Poster Session 2 – Chemistry

159
Synthesis and evaluation of tetralone derivatives: P450 enzyme inhibitors as differentiating agents for the treatment of hormone-refractory prostate cancer

S. W. Yee and C. Simons

Department of Medicinal Chemistry, Welsh School of Pharmacy, Cardiff University, King Edward Avenue VII, Cardiff, UK. E-mail: yeesw@cardiff.ac.uk

Prostate cancer is the most common malignancy among males in the US, with 230 110 estimated new cases and 29 900 deaths for 2004 alone (Jemal et al 2004). Hormonal therapy and surgical castration have prominent roles in the treatment of advanced prostate cancer. Unfortunately, hormonal therapy is not capable of producing durable responses in the majority of patients with advanced disease. Once the patient develops hormone-refractory prostate cancer (HRPC), his outlook is poor, with a median survival time of 9 months. Clearly, new effective treatment strategies are needed for the treatment of HRPC. One of the new therapeutic strategies is to employ a differentiating agent to suppress prostate cancer cell proliferation. Vitamin D and retinoic acid have antiproliferative and differentiating effect on prostate cancer cells (Peehl & Feldman 2003). The P450 enzymes that are responsible for the metabolism of vitamin D and retinoic acid are cytochrome 24 (CYP24) and 26 (CYP26), respectively. CYP 24 has been shown to be expressed in some prostate cancer cell lines. Liarozole and ketoconazole, inhibitors of CYP26, have shown promising data in the treatment of HRPC. Identification of potent inhibitors of CYP24 and CYP26 may be a new strategy for the treatment of androgen-independent prostate cancer. It has been shown in our own laboratory, that isoflavones and flavones, tetralones (Kirby et al 2003) and coumarins affect the activity of a variety of cytochrome P450 enzymes involved in hormone biosynthesis. In view of this, it was of interest to investigate the inhibitory activity of these compounds against CYP24 and CYP26. The biochemical evaluation of the synthesised tetralone compounds was undertaken using a modification of the method of Kirby et al (2003). The incubation mixtures (0.5 mL) containing NADPH generating system (50 μL) and substrate (10 μL, either [11,12-^{3}H] retinoic acid or 25-hydroxy[26,27-methyl-^{3}H]-vitamin D) in phosphate buffer (pH 7.4) and enzyme suspension (20 μL liver microsomal or 50 μL kidney mitochondrial fractions) were incubated at 37°C for 30 min in a shaking water bath. The solutions were quenched by the addition of 1 % acetic acid v/v (200 μL). Then ethyl acetate containing 0.02% butylated hydroxy anisole (2 mL) was added and the tube vortexed for 15 s. The organic layer (1.5 mL) was transferred into a clear tube and the solvent evaporated using a centrifuge connected to a vacuum pump and a multitrap at −80°C. The residue was reconstituted in methanol (50 μL) and was injected into a reverse-phase HPLC connected to an online scintillation detector. The separated [^{3}H]-metabolites were quantitatively calculated from the areas under the curves. The percentage inhibition was calculated from: 100[(metabolites (control) – metabolites (inhibitor)/(metabolites control)]%. The results showed that the synthesised tetralone compounds (at 100 μM and 20 μM) displayed greater or similar inhibitory activity than the standard compound for CYP24 and CYP26, namely ketoconazole. Homology models of CYP24 and CYP26 have been constructed using various templates, and molecular docking studies of substrate and inhibitors have been carried out to broaden the understanding of enzyme/inhibitor interactions.

Jemal, A., et al (2004) *CA Cancer J. Clin.* **54**: 8–29
Kirby, A. J., et al (2003) *J. Enzyme Inhib. Med. Chem.* **18**: 27–33
Peehl, D. M., Feldman, D. (2003) *Endocr. Relat. Cancer* **10**: 131–140

160
Categorical modeling of the carcinogenicity of organic compounds using neural network

K. Tanabe, N. Ohmori, S. Ono, T. Suzuki*, T. Matsumoto† and H. Uesaka‡

Department of Management Information Science, Chiba Institute of Technology, Tsudanuma 2-17-1, Narashino-shi, Chiba 275-0016, *Natural Science Laboratory, Toyo University, 2-11-10 Oka, Asaka, Saitama 351-8510, †Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai-shi, Miyagi 980-8577, and ‡Department of Regional Science, Toyama University of International Studies, Higashikuromaki 65-1, Ohyama-cho, Kamishinkawa-gun, Toyama 930-1292, Japan. E-mail: tanabe@pf.it-chiba.ac.jp

