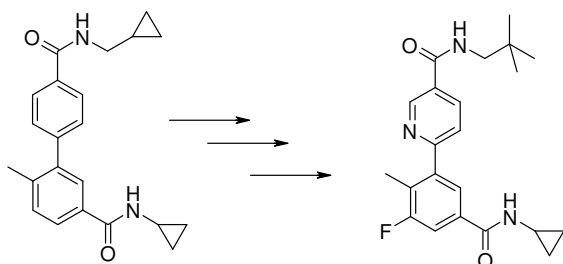
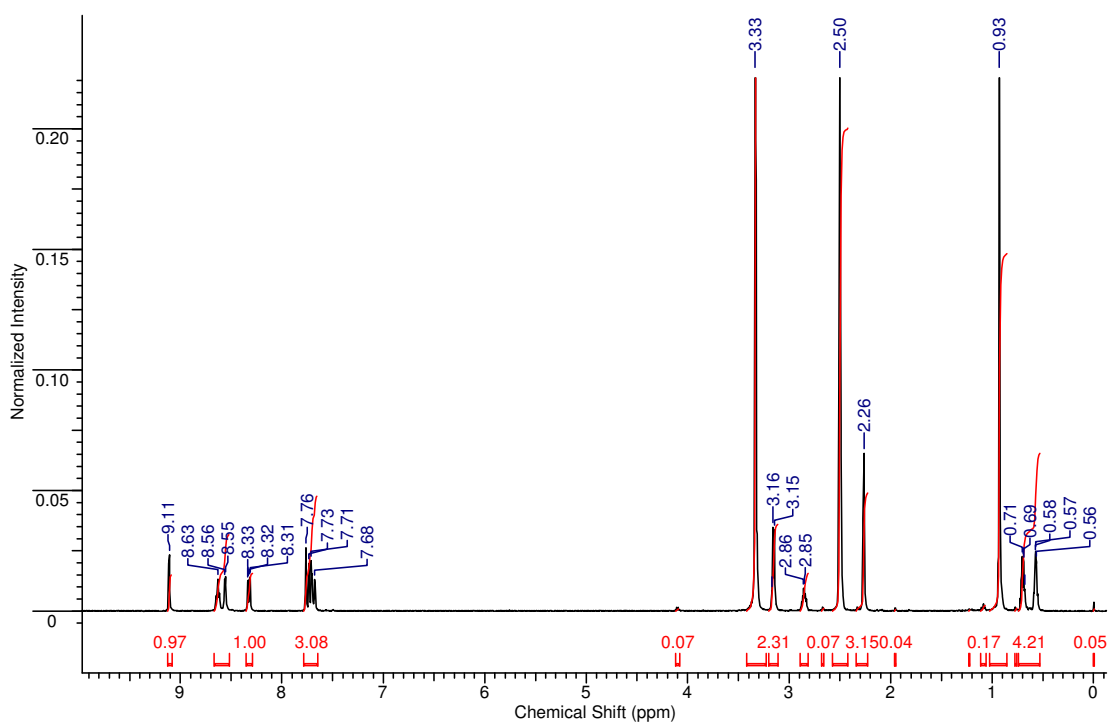


p38 α Mitogen-activated protein Kinase Inhibitors: Optimization of a series of biphenylamides, to give a molecule suitable for clinical progression

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NMR Spectrum of compound 2



Cassette Rat PK. The plasma pharmacokinetics of compounds were determined in male Lewis rats after dosing a mixture of three compounds plus standard compound **1** orally at 0.25mg/kg each. The compounds were formulated as a solution (1mg/ml total) in DMSO:PEG200:water 10:70:20 (v/v/v). Blood was collected over a 24hour time period. Plasma was prepared following centrifugation and compound extracted from 50 μ L plasma using protein precipitation with acetonitrile. Samples were then evaporated under nitrogen and re-dissolved in 100 μ L of 10:90 acetonitrile: water.

Analysis was performed using LC-MSMS on the API365 Sciex mass spectrometer with a 5min fast gradient comprising 0.1% formic acid in water and 0.1% formic acid in acetonitrile (mobile phases), 20µl injection volume, flow rate 0.4ml/min and ODS3 Prodigy column (50 x 2.1mm, 5µm). Pharmacokinetic data was generated using non compartmental analysis.

