Zones, Scores and Solvents: Reading the Quinazoline Antimicrobial Literature

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***Abstract*—The quinazoline nucleus is routinely described as a privileged scaffold, and the description is defensible: it carries natural alkaloids, two marketed kinase inhibitors, an α_1-adrenoceptor antagonist and a clinical antifolate. What it does not carry is a marketed antibacterial agent, despite three decades of papers reporting that newly synthesised quinazolines inhibit bacterial growth. This review asks why, and locates much of the answer in how the work is designed and reported rather than in the chemistry itself. Three synthetic entries to the ring system the Niementowski condensation, the benzoxazinone route and nucleophilic displacement on 4-chloroquinazoline are compared on balanced equations, and their theoretical atom economies (58.7–92.9%) are computed and set against the E-factor and process mass intensity that actually govern waste. Because solvents account for roughly 80–90% of the non-aqueous mass of a typical process, a route described as green because it is microwave-assisted or run in a deep eutectic solvent is an unquantified claim until work-up and purification are included. On the biological side, the dominant primary screen remains agar diffusion, whose readout depends on the diffusion coefficient of the test compound as much as on its potency; for the lipophilic 2,3-disubstituted quinazolinones that most series produce, this systematically rewards the wrong structural trend. Reported activities are tabulated, the substituent effects that survive minimum-inhibitory-concentration testing are separated from those that do not, and the role of molecular docking is bounded: it can rationalise, prioritise and generate hypotheses, but a favourable score is not evidence of a mechanism. A minimum reporting standard is proposed.**

***Index Terms*—Quinazoline; quinazolin-4(3H)-one; green synthesis; atom economy; minimum inhibitory concentration; structure–activity relationship; molecular docking**

I. INTRODUCTION

Few heterocycles are introduced as confidently as the quinazoline. The ring system appears in alkaloids isolated across at least four plant families, in actinomycete metabolites, and in drugs that reached the clinic by three unrelated mechanisms [1,2]. Reviews of its chemistry run to hundreds of pages [3–5]. Against that background, it is unsurprising that a synthetic chemistry group looking for a scaffold on which to hang new substituents should choose this one, and that the resulting compounds should be sent for antimicrobial screening.

What is surprising is the outcome. After decades of such papers, no quinazoline is marketed as an antibacterial drug. The scaffold yielded gefitinib and erlotinib against the epidermal growth factor receptor, prazosin at the α_1-adrenoceptor, and trimetrexate as an antifolate (Fig. 1), but nothing in the antibacterial pharmacopoeia. One reading is that the chemistry is simply unsuited to the target space. A second reading, which this review defends, is that a large part of the published antimicrobial evidence is not of a kind that could support a lead, whatever the compounds are worth.

Two claims recur in this literature and are examined here in turn. The first is that a synthesis is green. The second is that a compound is active and that docking explains how. Both are usually asserted rather than measured, and both can be measured without special equipment. The aim of what follows is not to dismiss the scaffold the data gathered in Table 3 include genuinely potent compounds but to separate the findings that would survive an independent repetition from those that would not.

