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Assessment of biological half life using *in silico* QSPkR approach: A self organizing molecular field analysis (SOMFA) on a series of antimicrobial quinolone drugs

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ABSTRACT

The quinolones belong to a family of synthetic potent broad-spectrum antibiotics and particularly active against gram-negative organisms, especially *Pseudomonas aeruginosa.* A 3D-QSPkR approach has been used to obtain the quantitative structure pharmacokinetic relationship for a series of quinolone drugs using SOMFA. The series consisting of 28 molecules have been investigated for their pharmacokinetic performance using biological half life ($t_{1/2}$). A statistically validated robust model for a diverse group of quinolone drugs having flexibility in structure and pharmacokinetic profile ($t_{1/2}$) obtained using SOMFA having good cross-validated correlation coefficient r^2_{cv} (0.6847), non cross-validated correlation coefficient r^2 values (0.7310) and high *F*-test value (33.9663). Analysis of 3D-QSPkR models through electrostatic and shape grids provide useful information about the shape and electrostatic potential contributions on $t_{1/2}$. The analysis of SOMFA results provide an insight for the generation of novel molecular architecture of quinolones with optimal half life and improved biological profile.

1. Introduction

Quinolones comprise a group of synthetic substances possessing antimicrobial activity against gram-negative organisms, especially *Pseudomonas aeruginosa*. The first quinolone to be marketed was nalidixic acid. Nalidixic acid and Cinoxacin were classified as the first generation quinolones and are mainly used for urinary tract infections. Other clinically used quinolones are norfloxacin, ciprofloxacin, ofloxacin, trovafloxacin, etc. Ciprofloxacin and Levofloxacin dominate the worldwide fluoroquinolone market. Due to enhanced antimicrobial activity, the role of fluoroquinolones has been extended beyond the traditional indications for quinolone antimicrobials in the treatment of urinary tract infections. Quinolone derivatives have effectively capitalized a tremendous role in a wider variety of infectious diseases including skin and respiratory infections (Ambrose et al., 1997).The mechanism of action of quinolones involves inhibiting the action of DNA gyrase and topoisomerase IV and kill bacteria by binding to these enzyme–DNA complexes, thereby disrupting DNA replication (Levine et al., 1998). The major limitations of the quinolones or other antimicrobials is due to increased administration of these drugs which led to the development of resistance in bacteria via various mechanisms such as alterations in target enzymes, bacterial cell permeability, and drug efflux (Ruiz, 2003). However, because of their excellent safety and tolerability, they have become popular alternatives to penicillins and cephalosporins in the treatment of various infections. Thus, the major impetus nowadays is search for the novel candidates having safety, low resistance and good bioavailability profile which further depend on the pharmacokinetic parameters. The rapid and complete absorption of drugs from the gastrointestinal tract and obtaining the peak serum concentrations obtained after oral administration close to intravenous administration has been one of the prime considerations among the researchers (Borcherding et al., 1996).

In a pharmaceutically driven drug discovery process, the major aim of the clinicians to target the molecule at the selective site in order to get maximum therapeutic efficacy without much adverse effects. However, with the increasing challenges in the pharmaceutical research, it has been estimated that the developmental cost of a new entity, starting from the clinical trials to the final approval requires about 8.5 years with a cost of $40 billion and only 21.5% of clinical success rate (http://csdd.tufts.edu/reports/description/rd_single_issues, accessed November 20, 2009) (TCSDD, 2008). Hence, prior to developmental stages of the drug discovery process, it is imperative to use suitable rational computational approaches such as

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in silico QSPkR/QSAR models to shorten the time as well as the precise prediction of the desirable properties required in a drug candidate (Hutter, 2009).

The study and evaluation of the basic pharmacokinetic parameters such as absorption, distribution, metabolism, and elimination (ADME) studies of the molecule in the particular class can help in the assessment of clinical parameters. QSPkR study also act as scientific tool to assess the early features of the pharmacokinetic characteristics of a drug molecule. Mostly quinolones exhibit a large volume of distribution and concentrate in tissues at levels that often exceed serum drug concentrations. Distribution of the quinolones into respiratory tract tissues and fluids is of particular interest due its inhibitory activity against common respiratory pathogens.

