Hybrid Azine Derivatives: A Useful Approach for Antimicrobial Therapy

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Abstract: Nowadays, infectious diseases caused by microorganisms are a major threat to human health, mostly because of drug resistance, multi-drug resistance and extensive-drug-resistance phenomena to microbial pathogens. During the last few years, obtaining hybrid azaheterocyclic drugs represents a powerful and attractive approach in modern antimicrobial therapy with very promising results including overcoming microbial drug resistance. The emphasis of this review is to notify the scientific community about the latest recent advances from the last five years in the field of hybrid azine derivatives with antimicrobial activity. The review is divided according to the main series of six-member ring azaheterocycles with one nitrogen atom and their fused analogs. In each case, the main essential data concerning synthesis and antimicrobial activity are presented.

Keywords: hybrid compounds; antimicrobial; pyridine; quinoline; isoquinoline; fused azine

1. Introduction

According to the WHO, infectious diseases caused by microorganisms represent a major threat that affects society and human health, exerting great pressure on health systems, individuals and communities [1]. In particular, overconsumption and widespread use and misuse of antimicrobial agents have resulted in the emergence of drug resistance, multi-drug resistance and extensive-drug-resistance phenomena to microbial pathogens and many other drawbacks (toxicity and non-specificity of drugs, high prices, etc.). So far, searching for new chemical entities with improved antimicrobial properties remains a very challenging and important task in medicinal chemistry.

During the last few years, molecular hybridization represents a powerful tool in drug design, by merging two or more drug pharmacophores in a single hybrid multi-functional molecule. Usually, the resulting hybrid entity has superior properties compared with conventional classic drugs, with dual or multiple target mechanisms, better biological activity and specificity, less side effects and toxicity, less drug–drug interactions, etc. [2,3]. As a result of this approach, important advances have been achieved in antimicrobial therapy, some of the present drugs from the market have a hybrid structure (Figure 1) and some hybrid structures are in different clinical trials (Figure 2) [2–8].
A literature survey revealed that azines are privileged scaffolds in current medicinal chemistry and drug discovery, possessing a large variety of biological activities, such as: antibacterial, antifungal, antimalarial, anthelmintic, antitubercular, antiviral, anticancer, anti-inflammatory, antihypertensive, diuretic, antithrombic, anticoagulant, antidepressant, anxiolytic, anticonvulsant, analgesic, antiulcer, antidiabetic, antihistaminic, etc. [4–8]. As a matter of fact, the greatest majority of the existing drugs from the market contain in their structure a nitrogen heterocycle, some of them being a
hybrid structure (Figure 1), which justifies the demand of the pharmaceutical industry for such drugs with nitrogen heterocycle skeleton.

Because of the above considerations, there is a large and urgent demand from the pharmaceutical industry for newer and better drugs with enhanced antimicrobial activity, with superior pharmacokinetic and pharmacodynamic properties, the hybrid drugs being a serious and preferential option.

In this review, we present an overview of the newest research concerning the synthesis and antimicrobial activity of hybrid azine derivatives. The main data reviewed in this paper are summarized in Table 1 presented below.
Table 1. Chemical structure, type of reactions and biological activity of the hybrids.

<table>
<thead>
<tr>
<th>Synthesized Hybrids</th>
<th>Type of Reactions</th>
<th>Antimicrobial Activity</th>
<th>Biological Activity</th>
<th>Strains Used</th>
<th>Effect Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>- thiazole-pyridine 2a–e and 4a–e</td>
<td>- cyclocondensation</td>
<td>- antibacterial; - antifungal</td>
<td>- B. cereus, S. aureus, E. coli, P. aeruginosa; - C. albicans</td>
<td>B. cereus, S. aureus</td>
<td>active on B. cereus, S. aureus</td>
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<td>- metal-pyridine derivatives 7</td>
<td>- complexation</td>
<td>- antibacterial; - antifungal</td>
<td>- B. cereus, S. aureus, E. coli, P. aeruginosa; - C. albicans</td>
<td>- M. tuberculosis</td>
<td>active on C. albicans</td>
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<tr>
<td>- thiazolidine-pyridine 11a–f and 12a–i</td>
<td>- condensation</td>
<td>- antitubercular</td>
<td>- S. aureus, S. mutans, E. coli, K. pneumoniae</td>
<td>- C. albicans</td>
<td>active on all strains</td>
</tr>
<tr>
<td>- thiazolidine-pyridine 14–20</td>
<td>- cyclocondensation</td>
<td>- antibacterial</td>
<td>- E. coli, P. aeruginosa, S. aureus, S. pyogenes; - C. albicans</td>
<td>active on all bacterial strains</td>
<td></td>
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<tr>
<td>- oxazino-pyridine 23a–j</td>
<td>- cyclocondensation, condensation</td>
<td>- antibacterial; - antifungal</td>
<td>- E. coli, K. pneumoniae, S. aureus, C. albicans</td>
<td>active on S. aureus, C. albicans</td>
<td></td>
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<tr>
<td>- tetrazolo-pyridine 25a–d and tetrazolo-quinoline 26a–e</td>
<td>- cyclocondensation</td>
<td>- antibacterial; - antifungal</td>
<td>- S. aureus, S. pyogenes, E. faecalis, E. coli, P. aeruginosa, A. baumannii</td>
<td>active on all strains</td>
<td></td>
</tr>
<tr>
<td>- oxadiazolo-imidazo-pyridine 28a–j</td>
<td>- cyclocondensation</td>
<td>- antibacterial; - antifungal</td>
<td>- E. coli, S. aureus, S. typhi</td>
<td>active on all strains</td>
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<tr>
<td>- triazolo-pyridine 30a–n and 31a–n</td>
<td>- cyclocondensation</td>
<td>- antibacterial</td>
<td>- E. coli, S. aureus, S. typhi</td>
<td>active on all strains</td>
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<tr>
<td>- triazolo-pyridine 35a–r</td>
<td>- cyclocondensation</td>
<td>- antibacterial; - antifungal</td>
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<td>active on all strains</td>
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<td>- pyrazole-pyridine 37–41, triazolo-pyridine 42–45 and triazino-pyridine 46</td>
<td>- cyclocondensation</td>
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<td>active on all strains</td>
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<tr>
<td>- piperine-pyridine 48a–h</td>
<td>- acylation</td>
<td>- antibacterial; - antifungal</td>
<td>- E. coli, K. pneumoniae, P. aeruginosa</td>
<td>active on E. coli, K. pneumoniae, P. aeruginosa</td>
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<tr>
<td>- triazolo-quinoline 58a–j and 59a–j</td>
<td>- reduction, N-alkylation, cyclocondensation</td>
<td>- antibacterial; - antifungal</td>
<td>- E. coli, P. aeruginosa, K. pneumoniae, E. faecalis, S. aureus, S. pneumoniae; B. subtilis</td>
<td>active on all strains</td>
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<tr>
<td>- piperidino-quinoline 61a,b and triazolo-piperidino-quinoline 62a–k</td>
<td>- N-alkylation, cyclocondensation</td>
<td>- antibacterial; - antifungal</td>
<td></td>
<td>active on all strains</td>
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<tr>
<td>- metal-quinoline 64a–d</td>
<td>- complexation</td>
<td>- antibacterial; - antifungal</td>
<td>- B. animalis, L. plantarum, B. subtilis</td>
<td>active on all strains</td>
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<tr>
<td>Hybrid Type</td>
<td>Method</td>
<td>Biological Activity</td>
<td>Bacteria/species</td>
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<tr>
<td>Triazole-benzothiazole-quinoline hybrids</td>
<td>Cyclocondensation</td>
<td>Antibacterial, Antifungal</td>
<td>S. aureus ATCC 663, S. aureus ATCC 25923, P. aeruginosa, C. albicans, A. terrei</td>
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<tr>
<td>Triazole-quinoline hybrids</td>
<td>Cyclocondensation</td>
<td>Antibacterial, Antifungal</td>
<td>E. coli, B. subtilis, P. aeruginosa, S. aureus, C. albicans, A. flavus, T. viridae, A. niger</td>
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<tr>
<td>Thiazole-quinoline</td>
<td>Condensation, Cyclocondensation</td>
<td>Antibacterial, Antifungal</td>
<td>E. coli, A. baumannii, K. pneumoniae, S. aureus, C. albicans, C. neoformans</td>
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<tr>
<td>Piperazin-quinoline</td>
<td>Alkylation, Condensation</td>
<td>Antibacterial, Antifungal</td>
<td>S. aureus, S. pyogenes, E. coli, P. aeruginosa, C. albicans, A. niger, C. clavatus</td>
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<tr>
<td>Pyridine-quinoline</td>
<td>Condensation</td>
<td>Antibacterial, Antifungal</td>
<td>- S. aureus, P. aeruginosa, C. albicans, A. niger, A. clavatus, M. tuberculosis</td>
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<tr>
<td>Triazole-quinoline</td>
<td>Cyclocondensation</td>
<td>Antibacterial, Antifungal</td>
<td>- S. aureus, S. pyogenes, E. coli, P. aeruginosa, C. albicans, A. niger, A. clavatus</td>
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<tr>
<td>Glycosylated-quinoline hybrids</td>
<td>Hydrolysis, Acylation</td>
<td>Antibacterial, Antifungal</td>
<td>E. coli, C. albicans, P. aeruginosa, A. baumannii, E. faecium, K. pneumonia, P. aeruginosa, E. coli, S. aureus, M. tuberculosis</td>
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<tr>
<td>Piperazine- and Morpholine-quinoline hybrids</td>
<td>Condensation, Alkylation</td>
<td>Antibacterial, Antitubercular</td>
<td>A. baumannii, E. faecium, K. pneumonia, P. aeruginosa, E. coli, S. aureus, M. tuberculosis, A. baumannii and M. tuberculosis</td>
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<tr>
<td>Imidazolium-quinoline</td>
<td>Substitution</td>
<td>Antibacterial</td>
<td>S. aureus, E. coli, P. aeruginosa, B. subtilis, A. baumannii and M. tuberculosis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Active on all strains</td>
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<td>- Active on S. aureus and E. coli</td>
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<td>- Active on S. aureus, E. coli, P. aeruginosa, C. albicans</td>
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<td>- Active on S. aureus, E. coli, P. aeruginosa, C. albicans</td>
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<td>- Active on S. aureus, E. coli, P. aeruginosa, C. albicans</td>
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<td>- Active on S. aureus, E. coli, P. aeruginosa, C. albicans</td>
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<td>- Active on S. aureus, E. coli, P. aeruginosa, C. albicans</td>
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</table>
- benzimidazole-quinoline and ferrocenyl-quinoline 106a–e and 107a–e - cyclocondensation, condensation - antifungal; - antitubercular B. subtilis - C. neoformans; - M. tuberculosis - antimalarial; - antitubercular P. falciparum, P. berghei - M. tuberculosis - active on P. falciparum, P. berghei
  - S. aureus ATCC 6538, S. aureus MRSA N315, S. epidermidis ATCC 14990, B. subtilis, E. coli, P. aeruginosa, P. vulgaris, S. aureus MRSA 6347, S. epidermidis MRSE, S. marcescens - active on all strains
  - S. aureus, E. coli, P. aeruginosa, B. subtilis, E. faecalis, S. typhi - A. baumanii, K. pneumonia, P. aeruginosa
- zwiterionic pyridine-fluoroquinolone and quinoline-fluoroquinolone 108a–h and 109a–h - Mannich-electrophilic amination - antibacterial - peptide-quinolone 113a–l and conjugates with ciprofloxacin, moxiﬂoxacin - solid-phase peptide synthesis - antibacterial
- oxazolidine- and triazole-fluoroquinolone 114a–b and 115a–j - one-pot three component Mannich reactions - antibacterial
- oxazolidine-fluoroquinolone 116a–t - alkylation - antibacterial; - antifungal
- benzimidazole-quinoline 117a–g, 118a–b and 119a–f - alkylation
- oxazolidine-quinoline 120a–g - cyclocondensation - antibacterial; - antifungal S. marcescens; - A. niger, T. mentagrophytes, C. albicans, C. parapsilosis - active on B. cereus
- oxazolidine-quinoline 121a–r - acylation, cyclocondensation, condensation - leishmanicidal - Leishmania major - active
- triazole-quinoline 122a–c - cyclocondensation - antitubercular - C. albicans clinical strains and laboratory - active
- pyrazole-isoquinoline 123a–g - cyclocondensation - antifungal; - antibacterial; - antitubercular - C. albicans, C. tropicalis, C. parapsilosis, C. parapsilosis - active on S. aureus, V. cholera, M. tuberculosis
<table>
<thead>
<tr>
<th>Structure/Reaction Type</th>
<th>Alkylation, Cycladdition/Complexation</th>
<th>Activity</th>
</tr>
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<tbody>
<tr>
<td>Piperazine- and pyrimidine-isoquinoline 126a-h and 127a-h</td>
<td>- N-alkylation, O-alkylation, S-alkylation</td>
<td>- antibacterial; - antifungal; - antitubercular</td>
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<tr>
<td>Imidazole- and benzimidazole-quinoline 128–134</td>
<td>- cycloaddition, N-alkylation</td>
<td>- antibacterial; - antifungal</td>
</tr>
<tr>
<td>Imidazole- and benzimidazole-pyridine 135–138</td>
<td>- cycloaddition, N-alkylation</td>
<td>- antibacterial; - antifungal</td>
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<td>Bis(imidazole)- and bis(benzimidazole)-pyridine 139–143</td>
<td>- N-alkylation</td>
<td>- antibacterial</td>
</tr>
<tr>
<td>Imidazole- and benzimidazole-pyridine and quinoline 144–147</td>
<td>- N-alkylation</td>
<td>- antibacterial</td>
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<tr>
<td>Quinoline-sulfonamide complexes 149–153</td>
<td>- acylation, complexation</td>
<td>- antibacterial; - antifungal</td>
</tr>
<tr>
<td>Pyrrolo-quinoline and pyrrolo-isooquinoline 154a–c and 155a–c</td>
<td>- cycloaddition</td>
<td>- antibacterial; - antifungal</td>
</tr>
<tr>
<td>Pyrrolo-phenanthroline 156a–c</td>
<td>- cycloaddition</td>
<td>- antibacterial</td>
</tr>
<tr>
<td>Mono-indolizine-pyridine 157a–e, salts of mono-indolizine-pyridine 159a–l and bis-indolizine-pyridine 160a–d</td>
<td>- N-alkylation, cycloaddition</td>
<td>- antibacterial</td>
</tr>
<tr>
<td>C. albicans, A. niger, A. oryzae, P. chrysogenum; M. tuberculosis</td>
<td></td>
<td></td>
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<tr>
<td>S. aureus, E. coli; C. albicans</td>
<td></td>
<td>- active on all strains</td>
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<tr>
<td>S. aureus, E. coli; C. albicans</td>
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<td>S. aureus, E. coli; C. albicans</td>
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<td>S. aureus, E. coli; C. albicans</td>
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<td>- active</td>
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<td>S. aureus, E. coli; C. albicans</td>
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<td></td>
<td>- active</td>
</tr>
<tr>
<td>S. aureus, E. coli; C. albicans</td>
<td></td>
<td>- active</td>
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</table>
2. Results and Discussion

