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QSAR MODELLING OF FLUOROQUINOLONE ANALOGUES AGAINST MULTIDRUG-RESISTANT STAPHYLOCOCCUS AUREUS

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Abstract

Fluoroquinolones remain among the most studied antibacterial scaffolds, yet resistance in methicillin-resistant Staphylococcus aureus has eroded their use against staphylococcal infection. This narrative review summarises what the published literature says about quantitative structure-activity relationship modelling of fluoroquinolone analogues against multidrug-resistant S. aureus. It covers the enzyme targets and resistance mechanisms that shape activity, the substituent effects established by medicinal chemistry, the descriptor and statistical approaches used in two-dimensional and three-dimensional models, and the validation standards expected of a credible model. No new models or data are reported. The main gaps are small, heterogeneous datasets, inconsistent minimum inhibitory concentration protocols and the scarcity of external prospective testing.

1. INTRODUCTION

Few antibacterial classes have been reworked as many times as the fluoroquinolones. Since nalidixic acid, medicinal chemists have added a fluorine, swapped amines, closed rings and trimmed side chains, producing agents from norfloxacin and ciprofloxacin to moxifloxacin and newer investigational compounds [1, 2, 3]. The effort was driven by a clear need. Staphylococcus aureus is a leading cause of skin, bone, bloodstream and device-related infection, and strains resistant to methicillin and to several other classes at once are now

common in hospitals and the community [4, 5, 6]. Each generation was introduced to improve some weakness of the last, whether poor Gram-positive activity, short half-life or a narrow spectrum. The result is a large body of structure and potency data, which is what makes quantitative modelling possible at all, and also what makes its heterogeneity such a problem [1, 3]. Against that background, a quantitative view of how structure drives activity in resistant strains is not an academic exercise but a way to decide which compounds are worth synthesising. Scaffold hopping away from quinolones has been tried, but the class keeps drawing attention because its mechanism is well characterised [7, 8].

Fluoroquinolone resistance in these strains is frequent, and it often travels with methicillin resistance, so that older agents such as ciprofloxacin are poorly reliable in staphylococcal disease [5, 6]. That poses a design question. If the scaffold is to be rescued, which structural features restore activity against organisms carrying target mutations and efflux pumps? Quantitative structure-activity relationship (QSAR) modelling tries to answer in numerical terms, by relating measured potency across a series of analogues to calculated properties of their structures [9, 10]. Put plainly, a QSAR model is a compact summary of a series. It can suggest which analogue to make next, but only if the series was measured consistently and the model has been tested honestly. Both conditions are harder to meet for resistant organisms than for susceptible laboratory strains [10, 11]. Published QSAR work on quinolones is spread across medicinal chemistry, microbiology and cheminformatics journals, and few papers speak to all three audiences at once [2, 10]. Small-molecule antibacterial discovery has been slow in recent decades, which increases the value of cheap computational filters [5, 10].

This article reviews the published work on that question. It is a narrative review of literature. It does not present new models, new calculations or new antibacterial testing. The sections that follow describe the biological target and resistance mechanisms, the structural features that govern activity, the QSAR techniques applied to quinolones, and the validation and data quality issues that decide whether a model deserves trust. The main findings are compiled in table 1 and grouped in fig. 1. Throughout, the aim is to separate what is firmly established from what is plausible but unproven. Readers who want a hands-on modelling protocol should consult the methodological sources cited here [11, 12]. The scope is deliberately limited to antibacterial activity and does not cover pharmacokinetic modelling.

