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Diacyl glyceryl ester prodrugs for slow release in the skin: synthesis and *in vitro* degradation and absorption studies for naproxen derivatives

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Diacyl glyceryl ester derivatives of naproxen were synthesized and tested for transdermal and dermal administration. Diacyl derivatives of aliphatic acids of various chain length were compared. The pharmaceutical properties of these compounds, such as lipophilicity, hydrolysis in a buffer solution at various pH values and degradation in human serum and hairless mouse skin homogenate, were investigated. All the diacyl derivatives were relatively stable in a neutral buffer solution, but were rapidly degraded to release naproxen in human serum and hairless mouse skin homogenate. The diacyl compounds could not penetrate hairless mouse skin *in vitro*. However, significant absorption into the skin could be measured, and this increased with increasing lipophilicity. A more than 100-fold difference in absorption was observed. The prodrugs were slowly hydrolyzed to naproxen inside the skin. The release of naproxen to the receptor compartment of diffusion cells showed that this type of prodrug could be used for controlled drug delivery.

1. Introduction

The dermal application of drugs can be targeted for the dermal tissue, local delivery of drugs or sustained systematic delivery. The desired properties of a drug for dermal application are that it will be absorbed into the skin, and that in transdermal delivery, the transport is relatively rapid. The skin is a lipophilic, biological membrane, and small non-polar molecules are therefore well absorbed into the skin and will have a relatively high diffusion rate. Highly polar and charged molecules are poorly absorbed into the skin, and large ones (MW $>$ 800) can only penetrate this barrier with difficulty. Crystalline lipophilic compounds must be dissolved into the aqueous phase or the lipophilic phase of the formulation before they can be absorbed into the skin.

A common approach to increase the dermal absorption of highly polar compounds, such as nucleic acid analogues, is to convert them into more lipophilic prodrugs [1]. The barrier function of the skin can also be reduced by the application of penetration enhancers, such as polyols [2], amides [2], fatty acids [2–5] and monoglyceryl ethers [6] in the drug formulation. The absorption of drugs can also be enhanced by the addition of cyclodextrin to enhance the solubility in the aqueous phase of a cream formulation [7].

The aim of the present work was to develop prodrugs that were well absorbed into the skin but were not transdermally transported. Then, the active component could be slowly released into the skin. Such an approach would allow fewer applications in the treatment of dermal disorders and the use of dermal formulations rather than dermal patches for sustained release.

Some diacyl glycerol prodrugs intended for oral application have previously been reported [8–10]. Using diacyl glycerol pro moiety for dermal prodrugs offers several advantages. The diacyl glycerol component is ubiquitous in the skin and should have little adverse effects after hydrolysis of the parent compound. The inclusion of a fatty acid in the structure could allow the compound to function similarly as a penetration enhancer to facilitate its own absorption. Furthermore, the structural similarity of the prodrug to the membrane lipids should improve binding in the lipophilic layers of the skin.

NSAID have been used in cream formulations to alleviate allergic skin reactions [11]. Naproxen is an NSAID that has mainly been used in the management of arthritis. Topical application of this drug has been suggested to avoid gastric ulcerogenic side effects [12, 13].

In the present work, we have investigated a range of synthesized diacyl glyceryl ester derivatives with varying acyl (fatty acid) moieties using naproxen as a model drug. The structural effects on non-enzymatic and enzymatic hydrolysis rates and the lipophilicity were investigated. The absorption, transdermal transport and release in hairless mouse skin was investigated for some of these compounds.