2.1. Six-Member Ring Azaheterocycles with One Nitrogen Atom. Hybrid Pyridine

In their attempt to identify new antimicrobial compounds, Eryilmaz et al. [9] designed and synthesized different hybrid pyridine derivatives bearing in the 2- and 4-position of the ring of a thiazole moiety. The synthesis was straight and efficient, involving a Hantzsch cyclocondensation of pyridine-2- and 4-carbothioamide 1 and 3 with acetophenone derivatives, when the desired hybrid 4-(R-2-yl)-2-(pyridin-2-yl)thiazole 2a–e and 4-(R-2-yl)-2-(pyridin-4-yl)thiazole 4a–e are obtained, Scheme 1. The synthesized compounds were tested for their antibacterial activity [four strains, Gram-positive (Bacillus cereus, Staphylococcus aureus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa)] and antifungal activity (one strain, Candida albicans) via minimal inhibitory concentration (MIC) method and DNA cleavage activity studies. The authors established interesting correlation structure-biological activity (SAR), the most relevant finding being that 4-pyridine thiazole hybrid compounds 4a–e showed more potent activity than 2a–e. The most promising compound was found to be 4c (MIC values 0.01 mM) exhibited on the bacterial strains Staphylococcus aureus and Bacillus cereus.

![Scheme 1](image)

In a subsequent paper, some of the above authors (Cinarli et al. [10]) synthesized different hybrid aroylhydrazone-pyridine-metal derivatives. The newly hybrid aroylhydrazone-pyridine metal derivatives [ZnL₂] 7 have been synthesized in two steps: an initial cyclocondensation of pyridine-2-acyl derivative 5 (with aroylhydrazone leading to pyridine-arylohydrazone ligand 6) is followed by complexation with M²⁺ metal (Zn²⁺), Scheme 2.

![Scheme 2](image)
The synthesized compounds were tested for their antibacterial activity (four strains, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*) and antifungal (one strain, *Candida albicans*) activity via minimal inhibitory concentration method. The [ZnL2]7 has been found to be more active than pyridine-arylohydrzone ligand 6 in all microorganisms (MIC= 11.71 μg/mL for bacteria and MIC= 23.43 μg/mL for *C. albicans*). The authors claim that the synthesized new complex acts on microorganisms by disrupting the cell wall structure. The DNA binding interactions was also determined experimentally by spectrophotometric and electrochemical methods. The obtaining data indicate that ligand 6 and hybrid [ZnL2]7 interact the most with guanine base, and charge transfer is from DNA guanine bases to the molecular structures. Moreover, antioxidant activity was determined, and the hybrid [ZnL2]7 acted as a scavenger against peroxide radicals.

Trotsko et al. [11] designed and synthesized different hybrid pyridine derivatives bearing at the 2-, 3- or 4- position of the ring of a thiazolidine-2,4-dione moiety. The synthesis involve a condensation reaction of hydrazonyl-pyridine 9a,b/10a-c with the corresponding (2,4-dioxo-1,3-thiazolidin-5-yl/ylidene) 11a-f, which are leading to the desired hybrid pyridine-2,4-dioxo-1,3-thiazolidin-5-yl derivatives 11a-f or pyridine-2,4-dioxo-1,3-thiazolidin-5-ylidene derivatives 12a-i, Scheme 3.

![Scheme 3. Reaction pathway to obtain hybrid thiazolidine-pyridine 11a-f and 12a-i.](image)

The in vitro antmycobacterial assay (*Mycobacterium tuberculosis*) of the newly obtained compounds reveals strong activity in the concentration range of 1–512 μg/mL and low cytotoxicity. Interesting SAR correlations have been performed, and the highest antimycobacterial activity (MIC= 1μg/mL) was demonstrated for the hybrid pyridine derivatives bearing the thiazolidine-2,4-dione moiety at the 4-position of the pyridine ring (hybrids 11a-c and 12g-i).

