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PARACETAMOL (ACETAMINOPHEN) ESTERS OF SOME NON-STEROIDAL ANTI-INFLAMMATORY CARBOXYLIC ACIDS AS MUTUAL PRODRUGS WITH IMPROVED THERAPEUTIC INDEX

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ABSTRACT

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Paracetamol (acetaminophen) esters [**4a–f**] of some acidic NSAIDs were synthesized and evaluated as mutual prodrug forms with the aim of improving the therapeutic index through prevention of the gastrointestinal toxicity. The structures of the synthesized esters were confirmed by IR and ^{1}H-NMR spectroscopy and their purity was established by elemental analyses and TLC. In-vitro stability studies revealed that the synthesized ester prodrugs **4a–f** are sufficiently chemically stable in non-enzymatic simulated gastric fluid (hydrochloric acid buffer of pH 1.3 ($t_{\frac{1}{2}} \sim$ 15–45 h)) and in phosphate buffer of pH 7.4 ($t_{\frac{1}{2}} \sim$ 4–40 h). In 80% human plasma and 10% rat liver homogenate, the mutual prodrugs were found to be susceptible to enzymatic hydrolysis releasing the corresponding NSAID and paracetamol at relatively faster rates ($t_{\frac{1}{2}} \approx$ 15–385 min and 1–140 min, respectively). Calculated log P values indicated that the prodrugs **4a–f** are more lipophilic than the parent drugs.

In-vivo experiments in rabbits showed higher plasma levels of ibuprofen after oral administration of its ester prodrug **4b** compared with those resulting from an equivalent amount of the corresponding physical mixture. Moreover, significant improvement in latency of pain threshold in mice has been observed up to 4 h after po administration of 0.02 mmol/kg of the prodrugs, compared with the corresponding physical mixtures. Gross observations and scanning electromicrographs of the stomach showed that the prodrugs induced very little irritancy in the gastric mucosa of mice after oral administration for 4 days. These results suggest that the synthesized mutual ester prodrugs were characterized by a better therapeutic index than the parent drugs.

Keywords: paracetamol (acetaminophen), NSAIDs, chemical and enzymatic stability, ulcerogenicity, pain threshold latency, prodrugs

INTRODUCTION

The potentially deleterious effects of the acidic non-steroidal anti-inflammatory drugs on the stomach are well known [1–3]. The direct contact mechanism was considered to play a determinant role in the formation of gastrointestinal lesions [4]. It is probably attributed to the free carboxylic group of the NSAIDs and to local inhibition of the cytoprotective action of prostaglandins on gastric mucosa [5]. On the other hand, paracetamol (acetaminophen) **1** is a commonly used analgesic, which is remarkably

safe at therapeutic doses. However several reports stated that large overdoses of paracetamol can produce a fulminant hepatic and renal tubular necrosis due to formation of the toxic metabolite, *N*-acetyl-*p*-benzoquinoneimine, NAPQI, **2** [6,7]. Ester prodrug forms have been shown to be one of the effective mechanisms that markedly reduce the ulcerogenic side-effects of some potent NSAIDs [8–11]. In addition, it has been reported that the aforementioned induced hepatotoxicity of paracetamol can be prevented through ester prodrug formation [12,13].

Thus, in these studies, we report the synthesis and evaluation of mutual ester prodrugs of some NSAIDs using paracetamol as masking group for the free carboxylic function to reduce their gastrointestinal side-effects. Benorylate **3**, the well-known mutual prodrug of aspirin and paracetamol [14,15] was also synthesized and investigated for comparison. The mutual prodrug form was selected as advantagenous, since the possible degradation products have well-established pharmacological, pharmacokinetic and biochemical profiles [16–19].

METHODS

Chemicals

Paracetamol and the studied non-steroidal anti-inflammatory drugs were provided by several pharmaceutical companies in Cairo (Kahira; El-Nasr; Misr and Eipico) and were used as received. All other chemicals and solvents used in the syntheses were of reagent grade and those used in kinetic studies were of analytical grade.

Analytical methods

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, England) and were uncorrected. IR spectra were recorded on a Schimadzu 200-91527 IR spectrometer as KBr disks. ^{1}H-NMR spectra were obtained on a Varian EM-360 NMR spectrometer (Varian, USA) using TMS as internal standard. Chemical shifts are given in δ ppm and multiplicity of the signals expressed as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and bs = broad singlet. Elemental analyses for C, H and N were carried out on a Perkin-Elmer 240C analyser in the Faculty of Science, Assiut University and were found to be within $\pm 0.4\%$ of the calculated values. Precoated silica gel plates (Kieselgel 60G, F254 nm, Merck, Darmstadt, Germany) were used for thin-layer chromatography (TCL) and, for column chromatography, silica gel 63–200 µm (Sigma, St Louis, USA). pH values were recorded on a Chekit Micro pH meter (England) at room temperature.

