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# Synthesis of 3-Substituted Benzamides and 5-Substituted Isoquinolin-1(2*H*)-ones and Preliminary Evaluation as Inhibitors of Poly(ADP-ribose)polymerase (PARP)

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Abstract—Inhibitors of poly(ADP-ribose)polymerase (PARP) inhibit repair of damaged DNA and thus potentiate radiotherapy and chemotherapy of cancer. 3-Substituted benzamides and 5-substituted isoquinolin-1-ones have been synthesised and evaluated for inhibition of PARP. Reduction of 3-(bromoacetyl)benzamide, followed by treatment with base, gave *RS*-3-oxiranylbenzamide. Reduction of 3-(hydroxyacetyl)benzonitrile with bakers' yeast gave the *R*-diol which was converted to *R*-3-(1,2-dihydroxyethyl)benzamide. Similar reduction of 3-(acetoxyacetyl)benzonitrile led towards the *S*-diol which was converted to its cyclic acetonide. *E*-2-(2,6-Dicyanophenyl)-*N*,*N*-dimethylethenamine was formed by condensation of 2,6-dicyanotoluene with dimethylformamide dimethyl acetal (DMFDMA); cyclisation under acidic conditions afforded 5-cyanoisoquinolin-1-one. Heck coupling of 5-iodoisoquinolin-1-one with propenoic acid formed *E*-3-(1-oxoisoquinolin-5-yl)propenoic acid. 3-Oxiranylbenzamide, 5-bromoisoquinolin-1-one and 5-iodoisoquinolin-1-one were among the most potent inhibitors of PARP activity in a preliminary screen in vitro. © 1998 Elsevier Science Ltd. All rights reserved.

## Introduction

Radiotherapy and many forms of chemotherapy of cancer kill proliferating malignant cells by damaging DNA. Efficient repair of this damage can lead to resistance to these therapeutic strategies.<sup>1</sup> Poly(ADP-ribose) polymerase (PARP) is the enzyme that is responsible for controlling excision repair processes and inhibitors of this enzyme have been shown to act as potentiators to the lethal effects of radiation<sup>2-7</sup> and DNA-damaging chemotherapeutic drugs.<sup>8-11</sup> PARP is abundant in most cell nuclei and ca. 90% of the PARP in cells is bound to chromatin. It comprises a 113 KDa protein with three domains: a 46 KDa DNA-binding domain, a 22 KDa automodification domain and a 54 KDa catalytic domain but acts catalytically as a homodimer. The protein binds to DNA strand-breaks through the two zinc fingers of the DNA-binding domain and must be bound

to DNA to have enzymatic activity.<sup>12,13</sup> PARP catalyses the transfer of ADP-ribose units from its substrate NAD<sup>+</sup> 1 (Figure 1) to several protein acceptors. Most of these are nuclear proteins involved in chromatin architecture and DNA metabolism (heteromodification) but the main protein to be poly(ADP-ribosyl)ated is PARP itself (automodification).<sup>14,15</sup>

Most known inhibitors of PARP mimic the nicotinamide moiety of the substrate **1**. The first enzyme-selective inhibitor<sup>16</sup> was 3-aminobenzamide **2** (Figure 1) (IC<sub>50</sub>  $22 \,\mu$ M<sup>17</sup>). Further structure-activity studies on substituted benzamides<sup>16–20</sup> showed that optimum activity in this monocyclic series was achieved with an electron-donating group at position 3 but with no substituent in positions 2 or 4–6 on the benzene ring. Recognition that the conformation of the benzamide pharmacophore was important<sup>20</sup> led to development of 5-substituted isoquinolin-1-ones<sup>17,20</sup> and 3,4-dihydroisoquinolin-1-ones<sup>20</sup> **3** and of 2,8-disubstituted quinazolin-4-ones<sup>17,21,22</sup> **4** (Figure 1), which are some 10- to 50-fold more potent as inhibitors (**3** R<sup>1</sup>=OH, R<sup>2</sup>=H,

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Figure 1. Structures of NAD<sup>+</sup> 1 and known inhibitors of PARP 2–5.

X=CH: IC<sub>50</sub> 0.14 $\mu$ M; 4 R<sup>1</sup>=OH, R<sup>2</sup>=Me, X=N: IC<sub>50</sub> 0.44 $\mu$ M). In 3 and 4, the heterocyclic ring constrains the conformation of the benzamide with the N–H bond *trans* to the carbonyl-arene bond. An interesting new approach to maintaining this required conformation was developed by Griffin et al.<sup>22</sup> who used a hydrogen-bond in the benzoxazolecarboxamides 5 (Figure 1) (5 R=Ph: IC<sub>50</sub> 2.1  $\mu$ M). A large study<sup>17</sup> of a

variety of cyclic amides and related compounds confirmed the need for the *trans*-arylamide structural element; large planar lactams such as 4-amino-1,8naphthalimide (IC<sub>50</sub> 0.18  $\mu$ M) and phenanthridin-6(5*H*)one (IC<sub>50</sub> 0.30  $\mu$ M) were particularly potent as inhibitors of PARP. In structures **2–5** (Figure 1), the consensus pharmacophore is shown in bold.

As part of our search for more active radiosensitising drugs,<sup>23,24</sup> we rationalised that design of a mechanismbased irreversible inhibitor of PARP may be more effective in inhibiting DNA repair in a therapeutic context. Scheme 1 shows a proposed PARP-catalysed mechanism in which the nicotinamide leaves the substrate NAD<sup>+</sup> 1, giving an intermediate oxonium ion 6. The incoming nucleophile (Nü), which is a carboxylate side-chain in the acceptor protein or the 2'-OH of the ribose of the growing poly(ADP-ribose) chain, then approaches from the  $\beta$ -face. A benzamide or isoquinolin-1-one 7 with an electrophilic substituent E (Scheme 1) may trap the nucleophile of the Glu or the poly(ADP-ribose) chain, capping the polymer, or may react with an appropriately placed nucleophile in the active site of PARP. We present here the synthesis and evaluation of benzamides and isoquinolin-1-ones with substituents in the 3- and 5-positions, respectively, which are electrophilic or which may make other interactions with the NAD<sup>+</sup>-binding site of PARP.



Scheme 1. Chemical mechanism for the PARP-catalysed formation of poly(ADP-ribose) from  $NAD^+ 1$  via the intermediate oxonium ion 6.

## **Chemical synthesis: Benzamides**

The simplest target benzamide with a benzylic electrophile in the 3-position for potential irreversible inhibition of PARP is 3-(chloromethyl)benzamide 9, which was prepared in high yield by treatment of the corresponding acyl chloride  $\mathbf{8}$  with ethereal ammonia, using a short contact time to avoid substitution at the benzylic position (Scheme 2).

Scheme 2 shows the synthetic routes to benzamides with more complex functional groups in the 3-position,

corresponding to the amine in the lead compound 3aminobenzamide 2 and to the 5-substituents in the isoquinolinones 3. Target compounds 11, 15*R*, 15*S*, 21, 22 and 24 all have an oxygen atom located at a site designed to be able to make hydrogen-bonding interactions with the active site of PARP corresponding to those of the ribose oxygen of the substrate NAD<sup>+</sup> 1. Since all these target compounds have a 2-carbon unit attached at the 3-position of the benzamide, 3-acetylbenzamide 11 appeared to be a suitable starting material, being also one of the targets. This is not commercially available but was readily synthesised by



Scheme 2. Synthesis of 3-substituted benzamides which are inhibitors of PARP. Reagents: (i) NH<sub>3</sub>, Et<sub>2</sub>O; (ii)  $H_2O_2$ , NaOH, EtOH,  $H_2O$ ; (iii) NaBH<sub>4</sub>, EtOH; (iv) PhI(OAc)<sub>2</sub>, CF<sub>3</sub>CO<sub>2</sub>H, MeCN, H<sub>2</sub>O; (v) bakers' yeast, sucrose, H<sub>2</sub>O; (vi) Br<sub>2</sub>, CHCl<sub>3</sub>; (vii) KOAc, NaI, EtOH; (viii) NH<sub>3</sub>, MeOH, H<sub>2</sub>O; (ix) Me<sub>2</sub>C(OMe)<sub>2</sub>, TsOH; (x) Br<sub>2</sub>, HOAc; (xi) NaOH, H<sub>2</sub>O.

hydrogen peroxide-assisted hydrolysis of the corresponding benzonitrile **10** under basic conditions. Straight forward reduction with sodium borohydride then afforded 3-(1-hydroxyethyl)benzamide **12**, which was required for later development of NMR methods for determination of optical purities of the enantiomers of the corresponding ethane-1,2-diols.

