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Report

Synthesis and Biological Evaluation of Ω-(*N,N,N*-Trialkylammonium)alkyl Esters and Thioesters of Carboxylic Acid Nonsteroidal Antiinflammatory Agents

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A series of novel ω-(*N,N,N*-trialkylammonium)alkyl ester and thioester derivatives [$RCOM(CH_2)_nNR_3^+ X^-$, M = O or S, n = 2–6, X = I or Cl] of 11 nonsteroidal antiinflammatory carboxylic acid agents (naproxen, ketorolac, indomethacin, ibuprofen, sulindac, ketoprofen, flufenamic acid, mefenamic acid, zomepirac, etodolac, and tifurac) was prepared and evaluated for their antiinflammatory, analgesic, and gastrointestinal erosive properties. In general, each prodrug retained the antiinflammatory activity characteristic of the corresponding parent drug but exhibited moderately to greatly reduced gastrointestinal erosive properties and significantly reduced analgetic potencies. This profile is likely due to a combination of factors including the rate of hydrolysis of the esters in the stomach, gut, and plasma, changes in the locus of absorption of the prodrug or nonsteroidal antiinflammatory drug (NSAID), and altered metabolic disposition patterns resulting from these changes. The results obtained from the compounds of this series indicate that esters of this general class may offer a means to modulate both the aqueous/lipid solubility and the hydrolytic/enzymatic cleavage indices of NSAID prodrugs which potentially possess a more favorable therapeutic ratio of antiinflammatory to gastrointestinal erosive activities.

KEY WORDS: nonsteroidal antiinflammatory drugs (NSAIDs); prodrugs; NSAID esters; gastrointestinal erosion; analgesic activity.

INTRODUCTION

The many acidic nonsteroidal antiinflammatory drugs (NSAIDs) constitute the principal class of agents for controlling the pain and inflammation of rheumatic disease (1). One approach to reduce the sometimes considerable gastrointestinal distress associated with chronic use of these compounds has been the investigation of NSAID prodrugs, designed to deliver the parent compound after various chemical or enzymatic transformations (2). While the range of these transformations extends from oxidation state alterations (3) to metabolic conversion to the active compound (4), most often the delivery systems involved have taken the form of simple alkyl and aryl esters which improve absorption and hydrolyze in plasma to the parent NSAID acid (5–7). Sometimes, however, these esters are not sufficiently labile *in vivo* to ensure a sufficiently high rate and extent of prodrug conversion (8). The need to incorporate increased chemical stability in formulation in proper balance with rapid drug release has been recently addressed by a number of functionalized ester derivatives, which combine improved aqueous solubility and enzymatic hydrolytic rate but maintain a high stability in solution. These appendages, most notably the N,N-disubstituted glycolamides, have been proposed to provide another means of varying both the aqueous/lipid solubility and the enzymatic/chemical hydrolytic indices (9).

The application of such functionalized esters to NSAID delivery might be expected to alter absorption and/or pharmacokinetic disposition of these prodrugs, potentially providing compounds with greatly improved gastrointestinal tolerances (9). Our interest in the prodrug approach was sparked by the observation that β-phenylpropionyl thiocholine iodide, previously reported to be an inhibitor of transglutaminase (10), exhibited marginal activity in antiinflammatory screens. Although attachment of this ester linkage to the NSAID naproxen (**1a**) did not yield an inhibitor of transglutaminase, the resulting profile of antiinflammatory versus gastrointestinal effects observed for **1b** prompted the synthesis and evaluation of the compounds described herein, a series of choline, thiocholine, and homologous esters of a range of NSAIDs currently in use. Although these compounds bear a resemblance to recently reported NSAID choline ester analogues designed for topical administration

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(11) and to fatty acid choline ester salts of NSAIDs intended for improved gastrointestinal absorption (12), the profile of *in vivo* activity observed for these compounds (vide infra) seems to be unique among this new class of trialkylammonium-containing NSAID prodrugs.

MATERIALS AND METHODS

Chemicals

Naproxen, ketorolac, and tifurac were obtained from Syntex Research; indomethacin, ibuprofen, sulindac, ketoprofen, flufenamic acid, mefanamic acid, and zomepirac were purchased from Sigma Chemical Co., St. Louis, Mo.; etodolac was obtained by extraction of commercial Lodine capsules obtained from Syntex Research, Maidenhead, Berkshire, Great Britain. *N,N*-Carbonyldiimidazole was obtained from Fluka Chemical Co., Ronkonkoma, N.Y., and was stored desiccated over Drierite at ambient temperature. 2-(*N,N*-Dimethyl)- and 2-(*N,N*-diethyl)aminoethanethiol HCl and the ω-(*N,N*-dialkyl)- or ω-(*N,N*-cycloalkyl)aminoalkanols were obtained from Aldrich Chemical Co., Milwaukee, Wis., or from Pfaltz and Bauer, Inc., Waterbury, Conn. Iodomethane and iodoethane (Aldrich) were passed through neutral alumina (ICN activity 0) immediately before use. *n*-Butyllithium (Aldrich; 1.6 *M* in hexane) was used as received and was transferred under positive nitrogen pressure or via syringe. Tetrahydrofuran (THF) was freshly distilled from sodium-benzophenone ketyl immediately prior to use.

