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Research Article

Improved Delivery Through Biological Membranes. XXXVII. Synthesis and Stability of Novel Redox Derivatives of Naproxen and Indomethacin[1]

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Several novel bioreversible redox derivatives of the nonsteroidal antiinflammatory drugs (NSAID) naproxen and indomethacin were synthesized. The stability of these dihydropyridine-NSAID derivatives their synthetic precursors, and predicted products of oxidative metabolism, the corresponding pyridinium salts, was determined in buffer, human and rat blood, and rat organ homogenate. The dihydropyridines exhibited the expected stability profiles in the media examined: oxidation, water addition, and/or ester hydrolysis. The corresponding pyridinium salts were quite stable in biomedia, ester hydrolysis being the primary route of decomposition. The results of this study may be useful in selecting suitable candidates for selective delivery of naproxen and indomethacin across the blood–brain barrier.

KEY WORDS: blood–brain barrier; selective delivery; bioreversible naproxen, indomethacin derivatives.

INTRODUCTION

The need for more effective and less toxic pharmacological agents is unquestionable. Safe and effective drugs are sought in an effort to improve on the treatments currently available. One area of particular need is in the effective delivery of drugs to the brain, a task that is often made difficult by the physical presence of the blood–brain barrier (1). This lipid bilayer allows only the most lipophilic of compounds to enter or leave the brain by passive diffusion, thereby severely limiting the usefulness of many drugs that may be used to treat brain disorders as diverse as Alzheimer's disease, cancer, inflammation, and neurosyphillis. In order to overcome this perplexing situation, various procedures have been attempted, including direct intrathecal or intraventricular injection or a large increase in the dosage of a particular drug in the hope that some small percentage of the active compound will reach the brain.

In recent years rational approaches to drug design (2–7) included the pyridinium salt dihydropyridine redox drug delivery system developed by Bodor (8,9). Ideally, a carrier system will increase the brain specificity of the compound and maintain a therapeutically active concentration of the drug in the brain over an extended period of time: peripheral tissues, meanwhile, should be spared unwanted prolonged exposure to the drug because of relatively rapid elimination from the general circulation. This concept has been successfully applied to several classes of drugs (10).

These objectives are achieved by administering the highly lipophilic chemical delivery system that rapidly distributes in both the blood and the brain. Oxidation of the dihydropyridine moiety of the chemical delivery system then occurs via the $NAD^+ \leftrightarrows NADH$ redox enzyme system, resulting in the formation of the corresponding pyridinium salt. This more hydrophilic compound is rapidly eliminated from the body, resulting in a lower circulating level of the parent drug. In the brain, however, the polar drug–carrier combination is locked in by the blood–brain barrier (BBB). This compound then serves as a source for the sustained release of the active parent drug in the brain.

The slow release of the drug depends on the rate of its enzymatic hydrolysis. The structures of both the carrier and the parent compound affect the rate of hydrolytic cleavage of the drug from the carrier. Once the drug–carrier combination is hydrolyzed the remaining carrier portion, due in part to its lower molecular weight, is actively transported out of the brain (11).

In previous examples of brain targeting of drugs using the chemical delivery system approach, the carrier moiety was a 1-methyl-1,4-dihydro nicotinic acid derivative, which upon oxidation and hydrolysis, yields trigonelline. Consequently only those drugs with derivatizable amine or hydroxy functions were studied, in the form of amide or ester derivatives, respectively, of trigonelline. The present work expands substantially the applicability of the redox delivery

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system to include carriers derivatized with hydroxyalkyl groups of various chain lengths and pyridine-ring substitution positions. The carriers were developed in order to effect brain delivery of drugs containing a carboxylic acid functional group. This goal is accomplished by first attaching the drug via an ester linkage to a carrier that contains a pyridine ring capable of participating in a pyridinium salt ⇆ dihydropyridine redox-type reaction.

The nonsteroidal agents naproxen and indomethacin are arylacetic acids and exhibit analgesic, antiinflammatory, and antipyretic behavior. At physiological pH, both naproxen and indomethacin exist primarily as carboxylate ions and consequently, do not readily cross the BBB. The antiinflammatory action of nonsteroidal compounds such as naproxen and indomethacin is due to peripheral mediation of prostaglandin synthesis. It has also been shown that these compounds inhibit prostaglandin synthesis at the central as well as the peripheral level. This central activity is responsible for the antipyretic properties of naproxen and indomethacin, effected by inhibition of prostaglandin synthesis within the hypothalamus (15).

The most common adverse effects associated with acidic nonsteroidal agents are gastrointestinal (GI) disturbances such as nausea, indigestion, heartburn, abdominal pain, and reactivation of latent peptic ulcers. Consequently, a brain-specific delivery system as described earlier could provide enhanced centrally mediated antipyretic activity while at the same time minimizing or eliminating peripheral (GI) toxicity. This study was performed to assess the feasibility of synthesizing brain-targeted delivery systems for naproxen and indomethacin (Fig. 1). The behavior of the dihydropyridines and the corresponding pyridinium salts was examined in various matrices in order to gain some insight into the comparative stability within this series of compounds, a new class of carrier molecules.

MATERIALS AND METHODS

Melting points were uncorrected and were determined using an Electrothermal melting point apparatus, equipped with the manufacturer's calibrated thermometer. Elemental analyses were performed by Atlantic Microlab Inc. (Atlanta, Georgia). Proton NMR spectra were obtained using a Varian EM 360A or EM 390 spectrometer. Chemical shifts are reported as parts per million units, downfield from tetramethylsilane used as an internal standard. Ultraviolet spectroscopy was performed on a Hewlett Packard 8451A diode array spectrophotometer. Chemicals were supplied by Aldrich Chemical Company except naproxen and indomethacin, which were purchased from Sigma Chemical Co.

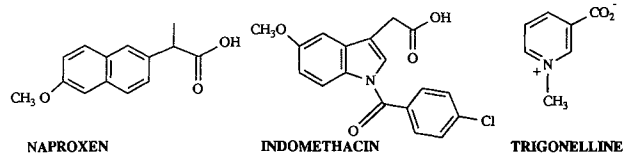

Fig. 1. Structures of key compounds naproxen, indomethacin, and trigonelline.

SYNTHESIS

3-Carbamoyl-1-(3-hydroxypropyl)pyridinium bromide (1)

Acetone (50 ml) was added to a flask containing 3-bromopropanol (13.9 g, 0.10 mol) and nicotinamide (12.2 g, 0.10 mol). The mixture was heated at reflux for 6 hr. Upon cooling the product crystallized and the off-white solid was collected and dried under nitrogen. Recrystallization from a mixture of 2-propanol:ethanol (3:1) gave the final product (12.2 g, 46.7%), mp 124–126°C.

UV (CH_3OH): 222 and 268 nm.

^1H NMR (d_6-DMSO) δ 9.7–9.9 (bs, 1H, pyridine H-2), 9.3–9.6 (d, 1H, pyridine H-6), 9.0–9.3 (m, 1H, pyridine H-4), 8.6–8.9 (bs, 1H, NH), 8.1–8.6 (m, 2H, pyridine H-5 and NH), 4.7–5.2 (t, 2H, N-CH_2), 4.3–4.6 (bs, 1H, OH), 3.4–3.7 (t, 2H, O-CH_2), 2.0–2.5 (p, 2H, CH_2).