The enantiomeric diols 15R and 15S were important synthetic intermediates and targets in their own right. In 15S, the secondary alcohol may occupy the binding site of the ribose oxygen of  $NAD^+$  1, while the primary alcohol has potential for mimicking the 2'-hydroxyl of that ribose. The crystal structure of the catalytic fragment of chicken PARP has been reported<sup>25</sup> and it is claimed that the binding site and interactions for  $NAD^+$  1 are very similar in PARP and in another ADP-ribosylating protein, diphtheria toxin.<sup>26–30</sup> In the latter, the 2'-OH of the nicotinamide ribose appears to make a weak hydrogen bonding interaction with an amino-acid residue. It was predicted that the enantiomer 15R should bind and inhibit PARP less efficiently and that comparison of the inhibitory data would enable a preliminary test of this hypothesis. Synthesis of the required enantiomeric diols 15R and 15S could, in principle, be achieved though several routes, including asymmetric dihydroxylation of a corresponding styrene and asymmetric reduction of a corresponding substituted acetylbenzamide. Although several methods of asymmetric reduction of alkyl-aryl ketones with 'chemical' reagents are known,<sup>31-33</sup> biotransformations frequently offer more reliably higher enantiomeric excesses. The reduction of ketones by bakers' yeast (Saccharomyces cerevisiae) is one of the most widely used biotransformations for the preparation of enantiomerically pure alcohols.<sup>34</sup> Aromatic α-hydroxyketones have been reduced asymmetrically<sup>35–37</sup> to diols by this organism. For example, (hydroxyacetyl)benzene is reduced to Rphenylethane-1,2-diol in high yield and high optical purity.<sup>35,36</sup> The opposite stereochemistry of reduction is observed when the hydroxy group is esterified.<sup>36</sup> However, the bakers' yeast reduction is intolerant<sup>37,38</sup> of some substituents on the aromatic ring, thus 3-(substituted acetyl)benzamides were discounted as suitable substrates. Relying on the efficient hydrogen peroxidemediated hydrolysis of nitriles to amides,<sup>39</sup> bioreductions of 3-(hydroxyacetyl)benzonitrile 13 and 3-(acetoxyacetyl)benzonitrile 18 were mooted as potentially enantiomerically efficient routes to the target enantiomeric diols 15R and 15S, respectively. 3-Acetylbenzonitrile 10 was hydroxylated directly at the methyl group using the hypervalent iodine reagent I,I-bis(trifluoroacetoxy)iodobenzene (iodosobenzene bis(trifluoroacetate)) developed by Moriarty et al.40 The modest yield of 13 is consistent with the observation<sup>40</sup> of generally inferior yields during hydroxylations of acetophenones

bearing electron-withdrawing groups on the benzene ring. Following the procedure of Manzocchi et al.,<sup>36</sup> 13 was reduced during 7 days in a fermenting preparation of bakers' yeast to the dihydroxyethylbenzonitrile 14*R* with  $[\alpha]_D^{20} = -46.6^\circ$ . In parallel, the ketone 13 was reduced with sodium borohydride in the usual manner to give the racemic diol 14*RS* for use in developing the methods for determination of *ee* by NMR (see below).

To form the S-diol 14S, the acetoxyacetylbenzonitrile 18 was required as substrate for the bioreduction. Bromination of 10 with bromine in chloroform<sup>41</sup> gave a 78%yield of the bromomethyl ketone 16 with 6% of the overoxidation product, the dibromomethylketone 17. Nucleophilic substitution of acetoxy for bromine was effected with potassium acetate in the presence of catalytic sodium iodide, according to the general method of Kaufmann and Müller.<sup>42</sup> In addition to the 64% yield of 18, two other products were recovered and characterised, the methyl ketone 10 and 3-(diethoxyacetyl)benzonitrile 19. The former arises from a reduction and the latter from an oxidation under the reaction conditions and proposed mechanisms for these processes are shown in Scheme 3. Clearly, the iodide acts as a nucleophilic catalyst, forming the iodomethyl ketone 25 as an intermediate. This can suffer three fates. Nucleophilic substitution with acetate to form 18 will be the main reaction early in the reaction, when large amounts of AcO<sup>-</sup> are present. When most of the acetate has been consumed, slower reactions become important, such as reduction of 25 by iodide to give 10 and elemental iodine and substitution by the weaker nucleophile



Scheme 3. Proposed mechanism of formation of the reduced product 10 and the oxidised product 19 during treatment of the 16 with KOAc and NaI.

ethoxide, giving the intermediate ethoxymethyl ketone 26. Formation of the enolate 27 is favoured by the presence of the ethoxy group; 27 can then react with the molecular iodine and substitution of ethoxide for iodine in 28 forms the acetal 19. Treatment of the acetoxyacetylbenzonitrile 18 with fermenting bakers' yeast for a much shorter contact time that that used for 13 afforded a high yield of the S-enantiomer of the monoester 20. This was separated from the trace of 3-(1,2-dihydroxyethyl)benzonitrile which was also formed; this was of unpredictable stereochemistry since it could have arisen from hydrolysis of the acetate ester followed by reduction to the *R*-diol **14***R* or hydrolysis of the acetate of **20** to give the S-diol 14S. The enantiomeric purity of 20 was assessed after conversion to its MTPA ester by treatment with the freshly prepared acyl chloride of R-Mosher's acid.<sup>43</sup> As a control, the derivatisation was repeated using racemic Mosher's acid chloride; the <sup>1</sup>H NMR signals from the R,R diastereoisomeric monoester were observed with identical intensity to those from the S, Rdiastereoisomer. Similar derivatisations of racemic 14RS with enantiomerically pure Mosher's acid chlorides gave identical spectra. Thus 20 was demonstrated to have been formed with >96% ee. The ester was cleaved with ammonia to reveal the S-diol 14S, which had  $[\alpha]_{\rm D}^{20} =$  $+46.6^{\circ}$ . Thus 14R also had ee > 96% (vide supra). As expected, the enantiomeric dihydroxyethylbenzonitriles 14R and 14S were efficiently converted to the corresponding 3-(1,2-dihydroxyethyl)benzamides 15R and 15S, respectively, by hydrogen peroxide-mediated hydrolysis.

The S-dihydroxyethylbenzamide **15S** was cyclised to the ketal **21** with acetone dimethyl acetal under acidic conditions. In this compound, the dioxolane may mimic the ribose ring in the PARP substrate NAD<sup>+</sup> **1**, with ether oxygens occupying the same relative positions in the five-membered rings.

In the synthetic sequence to the 3-oxiranylbenzamide 24, it was essential that the carboxamide be carried through the assembly of the heterocycle, as the conditions required for hydrolysis of the nitrile would be deleterious to the integrity of the oxirane. Bromination of 11 with bromine in acetic acid gave the bromomethyl ketone 22 that, surprisingly, is previously unreported in the literature. Attempts at asymmetric reduction of the ketone using bakers' yeast were unsuccessful but treatment with sodium borohydride, followed by ring-closure of the oxirane under basic conditions gave the racemic oxiranylbenzamide 24 in excellent yield in a one-pot process.

## Chemical synthesis: Isoquinolin-1-ones

The isoquinolin-1-ones have the benzamide core in their structure but have the amide as part of a heterocyclic

fused ring. By conformationally restricting the amide group in this way, the activity of these compounds as inhibitors of PARP is greatly increased.<sup>17,20</sup> An iso-quinolin-1-one with a carbon substituent at the 5-position would be a good precursor for a variety of candidate PARP inhibitors of this general type 7. However, there are few examples of such substituted iso-quinolinones in the literature.

The synthesis of 5-cyanoisoquinolinone 30 has been reported by Wenkert et al.44 This compound gives potential for elaboration of the 5-substituent by hydrolysis to the carboxylic acid 36 or by Pinner reaction a the corresponding ester; reduction and further elaboration could give a variety of benzylic electrophiles. Following the published procedure,<sup>44</sup> 2,6-dicyanotoluene **29** was condensed with ethyl formate under basic conditions, followed by acid hydrolytic workup, to give the isoquinolinone 30 in low yield, together with a significant amount of 5-cyanoisocoumarin 31. Higher yielding approaches to 30 were therefore sought (Scheme 4). The dinitrile 29 was unaffected by moderately forcing Pinner conditions but hydrogen peroxidemediated hydrolysis under controlled conditions gave selectively the monocyanobenzamide 32. Cyclocondensation with a formate synthon was envisaged but 32 failed to react with triethyl orthoformate and, on treatment with the more reactive dimethylformamide dimethyl acetal, afforded a high yield of the benzoylfor-



Scheme 4. Routes to 5-cyanoisoquinolin-1-one 30. Reagents: (i) EtOCHO, KOBu<sup>t</sup>; (ii) H<sub>2</sub>O<sub>2</sub>, NaOH, EtOH, H<sub>2</sub>O; (iii) (MeOH)<sub>2</sub>CHNMe<sub>2</sub>, DMF; (iv) TsOH, PhMe; (v) HCl, MeOH; (vi) KOH, EtOH.

mamidine 33 from condensation with the carboxamide only. An attempt to complete the cyclisation by condensation with the activated methyl group under acidic conditions gave the  $N^1$ ,  $N^3$ -diacylformamidine 34 as the sole isolable product. The mechanistic origin of this unsymmetrical formamidine is unclear but no isoquinolinones were found in the product mixture. Since the carboxamide was proving to be the sole nucleophile in 32, attention was turned to condensation of a formate synthon with the methyl group of 29, in which the nitriles cannot compete as nucleophiles. Condensation of 29 with dimethylformamide dimethyl acetal efficiently led to the E-dimethylaminostyrene 35. Cyclisation to the required 5-cyanoisoquinolinone 30 was then achieved in high yield by treatment with methanolic hydrogen chloride, followed by aqueous workup. The remaining nitrile was, interestingly, completely unaffected by these Pinner reagents. Treatment of 30 under more forcing acidic conditions failed to produce the corresponding ester. In contrast, the nitrile was hydrolysed with base to give the carboxylic acid 36. The insolubility of 36 in common solvents precluded efficient further elaboration from this sequence and alternatives were sought.

