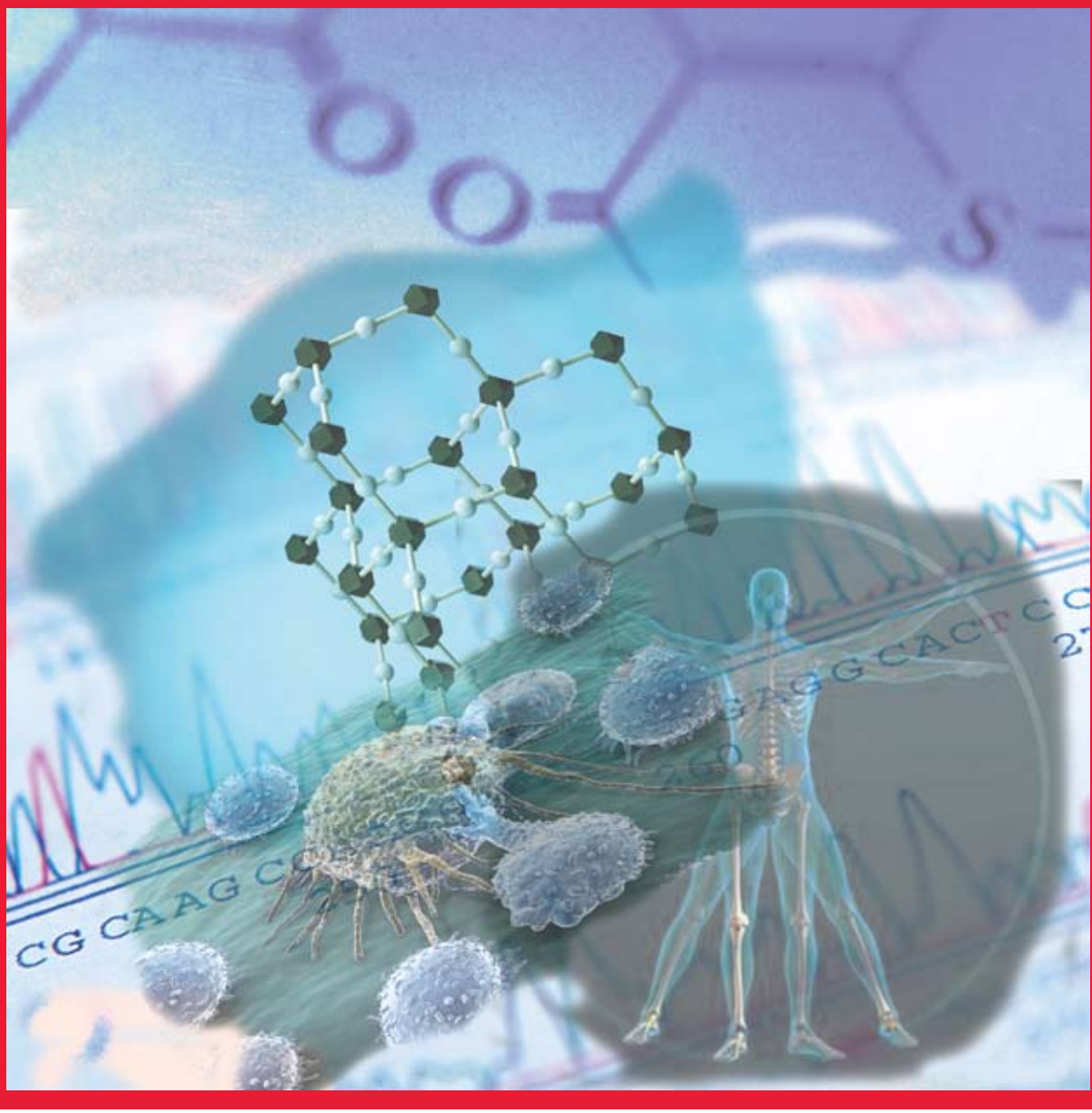
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# Design of dual action antibiotics as an approach to search for new promising drugs

**A N Tevyashova, E N Olsufyeva, M N Preobrazhenskaya (deceased)**

*Gause Institute of New Antibiotics*
*ul. B. Pirogovskaya 11, 119021 Moscow, Russian Federation*

The review is devoted to the latest achievements in the design of dual action antibiotics — heterodimeric (chimeric) structures based on antibacterial agents of different classes (fluoroquinolones, anthracyclines, oxazolidines, macrolides and so on). Covalent binding can make the pharmacokinetic characteristics of these molecules more predictable and improve the penetration of each component into the cell. Consequently, not only does the drug efficacy increase owing to inhibition of two targets but also the resistance to one or both antibiotics can be overcome. The theoretical grounds of elaboration, design principles and methods for the synthesis of dual action antibiotics are considered. The structures are classified according to the type of covalent spacer (cleavable or not) connecting the moieties of two agents. Dual action antibiotics with a spacer that can be cleaved in a living cell are considered as dual action prodrugs. Data on the biological action of heterodimeric compounds are presented and structure – activity relationships are analyzed.
The bibliography includes 225 references.

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## I. Introduction

Antibiotics (from ἀντι meaning against and βιος meaning life in ancient Greek) are substances of natural origin or their synthetic and semisynthetic analogues that suppress the growth of cells, most often, prokaryotic or protozoa cells. Traditionally, antibacterial agents were classified into natural (proper antibiotics, for example, penicillin), semisynthetic (modification products of natural molecules) and synthetic (for example, sulfamides and nitrofurans). Currently this classification is no longer valid, because a number of natural antibiotics are prepared chemically (levomycetin) and some agents called antibiotics (fluoroquinolones) are actually synthetic compounds.

The discovery of sulfamides and β-lactams in the 1930s was a real revolution in the treatment of infectious diseases. The views of incurability of bacterial infections such as tuberculosis, syphilis, sepsis and so on became a thing of the past. The golden age of research in the field of antibiotics lasted approximately 40 years in which virtually all main classes of antibiotics were discovered. However, from the early 1970s up to the end of the 20th century, only analogues of known drugs were manufactured and introduced into clinical practice. The only exception was mupirocin, which is a local action antibiotic active against gram-positive bacteria, authorized for use in 1985. The situation has improved since 2000, as five new classes of antibiotics were discovered: oxazolidinones (linezolid, 2000), cyclic lipopeptides (daptomycin, 2003), pleuromutilins (retapamulin, 2007), fidaxomicin (2010) and bedaquiline (2012).[1] However, the application of antibiotics of these classes has been limited to treatment of diseases caused by gram-

**A N Tevyashova** Ph.D. Senior Researcher at the Laboratory of Chemical Transformation of Antibiotics of the GINA. Telephone: (7-499) 246 06 36, e-mail: chulis@mail.ru
**Current research interests**: chemical modification of antibiotics, structure – activity relationships, mechanisms of action of antibiotics.
**E N Olsufyeva** Doctor of Chemical Sciences, Professor, Leading Researcher at the same laboratory. Telephone: (7-499) 246 06 36, e-mail: eolsufeva@list.ru
**Current research interests**: chemical modification of antibiotics, structure – activity relationships, mechanisms of action of antibiotics, organic synthesis, peptides, glycosides.
**M N Preobrazhenskaya (deceased)** Doctor of Chemical Sciences, Professor.
**Research area**: chemical modification of antibiotics, antitumour agents, antimicrobial agents, structure – activity relationships, macrolides, organic synthesis, glycosides.

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positive bacteria, whereas currently of high demand are agents having high activity against gram-negative microorganisms and fungi.

Depending on the action on microorganisms, antibacterial, antifungal, antiviral and antiparasitic antibiotics can be distinguished. In addition, some classes of antibiotics have antitumour activities (anthracyclines, aureolic acid group antibiotics, actinomycin, bleomycitin, bleomycin, mitomycin) or have antiinflammatory (doxycycline and minocycline)[2,3] or immunomodulatory action (cyclosporin A).[4] The therapeutic action of antibiotics is based on suppression of vital activity of the cell as a result of inhibition of a metabolic process more or less specific to the microbe (Table 1).

Despite the high efficiency of treatment of many infectious diseases, the scope of application of antibiotics is largely restricted by the side effects that accompany the use of these drugs. Mainly, these are allergic and toxic responses (examples are the cochlear neuritis caused by aminoglycosides,[8] hepatotoxic action of tetracyclines, rifampicin and erythromycin,[9] hemato- and nephrotoxicity caused by polyene antibiotics[10]) or chemotherapeutic side effects of taking antibiotics: bacteriolysis,[11] dysbacteriosis,[12] superinfecions and so on. The toxic effects of antitumour antibiotics are especially pronounced; in particular, anthracycline antibiotics exhibit high cardiotoxicity, mutagenicity and carcinogenicity.[13,14]

However, the key problem of modern antibacterial therapy is the permanently growing resistance of pathogenic bacteria to the antibiotics used (see Table 1).[15,16] The antibiotic resistance of microorganisms can be either natural[17] or acquired.[18,19] The major mechanisms of development of bacterial resistance to antibacterial agents are the following: decrease in the affinity of the agent for the antibiotic target, inactivation of the antibiotic in the cell, active efflux of the antibiotic from the microbial cell and deterioration of the permeability of the external structures of the microbial cell.[20,21]

The spread of antibiotic resistance decreases the efficiency of therapy and, hence, results in more severe and prolonged diseases, increases the frequency of hospital admissions of patients and the number of lethal outcomes; this also enhances the economic loss for the society. The spread of drug-resistant pathogenic bacteria has led to a steady increase in the frequency of bacterial diseases that were successfully treated quite recently. The death rate upon sepsis, tuberculosis and pulmonary fevers sharply increased because the pathogens acquired resistance to the antibacterial agents used. For example, more than 23 000 patients die annually in the USA due to infections caused by antibiotic-resistant bacteria.[22]

The side effects of taking antibiotics and especially the appearance and spread of antibiotic resistance of pathogens have resulted (and would result) in the loss of clinical significance of some drugs, which stimulated the search for

**Table 1**. Key chemical classes of antibiotics.[5]

| Class of compounds (representative) | Year of discovery | Year of commercialization | Year of resistance detection | Spectrum of action |
|---|---|---|---|---|
| Inhibition of peptidoglycan biosynthesis | | | | |
| β-Lactams (penicillin) | 1928 | 1939 | 1945 | broad |
| Glycopeptides (vancomycin) | 1953 | 1958 | 1960 | gram-positive bacteria |
| D-Cycloserine | 1955 | 1964 | 1972 | broad, including mycobacteria |
| Inhibition of protein biosynthesis | | | | |
| Macrolides (erythromycin) | 1948 | 1951 | 1955 | broad |
| Oxazolidinones (linezolid) | 1955 | 2000 | 2001 | gram-positive bacteria |
| Tetracyclines (tetracycline) | 1944 | 1952 | 1950 | broad |
| Aminoglycosides (streptomycin) | 1943 | 1946 | 1946 | " |
| Lincosamides (lincomycin) | 1962 | 1964 | 1968 | gram-positive bacteria, mycoplasma spp |
| Inhibition of DNA transcription and replication | | | | |
| Fluoroquinolones (ciprofloxacin) | 1961 | 1968 | 1968 | broad |
| Anthracyclines (daunorubicin) | 1950 | 1967 | | antitumour action |
| Aureolic acid group (mitramycin) | 1953 | 1970 | | " |
| Bleomycin (bleomycin $A_2$) | 1966 | 1973 | | " |
| Ansamycins (rifampin) | 1957 | 1958 | 1962 | gram-positive bacteria, mycobacteria |
| Inhibition of folic acid metabolism | | | | |
| Sulfamides (prontosil) | 1932 | 1936 | 1942 | gram-positive bacteria |
| Benzyldiaminopyrimidines (trimetoprim)[a] | 1962 | 1968 | 1972 | broad |
| Action on the cytoplasmic membrane | | | | |
| Lipopeptides (daptomycin) | 1986 | 2003 | 1987 | gram-positive bacteria |
| Polyenes (amphotericin B)[b] | 1953 | 1958 | | antifungal action |

[a] See Ref. 6, [b] see Ref. 7.

ways to overcome the difficulties. Solving the problem of treatment of infections caused by multi-drug resistant bacteria is related to both the development and implementation of decisive and adequate measures on the control of antibiotic resistance [23] and the search for new antimicrobial agents active against resistant bacteria.

The modern routes of the search for new antibacterial agents include:

(1) biotechnological approach based on the use of conventional or genetic engineering methods to create libraries of new natural compounds, activation of cryptic genes of bacteria, genome mining, synthetic biology;

(2) chemical (synthetic) approach, including rational drug design, combinatorial synthesis, total synthesis (this approach has an applied value mainly for compounds with moderate molecular mass);

(3) combined (semisynthetic) approach: modification of known natural antibiotics by chemical or genetic engineering methods.[15]

Each approach has its own advantages and drawbacks; however, currently, the last-mentioned approach is considered to be most promising. Among the 22 new antibiotics authorized for use since 2000, only two drugs (daptomycin and fidaxomicin) refer to natural antibiotics, ten drugs are synthetic (nine of these belong to the known class of quinolones and fluoroquinolones), and ten drugs are derivatives of natural antibiotics.[1]

A promising line of the combined (semisynthetic) approach is preparation of dual action antibiotics — heterodimers formed by drugs of different classes. The present review is devoted to achievements of the last 10–15 years in this area.

## II. Combination therapy and theoretical grounds of the development of dual action antibiotics. Overcoming the resistance

One of the ways for overcoming the drug resistance of microorganisms or tumour cells that has shown successful results is combination therapy, *i.e.*, simultaneous use of two or more therapeutic agents (Fig. 1).[24, 25]

The clinical efficacy of combination therapy for preventing development of antibiotic resistance was convincingly demonstrated in relation to the treatment of tuberculosis.[26] However, combination therapy has a number of serious drawbacks because the effect of a particular combination observed *in vitro* is not always manifested *in vivo*. This is a consequence of different pharmacokinetic characteristics of the drugs contained in the combination. Furthermore, this strategy is inapplicable to multi-drug resistant bacteria that are resistant to any of the antibiotics used separately. Therefore, an approach was proposed that is supposed to be able to overcome the drawbacks of combination therapy: the design of dual action antibiotics. In the literature, these structures are also referred to as 'chimeric † antibiotics'.

† Chimera (from Χιμαιρα meaning a young goat in ancient Greek): in Greek mythology, according to Homer, this is a monster having the lion head and neck and the goat body. However, in Theogony, Hesiod depicts it as having three heads: a goat head in the middle of the chine, a snake head on one end of the body, and a lion head on the other end of the body.

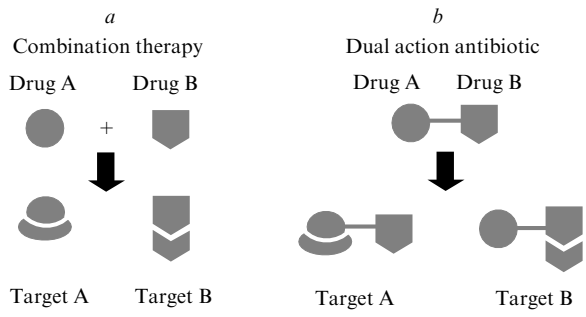



**Figure 1.** Two strategies for overcoming the resistance.[24]

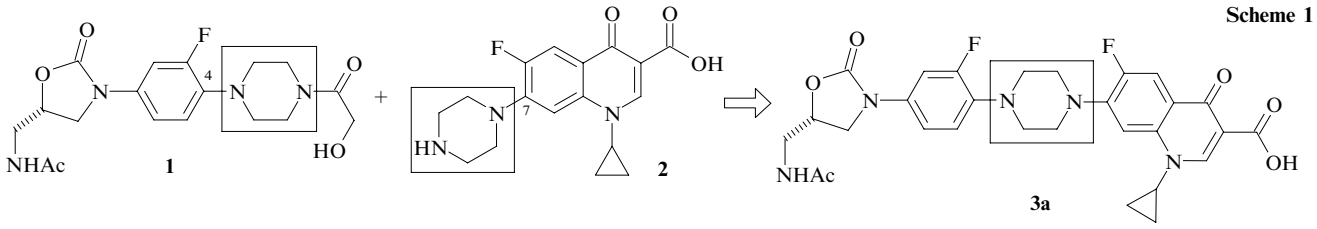
The development of dual action antibiotics refers to the general twin drug strategy, *i.e.*, the use of pharmaceuticals containing two pharmacophoric groups connected by a covalent bond to form a single molecule (see Fig. 1 *b*). The two moieties of the twin drug can be connected *via* a spacer either directly or with a slight overlap. In the literature, these compounds are also referred to as hybrid compounds or bivalent compounds. Presumably, the covalent bonding makes the pharmacokinetic characteristics of the resulting molecule more predictable. In addition, high penetrability of one of the antibiotics contained in this molecule can enhance the penetration and, hence, cellular accumulation of the other antibiotic, which in turn can lead to better inhibition of the targets of both antibiotics. As a result, the resistance to one or both antibiotics contained in the dual action molecule can be overcome.[27] The covalent bridge between two antibiotic molecules in the chimeric structure can be designed as a cleavable spacer, and therefore this structure can act as a prodrug. Meanwhile, a non-cleavable covalent spacer between two antibiotic molecules provides a dual mechanism of action.

Thus, the development of dual action antibiotics currently appears to be a promising strategy aimed at the manufacture of drugs active against resistant microorganisms, having a broad spectrum of action compared with the initial antibiotics and retarding the development of antibiotic resistance.

The data presented below summarize the synthetic approaches to the development of dual action antibiotics. The drugs are classified, first, according to the type of a covalent spacer (cleavable or non-cleavable) connecting the two drug molecules. Further division into classes was performed in terms of the chemical structure of one of the antibiotics contained in the molecule. This classification is rather arbitrary, and classes overlap to a considerable extent.

Reviews on dual action antibiotics can be found in the literature, including the reviews by Pokrovskaya and Baasov [27] and Bremner *et al.*[28] However, the authors of the former review mainly restricted themselves to consideration of the structures of dual action antibiotics with a non-cleavable spacer (without considering the syntheses), whereas the latter review is devoted to dual action antibiotics as prodrugs, while compounds of other types are only briefly discussed.

**Scheme 1**



## III. Dual action antibiotics containing a non-cleavable covalent spacer

### III.1. Antibiotics based on quinolones

Dual action antibiotics containing a quinolone moiety, most often, a fluoroquinolone moiety covalently bonded to another antibacterial agent are represented most extensively in the literature. This is due to several factors. First, quinolones have an intricate mechanism of action including inhibition of bacterial topoisomerase II or IV or DNA gyrase.[29] Thus, the possible weakening of the quinolone interaction with one of the targets due, for example, to steric hindrance caused by the attached linker and a molecule of another antibacterial agent can be counterbalanced by interaction with another target. Second, the structure–activity relationships have been thoroughly studied for quinolones and it was shown that the amino group of the piperidine residue in position 7 of the quinolone can be easily modified without the loss of antibacterial activity.[30] Quinolones are rather chemically stable and, hence, they are promising candidates for the design of chimeric antibiotics. Note, however, that resistance to this class of antibacterial agents is rapidly formed. The principal mechanisms of resistance to fluoroquinolones comprise chromosomic mutations of the genes encoding fluoroquinolone targets — DNA gyrase and topoisomerase IV and mutations of the genes responsible for regulation of the expression of the AcrAB-TolC transmembrane efflux pump accomplishing the active transport of fluoroquinolones out of the cell.[31–34]

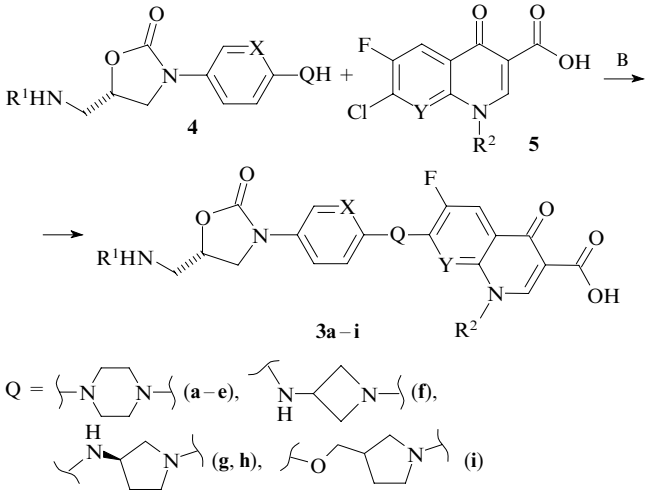
#### III.1.a. Combination of fluoroquinolones and oxazolidinones

The fluoroquinolone–oxazolidinone hybrid compounds are among the most numerous and successful types of dual action antibiotics containing fluoroquinolones.

Oxazolidinones inhibit the protein synthesis in a bacterial cell by being bonded to the domain V of the 23S ribosomal RNA (rRNA) in the peptidyl transferase site of the ribosomal 50S subunit.[35] Oxazolidinones are of low activity with respect to gram-negative bacteria because they poorly penetrate through their external membranes. Development of the resistance to oxazolidinone is related to the decrease in the affinity of 23S rRNA to antibiotics, most mutations being due to replacement of the guanine nucleic base by cytosine in the 2576 position of the peptidyl transferase site of 23S rRNA.[35] Since fluoroquinolones readily penetrate through a cellular membrane of gram-negative bacteria, it was assumed that preparation of a dual action antibiotic based on hydrophobic fluoroquinolone would improve penetration of oxazolidinone into the bacterial cell, extend the spectrum of antibacterial activity of the drugs and induce activity with respect to resistant strains.

In 2003, two research teams, Hubschwerlen *et al.*[36] and Gordeev *et al.*,[37] reported dual action antibiotics based on eperezolid (**1**) and ciprofloxacin (**2**). Since molecules of both drugs contain a piperazine moiety (in position 4 of eperezolid and in position 7 of ciprofloxacin), they were combined into one chimeric structure *via* these positions (Scheme 1). Not only the piperazine ring (compound **3**) but also other heterocycles (Q) have been used as spacers (Scheme 2).

**Scheme 2**

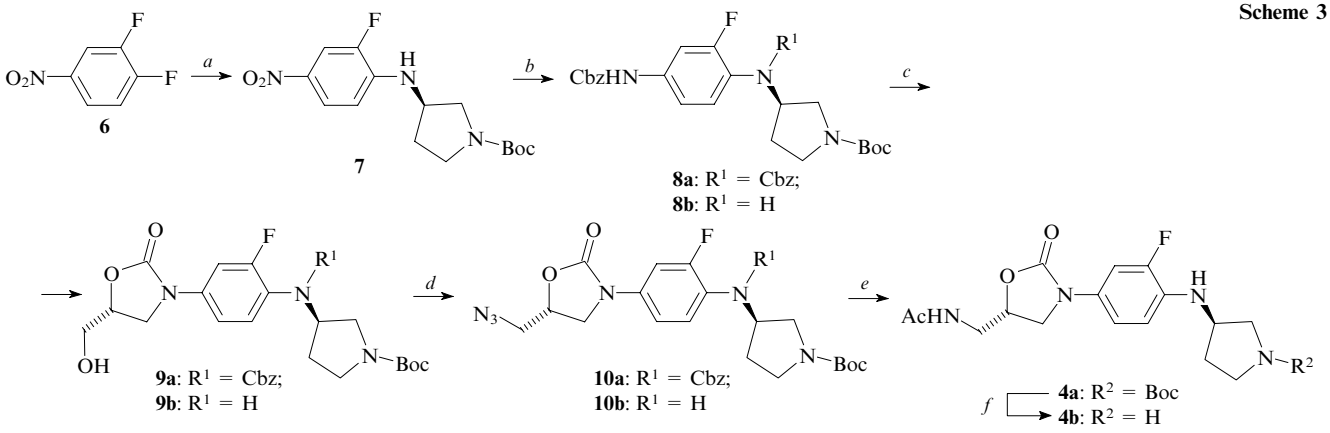


| Compound **3** | $R^1$ | X | Y | $R^2$ | Yield (%) |
|---|---|---|---|---|---|
| **a** | Ac | CF | CH | cyclo-$C_3H_5$ | 26 |
| **b** | Ac | CF | N | cyclo-$C_3H_5$ | 77 |
| **c** | Ac | N | N | cyclo-$C_3H_5$ | 47 |
| **d** | C(=S)OMe | CF | N | cyclo-$C_3H_5$ | 23 |
| **e** | Ac | CF | $COCH_2N(Me)$ | | 21 |
| **f** | Ac | CF | N | cyclo-$C_3H_5$ | 30 |
| **g** | Ac | CF | CH | cyclo-$C_3H_5$ | 5 |
| **h** | Ac | CF | N | cyclo-$C_3H_5$ | 5 |
| **i** | Ac | CF | N | cyclo-$C_3H_5$ | – |

The key step of the synthesis of oxazolidinone- and quinolone-based dual action antibiotics (compounds **3a–i**) was the condensation of compounds **4** containing the terminal basic nitrogen atom in the substituent in the aromatic ring with 7-chloro derivatives of quinolone **5**. The reaction was performed in the presence of organic bases (B) such as 1,4-diazabicyclo[2.2.2]octane (DABCO) and *N*,*N*-dimethyl-*p*-toluidine (see Scheme 2).[36,37] Quinolone **5** was pre-activated by either preparation of boron complexes, as reported in a patent,[38] or heating with chlorotriethylsilane in the presence of triethylamine, resulting in the corresponding trimethylsilyl derivative.[36]

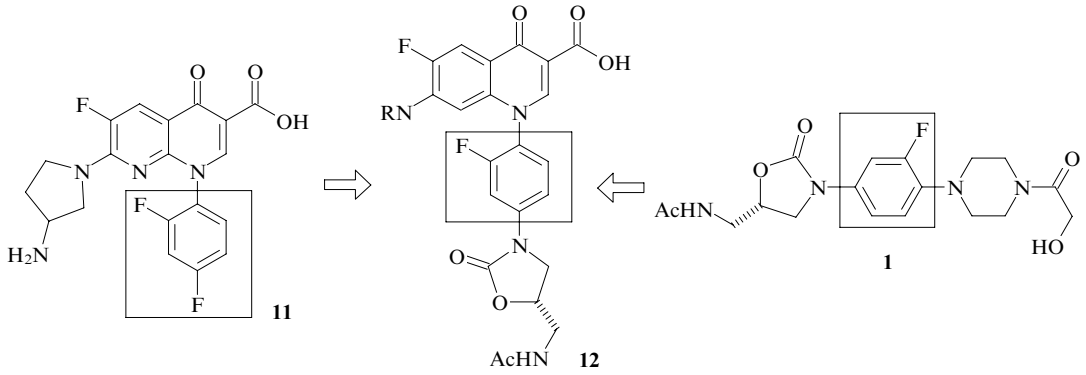
Compounds **4** were synthesized from 3,4-difluoronitrobenzene (**6**) by adjusting the procedure developed for the synthesis of eperezolid.[39] This is exemplified in Scheme 3, which depicts the synthesis of derivatives **4a**,**b** *via* successive transformations of intermediates **7–10**.[36] Note

**Scheme 3**



(*a*) (1) 3-(*R*)-Allyloxycarbonyl-3-aminopyrrolidine, $Et_3N$; 2) $Bu^n_3SnH$, AcOH, $PdCl_2(PPh_3)_2$; 3) $Boc_2O$; (*b*) 1) $H_2$, Pd/C; 2) CbzCl, $NaHCO_3$; (*c*) $Bu^nLi$, (*R*)-glycidyl butyrate; (*d*) 1) MsCl, $Et_3N$; 2) $NaN_3$; (*e*) 1) $H_2$, Pd/C; 2) $Ac_2O$, AcOH; (*f*) $Et_3SiH$, TFA; Boc is *tert*-butyloxycarbonyl, Cbz is benzyloxycarbonyl, Ms is methanesulfonate, TFA is trifluoroacetic acid

**Scheme 4**



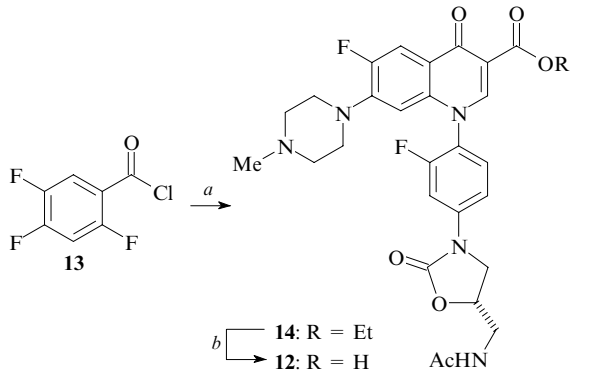
that the authors used inseparable mixtures of derivatives **8**–**10** containing secondary (**b**) and tertiary (**a**) amino groups.

