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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)

Corrine Y. Watson,[a] William J. D. Whish[b] and Michael D. Threadgill[a,*]

[a]*Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK*
[b]*Department of Biology & Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK*

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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.

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.[1] 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[2–7] and DNA-damaging chemotherapeutic drugs.[8–11] 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.[12,13] PARP catalyses the transfer of ADP-ribose units from its substrate NAD^+ 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).[14,15]

Most known inhibitors of PARP mimic the nicotinamide moiety of the substrate **1**. The first enzyme-selective inhibitor[16] was 3-aminobenzamide **2** (Figure 1) (IC_{50} 22 μM[17]). Further structure–activity studies on substituted benzamides[16–20] 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[20] led to development of 5-substituted isoquinolin-1-ones[17,20] and 3,4-dihydroisoquinolin-1-ones[20] **3** and of 2,8-disubstituted quinazolin-4-ones[17,21,22] **4** (Figure 1), which are some 10- to 50-fold more potent as inhibitors (**3** $R^1 = OH$, $R^2 = H$,

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*Corresponding author. E-mail: prsmdt@bath.ac.uk

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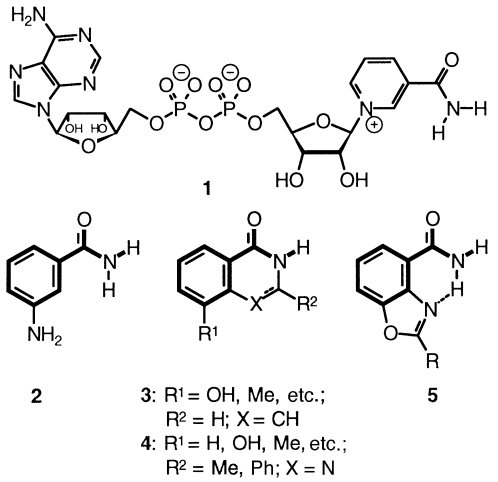
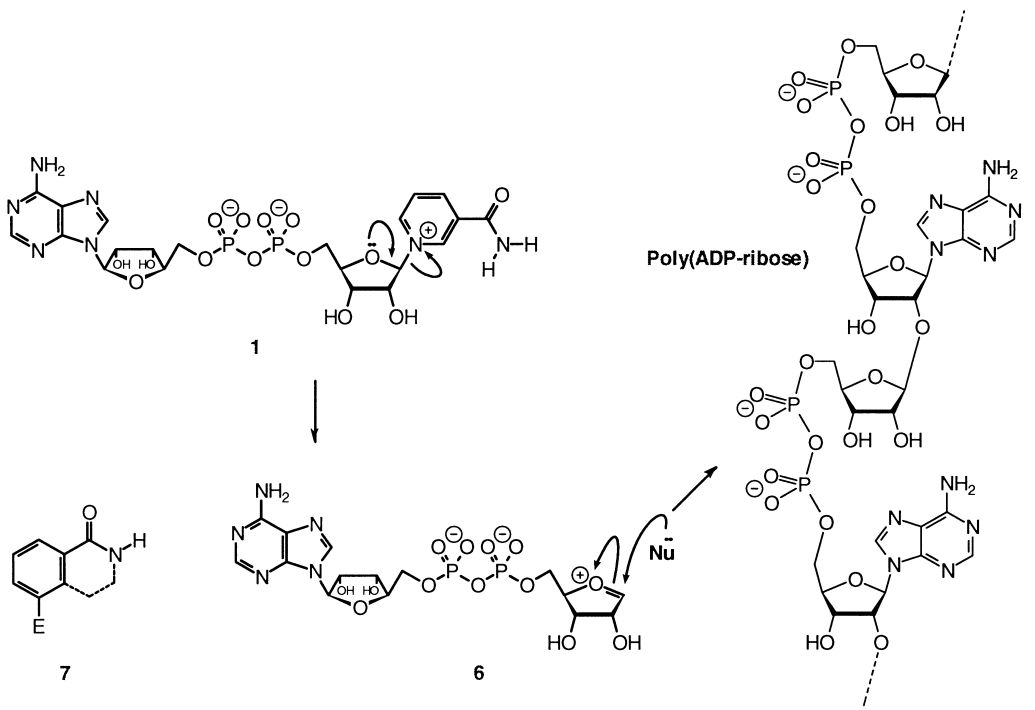


Figure 1. Structures of NAD^+ **1** and known inhibitors of PARP **2**–**5**.

