Full Paper

Unanticipated Acyloxymethylation of Sumatriptan Indole Nitrogen Atom and its Implications in Prodrug Design

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Sumatriptan is a potent and selective 5-HT_{1B} and 5-HT_{1D} agonist used in the symptomatic treatment of migraine; it shows poor oral bioavailability ascribed, in part, to its low lipophilicity. In an attempt to develop acyloxymethyl prodrugs of sumatriptan suitable for oral administration, we carried out the reaction of sumatriptan with chloromethyl esters. To our surprise, acyloxymethylation occurred preferentially at the indole nitrogen rather than at sulfonamide nitrogen, reflecting a difference either in product stability or in the nucleophilicities of the indole and sulfonamide anions. The hydrolysis of the corresponding N^1-acyloxymethyl derivatives was studied in aqueous buffers and in human plasma, by HPLC. N^1-Acyloxymethyl derivatives of sumatriptan are rapidly hydrolysed to the chemically stable N^1-hydroxymethylsumatriptan at pH 1-13. Slow formation of the parent drug was observed only at high pH values. Hydrolysis of sumatriptan derivatives is slower in human plasma than in phosphate buffer and also generates N^1-hydroxymethylsumatriptan rather than the parent drug. These results indicate that N^1-acyloxymethyl derivatives of sumatriptan cannot be considered as true prodrugs of sumatriptan.

Keywords: Acyloxymethylation / Indole / Prodrugs / Sumatriptan

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Introduction

Sumatriptan **1** is a potent and selective serotonin (5-HT) agonist that activates 5-HT_{1B} and 5-HT_{1D} subtype receptors used in the symptomatic treatment of migraine [1]. Although representing a significant achievement in migraine treatment over ergot alkaloids [2], sumatriptan presents several limitations, particularly in its pharmacokinetic profile, which may be ascribed to its low lipophilicity (log P = 0.93) and first-pass effect through the action of monoamine oxidase type A (MAO-A) [1, 3 – 6]. For example, sumatriptan exhibits poor oral bioavailability (14%), reaches peak plasma drug concentration (C_{max}) at 2.5 h and has a short half-life ($t_{1/2}$ of 2 h) [2]. In addition, sumatriptan appears to cross the blood-brain barrier only slowly and its distribution in the CNS is poor [2]. Moreover, *in-vivo* studies also suggest substantial inter-individual variability in plasma concentrations after oral administration, reflected by the fact that approximately 30% of patients report inadequate relief or do not respond to oral sumatriptan and a significant number of patients are prone to headache recurrence [5]. In order to address these shortcomings of sumatriptan, alternative drug formulations, such as subcutaneous injection, have been developed [2, 7]. Subcutaneous administration is the most effective and fastest-acting route of administration, but the resulting adverse event profile is higher than that of oral sumatriptan [2, 7].

The prodrug approach is widely used to improve drug delivery properties of numerous therapeutic agents by covalently attaching a pro-moiety to the parent drug that can be released *in vivo*, either chemically or enzymatically [8, 9]. Sumatriptan contains a secondary sulfonamide group and an indole group that are amenable to derivatisation into a prodrug. Only a few successful pro-

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Abbreviation: density functional theory (DFT)

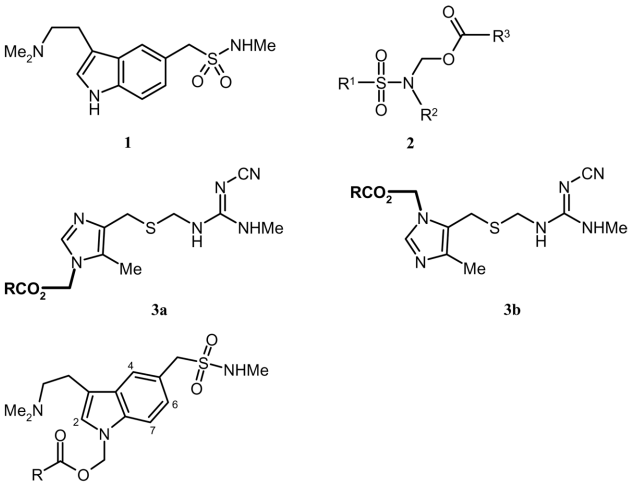


Figure 1. Structures of compounds **1 – 4**.

drugs for secondary sulfonamide agents have been reported [9 – 16]. From these, *N*-acyloxymethylsulfonamides **2** emerged as potential prodrugs for both secondary sulfonamides and carboxylic acid agents, displaying low reactivity in aqueous buffers yet being rapidly activated to the parent drug by human esterases [9, 15]. In contrast, prodrug derivatives for the indole moiety have been sparsely reported [17]. However, acyloxymethylation of the related imidazole NH group has been used to prepare *N*-acyloxymethyl derivatives of cimetidine **3** [18]. Both, cimetidine prodrug tautomers, **3a** and **3b**, are rapidly activated to the parent drug in human plasma [18].