Derivatives **3a**–**i** differ in the nature of the spacer and the structure of the initial fluoroquinolone **2**. As the quinolone antibiotics, ciprofloxacin (Y = CH, $R^2$ = cyclo-$C_3H_5$), norfloxacin (Y = CH, $R^2$ = Et) and naphthyridone (Y = N, $R^2$ = cyclo-$C_3H_5$) have been used.[36,37]

Gordeev *et al.*[37] attempted another strategy for combining the fluoroquinolone and oxazolidinone structures. In this case, the fluorophenyl substituent of tosufloxacin (**11**) and eperezolid (**1**) served as the common moiety (Scheme 4). Compound **12** was synthesized from 2,4,5-trifluorobenzoyl chloride (**13**) *via* intermediate ester **14** as shown in Scheme 5.

The prepared chimeric antibiotics **3a**–**h** and **12** were tested for antibacterial activities with respect to gram-positive and gram-negative bacteria, including linezolid- and ciprofloxacin-resistant strains. It was found that the mode of coupling the fluoroquinolone and oxazolidinone structures plays an important role in manifesting the biological activity. Indeed, compound **12** in which the oxazolidinone pharmacophore was attached to nitrogen in position 1 of fluoroquinolone *via* an aromatic spacer proved to be inactive.[37] Meanwhile, compounds **3a**–**h** having oxazolidinone and fluoroquinolone moieties connected by a heterocyclic spacer showed a high antibacterial activity.[36,37] For the series of derivatives **3a**–**h**, an important role of the spacer structure was ascertained: antibiotics **3a**–**e** containing a piperazine ring resembled linezolid in

**Scheme 5**



(*a*) (1) Ethyl 3-(dimethylamino)acrylate, $Et_3N$, dioxane, rt; (2) (*S*)-5-acetamidomethyl-3-(4-amino-3-fluorophenyl)oxazolidinone, 4-methylmorpholine, NMP, 110 °C (3) DBU, NMP, 85 °C; (4) *N*-methylpiperazine, NMP, 80 °C; (*b*) (1) aq. HCl, 80 °C; (2) $Ac_2O$, polyvinylpyridine, MeCN; NMP is *N*-methyl-2-pyrrolidone, DBU is 1,8-diazabicyclo[5.4.0]undec-7-ene

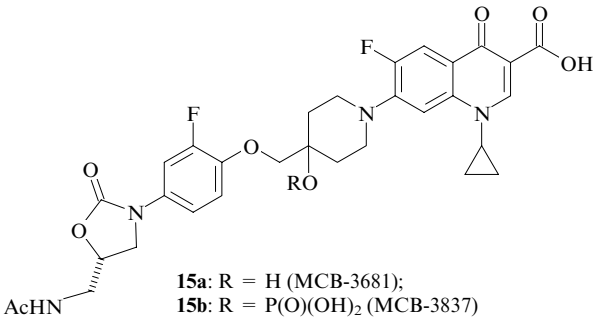
the spectrum of their antibacterial activity and were also active against quinolone-resistant strains but less active against linezolid-resistant strains. Meanwhile, the spectrum of antibacterial activity of chimeric structures **3f**–**h** containing an aminopyrrolidine or aminoazetidine spacer is similar to that of quinolones, *i.e.*, these compounds are

active against gram-negative bacteria and less active against quinolone-resistant strains.

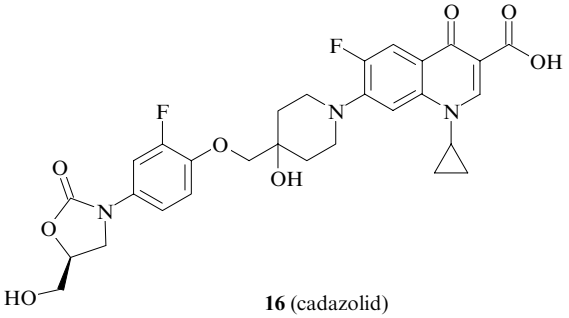
As a continuation of this research, Hubschwerlen *et al.*[40] studied the effect of the spacer between the ciprofloxacin (**2**) and eperezolid (**1**) molecules on the range and mechanism of action of the dual action antibiotics prepared according to Schemes 2 and 3. Heterocyclic bases, specifically, piperidine, pyrrolidine, azetidine, *etc.*, served as the spacers between molecules **1** and **2**. The activities of these antibiotics substantially increased when oxy- and oxymethyl-containing heterocycles were employed as the spacers. The most active derivative **3i** has a 3-oxymethylpyrrolidine spacer (both enantiomers are active). This compound shows a high activity with respect to both resistant and sensitive strains of *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *S. pneumonia*, *Enterococcus faecalis* and *E. faecium*. Chimeric antibiotic **3i** was found to efficiently inhibit protein synthesis [eperezolid (**1**) target] and the topoisomerase IV and DNA gyrase activities [ciprofloxacin (**2**) targets], *i.e.*, has a dual mode of action.[40]

The subsequent studies into the synthesis of antibiotics with dual mode of action based on fluoroquinolones and oxazolidinones were reported as patents.[41–43] It was demonstrated that among the fluoroquinolone and oxazolidinone hybrids, most promising is the antibiotic MCB-3681 (**15a**), which possesses high activity against linezolid- and fluoroquinolone-resistant gram-positive [MRSA, vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *S. pneumonia*] and gram-negative bacteria and also against *Bacillus anthracis*.[42] First, it was reported[27] that this antibiotic is going to be subjected to clinical trials as water-soluble phosphoric acid ester MCB-3837 (**15b**); however, cadazolid (**16**) having a similar structure proved to be the drug of choice. Like compounds **15a**,**b**, compound **16** contains a 4-hydroxypiperidine linker but differs in the configuration of the stereocentre and the nature of substituent in the oxazolidinone ring.

**Structures 15, 16**



**15a**: R = H (MCB-3681);
**15b**: R = P(O)(OH)$_2$ (MCB-3837)



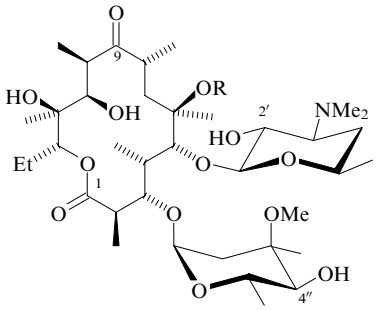
**16** (cadazolid)

Currently, cadazolid has successfully passed phase II clinical trials; in autumn 2013, the beginning of the phase III trials for treating *Clostridium difficile*-caused diarrhea was announced.

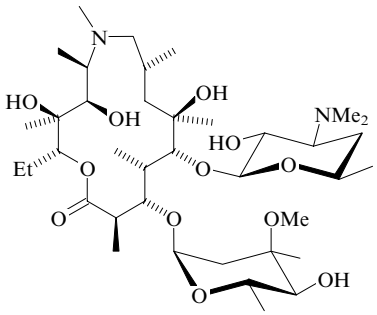
### III.1.b. Combination of quinolones and macrolides

Macrolides represent an extensive class of antibiotics with chemical structure comprising a macrocyclic lactone and one or several carbohydrate residues attached to it. Depending on the number of atoms in the ring, macrolides are subdivided into 14-membered [for example, erythromycin (**17**), clarithromycin (**18**) and roxithromycin], 15-membered [azalides, azithromycin (**19**)] and 16-membered (josamycin, midecamycin, spiramycin). Further, among 14-membered macrolides, it is customary to distinguish ketolides, which contain a keto group in position 3 of the lactone ring instead of the cladinose residue.[44–47]

**Structures 17–19**



**17**: R = H (erythromycin);
**18**: R = Me (clarithromycin)



**19** (azithromycin)

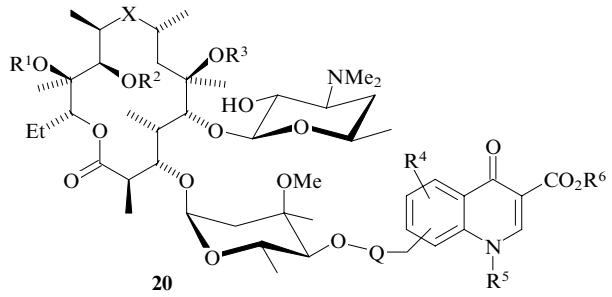
The mechanism of action of macrolides is related to disruption of the protein synthesis in the microbial cell upon interaction of the antibiotic with the domain V of 23S rRNA in the peptidyl transferase site of a ribosome. Macrolides suppress the motion of the growing peptide chain, thus inhibiting peptide chain translocation and elongation reactions.[48]

The most frequently encountered mechanisms of resistance to macrolides include the active efflux of the antibiotic out of the cell and the target modification *via* specific post-transcriptional modification (methylation) of 23S rRNA or mutations of the genes encoding either 23S rRNA or ribosomal proteins.[48]

The GlaxoSmithKline company in cooperation with other research centres carries out large-scale investigations on the synthesis and biological activity of dual action antibiotics based on macrolides and quinolones.[49–56] It was proposed to call these structures 'macrolones'.

In macrolones **20**, the macrolide and the quinolone are connected by a spacer in position 4 of the macrolide cladinose and the 6- or 7-position of the quinolone.

**Structures 20**



**20**

Various types of macrolones differing in the structures of the starting macrolide (most often, clarithromycin and azithromycin), quinolone and the spacer have been studied.[49–56]

The hydroxy groups of macrolide antibiotics considerably differ in the reactivity: the OH group in the 2-position of desosamine (3,4,6-trideoxy-3-dimethylamino-D-xylohexose) is most reactive; the second most reactive is the group in the 4-position of cladinose; and the hydroxy groups located in the macrocycle are least reactive. The difference between the reactivities of the macrolide hydroxy groups allows for their selective modification. Below we present examples of the synthesis of some macrolones.

Macrolone **20a** containing a 4″-*O*-acyl spacer with two additional ester groups was prepared in three steps from azithromycin. First, the OH group in the 2′-position of desosamine in macrolide **19** was protected, then intermediate **21** was acylated by dicarboxyl-containing quinolone **22** in the presence of water-soluble carbodiimide, and the final step was removal of the protecting 2′-*O*-acetyl group (Scheme 6).[49,50] The difference between the p$K_a$ values of the carboxy groups in quinolone derivative **22** is fairly high, which ensured the regioselective esterification.

Macrolone **20b** was synthesized in a similar way. Compound **23** served as the quinolone component. The carboxy group linked to the quinolone ring in macrolone **24** was converted to an ester group by treatment with isopropyl iodide in DMF in the presence of potassium carbonate (Scheme 7).[54]

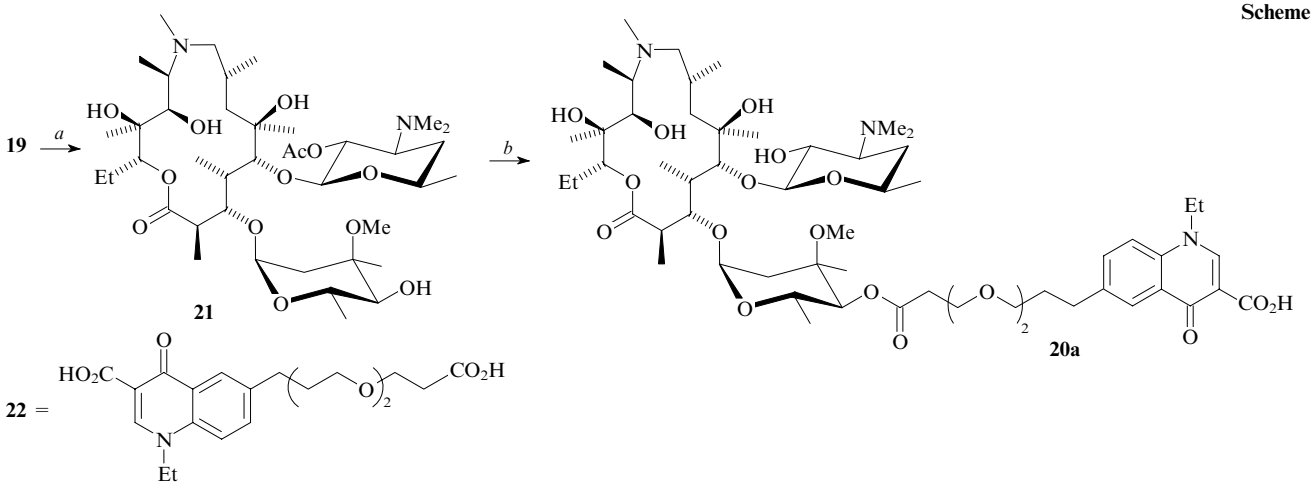
A large series of analogues of **20b** with a 4″-*O*-acyl spacer containing an ester group and another function (either an ester group or a secondary amino group) and various alkoxycarbonyl groups in position 3 of quinolone have been prepared. In this case, esterification of the carboxy group with alkyl iodides was the final step of the synthesis. Macrolides with 7-unsubstituted quinolone were prepared by catalytic hydrogenation of chloro derivatives, which was performed up to esterification of the carboxy group.

4″-*O*-Acrylic ester of the macrolide antibiotic (**25**) prepared by a reported procedure has been synthesized as the key intermediate for the synthesis of macrolones containing an alkylamine or piperazine spacer (Scheme 8).[51,53,55,56] Compound **25** was introduced into the Michael reaction with the corresponding quinolone amino derivative **26** or **27** in the presence of a base, $Et_3N$ or DBU, resulting in products **20c** or **28**. The triple bond of the latter compound was hydrogenated with hydrogen over a palladium catalyst to give compound **20d**.

2′-*O*-Acetylazithromycin 11,12-cyclic carbonate **29** served as the starting compound for the synthesis of macrolones **20e–k** containing a spacer with two ether groups (Scheme 9). The starting compound **29** was prepared from azithromycin by a reported procedure.[52]

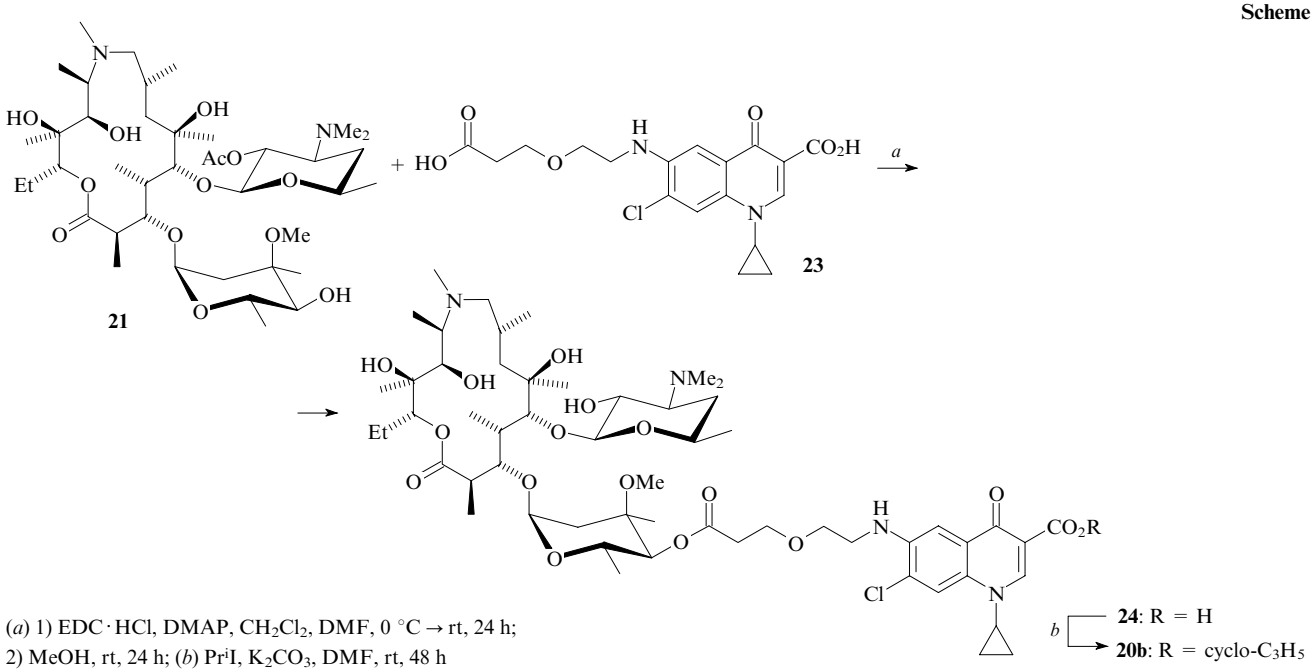
First, the 4″-*O*-hydroxy group in compound **29** was alkylated by the reaction with an excess of acrylonitrile in the presence of sodium hydride; this was followed by a series of transformations of the terminal cyano group in nitrile **30a** to the hydroxymethyl group *via* intermediate formation of derivatives **30b–f**. The central ether bond in the spacer was formed by alkylation of the terminal hydroxy group of compound **30f** with allyl *tert*-butyl carbonate in the presence of palladium(0) complexes. Then the 2′-*O*-acetyl group and the 11,12-cyclic carbonate protection in intermediate **31** were selectively removed yielding the key intermediate **32**, which was introduced in the Heck reaction with the corresponding 6-iodoquinolones **33** in the presence of $Pd(OAc)_2$ and tris(*o*-tolyl)phosphine. The final step of the synthesis was catalytic hydrogenation of the double bond in compounds **34** (see Scheme 9). The yields of the

**Scheme 6**

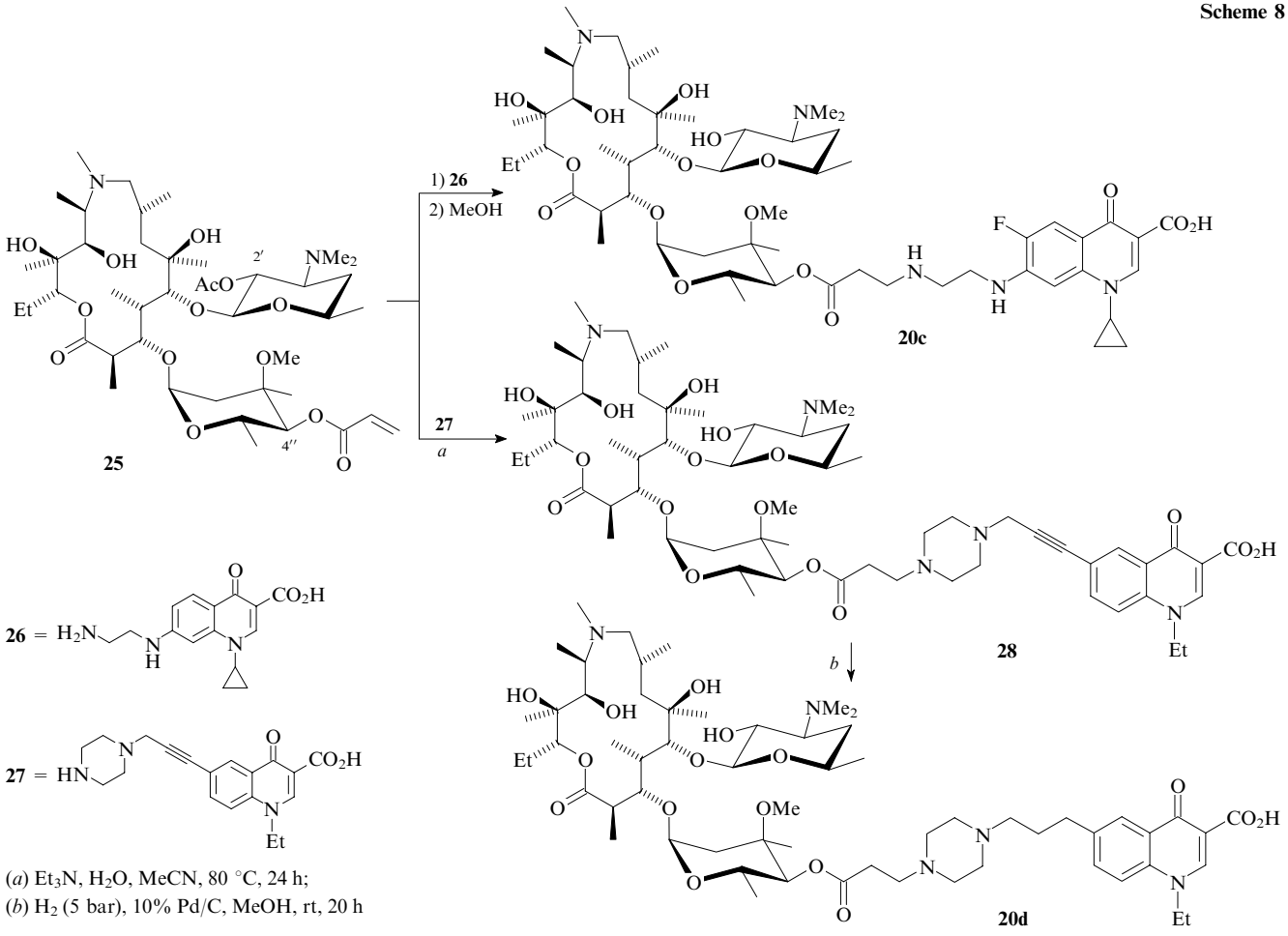


(*a*) $Ac_2O$, $NaHCO_3$, $CH_2Cl_2$, rt, 4 h; (*b*) 1) **22**, EDC·HCl, DMAP, $CH_2Cl_2$, 0 °C → rt; 2) MeOH, 55 °C, 24 h; EDC is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, DMAP is 4-dimethylaminopyridine