Anal. ($C_9H_{13}BrN_2O_2$). Calculated: C, 41.40; H, 5.02; Br, 30.60; N, 10.73. Found: C, 41.26; H, 5.07; Br, 30.66; N, 10.69.

3-[(2-Hydroxyethyl)carbamoylpyridine (2)

A neat mixture of 2-aminoethanol (6.1 g, 0.10 mol) and ethyl nicotinate (15.1 g, 0.10 mol) was heated at reflux overnight. As the mixture was cooled the product precipitated as a crystalline solid that was collected by filtration, washed with ether, and then recrystallized from 2-propanol/ether to give 10.7 g (64.5%) of 2, mp 88.5–89.5°C.

UV (CH_3OH): 222 nm.

^1H NMR (d_6-DMSO) δ 9.0–9.2 (bs, 1H, pyridine H-2), 8.5–8.9 (m, 2H, pyridine H-6 and NH), 8.2–8.4 (m, 1H, pyridine H-4), 7.4–7.7 (m, 1H, pyridine H-5), 4.8–5.0 (t, 1H, OH), 3.3–3.8 (m, 4H, $(CH_2)_2$).

Anal. ($C_8H_{10}N_2O_2$). Calculated: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.73; H, 6.11; N, 16.82.

3-[(3-Hydroxypropyl)carbamoylpyridine (3)

A mixture of ethyl nicotinate (15.12 g, 0.10 mol) and 3-aminopropanol (8.2 g, 0.11 mol) was heated at reflux in toluene (50 ml). An azeotrope of ethanol/toluene was removed under reduced pressure every 12 hr and an equal amount of fresh toluene was added. The reaction was continued for 2 days. The solvent was completely removed and the thick oily residue was distilled using a Gugelrohr apparatus. The compound that was collected, bp 145–155°C at 0.1-mm pressure, was a light yellow, viscous oil that solidified into a wax-like substance on freezing. The reaction yielded 16.0 g (88.8%) of 3.

UV (CH_3OH): 226 nm.

1H NMR ($CDCl_3$) δ 9.0–9.2 (s, 1H, pyridine H-2), 8.6–8.8 (d, 1H, pyridine H-6), 8.2–8.5 (bt, 1H, NH), 8.1–8.3 (d, 1H, pyridine H-4), 7.3–7.5 (m, 1H, pyridine H-5), 4.8–5.0 (s, 1H, OH), 3.4–3.9 (m, 4H, CH_2–O and CH_2–N), 1.6–2.1 (p, 2H, CH_2).

Anal. ($C_9H_{12}N_2O_2 \cdot \frac{1}{4}H_2O$). Calculated: C, 58.52; H, 6.82; N, 15.16. Found: C, 58.46; H, 6.94; N, 15.12.

(+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid, Ester with Chloromethanol (4)

Previously prepared chloromethyl chlorosulfate (12)

(1.9 g, 12 mmol) in methylene chloride (5 ml) was added dropwise to a mixture of naproxen (2.3 g, 10 mmol), sodium bicarbonate (3.2 g, 3.8 mmol), tetrabutylammonium hydrogen sulfate (0.68 g, 2.0 mmol) in water and methylene chloride (10 ml of each), using the method of Binderup and Hansen (12). The reaction was followed by thin-layer chromatography (Tlc) using hexanes:chloroform (3:2) as eluent. The product had an R_f of 0.35 in this system. The resulting white solid compound was dissolved in hot petroleum ether and filtered to remove any unreacted naproxen. The solvent was removed under reduced pressure and the solid product was recrystallized from 2-propanol giving 1.9 g (67%) of **4**, mp 67–68°C.

UV (CH_3OH): 224, 266, and 332 nm.

^1H NMR ($CDCl_3$) δ 7.0–7.8 (m, 6H, napthalene protons), 5.5–5.8 (s, 2H, CH_2), 3.7–4.1 (m, 4H, CH, and CH_3-O), 1.4–1.7 (d, 3H, CH_3).

Anal. ($C_{15}H_{15}ClO_3$). Calculated: C, 64.64, H, 5.42, Cl, 12.72. Found: C, 64.75, H, 5.45, Cl, 12.65.

(+)-6-Methoxy-α-methyl-2-naphthaleneacetic acid, Ester with *N*-(2-Hydroxyethyl)nicotinamide (5)

Naproxen (2.30 g, 10.0 mmol) was coupled with compound 2 (1.71 g, 10.0 mmol) using DCC (2.30 g, 11.0 mmol) and DMAP (122 mg, 1.00 mmol) in acetonitrile (150 ml). The reaction was stirred at room temperature for 48 hr. The precipitated DCU was removed by filtration. The solvent was removed under reduced pressure and the residual clear oil was stirred with anhydrous ether. The resulting white solid was vacuum-filtered, washed with ether, and air-dried. The compound was recrystallized from 2-propanol. The final product was filtered, washed successively with 0.5% aqueous sodium bicarbonate, water, and ether, and then dried in a desiccator over P_2O_5. The recrystallized material weighed 2.40 g, resulting in an overall yield of 63.4% of **5**, mp 79–82°C.

UV (CH_3OH): 226, 264, and 332 nm.

^1H NMR ($CDCl_3$) δ 8.8–9.0 (bs, 1H, pyridine H-2), 8.5–8.8 (d, 1H, pyridine H-6), 7.0–8.0 (m, 9H, pyridine H-4 and H-5, naphthalene protons, and NH), 4.1–4.4 (t, 2H, CH_2-O), 3.6–4.0 (m, 4H, CH and CH_3–O), 3.4–3.7 (t, 2H, CH_2–N), 1.4–1.7 (d, 3H, CH_3).

Anal. ($C_{22}H_{22}N_2O_4$). Calculated: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.92; H, 5.88; N, 7.39.

(+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid, Ester with *N*-(3-Hydroxypropyl)nicotinamide (6)

A reaction of naproxen (2.30 g, 10.0 mmol) and compound 3 (1.80 g, 10.0 mmol) was carried out in acetonitrile, using DCC (2.26 g, 11.0 mmol) and DMAP (122 mg, 1.00 nmol) as a coupling agent. After 24 hr the reaction mixture was filtered. The solvent was removed from the filtrate under reduced pressure, giving an oily residue. Column chromatographic separation using Mallinckrodt silica gel (100–200 mesh, 60 Å special) and a mobile phase of chloroform:tetradydrofuran (4:1) allowed isolation of the product as a clear oil that crystallized overnight in a vacuum desiccator, giving 2.60 g (66.7%) of **6**, mp 72–75°C.

UV (CH_3OH): 224, 264, and 332 nm.

