J Comput Aided Mol Des (2009) 23:97–103
DOI 10.1007/s10822-008-9242-3

Structural pairwise comparisons of HLM stability of phenyl derivatives: Introduction of the Pfizer metabolism index (PMI) and metabolism-lipophilicity efficiency (MLE)

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Received: 1 July 2008 / Accepted: 2 September 2008 / Published online: 18 September 2008

Abstract Data mining by pairwise comparison of over 150,000 human liver microsome (HLM) intrinsic clearance values stored within the internal Pfizer database has been performed by an automated tool. Systematic probability tables of specific structural changes on the intrinsic clearance of phenyl derivatives have been generated. From these data two new parameters, the Pfizer Metabolism Index (PMI) and Metabolism-Lipophilicity Efficiency (MLE) are introduced for each fragment. The findings are applied to a Topliss style analysis that focuses on metabolic stability.

Keywords Pairwise comparison · Human liver microsome · Pfizer metabolism index · Metabolism-lipophilicity efficiency · Topliss analysis

Introduction

A successful drug discovery programme includes the balancing of many parameters. Two key parameters are undoubtedly pharmacological potency and metabolic stability. These in vitro parameters are early indicators of the clinical effective concentration and unbound clearance and must be optimised to minimise the effective dose to a patient [1].

Over thirty years ago, Topliss proposed some operational schemes for optimising pharmacological potency [2] and this approach has been extensively applied throughout this time in the optimisation of chemical series through design [3].

While the structure activity relationships (SAR) within each chemical series may be very sensitive to the biological target in question, the metabolic turnover of the molecules are always subjected to the same set of human metabolic enzymes, albeit a large battery of enzymes with varied and overlapping specificities. In principle, general structural trends may exist for structure metabolism relationships [4] (SMR) that are applicable across chemical series.

What this paper aims to do is to identify what the probability is that a molecular change will either increase or decrease the metabolic stability of molecule. Furthermore, by arranging this data within Topliss schemes, the aim is to inform the designer of chances of improving metabolic stability at the same time as aiming to improve pharmacological potency.

Since automation of the human liver microsome (HLM) assay was introduced at Pfizer and the assay format globally standardised, a huge data set referencing over 150,000 compounds has been created with a high level of consistency that is ideal for data mining. A computational tool has been created that allows for the whole database to be scanned and data retrieved from pairs of compounds that differ only by one functional group. These examples, however, will be taken from a cross section of a multitude of chemical series and therefore will represent the overall probability that a functional group change will lead to an improvement in metabolic stability. A related approach has recently been applied to some other properties of drug molecules [5].

Methods

HLM assay

Human microsomal assays were performed using pooled microsomes from the Pfizer Global Supply (Gentest). In humans, variability in P450 expression is a significant

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factor, so a large number of liver microsome preparations were pooled to represent an 'average' array of P450s. All other reagents were obtained from Sigma.

HLMs that has been thawed for one minute in a water bath at 37 °C to give a protein concentration of 0.8 mg/mL and P450 concentration of 0.25 μM were added to 100 mM Phosphate buffer at pH 7.4, containing 1 mM of magnesium chloride at 37 °C. The NADPH regenerating system was added, comprised of 1 mM NADP+; 5 mM isocitric acid; 1 U/mL isocitric dehydrogenase. Substrate was added to give a final concentration of 1 μM and the rate of decline measured over 1 h.

Description of the data mining tool

The data used for this analysis was extracted from the Pfizer internal database with an in-house data mining tool known as 'Buy me Grease (v.2.0)'. This tool was designed to evaluate how specific alterations on chemical structure can affect in vitro ADME properties. It runs on a Pipeline Pilot (v.6.1.5) server and is exposed to end-users as an internal Webservice [6] which consists of an input form protocol and a working protocol. Via the form protocol, the user defines a generic molecular transformation with an RXN file, which is a standard file format created by MDL (formerly Molecular Design Ltd.) to represent chemical reactions, and can be uploaded or sketched on an ISIS/Draw java applet [7]. The user also specifies which in vitro ADME assays to include in the analysis, and whether censured data points (i.e., reported as '<'or '>') should be considered or not. For this work, only logD7.4 and HLM clearance were selected (see previous section for details about these particular in vitro assays). Before submitting the job to the Pipeline Pilot server, the user also has the option to describe in which way the final output should be sorted: by which in vitro property, of which chemical species (reactant, product or their relative ratio) and whether it should be increasing or decreasing.

