UNIVERSITY OF MEDICINE AND PHARMACY
„CAROL DAVILA”, BUCHAREST
DOCTORAL SCHOOL
PHARMACY FIELD

Complex study of molecular development and testing of Schiff bases with biological potential

ABSTRACT OF THE DOCTORAL THESIS

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I. State of the art

1. Schiff bases– compounds of interest for medicinal chemistry

Schiff bases belong to the class of functional derivatives of the carbonyl group, obtained by substituting the C=O group under the action of basic nucleophiles (ammonia and its derivatives) [1–3]. In medicinal chemistry, Schiff base-type agents are found among chemotherapeutic agents and antibiotics [4].

One economic strategy for the design and development of new therapeutic agents involves modifying existing agents to enhance their therapeutic effect and/or induce new biological actions [5] and molecular hybridization, which involves the binding of two or more pharmacophores into a single molecule for the purpose of enhancing pharmacological actions [6, 7] or the reduction of secondary reactions [8, 9].

In this context, the coupling of bacteriostatic sulphonamides with various aromatic aldehydes has led to obtaining Schiff bases with a complex biologic potential: antibacterial effect on Gram-positive cocci [5, 10–13], antifungal effect [5, 10–14], antimycobacterial activity [5] and antibiofilm potential [14], the introduction of halogen groups significantly improving the derivatives' actions [5, 10–13]. They also demonstrated anticancer effects on various cell lines, both as ligands [15], and as in metal complexes [16–18], as well as an inhibitor effect of certain enzymes with biologic importance, the most important of which being carbonic anhydrase (CA) (the isoforms overexpressed in cancer, CA IX, CA XII) [15, 19, 20].

Moreover, the scientific literature mentions hybrid molecules obtained by combining the quinazolin-4(3*H*)-one ring, a pharmacophore of significance among natural and synthetic compounds [21, 22], with various substituted aromatic rings, using the *N*-acylhydrazone linker. The resulting Schiff bases exhibited antibacterial and anti-biofilm activity [23], antimycobacterial [24], and, especially, anti-cancerous effect [25–28]. In particular, based on the procaspase-activator, PAC-1, a series of (*E*)-*N'*-benzylidene-2-(4-oxoquinazolin-3(4*H*)-yl)acetohydrazides were obtained, providing superior anti-cancerous properties, the substitution of the benzene core with 2-hydroxy-4methoxy and 2-hydroxy-3-allyl groups being the most beneficial for activity [25, 27, 28].

2. Microwave assisted organic synthesis

The synthetic protocols for obtaining this class of compounds include both conventional methods and modern, environmentally friendly methods, such as microwave-assisted synthesis [29]. The latter offers several advantages: rapid and efficient dielectric heating, accelerated reaction rates, higher yields, reduced side reactions indicating a cleaner kinetic profile, straightforward monitoring, and solvent-free operation for green synthesis. [30–32]. The interest of this particular type of synthesis comes from the context of international policies, which advocate for green chemistry, with a reduced impact on the environment [33].

II. Personal contributions

3. Working hypothesis and general objectives

Taking all of the above into account, this doctoral project aims to explore the potential of bacteriostatic sulfonamides, well-known drugs with antibacterial activity, as well as 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide, a pharmacophore with antimicrobial and anticancer potential, in the form of Schiff bases, with the aim of obtaining multifunctional compounds with improved pharmacological effects.

The main objective of the research is to design molecular compounds through a comprehensive review of specialized literature and databases, and to develop and optimize an original microwave-assisted synthesis method to obtain novel Schiff bases. The specific objectives include comprehensive physicochemical and spectral characterization, *in silico* analysis of the drug-like profile and molecular descriptors, *in vitro* testing to evaluate antimicrobial, anti-biofilm, anti-cancer, and antioxidant activities, *in vitro* acute toxicity testing on crustaceans, and conducting molecular docking studies on various relevant enzymes related to the targeted biological effects.