Considering the potential utility of *N*-acyloxymethylation in prodrug synthesis, it was therefore necessary to assess the applicability of this approach to sumatriptan. Herein, we report that *N*-acyloxymethylation of sumatriptan with chloromethyl esters occurs preferentially at the indole nitrogen rather than at sulfonamide nitrogen, leading to derivatives **4**. A kinetic study designed with the objective of evaluating the influence of the acyl carrier on the chemical reactivity of *N*-acyloxymethyl derivatives **4** in aqueous buffers and in human plasma is also presented. The mechanism of hydrolysis of the sumatriptan derivatives **4** and its implication in prodrug design are discussed. The structures of compounds **1 – 4** are presented in Fig. 1.

Results and discussion

Synthesis

Compounds **4** were synthesized, albeit in moderate yields, by reacting sumatriptan with the appropriate chloromethyl ester and sodium hydride in DMF. The structural assignment of derivatives **4** is based on several spectroscopic data. A characteristic feature of the ^{1}H-NMR spectra for these compounds is the resonance of the NCH_2O group that appears as a singlet at 6.2 – 6.4 ppm, which is similar to that for the previously reported *N*-protected indole, *N*-pivaloyloxymethylindole, *i.e.* 6.1 ppm [19], but shifted downfield when compared to those of *N*-acyloxymethyl-*N*-methylsulfonamides (ca. 5.5 ppm) [9, 15, 20]. The carbon chemical shifts for the NCH_2O signal of **4** are also similar to that of *N*-pivaloyloxymethylindole, *i.e.* 68 ppm [19]. Further confirmation that acyloxymethylation occurred at the indole moiety came from the signal of the sulfonamide *N*-methyl group, which appears as a doublet at ca. 2.5 ppm. Finally, nuclear Overhauser enhancement spectroscopy (NOESY) experiments revealed interactions through space between the NCH_2O protons with C-2 and C-7 indole protons.

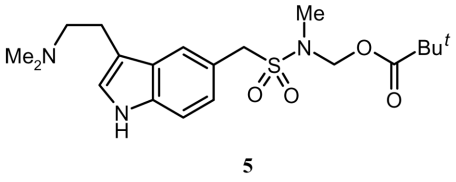


Figure 2. Structure of compound **5**.

The preferential acyloxymethylation of the sumatriptan indole over the sulfonamide is somewhat surprising, taking into account the acidity differences between the two NH groups in non-aqueous solvents (for example, the pK_a values in DMSO for indole and $MeSO_2NH_2$ are 20.96 and 17.5, respectively [21]). This difference would imply the major anion present in solution is the sulfonamide anion, by at least a ratio of 1000 : 1. That acyloxymethylation occurs at the indole nitrogen atom must be attributable to one (or both) of two factors (i) the greater nucleophilicity of the indole anion, and (ii) the greater stability of the substituted indole product. In an attempt to gain insight into the factors that affect the acyloxymethylation of sumatriptan, the energies for compound **4a** and its *N*-acyloxymethylsulfonamide counterpart **5**, (Fig. 2) were calculated using density functional theory (DFT). The energy-minimized structures of **4a** and **5** are depicted in Fig. 3. The DFT calculations indicate, that compound **4a** is ca. 7.5 kJ mol^{-1} more stable than its counterpart **5**. Thus, substitution at the indole nitrogen in the anion of **1** appears to be both kinetically and thermodynamically driven.

Hydrolysis in aqueous buffers

The hydrolysis of *N*-acyloxymethyl sumatriptan derivatives **4** in aqueous buffers proceeds with quantitative