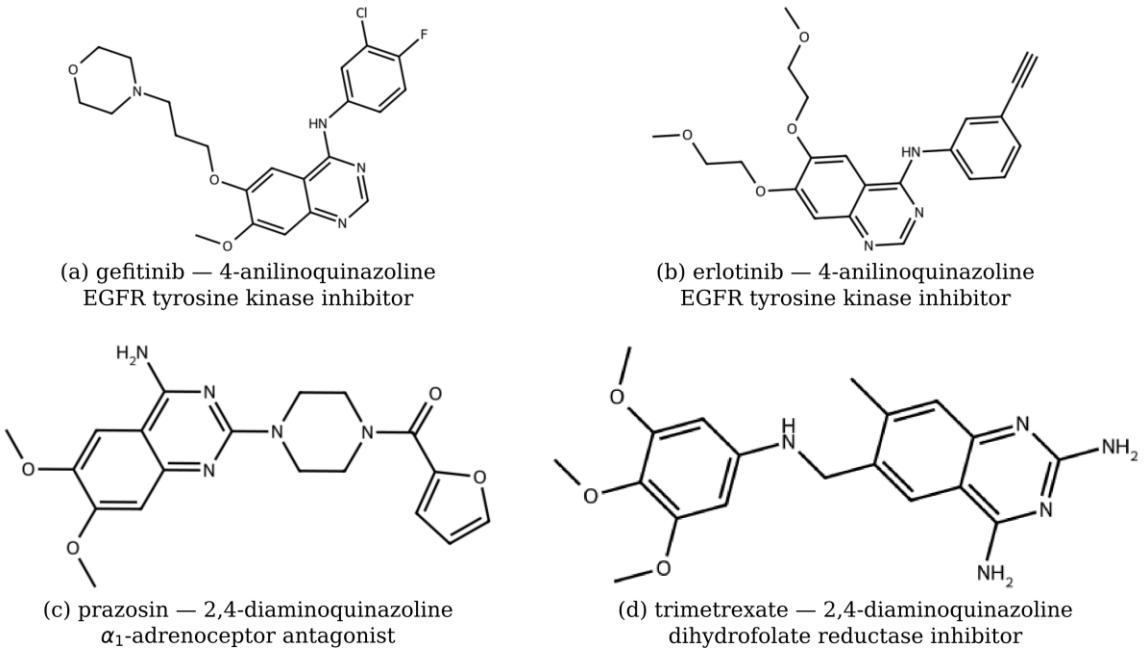


Fig. 1. Marketed drugs built on the quinazoline nucleus, showing how far substitution redirects the scaffold: (a, b) 4-anilinoquinazoline EGFR tyrosine kinase inhibitors; (c) a 2,4-diaminoquinazoline α_1-adrenoceptor antagonist; (d) a 2,4-diaminoquinazoline dihydrofolate reductase inhibitor. No quinazoline is marketed as an antibacterial agent.

II. THE SCAFFOLD AND WHAT IT HAS DELIVERED

2.1 Natural occurrence

Quinazoline alkaloids are widely distributed. Vasicine from Adhatoda vasica is a bronchodilator and oxytocic; febrifugine from Dichroa febrifuga is strongly antimalarial; luotonin A, tryptanthrin, rutaecarpine and chrysogine span antitumour, anti-inflammatory and antimicrobial activity [1–3]. Comprehensive surveys of the class describe over two hundred such molecules and their mechanisms [1,2]. Two observations follow from that body of work and are worth carrying forward. First, the biological activity of natural quinazolines is largely determined by what is fused or appended to the ring rather than by the ring alone. Second, several of these alkaloids are reported as antibacterial, yet none has been developed, which is the same pattern the synthetic literature shows.

2.2 The clinical footprint

The four marketed drugs in Fig. 1 illustrate how far substitution redirects the scaffold. Gefitinib and erlotinib are 4-anilinoquinazolines that occupy the adenine pocket of a kinase; prazosin is a 2,4-diaminoquinazoline acting at a G-protein-coupled receptor; trimetrexate is a 2,4-diaminoquinazoline antifolate that inhibits dihydrofolate reductase (DHFR). The last of these matters most to the present discussion, because DHFR is also a bacterial target, and the most convincing antibacterial quinazolines reported to date are structurally close to it.