3. General research methodology

Microwave-assisted synthesis was performed using the Biotage® Initiator Classic 2.0 reactor. Physicochemical characterization included the determination of melting points, the solubility in organic solvents, the retention factors, and the purity by high-performance thin-

layer chromatography (HPTLC). The structures of the compounds were confirmed by nuclear magnetic resonance spectroscopy, high-resolution mass spectrometry, and infrared and ultraviolet-visible spectroscopy.

The antioxidant activity was determined by the capacity of the compounds to reduce the 1,1-diphenyl-2-picrylhydrayl radical. The antimicrobial and anti-biofilm activity were evaluated on representative pathogens: Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*), Candida species (*Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida auris*), and two strains of *Mycobacterium tuberculosis*. The cytotoxic potential was evaluated on standardized cellular lines, cancerous (HT-29, LN229, A431) and non-cancerous (VERO). The pharmacologic profile was evaluated with the help of multiple online prediction programs (SwissADME [34], pkCSM [35], admetSAR2.0 [36], ADMETlab [37], SuperPred database [38], SwissTargetPrediction [39], Super-PRED [38] and PASS Online [40]), and the evaluation of the molecular descriptors and the docking studies was performed using dedicated programmes (Spartan'20 [41], CLC Drug Discovery Workbench 2.4 (2015) [42], Molegro Virtual Docker (2019) [43]). The crustacean species *Artemia franciscana* Kellogg was used to assess acute *in vivo* toxicity at 24 and 48 hours. The stability of the compounds in water was evaluated using the ultrasonic method [44, 45].

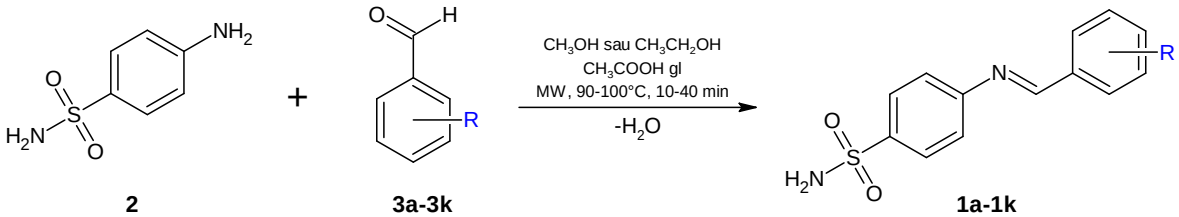
5. Contributions to the synthesis, spectral characterization, *in silico* study and biological evaluation of sulfanilamide Schiff bases

The first part of the doctoral research involved the development of a microwave-assisted synthesis method for the preparation of Schiff base derivatives starting from sulfanilamide and various substituted, halogenated, and non-halogenated benzaldehydes [46] and salicylaldehydes. The study aimed to synthesize novel compounds and to apply the newly developed microwave-assisted synthetic procedure to obtain known derivatives, for which a biological effect was highlighted.

All compounds were confirmed by spectroscopic methods and characterized physicochemically. An *in silico* study was performed to determine the molecular characteristics and the pharmacological profile. The biologic potential was evaluated through: (i) the determination of the antioxidant capacity, (ii) the evaluation of the antimicrobial and anti-biofilm activity on Gram-positive and Gram-negative bacteria, and

on the *Candida* pathogen species, (iii) the screening of the cytotoxicity on the cancerous cellular lines. For the most active derivatives, the stability of suspensions in water was evaluated *via* the ultrasound method. The molecular docking study aimed to correlate observed biological effects with potential activity mechanisms. The research involved a preliminary study of acute *in vivo* toxicity on the crustacean species *Artemia franciscana* Kellogg.

Among the obtained eleven derivatives (**1a-1k**), six compounds are novel (**1a, 1c-1g**) (Scheme 1). The synthetic procedure started from the classic reaction conditions: absolute methanol or ethanol as reaction medium, glacial acetic acid as catalyst, operating at 90-100 °C for 10-40 minutes under microwave irradiation in a closed system.



