Poster Session 1 – Medicinal Chemistry

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Synthesis, modelling and biophysical studies of amino-anthraquinones as inhibitors of telomerase

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Sequences of DNA rich in guanine can adopt unusual DNA secondary structural forms. In particular, four guanine bases can associate in a planar hydrogen-bonded assembly called a G-tetrad (for review, see Cairns *et al* 2002a). Successive layers of G-tetrads allow single stranded DNA to adopt a high-order foldback structure in solution termed a G-tetraplex (or quadruplex). Stabilisation of G-tetrad structures by intercalation or end-pasting has been shown to be useful for the inhibition of telomerase, an enzyme responsible for the elongation of telomere structures in DNA, (Cairns *et al* 2002b; Kim *et al* 2002). As part of a programme of work in our laboratories, several functionalised amino-anthraquinones (AAQ) have been examined as selective ligands to stabilise four-stranded DNA structures. Such complexes would prevent access of telomerase to its linear DNA substrate and thereby elicit a possible chemotherapeutic response for tumour control. Certain AAQs have previously been examined as intercalators for duplex DNA in the quest for cytotoxic agents (Agbandje *et al* 1992) but not examined in the context of high-order DNA.

Uncyclised and cyclised 1-mono, 1,5-di- and 1,8-di-AAQs of different complexion have been synthesised with differing side-chains (e.g. $-NH(CH_2)_2OH$, $-NH(CH_2)_2NH(CH_2)_2OH$ and $-NH(CH_2)_2N(CH_2CH_3)_2$). A cleaner, more flexible, route to these compounds has been developed in the course of our work.

Binding energies for novel cyclised compounds have been calculated using molecular modelling techniques (SGI Octane R12000 workstation using the Insight-II 2000 graphics interface and Discover 98.0 simulation software (Accelrys, Cambridge, UK)) with two reported (NMR and crystal) DNA tetraplex structures (Wang & Patel 1993; Parkinson *et al* 2002). Early findings show that energies appear superior for the NMR rather than the crystal structure (~ -700 vs ~ -400 kcal mol^{-1}) using one selected quinoxaline-functionalised AAQ ligand, and that the available binding sites differ markedly in affinity for ligand.

Biophysical examination of the interaction using thermal DNA denaturation methods (McConnaughie & Jenkins 1995) show stabilisation of calf thymus DNA, ([duplex]/[drug] ratio = 10:4) for uncyclised compounds with negligible effect for the cyclised AAQ series. For example, 1-[2-(2-hydroxyethylamino)ethylamino] anthraquinone, $\Delta T_m = 10°C$; 4-(2-hydroxyethyl)-1,2,3,4-tetrahydro-1,4-diaza-benzo[*a*]anthracene-7,12-dione, $\Delta T_m \leqslant 1°C$.

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039
Synthesis of azinomycin antitumour antibiotics: effect of stereochemistry on biological activity

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Azinomycins A and B were isolated from the culture broth of *Streptomyces griseofuscus* and were found to exhibit potent in-vitro cytotoxic activity and significant in-vivo anti-tumour activity (Nagaoka *et al* 1986). Together with the azinomycins, an additional metabolite devoid of the 1-aza-bicyclo[3.1.0]hexane ring system was isolated from the culture broths of *S. griseofuscus* and was later shown to possess potent cytotoxic activity against P388 murine leukaemia (Hashimoto *et al* 1994). The main objective of this work is to establish the mechanism of action of the azinomycins through the synthesis of structural analogues containing deep-seated structural modifications and the study of their DNA-binding and alkylation. The natural products possess (2*S*, 3*S*) stereochemistry. All the four possible stereoisomers (Mach43-51) were made to evaluate how the nature of stereochemistry influences cytotoxicity and antibiotic activity.

The initial steps of the synthesis followed the route developed by Shipman & co-workers to the left-hand portion of the azinomycins, based upon the Sharpless asymmetric dihydroxylation reaction. Commercially available 3,3-dimethylacrylic acid was converted into the corresponding benzyl ester under phase transfer conditions. Asymmetric dihydroxylation of this α,β-unsaturated ester using AD-mix-α or AD-mix-β gave a diol with (*R*) or (*S*) stereochemistry, respectively. The (*R*) and (*S*) diols were converted into (*R*)- and (*S*)- allylic alcohols in three steps involving selective mesylation, epoxidation and subsequent acid catalysed ring opening of the epoxide. The allylic alcohols were then successfully epoxidised with mCPBA, without stereochemical control, to give a mixture of the two diastereoisomers in a 45:55 ratio. Column chromatography allowed separation of the diastereoisomers. To complete the synthesis of epoxy amide, the various homo chiral epoxy alcohols were coupled with 3-methoxy-5-methylnaphthoic acid chloride. Hydrogenation of the benzyl ester gave the carboxylic acid, which was further converted to 3,4-epoxy-2-(3-methoxy-5-methyl-1-napthoyloxy)-3-methyl-butanamide by coupling with ammonium hydroxide. Preliminary cytotoxicity tests have shown that Mach43, which has the same stereochemistry as the natural product, is extremely active against the U2-OS ovarian sarcoma cell line whereas Mach44, with variation at the epoxide group is around 9 times less active. Mach50 and 51 were less active than the natural isomer, suggesting that a change in the sidechain stereochemistry causes a slight decrease in biological activity. However, in this case variation in the epoxide stereochemistry had no effect on activity. Interestingly, all compounds were quite potent against methicillin-resistant *Staphylococcus aureus* (MRSA), suggesting a new role for these compounds.

Table 1 Showing results from testing on tetracycline resistant bacteria, human osteosarcoma cell lines (U2-OS) and HoeR415

Compound	MRSA MIC (mg mL^{-1})	U2-OS (nM)	HoeR415 (nM)
Mach43	0.25	14	14
Mach44	2	123	123
Mach50	1	41	41
Mach51	1	41	41

These findings strongly suggest that analogues of the natural products do not need to crosslink DNA to retain potent anti-tumour activity. The compounds synthesised here are currently under investigation for their ability to alkylate DNA sequence selectively and for their antitumour activity against an extended set of cell lines. We are also designing new, more therapeutically useful agents.

