Poster Session 2 – Medicinal Chemistry

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Molecular modelling of novel ellipticine–amino acid inhibitors of topoisomerase I

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The inhibition of topoisomerase I activity by substituted ellipticines has previously been reported, with the most active inhibitors being the 9-hydroxy, N2-methyl and 5-ethyl derivatives (Foss 1992). In the course of our work to prepare ellipticine derivatives with greater topoisomerase I inhibitory activity, a number of compounds have been synthesised and their interaction with the enzyme modelled. Enhancement of the electrophilicity of the pharmacophore increases the topoisomerase cleavable complex stabilising activity and we have accomplished this by introduction of a formyl group at the 9-position.

Cummings et al (1996) have shown that amino-acid conjugates of common intercalators display a topoisomerase I and II inhibition which circumvents drug resistance. Amino-acid derivatives of ellipticine at the C9–C10 position have also previously been prepared which cyclise to give oxazolopyridocarbazoles, thus lengthening the intercalating moiety.

In the course of our work on substituted ellipticines, we have performed initial molecular modelling studies to establish the binding characteristics of ellipticine derivatives of this type with DNA.

Molecular modelling was carried out on an SGI Octane R12000 workstation using the Insight II 2000 graphics interface and Discover 98.0 simulation software (Accelrys, Cambridge, UK) employing a modification of the method of Cairns et al (2002). A fragment of canonical B-DNA d $(ATGCAT)_2$ with an intercalation gap of 7Å was generated incorporating explicit counterions. The amino acid–ellipticine conjugate was then docked manually into the intercalation gap in the four possible orientations with respect to both grooves. This complex was then solvated in a periodic box 35Å × 35Å × 35Å of explicit TIP3P water and minimised using separate nonbonded cutoffs for van der Waals and electrostatic interactions until an energy gradient of 0.01 kcal mol^{-1} $Å^{-1}$ was attained. Molecular dynamics was run on the entire system for 110ps (10ps equilibration) at a simulated temperature of 298K with the terminal nucleotides tethered at a force constant of 100 kcal mol^{-1} $Å^2$ and the minimised average structure taken from the simulation trajectory. The most favourable of the four average drug–DNA complexes was then inserted into the central DNA binding pore of topoisomerase I (Brookhaven 98S1504) in a non-covalent binary complex, and the entire complex minimized. The resultant structure showed a favourable interaction energy between the ellipticine derivative and the enzyme (-250.198 kcal mol^{-1}), with significant distortion of the active site residues. This distortion would prevent DNA strand scission, thus inhibiting topoisomerase activity.

Preliminary results from topoisomerase I inhibitory assays indicate that our compounds are indeed active against the enzyme as inhibitors as opposed to poisons, the mechanism usually associated with conventional topoisomerase I active agents (Pommier 1998).

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Cummings, J., Macpherson, J., Meikle, I., et al (1996) *Biochem. Pharm.* **52**: 979–990

Foss, P. (1992) *Mol. Pharmacol.* **42**: 590

Pommier, Y., Pourquier, P., Fan, Y., et al (1998) *Biochim. Biophys. Acta* **1400**: 83–106

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Comparison of a number of commercial software programs for the prediction of octanol–water partition coefficient

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Octanol–water partition coefficient (P) is a key property in drug design and development, since it controls drug transport within an organism, and is one of the drug properties specified in the Lipinski "rule of 5" (Lipinski et al 1997) relating to drug absorption. Partition coefficient is tedious to measure, and so, especially with the advent of high-throughput screening, methods for the rapid calculation of log P have become available commercially. Some years ago a survey was carried out (Mannhold & Dross 1996) of the predictive ability of some of the methods then available. Those methods have now been updated, and new methods have become available. We have therefore carried out a survey of nine such methods: ClogP (www.biobyte.com), KOWWIN (esc.syrres.com), SLIPPER (www.ipac.ac.ru/qsar), ABSOLV (www.sirius-analytical.com), ProLogP (www.compudrug.com), SPARC (ibmlc2.chem.uga.edu/sparc), Interactive Analysis (IA) (www.logp.com), KlogP (www.multicase.com) and ACD (www.acdlabs.com). To facilitate comparison with the Mannhold & Dross (1996) survey, we used the same 138 compounds (90 simple organics and 48 drugs) as they did, although it is possible that some of their measured log P values are not accurate because of assumptions made in the correction for ionisation. A few of the measured log P values have been updated from the values given by Mannhold & Dross (1996). Two of the methods that we surveyed, namely IA and SPARC, are available on-line, and IA is also available off-line. All the off-line methods can be operated in high-throughput batch mode. The results are shown in Table 1 as percentages of compounds within the error ranges ($\leqslant 0.5$), (> 0.5, $\leqslant 1.0$) and (> 1.0) log units from the measured values of log P.

Table 1 Percentage of compounds with log P (calc.) within error ranges

Method	$\leqslant 0.5$	$> 0.5 \leqslant 1.0$	>1.0
ACD	93.5	5.8	0.7
IA	93.5	5.1	1.4
KOWWIN	89.1	7.3	3.6
SPARC	88.5	9.0	2.5
ClogP	88.4	10.9	0.7
Klogp	81.9	15.9	2.2
ProLogP	81.2	15.2	3.6
SLIPPER	81.1	10.9	8.0
ABSOLV	67.4	17.4	15.2

With one exception, all the methods handled all 138 compounds; SPARC did not recognise the SMILES codes (Weininger 1988) of 16 of the 48 drugs. Since, overall, drug log P values were less well predicted than were those of simple organics, SPARC is therefore probably less accurate than the figures in Table 1 indicate. There has been considerable improvement in the prediction of log P since 1996, for the best method at that time yielded 90.6% with $\leqslant 0.5$ error, 5.8% with 0.5–1.0 error and 3.6% with > 1.0 error.

Lipinski, C. A., et al (1997) *Adv. Drug Deliv. Rev.* **23**: 3–25

Mannhold, R., Dross, K. (1996) *Quant. Struct.-Act. Relat.* **15**: 403–409

Weininger, D. (1988) *J. Chem. Inf. Comput. Sci.* **28**: 31–36

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Comparison of a number of commercial software programs for the prediction of aqueous solubility

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The aqueous solubility (S) of a drug candidate is of prime importance, for it affects dissolution rate and limits the blood level that can be achieved. The "rule of 5" proposed by Lipinski et al (1997) was in part aimed at solubility estimation. With the advent of combinatorial chemistry and high-throughput screening it is imperative that rapid calculation methods are available for aqueous solubility, since its experimental determination is tedious and often difficult (Ghose et al 1999).

In recent years, a number of computerised methods have become available for the prediction of aqueous solubility, and we have carried out a survey of seven of these, namely WSKOWWIN (esc.syrres.com), SLIPPER (www.ipac.ac.ru/qsar), ABSOLV (www.sirius-analytical.com), ACD (www.acdlabs.com), SPARC (www.ibmlc2.chem.uga.edu/sparc), Interactive Analysis (IA) (www.logp.com) and Accelrys (www.accelrys.com). The last two are available on-line, and IA is also available off-line. All the off-line methods can be operated in high-throughput batch mode. We selected 113 compounds (96 simple organics and 17 drugs and pesticides) from the compilation of Abraham & Le (1999), and generated predicted log S values with each of the prediction methods. The results are shown in Table 1 as percentages of compounds within the error ranges ($\leqslant 0.5$), ($> 0.5 \leqslant 1.0$) and (> 1.0) from the measured log S values (all S values in mol L^{-1}).

Table 1 Percentage of compounds with log S (calc.) within error ranges

Method	$\leqslant 0.5$	$> 0.5 \leqslant 1.0$	> 1.0
IA	75.0	17.0	8.0
ACD	72.6	18.6	8.8
Abraham & Le	72.6	18.6	8.8
WSKOWWIN	69.9	18.6	11.5
SPARC	68.1	18.6	13.3
ABSOLV	61.9	25.7	12.4
SLIPPER	50.4	23.0	26.6
Accelrys	48.7	31.0	20.3

All the methods handled all 113 compounds, with one exception: IA did not recognise the SMILES code (Weininger 1988) of methane. ABSOLV is a computerised version of the Abraham solvation method (Abraham et al 1999); the manual solvation method was used by Abraham & Le (1999), and their results are included in Table 1 for comparison.