Quantitative structure–property relationship (QSPkR) approaches represent one of the robust mathematical tools to analyze the correlation between molecular properties and pharmacokinetic parameters. QSPkR studies enable pharmaceutical scientist to alter the pharmacokinetic properties of a drug without compromising its pharmacodynamic competency. The major advantage of QSPkR studies lies in the fact that once such a relationship is established with adequate statistical degree of confidence, it can be of a valuable assistance in the prediction of the behaviour of new molecules even before they are actually synthesized. Several novel nonlinear machine learning methods have been applied for the prediction of pharmacodynamic and ADME properties. Recently, there have been a number of computer programs to correlate molecules in terms of molecular descriptors and pharmacokinetic parameters that are more meaningful to pharmaceutical scientists. Robinson et al. have developed such a novel three dimensional quantitative structure activity relationship technique called self-organizing molecular field analysis (SOMFA) similar to both comparative molecular field analysis (CoMFA) and molecular similarity studies (Robinson et al., 1999; Cramer et al., 1988).

It is also a grid-based approach however no probe interaction energies are required to be calculated and it predicts intrinsic molecular properties such as the molecular shape and electrostatic potential, which are used to develop QSAR/QSPkR models (Zheng and Li, 2006). It avoids complex statistical tools and variable selection procedures favored by other methods. A successful QSPkR model generates statistically significant relationships between chemical structure and pharmacokinetic properties. The underlying assumption is that the variations of pharmacokinetic properties within a series can be correlated with the changes in the measured or computed molecular features of the molecules. A validated 3D-QSPkR model not only helps in better understanding of the structure–activity relationships of any class of molecules, but also provides researcher an insight at molecular level about the lead molecules for further optimization. Thus, information obtained from 3D-QSPkR analysis provides important guidelines for drug design process.

A SOMFA 3D-QSPkR model could be based on any molecular property; in the present study molecular shape and electrostatic potential have been used. The inherent simplicity of this method allows the possibility of aligning the molecules as an integral part of the model derivation process and of aligning prediction molecules to optimize their predicted activities or pharmacokinetic properties (Du et al., 2003).

The half-life of a drug in plasma or serum is a clinically significant parameter frequently used for indicating the persistence of the drug in its volume of distribution. Furthermore, for designing the new dosage regimen, it is related with duration of clinical effects and frequency of dosing. Thus, clinical suitability of a drug candidate can be implicated through *in silico* approaches instead of using costly, time consuming and ethically stringent *in vivo* protocols. The prediction of $t_{1/2}$ using conventional pharmacokinetic approaches involves exorbitant cost and time-consuming protocols as compared to *in silico* procedures of quantitative structure pharmacokinetic relationship (QSPkR) (Duch et al., 2007).

A series of quinolone drugs consisting of 28 molecules have been investigated *in silico* for their pharmacokinetic performance using biological half life ($t_{1/2}$). Furthermore, the compounds in this class exhibit similar pharmacokinetic characteristics that include mechanism of action and degree of affinity with body tissues. Recently, numerous works for the optimization of molecular architecture using SOMFA (Aggarwal et al., 2010a,b; Thareja et al., 2010a,b,c) have been reported from our research group. The prime objective of the present study is to investigate the *in silico* QSPkR using SOMFA on a quinolone class of drugs extensively employed as antimicrobials for the predictive assessment of $t_{1/2}$.

Table 1
Actual and predicted biological half lives of the quinolone drugs.