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040
Pyrrolobenzodiazepine-polyamide libraries: synthesis and DNA binding selectivity

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The ability to modify gene transcription by inhibiting the activity of specific transcription factors or blocking transcription in the coding region of a gene has potential applications in the treatment of cancer. A sufficient number of base pairs must be recognised in order to obtain a specific effect. At the molecular level the minor-groove binding pyrrolobenzodiazepine (PBDs) recognise and covalently bind to Pu-G-Pu sequences (Thurston 1993; Hardy *et al* 2003), whereas heterocyclic polyamide (HPAs) interact reversibly with a variety of sequences depending on the nature of the heterocycle and the binding stoichiometry. The aim of this work is to synthesise hybrid molecules that combine the recognition characteristics of both molecules.

Solution phase peptide coupling methods have been used to synthesise a series of PBD-HPA hybrids with polyamide components (N-methylpyrroles) of varying length with a long-term view of studying the effect of polyamide length on DNA binding-site size and selectivity, binding affinity, cellular penetration and in-vitro cytotoxicity. GW6 consists of a PBD attached to three N-methylpyrrole heterocycles. Footprinting studies have shown that the molecule binds to a GATAATC sequence suggesting that it possesses DNA-recognition properties characteristic of both the PBD and HPA portions of the molecule. Using fluorescence microscopy we have shown that, unlike Dervan hairpin polyamides (Belitsky *et al* 2002) that bind to a similar number of base pairs, GW6 penetrates the nucleus of cultured MCF-7 cells. It also has in-vitro cytotoxic properties with a mean GI50 of < 10 nM in the NCI's 60 cell line screen.

Synthesis of the PBD-HPA conjugates has been successfully transferred to solid phase methodology using a variety of heterocyclic building blocks, and has been extended to the preparation of PBD-heterocycle combinatorial libraries. These libraries have been synthesised in conjunction with a peptide coding strand to enable identification of an individual DNA-binding molecule. A 100 000 member library has been screened against a number of rhodamine labelled gene fragments including one taken form *bcr-abl* and a number of hits have been obtained. Edman degradation of the tag sequences has revealed the structures of 10 lead molecules which are currently being resynthesized for further biological evaluation.

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041
Design and synthesis of duocarmycins as potential prodrugs

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The duocarmycins are naturally occurring antitumour antibiotics that derive their biological properties through sequence selective minor groove alkylation of duplex DNA. (+)-CC-1065 and duocarmycin A were the first members of this family synthesized, followed by duocarmycin SA, the latest and the most potent member of this group (Boger *et al* 2000). Reaction with DNA occurs through adenine N3 alkylation by the cyclopropane and subsequent ring opening and disruption of the cyclohexadienone system. Thus spirocyclopropane formation is crucial to biological activity of the duocarmycins. Removal of the hydroxy functionality on the duocarmycin analogues prevents spirocyclopropane formation and thereby gives potential viable prodrugs that can be bio-oxidatively activated by isoforms of cytochrome P-450 (CYPs) as is known for a number of anticancer drugs (Patterson *et al* 1999). There is growing evidence that certain CYPs may be over expressed in tumours and that intracellular activation of the duocarmycin prodrugs will lead to tumour selective agents.

The three main left hand sub-units under investigation are deshydroxy-N-Boc-CI (the minimum potent chromophore), deshydroxy-N-Boc-CBI (a synthetic analogue of the natural products) and deshydroxy-N-Boc-DSA (the alkylation subunit of duocarmycin SA). The left hand subunit is central to our studies and indeed examination of this subunit is dependant on reliable synthetic strategies.

The synthesis of deshydroxy N-Boc-CI involved a simple 3-step strategy originating from commercially available 2-Bromoaniline. Protection of the free amine was accomplished by refluxing with $(Boc)_2O$ and Et_3N in dioxane(100°C, 64%). This method showed significant improvement over more common Boc protection procedures where Di-Boc derivatives were found to be prominent. This was followed by N-alkylation (3 equiv NaH, 3 equiv 1,3-dichloropropene, DMF, 15 h, 25°C, 73%) to give the key substrate ready for free radical cyclization (90°C, 0.4 equiv AIBN, 1 equiv Bu_3SnH, 2 h, 89%)

Deshydroxy N-Boc-CBI was synthesized from 2-napthoic acid. Curtius rearrangement of the initial acid with the Shiori-Yamada reagent (t-BuOH, 1.7 equiv Et_3N, 4A molecular sieves, 1.2 equiv DPPA, reflux, 18 h, 77%) and deprotection primed the compound for regioselective iodination (THF, 0.4 equiv TsOH, 1 equiv NIS, 25°C, 4 h, 59%) at the required position. The amine was re-protected over 2 steps, N-alkylation and 5-exo-trig radical cyclisation (90°C, 0.4 equiv AIBN, 2 equiv Bu_3SnH, 4A molecular sieves, 2 h, 87%) then provided the required compound.

The synthesis of dehydroxy DSA originated from commercially available Ethyl-5-nitroindole-2-carboxylate. The indole nitrogen was protected with benzoyl chloride (DCM, 1 equiv Et_3N, 1 equiv DMAP, 1.6 equiv benzoyl chloride, 16 h, 86%) which then underwent reduction to provide the required amine (THF, 10% Pd/C, H_2, 16 h, 96%). Iodination was carried out as above along with Boc protection, N-alkylation and eventual free radical ring closure.

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042
Synthesis and characterisation of captopril carboxylate-ester prodrugs

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Most drugs are designed primarily for oral administration. The activity and stability profiles desirable for this route often make them unsuitable for transdermal delivery.