Krohn et al.<sup>45</sup> reported the synthesis of benzamide-3-(β-D-riboside) using addition of 3-(4,5-dihydro-4,4-dimethyloxazol-2-yl)phenyl lithium to the protected ribose-derived lactone **41** as the critical step of assembly. An analogueous addition of an aryl lithium derived from an isoquinolinone would represent a rapid entry into isoquinolinones with highly functionalised carbon chains in the 5-position. Lithiation of isoquinolinones is unfeasible, owing to the presence of the carbonyl group. 1-Lithioisoquinolines and 4-lithioisoquinolines are  $known^{46-48}$  to react with carbon electrophiles but 5lithioisoquinoline is hitherto unreported. A two-step sequence (N-oxidation and rearrangement with acetic anhydride) has been described<sup>44</sup> for conversion of isoquinolines to the corresponding isoquinolin-1-ones, although the conditions required are harsh. 5-Bromoisoquinoline 37 (prepared<sup>49</sup> from 5-aminoisoquinoline by diazotisation and treatment with copper (I) bromide) was lithiated by transmetallation with butyl lithium at -116 °C. Treatment of the intermediate **38** (Scheme 5) with a model carbon electrophile, benzaldehyde, gave a moderate yield of the alcohol 39, along with a small amount of isoquinoline 40, derived from quenching unreacted anion 38 during aqueous workup. However, treatment of 38 with 41 gave only products of degradation of the lactone, together with large quantities of isoquinoline 40, suggesting that the highly basic anion 38 had deprotonated the 2-position of the lactone.

The Heck reaction<sup>50,51</sup> was investigated as an alternative metal-mediated carbon–carbon bond forming process for attachment of functionalised carbon chains at the



Scheme 5. Reaction of 5-lithioisoquinoline 38 with benzaldehyde and structure of 41. Reagents: (i) BuLi,  $C_6H_{14}$ , THF; (ii) PhCHO, THF.

5-position of the isoquinolinone pharmacophore. 5-Iodoisoquinolin-1-one 42 was synthesised by one-pot Curtius rearrangement and cyclisation from 3-(2-iodophenyl)propenoic acid at 260 °C, as previously described.<sup>24</sup> The 5-bromo analogue 51 was prepared similarly<sup>24</sup> from 3-(2-bromophenyl)propenoic acid. Although Plevyak and Heck used acetonitrile<sup>51</sup> as the reaction solvent in a sealed tube at 100 °C, the procedure is facilitated by conducting the coupling of 42 with propenoic acid in the boiling homologue propanenitrile to give the isoquinolinone-5-propenoic acid 43 in excellent yield. Attempted extension of this procedure to coupling of more highly functionalised 5-carbon alkenes was less successful. Grignard reaction of R-glyceraldehyde acetonide with vinyl magnesium bromide52,53 gave the allylic alcohol 44 as a 10:7 mixture of diastereoisomers. This contains a five-carbon unit appropriately functionalised for potential later elaboration to mimics of the ribose unit of NAD<sup>+</sup> 1. Palladium-catalysed coupling of alkene 44 to 42 gave a low yield of the expected mixture of diastereoisomers of the alcohol 45 (Scheme 6) which could be separated chromatographically from the ketone 46. The latter arises from Pd-catalysed migration of the C = C double bond of the allylic alcohol 45 into the enolic position, followed by tautomerisation. Similar migration of a C = C double bond also took place during the coupling of 42 with the simpler five-carbon unit, pent-4-enol. The observed product mixture was also complicated in this case by poor regioselectivity during the initial Heck coupling. Product alcohols 47 and 48 were obtained as a chromatographically inseparable mixture and arise from reaction



Scheme 6. Heck couplings of 42 with various alkenes and structure of 51. Reagents: (i) CH<sub>2</sub>CHCO<sub>2</sub>H, Pd(OAc)<sub>2</sub>, Et<sub>3</sub>N, EtCN; (ii) 44, Pd(OAc)<sub>2</sub>, Et<sub>3</sub>N, EtCN; (iii) CH<sub>2</sub>CH(CH<sub>2</sub>)OH, Pd(OAc)<sub>2</sub>, Et<sub>3</sub>N, EtCN.

of the alkene at the less hindered and more hindered ends, respectively, in contrast to the usual sensitivity of the Heck coupling to steric effects.<sup>50</sup> Migration of the C = C double bond along the alkyl chain of **47** and **48** was terminated by enol keto tautomerisation, as for the ketone **46**, giving an inseparable mixture of the isomeric aldehydes **49** and **50**, respectively.

#### **Biological Evaluation and Discussion**

As a preliminary screen, the inhibitory effects of 3-substituted benzamides and 5-substituted isoquinolin-1ones on PARP were examined in vitro at ca. 10 µM concentration. The enzyme was extracted from nuclei isolated from L929 cells (mouse areolar and adipose tissue cells). PARP activity was estimated by incorporation of radioactivity from [3H]adenosine-NAD+ into acid-insoluble macromolecules, according to the method published previously.16 The acid-precipitated radioactivity of the test incubations was expressed as a percentage of the control to which the solvent buffer alone was added. The results are given in Table 1. All the 3-substituted benzamides inhibited PARP activity, as expected.<sup>16</sup> Interestingly, particularly potent inhibition was shown by 11 and 22, which have electronwithdrawing groups in the 3-position. Previous reports<sup>16</sup> have suggested that electron-withdrawing groups (e.g. nitro) are deleterious to activity. Also notably potent was the oxirane 24. All the isoquinolinones inhibited PARP strongly at ca.  $10 \,\mu$ M, with the activity of the enzyme being virtually abolished by 5-iodoisoquinolinone 42 and 5-bromoisoquinolinone 51. However, there was no evidence of increased inhibition of PARP activity by the potentially electrophilic benzamides 9 and 24 and isoquinolinone 43 when the nuclear preparation was incubated with the test compounds for different time intervals up to 30 min before the assay was started by addition of [<sup>3</sup>H]-NAD<sup>+</sup>. These observations suggest that these compounds are not acting as time-dependent irreversible inhibitors of PARP.

A preliminary study of the effect of concentration of **55** on the inhibition of PARP activity showed  $IC_{50}$  ca. 300 nM, which is comparable with the value reported by Suto et al.<sup>20</sup> using a different assay system employing a different source of the enzyme. Indeed, it is notable that a variety of enzyme preparations and assay method have been reported<sup>2–11,16–22</sup> to have been used in the development and evaluation of inhibitors of PARP. Compound **55** was also evaluated for its inhibition of the mono-ADP-ribosylating activity of diphtheria toxin.<sup>54,55</sup> The isoquinolinone (0–250  $\mu$ M) was incubated in buffer with diphtheria toxin (100 ng), its natural

 Table 1. Results of preliminary studies of inhibition of PARP activity by 3-substituted benzamides and 5-substituted isoquinolin-1(2H)-ones

Compd	Inhibition of PARP activity at ca. $10\mu M$
Benzamides	
9	62% @ 10.5 μM
11	83% @ 11.0 μM
15R	32% @ 9.3 μM
15S	44% @ 9.4μM
21	54% @ 10.0 μM
22	84% @ 10.7 μM
24	88% @ 9.2μM
Isoquinolinones	
36	79% @ 13.2μM
42	96% @ 8.0 μM
43	81% @ 11.6μM
51	95% @ 11.2μM
55	92% @ 4.0μM
	$[IC_{50} = ca. 300 \text{ nM}]$

substrate elongation factor 2 (eEF-2)<sup>56,57</sup> (4.0 µg) and  $[^{14}C]$ -NAD<sup>+</sup> in a total reaction volume of 55 µL for 45 min. Trichloroacetic acid was added to precipitate the macromolecular fraction including the eEF-2; this was collected and the incorporated radioactivity was assayed. Compound 55 inhibited this activity with  $IC_{50}$ ca. 80  $\mu$ M, giving an apparent > 200-fold selectivity for inhibition of PARP. Direct comparisons of structural requirements for inhibition of PARP and of diphtheria toxin have not previously been made but Banasik et al.<sup>17</sup> noted selectivities of > 1000-fold for PARP inhibition by 3-hydroxybenzamide and by phenanthridin-6(5H)-one over inhibition of an arginine-specific mono(ADP-ribose)transferase by these compounds. These observations tend to indicate that there are significant differences between the binding site for NAD<sup>+</sup> in PARP and those in the mono(ADP-ribosyl)ating enzymes, in contrast to the claim of similarity by Ruf et al.<sup>25</sup> Clearly, further studies are required in this area.