Drug	Actual biological $pt_{1/2}$	Predictive biological $pt_{1/2}$ (at 1.0 Å)	Residual biological $pt_{1/2}$
Amifloxacin (1)	−0.617	−0.442	0.175
Balofloxacin (2)	−0.892	−0.897	−0.005
Cinoxacin (3)	−0.255	−0.397	−0.142
Clinafloxacin (4)	−0.752	−0.822	−0.070
Ciprofloxacin (5)	−0.663	−0.718	−0.055
Difloxacin (6)	−1.433	−1.243	0.190
Enoxacin (7)	−0.792	−0.807	−0.015
Fleroxacin (8)	−1.033	−0.921	0.112
Flosequinan (9)	−0.161	−0.229	−0.068
Flumequin (10)	−0.978	−0.714	0.264
Gatifloxacin (11)	−0.873	−0.885	−0.012
Gemifloxacin (12)	−0.823	−0.932	−0.109
Grepafloxacin (13)	−0.716	−0.842	−0.126
Levofloxacin (14)	−0.869	−0.729	0.140
Lomefloxacin (15)	−0.803	−0.887	−0.084
Moxifloxacin (16)	−1.072	−0.999	0.073
Nalidixic acid (17)	−0.243	−0.462	−0.219
Norfloxacin (18)	−0.726	−0.794	−0.068
Ofloxacin (19)	−0.739	−0.838	−0.099
Oxolinic acid (20)	−0.740	−0.483	0.257
Pefloxacin (21)	−1.021	−0.990	0.031
Pipemidic acid (22)	−0.362	−0.558	−0.196
Rosoxacin (23)	−0.813	−0.579	0.234
Sitafloxacin (24)	−0.663	−0.827	−0.164
Sparfloxacin (25)	−1.301	−1.315	−0.014
Temafloxacin (26)	−0.898	−1.124	−0.226
Tosufloxacin (27)	−0.604	−0.639	−0.035
Trovafloxacin (28)	−0.892	−0.659	0.233

2. Experimental

2.1. Data set and pharmacokinetic profile

A dataset of 28 drug molecules belonging to quinolone class of drugs as broad spectrum antibiotics including first to fourth generation candidates were taken from the literature (Shargel et al., 2005; Maryadele and O'Neil, 2006; Bruton et al., 2006; Cheng et al., 2007; Hooper and Wolfson, 1993) and used for 3D-QSPkR study (Thareja et al., 2010b). Wide variation in structures and biological half life ($t_{1/2}$) of drug molecules qualify for the present study. The negative logarithm of the measured biological half life ($t_{1/2}$) as $pt_{1/2}$ ($\log 1/t_{1/2}$ or actual activity) was used as dependent variable, thus correlating the data linear to the free energy change (Sachan et al., 2007). The general structure of the molecules has been presented in Fig. 1 and their actual and predicted biological half life ($t_{1/2}$) is presented in Table 1.

2.2. Molecular Modeling

The three dimensional structures of the quinolone drugs were constructed with the Chemdraw Ultra 8.0 running on an Intel Core 2 Duo CPU T5270@1.40GHZ/Microsoft Win XP Home edition platform and were subjected to energy minimization using molecular mechanics (MM2). The minimization is continued until the root mean square (RMS) gradient value reaches a value smaller than 0.001 kcal/mol Å. The Hamiltonian approximations Austin model 1 (AM1) method (Aggarwal et al., 2010a) available in the MOPAC module (Thareja et al., 2010a) of Chem3D is adopted for re-optimization until the root mean square (RMS) gradient attains a value smaller than 0.001 kcal/mol Å. Unless otherwise indicated, all parameters were kept default.

2.3. SOMFA 3D-QSPkR models

In the SOMFA study, a 40 Å × 40 Å × 40 Å grid originating at (−20, −20, −20) with a resolution of 1 Å was generated around the aligned molecules (Thareja et al., 2010c). The best model resulted using 1 Å