Numerous chemicals of natural and synthetic origin have been produced and the adverse effects of most of those chemicals on human health and ecosystems are not known. 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 performed (Tanabe & Matsumoto 2002; Romualdo 2003). QSAR models of the relationships between structure and carcinogenicity of chemicals were constructed by applying a multilayer neural network with 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 training set of 324 chemicals and a testing set of 168 chemicals in the database of the PTC (the Predictive Toxicology Challenge contest (www.informatik.uni-freiburg.de/~ml/ptc/) were characterized by means of three sets of molecular descriptors, Dragon, tReymers and Helma. These descriptors were entered into the input layer of a three-layered NN, and the carcinogenicity data were entered into the output layer (0 for noncarcinogenic or 1 for carcinogenic chemicals). To avoid the over-learning which is serious in an NN, the original training set was equally divided into a learning set and a validation set. While an NN was trained by using the learning set, the errors between the output and teaching data for the learning, validation and test sets were counted in each cycle. At a sub-optimal model structure, the classification ability of the NNs with different descriptor sets was tested on the male rat data of 168 chemicals. The correct classification rates obtained were 67.7%, 72.5% and 74.9%, using 18 tReymers, 24 Helma, and 42 tReymers+24 Helma descriptors, respectively. The prediction accuracy is significantly improved compared with reported values by earlier attempts using such as a statistical method such as regression analysis and partial least squares. Most of earlier reported values for the correct classification rates were about 60%, and the best value was 67.6% reported by T. Okada to the best of our knowledge. Thus, our result demonstrated the superiority of the NN as a nonlinear modeling method. Development of an open system for predicting the carcinogenic activity of chemicals based on these results is under study. In conclusion, our model using a three-layered neural network and 42 tReymers+Helma descriptors showed a higher performance of 75% correct classification rates to predict the carcinogenicity than any of the PTC 2000–2001 contestants.

Acknowledgement: This work was supported by a Grant-in-Aid for Scientific Research (A) (14209022).

Romualdo, B. (2003) *Quantitative structure-activity relationship (QSAR) models of mutagens and carcinogens*. CRC Press , Boca Raton
Tanabe, K., Matsumoto, T. (2002) *J. Comput. Chem. Japan* **1**: 23–28
Zupan, J., Gasteiger, J. (1999) *Neural networks in chemistry and drug design*. 2nd edn,:Wiley-VCH, Weinheim

161
Synthesis and biochemical evaluation of a range of sulphamoylated compounds against oestrone sulphatase (ES) and an insight into the mechanism of inhibition of ES

K. Aidoo-Gyamfi, C. P. Owen and S. Ahmed

Deparment of Pharmacy, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston, Surrey, UK. E-mail: S.Ahmed@kingston.ac.uk

A number of steroidal and non-steroidal inhibitors has been investigated as potent inhibitors of oestrone sulphatase (ES), including oestrone-3-*O*-sulphamate (EMATE) and coumarin-7-*O*-sulphamate (COUMATE) — both compounds, along with other potent inhibitors, contain a sulphamate moiety, which is believed to be involved in the irreversible inhibition of ES. A potential mechanism for the inhibition of ES by sulphamoylated compounds (Woo et al 2000) was published, which proposed that the cleavage of the carbon backbone occurred after the production of an imine moiety (and which irreversibly inhibited the enzyme). From our study on pK_a, however, we postulated that the hydrolysis of the S-OR bond was an important step in the inhibition process (Ahmed et al 2000) and that without this step, irreversible inhibition of ES could not take place. As such, our earlier findings, and therefore the conclusions, were in direct contradiction to the mechanism proposed by Woo et al (2000). Here, we report our efforts to resolve the contradiction between our hypotheses derived from our earlier studies and the proposed mechanism. We therefore report the synthesis and biochemical evaluation of a range of alkyl- and phenyl-sulphamoylated compounds and a small range of methanesulphonate based compounds of straight chain alkyl alcohols. In the synthesis of the 4-sulphamoylated derivatives of 4-hydroxy phenol and alkyl alcohols, modified literature procedure was followed and found to proceed well and in good yield without any major problems. The synthesised compounds were then evaluated against human placental microsomal enzyme using standard literature methods (Selcer et al 1996). The results of the biochemical evaluation show that, in general, all of the compounds considered within the present study are less potent than COUMATE and EMATE (IC50 = 0.5 μM). Detailed consideration of the inhibitory activity shows that the phenyl sulphamate based compounds are found to possess good inhibitory activity (e.g. 3-nitrophenyl sulphamate was found to