## Conclusion

Routes have been developed for syntheses of series of 3substituted benzamides and 5-substituted isoquinolin-1ones. In a preliminary screen in vitro, all the test compounds inhibited PARP activity. Further studies are being undertaken to establish the nature of the inhibition by the more potent members of these series; the results of these studies will be published elsewhere. Modifications of the potent lead compounds identified here, by incorporating appropriate targetting moieties or by development of selectively-activated prodrugs, may lead to tumour-selective inhibition of DNA repair and to selective potentiation of radiotherapy and chemotherapy of cancer.

#### Experimental

# General methods

Solutions in organic solvents were dried with anhydrous MgSO<sub>4</sub>. The chromatographic stationary phase was silica gel. IR Spectra were obtained of samples as KBr discs, unless otherwise noted. NMR spectra were obtained of solutions in CDCl<sub>3</sub>, except where noted. Dilute  $H_2SO_4$  refers to a 10% aqueous solution, dilute HCl refers to a 2 M aqueous solution. The brine was saturated. The bakers' yeast was obtained from Phipps Bakery, Bath, UK. The sucrose was 'Silver Spoon' brand obtained from J. Sainsbury p.l.c., Bath, UK. 5-Iodoisoquinolin-1-one **42** and 5-bromoisoquinolin-1-one **51** were prepared as described previously by us.<sup>24</sup> 5-Aminoisoquinolin-1-one was prepared essentially by the method of Wenkert et al.<sup>44</sup>

**3-(Chloromethyl)benzamide (9).** NH<sub>3</sub> in dry Et<sub>2</sub>O (4% w/v, 200 mL) was added to 3-chloromethylbenzoyl chloride (8) (2.85 g, 15 mmol) in Et<sub>2</sub>O (25 mL) during 10 min. The mixture was stirred for 30 min then washed with water, with dilute H<sub>2</sub>SO<sub>4</sub> (2×) and with water and was dried. Evaporation and recrystallisation (EtOAc: hexane) gave **9** (2.27 g, 89%) as white needles: mp 118–120°C (lit.<sup>58</sup> mp 119–120°C); IR 3360, 3190, 1655, 1625, 705 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  4.64 (2 H, s, CH<sub>2</sub>), 6.34 (1 H, bs, NH), 7.30 (1 H, bs, NH), 7.44 (1 H, t, *J*=7.7 Hz, 5-H), 7.54 (1 H, d, *J*=7.7 Hz, 4-H), 7.84 (1 H, d, *J*=7.7 Hz, 6-H), 7.94 (1 H, s, 2-H); MS (EI) *m/z* 171/169 (M).

**3-Acetylbenzamide (11).** H<sub>2</sub>O<sub>2</sub> (27.5% in water, 15 mL, 121 mmol) was added during 30 min to 3-acetylbenzonitrile (10) (5.0 g, 34 mmol) and NaOH (340 mg, 8.5 mmol) in EtOH (50 mL) and water (17 mL) was added, keeping the temperature  $< 50 \,^{\circ}$ C. The mixture was stirred at 50 °C for 1 h. After neutralisation with dilute H<sub>2</sub>SO<sub>4</sub>, the EtOH was evaporated. The residue, in CH2Cl2, was washed with water and with brine and was dried. Evaporation gave 11 (5.48 g, 97%) as white crystals: mp 125.5-126.5 °C; Found: C, 66.1; H, 5.55; N, 8.20. C<sub>9</sub>H<sub>9</sub>NO<sub>2</sub> requires C, 66.25; H, 5.56; N, 8.58%; IR 3380, 3185, 1680, 1655, 1630 cm<sup>-1</sup>; 1H NMR  $\delta$  2.67 (3) H, s, Me), 5.93 (1 H, bs, NH), 6.30 (1 H, bs, NH), 7.58 (1 H, t, J=7.8 Hz, 5-H), 8.06 (1 H, dt, J=7.8, 1.5 Hz, 4-H), 8.12 (1 H, dt, J=7.8, 1.5 Hz, 6-H), 8.40 (1 H, t, J = 1.5 Hz, 2-H; MS (EI) m/z 164.0671  $(\mathbf{M})$  $({}^{13}C_{9}H_{9}NO_{2}$  requires 164.0667), 163.0636 (M)  $({}^{12}C_9H_8NO_2$  requires 163.0633), 148 (100%).

**RS-3-(1-Hydroxyethyl)benzamide** (12). 3-Acetylbenzamide (11) (500 mg, 3.1 mmol) was stirred with NaBH<sub>4</sub> (156 mg, 4.1 mmol) in EtOH (12 mL) at 0 °C for 30 min and at 20 °C for 1 h. The reaction was quenched by addition of dilute HCl and the solvent was evaporated. The residue, in EtOAc, was washed with water (2×) and was dried. Evaporation gave 12 (311 mg, 62%) as white crystals: mp 127–129 °C; IR 3360, 3190, 1670, 1620 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  1.33 (3 H, d, J=6.6 Hz, Me), 4.76 (1 H, q, J=6 Hz, CH), 5.24 (1 H, d, J=4.0 Hz, OH), 7.32 (1 H, bs, NH), 7.38 (1 H, t, J=7.7 Hz, 5-H), 7.49 (1 H, d, J=7.7 Hz, 4-H), 7.72 (1 H, d, J=7.7 Hz, 6-H), 7.86 (1 H, s, 2-H), 7.95 (1 H, bs, NH); MS (EI) m/z 166.0867 (100%) (M) (<sup>12</sup>C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> requires 166.0868).

**3-(Hydroxyacetyl)benzonitrile (13).** 3-Acetylbenzonitrile **10** (1.00 g, 6.9 mmol), PhI(O<sub>2</sub>CCF<sub>3</sub>)<sub>2</sub> (5.9 g, 13.8 mmol) and CF<sub>3</sub>CO<sub>2</sub>H (1.06 mL, 13.8 mmol) were boiled under reflux in water (8 mL) and MeCN (40 mL) for 8 h. The evaporation residue, in EtOAc, was washed with water. The aqueous phase was extracted with EtOAc (3×). The organic extracts were washed with saturated aq NaHCO<sub>3</sub> and were dried. Evaporation and chromatography (EtOAc:hexane, 1:1) gave **13** (310 mg, 28%) as white crystals: mp 102–105 °C; IR 3400–3600, 2200, 1650–1700 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  3.37 (1 H, bs, OH), 4.91 (2 H, s, CH<sub>2</sub>), 7.68 (1 H, t, *J*=7.7 Hz, 5-H), 7.92 (1 H, d, *J*=7.7 Hz, 4-H), 8.15 (1 H, d, *J*=7.7 Hz, 6-H), 8.22 (1 H, s, 2-H); MS (EI) *m/z* 161.0471 (M) (<sup>12</sup>C<sub>9</sub>H<sub>7</sub>NO<sub>2</sub> requires 161.0432) 130 (100%).

**R-3-(1,2-Dihydroxyethyl)benzonitrile (14R).** To a fermenting slurry of bakers' yeast (88 g), water (880 mL) and sucrose (88 g) was added 13 (600 mg, 3.7 mmol). The mixture was stirred at 30 °C for 7 days. Et<sub>2</sub>O (100 mL) and Celite<sup>®</sup> (100 g) were added and stirring continued for a further 15 min. The slurry was filtered and the solids were washed with  $Et_2O$  (2×50 mL). The filtrate was extracted with  $Et_2O$  (5×100 mL). Drying, evaporation and chromatography (EtOAc:hexane 1:1 3:2) gave 14R (268 mg, 44%) as a white solid: mp 64- $66 \,^{\circ}\text{C}; \, [\alpha]_{\text{D}}^{20} - 46.6^{\circ} \, (c \, 1.0, \, \text{CH}_2\text{Cl}_2); \,^1\text{H} \, \text{NMR} \, \delta \, 2.33 \, (2 \, \text{Cl}_2); \,^1\text{Cl}_2 + 100 \, \text{Cl}_2 + 100 \, \text{$ H, bs, OH), 3.62 (1 H, dd, J = 11.4, 7.7 Hz) and 3.81 (1 H, dd, J=11.4, 3.6 Hz)(CH<sub>2</sub>OH), 4.87 (1 H, dd, J=7.7, 3.6 Hz, ArCH), 7.48 (1 H, t, J=7.7 Hz, 5-H), 7.6 (2 H, m, 4,6-H2), 7.71 (1 H, s, 2-H); MS(CI) *m*/*z* 164 (M+H) (100%), 146.