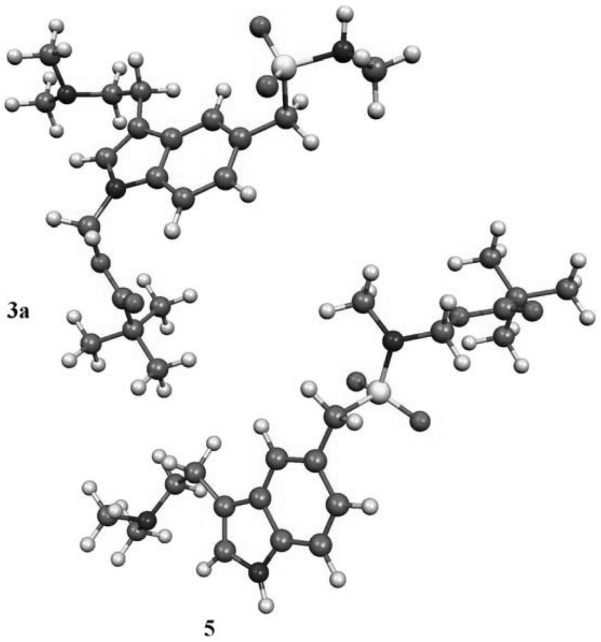
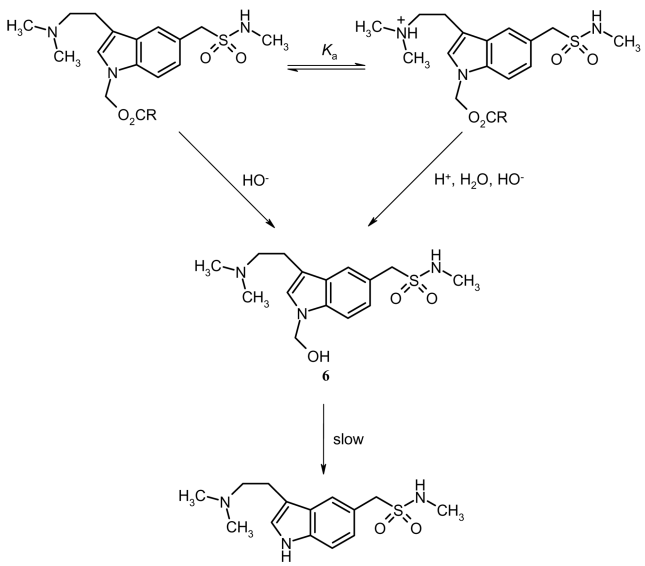


Figure 3. Geometries of **4a** and **5** using B3LYP/6-31+G(d,p).



Scheme 1. Hydrolysis of *N*-acyloxymethyl sumatriptan derivatives **4** in aqueous buffers.

formation of *N*-hydroxymethyl sumatriptan (**6**, Scheme 1) in the pH range 1 to 10, as revealed by HPLC and ESI-MS analysis of reaction mixtures. For example, Fig. 4 depicts the time course for the hydrolysis of compound **4a** at pH 7.2. Above pH 10, slow conversion of **6** into sumatriptan was observed. These results reflect the low nucleofugality of the resulting indole anion (a pK_a of 16 in aqueous solution can be calculated for the dissociation of the sumatriptan indole NH group [22]) and are

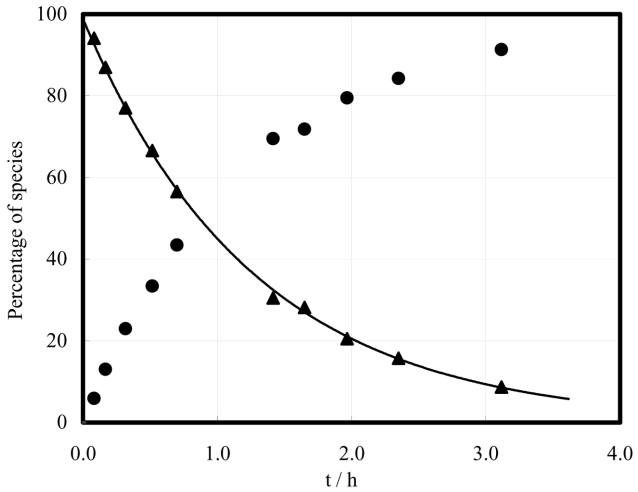
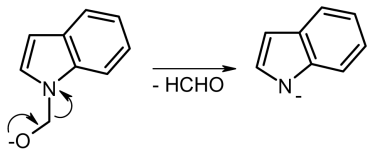


Figure 4. Time courses for *N*-pivaloyloxymethylsumatriptan **4a** (▲) and *N*- hydroxymethylsumatriptan (●) in the hydrolysis of **4a** at 37°C in pH 7.2 phosphate buffer.



Scheme 2. Loss of formaldehyde from indole anion via E1cb-type mechanism.

consistent with loss of formaldehyde from **6** occurring via an E1cb-type mechanism (Scheme 2) [23]. The stability displayed by **6** is consistent both with that of the indole *N*-hydroxymethyl derivative of the NK_1 receptor antagonist PD154075 (5% decomposition over 4 d at pH 9) [17] and with the observation of Buur *et al*. that the rate of decomposition of *N*-hydroxymethyl derivatives of amides and imides increases sharply with the acidity of the parent NH-acidic compound, as indicated by a Bronsted β_{lg} value of −0.8 [24]. Using this structure-reactivity relationship and a pK_a of 16 for the indole NH [22], a half-life of ca. 40 days can be calculated for the decomposition of *N*-hydroxymethylsumatriptan **6** in pH 7.4 buffer at 37°C, a value consistent with the lack of decomposition of **6** at this pH.

