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In silico prediction of acyl glucuronide reactivity

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Abstract Drugs and drug candidates containing a carboxylic acid moiety, including many widely used non-steroidal anti-inflammatory drugs (NSAIDs) are often metabolized to form acyl glucuronides (AGs). NSAIDs such as Ibuprofen are amongst the most widely used drugs on the market, whereas similar carboxylic acid drugs such as Suprofen have been withdrawn due to adverse events. Although the link between these AG metabolites and toxicity is not proven, there is circumstantial literature evidence to suggest that more reactive acyl glucuronides may, in some cases, present a greater risk of exhibiting toxic effects. We wished therefore to rank the reactivity of potential new carboxylate-containing drug candidates, and performed kinetic studies on synthetic acyl glucuronides to benchmark our key compounds. Driven by the desire to quickly rank the reactivity of compounds without the need for lengthy synthesis of the acyl glucuronide, a correlation was established between the degradation half-life of the acyl glucuronide and the half life for the hydrolysis of the more readily available methyl ester derivative. This finding enabled a considerable broadening of chemical property space to be investigated. The need for kinetic measurements was subsequently eliminated altogether by correlating the methyl ester hydrolysis half-life with the predicted ^{13}C NMR chemical shift of the carbonyl carbon together with readily available steric descriptors in a PLS model. This completely in silico prediction of acyl glucuronide reactivity is applicable within the earliest stages of drug design with low cost and acceptable accuracy to guide intelligent molecular design. This reactivity data will be useful alongside the more complex additional pharmacokinetic exposure and distribution data that is generated later in the drug discovery process for assessing the overall toxicological risk of acidic drugs.

Keywords Metabolite · Reactivity · Drug design · QSAR · QSPR · Prediction

Introduction

Many important pharmaceutical drugs possess a carboxylic acid moiety, including non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants (Valproic acid), hypolipidemic drugs (Clofibrate), and diuretics (Furosemide). The structures of a number of relevant carboxylic acid drugs are given in Fig. 1. Several carboxylic acid drugs have been withdrawn from the market as a consequence of rare but occasionally severe idiosyncratic adverse effects [1].

A common metabolite of many drugs containing carboxylic acid functionalities is the 1 β-*O*-acyl glucuronide (AG). AGs are formed via the reaction of the substrate with uridine diphosphate-glucuronic acid catalysed by uridine-diphosphate-glucuronotransferase. These metabolites are unstable at physiological pH and can undergo acyl migration and hydrolysis reactions as well as reacting with endogenous molecules such as proteins via trans acylation and glycation reactions. Acyl migration involves the

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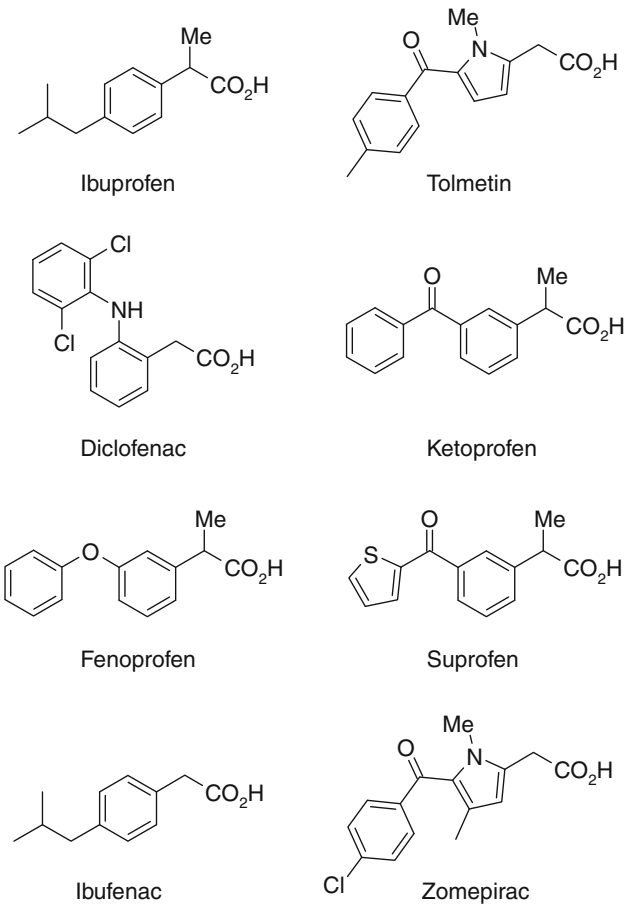