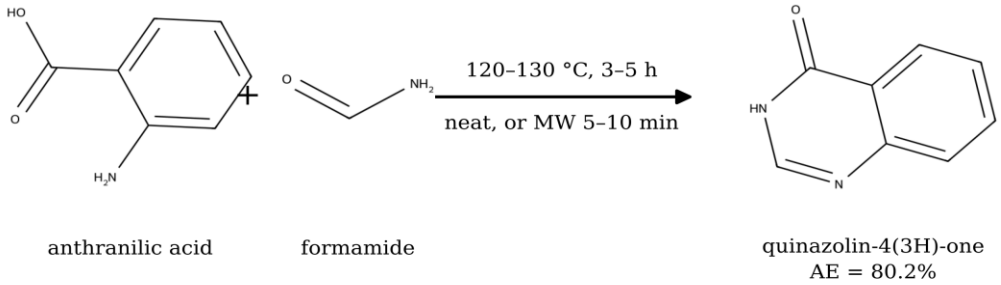
III. SYNTHETIC ACCESS TO THE RING

Three routes dominate the experimental sections of antimicrobial quinazoline papers. All three begin from anthranilic acid, which is inexpensive, and all three are old. Their differences are best seen in balanced form, because that is what fixes the atom economy.

3.1 The Niementowski condensation

Heating anthranilic acid with formamide at 120–130 °C gives quinazolin-4(3H)-one directly (Scheme 1) [6]. The reaction is a tandem amidation–cyclodehydration; formamide serves as reagent, one-carbon source and solvent, and the only by-products are water and ammonia. On the balanced equation the

atom economy is 80.2%, which is high for a ring-forming step. The route is also the one most often run under microwave irradiation, where reaction times fall from hours to minutes [7,8]. Its limitation is scope: it installs nothing at N-3 or C-2, so every subsequent substituent must be introduced in a separate operation.

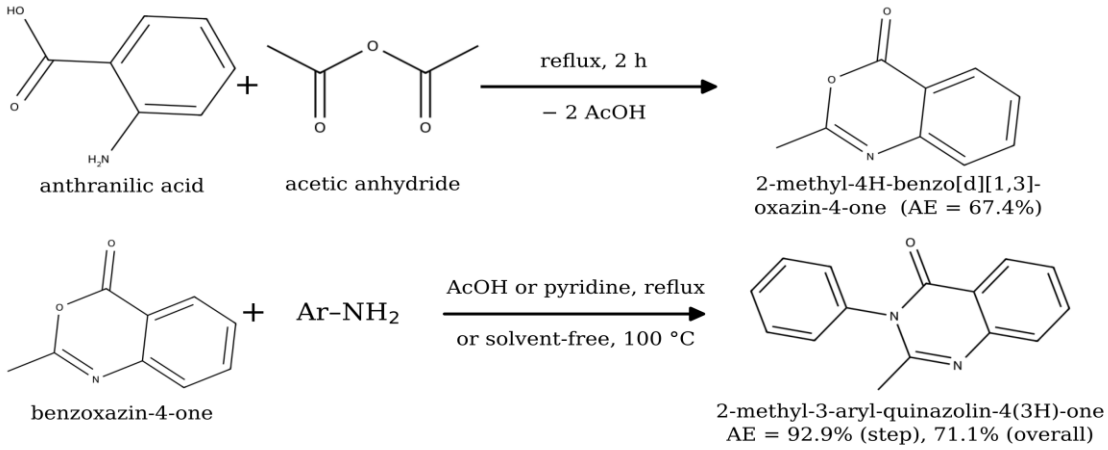


Scheme 1. The Niementowski condensation. Formamide acts as reagent, one-carbon source and solvent; water and ammonia are the only by-products. AE = atom economy.

3.2 The benzoxazinone route

Where 2,3-disubstituted quinazolin-4(3H)-ones are wanted and they are the commonest class in antimicrobial series the standard entry is two-step (Scheme 2). Anthranilic acid and excess acetic anhydride give 2-methyl-4H-benzo[d][1,3] oxazin-4-one, which is opened and reclosed by a primary amine to give the 2-methyl-3-substituted quinazolinone. The amine step is efficient on paper, at 92.9% atom economy, but the anhydride step is not: two equivalents of acetic acid are expelled, and the step scores 67.4%. The route overall is 71.1% atom-economical. The attraction is that the amine is the point of diversity, so a single intermediate serves a whole series which is precisely why so many published series share a 2-methyl group that nobody has varied.