The pseudo-first-order rate constants, k_{obs}, for the hydrolysis of derivatives **4a, b** were determined in HCl and NaOH solutions as well as aqueous buffers using several acetate-, phosphate- and borate-buffer concentrations. While pivaloyloxymethyl derivative **4a** was studied at 25°C, its 4-methoxybenzoyloxymethyl counterpart **4b** was studied at 15°C and between pH 1 and pH 9.5, due to its higher reactivity. For both compounds, no significant dependence of hydrolysis rates on buffer concentration

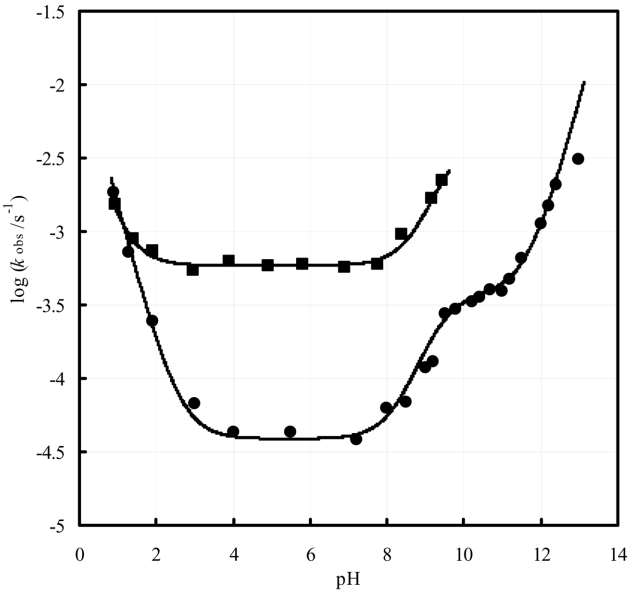


Figure 5. pH-Rate profiles for compounds **4a** at 25°C (•) and **4b** at 15°C (■).

was observed over a 7- to 10-fold buffer concentration range.

The influence of pH on the overall rates of hydrolysis of *N*-acyloxymethylsumatriptan derivatives **4** is presented in Fig. 5, where the logarithm of the k_{obs} values is plotted against the pH. The pH-rate profile for **4a** is sigmoidal, reflecting protonation of the dimethylamino group. The best computer fit (solid line) to the experimental data for **4a** in Fig. 5 was achieved using Equation (1) and is consistent with reaction pathway presented in Scheme 1.

$$k_{obs} = (k_0 + k_{H^+}[H^+ + k_{HO^-}[HO^-]) \frac{[H^+]}{k_a + [H^+]}$$

$$+(k'_{HO^-}[HO^-]) \frac{K_a}{k_a + [H^+]} \quad (1)$$

Equation 1. pH-Rate expression of hydrolysis of *N*-acyloxymethylsumatriptan derivative **4a**.

In Equation (1), where $[H^+]/(K_a + [H^+])$ and $K_a/(K_a + [H^+])$ are the fractions of the protonated and non-protonated forms of compound **4a**, respectively, K_a is the apparent ionisation constant, k_0 is the first-order rate-constant for the non-catalysed hydrolysis of the protonated form, k_{H^+} is the second-order rate-constant for the specific acid catalysed hydrolysis of the protonated form and k_{HO^-} and k'_{HO^-} are the second-order rate-constants for the specific base-catalysed hydrolysis of the protonated and non-protonated forms, respectively. The values derived are listed in Table 1. The good agreement between the calculated and

Table 1. Rate data for the hydrolysis of *N*-acyloxymethylsumatriptan derivatives **4a**, at 25°C, and **4b**, at 15°C, with ionic strength maintained at 0.5 M with addition of $NaClO_4$. The pK_a value for **4a** was determined from the pH-rate profile.

	4a	**4b**
k_0/s^{-1}	4.33×10^{-5}; 1.88×10^{-4} [a]; 4.32×10^{-4} [b]; 7.13×10^{-4} [c]	5.86×10^{-4}
k_{H^+}/$M^{-1}s^{-1}$	1.42×10^{-2}; 5.15×10^{-3} [d]; 2.63×10^{-2} [e]; 6.15×10^{-2} [a]	6.89×10^{-3}
k_{HO^-}/$M^{-1}s^{-1}$	20.5	90.4
k'_{HO^-}/$M^{-1}s^{-1}$	7.85×10^{-2}	–
pK_a	9.26	–

[a] 37°C.
[b] 45°C.
[c] 50°C.
[d] 15°C.
[e] 30°C.

