5-Benzamidoisoquinolin-1-ones and 5-(ω-carboxy-alkyl)isoquinolin-1-ones as isoform-selective inhibitors of poly(ADP-ribose)polymerase-2 (PARP-2)

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Abstract. PARP-2 is a member of the poly(ADP-ribose) polymerase family, with some activities similar to those of PARP-1 but with other distinct roles. Two series of isoquinolin-1-ones were designed, synthesised and evaluated as selective inhibitors of PARP-2, using the structures of the catalytic sites of the isoforms. A new efficient synthesis of 5-aminoisoquinolin-1-one was developed and acylation with acyl chlorides gave 5-acylaminoisoquinolin-1-ones. Examining isoquinolin-1-ones with carboxylates tethered to the 5-position, Heck coupling of 5-iodoisoquinolin-1-one furnished the 5-CH=CHCO₂H compound for reduction to the 5-propanoic acid. Alkylation of 5-aminoisoquinolin-1-one under mildly basic conditions, followed by hydrolysis, gave 5-(carboxymethylamino)isoquinolin-1-one, whereas it was alkylated at 2-N with methyl propenoate and strong base. Compounds were assayed in vitro for inhibition of PARP-1 and PARP-2, using FlashPlate and solution-phase assays, respectively. The 5-benzamidoisoquinolin-1-ones were more selective for inhibition of PARP-2, whereas the 5-(ω-carboxyalkyl)isoquinolin-1-ones were less so. 5-Benzamidoisoquinolin-1-one is the most PARP-2-selective compound (IC₅₀(PARP-1) / IC₅₀(PARP-2) = 9.3) to date, in a comparative study.

Keywords: Poly(ADP-ribose)polymerase-2, 5-aminoisoquinolin-1-one, 5-benzamidoisoquinolin-1-one, isoform selectivity, X-ray structure.

Introduction
Poly(ADP-ribosyl)ation of proteins, first reported in 1963,¹ involves the transfer of multiple ADP-ribose units from substrate NAD⁺ onto glutamate residues of the target proteins, resul-
ting in the formation of polyanionic poly(ADP-ribose) (PAR) polymers. There has been much research effort since to characterise the structures and functions of the poly(ADP-ribose)-polymerases (PARPs), the family of enzymes responsible for catalysis of this reaction.²⁻⁵

PARP-1 is responsible for most of the poly(ADP-ribosyl)ation activity in the cell and has a major role in regulating the repair of damaged DNA. It is a 116 KDa protein with three major domains: an N-terminal DNA-binding domain carrying three zinc fingers,⁶ a central automodification domain (containing also a nuclear localisation signal⁷ and sites for cleavage by caspase-3⁸) and a C-terminal NAD⁺-binding catalytic domain. The catalytic activity of the PARP-1 is activated by detection of a damaged site and PAR is built up on histone-1 and on PARP-1 itself. Inhibition of PARP-1 therefore inhibits repair of damaged DNA and several inhibitors of this enzyme are currently in clinical trial as sensitisers to the effects of cytotoxic DNA-damaging drugs in several tumours and as single-agent therapies in BRCA-mutant breast cancer.⁹⁻¹⁴ In addition to its role in regulating repair of damaged DNA, PARP-1 also regulates NF-κB and processes and molecules downstream therefrom. Thus inhibitors of the catalytic activity of PARP-1 have shown interesting activity in vivo in models of several disease states, including haemorrhagic shock, myocardial infarction, stroke and other ischaemia-reperfusion injuries, as well as in inflammatory disorders and reproductive health.¹⁵⁻²⁰ Recently, inhibitors of PARP-1 have also been shown to inhibit angiogenesis and metastasis in cancer.²¹⁻²³

PARP-2 is a 62 KDa protein with three functional domains.²⁴ The N-terminal DNA-binding domain lacks zinc fingers but is rich in basic amino-acids, which account for the binding to DNA. The differences in structure of the DNA-binding domains of PARPs 1 and 2 may also reflect the differences in the DNA structures which the two enzymes recognise and the fact that PARP-2 binds to single-strand breaks (SSBs) less efficiently than does PARP-1. The
The automodification domain is also responsible for the protein-protein interactions that PARP-2 shares with various partners, including PARP-1, XRCC1 and DNA ligase 3. The catalytic domain of mouse and human PARP-2 are highly conserved and both show high homology with the human PARP-1 catalytic domain (69% in the case of human PARP-2).

This isoform accounts for approximately 5-10% of the total cellular PARP activity. It can heterodimerise with PARP-1 and it shares many functions and targets. However, PARP-2 has some different protein targets and potential functions. Schreiber et al. showed that PARP-2-/- mouse embryonic fibroblasts have delayed repair of alkylated bases in DNA following treatment with N-methyl-N-nitrosourea (MNU) and suggest that the PARP-1 / PARP-2 heterodimer is important for efficient base-excision repair. By contrast, Fisher et al. found that a reduction in PARP-1 significantly reduced the rate of repair of single-strand breaks in DNA but reduction in PARP-2 had only a minor effect. PARP-2 interacts with TRF2, a telomere-binding regulatory protein, and affects its ability to bind to DNA both through a non-covalent interaction of PAR with the DNA-binding domain of TRF2 and through a covalent modification at the dimerisation domain. PARP-2 may have a functional role in the maintenance of telomeres but further work using selective inhibitors of this isoform are required to gain insights into the precise molecular mechanisms at work. Antisense knockdown of PARP-2 in a mouse model of colitis resulted in improvement in inflammation and normalisation of colonic function. Deletion of PARP-2 but not of PARP-1 leads to a significant diminution in CD4+CD8+ double-positive thymocytes, suggesting that PARP-2 has a role in survival of T-cells during thymopoiesis and may regulate the apoptosis of thymocytes. PARP-2 is widely expressed in the seminiferous epithelium, in contrast to limitation of PARP-1 to the peripheral cell layer, pointing to distinct roles in spermatogenesis and a more prominent role for PARP-2. PARP-2 knockout mice display lipodystrophy, probably due to regulation of the expression of peroxisome proliferator-activated receptor-γ by PARP-2. The identification of
these roles for PARP-2 which are distinct from those of the major isoform PARP-1 point to the need for selective inhibitors initially as pharmacological tools to help resolve and distinguish the roles of PARP-2 but also, in the longer term, possibly as drugs.

