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5-Aminoisoquinolin-1-one (5-AIQ), a Water-Soluble Inhibitor of the Poly-(ADP-Ribose)Polymerases (PARPs)

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Abstract: 5-Aminoisoquinolin-1-one (5-AIQ) is a water-soluble inhibitor of the poly(ADP-ribose)polymerases (PARPs), lacking isoform-selectivity. Although of only moderate potency *in vitro* against PARP-1, it is highly active in many assays in cells and in models *in vivo*, indicating excellent uptake. Optimisation of the several synthetic sequences to 5-AIQ has led to development of a short and efficient route from 1-chloroisoquinoline. It has been used widely as a biochemical and pharmacological tool to study the effects of inhibition of the PARPs. It ameliorates the damage to cells and tissues following reperfusion of ischaemic tissue, showing significant protective activity in a rodent model of haemorrhagic shock at the remarkably low dose of 30 μg Kg^{-1}. Protection is also seen in models of myocardial infarction, ischaemic kidney and liver disorders, stroke and organ transplantation. Inhibition of PARP-1 by 5-AIQ causes down-regulation of the activity of NF-κB, which then down-regulates the expression of several gene products. Thus 5-AIQ has anti-inflammatory activity *in vivo*, through modulating the expression of cytokines and adhesion molecules. This indirect inhibition of expression is relevant in the activity of 5-AIQ in models of arthritis, Parkinson's disease, multiple sclerosis, spinal cord injury, periodontitis and inflammatory conditions of the lung. Inhibition of expression of matrix metalloproteinases and other factors gives rise to anti-angiogenic activity and to remarkable anti-metastatic activity in a mouse model. Thus, although it has been overtaken by other PARP-inhibiting drugs in the oncological clinic, 5-AIQ remains a valuable tool to study the roles of PARPs in health and in diverse diseases.

Keywords: 5-AIQ, 5-Aminoisoquinolin-1-one, Cancer, Inflammation, Ischaemia-reperfusion injury, Metastasis, NF-κB, Poly(ADP-ribose)polymerase.

1. INTRODUCTION

1.1. 5-AIQ

5-Aminoisoquinolin-1-one (5-AIQ, **1**, Figure 1) is a small-molecule inhibitor of the catalytic activity of several isoforms in the PARP superfamily. As its hydrochloride salt, it is highly water-soluble, unlike many inhibitors. This solubility and good uptake into cells may contribute to its remarkable potency *in vivo*. In this review, the discovery, chemistry and biological activities of this compound are explored, particularly relating to its widespread use as a biochemical and biological tool.

1.2. The PARPs

The poly(ADP-ribose)polymerases (PARPs) comprise a superfamily of more than seventeen enzymes that catalyse the transfer of ADP-ribose units from NAD$^+$ onto glutamate side-chains on acceptor proteins, biosynthesising polyanionic poly(ADP-ribose) polymers [1]. The archetypal PARP, PARP-1, was discovered over fifty years ago [2] and it was the sole known member until 1999, when Amé *et al.* reported a second PARP, PARP-2 [3]. PARP-1 is responsible for
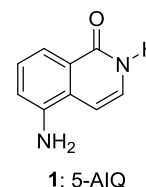
Figure 1. Structure of 5-aminoisoquinolin-1-one (**1**, 5-AIQ).

most of the poly(ADP-ribosyl)ation activity in the cell and has a major role in regulating the repair of damaged DNA. PARP-1 is a 116 KDa protein, comprising an N-terminal DNA-binding domain containing three zinc fingers [4], a central automodification domain containing a site for cleavage by caspase-3 [5] and a nuclear-localisation signal [6] and a C-terminal catalytic site, which binds the substrate NAD$^+$. Two of the zinc fingers are responsible for sensing damage in the DNA, leading to PARP-1 being referred to as a "molecular nick sensor" [7]. PARP-2 is a smaller protein (62 KDa), containing similar C-terminal NAD$^+$-binding catalytic domain but with an N-terminal DNA-binding domain which lacks zinc-fingers but is highly basic in nature and which is responsible for protein-protein interactions. This PARP also has a role in identifying damaged sites in DNA. Interestingly, one of the binding partners for PARP-2 is PARP-1 [8].

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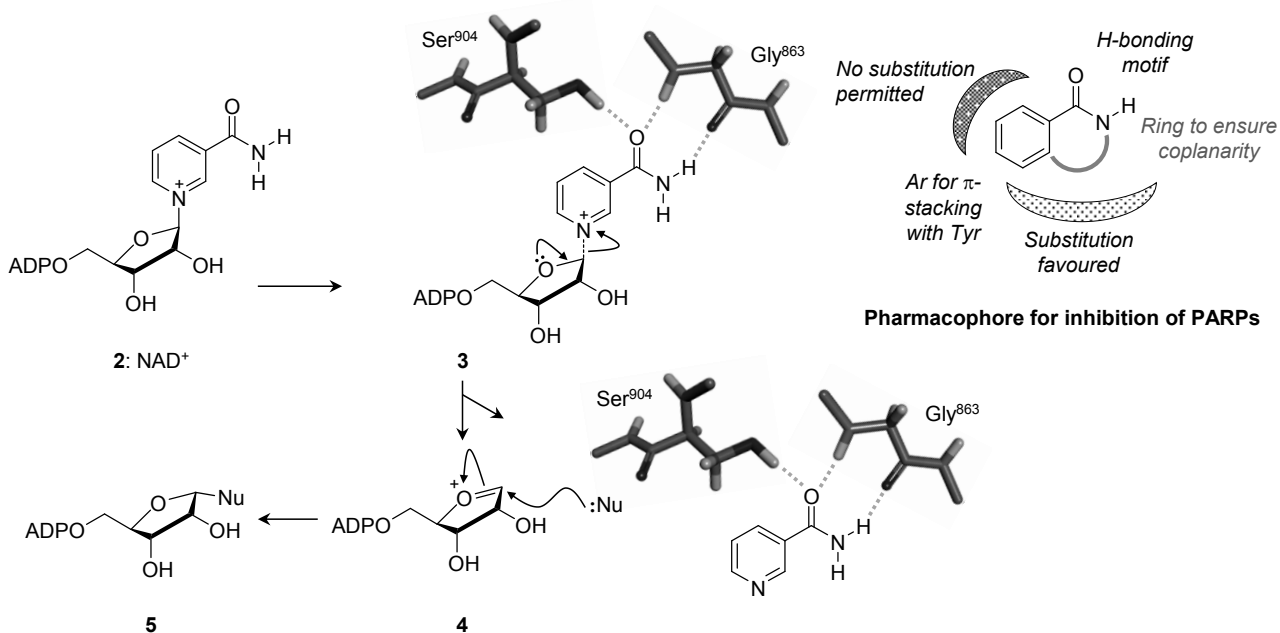
Scheme 1. General chemical mechanism for the poly(ADP-ribosyl)ation reaction catalyzed by PARPs, with amino-acids in the enzyme numbered as for PARP-1. General pharmacophore for compounds which inhibit PARPs.

