Chemistry

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The synthesis of a series of amides based upon 4-hydroxybenzoic acid in the development of potential inhibitors of the enzyme oestrone sulphatase

A. Waheed, K. Aidoo-Gyamfi, C. Owen and S. Ahmed

Kingston University, School of Pharmacy and Chemistry, Dept of Pharmacy, Penryn Road, Kingston upon Thames, Surrey, KT1 2EE, UK. E-mail: s.ahmed@kingston.ac.uk

Objective The production of oestrone in postmenopausal women has been shown to be catalysed by the enzyme oestrone sulphatase (ES). We have previously reported the synthesis of a number of 4-hydroxybenzoic acid based inhibitors (Ahmed et al 2001), in particular, a series of alkyl and cycloalkyl esters of 4-sulphamoylated benzoic acid, one of which has proved to be a highly potent inhibitor of ES (Patel et al 2004) and which was found to be more potent than 667-COUMATE. However, our ester-based compounds were found to have poor stability, in particular, the ester moiety was found to readily undergo hydrolysis; here, we report the results of the synthesis of a series of mono- and di-*N*-alkylated compounds based upon sulphamic acid 4-carbamoyl-phenyl ester; in particular, we report the attempted synthesis using a 'one-pot' approach.

Method In the synthesis of the target compounds, we initially considered a 'one-pot' synthesis of the *N*-alkyl derivatives of 4-hydroxy benzamide. In general, the procedure involved the reaction between 4-hydroxybenzoic acid with acetic anhydride in anhydrous toluene and the mixture refluxed for 7 h. The solvent was removed under vacuum and the appropriate amine, dissolved in anhydrous toluene, was then added to the resulting oil and the mixture refluxed for 16 h. After cooling, the organic layer was washed with dilute hydrochloric acid (HCl, 1 M), water (2×20 mL) and dried over anhydrous magnesium sulphate to give the target compound. The 4-hydroxy moiety was converted to the sulphamate involving a reaction with sulphamoyl chloride (formed *in situ* from the reaction between formic acid and chlorosulphonyl isocyanate) using dimethyl acetamide (DMA) as the solvent in the absence of a base (Okada et al 2000).

Results Our initial attempt at the 'one-pot' synthesis of the *N*-alkyl derivatives of 4-hydroxy benzamide was based on the initial activation of the carbonyl moiety and the protection of the 4-hydroxy functional group involving the reaction of the 4-hydroxybenzoic acid with acetic anhydride. The expected intermediate (the mixed anhydride as well as the 4-acetoxy functionality) would then be reacted with the appropriate amine to give the target compound. However, we discovered that the mixed anhydride did not yield the amide in sufficient quantity, as such, we synthesised the 4-acetoxybenzoic acid which was converted to the acyl chloride prior to conversion to the appropriate 4-hydroxy benzamide.

Conclusion The initial one-pot synthetic route did not result in the synthesis of the target compounds, presumably due to the insufficient activation of the C=O moiety within the mixed anhydride. However, the target amides were synthesised using the similar route, albeit via the acyl chloride.

Ahmed, S., et al (2001) *Bioorg. Med. Chem. Lett*. **11**: 3001–3006
Okada, M., et al (2000) *Tet. Lett*. **41**: 7047–7051
Patel, C. K., et al (2004) *Bioorg. Med. Chem. Lett*. **14**: 605–611

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Synthesis and biochemical evaluation of brominated derivatives of alkyl and cycloalkyl esters of 4-sulphamoylated benzoic acid

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

Kingston University, School of Pharmacy and Chemistry, Dept of Pharmacy, Penryn Road, Kingston upon Thames, Surrey, KT1 2EE, UK. E-mail: s.ahmed@kingston.ac.uk

Objective We have previously reported a series of compounds based on esters of 4-[(aminosulphonyl)oxy]benzoate, which were found to be good inhibitors of oestrone sulphatase (ES) (Ahmed et al 2001), but not as potent as 667-COUMATE. We initiated a series of studies where we attempted to increase the potency of our initial esters, and recently we reported cyclooctyl 4-[(aminosulphonyl)oxy]benzoate as being a potent inhibitor of ES with greater inhibitory activity than 667-COUMATE (Patel et al 2004). We have previously reported that the incorporation of groups into the phenyl ring system within the sulphamate-based compounds results in a change in potency. Here, we report the results of our efforts to further increase the potency of our compounds, and therefore report: the synthesis of a number of brominated derivatives of esters of 4-sulphamated benzoic acid; their *in vitro* biochemical evaluation; and the rationalisation of their inhibitory activity.

