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Acyloxymethyl as a Drug Protecting Group. Part 6: *N*-Acyloxymethyl- and *N*-[(Aminocarbonyloxy)methyl]sulfonamides as Prodrugs of Agents Containing a Secondary Sulfonamide Group

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Abstract—Tertiary *N*-acyloxymethyl- and *N*-[(aminocarbonyloxy)methyl]sulfonamides were synthesised and evaluated as novel classes of potential prodrugs of agents containing a secondary sulfonamide group. The chemical and plasma hydrolyses of the title compounds were studied by HPLC. Tertiary *N*-acyloxymethylsulfonamides are slowly and quantitatively hydrolysed to the parent sulfonamide in pH 7.4 phosphate buffer, with half-lives ranging from 20 h, for **7d**, to 30 days, for **7g**. Quantitative formation of the parent sulfonamide also occurs in human plasma, the half-lives being within 0.2–2.0 min for some substrates. The rapid rate of hydrolysis can be ascribed to plasma cholinesterase, as indicated by the complete inhibition observed at [eserine] = 0.10 mM. These results suggest that tertiary *N*-acyloxymethylsulfonamides are potentially useful prodrugs for agents containing a secondary sulfonamide group, especially with $pK_a < 8$, combining a high stability in aqueous media with a high rate of plasma activation. In contrast, *N*-[(aminocarbonyloxy)methyl]sulfonamides **7h–j** do not liberate the parent sulfonamide either in aqueous buffers or in human plasma and thus appear to be unsuitable for development as sulfonamide prodrugs.

Introduction

The use of a secondary sulfonamide group as a bioisosteric replacement for the amide and carboxylic acid groups, as well as an isostere of the transition-state for the hydrolysis of the peptide bond, is becoming an increasingly important tool in drug design.[1–3] In many cases, sulfonamide analogues often exhibit only poor-to-moderate oral activity as a result of unfavourable physicochemical properties such as extreme low or high lipophilicity. Numerous examples have been reported recently and include the HT_{1D} antagonist sumatriptan **1**, the leukotriene D_4 antagonists **2**, the human leukocyte elastase inhibitors **3** and the endothelin antagonist **4.**[4–8]

Several drugs containing a secondary sulfonamide group also exhibit undesirable side effects that affect their therapeutic effectiveness after oral administration. For example, although significantly better tolerated than typical cyclooxygenase-1 (COX-1) inhibitors such as indomethacin, nimesulide, **5**, the lead compound of the methanesulfonamide class of cyclooxygenase-2 (COX-2) inhibitors, still reveals gastric damage comparable to isoxicam.[9–12] Such gastric damaging effect is not restricted to nimesulide, and has also been reported with other, more recent, COX-2 methanesulfonamide inhibitors.[13]

A useful approach to improve drug delivery is to transform the drug molecule into a prodrug, which in this case might be achieved by linking the sulfonamide to an inactive carrier.[14] The main requisite is the quantitative release of the parent drug in vivo, either chemically or enzymatically. Also in the case of nimesulide and related COX inhibitors, the prodrug approach could be used in order to minimise the potential for gastrointestinal irritation due to topical COX inhibition and prostaglandin depletion by the parent drug. However, only few prodrugs for secondary sulfonamide agents have been reported.[15–17] From these, only *N*-acylsulfonamides, e.g. **6**, have shown to have some potential as prodrugs of secondary sulfonamides as a result of their high rate of in vivo activation.[16,18]

Recently, we reported that tertiary *N*-acyloxymethylsulfonamides, **7** ($X = OCOR^3$) are potential prodrugs for both secondary sulfonamides and carboxylic acid

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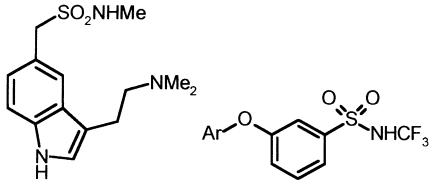
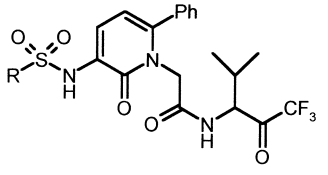
agents.[19] Compounds **7** (X = OCOR^3) undergo spontaneous hydrolysis at pH 7.4 by an S_N1-type mechanism, involving the formation of an *N*-sulfonyliminium ion in the rate-determining step.[19,20] Accordingly, reactivity of **7** increases sharply with electron donating substituents in the sulfonamide moiety (both R^1 and R^2), with the *N*-aryl derivatives presenting the highest stability.[19,20] Considering the potential utility of *N*-acyloxymethylsulfonamides **7** it was therefore necessary to assess the applicability of this prodrug approach with a wider range of compounds representative of therapeutic agents that contain the secondary sulfonamide group. We now report the synthesis of a series of *N*-acyloxymethylsulfonamides **7a–g** and a kinetic study designed with the objective of evaluating the influence of pK_a and structure of the sulfonamide on the chemical reactivity and stability in human plasma. Their *N*-aminocarbonyloxymethyl counterparts **7h–j** were also synthesised to assess the ability of a carbamoyloxymethyl group (**7**, X = OCONR^{3}R^4), based on amino acids, to be chemically and enzymatically activated to the parent sulfonamide. The data are compared with N^1-acetylsulfisoxazole, **6**, a well-known taste-masking prodrug.



1 **2**



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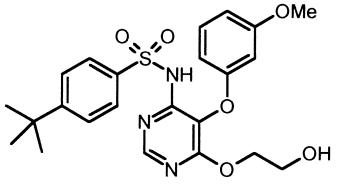

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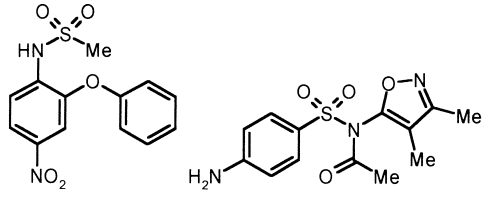
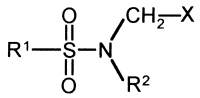


5 **6**



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Chemistry

Tertiary *N*-acyloxymethylsulfonamides **7a–g** were synthesised by reaction of the secondary sulfonamide with the appropriate chloromethyl ester.[19] The strategy for the synthesis of *N*-aminocarbonyloxymethylsulfonamides **7h–j** involves the preparation of *N*-[(chloromethyl)oxy]carbonyl derivatives **8** by reaction of the appropriate amino acid with chloromethyl chloroformate (Scheme 1, for **7h**). Compounds **8** were converted quantitatively to their iodomethyl counterparts **9** with sodium iodide at room temperature. Finally, reaction of intermediates **9** with the sodium salt of nimesulide, **5**, afforded **7** in ca. 30% yield.

