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β-Aminoketones as prodrugs with pH-controlled activation

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Abstract

N-Mannich bases have been widely applied as prodrugs of amine drugs. The analogous C-Mannich bases (β-aminoketones) have received rather less attention probably because they are not sufficiently susceptible to elimination at pHs encountered in vivo. Compounds in which there is a thermodynamic advantage to elimination may be an exception. In this study, the physicochemical characteristics of a series of Michael amino addition adducts of chalcone and other carbonyl compounds is explored. The ketone adducts rapidly eliminate at around pH 7.4 ($t_{1/2} < 15$ min) releasing the amine and the ketone but they are stable under acidic conditions. The Michael adducts are more lipophilic than the parent amines and have significantly suppressed ionisation characteristics at biologically relevant pH values.

Keywords: Prodrugs; Aminoketones; pH activated

1. Introduction

Mannich bases (Fig. 1) have been extensively studied as prodrug systems for amine, amide and imide drugs (Testa and Meyer, 2003). These compounds contain an X–CH_2–N fragment (where X– can be C–, O–, S– or N–) which undergoes a non-enzymatic cleavage at blood pH liberating the N- and X-containing fragments, along with formaldehyde in the case of N-Mannich bases (Fig. 1-path 1). Relative to their parent compounds, Mannich bases may have enhanced oral bioavailability (Bundgaard et al., 1982), increased water solubility (Bundgaard and Johansen, 1980) or increased skin permeation (Koch and Sloan, 1987). One clinically used Mannich base is rolitetracycline (Pitman, 1981), a water-soluble prodrug of tetracycline: hetacillin, another clinically used compound, may be regarded as a cyclic Mannich base of ampicillin (Tsuji and Yamana, 1974). Notwithstanding these and other promising programs, the approach has not met early expectations. Enthusiasm for N-Mannich bases is tempered by concerns about their stability in vitro, even under acidic conditions, and the effects of their typical formaldehyde by-product in vivo (Testa and Meyer, 2003), although the latter characteristic can be harnessed productively (e.g., Burke and Koch, 2004).

Relative to their O-, N-, S-Mannich base analogues, C-Mannich bases (β-aminoketones) have not received much attention as prodrug types. C-Mannich bases have at least the merit of not releasing formaldehyde, since fragmentation occurs by amine elimination (Fig. 1-path 2), rather than deaminomethylation. Moreover, the C-Mannich bases are likely to be more stable under acidic conditions, though it seems that they may not be sufficiently reactive at higher pH to be useful as prodrugs. Indeed, what little work there has been in this area has focussed on their potential application as prodrugs of unsaturated ketones, through elimination of the amino component (and not on the amino component as the drug). For example, some anti-microbial and cytotoxic C-Mannich bases have had their activity attributed to α,β-unsaturated ketones derived from them (Gul et al., 2002). Bis-Mannich bases of acrylophenones yield the active enone compound by deamination under simulated physiological conditions (Dimmock et al., 1987). In contrast, mono amino Mannich bases derived from acetophenone are reported to be stable (Dimmock et al., 1983).

We have recently reported that indanone derivatives of secondary amines tend to undergo rapid elimination at around neutrality yielding the parent amine along with indenone but are more stable at low pH (Gilmer et al., 2005). The

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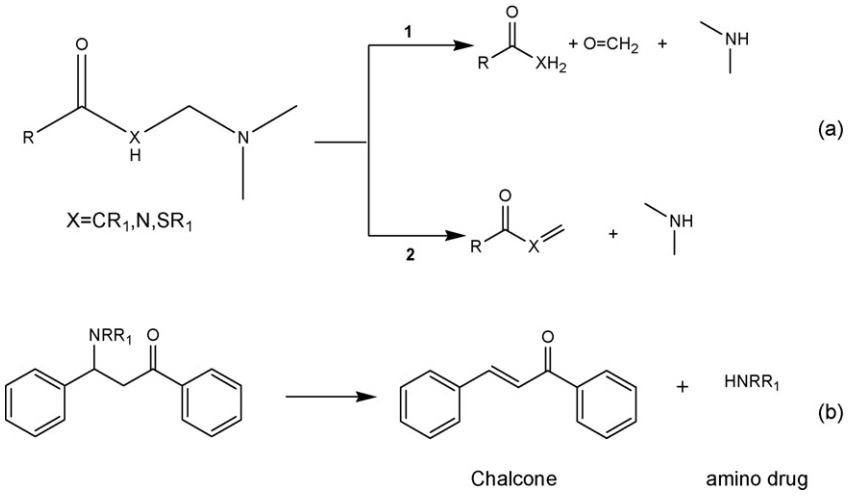


Fig. 1. (a) Cleavage reactions of Mannich bases (adapted from Tramontini and Angiolini, 1994). (b) Amino benzalacetophenones as prodrugs.

unexpectedly high rates of elimination of these tertiary prodrugs appeared to be connected to the extended conjugation available to the indenone side-product. In order to test this hypothesis and to extend the concept, we have evaluated amino benzalacetophenones (Fig. 1b) whose elimination product – chalcone – possesses more extended conjugation than indenone. Furthermore, chalcones are biologically important molecules in their own right as plant flavanoid precursors and cytoprotective agents, and the design may therefore also have application in chalcone protection. For comparative purposes, we present data for a number of other β-amino carbonyl compounds (Table 1).

