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PAPER

Electrostatic properties of nine fluoroquinolone antibiotics derived directly from their crystal structure refinements†

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This article discusses the relevance of the similarity of the molecular electrostatic potential for rational drug design and optimization of lead structures on the basis of a comparison of nine fluoroquinolone antibiotics. Molecular electrostatic potentials and dipole moments were calculated directly from the electron-density distribution after crystal structure refinement with invarioms, theoretical non-spherical scattering factors. Deposited, newly determined and re-measured single-crystal diffraction data of varying quality were evaluated in this manner. An *in silico* validation procedure for invariom database entries is presented. Despite differences in their substitution pattern, molecular electrostatic potentials for molecules with the same protonation states show a striking degree of similarity for the whole group of compounds studied. The rapid calculation of electrostatic potentials directly from the invariom database makes the procedure suitable for high-throughput screening.

1. Introduction

Detailed understanding of antibacterial action as well as multi-drug resistance mechanisms on the level of molecular structure is currently emerging for the fluoroquinolone class of anti-infective agents—an understanding that enables new success in structure-based drug design on novel bacterial topoisomerase inhibitors.¹ Such successes are urgently needed, since bacterial resistance to established broad-spectrum antibiotics is increasing.² A review article summarizes the development and classification of bacterial topoisomerase inhibitors up until 2005.³ Fluoroquinolone drug molecules inhibit the enzymes gyrase and topoisomerase IV, which are part of the topoisomerase IIA subfamily. Both enzymes occur only in bacteria and are not present in eucaryotes, which makes them ideal drug targets. The function of topoisomerases is to decatenate DNA and to relax positive super-coils.

Fluoroquinolone antibiotics are remarkable for several reasons. They are fully synthetic, rather than derived from a natural compound, exhibit a broad antibacterial spectrum based on a unique mechanism of action, show favorable pharmacokinetic properties and a low incidence of adverse reactions, and they evince good absorption and tissue distribution after oral administration. For structural and comparative studies it is favorable that they are similar in size and shape. Not only are their molecular structures well known, but macromolecular receptor binding sites were also recently studied.¹ Despite the fact that aspects of modeling their electron-density distribution play

a major role in this paper, our motivation is to study the link between drug-receptor interaction and the electrostatic properties of these molecules on a structural level.

We recently optimized the procedures involved in the invariom approach,^{4,5} extending coverage beyond oligopeptide and protein molecules to most chemical environments present in organic chemistry. ‘Invarioms’ are non-spherical scattering factors for use in crystal-structure refinement. They reproduce molecular electron density almost completely and replace the conventional scattering factors of the commonly used independent atom model (IAM). A fitting example of the potential chemical complexity that can now be dealt with are hetero-aromatic structures like the fluoroquinolone antibiotics. The invariom database⁶ was extended and now contains all entries required for such molecules. Achieving this coverage mainly required a modification of the invariom notation, especially for aromatic ring systems. The notation also allows higher success rates in automatic scattering factor assignment with the preprocessor program INVARIOMTOOL.⁷ The functionality of INVARIOMTOOL was implemented in the open-source molecular viewing program MOLECOOLQT,⁸ a graphical user interface which allows manually to change the bond distinguishing parameter χ . This facilitates scattering factor assignment. Full details on the improvements and the extension of the new database will be reported in a forthcoming paper.

For the research discussed here we used invarioms for least-squares structure refinement of several molecules of the fluoroquinolone type. They include ciprofloxacin (cipro),^{9–12} enoxacin (eno),^{13,14} lomefloxacin (lome),^{15,16} ofloxacin (oflox),^{17–20} norfloxacin (nor),^{21–27} sparfloxacin (spar),^{28,29} fleroxacin (fleo),³⁰ moxifloxacin (moxi)³¹ and nadifloxacin (nadi).³² A common atomic naming scheme was chosen for our structural

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Pharmacophore	Name	X ₈	R ₁	R ₅	R ₇
Ciprofloxacin	CH		H
	Enoxacin	N		H
	Fleroxacin	CF		H
	Lomefloxacin	CF		H
	Nadifloxacin			H
	Norfloxacin	CH		H
	Moxifloxacin			H
	Ofloxacin			H
	Sparfloxacin	CF		NH ₂

Fig. 1 Overview of different substitution patterns of the pharmacophore in nine fluoroquinolone antibiotics reviewed in this study.

studies. Fig. 1 summarizes how these molecules differ chemically in their functional groups at R₁, R₅, R₇ and X₈.

The aim of this study was twofold: We generated accurate structural information for several fluoroquinolone antibiotics by invariom refinement of the experimental Bragg data for comparison of derived dipole moments and electrostatic potentials. The comparison of molecular electrostatic potentials has important implications on their function as anti-infective agents. Secondly, we wanted to address how well the invariom database reproduces the electron density of a fluoroquinolone structure in the gas phase. For that purpose we geometry-optimized a subset of the molecules mentioned above on the B3LYP level of theory with the same (rather extended) basis set D95++(3df,3pd)³³ that is used for geometry optimization of model compounds in the invariom database. We also compared gas-phase and solid-state electrostatics. These comparisons allowed us to validate the performance of the invariom database in reproducing a theoretical electron density by pseudoatom fragments, thereby establishing a benchmark procedure. Ultimately we want to extend the reach and contribution of small-molecule crystallography and especially charge-density research to structure-based drug design. We intend to use the structural information gained here for future investigations on fluoroquinolone antibiotics on the drug-receptor level.