The ability to predict log S is much inferior to the ability to predict log P (partition coefficient); for example KOWWIN was able to predict 90% of log P values to within $\pm$ 0.5 log units of measured values (Mannhold & Dross 1996). This is almost certainly due to the relatively large entropic contributions to solubility, which are difficult to model.

Abraham, M. H., Le, J. (1999) *J. Pharm. Sci.* **88**: 868–880

Abraham, M. H., et al (1999) *Pestic. Sci.* **55**: 78–88

Ghose, A. K., et al (1999) *J. Comb. Chem.* **1**: 55–68

Lipinski, C. A., et al (1997) *Adv. Drug Deliv. Rev.* **23**: 3–25

Mannhold, R., Dross, K. (1996) *Quant. Struct.-Act. Relat.* **15**: 403–409

Weininger, D. (1988) *J. Chem. Inf. Comput. Sci.* **28**: 31–36

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QSAR analysis of P-glycoprotein-regulated brain penetration of a diverse group of drugs

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P-glycoprotein is a membrane-bound transporter protein responsible for inhibiting the penetration of drugs through various membranes, including the blood–brain barrier (Jolliet-Riant & Tillement 1999). Many drugs from different therapeutic areas have been identified as substrates for P-glycoprotein (Schinkel et al 1996).

An important tool for the investigation of the rôle of P-glycoprotein in blood–brain barrier penetration is the P-glycoprotein knockout mouse (mdr1a (−/−)) (Schinkel et al 1994), which completely lacks the form of P-glycoprotein expressed in the blood–brain barrier. Ayrton & Morgan (2001) have collected data on the ratio (R(−/+)) of brain levels of 22 diverse drugs, covering 13 therapeutic classes, in the brain of the mdr1a (−/−) mouse to those in the wild-type mdr1a (+/+) mouse, and we have carried out a quantitative structure-activity relationship (QSAR) analysis of those data.

QSAR analysis was carried out using the program QsarIS (www.scivision.com), which generates many physicochemical and structural descriptors and uses a genetic algorithm to select the best QSAR model. The QSAR that we obtained was:

$$\log R(-/+) = 0.113\ {}^{3}\chi^{v}_{p} + 22.6\ \alpha/V - 0.104\ Ct_{SdssC} - 0.0435\ N_{circ} - 0.317 \quad (1)$$

$n = 22$, $r^2 = 0.854$, $Q^2 = 0.788$, $s = 0.182$, $F = 24.9$

where ${}^{3}\chi^{v}_{p}$ = 3rd order valence path molecular connectivity; α/V = polarisability per unit volume; Ct_{SdssC} = no. of C atoms forming one double bond; N_{circ} = no. of circuits (all possible rings); n = no. of compounds; r = correlation coefficient; Q = cross-validated correlation coefficient (leave-one-out procedure); s = standard error of the estimate; and F = Fisher statistic.

We also carried out external validation by removing 5 compounds randomly, developing the QSAR on the remaining 17 compounds and using the QSAR to predict the activity of the 5 test compounds. This procedure was carried out 4 times, and the mean errors found each time were: 0.181, 0.167, 0.208 and 0.190. Since the standard error of the mean from equation 1 is 0.182, these errors indicate that the QSAR has good predictivity. We further carried out 100 randomisations of the biological data, to check for chance correlations; the mean r^2 found was 0.186, and the maximum r^2 found was 0.520. Thus the risk that equation1 is due to chance is $< 1\%$.

It is difficult to put a precise physicochemical meaning on the descriptors selected in equation 1, but they can be interpreted as modelling molecular bulk and weak (dispersive) interactive forces. This accords with the conclusions of a previous study (Dearden et al 2001) of P-glycoprotein-associated ATPase activity of a different group of drugs.

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Jolliet-Riant, P., Tillement, J. (1999) *Fund. Clin. Pharmacol.* **13**: 16–26

Schinkel, A. H., et al (1994) *Cell* **77**: 491–502

Schinkel, A. H., et al (1996) *J. Clin. Invest.* **97**: 2517–2524

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Rational design and synthesis of inhibitors of *Leishmania* NAD-dependent glycerol-3-phosphate dehydrogenase

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The Leishmaniases are parasitic diseases caused by different species belonging to the genus *Leishmania*, a protozoa transmitted by the phlebotomine sandfly.

Current chemotherapeutic treatments for the disease have appreciable toxicity and in some endemic areas, up to 40% of the parasites exhibit some degree of resistance. Currently available therapies involve either intravenous or intramuscular delivery and the need for an orally active agent has led to the development of miltefosine. However, there is still a need for alternative oral treatments that are affordable, efficacious and safe (TDR News 2000).

Trypanosomastid parasites are highly dependent on glycolysis as a source of ATP production (Opperdoes 1987). The glycolytic enzyme glycerol-3-phosphate dehydrogenase (G3PDH) is common to both parasite and host, and uses the same cofactor, NAD. On comparison of the three-dimensional structures of human and *Leishmania mexicana* GAPDH, there are exploitable differences for rational drug design. The NAD binding region is well conserved around the nicotinamide moiety of the cofactor, whereas the adenosine binding region shows structural differences between parasite and host enzymes, particularly in the vicinity of the adenosine ribose (Suresh et al 2000).

Modelling studies have shown that the topology of the NAD binding channel in G3PDH is compatible with the *R*-isomeric analogues of pyrrolobenzodiazepine antibiotics. The benzodiazepine moiety occupies the adenine region of the binding site, while the fused pyrrolo ring is positioned in the ribosyl region, and a carboxylic acid group binds to the phosphate binding region of the cofactor. The natural C11a *S*-isomers of these antibiotics are known cytotoxic agents (Jenkins et al 1994). The *R*-stereochemistry at position 11a, coupled with the presence of a bulky acetyl at position 10, is compatible with the NAD binding channel and prevents binding with DNA thus overcoming potential host cytotoxicity (Thurston 1993). Preparation of the *R*-isomer from D-proline, protected at the α-carboxylic acid prior to reaction with the acyl chloride of the 2-nitrobenzoic acid analogue (Smith et al 1988). Subsequent reduction of the α-ester to the aldehyde and the nitro group to the amine results in spontaneous intramolecular cyclisation to the desired pyrrolobenzodiazepine isomer (Kamal et al 1997).

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Kamal, A., et al (1997) *Tetrahedron* **53**: 3223–3230
Opperdoes, F. R. (1987) *Annu. Rev. Microbiol.* **41**: 127–151
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Suresh, S., et al (2000) *Structure*. **8**: 541–552
TDR News, No. 62, June 2000
Thurston (1993) In: Neidle, S., Waring, M. J., (eds) *Molecular aspects of anticancer drug-DNA interactions*. Macmillan, pp 1, 54–88

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Synthesis and calcium channel antagonist activity of 2-imidazolyl-substituted dihydropyridines

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Structurally diverse groups of compounds are known to be effective as calcium antagonists (Goldmann & Stoltefuss 1991). The most potent class of antagonists comprises derivatives of 1,4-dihydropyridine. The effect of various C-4 imidazolyl substituents in conjunction with different C-3, C-5 diesters on calcium-channel antagonist activity have been previously reported (Shafiee et al 1998; Hadizadeh et al 2002). We now report synthesis (Hantzch 1882; Archibald et al 1990) and calcium-channel antagonist activity of 12 new methyl and ethyl diester analogues of nifedipine in which the o-nitrophenyl group at 4 position is replaced by 1-benzyl-2-alkylthio-5-imidazolyl substituent, and its methyl group at 2 position is replaced by 2-(1*H*-imidazol-1-yl)ethyl or 2-(dimethylamino)ethyl substituent. Guinea-pig ileum contractile response to KCl, acetylcholine (Ach) and electrical stimulations of title compounds were determined according to Bolger et al (1983) and Rovnyak et al (1992). The IC50 values were determined as concentration needed to produce 50% relaxation of contracted guinea-pig ileum in different tests. The IC50 (μM) values of the lead compound were 67.0 , 66.1 and 16.0 in acetylcholine, KCl and electrical stimulation tests. The IC50 (nM) for nifedipine was 14.0 in the KCl test.