The submission of the input form triggers the working protocol in the server. The first steps of the data mining process are to query the Pfizer internal database for compounds that contain the moiety defined in the RXN file as a reactant, and then apply the reaction on that compound. The 'Perform Reaction on each Molecule' component in Pipeline Pilot carries out both operations in a single step. For each hit found in the database, it outputs the structure and ADME information on the reactant as textual information, and the structure of the product as a molecular object. The molecular object is subsequently used to search the Pfizer internal database again for an exact match. If a matching structure is found, then the ADME data of the corresponding compound is added to the data stream, and the complete pair of reactant and product compounds is indexed.

Once all the compound pairs and their ADME properties have been extracted from the Pfizer database, then the product : reactant ratios are calculated for each one of the in vitro ADME properties initially selected by the user. As an approximate estimation, and to account for the variability in the assays, the following criteria are applied to tag each pair of compounds: a ratio above 2-fold is considered a significant increase in the property effected by the structural change; less than 2-fold is considered a significant decrease; any value in between is considered an indication that the structural change has a neutral effect on the particular ADME property.

The final process performed by the working protocol is to write a PDF report [8] as a temporary file, and display it to the end user via the Webservice. The PDF file contains the following information:

A sketch of the RXN file submitted by the user to the Webservice.

A series of 2D plots representing the values found in 'product' compounds versus the values found for 'reactant' compounds for each ADME property requested by the user. Each point in the plot represents one pair of compounds that matches the transformation submitted as an RXN file.

A series of histogram plots representing the distribution of the product:reactant ratios between 0 and 3-fold for each in vitro assay requested. Below each histogram there is a summary of the number and percentage of cases where the change in the property is increasing, decreasing or neutral according to the criteria described above. These data are expressed in Table 1 as '% Increase', '% Decrease', and '% No Change'.

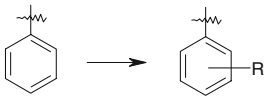
A table that contains specific information of each and every pair found in the Pfizer database that matches the input RXN file. Each row of the table corresponds to one unique pairwise example. It contains the structures of the reactant and the product, as well as the raw data and a radar chart, visually representing the effect of the structural change in the ADME properties of the compounds.

Although the database used for this work is proprietary, the data mining methodology described above is reproducible and can be applied to any database that contains chemical structure information linked to ADME information.

Results

Structure metabolism relationships (SMR) of single point changes are influenced by the whole structure of the molecule. As a result, it may become well established within a single chemical series that a particular molecular change often benefits the metabolic stability. However, it is commonly found that this knowledge is unique to this series and does not generally translate to different chemical series

Table 1 Analysis of change in HLM Clint upon addition of an atom or group when compared with the unsubstituted phenyl



R	No of pairs	Change is HLM Clint			PMI	Probability of same or better	ΔcLogP
		% Decrease	% No change	% Increase			
2-Cl	191	4.2	68.1	27.7	−23.6	72.3	0.72
3-Cl	237	4.6	65.4	30.0	−25.3	70.0	0.72
4-Cl	247	15.4	72.9	11.7	3.6	88.3	0.72
2-F	303	5.0	85.5	9.6	−4.6	90.4	0.15
3-F	278	4.0	87.1	9.0	−5.0	91.0	0.15
4-F	491	9.2	86.4	4.5	4.7	95.5	0.15
2-OMe	162	3.7	57.4	38.9	−35.2	61.1	−0.08
3-OMe	228	3.1	63.6	33.3	−30.3	66.7	−0.08
4-OMe	375	7.7	76.0	16.3	−8.5	83.7	−0.08
2-Me	156	6.4	53.8	39.7	−33.3	60.3	0.50
3-Me	184	1.6	57.6	40.8	−39.1	59.2	0.50
4-Me	239	3.8	67.4	28.9	−25.1	71.1	0.50
2-CF_3	43	4.7	51.2	44.2	−39.5	55.8	0.89
3-CF_3	69	7.2	59.4	33.3	−26.1	66.7	0.89
4-CF_3	73	17.8	60.3	21.9	−4.1	78.1	0.89
2-CN	42	11.9	71.4	16.7	−4.8	83.3	−0.56
3-CN	89	14.6	71.9	13.5	1.1	86.5	−0.56
4-CN	101	20.8	67.3	11.9	8.9	88.1	−0.56
2-OH	25	8.0	68.0	24.0	−16.0	76.0	−0.66
3-OH	30	10.0	80.0	10.0	0.0	90.0	−0.66
4-OH	47	36.2	55.3	8.5	27.7	91.5	−0.66
4-tBu	24	12.5	58.3	29.2	−16.7	70.8	1.83
4-COMe	10	20.0	70.0	10.0	10.0	90.0	−0.56
4-$CONH_2$	20	25.0	70.0	5.0	20.0	95.0	−1.49
4-SO_2NH_2	12	33.3	58.3	8.3	25.0	91.7	−1.84
4-SO_2Me	22	40.9	59.1	0.0	40.9	100.0	−1.64

