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-nitrosomercapto-benzimidazole 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-

litis, selective beta cell destruction, insulin deficiency and hyperglycaemia (Mordes et al 1996). Abnormality in the central (thymic) tolerance by clonal deletion of potentially autoreactive cells represents a possible contributory factor to the susceptibility of substrains of the BB rat. The abundance of cells expressing inducible nitric oxide synthase (iNOS), termed nitrergic, is proposed as one mechanism involved in the negative selection of self-reactive thymocytes necessary to initiate T-cell dependent beta cell destruction. Nitrergic cells are deficient in autoimmune-susceptible Lewis rat compared with resistant Fischer and Sprague Dawley strains (Downing et al 1998). We therefore examined nitrergic cell abundance in 2 substrains of BB rat – diabetes resistant non-diabetic (DRnd) and diabetes prone, which was subdivided between pre-diabetic (DPpd) and diabetic (DPdb) phenotypes. Beckman glucose Analyzer measurements of blood confirmed rats as either non-diabetic (glucose $< 200\,mg\,dL^{-1}$) or diabetic (glucose > 250) for at least 2 consecutive days. Five rats (~13 weeks old) were used for each group, males and females being pooled since there are no differences in autoimmune susceptibility. Paraformaldehyde-fixed thymi were stored frozen (−20°C) before parallel processing of batches containing all 3 groups for enzyme histochemistry in 100-micron sections. Abundance of medullary nitrergic cells stained positive by NADPH-diaphorase was used as a marker of iNOS. Counts and section surface area measurements were made from glycerol-mounted sections, where tissue shrinkage is less than other studies using dehydration and xylene-based mountant. An observer blind to substrain performed the counts. Mean nitrergic cell abundance (count/mm^2) ± s.e.m. was 0.798 ± 0.133 for DRnd, 0.506 ± 0.065 for DPpd and 0.117 ± 0.032 for DPdb. One-way analysis of variance with Tukey's follow-up test identified significant difference between both DP groups compared with DRnd and between the pre-diabetic and diabetic groups of the DP substrain ($P < 0.001$). Our results identify an association between the DP genotype and deficiency in nitrergic cell abundance and suggest that the diabetic phenotype exacerbates this deficiency, although the possible effect of gender needs to be further investigated.

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Poster Session 1 – Drug Metabolism

069
Metabolism of ibuprofen: synthesis and chromatographic resolution of 3-hydroxyibuprofen

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The metabolism of (*R,S*)-ibuprofen has been extensively investigated, the two major metabolites being 2-hydroxyibuprofen and carboxyibuprofen (Mills et al 1973). The urinary excretion of these metabolites, both free and conjugated, together with the unchanged drug account for approximately 75% of an oral dose following administration of the racemate. The corresponding 3-hydroxy metabolite, the metabolic precursor of carboxyibuprofen, has been identified as a minor urinary metabolite (Brooks & Gilbert 1974) and reported following incubation of the drug with human liver microsomes (Hamman et al 1997). The formation of 3-hydroxyibuprofen results in the introduction of a second stereogenic centre into the drug and thus four stereoisomeric forms of the metabolite are possible. Investigations into the stereoselectivity of ibuprofen oxidation are hampered due to the lack of stereochemically defined samples of the 3-hydroxy metabolite, the formation of which may partially, or totally, determine the stereochemistry of the major urinary metabolite carboxyibuprofen. To address this problem, we now report the synthesis, chromatographic resolution and partial stereochemical characterisation of this metabolic intermediate. The aryl aldehyde, ethyl 2-(4-formylphenyl)propionate, prepared by reaction of the corresponding 4-chloromethyl derivative with hexamine followed by acid hydrolysis (Sommelet reaction), was treated with the phosphrous ylid obtained by reaction of diethylphosphite with 2-bromopropionic acid. The resulting acrylic acid, 3-(4-(1-ethoxycarbonylethyl)phenyl)-2-methylacrylic acid, was converted to a mixed anhydride on treatment with isobutylchloroformate and reduced with sodium borohydride to yield the corresponding alcohol. Ester hydrolysis, followed by catalytic hydrogenation, palladium on charcoal, yielded the target compound, 3-hydroxyibuprofen. Chromatographic resolution of the four stereoisomers of 3-hydroxyibuprofen was achieved using an amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) chiral stationary phase (CSP) and a mobile phase consisting of hexane:methanol:ethanol (96:3:1% v/v/v), containing trifluoroacetic acid (0.05% v/v), at a flow rate of $1.0\,mL\,min^{-1}$ with UV detection at 220 nm. Under these conditions the stereoisomers were baseline resolved with retention times of 28.8, 34.1, 53.5 and 72.4 min and resolution factors of 2.33, 6.42 and 3.57. Examination of material isolated by semi-preparative chromatography, using the same CSP, by circular dichroism and comparison of the spectra obtained with those of the enantiomers of ibuprofen, indicated that the first and second eluting analyte pairs possessed *R*- and *S*-stereochemistry in the propionic acid moiety. Thus, we conclude that the faster and later eluting pairs of chromatographic peaks correspond to the *R,R*-/*S,R*- and *R,S*-/*S,S*-diastereoisomeric analytes respectively.