Whereas the NAD$^+$-binding catalytic domains of PARP-1 and PARP-2 have 69% similarity [3], there is an insertion of three contiguous amino-acids in PARP-2, possibly correlating with differences in the selection of acceptor proteins for the two isoforms. For example, whereas PARP-1 primarily poly(ADP-ribosyl)ates the linker histone H1, PARP-2 tends to modify core histones [9]. In addition to its role in opening the structure of chromatin around the site of DNA damage and recruitment of repair proteins, PARP-1 regulates the function of NF-κB in transcription [10,11], leading to implication in inflammation [12], and in angiogenesis [13] and metastasis [14,15] in cancer. PARP-1 is also over-activated in ischaemia-reperfusion injury, where the re-supply of molecular oxygen causes extensive DNA damage [16-21]. This over-activation leads to rapid depletion and exhaustion of the cellular supply of NAD$^+$, part of its energy currency, leading to necrotic cell death. Whereas the roles of PARP-1 and PARP-2 do overlap, particularly in response to DNA damage [22], some specific functions of PARP-2 have been identified [9], *e.g.* in spermatogenesis [23], thymopoeisis [24] and function of telomeres [25].

PARP-3 is a smaller protein, which, although its NAD$^+$-binding domain has some homology with those of PARP-1 and PARP-2, appears to be a mono-ADP-ribosyl transferase [26]. Unlike PARP-1 and PARP-2, it is not activated by damage to DNA. It may have functions in regulating PARP-1 [26], NuMA (at the mitotic spindle) and tankyrase-1 (PARP-5a; at the telomere) [27]. Vaults are one of the smallest organelles in mammalian cells [28]. Despite first being identified in 1986 [29], their precise role is still unclear, although they may be involved in trafficking of small molecules or proteins or in multi-drug resistance. The major protein component is MVP, with smaller amounts of telomerase-associated protein (TEP-1) and a large PARP, PARP-4 (193 KDa). When isolated, PARP-4 (also known as vPARP) binds

NAD$^+$ and is catalytically active [30] but it is not known whether this activity is relevant to its function in the vault or if PARP-4 is merely a structural protein.

The tankyrases (PARP-5a and PARP-5b) are a pair of closely related members of the PARP superfamily, with roles in regulating the lengths of telomeres, in the correct function of the mitotic spindle and in the *wnt* signalling pathway [31,32]. These two isoforms are almost mutually redundant, although some roles are currently thought to be fulfilled by tankyrase-1 alone. As most of the functions of the tankyrases are involved with proliferation of cells, there is considerable current interest in designing and developing selective and potent inhibitors for cancer therapy [33]. Other PARPs have received less attention as targets for drug design, although PARP-14 has recently been recognised as key to proliferation in multiple myeloma and in aggressive B-cell lymphoma [34-36].

1.3. Mechanism of poly(ADP-ribosyl)ation

Scheme 1 shows the general chemical mechanism for the poly(ADP-ribosyl)ation reaction catalysed by PARPs. Substrate NAD$^+$ **2** binds into the catalytic site of the enzyme principally through three strong hydrogen-bonds from the nicotinamide to Gly^{863} and Ser^{904} (human PARP-1 sequence numbering). Tyr^{907} also makes a π-stack with the nicotinamide. This strong binding to the carboxamide in complex **3** stretches and weakens the ribose-nicotinamide C–N bond, facilitating elimination of nicotinamide to form the intermediate oxonium species **4**. Glu^{988} is located adjacent to the cation to provide stabilisation. The potent electrophile **4** is trapped by attack of an oxygen nucleophile on the α-face; this nucleophile is either the carboxylate of the side-chain of Glu in a target protein (for attachment of the first monomer) or a ribose-OH (for subsequent ADP-ribosylation to build the polymer). The approach of the nucleophile is always from the α-
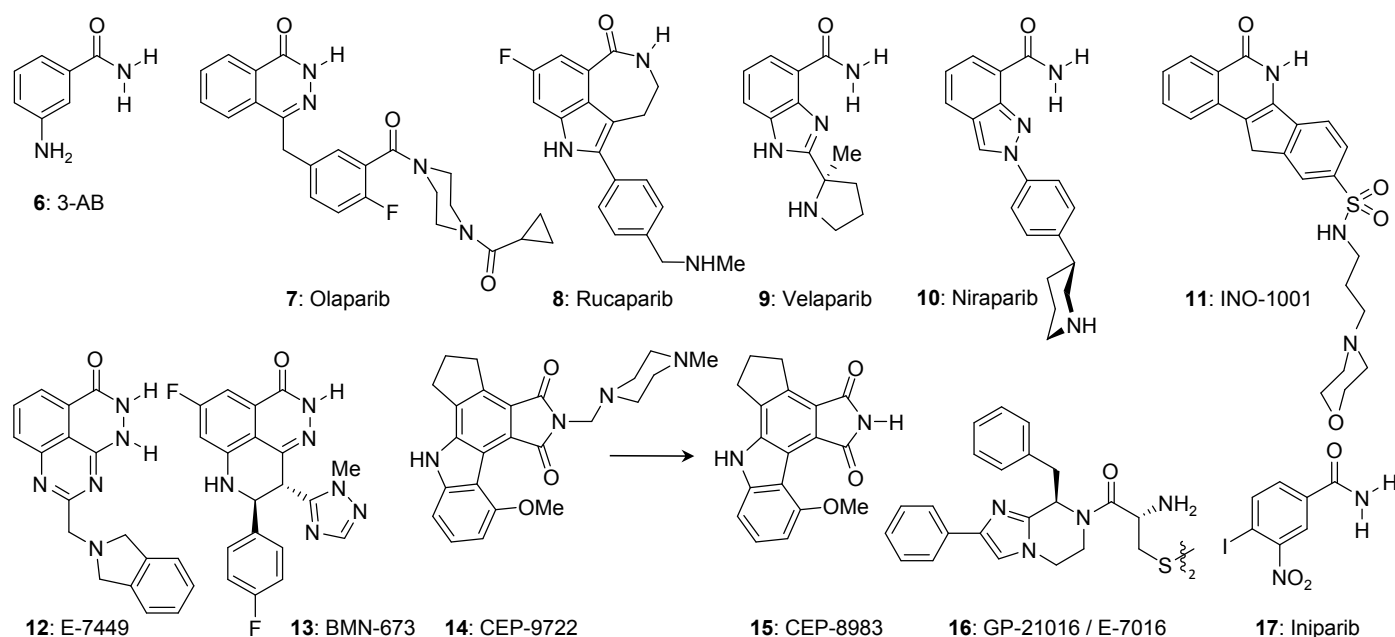
Figure 2. Structures of the first "gold-standard" selective inhibitor of PARP activity 3-AB (**6**) and the inhibitors which have entered clinical trial or which are of advanced interest (**7-16**). Iniparib **17** failed clinical trial and was later shown not to be a *bona fide* inhibitor of PARP-1.

face, generating the α-glycosidic bonds in **5**. The key feature of the non-covalent binding of NAD^+ is the set of three hydrogen bonds to the nicotinamide and this is exploited in the design of most true inhibitors of PARP-1.

This Scheme also shows the general pharmacophore for compounds which inhibit PARPs by binding as mimics of nicotinamide. The amide motif is essential for H-bonding with Ser and Gly at the active site. The carbocyclic ring should be aromatic, to make π-stacking interactions with one or more Tyr residues. Regions where additional substituents are preferred or disallowed are shown; these regions vary between the PARP isoforms, facilitating the development of selective isoform-selective inhibitors.

1.4. Inhibitors of PARPs

The first selective inhibitor of PARP activity in lysed cells was 3-aminobenzamide (3-AB, **6**, Figure 2), reported by Purnell and Whish in 1980 [37]. This compound contains the key pharmacophore for inhibition of PARPs, the aromatic carboxamide. Although of modest potency (IC_{50} = *ca.* 30 μM), its simplicity, selectivity for PARPs over other enzymes using NAD^+ and water-solubility led to it being the "gold standard" inhibitor for many years. Indeed, it is still used by some researchers.