Fig. 1 Chemical structures of relevant carboxylic acid drugs

transfer of the acyl group from the 1 β position to the C-2, C-3, or C-4 position of the glucuronic acid ring (Fig. 2b), which results in the formation of acyl glucuronide isomers [2]. Covalent binding of AGs to specific target proteins located in specific tissues may be related to some clinically relevant adverse drug reactions; however, the toxicological mechanisms are still unknown [3–6] and this assertion remains controversial. For example, Koga has recently failed to find any evidence for a direct toxic effect of AGs in liver cells [7], but an immune-modulated response has neither been proven nor ruled out to date. It is likely the balance between hydrolysis and migration as well as the inherent reactivity of the AG will be important in determining the overall level of toxicological risk that these species may pose. Indeed Sawamura has demonstrated a separation between safe and withdrawn or warning label carboxylic acid containing drugs based on AG half-life [8]. There is no simple relationship between structure and human toxicity. For example, ibuprofen remains on the market whereas the close analogue ibufenac was withdrawn (severely hepatotoxic). In this pair of structures, the former indeed has the more sterically congested α-methyl group adjacent to the acetic acid that forms the acyl glucuronide metabolite, rendering it less reactive. However similar findings also exist with Tolmetin (safe) compared to Zomepirac (removed from market) and Ketoprofen (safe) compared to Suprofen (removed from the market), and in these cases, the substructures around the acid are constant. In such cases, additional consideration of likely doses, pharmacokinetic properties or differing immune responses may be additional requirements to help explain their differing toxicities. Overall, when a thorough understanding of the reactivity as well as exposure and distribution of AG metabolites is attained one might hope to make comparative toxicity judgements.

The extent of covalent binding of AGs to tissue proteins may be expected to relate to the chemical reactivity of the reactive metabolite and associated isomers. Several studies have shown a positive correlation between the extent of covalent binding of AGs and their degradation rate parameters. Benet [9] found that a correlation existed for six drugs between the extent of covalent binding to a given peptide, and the degradation rate constant of their 1 *β-O*-AG metabolites, while Bolze [10] successfully correlated aglycone appearance rate to extent of covalent binding to human serum albumin.

The degradation rate of an AG will be dependent on migration and hydrolysis rates, which in part would be expected to relate to the electronic properties of the molecule. Vanderhoeven et al. [11] have shown that for sets of *para*-substituted benzoic acids, the AG reaction rate can be successfully predicted using a quantitative structure–activity relationship (QSAR) approach, from parameters including the ^{13}C NMR shift. More recently Yoshioka et al. [12] have widened this to include *ortho*- and *meta*-substituted derivatives and demonstrated correlations with both measured and calculated ^{1}H NMR shifts and electronic parameters.

Robust practical QSAR models for predicting AG reaction rate require large diverse datasets. One of the problems in generating large datasets is the difficulty in generating AGs either by chemical synthesis or by isolation from in vitro or in vivo systems. We have devised a method for studying the reactivity of a wide range of carboxylic acid containing molecules without the need for time-consuming synthesis of AGs. Our approach firstly involved using readily synthesised methyl esters of carboxylic acids as a surrogate for the corresponding AG and establishing a correlation between AG degradation rate and the hydrolysis rate of the corresponding methyl ester of the parent carboxylic acid. Secondly, a correlation was observed between methyl ester hydrolysis rates of a large structurally diverse set of compounds and the measured ^{13}C NMR shift of the carbonyl carbon. This work significantly extends the property space beyond the small sets of NSAIDs and