**Scheme 7**



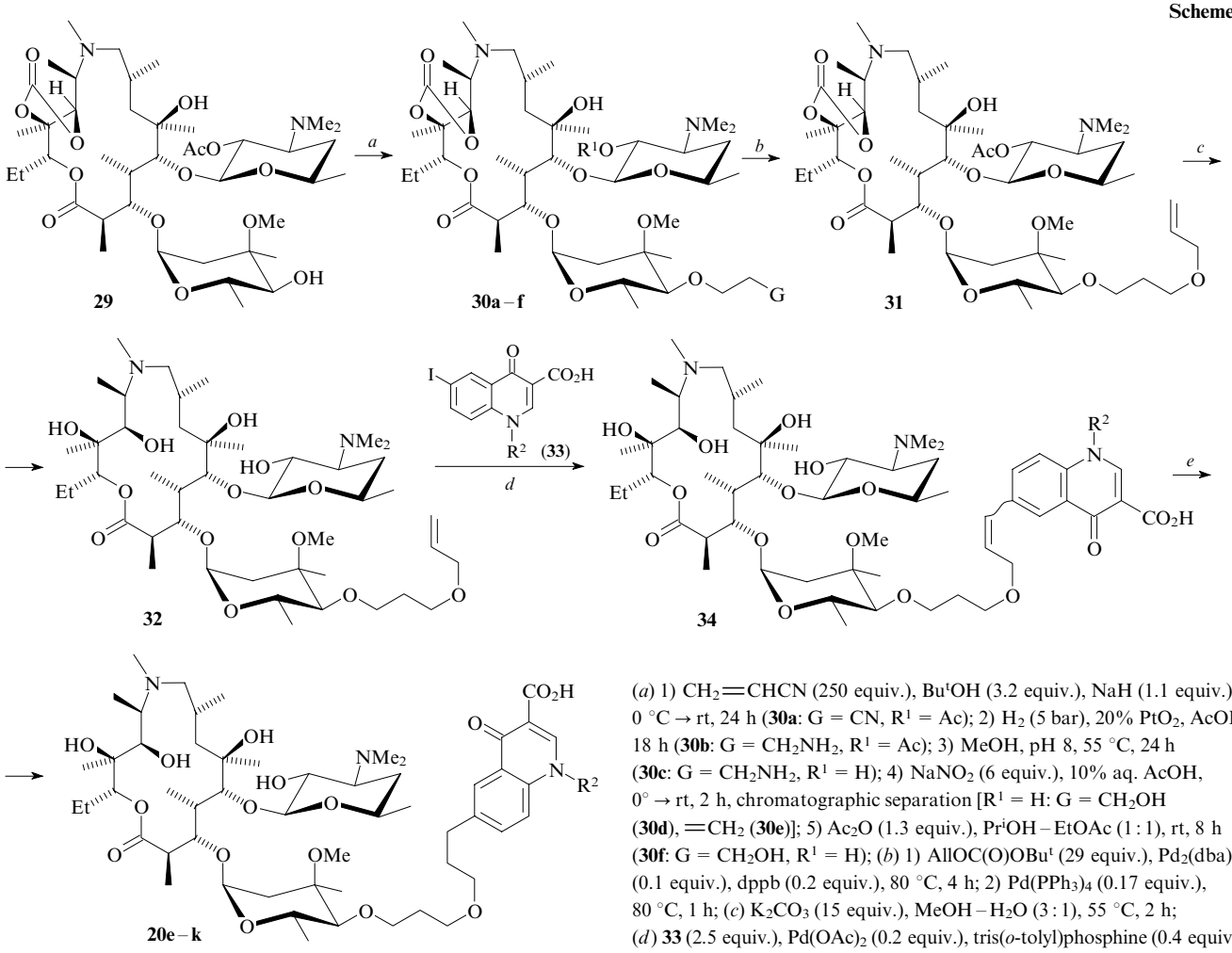

**Scheme 8**




final products were not indicated in the original publication.[52]

All of the synthesized macrolones **20** (Table 2) possessed high antibacterial activities, the minimum inhibitory concentrations (MIC) being $< 0.125\ \mu g\ ml^{-1}$ with respect to the gram-positive bacteria *S. aureus*, *S. pneumoniae* and *S. pyogenes* sensitive to erythromycin.[49–55] In most cases,

**Scheme 9**




**20**: $R^2$ = Et (**e**), $Pr^i$ (**f**), $Bu^t$ (**g**), cyclo-$C_3H_5$ (**h**), $NMe_2$ (**i**), $(CH_2)_2OH$ (**j**), $(CH_2)_2NHMe$ (**k**)

(*a*) 1) $CH_2{=}CHCN$ (250 equiv.), $Bu^tOH$ (3.2 equiv.), NaH (1.1 equiv.), 0 °C → rt, 24 h (**30a**: G = CN, $R^1$ = Ac); 2) $H_2$ (5 bar), 20% $PtO_2$, AcOH, 18 h (**30b**: G = $CH_2NH_2$, $R^1$ = Ac); 3) MeOH, pH 8, 55 °C, 24 h (**30c**: G = $CH_2NH_2$, $R^1$ = H); 4) $NaNO_2$ (6 equiv.), 10% aq. AcOH, 0° → rt, 2 h, chromatographic separation [$R^1$ = H: G = $CH_2OH$ (**30d**), $=CH_2$ (**30e**)]; 5) $Ac_2O$ (1.3 equiv.), $Pr^iOH$–EtOAc (1 : 1), rt, 8 h (**30f**: G = $CH_2OH$, $R^1$ = H); (*b*) 1) AllOC(O)$OBu^t$ (29 equiv.), $Pd_2(dba)_3$ (0.1 equiv.), dppb (0.2 equiv.), 80 °C, 4 h; 2) $Pd(PPh_3)_4$ (0.17 equiv.), 80 °C, 1 h; (*c*) $K_2CO_3$ (15 equiv.), MeOH–$H_2O$ (3 : 1), 55 °C, 2 h; (*d*) **33** (2.5 equiv.), $Pd(OAc)_2$ (0.2 equiv.), tris(*o*-tolyl)phosphine (0.4 equiv.), $Et_3N$ (4 equiv.), DMF, $N_2$, 65 °C, 2 h; then 75 °C, 18 h; (*e*) $H_2$ (3 bar), 10% Pd/C, MeOH, 15 h; dba is dibenzylideneacetone, dppb is 1,4-bis(diphenyl)phosphinobutane

these hybrid structures were active against the *S. pyogenes* strains resistant to macrolides.

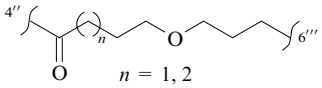
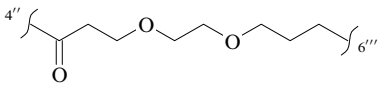
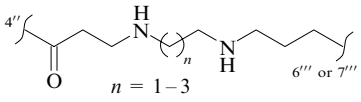
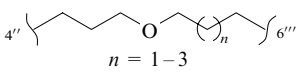
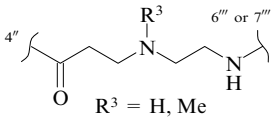
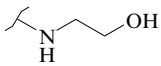
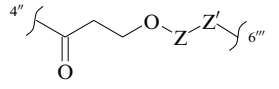
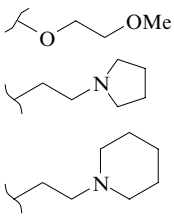
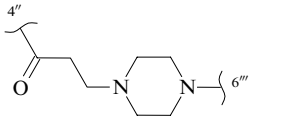
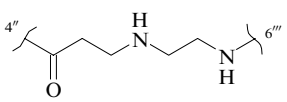
The structure–biological activity relationship was established for macrolones as a function of the macrolide structure, the length and the nature of the spacer, the site of spacer attachment to the quinolone moiety and the type of substituents at the nitrogen atom and in the 3-carboxy group of quinolone. It was found that elongation of the spacer results in a higher activity of the hybrid structure against pathogens that cause respiratory infections (especially the *S. pyogenes* strains resistant to macrolides) and also increases the oral bioavailability of the compound in *in vivo* experiments on rats.[51, 54] The use of a piperazine spacer did not increase the antibacterial activity; apparently, the spacer flexibility plays an important role in the antibacterial activity of macrolones, first of all, with respect to resistant strains.[55] Compounds containing a free carboxy group showed higher antibacterial activities than the corresponding esters; however, the latter had a higher oral bioavailability in rat experiments.[54] The macrolones in which the spacer was attached to the macrolide position 4 and that contained an ester bond were more active than analogous compounds with an ether bond.[55] Halogen-containing macrolones showed somewhat higher antibacterial activities.[51]

Macrolide antibiotics are known to be accumulated in some human organs and tissues. In particular, accumulation of macrolides in the macrophages and granulocytes is useful for their transport into the inflammation area, which provides an additional therapeutic effect.[58] For this reason, the ability to be accumulated in granulocytes (polymorphonuclear leukocytes) has been studied for the most active macrolones depending on the structure of macrolide and quinolone moieties and the nature of the spacer (Fig. 2).

These studies provided the conclusion that the presence of atoms or functional groups able to function as hydrogen bond donors or secondary amide groups in the molecule of macrolone **20** diminishes the cellular accumulation of the antibiotic, while an increase in the lipophilicity of the compound increases the accumulation of macrolones in granulocytes.

An alternative approach to the creation of dual action antibiotics based on macrolides and quinolones was proposed by the PLIVA company.[59] A previously developed method was used to prepare O-substituted ketolides **35a–f**.[60] The method comprised preparation of 6-*O*-allyl-8*a*-aza-8-homoerythromycin A derivative (**36**), epoxidation of the allylic moiety of **36** in the presence of *m*-chloroper-

**Table 2**. Structural units of macrolones **20**.

| X | $R^1$, $R^2$ | $R^3$ | Q [a] | $R^4$ | $R^5$ | $R^6$ | Synthetic route (example of the structure) | Ref. |
|---|---|---|---|---|---|---|---|---|
| C═O, N(Me)$CH_2$ | $R^1 = R^2 = H$ | H, Me |  $n = 1, 2$ | H | Et | H | – | 49 |
| C═O, N(Me)$CH_2$, C(O)NH, C(═NOEt) | $R^1 = R^2 = H$, $R^1–R^2$ = C(O)NH, C(O)NMe | H, Me |  | H | Et, cyclo-$C_3H_5$ | H | Scheme 6 (**20a**) | 50 |
| C═O, C(O)NH, N(Me)$CH_2$ | $R^1 = R^2 = H$, $R^1–R^2$ = C═O | H, Me |  $n = 1–3$ | 6‴-F, 7‴-H, 7‴-Cl | Me, Et, cyclo-$C_3H_5$ | H, Me | – | 51 |
| N(Me)$CH_2$ | $R^1 = R^2 = H$ | H |  $n = 1–3$ | H | Et, $Pr^n$, $Pr^i$, cyclo-$C_3H_5$, $Bu^t$, $NMe_2$ | H | Scheme 9 (**20e–k**) | 52 |
| N(Me)$CH_2$ | $R^1 = R^2 = H$ | H |  $R^3$ = H, Me | H, 6‴-F, 7‴-Cl | cyclo-$C_3H_5$ |  | Scheme 8 (**20c**) | 53 |
| N(Me)$CH_2$ | $R^1 = R^2 = H$ | H |  Z = $CH_2CH_2$, $CH_2CH_2OCH_2CH_2$; Z′ = NH, O | H, 7‴-Cl | cyclo-$C_3H_5$ |  | Scheme 7 (**20b**) | 54 |
| N(Me)$CH_2$ | $R^1 = R^2 = H$ | H, Me |  Z = C(O), $CH_2$ | H | Et, cyclo-$C_3H_5$ | H, Et | Scheme 8 (**20d**) | 55 |
| C(O)NH | $R^1 = R^2 = H$ | H, Me |   | 7‴-F | cyclo-$C_3H_5$ | H | | 56 |

[a] The numerals show the positions of substituents.

oxybenzoic acid (mCPBA) and reduction of the intermediate N-oxide. The epoxy derivative **37** thus formed was subjected to nucleophilic oxirane ring opening reaction with aminoquinolones in the presence of $LiClO_4$. This gave rise to diastereometric β-amino alcohols **35a–f** (Scheme 10).

A study of the antibacterial activity of these compounds demonstrated that the activity depends substantially on the position to which the quinolone moiety is attached. The antibacterial activity of product **35c** in which the macrolide substituent is linked to the piperazine ring in position 7 was 2–32 times higher than the activity of 6-substituted analogues **35b,e**.

Antibiotics containing a piperazine spacer were more active. Depending on the type of spacer, differences in the spectrum of antibacterial activity were observed: compounds with an ethylenediamine spacer resembled azalides (activity against gram-positive bacteria and reduced activity with respect to gram-negative bacteria), whereas compounds with a piperazine spacer were similar to quinolones (more active against gram-negative bacteria, especially *H. influenzae* and *M. catarrhalis*).[60] Esterification of the

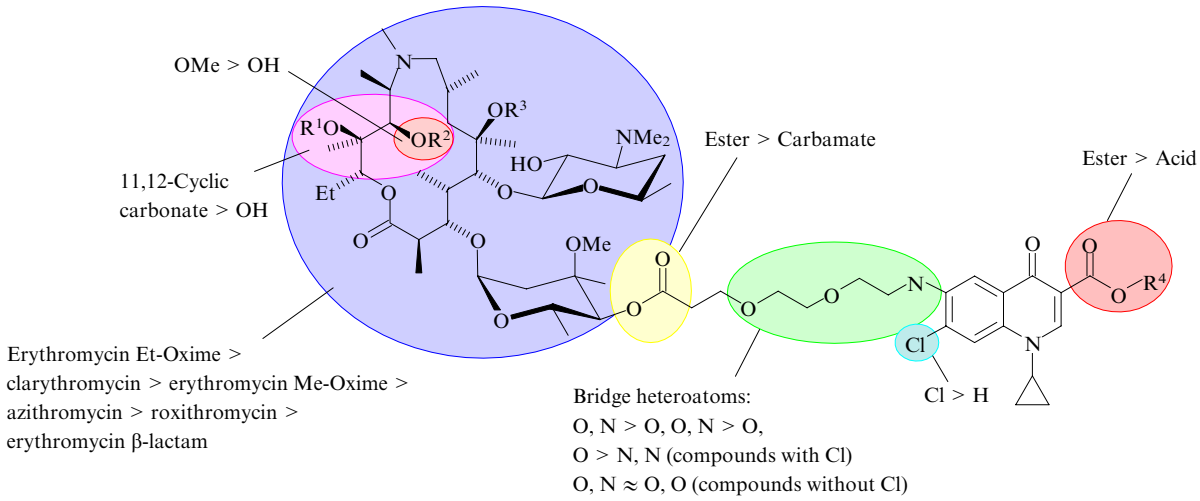
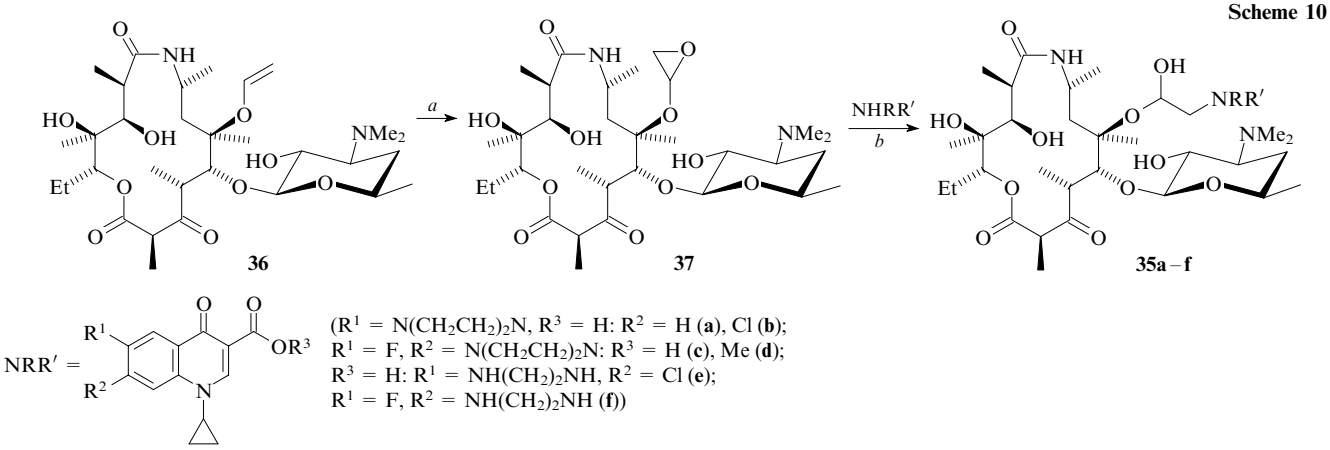



**Figure 2.** Structure–granulocyte accumulation relationship for the studied series of macrolones.[58]

**Scheme 10**




(*a*) 1) mCPBA, $CH_2Cl_2$, 0 °C; 2) Zn, $NH_4OH$, rt; (*b*) $LiClO_4 \cdot 3H_2O$, $Pr^iOH$, Δ

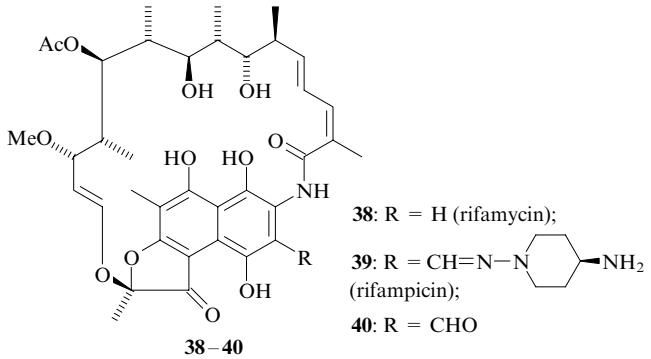
carboxy group resulted in a sharp decrease in the antibacterial activity (compound **35d**). All of the specimens were more active than azithromycin with respect to macrolide-resistant strains.

Thus, the design of macrolide- and quinolone-based antibiotics with a dual mode of action is a new, promising line of search for new antibacterial drugs being under vigorous development.

### III.1.c. Combination of quinolones and rifamycin

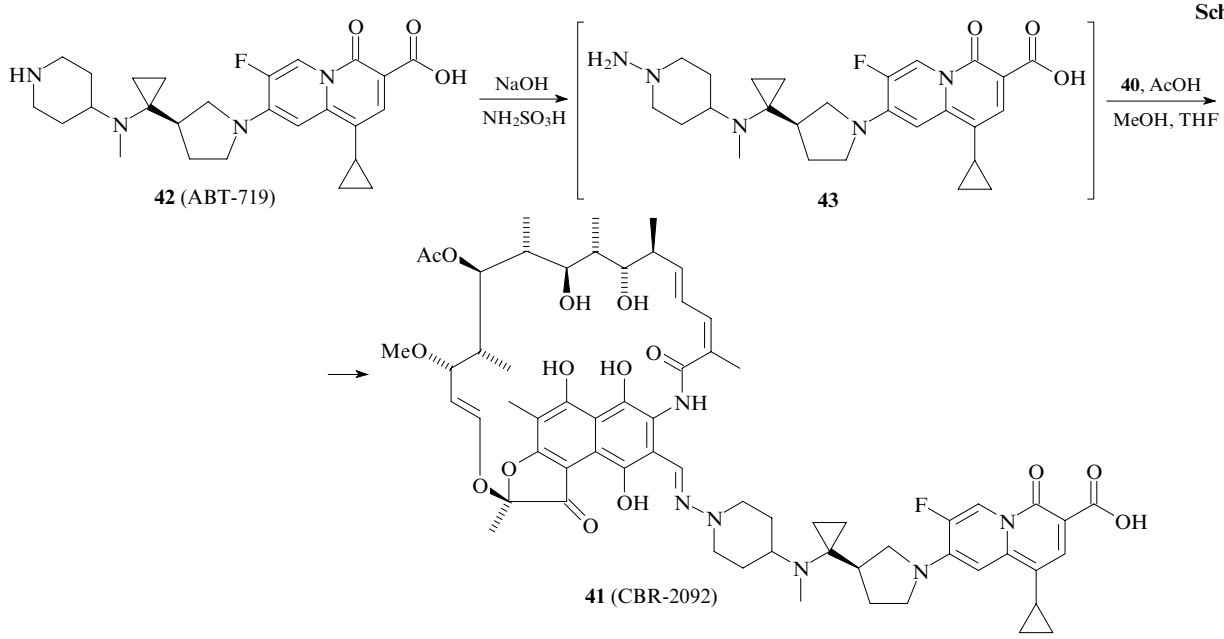
The antibiotic rifamycin (**38**), which belongs to the class of ansamycins, has a high activity against gram-positive bacteria including mycobacteria and is widely used to treat tuberculosis. The mechanism of action of this drug is related to inhibition of the DNA-dependent RNA polymerase of microorganisms. The rifamycin resistance of bacteria is caused most often by point mutations in the gene *rpoB* encoding the β-subunit of the enzyme, which changes the structure of RNA polymerase and decreases the enzyme affinity to the antibiotic.[61] Due to the high rate of development of the resistance, rifamycin is used only in combination with other antibacterial drugs, which restricts the scope of its use to some extent. Semisynthetic analogues of rifamycin, such as rifampicin (**39**) and 3-formyl derivative **40** have been prepared.[61]

**Structures 38–40**




In the beginning of the 21st century, Cumbre Pharmaceuticals carried out a large-scale investigation of rifamycin-, rifampin- and quinolizine-based antibiotics with a dual mode of action. The developed methods for the synthesis of these structures were patented.[62–66] In 2008, two publications appeared[67,68] describing the chimeric antibiotic CBR-2092 (**41**) including the mechanism of its

**Scheme 11**




action on *Staphylococcus aureus* and the activity *in vitro* with respect to some *Staphylococci* and *Streptococci* strains. This agent was selected out of more than 300 synthesized dual action antibiotics based on rifamycins and quinolizines, which differed in the structures of the quinolizine moiety, the spacer, the sites of the spacer attachment to rifamycin and so on. An important role in manifestation of the activity of these compounds against rifamycin-resistant strains is played by the structure of the quinolone moiety.[67] Antibiotic **41**, which belongs to the class of 4*H*-4-oxoquinolizines, combines rifampicin (**39**) and quinolone ABT-719 (**42**) *via* a piperidine moiety (Scheme 11).

For the synthesis of compound **41**, first, the hydrazine derivative of quinolizine **43** was prepared *in situ* and then it was made to react with 3-formylrifamycin (**40**) under weakly acidic conditions (see Scheme 11).[63] The antibiotic CBR-2092 (**41**) was shown to act in a dual mode by inhibiting the DNA-dependent RNA polymerase (like rifamycin) and DNA gyrase and topoisomerase IV (like quinolizines).[67] This derivative exhibited a high antibacterial activity (the MIC with respect to 300 clinical isolates of *Staphylococci* and *Streptococci* was in the range of $0.008-0.5\ \mu g\,ml^{-1}$). In addition, it was active against rifamycin- or quinolone-resistant strains.[68] It was found that CBR-2092 was not a substrate for NorA and MepA pumps, which ensure the efflux of xenobiotics from a bacterial cell and are thus responsible for the antibiotic resistance of bacteria. Antibiotic **41** was subjected to the whole set of preclinical trials,[69] after that, it successfully passed phase I clinical trials in 2006. However, no further advancement of this drug was reported.

### III.1.d. Combination of fluoroquinolones and tetracyclines

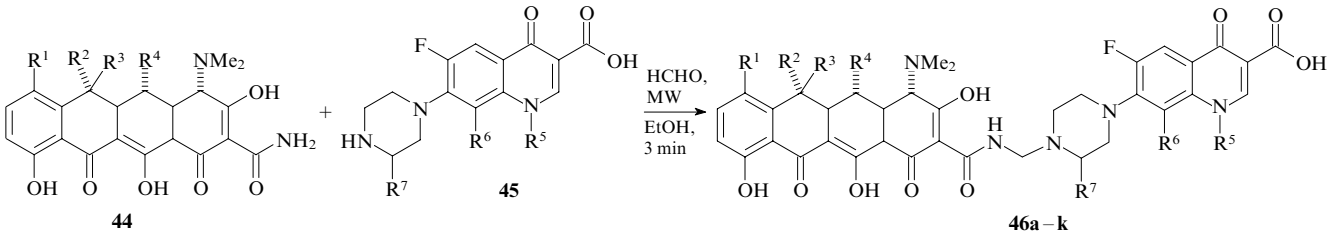
Tetracyclines are broad spectrum antibiotics corresponding to the class of polyketides. They are highly active *in vitro* with respect to numerous gram-positive and gram-negative bacteria. The antibacterial action of tetracyclines is based on suppression of protein synthesis. The antibiotic interacts with the ribosome 30S-subunit, which results in inhibition of binding of the aminoacylated transfer RNA (tRNA) to the ribosomal complex.[70] Three principal mechanisms of tetracycline resistance are known: (i) decrease in the intracellular concentration of the antibiotic due to its retarded penetration into the cell or activation of the efflux pump, which removes the drug from the cell by active transport; (ii) decrease in the tetracycline affinity to ribosomes due to attachment of protecting proteins to the latter; and (iii) inactivation of tetracyclines by bacterial enzymes.[71] Furthermore, the microbial resistance to one tetracycline is accompanied by cross-resistance to other antibiotics of this group, as their chemical structures and the mechanisms of action are similar. A principal way of preventing the spread of tetracycline-resistant bacterial strains is the therapy with combinations of antibiotics with different mechanisms of antimicrobial action.

Apart from the antibacterial action, tetracycline antibiotics show other types of biological activities. For example, many of them have an inflammatory action, and doxycycline is potent matrix metalloproteinase inhibitor.[72] It was shown that tetracyclines, especially those containing bulky substituents in the aminocarbonyl moiety in position 2, exhibit noticeable activity against the human immunodeficiency virus (HIV).[73]

Sriram *et al.*[74] described the synthesis of new chimeric antibiotics based on tetracyclines and fluoroquinolones and study of their antibacterial activity and the ability to inhibit the HIV-1 integrase. The starting compounds used were tetracycline derivatives **44** (tetracycline, oxytetracycline or minocycline) and fluoroquinolones **45** (norfloxacin, ciprofloxacin and gatifloxacin) containing a secondary amino group in the piperazine moiety. The reaction of these antibiotics in ethanol in the presence of formaldehyde for 3 min afforded derivatives **46a–k** (Scheme 12, Table 3). The reaction was initiated by microwave (MW) radiation.