^1H NMR ($CDCl_3$) δ 8.9–9.1 (bs, 1H, pyridine H-2), 8.5–8.8 (d, 1H, pyridine H-6), 7.9–8.2 (d, 1H, pyridine H-4), 7.5–7.8 (m, 3H, naphthalene protons), 7.0–7.5 (m, 5H, pyridine H-5, NH, and naphthalene protons), 4.0–4.3 (t, 2H, CH_2–O), 3.6–4.0 (m, 4H, CH_3–O, and CH), 3.1–3.5 (q, 2H, CH_2–N), 1.6–2.1 (p, 2H CH_2), 1.4–1.7 (d, 3H, CH_3).

Anal. ($C_{23}H_{24}N_2O_4$). Calculated: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.46; H, 6.18; N, 7.09.

1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic Acid, Ester with *N*-(2-Hydroxyethyl)nicotinamide (7)

A reaction of indomethacin (1.79 g, 5.00 mmol) and compound 2 (0.830 g, 5.00 mmol) was carried out using DCC (1.10 g, 5.50 mmol) as the coupling agent in acetonitrile. The precipitated CDU was removed by vacuum filtration after 48 hr. The solvent was removed from the filtrate under reduced pressure. The product solidified on stirring with anhydrous ether and was collected by filtration, air-dried, and recrystallized from ethanol/ether to give 1.65 g (65.2%) of **7**, mp 123–125°C.

UV (CH_3OH): 222 and 320 nm.

^1H NMR ($CDCl_3$) δ 8.7–8.9 (bs, 1H, pyridine H-2), 8.6–8.8 (d, 1H, pyridine H-6), 7.7–8.0 (bd, 1H, pyridine H-4), 7.2–7.7 (m, 5H, phenyl protons and pyridine H-5), 6.4–7.0 (m, 4H, indole protons and NH), 4.2–4.4 (t, 2H, CH_2–O), 3.5–3.9 (m, 7H, CH_3–O, CH_2–N, CH_2), 2.2–2.4 (s, 3H, CH_3).

Anal. ($C_{27}H_{24}ClN_3O_5 \cdot \frac{1}{2}H_2O$). Calculated: C, 62.98; H, 4.89; N, 8.16. Found: C, 63.27; H, 4.91; N, 8.49.

1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic Acid, Ester with *N*-(3-Hydroxypropyl)nicotinamide (8)

A reaction of indomethacin (3.58 g, 10.0 mmol) and compound 3 (1.80 g, 10.0 mmol) with DCC (2.26 g, 11.0 mmol) and DMAP (0.12 g, 1.0 mmol) was carried out using the same method as described for compound **6**. The white solid product was obtained without column chromatography and then recrystallized from 2-propanol/ether and again from 2-propanol. The reaction yielded 3.60 g (69.2%) of **8**, mp 122–123°C.

UV (CH_3OH): 222 and 320 nm.

^1H, NMR ($CDCl_3$) δ 8.9–9.2 (bs, 1H, pyridine H-2), 8.6–8.8 (bd, 1H, pyridine H-6), 7.9–8.3 (bd, 1H, pyridine H-4), 7.2–7.9 (m, 6H, phenyl protons, pyridine H-5, and NH), 6.5–7.1 (m, 3H, indole protons), 4.1–4.5 (t, 2H, CH_2–O), 3.2–4.0 (m, 7H, CH_3–O, CH_2–N, and CH_2–CO), 2.2–2.5 (s, 3H, CH_3).

Anal. ($C_{28}H_{26}ClN_3O_5$). Calculated: C, 64.68; H, 5.04; Cl, 6.82; N, 8.08. Found: C, 64.55; H, 5.10; Cl, 6.79; N, 8.03.

3-Carbamoyl-1-hydroxymethylpyridinium chloride, Ester with (+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid (9)

Nicotinamide (0.24 g, 2.0 mmol) was dissolved in acetone. The chloromethyl ester of naproxen, compound **4** (0.56 g, 2.0 mmol + 1% excess), was then added and the solution was stirred at reflux for 48 hr. The precipitated white product was vacuum filtered, washed with ether, and dried. The mother liquor was reduced to dryness and the resulting solid recrystallized from 2-propanol, giving a second crop of crystals. The yield of **9** was 0.49 g or 61% overall; mp 227–229°C.

UV (CH_3OH): 226, 268, and 332 nm.

^{1}H NMR (d_6-DMSO/CD_3OD) δ 9.5–9.6 (bs, 1H, pyridine H-2), 9.0–9.3 (m, 2H, pyridine H-6 and H-4), 8.1–8.4 (m, 1H, pyridine H-5), 7.6–7.9 (m, 4H, naphthalene protons, and NH), 7.1–7.5 (m, 4H, naphthalene protons, and NH), 6.5–6.6 (s, 2H, CH_2), 3.9–4.3 (q, 1H, CH), 3.8–3.9 (s, 3H, CH_3-O), 1.5–1.6 (d, 3H, CH_3).

Anal. ($C_{21}H_{21}ClN_2O_4$). Calculated: C, 62.92; H, 5.28; Cl, 8.84; N, 6.99. Found: C, 62.73; H, 5.30; Cl, 8.94; N, 6.94.

3-Carbamoyl-1-(3-hydroxpropyl)pyridinium Bromide, Ester with (+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid (10)

Naproxen (2.5 g, 11 mmol) and compound 1 (2.6 g, 10 mmol) were dissolved in a minimum amount of dimethylformamide (200 ml), to which DMAP (130 mg, 1.1 mmol) and DCC (2.3 g, 11 mmol) were added. The solution was stirred at room temperature for 2 days. The crude product was isolated using the same method as for compound 8. The solid was powdered and washed well with anhydrous ether. The crude product was recrystallized from ethanol, and upon partial cooling the mother liquor was decanted from a reddish brown impurity that first solidified. Additional cooling of the mother liquor, along with brief scratching, gave a large quantity of fluffy tan crystals, which were again recrystallized from ethanol, giving 4.0 g (85%) of **10**, mp 152–154°C.

UV (CH_3OH): 224, 266, and 332 nm.

^{1}H NMR (d_6-DMSO) δ 9.5–9.8 (bs, 1H, pyridine H-2), 9.1–9.4 (bd, 1H, pyridine H-6), 8.9–9.2 (bd, 1H, pyridine H-4), 8.5–8.8 (bs, 1H, NH), 8.0–8.5 (m, 2H, pyridine H-5, and NH), 7.0–8.0 (m, 6H, naphthalene protons), 4.6–5.0 (t, 2H, CH_2–N), 4.0–4.4 (t, 2H, CH_2–O), 3.6–4.0 (m, 4H, CH, and CH_3–O), 2.1–2.6 (m, 2H, CH_2), 1.3–1.6 (d, 3H, CH_3).

Anal. ($C_{23}H_{25}BrN_2O_4$). Calculated: C, 58.36; H, 5.32; Br, 16.88; N, 5.92. Found: C, 58.23; H, 5.34; Br, 16.95; N, 5.86.

