Poster Session 3 – Chemistry

221
QSAR modelling of hERG potassium channel inhibition with low-dimensional descriptors

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An increasing number of non-antiarrhythmic drugs are associated with prolongation of the QT interval of the electrocardiogram, leading to significant adverse effects such as bradycardia, electrolyte imbalance and impaired hepatic and renal function. A number of such drugs (e.g. terfenadine, cisapride, astemizole) have been withdrawn from the market because of this. The hERG (human ether-a-go-go-related gene) potassium channel is expressed in the human heart; it is a major contributor to cardiac repolarisation and contributes to the QT interval. Its inhibition (hERG K + CI) generally leads to prolongation of the QT interval. Several 3-dimensional (3D) QSAR studies of hERG K + CI have been published (Cavalli et al 2002; Ekins et al 2002; Pearlstein et al 2003). However, the results from 3D QSAR studies are often difficult to interpret, so we have carried out a low (0–2D) QSAR analysis of a diverse data-set of 60 drugs and drug candidates using published hERG K + CI IC50 values determined in mammalian cells. We calculated a total of 200 descriptors using QsarIS (now MDL QSAR; www.mdli.com), TSAR (www.accelrys.com) and MOLPRO (www.ibmh.msk.su/qsar). The step-wise linear regression routine in MINITAB ver. 13.1 statistics software was used to select the descriptors that best modelled the IC_{50} values. The following QSAR was obtained:

$$\log IC50 = 0.411\ nO - 1.20\ Ed_{max} - 0.683\ (^3\chi_c - {}^4\chi_{pc}) + 0.000148\ Iz + 0.000635\ TE + 2.12$$

$$n = 60 \quad r^2 = 0.842 \quad Q^2 = 0.797 \quad s = 0.614 \quad F = 57.5$$

where nO = number of oxygen atoms, Ed_{max} = maximum hydrogen bond donor energy, $(^3\chi_c - {}^4\chi_{pc})$ = difference between 3rd order cluster and 4th order path-cluster molecular connectivity, Iz = principal moment of inertia along z axis, TE = total molecular energy, n = number of compounds in training set, r = correlation coefficient, Q = cross-validated correlation coefficient (leave-one-out procedure), s = standard error of the estimate, and F = Fisher statistic. We also validated the QSAR by removing 20% of the compounds from the training set, regenerating the QSAR on the remaining 48 compounds, and then predicting the IC50 values of the 12 compounds that had been removed. This procedure was repeated five times, so that all compounds were removed in turn. The predicted and measured IC50 values correlated well ($r^2 = 0.806$), indicating that the QSAR has good predictive ability. Ed_{max} values are negative, so that high values of hydrogen bond donor energy will increase IC50 values (i.e. will reduce hERG K + CI). The nO term must be related, at least in part, to hydrogen bond acceptor capability. Hence hydrogen bonding clearly has a positive effect in reducing hERG K + CI. Both $(^3\chi_c - {}^4\chi_{pc})$ and Iz are shape descriptors, indicating the importance of molecular shape in hERG K + CI; this is also demonstrated by the pharmacophore model developed by Ekins et al (2002). TE appears to be a size term, as it correlates strongly ($r^2 = 0.930$) with molecular weight; however, TE values are negative, indicating that small molecules give rise to less hERG K + CI.

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222
Towards novel pro-drugs for CNS delivery of anti-trypanosomal drugs