Most attempts to identify PARP-2-selective inhibitors have relied on screening libraries of inhibitors of PARP-1 against both isoforms. Interestingly, most of the clinical candidate PARP-1 inhibitors and of the widely used pharmacological tool inhibitors display little or no selectivity and thus any pharmacological effect thereof must be regarded as the effect of pan-PARP inhibition or, at minimum, of PARP-1 and PARP-2 together. As shown in this paper, our lead PARP-1 inhibitor 5-aminoisoquinolin-1-one (5-AIQ, Figure 1), which is potently active in vivo, is devoid of isoform-selectivity in vitro. Similarly, two other standard PARP-1 inhibitors

![Chemical Structures](image-url)

**Figure 1.** Structures of reported potent non-isoform-selective inhibitors 1-7, PARP-1-selective inhibitor 8 and PARP-2-selective inhibitors 9-11.
inhibitors, DPQ 2 and PJ34 3 are reported to have little isoform-selectivity between PARP-1 and PARP-2.33 The latest clinical candidates veliparib 4 34 and olaparib 5,36 the biochemical tools BYK49187 6a,35 BYK236864 6b35 and the thiopyranopyrimidinone 737 also inhibit the two isoforms potently but approximately equally. However, in 2006, Ishida et al. reported the identification of a series of quinazolin-4-ones as selective inhibitors of PARP-1, of which 8 was the most selective, with IC$_{50}$ (PARP-1) = 13 nM and IC$_{50}$ (PARP-2) = 500 nM (selectivity ratio = 39); minor modifications to the substitution on the fluorophenyl ring were tolerated in the structure-activity relationship (SAR) but the long pendant 3-(4-aryl-1,2,5,6-tetrahydropyridin-1-yl)propyl group at the 3-position of the quinazolinone was shown to be essential in this series, as 5-chloro-2-methylquinazolin-4-one was non-isoform-selective and was 92-fold less active against PARP-1 than was 8.38 The same paper also reports that 9a was some twelve-fold more potent as an inhibitor of PARP-2 than it was of PARP-1; other varied substituents in the 4-position of the phenyl were tolerated with little loss of selectivity.38 The observed selectivities were rationalised by examining X-ray structures of co-crystals of PARP-1 with inhibitors and of homology models of PARP-2. Separately, this group also reported that a 2-aryl-quinoxaline-5-carboxamide 9b had 5-fold selectivity for inhibition of PARP-2.39 By far the most selective inhibitors of PARP-2 claimed to date are a series of 5-benzoyloxy and 5-phenacyloxy- isoquinolin-1-ones and 3,4-dihydroisoquinolin-1-ones, of which 10 and 11 appear to show 60-fold and 16-fold selectivity, respectively.33 These compounds, which pick up the usual PARP-binding contacts of the classical (3,4-dihydro)isoquinolin-1-one pharmacophore, achieve their selectivity by relatively weak micromolar binding to PARP-1, rather than great potency against PARP-2. This poor binding to PARP-1 is probably due to the 5-benzoyloxy and 5-phenacyloxy substituents being too large to be accommodated in the slightly smaller hydrophobic binding pocket of PARP-1, as compared to that of PARP-2 (see below).
In the present work, we report on two different approaches to the discovery of PARP-2-selective inhibitors, both based on the isoquinolin-1-one core which gives good binding to both isoforms through the hydrogen-bonding network to the lactam oxygen and N—H and through π-stacking of the aromatic ring to the adjacent Tyr residues (Tyr$^{907}$/Tyr$^{449}$).

**Chemical synthesis**

Noting the remarkable selectivity for inhibition of PARP-2 by 10 claimed by Pellicciari *et al.*, we sought to use this core to develop further selective inhibitors of this isoform. Inhibitors 10 and 11 both contain O-aryl esters, which are likely to be labile to hydrolysis in physiological media. To obviate this potential problem, the ester was replaced by an amide in target compound 15a (Scheme 1); this amide should be isosteric but may restrict rotation in this part of the molecule.

Synthesis of 15a and its analogues 15b-n required quantities of 1. This compound is commercially available but is prohibitively expensive for applications in synthesis. There are three previous published syntheses of 1 (where the critical steps are: a Polonowski rearrangement 5-nitroisoquinoline-N-oxide, reductive cyclisation of methyl 2-cyanomethyl-3-nitrobenzoate and by condensation of methyl-2-methyl-3-nitrobenzoate with dimethylformamide dimethylacetal) but all have severe limitations. Thus, for large-scale preparation of 1, a new synthesis was needed, with requirements for high yield and avoidance of scale-limiting steps such as column chromatography. Scheme 1 shows our development of this important new route. In this route, the 5-nitrogen substituent is introduced by nitration. However, the most nucleophilic position of isoquinolin-1-ones is 4-C, so direct nitration of isoquinolin-1-one was not feasible. The activating enamide function of isoquinolin-1-one was masked as the iminochloride in 12. Commercially available 1-chloroisoquinoline 12 was nitrated selectively in the 5-position in 92% yield; to achieve this regioselectivity, it was necessary to pre-dissolve 12 in
conc. sulfuric acid to ensure that the heterocyclic ring was fully protonated and deactivated before addition of the nitrating reagents. The imino-chloride in 13 was hydrolysed by heating in acetic acid to provide 14 in high yield and catalytic hydrogenation of the nitro group, under conditions previously reported,\textsuperscript{42} then furnished 1. This new sequence is reliable, high-yielding (54% overall), highly reproducible and involves only two intermediate recrystallisations and no chromatography for purification, giving the potential for it to be used on larger scales.

5-Benzamidoisoquinolin-1-one 15a was synthesised in good yield by acylation of 1 with benzoyl chloride in hot pyridine (Scheme 1); the relatively forcing conditions were required to overcome the poor nucleophilicity of the exocyclic amine in 1 and to ensure good solubility. To explore the structure-activity relationships around this the phenyl ring, a series of 5-(substituted-benzamido)isoquinolin-1-ones 15b-j were prepared similarly from 1 and substituted benzoyl chlorides. Following a classical medicinal chemical replacement of the benzene aromatic ring with thiophene led to design of 5-(thiophen-2-ylcarboxamido)isoquinolin-1-one 15k, which was prepared from 1 with thiophene-2-carbonyl chloride. The need for aromatic-
ity was tested by replacement with cyclohexyl in 15l and by 3-D bulky aliphatic groups in the pivalamido analogue 15m and the adamantylcarboxamido compound 15n.

