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The role of steric effects in the direct mutagenicity of *N*-acyloxy-*N*-alkoxyamides

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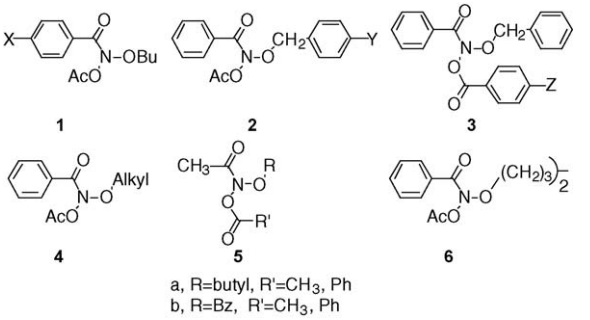
Abstract

Electrophilic *N*-acyloxy-*N*-alkoxyamides are mutagenic in *Salmonella typhimurium* TA100 without the need for S9 metabolic activation and they react with DNA at guanine—N7 at physiological pH. Since these are direct-acting mutagens, structural factors influence binding and reactivity with DNA. Mutagenicity in TA100 can be predicted by a QSAR incorporating hydrophobicity (log *P*), stability to substitution reactions at nitrogen (pK_a of the leaving acid) and steric effects of *para*-aryl substituents (E_s). A number of mutagens exhibit activities that deviate markedly from the predicted values and they fall into two classes: di-*tert*-butylated *N*-benzoyloxy-*N*-benzyloxybenzamides, which – because of their size – are most probably excluded from the major groove or are unable to achieve a transition state for reaction with DNA, and *N*-benzoyloxy-*N*-butoxyalkylamides with branching α-to the amide carbonyl, which are resistant to S_N2 reactions at the amide nitrogen.

Keywords: Mutagenicity; Direct-acting; Ames Test; Steric effects; QSAR; Electrophiles; Amides

1. Introduction

N-Acyloxy-*N*-alkoxyamides are a class of direct-acting mutagens that we have shown to react with DNA in the major groove at guanine—N7 (G—N7) at physiological pH [1–7]. Results have pointed to an S_N2 reaction with DNA, which involves displacement of the *N*-acyloxy group by the N7 lone pair of guanine [7]. The mutagenicity of a wide range of congeners (**1–6**) has been measured in *Salmonella typhimurium* strain TA100 and mutagenic activity has been found to be dependent upon a number of variables.



Activity correlates with stability or pK_a of the carboxylic acid leaving group in that mutagens that are the most reactive towards S_N2 reactions are the least

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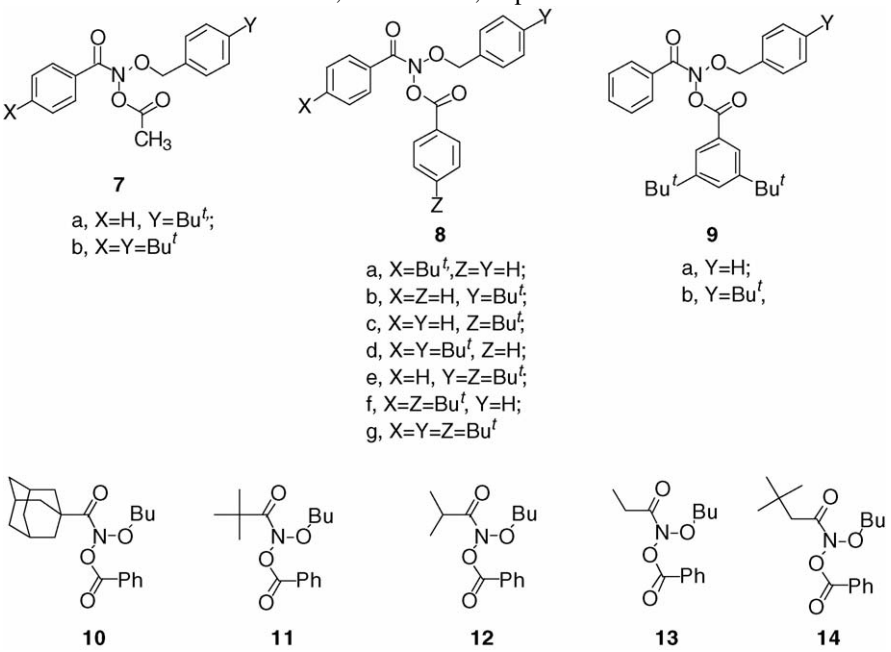
mutagenic [5,6]. Furthermore, there is an obvious dependence upon hydrophobicity of the intact mutagens (as estimated from log *P*, the log of the octanol/water partition coefficient). Since these mutagens react at G—N7 and require no metabolic activation, it is reasonable to propose that at physiological pH, the hydrophobic effect reflects binding of the molecules in the major groove of DNA. More recently, we have determined that steric effects also play a role and, using Taft steric parameters, E_s, for substituents attached to the *para* positions of benzene rings on each of the three side chains, the pK_a of the departing acid and log *P*, a quantitative structure–activity relationship has been derived, which predicts well the activity of a wide range of *N*-acyloxy-*N*-alkoxyamides (Eq. (1))[8].

Analysis of Eq. (1) indicates a strong dependence upon log *P*, significant correlations with pK_a and, where present, steric effects of groups at *para* positions on both the amide and benzyloxy side chains. However, statistically, steric effects due to substituents on the leaving group would appear to be less important. The dependence upon hydrophobicity contrasts with the hitherto prevailing view that a lack of correlation with log *P* is in fact a characteristic of direct-acting mutagens [9–11].

The relationship in Eq. (1) provides an excellent means of probing the effect of substructure upon mutagenic activity and therefore upon binding to DNA. In this paper, we describe two sets of mutagens for which the QSAR fails in that both are found to be significantly less mutagenic than predicted. The deviation from the predicted values can be ascribed on the one hand to distal and, on the other, to proximal steric effects.



$$\log \text{TA } 100^{1} = 0.29(\pm 0.03)\log P + 0.22(\pm 0.09)\text{p}K_a + 0.14(\pm 0.04)E_s^1 + 0.16(\pm 0.04)E_s^2 + 0.11(\pm 0.06)E_s^3 + 0.81(\pm 0.5)$$
$$n = 41, \quad \text{standard error} = 0.17, \quad r = 0.891, \quad F = 29.17 \qquad (1)$$

E_S^1, E_S^2 and E_S^3 represent steric effects on the benzamide, benzyloxy and benzoyloxy side chains, respectively.

[1] Log of mutagenic activity in *Salmonella typhimurium* TA100 at 1 μmol/plate taken from the gradients of revertant colonies versus dose in the linear range of dose response curves.

2. Materials and methods

2.1. Instrumentation

Melting points were determined on a Reichert Microscopic Hot-Stage and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 1725× FT spectrophotometer. Nuclear Magnetic Resonance spectra (300 MHz) were recorded in $CDCl_3$ on a Bruker AC 300P FT NMR spectrometer. ESI mass spectra were obtained on a Varian 1200L Mass Spectrometer. Micro-analytical data were obtained from the Research School of Chemistry, Australian National University, Canberra.