SYNTHESIS OF NON-STEROIDAL ANTIINFLAMMATORY DRUG ANALOGUES FOR SELECTIVE STUDIES ON THE COX-II ENZYME

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SUMMARY

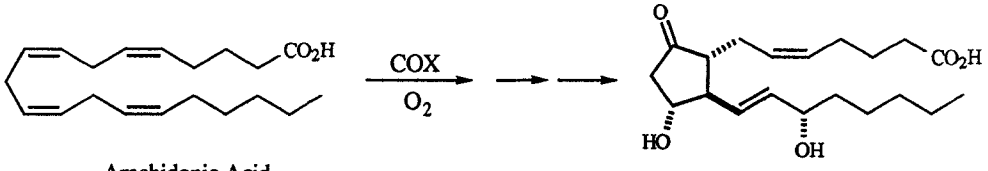
Synthesis of the azido substituted non-steroidal antiinflammatory drug 2-(2,6-dichloroanilino)phenylacetic acid and isotope labeling of this compound have been performed and are described. Initial evaluation of the binding ability and photoreactivity indicates that this compound has potential for photoaffinity labeling as well as enzyme selectivity studies.

Key words: Photoaffinity labeling, COX-II, azido diclofenac

INTRODUCTION

Vane (1) reported that NSAIDs inhibit the enzyme cyclooxygenase (COX) or prostaglandin synthase (PGHS) from producing prostaglandins (see Figure 1), and that these prostaglandins

Figure 1



Arachidonic Acid — Prostaglandin PGE_2

were directly responsible for the inflammatory response (2). A second form of cyclooxygenase has been proposed by several independent groups since 1972 and recently it (COX-II) has been cloned and characterized by Simmons (3).

The medicinal regulation of biological prostaglandin synthesis has been a target for obvious reasons. Prostaglandins have been implicated in: stimulation of smooth muscle; dilation of small arteries and bronchia; lowering of blood pressure; inhibition of gastric secretion, lipolysis, and platelet aggregation; induction of labor or abortion; menstruation; increase in ocular pressure;

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dysmenorrhea; inflammatory reactions; nasal vasoconstriction; kidney function; and in autonomic neurotransmission (4). Cyclooxygenase products have also been implicated in cancer (5) and colon cancer (6) in particular.

Since the NSAIDs have been shown to moderate the biological prostaglandin production, it is no surprise that there are several NSAIDs with demonstrated anticancer activity. On the other hand, it is intriguing that all NSAIDs are not chemotherapeutic toward cancer since they all inhibit COX. One possible explanation for this inconsistency is that the two forms of COX have differing activity, where one enzyme may have more involvement in pharmacological activities than the other. Could selective inhibition of COX-II, for example, lead to improved response to cancer or inflammation or any of the other numerous physiological activities related to prostaglandins?

Another intriguing question is whether differentiation of the medicinal properties of the two prostaglandin synthases can be accomplished by selective inhibition. In order for this to be examined, an assay for differential binding is required. Fortunately, this assay is now available (7). This provides a powerful tool for further research concerning NSAIDs and their mode of action on these enzymes. Rous sarcoma virus ($pp60^{v\text{-}src}$) and other mitogenic agents have been shown to significantly increase the expression of COX-II mRNA. Treatment of these cells with a small subset of the NSAID resulted in significant inhibition of foci formation (8). The most interesting result was the selective binding of diclofenac (2-(2,6-dichloroanilino)phenylacetic acid) to COX-II.