3-[(2-Hydroxyethyl)carbamoyl]-1-methylpyridinium Iodide, Ester with (+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid (11)

The quaternization of the naproxen ester, compound 5 (1.0 g, 2.6 mmol), was carried out using methyl iodide (2.3 g, 16 mmol) in acetone (45 ml). The solution was heated at reflux for 24 hr. The precipitated product was filtered and the off-white powder was dried. The material weighed 2.2 g and was found to be analytically pure without recrystallization. The solvent was removed from the acetone filtrate and the residue was solidified with anhydrous ether. The resulting dark yellow powder was dissolved in water and washed with ether (4 × 30 ml). The water was then removed under vacuum, giving 0.2 g of a light yellow powder. The overall yield of the reaction was 93%, mp 169–170°C.

UV (CH_3OH): 226, 266, and 332 nm.

^{1}H NMR (d_6-DMSO) δ 9.3–9.5 (bs, 1H, pyridine H-2), 9.0–9.3 (m, 2H, pyridine H-6, and NH), 8.7–9.0 (bd, 1H, pyridine H-4), 8.1–8.5 (m, 1H, pyridine H-5), 7.0–7.9 (m, 6H, naphthalene protons), 4.3–4.5 (s, 3H, CH_3–N), 4.1–4.4 (t, 2H, CH_2–O), 3.4–4.1 (m, 6H, CH, CH_3–O, and CH_2–N), 1.4–1.7 (d, 3H, CH_3).

Anal. ($C_{23}H_{25}IN_2O_4$). Calculated: C, 53.09; H, 4.84; I, 24.39; N, 5.38. Found: C, 52.98; H, 4.85; I, 24.29; N, 5.34.

3-[(3-Hydroxypropyl)carbamoyl]-1-methylpyridinium Iodide, Ester with (+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid (12)

The ester of naproxen, compound 6 (2.3 g, 5.9 mmol), was dissolved in acetone. Methyl iodide (4.0 g, 29 mmol) was added and the solution was heated at reflux for 48 hr. The reaction mixture was cooled, taken to an oily foaming residue upon removal of the solvent under reduced pressure, and then dried further on a vacuum pump. The product was dissolved in acetone (2 × 25 ml) and each time the solvent was removed under reduced pressure. The product was then dried using a vacuum pump, giving 2.9 g (93%) of **12**, mp 100–102°C.

UV (CH_3OH): 224, 266, and 332 nm.

^{1}H NMR (d_6-DMSO) δ 9.4–9.5 (s, 1H, pyridine H-2), 8.8–9.3 (m, 3H, pyridine H-6 and H-4, and NH), 8.2–8.4 (m, lH, pyridine H-5), 7.1–8.0 (m, 6H, naphthalene protons), 4.4–4.5 (s, 3H, CH_3-N), 4.1–4.3 (t, 2H, CH_2–O), 3.8–4.1 (m, 4H, CH, and CH_3–O), 3.2–3.5 (t, 2H, CH_2–N), 1.7–2.1 (p, 2H, CH_2), 1.4–1.7 (d, 3H, CH_3).

Anal. ($C_{24}H_{27}IN_2O_4$). Calculated: C, 53.94; H, 5.09; I, 23.75; N, 5.24. Found: C, 54.00; H, 5.13; I, 23.81; N, 5.22.

3-[(2-Hydroxyethyl)carbamoyl]-1-methylpyridinium iodide, Ester with 1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic Acid (13)

The quaternization of compound 7 (0.50 g, 1.0 mmol) was carried out in acetone using methyl iodide (1.7 g, 12 mmol) at reflux temperature overnight. The solvent was removed under reduced pressure and a yellow solid was obtained. The product was recrystallized using ethanol and a small amount of ether, giving 0.43 g (66%) of **13**, mp 178–179°C.

UV (CH_3OH): 220 and 320 nm.

^{1}H NMR (d_6-DMSO) δ 9.3–9.5 (bs, 1H, pyridine H-2), 9.1–9.3 (m, 2H, pyridine, H-6, and NH), 8.8–9.0 (bd, 1H, pyridine H-4), 8.1–8.4 (m, 1H, pyridine H-5), 7.6–7.8 (s, 4H, phenyl protons), 6.6–7.2 (m, 3H, indole protons), 4.4–4.6 (s, 3H, CH_3–N), 4.2–4.5 [t, 2H, CH_2–O], 3.5–4.0 (m, 7H, CH_3–O, CH_2–CO, and CH_2–N), 2.2–2.3 (s, 3H, CH_3).

Anal. ($C_{28}H_{27}ClIN_3O_5$). Calculated: C, 51.91; H, 4.20; Cl, 5.47; I, 19.59; N, 6.48. Found: C, 51.80; H, 4.24; Cl, 5.41; I, 19.46; N, 6.42.

3-[(3-Hydroxypropyl)carbamoyl]-1-methylpyridinium Iodide, Ester with 1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic Acid (14)

The ester of indomethacin, compound 8 (3.1 g, 6.0 mmol), was dissolved in a minimum of acetone. A twofold excess of methyl iodide (2.5 g, 18 mmol) was added to the solution, which was then heated to reflux. The reaction was continued for 24 hr. Approximately one-half of the reaction solvent was removed under reduced pressure and the precipitated light yellow product was collected by filtration, washed with ether, and dried in a vacuum desiccator, giving 3.9 g (97%) of **14**, mp 168–169°C.

UV (CH_3OH): 222 and 320 nm.

^{1}H NMR (d_6-DMSO) δ 9.3–9.5 (bs, 1H, pyridine H-2), 8.8–9.2 (m, 3H, pyridine H-6 and H-4, and NH), 8.2–8.4 (m,

1H, pyridine H-5), 7.5–7.8 (s, 4H, phenyl protons), 6.6–7.2 (m, 3H, indole protons), 4.3–4.5 (s, 3H, CH_3–N), 4.0–4.3 (t, 2H, CH_2–O), 3.6–4.0 (bs, 5H, CH_3–O, and CH_2CO), 3.2–3.5 (t, 2H, CH_2–N), 2.2–2.4 (s, 3H, CH_3), 1.7–2.1 (p, 2H, CH_2).

Anal. ($C_{29}H_{29}ClIN_3O_5$). Calculated: C, 52.62; H, 4.42; Cl, 5.36; I, 19.17; N, 6.35. Found: C, 52.55; H, 4.46; Cl, 5.29; I, 19.19; N, 6.34.

(+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid, Ester with 1,4-Dihydro-1-hydroxymethylnicotinamide (15)

The quaternary ester 9 of naproxen (200 mg, 0.5 mmol) was dissolved in a mixture of acetonitrile:2-propanol, 6:1. An excess of previously prepared 1-benzyl-1,2-dihydroisonicotinamide (14) (200 mg, 0.8 mmol) was added and the reaction was allowed to proceed for 1 hr at room temperature under a nitrogen atmosphere. When the reaction was complete the solvent was removed under reduced pressure and the resulting orange foam was dissolved in methylene chloride and filtered to remove the quaternary side product. The solvent was again removed at reduced pressure and the oily residue was dissolved in chloroform and passed down a short column of neutral alumina. The appropriate fraction was collected and the solvent removed under reduced pressure. The oily product was triturated with anhydrous ether and the ether was then removed at reduced pressure. This gave a hygroscopic yellow foam. However, NMR evidence indicated that the final compound had partially hydrolyzed on the column.

