and 0.12% and major constituents of the oils were *cis*-chrysanthenyl acetate (36.1%) and linalool (21.6%), respectively. Twenty-nine compounds of the essential oil of the flowering tops were identified and 37 of the 38 compounds in the essential oil of the non flowering tops were identified. Carvacrol, only in the essential oil of non flowering tops with Rf = 0.5 on TLC plates, showed antibacterial activity on all six bacterial strains and was identified by GC-MS after purification on PTLC. Antibacterial activity was also reported from other species of *Stachys*, such as *S. officinalis, S. germanica, S. sylvatica, S. plumose* and *S. reca* (Skaltsa et al 2003) but antibacterial compounds were not identified. Carvacrol was reported as a very potent antibacterial compound. Essential oils of another species of *Stachys* have chrysanthenyl acetate and linalool but not as major components. Germacrene-D in *S. laxa* and *S. sylvatica*, and linalyl acetate in *S. iberica* were the major components of the essential oils.

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Poster Session 1 – Chemistry

041
GRIND-based QSPR method to predict the enantiomeric excess in catalysis

I. Morao[1,2], P. D. Higginson[2], J. C. Mitchell[1] and M. J. Snowden[1]

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Quantitative Structure Property/Activity Relationship (QSP/AR) methodology is a chemo-informatic technique successfully applied to a wide range of chemical and biological problems. The generation of a mathematical model, based on previously tested molecules, capable of predicting the behaviour of new compounds can reduce considerable synthetic effort. To study chirality as a property, 3D-descriptors become the obvious choice. Of these, molecular interaction field (MIF) based descriptors have shown to work particularly well in drug design. To date, only a couple of papers have been published to predict the enantiomeric excess of chiral catalysts using the CoMFA analysis. This approach involves the alignment step of all the molecules, which is only straightforward when they all present a substructure in common. We have shown (Morao et al 2005) that GRid INdependent Descriptors (GRIND methodology) give comparable and even better results than ComFA analyses but with no need of the superimposition step. Two different asymmetric reactions are here presented and analysed. The first one consists of a Diels Alder cycloaddition that affords the formation of quinulidine derivates. The second reaction is the reduction of acetophenone with borane. The working procedure can be itemised into five different steps: geometry optimisation of the ligand-metal complex at PM3 semiempirical level; calculation of molecular interaction field of the ligand using standard probes (DRY, N1, O and TIP); filtered points derivation; generation of GRIND descriptors; and application of the diagnostic model (PLS). To carry out the whole process only two user-friendly packages are needed (SPARTAN and ALMOND). The results obtained using this new methodology are summarised in Table 1. GRIND descriptors are capable to fit the experimental data (training set of 18 and 24 ligands for reaction 1 and 2, respectively) given the cross correlation values (r^2) is over 0.9. The internal validation of these QSPR equations using three component random groups also presents a high prediction power (q^2). Finally, the external validation using four new chiral ligands in both reactions showed that the highest residual is less than 15 units. Therefore, we propose this fast and straightforward alignment-independent QSPR methodology for the prediction of the enantiomeric excess in asymmetric catalysis.

Table 1 Statistical information of the QSPR models generated

Reaction	Training set	r^2	q^2
1	18	0.9	0.5
2	24	0.9	0.8

Morao, I. et al (2005) *J. Am. Chem. Soc.* **127**: Submitted

042
Tight-binding inhibitors as the first active site titrant assays of thymidine phosphorylase from *Escherichia coli*

A. Gbaj, P. Reigan, P. N. Edwards, S. Freeman, M. Jaffar and K. T. Douglas

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Thymidine phosphorylase (TP, EC 2.4.2.4), by catalysing the reversible phosphorolysis of (substituted-)uracil 2'-deoxynucleosides to give 2'-deoxyribose-1-phosphate and (substituted-) uracil, provides both catabolic and salvage routes for pyrimidines. It also provides such metabolic routes for anti-pyrimidine therapeutic agents, such as anti-cancer drugs (e.g. 5-fluorouracil) and antivirals. The 2'-deoxyribose-1-phosphate is rapidly dephosphorylated in the cell to produce 2-D-deoxyribose (an angiogenic factor also known as endothelial cell chemoattractant), which is then exported from the cell (Brown & Bicknell (1998). The discovery that TP is identical with platelet-derived endothelial cell growth factor (PD-ECGF) and that TP has angiogenic activity has given major impetus to the design of strong, specific inhibitors of the human enzyme. TP is attracting attention as a cancer target as it plays a role in tumour angiogenesis (Cole et al 1999), and is expressed at higher levels in the plasma of patients with cancer and in solid tumours relative to normal tissues. The objective of this study was to understand the mode of action of some very strong inhibitors of the *E. coli* enzyme, and to determine if their mode of action differs for *E. coli* and human TP. In the course of our work we were also able to develop a convenient assay to determine the concentration of *E. coli* TP – in fact the first active site titration for it. Spectrophotometric assays for TP involve measurements of UV or visible absorbance differences between the nucleoside (thymidine or 5-nitro-2'deoxyuridine) and the base at pH 7.4. 5-Chloro-6-(2′-imino-pyrrolidin-1′-yl)methyl-uracil hydrochloride (TPI, 1) and its 5-bromo analogue (**2**), as well as 6-(2′-amino-imidazol-1′-yl)methyl-5-bromo-uracil (**3**) and its 5-chloro analogue (**4**) act as strong inhibitors of TP (Reigan et al 2005). However, the measured value of I_{50} (the concentration of inhibitor required to give 50% inhibition under standard conditions) apparently depends on the concentration of enzyme used in the case of TP from *E. coli*. A plot of activity against inhibitor concentration gave straight lines with intercepts on the inhibitor concentration axis proportional to the amount of enzyme added. We elucidated the origins of this effect here by demonstrating that compounds **1**–**4** act as stoichiometric inhibitors of recombinant *E. coli* TP even at enzyme concentrations of 1.3 nM. The compounds present the first active-site titrants of recombinant *E. coli* TP and can be used operationally in this way. The exocyclic guanidino analogues of compounds **1**–**4** show time-dependent behaviour: initial tight binding inhibition (not stoichiometric) increases slowly when enzyme is pre-incubated (over some hours) with the inhibitor at room temperature.

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043
Discovery of the novel prodrug DMU-943, a highly selective anticancer agent

S. Lodhi, P. C. Butler, G. A. Potter and K. J. M. Beresford

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Although the expression levels of many P450s differ between tumour and corresponding normal tissue, CYP1B1 is the only P450 isoform that is significantly and consistently over-expressed in tumours making it an excellent target for the tumour specific activation of anticancer prodrugs (Rooney et al 2004). We have recently patented a number of compounds based on the chalcone and stilbene structures, which show exciting potential as anticancer prodrugs (Potter et al 2001). A problem with using chalcones and stilbenes therapeutically arises from their high lipophilicity, which results in low water solubility and poor pharmacokinetics. One solution to this problem is to incorporate the functionality that has been shown by a structure activity study to be essential for bioactivation into a nitrogen containing heterocycle. This approach can lead to compounds with increased hydrophilicity and improved physicochemical properties (Wang et al 2002). The identification of selective CYP1B1 activated prodrugs is complicated by the down-regulation of P450 expression in most cultured cells. The cytotoxicity of the compounds synthesised in this study

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Poster Session 1 – Chemistry

041
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I. Morao[1,2], P. D. Higginson[2], J. C. Mitchell[1] and M. J. Snowden[1]

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042
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043
Discovery of the novel prodrug DMU-943, a highly selective anticancer agent

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were investigated using a human breast cell line panel that has been characterised for CYP expression in our laboratories. This panel consists of MCF7 ER+ tumour with very low levels of CYP expression, MCF7 induced with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to express high levels of CYP1A1, MDA-MB-468 ER- tumour that constitutively expresses CYP1B1 and MCF10A, a 'normal' breast cell line that has no basal CYP expression. Of the novel heterocyclic compounds synthesised, DMU-943 was found to give the most exciting results, showing very low toxicity to the normal MCF10A cells ($IC_{50} = 30\,\mu M$) but very high toxicity to MDA-MB-468 tumour cells, which express CYP1B1 ($IC_{50} = 0.015\,\mu M$). Thus, DMU943 is 2000-fold more toxic to the cancer cells, which express CYP1B1, than to the normal cells, which have no basal CYP expression. With the MCF7 cell line DMU-943 showed low toxicity ($IC_{50} = 8.0\,\mu M$ and $2.0\,\mu M$ after induction with TCDD). This suggests that DMU-943 is selectively metabolised by CYP1B1 as we have found that TCDD primarily induces CYP1A1 in MCF7 cells. Although the mechanism of activation of DMU-943 is currently unknown, our previous work (Potter et al 2001) indicates that the most likely mode of action will involve dealkylation of the methylenedioxy group in DMU-943 by CYP1B1 to generate a catechol, which then functions as a tyrosine kinase inhibitor. In conclusion, DMU-943 is metabolised to a highly cytotoxic compound by the tumour specific enzyme CYP1B1 and could prove to be a highly selective anticancer agent. Pre-clinical evaluation studies of DMU-943 are currently in progress.