Archibald, J. L., et al (1990) *J. Med. Chem.* **33**: 646–652
Bolger, G.T., et al (1983) *J. Pharmacol. Exp. Ther.* **225**: 291–309
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Goldmann, S., Stoltefuss, J. (1991) *Angew Chem. Int. Ed. Engl.* **30**: 1559–1578
Hadizadeh, F., et al (2002) *J. Sciences, Islamic Republic of Iran* **13**: 29–33
Hantzch, A. (1882) *Justus Liebigs Ann. Chem.* **215**: 1–85.
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Shafiee, A., et al (1998) *Pharm. Acta Helv.* **73**: 75–79

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Synthesis of S-nitroso captopril

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Angiotensin converting enzyme (ACE) inhibitors are a group of drugs developed during the 1970s. They are currently used extensively in the treatment of hypertension and cardiac failure and have recently been shown to improve prognosis after myocardial infarction.

Captopril is an ACE-inhibitor that has until recently dominated clinical practice. During the last few years, other new long-acting members have become available, including perindopril, lisinopril, cilazapril, quinapril, fosinopril, ramipril, trandolapril and zofenopril. None of these possess significantly different clinical properties to captopril. This study describes the synthesis of potential analogues of captopril, and is part of a wider study, which aims to enhance the therapeutic profile of this drug, particularly with regard to specific routes of administration.

Previous work by many groups has shown that the incorporation of a nitric oxide (NO) moiety into other classes of drugs have been shown to reduce side effect and enhance the therapeutic effects, in comparison with that of the parent drug. This effect was first observed in non-steroidal anti-lnflammatory drugs (Wallace et al 1994).

NO has a role in treating and preventing a diversity of cardiovascular conditions, both acute and chronic failures of the heart and circulatory functions. Its actions include vasodilatation (smooth muscle cell relaxation), as well as the inhibition of vascular muscle cell proliferation, platelet adhesion and aggregation, and local inflammation, all of which affect cardiovascular function. It is hoped that a molecule possessing both NO-donating properties and ACE-inhibitory properties would present a substantial synergy in their combined therapeutic activity. Therefore, we have synthesised S-nitroso captopril, a punitive NO-releasing derivative of captopril.

The synthesis of S-nitroso captopril was achieved by using established methodology (Garvey et al 1997). Captopril was dissolved in anhydrous methylene chloride and cooled to 0°C. *Tert*-butyl nitrite was added and the resulting mixture was stirred at 0°C. for 30 min. The reaction mixture was allowed to warm to room temperature and stirred at room temperature for 1 h. The solvent and excess of *tert*-butyl nitrite were evaporated to give S-nitroso captopril.

These initial results suggest that the NO-releasing moiety can be incorporation into captopril. On-going research in our laboratories is focused on evaluating the therapeutic benefits of S-nitroso captopril and other similarly modified derivatives.

Garvey, et al (1997) US Patent, 5703073
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Molecular modelling of the benzo[*c*]phenanthridine-DNA-topoisomerase complex

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DNA topoisomerase 1 (topo 1) is an enzyme that can prevent DNA tangling during replication, transcription and repair. It is, therefore, a key molecular target for anticancer drugs. Topo 1 poisons can be characterised by their suppression of DNA cleavage or their ability to stabilise the enzyme–DNA complex. Structural information on the interactions between DNA, drugs and topo 1 would provide valuable information for the discovery of new anticancer drugs.

Topo 1 activity of benzo[*c*]phenanthridines (BCPs) is dependent on the substitution pattern around the tetracylic nucleus. Fagaronine, a 2,3,8,9 substituted BCP, is a DNA intercalator (Pezzuto et al 1983) that stabilises the topo 1 cleavage complex at low concentrations (Ianoul et al 1999; Fleury et al 2000; Lynch et al 2001). Stabilisation at DNA cleavage sites by fagaronine was found to be non-specific. In contrast, the pattern and mechanism of topo-1-mediated DNA cleavage in the presence of ethoxidine, a synthetic 2,3,8,9 substituted BCP with an ethoxy group in position 12, is completely different. It exhibits inhibition of DNA relaxation at a 10-fold lower concentration than fagaronine, but by suppression of topo 1 and only at AT sites.

To investigate these different mechanisms of action against topo 1, we have performed modelling studies on DNA sequences representative of the DNA cleavage sites: 5'-d(GTTGGT)-3' and 5'-d(CTTAGA)-3' — both compounds can orientate in different positions relative to the base pair axis within the sequence. Essentially, the 12-ethoxy group of ethoxidine can be in the major or the minor groove, as can the 2-hydroxy group of fagaronine. Our studies indicate that there is a clear orientational preference shown by ethoxidine on binding with DNA that is absent with fagaronine (Tables 1 and 2). We are currently modelling the ternary complexes to see whether such orientational preferences can explain the different mechanisms of action.

Table 1 Binding energies for ethoxidine and fagaronine in the 5'-d(GTTGGT)-3' sequence

BCP	OH/OCH_2CH_3 position	Binding energy (kcal)
Ethoxidine	Minor groove	−50.91
Ethoxidine	Major groove	−74.76
Fagaronine	Minor groove	−54.27
Fagaronine	Major groove	−55.37

Table 2 Binding energies for ethoxidine and fagaronine in the 5'd(CTTAGA)-3' sequence

BCP	OH/CH_2CH_3 position	Binding energy (kcal)
Ethoxidine	Minor groove	−59.08
Ethoxidine	Major groove	−44.94
Fagaronine	Minor groove	−22.09
Fagaronine	Major groove	−22.75

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Pezzuto, J. M., Antosiak, S. K., Messmar, W. M., et al (1983) *Chem-Biol Interact.* **43**: 323–339

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Structural aspects of antisense oligonucleotide-based artificial ribonucleases

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The development of artificial ribonucleases is one of the most demanding aspects of RNA targeting (Podyminogin et al 1993; Beloglazova et al 2000). Their design has normally been based on imitation of the active sites of natural ribonucleases such as RNase A or T1, achieved by chemical conjugation of hydrolytically active groups (imidazole, aliphatic amino groups, etc.) that make up the active site of natural ribonucleases. The binding site of the artificial nucleases, designed to deliver a hydrolytic construct to the RNA target, normally contains intercalating or polycationic groups able to bind RNA. Alternatively, the binding site can be an oligonucleotide complementary to the target RNA, and recognising the RNA target sequence trough specific Watson-Crick hydrogen-bonding.

Recently, we have designed a number of molecular frameworks expected to provide artificial RNase activity against a chosen region of $tRNA^{Phe}$. We synthesised some examples of this new type of artificial ribonuclease containing bis- and tetra-imidazole cleaving constructs conjugated to antisense oligonucleotides via flexible linkers of different structures and lengths. Biochemical assays of the hydrolytic activity of these artificial ribonucleases showed that their ability to cleave $tRNA^{Phe}$ is governed by intrinsic properties of the respective cleaving constructs.

Molecular modelling (Molecular Mechanics/Molecular Dynamics) was used to determine the preferred orientation(s) of the cleaving group(s) in the vicinity of the cleavage site to provide a structural basis to explain the hydrolytic strengths of various conjugates (SYBYL6.6).

Bis-imidazole cleaving constructs were found to be conformationally highly flexible, with no preferred specific conformation regardless of their initial modelling position, implying the absence of interactions between cleaving groups and target able to stabilise an active conformation. The hydrolytic activity of bis-imidazole structures appears to be a random event, with no particular pre-scaffolded conformation or interactions with the target site. These data are in agreement with the experimentally determined limited hydrolytic activity ($\approx$ 25%) found for these compounds.

In contrast, for the tetra-imidazole containing compounds, molecular modelling showed that a prefered orientation(s) of cleaving constructs to form a pre-organized active conformation exists and this is strongly dependent on the chemical structure of the linker connecting these constructs to the antisense oligonucleotide. For example, the inclusion of deoxyribothymidine into the linker markedly decreased the probability of cleaving groups to reside near the cleavage site, presumably due to a semi-stacking interaction with the neighbouring nucleotide residue, while the inclusion of a cyclohexyl fragment allowed one of the constructs to successfully find an energetically favourable conformation for cleavage. These molecular mechanics/molecular dynamics calculations results are consistent with the observed hydrolytic activity measured for these ribonucleases (16% and 50%, respectively).