We are interested in designing analogues of currently used drugs for which the sustained steady-state blood plasma level associated with transdermal delivery — and which is unattainable orally — would be particularly beneficial. These prodrugs must be based on chemical bonds that are easily metabolised to the original, therapeutically active molecule. We have initially focused on ACE-inhibitors and calcium-channel blockers, designing a series of captopril analogues which QSAR modelling suggests would have significantly higher permeability than the parent drug.

Mathematical modelling studies (e.g. Potts & Guy 1992; Cronin *et al* 1999; Moss & Cronin 2002) have determined that the main predictors of percutaneous absorption are size and lipophilicity of the drug. For absorption from aqueous vehicle these may be modelled as MW and octanol/water partition coefficient (P): model:

$\log k_p$ (cm/s) = 0.74 log P – 0.0091 MW – 2.39 (Moss & Cronin 2002)

where k_p is the permeability coefficient.

We describe here the synthesis and characterisation of the first series of captopril prodrugs — a range of carboxyl esters.

Captopril (10 mmol, 2.18 g) was dissolved in excess (2 mL) of the series C1 to C6 *n*-alkanols and excess thionyl chloride (0.2 mL) was added dropwise at 0°C. In initial experiments the mixture was heated to 110°C for 4 h to complete the reaction (Tai *et al* 1995). Excess alcohol, HCl and SO_2 were removed in-vacuo to yield the esters. Purification, when required, was performed by flash chromatography using silica gel 60G (particle size 0.040–0.063 mm) with chloroform:methanol 80:20 mobile phase. All the prodrugs produced were oils at room temperature.

Acceptable yields (85–97%) were obtained. Characterisation was achieved by FT-IR, Raman, optical rotation, GC-MS and TLC methods. In particular, Raman spectroscopy indicated that the thiol ether was not produced, and FT-IR confirmed the presence of the carboxyl ester linkage and absence of free carboxylic acid. Diastereoisomers were observed by GC-MS and TLC in some cases. They were separated by preparative-TLC and their individual optical rotations determined. However, decreasing the reaction temperature to 60°C and time to 2 h prevented the isomerisation and retained the correct isomer. Stability and in-vitro metabolism studies are in progress.

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043
Synthesis of anti-infective quaternary ammonium salts for surface-immobilisation

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Despite major advances in techniques for the management of ventilator dependent patients and the routine use of effective disinfect respiratory equipment, ventilator associated pneumonia (VAP) continues to complicate the course of 8–28% of patients receiving mechanical ventilation (MV). Rates of pneumonia are considerably higher among patients hospitalised in intensive care unite (ICUs) compared with those in hospital wards, and the risk of pneumonia is increased 3- to 10-fold for the intubated patient receiving MV. VAP is defined as an inflammation of the lung parenchyma caused by infectious agents not present or incubating at time MV was started. Prolonged (more than 48 h) MV is the most important factor associated with nosocosmial pneumonia. However, VAP may occur within first 48 h after intubation (Chastre & Fagan 2002). A rational strategy to reduce the incidence of VAP is therefore to develop anti-infective polymeric biomaterial surfaces for the polymers used in endotracheal tubes to. This study focuses on the synthesis of membrane-disrupting molecules which can be irreversibly attached to such polymers.

Suitable molecules include quaternary ammonium compounds (QACs). QACs may be considered as being tetrasubstituted ammonium compounds in which the nitrogen atom has alkyl or heterocyclic substituents (R) with charge balance being provided by a small anion. The total number of carbon atoms in the four R groups is more than 10. For a QAC to have high antimicrobial activity at least one of the R groups must have a chain length in range C8 to C18. Pyridinium compounds such as cetylpyridinium chloride (CPC) are thus classified as QACs (Russell *et al* 1999). CPC is a cationic surface-active agent and is capable of absorbing to negatively charged bacterial cell membrane phosphates possibly disrupting the cell wall and increasing permeability. CPC has been shown to be bactericidal to Gram-positive bacteria and relatively uneffective against some Gram-negative bacteria (Smith *et al* 1991). It is employed pharmaceutically, for skin disinfection and for antiseptic treatment of small wound surfaces (0.1–0.5% solutions), as an oral and pharyngeal antiseptic (lozenges containing 1–2mg of the QAC) and as a preservative in emulsions (Russell *et al* 1999).

A multi-step synthetic route to derivatised CPC has been developed whereby functional groups appropriate for covalent attachment to PVC surfaces are incorporated remote from the pyridinium headgroup in order to allow the mechanism of action against bacterial membranes to be maintained when surface-immobilised. The route developed can be applied generally to explore the effect of structural variation.

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044
A novel approach to the synthesis of C-ring *Endo/Exo*-unsaturated pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) with potent in-vitro cytotoxicity

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There is currently interest in the pyrrolobenzodiazepine (PBD) antitumour antibiotics due to their potential to act as DNA sequence-selective gene regulators. SAR studies have revealed that the presence of a C2 substituent and *endo* or *exo* unsaturation in the C-ring are important for optimal DNA binding and antitumour activity.

Many published syntheses of PBDs rely on the presence of a 9-hydroxy substituent in the A-ring. This allows formation of a crucial O9-N10 benzal-protected PBD dilactam which can be reduced to afford the desired PBD target molecule. Unfortunately, the presence of a 9-hydroxy group is therapeutically undesirable, leading to acute cardiotoxicity (Hurley *et al* 1984). Thus a concise synthetic approach was required to allow the introduction of C2-substituents and C-ring *endo/exo* unsaturation that can be applied to *des*-9-hydroxy PBD targets.

Our previous attempts to prepare *endo/exo* unsaturated PBDs via reduction of N10-SEM protected dilactams were unsuccessfu (Thurston *et al* 1984). We therefore adopted a strategy whereby the desired C2 substituents were installed by Heck coupling to an N10-protected PBD carbinolamine, avoiding the need to reduce a dilactam intermediate. In addition, the approach had the advantage of directly introducing the C2 side chain avoiding the extensive functional group interconversions employed previously.