2. Investigations and results

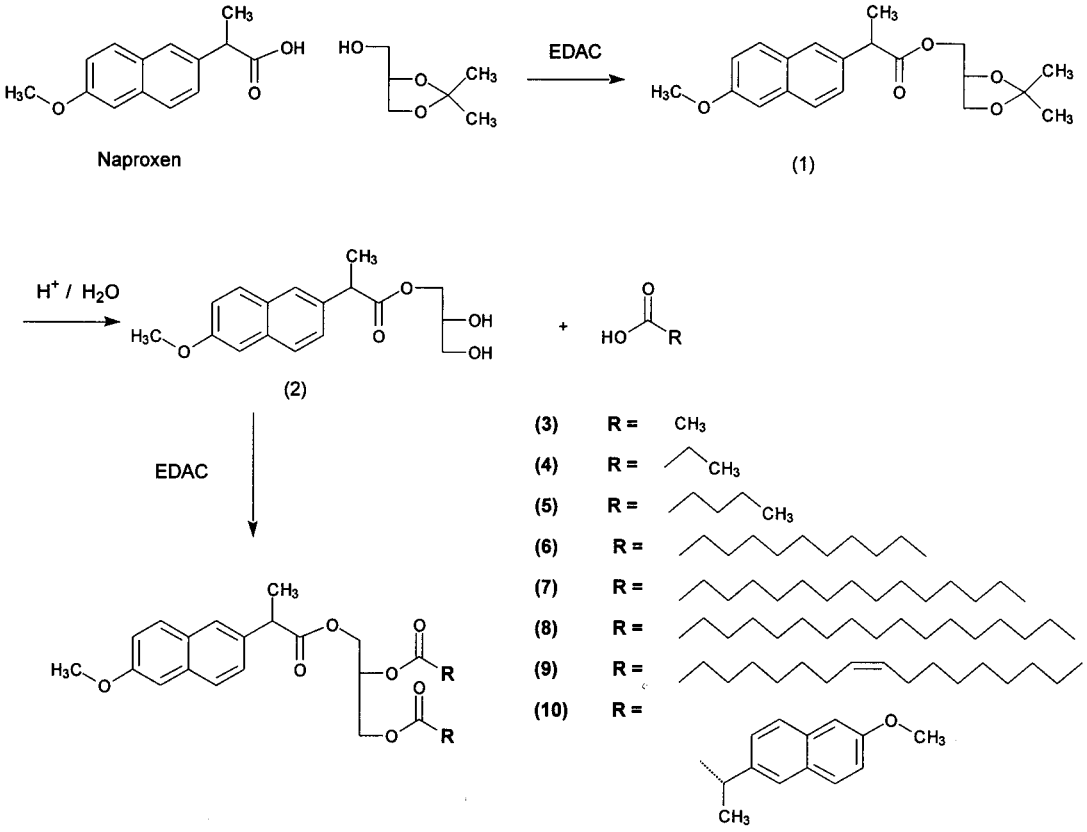
2.1. Synthesis of prodrugs

All the compounds were synthesized following the general route shown in Scheme 1. Naproxen was condensed with isopropylideneglycerol. After acidic removal of the isopropylidene protecting group, aliphatic esters were formed on the remaining 1,2 hydroxyl groups of the glycerol moiety. Following flash chromatography or crystallization, the compounds were obtained in 50–94% yield. All the derivatives were crystalline except dibutanoyl (**4**) and dioleoyl derivatives (**9**), which were obtained as oils. The isopropyliden glycerol was used as a racemic (D,L)-mixture and diastereomeric derivatives were therefore formed. Despite that the diastereomers did not separate in chromatographic procedures (flash chromatography, TLC and HPLC), the diastereomeric compounds had well defined melting points.

2.2. Apparent lipophilicity indices

All the derivatives, except compound **2**, were poorly soluble in water. The aqueous solubilities of compounds **3** and **4** were above 10 μg/ml. The aqueous solubility was so low that it could not be accurately determined by our experimental procedure. The apparent lipophilicity indices were therefore calculated from the TLC R_f values according to Sangster [14]. The method was based on extrapolation of the octanol/water partition coefficient for compounds **3** and **6**, which where measured by the shake flask method, and the value for naproxen and compound **2** had previously been determined [15, 16]. The lipophilic index for the oleyl derivative (**9**) was about 100 times higher than that for the acetyl derivatives (**3**) (Table 1).

Scheme 1



2.3. Degradation in aqueous buffers

All compounds had a maximum stability between pH 3 and 7, and showed similar pH dependence. This is illustrated in the pH-degradation rate profile for the dibutanoyl derivative (**4**), where there was a base-specific catalysis at high pH and acid-specific catalysis at low pH (Fig. 1). The degradation rate profile could thus be described by the following rate expression.

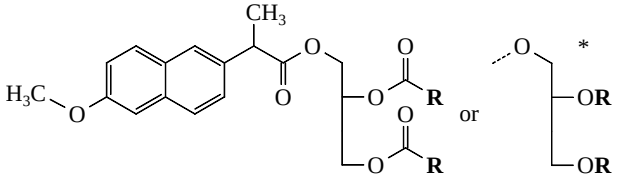
$$k_{obs.} = k_H[H^+] + k_0 + k_{OH}[OH^-]$$

where $[H^+]$ and $[OH^-]$ are the hydrogen ion and hydroxide ion concentration, k_H and k_{OH} are second-order rate constants for the specific acid- and base-catalyzed hydrolysis, and k_0 is a first-order rate constant for spontaneous or water-catalyzed hydrolysis. The following values were obtained from the profile: $k_H = 7.1\,M^{-1}\,h^{-1}$, $k_0 = 3.5 \times 10^{-2}\,h^{-1}$ and $k_{OH} = 6.7 \times 10^4\,M^{-1}\,h^{-1}$.

The degradation rates for compounds **1** and **4** could be determined in aqueous buffer solutions, but compounds **5** to **9** were insoluble in water. The degradation rate constants were therefore also determined in 50% aqueous, 50 mM HCl or 50% aqueous, 5 mM NaOH ethanaolic solutions to allow a comparison between various aliphatic chain lengths (Table 2). No degradation of naproxen could be detected under the experimental conditions.

Compound **1** was rapidly degraded under acidic conditions, and the primary product was **2**, due to hydrolysis of the ketal. Conversely, this compound degraded more slowly than the other compounds under basic conditions.

Table 1: Relative lipophilicity indices obtained from the TLC R_f values for the naproxen derivatives



Compd.	R	R_f	Log K_{app}
Naproxen	–	0.46	3.18
1	$>CH(CH_3)_2$*	0.61	4.23
2	–H*	0.13	2.65
3	$-CH_3$	0.56	3.78
4	$-C_3H_7$	0.67	4.84
5	$-C_5H_{11}$	0.77	5.68
6	$-C_{11}H_{23}$	0.81	6.08
7	$-C_{15}H_{31}$	0.84	6.28
8	$C_{17}H_{33}$	0.90	6.39
9	$C_{17}H_{35}$	0.85	6.84