Compound 22 (Scheme 2) extends the structure of 15a by introducing a methyl group at the 3-position of the isoquinolin-1-one ring system. The synthesis of this target was analogous to that of 15a, thus requiring 3-methylisoquinolin-1-one 21 as a starting material. As for 1, this educt is commercially available but prohibitively expensive (> £5000 g⁻¹). None of the synthetic sequences to 1 could be adapted to the 3-methyl analogue 21; reaction of methyl 2-methyl-3-nitrobenzoate with dimethylacetamide dimethylacetal gives 3-dimethylamino-1-methoxy-5-nitronaphthalene, rather than 19,43 and 1-chloro-3-methyl-5-nitroisoquinoline is only available by a low-yielding Polonowski rearrangement of 3-methyl-5-nitroisoquinoline-N-oxide.44 A new route was required, starting from a suitable 1,2,3-trisubstituted benzene. The Hurtley reaction involves Cu-catalysed displacement of the bromine of ortho-bromobenzoic acids by the enolates of β-diketones.45,46 However, we have previously shown that the reaction fails with 3-bromo-2-nitrothiophene-4-carboxylic acid.47 Despite this adverse omen, 2-bromo-3-nitrobenzoic acid 16 did react with the potassium enolate of pentane-2,4-dione in the presence of Cu powder. Under the reaction conditions (boiling t-butanol), the immediate Hurtley coupling product 17 was not observed; rather it was deacetylated to the monoketone 18, which cyclised to give the isocoumarin 19, achieving several synthetic steps in one pot. The isocoumarin 19 was converted to the isoquinolin-1-one 20 and reduction of the nitro group with tin(II) chloride furnished the required intermediate 21 for acylation to provide the target amide 22.

In 2004, the crystal structure of the catalytic fragment of murine PARP-2 was solved at 2.8 Å resolution, seven years after the data for the chicken PARP-1 catalytic fragment were made available.49 The high degree of homology of the PARP catalytic domain between different
species means that PARP inhibitors are unlikely to show a wide species-difference in their binding of inhibitors between chicken, mouse and human PARPs, for a specific isoform. The binding of inhibitors to PARP-1 was deduced by Ruf et al.\textsuperscript{50} to be due to hydrogen bonding from the carbonyl of lactams/amides in the inhibitors to Gly\textsuperscript{863}-NH and Ser\textsuperscript{904}-OH and from the lactam/amide N—H to Gly\textsuperscript{863}-O and hydrophobic or π-stacking interactions with Tyr\textsuperscript{907}. PARP-2 is a much smaller protein than is PARP-1 but the hydrogen-bonding and π-stacking motifs are retained in the nicotinamide-binding site, with Gly\textsuperscript{405} (PARP-2) corresponding to Gly\textsuperscript{863} (PARP-1), Ser\textsuperscript{446} (PARP-2) corresponding to Ser\textsuperscript{904} (PARP-1) and Tyr\textsuperscript{449} (PARP-2) corresponding to Tyr\textsuperscript{907} (PARP-1). Our inspection of the binding sites of the isoforms noted that Gln\textsuperscript{763} (neutral, polar) in PARP-1 is replaced by Lys\textsuperscript{308} (basic, polar); these residues are located slightly above the plane of the nicotinamide mimic and at the edge of the hydrophobic pocket; i.e. “south” of the 5-position of potentially bound isoquinoline-1-ones. Using molecular modelling to design further potentially selective inhibitors, the crystal structures of chicken PARP-1 with 8-hydroxy-2-methylquinazolin-4-one bound\textsuperscript{50} and murine PARP-2\textsuperscript{48} were used as starting structures, from which the binding pockets were established and com-
pared. The binding pocket of PARP-1 was measured and mapped, together with key interactions between the bound inhibitor and the pocket. Comparisons were also made to PARP-1 without inhibitor to confirm that the receptor binding pocket is relatively rigid and does not change conformation upon binding the substrate or an inhibitor ligand. The distances observed in PARP-1 were then used to dock a minimised and charged (Gastieger / Hückel) structure of 1 into the binding pocket of PARP-2. Once docked, restraints were added (as observed in the PARP-1 model) and the ligand was subjected to molecular dynamics (300 K for 5 ps) and then re-minimised. The ligand and binding pocket (5.0 Å from ligand) were then subjected to molecular dynamics (300 K for 5 ps) and the complete complex (enzyme and 1) was minimised to give the final model. In this model, the basic Lys$^{308}$ sat close (3.1 Å) to the 5-amine of 1. Two short series of compounds were designed to exploit this opportunity for isoform-selectivity by linking an anionic carboxylate to the 5-position of the isoquinolin-1-one.

In the first three compounds, the carboxylic acid was attached to the 5-position of the isoquinolin-1-one through all-carbon links (Scheme 3). Compound 24 was prepared by hydrolysis of 5-cyanoisoquinolin-1-one 23.$^{51,52}$ The targets 26 and 27 have a C$_2$ chain between the carboxylic acid and the isoquinoline, the former with a rigid link and the latter with a flexible link. These were approached by a Heck coupling of 5-iodoisoquinolin-1-one 25 to give 26.$^{52}$ Selective hydrogenation of the exocyclic double bond under acidic conditions led to 27.

**Scheme 3.** Synthesis of C-linked 1-oxoisquinoline-5-carboxylic acids 24, 26 and 27. Reagents and conditions: i, KOH, EtOH, reflux, then aq. HCl, 83%; ii, HO$_2$CCH=CH$_2$, Pd(OAc)$_2$, Et$_3$N, EtCN, reflux, 97%; iii, H$_2$, Pd/C, EtOH, 66%.
Exploiting the now ready availability of 1 as a starting material, it was planned to attach carboxylic acids through linkers to the exocyclic amine to provide target compounds such as 29 (Scheme 4). Heating 1 with ethyl bromoacetate in DMF in the presence of tertiary amine base alkylated the weakly nucleophilic amine, giving the ester 28 in modest yield. Hydrolysis then furnished the carboxylic acid 29. Attempting to access the homologous ester 30, 1 was treated with sodium hydride and methyl acrylate. Surprisingly, the sole isolable product was 31, where the CH₂CH₂CO₂Me unit is attached to the lactam 2-N, rather than the exocyclic amine. The location of this unit was confirmed by the ¹H NMR chemical shift of the NCH₂ protons (δ 4.09, more typical of CH₂ attached to the lactam rather than CH₂ attached to an aniline nitrogen) and by HMBC correlations between these CH₂ protons and the lactam carbonyl-¹³C at δ 161.2 and between these CH₂ protons and 3-C at δ 130.6; these correlations are incompatible with the alternatives 30 or 1-(MeO₂CH₂CH₂CO)-5-NH₂-isoquinoline (from alkylation at oxygen). Sodium hydride is a much stronger base than the diisopropylethylamine used in the synthesis of 28 and is capable of removing the lactam N—H proton. In the anion so generated, the N⁺ of the lactam is more nucleophilic than the neutral exocyclic -NH₂ and reacts with the electrophile. Anions derived from other 5-substituted isoquinolin-1-ones usually react through the 2-N with alkylating electrophiles,⁴²,⁵³,⁵⁴ although there are some examples of reaction at the exocyclic oxygen at the 1-position under Mitsunobu conditions.⁵² Hydrolysis of the ester of 31 with aqueous acid gave the corresponding carboxylic acid 32.