Several inhibitors of PARP-1 have entered clinical trial, as potentiators of conventional chemotherapy of cancer, as single-agent therapies in BRCA-mutant and triple-negative breast cancer and in ischaemia-reperfusion injury. Olaparib **7** was first disclosed by Astra-Zeneca in 2008 [38] and proceeded to Phase-1 clinical trial, where it was well tolerated and showed promise in some patients with BRCA-mutated breast, ovarian and prostate cancer [39-43]. Phase-2 trials showed little beneficial effect in Ewing's sarcoma [44] but good activity in BRCA-mutant ovarian carcinoma [45], leading to a successful Phase-3 trial in the latter disease and

approval by the FDA in late 2014. However, some resistance to therapy with olaparib has recently been noted [46,47].

Rucaparib **8** was developed by Pfizer-Agouron from pioneering work at the University of Newcastle. It is showing useful activity in Phase-2 clinical trials, including potentiation of the therapeutic effects of the DNA-methylating drug temozolomide in melanoma [48]. In this drug, the secondary aliphatic amine in the side-chain allows for the formation of water-soluble phosphate salts. Veliparib **9** is the product of research at Abbott Laboratories. Phase-1 clinical trials were reported in 2011 and 2012, using the drug as a potentiator of the cytotoxicities of topotecan and of cyclophosphamide [49,50], and Phase-2 trials are ongoing. Tesaro Inc. very recently reported the discovery of niraparib **10** [51]. This drug successfully completed Phase 1 trial, showing some activity in BRCA-mutant ovarian and mammary carcinomas; it is now in Phase-3 trial [52]. INO-1001 **11** was the subject of a clinical trial in 2009 in the context of addressing ischaemia-reperfusion injury during treatment for myocardial infarction [53]. E-7449 shows interesting antiproliferative effects in acute myeloid leukaemia cells from patients [54]. The binding of the rationally designed BMN-673 to PARP-1 has been studied in detail [55,56]. A dose-escalation Phase-1 study using CEP-9722 **14** to potentiate temozolomide achieved limited partial responses in melanoma [57], this compound is a water-soluble prodrug of CEP8983 **15**. GP-21016 / E-7016 **16** does not contain the usual NAD^+-binding pharmacophore but is claimed to inhibit PARP-1 and potentiate the cytotoxic effects of temozolomide and of radiation *in vitro* and *in vivo* [58]. Iniparib **17** was initially claimed to be an inhibitor of PARP-1 in reports of pre-clinical studies but it failed in clinical trial. It was subsequently shown to be a non-specific thiol-reactive agent, not a specific inhibitor of PARP-1 [59].
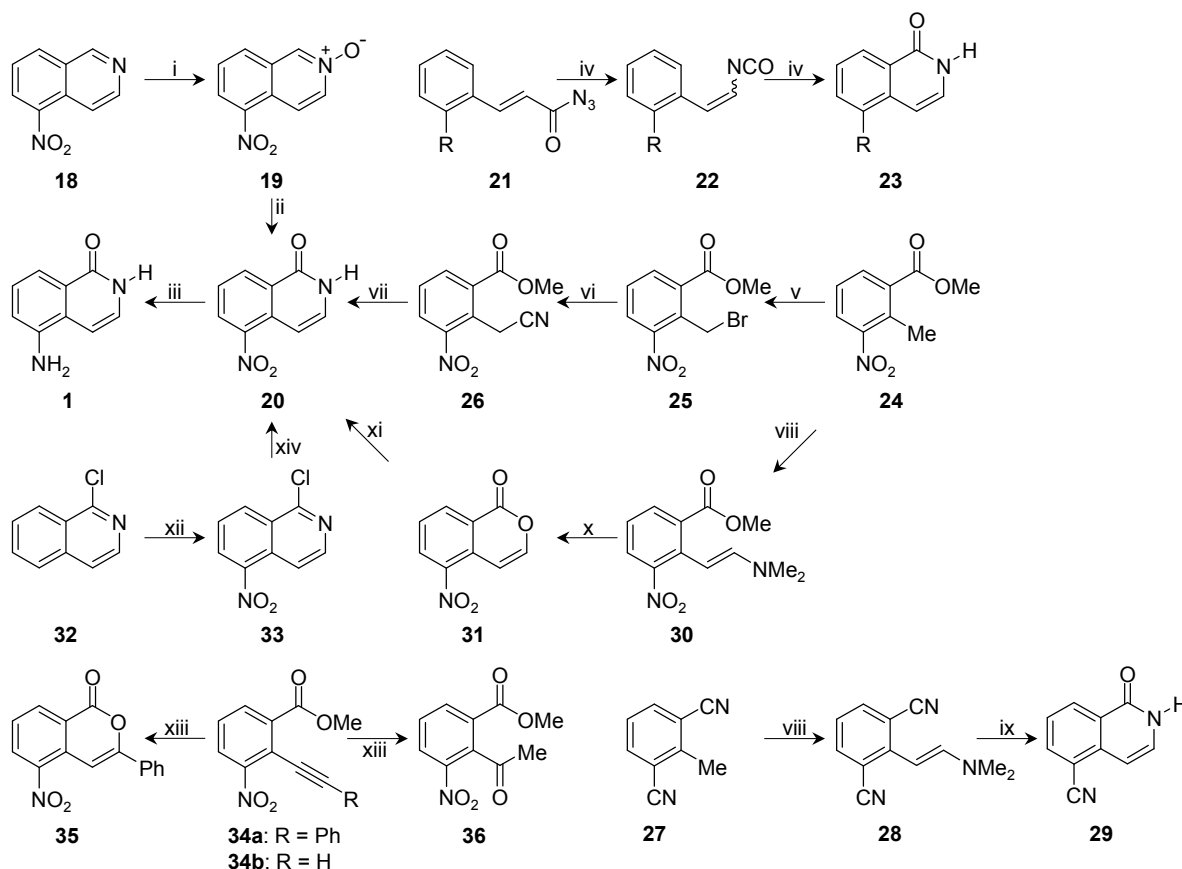
Scheme 2. Synthetic approaches to 5-AIQ **1**. *Reagents & conditions*: i, H_2O_2, AcOH, reflux [60,61,65,66]; ii, (a) Ac_2O, reflux, (b) aq. Na_2CO_3 [60,61,65]; iii, H_2, Pd/C, EtOH [60,61,65] *or* Zn, NH_4Cl, water, THF, EtOH [67] ; iv, Ph_2O, 230°C [68,69] *or* Bu_3N, Ph_2O, 230°C [70,71] *or* tetraglyme, 280°C [69]; v, Br_2, $(PhCO_2)_2$, CCl_4, hv, reflux [72]; vi, Et_4N^+ ^-CN, MeCN [72]; vii, DiBAL-H, CH_2Cl_2, -78°C [72]; viii, $(MeO)_2CHNMe_2$, reflux [65,67,73-75]; ix, (a) HCl, MeOH reflux, (b) aq. Na_2CO_3 [75]; x, SiO_2, hexane, EtOAc [67,73,74]; xi, NH_3, EtOH, autoclave [67] *or* NH_3, EtOH, ambient temperature [74] *or* NH_3, $MeOCH_2CH_2OH$, reflux [73]; xii, HNO_3, H_2SO_4, 0°C-20°C [76-78] *or* KNO_3, H_2SO_4 [79,80]; xiv, AcOH, 100°C [78] *or* aq. HCl, reflux [81]; xiii, $HgSO_4$, H_2SO_4, acetone, reflux [82].