2. Materials and methods

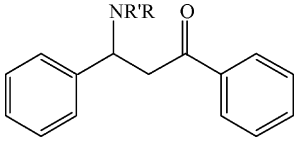
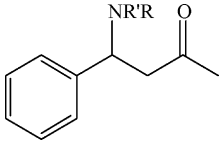
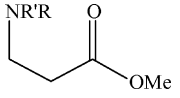
2.1. Materials

Desloratadine (micronised USP) was donated by Schering-Plough (Avondale) company. L-3,4-Dihydroxyphenylalanine 99%, 2-phenylethylamine 99%, 1-aminoindane, 2-methyl-1-indanone 99%, benzoyl peroxide (70% remainder water), acetophenone 99%, methyl 3-bromopropionate 97% and triethylamine were purchased from Aldrich. Benzalacetone >98%, *R*(−)-1-aminoindane >98%, benzalacetophenone >98%, were from Fluka; Merck silica gel 60 (particle size 0.040–0.063 mm) was used for flash column chromatography. *N*-Bromosuccinimide 98% was from BDH and carbon tetrachloride (HPLC grade) was from Riedel-de Haën. Phosphoric acid (>99%, Fluka), sodium dihydrogen orthophosphate (BDH) and tetrabuthylammonium dihydrogenphosphate (97%, Aldrich) were used for the preparation of running buffers for capillary electrophoresis. Citric acid monohydrate (99% ACS, Aldrich), boric acid (M&B) and tripotassium orthophosphate (BDH) were used for the preparation of buffers for kinetic studies. Acetonitrile for HPLC from Riedel-de Haën was used for the preparation of stock solutions. Aqueous solutions were prepared with distilled and deionised water (Milli-Q Water System, Millipore).

2.2. Chemistry

Infrared spectra (IR) were obtained using a Perkin-Elmer Paragon 1000 FT infrared spectrometer. ^{1}H and ^{13}C NMR were recorded at 20 °C on a Brucker DPX 400 spectrophotometer (400.13 MHz ^{1}H, 100.61 MHz ^{13}C) at the Department of Chemistry, Trinity College Dublin. Samples were dissolved in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide ($(CD_3)_2SO$). Chemical shifts are in ppm. Coupling constants are in Hertz. ^{1}H shifts were assigned relative to the tetramethylsilane (TMS) peak at 0.00 ppm and ^{13}C shifts were assigned relative to the central carbon of the $CDCl_3$ triplet at 77.0 ppm or relative to the middle $(CD_3)_2SO$ septet at 39.7 ppm. High resolution mass spectra (HRMS) were acquired on a Micro-

Table 1
Test compounds

Compound	Amine (NR′R)	Carrier group
1a	Piperidine	
1b	Propylamine	
1c	Hexylamine	
1d	*R*(−)-1-aminoindane	
1e	Cyclopentylamine	
1f	Phenylethylamine	
1g	Dopamine	
1h	Desloratadine	
2	Piperidine	
3a	2-Aminoindane	
3b	Desloratadine	

mass spectrometer (TOF, electrospray ionisation). The claim of quantitative yield for some compounds produced in solid-state reaction, refers to the fact that no residues of reactants or side products were detected by NMR.

Compounds **1a–f** (Table 1) were prepared from the unsaturated ketones, benzalacetophenone (chalcone) by dissolving them, with stirring, with an equimolar quantity of the amine at room temperature. Typically, after approximately 5–10 min the mixtures solidified affording the sought product.

2.2.1. 1,3-Diphenyl-3-piperidin-1-yl-propan-1-one

1a δ_H ($CDCl_3$) was prepared from piperidine (0.17 g, 2 mmol) and chalcone (0.42 g, 2 mmol). A white solid was obtained (0.59 g, 2 mmol) quantitatively. mp and δ_H ($CDCl_3$) was in agreement with literature (Toda et al., 1998) *m/z* 294.1861 (MH^+, expected: 294.1858).

2.2.2. 3-Propylamino-1,3-diphenyl-propan-1-one

1b was prepared from *n*-propylamine (0.12 g, 2 mmol) and chalcone (0.42 g, 2 mmol). Quantitative yield as a white solid. mp 40–42 °C. IR ν_{max} (Kerr) 1678 (C=O) cm^{-1}. δ_H ($CDCl_3$) 0.92 (3H, m, C$\underline{H}_3$), 1.48 (2H, m, C$\underline{H}_2$CH$_3$), 2.37 (^{1}H, br, s, N$\underline{H}$), 2.45 (2H, m, NHC$\underline{H}_2$CH$_2$), 3.30 (^{1}H, dd, J_{gem} = 17.6, J_{vic} = 4.5, NHCHC$\underline{H}_2$C=O), 3.38 (^{1}H, dd, J_{gem} = 17.6, J_{vic} = 8.0, NHCHC$\underline{H}_2$C=O), 4.32 (^{1}H, dd, *J* = 4.5, 8.0, NHC$\underline{H}$CH$_2$C=O), 7.28 (^{1}H, m, ArC$\underline{H}$), 7.36 (2H, m, ArC$\underline{H}$), 7.44 (4H, m, ArC$\underline{H}$), 7.54 (^{1}H, m, ArC$\underline{H}$), 7.93 (2H, d, *J* = 7.5, ArC$\underline{H}$). δ_C ($CDCl_3$) 11.7 (C$\underline{H}_3$), 23.1 (C$\underline{H}_2$CH$_3$), 47.2 (CHC$\underline{H}_2$C=O), 49.6 (C$\underline{H}_2$CH$_2$CH$_3$), 58.9 (NHC$\underline{H}$CH$_2$), 127.2 (3C, ArC$\underline{H}$), 128.0 (2C, ArC$\underline{H}$), 128.5 (2C, ArC$\underline{H}$), 133.1 (ArC$\underline{H}$), 136.8 (C$\underline{C}$HNH), 143.6 (C$\underline{C}$=O), 198.9 (C$\underline{=}$O). *m/z* 268.1710 (MH^+, expected: 268.1701).

2.2.3. 3-Hexylamino-1,3-diphenyl-propan-1-one

1c was prepared from *n*-hexylamine (0.20 g, 2 mmol) and chalcone (0.42 g, 2 mmol). Quantitative yield as a white solid. mp and δ_H ($CDCl_3$) are in agreement with literature (Toda et al., 1998). *m/z* 310.2183 (MH^+, expected: 310.2171).

