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SYNTHESIS OF DEUTERIUM LABELLED 4′-HYDROXYDICLOFENAC

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SUMMARY

Diclofenac is a potentially useful substrate for the study of drug-drug interactions caused by modulation of the activity of specific isoforms of cytochrome P450. The synthesis of a deuterium labelled version of its principal human metabolite, 4′-hydroxydiclofenac, for use as an internal standard in LC-MS-MS studies, is described.

Keywords: deuterium label, cytochrome P450 probe, diclofenac

INTRODUCTION

The prediction of clinically significant drug-drug interactions is becoming an increasingly important aspect of the development process in the pharmaceutical industry (1). Much of this work has concentrated on cytochrome P450 enzymes (CYP) which play a major role in the metabolism of xenobiotic agents. This family of oxidative enzymes consists of a number of isoforms, each of which exhibit differing degrees of substrate specificity. Probe substrates have now been identified for each of the major isoforms responsible for drug metabolism (2), and work has recently been published on the determination of inter-individual variations in the levels of the these enzymes using a mixture of such probes (3). Work is also in progress to assess if a suitable mixture of substrates can be used to determine the effects of drug candidates on CYP mediated metabolism and hence

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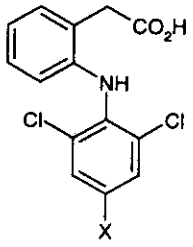
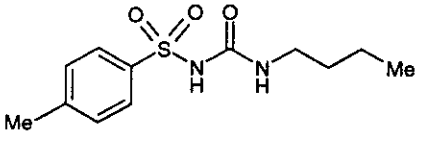


(1) X = H, Diclofenac
(2) X = OH, 4'-Hydroxydiclofenac



(3) Tolbutamide

identify potentially harmful drug-drug interactions (4). Such a strategy would involve the development of assay systems capable of simultaneously quantifying both the substrate and the major metabolite for each of these probes.

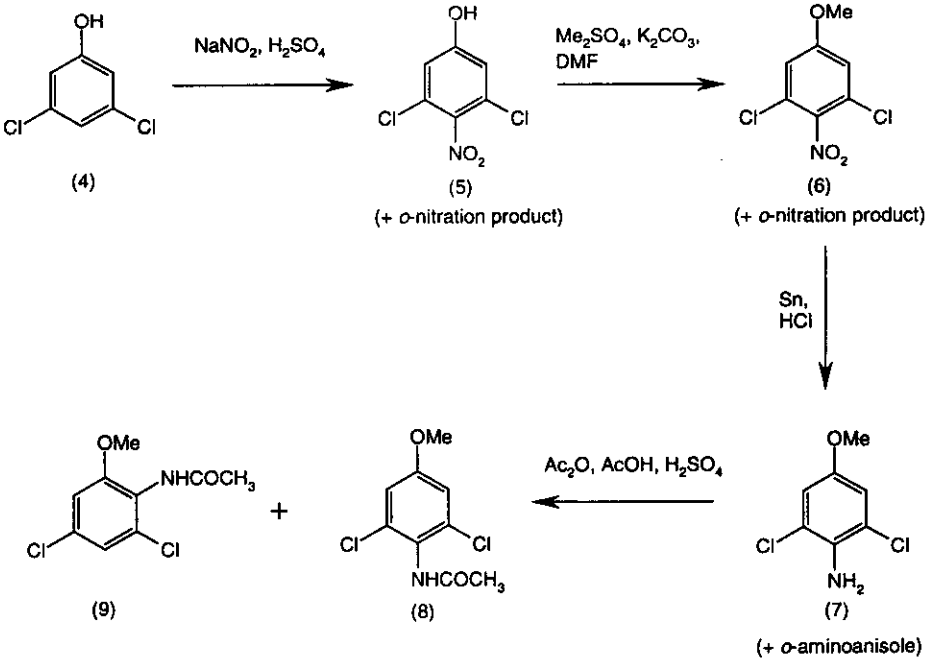
Both diclofenac (1) (5) and tolbutamide (3) (6) have been used as substrates for the CYP 2C9 isoform. Diclofenac has the advantage that it is more water soluble, has a higher affinity for the enzyme and a significantly faster turnover in human liver microsome preparations. A stable labelled version of the 4'-hydroxy derivative (2) (the major metabolite) was required for use as an internal standard in an LC-MS-MS assay for diclofenac metabolism. Unlabelled (2) had been previously prepared by a microbiological method (7), but this was felt to be unsuitable for the preparation of larger quantities of labelled material. A chemical synthesis of (2) had also been reported (8), but the preparation of a key intermediate was not described.