S-3-(1,2-Dihydroxyethyl)benzonitrile (14S). The S-ester 20 (500 mg, 2.4 mmol) was stirred with aq NH<sub>3</sub> (35%, 5.0 mL) in MeOH (15 mL) for 45 min. The volatile materials were evaporated. THF (50 mL) was added and the volatile materials were evaporated; this procedure was repeated twice.  $CH_2Cl_2$  (100 mL) was added. Drying and evaporation gave 14S (360 mg, 92%) as white

crystals: mp 64–66 °C;  $[\alpha]_{D}^{20}$  +46.6° (*c* 2.0, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR  $\delta$  2.86 (2 H, bs, 2×OH), 3.60 (1 H, dd, *J*=11.4, 7.9 Hz) and 3.78 (1 H, dd, *J*=11.4, 3.3 Hz)(CH<sub>2</sub>), 4.84 (1 H, dd, *J*=7.9, 3.3 Hz, ArCH), 7.46 (1 H, t, *J*=7.7 Hz, 5-H), 7.60 (2 H, m, 4,6-H<sub>2</sub>), 7.71 (1 H, s, 2-H); MS (FAB) *m*/*z* 164.0657 (100%) (M+H) (<sup>12</sup>C<sub>9</sub>H<sub>10</sub>NO<sub>2</sub> requires 164.0712).

*RS***-3-(1,2-Dihydroxyethyl)benzonitrile** (14*RS*). Compound 13 (80 mg, 500 µmol) was stirred with NaBH<sub>4</sub> (19 mg, 500 mmol) in EtOH (3 mL) for 3 h. The evaporation residue, in EtOAc, was washed with water. Drying and evaporation gave 14*RS* (70 mg, 86%) as a colourless viscous oil: IR 3340–3410, 2220 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  2.9 (1 H, bs, OH), 3.5 (1 H, bs, OH), 3.60 (1 H, dd, J=11.3, 3.2 Hz) and 3.78 (1 H, dd, J=11.3, 8.1 Hz)(CH<sub>2</sub>), 4.85 (1 H, dd, J=3.2, 8.1 Hz, ArCHOH), 7.47 (1 H, t, J=7.7 Hz, 5-H), 7.6 (2 H, m, 4,6-H<sub>2</sub>), 7.72 (1 H, s, 2-H).

*R***-3-(1,2-Dihydroxyethyl)benzamide (15***R***). The** *R***-diol <b>14***R* was treated with  $H_2O_2$  and NaOH, as for the synthesis of **15***S*, to give **15***R* (quant) as a hygroscopic viscous gum: <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO) 3.45 (2 H, d, J=6.4 Hz, CH<sub>2</sub>), 4.0 (2 H, bs, 2×OH), 4.57 (1 H, t, J=6.4 Hz, ArCHOH), 7.32 (1 H, bs, NH), 7.37 (1 H, t, J=7.7 Hz, 5-H), 7.48 (1 H, d, J=7.7 Hz, 4-H), 7.74 (1 H, d, J=7.7 Hz, 6-H), 7.85 (1 H, s, 2-H), 7.96 (1 H, bs, NH); MS (FAB) m/z 182.0816 (100%) (M+H) (<sup>12</sup>C<sub>9</sub>H<sub>12</sub>NO<sub>3</sub> requires 182.0817).

*S*-3-(1,2-Dihydroxyethyl)benzamide (15*S*). The *S*-diol 14*S* (330 mg, 2.2 mmol), in EtOH (6.5 mL), was stirred at 50 °C for 1 h with aq NaOH (0.5 M, 1.0 mL, 0.5 mmol) and aq H<sub>2</sub>O<sub>2</sub> (27.5%, 0.85 mL, 6.8 mmol). The solution was neutralised with dilute HCl. Filtration and evaporation gave 15S (366 mg, quant.) as a hygroscopic viscous gum: <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  3.45 (2 H, d, *J*=6.4 Hz, CH<sub>2</sub>), 4.0 (2 H, bs, 2×OH), 4.57 (1 H, t, *J*=6.4 Hz, ArCHOH), 7.32 (1 H, bs, NH), 7.37 (1 H, t, *J*=7.7 Hz, 5-H), 7.48 (1 H, d, *J*=7.7 Hz, 4-H), 7.74 (1 H, d, *J*=7.7 Hz, 6-H), 7.85 (1 H, s, 2-H), 7.96 (1 H, bs, NH); MS (FAB) *m*/*z* 182.0822 (100%) (M+H) (<sup>12</sup>C<sub>9</sub>H<sub>12</sub>NO<sub>3</sub> requires 182.0817).

**3-(Bromoacetyl)benzonitrile (16) and 3-(dibromoacetyl)benzonitrile (17).** Br<sub>2</sub> (1.1 mL, 21.3 mmol) was added dropwise to **10** (3.00 g, 20.7 mmol) in CHCl<sub>3</sub> (15 mL) at 0°C during 30 min. The mixture was stirred at 20°C for 2 h. Evaporation and chromatography (EtOAc:hexane, 1:9) gave **17** (500 g, 8%) as white crystals: mp 84.5– 86°C; Found: C, 35.65; H, 1.62; N, 4.58. C<sub>9</sub>H<sub>5</sub>Br<sub>2</sub>NO requires C, 35.68; H, 1.66; N, 4.62%; IR 2240, 1705 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  6.56 (1 H, s, CHBr<sub>2</sub>), 7.68 (1 H, t, *J*=7.9 Hz, 5-H), 7.92 (1 H, dt, *J*=7.9, 1.5 Hz, 6-H), 8.37 (1 H, dt, *J*=7.9, 1.5 Hz, 4-H), 8.42 (1 H, t, *J*=1.5 Hz, 2-H); MS (CI) *m/z* 306/304/302 (M). Further elution gave **16** (3.60 g, 78%) as white needles: mp 70–71 °C (lit.<sup>59</sup> mp 65.5–66.5 °C); IR 2240, 1710 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  4.43 (2 H, s, CH<sub>2</sub>Br), 7.67 (1 H, t, *J*=7.9 Hz, 5-H), 7.90 (1 H, dt, *J*=7.9, 1.5 Hz, 6-H), 8.22 (1 H, dt, *J*=7.9, 1.5 Hz, 4-H), 8.28 (1 H, t, *J*=1.5 Hz, 2-H); MS(CI) *m/z* 224/222 (M).

3-(Acetoxyacetyl)benzonitrile (18), 3-acetylbenzonitrile (10) and 3-(diethoxyacetyl)benzonitrile (19). Compound 16 (5.50 g, 24.5 mmol) was boiled under reflux with KOAc (2.40 g, 24.5 mmol) and NaI (360 mg, 2.4 mmol) in EtOH (100 mL) for 6 h. The evaporation residue, in EtOAc, was washed with water  $(3\times)$ . Drying, evaporation and chromatography (hexane:EtOAc, 4:1) gave 19 (160 mg, 3%) as a pale-yellow oil: IR (film) 2240, 1705 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  1.26 (6 H, t, J=7.2 Hz, 2 ×Me), 3.65 (2 H, dq, J=9.5, 7.2 Hz) and 3.81 (2 H, dq, J=9.5, 7.2 Hz)  $(2 \times CH_2)$ , 7.59 (1 H, t, J = 7.7 Hz, 5-H), 7.83 (1 H, dt, J=7.7, 1.5 Hz, 6-H), 8.39 (1 H, dt, J=7.7, 1.5 Hz, 4-H), 8.51 (1 H, t, J = 1.1 Hz, 2-H); <sup>13</sup>C NMR  $\delta$  15.00, 64.01, 103.68, 112.58, 117.92, 129.14, 133.71), 133.78, 134.07, 135.97, 192.03; MS (FAB) *m*/*z* 234.1137 (M + H) (100%) (<sup>12</sup>C<sub>13</sub>H<sub>16</sub>NO<sub>3</sub> requires 234.1130). Further elution gave 10 (1.15 g, 32%). Further elution gave 18 (3.20 g, 64%) as white crystals: mp 70–72 °C; Found: C, 64.9; H, 4.42; N, 6.76. C<sub>11</sub>H<sub>9</sub>NO<sub>3</sub> requires C, 65.02; H, 4.46; N, 6.89%; IR 2240, 1755, 1710 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$ 2.24 (3 H, s, Me), 5.31 (2 H, s, CH<sub>2</sub>), 7.66 (1 H, t, J = 7.8 Hz, 5-H), 7.90 (1 H, dt, J = 7.8, 1.3 Hz, 6-H), 8.14 (1H, dt, J = 7.8, 1.3 Hz, 4-H), 8.20 (1 H, t, J = 1.3 Hz, 2-H); MS(CI) m/z 204 (M + H) (100%).