Methods

Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on either an EM-390 (90-MHz) or a Bruker WM 300 (300-MHz) instrument. Infrared spectra were recorded as KBr pellets with a Perkin-Elmer 237 grating spectrometer. All compounds exhibited NMR and IR spectral data consistent with the proposed structures. Elemental analyses were performed by either Atlantic Microlabs, Norcross, Ga., or by the Analytical and Environmental Research Department, Syntex Research, on samples dried 24 hr at ambient temperature and high vacuum. Results were within 0.4% of theoretical values, unless otherwise stated. All reactions were carried out under positive nitrogen pressure. All organic extracts were dried over sodium sulfate prior to evaporation.

General Procedures for Synthesis of NSAID Prodrugs

The NSAID ω-(*N,N,N*-trialkylammonium)alkyl esters (**1b** and **h–o**, **2b** and **3–10b**) and thioesters (**1c–f**, **2–11c**) were each prepared by activation of the parent NSAID acid **1–11a** (Fig. 1) with *N,N*-carbonyldiimidazole, reaction of the resulting imidazolide with the appropriate ω-(*N,N*-dialkylamino)alcohol or thiol, and quaternization (Fig. 2). This method provided an efficient way to synthesize and isolate the desired esters in a high yield and purity. Yields, melting points, and analytical data for target compounds are summarized in Table I.

NSAID Choline Ester Iodides and Homologues (M = O, n = *2–6*). A solution of the carboxylic acid NSAID (50 mmol) and *N,N*-carbonyldiimidazole (8.9 g, 55 mmol) in THF (100 ml) was stirred at ambient temperature for 1–2 hr. This mixture was then treated in a dropwise fashion with a solution prepared from the desired ω-(*N,N*-dialkyl)- or ω-(*N,N*-cycloalkyl)aminoalkanol (50 mmol) and *n*-butyllithium (3.0 ml, 5 mmol) in THF (100 ml). The resulting mixture was stirred at ambient temperature for 2 hr, and then the THF was removed by evaporation. The residue was diluted with anhydrous diethyl ether (300 ml), and the solution was washed with water (5 × 200 ml) and with brine (2 × 200 ml). The organic extract was dried, filtered, and evaporated to give a thick oil, which was dried at high vacuum overnight. The intermediate ester was then dissolved in diethyl ether (200–500 ml) and was treated with the desired alkyl iodide (150 mmol) at ambient temperature. After stirring overnight, the resulting thick white precipitate was collected by filtration, washed with additional diethyl ether, and was air dried, with care to protect it from light. The resulting solid was then further dried over phosphorus pentoxide at 10 mm Hg. Recrystallization from refluxing acetone, with hot filtration, was used to obtain analytical samples.

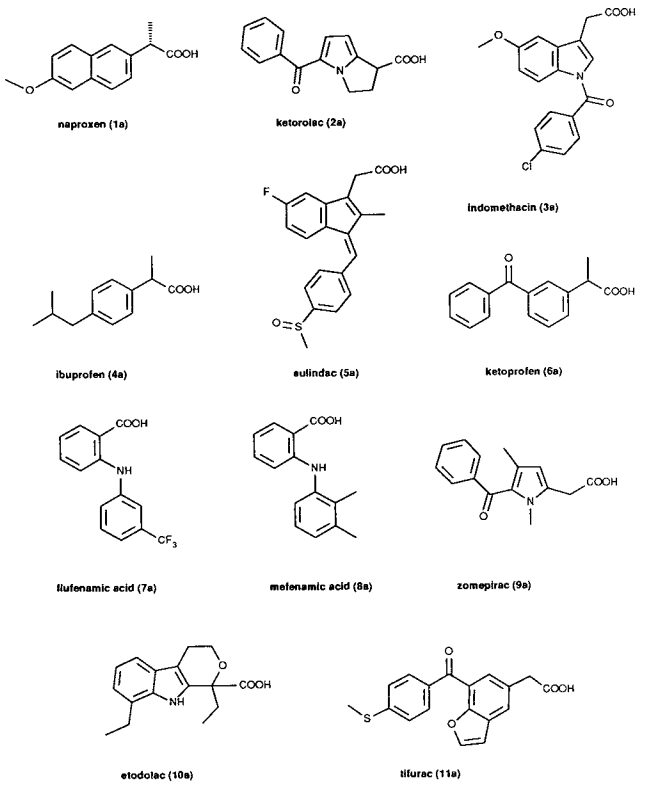

Fig. 1. Chemical structures of carboxylic acid NSAIDs **1–11a**.