possess an IC50 value of 120 μM) whereas the alkyl sulphamates are, in general, non-inhibitors. Within the alkyl sulphamates, however, the α-substituted compounds, are found to possess greater inhibitory activity than the non-α-substituted derivatives (e.g. 2,2,2-trichloroethylsulphamate possessed an IC50 value of 750 μM), furthermore, the inhibitory activity of all of the inhibitors correlate well with the pK_a of the parent alcohols. The investigation into the mode of action of all of the inhibitors shows that the sulphamate-based inhibitors (both phenolic and alkyl alcohol based compounds) are irreversible (i.e. the enzyme did not recover activity after incubation with the synthesised sulphamate based compounds), whereas the methanesulphonate based compounds, although weak inhibitors, are found to be reversible in nature. The observation that only the phenyl sulphamate based compounds are inhibitors of ES whereas the non-α-substituted alkyl sulphamate based compounds are not, clearly demonstrates that the cleavage of the S-OR bond is an important step in the irreversible inhibition of this enzyme.

Ahmed, S., et al (2000) *Biochem. Biophys. Res. Commun.* **272**: 583–587
Selcer, K. W., et al (1996) *J. Steroid Biochem. Mol. Biol.* **59**: 83–91
Woo, L. W. L., et al (2000) *Chem. Biol.* **7**: 773–791

162
Anti-inflammatory activity of nitroxybutyl ester of mefenamic acid

M. Aneja and D. P. Pathak

Dept of Pharmaceutical Sciences, GJU, Hisar-125001 India.
E-mail: dppathak@yahoo.com

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly self administered class of drug and gastrointestinal side effects cause an estimated 16 000 deaths and 107 000 cases of hospitalization each year in the USA. Gastrointestinal damage is an important side effect of these drugs that has been attributed to the inhibition of gastric COX-1 activity leading to loss of cytoprotective prostaglandins (mainly PGE_2 and PGI_2) formation. All the applications of these drugs (i.e. antipyretic, ant-inflammatory and analgesic) are related to primary action of drugs inhibition of arachidonate cyclooxygenase (COX) and thus inhibition of production of prostaglandins and thromoboxanes. There are many methodologies for counteracting this side effect of NSAIDs. These include the use of antacids, gastric acid pump inhibitors, H_2-receptor antagonists and barrier agents. These are effective only in the treatment of duodenal ulcer but not for the treatment of gastric ulcer caused by NSAIDs. Another approach is synthetic prostaglandins analogues (e.g. misoprostol) which reduce gastric acid secretion. This is effective in treatment of gastric ulcer but causes diarrhoea, abdominal pain and act as abortifacient in pregnant women. There is another choice for prevention of gastric side effect of NSAIDs — COX-2 inhibitor NSAIDs (e.g. celecoxib and rofecoxib). They causes fluid retention and induces renal failure. Nitric oxide (NO) decreases the NSAID-induced damage by increasing mucosal blood flow and mucous secretion by gastric epithelial cells, which results in decrease in gastric toxicity. NO-donating NSAIDs hybrid compound such as nitroxybutyl ester of NSAIDs show reduced ulcerogenic activity while maintaining anti-inflammatory activity. There are many compounds which are already syntherized by Nicox & Nitromed; these are as follows: NO-aspirin, NO-diclofenac, NO-indomethacin, NO-naproxen, NO-flurbiprofen, NO-ibuprofen, NO-ketoprofen, which are now in different phases of clinical trials. NO-NSAIDs may be of therapeutic benefits in a wide variety of disease states, including pain, inflammation, thrombosis, neurodegenerative disease of CNS, cancer, liver disease, impotence, bronchial asthma and osteoporosis. In this work we have synthesized one compound of this type (i.e. NO-releasing mefenamic acid). The synthesis was done by a two-step reaction. In first step, the chlorobutyl ester of mefenamic acid was prepared by esterification of the sodium salt of mefenamic acid by 1,4 dichlorobutane. In second step, product was prepared by further esterification of the chlorobutyl ester of mefanamic acid with silver nitrate. The nitroxy butyl ester of mefenamic acid was screened by in-vitro and in-vivo methods. Melting point study, TLC, IR, NMR and HPLC were used for in-vitro studies. In-vivo studies were carried out for anti-inflammatory and anti-ulcer activity on rats. The result was compared with that of pure drug.