The key intermediate is a PBD enol triflate capable of taking part in a Heck coupling reaction. Three novel PBDs and a natural product (porothramycin) have been successfully synthesized via this concise approach. It demonstrates the versatility of this novel convergent route which should be applicable to a range of C2-substituted *endo/exo*-unsaturated PBDs.

Preliminary biological evaluation has revealed that these three novel PBDs have significant in-vitro cytotoxicity.

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045
Application of pyrrolo[2,1-*c*][1,4]benzodiazepine – isocyanate precursors to the preparation of prodrugs

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Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are low molecular weight molecules that recognise and bind to specific sequences of DNA blocking transcription and in the case of dimers, replication. Previous SAR studies have shown that blocking the N10 position abolishes the ability of the PBD to bind covalently to guanine in the minor groove of DNA.

This property of PBDs can be exploited in order to produce prodrugs capable of targeting tumour cells. In this instance photolabile protecting groups have been employed at the N10 position to effectively block the activity of the PBD. By using a light source of suitable wavelength the N10 protecting groups can be cleaved and the PBD prodrugs activated. PBD synthesis followed the approach originated by Fukuyama *et al* (1993), which relies on the use of N10 protecting groups to control the critical B-ring cyclisation step.

The photolabile protecting groups chosen were based on 6-nitroveratryloxycarbonyl alcohol (NVOC), 2-nitrobenzylalcohol, α-methyl-2-nitrobenzylalcohol and 4-(4-hydroxymethyl-2-methoxy-5-nitrophenoxy)-butyric acid allyl ester, which can also act as a photolabile linker to join a PBD to an antibody conjugate.

The first two alcohols were commercially available. α-Methyl-2-nitrobenzylalcohol was synthesised via the reduction of 2'-nitroacetophenone. The final linker was synthesised following a five step synthetic route reported by Holmes (1997). Attachment of the photolabile compounds to the PBD core was successfully performed via an isocyanate intermediate which formed the desired carbamate. Oxidation of these compounds afforded the protected PBDs in good yield.

The protected PBDs were then exposed to light at 365 nm for a period of 9–19 h and release of free PBD monitored by HPLC.

Future work will investigate photolytic conditions to optimise release of free PBD in the photolysis step.

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046
Neural network prediction of carcinogenicity of organic compounds

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A large variety of synthetic chemicals have been produced and the adverse effects of most of these chemicals on human health and ecosystems are unknown. For carcinogenicity, only limited data are available and the experimental determinations using animals are very costly and time consuming. Therefore, an attempt on a basis of Quantitative Structure–Activity Relationship (QSAR) models for estimating the carcinogenicity has been made (Tanabe & Matsumoto 2002; Romualdo 2003). The primary purpose of present study was to develop a QSAR model of carcinogenicity for diverse chemicals from the structures of molecules.

QSAR models of relationships between structure and carcinogenicity of chemicals were constructed by applying a multilayer neural network using the back-propagation algorithm (Zupan & Gasteiger 1999). The neural network (NN) was used to classify the chemicals studied into two categories, namely inactive or active. A learning set of 324 chemicals and a testing set of 168 chemicals in the database of the Challenge contest (www.informatik.uni-freiburg.de/~ml/ptc/) were characterized by means of the three sets of molecular descriptors, Dragon, tReymers and Helma. These descriptors were entered into the input layer of a three-layered NN, and carcinogenicity data were into the output layer (0 for non-carcinogenic or 1 for carcinogenic compounds).

After the training phase (5000 training cycles), the classification ability of the NNs with different descriptor sets was tested on the male rat data of 324 compounds. The correct classification rates obtained were 70.3%, 76.4% and 76.4%, using 839 Dragon, 22 Helma and 16 tReymers descriptors, respectively. The model with Dragon descriptor was discarded and the predictive performances of the three optimal NN models with descriptors of Helma, tReymers and their combination were evaluated for the data of four kinds of test animals (Table 1).

Table 1 Comparison of correct classification rate in % with different descriptor sets for test animals

Descriptor set	No. descriptors	MR	FR	MM	FM
Helma	22	76.4	84.0	87.3	82.9
tReymers	16	75.5	84.6	88.5	84.6
Helma + tReymers	37	77.8	85.3	88.4	84.5

MR: male rats, FR: female rats, MM: male mice, FM: female mice

The prediction accuracy is significantly improved with compared with reported values (60–70%) by earlier attempts using a statistical method such as regression analysis and partial least squares. It demonstrates the superiority of a NN as a nonlinear modeling method. Development of an open system for predicting the carcinogenic activity of chemicals based on the present results is under study.

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047
Shape changes, H..H contacts and hydrogen bonds in derivatives of antimycobacterial carboxamidrazones

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Previously investigated antimycobacterial N^1-benzylidene pyridine-2-carboxamidrazones had in their central region only an amino group as a hydrogen bond donor, along with two nitrogen atoms in the chain and one in the ring as potential acceptors. In the present study one or more NH groups have been introduced into the central chain along with a carbonyl or sulphonyl group. Specifically, the R groups in the general structure 2-Py-C(NH_2)=N-R are (I) the amide NH-CO-Ar, (II) the urea NH-CO-NH-Ar, (III) the sulphonamide NH-SO_2-Ar, where in each case Ar = p-(t-butyl)phenyl. While both the unmodified parent compound where R is N=CH-Ar and (I) are active against *Mycobacterium fortuitum* with MIC 8–16 μg mL^{-1}, (II) and (III) showed no activity. Calculated log P values (TSAR, version 3.3, Oxford Molecular Ltd.) cannot explain this difference since they are 3.5, 3.2, 3.2 for (I)–(III).