The resulting series of chimeric antibiotics **46a–k** was tested for the ability to inhibit the HIV-1 integrase and to act against *Mycobacterium tuberculosis*. Among the tested

**Scheme 12**




**44** **45** **46a–k**

**Table 3**. Chimeric antibiotics **46a–k** based on tetracyclines and fluoroquinolones (see Scheme 12).[74]

| Compound **46** | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ | $R^7$ | Yield (%) |
|---|---|---|---|---|---|---|---|---|
| **a** | H | Me | OH | H | Et | H | H | 78 |
| **b** | H | Me | OH | H | Et | F | Me | 54 |
| **c** | H | Me | OH | H | cyclo-$C_3H_5$ | H | H | 61 |
| **d** | H | Me | OH | H | cyclo-$C_3H_5$ | OMe | Me | 69 |
| **e** | H | Me | OH | OH | Et | H | H | 43 |
| **f** | H | Me | OH | OH | Et | F | Me | 41 |
| **h** | H | Me | OH | OH | cyclo-$C_3H_5$ | H | H | 66 |
| **g** | H | Me | OH | OH | cyclo-$C_3H_5$ | OMe | Me | 41 |
| **h** | $NMe_2$ | H | H | H | Et | H | H | 62 |
| **i** | $NMe_2$ | H | H | H | Et | F | Me | 49 |
| **j** | $NMe_2$ | H | H | H | cyclo-$C_3H_5$ | H | H | 52 |
| **k** | $NMe_2$ | H | H | H | cyclo-$C_3H_5$ | OMe | Me | 58 |

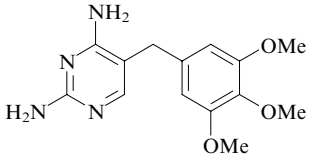
compounds, compound **46i** was found to be the most effective inhibitor of HIV-1 replication: the half-maximal effective concentration ($EC_{50}$) was 5.2 μmol ml$^{-1}$. It had a high antibacterial activity against *Mycobacterium tuberculosis* (MIC = 0.2 μg ml$^{-1}$), including tetracycline-resistant strains.

The HIV-1 infection is a serious risk factor for development of tuberculosis. Apart the antiretroviral action, compound **46i** is active against *Mycobacterium tuberculosis*; hence, it can prevent tuberculosis in HIV-1 patients and can be used to treat tuberculosis in these patients.[74]

### III.1.e. Combination of fluoroquinolones and benzylpyrimidine

The mechanism of action of benzylpyrimidines is related to inhibition of dihydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate. A representative of this class, trimethoprim (**47**), is used as a broad spectrum antibiotic for prevention and treatment of infectious diseases, in particular, urogenital infections.[75]

**Structure 47**

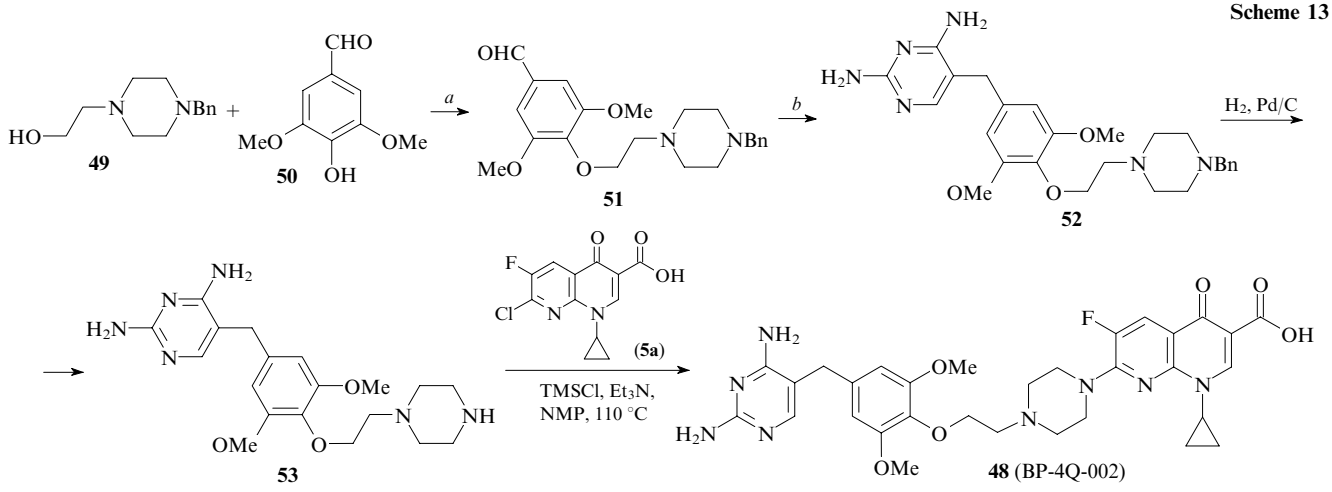


**47** (trimethoprim)

In order to eliminate the limitations of the combination therapy by trimethoprim (**47**) and ciprofloxacin (**2**) related to different pharmacokinetic characteristics of these antibiotics, a series of chimeric antibiotics has been prepared in which benzylpyrimidine is attached to position 7 of quinolone or 4*H*-4-oxoquinolizine *via* a spacer. The general synthetic route to these chimeric antibiotics comprised the preparation of benzylpyrimidine having a spacer and its subsequent reaction with 7-chloro-substituted quinolone.

The synthesis of the most active antibiotic of this series — BP-4Q-002 (**48**) — based on trimethoprim and ciprofloxacin connected by an ethylene bridge is shown in Scheme 13.[76] The first step was Mitsunobu alkylation of the phenol group of syringaldehyde (**50**) with alcohol **49**; this was followed by pyrimidine ring formation from the alde-

**Scheme 13**




**49** **50** **51** **52** **53** **48** (BP-4Q-002)

(*a*) $Ph_3P$, $Et_3N$, DEAD, $CH_2Cl_2$; (*b*) 1) $PhNH(CH_2)_2CN$, $Bu^tOK$, DMSO; 2) $(NH_2)_2C{=}NH$, EtOH;
DEAD is diethyl acetylenedicarboxylate, TMS is trimethylsilyl

hyde group of molecule **51**, 3-aminonitrile and guanidine. The benzyl protection in compound **52** was removed by palladium-catalyzed hydrogenation. The final step was hetarylation of piperazine **53** with 7-chloroquinolone **5a**.

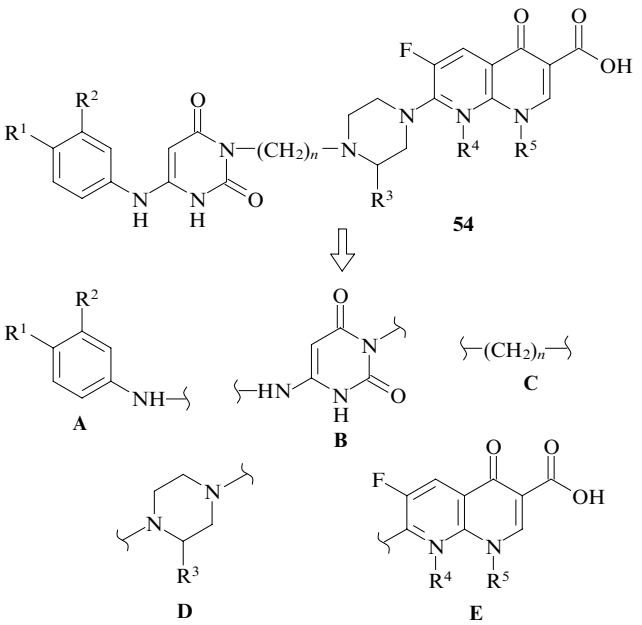
The compound BP-4Q-002 was 30 times more active than ciprofloxacin (**2**) or a ciprofloxacin– trimethoprim (1 : 1) combination against the *S. aureus* NRS strain resistant to ciprofloxacin.[75] The antibacterial activity of derivative **48** against the sensitive *E. coli* and *B. subtilis* strains was close to the activity of trimethoprim (**47**) but lower than the activity of ciprofloxacin (**2**). Unfortunately, no data on further development of the research along this line have been reported.

### III.1.f. Combination of fluoroquinolones and anilinouracils

6-Anilinouracils are new antibacterial agents; the mechanism of their action is related to the inhibition of bacterial DNA polymerase IIIC, the enzyme involved in the replication of gram-positive bacteria and mycoplasms.[77] However, anilinouracils do not always efficiently inhibit the DNA polymerase activity, possibly, due to relatively low penetration into the cell, active efflux of the agents out of the cell or a decrease in the enzyme sensitivity to the inhibitor.[78] The attachment of a fluoroquinolone molecule to anilinouracils can enhance the penetration of antibiotics into the bacterial cell and extend the spectrum of antibacterial activity by affecting two stages of DNA replication at once.

A method for preparing dual action antibiotics based on anilinouracils and fluoroquinolones, compounds **54** in which different fluoroquinolones are attached to the N(3) atom of 6-anilinouracils, has been developed.[79,80] Considering the nature of substituents and accessibility of the starting compounds, retrosynthetic analysis of structure **54** resulted in the development of three approaches to the synthesis of such antibiotics (Scheme 14). These approaches differ in the combination of moieties **A**–**E**.[80]

**Scheme 14**



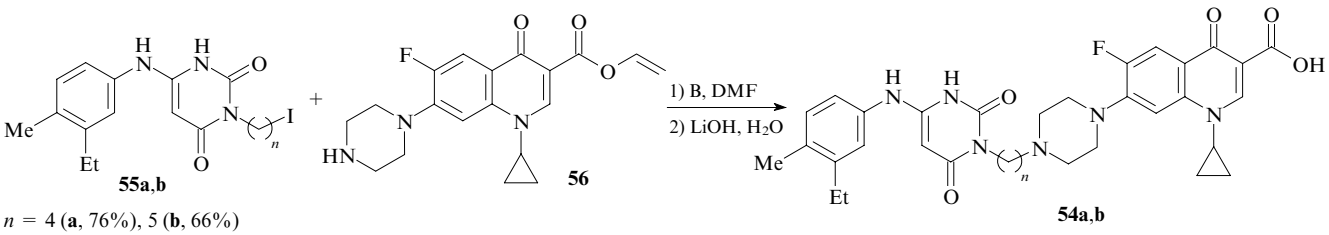
The simplest method (option **A**–**B**–**C** + **D**–**E** in Scheme 14) involved the reaction of 3-(iodoalkyl)anilinouracils **55a**,**b** with 7-piperazino-6-fluoroquinolone **56** in DMF in the presence of bases (B) such as $K_2CO_3$ or $NaHCO_3$ (Scheme 15). In order to exclude the presence of a starting fluoroquinolone impurity in the final products **54a**,**b**, vinyl esters of the fluoroquinolones were used and then the ester group was hydrolyzed with LiOH.

The second method (option **A**–**B**–**C**–**D** + **E** in Scheme 14) was based on the reaction of 6-(3-ethyl-4-methylanilino)-3-(4-piperazinobutyl)uracil (**57**) with ethyl 6,7,8-trifluoroquinolone-3-carboxylate **58a**; this gave antibiotic **54c** in a high yield (Scheme 16).

Finally, the third method (option **A**–**B**–**C**+**D**+**E** in Scheme 14) comprised three steps: replacement of the fluorine atom in position 7 of trifluoroquinolone **58b** by a chiral piperazine moiety, the subsequent condensation of intermediate **59** with compound **55a** and hydrolysis of the ester group. The use of stereoisomeric piperazines afforded *S*- and *R*-isomers of compound **54d** (Scheme 17).

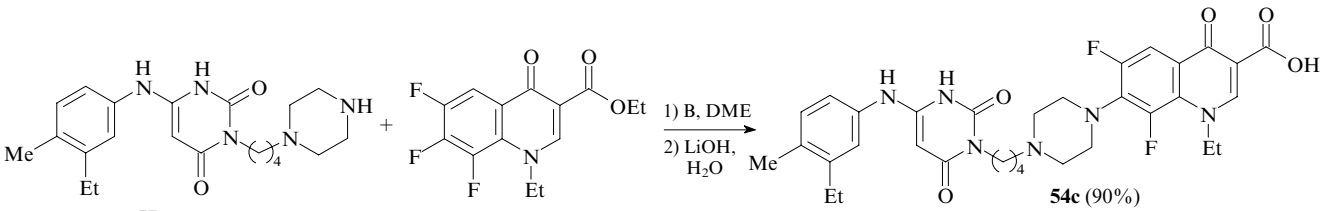
This procedure (see Scheme 14) afforded dual action antibiotics based on anilinouracil and fluoroquinolones containing different substituents in the piperazine moieties and spacers based on other cyclic and bicyclic diamines. All of the synthesized antibiotics **54a**–**d** selectively inhibited the DNA polymerase IIIC activity. The antibacterial activity of these compounds against gram-positive bacteria was much higher than that of anilinouracils but lower than in the case of ciprofloxacin against the fluoroquinolone-sensitive strains.[80] The fluoroquinolone-resistant MRSA 1094, *E. faecium* 19434 and VRE 700802 strains were highly sensitive to new antibiotics. Chiral compounds **54d** containing a 3-methylpiperazine moiety proved to be most active, their activity being comparable with that of ciprofloxacin; the *R*-enantiomer was somewhat more active than the *S*-form. The mechanism of action and the spectrum of antibacterial activity of racemate **54d** has been studied.[81] This derivative was shown to be a potent inhibitor of DNA polymerase IIIC, topoisomerase and gyrase, as it selectively affected the DNA synthesis in bacterial cells. The antibiotic (*R*,*S*)-**54d** had a high antibacterial activity against gram-positive and gram-negative bacteria, including anilinouracil- and fluoroquinolone-resistant strains. Note that the *R*-enantiomer (MBX-500) was chosen for subsequent investigations; it showed high efficiency in *in vivo* experiments (in mice and guinea pigs) for treating *Clostridium difficile*-

**Scheme 15**



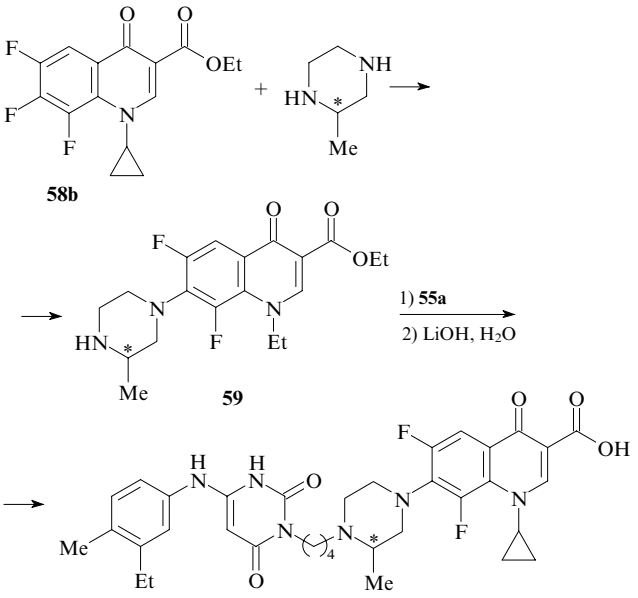
$n$ = 4 (**a**, 76%), 5 (**b**, 66%)

**Scheme 16**




**57** **58a** **54c** (90%)

**Scheme 17**




**58b** **59**

(*R*,*S*)-**54d** (251D);
(*R*)-**54d** (MBX-500, 65%);
(*S*)-**54d** (41%)

caused infections.[82,83] Thus, the creation of chimeric antibiotics based on anilinouracils and fluoroquinolones furnished the promising drug MBX-500 [(*R*)-**54d**], which was efficient in *in vivo* experiments on the treatment of infections caused by gram-positive anaerobic bacteria, including *Clostridium difficile*, and accompanied by diarrhea and pseudomembranous colitis.

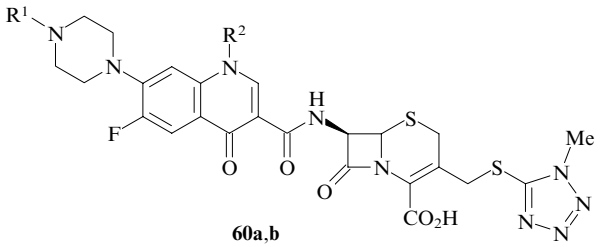
### III.1.g. Combination of fluoroquinolones and cephalosporins

Cephalosporins are β-lactam antibiotics with the chemical structure based on the 7-aminocephalosporinic acid. Cephalosporins are among the most widely used classes of antibiotics; they act through suppression of the synthesis of the peptidoglycan layer of the bacterial cell wall, resulting in the cell lysis.[84–86]

According to many researchers, fluoroquinolones and β-lactams present a perfect combination for being joint into a chimeric molecule, which should possess a broad spectrum of antibacterial activity based on different mechanisms of action of the components. Fluoroquinolones have a high antibacterial activity against gram-negative strains (including β-lactam-resistant strains and MRSA), whereas β-lactams are active against gram-positive bacteria. One more important advantage of the creation of dual action antibiotics based on β-lactams and fluoroquinolones is increasing solubility of the latter.

Johnson and Jones[87] described dual action antibiotics based on fluoroquinolones (enrofloxacin and norfloxacin) and cephalosporin (cefamandole). These hybrid molecules **60a**,**b** contained an amide bond formed by the quinolone carboxy group and β-lactam 7-amino group.

**Structures 60**



**60a**,**b**

$R^1$ = Et, $R^2$ = cyclo-$C_3H_5$ (**a**); $R^1$ = H, $R^2$ = Et (**b**)

Compounds **60a**,**b** had a high activity against various strains of *Enterobacter* (the $MIC_{90}$ values were $\sim$0.5 μg ml$^{-1}$); however, they were inactive against the fluoroquinolone-resistant strains. Apparently, this was due to the fact that such derivatives are substrates of efflux pump proteins, which remove xenobiotics from the cell.[87]

### III.1.h. Combination of fluoroquinolones and aminoglycosides

Aminoglycosides form an extensive group of antibiotics active with respect to both gram-positive and gram-negative bacteria.[88–90] These drugs interact with the decoding site (A-site) of the 16S subunit of rRNA, which disrupts translation and, hence, protein synthesis and finally induces cell death.[91,92] However, development of the resistance to aminoglycosides and pronounced toxicity of these drugs are serious problems.[93,94]

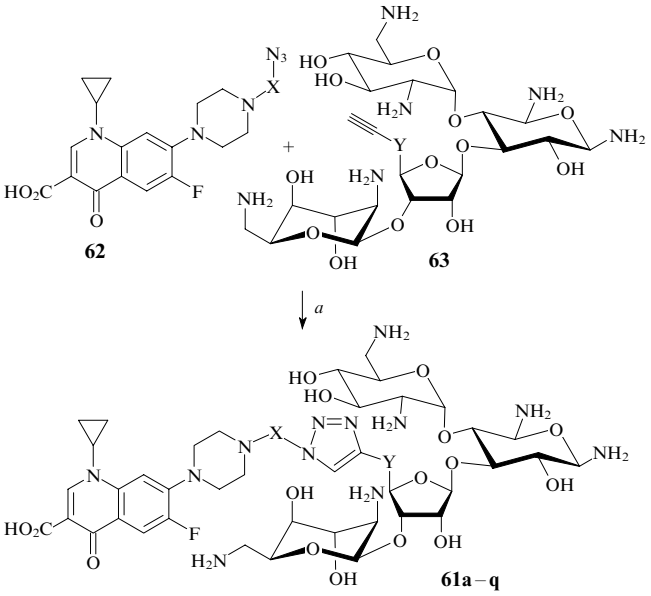
Pokrovskaya *et al.*[95,96] described ciprofloxacin- and neomycin-based antibiotics **61a**–**q** with a dual mode of action in which the components were connected by various 1,2,3-triazole-containing spacers. The syntheses of these compounds are based on click-chemistry techniques: organic azides react with acetylenes in the presence of copper(I) salts giving rise to a triazole ring.

As the starting compounds, the authors used azide derivatives of ciprofloxacin **62** and alkyl derivatives of neomycin **63**. The key condensation step was performed under microwave radiation in the presence of an organic base and a copper(I)-based catalyst (Scheme 18). Antibiotics **61a**–**q** differ in the nature and the length of the spacer.

The prepared antibiotics **61a**–**q** showed high antibacterial activities. The activity of **61a**–**q** was higher than that of neomycin (especially against aminoglycoside-resistant *E. coli* strains) but lower than that of ciprofloxacin. The nature and the structure of the spacer markedly affected the antibacterial action: antibiotics **61i**,**q** were most promising

having a 32–250 times higher activity than neomycin against some *E. coli* strains and some gram-positive strains including MRSA.[95]

**Scheme 18**




(*a*) $[(MeCN)_4Cu]PF_6$, 7% $Et_3N$ in $H_2O$, MW, 40 s

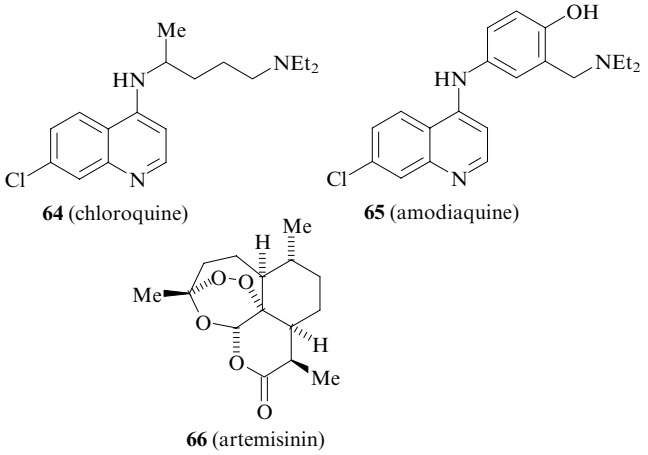
| Compound **61** | X | Y | Yield (%) |
|---|---|---|---|
| **a** | $(CH_2)_2$ | 4-$C_6H_4NHC(O)$ | 63 |
| **b** | $(CH_2)_3$ | 4-$C_6H_4NHC(O)$ | 90 |
| **c** | $(CH_2)_4$ | 4-$C_6H_4NHC(O)$ | 92 |
| **d** | $(CH_2)_5$ | 4-$C_6H_4NHC(O)$ | 94 |
| **e** | $(CH_2)_6$ | 4-$C_6H_4NHC(O)$ | 96 |
| **f** | $CH_2CH(OH)CH_2$ | 4-$C_6H_4NHC(O)$ | 92 |
| **g** | $(CH_2)_2O(CH_2)_2$ | 4-$C_6H_4NHC(O)$ | 95 |
| **h** | *m*-$CH_2C_6H_4CH_2$ | 4-$C_6H_4NHC(O)$ | 95 |
| **i** | *p*-$CH_2C_6H_4CH_2$ | 4-$C_6H_4NHC(O)$ | 91 |
| **j** | $(CH_2)_2$ | $CH_2NHC(O)$ | 97 |
| **k** | $(CH_2)_3$ | $CH_2NHC(O)$ | 93 |
| **l** | $(CH_2)_5$ | $CH_2NHC(O)$ | 96 |
| **m** | $CH_2CH(OH)CH_2$ | $CH_2NHC(O)$ | 85 |
| **n** | $(CH_2)_2O(CH_2)_2$ | $CH_2NHC(O)$ | 90 |
| **o** | *m*-$CH_2C_6H_4CH_2$ | $CH_2NHC(O)$ | 93 |
| **p** | *p*-$CH_2C_6H_4CH_2$ | $CH_2NHC(O)$ | 96 |
| **q** | $(CH_2)_2$ | $CH_2OCH_2$ | 97 |

It is noteworthy that chimeric antibiotics **61i**,**q** acted in a dual mode and their inhibiting action on DNA-gyrase and topoisomerase IV (molecular targets of fluoroquinolones) was several times higher than that of ciprofloxacin. As the most significant feature of antibiotics **61i**,**q**, the authors indicated the ability to substantially retard the development of resistance in sensitive *E. coli* and *B. subtilis* strains as compared with the ciprofloxacin–neomycin (1 : 1) combination.[95] Currently, the research into fluoroquinolone- and aminoglycoside-based chimeric antibiotics is being continued.

### III.2. Dual action antibiotics containing aminoquinolines

Aminoquinolines are a group of highly efficient antiparasitic drugs used to treat malaria. This disease is caused by *Plasmodium* parasites, which are transferred to people *via* the bite of infected *Anopheles* mosquitoes. Over decades, aminoquinolines, in particular, chloroquine (**64**) and amodiaquine (**65**), have been regarded as primary drugs for treating malaria. However, back in the 1970–1980s, resistance to these drugs was already a common case. Currently, according to World Health Organization (WHO) recommendations, the natural lactone artemisinin (**66**) is the best drug for treating malaria, especially in the case of *P. falciparum* species. However, to prevent development of resistance, this drug is used only in combination with other antimalarial drugs, for example, aminoquinolines.[97]

**Structures 64–66**




For this reason, development of new available antimalarial drugs active against resistant types of *Plasmodium* parasites is a topical task.

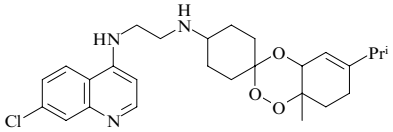
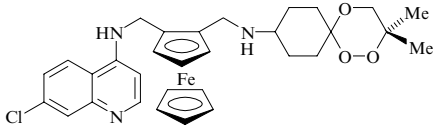
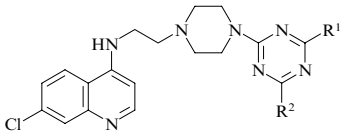
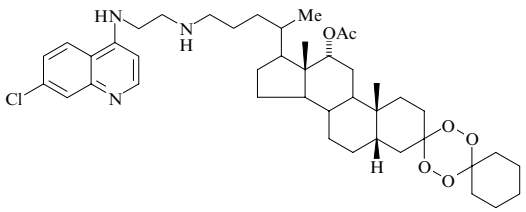
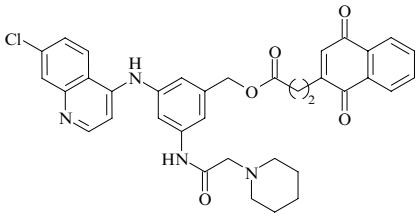
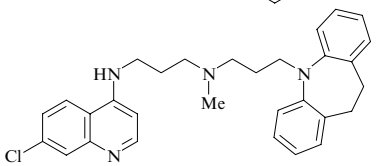
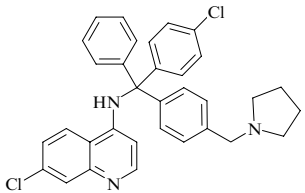
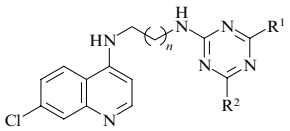
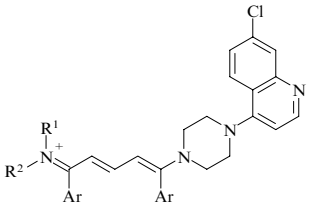
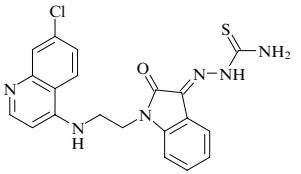
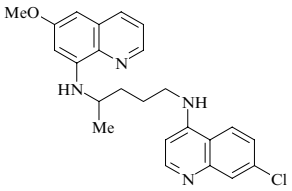
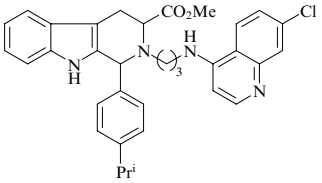
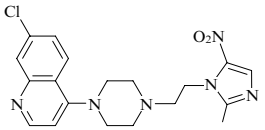
A particular line of this research is the conjugation of the pharmacophoric group of aminoquinolines with another antiparasitic drug in order to prepare dual action antibiotics.[98] Chimeric structures based on 4-aminoquinolines and chemotherapeutic drugs of other classes are under vigorous research; however, we will restrict ourselves to presenting only some of these structures with indicating the targets on which they act (Table 4).