Sanad et al. [12] have performed an interesting study concerning the in vitro antimicrobial activity of some newly hybrid thieno-pyrimidine-pyridine derivatives. The synthesized compounds belonged to different classes of substituted pyridine: thiophen-dihydropyridine 14, thiophen-pyridino-pyrimidin-4(1H)-one 15, and fused pyridine: pyrido-thiophen-triizolo-pyrimidine 16a-c, thiophen-pyrido-thieno derivative 17, thiophen-pyrido-thieno-pyrimidin-4-one 18, thiophen-pyrido-thieno-pyrimidin-2,4-dione 19, thiophen-pyrido-thieno-pyrimidin-2-R-4-one 20, Scheme 4.
The synthetic approach is straight and efficient, involving typical organic chemistry reactions, mostly cyclocondensations. The synthesized compounds were tested in vitro for their antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae* as Gram-negative bacterial strains as well as against *Staphylococcus aureus* and *Streptococcus mutans* as Gram-positive bacterial strains. The obtained results (expressed as the diameter of inhibition zones (DIZ) and MIC) reveal that the thio-phen-pyrid-thieno-pyrimidin-2-R-4-one 20a,b exhibit the strongest antibacterial activities against all the tested bacteria, in the range of 40–60 mm for inhibition zones, respectively, 4–16 μg/mL for MIC values.

Desai et al. [13] have studied the in vitro antimicrobial activities of some newly hybrid oxazino-pyridine derivatives. The desired compounds, oxazin-3(4H)-yl(phenyl)ethyldene)amino)-6-(arylidene)amino)-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine 23a–j, were synthesized in two steps, by cyclocondensation of oxazine 21 followed by condensation of the intermediate 22, Scheme 5.
Scheme 5. Reaction pathway to obtain hybrid oxazino-pyridine 23a–j.

The synthesized hybrid compounds were tested for their in vitro antibacterial activity against various bacteria (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes) and fungus (Candida albicans, Aspergillus niger, Aspergillus clavatus) via the MIC method. Some compounds have proved to have a very powerful activity against bacteria E. coli (23h, MIC = 25 μg/mL) and against fungus C. albicans (23f, MIC = 50 μg/mL), respectively, A. clavatus (23h, MIC = 25 μg/mL).

Sribalan et al. [14] have studied the in vitro antimicrobial activity of some triazole-heterocycle hybrid derivatives. The synthesis supposes a cyclocondensation reaction of amide precursors 24 with sodium azide, when the corresponding tetrazolo-pyridine 25a–d and tetrazolo-quinoline 26a–e hybrids are obtained, Scheme 6.

Scheme 6. Reaction pathway to obtain the tetrazolo-pyridine 25a–d and tetrazolo-quinoline 26a–e hybrids.

The synthesized tetrazolo-pyridine 25a–d and tetrazolo-quinoline 26a–e hybrids were tested for their in vitro antibacterial activity against various bacteria (Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes) and the fungus Candida albicans. An interesting SAR correlation has been performed. The compound 25a (the pyridyl ring is decorated with n-butyl) proved to be the most active from the tetrazolo-pyridine series against all bacteria (DIZ in the range of 4–15 mm), having a superior inhibition to the standard drug (amikacin). The compound 26d (the quinoline ring is decorated with a piperidyl-sulfonamide moiety) proved to be the most active from the tetrazolo-quinoline series against all bacteria (DIZ in the range of 4–10 mm), having a comparable inhibition to the standard. The antifungal activity was negligible.

Kuthyala et al. [15] have studied the in vitro antimicrobial activity of some oxadiazolo-imidazopyridine hybrid derivatives. The synthesis was straight, involving a cy-
clocondensation reaction of hydrazonyl-imidazopyridine 27 with different benzoic acids, when the corresponding oxadiazolo-imidazopyridine hybrids 28a–j were obtained, Scheme 7.

![Scheme 7. Reaction pathway to obtain oxadiazolo-imidazopyridine hybrids 28a–j.](image)

The synthesized oxadiazolo-imidazopyridine hybrids 28a–j were tested for their in vitro antibacterial activity against various bacterial pathogens (Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis) and the fungus Candida albicans and Aspergillus niger. An interesting SAR correlation has been performed. The compounds 28f and 28g have high activity against Gram-positive bacteria S. aureus (MIC = 3.12 μg/mL), while compound 28f proved to have high activity against fungus C. albicans (MIC = 12.5 μg/mL).

Ahirwaret et al. [16] synthesized two new series of some 1,3,4-triazolo-pyridine hybrid derivatives and studied their antimicrobial activities. The synthesis was conducted in two steps: a cyclocondensation reaction of dithiocarbazate 29 with ammonia leading to the first class of hybrids triazolo-pyridine 30a–n, then an alkylation reaction of 30a–n with benzyl halide takes place leading to the second class of hybrids triazolo-pyridine 31a–n, Scheme 8.

![Scheme 8. Reaction pathway to obtain 1,3,4-triazolo-pyridine hybrids 30a–m and 31a–m.](image)

The synthesized triazolo-pyridine hybrids 30a–n and 31a–n were evaluated for their in vitro antibacterial activity against Gram-positive bacteria (three strains: Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis) and Gram-negative bacteria (three strains: Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii) by MIC assay. From the tested compounds, two of them, 31h and 31i, have excellent activity against all strains (MIC in the range of 0.91–11 μg/mL).

Jaabil et al. [17] have studied the in vitro antimicrobial activities of some newly hybrid 1,2,3-triazolo-pyridine derivatives. The synthesis was green and efficient, under grinding strategy at room temperature, involving one-pot sequential multicomponent reactions of aryl aldehydes 32a–r, malonitrile 33, methanol and 1,2,3-triazolyl ketone 34, when the corresponding 1,2,3-triazolyl-pyridine/cyanopyridine hybrids 35a–r were obtained, Scheme 9.
The synthesized 1,2,3-triazolo-pyridine hybrids 35a–r were screened for their in vitro antibacterial activity against three human bacterial strains, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*, using the MIC method. Some of the 1,2,3-triazolyl cyanopyridine hybrids displayed a remarkable activity against the tested germs, better than tetracycline (standard drug), according to the R-substituent from the phenyl ring. The most active compounds were 35c (with R= –4-chloro; MIC in the range of 50–90 μg/mL), 35e (with R= –2-methyl; MIC in the range of 40–90 μg/mL) and 35r (with R= –2-thienyl; MIC in the range of 70–120 μg/mL). The hybrid 1,2,3-triazolo-pyridine compounds were also tested for their antioxidant activity in the assay by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, showing promising results.

Felefel et al. [18] synthesized three new series of some pyridine hybrid derivatives (namely pyrazole-pyridine 37–41, triazolo-pyridine 42–45 and triazino-pyridine 46) and studied their antimicrobial activities. The synthesis is using as starting material 6-(3,4-dimethylphenyl)-2-hydrazinyl-4-(thiophen-2-yl)-pyridine-3-carbonitrile 36 which react with different compounds with methylene active group (namely acetyl acetone, diethylmalonate, ethyl cyanoacetate, ethyl benzoylacetate and/or ethyl acetooacetate) to produce the desired pyrazole-pyridine hybrid derivatives 37–41, Scheme 10.
The synthesis of triazolo-pyridines 42–45 and tetrazolo-pyridines 46 use as starting material the same intermediate, the 6-(3,4-dimethylphenyl)-2-hydrazinyl-4-(thiophen-2-yl)-pyridine-3-carbonitrile 36, which react with the appropriate formic acid, acetic acid, benzoyl chloride, carbon disulfide, respectively, sodium nitrite, to produce the desired hybrid derivatives 42–45 and 46, Scheme 11.

![Scheme 11. Reaction pathway to obtain triazolo-pyridines 42–45 and tetrazolo-pyridine 46 hybrids.](image_url)

The synthesized pyridine hybrids 37–46 were screened for their in vitro antibacterial activity against Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), Gram-negative bacteria (Salmonella typhi and Escherichia coli) and fungus (Aspergillus flavus and Candida albicans) using the disk diffusion agar technique. Some of the hybrids have significant antimicrobial activity, the most active compounds being 37 with a DIZ in the range of 10–17 mm. The antioxidant activity was also tested.

Amperayani et al. [19] synthesized a library of piperine-pyridine hybrid derivatives and studied their antimicrobial activities. The reaction pathway is straight, in one step, involving an acylation reaction of various amino-pyridine derivatives 47a–h, when the corresponding hybrids piperine-pyridine derivatives 48a–h are obtained, Scheme 12.
Scheme 12. Reaction pathway to obtain piperine-pyridine hybrids 48a–h.

The synthesized piperine-pyridine hybrid derivatives 48a–h were tested for their in vitro antibacterial activity against some Gram-positive and Gram-negative bacterial strains (Bacillus subtilis, Streptobacillus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis and Salmonella typhi) and fungus strains (Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and Candida albicans) using the disk diffusion agar technique. The piperine-pyridine hybrids 48a, 48d and 48h have very good activity against the Gram-negative strains E. coli, K. pneumoniae, E. faecalis and P. aeruginosa, having a DIZ in the range of 22–26 mm, superior to control standard drug. The antifungal activity of hybrids was moderate.

2.2. Six-Member Ring Azaheterocycles with One Nitrogen Atom. Hybrid Quinoline and Isoquinoline

In their attempt to obtain new quinoline derivatives with antimicrobial activity, Albayrak et al. [20] synthesized a library of 20 new triazolo-quinoline hybrid derivatives and studied their antimicrobial activities. The reaction pathway involves several steps (Scheme 13), starting from 8-nitroquinoline 53. The initial reduction reaction of 53 is leading to 8-aminoquinoline 54, which is suffering a subsequent N-alkylation with azido-iodo-propane 52a,b (generated from the corresponding bromo-alkyl alcohol) leading to alkyl-azide-quinolines 55 and 56. Finally, the alkyl-azole-quinoline derivatives are treated with the corresponding alkyne 57a–j leading to the desired products, the triazolo-quinoline hybrid derivatives 58a–j and 59a–j.

