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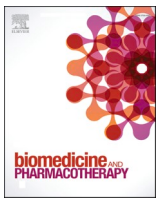

Review

Hidden potential of hydrazinecarboxamides (semicarbazides) as potential antimicrobial agents: A review

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ABSTRACT

Hydrazinecarboxamides (semicarbazides) are increasingly recognized as a versatile scaffold in developing potential antimicrobial agents. In addition to a brief overview of the synthetic methods to prepare them, this review comprehensively analyses their antimicrobial properties. These derivatives have demonstrated potent activity against a broad spectrum of mycobacteria, bacterial and fungal pathogens, highlighting their potential to address critical human health challenges, including neglected diseases, and to combat growing antimicrobial resistance. They have also been investigated for their antiviral and antiparasitic properties. The review also summarizes structure-activity relationships, known mechanisms of action and emphasizes the crucial role of the hydrazinecarboxamide moiety in facilitating interactions with biological targets. The combination of hydrazinecarboxamides with other bioactive scaffolds (primaquine, isoniazid, etc.) has led to an identification of promising drug candidates, including those active against resistant strains, offering a promising approach for future innovations in the field of antimicrobial therapy. Attention is also drawn to limitations of hydrazinecarboxamides (poor physicochemical properties, cytotoxicity to human cells, and insufficient target selectivity), which may hinder their clinical application.

1. Introduction

Novel drugs often address previously unmet medical needs or contribute to advancements in patient care and public health. They include structural modifications of existing products or new entities with different mechanisms of action (MoA) and completely distinct structures. These new products contribute to improving treatment quality, better access to medicines, more choices for consumers, and a competitive market that improves affordability and public health [1]. In pharmacology and drug development, the molecules used can be divided into small and large molecules. More than 90 % of drugs on the market today are small molecules, but large molecules, known as biologics, are rapidly growing in popularity and importance [2]. Small-molecule drugs have several advantages, such as oral delivery and cell membrane permeability, which allow them to reach specific tissues and targets. The small size allows easy passage through the gastrointestinal tract and good penetration of cell membranes, enabling the active molecule to rapidly absorb into the bloodstream, distribute throughout the body, and reach intracellular targets. For this purpose, small-molecule drugs can be designed to acquire specific affinity and selectivity [3].

Biologics are classified as proteins that have therapeutic effects. In contrast to small-molecule drugs, most large molecule drugs are complex, composed of more than 1300 amino acids, and are identical or analogous versions of human proteins. These large-molecule pharmaceutical drugs are developed through complex processes, often requiring more than 1000 steps [2].

In recent years, the research has revolutionized drug development and offered treatment options for previously incurable medical conditions.

However, despite recent advances in the field of biologics, the design, synthesis, evaluation, and further development of small-molecule drugs still play a crucial role in introducing new drugs. One of the "classical" approaches in medicinal chemistry involves modifications of known, well-established, and clinically validated scaffolds. Interestingly, several scaffolds can be found in small-molecule and peptide drugs, including hydrazinecarboxamides (semicarbazides).

Semicarbazide (also known as carbamoylhydrazine) is soluble in water but insoluble in organic solvents such as ether and ethanol. It has been used as a synthetic intermediate to produce various industrial

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chemicals for many applications and drugs and to determine carbonyl compounds. The review of ecotoxicological and toxicity issues of free semicarbazide has been published recently [4].

The examples of hydrazinecarboxamide- and hydrazinethiocarboxamide-based drugs are shown in Fig. 1. This motif is present in azapeptides utilized as close surrogates of natural peptides, where α-carbon(s) of amino acids is replaced by nitrogen to result in a hydrazinecarboxamide moiety [5]. Goserelin is an example of a successful azapeptide drug containing the hydrazinecarboxamide group [6,7]. Some nitrofurans contain this moiety (nitrofurazone **a**, Fig. 1, nitrofurantoin **b**, Fig. 1). They are a potent prodrug class used for urogenital and topical infections, typically caused by Gram-negative and Gram-positive bacteria. However, they are also capable of inhibiting fungi and mycobacteria [8,9]. Phenicarbazide (**c**, Fig. 1) was used as an analgesic and antipyretic drug [10,11]. Among thio analogues, antitubercular agent thioacetazone (**d**, Fig. 1), antiviral methisazone (**e**, Fig. 1), and antiseptic drug ambazone can be mentioned (**f**, Fig. 1) [12]. The hydrazinecarboxamide moiety can also be incorporated into heterocycle, both partly (nitrofurantoin; hypoglycaemic antidiabetic drug gliclazide [13] **g**, Fig. 1) or entirely (nefazodone [14] **h**, Fig. 1).

Hydrazinecarboxamides have also been proposed as valuable tools for bioorthogonal conjugation chemistry *via* fast reaction with an aryl aldehyde or ketone with an *ortho*-boronic acid substituent. Initially, a semicarbazone intermediate is formed, rearranging into a stable diazaborine (Fig. 2). This approach was successfully employed to label bacterial pathogens in blood serum [15].

Hydrazinecarboxamide is also a structural part of fluorescent Lucifer yellow dye used, e.g., to stain nerve cells (Fig. 3) [16]. Oligo (hydrazinecarboxamide)-based cyclophane macrocycles and their acyclic analogues have also been investigated for various purposes [17–19].

In addition, hydrazinecarboxamides and their derivatives have been investigated concerning other potential technical and technological applications [20,21], and complexation properties [22].

The scope of this article is to review antimicrobial activity and recent trends in the group of hydrazinecarboxamides (semicarbazides). From a structural point of view, both acyclic and cyclic semicarbazones (Fig. 4) can also be considered hydrazinecarboxamide derivatives. The compounds are usually synthesized from corresponding carbonyl compounds and semicarbazide (or their salts with acids) analogously to hydrazones and imines. The syntheses and antimicrobial properties of semicarbazones are not in the scope of this review.

The anticancer, antioxidant, enzyme inhibiting, anticonvulsive, and anti-inflammatory activities of hydrazinecarboxamides were summarized elsewhere [23].

2. Brief overview of synthesis

Various synthetic pathways have been reported to synthesize hydrazinecarboxamides. In this review, we summarized the most frequent and efficient methods encountered in the literature. In general, the synthesis of targeted derivatives is straightforward, enabling the preparation of a wide range of derivatives to evaluate structure-activity relationships (SAR) effectively and utilize them in further drug design.

The preparation of hydrazinecarboxamides is generally performed by the reaction of hydrazides or hydrazines [24] with various isocyanate derivatives (Fig. 5; it also shows trivial numbering of semicarbazide) [25–27]. The reaction can occur at room temperature, under reflux, or with cooling, depending on the nature of the substrates. Different solvents, such as acetonitrile and alcohols (typically methanol and ethanol), may also be used. This approach is typically fast and yields good to excellent results.

The used hydrazides may be commercially available or prepared in-house, usually by hydrazinolysis of corresponding methyl or ethyl esters. These esters are predominantly obtained by Fischer esterification. Isocyanates that are not for commercially available can be synthesized using several methods that were reviewed, e.g., by Kreye et al. [28]. The most frequent approach is based on the reaction of appropriate amine and phosgene derivatives in the presence of a tertiary base, nucleophilic substitution from inorganic cyanates, or rearrangement reactions from functional carboxylic acid derivatives (Curtius, Hofmann, and Lossen rearrangements). Due to the notorious toxicity of methyl isocyanate [29], *N*-methylated hydrazinecarboxamides have also been prepared by a reaction of hydrazides with *N*-succinimidyl *N*-methylcarbamate (also known as "methyl isocyanate substitute") in the presence of a non-nucleophilic tertiary base, such as *N*-ethyl-*N*,*N*-diisopropylamine (DIPEA), triethylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1,4-diazabicyclo[2.2.2]octane (DABCO), or pyridine) to provide *N*-methylhydrazinecarboxamides [30,31].

Recently, formamides were introduced as surrogates for isocyanates. The reaction is catalysed by a ruthenium-based complex (Fig. 6) [32].

Readily available phenyl 2-substituted hydrazine-1-carboxylates can also be substitutes for isocyanates *via* aminoisocyanate as an intermediate [33]. Later, this methodology was expanded using *tert*-butyl 2,

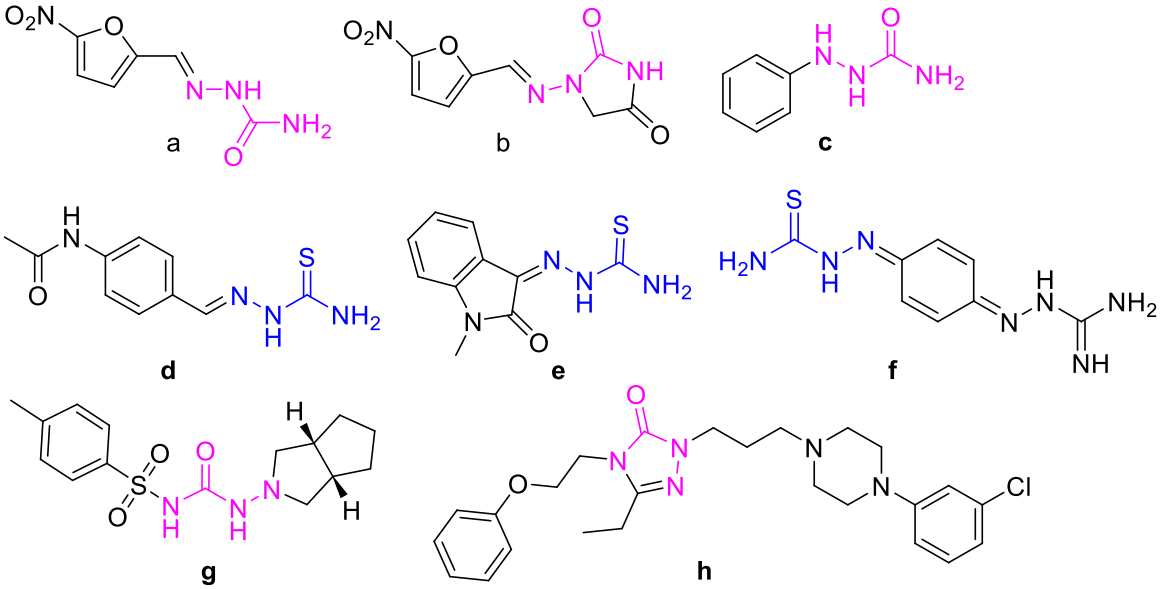

Fig. 1. Hydrazinecarboxamide- and hydrazinethiocarboxamide-based drugs.

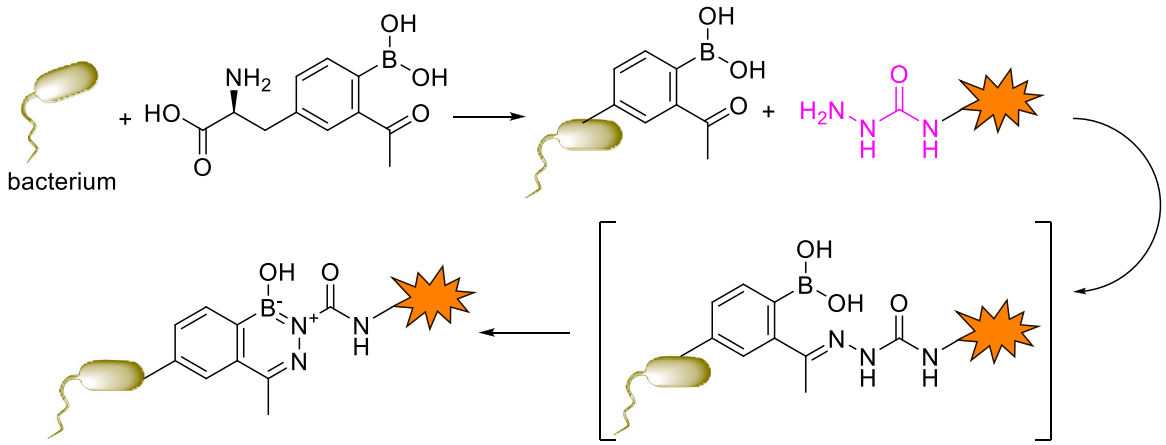

Fig. 2. The bioorthogonal conjugation approach based on carbonylated boronic acid and semicarbazide.

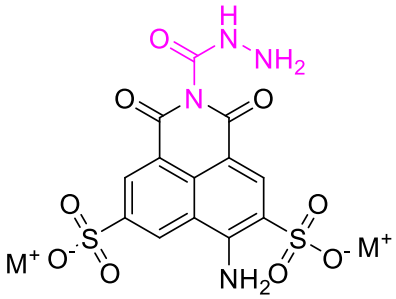

Fig. 3. Lucifer yellow dye.

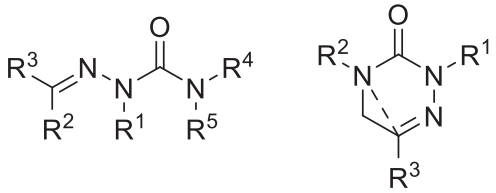

Fig. 4. General structures and substitution patterns of semicarbazones.