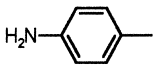
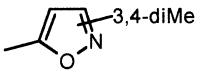
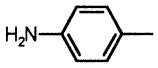
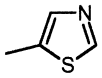
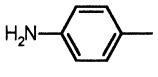
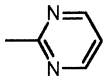
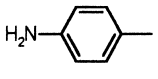
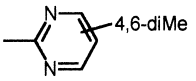
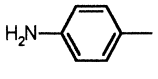
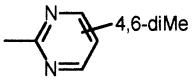
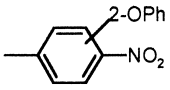
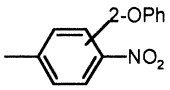
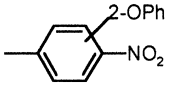
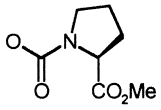
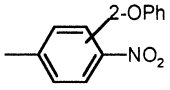
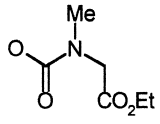
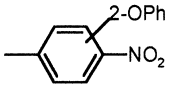
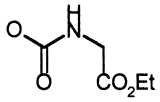
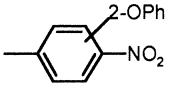
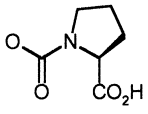
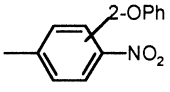
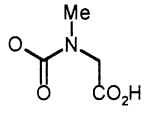
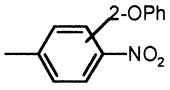
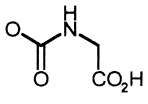
The structure of compounds **7a–j** follows from their spectroscopic and analytical data. The ^{1}H NMR spectra of *N*-acyloxymethylsulfonamides **7a–g** and derivative **7j** exhibit a characteristic singlet at ca. δ 5.8–6.6 ppm due to the NCH_2O group. The carbamoyloxymethyl derivatives **7h–i**, which contain a tertiary carbamate group, appear to be a mixture of E and Z rotamers, as revealed by their ^{13}C and ^{1}H NMR spectra. Interestingly, for the proline derivative, **7h**, the NCH_2O signal appears as two pairs of doublets, reflecting the diastereotopic nature of the methylene protons (^2J = 11.7 and 11.4 Hz) as well as the presence of two rotamers due to restricted rotation about the carbamate C–N bond. The spectroscopic detection of rotamers has been reported for other prolyl carbamates and is consistent with a barrier to rotation for the carbamate N–C=O framework similar to the barrier of acyclic tertiary amides.[21]

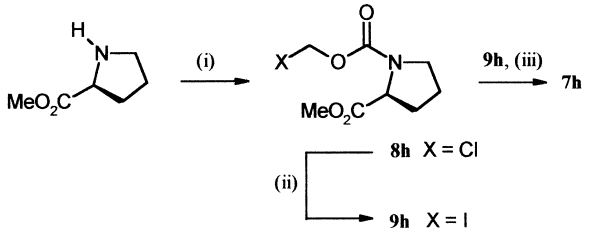
Results and Discussion

Chemical hydrolysis

Products of hydrolysis. As revealed by the HPLC analysis of reaction mixtures, the hydrolyses of *N*-acyloxymethylsulfonamides **7a–g** proceed with quantitative formation of the parent sulfonamide at all pH values studied. No lag time was observed for the formation of the parent sulfonamide at any pH value, indicating that the corresponding *N*-hydroxymethylsulfonamide (**7**, X = OH) decomposes very rapidly to the sulfonamide. A similar behaviour has been reported for other *N*-hydroxymethyl derivatives of NH-acidic drugs.[22] The rate of decomposition of *N*-hydroxymethylamides and imides increases sharply with the pK_a of the parent NH-compound, as indicated by the Bronsted β_{lg} value of −0.8.[23] Using this structure–reactivity relationship, a half-life of 0.01 s can be calculated for the decomposition of the proposed *N*-hydroxymethyl derivative of nimesulide (pK_a 5.9) at pH 7.4. In contrast to the *N*-acyloxymethyl

Table 1. Structure, melting points, elemental analyses and IR for compounds **7a**–**m**

Compound	R^1	R^2	X	m.p. (°C)	C% (calc.)	H% (calc.)	N% (calc.)	ν_{max} (cm^{-1})
7a			$OCOC_6H_5$	122–124	57.1 (56.8)	4.7 (4.8)	10.4 (10.5)	3474, 3362, 1742
7b			$OCOC_6H_5$	184–187	53.0 (52.4)	4.0 (3.9)	10.3 (10.8)	3460, 3360, 1725
7c			$OCOC_6H_5$	184–186	56.6 (56.2)	4.6 (4.2)	14.2 (14.6)	3451, 3360, 1708
7d			$OCOC_6H_5$	132–134	58.3 (58.2)	4.9 (4.9)	13.5 (13.6)	3474, 3375, 1726
7e			$OCOCMe_3$	158–161	55.3 (55.1)	6.2 (6.2)	14.0 (14.3)	3463, 3371, 1728
7f	Me		$OCOC_6H_5$	120–122	57.1 (57.0)	4.1 (4.1)	6.3 (6.3)	1719
7g	Me		$OCOCMe_3$	Oil	54.3 (54.0)	5.3 (5.2)	6.7 (6.6)	1740
7h	Me			79–81	51.5 (51.1)	4.9 (4.7)	8.2 (8.5)	1754, 1719
7i	Me			Oil	50.0 (49.9)	4.3 (4.8)	9.2 (8.7)	1749, 1720
7j	Me			Gum	48.9 (48.8)	4.2 (4.5)	9.2 (9.0)	3399, 1754, 1721
7k	Me			Oil	49.9 (50.1)	4.5 (4.4)	9.0 (8.8)	3605 (br),1722
7l	Me			Oil	48.0 (47.7)	4.2 (4.2)	9.2 (9.3)	3555 (br),1720
7m	Me			Oil	46.7 (46.5)	4.3 (3.9)	9.9 (9.6)	3376, 2856, 1725