Scheme 13. Reaction pathway to obtain triazolo-quinoline hybrids 58a–j and 59a–j.
The synthesized triazolo-quinoline hybrid derivatives 58a–j and 59a–j were tested for their in vitro antibacterial activity against some Gram-positive and Gram-negative bacterial strains (Bacillus subtilis, Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterococcus faecalis) and fungus strains (Candida parapsilosis and Candida albicans) using the disk diffusion agar technique. The triazolo-quinoline hybrid derivatives 58a–j and 59a–j manifest good activity against the tested strains. The most active compound was 58a, having excellent activity against E. coli, P. aeruginosa, K. pneumonia, E. faecalis, S. aureus, S. pneumoniae, B. subtilis, C. albicans and C. parapsilosis. In some cases, the activity was several orders of magnitude superior to control drugs (DIZ of 58a was in the range of 35–250 mm; control, ampicillin, respectively, fluconazole have had a DIZ of 35 mm).

Hryhoriv et al. [21,22] synthesized two new classes of hybrid derivatives analogous to fluoroquinolones, namely piperidino-quinoline 61a,b and 1,2,3-triazolo-piperidino-quinoline 62a–k, and studied their antimicrobial activities. The first class of hybrids was obtained via an N-alkylation reaction of piperidino-quinoline 60a,b, when the N-substituted-piperidino-quinoline hybrids 61a,b are obtained. A click cyclocondensation reaction of 61a,b occurs to the second class of hybrids, the 1,2,3-triazolo-piperidino-quinoline 62a–k, Scheme 14.

Scheme 14. Reaction pathway to obtain N-substituted-piperidino-quinoline hybrids 61a,b and 1,2,3-triazolo-piperidino-quinoline hybrids 62a–k.

The synthesized hybrid derivatives piperidino-quinoline 61a,b and 1,2,3-triazolo-piperidino-quinoline 62a–k were tested for their in vitro antibacterial activity against standard bacterial strains Staphylococcus aureus and Escherichia coli, respectively, and the fungus Candida albicans using the disk diffusion agar technique. The antimicrobial assay was also made by some clinical bacterial strains S. aureus and E. coli, respectively, and fungus C. albicans using the same method. The hybrid, 1,2,3-triazolo-piperidino-quinoline 62c have a very good activity against the tested standard strains (DIZ in the range of 25–35 mm), having a superior inhibition zone to control (DIZ = 25 mm). Against clinical microbial strains, the activity was negligible.

Drweesh et al. [23] synthesized hybrid organic-inorganic derivatives and studied their antimicrobial activities, antiproliferative activity, and radical scavenging properties. In order to synthesize the desired palladium-quinoline derivatives 64a–d, they used organic cation modulation, doing a complexation reaction with PdCl₂ of the quinolines 63a–d, Scheme 15.
The synthesized palladium-quinoline derivatives hybrids 64a–d and the free ligands 63a–d, were tested for their in vitro antimicrobial activity against 14 standard microbial strains (Gram-positive and Gram-negative bacteria, fungus: Bifidobacterium animalis, Lactobacillus plantarum, Bacillus subtilis, Staphylococcus aureus ATCC 663, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Salmonella enterica, Candida albicans, Saccharomyces boulardii, Aspergillus flavus, Trichoderma viridae, Aspergillus niger). All hybrid compounds 64a–d have high antimicrobial activity against all tested strains, with minimum inhibitory concentration values ranging from 1.95 to 250 μg/mL. The results of DNA interaction studies indicate that the hybrids 64a–d and the free ligands 63a–d, interact with the DNA via an intercalation mechanism (the aromatic chromophore intercalates the base pairs of DNA; compound 64a has the highest binding affinity). The anticancer activity was also studied, with compounds 64a and 64b having selective and high cytotoxicity against human lung and breast cancer cells.

Nehra et al. [24] synthesized a series of triazole-benzothiazole-quinoline hybrids and studied their antimicrobial properties. The reaction pathway is straight and efficient (Scheme 16), involving a click cyclocondensation reaction of azido-alkyl-benzothiazole 65a–f (generated in situ from the corresponding bromo-alkyl derivative) with the corresponding alkyne-quinoline, leading to the desired products, triazole-benzothiazole-quinoline hybrids 66a–f.

The synthesized hybrids 66a–f were evaluated for their in vitro antimicrobial activity against two Gram-positive strains (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative strains (Escherichia coli and Pseudomonas aeruginosa) and two fungal strains (Candida tropicalis and Aspergillus terreus). The tested hybrids have good antimicrobial activity against both bacteria and fungus. The most promising compound was proved to be 66a, with an antibacterial (DIZ in the range of 15–17 mm) and antifungal (DIZ in the range of 21–34 mm) activity superior to reference ciprofloxacin (DIZ = 22 mm) and fluconazole (DIZ = 20 mm), respectively. Interesting molecular docking studies were also performed.

Awolade et al. [25] synthesized a library of triazole-quinoline hybrids and studied their antimicrobial properties. The reaction pathway is straight involving click chemistry of various azides with triple bond derivatives, via copper(I)-catalyzed azide-alkyne 3 + 2 dipolar cycloaddition reactions, Scheme 17.
Scheme 17. Reaction pathway to obtain triazole-quinoline hybrids 67a–u, 68a–z, 69a–n and 70a,b.

The synthesized hybrids 67a–u, 68a–z, 69a–n and 70a,b were evaluated for their in vitro antimicrobial activity against ESKAPE microbial strains (bacteria and fungus: Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Candida albicans and Candida neoformans). Some of the compounds proved to have a good and broad-spectrum of antibacterial activity, against methicillin-resistant *S. aureus* (MRSA), *E. coli*, *A. baumannii*, multidrug-resistant *K. pneumoniae* and the fungus *C. albicans* and *C. neoformans* (superior to control, fluconazole). The most promising antibacterial compound was proved to be 70b with an MIC= 75.39 μM against MRSA, *E. coli*, *A. baumannii*, and multidrug-resistant *K. pneumoniae*. The hybrid 70b also has a very good antifungal activity against *C. albicans* and *C. neoformans* with an MIC of 37.69 and 2.36 μM, respectively, superior to control fluconazole.

Ammar et al. [26] synthesized a series of thiazole-quinoline hybrids and studied their antimicrobial properties. In order to synthesize the desired compounds, they used the condensation reaction between formil-quinoline derivatives with amino-thiazole or sulfathiazole, when the desired Schiff’s base thiazole-quinoline 71 and 72, are obtained, Scheme 18.

Scheme 18. Reaction pathway to obtain thiazole-quinoline hybrids 71 and 72.

Further, the condensation reaction between formil-quinoline derivatives with different thiazolone derivatives lead to hybrid thiazole-quinoline derivatives 73–76, Scheme 19.
Finally, the cyclization of different quinoline-thiosemicarbazone derivatives with the halogenated compounds lead to other hybrid thiazole-quinoline derivatives 77–82, Scheme 20.

Scheme 19. Reaction pathway to obtain thiazone-quinoline hybrids 73–76.

Scheme 20. Reaction pathway to obtain thiazole-quinoline hybrids 77–82.

The synthesized hybrids 71–82, were evaluated for their in vitro antimicrobial activity against eight standard microbial strains, three Gram-positive bacteria (Staphylococcus aureus, Bacillus faecalis and Bacillus subtilis), three Gram-negative bacteria (Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa), and two fungi (Candida albicans and Fusarium oxysporum). Some of the compounds have good antimicrobial activity, with MIC and MBC values ranging between 0.95 and 62.5 μg/mL, and 1.94 and 118.7 μg/mL, respectively. Two compounds, namely 77b and 73a, proved to be the most active of the se-
ries against *S. aureus* and *E. coli* having an MIC between 0.95 and 7.81 μg/mL, respectively a MBC between 3.31 and 15.62 μg/mL.

Using a similar strategy, some of the above authors (Eissa et al. [27]) synthesized a new series of thiazole-quinoline hybrids and studied their antimicrobial properties. In order to synthesize the desired compounds, they used the cyclization of quinoline-thiosemicarbazone derivatives with the halogenated compounds, when the corresponding hybrid thiazole-quinoline derivatives, 83a–f, 84a–f and 85a–f are obtained, Scheme 21.

![Scheme 21. Reaction pathway to obtain thiazole-quinoline hybrids 83–85a–f.](image)

The synthesized hybrids 83a–f, 84a–f and 85a–f, were evaluated for their in vitro antimicrobial activity against *Gram-positive* (five strains: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Bacillus subtilis* and *Enterococcus faecalis*) and *Gram-negative* bacteria (five strains: *Neisseria gonorrhoeae, Proteus vulgaris, Klebsiella pneumoniae, Shigella flexneri* and *Pseudomonas aeruginosa*), as well as fungus (five strains: *Aspergillus fumigatus, Aspergillus clavatus, Candida albicans, Geotrichum candidum* and *Penicillium marneffei*). Some of the compounds displayed good antimicrobial activity, superior to the used control. The most active compound was found to be 85e, having a two-fold potency compared with gentamycin for inhibition of *N. gonorrhoeae*, four-fold potency compared with amphotericin B for the inhibition of *A. fumigatus*, equipotent activity compared with the reference drugs for inhibition of *S. flexneri, S. pyogenes, P. vulgaris, A. clavatus, G. candidum* and *P. marneffei*.

Lagdhir et al. [28] synthesized a library of piperazin-quinoline hybrids and studied their antimicrobial properties. The reaction pathway involves two steps (an alkylation and a condensation reaction), leading to the piperazin-quinoline hybrids 86a–l, Scheme 22.