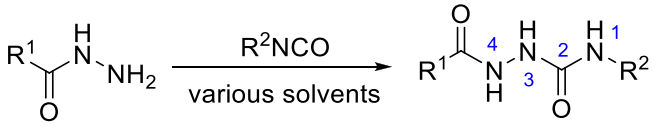

Fig. 5. Synthesis of hydrazinecarboxamides from hydrazides and isocyanates.

2-dibenzylhydrazine-1-carboxylate as a starting material. This reaction tolerated other leaving groups, as well as *N*,*N*-disubstitution. When, instead of a simple primary or secondary amine, an ester of an amino acid is used, this reaction leads to aminohydantoins, i.e., partially cyclic hydrazinecarboxamides [34]. However, the reaction's scope is broader: treating phenyl hydrazine-1-carboxylate with primary or secondary amines in the presence of a tertiary base provides *N*,*N*-disubstituted hydrazinecarboxamides under mild conditions [35]. These reactions are summarized in Fig. 7.

Another widely used method involves the reaction of aldehydes or ketones with commercially available semicarbazides (often in the form of salts, typically hydrochlorides) (Fig. 8) [36–40]. The use of an acidic catalyst (hydrochloric acid) has also been reported [37]. A modification of this method with the use of ultrasound irradiation was performed by Kumar et al. [41]. The double bond present in semicarbazone intermediates can be subsequently reduced by various reducing agents [42,43] or directly without isolation of these intermediates *via* reductive amination.

Monosubstituted hydrazinecarboxamides can be obtained by the reaction of carbamates with hydrazine hydrate under reflux (Fig. 9) [44], using various solvents, mainly alcohols (methanol, ethanol, propan-2-ol) [45,46]; an acidic catalyst (e.g., glacial acetic acid) can be used alternatively [47]. Hydrazine can also be substituted, leading to polysubstituted derivatives. The synthesis can also start from other functional derivatives of carbamic acid, e.g., carbamoyl halides (Fig. 9) [48], or ureas (Fig. 10) [49], in this case advantageously with a good leaving group, e.g., 1*H*-imidazole introduced from 1,1′-carbonyldiimidazole [50] or 1-hydroxybenzotriazole [51,52].

Alternatively, hydrazinecarboxamides are synthetically available from iminodiaziridines and carboxylic acids (Fig. 11). These unusual iminodiaziridine intermediates can be synthesized in high yields by cyclization of 1,2,3-trisubstituted guanidines using *tert*-butyl hypochlorite and potassium *tert*-butoxide or by base-mediated elimination of sulfuric acid from 1,2,3-trisubstituted *N*-hydroxyguanidine *O*-sulfonic acids [53,54].

Hydrazinecarboxamides can also be directly prepared from guanidines substituted by an electronegative substituent (chlorine, sulfonic acid) by treatment with a strong hydroxide (Fig. 12) [55].

Alkylation can further modify the formed hydrazinecarboxamides (as well as semicarbazones). Depending on the conditions, selective N^2 alkylation or N^1,N^2 dialkylation can be achieved (Fig. 13) [56].

Ivanovich et al. [43] described a valuable synthetic approach for transforming unsaturated acyclic hydrazinecarboxamides into cyclized forms (Fig. 14), although the course of the reaction is not equivocal.

3. Overview of antimicrobial activity

The rise of drug-resistant pathogens has become a critical public

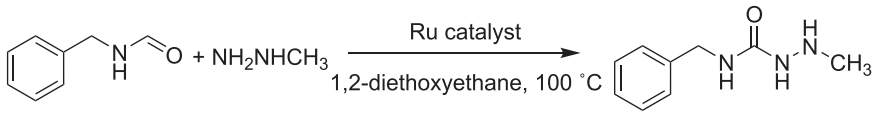

Fig. 6. Synthesis of hydrazinecarboxamides from formamides.

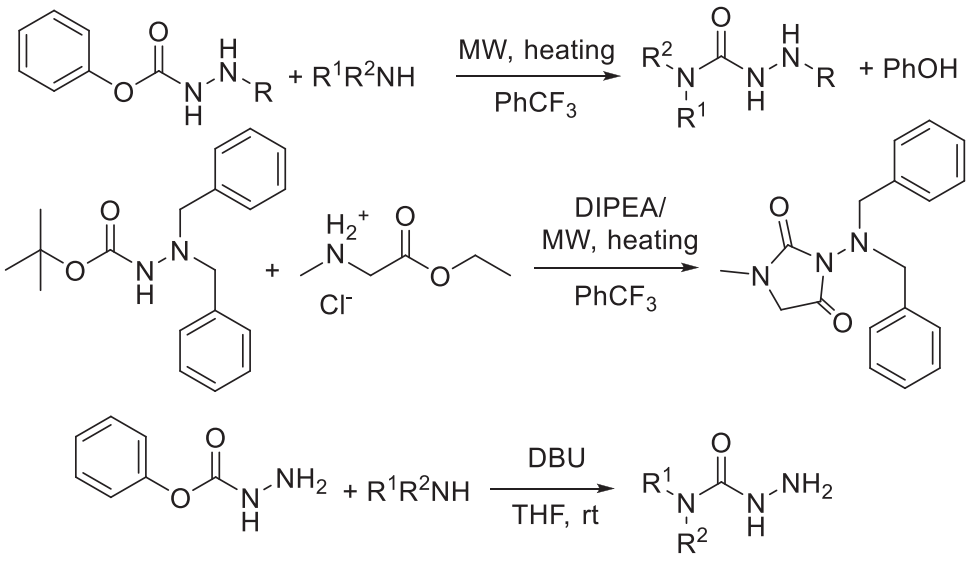

Fig. 7. Synthesis of hydrazinecarboxamides utilising hydrazinecarboxylates.

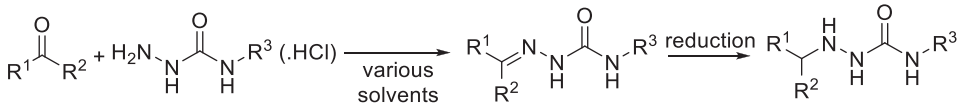

Fig. 8. Synthesis of hydrazinecarboxamides from aldehydes/ketones and semicarbazides (R^1, R^2 = alkyl or aryl, H).

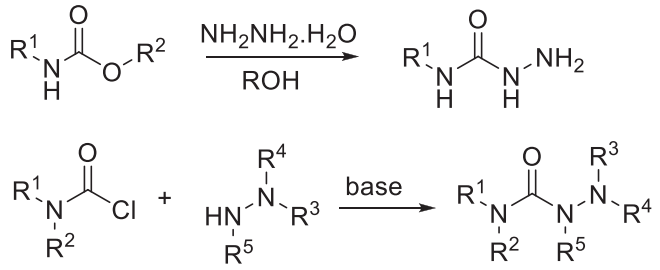

Fig. 9. Synthesis of hydrazinecarboxamides from carbamic acid derivatives and hydrazines (R^1, R^2 = alkyl, aryl).

health challenge, prompting an urgent need to develop new antimicrobial molecules to target bacteria, fungi, parasites, and viruses. Factors contributing to microbial resistance include the overuse and misuse of antibiotics in human medicine and agriculture, as well as an insufficient development of new antibiotics. The World Health Organization (WHO) has identified drug resistance as one of the top ten global public health threats, necessitating immediate and sustained efforts to develop new antimicrobial agents [57]. Consequently, there is a pressing need for new compounds that can target resistant pathogens and reduce the spread of infections [58]. The development of these agents involves several key challenges: (1) Discovery of new targets: Identifying novel microbial targets that are essential for survival or pathogenicity but are absent or significantly different in human cells; (2) Minimizing toxicity:

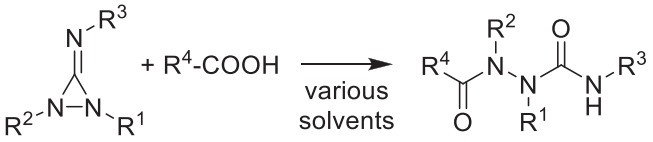

Fig. 11. Synthesis of hydrazinecarboxamides from iminodiaziridines and carboxylic acids.

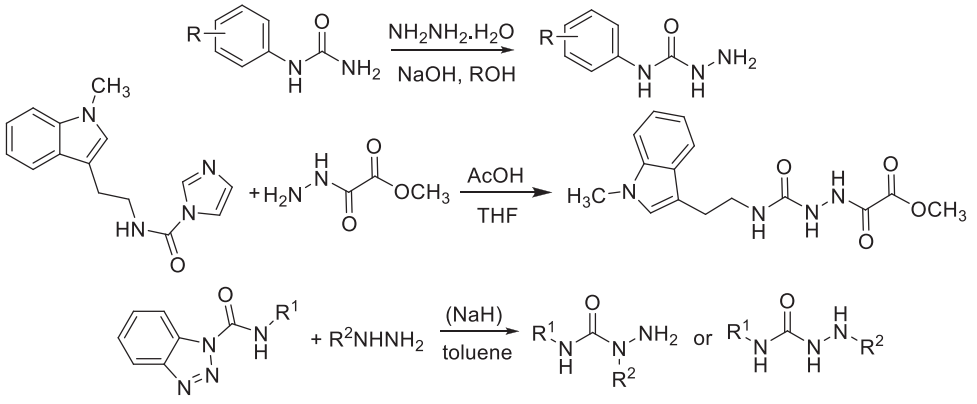

Fig. 10. Examples of hydrazinecarboxamides preparation from urea derivatives.

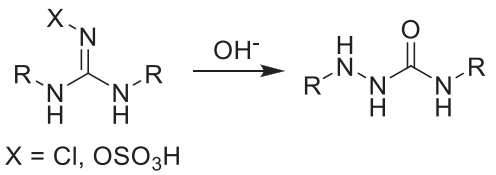

Fig. 12. Synthesis of hydrazinecarboxamides from guanidines.

Ensuring that new biocides agents are effective against microbes without causing harm to human cells; (3) Combatting resistance mechanisms: Designing molecules that can evade or inhibit the mechanisms by which microbes develop resistance.

From a historical point of view, hydrazinecarboxamides have been investigated for their antimicrobial activity for a long time. For example, in 1988, a group from the USSR investigated three chlorinated acridine-hydrazinecarboxamide conjugates (Fig. 15; **1**), and they identified their microbistatic activity against *Staphylococcus aureus*, spores of *Bacillus anthracis*, and *Candida albicans* within the range of 15.6–31.2 μg/mL [59].

Similarly, Demchenko et al. [60] investigated a series of 1,2-disubstituted hydrazines including several hydrazinecarboxamides (Fig. 15; **2**) against bacteria causing human infections (*S. aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Escherichia coli*), human- and phytopathogenic fungi (*Fusarum oxysporum*, *Penicillium rubrum*, *Botrytis cinerea*, *Helminthosporium sativum*, and *Aspergillus niger*), and phytopathogenic bacteria (*Xanthomonas malvacearum*, *Erwinia phytophthora*, *Pseudomonas cerasi*). All the compounds generally have lower fungicidal and bactericidal activity compared to the reference drugs, tetramethylthiuramdisulfide, benomyl, and chloramine. They showed fungicidal/bactericidal activity of 40–100 % of the control at concentrations of 0.05 or 0.01 %, but inhibition of human bacteria was negligible.

Thus, the research on hydrazinecarboxamides as potential antimicrobial agents is founded on three primary approaches. First, untargeted screening of derivatives and their libraries as part of a comprehensive pharmacological characterization of these compounds, referred to as "activity fishing". Second, these two papers illustrated the rational design and evaluation of hydrazinecarboxamides as potential broad-spectrum antimicrobial agents. Third, the development of compounds with a specific and narrow spectrum of antimicrobial activity, such as antibacterial, antimycobacterial, antifungal, antiprotozoal, and antiviral properties. This targeted approach can be further enhanced by preparing hybrid molecules that combine semicarbazide with other scaffolds known for their antimicrobial activities. This approach may even yield compounds with unexpected activity.

3.1. Antimycobacterial activity

Isoniazid (INH, isonicotinoyl hydrazide) remains still one of the most efficient and used first-line anti-tubercular drugs with a selective action. This prodrug must be activated by the mycobacterial catalase-peroxidase (KatG) enzyme. The main target of this drug is enoyl-acyl carrier protein reductase (InhA). Its blockade inhibits the synthesis of mycolic acids, key and highly specific components of mycobacterial cell wall responsible for its lipophilic character. InhA is a proven and validated target for the treatment of tuberculosis (TB). Most cases of INH resistance are mediated by mutations in the *katG* gene, leading to the abolished activation of the drug. The newly designed molecules combined increased lipophilicity to cross biological barriers, protection against INH metabolisms at N^2, and facilitated activation even in the absence of KatG [31,61].