Discrete Rat PK. The plasma pharmacokinetics in male Lewis rats were determined after dosing intravenously and orally at 1mg/kg. The compound was formulated as a solution (1mg/ml) in DMSO:PEG200:water 10:70:20 (v/v/v). Blood was collected over a 24hour time period. Plasma was prepared following centrifugation and compound extracted from 50µL plasma using protein precipitation with acetonitrile. Samples were then evaporated under nitrogen and re-dissolved in 100µl of 10:90 acetonitrile: water. Analysis was performed using LC-MSMS on the API365 Sciex mass spectrometer with a 5min fast gradient comprising 0.1% formic acid in water and 0.1% formic acid in acetonitrile (mobile phases), 20µl injection volume, flow rate 0.4ml/min and ODS3 Prodigy column (50 x 2.1mm, 5µm). Pharmacokinetic data was generated using non compartmental analysis.

Cynomolgus monkey PK. The pharmacokinetics of **2** were determined in three male Cynomolgus monkeys, with a cross-over study at 1.7 mg/kg intravenous and 1.4 mg/kg oral. **2** was formulated as a solution in DMSO:20% aq Cavitron 1:99 (v/v) for the intravenous arm (60minutes infusion) and in DMSO:6% aq Cavitron 1:99 (v/v) for the oral arm.

PG-PS model. Male Lewis rats, weighing 125-150g at the start of the study, were purchased from Harlan Olac (Bicester, UK). Each treatment group consisted of 8 rats housed in two cages of four, fed on standard rodent chow with free access to water.

Induction of arthritis. Under general anaesthesia, the rats were given an intra-articular injection of 5µg peptidoglycan-polysaccharide from *Streptococcus pyogenes* (PG-PS 100P from Lee Laboratories, Batch 147884) in a volume of 10µl sterile PBS into the right ankle joint. Fourteen or fifteen days later, the rats were injected intravenously with 200µg PG-PS in a volume of 0.5mls sterile PBS. This systemic injection of PG-PS reactivates the inflammation in the previously injected ankle joint and is designated Day 0.

Dosing schedule. The rats were dosed orally, twice daily, on days 0, 1 and 2, with 8hours between the daily doses. The first dose was given 1hour prior to reactivation of arthritis on Day 0. The dosing volume was 4mls/kg body weight. Treatments were randomized between the groups and coded so that the assessments were performed blind. Each study contained an arthritic control group dosed with vehicle 0.5% methyl cellulose in water and in most studies, a group dosed with prednisolone (2mg/kg as a positive treatment control, together with three or four dose levels of compound. The experiment was terminated on Day 3.

Assessments (Ankle swelling). The diameter of the injected (right) ankle was measured on Days 0, 1, 2 and 3 using calipers. The Day 0 measurement represents the starting diameter and was subtracted from the subsequent measurements so that the results were expressed as change in ankle diameter.

Assessments (Pain). The hypersensitivity to pain was determined by measuring weight bearing using an instrument made in-house (dual channel weight averager).

Normal rats distribute their body weight equally between the two hind paws, but when the left hind paw is inflamed and /or painful, the weight is re-distributed so that less weight is put on the affected paw (decrease in weight bearing). Assessment of this change is a sensitive method for measuring 'incident pain' and allodynia. Baseline weight bearing readings were taken on Day -14 just before sensitisation, and Day -1 (the day before the reactivation injection). After reactivation on Day 0 and the induction of inflammation and pain, the weight bearing measurements were taken on Days 1, 2 and 3 post reactivation. The results were expressed as the difference between the weight applied to the non-inflamed paw compared with the inflamed paw. This number becomes larger as the pain increases.

Data Analysis. The ankle swelling data are expressed as mean change in ankle diameter (ankle swelling) + standard error of the mean (SEM), and % inhibition of ankle swelling compared with the vehicle control, for each treatment group. Statistical analysis was carried out on the ankle swelling data collected on Day 3, when the experiment was terminated, using a Kruskal-Wallis test, and comparing each drug treated group with the vehicle treated group in individual experiments. The ED50 was calculated as the dose estimated to induce 50% inhibition compared with the vehicle control, by linear regression. For the weight-bearing assessment of pain, statistical analysis was carried out using a Kruskal-Wallis test, and comparing each drug treated group with the vehicle treated group.