![Scheme 22. Reaction pathway to obtain piperazin-quinoline hybrids 86a–l.](image)

The synthesized hybrids 86a–l were evaluated for their in vitro antibacterial (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa*) and antifungal (*Aspergillus clavatus, Aspergillus niger* and *Candida albicans*) activity, antimalarial (*Plasmodium falciparum*) and antituberculosis (*Mycobacterium tuberculosis*) activity. Some of the compounds have good antibacterial and antifungal activity against *S. aureus* and *C. albicans*. The hybrids 86a, 86b, 86d, 86j and 86k, are the most active as an antimicrobial against *S. aureus*, having an MIC= 100 μg/mL, equal to the control drug ampicillin. The hybrid 86k has excellent antifungal activity against *C. albicans*, having an MIC= 250 μg/mL, two folds higher compared with the control drug griseofulvin. The antimalarial and antitubercular activity proved to be moderate for the majority of compounds.
Desai et al. [29] synthesized a series of pyridine-quinoline hybrids and evaluated it for their antimicrobial properties. The reaction pathway involves a cyclocondensation reaction of quinoline derivative with benzylidene-malononitril, when the corresponding pyridine-quinoline hybrids 87a–j were obtained, Scheme 23.

**Scheme 23.** Reaction pathway to obtain pyridine-quinoline hybrids 87a–j.

The synthesized hybrids 87a–j were evaluated for their in vitro antimicrobial activity against Gram-positive (two strains: *Staphylococcus aureus* and *Staphylococcus pyogenes*) and Gram-negative (two strains: *Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, as well as to fungus (three strains: *Aspergillus clavatus*, *Aspergillus niger* and *Candida albicans*). Some of the compounds displayed promising antimicrobial activity. The hybrid 87i has the best antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* strains, with an MIC = 12.5 μg/mL, two folds higher compared with the control drug ciprofloxacin (MIC = 25 μg/mL). The most active compound against *C. albicans* was found to be 87e, having an MIC = 25 μg/mL, much better compared with the control drug griseofulvin (MIC = 500 μg/mL).

Vishnuvardhan et al. [30] synthesized a library of triazole-quinoline hybrids and studied their antimicrobial properties. The reaction pathway involves a typical click cyclocondensation reaction of quinoline with a triple bond with aryl-azide derivatives, when the corresponding triazole-quinoline hybrids 88a–l, Scheme 24.

**Scheme 24.** Reaction pathway to obtain triazole-quinoline hybrids 88a–l.

The synthesized hybrids 88a–l were evaluated for their in vitro antimicrobial activity against Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, as well as to fungus (*Aspergillus niger* and *Candida albicans*). Most of the hybrid compounds have good antimicrobial activity. The best antibacterial activity reveals the hybrids 88d, 88h and 88i, having a DIZ in the range of 16–21 mm, superior to control ampicillin (DIZ = 15 mm). The best antifungal activity reveals the hybrids 88d, 88h and 88k, having a DIZ in the range of 18–27 mm, superior to control griseofulvin (DIZ = 17 mm).

Abdel-Rahman et al. [31] synthesized a series of piperazin-quinoline hybrids derived from ciprofloxacin and studied their antimicrobial and anticancer properties. The
reaction pathway involves the reaction of ciprofloxacin with the corresponding phenolic derivatives with an excess of formaldehyde, when the piperazin-quinoline hybrids 89a–j are obtained, Scheme 25.

![Scheme 25. Reaction pathway to obtain piperazin-quinoline hybrids 89a–j.](image)

The synthesized hybrids 89a–j were evaluated for their antimicrobial and anticancer activity. The antibacterial screening was preconformed on Gram-positive and Gram-negative strains: *Staphylococcus aureus*, MRSA clinical strain, MRSA reference strain, *Escherichia coli* and *Pseudomonas aeruginosa*. The obtained results reveal that the hybrid 89d has the best antibacterial activity against *S. aureus*, MRSA (reference strain) and MRSA (clinical strain) with an MIC of 0.57, 0.52, and 0.082 μg/mL, respectively, (compared with the reference standard drug ciprofloxacin which has an MIC of 1.63 μg/mL against *S. aureus*, an MIC of 1.45 μg/mL against MRSA reference, and an MIC of 0.84 μg/mL against MRSA clinical). The hybrid 89j exhibited the best antimicrobial activity against *E. coli* and *P. aeruginosa*, with an MIC of 0.036 and 0.043, respectively, (compared with the reference standard drug ciprofloxacin which has an MIC of 0.056 μg/mL against *E. coli* and an MIC of 1.27 μg/mL against *P. aeruginosa*).

Mohammed et al. [32] synthesized a series of glycosylated-quinoline hybrids derived from fluoroquinolone and studied their antimicrobial properties. The reaction pathway involves the reaction of ciprofloxacin with the corresponding phenolic derivative with an excess of formaldehyde, when the glycosylated-quinoline hybrids 90–94 are obtained, Scheme 26.

![Scheme 26. Reaction pathway to obtain glycosylated-quinoline hybrids 90–94.](image)
The synthesized glycosylated-quinoline hybrids 90–94 were evaluated for their antibacterial activity against various Gram-positive and Gram-negative bacteria: *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *E. coli* clinical isolate (resistant to nalidixic acid, ciprofloxacin HCl and norfloxacin antibiotics), methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA). The hybrids were also tested for their antifungal activity against fungi: *Candida albicans*, *Aspergillus flavus*, *Fusarium solani*, *Stachybotrys chartarum* and *Penicillium chrysogenum*. The hybrid compounds 90, 91 and 94a have excellent antimicrobial activity against a fluoroquinolone-resistant *E. coli* clinical isolate, comparable to controls ciprofloxacin and norfloxacin. The hybrid compound 91 also has good antifungal activity against *C. Albicans* and *P. chrysogenum*.

Shruthi et al. [33] synthesized a series of piperazine-quinoline hybrids 95a–e and morpholine-quinoline hybrids 96a–f and evaluate them for their antimicrobial properties. The reaction pathway is depicted in Scheme 27.

![Scheme 27. Reaction pathway to obtain piperazine- and morpholine-quinoline hybrids 95a–e and 96a–f.](image)

The synthesized hybrids 95a–e and 96a–f were evaluated for their antibacterial (*Acinetobacter baumanii*, *Enterococcus faecium*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) and antitubercular (*Mycobacterium tuberculosis*) activity. Hybrid 95b has the best antibacterial activity against *E. coli* and *S. aureus* strains with an MIC of 4, respectively, 2 μg/mL, compared to standard drug vancomycin (MIC of 16, respectively, 0.5 μg/mL). Hybrids 95d, 95e and 96f exhibited the best antibacterial activity against *A. baumanistains* with an MIC in the range of 1–2 μg/mL, compared to standard drug vancomycin (MIC=0.5 μg/mL). Hybrids 95b, 95d and 95e also have promising antitubercular activity with an MIC of 4 μg/mL.

Kaur et al. [34] synthesized a series of 3- and 7- substituted-quinoline hybrids derived from fluoroquinolone and studied their antimicrobial properties. The reaction pathway involves the reaction of fluoroquinolone derivatives with the corresponding reagents, when the quinoline hybrids 97–104a,b are obtained, Schemes 28 and 29.
The synthesized quinoline hybrids 97–104a,b were evaluated for their antibacterial activity against four bacterial strains: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. All hybrids 97–104a,b have proved to be active against all bacterial strains, with an MIC value of 25 μg/mL which is fourfold more active compared to the standard drug ciprofloxacin (MIC= 100 μg/mL).

Insuasty et al. [35] synthesized a series of imidazolium-quinoline hybrids and studied their antimicrobial properties. The reaction pathway involves the reaction of 3-formyl-quinolone derivatives with the corresponding imidazolium salts, when the imidazolium-quinoline hybrids 105a–h are obtained, Scheme 30.
The synthesized imidazolium-quinoline hybrids 105a–h were evaluated for their antibacterial (Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus), antifungal (Cryptococcus neoformans) and antitubercular (Mycobacterium tuberculosis H37Rv and Mycobacterium bovis BCG) activities. Hybrid derivatives 105c,d demonstrated a remarkable antifungal activity against C. neoformans (MIC in the range of 15 μg/mL) while for the other fungal strains the activity is weak. The hybrids have modest antibacterial activity (both against Gram-positive and Gram-negative bacteria) as well as antitubercular activity.

Baartzes et al. [36] synthesized a series of benzimidazole-quinoline and ferrocenyl-quinoline hybrids and studied their antimicrobial properties. The reaction pathway involves the reaction of amino-quinoline derivatives with the corresponding formyl derivatives, when the benzimidazole-quinoline hybrids 106a–e and ferrocenyl-quinoline hybrids 107a–e are obtained, Scheme 31.

The synthesized quinoline hybrids 106a–e and 107a–e were evaluated for their antimalarial (Plasmodium falciparum and Plasmodium berghei) and antitubercular (Mycobacterium tuberculosis) activity. All hybrid derivatives are active against tested malaria strains and have modest activity against them. The most active hybrids against malarial strains have proved to be 106c and 107b, with an IC₅₀ of 0.43, respectively, 0.32 μM, compared with the standard drug chloroquine (IC₅₀= 0.01 μM).
Fedorowicz et al. [37] synthesized a series of zwiterionic hybrids pyridine-fluoroquinolone 108a–h and quinoline-fluoroquinolone 109a–h and studied their antimicrobial properties. The reaction pathway involves a tandem Mannich-electrophilic amination reaction of isoxazolones derivatives and fluoroquinolone bearing a secondary amino group at position 7 of the quinoline ring, Scheme 32.

The synthesized quinoline hybrids 108a–h and 109a–h were evaluated for their antibacterial activity against Gram-positive and Gram-negative bacterial strains (laboratory and clinical: Staphylococcus aureus ATCC 6538, Staphylococcus aureus MRSA N315, Staphylococcus epidermidis ATCC 14990, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Proteus vulgaris NCTC 4635, Staphylococcus aureus MRSA 6347, Staphylococcus epidermidis MRSE 13,199 and Serratia marcescens 12795) as well as for antibiofilm activity. The hybrid derivatives proved to have bactericidal and antibiofilm activity. The most active hybrids were found to be 109d and 109e, exhibiting good inhibition against all strains, with the IC₅₀ values in the low micromolar range.