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044
The development of novel prodrugs for the treatment of melanoma

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Malignant melanoma is a highly aggressive and often fatal cancer that affects the pigment cells of the skin. Although malignant melanoma accounts for only 11% of skin cancers, it accounts for more than 90% of skin cancer deaths. Over the past two decades the incidence of melanoma has risen at a higher rate than for any other type of cancer, and indications are that the incidence of melanoma will continue to rise. This, together with the knowledge that many melanoma tumours are difficult to treat with chemotherapeutic agents, due to the drug resistant characteristics of the disease, has led us (Jordan et al 2002), and others (e.g., Simoneva et al 2000), to seek new targeted methods for treating melanoma. Melanoma cells contain high levels of the tyrosinase enzyme (Prota et al 1994) and since this enzyme is virtually absent from other cells, it provides a good target for the in situ activation of prodrugs. The objective of this study was to synthesise and analyse two new series of prodrugs for the treatment of melanoma, that rely upon novel tyrosinase mediated bioprocessing to effect selective drug release. The prodrugs have been synthesised and characterised using a range of chemical and analytical techniques. Data has been accumulated using oximetry, HPLC assays and cytotoxicity studies to assess whether: the prodrugs are substrates for tyrosinase; the prodrugs are stable in aqueous and biological media; drug release is effected upon treatment with tyrosinase; and enhanced toxicity is evident in melanotic as opposed to amelanotic cell lines. Oximetry studies have proved that members of both series of prodrugs are good substrates for tyrosinase. HPLC studies have illustrated that, in general, urea linked prodrugs display more therapeutically useful stabilities in aqueous and biological media than thiourea linked prodrugs. In addition, HPLC studies have demonstrated that tyrosinase mediated drug release is effective for urea linked prodrugs from both series of prodrugs. Preliminary cytotoxicity studies using a melanotic and an amelanotic cell line have illustrated that the urea linked prodrugs display enhanced toxicity in the melanotic cell line, as required. This study has suggested that tyrosinase can indeed be utilised for the release of cytotoxic agents from prodrugs. Since tyrosinase is present in high levels within melanoma tumours, and is virtually absent from other cells, this suggests that tyrosinase-labile prodrugs may be of use within targeted strategies for the treatment of melanoma.

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045
Preparation, cytotoxicity and skin delivery of temozolomide esters, an alternative to temozolomide for treatment of skin tumours

P. Suppasansatorn[1], G. Wang[1,2], W. Wang[2], B. R. Conway[1] and Y. Wang[1]

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Temozolomide (TMZ), a DNA methylating agent, has been approved for treatment of primary or secondary brain tumours with oral administration. TMZ has shown equal efficacy to dacarbazine for patients with advanced metastatic melanoma in Phase II and II clinical trials (Middleton et al 2000). For treatment of skin tumours with TMZ, particularly for after surgery and early stages of cutaneous melanoma, skin delivery could provide a means of reducing systematic toxicity and improving the therapeutic benefit of the drug. Preparation of an ester prodrug is one method to enhance the skin absorption of a topically applied drug (De Rosa 2003). Since there is only one report describing the cytotoxicity of temozolomide acid (TMZA) compared with TMZ in TLX5 lymphoma cells (Tsang et al 1990), we conducted an in vitro assay to compare the activity of TMZA and TMZ against a panel of melanoma and glioma cells to verify validity of using TMZA in the skin deliverable ester prodrugs. TMZA and TMZ displayed similar cytotoxicity against the panel of the cancer cells (Table 1), indicating that TMZA could serve as the parent drug for the ester prodrugs. TMZ alkyl esters (methyl to octyl) were prepared by a direct esterification of TMZA with a bromo-alkane in the presence of a base or with an alcohol in the presence of a coupling agent such as PyBroP. The TMZ esters were characterized by IR, NMR, MS spectroscopic technology and microanalysis. In vitro, TMZ esters were readily hydrolysed into TMZA by a pig liver esterase under incubation conditions and monitored by ^{1}H NMR. Silicone membranes, rat skin and human skin were used to investigate permeation of TMZ and the ester prodrugs using a Franz-cell apparatus, and the drug in the receiver compartment was detected by HPLC. TMZ was unable to permeate through either the silicone membrane or the skin to appreciable levels, while the permeating ability of the ester prodrugs increased with extending length of the alkyl carbon chain from methyl to hexyl, after that permeating ability decreased. Furthermore in experiments with silicone membrane, TMZ esters were detected in the receptor compartment; while with skins, TMZA was found. The results demonstrated that esterases within skins are capable of converting TMZ esters into TMZA, so the ester prodrug strategy is feasible. TMZ hexyl ester (TMZ-HE) was chosen to conduct an in vivo antitumour study. BALB/c nude mice were subcutaneously inoculated with MV3 human melanoma on their right axilla and were treated with freshly made 5% (w/v) DMSO solution of TMZ-HE twice a day (total 20 mg a day). Over a two-week period, tumour growth in the mice was significantly inhibited, indicating that TMZ-HE is a promising candidate for development of a skin deliverable prodrug for treatment of skin tumours.

Table 1 In vitro cytotoxicity of temozolomide acid (TMZA) and temozolomide (TMZ) against cancer cells

Compounds	IC_{50} ($\mu g\,mL^{-1}$)				
	MV3[a]	M14[b]	B16[c]	B16-BL6[d]	SHG-44[e]
TMZA	36.047	99.020	36.170	43.500	7.067
TMZ	7.860	> 100	61.905	100	9.043

[a,b]human melanoma; [c,d]mouse melanoma; [e]human glioma.

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046
A comparison of catalysts to promote imidazolide couplings including the identification of 2-hydroxy-5-nitropyridine as a new, safe and effective catalyst

P. J. Dunn[1], W. Hoffmann[1], Y. Kang[1,2], J. C. Mitchell[2] and M. J. Snowden[2]

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The use of *N*,*N*′-carbonyldiimidazole (CDI) as a coupling agent for amide bond formation has recently been applied to the large-scale synthesis of a

number of pharmaceutical products. In addition, a survey of 128 chemical processes recently developed by AstraZeneca, GSK and Pfizer revealed that CDI is the third most common reagent used for *N*-acylation being used in 9 out of 84 examples. Although the relatively low cost, high yields and clean environmental conditions are advantages of this reagent, the drawback of CDI is that the resulting imidazolide is less reactive than the corresponding acid chloride. To accelerate acylation with hindered carboxylic acids or weakly nucleophilic amine, a catalyst is a necessary option. One of the most common catalysts is 1-hydroxybenzotrizole hydrate (HOBt). However, this reagent is known to explode when heated beyond its melting point (around 160°C) and the transportation of this catalyst is subject to restriction in Europe because of this explosive potential. In a previous investigation (Bright et al 2004), 2-hydroxypyridine proved to be a possible option as it is relatively cheap and safe to use according to Differential Scanning Calorimetry (DSC). Three additional catalysts were added to this current study, which concerns both catalytic effectiveness and safety for promoting imidazolide coupling. Of these catalysts, 1-hydroxy-6-chlorobenzotriazole is commercially available and with a lower pKa value compared with HOBt (4.15 vs 4.60) (Koppel et al 1993), which should be more effective. However, this catalyst, which has been described in the literature as a safe catalyst (Marder & Albericio 2003), was found to be shock sensitive. 1-Hydroxy-7-azabenzotriazole has an even lower pKa (3.47) (Marder & Albericio 2003) and hence should be a very effective catalyst. However, this reagent is reported to have significant safety drawbacks and is shock sensitive (Marder & Albericio 2003). Finally, 2-hydroxy-5-nitropyridine was found to be the best when both criteria were considered. As the pKa of the catalyst was thought to be related to its catalytic effectiveness, 2-hydrox-5-nitropyridine is a new catalyst for this type of reaction, which is significantly more acidic than 2-hyroxypyridine. It is safe, effective, readily available and a similar price to the 1-hydroxybenzotriazole hydrate.

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047
The synthesis of novel 6-azapyrimidine-thio-nucleoside analogues

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4′-Thionucleoside analogues have been of considerable interest since Bobek et al (1975) reported remarkable findings in the 1970s, where thionucleoside analogues were shown to be more potent in vitro than oxonucleoside analogues. There have been numerous publications concerning the synthesis of thionucleoside analogues with modified bases and sugar moieties. 6-Azauridine was among the earliest 6-azapyrimidine-nucleoside analogue synthesised and has shown beneficial effects in leukaemia and some hyperplastic diseases. In general, nucleoside analogues act as competitive inhibitors or alternative substrates to a specific viral enzyme involved in the biosynthesis of the viral RNA and DNA and thus disrupt the viral replication process. However, to some extent, the active antiviral agents were also incorporated into the host cells, which led to cell death or toxicity. L-nucleoside analogues are the promising solution to this problem, as the L-nucleosides are not generally recognised by host enzymes and thus provide good antiviral activity with minimal toxicity. The group research interest in this area is concerned with the synthesis of novel thionucleoside analogues with modified bases; 6-azapyrimidines and modified sugar moieties; acyclic, dideoxy and L-nucleosides. The novel analogues synthesised will be evaluated biologically at the Rega Institute in Leuven, Belgium as potential antiviral agents using a wide screen of viral assays, including Human Immunodeficiency Virus type 1 and 2 (HIV 1 and HIV 2), Herpes simplex Virus type 1 and 2 (HSV 1 and HSV 2) and Varicella Zoster Virus (VZV). An efficient method of protection and deprotection of the nucleoside analogues is also under investigation. The sugar moieties used in the experiment were either available commercially or synthesised using literature methods. The bases used were synthesised according to the described methodology. The Vorbrüggen coupling method was used, involving reaction of persilylated heterocyclic bases with the appropriate sugar moiety in the presence of a Lewis acid catalyst (Vorbrüggen et al 1981). Before the coupling procedure, the heterocyclic bases were silylated using *N*, *O*-bis(trimethylsilyl)acetamide (BSA). This was followed by the deprotection of the hydroxyl group according to the described methodology to obtain the desired nucleoside analogues. The synthesis of the novel 2′,3′-dideoxy thionucleoside analogues was achieved by applying the Barton-McCombie deoxygenation procedures (Hartwig 1983) on the 2′-deoxythionucleosides synthesised earlier. A series of novel acyclic and dideoxy thionucleoside analogues have been prepared for biological testing. The synthesis of both series will be described. A number of synthetic routes for the preparation of the L-thio sugar have been investigated.