due to the different molecular properties of these new series. The objective of these studies was to capture the SMR of single point changes across a wide diversity of chemical series and, therefore, to average out the series-specific effects. As a result the summarised information gives a probability guide to the user of the effect the molecular change will make when applied to a novel chemical series. As a result, those changes with the highest probability of success can be targeted by the designer.

For each structural change, the computational tool pulls from the database the human liver microsome (HLM) value for both the starting structure and the finishing structure. Each HLM datapoint is a measure of the intrinsic clearance of the compound (Clint) in units of μL/min/mg of protein, so low values indicate lower metabolic turnover than high values. For the list of pairs of Clint values a count is made of the number of examples where the Clint values decreased by more than 2-fold, and the number of examples where the Clint increased by more than 2-fold. In order to recognise the experimental variability of the assay, pairs that showed a less than 2-fold difference in the results were considered not to be a significant change and were totalled as a third count.

Table 1 summarises the effect of the addition of an atom or group when compared with the base situation of an unsubstituted phenyl group.

Column 1 describes *R*, the atom or group added to the unsubstituted phenyl. The remainder of the molecule is always placed in the 1-position.

Column 2 gives the total of the *number of pairs* found for the transformation in the Pfizer global database that have HLM Clint values generated. Data generated from less than 20 pairs may not be entirely representative of drugable chemical space.

Column 3 shows the *% decrease* of the dataset that has a >2-fold decrease in HLM Clint representing an experimentally significant improvement in metabolic stability.

Column 4 shows the *% no change* of the dataset that is considered to be within experimental variability.

Colum 5 shows the *% increase* of the dataset that has a >2-fold increase in HLM Clint representing an experimentally significant reduction in metabolic stability.

Column 6 is the *Pfizer Metabolism Index (PMI)* which is a summary parameter calculated by subtracting the % increase from the % decrease. By definition a PMI value of +100 would be for a group that always gives a >2-fold increase in the HLM value, while a PMI value of −100 would always give a >2-fold decrease in the HLM value. A PMI value of 0 would come from a change that always has no effect, or that has an equal amount of increases and decreases.

Column 7 is the total of Columns 3 and 4 and is the probability that the structural change will result in an HLM Clint that is either the same or better. It was found that this parameter was strongly correlated with PMI.

Column 8 describes the change in calculated LogP for the change. A positive value shows that the modification adds lipophilicity compared to an unsubstituted phenyl, while a negative value shows that the change adds polarity.

Discussion

The analysis of the averaged effect of a single molecular change upon the HLM Clint for a large number of pairs of compounds is summarised in Table 1. The most frequent molecular change in the data set was the addition of a *para*-fluorine with 491 pairs. The lowest number of pairs has around 10–20 examples, and so the findings from these changes may be less applicable to novel examples from druggable chemical space.

As discussed above the PMI is a parameter formed from combining the columns for the percentage changes to the HLM values. Figure 1 shows how the PMI inversely correlates with the change in the cLogP [9] value for the molecular change. This is to be expected given the role of P450 enzymes in converting lipophilic xenobiotics into more polar compounds that can be further metabolised or excreted. As the PMI score is compiled from a huge variety of structures, it is interesting that there is not a more pronounced effect of the exact structural change on the PMI score. For further analysis of these data, two scenarios should be considered, (a) where the site of metabolism occurs remotely to the phenyl group (b) where the unsubsitituted phenyl moiety is the site of metabolism.