Borazjani et al. [38] synthesized a library of quinoline hybrids (benzothiazole-benzo-quinoline 110, imino-benzothiazole-benzo-quinoline 111a–d, β-lactam-benzothiazole-benzo-quinoline 112a–m) and studied their antimicrobial properties. The reaction pathway involves a [2+2]-cycloaddition reaction of imines 111a–d and ketenes derived from substituted acetic acids, Scheme 33.
The synthesized quinoline hybrids 110–112 were evaluated for their antimicrobial activity against Gram-positive and Gram-negative bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*. From the β-lactam class, the assay indicates that the most active hybrids against *E. coli* and *P. aeruginosa*, are 112k and 112m, with an MIC of 42, respectively, 20 μg/mL, compared to standard drug gentamycin (MIC of 90, respectively, 5 μg/mL). From the imino-benzothiazole-benzo-quinoline class, the most active hybrids against *P. aeruginosa* and *S. aureus*, are 111a–c, with an MIC of 42 μg/mL, compared to standard drug gentamycin (MIC of 5, respectively, 90 μg/mL).

Berry et al. [39] synthesized a series of peptide-fluoroquinolone hybrids and studied their antimicrobial properties. In order to synthesize the desired hybrids, the authors used solid-phase peptide synthesis, from levofloxacin fluoroquinolone with the corresponding peptide (oligopeptide), when the desired peptide-fluoroquinolone hybrids 113a–l are obtained, Scheme 34.

![Scheme 34. Reaction pathway to obtain peptide-quinolone hybrids 113a–l.](image)

The synthesized peptide-fluoroquinolone hybrids 113a–l were evaluated for their antimicrobial activity against MDR bacterial strains, *Gram-negative* and *Gram-positive*: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), *Enterococcus faecalis*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*. The assay indicates that all the peptide-hybrids have weak antibacterial activity. If the hybrids are mixed with fluoroquinolone (ciprofloxacin, levofloxacin and moxifloxacin) drugs, the resulting conjugates possess antimicrobial activity against MDR *Gram-negative* bacteria (clinical isolates, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *A. baumannii*), superior to reference levofloxacin.

Mermer et al. [40] synthesized a library of triazole- and oxadiazole-fluoroquinolone hybrids and studied their antimicrobial properties. The reaction pathway took place in several steps of sequential reactions, starting from phenyl piperazine. Finally, the corresponding triazole-fluoroquinolone 114a–b and oxadiazole-fluoroquinolone 115a–j hybrids were obtained via a one-pot three-component Mannich reaction, Scheme 35. The reactions were performed both under conventional thermal heating and microwave, the last pathway being more advantageous.
Scheme 35. Reaction pathway to obtain oxadiazole- and triazole-fluoroquinolone hybrids 114a–b and 115a–j.

The synthesized hybrids 114a–b and 115a–j were tested for their antimicrobial activity (against Gram-positive and Gram-negative strains: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter haemolyticus*), DNA gyrase and Topoisomerase IV inhibition potentials. The hybrids have good antimicrobial activity and displayed excellent DNA gyrase inhibition. The hybrids 114b, 115b and 115h exhibited the best antimicrobial activity against the tested strains. Thus, the hybrids have excellent activity against *K. pneumoniae* with an MIC of 0.25 μg/mL, compared with the standard drug gentamycin (MIC= 0.25 μg/mL). The hybrids also have excellent activity against *A. haemolyticus* and *P. aeruginosa* with an MIC in the range of 0.5–2 μg/mL, compared with the standard drug gentamycin (MIC= 0.78 μg/mL, respectively, MIC= 1.56 μg/mL). Against *Gram-positive* strain *E. faecalis* the hybrids have excellent activity with an MIC in the range of 0.5–8 μg/mL, compared with the standard drug ampicillin (MIC= 12.5 μg/mL).

Guo et al. [41] synthesized a library of oxadiazole-quinoline hybrids and studied their antibacterial properties. The reaction pathway is straight, involving an alkylation reaction of fluoroquinolone with the corresponding oxadiazole, when the desired oxadiazole-fluoroquinolone hybrids 116a–t were obtained, Scheme 36.

Scheme 36. Reaction pathway to obtain oxadiazole-fluoroquinolone hybrids 116a–t.

The synthesized oxadiazole-fluoroquinolone hybrids 116a–t were tested for their antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and laboratory *Staphylococcus aureus*. The hybrids displayed good antibacterial activity, one of
the compounds 116k exhibited excellent antibacterial activity against both methicillin-resistant *S. aureus* and laboratory *S. aureus*, with an MIC in the range of 0.25–2 μg/mL, superior to control drug vancomycin (MIC= 2 μg/mL).

Wang et al. [42] synthesized a series of benzimidazole–quinoline hybrids and studied their antibacterial and antifungal properties. The reaction pathway involves an *N*-alkylation reaction of fluoroquinolone with the corresponding benzimidazole, when the desired benzimidazole-fluoroquinolone hybrids 117a–g, 118a–b and 119a–f, were obtained, Scheme 37.

![Scheme 37. Reaction pathway to obtain benzimidazole-quinoline hybrids 117a–g, 118a–b and 119a–f.](image)

The synthesized benzimidazole-fluoroquinolone hybrids 117a–g, 118a–b and 119a–f were screened against Gram-positive and Gram-negative bacteria, respectively, fungus (methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus aureus* ATCC25923, *Staphylococcus aureus* ATCC29213, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, *Candida albicans*, *Candida tropicalis*, *Aspergillus fumigatus*, *Candida albicans* ATCC90023, *Candida parapsilosis* ATCC22019). The results of the assay were promising, with some hybrids having excellent antibacterial activity. The most active hybrids against *K. pneumonia* are 117a and 117c, with an MIC of 8 μg/mL, compared to the standard drug norfloxacin (MIC > 512 μg/mL). The most active hybrids against *S. aureus* are 119a and 119f, with an MIC of 4 μg/mL, compared to the standard drug norfloxacin (MIC= 64 μg/mL).

Bharadwaj et al. [43] synthesized a series of oxadiazole–quinoline hybrids and studied their antibacterial and antifungal properties. The reaction pathway involves a cyclocondensation reaction of hydrazinyl-quinoline derivative with the corresponding aromatic acids, when the desired oxadiazole–quinoline hybrids 120a–g were obtained, Scheme 38.
The synthesized oxadiazole–quinoline hybrids 120a–g were tested against clinical isolates Gram-positive and Gram-negative bacteria (Staphylococcus aureus, Bacillus cereus, Escherichia coli, Serratia marcescens), respectively, fungus (Aspergillus niger, Trichophyton mentagrophytes, Candida albicans, Candida parapsilosis). The antimicrobial activity of oxadiazole–quinoline derivatives was good, the hybrids 120a and 120f having the best antimicrobial activity against B. cereus with an MIC of 17, respectively, 24 μg/mL, compared to standard drug ampicilin (MIC= 16 μg/mL).

Tahaab et al. [44] synthesized a series of oxadiazole–quinoline hybrids and studied their leishmanicidal potential. The reaction pathway to obtain the oxadiazole–quinoline hybrids 121a–r is depicted in Scheme 39.

The synthesized oxadiazole–quinoline hybrids 121a–r were tested for their leishmanicidal activity against Leishmania major promastigote. Most of the synthesized hybrids have a good leishmanicidal activity, compound 121r was found to be the most active (IC50 = 0.10 μM) from the series, being 70 times more active than the standard drug (pentamidine, IC50 = 7 μM).

Irfan et al. [45] synthesized a series of triazole–quinoline hybrids and studied their antifungal properties. The reaction pathway involves a typical click cyclocondensation reaction of azide with a compound with a triple bond, when the desired triazole–quinoline hybrids 122a–c were obtained, Scheme 40.
The synthesized triazole–quinoline hybrids 122a–c were tested against fungus Candida albicans, both clinical isolates and laboratory strains [three FLC susceptible strains (C. albicans D27, C. albicans D31 and C. albicans D39) and one FLC resistant strain (C. albicans D15.9)]. The best antifungal activity was found for the hybrids 122a and 122b, having an MIC of 25 μg/mL for 122a and an MIC of 250 μg/mL for 122b, compared to control FLC (MIC>1 μg/mL).

Pandya et al. [46] synthesized a library of pyrazole–isoquinoline hybrids and studied their antimicrobial properties. The reaction pathway involves a palladium-catalyzed reaction of pyrazole derivatives with t-butyl-isocyanide, when the corresponding pyrazole–isoquinoline hybrids 123a–g, were obtained, Scheme 41.

The synthesized pyrazole–isoquinoline hybrids 123a–g were evaluated for their antimicrobial activity against different pathogenic strains: bacterial strains (Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Streptococcus pyogens and Vibrio cholera), fungal strains (Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis and Candida parapsilosis) and tubercular strain (Mycobacterium tuberculosis). The antimicrobial activity of hybrids was very good, the hybrids 123e and 123g having the best antimicrobial activity, compared to standard drugs kanamycin and amphotericin B. Thus, the most active hybrids against S. aureus are 123e and 123g, having an MIC of 20 μM, respectively, 37 μM, compared to standard drug kanamycin (MIC of 31 μM). The most active hybrids against V. cholera are 123e and 123g, having an MIC of 41 μM, respectively, 90 μM, compared to the standard drug kanamycin (MIC of 62 μM). The hybrids 123e and 123g have the best antitubercular activity against M. tuberculosis with an MIC of 30 μg/mL, respectively, 32 μg/mL, compared to standard drugs rifampicin and isoniazide (MIC of 90 μg/mL).