2.2.4. 3-(Indan-1-ylamino)-1,3-diphenyl-propan-1-one

1d was produced from *R*-1-aminoindane (0.13 g 1 mmol) and chalcone (0.21 g, 1 mmol). A mixture of two diastereomers was obtained with a ratio of 25:75 as confirmed by NMR. 1.6–1.8 (^{1}H, m, NHCHC$\underline{H}_2$CH$_2$), 1.84 (^{1}H, s, br, N$\underline{H}$), 1.95–2.04* (^{1}H, m, NHCHC$\underline{H}_2$CH$_2$), 2.12–2.20 (^{1}H, m, NHCHC$\underline{H}_2$CH$_2$), 2.44–2.51* (^{1}H, m, NHCHC$\underline{H}_2$CH$_2$), 2.65–2.7 (^{1}H, m, NHCHCH$_2$C$\underline{H}_2$), 2.7–2.8* (^{1}H, m, NHCHCH$_2$C$\underline{H}_2$), 2.8–2.90 (^{1}H, m, NHCHCH$_2$C$\underline{H}_2$), 2.98–3.05 (^{1}H, m, NHCHCH$_2$C$\underline{H}_2$), 3.29–3.45* (2H, m, NHCHC$\underline{H}_2$C=O), 4.01*, 4.13 (^{1}H, t, *J* = 6.8, NHC$\underline{H}$CH$_2$CH$_2$), 4.59* (^{1}H, dd, J_{gem} = 8.8, J_{vic} = 3.4 NHC$\underline{H}$CH$_2$C=O), 4.68 (^{1}H, dd, J_{gem} = 8.2, J_{vic} = 4.8 NHC$\underline{H}$CH$_2$C=O), 7.19–7.91 (12H, m, Ar$\underline{H}$), 7.96 (2H, d, *J* = 7.5, Ar$\underline{H}$). δ_C ($CDCl_3$) (for the dominant diastereomer) 30.1 (C$\underline{H}_2$), 37.3 (C$\underline{H}_2$), 47.6 (C$\underline{H}_2$), 58.6 (C$\underline{H}$), 60.4 (C$\underline{H}$), 122.4 (ArC$\underline{H}$), 122.6 (ArC$\underline{H}$), 124.3 (ArC$\underline{H}$), 125.3 (ArC$\underline{H}$), 125.5 (ArC$\underline{H}$), 125.6 (2C, ArC$\underline{H}$), 126.1 (ArC$\underline{H}$), 126.6 (2C, ArC$\underline{H}$), 126.7 (2C, ArC$\underline{H}$), 127.1 (ArC$\underline{H}$), 130.9 (ArC$\underline{H}$), 135.1 (C$\underline{}$), 141.4 (C$\underline{}$), 141.8 (C$\underline{}$), 144.1 (C$\underline{C}$=O), 197.04 (C$\underline{=}$O). *m/z* 342.1848 (MH^+, expected: 342.1858).

2.2.5. 3-Cyclopentylamino-1,3-diphenyl-propan-1-one

1e the reaction of cyclopentylamine (0.17 g, 2 mmol) with chalcone (0.42 g, 2 mmol) produced a white solid. Seventy three percent (27% remained unreacted as confirmed by NMR). IR ν_{max} (KBr) 1603 (C=O), 2857, 2954 (aliphatic C–H) cm^{-1}. δ_H ($CDCl_3$) 1.46 (^{1}H, s, br, N$\underline{H}$), 1.46–1.89 (8H, m, NHCHC$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$), 2.89 (^{1}H, t, *J* = 6.8 NHC$\underline{H}$CH$_2$CH$_2$), 3.28 (^{1}H, dd, J_{gem} = 17.0, J_{vic} = 4.5, NHCHC$\underline{H}_2$C=O), 3.36 (^{1}H, dd, J_{gem} = 17.0, J_{vic} = 8.0, NHCHC$\underline{H}_2$C=O), 4.39 (^{1}H, dd, *J* = 4.5, 8.0, NHC$\underline{H}$CH$_2$C=O), 7.29–7.66 (8H, m, ArH), 8.04 (2H, m, ArH). δ_C ($CDCl_3$) 23.5, 23.7 (NHCHCH$_2$C$\underline{H}_2$C$\underline{H}_2$), 32.0, 33.8 (NHCHC$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}_2$), 47.3 (NHCHC$\underline{H}_2$C=O), 57.0 (NH$\underline{C}$HCH$_2$C=O), 57.2 (NH$\underline{C}$HCH$_2$CH$_2$), 127.1 (Ar$\underline{C}$H), 127.2 (2C, Ar$\underline{C}$H), 127.9 (2C, Ar$\underline{C}$H), 128.4 (2C, Ar$\underline{C}$H), 128.4 (2C, ArC$\underline{H}$), 133.0 (ArC$\underline{H}$), 136.8 (C$\underline{C}$HNH), 143.6 (C$\underline{C}$=O), 198.8 (C$\underline{=}$O). *m/z* 294.1857 (MH^+, expected: 294.1858).

