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^{1}H NMR studies on the reductively triggered release of heterocyclic and steroid drugs from 4,7-dioxoindole-3-methyl prodrugs

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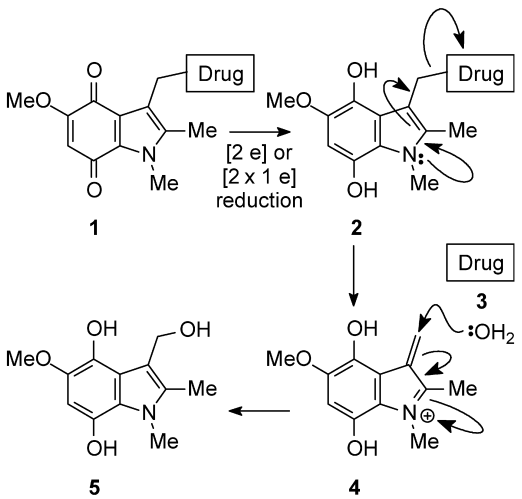
Abstract—Hypoxia is a feature of several disease states, including cancer and rheumatoid arthritis. Prodrug systems which, after bioreduction, selectively release active drugs in these tissues may be important in therapy. An improved preparation of 1,2-dimethyl-3-hydroxymethyl-5-methoxyindole-4,7-dione was developed. Mitsunobu coupling with (5-substituted) isoquinolin-1-ones (potent inhibitors of poly(ADP-ribose)polymerase) gave 1-(1,2-dimethyl-4,7-dioxo-5-methoxyindol-3-ylmethoxy)isoquinolines and *N*-(1,2-dimethyl-4,7-dioxo-5-methoxyindol-3-ylmethyl)isoquinolin-1-ones. Similar coupling with the anticancer drug pentamethylmelamine gave its potential prodrug 1,2-dimethyl-3-(*N*-(4,6-bis(dimethylamino)-1,3,5-triazin-2-yl)-*N*-methylaminomethyl)-5-methoxyindole-4,7-dione. Treatment of sodium prednisolone hemisuccinate with 3-chloromethyl-1,2-dimethyl-5-methoxyindole-4,7-dione gave an analogous candidate prodrug of the anti-inflammatory drug prednisolone. In a chemical model system for bioreduction, $SnCl_2$ in $CDCl_3/CD_3OD$ triggered rapid stoichiometric release of isoquinolin-1-ones from the O-linked prodrugs but not from the N-linked analogues. Use of this system allowed the release process to be monitored in situ by ^{1}H NMR spectroscopy. Diethyl hydrazine-1,2-dicarboxylate was found to reduce Sn^{IV} to Sn^{II}, making the overall reductive release catalytic in tin. The reduced (hydroquinone) prodrug may have a short lifetime under these reductive conditions, meaning that only good leaving groups are expelled. Thus 1-(1,2-dimethyl-4,7-dioxo-5-methoxyindol-3-ylmethoxy)isoquinolines and analogues may be useful as reductively triggered prodrugs.

1. Introduction

Hypoxia, whether acute or chronic, is a feature of several disease states, including cancer and rheumatoid arthritis.[1–4] In the hypoxic regions of solid tumours, viable cells are relatively resistant to radiotherapy and to many chemotherapeutic strategies.[1,2] We[5–8] and others[9–13] have proposed the concept of prodrug systems in which the pharmacophores of drugs are masked by reductively cleaved groups. Thus, in hypoxic tissue, bioreduction of appropriate functional groups triggers release of active drugs selectively in that tissue. These masking groups have included nitroheterocycles[5–8] and indole-4,7-diones (indolequinones).[9,10] In particular, Naylor et al.[10,14] carried out a study on reductively triggered release of a variety of oxygen-based and sulfur-based leaving groups from their (substituted) 4,7-dioxoindole-3-methyl derivatives. Radiolysis was used for the reduction of the quinones in this study; elimination of the leaving groups was found[10] to be predominantly from the two-electron reduction intermediate, the hydroquinone (4,7-dihydroxyindole-3-methyl) derivative. Scheme 1 shows a proposed mechanism for the release of drugs following reduction of the 4,7-dioxoindole-3-methyl prodrugs **1**. When **1** is reduced by two electrons to the dihydroxyindole **2**, the electron-density at the indole nitrogen increases markedly, triggering expulsion of the leaving group **3** ('Drug'), in a reverse-Michael-like process. This expulsion generates an alkenyliminium electrophile **4**,



Scheme 1. Proposed mechanism of reductively triggered release of drugs from general 4,7-dioxoindole-3-methyl prodrugs **1**.

Keywords: prodrugs; indoledione; ^{1}H NMR spectrum; isoquinolin-1-one.

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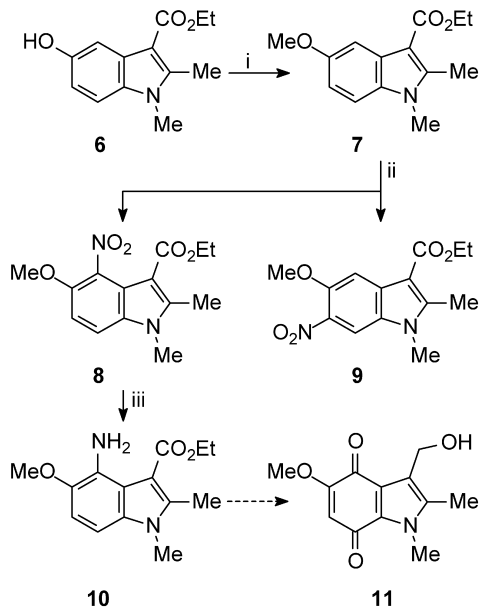


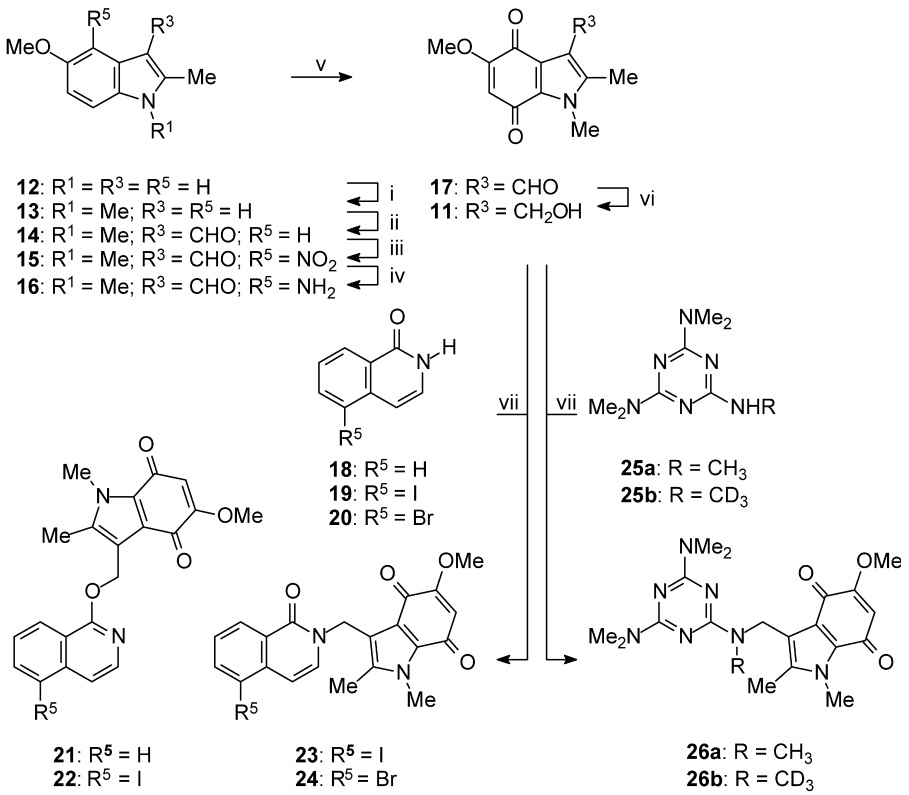
Scheme 2. Investigation of a synthetic route to 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione **11** from the ester **6**. *Reagents and conditions*: (i) KH, MeI, DMF, N_2, 0°C; (ii) HNO_3, AcOH, −10°C→20°C; (iii) Sn, aq. HCl, EtOH, reflux.