2. Experimental

Twelve crystal structures were studied. In cases of ciprofloxacin, enoxacin and norfloxacin two structures of the same molecule, differing only in solvation or protonation state, were investigated. The single-crystal structure of lomefloxacin hydrochloride tetrahydrate included in our analysis has not been reported before. Due to the minuscule crystal dimensions, structure determination required the high primary beam intensity of synchrotron radiation. The measurement was carried out at the

Swiss Light Source (SLS) beam line X10SA. The wavelength was chosen to be 0.8000 Å and data were measured up to a resolution of $\sin\theta/\lambda_{\max} = 0.54 \text{ \AA}^{-1}$. Diffraction data were re-measured in-house for enoxacin 1.7 H₂O, nadifloxacin 0.5 H₂O, norfloxacin anhydrate, norfloxacin 2 H₂O and ofloxacin anhydrate. Three different diffractometers were used: a Bruker APEX II diffractometer with an Incoatec microsource with Ag-K α radiation, a similar machine with Mo-K α radiation and a Bruker SMART 6000 diffractometer with a Cu-K α rotating anode. All three machines have focusing optics from Incoatec and are equipped with an open-flow nitrogen gas stream cooling device. All measured data sets were collected at a sample temperature of 100 K. For enoxacin 1.7 H₂O it turned out that the published structural model by Yoon *et al.*¹³ was inadequate (CSD/cif-code: ITEKUN). We found that the previously reported methanol solvate is in fact a water solvate with two full, two half and one 40% occupied water site. This finding was substantiated by re-collecting low-temperature data to high resolution, which were measured up to a resolution of $\sin\theta/\lambda_{\max} = 1.02 \text{ \AA}^{-1}$ with an overall coverage of 99.8% and an internal *R*-factor of 4.2%. Crystallographic data of all re-measured datasets and of the structure determination of lomefloxacin are listed in Table 1. Additional information on the measured structures can be found in the CIF files and the supplementary information.† Deposited X-ray single-crystal diffraction data from the original authors were used in re-refinements with invarioms. Intensity data for enoxacin 3 H₂O, fleroxacin H₂O HCl, moxifloxacin HCl 0.5 H₂O 0.5 CH₃OH and sparfloxacin 3 H₂O were downloaded from the web site of the IUCr journals *Acta Crystallographica, Section C* or *E*. In the case of ciprofloxacin hexahydrate, the data fulfil the requirements for a charge density study. Such a study was recently reported³⁴ together with a re-determination of ciprofloxacin hydrochloride 1.4 H₂O. Tables of IAM/invariom *R*-factor comparisons, data resolution, difference electron density

Table 1 Crystal and structure refinement data of (formerly unknown) lomefloxacin solvate and five re-determinations of fluoroquinolone antibiotics. Enoxacin 1.7 H₂O has previously been reported as the methanol solvate.¹³ (CSD/cif-code: ITEKUN)