S-3-(2-Acetoxy-1-hydroxyethyl)benzonitrile (20). Bakers' yeast (7.5 g) was suspended in water (56 mL) at 30 °C and sucrose (11.2g) was added. To this fermenting slurry, 18 (760 mg, 3.7 mmol) was added and the mixture was stirred for 8h before being filtered through Celite<sup>®</sup>. The filtrate was extracted with Et<sub>2</sub>O  $(4 \times 30 \text{ mL})$ . Drying, evaporation and chromatography (EtOAc:hexane 2:3) gave 20 (650 mg, 85%) as a colourless oil:  $[\alpha]_{D}^{20} + 34.9^{\circ}$  (c 2.9, CH<sub>2</sub>Cl<sub>2</sub>); IR (film) 3400-3500, 2240, 1740 cm<sup>-1</sup>; <sup>1</sup>H NMR δ 2.11 (3 H, s, Me), 3.04 (1 H, bs, OH), 4.12 (1 H, dd, J = 11.6, 7.9 Hz) and 4.30 (1 H, dd, J=11.6, 3.5 Hz)(CH<sub>2</sub>O), 5.01 (1 H, dd, J=7.9, 3.5 Hz, ArCH), 7.49 (1 H, t, J=7.7 Hz, 5-H), 7.62 (2 H, m, 4,6-H<sub>2</sub>), 7.73 (1 H, t, J=1.6 Hz, 2-H); MS (FAB) m/z 206.0869 (M+H) ( ${}^{12}C_{11}H_{12}NO_3$  requires 206.0817), 188 (100%). Further elution gave 3-(1,2dihydroxyethyl)benzonitrile of unknown stereochemistry (14 mg, 2%) as a colourless oil.

S-3-(4,5-Dihydro-2,2-dimethyl-1,3-dioxol-4-yl)benzamide (21). Freeze-dried 15S (62 mg,  $34 \mu \text{mol}$ ) was stirred with 2,2-dimethoxypropane (1.0 mL) and TsOH (1.0 mg) for 3 days. EtOAc (50 mL) was added and the solution was

washed with 10% aq Na<sub>2</sub>CO<sub>3</sub> and with brine. Drying and evaporation gave **21** (55 mg, 82%) as a white solid: mp 128–130 °C;  $[\alpha]_{D}^{20}$  + 39.2° (*c* 2.5, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR  $\delta$  1.50 (3 H, s, Me), 1.57 (3 H, s, Me), 3.70 (1 H, t, J=8.0 Hz, dioxole 5-H), 4.35 (1 H, dd, J=8.0, 6.2 Hz, dioxole 5-H), 5.13 (1 H, dd, J=8.0, 6.2 Hz, dioxole 4-H), 5.71 (1 H, bs, NH), 6.11 (1 H, bs, NH), 7.57 (1 H, t, J=7.6 Hz, Ar 5-H), 7.55 (1 H, dt, J=7.6, 1.5 Hz, Ar 4-H), 7.73 (1 H, dt, J=7.6, 1.5 Hz, Ar 6-H), 7.82 (1 H, t, J=1.5 Hz, 2-H); MS (FAB) m/z 222.1153 (M+H) (100%) (<sup>12</sup>C<sub>12</sub>H<sub>15</sub>NO<sub>3</sub> requires 222.1130), 164.

3-(Bromoacetyl)benzamide (22). Br<sub>2</sub> (2.0 g, 12 mmol) was added to 11 (2.0 g, 12 mmol) in AcOH (180 mL) at 60 °C. The mixture was stirred for 2.5 h. Evaporation and recrystallisation (MeCN) gave 22 (1.74 g, 48%) as white crystals: mp 133–135 °C; Found: C, 44.8; H, 3.33; N, 5.78. C<sub>9</sub>H<sub>8</sub>BrNO<sub>2</sub> requires C, 44.66; H, 3.33; N, 5.79%; <sup>1</sup>H NMR δ 4.67 (2 H, s, CH<sub>2</sub>), 6.82 (1 H, bs, NH), 7.59 (1 H, t, J=7.8 Hz, 5-H), 8.01 (1 H, bs, NH), 8.12 (1 H, dt, J=7.8, 1.5 Hz) and 8.21 (1 H, dt, J=7.8, 1.5 Hz) (4,6-H<sub>2</sub>), 8.57 (1 H, t, J=1.5 Hz, 2-H); MS (FAB) m/z 243.9796 (100%) (M+H) ( ${}^{12}C_9H_9{}^{81}BrNO_2$ requires 243.9796). 241.9810 (100%) (M + H) $({}^{12}C_9H_9{}^{79}BrNO_2$  requires 241.9817).

RS-3-(Oxiran-2-yl)benzamide (24). Compound 22 (500 mg, 2.1 mmol) was stirred with NaBH<sub>4</sub> (47 mg, 1.2 mmol) in EtOH (6 mL) at 0 °C for 30 min and at 20 °C for 2 h. Aq NaOH (1.0 M, 1.5 mL, 1.5 mmol) was added and the mixture was stirred at 20  $^{\circ}\mathrm{C}$  for 20 min and at 50  $^{\circ}\mathrm{C}$  for 10 min. Water (30 mL) was added and the mixture was extracted with  $CH_2Cl_2$  (2×). The extracts were washed with brine. Drying and evaporation gave 24 (331 mg, 98%) as white crystals: mp 95.5–97 °C; Found: C, 66.0; H, 5.67; N, 8.38. C<sub>9</sub>H<sub>9</sub>NO<sub>2</sub> requires C, 66.25; H, 5.56; N, 8.58%; <sup>1</sup>H NMR δ 2.89 (1 H, m, oxirane 3-H), 3.15 (1 H, t, J=4.8 Hz, oxirane 3-H), 3.99 (1 H, m, oxirane 2-H), 7.40 (1 H, bs, NH), 7.44 (2 H, m, 4,5-H<sub>2</sub>), 7.79 (2 H, m, 2,6-H<sub>2</sub>), 8.01 (1 H, bs, NH); MS (FAB) m/z 164.0736 (100%) (M+H) ( ${}^{12}C_9H_{10}NO_2$  requires 164.0712).

5-Cyanoisoquinolin-1-one (30). Method A. 2,6-Dicyanotoluene 29 (2.0 g, 14 mmol) was stirred at 0–5 °C with KOBu<sup>t</sup> (8.7 g, 77 mmol) in freshly distilled EtOCHO (50 mL) for 25 min. Et<sub>2</sub>O (100 mL) was added and the suspension was filtered. The solid was dissolved in water (20 mL). The solution was acidified with AcOH, saturated with NaCl and extracted with CHCl<sub>3</sub>. Drying, evaporation, chromatography (CHCl<sub>3</sub>:EtOAc, 2:1) and chromatography (hexane:EtOAc, 4:1) yielded 31 (330 mg, 14%) as white crystals: mp 212–214 °C; IR 2240, 1735 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  6.88 (1 H, d, *J*=5.7 Hz, 4-H), 7.46 (1 H, d, *J*=5.7 Hz, 3-H), 7.64 (1 H, t, *J*=7.7 Hz, 7-H), 8.05 (1 H, d, *J*=7.7 Hz, 6-H), 8.52 (1 H, d, J=7.7 Hz, 8-H); MS (EI) m/z 172.0353 (M) ( ${}^{13}C^{12}C_{9}H_5NO_2$  requires 172.0353), 171.0319 (M) ( ${}^{12}C_{10}H_5NO_2$  requires 171.0320), 143 (100%). Further elution gave **30** (300 mg, 13%) as a pale-yellow solid: mp 296–300 °C (lit.<sup>44</sup> mp 275–276 °C); IR 3500, 3300, 2230, 1690, 1660 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  6.60 (1 H, J=7.0 Hz, 4-H), 7.47 (1 H, d, J=7.0 Hz, 3-H), 7.63 (1 H, t, J=7.7 Hz, 7-H), 8.26 (1 H, d, J=7.7 Hz, 6-H), 8.47 (1 H, d, J=7.7 Hz, 8-H), 11.72 (1 H, bs, NH); m/z (EI) 171.0512 (M) ( ${}^{13}C^{12}C_9H_6N_2O$  requires 171.0514), 170.0480 (M) ( ${}^{12}C_{10}H_6N_2O$  requires 170.0480).

**5-Cyanoisoquinolin-1-one (30).** Method B. Compound **35** (300 mg, 1.5 mmol) in dry MeOH (50 mL) was saturated with hydrogen chloride. The mixture was boiled under reflux for 3 days. The evaporation residue was stirred with 10% aq NNa<sub>2</sub>CO<sub>3</sub> for 30 min. The suspension was extracted with EtOAc (4×). Drying, evaporation, and recrystallisation, followed by chromatography of the mother liquor (hexane:EtOAc, 4:1), gave **30** (141 mg, 55%) as an off-white solid, mp 296–300 °C. Also isolated by chromatography was **31** (13 mg, 5%).

