method). Chemical stability was assessed using a validated stability-indicating high-performance liquid chromatography method.

Results The main results from physical and chemical stability testing of paclitaxel infusions during the shelf-life periods are shown in Table 1. The stability of paclitaxel infusions during storage was mainly limited by physical stability due to the precipitation of paclitaxel. This was concentration-dependent and reflects the fact that commercially available paclitaxel concentrates are solubilized with Cremophor EL and ethanol. Sub-visual particulate counts were within the British Pharmacopoeia limits. An adequate stability period ($\geq$17 days) was obtained with both 0.3 and 1.0 mg/mL paclitaxel infusions, which facilitates the dose banding of paclitaxel chemotherapy.

Conclusions Paclitaxel demonstrated robust stability in 0.9% sodium chloride over at least 17 days, which facilitates the feasibility of dose-banding strategy. Further pharmacokinetic studies will be performed by *ex vivo* simulations and clinical studies to evaluate the role of this dose-banding strategy compared with standard BSA-based dosing and flat-fixed dosing.

Plumridge, R., Sewell, G. J. (2001) *Am. J. Health-Syst. Pharm.* **58**: 1760–1764

125
Stability of 500 mg/50 mL and 250 mg/50 mL vancomycin B in CIVAS batch-manufactured pre-filled syringes

S. Polli[1], P. Tunstell[2], J. Petit[2], O. Rabui[2] and B. Forbes[1]

[1]King's College London and [2]St Thomas' Hospital, London, UK.
E-mail: ben.forbes@kcl.ac.uk

Objectives Vancomycin is used as an infusion to treat serious infections in hospitalized patients. Pre-filled syringes for infusion were multi-dispensed by diluting the concentrated vancomycin with diluent in each individual syringe within the aseptic manufacturing unit at Guy's and St. Thomas' Hospitals and assigned 28-day expiry. Following a review of this method of manufacture, improvement in the quality assurance of the product was recommended by production of an aseptically prepared diluted bulk solution that was then packed into 50 mL syringes with no further dilution. The final batch of syringes could be assayed for vancomycin content prior to batch release using a representative sample from the batch. The aim of this study was to establish a stability-indicating assay to validate the new production method and to verify the stability of the vancomycin product.

Methods The high-performance liquid chromatography (HPLC) assay (Figure 1) conformed to International Conference on Harmonisation (ICH) guidelines (ICH 1994) for a stability-indicating assay with regard to specificity, linearity, accuracy and precision. Vancomycin hydrochloride pre-filled syringes (250 mg/50 mL and 500 mg/50 mL) were prepared using the new production method. The pre-filled syringes were evaluated for vancomycin B content (by HPLC assay), pH and clarity. Vancomycin B content was measured for over 63 days under conditions of 2–8°C, 25°C with or without protection from light, and 40°C.

Results No loss of vancomycin B content was measured in syringes stored at 2–8°C over the test period. Syringes stored at 25°C protected from light showed the vancomycin B content to decline at rates of 0.20 and 0.15%/day for 250 mg/50 mL and 500 mg/50 mL, respectively. When exposed to light at 25°C, the rate of vancomycin B decline was 0.23 and 0.17%/day for 250 mg/50 mL and 500 mg/50 mL. Syringes stored at 40°C had the fastest decline in vancomycin B content, at a rate of 1.49 and 1.47%/day for 250 mg/50 mL and 500 mg/50 mL, respectively. Decline in vancomycin concentration was associated with an increase in pH. A white precipitate appeared in the least stable syringes (those stored at 40°C).

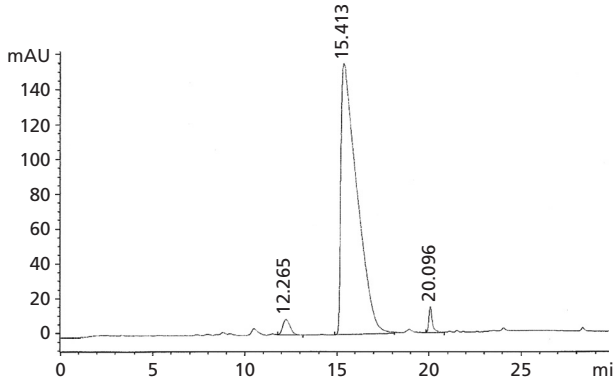

Figure 1 A chromatogram of 2 mg/mL vancomycin B standard. Retention time = 15.4 minutes (vancomycin peak area = 95.4% of area).