Scheme 1. (i) $ClCH_2OCOCl/Et_3N/CH_2Cl_2$; (ii) NaI/acetone; (iii) **5** (sodium salt)/THF.

derivatives **7f–g**, the carbamates **7h–m** did not decompose to nimesulide over the pH 7.4–13 range. Large-scale hydrolyses of compounds **7h–j** afforded only the product from the hydrolysis of the amino acid methyl/ethyl ester functionality, as shown by comparison with the standards **7k–m**.

pH-Rate profiles. The pseudo-first-order rate constants, k_{obs}, for the hydrolysis of derivatives **7** were determined in HCl and NaOH solutions, as well as in aqueous buffers using several acetate, phosphate and borate buffer concentrations. The rates of hydrolysis of the more reactive compounds, e.g. **7d**, were not subject to significant buffer catalysis over at least a 10-fold buffer concentration range. In contrast, the rates of decomposition for the less reactive compounds, e.g. **7b**, presented a slight dependence on buffer concentration over the same concentration range. Using the intercepts of plots of k_{obs} versus [buffer], together with the k_{obs} values determined in HCl and NaOH solutions, the pH-rate profiles presented in Figure 1 were constructed. In the acidic region, compounds **7b–d** exhibit a sigmoidal pH-rate profile, which reflects protonation of the 4-amino group, rather than one of the heterocyclic nitrogen atoms (see below). The profile can be described by eq (1)

$$k_{obs} = \left\{k_o + k_{OH^-}[OH^-] + k_{H^+}^{Neut}[H^+]\right\}\frac{K_a}{K_a + [H^+]} + k_{H^+}^{Prot}[H^+]\frac{[H^+]}{K_a + [H^+]} \quad (1)$$

where $K_a/(K_a + [H^+])$ and $[H^+]/(K_a + [H^+])$ are the fraction of the unionised and ionised forms of the compound, respectively, and are the acid-catalysed second-order rate constants for these species, k_o is the first-order rate constant for the pH-independent decomposition of the unionised form, k_{OH^-} the OH^- catalysed second-order rate constant for the unionised form and K_a is the apparent ionisation constant of the compound (Scheme 2). In contrast, the pH-rate profile of compound **7f**, which does not contain any basic function, displays a simple U-shape, indicative of the presence of acid-catalysed, base-catalysed, and pH-independent processes (eq. (2)). Similar pH-rate profiles have been previously described for other neutral *N*-acyloxymethyl derivatives of sulfonamides.[20]

$$k_{obs} = k_o + k_{OH^-}[OH^-] + k_{H^+}^{Neut}[H^+] \quad (2)$$

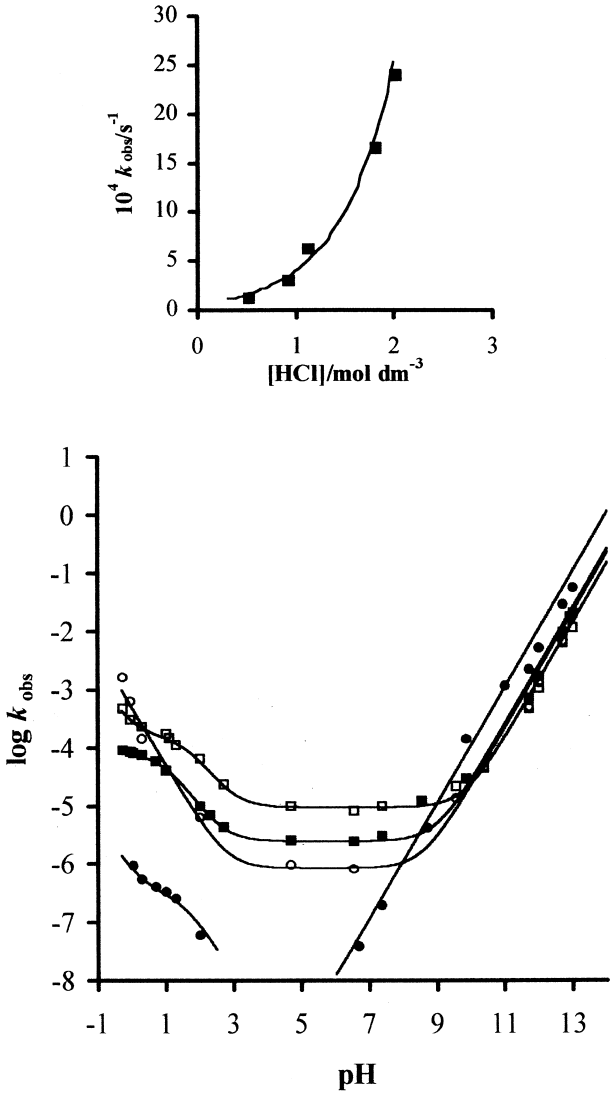
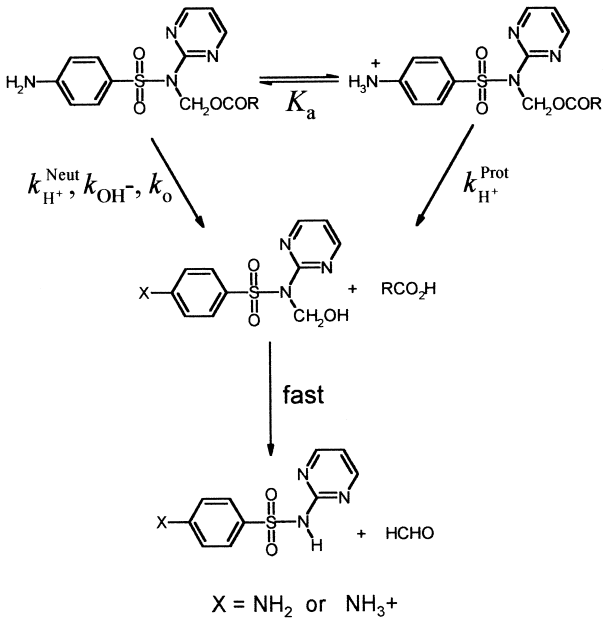


Figure 1. pH-Rate profiles for compounds **7b** (●), **7c** (■), **7d** (□) and **7f** (○) at 37 °C. Insert: dependence of k_{obs} with [HCl] for **7f**, at 37 °C.