NSAID Thiocholine Ester Iodides (M = *S*, n = *2*). A solution of the carboxylic acid NSAID (50 mmol) and *N,N*-carbonyldiimidazole (8.9 g, 55 mmol) in THF (100 ml) was

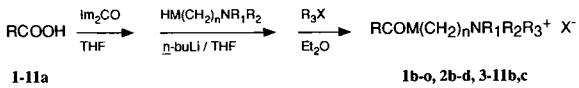

Fig. 2. Synthesis of ω-(*N,N,N*-trialkylammonium)alkyl ester and thioester prodrugs of NSAIDs **1–11a**.

Table I. Physical Data for NSAID Prodrugs (1b–o, 2b–d, 3–10b and c and 11c)

No.	% yield	mp, °C	Formula (MW)	Elemental analysis, calculated found		
				C	H	N
1b	83	194–195	$C_{19}H_{26}NO_3I$ (443.38)	51.47 (51.38)	5.91 (5.93)	3.16 (3.21)
1c	96	125–126	$C_{19}H_{26}NO_2SI \cdot H_2O$ (477.43)	47.79 (47.52)	5.48 (5.78)	2.93 (3.28)
1d	41	110–112	$C_{19}H_{26}NO_2SCl$ (367.93)	—[a]		
1e	84	161–162	$C_{18}H_{24}NO_2SCl \cdot 0.1\ H_2O$ (355.7)	60.78 (60.72)	6.86 (6.93)	3.94 (3.96)
1f	92	66–67[b]	$C_{22}H_{32}NO_2SI \cdot H_2O$ (519.48)	50.86 (50.76)	6.60 (6.43)	2.70 (3.04)
1g	95	54–56[b]	$C_{22}H_{32}NO_3I \cdot 0.25\ H_2O$ (489.9)	53.93 (53.99)	6.69 (6.60)	2.86 (2.64)
1h	38[c]	115–116	$C_{21}H_{28}NO_3I$ (469.35)	53.74 (53.99)	6.01 (6.06)	2.98 (2.86)
1i	98	114–115	$C_{22}H_{30}NO_2I \cdot 1.5\ H_2O$ (510.4)	51.77 (51.66)	6.52 (6.13)	2.74 (3.05)
1j	40[c]	144–145	$C_{21}H_{28}NO_4I \cdot 0.5\ H_2O$ (494.36)	51.02 (51.30)	5.91 (5.70)	2.83 (2.76)
1k	82	74–76	$C_{25}H_{38}NO_3I \cdot H_2O$ (545.49)	55.04 (55.29)	7.39 (7.36)	2.57 (2.82)
1l	96	222–224	$C_{20}H_{28}NO_3I$ (457.34)	52.52 (53.02)	6.17 (6.08)	3.06 (3.08)
1m	50[c]	139–140	$C_{21}H_{30}NO_3I$ (471.37)	53.51 (53.87)	6.42 (6.30)	2.97 (3.16)
1n	82	105–106	$C_{22}H_{32}NO_3I$ (485.39)	54.43 (54.32)	6.65 (6.71)	2.89 (2.90)
1o	6[c]	79–81	$C_{23}H_{34}NO_3I$ (499.42)	55.31 (55.22)	6.86 (6.53)	2.80 (3.32)
2b	72	80–82	$C_{20}H_{25}N_2O_3I \cdot 0.5\ H_2O$ (468.33)	50.32 (50.41)	5.49 (5.33)	5.87 (6.06)
2c	87	118–120	$C_{20}H_{25}N_2O_2SI$ (484.39)	49.59 (49.64)	5.20 (5.31)	5.78 (5.51)
2d	82	138–140	$C_{22}H_{29}N_2O_3I$ (496.38)	53.23 (53.62)	5.89 (5.93)	5.64 (5.58)
3b	45[c]	209–210	$C_{24}H_{28}N_2O_4ClI$ (570.9)	50.49 (50.53)	4.94 (4.83)	4.91 (4.93)
3c	98	180–181	$C_{24}H_{28}N_2O_3SClI \cdot 0.5\ H_2O$ (595.92)	48.37 (48.54)	4.91 (4.88)	4.70 (4.66)
4b	86	127–128	$C_{18}H_{30}NO_2I$ (419.34)	51.55 (51.37)	7.21 (7.18)	3.34 (3.71)
4c	98	143–144	$C_{18}H_{30}NOSI \cdot 0.25\ H_2O$ (439.91)	49.14 (49.17)	6.99 (6.90)	3.18 (3.13)
5b	47[d]	209–210	$C_{25}H_{29}NO_3SFI \cdot H_2O$ (579.48)	51.81 (51.82)	5.09 (5.02)	2.42 (2.83)
5c	97[d]	148–150	$C_{25}H_{29}NO_2S_2FI \cdot 2.5\ H_2O$ (630.57)	47.62 (47.80)	5.44 (5.08)	2.22 (2.55)
6b	88	60–62	$C_{21}H_{26}NO_3I \cdot 0.25\ H_2O$ (471.8)	53.45 (53.52)	5.66 (5.55)	2.97 (3.30)
6c	70	126–128	$C_{21}H_{26}NO_2SI \cdot 0.5\ H_2O$ (492.41)	51.22 (51.04)	5.53 (5.39)	2.85 (2.84)
7b	68	138–139	$C_{19}H_{22}N_2O_2F_3I \cdot 0.5\ H_2O$ (503.3)	45.34 (45.56)	4.61 (4.52)	5.57 (5.66)
7c	36[c]	100–101	$C_{19}H_{22}N_2OF_3SI$ (510.36)	44.71 (44.92)	4.35 (4.41)	5.49 (5.27)
8b	44[c]	181–183	$C_{20}H_{27}N_2O_2I \cdot 0.5\ H_2O$ (463.35)	51.84 (51.80)	6.09 (5.98)	6.05 (6.10)
8c	34[c]	215–216	$C_{20}H_{27}N_2OSI \cdot 0.25\ H_2O$ (474.91)	50.58 (50.63)	5.84 (5.65)	5.90 (5.95)
9b	85	170–172	$C_{20}H_{26}N_2O_3ClI$ (504.79)	47.58 (47.32)	5.19 (5.10)	5.55 (5.51)
9c	32[c]	162–163	$C_{20}H_{26}N_2O_2SClI$ (520.86)	46.12 (45.95)	5.03 (4.90)	5.38 (5.37)
10b	25	104–106[b]	$C_{22}H_{33}N_2O_3I \cdot 0.25\ H_2O$ (504.9)	52.33 (52.19)	6.69 (6.76)	5.55 (5.34)
10c	67	126–128[b]	$C_{22}H_{23}N_2O_3SI\ H_2O$ (534.49)	49.43 (49.64)	6.60 (6.36)	5.24 (4.89)
11c	75	146–147	$C_{23}H_{26}NO_3S_2I$ (555.49)	49.72 (49.38)	4.72 (4.67)	2.52 (2.44)

