Cyclic amide derivatives as potential prodrugs II: N-hydroxymethylsuccinamide-/isatin esters of some NSAIDs as prodrugs with an improved therapeutic index. Eur J Med Chem

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JULY 1999

Impact Factor: 3.45 · DOI: 10.1016/S0223-5234(00)80025-2 · Source: PubMed

3 AUTHORS, INCLUDING:

Farghaly Abdelhamid Omar
Assiut University
40 PUBLICATIONS **287** CITATIONS
SEE PROFILE

Tarek Aboul-Fadl
Assiut University
74 PUBLICATIONS **795** CITATIONS
SEE PROFILE

Eur. J. Med. Chem. 34 (1999) 551–562

Original article

Cyclic amide derivatives as potential prodrugs II: *N*-hydroxymethylsuccinimide- / isatin esters of some NSAIDs as prodrugs with an improved therapeutic index

Nadia M. Mahfouz, Farghaly A. Omar*, Tarek Aboul-Fadl

Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

(Received 15 October 1998; accepted 29 December 1998)

Abstract – Ester prodrugs of aspirin **1a**, ibuprofen **1b**, naproxen **1c** and indomethacin **1d** were synthesized using *N*-Hydroxymethylsuccinimide (HMSI) **3** and *N*-hydroxymethylisatin (HMIS) **4** as promoieties to reduce their gastrointestinal toxicity and improve bioavailability. Additionally, the kinetics of hydrolysis of the synthesized prodrugs **5a–d** and **6a–d** were studied at 37 °C in non-enzymatic simulated gastric fluid (SGF; hydrochloric acid buffer pH = 1.2); 0.02 M phosphate buffer (pH = 7.4); 80% human plasma and 10% rat liver homogenate. The results indicate higher chemical stability of the ester prodrugs in non-enzymatic SGF ($t_{1/2} \cong 6.5$–18.6 h) and rapid conversion to the parent drugs in 80% human plasma ($t_{1/2} \cong 11.4$–235 min) as well as in 10% rat liver homogenates ($t_{1/2} \cong 12.0$–90.0 min). As a general pattern, the HMSI esters **5a–d** revealed higher chemical stability than the corresponding HMIS analogues **6a–d.** The pH-rate profile of **5c** and **6a** indicated maximum stability of the former at pH = 1.2–8.0 and of the latter at pH = 1.2–4.0. The distribution coefficient ($D_{7.4}$) values of the prodrugs **5a–d, 6a–d** and the parent drugs **1a–d** in an n-octanol/phosphate buffer (pH =7.4) system indicated enhanced lipophilic properties of the prodrugs. Furthermore, the HMIS ester prodrugs **6a–d** are more lipophilic than the corresponding HMSI derivatives **5a–d**. In vivo ulcerogenicity studies using scanning electron microscopy on stomach specimens of rats treated with an oral dose for 4 d revealed that the synthesized ester prodrugs are significantly less irritating to gastric mucosa than the parent drugs. These results suggested HMSI and/or HMIS esters possess good potential as prodrugs with an improved therapeutic index for oral delivery of NSAIDs. © 1999 Éditions scientifiques et médicales Elsevier SAS

NSAI prodrugs / *N*-hydroxymethylsuccinimide (HMSI) / *N*-hydroxy methylisatin (HMIS) / in vitro stability / lipophilicity / ulcerogenicity

1. Introduction

The gastrointestinal toxicity of the acidic NSAIDs is one of the most challenging problems in medicinal chemistry, since these side effects are usually related to the intrinsic mechanism responsible for the desired activity [1, 2]. The direct contact mechanism appears to play the major role in the production of the gastrointestinal lesions [3]. It is probably caused by a combination of local irritations produced by the free carboxylic group of the NSAIDs and by local inhibition of the cytoprotective action of prostaglandin on gastric mucosa [4]. One of the recent approaches for overcoming such therapeutic problems is the concept of retrometabolic drug design, that incorporates targeting and metabolic considerations into the design process. This has been used for improving the therapeutic properties of a wide variety of drugs through development of prodrugs or soft drugs [5]. Accordingly, the carboxylic group of the NSAID can thus be temporarily masked and its direct effect on the gastric mucosa will be prohibited [6].

We have recently shown the *N*-hydroxymethylphthalimide ester prodrugs of some NSAIDs to be almost devoid of the gastric ulcerogenic activity of the parent drugs [7]. In a trial for optimization of this prodrug system, two additional analogous cyclic amides: N-hydroxymethylsuccinimide (HMSI) **3** and *N*-hydroxymethyl isatin (HMIS) **4** were synthesized, to be used as alternative promoieties for *N*-hydroxymethyl-phthalimide (HMPhI). The new promoieties **3** and **4** having advantages as derivatives of endogenous substances [8, 9]. Moreover, several related succinimide derivatives were reported as promoieties for improving the physicochemi-

*Correspondence and reprints

cal and/or therapeutic properties of some drugs [10, 11]. This paper describes the synthesis, physicochemical properties, kinetics of the chemical and enzymatic degradation of HMSI and HMIS esters of aspirin, ibuprofen, naproxen and indomethacin for potential use as prodrugs with an improved therapeutic index.

2. Chemistry

The promoieties N-hydroxymethylsuccinimide (HMSI) **3** and N-hydroxymethylisatin (HMIS) **4** were prepared by reaction of formaldehyde (40% w/v) and succinimide or isatin in boiling ethanol. The selected NSAIs **1a–c** were allowed to react with ethyl chloroformate in the presence of triethylamine in carefully dried dichloromethane to give the mixed anhydrides **2a–c** as illustrated in *figure 1*. Treatment with HMSI **3** or HMIS **4** at ambient temperature gave the corresponding ester prodrugs **5a–c** and **6a–c** respectively. Attempts to react indomethacin **1d** and ethyl chloroformate in the same manner failed, since such treatment led to the formation of indomethacin ethylester, as evidenced from the ^{1}H-NMR spectrum of the product. This is probably due to the presence of a methyl group at C-2 of the indole nucleus that provides sufficient steric hindrance to discourage the attack of the bulky HMSI **3** or HMIS **4** at the carboxylic carbonyl. The mixed anhydride undergoes simultaneous rearrangement to afford the sterically favoured ethyl ester of indomethacin. However, **1d** could be esterified to **5d** and **6d** *(figure 1)* by conversion to the corresponding acid chloride **2d,** that is then treated with the respective promoiety in the presence of triethylamine. The physical constants, yields and elemental analysis of the ester prodrugs **5a–d** and **6a–d** are listed in *table I.*

The assignment of the structures was established on the basis of the characteristic ^{1}H-NMR and Mass spectral data and the purity was ascertained by elemental analyses, TLC and HPLC. The ^{1}H-NMR data of the promoieties **3** and **4** as well as the prodrugs **5a–d** and **6a–d** are summarized in *table II.* As evidenced from the table, the promoieties HMSI **3** and HMIS **4** showed the characteristic pattern of the methylene and hydroxyl protons along with the signal of the aliphatic protons in the former and the aromatic multiplet in the latter. The methylene protons appeared at nearly equal chemical shifts in both compounds ($\delta \cong 5.0$ ppm), whereas the chemical shift of the hydroxyl proton in HMSI ($\delta = 4.2$ ppm) differ from that in HMIS ($\delta = 6.3$ ppm). The observed downfield shift of the hydroxyl proton in the latter might be explained on basis of its orientation relative to the deshielding anisotropic effects of the neighbouring phenyl nucleus in addition to the effect of d_6-DMSO used as a solvent. It is