HPLC

An analytical method was developed for quantitative determination of the ester prodrugs **4a–f** and the corresponding parent drugs **3a–f** throughout the in-vitro and in-vivo studies. A Knauer HPLC (Fa. Knauer, Berlin, Germany) was used, consisting of a pump (Knauer M-64), a tunable UV detector (Knauer V0890), Shimadzu CR6A Chromatopac data module integrator and a 20-μl loop injector valve. The column was a reversed-phase Hypersil BDS C18 (250 × 4.6 mm; 5 μm particle) in conjunction with a cartridge guard precolumn. The chromatographic conditions for each of the studied compounds are summarized in Table 1. Least-square equations derived by correlating the recorded peak areas with known concentrations of each compound, were then used for calculation of unknown concentrations in the studied samples.

*Synthesis of the 4-(acetamido)phenyl ester prodrugs **4a–e***

Ethyl chloroformate (1.1 g, 0.01 mol) was added dropwise to a cooled solution (0–5°C) of the respective non-steroidal anti-inflammatory carboxylic acid (0.01 mol) and triethyl amine (1.02 g, 0.01 mol) in methylene chloride (50 ml). The mixture was stirred for a further 30 min, then paracetamol (1.51 g, 0.01 mol) was added over a period of 30 min and stirring was continued overnight at room temperature. The reaction mixture

TABLE 1
Analytical HPLC data[a] and C_{logP} values for paracetamol, NSAIDs **3a–f** and the ester prodrugs **4a–f**

Compound	Eluent[b]	RT (min)	$C_{log\ P}$	log P[c]
1	60:40	2.6	0.49	0.51
3a	70:30	3.0	1.02	1.02
3b	90:10	3.6	3.68	3.50
3c	80:20	3.7	2.82	3.34
3d	90:10	2.5	3.03	4.40
3e	90:10	3.4	5.51	5.25
3f	90:10	3.2	4.18	4.27
4a	65:35	5.4	2.15	–
4b	90:10	4.4	4.78	–
4c	80:20	6.4	3.92	–
4d	90:10	3.9	4.13	–
4e	90:10	4.7	5.51	–
4f	90:10	5.6	6.19	–

[a]Detection at λ_{max} = 254 nm; for **3a** and **4a** at 270 nm. Flow rate 1 ml/ml
[b]Methanol:phosphate buffer: 0.2 mol/L, pH 4
[c]Reported values

was then washed with water and a cold solution (5%) of sodium hydrogen carbonate, dried over anhydrous sodium sulphate and filtered. The solvent was removed under reduced pressure and residue recrystallized from the appropriate solvent or purified by column chromatography.

2-{[4-(Methylcarboxamido)phenyl]oxycarbonyl}phenyl acetate **4a**

2.4 g (77%); mp 183–5°C (ethyl acetate); (reported 178–81°C [20]); IR: (KBr), νcm^{-1}: 3310(*NH*$COCH_3$); 1766(*CO*CH_3); 1738(*CO*OC_6H_4); 1663(NH*CO*CH_3); ^{1}H-NMR, $CDCl_3$; δ_H: 2.1(3H, s, NHCOCH_3); 2.3(3H, s, OCOCH_3); 6.9–7.7(8H, m, phenyl-H and *NH*CO-); 8.1(1H, dd, $J_{3,4}$ = 6 Hz, $J_{3,5}$ = 2 Hz, C-3-H). Analysis calculated for $C_{17}H_{15}NO_5$ (313.31): C, 65.17; H, 4.83; N, 4.47. Found: C, 65.43; H, 4.78; N, 4.36.

4-(Methylcarboxamido)phenyl 2-(4-isobutylphenyl)propanoate **4b**

3.1 g (90%); mp 87–9°C (ethyl acetate/petroleum ether); IR, (KBr) νcm^{-1}: 3370(*NH*$COCH_3$); 1720(*CO*OC_6H_4); 1680(NH*CO*CH_3); ^{1}H-NMR, $CDCl_3$, δ_H: 0.9(6H, d, J = 7 Hz, $(CH_3)_2$CH-); 1.55(3H, d, J = 7 Hz, CH_3CH-); 1.7(1H, m, $(CH_3)_2$*CH*-); 2.1(3H, s, NHCOCH_3); 2.5(2H, d, J = 7 Hz,-CH CH_2-); 6.9(2H, d, J = 8 Hz, H ortho to NHCO-); 7.5(2H, d, J = 7 Hz, aromatic H ortho to OCO-); 7.6(1H, bs, CO*NH*-); 7.1(4H, bs, phenyl H). Analysis calculated for $C_{21}H_{25}NO_3$ (339.18): C, 74.31; H, 7.42; N, 4.13. Found: C, 74.65; H, 7.00; N, 4.37.

