Poster Session 2 – Medicinal Chemistry

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QSAR study of reversal of multidrug resistance by phenothiazines

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An adverse effect of great concern is multidrug resistance (MDR), whereby drugs are effluxed from cells via over-expression of membrane-bound proteins such as P-glycoprotein and multidrug resistance-associated protein (Stouch & Gudmundsson 2002). Such efflux is considered to be one of the major causes of failure of cancer chemotherapy (Ambudkar *et al* 1999). Various classes of chemicals have, however, been found to exhibit MDR reversal (MDRR) (Wiese & Pajeva 2001). One such class is the phenothiazines, and Ramu & Ramu (1992) have reported the MDRR ability of 232 phenothiazines and related compounds. We have carried out a quantitative structure–activity relationship (QSAR) investigation of their results.

QSAR analysis was performed using MDLQSAR (www.mdli.com) and TSAR (www.accelrys.com) software to generate 184 descriptors, and Minitab statistical software to develop QSARs, using stepwise regression. We could utilise MDRR values for only 157 phenothiazines, as the remainder had results reported as, for example, > 100. Despite all the compounds being of the same general chemical class, we could not obtain a good QSAR for all the compounds together. However, when we used sub-sets of more specific chemical class, we could generate good QSARs. Two examples are:

Subsets 1 and 2

$\log MDRR = 0.00154\ IM1_S + 0.0989\ D_Y - 0.0249\ P_{XY} + 0.00586\ AE + 0.146\ I_2 + 0.152$

$n = 36\ r^2 = 0.813\ r^2_{adj} = 0.782\ Q^2 = 0.752\ s = 0.160$

Sub-sets 14–25

$\log MDRR = 0.195\ {}^5\chi_p + 0.147\ SsssN + 0.00597\ PSA - 0.00240\ TSA - 0.255\ N_{5\text{-}AR} + 0.094$

$n = 37\ r^2 = 0.864\ r^2_{adj} = 0.842\ Q^2 = 0.820\ s = 0.168$

where MDRR = ability to reverse MDR, $IM1_S$ = inertia moment 1 (size), D_Y = dipole Y component, P_{XY} = polarisation (XY plane), AE = angle energy, I_2 = indicator variable to distinguish sub-sets 1 and 2, ${}^5\chi_p = 5^{th}$ order path molecular connectivity, SsssN = sum of electrotopological state indices for singly-bonded nitrogen, PSA = polar surface area, TSA = total surface area, $N_{5\text{-}AR}$ = no. of 5-membered aliphatic rings, n = no. of compounds in training set, r = correlation coefficient, r_{adj} = correlation coefficient adjusted for degrees of freedom, Q = cross-validated correlation coefficient, s = standard error.

There are no common descriptors in the two QSARs, and this was the case throughout our analysis, for the six QSARs that we obtained for various sub-sets. We interpret this as indicating that, despite the overall structural similarity of the compounds, different sub-sets exert their MDR reversal effect by different mechanisms. Clearly, therefore, it will not be possible to devise a global QSAR for MDRR.

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In-vitro studies on novel oestrogenic polyamine drug conjugates

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Polyamines are low molecular weight compounds, found in all mammalian cells, which have been established to play key roles in cellular growth and proliferation. In addition to biosynthesis, polyamines enter cells via the specific polyamine transport system (PTS) whose expression is found to be upregulated in rapidly growing tumours making them a popular target for antitumour drug design (Delcros *et al* 2002). Oestrogen receptor (ER) positive (> 10 fmol receptor/mg cytosolic protein) (Whittliff 1984) breast tumours are known to display a dependency on oestrogens to maintain cell growth. We have previously exploited this requirement by synthesising bifunctional prodrugs composed of oestrone linked to the anthracycline antitumour drug doxorubicin (DOX) via hydrocarbon chains. DOX, a highly potent topoisomerase II inhibitor used in the chemotherapy of advanced breast cancer, is relatively unselective, and can cause a potential fatal cardiomyopathy. It was found that a lead bifunctional compound, CCRL 1046, although less potent than DOX alone, was more selective towards ER positive cell lines. To further improve selectivity and cellular uptake, the hydrocarbon linker was replaced with the polyamine spermine giving a trifunctional prodrug. DOX was replaced with the intercalating acridine moiety as a model drug with a view to lowering the overall toxicity of the conjugate.

Spermine was first protected with butoxycarbonyl (BOC) on each of its secondary amine groups. One free primary amine group was linked to oestone via a reductive amination reaction at the C_{17} carbonyl. The acridine moiety was incorporated by first reacting the other free terminal amine of spermine with succinic anhydride to give a carboxylic acid functionality. This intermediate was then reacted with acridine, functionalised at the 9 position, to give the required conjugate after deprotection.

These compounds were evaluated in chemosensitivity tests using the cultured human breast cancer cell lines MCF-7 (ER positive) and Hs-578t or MT-1 (ER negative) in supplemented RPMI 1640 medium. Percentage cell survival was determined after 96-h exposure, using drugs in the range 0.001–100 μM via the colorimetric MTT assay (Jabbar *et al* 1989).

These data indicate that when DOX is combined in the prodrug CCRL 1046, there is some selectivity for the ER positive cell line (1.0 μm for MCF-7 vs 2.1 μm for MT-1). The trifunctional acridine-containing compound is, as expected, less potent however it also shows selectivity for the ER positive cell line (31 μm for MCF-7 vs 48 μm for Hs578T), hence, making it a suitable lead compound for further investigation.

