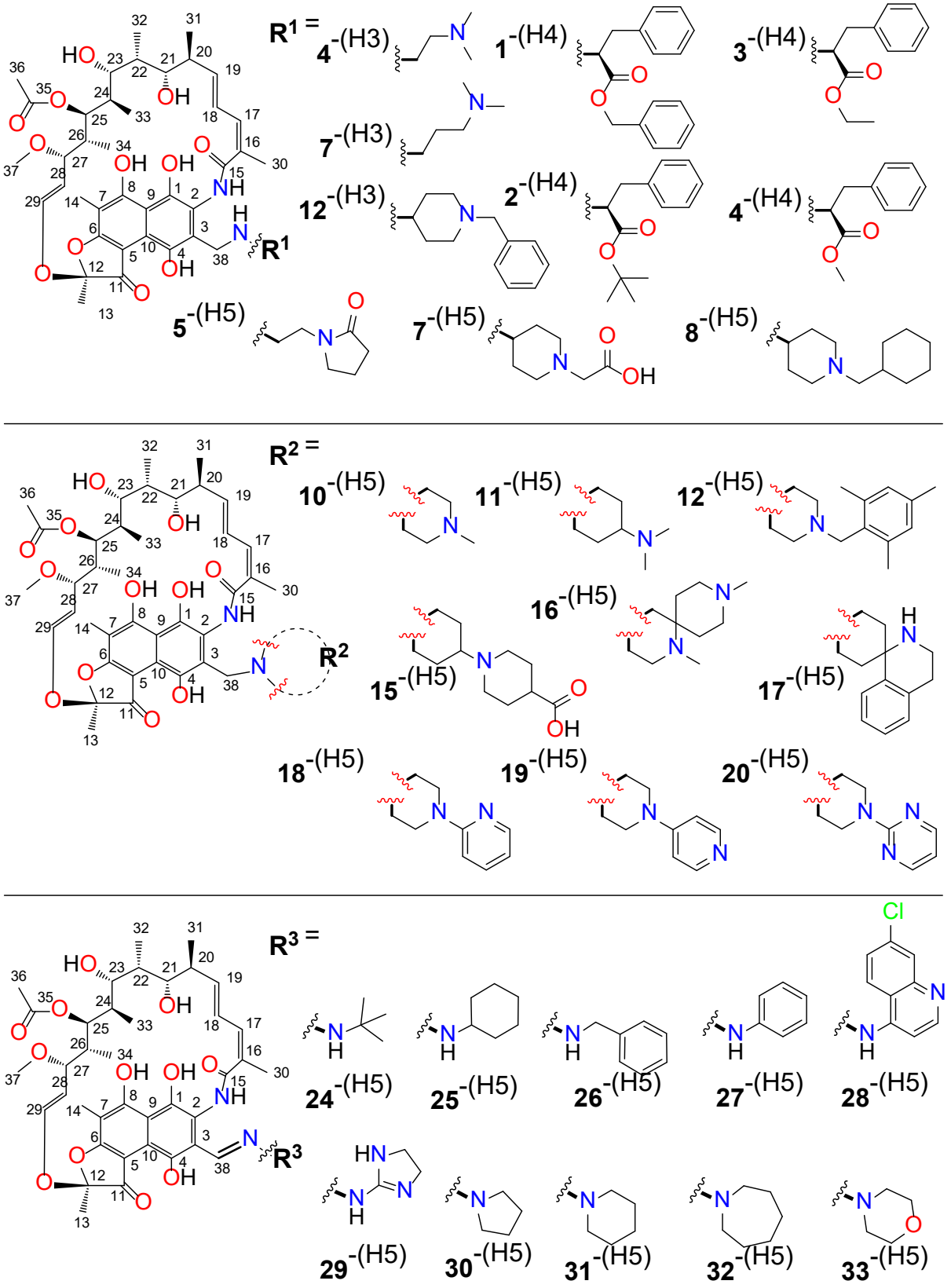
A perfect example is our observation, showing that for **Rif** purchased from one source but from different series of packaging, we recorded different spectroscopic images in the solid state. One package contained nonionic **Rif**, while another package contained a mixture of ionic and nonionic **Rif**, suggesting "contamination" (Figure 4).

4.3.2.2 Discussion of the works H3, H4 and H5

The biological activity of **Rif** is related to the unique structure of this molecule. As mentioned in the introduction, the conformation of the ansamycin bridge plays a very important

role in the interaction of this class of antibiotics with bacterial RNA polymerases. However, although the matching of the ansamycin bridge itself is a necessary condition, it is not a sufficient condition for the occurrence of high microbiological activity of rifamycins. In this case, the substituent in the C(3) position plays an equally important role, which is perfectly demonstrated by the case of **Ral**, for which significantly lower MIC values are recorded in antibacterial activity tests. In works **H3**, **H4** and **H5** new amine derivatives of **Ral** containing various functional groups were presented, such as: tertiary amines, substituted triazole systems, amino acid derivatives and new hydrazones. Based on the obtained spectroscopic results and biological tests, a discussion of the structure-activity relationships for these compounds was also presented. Not wanting to cause unnecessary confusion by adding a different numbering than in the source materials, I will leave the derivative designations in accordance with those contained in works **H3** - **H5**, adding each time when using the designation of a given compound, information about which work this compound comes from, giving the publication designation in brackets [e.g. **1**-(H3)].

It is known from previous scientific works that the high **Rif** activity is caused by the possibility of protonation of the N(43) nitrogen atom, which is carried out in the ionic form in protic solvents (possibility of interaction with the negatively charged, highly conservative E_{445} glutamate).[4,5] In order to check the influence of the substituent in position C(3) on the structure and activity of new amine analogues of **Rif**, a series of derivatives containing basic substituents on ethyl or propyl linkers **4**-(H3)–**11**-(H3), **13**-(H3) and **14**-(H3) were first synthesized and a piperidine moiety with a benzyl-substituted nitrogen atom **12**-(H3) (Figure 5, Fig. 4 in work **H3**). In work **H4**, ester derivatives of various amino acids **1**-(H4)–**12**-(H4) were obtained (Figure 5, Scheme 2 in work **H4**,). In turn, work **H5** presents several series of amine derivatives of Ral containing basic heterocyclic systems in their structure, including various amide systems **2**-(H5)–**23**-(H5) (Figure 5, Scheme 1a in work **H5**,) and **10** derivatives being **Ral 24**-(H5)–**33**-(H5) hydrazones (Figure 5, Scheme 1b in work **H5**). Based on the spectroscopic analysis of these derivatives, it turned out that almost all amine analogues of **Rif** exist in the zwitterionic form, and the proton from the phenol group O(8)H was transferred to the nitrogen atom of N(38), as evidenced by the characteristic positions of the signals from the proton/ N+(38)-H and C(8) and C(11) carbon atoms in ^{1}H and ^{13}C NMR spectra. Only for derivatives **10**-(H4)–**12**-(H4) were ionic structures observed, in which a proton was transferred to the basic center of the introduced substituent (Scheme 2 in work **H4**).



Rysunek 5 Struktury wybranych aminowych analogów **Rif** i pochodnych hydrazonowych **Ral** z prac H3-H5.

This fact was unfavorable from the point of view of potential molecular matching, such as in the case of **Rif** with RNAP (interaction with E_{445}). Apart from the protonation site, another factor that could adversely affect the biological activity of the obtained derivatives were physicochemical parameters, such as water solubility and logP values, which have better values for **Rif** compared to the tested derivatives (Table 4 in work **H3**, Table 1 in work **H4** , Table 9S in the supplement to paper **H5**). Another structural factor that had an unfavorable impact on the results of microbiological tests was the formation of intramolecular hydrogen bonds, which involved nitrogen atoms in the substituent attached to the C(3) carbon atom. These interactions were hindered by binding to RNAP polymerases, which concerned primarily the derivatives obtained in papers **H3** and **H4** (Fig. 6 in paper **H3** and Fig. 2a and b in paper **H4**). These facts, among others, meant that the vast majority of compounds of this type did not show spectacular microbiological activity compared to **Rif**, and it should be noted that the **4**-(H3) - **11**-(H3) derivatives usually showed higher activity than the substances starting point **Ral** (Table 3, Table 3 in work **H3**,). The exception in this series of derivatives was compound **12**-(H3), which in the case of Gram-positive bacteria showed activity of MIC = 0.25 μg/ml, while after additional protonation, the MIC values decreased by one order of magnitude for *S. epidermidis* bacterial strains. The **12**-(H3) derivative and its salt showed identical antimicrobial activity to **Rif** in the case of tubercle bacilli (MIC = 0.005 μg/ml), and the MIC values in the case of the *M. Bovis* strain were slightly better (MIC = 0.125 μg/ml). The high activity of the protonated compound **12+H**-(H3) results from the analogous manner of interaction of this compound with bacterial RNA polymerase as **Rif**, with the simultaneous implementation of a key interaction by this compound with E_{445} glutamate, which is perfectly visualized by the theoretical model based on molecular docking (Figure 6a, Fig .13a in work **H3**). However, a question arises here: what factor is responsible for the very interesting antibacterial activity of ester derivatives of amino acids that do not contain basic groups in their structure. In the case of these derivatives, which are amino analogues of **Rif** with esterified amino acids Phe, Trp and Tyr, a dependence related to the size of the ester substituent was visible. Smaller substituents (Me, Et) contributed to these compounds obtaining lower MIC values, i.e. higher activity, which in many cases were only one order of magnitude higher than **Rif** (Table 3, Table 1 in work **H4**). In the case of these derivatives, their interaction model with RNAP at the allosteric site is very similar to that implemented for other rifamycins. The key difference is the possibility of forming an intermolecular hydrogen bond between the carbonyl ester group of the amino substituent in the C(3) position and the peptide NH group of alanine A_{447} (Figure 6b, Fig. 3 in work **H4**). This

Table 3 Selected test results for the antibacterial activity of **Rif**, **Ral** and selected **Ral** derivatives. MIC values are given in μg/ml. For **Rif** and **Ral**, the publication from which the MIC value was taken is given in the superscript.

Compound	Bacteria strain					
	S. aureus ATCC 4163	*S. aureus* ATCC 6538	*S. epidermidis* ATCC 12228	*S. epidermidis* ATCC 49134	*M tuberculosis* H37Rv	*M. bovis*
Ral	2^{H4}	4^{H5}	4^{H5}	2^{H5}	$>1^{H3}$	$>1^{H3}$
Rif	0.008^{H3}	0.0156^{H5}	0.0156^{H5}	0.0156^{H5}	0.005^{H3}	0.25^{H3}
4-(H3)	1	1	0.5	0.5	1	1
12-(H3)	025	0.25	0.25	0.25	0.005	0.125
12-(H3) + H^+	0.125	0.125	0.06	0.03	0.005	0.125
20-(H3)	1	1	0.5	0.5	0.5	0.5
31-(H3)	1	>1	>1	>1	0.5	0.5
1-(H4)	0.5	0.5	0.125	0.25	-	-
2-(H4)	0.5	0.5	0.125	0.25	-	-
3-(H4)	0.063	0.0125	0.063	0.063	-	-
4-(H4)	0.063	0.063	0.031	0.031	-	-
6-(H4)	0.5	0.25	0.063	0.063	-	-
7-(H4)	0.031	0.031	0.016	0.016	-	-
5-(H5)	-	2	2	1	-	-
7-(H5)	-	0.25	0.0625	0.0625	-	-
11-(H5)	-	0.25	0.125	0.125	-	-
15-(H5)	-	0.0078	0.0078	0.0078	-	-
16-(H5)	-	0.25	0.0625	0.0625	-	-
29-(H5)	-	0.0078	0.0078	0.0078	-	-
30-(H5)	-	0.25	0.25	0.25	-	-
31-(H5)	-	0.0156	0.0156	0.03125	-	-
32-(H5)	-	0.0625	0.03125	0.03125	-	-

additional interaction stabilizing the amino acid derivatives generates a steric hindrance between the ester substituent and the methyl group of A_{447}. Taking into account this result of molecular docking, the SAR relationship seemed justified - why smaller substituents are

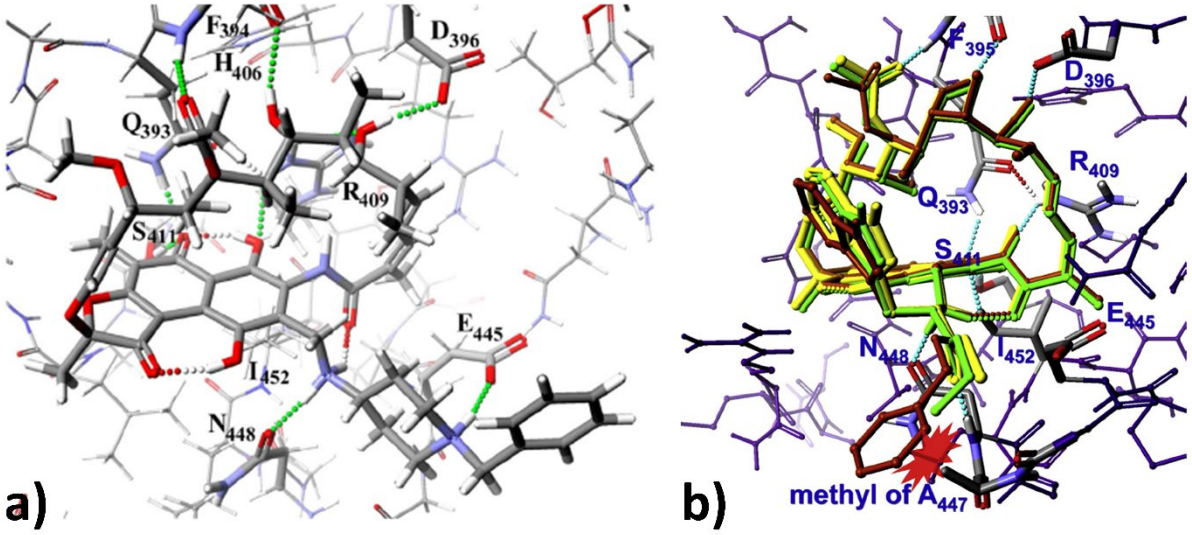


Figure 6 a) the most energetically favorable intermolecular interactions between the key amino acids RNAP and **12+H**$^{+}$-(H3)^{H3}; b) Superposition of docking models for new rifamycins containing L-Trp methyl (**7**-yellow), L-Trp ethyl (**6**-green) and L-Trp benzyl (**5**-brown) esters at the binding site of DNA dependent RNA polymerase of Thermus aquaticus (RNAP-violet).^{H4}

preferred in the structure of this type of derivatives in the context of higher microbiological activity.