The best computer fit (solid lines) to the experimental data (individual points) in Scheme 2 was achieved using eqs (1) and (2), the experimentally determined values for k_{obs} and pH, and the values for $k_{H^+}^{Neut}$, $k_{H^+}^{Prot}$ k_{OH^-}, k_o and pK_a listed in Table 2. For compound **7b**, no reproducible rate data was obtained between pH 3 and 5 and thus $k_{H^+}^{Neut}$ and $k_{H^+}^{Prot}$ were determined from eq. (1) assuming that no spontaneous or alkaline hydrolyses occur at pH < 2.

pH-Independent pathway. The k_o data presented in Table 2 indicates that ester **7d** is more reactive than **7c** by a factor of 4, suggesting that the pH-independent pathway is accelerated by electron-donating substituents in the *N*-aryl moiety. This is consistent with an S_N1-type mechanism involving the formation of an *N*-acyliminium ion in the rate-determining step.[20] Further evidence of an S_N1-type mechanism for the pH-independent hydrolysis of acyloxymethylsulfonamides **7c–d** is provided by the temperature dependence study with **7d** at pH 7.4, from which $\Delta S^{\neq}$ and $\Delta H^{\neq}$ values of -25 ± 8 JK^{-1} mol^{-1} and 98.1 ± 0.5 kJ mol^{-1}, respectively,



Scheme 2.

were calculated. The slightly negative value of $\Delta S^{\neq}$ is well within the range observed for unimolecular reactions carried out in the presence of an organic co-solvent.[20,24] The S_N1-type mechanism is also supported by the absence of buffer catalysis in the pH-independent region of **7d**. Ester **7f** is much less reactive in this pH-rate profile region, with a k_o value closer to that reported for *N*-acetoxymethyl-5-fluorouracil.[22] The value of $\Delta S^{\neq}$ for the hydrolysis of **7f** at pH 7.4 is -85 ± 4 kJ mol^{-1}, typical of a bimolecular process[20] involving nucleophilic attack of the solvent at the ester carbonyl carbon atom of **7f**. Such a change in mechanism might arise from highly unfavourable non-bonding interactions between the *ortho* phenoxy group and the $N{=}CH_2$ protons in the S_N1 transition-state.

Acid-catalysed pathway. The $k_{H^+}^{Neut}$ values in Table 2 indicate that the acid-catalysed hydrolysis of the neutral forms is also enhanced by electron-donating substituents in the *N*-aryl group, with ester **7d** being more reactive than **7c** by a factor of 8. This is consistent with a dissociative mechanism, identical to that for the pH-independent pathway, involving protonation of the substrate prior to iminium ion formation.[20] Compound **7f** is also most likely to hydrolyse by a S_N1-type mechanism as indicated by the monotonic increase of k_{obs} with [HCl] (insert in Fig. 1). A similar effect has been reported for *N*-acyloxymethylamides.[25]

The calculated pK_a values for compounds **7b–d** are ca. 0.8 units below the values reported for the parent sulfonamides (Table 2). Such a difference might be ascribed to the electron-withdrawing effect of the acyloxymethyl moiety on the sulfonamido group. The range of pK_a values for **7b–7d** is 1.1–1.7, which is consistent with protonation occurring at the 4-NH_2 group rather than at the heterocyclic nitrogen atom. Substantially lower pK_a values would be expected if protonation occurred in the heterocyclic moiety. For example, the pK_a of 2-(*N*-benzyl-*N*-*p*-tosylamino)thiazole is -0.53,[27] while that estimated for compound **7c** is ca. -0.1.[28] Not surprisingly, the protonated forms of *N*-acyloxymethylsulfonamides **7b–7d** are ca. 10- to 60-fold less reactive than the neutral forms in the acid-catalysed pathway, reflecting the difficulty of the second, catalytic protonation. However, the acid-catalysed hydrolysis of the ionised forms is also enhanced by electron-donating substituents in the *N*-aryl group (Table 2), suggesting that the ionised forms also decompose via an S_N1-type mechanism.

Base-catalysed pathway. Inspection of the k_{OH^-} values in Table 3 suggests that the alkaline hydrolysis of *N*-acyloxymethylsulfonamides **7a–g** is dependent on the steric effect exerted by the sulfonamide and ester moieties. Indeed, esters **7a** and **7d**, which contain two methyl groups in the heterocyclic ring, hydrolyse ca. 2-fold more slowly than unsubstituted compounds **7b** and **7c**. Also, the *N*-pivaloyloxymethylsulfonamides **7e** and **7g** are 3-fold less reactive toward OH^- than their *N*-benzoyloxymethyl counterparts **7d** and **7f**, respectively. The dependence of reactivity on the steric properties suggests that the mechanism of alkaline hydrolysis of *N*-acyloxymethylsulfonamides occurs via direct OH^- attack at the carbonyl carbon atom.[29] Unexpectedly, both tertiary, **7h–i**, and secondary, **7j**, *N*-(aminocarbonyloxy)methyl derivatives undergo hydrolysis exclusively at the ester, rather than the carbamate, group. Secondary carbamates with alcohol leaving groups with p$K_a < 12.5$ are known to hydrolyse via a facile elimination–addition mechanism (E1cB).[30] We have recently reported that the kinetically determined pK_a value for *N*-hydroxymethyl-*N*-methylsulfonamides is ca. 12.5,[20] and we

Table 2. Second-order rate constants, $k_{H^+}^{Neut}$, $k_{H^+}^{Prot}$ and k_{OH^-}, for the acid- and base-catalysed, and pseudo-first-order rate constants, k_o, for the pH-independent hydrolyses of *N*-acyloxymethylsulfonamides **7b–d** and **7f**, in aqueous buffers containing 10% acetonitrile (v/v), at 37 °C

Compound	k_{OH^-} (dm^3 mol^{-1} s^{-1})	k_o (10^{-7} s^{-1})	$k_{H^+}^{Neut}$ (10^4 dm^3 mol^{-1} s^{-1})	$k_{H^+}^{Prot}$ (10^{-7} dm^3 mol^{-1} s^{-1})	pK_a
7b	0.562	[a]	0.115	5.52	1.55 (2.36[e])
7c	0.152	25.0	9.00	130	1.07 (2.00[e])
7d	0.095	96.9, 325[b], 574[c], 1400[d]	75.0	1450	1.67 (2.36[e])
7f	0.134	9.66, 15.9[b], 31.8[c], 91.6[d]	5.02	—	—

[a]Not determined.
[b]45 °C.
[c]50 °C.
[d]60 °C.
[e]From ref 26.