which is trapped by water (giving the hydroxymethylindole **5**) or by other ambient nucleophiles. In this process, the rate of release of the drug **3** is predicted to depend on its leaving group ability (as reported by the pK_a of its conjugate acid)[10,14] and on the lifetime of the reduced species **2**.

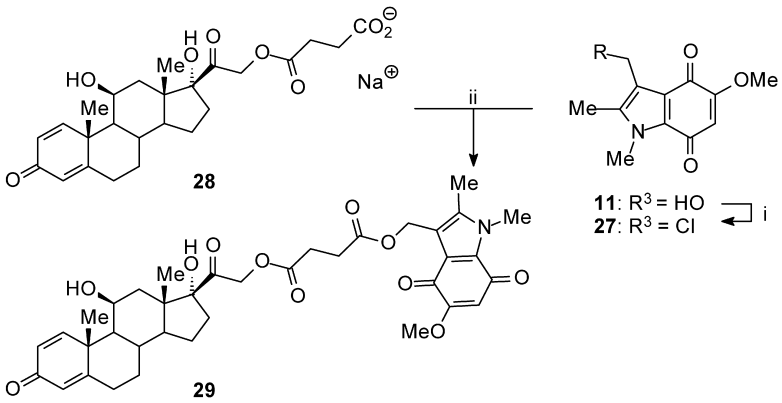
In our previous paper,[8] we reported on the successful release of (5-substituted) isoquinolin-1-ones from 1-(5-nitrothien-2-ylmethoxy)isoquinolines upon reductive triggering with a sodium borohydride/palladium/aqueous propan-2-ol system. These 5-substituted isoquinolin-1-ones are potent inhibitors of poly(ADP-ribose) polymerase (PARP),[15] an enzyme which is important in the control of repair of DNA and inhibition of which is potentially therapeutic in cancer,[16] inflammation,[17,18] haemorrhagic shock,[19] stroke[20] and HIV infection.[21] Isoquinolin-1-ones are also released from their 2-(5-nitrofuran-2-ylmethyl)- and 2-(1-methyl-2-nitroimidazol-5-ylmethyl)- derivatives[5,7] by treatment with the same reductant and with zinc/ammonium chloride, respectively. It is notable that all the reductively triggered release studies reported to date have used HPLC, TLC or ex situ NMR analysis of the products to monitor the course of the release process. In this paper, we report the results of a series of biomimetic reductively triggered release studies on 4,7-dioxoindole-3-methyl prodrugs of isoquinolin-1-ones and of an anti-inflammatory steroid, in which the release of the parent drugs is monitored in situ by ^{1}H NMR spectroscopy.

2. Results

The first aspect of the present work was to develop an efficient synthesis of the indole-4,7-dione trigger unit in a form suitable for attachment to the drug moieties. Synthesis of the initial target, 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione **11** has been reported by Naylor



Scheme 3. An efficient synthetic route to **11** and Mitsunobu couplings giving the isoquinolinone-derived prodrugs **21**–**24** and the pentamethylmelamine-derived prodrugs **25**,**26**. *Reagents and conditions*: (i) NaH, MeI, DMF, Ar, 60°C; (ii) $POCl_3$, DMF, reflux; (iii) fuming HNO_3, AcOH, 5°C→20°C; (iv) Sn, aq. HCl, EtOH, reflux; (v) $K_2ON(SO_3)_2$, NaH_2PO_4, Na_2HPO_4, water, acetone; (vi) $NaBH_4$, MeOH, Ar, then air; (vii) Ph_3P, EtO_2CNNCO_2Et, THF, Ar.



Scheme 4. Synthesis of 4,7-dioxoindol-3-methyl prodrug **28** of prednisolone. *Reagents and conditions*: (i) $SOCl_2$; (ii) DMF, reflux.

et al.[10] from ethyl 1,2-dimethyl-4,7-dioxo-5-hydroxyindole-3-carboxylate **6**, in which most of the required substituents are in the required locations on the indole. However, several problems were encountered when this sequence was used (Scheme 2). The phenolic hydroxyl of **6** was deprotonated with potassium hydride and methylated with iodomethane in high yield, as expected. Optimisation of the conditions for nitration of **7** led to a maximum yield of 63% of the required 4-nitro isomer **8**, which could be separated only by careful chromatography from its 6-nitro regioisomer **9** (14% yield). Reduction of the nitro group of **8** with tin under acidic conditions gave the amine **10**; this aminoindole, bearing an *ortho*-electron-donating group, was highly unstable as the free base and decomposed rapidly. Thus, although the later steps of Naylor's route[10] to **11** were found to be efficient, the difficult separation of **8** from **9** and the instability of **10** led to low and unreliable overall yields from this sequence.

Much more effective in reliably delivering good overall yields of **11** was a route modified significantly from an earlier published sequence[22] (Scheme 3). 5-Methoxy-2-methylindole **12** was deprotonated at nitrogen then methylated with iodomethane under moderately forcing conditions to give the intermediate **13**. Vilsmeier formylation with the dimethylformamide/phosphorus oxychloride reagent was found to give a higher yield of the 3-carboxaldehyde **14** with an easier work-up than did the *N*-methylformanilide/phosphorus oxychloride reagent.[22] Nitration with fuming nitric acid in acetic acid was now selective for the 4-position and the intermediate **15** was reduced with tin to **16**. This aminoindole was much more stable and tractable than the analogous ester **10** and could be isolated and stored satisfactorily. Oxidation with Fremy's salt gave the quinone **17**, then reduction of the aldehyde (and, simultaneously, the quinone), followed by air oxidation in situ of the intermediate hydroquinone gave the required 3-hydroxymethylindole quinone **11**. As reported previously,[23] Mitsunobu coupling of **11** with the isoquinolin-1-ones **18**–**20** gave the 1-(4,7-dioxoindolylmethoxy)isoquinolines **21** and **22** and the 2-(4,7-dioxoindolylmethyl)isoquinolin-1-ones **23** and **24**. A similar coupling of **11** with pentamethylmelamine **25a**, an anticancer drug,[24] gave the potential prodrug **26a**, in which the 4,7-dioxoindolylmethyl unit is attached to an exocyclic nitrogen, in much higher yield than was obtained for the trideuterio isotopomer **26b**.[25]