Crystal structures have been determined for (I)–(III), taking note of the degree of planarity and general shape of each molecule, the H..H contacts and the intermolecular hydrogen bonds. The angle between the pyridyl ring at one end and the phenyl ring at the other end of the molecule is 53°, 44°, 59° and 86° in (I), (II), and the two independent molecules of (III) respectively. In (I) and (II) there are twists along the chain, the greater twists about the longer bonds, while in (III) the N-S and S-C bonds are gauche. The amino group and the NH in the R group are in close proximity, creating N-H..H-N contacts significantly shorter than the 2.4 Å expected from van der Waals radii: 1.90 Å in (I), 1.93 Å in (II), 2.18 and 2.30 Å in (III). Rehybridisation of the nitrogen atom in the NH group from sp^2 to sp^3 appears to be of some importance in (II) and very significant in (III) as a means to relieve the H..H contact. The preferred acceptor for intermolecular hydrogen bonding is a carbonyl or sulphonyl O atom.

A model previously developed (Schwalbe *et al* 2000) to explain activity differences among carboxamidrazones postulated that the pyridyl nitrogen atom is anchored by a hydrogen bond donor at the putative active site, and there is an optimum distance from this point to the furthest extremity of the lipophilic arylidene substituent. In the present study, although none of the molecules is as flat as the parent compound, (I) is reasonably linear and consistent with such a model. Interposition of the extra NH group in (II) imparts a scimitar-like bend, so that one extremity of the molecule must contact a different part of the receptor. The staggered bonds to sulphur make (III) an L-shaped molecule. It is therefore not surprising that (II) and (III) are inactive.

Schwalbe, C. H., *et al.* (2000) *J. Pharm. Pharmacol.* **52** (Suppl.): 105

048
Changes in antimycobacterial carboxamidrazones upon N-oxidation

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A series of pyridylcarboxamidrazones show promise as antimycobacterial agents. In the particular set with formula Py-C(NH_2)=N-N=CH-[p-(t-Bu)]Ph , MIC values against *Mycobacterium fortuitum* are similar (8–16 μg mL^{-1}) for both the 2-pyridyl (I) and 4-pyridyl (II) isomers. However, compound (III), the N-oxide of (II), is inactive.

We report here the crystal structures of (I), (II) and (III) along with the results of 6–31G* ab-initio molecular orbital calculations for simplified analogues without the t-butyl substituent. For maximum conjugation the molecules should remain flat. In the crystal of (I), as in related active molecules (Schwalbe *et al* 1999) there is no serious obstacle to this, since the pendant amino group can form favourable N-H..N interactions to either side. The pyridine and benzene ring planes intersect at 20°, and no bond linking them is twisted away from planarity by more than 9°. In (II) the repositioning of the ring N atom creates a C-H..H-N clash. In the two independent molecules found in the crystal the angle between ring planes increases to 31° and 34°, with the maximum bond twist now 26° (about the exit bond from the pyridyl ring). In (III) these values resemble (I) more than (II), and thus the imposed twist is unlikely to be responsible for the inactivity. In the transformation of (II) to (III) one hydrogen bond acceptor (N) is replaced by a stronger one (O), albeit with the long axis of the molecule extended by 1.27 Å. If this atom serves an anchor point to the receptor by interacting with a hydrogen bond donor, the stronger interaction may be outweighed by steric clashes at the far end of the binding pocket.

The electronic properties may provide a further explanation. In (I) and (II) the Löwdin charges on the ring nitrogen atom are –0.164 and –0.179, respectively. In (III) this charge reverses sign and becomes +0.248, while a negative charge of –0.502 migrates to the attached oxygen atom. However, the strong local dipole in the vicinity of the ring N atom in (III) has no precedent in the other two molecules. This local change has a considerable effect on the log P calculated for the whole molecule with TSAR (version 3.3, Oxford Molecular Ltd.): c log P is 4.9 for (I), 4.5 for (II) but only 1.5 for (III). Possibly this enhanced polarity stops penetration of the drug through the nonpolar coating of the bacterium.

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049
Development of a binding model for anti-cancer cyclohexadienones

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4-Hydroxy-4-(benzothiazol-2-yl)cyclohexadienone (AW464) is a highly active member of a set of quinols that show selectivity against renal and colon cancer cell lines (Wells *et al* 2003). Database mining of the NCI 60 cell screening panel suggests that the protein thioredoxin is its target. Human thioredoxin (1ERT in the Protein Data Bank) has two active-site cysteine residues at positions 32 and 35 with S atoms only 3.9 Å apart. Their proximity suggests that they could attack the cyclohexadienone ring of the drug in a 'double Michael addition.' The present study starts from the crystal structure of AW464 (Schwalbe *et al* 2002) and seeks to address the following issues: the geometry of the cyclohexadienone (CH) ring; the steric relationship between this ring and the benzothiazole (BT) ring; the geometry and stability of model adducts; and the geometry and thermodynamics of thioredoxin binding.

The CH ring is not completely flat. The carbonyl C atom deviates by 0.03 Å from the plane through the double bonds, and the bridgehead carbon C8 deviates by 0.06 Å so as to bring the OH group closer to the plane, as is observed in 11 of 12 related structures in the Cambridge Structural Database. In AW464 the BT plane roughly bisects the CH ring: torsion angle N3-C2-C8-O14 is –168.8(2)° (i.e. N3 is far from O14). This torsion angle approaches 90° in a related indole derivative, suggesting free rotation.