Table 3. Lipophilicity data, molar refractivities, rate data for base-catalysed pathway at 37 °C and half-lives in human plasma at 37 °C and pH 7.4 (phosphate saline buffer) for compounds **6** and **7a–j**

Compound	Log P	MR (cm^3)	k_{OH^-} ($10\ M^{-1}\ s^{-1}$)	pH 7.4 buffer		Human plasma	
				$k_{pH\ 7.4}$ ($10^{-7}\ s^{-1}$)	$t_{1/2}$ (min)	k_{plasma} ($10^{-5}\ s^{-1}$)	$t_{1/2}$ (min)
7a	2.83	103.13	2.05	18.7	6.26×10^3	199	5.78
7b	1.47	99.55	5.62	25.0	4.65×10^3	9580	0.12
7c	2.15	99.20	1.52	108	1.18×10^3	833	1.38
7d	3.59	108.45	0.950	82.9	1.40×10^3	28.1	41.4
7e	3.07	102.27	0.320	30.1	3.83×10^3	4.71	244
7f	3.97	112.39	1.34	8.52	13.5×10^3	596	1.93
7g	3.96	106.20	0.523	2.48	46.6×10^3	11.3	102
7h	2.85	119.14	0.565	2.45	47.1×10^3	ND[a]	ND
7i	2.20	116.57	2.65	5.66	20.3×10^3	0.390	2.95×10^3
7j	2.28	111.71	9.60	16.4	7.03×10^3	1.20	954
6	0.82[b]	76.68	56.5	135	834	3630	0.3

[a]ND, no decomposition during 72 h.
[b]CLOGP from ref 26.

would expect a $pK_a < 12.5$ for the *N*-hydroxymethyl derivative of **5**. Thus, we expected compound **7j** to undergo hydrolysis of the carbamate group. The preferred ester hydrolysis observed with **7j** may be the result of a strong electron-withdrawing effect of the carbamoylmethyl moiety on the ester function. Further evidence in support of a common pathway for the alkaline hydrolysis of **7h–j** is provided by the good correlation between log k_{OH^-} values and the corresponding molar refractivities (Table 3), MR ($r^2 = 0.95$), indicating that for these compounds reactivity depends on the steric properties of the amino acid residue.[31]

Hydrolysis in human plasma

The hydrolysis in human plasma followed strict first-order kinetics for at least four half-lives, with $r^2 > 0.95$ in all cases where a reaction could be monitored. Product analysis by HPLC reveals that the plasma catalysed hydrolysis of esters **7a–g** leads to the quantitative formation of the corresponding sulfonamide (Fig. 2), which is consistent with the instantaneous decomposition of the *N*-hydroxymethyl intermediate (**7**, X = OH) to the parent sulfonamide. In contrast, *N*-(aminocarbonyloxy)methyl derivatives **7i**,**j** undergo decomposition in plasma exclusively via ester hydrolysis while, unexpectedly, **7h** is stable to cleavage of either functionality.

The susceptibility of compounds **7** to undergo enzymatic plasma activation was assessed in vitro by comparing the rates of hydrolysis in 80% human plasma and in pH 7.4 phosphate buffer at 37 °C. From the rate data presented in Table 3, it is clear that human plasma enzymes markedly accelerate the rate of hydrolysis of *N*-acyloxymethylsulfonamides **7a–g**, with half-lives ranging from 0.1 to ca. 240 min. The plasma hydrolysis of *N*-acyloxymethylsulfonamides **7a–g** appears to be markedly affected by the steric properties of the sulfonamide and ester moieties. Indeed, compounds **7b**,**c** hydrolyse 40 times faster than the more sterically hindered esters **7a**,**d**. Similarly, the *N*-benzoyloxymethyl derivatives **7d** and **7f** are more susceptible to plasma hydrolysis than their *N*-pivaloyloxymethyl counterparts **7e** and **7g**. Similar dependence of plasma hydrolysis on the steric hindrance of the ester moiety has also been reported for *N*-acyloxymethyl derivatives of other NH-acidic drugs.[22,32,33] The reactivity of N^1-acetylsulfisoxazole, **6**, in pH 7.4 buffer and in human plasma is also presented in Table 3. The most remarkable feature is the 7-fold greater reactivity of **6** at pH 7.4 when compared with **7a**. Such a difference cannot be ascribed to the different acyl moieties, as *N*-benzoyl-*N*-methyl-*p*-toluenesulfonamide is ca. 1.5 times more reactive than its *N*-acetyl analogue both at pH 4.0 and pH 7.4.[16] Thus, *N*-acyloxymethylsulfonamides appear to be more stable than their *N*-acyl counterparts, and therefore are more amenable to liquid pharmaceutical formulations.

The rate data in Table 3 were analysed for quantitative relationships with several physicochemical parameters, including the molar refractivity (MR) of the prodrug, the pK_a of the parent sulfonamide and the log *P* values. No clear correlation between the rate data encompassing all *N*-acyloxymethylsulfonamides **7a–g** and the

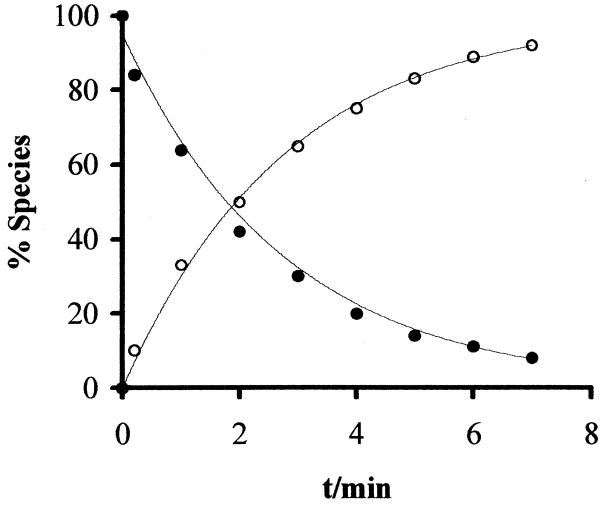


Figure 2. Time-courses for *N*-benzoyloxymethylnimesulide **7f** (●) and nimesulide **5** (○) in the hydrolysis of **7f** in 80% human plasma at 37 °C.