This is also the step where solvent choice has been most actively revisited. Twenty choline chloride-based deep eutectic solvents were screened for the related synthesis of 2-mercaptoquinazolin-4(3H)-ones from anthranilic acids and isothiocyanates; choline chloride:urea (1:2) was the most effective and acted as both solvent and catalyst [7]. The same eutectic was identified as the best of twenty natural deep eutectic solvents for 2-methyl-3-substituted quinazolinones, while 3-aryl quinazolinones were obtained in a one-pot microwave reaction of anthranilic acid, an amine and trimethyl orthoformate in ethanol [8].

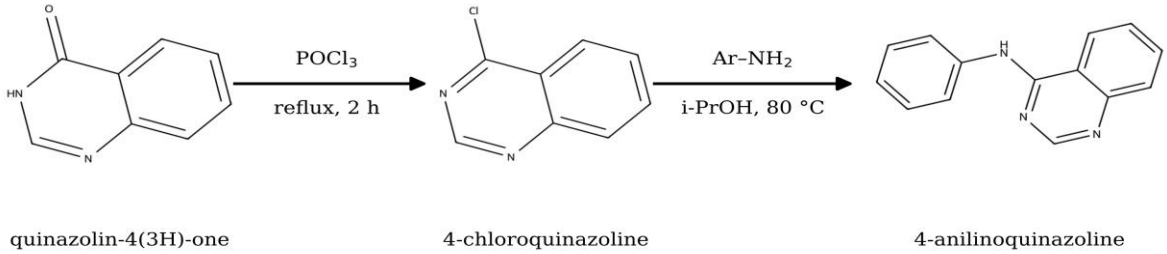


Scheme 2. The benzoxazinone route to 2-methyl-3-substituted quinazolin-4(3H)-ones. The amine introduced in the second step is the point of diversity in most published series; the unvaried 2-methyl group is inherited from the first step.

3.3 Displacement at C-4

The third route converts the quinazolin-4(3H)-one to 4-chloroquinazoline with phosphorus oxychloride, then displaces chloride with an amine (Scheme 3). It is the route to the 4-anilinoquinazolines of Fig. 1, and to 2,4-diaminoquinazolines when the 2-position is also activated. Atom economy is a poor description of this sequence, because $POCl_3$ is used in large excess and quenched; its waste burden is dominated by the quench and the aqueous work-up, not by the stoichiometry. That is the general lesson of the next section.

One-pot three-component variants that combine anthranilic acid, an amine and an orthoester under Lewis's acid, heteropolyacid, ionic liquid or solvent-free conditions shorten the sequence considerably [8]. They are frequently described as the greenest option, yet their theoretical atom economy is the lowest of the four entries computed here, at 58.7%, because three equivalents of ethanol or methanol leave with the orthoester. Fewer steps and lower atom economy are not contradictory; they measure different things, which is the point of Table 1.



Scheme 3. Chlorodehydroxylation and nucleophilic displacement at C-4, the entry to 4-anilinoquinazolines. Atom economy describes this sequence poorly because $POCl_3$ is used in excess and quenched.

Table 1. Green chemistry metrics applicable to a quinazoline synthesis, and the boundary of each. A route may be excellent on atom economy and poor on process mass intensity; the two answer different questions [9–14].

Metric	Definition	What it captures	What it ignores
Atom economy (AE)	Molar mass of product / summed molar masses of reactants in the balanced equation × 100	Intrinsic efficiency of the chemical transformation; comparison between route designs before any experiment	Yield, solvent, catalyst, reagent excess, work-up, purification, energy
Reaction mass efficiency (RME)	Mass of isolated product / total mass of reactants × 100	Atom economy combined with actual yield and stoichiometric excess	Solvents and auxiliaries, which usually dominate the mass balance
E-factor	kg of waste generated / kg of product isolated	All material leaving the process, including solvent, aqueous waste and purification losses	Relative toxicity or persistence of the different waste streams
Process mass intensity (PMI)	Total mass of materials used / mass of product; numerically E-factor + 1	The same information as the E-factor, counted as input rather than waste	Hazard, energy and the relative impact of individual solvents
Solvent intensity	kg of solvent used / kg of product	The term that typically accounts for 80–90% of non-aqueous process mass	Everything outside the solvent stream