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Geometry and hydrogen bonding in two anti-cancer benzothiazoles

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Crystal structure determinations and ab-initio molecular orbital calculations (3-21G) have been carried out for two anti-cancer drugs, each containing a benzothiazole ring but affecting different biomolecular targets.

2-(4-Amino-3-methylphenyl)-5-fluorobenzothiazole (5F203) is a ligand for the arylhydrocarbon receptor (AhR), by which it is transported into tumour cells and translocated to the nucleus (Loaiza-Perez et al 2002). The ligand-bound receptor heterodimerises with the nuclear transporter (ARNT) and triggers transcription of the CYP1A1 gene. 5F203 is metabolised to a reactive chemical intermediate by CYP1A1 (Chua et al 2000) and forms DNA adducts within sensitive cells. Tumour types which have the necessary characteristics (a cytosolic AhR and inducible CYP1A1) including breast, ovarian and lung tumours, are exquisitely sensitive to the action of the drug.

4-Hydroxy-4-(benzothiazol-2-yl)cyclohexadienone (AW464) is a lead structure of a family of quinols with selective actions against renal and colon cell lines. Structure–activity relationships indicate that a 6/5-bicyclic nucleus at the 4-position is optimal for activity and the cyclohexadienone moiety acts as a double Michael acceptor. Database mining of the NCI 60 cell screening panel suggests that the protein thioredoxin, with two active-site cysteine residues, is the target for the drug.

The affinity for different targets can be rationalised in terms of the different arrangement of ring planes. In the crystalline state the benzothiazole and phenyl ring planes of the flat 5F203 are misaligned by only 3.6(1)°, while in AW464 the 81.4(1)° angle between heterocycle and cyclohexadienone creates an L-shaped molecule. Corresponding crystallographic bond distances in the benzothiazole rings of the two molecules generally agree within 0.013 Å. However, the exit bond C2-C8 is much longer (by 0.069(3) Å) in AW464 reflecting the loss of conjugation and altered hybridisation at C8, partially compensated with a shortening of S1-C2 by 0.029(2) Å. These differences at C2 and similarities elsewhere are corroborated by the results of optimisation. Both compounds have polar H atoms, which associate intermolecularly, in substituents attached to the phenyl or cyclohexadienone ring. In 5F203 significantly negative Löwdin calculated charges are found on benzothiazole N3 (−0.226) followed by F14 (−0.178). Correspondingly, in the crystal structure one amino H atom forms a hydrogen bond with N3 and the other approaches F14. In AW464 the Löwdin charge on the benzothiazole ring N atom is less negative (−0.187), but carbonyl O15 is a powerful new hydrogen bond acceptor (−0.259). In its crystal structure the hydroxyl H atom recruits water O16 as acceptor, and the H atoms of water hydrogen bond to N3 and O15.

We thank Drs T. D. Bradshaw and A. W. Westwell for synthesis of samples.

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Loaiza-Perez, A. I., et al (2002) *Mol. Pharmacol.* **61**: 13–19

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Inhibitors of seprase: putative modulators of metastasis and novel targeting agents

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The most devastating and life-threatening characteristic of malignant neoplasia is their ability to form metastatic foci, which are often resistant to conventional therapies are the cause of most cancer deaths. The metastatic potential of malignant neoplasia has been shown to be related to their ability to contact and degrade the extracellular matrix (ECM). In particular the correlation between invasiveness and the upregulation of various proteases at specialized invadopodia, finger-like protrusions of the cell membrane which make contact with the extracellular matrix (Kelly et al 1994; Monsky et al 1994), suggests that blockade of this activity could, wholly or in part, arrest the process of metastasis.

Seprase (*S*urfaced *expr*essed prote*ase*), is a serine protease integral to the cell membrane (Kelly 1999), which has been associated with the invasive nature of a number of neoplastic cell lines in-vitro, in particular the highly invasive human melanoma cell line, LOX, which exhibit aggressive metastatic behaviour. It is in these cells that the correlation between seprase upregulation and invasiveness has been demonstrated (Kelly 1999).

Seprase comprises a carboxyl terminus catalytic region of approx. 200 residues which is homologous (68% identity) (Ariga et al 2001), to another non-classical serine protease dipeptidyl peptidase IV (DPP-IV). DPP-IV and seprase both exhibit well-characterized post-proline cleavage, cleaving N-terminal dipeptides from bioactive peptides/proteins where proline is the penultimate residue. This X-Pro-aa (aa = amino acid) cleavage is both highly specific and unusual. Exploitation of this characteristic recognition motif leads toward the development of active-site directed seprase-specific inhibitors as putative anti-metastatic agents and potential targeting of seprase as a pro-drug activator. Highly specific inhibitors can be modified further to yield novel imaging and therapeutic agent based on the covalent capture of said inhibitors incorporating radionucleotides, fluorescent markers or photoactivatible groups, at the tumour site.

We have synthesized X-Pro^{P}-$(OPh)_2$ templates, these have been based on the known reactivity of amino acid derived diphenyl phosphonate esters toward serine proteases (Belyaev et al 1999). We have successfully completed synthesis of Ala-Pro^{P}-$(OPh)_2$ and have demonstrated its activity as an irreversible inhibitor of DPP-IV with a second order rate constant of $5\,\text{M}^{-1}\text{min}^{-1}$. A series of such analogs are presently being characeterised for their inhibitory activity against DPP-IV and seprase-like activity of LOX cells in culture.

Ariga, N., et al (2001) *Int. J. Cancer (Pred. Oncol.)* **95**: 67–72
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Studies on the chemosensitivity of cultured human breast tumours to novel oestrogenic prodrugs

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The dependence on oestrogens to stimulate cell growth in oestrogen receptor (ER) positive (> 10 fmol receptor/mg cytosolic protein) (Wittliff 1984) breast tumours has been exploited by synthesising prodrugs which incorporate both oestrogenic and anticancer moieties. Doxorubicin (DOX), an anthracycline antitumour antibiotic, is a highly potent topoisomerase II inhibitor used in the chemotherapy of advanced breast cancer, but is relatively unselective, and causes potential fatal cardiomyopathy. To improve selectivity for breast tumours containing ER, prodrugs were synthesised which incorporate both steroidal and anticancer drug (DOX) moieties. In the first series, hydrocarbon spacer groups were covalently linked to oestrone (E) at the 17 position. In the second series, compounds from the first series were covalently amide-linked to DOX via the daunosamine amino group.

These compounds were evaluated in chemosensitivity tests using cultured human cell lines including ER positive MCF-7, ER negative MT-1 and MCF-7ADR breast cancer lines, and the K562 leukaemia cell line in supplemented RPMI 1640 medium. Percentage cell survival was determined after 96 h exposure, using drugs in the range 0.001–100 μM via the colorimetric MTT assay (Jabbar et al 1989) using a Labsystems Multiskan Plus ELISA reader, set at 550 nm.

Table 1 shows the IC50 values for the first series of compounds which incorporated 14 or 16 carbon spacer groups terminating in a carboxylic acid group. The prodrug CCRL1046 contained E linked at the 17- position via amide linkage and a 14 methylene hydrocarbon spacer group to DOX also via amide linkage to the daunosamine amino group.

Table 1 IC50 values (μM ± s.d.) for oestrone-hydrocarbon derivatives and for DOXO–linked oestrogen prodrugs

Compound	MCF-7	MT-1	MCF-7ADR	K562
DOX	0.45	0.60	1.08	0.91
1st series	> 10	> 10	> 10	> 10
CCRL1046	1.0	2.1	2.5	2.0

These data indicate that when DOX is combined in the prodrug CCRL1046, there is some loss in potency, but increased selectivity for the ER positive MCF-7 cell line.
Hence compounds from the second series were shown to have selectivity for the ER positive cell lines making them suitable lead compounds for further studies.