X = CH: IC_{50} 0.14 μM; **4** R^1 = OH, R^2 = Me, X = N: IC_{50} 0.44 μM). 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.[22] who used a hydrogen-bond in the benzoxazolecarboxamides **5** (Figure 1) (**5** R = Ph: IC_{50} 2.1 μM). A large study[17] 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-1,8-naphthalimide (IC_{50} 0.18 μM) and phenanthridin-6(5*H*)-one (IC_{50} 0.30 μM) 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,[23,24] we rationalised that design of a mechanism-based 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^+ **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 β-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^+-binding site of PARP.

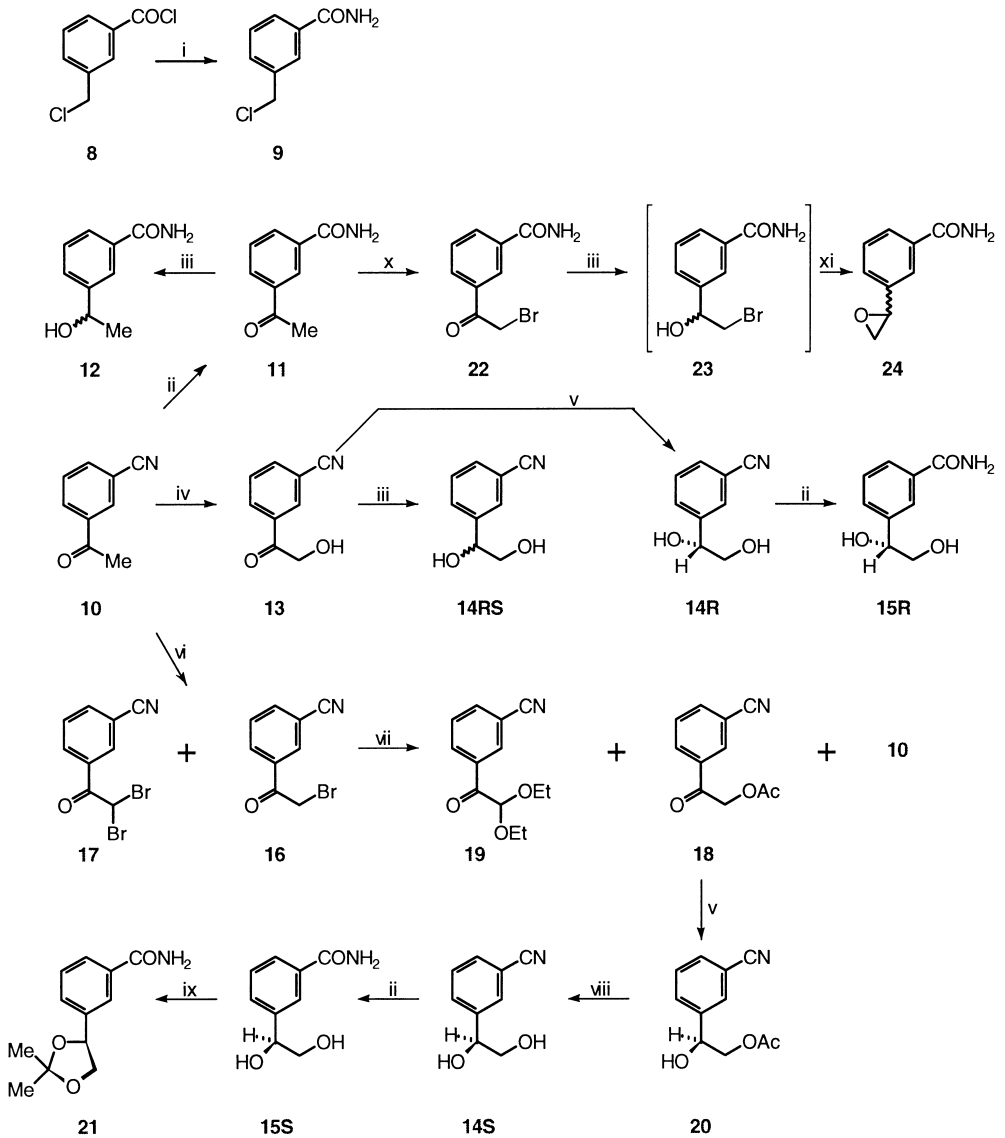


Scheme 1. Chemical mechanism for the PARP-catalysed formation of poly(ADP-ribose) from NAD^+ **1** via the intermediate oxonium ion **6**.

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 **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 3-aminobenzamide **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^+ **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

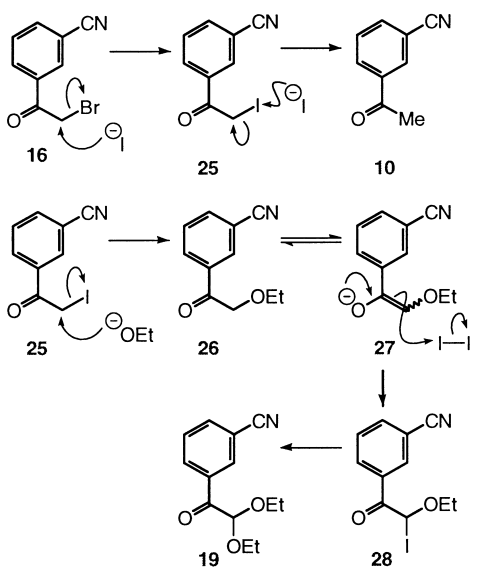


Scheme 2. Synthesis of 3-substituted benzamides which are inhibitors of PARP. Reagents: (i) NH_3, Et_2O; (ii) H_2O_2, NaOH, EtOH, H_2O; (iii) $NaBH_4$, EtOH; (iv) $PhI(OAc)_2$, CF_3CO_2H, MeCN, H_2O; (v) bakers' yeast, sucrose, H_2O; (vi) Br_2, $CHCl_3$; (vii) KOAc, NaI, EtOH; (viii) NH_3, MeOH, H_2O; (ix) $Me_2C(OMe)_2$, TsOH; (x) Br_2, HOAc; (xi) NaOH, H_2O.

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 **15*R*** and **15*S*** were important synthetic intermediates and targets in their own right. In **15*S***, 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[25] 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.[26–30] 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 **15*R*** 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 **15*R*** and **15*S*** 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,[31–33] 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.[34] Aromatic α-hydroxyketones have been reduced asymmetrically[35–37] to diols by this organism. For example, (hydroxyacetyl)benzene is reduced to *R*-phenylethane-1,2-diol in high yield and high optical purity.