Conclusions A new batch production process was implemented with a shelf life of 56 days assigned to 250 mg/50 mL and 500 mg/50 mL vancomycin pre-filled syringes when stored at 2–8°C. The HPLC assay is now used as a quality-control test for vancomycin B content prior to batch release.

International Conference on Harmonisation (1994) *Technical requirements for registration of pharmaceuticals for human use*. ICH-Q2A. International Conference on Harmonisation

Chemistry

126
Quantitative structure–activity relationship analysis of local anaesthetic activity of a series of 3-aminobenzo[*d*]isothiazole derivatives

J. C. Dearden[1], M. Hewitt[1], H. Modarresi[1], A. Geronikaki[2], P. Vicini[3], N. Dabarakis[2], V. Poroikov[4], A. Lagunin[4] and G. Theophilidis[2]

[1]Liverpool John Moores University, Liverpool, UK, [2]Aristotle University, Thessaloniki, Greece, [3]University of Parma, Parma, Italy and [4]Institute of Biomedical Chemistry of Russian Academy of Medical Sciences, Moscow, Russian Federation.
E-mail: j.c.dearden@ljmu.ac.uk

Objectives To test a number of 3-aminobenzo[*d*]isothiazole derivatives as potential local anaesthetics, and to develop a quantitative structure–activity relationship (QSAR) for the local anaesthetic activity of the compounds.

Methods Synthesis of most of the compounds has been reported previously (Geronikaki et al 2003). Local anaesthetic activity of 30 compounds, together with that of lidocaine as a reference compound, was determined in the rat sciatic nerve model using Wistar rats. For the QSAR analysis, descriptors were calculated using TSAR (www.accelrys.com), ADMEWORKS Predictor (www.fqs.pl) and HYBOT (http://software.timtec.net/hybot-plus.htm) software. We used MOBYDIGS (www.talete.mi.it/mobydigs.htm) software to eliminate descriptors with very poor correlation with local anaesthetic activity and those with high pairwise collinearity, leaving a total of 411 descriptors. The MOBYDIGS genetic algorithm procedure was used to select the best descriptors. Cross-validation was carried out using the LOO (leave-one-out) procedure.

Results A six-descriptor QSAR was developed with reasonably good statistics:

$$\log \mathrm{RA}_{100} = -0.961\ \mathrm{FVMX} + 0.103\ \mathrm{ELOW1} - 0.00524\ \mathrm{ECCN} + 28.0\ \mathrm{CARB\text{-}1} - 0.0382\ \mathrm{DPM_Z} + 2.11\ \Sigma\mathrm{Q}^{+} - 7.56$$

$$\mathrm{n} = 31 \quad R^2 = 0.772 \quad Q^2 = 0.648 \quad s = 0.101 \quad F = 13.5$$

where RA_{100} = activity of compound relative to lidocaine activity (= 100), FVMX = maximum free valence value (an indicator of polarity/reactivity), ELOW1 = difference between minimum and maximum electrotopological state values (size/polarity), ECCN = whole molecule eccentric connectivity index (molecular shape), CARB-1 = average charge on carbonyl carbon atoms (polarity), $\mathrm{DPM_Z}$ = dipole moment in Z direction (polarity), $\Sigma\mathrm{Q}^{+}$ = sum of positive charges on atoms (polarity/hydrogen bonding), n = number of compounds in training set, R = multiple correlation coefficient, Q = cross-validated multiple correlation coefficient, s = standard error of estimate and F = Fisher statistic. All P values were <0.02, indicating that each descriptor had a less than 2% chance of having been selected by chance. There have been but few previous QSAR studies of local anaesthetic activity. Recanatini et al (1988) found a weak correlation (r^2 = 0.652) with distribution coefficient for a series of lidocaine derivatives, and Caliendo et al (1996) obtained a reasonable correlation (r^2 = 0.750) with partition coefficient for a series of isobutyramides.

Conclusions The QSAR results are in accord with our previous conclusions (Geronikaki et al 2003) that molecular size/shape, polarity and hydrogen bonding are largely responsible for local anaesthetic activity. Unlike other workers, we did not find that local anaesthetic activity correlated with partition/distribution coefficient, although it should be pointed out that the descriptors that we have found important all contribute to lipophilicity. The QSAR should be useful in the search for more potent 3-aminobenzo[*d*]isothiazole local anaesthetics.