The simplest hydrazinecarboxamide derived from INH, 2-isonicotinoylhydrazine-1-carboxamide (Fig. 16; **3**, R = H), was investigated *in silico* utilizing molecular docking and molecular dynamic studies to study its interactions with *Mycobacterium tuberculosis* (*Mtb.*). InhA. It was confirmed that this compound can create stable interactions with this target enzyme, meeting Lipinski's Rule of Five (Ro5). The predicted binding energy was even lower than for parent INH [62]. Its minimum inhibitory concentration (MIC) for *Mtb.* strain $H_{37}Rv$ (that is fully drug-susceptible to all drugs) was 1 μg/mL [63]. However, INH is a prodrug and not a direct inhibitor of InhA.

2-Isonicotinoyl-*N*-(substituted phenyl)hydrazine-1-carboxamides

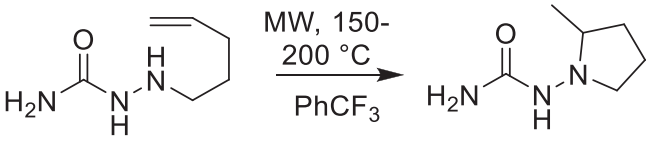

Fig. 14. Thermal cyclization of unsaturated hydrazinecarboxamides.

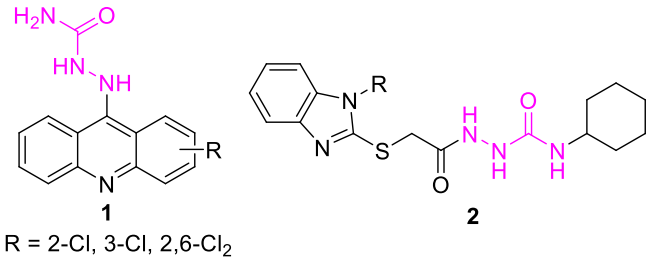

Fig. 15. Examples of older hydrazinecarboxamides studied as broad-spectrum antimicrobial agents.

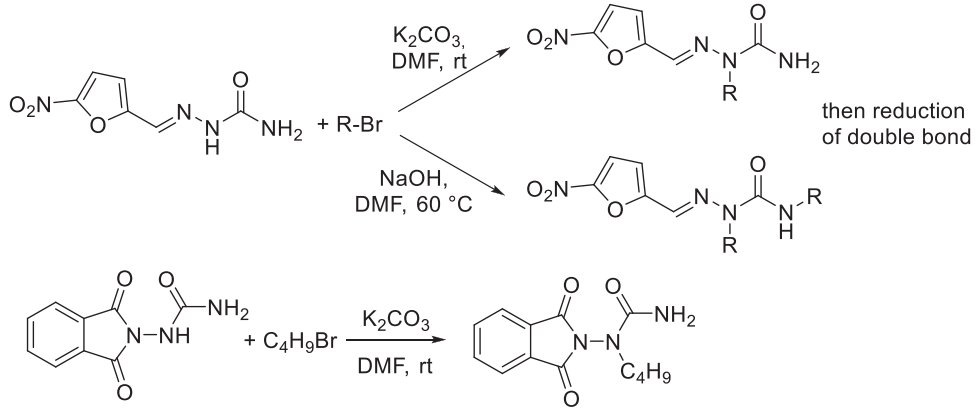

Fig. 13. Selective alkylation of hydrazinecarboxamides and semicarbazones.

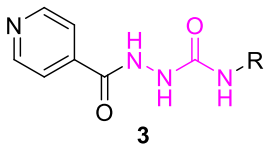

Fig. 16. INH-based hydrazinecarboxamides.

(Fig. 16; **3**, R = substituted phenyl), were synthesized and evaluated against *M. tuberculosis* 331/88 (i.e., $H_{37}Rv$), *Mycobacterium avium* 330/88, *Mycobacterium kansasii* 235/80 and 6509/96 (a clinical isolate). The substituents of the phenyl ring cover 4-alkyls (both branched and linear), various halogens, CF_3, and OMe groups, including polysubstitution. The compounds share the highest activity for *Mtb.* Among them, *N*-(4-octylphenyl) derivative was the most potent with MIC comparable to those of INH (1–2 μM vs. 0.5–1 μM), followed by lipophilic halogenated molecules (R = 2,4,6-Cl_3-Ph, 4 μM; 2,4-Br_2 and 3-CF_3-4-Br substituted phenyls with MIC of 8 μM). The situation for non-tuberculous mycobacteria (NTM) was different. Eleven hydrazinecarboxamides showed significantly lower MIC for *M. avium* than INH, finding that the optimal substitution pattern for the phenyl moiety is a small 4-alkyl group followed by 4-methoxy and 4-heptyl groups. For both strains of *M. kansasii*, the alkyl substituted hydrazinecarboxamides showed a higher *in vitro* potency than the halogenated derivatives. Generally, 4-isopropylphenyl derivative was the most active hydrazinecarboxamide against all three NTM strains (MIC of 8–32 μM). A range of these compounds showed notable micromolar activity against the INH-resistant NTM strains. In the case of *M. avium*, due to the absent KatG, this observation can be explained based on the facilitated liberation of isonicotinoyl radical from the less stable hydrazide derivative prodrugs. Another explanation of their activity was provided by molecular docking study with *N*-(4-alkylphenyl) and *N*-(halogenated phenyl) hydrazinecarboxamides. The investigation of the binding of the derivatives to the active site of InhA showed differences between alkyl and halogenated derivatives, favouring longer alkyl substituents in the substrate cavity and creating more interactions. In this way, these derivatives can inhibit InhA competitively without the need for previous bioactivation [61].

N-Alkyl-2-isonicotinoylhydrazine-1-carboxamides (Fig. 16; **3**, R = *n*-alkyl from C_1 to C_{18}), along with their cyclic oxadiazole analogues (*N*-alkyl-5-(pyridine-4-yl)-1,3,4-oxadiazole-2-amines), were evaluated against *Mtb.* 331/88 (fully drug-susceptible) and three identical NTM strains. All hydrazinecarboxamides and oxadiazoles showed significant activity with MIC of ≥0.5 μM. *Mtb.* was the most susceptible strain for 2-isonicotinoylhydrazine-1-carboxamides. On the other hand, *M. avium* showed the highest tolerance rate (MIC ≥32 μM). An optimal activity for *Mtb.* is conferred by a small alkyl (methyl, ethyl, and propyl with MIC ≤2 μM); further elongation of the alkyl chain decreases potency up to 125 μM (C_8, C_9) with the following drop of MIC. *N*-Decyl derivative exhibited the best activity for *Mtb.* among derivatives with a long aliphatic chain (16–32 μM). For *M. avium*, the most potent carboxamides are those highly lipophilic substituted from C_{11} to C_{16} alkyls (MIC ≤125 μM) with C_{12} superiority (32–62.5 μM). Contrarily to *Mtb.*, shorter alkyls resulted generally in virtually inactive compounds (MIC ≥1000 μM), the propyl derivative being an exception (250/500 μM). This molecule was also the best inhibitor of both *M. kansasii* strains (4–16 μM). Drawing a comparison to parent INH, three derivatives led to an identical *in vitro* inhibition of *Mtb.* Overall, the majority was superior against *M. avium*, and their activity against *M. kansasii* was not uniform. Then, the most active hydrazinecarboxamides underwent screening against multidrug-resistant tuberculosis (MDR-TB) strains with different resistance patterns. MDR-TB refers to strain of *Mtb.* that is resistant to at least the two most potent first-line anti-tuberculosis drugs: INH and rifampicin (RIF). However, their MIC values are significantly higher than those obtained for drug-susceptible *Mtb.* $H_{37}Rv$ (up to 125 times). The highest activity was observed for 2-isonicotinoyl-*N*-propylhydrazine-1-carboxamide (a uniform MIC value of 16 μM). These results indicate the cross-resistance to parent INH, although they are more active than this drug. Notably, the compounds were nontoxic for eukaryotic HepG2 cells; they had excellent selectivity expressed as selectivity indexes (SI). 2-Isonicotinoyl-*N*-methylhydrazine-1-carboxamide was investigated to identify its MoA. It was experimentally confirmed that it targets InhA and inhibits the synthesis of all types of mycolic acids similarly to INH, thus being its prodrug [31]. *N*-Butyl-2-isonicotinoylhydrazine-1-carboxamide (Fig. 16; **3**, R = *n*-butyl) was further investigated for its spectroscopic behaviour [64].

Gavadia et al. [63] reported several INH-based aliphatic carboxamides and their thio isosteres. Three of them were identical to those in [31] (Fig. 16; **3**, R = Me, Et, *n*-butyl), two compounds were new (R = H, cyclohexyl). Evaluation against *Mtb.* $H_{37}Rv$ showed a uniform MIC value of 1 μg/mL, while free INH did MIC of 0.03 μg/mL. Interestingly, thio isosteres were less active (MIC 2–4 μg/mL). The molecular docking with InhA displays weak binding interactions with the receptor, absent essential interactions specific to direct inhibitors, and low binding energy. On the other hand, the results indicate a binding affinity with KatG, thus suggesting analogous behaviour and MoA of the parent INH. ADMET *in silico* analysis confirmed an acceptable profile, drug-likeliness, and low toxicity [63]. However, no activity against drug-resistant strains and *in vitro* toxicity was reported.

Using molecular hybridization and isostery (pyridine replaced by pyrimidine) concepts, novel antimycobacterial *N*-alkyl-2-(pyrimidine-5-carbonyl)hydrazine-1-carboxamides (Fig. 17) and their cyclic analogues, *N*-alkyl-5-(pyrimidine-5-yl)-1,3,4-oxadiazol-2-amines, were prepared. Forty-eight compounds were tested against *Mtb.* $H_{37}Rv$, *M. avium*, and *M. kansasii*. In general, oxadiazoles and C_8-C_{12} alkyls are the most effective, with MIC values starting from 2 μM with no cross-resistance to clinically used drugs (INH, ansamycins, aminoglycosides, ethambutol, EMB, fluoroquinolones, clofazimine) as MDR-TB strains were inhibited at same concentrations as the susceptible strain. Generally, *Mtb.* was the most sensitive strain, followed by *M. kansasii*. Unfortunately, hydrazinecarboxamide precursors are predominantly less active against *Mtb.* than oxadiazoles, for some pairs up to 16-fold, but not uniformly for *M. kansasii*. Considering pyrimidyl isomers, 4-yl derivative showed a good activity for *Mtb.* and NTM, including *M. avium*. The majority of compounds also met the criteria for drug-likeness [65].

Among a larger series of indole–pyridine derived hydrazides, hydrazide–hydrazones, and thiosemicarbazones, also two hydrazinecarboxamides (Fig. 18; **4** and **5**) derived from INH were investigated as potential antimycobacterial agents, but both were inactive. Removing the 4-chlorophenylaminocarbonyl group resulted in submicromolar activity against *Mtb.* $H_{37}Rv$ in both cases [66].

Other heterocycles used for hybridization with hydrazinecarboxamide moiety in one molecular entity were quinoline and benzothiazole [67]. This study covers, among other linkers, also four hydrazinecarboxamides of a total of 25 compounds (Fig. 19; **6**, R = H, CF_3, F, NO_2). These *N*-(benzo[*d*]thiazol-2-yl)-2-(7-chloroquinolin-4-yl)hydrazine-1-carboxamide hybrids demonstrated significant antitubercular activity against *Mtb.* $H_{37}Rv$ strain favouring the most lipophilic trifluoromethyl group with MIC_{90} of 4.9–6.9 μM, followed by unsubstituted derivative. On the other hand, fluorine and nitro derivatives

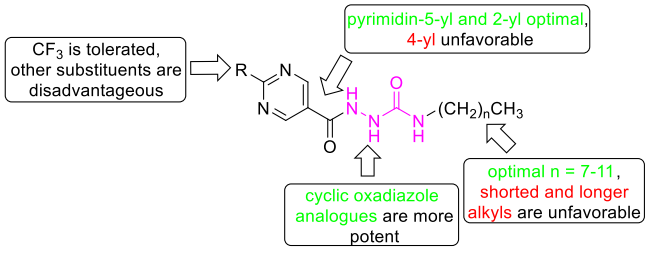

Fig. 17. Structure and SAR of pyrimidine derivatives.

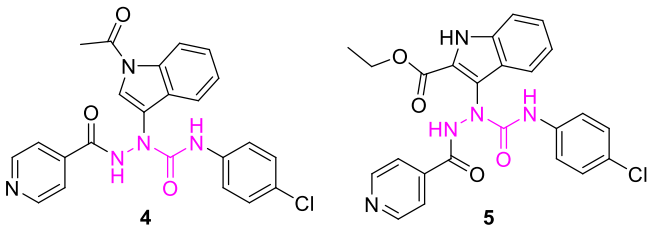

Fig. 18. Inactive INH-based hydrazinecarboxamides.