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070
In vitro metabolism of captopril ester prodrugs: experimental and molecular modelling approaches

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Captopril is an orally administered ACE-inhibitor that could benefit from the zero order kinetics afforded by percutaneous absorption. A series of straight chain and branch chain esters prodrugs of captopril has been developed as potential candidates for transdermal delivery. These carboxyl ester prodrugs have similar or higher flux to the parent drug in vitro (Gullick et al 2004; Moss 2004). For bioavailabilty issues, it is necessary to investigate the metabolism of the prodrugs in the skin. In vitro metabolism experiments were undertaken to assess the degree of metabolism that the prodrugs undergo, and to assess if structural changes can influence metabolism. The latter issue was also investigated via molecular modelling/docking approaches. In vitro experiments were adapted from a previously published method (Woolfson et al 1990). This method involved incubation of the ester 37°C with porcine liver esterase. Samples were taken at time-points, at which time hydrolysis was quenched using acetone (to de-nature the enzyme). Samples were then filtered and analysed by LC-MS to determine the concentration of captopril and prodrug at each time-point. Molecular modelling experiments were performed to determine the lowest mean docked energy ($kJ\,mol^{-1}$) between each prodrug and two individual proteins known to reside in the skin. The proteins used for docking studies were Cytochrome P450 2c1 (1BBC) and RNAse (1OG5). Experimentally, the metabolism of each prodrug appears to follow pseudo-first order kinetics. The plots ln(percentage remaining drug) against time yielded straight lines, from which the rate constants and half-lives were determined. Rate of metabolism reached a peak at the ethyl and propyl prodrugs, and decreased substantially thereafter. Further, the difference in rate of metabolism observed between straight chain and branch chain is not significant, indicating very little change in the rate constant due to adjustments in the alkyl chain, and this is supported by the findings from the molecular modelling experiments. Molecular modelling studies indicated that the lowest mean docked energy decreases as ester chain length increases, from –10 to $-16\,kJ\,mol^{-1}$. It is predicted that larger increases in chain length will lead to a greater reduction of the docking energy. However, this is not a significant issue with prodrug candidates due to the substantial decrease in aqueous solubility and bioavailability as chain length increases.

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071
Oxidatively modified LDL modulate heme oxygenase-1 and glutathoine levels in human aortic smooth muscle cells in response to oxidative stress via the mitogen activated protein kinases

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Atherosclerotic vascular disease is a major cause of death in the western world and involves retention of oxidised LDL in the vessel wall (Ross 1999), leading to endothelial and smooth muscle cell (SMC) dysfunction. Oxidised LDLs induce antioxidant stress proteins such as heme oxygenase-1 (HO-1) (Siow et al 1999) and elevate levels of the endogenous antioxidant glutathoine (GSH) as an adaptive response to increased oxidative injury. HO-1 catabolises