In the case of all **Rif** amine analogues, they occur in the zwitterion form, and the vast majority of them undergo protonation of the N(38) nitrogen atom to form a phenolate in the O(8) position. In the case of derivatives obtained in work **H5**, two derivatives **7**-(H5) and **15**-(H5) deserve special attention (Figure 5). These compounds have one common feature, which is the presence in their structure of a carboxyl group with a rigid substituent, which, due to its acidic nature, affects the structure of these derivatives - these are "double" zwitterions. In addition to the N(38) nitrogen atom, the introduced basic centers in the substituents are also protonated. Such a double zwitterion structure results in a very large increase in antibacterial activity compared to other **Ral** derivatives (Table 3, Table 2 in work **H5**), which is the result of the perfect molecular match of bacterial RNA polymerase inhibitors. In both cases, a stabilizing bond with E_{445} (RNAP) can be implemented, and in the case of the **15**-(H5) derivative, the presence of the carboxylate group allows the formation of an additional hydrogen interaction with the NH group of the N_{448} asparagine side chain (Figure 7a and b, Fig. 3a and b in work **H5**). Comparing the MIC values for **15**-(H5) it was clearly visible that a significant improvement in solubility at the expense of lipophilicity and improved stabilization of the antibiotic in the binding site with RNAP results in an increase in antibacterial activity (MIC = 0.0078 μg/ml), even compared to the results obtained for **Rif**. In the case of the **7**-(H5) derivative, promising results were obtained for *S. epidemidis* strains (MIC = 0.0625 μg/ml) - similar results were obtained for the **16**-(H5) derivative, for which the theoretical binding model

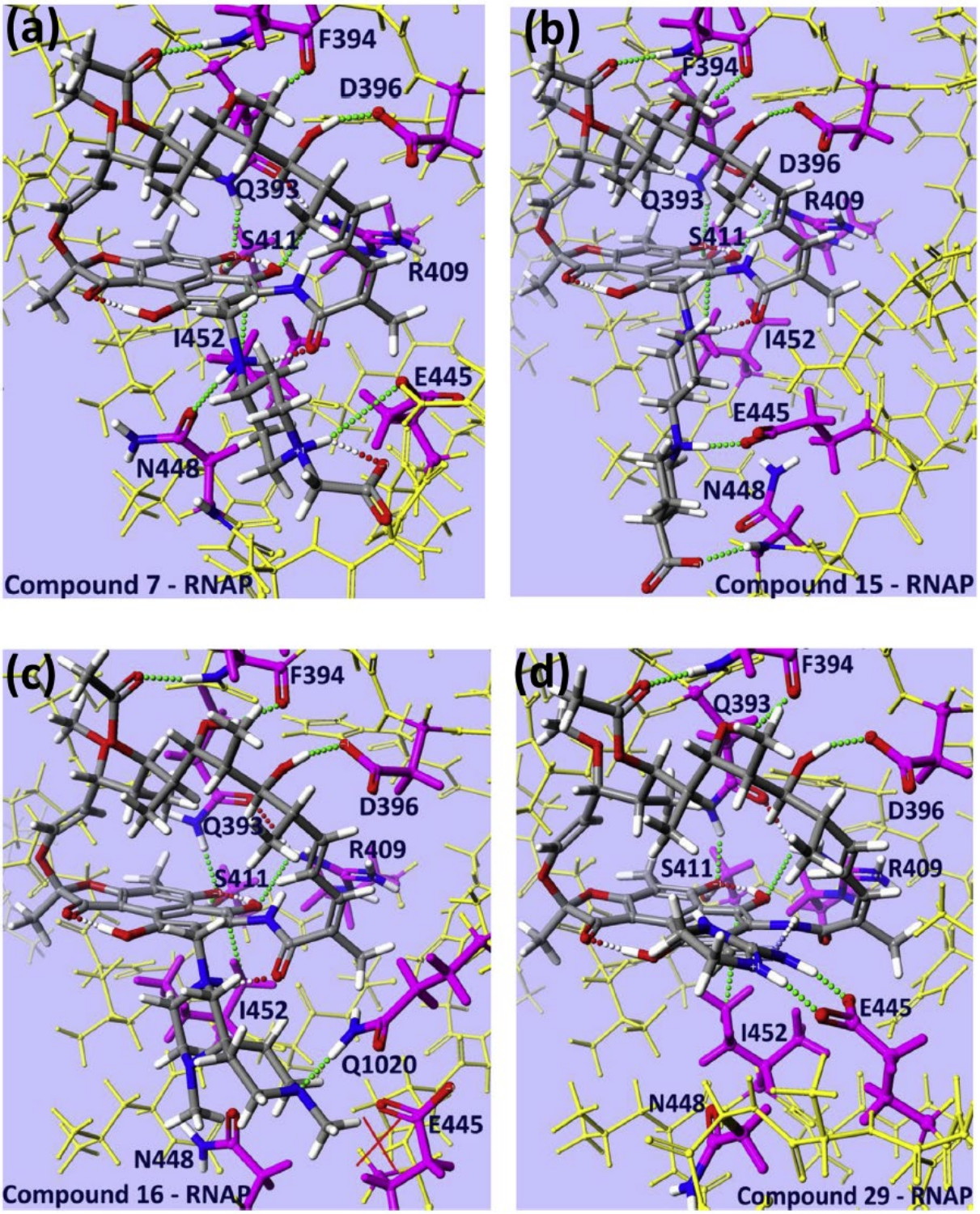


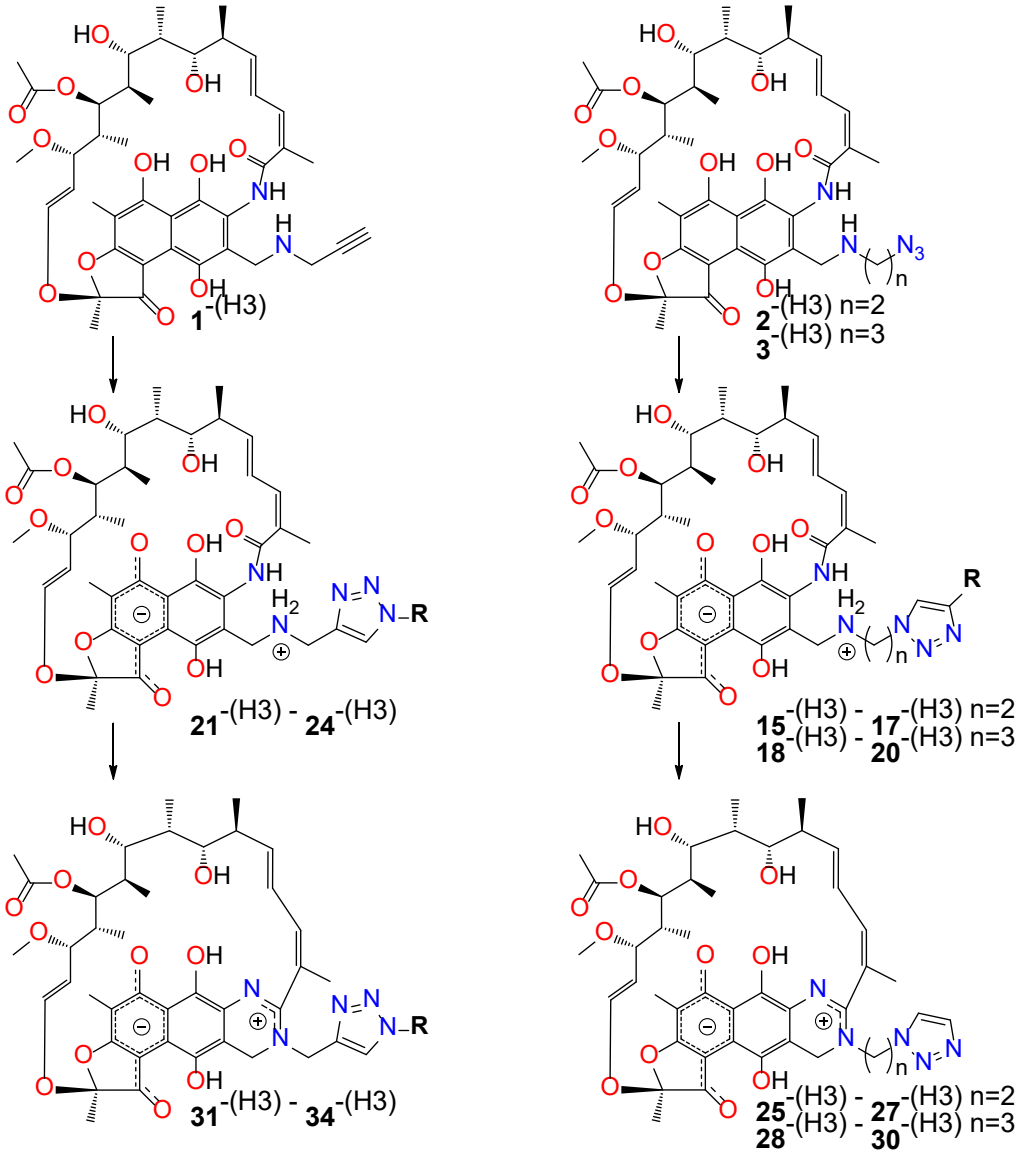
Figure 7 Docking models for new rifamycin congeners (grey): **7** (a), **15** (b), **16** (c) and **29** (d) at the binding site of RNAP from *T. aquaticus*[19].[H5]

in the RNAP binding site shows an additional stabilization through hydrogen interaction with glutamine Q_{1020} (Figure 7c, Figure 3c in work **H5**).

Among the obtained **Ral** hydrazones (Figure 5, Scheme 1 in work **H5**), two of them existed in solution in the ionic form **28**-(H5) and **29**-(H5), and this was due to the fact that only

these derivatives contained in their structure a basic moiety capable of protonation. The most interesting observations were provided by the analysis of the antibacterial activity results, because among the tested **24**-(H5)–**33**-(H5) hydrazones, only 3 compounds were distinctive (Table 3, Table 2 in work **H5**). Two of them, being isostructural with **Rif** and containing cyclic systems: piperidine **31**-(H5) and azepane **32**-(H5), were characterized by activity comparable to that obtained for rifaximin (**Rifx**) and only slightly weaker than **Rif**. In turn, compound **29**-(**H5**) was as active as compound **15**-(H5), which was explained by its well-balanced physicochemical properties (water solubility 4.25 mg/ml and logP = 1.33) and favorable binding model with RNAP, in which the highly conserved glutamate E_{445} is involved (Figure 7d, Fig. 3 in work **H5**).

From the point of view of an organic chemist, it was interesting to check the stability of **Rif** amine analogues in a situation where these compounds will constitute a platform for further development. In publication **H3**, a series of triazole derivatives **15**-(H3)–**24**-(H3) was obtained (Scheme 6, Fig. 1 in publication **H3** and Fig. 3 in publication **H3**), which were synthesized using appropriately functionalized amine analogues of **Rif** having azide or alkyne groups. The synthetic method was selected on the basis of Huisgen's 1,3-dipolar cycloaddition (CuAAc) reaction tests, in which various catalyst equivalents (CuOAc) were used in the temperature range 0° - 60°C. The obtained results showed that the best results (% substrate conversion and reaction time) were achieved using 10% of the catalyst and a temperature of 60°C (Table 2 in work **H3**). For volatile alkyne reagents that were used to synthesize **15**-(H3)–**20**-(H3) derivatives, the best solution was to lower the temperature to 22°C and extend the reaction time. However, too long exposure of **Rif** amine analogues to the reaction environment over time unexpectedly led to the obtainment of new heterocyclic derivatives containing the 3,4-dihydrobenzo[g]quinazoline system **25**-(H3)–**34**-(H3) (Scheme 6, Fig. 1 in the work **H3** and Fig 3 in work **H3**). However, the antibacterial activity results for all derivatives containing the **15**-(H3)–**34**-(H3) triazole moiety were not very attractive compared to **Rif** (Table 3 in **H3**).

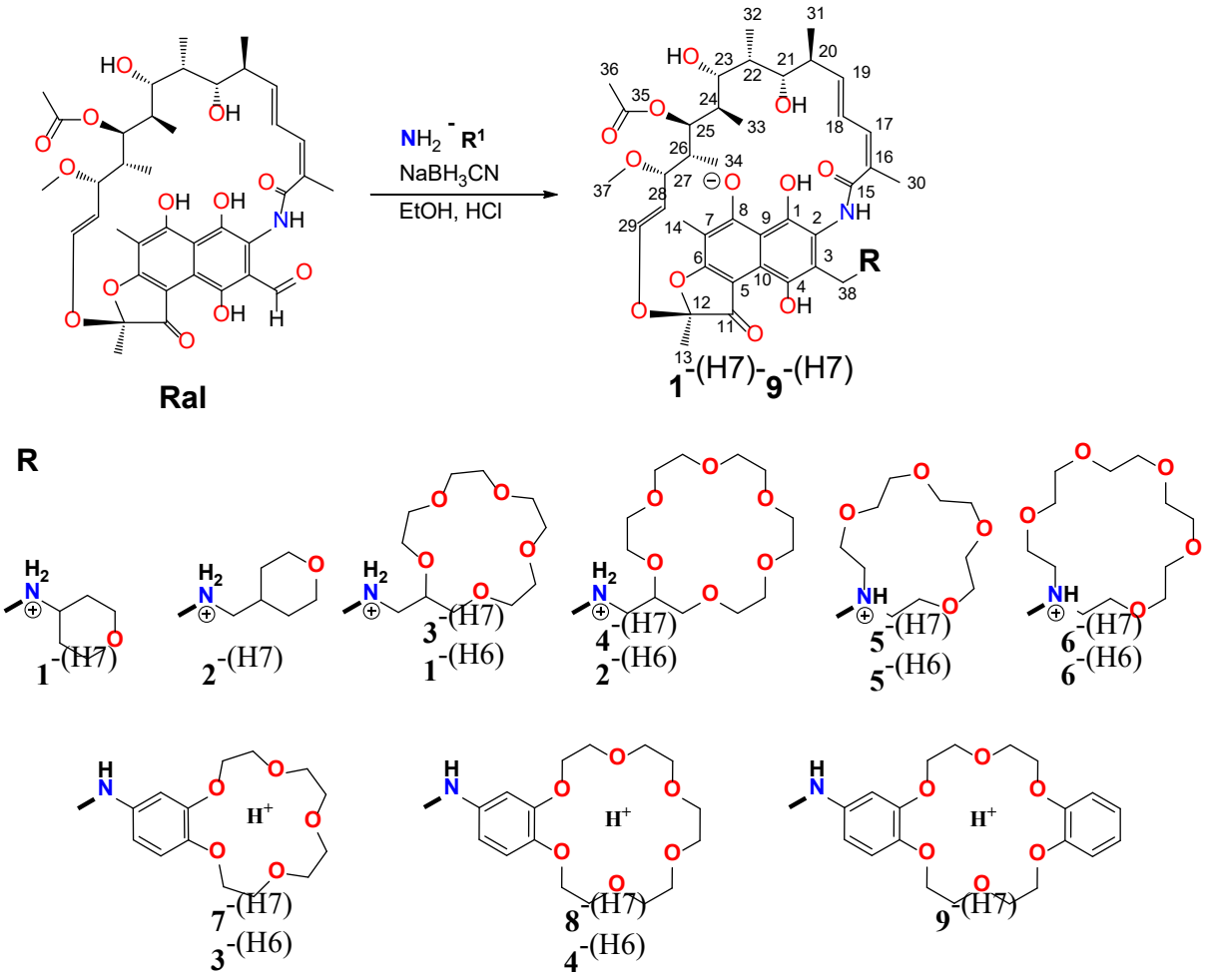


Scheme 6 Scheme of the synthesis of triazole amine derivatives of **Rif** analogues **15**-H(3) – **24**-(H3) and their heterocyclic derivatives **25**-H(3) – **34**-(H3).