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048
Trends in teaching of pharmacognosy and related subjects: a cross-sectional study of UK schools of pharmacy

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In the UK, herbal medicines and related products are widely available for purchase from community pharmacies, and pharmacists are a source of information and advice on these products for the consumer (Barnes 2001). Academic pharmacognosists in the UK continue to make calls for increased emphasis on the teaching of pharmacognosy and herbal medicinal products in undergraduate Masters of Pharmacy (MPharm) programmes at UK schools of pharmacy (SOPs) (Houghton & Barnes 2004). The aim of this study was to investigate trends in the teaching of pharmacognosy at UK SOPs. This was a cross-sectional study involving a postal questionnaire. Data were collected using the instrument developed for a previous study conducted in the year 2000 (Barnes 2001), with some modifications. The sampling frame comprised all 'old' (established more than 5 years ago), 'new' (first cohort of students within last 5 years) and proposed SOPs in the UK (n = 16, 3 and 7, respectively). In November 2004, a copy of the questionnaire and a covering letter were posted to academic staff known to be involved with pharmacognosy/related subjects, or to the head of school or other appropriate individual; 4 follow-up contacts were made to non-responders at approximately 4-week intervals. Data were entered into Microsoft Excel version 10 for storage and were analysed using SPSS version 12.0.1. To date, responses from 17 (65%) SOPs have been received (11 'old', 1 'new' and 5 proposed SOPs). Of the 12 respondents from existing SOPs, 11 (92%; 10 'old' and 1 'new' SOP) stated that their MPharm programme includes teaching of pharmacognosy and related subjects as part of the core curriculum. There is no statistically significant difference between the proportions for 2000 and 2004–2005 (13/15 vs 11/12 for 2000 and 2004–2005, respectively; $P = 1.000$; Fisher's exact test; 2-tailed). For 2004–2005, for the 11 SOPs teaching pharmacognosy/related subjects (e.g. complementary medicines) on the core curriculum, the median (lower and upper quartiles; Q_L, Q_U) total number of hours teaching (including self-directed study) and number of contact hours (lectures, tutorials, practical classes, including field visits) were 64 h (25,96) and 50 h (25,57), respectively. Data for both 2000 and 2004–2005 were available for 10 SOPs; there were no statistically significant differences in the total number of hours teaching, number of contact hours, number of lecture hours and number of practical class hours for 2004–2005 compared with 2000 ($P > 0.05$ for all; Wilcoxon Signed Rank Test for two related samples). The extent of teaching of pharmacognosy and related subjects on the MPharm core curriculum has not increased since the year 2000 despite inclusion of these subjects in the Royal Pharmaceutical Society of Great Britain's indicative syllabus (RPSGB 2001) published in 2001 and continued public interest in plant-based medicines. Heads of SOPs, including new and proposed SOPs, may need to consider whether or not their MPharm programme satisfies the RPSGB requirements and adequately prepares future pharmacists to advise on the safe, effective and appropriate use of herbal and complementary medicines.

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049
Prediction of Henry's law constant of organic chemicals

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Henry's law constant *H* (air-water partition coefficient) controls the absorption of inhalation ansthetics and other volatile drugs, affects the taste of

medicines by controlling vaporisation of volatile flavourings and is a factor in the distribution of drugs released into the environment (e.g. by excretion). There have been a number of quantitative structure–property relationship (QSPR) studies of H (Dearden & Schüürmann 2003), but none with such a large and diverse data-set as we have used here. We collected a total of 858 measured H values of organic compounds from literature. Structures were optimized in AMPAC using the PM3 hamiltonian, and CODESSA software was used to calculate 327 molecular descriptors. The data were split into a training set of 748 compounds and an external test set of 110 compounds. The step-wise regression in Minitab-14 was used to generate the following QSPR:

$$\log H = 0.517n_F - 0.244n_O - 1.25n_N - 0.591n_{ring} + 0.0046DPSA - 0.469PPSA - 5.66HADSA - 0.242\mu - 0.171$$
$$n = 748 \quad r^2 = 0.911 \quad s = 0.61 \quad r^2_{cv} = 0.910 \quad s_{cv} = 0.63 \quad F = 953.9$$

where n_F, n_O, and n_N are number of fluorine, oxygen and nitrogen atoms respectively, n_{ring} is number of rings, $DPSA$, $PPSA$, $HADSA$, and μ are difference between positively and negatively charged surface area, atomic charge-weighted partial positively charged surface area, hydrogen atom-dependent hydrogen bond donor surface area and dipole moment respectively, n is number of compounds in training set, r and s are correlation coefficient and standard error of estimate, r_{cv} and s_{cv} are cross-validated (leave-one-out procedure) correlation coefficient and standard error of estimate, and F is the Fisher statistic. The most important descriptors of the model are $HADSA$ ($r^2 = 0.44$), $PPSA$ ($r^2 = 0.34$), n_O ($r^2 = 0.29$), and μ ($r^2 = 0.20$). The inclusion of $HADSA$, n_O and n_N demonstrates the importance of hydrogen bonding on H, while $PPSA$ and μ model the effect of coulombic forces between solute and solvent molecules on H; the Onsager equation relates μ^2 directly with the solvation energy of a solute, which controls H. The QSPR was used to predict the Henry's law constants of the 110 external test-set compounds, and these were found to agree well with measured values ($r^2_{test} = 0.921$, $s_{test} = 0.52$). Hence the QSPR has excellent predictive ability.

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050
QSPR prediction of melting points of drug compounds

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Melting point (MP) is an important drug property, affecting drug solubility and formulation. There have been numerous attempts to model MP using quantitative structure-property relationship (QSPR) approaches (Dearden 1999, 2003), but none has proved very successful for drugs, probably because most drugs are not simple organic compounds. The aim of this study was to develop a QSPR model for the MP of drugs and medicinal compounds and to propose a mechanistic basis for the relation between molecular descriptors and their effects on MP. The MPs of 182 structurally diverse drugs and medicinal compounds, with MPs ranging from −118°C to 330°C, were retrieved from literature. 460 descriptors were generated in TSAR and CODESSA, following optimisation in TSAR and AMPAC using the AM1 hamiltonian. Using Minitab-14, a combination of step-wise regression and subset selection was used to select the best descriptors. The model was developed on 165 training compounds and was validated with the leave-one-out (LOO) cross-validation method and with the 17-compound external test-set. The best QSPR obtained was:

$$MP(^\circ C) = 290 + 592RNR + 399FHBS + 1571HADC + 31.5{}^3\chi^v_c - 820MPC - 195RNC - 16.7HLG$$
$$n = 165 \quad r^2 = 0.763 \quad RMSE = 44^\circ$$

where RNR is relative number of rings, $FHBS$ is fractional hydrogen bonding surface area, $HADC$ is number of hydrogen atoms with H-bond donor ability, ${}^3\chi^v_c$ is 3rd-order valence cluster molecular connectivity, MPC is maximum partial charge, RNC is relative negative charge, HLG is HOMO-LUMO gap, n is number of compounds in the training set, r is correlation coefficient and $RMSE$ is root mean square error of prediction. Cross-validation yielded $r^2 = 0.753$, $RMSE = 45^\circ$, whilst the external test-set yielded $r^2 = 0.705$, $RMSE = 38^\circ$. These are acceptable values considering the wide range of pharmaceutically relevant compounds included in this data-set, and are better than those obtained by Bergström et al (2003). By interpreting the descriptors, one can gain some insight into factors likely to govern the melting point of drug compounds. The most important descriptors in the model are RNC ($r^2 = 0.30$), HLG ($r^2 = 0.28$) and $HADC$ ($r^2 = 0.28$). RNC and its sign indicate that although the charged areas of molecules give rise to stronger attraction, the sharp charged spots on the molecules cause instability in the crystal lattice, leading to vibrational resonance of lattice points and low melting point. HLG is related to molecular hardness of a molecule. Soft molecules can easily change both their number of electrons and the distribution of charge within the molecule; therefore two such molecules can interact with each other in several ways. The sign of HLG in the model is completely consistent with this theory as the soft molecules are more diverse in attraction sites and should have high melting point. $HADC$ and also $FBSA$ ($r^2 = 0.22$) show the importance of H-bonding on melting point. RNR ($r^2 = 0.17$) is indicative of crystal packing ability, and ${}^3\chi^v_c$ ($r^2 = 0.16$) reflects the complexity and atomic diversity of a molecule.