Our simplest model adduct has one S-Me group and one H atom attached to each double bond of CH. The S-Me can be on the same side as the BT ring ("up") or opposite ("down"). Semi-empirical molecular orbital calculations with PM3 parameters unsurprisingly show down-down to have a 5 kcal mol^{-1} lower heat of formation than up-up, although up-down achieves equal stability with ca. 90° rotation of the BT ring. A down-down adduct of 1,2-ethanedithiol with symmetrical C-S bonds and S..S contact of 3.29 Å can be built, although a less symmetrical structure with wider S..S contact is more stable. Construction and optimisation with PM3 of an adduct with the relevant Cys-Gly-Pro-Cys fragment down-down yields a structure with N3-C2-C8-O14 torsion angle −122° and S..S contact 4.45 Å. Finally, a hybrid PM3/MM optimisation of the full thioredoxin molecule, free drug and the thioredoxin adduct gives S..S contact of 4.41 Å and positions the BT ring in a channel near an indole ring of tryptophan. The calculated enthalpy change is +35 kcal mol^{-1} (only +11 kcal mol^{-1} with AM1 parameters), mainly due to distortion of the drug. However, the entropy increase from release of water may yet favour the reaction.

Schwalbe, C. H., *et al.* (2002) *J. Pharm. Pharmacol.* **54** (Suppl.): 162
Wells, G., *et al.* (2003) *J. Med. Chem.* **46**: 532–541

050
Synthesis and evaluation of a range of phenyl alkyl azoles as inhibitors of 17α-hydroxylase (17-OHase) and 17,20-lyase

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The conversion of pregnanes to androgens is catalysed by a single enzyme and involves two distinct steps potentially involving two components within the same enzyme: an initial 17α-hydroxylation (presumed to be carried out by the 17α-hydroxylase (17α-OHase) component); followed by the cleavage of the C17-C20 bond (presumed to be carried out by the 17,20-lyase). No crystal structure exists for 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$), however, workers within the field have utilised homology modelling in an attempt to elucidate further information regarding the active site. In our efforts we have developed a novel technique (namely, the substrate-haem complex (SHC) approach), which we have utilised to produce a representation of the essential components within the active site of the overall enzyme complex (Ahmed *et al* 1999). 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 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, evaluation and molecular

modelling of inhibitors of both components. The synthesis of the final compounds was achieved through the *N*-alkylation of the azole using the appropriate (4-substituted phenyl)-alkyl-bromide and suitable base. In general, 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 — here we describe the general assay for both components. The assay mixture consisted of NADPH-generating system (50 μL), the inhibitor and substrate (10 μL), in phosphate buffer (915 μL, pH 7.4). The assay was initiated by the addition of testicular microsomes (15 μL) — warmed to 37°C before addition. 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. 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); the most potent imidazole-based compound was found to be 10 and 6 times more potent than KTZ against 17,20-lyase and 17α-Ohase, respectively. Detailed consideration of the inhibitory data for the compounds shows that there is a good correlation between IC50 and logP and the inhibitory activity has been rationalised using the SHC approach which suggests that H-bonding interaction with the active site of $P450_{17\alpha}$ (corresponding to the steroid C(3)=O) results in increased potency. The compounds synthesised within the present study are therefore good lead compounds in the design of further novel inhibitors of $P450_{17\alpha}$.

Ahmed, S., *et al.* (1999) *Bioorg. Med. Chem.* 7: 1487–1496

051
The structure–activity relationship determination of a series of sulphamated compounds based upon cinnamic acid

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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 reported within the literature 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 — in particular, we hypothesised that compounds based on cinnamic acid would be good monocyclic-based mimics of the coumarin backbone. Here, we report the results of the synthesis and in-vitro biochemical evaluation (using literature based assay procedures (Table 1)) of compounds based upon 4-hydroxycinnamic acid. The inhibitory activity obtained was then rationalised using a theoretical model of the active derived from the consideration of the transition-state (TS) of the reaction catalysed by ES and involved the superimpositioning of the coumarin based compounds (and our potential inhibitors) onto the derived TS.

Table 1 IC50 data for compound under study

Alkyl chain	Compound no.	IC50 (μM)
Me	1	790.5 ± 13.4
Et	2	333.7 ± 1.4
Pr	3	273.9 ± 8.4
Bu	4	64.5 ± 2.1
Pe	5	992 ± 12
EMATE	—	0.5 ± 0.001
COUMATE Reduced	—	13.8 ± 0.07
COUMATE	6	203 ± 5.7
667-COUMATE	—	0.23 ± 0.01

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 which is observed to exist within the coumarin based compounds. In an effort to determine the unknown factors we synthesised a derivative of COUMATE (6) 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). Furthermore, from the modelling of the above compounds onto the TS, we discovered that the weaker inhibitors based on the cinnamic acid backbone underwent steric interaction with the hydrogen bonding group which binds to the C(17)=O of estrone sulphate. In conclusion, we have highlighted the factors which may be taken into consideration in the design of further novel inhibitors of ES.

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

052
Testosterone based compounds as probes for the active site of oestrogen synthetase

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The biosynthesis of oestrogens from androgens is mediated by the oestrogen synthetase enzyme aromatase (AR) and requires O_2 and NADPH to function. Here, we report the results of a study into the synthesis, biochemical evaluation and the molecular modelling of compounds based upon C(17) derivatives of testosterone as probes for the active site of AR, in particular, the area of the active site which would normally bind to the C(17)=O of androstenedione — it has previously been suggested that this area is highly constrained and is not able to accommodate large groups (Banting *et al* 1988).

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 inhibitor. The results of the biochemical evaluation for a small range of compounds are shown in Table 1.

Table 1 Testosterone based compounds synthesised and their IC50 values (mean of triplicate values)

Compound	IC50 (μM)	Relative potency
Testosterone	20.6 ± 0.2	2.7
Testosterone acetate	48.7 ± 0.4	1.1
Testosterone butanoate	58.3 ± 0.3	0.9
Testosterone pentanoate	63.1 ± 0.2	0.9
Testosterone octanoate	88.7 ± 0.1	0.6
Testosterone 4-nitrobenzoate	8.5 ± 0.05	6.4
AG	54.8 ± 0.06	1

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.