Table 1: Published findings relevant to QSAR of fluoroquinolones against S. aureus

Topic	Reported finding	Ref.
Cleavage complex binding	Drug stabilises DNA breaks through a metal ion bridge and keto-acid binding	[7, 8, 13]
Primary target in S. aureus Ciprofloxacin	Topoisomerase IV is the first target, with gyrase secondary	[14, 15]
Balanced targeting C-8 methoxy agents	Moxifloxacin-type compounds act on both enzymes with less mutant selection	[16, 17]
Efflux by NorA	Hydrophilic quinolones are better substrates than hydrophobic ones	[18, 19]
C-7 substituent	Bulkier lipophilic rings favour Gram-positive activity and reduce efflux	[1, 2]
N-1 and C-6 groups	Cyclopropyl and fluorine raise potency and penetration	[1, 2]
Physicochemical balance	Zwitterionic character, lipophilicity and solubility set permeability and accumulation	[2, 20]
Hansch analysis	Potency related linearly to lipophilic, electronic and steric terms	[9, 21]
CoMFA and CoMSIA	Field-based 3D models depend heavily on molecular alignment	[22, 23]
External validation	Predictive power must be shown on compounds withheld from training	[24, 25, 26]
OECD principles	Defined endpoint, algorithm, applicability domain and mechanistic interpretation	[12]

2. TARGETS AND RESISTANCE

Fluoroquinolones act by trapping bacterial type II topoisomerases, DNA gyrase and

topoisomerase IV, on DNA in a cleavage complex. The drug does not simply block the enzyme. It stabilises a break in the DNA, and the accumulation of such complexes halts replication and leads to cell death [7, 27, 28]. Structural studies show a water-metal ion bridge, usually involving magnesium, that links the keto-acid group of the drug to the enzyme, and the drug intercalates between base pairs at the cleaved site [8, 13]. This binding mode explains why changes at the keto-acid core are rarely tolerated. Eukaryotic topoisomerase II is much less sensitive to these drugs, which is the basis of their selective toxicity, though some analogues do inhibit it at higher concentrations [2, 7]. The geometry of this complex is the reason that cross-resistance between quinolones is so common. A mutation that weakens the water-metal bridge affects the whole class, albeit unevenly, and newer analogues are designed to contact the enzyme at additional points to compensate [8, 13]. Such structural detail makes quinolones one of the better understood antibacterial classes at the atomic level [13, 29].

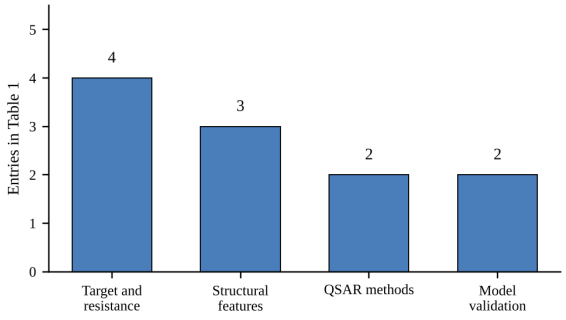


Fig. 1: Number of entries in table 1 by topic group

In S. aureus the preferred target depends on the compound. For ciprofloxacin, topoisomerase IV (GrlA and GrlB) is the primary target and gyrase is secondary, so the first-step resistance mutation is usually in grlA, with gyrA changes adding further resistance later [14, 15]. Some newer agents with a C-8 methoxy group, moxifloxacin among them, show more balanced targeting of the two enzymes, and this is thought to make single mutations less effective at producing resistance [16, 17]. Dual targeting is therefore a design goal, and one that a QSAR study has to be able to represent. Clinical resistance in staphylococci usually arises by stepwise accumulation of such mutations, with each step raising the inhibitory concentration a few fold, so that resistant isolates often carry several changes [14, 15]. Differences in the order of target preference between compounds were established by selecting mutants step by step and sequencing the genes involved, and they have been cited repeatedly as a rationale for choosing particular C-7 and C-8 substituents [14, 15, 17]. This evidence supports the view that no single descriptor can stand for target preference [15, 17].

Mutation is only part of the story. The NorA multidrug efflux pump, and other staphylococcal pumps, expel hydrophilic fluoroquinolones such as norfloxacin and ciprofloxacin more efficiently than hydrophobic ones [18, 19]. Overexpression of such pumps raises minimum inhibitory concentrations modestly, but in combination with target mutations it can tip a strain into clinical resistance. For modelling, the consequence is that activity in resistant strains depends on at least three things at once: affinity for the mutated enzyme, penetration into the cell and susceptibility to efflux. Descriptors that capture only one of these will explain only part of the variance [10, 19]. Other mechanisms exist, including plasmid-borne protective proteins in some bacteria, but in S. aureus the chromosomal mutations and efflux dominate the literature [7, 19]. Genetic work has also shown that regulatory changes can raise pump expression without any change in the pump protein itself. Inhibitors of efflux have been explored as adjuvants, although none had reached routine clinical use by 2021 [18, 19]. Taken separately, each mechanism is modest, which is why combinations drive clinical failure [5, 19].