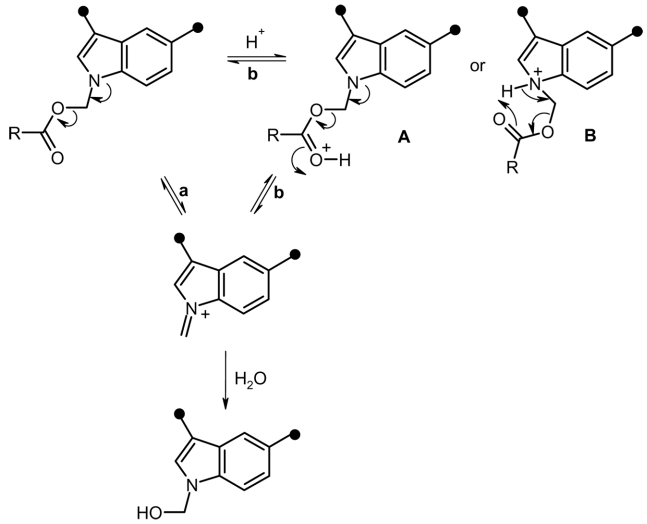
experimentally determined first-order rate constants suggests that the degradation pathway presented in Scheme 1 and Eq. (1) adequately describes the degradation kinetics of compound **4a**. The kinetically determined pK_a value is 9.26, which is close to, but lower than that for ionisation of the dimethylamino group in sumatriptan (pK_a 9.63 [25]), and thus is also consistent with incorporation of the acyloxymethyl moiety at the indole nitrogen atom.

The pH-rate profile for derivative **4b** (Fig. 5) is U-shaped with a broad plateau from pH 3 to pH 8, indicating the occurrence of specific acid and base catalysis as well as a non-catalysed reaction according to the rate expression shown in Eq. (2), where k_0, k_{H^+} and k_{HO^-} (Table 1) have the usual meaning and refer to the hydrolysis of the protonated form of **4b**. The higher reactivity of **4b** is such that we were unable to follow reactions by the HPLC method at pH values greater than 9, so were unable to detect reaction of the unprotonated form of this compound.

$$k_{obs} = k_0 + k_{H^+}[H^+] + k_{HO^-}[HO^-] \quad (2)$$

Equation 2. pH-Rate expression of hydrolysis of *N*-acyloxymethylsumatriptan derivative **4b**.

Inspection of the data in Table 1 indicates that the protonated form of compound **4b** hydrolyses via the pH-independent pathway at a rate ca. 25 times faster than its pivaloyloxymethyl counterpart **4a** (assuming that k_0 for **4b** increases two times when temperature increases 10°C). Such difference in reactivity, together with the unusually high k_0 values displayed by compounds **4** when compared with analogous esters derived from simple aliphatic alcohols (usually in the range of 10^{-10} to 10^{-7} s^{-1} at 25°C; [26]), is consistent with an S_N1-type mechanism that



Scheme 3. Hydrolysis mechanism explained in the text.

involves departure of the carboxylate leaving group in the rate-limiting step, followed by trapping of the resulting iminium ion by water (Scheme 3, path a). According to this mechanism, which has been reported for a wide range of *N*-acyloxymethyl derivatives of weakly NH-acidic compounds such as amides [27–29] and sulfonamides [9, 15, 20], the rates of hydrolysis increase with acidity of the carboxylic acid (the pK_as for pivalic acid and 4-methoxybenzoic acid are 5.04 and 4.51, respectively [22]). Further support for the unimolecular mechanism of the pH-independent hydrolysis of compounds **4** comes from the temperature dependence of k_0 for **4a** (Table 1), which yielded an entropy of activation, $\Delta S^{\ddagger}$, value of -35.9 J K^{-1} mol^{-1}. Despite being negative, this $\Delta S^{\ddagger}$ value is close to zero and thus consistent with a unimolecular mechanism [15, 30].