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Parasitic trypanosomes and leishmanias of the order kinetoplastida are the causative agents of several debilitating third world diseases, including African sleeping sickness (causative agent *Trypanosoma brucei rhodesiense* or *T. b. gambiense)*, Chagas' disease (*Trypanosoma cruzi*), in South America and leishmaniasis (*Leishmania donovani)*, mostly in Asia. They cause millions of deaths each year. Often the victims are children, and always in poorer countries of the developing world. These parasites do not use the ubiquitous glutathione/glutathione reductase system (GR) for redox protection. Instead, they rely on trypanothione and trypanothione reductase (TR) to protect them from oxidative stress. The two enzymes (TR of the parasite and GR of the host) are mutually exclusive in substrate use, which makes the parasite enzyme a potential target for antitrypanosomal drug design. TR was isolated and purified from *E. coli* containing the recombinant *T. cruzi* TR gene and characterized by specific activity and k_{cat} measurment against its natural substrate trypanothione and synthetic substrate $[ZCG\text{-}dmapa]_2$. Several TR-specific inhibitors, (both open chain and closed chain tri-cyclic compounds), have been synthesized and studied biochemically. They showed very strong inhibition of TR (e.g., compound **I**, **II** and **III**, gave I50 values from 660 nM to 34 μM against TR). The in-vitro antiprotozoal activity of these compounds was then tested against *Leishmania donovani, Trypanosoma cruzi, Trypanosoma brucei rhodesiense* (strain STIB 900) and malarial parasite *Plasmodium falciparum* (strain 3D7) in the London School of Hygiene and Tropical Medicine. These compounds were also found to exhibit very strong inhibition of the growth of all parasites tested. Compound **III** had ED50 values (concentration required to inhibit parasite growth by 50%) of 8, 6, 1.1 and 0.02 $\mu g\,mL^{-1}$ against *L. donovani, T. cruzi, T. b. rhodesiense* (strain STIB 900) and *P. falciparum* (strain 3D7), respectively, whereas compound **II** showed ED50 values of 30, 16, 1.1 and 0.13 $\mu g\,mL^{-1}$ against these parasites. These results not only are exciting in our attempts to find new drugs against African sleeping sickness and leishmaniasis, as well as Chagas' disease of South America, but they also encourage us to follow up the unexpected, and powerful, antimalarial activity of these compounds and to determine the structural origin of activity against *P. falciparum*.

223
Molecular modelling of drug interactions with a chiral stationary phase

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Hexahelicene is a polycyclic aromatic compound comprising six fused benzene rings. As a result of steric crowding in the molecule, the aromatic rings are not permitted to lie flat and instead adopt a helical twist that may be right handed, [P(+)] or left handed [M(−)]. Compounds such as P (+) and M (−) hexahelicene are non-superimposable mirror image forms of each other and exhibit chirality. While hexahelicenes have found use as chiral stationary phases (CSP) for the separation of enantiomers by HPLC, their use has been limited (Matlin et al 1988). There is now a need for more complex selectors and, with trends towards miniaturisation, complex selectors such as hexahelicenes will become more accessible (Lough 2003). In an attempt to model the interactions occurring with CSP, binding energies for novel chiral compounds were calculated using molecular modelling techniques (SGI Octane R12000 workstation using Insight-II 2000 graphics interface and Discover 98.0 simulation software; Accelrys, Cambridge, UK) and compared with retention times and retention factors (k) obtained when these compounds were analysed chromatographically. Models for each test molecule were constructed (Insight-II) and partial atomic charges approximated from a single-point PM3 calculation using MOPAC. Atom potentials were assigned according to parameters defined within the cvff force-field. Each drug was individually docked and optimal orientations obtained by computing non-bonded interactions between the reactants. Each complex was minimized using a conjugate gradients algorithm until an energy convergence criterion of 0.1 kcal mol^{-1} A^{-1} was reached. Molecular dynamics (MD) at 300 K were performed on the system for 10 ps equilibration and 100 ps of production employing a 1-fs time step, from which 100 structures were sampled at 1-ps intervals and averaged. The final averaged structure was then minimized as described. Simulations were repeated after solvation with two 10-Å layers of water to prevent evaporation during simulations. Ligand structures were sampled for conformational averaging in water by immersion in a solvent box of 25 $Å^3$ and MD subject to periodic boundary conditions while non-bonded interactions for van der Waals were accounted for by the Ewald-summation method. An MD equilibration period of 5 ps at 300 K was used followed by a 50-ps production phase with a 1-fs time step. Atomic trajectories saved every 1 ps were used for conformational averaging. The latter structures were finally minimised to an energy convergence criterion of 0.1 kcal mol^{-1} $Å^{-1}$ (Cairns et al 2002). The energies obtained from the modelling show good agreement with the experimentally derived data with the most negative (i.e. most favourable) energy corresponding to compounds with the longest retention times. When the various components of the total interaction energy were examined, it was found that the major interaction in-vacuo was van der Waals between non-polar regions of the drug and the aromatic surface of the hexahelicene molecule, while in the solvated simulation, van der Waals interactions were less apparent. These data suggest that