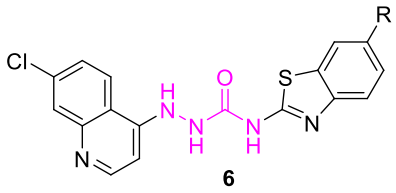

Fig. 19. *N*-(Benzo[*d*]thiazol-2-yl)-2-(quinolin-4-yl)hydrazine-1-carboxamide hybrids.

were inactive. The majority of compounds exhibited low cytotoxicity for HepG2. *In silico* studies were focused on profiling ADME (absorption, distribution, metabolism, and excretion). Most of these hybrids possess favourable drug-like properties, aligning well with established pharmacokinetic parameters. They were predicted to have no violation of Ro5, high human oral absorption (≥71 %), no concerns with cardiotoxicity (HERG K^+ channels), and a neutral profile for brain-blood partition coefficient and binding to human serum albumin.

Quinoline is a popular scaffold for potential anti-TB drugs. In study [68], their design was inspired by the structure of mefloquine, a well-known antimalarial agent. Focusing on mycobacteria, five hydrazinecarboxamides (Fig. 20; **7**), as a part of a more extensive compounds collection, were evaluated against *Mtb.* H_{37}Rv and one MDR-TB strain resistant to INH, RIF, and EMB. The highest activity was associated with the most lipophilic dimethyl derivative (6.25 μM for both strains), followed by fluorinated and methoxy derivatives (12.5 μM). Cyano- and pentyl derivatives inhibited only the susceptible strain with MIC of 25 μM, but against MDR-TB, they were inactive at 50 μM.

2-[4-Hydroxy-8-(trifluoromethyl)quinoline-3-carbonyl]-*N*-phenylhydrazine-1-carboxamides (Fig. 20; **8**) were introduced as 4-hydroxyquinoline derivatives to boost its antibacterial properties. Their antimycobacterial activity was screened using *Mtb.* H_{37}Rv, MDR-TB strains (resistant to INH, RIF, and EMB), and NTM: *M. smegmatis* and *M. fortuitum*. All five hydrazinecarboxamides inhibited drug-susceptible *Mtb.* H_{37}Rv with MIC of 1.25–10 μg/mL favouring 4-methyl/methoxy derivatives (MIC = 1.25 μg/mL). However, these two derivatives substituted with small lipophilic electron-donating groups also inhibited MDR-TB and NTM strains at slightly higher concentrations (10–25 μg/mL). Ethyl derivative was the lowest active, inhibiting only the H_{37}Rv strain at 12.5 μg/mL. Interestingly, analogous thiosemicarbazides share somewhat higher activity. Based on molecular docking, InhA was proposed as their target structure, but this investigation was not performed on hydrazinecarboxamides [69].

The antimycobacterial activity of many primaquine (PQ)-hydrazinecarboxamides and bis-urea hybrids (i.e., both series contained hydrazinecarboxamide moiety) against *Mtb.* H_{37}Ra and two clinical isolates of *M. avium* complex and *M. avium* subsp. *paratuberculosis* was summarized in the manuscript of Pavic et al. [70] Some of these modifications improved antimycobacterial activity of the parent antimalarial drug (MIC = 32 μg/mL), up to 16×, favouring *N*-{[4-chloro-3-(trifluoromethyl)phenyl]carbamoyl}-2-{4-[(6-methoxyquinolin-8-yl) amino]pentyl}hydrazine-1-carboxamide (Fig. 21; **9**) with acceptable selectivity (SI = 15 in L6 cell line). On the other hand, many modifications led to less active, nonselective, or both analogues. Interestingly, similarly to INH, the conversion of the terminal amino group to unsubstituted hydrazinecarboxamide did not lead to a change in biological activity.

Six PQ-based compounds linked by a urea and bis-urea spacer with various amino alcohols were designed as potential antimycobacterial agents. In general, bis-ureas (i.e., compounds containing hydrazinecarboxamide moiety) exhibited lower antimycobacterial activity than "simplified" ureas. The racemic derivatives of 1-(hydroxyethyl)cyclopropane-1-amine and tyramine were the most active (Fig. 21; **10** and **11**), with MIC values of 16 and 32 μg/mL for *Mtb.* H_{37}Ra and 8 and 16 μg/mL for *M. marinum*, respectively. The introduction of fluorine atom(s) and the expansion of cycloalkyl moiety were not beneficial. However, they were inactive against *M. smegmatis* and *M. kansasii*. In addition, these compounds do not have optimal properties for drug-likeness (higher number of rotatable bonds and polarity) [52].

Patel et al. [26] synthesized and evaluated various semicarbazide derivatives of 4-(adamantan-1-yl)quinoline; adamantane is a well-established scaffold for development of anti-TB agents. The activity against *Mtb.* H_{37}Rv was various amongst the different groups of derivatives. The third group of derivatives, the hydrazinecarboxamides (Fig. 22; **12**), also showed promising activity. The most active 4-CF_3O-Ph derivative had an inhibitory activity of 99 % at the concentration of 3.125 μg/mL. In this series, cycloalkyl derivatives demonstrated more significant activity than their aliphatic counterparts, as evidenced by the inhibition rates at this concentration. Specifically, cyclopentyl and cyclododecyl derivatives both showed 87 % inhibition, compared to 55 % for the pentyl derivative and 47 % for the hexyl derivative. None of the derivatives from all three groups were more active than INH and RIF used as comparators, which had an inhibitory activity of 99 % at MIC values of 0.1 and 0.2 μg/mL, respectively.

N-(Adamantan-1-yl)-2-(4,6-difluoro-1*H*-indole-2-carbonyl)hydrazine-1-carboxamide (Fig. 22; **13**) inhibited *Mtb.* H_{37}Rv with MIC of 32 μg/mL (82.38 μM) [71].

The nitro group and pyrrole are well-known pharmacophores for antimycobacterial activity, which led to the design of 4-nitropyrrole-based hydrazinecarboxamides (Fig. 23; **14**). They exhibited a range of activity against *Mtb.* H_{37}Rv with MIC varied, indicating differing potency levels (0.80–59.2 μg/mL). The best activity was identified for compounds with furfuryl and 4-substituted phenyls (MeO, Br, Cl, and especially NO_2) whose activity was close to INH. For this action, *N*-methylation of pyrrole was generally detrimental despite enhanced lipophilicity, which helps in crossing barriers, including the cell wall of *Mtb.* [72].

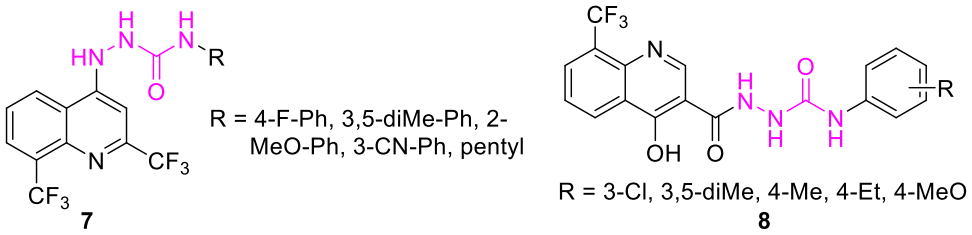

Fig. 20. Quinoline-based hydrazinecarboxamides.

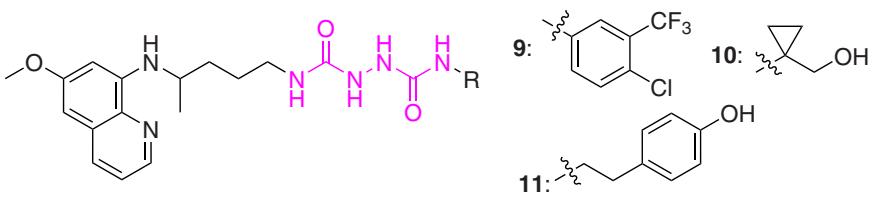

Fig. 21. Primaquine-hydrazinecarboxamide hybrids.

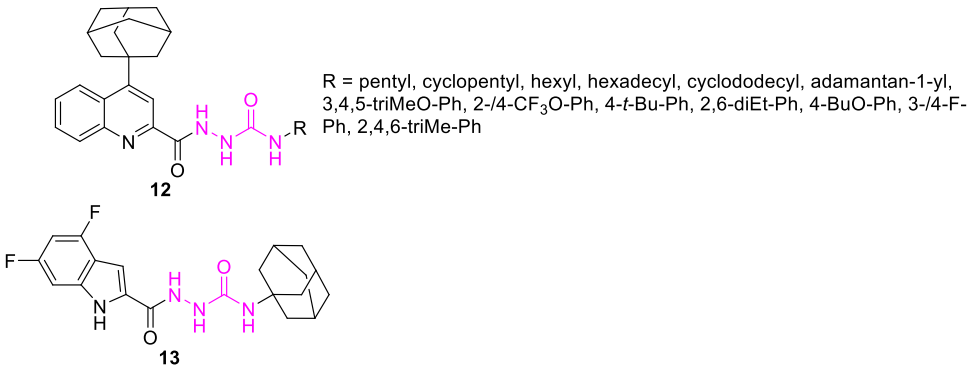

Fig. 22. Adamantane-based antitubercular compounds containing hydrazinecarboxamide moiety.

Fig. 23. Nitrogen-containing heterocycles based hydrazinecarboxamides.

2-Amino-4-[2-(2-carbamoylhydrazineyl)-2-oxoethoxy]-6-(1-phenylprop-1-en-1-yl)pyrimidine-5-carboxamides (Fig. 23; **15**) were designed as pyrimidine hybrids and evaluated against *Mtb.* $H_{37}Ra$ strain. Five of the nine compounds were active with MIC values of 3.90–15.62 μg/mL. The order of substituents R contributing to the activity is as follows: 2-F = 3-Cl > 4-NO_2 = 4-Br > H. Obviously, the activity was notably influenced by the lipophilicity of the substituents. *In silico* predictions of ADME and drug-likeness provided satisfactory results but with a higher number of hydrogen bond donors. The molecular docking studies focused on two protein targets associated with *Mtb.*: glutamine synthetase and RNA polymerase. For glutamine synthetase, the compounds with 3-Cl a 2-F substitution exhibited particularly high docking scores, as well as for RNA polymerase. The proposed interactions primarily involved hydrogen bonds and hydrophobic interactions [73].

Krátký et al. [30] designed and synthesized *N*-alkyl-2-[4-(trifluoromethyl)benzoyl]hydrazine-1-carboxamides (Fig. 24; **16a**) as potential antimicrobial agents and cholinesterases inhibitors. The compounds were evaluated against *Mtb.* $H_{37}Rv$ and NTM (*M. avium*, *M. kansasii*). Cyclization to 1,3,4-oxadiazoles (Fig. 24; **16b**) increased the potency against *M. tuberculosis* and *M. kansasii*. *N*-Hexyl-5-[4-(trifluoromethyl)phenyl]-1,3,4-oxadiazol-2-amine (Fig. 24; **16b**, R = *n*-hexyl) exhibited the lowest MIC within this study (MIC ≥62.5 μM); however, this activity is relatively mild. For most hydrazinecarboxamides, the exact determination of MIC was impossible due to their poor solubility; their MIC values exceeded 250 μM.

N-(4-Methoxyphenyl)-2-[3-(morpholinomethyl)benzofuran-2-carbonyl]hydrazine-1-carboxamide (Fig. 25; **17**) exhibited mild inhibition of drug-susceptible strain *Mtb.* (ATCC 27294) with MIC of 125 μg/mL [74]. *N*-Acyl-4-allylsemicarbazide derivatives (Fig. 25; **18**) and their cyclic analogues 4-allyl-1,2,4-triazoline-5-ones were investigated *in vitro* for antimicrobial activity against a panel of bacteria, including *Mtb.* $H_{37}Ra$, *M. smegmatis* and *M. phlei*. However, they were inactive [75].

Also, azapeptides and their drug conjugates can help combat TB. The bactericidal properties of macrophages are linked to their ability to deliver bacteria to hydrolytic lysosomes, thereby triggering the process of autophagy. This process is augmented with ubiquitin-derived

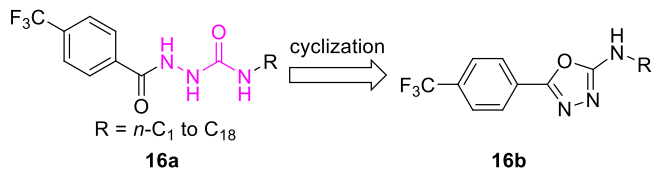

Fig. 24. 4-(Trifluoromethyl)benzohydrazide-based hydrazinecarboxamides and oxadiazoles.