2. 5-AIQ

2.1. Discovery of 5-AIQ

5-AIQ **1** was first synthesised by Wenkert *et al.* in 1964, as part of a chemical study on isoquinolinones, isocoumarins and related compounds, although the biological and pharmacological effects were not investigated [60]. The first report of the PARP-inhibitory activity of **1** came in 1991, when Suto *et al.* developed a series of simple 5-substituted isoquinolin-1-ones and 3,4-dihydroisoquinolin-1-ones [61,62], with the aim of inhibiting repair of DNA damaged by ionising radiation. The compounds were proposed as potential sensitisers of tumour cells to the cytotoxic effects of radiotherapy of cancer. The rationale was that inhibition of the repair of DNA damaged by the X-radiation would allow the tumour cells to proceed in the cell cycle carrying the unrepaired damage and be arrested at checkpoints or fail in S-phase, leading to apoptosis. 5-AIQ **1** was shown to have IC_{50} = 240 nM against the activity of a semi-purified preparation of PARP-1 (the only PARP known at the time) but it was not evaluated for radiosensitisation. Thus **1** was shown to be *ca.* 100-fold more potent than the standard at the time, 3-aminobenzamide. This seminal paper also helped to define the

pharmacophore for inhibition of PARP-1, setting out the requirement for the secondary amide motif to be held coplanar with the benzene ring (Scheme 1) and identifying the need for a substituent at the 5-position of the isoquinolinones (but not at the 6- and 7-positions). Problems of very poor solubility were noted for the analogue (5-methyl-3,4-dihydroisoquinolin-1-one; PD 128763) taken forward to pre-clinical development [63,64] and most of the other isoquinolin-1-ones.

2.2. Chemistry of 5-AIQ

2.2.1. Synthesis of 5-AIQ

Scheme 2 shows the approaches that have been investigated for the synthesis of 5-AIQ **1**. Oxidation of 5-nitroisoquinoline **18** with hydrogen peroxide under acidic conditions gives the N-oxide **19** [60,61,65,66]. In the original synthesis of **1** by Wenkert *et al.* [60], a Polonovski rearrangement using boiling acetic anhydride was reported to provide 5-nitroisoquinolin-1-one **20** in 74% yield. From here, reduction of the nitro group with hydrogen and palladium catalyst was straightforward. Work-up with hydrochloric acid was essential to convert **1** into its water-soluble hydrochloride salt to allow isolation and purification. This reduction has also been
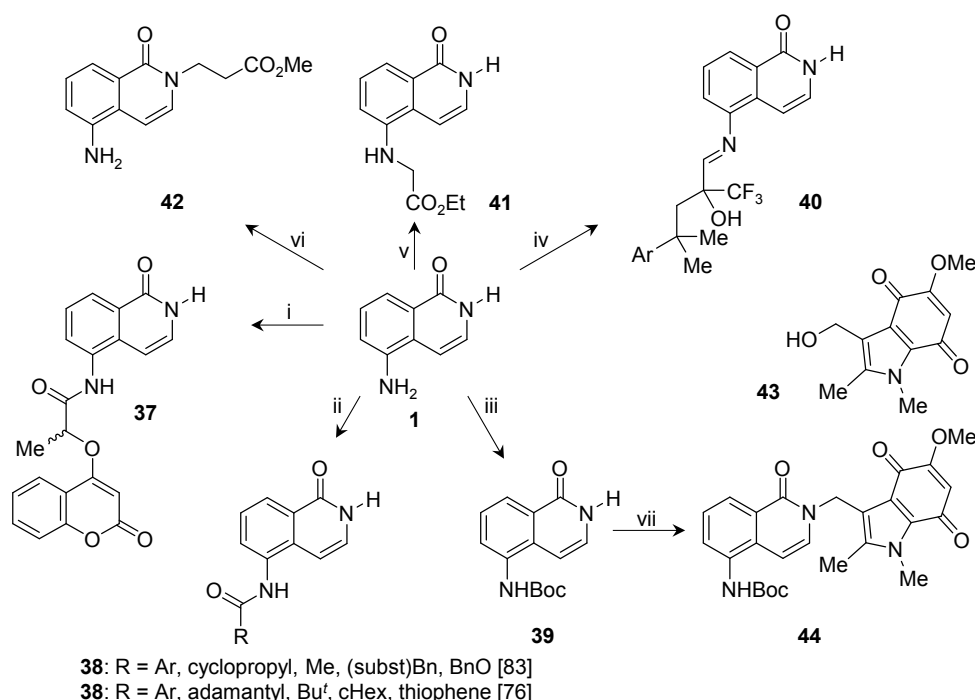
Scheme 3. Reactivity of 5-AIQ **1**. *Reagents & conditions*: i, coumarin-4-OCHMeCO₂H, HATU, DMF [84]; ii, RCOCl, pyridine, acetone [85] *or* RCOCl, pyridine, 90°C [78]; iii, Boc₂O, Et₃N, DMF [69]; iv, ArCMe₂CH₂C(OH)(CF₃)CHO, Ti(OPr^i)₄, xylene, 120°C [86] *or* ArCMe₂CH₂C(OH)(CF₃)CHO, Ti(OEt)₄, xylene, 150°C [87] *or* ArCMe₂CH₂C(OH)(CF₃)CHO, AcOH, 20°C [67]; v, BrCH₂CO₂Et, Pr^{i}₂NEt, NaI, DMF, 80°C [78]; vi, H₂C=CH₂CO₂Me, NaH, THF [78]; vii, **43**, Ph₃P, DEAD [69].

achieved with zinc under mildly acidic conditions [67]. This general route was also used by the Warner-Lambert Parke-Davis group [61] but Watson *et al.* found the Polonovski re-arrangement to be capricious and unreliable [65].

Eloy and Deryckere [68] devised a new approach to 5-substituted isoquinolin-1-ones **23** through formation of *ortho*-substituted cinnamoyl azides **21**, which were then thermolysed in diphenyl ether at 230°C, triggering a Curtius rearrangement to the isocyanates **22** and electrophilic cyclis-ation. This process is good when the substituent R is elec-tron-donating and is satisfactory for electron-neutral groups. This group [68] subsequently published an enhancement of adding tributylamine as a high-boiling basic and nucleophilic catalyst. Berry *et al.* improved the method still further by using tetraglyme at 280°C, facilitating isolation of the iso-quinolinone products [69]. However, the rearrangement / cyclisation fails for R being electron-withdrawing (*e.g.* nitro) and the starting cinnamoyl azide is inaccessible for R = NH₂, precluding access to **1** by this approach.

Radical bromination at Ar-CH₃ provided the bromomethyl derivative **25**. Substitution with tetraethylammonium cyanide introduced the nitrile in **26**, which was reduced selectively with DiBAL-H at low temperature to afford **20** in 23% yield [72]. This route was not adaptable to the synthesis of analo-gues and was intolerant of other esters (*e.g.* Pr^i).