Caliendo, G. et al (1996) *Eur. J. Med. Chem.* **31**: 99–110
Geronikaki, A. et al (2003) *SAR QSAR Environ. Res.* **14**: 485–495
Recanatini, M. et al (1988) *Quant. Struct. Act. Relat.* **7**: 12–18

127
A quantitative structure–toxicity relationship study of benzodiazepine drugs

T. Suzuki[1], S. Funar-Timofei[2] and D. Ionescu[3]

[1]Toyo University, Tokyo, Japan, [2]Institute of Chemistry, Romanian Academy, Timişoara, Romania and [3]University of Medicine and Pharmacy "Victor Babeş", Timişoara, Romania. E-mail: azul@oak.ocn.ne.jp

Objectives Benzodiazepines are defined as a large family of drugs used as hypnotics, anxiolytics, tranquillizers, anti-convulsants and pre-medication, and for intravenous sedation. This paper presents a structure–toxicity study applied to a series of 58 benzodiazepine derivatives to model the benzodiazepine derivative toxicity by statistical methods.

Methods As dependent variable the lethal oral dose for mouse LD_{50}, expressed in mg/kg (mg/kg transformed into mol/kg), retrieved from the RTECS database (RTECS Database, MDL Information Systems, San Leandro, CA, USA) was used. The molecular structure of the benzodiazepine derivatives was built by the Chem3D Ultra (Chem3D Ultra 6.0, CambridgeSoft.Com, Cambridge, MA, USA) package and energetically optimized using the molecular mechanics method and quantum chemical calculations (RM1 approach). To the lowest-energy conformations obtained by molecular mechanics calculations the MOPAC 2007 (Stewart 2007) semi-empirical molecular orbital program was applied to finally optimize the structures. Several structural descriptors, volumes, molecular surface area and hydrophobicities were calculated from the minimized structures by the Dragon (Dragon 2.1-2002, Talete SRL, Milano, Italy), Winmostar (Winmostar v.3.59c, Winmostar by Delphi, Norio Senda) and ALOGPS 2.1 (Tetko and Tanchuk 2002) programs. Also, quantum chemical descriptors were derived from RM1 (Rocha et al 2006) calculations. Multiple linear regression (MLR), artificial neural networks and support vector machines were used to develop QSAR models.

Results The calculated structural parameters were then related to the log LD_{50} values by MLR. Variable selection was performed by the genetic algorithm, implemented in the MOBYDIGS software package (Todeschini et al 2003; version 1; www.talete.mi.it/mobydigs.htm), with the leave-one-out (LOO) cross-validated q^2 value as a fitness function. Several MLR equations were thus found and good correlations and predictable models were obtained. Presence of nine-membered heterocycles, of unsubstituted aromatic carbon atoms, of the number of primary aromatic amines in the benzodiazepine derivatives and higher HOMO and LUMO molecular orbital values increased the benzodiazepine toxicity. Higher unsaturation index values and benzodiazepine COSMO molecular volumes decreased the LD_{50} values. Increased sum of atom self-polarizability, molecular hardness, Mulliken electronegativity, electrophilic delocalizability, atomic minimum charge and number of ring secondary carbon atoms decreased the benzodiazepine toxicity. Non-linear modelling methods of artificial neural networks and support vector machines gave somewhat better models than those obtained by MLR using the same set of descriptors.

Conclusions MLR analysis was used to model benzodiazepine mouse lethal oral toxicity. Specific benzodiazepine structural features influencing the toxicity were found. The benzodiazepine electron donor/acceptor ability increased the drug toxicity. Increased local soft reactivity within the molecule, molecular electron-attraction tendency and susceptibility of benzodiazepine molecules to electrophilic attack decreased benzodiazepine toxicity.