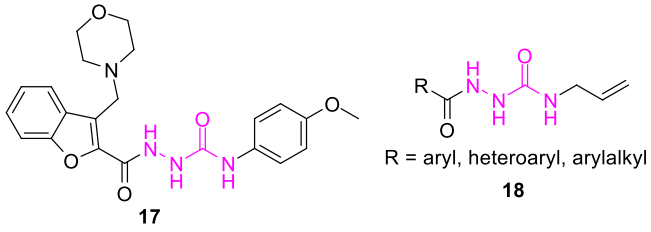

Fig. 25. Hydrazinecarboxamides with limited (**17**) or no (**18**) antimycobacterial activity.

peptides (Ub2) that stimulate the fusion of phagosomes to lysosomes to eliminate mycobacteria from infected macrophages. It was shown that a synthetic peptide Ub2 (STLHLVLRLRGG) has potent mycobactericidal activity *via* direct killing by disrupting the membrane integrity, in addition to their ability of lysosomal-targeted killing and elimination of mycobacteria. Thus, a novel series of Ub2 peptides was prepared, wherein Gly residues were replaced with azaGly to improve metabolic stability and SAR study. Both glycine residues at the C-terminus were replaced with azaGly; moreover, the N-terminus was also modified *via* attachment of azaGly. N-terminal azaGly was also used as a linker for binding cargo – nitrogen-containing heterocyclic scaffolds (Fig. 26; **19**) to improve metabolic stability, boost cationic charge at the N-terminus even more, and enhance lipophilicity. The prepared peptides have a direct antimycobacterial activity against *Mtb*. $H_{37}Ra$, favouring aza-peptides, especially those with N-terminal azaGly. The peptides having heteroaryl moieties at the N-terminus failed to inhibit bacterial growth significantly. On the other hand, all peptides and conjugates inhibit mycobacterial growth in infected J774 A.1 macrophages. Ub2 peptide significantly reduced the survival of mycobacteria in a concentration-dependent manner. The peptides with the substitution of azaGly at the 12th position and at the N-terminus exhibited similar behaviour, but at higher concentrations. Regarding N-terminal conjugates, 6-chloronicotinoyl and 6-bromopicolinoyl moieties (Fig. 26; **19**, R = Cl, Br) have shown the best activity for intracellular killing of mycobacteria, significant even at 0.195 μM. Treating macrophages with Ub2 peptide derivatives led to an acidification of lysosomes, thus promoting intracellular clearance of mycobacteria. The cytotoxicity was generally negligible for all derivatives (>500 μM) [76].

Key structural features influencing antimycobacterial activity include 1,4-disubstituted semicarbazides, where the N^1 substituent is preferably an acyl group derived from heteroaromatic or *ortho*-condensed heteroaromatic acids, ideally containing nitrogen. This acyl group can also be substituted by a benzoyl group bearing lipophilic and/or electron-withdrawing substituents, such as halogens, CF_3, or NO_2. At the N^4 position, activity is typically associated with the presence of longer alkyl chains, cycloalkyls (including adamantan-1-yl), or phenyl groups substituted with lipophilic moieties. Additionally, the incorporation of scaffold with inherent antimycobacterial properties elsewhere in the molecule, such as INH, quinolines, or PQ, is particularly advantageous.

3.2. Antibacterial activity

Six pyrazole derivatives (Fig. 27; **20**, R = H, Cl, OH, NO_2, OCH_3, N$(CH_3)_2$) were tested against a panel of Gram-negative (*E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*) and Gram-positive bacteria (*S. aureus*, *Staphylococcus epidermidis*). All of them were active against *E. coli* and *S. epidermidis* in terms of inhibition zones (within the range of 12–28 mm and 10–18 mm, respectively, at the concentration of 100 μg/mL), and the remaining strains were inhibited by two or three compounds. The phenolic derivative (Fig. 27; **20**, R = OH) was highly active against *E. coli* (MIC value of 0.25 μg/mL) based on a comparison to ciprofloxacin (CIP; MIC = 0.5 μg/mL). The best results against *S. epidermidis* were obtained with nitro compound (MIC 2 μg/mL) and matched up with CIP (MIC of 4 μg/mL). MIC of chloro derivative for *K. pneumoniae* was 1 μg/mL. *P. aeruginosa* and *S. aureus* were inhibited with MIC values of ≥32 μg/mL, higher than CIP [41].

In 2001, hydrazinyl urea-based antibacterial agents (Fig. 28; **21**-**23**)

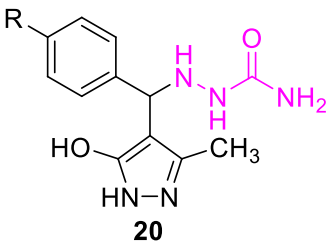

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Fig. 27. Pyrazole-hydrazinecarboxamide derivatives.

were identified to combat drug-resistant Gram-positive pathogens *via* targeting peptidoglycan synthesis, a critical pathway for bacterial cell wall formation. These compounds were discovered through a combinatorial chemistry approach. They were evaluated against two strains of *S. aureus* (one was methicillin-, MRSA, and CIP-resistant), vancomycin-resistant *Enterococcus faecium* (VRE), *Enterococcus faecalis*, and two strains of *Streptococcus pneumoniae*, including one penicillin-resistant strain. The identified hits exhibited broad-spectrum antibacterial activity, particularly against MRSA and VRE in a low micromolar range, outperforming standards in some cases. The monosubstituted hydrazinecarboxamide (Fig. 28; **21**) showed mild activity with MIC of 101 μM, while disubstituted analogue (Fig. 28; **22**) exhibited a geometric mean of MIC of 3 μM. It also showed inhibition of cell wall biosynthesis (IC_{50} of 17 μM) at a level like the MIC values and bactericidal action. MIC of 1.1 μM was found for *Legionella* sp., showing that its antibacterial efficacy extends to certain Gram-negative organisms, particularly those involved in respiratory infections. It was extremely potent with a MIC value of 0.004 μM against anaerobic *Bacteroides fragilis*. However, the molecule did not show significant activity against typical Gram-negative *Enterobacteriaceae* such as *E. coli*, *Klebsiella* sp., *Proteus* sp., and *Enterobacter* sp. with MIC values exceeding 300 μM. This suggests that the compound's mechanism or structural features may prevent effective penetration of the Gram-negative outer membrane or result in active efflux or inactivation by these bacteria. The compound was also tested for cytotoxicity on eukaryotic cells using *Saccharomyces cerevisiae*, where it showed no inhibitory activity at 300 μM, indicating a lack of toxicity at antibacterial concentrations, along with no haemolytic activity at this concentration on sheep red blood cells. However, it suffers from low aqueous solubility. That is why additional analogues were prepared by combinatorial synthesis. The preserved activity, along with sufficient water solubility, was mainly associated with the presence of 4(3*H*)-quinazolinone scaffold (Fig. 28; **23**) [77].

4-Nitropyrrole-based hydrazinecarboxamides (Fig. 23; **14**) were also screened against various Gram-positive and Gram-negative organisms. The compounds were also assessed for their cytotoxic effects (Vero cell line) to determine their safety profile for therapeutic use. The compounds showed antibacterial activities with a narrow MIC range of 1.56–6.25 μg/mL despite the strain used (MRSA, methicillin-susceptible *S. aureus*, MSSA, *E. coli*, *K. pneumoniae*). This suggests that substitution of the parent scaffold had no significant influence. These MIC values were analogous to CIP. The cytotoxicity ranged more (IC_{50} of 211.3–633.0 μg/mL); 4-nitrophenyl congener was the most toxic here. *N*-Methylation of pyrrole generally decreased cytotoxicity. Nevertheless, all the compounds showed a sufficient selectivity for microbial pathogens [72].

2-[2,8-Bis(trifluoromethyl)quinolin-4-yl]hydrazine-1-carboxamides (Fig. 20; **7**) also exhibited antibacterial activity against various

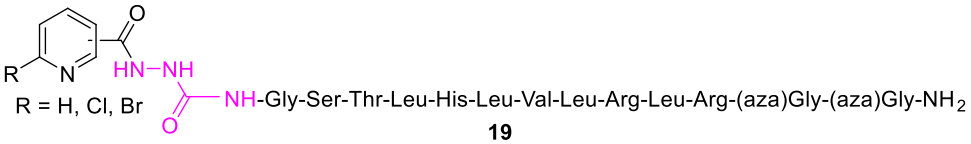

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Fig. 26. Antitubercular azapeptide conjugates.

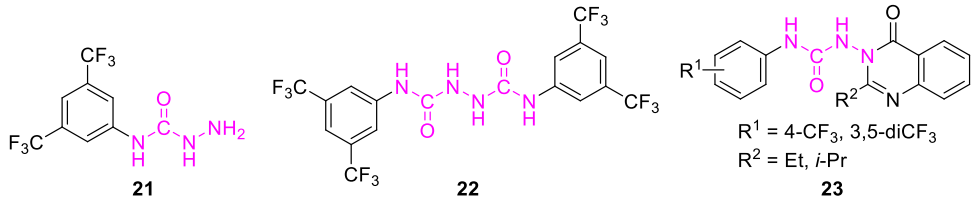

Fig. 28. Trifluoromethyl substituted hydrazinecarboxamides.

pathogens (*S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*). In general, electron-donating groups (Me, MeO) enhanced antibacterial activity, as illustrated by a uniform MIC value of 6.25 μg/mL for all four strains. These values are like those of CIP used as a comparator. Then, pentyl and 4-fluorophenyl derivatives inhibited *S. aureus*, *E. coli*, and *P. aeruginosa* at 12.5 μg/mL but with lower activity for KP. The presence of cyano group was translated into the lowest antibacterial action (MIC = 50 μg/mL) [68].

In addition to inhibition of mycobacteria, five 2-[4-hydroxy-8-(trifluoromethyl)quinoline-3-carbonyl]-*N*-phenylhydrazine-1-carboxamides (Fig. 20; **8**) were found also as potent antibacterial agents. This activity was assessed using two Gram-positive (*S. aureus*, *S. pyogenes*) and three Gram-negative species (*P. aeruginosa*, *K. pneumoniae*, *E. coli*). All of them showed a broad-spectrum antibacterial activity; again, the best activity was associated with 4-Me and 4-MeO substitution (MIC of 0.1–0.8 μg/mL that are comparable to CIP) followed by 3-Cl and 3,5-diMe derivatives. 4-Ethyl molecule was again at least active (MIC 3.12–12.5 μg/mL) [69].

Antibacterial activity of *N*-acyl-4-allylsemicarbazides (Fig. 25; **18**) and their cyclic analogues 4-allyl-1,2,4-triazoline-5-ones was investigated against *E. coli*, *S. aureus*, and *S. epidermidis*. Only one derivative (R = 4-NO_2-phenyl) was active against *S. epidermidis* with an inhibition zone of 22 mm but a high MIC value of 2048 μM [75].

2-[2-(2-Benzyl-1*H*-benzimidazol-1-yl)acetyl]-*N*-phenylhydrazine-1-carboxamide (Fig. 29; **24**) was as a member of a larger series of 2-benzylbenzimidazoles screened for its antibacterial activity. It inhibits the growth of a majority of Gram-positive and Gram-negative bacteria (*Salmonella enterica*, *S. aureus*, *P. vulgaris*, *Enterobacter aerogenes*, *Bacillus subtilis*, and *Gordonia rubripertincta*), although at comparatively high MIC values (1.25–2.5 mg/mL), while two clinical isolates (*Acinetobacter lwoffii* and *Bacillus megaterium*) were resistant [78].

A series of benzimidazole derivatives with a hydrazinecarboxamide linker (Fig. 29; **25**) were synthesized and evaluated as potential inhibitors of *S. aureus* and *E. coli* growth. Several analogues exhibited low MIC values against both strains. Semicarbazides inhibited *S. aureus* with MIC values ranging from 6 to 100 μM and *E. coli* with MIC values starting from 12 μM. In this series, various functional moieties with different properties were tolerated; the lowest activity was associated with the unsubstituted phenyl ring. Here, thio analogues showed significantly lower activities. Extended screening of antibacterial activity was performed against a panel of clinically relevant bacteria for three of the most potent semicarbazides (Fig. 29; **25**, R = adamantan-1-yl, 3,5-diMeO-phenyl, and 2-naphthyl). Dimethoxy derivative was identified as the most potent compound against Gram-positive strains (*S. aureus*, *E. hirae*, *S. pyogenes*, and *S. pneumoniae* with MIC of 1–25 μM); the remaining two compounds were almost identically potent. However, this derivative inhibited only two of four Gram-negative strains (*E. coli*, *K. pneumoniae*), while *P. aeruginosa* and *P. vulgaris* were resistant. At the same time, the naphthyl analogue exhibited MIC of 6–50 μM for all Gram-negative species. The investigation of MoA revealed that these compounds did not show appreciable inhibitory activities in the transcription/translation assay, suggesting a different MoA [79].

1,4-Disubstituted semicarbazide (Fig. 30; **26**) and 4,4′-bis[1-substituted semicarbazide]diphenylmethane derivatives (Fig. 30; **27**) were prepared to evaluate their antibacterial activity. However, only one compound, *N*-(4-bromophenyl)-2-[2-(pyridine-4-yl)acetyl]hydrazine-1-carboxamide (Fig. 30; **26**, R^1 = 4-pyridyl, R^2 = 4-bromophenyl), from a total of eighteen was significantly active; the remaining ones did not completely inhibit the growth of the used reference strains of Gram-positive and Gram-negative bacteria even at a concentration of 1000 μg/mL. A partial inhibitory effect was found for 4-pyridyl-containing compounds but at high concentrations. *N*-Methylpyrrolyl and diphenylmethane analogues were utterly inactive. The title hydrazinecarboxamide exhibited inhibition of Gram-positive bacteria (coagulase-positive *S. aureus*, coagulase-negative *S. epidermidis*, *B. subtilis*, *B. cereus*, *Micrococcus luteus*) with MIC values of 15.63–125 μg/mL, but with no activity against Gram-negative pathogens [80].