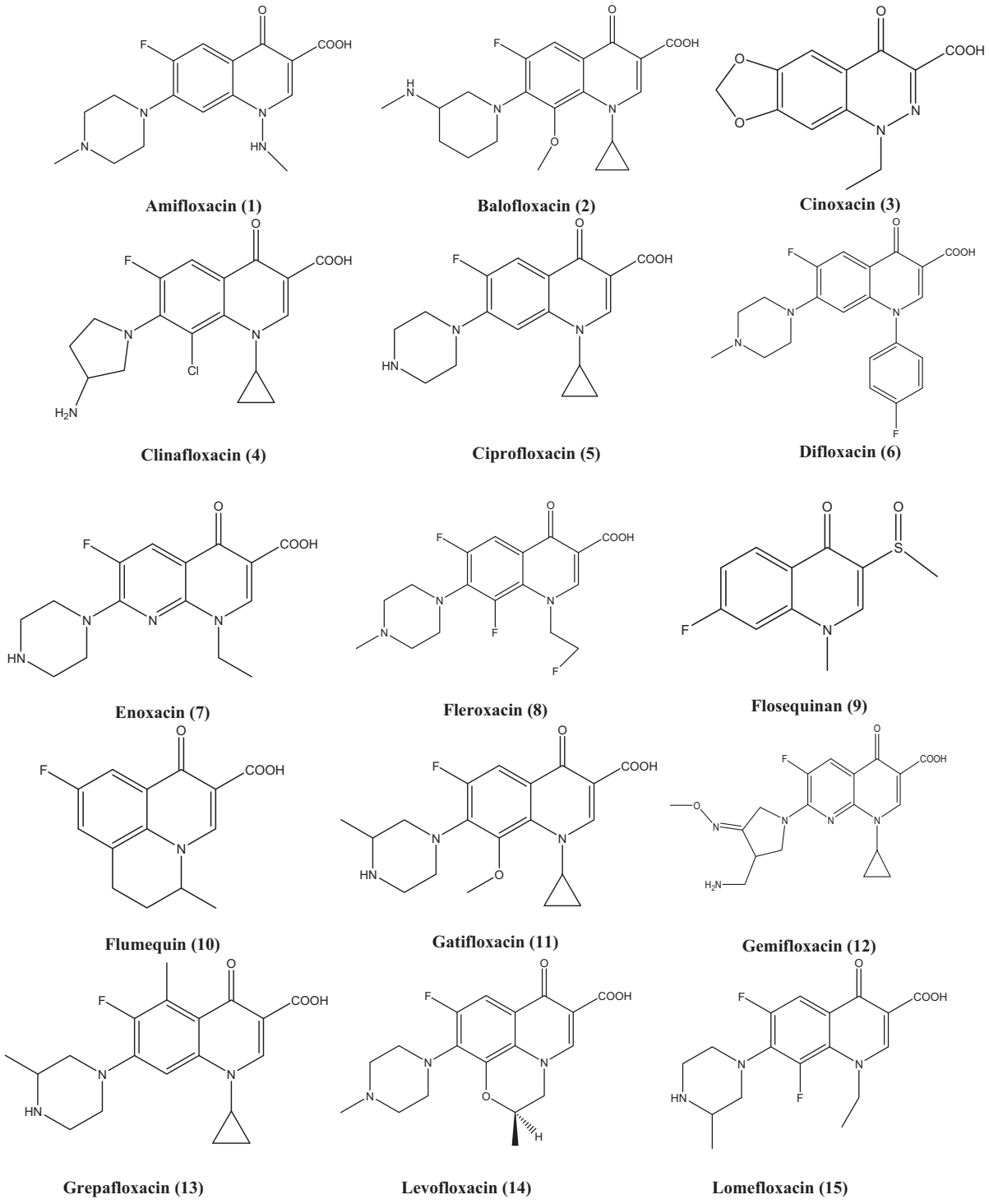

Fig. 1. Chemical structures of quinolone drugs.