163
Synthesis of isoflavone derivatives, and the biochemical evaluation of their effect on the growth of MCF-7 breast cancer cells

K. E. James and M. Saunders

University of Portsmouth, School of Pharmacy and Biomedical Sciences, St Michaels Building, White Swan Road, PO1 2DT, UK. E-mail: Karen.James@port.ac.uk

A vast number of epidemiological studies have indicated that isoflavones exhibit anti-cancer properties (Adlercreutz et al 2002). They have been postulated to act via a number of different mechanisms, including acting as oestrogen receptor agonists/antagonists (Messina & Loprinzi 2001), acting as anti-oxidants (Boruns 2002) and inhibiting enzymes such as glycosidase (Lee & Lee 2001) and DNA topoisomerases. Despite their apparent therapeutic benefits there has been limited development of isoflavones as drugs. Rapid in-vivo metabolism and poor bioavailability seems to be a major factor influencing this. In this study we have synthesised a range of isoflavone derivatives using a simple one-pot procedure (Balasubramanian & Nair 2000). The isoflavones were designed to investigate structural features required for oestrogen receptor binding. A parallel study was conducted to investigate the in-vitro metabolism of each derivative in liver microsomes. The aim of this study was to determine if the beneficial biological properties could be maintained or improved, while reducing the possibility for metabolism. The effect of the isoflavones synthesized on the growth of MCF-7 cells (hormone dependent breast cancer cells) was determined. The results from one of these derivatives is shown in Table 1. From these results it can be seen that as the concentration of compound 1 increases to 10 μM, cell growth is stimulated. At higher concentrations a reduction in cell growth is observed. Daidzien, a natural isoflavone with anti-cancer properties, also acts in this way, indicating that compound 1 is acting via a similar mechanism. Compound 1 differs from daidzein in that it has fewer sites available for phase II metabolism. Further studies are underway to see if this influences in-vitro metabolism.

Table 1 Effect of compound 1 on MCF-7 cells

Compound 1 (μM)	No. cells per well	% change in cell no. compared with 0 μM
0	960 000	—
0.1	480 000	−50
1	1 340 000	39.6
10	6 020 000	527.1
100	3 040 000	216.7
1000	4000	−95.8

Adlercreutz, H. (2002) *Lancet Oncol.* **3**: 384–373
Balasubramanian, S., Nair, M. G. (2000) *Synth. Comm.* **30** 469–484
Boruns, F. (2002) *Food Res. Int.* **35**: 187–193
Messina, M. J., Loprinzi, C. L. (2001) *J. Nutr.* **131** (Suppl.): 3095S–3108S
Lee, D. S., Lee, S. H. (2001) *FEBS Lett.* **501**: 84–86

164
The structure–activity relationship determination of a series of compounds based upon 4-hydroxybenzoic acid

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

Department of Pharmacy, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston, Surrey, UK. E-mail: S.Ahmed@kingston.ac.uk

Oestrone sulphatase (ES) is 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*-sulphamate (COUMATE) and its tricyclic derivatives such as 667-COUMATE, the latter being in Phase II clinical trials (Woo et al 2000)). In an effort to synthesise highly potent inhibitors of this enzyme, we have undertaken extensive structure–activity relationship determination and molecular modelling studies. Using a theoretical model of the active site derived from the consideration of the transition state (TS) of the reaction catalysed by ES, we superimpositioned both known steroidal and non-steroidal inhibitors (and our potential inhibitors) onto the derived TS (Ahmed et al 2001). From our studies, we concluded that the benzoic acid moiety together with a sulphamate group may result in compounds which possess potent inhibitory activity; here, we report the results of the synthesis, in-vitro biochemical evaluation (using literature based assay procedures (Table 1)) and the rationalisation of the structure–activity relationship of a series of straight chain and cyclic esters based upon 4-hydroxybenzoic acid. The results of the current study show that the synthesised compounds are, in general, good inhibitors of ES in comparison with the three standard compounds, namely EMATE, COUMATE and 667-COUMATE (Table 1). However, a small number of compounds show extremely potent inhibitory activity, with compound 3 ($IC50 = 0.17$ μM) possessing the most potent inhibitory activity of all of the synthesised compounds, being ~81 times more potent than COUMATE

and equipotent to 667-COUMATE. Detailed consideration of the inhibitory activity shows that there is a good correlation between the logP of the inhibitors and the IC50 values — a plot of logP vs IC50 shows that there is a decrease in IC50 values with increasing logP up to 3.7 (compound 3 was found to possess a logP of 3.87, close to that of EMATE) beyond which the potency of the compounds begins to decrease; compound 4, possessing a large alkyl chain was found to possess weak inhibitory activity (IC50 = 4.8 μM). Using the derived TS for the reaction catalysed by ES, we were able to rationalise the observed trend — that is, with an increase in alkyl group length (e.g. with a nonyl side chain as in compound 4), steric interactions between the inhibitor and enzyme active site increased resulting in a decrease in the inhibitory activity. In conclusion, we have provided an important factor (overall length of inhibitor) for consideration in the design of novel inhibitors, as well as synthesising an extremely potent inhibitor of ES.