Rocha, G. B. et al (2006) *J. Comput. Chem.* **27**: 1101–1111
Stewart, J. J. P. (2007) *J. Mol. Model.* **13**: 1173–1213
Tetko, I. V., Tanchuk, V. Y. (2002) *J. Chem. Inf. Comput. Sci.* **42**: 1136–1145

128
Redox-activated fluorophores

S. Passmore[1], S. Freeman[1], E. Bichenkova[1], N. Rattray[1] and M. Jaffar[2]

[1]University Of Manchester, Manchester and [2]Morvus Technology, Llanarthne, Carmarthen, UK. E-mail: sarah_passmore@hotmail.com

Objectives To design, synthesize and evaluate a tripartite redox-activated fluorescent biomarker system. Redox-activated prodrugs have been developed to selectively target the physiological differences that are present in the disease state, for example wound healing and pancreatitis. Upon redox activation, the prodrug is reduced, which results in the release of the fluorophore as shown in Figure 1.

Methods A number of substituted 3,6-dioxocyclohexa-1,4-dienylpropanoic acid derivatives have been synthesized. Several derivatives with methyl groups on the quinone ring and in the side chain have been prepared to vary the kinetics of cyclization of the prodrug to release the fluorophore. The quinones have been attached to fluorescein and 7-hydroxycoumarin in the presence of dicyclohexyl-carbodiimide and 4-*N*,*N*-dimethylaminopyridine in stoichiometric ratios to produce mono- and bis-linked prodrugs. Upon attachment, the fluorescence properties of fluorescein and 7-hydroxycoumarin are quenched. Release of the fluorophore from the system by reduction of the quinone allows fluorescence to occur. The prodrugs were chemically activated (using sodium dithionite as the reductant) and release of the fluorophore was monitored by thin-layer chromatography, UV and fluorimetric methods.

Results Three mono- and three bis-linked prodrugs were synthesized and characterized by nuclear magnetic resonance and mass spectrometry. The rate of release (after activation) of the fluorophore was dependent upon the R-group substituents. Release of the fluorophore from the derivative with methyl groups at all R positions was at the highest rate. This may be due to the increased strain in the system caused by this 'trimethyl lock' phenomenon, which results in the closer proximity of the hydroxyl group on the ring to the carbonyl ester of the linked fluorophore.

Conclusions Novel mono- and bis-linked fluorophore prodrugs were successfully synthesized. Evaluation of these compounds suggests that under chemical reduction the quinone is reduced to the hydroquinone, which undergoes intramolecular cyclization to release the fluorophore. This prodrug system can be developed further to enhance its physiochemical properties and may be of potential use as a redox-activated system for wound healing and treating pancreatitis.

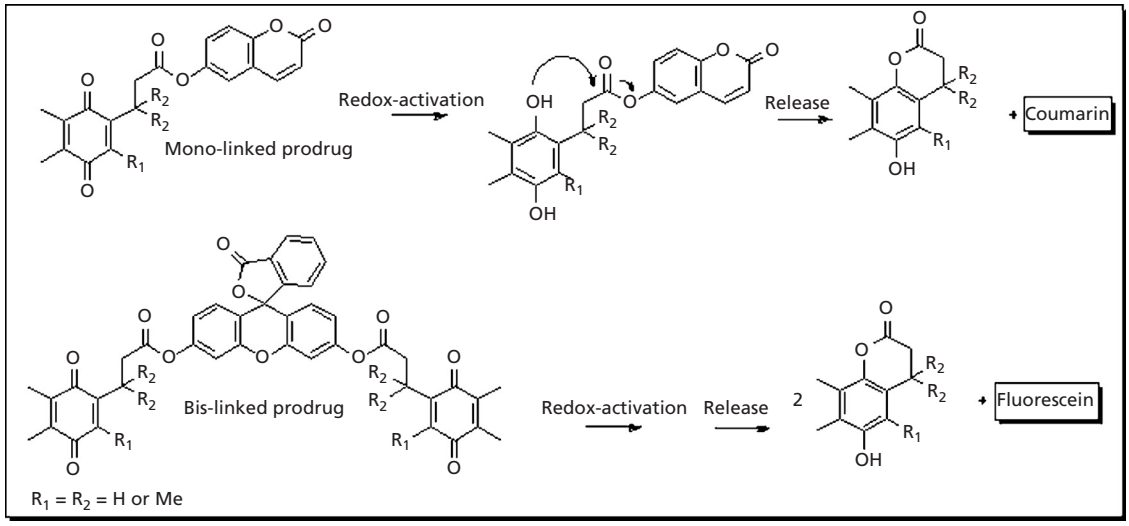

Figure 1 Proposed release of fluorophore from the quinone prodrug system.