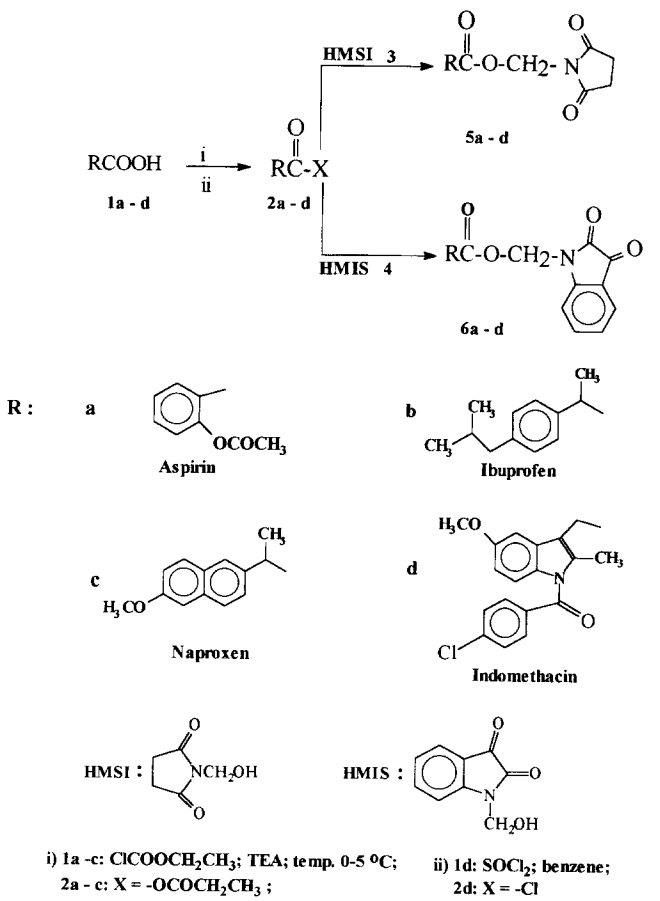

Figure 1. Synthesis of the ester prodrugs **5a–d** and **6a–d.**

also worth noting that the methylenic protons of the HMIS-derived ester prodrugs **6a–d** are downfield shifted ($\delta \cong 6.0$ ppm) relative to their corresponding ones of the HMSI-derived series **5a–d.** This could also be attributed to the possible conjugation of the lone pair of electrons of the isatin-nitrogen atom with the adjacent aromatic system resulting in a subsequent deshielding of the methylene group.

The mass spectral data *(table II)* revealed the parent peaks, base peaks in addition to some other peaks of the investigated ester prodrugs in different relative intensities. In the case of the HMSI-derived esters of aspirin **5a** and indomethacin **5d,** the base peak at m/z = 112 could be attributed to the ion fragment $[C_5H_6NO_2]^+$. A plausible fragmentation pattern would be the cleavage of the O-CH_2- bond resulting in the formation of *N*-methylenesuccinimide, which corresponds to the aforementioned ion fragment. A similar fragmentation pattern was also

Table I. Physical constants; yields and elemental analysis of the ester prodrugs **5a–d** and **6a–d.**

Compound	% Yield	M.p. °C (solvent)	Mol. formula (MW)
5a	65	114–16* a	$C_{14}H_{13}NO_6$ (291.26)
5b	63	66–68 b	$C_{18}H_{23}NO_4$ (317.16)
5c	64	148–50 c	$C_{19}H_{19}NO_5$ (341.13)
5d	60	120–23 d	$C_{24}H_{21}ClN_2O_6$ (468.11)
6a	86	165–67 e	$C_{18}H_{13}NO_6$ (339.30)
6b	82	100–103 d	$C_{22}H_{23}NO_4$ (365.43)
6c	85	161–63 e	$C_{23}H_{19}NO_5$ (389.41)
6d	75	92–94 f	$C_{28}H_{21}ClN_2O_6$ (516.94)

a: ethyl acetate/petroleum ether; b: ether/petroleum ether; c: acetone; d: methanol; e: methanol/ethyl acetate; f: acetone/ petroleum ether.
* reported [Lit. 11]: 117–118 °C.

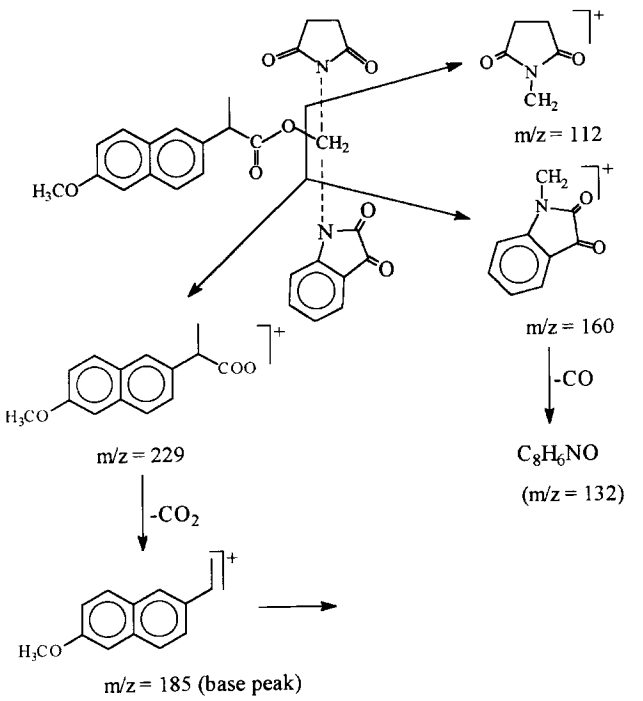

Figure 2. Suggested fragmentation pattern for the ester prodrugs **5c** and **6c**.

observed in the HMIS-derived esters of aspirin **6a**, ibuprofen **6b** and indomethacin **6d**, leading to the ion fragment at m/z = 160 for $[C_9H_6NO_2]^+$, which is best represented by *N*-methylene-isatin, the promoiety of this series. This latter ion fragment was further degraded in the usual pathway reported for the isatin derivatives [12], whereby loss of CO affords the most stable ion fragment at m/z = 132. Moreover, the aspirin prodrugs **5a** and **6a** are characterized also by rapid cleavage of the acetyl moiety $[CH_2CO]^+$, as shown in *table II*. On the contrary, the base peak in the case of naproxen prodrugs **5c** and **6c** at m/z = 185 may be resulting from ion fragments of the parent. The suggested fragmentation pattern, as illustrated in *figure 2*, involves also cleavage of the O-CH_2 bond to give naproxen and the previously observed ion fragments of the promoieties. The most stable ion fragment was produced in this case by loss of a CO_2 molecule from $[nap\text{-}COO]^+$, whereas the ion fragments of the promoieties at m/z = 112 and 132 were found to be less abundant.