Watson *et al.* achieved condensation of the activated Ar-Me in 2,6-dicyanotoluene **27** with dimethylformamide di-methylacetal (DMFDMA) to give the enamine **28** [65]. Reaction with acid effected the hydration / cyclisation / hyd-rolysis to 5-cyanoisoquinolin-1-one **29**. Adapting this appr-

oach to the synthesis of **31**, and hence **1**, condensation of the Ar-CH₃ of **24** with DMFDMA gave the corresponding enamine **30** [67,73-75]. From here, the enamine can be hydrolysed with aqueous acid, whereupon the intermediate enol cyclises to form the isocoumarin **31** [75]. Alternatively, this hydrolysis / cyclisation can be achieved by simply passing **30** through a column of silica gel [67,73,74]; pre-sumably, the silica provides the necessary acid-catalysis. Replacement of the ring-oxygen with nitrogen (forming 5-nitroisoquinolinone **20**) has been effected with ethanolic ammonia at high temperature in an autoclave [67] and with ammonia in refluxing 2-methoxyethanol [73], avoiding the need for a pressure vessel. There is one curious report of the reaction with ammonia occurring at ambient temperature [74]. The sequence **24** → **30** → **31** → **20** → **1** proceeds in 22% overall yield.

The above sequence was the most efficient synthesis of **1** for some years until Sunderland *et al.* disclosed a three-step process. Nitration of 1-chloroisoquinoline **32** occurs select-ively at the 5-position [76-80], in contrast to nitrations of 2-substituted isoquinolin-1-ones which tend to occur at the 4-position [69]. Acetolysis of the 1-chloro of **33** and hydrolysis of the ester upon work-up forms 5-nitroisoquinolin-1-one **20**, from which the target **1** can be obtained readily [77]. The hydrolysis of **33** to **20** can also be achieved in lower yield with aqueous hydrochloric acid [81]. This new sequence **32** → **33** → **20** → **1** proceeds in 54% overall yield [78].

Hg²⁺-catalysed 6-*endo*-dig cyclisation of the disubstituted alkyne **34a** smoothly led to 5-nitro-3-phenylisocoumarin **35**. However, the reaction could not be adapted to the synthesis
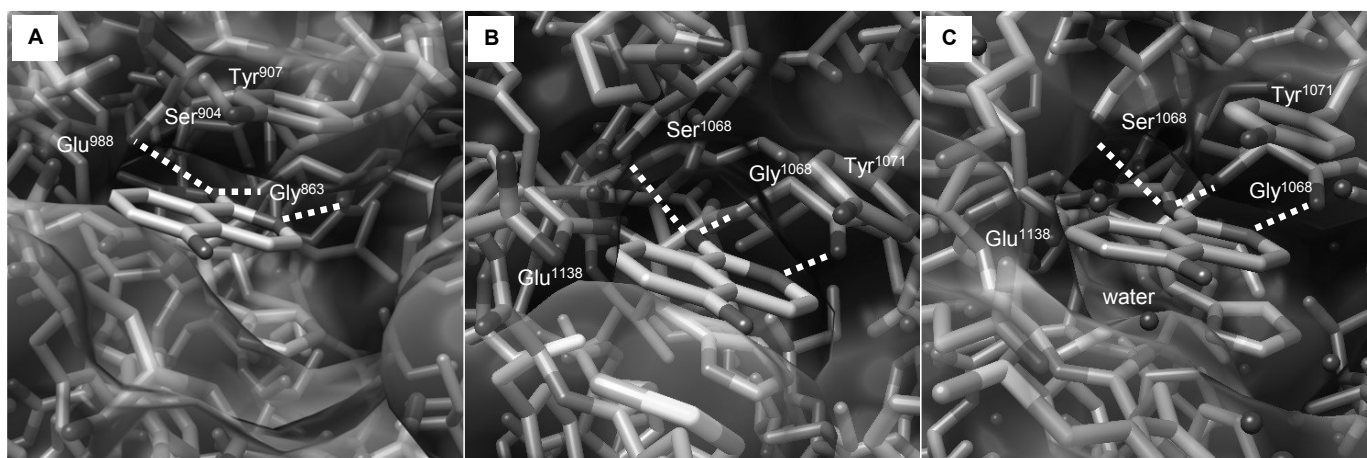
Figure 3. Structures of 5-AIQ **1** bound into **A**: the catalytic domain of chicken PARP-1 (model from docking study) [1,96], **B,C**: the catalytic domain of tankyrase-2 (PARP-5b) (crystal structures **B**: [90], **C**: [88]).

of the 3-unsubstituted analogue **31**, as treatment with Hg^{2+} gave only the acetyl compound **36**, the product of hydration of the terminal alkyne of **34b**, *via* a 5-*exo*-dig first step [82].

2.2.2. Physical chemistry of 5-AIQ

The hydrochloride salt of 5-AIQ **1** is a high-melting off-white to buff solid, which is soluble to >5% in water but slightly less soluble in buffers. The free base is very poorly soluble in water and many other solvents. The calculated logP has been reported as -0.4 [83].

2.2.3. Chemical reactivity of 5-AIQ

The structure of **1** contains three potentially nucleophilic sites (5-NH_2, 1-C=O, 2-N) but no reactive electrophiles (Scheme 3). The 5-amino group is weakly nucleophilic and has been acylated with a carboxylic acid activated by 1-(bis-(dimethylamino)methylene)-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU), forming **37** [84]. A range of amides **38** has also been formed using acyl chlorides at ambient temperature in the presence of pyridine [85] or, more effectively, with acyl chlorides in hot pyridine [78]. *t*-Butoxycarbonyl protection has been introduced in **39** with di-Bu^t dicarbonate in the presence of a tertiary amine base. At one oxidation level lower, imines **40** have also been formed from **1** and aldehydes under forcing conditions with Lewis-acid catalysis (Ti(IV)) [86,87] or prolonged treatment in acetic acid at ambient temperature [67]. At one further oxidation level lower, **1** was alkylated at the 5-amine with ethyl bromoacetate under mildly basic conditions to form **41** in low yield [78]. By contrast, use of the much stronger base sodium hydride deprotonated at 2-N and the resultant anion was alkylated by the conjugate electrophile methyl propenoate to give **42** with complete regioselectivity [78]. Protection of the 5-amine with Boc in **39** allowed Mitsunobu alkylation with **43** at 2-N to provide the putative (bio)reductively activated prodrug **44** [69].

Isotopomers of **1** have yet to be reported.

2.3. Inhibition of isolated enzymes by 5-AIQ

5-AIQ **1** was designed as an inhibitor of PARP-1, at a time when this was the only isoform known. The IC_{50} value for this inhibition has variously been reported as 240 nM (against semi-purified enzyme from calf thymus) [61], 300 nM (against PARP activity in the lysate of nuclei from murine L929 cells) [65], 300 nM (against recombinant human PARP-1 using a radiochemical assay) [88], 1.17 μM (using recombinant human PARP-1 in a scintillation proximity assay) [83], 940 nM (using recombinant human enzyme in a FlashPlate assay) [78], 250 nM [89] and 1.8 μM (using recombinant human PARP-1 in the Trevigen assay) [90,91]. The diversity in values is probably due to the different assay conditions used, as the PARP reaction is sensitive to detailed changes in conditions. IC_{50} values for inhibition of the isoform PARP-2 are very similar, indicating no selectivity in binding. A value of IC_{50} = 1.84 μM was reported for inhibition of recombinant mouse PARP-2 in a scintillation proximity assay [83] and a similar study reported IC_{50} = 1.05 μM, again using recombinant mouse PARP-2 [78]. No similar studies against the isoforms PARP-3 and PARP-4 have been carried out. However, a recent investigation by Paine *et al.* [93] revealed only modest inhibition of tankyrase-2 (PARP-5b) at 1.0 μM, again showing little selectivity between the isoforms of PARP. Qiu *et al.* confirmed this relatively weak binding to tankyrase-2 in determining IC_{50} = 10 μM [89].