RESULTS AND DISCUSSION

The route to deuterated 4'-hydroxydiclofenac (14) described herein, is based on a synthesis of deuterated diclofenac reported previously (9). The key intermediate required was acetanilide (8) derived from 2,6-dichloro-4-methoxyaniline (7) (Scheme 1). It was anticipated that a late stage O-demethylation would provide the 4'-hydroxy function.

Treatment (10) of 3,5-dichlorophenol (4) with sodium nitrite in sulphuric acid gave the required nitro compound (5) together with the *o*-nitration product (*ca.* 1:1) in 76% yield (Scheme 1). Direct nitration of (4) using sodium nitrate in sulphuric acid gave a similar mixture in lower yield.

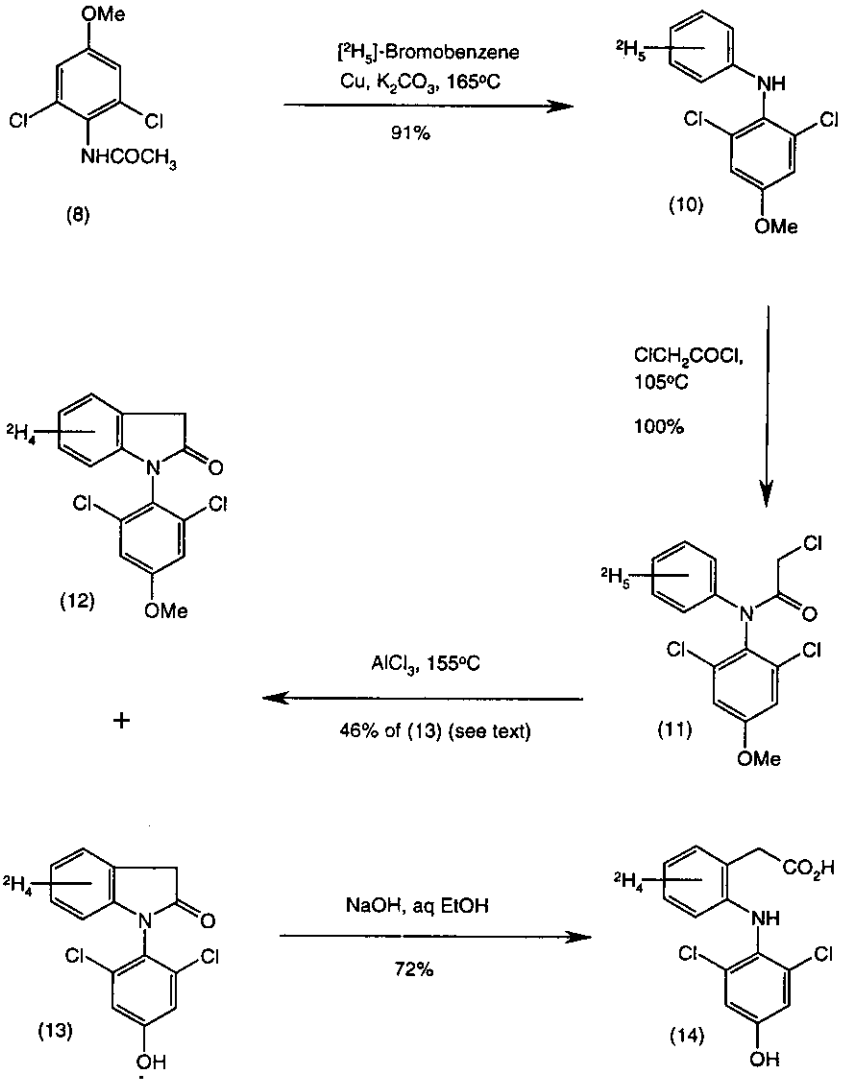
Scheme 1



These isomers were not easily separable hence the mixture was methylated, reduced with metallic tin and then acetylated. The regioisomeric acetanilides (8) and (9) were readily separated chromatograpically to give the desired isomer (8) in 12% overall yield from (4). Copper-mediated N-arylation of amide (8) with [$^2H_5$]bromobenzene (Scheme 2) proceeded smoothly to give, after basic hydrolysis, an excellent yield of diphenylamine (10); no loss or scrambling of the deuterium label was observed.

Acylation of amine (10) with chloroacetyl chloride yielded crude amide (11), which was then cyclised with aluminium chloride. The reaction conditions for this cyclisation were critical; if the neat mixture was maintained at 150-155°C for 75min, the initially formed ether (12) was also demethylated to yield the desired phenol (13), together with a more polar impurity.

Scheme 2



Attempts to limit the formation of this impurity by carrying out the reaction either at a lower temperature or for a shorter time did not result in complete demethylation. The use of a solvent (tetrachloroethane) inhibited demethylation and also gave rise to deuterium/hydrogen exchange. The observation of *in situ* demethylation was fortuitous since it was subsequently found difficult to

efficiently convert isolated (12) into phenol (13). Treatment of (12) with boron tribromide gave very little reaction, whilst attempted reaction with sodium ethylthiolate resulted in the displacement of both chloride atoms by the ethylthio moiety. An alternative method (8) entailed heating (12) in molten pyridine hydrochloride at 170°C but gave only a low yield (15%) of the phenol (13), accompanied by a large amount of insoluble material.