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051
QSAR analysis of 5-arylidene-2,4-thiazolidinediones as aldose reductase inhibitors

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Diabetes mellitus is a chronic disease characterized by hyperglycaemia. Diabetic patients are at risk for developing long-term complications including neuropathy, nephropathy, retinopathy and cardiovascular diseases. At present, there is no specific therapy available for diabetic complications. Aldose reductase inhibitors (ARI) showed promising result in both preclinical and early clinical trials in management of diabetic complications. In the search for potent ARIs, many compounds of diverse structure have been identified. Here we report quantitative structure activity relationship (QSAR) studies of arylidene-2,4-thiazolidinediones (Bruno et al 2002) as ARIs. In this work we have tried to identify the associated molecular properties and exploited them to optimize ARI activity. The biological activity data (IC_{50} in μM) was converted to negative logarithmic mole dose (pIC_{50}) for QSAR analysis. The series was subjected to Fujita-Ban approach to estimate the de-novo contribution of substituents to the activity of the molecules.

$$pIC_{50} = 0.672\ (\pm 0.167)[3 - OC_6H_5] + 0.854\ (\pm 0.279)[3 - CH_3 + 1.332\ (\pm 0.123)[CH_2COOH] + 4.877$$
$$n = 23, r = 0.937, SE = 0.269, F = 45.271 \qquad (Model - 1)$$

The series was divided into training set of 18 compounds and test set of 5 compounds for 2D and 3D QSAR analysis on the basis of structural diversity and biological activity. The series was subjected to Hansch analysis to find the contribution of various substituents constants at position R_1 and R_2 of molecule.

$$pIC_{50} = 1.338\ (\pm 0.161)\ HD_2 + 0.012\ (\pm 0.007)\ MR_1 + 4.862$$
$$n = 18, r = 0.907, SE = 0.322, F = 34.905, ICAP < 0.090,$$
$$Q^2 = 0.659, S_{PRESS} = 0.447, S_{DEP} = 0.407,$$
$$Chance\ 0.001, r^2_{pred} = 0.673 \qquad (Model - 2)$$

Molecular modelling studies were carried out using MOE (MOE Users Manual) and VALSTAT (Gupta et al 2004). The energy minimization of the molecules was carried out via steepest descent, conjugative gradient and truncated Newton method in sequence using MMFF94 force field with energy tolerance value of root mean square gradient 0.001 kcal mol^{-1} and maximum number of iteration set to 1000. Conformational search of each energy-minimized structure was performed using stochastic approach. All conformers generated for each structure were analysed in conformational geometries panel with great care and the lowest energy conformation of each structure was selected and added to a molecular database to compute various physicochemical properties. Two models were selected on the basis of statistical criteria.

$$pIC_{50} = 0.029\ (\pm 0.006)PEOE\_VSA - 0 + 0.454\ (\pm 0.083)\ a\_don - 0.412(\pm 0.047)E\_ang + 14.511$$
$$n = 18, r = 0.955, SE = 0.234, F = 48.639, ICAP < 0.590,$$
$$Q^2 = 0.843, S_{PRESS} = 0.314, S_{DEP} = 0.277, r^2_{bs} = 0.917,$$
$$S_{bs} = 0.055, Chance < 0.001, r^2_{pred} = 0.728 \qquad (Model - 3)$$

$$pIC_{50} = 0.243(\pm 0.082)\text{chi1\_C} - 0.343(\pm 0.052)\text{E\_ang} + 0.544(\pm 0.097)\text{a\_don} + 13.138$$
$$n = 18, r = 0.928, SE = 0.296, F = 28.731 ICAP < 0.433, Q^2 = 710$$
$$S_{PRESS} = 0.426, S_{DEP} = 0.376, r^2_{bs} = 0.869, S_{bs} = 0.091,$$
$$Chance < 0.001, r^2_{pred} = 0.870 \qquad (Model - 4)$$

a_don and PEOE_VSA-0 contributed positively, while E_ang contributed negatively to Model-3, wheres chi1_C and a_don contributed positively while E_ang contributed negatively to Model-4. The QSAR analysis gave insight to some common important structural features (i.e. distal end of hydrogen donor groups at nitrogen atom of thiazolidinediones is important for ARI activity and could interact through hydrogen bond formation with the enzyme while the molar refractivity at the phenyl ring of the nucleus plays a significant role in the hydrophobic interaction with enzyme). De-novo analysis inferred that the 3 position of the phenyl ring can be explored for optimization of the analogues.

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052
Fluorescent polymeric binding mimics of CYP2D6

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Studies are described whereby fluorescent molecularly imprinted polymers (MIPs) have been prepared as binding mimics of cytochrome P450 isoform CYP2D6 and which have the potential to form the sensing element in a high-throughput assay for the prediction of CYP2D6 affinity. Most drugs are metabolised by the cytochrome P450 family of enzymes and as a result the half-life of a drug within the body is linked with its rate of cytochrome P450 mediated biotransformation. Some P450s, such as CYP2D6, are subject to population-based polymorphisms and hence it is essential to the drug discovery process to know the P450 metabolic fate of any drug candidate. MIPs are crosslinked polymers containing bespoke functionalised cavities arising from the inclusion of template molecules in the polymerisation mixture and their later extraction. With the inclusion of appropriate functional monomers, binding sites are created, which have a memory for the templates both in terms of shape and matching functionality. Fluorescent MIPs have the added benefit of a fluorophore in their cavities, which may respond to the presence of bound test compound by a change in their fluorescence output (Rathbone & Ge 2001; Rathbone et al 2005). Based upon earlier modelling studies (Islam et al 1991), a series of relatively rigid templates was designed, which potentially represent the "negative" of the CYP2D6 active site. These were incorporated into crosslinked polymers along with a fluorescent functional monomer to give fluorescent MIPs. After extraction of the templates the MIPs were challenged with a panel of drugs to test for any discriminatory recognition of known CYP2D6 binders. Entry of a test compound into an MIP cavity resulted in quenching of the fluorescence of the MIP-bound fluorophore. Calibration studies were also performed using a soluble linear polymer containing the fluorophore to determine the relative quenching abilities of the test compounds towards the fluorophore in a non-MIP environment. The MIPs re-bound their templates and various imprinting effects were encountered for test compound/drug recognition. One MIP in particular exhibited a rational discrimination amongst the related synthetic templates and was reasonably successful in recognising CYP2D6 substrates from a drug panel. The template used to produce this MIP is the subject of ongoing studies to optimise the CYP2D6 substrate recognition profile of the imprinted polymers.

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053
Discovery of a N^1-Benzylidene-pyridine-4-carboxamidrazone with potent and selective activity against Gram-positive bacteria

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Certain N^1-benzylidene-pyridinecarboxamidrazones are known to have antimycobacterial activity and this constitutes most of the published work for these compounds in the antimicrobial area (e.g. Mamolo et al 1993; Billington et al 1998). Most of the published compounds of this type that have been examined for biological activity contain bezylidene moieties substituted with relatively non-polar functionalities (halogen, alkyl, alkoxy) and very little indeed by way of hydrogen bond donor functionality. Therefore, to explore this neglected area of possible bioactivity, we examined a set of phenolic N^1-benzylidene-heteroarylcarboxamidrazones. The compounds were prepared by condensation of the appropriate aldehyde and pyridine-, pyrazine- or quinolyl-heteroarylcarboxamidrazone. The latter building blocks were prepared by the action of ethanolic hydrazine hydrate upon the corresponding cyano compounds. Each compound was initially tested for a zone of inhibition on agar, against both a methicillin-sensitive strain of *Staphylococcus aureus* (reference strain NCTC 6571) and a clinical isolate of MRSA (96-7475). If a zone of inhibition was observed against the MRSA strain, the MIC for that compound was measured against a panel of organisms, using a multi-point innoculator and the agar diffusion method. The panel of Gram-positive organisms used comprised three methicillin-sensitive *S. aureus* strains, 10 MRSA clinical isolate strains, two *E. faecium* strains and seven strains of *E. faecalis* (including six clinical isolates). Eight different Gram-negative bacteria were also tested to investigate the possibility of any broad-spectrum activity. One compound in particular, an N^1-benzylidene-pyridine-4-carboxamidrazone, gave an intriguing and very sharp structure–activity profile. The compound in question contained a phenolic hydroxyl, as well as two bulky lipophilic alkyl, substituents in the benzylidene portion. This compound exhibited the most potent activity of the set against Gram-positive bacteria (MIC 2–4 $\mu g\,mL^{-1}$ against all strains tested). The same high activity (2–4 $\mu g\,mL^{-1}$) was also observed against a panel of seven vancomycin-resistant enterococci clinical strains. Any change made to the substitution pattern in this compound or the deletion or modification of substituents resulted in much reduced or completely abolished antimicrobial activity. Although the compound exhibited no activity against *Mycobacterium fortuitum*, it proved to be highly active against *M. tuberculosis* $H_{37}Rv$ (100% inhibition at 6.25 $\mu g\,mL^{-1}$, Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Birmingham, AL). In stark contrast to the Gram-positive results, no activity was observed against any Gram-negative bacteria. It is this dramatic selectivity that is the focus of current investigations.

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054
Synthesis of novel imidazolyl analogues of moclobemide as monoamine oxidase inhibitors

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MAO (EC 1.4.3.4) is an outer mitochondrial membrane FAD containing enzyme found in nearly all tissues. On the basis of their substrate and inhibitor specificities, two major isoforms have been described, the MAO-A and the MAO-B, made up of different polypeptides. MAO-A preferentially catalyses the oxidative deamination of serotonin (5-HT), adrenaline (A) and noradrenaline (NA) and is selectively inhibited by moclobemide or 4-chloro-N-(2-morpholinoethyl)benzamide (Silvestri 2003). The early MAO inhibitors, such as isocarboxazide and tranylcypromine, were nonselective and irreversible. Because of their adverse actions, the therapeutic applications of this first generation MAO inhibitors have been diminished. Today efforts towards the development of monoamine oxidase inhibitors are focused on selective MAO-A or MAO-B inhibitors. Selective MAO-B inhibitors, such as deprenyl, are being examined in the treatment of disorders such as schizophrenia, Alzheimer's disease and Parkinson's disease. The MAO-A inhibitors, such as moclobemide, are effective in the treatment of depression (Hadizadeh & Ghodsi 2005). Our interest in the chemistry of the nitrogen-containing ring, specially imidazole, which provides a site for binding with amino acids within the proteins and enzymes, motivated us to design a similar structure to moclobemide by replacing the moclobemide phenyl ring with substituted imidazole. First, an in-silico study was performed. So, molecules of moclobemide and designed structures were simulated in Hyperchem 7 under semi-empirical method using AM1, closed shell gradient 0.01. Superposing the molecules, not surprisingly, showed high similarity (RMS 0.46 Å) between the molecules, which suggests similar interaction in the active site of MAO. Then novel 1-benzyl-2-(alkylthio)-N-(2-morpholinoethyl)-1H-imidazole-5-carboxamides were synthesized. Initially, a mixture of benzylamine hydrochloride,