Thiazole-based hydrazinecarboxamides were prepared and investigated against MSSA, *B. subtilis*, and *B. cereus*, among a larger series of trisubstituted thiazoles. Cyclohexyl acetamide derivative (Fig. 31; **28**, R = CH_3) exhibited moderate antimicrobial activity against *S. aureus* with MIC of 25 μg/mL but not against other bacteria. Its aromatization led to a decrease in activity. Replacing of acetamide moiety with ureido group (Fig. 31; **28**, R = $C_6H_{11}NH$) improved the activity of the cyclohexyl derivative (12.5 μg/mL), but not its phenyl analogue, and still does not broaden the spectrum of activity. Analogous thiosemicarbazide derivatives were more potent and showed a much broader spectrum of activity [81].

The compound **29** (Fig. 31) was part of a series utilizing combination of 4-aminoquinoline and 1,2,4-triazole scaffolds. It displayed moderate to low antibacterial activity across a range of tested bacteria with MIC values of 6.25–50 μg/mL against *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli*,

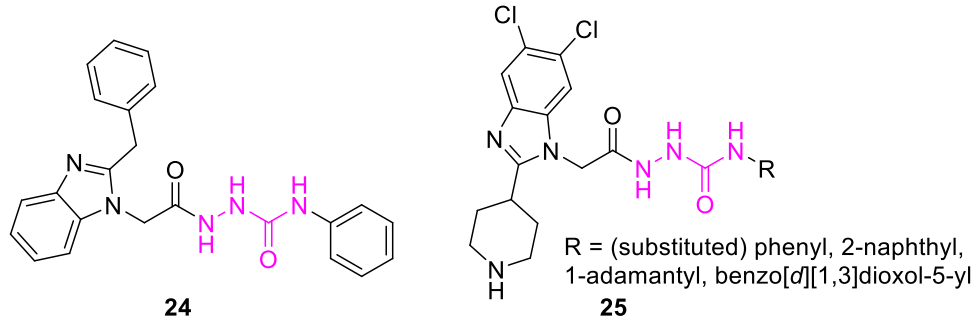

Fig. 29. Benzimidazole-hydrazinecarboxamide derivatives.

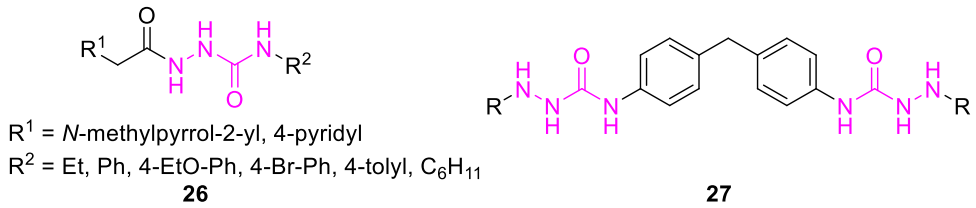

Fig. 30. 1,4-Disubstituted semicarbazides containing nitrogen heterocycles (**26**) and 4,4′-bis[1-substituted semicarbazide]diphenylmethane derivatives (**27**).

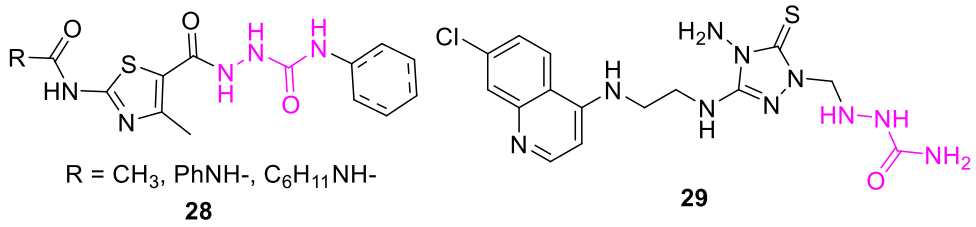

Fig. 31. Sulphur-containing hydrazinecarboxamides.

P. aeruginosa, *Proteus mirabilis*, and *P. vulgaris*. Gram-positives were generally more susceptible (MIC of 6.25–12.5 μg/mL) [82].

The hydrazinecarboxamide MAC-0047200 (Fig. 32; **30**) was identified in a library screening campaign as a molecule active against *B. subtillis*, a model of Gram-positive bacteria, with EC_{50} of 16.61 μM, but no activity on bacterial membrane or cell wall was recorded [83].

Patel et al. [84] focused on quinazolinyl-triazinyl hydrazinecarboxamides (2-[4-({2-[(6,8-dibromoquinazolin-4-yl)amino]ethyl}amino)-6-morpholino-1,3,5-triazin-2-yl]hydrazine-1-carboxamides; Fig. 32; **31**). These compounds were designed to combine quinazoline and *s*-triazine pharmacophores through an ethylene diamine linkage, a novel structural motif aimed at enhancing antimicrobial efficacy. They were tested against a range of bacterial strains, including *S. aureus*, *B. cereus* (Gram-positive), *P. aeruginosa*, and *K. pneumoniae* (Gram-negative), with MIC values of 6.5–100 μg/mL. For *S. aureus* and *P. aeruginosa*, the optimal substituent (Fig. 32; **31**) was 4-methylcyclohexyl (6.5 and 12.5 μg/mL, respectively), while against *B. cereus* were preferred 2- and 4-chlorophenyl moieties (25 μg/mL). Small alkyls (Et, Pr) led to the best activity against *K. pneumoniae* (12.5 μg/mL), followed by cyclohexyl. Electron-donating groups generally enhanced activity, while bulky or electron-withdrawing groups tended to reduce efficacy.

Fourteen isoquinolinedione-urea hybrid compounds represent hydrazinecarboxamides with one nitrogen incorporated into the cycle (Fig. 32; **32**). The heterocyclic part was fixed, while the *N*-substitution of carboxamide was different. Most substituents cover substituted phenyls (various substituents and positions), *tert*-butyl, and cyclohexyl. MIC values for Gram-positive pathogens (MSSA, MRSA, *E. faecalis*) ranged from 8 μg/mL to 512 μg/mL with two CF_3 derivatives **32** superiority (8–32 μg/mL). Interestingly, MRSA was more susceptible than MSSA strains. This was significantly better than the reference antibiotic gentamicin, which had a MIC of 32 μg/mL against MRSA. MIC values for the Gram-negative bacteria were generally higher, ranging from 256 μg/mL to 1024 μg/mL (*E. coli*, *P. aeruginosa*) with no clear superiority of any derivative. In general, higher log*P* values correlated with better antibacterial activity. The most active chlorinated compound was docked into the ATP-binding pocket of *S. aureus* DNA gyrase B (GyrB) subunit, revealing critical hydrogen bonds with Asp81 and hydrophobic interactions of halogens with the enzyme. The phenyl ring of the isoquinolinedione moiety engaged in π-cation interactions with Arg84. *In silico* predictions indicated that the compounds possess favourable physicochemical properties for oral bioavailability and drug-likeness. They adhered to Lipinski's Ro5 and exhibited good absorption potential [85].

Three racemic 2-[(2-hydroxyphenyl)(phenyl)methyl]hydrazine-1-carboxamides (Fig. 32; **33**, R = H, NO_2, NH_2) were investigated in terms of diameter of inhibition zone at a single concentration of 10 μg/mL against Gram-positive bacteria (*S. aureus* and *B. subtilis*) with zones of 10–17 mm with increasing activity in this order of R: H < NO_2 < NH_2. The latter strain was more susceptible. The inhibition of Gram-negative bacteria (*E. coli*, *P. aeruginosa*) was almost identical for amino derivative

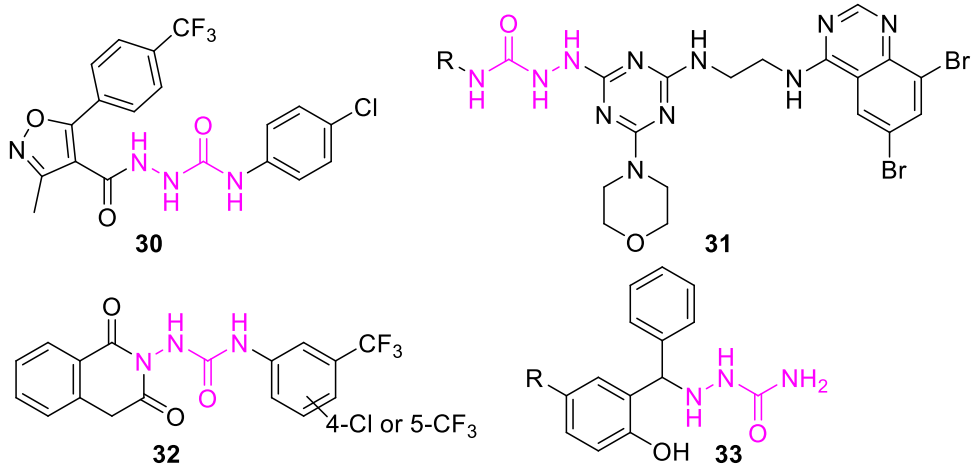

Fig. 32. Other antibacterial hydrazinecarboxamides.

but lower in the case of the remaining two ones (from inactivity to 12 mm) [86].

Four *N*-(4,7-dimethyl-2-oxo-2*H*-chromen-6-yl)-2-(2-oxo-2*H*-chromene-3-carbonyl)hydrazine-1-carboxamides (Fig. 33; **34**, R = H, 6-/7-/8-Me) were synthesized as precursors of corresponding 1,3,4-oxadiazoles and thiadiazoles. These intermediates exhibited mild antibacterial activity against *S. aureus*, *E. coli*, and *S. typhi* with MIC values of 100–200 μg/mL. The activity was slightly increased after cyclization [87].

Six hydrazinecarboxamides derived from *p*-aminobenzoic acid (Fig. 33; **35**) were assessed against bacterial strains such as *S. aureus* and *E. coli*, both collection strains and clinical isolates. MIC values against MSSA and MRSA strains were low, at 256–512 μg/mL, while against *E. coli*, including an extended-spectrum β-lactamase producing strain, were 128–256 μg/mL. There were no differences in activity against resistant strains despite substitution patterns. However, it was generally low. Isosteric hydrazinecarbothioamides produced an enhancement in antimicrobial activity [88].

A weak antibacterial activity was found for *N*-(2-chlorophenyl)-2-(2-phenoxyacetyl)hydrazine-1-carboxamide (Fig. 33; **36**), the only one from ten hydrazinecarboxamide precursors of 5-(phenoxymethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones. Its MIC against three strains of *S. aureus*, *S. epidermidis*, *B. subtilis*, *B. cereus*, and *M. luteus* were 500–1000 μg/mL and minimal bactericidal concentration of ≥1000 μg/mL [89].

Ten PQ derivatives, phenyl substituted N^1-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-N^2-phenylhydrazine-1,2-dicarboxamides avoided any activity against a range of bacterial strains: MSSA and MRSA strains, *E. faecalis* including VRE, *Bacillus pumilus*, *B. cereus*, *B. subtilis*, *P. aeruginosa* including multidrug-resistant strain, and *E. coli* [90].

Also, complexes derived from semicarbazides have been investigated as potential bioactive agents. A study was centred on synthesizing and characterizing chromium(III) complexes using various ligands derived from diketohydrazides. Among them, 2-[2-oxo-2-(phenylamino)acetyl]-*N*-phenylhydrazine-1-carboxamide (Fig. 34; **37**, abbreviated as Lig) and its complex with $Cr(III)Cl_3$ were involved. They were tested against Gram-positive *B. subtilis* and Gram-negative *E. coli*. The $[Cr(III)(Lig)Cl_3]$ complex showed enhanced biological activity compared to the free ligand. At a high concentration of 1 mg/mL, the ligand and complex provided inhibition zones of 8 and 13 mm for *E. coli*, but they were inactive against *B. subtillis* [91]. Also complexation with Cu(II) ions to form $[Cu(Lig)Cl_2(H_2O)]$ [92], with Ni(II) to provide $[Ni_2(Lig)(H_2O)_2].2H_2O$ [93], Co(II) to obtain $[Co(Lig)Cl(H_2O)_2]\cdot H_2O$ [94] and with Mn(II) to obtain $[Mn(Lig)_2]$ [95] improves the activity of this ligand against *E. coli* with almost identical activity values.