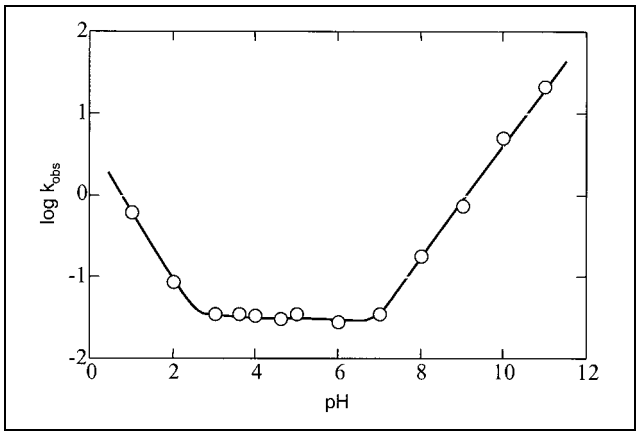


Fig. 1: pH degradation rate profile for compound **4**

Table 2: Hydrolytic degradation rates and $t_{1/2}$

	Aqueous buffer solutions				50% aqueous ethanolic solutions			
	pH2		pH 9		59 mM HCL		0.5 mM NaOH	
Compd.	k_{obs} (h^{-1})	$t_{1/2}$ (h)	k_{obs} (h^{-1})	$t_{1/2}$ (h)	k_{obs} (h^{-1})	$t_{1/2}$ (h)	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1	0.500	1.4	0.1831	3.8	>20*	<0.04	0.016 ± 0.001	44 ± 3
2	0.078	8.9	0.2720	2.5	0.021 ± 0.001	33 ± 2	0.029 ± 0.001	24 ± 1
3	0.085	8.2	0.8128	0.8	0.247 ± 0.003	2.8 ± 0.1	0.230 ± 0.010	3.0 ± 0.15
4	0.072	9.6	0.7610	0.9	0.102 ± 0.003	6.8 ± 0.2	0.061 ± 0.005	11.3 ± 1.0
5					0.109 ± 0.004	6.3 ± 0.3	0.049 ± 0.001	14.0 ± 0.2
6					0.070 ± 0.000	9.9 ± 0.0	0.051 ± 0.000	13.6 ± 0.0
7					0.080 ± 0.004	8.6 ± 0.5	0.056 ± 0.003	12.4 ± 0.7
8					0.101 ± 0.003	6.9 ± 0.4	0.047 ± 0.002	14.7 ± 0.7
9					0.098 ± 0.009	7.0 ± 0.7	0.033 ± 0.002	21.0 ± 1.5

* **1** was completely degraded after 12 min

Compound **2** degraded 8 to 12 times more slowly than the acetyl derivative (**3**). The same trends, but with much smaller differences, could be observed for aqueous solutions. The longer alkyl chain compounds could only be compared in ethanolic solutions. The degradation was fastest for **3** but was very similar for the derivatives with longer aliphatic chains, and decreased slightly with increased length of the alkyl chain in the alkaline solutions.
Formation of monoacyl-derivatives was observed for the degradation of the aliphatic acid derivatives in acidic solutions, followed by the appearance of naproxen. In alkaline solutions, the compounds were hydrolyzed directly to naproxen.

2.4. Bioconversion

Figs. 2 and 3 show the degradation of **2** and **4**, respectively, along with the release of naproxen and other degradation products. The conversion of these compounds in serum was 100% and the degradation rates were at least 100 times faster than in aqueous buffer solutions. The degradation rate constants for compounds **2** and **4** were $k_{obs} = 0.46\ h^{-1}$ ($t_{1/2} = 1.5$ h) and $k_{obs} = 1.3\ h^{-1}$ ($t_{1/2} = 0.5$ h), respectively. Naproxen was the only detectable degradation product of **2**. Naproxen was also the main initial degradation product of **3**, with the monoacyl derivative present for the first 2 h and the glycerol derivative present for 4 h. Compound **3** was tested in human plasma, and its behavior was similar to **4** with $k_{obs} = 0.64\ h^{-1}$ ($t_{1/2} = 65$ min). Serum degradation of the more lipophilic compounds **6** to **10** could not be measured under the experimental conditions used.
The degradation of compounds **3**, **5** and **6** was measured in hairless mouse skin homogenate. The k_{obs} in skin homogenate was within the same range as that in serum, and compound **6** degraded fastest in this case. The compounds degraded rapidly in fresh homogenate, and the degradation was first-order (Table 3). However, after 5 h, the hydrolytic enzymes were no longer active, and no further degradation could be detected.

Table 3: Degradation rate in hairless skin homogenate

Compd.	k_{obs} (h^{-1})	$t_{1/2}$ (h)
Naproxen	0	–
3	0.509 ± 0.047	1.4 ± 0.1
5	0.284 ± 0.003	2.4 ± 0.0
6	0.765 ± 0.048	0.9 ± 0.1

2.7. Adsorption on hairless mouse skin

Propyleneglycol was used as the donor phase for the skin permeation experiments as the acyl derivatives were poorly soluble or insoluble in water. Previously, it has been reported that naproxen and compound **2** can be transdermally transported [16]. This was confirmed in the present study. However, it was also observed that compound **2** was partially degraded, and 5–10% of the total naproxen that emerged into the receptor phase had been released from the glycerol moiety. Although the diacyl glyceryl esters of naproxen did not penetrate the skin, some significant amount of naproxen was detected in the acceptor phase. The concentration of naproxen increased with time (Fig. 4). Trinaproxyl glycerol was only slowly degraded in serum, and when this compound was applied to the skin, no naproxen could be detected in the donor phase.

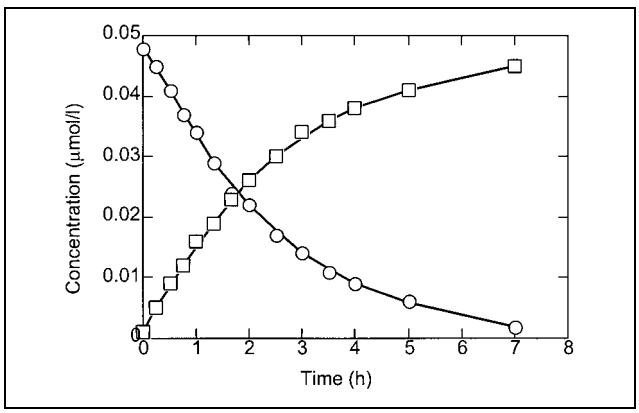


Fig. 2: Degradation of compound **2** at pH 9, 60 °C. Concentrations of **2** (○) and naproxen (□) were measured by HPLC