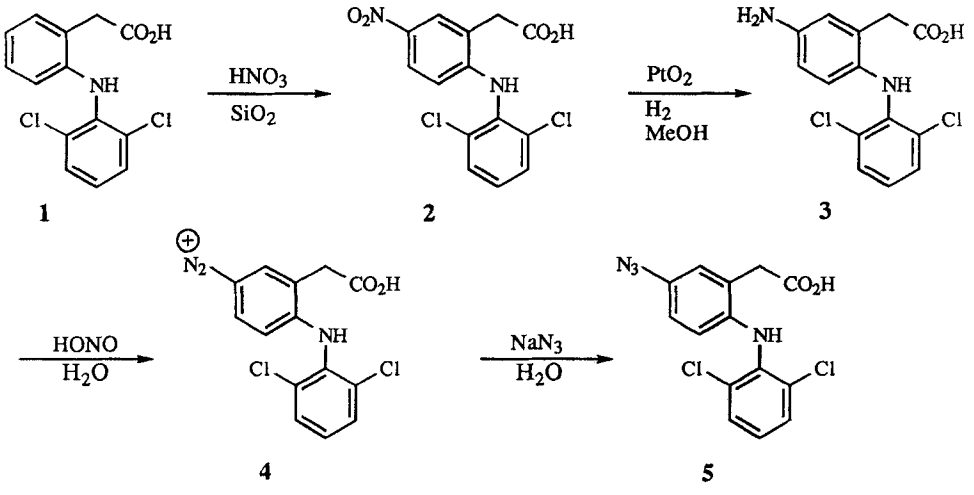
Photoaffinity labeling is one tool for determining active binding sites in enzyme-substrate complexes. Typically the modified substrate is also radiolabeled so that analysis and identification of the substituted, digested enzyme fragments is simplified (9). As a result of the NSAID screening, we have chosen to investigate modification of diclofenac. We report here, the synthesis and photochemical properties of labeled diclofenac.

RESULTS

Azidodiclofenac (see Figure 2), was our initial target compound. We have carried out its synthesis as shown in Figure 2 and we are encouraged by the fact that it has been shown to retain activity with COX-II. In fact, preliminary results indicate that the azido compound in the absence of light would be an effective NSAID itself. Therefore the diclofenac derivatives presumably have potential as pharmaceutical drugs as well. We expect that the substituted compounds synthesized

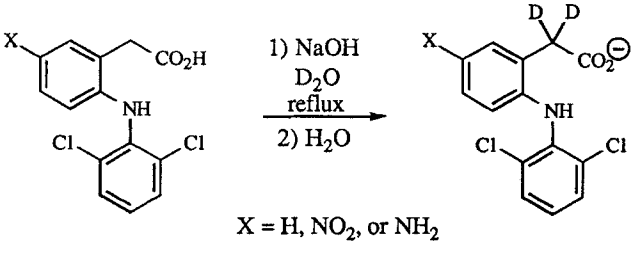
along the pathway (**2** and **3**, Figure 2) to the desired structure will show activity against COX-II. Further testing for drug use is currently underway.

Figure 2



Use of a radiolabeled reagent is standard procedure for the photoaffinity labeling experiment. We have successfully synthesized the isotopically labeled model compound shown in Figure 3. Due to the excellent results for deuteration, we are prepared to apply the same methodology to tritiation.

Figure 3



We have investigated the photolysis of the azide and our preliminary results have been presented (10). Irradiation in CH_2Cl_2/n$BuNH_2$ with wavelength above 300 nm results in conversion of the azido group to a reactive nitrene which ultimately produces a covalent bond with the butyl amine. We have not explored the photobehavior of the substrate-enzyme complex. This aspect of the project should provide information about the COX-II binding site and also provide further data for determining the COX-I/COX-II selectivity of diclofenac derivatives.

In summary, we have: 1) synthesized azidodiclofenac, 2) determined its binding ability with COX-II, 3) synthesized the deuteriolabeled azidodiclofenac precursor, and 4) evaluated the photochemical reactivity of the azide. We will be able to report the results from irradiation of the photoactive analogue and analysis of its photoaffinity labeling in the near future.

We view these results as a significant contribution to the fields of medicinal chemistry and cancer research. Our efforts also improve the basic understanding of the role of cyclooxygenase in a variety of biological functions.