Hydrazinecarboxamides have demonstrated antibacterial activity against both Gram-positive and Gram-negative species, with a distinct SAR compared to their antimycobacterial action. In this case, disubstitution of this scaffold is not necessary, as many N^4-monosubstituted

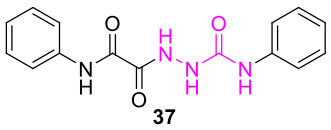

Fig. 34. Hydrazinecarboxamide-based ligand used for complexation with various metal ions.

derivatives have shown promising activity. Additionally, acylation at the N^4 position is not crucial for bacterial inhibition. The incorporation of a nitrogen into an *ortho*-condensed heterocycle can also be effective. For N^1 substitution, aryl groups are particularly advantageous. Regarding the substituents on aromatic systems, CF_3 group and electron-donating lipophilic substituents (such as alkyls and alkoxyls) are of particular interest for enhancing activity. The presence of a quinoline ring within the structure further contributes to antibacterial properties, however, PQ itself has not enhanced this activity.

3.3. Antifungal activity

In addition to antibacterial properties, bis-chromene hydrazinecarboxamide derivatives (Fig. 33; **34**) were able to inhibit *C. albicans* and *A. niger* with MIC of 75–200 μg/mL, too. Here, the methylation of the parent scaffold was advantageous [87].

Pyrazole-hydrazinecarboxamide hybrids (Fig. 27; **20**) also exhibited uniform antifungal potency against *A. niger* (inhibition zones of 10–24 mm at the concentration of 100 μg/mL; the most active was chloro derivative with MIC of 1 μg/mL) and *Microsporum audouinii* (10–22 mm, favouring hydroxy derivative, MIC = 0.5 μg/mL). Here, these activities were fully comparable to clotrimazole, an imidazole drug used as a standard. *C. albicans* was inhibited by four derivatives (MIC ≥64 μg/mL) and *Cryptococcus neoformans* only slightly by two derivatives with nitro analogue superiority (MIC of 64 μg/mL) [41].

In addition to antibacterial activity, nitropyrroles (Fig. 23; **14**) were identified also as potent inhibitors of *C. albicans*. They demonstrated uniform MIC values for *N*-methylpyrroles (1.56 μg/mL) and desmethyl analogues (3.12 μg/mL), regardless of the hydrazinecarboxamide substitution pattern. These values were identical to amphotericin B [72].

In addition, quinazolinyl-triazinyl hydrazinecarboxamides (Fig. 32; **31**) could also inhibit *C. albicans* and *Aspergillus clavatus*, showing MIC values of 12.5–100 μg/mL. *N*-Phenyl derivative was the most potent inhibitor of both *A. clavatus* (12.5 μg/mL; remaining analogues shared clearly higher MIC values of ≥50 μg/mL) and *C. albicans* (25 μg/mL), here together with 3-chlorophenyl compound, followed by cyclohexyl and 2-phenethyl derivatives [84].

Among three 2-[(2-hydroxyphenyl)(phenyl)methyl]hydrazine-1-carboxamides (Fig. 32; **33**), the presence of nitro group was beneficial against *C. albicans* (inhibition zone of 13 mm), followed by amino

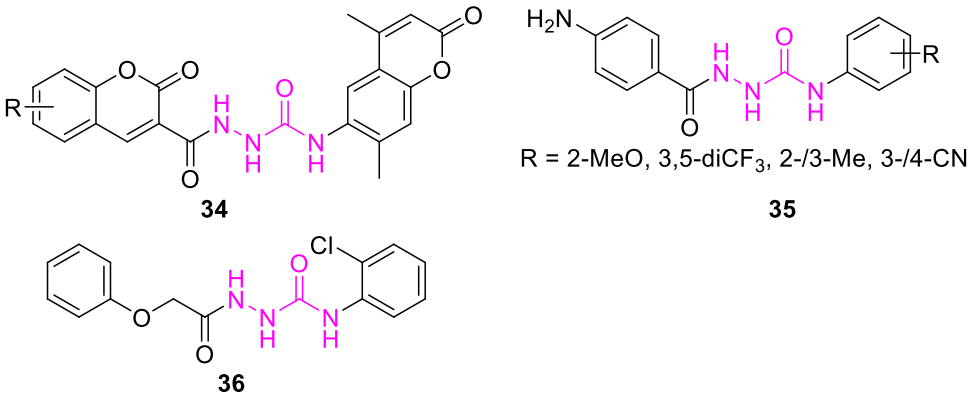

Fig. 33. Hydrazinecarboxamides of various structures with a mild antibacterial activity.

analogue (10 mm) and unsubstituted congener (7 mm). These results were comparable to clotrimazole (12 mm). In all cases, the concentration of the investigated compounds was 10 μg/mL [86].

The study of *p*-aminobenzoic acid-based hydrazinecarboxamide derivatives [88] (Fig. 33; **35**) also covered two *C. albicans* strains. All six molecules demonstrated some degree of antifungal activity with almost uniform IC_{50} values of 128–512 μg/mL against both collection and clinically isolated strains, mildly favouring 4-cyano derivative.

To address the rising challenge of antifungal resistance by a combination of benzofuran and hydrazinecarboxamide functionalities, novel hybrids were screened on six fungal strains: two fluconazole (FLU)-susceptible *C. albicans* strains, *A. fumigatus*, *Trichophyton rubrum*, *Candida krusei*, and *Candida parapsilosis*; the last strain showed a complete resistance to all compounds studied. In the first series (Fig. 35; **38**, n = 1), 2-methyl derivative showed notably lower MIC values compared to others, indicating higher and broad-spectrum potency (MIC of 16–64 μg/mL), followed by R = H, 4-Me, 4-EtO, and 2,3-diMe (Fig. 35; **38**, n = 1). Other substituents led to a narrower spectrum of activity. The shorter linker (Fig. 35; **38**, n = 0) was tolerated only for 4-Me and 4-EtO substituents, while for the remaining derivatives, it was disadvantageous. Then, a three-carbon linker was fixed and 4-fluorobenzoyl group was replaced with picolinoyl group (Fig. 35; **39**). This change significantly improved the activity, decreasing MIC to 2 μg/mL. Here, the most active was unsubstituted analogue (2–32 μg/mL), closely followed by 4-Me and 4-EtO derivatives, that were superior or comparable to FLU for one strain of *C. albicans*, *A. fumigatus*, *T. rubrum*, and *C. krusei*. To simplify the structure of benzofuran, cut off the furan ring of the benzofuran structure, and connect two hydrophobic fragments *via* antimicrobial resorcinol, corresponding 1,3-dialkoxybenzene hybrids were synthesized, preserving a three-carbon linker (Fig. 35; **40**). This series showed notable antifungal activities, generally improving upon the activity profiles seen in the benzofuran series **38** and **39**. This suggests the simplified core structure may improve interaction with fungal targets or enhance cellular uptake. The presence of a second fluorine in benzoyl portion of the molecule often enhanced the antifungal activity. In *N*-phenyl group, hydrogen, 2-/4-Me, and 2,5-diMe groups tended to show better activity than those with more substantial electron-withdrawing groups. In this group, MIC started from 0.5 μg/mL and they were also active against *C. parapsilosis*. In contrast to benzofurans, these antifungal agents were predominantly superior or fully comparable to FLU against *A. fumigatus*, *T. rubrum*, *C. krusei*, and *C. parapsilosis*. For *C. albicans*, the most active derivatives exhibited analogous MIC values against one of the two strains. Then, five of the most active agents from picolinoyl (**39**) and resorcinol (**40**) groups were investigated against two strains of FLU-resistant *C. albicans* isolated from patients suffering from AIDS. The compounds preserved mainly excellent antifungal activity when compared to susceptible strain of 2–32 μg/mL (MIC of FLU ≥128 μg/mL), thus indicating no cross-resistance. Docking analysis of 2-{3-[(3-methyl-2-picolinoylbenzofuran-4-yl)oxy]propyl}-*N*-(*p*-tolyl)hydrazine-1-carboxamide (Fig. 35; **39**, R = 4-Me) with *N*-myristoyltransferase from *C. albicans* suggested its binding mode. The benzofuran ring located at the centre of the active site was surrounded by hydrophobic residues (Tyr225, Tyr354, and Leu394) and formed π-π interaction with Tyr225. The pyridine ring formed hydrophobic interactions with Phe115, Phe240, and Phe339. The phenyl hydrazinecarboxamide moiety stretched into the hydrophobic pocket constituted by Phe117, Tyr119, and Phe176 and the oxygen of the benzofuran formed a hydrogen bond with His227 [96].

On the other hand, triazine derivatives also bearing hydrazinecarboxamide linker (Fig. 36; **41**) avoided any antifungal action alone or in combination with FLU (MIC >64 μg/mL) against FLU-resistant *C. albicans* strains. Notably, isosteric carbothioamides were active [97].

Similarly, four 2-(2-amino-4-methylthiazole-5-carbonyl)hydrazine-1-carboxamide derivatives (Fig. 31; **28**) lacked activity against *C. albicans* [81]. Analogous results were found for hydrazinecarboxamide-PQ hybrids (MIC >100 μg/mL, while MIC of PQ = 350 μg/mL) [90].

Fifteen *N*-benzoyl-*N'*-[5-(2′-substituted phenyl)-2-furoyl] semicarbazides (Fig. 37; **42**) were designed to combat, e.g., phytopathogenic fungi. In contrast to their insecticidal activity, they had lower fungicidal activity against important agricultural fungi (*Phytophthora capsicii*, *Botrytis cinerea*, *Fusarium oxysporum*, *Rhizoctonia solanii*, and *Corynespora cassiicola*). At a single concentration of 50 μg/mL, inhibition rates ranged from 0 % to 50 %, here being less effective than various commercial fungicides. For *P. capsicii* and *B. cinerea*, the optimal substitution was R^1 = 2,6-Cl_2, R^2 = 4-CH_3 (49 % inhibition), while for *F. oxysporum*, *R. solanii*, and *C. cassiicola* the optimal patterns were R^1 = 2,6-F_2, R^2 = 4-F (37.5 %), R^1 = 2,6-F_2, R^2 = 2,4-F_2 (50 %) and R^1 = 2,6-

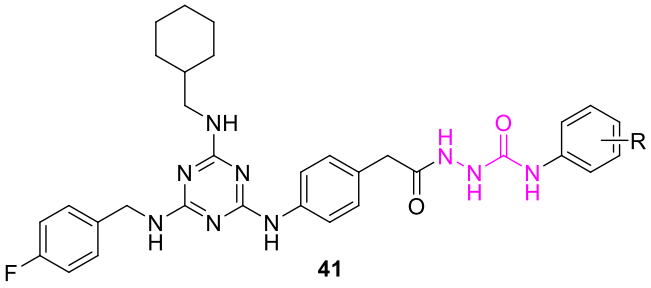

Fig. 36. Triazine-hydrazinecarboxamide derivatives with low antifungal activity.

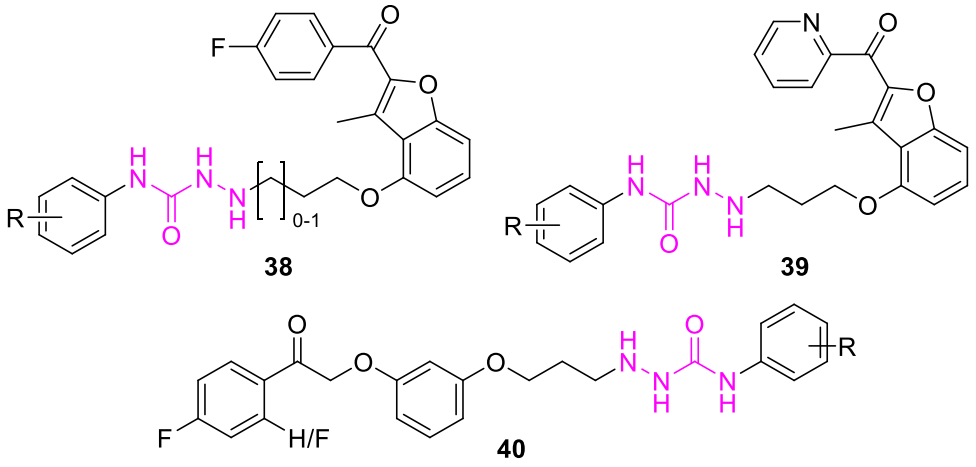

Fig. 35. Hydrazinecarboxamide-benzofuran hybrids (**38** and **39**) and their acyclic analogues **40**.

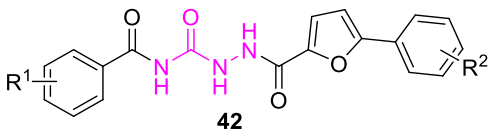

Fig. 37. Diacylhydrazinecarboxamides with activity against phytopathogenic fungi.

Cl_2, R^2 = 2,4-F_2 (20 % inhibition) [98].