4-(Methylcarboxamido)phenyl 2-(6-methoxy-2-naphthyl)propanoate **4c**

3.5 g (95%); mp 176°C (acetone); IR, (KBr) νcm^{-1}: 3370(*NH*$COCH_3$); 1739 *CO*OC_6H_4); 1679(NH*CO*CH_3); ^{1}H-NMR, $CDCl_3$, δ_H: 1.8(3H, d, J = 7 Hz, CH_3CH-); 2.1(3H, s, -NHCOCH_3); 3.9(3H, s, -OCH_3); 4.0(1H, q, J = 7 Hz, CH_3*CH*-); 6.9–7.9(11H, m, NHCO-, naphthyl H, phenyl H). Analysis, calculated for $C_{22}H_{21}NO_4$ (363.15): C, 72.70; H, 5.83; N, 3.86. Found: C, 72.80; H, 5.40; N, 4.21.

4-(Methylcarboxamido)phenyl 2-[2-(2,6-dichloroanilino)phenyl]acetate **4d**

2.3 g (55%); mp 188–90°C (acetonitrile); ^{1}H-NMR, d_6-DMSO, δ_H: 2.1(3H, s, NHCOCH_3); 4.0(2H, s, -CH_2COO-), 6.8(2H, d, J = 8 Hz, H ortho to -NHCO-); 7.2(2H, d, J = 7 Hz, H ortho to -OCO-); 6.5–7.5(11H, m, phenyl+NH). Analysis, calculated for $C_{22}H_{18}Cl_2N_2O_3$ (428.07): C, 61.67; H, 4.24; N, 6.54. Found: C, 61.20; H, 4.04; N, 7.15.

4-(Methylcarboxamido)phenyl 2-[3-(trifluoromethyl)anilino]benzoate **4e**

2.5 g (60%) mp 153–5°C (acetonitrile); IR, (KBr) νcm^{-1}: 3370(*NH*COCH$_3$), 3315 (-NH-); 1695(*CO*OC$_6$H$_4$); 1670(-NH*CO*CH$_3$). ^{1}H-NMR, d$_6$-DMSO, δ_H: 2.1(3H, s, NHCO*CH*$_3$); 6.8(1H, m, H-6 of CF$_3$C$_6$H$_4$-); 7.0–7.6[8H, m, H-2,4,5 of CF$_3$C$_6$H$_4$-; H-3,4,5 of C$_6$H$_4$COO- and H ortho to -OCO-], 8.1(1H, d, H-6 of C$_6$H$_4$COO-); 9.4(1H, m, NHCO). Analysis, calculated for $C_{22}H_{17}F_3N_2O_3$ (414.12): C, 63.75; H, 4.14; N, 6.76. Found: C, 63.60; H, 4.05; N, 6.65.

*Synthesis of 4-(methylcarboxamido)phenyl 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indolyl]acetate **4f***

Thionyl chlorde (3.84 g 0.03 mol) was added dropwise to a stirred suspension of indomethacin (3.57 g, 0.01 mol) in dried benzene (150 ml) and the mixture was refluxed for 3 h. The solvent and excess thionyl chloride were removed under reduced pressure and the resulting acid chloride was then dissolved in methylene chloride (50 ml) and added dropwise to a cooled solution of paracetamol (1.5 g, 0.01 mol), triethylamine (1.1 ml, 0.01 mol) and 4-dimethylaminopyridine 0.01 g in methylene chloride (50 ml). The reaction mixture was stirred for 10 h at room temperature and the precipitated product was then filtered, dried and recrystallized from acetonitrile. Yield 3.4 g (70%); mp: 189–91°C; IR, νcm^{-1}: 3340 (*NH*COCH$_3$), 1726(*CO*OC$_6$H$_4$-), 1672(NH*CO*CH$_3$), 1600(-*CO*NHC$_6$H$_4$Cl); ^{1}H-NMR, d$_6$-DMSO, δ_H: 2.5(3H, s, C-2-CH$_3$); 2.0(3H, s, -NHCO*CH*$_3$), 3.9(2H, s, CH$_2$COO-), 3.8(3H, s, -OCH$_3$), 6.8–7.8(m, 11H, phenyl protons), 8.2(bs, 1H, NHCO-). Analysis, calculated for $C_{27}H_{23}ClN_2O_5$ (490.94): C, 66.06; H, 4.72; N, 5.71. Found: C, 66.40; H, 4.70; N, 5.71.

Calculation of log P values

The apparent partition coefficient log P values of the synthesized derivatives as well as the parent compounds were computed using a PC-software package (MacLogP 2.0, BioByte Corp., CA, USA).

In-vitro experiments

Chemical hydrolysis

The hydrolysis of the synthesized ester prodrugs **4a–f** was studied in 0.02 mol/L hydrochloric acid and phosphate buffers of pH 1.2 and 7.4, respectively. Buffer solutions containing the ester prodrugs (2×10^{-5} mol/L) were kept at a constant temperature of 37 ± 0.1°C in a water bath and, at appropriate time intervals, samples of 20 µl were taken and analysed immediately by HPLC for the remaining ester prodrug. Pseudo-first-order rate constants (k_{obs}) for the hydrolysis of the ester

prodrugs **4a–f** were then calculated from the slopes of the linear plots of log % residual prodrug vs. time. The experiments were run in triplicate for each ester and the mean values of the rate constants were calculated.