[a] Hygroscopic solid; no analysis obtained.
[b] Amorphous solid, isolated by trituration of the evaporated reaction residue.
[c] Recrystallized from acetone.
[d] Orange solid.
[e] Tifurac choline ester (11b) not prepared.

stirred at ambient temperature for 1–2 hr. This mixture was then treated in a dropwise fashion with a solution prepared from the desired ω-(*N,N*-dialkyl)ethanethiol HCl (60 mmol) and *n*-butyllithium (41 ml, 66 mmol) in THF (250 ml). This mixture was worked up and treated as above to obtain the title compounds.

Naproxen Thiocholine Ester Chloride (1d). Naproxen 2-(*N,N*-dimethylamino)ethanethiol ester (free base), obtained as above, was treated with condensed methyl chloride in a Parr pressure apparatus at 80°C for 48 hr. After cooling, the resulting solid was triturated with diethyl ether and filtered three times, to remove unreacted starting material. The hygroscopic solid was dissolved in absolute ethanol and was evaporated at high vacuum to yield a glass. Trituration with diethyl ether provided a solid after drying at high vacuum.

*Naproxen 2-(*N,N*-Dimethylamino)ethanethiol Ester Hydrochlodride (1e).* A solution of naproxen 2-(*N,N*-dimethylamino)ethanethiol ester in diethyl ether, obtained as above, was treated with a saturated solution of hydrogen chloride in ethyl acetate. The resulting white precipitate was collected by filtration, washed with diethyl ether, and dried as above.

Biological Evaluation Procedures (13)

Rat Adjuvant Arthritis Assay (ADAR). Female Crl:CD (SD)BR rats weighing 160–180 g were randomly distributed to treatment groups of 12 animals and were given food and water ad libitum. Test materials for oral administration were prepared fresh daily as suspensions by homogenization in an aqueous vehicle consisting of 0.9% NaCl, 0.5% sodium carboxymethyl cellulose (CMC), 0.4% polysorbate 80, 0.9% benzyl alcohol, and 97.3% distilled water. Animals were orally dosed with volumes of 1 ml twice per day Monday

through Friday and with 2 ml once per day on Saturdays and Sundays. Positive control was provided by administration of drug-free vehicle (CMC). At time 0, animals were injected intradermally in the proximal quarter of the tail with 0.1 ml of a mineral oil (Sigma) suspension of heat-killed and dried *Mycobacterium butyricum* (Difco) at a concentration of 1 mg/0.1 ml. After sacrifice (day 17), the hind paws of each animal were removed and weighed. The data are reported in Table II as percentage inhibition of hind paw weight increase relative to positive control for $n = 12$.