3. Results and discussion

3.1. In vitro stability

It was expected that the synthesized ester prodrugs **5a–d** and **6a–d** would be hydrolysed in-vivo before, during or after absorption to release the corresponding free NSAIDs which would then exert their characteristic pharmacological actions. Therefore, it was desirable to determine the relative susceptibility of these compounds at 37 °C in simulated gastric fluid (hydrochloric acid buffer, pH 1.2), phosphate buffer of pH 7.4, 80% human plasma and 10% rat-liver homogenate. The use of 10% liver homogenate in hydrolysis studies instead of an undiluted state was based on the fact that liver homogenates are much more enriched with esterases than plasma [13] and the need to obtain comparable data for all the studied esters.

All prodrugs were found to be converted quantitatively to the parent drugs in both chemical and enzymatic hydrolysis as revealed by HPLC analysis of the reactions. It is worthwhile mentioning that in case of the prodrugs **5a** and **6a**, the resulting aspirin was subjected to subsequent degradation into salicylic acid at the studied pH values. *Figure 3* illustrates a representative HPLC chromatogram for the hydrolysis of aspirin ester prodrug **6a** after 20 min incubation in citrate buffer of pH = 6 at 37 °C. The progress of both chemical and enzymatic hydrolysis of the esters **5a–d** and **6a–d** followed pseudo first-order kinetics over several half-lives. The rate constants (k_{obs}) for the individual reactions were calculated

Table II. ^{1}H-NMR and MS data of the N-hydroxymethyl cyclic amides **3**, **4** and the ester prodrugs **5a–d** and **6a–d.**

	^{1}H-NMR δ ppm(m; *J* Hz)	M$^+$; (%)	Mass 100 %	others(%)
3	2.9 (4H, s, succinim-H), 4.2 (1H, bs, -OH), 5.1 (2H, s, N-CH_2-)			
4*	5.0 (2H, d, *J* = 7 Hz, N-CH_2), 6.3 (1H, t, *J* = 7 Hz, -OH), 6.8–7.8 (4H, m, isat-H)			
5a	2.3 (3H, s, -CH_3), 2.8 (4H, s, succinim-H), 5.7 (2H, s, N-CH_2-) 7.0–8.0 (4H, m, ph)	291; (0.14)	112	249 (91): M-C_2H_2O
5b	0.9 [6H, d, *J* = 7 Hz, -$(CH_3)_2$], 1.4 (3H, d, *J* = 7 Hz, -$CHCH_3$), 1.9 [1H, m, -$CH(CH_3)_2$], 2.5 (2H, d, *J* = 7 Hz, -CH_2-CH-), 2.8 (4H, s, succinim-H), 3.6 (1H, q, *J* = 7 Hz,-$CHCH_3$), 5.5 (2H, s, -N-CH_2-), 7.1 (4H, bs, ph-H)	–	–	
5c	1.7 (3H, d, *J* = 7 Hz, -$CHCH_3$), 2.6 (4H, s, succinim-H), 3.8 (1H, q, *J* = 7 Hz, -$CHCH_3$), 3.9 (3H, s, 3H, OCH_3), 5.5 (2H, s, -N-CH_2-), 7.0 - 7.8 (6H, m, naphthyl-H)	341; (17.4)	185	229 (8.8) (M-112)
5d	2.3 (3H, s, -CH_3), 2.6 (4H, s, succinim-H), 3.6 (2H, s, CH_2CO-), 3.9 (3H, s, OCH_3), 5.5 (2H, s, -NCH_2), 6.6–7.1 (3H, m,indol-H) 7.3–7.8 (4H, m, ph-H)	468; (6.2)	112;	249; 189; 139(51.6): C_7ClH_4O
6a	2.3 (3H, s, CH_3), 6.0 (2H, s, -N-CH_2-), 6.9–7.8(7H, m, isat-4H + ph-H4,5,6), 8.0 (1H, dd, $J_{3,4}$ = 8Hz, $J_{3,5}$ = 3Hz, ph-H3)	339; (4.3)	132	297 (5.5): M-C_2H_2O 160 (17.8)
6b	0.9 [6H, d, *J* = 7 Hz, $CH(CH_3)_2$], 1.5 (3H, d, *J* = 7Hz, -$CHCH_3$), 1.9 (1H, m, -$CH(CH_3)_2$), 2.3 (2H, d, *J* = 7 Hz, -CH_2CH-), 3.8 (1H, q, *J* = 7 Hz, -CHCH3), 5.7 (2H, s -N-CH_2-), 6.9–7.8 (4H, m, isat-H), 7.1 (4H, bs, ph-H)	365; (6.0)	132	161 (82): $[CH_3$-isat$]^+$
6c	1.8 (3H, d, *J* = 7 Hz, -$CHCH_3$), 3.9 (1H, q, *J* = 7 Hz, -$CHCH_3$), 3.9 (3H, s, -OCH_3), 5.8 (2H, s -N-CH_2-), 6.5–7.7 (10 H, m, naphthyl + isat-H)	389; (11.2)	185	161 (16.5); 132 (80.2)
6d*	2.2 (3H, s, -CH_3), 3.8 (2H, s, CH_2COO-), 4.0 (3H, s, -OCH_3) 5.9 (2H, s -N-CH_2-), 6.6–7.4 (7H, m, indolyl-H + isatin-H), 7.7 (4H, bs, ph-H)	516; (7.7)	132	161 (49.3) 139 (85.5): $[C_7ClH_4O]^+$

^{1}H-NMR: solvent $CDCl_3$; *compounds **4** and **6d** in d_6-DMSO; bs: broad singlet; dd: doublet of doublet; MS: M$^+$ = molecular ion peak; base peak (100%); $[C_7ClH_4O]^+$: p-chlorobenzoyl.

from the linear regression equations correlating the residual ester concentrations vs. time. The corresponding half-life for the respective prodrugs **5a–d** and **6a–d** were then calculated. The results are summarized in *table III.*

The kinetic data revealed that HMSI and HMIS esters **5a–d** and **6a–d** of the selected NSAIDs were hydrolysed more slowly at pH 1.2 ($t_{1/2}$ ~ 6.5–18.6 h) than at pH 7.4 ($t_{1/2}$ ~ 10.7–85 min). Moreover, it has been observed that, at constant pH and temperature the HMIS-derived ester prodrugs **6a–d** are more susceptible to chemical hydrolysis than their respective analogues of HMSI series **5a–d.** As previously evidenced in ^{1}H-NMR spectra, the ester linkage in the former group of compounds is relatively activated due to the neighbouring phenyl moiety of isatin.

The observed acid stability of the ester prodrugs **5a–d** and **6a–d** fulfills the essential requirements for the oral delivery system of NSAIDs [14], whereby the masking group should be acid-stable to prevent the direct contact effects with the stomach mucosa as well as the local inhibition of the protective prostaglandins.