The selectivity of **1** for inhibition of PARPs, compared with mono-ADP-ribosyl transferases also using NAD^+ as substrate, was shown by the higher IC_{50} for inhibition of the catalytic activity of diphtheria toxin (IC_{50} = 80 μM) [65]. The compound is also reported to bind to and inhibit the PE24H catalytic domain of *Pseudomonas aeruginosa* exotoxin A, another bacterial mono(ADP-ribosyl)ating toxin, with IC_{50} = 23 μM [93].

Weak off-target inhibition of matrix metalloproteinase-2 (MMP-2) by 5-AIQ **1** and other agents designed to inhibit PARP-1 has been observed, with IC_{50} = 102 μM for **1**. Inhibitory potency against MMP-2 for the compounds broadly followed their potencies against PARP-1 [94]. However, **1** has been reported not to inhibit the activity of human sirtuin-1 [95].

2.4. Binding of 5-AIQ to PARPs

Structural studies showing the mode of binding of 5-AIQ **1** to the target PARPs have revealed the designed hydrogen-bonding interactions with the Ser / Gly motifs and π-stacking with Tyr residues in the active site. Figure 3 illustrates the structures and binding.

No crystal structures have yet been reported for the binding of 5-AIQ **1** into PARP-1 but a docking / molecular modelling structure is in the public domain [1,96]. This structure, shown in Figure 3A, was prepared by replacing the ligand PD 128763 (5-methyl-3,4-dihydroisoquinolin-1-one) in the first crystal structure published for the NAD^+-binding catalytic domain of chicken PARP-1 [97] with the structure of **1**, followed by molecular dynamics calculations and minimisation of energy. Ligand **1** binds as a strict analogue of the nicotinamide of NAD^+. Hydrogen bonds are formed from the N–H of **1** to the carbonyl oxygen of Gly^{863} and from the carbonyl oxygen of **1** to the backbone NH of this Gly and to the side-chain OH of Ser^{904}. The aromatic ring of Tyr^{907} forms a π-stack with the ligand. The carboxylate of Glu^{988}, involved in stabilising the intermediate oxonium ion, is within 5 Å of the 5-NH_2.

Two structures have been reported with **1** bound into constructs containing the catalytic domain of tankyrase-2 (PARP-5b); these structures are broadly similar but differ in detail. Both structures [89,92] show the expected hydrogen bonds from the carbonyl of **1** to the side-chain OH of Ser^{1068} and to the backbone N-H of Gly^{1032}. The lactam N-H of **1** forms a hydrogen bond with the backbone C=O of Gly^{1032}, completing the classical hydrogen-bonding motif for binding of inhibitors to PARPs. π-Stacking to the aromatic side-chain of Tyr^{1071} is also evident. Qiu *et al.* [89] claim an additional hydrogen bond from the 5-NH_2 to a conserved water molecule, whereas this water is absent from the structure reported by Paine *et al.* [92] and the side-chain carboxylate oxygen of Glu^{1138} is coplanar with **1** and located 4.6 Å from the 5-amine nitrogen.

2.5. Cellular and *in vivo* pharmacology of 5-AIQ

2.5.1. PARP-1 and repair of damaged DNA

Inhibitors of PARP-1 sensitise cells to killing by ionising radiation by slowing the rate of repair of the damage caused to DNA [98]. Although 5-AIQ **1** is a potent inhibitor of PARP-1, it showed little radiosensitising effect on V79 Chinese hamster lung cells [62]. Conventional chemotherapy of cancer by drugs which cause single-strand breaks in DNA (*e.g.* alkylating agents, topoisomerase-2 poisons) is also potentiated by inhibition of PARP-1 [99-101] but there are no reports of evaluation of **1** in this context.

2.5.2. 5-AIQ and ischaemia-reperfusion damage

The role of PARP-1 in the cellular damage and death consequent to reperfusion with molecular oxygen following ischaemia / hypoxia was identified as long ago as 1986 [102]. Briefly, this key involvement is as follows [102]: Occlusion of a blood vessel interrupts the supply of oxygenated blood to an organ. Restoration of the blood supply (spontaneously or through medical intervention) causes a rapid influx of molecular oxygen (an oxidising diradical) and generation of

other reactive oxidising species, leading to extensive damage to DNA. PARP-1 is over-activated as part of the repair response and quickly (<10 min) consumes the cytosolic reserve of NAD^+. The drastic fall in the concentration of intracellular NAD^+ leads to unavailability of this essential component of the energy-generating pathways, including glycolysis and mitochondrial respiration. The cell then runs out of energy, leading to necrotic death. PARP-1 is also involved in the inflammatory response to reperfusion, exacerbating the lethal effects on the cell. Pharmacological inhibition of PARP-1 reduces or abolishes the cell death and organ damage consequent to reperfusion injury [88,103,104].

Water-solubility and good bioavailability are key to successful intervention with inhibitors of PARP-1 in reperfusion injury. The former is reflected in the remarkable potency of 5-AIQ **1** in a rodent model of haemorrhagic shock [73]. 5-AIQ inhibited the intracellular activity of PARP-1 in human cardiac myoblasts, in which oxidative damage had been caused by hydrogen peroxide, with $IC_{50} = 10$ μM. Administration of **1** to rats, which had suffered haemorrhage, shortly before reperfusion mitigated the multiple organ failure seen in untreated animals in a dose-dependent manner, with significant responses seen at the very low dose of 30 μg Kg^{-1}.

Ischaemia / reperfusion injury is also a major component of damage in myocardial infarction. 5-AIQ **1** inhibited PARP-1 activity in H9c2 cardiac cells challenged with hydrogen peroxide, with $IC_{50} = 4.5$ μM, and dose-dependent significant reduction in the size of the infarct was noted with **1** in a rat model of myocardial infarction (occlusion of the descending coronary artery, followed by removal of the occlusion) [105]. Good activity was seen at doses down to 100 μg Kg^{-1}. Sodhi *et al.* reproduced this effect in isolated perfused rat hearts [106]. Significant attenuation of the size of the infarct and of release of the necrotic markers lactate dehydrogenase and creatine kinase was achieved at a 5.0 μM concentration of **1**, indicating good uptake into the tissue and cells. Interestingly, Park *et al.* suggest that at least part of the protective effect of **1** in H9c2 cells is due to up-regulation of the activities of superoxide dismutase and catalase and to activation of Akt and GSK-3β [107]. Balakumar and Singh used 5-AIQ **1** (erroneously described as 5-aminoisoquinoline) as a tool to demonstrate that PARP-1 is involved in cardiac hypertrophy consequent to partial abdominal aortic constriction but is not a component of the cardiac hypertrophy associated with chronic swimming training in rats [108]. This reinforces the link between the protective action of **1** and ischaemia / reperfusion in the heart.