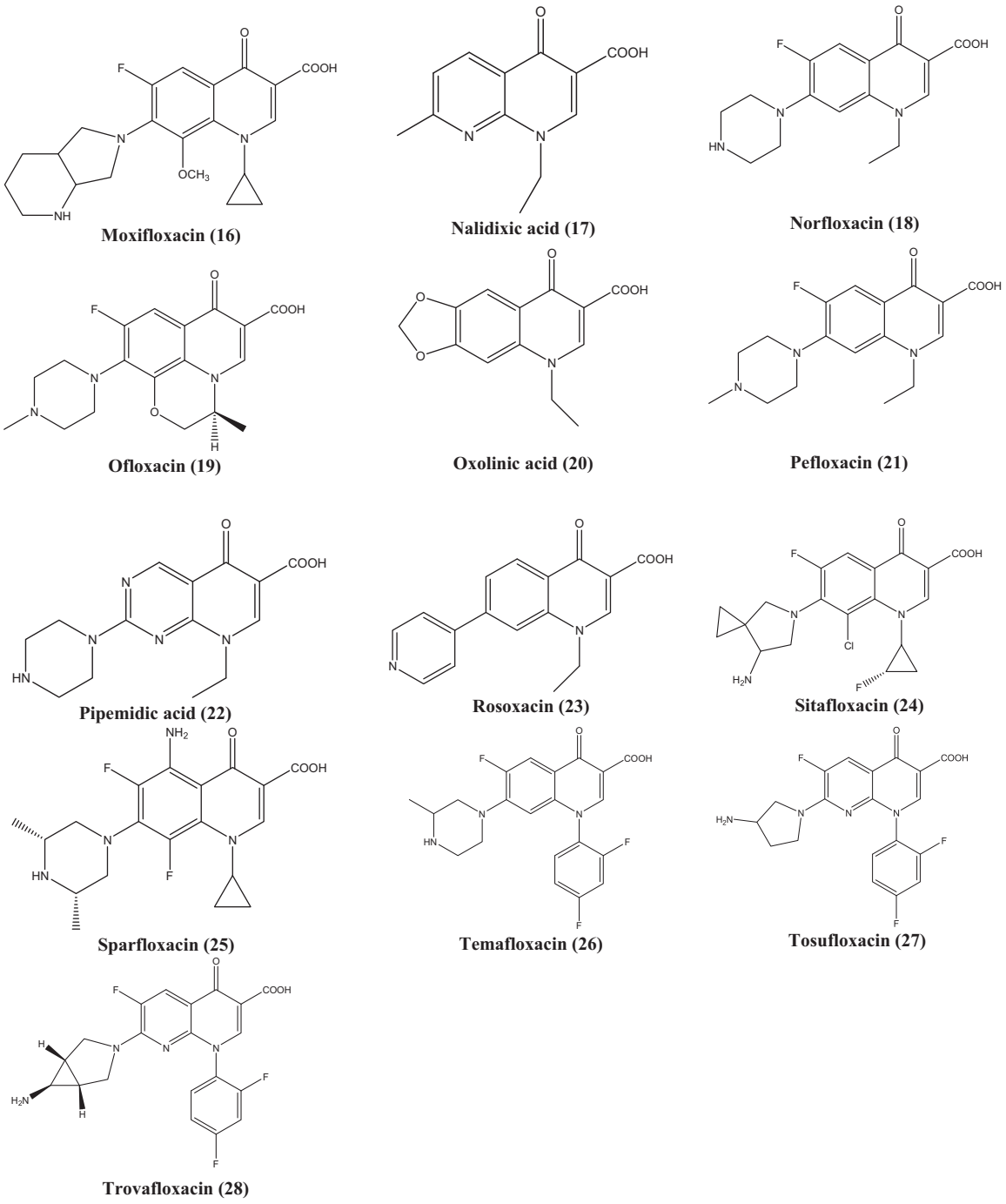

Fig. 1. (*Continued*).

grid resolution under exploration has been depicted in Table 2. The multiple linear regression (MLR) algorithm was used in conjugation with leave one out (LOO) cross-validation to develop the final model. In this analysis, one compound was dropped in turn and a model was generated from the remaining molecules. This model was then used to predict the activity of the dropped compound. This procedure was repeated until all the molecules were predicted. This MLR analysis gave the optimum number of components that was used to generate the final models without cross-validation. The result from a cross-validation analysis was expressed as r^2_{cv} (q^2) value, which is defined as

$$r^2_{cv} = \frac{1 - PRESS}{\sum (Y - Y_{mean})^2}$$

where $PRESS = \sum (Y - Y_{pred})^2$.

The result from a cross-validation analysis was expressed as r^2_{cv} value, which can take up values in the range from 1, suggesting a

Table 2
Multiple linear regression analysis at 1.0 Å.