2 (sulfanilamidă) **3a-3k** **1a-1k**

*Scheme 1. Synthesis of sulfanilamide Schiff **1a-1k**. R is: 4-OPh (**a**), 2-Br (**b**), 2,6-diCl (**c**), 3,5-diCl (**d**), 2,3,5-triCl (**e**), 2-Cl-4-NO_2 (**f**), 3,5-diBr (**g**), 2-OH (**h**) ,2-OH-4-OCH_3 (**i**), 2-OH-5-Br (**j**), H (**k**).*

For the compounds mentioned in the literature (**1h–1k**), the yields obtained using the original protocol were comparable to those reported [20, 47], but the advantage of a shorter reaction time remains, from over 3–4 hours in the conventional methods to 10–40 minutes in the present method. The synthesis method presented in the doctoral thesis for compound **1b** offers the advantage of replacing the toxic reaction medium (THF) [48] using a solvent with a high safety profile (ethanol).

The *in silico* analysis of the pharmacological profile has indicated that Schiff bases **1a-1k** exhibit a drug-like character, high gastrointestinal absorption, but possibly hepatotoxic and nephrotoxic effects. Among the biological targets, the enzymes specific to sulphonamide-type molecules stand out: carbonic anhydrase, cyclooxygenase-2 (COX-2), and the amyloid-binding protein. The calculation of the molecular descriptors allowed the observation of the effects of substitution of the benzene ring on the electronegativity and reactivity of the Schiff bases, the substitution with electron-withdrawing groups (halogen atoms and nitro groups) raising the electronegativity, while the electron-donating groups (hydroxy, methoxy, phenoxy) had the opposite effects. In addition, it allowed the identification of structural elements predisposed to chemical attacks, the azomethine group acting as a substrate for nucleophilic attacks.

The maximum total antioxidant capacity was observed for the phenoxy derivative **1a**, while the lowest was for the trichloro derivative **1e**. The biological evaluation results indicate selectivity toward Gram-positive bacteria, with (*E*)-4-{[(2-bromophenyl)methyldene]amino}benzen-1-sulfonamide (**1b**) and (*E*)-4-{[(3,5-dichlorophenyl)methyldene]amino}benzen-1-sulfonamide (**1d**) showing anti-staphylococcal activity against both planktonic and sessile forms (MIC 0,156 mg/mL). Regarding antifungal activity, the mono- and dihalogenated derivatives **1b-1d** stand out against *C. parapsilosis* (MIC 0,7813-0,3906 mg/mL). For anticancer activity, compounds **1c** and **1d** significantly reduced cellular viability at 5 μg/mL after 24 hours of exposure (**1d** – HT-29, **1c** – LN229), exceeding the effect of sulphanilamide.

The acute *in vivo* toxicity on *Artemia franciscana* Kellogg varies depending on the substituents present in the molecular structure. Substitution with the *para*-phenoxy group and two *meta*-bromine atoms relative to the azomethine group results in the most toxic compounds, **1a** and **1g** (lethal concentration 50%, LC_{50} < 62,5 μM), followed by the 3,5-dichloroderivative **1d** and the 2,3,5-trichloroderivative **1e**, with LC_{50} values of 145 μM and 566 μM, respectively. Substitution at positions 2 and 6 with chlorine (**1c**) or at positions 2 and 4 with chlorine and a nitro group (**1f**) produces compounds with reduced toxicity, with LC_{50} values exceeding 1000 μM.

The docking study on dihydropteroate synthase showed a preference for the binding of Schiff bases **1a-1e** to the enzyme in *S. aureus* compared to the one from *E. coli*. Besides the sulfonamide group, the main structural element involved in interactions with the amino acids of the binding site, the nature and position of substituents on the second benzene ring influenced the interaction with the enzyme. The azomethine group can also contribute to hydrogen bonding with amino acids in the active site (Fig. 1).