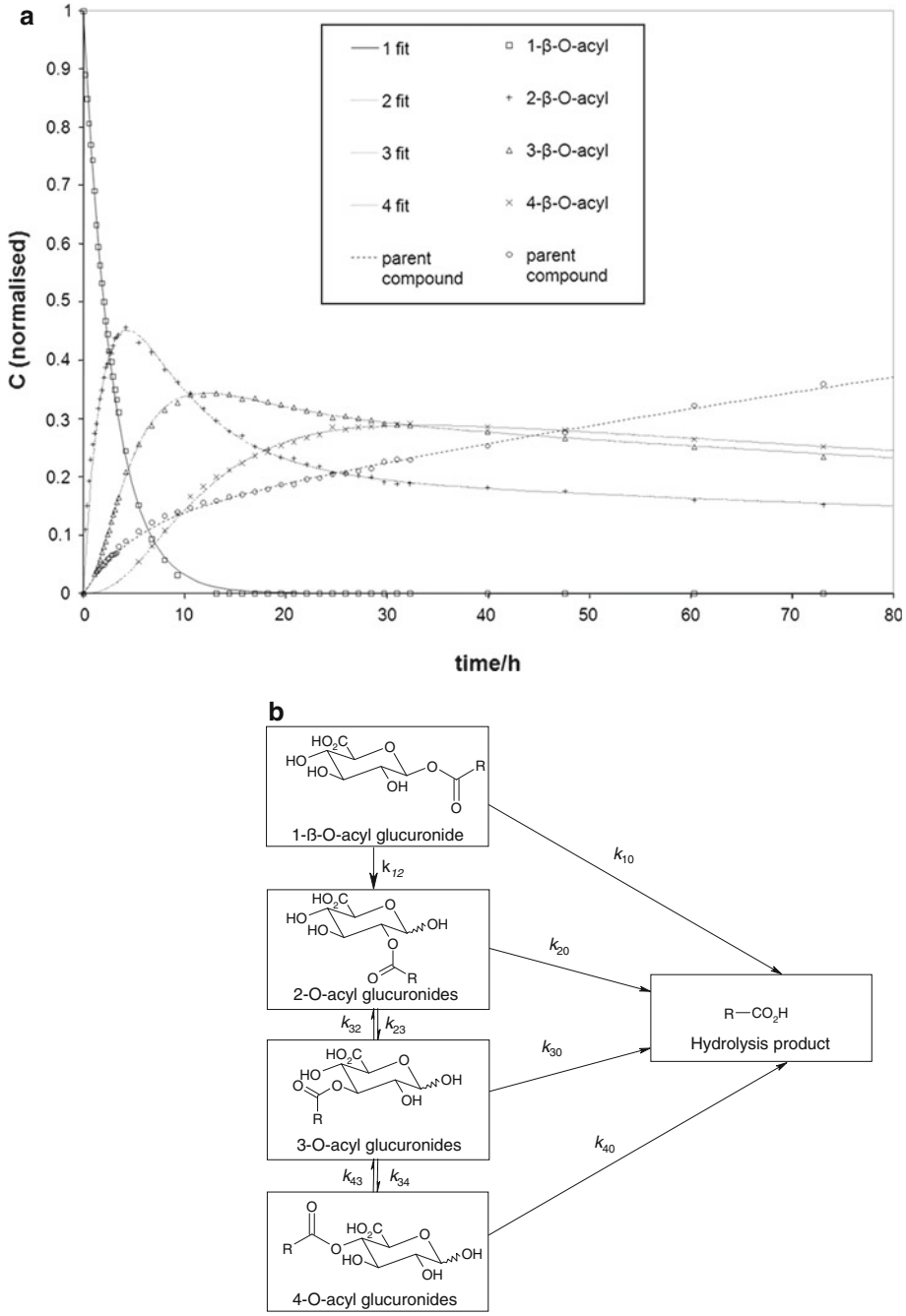

Fig. 2 **a** Rearrangement and hydrolysis kinetics for Tolmetin acyl glucuronide, showing experimental data (*points*) and fit (*lines*). **b** General rearrangement and hydrolysis scheme for acyl glucuronides

simple benzoic acids studied previously. Thirdly, a partial least squares (PLS) model was built that successfully predicted the rate of methyl ester hydrolysis for the compounds of interest, using calculated steric parameters and calculated ^{13}C NMR carbonyl carbon shift. Finally a correlation was established between the PLS predicted methyl ester hydrolysis rate and the AG degradation rate allowing a fully in silico prediction of AG reactivity and potentially a more informed choice when designing future novel candidate drugs.

Methods

Compound source

All chemicals and reagents were used as received without additional purification. Synthetic acyl glucuronides were prepared from the corresponding carboxylic acid via Mitsunobu coupling with the mono *O*-benzyl glucuronic acid derivative (2*S*,3*S*,4*S*,5*R*)-benzyl 3,4,5,6-tetrahydroxy-tetrahydro-2*H*-pyran-2-carboxylate, followed by deprotec-

tion as described [13] by or by biosynthesis as described below.

Kinetic measurements

Rates of reaction of methyl esters and acyl glucuronides were measured using an HPLC–UV method. Ten microlitre of a 5 mM solution of compound in DMSO was added to 1 mL of pH 11 sodium hydroxide solution in the case of methyl esters or 0.1 M pH 7.4 sodium phosphate buffer solution in the case of acyl glucuronides, and mixed thoroughly on a vortex mixer. The solution was injected onto an Agilent 1100 HPLC system at appropriate time intervals and the solution was maintained at 25 °C. The chromatographic peak corresponding to the un-reacted starting material was quantified over the time course of the experiment. Plotting of the natural log of peak area against time resulted in pseudo-first order rate constants for the disappearance of methyl ester or acyl glucuronide. Acyl glucuronide degradation profiles were fitted using ModelMaker v4.0 (Modelkinetix). The program used the Runge–Kutta method, and the parameters were obtained using a least squares estimation.

In vivo studies

All in vivo work was subject to internal ethical review and conducted in accordance with Home Office requirements under the Animals Scientific Procedures Act (1986) and allowed a minimum of 1 week acclimatisation. Healthy virus antibody-free male Sprague–Dawley rats were obtained from Charles River (Margate, UK). They were housed in a light-controlled room, kept at a temperature of 19 °C ± 2 °C and 55% ± 10% humidity. They received a Teklad 2021 diet (Harlan) and had access to water ad libitum.