The stability of the ester prodrugs **5c** and **6a** as representative examples for each series was further investigated over the pH range 1.2–10 at 37 °C to evaluate the effect of pH on the degradation rate and to determine the pH of maximum stability. The values of the pseudo first-order rate constants (k_{obs}), for hydrolysis of the two compounds **5c** and **6a**, at the respective pH are given in *table IV.* The pH-rate profiles are shown in *figure 4* in

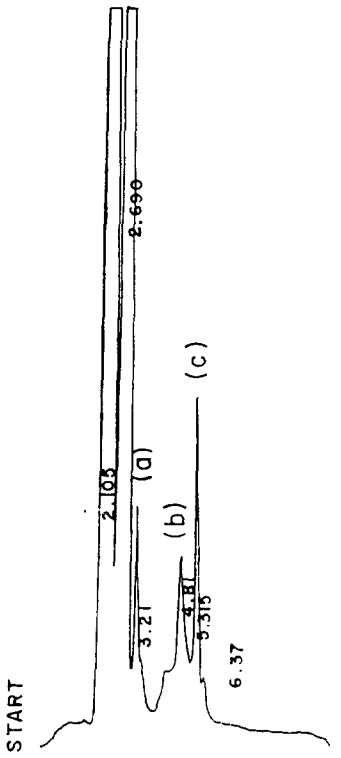

Figure 3. HPLC chromatogram showing the degradation of the prodrug **6a** after 20 min in citrate buffer of pH 6 at 37 °C: mobile phase, acetonitrile:water (45:55). a) aspirin, b) salicylic acid, c) remaining ester prodrug **6a**.

which the logarithms of the observed pseudo-first-order rate constants (k_{obs}) are plotted against pH. The ester **5c** exhibits a broad U-shape hydrolysis profile, indicative of the presence of acid-catalysed (k_{H^+}), base-catalysed (k_{OH^-}) and pH-independent (k_o) processes according to the following rate expression:

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

This profile is analogous to those reported for simple acyloxymethylamides [15]. It could be observed that the HMSI-derived ester prodrugs **5a–d** represented by **5c** are significantly stable at the pH range 2–8.

The hydrolysis rate profile of **6a** deviates from the simple U-shape *(figure 4)* and shows two narrow pH-independent regions, with different rates, between pH 2.2–4 as well as at pH 6–7.4. Maximal stability of this ester prodrug could be attained at pH-values < 4. Significant acid catalysis of hydrolysis has been observed at pH 4–6. This complex profile might be due to the possible hydrolysis of the acetyl group along with the ester function of the prodrug **6a**. As seen from *figure 4*, the ester **5c** is much more stable than **6a** and both of them show minimal degradation rates at pH 1–2, which is the normal pH range of the stomach.

The rate data for hydrolysis in 80% human plasma *(table III)* indicated an increase in the observed rates of hydrolysis of most prodrugs (prodrug **6d** exempted) by factors ranging from 2–7-fold ($t_{1/2}$ ~ 11.4–35 min) relative to their respective values in aqueous buffer solution of pH 7.4. In 10% rat liver homogenate ($t_{1/2} \cong$ 12.0–110.0 min), the rates of hydrolysis are much more accelerated, taking into account the low enzymatic concentration due to dilution. In addition, no appreciable differences could be observed between the two series **5a–c** and **6a–c** with respect to their susceptibility to enzymatic hydrolysis. The ester prodrugs of indomethacin **5d** and **6d** exhibit low rates of enzymatic hydrolysis ($t_{1/2}$ = 3.5 and 1.5 h respectively). As previously observed for the N-hydroxyymethylphthalimide analogue [7], the bulkiness of these ester prodrugs interfered with their ability to bind to the esterase active sites.

3.2. Lipophilicity

Lipophilicity is well known as a prime physicochemical descriptor of drugs with relevance to their biological properties. Consequently, there must be a valid and quick procedure to quantify molecular lipophilicity. In this context calculative approaches are superior to experimental procedures [16]. Validity studies of the most commonly used calculation methods confirm the superiority of the fragmental methods over the atom-based approaches [16]. The partition coefficients of the synthesized prodrugs **5a–d** and **6a–d** as well as those of the parent drugs **1a–d** were calculated using a PC-software package, based on the fragmental method developed by Leo [17] and the values are listed in *table V*.

It is worthwhile mentioning that the parent drugs **1a–d** are weak acids and are partially ionized at pHs higher than their pK_a values. The effect of the pH on the partitioning behaviour of such compounds has been documented and must be taken into consideration [18]. The adopted calculation method cannot adequately deal with differing contributions of their mesomeric forms at the pH of the intestine (pH > 7). Consequently, the distribution coefficients (D) of the synthesized prodrugs **5a–b** and **6a–b,** as well as their parent drugs **1a–d** were determined in an n-octanol/phosphate buffer (pH = 7.4) system. Alternatively, a chloroform/phosphate buffer (pH = 7.4) system was used for determination of the $D_{7.4}$ values of the ester prodrugs **5c–d** and **6c–d** due to their

Table III. In vitro kinetic data for chemical and enzymatic degradation of the prodrugs **5a–d** and **6a–d**.

PD	K_{obs} min^{-1}; ($t_{1/2}$) pH 1.2[a]	pH 7.4[b]	80 % h. plasma	10 % rat liver
5a	1.4615×10^{-3} (7.90 h)	1.9457×10^{-2} (35.6 min)	4.6014×10^{-2} (15.1 min)	3.3340×10^{-2} (20.8 min)
5b	0.7689×10^{-3} (15.02 h)	1.5028×10^{-2} (46.1 min)	3.6093×10^{-2} (19.2 min)	1.3653×10^{-2} (50.8 min)
5c	0.9324×10^{-3} (12.39 h)	0.8387×10^{-2} (1.37 h)	6.0897×10^{-2} (11.4 min)	3.0499×10^{-2} (22.7 min)
5d	0.6197×10^{-3} (18.64 h)	1.6644×10^{-2} (59.4 min)	1.9866×10^{-2} (34.9 min)	3.0499×10^{-2} (22.7 min)
6a	1.7645×10^{-3} (6.55 h)	2.884×10^{-2} (24.03 min)	n.d	5.7644×10^{-2} (12 min)
6b	1.0995×10^{-3} (10.5 h)	6.4683×10^{-2} (10.71 min)	4.0635×10^{-2} (17.05 min)	2.7127×10^{-2} (25.55 min)
6c	1.2757×10^{-3} (9.05h)	2.8838×10^{-2} (24.03 min)	5.5898×10^{-2} (12.4 min)	3.3240×10^{-2} (36.00 min)
6d	1.4876×10^{-3} (7.76 h)	3.9817×10^{-2} (17.4 min)	3.2480×10^{-3} (3.55 h)	7.6769×10^{-3} (1.50 h)

PD: Prodrug; [a]: hydrochoric acid buffer (0.2 M); [b]: phosphate buffer (0.02 M).

poor solubility in n-octanol. The latter D-values were corrected to the respective ones in the n-octanol system on the basis of a reported regression equation correlating the two solvent systems [19].

$$LogP_{chloroform} = 1.10\ (\pm 0.12)logP_{oct} - 0.649\ (\pm 0.18)$$

Table IV. Pseudo-first-order rate constants (k_{obs}) and the corresponding ($t_{1/2}$) for the hydrolysis of the prodrugs **5c** and **6a** in aqueous buffer solutions at 37°C.