EXPERIMENTAL

Nuclear magnetic resonance (FTNMR) were obtained using a Varian Gemini-200 MHz spectrometer. Spectra were obtained using $CDCl_3$, D_2O or DMSO-d_6 as indicated. Infrared spectra (IR) were obtained on a Perkin Elmer Model 1600 FTIR spectrophotometer. Ultraviolet (UV) spectra were recorded in methanol on a Perkin Elmer Model 320 spectrophotometer. Melting points were determined using a Fisher-Johns hot-stage apparatus and are uncorrected.

Solvents used for photolyses were dried and distilled from CaH_2. Ethyl acetate was distilled from P_2O_5. Pentane was distilled and stored over $CaCl_2$. Chloroform, acetic acid, toluene, methylene chloride, spectrograde acetone, and spectrograde *i*-propyl alcohol were purchased and used without further purification. 2-(2,6-Dichloroanilino)phenylacetic acid sodium salt (Diclofenac Sodium) was purchased from Sigma Chemical Co. and used without further purification.

2-(2,6-Dichloroanilino)-5-nitrophenylacetic acid (2). The diclofenac sodium (0.330 g, 1.04 mmol) was dissolved in 3.0 mL methylene chloride then 0.59 g of nitrated silica gel (11) was added at room temperature. The reaction stirred for 5 minutes then the silica gel was filtered and washed with methylene chloride and the organic layer was concentrated to give a crude yield of 0.291 g (77%) of nitrated diclofenac (**2**). This material as a sodium salt decomposes at high temperatures. The melting point for the protonated acid is 184-187 °C.

The spectral data for compound **2** were: ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1 H, $HNAr_2$), 8.11 (d, J = 2.5 Hz, 1 H, ArH), 7.93 (dd, J = 2.5 and 10 Hz, 1 H, ArH), 7.63 (d, 2 H, J = 10 Hz, ArH), 7.40 (t 1 H, J = 10 Hz, ArH), 6.12 (d, J = 10 Hz, 1H, ArH), 3.84 (s, 2 H, CH_2).

2-(2,6-Dichloroanilino)-5-aminophenylacetic acid (3). In 50 mL methanol was placed 84.0 mg (0.23 mmol) of the nitro substituted diclofenac **2**, 21.5 mg K_2CO_3, and 20 mg PtO_2 (12).

The mixture was pressurized to 45 psi with H_2 and shaken 3 h. The catalyst was then filtered off and the solution concentrated under reduced pressure. The resulting crude product was extracted with methylene chloride then purified by column chromatography on silica gel with ethyl acetate:pentane:acetic acid (30:69.5:0.5) to yield pure amino-diclofenac **3** (95%). Attempts to recrystallize this material were unsuccessful.

The spectral data for compound **3** were: ^{1}H NMR (DMSO-d_6) δ 7.35 (d, 2 H, J = 10 Hz, ArH), 6.88 (t, 1 H, J = 10 Hz, ArH), 6.6 (variable) (bs, 3 H, NH), 6.35 (d, J = 2.9 Hz, 1 H, ArH), 6.19 (dd, J = 2.9 and 8.6 Hz, 1 H, ArH), 6.05 (d, J = 8.6 Hz, 1 H, ArH), 3.55 (s, 2 H, CH_2); IR (KBr) 3500-2500 (br), 2950, 1580, 1510, 1380, 770 cm^{-1}.

2-(2,6-Dichloroanilino)-5-azidophenylacetic acid (5). The amino-diclofenac **3** (84.5 mg, 0.25 mmol) was dissolved in 10% HCl with 0.0235 g (0.341 mmol) sodium nitrite. This was followed by 0.0225 g (0.346 mmol) of sodium azide. The aqueous phase was then made basic with 10% NaOH and washed with methylene chloride. The organic phase from the basic wash was then dried over Na_2SO_4. The solvent was evaporated to give 24.7 mg (32%) of the solid azido subsituted diclofenac, m.p. 80-82 °C.