Enzymatic hydrolysis

Decomposition studies in 80% human plasma containing isotonic phosphate buffer of pH 7.4 at 37°C were performed by adding the appropriate amount of the stock methanolic solution (1×10^{-5} mol/l) of the derivatives to plasma solution. At appropriate times, samples of 50 µl were withdrawn and mixed with 50 µl of acetonitrile for deproteinization and centrifuged at 1×10^{4} rpm for 5 min. The clear supernatant (20 µl) was analysed by HPLC as described above.

Male Wistar rat livers were homogenized with ice-cooled saline to give a concentration of 40% w/v, and were then centrifuged at 15 000 rpm for 15 min. The supernatants were collected and stored at –40°C until use. Homogenate was thawed 10 min before the experiments and diluted with saline to give a preparation of 10% w/v. The hydrolysis studies in rat liver homogenate were performed as described for 80% human plasma solution above. The resulting kinetic data, average of three experiments, are given in Table 2.

In-vivo evaluation

Bioavailability studies

Two groups, each of three rabbits (1.5 kg weight), were used. The first group was administered a dose of 0.025 mmol/kg orally of ibuprofen prodrug **4b** as a suspension in 0.1% carboxymethylcellulose (CMC). An equivalent dose as a physical mixture of ibuprofen **3b** and paracetamol **1** in 0.1% CMC was given to the rabbits of the second group. Blood samples, each of 0.5 ml, were withdrawn from the marginal ear vein at time intervals of 30, 60, 120 and 240 min, and continued every 4 h up to 24 h. The blood samples were then centrifuged to separate plasma, from which aliquots of 50 µl were treated as mentioned above for 80% human plasma and were analysed for ibuprofen **3b** and paracetamol **1** contents using HPLC.

Ulcerogenicity studies

A JEOL, JSM-5400LV scanning electron microscope (Electron Microscope Unit, Assiut University) was used for observing mucosal injury from scanning micrographs of stomach specimens. Three groups, each of 4 mice, were used and fasted for 12 h prior to the administration of drug solutions. The first group was administered a daily oral dose, (0.025 mmol/kg) as a 1-ml suspension of the prodrug **4d** in 0.5% carboxymethylcellulose solution, for 4 successive days. In a similar manner, the second

TABLE 2
Kinetic parameters of the prodrugs **4a–f**

Compound	pH 1.3[a]		pH 7.4[b]		Plasma[c]		Liver homogenate	
	$k_{obs.}$ (h^{-1})	$t_{\frac{1}{2}}$ (h)	$k_{obs.}$ (h^{-1})	$t_{\frac{1}{2}}$ (h)	$k_{obs.}$ (min^{-1})	$t_{\frac{1}{2}}$ (min)	$K_{obs.}$ (min^{-1})	$t_{\frac{1}{2}}$ (min)
4a	0.016	43.0	0.160	4.3	0.0497	13.9	–[e]	–
4b	0.026	26.8	0.028	24.8	0.0056	120	0.0549	12.6
4c	0.044	15.8	0.036	19.3	0.0065	108	0.0243	28.5
4d	0.1034	6.7	0.846	0.8	0.0018	384	0.0179	38.5
4e	0.1019	6.8	0.0713	9.7	0.0029	234	0.0175	39.6
4f	0.0721	9.6	0.5700	1.22	0.0063	110	0.0162	42.9

[a]Hydrochloric acid buffer (0.02 mol/L); [b]phosphate buffer (0.02 mol/L); [c]80% human plasma; [d]10% rat liver homogenate; [e]not detected

group received equivalent doses of the corresponding physical mixture. The third group was administered equivalent amounts of the vehicle and considered the control group. Food was withdrawn from all groups until 24 h after the last dose. The mice were then sacrificed, so that the stomach could be removed, opened along the greater curvature and cleaned gentle by dipping in saline. Randomly selected specimens were then taken and prepared for scanning in an electron microscope. Specimen were fixed by soaking in glutaraldehyde solution (5% in cacodylate buffer; pH 7.2) for 24 h followed by three washings each for 20 min with cacodylate buffer. The specimen were then treated with osmium tetraoxide (1% solution) for 2 h and washed with cacodylate buffer as shown above. The specimen were then subjected to dehydration by treatment for 30 min with each of 30%, 50% and 70% ethanolic solutions followed by 90% ethanol for 1 h and finally in absolute ethanol for 2 days. After discharge of the alcohol the specimen were soaked in amyl acetate solution for 2 days, dried under reduced pressure mounted on holders and coated for scanning in a scanning electron microscope (SEM).