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165
Synthesis of taste-free and stable erythromycin pro-drugs

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Erythromycin A exhibits poor patient-compliance, especially in children, owing to its vile taste (Graham 1992). Erythromycin undergoes intramolecular cyclisation in the acidic conditions of the stomach leading to formation of substantially inactive degradation products causing the adverse effects (Atkins et al 1986). Erythromycin B, however, was found to be acid-resistant (Mordi et al 2000).
Taste-free esters of erythromycin A such as erythromycin 2'-ethyl succinate, are commonly used as paediatric formulations. Unfortunately, hydrolysis of the esters follows the same (base-catalysed) mechanism in the bottle as in the body. This poster describes the synthesis of derivatives of erythromycin A (1) and B (2) intended to hydrolyse selectively in the body rather than the bottle. Although this objective was achieved in both series, the behaviour of the esters in solution is different, the A derivative being more stable to stomach acid, the B derivative being more stable in storage conditions.
The key intermediates in our synthesis, erythromycin A and B enol ethers (3) and (4), respectively, were readily prepared using the published protocol (Kurath et al 1971). Treatment of (3) and (4) with the appropriate acid chloride (Murphy 1953–1954), yielded the 2'-esters, erythromycin A and B enol ether ethyl succinates (5) and (6), respectively.
Both (5) and (6) were stored in suspension (25 mg mL^{-1}) for 21 days at 4°C at both pH 6.0 and 8.0. NMR studies indicated that 20% of (5) hydrolysed to erythromycin A at pH 6.0 and only 10% at pH 8.0. However, (6) indicated negligible hydrolysis to free erythromycin B, at pH 6.0 and 8.0. At pH 2.0, both (5) and (6) equilibrated within 5–10 min with their respective erythromycin 2'-ethyl succinates, with the equilibrium lying overwhelmingly (95%+) on the side of the erythromycin 2'-ethyl succinates. However, whereas erythromycin B 2'-ethyl succinate degraded slowly under these conditions by loss of the cladinose sugar, erythromycin A 2'-ethyl succinate was essentially stable.
Erythromycin enol ether 2'-ethyl succinates are therefore stable and taste free in conditions resembling the formulation conditions and are converted rapidly to pro-drugs of erythromycin on contact with the stomach acid. Erythromycin A enol ether 2'-ethyl succinate is more stable to acid, which may permit the use of smaller doses or a less rigid dosage regime, especially beneficial when the patient is a school-age child. Erythromycin B enol ether 2'-ethyl succinate is still more stable in the bottle and will allow complete taste masking, this may be the better drug for very young children.

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Graham, E. M. (1992) *Obst. Gynec. Clin. N. Am.* **3**: 539–549
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166
The biological evaluation of a range of novel inhibitors of oestrone sulphatase against the MCF-7 breast cancer cells

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Recently, the enzyme oestrone sulphatase has become the target of intensive research in the treatment of hormone-dependent breast cancer and the steroidal compound oestrone-O-sulphamate (EMATE) and the non-steroidal coumarin derived 667-COUMATE are potential drugs. In our search for potent inhibitors of oestrone sulphatase, we have reported the results of our investigation into the role of physicochemical properties such as pK_a (Ahmed et al 2000) as well as the synthesis and in-vitro evaluation of a range of novel inhibitors of this enzyme (Ahmed et al 2001).
Potential anti-cancer drugs must show a high degree of potency and selectivity. However, these compounds must not produce any stimulation of the breast cancer cells. As previously mentioned, in our search for potent inhibitors of oestrone sulphatase, we have synthesised a number of compounds which have been shown to possess potent irreversible inhibition against this enzyme, although their oestrogenic property has not been evaluated. Here, we present the results of a study whereby MCF-7 cells were utilised in a study of a range of compounds so as to observe their effect on breast cancer cells. EMATE, COUMATE and 667-COUMATE were also synthesised and evaluated as standard compounds.
MCF-7 breast cancer cells were plated into 6-well plates (50 000 cells per well) and incubated (37°, 5% CO_2) in standard media (Warmed IMEM + 10% v/v fetal bovine serum (FBS) + 1% v/v L-glutamine + 1% v/v non-essential amino acids (NEAA)). After 24 h, the media was changed to oestrogen free media (Warmed IMEM without phenol red + 10% charcoal stripped FBS + 1% v/v L-glutamine + 1% v/v NEAA) and the cells incubated for 5 days to allow steroid levels to decline. The media was then changed to oestrogen free media + the test compounds (10 μM) in duplicate. After a further 5 days incubation the cells were detached from the wells (using trypsin-EDTA), re-suspended in standard media, stained (Trypan blue), and counted with a haemocyotometer.
The results of the present study suggest that the compounds considered, in general, do not stimulate the MCF-7 cells – as such, they may be considered to possess little or no oestrogenic activity. However, EMATE was observed to stimulate the cells, this therefore correlates with previous reports. The recently reported inhibitors of oestrone sulphatase (based upon 4-sulphamated derivatives of phenyl ketone and benzoic acid esters) were found to have no stimulatory activity against the MCF-7 cell line, furthermore, the compounds were observed to be potent suppressors of division when compared with the standard compounds (possessing an IC50 value of 3.4 ± 0.13, as such, proved to be some 3.5 times more potent than COUMATE under similar conditions). 667-COUMATE, however, proved to be slightly oestrogenic when compared to the control. In conclusion, our study allows the rapid determination of the potential of compounds to stimulate breast cancer cells.

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Molecular modelling and structure–activity relationship determination study of a range of inhibitors of 5α-reductase

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5α-Reductase (5AR) is responsible for the conversion of testosterone to dihydrotestosterone (the more potent androgen) and is a major therapeutic target in the treatment of prostate diseases. In our search for potent inhibitors of 5AR, we have previously undertaken the superimpositioning studies where known inhibitors

were fitted onto the substrate and structural requirements determined (Ahmed & Denison 1998). Here, we report the results of a study into the derivation of a transition-state (TS) for the reaction catalysed by 5AR. The TS was then used to model known inhibitors of this enzyme and to elucidate the role of: the logP; conformational analysis of the side chains (to investigate the available space); and molar refractivity of the C(20) side chains of a range of known steroidal inhibitors with the biological activity.

In the derivation of the TS, we considered the proposed reaction mechanism (Holt et al 1991). The structures and TS were all constructed within the molecular modelling software CaChe. Physicochemical properties were determined using the procedures available within Project Leader. Alchemy III was used in the superimpositioning studies of the inhibitors onto the TS and the investigation of conformational space about the C(17) region, respectively.

We have previously published a report where we superimposed a range of inhibitors of this enzyme (Ahmed 1996) — we concluded that the main feature required by the non-steroidal inhibitors is the mimicking of the C(3)=O group of the steroidal A ring and that the C(20) region was not involved in any interaction. The role of the C(20) region remains unclear, although several workers have presumed the existence of a binding group at the active site which would bind any hydrogen bonding groups within this area. It is our hypothesis that it is the hydrophobicity of functional groups about the C(20) area of the steroidal inhibitors that have resulted in their potent inhibitory activity. That is, the contribution towards an increase in the hydrophobicity of these groups is responsible for the increase in the biological activity rather than any hydrogen bonding interactions.

From the results of our investigation into the properties of the side chain, we observe that there is a relationship between the inhibitory activity and physicochemical property of the inhibitors. For example, it can be observed that there is an increase in potency with logP of the side chain. When the conformations of the inhibitor side chains are considered, we discovered that a large number of conformers are possible. These conformers would be expected to be involved in steric interactions with any group about the C(20)=O area of the active site.

In conclusion, this study supports our hypothesis that the increased inhibitory activity is as a result of the hydrophobic nature of the inhibitor's side chain as opposed to any interaction between the inhibitor and the active site.

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Ahmed, S., Denison, S. (1998) *Bioorg. Med. Chem. Lett.* **8**: 409–415
Holt, D. A., et al (1991) *Bioorg. Med. Chem. Lett.* **1**: 27–32

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A novel approach to the computer-based screening of compounds against the P-450 family of enzymes

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In the treatment of hormone-dependent tumours, a number of cytochrome P450 enzymes have been considered as potential therapeutic targets. For example, the enzymes 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$) and aromatase (AR) have both been considered in the treatment of prostate and breast cancers respectively, resulting in the synthesis of a large number of potent inhibitors — a number of these compounds are known to inhibit more crucial P-450 enzymes such as cholesterol side-chain cleavage enzyme (CSCC), and as a result, compounds such as aminoglutethimide (AG) are administered with supplements of cortisol to overcome this. The ability of these compounds to inhibit other P-450 enzymes is an important factor, as such, a system which could predict the ability of such compounds to inhibit a range of enzymes in-vivo would be very useful in the drug design discovery process.