A special class of dual action agents based on aminoquinolines is represented by aminoquinoline conjugates with antibiotics, in particular, penicillins (compound **67**), cephalosporins (**68**), fluoroquinolones (**69**), streptogramin (**70**), macrolides (**71**), glycopeptides (**72**) and oxazolidinones (**73**).[113]

Note that in this case, the pharmacophoric group of aminoquinoline served as an inhibitor of membrane efflux pumps, which account for the multi-drug resistance of the clinical isolates of gram-negative bacteria.[114] Earlier, it has been shown[115] that some aminoquinolines restore the sensitivity of multi-drug resistant (MDR) bacterial strains to antibiotics of various classes. Thus, while designing chimeric antibiotics, the patent's authors[113] assumed that the introduction of an aminoquinoline moiety would endow the final structure with activity against resistant bacterial strains owing to inhibition of the operation of membrane efflux pumps. Most of the chimeric antibiotics they studied, except for compound **67**, showed high antibacterial activities with respect to both sensitive and resistant gram-positive strains, whereas the activity against gram-negative bacteria was moderate.[113]

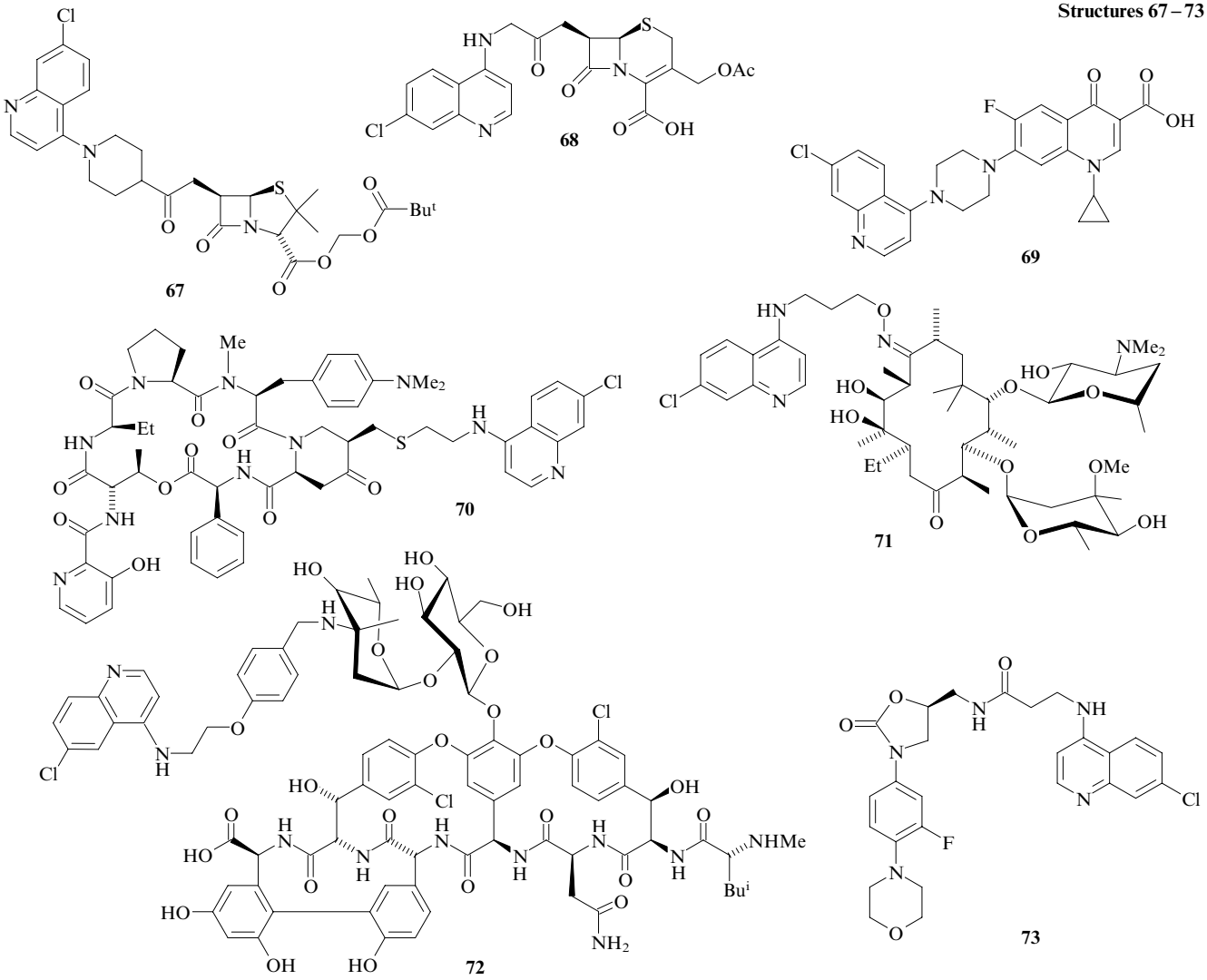
A combination of two described approaches was applied in a series of works[116–119] devoted to dual action anti-

**Table 4**. Antimalarial chimeric drugs based on aminoquinolines.

| Example of the structure | Additional pharmacophore groups (action target) | Example of the structure | Additional pharmacophore groups (action target) |
|---|---|---|---|
|  | trioxane (haem polymerization) [99, 100] |  | ferrocene + trioxane (haem polymerization) [107] |
|  | triazine (haem polymerization and dihydrofolate reductase) [101] |  | tetraoxane (haem polymerization) [108] |
|  | napthtoquinone (haem polymerization and glutathione reductase) [102] | | |
|  | imipramine (haem polymerization and interaction with the PfCRT protein [a]) [103] |  | clotrimazole analogue (haem polymerization) [109] |
|  | triazine (haem polymerization and dihydrofolate reductase) [104] |  | streptocyanine (haem polymerization) [110] |
|  | thiosemicarbazone (haem polymerization and cysteine proteases) [105] |  | primaquine (haem polymerization and interaction with DNA) [111] |
|  | tetrahydro-β-carboline (haem polymerization and DNA synthesis) [106] |  | metronidazole (haem polymerization interaction with DNA) [112] |

[a] PfCRT is the transport protein responsible for the resistance to chloroquine.

**Structures 67–73**



biotics based on aminoquinolines and macrolides, including azalides.

Apart from the broad antibacterial spectrum, azithromycin (**19**) has also antimalarial activity, the mechanism of action being related to inhibition of the protein synthesis in the *Plasmodium* ribosomes, which bear a resemblance to the ribosomes of prokaryotes.[120–124] Azithromycin has been extensively clinically studied for treating and preventing malaria as a single agent therapy and as a combination with other antimalarial agents. However, analysis of the results of 15 clinical trials provided the conclusion that the use of azithromycin for treating infections caused by *P. falciparum* or *P. vivax* has no advantages over the currently accepted standard protocol.[125–134]

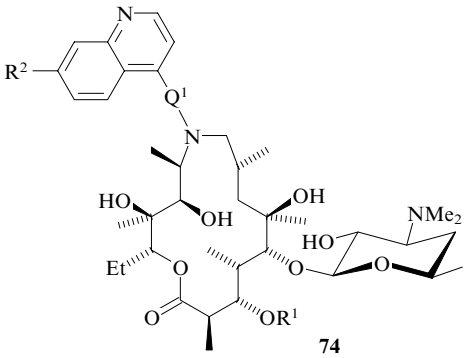
The synthesis of dual action antibiotics based on macrolides and aminoquinolines was aimed at the extension of the potential of macrolides as antimalarial agents and design of drugs active against resistant *Plasmodium* species and having reduced antibacterial activity. Three modes of attachment of the quinoline moiety to the azithromycin molecule were used: at the nitrogen atom in the macrocycle (9a-N-substituted macrolides **74**)[118] and at the 2′-hydroxy group and 3′-amino group of the desosamine residue [compounds **75** (Ref. 117) and **76** (Ref. 119), respectively].

These series of derivatives differed in the length and structure of the spacer, the presence of the amino group and chlorine atom in the quinoline residue and also the presence or the absence of an L-cladinose moiety.

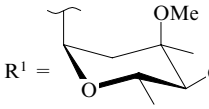
A study of the biological activities of compounds **74**–**76** demonstrated that the attachment of a quinoline moiety, especially 4-amino-7-chloroquinoline, to the azithromycin molecule substantially enhances the antimalarial activity of chimeric structures with a pronounced decrease in the antibacterial activity. Antibiotics containing a cladinose residue had a more pronounced antimalarial action. In some cases, new derivatives were active against aminoquinoline-resistant *Plasmodium*. However, one of the most active new derivatives — compound **76** (azithromycin modified at the desosamine 3′-amino group) — showed a decrease in the oral bioavailability in *in vivo* experiments.[119] This restricts the possible applicability of these derivatives as antimalarial drugs. According to the authors,[117,118] further studies of the biological activity and mechanisms of action of azithromycin- and aminoquinoline-based antibiotics of types **74** and **75** are required.

Thus, the preparation of dual action antibiotics based on aminoquinolines is a promising line of research; moreover, depending on the task set, drugs with a specified type of activity (antimalarial or antibacterial) can be designed.

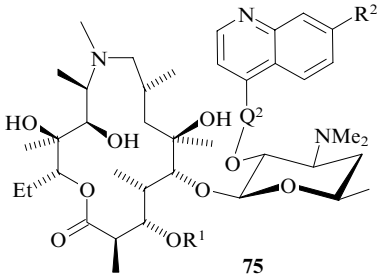
**Structures 74–76**



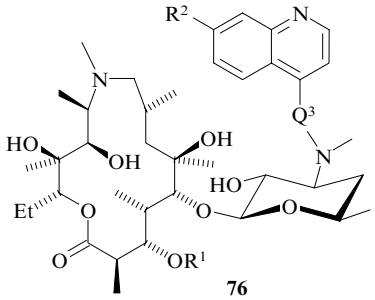
**74**



$R^1$ = or H; $R^2$ = H, Cl;

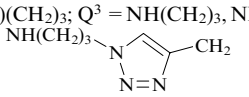


**75**



**76**

$Q^1 = CH_2NH(CH_2)_3$, $C(O)(CH_2)_2$, $NH(CH_2)_3$, $NHC(O)(CH_2)_2$, $C(O)NH(CH_2)_3$;
$Q^2 = NH(CH_2)_3$, $NH(CH_2)_3C(O)NH(CH_2)_3$, $NH(CH_2)_2C(O)NH(CH_2)_3$, $CH_2NH(CH_2)_3$, $CH_2N(Me)(CH_2)_3$; $Q^3 = NH(CH_2)_3$, $NH(CH_2)_2C(O)$, $NH(CH_2)_3C(O)$, $NH(CH_2)_3C(O)NH(CH_2)_3$,



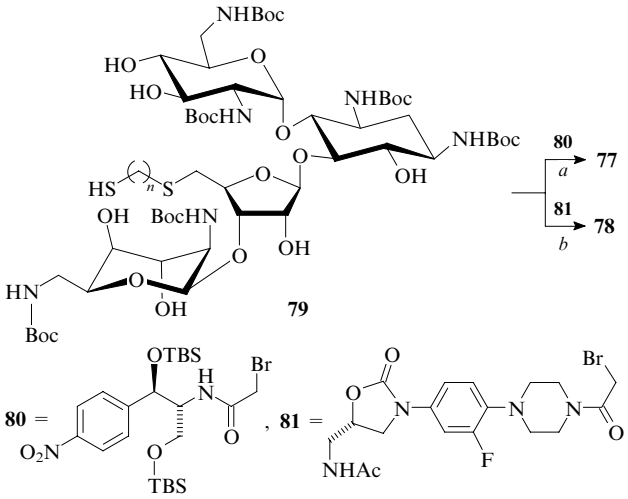
## III.3. Dual action antibiotics containing aminoglycosides

As has already been noted, the molecular mechanism of action of aminoglycosides is based on the interaction with rRNA; further, various classes of aminoglycosides bind to different sites of rRNA.[135] It was shown that aminoglycosides have an approximately 10 times higher affinity to the prokaryotic rRNA than to the eukaryotic rRNA.[136, 137] However, this specificity difference, which is not great, is apparently partly responsible for the toxicity of aminoglycosides in mammals.[89, 135] Several ways of increasing the specificity of binding of aminoglycosides to rRNA were studied. One of the strategies being developed is the construction of heteroconjugates of aminoglycoside antibiotics with compounds having RNA affinity, in particular, with amino acids,[138] simple hydrophobic[139] or hydrophilic molecules,[140] peptides,[141] and other antibacterial agents.

Yu and co-workers[142–144] published a series of works investigating a number of antibiotics with a dual mode of action based on aminoglycosides, neomycin and chloramphenicol (compounds **77a–c**) or linezolid (compound **78**).

The key step of the synthesis of chimeric antibiotics **77** and **78** was the alkylation of Boc-protected neomycin thio derivative **79** with bromo derivatives of chloramphenicol (**80**) and linezolid analogue (**81**) in the presence of caesium carbonate (Scheme 19).

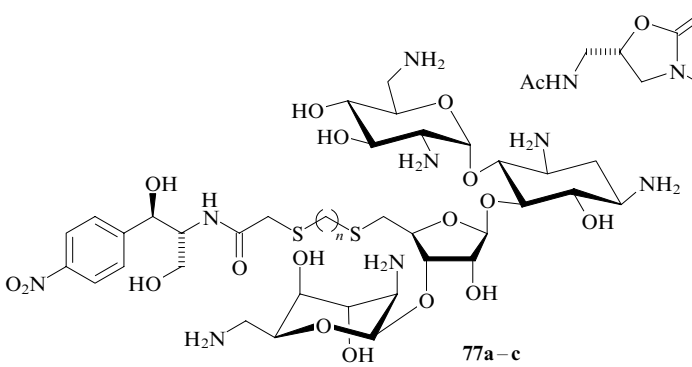
**Scheme 19**



(*a*) 1) $Cs_2CO_3$, DMF, Δ; 2) TBAF, THF; 3) TFA; (*b*) 1) $Cs_2CO_3$, DMF, Δ; 2) TFA; TBS = $Bu^n_3Si$, TBAF = $Bu^n_4NF$

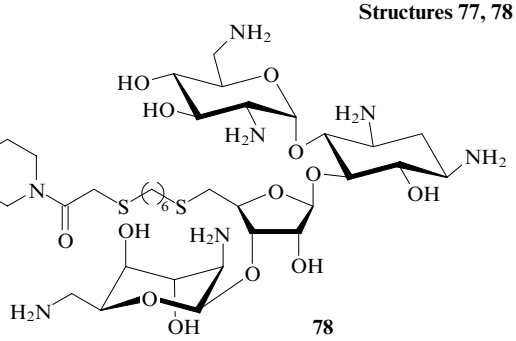
Chimeric antibiotic **78** based on neomycin and linezolid demonstrated a substantial increase in the affinity to the A-site of the 16S subunit of rRNA with respect to that of neomycin, while the chimeric antibiotic **77b** based on neomycin and chloramphenicol had the highest specificity of binding to the A-site of bacterial 16S rRNA compared to

**Structures 77, 78**



**77a–c**

$n$ = 3 (**a**), 6 (**b**), 9 (**c**)



**78**

the A-site of the 18S subunit of human rRNA.[143] However, the MIC values for chimeric antibiotics **77** and **78** with respect to a panel of bacterial strains were not correlated with their binding constants to rRNA, and their antibacterial activity was lower than that of neomycin.

### III.4. Dual action antibiotics containing glycopeptides

Glycopeptides (glycopeptide antibiotics) are a group of highly active antibiotics based on glycosylated non-ribosomal peptides with macrocycles as side groups. For many years, the group of clinically significant glycopeptide antibiotics has included only two drugs — vancomycin and teicoplanin.[145, 146]

Glycopeptides are active against gram-positive aerobic and anaerobic microorganisms and are the drugs of choice to treat infections caused by MRSA, methicillin-resistant *St. epidermis* (MRSE) and ampicillin- and aminoglycoside-resistant enterococci. Since the glycopeptide antibiotics are 'reserve drugs', recommendations restricting application of these agents have been developed in most countries in order to prevent the spread of resistance to this class of antibiotics.[147] Nevertheless, the propagation of resistant gram-positive microorganisms, including vancomycin-resistant enterococci (VRE) is still a considerable problem.[148, 149] The infections caused by the vancomycin-resistant *Staphylococci* strains are still encountered much more rarely.[150] However, in summer of 2013, the first clinically documented case of a disease caused by vancomycin-resistant *St. aureus* (VRSA) in Europe (in Portugal) was reported.[151] Note that the VRE strains, especially VRSA, are resistant to the vast majority of antibiotics used. Therefore, the spread of these pathogens poses a very serious problem.

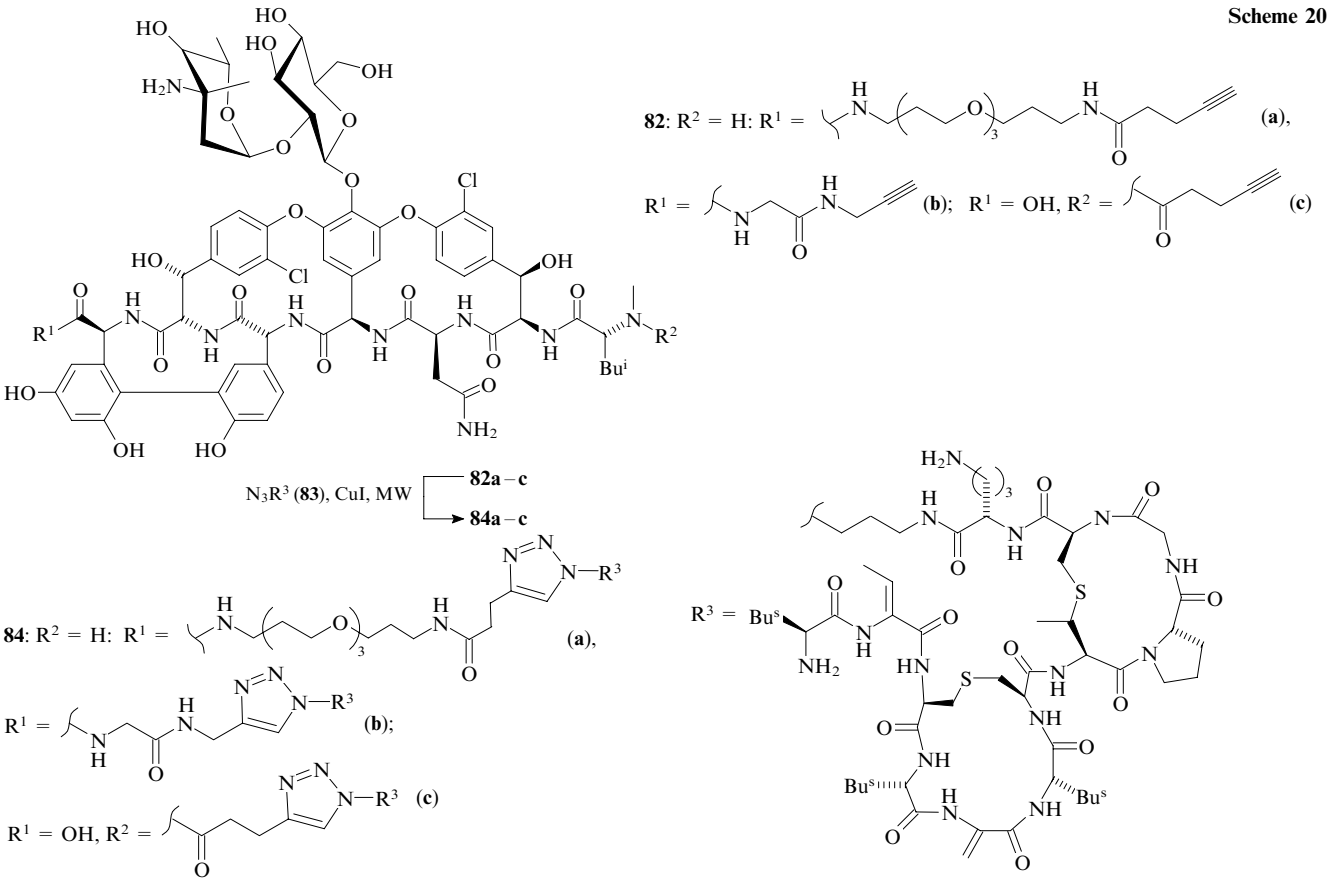
The mechanism of action of glycopeptide antibiotics is related to inhibition of the biosynthesis of the bacterial cell wall by non-covalent binding to the D-alanine-D-alanine dipeptide-containing peptidoglycan precursor located in lipid II.[27, 152] It was ascertained that hydrophobic derivatives of glycopeptides inhibit the transglycosylation step in a different way than natural antibiotics, as they have an additional binding site to the target[153] and, hence, they overcome the vancomycin resistance of bacteria.

Hence, the design of dual action antibiotics based on glycopeptides and other antibacterial agents appears especially promising.

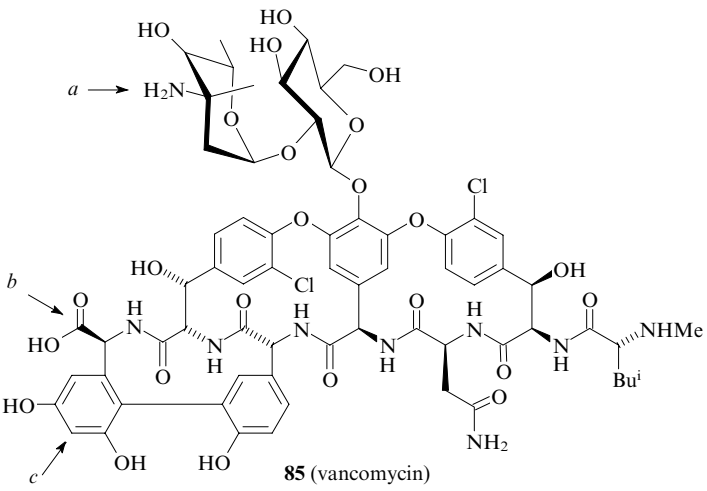
#### III.4.a. Combination of glycopeptides and nisin

Breukink and co-workers[154] described dual action antibiotics based on vancomycin and a fragment of nisin, a natural peptide, having antimicrobial properties.[155] Although vancomycin and nisin belong to different classes of antibiotics and have different mechanisms of action, the cell wall precursor, lipid II, is the molecular target for both antibiotics.[156] Vancomycin binds to the tripeptide part (Lys-D-Ala-D-Ala) of lipid II,[153] while the N-terminal fragment of nisin (1–12 residues) binds to the unique pyrophosphate part present in the lipid II structure.[157] The peptide fragment of nisin (1–12 residues) almost loses the antimicrobial activity but retains the ability to bind to lipid II in model experiments.[158] Presumably, a combination of vancomycin and nisin in one molecule would result in enhanced affinity of this molecule to lipid II owing to the presence of two moieties interacting with the target. This, in turn, may increase the activity of the resulting chimeric structure against the VRE.

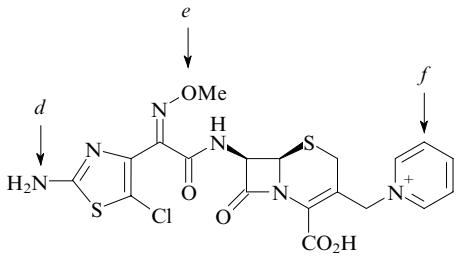
**Scheme 20**



**Structures 85, 86**



**85** (vancomycin)



**86** (cephalosporin)

The sites of attachment of vancomycin and the N-terminal nisin fragment (1–12 residues) and the optimal length of the spacer were found by computer simulation. From acetylene vancomycin derivatives **82a–c** and azide-containing nisin **83** in the presence of copper sulfate, three chimeric molecules **84a–c** were prepared using click-chemistry techniques and microwave radiation (Scheme 20).[154]

New antibiotics **84a–c** showed high activities against the VRE (the most promising compound **84a** was 40 times more active than vancomycin or nisin) and the *K. pneumonia* and *Moraxella catarrhalis* strains resistant to both vancomycin and nisin. However, hybrid compounds were slightly less active than vancomycin with respect to sensitive strains of enterococci.[154]

### III.4.b. Combination of glycopeptides and β-lactams

A strategy resembling that described above was used to design antibiotics with a dual mode of action based on vancomycin (**85**) and β-lactams.[159, 160] It was assumed that two targets, the vancomycin (lipid II intermediate) and β-lactam (transpeptidase domain of penicillin-binding proteins), can be hit simultaneously in this way. Since in a bacterial cell, these targets are proximate and are responsible for successive stages of cell wall biosynthesis, it was expected that the chimeric structures based on glycopeptides and β-lactams would possess an enhanced antibacterial activity.