Determination of pain threshold latency

Groups of 6 mice each were used for each of the studied prodrugs and the corresponding physical mixtures. The preparations were given ip, as suspensions in 0.5% CMC, at a dose of 0.02 mmol/kg and the control group received an equivalent amount of the vehicle. The mice were placed individually on a hot plate that was preheated to a fixed temperature of 54 ± 0.5°C and the time in seconds which elapsed before the mouse started jumping was recorded. The latency to jump was determined for each mouse after 15, 30, 60, 120, 180 and 240 min. The differences in latencies, with respect to the control group, were used as an indication of analgesia. Student's *t*-test was performed on the data for the prodrugs and their corresponding physical mixture vs. control as well as for the respective prodrugs vs. the physical mixtures.

RESULTS AND DISCUSSIONS

Chemistry

The synthesis of the ester prodrugs **4a–f** was performed as outlined in Figure 1. Interaction of the mixed carboxylic–carbonic anhyrdride intermediate, resulting from the respective NSAI carboxylic acid **3a–e** and ethyl chloroformate in the presence of triethylamine, with paracetamol **1** affords the corresponding esters **4a–e** in good yields. Application of this method for synthesis of indomethacin–paracetamol ester prodrug **4f** results in the formation of indomethacin ethyl ester as a sterically favoured product. The targeted prodrug **4f** has been alternatively synthesized by conversion of indomethacin to the corresponding acid chloride followed by esterification with paracetamol. The structures of the synthesized compounds **4a–f** were confirmed by IR and ^{1}H-NMR spectroscopy and their purity was ascertained by elemental analysis, TLC and HPLC. The results of elemental analyses (C, H and N) of the synthesized compounds were in all cases within $\pm 0.04\%$ of the theoretical value.

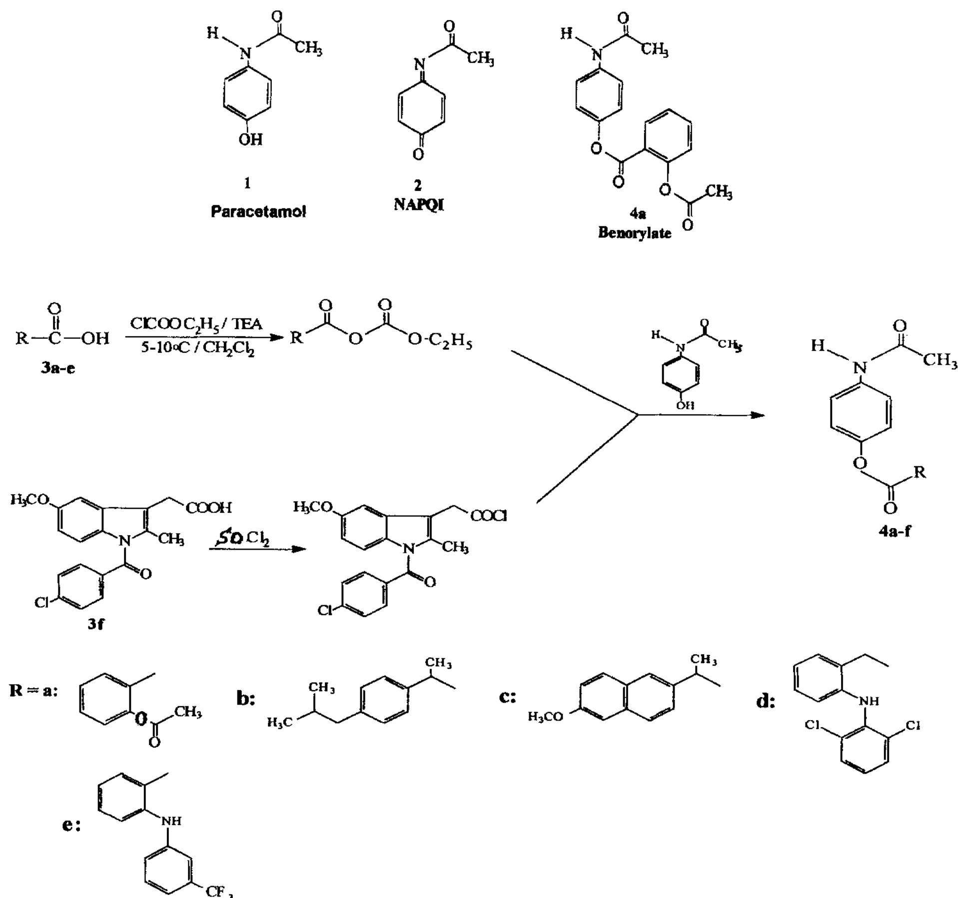

Figure 1. Synthetic route for the prodrugs **4a–f**

The log P values of the synthesized prodrugs **4a–f** and the parent drugs **1** and **3a–f** were calculated using a PC-software package, which enables calculation of log P on the basis of the fragment method developed by Leo [21]. The calculated log P for the prodrugs **4a–f** and their parent drugs are listed in Table 1. The validity of the method was ascertained through conformity of the calculated log P-values for the parent drugs with reported values determined by conventional methods. The results in Table 1 reveal that the prodrugs are more lipophilic than the parent drug; absorption from the GIT could therefore be enhanced.