Biosynthesis of acyl glucuronides

Rats (250–350 g) were surgically prepared under non-recovery isoflurane anaesthesia. The bile duct was cannulated and a cannula was also implanted into the jugular vein for the dosing of compound. Carboxylic acid precursors were prepared at 10 mg/mL in bicarbonate buffer (pH 10):dimethylacetamide (DMA) (80:20) and were given as a bolus dose at 10 mg/kg. Bile was collected into vials containing 1 µL of phosphoric acid and immersed in dry ice in order to stabilise any acyl glucuronide formed. Collection occurred over 3 h at 0–15, 15–30, 30–60 min then every hour thereafter, to minimise bile salt contamination. Purification by reverse phase HPLC gave the pure 1 *β-O*-acyl glucuronide (AG).

Quantitative structure–property relationship (QSPR) modelling of reactivity

The statistical method employed to generate the QSPR model was Partial Least Squares (PLS) using SIMCA-P software (Umetrics) [14]. The molecular structure of 43 compounds was used to calculate ^{13}C NMR chemical shift and other electronic parameters as described below. Steric descriptors for the model were generated by stripping the R-groups from the compounds in the training and test sets according to the generic structure shown in Fig. 5 which was common to all compounds. Sterimol parameters [15] were then calculated for each of the R-groups and included with the electronic descriptors as *x* variables for PLS analysis. The number of components fitted to the methyl ester hydrolysis rates (*y* data) was determined automatically in SIMCA using a leave many out procedure to assess their individual significance (leave 1/7 of the data points out, rebuilding the model 20 times). To test the robustness of the PLS model a randomisation test was performed 999 times (within SIMCA-P) on the initial observed *y* data.

Calculation of electronic descriptors including carbonyl ^{13}C NMR shift

All calculations were performed using Gaussian 03 (Revision W.06.1, Frisch, M. J et al.; Gaussian, Inc., Wallingford CT, 2004). The structure of the methyl ester was minimised using DFT/B3YLP theory and the 6–31 g(d,p) basis set. In cases where it was not clear that a global minimum had been reached, the minimisation was repeated from different starting geometries to give confidence that a global minimum had been found. Calculation of NMR and electronic parameters (Mulliken and electrostatic charge potential) was carried out using the GIAO method using DFT/B3YLP theory and the 6–31 + g(d,p) basis set. All calculations were performed in the gas phase as a reasonable approximation to chloroform solution. Referencing of the NMR shifts was to tetramethylsilane, calculated under the same conditions. Minimisations typically took around 12 h and chemical shift calculations around 6 h on a dual core PC running at 3 GHz.

NMR measurements

Carbonyl ^{13}C chemical shifts were measured in chloroform solution at a concentration between 2 and 10 mg/mL typically using a Varian UnityInova spectrometer at a carbon frequency of 125 MHz and a probe temperature of 25 °C. Spectra were referenced to tetramethylsilane at 0.00 ppm.

Results

Reactivity of AGs

To investigate the reactivity of the series of interest, Tolmetin and Diclofenac AGs as well as two AGs of proprietary AZ compounds A and B conforming to the general structure shown in Fig. 5 were synthesized. The observed disappearance of parent AG followed 1st order kinetics and was fitted to give the degradation rate constants shown in column 1 of Table 1. These studies were performed under standard physical chemistry conditions of 298 K rather than at physiological temperature to give a wide dynamic range of measurements. We obtained the full degradation profile of the four compounds, an example of which is shown in Fig. 2a. The experimental points could be fitted to the known degradation pathways of AGs (Fig. 2b), to yield the rate constants for the first rearrangement k_{12} and pseudo first order rate constant for hydrolysis k_{10}, Table 1. The overall AG degradation process (k_{obs}) is dominated by the first rearrangement (k_{12}) in all four cases, with k_{12} being faster that k_{10} by a consistent ratio of 12–15. As the three parameters k_{obs}, k_{10} and k_{12} correlate strongly with the each other (k_{12} with k_{10}, $r^2 = 0.999$; k_{obs} with k_{10} $r^2 = 0.984$ and k_{obs} with k_{12} $r^2 = 0.988$), any of the rate constants could be used to rank reactivity, with k_{obs} being the most convenient to determine. It was therefore concluded that measuring further full degradation profiles was unnecessary.

An additional set of AGs derived from marketed NSAIDs were biosynthesised and used as reactivity benchmarks. When combined with the synthesised AGs above, this full set included both compounds not known to be associated with suspected reactive metabolite mediated idiosyncratic toxicity such as Ibuprofen, and those that have already been withdrawn from the market due to adverse events such as Suprofen. Due to difficulties encountered in purification, Zomepirac was ultimately excluded from this set, leaving eight compounds. The degradation rate of these AGs were measured at 298 K in pH 7.4 phosphate buffer which represented convenient conditions for the range of reactivities observed (Table 2).