Table 1 IC50 data for compounds under study

Compound	% inhibition	IC50 (μM)
1	84 ± 4	1.70 ± 0.07
2	87 ± 4	0.500 ± 0.028
3	82 ± 1	0.170 ± 0.007
4	76 ± 5	4.80 ± 0.17
667-COUMATE	56 ± 1	0.21 ± 0.01
EMATE	69 ± 4	0.500 ± 0.001

Ahmed, S., et al (2001) *Bioorg. Med. Chem. Lett.* **11**: 3001–3006
Woo, L. W. L., et al (2000) *Chem. Biol.* **7**: 773–791

165
Synthesis and biochemical evaluation of a range of phenyl alkyl azoles as inhibitors of 17α-hydroxylase (17-OHase) and 17,20-lyase (lyase)

*S. Dhanani, C. H. Patel, C. P. Owen and S. Ahmed

Department of Pharmacy, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston, Surrey and *School of Life Sciences, Kingston University, Penrhyn Road, Kingston, Surrey, UK. E-mail: S.Ahmed@kingston.ac.uk

The biosynthesis of androgens is catalysed by a single enzyme and involves two distinct steps: 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 ($P450_{17\alpha}$) though 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 (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 1999). In an effort to add support to the SHC, we have undertaken the design and synthesis of a range of compounds based upon phenyl-alkyl-azoles (imidazole and triazole) 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, biochemical evaluation and molecular modelling of inhibitors of both components. The synthesis of the final azole compounds was achieved through the *N*-alkylation of the azole using the appropriate phenyl-alkyl-bromide and a suitable mild 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 procedure (Li et al 1996). The results show that the compounds were, in general, equipotent or more potent than the standard compound for $P450_{17\alpha}$, namely ketoconazole (KTZ) (Table 1); the most potent imidazole-based inhibitor, compound 6, was found to be ~11 and ~4 times more potent than KTZ against 17α-OHase and 17,20-lyase, respectively. Detailed consideration of the inhibitory data for the compounds shows that there is a good correlation between IC50 and logP. The compounds were modelled onto the SHC for the overall $P450_{17\alpha}$ enzyme which showed that the synthesised compounds were able to fit within the active site without undergoing any unfavourable steric interactions. The compounds synthesised within this study are therefore good lead compounds in the design (using the SHC approach) of further novel inhibitors of $P450_{17\alpha}$. Furthermore, the increase in inhibitory activity with increasing alkyl chain length add further support to the SHC approach, that is the requirement of both the hydrogen bonding interaction (with the active site) and the Fe-imidazole interactions.

Table 1 Inhibitory data obtained for a range of phenyl alkyl imidazole compounds ([I] = 10 μM)

Compound no.	17-OHase		Lyase	
	% inhibition	IC^{50} (μM)	% inhibition	IC50 (μM)
1	23.35 ± 0.97	30.95 ± 0.68	39.95 ± 0.89	10.30 ± 2.05
2	40.69 ± 0.72	8.65 ± 1.37	60.12 ± 0.25	2.17 ± 0.14
3	59.74 ± 0.63	2.20 ± 0.25	65.95 ± 0.31	1.36 ± 0.12
4	61.28 ± 0.58	0.87 ± 0.03	72.59 ± 1.29	0.55 ± 0.03
5	67.45 ± 0.48	0.32 ± 0.05	94.14 ± 0.32	0.09 ± 0.01
6	70.17 ± 0.63	0.25 ± 0.01	84.53 ± 0.14	0.22 ± 0.02
7	64.52 ± 0.29	1.06 ± 0.03	79.13 ± 2.68	0.35 ± 0.03
KTZ	64.28 ± 0.80	3.76 ± 0.01	56.11 ± 0.18	0.8 ± 0.05

Ahmed, S. (1999) *Bioorg. Med. Chem.* **7**: 1487–1496
Li, J. S., et al (1996) *J. Med. Chem.* **39**: 4335–4339

166
Synthesis, biochemical evaluation and molecular modelling of a range of compounds as potential inhibitors of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD)