Scheme 4. Synthesis of 5-(ω-carboxyalkylamino)-isoquinolin-1-ones 29 and 32. Reagents & conditions: i, EtO₂CH₂Br, Pr₂NEt, NaI, DMF, 80°C, 19%; ii, aq. HCl, reflux, 87% (29), 85% (32); iii, NaH, THF, MeO₂CCH=CH₂, 67%.
In view of the unexpected regioselectivity of the reaction of 1 with methyl propenoate under strongly basic conditions, it was necessary to prove the structure of 28 or 29 through an X-ray crystal structure determination. Careful recrystallisation of the carboxylic acid 29 from methanol provided diffraction-quality crystals of the corresponding methyl ester 33. The crystal structure (Figure 2) confirmed the expected structure, with the CH₂CO₂Me attached to the exocyclic amine. The isoquinolin-1-ones formed hydrogen-bonded pairs in the crystal, with the lactam N—H bonded to the carbonyl oxygen of the partner molecule. The 5-NHCH₂CO₂Me unit adopted an extended conformation but appears not to be hydrogen-bonded; the exocyclic nitrogen was planar, as expected, and the methylene was orientated away from the peri 4-H.

Biochemical and cell biological evaluation

The two series of 5-substituted isoquinolin-1-ones were evaluated *in vitro* for inhibition of the catalytic activities of full-length active human PARP-1 isolated from HeLa cell nuclear extract (using a FlashPlate assay previously developed by us) and full-length mouse PARP-2 (using a solution-phase assay); the results are presented in Table 1 for 15a-n, 22, 24, 26, 27, 29 and 32. 1 and the two claimed PARP-2-selective inhibitors 9b and 10 from the literature were assayed as controls.
As expected, 1 showed no selectivity towards either isoform, with IC\textsubscript{50} = \textit{ca.} 1 \mu M for each. The 5-benzamido compound 15a was approximately equipotent with 1 against PARP-2 but the increased bulk of the benzoyl group and loss of the basicity of the amine diminished the inhibition of PARP-1 dramatically, leading to a 9.3-fold selectivity towards inhibition of PARP-2. Exploration of the SAR around the phenyl ring indicated that introducing a \textit{para}-substituent reduced the selectivity, mostly through decreasing the potency against PARP-2 (15b,d-f), rather than by increasing the inhibition of PARP-1; the 4-nitrobenzamido and 4-iodobenzamido analogues 15c,h were the exception, where the compounds were more potent against PARP-1 than was 15a. Moving the methyl group to the ortho-position of the benzamide, in 15i, reduced inhibition against both isoforms but the corresponding ortho-iodo compound 15j was non-selective but had fairly good potency against PARP-1 and PARP-2. The common medicinal chemical replacement of a benzene ring with a thiophene, in 15k, reduced potency against both isoforms and replacement with bulky aliphatic groups, in 15l-n, continued the trend of diminishing activity without gain of selectivity. Adding a methyl group at the 3-position, in 22, caused loss of potency against PARP-2 relative to that of 15a but retention of activity against PARP-1, leading to a loss of selectivity towards the former enzyme.

Curiously, the benzoate ester 10, which is claimed to be the most selective for inhibition of PARP-2 with a reported ratio of IC\textsubscript{50} values \textit{ca.} 60\textsuperscript{33} had activity against PARP-2 very similar to that of the close analogue, the benzamide 15a, as expected, but proved to be potent in our assay of inhibition of PARP-1 catalytic activity, giving a selectivity ratio of only 2.75. The quinoxaline-5-carboxamide 9b was remarkably potent against both isoforms, with IC\textsubscript{50} = 30 nM \textit{vs.} PARP-1 and IC\textsubscript{50} = 90 nM \textit{vs.} PARP-2. These values give an approximate 3-fold selectivity for inhibition of PARP-1, in contrast to the claimed 5-fold selectivity for PARP-2.\textsuperscript{39} These marked differences in apparent selectivities for the different isoforms probably arise from the different assays used and different sources of the enzymes used. Pellicciari \textit{et
al.\textsuperscript{33} compared bovine PARP-1 with murine PARP-2 using the incorporation of radioactivity from $^3$H-NAD$^+$ into trichloroacetic acid-insoluble material as an assay of enzymic activity, whereas Iwashita \textit{et al.}\textsuperscript{39} compared human PARP-1 with murine PARP-2 using the same method.\textsuperscript{60} We compared human PARP-1 with murine PARP-2 using different assay methods. Thus it is highly likely that our comparative assays are a much more stringent test of selectivity for inhibition of PARP-2 than the assay pairs previously used or, alternatively, tend to bias towards reporting selectivity for inhibition of PARP-1.