IV. GREEN CHEMISTRY IS A MEASUREMENT

4.1 The metrics and what each one sees

Atom economy, introduced by Trost, is the ratio of the molar mass of the desired product to the summed molar masses of all reactants in the balanced equation [9]. It is computed before any experiment is done and is therefore a property of the route, not of the run. It ignores yield, solvent, catalyst, reagent excess, work-up and purification. The E-factor, defined by Sheldon as kilograms of waste per kilogram of product, captures all of those, and the gap between the two numbers is the gap between a route and a process [10,11]. Pharmaceutical manufacture sits at the unfavourable end of the reported range, typically 25 to over 100, against 1–5 for bulk chemicals and below

0.1 for oil refining (Fig. 2b) [11]. Process mass intensity, adopted by the ACS Green Chemistry Institute Pharmaceutical Roundtable, is the same quantity counted forwards total mass in per mass of product and is numerically the E-factor plus one [12,13]. Reaction mass efficiency combines atom economy with actual yield and stoichiometry, and is the most informative single number a student laboratory can report [14]. Table 1 sets out what each metric does and does not include.

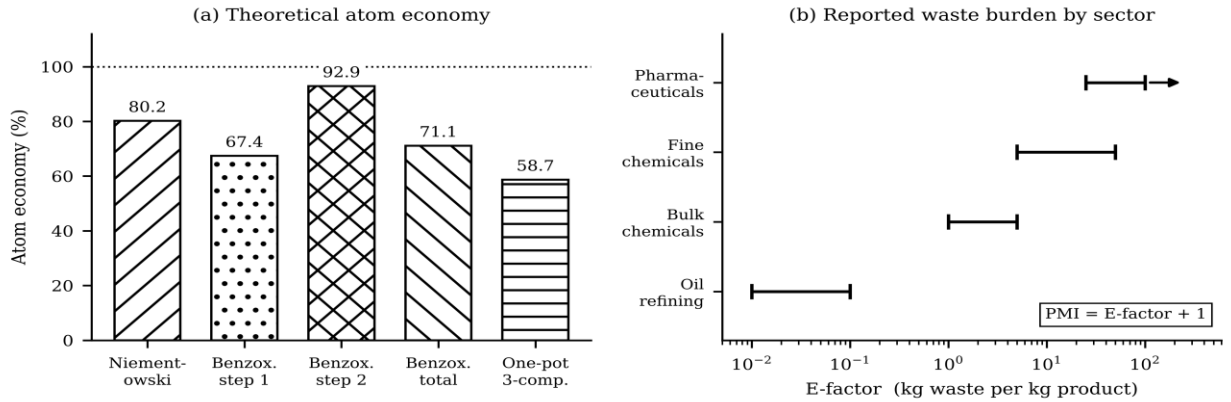


Fig. 2. (a) Theoretical atom economies computed from the balanced equations of the routes in Schemes 1–3 and of the one-pot three-component variant. (b) Reported E-factor ranges by industrial sector on a logarithmic scale; the arrow indicates that pharmaceutical values commonly exceed 100. Atom economy is a property of the equation; the E-factor is a property of the process, and the two differ mainly by solvent.

4.2 Why the solvent decides

Solvents account for the majority of the mass in a fine-chemical process, on the order of 80–90% of the non-aqueous material, and they dominate the life-cycle environmental impact of most syntheses [11,15]. A consequence follows immediately, and it is uncomfortable for the way green claims are usually made. Improving the atom economy of a step from 67% to 93% changes a small part of the total mass balance; replacing dichloromethane with ethanol, or eliminating a chromatographic purification, changes a large part of it. Solvent selection guides exist precisely so that this choice can be defended rather than asserted [15].