Verma et al. [47] obtained a series of piperazine- and pyrimidine-isoquinoline hybrids and studied their antimicrobial properties. The piperazine-isoquinoline hybrids 126a–h were synthesized by condensation of the carboxylic acid intermediates 124a–d with appropriate aryl-piperazines, Scheme 38. The pyrimidine-isoquinoline hybrids 127a–h were synthesized in two steps: an O-alkylation of the carboxylic acid intermediates 124a–d (with ethylene dichloride), followed by an S-alkylation of the obtained compounds 125a–d (with thio-pyrimidine), Scheme 42.
The synthesized piperazine- and pyrimidine-isoquinoline hybrids 124a–h and 125a–h were evaluated for their antibacterial and antifungal (Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Aspergillus niger, Aspergillus oryzae, Candida albicans and Pencillium chrysogenum), antioxidant, anticancer and antituberculosis (Mycobacterium tuberculosis) activities. The antibacterial assay indicates that three hybrids, namely 124a, 125a and 126c have the best activity against E. coli (with an MIC in the range of 1–3 μg/mL) and K. pneumoniae (with an MIC in the range of 1.5–3 μg/mL), compared with the standard drug ciprofloxacin (MIC= 1.5 μg/mL). The hybrids 125a, 126a and 127a also have excellent activity against S. aureus (with an MIC in the range of 1–3 μg/mL) and B. subtilis (with an MIC in the range of 1.5–3 μg/mL), compared with the standard drug ciprofloxacin (MIC= 1.5 μg/mL, respectively, MIC= 3 μg/mL). The hybrids 125a, 126a and 127a have excellent activity against fungus A. niger, C. albicans, A. oryzae, and P. chrysogenum (with an MIC of 1.5 μg/mL), compared with the standard drug fluconazole (MIC= 1.5 μg/mL for A. niger and C. albicans, respectively, MIC= 3 μg/mL for A. oryzae, and P. chrysogenum). The hybrids 127b and 127e have the best activity against M. tuberculosis (MIC 1.0 mg/mL), compared with the standard drug rifampicin (MIC= 0.1mg/mL). The antioxidant and anticancer activity proved to be modest.

2.3. Our Group Recent Contributions

Our concern for obtaining new six-member ring azaheterocycle entities with antimicrobial activity for medicinal chemistry applications was started three decades ago [48–51] when we tried to obtain new diazines with good to excellent antibacterial and antifungal activities. Further, we will present some recent results obtained by us in the field of hybrid azines with antimicrobial activity.

In continuation of our concern for new compounds with antimicrobial activity, Diaconu et al. [52] synthesized a large library of hybrid imidazole- and benzimidazole-quinoline derivatives and studied their antimicrobial properties. The reaction pathway (Scheme 42) involves an initial N-acylation reaction of 8-aminoquinoline, followed by an N-alkylation of the -NH- amino group from imidazole/benzimidazole heterocycle, when the key imidazole-quinoline 128a,b and benzimidazole-quinoline 129a,b hybrids are obtained. Next, a quaternization reaction of N-imidazole atom with activated halogenated compounds leads to a second class of hybrids, the quaternary salts of imidazole-benzimidazole-quinolines, 130a–k and 132a–k (with one methylene group as linker) and 131a–k and 133a–k (with two methylene groups as linker). Finally, imidazolium and benzimidazolium ylides (generated in situ from the corresponding salts) react with dimethyl acetylenedicarboxylate (DMAD), generating another class of hybrid quinoline derivatives, the benzimidazole-quinoline cycloadducts 134a–k, Scheme 43.
The synthesized hybrids were evaluated for their antimicrobial (Staphylococcus aureus, Escherichia coli and Candida albicans) and anticancer activities. The results of the antibacterial assay indicate that some hybrid compounds are biologically active in the range of nano-molar, five benzimidazole-quinoline hybrid salts (133c, 133d, 133f, 133h, 132h) have excellent activity against Gram-negative bacteria E. coli (DIZ in the range of 20–24 mm) superior to control gentamicin (DIZ of 12 mm) and one compound (133i) have excellent activity against Gram-positive bacteria S. aureus (DIZ of 20 mm) superior to control gentamicin (DIZ of 14 mm). The anticancer assay indicates that some benzimidazole-quinoline hybrid salts (133h, 132h, 133c, 133f) have excellent anticancer activity in the range of nano-molar, against some cancer cells (Leukemia, Breast cancer, Lung cancer and Ovarian cancer). Interesting SAR correlations have been performed.

In another research work, Diaconu et al. [53] synthesized a new series of hybrid imidazole- and benzimidazole-pyridine derivatives and studied their antimicrobial properties. The reaction pathway (Scheme 43) involves an N-acylation reaction of 2-aminopyridine, followed by an N-alkylation of the -NH- amino group from imidazole/benzimidazole heterocycle when the corresponding imidazole-pyridine hybrids are obtained. Finally, a quaternization reaction of N-imidazole atom with activated halogenated compounds leads to a second class of hybrids, the imidazole-pyridine 137a,b and benzimidazole-pyridine 138a,b salts, Scheme 44.

Scheme 43. Reaction pathway to obtain imidazole- and benzimidazole-quinoline hybrids 128–134.

Scheme 44. Reaction pathway to obtain imidazole- and benzimidazole-pyridine hybrids 135–138.
The synthesized hybrids were tested for their antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The results of the antibacterial assay indicate that the imidazole- and benzimidazole-pyridine hybrids have interesting antimicrobial properties, especially the hybrid benzimidazole-pyridine salt 138a have a powerful antibacterial activity against Gram-positive strain *S. aureus* and Gram-negative germ *E. coli* (DIZ of 30 mm), superior to control drug gentamicin (DIZ of 18 mm).

Antoci et al. [54] synthesized a new series of hybrid bis(imidazole)- and bis(benzimidazole)-pyridine derivatives and studied their antimycobacterial activity. The reaction pathway (Scheme 44) involves an N-alkylation of the -NH- amino group from imidazole/benzimidazole heterocycle when the corresponding bis(imidazole)-pyridine 139, 140 and bis(benzimidazole)-pyridine 141 hybrids are obtained. In the next step, a quaternization reaction of N-imidazole atom with activated halogenated compounds leads to a second class of hybrids, the bis(imidazole)-pyridine 142a–g and bis(benzimidazole)-pyridine 143a–g salts, Scheme 45.

![Scheme 45](image)

Scheme 45. Reaction pathway to obtain bis(imidazole)- and bis(benzimidazole)-pyridine hybrids 139–143.

The synthesized hybrids were tested in a primary screening for their antimycobacterial activities against *M. tuberculosis* H37Rv under aerobic conditions, eight hybrids (namely 140, 141, 142e,f and 143c,e,f,g) having excellent activity against *Mtb* H37Rv, with an MIC in the range of 17–92 μM. The most active antmycobacterial five compounds (namely 140, 141, 142f and 143c,f) were subjected to the secondary antimycobacterial assay. The obtained results indicate that our compounds are very active against both replicating and non-replicating *Mtb* (superior to control metronidazole), exhibited excellent intracellular activity, are active against drug-resistant *Mtb* strains, have no cytotoxicity, and three of them (142f and 143c,f) have a bactericidal mechanism of action. The results of ADMET pharmacokinetic experimental studies for hybrid 143f, reveal that this compound is truly a candidate for a future drug: a lower clearance rate, a great half-time in vivo, a low potential for drug–drug interactions with a high duration of action and lack of cytotoxicity. The best antitubercular activity has the hybrid 143e with an MIC of 17 μM, MBC of 50 μM, IC50 of 9 μM. Under anaerobic conditions (LORA) the hybrid 143e have the MIC of 120 μM and IC50 of 9 μM. Against resistant isolates of *Mtb* strains [five strains, INH-R1 and INH-R2 (strains resistant to isoniazid), RIF-R1 and RIF-R2 (strains resistant to rifampicin), FQ-R1 (strain resistant to fluoroquinolone)] the hybrid 143e have
the MIC in the range of 10–30 μM and IC50 in the range of 10–20 μM. Against nontuberculous mycobacteria *Mycobacterium avium* and *Mycobacterium abscessus*, the hybrid 143e has the MIC in the range of 50–80 μM and IC50 in the range of 30–50 μM. The intracellular activity and cytotoxicity of the hybrid 143e were IC50 of 14 μM, respectively, and IC50 of 50 μM.

Furthermore, in a subsequent paper [55], some of the above authors performed a thorough molecular docking study in order to determine the binding sites and ADMET properties of the hybrid bis(imidazole)- and bis(benzimidazole)-pyridine derivatives. The obtained results indicate the most probable binding sites the G-quadruplex DNA string and DNA strain in complex with dioxygenase. The predicted ADMET properties are in accordance with the experimental one presented above [54].

Continuing our studies in the field of hybrid pyridine and quinoline derivatives, Mantu et al. [56] synthesized a new series of hybrid imidazole- and benzimidazole-pyridine and quinoline derivatives and studied their antimicrobial properties. The reaction pathway involves a quaternization reaction of N-imidazole atom with activated halogenated compounds, when the corresponding salts of imidazole- and benzimidazole-pyridine and quinoline hybrids are obtained, Scheme 46.

![Scheme 46. Reaction pathway to obtain salts of imidazole- and benzimidazole-pyridine and quinoline hybrids 144–147.](image)

The synthesized hybrids were tested for their antimycobacterial and anticancer activities. The antimycobacterial assay reveals that our hybrids have modest activity against *Mtb* strains. The anticancer assay indicates that one of the hybrids, namely 129a, has a very good and selective antitumor activity against Renal Cancer A498 and Breast Cancer MDA-MB-468.