2.2.6. 3-Phenethylamino-1,3-diphenyl-propan-1-one

1f was prepared from chalcone (0.42 g, 2 mmol) and phenylethylamine (0.24 g, 2 mmol) quantitative yield as a white solid. mp 74–76 °C, IR ν_{max} (KBr) 1671 (C=O) cm^{-1}. δ_H ($CDCl_3$) 2.69–2.82 (4H PhC$\underline{H}_2$C$\underline{H}_2$NH), 3.29 (^{1}H, dd, J_{gem} = 17.1, J_{vic} = 5.0, NHCHC$\underline{H}_2$C=O), 3.36 (^{1}H, dd, J_{gem} = 17.1, J_{vic} = 8.0, NHCHC$\underline{H}_2$C=O), 4.36 (^{1}H, dd, *J* = 4.5, 8.0, NHC$\underline{H}$CH$_2$), 7.15–7.30 (6H, m, Ar$\underline{H}$), 7.37 (4H, m, Ar$\underline{H}$), 7.45 (2H, m, Ar$\underline{H}$), 7.58 (^{1}H, m, Ar$\underline{H}$), 7.92 (2H, m, Ar$\underline{H}$). δ_C ($CDCl_3$) 36.4 (Ph-C$\underline{H}_2$), 47.2 (CHC$\underline{H}_2$C=O), 48.9 (C$\underline{H}_2$NH), 58.9 (NHC$\underline{H}$CH$_2$), 126.0 (ArC$\underline{H}$), 127.2 (2C, Ar$\underline{C}$H), 127.3 (ArC$\underline{H}$), 128.0 (2C, Ar$\underline{C}$H), 128.3 (2C, Ar$\underline{C}$H), 128.5 (4C, ArC$\underline{H}$), 128.6 (2C, Ar$\underline{C}$H), 132.8 (ArC$\underline{H}$), 136.4 (C$\underline{C}$HNH), 139.6 (C$\underline{C}$H$_2$CH$_2$), 142.9 (C$\underline{C}$=O), 198.4 (C$\underline{=}$O). *m/z* 330.1874 (MH^+, expected: 330.1858).

2.2.7. 3-[2-(3,4-Dihydroxy-phenyl)-ethylamino]-1,3-diphenyl-propan-1-one hydrochloride salt

1g was prepared from dopamine hydrochloride (0.19 g, 1 mmol) and benzalacetophenone (2.29 g, 11 mmol). The amine was dissolved in DMF with a large excess of benzalacetophenone. One millimolar of triethylamine was added and the reaction mixture was stirred at room temperature until complete consumption of the amine (followed by capillary electrophoresis, CE). The solvent was evaporated and the residue was redissolved in water containing 1 mmol of HCl. This solution was extracted with DCM to remove the excessive ketone, and the aqueous solution was evaporated to dryness. The residue was washed up with a mixture of hexane and methanol (50:50). An off-white solid was obtained (0.25 g, 0.63 mmol) 63%. mp 220–222 °C. IR ν_{max} (KBr) 3328 (OH), 1686 (C=O), cm^{-1}. δ_H ($(CD_3)_2SO$) 2.71 (2H, m, NHCH$_2$C$\underline{H}_2$), 2.87 (2H, m, NHC$\underline{H}_2$CH$_2$), 4.03 (^{1}H, dd, J_{gem} = 17.6, J_{vic} = 9.0, NHCHC$\underline{H}_2$C=O), 4.11 (^{1}H, dd, J_{gem} = 17.6, J_{vic} = 4.5, NHCHC$\underline{H}_2$C=O), 4.83 (^{1}H, m, br, NHC$\underline{H}$CH$_2$C=O), 6.38 (^{1}H,

d, $J = 8.0$, $CH_2CC\underline{H}CHCOH$), 6.54 (^1H, s, $CH_2CC\underline{H}COH$), 6.65 (^1H, d, $J = 7.5$, $CH_2CCHC\underline{H}COH$), 7.34–7.44 (3H, m, Ar$\underline{H}$), 7.53 (2H, t, $J = 7.8$, Ar$\underline{H}$), 7.64 (^1H, m, Ar$\underline{H}$), 7.69 (2H, m, Ar$\underline{H}$), 7.92 (2H, d, $J = 8.0$, Ar$\underline{H}$), 8.80 (^1H, s, NH^+), 9.69 (^1H, s, O$\underline{H}$), 9.91 (^1H, s, O$\underline{H}$). δ_C ($(CD_3)_2SO$) 31.0 ($\underline{C}H_2CH_2NH$), 41.7 ($CH\underline{C}H_2C{=}O$), 46.5 ($CH_2\underline{C}H_2NH$), 57.6 ($\underline{C}HCH_2C{=}O$), 115.9 ($HOC\underline{C}HCCH_2CH_2NH$), 116.0 ($\underline{C}HCHCCH_2CH_2NH$), 119.2 ($CH\underline{C}HCCH_2CH_2NH$), 128.1 (2C, Ar$\underline{C}$H), 128.6 (Ar$\underline{C}$H), 128.8 (2C, Ar$\underline{C}$H), 128.9 (2C, Ar$\underline{C}$H), 129.2 (2C, Ar$\underline{C}$H), 133.9 (Ar$\underline{C}$H), 135.2 ($\underline{C}CH_2CH_2NH$), 136.1 ($\underline{C}CHNH$), 144.2 ($\underline{C}C{=}O$), 145.4 (2C, $HO\underline{C}\underline{C}OH$), 195.8 ($\underline{C}{=}O$). *m/z* 362.1758 ($M(-Cl^-)$, expected: 362.1756).

2.2.8. 3-[4-(8-Chloro-5,6-dihydro-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-piperidin-1-yl]-1,3-diphenyl-propan-1-one hydrobromide salt