Table 1 Degradation, hydrolysis and migration rates of AGs at pH 7.4, 298 K

Compound	k_{obs}/h^{-1}	k_{12}/h^{-1}	k_{10}/h^{-1}	Ratio k_{12}/k_{10}
AZ1	1.62	1.52	0.10	15
AZ2	2.60	2.93	0.19	15
Tolmetin	0.33	0.32	0.03	12
Diclofenac	0.22	0.22	0.02	12

Table 2 Half lives for methyl ester hydrolysis at 298 K (pH 11), AG degradation rate pH 7.4, 298 K, calculated AG half-life at 310 K (37 °C) using the Arrhenius equation, and known literature AG degradation half-lives

Compound	Methyl ester $T_{1/2}$ (min)	AG $T_{1/2}$ (h)	AG $T_{1/2}$ (h) 310 K (Calc)	AG $T_{1/2}$ (h) 310 K (Lit)
Ibuprofen	36.5	18.1	3.8	3.3
Tolmetin	3.7	2.1	0.4	0.26
Diclofenac	3.7	3.1	0.6	0.51
Ketoprofen	14.5	8.7	1.8	1.45
Fenoprofen	29.0	16.3	3.4	1.93
Suprofen	11.6	7.1	1.5	–
AZ1	0.6	0.27	N/A	–
AZ2	2.9	0.45	N/A	–

Use of methyl esters as AG surrogates

The hydrolysis rates of the corresponding methyl ester derivatives were examined to see if these simpler derivatives could act as useful kinetic surrogates. The hydrolysis rates of methyl esters derived from the same NSAID acids used to synthesise the above AGs were measured (298 K, pH 11 NaOH) and compared to the AG degradation kinetics above (Table 2, Fig. 3). The higher pH used for ester hydrolysis kinetics gave convenient conditions for the range of half lives to be measured. The AG degradation half lives were well correlated to the methyl ester hydrolysis half lives ($r^2 = 0.954$) and showed that the simpler methyl ester derivatives were indeed useful AG reactivity surrogates (Fig. 4).

Relationship between ^{13}C NMR shifts and methyl ester hydrolysis rates

Given the observation that methyl ester hydrolysis rates correlate with AG degradation, a larger set of methyl esters conforming to the general structure shown in Fig. 5 was synthesised, and hydrolysis rates determined. These simpler derivatives enabled study of a wider selection of substructures across diverse physicochemical property space demonstrating the importance of both electronic and steric factors to the rate of ester hydrolysis. Additionally, the ^{13}C NMR shift of the ester carbonyl carbon was measured as previous reports have illustrated a good correlation between ester hydrolysis rates and the corresponding ^{13}C NMR shifts [16, 17]. The methyl ester hydrolysis rates/^{13}C NMR shift (measured) correlation for this new data set is shown in Fig. 4a and b.

Predictions of ^{13}C NMR shifts

Ab initio calculation of NMR chemical shifts is becoming widely established [18–21]. Successful calculation of ^{13}C

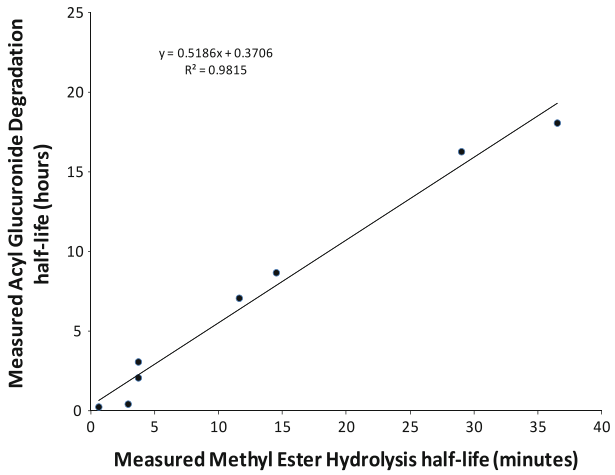

Fig. 3 Correlation of measured methyl ester hydrolysis half life with AG degradation half life

shifts would allow us to build a complete in silico model removing the need for synthesis of unsuitable compounds. Figure 6 shows the highly significant correlation between measured and calculated NMR shifts for the methyl esters in this study ($R^2 = 0.87$).

In silico prediction of glucuronide degradation rate

With the eight methyl esters in Table 2 reserved to test the models predictivity, the remainder were used as the training set to build a partial least squares model for the prediction of ester hydrolysis rate. The descriptors in the final model included calculated ^{13}C NMR carbonyl shift and steric parameters (Supplemental data). This model is shown in Fig. 7 and has a root mean square error (RMSE) of 0.4 and $r^2 = 0.73$. To test the predictivity of the model we used the methyl esters detailed in Table 2 (and Zomepirac) as an independent test set, and an RMSE of 0.17 (and $r^2 = 0.75$) was obtained in prediction of their ester hydrolysis rates. Thus, predicted methyl ester hydrolysis rate can be used as a surrogate for measured methyl ester hydrolysis rate and be used to predict the AG degradation rate of a compound based upon the correlation obtained between predicted ester hydrolysis rate and AG degradation rate (Fig. 8). The 95% confidence interval in Fig. 8 on prediction of AG degradation rate is relatively large due to ibuprofen being a significant outlier, however, for a compound with a predicted methyl ester hydrolysis half life of 30 min there is a 95% chance that the compound will have an AG degradation half-life of at least 5 h. With the ability to predict methyl ester hydrolysis rates based on structure alone, it is now possible to obtain meaningful in silico prediction of AG degradation rates for novel compounds without synthesis.