Scheme 4 shows the approach to the synthesis of the prodrug **29** for the anti-inflammatory steroid prednisolone. The 3-hydroxymethylindole-4,7-dione **11** was converted to the 3-chloromethyl analogue **27** in high yield with thionyl chloride. This compound did not alkylate any of the three hydroxy groups of prednisolone under a variety of basic conditions, an observation which correlates with the lack of reactivity of this steroid with chloromethylthiophenes.[8] Prednisolone hemisuccinate **28**, the monoester of prednisolone at the primary alcohol with butanedioic acid, is a known prodrug of the therapeutic steroid in its own right,[26] being cleaved efficiently to **11** in vivo by esterases. Alkylation of the sodium salt of **28** with the chloromethylindole **27** gave the required candidate prodrug **29**. The ^1H NMR spectrum of **29** was somewhat complex. However, it provided confirmation that the dioxoindol-3-ylmethyl group had become attached as an ester at the carboxylate, rather than as an ether at either of the two hydroxyls of **28**. The signal for the indole-3-CH_2 protons moved downfield from δ 4.86 to 5.16 in the spectrum of samples in $(CD_3)_2SO$. In the spectra of samples of **29** in $(CD_3)_2SO$ and in $CDCl_3$, these CH_2 protons resonated as a singlet; in contrast, the spectrum run in CD_3OD showed separate doublet signals for these protons, indicating that they are magnetically inequivalent and demonstrating that the indoledione unit was attached to a chiral molecule.

Several reductant systems have been used in 'biomimetic' studies of the reductively triggered release of drugs and other leaving groups from prodrugs. These include $NaBH_4$/Pd in aqueous propan-2-ol,[5–7] $Na_2S_2O_4$,[14] Zn/NH_4Cl[7] and $SnCl_2$,[7] in addition to radiolytic reduction.[14] In particular, Naylor and co-workers have used radiolysis and sodium dithionite in their studies on reductively triggered release from indole-4,7-dione derivatives.[10,22,27] However, all of these studies of release from indolediones and nitroheterocycles have employed either HPLC analysis or ex situ NMR spectroscopy to follow the release profile.

In the present study, $SnCl_2$, which has not previously been used to reduce indolequinones, was used as the reductant; initial experiments used chromatographic methods to report the outcomes of the reductions. Treatment of the potential prodrug **21** with $SnCl_2$ in dilute solution in methanol (Method A) gave a major fully resolved peak in the HPLC chromatogram (Fig. 1) with a retention time (Rt) 4.9 min,

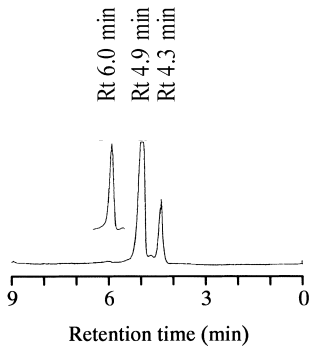


Figure 1. HPLC chromatogram of the reaction mixture after treatment of prodrug **21** with $SnCl_2$ (Method A). The main trace shows the reaction mixture and the inset shows the control peak corresponding to authentic **21** without addition of $SnCl_2$. The major product peak at Rt=4.9 min corresponds to **18**.

after 10 min reaction time, and **21** (Rt=6.0 min) had been completely consumed. This product peak corresponded to the released isoquinolin-1-one **18**. Under the same HPLC conditions, the 3-hydroxymethylindole-4,7-dione **11** gave a peak at Rt=4.6 min.

Having established for the first time in this preliminary experiment that tin(II) chloride is capable of reducing the indole-4,7-dione unit in **21** and hence triggering rapid release of **18**, a series of experiments was devised to study this release in situ by ^{1}H NMR spectroscopy. Owing to its limited solubility in methanol, a solution of **21** in $CDCl_3$ was prepared and $SnCl_2$ was added in portions as a solution in $CDCl_3$. ^{1}H NMR spectra were recorded after each addition. These spectra showed conversion of the prodrug **21** to the isoquinolin-1-one **18** and material which had signals very similar to 3-hydroxymethylindole-4,7-dione **11**. The spectra run after addition of less than 1 equiv. of the reductant showed mixtures of **21**,**18** and this material with integral ratios corresponding to the amount of tin(II) added. Figure 2 shows three spectra from this experiment, corresponding to the original sample of **21**, the sample after addition of 0.5 equiv. of $SnCl_2$ and that after addition of 1 equiv. of the reductant. Upon reduction, the signals for the isoquinoline 3-H and 4-H were changed most markedly, with upfield shifts of 1.1 and 0.8 ppm, respectively. A smaller upfield shift was seen for 5-H (0.3 ppm) whereas the signals for 6-H, 7-H and 8-H were moved by less than 0.1 ppm. The greater effect in the heterocyclic ring probably reflects the change from the 'alkoxypyridine' structure in the prodrug **21** to the bioactive carbonyl 'pyridinone' tautomer in **18**. The signal for the methylene group at position-3 of the indole ring, which was also the linking point between the two subunits of **21**, shifted from δ 5.7 to 4.4, corresponding to 4,7-dioxoindole-3-CH_2OH(D) or 4,7-dioxoindole-3-CH_2O alkyl and giving further evidence that release of the drug moiety **18** had occurred. The signals for the ring protons of the indole unit formed indicated clearly that it was a quinone, with 6-H at δ 5.4, rather than a 4,7-dihydroxyindole.