Method The initial synthesis of the alkyl and cycloalkyl esters of 4-hydroxybenzoic acid was undertaken successfully using modified literature procedure (Ahmed et al 2001). In the synthesis of the target compounds, the synthesised esters were initially brominated followed by aminosulphonation to give the desired compounds. The synthesised compounds were then evaluated (initial screening and IC_{50} values) for ES inhibition using standard literature methods. In an effort to compare previously reported inhibitors to the brominated derivatives, a series of straight chain containing esters of 4-hydroxybenzoic acid was also synthesised, as were two of the potent cycloalkyl based inhibitors. EMATE, COUMATE and 667-COUMATE were used as standard compounds within the assay.

Results In the synthesis of the initial esters of 4-hydroxybenzoic acid, modified literature procedure was followed and was found to proceed in good yield without any major problems (yield ranging from 50% to 80%). The synthesis of the target compounds involved the initial synthesis of the brominated derivative followed by the sulphonation of the 4-hydroxy moiety. Both steps proved to be troublesome, with the aminosulphonation step resulting in very low yield for the final target compounds, presumably due to the steric hindrance caused by the two bromine atoms which are ortho to the 4-hydroxy functionality. Consideration of the initial screening data obtained for the final compounds shows that the brominated compounds are, in general, weaker inhibitors of ES than the non-brominated derivatives, e.g. the octyl ester possessed 76% inhibition whilst the dibromo derivative was found to possess 34% inhibition under similar conditions. This would therefore appear to contradict our previous reports. However, a detailed analysis of the brominated compounds showed that these had undergone degradation. That is, investigation of the ester moiety showed that the ester functionality had undergone some hydrolysis, thereby reducing overall inhibitory activity.

Conclusion In conclusion, the brominated compounds possess good inhibitory activity; however, they were not as potent as expected due to the ester moiety having undergone hydrolysis.

Ahmed, S., et al (2001) *Bioorg. Med. Chem. Lett*. **11**: 2525–2529
Patel, C. K., et al (2004) *Bioorg. Med. Chem. Lett*. **14**: 605–609

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Synthesis and biochemical evaluation of a series of aryl ketones as potent and specific inhibitors of type 3 isozyme of 17β-hydroxysteroid dehydrogenase (17β-HSD)

M. S. Olusanjo, R. Lota, C. P. Owen and S. Ahmed

Kingston University, School of Pharmacy and Chemistry, Dept of Pharmacy, Penryn Road, Kingston upon Thames, Surrey, KT1 2EE, UK. E-mail: s.ahmed@kingston.ac.uk

Objectives In the fight against hormone-dependent prostate cancer, isozymes of 17β-hydroxysteroid dehydrogenase (17β-HSD), in particular type 3 (17β-HSD3), have been considered as a potential biochemical target. In an effort to design novel inhibitors of this enzyme, we concluded that since the crystal structure of 17β-HSD3 is not available, the derivation of the transition-states (TS) of the reduction reaction (based on the previously published mechanism of Penning (1997)) may aid the drug design process. Here, we report the initial results of our molecular modelling study and the subsequent

synthesis (of a range of compounds based on the 4-hydroxyphenyl ketone backbone) and biochemical evaluation (using radiolabelled androstenedione (AD) (for 17β-HSD3 activity) and oestrone (for 17β-HSD1 activity)) of the synthesised compounds.

Method The structures of AD, NADPH (partial structure was used due to a limitation of the software) were all constructed within the CACHE molecular modelling software suite on an Intel microprocessor based IBM PC compatible microcomputer. Using atoms, available fragments and amino acids (presumed to be present at the active site) were also constructed and refined using PM3 parameters. To determine the TS, the various groups involved in the reduction reaction were also connected to each other and the structure. A 'product file' was also created and the structures minimised as previously described. The saddle point for the reaction was calculated and the resulting TS refined using a minimise gradient calculation. In the design of the potential inhibitors, compounds were superimposed onto the TS so as to mimic the steroid C(17)=O moiety and the 'degree of superimposition' evaluated. In the synthesis of the 4-hydroxyphenyl ketone based inhibitors, Friedel-Crafts acylation of phenol was undertaken using a range of acid chlorides from acetyl chloride to dodecanoyl chloride, as well as some cyclic acyl chlorides. The synthesised compounds were then evaluated for inhibitory activity against both 17β-HSD1 and 17β-HSD3 using modified literature procedures.