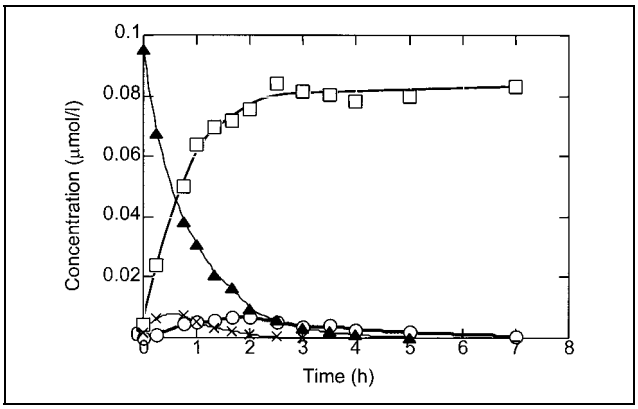


Fig. 3: Degradation of compound **4** in serum at 37 °C. The concentrations of compound **4** (△), the mono acyl naproxen derivative (×), compound **2** (○) and naproxen (□) were measured by HPLC

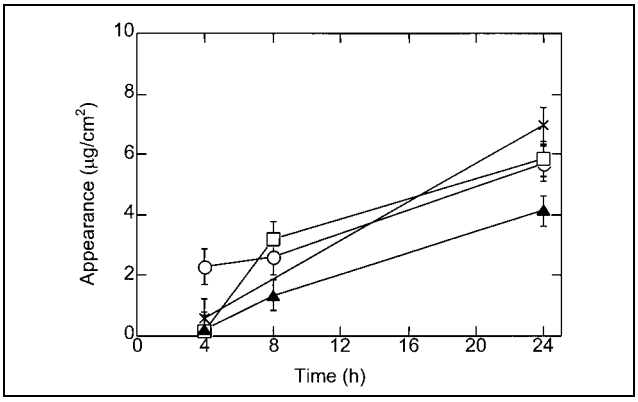


Fig. 4: Appearance of naproxen in the donor phase with 10 mg/ml of compound **3** (×), **5** (△), **6** (□) or **9** (○) in the donor phase

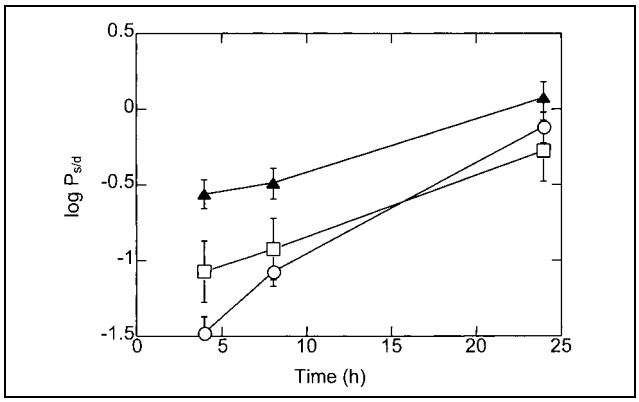


Fig. 5: Partition of compounds **5** (×), **6** (□) and **9** (△) from the donor phase into hairless mouse skin

Apparently the diacyl glycerol esters were absorbed into the skin and degraded to release naproxen in the acceptor phase. A second experiment was therefore undertaken to measure the partition of three of these compounds into the skin and the simultaneous release of naproxen into the acceptor phase.

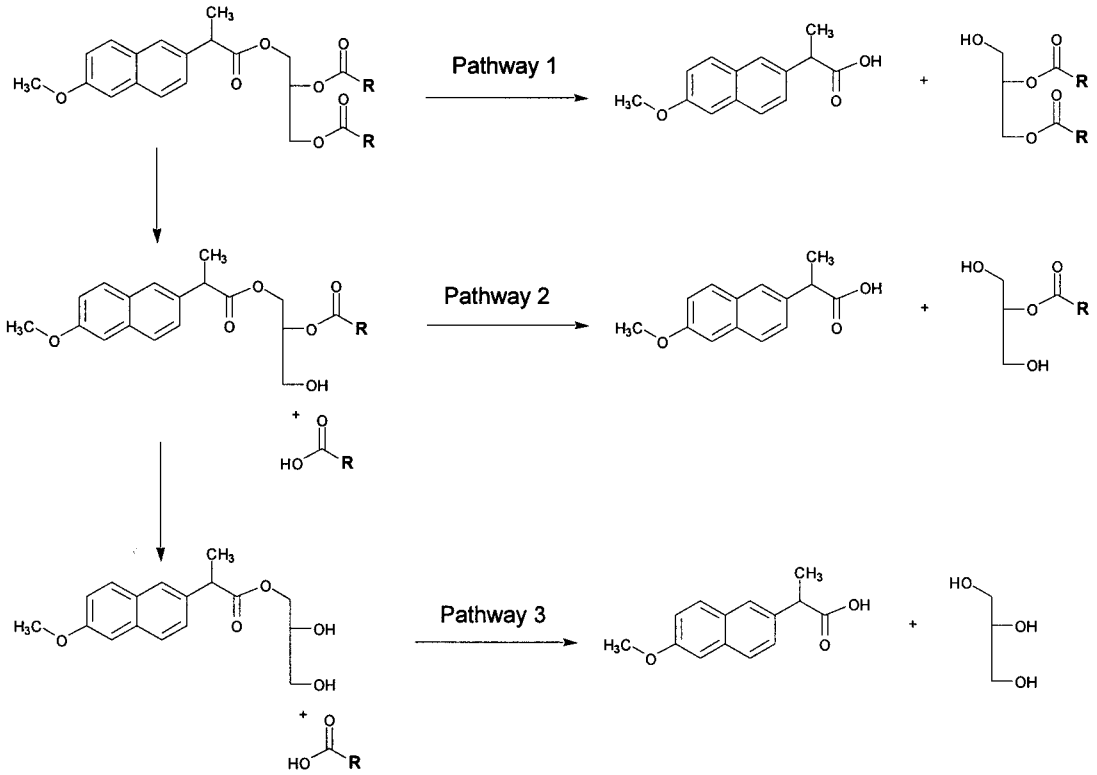
There was a continuous buildup of the compounds in the skin for 24 h (Fig. 5) with an almost linear increase of the compounds in the skin. However, the difference between the compounds decreased with time. After 4 h of incubation, there was about 10 times more dioleoyl derivative than dihexanoyl derivative. After 24 h the difference between the derivatives was only about 1.5-fold. Naproxen accumulated poorly in the skin, about 10 times less than the dihexanoyl derivative.