[35,36] The opposite stereochemistry of reduction is observed when the hydroxy group is esterified.[36] However, the bakers' yeast reduction is intolerant[37,38] of some substituents on the aromatic ring, thus 3-(substituted acetyl)benzamides were discounted as suitable substrates. Relying on the efficient hydrogen peroxide-mediated hydrolysis of nitriles to amides,[39] bioreductions of 3-(hydroxyacetyl)benzonitrile **13** and 3-(acetoxyacetyl)benzonitrile **18** were mooted as potentially enantiomerically efficient routes to the target enantiomeric diols **15*R*** and **15*S***, 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[40] of generally inferior yields during hydroxylations of acetophenones bearing electron-withdrawing groups on the benzene ring. Following the procedure of Manzocchi et al.,[36] **13** was reduced during 7 days in a fermenting preparation of bakers' yeast to the dihydroxyethylbenzonitrile **14*R*** with $[\alpha]_D^{20} = -46.6°$. 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 **14*S***, the acetoxyacetylbenzonitrile **18** was required as substrate for the bioreduction. Bromination of **10** with bromine in chloroform[41] 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.[42] 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^- 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 **14*S***. 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.[43] As a control, the derivatisation was repeated using racemic Mosher's acid chloride; the ^{1}H NMR signals from the *R*,*R* diastereoisomeric monoester were observed with identical intensity to those from the *S*,*R* diastereoisomer. Similar derivatisations of racemic **14*RS*** 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 **14*S***, which had $[\alpha]_D^{20} = +46.6°$. Thus **14*R*** also had *ee* > 96% (vide supra). As expected, the enantiomeric dihydroxyethylbenzonitriles **14*R*** and **14*S*** were efficiently converted to the corresponding 3-(1,2-dihydroxyethyl)benzamides **15*R*** and **15*S***, respectively, by hydrogen peroxide-mediated hydrolysis.