H. Abdulkadir, R. Lota, C. P. Owen, *S. Dhanani and S. Ahmed

Department of Pharmacy, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston, Surrey and *School of Life Sciences, Kingston University, Penrhyn Road, Kingston, Surrey, UK. E-mail: S.Ahmed@kingston.ac.uk

The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) is responsible for the conversion of C(17) = O groups to the reduced hydroxy moiety. As such, this enzyme is responsible for the formation of a potent oestrogen, namely oestradiol from oestrone. 17β-HSD has come under consideration as a potential target in the fight against hormone dependent cancer such as breast cancers. In an effort to aid the drug design process, we concluded that the derivation of the transition states (TS) of the catalysed reactions may lead us to the rationalisation of the inhibitory activity of a range of inhibitors of this enzyme. Here, we report the initial results of the development of a model (involving the determination of the TS of the reduction reaction (Penning 1997)) as a simplified representation of its active site and the rationalisation of the inhibitory activity of a number of known flavone based inhibitors of 17β-HSD (Lelain et al 1999). Using the derived TS, we undertook the design of a number of compounds that were synthesised and subsequently evaluated against rat testicular microsomal enzyme (using radiolabelled oestrone as the substrate). The determination of the TS and the design process were undertaken using the molecular modelling software CaChe. In the design of the potential inhibitors, compounds were superimposed onto the TS and the 'degree of superimposition' evaluated. It was concluded from our modelling studies that compounds based upon the biphenyl backbone and possessing a carbonyl moiety to mimic the steroidal C(17) = O group would be expected to show some inhibition. As such, the proposed inhibitors were synthesised involving Friedel-Crafts acylation of the biphenyl ring system — a range of acid chlorides (from acetyl chloride to heptanoyl chloride as well as benzoyl chloride) were used so as to allow us to obtain some useful structure-activity relationships. The reactions, in general, proceeded in good yield (typically 60%) and without any major problems. The synthesised compounds were evaluated for inhibitory activity against 17β-HSD using modified standard literature procedures. Furthermore, we used previously reported flavones as standard compounds in an effort to produce a comparison between the synthesised compounds and known inhibitors of 17β-HSD. From the results of our modelling study, we conclude that with the small range of flavone derivatives considered, the ability of these compounds to fit within the active site (or indeed occupy similar area/volume of space as the natural substrate) is the major factor in their inhibitory activity. Furthermore, the area corresponding to the C(17) area of the steroid backbone is considered to be sterically hindered. As such, any interactions between the inhibitor and the NADPH moiety results in a further unfavourable interaction that results in a decrease in the inhibitory activity (e.g., 7-hydroxyflavone, the more potent inhibitor and possessing an IC50 value of 9.0 ± 0.1 μM, is not involved in any unfavourable interactions with the active site whereas 6-hydroxyflavone, IC50 = 16.4 ± 0.5 μM, undergoes steric interaction and is thus the weaker inhibitor). That the results of the modelling study may have some validity is clearly supported when we consider the inhibitory activity of the range of biphenyl based compounds (i.e. inhibitory activity decreases with an increase in chain length or volume of the substituent acyl group).

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

167
Synthesis of 3-alkylated-(4′-aminobenzyl)-2-oxazolidinones as probes in the investigation of the active site of 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$)

C. H. Patel, S. Dhanani*, H. Abdulkadir, C. P. Owen and S. Ahmed

Department of Pharmacy, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston, Surrey and *School of Life Sciences, Kingston University, Penrhyn Road, Kingston, Surrey, UK. E-mail: S.Ahmed@kingston.ac.uk