Chemical name	Enoxacin	Lomefloxacin	Nadifloxacin	Norfloxacin	Norfloxacin	Ofloxacin
Empirical formula	C ₁₅ H ₁₇ FN ₄ O ₃ ·1.7H ₂ O	C ₁₇ H ₂₀ F ₂ N ₃ O ₃ ·2H ₂ O·0.95Cl	C ₁₉ H ₂₁ FN ₂ O ₄ ·0.5H ₂ O	C ₁₆ H ₁₈ FN ₃ O ₃	C ₁₆ H ₁₈ FN ₃ O ₃ ·2H ₂ O	C ₁₈ H ₂₀ FN ₃ O ₄
Formula weight	351.28	422.21	369.385	319.33	355.37	361.37
Crystal system	Monoclinic	Monoclinic	Monoclinic	Triclinic	Monoclinic	Monoclinic
Space group	<i>P</i> ₂ ₁ / <i>c</i> (No. 14)	<i>P</i> ₂ ₁ / <i>c</i> (No. 14)	<i>P</i> ₂ ₁ / <i>n</i> (No. 14)	<i>P</i> ₁ (No. 2)	<i>P</i> ₂ ₁ / <i>c</i> (No. 14)	<i>C</i> ₂ / <i>c</i> (No. 15)
<i>Z</i>	8	4	4	2	4	8
<i>a</i> /Å	8.4124(6)	5.200(1)	10.7365(5)	4.2600(6)	8.2477(6)	30.073(6)
<i>b</i> /Å	22.0645(15)	39.960(8)	17.1268(8)	9.7217(12)	21.6745(17)	6.7129(14)
<i>c</i> /Å	17.1587(12)	9.380(2)	18.7756(8)	17.862(3)	9.5199(7)	16.722(3)
α [°]	90	90	90	78.225(8)	90	90
β [°]	100.896(1)	90.55(3)	91.203(3)	87.712(9)	110.692(1)	104.76(3)
γ [°]	90	90	90	79.626(7)	90	90
<i>V</i> /Å ³	3127.5(4)	1949.0(7)	3451.7(3)	712.34(18)	1592.0(2)	3264.4(12)
Density [g cm ⁻³]	1.492	1.439	1.422	1.489	1.483	1.471
μ [mm]	0.120	0.249	0.912	0.949	0.118	0.069
<i>F</i> (000)	1481	885	1560	336	752	1520
Crystal size/mm	0.08 × 0.11 × 0.21	0.01 × 0.01 × 0.05	0.05 × 0.07 × 0.12	0.04 × 0.04 × 0.10	0.05 × 0.05 × 0.20	0.17 × 0.19 × 0.20
Data collection						
<i>T</i> /K	100	100	100	100	100	100
Radiation type, λ /Å	Mo-K α , 0.7107	Synchrotron, 0.8000	Cu-K α , 1.5418	Cu-K α , 1.5418	Mo-K α , 0.7107	Ag-K α , 0.5608
$\theta_{\min}$, $\theta_{\max}$ [°]	1.5, 46.5	2.3, 25.8	3.5, 71.7	2.5, 67.3	1.9, 33.3	1.1, 26.0
($\sin\theta/\lambda$) _{max} [Å ⁻¹]	1.02	0.54	0.62	0.60	0.77	0.78
Tot., Uniq. reflections	493757, 27877	18001, 2299	61505, 6659	16718, 2479	32868, 6042	84774, 6517
Observed refl. [<i>I</i> > 3 σ (<i>I</i>)]	17859	2141	4901	1990	4059	4908
Overall completeness [%]	99.8	87.4	98.6	96.3	98.6	99.8
Redundancy	17.38	6.69	8.99	6.21	5.32	12.27
<i>R</i> _{int}	0.042	0.086	0.033	0.075	0.037	0.054
<i>N</i> _{ref} , <i>N</i> _{par}	17859, 602	2141, 243	4901, 478	1990, 226	4059, 292	4908, 255
<i>R</i> _w , <i>wR</i>	0.0273, 0.0172	0.0703, 0.1056	0.0484, 0.0557	0.1155, 0.1918	0.0311, 0.0261	0.0346, 0.0344
<i>S</i>	2.78	3.91	3.09	1.59	2.18	2.73
$\Delta\rho_{\min}$, $\Delta\rho_{\max}$ [e Å ⁻³]	−0.27, 0.38	−0.44, 0.31	−0.25, 1.03	−0.50, 0.89	−0.22, 0.29	−0.29, 0.43

and the weighting scheme[‡] used are listed for all twelve datasets in the supplementary information.[†]

3. Methods

3.1. Structure-factor modeling and least-squares refinements

The structure of the new solvent of lomefloxacin was solved by direct methods with the program SHELXS³⁶ and crystallizes in the space group *P*₂₁/*c*. Due to disorder of the piperazine ring as characterized by an overlay of both enantiomers, the structure was also solved and tentatively refined in space group *P*₂₁. However, the disorder remained present in the lower symmetry space group and rendered the refinement of anisotropic displacement parameters (ADPs) instable. Hence, space group *P*₂₁/*c* is the correct choice. Structures that were known existing from literature were initially re-refined with the program SHELXL.³⁶ A common atomic naming scheme for the pharmacophore—as present in all antibiotics—was employed. This naming scheme is based on IUPAC notation of unsubstituted quinoline. The program XD³⁷ was used for further spherical and invariom full-matrix least-squares refinements. Values for anomalous

dispersion were adjusted manually. For all atoms including chloride ions the radial electron-density distribution of atomic wavefunctions as tabulated by Clementi and Roetti³⁸ was used for the scattering factor. The criterion for observed reflections was *I* > 3 σ (*I*), the default value in the program XDLSM, the least-squares program of the XD package.³⁷ After initially repeating the independent atom model (IAM) refinement in XDLSM, non-spherical scattering factors of a revised version of the invariom database were assigned to each atom in the crystal structures studied by using the program INVARIOMTOOL.⁷ Details of all model compounds used to obtain invarioms can be found in the supplementary information[†] and are illustrated in Fig. 2 using the example of the pharmacophore of enoxacin. The relevant INVARIOM, SMILES[§] and IUPAC names are also given.

Invariom names contain only information on the local chemical environment of an atom. This is in contrast to SMILES and IUPAC notations which generally describe the whole molecule¶.

§ The abbreviation SMILES stands for ‘Simplified Molecular Input Line Entry System’ and was introduced by D. Weininger.³⁹

¶ In the invariom approach we generate model compounds based on local chemical environments. Empirical rules are used for that purpose. The scattering factor database for the fluoroquinolone compounds studied in this paper contained several modifications and improvements that have not yet been published. This revision of the invariom database will be the subject of a subsequent paper. A cross-validation of the empirical rules is presented in section 3.3.

‡ Refinement weight parameters were manually adjusted for invariom refinement with the program DRKPLOT, which is implemented in the program WINGX.³⁵

Fig. 2 A depiction of model compounds used to reproduce aspherical electron density for enoxacin from pseudoatom fragments. Three types of linear notations are listed: INVARIOM, SMILES and IUPAC. While SMILES and IUPAC name a whole molecule, the invariom notation describes the chemical environment of an atom. A suitable model compound reproduces the atomic environment on the level of the electron-density distribution.