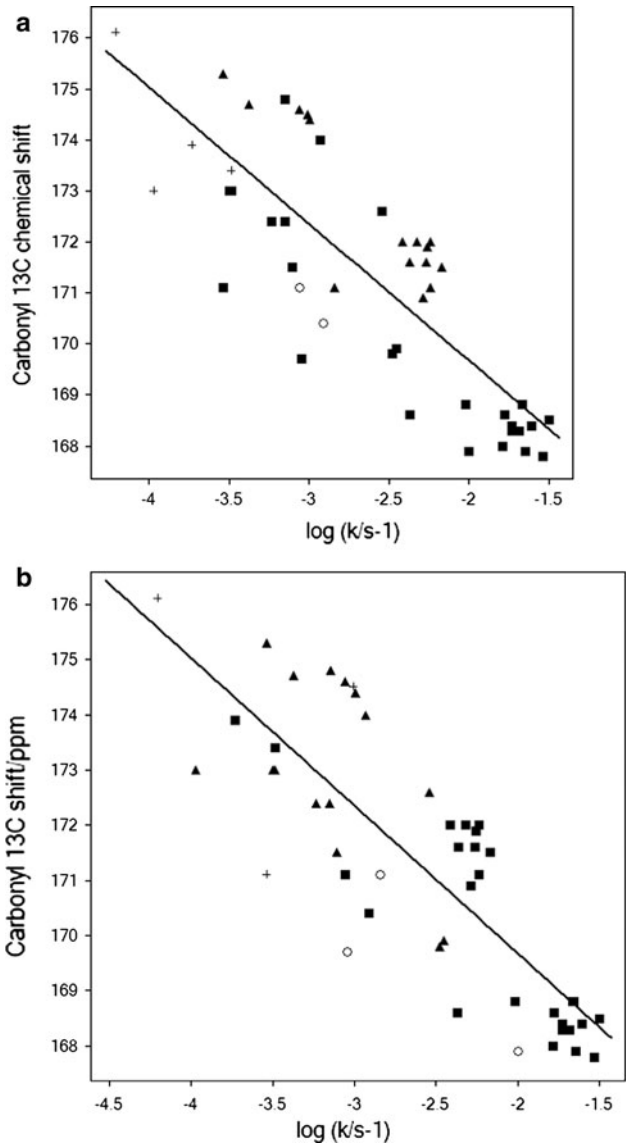

Fig. 4 **a** Correlation of methyl ester hydrolysis rate with ^{13}C NMR shift of carbonyl carbon showing influence of steric effects. Shape is according to α-substitution: *filled square* $R^2 = R^3 =$ H; *filled triangle* $R^2 =$ methyl $R^3 = H$; *open circle* $R^2 =$ ethyl $R^3 =$ H; *plus* $R^2 = R^3 =$ methyl. **b** Correlation of methyl ester hydrolysis rate with ^{13}C NMR shift of carbonyl carbon showing influence of electronic effects. *Shape* is according to R^3 link atom: *filled square* oxygen; *filled triangle* carbon; *open circle* sulphur; *plus* nitrogen

Discussion

The realization that both the well established migration rate but also the hydrolysis rate can be used to rank AG reactivity prompted us to explore the use of methyl esters as surrogates for the AGs. Demonstrating the validity of this simplification for the purpose of ranking the reactivity of potential drug candidates removes the need for complex synthesis of the AG. The measured half lives for degradation of the AGs are in good agreement with previously

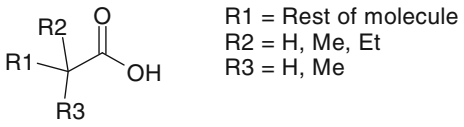

Fig. 5 General structure of compounds for which ester hydrolysis rate was measured