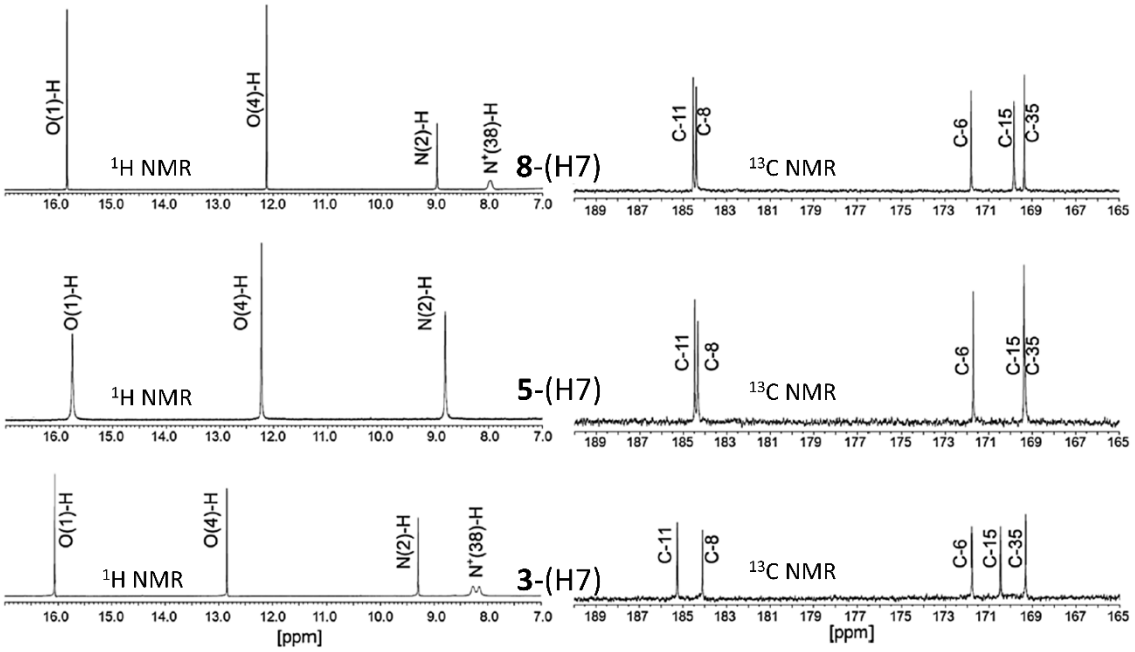
4.3.2.3 Discussion of the works H6 and H7

SAR studies for the role of a substituent in the C(3) position of **Rif** amino analogues were complemented by the preparation of derivatives containing crown ethers in their structure (Scheme 7, Fig. 1 in paper **H6** and Fig. 1 in paper **H7**). In the case of aminomethylcrown ethers **3**-(H7) and **4**-(H7) and azacrown systems **5**-(H7) and **6**-(H7), the spectroscopic characteristic did not differ from that observed for the remaining amine derivatives of **Ral 1**-(H7) and **2**-(H7), and therefore they existed in an ionic form with a protonated nitrogen atom N(38) (Figure 8, Fig. 2 in work **H6** and Fig. 2a in work **H7**). The derivatives **7**-(H7)–**9**-(H7) differed in that a

4'-aminobenzocrown was used to obtain them. The use of such an aminobenzocrown system was associated with reduced basicity of the nitrogen atom in the context of the analyzed process of proton transfer from the phenol group O(8)-H. This assumption was confirmed by the lack of an appropriate signal in their ^{1}H NMR spectra from the N^{+}(38)H proton (Figure 8, Fig. 1 in paper **H6** and Fig. 1 in paper **H7**). However, data collected from ^{13}C NMR spectra indicated that the phenolic O(8)-H group was deprotonated, but there was no signal in the proton spectra to confirm its location. Solving this puzzle was possible thanks to the use of FT-IR measurements with the addition of water molecules (Figure 9, Fig. 3 and 5 in work **H6** and Fig. 3 in work **H7**). Absorption continua appear in these spectra, which indicate the presence of H_3O^{+} cations or Zundel $H_5O_2^{+}$ cations in the **7**-(H7)–**9**-(H7) structures.[29,30] Therefore, the O(8)H proton transfer was carried out to the benzocrown ether cavity for discussed derivatives (Scheme 7, Fig. 1 in work **H6** and Fig. 1 in work **H7**).



Scheme 7 Method of synthesis and structure of new amine derivatives of **RaI** derivatives containing corona systems in their structure. In the case of derivatives shown in work **H6**, additional notation was used under the numbering used in the work **H7**.



Fugure 8 Comparison of fragments of ^{1}H and ^{13}C NMR spectra for selected derivatives from papers **H6** and **H7**.

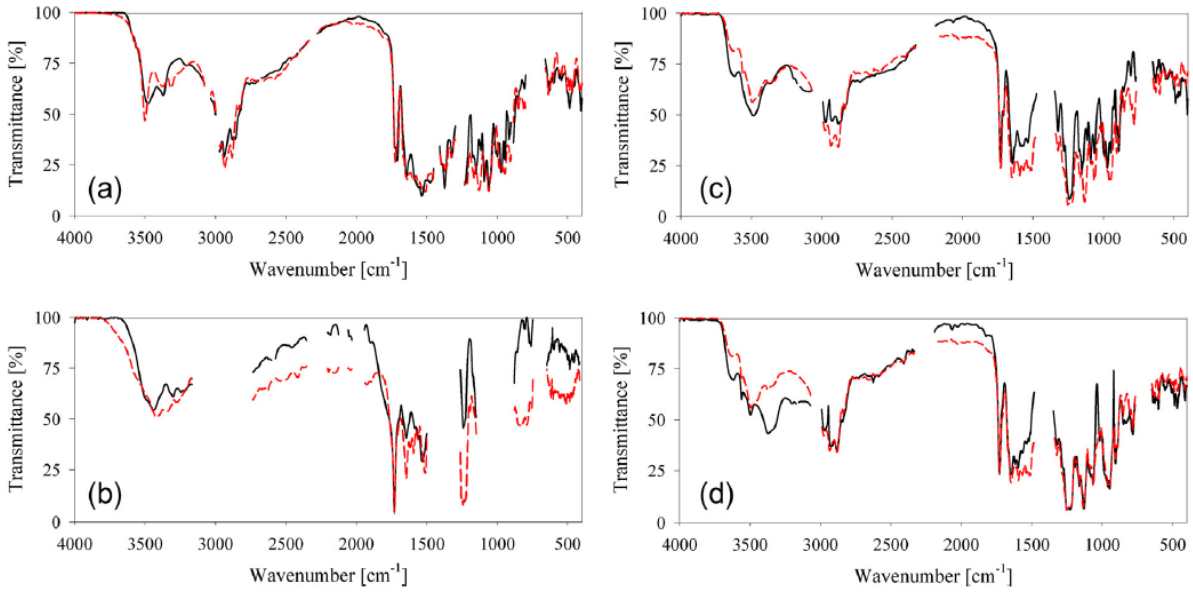


Figure 9 Comparison of FT-IR spectra of: (a) **1**-(7) (black solid) and **9**-(7) (dashed) in CH_2Cl_2; (b) **1**-(7) (black solid) and **9**-(7) (dashed) in DMSO; (c) **1**-(7) (black solid) and **9**-(7) (dashed) in CH_3CN; (d) **9**-(7) (dashed) and **9**+histamine-(7) (black solid) in CH_3CN.

Antibacterial activity tests were performed for derivatives **1**-(H7)–**9**-(H7) (Table 2 in work **H7**), which included complexes with lithium or potassium for derivatives **3**-(H7) and **4**-(H7) and complexes benzocorona systems **7**-(H7)–**9**-(H7) with selected amines. The analysis of the obtained results showed that the transformation of compounds with ordinary aminomethyl crown systems **3**-(H7) and **4**-(H7) into their complexes with Li+ and Na+ cations increases or maintains the MIC values (Table 2 in work **H7**). However, the situation was similar for **7**-(H7)–**9**-(H7) derivatives, for which the greatest gain in the increase in antibacterial activity was

observed for complexes with histamine - a reduction in the MIC value of up to 8-fold (Table 2 in work **H7**). Complexes **7**-(H7)–**9**-(H7) with 2ClBzA and 4FbzA turned out to be only slightly weaker than those with histamine in relation to the tested strains of Gram-positive bacteria. However, the smallest increase in activity in relation to the initial derivatives was recorded for complexes with isoniazid, which was most likely related to the lowest stability of complexes of compounds **7**-(H7)–**9**-(H7) with the tested amines. The results of molecular modeling of the obtained derivatives indicated that in the case of benzocrown ethers, the presence of a positively charged molecular entity in the corona system (e.g. a metal cation) may additionally stabilize this type of compounds in the binding site with RNAP (Figure 10, Fig. 4 in paper **H7**).

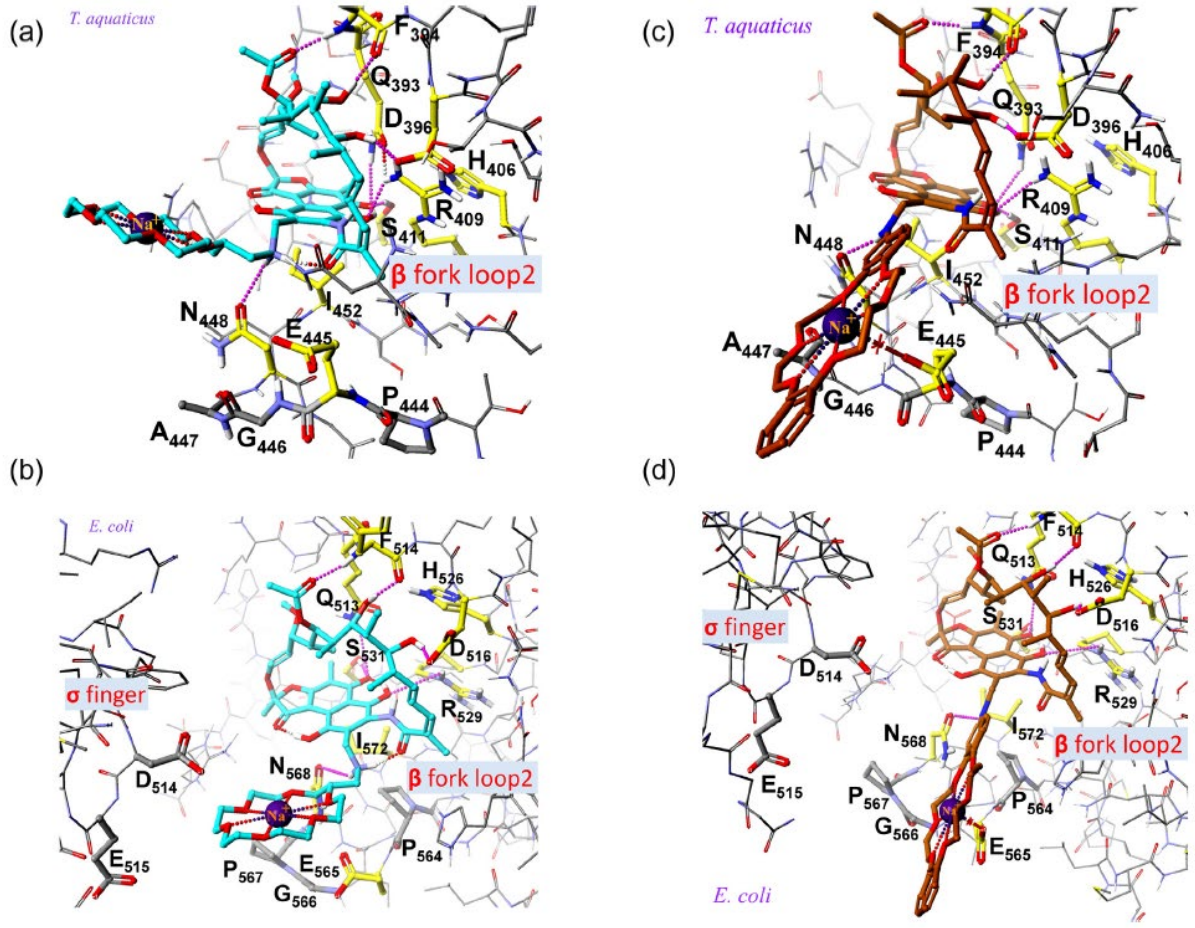


Figure 10 Docking models of new rifamycins: **4**-Na+ (a and b) and **9**-Na+ (c and d) at the binding sites of *Thermus aquaticus* and *Escherichia coli* DNA dependent RNA polymerases (RNAP)[H7]

4.3.2.4 Discussion of the work H8

Rifamycins, as antibiotics, are characterized by a very broad spectrum of antibacterial activity, especially in the case of G+ bacteria and Mycobacteria.[17,23] In the case of G– bacteria,

however, the situation is slightly more diverse. **RMP** and rifapentine (**RFP**) do not have as high activity against G− bacteria as e.g. rifabutin (**RFB**) or rifaximin (**RFX**) (Figure 11, Fig. 1 in work **H8**).[17] Information could be found in the literature that this difference may be related to the ability of **RFB** and **RFX** to more easily penetrate the bacterial cell wall and the cell membrane system of G− bacteria.[23,31] It was, of course, expected that such a difference would be related to differences in the structure of the walls and cell membranes of G+ and G− bacteria.[32,33] Bacterial RNA polymerases, regardless of whether they come from G+ or G− bacteria, have a very similar structure and bind to rifamycin antibiotics in the same way. This fact only further suggested that one of the reasons for the observed difference in activity between these antibiotics may be the way they reach the interior of individual bacterial cells. In the work **H8**, the dynamic conformational equilibrium of the ansamycin bridge in solution was experimentally proven for the first time for **RFB** and **RFX**, as opposed to **RMP** and **RFP**, facilitating the understanding of the larger spectrum of antibacterial activity for **RFB** and **RFX** (both G+ and G−) compared to **RMP** and **RFP** (G+ only).

In the ^{13}C NMR and ^{1}H NMR spectra recorded in different $CDCl_3$ or DMSO-d_6+H_2O, both similarities and differences between **RMP**, **RFP**, **RFB** and **RFX** were visible (Figure 12, Fig. 2 in work **H8**). In the case of the spectra measured in $CDCl_3$ for **RFP** and **RFB**, signals above 190 ppm from the C(11) carbon atom and below 170 ppm for the C(8) carbon atom and a signal from the proton of the phenolic group O(8)H were visible, a similar situation occurred for **RMP**, therefore these three antibiotics are in the non-ionic form in the aprotic solvent. In the NMR **RFB** spectra measured in DMSO-d_6+H_2O, similar relationships could be observed as in $CDCl_3$, so in the case of this antibiotic there was no intramolecular proton transfer from the phenolic group O(8)H to the basic substituent in the C(3)/C position (4) and **RFB**, regardless of the type of solvent, existed in a non-ionic form. The opposite is the case with **RFX**, both in protic and non-protic systems, in the ^{13}C NMR spectra there were signals from C(11) and C(8) carbon atoms between 190 and 180 ppm and in the ^{1}H NMR spectrum there was no corresponding signal from the phenol group O(8)-H (Figure 12, Fig. 2 in work **H8**), so as in the case of **RMP** and **RFP** in ionic form. This proves that the **RFX** molecule in solution exists only in the ionic form with a proton located on the amidine system "connecting" the C(3) and C(4) positions. The next step was to examine the conformation of the asnamycin bridge for **RMP**, **RFP**, **RFB** and **RFX** depending on the polarity of the solvent. For **RMP** and **RFP**, the conformation of the ansamycin bridge was almost identical and independent of the solvent - for this reason, the structure was classified as a type-A conformer.