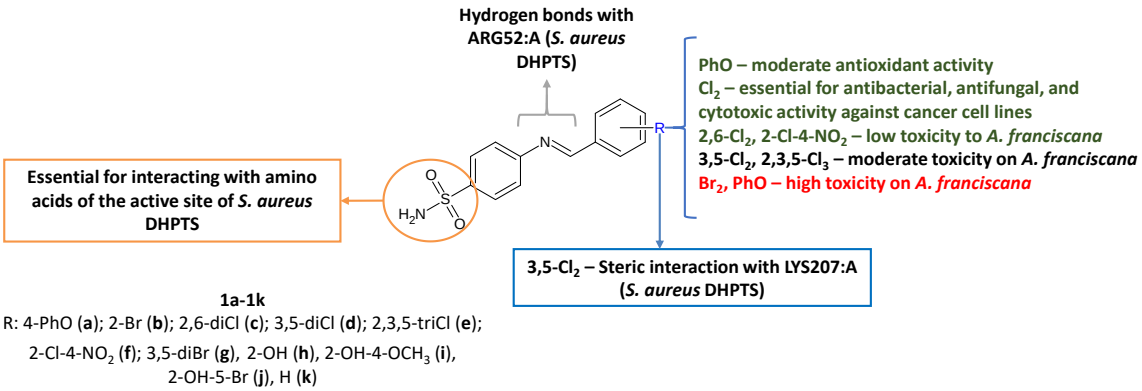


*Figure 1. Chemical structure–biological activity relationships for sulfanilamide Schiff bases **1a–1k**.*

The stability of the suspensions of the most active Schiff bases (**1b, 1d, 1e**) was determined using the ultrasound method. They showed high stability, comparable to that of the sulfanilamide used as a reference; the compounds substituted with chlorine atoms (**1d, 1e**) were more stable than the bromine derivative (**1b**), which supports their incorporation into nanomaterials.

Based on this information, the series of sulfanilamide Schiff bases constitutes a starting point for further development and optimization, and for aiming research towards an unexplored branch in this study, which is the obtaining of metal complexes and their biological testing, as well as obtaining functionalized materials for antimicrobial treatment.

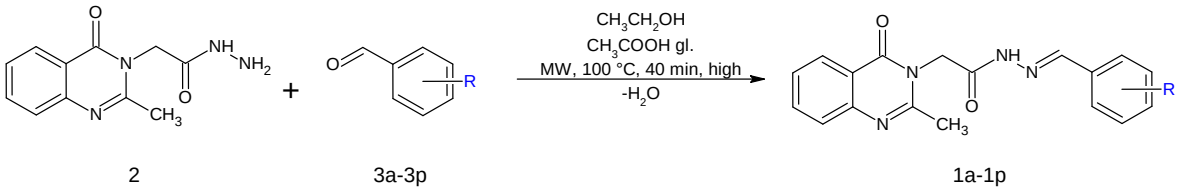
6. Contributions to the study of the molecular development and biological evaluation of *N*-acyl-hydrazone Schiff bases of 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide

The second study within the doctoral research aimed to design *N*-acyl-hydrazone Schiff bases starting from 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide. The molecular design rationale was based on a review of the literature and considered the following aspects: (i) maintaining the (*E*)-*N*′-benzylidene-2-(4-oxoquinazolin-3(4*H*)-yl)acetohydrazide pharmacophore; (ii) keeping a small-volume lipophilic group (methyl) at position 2 of the quinazolinone ring; (iii) varying the substituents on the benzene ring to adjust the biological profile [49].

The new compounds were synthesized using the microwave-assisted method previously developed for Schiff bases, with adaptations. Spectral and physicochemical analyses, complemented by high-performance thin-layer chromatography, confirmed purity. *In silico* assessment of molecular descriptors and pharmacological profiles was then performed. Subsequent testing evaluated antibacterial activity against Gram-positive and Gram-negative bacteria, *M. tuberculosis*, antifungal activity against *Candida* species, and cytotoxicity against tumor and non-tumor cell lines. Antioxidant capacity was included due to the presence of the hydroxyphenylimino group, which can impart redox properties [50]. To investigate a potential mechanism of action, a molecular docking study was conducted on biological targets relevant to the actions under investigation. Finally, the research

concluded with an *in vivo* acute toxicity assessment using the crustacean species *Artemia franciscana* Kellogg.

The synthesis was carried out using microwave irradiation, with absolute ethanol as the solvent and glacial acetic acid as the catalyst; the mixture was heated to 100 °C for 40 minutes, preceded by a 5-minute pre-stirring, with the absorption level set to "high." This yielded a series of 16 original derivatives (**1a–1p**) (Scheme 2).