Extensive research has been undertaken to produce compounds that are both potent and selective inhibitors of the enzyme 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$). Compounds that have shown potent inhibitory activity against this enzyme have been based upon an azole moiety, which is postulated to undergo reversible co-ordinate bond formation with the Fe atom of the cytochrome P-450 porphyrin haem structure. Here, we report the synthesis and screening of a range of oxazolidinone-based compounds (and therefore the use of a phenylamine moiety as the Fe-ligating group) as probes of the active site of this enzyme in an effort to elucidate further information regarding the active site of P-450$_{17\alpha}$. In the general use of the "Evans" oxazolidinone, the most widely synthesised derivatives have been the *N*-acyl compounds. *N*-Alkylation has been somewhat ignored, nevertheless, synthesis has been undertaken using strong bases such as LDA. In our hands, the use of LDA proved to be unsuccessful and a search for an appropriate base led us to sodium hydride (NaH) using anhydrous *N,N*-dimethylformamide (DMF) as the solvent, and this provided the range of alkylated compounds in high yield (typically 60–85%). In the synthesis of the phenylamine derivative, we considered the nitration (followed by reduction of the nitro group to the desired amine functionality) of the phenyl ring system; the reaction was undertaken using nitric acid (5 M) in dichloromethane (DCM) at room temperature. In general, the reaction proceeded without any major problems, however, problems were encountered with the larger alkyl chain derivatives and the synthesis of the heptyl, octyl, nonyl and decyl derivatives was achieved involving an increase in reaction time (yield 80%). The target compounds were obtained through the use of hydrogen gas and palladium on activated charcoal (yield 70%). The synthesised compounds were screened for inhibitory activity using the standard literature method (Li et al 1996) using ketoconazole (KTZ) as the standard (an imidazole-based inhibitor). From the results of the initial screening against P-450$_{17\alpha}$, we observe that the majority of the novel inhibitors possess good inhibitory activity but are less potent than the standard compound, KTZ (possessing 90% [KTZ] = 10 μM). Comparison of the inhibitory activities of the *R*- and *S*-forms show that the two series of inhibitors appear to behave in a totally different manner. That is, the *R*-enantiomer, in general, appears to be weaker than the *S*-form, the most potent compound within the former range being the nonyl which possesses 69% inhibition, where as the most potent within the *S*-form is the heptyl and which possesses 85% inhibition. In an effort to consider the possibility of these compounds possessing inhibitory activity against alternative cytochrome P-450 enzymes, the compounds were also evaluated against oestrogen synthetase (AR) and were found to be extremely good inhibitors of this enzyme. Modelling of these compounds using the novel substrate-haem complex (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 readily possible. However, within the SHC representation of the AR active site, hydrogen bonding [corresponding to the C(17) area of the steroid backbone] appears to be possible, thereby resulting in a better inhibitory profile against the latter enzyme in comparison to P-450$_{17\alpha}$.

Li, J. S., et al (1996) *J. Med. Chem.* **39**: 4335–4339

168
The structure–activity relationship determination of a series of compounds based upon 4-hydroxyphenyl ketones as potential inhibitors of the enzyme oestrone sulphatase

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

Department of Pharmacy, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston, Surrey, UK. E-mail: S.Ahmed@kingston.ac.uk

The production of oestrone in postmenopausal women has been shown to be catalysed by the enzyme oestrone sulphatase (ES), in particular, ES is responsible for the conversion of the stored and inactive form of oestrone (oestrone sulphate) to the active form through a hydrolysis reaction. A number of steroidal (for example, 3-*O*-oestronesulfamate, EMATE) and non-steroidal (for example, 4-methylcoumarin-7-*O*-sulphamate, COUMATE) inhibitors of this enzyme have been reported within the literature and have shown potent inhibitory activity. The success of the non-steroidal compounds has resulted in one of the coumarin derivatives, the tricyclic derivatives of COUMATE, namely 667-COUMATE, having entered clinical trials (Woo et al 2000). In an effort to synthesise potent inhibitors of ES, we have undertaken extensive structure-activity relationship determinations and molecular modelling studies. Using the derived transition-state (TS) of the reaction catalysed by ES as a representation of the active site, we superimpositioned both known steroidal and non-steroidal inhibitors (and our potential inhibitors) onto the derived TS (Ahmed et al 2001). From our studies, we concluded that sulphamoylated derivatives of 4-hydroxyphenyl ketones may result in compounds which possess good inhibitory activity; here, we report the results of the synthesis, in vitro biochemical evaluation (using literature based assay procedures) and the rationalisation of the structure-activity relationship of a series of straight chain and cyclic containing compounds based upon 4-hydroxyphenyl ketones and 3,5-dibromo-4-hydroxyphenyl ketones. The results of this study show that the synthesised compounds are, in general, good inhibitors of ES, although all of the synthesised compounds are weaker inhibitors than the three standard compounds, namely EMATE, COUMATE and 667-COUMATE. The most potent compound is the nonophenone derivative (sulphamic acid 4-nonanoylphenyl ester), which is found to possess 67% inhibition at 10 μM concentration – under similar conditions, 667-COUMATE was found to possess 56% inhibition at 0.25 μM concentration. Consideration of the di-brominated compounds show that they are much weaker inhibitors than the non-brominated compounds, the most potent compound being sulphamic acid 2,6-dibromo-4-nonanoylphenyl ester, which was found to possess 93% inhibition but at a much higher increased inhibitor concentration (50 μM). Detailed consideration of the inhibitory activity shows that there is a good correlation between the logP of the inhibitors and the IC50 values. That is, as the length of the alkyl chain length increases, the inhibitory activity increases upto the optimum logP (approximately 3.5) beyond which inhibitory activity decreases with increasing alkyl chain length. Using the TS obtained, we have rationalised the decrease in inhibitory activity being due to an increase in the steric interaction between the alkyl chain and the active site wall. Furthermore, using molecular modelling, we have rationalised the trends in the brominated derivatives – which is due to the increased interaction between the active site wall and the substitution at the 3,5-positions on the phenyl ring with the inhibitors. In conclusion, we have provided two further factors (the overall length of inhibitor and the steric hindrance close to the steroid A ring) for consideration in the design of novel inhibitors, as well as adding further support to the representation of the ES active site obtained from the consideration of the reaction catalysed by this important enzyme.