In summary, hydrazinecarboxamides have demonstrated antifungal properties, although to a lesser extent than their antibacterial and antimycobacterial activities. Limited number of active analogues restricts the scope of SAR analysis for hydrazinecarboxamides. Nevertheless, their antifungal activity of hydrazinecarboxamides is enhanced when conjugated with nitrogen-containing heterocycles (such as pyrrole, pyrazole, and triazine) or oxygen-containing heterocycles like (benzo)furan. Hydrazinecarboxamides can be either mono- or disubstituted, and acylation is not essential for activity. For the N^1 position, substitution with aryl is particularly beneficial. Small alkyls, NO_2 group, and fluorine atoms have also contributed to increased antifungal activity. The most promising derivatives (**38**-**40**) features a unique structural motif: an arylated and methylated benzofuran (or its open analogue) linked *via* a shorter oxyalkyl chain to *N*-phenyl hydrazinecarboxamide.

3.4. Antiviral activity

Quinazoline-triazine hybrids (Fig. 32; **31**) were also screened against HIV-1 (IIIB) and HIV-2 (ROD) cell cultures. The assay assesses the ability of the compounds to inhibit viral-induced cytopathic effects in MT-4 cells. However, most compounds from this series exhibited high IC_{50} values, indicating a lower antiviral potency. Three compounds (R = phenyl, 3-Cl-phenyl, and allyl) demonstrated relatively lower IC_{50} values for ROD strain (33.65, 36.45, and 51.98 μg/mL, respectively). However, the activity is associated with no selectivity [84].

PQ-cinnamic acid mutual derivatives linked *via* hydrazinecarboxamide bond (Fig. 38; **43**) were evaluated against a range of viruses, including herpes simplex virus (HSV) type 1 (KOS), HSV type 2 (G), thymidine kinase-deficient HSV-1, vaccinia virus, adenovirus 2, and human coronavirus (229E) in HEL cell cultures. Five compounds exhibited moderate activity against coronavirus with EC_{50} values of 8.4–15.0 μM. Notably, the three most active compounds contained a trifluoromethyl group, which appeared to enhance their antiviral properties. These compounds did not alter the normal cell morphology at a concentration of 100 μM, indicating a sufficient selectivity. Notably, replacing acylsemicarbazide linker with a simple amide bond led to a completely abolished activity [99].

2-Isonicotinoyl-*N*-phenylhydrazine-1-carboxamide (Fig. 38; **44**) was investigated as a potential agent to combat dengue virus [100], while *N*-(3-chlorophenyl)-2-[2-(trifluoromethyl)quinolin-4-yl]hydrazine-1-carboxamide (Fig. 38; **45**) was evaluated against Coxsackie virus [101]. On the other hand, 2-(1*H*-benzo[*d*][1,2,3]triazole-1-carbonyl)hydrazine-1-carboxamides (Fig. 38; **46**) and *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}hydrazine-1,2-dicarboxamides (Fig. 38; **47**) avoided any activity against a wide variety of viruses, including HSV-1 and HSV-2, vaccinia virus, and vesicular stomatitis virus in HEL cell cultures, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures, vesicular stomatitis virus, Coxsackie B4 virus, and respiratory syncytial virus in HeLa cell cultures, feline coronavirus and feline herpes virus in CRFK cell cultures, influenza A (H1N1 and H3N2) and B in MDCK cell cultures and HIV-1 (IIIB) and HIV-2 (ROD) in CEM cell cultures [102]. Also, hydrazinecarboxamides derived from nonsteroidal anti-inflammatory drugs ibuprofen, ketoprofen, and fenoprofen were inactive against a panel of DNA and RNA viruses [103].

Hydrazinecarboxamides have demonstrated some potential in antiviral applications, but their efficacy appears limited based on the available data, as several derivatives were either inactive or non-selective. Even the incorporation of another bioactive scaffold did not consistently produce an active compound, as shown with the contrasting activity of PQ derivatives, such as the active derivatives (**43**) and the inactive compound (**47**). Given these inconsistencies, hydrazinecarboxamides are currently not strong candidates for broad-spectrum antiviral drug development without extensive further modifications. Consequently, it is not possible to provide a comprehensive SAR for their antiviral activity due to the limited and inconsistent data.

3.5. Antiprotozoal activity

The study of Kedzierska et al. [104] aimed to evaluate three new primaquine-hydrazinecarboxamide derivatives (Fig. 39; **48**) for their antimalarial activity *in vitro* against the erythrocytic stages of *Plasmodium falciparum* (drug-susceptible strain) *in vitro*. The results demonstrated varying degrees of antimalarial effectiveness among the tested compounds with IC_{50} values of 3.7–41.4 μM and SI values of 1–20, favouring bis-primaquine derivative. Cytotoxicity was evaluated in L6 rat skeletal myoblasts cell line. Nevertheless, this activity is beyond chloroquine used for comparison.

Pavic et al. [70] published a comprehensive overview of the antimalarial activity for *Plasmodium berghei* of a range of PQ-hydrazinecarboxamides and bis-urea hybrids (Fig. 21). Some of these compounds exhibited nanomolar IC_{50} values.

Hydrazinecarboxamides coupled with methoxylated arylnicotinic acids (Fig. 39; **49**) were investigated for their potential as anti-leishmanial agents by targeting the folate metabolic pathway by inhibiting pteridine reductase 1. They were tested for their anti-promastigote action against *Leishmania major* with IC_{50} values in a narrow concentration range of 18.6–23.9 μM, favouring unsubstituted analogue. Isosteric thiosemicarbazides were more potent, so advanced studies were

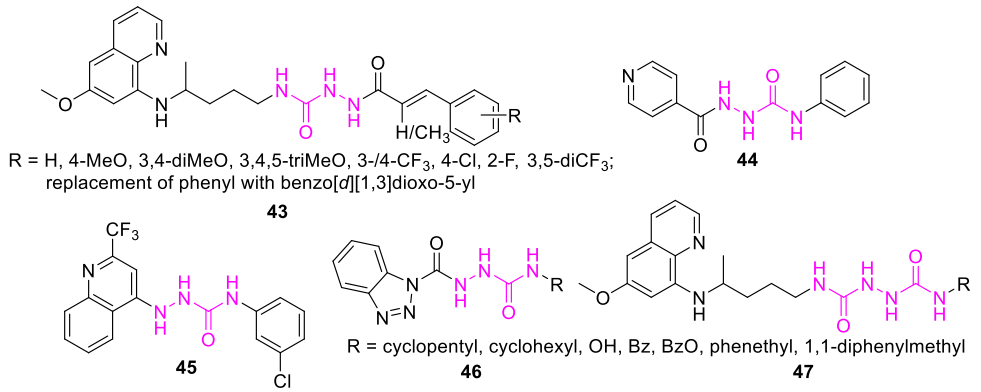

Fig. 38. Hydrazinecarboxamides investigated as potential antiviral agents.

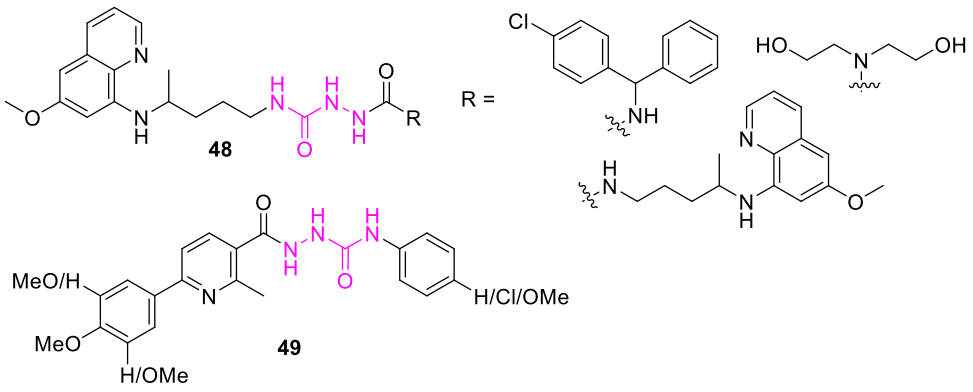

Fig. 39. Antiprotozoal hydrazinecarboxamides.

performed on this group of derivatives [105].

Hydrazinecarboxamides have shown potential in antiprotozoal applications for the treatment of diseases caused by *Plasmodium* and *Leishmania* species. However, the activity of the hydrazinecarboxamides presented here can be attributed primarily to the parent scaffolds (PQ, arylnicotinic acids), with the hydrazinecarboxamide moiety merely contributing to their modulation.

3.6. Other types of activity related to infectious diseases: insecticidal and immunomodulating action

N-Benzoyl-2-(5-phenylfuran-2-carbonyl)hydrazine-1-carboxamides (Fig. 37; **42**) were, i.a., designed to combat insects. Their *in vivo* activity was confirmed at the concentration of 500 μg/mL, especially towards diamondback moth (*Plutella xylostella*) with the superiority of compounds, where R^1 = 3-CH_3, R^2 = 2-Cl and R^1 = 2-OCH_3, R^2 = 4-Cl (95 % and 85 % response rate, respectively), along with a lower suppression of cotton aphid (*Aphis gossypii*) of 38 % produced by R^1 = 2,6-Cl_2, R^2 = 2,4-F, and no effect on red spider mite (*Tetranchus urticae*). Except for the last-mentioned species, the activity was comparable to the commercial pesticides (RH-5849, hexaflumuron) [98].

N-(5-Substituted 1,3,4-thiadiazol-2-yl)hydrazinecarboxamides (Fig. 40; **50**) were investigated for their immunomodulatory activity. Two compounds **50** (n = 0, R = H, MeO) share immunostimulant activity. At the same time, the remaining derivatives produced immunosuppressive responses expressed as changes in IgM and IgG levels, total mesenteric lymph nodes lymphocyte count, and histopathological examination of liver sections [106].

4. Conclusions

This manuscript comprehensively evaluates biological activities of hydrazinecarboxamides (semicarbazides), revealing significant antimicrobial properties. This scaffold has demonstrated potent activity against various bacterial, mycobacterial, and fungal strains, as well as viruses and protozoa, indicating its potential to serve as a versatile cornerstone for antimicrobial agents.

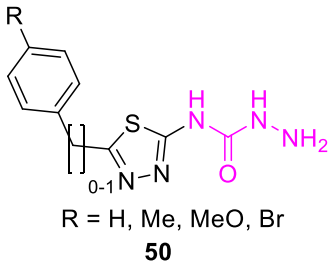

Fig. 40. Hydrazinecarboxamide-thiadiazole hybrids with proposed immunomodulatory activity.

The hydrazinecarboxamide group itself plays a crucial role in enhancing the biological activity of the compounds. This moiety can facilitate strong interactions with biological targets, thereby improving the potency and selectivity of the derivatives. Its inclusion in the molecular structure enables hydrogen bonds formation and other interactions essential for target binding and, thus, final bioactivity. Consequently, hydrazinecarboxamides have been established as a valuable scaffold in the design of new potential drugs. This moiety plays three distinct roles. For some compounds, its presence is absolutely essential for activity. In others, it is advantageous by enhancing activity compared to other (bio)isosteric or similar functional groups. In the last group of compounds, it is studied as one of many substituents to investigate chemical space, serving for modification of another fundamental biologically active scaffold. Here, in some cases, its presence may not necessarily lead to effective derivatives and it is not considered an "automatic" pharmacophore. On the contrary, hybridization with other bioactive compounds into a single molecular entity can be a highly advantageous approach.

Despite their promising results, hydrazinecarboxamides have exhibited several limitations. Some derivatives suffer from poor physicochemical properties, which can hinder their bioavailability and therapeutic efficacy. Additionally, the potential cytotoxicity of these compounds, especially at higher concentrations, raises concerns about their safety profiles. Because some compounds are multitargeting, this can complicate their development as selective drugs. In addition, only a limited number of papers report the results from *in vivo* studies.

Future research should focus on optimizing the structures to identify SAR in more details, on elucidation the exact MoA and on *in vivo* evaluation. The involvement of computational approaches will aid in the rational design of advanced derivatives. Additionally, novel synthetic methods for their selective and highly efficient preparation are of special interest, as hydrazinecarboxamides have become a versatile and tuneable building block. Future research directions will focus on the design of hybrid molecules that combine the hydrazinecarboxamide moiety with other well-established bioactive scaffolds known for their antimicrobial properties, particularly to combat drug-resistant strains. This includes approved drugs such as isoniazid and quinolines (e.g., primaquine, hydroxyquinoline), whose mutual derivatives have demonstrated synergistic effects in early *in vitro* studies. Additionally, heterocyclic scaffolds, well-known for their specific antimicrobial actions, will be utilized, particularly nitrogenous heterocycles, or adamantane for its antimycobacterial activity. Furthermore, the introduction of specific substituents will be explored. Alkyl, alkoxyl, halogen (F, Cl), and trifluoromethyl groups are of particular interest for enhancing antimicrobial efficacy. Under certain conditions, especially against mycobacteria, nitro group may also be considered. The incorporation of lipophilic substituents will aim to enhance biological barriers penetration, thereby improving the compounds' ability to cross biological barriers and reach

intracellular targets. Azapeptides and their conjugates with other antimicrobial agents will also be studied.

CRediT authorship contribution statement

Martin Krátký: Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Neto-Honorius Houngbedji:** Writing – original draft, Investigation. **Jarmila Vinšová:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 4 in order to improve the readability, style, and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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