Strain choice complicates interpretation. Studies that report activity against a panel of resistant clinical isolates, each with its own mutations and pump expression, give a mixed signal, whereas studies using isogenic mutants offer cleaner mechanistic insight but fewer strains [14, 15, 30]. A model trained on one type of data cannot be assumed to predict the other. Analysts working with clinical isolates should record the genotype of each

strain whenever possible. Isogenic panels are especially informative for this purpose. Genotyping is now cheap enough to be routine. Reports differ.

3. STRUCTURE AND ACTIVITY

The classical structure-activity relationships of quinolones were worked out well before computational modelling matured. The 3-carboxylic acid and 4-oxo groups are essential for binding. A fluorine at C-6 improves gyrase affinity and cell penetration, a cyclopropyl group at N-1 raises potency against many species, and an amine-containing ring at C-7 broadens spectrum and shapes pharmacokinetics [1, 2]. Together these rules supplied the first rational basis for design, and they remain the starting point for any set of analogues that a modeller chooses to study. Early comparative work also established that small changes at N-1 can shift activity several-fold, so much so that the substituent at this position is often held constant in a series to simplify modelling [1, 21]. A useful consequence is that a modeller can treat these positions as separate blocks, each described by its own descriptors, and can ask which block explains most of the variation in activity across the series [2, 9]. Substituent effects are therefore additive only to a first approximation [1, 2]. Later work refined rather than replaced them.

Position C-7 deserves special attention for Gram-positive activity. Bulky or lipophilic substituents, such as substituted pyrrolidines and diazabicyclic rings, tend to improve potency against staphylococci and to reduce susceptibility to efflux, while small hydrophilic piperazines favour Gram-negative activity [1, 18, 19]. A methoxy group at C-8 is associated with lower selection of resistant mutants in S. aureus, though it was also linked to phototoxic and cardiac concerns in some analogues, a reminder that potency alone cannot guide design [3, 16, 17]. For a modeller this means that substituent descriptors at C-7 should include volume and flexibility, not only lipophilicity, since the shape of the ring controls how it sits near the enzyme surface and in the efflux pump [2, 19]. Reviews of structure-side-effect relationships make the same point from the other direction, showing that features that improve staphylococcal potency are not always the ones that keep toxicity low [1, 3]. Ring size, basicity and stereochemistry all contribute and are not captured by a single lipophilicity term [2, 3].

Physicochemical balance sets the boundaries. Quinolones are zwitterionic at physiological pH, and the ionisation state of the C-7 amine and the carboxylic acid affects permeability, protein binding and accumulation in the cell [2, 20]. Lipophilicity helps penetration into staphylococci but, if excessive, increases toxicity and lowers solubility. Simple rule-based property filters give a rough check, although the quinolones sit near the edges of some of these rules and several successful agents would fail a strict reading [2]. This matters because most successful analogues were developed empirically and only later rationalised, which limits how much prospective guidance the early models could give [2, 3]. For this reason published models that predict potency alone need to be read next to the side-effect literature, rather than in isolation [1, 3]. Practical drug design depends on weighing several of these properties at once [2, 20].

4. QSAR APPROACHES

Hansch and Fujita introduced the idea that biological potency can be expressed as a linear function of lipophilic, electronic and steric parameters, and this extrathermodynamic approach remains the conceptual root of the field [9, 21]. For fluoroquinolones, early models relied on the partition coefficient, molar refractivity, Hammett constants and indicator variables for the presence of particular substituents. Potency was expressed as the negative logarithm of the minimum inhibitory concentration, so that larger values mean stronger activity and the relationship with descriptors is roughly linear [9, 21]. The strength of the method is that it is transparent. Each term in the equation can be read as a statement about the chemistry, and a chemist can check whether it agrees with experience [9, 21]. Its limits are equally clear. A linear equation assumes that each descriptor acts independently and additively, and in quinolones that is rarely true, since

lipophilicity and charge interact through the zwitterionic centre [2, 21]. Many early quinolone models followed this pattern with a handful of terms [9, 21].