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Novel phenylamine-based compounds as potential inhibitors of the enzyme complex 17α-hydroxylase/17,20-lyase (P450$_{17\alpha}$)

M. Akbarzadeh, P. Acharya, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK.
E-mail: s.ahmed@kingston.ac.uk

Objectives In the treatment of androgen-dependent prostate cancer, extensive research has been undertaken to produce compounds that are potent inhibitors of the P450 enzyme complex 17α-hydroxylase/17,20-lyase (P450$_{17\alpha}$), which is responsible for the conversion of C_{21} steroids to C_{19} androgens. The overall conversion involves the initial 17α-hydroxylation (involving the 17α-hydroxylase (17α-OHase) component) of progesterone or pregnenolone, followed by the cleavage of the C-17–C-20 bond (involving the 17,20-lyase (lyase) component) to produce the appropriate androgen. We have reported previously the substrate–haem complex approach for P450$_{17\alpha}$, which is an approximate representation of the essential components in the active site of P450$_{17\alpha}$. In an effort to design potent and, in particular, specific inhibitors of P450$_{17\alpha}$, we undertook the design of compounds based upon *N*-alkyl-4-methyl-5-(4-aminophenyl)-2-oxazolidinone. We hypothesized that the oxazolidinone moiety would undergo hydrogen-bonding interactions while the phenylamine moiety would ligate to the iron of the haem system. Here we report the initial results of our study into the synthesis, evaluation and molecular modelling of novel compounds against P450$_{17\alpha}$. The compounds were also evaluated against aromatase in an effort to determine their specificity.

Methods The target compounds were synthesized through initial N-alkylation of the oxazolidinone moiety using the appropriate alkyl bromide and suitable base (e.g. sodium hydride, NaH) in anhydrous dimethyl formamide (DMF). The N-alkylated compound was nitrated, followed by reduction of the nitro group using Pd/C and hydrogen gas atmosphere. Biochemical evaluation of the compounds was undertaken using a literature assay procedure with rat testicular homogenate for both P450$_{17\alpha}$ and aromatase activity (Owen et al 2006).

Results The use of NaH in anhydrous DMF provided us with a range of alkylated compounds in high yields (ranging from 60 to 80%). The nitration method involved the use of (5 M) nitric acid at 0°C: it was found that in the synthesis of the heptyl, octyl, nonyl and decyl derivatives the time of reaction needed to be increased to obtain high yields. The nitro compounds were then reduced to give the target amino compounds in good yield. The synthesized compounds were screened for inhibitory activity using a standard literature method, and using ketoconazole (KTZ) as the standard. From the results of the initial screening against P450$_{17\alpha}$, we observe that the majority of novel inhibitors possessed poor inhibitory activity in comparison with KTZ (found to possess 62 and 79% inhibitory activity against 17α-OHase and lyase respectively). The most potent compound was the N-pentyl derivative, which showed 55 and 70% inhibitory activity against 17α-OHase and lyase respectively under similar conditions. Against aromatase, the compounds were also found to be poor inhibitors in comparison to the standard compound, aminoglutethimide. The weak inhibitory activity is postulated to be due to the weak ligating property of the phenylamine moiety as opposed to the azole functionality within KTZ.

Conclusions The compounds have proven to be weak inhibitors of P450$_{17\alpha}$; however, they have provided us with some insight into the design of specific inhibitors of P450$_{17\alpha}$.

Owen, C. P. et al (2006) *Bioorg. Med. Chem. Lett.* **16**: 4011–4015

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Synthesis and biological evaluation of a series of thiosemicarbazone-based compounds as potential inhibitors of oestrone sulphatase

H. Bordbar, T. Cartledge, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK.
E-mail: s.ahmed@kingston.ac.uk

Objectives The enzyme oestrone sulphatase (ES) is a therapeutic target in the treatment of hormone-dependent breast cancer. We have investigated a number of physico-chemical properties which we considered to be important in the design of potent irreversible sulphamate-based inhibitors of ES (Ahmed et al 2000, 2001). Recently, a series of thiosemicarbazone-based compounds has been reported as potent inhibitors of this enzyme (Jütten et al 2002). However, the thiosemicarbazone-based inhibitors were found to possess allosteric inhibition against ES and no structure–activity relationship (SAR) for these compounds has been fully rationalized. We report here the initial results of our study into the synthesis and biochemical evaluation of a series of thiosemicarbazone-based compounds as potential inhibitors of ES and thereby attempt to provide an insight into the SAR for these compounds.