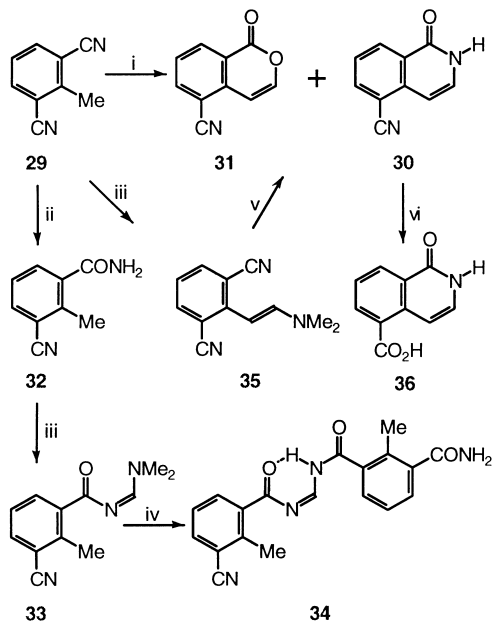
The *S*-dihydroxyethylbenzamide **15*S*** was cyclised to the ketal **21** with acetone dimethyl acetal under acidic conditions. In this compound, the dioxolane may mimic the ribose ring in the PARP substrate NAD^+ **1**, with ether oxygens occupying the same relative positions in the five-membered rings.

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

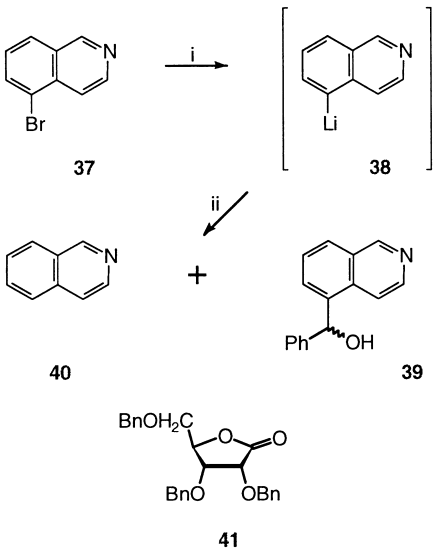
The isoquinolin-1-ones have the benzamide core in their structure but have the amide as part of a heterocyclic fused ring. By conformationally restricting the amide group in this way, the activity of these compounds as inhibitors of PARP is greatly increased.[17,20] An isoquinolin-1-one with a carbon 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 isoquinolinones 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,[44] 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 peroxide-mediated 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^t; (ii) H_2O_2, NaOH, EtOH, H_2O; (iii) $(MeOH)_2CHNMe_2$, DMF; (iv) TsOH, PhMe; (v) HCl, MeOH; (vi) KOH, EtOH.