	5c		**6a**	
PH*	k_{obs} (min^{-1})	$t_{1/2}$	k_{obs} (min^{-1})	$t_{1/2}$
1.2	0.932×10^{-3}	12.40 h	1.765×10^{-3}	6.50 h
2.2	0.996×10^{-3}	11.50 h	2.226×10^{-3}	5.20 h
3.0	2.553×10^{-3}	4.50 h	2.103×10^{-3}	5.00 h
4.0	3.037×10^{-3}	3.80 h	2.469×10^{-3}	4.70 h
5.0	2.815×10^{-3}	4.10 h	8.846×10^{-3}	1.31 h
6.0	3.978×10^{-3}	2.90 h	2.701×10^{-2}	25.70 min
7.4	0.8387×10^{-2}	1.38 h	2.884×10^{-2}	24.00 min
8.0	4.522×10^{-3}	2.60 h	–	–
9.0	1.590×10^{-2}	43.60 min	–	–
10.0	0.932×10^{-2}	74.40 min	–	–

* pH =1.2 (hydrochloric acid buffer); pH = 2.2–6.0 and 8.0–9.0 (citrate buffer); pH = 7.4 (phosphate buffer); pH = 10.0 (borate buffer).

The measured $D_{7.4}$-values as well as the calculated partition coefficients of the prodrugs **5a–d, 6a–d** and the parent drugs **1a–d** are listed in *table V.*

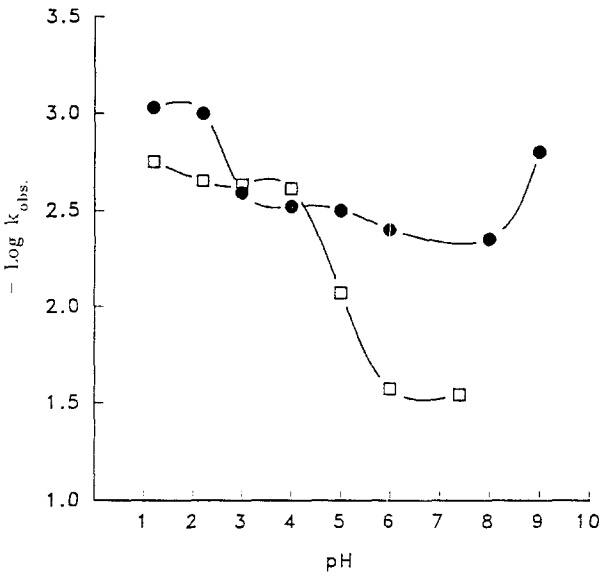

Figure 4. pH rate profiles for the hydrolysis of prodrugs 5c (●) and 6a (□) in aqueous buffers at 37 °C.

Table V. Chromatographic data for analytical HPLC and the lipohilicity parameters of the prodrugs: **5a–d**; **6a–d** and the parent drugs **1a–d.**

PD	Eluent	Detection	Rt (min)	Lipophilicity [$D_{7.4}$; (Clog p)]		
		λmax (nm)	PD	D	PD	D
5a	ACN/H_2O (45:55)	230	6.7	4.5	1.01 (0.67)*	0.18 (1.02)
5b	ACN/H_2O (75:25)	228	5.3	3.7	2.62 (3.37)	1.28 (3.50)
5c	ACN/H_2O (70:30)	232	5.7	3.3	2.46 (2.51)	1.48 (3.34)
5d	ACN/H_2O (75:25)	257	5.9	4.8	5.47 (4.64)	2.55 (4.27)
6a	ACN/H_2O (65:35)	230	5.3	3.2	3.72 (1.81)	0.18 (1.02)
6b	ACN/H_2O (78:22)	254	6.6	3.4	4.23 (4.51)	1.28 (3.50)
6c	ACN/H_2O (65:35)	232	7.0	4.3	4.84 (3.65)	1.48 (3.34)
6d	ACN/H_2O (80:20)	257	6.3	4.5	5.13 (5.78)	2.55 (4.27)

$D_{7.4}$: distribution coefficient in n-octanol/ phosphate buffer (pH = 7.4). Clog P: calculated using PC-software package based on Lit. 17; PD: Prodrug; D: Drug; *Reported Log P = 0.500 [11]

Unlike the calculated values, the results in *table V* revealed that there are appreciable differences in the $D_{7.4}$ values of the parent drugs and their prodrugs. This is because the drug will be negatively charged, whereas, the respective prodrug is still neutral at pH = 7.4. It is evident that the prodrugs **5a–d** and **6a–d** are more lipophilic and might be characterized by enhanced intestinal absorption relative to the parent drugs **1a–d**. Moreover, the N-hydroxymethylisatin-derived (HMIS) ester prodrugs **6a–d** are more lipophilic than their respective ones of the HMSI series **5a–d.** This could be attributed to the higher CH index [20] of promoiety in the HMIS-derived esters as compared with that of the corresponding ones of the HMSI series.

3.3. Ulcerogenic activity

Gastrointestinal ulceration is a serious side effect associated with many acidic NSAIDs. The clinical uses of many potent anti-inflammatory agents are strongly limited by its gastrointestinal toxicity. To diminish these deleterious effects, ester prodrugs were synthesized and ulcerogenic activities were tested after repeated dosing (once daily for four days) of the prodrugs in comparison to the parent carboxylic acid drugs. The ulcerogenic activity was routinely determined through counting the number of ulcers of the stomach and intestine by visual examination using a binocular magnifier. The severity of the mucosal damage was then assessed on the basis of the frequency and size of the observed ulcers [21, 22]. Alternatively, we reported the application of the scanning electron microscope for investigation of the ulcerogenic activity of NSAI agents as well as their prodrugs [7, 23, 24]. This new technique represents a highly accurate and self-explanatory description for the effects of the investigated compounds on the protective as well as the mucosal layer of the gastrointestinal tract.

Figures 5 and *6* illustrate scanning-electromicrographs for stomach as well as duodenum specimens of rats treated with a daily oral dose (4 d) of aspirin **1a** *(figure 5A)* and indomethacin **1d** *(figure 6A)* and their respective prodrugs **5a, 6a, 5d** and **6d** (*figures 5B, 5C, 6B and 6C*) respectively. The figures revealed damage of the protective mucous layer of the stomach in the case of the drugs-treated groups (*figure 5A* and *6A*). The bared surface epithelial cells of the mucosa were then subjected to the direct effect of the acid resulting in ulceration as can be seen in the rats treated with indomethacin *(figure 6A)*. As previously observed [7, 24], the aforementioned toxic effects are not detected in stomach specimens of the prodrugs-treated groups (*figures 5B, 5C, 6B and 6C*) and the scanning-electromicrographs are almost identical to that of the control group *(figure 5D)*. It is also worth noting that neither of the parent drugs **1a**, **1d** nor the prodrugs **5a**, **6a**, **5d** and **6d** exerted any ulcerogenic activity on the upper intestine under the used dosing schedule. The corresponding scanning-electron micrographs of the duodenum specimen are illustrated in *figures 5D, 6D, 5E, 6E, 5F* and *6F*.

4. Conclusion

The results indicated that the suggested ester prodrugs **5a–d** and **6a–d** were sufficiently chemically stable in simulated-gastric fluid and characterized by enhanced lipophilicity at pHs simulating that of the intestine that attain intact absorption at an appreciably higher rate than the parent drugs. In vitro and in vivo studies indicated that the prodrugs were rapidly bioconverted to the parent drugs in 80% human plasma and were appreciably less irritating to the stomach than the parent drugs.