Where the site of metabolism is remote to the phenyl group, then for the majority of pairs, the only effect of the molecular change is the effect it has on the physicochemical properties of the molecule, the most relevant of these being lipophilicity and microsomal binding (see below). For these cases a strong correlation between PMI and ΔcLogP would be expected. There is also the potential that the molecular change introduces a new, more rapid site of metabolism and so this structural change would result in a PMI score that is lower that would be normally seen for the corresponding change in cLogP. Within Fig. 1, the addition

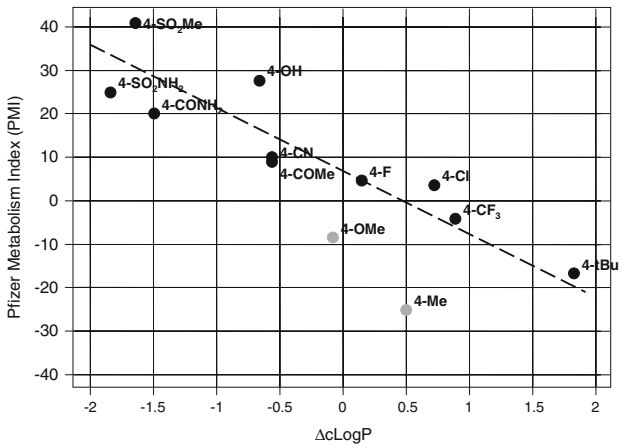


Fig. 1 PMI v. for ΔcLogP *para*-derivatives

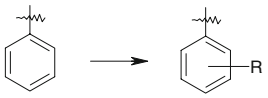
of *para*-methoxy and *para*-methyl are well below the correlation line and *O*-demethylation and benzylic oxidation are common metabolic routes.

Where the unsubsitituted phenyl moiety is the major site of metabolism then it would be expected that the structural change will have a more pronounced effect. For example in the cases where fluorine replaces a hydrogen that is a metabolised, this is the classic example of blocking the site of metabolism. Interestingly the PMI score for *para*-fluoro is relatively modest, however, this change is often favoured by medicinal chemists as the small change in physicochemical parameters is unlikely to detrimentally affect other properties of the molecule such as permeability and oral absorption, unlike the other polar groups with higher PMI scores, e.g., primary sulphonamides and carboximides.

The influence of microsomal binding should also be considered. The HLM data on which the tables have been compiled are a measure of the apparent Clint and, therefore, an increase in microsomal binding of 2-fold would decrease the apparent Clint by 2-fold without there being a real change in the Clint. Fortunately, for the data set considered, such large changes in microsomal binding are only possible for some of the examples when the unsubstituted phenyl has a cLogP > 4.5, which is only a minor component of the dataset. As a result, microsomal binding will not be considered further in the discussion.

Table 2 summarised the positional effect of the structural change with PMI scores compared for *ortho*, *meta* and *para*. In all cases placing the group in the *para* position gives an improved PMI score compared to the *ortho* and *meta* positions which are usually comparable. The enhanced PMI values for *para*-substitution is presumably due to blocking metabolism at this position in the unsubstituted phenyl. Again, throughout the dataset there is a trend towards a more negative PMI score for the groups that increase cLogP the most. There are two notable exceptions to this: (i) a

Table 2 A positional analysis of PMI values compared to unsubstituted phenyl



ΔcLogP		PMI		
		ortho	*meta*	*para*
0.89	CF_3	−39.5	−26.1	−4.1
0.72	Cl	−23.6	−25.3	3.6
0.50	Me	−33.3	−39.1	−25.1
0.15	F	−4.6	−5.0	4.7
−0.08	OMe	−35.2	−30.3	−8.5
−0.56	CN	−4.8	1.1	8.9
−0.66	OH	−16.0	0.0	27.7

methoxy group in all positions shows negative PMI scores, despite reducing cLogP, and as discussed above, this is likely due to the effect of introducing a potential *O*-demethylation process. (ii) *ortho*-hydroxy groups also give a negative PMI, out of step with the *meta*-hydroxy value, however, potentially this may be explained by 25 pairs being a relatively small sample number and not truly representative. It must also be stressed that the PMI score only reflects P450 metabolism and potentially hydroxy compounds could be susceptible to rapid Phase II metabolism.