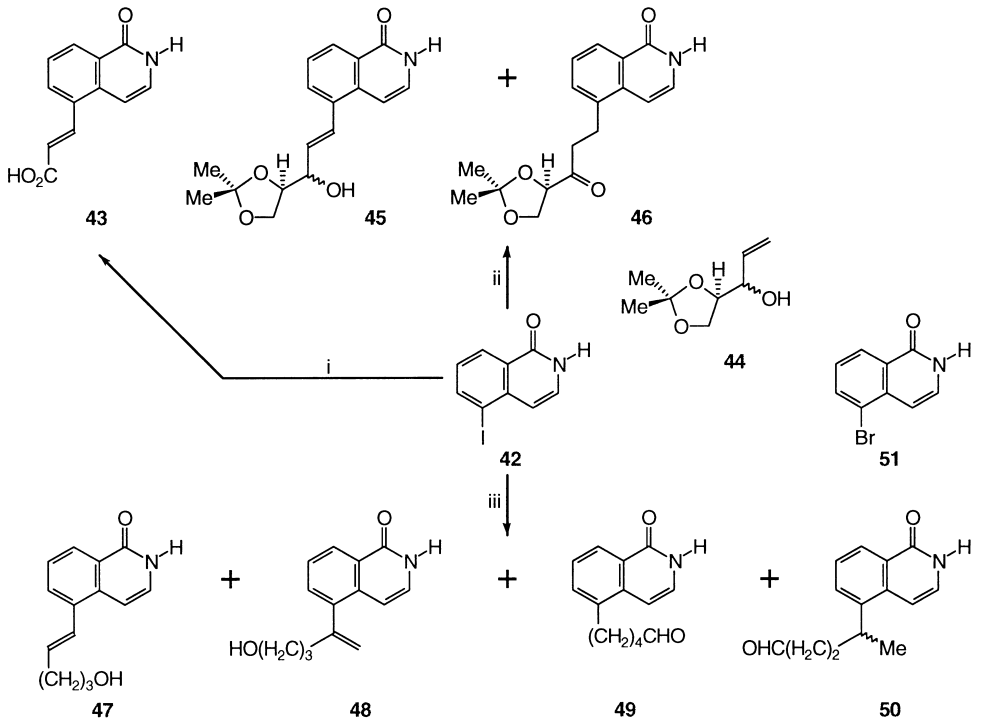
mamidine **33** from condensation with the carboxamide only. An attempt to complete the cyclisation by condensation with the activated methyl group under acidic conditions gave the N^1,N^3-diacylformamidine **34** as the sole isolable product. The mechanistic origin of this *unsymmetrical* formamidine 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.



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

Krohn et al.[45] 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 5-lithioisoquinoline is hitherto unreported. A two-step sequence (*N*-oxidation and rearrangement with acetic anhydride) has been described[44] for conversion of isoquinolines to the corresponding isoquinolin-1-ones, although the conditions required are harsh. 5-Bromoisoquinoline **37** (prepared[49] 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[50,51] was investigated as an alternative metal-mediated carbon–carbon bond forming process for attachment of functionalised carbon chains at the 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.[24] The 5-bromo analogue **51** was prepared similarly[24] from 3-(2-bromophenyl)propenoic acid. Although Plevyak and Heck used acetonitrile[51] 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 bromide[52,53] 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^+ **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_2CHCO_2H, $Pd(OAc)_2$, Et_3N, EtCN; (ii) **44**, $Pd(OAc)_2$, Et_3N, EtCN; (iii) $CH_2CH(CH_2)OH$, $Pd(OAc)_2$, Et_3N, 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.[50] 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-1-ones 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 [^{3}H]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.[16] Interestingly, particularly potent inhibition was shown by **11** and **22**, which have electron-withdrawing groups in the 3-position. Previous reports[16] 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 μM, 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 [^{3}H]-NAD$^+$. 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.[20] 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[2–11,16–22] 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.[54,55] The isoquinolinone (0–250 μM) 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(2*H*)-ones

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

substrate elongation factor 2 (eEF-2)[56,57] (4.0 μg) and [^{14}C]-NAD^+ 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 μM, 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.[17] noted selectivities of >1000-fold for PARP inhibition by 3-hydroxybenzamide and by phenanthridin-6(5*H*)-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^+ in PARP and those in the mono(ADP-ribosyl)ating enzymes, in contrast to the claim of similarity by Ruf et al.[25] Clearly, further studies are required in this area.