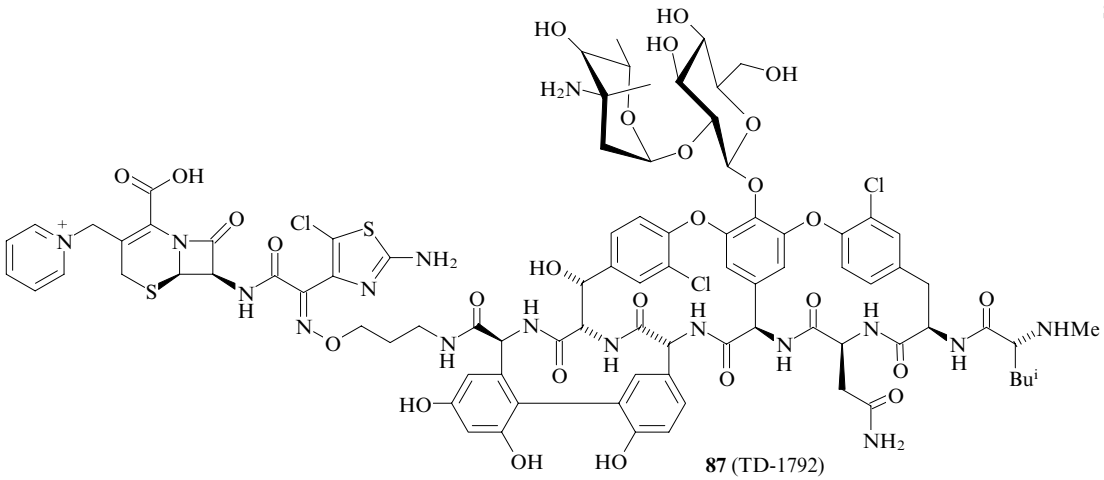
Several options of coupling of vancomycin and cephalosporin molecules have been studied.[160] The amino group of vancosamine (path *a*), the terminal carboxy group (path *b*) and the 4′-position of the side aromatic ring of the amino acid (path *c*) were chosen as the attachment points in vancomycin (**85**). Cephalosporin (**86**) was modified at three positions: the aminothiazole moiety (path *d*), the oxime function (path *e*) and the pyridinium C(3) atom (path *f*).

Combinations of various points of attachment to vancomycin and cephalosporin gave rise to nine different chimeric antibiotics; amidation was the key step of conjugation of the derivatives.

All antibiotics synthesized in this way showed synergistic antibacterial activities (more than 15 times higher than the activity of the initial drugs) with respect to a panel of sensitive and resistant gram-positive bacterial strains including MRSA and VRE.[160] Since there was no significant effect of the conjugation points of vancomycin and cephalosporin on the antibacterial activities of chimeric antibiotics, it was concluded that the conjugates do not bind simultaneously to both cell targets and the high antibacterial activity is due to the synergistic action on the cell wall biosynthesis.

Some compounds were tested in *in vivo* experiments. The chimeric antibiotic TD-1792 (**87**) — the amide formed by the terminal carboxy group of vancomycin (path *b*, see structure **85**) and the amine-containing

**Structure 87**



**87** (TD-1792)

spacer attached to the cephalosporin oxime function (path *e*, see structure **86**) — was chosen as the candidate drug for clinical trials.

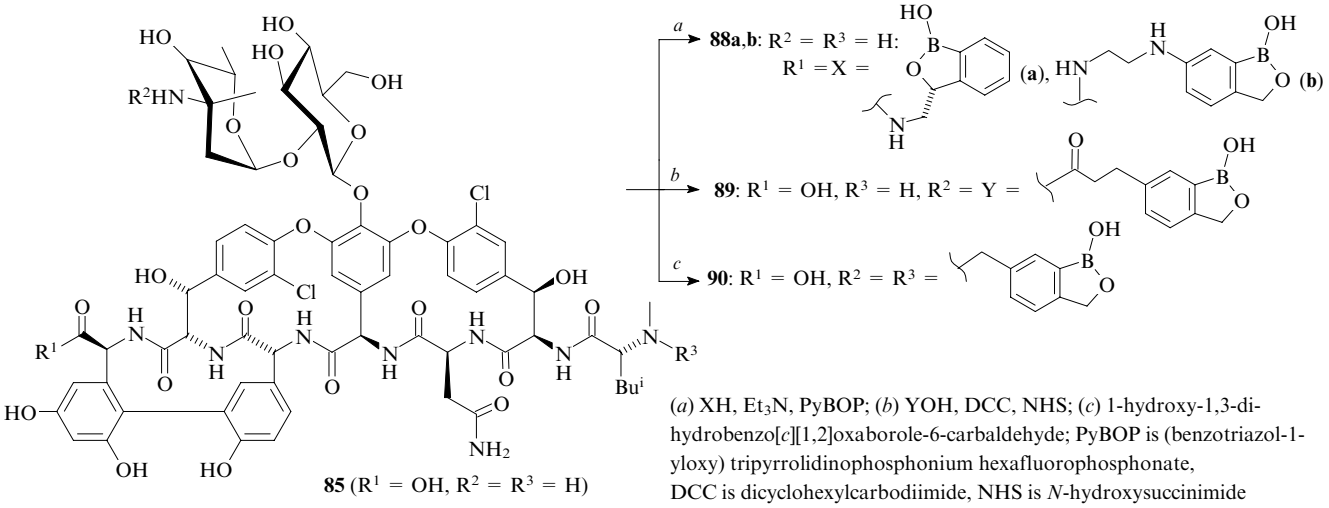
The activity spectrum of TD-1792 (**87**) was studied against a broad panel of bacterial strains.[161,162] It was shown to be highly active in relation to gram-positive bacteria, including MRSA, overcomes the penicillin resistance in pneumococci and fluoroquinolone resistance in streptococci and has a sufficient stability to the action of β-lactamases.[163]

Drug **87** has passed preclinical trials,[164] phase I and II clinical trials (in comparison with vancomycin) for the treatment of complicated skin infections.[165] The results of phase II attest to good prospects of further advancement of the vancomycin- and cephalosporin-based chimeric antibiotic for treating severe infections, in particular, MRSA-caused diseases. In October, 2012, the Therevance company promoting TD-1792 concluded a partner agreement with the Russian R-Pharm company transferring the exclusive rights for advancement and commercialization of the drug in Russia, C.I.S. countries, Georgia and Ukraine.

### III.4.c. Combination of glycopeptides and benzoxaboroles

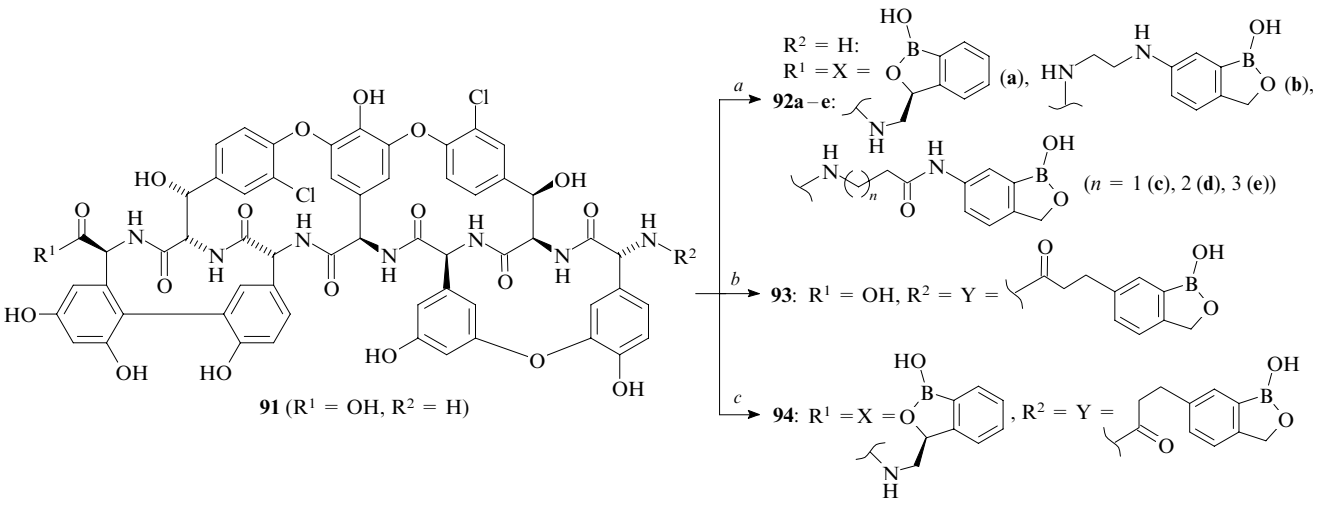
Boronic acids and benzoxaboroles are known to react with various biologically important compounds of the living cell — alcohols, amino alcohols, carbohydrates, RNA and some peptides.[166,167] This feature can underlie the biological activity of their derivatives and enhance the transport of macromolecules into the cell as a result of interaction of polysaccharide 1,2- and 1,3-diols located on the cell surface.[168] The attachment of a boronic acid fragment to an antibiotic molecule can substantially change the chemical and biological properties of the drug. Of particular interest for conjugation with antibiotics are heterocyclic boronic acids, benzoxaborole derivatives, which are being intensively developed by Anacor Pharmaceuticals in the USA. Indeed, clinical trials have been performed for the drug AN3365 (GSK2251052) with the chemical name (*S*)-3-aminomethyl-7-(3-hydroxypropyl)benzo[*c*][1,2]oxaborol-1-(3-*H*)-ol. This is a new boronic antibiotic active against multidrug resistant gram-negative bacteria. The mechanism of its action is based on inhibition of bacterial aminoacyl-transfer RNA synthase.[169] Compounds having high antifungal, antimalarial or antitrypanosomal activity have been found among benzoxaboroles.[170–172]

**Scheme 21**




(*a*) XH, $Et_3N$, PyBOP; (*b*) YOH, DCC, NHS; (*c*) 1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carbaldehyde; PyBOP is (benzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphonate, DCC is dicyclohexylcarbodiimide, NHS is *N*-hydroxysuccinimide

**Scheme 22**




(*a*) XH, $Et_3N$, PyBOP; (*b*) YOH, DCC, NHS; (*c*) 1) XH, $Et_3N$, PyBOP; 2) YOH, DCC, NHS

Printsevskaya *et al.*[173] described antibiotics based on glycopeptides and benzoxaboroles. The authors studied three possible options of attachment of benzoxaborole to a vancomycin molecule (**85**) to give the following derivatives: amides **88a**,**b** at the terminal carboxy group and aminobenzoxaborole; N-acyl derivative **89** at the vancosamine amino group and benzoxaborole containing a carboxy group; and dialkyl derivative **90** at the N-terminal nitrogen atom and the vancosamine amino group (Scheme 21).

Also, using teicoplanin aglycone (**91**) and benzoxaboroles, a series of structures **92**–**94** has been obtained (Scheme 22). For example, the researchers synthesized amides in which the benzoxaborole residue was attached to the teicoplanin aglycone *via* a spacer (compounds **92b**–**e**) and the N-acyl derivative **94** containing two benzoxaborole residues.

Chimeric antibiotics in which the glycopeptide and benzoxaborole molecules are separated by a spacer showed high antibacterial activities against gram-positive bacteria, including those possessing moderate resistance to glycopeptides. Printsevskaya *et al.*[173] concluded that researcher along this line should be continued.

## III.5. Dual action antibiotics based on amphotericin B and benzoxaboroles

A polyene macrocyclic antibiotic, amphotericin B (AmB, **95**), is the drug of choice for treating systemic fungal infections.[174] It suppresses the growth of yeast-like fungi, dermathophytes, mildews, some plant pathogenic fungi and causative agents of deep mycoses (coccidiosis and histoplasmosis). Amphotericin B is meant for systemic use and is administered intravenously. This antibiotic is especially significant for treating fungal diseases that complicate AIDS. The mechanism of action of polyene antibiotics, including amphotericin B, is based on complex formation between the antibiotic and sterols contained in cell membranes and assembly of several such complexes to form a pore in the membrane.[175] The efficiency of polyenes against fungal pathogens is dictated by more selective binding of the agent to ergosterol contained in the fungal membrane rather than to cholesterol characteristic of the human and animal cell membranes. However, high toxicity of AmB (nephrotoxicity and hemolytic toxicity), side responses (mainly allergic) and poor absorbability from the gastro-intestinal tract restrict the clinical use of this drug.[176] This stimulated the search for semisynthetic derivatives of macrocyclic polyene antibiotics.[177]

Since the action of polyene antibiotics is based on interaction with cell membrane components and the above-mentioned benzoxaboroles can potentially enhance the interaction with various cell structures, our research group prepared antibiotics with amphotericin B and benzoxaborole fragments of various structures.[178]

Derivatives **96**–**98** result from modification of compound **95** at the amino group of the 3-amino-3,6-dideoxy-β-D-mannopyranose moiety: amides **96a**–**c** were synthesized by acylation with the corresponding acids in the presence of *N*,*N*′-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (HOBt); sulfamide **97** was formed in the reaction with benzoxaborole sulfochloride; and amine **98** was obtained by reductive alkylation with 1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carbaldehyde in the presence of sodium cyanoborohydride. Amide **99**, the product of functionalization of AmB at the carboxy group of the polyene macrocycle, was prepared by amidation of compound **95** in the presence of PyBOP (Scheme 23).

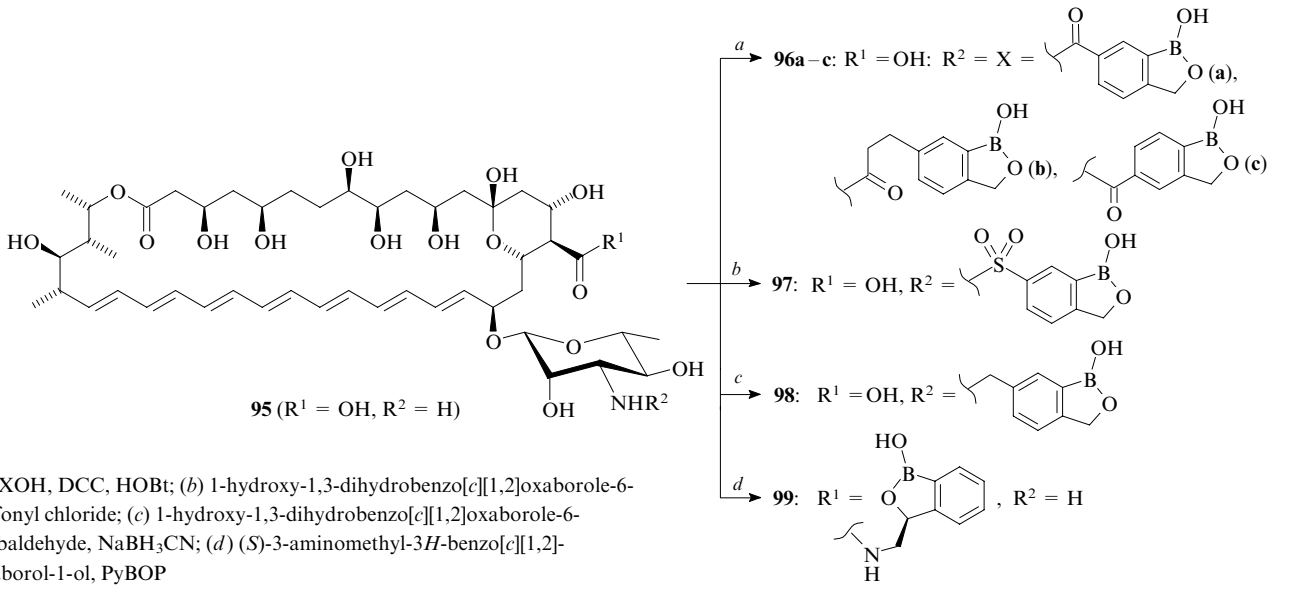
The highest antifungal activity was found for derivative **98** (at the activity level of amphotericin B); the activity of other conjugates was somewhat lower. Further studies along this line are aimed at introduction of a spacer between the polyene antibiotic and the benzoxaborole moiety.

## III.6. Miscellaneous examples of dual action antibiotics

As mentioned above, one approach to antibiotics with a dual mode of action implies the design of molecules by coupling two antibacterial agents or their structural units. This section deals with hybrid molecules designed using only pharmacophoric groups (pharmacophores) of antibiotics to form the new molecule rather than by connecting two antibacterial agents directly or *via* a spacer.

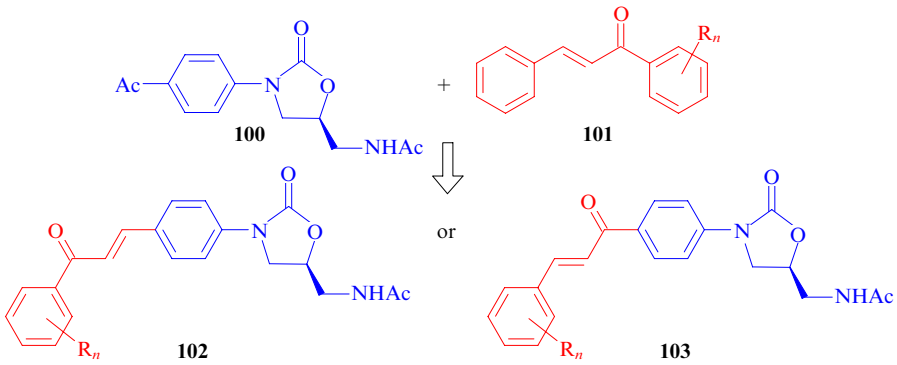
A series of dual action antibiotics prepared by combining pharmacophores of oxazolidinone **100** and chalcones

**Scheme 23**




(*a*) XOH, DCC, HOBt; (*b*) 1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-sulfonyl chloride; (*c*) 1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborole-6-carbaldehyde, $NaBH_3CN$; (*d*) (*S*)-3-aminomethyl-3*H*-benzo[*c*][1,2]-oxaborol-1-ol, PyBOP

**Scheme 24**



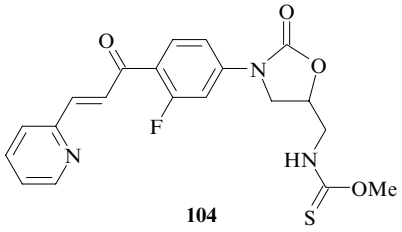

$R_n$ = H, 4-Me, 4-OMe, 3-F, 4-$NO_2$, 2,2-$Cl_2$-6-F

**101** have been described (Scheme 24).[179] Chalcones are precursors of flavonoids and isoflavonoids possessing a broad spectrum of biological activity, in particular, antimicrobial activity.[180] In their structure, two aromatic rings are connected by a α,β-unsaturated carbonyl bridge, which is necessary for antibacterial activity to be manifested.[181]

A series of hybrid structures based on oxazolidinones and chalcones have been prepared, in particular, regioisomers **102** and **103**, which differ in the position of the α,β-unsaturated carbonyl group (see Scheme 24) and by substituents in the chalcone aromatic ring.

Unfortunately, these compounds did not virtually show antibacterial activity with respect to the panel of gram-positive bacterial strains. Further structure modification comprising introduction of heteroatoms into the aromatic group of oxazolidinone, replacement of the acetamide group by a thiocarbamate group and replacement of the chalcone benzene ring by a heterocycle resulted in the synthesis of antibiotic **104**.

**Structure 104**




**104**

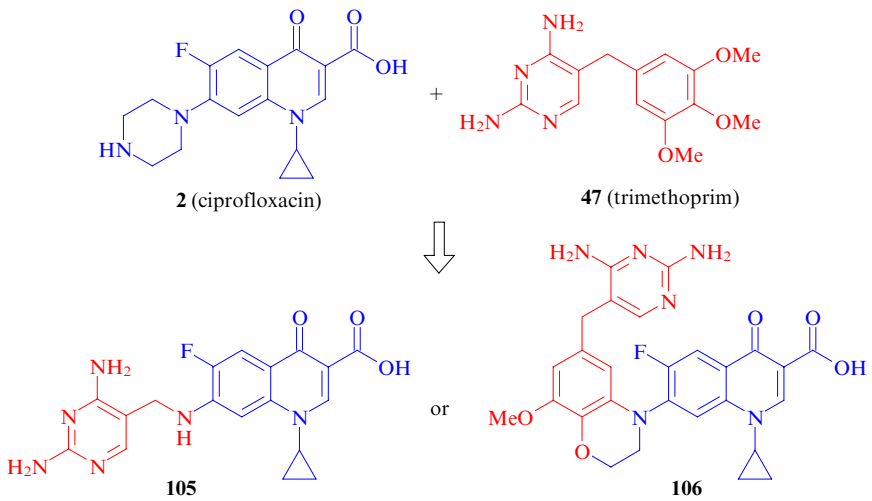
This compound showed a high antibacterial activity, in particular, against *S. aureus* and MRSA (MIC = 0.25–1 μg $ml^{-1}$, which is at the level of linezolid or vancomycin) and against the *Enterococcus faecium* strains both vancomycin-sensitive (VSEF) and vancomycin-resistant (VREF) (it is 70 times more active than vancomycin).

However, since the antibacterial activity of the respective initial chalcone is unknown, it is difficult to draw conclusions whether or not the components of compound **104** show a synergistic behaviour.

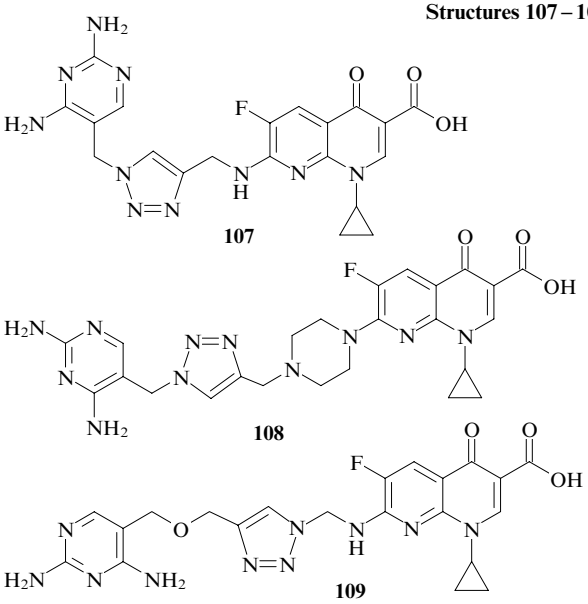
One more example of dual mode of action brought about by coupling of the pharmacophores of two antibacterial agents are the hybrid molecules based on fluoroquinolones and benzylpyrimidines. The design of molecules **105** and **106** by coupling of the structural fragments of ciprofloxacin (**2**) and trimethoprim (**47**) is presented in Scheme 25.[182]

In the design of heterodimers differing in the degree of overlap of the pharmacophores and in the molecular mass, Karoli *et al.*[182] used data on the structure–activity relationships for fluoroquinolones and benzylpyrimidines. However, the researchers faced considerable synthetic difficulties: whereas antibiotic **105** was isolated by HPLC in a moderate amount, in the case of **106**, several tested synthetic charts did not lead to the desired outcome. Antibiotic **105** proved to be inactive against a panel of gram-positive and gram-negative strains. The lack of activity led to the assumption that the overlap of the ciprofloxacin and trimethoprim pharmacophores was excessive.

**Scheme 25**



**Structures 107–109**



**107**



**108**



**109**

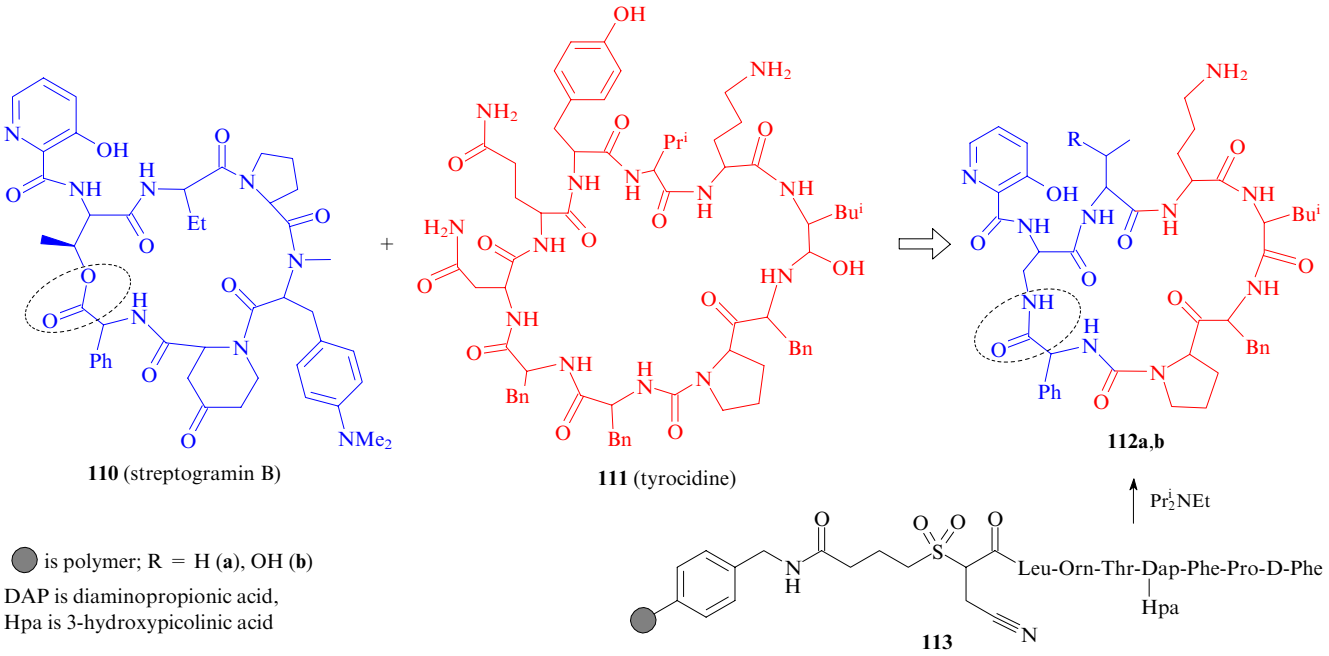
Subsequently, *in silico* computer simulation techniques have been employed for the design of chimeric structures in order to verify, by quantum chemical calculations, whether the desired structure would be able to interact with the targets of both antibacterial agents. Hence, studies of structures **107**–**109** prepared by click-chemistry methods were more successful.