Murine CIA model

Male DBA/1 mice, at the age of 10-12 weeks, were purchased from Janvier Elevage (France). Each treatment group consisted of 10 mice housed in a single cage with free access to food and water at all times.

Induction of arthritis. Arthritis was induced using a two-step immunization protocol. On Day 0, the mice were immunized subcutaneously at the base of the tail with bovine collagen type II emulsified in Freund's Complete Adjuvant. On Day 21, the mice were boosted with an intra-peritoneal injection of bovine collagen diluted in PBS.

Dosing schedule. On the first day of dosing mice showing initial signs of mild disease were divided evenly between the individual dosing groups such that each group included mice already showing symptoms with the remaining mice not yet showing symptoms. The mice were dosed orally, twice daily, on days 22 to 36 inclusive with compound in a 1% DMSO : 99% methyl cellulose (0.5% in water) vehicle. A control group was dosed with vehicle po b.i.d. as a positive control for the disease induction. The experiment was terminated on Day 36, when the following assessments were made.

Clinical score. The clinical score provided an estimate of inflammation. This was recorded throughout the 14 day study period at 2-3 day intervals. A sliding scale of 0 (no inflammation) to 2 (severe swelling) was applied to each of the four paws, thus the maximum score attainable per animal was 8.

Histological analysis. At the end of the study, on Day 36, the mice were killed and one knee joint removed and placed in formalin for subsequent histological analysis. Paraffin blocks were made and slides cut and stained with H&E and Safranin O. Six separate sections of each joint were examined and the following histological

characteristics were assessed and scored (blinded to the treatments): infiltrate (cellular infiltrate into the synovium), exudate (cellular migration into the joint space), chondrocyte death and proteoglycan loss (markers of cartilage damage) and bone apposition (new bone formation). Pathological changes were graded from 0 (no effect) to 3 (very marked).

In silico ADME modelling Rules! – Directing SAR in lead optimisation.

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Introduction

The main CMRK issues identified within a lead optimisation programme were (i) achieving good oral exposure in the rat to ensure systemic activity in the animal disease model and to increase confidence with respect to developability and (ii) limiting the inhibition of human CYP450 isoenzymes (focusing on CYP2C9 as the isoenzyme most prone to inhibition by this template) therefore reducing the risk of drug-drug interactions.

In vivo oral PK data was available for 83 compounds whereas in vitro CYP2C9 inhibition data had been collected for 342 compounds, all from the same template. This data was analysed along with in vitro clearance data in rat liver microsomes (from an in-house screen) and in silico descriptors to generate guidelines for predicting CMRK properties. These guidelines helped to determine structure activity relationships (SAR) within the series and direct the chemistry to make molecules with good oral exposure in the rat and limited inhibitory effect on CYP2C9.

Methods

In vivo PK data was collected following either discrete or cassette (4 compounds) administration to male Lewis rats at a total dose of 1mg/kg formulated in 10% DMSO/70% PBQ 200/20% water. Blood was sampled out to 12 hours. The area under the concentration time curve (AUC) was calculated and normalised for an oral dose level of 1mg/kg. The AUC was then classified as 'poor' (<758hr.ng/ml, 58 comp's), 'good' (>758hr.ng/ml, 20 comp's) or 'very good' (>2858hr.ng/ml, 5 comp's). IC50s against CYP2C9 from an in-house high throughput fluorescence screen on cDNA expressed isoenzymes were classified as <1µM (45), 1-10µM (170) or >10µM (127), with the latter being desirable.

An in-house software package was used to calculate in silico descriptors, which were used as input for models built from actual data and developed to predict ADME properties. Two such models used in this analysis are (i) 'PQP substrate model' which predicts a compound to be classified as a 'substrate' or a 'non substrate' for the active efflux transporter P-glycoprotein (PQP) and (ii) 'CYP450 inhibition model' which generates predictor scores (labeling 1) across the IC50 categories <1µM, 1-10µM and >10µM against CYP2C9, 2C8 and 3A4.