The inhibitory activity (and in particular the highly potent inhibitory activity of testosterone 4-nitrobenzoate) obtained for the compounds synthesised within the present study appear to contradict the earlier homology based studies regarding the volume of space available within the active site of AR corresponding to the C(17) area of the steroid backbone. For example, the acetate derivative is only ~3.3 times more potent than the decanoate, however, since the conformational space of the latter would be expected to be extremely large, steric hindrance would be expected to greatly affect the inhibitory activity, which is not observed. In conclusion, we suggest that large groups may indeed 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.

Banting, L., *et al*. (1988) *J. Enzyme Inhib*. **2**: 215–229
Thompson, E. A., Siiteri, P. K. (1974) *J. Biol. Chem*. **249**: 5373–5378

053
In vitro evaluation of a range of straight chain and cyclic esters of benzoic acid as inhibitors of oestrone sulphatase

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It has been shown that the inhibition of the enzyme oestrone sulphatase (ES) may be a new therapeutic target in the treatment of hormone-dependent breast cancer. We have investigated a number of physicochemical properties which we considered to be important in the design of highly potent irreversible inhibitors of this enzyme. In general, the potent inhibitors contain a sulphamoylated phenyl ring containing electron-withdrawing functionality. We have recently proposed a mechanism for the irreversible inhibition of ES and concluded that the potent inhibitory activity observed within compounds containing the sulphamate moiety was probably related to the stability of the anion which has been presumed to exist during the course of the ES catalysed reaction. Here, we report the results of our study into a series of compounds where we have attempted to optimise the pKa and logP of the molecules so as to produce potent inhibitors of ES. Furthermore, we have utilised molecular modelling techniques, involving the determination of the transition-state for the hydrolysis of oestrone sulphate, to reduce steric interaction between the inhibitor and hydrogen bonding groups at the active site.

In the synthesis of the inhibitors, we used a standard literature procedure involving the reaction between aminosulphonyl chloride with the ester of 4-hydroxybenzoic acid in dimethyl acetamide at room temperature. The biochemical evaluation of the series of compounds was undertaken using both microsomal enzyme and MCF-7 cell line using the previously reported method of Selcer *et al* (1996). The pKa of the parent phenols were determined using a spectrophotometric method, while logP was calculated using Projectleader within the CaChe molecular modelling program.

The results of the biochemical evaluation show in general, that all the compounds were potent inhibitors of ES, in particular, the long chain esters were more potent than the two standard compounds, EMATE and COUMATE, but less potent than 667-COUMATE. Furthermore, the compounds were found to be potent inhibitors of MCF-7 cells. Consideration of the inhibitory activity with respect to the physicochemical properties shows that a correlation exists between logP and IC50. Modelling of the compounds on the transition-state shows that with the large alkyl chain compounds, such as the decyl ester, steric interaction may be possible between the hydrogen bonding group at the active site which would normally interact with the C(17)=O of oestrone. On modelling the cyclic esters onto the transition-state, we observed that the cyclooctyl ester was able to fit within the transition-state without undergoing steric interaction. On evalutaing the cyclooctyl ester, we discovered that this compound was indeed a highly potent inhibitor, in fact, the compound was some 3 times more potent than 667-COUMATE, which has recently entered clinical trials.

From the results of the current study, we are able to provide a number of important structural features and physicochemical properties which may be utilised in the development of further novel compounds against ES.

Selcer, K. W., *et al*. (1996) *J. Steroid Biochem. Mol. Biol*. **59**: 83–91

054
PLGA surrogate polymer libraries

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Polymers are ubiquitous in pharmaceutical formulations and feature in the majority of strategies (e.g. conjugates, particulates, hydrogels, implants, etc.) in the delivery of sensitive or toxic pharmacologically active molecules. Polyesters, especially poly(lactide co-glycolide) (PLGA), have been extensively studied (Edlund & Albertsson 2003). To better exploit the properties of these polymers we are developing PLGA surrogate polymers that possess chemical moieties for functionalisation. An important component of research in medicines development is the use of combinatorial strategies. We are developing analogous strategies to systematically optimise biomedical polymer properties. Combinatorial libraries of polymers (Brocchini *et al* 1997) have been used (a) to increase in unique relationships the number of candidate polymers for medical applications, (b) to efficiently systematise the study of structure-property correlations and (c) to incrementally modulate specific polymer properties (e.g. solubility with actives, thermal properties) over a wide range while keeping other defined properties unchanged (e.g. mechanism of degradation, molecular weight characteristics). This paradigm for development makes it possible to examine broadly similar polymers that together span a large range of properties.

Two classes of polyester have been prepared (1) A-B strictly alternating aliphatic polyesters derived from either protected glycerol or serinol and diacid monomers and (2) polyester-amides that are derived from serinol and diacid monomers. Both sets of polymers are prepared by condensation polymerisation. A library of A-B strictly alternating polyesters is being constructed by parallel synthesis using a range of 2-glycerol substituted analogues and a range of diacid monomers. These polymers have been prepared in dichloromethane or THF by carbodiimide mediated coupling catalysed by dimethylamine pyridine-*p* toluene sulfonic acid complex. Polymers with molecular weights ranging from 10 000 to 25 000 g mol^{-1} are isolated by solvent evaporation followed by precipitation in cold methanol.

The polyester-amide polymers are prepared using polymeric precursors that are then functionalised to provide the library polymers. Polymerisation is conducted with Fmoc-serinol and diacids to provide polyesters with molecular weights up to 35 000 g mol^{-1}. The Fmoc group is removed with mild base to generate the serinyl-2-amino group. The free amine readily undergoes reaction with polyester

moieties along the polymer mainchain to product hydroxy-pendent polyesteramides. The molecular weight of the polymer is predominantly maintained after scrambling. The hydroxy group is then functionalised with electrophilic reagents (e.g. acid chlorides) to produce each library polymer. The advantage of this synthetic strategy is a minimal number of polymerisation reactions are required since each precursor polymer is used to generate a large number of library polymers.