Conclusion

Routes have been developed for syntheses of series of 3-substituted benzamides and 5-substituted isoquinolin-1-ones. 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 $MgSO_4$. 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 $CDCl_3$, 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.[24] 5-Aminoisoquinolin-1-one was prepared essentially by the method of Wenkert et al.[44]

3-(Chloromethyl)benzamide (9). NH_3 in dry Et_2O (4% w/v, 200 mL) was added to 3-chloromethylbenzoyl chloride (**8**) (2.85 g, 15 mmol) in Et_2O (25 mL) during 10 min. The mixture was stirred for 30 min then washed with water, with dilute H_2SO_4 (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.[58] mp 119–120 °C); IR 3360, 3190, 1655, 1625, 705 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 4.64 (2 H, s, CH_2), 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_2O_2 (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 °C. The mixture was stirred at 50 °C for 1 h. After neutralisation with dilute H_2SO_4, the EtOH was evaporated. The residue, in CH_2Cl_2, 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. $C_9H_9NO_2$ requires C, 66.25; H, 5.56; N, 8.58%; IR 3380, 3185, 1680, 1655, 1630 cm^{-1}; 1H NMR δ 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 (M) ($^{13}C^{12}C_9H_9NO_2$ 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 $NaBH_4$ (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^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 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) ($^{12}C_9H_{11}NO_2$ requires 166.0868).

3-(Hydroxyacetyl)benzonitrile (13). 3-Acetylbenzonitrile **10** (1.00 g, 6.9 mmol), $PhI(O_2CCF_3)_2$ (5.9 g, 13.8 mmol) and CF_3CO_2H (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 $NaHCO_3$ 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^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 3.37 (1 H, bs, OH), 4.91 (2 H, s, CH_2), 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) ($^{12}C_9H_7NO_2$ requires 161.0432) 130 (100%).

R*-3-(1,2-Dihydroxyethyl)benzonitrile (14*R*).** 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_2O (100 mL) and Celite® (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 **14*R (268 mg, 44%) as a white solid: mp 64–66 °C; $[\alpha]_D^{20}$ −46.6° (*c* 1.0, CH_2Cl_2); ^1H NMR δ 2.33 (2 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_2OH), 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 (14*S*).** The *S*-ester **20** (500 mg, 2.4 mmol) was stirred with aq NH_3 (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_2Cl_2); ^1H NMR δ 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_2), 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_2), 7.71 (1 H, s, 2-H); MS (FAB) m/z 164.0657 (100%) (M + H) ($^{12}C_9H_{10}NO_2$ requires 164.0712).

RS*-3-(1,2-Dihydroxyethyl)benzonitrile (14*RS*).** Compound **13** (80 mg, 500 μmol) was stirred with $NaBH_4$ (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^{-1}; ^1H NMR δ 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_2), 4.85 (1 H, dd, $J=3.2$, 8.1 Hz, ArC*H*OH), 7.47 (1 H, t, $J=7.7$ Hz, 5-H), 7.6 (2 H, m, 4,6-H_2), 7.72 (1 H, s, 2-H).

R*-3-(1,2-Dihydroxyethyl)benzamide (15*R*).** The *R*-diol **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: ^1H NMR ($(CD_3)_2SO$) 3.45 (2 H, d, $J=6.4$ Hz, CH_2), 4.0 (2 H, bs, 2×OH), 4.57 (1 H, t, $J=6.4$ Hz, ArC*H*OH), 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) ($^{12}C_9H_{12}NO_3$ 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_2O_2 (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: ^1H NMR ($(CD_3)_2SO$) δ 3.45 (2 H, d, $J=6.4$ Hz, CH_2), 4.0 (2 H, bs, 2×OH), 4.57 (1 H, t, $J=6.4$ Hz, ArC*H*OH), 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) ($^{12}C_9H_{12}NO_3$ requires 182.0817).