Results

Predicting oral exposure in the rat

Figure 1 shows calculated molar refractivity (CMR) calculated using the Daylight program, plotted against ACLogD(pH7.4) calculated from the ACOLabio suite of software. Markers are coloured by oral exposure in the rat as 'poor' (red), 'good' (yellow) or 'very good' (green). If compounds are removed with ACLogD(pH7.4) >3.5 (purple shaded area) and with CMR >13 (blue shaded area) then 32/58 (55%) of poor compounds can be removed without losing any compounds with good oral exposure. Figure 2 shows that the optimum distance from the linear discriminant function (LDF-distance) derived from the CMR/ACLogD(pH7.4) plot for good oral exposure in the rat is approximately 2. LDF-distance can therefore be used to focus on compounds with optimum oral exposure in the rat.

Figure 1. CMR vs. ACLogD(pH7.4)

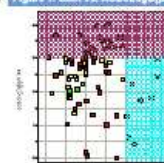
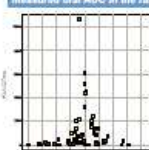


Figure 2. LDF distance vs. measured oral AUC in the rat

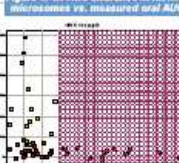


Further filtering, by removing compounds predicted to be substrates for PQP, removes a total (in combination with the CMR/ACLogD(pH7.4) rules in figure 1) of 39/58 (57%) of poor compounds, this is represented in figure 3, which shows that none of the good compounds are lost, they are all within the circled area and marked for progression. Figure 4 shows that compounds from this template with an in vitro clearance >3ml/min/g tissue in rat microsomes (purple shaded area) have poor oral exposure, combining this in vitro measurement with the in silico guidelines results in the removal of 41/58 (71%) of poor compounds without losing any with good oral exposure.

Figure 3. PQP substrate prediction vs. oral AUC prediction in rat



Figure 4. In vitro clearance in rat liver microsomes vs. measured oral AUC



Predicting human CYP2C9 inhibition in vitro

If a molecule meets any of the following descriptor thresholds it is predicted to have an IC50 <10µM against CYP2C9 in vitro and therefore classed as 'poor': When over half of the predictive score (>0.5) falls into the category of 'IC50 <1µM' using the in-house CYP2C9 inhibition in silico model. If ACLogD(pH7.4) is >4.5, if the LDF-distance is >3, or if polar surface area (PSA) is <50 (calculated as described by Clark, 1999).

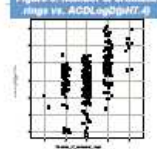
If none of the above are true the molecule is classed as 'good'.

Figure 5 shows compounds predicted as 'poor' or 'good' based on the above descriptors and plotted against measured CYP2C9 inhibition IC50 values are classified as <1µM (red), 1-10µM (yellow) or >10µM (green). The circled area shows that 74/215 (34%) compounds with IC50 values <10µM are filtered out using these descriptors and that no good compounds (>10µM) are lost. Figure 6 shows that there is a trend for CYP2C9 inhibition to increase with the number of aromatic rings in a compound with sensitivity to lipophilicity in each group.

Figure 5. Model prediction vs. measured CYP2C9 IC50



Figure 6. Number of aromatic rings vs. ACLogD(pH7.4)



Discussion and Conclusions

- Descriptors have been identified which influence oral exposure in the rat and inhibition of human CYP2C9.
- Using these in silico descriptors in combination with in vitro measurements 71% of compounds from this series with poor oral exposure in the rat can be filtered out without losing any compounds with good oral exposure.
- 34% of compounds from this series with measured IC50 values <10µM against CYP2C9 can be filtered out, without losing any compounds with IC50 values >10µM.
- These in silico guidelines developed from a training set have been successfully applied to the same series within a lead optimisation programme (see figures 7 and 8), resulting in the synthesis of fewer compounds but with a greater percentage having desirable CMRK properties.

Figure 7. Impact of in silico modelling on oral exposure in the rat

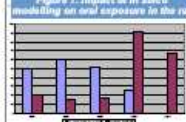
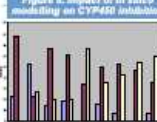


Figure 8. Impact of in silico modelling on CYP450 inhibition



References

- D.E. Clark (1999) Rapid Calculation of Polar Molecular Surface Area and Its Application to the Prediction of Transport Phenomena: 1. Prediction of Intestinal Absorption. J. Pharm. Sci. 88(5), 507-514.

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