Mouse Phenylquinone-Induced Writhing Analgesic Assay (PQWM). Female Sim:(SW)f BR mice weighing 19–22 g were randomly distributed to treatment groups of eight animals. At hour −1, test compounds were administered as suspensions in the aqueous vehicle used above in the amounts and by the routes indicated (Table II). Administration of the aqueous vehicle alone was used as positive control. At hour 0, animals were injected i.p. with 0.25 ml of a 0.02% solution of phenylquinone in 97:3 distilled water:absolute ethanol. Animals were placed in separate compartments, and the number of writhes for each animal was recorded for 10 min utilizing a Diffcount eight-key recorder. The percentage inhibition of writhing vs positive control is reported in Table II.

Gastrointestinal Erosion Assay (GIER). Male Crl:CD (SD)BR or HSD:(SD)BR rats weighing 185–200 g were acclimated in-house for approximately 1 week after arrival from the supplier. Each animal was individually identified, and body weights were obtained on day 1 and at autopsy. The test materials were given orally, either in a single daily dose (regimen A) or in split-daily dose fashion (regimen B). The total daily dose by either of these dosing regimens is indicated, along with the dosing pattern used, in Table II. The compounds were administered as a suspension in the aqueous vehicle used above in a volume of 2 ml per 200 g body weight, for 4 days, followed by sacrifice on the day of the last dose. Food was removed following the last dose. At necropsy, the stomach and small intestine were removed and examined for lesions, which were graded [0–5] as follows: [0]—no detectable gastrointestinal lesions; [1]—small ulcerations (1–15) less than 1 mm in diameter; [2]—small ulcerations plus medium (1–10) ulcerations 2–3 mm in diameter; [3]—predominantly medium ulcerations plus severe ulcers (2–5) of diameter >4 mm, without evidence of perforation; [4]—many (>20) medium and severe ulcerations with evidence of perforations; and [5]—score for animals which died during the course of the assay as a consequence of intestinal perforation and septicemia. The data are reported as the mean ulcer score (0–5) ± standard deviation ($n = 5$) for each test compound.

Determination of NSAID Prodrug Hydrolysis Rates

The chemical hydrolysis rates of **1b**, **c**, and **m**, **2c**, and **3c** were measured at pH 7.4 and 37°C. Samples for hydrolysis were prepared by adding the compound to a 0.02 *M* phosphate buffer solution (pH 7.4) containing sufficient 1.0 *M* KCl to yield a final ionic strength of 0.15 *M*. The solutions were sealed in amber glass ampoules and were reacted at 37°C for up to 11 days. Samples were removed at appropriate intervals and were quenched by adjusting the pH to 3.0 with 1 *M* HCl. After dilution in mobile phase, samples were assayed for the presence of ester and hydrolysis product by HPLC. The method utilized an Altex Ultrasphere ODS 5-μm column (4.6-mm I.D. × 25 cm) and a mobile phase of 55% methanol and 45% 0.0025 *M* *N,N*-dimethyl-*N*-octyl ammonium phosphate buffer, adjusted to pH 3.0 with phosphoric acid. The flow rate was 1.0 mL/min. Detection was carried out at 230 nm, using a Schoefel Model 770 variable-wavelength UV detector. The sample volume was 50 μl (typically 1–2 μg drug), and the compounds were detected by UV at 230 nm. Hydrolysis data are reported as K_{obs} (rate constant) and T_{50} (time to half-consumption; hr) in Table III.

RESULTS AND DISCUSSION

The NSAID prodrugs were evaluated for their biological activities in three standard assays used to gauge the parent agents: (i) inhibition of adjuvant-induced arthritis in the rat (ADAR), for antiinflammatory activity; (ii) phenylquinone-induced writhing in the mouse (PQWM), for analgetic activity; and (iii) gastrointestinal (GI) erosive activity in the rat (GIER). These data are reported in Table II, as the percentage inhibition for ADAR and PQWM, and as the ulcer score, on a scale of 0–5, for GIER. All doses are recorded as micromoles per kilogram to facilitate comparison with the parent NSAIDs.