Parameter	Resolution 1.0 Å
r^2	0.7310
r^2_{cv} (q^2)	0.6847
F	33.9663
r^2_{se}	0.1546
q^2_{se}	0.1674

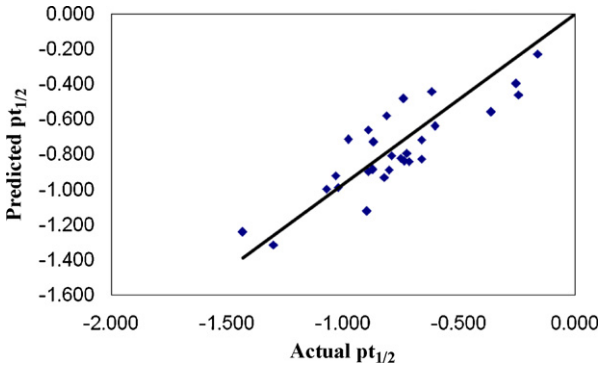

Fig. 2. Graph of actual vs. predicted $t_{1/2}$ of all quinolone drugs from the best predictive SOMFA model.

perfect model, to less than 0 where errors of prediction are greater than the error from assigning each compound mean activity of the model (Golbraikh and Tropsha, 2002).

Fischer statistics (*F*-test) is the ratio between explained and unexplained variance for a given number of degrees of freedom. The larger the value of *F*, the greater the probability that the QSPkR models will be statistically significant (Kulkarni et al., 1999). Since the final equations are not very useful to represent efficiently the SOMFA models, 3D master grid maps of the best models are displayed by Grid-Visualizer program. These grids represent area in space where steric and electrostatic field interactions are responsible for the observed variations in the $t_{1/2}$.

3. Results and discussion

In the present QSPkR study, SOMFA was employed with data set composed of clinically used 28 quinolone drug molecules whose biological half life are known to find out molecular features responsible for optimal half life. Statistical results of SOMFA models obtained by MLR analysis, i.e. cross-validated r^2_{cv}, non cross-validated r^2, *F*-test value serves as a quantitative measure of the predictability of the SOMFA.

During SOMFA studies, grid spacing of 1.0 Å was investigated. The best SOMFA model obtained showed good cross-validated correlation coefficient r^2_{cv} (0.6847), non cross-validated correlation coefficient r^2 values (0.7310), high *F*-test value (33.9663) with satisfied statistical correlation and predictive ability. The actual and predicted biological half lives of the molecules are reported in Table 1 using best Model A. Figs. 2 and 3 show a good linear correlation and moderate difference between actual and predicted values of dataset molecules.

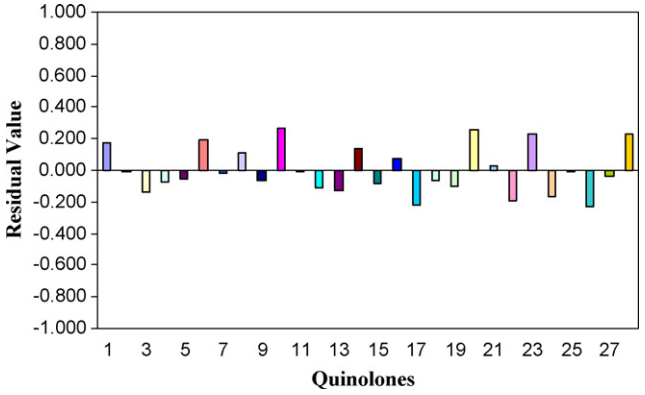

Fig. 3. Histogram of SOMFA residual $t_{1/2}$ of all quinolone drugs.

Fig. 4. Electrostatic grid showing drug Difloxacin (6) having maximum biological half life in the background at 1.0 Å resolution.

SOMFA calculation for both shape and electrostatic potentials were performed which have been presented as master grids (Figs. 4 and 5) using resolution of grid at 1.0 Å. The master grid maps derived from the best model were used to display the contribution of shape and electrostatic potential. The master grid maps gave a direct visual indication regarding structural features responsible to differentiate the biological half life of drugs in the data set under study. The master grid also offered an interpretation as to design and optimize novel molecules with much improved biological half life. Each master grid map was colored in two different colors for favorable and unfavorable effects. In other words, the electrostatic features were red (more positive charge increases $t_{1/2}$ or more negative charge decreases $t_{1/2}$) and blue (more negative charge increases $t_{1/2}$ or more positive charge decreases $t_{1/2}$), and the shape feature are red (more steric bulk increases $t_{1/2}$) and blue (more steric bulk decreases $t_{1/2}$), respectively (For interpretation of the references to color in this text, the reader is referred to the web version of the article.) (Fig. 6).