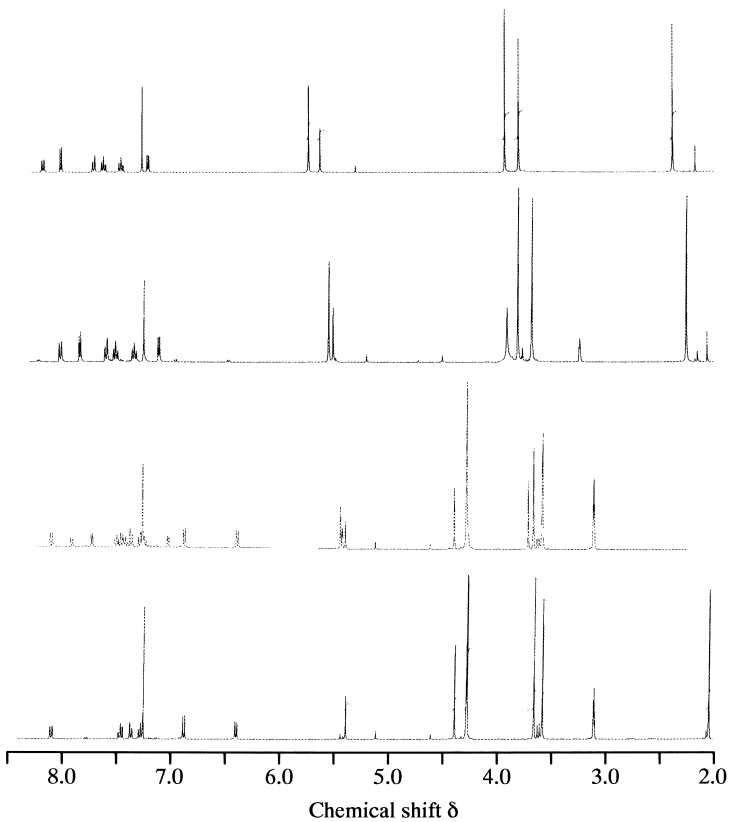


Figure 2. ^{1}H NMR spectra of samples of **21** treated with $SnCl_2$ in $CDCl_3/CD_3OD$, showing stoichiometric and quantitative release of **18**, by Method B. The top spectrum shows **21** in $CDCl_3$ before addition of $SnCl_2$, the upper middle trace shows the spectrum after addition of 0.1 equiv. $SnCl_2$ in CD_3OD and illustrates the effect of the solvent on the chemical shift, the lower middle trace shows the spectrum after addition of 0.5 equiv. $SnCl_2$ and the bottom trace shows the spectrum after addition of 1.0 equiv. $SnCl_2$. The peak at δ 7.3 corresponds to $CHCl_3$, the peak at δ 4.3 corresponds to CD_3OH and the peak at δ 3.3 corresponds to CD_2HOD.

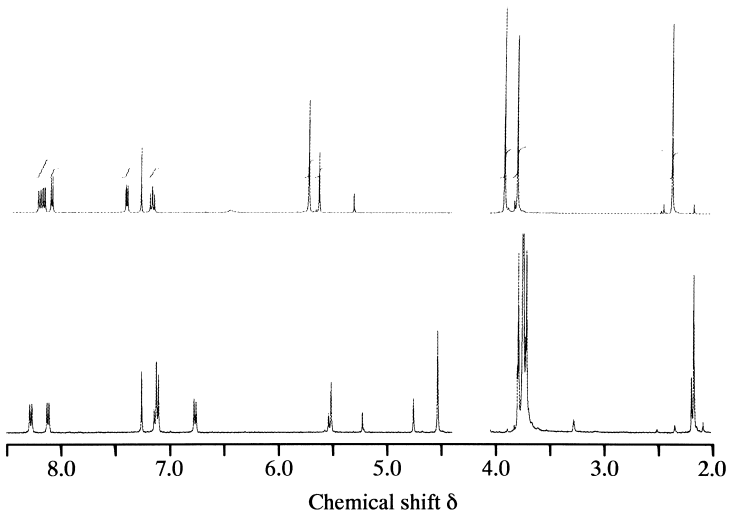


Figure 3. ^{1}H NMR spectra of samples of **22** treated with $SnCl_2$ in $CDCl_3/CD_3OD$, showing stoichiometric and quantitative release of **19**, by Method B. The upper spectrum shows **22** in the solvent mixture before addition of $SnCl_2$ and the lower trace shows the spectrum after addition of 1.0 equiv. $SnCl_2$. The peak at δ 7.3 corresponds to $CHCl_3$ and that at δ 3.3 corresponds to CD_2HOD.

At this point, two control experiments were conducted. First, since the chemical shifts of the protons in the mixture of **11** and **18** produced by the treatment of **21** with $SnCl_2$ did not exactly match those of authentic samples in $CDCl_3$, the spectra were recorded of individual samples of **11** and **18** in a mixture of $CDCl_3$ and CD_3OD of composition corresponding to the solvent mixture after addition of 1 equiv. of reductant in CD_3OD in the reductively triggered release experiment. The chemical shifts now corresponded exactly, indicating that the previous small discrepancies had been due to solvent polarity and dipole effects. Second, it may be postulated that Sn^{2+}, being a Lewis acid, may have catalysed the cleavage of the carbon–oxygen bond by nucleophilic attack of chloride, water or methanol at the methylene, rather than the desired quinone reduction triggering the release of the drug moiety. As a control, a similar NMR-monitored experiment was then carried out using $SnCl_4$, which is a stronger Lewis acid but exhibits no reductive properties. No release of **18** was observed, demonstrating that the release by $SnCl_2$ is, indeed, reductively triggered.

Thus the reductively triggered release from the indolequinone prodrug **21** occurs with 1:1 stoichiometry, implying that a two-electron reduction is required for expulsion of the leaving group under these conditions. No paramagnetic effects were seen in any of the NMR spectra.

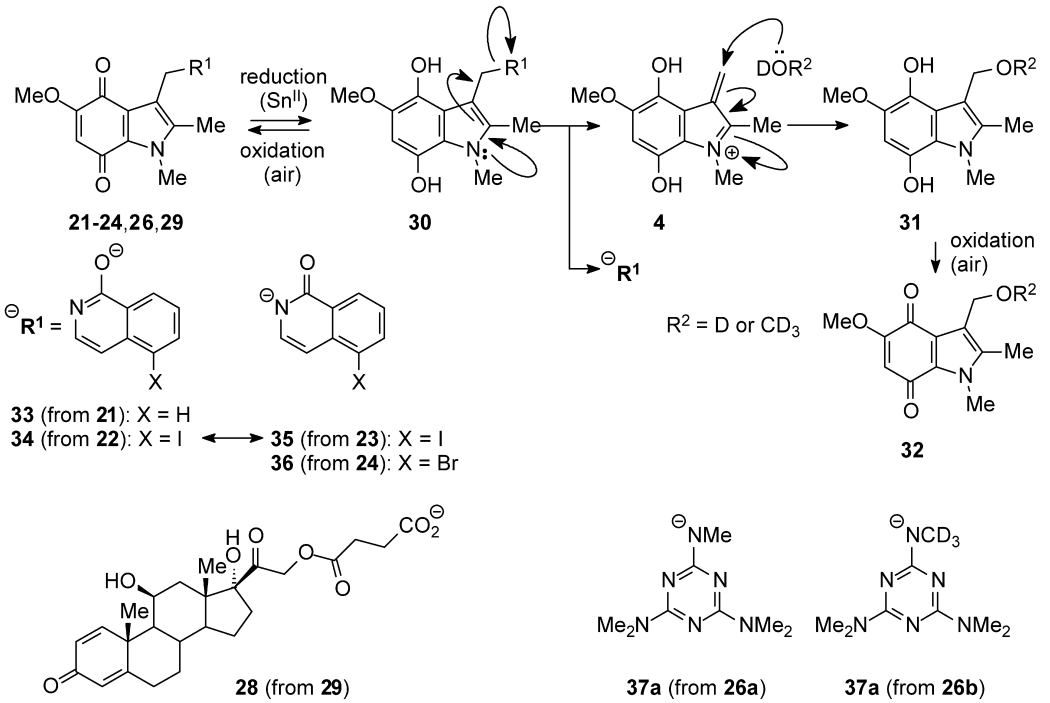
In stark contrast with the above strictly stoichiometric reductively triggered release from **21** with tin(II), an analogous NMR experiment in which **21** was treated with $SnCl_2$ in the presence of ca. 3 equiv. of diethyl hydrazine-1,2-dicarboxylate showed complete release of **18** after the addition of only 0.1 equiv. of the tin reagent. Repetition of the experiment with other ratios of $SnCl_2$ to **21** (0.2–1.0) gave similar NMR evidence of complete reaction. In a control experiment, no reaction was observed in the absence of $SnCl_2$. Thus, in these experiments, the process is apparently catalytic in tin.