Although among these compounds, only compound **109** had a dual mode of action and interacted with the targets of both antibiotics — topoisomerase IV and gyrase (fluoroquinolone targets) and dihydrofolate reductase (trimethoprim target), it still exhibited a moderate antibacterial activity. Antibiotic **108** was most active in this series; however, it inhibited only topoisomerase IV and gyrase but did not affect the activity of dihydrofolate reductase. Thus, in the case of trimethoprim and ciprofloxacin, the approach based on the coupling of pharmacophores proved to be of low utility. Meanwhile, the use of a spacer between the pharmacophore groups may give rise to compounds having antibacterial activities, which is in good agreement with the above examples.

Chimeric structures obtained by coupling fragments of antibacterial agents, streptogramin B (**110**) and tyrocidine (**111**), have been described (Scheme 26).[183] The mechanism of action of streptogramin B group antibiotics comprises inhibition of the translation *via* binding to a ribosome.[184] Despite the relatively recent introduction of these antibiotics into clinical practice, the resistance to them is encountered quite often.[185] The mechanisms of resistance to streptogramins B are related to modification of the target (methylation of ribosomal RNA with methyltransferases) and enzymatic hydrolysis of the ester bond by the Vgb lyase. Johnston *et al.*[184] proposed using the isosteric replacement of the ester group (marked by a dashed oval in Scheme 26) by a more stable amide bond and coupled the streptogramin B structure with tyrocidine, a cyclic peptide type antibiotic, which functions by damaging the structure of the cell membrane and inhibiting the RNA synthesis.

Cyclic peptide **112a** and its analogues differing in the number and composition of amino acids were prepared by solid-phase peptide synthesis. The final cyclization step was accomplished using two strategies: enzymatic cyclization induced by the immobilized recombinant enzyme tyrocidine thioesterase and chemical cyclization in the presence of a base. However, in the former case, the yield of the target product was very low, while cyclization of biopolymer **113** with a sulfamide linker in THF in the presence of 20 mass % of diisopropylethylamine afforded 10 mg of peptide **112b** (see Scheme 26). Antibiotic **112b** proved to be virtually inactive with respect to a panel of gram-negative bacteria but highly active against gram-positive bacteria, including *Bacillus subtilis*, *Enterococcus faecium*, *E. faecalis*, *Str. pneumoniae*, *St. epidermidis*, *St. saprophyticus* and *Micrococcus luteus*. Thus, like many peptide and polyketide antibiotics, chimeric antibiotic **112b** does not apparently penetrate through the cell wall of gram-negative bacteria.

**Scheme 26**



**110** (streptogramin B)

**111** (tyrocidine)

**112a,b**

**113**

is polymer; R = H (**a**), OH (**b**)

DAP is diaminopropionic acid,
Hpa is 3-hydroxypicolinic acid

Unexpectedly, it turned out that the mechanism of action of antibiotic **112b** differs from the mechanisms of action of both streptogramin and tyrocidine, *i.e.*, the authors succeeded in preparing a peptide antibiotic with a new (yet unknown) mechanism of action.

Thus, the preparation strategy of antibiotics based on coupling of two antibacterial agents, although provides in some cases unexpected and interesting results, is associated with considerable synthetic problems and complexity of predicting the biological activity of the resulting compounds. In most cases, the introduction of a spacer between the pharmacophoric groups of two molecules is the preferred strategy for the preparation of dual action antibiotics.

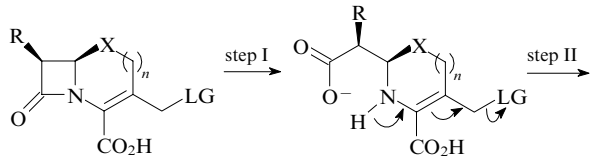
## IV. Dual action antibiotics containing a cleavable spacer as prodrugs

Dual action antibiotics containing a cleavable spacer can also be considered as dual action prodrugs that contain two pharmaceutical agents connected by a spacer. In the living cell (body), the spacer is chemically or enzymatically cleaved, thus releasing two active components. At least one of the components should possess antibacterial activity. Another possible option is that one compound increases the activity of the other one owing to inhibition of certain enzymes or operation of efflux pump proteins providing active transport of antibiotics out of the cell.

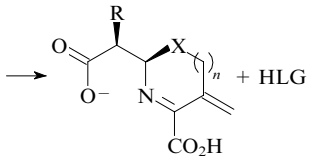
### IV.1. Dual action antibiotic prodrugs based on β-lactams

The development of antibacterial prodrugs with a dual mode of action was triggered by the discovery of the fact that cephalosporins containing a good leaving group in the methylene substituent in position 3 give off this group after enzymatic hydrolysis of the β-lactam amide bond induced by β-lactamases (Scheme 27).[186]

**Scheme 27**




Cephalosporin (X = S, $n$ = 1); penem (X = S, $n$ = 0) or carbopenem (X = $CH_2$, $n$ = 0)



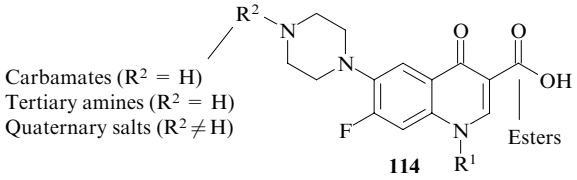

LG is leaving group; step I is cleavage of the β-lactam amide bond; step II is LG elimination

Thus, while designing dual action prodrugs based on β-lactams, one should take into account the fact of lactam ring opening to release the second antibacterial agent located in position 3 of cephalosporin or in a similar position of other lactams, *e.g.*, penem or carbopenem.

#### IV.1.a. Dual action antibiotic prodrugs based on β-lactams and fluoroquinolones

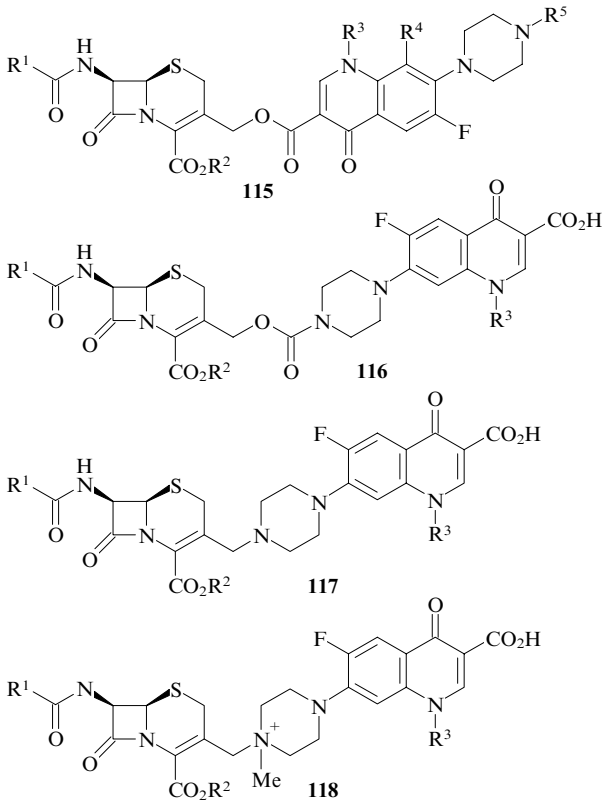
The greatest number of studies concerning dual action antibiotic prodrugs describe the design of molecules based on β-lactams and fluoroquinolones. The molecules of fluoroquinolones **114** have two sites suitable for relatively easy attachment of β-lactams to form a chimeric compound, namely, the carboxy group in position 3 and the secondary nitrogen atom in piperazine.

**Structure 114**



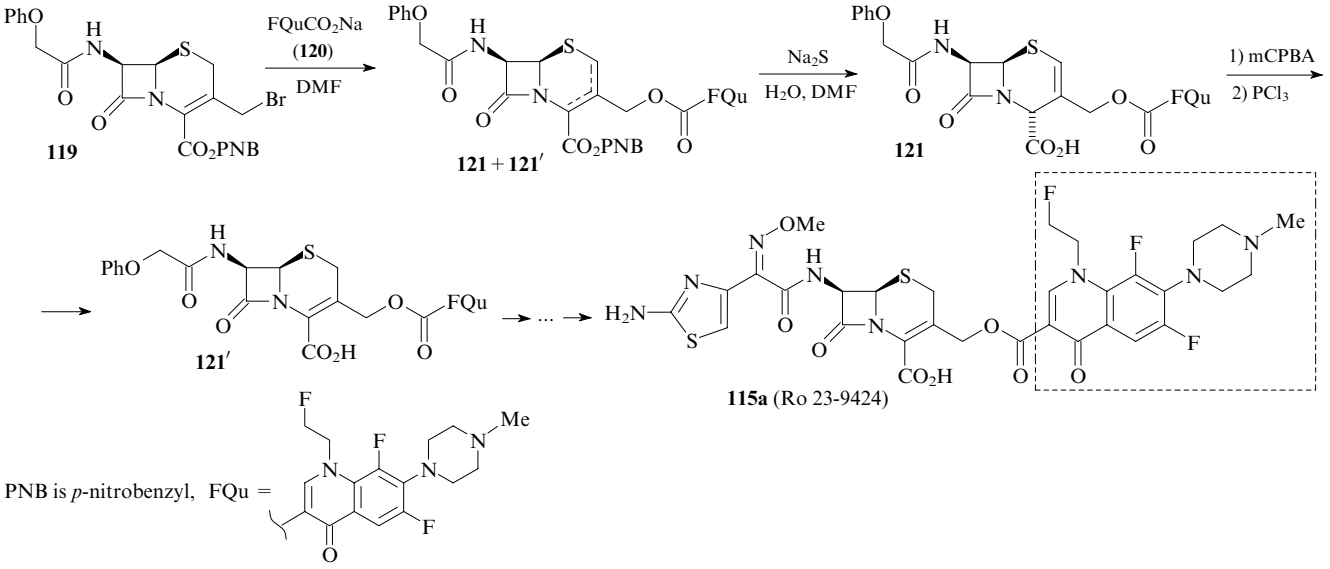

A most extensive series of the dual action antibiotic prodrugs based on β-lactams and fluoroquinolones was described by Albrecht *et al.*[187–190] In these structures, the cephalosporin molecule was attached to fluoroquinolone *via* an ester (compounds **115**) [187] or carbamate bond (**116**) [188] or *via* a tertiary (**117**) [189] or quaternary nitrogen atom (**118**).[190]

**Structures 115–118**




Antibiotic **115a** (Ro 23-9424), which contains a third-generation cephalosporin (desacetyl cefotaxime) and fleroxacin connected by an ester bond has become the leader compound of this series. The key step of the synthesis of compound **115a** has been the reaction of bromo derivative **119** with the sodium salt of a substituted nalidixic acid **120** (Scheme 28). This gave an intermediate existing as a mixture of two isomers with different positions of the double bond [at C(2) (**121**) or C(3) (**121′**) atom, the required **121′** isomer being formed in a lower amount]. The removal of the PNB protective group was followed by the standard oxidation and reduction reaction sequence to give the desired product **115a**.

**Scheme 28**




The antibiotic Ro 23-9424 had a broad spectrum of antibacterial activity *in vitro* and *in vivo* with respect to gram-positive (MIC = 1 μg ml$^{-1}$ against *St. aureus*) and gram-negative bacteria (MIC = 0.125 μg ml$^{-1}$ against *E. coli*).[191, 192] This drug has been vigorously investigated in *in vivo* experiments. The compound has typical pharmacokinetic characteristics of cephalosporins,[193] it is stable in blood and is mainly excreted from the body unchanged.[194] The antibiotic Ro 23-9424 has passed phase I clinical trials;[195] however, no further advancement has been reported. Probably, the use of compound **115a** does not prevent the development of resistance.[196]

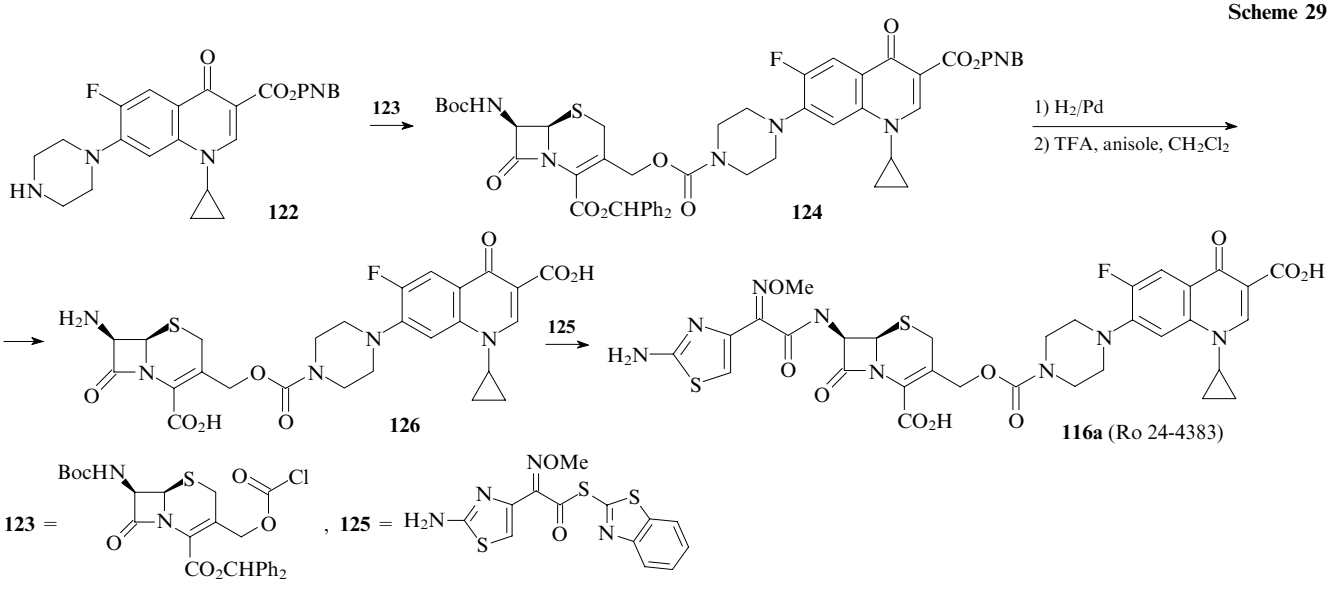
Georgopapadakou and McCaffrey[197] investigated the stability of Ro 23-9424 to various buffer solutions and to β-lactamases. They demonstrated that this antibiotic is hydrolyzed rather fast in solutions and in blood serum (the half-life was 3 to 6.9 h) but it is rather stable to the action of β-lactamase (compound **115a** was hydrolyzed to a noticeable extend under the action of only one of three β-lactamases studied). Thus, it was concluded that chemical hydrolysis of Ro 23-9424 can occur *in vitro* to give fluoroquinolone and cephalosporin.

To improve the stability, solubility and synthetic accessibility of chimeric antibiotics based on fluoroquinolones and β-lactams, compounds with a carbamate bond [188] and a tertiary [189] or quaternary [190] nitrogen atom as the spacer have been prepared.

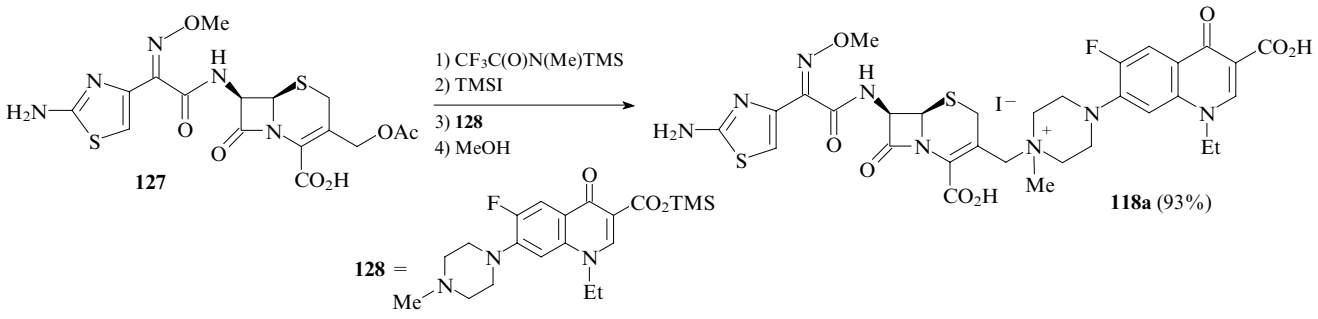
The carbamate analogues, for example, Ro 24-4383 (**116a**), were synthesized by acylation of the piperazine N(4′) atom of fluoroquinolone **122** with appropriate chloroformate (*e.g.*, compound **123**) (Scheme 29). The deprotection of intermediate **124** and acylation with thioester **125** of the amino groups of β-lactam **126** furnished the desired product.

Most active among this series was the antibiotic Ro 24-4383 (**116a**), which showed high antibacterial activity *in vitro* with respect to gram-negative (MIC = 0.031 μg ml$^{-1}$ against *E. coli*) and gram-positive bacteria (MIC = 2 μg ml$^{-1}$ against *St. aureus*). Carbamate **116a** also had an enhanced chemical stability (the half-life in

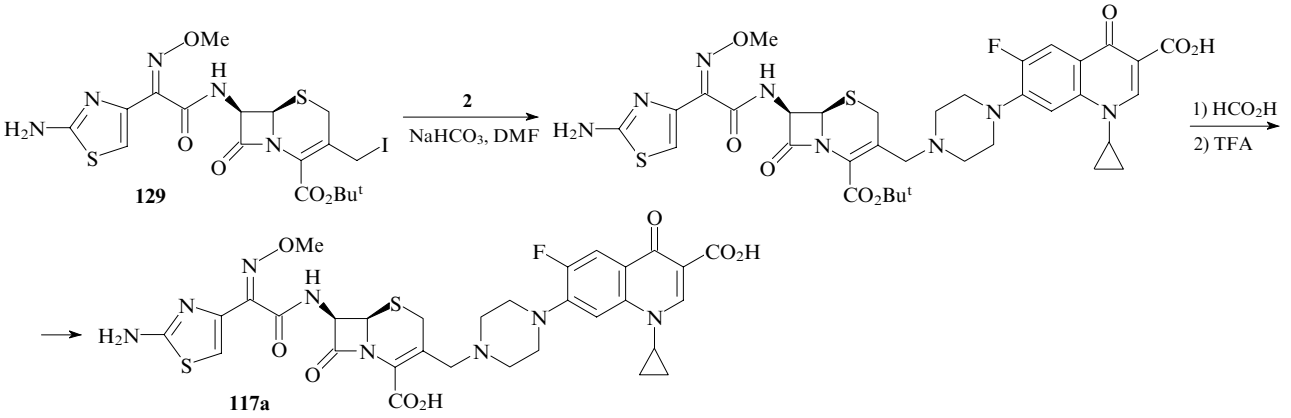
**Scheme 29**



**Scheme 30**




**Scheme 31**




the phosphate buffer at pH 7.4 at 37 °C was ~10.5 h). The carbamate derivatives proved to be fairly sensitive to the β-lactamase-induced hydrolysis. In view of the high stability in solutions and sensitivity to β-lactamases, Albrecht *et al.*[188] deem that in β-lactamase-producing microorganisms, Ro 24-4383 acts by only one mechanism, namely, due to the fluoroquinolone, which is released upon hydrolysis of the β-lactam ring.

Chimeric antibiotics containing quaternary nitrogen in the spacer were prepared by alkylating the piperazine N(4) atom. In the initial stage of the synthesis of, for example, compound **118a**, cephalosporin analogue **127** was treated with a silylating reagent to protect the labile groups, and then protected fluoroquinolone **128** was added *in situ*. Methanol solvolysis resulted in precipitation of product **118a** as the quaternary ammonium salt (Scheme 30).[190]

Dual action prodrugs containing a quaternary amino group possessed a much lower antibacterial activity than the compounds with an ester or carbamate type spacer; however, they were exceptionally stable in solutions (the half-life was ~12 days).

Derivatives with a tertiary amino group in the spacer were prepared by N-alkylation of the piperazine ring of fluoroquinolone with various β-lactams having a haloalkyl group as a substituent. For example, compound **117a** was formed upon the reaction of ciprofloxacin (**2**) with iodo derivative of cephalosporin **129** (Scheme 31).[189]

Dual action antibiotics with a tertiary amino group in the spacer possessed a high antibacterial activity *in vitro* with respect to gram-negative (compound **117a**: MIC = 0.125 μg ml$^{-1}$ against *E. coli*) and gram-positive strains (compound **117a**: MIC = 4 μg ml$^{-1}$ against *St. aureus*). However, a study of the mechanisms of action of chimeric structures of this type showed that they are likely to act as whole chimeric molecules with a dual mode of action rather than as prodrugs.[189]

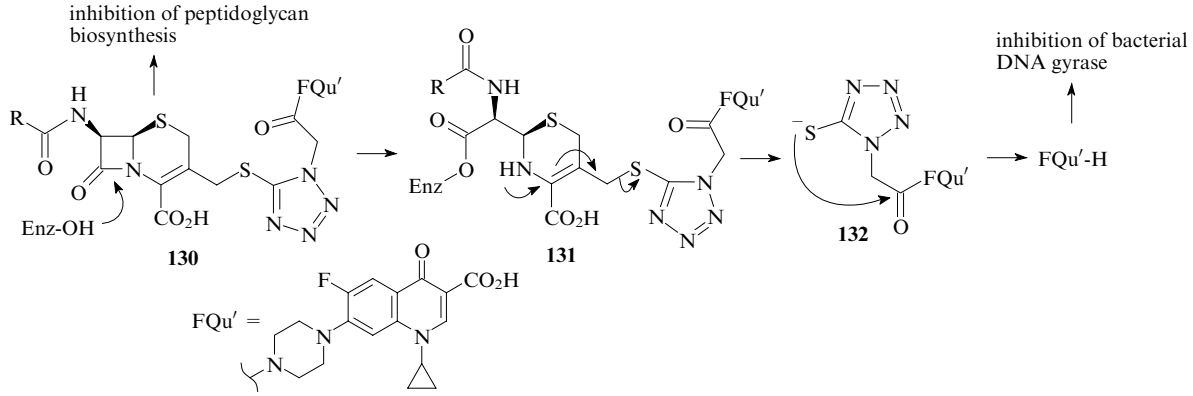
The studies of dual action antibiotics based on fluoroquinolones and β-lactams were continued by an attempt to obtain structures in which two antibiotics are connected by a tetrazole-containing spacer.[198]

The design of such a chimeric antibiotic prodrug **130** was based on the assumption that β-lactam ring opening induced by an enzyme (Enz-OH) is followed by cleavage of the C–S bond in intermediate **131**. The subsequent intramolecular nucleophilic attack of the resulting thiolate anion **132** by the amide carbonyl group leads to the release of the fluoroquinolone molecule (Scheme 32).[198]

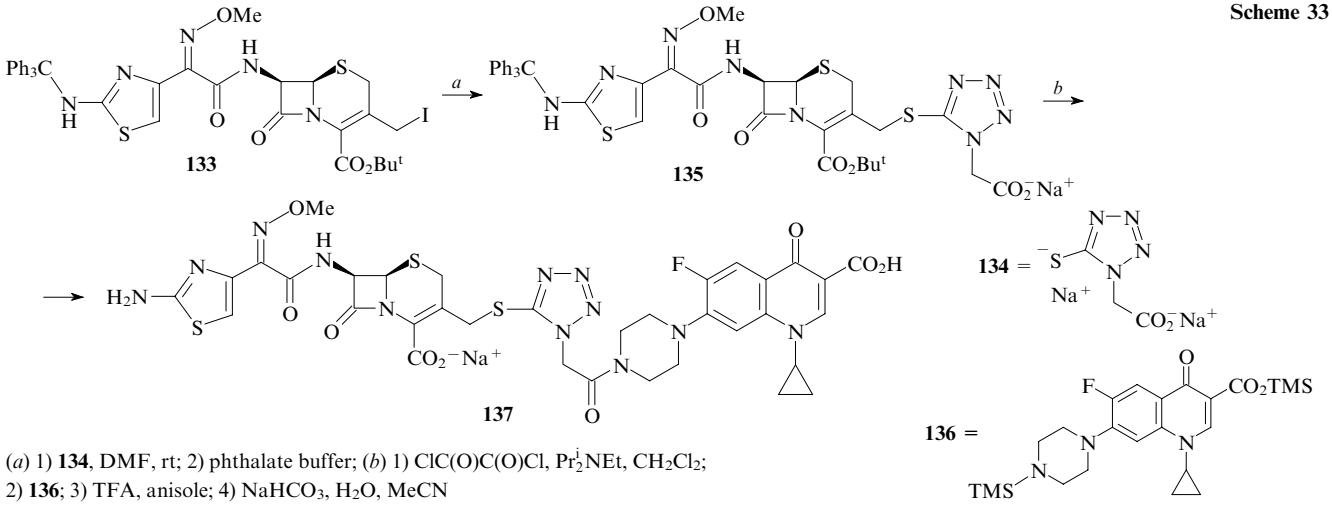
The tetrazole ring was introduced into 3-iodomethylcephalosporin molecule **133** by nucleophilic substitution involving tetrazole **134**. Then intermediate **135** was activated *in situ* with oxalyl chloride and made to react with disilylated ciprofloxacin derivative **136**. The final step of the synthesis is elimination of the protecting (trimethylsilyl, N-trityl and ester) groups to give heterodimeric structure **137** (Scheme 33).[198]

Antibiotic **137** had a moderate activity *in vitro* against gram-negative (MIC = 4 μg ml$^{-1}$ against *E. coli*) and gram-positive strains (MIC = 4 μg ml$^{-1}$ against *St. aureus*). It was highly stable in solutions (the half-life was 11 to 16 days) and was efficiently hydrolyzed by β-lactamases. However, hydrolysis of the amide bond between the piperazine ring and the tetrazole acyl group proceeded to a noticeable extent only at high pH values (11–13).