dihydroxyacetone dimmer and potassium thiocyanate in n-butanol and glacial acetic acid was stirred for 3 days. After that a precipitate was formed and filtered to give 1-benzyl-2-mercapto-imidazole-5-methanol (**1**). Then it was reacted with alkyl halide (RX) in alcoholic solution of sodium hydroxide to give 2-alkylthio-1-benzylimidazole-5-methanol (**2**). Compound **2** was refluxed in chloroform overnight at the presence of manganese dioxide to give 2-alkylthio-1-benzylimidazole-5-carbaldeyde (**3**). Further oxidation of **3** with aqueous alkaline solution of silver nitrate (tollens reagent) afforded 2-alkylthio-1-benzylimidazole-5-carboxylic acid (**5**). Compound **5** was reacted with thionyl chloride to give the corresponding 2-alkylthio-1-benzylimidazole-5-carbonyl chloride (**6**). Condensation of **6** with 2-morpholinoethylamine in tetrahydrofuran at the presence of pyridine gave the title 1-benzyl-2-(alkylthio)-N-(2-morpholinoethyl)-1H-imidazole-5-carboxamides (**7**). The title compounds and intermediates were characterized by ^{1}H NMR and IR spectroscopy.

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055
Prediction of chemical carcinogenicity based on an evaluated chemical safety database CAESAR

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Numerous chemical substances of natural and synthetic origin have been produced for our use such as various drugs, foods, personal care products, household cleaners, and agricultural chemicals. However, the adverse effects of most of those agents on human health and global environment are not known. Among chemicals currently in commerce, quite a few are ascertained on their safety, and reliable data on chemical hazard assessment are quite limited. Therefore, an attempt on a basis of Quantitative Structure–Activity Relationship (QSAR) models for estimating carcinogenicity has been performed (Tanabe & Matsumoto 2002; Romualdo 2003; Tanabe et al 2004). To construct a database system having reliable data on chemical toxicity, a CAESAR (Computer-aided Evaluation of Chemical Safety with QSAR) system has been developed in this work. It consists of two databases. One contains reliable, critically reviewed, experimental hazard data on selected chemicals. For example, carcinogenicity data on about 1000 chemical substances have been collected from various sources, such as NTP, NCI and others, and ranked into five categories according to the reliability of the risk of incidence of cancer. Another contains hazard data predicted from QSAR models, which relate toxicity of molecules to chemical structure on the basis of available biological properties of more than 100 000 chemical substances in commerce. This system predicts chemical carcinogenesis by the artificial neural network (ANN) with back-propagation method. For the ANN modelling, a three-layered neural network model to predict the carcinogenicity of a variety of compounds was developed. For the output, the data of 454 compounds with the carcinogenic activity of male rats from the database were employed. The ANN was used to classify the chemicals studied into two categories, namely inactive or active. The set of 454 compounds was split into training (144 compounds), validation (143) and test (167) sets. The carcinogenicity data were entered into the output layer (0 for noncarcinogenic or 1 for carcinogenic chemicals). The inputs were 10 principal components from 37 kinds of molecular descriptors, including quantum chemical descriptors. To solve the problems, such as over-training, over-fitting and local minimum in training, the neural network with the error-back-propagation algorithm, various conditions of the network such as the training cycles and neuron numbers of the intermediate layer were optimized. While an NN was trained by using the learning set, the errors between the output and teaching data for the learning, validation and test sets were counted in each cycle. The optimum model showed a correct classification rate close to 74%, which is higher than any reported values in the Predictive Toxicology Challenge 2000–2001 contestants (www.informatik.uni-freiburg.de/~ml/ptc/). The present result demonstrated the superiority of the ANN as a nonlinear modelling method, such as multiple linear regression.

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056
Dipeptidyl peptidase IV deficiencies are associated with low relative abundance of nitrergic cells in the thymus of sub-strains of Fischer F344 rat

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Dipeptidyl peptidase IV (Dpp4) activity of CD26 is associated with the co-activation of T-cells alongside T-cell receptor mediated stimulation. Pharmacological inhibitors of Dpp4 offer a therapeutic possibility in the suppression of autoimmune activity of peripheral T-cells (Mathisen 2003). However, the same signalling mechanism, through T-cell receptor and co-stimulatory molecules, such as Dpp4/CD26, may underlie central (thymic) tolerance by clonal deletion of potentially autoreactive cells. The abundance of cells expressing inducible nitric oxide synthase (iNOS) coined nitrergic, is proposed as one mechanism involved in the negative selection of self-reactive thymocytes. Nitrergic cells are deficient in autoimmune-susceptible Lewis rat compared with resistant Fischer and Sprague Dawley strains (Downing et al 1998). Genetic or pharmacological disruption of T-cell co-stimulation within the thymus may also lead to reduced nitergic cell activation in thymus. We examined nitrergic cell abundance in two sub-strains of the F344 Fischer rat (GER and JAP) characterised for inactivity of Dpp4 compared with wild type (USA) (Karl et al 2003). All rats were subject to prior periodontal treatment, females and aged (~22 months) with JAP and GER being 9 and 14 days younger than USA, respectively. It was hypothesised that either or both inactive Dpp4$^-$ mutant sub-strains of F344 Fischer rat, GER and JAP, would have reduced co-activatory signalling; this would lead to reduced nitergic cell activation in thymus compared with wild type (Dpp4$^+$) USA. Paraformaldehyde-fixed thymi were stored frozen (−20°C) before parallel processing of batches containing all three strains for enzyme histochemistry in 100-micron sections (four sections from four rats per strain). Abundance of medullary nitrergic cells (mean count/mm^2 ± s.e.m.) stained positive by NADPH-diaphorase was used as a marker of inducible nitric oxide synthase (iNOS). Counts and section surface area measurements were made from glycerol-mounted sections. Results confirmed lower abundance of nitrergic cells in both JAP (2.27 ± 0.29) and GER (1.92 ± 0.32) thymus compared with the USA sub-strain (6.88 ± 0.84; one way analysis of variance $P < 0.001$). There was no significant difference between nitrergic cell abundance of the Dpp4$^-$ sub-stains (GER and JAP) according to Tukey's follow up test. Our results are consistent with the notion that Dpp4 activity of CD26 contributes to co-stimulatory function during central thymic tolerance via the expression of iNOS. Although JAP and GER Dpp4$^-$ sub-strains may have an increased risk of autoimmune T-cell repertoires, these cells may be deficient in their ability to activate. The use of inhibitors of Dpp4 to treat multiple sclerosis should be monitored for their ability to cause autoimmune complications following withdrawal, as has been reported for cyclosporine A (Kosaka et al 1990).

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057
Structural properties of andrographolide derivatives with anti-cancer activity

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Andrographolide (A), a labdane diterpenoid from *Andrographis paniculata*, is cytotoxic against cancer cells but has additional activity (e.g. hypotensive and anti-inflammatory). To eliminate side-effects it is desirable to define the structural features that endow A with one particular type of activity. Nanduri et al (2004) prepared an epoxy derivative of A and investigated the effect on cytotoxic activity of making acetyl or larger esters out of one or more of its three hydroxyl groups. Surprisingly, mono- and diacetylation reduce activity but triacetylation enhances it. By determining the crystal structure of triacetylandrographolide (TAA) and comparing it with the similarly acetylated epox-

ide (TAAO; Nanduri et al 2004) and 8-chloro derivative (TAACl; Roengsumran et al 2002) as well as A itself (Spek et al 1987) and a model for its epoxide AO, by using CAChe to seek preferred conformations, and by optimising preferred structures ab-initio in the 6–31G* basis set, we have investigated the effects on geometry of addition to the exocyclic double bond and of acetylation. Despite identical space groups, the unit cell dimensions of TAA ($a = 8.044(2)$, $b = 14.805(3)$, $c = 21.680(4)$ Å) differ from those for TAAO (9.532(1), 32.287(6), 8.510(2) Å, respectively), suggesting different molecular dimensions. Torsion angles about the two single bonds in the chain linking the decalin and gamma-butyrolactone ring systems define their orientation (Table 1). As numbered by Spek et al (1987) they are T1 = C10-C9-C11-C12 and T2 = C9-C11-C12-C13. They change little between TAA and AG, but TAAO and TAACl show drastic changes. The orientation of the hydroxymethyl group is defined by T3 = C3-C4-C19-O4. The considerable differences between crystallographically observed conformations and calculated global minima suggest that several low-energy conformations are available, from which crystal packing forces select one for the solid state that may differ from the minimum in solution. Calculations of molecular descriptors in CAChe have not given strong correlation with activity: substituent-based log*P* values, unlike activity, increase monotonically upon successive acetylation of A, and HOMO and LUMO energies as well as their difference appear unrelated to activity.

Table 1 Key torsion angles (°) by X-ray and modelling

Torsion angle*	TAA	A	TAAO	TAA Cl	AO
T1 (X)	164.2	170.6	90.2	95.7	
T2 (X)	158.9	158.1	−137.3	−138.2	
T3 (X)	−156.0	−62.2	−79.9	−81.4	
T1 (C)	81.2	79.6	150.6		140.8
T2 (C)	−145.7	−144.1	−118.5		128.4
T3 (C)	−80.9	−77.1	−80.1		−63.9

*(X) = X-ray data; (C) = calculated values.