The 5-iodo analogue **22** gave similar results when treated with $SnCl_2$ in a mixture of $CDCl_3$ and CD_3OD. The ^{1}H NMR spectra (Fig. 3) of the reaction mixture after additions of the reductant corresponded to mixtures of the starting material **22**, 5-iodoisoquinolin-1-one **19** and the material which had signals very similar to those of **11**. Again, the reduction showed 1:1 (**22**: Sn(II)) stoichiometry. No reductive deiodination took place, in that no signals corresponding to **18** were observed, in contrast with the reductive debromination which takes place during treatment of 5-bromo-2-(1-methyl-2-nitroimidazol-5-ylmethyl)isoquinolin-1-one with an alternative 'biomimetic' reductant system, $NaBH_4$/Pd/aqueous propan-2-ol.[7]

Application of the $SnCl_2/CDCl_3/CD_3OD$ reductant system to the N-linked dioxoindol-3-ylmethyl isoquinolin-1-one candidate prodrugs **23** and **24** gave a markedly different outcome. No changes in the ^{1}H NMR spectra were seen after addition of 1 equiv. of $SnCl_2$, indicating that release of **19** and **20**, respectively, does not occur. More forcing reaction conditions (5 equiv. of $SnCl_2$ and 24 h reaction time; Method C) also gave no release of the isoquinolinones, as shown by the NMR spectra.

Treatment of the potential prodrug **26a** of pentamethylmelamine with $SnCl_2$ by Method B gave mixed results. The ^{1}H NMR spectrum obtained after addition of $SnCl_2$ in CD_3OD comprised only broad peaks. Addition of deuterium oxide caused the spectrum to sharpen enough to allow the identification of individual peaks, by breaking up tin complexes and allowing extraction of the tin salts to the upper aqueous phase. The spectrum of the lower (largely $CDCl_3$ and CD_3OD) phase now showed the presence of unreacted **26a** but also contained signals corresponding to free pentamethylmelamine **26a** and to the 4,7-dioxoindole-3-CH_2OR derivative observed in the release experiments on **21** and **22**, above. However, the spectrum was of too poor resolution to allow quantification of the species present. Reduction of the trideuterio analogue **26b** gave similar results.

Subjection of the indoledione–prednisolone potential



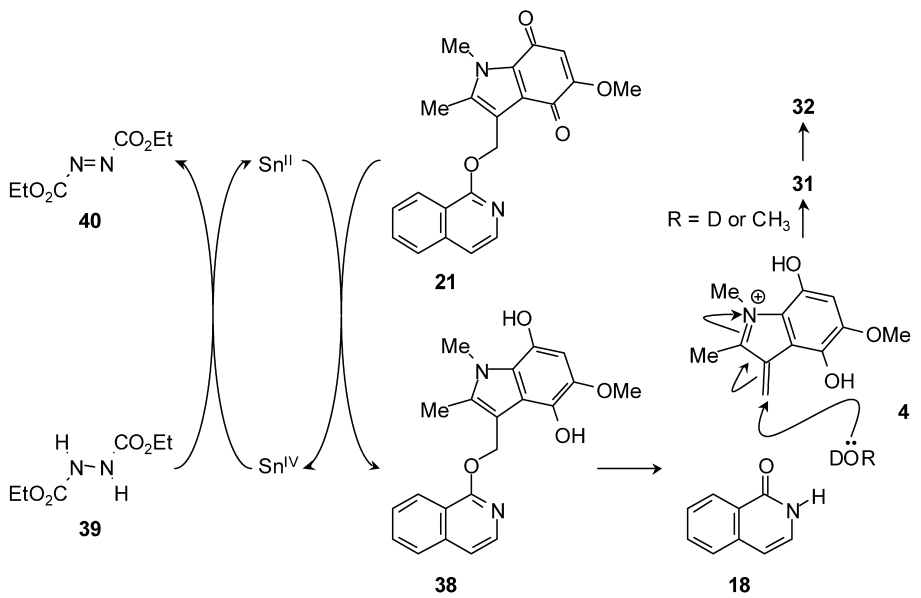
Scheme 5. Proposed mechanism of release of drugs from prodrugs **21**–**24**, **26** and **29**, reductively triggered by stoichiometric $SnCl_2$ in a solvent mixture of $CDCl_3$ and CD_3OD, and illustration of the natures of the respective leaving groups.

prodrug **29** (containing the succinate linker) to the same conditions (Method B) gave spectra in which the chemical shifts were not changed upon addition of the $SnCl_2$. Whereas little change in the chemical shifts of most of the protons in the indole and steroid units would be expected, cleavage of the CH_2–O linkage should change the chemical shift of the indole-3-CH_2 protons and change their multiplicity, since this methylene is no longer in a chiral molecule once cleavage has taken place (note that CD_3OD was the solvent required for this magnetic inequivalence of the CH_2 protons to be evident). A similar lack of reductively triggered release was observed when an alternative reductant, $Na_2S_2O_4$ (a reductant previously used for triggering reductive release from 4,7-dioxoindole-based prodrugs[14]), was used (Method D). Interestingly, hydrolysis/methanolysis of the esters in the linker butanedioate unit was also not observed under both sets of conditions, in contrast to the effects of the $SnCl_2$/MeOH system on the analogous nitrothiophene-based candidate prodrug for prednisolone ((5-nitrothien-2-yl)methyl prednisolone-21-yl butanedioate).

3. Discussion

Schemes 5 and 6 show the rationalisation and proposed mechanisms underlying these results. In the first experiments, the tin(II) reduces the substrates **21**–**24**, **26a**,**b** or **29** (Scheme 5) to the corresponding dihydroxyindole **30**. This can have two possible fates: if the expulsion of the drug-based anion $^{-}R^1$ is rapid, it will leave to give the intermediate **31** and $^{-}R^1$; if not, intermediate **30** may have sufficient lifetime to be re-oxidised by atmospheric oxygen back to the candidate indole-4,7-dione prodrug. Prodrugs **21** and **22** lead to the isoquinoline-1-alkoxide anions **33** and **34**, whereas the release of isoquinolin-1-ones from N-linked candidate prodrugs **23** and **24** would need the corresponding nitrogen-centred isoquinolin-1-one anions to act as leaving groups. However, the isoquinoline-1-alkoxides and nitrogen-centred isoquinolin-1-one anions are only resonance forms of one another and must therefore, be of the same energy. Hence, the difference in the fate of the two types of reduced prodrug cannot have a thermodynamic explanation but must be kinetic; the precise origin of the difference in outcome remains unclear. The indole moiety forms the electrophilic intermediate **4**, which is trapped by an ambient nucleophile. In this case, the ambient nucleophile will either be adventitious D_2O or, more likely, CD_3OD, leading to 4,7-dihydroxyindole intermediate **31**. This, in turn, is oxidised by air to the corresponding indole 4,7-dione **32**. Thus the indole co-product observed in the ^1H NMR experiments is likely to be 1,2-dimethyl-5-methoxy-3-(trideuteriomethoxymethyl)indole-4,7-dione.