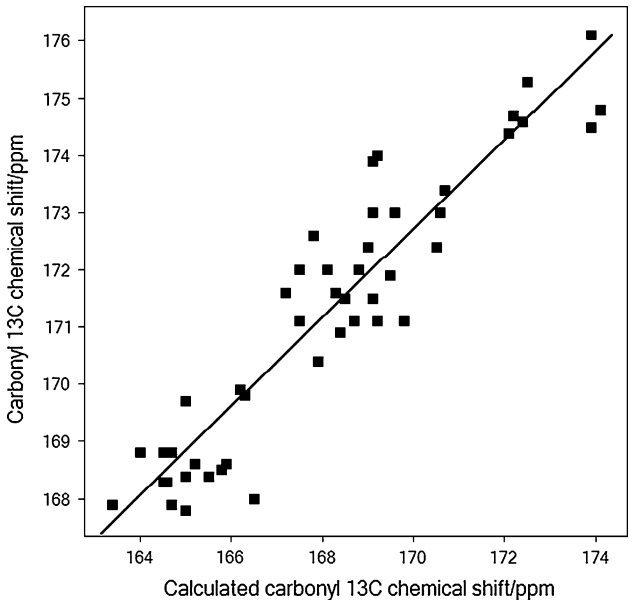

Fig. 6 Measured versus predicted carbonyl ^{13}C NMR chemical shifts

reported values [22], once the half-life measured at 298 K is converted using the Arrhenius equation to a half-life predicted at 310 K (37 °C). This conversion is based on an activation energy of 100,000 Jmol^{-1}, as reported [23] for the dominating acyl migration (noted previously). The correlation between AG degradation rate and measured methyl ester hydrolysis rate is excellent with an r^2 value for the line of fit (forced through the origin) of 0.954. It was therefore considered that ester hydrolysis estimates would allow successful ranking of the AG reactivity of new potential drug candidates. This relationship overcomes the complexity of synthesising AGs which places limitations on chemical diversity and the quantity of data available to build robust QSPR models. Indeed our work includes hydrolysis data on a wider range of chemical structures than previously reported and includes hetero atom containing linkers and a range of mono- and di- substituents adjacent to the acid, as well as the more well known NSAID substructures.

Correlations have been established between the ^{13}C NMR carbonyl shift and AG reactivity for small series' of model benzoic acids and arylalkyl carboxylic acids [11, 12, 24]. Figure 4a and b showed that for our set of methyl esters, measured ^{13}C NMR is correlated with reactivity

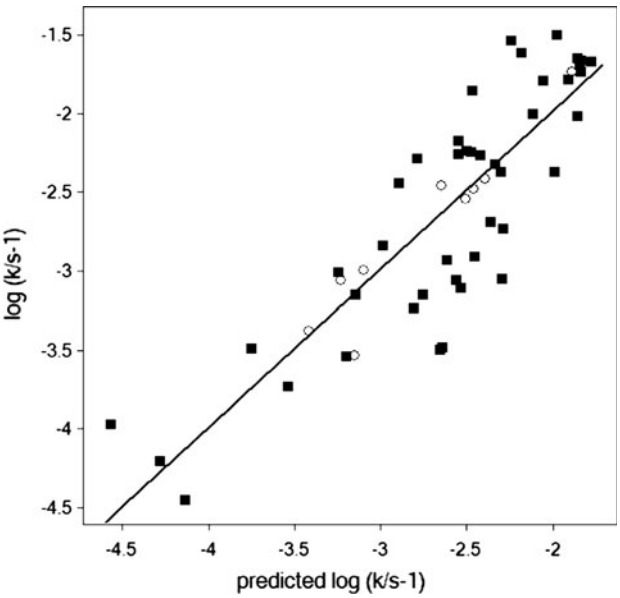

Fig. 7 QSPR model for prediction of methyl ester hydrolysis rate (*filled square* training set, *open circle* test set), training set RMSE = 0.4 (r^2 = 0.73); test set RMSE = 0.17 (r^2 = 0.75)

explaining approximately 60% of the variance. Given the importance of this descriptor, it was encouraging that the measured ^{13}C NMR shift could be also calculated from structure alone using ab initio methods negating the need for chemical synthesis.

Figure 4a and b show the correlation between measured methyl ester hydrolysis rate and ^{13}C chemical shift with points differentiated to show steric effects (Fig. 4a) and electronic effects (Fig. 4b). Figure 4a shows that increasing steric bulk adjacent to the acid reduces hydrolysis rate as expected (a left shift with respect to the x axis for a consistent linker type), whereas in Fig. 4b the more electronegative hetero atom containing linkers display faster rates of hydrolysis (a right shift with respect to the x axis for a consistent substitution pattern). The combination of a less electronegative linker atom such as nitrogen or carbon with two methyl groups adjacent to the acid resulted in some of the least reactive analogues. These were up to 1,000 fold less reactive than the equivalent unsubstituted oxygen linked analogues. Figure 4b also shows that compounds tend to cluster depending on linking atom suggesting that more remote structural changes have a diminishing effect. These qualitative structure reactivity relationships can be used to modulate the potential reactivity of AG metabolites of future candidate drugs.

While ^{13}C NMR shift is an important reactivity descriptor, a substantial component of reactivity remained unexplained. A multivariate PLS model was therefore constructed that included other electronic and steric descriptors in its input. The optimised QSPR model