Table 1 also shows the evaluation of the isoquinolin-1-ones bearing a carboxy-terminated function at the 5-position; these carboxylates had been designed to interact with the positively charged side-chain of Lys$^{308}$ near the PARP-2 NAD$^+$-binding site. Using the current assays for PARP-1 and for PARP-2, isoquinolinone-5-carboxylic acid 24 showed little inhibition of either isoform, a surprising result in view of the observation of IC$_{50}$ < 13 μM in an earlier assay using broken cell nuclei as the source of PARP activity.\textsuperscript{51} The isoquinolin-1-ones 26, 27 and 29 carrying the carboxy group attached to the 5-position through a more-or-less flexible linker were active against both isoforms but with modest selectivity. The isoquinolinone-5-propenoic acid had previously shown IC$_{50}$ < 11 μM in the broken nuclei assay against mixed PARPs, broadly consistent with the presently observed values in the purified enzyme assays.\textsuperscript{51} Curiously, 32, which bears the carboxy group attached through a tether to the 2-N of the ring system, was potent against both isoforms, with ca. 3-fold selectivity for inhibition of PARP-1 over PARP-2. This compound appears to be slightly more potent against PARP-1 than is the lead PARP inhibitor 1 but lacks the N–H generally taken\textsuperscript{3} to be part of the essential pharmacophore for binding to the PARPs (through an H-bond to Gly$^{863}$-O in PARP-1 and to Gly$^{405}$-O in PARP-2) but Eltze \textit{et al.} have recently reported some inhibitors of PARP lacking this N–H (fused 2,3-dihydroquinolin-4-ones 5, thus with CH$_2$ replacing the usual NH).\textsuperscript{35} The precise binding mode of 32 to the PARPs will be the subject of later studies.
The most selective inhibitor of PARP-2 identified in our study, 5-benzamidoisoquinolin-1-one 15a, together with the non-isoform-selective lead inhibitor 1 and examples 15l-n of 5-(bulky alkyl)amidoisoquinolin-1-ones which are poorly active against both isoforms, were evaluated for cytotoxicity towards a panel of three diverse human carcinoma cell lines and one human fibroblast cell line in vitro using the MTS colorimetric assay. None of the isoquinolin-1-ones were active against HT29 human colon carcinoma cells or the FEK4 normal human fibroblasts (Table 2). Two compounds, 1 and the most selective PARP-2 inhibitor 15a were very weakly cytotoxic towards the MDA-MB-231 human breast carcinoma cells and two, 15a and 15n, were weakly cytotoxic towards the LNCaP human prostate carcinoma cells. These results are consistent with our previous observation that 1 caused only 40% inhibition of the proliferation of CT26 murine colon carcinoma cells at the very high concentration of 1 mM.57 None of the cell lines used here have mutant BRCA, so the lack of cytotoxicity is unsurprising.

**Post facto structural studies**

*Post facto* molecular modelling studies were undertaken to rationalise the strong selectivity for inhibition of PARP-2 observed for the 5-benzamidoisoquinolin-1-one 15a and for the smaller but significant selectivity observed for the carboxylic acid 29; these represent the most isoform-selective examples from each of the two series originally designed. These *post facto* studies were aided by the disclosure in 2009 (after the current experimental work had been completed) of a crystal structure of human PARP-158 and in 2010 of a crystal structure of human PARP-2 complexed with ABT888 4.59 Starting structures were taken from published X-ray crystallographic structures of human PARP-1 complexed with a quinoxalinone inhibitor (A861696)58 and of murine PARP-2 without an inhibitor ligand.48 The latter was compared with the more recent data for human PARP-2 obtained with 3-aminobenzamide bound;59 this comparison was used to drive docking and to refine the nicotinamide / inhibitor-
binding pocket of PARP-2. Non-selective inhibitor 1 and PARP-2-selective inhibitors 15a and 29 were then docked into the models using the existing bound inhibitor (for PARP-1) and the docked inhibitor (for PARP-2) as templates. Once docked, the inhibitors were subjected molecular mechanics and dynamics calculations to establish optimal docking conformations; during these calculations, the receptor was restrained to its original conformation. Lastly, both the inhibitors and binding pockets (radius 10Å) were subjected to molecular dynamics and finally molecular mechanics calculations to give the final structures (Figure 3). The structures of 15a and of 29 made the expected hydrogen-bonding contacts of the lactam with the Ser and Gly residues and π-stacks of the isoquinoline-1-one cores with Tyr107 (PARP-1) and Tyr449 (PARP-2).

Differences between the binding pockets of PARP-1 and PARP-2 can be observed from the models. The binding pocket in PARP-1 is smaller than that of PARP-2. Furthermore, the
cavity which accepts groups attached to the 5-position of isoquinolin-1-ones is arranged essentially in plane with the bound 1 in PARP-2 but lies significantly above the plane of the bound ligand in PARP-1. This means that any bulky substituent in this position should lead to selectivity for binding to PARP-2 and inhibit binding to PARP-1. Compound 1 has a very small amino group in this location, which can easily be accommodated in both structures. By contrast, the 5-benzamido group in 15a can only be accommodated by the larger and in-plane void of the PARP-2 structure and is sterically excluded by PARP-1 (Figure 3). Indeed, it was not possible to generate a corresponding model for binding of 15a to PARP-1, owing to steric clash of the benzamide unit with the walls of the pocket. Now, the apparent SAR requirement for a benzamide in the 5-position, rather than an equivalent aliphatic amide, can be rationalised by the establishment of an additional aromatic π-stacking interaction onto a Tyr residue (Tyr438) in the wall of the pocket in PARP-2. The structure of human PARP-2 contains a captive water molecule near the 3-amino group of the bound inhibitor 3-aminobenzamide and it has been proposed that this mediates hydrogen bonding to the amine. A similar water-mediated hydrogen bond to the 5-NH₂ could also be responsible, in part, for the good binding of 1 to both PARP-1 and PARP-2. The facility for hydrogen bonding (now to the secondary amide N-H) is retained in bound 15a.

Similar docking / modelling of the less selective inhibitor 29 into the active site of murine PARP-2 was carried out. The above putative water-mediated hydrogen bond is also available in this structure (from the 5-NH). The side-chain is located within the “in plane” pocket of the PARP-2 protein, although the π-stacking is, of course, not possible. The terminal carboxylate can also access the ammonium side-chain of Lys308. The smaller size and the flexibility of the side-chain of 29 permit access to both the larger pocket of PARP-2 and the smaller pocket of PARP-1, leading to more modest isoform-selectivity than for 15a.
Conclusions

In this paper, we report the design, synthesis and biochemical evaluation of new 5-substituted isoquinolin-1-ones as selective inhibitors of PARP-2. Compounds were designed partly by modelling using the reported crystal structures of the catalytic NAD$^+$-binding sites of PARP-1 and PARP-2.\textsuperscript{48,49} A new high-yielding synthesis of our lead PARP inhibitor 1 has been developed, which has the potential for large-scale preparations of this important compound to make it more economically available for biological and chemical studies. A Hurtley coupling to 2-methyl-3-nitrobenzoic acid 16 led directly to the isocoumarin 19; conversion of 19 to the isoquinolin-1-one 20 and reduction of the nitro group provided a good route to 21. Acylation of the exocyclic amines of 1 and 21 gave the corresponding 5-amidoisoquinolin-1-ones 15a-n and 22 in good yields. Four isoquinolin-1-ones carrying carboxylates at to 5-position (linked directly to the ring or through tethers) were synthesised by hydrolysis of 5-cyanoisoquinolin-1-one 23, by a Heck coupling to 5-iodoisoquinolin-1-one 25 or by chain-extension from the amine of 1. Under strongly basic conditions, 1 was alkylated at the ring 2-N, rather than at the exocyclic amine.