UV (CH_3OH): 228, 264, 334, and 346 nm.

^1H NMR ($CDCl_3$) δ 7.0–7.8 (m, 7H, naphthalene protons and dihydropyridine H-2), 5.5–5.9 (m, 1H, dihydropyridine H-6), 5.1–5.5 (bs, 2H, NH_2), 4.6–5.0 (m, 1H, dihydropyridine H-5), 4.4–4.6 (s, 2H, CH_2), 3.8–4.0 (s, 3H, CH_3–O), 3.3–3.7 (q, 1H, CH), 3.0–3.3 (bs, 2H, dihydropyridine H-4), 1.4–1.7 (d, 3H, CH_3).

Anal. ($C_{21}H_{22}N_2O_4 \cdot H_2O$). Calculated: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.50; H, 6.68; N, 7.99.

(+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid, Ester with 1,4-Dihydro-1-(3-hydroxypropyl)nicotinamide (16)

The quaternary ester, compound 10 (470 mg, 1.0 mmol), was dissolved in degassed water (70 ml), and sodium dithionite (520 mg, 3.0 mmol) and sodium bicarbonate (420 mg, 5.0 mmol) were added at once. A layer of ether was added and the reaction was allowed to proceed under a nitrogen atmosphere for 60 min. The reaction mixture was extracted with methylene chloride (4 × 30 ml). The extracts were combined and washed with water (50 ml) and dried over magnesium sulfate. The solvent was removed under reduced pressure giving an oily foam. The product was repeatedly redissolved in methylene chloride and dried on a vacuum pump until the compound was obtained as a light yellow solid foam. The final yield was 180 mg (46%) of **16**, mp 42–46°C.

UV (CH_3OH): 224, 264, 334, and 356 nm.

^1H NMR ($CDCl_3$) δ 6.8–7.8 (m, 7H, naphthalene protons, and dihydropyridine H-2), 5.2–5.5 (m, 3H, dihydropyridine H-6 and NH_2), 4.3–4.7 (m, 1H, dihydropyridine H-5), 3.7–4.3 (m, 6H, CH_2–O, CH_3–O, and CH), 2.7–3.2 (m, 4H, dihydropyridine H-4, and CH_2–N), 1.3–2.0 (m, 5H, CH_2 and CH_3).

Anal. ($C_{23}H_{26}N_2O_4 \cdot H_2O$). Calculated: C, 66.97; H, 6.84; N, 6.79. Found: C, 67.02; H, 6.57; N, 6.74.

(+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid, Ester with 1,4-Dihydro-*N*-(2-hydroxyethyl)-1-methylnicotinamide (17)

The quaternary salt 11 (780 mg, 1.5 mmol) was dissolved in degassed, deionized water (200 ml) and acetonitrile (10 ml). Sodium dithionite (780 mg, 4.5 mmol) and sodium bicarbonate (630 mg, 7.5 mmol) were combined and added to the solution at room temperature. The reaction was continued for 1 hr, and nitrogen gas was slowly bubbled through the reaction mixture. The partially precipitated product was extracted with ether (8 × 30 ml) and then isolated in the same way as described for **16**. The resulting foam was rinsed with anhydrous ether (3 ml) and the solvent was removed under vacuum, leaving 390 mg (66%) of 17, as an oil.

UV (CH_3OH): 224, 264, 334, and 358 nm.

^1H NMR ($CDCl_3$) δ 7.0–7.9 (m, 6H, naphthalene protons), 6.7–7.0 (bs, 1H, dihydropyridine H-2), 5.1–5.7 (m, 2H, dihydropyridine H-6, and NH), 4.2–4.6 (m, 1H, dihydropyridine H-5), 4.0–4.3 (t, 2H, CH_2–O), 3.6–4.0 (m, 4H, CH_3–O, and CH), 3.3–3.7 (t, 2H, CH_2–N), 2.6–3.0 (s, 3H, CH_3–N), 2.4–2.6 (bs, 2H, dihydropyridine H-4), 1.3–1.7 (d, 3H, CH_3).

Anal. ($C_{23}H_{26}N_2O_4 \cdot H_2O$). Calculated: C, 66.97; H, 6.84; N, 6.79. Found: C, 67.11; H, 6.69; N, 6.63.

(+)-6-Methoxy-α-methyl-2-naphthaleneacetic Acid, Ester with 1,4-Dihydro-*N*-(3-hydroxypropyl)-1-methylincotinamide (18)

Compound 12 (530 mg, 1.0 mmol) dissolved in degassed water (150 ml) was washed with ether (50 mmol), and the organic phase was discarded. The aqueous layer was transferred to a flash and sodium dithionite (520 mg, 3.0 mmol) and sodium bicarbonate (420 mg, 5.0 mmol) were added at once. The reaction was run and the crude product isolated in the same way as described for **16**. The crude product was an orange oil. Chloroform was used to dissolve and then elute the final product from a short column of neutral alumina. The fraction which contained the dihydro compound was collected and the solvent was removed under reduced pressure. The product was then dried on a vacuum pump, yielding 300 mg (73%) of **18** as a yellow hygroscopic foam.

UV (CH_3OH): 224, 264, 334, and 354 nm.

^1H NMR ($CDCl_3$) δ 7.0–7.9 (m, 6H, naphthalene protons), 6.9–7.1 (bs, 1H, dihydropyridine H-2), 5.3–5.8 (m, 2H, dihydropyridine H-6, and NH), 4.4–4.8 (m, 1H, dihydropyridine H-5), 4.0–4.3 (t, 2H, CH_2–O), 3.6–4.0 (m, 4H, CH_3–O, and CH), 3.1–3.4 (t, 2H, CH_2–N), 2.8–3.1 (bs, 2H, dihydropyridine H-4), 2.8–3.0 (s, 3H, CH_3–N), 1.4–2.0 (m, 5H, CH_2, and CH_3).

Anal. ($C_{24}H_{28}N_2O_4 \cdot \frac{1}{2}H_2O$). Calculated: C, 69.04; H, 7.00; N, 6.71. Found: C, 69.40; H, 7.23; N, 6.39.

1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic Acid, Ester with 1,4-dihydro-*N*-(2-hydroxyethyl)-1-methylnicotinamide (19)

The indomethacin quaternary carrier, 13 (140 mg, 0.22 mmol), was dissolved in a minimum amount of degassed

water:acetonitrile (8:2). Sodium bicarbonate (91 mg, 1.1 mmol) and sodium dithionite (110 mg, 0.65 mmol) were added to the solution while stirring at 0°C. The solution was then allowed to warm to room temperature. The reaction was continued and the product isolated in the same way as described for **17**. The resulting oil was dissolved in acetone and the solvent was removed (2 × 10 ml) under reduced pressure, giving **19** as a dry foam, 92 mg (82%), mp 60–65°C.

UV (CH_3OH): 220 and 332 nm.