After having assigned suitable scattering factors and their local atomic coordinate systems, least-squares refinement was repeated, giving better figures of merit (see Table 1 and the supplementary information†), an increased accuracy for geometries, smaller standard deviations of refined parameters and physically more meaningful anisotropic displacement parameters, as has been shown for a number of examples in earlier studies.^{5,40}

3.2. Parameterisation

Depending on the number of reflections collected for the individual datasets and the stability of refinements, a varying number of parameters could be afforded. Hence, models used for the different datasets differ in their sophistication. Only for the two structures of ciprofloxacin 6 H₂O and enoxacin 1.7 H₂O a high resolution (exceeding $(\sin\theta/\lambda)_{\max} = 1.0 \text{ \AA}^{-1}$) was reached. A free multipole refinement would have been possible and was only carried out for enoxacin (see section 4.5). Details of an earlier multipole refinement of ciprofloxacin 6 H₂O were given before.³⁴ Nuclear positions as well as displacement parameters of non-hydrogen atoms were refined anisotropically for both molecules. In addition, the anisotropic displacements of hydrogen atoms were estimated for both datasets with the SHADE^{241,42} server. For hydrogen-atom treatment in enoxacin 1.7 H₂O, both main molecules present in the asymmetric unit were divided into two fragments. Whereas the first fragment consisted of the pharmacophore and the rest groups R1, R5 and X8, the piperazine ring was chosen to be the second rigid group (see Fig. 1). For each rigid group a TLS-analysis (translation, libration, screw) was carried out separately. This allowed the estimation of anisotropic displacement parameters of hydrogen atoms attached to the respective rigid group. For the low-resolution data set of lomefloxacin only positional and displacement parameters of the

non-disordered part were refined in XD, while maintaining the restrained displacement parameters from a preceding SHELXL-refinement for the disordered part. X–H distances were set to values stored in the invariom database and kept fixed. This procedure is equivalent to setting X–H bond distances to standard values from neutron diffraction,⁴³ but has the additional advantage of being fully automated in INVARIOMTOOL. For the data set of nadifloxacin, isotropic displacement parameters for hydrogen atoms were not refined, but constrained to values of 1.5 (OH and CH₃ groups) or 1.2 (CH₂, C_{arom}H and CH groups) times the value of the U_{equiv} of the parent atom they were riding on. Non-hydrogen atoms were refined anisotropically. X–H distances were set to values obtained from the invariom database, constraining H-atom shifts to their parent atom and maintaining the relative starting positions from SHELXL. For the dataset of moxifloxacin X–H distances were also set to theoretical values from the invariom database and kept fixed. Displacement parameters of hydrogen atoms were treated as isotropic, those of non-hydrogen atoms as anisotropic. For all remaining datasets (ciprofloxacin HCl 1.4 H₂O, enoxacin 3 H₂O, fleroxacin H₂O HCl, moxifloxacin HCl 0.5 H₂O 0.5 CH₃OH, ofloxacin anhydrate and sparfloxacin 3 H₂O) X–H distances were likewise set to values from the invariom database. Shifts of hydrogen positions were constrained to their parent non-hydrogen atoms. Displacement parameters of all non-hydrogen atoms were refined anisotropically; hydrogens were treated as isotropic.

3.3. Cross-validation of database entries

The invariom formalism relies on geometry optimizations of model compounds, which are representative for an atom of interest in a particular chemical environment. Model compounds are selected by the characteristics of the environment the atom of

interest is situated in—either one or two spheres of neighboring atoms are included, or, in case of de-localization, a suitable de-localized system is selected. Electron-density partitioning into pseudoatom fragments relies on the Hansen/Coppens multipole model⁴⁴ and involves a ‘projection’ of the quantum-mechanical molecular electron density into structure factors.⁴⁵ Partitioning into pseudoatoms makes the electron-density transferable so that it can be used in different molecules with changing conformations.

In each of the steps involved approximations are made and numerical inaccuracies can occur. Although transferability and reproducibility have been studied, *e.g.* by bond-topological and atomic properties for tripeptides,⁴⁶ a proof for the accuracy of the empirical approach using model compounds in complex chemical environments—like that found in the heteroaromatic ring systems of the fluoroquinolone antibiotics—was lacking. Especially the choice of fragments is non-trivial, since these systems are aromatic and contain heteroatoms N, O and F, which give rise to inductive and mesomeric effects. The extent of de-localization is increased by the zwitterionic nature of the fluoroquinolones.³ Our method of general selection of model compounds for complex chemical environments, as present *e.g.* in fluoroquinolone antibiotics, will be explained in detail in a forthcoming paper.

We next studied the precision of the invariom formalism in reproducing molecular electron density by summing up pseudoatom contributions. The following procedure was used: Initially we performed a geometry optimization of the whole fluoroquinolone molecule. We chose the same basis set and level of theory as for generating the invariom database entries. After having obtained an energy minimum for the geometry, a frequency calculation ensured the location of the global minimum. Such calculations can be quite time consuming. They can take weeks on current computers, even when multiple processors are used. Subsequently, the electron density from the optimization (as stored in a wavefunction file) was placed in an artificial unit cell with $\vec{a} = \vec{b} = \vec{c} = 30 \text{ \AA}$ in space group $P\bar{1}$. An analytical Fourier transform⁴⁷ with the program TONTO⁴⁸ was subsequently carried out to give a set of structure factors. A multipole model was fitted to these structure factors, yielding an analytical model for the molecular electron-density distribution within the Hansen/Coppens multipole model. It is this electron-density distribution that we aim to reproduce on the basis of pseudoatoms of the invariom database, thereby allowing database validation by difference electron-density calculation of multipole refinement minus invariom refinement on a grid. For enoxacin, where high-resolution diffraction data were measured, Bragg data were also evaluated in this manner, serving as experimental reference (see section 4.5).