In contrast to the pH-independent region, the difference in reactivity between **4a** and **4b** in the specific-acid catalysed region is negligible. The temperature dependence of k_{H^+} for **4a** (Table 1) yielded an $\Delta S^{\ddagger}$ value of -7.73 J K^{-1} mol^{-1}. This value is also consistent with a dissociative mechanism, identical to that of the pH-independent pathway, other than an extra step involving protonation of the substrate at the ester or indole moieties prior to iminium ion formation (Scheme 3, path b). Protonation at the carbonyl oxygen atom (Scheme 3, **A**) and iminium-ion formation have opposite electronic requirements, and thus the overall effect exerted by the acyloxymethyl moiety on the rate of hydrolysis is expected to be very small, an outcome consistent with the small reactivity difference between **4a** and **4b** for the acid-catalysed pathway. Alternatively, *N*-protonation of the indole (Scheme 3, **B**) is likely to be a fast process [31], but it would imply a strained six-membered transition state for proton transfer.

Table 2. Pseudo-first-order rate constants, k_{obs}, and half-lives, $t_{1/2}$, for the hydrolysis of N^1-acyloxymethyl derivatives of sumatriptan, **4**, in isotonic phosphate buffer solution at pH 7.4 and 80% human plasma at 37°C.

Compound	pH 7.4 Buffer		80% Human plasma	
	$10^4 k_{obs}$/s^{-1}	$t_{1/2}$/min	$10^4 k_{obs}$/s^{-1}	$t_{1/2}$/min
4a	2.10	58	1.83	65
4b	22.3	5.2	8.05	15

Hydrolysis in human plasma

The susceptibility of the sumatriptan derivatives **4** to undergo enzyme-catalysed hydrolysis was studied *in vitro* at 37°C in pH 7.4 isotonic phosphate buffer containing 80% human plasma. Under the experimental conditions used, the reaction displayed first-order kinetics for at least four half-lives and led to the quantitative formation of *N*-hydroxymethylsumatriptan **6**. The observed half-lives for the hydrolysis in human plasma together with those of hydrolysis in pH 7.4 phosphate buffer are presented in Table 2. From these data, it is possible to conclude that hydrolysis of compounds **4** is slower in human plasma than in phosphate buffer. This might be ascribed to strong binding of compounds **4** to non-catalytic proteins, thus preventing their hydrolysis, an effect which has been also reported for other acyloxymethyl derivatives of NH-acidic compounds [28] as well as for several alkyl esters [32].

Conclusion

In conclusion, classical *N*-acyloxymethylation of sumatriptan leads to alkylation of the indole nitrogen (N^1) atom. The N^1-acyloxymethyl derivatives of sumatriptan are rapidly hydrolysed, both in aqueous buffers and in human plasma, to the corresponding alcohol (N^1-hydroxymethylsumatriptan), which is stable in neutral and slightly alkaline solutions. Thus, N^1-acyloxymethyl derivatives of sumatriptan cannot be considered as true prodrugs of sumatriptan. These results indicate that future prodrug development for sumatriptan and other indole-containing drugs based on the derivatisation of indole NH group, must take in account the activation chemistry and whether this can lead to stable N^1-hydroxymethylsumatriptan.

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The authors have declared no conflict of interest.

Experimental

General procedures

^{1}H- and ^{13}C-NMR, COSY, HMQC and NOESY spectra were recorded as $CDCl_3$ or d_6-DMSO solutions on a Bruker AM 400 WB (Bruker Bioscience, Billerica, MA, USA); chemical shifts are given in ppm and coupling constants, *J*, are quoted in Hz. The LC – MS system consisted of a Waters 2695 Separation Module, Waters 2996 photodiode array detector (Waters Corporation, Milford, MA, USA), Atlantis dC18 5 μm (2.1 × 150 mm) column (Waters) and a Micromass Quattro Micro API spectrometer (Micromass, Manchester, UK). The high-resolution mass spectra (HRMS) were obtained on a Bruker MicroTOF ESI-TOF MS system (Bruker). FTIR spectra were recorded on a Nicolet Impact 400 spectrophotometer (Nicolet, Madison, WI, USA). Melting points were determined using a Bock Monoscop M. Instrument and are uncorrected. HPLC was performed using a system comprising a Merck Hitachi LaChrom L-7100 pump (Merck, Darmstadt, Germany) coupled to a Shimadzu SPD-6AV UV-vis detector (Shimadzu, Tokyo, Japan)), a Rheodyne 10 mL injector (Rheodyne Europe, Alsbach, Germany), a Merck Hitachi D-2500A integrator, and a Merck LiChrospher® 100 RP-8 5 μm 125 × 4 mm column. Water was distilled and deionized using a Millipore apparatus (Millipore Iberica S.A.U., Madrid, Spain). All chemicals used were of reagent grade, without further purification, except those for kinetic and HPLC studies, which were of analytical or LiChrosolv® (Merck) grade. Sumatriptan succinate Ph. Eur. was obtained from SMS Pharmaceuticals Limited (Hyderabad, India).