To this end, workers have previously used molecular modelling to discover the specific nature of the active sites of the more important enzymes (e.g. homology modelling) (Burke et al 1997), as well as superimpositioning studies. However, these processes can be complex and have the potential to produce inaccurate models. Here, we report the development of simpler and more accurate models of the active site of these enzymes and use these models in the prediction of the biochemical profile of compounds such as pyridogluethimide (PYG), bifonazole and miconazole.

In the development of the models, the molecular structures (e.g. the four inhibitors under study, the steroid substrates, haem and the possible amino acids involved at the active site) were all constructed and minimised (using the fastest minimisation routine available — cycles of 300 iterations were initially attempted until the gradient dropped below 10^{-3}) within the CACHE molecular modelling software. The minimsied structures were connected and the overall structures minimised using Zindo within Mopac — resulting in the substrate-haem (SH) complex for each enzyme (all atoms, except those for the substrate, were then locked).

To study the interactions of the inhibitors and the active site, the substrate within the SH complex was deleted and replaced with the minimised structure of the inhibitor (e.g. miconazole was bonded to the haem and the appropriate hydrogen bonds formed between groups at the active site and the inhibitor). The overall inhibitor-haem (IH) complex was minimised and the final energy noted.

Table 1 Prediction of inhibitory activity

Compound	AR	CSCC	P-450$_{17\alpha}$
Bifonazole		X	√
Miconazole	√	X	√

The results for PYG correlate well with that reported in the literature, although, for miconazole and bifonazole, there appears to be a slight discrepancy in that both of these two compounds are known inhibitors of all three enzymes, as such, some 'fine tuning' is required. However, we believe that, through the consideration of the inhibitor/substrate-haem complex and the novel approach, it is possible to derive a computer based system which allows the initial non-biochemical evaluation of novel compounds.

Burke, D. F., et al (1997) *Anti-Cancer Drug Des.* **12**: 113–123

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Synthesis of 3-alkylated-4-(4-aminobenzyl)-2-oxazolidinones as probes in the investigation of the aromatase active site

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Extensive research has been undertaken to produce compounds that are both potent and selective inhibitors of the enzyme aromatase (AR). Here, we report the synthesis and screening of a range of oxazolidinone based compounds as chiral probes in the effort to elucidate further information regarding the active site or AR.

In the general use of the Evans oxazolidinone, the most widely synthesised derivatives have been the N-acyl compounds. N-alkylation has been somewhat ignored, nevertheless, synthesis has been undertaken using strong bases such as LDA. In our hands, the use of LDA proved to be unsuccessful and a search for an appropriate base led us to NaH using DMF as the solvent, and this provided the range of alkylated compounds in high yields. The 4-amino compounds are required for the ligation to the Fe atom of the cytochrome P-450 haem. Conversion of the nitro derivative to the amino affords an easy route to the phenylamine derivative. Nitration was undertaken using dilute nitric acid (5 M) at 0°C. Problems were encountered with the larger alkyl chain derivatives and the synthesis of the heptyl, octyl, nonyl and decyl derivatives was achieved involving an increase in reaction time (yields 80%). The target compounds were obtained through the use of hydrogen gas and palladium on activated charcoal with yields of 70%. The synthesised compounds were screened for inhibitory activity using the standard literature method (Thompson & Siterii 1974) and using aminoglutethimide (AG) as the standard. The results of the biochemical evaluation are shown in Table 1.

From the results of the initial screening, we observe that the majority of the novel

inhibitors possessed good inhibitory activity. However, comparison of the inhibitory activities of the *R*- and *S*-forms show that the two series of inhibitors behave in a totally different manner. Table 1 shows the effect of increasing alkyl chain length on the inhibitory activity value of the final compounds of the *R*- and *S*-forms, respectively.

Table 1 Initial screening data (mean of triplicate values) for a small range of synthesised compounds ([I] = 100 μM)

Alkyl substituent	Chirality	Compound number	Percentage inhibition
Bu	*R*	10	86
Pent	*R*	11	93
Hex	*R*	12	87
Hept	*R*	13	86
Oct	*R*	14	76
Me	*S*	17	65
Et	*S*	18	60
Bu	*S*	20	42
Hex	*S*	22	21
Non	*S*	25	10
AG	–	–	53

Detailed consideration of Table 1 shows a clear correlation between decreasing potency (of the compounds derived from the *S*-form) with increasing alkyl chain length — this has been rationalised through the use of the molecular modelling which suggests that the decrease in potency is due to steric interaction between the protein back bone and the alkyl chain.

Thompson, E. A., Siterii, P. K. (1974) *J. Biol. Chem.* **249**: 5373–5378

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The structure–activity relationship determination of a series of sulphamated compounds based upon cinnamic acid

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In the treatment of hormone-dependent breast cancer, extensive research has been undertaken to produce compounds which are both potent and selective inhibitors of oestrone sulphatase (ES), the enzyme responsible for the conversion of the stored form of oestrogens to the active form. A number of compounds (both steroidal and non-steroidal) have been synthesised and have shown potent inhibitory activity. The most potent non-steroidal compounds are based upon the coumarin backbone (e.g. 4-methylcoumarin-7-*O*-sulfamate (COUMATE) and its tricyclic derivatives such as 667-COUMATE, the latter being in Phase II clinical trials (Woo et al 2000)). The highly potent inhibitory activity possessed by this family of compounds is intriguing and in an effort to rationalise the potency of these compounds, we initiated a series of studies involving: molecular modelling (in particular the derivation of the transition-state), and the synthesis and biochemical evaluation of derivatives of the coumarin backbone. Here, we report the results of our study.

Molecular Modelling study involved: the derivation of the transition-state (TS) for the reaction catalysed by ES and; the superimpositioning of the coumarin based compounds (and our potential inhibitors) onto the derived TS. The results of the modelling study suggested that compounds based upon the cinnamic acid backbone (i.e. presence of the C=C and C=O conjugated to the aromatic ring system) may possess inhibitory activity. As such, we synthesised a number of cinnamic-acid-based compounds. The compounds were then evaluated for their in-vitro biochemical activity using literature based assay procedures (Table 1).

Table 1 IC50 data for compounds under study

Alkyl chain length	IC50 (μM)
1	781
2	337
3	267
4	113
5	993
EMATE	2
COUMATE	12
667-COUMATE	0.2

The results of the current study show that the cinnamic-acid-based compounds are very poor inhibitors of ES (the best inhibitors is ~565 and ~10 times weaker than 667-COUMATE and COUMATE, respectively). As such, the study suggests that alternative factors may be involved in the potent inhibitory activity displayed by these compounds rather than simply the mimicking of the conjugated system that is observed to exist within the coumarin based compounds. In an effort to determine the unknown factors, we synthesised a derivative of 667-COUMATE and COUMATE where the C(3) to C(4) double bond was reduced. The biochemical evaluation of the reduced compounds showed that the C=C bond is crucial for inhibitory activity due to its impact on the acidity of the parent phenol (i.e. pK_a, which we have shown to be important in determining inhibitory activity against ES). In conclusion, we have highlighted the factors required for potent inhibitory activity within the COUMATE series of compounds

Woo, L. W. L., et al (2000) *Chem. Biol* **7**: 773

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Comparison of the theoretically derived model of the active site of the cytochrome P-450 enzyme camphor hydroxylase with its crystal structure

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Although the general substrate–haem complex (SHC) approach has proved successful, the accuracy of the theoretical model has been unclear with respect to the crystal structure of the enzyme active site, since most of the eukaryotic P-450 enzymes are membrane bound and therefore difficult to crystallise. We considered the enzyme for which crystal structures are available, namely camphor hydroxylase ($P\text{-}450_{CAM}$) (from *Pseudomonas putida*). In this report, we derive the SHC representation for $P\text{-}450_{CAM}$ and compare the theoretical model with the available crystal structures of this enzyme.