3.4. Dipole moment comparison of experiment and theory, electrostatic potentials

Dipole moments⁴⁹ can be obtained directly after invariom refinement from the multipole parameters assigned. Quantum chemical single-point energy calculations can give values for comparison. Using the experimental geometry such calculations were performed with the density functional B3LYP and the basis set D95++(3df,3pd). Since some of the molecules studied were

positively charged, and since the dipole moment is origin-dependent for charged species,⁵⁰ we used the program TONTO⁴⁸ in order to ‘project’ their isolated-molecular density onto theoretical structure factors.⁴⁵ A multipole refinement was subsequently carried out against those theoretical structure factors and solvent molecules or counter-ions were omitted in this procedure. This sequence ensured that the same density model and origin were used to calculate dipole moments from the single-point calculations. The three-dimensional electron-density distribution from invariom refinement was evaluated according to the method of Su and Coppens in calculations of the molecular electrostatic potential.⁵¹

4. Results and discussion

4.1. Non-spherical atom refinements

Non-spherical atomic scattering factors (invarioms) were automatically assigned from the invariom database for all nine chemically different fluoroquinolone molecules. The number of parameters in the least-squares refinement did not increase in this procedure. Hence, using invarioms was possible also for middle- or low-resolution datasets of varying quality. Even in the borderline case of the disordered structure of lomefloxacin invarioms were used successfully. The precise reconstruction of molecular electron density from invariom fragments leads to a better fit of calculated and observed structure factors in all twelve structures studied (see Table 1 and the supplementary information†). Our main objective was to calculate molecular properties from the invariom electron density. We will discuss those properties in the following sections||.

4.2. Molecular electrostatic potentials

Fig. 3, 4 and 5 show the electrostatic potentials (ESP) of the twelve fluoroquinolone antibiotics, which are neutral, cationic or zwitterionic in the crystalline solid state. The similarity of molecular dimensions and—more importantly—of the ESP for each ionization state is striking. Having studied only active molecules of the same class of compound that share a very similar activity,⁵² we can conclude that high similarity in both molecular dimensions and ESP are particular features and a prerequisite of biological activity of these drug molecules**. This finding leads us to suspect that being able to design a similar alternative to a known active drug molecule or drug candidate therefore would primarily require matching its molecular dimensions and ESP. Hence, this study provides an example for the rationale behind the common ‘me-too’ approach in the pharmaceutical industry,⁵³ where the aim is to match (and possibly improve) the properties of an existing drug molecule without having to repeat the resource-hungry development process. This aspect is also highly relevant for research on the problem of bacterial resistance where existing drugs need to be

|| It should be mentioned that the calculation of molecular properties within the invariom approach is not limited to crystallographic studies. Geometries generated from 2D-NMR, force field or low-level quantum chemical calculations are alternative starting points.

** This is remarkable, because the presence of different chemical groups usually leads to different electrostatics. We see that this is not the case for the fluoroquinolones.

Fig. 3 Uncharged molecules: ESP mapped on the van der Waals surface for structures of (a) nadi 0.5 H₂O, (b) nor anhydrate and (c) oflox anhydrate. The solvent water molecule of nadi 0.5 H₂O was excluded from the calculation. Values of the ESP are given in e Å⁻¹.

modified in response to increasing resistance against anti-infective agents.² To match dimensions and ESP of an existing drug can therefore be seen as a strategy in rational drug design. Empirically this recipe is already being followed when improving bio-availability and pharmacokinetic properties of drug molecules during lead optimization. These aspects are

fittingly illustrated with the example of the fluoroquinolone antibiotics.

Evidently, the ESP may also be generated from high-level *ab initio* calculations. The experimental approach followed here still has the advantage that the conformation observed in the crystal can be directly investigated—almost as a by-product—of

Fig. 4 Cationic molecules: ESP mapped on the van der Waals surface for structures of (a) cipro HCl 1.4 H₂O, (b) flero 3H₂O HCl, (c) lome 4 H₂O HCl and (d) moxi HCl 0.5 H₂O 0.5 CH₃OH. Solvent molecules/ions were excluded from calculations. Values of the ESP are given in e Å⁻¹.

Fig. 5 Zwitterionic molecules: ESP mapped on the van der Waals surface for structures of (a) cipro 6 H₂O, (b) enox 1.7 H₂O, (c) enox 3 H₂O, (d) nor 2 H₂O and (e) spar 3 H₂O. Solvent water molecules were excluded from calculations. Values of the ESP are given in e Å⁻¹.

invariom refinement itself. Moreover, ESP calculations from pseudoatoms are rapid. By contrast, the molecular size of fluoroquinolones alone makes geometry optimization, where extended basis-sets are desired, computationally an expensive proposition^{††}. Last but not least, theoretical approaches meet problems in the charged or zwitterionic nature of fluoroquinolone molecules. In such cases, often conformations are obtained that must be considered unrealistic in biological environments with high dielectric constants.