corresponding physicochemical parameters emerged. However, a good correlation ($r^2 = 0.89$) was observed between log k_{OH^-} and log k_{plasma}, suggesting that both alkaline and plasma hydrolyses follow similar pathways and thus have the same structural requirements (Fig. 3). To assess whether the rate-enhancement observed with human plasma, compared to hydrolysis in pH 7.4 buffer, could be ascribed to the action of plasma esterases, the effect of eserine on the rate of hydrolysis was also studied. Eserine is a specific inhibitor of the serine hydrolase cholinesterase (E.C. 3.1.1.8, also called pseudocholinesterase, plasma cholinesterase or butyrylcholinesterase).[34] Most significantly, a 220-fold decrease in reactivity was observed upon incubation with 10 μM eserine, and a complete inhibition of hydrolysis was observed with 100 μM eserine (Table 4). This strongly suggests that plasma cholinesterase is the major enzyme responsible for the rapid hydrolysis of the *N*-acyloxymethylsulfonamides **7a**–**g**. A similar inhibition profile by eserine has been reported for the esterase-catalysed hydrolysis of glycolamide esters in 50% human plasma.[35]

Conclusions

The rate data for the pH-independent pathway clearly indicate that highly stable *N*-acyloxymethylsulfonamides can be prepared when the parent sulfonamide contains either an *N*-heterocyclic ring or an *N*-aryl moiety bearing electron-withdrawing substituents. The *N*-acyloxymethyl derivatives of sulfonamides combine a high stability in aqueous buffers together with a high susceptibility to enzyme-catalysed hydrolysis. Thus, *N*-acyloxymethylation is a more attractive approach than *N*-acylation to obtain potentially useful prodrug systems for agents containing the secondary sulfonamide function. Hydrolysis of this new prodrug type in human plasma is reduced by the steric hindrance exerted by substituents in the sulfonamide and ester moieties. In contrast, the *N*-aminocarbonyloxymethylsulfonamides do not hydrolyse to the parent sulfonamide in aqueous buffers or in human plasma, rendering them unsuitable as sulfonamide prodrugs. Studies are in progress to examine the utility of *N*-acyloxymethylsulfonamides as potential prodrug systems for carboxylic acid agents.

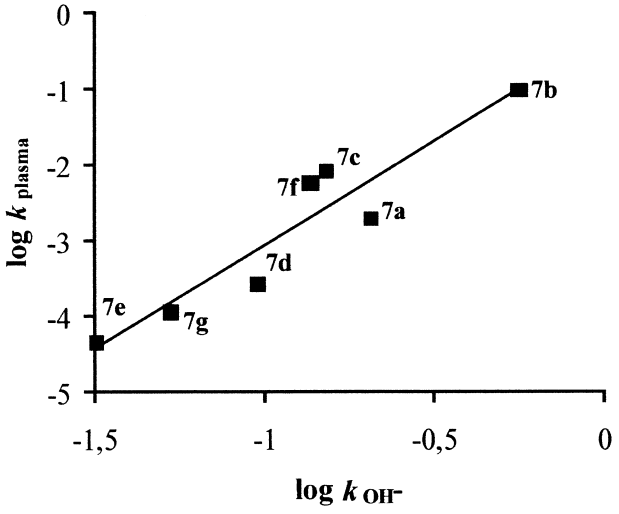


Figure 3. Plot of log k_{OH^-} *versus* log k_{plasma} for *N*-acyloxymethylsulfonamides **7a**–**g**. The data are from Table 3.

Table 4. Effect of eserine on the pseudo-first-order rate constants, k_{obs}, and half-lives, $t_{1/2}$, for the hydrolysis of **7f** in 80% human plasma at 37 °C

[Eserine] (μM)	k_{plasma} (10^{-5} s^{-1})	$t_{1/2}$ (min)
0	596	1.93
5	4.08	282
10	2.72	423
100	ND[a]	ND

[a]ND, no decomposition during 48 h.

Experimental

Elemental analyses were obtained from ITQB (Oeiras, Portugal) and from Medac (Egham, UK) laboratories. ^1H and ^{13}C NMR spectra were recorded as $CDCl_3$ solutions on a General Electric QE-300 or Jeol JNM-EX400 spectrometers using Me_4Si as internal standard; coupling constants, *J*, are quoted in Hz. FTIR spectra were recorded on a Nicolet Impact 400 spectrophotometer. Electron-impact ionisation (EI) and fast-atom bombardment (FAB) mass spectra were recorded on a VG Mass Lab 25–250 spectrometer. Melting points were determined using a Buchi 510 instrument and are uncorrected. HPLC was performed using a system comprising a Shimadzu LC-9A pump coupled to a variable wavelength Shimadzu SPD-6AV UV–vis detector, a 20 μl loop injection valve and a Merck LiChrospher® 100 RP-8 5 μm 125×4 mm column. The sulfonamides, amino acids, chloromethyl pivalate and chloromethyl chloroformate were purchased commercially. All chemicals were reagent grade except those for kinetic studies and HPLC, which were analytical or LiChrosolv (Merck) grade. Chloromethyl benzoate was prepared according to a literature method.[36] N^1-Acetylsulfisoxazole was a kind gift of Professor Maria J. Trigo (University of Oporto, Portugal).

4-Amino-*N*-benzoyloxymethyl-*N*-(3,4-dimethyl-3-isoxazolyl)benzenesulfonamide 7a. A solution of chloromethyl benzoate (1 mol equiv) in dry THF (1 mL) was added dropwise to a suspension of the sulfisoxazole, sodium salt (1 mol equiv) in dry THF (5 mL). When the reaction was complete, as indicated by TLC, the solvent was removed under vacuum and CH_2Cl_2 (20 mL) was added to the residue. The resulting solution was washed with sodium hydrogen carbonate, water and then evaporated to afford the crude **7a**, which was purified by column chromatography on silica gel (SiO_2) using diethyl ether as eluent; 44%; δ_H (ppm): 1.95 (3H, s, isox-Me), 2.23 (3H, s, isox-Me), 4.22 (2H, s, NH_2), 5.78 (2H, s, NCH_2O), 6.59–7.83 (9H, m, ArH); *m*/*z* 401 (M^+).