3-(Bromoacetyl)benzonitrile (16) and 3-(dibromoacetyl)-benzonitrile (17). Br_2 (1.1 mL, 21.3 mmol) was added dropwise to **10** (3.00 g, 20.7 mmol) in $CHCl_3$ (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. $C_9H_5Br_2NO$ requires C, 35.68; H, 1.66; N, 4.62%; IR 2240, 1705 cm^{-1}; ^1H NMR δ 6.56 (1 H, s, $CHBr_2$), 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.[59] mp 65.5–66.5 °C); IR 2240, 1710 cm^{-1}; ^1H NMR δ 4.43 (2 H, s, CH_2Br), 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×). Drying, evaporation and chromatography (hexane:EtOAc, 4:1) gave **19** (160 mg, 3%) as a pale-yellow oil: IR (film) 2240, 1705 cm^{-1}; ^1H NMR δ 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×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); ^{13}C NMR δ 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%) ($^{12}C_{13}H_{16}NO_3$ 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. $C_{11}H_9NO_3$ requires C, 65.02; H, 4.46; N, 6.89%; IR 2240, 1755, 1710 cm^{-1}; ^1H NMR δ 2.24 (3 H, s, Me), 5.31 (2 H, s, CH_2), 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 (11.2 g) was added. To this fermenting slurry, **18** (760 mg, 3.7 mmol) was added and the mixture was stirred for 8 h before being filtered through Celite®. The filtrate was extracted with Et_2O (4×30 mL). Drying, evaporation and chromatography (EtOAc:hexane 2:3) gave **20** (650 mg, 85%) as a colourless oil: $[\alpha]_D^{20}$ +34.9° (*c* 2.9, CH_2Cl_2); IR (film) 3400–3500, 2240, 1740 cm^{-1}; ^1H 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_2O), 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_2), 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-(1,2-dihydroxyethyl)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 **15*S (62 mg, 34 μmol) 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_2CO_3 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_2Cl_2); ^1H NMR δ 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%) ($^{12}C_{12}H_{15}NO_3$ requires 222.1130), 164.

3-(Bromoacetyl)benzamide (22). Br_2 (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. $C_9H_8BrNO_2$ requires C, 44.66; H, 3.33; N, 5.79%; ^1H NMR δ 4.67 (2 H, s, CH_2), 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_2), 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 $NaBH_4$ (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 °C for 20 min and at 50 °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. $C_9H_9NO_2$ requires C, 66.25; H, 5.56; N, 8.58%; ^1H 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_2), 7.79 (2 H, m, 2,6-H_2), 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 $KOBu^t$ (8.7 g, 77 mmol) in freshly distilled EtOCHO (50 mL) for 25 min. Et_2O (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 $CHCl_3$. Drying, evaporation, chromatography ($CHCl_3$: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^{-1}; ^1H NMR δ 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) ($^{13}C^{12}C_9H_5NO_2$ 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.[44] mp 275–276 °C); IR 3500, 3300, 2230, 1690, 1660 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 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_2CO_3 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 1 h 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^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 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%) ($^{12}C_9H_8N_2O$ requires 160.0637).

3-Cyano-*N*-(dimethylaminomethylene)-2-methylbenzamide (33). 3-Cyano-2-methylbenzamide **32** (102 mg, 640 μmol) was boiled under reflux with $Me_2NCH(OMe)_2$ (164 mg, 1.25 mmol) in DMF (1 mL) for 3 h. Water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (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; ^{1}H NMR δ 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%) ($^{12}C_{12}H_{14}N_3O$ 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_2CO_3. Drying, evaporation and recrystallisation CH_2Cl_2) gave **34** (132 mg, 27%) as white crystals: mp 160–162 °C; Found: C, 64.25; H, 4.55; N, 15.6. $C_{19}H_{16}N_4O_3.0.5H_2O$ requires C, 63.95; H, 4.8; N, 15.7%; IR 3370, 3190, 2210, 1735, 1690, 1655 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 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, $CONH_2$), 7.90 (2 H, d, $J=7.7$ Hz, 4,6-H_2), 8.00 (1 H, bs, $CONH_2$), 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_2NOC(-Me)C_6H_5CON), 161 (NC(Me)$C_6H_5CONH_2$ + 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 $Me_2NCH(OMe)_2$ (10 mL) for 4 days. Evaporation and recrystallisation (EtOH) gave **35** (973 mg, 71%) as a yellow solid: mp 117–119 °C; ^{1}H NMR δ 3.00 (6 H, s, NMe_2), 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_2), 7.77 (1 H, d, $J=13.6$ Hz, C = CHN); MS (EI) m/z 197.0960 (100%) (M) ($^{12}C_{12}H_{11}N_3$ 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_2 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, 3400–2700, 1705, 1655, 1625 cm^{-1}; ^{1}H NMR δ (CD_3OD) 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) ($^{13}C^{12}C_9H_7NO_3$ requires 190.0459), 189.0425 (100%) (M) ($^{12}C_{10}H_7NO_3$ 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_2 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_4Cl (20%, 1.0 mL) was added. After 5 min, the solvents were evaporated. The residue, in CH_2Cl_2, was washed with saturated aq $NaHCO_3$ and was dried. Evaporation and chromatography (hexane:EtOAc, 3:2) gave **40** (4 mg, 3%) as a pale-yellow oil: ^{1}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; ^{1}H NMR δ 1.7 (1 H, bs, OH), 6.48 (1 H, s, C*H*OH), 7.36