molecular dynamics simulations of the type described are useful for modelling the separations of enantiomers during CSP.

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224
QSPR study of silicone with respect to the validity of Log P as hydrophobicity descriptor and the correlation to skin permeation

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Quantitative structure-permeability relationships studies, QSPR, on skin have been utilised to model percutaneous permeation of exogenous chemicals by various researchers throughout the last 30 years. From these studies the main determinants were found to be molecular size and hydrophobicity. However, from silicone QSPR studies it was determined that the hydrogen bonding capacity of the diffusing compound was the main determinant in permeation. Various attempts have been made to extrapolate the hydrogen-bonding component from the hydrophobicity descriptor, usually expressed as the logarithm of the octanol-water partition coefficient, Log P, partly to determine a correlation between silicone and skin permeation models. Other lipophilicity descriptors have since been investigated to find a closer fit for membrane permeation relationships. Therefore an investigation of various lipophilicity descriptors, such as solubility, hydrogen bonding and hydrophobicity in silicone permeation is presented. This has been achieved through establishing a silicone permeation database, acquired from literature searches on silicone permeation data. These descriptors have been supplemented with an array of energetic (potential, heat of formation, dipole moment, EHomo, ELumo . . .) and steric (volume, surface, solvent accessible surface . . .) descriptors to provide as close a relationship as possible. Most of these descriptors can be quantified through computational chemistry, whereas the others have been acquired from literature searches. Difficulties in providing accurate models arise from the variability within the data used for these QSPR studies. Most databases employed are compilations of various different permeation sources, which present a considerable amount of error. To this extent, data cleaning tools are employed to reduce the error incorporated within the studies. The QSPR models are then established through various statistical analysis tools (e.g., principle component analysis and least squares regression analysis). Validations of the resulting relationships are supplied through further statistical tools, primarily correlation coefficients, standard errors and Fischer's statistics. Evaluation of the proposed QSPR model is achieved by predicting permeation parameters of previously unknown compounds. These predicted permeation coefficients are then validated through diffusion studies. Franz Cell or recently Novel Diffusion Cell studies are the main experimental techniques utilised to determine the permeability coefficient of compounds through silicone and excised skin. The experimentally determined permeability coefficients can then be compared with the predicted values.

225
Identification of a better catalyst in amide preparation and a demonstration of a new optimisation tool

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The formation of amides from carboxylic acid via imidazolide, formed by reaction with *N,N′*-carbonyldiimidazole (CDI) is often encountered in the preparation of pharmaceutical compounds and intermediates. Although the intermediate, imidazolide, is not as active as the corresponding acid chloride, the reaction can be accelerated by the use of a catalyst, 1-hydroxybenzotriazole (HOBt) (Dunn et al 2003). The clean environmental conditions and good yield in this method have attracted significant attention. As a catalyst, HOBt has many advantages — reduction of racemisation in the carbodimide method of amide bond formation (Windridge et al 1971) and increase of the coupling rate both in solution method (König et al 1973) and on solid supports (Khan et al 1976). However, HOBt can explode when heated to high temperature (Urben 1999) and is subject to potential transport restrictions, hence these are constraints if considering HOBt for large scale manufacture. Therefore, the aim of this investigation is to reduce the amount of HOBt as much as possible, while maintaining acceptable yield. To reach this goal, three strategies were applied in this research: reduction of HOBt amount required in the process to different levels; omission of HOBt in the preparation — the experiments were carried out with different methods without any catalysts; and replacement of HOBt with an alternative catalyst — reaction with two catalysts, 2-hydroxypyridine and *endo-N*-hydroxy-5-norbornene-2,3-dicaroximide, giving the highest yield among a group of catalysts were further tested under different temperature and at different catalyst level. To our knowledge, this research also provided the quantitative data on the rate of acceleration for three different catalysts for the first time. Another objective of this investigation is to demonstrate the use of kinetic modelling to optimise reactions. To the academic researchers, optimisation is to devise a set of conditions, which could lead to the best yields. However, to the industrial chemists and engineers involved in R&D, scale-up and production, optimisation means a range of goals relating to yield, quality, cost, efficiency and throughput. The optimisation tool used in this study is Dynochem, a kinetic modelling software, which was developed for providing the kinetic fitting, dynamic simulation and mixing assessment in one package. The kinetic models built for this research were based on HPLC data obtained during the reaction period. This method is able to predict the yield under different conditions and it will surely become a very useful optimisation technique.