^{1}H NMR analysis of (13) revealed that during the cyclisation reaction there was some scrambling of the deuterium label into the other aryl ring (average of *ca.* one deuterium per molecule). Finally, base hydrolysis of the N-arylindolone (13) yielded deuterated 4′-hydroxydiclofenac (14) as a crystalline solid. Mass spectrometry indicated the following distribution of mass ions (MH^+): 313 (M+0), 3%; 314 (M+1), 7.7%; 315 (M+2) 15.5%; 316 (M+3), 22.9%; 317 (M+4), 19.4%; 318 (M+5), 15.7%; 319 (M+6) 9.2%; 320 (M+7) 4.4%. Despite this scrambling, the label was suitable for use as an internal standard and will be used in continuing LC-MS-MS studies to evaluate CYP mediated metabolism.

This route has also been used to prepare unlabelled 4′-hydroxydiclofenac (2) in similar overall yield, and could readily be scaled up to provide gram quantities of material.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian Unity spectrometer at 400MHz. Mass spectra were recorded on a Hewlett Packard 5989A instrument. All column chromatography was carried out over Merck Kieselgel 60 (9385) silica gel. HPLC analyses were performed on a Gilson gradient system equipped with a Gilson 118 UV detector.

N-(2,6-Dichloro-4-methoxyphenyl)acetamide (8).

To a well-stirred mixture of 3,5-dichlorophenol (4) (4.00g; 24.54mmol) and sodium nitrite (3.0g; 43.5mmol) in water (45ml) was slowly added a solution of concentrated sulphuric acid (2ml) in water (10ml). The mixture was then heated

under reflux for 2h, allowed to cool, added to aqueous sodium carbonate and extracted with ethyl acetate. The combined extracts were dried and evaporated and the residue purified by chromatography over silica gel eluted with isohexane-ethyl acetate (4:1). The isomeric nitro compounds (5) were obtained as a yellow oil (3.89g; 76%), which was then treated with a mixture of dimethylsulphate (3ml; 31.5mmol) and potassium carbonate (5g; 36.2mmol) in dimethylformamide (25ml) at 60°C for 1h. The resulting mixture was added to 2M hydrochloric acid and extracted with diethyl ether. The residue obtained on evaporation of the dried extracts was purified by chromatography over silica gel, eluted with isohexane-ethyl acetate (4:1), to give the mixture of methyl ethers (6) as a pale red solid (3.43g). An aliquot (1.01g) of this material in concentrated hydrochloric acid (30ml) was treated with granulated tin (2g) then heated under reflux for 1h. A further quantity of tin (2g) was cautiously added and heating continued for a further 1 hour. After cooling to room temperature, the mixture was carefully neutralised with 10M sodium hydroxide and extracted with diethyl ether. The combined extracts were dried and evaporated and the residue taken into acetic acid (12ml) then treated with acetic anhydride (2ml) followed by concentrated sulphuric acid (200mg). After 2h at 20°C the mixture was added to an excess of aqueous sodium carbonate and extracted with ethyl acetate. Evaporation of the dried extract gave a brown solid which was purified by chromatography over silica gel eluted with isohexane-ethyl acetate (3:2 then 1:1). The *acetanilide* (8) (206mg; 12% from (4)) was obtained as a mauve solid, mp 179-182°C. δ_H ($CDCl_3$) 6.92 (2H, s, Ar<u>H</u>), 6.80 (1H, br, N<u>H</u>), 3.79 (3H, s, ArOC<u>H</u>$_3$), 2.22 (3H, s, C<u>H</u>$_3$CONH).

***(2,6-Dichloro-4-methoxyphenyl)[$^2H_5$]phenylamine* (10).**

A mixture of N-(2,6-dichloro-4-methoxyphenyl)acetamide (8) (1.12g; 4.78mmol), potassium carbonate (478mg; 3.46mmol) and copper powder (180mg; 2.83mmol) in [$^2H_5$]bromobenzene (99.5 atom % ^2H) (8ml) was heated under nitrogen in an oil bath maintained at 160-165°C for 19h. The temperature was then reduced to 80°C and potassium hydroxide (1.5g) and ethanol (15ml) were added. The mixture was

heated under reflux for a further 1.5h, cooled, diluted with water (100ml) and extracted with diethyl ether.