Methods In the synthesis of the target compounds, we undertook a reaction between a carbonyl moiety (e.g. benzaldehyde) and cyclohexylthiosemicarbazone in ethanol. The mixture was left to reflux for 24 hours and allowed to cool to room temperature. The resulting solid was filtered and re-crystallized from aqueous ethanol to give the target compounds. In the biochemical evaluation we used a modified literature procedure (Ahmed et al 2001), with the modification being the use of rat liver microsomal enzyme.

Results The target compounds were obtained in relatively good yield (ranging from 50 to 90%) and without any major problems where benzaldehyde was the carbonyl-containing reactant. However, the use of ketones (e.g. acetophenone) did not yield any product. Through the use of harsher conditions the target compounds were obtained; however, the yields were relatively smaller (ranging from 15 to 40% when acetophenone was reacted with cyclohexylthiosemicarbazone). In general, the thiosemicarbazone-based compounds proved to be weaker inhibitors than the standard used, namely EMATE (which was found to possess 99% inhibition at an inhibitor concentration of 50 μM). The most potent compound was found to be where the starting benzophenone-containing reactant possessed a polar group *ortho* on the phenyl ring of the target compound (possessing 65% inhibition under similar conditions). With regards to SAR, from an initial consideration of the initial screening data, it would appear that the meta-substitution of the phenyl ring within the target compounds results in an initial decrease in inhibitory activity: this is presumably due to steric factors since the presence of any functionality at this position results in a decrease in inhibitory activity. *Ortho*- and *para*-substitution did not appear to have a major impact on the inhibitory activity.

Conclusions We have provided some novel ES inhibitors with limited SAR; however, these compounds have allowed us to consider some aspects of the structural features so as to allow us to design further novel inhibitors of ES.

Ahmed, S. et al (2000) *Biochem. Biophys. Res. Commun.* **272**: 583–585
Ahmed, S. et al (2001) *Bioorg. Med. Chem. Lett.* **11**: 899–902
Jütten, P. et al (2002) *Bioorg. Med. Chem. Lett.* **12**: 1339–1342

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Use of the transition-state of the reaction catalysed by 5α -reductase (5AR) in the modelling of known inhibitors of 5AR: an insight into the conformational space for the side chains of steroidal inhibitors of 5AR

O. Hassan, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK.
E-mail: s.ahmed@kingston.ac.uk

Objective The enzyme 5α-reductase (5AR) is responsible for the conversion of testosterone to dihydrotestosterone and has been the major therapeutic target in the treatment of benign prostate hyperplasia. We have previously reported the construction of the transition state of the reaction catalysed by 5AR as an approximate representation of its active site (Ahmed and Denison 1998). 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. The role of the C-20 area of the steroid backbone remains unclear; however, several workers have presumed the existence of a binding group at the active site which would bind any hydrogen-bonding groups in this area. It is our hypothesis that it is the hydrophobicity of functional groups about the C-20 area of the steroidal inhibitors that has resulted in their potent inhibitory activity and not the polar–polar interaction, as hypothesized by other workers. 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. Here we report the results of an initial investigation into our hypothesis involving the analysis 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 inhibitors with biological activity.

Methods The construction of the transition state has been described previously (Ahmed and Denison 1998) and will therefore not be discussed here. The construction, minimization and conformational analysis of the steroidal inhibitors (in particular, the groups about the C-20 area of the steroid backbone) were undertaken in the molecular modelling software CaChe whereas logP and other physico-chemical parameters were determined using the procedures available in Project Leader software.

Results From the results of our investigation into the properties of the side chain, we observed that there is a correlation between the inhibitory activity and physico-chemical property of the inhibitors. For example, we observed an increase in potency with logP of the side chain. The conformational analysis provides further support to our hypothesis. That is, when the conformations of the inhibitors' side chain are considered, we discovered that a large number of conformers are possible. Furthermore, these conformers would be expected to be involved in steric interactions (as a result of the rotation of the single bonds) with potential hydrogen-bonding group (s) about the area of the active site corresponding to the C-20 of the steroid backbone.