Results The reactions, in general, proceeded in good yield (typically 60%) and without any major problems. Consideration of the initial screening data shows that the compounds based on the 4-hydroxyphenyl ketone are good and specific inhibitors of 17β-HSD3, e.g. 4-hydroxynonaphenone (1) was found to be the most potent inhibitor possessing an inhibitory activity of ~84% inhibition at [I] = 100 μM. In comparison, compound 1 was found to possess ~36% inhibition against 17β-HSD1 under similar conditions. Consideration of these compounds against other enzymes within the HSD family of enzymes, in particular, 3β-HSD, showed that the compounds are specific inhibitors of 17β-HSD3, e.g. 1 was found to possess <10% inhibitory activity against 3β-HSD.

Conclusion The results of our study (in particular the observed inhibitory activity) have added further support to our initial model of the 17β-HSD3 active site [the use of a carbonyl moiety to mimic the C(17) = O group within the natural substrate] and to the derived TS. These compounds are therefore good lead compounds in the search for more potent inhibitors of 17β-HSD3.

Le Lain, R., et al (1999) *J. Pharm. Pharmacol*. **51** (Suppl.): 23
Penning, T. M. (1997) *Endocrine Rev*. **18**: 281–305

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Synthesis of a series of esters of oestrone and dehydroepiandrosterone to map the area of the active site of the type 3 isozyme of 17β-hydroxysteroid dehydrogenase corresponding to the C(3) area of the steroid backbone

F. Masri, M. S. Olusanjo, C. P. Owen and S. Ahmed

Kingston University, School of Pharmacy and Chemistry, Dept of Pharmacy, Penryn Road, Kingston upon Thames, Surrey, KT1 2EE, UK. E-mail: s.ahmed@kingston.ac.uk

Objective The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) is a biochemical target in the fight against hormone-dependent cancers. The type 3 isozyme (17β-HSD3) is responsible for the conversion of weak androgens (e.g. androstenedione (AD)) into the more potent steroids (e.g. testosterone) involving the reduction of the C(17) = O group to the C(17)-βOH moiety. Here, we report the initial results of a molecular modelling study where we have attempted to map the area about the active site corresponding to the C(3) of the substrate. As such, we have undertaken the synthesis of a series of esters of oestrone (E1) and dehydroepiandrosterone (DHEA). The compounds were evaluated using rat testicular microsomal enzyme, using radiolabelled androstenedione.

Method In the synthesis of the esters, the starting steroids were reacted with the appropriate acyl chloride and the reactions proceeded in good yield (ranging from 50% to 70%) and without any major problems. In the molecular modelling study, we undertook the derivation of the transition-states (TS) of the reaction catalysed by 17β-HSD3 based on the previously published mechanism of Penning (1997) (since the crystal structure of the type 3 isozyme is not available). The structures of AD, the synthesised compounds, and the partial structure of NADPH (due to software limitation) were all constructed within the CACHE molecular modelling software. Amino acids presumed to be present at the active site were also constructed. To determine the TS, the various groups involved in the reduction reaction were also connected together and the structures minimised using a conjugate gradient calculation – a 'reactant' and a 'product' file were produced. The saddle point for the reaction was calculated from the two files and the resulting TS refined. In the modelling process, the low energy conformers were determined and superimposed onto the steroid backbone.

Results Consideration of the initial screening data shows that the compounds based on the corresponding esters of E1 are in general weaker than the esters based on DHEA, for example, the pentyl ester of E1 was found to possess 38% inhibitory activity whilst the pentyl ester of DHEA was found to possess 58% inhibitory activity. In comparison to our previously published compounds based on 4-hydroxyphenyl ketone, all the ester based compounds are weak inhibitors of 17β-HSD3. However, consideration of the structure-activity relationship suggests that there is very little conformational space about the area of the active site corresponding to the C(3) area of the steroid backbone. That is, with an increase in the alkyl chain length, there is a decrease in the inhibitory activity; as such, the pentyl esters of both E1 and DHEA possess weaker inhibitory activity than the acetyl derivatives.

Conclusion In summary, although the compounds within the current study have shown poor inhibitory activity, the results suggest that the conformational space about the area of the active site corresponding to the C(3) of the steroid backbone is restricted. These compounds are therefore good lead compounds in the search for more potent and specific inhibitors of 17β-HSD3.