Thus the ability of the O-linked prodrugs **21** and **22** to release isoquinolin-1-ones upon reductive triggering, whereas the N-linked analogues **23** and **24** do not under these conditions, can be rationalised. Comparison with the fates of nitroheterocycle-based prodrugs of isoquinolin-1-ones can be made. Reduction of the O-linked prodrugs 1-(5-nitrothien-2-ylmethoxy)isoquinoline, 5-iodo-1-(5-nitrothien-2-ylmethoxy)isoquinoline and 5-bromo-1-(5-nitrothien-2-ylmethoxy)isoquinoline with $NaBH_4$/Pd triggers rapid release of the corresponding isoquinolin-1-ones.[8] However, selective reduction of the nitro groups of the N-linked prodrugs *N*-(5-nitrofuran-2-ylmethyl)isoquinolin-1-one and 5-bromo-*N*-(1-methyl-2-nitroimidazol-5-ylmethyl)isoquinolin-1-one also leads[8] to release of the corresponding isoquinolin-1-ones. This latter observation can be rationalised in that the reduction of the nitro group to



Scheme 6. Proposed mechanism of release of **18** from prodrug **21** in a process which is apparently catalytic in $SnCl_2$, in which **39** is the stoichiometric reductant.

an amine or a hydroxylamine is effectively irreversible. Thus the reduced intermediates have a long lifetime and even poor leaving groups can be released. This is particularly evident in the case of 5-nitrofuran-2-ylmethyl ethers which release even unstabilised alkoxides (notably poor leaving groups) upon reductive triggering.[6]

The apparently catalytic role of tin in the reductively triggered release of **18** from **21** in the presence of excess diethyl hydrazine-1,2-dicarboxylate **39** is rationalised as shown in Scheme 6. In this process, the tin(II) reduces the prodrug **21** to the dihydroxyindol-3-ylmethoxyisoquinoline **38**, as before. This fragments in the usual way, giving the isoquinolin-1-one **18** and the electrophilic intermediate **4** which captures (deuterated) water or methanol to give **31**, which is in turn autooxidised to give the observed 4,7-dioxoindole co-product **32**. The tin(IV) produced is reduced by **39** back to Sn(II) which is then available for another catalytic cycle; diethyl azodicarboxylate **40** is the product of the oxidation of **39**. Tin(IV) has not previously been reported to oxidise hydrazine-1,2-dicarboxylates but this oxidation has been carried out with the analogous lead(IV).[29]

4. Conclusions

Several conclusions can be drawn from this study. First, $SnCl_2$ is a good reductant for biomimetic reduction of indole–quinone prodrugs in chemical model systems. It can also be used catalytically in the presence of a co-reductant, a hydrazine-1,2-dicarboxylate ester. Second, this reductant can be used in a chloroform/methanol solvent system, which dissolves many candidate prodrugs and enables the progress of the reductively triggered release of drugs from the prodrugs to be studied in situ by NMR. Third, the dependence of the outcome on the kinetics of release is confirmed. This system is a much more rigorous test of reductively triggered indolequinone-based prodrugs than are the radiolysis and sodium dithionite systems in which a large excess of reductant is present, effectively prolonging the lifetime of the reduced intermediate and allowing weaker leaving groups to leave. Finally, the O-linked prodrugs **21** and **22** are shown to undergo very rapid and quantitative release of the therapeutically useful PARP inhibitory isoquinolin-1-ones, triggered by reduction. Further work in our laboratory will seek to exploit and adapt this finding to other pharmacologically active agents.

5. Experimental

5.1. General

NMR spectra were recorded using $CDCl_3$ as solvent, except where noted. Melting points were determined using a Reichert-Jung Thermo Galen Koffler block and are uncorrected. Infra-red spectra were recorded as KBr discs. Mass spectra were obtained in the FAB positive ionisation mode. Experiments were conducted at room temperature, unless otherwise stated. Solutions in organic solvents were dried with $MgSO_4$. Solvents were evaporated under reduced pressure. The stationary phase for flash column chromatography was silica gel. HPLC was performed using a semi-preparative column Kromasil 10C18, a Jasco PU-986 preparative pump and Jasco UV-975 detector. MeOH was used as the eluant, with a flow rate of 5.0 mL min^{-1}, the injection volume was 20 μL. 1,2-Dimethyl-3-(isoquinolin-1-yloxymethyl)indole-4,7-dione **21**,[23] 1,2-dimethyl-3-(5-iodoisoquinolin-1-yloxymethyl)indole-4,7-dione **22**,[23] 1,2-dimethyl-3-(5-iodo-1-oxoisoquinolin-2-ylmethyl)-5-methoxyindole-4,7-dione **23**,[23] 1,2-dimethyl-3-(5-bromo-1-oxoisoquinolin-2-ylmethyl)-5-methoxyindole-4,7-dione **24**[23] and 1,2-dimethyl-3-(*N*-(4,6-bis(dimethylamino)-1,3,5-triazin-2-yl)-*N*-trideuteriomethylaminomethyl)-5-methoxyindole-4,7-dione **26b**[25] were prepared as previously described by us.

5.1.1. Ethyl 1,2-dimethyl-5-methoxyindole-3-carboxylate 7. Ethyl 5-hydroxy-2-methylindole-3-carboxylate **6** (6.0 g, 27 mmol) in dry DMF (50 mL) was added under N_2 to a stirred suspension of KH (3.3 g, 82 mmol) in dry DMF (200 mL) at 0°C. The mixture was stirred for 45 min. Iodomethane (11.7 g, 82 mmol) was added dropwise at 0°C and the mixture was warmed to 20°C during 4 h. Saturated aq. NH_4Cl was added and the mixture was extracted thrice with EtOAc. The combined extracts were washed with water. Drying, evaporation and chromatography (EtOAc) gave **7** (5.4 g, 81%) as a white solid: mp 119–120°C (lit.[10] mp 119–121°C); NMR δ_H 1.45 (3H, t, J=7.1 Hz, CH_2CH_3), 2.74 (3H, s, 2-Me), 3.66 (3H, s, NMe), 3.88 (3H, s, OMe), 4.39 (2H, q, J=7.1 Hz, CH_2), 6.87 (1H, dd, J=8.8, 2.5 Hz, 6-H), 7.17 (1H, d, J=8.8 Hz, 7-H), 7.66 (1H, d, J=2.5 Hz, 4-H).