No single trend of activity was discerned for the NSAID esters and thioesters of this study; rather, a spectrum of activity profiles was observed. Qualitative analysis of the data generated in this battery of assays reveals that the NSAID prodrugs of this study fall into three general categories. The first group is composed of compounds which maintain the levels of activity vs side effects, as compared on a molar basis, of the parent NSAID in all assays. These compounds cannot be considered as prodrugs, since they offer no significant therapeutic ratio improvement over the parent agent, likely due to rapid hydrolytic conversion. The derivatives of ketoprofen (**6a**) and zomepirac (**9a**), the esters of which seem to deliver the parent agent without any change in overall profile of action, are characteristic of this category. Conversely, compounds which show significantly depressed activities in all assays either do not hydrolyze to the parent NSAID efficiently, are poorly absorbed, or are subject to alternative metabolic disposition. For example, both the esters and thioesters of sulindac (**5a**) and mefenamic acid (**8a**) are nearly inactive in the three assays. Intermediate between these two classes are compounds which exhibit an altered activity profile in comparison with the parent NSAID. These compounds can be considered as potential prodrugs when the desired activities of the parent NSAID are maintained, while untoward side effects are reduced or eliminated, resulting in exploitable improvements in the therapeutic ratio(s). A general trend within this series is that, contrary to expectations, the thioesters are seemingly hydrolytically more stable than their corresponding oxy analogues, as measured by their diminished propensity to induce GI erosion. This difference results in a useful shift in the activity profile for most of the thioesters, as exemplified by comparison of the pairs derived from indomethacin (**3a**), flufenamic acid (**7a**), and etodolac (**10a**).

The prodrug esters of this last category maintain rea-

Table II. Evaluation of $RCOM(CH_2)_nNR_1R_2R_3^+ X^-$ (1b–o, 2b–d, and 3–11b and c) as NSAID Prodrugs

Parent NSAID *R*COOH	No.	*M*	*n*	R_1	R_2	R_3	*X*	Biological evaluation[a] ADAR[b] % inhibition (μmol/kg)	PQWM[c] % inhibition (μmol/kg)	GIER[d] Ulcer score (μmol/kg)	Dosing regimen[e]
Naproxen	1a							770 (30)	90 (95)	3.4 ± 0.6 (127)	A
										2.2 ± 0.9 (108)	A
										0.4 ± 0.6 (87)	A
	1b	O	2	Me	Me	Me	I	77 (27)	33 (112)	3.8 ± 0.8 (451)	B
										1.0 ± 0.7 (225)	A
										0.0 ± 0.0 (56)	A
	1c	S	2	Me	Me	Me	I	64 (30)	17 (109)	3.6 ± 0.6 (838)	A
										1.5 ± 0.7 (628)	
										0.2 ± 0.5 (418)	A
	1d	S	2	Me	Me	Me	Cl	59 (54)	8 (136)	2.8 ± 1.1 (1087)	A
										0.8 ± 0.5 (815)	A
										0.2 ± 0.5 (544)	A
	1e	S	2	Me	Me	H	Cl	81 (70)	61 (141)	3.4 ± 0.5 (281)	A
	1f	S	2	Et	Et	Et	I	48 (27)	29 (193)	1.2 ± 1.1 (193)	B
										1.2 ± 0.4 (96)	A
	1g	O	2	Et	Et	Et	I	41 (20)	40 (102)	2.4 ± 0.9 (510)	B
										0.0 ± 0.0 (204)	A
	1h	O	2	$-(CH_2)_4-$		Me	I	52 (21)	52 (106)	3.6 ± 0.5 (533)	B
										0.0 ± 0.0 (213)	A
	1i	O	2	$-(CH_2)_5-$		Me	I	49 (20)	27 (98)	2.2 ± 0.8 (490)	B
										0.0 ± 0.0 (196)	A
	1j	O	2	$-(CH_2)_2O$		Me	I	31 (20)	50 (101)	0.8 ± 0.8 (505)	B
										0.0 ± 0.0 (202)	A
	1k	O	2	n-Bu	n-Bu	Me	I		38 (92)	— (458)[f]	
	1l	O	3	Me	Me	Me	I	76 (54)	58 (109)	3.2 ± 0.5 (546)	B
										0.4 ± 0.6 (219)	B
	1m	O	4	Me	Me	Me	I	70 (53)	56 (106)	0.0 ± 0.0 (530)	B
										0.0 ± 0.0 (212)	A
	1n	O	5	Me	Me	Me	I	96 (51)		5.0 ± 0.0 (515)	B
										0.4 ± 0.5 (51)	B
	1o	O	6	Me	Me	Me	I		54 (100)		
Ketorolac	2a							86 (31)	90 (2)	3.6 ± 0.5 (78)	B
								58 (15)		2.6 ± 0.5 (39)	B
								53 (8)		0.8 ± 0.8 (20)	B
	2b	O	2	Me	Me	Me	I	69 (11)	95 (11)	3.2 ± 0.4 (53)	B
								20 (2)		0.6 ± 0.9 (21)	A
	2c	S	2	Me	Me	Me	I	68 (33)	100 (21)	3.6 ± 0.6 (155)	A
								65 (17)	71 (10)	3.2 ± 0.5 (103)	A
								41 (8)		0.8 ± 0.9 (52)	A
	2d	O	4	Me	Me	Me	I	83 (40)	31 (6)	4.4 ± 0.5 (201)	B
								73 (10)	18 (2)	4.0 ± 0.0 (100)	B
								53 (5)		2.6 ± 0.5 (50)	B
Indomethacin	3a							75 (5.6)	50 (4.2)	4.4 ± 0.5 (42)	A
										3.2 ± 1.1 (11)	B
										1.8 ± 0.8 (8)	B
	3b	O	2	Me	Me	Me	I	87 (26)	62 (8.8)	4.4 ± 0.5 (175)	B
										0.0 ± 0.0 (44)	B
	3c	S	2	Me	Me	Me	I	59 (23.5)	19 (42)	0.0 ± 0.0 (168)	A
Ibuprofen	4a							58 (97)	97 (242)	0.0 ± 0.0 (242)	B
	4b	O	2	Me	Me	Me	I	50 (238)	95 (238)	0.0 ± 0.0 (596)	B
	4c	S	2	Me	Me	Me	I	59 (227)	62 (227)	0.0 ± 0.0 (568)	A
Sulindac	5a							42 (56)	59 (42)	3.8 ± 0.5 (280)	B
	5b	O	2	Me	Me	Me	I	0 (69)	0 (86)	0.0 ± 0.0 (345)	A
	5c	S	2	Me	Me	Me	I	36 (63)	0 (79)	0.0 ± 0.0 (317)	A
Ketoprofen	6a							85 (39)	76 (6)	4.4 ± 0.5 (80)	B
										2.4 ± 0.5 (20)	B
	6b	O	2	Me	Me	Me	I	90 (53)	98 (53)	4.0 ± 0.0 (53)	B