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

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

W.-Y. Lee, H. Abdulkhadir, C. Owen and S. Ahmed

Kingston University, School of Pharmacy and Chemistry, Department of Pharmacy, Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE, UK.
E-mail: s.ahmed@kingston.ac.uk

Objective Extensive research has been undertaken to produce compounds that are both potent and selective inhibitors of the enzyme 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$). Compounds containing an azole group have shown potent inhibitory activity against this enzyme involving co-ordinate bond formation with the haem at the active site. Here, we report the synthesis and screening of a range of oxazolidinone-based compounds which use a phenylamine moiety as the Fe-ligating group. Furthermore, in an effort to add further support to the substrate-haem complex (SHC) approach developed by us (and the methodology used in the design of these compounds) (Ahmed & Davis 1995) we have attempted to use the target compounds as probes of the active site of this enzyme.

Method The use of the oxazolidinone-based chiral auxiliaries has involved the synthesis of the *N*-acyl derivatives, whereas *N*-Alkylation has been some what ignored. In general, the synthesis of the *N*-acyl derivatives has been undertaken using strong bases such as LDA; in our hands, the use of LDA in the alkylation reaction proved to be unsuccessful. The use of sodium hydride (NaH) using anhydrous *N,N*-dimethylformamide (DMF) as the solvent provided us with the desired range of *N*-alkylated compounds in high yield (typically 40–70%). In the synthesis of the phenylamine derivatives, we considered the nitration of the phenyl ring system, followed by the subsequent reduction of the nitro group to the desired amine functionality. The reaction was undertaken using nitric acid (5 M) in dichloromethane (DCM) at room temperature—the reaction proceeded without any major problems (yield 65%). The target phenylamine-based compounds were obtained through the use of hydrogen gas and palladium on activated charcoal (yield 80%). The synthesised compounds were screened for inhibitory activity using the standard literature method using ketoconazole (KTZ) as the standard inhibitor (Li et al 1996).

Results From the results of the initial screening against P-450$_{17\alpha}$, we observe that the novel inhibitors possess poor inhibitory activity and are weaker than the standard compound, KTZ (90% inhibitory activity at [KTZ] = 10 μM), the most potent compound being the *N*-heptyl derivative which was observed to possess some 60% inhibition at [I] = 50 μM. Comparison of the biochemical evaluation data shows that the inhibitory activity appears to be related to the alkyl chain length. Modelling of these compounds using the SHC approach suggests that within the P-450$_{17\alpha}$ active site, hydrogen bonding interaction between the active site (corresponding to the C(3) area of the steroid backbone) and the C = O group within the oxazolidinone moiety is not possible. Furthermore, steric interaction between the inhibitor and the enzyme active site is possible, and may be a factor in the poor inhibitory activity observed within the compounds under study.

Conclusion We have provided a new series of lead compounds which may allow for the specific inhibition of P-450$_{17\alpha}$ as a result of the phenylamine moiety binding poorly to the haem.

Ahmed, S., Davis, P. J. (1995) *Bioorg. Med. Chem. Lett*. **5**: 1673–1678
Li, J. S., et al (1996) *J. Med. Chem*. **39**: 4335–4339

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Design, synthesis and biological evaluation of chalcone derivatives possessing a methanesulfonamido pharmacophore as selective COX-2 inhibitors

T. Zebardast and A. Zarghi

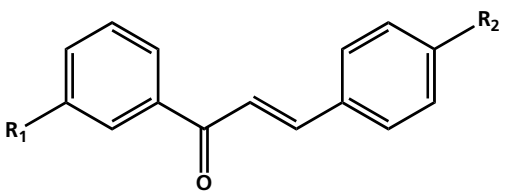
Shaheed Beheshti University of Medical Sciences, School of Phamacy, #105, Shams Alley, Valiasr Street, Iran. E-mail: tannazzebardast@yahoo.com

Objectives The clinical use of traditional nonsteroidal anti-inflammatory drugs (NSAIDS) such as aspirin for the treatment of inflammation and pain is often accompanied by adverse gastrointestinal side effects. Their anti-inflammatory activity is due to inhibition of cyclooxygenases (COXs), which catalyze the bioconversion of arachidonic acid to inflammatory prostaglandins (PGs). PGs that are produced via the inducible COX-2 isozyme are responsible for inflammation, pain and fever, whereas the constitutively expressed COX-1 isozyme produces PGs that exhibit beneficial cytoprotective properties. The recent withdrawal of diaryl heterocyclic selective COX-2 inhibitors such as rofecoxib and valdecoxib due to their cardiovascular side effects clearly delineates the need to explore and evaluate new structural ring templates (scaffolds) possessing COX-2 inhibitory activity.