The SOMFA electrostatic potential map (Fig. 4) shows some important features (For interpretation of the references to color in this text, the reader is referred to the web version of the article.): high density of red points around the C-7 and C-3 of quinolone skeleton indicating presence of electropositive groups favorable

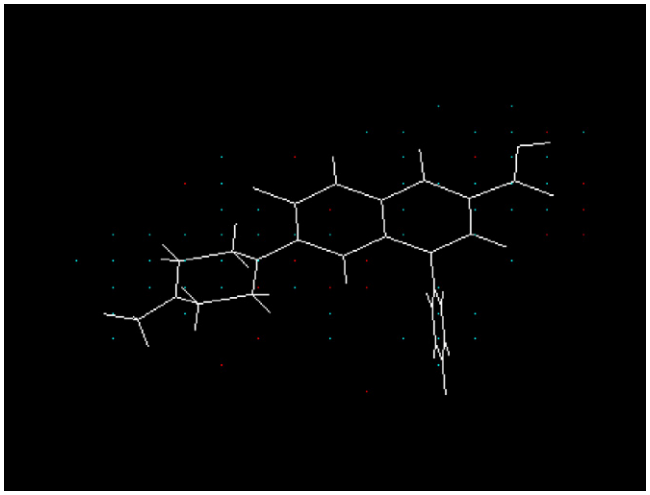

Fig. 5. Shape grid showing Difloxacin (6) having maximum biological half life in the background at 1.0 Å resolution.

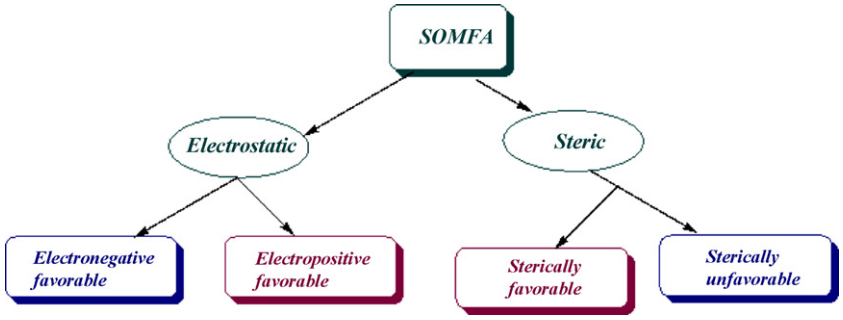

Fig. 6. Color code representation of SOMFA grids.

for optimal biological half life while few blue points around N-1 indicating the presence of electronegative groups favorable for improved biological half life. Meanwhile in SOMFA shape potential map (Fig. 5), high density of blue points around N-1 and C-7 of quinolone molecules suggesting unfavorable steric interaction while around C-8 showed favorable steric interactions for improving biological half life (For interpretation of the references to color in this text, the reader is referred to the web version of the article.). These features are essential while designing new quinolone analogues in order to have optimal biological half life.

4. Conclusion

A statistically validated robust SOMFA 3D-QSPkR models for a diverse set of quinolone drugs having flexibility in structure and pharmacokinetic profile capable of predicting the half life of new chemical moieties have been developed. The master grid obtained from the present SOMFA models indicated electrostatic and shape potential contributions on biological $t_{1/2}$. These features can be mapped back onto the structural features relating to trends in half life of the quinolones. Shape and electrostatic potential contributions calculations will be helpful in designing of novel quinolones with optimal half life period which will improve biological profile.