Deep eutectic solvents, microwave heating and solvent-free grinding are all reasonable interventions, and the quinazolinone literature has tested them seriously [7,8]. But a choline chloride:urea medium that is subsequently washed out with several volumes of ethyl acetate, and a product that is then recrystallised twice, may carry a higher process mass intensity than the conventional reflux it replaced. None of the papers surveyed for this review that describe a quinazoline synthesis as green report an E-factor or a PMI. The measurement requires only the masses already recorded in a laboratory notebook.

V. THE ASSAY DECIDES THE STRUCTURE–ACTIVITY RELATIONSHIP

5.1 Agar diffusion and what it actually measures

The overwhelming majority of antimicrobial quinazoline papers report zones of inhibition from agar disc or well diffusion. The format is cheap, visual and genuinely useful as a qualitative screen. It is not a potency measurement. The diameter of a zone is set jointly by the compound's intrinsic activity and by how fast it moves through the gel; a less polar compound diffuses more slowly and produces a smaller zone at equal potency, while cationic compounds are retarded further by electrostatic binding to acidic groups in the agar [20,21]. Zone size also varies with agar depth, inoculum density, molecular weight and the growth rate of the organism, which is why standardised diffusion methods are validated drug by drug and are not transferable to novel chemistry [22,23].

For this scaffold the consequence is specific rather than general. 2,3-Disubstituted quinazolin-4(3H)-ones become more lipophilic as aryl and halogenated substituents are added exactly the substituents a medicinal chemist would add to improve target affinity. Those compounds diffuse more slowly. A series ranked by zone diameter will therefore tend to

rank the more lipophilic, often more potent, members lower. The resulting structure–activity relationship is partly a diffusion relationship wearing a biological label, and it will not reproduce when the same compounds are tested in broth.

Table 2. The two antimicrobial screening formats compared on the attributes that matter for a novel quinazoline series [20–26].

Attribute	Agar disc / well diffusion	Broth microdilution
Primary readout	Zone diameter (mm)	Minimum inhibitory concentration (μg/mL or μM)
Depends on compound diffusion in gel	Yes, strongly	No; compound is dissolved throughout the well
Effect of increasing lipophilicity	Slower diffusion, smaller zone at equal potency potency is systematically underestimated	None beyond solubility limits, which are detectable as precipitation
Other sources of variance	Agar depth, inoculum density, molecular weight, organism growth rate, electrostatic binding of cations to agar	Inoculum density, medium composition, reading endpoint
Regulatory status for novel compounds	Qualitative screen; standardised only for validated drug–organism pairs	Reference method of CLSI and EUCAST (ISO 20776-1)
Supports MBC and selectivity index	No	Yes, from the same plate series

5.2 What the reference methods require

Both CLSI and EUCAST designate broth microdilution as the reference method for determining the minimum inhibitory concentration (MIC) of a compound against rapidly growing aerobic bacteria, with agar dilution accepted for particular organisms [22,24,25]. The method is not expensive: a 96-well plate, a two-fold dilution series, a standardised inoculum of approximately 5×10^5 CFU/mL and an overnight read. Reviews of in vitro antimicrobial evaluation set out the procedure for laboratories unfamiliar with it [26]. Table 2 compares the two formats on the attributes that matter for a new series.

Two additions convert an MIC into evidence. The first is a minimum bactericidal concentration, which distinguishes a compound that kills from one that merely delays growth. The second is a mammalian cytotoxicity counter-screen on the same compounds at the same time, from which a selectivity index can be computed. Without it, an MIC of 32 μg/mL is uninterpretable: it may indicate a selective antibacterial or a general cytotoxin. The quinazoline series that have progressed furthest are precisely those that reported both. In the N^2, N^4-disubstituted quinazoline-2,4-diamine work, MICs as low as 0.5 μM against MRSA and multidrug-resistant Acinetobacter baumannii were accompanied by HepG2 LD_{50} values and an explicit activity index, bactericidal confirmation, a DHFR rescue experiment with tetrahydrofolate, resistance-development studies over eight days and an in vivo model [17].