Methods Docking experiments were performed using insight II Software Version 2000. A one-step Claisen-Schmidt condensation was used to prepare the target 1, 3-diarylprop-2-en-1-ones in which a methanesulfonamido substituent was attached to the meta position of C-1 phenyl ring. The sodium hydroxide catalyzed condensation of a corresponding acetophenone with a para-substituted benzaldehyde afforded the 1, 3-diarylprop-2-en-1-ones (Figure 1) in moderate to high yield.

Results Docking studies of these compounds showed that the designed molecules were well attached into the active site of COX-2 enzyme. COX-1 and COX-2 inhibition studies showed that all compounds were selective COX-2 inhibitors since no inhibition of COX-1 was observed at a concentration of 100 μM. The order of COX-2 potency, and COX-2 selectivity index, for the C-1 meta-methanesulfonamidophenyl compounds, with respect to the nature of the C-3 phenyl substituent was Me > F > H > OMe.

Conclusions This study indicates that (i) a new class of acyclic (*E*)-1,3-diphenylprop-2-en-1-ones could be prepared via a simple one-step stereoselective Claisen-Schmidt condensation, (ii) the propenone moiety is a suitable scaffold (template) to design COX-2 inhibitors, (iii) in this chalcone class of compounds, the meta-MeSO2NH moiety has been proved to be a suitable COX-2 pharmacophore, and that COX-2 inhibition is sensitive to the nature of the C-3 phenyl substituents.



A, R1 = NHSO2Me, R2 = H
B, R1 = NHSO2Me, R2 = Me
C, R1 = NHSO2Me, R2 = F
D, R1 = NHSO2Me,R2 = OMe

Figure 1 Structure of the 1, 3-diarylprop-2-en-1-ones.

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The role of physicochemical properties in determining the overall inhibitory activity of sulphamate based compounds against the enzyme oestrone sulphatase (ES)

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

Kingston University, School of Pharmacy and Chemistry, Department of Pharmacy, Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE, UK.
E-mail: s.ahmed@kingston.ac.uk

Objectives The synthesis of sulphamated compounds involve the reaction between the phenol derivative and aminosulphonyl chloride. The synthesis is often aided through the use of a base, e.g. sodium hydride (NaH). However, the use of NaH with dimethyl formamide (DMF) as the solvent results in the formation of the so-called 'DMF-adduct' Okada et al (2000) developed the use of dimethyl acetamide (DMA) in the absence of base. We have utilised this route to good effect and have synthesised numerous highly potent compounds. However, when we attempted to synthesise a range of mono- and di-halogenated derivatives of esters of 4-hydroxybenzoic acid, the synthesis of the sulphamoylated product proved to be difficult – an initial investigation suggested that the acidity of the phenol may be one factor. Here, we report the results of our study in an effort to rationalise (using molecular modelling and pKa determination) the lack of any sulphamoylated product involving the determination of the acidity of the phenolic moiety.

Method The pKa values of the parent phenols were evaluated using a standard literature method (Harwood & Moody 1989) involving the change in UV absorption under: acidic; buffer; and basic conditions. Approximately 3–4 mg of the compound was added to 100 ml of borax buffer and the UV spectra determined (between 350 nm and 250 nm). The absorbance value was adjusted to approximately 1 involving the addition of buffer or the compound. The solution was filtered and 20 ml of the stock solution made up to 25 ml with either: HCl (2 M); borax buffer; or NaOH (2 M). The UV spectrum of each solution was determined and using the absorption values at a single wavelength, the mole fraction was calculated. The pKa was then calculated.

Results From the pKa determination, we discovered that the presence of the bromine atoms on the phenolic moiety resulted in a decrease in pKa, e.g. the pKa values of the ethyl esters of 4-hydroxy benzoate, 3-bromo-4-hydroxybenzoate and 3,5-bromo-4-hydroxybenzoate were found to be 9.3, 8.9 and 8.5, respectively. The charge density calculations showed that the insertion of the halogen functionality resulted in a decrease in the charge on the oxygen atom of the phenoxide ion. We also discovered that the fluoro-derivative possessed the highest pKa value within the halogen derivatives. The observation that the charge density decreased from fluorine to bromine can be rationalised in terms of the atomic volume of the halogen and therefore the effective distribution of the charge, that is, the greater the charge density on the phenoxide oxygen, the greater the stability of the S-OPh bond. Increase in the stability of the sulfamoylated derivative resulted in an increase in the yield (with a corresponding decrease in inhibitory activity).