*Schema 2. General synthetic procedure for the preparation of N-acylhydrazones **1a–1p**. R represents: 4-F (**a**); 4-Br (**b**); 2-Br (**c**); 2,4-diCl (**d**); 2,6-diCl (**e**); 3,5-diCl (**f**); 2,3,5-triCl (**g**); 2-Cl-4-NO_2 (**h**); 2-OH-5-Br (**i**); 2-OH-3,5-diBr (**j**); 2-OH-3-OCH_3 (**k**); 2-OH-4-OCH_3 (**l**); 2-OH-5-OCH_3 (**m**); 3,4-diOCH_3 (**n**); 3-OCH_3-4-OH-5-NO_2 (**o**); 4-OPh (**p**).*

Nuclear magnetic resonance analysis revealed the presence of isomers due to the *N*-acyl-hydrazone group. The ^{1}H-NMR spectra recorded in DMSO-d6 for the analyzed compounds show a single signal at δ_H 8 ppm, corresponding to the azomethine group, characteristic of the *E* isomer. At the same time, the signals for the azomethine group (δ_H 8.0–8.6 ppm, δ_C 141.9–147.5 ppm), the methylene group (δ_H 4.8–5.3 ppm, δ_C 45.2–46.2 ppm), methyl (δ_H 2.50–2.55 ppm, δ_C 22.8–23.5 ppm), and the amide group's N_H (δ_H 11.6–12.0 ppm) highlighted the presence of the two conformational isomers, *syn-E* and *anti-E*. HPTLC analysis confirmed the purity of the obtained compounds by the presence of a single chromatographic spot, and the developing system (chloroform:methanol 9:1 v/v) allowed the separation of salicylaldehyde derivatives from polyhalogenated ones, with a few exceptions (**1a**, **1h**, **1f**, **1k**).

In silico analysis of the pharmacodynamic profile confirmed activities in oncology and infectious diseases, and highlighted potential applications in the treatment of metabolic and neurodegenerative diseases.

Regarding biological evaluations, *N′*-[(*E*)-(3,5-bromo-2-hydroxyphenyl)-methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)aceto-hydrazide (**1j**) exhibited both qualitative and quantitative activity against *S. aureus* (DIZ 12.33 ± 0.58 mm; MIC 0.3125 mg/mL; IC50 0.0857 mg/mL), underscoring the importance of halogen substitution on the salicylaldehyde nucleus.

Furthermore, derivatives **1b**, **1c**, **1k**, **1l**, and **1m** exhibited high antitubercular activity at a concentration of 1–2 mg/mL against both rifampicin- and isoniazid-sensitive and -resistant strains of *M. tuberculosis*.

Following cytotoxicity analysis, three of the halogenated derivatives stood out for their potency and selectivity against tumor cell lines. *N'*-[(*E*)-(4-bromophenyl)methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**1b**) exhibited potency against both cell lines (IC_{50} 3.125–12 μM), while *N'*-[(*E*)-(4-fluorophenyl)methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**1a**) and *N'*-[(*E*)-(2,4-dichlorophenyl)-methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**1d**) exhibited activity specifically against A431 (IC_{50} 6.25–12 μM). Furthermore, the antioxidant capacity of the derivatives containing nitro (**1h**, **1o**), hydroxy (**1i**, **1k**, **1j**, **1o**), and phenoxy (**1p**) groups was twice that of 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide. At the same time, the acute *in vitro* toxicity of the derivatives on the crustacean species *Artemia franciscana* Kellogg was lower than that of the starting material (Fig. 2), confirming the benefit of derivatization in the form of Schiff bases.