3. Discussion

The general method of preparing the diacyl glyceryl derivatives could also be used to prepare similar derivatives of other drug compounds containing a free carboxylic acid moiety. The lipophilicity of diacyl glyceryl esters of naproxen increased with their chain length, as would be expected. The largest difference between the diacyl derivatives was 100-fold. However, this is only a rough estimate of values that could not be directly measured. Best results are usually obtained between R_f values 0.2–0.8 [14].

When the diacyl derivatives were hydrolyzed, three pathways leading to free naproxen could be identified (Scheme 2). Esters of the relatively acidic acetic acid were more easily hydrolyzed than butyl or naproxyl esters. Compound **3** was therefore degraded faster than the other diacyl glyceryl compounds. However, the difference could also be explained by less self-association. In partially ethanolic HCl solutions, the aliphatic acid moieties tended to be released first (pathway 2 and 3), whereas in degradation in partially ethanolic alkaline solutions, the naproxen moiety was removed first (pathway 1). Although the compounds were stable in neutral buffer solutions, they were rapidly degraded by hydrolytic enzymes in biological sam-

Scheme 2



ples, showing that these types of compounds could also be appropriate as prodrugs for dermal applications. The bioconversion showed that these derivatives were primarily converted by pathway 1 to naproxen. In both human serum and hairless mouse skin homogenate, the degradation proceeded faster with longer alkyl chain derivatives. This chain-length dependence has previously been observed in several studies [17–19] when single aliphatic chain prodrugs were tested. The degradation rates in human serum were similar to what has recently been reported by Rautio et al. [20] for simple hydroxyalkyl esters of naproxen. This information can be useful in determining the suitable length of fatty acid derivatives, where a long- or short-term drug release is required. However, protein binding and absorption of the more lipophilic derivatives may decrease their degradation rate *in vivo*.

The prodrugs were structurally similar to triacylglycerol and some skin permeation enhancers. Initially, the highly lipophilic dioleoyl derivative partitioned better into the skin than the derivatives with shorter aliphatic side chains. This is consistent with the observation that oleic acid can function as a skin permeation enhancer [4]. These compounds did not penetrate the skin as such but were degraded to continuously release the naproxen. The limiting factor for the absorption of the longer chain length derivatives would be their solubiltity in the propylene glycol donor phase. However, such disadvantages can be overcome with derivatives of unsaturated fatty acids, such as oleic acid, which are present as oils at room temperature. Such oily compounds could be applied directly to the skin, and in further work, this approach will be investigated.

The use of prodrugs that accumulate in the skin and release the drug slowly can be considered as an alternative to dermal patches for potent drugs. This approach can also have value for local treatment with drugs acting against dermal diseases, such as inflammation, bacterial and viral infection, where the prodrug will accumulate at the site of action and release the active compound over a long period.

4. Experimental

4.1. Synthesis of naproxen derivatives

4.1.1. (D,L)-*1,2-Isopropylidene-3-naproxenoglycerol* (**1**)

Naproxen (4.0 g, 17 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, 3.6 g, 18 mmol) in dichloromethane (45 ml) was stirred for 15 min. 1,2-Isopropylideneglycerol (2.4 g, 18 mmol) and 4-dimethylaminopyridine (DMAP, 50–100 mg) was added to the solution and refluxed for 16 h. The mixture was concentrated under reduced pressure, diluted with $(C_2H_5)_2O$ (100 ml) and washed, first with H_2O (100 ml), then with aqueous 5% Na_2CO_3 (100 ml) and finally again with H_2O (100 ml). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure; the oily residue was purified by silica gel 60 (0.063–0.200 mm) column chromatography, eluted with a mixture of toluene-THF-acetic acid (96/3/1), followed by crystallization from CH_3OH/H_2O (98/2). The title compound was obtained as a white powder (4.3 g, 72% yield), m.p. 62–64 °C.

^{1}H NMR ($CDCl_3$, δ: 7.71–7.10 (m, 6 H); 4.24–4.07 (m, 3 H); 3.98–3.88 (m, 5 H); 3.66–3.59 (m, 1 H); 1.58 (d, J = 7.15 Hz, 3 H); 1.34 (s, 3 H) and 1.32 (s, 3 H) ppm.

4.1.2. (D,L)-*1-Naproxenoylglycerol* (**2**)

A solution of (D,L)-isopropylidene-3-naproxenoyl glycerol (**1**) (2.5 g 7 mmol) in a mixture of water-acetic acid-acetone (90/9/1) was heated up to 70 °C, allowed to cool and stirred at room temperature for 4 h. The reaction mixture was then concentrated under reduced pressure; the oily residue was purified by silica gel 60 (0.063–0.200 mm) column chromatography, eluted with a mixture of CH_2Cl_2/CH_3OH (97/3), which was followed by crystallization from CH_3OH. This afforded the desired product as a white solid (1.77 g, 81% yield); m.p. 61–63 °C.