^1H NMR ($CDCl_3$) δ 7.3–7.9 (q, 4H, phenyl protons), 6.5–7.1 (m, 4H, indole protons, and dihydropyridine H-2), 5.5–5.8 (m, 1H, dihydropyridine H-6), 5.2–5.4 (bs, 1H, NH), 4.4–4.8 (m, 1H, dihydropyridine H-5), 4.1–4.4 (t, 2H, CH_2–O), 3.8–4.0 (s, 3H, CH_3–O), 3.6–3.8 (s, 2H, CH_2–CO), 3.4–3.7 (m, 2H, CH_2–N), 2.8–3.0 (s, 3H, CH_3–N), 2.6–2.9 (bs, 2H, dihydropyridine H-4), 2.3–2.5 (s, 3H, CH_3).

Anal. ($C_{28}H_{28}ClN_3O_5 \cdot 2H_2O$). Calculated: C, 60.27; H, 5.78; Cl, 6.35; N, 7.53. Found: C, 60.14; H, 5.73; Cl, 6.11; N, 7.24.

1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic Acid, Ester with 1,4-Dihydro-*N*-(3-hydroxypropyl)-1-methylnicotinamide (20)

The quaternary ester, **14** (660 mg, 1.0 mmol), was dissolved in degassed, deionized water (80 ml) and acetonitrile (5 ml). A mixture of sodium dithionite (520 mg, 3.0 mmol) and sodium bicarbonate (420 mg, 5.0 mmol) was added to the solution. The reaction was continued and the product isolated and solidified using the same method as described for **16**. Residual **14** was removed by dissolving the product in chloroform and eluting from a short column of neutral alumina. The purified product was recovered, the solvent removed, and the resulting oil dissolved in methylene chloride. Again the solvent was removed under reduced pressure and the resulting foam was triturated with anhydrous ether. The ether was removed using a vacuum pump, giving 300 mg (56%) of **20** as a dry, light yellow foam, mp 52–56°C.

UV (CH_3OH): 212 and 332 nm.

^1H NMR ($CDCl_3$) δ 7.3–7.8 (q, 4H, phenyl protons), 6.5–7.1 (m, 4H, indole protons, and dihydropyridine H-2), 5.2–5.9 (m, 2H, dihydropyridine H-6, and NH), 4.5–4.8 (m, 1H, dihydropyridine H-5), 3.9–4.3 (t, 2H, CH_2–O), 3.7–3.9 (s, 3H, CH_3–O), 3.5–3.8 (s, 2H, CH_2–CO), 3.2–3.5 (t, 2H, CH_2–N), 2.9–3.2 (bs, 2H, dihydropyridine H-4), 2.8–3.0 (s, 3H, CH_3–N), 2.2–2.5 (s, 3H, CH_3), 1.6–2.2 (p, 2H, CH_2).

Anal. ($C_{29}H_{30}ClN_3O_5 \cdot \frac{3}{4}H_2O$). Calculated: C, 63.38; H, 5.78; Cl, 6.45; N, 7.65. Found: C, 63.42; H, 5.78; Cl, 6.53; N, 7.65.

ANALYTICAL METHODS

High-pressure liquid chromatography systems were developed for the analysis of the dihydropyridine drug–carrier delivery systems as well as their corresponding pyridinium salt derivatives. The chromatographic analyses were performed using a component system consisting of a Kontron System 600 pump and auto sampler, a Perkin–Elmer LCI-100 integrator, and a Kratos Model 757 UV/visible, variable-wavelength detector. A 25-cm reversed-phase Toyasoda column, 5-μm ODS-C-18, was used at ambient temperature in combination with a guard column packed with Whatman pellicular ODS-C-18 media.

The quaternary compounds **9–14** were analyzed using a mobile phase consisting of acetonitrile and monobasic potassium phosphate buffer (15–50 m*M*), at a one-to-one ratio. The concentration of the buffer was adjusted for each of these compounds in order to obtain a retention time of 5 to 6 min. The parent drugs naproxen and indomethacin could also be detected using each of these systems. The dihydropyridine compounds (**15–20**) were analyzed using a mobile phase consisting of various ratios of acetonitrile and water. Mixtures containing as much as 80% of the organic solvent were used in order to obtain retention times of 4 to 6 min.

Stability of Quaternary Drug–Carrier Compounds (9–14) and Dihydropyridine CDS Compounds (15–20) in pH 7.4 Phosphate Buffer

Phosphate buffer (40 mm) was equilibrated in a water bath at 37°C. A 5×10^{-3} *M* stock solution in DMSO was freshly prepared for each compound before use. Ten microliters of the stock solution was added per milliliter of buffer used in each experiment. Aliquots of 100 μl were withdrawn at various time points and pipetted into 400-μl portions of ice-cold acetonitrile. The samples were centrifuged for 3 min at 10,000 rpm and the supernatant was sampled to determine the rate of disappearance using high-pressure liquid chromatography.

Stability of Quaternary Drug–Carrier Compounds (9–14) in 100% Whole Human Blood and 100% Whole Rat Blood

Blood was withdrawn from a volunteer shortly before beginning each experiment. The blood was placed in heparinized tubes and stored on ice until needed, at which time it

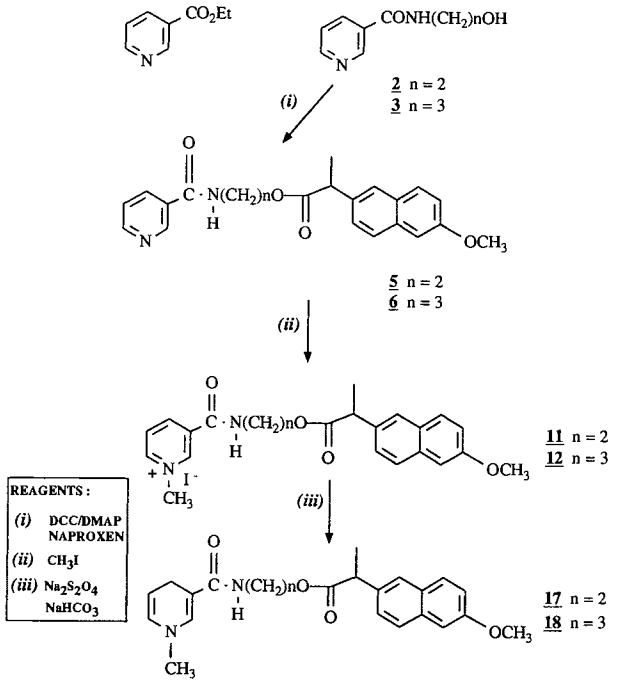

Fig. 2. Synthesis of the naproxen chemical delivery systems **17** and **18**.