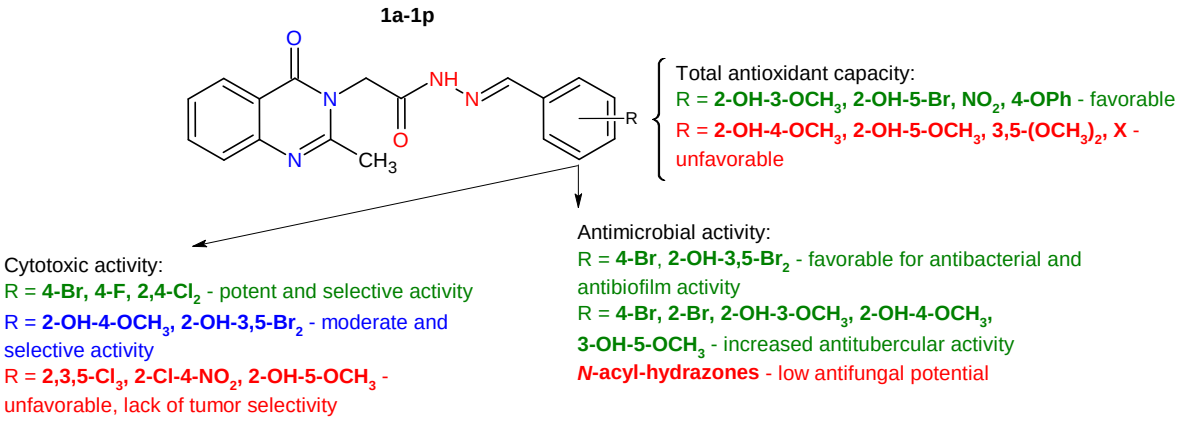


*Figure 2. Correlation between the chemical structure and the various biological properties observed in the series of N-acylhydrazones **1a–1p**. R represents: 4-F (**a**); 4-Br (**b**); 2-Br (**c**); 2,4-diCl (**d**); 2,6-diCl (**e**); 3,5-diCl (**f**); 2,3,5-triCl (**g**); 2-Cl-4-NO_2 (**h**); 2-OH-5-Br (**i**); 2-OH-3,5-diBr (**j**); 2-OH-3-OCH_3 (**k**); 2-OH-4-OCH_3 (**l**); 2-OH-5-OCH_3 (**m**); 3,4-diOCH_3 (**n**); 3-OCH_3-4-OH-5-NO_2 (**o**); 4-OPh (**p**).*

In line with the *in vivo* results, the molecular docking study supports the observed effects. Thus, the binding mode of derivative **1j** at the active site of *S. aureus* gyrase B is consistent with the orientations inferred for other quinazolone-type inhibitors. With regard to EGFR kinase, the substitution on the phenyl group influences the orientation within the binding pocket. In the case of the 2,3- and 2,4-disubstituted derivatives (**1k** and **1l**), the quinazolinone ring occupies the adenine pocket, while the phenyl group is located in the K pocket. Regarding the enoyl reductase in *M. tuberculosis*, with the exception of derivatives

1m–1p, all *N*-acylhydrazones orient themselves in the binding pocket in a manner similar to the co-crystallized ligand.

The pharmacological potential of the new derivatives must be complemented by in-depth studies of the mechanism of action and antitubercular activity. At the same time, the compounds lend themselves to further optimization, aiming to derivatize the quinazolinone ring and generate Schiff bases with a broader range of carbonyl compounds.

7. Conclusions and personal contributions

The main objective of this doctoral thesis was to develop an original microwave-assisted synthesis method for the preparation of Schiff bases starting from sulfanilamide, specifically 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide. This goal was achieved by adapting conventional synthetic protocols and optimizing the method. As a result, 27 derivatives were obtained, 22 of which were original, with molecular designs conceived based on a review of the specialized literature.

The main advantage of microwave-assisted synthesis was the significant reduction in reaction time, from at least 3–4 hours, as is typical for conventional methods, to 10–40 minutes. At the same time, the process is carried out in a closed system, which reduces exposure to solvent vapors, and reaching high temperatures (90–100 °C) allows for a cleaner kinetic profile, with products obtained at high purity, particularly in the *N*-acyl-hydrazone series. A disadvantage is the limited yield, as the process is carried out in 2–5 mL reaction vials. Reaction vials with a maximum capacity of 10–20 mL may be used for optimized processes.

The specific objectives focused on the physicochemical and spectral characterization of the obtained compounds, achieved through a combination of specific techniques, as well as the investigation of the biological profile and acute *in vivo* toxicity. In the latter case, differences were observed between the actions of the derivatives and those of the raw materials from which the series of Schiff bases was obtained.