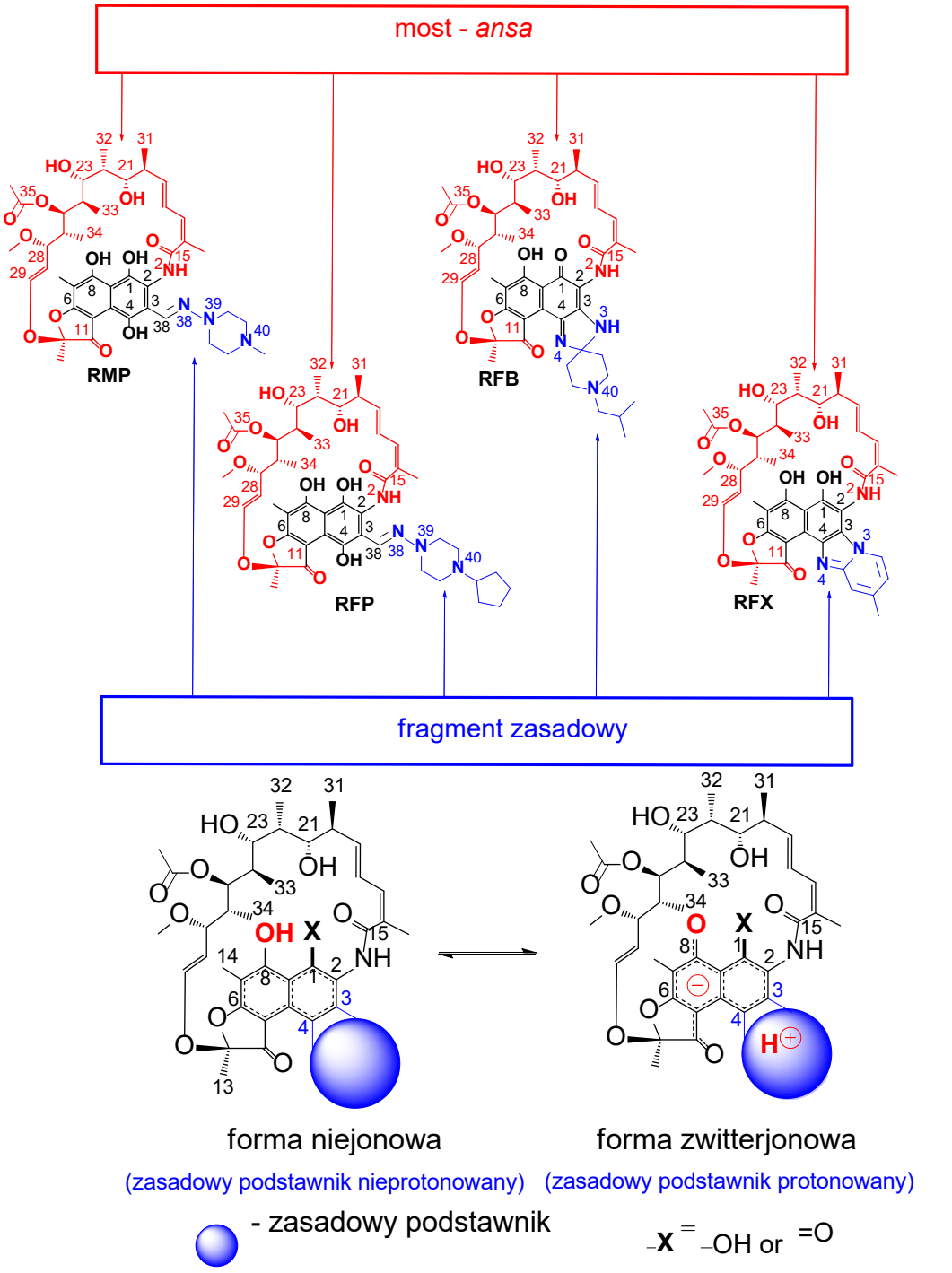
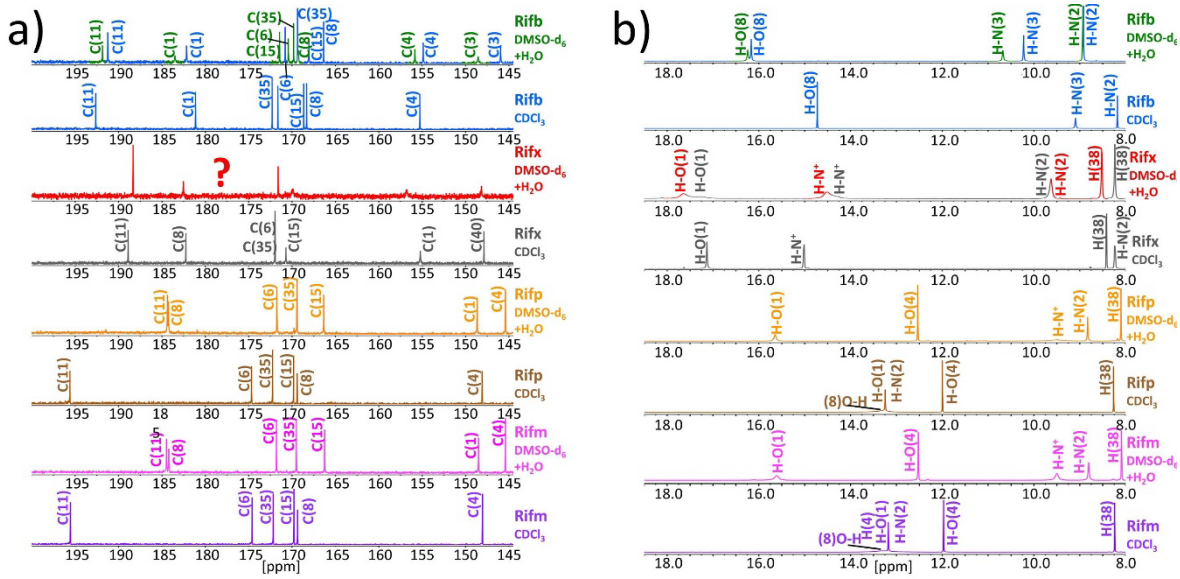


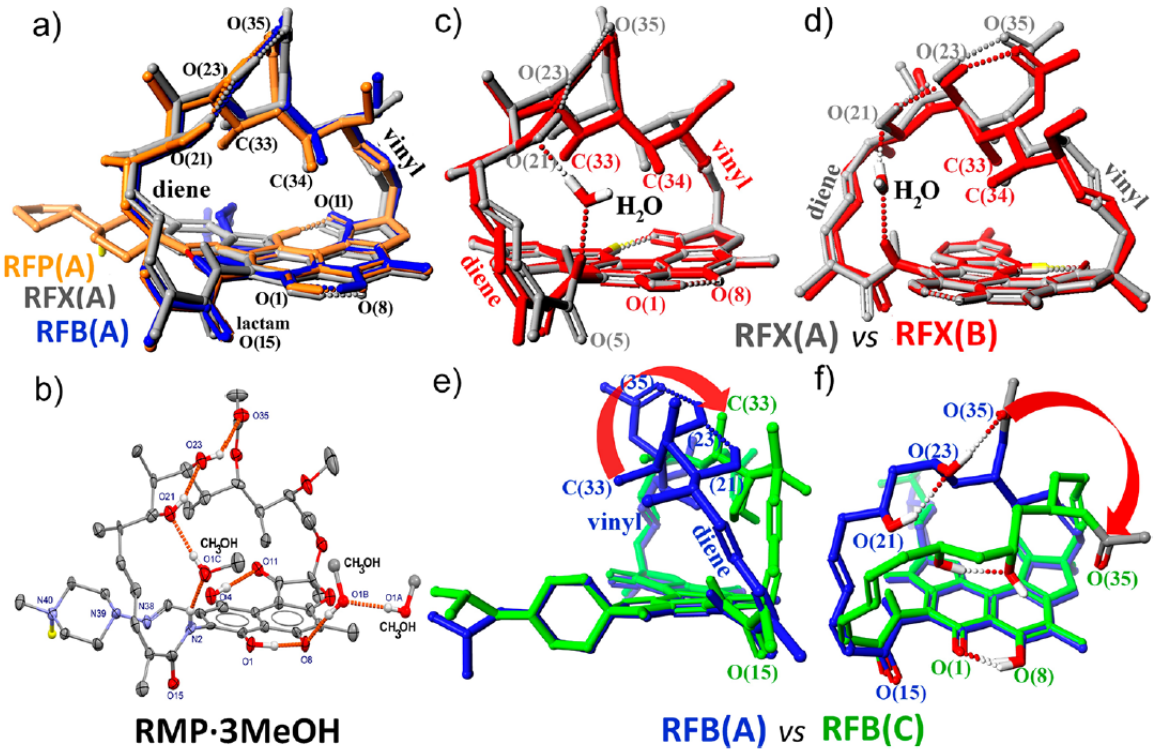
Figure 11 Structural formula of clinically used rifamycin-type antibiotics and their nonionic and zwitterionic forms illustrated at the bottom.[H8]



Rysunek 12 (a) Comparison of ^{13}C NMR spectra in the range of 145–200 ppm of **RMP**, **RFP**, **RFX**, and **RFB** measured in $CDCl_3$ and $DMSOd_6$+H_2O at room temperature: blue, used to show signals for **RFB**(A); green, used to show signals for **RFB**(C), gray, used to show signals for **RFX**(A); red, used to show signals for **RFX**(B). (b) Comparison of ^{1}H NMR spectra in the range of 8.0–19.0 ppm of **RMP**, **RFP**, **RFX**, and **RFB** measured in $CDCl_3$ and DMSO-d_6+H_2O at room temperature: blue, used to show signals for **RFB**(A); green, used to show signals for **RFB**(C); gray, used to show signals for **RFX**(A); red used to show signals for **RFX**(B).[H8]

The analysis of **RFX** spectra in various solvents showed that in $CDCl_3$ this compound also occurs in the type-A conformation [**RFX**(A)] (Figure 13a, Fig. 4a in work **H8**). In turn, the spectra of this antibiotic in DMSO-d_6+H_2O were difficult to interpret and for this reason a series of measurements were performed in a temperature gradient including the additional solvent system CD_3CN+H_2O (Fig. 2 c and d in work **H8**). In measurements at -30°C in systems with H_2O, it turned out that **RFX** is present in equilibrium in the form of two conformers: **RFX**(A) and a new type of conformer **RFX**(B). Comparative analysis of these two conformers showed that the greatest differences are observed for the diene system, vinyl system and methyl groups C(33) and C(34). At this point, the question arises as to what is the driving force behind the creation of the **RFX**(B) structure. FT-IR measurements came to the rescue and were performed in aprotic systems and in DMSO-d_6+H_2O (Fig. 3c in paper **H8**). The most important difference is the visible absorption continuum for the protic system, which proves that the water molecule is responsible for stabilizing this form. The newly developed crystallographic structure of RMP with methanol shed light on how the water molecule stabilizes the **RFX**(B) conformer (Figure 13b, Fig. 4b in work **H8**). In this structure, there was a collective system of hydrogen bonds that connected the N(2)-H group with the acetyl group by including the O(21)H hydroxyl group and a methanol molecule into the system. In order to build a theoretical model, the methanol molecule from this crystallographic structure was replaced with a water molecule, which resulted in obtaining an arrangement of the ansamycin bridge in the calculations, which

correlated very well with the data obtained in the NMR spectra for the **RFX**(B) conformer (Figure 13 c and d, Fig. 4 c and d). Based on the NMR temperature spectra, the energy barrier for the transformation of one conformer into another was determined and was approximately 0.5 kcal/mol. Such a small energy barrier for the conformation change indicates that this method of arranging the ansamycin bridge is also possible to form in solution for other rifamycins, but only in the case of compounds that cannot form an intramolecular N(2)H•••X hydrogen bond in solution (group in position C(3) (e.g. for RFP N(2)-H•••N(38).



Rysunek 13 (a) Structures of **RFP**(A), **RFX**(A), and **RFB**(A) calculated by the B88 LYP (GGA) DFT method. (b) X-ray structure of the **RMP**· 3MeOH solvate. (c) DFT calculated and superimposed structures of **RFX**(A) and **RFX**(B) from the lactam-diene side. (d) DFT calculated and superimposed structures of **RFX**(A) and **RFX**(B) viewed through the cavity between the ansa-bridge and the aromatic core. (e) DFT calculated and superimposed structures of **RFB**(A) and **RFB**(C) from the lactam-diene side; the movement of the bridge is marked by the red arrow. (f) DFT calculated and superimposed structures of **RFB**(A) and **RFB**(C) viewed through the cavity between the ansa-bridge and the aromatic core where all peripheral groups of the ansa-bridge are omitted for clarity; the movement of the bridge is marked by the red arrow.

In the spectra recorded for **RFB** in CDCl3 (Figure 12, Fig. 2 in **H8**), there was one form that was coincident with **RFP**(A) and **RFX**(A), so it was named **RFB**(A) (Figure 13a, Fig. 4a in work **H8**). Type A and B conformers had an "open" structure in which the hydroxyl groups from the ansamycin bridge O(21)H and O(23)H were directed "outward" - therefore these conformers have the ability to bind to RNAP (Figure 14c, Fig .5c in work **H8**). Two sets of signals were visible in the **RFB** spectrum in DMSO-d_6+H_2O at room temperature (Figure 12, Fig. 2 in work **H8**). One of them corresponded to the **RFB**(A) conformer, while the other one

was completely different and was assigned to the **RFB**(C) conformer. Analyzing chemical shifts in ^{1}H and ^{13}C NMR spectra, differences could be seen in virtually every part of the ansamycin bridge. The difference in the position of the signal from the H(34) proton for **RFB**(A) ($\delta = -0.24$ ppm) and **RFB**(C) ($\delta = 0.90$ ppm) indicated that the C(34) methyl group for **RFB**(C) is located away from the aromatic core. Moreover, signals from H17−H19 protons indicated a different orientation of this fragment in **RFB**(C) and **RFB**(A). For **RFB**(C), the signal from the C(15) carbonyl carbon atom was exposed and shifted by 5 ppm compared to **RFB**(A), indicating a weaker or complete loss of coupling between the diene system and the lactam in **RFB**(C). The analysis of contacts in the ^{1}H-^{1}H NOESY spectra (Fig. 3e in work **H8**) clearly confirmed that the **RFB**(C) conformation takes a closed form, because only hiding the aliphatic hydroxyl groups "inside" allows the theoretical model to be consistent with the experimental data (Figure 14b , Fig. 5b in work **H8**). Once again, the question arose as to how such a structure is stabilized. A partial answer to this question was provided by the type-B conformation, in which a water molecule is present. However, one such molecule is not enough to stabilize the **RFB**(C) structure, which was confirmed by the results obtained from the analysis of FT-IR spectra, in which the maximum absorption continuum was achieved for two water molecules (Fig. 3f and g in paper **H8**). With such information at our disposal, it was possible to build a theoretical model that allowed us to visualize the structure of **RFB**(C), showing that the role of the first water molecule is similar to that in the type-B conformer (Figure 13e, Figure 14b, Figures 4e and 5b in work **H8**). However, the second water molecule is necessary to maintain the collective system of hydrogen bonds between the N(2)H group and the carbonyl oxygen atom O(35), connecting the O(23)H group with the acetyl group. Additionally, such location of the second water molecule in this structure allows the extension/forking of the collective hydrogen bond system with the phenol group O(8)H and the quinone oxygen atom O(1). The barrier value of the transformation from **RFB**(A) to **RFB**(C) determined on the basis of NMR measurements is between 3.5 and 6.5 kcal/mol and this value depends on the solvent. It is worth adding here that the ratio of these two conformers is strictly temperature-dependent and at 65°C the A form predominates (1:0.38), while at -25°C the C form predominates (2.28:1) (Fig. 3i in work **H8**). As in the case of the type-B conformer for **RFX**, the type-C conformer can be implemented by other rifamycins (without the presence of the intramolecular hydrogen bond N(2)H•••X (group in the C(3) position) in the structure, which was presented in the theoretical model for **RFX** (Fig. 5f in work **H8**). From the point of view of better activity of **RFB** and **RFX** in relation to Gram-negative bacteria, the possibility

of adopting a closed conformation shows that these antibiotics can smoothly change their structure, affecting the lipophilic- hydrophobic and thus penetrate the interior of not only Gram-positive but also Gram-negative bacteria, unlike **RMP** and **RFP** that are active only against Gram-positive strains (Figure 14b).