A third addition costs nothing and is almost universally omitted: a statement of what the compound was dissolved in, at what final concentration, and what the vehicle alone did to the organism. Dimethyl sulfoxide above roughly 1% is itself inhibitory for several test species, and a series reported without a vehicle control cannot be distinguished from a series that is reporting its solvent. It is also worth screening a new set against the published interference substructures before investing in it, because compounds that aggregate, redox-cycle or react covalently give reproducible but uninformative activity across unrelated assays [41].

VI. SUBSTITUENT EFFECTS THAT SURVIVE MIC TESTING

Filtering the reported data for studies that used dilution methods leave a smaller but more consistent picture, summarised in Table 3.

Position 2 and position 3 together define the class. 2-Methyl-3-aryl quinazolin-4(3H)-ones are the most frequently made and among the least potent; substitution at N-3 is necessary for activity in most series, but an unvaried 2-methyl group appears to be a limitation inherited from the benzoxazinone route rather than an optimum. Replacing the 2-substituent

with a thioamido or guanidino group raised antitubercular potency to an MIC of 6.25 µg/mL against Mycobacterium tuberculosis H37Rv in a set of fourteen 2,3-disubstituted quinazolinones [27].

Position 6 is the most informative. In the quinazoline-2,4-diamine series, strong antibacterial activity against multidrug-resistant A. baumannii required a halide or small alkyl group at C-6 [17]. A larger campaign of fifty-three quinazolinones across three structural types found selective antimycobacterial activity with MICs down to 6.25 µg/mL, retained against drug-resistant strains, and tracked potency against calculated log P across the series [16].

The 2,4-diamino pattern itself is the clearest signal in the field. It is the pattern of trimetrexate, it is the pattern of the most potent antibacterial quinazolines reported, and in both cases the target is DHFR. Against that, the 4-oxo compounds that dominate the synthetic literature are a different pharmacophore, and the enzyme most often invoked for them is DNA gyrase, where quinazolin-4(3H)-ones bearing hydrazone or pyrazole substituents inhibited the Escherichia coli enzyme with IC_{50} values of 3.19–4.17 µM [18]. Gyrase is a well-validated antibacterial target, addressed clinically at the GyrA cleavage complex by the fluoroquinolones and at the GyrB ATPase site by novobiocin, and the two sites behave differently enough that a paper should state which one it means [39,40]. Most quinazolinone docking studies address the ATPase domain without saying so explicitly.

Table 3. Representative quinazoline antimicrobial series, ordered by the strength of the evidence rather than by potency. The first two rows report dilution-based MICs with counter-screens; the last reports docking against a diffusion-based screen.

Compound class	Organism(s)	Reported activity	Proposed target	Ref.
N^2,N^4-Disubstituted quinazoline-2,4-diamines; 6-halide or 6-alkyl	MRSA; multidrug-resistant A. baumannii	MIC as low as 0.5 µM; bactericidal; HepG2 LD_{50} and activity index reported; in vivo efficacy	DHFR, supported by tetrahydrofolate rescue	[17]
Quinazolinones, 53 compounds across three structural types	M. tuberculosis H37Rv, H37Ra and drug-resistant strains	MIC as low as 6.25 µg/mL; activity retained against resistant strains	Penicillin-binding protein 1A (in silico)	[16]
2,3-Disubstituted quinazolinones with thioamido or guanidino groups	M. tuberculosis H37Rv (Alamar blue)	MIC 6.25–100 µg/mL across the series	Not experimentally assigned	[27]
Quinazolin-4(3H)-ones bearing hydrazone or formyl-pyrazole groups	E. coli; Gram-positive panel	Enzyme IC_{50} 3.19–4.17 µM for the four most potent members	DNA gyrase B, assayed as well as docked	[18]
Quinazoline–piperazine phosphorodiamidate hybrids	Gram-positive and Gram-negative panel	Zone-based primary screen; docking scores exceeding ciprofloxacin	DNA gyrase (docking only)	[19]

VII. WHAT DOCKING CAN AND CANNOT ESTABLISH

Molecular docking appears in almost every recent quinazoline antimicrobial paper, usually against DNA gyrase and usually reported as a binding energy in kcal/mol alongside a list of contacted residues [18,19]. The technique is sound and the software is well validated for what it was built to do. Established docking programs recover a crystallographic binding pose to within 2 Å root-mean-square deviation in roughly 70–80% of cases [28,29]. Ranking compounds correctly by affinity is substantially harder, and predicting absolute binding free energy from a docking score is harder still; comparative assessments of scoring functions report modest and target-dependent correlation with measured affinity [30,31].