The structures of the porphyrin and camphor required for the construction of the substrate–haem complex were all constructed within Alchemy III molecular modelling software on an IBM PC compatible microcomputer using atoms/fragments available. The completed structures were then subjected to an initial minimisation using the conjugate-gradient algorithm until the gradient fell to below 10^{-5}. Conformational analysis was performed on flexible parts of the inhibitors using the conformational analysis software Powersearch. The low energy conformers produced were retained for further study. In the construction of the SHC, we used the previously reported approach (Ahmed 1995; Ahmed & Davis 1995). It is postulated that there is an involvement of a ferroxy radical in an exo manner. As such, the attacking species would be expected to be positioned at an appropriate distance and angle about the steroid such that the C(5) position can be attacked. We therefore attached the haem to the C(5) of camphor, via the ferroxy oxygen, and carried out minimisation of the complex until the gradient fell to below 10^{-5}. This resulted in the $P\text{-}450_{CAM}$ SHC. To compare the theoretically derived model with the crystal structure, three crystal structures were obtained from the Brookhaven Protein Databank and using Rasmenu, the structures were simplified, involving the extraction of the ligand and the prosthetic haem groups from the protein backbone. The files were then read into Alchemy III and

superimposed onto the theoretically derived model using the four N atoms of the porphyrin ring of each model and the root mean square distance determined as a measure of the fit. The distance between the camphor carbonyl group and C(5) of each model was determined.

Comparing the distances, we observe that the theoretical model appears to be a very good approximation to the situation observed in the three crystal structures, with the C(5) of the crystal structure-derived camphor to the C(5) of the theoretically derived SHC camphor distances being in the range of 0.75–1.28Å, with RMS fit less than 0.2. From the consideration of the differences in distances between groups, the SHC appear to be a good approximation of the active site of P-450$_{CAM}$. In conclusion, the preliminary results of the comparison of the theoretically derived SHC and the reported crystal structures appear to be similar with both camphor molecules being in close proximity. The SHC approach therefore appears to be a very good approximation.

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Ahmed, S., Davis, P. J. (1995) *Bioorg. Med. Chem. Lett.* **5**: 1673–1678

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Synthesis and biochemical evaluation of novel inhibitors of the enzyme 5α-reductase (5AR)

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The enzyme 5α-Reductase (5AR) is responsible for the conversion of testosterone (T) to dihydrotestosterone (DHT) and has been the major therapeutic target in the treatment of benign prostate hyperplasia (bph). We have recently published a report where we considered the initial modelling of some inhibitors of this enzyme, and from which we were able to hypothesise factors required for inhibition (Ahmed 1996). From the results of this report, we concluded that the main feature required by the non-steroidal inhibitors is the mimicking of the steroidal A ring and in particular the C(3)=O carbonyl group.

We undertook an extensive molecular modelling study and concluded that the pyrrolidine-2,5-dione ring can be used as a possible mimic for the steroidal A-ring. We therefore designed several novel inhibitors based upon phenyl-alkyl pyrrolidine-2,5-diones. Here we report the synthesis and the biochemical evaluation of some of these novel inhibitors for activity against 5AR.

The synthesis of the target compounds were undertaken involving the reaction between the appropriate alkyl bromide and N-substituted succinimide, in the presence of sodium hydride and anhydrous tetrahydrofuran (THF) as the reaction solvent. The reactions proceeded successfully and without any major problems.

In the evaluation of these compounds, standard literature procedure was used involving the use of appropriate pH so as to target the type II isozyme: into duplicate tubes was placed (1,2,6,7-^3H)-testosterone (99 mCi μM^{-1}) in ethanol (50 μM final concentration) with and without inhibitor (100 μM dissolved in ethanol) and the solvent evaporated. NADPH (1 μM) in Tris-citrate buffer (215 μL, 0.1 M, pH 5.5) was added, followed by liver microsome. The mixture was incubated at 25°C for 2 h and then extracted with ethyl acetate (3 × 2 mL). The pooled organic extract was evaporated and the residue dissolved in ethanol (20 μΛ). A sample (2 μL) was mixed with an equal sample of carrier steroids (testosterone (R_f = 0.44); dihydrotestosterone (0.56); epiandrosterone (0.71); and androstenedione (0.64)) and applied on a silica TLC plate and developed using dichloromethane:1,4-dioxane (94:6) over 2 h. Steroids were visualised (I_2), removed, dissolved in 1 mL of acetone before the addition of scintillation fluid T (3 mL) and measured for ^{3}H by liquid scintillation.

The results of initial screening suggest that the pyrrolidine-2,5-dione-based compounds are weak inhibitors of 5AR and are therefore good lead compounds in the development of more potent non-steroidal inhibitors. For example, the compound such as 3-[3'(4"-aminophenyl)propyl]pyrrolidine-2,5-dione was found to possess 10% inhibition at 100 μM. The inhibition data would therefore appear to support the previously reported model for 5AR.

Ahmed, S. (1996) *Pharm. Sci.* **2**: 251–253

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Synthesis and biochemical evaluation of testosterone-based inhibitors of aromatase (AR)

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The conversion of androgens (C_{19}) to oestrogens (C_{18}) occurs in a number of cell and tissue types, such as ovarian granulosa, adipose tissue and placenta. The extragonadal synthesis of oestrogens has great pathophysiological importance. For example, those produced by adipose tissue have been shown to play a rôle in the pathogenesis of certain forms of breast cancer and endometrial adenocarcinoma. The biosynthesis of oestrogens is mediated by the enzyme aromatase (AR) and requires O_2 and NADPH to function (Thompson & Siiteri 1974). AR has been the target of extensive research for some time, resulting in the design and synthesis of a variety of inhibitors for the possible treatment of breast cancer. Here, we report the results of a study into the synthesis and biochemical evaluation of inhibitors based upon C(17) derivatives of testosterone and the rationalisation of the inhibitory activity through molecular modelling.

The compounds were synthesised involving reacting a range of acid chlorides (from acetyl chloride to decanoyl chloride) and testosterone under reflux in anhydrous toluene. The reactions did not prove to be troublesome and gave the desired compounds in high yields (in general, 70% yield). The synthesised compounds were screened for inhibitory activity using the standard literature method (Thompson & Siiteri 1974), using aminoglutethimide (AG) as the standard. The results of the biochemical evaluation are shown in Table 1.

Table 1 Testosterone-based compounds synthesised and their IC50 values

Compound	IC50 (μM)
Testosterone	20.6 ± 0.2
Testosterone acetate	48.7 ± 0.4
Testosterone propionate	55.9 ± 0.1
Testosterone butanoate	58.3 ± 0.3
Testosterone pentanoate	63.1 ± 0.2
Testosterone heptanoate	83.1 ± 0.1
Testosterone octanoate	88.7 ± 0.7

Data are presented as the mean ± s.d. of triplicate values

The molecular structures of androstenedione (AD), the haem and part of the NADPH molecule, were all constructed and minimised (using the fastest minimisation routine available — cycles of 300 iterations were attempted until the gradient dropped below 10^{-3}) within the CaChe molecular modelling software. The structures were refined using ZINDO procedures. For the superimpositioning study, Alchemy III was used.

It has been previously reported that the area of the active site of AR [corresponding to the C(17) area of the steroidal backbone] is hindered (Banting et al 1988). Furthermore, it has been suggested that compounds possessing large groups about this area may prove to be very weak inhibitors of AR. The results of this study appear to contradict the earlier studies. For example, the acetate derivative is only ~3.3 times more potent than the decanoate. Consideration of the results of the molecular modelling, however, suggest that the conformational space of the decanoate is extremely large, as such, steric hindrance would be expected to greatly affect the inhibitory activity, which is not observed. In conclusion, we propose that

large groups may be able to occupy the area corresponding to the C(17) area of the steroidal backbone. As such, large alkyl groups could be used in the design of potential inhibitors to increase logP and therefore possibly potency.