The spectral data for compound **5** were: ^{1}H NMR (DMSO-d_6) δ 7.53 (d, 2 H, J = 10 Hz, ArH), 7.31 (variable) (s, 1 H, NH), 7.22 (t, 1 H, J = 10 Hz, ArH), 7.14 (d, J = 2.6 Hz, 1 H, ArH), 7.00 (dd, J = 2.6 and 8.3 Hz, 1 H, ArH), 6.27 (d, J = 8.3 Hz, 1 H, ArH), 3.71 (s, 2 H, CH_2); IR (neat) 3200-2500 (br), 2950, 2120, 1500, 1460, 1270, 1000, 730 cm^{-1}.

Photolysis of Azido Diclofenac (5). A CH_2Cl_2:n-$BuNH_2$ (400:1) solution of azide **5** (45 mg in 100 mL) was irradiated with a 450 W Hg Hanovia immersion lamp through quartz for 30 min. The solvent was evaporated to give a dark oil. Analysis by tlc and NMR indicate total consumption of the initial azide and the presence of several new polar products.

The spectral data for the putative amino trapped compound were: ^{1}H NMR (DMSO-d_6) δ 7.5-7.2 (m, 3 H, ArH), 6.87 (m, 1 H), 6.52 (dd, 1 H, J = 16 and 7 Hz, =CH), 6.35 (m, 1 H, =CH), 5.5 (bs, 4 H, NH), 3.65 (bs, 2 H, CH_2), 2.70 (q, 2 H, N-CH_2), 1.6-1.3 (m, 4 H, CH_2-CH_2), 0.95 (t, 3 H, CH_3).

2-(2,6-Dichloroanilino)-5-aminophenyldideuterioacetic acid. Amino-diclofenac **3** (0.110 g, 0.351 mmol) was dissolved in 8 mL D_2O with 5% (w/v) NaOH and refluxed 3 h (14).

The solution was then cooled and acidified with 10% HCl until precipitation of a white solid ceased. The suspension was then extracted with CH_2Cl_2 and the organic phase dried over anhydrous sodium sulfate, then filtered and concentrated to give a white solid. ^1H NMR (200 MHz, $CDCl_3$) showed that the substitution of deuterium at the α position was essentially quantitative. This same procedure successfully deuterated 2-(2,6-dichloroanilino)-5-nitrophenylacetic acid (**2**) and diclofenac (**1**).

2-(2,6-Dichloroanilino)-5-azidophenyldideuterioacetic acid. Deuteration of azido-diclofenac using identical conditions (NaOH, D_2O, reflux) as indicated in the previous procedure gave only decomposition. Stirring at room temperature for 2 weeks resulted in no conversion to the desired dideuterio azide.

ACKNOWLDGEMENT

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REFERENCES

1. Vane, J. R. — Nature 231: 232 (1971).
2. Willis, A. L. — Parmacol. Res. Commun. 2: 297 (1970).
3. Xie, W.; Robertson, D.L.; Simmons, D. L. — Drug Development Research 25: 249 (1992).
4. Merck Index (9th Ed.), Merck & Co., Rahway, New Jersey, 1976.
5. Bennet, A. — The Prostaglandin System, Berti, F. and Velo, G. P. eds, Plenum Press, New York, 417, 1981.
6. Marnett, L. J. — Cancer Res. 52: 5575 (1992).
7. Evett, G. A. Ph.D. Thesis, Brigham Young Univeristy, 1993.
8. Xie, W.; Chipman, J.; Robertson, D. L.; Erikson, R. L.; Simmons, D. L. — Proc. Natl. Acad. Sci. USA 88: 2692 (1991).
9. Bayley, H. — Photogenerated Reagents in Biochemistry and Molecular Biology, Elsevier, Amsterdam, 1983.
10. Fleming, S. A.; Reber, J. Abstract CHED # 0254, — 209th American Chemical Society National Meeting, Anaheim, CA, 1995.
11. Tapia, R.; Torres, G.; Valderrama, J. A. — Syn. Commun. 16: 681 (1986).
12. Skiles, J. W.; Cava, M. P. — J. Org. Chem. 44: 409 (1979).
13. Castell, J. V.; Martinez, L. A.; Miranda, M. A.; Tárrega, P. — Journal of Labelled Compounds & Radiopharmaceuticals 34: 93 (1993).