4.3. Politzer analysis

We performed a quantitative analysis of the ESP following the procedure of Politzer and coworkers.⁵⁴

^{††} For molecules having a high degree of conformational flexibility it is generally difficult to find the global minimum. Starting with the conformation present in the crystal is therefore attractive for geometry optimizations. This phenomenon can best be illustrated for moxifloxacin, where pre-optimizations did not allow us to find the global energetic minimum, whereas starting from the geometry present in the crystal after invariom refinement did.

$$\overline{V}_S^+ = \frac{1}{n} \sum_{i=1}^n V_S^+(\mathbf{r}_i), \quad (1)$$

$$\overline{V}_S^- = \frac{1}{m} \sum_{i=1}^m V_S^-(\mathbf{r}_i), \quad (2)$$

$$\overline{V}_S = \frac{1}{m+n} \left[\sum_{i=1}^n V_S^+(\mathbf{r}_i) + \sum_{i=1}^m V_S^-(\mathbf{r}_i) \right]. \quad (3)$$

The positive, negative and overall average potential values $\overline{V}_S^+$, $\overline{V}_S^-$ and $\overline{V}_S$ on the surface were calculated as given in eqn (1)–(3), where n and m are the number of grid points with positive and negative potential. The average deviation from the overall potential on the surface is Π :

$$\Pi = \frac{1}{m+n} \sum_{i=1}^{m+n} |V_S(\mathbf{r}_i) - \overline{V}_S|. \quad (4)$$

The total variance of the surface potential is defined as a sum of the positive and negative variance of the surface potential:

Table 2 The ESP was mapped on the van der Waals surface. Twelve fluoroquinolone structures were compared following the method developed by Politzer *et al.* Structures are grouped according to their formal charges. $\bar{V}_S^+$ is the average positive, $\bar{V}_S^-$ the average negative overall potential. Π denotes the average deviation of the overall potential and σ_{tot}^2 the total variance of the surface potential

Politzer Analysis	Fluoroquinolone Solvate	Uncharged structures				Cationic structures				Zwitterionic structures				
		nadi 0.5 H ₂ O	nor —	oflox —	cipro HCl	flero H ₂ O HCl	lome 4 H ₂ O HCl	moxi HCl 0.5 H ₂ O 0.5 CH ₃ OH		cipro 6 H ₂ O	eno 3.4 H ₂ O	eno 3 H ₂ O	nor 2 H ₂ O	spar 3 H ₂ O
	Formal Charge	0	0	0	+	+	+	+		+-	+-	+-	+-	+-
	Distribution													
	$\bar{V}_S^+[\text{e } \text{\AA}^{-1}]$	0.069	0.073	0.057	0.087	0.083	0.083	0.072		0.132	0.121	0.127	0.136	0.105
	$\bar{V}_S^-[\text{e } \text{\AA}^{-1}]$	-0.060	-0.060	-0.059	-0.084	-0.076	-0.076	-0.074		-0.138	-0.131	-0.136	-0.138	-0.126
	$\Pi[\text{e } \text{\AA}^{-1}]$	0.064	0.067	0.058	0.085	0.080	0.079	0.073		0.135	0.125	0.130	0.137	0.114
	$\sigma_{\text{tot}}^2[\text{e}^2 \text{\AA}^{-2}]$	0.011	0.012	0.009	0.022	0.017	0.020	0.017		0.053	0.049	0.052	0.053	0.043

Table 3 Dipole moment magnitudes of twelve fluoroquinolone structures from (a) invariom pseudoatom density fragments (INV) (b) single-point calculations (SP) from experimental geometries (c) multipole projections (MP) of these single-point calculations. All values were calculated for the molecular center of mass as the choice of origin and are given in Debye. Solvent molecules were excluded from calculations

Dipole moment	Fluoroquinolone Solvate	Uncharged structures				Cationic structures				Zwitterionic structures				
		nadi 0.5 H ₂ O	nor —	oflox —	cipro HCl	flero H ₂ O HCl	lome 2 H ₂ O HCl	moxi HCl 0.5 H ₂ O 0.5 CH ₃ OH		cipro 6 H ₂ O	eno 1.72 H ₂ O	eno 3 H ₂ O	nor 2 H ₂ O	spar 3 H ₂ O
	Formal Charge	0	0	0	+	+	+	+		+-	+-	+-	+-	+-
	Distribution													
	Invariom	13.9	13.8	13.4	24.1	22.4	23.4	24.0		39.4	37.5	37.6	38.4	37.4
	Single Point (SP)	10.3	11.0	10.8	—	—	—	—		40.9	40.4	40.7	41.0	42.9
	Calculation													
	Multipole Projection (MP) of SP	15.3	12.8	12.6	28.7	28.1	26.9	29.6		37.7	36.6	37.4	37.7	37.7
	Difference (INV - MP)	-1.4	1.0	0.8	-4.6	-5.7	-3.5	-5.6		1.7	0.9	0.2	0.7	-0.3
	Divergence	-9.2	7.8	6.3	-16.0	-20.3	13.0	-18.9		4.5	2.4	0.5	1.9	-0.8
	[(INV - MP)/MP] [%]													

$$\sigma_{\text{tot}}^2 = \sigma_+^2 + \sigma_-^2 = \frac{1}{n} \sum_{i=1}^n [V_S^+(r_i) - \bar{V}_S^+]^2 + \frac{1}{m} \sum_{i=1}^m [V_S^-(r_i) - \bar{V}_S^-]^2 \quad (5)$$