In-vitro hydrolysis studies

Essential requisites for a prodrug designed for oral delivery are its chemical stability at pH values simulating the gastric fluids and its ability to readily release the parent drug after absorption. Consequently, the kinetics of chemical and enzymatic hydrolysis of the paracetamol ester prodrugs of the selected NSAIDs were studied at 37°C in aqueous buffer solutions of pH 1.3 and pH 7.4 as well as in 80% human plasma and 10% rat liver homogenate. The reactions were monitored by HPLC for the decrease in ester concentration vs. time and were found to display pseudo-first-order kinetics over several half-lives. The rate constant (k_{obs}) for hydrolysis and the corresponding half-life for each of the studied prodrugs are listed in Table 2.

The results in Table 2 reveal that all the synthesized prodrugs are sufficiently stable at pH 1.3, which is considered as a non-enzymatic simulated gastric fluid (SGF), so that no appreciable hydrolysis to the free acids might occur in the stomach. Similarly, the chemical stability of the ester prodrugs **4a–f** at pH 7.4 ($t_{\frac{1}{2}} \cong$ 1.5–25 h) suggests that they are absorbed almost unchanged from the intestine.

The kinetic data (Table 2) show that the synthesized derivatives are more susceptible to rat liver homogenate enzymes than the human plasma. This observation may be due to species differences as well as the enrichment of liver homogenate with esterases relative to plasma, which is consistent with previously reported investigations [22]. As a general pattern, the rates of hydrolysis of the ester prodrugs **4a–f** in human plasma and rat liver homogenate are markedly accelerated compared with those in aqueous buffers. It is noteworthy that the susceptibility for enzymatic hydrolysis was found to be affected by the type of NSAID attached to paracetamol. Aspirin prodrug **4a**, with the smallest acyl moiety, was found to be the most labile one in both of the investigated enzyme systems. In contrast, the prodrugs **4d–f** were the least susceptible ones due to the bulkiness of the acyl moiety that postponed proper fitting to receptor sites of the enzyme.

Bioavailability studies

Based on in-vitro evaluation, prodrug **4b** was chosen for further evaluation with respect to chemical stability. A limited experiment in rabbits using **4b** and an equivalent dose of the corresponding physical mixture of ibuprofen **3b** and paracetamol has shown that the ester **4b** is efficiently absorbed after oral administration (Figure 2). No remarkable concentrations of the intact prodrug were detected, indicating very rapid ester hydrolysis in vivo compared with the in-vitro plasma hydrolysis data (Table 2). Moreover, Figure 2 illustrates also that the ester prodrug **4b** produced higher ibuprofen plasma levels up to 24 h, compared with those resulting from the physical mixture. This indicates that the prodrug is more bioavailable orally as its rate of absorption, due to improved lipophilicity, is faster than the parent drugs in physical mixture.

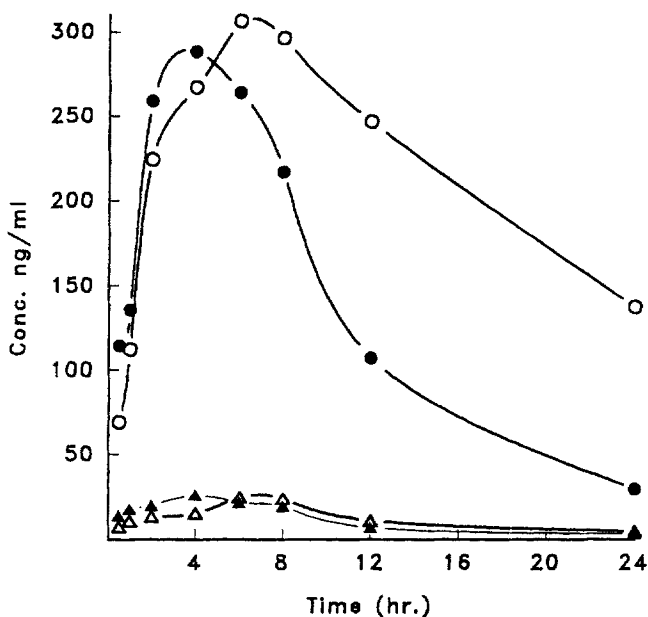

Figure 2. Plasma levels of ibuprofen (**3b**) (●, ○) and paracetamol (1) (▲, △) after oral administration of **4b** (open symbols) and the corresponding physical mixture (solid symbols) to rabbits (dose 0.025 mmol/kg). Each point represents the mean ± SE ($n = 3$)