Table II—*Continued*

Parent NSAID RCOOH	No.	M	n	R_1	R_2	R_3	X	Biological evaluation[a] ADAR[b] % inhibition (μmol/kg)	PQWM[c] % inhibition (μmol/kg)	GIER[d] Ulcer score (μmol/kg)	GIER[d] Dosing regimen[e]
	6c	S	2	Me	Me	Me	I	90 (51)	98 (102)	4.6 ± 0.6 (203)	B
										2.0 ± 0.0 (51)	B
Flufenamic acid	7a							82 (96)	33 (355)	2.8 ± 0.8 (355)	B
										1.6 ± 0.5 (178)	B
	7b	O	2	Me	Me	Me	I	75 (199)	34 (199)	3.0 ± 0.7 (496)	A
										3.0 ± 0.0 (199)	B
	7c	S	2	Me	Me	Me	I	56 (196)	7 (196)	0.0 ± 0.0 (490)	A
Mefenamic acid	8a							60 (83)		0.0 ± 0.0 (415)	B
	8b	O	2	Me	Me	Me	I	36 (216)	13 (108)	0.0 ± 0.0 (216)	A
	8c	S	2	Me	Me	Me	I		0 (105)	0.0 ± 0.0 (526)	A
Zomepirac	9a								86 (17)	4.8 ± 0.5 (68)	A
										3.2 ± 0.5 (34)	A
										0.4 ± 0.5 (17)	A
	9b	O	2	Me	Me	Me	I	68 (10)	99 (20)	4.2 ± 0.5 (198)	A
										4.2 ± 0.4 (99)	B
	9c	S	2	Me	Me	Me	I	87 (38)	95 (38)	4.2 ± 0.5 (480)	A
										5.0 ± 0.0 (192)	B
Etodolac	10a								10 (35)	4.4 ± 0.5 (348)	B
	10b	O	2	Me	Me	Me	I		7 (50)	0.0 ± 0.0 (400)	B
	10c	S	2	Me	Me	Me	I	17 (47)	10 (47)	0.0 ± 0.0 (387)	B
Tifurac	11a							57 (30)	87 (15)	0.6 ± 0.6 (270)	A
	11c	S	2	Me	Me	Me	I	37 (36)	76 (36)	0.0 ± 0.0 (270)	B

[a] Biological evaluation protocols are described under Materials and Methods; for comparison purposes, all dose levels given in parentheses are in terms of μmol/kg compound delivered; waters of hydration are accounted for in molecular weights used for this conversion.
[b] ADAR = adjuvant-induced arthritis in the rat; data are presented as the % inhibition of hind paw weight increase, $P < 0.05$ ($n = 12$) by Student's t test unless otherwise noted.
[c] PQWM = phenylquinone-induced writhing in the mouse; data are presented as the % inhibition of the writhing response; $P < 0.05$ ($n = 8$) using Dunnetts test.
[d] GIER = gastrointestinal erosion induction assay in the rat; data are presented as the mean ulcer score (0–5) ± SD ($n = 5$).
[e] Dosing regimens: (A) single daily dose; (B) split daily dose (see Materials and Methods for details).
[f] Insoluble.