The isoform-selectivities of compounds 24, 26, 27 and 29, carrying carboxylates designed to bind electrostatically to basic Lys$^{308}$ in the PARP-2 structure, which replaces neutral Gln$^{763}$ in PARP-1, were disappointing. Reasonable potency against PARP-2 was observed for 26, 27 and 29 but inhibition of PARP-1 activity was also strong. The modest selectivity was rationalised by \textit{post facto} modelling studies.

The 5-amidoisoquinolin-1-ones were investigated as more hydrolytically stable analogues of the benzoate ester 10, for which Pellicciari \textit{et al.} claimed 60-fold selectivity for inhibition of PARP-2.\textsuperscript{33,56} Of this series, the simple benzamide 15a, the closest analogue of 10, proved to be the most selective (IC$_{50}\text{(PARP-1)}$ / IC$_{50}\text{(PARP-2)}$) = 9.3. In our comparative assays, 15a had act-
ivity equal to that of 10 against PARP-2 but 10 was much more potent than was 15a against PARP-1, leading to an observed selectivity of only 2.75-fold for 10. Similarly, the quinoxaline-5-carboxamide 9b showed a 3-fold selectivity for PARP-1 in our comparative assays, contrasting with a claimed 5-fold selectivity for PARP-2.39 In the light of these results, it is now evident from our direct comparisons that 5-benzamidoisoquinolin-1-one 15a is the most isoform-selective inhibitor of PARP-2 reported to date. Post facto modelling studies rationalised the structural basis of the observed selectivity.

Acknowledgements

We wish to thank Dr. Timothy J. Woodman (University of Bath) for many of the NMR spectra, Dr. Anneke Lubben (University of Bath) for the mass spectra and Professor Rex M. Tyrrell (University of Bath) for the kind gift of the FEK4 cells. We are very grateful to KuDOS Pharmaceuticals Ltd., Cancer Research UK and the University of Bath for financial support. MDT, MDL and AST are members of Cancer Research at Bath (CR@B).

Experimental section

General

NMR spectra were recorded on JEOL Delta 270 and Varian Mercury 400 spectrometers. Mass spectra were obtained using VG7070E and Bruker microTOF™ spectrometers. IR spectra were measured on a Perkin-Elmer RXI FTIR spectrometer. The stationary phase for chromatography was silica gel. All reactions were carried out at ambient temperature, unless otherwise stated. Solvents were evaporated under reduced pressure. Melting points were determined using a Reichert-Jung Thermo Galen instrument and are uncorrected. Target compounds for biochemical evaluation were >95% pure, as shown by CHN combustion microanalyses (carried out at the School of Pharmacy, University of London) and TLC for novel
compounds and by identity of mp and TLC for known compounds. Compound 12 was obtained from the Aldrich Chemical Co.

5-Aminoisoquinolin-1-one hydrochloride (1). Compound 14 (1.6 g, 8.4 mmol) was stirred with Pd/C (10%, 1.0 g) in EtOH (120 mL) and aq. HCl (9 M, 4 mL) under H₂ for 2 h. The suspension was then filtered through Celite®. The Celite® pad and residue were suspended in water (1000 mL) and heated. The hot suspension was filtered through a second Celite® pad. Concentration of the filtrate and drying gave 1 (1.2 g, 71%) as white crystals: mp 248–252°C (decomp.) (lit.¹⁵ 250–260°C (decomp.)).

1-Chloro-5-nitroisoquinoline (13). Aq. HNO₃ (70%, 850 mg, 13.4 mmol) in conc. H₂SO₄ (5 mL) was added dropwise to 12 (2.00 g, 12.2 mmol) in conc. H₂SO₄ (10 mL) at 0-5°C. The mixture was stirred at 0°C for 2 h, then poured onto ice. The precipitate was collected, washed (H₂O), dried and recrystallised (EtOAc / hexanes) to give 13 (2.34 g, 92%) as pale yellow crystals: mp 181–183°C (lit.⁶¹ mp 183–184°C).

5-Nitroisoquinolin-1-one (14). Compound 13 (5.00 g, 24.0 mmol) was stirred at 100°C in AcOH (100 mL) for 40 h. The cooled suspension was then poured onto ice. The solid was collected, washed (H₂O) and recrystallised (EtOH) to give 14 (3.74 g, 82%) as pale yellow crystals: mp 247–249°C (decomp.) (lit.¹⁵ mp 247–249°C).

5-Benzamidoisoquinolin-1-one (15a). Compound 1 (50 mg, 0.25 mmol) was stirred with PhCOCl (39 mg, 0.28 mmol) in pyridine (2.0 mL) at 90°C for 16 h. Evaporation and recrystallisation (EtOAc) gave 15a (57 mg, 86%) as an off-white solid: mp >310°C (decomp.).

3-Methyl-5-nitroisocoumarin (19). 2-Bromo-3-nitrobenzoic acid 16⁶² (2.5 g, 10 mmol) and Cu powder (67 mg, 1.1 mmol) were added to pentane-2,4-dione (5.3 g, 53 mmol) and KOBu’ (2.3 g, 20 mmol) in Bu’OH (50 mL). The mixture was boiled under reflux for 16 h, then
poured into H₂O (350 mL) and acidified with aq. HCl (2 M). Extraction (Et₂O), evaporation and chromatography (hexane / EtOAc 3:2) gave 19 (470 mg, 23%) as yellow crystals: mp 199–200°C.

3-Methyl-5-nitroisoquinolin-1(2H)-one (20). A solution of 19 (470 mg, 2.3 mmol) in MeO(CH₂)₂OH (100 mL) was saturated with NH₃, boiled under reflux for 4 h, then evaporated until 10 mL remained. The concentrate was stored at 4°C for 16 h. The solid was collected, washed (H₂O, EtOH) and recrystallised (MeOH) to give 20 (320 mg, 68%) as bright yellow crystals: mp 231–232°C (decomp.).
5-Amino-3-methylisoquinolin-1(2H)-one (21). Compound 20 (320 mg, 1.6 mmol) was heated with SnCl₂ (900 mg, 4.7 mmol) in EtOH (20 mL) at 70°C for 4 h, then carefully poured into ice-H₂O (200 mL). The suspension was made alkaline with aq. NaOH and the precipitate was filtered. Extraction of the filtrate (EtOAc), evaporation and recrystallisation (hexane, EtOAc) gave 21 (160 mg, 59%) as yellow crystals: mp 183–184°C.