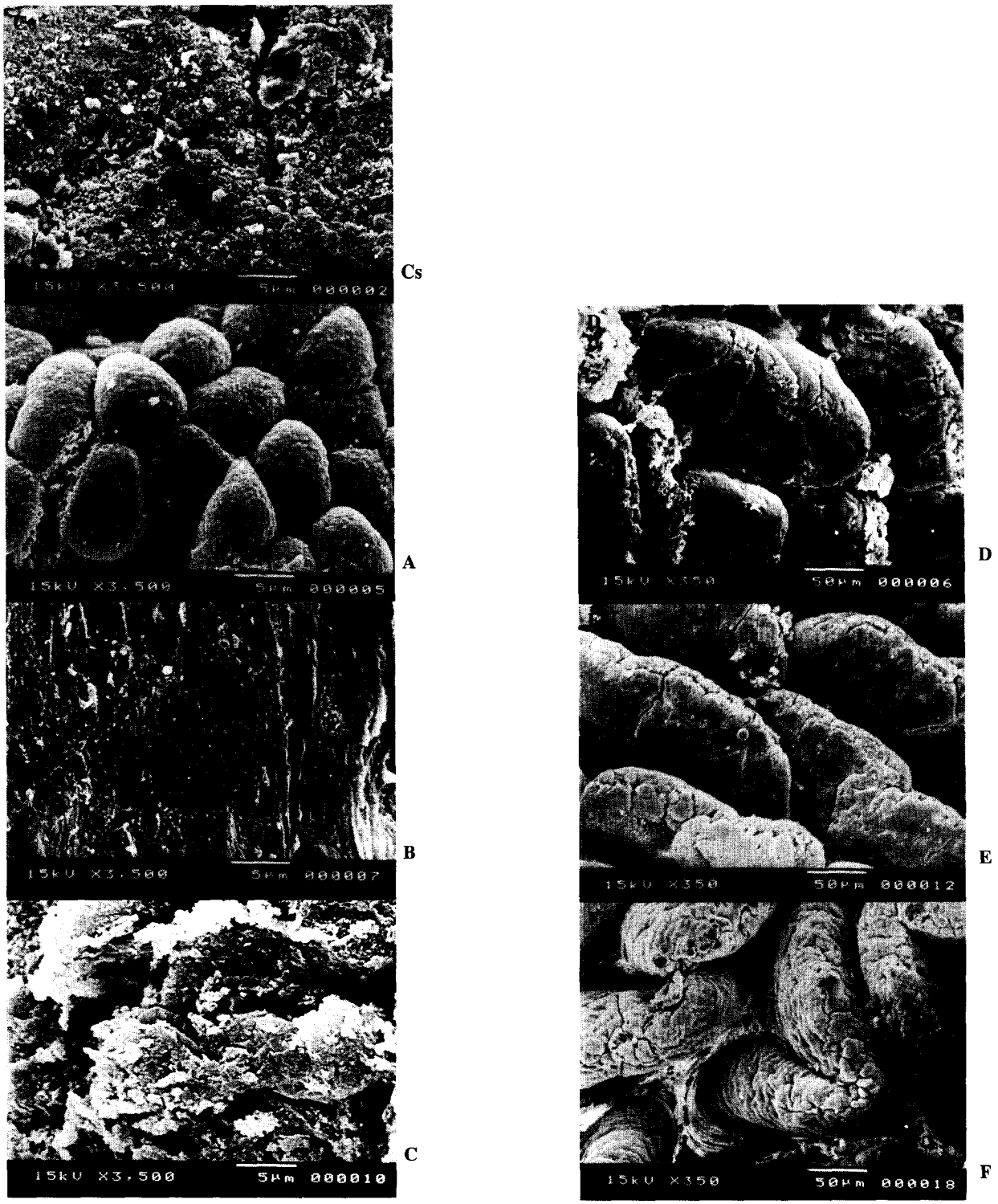

Figure 5. Scanning electron micrographs following a daily oral dose for 4 d of aspirin **1a** and its prodrugs **5a** and **6a** in rats. Stomach: **A.** 1a; **B.** 5a; **C.** 6a; **Cs.** control. Intestine: **D.** 1a; **E.** 5a; **F.** 6a; Ci. control.