Conclusion The results of the current study can therefore be used to allow us to determine the degree of difficulty in the synthesis of the sulphamoylated compound as well as giving us an indication of the level of inhibitory activity.

Harwood, L. M., Moody, C. J. (1989) *Exp. Org. Chem.* **7**: 16–719
Okada, M., et al (2000) *Tet. Lett.* **41**: 7047–7051

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Molecular modelling of the interactions between cysteamine and cystamine prodrugs and β cyclodextrin

D. Cairns, A. Benylles, B. McCaughan and G. Kay

The Robert Gordon University, Schoolhill, Aberdeen, UK. E-mail: d.cairns@rgu.ac.uk

Objectives As part of our programme to design and synthesise novel agents for the treatment of nephropathic cystinosis (Cairns et al 2002a; Gahl et al 2002), molecular modelling studies were undertaken to evaluate the ability of β cyclodextrin (CD) to enhance the aqueous solubility of a series of alkyl and aryl derivatives of cysteamine and cystamine.

Methods Dynamic molecular modelling was carried out using the Insight-II 2000 graphics interface and Discover 98.0 simulation software (Accelrys, Cambridge, UK) using a modification of the method of Cairns et al (2002b). The cvff91 force field was used throughout with explicit solvent effects represented by the TIP3P protocol. Models for each prodrug and β cyclodextrin were constructed (Insight-II) and partial atomic charges approximated from a single-point PM3 calculation using MOPAC. Each drug-CD complex was solvated and minimised. The optimised models were subjected to MD simulation (10 ps equilibration and 100 ps production, 1-fs timesteps) with initial atomic velocities taken from a Maxwell-Boltzmann distribution at 300 or 600 K. The time-averaged structures from 100 samples taken at 1-ps intervals were minimised. Ligand structures were sampled for conformational averaging in water by immersion in a solvent box of 25 $Å^3$ and MD subject to periodic boundary conditions. Atomic trajectories saved every 1 ps were used for conformational averaging, and these structures were finally minimised to an energy convergence criterion of 0.1 kcal mol^{-1} $Å^{-1}$. Drug-CD binding enthalpies were obtained by subtraction of the potential energy of the drug and CD from that of the complex. The computed binding energies obtained for both the 1:1 and 1:2 drug to CD models are shown in Table 1.

Conclusions These results suggest that β cyclodextrin may be a good enhancer of aqueous solubility for this series of prodrugs, and that increasing the number of CD molecules in the model yields a more favourable binding enthalpy. In addition, Prodrug 1, which is the most hydrophobic of the compounds studied (calculated partition coefficient, data not shown) displayed the most favourable binding enthalpy. Studies are currently underway in our laboratories to evaluate the effect of larger CD ring size (γ cyclodextrin) and the effects of a 'sandwich-type' model, where the drug is docked between two, or more, molecules of cyclodextrin rather than occupying the central cavity of the CD ring. Initial results suggest the binding enthalpies for this orientation are more favourable than the values quoted above.

Table 1 Computed binding energies for the 1:1 and 1:2 drug to CD models

	Prodrug 1	Prodrug 2	Prodrug 3	Prodrug 4	Prodrug 5
1:1 model	−234.6	−166.3	−14.32	−96.63	−44.54
1:2 model	−268.3	−227.2	−61.45	−119.1	−47.93

Data are expressed as binding enthalpies (kcal mol^{-1}).

Cairns, D., et al (2002a) *Pharm. J.* **269**: 615–616
Cairns, D., et al (2002b) *Bioorg. Med. Chem.* **10**: 803–807
Gahl, W. A., et al (2002) *N. Engl. J. Med.* **347**: 111–121

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Design and synthesis of nitrogen heterocyclic diacyl lipospermines for gene delivery