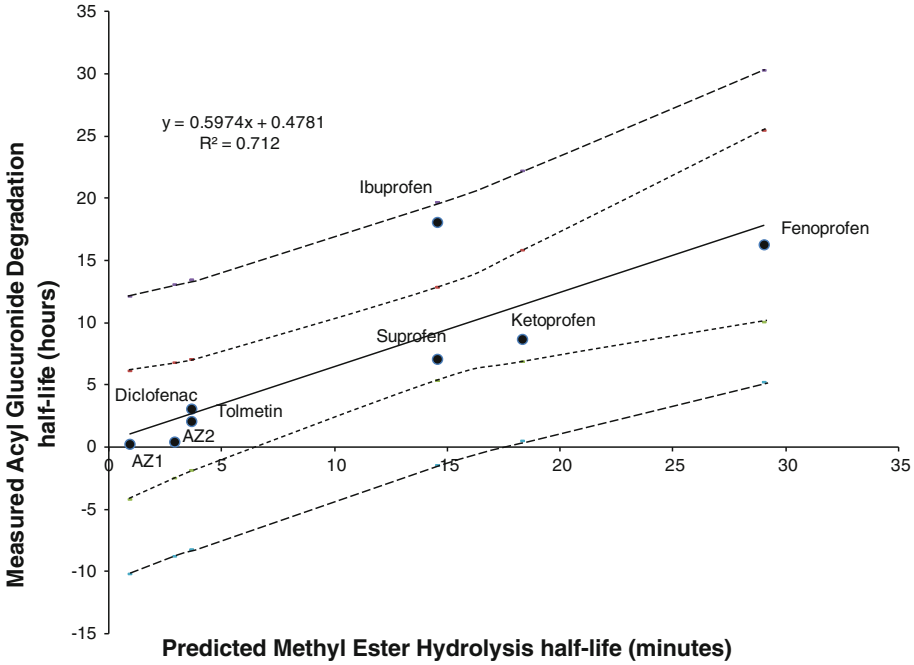

Fig. 8 Measured AG degradation half-life versus predicted methyl hydrolysis half-life (from columns 2 and 3, Table 2) for six NSAIDs and two in house compounds. *Circles* measured data; *solid line* line of best fit; *short dash* 95% confidence interval on mean; *long dash* 95% confidence interval on prediction

generated (Fig. 7) to predict methyl ester hydrolysis rate shows good predictivity over a 2.5 log unit range in reactivity, and the independent test set of NSAIDs is well predicted. Interestingly, no further electronic parameters other than calculated ^{13}C NMR shift were significant in the final model. The work of Yoshioka and Baba highlighted use of the pK_a, calculated partial atomic charge on the acidic proton of the parent acid, and proton and carbonyl NMR chemical shift as important electronic descriptors of AG reactivity depending on the sub series studied [12, 24]. Interestingly Yoshioka found that ^{13}C carbonyl shift correlated with other steric rather than electronic parameters. However, given that for our compound set, the correlation was improved by addition of five steric (Sterimol) descriptors only and no further electronic parameters (Fig. 7 vs. Fig. 4), this suggests that the calculated ^{13}C carbonyl shift descriptor may predominantly describe the electronic properties of our compounds. Steric descriptor PLS coefficients for R^2 and R^3 substituents adjacent to the acid are negative with respect to the hydrolysis rate constant, reflecting that increase in steric bulk around the carbonyl centre leads to a decreased reaction rate. The scaled PLS model coefficients are tabulated in the Supplemental material.

It should be noted that as our correlations are based on methyl ester hydrolysis rates, they do not take account of the stereochemical implications and therefore cannot predict the small differences ($\sim$2 fold) in degradation rate that have been observed for AG metabolites of some acid enantiomers [23].

The observation that (a) ^{13}C NMR shift can be reliably predicted; (b) in combination with steric descriptors, ester hydrolysis rates can be predicted and (c) this is correlated with AG degradation rate led us to conclude that a purely in silico prediction of AG reactivity could be achieved. Due to the limited data sets of AG degradation data available in the literature, the novel methodology that we have described allows the ranking of future diverse chemistry targets with a strong association to gold-standard AG reactivity data. Errors associated with new predictions using this approach will arise from the regression line in Fig. 8, however, for a compound with a predicted methyl ester hydrolysis half life of 30 min there is a 95% chance that the compound will have an AG degradation half-life of at least 5 h. Increasing the dataset of AG degradation and predicted methyl ester hydrolysis half-life may reduce the confidence intervals on prediction.

It has been possible to predict the AG degradation rate from calculated descriptors and the use of a simple regression for 6 NSAIDs and two in-house compounds. The model contains a useful range of mono- and di-substituents alpha to the carboxylic acid group and also C, S, O & N based linkers (beta groups) giving substantial broadening beyond more limited sets of NSAIDs disclosed in previous literature. Nevertheless, we would expect the applicability of our method to be limited to the chemical scope used to build the model and not be necessarily relevant, for example, to benzoic acid derivatives. The range of descriptor values used to build the PLS model (Supplemental data) provides additional guidance regarding the property space covered. Ongoing work could expand the dynamic range of the model still further. The possibility of the use of classification models for early triaging of virtual libraries could also be considered.

This approach has proved highly successful in the ranking of potential drug candidates, and demonstrates the principle that for ranking and prioritization purposes it is not always necessary to conduct lengthy AG synthesis and degradation measurements. Synthesis of important examples should be enough for the medicinal chemist to assess series of compounds, with the bulk of reactivity tuning taking place on an in silico basis.

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