**3-Cyano-2-methylbenzamide (32).** 2,6-Dicyanotoluene **29** (13.0 g, 21 mmol) was stirred with aq  $H_2O_2$  (27.5% w/v, 7.0 mL, 57 mmol) and NaOH (210 mg, 5.25 mmol) in EtOH (75 mL) and water (10 mL) for 1h before being extracted with EtOAc (4×100 mL). Drying, evaporation, and recrystallisation (EtOAc) gave **32** (1.62 g, 48%) as white crystals: mp 186–188 °C; IR 3420, 3350, 2218, 1680, 1625 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  2.65 (3 H, s, Me), 6.94 (1 H, bs, NH), 7.36 (1 H, t, *J*=7.7 Hz, 5-H), 7.52 (1 H, bs, NH), 7.65 (2 H, m, 4,6-H2); MS (EI) *m*/*z* 160.0634 (M) (100%) (<sup>12</sup>C<sub>9</sub>H<sub>8</sub>N<sub>2</sub>O requires 160.0637).

**3-Cyano-***N***-(dimethylaminomethylene)-2-methylbenzamide** (33). 3-Cyano-2-methylbenzamide 32 (102 mg, 640 µmol) was boiled under reflux with Me<sub>2</sub>NCH (OMe)<sub>2</sub> (164 mg, 1.25 mmol) in DMF (1 mL) for 3 h. Water (20 mL) was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4×). The combined extracts were washed with water and with brine. Drying and evaporation gave 33 (114 mg, 83%) as a yellow solid: mp 98–100 °C; <sup>1</sup>H NMR  $\delta$  2.81 (3 H, s, Ar-Me), 3.19 (3 H, s, NMe), 3.23 (3 H, s, NMe), 7.33 (1 H, t, *J* = 7.7 Hz, 5-H), 7.65 (1 H, d, *J* = 7.7 Hz, 4-H), 8.18 (1 H, d, *J* = 7.7 Hz, 6-H), 8.62 (1 H, s, NCH); MS (FAB) *m/z* 216.1139 (M+H) (100%) (<sup>12</sup>C<sub>12</sub>H<sub>14</sub>N<sub>3</sub>O requires 216.1137).

*N*-(3-Carbamoyl-2-methylbenzoyl)-*N*-(3-cyano-2-methylbenzoyl)formamidine (34). Compound 33 (300 mg, 1.4 mmol) was boiled under reflux with TsOH (361 mg, 2.1 mmol) in PhMe (10 mL) for 13 h. The evaporation residue, in  $CH_2Cl_2$ , was washed with water and with

10% aq NNa<sub>2</sub>CO<sub>3</sub>. Drying, evaporation and recrystallisation CH<sub>2</sub>Cl<sub>2</sub>) gave **34** (132 mg, 27%) as white crystals: mp 160–162 °C; Found: C, 64.25; H, 4.55; N, 15.6. C<sub>19</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>.0.5H<sub>2</sub>O requires C, 63.95; H, 4.8; N, 15.7%; IR 3370, 3190, 2210, 1735, 1690, 1655 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  2.59 (3 H, s, Me), 2.63 (3 H, s, Me), 7.50 (1 H, t, *J* = 7.7 Hz, 5-H), 7.60 (1 H, t, *J* = 7.7 Hz, 5'-H), 7.71 (1 H, d, *J* = 7.7 Hz, 4'-H), 7.72 (1 H, bs, CONH<sub>2</sub>), 7.90 (2 H, d, *J* = 7.7 Hz, 4/6-H<sub>2</sub>), 8.00 (1 H, bs, CONH<sub>2</sub>), 8.04 (1 H, d, *J* = 7.7 Hz, 6'-H), 9.22 (1 H, s, formamidine CH), 11.78 (1 H, bs, formamidine NH); MS (FAB) *m*/*z* 349 (M+H), 172 (M- H<sub>2</sub>NOC(-Me)C<sub>6</sub>H<sub>5</sub>CON), 161 (NC(Me)C<sub>6</sub>H<sub>5</sub>CONH<sub>2</sub> + H).

*E*-2-(2,6-Dicyanophenyl)-*N*,*N*-dimethylethenamine (35). 2,6-Dicyanotoluene **29** (1.00 g, 7.0 mmol) was boiled under reflux in Me<sub>2</sub>NCH(OMe)<sub>2</sub> (10 mL) for 4 days. Evaporation and recrystallisation (EtOH) gave **35** (973 mg, 71%) as a yellow solid: mp 117–119 °C; <sup>1</sup>H NMR  $\delta$  3.00 (6 H, s, NMe<sub>2</sub>), 5.36 (1 H, d, *J*=13.6 Hz, ArCH=C), 6.94 (1 H, t, *J*=7.7 Hz, 5-H), 7.63 (2 H, d, *J*=7.7 Hz, 4,6-H<sub>2</sub>), 7.77 (1 H, d, *J*=13.6 Hz, C=CHN); MS (EI) *m*/*z* 197.0960 (100%) (M) (<sup>12</sup>C<sub>12</sub>H<sub>11</sub>N<sub>3</sub> requires 197.0953), 182 (M-Me), 155.

**Isoquinoline-5-carboxylic acid (36).** Compound **30** (427 mg, 2.5 mmol) was boiled under reflux with KOH (2.4 g, 43 mmol) in EtOH (10 mL) under N<sub>2</sub> for 3 days. The mixture was acidified with aq HCl (9 M). The evaporation residue, in MeOH, was filtered. Evaporation of the solvent from the filtrate gave **36** (394 mg, 83%) as a white solid: mp > 300 °C; IR 3400–2700, 1705, 1655, 1625 cm<sup>-1</sup>; <sup>1</sup>H NMR δ (CD<sub>3</sub>OD) 7.26 (1 H, d, J=7.7 Hz, 4-H), 7.58 (1 H, t, J=7.7 Hz, 7-H), 7.75 (1 H, d, J=7.7 Hz, 3-H), 8.41 (1 H, d, J=7.7 Hz, 8-H), 8.55 (1 H, d, J=7.7 Hz, 6-H); MS (EI) *m/z* 190.0458 (M) (<sup>13</sup>C<sup>12</sup>C<sub>9</sub>H<sub>7</sub>NO<sub>3</sub> requires 190.0459), 189.0425 (100%) (M) (<sup>12</sup>C<sub>10</sub>H<sub>7</sub>NO<sub>3</sub> requires 189.0426).

RS-Isoquinolin-5-ylphenylmethanol (39). BuLi in hexanes (2.5 M, 0.40 mL, 1.0 mmol) was added to 37 (208 mg, 1.0 mmol) in dry THF (6.2 mL) at -116 °C under N<sub>2</sub> and stirring continued for 10 min. PhCHO (104 mg, 980 µmol) in dry THF (1.0 mL) was added and the mixture was allowed to warm to 20 °C during 30 min. Aq NH<sub>4</sub>Cl (20%, 1.0 mL) was added. After 5 min, the solvents were evaporated. The residue, in CH<sub>2</sub>Cl<sub>2</sub>, was washed with saturated aq NaHCO<sub>3</sub> and was dried. Evaporation and chromatography (hexane:EtOAc, 3:2) gave 40 (4 mg, 3%) as a pale-yellow oil: <sup>1</sup>H NMR δ 7.66 (3 H, m, 4,6,7-H3), 7.82 (1 H, d, J=8.1 Hz, 5-H), 7.98 (1 H, d, J=8.1 Hz, 8-H), 8.53 (1 H, d, J = 5.9 Hz, 3-H), 9.26 (1 H, s, 1-H). Further elution gave recovered 37 (26 mg, 13%). Further elution gave 39 (79 mg, 34%) as white crystals: mp 124–126 °C; <sup>1</sup>H NMR δ 1.7 (1 H, bs, OH), 6.48 (1 H, s, CHOH), 7.36 (5 H, m, Ph-H<sub>5</sub>), 7.65 (1 H, t, J = 7.7 Hz, isoquinoline 7-H), 7.78 (1 H, d, J = 6.1 Hz, isoquinoline 4-H), 7.9 (2 H, m, isoquinoline 6,8-H<sub>2</sub>), 8.41 (1 H, d, J = 6.1 Hz, isoquinoline 3-H), 9.20 (1 H, s, isoquinoline 1-H); MS (EI) m/z 236.1030 (M) ( $^{13}C^{12}C_{15}H_{13}NO$  requires 236.1030), 235.0993 (M) ( $^{12}C_{16}H_{13}NO$  requires 235.0997).