^{1}H NMR ($CDCl_3$), δ 7.73–7.12 (m, 6 H); 4.25–4.05 (m, 2 H); 3.97–3.80 (m, 5 H); 3.62–3.40 (m, 2 H); 2.05–2.15 (m, 2 H); 1.58 (d, J = 7.15, 3H) ppm.

4.1.3. (D,L)-*1,2-Diacetyl-3-naproxenoylglycerol* (**3**)

To a (D,L)-1-naproxenoyl glycerol (**2**) (0.21 g 0.69 mmol) in CH_2Cl_2 (20 ml) solution, acetic anhydride (0.1 g 98 mmol), 4-dimethylaminopyridine (DMAP, 0.05 g 0.4 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, 0.02 g 0.1 mmol) was added. The mixture was refluxed for 3 h; washed with H_2O (3 × 10 ml), and evaporated under reduced pressure; the oily residue was purified by silica gel 60 (0.063–0.200 mm) column chromatography, eluted with a mixture of toluene-methanol (98/2), which was followed by crystallization from CH_3OH (7–10 days). The title compound was obtained as a white powder (253 mg, 94% yield); m.p. 60 to 63 °C.

^{1}H NMR ($CDCl_3$), δ 7.75–7.10 (m, 6 H); 5.25–5.15 (m, 1 H) 4.35–3.80 (m, 8 H); 2.05 (s, 3 H); 1.89 (s, 1.5 H*), 1.88 (s, 1.5 H*) (d, 3 H); 1.58 (J = 7.2 Hz 3 H) ppm. * The diastereomers were resolved in these peaks.

4.1.4. (D,L)-*1,2-Dibutanoyl-3-naproxenoylglycerol* (**4**)

The title compound was obtained as a yellowish oil (81% yield) by acylation of (D,L)-1-naproxenoylglycerol (**2**) with butaric anhydride following the same general experimental procedure as in 4.1.3.

^{1}H NMR ($CDCl_3$), δ 7.75–7.15 (m, 6 H); 5.20–5.10 (m, 1 H); 4.20–4.10 (m, 2 H); 3.95–3.70 (m, 6 H); 2.40–2.30 (m, 4 H); 1.75–1.60 (m, 7 H); 1.00–0.90 (m, 6 H) ppm.

4.1.5. (D,L)-*1,2-Dihexanoyl-3-naproxenoylglycerol* (**5**)

Hexanoic acid (0.08 g, 0.7 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC, 0.13 g 0.7 mmol) in CH_2Cl_2 (15 ml) was stirred for 15 min. (D,L)-1-Naproxenoylglycerol (**2**) (0.12 g 0.4 mmol) and 4-dimethylaminopyridine ethyl amine (DMAP, 30–100 mg) was added to the solution and refluxed for 16 h. The mixture was concentrated under reduced pressure, diluted with ether (20 ml), washed first with H_2O (20 ml), then with aqueous 1% Na_2CO_3 (20 ml) and finally with H_2O (20 ml). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure; the oily residue was purified on silica gel 60 (0.063–0.200 mm) column chromatography, eluted with a mixture of toluene-THF-acetic acid (96/3/1), which was followed by crystallization from C_2H_5OH. The title compound was obtained as a white powder (100 mg, 50% yield); m.p. 83–85 °C.

^{1}H NMR ($CDCl_3$, δ 7.75–7.15 (m, 6 H); 5.20–5.10 (m, 1 H); 4.25–4.10 (m, 2 H); 3.95–3.70 (m, 6 H); 2.40–2.30 (m, 4 H); 1.70–1.55 (m, 7 H); 1.35–1.25 (m, 8 H); 0.95–0.85 (m, 6 H) ppm.

4.1.6. (D,L)-*1,2-Dilauroyl-3-naproxenoylglycerol* (**6**)

The title compound was obtained as a white powder (68% yield) by acylation of **2** with lauric acid following the same general experimental procedure as for **5**; m.p. 36–38 °C.

^{1}H NMR ($CDCl_3$), δ 7.75–7.15 (m, 6 H); 5.20–5.10 (m, 1 H); 4.25–4.10 (m, 4 H) 3.95–3.70 (m, 4 H); 2.40–2.30 (m, 4 H); 1.65–1.55 (m, 7 H); 1.35–1.20 (m, 32 H); 0.95–0.85 (m, 6 H) ppm.

4.1.7. (D,L)-*1,2-Dipalmitoyl-3-naproxenoylglycerol* (**7**)

The title compound was obtained as a white solid (69% yield) by acylation of **2** with palmitic acid following the same experimental procedure as described above, followed by crystallization from CH_3OH; m.p. 52–54 °C.

^{1}H NMR ($CDCl_3$), δ 7.70–7.05 (m, 6 H); 5.30–5.20 (m, 1 H); 4.25–4.00 (m, 4 H); 3.90–3.65 (m, 4 H); 2.30–2.20 (m, 4 H); 1.65–1.55 (m, 7 H); 1.35–1.20 (m, 48 H); 0.95–0.85 (m, 6 H).

4.1.8. (D,L)-*1,2-Distearoyl-3-naproxenoylglycerol* (**8**)

The title compound was obtained as a white solid (70% yield) by acylation of **2** with stearic acid following the same experimental procedure as described above, followed by crystallization from C_2H_5OH; m.p. 53–55 °C.

^{1}H NMR ($CDCl_3$), δ 7.70–7.05 (m, 6 H); 5.30–5.20 (m, 1 H); 4.25–4.00 (m, 4 H); 3.90–3.65 (m, 4 H); 2.30–2.00 (m, 4 H); 1.65–1.55 (m, 7 H); 1.35–1.20 (m, 56 H); 0.95–0.85 (m, 6 H) ppm.

4.1.9. (D,L)-*1,2-Dioleoyl-3-naproxenoylglycerol* (**9**)

The title compound was obtained as a clear oil (84% yield) by acylation of **2** with oleic acid as described before.