sonable antiinflammatory activity (ADAR), while exhibiting a reduced tendency to cause GI erosion (GIER) and significantly diminished analgetic properties (PQWM). This profile probably reflects an alteration in the conventional delivery mode for the active NSAID, by either a change in the locus of absorption or slow plasma release after absorption. In either case, however, local irritation due to the parent agent seems to be minimized. These effects are obtained, however, at the expense of the loss of acute analgetic effects. For example, comparison of naproxen (**1a**) with its thiocholine ester (**1c**) shows that antiinflammatory activity in ADAR is nearly identical (70 vs 64% at 30-μmol/kg dose), but analgetic activity is drastically decreased (90 vs 33% at 95- and 112-μmol/kg doses, respectively). However, the dose of **1c** required to induce significant GI erosion is significantly elevated (3.4 at 127 μmol/kg **1a** vs 3.6 at 838 μmol/kg **1c**), resulting in an almost sevenfold increase in the therapeutic index, as measured by antiinflammatory vs GI erosion potencies. This pattern of changes in the antiinflammatory/analgetic vs GI erosion potencies, in comparison with the parent NSAIDs, indicates that only compounds of the category may successfully qualify as useful prodrugs.

The magnitude of the therapeutic ratio shift does vary across the range of NSAIDs also, indicating that, as at the extremes of the spectrum of activity observed over all groups, the nature of the NSAID itself does play a significant role in the absorption and metabolism pattern observed for

Table III. Kinetic Data for the Hydrolysis of NSAID Esters at 37°C, pH 7.4[a]

Compound	$K_{obs} \times 10^6$ (sec^{-1})	T_{50} (hr)
1b	2.37	82
1c	1.65	117
1m	0.16	1260
2c	61.1	3.2
3c	1.49	129

[a] See Materials and Methods for protocol and HPLC conditions used to monitor hydrolyses.

each pair. The variability among the ester derivatives is reflected in the kinetic rates of hydrolysis, determined at pH 7.4 and 37°C, for a selected set of compounds (Table III). A number of these prodrugs, especially the naproxen esters (vide infra), maintain a great deal of antiinflammatory activity while exhibiting significant reductions in GI erosive properties. This may indicate that diversion of the delivery of the NSAID to alternative sites of absorption, such as the lower GI tract, may result in a more beneficial therapeutic ratio shift than increased stomach absorption, the usual prodrug goal, does.

In an attempt to determine the functional characteristics responsible for the observed profile, and the associated limitations, the series of naproxen esters (**1b–o**) was prepared. As noted above, the thiocholine ester (**1c**) offered a more favorable shift than did the choline ester (**1b**), probably due to somewhat increased hydrolytic stability (Table III). Replacement of iodide as counterion with chloride (**1d**) did not significantly alter activity. Removal of one methyl group of the quaternary ammonium moiety (**1e**) did markedly increase GI erosive potency, due to the increased instability of the ester in this compound, a characteristic attributable to the assisted hydrolysis provided by the β-dimethylamino functionality. Increased bulk in the quaternary ammonium group, either acyclic (**1f, g, k**) or cyclic (**1h–j**), decreased potency uniformly across the bioassay panel but still resulted in useful therapeutic ratios for these compounds. Lengthening of the alkyl chain of the ester group from two to six carbons gave mixed results in terms of both hydrolytic stability and alteration of the therapeutic profile, thus offering no advantage over the choline or thiocholine esters.

The preliminary biological and pharmaceutical evaluation of the ω-(trialkylammonium)alkyl(thio)esters of a series of nonsteroidal antiinflammatory agents reported here indicates that addition of the charged ester group to the parent agents can, in most cases, alter the therapeutic indices derived from antiinflammatory, analgestic, and GI erosive potencies. The observed shifts in activities may be due to changes in the absorption and metabolism pattern of the prodrug versus its parent, resulting in the release of the active agent in a manner which significantly diminishes both local irritation and systemic GI erosion. This profile could be due to a combination of factors including the rate of hydrolysis of the esters in the stomach, gut, and plasma, changes in the locus of absorption of the prodrug or NSAID, and altered metabolic disposition patterns resulting from these changes. Differences between agents may be attributable to differential rates of chemical or enzymatic hydrolysis of the esters. The results obtained from the compounds of this series indicate that esters of this general class may offer a means to modulate both the aqueous/lipid solubility and the hydrolytic/enzymatic cleavage indices of NSAID prodrugs which potentially possess a more favorable therapeutic ratio of antiinflammatory to GI erosive activities. Further detailed investigation into the actions of compounds of this family, in particular **1c** and **2c**, including additional studies of their biological activities, a summary of their cleavage to the respective parent NSAIDs by a variety of biologically relevant media, and the investigation of their mechanism and locus of action, will be reported subsequently.

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