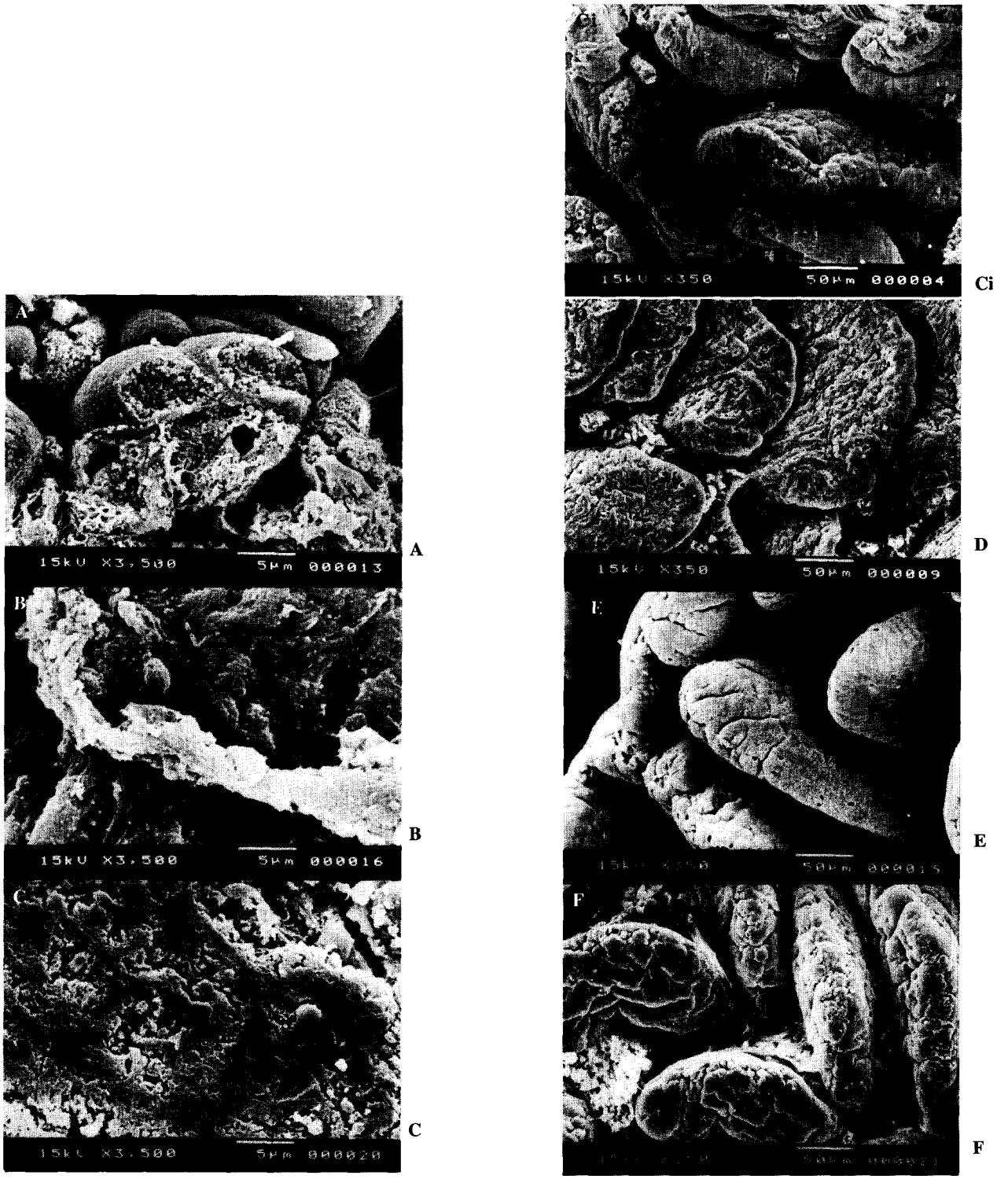

Figure 6. Scanning electron micrographs following a daily oral dose for 4 d of indomethacin **1d** and its prodrugs **5d** and **6d** in rats. Stomach: **A.** 1d; **B.** 5d; **C.** 6d. Intestine: **D.** 1d; **E.** 5d; **F.** 6d.

5. Experimental protocols

5.1. Chemistry

Aspirin **1a**, ibuprofen **1b**, naproxen **1c** and indomethacin **1d** were kindly provided by several pharmaceutical companies in Cairo and used as received. All other chemicals and reagents used in the synthesis were of reagent-grade and those for the kinetic studies were of analytical grade. Fresh doubly distilled water was used in the preparation of the solutions.

5.1.1. Synthesis of N-hydroxymethylsuccinimide 3

A solution of succinimide 5.0 g (0.05 mol) and 3.0 mL formaldehyde solution (40% w/v) in 100 mL ethanol (90%) was refluxed for 1 h. The solvent was removed under reduced pressure and the oily residue was crystallized from ether/petroleum ether. Yield 4.0 g 60%. m.p. 55–58 °C (ether/petroleum ether). Lit. [25] no specific constants were cited; ^{1}H-NMR data are listed in *table II.*

5.1.2. Synthesis of N-hydroxymethylisatin 4

A suspension of isatin 7.5 g (0.05 mol) and 3.0 mL formaldehyde solution (40% w/v) in water (100 mL) was refluxed for 2 h. The hot solution was filtered, cooled overnight and the separated crystalline product was filtered and dried in air. Recrystallization from ethylacetate affords a pure product. Yield 6.2 g (70%), m.p 150–52 °C (ethyl acetate). Lit. [26] m.p. 148–150 °C; ^{1}H-NMR data are listed in *table II.*

5.1.3. General method for synthesis of the ester prodrugs 5a–c and 6a–c

To a cooled solution (0–5 °C) of the respective non-steroidal anti-inflammatory carboxylic acid **1a–c** (0.01 mol) and triethylamine 1.02 g (0.01 mol) in methylene chloride (50 mL) was added dropwise ethyl chloroformate 1.1 g (0.01 mol) and the mixture was stirred for a further 30 min. The respective cyclic amide (0.01 mol) was then added portionwise over a period of 30 min and stirring was continued overnight at room temperature. The reaction mixture was then washed successively with water and 5% sodium hydrogen carbonate solution, dried over anhydrous sodium sulfate and filtered. The solvent was then removed under reduced pressure and the residues recrystallized from the appropriate solvent. Physical constants and yields are in *table I.* ^{1}H-NMR and mass spectral data are summerized in *table II.*

5.1.4. Synthesis of indomethacin ester prodrugs 5d and 6d

Thionyl chloride (3.84 g, 0.03 mol) was added to a 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 then removed under reduced pressure. The resulting acid chloride was dissolved in methylene chloride (50 mL) and added dropwise to a cooled solution containing the appropriate cyclic amide (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 acetone/petroleum ether.

5.2. Analytical methods

Melting points were determined with an electrothermal apparatus (Stuart Scientific, England) and were uncorrected. Precoated silica gel plates (Kieselgel 60G F254 nm, Merck, Germany) were used for TLC. ^{1}H-NMR spectra [δ (ppm) J (Hz)] were obtained on a Varian EM-360L (60 MHz) spectrometer (reference TMS). Mass spectra were determined on a Shimadzu GC/MS QP-5000 (Shimadzu Co., Kyoto, Japan) at the Institute of Pharmaceutical Chemistry, Vienna University, Vienna, Austria. Elemental analysis for C, H and N were obtained on a Perkin-Elmer 240 °C analyser and were within ± 0.4% of the theoretical values.

The HPLC system consisted of a pump [Knauer pump 64, Germany], a variable-wavelength detector [Knauer], a reverse-phase C18 column (Eurospher 80 RP-18, 25 × 0.5 cm i.d.) equipped with a cartridge guard column, a Shimadzu C-R 6A chromatopac recording integrator, and a 20 µL injection loop. Chromatographic separations were achieved using a mobile phase of acetonitrile and water containing 1% triethylamine at a flow rate of 1.0 mL/min. The relative ratio of acetonitrile and water *(table V)* has been adjusted in each case to attain separation of the peaks of the prodrug, parent drug as well as the expected degradation products *(figure 3).* The column effluent was monitored at the convenient wavelengths *(table V).*

5.3. In vitro hydrolysis kinetics

5.3.1. Chemical hydrolysis

The hydrolysis of the studied HMSI (**5a–d**) and HMIS (**6a–d**) ester prodrugs was studied at pH 1.2 using a buffer solution of hydrochloric acid and potassium chloride and in pH 7.4 phosphate buffer. A constant ionic strength of 0.5 µ at 37 °C was maintained in both buffers by adding a calculated amount of potassium chloride. Buffer solutions containing 0.02% w/v tween 80 and the ester prodrug (1×10^{-4} M) were kept at a constant temperature of 37 °C in a water bath. At appropriate time intervals,

samples of 20 μL were taken and analysed immediately by HPLC for the respective NSAID and the remaining ester prodrug. Least-square equations, derived by correlating peak areas in HPLC chromatograms to known concentrations of each compound were used for calculation of the residual ester concentrations in the studied samples. The correlation coefficients of the standard curves were ≅ 0.999.

The pH profile: the hydrolysis of the ester prodrugs **5c** and **6a** was studied in aqueous buffer solutions of pH 1.2–10 at 37 ± 0.1 °C. The buffers used were hydrochloric acid, citrate, phosphate and borate. The total buffer concentration was 0.02 M and a constant ionic strength of 0.5 μ for each buffer was maintained by adding a calculated amount of potassium chloride. Degradation of the studied compounds was followed by an HPLC procedure capable of determining intact ester and all products of hydrolysis. The reactions were initiated by adding 0.5 mL of stock solutions to 5 mL of the preheated buffer solutions in screw capped tubes, the final concentrations of the compounds being about 1×10^{-4} M. At appropriate intervals 20 μL samples of the reaction solutions were injected into the chromatographic system and analysed for the remaining ester prodrug. Pseudo-first-order rate constants for the hydrolysis were calculated from the slopes of the linear plots of log (% residual prodrugs) vs. time.