Procedure for obtaining neutral sumatriptan

Sumatriptan succinate was dissolved in water and sodium hydrogen carbonate was added until pH 9.5. Sumatriptan was then extracted with ethyl acetate. Crystallisation from absolute ethanol afforded **1** as a pale yellow powder; Mp. 169 – 171°C. IR ν_{max}/cm^{-1}: 3354, 1312, 1122. ^{1}H-NMR δ_H (d_6-DMSO): 2.24 (6H, s, N*Me*$_2$), 2.51 (2H, t, *J* = 7.5, C*H*$_2$Ar), 2.55 (3H, d, *J* = 4.8, N*Me*), 2.82 (2H, t, *J* = 7.5, NC*H*$_2$), 4.31 (2H, s, C*H*$_2$SO$_2$), 6.79 (1H, q, *J* = 4.8, N*H*), 7.06 (1H, d, *J* = 8.4, Ar*H*), 7.11 (1H, s, Ar*H*), 7.30 (1H, d, *J* = 8.4, Ar*H*), 7.49 (1H, s, Ar*H*), 10.78 (1H, s, N*H* (Ar)). ^{13}C-NMR δ_C (d_6-DMSO): 23.0 (*C*H$_2$Ar), 28.9 (NC*H*$_3$), 45.1 (*Me*$_2$N), 56.7 (*C*H$_2$SO$_2$), 59.9 (NC*H*$_2$CH$_2$), 111.1 (*C*H (Ar)), 112.6 (*C*CH$_2$SO$_2$), 119.4 (CH$_2$*C*H$_2$C), 120.6 (*C*H (Ar)), 122.9 (*C*H (Ar)), 123.6 (*C*H (Ar)), 127.2 (C_{quat} (Ar)), 135.9 (C_{quat} (Ar)).

Procedure for the synthesis of N-acyloxymethylsumatriptan derivatives

To a solution of sumatriptan (1 mol eq.) in anhydrous DMF (1 mL/sumatriptan mol) sodium hydride was added (1.1 mol eq.). When liberation of hydrogen was complete, a solution of the appropriate chloromethyl ester [33] was added and the mixture left reacting at room temperature. When the reaction was complete (TLC) the solvent was removed under vacuum. Dichloromethane (50 mL) was added to the residue and the resulting solution washed with water and then evaporated to afford crude **4** which was subjected to column chromatography on silica gel using dichloromethane/methanol (4 : 1) as eluent. Subsequent recrystallisation from dichloromethane and ethyl acetate afforded compounds **4**. Alternatively, the sodium salt of sumatriptan was prepared directly from sumatriptan succinate (1 mol eq.) with sodium hydride (3 mol eq.).

*N^1-Pivaloyloxymethylsumatriptan **4a***

White powder, yield: 56%, mp. 161 – 164°C. IR ν_{max}/cm^{-1}: 3432, 1737, 1317, 1132. ^{1}H-NMR δ_H (d_6-DMSO): 1.08 (9H, s, C*Me*$_3$), 2.39 (6H, s, NC*H*$_3$), 2.59 (3H, d, *J* = 4.8, NC*H*$_3$), 2.74 (2H, t, *J* = 7.6, C*H*$_2$Ar), 2.88 (2H, t, *J* = 7.6, NC*H*$_2$), 4.38 (2H, s, C*H*$_2$SO$_2$), 6.15 (2H, s, NC*H*$_2$O), 6.87 (1H, q, *J* = 4.8, N*H*Me), 7.21 (1H, d, *J* = 8.4, Ar*H*), 7.32 (1H, s, Ar*H*), 7.53 (1H, d, *J* = 8.4, Ar*H*), 7.56 (1H, s, Ar*H*). ^{13}C-NMR δ_C (d_6-DMSO): 22.8 (*C*H$_2$Ar), 26.9 (C*Me*$_3$), 29.7 (NC*H*$_3$), 38.9 (*C*Me$_3$), 45.0 (*Me*$_2$N), 57.7 (*C*H$_2$SO$_2$), 59.4 (NC*H*$_2$CH$_2$), 68.6 (NC*H*$_2$O), 110.0 (*C*H, Ar), 114.5 (*C*CH$_2$SO$_2$), 121.3 (CH$_2$*C*H$_2$C), 121.4 (*C*H, Ar), 125.1 (*C*H, Ar), 126.3 (*C*H, Ar), 128.9 (*C*, Ar), 136.5 (*C*, Ar), 178.2 (*C*=O). MS m/z (MW) = 409.5275 (Calcd. 409.5498).