3-(1-Oxoisoquinolin-5-yl)propanoic acid (27). Compound 26 (160 mg, 8.4 mmol) in EtOH (25 mL) and aq. HCl (34%, 4 mL) was stirred vigorously with Pd/C (10%, 100 mg) under H₂ for 2 h. Filtration (Celite®) and evaporation of the solvent from the filtrate 27 (1.1 g, 66%) as white crystals: mp 260–263°C.

Ethyl 2-(1-oxoisoquinolin-5-ylamino)acetate (28). Compound 1 (1.0 g, 4.4 mmol), Pr₂NEt (1.4 g, 11 mmol), ethyl bromoacetate (885 mg, 5.3 mmol) and NaI (100 mg, 0.7 mmol) were stirred at 80°C in DMF (60 mL) for 16 h. Evaporation and recrystallisation (MeOH) gave 28 (121 mg, 19%) as pale buff crystals: mp 199–201°C.

5-(Carboxymethylamino)isoquinolin-1-one hydrochloride (29). Ester 28 (94.0 mg, 0.39 mmol) was boiled under reflux in aq. HCl (6.0 M, 4.0 mL) for 3 h. Evaporation gave 29 (86 mg, 87%) as a pale amber solid: mp 275–280°C (decomp.).

Crystal data for 33. All data were collected at 150 K on a Nonius kappaCCD diffractometer. The structure was solved using SHELXS-97 and refined using full-matrix least squares in SHELXL-97. C₁₂H₁₂N₂O₃, M = 232.24, λ = 0.71073 Å, triclinic, space group = P-1 (No. 2) a = 7.7290(3), b = 7.7930(3), c = 9.2330(4)Å, α = 103.760(2), β = 93.294(2), γ = 94.054(2°), U = 537.23(4) Å³, Z = 2, Dₐ = 1.436 g cm⁻³, μ = 0.105 mm⁻¹, F(000) = 244. Crystal size = 0.25 × 0.25 × 0.10 mm, unique reflections = 2405 [R(int) = 0.0494], observed (I > 2σ(I)) = 1503, data / restraints / parameters = 2405 / 1 / 160. Final R indices [I > 2σ(I)], R1 = 0.0547, wR2
$R = 0.1355; R$ indices (all data) $= R1 = 0.1010, wR2 = 0.1589$. Max. peak / hole, 0.718 and -0.309 eÅ$^-3$. Crystallographic data for 33 have been deposited at the Cambridge Crystallographic Data Centre: CCDC 781071. Requests for data should be addressed to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.

**PARP-1 inhibition assay.** Compounds were assayed for inhibition of the catalytic activity of PARP-1 using the FlashPlate scintillation proximity assay previously developed at KuDOS.$^{55}$ Compounds were evaluated at eight different concentrations (0.75 nM – 1.5 μM + further concentrations to 10 μM if necessary) in triplicate, using full-length human PARP-1 (ca. 50 ng) isolated from HeLa cell nuclear extract. Compounds were dissolved in DMSO prior to addition, to give a final concentration of 2% in the assay mixture, a concentration which had been shown to have no effect on the activity of the enzyme. Oligonucleotides 5’-ACTTGATTAGTTACGTAACGTTATGATTGA-3’ / 5’-TCAATCATAACGTTACGTAACTAATCAAGT-3’ were used as the DNA ligand. The concentration of NAD$^+$ was 5.0 μM. The reaction buffer was HEPES (1.0 M, 12.5 mL), MgCl$_2$ (1.0 M, 6.25 mL), KCl (3.0 M, 8.3 mL), dithiothreitol (77 mg), glycerol (propane-1,2,3-triol, 50 mL), NP-40 (50 μL) made up to 500 mL with milliQ water, then adjusted to pH 7.6 with aq. KOH (3.0 M). Compounds were incubated with the enzyme for 10 min, then the other components were added and the reaction allowed to proceed for 45 min. Data were fitted using a logarithmic concentration scale to a dose-response curve using SigmaPlot 11; IC$_{50}$ values were measured usually in two or three independent experiments and the mean values are reported.

**PARP-2 inhibition assay.** Compounds were assayed for inhibition of the catalytic activity of PARP-2 using a method in which recombinant full-length murine PARP-2 protein (Alexis) (ca. 50 ng) was bound down by a PARP-2-specific antibody in a 96-well white-walled plate. PARP-2 activity was measured following addition of $^3$H-NAD$^+$ and DNA.$^{36}$ After washing,
scintillant was added to measure the $^3$H-incorporated. Compounds were evaluated at eight different concentrations (0.75 nM – 1.5 μM + further concentrations to 10 μM if necessary) in triplicate. Compounds were dissolved in DMSO prior to addition, to give a final concentration of 2% in the assay mixture, a concentration which had been shown to have no effect on the activity of the enzyme. Oligonucleotides 5'-ACTTGATTAGTTACGTAACGTTATGATTGA-3' / 5'-TCAATCATAACGTTACGTAACTAATCAACTAAGT-3' were used as the DNA ligand. The concentration of NAD$^+$ was 2.5 μM. The reaction buffer was HEPES (1.0 M, 12.5 mL), MgCl$_2$ (1.0 M, 6.25 mL), KCl (3.0 M, 8.3 mL), dithiothreitol (77 mg), glycerol (propane-1,2,3-triol, 50 mL), NP-40 (50 μL) made up to 500 mL with milliQ water, then adjusted to pH 7.6 with aq. KOH (3.0 M). Compounds were incubated with the enzyme for 10 min, then the other components were added and the reaction allowed to proceed for 45 min. Data were fitted using a logarithmic concentration scale to a dose-response curve using SigmaPlot 11; IC$_{50}$ values were measured usually in two or three independent experiments and the mean values are reported.

**Supporting Information Available:** Synthetic procedures and spectroscopic data for 15b-n, 22, 24, 26, 31 and 32, spectroscopic data for 1, 13, 14, 15a, 19, 20, 21 and 27-29, biochemical and cell biological evaluation procedures and dose-response curves, elemental combustion analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

**References**


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63. Sheldrick, G. M. SHELXL-97, a computer program for crystal structure refinement; University of Göttingen: Göttingen, 1997.
Table 1. Inhibition of the activities of PARP-1 and PARP-2 by 5-amidoisoquinolin-1-ones 15a-n and 22, by 5-(ω-carboxyalkyl)- and 5-(ω-carboxyalkylamino)-isoquinolin-1-ones 24, 26 and 29 and by 2-(2-carboxyethyl)isoquinolin-1-one 32. Data for 1, 5-benzoyloxyisoquinolin-1-one 10 and 2-(4-chlorophenyl)quinoxaline-5-carboxamide 9 are shown for comparison.