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The derivation of the transition state for the reduction reaction of 17β-hydroxysteroid dehydrogenase as a representation of the active site

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The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) is responsible for the conversion of C(17)=O groups to the reduced hydroxy moiety. As such, this enzyme is responsible for the formation of both potent androgens and oestrogens. The reverse reaction, the oxidation of C(17)-OH to the carbonyl group, is also known to be undertaken by this enzyme. 17β-HSD has come under consideration as a potential target in the fight against hormone dependent cancers such as breast and prostate cancers. For example, this enzyme catalyses the conversion of the weak oestrogen, namely oestrone, to the more potent (and mitogenic) oestrogen, oestradiol. This enzyme is known to exist in at least 12 different isozymes (each uses a different substrate and undertakes either the forward reduction or the reverse oxidation reactions).

In an effort to aid the drug design process, we concluded that the derivation of the transition-states of the catalysed reactions may lead us to the rationalisation of the inhibitory activity of a range of inhibitors of this enzyme. Here, we report the initial results of the development of a model [involving the determination of the transition-state of the reduction reaction (Penning 1997)] for the active site of this enzyme and the rationalisation of the inhibitory activity of a number of inhibitors of 17β-HSD.

The determination of the transition-state was undertaken using the molecular modelling software CaChe on an IBM compatible microcomputer. Using atoms and available fragments from the structure library for amino acids, the tyrosine, serine and threonine amino acids presumed to be at the active site were constructed. Testosterone and the partial structure of NADPH (due to a limitation of the software) were also constructed and two files (starting point and end point) were developed. The files/structures were minimised using augmented MM2 calculations. The two files were then used in the final series of MOPAC calculations leading to the transition-state, which was determined using PM3 parameters. The transition-state was verified involving the determination of the negative frequency for the transition-state (−476.11) within MOPAC. Alchemy III was then used to undertake the superimpositioning of a range of literature based inhibitors including the recently reported flavenoid based inhibitors (Lelain et al 1999) of this enzyme.

From the results of our superimpositioning study, we conclude that with the small range of flavone derivatives considered, the ability of these compounds to fit within the active site (or indeed occupy similar area/volume of space as the natural substrate) is the 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. As such, any interactions between the inhibitor and the NADPH moiety results in a further unfavourable interaction that results in a decrease in the inhibitory activity. For example, the superimpositioning of two flavone based inhibitors suggest that 7-hydroxyflavone (the more potent inhibitor) is not involved in any unfavourable interactions with the active site whereas 6-hydroxyflavone undergoes steric interaction and is the weaker inhibitor.

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Penning, T. M. (1997) *Endocrine Rev.* **18**: 281–305

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Derivation and use of a representation of the active site of $P450_{17\alpha}$ using a novel approach

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The P-450 enzyme 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) mediates the conversion of the pregnanes to the androgens. The specific characteristics of the active site of this enzyme are not known, as it is membrane bound, although homology modelling of the P-450 enzyme camphor hydroxylase ($P\text{-}450_{CAM}$), have caused a number of hypotheses to be forwarded (Burke et al 1997). In an effort to produce a simplified representation of the active site of $P450_{17\alpha}$, we have previously constructed individual substrate–haem complexes for each component (Ahmed 1995): the 17,20-lyase and the 17α-hydroxylase. Here, we report the results of a study where we have combined our individual structures to give an overall complex. Using the resulting complexes, we have produced a simplified representation of the active site using which we have modelled known inhibitors of $P450_{17\alpha}$ in an attempt to gain a greater insight into this enzyme.

Both substrate-haem complexes were constructed using the previously reported novel approach (Ahmed & Davis 1995) and the molecular modelling software CaChe and Alchemy III. In the construction of the substrate–haem complexes, we hypothesised that the attacking ferroxy oxygen species must be positioned within approximate attacking distance (and angle) to the appropriate C atom. Thus in the case of the lyase, the ferroxy based haem was presumed to be close to the C(20)=O such that attack on the C(20) carbonyl group could take place. We therefore attached the terminal oxygen of the Fe^{IV}-O• species to the C(20) carbonyl carbon atom of 17α-hydroxypregnenolone and carried out an initial minimisation of the complex using MM3. This then resulted in the ferroxy haem-based 17,20-lyase substrate–haem complex (a similar technique was utilised to give the peroxy based substrate-haem complex). Conformational analysis was performed on flexible parts of the substrate–haem complex (i.e. about the Fe-O• and O-C(20) in the case of the ferroxy substrate–haem complex) using CaChe. Points on the haem were then used for the superimpositioning of the two substrate-haem complexes for the lyase and hydroxylase components, resulting in the overall representation of $P450_{17\alpha}$.

From the consideration of the results of this study, we observe that the overall structure is an approximate slanted L shape (i.e. the substrate–haem complex for the lyase component is found to be greater in length than the complex for the hydroxylase component). This result is supported by a previous homology-based study where a similar two-lobed structure was observed.

Undertaking the modelling of known inhibitors of $P450_{17\alpha}$, we discovered that a large number of the more potent inhibitors possessed low energy conformers that adopted the L-shape, thereby allowing the inhibitors containing moieties able to interact effectively with the two hydrogen bonding groups within the active site. Using the derived active site representation, we have undertaken the design of novel compounds that have proved to be potent inhibitors of $P450_{17\alpha}$.

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Burke, D. F., et al (1997) *Anti-Cancer Drug Des.* **12**: 113–123

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Synthesis and evaluation of derivatives of phenyl alkyl azoles as inhibitors of 17α-hydroxylase/17,20-lyase

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A number of inhibitors of 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) have been previously reported (Ahmed et al 1995a, b), in particular, compounds based upon 4-substituted phenylethyl and phenylpropyl based azoles (imidazole and triazole), which at the time were shown to possess weak inhibitory activity against $P450_{17\alpha}$.

However, a major problem exists with these compounds in that they are able to inhibit other cytochrome P-450 enzymes, such as cholesterol side chain cleavage and as a result severe side effects can be observed. In an effort to synthesise compounds that are able to specifically inhibit $P450_{17\alpha}$, we expanded the work initially carried out by Ahmed so as to consider compounds containing larger alkyl chains. We therefore considered the design and synthesis of compounds based upon (4-substituted phenyl)-alkyl-azoles (imidazole, triazole and tetrazole) where the alkyl spacer group ranged in length from the methyl to decyl. Here, we report the initial results of our study into the synthesis and evaluation of inhibitors of $P450_{17\alpha}$. The inhibitors were designed using the substrate-haem complex approach. The synthesis of the target compounds was achieved through the N-alkylation of the azole using the appropriate (4-substituted phenyl)-alkyl-bromide and suitable base so as to produce the azole-based anion. The reactions proceeded in good yield and no major problems were encountered.

The biochemical evaluation of the synthesised compounds was undertaken using standard literature assay procedures. The assay mixture consisted of NADPH-generating system (50 μL), the inhibitor and substrate (10 μL), in phosphate buffer (925 μL, pH 7.4). The testicular microsomes (15 μL, final concentration of 0.23 μM) were warmed to 37°C before addition to the assay mixture. The assay was initiated by the addition of the microsomes. After 30 min incubation at 37°C, the assay was quenched by the addition of ether (2 mL) and placed on ice. The solutions were vortexed and the ether phase extracted and evaporated. Acetone (30 μL) was then added to each tube followed by steroid carriers (androstenedione, 17α-hydroxyprogesterone, and testosterone). The mixture was spotted onto TLC plates and run (approximately 2 h), mobile phase consisted of chloroform (80 mL), ethyl acetate (10 mL), cyclohexane (10 mL) and methanol (4 mL). After development, the separated steroids were identified, cut from the plate and placed into a scintillation tube with acetone (1 mL) and scintillation fluid (Cocktail T). The samples were vortexed and then read for tritium.

The results show that the compounds were, in general, equipotent or more potent than the standard compound for $P450_{17\alpha}$, namely ketoconazole (KTZ). Detailed consideration of the inhibitory data for the compounds shows that there is a good correlation between inhibitory activity and logP. This has been rationalised using molecular modelling and suggests that H-bonding interaction with the active site of $P450_{17\alpha}$ [corresponding to the steroid C(3)=O] may result in an increase in potency. The compounds synthesised within this study have therefore proved to be good lead compounds.

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Ahmed, S., et al (1995b) *Drug Des. Disc.* **13**: 27–41