Three practices separate a useful docking section from a decorative one. Redock the co-crystallised ligand and report the RMSD, which establishes that the protocol reproduces a known answer before it is applied to an unknown one; one of the quinazolinone studies surveyed does exactly this [19]. State the correlation, if any, between docking score and measured MIC across the whole series rather than for

the best compound alone. And do not let the docking target stand in for a mechanism: if DNA gyrase is the proposed target, a supercoiling or ATPase inhibition assay is the evidence, and such assays have been run on quinazolinones [18]. A compound with a favourable score and no enzyme assay has a hypothesis, not a mechanism.

The scaffold also sits awkwardly against what is known about antibacterial physicochemistry. Compounds that accumulate in Gram-negative bacteria tend to be relatively polar, to carry an ionisable amine and to be conformationally restricted properties that differ systematically from those of drug-like molecules optimised for mammalian targets [32–34]. Lipophilic 2,3-disubstituted quinazolinones are close to the latter description and far from the former, which may explain both their weak Gram-negative activity and the repeated finding that activity is better against Gram-positive organisms [35,36]. The diaminoquinazolines, which are basic and compact, fit the accumulation rules better and perform better [17].

There is a practical corollary for a dissertation-scale project. If a series is lipophilic, neutral and built on a 4-oxo core, the realistic expectation is Gram-positive activity in the tens of micrograms per millilitre, and the interesting question becomes selectivity rather than breadth. A screening panel designed around that expectation two Gram-positive strains, one Gram-negative, one yeast, read as MICs and paired with a single mammalian cell line yields more usable information than a wider panel read as zones, and costs less.

VIII. A MINIMUM STANDARD FOR THE NEXT SERIES

Nothing proposed here requires equipment beyond a standard pharmaceutical chemistry laboratory with access to a microbiology bench. For the chemistry: report the balanced equation and atom economy of each step, record solvent volumes for reaction, work-up and purification, and compute an E-factor or process mass intensity for the sequence as performed; if a green medium is claimed, give the number for both the new and the displaced route. For the biology: use broth microdilution to obtain MICs against reference strains, add minimum bactericidal concentrations, include a mammalian cell line and report the selectivity index, and include a solvent control at the DMSO concentration used. For the modelling: redock the native ligand, report the RMSD, correlate scores against measured MICs across the series, and treat a predicted target as a hypothesis until an enzyme assay tests it.

Applied to the existing literature, this standard would discard few compounds and a great deal of apparent structure–activity information. That is the intended effect. The priority pathogens for which new agents are needed are defined [37,38], the attrition rate of antibacterial discovery programmes is well documented [35,36], and a scaffold that has produced four marketed drugs deserves to be tested against the same evidentiary bar as any other.

IX. CONCLUSION

The quinazoline is a capable scaffold with a thin antibacterial record, and the thinness is at least partly an artefact of how it has been studied. Three classical routes give ready access to the ring with theoretical atom economies between 58.7% and 92.9%, but atom economy is the smallest term in the waste balance, and the green claims attached to deep eutectic and microwave protocols remain unquantified in the papers that make them. On the biological side, agar diffusion has supplied most of the published structure–activity data for a compound class whose lipophilicity makes that assay systematically misleading, and docking has frequently been asked to carry an evidentiary weight it cannot bear. The compounds that depart from this pattern the 2,4-diaminoquinazoline DHFR inhibitors with micromolar and submicromolar MICs, selectivity indices, target rescue experiments and animal data are also the ones that look like leads. The difference between the two bodies of work is not chemical talent. It is measurement.

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