1h was prepared from desloratadine (0.31 g, 1 mmol) and benzalacetophenone. The amine was dissolved in water/acetonitrile/ethanol (95:2.5:2.5) with benzalacetophenone. One millimolar of hexadecyltrimethylamonnium bromide was added and the reaction mixture was stirred at room temperature overnight. The precipitated product was separated by filtration and dried under vacuum. An off-white solid was afforded (0.25 g, 0.42 mmol) 42%. mp 116–118 °C. IR ν_{max} (KBr) 1683 (C=O), 1664 (C=C), cm^{-1}. δ_H ($CDCl_3$) 1.66 (N$\underline{H}$), 1.98–2.03 (^1H, m, CH_2), 2.16–2.48, 2.02–2.29 (5H, m, $C{=}C\underline{C}H_2$ and $C{=}CCH_2C\underline{H}_2N$), 2.69–2.82 (4H, m, $C{=}CCH_2C\underline{H}_2N$ and $C\underline{C}H_2CH_2C$), 3.26–3.38 (3H, m, $CCH_2\underline{C}H_2C$ and $NCHC\underline{H}_2C{=}O$), 3.66 (^1H, dd, $J_{gem} = 16.4$, $J_{vic} = 6.8$, $NCHC\underline{H}_2C{=}O$), 4.26 (^1H, t, $J = 6.5$, N$\underline{C}$H), 7.04–7.40 (7H, m, Ar$\underline{H}$), 7.41–7.67 (6H, m, Ar$\underline{H}$), 7.92 (2H, d, $J = 8.2$, $C\underline{H}CC{=}O$). δ_C ($CDCl_3$) 31.3 (2C, Ph-$\underline{C}H_2$), 31.7 (2C, $C\underline{C}H_2CH_2N$), 44.3 (2C, $CH\underline{C}H_2C{=}O$), 44.3 ($NH\underline{C}HCH_2$), 51.2, 52.3 (2H, $\underline{C}H_2NH$), 121.9 (Ar$\underline{C}$H), 125.8 (Ar$\underline{C}$H), 127.2 (Ar$\underline{C}$H), 127.9 (2C, Ar$\underline{C}$H), 128.1 (4C, Ar$\underline{C}$H), 128.5 (2C, Ar$\underline{C}$H), 128.8 (Ar$\underline{C}$H), 128.9 (Ar$\underline{C}$H), 130.8 (Ar$\underline{C}$H), 132.3 ($\underline{C}$), 132.5 ($\underline{C}$), 132.9 (Ar$\underline{C}$H), 133.3 ($\underline{C}$), 137.2 (Ar$\underline{C}$H), 137.7 ($\underline{C}$), 139.0 ($\underline{C}$), 139.4 ($\underline{C}$), 139.8 ($\underline{C}$), 146.5 (Ar$\underline{C}$H), 157.5 ($\underline{C}$), 198.6 ($\underline{C}{=}O$) *m/z* 519.2206 (MH^+, expected: 519.2203).

2.2.9. 4-Phenyl-4-piperidin-1-yl-butan-2-one

2 was prepared in solid-state from benzalacetone (0.30 g, 2 mmol) and piperidine (0.17 g, 2 mmol), by stirring the two compounds for 30 min. It was obtained as yellowish oil (0.23 g, 2 mmol) that was confirmed to be pure by NMR 100%. IR ν_{max} (NaCl plate) 1715 (C=O), δ_H ($CDCl_3$) 1.28 (2H, qi, $J = 5.2$, $NCH_2CH_2C\underline{H}_2$), 1.48 (4H, m, $2 \times NCH_2C\underline{H}_2CH_2$), 2.06 (3H, s, C$\underline{H}_3$), 2.24 (2H, m, $NC\underline{H}_2CH_2$), 2.36 (2H, m, $NC\underline{H}_2CH_2$), 2.75 (^1H, dd, $J_{gem} = 15.5$, $J_{vic} = 7.5$, $NCHC\underline{H}_2C{=}O$), 3.04 (^1H, dd, $J_{gem} = 15.5$, $J_{vic} = 7.5$, $NCHC\underline{H}_2C{=}O$), 3.99 (^1H, t, $J = 7.52$, $NC\underline{H}CH_2C{=}O$), 7.18–7.21 (3H, m, $CC\underline{H}CHC\underline{H}CHC\underline{H}$), 7.26 (2H, m, $CCHC\underline{H}$). δ_C ($CDCl_3$) 24.2 ($NCH_2CH_2\underline{C}H_2$), 26.0 (2C $NCH_2\underline{C}H_2$), 30.0 ($\underline{C}H_3$), 46.6, 50.6 (2C $N\underline{C}H_2CH_2$), 65.5 (N$\underline{C}$H), 126.9 (^1C, Ar$\underline{C}$H), 127.6 (2C, Ar$\underline{C}$H), 128.0 (2C, Ar$\underline{C}$H), 138.3 ($\underline{C}CHNH$), 207.0 ($\underline{C}{=}O$). *m/z* 232.1703 (MH^+, expected: 232.1701).

2.2.10. 3-(Indan-2-ylamino)-propionic acid methyl ester

3a was prepared from 2-aminoindane hydrochloride (0.17 g, 1 mmol) and 3-bromopropionic acid methyl ester (0.16 g, 2 mmol) in dry DCM (20 ml) at 0 °C to which was added triethylamine (1.9 mmol eq.). The reaction was stirred for 3 h. The solvent was evaporated and the reaction mixture was cleaned up by flash chromatography with DCM/methanol (92.5:7.5). A brown oil was obtained (0.11 g, 0.51 mmol) 51%. IR ν_{max} (NaCl plate) 1736 (C=O) cm^{-1}. δ_H ($CDCl_3$) 1.62 (^1H, s, br, NH), 2.56 (2H, t, $J = 6.5$, $NHCH_2CH_2C{=}OOCH_3$), 2.78 (2H, dd, $J_{gem} = 15.6$, $J_{vic} = 6.5$, CCH_2CHNH), 2.97 (2H, t, $J = 6.5$, $NHCH_2CH_2C{=}OOCH_3$), 3.19 (2H, dd, $J_{gem} = 15.6$, $J_{vic} = 7.0$, CCH_2CHNH), 3.66 (^1H, m, CCH_2CHNH), 7.14–7.17 (2H, m, ArH), 7.19–7.23 (2H, m, ArH). δ_C ($CDCl_3$) 34.7 (CH_2COOCH_3), 40.0 (2C, $NHCHCH_2$), 43.3 ($CH_2CH_2COOCH_3$), 51.5 (CH3), 59.5 (NHCH), 124.6 (2C, $CHCHCCH_2CNH$), 126.3 (2C, $CHCHCCH_2CNH$), 141.6 (2C, $CHCCH_2CNH$), 173.1 (C=O). *m/z* 220.1345 (MH^+, expected: 220.1338).