**Scheme 32**




**Scheme 33**




(*a*) 1) **134**, DMF, rt; 2) phthalate buffer; (*b*) 1) ClC(O)C(O)Cl, $Pr^i_2NEt$, $CH_2Cl_2$; 2) **136**; 3) TFA, anisole; 4) $NaHCO_3$, $H_2O$, MeCN

### IV.1.b. Miscellaneous examples of dual action antibiotic prodrugs based on β-lactams

β-Lactams have served as the base for the design of some other dual action antibiotics functioning by the prodrug principle.

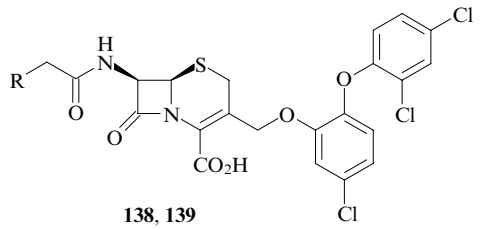
According to author's intention, compound **138** (NB2001) was expected to have a dual mode of action and enhanced activity against β-lactamase-producing strains owing to combination of cephalosporin and triclosan molecules.[199] It was ascertained that this derivative is a substrate for β-lactamase. Formation of triclosan was observed in β-lactamase-producing *E. coli* strains. This indicated that as regards the mechanism of action, antibiotic **138** is a prodrug.

A tetrazole analogue of antibiotic **138**, compound **139** (NB2030), has also been studied.[200] It was shown that the major contribution to the antibacterial activities of both the NB2001 and NB2030 drugs is due to hydrolysis of the β-lactam ring and release of triclosan, which inhibits enoyl-ACP reductase.

Wang *et al.*[201] reported a series of dual action prodrugs in which a cephalosporin or penicillin antibiotic is connected *via* position 3 or 7 of the β-lactam ring to a cytotoxic agent — mitozolomide (**140**) or temozolomide (**141**).

The mechanism of action of the antitumour drugs, mitozolomide and temozolomide, is related to cross-linking of DNA molecules and alkylation of DNA nitrogen bases.[202–204] It was assumed that combination of the specificity of the β-lactam and the cytotoxic action of mitozolomide or temozolomide would result in highly active antibacterial drugs. However, the results of testing of antibacterial activity of chimeric antibiotics **140** and **141** were negative; furthermore, these compounds proved to be inactive against MRSA and other β-lactamase-producing strains.

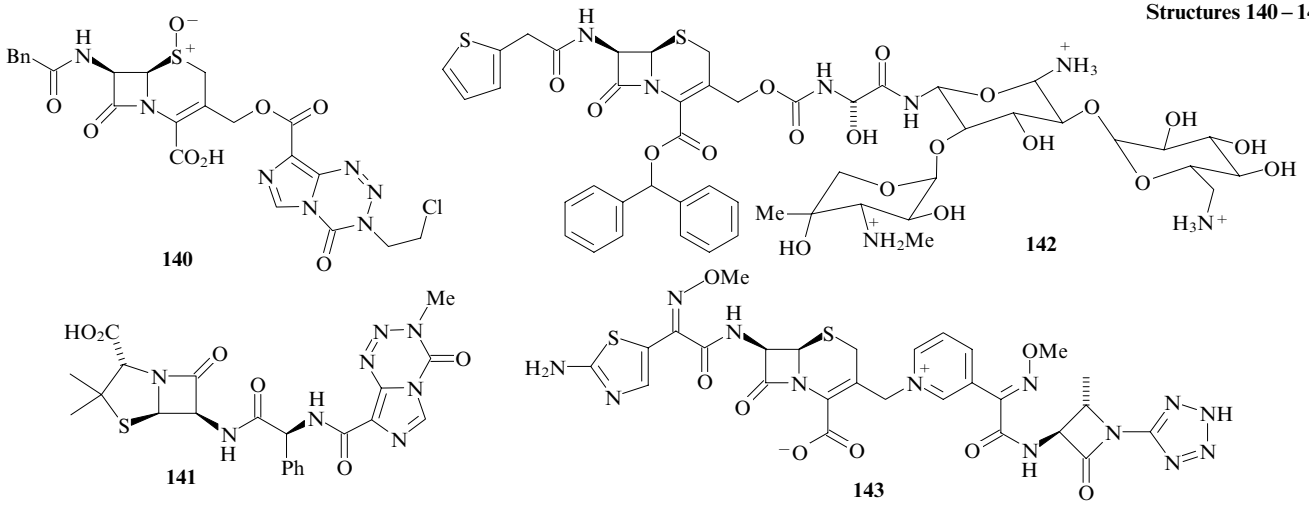
**Structures 138, 139**




R = 2-Th (**138**, NB2001), tetrazol-1-yl (**139**, NB2030); Th is thienyl

Mobashery and co-workers[205] developed approaches to dual action structures based on β-lactams and aminoglycosides. These antibiotics were designed for reducing the toxicity of aminoglycosides in humans and for the delivery of aminoglycosides into a bacterial cell. It was assumed that after a non-toxic conjugate has penetrated into the cell, β-lactam would be hydrolyzed and aminoglycoside would split off. The authors noted substantial experimental difficulties faced in the synthesis of these chimeric products. Nevertheless, they were able to prepare three conjugates that differed in the structure of aminoglycoside and in

**Structures 140 – 143**



which cephalosporin and aminoglycoside were connected by a carbamate spacer. An example of such antibiotics is compound **142**. The key stage of the synthesis was condensation of the corresponding protected derivative of aminoglycoside with cephalosporin containing an activated carbonate group.

Preliminary biological tests of the resulting antibiotics **142** demonstrated that they are good substrates for β-lactamase and have lower toxicity than the initial aminoglycosides. Data on the antibacterial activity have not been published as yet.

A cephalosporin antibiotic, cefotaxime, served as the basis for a series of dual action drugs containing monobactam as the second antibacterial agent.[206] The monobactam used in the study had a relatively low antibacterial activity in *in vitro* experiments, presumably due to low penetrability into the cells. However, attachment of this agent to a cephalosporin molecule gave rise to conjugates with a pronounced antibacterial activity; furthermore, some of them, for example, antibiotic **143**, were more active than the initial cefotaxime against some *E. coli* and *K. pneumonia* strains.

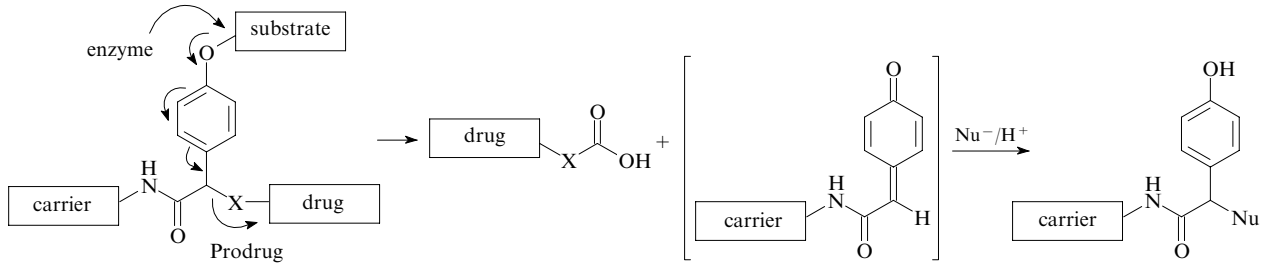
## IV.2. Other examples of dual action antibiotic prodrugs

Lee *et al.*[207] developed a general structure of the dual action prodrug based on 4-hydroxymandelic acid in which combination of different biologically active compounds is theoretically possible. Scheme 34 presents the general mechanism of action of this drug. The prodrug undergoes a cascade of transformations induced by enzymes, resulting in the release of two components.

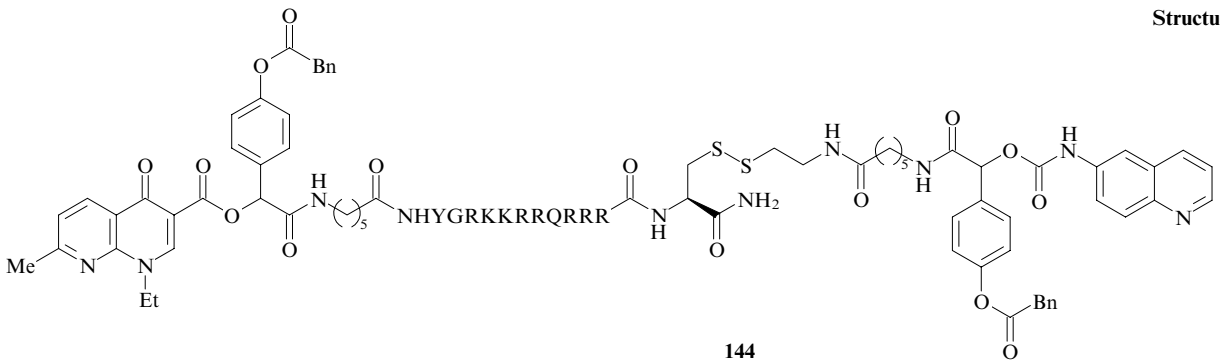
To validate this concept, antibiotic **144** based on nalidixic acid and 6-aminoquinoline attached to different ends of the peptide carrier *via* a spacer containing 4-hydroxymandelic acid has been prepared.

The incubation of antibiotic **144** with penicillin G amidase from *E. coli* — an enzyme that is also able to hydrolyze the ester bond — resulted in the release of

**Scheme 34**




**Structure 144**



nalidixic acid and 6-aminoquinoline, indicating that enzyme-catalyzed cleavage of the compound has taken place. The antibacterial activity of chimeric antibiotic **144** with respect to the *E. coli* strains that had been transformed by the penicillin G amidase-encoding gene was 70 times higher than the activity of nalidixic acid (half-maximum inhibitory concentrations $IC_{50}$ = 1.9 and 138 μmol $ml^{-1}$, respectively).

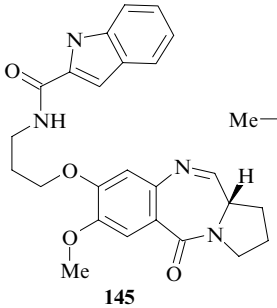
## V. Dual action therapeutic agents based on antitumour antibiotics

The synthesis of hybrid or chimeric structures that combine two antitumour agents with the same or different mechanisms of action is widely used for the search for new promising antitumour agents. The achievements in this field were exhaustively described by Viegas Jr. *et al.*[208] in 2007 and by Gediya and Njar[209] in 2009. The latest information on this issue is reported in the review by Fortin and Berube.[210] Note that although quite a number of hybrid and chimeric antitumour agents have been prepared and studied, there are still few compounds based on antitumour antibiotics.

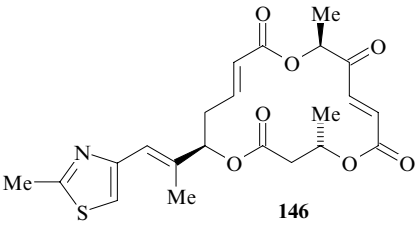
Some examples of incorporation of antitumour antibiotics into structures with a presumed dual mode of action or hybrid molecules of this type are presented below.

Conjugates of pyrrolo[2,1-*c*]benzo-1,4-diazepines (PBD) with indoles have been obtained.[211] Pyrrolo[2,1-*c*]benzo-1,4-diazepines are natural antitumour antibiotics, which act *via* DNA alkylation and inhibition of nucleic acid synthesis. The introduction of an indole moiety is known to enhance the cytotoxic properties of antitumour agents. Wang *et al.*[212] described the PBD- and indole-based structures, for example, compound **145**. These derivatives show high antiproliferative activity (in the nanomolar concentration range), cause apoptosis of the A2058 cells, bind to DNA and have high antitumour activity *in vivo*.

**Structures 145, 146**



**145**

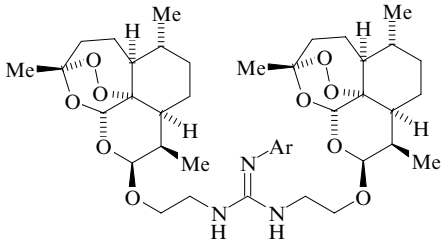


**146**

Hybrids of macrosphelide A (macrolide antibiotic) and epothilone (antitumour agent), for example, compound **146** (MSt-2) result from combination of structures *via* introduction of the 2-methyl-4-(prop-1-enyl)thiazole moiety present in epothilone into the macrosphelide molecule.[213] Compound **146** exhibits antiproliferative activity with respect to the HCT116 and AGS tumour cells and no cytotoxic activity against human fibroblasts. It also gives rise to reactive oxygen species, activates Jun-kinases and induces the apoptosis of the lymphoma cells of the U937 line.[214]

In order to improve the pharmacokinetic characteristics (increase the solubility and the lifetime in the blood plasma), artemisinin and guanidine hybrids — compounds **147a**,**b** — were obtained.[215] They consisted of two artemisinin molecules attached to the two different ends of the spacer containing a guanidine moiety. Derivatives **147a**,**b** showed antiproliferative activity with respect to the the HT-29 tumour cell line.

**Structures 147**

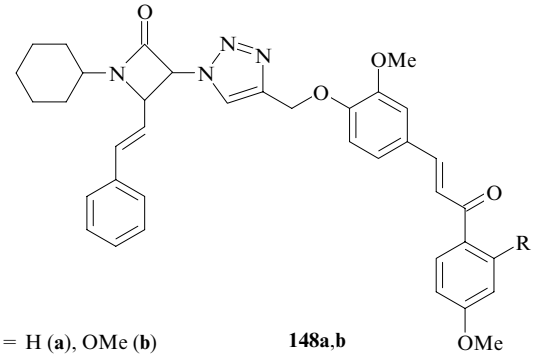


**147a**,**b**

Ar = 2,4-$Cl_2C_6H_3$ (**a**), 4-$F_3CC_6H_4$ (**b**)

The use of click-chemistry methods provided the synthesis of heterofunctional hybrids **148a**,**b** based on β-lactams and chalcones connected by a spacer to a 1,2,3-triazole moiety.[216] Compounds **148a**,**b** exhibited antiproliferative activity in the micromolar concentration range with respect to the A-549, THP-1 and Caco-2 tumour cell lines.

**Structures 148**



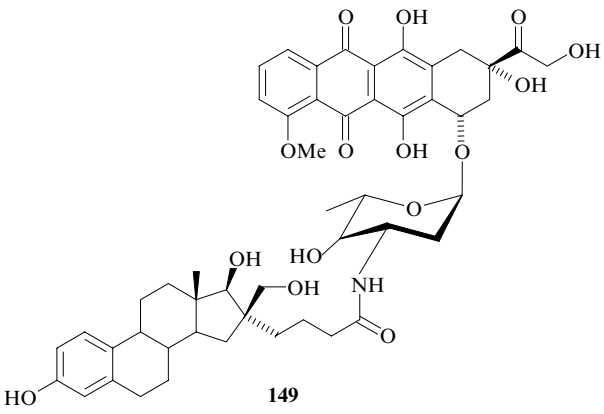
R = H (**a**), OMe (**b**) **148a**,**b**

In order to reduce the cytotoxicity of antracyclic antibiotics, in particular, doxorubicin, Saha *et al.*[217] prepared doxorubicin- and estrogen-based conjugates. Compound **149** showed a high affinity to estrogen receptors and had antiproliferative activity in the micromolar concentration range with respect to the HT-29 and MCF-7 tumour cell lines, which, however, was two orders of magnitude lower than that of doxorubicin.

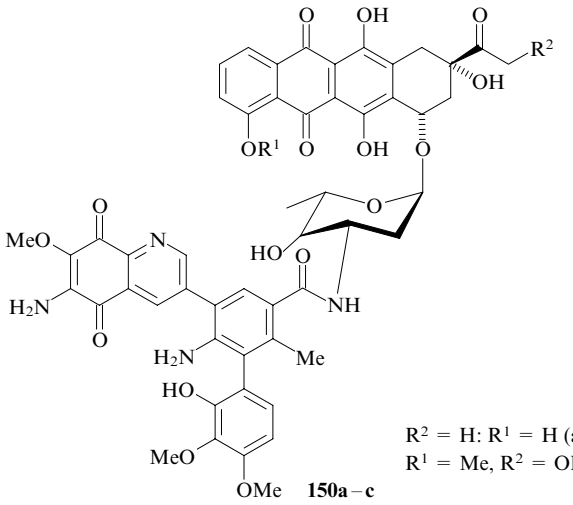
Amides of the antitumour antibiotic streptonigrin (bruneomycin) with the antracyclic agents — carminomycin (**150a**), daunorubicin (**150b**) and doxorubicin (**150c**) — have been reported.[218] Chimeric antibiotics **150a**–**c** proved to be much less active than the initial antitumour agents with respect to the tumour cells of various lines. This is likely to attest to steric hindrance in the receptor recognition processes and enzymatic cleavage of the amide bond in the biological systems *in vitro* and *in vivo*.

Conjugates of doxorubicin with various cephalosporins (compounds **151a**–**f**) were designed according to the above-described principle (see Scheme 27) in such a way that the

**Structures 149 – 151**

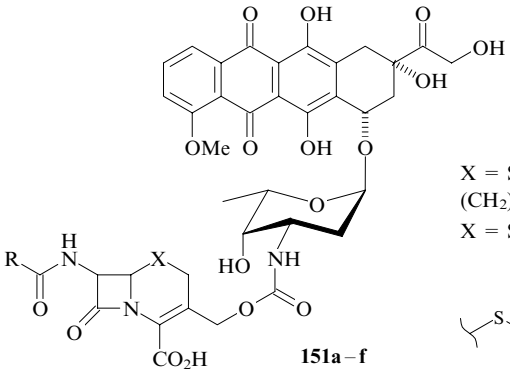


**149**

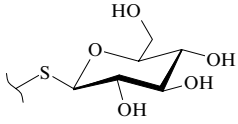


$R^2$ = H: $R^1$ = H (**a**), Me (**b**);
$R^1$ = Me, $R^2$ = OH (**c**)

**150a – c**



X = S: R = H (**a**), Bn (**b**),
$(CH_2)_3CO_2H$ (**c**), $CH(SO_3H)Ph$ (**d**);
X = S(=O): R = 2-Th (**e**),



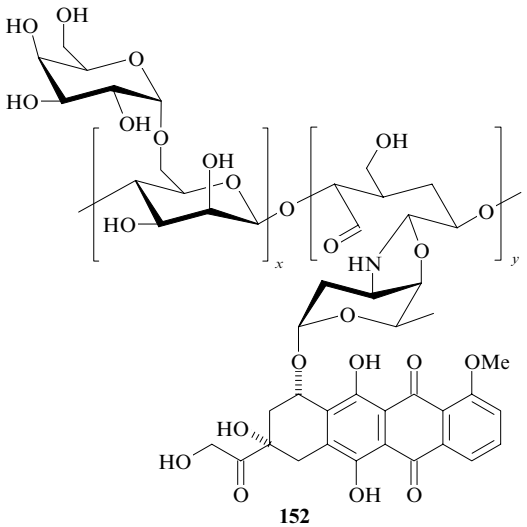
(**f**)

**151a – f**

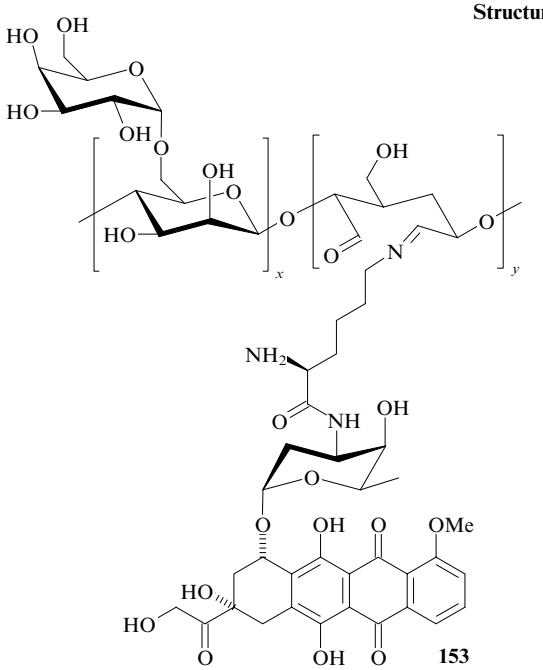
doxorubicin molecule is released after β-lactamase-induced cleavage of the cephalosporin moiety.[219,220] These structures were considered within the framework of the two-stage strategy of antibody-directed enzyme prodrug therapy (ADEPT). In the first stage, an enzyme attached to monoclonal antibodies specific to the particular tumour is administered into the body. In the second stage, a non-toxic prodrug is introduced and then selectively cleaved under the action of the antibody-delivered enzyme to give the active cytostatic.[221]

Compounds **151a – f** were much less cytotoxic in *in vitro* experiments than doxorubicin with respect to human adenocarcinoma H2981 cells, and the β-lactamase conjugate with the antibody efficiently activated these prodrugs with the release of free doxorubicin. In the authors' opinion, of most interest was compound **151b**, which was 10 times less cytotoxic than doxorubicin. This derivative was actively studied *in vivo* and, in some experiments, prodrug **151b** in combination with the β-lactamase – antibody conjugate was

**Structures 152, 153**



**152**



**153**

superior over doxorubicin, for example, for treating intestinal tumours.[222]

Conjugates of doxorubicin with the polysaccharide DAVANAT (or GM-CT-01) have been reported.[223] This polysaccharide, resulting from controlled partial hydrolysis of the polysaccharide from the *Cyamopsis tetragonoloba* or *Guar gum* seeds, is 1,4-β-D-galactomannan consisting of (1→4)-β-D-mannopyranosyl subunits bearing α-D-galactopyranose residues.[224] This compound has passed phase II clinical trials as a drug enhancing the action of cytotoxic agents and mitigating side effects of chemotherapy. Conjugates of doxorubicin with DAVANAT® have been prepared differing in the antibiotic load and mode of attachment to the polysaccharide, either directly (**152**) or *via* a spacer (**153**).[225]

Water-soluble conjugate **152** retained the antiproliferative activity with respect of three tumour cell lines: B16-F1 mouse melanoma, MCF-7 breast cancer and HT-29 (HTB-38) colon cancer cell lines.

## VI. Conclusion

The search for new dual action antibiotics based on known antibacterial agents is a topical area of modern research. The key advantages of dual action drugs include the potential activity against resistant microorganisms and extension of the spectrum of action as compared with the component antibiotics contained in the new molecule. In some cases, this can solve the solubility problem of one of the components, change the ability to penetrate into the bacterial or tumour cell or reduce the toxicity (this is especially important for antitumour drugs) (Table 5). The design of structures of this sort should obviously rely on the knowledge of the mechanisms of action and structure–biological activity relationships for each of the components.

However, the preparation of chimeric structures based on antibiotics is faced with considerable synthetic difficulties. Often, antibiotics of different classes have different stability ranges. These ranges determine the applicability of chemical reactions, which may not coincide for different classes of drugs. Furthermore, each of the components is a sophisticated polyfunctional compound, which complicates the chemical modification of particular functional groups in the presence of other reaction sites. Of special note are purification methods of dual action antibiotics because in this case, the absence of traces of initial compounds in the final product is of prime importance.

In some cases, antibiotics designed as dual action drugs lose the ability to interact with one of the targets of the drugs contained in the chimeric molecule. The increase in the molecular mass can markedly change the bioavailability of the drug due to the change in the pharmacokinetic properties. As a result, the drug delivery to organs and tissues may pose a problem and also the drug penetrability into the bacterial cell may decrease.

Apparently, the circumstances listed above account for the small number of successful examples of commercialization of dual action antibiotics. Currently, only two drugs of this type — cadazolid (**16**) and TD-1792 (**87**) — are under clinical trials.

Many researchers believe that the future of dual action drugs is related to the preparation of antibiotics by combining the structural units of molecules responsible for binding to the target, for penetration into the cell and for the optimal pharmacological characteristics. However, this approach requires detailed investigation of the structure–biological activity relationships, the use of various simulation techniques and the knowledge of the mechanisms of action of antibiotics at the molecular and cellular levels.

**Table 5**. Comparative analysis of combined therapy by dual action drugs and dual action antibiotic prodrugs.

| Type | Advantages | Disadvantages |
|---|---|---|
| Combined therapy (separate administration of two therapeutic agents) | 1. Administration of known components<br>2. To achieve the optimal action, the dosage of each component can be adjusted separately | 1. Problem of adjustment of the optimal dosage form and administration schedule<br>2. Different pharmacokinetic characteristics of each drug<br>3. Many side effects |
| Dual action drugs | 1. Simultaneous delivery of the drugs into the bacterial cells to the targets and high concentration of two drugs simultaneously<br>2. Overcoming the resistance and possible retardation of development of the resistance<br>3. Easy selection of the dosage form<br>4. Decrease in the toxicity | 1. Difficulty of the synthesis<br>2. Activity of each component against the target may be reduced<br>3. Possible problems of drug delivery to the targets<br>4. High molecular mass and, as a consequence, decrease in the bioavailability |
| Dual action prodrugs | 1. Simultaneous delivery of the drugs into the bacterial cells to the targets and high concentration of two drugs simultaneously<br>2. Overcoming the resistance and retardation of development of the resistance<br>3. Easy selection of the dosage form<br>4. Increase in the chemical stability of the drug<br>5. Decrease in the toxicity | 1. Difficulty of the synthesis<br>2. Possible problems of drug delivery to the targets<br>3. High molecular mass and, as a consequence, decrease in the bioavailability<br>4. Possible problems of penetration into the cell |

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