M. K. Soltan and I. S. Blagbrough

University of Bath, Bath BA2 7AY, UK. E-mail: prsisb@bath.ac.uk

Objectives Efficient vectors are urgently required for the delivery of poly-nucleic acids (DNA or siRNA) for a successful therapy in difficult-to-treat diseases e.g. cancer, inflammation, cystic fibrosis. Among non-viral gene delivery systems, non-liposomal cationic lipids are promising, non-toxic gene carriers (Ahmed et al 2006). Our conjugates are based on the naturally occurring tetra-amine spermine (Ahmed et al 2006). To improve the efficiency of gene delivery, we are investigating *N*-heterocyclic derivatives of novel spermine-based cationic lipid formulations. Our model for designing these diacyl lipospermine vectors for gene delivery consists of three moieties: a hydrophobic domain, linkers, and cationic head groups – in these cases nitrogen heterocycles pendant from the primary amines of spermine. We are investigating *N*-heterocyclic cationic head groups (e.g. imidazole, pyridine, and quinoline) aiming for a final amine pK_a of around 6.5 (5–7) to assist in swelling via the proton sponge effect. Here the import of protons with water molecules, and possibly with chloride counterions, leads to swelling and eventual rupture of the sub-cellular organelle, the endosome, thus helping to avoid lysosomal degradation and leading to more efficient transfection. As we control the functional groups in and around the *N*-heterocycles, we can make them more (or less) basic. We predict that this control of the pK_a will be important in facilitating endosome escape. Basic character increases across substituted *N*-heterocycles: pyridine 5.19, with 2-methyl-5.97, and 2-amino-6.82; and presumably analogously for imidazole 6.96, benzimidazole 5.53, and quinoline 4.90 (Albert & Serjeant 1971).

Methods In our synthesis we used different primary amine protecting groups e.g. phthalimide (via *N*-carbethoxyphthalimide), 2,5-dimethylpyrrole (via hexan-2,5-dione, acetonylacetone), and trifluoroacetyl (via ethyl trifluoroacetate). Then different hydrophobic domains were introduced by reacting these diprotected spermines, e.g. N^1,N^{12}-diphthalimidospermine with decanoyl, lauroyl, myristoyl, stearoyl, and oleoyl chlorides finally removing the protecting group by heating under reflux with ethanolic hydrazine hydrate to yield N^4,N^9-didecanoyl, N^4,N^9-dilauroyl, N^4,N^9-dimyristoyl, N^4,N^9-distearoyl, and N^4,N^9-dioleoyl spermines, respectively. Then reaction of these diacyl spermines with different aromatic heterocyclic aldehydes (4(5)-imidazole-carboxaldehyde, 2-pyridine-carboxaldehyde, and 2-quinoline-carboxaldehyde) and reduction of the formed imines with sodium borohydride gave (4(5)-imidazolylmethyl, 2-pyridylmethyl, and 2-quinolinylmethyl)-N^4,N^9-didecanoyl, N^4,N^9-dilauroyl, N^4,N^9-dimyristoyl, N^4,N^9-distearoyl, and N^4,N^9-dioleoyl spermines, respectively. Also, to conjugate directly to the *N*-heterocycle, we investigated aromatic nucleophilic substitution with 2-bromopyridine, 2-chlorobenzimidazole, and 2-methylthiobenzimidazole, heated under reflux in high boiling point solvents and with a variety of basic catalysts, but without success.

Results Following from the promising results we obtained with N^4,N^9-diacyl spermine conjugates (Ahmed et al 2006), we have designed and synthesized N^1,N^{12}-di(2-imidazolyl)-N^4,N^9-dioleoyl spermine, by a route using thiomethanol nucleophilic displacement from 2-methylthio-2-imidazoline followed by aromatization using barium manganate oxidation (Hughey et al 1980).

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Hughey, J. L., et al (1980) *Synthesis* **6**: 489–490

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Synthesis and evaluation of a range of substituted benzyl azoles as inhibitors of 17α-hydroxylase (17α-OHase) and 17,20-lyase

I. Shahid, S. Dhanani, C. P. Owen and S. Ahmed

Kingston University, School of Pharmacy and Chemistry, Dept of Pharmacy, Penryn Road, Kingston upon Thames, Surrey, KT1 2EE, UK. E-mail: s.ahmed@kingston.ac.uk

Objectives The conversion of pregnanes to androgens involves an initial 17α-hydroxylation (involving the 17α-hydroxylase (17α-OHase)) followed by the cleavage of the C(17)-C(20) bond (involving 17,20-lyase (lyase)). We have previously developed a novel technique, the substrate-haem complex (SHC) approach, for 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) which has been used to produce a representation of the essential components within the active site of the overall enzyme complex (Ahmed 1999). Using the SHC, we have designed a number of compounds based upon the benzyl azole backbone; we have previously reported the synthesis and biochemical evaluation of halogenated derivatives of benzyl imidazoles which were found to be highly potent inhibitors of $P450_{17\alpha}$. Here, we report the initial results of our study into the synthesis, evaluation and molecular modelling of the non-halogenated derivatives of benzyl azole.