In the case of sulfanilamide derivatives, (*E*)-4-{[(2-bromophenyl)methylidene]amino}benzene-1-sulfonamide (**1b**) and (*E*)-4-{[(3,5-dichlorophenyl)methylidene]amino}benzene-1-sulfonamide (**1d**) exhibited superior antistaphylococcal and antibiofilm activity compared to sulfanilamide (MIC 0.014 mg/mL and 0.019 mg/mL, compared to 0.39 mg/mL), while (*E*)-4-{[(2,3,5-

trichlorophenyl)methylidene]amino}benzene-1-sulfonamide (**1e**) was active against *E. faecalis* (MIC 0.156 mg/mL compared to 1.25 mg/mL for sulfanilamide). Furthermore, (*E*)-4-{[(3,5-dichlorophenyl)methylidene]amino}benzene-1-sulfonamide (**1d**) and (*E*)-4-{[(2,6-dichlorophenyl)methylidene]amino}benzene-1-sulfonamide (**1c**) significantly reduced the viability of HT-29 colorectal adenocarcinoma and LN229 glioblastoma tumor cells, respectively (5 μg/mL at 24 hours and 1.6 μg/mL at 48 hours) at concentrations more than eight times lower than those required for sulfanilamide. This highlights the importance of halogen substitution for biological effects, with two chlorine atoms being ideal. On the other hand, this may increase acute toxicity in the crustacean species *Artemia franciscana* Kellogg; therefore, substitution with two bromine atoms, three chlorine atoms, or a phenoxy group should be avoided.

With regard to the *N*-acyl-hydrazone Schiff bases **1a–1p**, the selective cytotoxic effect on cancer cell lines (HT-29, A431) was the main characteristic of the series. *N′*-[(*E*)-(4-bromophenyl)methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**1b**) exhibited potency against both cell lines (IC_{50} 3.125–12 μM), while *N′*-[(*E*)-(4-fluorophenyl)methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**1a**) and *N′*-[(*E*)-(2,4-dichlorophenyl)methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)aceto-hydrazide (**1d**) exhibited activity specifically against A431 (IC_{50} 6.25–12 μM), outperforming 5-FU (IC_{50} > 50 μM). The cytotoxic effect varied significantly depending on the substituents present on the benzene ring, with groups such as 2,3,5-trichloro, 2-chloro-4-nitro, and 2-hydroxy-5-methoxy inducing non-selective cytotoxicity.

Microbiological analysis revealed the selectivity of *N*-acylhydrazones **1a-1p** against Gram-positive bacteria, as evidenced by inhibition of bacterial growth (*S. aureus, E. faecalis, C. albicans*) and a possible microbicidal effect, whereas 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide was inactive in this context.

N′-[(*E*)-(3,5-bromo-2-hydroxyphenyl)methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**1j**) exhibited both qualitative and quantitative activity against *S. aureus* (DIZ 12.33 ± 0.58 mm; MIC 0.3125 mg/mL; IC_{50} 0.0857 mg/mL), highlighting the importance of halogen substitution on the salicylaldehyde nucleus, as derivatives lacking hydroxyl groups (**1a–1h**) or methoxy/ phenoxy-derivatives (**1k–1p**) exhibited reduced or insignificant activity. However, the antifungal effect of 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide exceeded that of its derivatives, indicating that conversion to *N*-acyl-hydrazone Schiff bases is unfavorable for activity against *C. albicans*. The antitubercular

activity is notable for five derivatives (**1b**, **1c**, **1k**, **1l**, and **1m**) that exhibited activity against both the susceptible and resistant strains of *M. tuberculosis*.

The antioxidant capacity of *N*-acyl-hydrazones with nitro (**1h**, **1o**), hydroxy (**1k**, **1i**, **1j**, **1o**), and phenoxy (**1p**) groups was twice that of 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide, and the acute *in vitro* toxicity of the derivatives to the crustacean species *Artemia franciscana* Kellogg was lower than that of the parent compound.