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058
Polarization of differentiated U937 cells by cytokine fragments

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Leukocytes exposed to chemoattractants, have been observed to adopt polarized morphologies and undergo chemotaxis. Cell polarization may not necessarily indicate chemotaxis, but can be used as a screening assay to detect potential chemotactic compounds (Haston & Wilkinson 1988). Cellular polarization was used to screen two cytokine fragments, Interleukin-1 beta [163-171] (β163) and Platelet Factor 4 [47-70] (PF4770) (Forni et al 1989; Jouan et al 1999), for chemotactic properties. Peptide fragments were synthesized by solid-phase Fmoc-based techniques and tested in vitro against dibutyryl cAMP differentiated U937 (dU937). dU937 cells (10^4–10^5cells/mL) were incubated (20 min at 37°C) with equal volumes of buffer (Hanks' balanced salt solution, 1% BSA and 10 mM HEPES), 10^{-8} M N-Formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLF, positive control), PF4770 (1.6×10^{-9} to 8.0×10^{-5} M) or β163 (4.5×10^{-7} M, 4.5×10^{-5} M and 2.2×10^{-4} M). Cells were then fixed with 2.5% glutaraldehyde, stained (Wright's stain) and air-dried before microscopy at 400× magnification. Changes in cell morphology were quantified by an image analysis programme (Carnoy 2.0, Laboratory Plant Systematics). Data comparisons were made using paired T-test, two-tailed at 95% CI (Minitab, Minitab Inc.). dU937 cells showed evidence of polarization when stimulated by fMLF. Subjective analysis of the micrographs seems to indicate that PF4770 produces polarization at 4.0×10^{-6} M and 1.6×10^{-5} M, whereas β163 elicited change in cell morphology at the lowest concentration tested. Cell polarization appeared the most evident for β163; in addition the cells adopt an aggregated appearance in comparison to control cells. PF4770 has been documented to be anti-angiogenic in vitro at 10 μM (Jouan et al 1999), within the concentration range where polarization was observed. Significant changes in cell surface area and perimeter were noted for fMLF ($p < 0.005$), PF4770 at 4.0×10^{-6} M, 8.0×10^{-7} M and 8.0×10^{-9} M ($P < 0.03$). β163 only caused a significant change in cell perimeter at 4.5×10^{-7} M ($P < 0.02$), which was also obtained for buffer alone ($P = 0.049$). This suggests that the polarization seen for β163 could be induced by the buffer. Results seem to indicate that both peptides have chemotactic properties. To determine whether polarization is indicative of chemotaxis, further analysis using a filter migration assay or an orientation assay is required (Haston & Wilkinson 1988). Demonstration of chemotaxis would be beneficial as it would highlight the peptides potential as candidates for cancer immunotherapeutic agents.

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059
The effect of hyperglycaemia on EGFR signalling and its potential role in diabetes-induced vascular dysfunction

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Diabetes mellitus is a well known risk factor in the development of micro- and macrovascular disease (Tognini et al 2003), more specifically it has been shown that hyperglycaemia associated with type I and type II diabetes is a key contributor to the development of these vascular complications (Gordon 2004). However, the underlying molecular mechanisms involved in the development and progression of these complications remain unclear. Since hyperglycaemia is known to affect many cell signalling molecules, including the epidermal growth factor receptor (EGFR) and molecules downstream of EGFR (Obata et al 1998), we hypothesize that EGFR plays an important role in mediating diabetes-induced vascular dysfunction through its control over the growth, migration, proliferation and hypertrophy of vascular cells such as endothelial cells. Western blotting and PCR techniques were used to identify changes in protein and gene expression of EGFR and molecules of the EGFR pathway from the ECV-304 cell culture model grown in high glucose (25.5 mM) compared with control cells grown in normal glucose (5.5 mM). Also, vasoconstriction response was measured from the mesenteric vascular bed of untreated, genistein treated or AG1478 treated STZ-induced diabetic rats. Also, gene expression changes in diabetic rats and the effects of the two inhibitors were assessed using microarray gene expression profiling technology. Initial studies in the mesenteric vascular bed of streptozotocin (STZ) diabetic rats showed higher levels of phosphorylated (p)EGFR compared with non-diabetic controls. We also showed that impairment in vasoconstriction response to noradrenaline in the STZ-diabetic rats could be normalized by both genistein (a broad spectrum receptor tyrosine kinase (RTK) inhibitor) and AG1478 (a selective EGFR inhibitor). Microarray results showed that the number of gene expression changes induced by diabetes was reduced in drug-treated animals. Studies in the endothelial-like human ECV-304 cell line grown in high glucose media also showed alterations in EGFR signalling. Upregulation of (p)EGFR levels by high glucose were accompanied by an increase in the gene expression of the EGFR ligands HB-EGF and TGFα. High glucose concentration increased phosphorylation of c-src and increased the activation of ADAM10, which are both involved in the EGFR transactivation machinery. Also, the protein expression of EGFR downstream pathway molecules ras and p38mapk were increased by high glucose and the phosphorylation of akt, an antiapoptotic molecule was decreased by high glucose. These results suggest an important role of EGFR signalling in mediating diabetes-induced vascular dysfunction. The changes in EGFR ligands and in molecules of the EGFR transactivation machinery by high glucose suggest that glucose may not directly alter the phosphorylation of EGFR but may alternatively modulate the expression of upstream molecules. The altered phosphorylation of molecules such as ras, p38mapk and akt, which are all part of particular EGFR pathways, suggest that the effect of high glucose is complicated, involving much cross-talk between these pathways. From these results we conclude that inhibition of EGFR may represent a novel therapeutic strategy for the treatment of diabetes-induced vascular dysfunction.

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060
Use of the steroid backbone in the search for a range of compounds as potential inhibitors of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD)

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Isozymes of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) are responsible for the conversion of C(17) = O groups to the reduced hydroxy moiety and, as such, they are responsible for the formation of the potent androgen testosterone from androstenedione. 17β-HSD has been considered as a potential target in the fight against hormone-dependent cancers, such as prostate cancer. Here, we report the initial results of the use of our derived transition-states (TS) (Owen & Ahmed 2004) for the forward reduction reaction (Penning 1997) in the design and synthesis (and subsequent biochemical evaluation) of a range of novel inhibitors of this enzyme. We also rationalise the structure–activity relationship for the synthesised compounds in comparison with a small range of known flavone-based inhibitors of 17β-HSD (Le Lain et al 1999). In the initial design process, we concluded that compounds which are able to mimic the steroid structure would possess inhibitory activity. More specifically, we concluded that groups able to mimic the steroidal C(17) = O group would be expected to act as a substrate and would therefore undergo the reduction step, whereas the C(3) = O moiety of the steroid backbone would allow the molecule to bind to the enzyme active site via hydrogen bond interaction. As such, the proposed inhibitors were synthesised involving the initial oxidative ring cleavage of the steroid backbone, leading to a 'keto-acid', which may be derivatised through the reaction with a range of alcohols (from methanol to octanol) so as to give the appropriate ester. The reactions, in general, proceeded in good yield and without any major problems (typically the yield for the ring cleavage reaction was 80%, while for the esterification reaction it was approximately 70%). In an effort to show that the C(17) = O was important in the inhibition process, we synthesised a small range of compounds where the C(17) = O group within the A-ring cleaved product (after esterification of the carboxylic moiety) was reduced with sodium borohydride to give the C(17)-OH functionality. The synthesised compounds were then evaluated for inhibitory activity against 17β-HSD using modified standard literature procedures. Furthermore, we used previously reported flavones as standard compounds in an effort to produce a comparison between the synthesised compounds and known inhibitors of 17β-HSD. Consideration of the initial screening data shows that the compounds are, in general, weak inhibitors of 17β-HSD. For example, the most potent compound is the pentyl ester (namely, 3-(3a,6-dimethyl-3,7-dioxo-dodecahydro-cyclopenta[a]-naphthalen-6-yl)-propionic acid pentyl ester) possessing an inhibitory activity of 29% at [I] = 100 μM. On evaluating the C(17) reduced form of this compound, and subsequently evaluating it against 17β-HSD, we discovered that the compound possessed no inhibitory activity against 17β-HSD. As such, the initial results of our study appear to support our previous hypothesis, in particular, that compounds which are able to mimic the C(17) = O group within the steroid substrate may act as potential inhibitors.

Le Lain, R. et al (1999) *J. Pharm. Pharmacol*. **51** (Suppl.): 23
Owen, C. P., Ahmed, S. (2004) *Biochem. Biophys. Res. Comm*. **318**: 131–134
Penning, T. M. (1997) *Endocrine Rev*. **18**: 281–305

061
Synthesis, biochemical evaluation and molecular modelling of a range of 4-substituted phenyl alkyl azoles as inhibitors of 17α-hydroxylase/17,20-lyase (P450$_{17\alpha}$)