4-Amino-*N*-benzoyloxymethyl-*N*-(2-thiazolyl)benzesulfonamide 7b. Purified by column chromatography (SiO_2, diethyl ether:ethyl acetate (9:1)); crystallized from ethyl

ether; 52%; δ_H (ppm): 4.02 (2H, s, NH_2), 6.06 (2H, s, NCH_2O), 6.61–7.43 (11H, m, ArH); *m/z* 389 (M^+), 268 ($M-C_6H_5CO_2$).

4-Amino-*N*-benzoyloxymethyl-*N*-(2-pyrimidinyl)benzenesulfonamide 7c. Purified by column chromatography (SiO_2, diethyl ether:ethyl acetate (9:1)); 30%; δ_H (ppm) (d_6-DMSO): 6.21 (2H, s, NH_2), 6.44 (2H, s, NCH_2O), 7.18–8.64 (12H, m, ArH); *m/z* 369 ($M-NH_3$), 263 ($M-C_6H_5CO_2$).

4-Amino-*N*-benzoyloxymethyl-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide 7d. Purified by column chromatography (SiO_2, diethyl ether:ethyl acetate (9:1)); 58%; δ_H (ppm): 2.32 (6H, s, pyr-Me), 4.18 (2H, s, NH_2), 6.60 (2H, s, NCH_2O), 6.65–8.01 (10H, m, ArH); *m/z*: 291 ($M-C_6H_5CO_2$).

4-Amino-*N*-pivaloyloxymethyl-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide 7e. Purified by column chromatography (SiO_2, diethyl ether:ethyl acetate); 47%; δ_H (ppm): 1.15 (9H, s, CMe_3), 2.31 (6H, s, pyr-Me), 4.14 (2H, s, NH_2), 6.31 (2H, s, NCH_2O), 6.61–7.96 (5H, m, ArH); *m/z* 291 (M-Bu^tCO_2).

***N*-benzoyloxymethyl-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7f.** Purified by column chromatography (SiO_2, diethyl ether:petroleum b.p. 40–60 (7:3)); 29%; δ_H (ppm): 3.26 (3H, s, CH_3S), 5.98 (2H, s, NCH_2O), 6.98–8.01 (13H, m, ArH); *m/z*: 442 (M^+), 412 ($M-NO$), 321 ($M-C_6H_5CO_2$).

***N*-Pivaloyloxymethyl-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7g.** Purified by column chromatography (SiO_2, diethyl ether:petroleum b.p. 40–60 (7:3)); 23%; δ_H (ppm): 1.16 (9H, s, CMe_3), 3.20 (3H, s, CH_3S), 5.73 (2H, s, NCH_2O), 7.12–7.96 (8H, m, ArH); *m/z* 422 (M^+), 392 ($M-NO$), 321 (M-Bu^tCO_2).

***N*-{[(*S*)-(2-Carbomethoxypyrrolidin-1-yl)carbonyloxy]methyl}-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7h. Step A**. A solution of the (*S*)-proline methyl ester hydrochloride (11.9 mmol) and triethylamine (21.6 mmol) in CH_2Cl_2 (20 mL) was added to a stirred solution of chloromethyl chloroformate (10.8 mmol) in CH_2Cl_2 (25 mL) at −10 °C. After 25 min at −10 °C, the reaction was allowed to reach room temperature. The precipitate was filtered and the filtrate washed with water, then brine, and dried with magnesium sulfate. Evaporation of the solvent gave the corresponding pure *N*-[(chloromethyloxy)carbonyl]proline methyl ester **8h** (1.64 g, 68%); δ_H (ppm): 1.92–2.38 (4H, m, $ProCH_2$), 3.47–3.70 (2H, m, CH_2N), 3.74 and 3.75 (3H, 2×s, MeO), 4.36–4.43 (1H, m, α-CH), 5.69 and 5.88 (1H, 2×d, $ClCH_2O$, $J=6.0$), 5.74 (1H, s, $ClCH_2O$).

Step B. A solution of *N*-[(chloromethyloxy)carbonyl]-proline methyl ester **8h** (2 mmol) and NaI (4 mmol) in dry acetone (2 mL) was stirred at room temperature. This reaction was monitored by ^1H NMR, following the disappearance of the OCH_2Cl signals at δ 5.7 ppm and the appearance of the OCH_2I signals at δ 5.8 ppm (ca. 1 h). Filtration and evaporation of the solvent gave the corresponding *N*-[(iodomethyloxy)carbonyl]proline methyl ester **9h**. The sodium salt of nimesulide (**5**, 1 mmol) was added to a solution of *N*-[(iodomethyloxy)-carbonyl]proline methyl ester **9h** (1.1 mmol) in dry THF (3 mL) at room temperature. When the reaction was complete, as indicated by TLC, the solvent was removed under vacuum and CH_2Cl_2 (20 mL) was added to the residue. The resulting solution was washed with sodium hydrogen carbonate, water and then evaporated to afford the crude product **7h** which was purified by column chromatography on SiO_2 using diethyl ether: petroleum b.p. 40–60 (7:3) as eluent. Crystallized from ethyl ether:petroleum; 42%; δ_H (ppm): 1.79–2.31 (4H, m, $ProCH_2$), 3.22 and 3.16 (3H, 2×s, MeS), 3.32 and 3.60 (2H, m, CH_2N), 3.69 and 3.72 (3H, 2×s, MeO), 4.27–4.31 (1H, m, α-CH), 5.62 and 5.76 (1H, 2×d, NCH_2O, $J=11.7$ Hz), 5.68 and 5.79 (1H, 2×d, NCH_2O, $J=11.4$ Hz), 7.15–7.96 (8H, m, ArH); δ_C (ppm): 23.31 and 24.09 ($ProCH_2$), 29.67 and 30.78 ($ProCH_2$), 41.80 and 42.05 (MeS), 46.47 and 46.93 (CH_2N), 52.27 and 52.39 (MeO), 58.76 and 59.05 (CH), 74.12 and 74.13 (NCH_2O), 112.30 and 112.38 (ArCH), 117.78 and 117.84 (ArCH), 120.09 (ArCH), 125.78 (ArCH), 130.57 (ArCH), 133.04 and 133.23 (ArCH), 134.38 (ArC), 148.56 (ArC), 153.18 and 153.62 (NCO), 154.47 (ArC), 155.54 and 155.72 (ArC), 172.53 and 172.90 (OCO); *m/z* 493 (M^+), 463 ($M-NO$), 321 ($M-O_2CProOMe$).