2.2.11. 3-[4-(8-Chloro-5,6-dihydro-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-piperidin-1-yl]-propionic acid methyl ester

3b was prepared from desloratadine (0.31 g, 1 mmol) and 3-bromopropionic acid methyl ester (0.088 mg, 1 mmol) in dry DCM (20 ml) at 0 °C to which was added triethylamine (1.9 mmol eq.). The reaction was stirred for 3 h. The solvent was evaporated and the reaction mixture was cleaned up by flash chromatography with DCM/methanol (92.5:7.5). A reddish oil was obtained (0.35 g, 0.88 mmol, 88%). IR ν_{max} (NaCl plate) 1738 (C=O) cm^{-1}. H–H and C–H COSY were used for assignment of the NMR shifts. δ_H ($CDCl_3$) 2.15 (2H, m, $NCH_2C\underline{H}_2C{=}O$), 2.36 (3H, m, C$\underline{H}_2$), 2.49 (3H, m, C$\underline{H}_2$), 2.69–2.90 (6H, m, C$\underline{H}_2$), 3.38 (2H, m, C$\underline{H}_2$), 3.66 (3H, s, $OC\underline{H}_3$), 7.07 (^1H, m, ArC$\underline{H}$), 7.14 (3H, m, ArC$\underline{H}$), 7.4 (^1H, m, ArC$\underline{H}$), 8.9 (^1H, m, ArNC$\underline{H}$). δ_C ($CDCl_3$) 30.6, 30.8 (2C, $\underline{C}H_2$), 31.4, 31.7 (2C, $\underline{C}H_2$), 32.1 ($NCH_2\underline{C}H_2C{=}O$), 51.5 ($O\underline{C}H_3$), 53.2 ($N\underline{C}H_2CH_2C{=}O$), 54.4, 54.5 (2C, $CCCH_2\underline{C}H_2N$), 122.0 (Ar$\underline{C}$H), 125.9 (Ar$\underline{C}$H), 128.9 (Ar$\underline{C}$H), 130.7 (Ar$\underline{C}$H), 132.6 ($\underline{C}$), 132.7 ($\underline{C}$), 133.3 ($\underline{C}$), 137.1 (Ar$\underline{C}$H), 137.8 ($\underline{C}$), 138.5 ($\underline{C}$), 139.4 ($\underline{C}$), 146.5 (Ar-N$\underline{C}$H), 157.5 ($N\underline{C}C{=}C$), 172.9 ($\underline{C}{=}O$). *m/z* 397.1693 (MH^+, expected: 397.1683).

2.3. Kinetics experiments

2.3.1. Aqueous buffer kinetics

The disappearance of the prodrugs was studied in the pH range of 0.5–11.7. For the preparation of the working buffers in the range 2–11.7, two stock solutions were used to prepare a universal buffer. Solution A was 0.05 M of citric acid monohydrate and 0.2 M of boric acid in distilled and deionised water. Solution B was 0.1 M in tripotassium-orthophosphate in distilled and deionised water. The two solutions were mixed and diluted in the necessary proportions to achieve the desired pHs and ionic strength of 0.154 without further addition of acid, base or salt (Albert and Serjeant, 1984). For pH under 2, HCl solutions were

used: ionic strength was set by addition of NaCl where appropriate. Some tests were performed at different ionic strengths to evaluate buffer catalysis (results not presented). Although not all compounds were evaluated, no important differences were detected for ionic strengths in the range of 0.05–0.6 for the compounds tested.

Typically, stock solutions of the compounds under investigation of approximately 1–5 mg/ml in acetonitrile were prepared. Twenty to 100 μl of the stock solutions were diluted in 1 ml of the working buffer warmed at 37 °C to obtain solutions with a final concentration of approximately 100 μg/ml. Each solution was introduced in the autosampler of the CE warmed at 37 °C and injections started immediately using the method described in Section 2.3.2.

2.3.2. Capillary electrophoresis

Capillary electrophoresis was performed in a Beckman P/ACE system 5510 equipped with a UV filter detector set at 200 or 214 nm. The system also had an autosampler with cooling possibility that was modified by connection to a water circulator. For kinetic tests, the water temperature was set so that, inside the sample vials, a temperature of 37 ± 1 °C was achieved. The fused silica capillary was of 12 cm effective length (20 cm total length) and an internal diameter of 50 μm. Samples were loaded by pressure injection for 5 s. Runs were carried out at 25 °C and at constant current of 100 or 150 μA in the direction of the cathode. New capillaries were conditioned beforehand with 0.1 M NaOH followed by deionised water for 5 min and the running buffer for 5 min. Before each run, the capillary was rinsed with the running buffer for 1 min. The running buffer was a phosphate buffer (pH 3, 100 mM) to which 100 mM of tetrabutylammonium phosphate (TBA) was added. The buffer was filtered through a 0.45 μm membrane filter before use. Data acquisition was performed by the system Gold and peak areas were recorded at 200 or 214 for the original compound and the parent amine (when applicable).

For pK_a determination, capillary electrophoresis and the method described in Simplício et al. (2004) was used.

2.3.3. HPLC

High performance liquid chromatography was performed using a system consisting of a Spectra System SCM1000 ultrasound, Spectra System P4000 pump and controller, Spectra System AS3000 autosampler and a Spectra System UV1000 detector controlled by Chromquest Chromatography Manager. The stationary phase was a C18 (4.6 mm × 250 mm) Waters Spherisorb 10 μm particle size column. A mixed gradient-isocratic mobile phase was employed which consisted of aqueous 42.5 mM orthophosphoric acid/6.1 mM triethylamine and acetonitrile in a ratio of 80:20 for 5 min then graded to 20:80 (aqueous: MeCN) over 10 min, maintained at that for 5 min then stepped back to initial conditions over 1 min and re-equilibrated for 9 min. The flow rate was 1.2 ml/min.

2.3.4. Non-linear regression

Graph Pad Prism® was used for fitting experimental data of the pH/rate profiles and the pH/mobility profiles.