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Measurement of molecular mobility of molecules in amorphous state using enthalpy relaxation data measured by StepScan DSC

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Molecular mobility has been reported as being vital in estimating physical and chemical stability in the amorphous state. One of the approaches for estimating molecular mobility is by using the empirical Kohlrausch-Williams-Watts equation (KWW) as described by Williams & Watts (1970) with measurement of the enthalpy relaxation accompanied with the glass transition event. Normal differential scanning calorimetry cannot separate the two events (glass transition and relaxation). Modulated DSC has been reported by Craig *et al* (2000) to measure enthalpy relaxation and hence molecular mobility of freeze dried lactose. In this work we are reporting the use of StepScan DSC, which, unlike modulated DSC, does not utilize a cooling step during the cycle. The purpose of this work was, firstly, to study the use of StepScan DSC to separate and measure the enthalpy relaxation response from the step change in specific heat at the glass transition temperature (Tg) and, secondly, to compare molecular relaxation processes for the amorphous forms of indomethacin and nifedipine.

The amorphous state of the drugs was prepared by a standard quench-melt method for indomethacin and nifedipine separately in the DSC furnace. Quenched sample was then annealed for 1, 2, 3, 12, 16 and 24 h at 35, 30, 25 and 15°C. Annealed samples were scanned using StepScan DSC (Perkin-Elmer) with optimised parameters of step-up temperature of 1°C, heating rate 2°C min^{-1}, isothermal time of 30 s (from 15 to 70°C). Area of the peak on IsoK baseline (for irreversible or kinetic changes) was used as an estimate of enthalpy relaxation. Values of 6Cp and Tg were measured from the specific heat line. Average molecular relaxation time (τ) and distribution parameter (β) values were calculated by fitting enthalpy relaxation values measured after annealing over time periods at different temperatures to the KWW equation using non-linear regression analysis, option provided by Origin 5.0.

Table 1 Values of τ and β obtained by curve fitting in empirical KWW equation for indomethacin and nifedipine

	Indomethacin		Nifedipine	
	τ (min)	β	τ (min)	β
35°C	1016 ± 142	0.29 ± 0.02	51 ± 6	0.5 ± 0.06
30°C	1055 ± 64	0.7 ± 0.04	1519 ± 139	0.5 ± 0.03
25°C	2048 ± 186	0.98 ± 0.1	12588 ± 5004	0.5 ± 0.06
15°C	$10^8 \pm 8 \times 10^8$	0.19 ± 0.1	$5 \times 10^{30} \pm 10^{33}$	0.04 ± 0.1

It is clear from the τ values obtained that nifedipine shows greater temperature dependence on relaxation than indomethacin, although both show the same Tg (ca. 46°C, half Cp extrapolated). It can be concluded from the above results that

1. StepScan DSC can be used in the measurement of enthalpy relaxation.
2. The empirical KWW equation can differentiate between the molecular mobility and relaxation processes of indomethacin and nifedipine in amorphous states hence could be of potential use in predicting the relative stability of the amorphous form of different drugs.

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Acute toxicity prediction with computer program PASS

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A large number of in-vivo toxicological assessments are necessary for drug development. Computer-aided toxicity prediction methods are designed to reduce time and financial costs of such experiments. The main aim of the study was to investigate whether computer program PASS (http://www.ibmh.msk.su/PASS) could be applied for predicting an acute toxicity. On the basis of structural formulae the present version of PASS predicts simultaneously 900 types of biological activity for compounds with mean accuracy about 85%. High efficacy of PASS in prediction of biological activity for chemical compounds has been demonstrated earlier (Poroikov *et al* 2003; Stepanchikova *et al* 2003).

As the PASS training set we used data on the relationships between structures and LD50 values for compounds from different chemical classes of the MDDR 2001.1 and Merck Index (thirteenth edition) databases. LD50 values were divided into categories according to the types of animals, route of administration and values of LD50 (Table 1). The structure of each compound is represented in PASS as a list of MNA (Multilevel Neighborhoods of Atoms) descriptors (Filimonov *et al* 1999). Using the compounds from the training set, the probability value for each class of acute toxicity is calculated on the basis of MNA descriptors statistics.

Table 1 Accuracy of LD50 prediction by PASS

Number	AP (%)	LD50 value
19	63.4	LD50, rat, i.v. < 10 mg kg^{-1}
78	64.5	LD50, rat, i.v. 10–100 mg kg^{-1}
66	68.5	LD50, rat, i.v. 100–1000 mg kg^{-1}
31	71.8	LD50, rat, i.v. >1000 mg kg^{-1}
22	87.5	LD50, rat, p.o. < 10 mg kg^{-1}
108	86.1	LD50, rat, p.o. 10–100 mg kg^{-1}
303	79.4	LD50, rat, p.o. 100–1000 mg kg^{-1}
679	78.6	LD50, rat, p.o. >1000 mg kg^{-1}

Number = number of compounds corresponding to LD50 value; AP = accuracy of prediction

It appeared that prediction accuracy calculated by leave-one-out cross-validation procedure (Table 1) for p.o. route of administration is better than that for i.v. one (about 83% and 70%, respectively). It may be because of incompleteness or less accuracy of data on "LD50, rat, i.v.". In general, moderate accuracy of prediction may be due to the use of compounds from different chemical series in the training set and to the relatively small number of compounds included into each class of toxicity. Based on existing experience, we expect that the further increase in number of data used for PASS training set will enhance the accuracy of prediction.

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