This type of analysis was implemented in the program MOLISO and previously carried out in charge density studies of the nucleoside thymidine⁵⁵ and the macrolide antibiotic roxithromycin.⁵⁶ We can see from values given in Table 2 that results from Politzer analysis are strongly correlated with the protonation state of the molecules. Values for the same protonation state agree quite well for $\bar{V}_S^+$, $\bar{V}_S^-$ and Π . These numbers can hence be used for preliminary high-throughput screening of a larger number of molecules. However, stereochemical information can not be entirely ignored. An example would be 17- α or 17- β -estradiol,⁵⁷ where inversion of one stereocenter can lead to significantly different biological activity. More generally, pairs of enantiomers might have a very similar range of $\bar{V}_S^+$, $\bar{V}_S^-$ and Π , so that the 3D-information of the ESP remains highly relevant. An important point to make is that the procedures involved generate same quality results for data sets of good as well as low quality, and are independent of data resolution.

4.4. Dipole moments

Dipole moments were calculated directly from the multipole parameters using the geometry after invariom refinement. In order to assess the quality of the dipole moments obtained,

single-point energy calculations were performed for comparison. In addition, the molecular electron density from the single-point energy calculation was projected onto the multiple model. Hence a set of three different dipole moments, invariom (INV), single-point (SP) and multipole projection of the single-point calculations (MP), were obtained for each molecule. Since the dipole moment of the entity considered is only independent of the choice of origin when the entity is neutral,⁵⁸ only two values, INV and MP are given for cationic structures^{††}. A projection of molecular electron density onto the multiple model circumvents that problem. An additional advantage of the projection procedure is that we can compare the influence of the density representation, either the multiple model or a quantum chemical basis set, in this manner.

A comparison of the dipole moment magnitude as given in Table 3 can be carried out either directly, comparing the absolute value of the dipole moment of the respective molecule, or in terms of the percentage difference between invariom (INV) and single-point multipole projection (MP). The direct comparison shows a good agreement of the magnitude of the dipole moment in each case. As before in the analysis of the electrostatic potential, dipole moments of different fluoroquinolone

^{††} This is due to the fact that for charged species the program GAUSSIAN does not follow the convention to use the center of mass, and that is why SP values are not given in these cases.

antibiotics agree especially well in those cases, where the molecular charge is alike, *i.e.* for neutral, cationic or zwitterionic molecules.

For the difference in percent we see a favorable agreement especially for zwitterionic structures, where the agreement is below 5%. This difference increases to better than 10% for uncharged molecules. For cationic structures the difference can be up to 20%. This finding seems to be directly related to the precision with which we manage to reproduce the molecular electron density by invariom fragments. Especially the unmodeled density of the carboxyl group will make a contribution to the differences seen. A trend that structures of higher quality (better *R*-factor) show a better agreement can also be observed. Furthermore, the relative difference between the two values compared will be influenced by the magnitude of the dipole moment itself, which is highest for zwitterionic molecules. For the purpose of high-throughput screening of a larger number of compounds the accuracy reached by invarioms is acceptable, especially since the time required for obtaining the dipole moment compares very favorably with that of a single-point energy calculation.

4.5. Verification of invariom database entries by experiment

For the validation of database entries we had already used difference electron density plots. A validation was first performed for L-ornithine hydrochloride.⁴⁰ It indicated that the database allowed density reconstruction with a precision better than the experimental noise. The same approach was subsequently used for the methylated non-standard amino acid aminoisobutyric acid⁵⁹ and the macrolide antibiotic roxithromycin.⁵⁶ In the latter two cases difference-density plots turned out to be even more useful, since they enabled characterization of dynamic processes in these crystal structures. Given confidence about the precision of database prediction, the study of interaction density, the difference between the electron density in the gas phase and the solid state, also becomes a possibility. In spite of shortcomings of the Hansen/Coppens multipole model,⁶⁰ which might not allow a chemical interpretation of difference-density features as interaction-density at the current stage,⁶¹ at least a qualitative picture of interaction-density features arises. Difference-density plots are therefore useful for a number of purposes of current interest, similar to the now mostly redundant deformation-density plots.

We carried out a multipole refinement based on the geometry from invariom refinement to calculate such a difference density map. As can be seen in Fig. 6 dynamic processes do not occur for enoxacin in the crystal at a measurement temperature of 100 K. Fig. 6 indicates qualitative agreement between invariom-database electron density and the density obtained from a refinement of multipole parameters as apparent in several transparent iso-surfaces with maximum and minimum values of (only) $0.2 \text{ e } \text{\AA}^{-3}$. One has to be aware that this choice of iso-surface highlights rather small differences—in contrast to common deformation electron-density features that easily exceed $0.7 \text{ e } \text{\AA}^{-3}$. As in the case of L-ornithine hydrochloride, difference-density features seen for enoxacin 1.7 H₂O do not exceed the minimal and maximal residual electron density, which is in the range of $\pm 0.4 \text{ e } \text{\AA}^{-3}$.