^{1}H NMR ($CDCl_3$), δ 7.75–7.05 (m, 6 H); 5.40–5.30 (m, 4 H); 4.25–4.00 (m, 3 H); 4.00–3.50 (m, 6 H); 2.30–2.20 (M, 12 H); 1.70–1.50 (m, 7 H); 1.40–1.20 (m, 40 H); 0.95–0.85 (m, 6 H) ppm.

4.1.10. (D,L)-*1,2,3-Trinaproxenoylglycerol* (**10**)

Naproxen (0.75 g, 3 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, 0.4 g, 2 mmol) in CH_2Cl_2 (15 ml) was stirred for 15 min. Glycerol (0.05 g, 0.5 mmol) and 4-dimethylaminopyridine (DMAP, 20–50 mg) was added to the solution and refluxed for 3 h. The mixture was washed three times with H_2O (10 ml). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure; the oily residue

was purified on silica gel 60 (0.063–0.200 mm) column chromatography, eluted with toluence, which was followed by crystallization from CH_3OH. The title compound was obtained as a white powder (0.25 g, 67% yield); m.p. 102–104 °C.
^{1}H NMR ($CDCl_3$) δ 7.75–7.05 (m, 18 H); 5.20–5.10 (m, 1 H); 4.30–4.00 (m, 2 H); 3.90 (s, 9 H); 3.80–3.70 (q, J = 7.14, 1 H); 3.55–3.45 (m, 2 H); 1.49 (d, J = 7.16, 3 H) 1.36 (d, J = 7.14, 3 H) and 1.34 (d, J = 7.14, 3 H) ppm.

4.2. HPLC measurements

The HPLC system consisted of a constaMetric 3000 (Milton Roy) solvent delivery system with a SP8450 (Spectra-Physics) variable wavelength detector, using a 150 mm, 4.6 mm i.d., 5 μm bead, C18 reverse-phase column.
The detection was performed at 230 nm wavelength and the flow rate was 1.5 ml/min. The mobile phases and retention times for each compound were as follows: naproxen acetonitrile, acetic acid, H_2O (40 : 1 : 59), 3.5 min; (**2**) acetonitrile, acetic acid, H_2O (40 : 1 : 59), 2.5 min; (**1**) acetonitrile, acetic acid, H_2O (70 : 1 : 29), 4.2 min; (**3**) acetonitrile, acetic acid, H_2O (70 : 1 : 29), 3.4 min; (**4**) acetonitrile, acetic acid, H_2O (80 : 1 : 19), 5.2 min; (**5**) acetonitrile, acetic acid, H_2O (90 : 1 : 9), 5.4 min; (**6**) acetonitrile, isopropanol (80 : 20), 3.6 min; (**7**) acetonitrile, isopropanol (60 : 40), 5.2 min; (**9**) acetonitrile, isopropanol (60 : 40), 4.8 min; (**8**) acetonitrile, isopropanol (50 : 50), 3.6 min; (**10**) acetonitrile, H_2O (90 : 10), 4.0 min.

4.3. Degradation rate studies

The degradation was measured by periodical HPLC analysis of buffered solutions heated on the sample rack. The degradation rate constants (k_{obs}) were obtained by linear regression of the peak integration. The degradation rates of **1** to **4** were determined at various pH in a Theorell-Stenhagen buffer system [21] (citrate, phosphate, borate, NaOH) and Na/citric buffer at 65 °C. Degradation rates at pH 2 (0.01 M HCl) and pH 10 (0.001 M NaOH) were determined for these compounds, which possessed sufficient solubility in aqueous buffer solution. Degradation rates of all the compounds were determined in 50% C_2H_5OH (5 mM HCl and 0.5 mM NaOH), at 65 °C.

4.4. Determination of R_f-value and log K_{app}

R_f-values were determined on TLC aluminum sheets silica gel 60 F_{254} from Merck. All the compounds were eluted with toluene/acetic acid/ethanol (24 : 1 : 1). The compounds were detected as black spots in UV-Betrachter from CAMAG at 254 nm. Apparent lipophilicity indices (log K_{app}) were calculated by the method of TLC correlation to the shake flask method [14]. Compounds which had sufficient aqueous solubility so that the octanol-water partition coefficients could be measured directly were used to determine the correlation coefficient. Two of the most hydrophilic compounds had been determined before [15, 16] and their o/w partition coefficients were also extrapolated in this fashion.

4.5. Bioconversion

4.5.1. Degradation in human serum

Degradation of **2**, **3**, **4** and **6** was measured in 97% human serum at 37 °C by dissolving the compound in C_2H_5OH. Samples were withdrawn (100 μl) at selected time intervals and the reaction quenched by addition of 100 μl 0.5 M perchloric acid. This was followed by dilution with 800 μl of mobile phase and centrifugation at 4000 rpm for 20 min. Aliquots of the clear supernatant were assayed by HPLC. An experimental standard was prepared by adding one of the compounds in phosphate buffer at pH 7.4, and no degradation was observed during the experiment.

4.5.2. Degradation in hairless mouse skin homogenate

Degradation of naproxen, **3**, **5** and **6** was measured in 97% hairless mouse skin homogenate by homogenizing 6 g of the skin in an 80-ml phosphate buffer at pH 7.4. The homogenate was maintained at 37 °C, and the compounds were dissolved in C_2H_5OH. Samples were withdrawn (100 μl) at selected time intervals and the reaction quenched by addition of 100 μl 0.5 M perchloric acid. This was followed by dilution with 800 μl of acetonitrile and centrifugation at 4000 rpm for 20 min. Aliquots of the clear supernatant were assayed by HPLC. When the compounds were treated in a phosphate buffer at pH 7.4, no degradation was observed during the experiment.