Ahmed, S., et al (2001) *Bioorg. Med. Chem. Lett.* **11**: 3001–3006
Woo, L. W. L., et al (2000) *Chem. Biol.* **7**: 773–791

169
Synthesis and biological evaluation of a series of phenyl alkyl imidazole based compounds as potential anti-bacterial agents

*M. Tsang, C. H. Patel, C. P. Owen and S. Ahmed

Department of Pharmacy, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston, Surrey; and *School of Life Sciences, Kingston University, Penrhyn Road, Kingston, Surrey, UK. E-mail: S.Ahmed@kingston.ac.uk

The use of azole-based compounds in the treatment of fungal infections is well documented (Martin 1999). In the search for novel antifungal agents, we undertook the design and synthesis of phenyl alkyl imidazole based compounds as potential 14α-demethylase inhibitors. These compounds were shown to be good anti-fungal agents; however, we also considered the potential use of these compounds against various strains of bacteria in an effort to determine their anti-bacterial properties. Here, we report the synthesis and evaluation of these compounds as potential anti-bacterial agents. In the synthesis of the compounds, the azole functionality was reacted with a phenyl alkyl halide in the presence of a suitable base. However, in most cases, in particular, the long chain containing compounds, the phenyl alkyl bromides were not readily available, and as such, these compounds were required to be synthesised. For example, in the synthesis of 5-(4-bromophenyl)-pentyl bromide, 4-bromocinnamic acid was initially esterified (using ethanol and concentrated sulphuric acid under reflux) and subsequently reduced to the ethyl 3-(4-bromophenyl)-propionate (using hydrogen gas over palladium on activated charcoal). The ester was reduced to the corresponding alcohol [3-(4-bromophenyl)-propan-1-ol] using lithium aluminium hydride ($LiAlH_4$), followed by bromination using phosphorus tribromide (PBr_3) to give 3-(4-bromophenyl)-propyl

bromide. The latter compound was then reacted with diethyl malonate to give 2-[3-(4-bromophenyl)-propyl]-malonic acid diethyl ester (using potassium tertiary butoxide dissolved in anhydrous tetrahydrofuran (THF) under reflux). Following acid hydrolysis, the resulting 5-(4-bromophenyl)-pentanoic acid was reduced using $LiAlH_4$ to give the appropriate alcohol (5-(4-bromophenyl)-pentan-1-ol), bromination of which (using PBr_3) resulted in the synthesis of 5-(4-bromophenyl)-pentyl bromide. Conversion of the latter compound to the azole derivative involved the reflux (in anhydrous THF) with imidazole in the presence of anhydrous potassium carbonate. The synthesised compounds were screened for minimum inhibitory concentration (MIC) using in-vitro agar diffusion and broth dilution assay against a range of bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli, Proteus mirabilis* and *Bacillus subtilis* (Table 1 shows the MIC for *S. aureus* and *E. coli*). From the results of the initial screening against the range of bacteria, we observe that the compounds are good biostatic compounds — all of the compounds considered here were found to inhibit the growth of the range of bacteria considered within the current study. In general, the compounds were found to inhibit *E. coli* at or below 5 μM, while the same compounds inhibited the growth of *S. aureus* at or below 10 μM. As such, these compounds are good lead compounds in the design of more potent azole-based antibacterial agents.

Table 1 MIC data (in triplicate) for a range of compounds evaluated against *E. coli* (EC) and *S. aureus* (SA)

Compound	**MIC (μM)**	
	EC	SA
1	2	10
2	5	1
3	5	10
4	1	10

Martin, M. V. (1999) *J. Antimicrob. Chemother*. **44**: 429–437