Modern two-dimensional models use hundreds or thousands of calculated descriptors, covering constitutional, topological, geometric, electrostatic and quantum chemical properties [31]. Selection of a handful from this pool is the critical step, and it is done by genetic algorithms, stepwise methods or partial least squares regression [32]. Machine learning methods, including support vector machines and random forests, were applied to antibacterial datasets, with the usual caution that flexible algorithms can fit noise when the number of compounds is small [10, 11]. A smaller model with interpretable descriptors is often more useful to a chemist than a more accurate black box. Descriptor pools can also be reduced by removing those that are nearly constant or highly intercorrelated before any model is fitted, which lowers the risk of spurious selection [31, 32]. Overfitting is the persistent risk, and as a rule of thumb the number of descriptors should stay small compared with the number of compounds in the training set [11, 24]. Cross-validation is used to choose the number of components in partial least squares models [24, 32].

Three-dimensional methods align the molecules and sample interaction fields around them. Comparative molecular field analysis uses steric and electrostatic probes, and comparative molecular similarity indices analysis adds hydrophobic and hydrogen-bonding fields [22, 23]. Both rely on partial least squares to relate the fields to activity. Contour maps then indicate where bulk or charge is favoured, which can be compared with the crystal structures of quinolone-enzyme complexes [13, 23, 29]. The weakness is alignment, since a wrong superposition of flexible C-7 side chains produces a plausible-looking but misleading model [22, 33]. Contour maps from such models have, in published analyses of quinolones, tended to highlight the C-7 region as the place where steric and electrostatic changes alter potency, in keeping with older chemical knowledge [2, 23]. Different alignment rules, using either a common core, a pharmacophore or the crystal conformation, give different fields, so the reported results should always state the rule applied [22, 33]. Field-based models are more demanding to build but give visual guidance [22, 23].

Table 1 brings together representative findings from the structural, mechanistic and methodological literature, and fig. 1 shows how the entries are distributed between groups. The balance is revealing. Mechanistic and chemical knowledge about quinolones is far more extensive than quantitative models built on resistant S. aureus data, which is why this review leans on the first two when interpreting the third [1, 2, 8, 18]. The honest reading is that many of the best-supported statements in this area come from chemistry and structural biology, with the models serving as a framework for organising them [13, 29]. A fair summary is that two-dimensional approaches suit large, diverse sets and three-dimensional approaches suit small, congeneric ones. Neither type can substitute for mechanistic understanding [10, 29]. Such a split of knowledge is typical of mature scaffolds.

5. VALIDATION AND DATA QUALITY

A QSAR model is only worth the care taken in validating it. Internal measures such as the leave-one-out cross-validated coefficient q2 test stability, yet a high q2 does not guarantee predictive power [24, 25]. Golbraikh and Tropsha showed that external prediction on compounds withheld from training is the decisive test, and that several statistical criteria should be satisfied together [25]. Randomisation of the activity values, in which the model should collapse, helps to exclude chance correlation [12, 26]. A model with a high fit to the training set but poor prediction for new compounds is overfitted, and the gap between the two is the most informative single number in a report [24, 25]. Y-randomisation and bootstrapping add confidence, and recent consensus papers suggest that the combination of several criteria is more reliable than any single threshold [24, 26]. Reporting only the best training statistic is a common weakness [24, 26].

The OECD principles ask for a defined endpoint, an unambiguous algorithm, a defined applicability domain, appropriate measures of fit and predictivity, and a mechanistic interpretation where possible [12]. Of these, the applicability domain is the most often ignored. A model trained on 6-fluoro-7-piperazinyl analogues says little about a molecule with an unusual bicyclic substituent, and a prediction for it should carry a warning rather than a number [10, 11, 12]. Domain definitions based on descriptor ranges or on distance to training compounds are simple to compute and should accompany every published model [10, 12]. Reporting the descriptor values for each prediction, so that another group can reproduce it, is a low-cost step that few papers take [11, 12]. This check is simple, yet often omitted from published studies [10, 12].