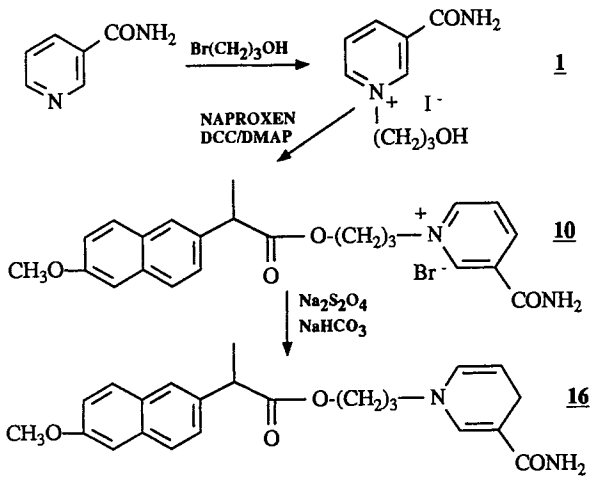

Fig. 3. Synthesis of the naproxen chemical delivery system 16.

was incubated at 37°C. A 5×10^{-3} *M* stock solution in DMSO was prepared for each compound before use. Ten microliters of the stock solution was added per milliliter of blood used in a given experiment. Aliquots of 100 μl were withdrawn at various time intervals and pipetted into 400 μl of ice-cold acetonitrile containing 5% DMSO by volume. The same procedure was used for fresh rat blood that was withdrawn into a heparinized syringe via heart puncture. The blood was collected just prior to the start of each experiment. Aliquots of 100 μl were withdrawn at various time intervals and pipetted into 400 μl of ice-cold acetonitrile. Samples were vortexed for 5 sec and centrifuged at 10,000 rpm for 5 min. The supernatant was used to determine the rate of disappearance of the compound by high-pressure liquid chromatography.

Stability of Quaternary Drug–Carrier Compounds (9–14) in 20% Rat Brain Homogenate and 20% Rat Kidney Homogenate

One gram of freshly obtained rat brain was homogenized with 4 ml of 40 mm phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 5 min. The supernatant was removed and incubated in a water bath at 37°C. A 5×10^{-3} *M* stock solution in DMSO was prepared for each compound before use. Ten microliters of the stock solution was added per milliliter of homogenate used in a particular experiment. Aliquots of 100 μl were withdrawn at various time intervals and pipetted into 400 μl of ice-cold acetonitrile. The samples were vortexed for 5 sec and centrifuged at 10,000 rpm for 5 min. The supernatant was used to measure the rate of disappearance of the compound by high-pressure liquid chromatography. The same procedure was used for freshly obtained rat kidney.

Stability of Dihydropyridine CDS Compounds (15–20) in 20% Rat Brain Homogenate

The same procedure was used as in the case of compounds (9–14) in 20% rat brain homogenate.

RESULTS AND DISCUSSION

The quaternary drug–carrier compounds (9–14) were synthesized using a variety of multistep sequences. The compounds in which the hydroxyalkyl group was attached to the amide nitrogen atom of nicotinamide were synthesized according to the scheme shown in Fig. 2. This route required that the hydroxyalkyl link be first attached to the basic nicotinamide structure. The desired drug was then esterified with the hydroxyalkylpyridine carrier. The resulting drug–carrier combination was then quaternized using methyl iodide and reduced using sodium dithionite, giving exclusively the corresponding 1,4-dihydropyridine.

The original attempts to synthesize the quaternary compounds possessing the hydroxyalkyl link at the 1-position of the pyridine ring were complicated by the low solubility of the pyridinium salts in most organic solvents (see Fig. 3). The quaternization of nicotinamide with appropriate haloalcohols was successful. However, these polar compounds were not sufficiently soluble in acetone, methanol, or aceto-

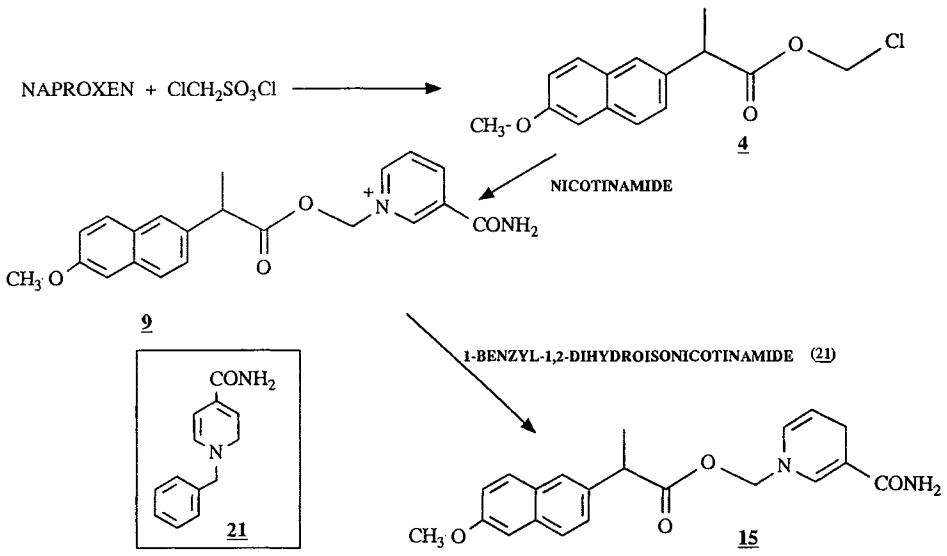

Fig. 4. Synthesis of the naproxen chemical delivery system 15.

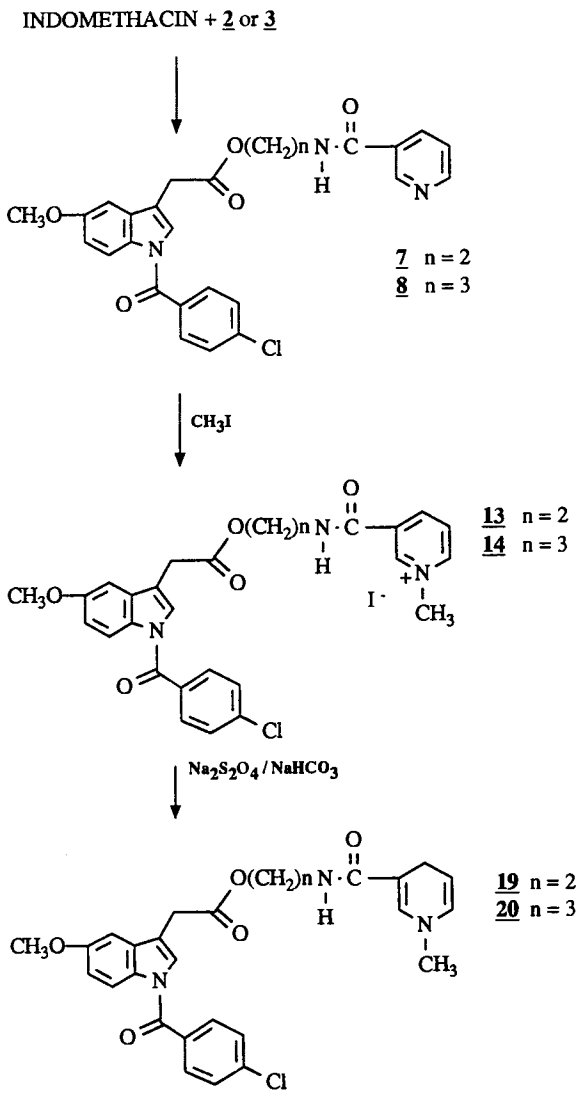

Fig. 5. Synthesis of the indomethacin delivery systems 19 and 20.

nitrile to allow their use in the esterification of either naproxen or indomethacin.