5-AIQ **1** also protects against ischaemia / reperfusion damage in other organs. PARP-1 activity was inhibited in a concentration-dependent manner with $IC_{50} = ca.$ 30 μM by **1** in primary proximal tubule cells from rat kidney after oxidative challenge with hydrogen peroxide, protecting the cells from necrotic death [109]. Moving to the situation *in vivo*, **1** (300 μg Kg^{-1}) diminished the damaging effects of acute occlusion of the arteries supplying the rat kidneys, followed by reperfusion, as shown biochemically and histologically. Curiously, **1** also protects the kidney against cisplatin-induced toxicity and restores function [110]. However, there is some dichotomy in the reports of the effects of inhibition of PARP-1 with **1** on ischaemia / reperfusion and oxidative

injury in the liver. 5-AIQ **1** was very effective at 3.0 mg Kg^{-1} in ameliorating the damage to the microcirculation and function in the livers of rats which had undergone haemorrhagic shock and resuscitation [111]. Khandoga *et al*. confirmed the protective effect on the microvasculature of the liver in the mouse but noted no overall effect on survival of the mice after occlusion and re-establishment of the hepatic blood supply [112]. 5-AIQ **1** was used as an inhibitor of PARP-1 to demonstrate that this enzyme is not involved to a major extent in the hepatotoxicity of acetaminophen / paracetamol [113]. Splanchnic artery occlusion shock in rats causes damage to the ileum and a lowering of mean arterial blood pressure. Both of these were diminished by treatment of the rats with **1** at 3.0 mg Kg^{-1} [114]. Interestingly, this splanchnic artery occlusion also caused an increase of the inflammatory molecule ICAM-1, which was also diminished by treatment with 5-AIQ.

A serious manifestation of ischaemia / reperfusion injury occurs in the transplantation of organs. Organs become hypoxic when removed from the donor and are reperfused when installed in the recipient and it was proposed that treatment of the donor with erythropoietin and of the recipient with 5-AIQ **1** was optimal for protection of the function of the organ [115]. A study by Szabó *et al*. showed that the PARP-1 inhibitors PJ34 and **1** (wrongly named as 5-aminoisoquinoline) improved cardiac function in hearts transplanted between rats, along with protecting against tissue damage demonstrated by immunohistochemistry [116]. Reperfusion-induced graft coronary endothelial dysfunction was ameliorated. Inhibition of PARP-1 with these agents also decreased expression of P-selectin and ICAM-1. Similar effects of **1** were seen in rodent models of transplantation of kidneys [117,118]. Additionally, a protective effect of **1** has been demonstrated against acute rejection in cardiac transplantation, which was attributed to inhibition of the inflammatory response (*vide infra*) [119].

Módis *et al*. used the inhibition of PARP-1 by 5-AIQ **1** human A549 cells in their detailed study [120], which concluded that PARP-1 regulates bioenergetics both under normal conditions and in oxidative stress. This effect may be due to the effect of PARP-1 on the intracellular concentration of NAD$^+$, rather than to regulation through poly(ADP-ribosyl)ation of a target protein.

2.5.2. 5-AIQ and cytotoxicity

The importance of over-activation of PARP-1 in the killing of human leukaemia HL60 cells by the antibiotic antimycin A was demonstrated by Ogita *et al*. using 5-AIQ **1** (wrongly named as 5-aminoisoquinoline) [121]. Antimycin A inhibits mitochondrial electron transport and induces the generation of reactive oxygen species and of nitric oxide. Reduction by **1** of the cytotoxicity caused by antimycin A, along with other data, indicated the role of PARP-1.

By contrast, PARP-1 has been shown not to be involved in the repair of DNA damage and genotoxicity induced by boron neutron capture therapy (BNCT) [122]. This therapy relies on delivering the stable ^{10}B isotope selectively to the tumour cells. The tumour is then irradiated with low-energy neutrons, which are captured by the ^{10}B, leading to formation of a ^{7}Li nucleus and an α-particle. The latter causes lethal damage to DNA. 5-AIQ **1** and other PARP-1 inhibitors did not diminish this damage in V79 Chinese hamster cells, which is consistent with the formation of double-strand breaks whereof PARP-1 is not involved in repair.

2.5.3. 5-AIQ and inflammation

As noted above, PARP-1 is involved in inflammatory processes; this role has recently been reviewed [12]. Potent anti-inflammatory activity has been shown for 5-AIQ **1** in a wide variety of models of inflammation with different triggers in different tissues and organs.

Cuzzocrea *et al*. proposed the use of 5-AIQ **1** in the treatment of inflammatory bowel disease, on the basis of its beneficial effect in mice with colitis induced by dinitrobenzenesulfonic acid [123]. In these mice, **1** (3.0 mg Kg^{-1}) reduced the incidence and severity of diarrhoea and loss of weight. Infiltration by neutrophils, myeloperoxidase activity and up-regulation of ICAM-1 were also reduced markedly by this dose.

The same group also noted an anti-inflammatory effect of **1** in a rat model of periodontitis [124]. Periodontitis was induced with a ligature around a tooth, leading to increased infiltration by neutrophils in the inflamed tissue, along with increased local myeloperoxidase activity and resorption of bone matrix. 5-AIQ 1 (5.0 mg Kg^{-1}) significantly reduced these and other parameters of inflammation.

In the kidney, 5-AIQ **1** (3.0 mg Kg^{-1}) markedly reduced thrombotic lesions and glomerulosclerosis *in vivo* in mice with nephrotoxic serum-induced immune-mediated nephritis [125]. The kidneys from 5-AIQ-treated animals showed less poly(ADP-ribose), showing that the drug addressed the target PARP-1, and lower expression of TNF-α. Similarly, in a model of accelerated lupus nephritis, treatment with **1** depressed the concentration of blood urea nitrogen (BUN) and histopathological examination showed lower lymphocyte infiltration, cellular proliferation and necrotic cell death. This led to a suggestion that **1** could act to block the development of spontaneous lupus nephritis through inhibiting PARP-1.

The beneficial effects of inhibition of PARP-1 with 5-AIQ **1** on inflammation in the lung have been extensively studied. Idiopathic pulmonary fibrosis is an inflammatory disease in humans in which there are excessive deposits of extracellular matrix in the lung. It does not respond well to current treatments with corticosteroids and cytotoxins. Genovese *et al*. developed a mouse model of the condition, in which the fibrosis is induced by the antineoplastic drug bleomycin [126]. Treatment with 5-AIQ **1** ameliorated the fibrosis and inflammation at a dose of 3.0 mg Kg^{-1}. Reductions in PARP activity and in formation of nitrotyrosine were observed, along with benefits in lower mortality and in maintenance of body weight. Thus **1** is confirmed as an effective inhibitor of PARP-1 in lung tissue in this model. Kawasaki *et al*. reproduced these effects in a similar model of bleomycin-induced pulmonary fibrosis in the mouse [127], comparing **1** with the known antifibrotic drugs valsartan and erythromycin. In an earlier study, acute inflammatory lung injury was induced with zymosan-activated plasma in BALBc mice [128]. Inhibition of up-regulation of ICAM-1 and P-selectin

was observed with administration of **1** at 3.0 mg Kg^{-1}, demonstrating an anti-inflammatory effect *in vivo*, as well as a diminution of lung injury. These results were paralleled *in vitro* in human endothelial cells challenged with oxidative stress or a pro-inflammatory cytokines (TNF-α), with significant dose-dependent reduction in release of ICAM-1 and of P-selectin from as low as 3.0 μM of **1**. In a model of chronic asthma in guinea pig, where the animals were sensitised with ovalbumin, 5-AIQ **1** was protective at the low dose of 500 μg Kg^{-1} [129]. Cough and dyspnoea were significantly reduced, along with PARP-1 activity in the lung and markers of inflammatory lung damage (*e.g.* TNF-α). Very recently, Ahmad *et al.* reported a study in which inflammation was induced in mouse lung with carrageenan [130]. Treatment with 5-AIQ **1** (1.5 mg Kg^{-1}) diminished the over-expression of a wide range of molecules associated with inflammation, including COX-2, STAT-3, NF-κB, p65 and PARP-1 itself and relieved the suppression of IκB-α and IL-4. The authors concluded that **1** is a potent anti-inflammatory agent.