Table 3 summarises the effect of two groups added to the phenyl. There are six combinations that two identical groups can be added to a molecule with an unsubstituted phenyl. Only sufficient pairs of examples exist for this to be meaningfully exemplified for fluorine. Interestingly only the 2,4-F_2 pattern gave a positive PMI score and this was also the best performing substitution pattern for addition of chlorine, but in both cases the PMI was lower than a the single *para*-substituent (Table 2).

For the methoxy group the situation is very different, with the 3,4-$(OMe)_2$ group being the best overall performing group and notably performing much better than a single *para*-methoxy, in contrast to the halogen examples above. Unlike the spherical halogens, the methoxy group is able to rotate into many conformations and the interplay of these two groups is speculated to be the cause of the extra stability.

The concepts of Ligand Efficiency (LE) and Ligand-Lipophilicity Efficiency (LLE) have recently being discussed [10]. LE is the ratio of potency and the number of heavy atoms. LLE is the potency from which the lipophilicity is subtracted.

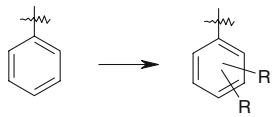
In a similar manner the above data can be used to generate Metabolism-Lipophilicity Efficiency (MLE) in which the PMI score has the change in lipophilicity of the fragment subtract according to the formula.

$$\mathrm{MLE} = \mathrm{PMI} + (25 \times \Delta\mathrm{cLogP})$$

The scaling factor of 25-fold is added to give a balanced weighting of the two factors.

Fragments with a high MLE are those that can accommodate lipophilicity while maintaining or improving metabolic stability. This concept is important as lipophilicity often correlates with potency and so the addition of polar groups to improve metabolic stability can often be a futile exercise due to the drop in potency that accompanies it. Polar groups will not obtain a good MLE score unless the increase in metabolic stability is significantly better that other groups of the same polarity.

Table 3 Analysis of change in HLM Clint upon bis-addition of an atom or group when compared with the unsubstituted phenyl



R	No of pairs	Change is HLM Clint			PMI	Probability of same or better	ΔcLogP
		% Decrease	% No change	% Increase			
2,4-Cl_2	41	5	85	10	−4.9	90.2	1.4
3,4-Cl_2	46	11	63	26	−15.2	73.9	1.3
3,5-Cl_2	24	8	50	42	−33.3	58.3	1.4
2,3-F_2	40	8	83	10	−2.5	90.0	0.2
2,4-F_2	119	12	78	10	1.7	89.9	0.3
3,4-F_2	106	15	62	23	−7.5	77.4	0.2
3,5-F_2	73	7	70	23	−16.4	76.7	0.3
2,5-F_2	60	5	80	15	−10.0	85.0	0.3
2,6-F_2	51	14	67	20	−5.9	80.4	0.3
2,4-Me_2	15	7	67	27	−20.0	73.3	1.0
3,4-Me_2	22	5	64	32	−27.3	68.2	1.0
2,3-$(OMe)_2$	15	0	80	20	−20.0	80.0	−0.3
2,4-$(OMe)_2$	24	17	58	25	−8.3	75.0	0.0
3,4-$(OMe)_2$	46	17	80	2	15.2	97.8	−0.3