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References

Aggarwal, S., et al., 2010a. Self organizing molecular field analysis on pregnane derivatives as human steroidal 5α-reductase inhibitors. Steroids 75, 411–418.
Aggarwal, S., et al., 2010b. 3D-QSAR studies on unsaturated 4-azasteroids as human 5α-reductase inhibitors: a self organizing molecular field analysis approach. Eur. J. Med. Chem. 45, 476–481.
Ambrose, P.G., Owens, R.C., Quintiliani, R., Nightingale, C.H., 1997. New generations of quinolones: with particular attention to levofloxacin. Conn. Med. 61, 269–272.
Borcherding, S.M., Stevens, R., Nicholas, R.A., Corley, C.R., Self, T., 1996. Quinolones: a practical review of clinical uses, dosing considerations, and drug interactions. J. Fam. Pract. 42, 69–78.
Bruton, L.L., Laza, J.S., Parker, K.L., 2006. Goodman Gilman's: The Pharmacological Basis of Therapeutics, 11th ed. McGraw-Hill Medical Publishing Division, New York, pp. 940–950.
Cheng, D., Xu, W.R., Liu, C.X., 2007. Relationship of quantitative structure and pharmacokinetics in fluoroquinolone antibacterials. World J. Gastroenterol. 13, 2496.
Cramer, R.D., Patteerson, D.E., Bunce, J.D., 1988. Comparative molecular field analysis (COMFA) effect of shape on binding of steroids to carrier proteins. J. Am. Chem. Soc. 110, 5959–5967.
Du, M., Li, L., Wu, B., Xia, L., 2003. Self-organizing molecular field analysis on [α] 1a-adrenoceptor dihydropyridine antagonists. Bioorg. Med. Chem. 11, 3945–3951.
Duch, W., Swaminathan, K., Meller, J., 2007. Artificial intelligence approaches for rational drug design and discovery. Curr. Pharm. Des. 13, 1497–1508.
Golbraikh, A., Tropsha, A., 2002. Beware of q^2! J. Mol. Graph. Model. 20, 269–276.
Hooper, D.C., Wolfson, J.S., 1993. Quinolone Antimicrobial Agents, 2nd ed. American Society for Microbiology, Washington, pp. 195–223.
Hutter, M.C., 2009. In-silico prediction of drug properties. Curr. Med. Chem. 16, 189–202.
Thareja, S., et al., 2010a. Sulphonamides as inhibitors of protein tyrosine phosphatase 1B (PTP 1B): a 3D-QSAR study using SOMFA approach. Chem. Pharm. Bull. 58, 526–532.
Thareja, S., et al., 2010b. 3D-QSAR studies on arylidine thiazolidinedione as aldose reductase inhibitors: a self organizing molecular field analysis approach. Med. Chem. 6, 30–36.
Thareja, S., et al., 2010c. Self organizing molecular field analysis on a series of human PTP 1B inhibitors. Lett. Drug Des. Discov. 6, 395–401.
Kulkarni, S.S., Gediya, L.K., Kulkarni, V.M., 1999. Three-dimensional quantitative structure activity relationships (3D-QSAR) of antihyperglycemic agents. Bioorg. Med. Chem. Lett. 7, 1475–1485.
Levine, C., Hiasa, H., Marians, K.J., 1998. DNA gyrase and topoisomerase. IV: biochemical activities, physiological roles during chromosome replication, and drug sensitivities. Biochim. Biophys. Acta 1400, 29–43.
Maryadele, J., O'Neil, 2006. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 14th ed. Merck Research Laboratories, Division of Merck and Co. Inc., USA.
Robinson, D.D., Winn, P.J., Lyne, P.D., Richards, W.G., 1999. Self-organizing molecular field analysis: a tool for structure–activity studies. J. Med. Chem. 42, 573–583.
Ruiz, J., 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J. Antimicrob. Chemother. 51, 1109–1117.
Sachan, N., Kadam, S.S., Kulkarni, V.M., 2007. Human protein tyrosine phosphatase 1B inhibitors: QSAR by genetic function approximation. J. Enz. Inhib. Med. Chem. 22, 267–276.
Shargel, L., Wu-Pong, S., Andrew, Y.U., 2005. Applied Biopharmaceutics and Pharmacokinetics, vol. 259., 5th ed. McGraw Hill Companies Inc, New York, pp. 864–866.
Tufts Center for the Study of Drug Development, 2008. Optimizing Protocol Design Strategies to Improve Clinical Research Performance. Tufts University School of Medicine, Boston, http://csdd.tufts.edu/reports/description/rd_single_issues (accessed 20.11.09).
Zheng, S., Li, Y., 2006. Self-organizing molecular field analysis on a new series of COX-2 selective inhibitors: 1,5-diarylimidazoles. Int. J. Mol. Sci. 7, 220–229.