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The biosynthesis of androgens is catalysed by a single enzyme and involves two distinct steps. The initial 17α-hydroxylation is undertaken by the 17α-hydroxylase (17α-OHase) component to give the 17α-hydroxylated intermediate. The cleavage of the C17-C20 bond is carried out by the 17,20-lyase component resulting in the formation of the appropriate androgen. No crystal structure exists for 17α-hydroxylase/17,20-lyase (P450$_{17\alpha}$); however, a number of techniques have used in an attempt to elucidate further information regarding the active site (e.g. homology modelling). In our efforts we have developed a novel technique (the substrate-haem complex (SHC) approach), which we have utilised to good effect to produce a representation of the active site of the overall enzyme complex (Ahmed et al 1999) and the essential components required for the catalysis process. In an effort to add support to the SHC, we have undertaken the design and synthesis of a range of compounds based upon 4-substituted phenylalkyl azoles (imidazole and triazole) where the alkyl 'spacer group' ranged in length from the methyl to pentyl and the substitution on the phenyl ring ranged from H to halogens. Here, we report the initial results of our study into the synthesis, biochemical evaluation and molecular modelling of inhibitors of both components. The synthesis of the final azole compounds was achieved through the *N*-alkylation of the azole using the appropriate 4-substituted phenylalkyl bromide and a suitable mild base. In general, the reactions proceeded in good yield and no major problems were encountered. However, in the case of the larger alkyl chain containing compounds, where the alkyl chain was greater than propyl, it was necessary to synthesise the appropriate 4-substituted phenylalkyl bromide. The biochemical evaluation of the synthesised compounds was undertaken using standard literature assay procedure (Li et al 1996). The results show that the compounds were, in general, equipotent or more potent than the standard compound for P450$_{17\alpha}$, namely ketoconazole (KTZ); the most potent imidazole-based inhibitor 4-fluorophenyl heptyl imidazole (IC50 = 57.5 ± 1.5 nM against lyase and IC50 = 173.62 ± 0.1 nM against 17α-OHase) was found to be ~29 and ~22 times more potent than KTZ (IC50 = 1660 ± 150 nM against lyase and IC50 = 3760 ± 10 nM against 17α-OHase). As a comparison, the 4-bromo-phenyl pentyl imidazole compound was found to possess IC50 = 58.1 ± 5.2 nM against lyase and IC50 = 500.0 ± 40 nM against 17α-OHase (i.e. it was also found to be more potent than KTZ). Detailed consideration of the inhibitory data for the compounds shows that there is a good correlation between IC50 and logP. The compounds were modelled onto the SHC for the overall P450$_{17\alpha}$ enzyme, which showed that the synthesised compounds were able to fit within the active site without undergoing any unfavourable steric interactions. Furthermore, the increase in inhibitory activity with increasing alkyl chain length adds further support to the requirement of both a hydrogen-bonding interaction (with the active site) and the Fe-imidazole interactions. The compounds synthesised within this study are therefore excellent lead compounds in the design (using the SHC approach) of further novel inhibitors of P450$_{17\alpha}$.

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062
The role of pKa in determining the synthesis of a wide range of phenolic compounds

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The synthesis of sulphamated compounds is well documented and involves the reaction between the phenol derivative and aminosulphonyl chloride (from formic acid and chlorosulphonyl isocyanate) in the presence of a suitable solvent. However, due to its instability, aminosulphonyl chloride is prepared in situ prior to the addition of the phenolic starting material. The synthesis is often aided through the use of a base, as such, a number of bases have been used although sodium hydride (NaH) is often preferred. However, the use of NaH, using dimethyl formamide (DMF) as the solvent, results in a major problem and involves the reaction between the sulphamated product and DMF, resulting in the so-called 'DMF-adduct' as well as poor yield. In an effort to overcome these problems, Okada et al (2000) developed the use of dimethyl acetamide (DMA) in the absence of base, an approach that led to an increase in the yield of the sulphamated compound. We have utilised this route to good effect and have synthesised a number of potent compounds, including 4-sulfamoyloxy-benzoic acid cycloheptyl ester (Patel et al 2004). Furthermore, our studies have also suggested that halogen atoms may be added to the phenyl moiety so as to increase the inhibitory activity (Ahmed et al 2001). However, when we attempted to synthesise a range of mono- and di-halogenated derivatives of 4-hydroxybenzoic acid, we discovered that the synthesis proved to be difficult; indeed, for the bromo-substituted derivative, none of the sulphamated compounds could be synthesised. Here, we report the results of our study in an effort to rationalise the lack of any sulphamated product. In our study, the molecular modelling software CaChe was used in the construction of the inhibitors and to determine the charge density on the phenoxide and phenol derivatives. The pKa of the esters of 4-hydroxybenzoic acid was determined using a spectroscopic technique and involved the determination of the UV spectra under acidic, neutral and basic conditions. From the pKa determination, we discovered that the presence of the bromine atoms on the phenolic moiety resulted in a decrease in pKa of the corresponding phenol (e.g., the pKa value of ethyl 4-hydroxy benzoate, ethyl 3-bromo-4-hydroxybenzoate and ethyl 3,5-bromo-4-hydroxybenzoate was found to be 9.3, 8.9 and 8.5, respectively). From the charge density calculations, we observed that the insertion of the halogen group resulted in a decrease in the charge on the oxygen atom of the phenoxide ion. For example, we discovered that the fluoro-derivative possessed the highest pKa values within the halogen

derivatives. The observation that the charge density decreased from fluorine to bromine can be rationalised in terms of the atomic volume of the halogen and therefore the effective distribution of the negative charge. The results of the current study can therefore be used to allow us to determine the degree of difficulty in the synthesis of the sulphamoylated compound.

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063
The molecular modelling of a series of steroidal and non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase (17β-HSD)

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The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) is responsible for the conversion of the C(17) carbonyl group to the reduced 17β-hydroxy moiety. As such, this enzyme is responsible for the formation of potent androgens and oestrogens (e.g. the conversion of the weak oestrogen, oestrone, to the more potent oestrogen, oestradiol). The reverse reaction, the oxidation of C(17)-OH to the carbonyl group, is also known to be undertaken by this enzyme, albeit by different isozymes – 17β-HSD is known to exist in at least 12 different isozymes. In the fight against hormone-dependent cancers, such as breast cancer, 17β-HSD is considered to be an important potential biological target. Here, we report the initial results of the development of a model for the active site of this enzyme through the rationalisation of the inhibitory activity of a number of inhibitors of 17β-HSD — it should be noted that we considered the reduction of oestrone to oestradiol, which is undertaken by type 1, so as to determine an initial pharmacophore that we can begin to modify the inhibitor backbone, allowing us to determine factors responsible for isozyme specificity. The structures of the natural substrate (namely oestrone) and the known steroidal (e.g. 5-androstene-3,17-dione and atamastane) and non-steroidal (e.g. 7-hydroxyflavone and 2,5-diphenyl-p-benzoquinone) inhibitors were all constructed within the Alchemy III molecular modelling software. The completed structures were then subjected to an initial minimisation using the conjugate-gradient algorithm until the gradient fell below 10^{-6}. Conformational analysis was performed (using the systematic search method with energy windows of 20–40 kcal mol^{-1} and bond rotation between 15–30°) on flexible parts of the inhibitors using Powersearch to determine the low energy conformers. On the assumption that the shape of the natural substrate would reflect the nature of the binding site of 17β-HSD, the lowest energy conformer(s) were superimposed by specification of three points on both the inhibitor and ring A or ring D of the natural substrate, using the polar groups in the fitting process. From the results of our modelling study, we conclude that within the range of inhibitors considered, the ability of these compounds to occupy similar area/volume of space as oestrone is a major factor in their inhibitory activity. Furthermore, the area corresponding to the C(17) area of the steroid backbone is considered to be sterically hindered (Penning, 1997), as such, any interactions between the inhibitor and the NADPH moiety resulting in steric interactions result in a decrease in the inhibitory activity. For example, 7-hydroxyflavone (IC50 = 9.0 $\mu\mu$ (Le Lain et al, 1999)), when superimposed onto the oestrone backbone, does not appear to possess any unfavourable conformations that would result in steric interactions with the active site. 6-Hydroxyflavone (IC50 = 16.4 $\mu\mu$) is found to possess low energy conformers that undergo steric interaction with the NADPH moiety and therefore possesses weak inhibitory activity. In conclusion, we believe this initial molecular modelling has provided us with a good model with which to design novel inhibitors of 17β-HSD.

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064
Synthesis and biochemical evaluation of a series of phenyl ketones as potential inhibitors 17β-hydroxysteroid dehydrogenase (17β-HSD)

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In the fight against hormone-dependent breast cancer, 17β-hydroxysteroid dehydrogenase (17β-HSD) has been considered as a potential target. In an effort to design novel inhibitors of this enzyme, we have previously undertaken the derivation of the transition-states (TS) of the reduction reaction (Penning 1997; Owen & Ahmed 2004). Here, we report the initial results of the use of our derived model in the design and synthesis (and the subsequent biochemical evaluation against rat testicular microsomal enzyme, using radiolabelled substrate) of a range of novel inhibitors of this enzyme and the rationalisation of their inhibitory activity against 17β-HSD (Le Lain et al 1999). In the design of the potential inhibitors, compounds were superimposed onto the TS and the 'degree of superimposition' evaluated. It was concluded from our modelling studies that compounds based upon a phenyl backbone and possessing a carbonyl moiety to mimic the steroidal C(17) = O group should possess some inhibition. As such, a range of compounds, based upon 4-hydroxyphenyl ketones were synthesised involving Friedel-Crafts acylation of phenol – a range of acid chlorides from acetyl chloride to dodecanoyl chloride were used as well as some cyclic acyl chlorides. Furthermore, we considered the role of the 4-hydroxy moiety by derivatisation through the synthesis of the corresponding ester, involving the reaction between the range of 4-hydroxyphenyl ketones with acetyl chloride. The reactions, in general, proceeded in good yield (typically 60%) and without any major problems. The synthesised compounds were evaluated for inhibitory activity against 17β-HSD using modified standard literature procedures. Consideration of the initial screening data shows that the compounds based on the 4-hydroxyphenyl ketone are weak inhibitors of this enzyme (e.g. 4-hydroxyhexaphenone was found to possess 24% inhibition at [I] = 100 $\mu\mu$). In comparison, the known flavonoid based inhibitors were also observed to possess poor inhibitory activity (i.e., 7-hydroxyflavone was found to possess 14% inhibition at similar inhibitor concentration). In a previous study, Le Lain et al (1999) reported the IC50 of 7-hydroxyflavone to be 9 μM. In summary, although the compounds within the current study have shown poor inhibitory activity, the results of our study suggest that there may be some validity to our initial model of the 17β-HSD active site, particularly the use of a carbonyl moiety to mimic the C(17) = O group within the natural substrate. These compounds are therefore good lead compounds in the search for more potent inhibitors of 17β-HSD.