Conclusions In conclusion, the above data support our initial hypothesis that the increased inhibitory activity is a result of the hydrophobic nature of the

inhibitor's side chain as opposed to any interaction between the inhibitor and the active site corresponding to the C-20 area.

Ahmed, S., Denison, S. (1998) *Bioorg. Med. Chem. Lett.* **8**: 2615–2620

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Novel inhibitors of oestrone sulphatase based on the derivatives of 4-aminophenol

A. Whaeed, T. Cartledge, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK.
E-mail: s.ahmed@kingston.ac.uk

Objectives We have previously reported the design and synthesis of a number of compounds against the enzyme oestrone sulphatase (ES) and have suggested a number of physico-chemical properties that we considered to be important in the design of potent irreversible sulphamate-based inhibitors of ES (Ahmed et al 2000, 2001). However, the compounds synthesized were found to be highly unstable and were prone to rapid degradation. In an effort to improve the stability, we have investigated a number of derivatives of benzamides, which, although they were found to be weak inhibitors, were more stable than the ester-based compounds previously reported by us. We report here the initial results of our study into the synthesis and biochemical evaluation of a series of sulphamated derivatives of 4-aminophenol as potential inhibitors of ES.

Methods In the synthesis of the target compounds, we followed literature procedure (Ahmed et al 2002). That is, following the derivatization of the amino functionality involving the reaction between 4-aminophenol and an anhydride, the resulting benzamide compound was aminosulphonated using aminosulphonyl chloride. In the biochemical evaluation, we used a modified literature procedure (Ahmed et al 2001), the modification being the use of rat liver microsomal enzyme.

Results The target compounds were obtained in relatively moderate yield (ranging from 40 to 60%) without any major problems. In general, the potential inhibitors proved to be weaker inhibitors than the standards used, namely EMATE (which was found to possess 99% inhibition at an inhibitor concentration of 50 μM) and 667-COUMATE (which was also found to possess 99% inhibition under similar conditions). The most potent compound was found to be the sulphamic acid 4-butyrylamino-phenyl ester (possessing 55% inhibition under similar conditions). With regards to the structure–activity relationship, from an initial consideration of the initial screening data, it would appear that, as previously shown by us, the logP of the inhibitor (and in particular, the carbon backbone) is an important physico-chemical factor in the inhibition of ES by the compounds under consideration. The compounds also proved to be somewhat more stable (chemically) than the previously reported benzoic acid esters which were highly potent inhibitors. In particular, the amide moiety was found to be resistant to degradation: it should be noted that the sulphamate moiety has always proved to be unstable and would appear to be an important characteristic for the compounds to possess inhibition.

Conclusions We have provided some novel ES inhibitors with limited stability so as to allow us to design further novel, and more potent, inhibitors of ES.

Ahmed, S. et al (2000) *Biochem. Biophys. Res. Commun.* **272**: 583–585
Ahmed, S. et al (2001) *Bioorg. Med. Chem. Lett.* **11**: 899–902
Ahmed, S. et al (2002) *Bioorg. Med. Chem. Lett*. **12**: 2391–2394

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Novel inhibitors of the enzyme complex 17α-hydroxylase (17α-OHase) and 17,20-lyase based on a phenyl acyl azole backbone

W.-Y. Lee, I. Shahid, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK.
E-mail: s.ahmed@kingston.ac.uk

Objectives Conversion of pregnanes to androgens is catalysed by the enzyme complex 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$). The overall process involves 17α-hydroxylation of the C-17 position of the steroid backbone (involving the 17α-hydroxylase (17α-OHase) component) followed by the cleavage of the C-17–C-20 bond (involving the 17,20-lyase (lyase) component). We have previously reported the substrate–haem complex (SHC) approach for $P450_{17\alpha}$, which allowed the production of an approximate model for the active site of $P450_{17\alpha}$ since the crystal structure is currently unavailable (Ahmed 1999). Using the SHC approach, we undertook the design of compounds based upon the phenyl acyl backbone. We hypothesized that the C=O moiety would utilize potential hydrogen-bonding interactions, thereby increasing inhibitory activity, a characteristic that was lacking in the previously synthesized compounds (Owen et al 2006, Patel et al 2006). Here, we report the synthesis and evaluation (against the two components of $P450_{17\alpha}$) of a range of phenyl acyl imidazole-based inhibitors.