<table>
<thead>
<tr>
<th>Cpd. No.</th>
<th>Isoquinolinone 3-substituent</th>
<th>Isoquinolinone 5-substituent</th>
<th>PARP-1 IC₅₀ (μM)</th>
<th>PARP-2 IC₅₀ (μM)</th>
<th>Observed selectivity (IC₅₀ (PARP-1) / IC₅₀ (PARP-2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H₂N-</td>
<td>0.94 ± 0.10</td>
<td>1.05 ± 0.11</td>
<td>0.9</td>
</tr>
<tr>
<td>15a</td>
<td>H</td>
<td>PhCONH-</td>
<td>13.9 ± 1.2</td>
<td>1.5 ± 0.2</td>
<td>9.3</td>
</tr>
<tr>
<td>15b</td>
<td>H</td>
<td>4-MePhCONH-</td>
<td>13.4 ± 1.2</td>
<td>6.5 ± 0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>15c</td>
<td>H</td>
<td>4-O₂NPhCONH-</td>
<td>3.0 ± 0.4</td>
<td>1.6 ± 0.2</td>
<td>1.9</td>
</tr>
<tr>
<td>15d</td>
<td>H</td>
<td>4-F₃CPhCONH-</td>
<td>10.7 ± 1.5</td>
<td>3.3 ± 1.0</td>
<td>3.2</td>
</tr>
<tr>
<td>15e</td>
<td>H</td>
<td>4-FPhCONH-</td>
<td>18.0 ± 1.2</td>
<td>3.6 ± 0.4</td>
<td>5.0</td>
</tr>
<tr>
<td>15f</td>
<td>H</td>
<td>4-ClPhCONH-</td>
<td>11.2 ± 1.3</td>
<td>3.9 ± 0.3</td>
<td>2.9</td>
</tr>
<tr>
<td>15g</td>
<td>H</td>
<td>4-BrPhCONH-</td>
<td>ND⁵⁹</td>
<td>ND⁵⁹</td>
<td>-</td>
</tr>
<tr>
<td>15h</td>
<td>H</td>
<td>4-IPhCONH-</td>
<td>7.6⁴</td>
<td>1.3⁴</td>
<td>5.8</td>
</tr>
<tr>
<td>15i</td>
<td>H</td>
<td>2-MePhCONH-</td>
<td>31.6 ± 3.4</td>
<td>5.6 ± 0.4</td>
<td>5.6</td>
</tr>
<tr>
<td>15j</td>
<td>H</td>
<td>2-IPhCONH-</td>
<td>4.5 ± 1.2</td>
<td>3.2 ± 1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>15k</td>
<td>H</td>
<td>(thiophen-2-yl)-CONH-</td>
<td>22.4 ± 3.4</td>
<td>7.0 ± 1.2</td>
<td>3.2</td>
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<tr>
<td>15l</td>
<td>H</td>
<td>cHexCONH-</td>
<td>&gt;80</td>
<td>27.9 ± 4.5</td>
<td>&gt;2.9</td>
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<tr>
<td>15m</td>
<td>H</td>
<td>Bu¹CONH-</td>
<td>&gt;100</td>
<td>29 ± 3.4</td>
<td>&gt;3.4</td>
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<tr>
<td>15n</td>
<td>H</td>
<td>(adamantan-1-yl)CONH-</td>
<td>&gt;50</td>
<td>19.9 ± 3.0</td>
<td>&gt;2.5</td>
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<tr>
<td>22</td>
<td>Me</td>
<td>PhCONH-</td>
<td>16.6 ± 1.2</td>
<td>6.3 ± 0.3</td>
<td>2.6</td>
</tr>
<tr>
<td>24</td>
<td>H</td>
<td>HO₂C-</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>H</td>
<td>HO₂CHC≡HC-</td>
<td>6.6 ± 1.0</td>
<td>4.7 ± 0.4</td>
<td>1.4</td>
</tr>
<tr>
<td>27</td>
<td>H</td>
<td>HO₂CCH₂CH₂-</td>
<td>8.6 ± 1.1</td>
<td>3.0 ± 0.2</td>
<td>2.9</td>
</tr>
<tr>
<td>29</td>
<td>H</td>
<td>HO₂CCH₂NH-</td>
<td>1.6 ± 0.1</td>
<td>0.55 ± 0.1</td>
<td>2.9</td>
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<tr>
<td>32⁵</td>
<td>H</td>
<td>H₂N-</td>
<td>0.55 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>0.4</td>
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<tr>
<td>10</td>
<td>H</td>
<td>PhCO₂⁻</td>
<td>4.1 ± 0.5</td>
<td>1.5 ± 0.4</td>
<td>2.75</td>
</tr>
<tr>
<td>9b</td>
<td>[2-(4-ClPh)quinoxaline-5-CNH₂]</td>
<td></td>
<td>0.03 ± 0.006</td>
<td>0.09 ± 0.02</td>
<td>0.33</td>
</tr>
</tbody>
</table>

⁵⁹ Not determined owing to limited solubility.
⁴ Measured once only.
⁵ Also carries –CH₂CH₂CO₂H at 2-position.
### Table 2. Inhibition of growth of HT29 human colon carcinoma cells, MDA-MB-231 human breast carcinoma cells, LNCaP human prostate carcinoma cells and FEK4 human dermal fibroblast cells by 1 and selected 5-amidoisoquinolin-1-ones 15a-l-n. Data are from single experiments using duplicates for each data point.

<table>
<thead>
<tr>
<th>Cpd No.</th>
<th>HT29 IC_{50} (μM)</th>
<th>MDA-MB-231 IC_{50} (μM)</th>
<th>LNCaP IC_{50} (μM)</th>
<th>FEK4 IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;200</td>
<td>170</td>
<td>&gt;200</td>
<td>&gt;200</td>
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<tr>
<td>15a</td>
<td>&gt;200</td>
<td>139</td>
<td>98</td>
<td>&gt;200</td>
</tr>
<tr>
<td>15l</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>15m</td>
<td>&gt;200</td>
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<td>&gt;200</td>
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<tr>
<td>15n</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>42</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>
15a: $R = \text{Ph, } \frac{IC_{50} (\text{PARP-1})}{IC_{50} (\text{PARP-2})} = 9.3$