4.5.3. Adsorption on hairless mouse skin

Female hairless mice, (3 CH/Tif hr/hr) obtained from Bommice (Denmark), were sacrificed by cervical dislocation and their full-thickness skins removed. The outer surface of the skin was rinsed with 35% (v/v) CH_3OH in H_2O and subsequently with distilled H_2O to remove any contamination. The skin was placed in Franz diffusion cells of type FDC 400 15 FF (Vangard International Inc., USA). The receiver compartment had a volume of 12.3 ml. The surface area of the skin in the diffusion cell was 1.77 cm^2. The receptor phase consisted of a phosphate buffer saline pH 7.4 containing 0.3% (w/v) Brij-58 to ensure sufficient drug solubility in the receptor phase. The receptor phase was sonicated under vacuum prior to use to remove dissolved air. The skin diffusion cells were stirred with a magnetic bar and kept at 37 °C by circulating H_2O through an external jacket. The donor phase consisted of suspension or solution of the prodrug in propyleneglycol that had been heated in a sonic water bath (50 ° for 30–40 min). After equilibration at room temperature, 2.0 ml of the donor phase were applied to the skin surface and the donor chamber covered with parafilm. Samples (100 μl) of the receptor phase were removed from the cells at various time intervals of up to 48 h and replaced with fresh buffer solution. The samples were kept frozen until analyzed by HPLC.
The same procedure was used to measure skin absorption except that the skin was removed after 4, 8 and 24 h, washed thoroughly with 50% (v/v) CH_3OH in H_2O and subsequently with distilled H_2O, dried with a paper wipe and kept frozen until analyzed. Parts of the skin were cut out, weighed, dissolved in 1 ml 5M HCl and heated to 70–80 °C until the skin was completely dissolved and all prodrugs degraded (16–24 h). The solution was neutralized with 1 ml 5M NaOH, and centrifuged at 4000 rpm for 30 min. Aliquots of the clear supernatant were assayed by HPLC. The total concentration of naproxen in the skin was measured. An experimental standard and recovery were prepared by adding a known amount of compounds to a clean skin sample and using the same procedure as described above. The recovery was 90% and naproxen was not degraded. The naproxen concentration in the receptor phases was also measured. Each experiment was repeated three or four times, and the results reported are the mean values ±standard error of the mean. The partition of the drug from the donor phase into the skin ($P_{s/d}$) was calculated by dividing the molar concentration in the skin by the molar concentration in the donor phase. The specific gravity of the skin was 1 g/ml.

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References

1 Bando, H.; Takagi, T.; Yamashita, F.; Takakura, Y.; Hashida, M.: Pharm. Res. **13**, 427 (1996)
2 Smith, E. W.; Maibach, H. I.: Percutaneous Penentration, p. 1, CRC Press, Inc, New York 1995
3 Cooper, E. R.; Merritt, E. W.; Smith, R. L.: J. Pharm. Sci. **74**, 688 (1985)
4 Loftsson, T.; Bodor, N.: Percutaneous penetration enhancer of oleic aacid and 2-ethyl-1,3-hexanediol, USA Patent 1991
5 Loftsson, T.; Gudmundsdottir, T. K.; Fridriksdottir, H.; Sigurdardottir, A. M.; Thorkelsson, J.; Gudmundsson, G.; Hjaltason, B.: Pharmazie **50**, 188 (1995)
6 Loftsson, T.; Masson, M.; Sigurdsson, H. H.; Magnusson, P.; Goffic, F. L.: Pharmazie **53**, 137 (1997)
7 Loftsson, T.; Bodor, N.: Percutaneous Penetration Enhancers, p. 335, CRC Press, Boca Rato, Florida, 1995, pp. 335.
8 Cordi, A.; Lacoste, J. M.; Duhault, J.; Espinal, J.; Boulanger, M.; Broux, O.; Husson, B.; Volland, J. P.; Mahieu, J. P.: Arzneim.-Forsch. **45**, 997 (1995)
9 Scriba, G. K.: Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry, Pharm. Res. **10**, 1181 (1993)
10 Sugihara, J.; Furuuchi, S.; Ando, H.; Takashima, K.; Harigaya, S.: J. Pharmacobio-Dyn. **11**, 555 (1988)
11 Loftsson, T.; Bodor, N.: J. Pharm. Sci.**70**, 756 (1981)
12 Harrison, I. T.; Lewis, B.; Nelson, P.; Rooks, W.; Roszkowski, A.; Tomolonis, A.; Fired, J. H.: J. Med. Chem. **13**, 203 (1970)
13 Roszkowski, A. P.; Rooks, W. H.; Tomolonis, A. J. and Miller, L. M.: J. Pharmacol. Exp. Ther. **179**, 114 (1971)
14 Sangster, J.: John Wiley and Sons Ltd. Chichester 1997
15 Dunn, W. J.: J. Med. Chem. **46**, 484 (1973)
16 Weber, H.; Meyer-Trumpener, K.; Lippold, B. C.: Arch. Pharm. **327**, 681 (1994)
17 Redden, R.; Douglas, J. E.; Burke, M. J. and Horrobin, D. F.: Int. J. Pharm. **165**, 87 (1998)
18 Silverman, R. B. (Ed.): The organic Chemistry of Drug Design and Action, Academic Press, New York 1992
19 Yamaoka, Y.; Roberts, R. D.; Stella, V. J.: J. Pharm. Sci. **72**, 400 (1983)
20 Rautio, J.; Taipale, H.; Gynther, J.; Vepsalainen, J.; Nevalainen, T.; Jarvinen, T. J.: J. Pharm. Sci **87**, 1622 (1998)
21 Fasman, G. D. (ED.): Practical Handbook of Biochemistry and Molecular Biology, CRC Press, Boca Raton 1990

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