(5 H, m, Ph-H_5), 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_2), 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), $Pd(OAc)_2$ (8.0 mg, 37 μmol) and Et_3N (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^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 6.57 (1 H, d, $J=15.8$ Hz, 2-H), 6.76 (1 H, 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_2); MS (FAB) m/z 216.0616 (M + H) ($^{12}C_{12}H_{10}NO_3$ requires 216.0661), 215.0584 (M) ($^{12}C_{12}H_9NO_3$ requires 215.0582).

5-(1*E*-3*RS*-3-(4*R*-2,2-Dimethyldihydro-1,3-dioxol-4-yl)-3-hydroxyprop-1-enyl)isoquinolin-1-one (45) and 5-(3-(4*R*-2,2-dimethyldihydro-1,3-dioxol-4-yl)-3-oxopropyl)isoquinolin-1-one (46). 5-Iodoisoquinolin-1-one **42**[24] (400 mg, 1.5 mmol) was boiled under reflux with 4*R*-2,2-dimethyl-4-(1*RS*-1-hydroxyprop-2-enyl)dihydro-1,3-dioxole **44**[52,53] (315 mg, 1.9 mmol), $Pd(OAc)_2$ (32 mg, 140 μmol) and Et_3N (370 mg, 3.7 mmol) in EtCN (1.5 mL) for 2 h. The evaporation residue, in EtOAc, was washed with dilute HCl (2 M) and with brine. Drying, evaporation and chromatography (EtOAcMe_2CO:-hexane, 2:1) gave **45** (76 mg, 18%) as an off-white solid: mp 164–166 °C; ^1H 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_2, 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/z 302.1373 (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^{-1}; ^1H 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_4), 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-(4-hydroxy-1-methylenebutyl)isoquinolin-1-one (48), 5-(5-oxopentyl)isoquinolin-1-one (49) and 5-(*RS*-4-oxo-1-methylbutyl)isoquinolin-1-one (50). 5-Iodoisoquinolin-1-one **42**[24] (400 mg, 1.5 mmol) was boiled under reflux with pent-4-enol (170 mg, 1.9 mmol), $Pd(OAc)_2$ (32 mg, 140 μmol) and Et_3N (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**: ^1H NMR ($(CD_3)_2SO$) 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_2), 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_2), 8.30 (1 H, m, isoquinoline 8-H), 9.78 (1 H, br, CHO), 11.37 (1 H, br, NH). **50**: ^1H NMR ($(CD_3)_2SO$) δ 1.30 (5 H, m, Me + butyl 2-H_2), 2.40 (2 H, t, $J=7.7$ Hz, butyl 2-H_2), 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_3), 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**: ^1H NMR ($(CD_3)_2SO$) δ 1.60 (2 H, quintet, $J=6.8$ Hz, pentenyl 2-H_2), 2.30 (2 H, m, pentenyl 3-H_2), 3.50 (2 H, m, pentenyl 1-H_2), 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**: ^1H NMR ($(CD_3)_2SO$) δ 2.10 (2 H, m, butyl 3-H_2), 2.50 (2 H, m, butyl 2-H_2), 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_2), 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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