Gastrointestinal tolerance

The ulcerogenicity of the prodrug **4d**, as a representative example for the synthesized oral delivery system, was tested in comparison with a physical mixture containing equivalent amounts of the parent drugs following oral administration for 4 days in mice. As can be seen from the gross observation, pale stomachs with thin structure and discrete ulcers are characteristic for mice treated with the physical mixture of diclofenac **3d** and paracetamol **1**. In contrast, the mice treated with chronic doses of the corresponding prodrug **4d** showed no significant gastric mucosal injury and were observed to be similar to the controls. In continuation of our previous reports [23] examination of stomach specimens of the treated experimental animals under scanning electron microscope affords a highly precise method for investigation of the ulcerogenic potential of NSAIDs. Figure 3, represents scanning electromicrographs, at a constant magnification power, for stomach specimens of mice treated with chronic doses of the prodrug 4d, (Figure 3A); the corresponding physical mixture of the parent drugs (Figure 3B) and the control group, (Figure 3C), which receive only the vehicle. As shown in Figure 3, the physical mixture-treated group (3B) was characterized by complete damage of the mucous layer besides ulceration of the submucosal cells. These

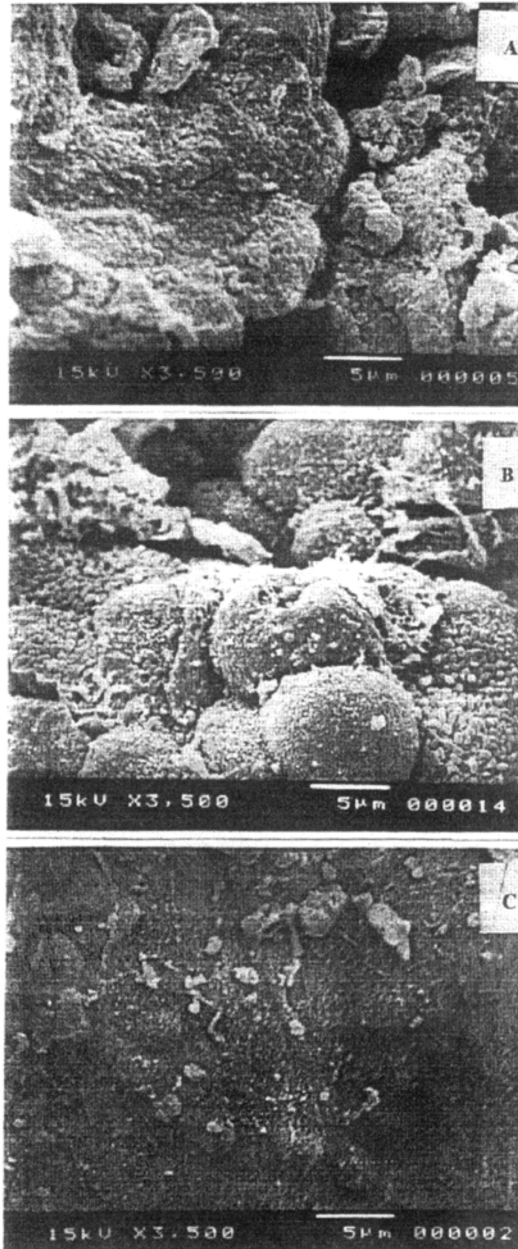

Figure 3. Scanning electromicrographs of mouse stomach specimen after chronic doses (4 days) of: **A** prodrug **4d**; **B** diclofenac/paracetamol mixture; **C** control

TABLE 3
The latencies of pain threshold (s) in mice treated with the prodrugs **4a–f** and the corresponding physical mixtures (mix 1–6)