5.1.2. Ethyl 1,2-dimethyl-5-methoxy-4-nitroindole-3-carboxylate 8 and ethyl 1,2-dimethyl-5-methoxy-6-nitroindole-3-carboxylate 9. Conc. HNO_3 (11 mL) in AcOH (41 mL) was added to **7** (5.1 g, 21 mmol) in AcOH (80 mL) at −10°C. The mixture was stirred for 2 h, while warming to 20°C. The suspension was poured onto ice/water and, after 15 min, the solid was collected by filtration and dried. Chromatography (EtOAc/hexane 1:1) yielded **9** (800 mg, 14%) as a pale yellow solid: mp 138–139°C (lit.[28] mp 139–141°C); NMR δ_H 1.46 (3H, t, J=7.0 Hz, CH_2CH_3), 2.79 (3H, s, 2-Me), 3.73 (3H, s, NMe), 4.02 (3H, s, OMe), 4.40 (2H, q, J=7.0 Hz, CH_2), 7.79 (1H, s, 4-H), 7.96 (1H, s, 7-H). Further elution gave **8** (3.8 g, 63%) as a pale yellow solid: mp 187–188°C (lit.[10] mp 189–192°C); NMR δ_H 1.36 (3H, t, J=7.1 Hz, CH_2CH_3), 2.67 (3H, s, 2-Me), 3.66 (3H, s, NMe), 3.91 (3H, s, OMe), 4.29 (2H, q, J=7.1 Hz, CH_2), 6.93 (1H, d, J=9.1 Hz, 6-H), 7.30 (1H, d, J=9.1 Hz, 7-H).

5.1.3. Ethyl 4-amino-1,2-dimethyl-5-methoxyindole-3-carboxylate 10. Compound **8** (2.18 g, 7.5 mmol) was stirred under reflux with Sn powder (4.0 g, 34 mmol) in EtOH (190 mL) and aq. HCl (9 M, 10 mL) for 30 min. The cooled solution was decanted off and was neutralised with aq. $NaHCO_3$. The suspension was added to an equal volume of water, was stirred overnight with CH_2Cl_2 (200 mL) and was filtered (Celite®). The organic layer was dried. Evaporation and chromatography (EtOAc) yielded **10** (1.56 g, 80%) as pale yellow crystals: mp 96–97°C (lit.[10] mp 96–98°C); NMR δ_H 1.41 (3H, t, J=7.0 Hz, CH_2CH_3), 2.64 (3H, s, 2-Me), 3.58 (3H, s, NMe), 3.87 (3H, s, OMe), 4.36 (2H, q, J=7.0 Hz, CH_2), 5.68 (2H, br s, NH_2), 6.52 (1H, d, J=8.7 Hz, 6-H), 6.88 (1H, d, J=8.7 Hz, 7-H).

5.1.4. 1,2-Dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione 11. $NaBH_4$ (160 mg, 4.3 mmol) was added to a suspension of **17** (100 mg, 0.43 mmol) in dry MeOH (40 mL, degassed) under Ar. The mixture was stirred for 1 h and aerated prior to the addition of water (20 mL). The mixture was extracted thrice with CH_2Cl_2. Drying, evaporation, chromatography (EtOAc) and recrystallisation (EtOAc) afforded **11** (50 mg, 33%) as an orange-red solid: mp 200–201°C (lit.[30] mp 199–200°C); NMR δ_H 2.21 (3H, s, 2-Me), 3.81 (3H, s, NMe), 3.88 (3H, s, OMe), 3.87 (1H, t, J=6.7 Hz, OH), 4.59 (2H, d, J=7.0 Hz, CH_2OH), 5.60 (1H, s, 6-H).

5.1.5. 1,2-Dimethyl-5-methoxyindole 13. 2-Methyl-5-methoxyindole **12** (100 mg, 620 μmol) was added slowly to a stirred suspension of NaH (27 mg, 60% in oil, 680 μmol) in dry DMF (15 mL) under Ar. The suspension was heated at 45°C for 10 min and cooled to 20°C. Iodomethane (750 mg, 5.3 mmol) was added during 5 min. The mixture was heated at 60°C for 1 h and poured onto cold aq. $NaHSO_4$ (10%). The mixture was extracted thrice with EtOAc. Drying, evaporation and chromatography (EtOAc/hexane 3:97) gave **13** (90 mg, 80%) as a pale buff solid: mp 70–73°C (lit.[22] mp 73–74°C); NMR δ_H 2.37 (3H, s, 2-Me), 3.59 (3H, s, NMe), 3.82 (3H, s, OMe), 6.16 (1H, s, 3-H), 6.73 (1H, dd, J=2.4, 8.8 Hz, 6-H), 6.93 (1H, d, J=2.4 Hz, 4-H), 7.06 (1H, d, J=8.8 Hz, 7-H).

5.1.6. 1,2-Dimethyl-5-methoxyindole-3-carboxaldehyde 14. $POCl_3$ (169 mg, 1.1 mmol) was added dropwise to dry DMF (80 mg, 1.1 mmol) and **13** (100 mg, 570 μmol) in CH_2Cl_2 (3 mL) and the mixture was heated under reflux for 2 h. Aq. NaOAc (1.0 M, 10 mL) was added and the mixture was stirred for 2.5 h before being extracted with EtOAc. Drying, evaporation and chromatography (EtOAc/hexane 1:1) gave **14** (50 mg, 43%) as an off-white solid: mp 115–117°C (lit.[22] mp 108–110°C); NMR δ_H 2.62 (3H, s, 2-Me), 3.63 (3H, s, NMe), 3.89 (3H, s, OMe), 6.16 (1H, s, 3-H), 6.89 (1H, dd, J=2.3, 8.9 Hz, 6-H), 7.17 (1H, d, J=8.9 Hz, 7-H), 7.80 (1H, d, J=2.3 Hz, 4-H), 10.10 (1H, s, CHO).

5.1.7. 1,2-Dimethyl-5-methoxy-4-nitroindole-3-carboxaldehyde 15. Fuming HNO_3 (90%, 2.0 mL) in AcOH (8.2 mL) was added to **14** (700 mg, 3.4 mmol) in AcOH (55 mL) at 5°C during 5 min. The temperature was allowed to rise to 20°C during 3 h, the mixture was poured onto ice and the precipitate was collected by filtration. Chromatography (EtOAc/hexane 2:1) gave **15** (430 mg, 60%) as a pale yellow solid: mp 235–237°C (lit.[22] mp 236–238°C); NMR δ_H ($(CD_3)_2SO$) 2.71 (3H, s, 2-Me), 3.76 (3H, s, NMe), 3.89 (3H, s, OMe), 7.25 (1H, d, J=9.0 Hz, 6-H), 7.75 (1H, d, J=9.0 Hz, 7-H), 9.90 (1H, s, CHO).