***N*-{[(*N*′-Ethoxycarbonylmethyl-*N*′-methyl)aminocarbonyloxy]methyl}-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7i.** Purified by column chromatography (SiO_2, diethyl ether:petroleum b.p. 40–60 (7:3)); 24%; δ_H (ppm): 1.26 and 1.27 (3H, 2×t, CH_3CH_2, $J=7.2$ Hz), 2.93 and 2.96 (3H, 2×s, N-Me), 3.15 and 3.21 (3H, 2×s, CH_3S), 3.93 and 3.94 (2H, 2×s, CH_2N), 4.17 and 4.21 (2H, 2×q, CH_3CH_2, $J=7.2$ Hz), 5.71 and 5.75 (2H, 2×s, NCH_2O), 7.12–7.98 (8H, m, ArH); δ_C (ppm): 14.15 and 14.16 (CH_3CH_2), 35.45 and 36.05 (N–Me), 41.86 and 42.05 (MeS), 50.48 and 50.64 (CH_2N), 61.35 and 61.49 (CH_2O), 74.49 and 74.70 (NCH_2O), 112.36 and 112.42 (ArCH), 117.79 and 117.89 (ArCH), 120.16 (ArCH), 125.84 (ArCH), 130.62 (ArCH), 133.00 and 133.12 (ArCH), 134.28 and 134.32 (ArC), 148.46 (ArC), 153.45 and 153.47 (NCO), 155.02 (ArC), 155.58 and 155.71 (ArC), 168.98 and 169.12 (OCO); *m/z* 481 (M^+), 321 ($M-EtOCOCH_2N(Me)CO_2$).

***N*-{[(*N*′-Ethoxycarbonylmethyl)aminocarbonyloxy]methyl}-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7j.** Purified by column chromatography (SiO_2, diethyl ether: petroleum b.p. 40–60 (7:3)); 7%; δ_H (ppm): 1.28 (3H, t, CH_3CH_2, $J=7.2$ Hz), 3.21 (3H, s, CH_3S), 3.86 (2H, d, CH_2N, $J=5.4$ Hz), 4.20 (2H, q, CH_3CH_2, $J=7.2$ Hz), 5.27 (1H, t, NH, $J=5.4$ Hz), 5.75 (2H, s, NCH_2O), 7.14–7.96 (8H, m, ArH); *m/z* 467 (M^+), 321 ($M-EtOCOCH_2\ NHCO_2$).

***N*-{[(*S*)-(2-Carboxypyrrolidin-1-yl)carbonyloxy]methyl}-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7k.** A solution of **7h** (50 mg) and NaOH 1 M (1 mL) in acetonitrile–water (1:1 mixture, 2 mL) was stirred at room temperature for 1 h. The reaction mixture was acidified with HCl 1 M until pH 1 and then extracted with ethyl

acetate (2×10 mL). Evaporation of the solvent gave the pure **7k**; δ_H (ppm): 1.78–2.29 (4H, m, $ProCH_2$), 3.10 and 3.18 (3H, 2×s, MeS), 3.23–3.63 (2H, m, CH_2N), 4.22–4.29 (1H, m, α-CH), 5.60 and 5.73 (1H, 2×d, NCH_2O, $J=11.5$), 5.65 and 5.76 (1H, 2×d, NCH_2O, $J=11.2$), 7.10–7.95 (8H, m, ArH).

***N*-{[(*N*′-Carboxymethyl-*N*′-methyl)aminocarbonyloxy]-methyl}-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7l.** Prepared from **7i**; δ_H (ppm): 2.86 and 2.90 (3H, 2×s, N–Me), 3.09 and 3.14 (3H, 2×s, CH_3S), 3.87 and 3.88 (2H, 2×s, CH_2N), 5.66 and 5.70 (2H, 2×s, NCH_2O), 7.01–7.92 (8H, m, ArH); *m*/*z* (FAB^-): 452 (M−H), 307 (**5**-H).

***N*-{[(*N*′-Carboxymethyl)aminocarbonyloxy]methyl}-*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide 7m.** Prepared from **7j**; δ_H (ppm): 3.19 (3H, s, MeS), 3.89 (2H, d, $glyCH_2$), 5.10 (1H, brs, NH), 5.72 (2H, s, NCH_2O), 7.06–7.92 (8H, m, ArH); *m*/*z* (FAB-) 438 (M−H), 307 (**5**−H).

Kinetic measurements

Reactions were monitored using HPLC, following either the loss of substrate or the formation of products. The ionic strength was maintained at 0.5 M with $NaClO_4$. Reactions were initiated by injecting ca. 50 μL of the appropriate substrate stock solution to 5 mL of the buffer solution. At regular intervals, samples of the reaction mixture were analysed using acetonitrile–water containing 0.2 M sodium acetate buffer (55:45 to 70:30%) as mobile phase, and a 1.0 mL min^{-1} flow. Quantitation of the prodrug and corresponding parent drug was obtained from measurements of the peak areas in relation to those of corresponding standards chromatographed under the same conditions.

Plasma studies

The hydrolyses of prodrugs in human plasma were studied by the HPLC method described above. Plasma was obtained from heparinised blood of healthy donors, pooled, and frozen at −70 °C before use. The prodrugs were incubated at an initial concentration of 5×10^{-5} M at 37 °C in human plasma diluted to 80% (v/v) with pH 7.4 isotonic phosphate buffer. At appropriate intervals, 200 μl aliquots were added to 400 μl of acetonitrile in order to quench the reaction and deproteinise the plasma. These samples were centrifuged for 5 min at 13,000 rpm and the supernatant was analysed by HPLC for the presence of the substrate and parent drug.

Partition coefficients

These were determined in octanol–pH 7.4 phosphate buffer at 22 °C. Each phase was mutually saturated before the experiment. The volumes of each phase were chosen so that solute concentration in the aqueous phase after distribution could readily be measured. The compounds were dissolved in octanol and the octanol–pH 7.4 phosphate mixtures were shaken for 30 min to reach an equilibrium distribution; each phase was analysed separately by the HPLC method described above. The partition coefficients, *P*, were calculated from the ratio of the peak area in octanol to the peak area in buffer.

Computations

Molecular refractivities, MR, were calculated with the software ChemSketch v.3.0 from Advanced Chemistry Development Inc. Calculation of the kinetic parameters from eq (1) was performed using Excel® (Microsoft) and the Solver option.

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