Evaporation of the combined, dried extracts gave an oil, which was purified by chromatography over silica gel eluted with isohexane-ethyl acetate (5:1). The *amine* (10) (1.19g; 91%) was obtained as a pale brown solid, mp 124-127°C. δ_H ($CDCl_3$) 6.97 (2H, s, ArH), 5.48 (1H, br, NH), 3.82 (3H, s, $ArOCH_3$).

***2-Chloro-N-(2,6-dichloro-4-methoxy[$^2H_5$]phenyl)-N-phenylacetamide* (11).**

A solution of (2,6-dichloro-4-methoxyphenyl)[$^2H_5$]phenylamine (10) (1.14g; 4.18 mmol) in chloroacetyl chloride (7ml) was heated at 105°C for 1h. Solvent was removed under high vacuum to leave crude *chloroamide* (11) (1.60g, >100%) as a purple solid. δ_H ($CDCl_3$) 7.02 and 6.95 (2H, 2 x br s, ArH) (restricted rotation), 4.18 and 3.97 (2H, 2 x br s, CH_2Cl), 3.85 and 3.79 (3H, 2 x br s, $ArOCH_3$).

***1-(2,6-Dichloro-4-hydroxyphenyl)-1,3-dihydro[4,5,6,7-$^2H_4$]indol-2-one* (13).**

An intimate mixture of 2-chloro-N-(2,6-dichloro-4-methoxy[$^2H_5$]phenyl)-N-phenyl acetamide (11) (206mg; 0.589mmol) and finely powdered aluminium chloride (265mg; 2mmol) was heated in a nitrogen atmosphere at 150-155°C (oil bath temperature) for 75min (the mixture frothed and rapidly formed a dark solid mass). After cooling, the mixture was partitioned between ethyl acetate and 2M hydrochloric acid. The aqueous layer was extracted with more ethyl acetate and the combined extracts were dried and evaporated. Chromatographic purification of the residue over silica gel eluted with isohexane-ethyl acetate (3:2) followed by trituration of the product with isohexane-diethyl ether (1:1; 3ml) gave the *indolone* (13) as a white crystalline solid (81mg; 46%), mp 275-277°C. δ_H ($CDCl_3$) 7.07 (s, ArH), 7.35, 7.20 and 6.40 (3 multiplets resulting from deuterium scrambling/exchange, ArH), 3.83 (2H, s, $CH_2C{=}O$).

***[2-(2,6-Dichloro-4-hydroxyphenylamino)[$^2H_4$]phenyl]-acetic acid* (14).**

A mixture of 1-(2,6-dichloro-4-hydroxyphenyl)-1,3-dihydro[4,5,6,7-$^2H_4$]indol-2-one (13) (153mg; 0.513mmol) and 10M sodium hydroxide solution (4ml) in

ethanol (5ml) was heated under reflux in a nitrogen atmosphere for 11.5h. The dark solution was added to water (60ml), washed with diethyl ether, acidified with 5M hydrochloric acid and extracted with diethyl ether. These extracts were washed with dilute aqueous pH6.5 phosphate buffer, then dried and evaporated. The residue was purified by chromatography over silica gel eluted with isohexane-ethyl acetate (3:2) containing 1% glacial acetic acid.

Trituration of the product with isohexane-ethyl acetate (5:1; 6ml) gave the *title compound* (14) (117mg; 72%), as a pale mauve solid, mp 180-187°C if heated rapidly from 175°C (gas evolved); mp >250°C (dec) if heated slowly from ambient. HPLC: 5μ Prodigy ODS2 column (15x0.46cm) eluted at 1ml/min with a linear gradient over 27min from 35% to 70% of acetonitrile-water (9:1) in 0.1% (v/v) aqueous trifluoroacetic acid, UV detection at 280nm; purity 97.1% (UV, a/a), retention time 14.1min. δ_H (d_6-DMSO) 6.92 (s, ArH), 7.12, 6.98, 6.72 and 6.40 (4 multiplets resulting from deuterium scrambling/exchange, Ar<u>H</u>), 3.64 (2H, s, C<u>H</u>$_2$C=O).

[2-(2,6-Dichloro-4-hydroxyphenylamino)phenyl]acetic acid (2).

Unlabelled diclofenac (2) was prepared using methodology similar to that described for the deutero analogue above and was obtained as a pale pink solid, mp 185-189°C if heated rapidly from 180°C (gas evolved); mp >250°C (dec.) if heated slowly from ambient (lit. (8) mp 185-188°C°). δ_H (d_6-DMSO) 7.12 (dd, J = 6 and 1.5 Hz, 1H, Ar<u>H</u>), 7.00 (t of d, J = 6 and 1.5 Hz, 1H, Ar<u>H</u>), 6.94 (s, 2H, Ar<u>H</u>), 6.10 (br d, J = 6Hz, 2H, Ar<u>H</u>), 3.65 (s, 2H, C<u>H</u>$_2$C=O).

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