5.3.2. Enzymatic hydrolysis

In 80% human plasma: the rate of enzymatic hydrolysis of the ester prodrugs **5a–d** and **6a–d** was studied in 80% human plasma containing isotonic phosphate buffer of pH 7.4 at 37 ± 0.1 °C. The reaction was initiated by adding 100 μL of stock methanolic solution (1×10^{-4} M) of the respective derivative to 1 mL of plasma solution. At appropriate time intervals, samples of 50 μL were withdrawn, mixed with 50 μL of acetonitrile for deproteinization and centrifuged at 1×10^4 rpm for 10 min. 20 μL of the clear supernatant was analysed by HPLC for the remaining ester prodrug as described above.

In 10% rat liver homogenate: male Wistar rat livers were homogenized with ice-cooled saline to give a concentration of 40% w/v, and were then centrifuged at 1×10^4 rpm for 15 min. The supernatant was 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 concentration. The hydrolysis studies in rat liver homogenate were performed as described for 80% human plasma solution. The kinetic data are the average of three experiments and are given in *table III.*

5.4. Determination of distribution coefficients

The distribution coefficient (D) of the prodrugs **5a–d**, **6a–d** and the parent drugs **1a–d** were determined by dissolving 10 mg of the respective compound in 10 mL of n-octanol or chloroform, in the case of **5c–d** and **6c–d,** 2 mL aliquots were then taken and added to an equal volume of phosphate buffer (pH = 7.4) in screw-capped test tubes. The mixtures were then vortexed for 15 min. and centrifuged at 1×10^4 rpm for 5 min. The layers were separated and aliquots of 100 μl were diluted to 10 mL in volumetric flasks. The absorption of the diluted solutions were then measured at the convenient λ-max. The concentration in each of the organic and aqueous layers were then calculated for each compound by virtue of calibration curves correlating absorbances and known concentrations. All experiments were conducted in triplicate and the mean values were taken. The values of $D_{7.4}$ for the respective compounds were then calculated by the usual method and the results are listed in *table V.*

5.5. In vivo ulcerogenicity study

A Joel, JSM-54000LV scanning electron microscope (electron microscope unit Assiut University, Assiut, Egypt) was used for the scanning micrographs. Wistar rats (200–250 g) from the animal house, Faculty of Medicine, Assiut University. Seven groups, each of 3 rats were used.

The tested drugs **1a** and **1d,** as well as their respective ester prodrugs **5a**, **6a**, **5d** and **6d** were suspended in 0.5% carboxymethylcellulose solution. The dose (1 mL suspension) was equivalent to: 100 mg/kg of **1a**; 6.75 mg/kg of **1d** or the equivalent amounts of the respective prodrugs **5a**, **6a**, **5d** and **6d**.

The animals were fasted for 12 h and then the first 6 groups were administered a daily oral dose of 1 mL of the drug suspensions for 4 successive days. The seventh group received an equal amount of the dispersion medium and was considered as control. All groups were denied access to food throughout this period and 24 h after the last dosing. The rats were sacrificed by spinal decapitation, the stomach and duodenum were removed, opened along the greater curvature and cleaned gently by dipping in saline. Randomly selected specimens were then taken and prepared for scanning in an electron microscope. The preparation method involves, firstly, fixation 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 specimens were then treated with osmium tetraoxide (1% solution) for 2 h and washed in the same sequence with cacodylate buffer solution. Secondly, dehydration of the

specimen by treatment for 30 min with each of 30%, 50% and 70% ethanolic solution was followed by 90% ethanol for 1 h and finally in absolute ethanol for 2 d. After discharge of the alcohol the specimens were soaked in amyl acetate solution for 2 d, dried under reduced pressure, mounted on holders and coated for scanning in electron microscopy.

Acknowledgements

The authors appreciate the assistance of Dr E. Urban, Institute für phamazeutische Chemie der Univesität Wien, in performing the mass spectra presented in this report.

References

[1] Vane J.R., Nature 231 (1971) 232–235.

[2] Shoen R.T., Vender R.J., Am. J. Med. 86 (1989) 449–458.

[3] Cioli V., Putzolu S., Rossi V., Barcellonu P.S., Corradino C., Toxicol. Appl. Pharmacol. 50 (1979) 283–289.

[4] Shanbhag V.R., Harpalani A., Dick R.M., J. Pharm. Sci. 81 (1992) 149–154.

[5] Bodor N., Pharmazie. 52 (1997) 491–494.

[6] Moreira R., Calheiros T., Mendes E., Pimentel M., Iley J., Pharm. Res. 13 (1996) 70–75.

[7] Omar F.A., Eur. J. Med. Chem. 33 (1998) 123–131.

[8] Glover V., Halket J.M., Watkins P.J., Clow A., Goodwin B.L., Sandler M.J., Neurochem. 51 (1988) 656–659.

[9] vonMuller M., Med. Exp. 7 (1962) 155–160.

[10] Saari W.S., Freedman M.B., Hartman R.D., King S.W., Raab A.W., Randall W.C., Engelhardt E.L., Hirschmann R., J. Med. Chem. 21 (1978) 746–753.

[11] Nielsen N.M., Bundgaard H., J. Med. Chem. 32 (1989) 727–734.

[12] Butcher M., Org. Mass Spectrom. 5 (1971) 759–763.

[13] Khans A.H., Bundgaard H., Int. J. Pharm. 62 (1990) 193–205.

[14] Bundgaard H., Nielsen N.M., Int. J. Pharm. 44 (1988) 151–158.

[15] Iley J., Moreira R., Ross E., J. Chem. Soc. Perkin Trans. 2 (1991) 563–570.

[16] Mannhold R., Rekker R.F., Sonntag C., TerLaak A.M., Dross K., Polymeropoulus E.E., J. Pharm. Sci. 84 (1995) 1410–1419.

[17] Leo A.J., Chem. Rev. 93 (1993) 1281–1306.

[18] Kubinyi H., Prog. Drug Res. 23 (1979) 97–198.

[19] Leo A.J., Hansch C., J. Org. Chem. 36 (1971) 1539–1545.

[20] Moriguchi I., Hirono S., Liu Q., Nakagome I., Matsushita Y., Chem. Pharm. Bull. 40 (1992) 127–130.

[21] Tammara V.K., Narurkar M.M., Crider A.M., Khan M.A., Pharm. Res. 10 (1993) 1191–1199.

[22] Ogiso T., Iwaki M., Tanino T., Nagai T., Ueda Y., Muraoka O., Tanabe G., Biol. Pharm. Bull. 19 (1996) 1178–1183.

[23] Omar F.A., Mahfouz N.M., Rahman M., Eur. J. Med. Chem. 31 (1996) 819–825.

[24] Fadl T.A., Omar F.A., Inflammopharmacology 6 (1998) 143–157.

[25] Borrows E.T., Johnson J.M., Brit. Patent 1, 220, 447, (1971); through ref. 11.

[26] Buu-Hoi N.P., Saint-Ruf G., Perche J.C., Bourgeade J.C., Chem. Ther. 3 (1968) 110–113.