E-3-(1-Oxoisoquinolin-5-yl)propenoic acid (43). 5-Iodoisoquinolin-1-one  $42^{24}$  (100 mg, 370 µmol) was boiled under reflux with propenoic acid (35 mg, 490 µmol), Pd(OAc)<sub>2</sub> (8.0 mg, 37 µmol) and Et<sub>3</sub>N (93 mg, 920 µmol) in EtCN (0.3 mL) for 1 h. Dilute HCl (10 mL) was added. The precipitate was washed with water and was dried to give 43 (76 mg, 97%) as an off-white solid: mp 315-318 °C; IR 3550, 1695, 1660 cm<sup>-1</sup>; <sup>1</sup>H NMR  $((CD_3)_2SO) \delta 6.57 (1 H, d, J = 15.8 Hz, 2-H), 6.76 (1 H, d, J$ d, J = 7.3 Hz, isoquinoline 4-H), 7.28 (1 H, d, J = 7.3 Hz, isoquinoline 3-H), 7.52 (1 H, t, J=7.7 Hz, isoquinoline 7-H), 8.10 (1 H, d, J=15.8 Hz, 3-H), 8.12 (1 H, d, J = 7.7 Hz) and 8.27 (1 H, d, J = 7.7 Hz) (isoquinoline 6, 8-H<sub>2</sub>); MS (FAB) m/z 216.0616 (M + H) ( ${}^{12}C_{12}H_{10}NO_3$ requires 216.0661), 215.0584 (M) (<sup>12</sup>C<sub>12</sub>H<sub>9</sub>NO<sub>3</sub> requires 215.0582).

5-(1E-3RS-3-(4R-2,2-Dimethyldihydro-1,3-dioxol-4-yl)-3-hydroxyprop-1-enyl)isoquinolin-1-one (45) and 5-(3-(4R-2,2-dimethyldihydro-1,3-dioxol-4-yl)-3-oxopropyl)iso-5-Iodoisoquinolin-1-one **42**<sup>24</sup> quinolin-1-one (46). (400 mg, 1.5 mmol) was boiled under reflux with 4R-2,2dimethyl-4-(1RS-1-hydroxyprop-2-enyl)dihydro-1,3dioxole 44<sup>52,53</sup> (315 mg, 1.9 mmol), Pd(OAc)<sub>2</sub> (32 mg, 140 µmol) and Et<sub>3</sub>N (370 mg, 3.7 mmol) in EtCN (1.5 mL) for 2h. The evaporation residue, in EtOAc, was washed with dilute HCl (2 M) and with brine. Drying, evaporation and chromatography (EtOAcMe<sub>2</sub>CO:hexane, 2:1) gave 45 (76 mg, 18%) as an off-white solid: mp 164–166 °C; <sup>1</sup>H NMR δ 1.40 (3 H, s, Me), 1.50 (3 H, s, Me), 2.17 (1 H, br, OH), 3.93 (0.5 H, dd, J=8.5, 5.5 Hz, dioxole 5-H, diastereoisomer A), 4.04 (1 H, m, dioxole 5-H<sub>2</sub>, diastereoisomer B), 4.1 (0.5 H, m, dioxole 5-H, A), 4.20 (0.5 H, m, dioxole 4-H, A), 4.28 (0.5 H, m, dioxole 4-H, B), 4.34 (0.5 H, m, propenyl 3-H, A), 4.56 (0.5 H, m, propenyl 3-H, B), 6.18 (1 H, m, propenyl 4-H), 6.81 (0.5 H, d, J = 7.3 Hz, isoquinoline 4-H, B), 6.82 (0.5 H, d, J=7.3 Hz, isoquinoline 4-H, A), 7.19 (1 H, d, J=7.3 Hz, isoquinoline 3-H), 7.24 (1 H, m, propenyl 1-H), 7.48 (1 H, t, J=7.8 Hz, isoquinoline 7-H), 7.78 (1 H, d, J = 7.8 Hz, isoquinoline 6-H), 8.37 (1 H, d, J = 7.8 Hz, isoquinoline 8-H), 10.6 (1 H, br, NH); MS (FAB) m/z302.1373 (M+H) ( ${}^{12}C_{17}H_{20}NO_4$  requires 302.1392). Further elution gave 46 (176 mg, 42%) as an off-white solid: mp 138-141 °C; IR 3300, 3160, 1715, 1660, 1630 cm<sup>-1</sup>; <sup>1</sup>H NMR δ 1.38 (3 H, s, Me), 1.43 (3 H, s, Me), 2.99 (1 H, t, J=7.6 Hz) and 3.00 (1 H, t, J = 7.6 Hz) and 3.20 (2 H, m) (propyl 1,2-H<sub>4</sub>), 3.96 (1 H, dd, J = 8.5, 5.5 Hz, dioxole 5-H), 4.19 (1 H, dd, J = 8.5,

7.8 Hz, dioxole 5-H), 4.45 (1 H, dd, J=7.8, 5.5 Hz, dioxole 4-H), 6.87 (1 H, d, J=7.3 Hz, isoquinoline 4-H), 7.29 (1 H, d, J=7.3 Hz, isoquinoline 3-H), 7.50 (1 H, t, J=7.9 Hz, isoquinoline 7-H), 7.61 (1 H, d, J=7.9 Hz, isoquinoline 6-H), 8.34 (1 H, d, J=7.9 Hz, isoquinoline 8-H), 11.15 (1 H, br, NH); MS (FAB) m/z 302.1384 (M+H) ( ${}^{12}C_{17}H_{20}NO_4$  requires 302.1392).

5-(E-5-Hydroxypent-1-enyl)isoquinolin-1-one (47), 5-(4hydroxy-1-methylenebutyl)isoquinolin-1-one (48), 5-(5oxopentyl)isoquinolin-1-one (49) and 5-(RS-4-oxo-1methylbutyl)isoquinolin-1-one (50). 5-Iodoisoquinolin-1one  $42^{24}$  (400 mg, 1.5 mmol) was boiled under reflux with pent-4-enol (170 mg, 1.9 mmol), Pd(OAc)<sub>2</sub> (32 mg, 140 µmol) and Et<sub>3</sub>N (370 mg, 3.7 mmol) in EtCN (1.5 mL) for 2 h. The evaporation residue, in EtOAc, was washed with dilute HCl, with water and with brine, and was dried. Evaporation and chromatography (EtOAc:MeOH, 99:1) gave unreacted 42 (221 mg, 55%). Further elution gave a mixture of 49 (5% by NMR) and 50 (2.5% by NMR). 49: <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO) δ 1.70 (4 H, m, pentyl 2,  $3-H_4$ ), 2.50 (2 H, m, pentyl  $4-H_2$ ), 2.90 (2 H, m, pentyl 1-H<sub>2</sub>), 6.73 (1 H, d, J=7.7 Hz, isoquinoline 4-H), 7.23 (1 H, d, J = 7.7 Hz, isoquinoline 3-H), 7.50 (2 H, m, isoquinoline 6, 7-H<sub>2</sub>), 8.30 (1 H, m, isoquinoline 8-H), 9.78 (1 H, br, CHO), 11.37 (1 H, br, NH). 50: <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  1.30 (5 H, m, Me + butyl 2-H<sub>2</sub>), 2.40 (2 H, t, J = 7.7 Hz, butyl 2-H<sub>2</sub>), 3.40 (1 H, m, butyl 1-H), 6.83 (1 H, d, J=7.7 Hz, isoquinoline 4-H), 7.50 (1 H, m, isoquinoline 3,6,7-H<sub>3</sub>), 8.30 (1 H, m, isoquinoline 8-H), 9.74 (1 H, br, CHO), 11.37 (1 H, br, NH); MS (FAB) m/z 230.1184 (M+H) ( ${}^{12}C_{14}H_{16}NO_2$  requires 230.1181). Further elution gave a mixture of 47 (13%) by NMR) and 48 (11% by NMR). 47: <sup>1</sup>H NMR  $((CD_3)_2SO) \delta 1.60 (2 H, quintet, J = 6.8 Hz, pentenyl 2-$ H<sub>2</sub>), 2.30 (2 H, m, pentenyl 3-H<sub>2</sub>), 3.50 (2 H, m, pentenyl 1-H<sub>2</sub>), 6.3 (1 H, m, pentenyl 4-H), 6.75 (1 H, d, J = 7.5 Hz, isoquinoline 4-H), 6.93 (1 H, d, J = 15.6 Hz, pentenyl 5-H), 7.18 (1 H, d, J=7.5 Hz, isoquinoline 3-H), 7.48 (1 H, m, isoquinoline 7-H), 7.80 (1 H, d, J = 7.5 Hz, isoquinoline 6-H), 8.10 (1 H, m, isoquinoline 8-H). 48: <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO) δ 2.10 (2 H, m, butyl 3-H<sub>2</sub>), 2.50 (2 H, m, butyl 2-H<sub>2</sub>), 3.50 (2 H, m, butyl 1- $H_2$ ), 5.5 (2 H, m,  $H_2C=C$ ), 6.62 (1 H, d, J=7.7 Hz, isoquinoline 4-H), 7.18 (1 H, d, J=7.7 Hz, isoquinoline 3-H), 7.48 (2 H, m, isoquinoline 6,7-H<sub>2</sub>), 8.10 (1 H, m, isoquinoline 8-H); MS (FAB) m/z 230.1178 (M+H)  $({}^{12}C_{14}H_{16}NO_2$  requires 230.1181).

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