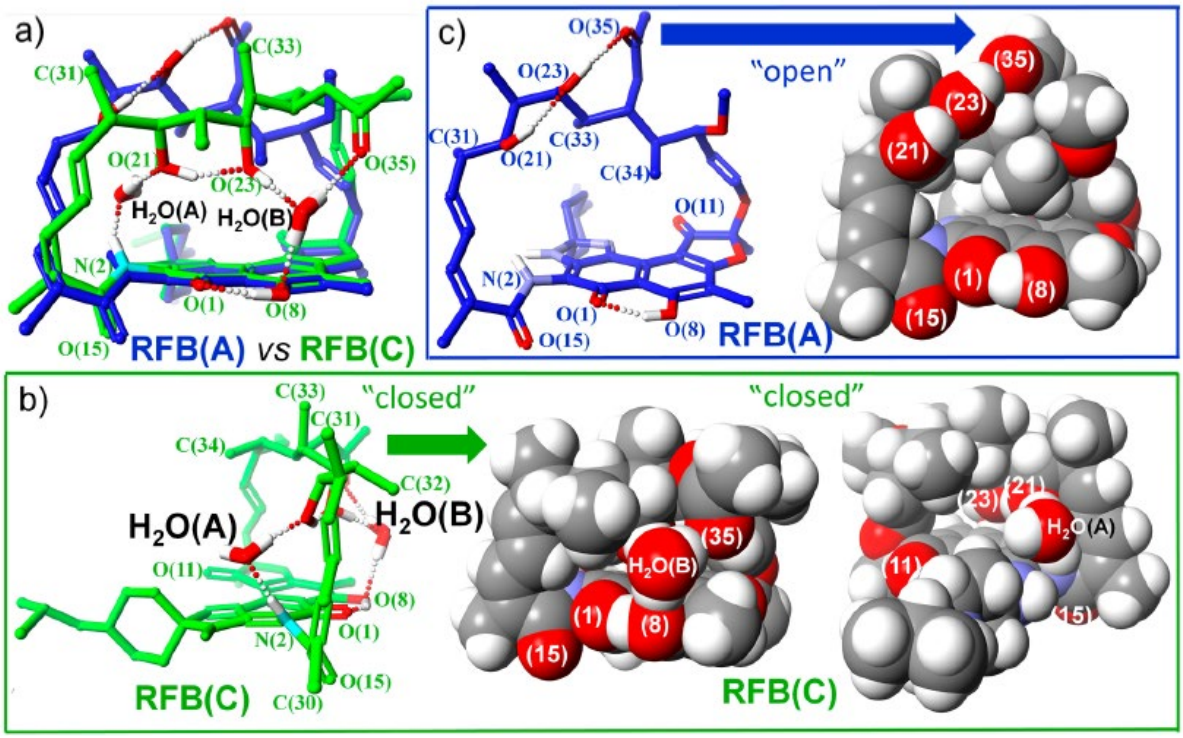


Figure 14 (a) DFT calculated and superimposed structures of **RFB**(A) and **RFB**(C)+2H_2O molecules viewed through the cavity between the ansabridge and the aromatic core. (b) DFT calculated structure of **RFB**(C)+2H_2O, where "closed" conformation of the ansa-bridge exists, viewed through the lactam-diene, H_2O(B), and H_2O(A) sides and visualized by stick-balls and space-filling (van der Waals radius) models. (c) DFT calculated structure of **RFB**(A) in stick-balls and space-filling projections viewed through the "basket's" cavity.

4.3.3 Summary

The submitted study, which is the basis for the habilitation procedure, fits perfectly into current trends and the need for continuous improvement of the arsenal of new or modified antibiotics. The extremely fast process of bacterial reproduction and the ability and ease of these microorganisms to adapt to new conditions mean that our species is constantly exposed to their attack. An additional factor that causes this problem to increase is drug resistance of bacteria (antibiotic resistance). Unfortunately, this phenomenon is caused primarily by the overuse of antibiotics (by doctors and patients). Microorganisms become resistant to drugs through acquired mechanisms, which makes antibiotics ineffective in the treatment of bacterial diseases. The forecasts are terrifying, indicating that in 2050 people will die more often from infections caused by antibiotic-resistant bacteria, and this will be one of the main causes of death,

comparable to the statistics of mortality caused by cancer.[34,35] For this reason, it is very important to search for new antibacterial agents and modification/obtaining antibiotic analogues that will oppose bacteria resistant to the antibiotics used.

The most important achievements of the presented study include:

I. Demonstration that in solutions containing water molecules, rifamycins (**RFB** and **RFX**) acquire conformational lability of the ansamycin bridge and are in a state of equilibrium between the "closed" conformer (hydrophobic nature) and "open" conformer (hydrophilic nature), which probably results in higher activity **RFB** and **RFX** against Gram-negative bacteria, compared to **RMP** and **RFP**

II. Determination based on the analysis of NMR spectra that **RFB** occurs only in a non-ionic form in solutions, while **RFX** occurs in an ionic form with a proton transferred from the phonol group O(8)H to the amidine system spanning the C(3)/C(4) positions

III. Preparation of a series of new amine analogues of **RMP**, including 63 amine analogues of rifampicin containing various substituents in the C(3) position in their structure, 10 heterocyclic derivatives containing the 3,4-dihydrobenzo[g]quinazoline system and 10 **Ral** hydrazones.

IV. Determination of the structures of the obtained derivatives based on spectroscopic data

V. Demonstration based on FT-IR analysis that benzocorona systems can act as a base in the intramolecular proton transfer process in amine analogues of **Rif**.

VI. Conducting SAR analysis for the obtained compounds from the ansamycin group and demonstrating, based on calculated models, that the high antibacterial activity is due to the possibility of interaction with E_{445} glutamate.

VII. Determining that for interaction with E_{445} glutamate, the protonated basic center should be located on a relatively stiff arm starting from the C(3) position.

VIII. Demonstration based on CP/MAS NMR and FT-IR studies that the method of purification/isolation of **Rif** affects the structure of this antibiotic in a solid and this fact should be taken into account when quantitatively and qualitatively determining this compound.

IX. Designing the synthesis of triazole derivatives of gossypol and obtaining 8 new Schiff Bases of gossypol.

X. Demonstration that the obtained gossypol derivatives have interesting antifungal activity.

XI. Establishing that the inhibition of ergosterol biosynthesis may be responsible for the antifungal activity of gossypol and its Schiff bases.

4.3.4 List of abbreviations used in alphabetical order

ACN – acetonitryl

^{13}C NMR – Carbon-13 nuclear magnetic resonance

CP/MAS – "Cross Polarization Magic Angle Spinnining",

F. acuminatum – Fusarium acuminatum

FT-IR – Fourier-transform infrared spectroscopy

G+ – Gram-positive bacteria

G– – Gram-negative bacteria

logP – lipophilicity coefficient

MeOH – methanol

MIC – Minimum inhibitory concentrations

NMR – Nuclear magnetic resonance

^{1}H NMR – Proton nuclear magnetic resonance

R. stolonifer - *Rhizopus stolonifer*

Ral – 3-formylorifamycin SV

RFB – Rifabutin

RFP – Rifapentine

RFX – Rifaximin

Rif – Rifampicine

RMP - Rifampicine

RNAP – Bacterial multisubunit DNA-dependent RNA polymerase

SAR – structure-activity relationship

S. epidermidis – Staphylococcus epidermidis

4.3.5 List of cited literature:

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5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

In the period from July to the end of December 2022, I was a visiting scientist at the Department of Biophysical Chemistry at the Institute of Biology at Humbolt University in Berlin (1/07/2022-31/12/2022, project number: D.00506.02.211200). The result of this international cooperation is the publication "Cascade transformation of the ansamycin benzoquinone core into benzoxazole influencing anticancer activity and selectivity", which was published in 2023 in The Journal of Organic Chemistry (J. Org. Chem., 87, (2023) , DOI: 10.1021/acs.joc.3c00493 - link) (**copy of the publication in Annex 8**). In this publication I have a double affiliation, the Faculty of Chemistry of Adam Mickiewicz University and the Institut für Biologie, Biophysikalische Chemie Humboldt-Universität zu Berlin.

In the post-doctoral period, I completed several short-term training courses in the use of differential infrared spectroscopy to study retinal photoreceptors. Training organized by Prof. Dr. Franz Bartel; Germany; Institut für Medizinische Physik und Biophysik, Charité – Universitätsmedizin Berlin; 15/11-20/11/2015, 11/02-18/02/2017, 04/10-08/10/2017 and 16/02-24/02/2018. (**confirmation in Annex 8**)

5.1 Cooperation with scientists from domestic and foreign research centres

As my research covers multiple disciplines, collaborating with scientists from both domestic and foreign research centers is crucial. I have had the privilege of working with various team leaders, listed below in order of the quantity of works accomplished:

1) Prof. Dr. Franz Bartl from the Department of Biophysical Chemistry, Institute of Biology at Humbolt University in Berlin (formerly Department of Medical Physics and Biophysics at the Medical University-Charité in Berlin) - extensive cooperation including, among others, spectroscopic consultations, 11 joint publications (2 before the doctorate, 9 after doctorate),
2) Prof. Ph.D. Marzena Gajęcka from the Department of Genetics and Pharmaceutical Microbiology, Medical University of Karol Marcinkowski in Poznań - antibacterial and antifungal activity tests, 6 joint publications (all post-doctoral),
3) Dr. Piotr Ruszkowski from the Medical University of Poznań - anticancer activity tests, 6 joint publications (all post-doctoral),
4) Prof. ICHO Ph.D. Wojciech Schilf from the Institute of Organic Chemistry of the Polish Academy of Sciences - NMR research, 6 joint publications (2 before the doctorate, 4 after the doctorate),
5) Ph.D. n. farm Joanna Stefańska from the Department of Pharmaceutical Microbiology, Medical University of Warsaw - antibacterial activity tests, 4 joint publications (all before PhD),
6) Prof. Ph.D. Eugeniusz Grech from the Faculty of Inorganic and Organic Chemistry, West Pomeranian University in Szczecin - research on Schiff bases and their complexes, 3 joint publications (2 before PhD, 1 after PhD),
7) Prof. Ph.D. Marek Murias from the Faculty of Toxicology, Poznań University of Medical Sciences - anticancer activity tests, 1 joint publication (post-doctoral)
8) Prof. Ph.D. Krzysztof Woźniak from the Faculty of Chemistry, University of Warsaw - measurements of crystallographic structures, 1 joint publication (post-doctoral)

6. Presentation of teaching and organizational achievements as well as achievements in popularization of science or art

I have been serving on the Scientific Council for the Chemical Sciences Discipline at the Faculty of Chemistry in the University of Adam Mickiewicz since 2020 for a term that runs until 2024. Throughout my academic career, starting from my first year as a doctorate student, I have been actively involved in teaching various classes such as laboratory classes, exercises, and proseminars to students studying chemistry and biology at the University of Adam Mickiewicz in Poznań (Table 4).

Table 4 Conducted teaching classes for students.

Before obtaining a Ph.D
i. Biochemistry with elements of biology - laboratory classes and seminars ii. Basics of organic chemistry – laboratory classes
After obtaining a Ph.D
i. Basics of organic chemistry - exercises ii. Biochemistry with elements of biology - exercises and laboratory classes iii. Organic chemistry (I MU) – laboratory classes iv. Biochemistry – exercises and laboratory classes v. Biochemistry II – laboratory classes vi. Forensic biochemistry – laboratory classes vii. Molecular spectroscopy – laboratory classes viii. Basics of natural product chemistry – laboratory classes

Since 2012, I have been the head of the Biochemistry and Biochemistry with elements of biology laboratories. I have developed laboratory exercises for laboratory classes in Biochemistry II and Forensic Biochemistry - I also act as a supervisor during these classes. Additionally, I am the author of the syllabus on *"Drug Toxicology"* (lecture) - a subject intended for students of *"Medical Chemistry with Drug Design"*, the opening of this field is planned for the coming academic year.

I was a research supervisor for my master's theses (Table 5). I also supervised Mrs. Klaudia Tokarska's master's thesis entitled: "Structure and physicochemical properties of heterocyclic derivatives at the C(17) position of the natural product geldanamycin." I am also an auxiliary supervisor of three doctoral theses that were defended with distinction (Table 5). I want to clarify that none of the scientific publications from these doctoral theses are included in my habilitation application.

Table 5 List of promoted doctoral and master's theses. Scientific supervision of students

Assistant supervisor of doctoral theses
I. Dr. Katarzyna Klich, date of awarding the degree: July 8, 2016 - thesis distinguished by both reviewers and RWCH UAM *"Functionalization of Spiramycin aglycone by using regio- and stereoselective cascade reactions"*
II. Dr. Joanna Domagalska, date of awarding the degree: July 8, 2016 - thesis distinguished by both reviewers and RWCH UAM *"Modification of josamycin aglycone using a regioselective nucleophilic substance of the S_N1' type and dipolar Huisgen cycloaddition"*
III. Dr. Anna Janas, date of awarding the degree: May 21, 2021 - thesis distinguished by both reviewers and RWCH UAM

"Synthesis and establishment of structure-biological activity correlation of new derivatives of 14- and 15-membered macrolide antibiotics containing rebuilt saccharide arms"

Master's thesis supervisor

I. Klaudia Tokarska, M.A., date of awarding the degree: June 14, 2023
"Structure and physicochemical properties of heterocyclic derivatives at the C(17) position of the natural product geldanamycin"

Supervision of master's students

Before obtaining a Ph.D

I. Katarzyna Klich, M.A., 2012, title of thesis: *"Synthesis and spectroscopic properties of new derivatives of 3-formylrifamycin SV"*

II. Anna Olsztyńska, M.A., 2012, title of work: *"The application of copper (I) catalysed Huisgen cycloaddition in synthesis of novel 3-formylrifamycin derivatives"*

After obtaining a Ph.D

I. Joanna Domagalska, M.A., 2013, title of thesis: *"Regioselective proton transfer in derivatives of 3-formyllorifamycin SV"*

II. Alicja Matuszak, M.A., 2013, title of thesis: *"Synthesis and characterization of secondary dibenzyl amines containing halogen atoms in their structures in the meta and para positions of the phenyl ring"*

III. Katarzyna Siwiak, M.A., 2014, title of thesis: *"Synthesis and physicochemical properties of new derivatives of 3-formylrifamycin SV containing crown ethers in the structure"*

IV. Marietta Blecha, M.A., 2015, title of thesis: *"Synthesis and structure of new triazole derivatives of gossypol"*

V. Katarzyna Barczak, M.A., 2015, title of thesis: *"Modification of 3-formylrifamycin SV using reductive amination reactions with amines containing an amide group in the structure"*

VI. Anna Buła, M.A., 2015, title of thesis: *"Synthesis and spectroscopic studies of new rifamycins containing anilines in their structure"*

VII. Grzegorz Iwanowicz, M.A., 2016, title of work: *"Synthesis of new hydrazone derivatives of semi-synthetic 3-formylrifamycin SV"*

VIII. Paulina Roesler, M.A., 2017, title of thesis: *"Synthesis and structural analysis of new rifamycin derivatives having basic centers in the structure"*

IX. Natalia Skrzypczak, M.A., 2019, title of thesis: *"New triazole derivatives of colchicein at the C-9 and C-10 position of the tropolone ring as potential anticancer agents"*

X. Monika Szukowska, M.A., 2020, title of thesis: *"Synthesis and structure of new rifamycin analogues containing tertiary amine groups"*

XI. Patryk Kalinowski, M.A., 2021, title of thesis: *"Functionalization of 16-membered aglycone of spiramycin via intramolecular cascade reactions"*

XII. Ewelina Smolarz, M.A., 2023, title of thesis: *"Functionalization of 16-membered aglycone of spiramycin via intramolecular cascade reactions"*

XIII. Anna Smoczyńska, M.A., 2023, title of thesis: *"Synthesis of new Spiramycin derivatives containing functionalized pyrrolidine-triazole arms"*

The supervisor of all diploma theses was prof. Ph.D. Piotr Przybylski

In addition to serving as an auxiliary supervisor for doctoral theses and a scientific supervisor for the laboratory work of students conducting their research for master's theses, I was also a reviewer of several bachelor's theses carried out at the Faculty of Chemistry of the University of Adam Mickiewicz in Poznań (Table 6). I was selected to review scientific articles that was sent to various publishing houses (Table 6).