Furthermore, since five derivatives (**1a–1e**) from the sulfanilamide Schiff base series and five compounds (**1c–1g**, **1p**) from the *N*-acylhydrazone series are derived from the reaction with the same aldehyde, a comparison can be made between the properties of these derivatives to determine the influence of the pharmacophore on biological effects. The antioxidant activity is more uniform in the case of the sulfanilamide derivatives (TAC 35–43%) compared to those of 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide-derived compounds (TAC 16.6–19.7%, respectively 42.3%). Furthermore, the sulfanilamide Schiff bases exhibit superior activity against *S. aureus* (MIC 0.014–0.059 mg/mL), being ten to a hundred times more potent than their *N*-acyl-hydrazone analogs (MIC 0.625–5 mg/mL). The cytotoxic activity on the HT29 cell line is difficult to assess, as the values are expressed differently; however, (*E*)-4-{[(3,5-dichlorophenyl)methylidene]amino}benzene-1-sulfonamide has a first dose with a significant effect at 15 μM (5 μg/mL), while *N'*-[(*E*)-(3,5-dichlorophenyl)methylidene]-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide exhibits an IC_{50} of 25 μM (9.7–9.95 μg/mL). In contrast, quinazolin-4(3*H*)-one derivatives exhibit lower acute *in vivo* toxicity in *Artemia franciscana* Kellogg than their sulfanilamide analogs.

Among the unresolved issues are the accurate estimation of the LC_{50} in the *in vivo* acute toxicity assay, since the concentration range of 62.5–1000 μM used and the mechanical phenomena did not allow for its calculation, as well as elucidating the cytotoxic mechanism of action for the two series (enzyme inhibition studies on representative enzymes, carbonic anhydrase for the sulfonamide series, and caspase 3 and EGFR kinase for the quinazolones).

Future directions for this research include further investigation of the mechanisms of action, in-depth microbiological analyses of the series of *N*-acyl-hydrazones with a quinazolin-4 (3*H*)-one structure in the context of tuberculosis, experimental determination of the solubility and lipophilicity of the most active compounds for Biopharmaceutical classification, development of a microwave-assisted synthesis technique for obtaining metal complexes, and evaluation of their biological profile in relation to the original ligands. Additionally, structural optimization of the most potent derivatives by substituting the

quinazoline ring, *in vivo* testing in animal models, and incorporation into nanomaterials are planned.

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1. **Coandă M**, Limban C, Nuță DC. Small Schiff Base Molecules—A Possible Strategy to Combat Biofilm-Related Infections. *Antibiotics* 2024; 13: 75. Journal indexing: Q1. Impact factor: 4.6 (2024). doi: 10.3390/antibiotics13010075. Available at: https://www.mdpi.com/2079-6382/13/1/75/htm.

   (Paper included in the chapter 1, pag. 19-25)

2. **Coanda M**, Limban C, Draghici C, Ciobanu A-M, Grigore GA, Popa M, Stan M, Larion C, Avram S, Mares C, et al. Current Perspectives on Biological Screening of Newly Synthetised Sulfanilamide Schiff Bases as Promising Antibacterial and Antibiofilm Agents. *Pharmaceuticals* 2024; 17: 405. Journal indexing: Q1. Impact factor: 4.8 (2024). doi: 10.3390/ph17040405. Available at: https://www.mdpi.com/1424-8247/17/4/405.

   (Paper included in the chapter 1, pag. 19-27, chapter 3, pag. 45-48, and chapter 4, pag. 56-77)

3. **Coandă M**, Drăghici C, Pintilie L, Kapronczai E-E, Chiriță C, Marinaș I-C, Ancuceanu R-V, Zarafu I, Ioniță P, Crăciun D-I, et al. Design, Synthesis, and Biological Evaluation of N-Acyl-Hydrazone-Linked Quinazolinone Derivatives with Antioxidant, Antimicrobial, and Anticancer Potential. *Pharmaceuticals* 2026; 19: 57. Journal indexing: Q1. Impact factor: 4.8 (2024). doi: 10.3390/ph19010057. Available at: https://www.mdpi.com/1424-8247/19/1/57/htm.

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