3. Results

3.1. Synthesis

Synthesis of amino benzylacetophenone compounds was accomplished by direct Michael addition of the amine to the unsaturated ketone (essentially the reverse reaction to the prodrug elimination and amine releasing reaction). The alternative Mannich reaction using the benzaldehyde, the amine and acetone failed to give reproducible results. The Michael reaction worked particularly well when equimolar quantities of the amine and ketone were mixed at room temperature in solventless conditions. Usually, the liquid mixture solidified in <10 min affording the target compound in quantitative yield. Surprisingly, single addition occurred exclusively even with primary amines. Derivatives of piperidine, *n*-propylamine, *n*-hexylamine, *R*-1-aminoindandone and phenylethylamine were prepared in this way. However, we were unable to isolate dopamine or desloratidine adducts using these conditions. The dopamine compound (**1g**) was obtained by treatment with a large excess of chalcone and excess enone removed by DCM extraction. The desloratadine derivative (**1h**) was prepared in aqueous/organic solution in the presence of a surfactant. Reaction of benzyl acetone with primary amines usually afforded mixtures which could consist of the compound resulting from single addition of the ketone and the amine, as well as the diasteriomeric mixture of the compounds resulting from double addition of the ketone to the amine. Attempts to purify the different compounds usually lead to degradation, which suggests fast amine elimination in organic solvents. Reaction with the secondary amine piperidine afforded the pure product (**2**) as a clear oil without further purification. Amino-propionates **3a** and **3b** were obtained by treating 3-bromopropionc acid methyl ester with amino indane or desloratidine in the presence of a tertiary base. The aminoketones were characterised by ^{1}H and ^{13}C NMR, IR, HRMS, capillary electrophoresis, TLC and HPLC.

3.2. Kinetic experiments

The disappearance of test compounds at 37 °C in aqueous solution at selected pHs over the range 0.5–11.7 was monitored using CE or HPLC as appropriate; for some compounds both HPLC and CE were used. The disappearance of the amino acetophenones in buffered aqueous solutions generally followed pseudo first-order kinetics over several half-lives. Evolution of the parent amines was observed for compounds **1d**, **1f**, **1g**, **1h** for which UV detection was possible with the methods used. Rates of degradation of compound **1h** were not determined because the peak of the compound in CE was wide and unsymmetrical and was difficult to quantify, probably as a result of low solubility. Nevertheless, the formation of desloratadine was observed as expected.

Pseudo first-order plots were constructed from the logarithm of remaining compound versus time and the half-lives estimated

Table 2
Observed rates of degradation and pK_as as obtained by fitting of data to Eq. (2) by non-linear regression (compounds **1c**, **1f**, **1g**), or observed rates of degradation at pH 3[§] and pH 7.4[¥] (compounds **1a**, **1b**, **1d**, **1e**, **2**) and respective half-lives at low and high pH; pK_as as determined by capillary electrophoresis

Compound	k_1 (min^{-1}) or k_{obs} (pH 3)[§]	k_2 (min^{-1}) or k_{obs} (pH 7.4)[¥]	pK_a	$t_{1/2}$ (pH 3)	$t_{1/2}$ (pH 7.4) (min)	pK_a
1a	0[§]	0.12[¥]	n.d.	–	5.8	7.7 ± 0.1
1b	0[§]	0.052[¥]	n.d.	–	13	8.4 ± 0.2
1c	0.0026	0.22	6.7 ± 0.3	4.4 h	3.2	7.0 ± 0.2
1d	0[§]	0.054[¥]	n.d.	–	13	5.8 ± 0.4
1e	0[§]	0.14[¥]	n.d	–	5.0	7.8 ± 0.1
1f	0.00043	0.14	6.3 ± 0.04	27 h	4.9	6.8 ± 0.2
1g	0.0003	0.0064	5.0 ± 0.4	4.8 days	14	7.0 ± 0.1, 8.7 ± 0.2
1h	n.d.	n.d.	n.d.	–	–	3.7 ± 0.4
2	0[§]	0.21[¥]	n.d.	–	3.3	8.5 ± 0.2

using Eq. (1), as illustrated for compound **1c** in Fig. 2:

$$t_{1/2} = \frac{0.693}{k_{obs}} \quad (1)$$

Generally, the compounds degraded rapidly at pH 7.4 ($t_{1/2}$ < 2–15 min) but they were stable under acidic conditions. Half-lives at pH 3 and 7.4 are presented in Table 2. In marked contrast to the amino indanone series, there were no significant differences in elimination rates between the tertiary derivatives of the secondary amines and the secondary derivatives of the primary amino compounds at pH 7.4. Furthermore, there was no evidence of the reverse Michael addition reaction for the benzyl acetophenone or benzyl acetone derivatives contrary to what was found with the indanones (Gilmer et al., 2005). This may be due to the fact that, in indenone, the double bond is necessarily in the cis configuration whereas the chalcone may be cis or trans. However, trans configuration should be preferred and this may hinder the available β position towards addition.

Recovery of the free amine was estimated in the case of the dopamine derivative **1g**. These tests were performed in buffer solutions and in plasma samples. Aliquots of the solutions kept at 37 °C were taken at time intervals and quenched with 5% perchloric acid solution to facilitate protein precipitation from plasma samples and, at the same time, stop the elimination reaction. A maximum recovery of dopamine of 69 ± 3% and 62 ± 4% (average of three determinations) was observed in buffer and plasma samples, respectively, as illustrated in Fig. 3. Low recovery may be related to further metabolism and/or precipitation of dopamine: no compounds other than dopamine or chalcone were detected by HPLC but there was a substantial decrease in dopamine concentration over the course of the experiments following its initial peak, indicating instability in the study media (Fig. 3).