Methods The target compounds were synthesized through the N-alkylation of the azole functionality involving the use of the appropriate derivatives of 2-bromo-1-phenyl-ethanone, a suitable base and anhydrous tetrahydrofuran as solvent. Biochemical evaluation of the compounds was undertaken using literature assay procedure (Owen et al 2006).

Results The reactions in the synthesis of the target compounds proceeded in good yield (ranging from 50 to 80%) and no major problems were encountered. The biochemical evaluation shows that the compounds were, in general, weaker inhibitors than the standard compound, namely ketoconazole (KTZ) (found to possess 62 and 79% inhibitory activity against 17α-OHase and lyase respectively), although a few were found to be equipotent to KTZ. The most potent was 4-bromophenyl acyl imidazole, which was found to possess 55 and 70% inhibitory activity against 17α-OHase and lyase respectively. The compounds were also found to possess slightly greater inhibitory activity against the lyase component than the 17α-OHase: this is a useful feature as it suggests that these compounds would lack major side effects associated with the inhibition of corticosteroid biosynthesis. The inhibitory activity observed has been rationalized using molecular modelling and suggests that interaction between the substituent on the phenyl moiety and the enzyme active site of $P450_{17\alpha}$ results in increased potency and not the C=O moiety: the carbonyl functionality was positioned such that any groups interacting with this group would undergo steric interaction with the haem, thereby lowering the inhibitory activity.

Conclusions The compounds synthesized in the present study are therefore good lead compounds in the design of further novel and specific inhibitors of $P450_{17\alpha}$.

Ahmed, S. (1999) *Bioorg. Med. Chem.* **7**: 1487–1496
Owen, C. P. et al (2006) *Bioorg. Med. Chem. Lett*. **16**: 4011–4015
Patel, C. H. et al (2006) *Bioorg. Med. Chem. Lett.* **16**: 4752–4756

Drug Delivery

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Development of oral sustained-release formulation of isoniazid/alginate/chitosan-blend microspheres for intestinal delivery

A. Samad, Y. Sultana, R. K. Khar, M. Aqil and A. K. Misha

Jamia Hamdard, New Delhi, India. E-mail: samadpharma@gmail.com

Objectives The objective of this work was the development of an oral sustained-release formulation of alginate/chitosan blend microspheres of isoniazid to increase the bioavailability of the drug by targeting the intestine. This would improve the absorption and reduce the toxicity of isoniazid and improve the compliance of the patient by decreasing the frequency of dosing.

Methods Microspheres were prepared by water in the oil-emulsification method. The concentration of polymer and crosslinker as well as crosslinking time were varied to note the effects on microsphere characteristics. The shape and surface characteristics were determined by scanning electron microscopy using a gold-sputter technique (Leo435P microscope). Particle sizes of both placebo- and drug-loaded formulations were measured using scanning electron microscopy and the particle-size distribution was determined using an optical microscope. The entrapment was measured using UV methods. (Drug-loaded microspheres were sonicated for 1 hour in simulated intestinal fluid, pH 7.4, to lyse the particles. The extent of drug loading was determined by measuring the absorbance.) The bioadhesive potential of the microspheres was measured in the rat intestine using 50 mg of the microspheres. The adhered microspheres were allowed to hydrate for 20 minutes and dried. After 20 minutes the adhered microspheres were separated and weighed. Percentage bioadhesion was then calculated. The physical state of the drug in the formulation was determined by differential scanning calorimetry (DSC). The release profiles of isoniazid from microspheres were examined in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). γ-Scintigraphic studies were carried out to determine the location of ^{99m}Tc-labelled microspheres, following oral administration to New Zealand white rabbits, and the extent of transit through the gastrointestinal tract.

Results The microspheres had a smooth surface and were found to be discreet and spherical in shape. Heterogeneous size distribution was found with an average diameter of 3.849 μm. Results indicated that the mean particle size of the microspheres increased with an increase in polymer and crosslinker concentration as well as the crosslinking time. The entrapment efficiency was found to be in the range of 52–94% w/w. Concentration of the crosslinker up to 7.5% caused an increase in the entrapment efficiency and the extent of drug release. Optimized isoniazid/alginate/chitosan-blend microspheres were found to possess good