Method The synthesis of the target compounds was achieved through the *N*-alkylation of the azole functionality involving the use of the appropriate 4-substituted benzyl bromide and suitable base. In general, the reactions proceeded in good yield (ranging from 60% to 90%) and no major problems were encountered. The biochemical evaluation of the synthesised compounds was undertaken using modified literature assay procedure (Li et al 1996). In general, the assay mixture consisted of NADPH-generating system (50 μl), the inhibitor and substrate (10 μl), in phosphate buffer (915 μl, pH7.4). The assay was initiated by the addition of rat 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 non-radiolabelled steroid carriers, spotted onto TLC plates and run (approximately 2 h) (mobile phase consisted of dichloromethane (70 ml) and ethyl acetate (30 ml)). After development, the separated steroids were cut from the plate and placed into a scintillation tube with acetone (1 ml) and scintillation fluid (HiSafe). The samples were vortexed and then read for tritium.

Results The results show that the compounds were, in general, weaker inhibitors than the standard compound for $P450_{17\alpha}$ 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 found to be 4-cyanobenzyl imidazole which was found to possess 29% and 55% inhibitory activity against 17α-OHase and lyase respectively under similar conditions. Furthermore, all of the compounds were more potent against the lyase component than the 17α-OHase – the weaker inhibitory activity observed against 17α-OHase (also involved in corticosteroid biosynthesis) would be expected to have reduced side-effects. Using the SHC approach, the inhibitory activity observed has been rationalised and suggests that H-bonding interaction with the active site of $P450_{17\alpha}$ results in increased potency.

Conclusion The compounds synthesised within the present study are therefore good lead compounds in the design of further novel inhibitors of $P450_{17\alpha}$.

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Synthesis and biophysical studies of chemical ribonucleases containing phenazinium anchor groups

J. K. Rattu, D. A. Stetsenko and E. V. Bichenkova

Manchester University, Stopford Building, Oxford Rd, Manchester University, M13 9PL, UK. E-mail: j_rattu@hotmail.com

Objective Artificial ribonucleases development is one of the most challenging approaches towards RNA targeting. Research into the design of novel artificial ribonucleases has been prompted by their potential use in both therapy and as tools in molecular biology (Crook et al 2004). The artificial ribonuclease design approach normally includes conjugation of functional groups, which act as binding and cleaving moieties (Fabani et al 2004; Kuznetsova et al 2004). The cleaving moiety design has normally been based on the imitation of the catalytic active centre of RNases with hydrolytically active functional groups. Other parts that are responsible for binding may consist of (antisense) oligonucleotides for specific binding and small molecules with non-specific affinity for RNA such as phenazinium.

Method This research deals with the two particular aspects of synthetic artificial ribonucleases. The first aspect was to study the effects a phenazinium anchor group on the binding of a model cleaving construct (1) to nucleic acids. Second aspect was to develop a novel and flexible approach to the synthesis of RNA cleaving constructs possessing both a catalytic moiety and an intercalating anchor group. Binding studies were conducted using UV-visible spectroscopy and thermal denaturation experiments to monitor any changes to the absorption of phenazinium chromophore. These changes were used to determine mode of interactions between phenazinium anchor group and target nucleic acid molecule.

Results The phenazinium compound in construct (1) (Figure 1) was found to bind externally, electrostatically and independently of secondary structure. Chemical ribonucleases have been successfully synthesised for biochemical investigation of their RNA cleaving efficacy.

Conclusions Novel chemical routes were developed for simple and robust synthesis of chemical ribonucleases and the nature of the phenazinium anchor's binding to nucleic acids was revealed.

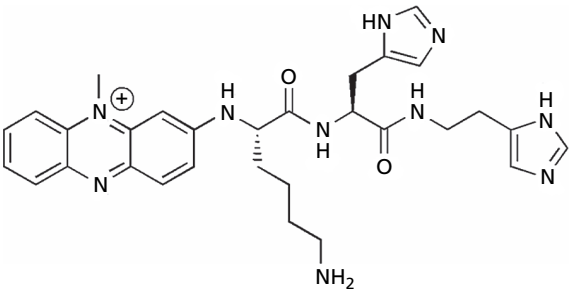

Figure 1 Chemical structure of bis-imidazole construct 1 with covalently attached phenazinium anchor group.

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