Diaconu et al. [57] synthesized two new series of hybrid quinoline-sulfonamide complexes and studied their antimicrobial activity. The reaction pathway involves a straight and efficient two-step procedure. In the first step, an acylation reaction of (3-, 4- or 8-)aminoquinoline derivatives with the corresponding benzenesulfonyl chlorides 148a–c or quinolylsulfonyl chloride 148d took place, the desired ligands quinoline-sulfonamide type 149a–d being obtained. In the second step, a complexation reaction of ligands 149a–d with metal acetate (Cu2+, Co2+, Cd2+) or chloride (Zn2+) took place, with the desired hybrids quinoline-benzene-sulfonamide complexes (150a–d, 151a–d and 152a–d) and quinoline-quinolinyl-sulfonamide complexes 153a–d being obtained. The reaction pathway is depicted in Scheme 47 for the complexes derived from 8-aminoquinoline.

![Scheme 47. Reaction pathway to obtain hybrids 150a–d, 151a–d, 152a–d and 153a–d.](image)
The synthesized hybrids were tested for their antimicrobial activity, with some of them having a very good antibacterial (Staphylococcus aureus, Escherichia coli) and antifungal (Candida albicans) activity. For instance, the hybrid N-(quinolin-8-yl)-4-chloro-benzenesulfonamide cadmium 153d has the best antibacterial activity, with a DIZ of 21 mm and an MIC of 19.04 × 10^{-5} mg/mL against S. aureus, a DIZ of 19 mm and an MIC of 609 × 10^{-5} mg/mL against E. coli, and an excellent antifungal activity against C. albicans, with a DIZ of 25 mm and an MIC of 19.04 × 10^{-3} mg/mL.

Al-Matarneh et al. [58] synthesized two new series of pyrrolo-quinoline and pyrrolo-isoquinoline hybrids and studied their antimicrobial activity. The reaction pathway involves a 3 + 2 dipolar cycloaddition reaction of the quinolinium and isoquinolinium ylides (generated in situ from the corresponding salts) with N-ethyl- or N-phenyl-maleimide, when the hybrid pyrrolo-quinoline 154a–c and pyrrolo-isoquinoline 155a–c are obtained, Scheme 48.

The synthesized hybrids pyrrolo-quinoline 154a–c and pyrrolo-isoquinoline 155a–c were tested for their antimicrobial activities but, unfortunately, the hybrids have no significant activity.

Danac et al. [59] synthesized a series of pyrrolo-phenanthroline hybrids and studied their antimycobacterial activity. The reaction pathway involves a 3 + 2 dipolar cycloaddition reaction of the phenanthroline ylides (generated in situ from the corresponding salts) with N-ethyl- or N-phenyl-maleimide, when the pyrrolo-phenanthroline hybrids 156a–c are obtained, Scheme 49.
The synthesized hybrids 156a–c were tested for their antimycobacterial activities. The antimycobacterial assay reveals that one hybrid, 156a, has a strong activity against the Mtb strain, with an IC50 of 56 μM.

Danac, Olaru et al. [60,61] synthesized a library of indolizine-pyridine hybrids and studied their antimycobacterial activity. The reaction pathway involves a 3 + 2 dipolar cycloaddition reaction of the 4,4′-bipyridinium mono-ylides (generated in situ from the corresponding salts) with ethyl propiolate, when the mono-indolizine-pyridine hybrids 157a–e are obtained, Scheme 49. Next, a quaternization reaction of pyridine nitrogen atom with activated halogenated compounds 158a–l is leading to the salts of mono-indolizine-pyridine hybrids 159a–l. Finally, another 3 + 2 dipolar cycloaddition reaction with ethyl propiolate is leading to bis-indolizine-pyridine hybrids 160a–d, Scheme 50.

The synthesized hybrids were tested in a primary screening for their antimycobacterial activities against M. tuberculosis H37Rv under aerobic conditions, the salts of mono-indolizine-pyridine hybrids 159a–l displaying an excellent activity against Mtb H37Rv, superior to the second-line antitubercular drugs cycloserine and pyrimethamine and, equal as the first line anti-TB Ethambutol. The most active antimycobacterial five compounds (namely 159a, 159c, 159d, 159h and 159i) were subjected to the secondary antimycobacterial assay (MIC, MBC, LORA, intracellular (macrophage) drug screening, and MTT cell proliferation). These mono-indolizine-pyridine hybrids have proved to be...
very active against replicating and non-replicating *M. tuberculosis*, are active against both extracellular and intracellular organisms, have a bactericidal mechanism of action, and had basically no toxicity. The best antitubercular activity has the hybrid 159i with an MIC of 8 μM, MBC of 3 μM, IC50 of 7 μM. Under anaerobic conditions (LORA) the hybrid 159i have the MIC of 63 μM and IC50 of 1.9 μM. Against resistant isolates of *Mtb* strains [five strains, INH-R1 and INH-R2 (strains resistant to isoniazid), RIF-R1 and RIF-R2 (strains resistant to rifampicin), FQ-R1 (strain resistant to fluoroquinolone)] the hybrid 159i have the MIC in the range of 6–22 μM and IC50 in the range of 6–12 μM. Against nontuberculous mycobacteria *Mycobacterium avium* and *Mycobacterium abscessus*, the hybrid 159i have the MIC in the range of 23–50 μM and IC50 in the range of 14–18 μM. The intracellular activity and cytotoxicity of the hybrid 159i were IC50 of 5 μM, respectively IC50 of 2 μM.

3. Perspectives and Conclusions

To conclude, we report herein the latest recent advances concerning the synthesis and antimicrobial properties of hybrid azine derivatives. The literature data presented in this review indicate that there is a great urgency in society and pharmaceutical industry to develop new antimicrobial drugs for the treatment of infectious diseases. Moreover, the data indicate that a modern approach used to overcome the drawbacks of infectious diseases is to use molecular hybridization strategy, as a new modern approach in drug discovery. The hybrid pyridine, quinoline, isoquinoline and their fused derivatives have invaluable importance in modern antimicrobial therapy, the results presented in this review indicate that they have a large variety of antimicrobial activities, including antibacterial, antifungal, antimycobacterial, antileishmanial, antimalarial, antiviral, etc.

We show that the best methods for the synthesis of hybrid compounds are cyclocondensation, condensation and simple typical organic chemistry reactions such as alkylation, acylation, etc. We also show that many hybrid compounds have excellent antimicrobial activity, the combination of an azine moiety with a five-member ring azaheterocycle being the best approach to obtain drugs with improved and superior antimicrobial properties. A special mention has to be made about the obtained results in antituberculosis therapy, where the use of hybrids with pyridine or by pyridine merged with an imidazole or benzimidazole moiety seems to be a very efficient approach in treatment, in some cases, the hybrids had a spectacular antitubercular activity, including a bactericidal mechanism of action. Moreover, the fact that some of these hybrids are in different clinical trials is a good and solid argument for further research in this field.

Finally, having in view the above consideration, we encourage and underline further studies in the field of hybrid azine merged with a five-member ring azaheterocycle, which appears to be the most promising field of research within this area.

Author Contributions: Design, conception and writing were performed by V.M. and I.I.M. D.A.-M., I.B. and A.A. were involved in conception of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS—UEFISCDI, project number PN-III-P4-ID-PCE-2020-0371, within PNCDI III.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are thankful to Romanian Ministry of Research, Innovation and Digitization, within Program 1—Development of the national RD system, Subprogram 1.2—Institutional Performance—RDI excellence funding projects, Contract no.11PFE/30.12.2021 and project POC/448/1/1 Research Centre with Integrated Techniques for Atmospheric Aerosol Investigation in Romania-RECENT AIR (grant agreement MySMIS no. 127324) and CERNESIM Center, within the Institute for Interdisciplinary Research at the Alexandru Ioan Cuza University of Iasi, for infrastructure used.

Conflicts of Interest: The authors declare no conflict of interest.
Abbreviations

ADMET Absorption, Distribution, Metabolism, Excretion, Toxicity
ESKAPE an acronym for the six highly virulent and antibiotic-resistant bacterial pathogens: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.

MIC minimal inhibitory concentration
MBC minimal bactericidal concentration
MRSA methicillin-resistant Staphylococcus aureus
diameter of inhibition zones
desoxeryrubonucleic acid
SAR structure activity relationship
IC₅₀ inhibitory concentration at 50%
LORA Low oxygen recovery assay
strains resistant to isoniazid
strains resistant to rifampicin
strain resistant to fluoroquinolone
nontuberculous mycobacteria
O-alkylation oxigen-alkylation
N-alkylation nitogen-alkylation
S-alkylation sulphur-alkylation
tert (eg, t—butyl means tert-butyl)
iso (eg, i—propyl means iso-propyl)
normal (eg, n—propyl means normal-butyl)
ortho, meta, para
Me methyl
Et ethyl
Pr propyl
Bu butyl
Ph phenyl
OMe methoxy
Bacillus cereus B. cereus
Bifidobacterium animalis B. animalis
Bacillus subtilis B. subtilis
Enterococcus faecalis E. faecalis
Lactobacillus plantarum L. plantarum
Staphylococcus aureus S. aureus
Staphylococcus epidermidis S. epidermidis
Streptococcus pneumoniae S. pneumoniae
Streptococcus mutans S. mutans
Streptococcus pyogenes S. pyogenes
Acinetobacter baumannii A. baumannii
Escherichia coli E. coli
Klebsiella pneumoniae K. pneumoniae
Neisseria gonorrhoeae N. gonorrhoeae
Proteus mirabilis P. mirabilis
Proteus vulgaris P. vulgaris
Pseudomonas aeruginosa P. aeruginosa
Salmonella enterica S. enterica
Salmonella typhi S. typhi
Shigella flexneri S. flexneri
Aspergillus clavatus  
Aspergillus niger  
Aspergillus flavus  
Aspergillus fumigatus  
Candida albicans  
Candida parapsilosis  
Cryptococcus neoformans  
Geotrichum candidum  
Penicillium marneffei  
Saccharomyces boulardii  
Trichoderma viridae  
Mycobacterium tuberculosis  
Mycobacterium avium  
Mycobacterium abscessus  
WHO

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