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065
The structure-activity relationship determination of a series of amides based upon 4-hydroxybenzoic acid as potential inhibitors of the enzyme oestrone sulphatase

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The production of oestrone in postmenopausal women has been shown to be catalysed by the enzyme oestrone sulphatase (ES). We have reported the synthesis of a number of 4-hydroxybenzoic acid based inhibitors (Ahmed et al 2001), in particular, a series of alkyl and cycloalkyl esters of 4-sulphamoxylated benzoic acid, one of which has proved to be a highly potent inhibitor of ES, namely 4-sulphamoyloxy-benzoic acid cycloheptyl ester (Patel et al 2004) and which was found to be more potent than 667-COUMATE which is currently undergoing clinical trials. However, our ester-based compounds were found to have poor stability; in particular, the ester moiety was found to readily undergo hydrolysis. Here, we report the results of the synthesis, in vitro biochemical evaluation and the rationalisation of the structure-activity relationship of a series of mono- and di-*N*-alkylated compounds based upon sulphamic acid 4-carbamoyl-phenyl ester. In the synthesis of the target compounds, the first step involved the synthesis of the alkylated derivatives of *N*-alkyl-4-hydroxy benzamide involving a one-pot synthesis of the *N*-alkyl derivatives of 4-hydroxy benzamide. The 4-hydroxy moiety was converted to the sulphamate group, involving a reaction with sulphamoyl chloride (formed in situ from the reaction between formic acid and chlorosulphonyl isocyanate) using dimethyl acetamide (DMA) as the solvent in the absence of a base (Okada et al 2000). The synthesised compounds were evaluated against human placental microsomal enzyme using standard literature method, involving the determination of the conversion of radiolabelled oestrone sulphate to oestrone – for comparison EMATE, COUMATE, 667-COUMATE and 4-sulphamoyloxy-benzoic acid cycloheptyl ester were used as standard compounds. The results of this study show that the synthesised compounds are, in general, poor inhibitors of ES. The most potent compound is the di-pentyl derivative (namely sulphamic acid 4-dipentylcarbamoyl-phenyl ester) and which is found to possess 40% inhibition at 50 μM concentration – under similar conditions, 667-COUMATE was found to possess 56% inhibition at 0.25 μM concentration.

Detailed consideration of the inhibitory activity shows that there is a good correlation between the logP of the inhibitors and the observed inhibitory activity. That is, as the length of the alkyl chain increases, the inhibitory activity increases upto the optimum logP (approximately 3.0) beyond which inhibitory activity decreases with increasing alkyl chain length. Using the TS obtained, we have rationalised the decrease in inhibitory activity being due to an increase in the steric interaction between the alkyl chain and the active site wall – the dialkyl side chain appears to further increase the steric interactions, as such, we propose that the increased interactions result in these compounds possessing poor inhibitory activity when compared with the standard compounds. In conclusion, we added further support to our representation of the active site and the hypothesis that steric interactions with the hydrogen bonding group, which normally binds to the steroid C(17) = O group, results in a decrease in inhibitory activity.

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066
Synthesis of alkylated derivatives of (4*S*,5*R*)-(−)-4-methyl-5-phenyl-2-oxazolidinone as probes in the investigation of the active site of 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$)

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Extensive research has been undertaken to produce compounds that are both potent and selective inhibitors of the enzyme 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$). Compounds that have shown potent inhibitory activity against this enzyme have been based upon an azole moiety, which is postulated to undergo reversible co-ordinate bond formation with the Fe atom of the cytochrome P-450. However, due to the non-selectivity of these compounds, workers within the field have undertaken extensive searches for compounds with increased selectivity. Here, we report the synthesis and screening of a range of oxazolidinone-based compounds, which use a phenylamine moiety as the Fe-ligating group – the phenylamine moiety binds poorly to the Fe and as such should possess less side-effects. Furthermore, in an effort to add further support to the substrate-haem complex (SHC) approach developed by us (and the methodology used in the design of these compounds), we have attempted to use the target compounds as probes of the active site of this enzyme. The use of the oxazolidinone-based chiral auxiliaries has involved the synthesis of the *N*-acyl derivatives. *N*-Alkylation has been somewhat ignored although we have previously reported the *N*-alkylated derivatives of the Evan's chiral auxiliary. In general, the synthesis of the *N*-acyl derivatives has been undertaken using strong bases such as LDA. In our hands, the use of LDA in the alkylation reaction proved to be unsuccessful. The use of sodium hydride (NaH), using anhydrous *N*,*N*-dimethylformamide (DMF) as the solvent, provided us with the range of *N*-alkylated compounds in high yield (typically 40–70%). In the synthesis of the phenylamine derivative, we considered the nitration of the phenyl ring system, followed by the subsequent reduction of the nitro group to the desired amine functionality. The reaction was undertaken using nitric acid (5 M) in dichloromethane (DCM) at room temperature – the reaction proceeded without any major problems (yield 65%). The target phenylamine-based compounds were obtained through the use of hydrogen gas and palladium on activated charcoal (yield 80%). The synthesised compounds were screened for inhibitory activity using the standard literature method (Li et al 1996) using ketoconazole (KTZ) as the standard inhibitor. From the results of the initial screening against P-450$_{17\alpha}$, we observe that the novel inhibitors possess poor inhibitory activity and are weaker than the standard compound, KTZ (90% inhibitory activity at [KTZ] = 10 μM), the most potent compound being the *N*-heptyl derivative, which was observed to possess some 60% inhibition at [I] = 50 μM. Comparison of the biochemical evaluation data shows that the inhibitory activity appears to be related to the alkyl chain length. Modelling of these compounds using the SHC approach suggests that within the P-450$_{17\alpha}$ active site, hydrogen bonding interaction between the active site (corresponding to the C(3) area of the steroid backbone) and the C = O group within the oxazolidinone moiety is not possible. Furthermore, steric interaction between the inhibitor and the enzyme active site is possible, and may be a factor in the poor inhibitory activity observed within the compounds under study.

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Poster Session 1 – Pharmacology

067
Synthesis of S-nitrosothiols as tracheal smooth muscle relaxants

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The S-nitrosothiols are the biological metabolites of nitric oxide. They represent a more stable metabolite of nitric oxide that can either be stored, or transported in the form of S-nitrosothiols (e.g. S-nitrosocysteine in the body). S-Nitrosothiols have remarkable therapeutic use as vasodilatory drugs and also they could be involved in vivo in some of the physiological processes. Various S-nitrosothiols, such as S-nitrosocaptopril, S-nitrosomercaptobenzothiazole, S-nitrosomercaptobenzimidazole and S-nitroso-*N*-acetylpenicillamine, have been synthesized in the laboratory by reported methods (Rodney & Kerr 1993). For the synthesis, a suspension of thiols, sodium acetate and hydrobromic acid in water was kept aside for 15 min, treated with acetic anhydride, stirred for 1 h and then kept undisturbed for the next 3 h. The resulting precipitates were collected by filtration and dried. The precipitates collected were then identified by means of TLC and quantified by UV spectroscopy and Griess reagent (Griess Reagent Kit for Nitrite Determination (G-7921) 2003). Smooth muscle relaxant activity for synthesized S-nitrosothiols was tested in vitro on rat tracheal chains. In this study, after the anaesthetisation of male albino rats (100–150 g) with chloroform, tracheal muscle was removed; after the removal of fat and connective tissue, the trachea was opened longitudinally opposite the trachealis and transverse strips consisting of two adjacent cartilage rings were prepared and suspended in a 25-mL organ bath containing a modified Kreb's solution previously maintained at 37°C. After the stabilization period of 45 min the trachea was allowed to relax with molar concentration 40.8×10^{-4} of adrenaline and S-nitrosothiols were added in different doses to the organ bath, no response was seen. In a separate experiment, tracheal chains were allowed to contract with a molar concentration 33.8×10^{-4} of histamine; relaxation with synthesized compounds was observed and recorded on the kymograph. The endothelium was removed by gently rubbing tracheal chain; complete lack of relaxation to a dose of adrenaline was demonstrated (Emmerson & Mackay 1979). Then, cumulative dose response curves were obtained for the various S-nitrosothiols by adding increasing concentrations of the synthesized compounds to tissue baths and relaxation was recorded and compared with the reported derivative (i.e. S-nitroso-*N*-acetylpenicillamine (SNAP)). As shown in Table 1, S-nitrosomercaptobenzimidazole (SNMBI) and S-nitrosomercaptobenzothiazole (SNMBT) produced a dose-dependent relaxation of the muscle (Sata et al 1990).

Table 1 Dose-dependent % relaxation produced by synthesised products such as S-nitrosomercaptobenzothiazole and S-nitrosomercaptobenzimidazole

Sr. no.	Molar concn	S-Nitroso-*N*-acetylpenicillamine (% relaxation)
1	16.3×10^{-4}	58.33 ± 4.10
2	24.4×10^{-4}	50.00 ± 3.84
3	36.3×10^{-4}	41.66 ± 2.44

Values of s.e.m. have been shown in the table.

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068
Relative abundance of nitrergic cells in the thymus of diabetes resistant non-diabetic (DRnd), diabetes prone pre-diabetic (DPpd) and diabetic (DPdb) substrains of BioBreeding (BB) rat

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The BioBreeding (BB) rat is an important animal model of human insulin-dependent diabetes mellitus (IDDM), developing spontaneous pancreatic insu-