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Brocchini, S., *et al*. (1997) *J. Am. Chem. Soc*. **119**: 4553–4554
Edlund, U., Albertsson, A. C. (2003) *Adv. Drug Del. Rev.* **55**: 585–609

055
A library of polycationic excipients

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Soluble polymers are actively being studied as co-excipients to complex with DNA in the search for formulations that can act as non-viral vectors. Such formulations would be expected to display properties for cellular uptake into the cytoplasm and thus may be useful for delivering other molecules intracellularly (e.g. proteins or toxins). Homopolycations and block copolymers composed of a cationic block and a hydrophilic segment can complex DNA providing high colloidal stability and reduced interaction with blood components.

We have recently prepared a narrow molecular weight distribution (MWD) homopolymeric precursor (Godwin *et al* 2001) that is used as a common intermediate for the preclinical development of water-soluble conjugates and other functionalised biomedical polymers. One advantage of using an active ester polymer as a precursor (Batz *et al* 1972; Ferruti *et al* 1972; Mammen 1995) is that only one polymer is needed to give families of functional polymers for study, all with the same molecular weight characteristics and with defined pendent chain structure (Godwin *et al* 2001). Recently we have also developed a narrow (MWD) block copolymer precursor (Pedone 2002) with one block being poly(ethylene glycol) and the other block being active ester that can be subsequently functionalised. Block copolymer precursors have been prepared with PEG2000 and PEG5000 blocks. Conjugation reactions with amines to give functionalised block copolymers are possible where little or no cleavage of the ester bond linking the two blocks is observed.

Using both the homopolymer and block copolymer precursors and knowing that the imidazole group of 2-(1*H*-Imidazol-4-yl)-ethylamine has a pK around 6.0 and thus becomes cationic in a slightly acidic medium that would be expected in the endosome, we have prepared cationic copolymers and block-copolymers that may form interpolyelectrolyte complexes with DNA and mediate an acid-dependent escape from intracellular vesicles (Putnam *et al* 2001). A library of 20 cationic copolymer and block copolymeric cations were prepared from 3 precursor polymers by the sequential reaction of the homo and block copolymeric precursors with (2-aminoethyl)trimethylammonium chloride hydrochloride, 3-(dimethylamino)propylamine and/or histamine followed by the addition of 1-amino-2-propanol. To probe the effects of PEG block, the polymeric precursors were selected so that the functionalised regions of the polymers that were produced were similar.

The conjugates contained different amounts of tertiary amine, quaternary ammonium, imidazole and hydroxypropyl methacrylamide side chains. These conjugates were assessed for their ability to complex plasmid DNA (pcDNA/luc) at pH 7.4 and 4.5 by agarose gel electrophoresis using polymer/DNA weight ratios ranging from 1.25:1 to 5:1. All polycations containing quaternarium ammonium groups were able to form complexes with DNA at low charge ratios. Photon correlation spectroscopy indicated that some block polycations containing tertiary amine/imidazole groups formed particles below 100 nm at pH 4.5. GPC and titration studies were also used to evaluate how polymer size in solution changes as a function of pH. In contrast to many polycations, in-vitro cytotoxicity studies (MTT assay) indicated that several block copolymers were only mildly toxic IC50 values of 5 mg mL^{-1}. Overall, the amount of PEG in the block polycations may be important for the stabilisation and solution properties of the DNA complexes.

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056
Solid-phase synthesis of DNA-intercalating cyclic depsipeptides

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The quinoxaline antibiotics are a class of naturally occurring, two-fold symmetrical bicyclic depsipeptides demonstrating both antibacterial and cytotoxic activity (Katagiri *et al* 1975). They are typified by echinomycin and triostin A, cyclic octadepsipeptides comprising two residues each of D-serine, L-alanine, *N*-methyl-L-cysteine and *N*-methyl-L-valine. Two depsipeptide bonds (between the D-serine hydroxyl and the L-valine carboxyl groups) are present in both echinomycin and triostin A, and the cyclic structures are made more rigid by a thioacetal or disulphide bridge respectively between the two cysteine residues.

The quinoxaline antibiotics derive their biological activity from bis-intercalation (Wakelin 1986). A 2-quinoxalinecarbonyl chromophore is attached to each side of the cyclic depsipeptide structure, via the D-serine amino groups. These chromophores have been shown to insert themselves between the base pairs of specific DNA sequences, forming two base pair sandwiches that orient the cyclic peptide in the minor groove. The long-lived complex has fatal consequences for the cell, possibly via the inhibition of recognition by RNA polymerase.

An analogue of triostin A, TANDEM (Triostin A *N*-DEMethylated), has been synthesised using solution-phase methodology and has proved useful in the investigation of the mechanism of bis-intercalating depsipeptides (Ciardelli *et al* 1978). The solution-phase synthesis was hampered by the need to perform often lengthy and cumbersome purification steps after each coupling and/or deprotection step.

We have employed a linear solid-phase approach to the synthesis of TANDEM using classical solid-phase peptide synthetic methodology. The key features of the synthesis are the formation of both depsipeptide bonds and the introduction of both chromophores on the solid-phase, and the flexibility afforded in the final cyclisation and disulphide bridge formation. We found that the disulphide bridge could be formed in-situ with the depsipeptide still attached to the solid-support, followed by cleavage and cyclisation; or, that the linear depsipeptide could be cleaved with the cysteine protection still in place for solution phase cyclisation and subsequent disulphide formation. Since the growing depsipeptide is immobilised on the solid support throughout the synthesis, reactants and by-products from each coupling and deprotection step are removed by simple filtration resulting in a substantially more rapid and efficient synthesis. This approach also lends itself well to the synthesis of analogues and libraries in which the amino acids and/or the chromophores (one or both) are substituted.

We are also investigating a convergent approach to the synthesis of TANDEM in which a suitably protected didepsipeptide building block is prepared in solution and utilised directly during the solid-phase assembly. We are also optimising the conditions for final solution-phase cyclisation.

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