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226
Relation of low solubility to crystal structure of the cyclohexylammonium salt of flurbiprofen

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Flurbiprofen is a nonsteroidal anti-inflammatory agent containing a carboxyl group. As the free acid its solubility in water is only $0.03\,mg\,mL^{-1}$. Salt formation is a common method for modifying the solubility of drugs. Comparison of the t-butylammonium salt with analogues having one, two or all three methyl groups replaced by the more hydrophilic hydroxymethyl did not reveal a simple correlation of aqueous solubility with hydrophilicity: in fact the $(HOCH_2)_2C(CH_3)NH_3^+$ salt is the most soluble (Anderson & Conradi 1985). Our study of a new series of flurbiprofen salts has extended the range. The irregular trend of solubility with alkyl chain length provides further confirmation that hydrophilicity of the counter ion does not control solubility. Thus the most hydrophilic butylammonium salt provides low solubility, which increases with pentylammonium and decreases again with greater chain length. The solubility of the cyclohexylammonium salt (CycFlur) is exceptionally low compared with the hexyl and benzyl analogues. Since the melting point (233.5–237.0°C) and solubility ($0.17\,mg\,mL^{-1}$) of the adamantylammonium salt are somewhat similar (Anderson & Conradi 1985), there may be a structural similarity between this salt and CycFlur. CycFlur also has the highest enthalpy of fusion of all the salts in our study,

Table 1 Melting point, enthalpy of fusion and aqueous solubility for flurbiprofen free acid and salts

Ammonium counter-ion	Mp (°C)	ΔH_f ($J\,g^{-1}$)	Solubility ($mg\,ml^{-1}$)
None	115.3–116.7	105.7	0.03
Butyl	139.1–141.4	93.8	0.19
Pentyl	97.2–99.1	62.8	7.51
Hexyl	92.3–95.9	81.0	2.80
Octyl	103.3–104.7	102.3	0.64
Benzyl	136.2–138.1	111.3	2.85
Cyclohexyl	215.9–221.0	159.0	0.37

implying exceptional stability of the crystal structure, which we sought to investigate. Racemic CycFlur crystallised from methanol as fine white needles in the monoclinic system. As found in crystal structures of flurbiprofen with bipyridyl species (Bailey Walsh et al 2003), the phenyl ring bearing the fluorine atom is disordered by twofold rotation. The ammonium and carboxylate groups together form a polar domain, allowing the nonpolar rings to associate elsewhere. Although there appears to be an imbalance of hydrogen bond donors and acceptors with three polar hydrogen atoms and just two carboxylate oxygen atoms, O1 accepts two hydrogen bonds at a reasonable H..O..H angle of 97° while O2 accepts a third. The roughly similar size of the cyclohexane ring and one phenyl ring of flurbiprofen are conducive to efficient packing. The two phenyl rings intersect at 46°, compared with 54° in the free acid (Flippen & Gilardi 1975) and 41° after molecular orbital optimisation of the anion in the 6-31G* basis set.

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