Tabela 6 List of reviews of bachelor's theses and scientific articles

Reviews of bachelor's theses

I. lic. Ewelina Nowak, 2021, title of thesis: *"Modification at the C-17 position of geldanamycin in the design of new anticancer agents"*
II. license Patryk Kalinowski, 2019, title of thesis: *"Resistance of various strains of bacteria to lactone macrolide antibiotics"*
III. lic. Daniel Walczak, 2018, title of thesis: *"Ansamycin antibiotics and leukomycins - structure and mechanisms of action"*
IV. Lic. Monika Szukowska, 2018, title of thesis: *"Mechanisms of bacterial resistance to used antibiotics from the macrolide group"*
V. lic. Maria Szyszka, 2017, title of thesis: *"Macrolides of natural origin - structure and biological properties"*
VI. Lic. Oskar Kubiczek, 2016, title of thesis: *"Intra- and intermolecular processes of proton transfer"*
VII. license Marta Biernaczyk, 2016, title of thesis*: "The use of 1 and 2D NMR spectroscopy on selected natural compounds"*
VIII. lic. Barbara Stańska, 2015, title of thesis: *"The use of macrolides in antibacterial therapy - selected examples"*
IX. lic. Paulina Roesler, 2015, title of thesis: *"Flavonoids - structure and biological properties"*
X. Lic. Dominika Czerwonka, 2015, title of thesis: *"Ansamacrolides - structure and biological activity"*

reviews of scientific articles

Journal name	$IF_{5\text{-years}}$	number of reviews
Bioorganic & Medicinal Chemistry Letters	2.6	3
Journal of Spectroscopy	2.4	3
Molecules	4.9	2
Pharmaceuticals	4.9	1
Current Organic Synthesis	2.0	1
Future Medicinal Chemistry	4.2	1
Journal of Molecular Structure	3.2	1
Letters in Organic Chemistry	0.7	1
	In total	**13**

The results of the research in which I participated were also presented at numerous national and international scientific conferences in the form of oral or poster presentations. (Table 7).

Tabela 7 List of conference presentations.

Befor obtaining a Ph.D

1. *Central European School on Physical Organic Chemistry*, 08-12 czerwiec **2008**, Karpacz, P. Przybylski, J. Kira, K. Pyta, A. Huczyński, G. Schroeder, B. Brzezinski, P. Barczyński *„Complexation properties of aza-derivatives of gossypol toward silver (I) cations investigated by potentiometric, ESI MS, spectroscopic and semiempirical methods”*, POSTER
2. Konwersatorium krystalograficzne **czerwiec 2008 Wrocław**, B. Wicher, K. Pyta, M. Gdaniec, P. Przybylski, *„Solwaty rifampicyny”*, POSTER
3. *Central European School on Physical Organic Chemistry*, 02-06 czerwiec **2009**, Przesieka. K. Pyta, P. Przybylski, D. Remlein-Starosta, G. Schroeder, B. Brzezinski, *„Antifungal activity of alkyl and heterocyclic aza-derivatives of gossypol as well as their complexes with $NaClO_4$”*, POSTER
4. *42nd IUPAC CONGRESS Chemistry Solutions; 2-7 Augest 2009; SECC, Glasgow, Scotland, UK,* P. Przybylski, K. Pyta, B. Brzezinski, *„Synthesis, spectroscopic, mass spektrometry and semi-empirocal studies of new type aminoalkyl-2,3-unsaturated derivatives of 16-membered natural macrolide antibiotic – Josamycin”*, POSTER
5. *Central European School on Physical Organic Chemistry*, 08-12 czerwiec **2010**, Przesieka. K. Pyta, P. Przybylski, J. Czupryniak, B. Wicher, M. Gdaniec, T. Ossowski, W. Schilf, E. Grech, B. Kołodziej, A. Szady-Chełmieniecka, B. Brzezinski, *„Spectroscopic and physico-chemical properties of aza-derivaties present in different tautomeric forms”*, POSTER
6. 18th International Conference on Organic Synthesis, Section: Natural Product Chemistry, 1-6 August, **2010**, Bergen, Norway, P. Przybylski, K. Pyta, B. Brzezinski, *„Synthesis and structural investigations of new aza-derivatives of 16-membered macrolide antibiotic – josamycin”*, POSTER
7. *Central European School on Physical Organic Chemistry*, 08-12 czerwiec **2011**, Przesieka. K. Pyta, P. Przybylski, *„Spectroscopic and physico-chemical properties of aza-derivaties present in different tautomeric forms”*, POSTER
8. Polish School of Crystallography Chemical Crystallography of the XXI-st Centary, 5-11 September **2011**, Gierłoż, B. Wicher, K. Pyta, P. Przybylski, M. Gdaniec, *„Tautomeric forms and conformers of rifampicin in the solid state”*, POSTER
9. Central European School om Physical Organic Chemistry, 07-11.05.**2012**, Przesieka, K. Klich, K. Pyta, A. Olsztyńska, P. Przybylski, J. Stefańska, *„Synthesis and structure of new rifampicin analogues – spectroscopic evidence of the presence of zwitterionic forms”*, POSTER
10. Central European School om Physical Organic Chemistry, 07-11.05.**2012**, Przesieka, A. Olsztyńska, K. Pyta, K. Klich, P. Przybylski, *„Synthesis and structure of new Rifampicin analogues – spectroscopic evidence of the presence of zwitterionic forms”*, Anna Olsztyńska, Krystian Pyta, Katarzyna Klich, Piotr Przybylski, POSTER

After obtaining a Ph.D

11. Central European School on Physical Organic Chemistry, 27-31.05.**2013**, Przesieka, K. Klich, K. Pyta, P. Przybylski, *„The E1cB elimination as an efficient method leading to obtain of novel derivatives of leucomycin A3”*, POSTER
12. BIT's 4th Annual International Congress of Medichem-2013, Chiny, Haikou, 13-16.11.**2013**, K. Pyta, P. Przybylski, K. Klich, J. Domagalska, *„From the Proton Transfer within Structure of Rifamycin Antibiotics to Mechanism of Inhibition of Bacterial RNA Polymerase Dependent on DNA”*, POSTER

13. BIT's 4th Annual International Congress of Medichem-2013, Chiny, Haikou, 13-16.11.**2013**, K. Pyta, P. Przybylski, K. Klich, J. Domagalska, W. Schilf, B. Kamieński, *„Investigation of proton transfer process in rifampicin in the solid state"*, POSTER
14. Chiralność: od cząsteczki elementarnej do uniwersum, Polska, Poznań, Wydział Chemii UAM, 7.06.**2013**, P. Przybylski, K. Pyta, K. Klich, *„Transformation of josamycin in solution – Michael addition or SN2 substitiution"*, POSTER
15. Chiralność: od cząteczki elementarnej do uniwersum, Polska, Poznań, Wydział Chemii UAM, 7.06.**2013**, P.Przybylski, K. Pyta, J. Domagalska, K. Klich, *„Regioselective proton transfer within 3-formylrifamicin SV derivatives"*, POSTER
16. VIIIth SYMPOSIUM: Nuclear magnetic resonance in chemistry, physics and biological sciences, Warszawa, 24 – 26 .09.**2014**, K. Klich, K. Pyta, P. Przybylski, *„Aplication of 1D and 2D NMR spectroscopy to determination of novel spiramycin derivatives structures"*, POSTER
17. VIIIth SYMPOSIUM: Nuclear magnetic resonance in chemistry, physics and biological sciences, Warszawa, 24 – 26 .09.**2014**, J. Domagalska, K. Pyta, P. Przybylski, *„Applictaion of 1D and 2D NMR spectroscopy to determination of novel josamycin derivatives structures"*, POSTER
18. TETRAHEDRON SYMPOSIUM, 16-19.06.**2015**, Berlin, Niemcy, K. Pyta, M. Blecha, P. Przybylski, *„New Gossypol Schiff bases containing 1,2,3-triazole rings"*, POSTER
19. TETRAHEDRON SYMPOSIUM, 16-19.06.**2015**, Berlin, Niemcy, J. Domagalska, K. Pyta, P. Przybylski, *„Diastereo- and regioselective functionalisation of josamycin aglycone using untypical reaction in dienol sysytem"*, POSTER
20. TETRAHEDRON SYMPOSIUM, 16-19.06.**2015**, Berlin, Niemcy, K. Klich, K. Pyta, P. Przybylski, *„Regio- and Stereospecific functionalization of 16-membered Lactone Aglycone of Macrolide type Antibiotics via Intramolecular cascade reactions"*, POSTER
21. Frontiers in Medicinal Chemistry 2015**,** 14-16.09.**2015**, Antwerpia, Belgia, K. Pyta, M. Blecha P. Przybylski, *„New Gossypol Schiff bases containing 1,2,3-triazole rings"*, POSTER
22. Frontiers in Medicinal Chemistry 2015**,** 14-16.09.**2015**, Antwerpia, Belgia, P. Przybylski, K. Klich, J. Domagalska, K. Pyta, *„Stereo- and Regioselective modification of spiramycin and josamycin lactone aglycones as a source of novel type inhibitors of 50S ribosomal subunit"*, POSTER
23. TRAMECHVIII 2015**,** 11-15.11.**2015**, Antalya, Turcja, K. Pyta, J. Domagalska, P. Przybylski, *„Synthesis of new heterocyclic derivatives of josamycin via S_N2` and dipolar cycloaddition of CUAAC type"*, POSTER
24. TRAMECHVIII 2015**,** 11-15.11.2015, Antalya, Turcja, K. Klich, K. Pyta, P. Przybylski, *„Regio- and stereospecific functionalization of 16-membered lactone aglycone of macrolide type antibiotics via intramolecular cascade reactions"*, POSTER
25. TRAMECHVIII 2015**,** 11-15.11.**2015**, Antalya, Turcja, P. Przybylski, K. Klich, J. Domagalska, K. Pyta, *„Regio- and steroselective "cascade" and "click" modifications of macrolide antibiotics as a source of new heterocyclic antibacterial agents"*, PREZENTACJA USTNA
26. XIII Seminarium Doktorantów „Na pograniczu chemii i biologii", 31.05–3.06.**2015** Karpacz, Poland, K. Klich, K. Pyta, P. Przybylski, *„Funkcjonalizacja aglikonu Spiramycyny przez zastosowanie regio- i stereoselektywnych reakcji kaskadowych"*, PREZENTACJA USTNA
27. XIII Ogólnopolskie Seminarium Doktorantów, *„Na pograniczu Chemii i Biologii"*, 31.05-01.06.**2015** Karpacz, POLAND, J. Domagalska, K. Pyta, P. Przybylski, , PREZENTACJA USTNA *„Modyfikacja aglikonu Josamycyny z wykorzystaniem regioselektywnej substytucji nukleofilowej typu SN1' i dipolarnej cykloaddycji Huisgena"*, PREZENTACJA USTNA
27. XIII Ogólnopolskie Seminarium Doktorantów, „Na pograniczu Chemii i Biologii", 31.05-01.06.**2015** Karpacz, POLAND, D. Czerwonka, P. Przybylski, K. Pyta, M. Kubicka, M. Gajęcka, F. Bartl, *„Makrolidy - struktura i właściwości koordynacyjne a aktywność biologiczna"*, PREZENTACJA USTNA
28. 58 Zjazd naukowy PTChem, 21-25 wrzesień 2015 Gdańsk, POLAND, D. Czerwonka, J. Domagalska, M. Kubicka, M. Gajęcka, K. Pyta, P. Przybylski, *„Structure and antimicrobial activity of new derivatives of 3-formylryfamycin SV containing crown-ethers and L-amino acids"*, POSTER
29. European Congress on Magnetic Resonance, 5-10.07.**2015**, Praga, Republika Czeska, J. Domagalska, K. Pyta, P. Przybylski, *„Modyfication of the aglycone Josamycin using the regioselective nucleophilic substitution SN1` type and dipolar Huisgen cycloaddition"*, POSTER

30. 59 Zjazd naukowy PTChem i SITPChem, 19-23 wrzesień 2016, Poznań, POLAND, P. Przybylski, K. Pyta, K. Klich, J. Domagalska, A. Janas, M. Gajecka, P. Pecyna, L. Celewicz, P. Ruszkowski, „*Regio- and stereoselective „Cascade" and „Click" modifications of macrolide lactone antibiotics*", S01W09 (wykład sekcyjny)

31. 59 Zjazd naukowy PTChem i SITPChem, 19-23 wrzesień 2016, Poznań, POLAND, " J. Domagalska, A. Janas, K. Pyta, P. Pecyna, P. Ruszkowski, L. Celewicz, M. Gajecka, F. Bartl P. Przybylski, „*Synteza i struktura nowych eterowych C(13)-podstawionych pochodnych leukomycyn"*, POSTER

32. 59 Zjazd naukowy PTChem i SITPChem, 19-23 wrzesień 2016, Poznań, POLAND, D. Czerwonka, K. Pyta, M.M. Kubicka, M. Gajecka, P. Pecyna, P. Przybylski, J. Domagalska, „*Structure and antibacterial activity of new amino derivatives of 3-formylrifamycin SV containing crow ethers and L-aminoacids*", POSTER

33. 60 Zjazd Naukowego Polskiego Towarzystwa Chemicznego, 17-21 IX 2017, Wrocław; A. Janas, K. Pyta, P.Przybylski*, „Modyfikacje aglikonu erytromycyny poprzez reakcje dipolarnej cykloaddycji Huisgena"*, POSTER

34. 6th IAPC Meeting, Sixth World Conference on Physico-Chemical Methods in Drug Discovery & Third World Conference on ADMET and DMPK, 4-7 IX 2017, Zagrzeb, A.Janas, K.Pyta, K. Pyta-Klich, J. Domagalska P.Przybylski, „*Synthesis, structure, antibacterial and anticancer activity of new macrolide antibiotics analogs"*;; POSTER

35. 2nd International Conference on Pharmaceutical Chemistry, 2-4 X 2017, Barcelona, K. Pyta, K.Pyta-Klich, A. Janas, J. Domagalska, P. Przybylski, „*Cascade approach to modification of lactone macrolide antibiotics*", POSTER