Compound	Mean ± SE[†] of the pain threshold (s)					
	15 min	30 min	1 h	2 h	3 h	4 h
Control	45.9 ± 1.73	50.25 ± 2.34	55.45 ± 1.56	56.24 ± 1.05	59.04 ± 0.91	62.48 ± 7.26
4a	$66.68 \pm 4.83^{b,*}$	$63.88 \pm 4.97^{c*}$	$89.66 \pm 4.66^{a*}$	$115.6 \pm 5.17^{a*}$	$107.4 \pm 5.44^{a*}$	$83.02 \pm 2.60^{c*}$
Mix 1	$57.7 \pm 2.11^{a*,d**}$	$79.42 \pm 5.66^{b*,c**}$	$81.08 \pm 4.57^{b*,d**}$	$106.72 \pm 5.65^{a*,d**}$	$105.75 \pm 10.46^{b*,d**}$	$55.87 \pm 1.05^{d*,a**}$
4b	$77.8 \pm 1.95^{a*}$	$118.93 \pm 8.99^{a*}$	$117.35 \pm 8.97^{a*}$	$146.18 \pm 19.50^{b*}$	$158.48 \pm 23.42^{b*}$	$154.35 \pm 11.67^{c*}$
Mix 2	$124.45 \pm 7.61^{a*,a**}$	$125.1 \pm 10.82^{a*,d**}$	$151.46 \pm 24.74^{b*,d**}$	$140.95 \pm 7.21^{a*,b**}$	$123.17 \pm 5.26^{a*,d**}$	$105.2 \pm 9.41^{c*,d**}$
4c	$54.53 \pm 2.22^{d*}$	$86.3 \pm 5.59^{a*}$	$87.63 \pm 0.59^{a*}$	$101.88 \pm 2.22^{a*}$	$99.73 \pm 1.96^{a*}$	$75.18 \pm 4.51^{d*}$
Mix 3	$73.93 \pm 2.58^{a*,a**}$	$77.98 \pm 8.21^{c*,d**}$	$96.20 \pm 4.68^{a*,d**}$	$106.23 \pm 4.55^{a*,d**}$	$106.22 \pm 4.29^{a*,d**}$	$94.83 \pm 2.46^{b*,c**}$
4d	$75.66 \pm 5.43^{b*}$	$94.14 \pm 11.39^{b*}$	$97.53 \pm 6.10^{a*}$	$113.63 \pm 8.86^{a*}$	$114.56 \pm 5.55^{a*}$	$145.88 \pm 7.40^{a*}$
Mix 4	$64.00 \pm 7.45^{c*,d**}$	$88.66 \pm 5.60^{a*,d**}$	$90.35 \pm 3.35^{a*,d**}$	$104.7 \pm 8.78^{b*,d**}$	$125.55 \pm 9.70^{a*,d**}$	$75.6 \pm 7.29^{d*,a**}$
4e	$43.67 \pm 0.99^{d*}$	$95.1 \pm 7.02^{a*}$	$115.7 \pm 9.77^{a*}$	$100.7 \pm 8.13^{b*}$	$105.31 \pm 1.75^{a*}$	$114.33 \pm 6.91^{a*}$
Mix 5	$62.25 \pm 3.91^{b*,b**}$	$72.26 \pm 1.83^{a*,c**}$	$100.07 \pm 7.49^{a*,d**}$	$114.32 \pm 10.52^{b*,d**}$	$113.55 \pm 9.47^{b*,d**}$	$77.68 \pm 3.65^{d*,b**}$
4f	$40.35 \pm 2.91^{d*}$	$105.36 \pm 5.62^{a*}$	$125.21 \pm 7.84^{a*}$	$128.15 \pm 3.67^{a*}$	$135.32 \pm 4.17^{a*}$	$116.15 \pm 6.34^{a*}$
Mix 6	$57.48 \pm 3.66^{c*,c**}$	$89.62 \pm 9.49^{b*,d**}$	$106.08 \pm 7.40^{a*,d**}$	$105.52 \pm 4.86^{a*,c**}$	$113.38 \pm 5.62^{a*,b**}$	$78.88 \pm 4.94^{d*,a**}$

[†] $n = 6$ mice; dose 0.02 mmol/kg; [*] t-test for prodrugs and their corresponding mixtures vs. control; [**] t-test for prodrugs vs. corresponding mixture; [a] significant at $p < 0.001$; [b] significant at $p < 0.01$; [c] significant at $p < 0.05$; [d] non-significant; ANOVA within each column is highly significant at $p < 0.0001$

effects are not observed in the prodrug-treated group (3A), which shows great similarity to the control group (3C). The previous observation affords good evidence for the safety of the suggested oral delivery system of NSAIDs compared with the conventional use of the parent drugs.

Analgesic activity

The analgesic activity of the synthesized mutual prodrugs **4a–f** and the corresponding physical mixtures, expressed as the latency in pain threshold, was investigated in mice according to the reported method [24]. In this procedure, the rodent is placed on a hot plate that is preheated to a temperature that is presumed to be aversive. The latency to lick a paw and/or escape (jump) is recorded. Differences between the latencies and those of controls are used as a measure of the degree of analgesia. Results are illustrated in Table 3. As a general pattern, the analgesic activity of the prodrugs significantly improves over time. This means that the prodrugs per se are devoid of analgesic activity and that the observed latent analgesia results from hydrolysis to the parent drugs. The results also showed significant differences between the prodrugs, **4b** and **4c**, and their corresponding physical mixtures at 15 min post-dosing. Meanwhile, prodrugs **4d–f** only exhibited a significant difference from the corresponding physical mixtures after a longer duration (2–4 h). In all cases, the synthesized prodrugs are significantly different from the control groups.

CONCLUSION

In-vitro and in-vivo evaluation of the synthesized mutual ester prodrugs **4a–f** of paracetamol and some NSAIDs revealed improvement in the therapeutic index of the parent drugs. The derivatives are characterized by adequate chemical stability, reduced ulcerogenic liability, enhanced lipophilicity and improved bioavailability compared with the corresponding physical mixtures and the parent drugs.

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