As previously stated (Gilmer et al., 2005), pH rate profiles obtained for these deamination reactions of C-Mannich bases can be accounted for by assuming unimolecular decomposition of the protonated and unionised forms of the original compound and may be mathematically represented in the form of a particular case of a Boltzmann sigmoid:

$$k_{obs} = k_2 - \frac{k_2 - k_1}{10^{(pH - pK_a)} + 1} \quad (2)$$

where k_1 and k_2 represent, respectively, the elimination rates from the protonated and the non-protonated amino indanone. pK_a is the ionisation constant of the compound and k_{obs} is the observed degradation rate constant at a particular pH.

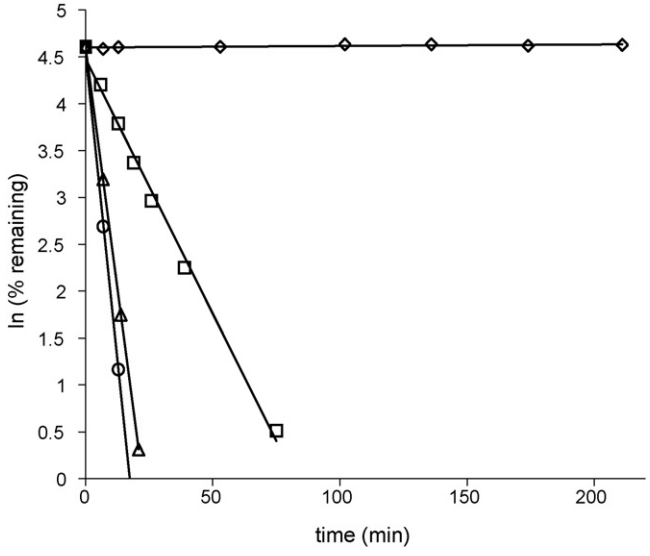


Fig. 2. Plot showing the pseudo first-order curves for the disappearance of **1f** in various solutions at 37 °C (◊, pH 3.0; □, pH 6.2; ▲, pH 7.8; ◯, pH 11.6).

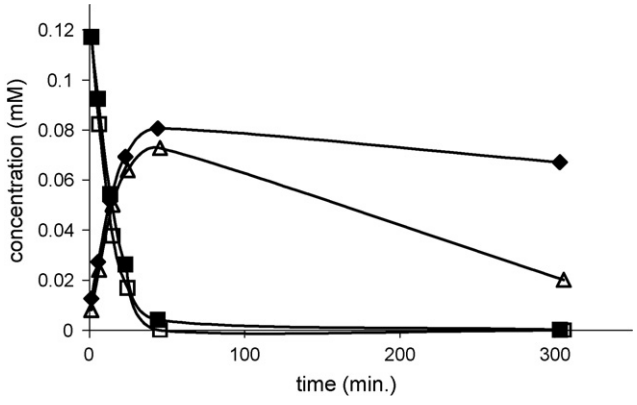


Fig. 3. Degradation profiles of **1g** in buffer and plasma (♦, dopamine in buffer pH 7.4; ■, **1g** in buffer pH 7.4; ▲, dopamine in plasma; □, **1g** in plasma).

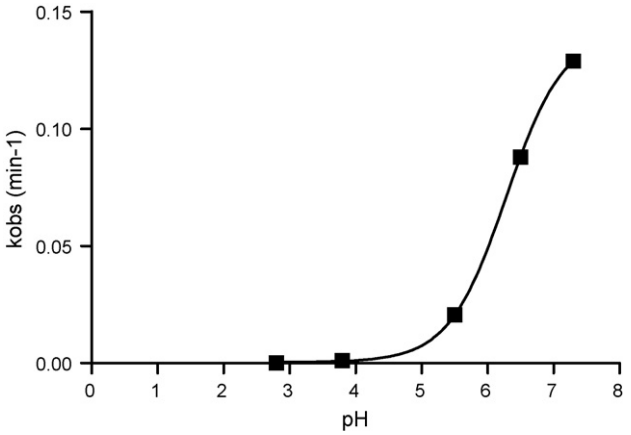


Fig. 4. Fitting of Eq. (2) to the experimental data of **1f**. The points correspond to experimentally derived data.

Graphical representation of the fitting is presented in Fig. 4 for compound **1f**. Non-linear regression applied to the pH/rate profiles obtained for compounds **1c**, **1f**, **1g** afforded, for Eq. (2), the parameters presented in Table 2. For the other compounds, the number of pHs tested was not sufficient for adequate equation fitting and therefore only the rates of degradation at pH 3 and 7.4 are presented. pK_a were also determined based on electrophoretic mobilities according to the procedure described in Simplício et al. (2004). Results of these determinations are also presented in Table 2.

Reductions of three to four pH units were observed for the β-aminoindanones in comparison with the free amines. This is advantageous for prodrug absorption as, at the pH of the intestine, larger amounts of the prodrug are in the neutral form, and consequently available for absorption, in comparison to the corresponding free amine.

Both ester derivatives (**3a–3b**) degraded in buffered solution at physiological pH, but not to the original amines. The major degradation products were detected by CE in both cases at higher migration times than the amino propionates and this was attributed to slow ester hydrolysis with formation of the corresponding acid. In this form, deamination is not likely to occur at pHs where the carboxylic function is ionised. However, the failure of the elimination reaction to compete effectively with hydrolysis in this series is consistent with the contention that conjugation in the product is important in driving elimination.

4. Discussion

As previously observed for analogous aminoindanone derivatives (Gilmer et al., 2005; Simplício et al., 2004), aminobenzyl acetophenone compounds are stable under aqueous acidic conditions but undergo rapid elimination at physiological pH. Elimination half-lives at neutral to basic pH were in the range 2–15 min for both secondary and tertiary adducts and were shorter than the half-lives of the corresponding indanone derivatives reported in the previous paper (Gilmer et al., 2005). This may be related to steric hindrance of the acetophenone derivatives. Similar observations were made about the benzyl acetone derivative.

In comparison to N-Mannich bases, C-Mannich bases seem to have the advantage of being more stable in acidic conditions and avoid the release of formaldehyde in physiological conditions since degradation seems to occur by deamination rather than deaminomethylation.

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