36. 2nd International Conference on Pharmaceutical Chemistry, 2-4 X 2017, Barcelona; P. Przybylski, A. Janas, K.Pyta-Klich, J. Domagalska, K. Pyta, Franz Bartl, „*Synthesis, antibacterial and anticancer potency of new lactone and lactam macrolide derivatives*", PREZENTACJA USTNA

37. 10th World Congress on Medicinal Chemistry and Drug, 14-15 VI 2018, Barcelona, A. Janas, K. Pyta, P. Przybylski, *„Synthesis and structure-activity relationship of a new derivatives of 14- and 15-membered macrolide antibiotics containing rebuilt saccharide arms"*, PREZENTACJA USTNA

38. Chemistry Beyond Nature, 21-22 VI 2018, Poznań, M. Szukowska, K. Pyta, A. Janas, P. Pecyna, M. Jaworska, M. Gajęcka, F. Bartl, P. Przybylski, *„Ansa-bridge movement implicated by the presence of different substituents at quinone ring of geldanamycins"*, POSTER

39. 24th Conference on Isoprenoids, 9-12 IX 2018, Białystok, A. Janas, K. Pyta, P. Przybylski, *„Synthesis and structure-activity relationship of a new derivatives of 14-,15- and 16-membered macrolide antibiotics containing rebuilt saccharide arms*", POSTER

40. 24th Conference on Isoprenoids, 9-12 IX 2018, Białystok, K. Pyta, M. Szukowska, N. Skrzypczak, A. Janas, M. Gdaniec, P. Przybylski, *„ Influence of C-17 substituents at quinone ring on conformational flexibility of geldanamycins"*, POSTER

41. 24th Conference on Isoprenoids, 9-12 IX 2018, Białystok, P. Przybylski, N. Skrzypczak, M. Szukowska, K. Pyta, A. Janas, B. Wicher, M.Gdaniec, P. Pecyna, M. Gajęcka, „*Basket-like lactam macrolides – modifications and structure – activity relationship studies",* PREZENTACJA USTNA

42. 61 Zjazd Naukowego Polskiego Towarzystwa Chemicznego, 17-21 IX 2018, Kraków, N. Skrzypczak, M. Szukowska, K. Pyta, A. Janas, M. Gdaniec, P. Przybylski, „*New quinone-functionalized derivatives of geldanamycin- structure and biological implications"*, POSTER

43. X Poznańska Konferencja Naukowa „Chemia i przemysł", 30 XI 2018, Poznań, N. Skrzypczak, M. Szukowska, K. Pyta, M. Gdaniec, A. Janas, P. Przybylski, *„Blokowanie funkcji białek chaperonowych (Hsp) przez pochodne geldanamycyny jako efektywna droga do uzyskania środków przeciwnowotworowych"*, POSTER

44. X Poznańska Konferencja Naukowa „Chemia i przemysł", 30 XI 2018, Poznań, M. Szukowska, N. Skrzypczak, K. Pyta, A. Janas, M. Gdaniec, P. Przybylski, *„Nowe ryfamycynowe inhibitory bakteryjnych polimeraz RNA - synteza i właściowości, aktywność przeciwbakteryjna i molekularny mechanizm działania"*, POSTER

45. IV Ogólnopolskie Sympozjum Chemii Bioorganicznej, Organicznej i Biomateriałów „BioOrg 2022", 3.12.2022, Poznań, E. Smolarz, A. Smoczyńska, K. Pyta, P. Przybylski, *„Synteza nowych-pirolidynowych pochodnych spiramycyny zawierających sfunkcjonalizowane ugrupowanie heterocykliczne wykorzystane w reakcji sprzęgania Hecka",* POSTER

46. IV Ogólnopolskie Sympozjum Chemii Bioorganicznej, Organicznej i Biomateriałów „BioOrg 2022", 3.12.2022, Poznań, A. Smoczyńska, E. Smolarz, K. Pyta, P. Przybylski, *„Synteza nowych bicyklicznych-pirolidynowych pochodnych spiramycyny zawierających sfunkcjonalizowane ugrupowanie heterocykliczne wykorzystywane do reakcji cykloaddycji Huisgena"*, POSTER

7. Apart from information set out in 1-6 above, the applicant may include otherinformation about his/her professional career, which he/she deems important.

7.1 Scientific achievements

Throughout my scientific career, I focused on researching various compounds derived from natural sources or synthesized in labs. The majority of my work centered on macrocyclic antibiotics, such as rifampicin and its analogues, geldanamycin, and clarithromycin - a 14-membered lactone macrolide. Additionally, I explored the properties of 15-membered azithromycin and 16-membered josamycin and spiramycin. Apart from antibiotics, I also investigated gossypol - a polyphenolic bisesquiterpene - and colchiceine - a tropolone alkaloid.

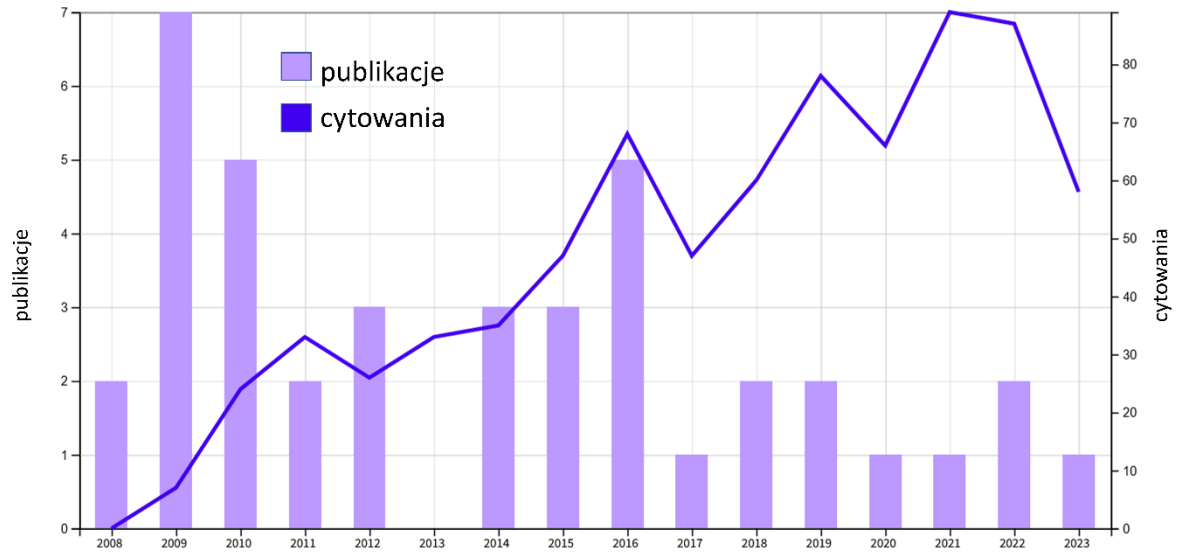
Before obtaining a Ph., I had the pleasure of working on the 16-membered lactone antibiotic josamycin. The research results concerned were the first to obtain an α,β-unsaturated lactone system for this type of antibiotic and provide a mechanism for this process (publication no. 9, Table 8). I also took part in the search for inhibitors of the bacterial 50S ribosomal subunit, this subject was a valid financial result of the National Science Center as part of the "SONATA BIS" (I was the contractor). This project aimed to modify a macrolide within a macrocyclic lactone system called aglycone. A 2015 paper in the Journal of Organic Chemistry describes the process of modifying spiramycin aglycone through selective reactio: two reactions of the E1cB type leading to the occurrence of α,β,δ,γ-dinunsaturated spiramycin aglycone and an addition reaction of the 1,2-type and the next 1,6-type addition reaction to the unsaturated lactone system. The above transformations led to obtaining a new type of spiramycin derivatives (doctoral thesis of Dr. Katarzyna Klich - I acted as an auxiliary supervisor) (publication no. 25, Table 8). A research paper published in 2016 revealed the selective reaction of $S_N1`$ and its ability to produce new leukomycin derivatives at the C-13 position in the aglycone ring of spiramycins (publication no. 26, Table 8). The study was part of Dr. Joanna Domagalska's doctoral thesis, where I acted as an auxiliary promoter. In subsequent studies, a series of new derivatives were discovered, which were an extension of the previous works. Biological activity tests conducted showed that this type of compound had interesting anticancer activity, a first in this field. This was documented in publications no. 28 and 29, Table 8.

As a contractor for the OPUS 10 project, I conducted research on semi-synthetic macrolide antibiotics. Our findings showed that the chemistry of these antibiotics is fascinating and involves intramolecular reactions. The key compound we discovered was an unusual β-keto-

ketene-acetal that has an inverted configuration on the C(13) carbon atom (doctoral thesis of Dr. Anna Janas, in which I was an auxiliary supervisor). Through subsequent transformations, we were able to open this compound and obtain a ketolide, which is an epimer of the intermediate compound. However, when we conducted the experiment under basic conditions, we obtained a mixture of two isomers that have an unsaturated system conjugated to the ketone moiety in the C(3) position. Our publication number 38 and Table 8 provide further details on this experiment.

I took part in a fascinating project, OPUS 13, that involved modifying the benzoquinone ansamycin - geldanamycin. Through our research, we were able to produce a new set of derivatives for this group of compounds. During the cascade reaction, we transformed the benzoquinone core of the amine derivatives of ansamycins into the benzoxazole system. This type of transformation provides possibilities for more modifications and has an intriguing effect on anticancer activity. Our work was published in publication no. 40, Table 8.

To date, I have co-authored 40 scientific publications from the JCR list (Table 8), with 8 of these publications [**H1**-**H8**] serving as the foundation for my habilitation procedure. I am the lead or corresponding author on 12 of the 40 publications. According to the Web of Science (Fig.), the total number of citations for my publication is 758, with 673 of these being non-self citations, resulting in a Hirsh index of 13.



Figere 15 Scientific achievements and citations per year. Data source: Web of Science.

Tabele 8 List of publications before and after obtaining a doctoral degree. All magazines are available in the JCR database. Scientometric data for IF5-year taken for 2022, for two publications the year of the last known value is given in brackets.

before obtaining a Ph.D		
publication	IF	$IF_{5\text{-years}}$
1. Przybylski, P., **Pyta, K.**, Ratajczak-Sitarz, M., Katrusiak, A., Brzezinski, B., „X-ray, FT-IR, ESI MS and PM5 studies of Schiff base of gossypol with allylamine and its complexes with alkali metal cations and perchlorate anion" *Struct. Chem.* 19, (**2008**), 983-995 DOI: 10.1007/s11224-008-9385-9, link	1.433	1.4
2. Przybylski, P., **Pyta, K.**, Wicher, B., Gdaniec, M., Brzezinski, B., *„Structure of a new Schiff base of gossypol with 1-(3-aminopropyl)-2-pyrrolidinone studied by the X-ray, FT-IR, NMR, ESI-MS and PM5 methods" J. Mol. Struct.* 889, (**2008**), 332-343 DOI: 10.1016/j.molstruc.2008.02.028, link	1.594	3.2
3. Przybylski, P., Huczynski, A., **Pyta, K.**, Brzezinski, B., Bartl, F., „Biological properties of Schiff bases and azo derivatives of phenols" *Curr. Org. Chem.* 13, (**2009**), 124-148 DOI:10.2174/138527209787193774, link	2.879	2.3
4. Przybylski, P., **Pyta, K.**, Remlein-Starosta, D., Schroeder, G., Brzezinski, B., Bartl, F., „Antifungal activity of alkyl and heterocyclic aza-derivatives of gossypol as well as their complexes with $NaClO_4$ against *Fusarium oxysporum* f. sp. lupini" *Bioorg. Med. Chem. Lett.* 19, (**2009**), 1996-2000 DOI:10.1016/j.bmcl.2009.02.051, link	2.650	2.6
5. Przybylski, P., **Pyta, K.**, Brzezinski, B., „Complexes of heterocyclic aza-derivatives of phytoalexin from cotton plant-gossypol with alkali metal cations and perchlorate anion studied by ESI mass spectrometric method in the positive and negative modes" *J. Mass. Spectrom.* 44, (**2009**), 838-846 DOI:10.1002/jms.1559, link	3.411	2.0
6. Przybylski, P., **Pyta, K.**, Ratajczak-Sitarz, M., Katrusiak, A., Brzezinski, B., „Structure of a New Schiff Base of Gossypol with Ethyl 4-Amino-1-piperidine Carboxylate in the Solid and in the Solution" *Polish J. Chem.* 83, (**2009**), 747-759	0.523	0.38 (2011)
7. Przybylski, P., **Pyta, K.**, Brzezinski, B., „Fragmentation pathways of new aza derivatives of 16-membered macrolide antibiotic – analog of Josamycin investigated by ESI and FAB mass spectrometric methods" *J. Mass. Spectrom.* 44, (**2009**), 1395-1401 DOI:10.1002/jms.1612, link	3.411	2.0
8. Przybylski, P., **Pyta, K.**, Stefańska, J., Ratajczak-Sitarz, M., Katrusiak, A., Huczyński, A., Brzezinski, B., „Synthesis, crystal structures and antibacterial activity of Aza-derivatives of bissesquiterpene from cotton plants - gossypol" *Eur. J. Med. Chem.* 44, (**2009**), 4393-4403 DOI:10.1016/j.ejmech.2009.05.032, link	3.269	6.5
9. Przybylski, P., **Pyta, K.**, Brzezinski, B., „Unexpected α,β-unsaturated products of reductive amination of the macrolide antibiothic josamycin" *Tetrahedron Lett.* 50, (**2009**), 6203-6207 DOI:10.1016/j.tetlet.2009.08.118, link	2.660	1.8
10. **Pyta K.**, Przybylski P., Schilf W., Kołodziej B., Szady-Chełmieniecka A., Grech E., Brzezinski B., „Spectroscopic and theoretical studies of the protonation of N-(5-nitrosalicylidene)-ethylamine" *J Mol Struct*. 967, (**2010**), 140-146 DOI:10.1016/j.molstruc.2010.01.002, link	1.599	3.2
11. **Pyta K.**, Przybylski P., Huczyński A., Hoser A., Woźniak K., Schilf W., Kamieński B., Grech E., Brzezinski B. „X-ray, spectroscopic and computational studies of the	1.599	3.2

tautomeric structure of a new hydrazone of 5-nitrosalicylaldehyde with indole-3-acetic hydrazide" *J Mol Struct*. 970, (**2010**), 147-154 DOI:10.1016/j.molstruc.2010.02.068, link		
12. Przybylski, P., **Pyta, K.**, Stefańska, J., Brzezinskia, B., Bartl, F. „Structure elucidation, complete NMR assignment and PM5 theoretical studies of new hydroxy-aminoalkyl-α,β-unsaturated derivatives of the macrolide antibiotic josamycin" *Magn. Reson. Chem*. 48, (**2010**), 286-296 DOI:10.1002/mrc.2574, link	1.247	1.8
13. Przybylski P., Kwit M., **Pyta K.**, Pankiewicz R., Schroeder G., Gawroński J., Brzezinski B., „Structure and atropoisomerisation of diastereoisomeric gossypol Schiff base with (R)-(+)-2-Amino-3-benzyloxy-1-propanol" *Tetrahedron:Asymmetry* 21, (**2010**), 973-981 DOI:10.1016/j.tetasy.2010.05.034, link	2.484	1.858 (2016)
14. Przybylski P., **Pyta K.**, Czupryniak J., Wichera B., Gdaniec M., Ossowski T., Brzezinski B., „The influence of protonation on molecular structure and physico-chemical properties of gossypol Schiff bases" *Org. Biomol. Chem.* 8 (24), (**2010**), 5511-5518 DOI:10.1039/c0ob00288g, link	3.451	3.0
15. **Pyta K.**, Przybylski P., „MALDI-TOF tandem mass spectrometric analysis of novel aza-analogues of semi-synthetic ansamycin antibiotic – rifampicin" *J. Mass. Spectrom.* 46, (**2011**), 751-756 DOI:10.1002/jms.1954, link	3.268	2.0
16. Przybylski P., **Pyta K.**, „Transformation of josamycin in alkaline solution - Intramolecular S_N2 substitution or E1cB elimination and intramolecular Michael addition?" *Tetrahedron Letters* 52 (47), (**2011**), 6275-6280 DOI:10.1016/j.tetlet.2011.09.086, link	2.683	1.8
17. **Pyta K.**, Przybylski P., Wicher B., Gdaniec M., Stefańska J., "Intramolecular proton transfer impact on antibacterial properties of ansamycin antibiotic rifampicin and its new amino analogues" *Org. Biomol. Chem.* 8, (**2012**), 2385-2388 DOI:10.1039/c2ob00008c, link	3.568	3.0
18. Wicher B., **Pyta K.**, Przybylsk, P., Tykarska E., Gdaniec M. „Redetermination of rifampicin penta-hydrate revealing a zwitterionic form of the anti-biotic" *Acta Crystallographica Section C: Crystal Structure Communications*, 68 (5), (**2012**), o209-o212. DOI:10.1107/S0108270112015296, link	0.492	0.7
In total, before the Ph.D	**42.221**	**42.738**
Average/publication	**2.346**	**2.374**