5.1.8. 4-Amino-1,2-dimethyl-5-methoxyindole-3-carboxaldehyde 16. Compound **15** (150 mg, 0.60 mmol) was heated under reflux with Sn powder (350 mg, 2.9 mmol) in EtOH (15 mL) and aq. HCl (3.0 M, 5 mL) for 1 h. The solution was neutralised with aq. $NaHCO_3$ and was extracted thrice with $CHCl_3$. Drying, evaporation and chromatography (EtOAc/hexane 1:1) gave **16** (100 mg, 62%) as a pale yellow solid: mp 155–157°C (lit.[22] mp 152–153°C); NMR δ_H ($(CD_3)_2SO$) 2.61 (3H, s, 2-CH_3), 3.59 (3H, s, NCH_3), 3.75 (3H, s, OCH_3), 6.0 (2H, s, NH_2), 6.56 (1H, d, J=9.0 Hz, 7-H), 6.85 (1H, d, J=9.0 Hz, 6-H), 9.71 (1H, s, CHO).

5.1.9. 1,2-Dimethyl-3-formyl-5-methoxyindole-4,7-dione 17. Potassium nitrosodisulfonate (920 mg, 3.4 mmol) in aq. NaH_2PO_4/Na_2HPO_4 buffer (30.5 mL, 0.3 M, pH 6.0) was added to **16** (150 mg, 680 μmol) in acetone (30.5 mL) and the mixture was stirred for 1 h. The acetone was evaporated. The precipitate was washed with water and cold MeOH and dried to give **17** (120 mg, 75%) as an orange solid: mp 240–242°C (lit.[30] mp 246–247°C); NMR ($(CD_3)_2SO$) δ_H 2.50 (3H, s, 2-Me), 3.82 (3H, s, NMe), 3.88 (3H, s, OMe), 5.89 (1H, s, 6-H), 10.37 (1H, s, CHO).

5.1.10. 1,2-Dimethyl-3-(*N*-(4,6-bis(dimethylamino)-1,3,5-triazin-2-yl)-*N*-methylaminomethyl)-5-methoxyindole-4,7-dione 26a. Diethyl azodicarboxylate (52 mg, 300 μmol) was added dropwise to 2,4-bis(dimethylamino)-6-methylamino-1,3,5-triazine **25a**[24] (59 mg, 300 μmol) and Ph_3P (79 mg, 300 μmol) in dry THF (15 mL) under dry Ar. The mixture was stirred for 15 min. Compound **11** (71 mg, 300 μmol) was added and the mixture was stirred for 16 h. Evaporation and chromatography (EtOAc) afforded **26a** (49 mg, 40%) as an orange glass: IR ν_{max} 1680, 1690, 1470 cm^{-1}; NMR δ_H 2.21 (3H, s, indole 2-Me), 2.94 (3H, s, melamine NMe), 3.11 (12H, s, 2×NMe_2), 3.81 (3H, s, indole 1-Me), 3.88 (3H, s, OMe), 5.12 (2H, m, CH_2), 5.61 (1H, s, indole 6-H); δ_C 9.9, 29.8, 32.8, 35.9, 39.6, 56.4, 106.6, 120.3, 122.5, 137.8, 159.4, 165.2, 178.6; MS *m/z* 414.2253 (M+H) ($C_{20}H_{27}N_7O_3$ requires 414.2256).

5.1.11. 3-(Chloromethyl)-1,2-dimethyl-5-methoxyindole-4,7-dione 27. Compound **11** (500 mg, 2.4 mmol) was stirred with $SOCl_2$ (5 mL) for 30 min. Evaporation and recrystallisation (EtOAc) gave **27** (420 mg, 79%) as an orange solid: mp 203–204°C (lit.[10] mp 204–205°C); NMR δ_H 2.28 (3H, s, 2-Me), 3.80 (3H, s, NMe), 3.89 (3H, s, OMe), 4.86 (2H, s, CH_2), 5.62 (1H, s, 6-H).

5.1.12. (1,2-Dimethyl-4,7-dioxo-5-methoxyindol-3-yl)-methyl prednisolone-21-yl butanedioate 29. Prednisolone-21-hemisuccinate sodium salt **28** (380 mg, 790 μmol) was heated under reflux with **27** (200 mg, 790 μmol) in DMF (50 mL) for 24 h. Evaporation and chromatography (EtOAc/CH_2Cl_2 4:1) gave **29** (210 mg, 40%) as a bright orange glass: IR ν_{max} 3450, 1730, 1650 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 0.76 (3H, s, prednisolone 18-H_3), 1.05 (1H, m, prednisolone 14-H), 1.13 (1H, m, prednisolone 8-H), 1.38 (3H, s, prednisolone 19-H_3), 1.45 (2H, m, prednisolone 7-H_2), 1.75 (2H, m, prednisolone 12-H_2), 2.05 (1H, m, prednisolone 9-H), 2.15 (2H, m, prednisolone 15-H_2), 2.24 (3H, s, indole 2-Me), 2.40 (2H, m, prednisolone 16-H_2), 3.65 (2H, m, prednisolone 6-H_2), 3.85 (3H, s, indole 1-Me), 3.98 (3H, s, OMe), 4.27 (1H, s, prednisolone 11-H), 4.71 (1H, d, *J*=17.5 Hz, prednisolone 21-H), 5.04 (1H, d, *J*=17.5 Hz, prednisolone 21-H), 5.16 (2H, s, indole-CH_2), 5.92 (1H, s, prednisolone 4-H), 5.77 (1H, s, indole 6-H), 6.17 (1H, d, *J*=10.6 Hz, prednisolone 1-H), 7.32 (1H, d, *J*=10.5 Hz, prednisolone 2-H); δ_C 9.9, 17.7, 21.4, 24.3, 29.4, 31.0, 32.5, 34.0, 34.6, 39.8, 44.6, 48.0, 51.7, 55.8, 56.7, 57.3, 68.5, 70.3, 89.9, 106.8, 115.7, 121.8, 122.3, 127.7, 129.3, 138.2, 157.1, 159.7, 170.9, 172.0, 178.0, 186.1, 204.9; MS *m/z* 678 (M+H). An HRMS determination could not be made owing to the extremely low abundance of the M+H ion.

5.2. Release studies

Method A. $SnCl_2$ (0.5 mg) was added to **21** (0.5 mg) in MeOH (2.0 mL). The mixture was stirred at 20°C. Aliquots (0.1 mL) were removed at regular time points and analysed by HPLC.

Method B. Compounds **21**–**26** and **29** (10 mg) were dissolved in $CDCl_3$ (0.6 mL) and transferred to an NMR tube. The ^1H NMR spectrum of each sample was recorded. $SnCl_2$ (10% in CD_3OD) was added in portions of 10 mol%. A ^1H NMR spectrum was run after each addition.

Method C. Compound **24** (10 mg) was dissolved in $CDCl_3$ (0.6 mL) and transferred to an NMR tube and the ^1H NMR spectrum was recorded. $SnCl_2$ (5.0 equiv., 10% in CD_3OD) was added and a ^1H NMR spectrum was run. A further ^1H NMR spectrum was recorded after the homogeneous mixture had been allowed to stand for 24 h.

Method D. Compound **29** (10 mg) was dissolved in $CDCl_3$ (0.6 mL) and transferred to an NMR tube. The ^1H NMR spectrum of each sample was recorded. $Na_2S_2O_4$ (10% in CD_3OD) was added in portions of 10 mol%. A ^1H NMR spectrum was run after each addition.

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