One ester (compound 10) was successfully prepared by this sequence using DMF as the final reaction solvent. Compound 1 was dissolved in a minimum amount of DMF and then coupled with naproxen in the presence of DCC and DMAP. The remaining naproxen quaternary drug–carrier combination (9) was synthesized following the scheme shown in Fig. 4. This involved the quaternization of nicotinamide with a previously prepared naproxen ester (4).

The indomethacin delivery systems 19 and 20 were prepared according to the scheme shown in Fig. 5.

The reduction of these pyridinium compounds to the corresponding 1,4-dihydropyridine delivery systems was generally carried out using sodium dithionite under slightly basic conditions. The lone exception came in the synthesis of compound 15. The quaternary salt was too labile to withstand the alkaline conditions of the aforementioned reduction. Therefore, an activated 1,2-dihydroisonicotinamide compound was successfully utilized as the reducing agent. Use of this reducing agent was first described by Nuvole *et al.* (14). The 1,2-dihydroisonicotinamide molecule, 21, may be considered activated in the sense that it is less stable than 1,4-dihydronicotinamides. Therefore, on mixing 21 with 9 there is a net hydride exchange to give the more energetically favored 1,4-dihydropyridine 15 along with 1-benzyl-4-carbamoylpyridinium chloride as an easily removed by-product.

The pyridinium salts were quite stable in pH 7.4 phosphate buffer, with the expected exception of compound 9 (see Table I). The primary route of decomposition of these compounds was the hydrolysis of the ester bond. The dihydropyridine compounds were generally less stable than the corresponding quaternary derivatives and followed a more complicated decomposition pattern: thus, the dihydropyridines were subject to water addition across the 5,6 bond, yielding transient intermediates that were detected only by their characteristic 290-nm absorption in the UV spectrum. The water addition products decomposed further and could not be detected by HPLC. Oxidation of the dihydropyridines resulted in formation of the corresponding pyridinium salts, detected by HPLC. Ester hydrolysis from either the dihydropyridines, the water addition products, the quaternary salts, or a combination of each of these gave the free drug, again detected by HLPC.

The quaternary compounds were sufficiently stable in both human and rat blood and quite stable in 20% rat brain or rat kidney homogenates, again with the exception of compound 9 (see Tables II and III). Hydrolysis of the ester was the primary path of decomposition. The most surprising finding of this particular work was the quaternary compounds' relative stability in rat blood compared to human blood; in general, much higher enzymatic activity, particularly esterase activity, is observed in rat than in human blood. This phenomenon is apparently due to some type of specificity of one or more of the enzymes contained in human blood toward compounds 10–13. The dihydropyridine derivatives, however, behaved in a more predictable manner and were generally more stable in human blood than in rat blood.

Decomposition of the dihydropyridines occurred both

Table I. Half-Life of Disappearance and Correlation Coefficient for Each Dihydropyridine and Quaternary Pyridinium Compound, in pH 7.4 Phosphate Buffer

Compounds	$t_{1/2}$ (r)	
	Dihydro.	Quat.
20 and 14	24 min	35 hr[a]
(0.99)	(0.998)	
19 and 13	32 min	26 hr[a]
(0.97)	(0.92)	
18 and 12	9.0 min	550 hr[a]
(0.99)	(0.88)	
17 and 11	16 min	58 hr[a]
(0.97)	(0.75)	
16 and 10	52 min	750 hr[a]
(0.998)	(0.98)	
15 and 9	5.1 hr	8.6 min
(0.98)	(0.998)	

[a] Parent drug was detectable but not within the first hour of each experiment.

Table II. Half-Life of Disappearance and Correlation Coefficient for Each Dihydropyridine and Quaternary Pyridinium Compound, in 100% Whole Rat and 100% Whole Human Blood

	$t_{1/2}$ (r)			
Compounds	Rat dihydro.	Human dihydro.	Rat quat.	Human quat.
20 and 14	24 min	27 min	18 min	37 min
	(0.999)	(0.98)	(0.999)	(0.993)
19 and 13	5.2 min	60 min	84 min	15 min
	(0.98)	(0.97)	(0.995)	
18 and 12	5.1 min	52 min	6.2 hr[a]	1.8 min
	(0.99)	(0.95)	(0.97)	
17 and 11	5.5 min	61 min	15 hr[a]	5.4 min
	(0.98)	(0.91)	(0.995)	
16 and 10	10 min	30 min	Stable[b]	3.2 min
	(0.97)	(0.998)		
15 and 9	3.7 hr	2.7 min	0.4 min	0.2 min
	(0.93)	(0.98)	(0.999)	

[a] Experiment was followed for less than one half-life.
[b] Compound very stable; parent drug was not detectable over the course of the experiment.

by oxidation back to the pyridinium salt and by hydrolysis of the ester bond. The only possible exception to this was in the case of compound 15, where the oxidation product could not be detected due to its relative instability.

The dihydropyridine compounds were readily oxidized in rat brain homogenate, again with the exception of compound 15. The resulting pyridinium salts were then slowly hydrolyzed to give the parent drug. The tissue homogenate studies show, then, that brain targeting should be possible with these compounds. The dihydropyridine derivatives are relatively stable in human blood, allowing time for distribution of the brain. The half-lives of the dihydropyridines in (rat) brain homogenate are substantially shorter than are half-lives of the corresponding quaternary salts. Consequently, the quaternary salts, now "locked" in the brain, will provide a sustained release form of the active drug. The results of these *in vitro* stability tests were used to compare and evaluate each chemical delivery system to determine which compound met the requirements of the ideal delivery system as outlined in the Introduction. In this way the present studies were used as a stepping stone to assess the likelihood of success in *in vivo* studies. The results of distribution and activity studies will be published in a subsequent article.

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Table III. Half-Life of Disappearance and Correlation Coefficient for Each Dihydropyridine and Quaternary Pyridinium Compound in 20% Rat Brain Homogenate and for Each Quaternary Pyridinium Compound in 20% Rat Kidney Homogenate

	$t_{1/2}$ (r)		
Compounds	Brain dihydro.	Brain quat.	Kidney quat.
20 and 14	1.9 min, stable[c]	16 hr[b]	
	(0.99)	(0.89)	
19 and 13	3.5, 11 hr[a]	35 hr[a]	
	(0.999)	(0.97)	(0.97)
18 and 12	4.5, 6.8 hr[a]	9.7 hr[a]	
	(0.998)	(0.94)	(0.79)
17 and 11	1.2, 5.8 hr[b]	Stable[c]	
	(0.99)	(0.77)	
16 and 10	1.6 min, stable[c]	Stable[c]	
		(0.98)	
15 and 9	37, 1.9 min	2.3 min	
	(0.97)	(0.998)	(0.997)

[a] Experiment was followed for less than one half-life.
[b] Parent drug was detectable but not within the first hour of each experiment.
[c] Compound very stable; parent drug was not detectable over the course of the experiment.

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