After obtaining a Ph.D

publication	IF	$IF_{5\text{-years}}$
19. **Pyta K.**, Przybylski P., Klich K., Stefańska J., „A new model of binding of rifampicin and its amino analogues as zwitterions to bacterial RNA polymerase" *Org. Biomol. Chem.* 10, (**2012**), 8283-8297 DOI:10.1039/c2ob26317c, link	3.568	3.0
20. Przybylski P., **Pyta K.**, Klich K., Schilf W., Kamieński B., „^{13}C and ^{15}N CP/MAS, ^{1}H-^{15}N SCT CP/MAS and FTIR spectroscopy as tools for qualitative detection of the presence of zwitterionic and nonionic forms of ansa-macrolide 3-formylrifamycin SV and its derivatives in solid state" *Magn. Reson. Chem*. 52 (1-2), (**2014**), 10-21, [**H2**] DOI:10.1002/mrc.4028, link	1.179	1.8

21. **Pyta K.**, Przybylski P., Klich K., Schilf W., Kamieński B., Grech E., Kołodziej B., Szady-Chełmieniecka A., Brzezinski B., „Impact of metal cation complexation and protonation on tautomeric and resonance forms of the oxaalkyl Schiff bases derived from 5-substituted salicylaldehyde and 2-hydroxy-1-naphthlaldehyde" *Struct. Chem.* 25 (6), (**2014**), 1733-1746 DOI:10.1007/s11224-014-0447-x, link	1.837	1.4
22. **Pyta K.**, Klich K., Domagalska J., Przybylski P., „Structure and evaluation of antibacterial and antitubercular properties of new basic and heterocyclic 3-formylrifamycin SV derivatives obtained via 'click chemistry' approach" *Eur. J. Med. Chem.* 84, (**2014**), 651-676, [**H3**] DOI:10.1016/j.ejmech.2014.07.066, link	3.447	6.5
23. **Pyta K.**, Przybylski P., Bartl F., „Regioselective long-range proton transfer in new rifamycin antibiotics: A process in which crown ethers act as stronger brønsted bases than amines" *ChemPhysChem*, 16 (5), (**2015**), 938-942, [**H6**] DOI:10.1002/cphc.201402892, link	3.138	3.0
24. Przybylski P., **Pyta K.**, Czerwonka D., Kubicka M.M., Gajecka M., „The effect of complexation of 3-formylrifamycin SV macrocyclic ether derivatives with metal cations and small nitrogen-containing organic molecules on antibacterial activity against *S. aureus* and *S. epidermidis*" *Bioorg. Med. Chem. Lett.* 25 (18), (**2015**), 3903-3909, [**H7**] DOI:10.1016/j.bmcl.2015.07.043, link	2.486	2.6
25. Klich K., **Pyta K.**, Przybylski P., „Regio- and Stereoselective Functionalization of 16-Membered Lactone Aglycone of Spiramycin via Cascade Strategy" *J. Org. Chem.* 80 (14), (**2015**), 7040-7049 DOI:10.1021/acs.joc.5b00847, link	4.785	3.4
26. Domagalska J., **Pyta K.**, Przybylski P., „Conversion of leucomycin-A3 antibiotic into novel triazole analogues via regio- and diastereoselective SN1′ substitution with allylic rearrangement and 1,3-dipolar cycloaddition of CuAAC type" *Tetrahedron Lett.* 57 (15), (**2016**), 1661-1666 DOI:10.1016/j.tetlet.2016.02.113, link	2.193	1.8
27. Czerwonka D., Domagalska J., **Pyta K.**, Kubicka M.M., Pecyna P., Gajecka M., Przybylski P., „Structure-activity relationship studies of new rifamycins containing l-amino acid esters as inhibitors of bacterial RNA polymerases" *Eur. J. Med. Chem.*, 116, (**2016**), 216-22, [**H4**] DOI:10.1016/j.ejmech.2016.03.061, link	4.519	6.5
28. Klich K., **Pyta K.**, Kubicka M.M., Ruszkowski P. Celewicz L. Gajecka M. Przybylski P., „Synthesis, Antibacterial, and Anticancer Evaluation of Novel Spiramycin-Like Conjugates Containing C(5) Triazole Arm" *J. Med. Chem.*, 59 (17), (**2016**), 7963-7973 DOI:10.1021/acs.jmedchem.6b00764, link	5.589	7.3
29. Domagalska J., Janas A., **Pyta K.**, Pecyna P., Ruszkowski P., Celewicz L., Gajecka M., Bartl F., Przybylski P. „16-Membered Macrolide Lactone Derivatives Bearing a Triazole-Functionalized Arm at the Aglycone C13 Position as Antibacterial and Anticancer Agents" *ChemMedChem*, (**2016**), 1886-1891 DOI:10.1002/cmdc.201600250, link	3.225	3.3
30. **Pyta K.**, Blecha M., Janas A., Klich K., Pecyna P., Gajecka M., Przybylski P., „Synthesis, structure and antimicrobial evaluation of a new gossypol triazole conjugates functionalized with aliphatic chains and benzyloxy groups" *Bioorg. Med. Chem. Lett*, 26 (17), (**2016**), 4322-4326, [**H1**] DOI:10.1016/j.bmcl.2016.07.033, link	2.454	2.6
31. Rogalski S., Zak P., Tadeuszyk N., **Pyta K.**, Przybylski P., Pietraszuk C., „The mechanism of activation of amidobenzylidene ruthenium chelates-latent catalysts of olefin metathesis" *Dalton Transactions*, 46 (4), (**2017**), 1277-1282 DOI:10.1039/c6dt04290b, link	4.099	3.7

32. Wicher B., **Pyta K.**, Przybylski P., Gdaniec M., „Solvates of zwitterionic rifampicin: recurring packing motifs via nonspecific interactions" *Cryst. Growth Des.*, 18 (2), (**2018**), 742–754 DOI:10.1021/acs.cgd.7b01121, link	4.153	3.6
33. Przybylski P., Pyta-Klich K., **Pyta K.**, Janas A., „Cascade reactions as efficient and universal tools for construction and modification of 6-, 5-, 4- and 3-membered sulfur heterocycles of biological relevance" *Tetrahedron*, 74(44), (**2018**), 6335-6365 DOI:10.1016/j.tet.2018.09.022, link	2.379	1.9
34. **Pyta K.**, Janas A., Szukowska M., Pecyn P., Jaworska M., Gajecka M., Bartl F., Przybylski P., „Synthesis, docking and antibacterial studies of more potent amine and hydrazone rifamycin congeners than rifampicin" *Eur. J. Med. Chem.*, 167, (**2019**), 96-104, [**H5**] DOI:10.1016/j.ejmech.2019.02.009, link	5.573	6.5
35. **Pyta K.**, Janas A., Skrzypczak N., Schilf W., Wicher B., Gdaniec M., Bartl F., Przybylski P., „Specific Interactions between Rifamycin Antibiotics and Water Influencing Ability To Overcome Natural Cell Barriers and the Range of Antibacterial Potency" *ACS Infectious Diseases Article* 5(10), (**2019**), 1754-1763 DOI:10.1021/acsinfecdis.9b00176, link	4.614	5.1
36. Skrzypczak N., **Pyta K.**, Ruszkowski P., Gdaniec M., Bartl F., Przybylski P., „Synthesis, structure and anticancer activity of new geldanamycin amine analogs containing C(17)- or C(20)- flexible and rigid arms as well as closed or open *ansa*-bridges" *Eur. J. Med. Chem.*, 202, (**2020**), Article 112624 DOI:10.1016/j.ejmech.2020.112624, link	6.514	6.5
37. Skrzypczak N., **Pyta K.**, Ruszkowski P., Mikołajczak P., Kucińska M., Murias M., Gdaniec M., Bartl F., Przybylski P., „ Anticancer activity and toxicity of new quaternary ammonium geldanamycin derivative salts and their mixtures with potentiators" *J. Enz. Inhibit. Med. Chem.*, 36, (**2021**), 1898-1904 DOI:10.1080/14756366.2021.1960829, link	5.756	5.2
38. Janas A., **Pyta K.**, Gdaniec M., Przybylski P., „ An Approach to Modify 14-Membered Lactone Macrolide Antibiotic Scaffolds" *J. Org. Chem.*, 87, (**2022**), 3758-3761 DOI:10.1021/acs.joc.1c02799, link	3.6	3.4
39. **Pyta K.**, Skrzypczak N., Ruszkowski P., Bartl F., Przybylski P., „Regioselective approach to colchiceine tropolone ring functionalization at C(9) and C(10) yielding new anticancer hybrid derivatives containing heterocyclic structural motifs" *J. Enz. Inhibit. Med. Chem.*, 37, (**2022**), 597-605 DOI:10.1080/14756366.2022.2028782, link	5.6	5.2
40. Skrzypczak N., **Pyta K.**, Bohusza W, Leśniewska A., Gdaniec M., Ruszkowski P., Schilf W., Bartl F., Przybylski P., „Cascade transformation of the ansamycin benzoquinone core into benzoxazole influencing anticancer activity and selectivity" *J. Org. Chem.*, 87, (**2023**), DOI: 10.1021/acs.joc.3c00493 DOI:10.1021/acs.joc.3c00493, link	3.6	3.4
In total, after the Ph.D	**84.308**	**87.7**
Average/publication	**3.803**	**3.955**
In total	**126.529**	**130.438**
Total average	**3.163**	**3.261**

7.2 Granty i projekty naukowe

before obtaining a Ph.D

1. „IUVENTUS PLUS" financed by the Ministry of Science and Higher Education, grant number: 0366/IP3/2013/72, grant title: "Design and synthesis of a new class of triazole derivatives of gossypol as effective fungicides, using click chemistry", implementation period: 2013-2015 , funding amount: PLN 227.650, **MANAGER OF THE PROJECT**.
2. „Miniatura" financed by the National Science Center, grant number: Nr DEC-2023/07/X/ST5/00689, grant title: "Study of the reactivity of the modified aglycone of 14-membered lactone macrolides", implementation period from October 5 2023, subsidy amount: PLN 49,991, **MANAGER OF THE PROJECT**.
3. „SONATA BIS 1" financed by the National Science Center, grant number: 2012/05/E/ST5/03792, grant title: "Modifications of leukomycin aglycone by Michael addition and dipolar Huisgen cycloaddition as new ways to obtain inhibitors of the 50S ribosomal subunit", implementation period: in 2013-2016, funding amount: PLN 986,800, **CONTRACTOR**.
4. „OPUS 3" financed by the National Science Center, grant number: 2011/03/B/ST5/01014, grant title: "Design and synthesis of new rifampicin analogues as a source of effective inhibitors of bacterial RNA polymerase", implementation period: in 2012-2015, funding amount: 615 000 PLN, **MAIN CONTRACTOR**.
5. „OPUS 10" financed by the National Science Center, grant number: UMO-2015/19/B/ST5/00231, grant title: "Functionalization of the 14-membered lactone ring of Erythromycins as a new source of semi-synthetic macrolide antibiotics", implementation period: in 2016-2020, funding amount: 745 500 PLN, **CONTRACTOR**.
6. OPUS 13 financed by the National Science Center, grant number: UMO-2017/25/B/ST5/00291 grant title: "Chemical modifications of the macrocyclic polyketide -geldanamycin opening access to new alternative anticancer agents blocking the activity of Hsp90 chaperone proteins", implementation period: in 2018-2021, funding amount: 1 175 000 PLN, **CONTRACTOR**.

Confirmation of granting, conducting and participating in the project in Annex 8.

7.3 Awards and distinctions

before obtaining a Ph.D

1. 2011 Laureat stypendium Fundacji na Rzecz Nauki Polskiej „START – Stypendium dla młodych utalentowanych naukowców"
2. 2011 Stypendysta Fundacji im. Adama Mickiewicza
3. 2012 Stypendysta Miasta Poznania

after obtaining a Ph.D

1. **2020** Nagroda II klasy Rektora UAM

Confirmation of Awards and distinctions in Annex 8.

7.4 Future plans

Due to the fact that I became a winner of the "Miniatura edition 7" program (confirmation in Annex 8), my attention in the near future will be focused on 14-membered macrolide antibiotics. The main assumption of this project is to investigate the reactivity of the compounds obtained and described in the work (Janas A., Pyta K., Gdaniec M., Przybylski P., "An Approach to Modify 14-Membered Lactone Macrolide Antibiotic Scaffolds" J. Org. Chem., 87, (2022), 3758-3761, DOI:10.1021/acs.joc.1c02799, link). The synthesized compounds may turn out to be precursors of the next generation of antibiotics from the group of 14-membered macrolides and for this reason they will be tested for biological activity - antibacterial and anticancer tests. However, determining the reactivity of the modified aglycone will allow for precise planning of the type of expected derivatives with a specific biological activity profile. As a result, the results of these studies will make it possible to rationally plan the type of substituent introduced into the aglycone part of macrolide antibiotics in order to maximize the biological effect (antibacterial and anticancer activity).

Taking into account the experience gained in the field of ansamycin antibiotics, I would like to use this knowledge to design and obtain a new class of ansamycins with a broad spectrum of activity (especially against Gram-negative bacteria). At this point, I would like to point out that proving the conformational lability of the ansamycin bridge of rifamycins in solutions containing water molecules (**RFB** and **RFX**) sheds new light on the process of transport of these antibiotics through cell membranes and bacterial cell walls. Based on these observations, the structures of these antibiotics can be planned more effectively to highlight as much as possible the structural elements contributing to high antibacterial activity and to improve the process of migration through the biological barriers of these antibiotics.

However, what has inspired me the most in my adventure with science so far concerns broadly understood intramolecular reactions that drive fascinating transformation processes (from my experience: 14- and 16-membered macrolides and their reactions and the formation of heterocyclic ansamycin systems: benzoxazole for amine derivatives of geldanamycin and benzoquinazoline system for amine derivatives of **Ral**). In the future, I would like to develop

and test the reactivity of systems of this type and similar ones, not only to develop chemistry, but above all to search for new bioactive compounds.

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(podpis wnioskodawcy)