The input data limit everything. Minimum inhibitory concentrations are determined by twofold dilution, so that a single measurement is uncertain by one dilution either way, and values from different laboratories may differ further when media, inoculum and endpoint reading are not harmonised [30]. Models that combine data from many papers inherit this noise. Testing under a single standard method, with reference strains and quality control organisms run alongside, would raise the ceiling on what any model can achieve [10, 30]. Docking should be treated as a hypothesis generator, not a verdict. Scoring functions often fail to rank analogues that differ by a single methylene group. Entropy and desolvation terms are particularly hard to estimate.

6. LIMITATIONS AND FUTURE DIRECTIONS

The available QSAR studies share several weaknesses. Datasets are often small, usually fewer than fifty compounds, and drawn from a narrow chemical series, which limits extrapolation [10, 11, 24]. Activity against resistant organisms is frequently reported for a single strain rather than for a defined panel with known genotype, and the link between descriptors and resistance mechanism is therefore inferred more than demonstrated [14, 15, 19]. Few studies test their predictions by synthesis and measurement of new compounds [10, 26]. Reviews of the field have also noted publication bias, in that models with disappointing performance rarely appear in print [10, 11]. Data sharing in standard formats, with structures, strain identifiers and the exact assay method for each value, would let groups pool results sensibly [10, 30]. These weaknesses are not unique to quinolone work but are particularly acute here [10, 11].

Progress will likely come from larger and better-curated datasets, from models that treat efflux and target affinity as separate contributions, and from combination of QSAR with structure-based methods [10, 13, 29]. Prospective testing against genotyped MRSA panels, with published protocols and negative results, would give the field the evidence it still lacks. Until then, QSAR is best treated as a guide for prioritising synthesis, not as a replacement for microbiological testing [10, 11, 30]. Until these steps become routine, comparisons across published models will remain difficult to interpret [10, 24]. Regulators and clinicians will judge any new analogue by animal and clinical data, not by prediction, so QSAR remains a first filter and never a final one [3, 12]. A pragmatic plan would combine modelling, synthesis and standard susceptibility testing in one cycle [10, 30].

An additional point concerns stewardship. Even a potent new analogue would face the same selective pressure that eroded its predecessors, so design for resistance suppression, such as balanced dual targeting, should be considered at the outset and not after clinical use begins [5, 16]. Quality control strains should be tested in every batch. Reporting the original method alongside each value is essential. Resistance suppression is as important as raw potency.

7. CONCLUSION

The literature suggests that QSAR can usefully organise what is already known about fluoroquinolone structure and activity, but that models of potency against multidrug-resistant S. aureus are still limited by data, not by statistics. Substituents at N-1, C-6, C-7 and C-8 are well understood qualitatively, and descriptors for lipophilicity,

charge and shape can capture much of the series trend [1, 2, 9]. What remains poorly represented is the interplay between mutant targets and efflux that defines resistant isolates [14, 18, 19].

Anyone reading or building such a model should ask a short list of questions. Were the activity data measured by one standard method against defined strains [30]? Was external validation done and was an applicability domain declared [12, 25]? Did the descriptors have a plausible mechanistic meaning [10, 11]? Where the answers are yes, the model may be a sensible aid to design. Where they are not, it should be read as a hypothesis. Wider adoption of shared data and common protocols would make that distinction easier to apply.

8. ABBREVIATIONS

QSAR-Quantitative structure-activity relationship, MRSA-Methicillin-resistant Staphylococcus aureus, MIC-Minimum inhibitory concentration, CoMFA-Comparative molecular field analysis, CoMSIA-Comparative molecular similarity indices analysis, OECD-Organisation for Economic Co-operation and Development, PLS-Partial least squares, DNA-Deoxyribonucleic acid, CLSI-Clinical and Laboratory Standards Institute

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Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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