Fig. 6 Experimental difference density: multipoles adjusted to the experimental data minus invariom prediction using experimental structure factors of enoxacin 1.7 H₂O using the geometry from invariom refinement in both cases. Difference density plotted as iso-surfaces from -0.2 to $0.2 \text{ e } \text{\AA}^{-3}$ with steps of $0.05 \text{ e } \text{\AA}^{-3}$.

4.6. Verification of invariom database entries by theory

The capacity of the invariom database in reproducing molecular electron density is easily verified by performing an invariom transfer for the optimized molecular geometry of the fluoroquinolone as outlined in section 3.3. Ideally we expect to see almost no difference between the whole-molecular density and the sum of invariom fragments. Fig. 7, 8 and 9 show the difference density of the two distributions on a grid. Since there is no experimental error involved and since we used the same level of the multipole expansion in both cases only the performance of the invariom database is investigated. The results show that the complex chemical environment of the fluoroquinolones can be successfully reproduced by fragments within a precision of $\pm 0.2 \text{ e } \text{\AA}^{-3}$. Highest difference densities occur at the sites of the oxygen atoms. Three explanations are possible:

- Known deficiencies of the multipole model in reproducing fine features near the oxygen atom.^{60–63}
- Intramolecular hydrogen bonds (in case of uncharged and cationic molecules).
- The approximation of invoking nearest-neighbor model compounds for some oxygen atoms.

We assume that approximations of density functional theory can also play a role in charged model compounds. Future studies should show which of these contributions play a significant part. In any case the precision reached is better or equal to the experimental accuracy that can be achieved with the best current high-resolution experiments when the Hansen/Coppens multipole model is used. We consider the invariom approach sufficiently precise to model X-ray diffraction data and to calculate molecular properties within the given error range—particularly since most of today's structure determinations still rely on the dated IAM§§.

5. Conclusion

Electrostatic properties of nine different fluoroquinolone antibiotics were investigated directly from invariom refinements of their crystal structures; the structure of lomefloxacin hydrochloride tetrahydrate is reported for the first time. In the case of

§§ E.s.d.'s of refined parameters are significantly smaller than those from IAM refinement.

Fig. 7 Uncharged molecules: difference densities were calculated from refined minus invariom database pseudoatom parameters. Refinement used theoretical structure factors from a projection of molecular electron density of the whole fluoroquinolone molecule after geometry optimization. Calculated structures have the same protonation state as experimental structures of nadi 0.5 H₂O (a), nor anhydrate (b), and oflox anhydrate (c). Solvent molecules present in the crystal structures were not included in the calculation. Difference density is plotted as iso-surfaces from -0.2 to 0.2 e Å⁻³ with steps of 0.05 e Å⁻³.

enoxacin 1.7 H₂O the structure does not contain methanol (as originally reported by Yoon *et al.*) but water only. Twelve different crystal structures were evaluated in total. Apart from

the structure determination of lomefloxacin, five structures were re-determined while six were obtained from the IUCr archives. In all cases the structural model was improved by using

Fig. 8 Difference-density plots (refined minus invariom) for cationic molecules. The refinement procedure was described in the caption of Fig. 7. Calculated structures have the same protonation state as experimental structures of fero 3H₂O HCl (a), lome 4 H₂O HCl (b), moxi HCl 0.5 H₂O 0.5 CH₃OH (c). Solvent molecules present in the crystal structures were omitted. Difference density is plotted as iso-surfaces from -0.2 to 0.2 e Å⁻³ with steps of 0.05 e Å⁻³.

Fig. 9 Difference-density plots (refined minus invariom) for zwitterionic molecules. The refinement procedure was described in the caption of Fig. 7. Calculated structures have the same protonation state as experimental structures of cipro 6 H₂O (a), enox (1.7H₂O and 3 H₂O) (b), nor 2 H₂O (c) and spar 3 H₂O (d). Solvent molecules present in the crystal were omitted. Difference density is plotted as iso-surfaces from -0.2 to 0.2 e $\text{\AA}^{-3}$ with steps of 0.05 e $\text{\AA}^{-3}$.

non-spherical scattering factors of the invariom database, leading to more accurate bond distances, de-convoluted atomic displacement parameters as well as lower parameter standard deviations and figures of merit. More importantly, the electron-density distribution after invariom refinement allowed a rapid calculation of molecular properties. Dipole moments and molecular electrostatic potentials were calculated from the revised geometries for all fluoroquinolones studied. The similarity of the molecular electrostatic potential for a respective ionization state is striking. We think that such a similarity is the underlying factor for their common biological activity, especially since different conformations do not need to be taken into account due to similar molecular dimensions. Property calculation from invarioms was achieved in a far shorter time period than is required for geometry optimization of whole molecules. Quantitative results from Politzer analysis allow a rapid comparison without the need visually to assess ESP similarity.

Experimental and theoretical validation show that molecular electron density can be reproduced within a precision of ± 0.2 e $\text{\AA}^{-3}$ by invariom refinement, which matches roughly the precision of the Hansen/Coppens multipole model. Results from invariom refinements can hence be considered to be of similar accuracy compared to what can be reached with the best current high-resolution experiments. They are accurate enough to be used for calculation of electrostatic molecular properties. Invariom refinement is possible also for middle- or low-resolution datasets of varying quality. When combining Politzer analyses, the magnitude of the dipole moment and an evaluation of molecular dimensions, procedures employed here are amenable to high-throughput screening of hundreds of compounds in a short time span.

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