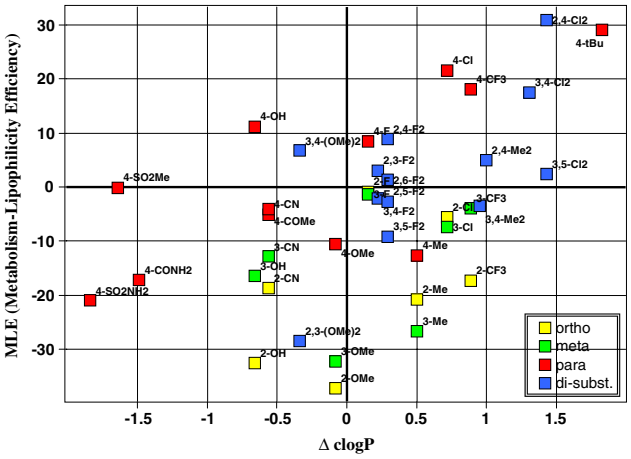


Fig. 2 MLE versus change in cLogP

Figure 2 shows MLE plotted against ΔcLogP. This representation is useful for highlighting the positional influence of a group. For example the *para*-trifluoromethyl group has a strongly positive MLE, while the same group in the *ortho* position has a negative MLE. The methylsulfone likewise is clearly shown to a have a better MLE than the primary sulphonamide despite both being polar groups placed in the *para* position.

How can PMI and MLE be used to aid the design process?

Figure 3 shows the Topliss scheme for the substitution of phenyl, that has been modified to include a symbol to indicate which PMI bucket a change belongs to, as indicated in the key. For a number of structural changes there is insufficient data to generate a PMI score, presumably as most of these are changes that introduce anilines or nitroaryls which are structural alerts within Pfizer.

The Topliss scheme was designed to allow the efficient mapping out of potency, and does not focus on metabolic stability. As a result beyond the *para*-chloro layer, there are many layers in which the scheme would lead to a high likelihood of an increase HLM Clint value. For a project that has already achieved sufficient potency, but requires improved metabolic stability, then targeting compounds with a high PMI would be the recommended approach.

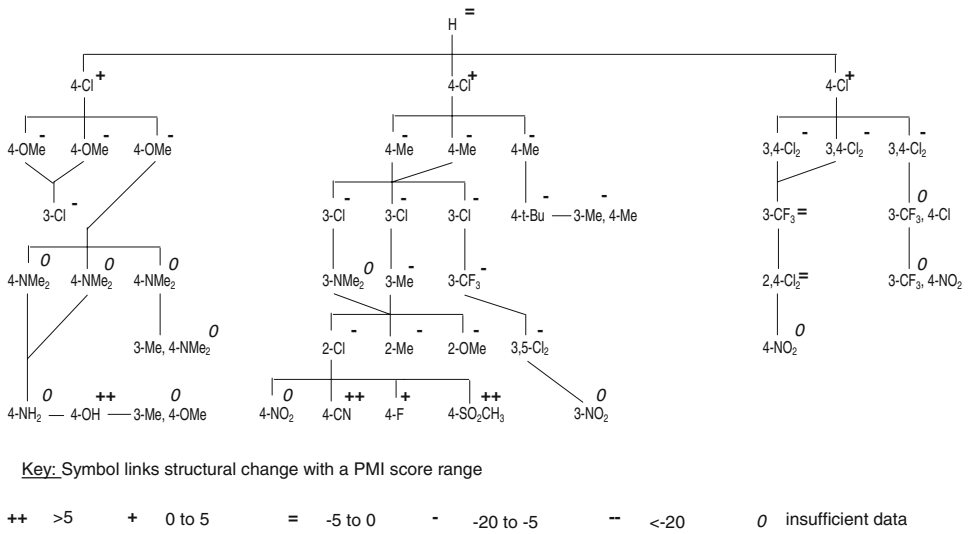
The application of the plot of MLE v. ΔcLogP (Fig. 2) is likely to depend upon the objectives of the new compounds being designed.

While a programme is in early lead development, usually potency increases will be the goal, and the groups in the top right quadrant of the plot are the most likely to achieve this given the positive ΔcLogP (due to the correlation of potency and lipophilicity) without introducing a major metabolic liability due to the positive MLE.

For the final stages of optimisation of a series the objectives will be different and the groups in the centre of the plot will be of most interest as the optimum lipophilicity will already have been achieved and the objective is to derivatise without re-introducing metabolic vulnerability.

As shown in Tables 1 and 3 the groups in the bottom half of the Fig. 2, can sometimes also yield improved profiles, however, the designer is informed that this will occur at a reduced rate.

Fig. 3 Modified Topliss Scheme including PMI values



Acknowledgements We thank Kevin Beaumont, Alan Brown, Iain Gardner, David Hepworth and Rob Webster for helpful discussions.

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