*N^1-(4-Methoxy)benzoyloxymethylsumatriptan **4b***

Yellow amorphous solid, yield 59%, mp. 125–128°C. IR ν_{max}/cm^{-1}: 3420, 1712, 1321, 1170. ^{1}H-NMR δ_H (d_6-DMSO): 2.45 (6H, s, NC*H*$_3$), 2.58 (3H, d, *J* = 4.8, NC*H*$_3$), 2.84 (2H, t, *J* = 6.5, C*H*$_2$Ar), 2.91 (2H, t, *J* = 6.5, NC*H*$_2$), 3.81 (3H, s, OC*H*$_3$), 4.40 (2H, s, C*H*$_2$SO$_2$), 6.39 (2H, s, NC*H*$_2$O), 6.85 (1H, q, *J* = 4.8, N*H*Me), 7.02 (2H, d, *J* = 8.8, Ar*H*) 7.23 (1H, d, *J* = 8.4, Ar*H*), 7.43 (1H, s, Ar*H*), 7.58 (1H, s, Ar*H*), 7.67 (1H, d, *J* = 8.4, Ar*H*), 7.87 (2H, d, *J* = 8.8, Ar*H*). ^{13}C-NMR δ_C (d_6-DMSO): 22.4 (*C*H$_2$Ar), 29.5 (NC*H*$_3$), 44.5 (*Me*$_2$N), 55.2 (O*Me*), 57.3 (*C*H$_2$SO$_2$), 58.9 (NC*H*$_2$CH$_2$), 68.4 (NC*H*$_2$O), 110.0 (*C*H, Ar), 113.1 (*C*CH$_2$SO$_2$), 121.2 (CH$_2$*C*H$_2$C), 121.4 (*C*H, Ar), 125.1 (*C*H, Ar), 126.8 (*C*H, Ar), 128.7 (*C*, Ar), 136.3 (*C*, Ar), 165.6 (*C*=O). MS m/z (MW) = 459.5801 (Calcd. 459.5663).

Hydrolysis in aqueous buffers and ESI-MS experiments

The kinetic studies were carried out using HPLC, following the loss of substrate and formation of *N*^{1}-hydroxymethylsumatriptan at a wavelength of 230 nm, at different temperatures. The ionic strength of the buffers was maintained at 0.5 M using $NaClO_4$. In a typical run, a reaction was initiated by adding 5 μL aliquot of a 10^{-3} M stock solution of substrate in acetonitrile to an Eppendorf containing 495 μL of thermostated buffer, resulting in a final concentration of 10^{-5} M. At regular intervals, samples of the reaction mixture were analysed by direct injection, except for solutions of pH above 11.5 in which they were previously neutralized with HCl. The mobile phase consisted of acetonitrile / aqueous buffer (sodium hexanesulfonate 10 mM, phosphoric acid 2.5 mM, sodium acetate 2.5 mM) in 60 : 40 (v/v) with a 1.0 mL min^{-1} flow. Pseudo-first-order rate constants for the hydrolytic degradation were determined from the slope of linear plots of ln (peak area) *vs*. time. Hydrolysis in 0.01 M pH 7.4 PBS was carried out at 37°C as described above.

For selected reactions of **4a**, aliquots were analysed by LC-ESI-MS in positive mode (collision energy 20 V) , which revealed the disappearance of substrate (m/z 410, 14%, MH$^+$; m/z 308, 100%, MH$^+$-OCOCMe$_3$) and formation of *N*^{1}-hydroxymethylsumatriptan (m/z 326, 32%, MH$^+$; m/z 296, 11%, MH$^+$-CH$_2$OH; m/z 281, 100%, MH$^+$-NHMe$_2$; m/z 187, 37%, MH$^+$-NHMe$_2$-SO$_2$NHMe; m/z 58, 18%, $CH_2NMe_2^+$).

Hydrolysis in human plasma

Compounds **4** were incubated at an initial concentration of 5×10^{-5} M at 37°C in human plasma [28] 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 deproteinize the plasma. The samples were centrifuged 15 000 rpm and the supernatant was analysed by HPLC for the presence of the substrate and product of hydrolysis.

Computational methodology

Density functional theory (DFT) [34, 35] calculations were carried out with the Gaussian 03 suite of programs [36]. The structures of **4a** and **5** were optimized without symmetry constraints using the B3LYP hybrid functional, which is a combination of the Becke's three-parameter (B3) exchange functional [37] with the Lee, Yang, and Parr (LYP) correlation functional [38], in conjunction with B3LYP/6-31+G(d,p) [39, 40] basis set. Frequency calculations confirmed that all the geometries found were minima on the respective potential energy surfaces. In the evaluation of data, the zero-point and thermal energy corrections were added to the minimum electronic energies.

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