2.5.4. 5-AIQ protects against neuronal disfunction

Inflammatory processes subsequent to the injury play an important role in neuronal death and malfunction after spinal cord injury. As noted above, 5-AIQ **1** has strong anti-inflammatory effects, owing to its inhibition of PARP-1 activity and its good uptake into cells. Genovese *et al.* explored the extension of the applications of this agent into treatment of spinal cord injury [131]. In a mouse model involving temporary compression of the spinal cord, 5-AIQ **1** (3.0 mg Kg^{-1}) inhibited the formation of poly(ADP-ribose) and reduced inflammation and spinal cord injury (shown histologically) and diminished apoptosis. A reduction of the binding of NF-κB to DNA was also observed, along with preservation of IκB, re-inforcing the concept that PARP-1 may regulate the function of NF-κB. In a truly functional assay, the motor score of the hind limbs of the animals also recovered well after treatment with **1**. This study was backed up later by an observation that inhibition of PARP-1 with **1** modulates cellular autophagy in spinal cord trauma [132].

Ischaemic stroke results from restricted flow of oxygenated blood in one or more arteries supplying the brain. Cells die in two phases, initially from deficiency of oxygen and, after restoration of the supply of blood, from excitotoxicity, inflammation and oxidative stress [133,134]. PARP-1 has been identified as a major player in the cell death consequent to reperfusion in stroke, *inter alia* through its regulation of NF-κB. PARP-1 can also cause neuronal cell death through parthanatos [135], a caspase-independent form of apoptosis, as over-activated PARP-1 causes translocation of apoptosis-inducing factor (AIF) to the nucleus [136], where it triggers condensation of chromatin and fragmentation into large pieces [137]. Microglial activation can take hours to days to develop after a stroke [138], giving a time-window for therapy. However, Yennari *et al.* point out that the immune response can have beneficial effects as well as deleterious outcomes [139]; this is of relevance to inhibition of PARP-1, as this enzyme is involved in angiogenesis (*vide infra*) and inhibition of vascular remodelling may be undesirable. In this context, 5-AIQ **1** (3.0 mg Kg^{-1}) has been shown to diminish significantly the oxidative activity of infiltrating neut-

rophils in a rat model of chronic ischaemic stroke but not in an acute model [139].

Other neurological disorders have also responded to treatment with 5-AIQ **1**. Parkinson's disease is characterised by degeneration of dopaminergic neurons of the substantia nigra in the brain. Dopaminochrome, an oxidised derivative of dopamine, is cytotoxic to these neurons, causing apoptosis. This apoptosis is mediated through translocation of apoptosis-inducing factor (AIF) to the nucleus, a process triggered by PARP-1 activity [140]. 5-AIQ **1** protects against this apoptosis in the murine mesencephalic cell line MN9D; the lowest concentration used was 50 μM, which may have been unnecessarily high. Farez *et al.* noted that the activity of PARP-1 is higher in patients in the progressive phase of multiple sclerosis (secondary progressive multiple sclerosis, SPMS) [141]. Higher serum concentrations of 15α-hydroxycholestene are also found in these patients and in mice with secondary progressive experimental autoimmune encephalomyelitis (EAE). 5-AIQ **1** (3.0 mg Kg^{-1}) inhibited the clinical signs of EAE. A detailed study confirmed toll-like receptor-2 (TLR2) and PARP-1 as a pathway in the pathology of the disease. One may speculate that this pathway could be a useful point of therapeutic intervention in this devastating progressive disease.

2.5.5. 5-AIQ and arthritis

Interesting anti-inflammatory activity is also seen for 5-AIQ **1** in model of arthritis. Ahmad *et al.* induced arthritis in mice using Complete Freund's Adjuvant containing heat-killed *M. tuberculosis*. Significant relief of the symptoms of the arthritis (*e.g.* oedema, erythema, joint function) were seen after treatment with **1** (1.5 mg Kg^{-1}) [142]. At the cellular level, **1** decreased the number of pro-inflammatory subsets of T-cells, while increasing the number of Treg cells; reduction of expression of NF-κB and other inflammatory mediators was observed, along with up-regulation of IκB-α and mRNA for anti-inflammatory mediators. This detailed study followed earlier work in collagen-induced arthritis by Gonzalez-Rey *et al.*, in which **1** (1.5 mg Kg^{-1} and 3.0 mg Kg^{-1}) strongly relieved the damage to tissue and bone caused by the arthritis [143]. Down-regulation of pro-inflammatory cytokines was observed, along with stimulation of IL-10, an anti-inflammatory cytokine. The authors concluded that PARP-1 could be an important target protein for the design of new drugs for the treatment of rheumatoid arthritis.

2.5.6. 5-AIQ and cancer

Many of the above observations strongly suggest that PARP-1, in addition to its role in regulating the repair of damaged DNA, regulates the expression of a number of genes and that this latter regulation can be modulated effectively by 5-AIQ **1** in whole cells and tissues. In direct studies on the modulation of expression of genes by **1**, it has been shown that, in T cells, the expression of transforming growth factor-β (TGF-β) receptor sub-type I is up-regulated by this agent [144]. TGF-β receptor-II is also regulated by PARP-1 but by protein-protein interaction rather than through enzymatic activity. Cai *et al.* found that 5-AIQ **1** inhibits the expression of PARP-1 itself in CT26 colon carcinoma cells, although **1** was used at the very high concentration of 100

µM which may possibly have led to inhibition of enzymes other than PARP-1 [145]. The authors proposed that this effect on expression of PARP-1 was due to inhibition of PARP-1 activity, leading to down-regulation of the activity of NF-κB. Following on from this initial study, the same group confirmed the effect on the expression of PARP-1 but also observed that the expression of vascular endothelial growth factor-C (VEGF-C) was down-regulated by **1**, though inhibition of PARP-1 and down-regulation of NF-κB [146]. VEGF-C is involved in angiogenesis, so the authors proposed that PARP-1 may regulate angiogenesis and inhibition thereof with **1** may inhibit this process. Rajesh *et al.* confirmed that **1** does inhibit the processes of angiogenesis, in common with other inhibitors of PARP-1 [147]. The proliferation of HUVEC human umbilical cells triggered by VEGF and by fibroblast growth factor (FGF) was inhibited significantly by **1** at 600 nM concentration. Inhibition of proliferation was also seen in the *ex vivo* rat aortic ring model at 1.2 µM concentration, a lower concentration than those required for other inhibitors of PARP-1.

Having established that primary samples of human colon carcinoma, especially metastatic cancer, had higher levels of poly(ADP-ribosyl)ation than adjacent normal colon and that these higher levels correlated with higher expression of P-selectin and ICAM-1, Wang and Hao noted that inhibition of PARP-1 with 5-AIQ **1** down-regulated the expression of these adhesion molecules in the HT29 human colon carcinoma line and diminished their ability to adhere to HUVEC cells [148,149]. The authors suggested that **1** may have anti-metastatic properties. Similar results were seen in CT26 murine colon carcinoma cells, with treatment with **1** reducing the levels of expression of NF-κB, integrin-β_1, MMP-2 and MMP-9. Interestingly, the expression of PARP-1 was also lower. The protease activities of the MMPs were also diminished, as shown by zymography [150], although 1 does have a weak direct inhibitory effect on MMP-2 [94]. Cells treated with **1** migrated significantly less through Matrigel and were less invasive *in vitro*, leading to the conclusion that **1** exerts its anti-migration effect through inhibition of PARP-1, which then down-regulates NF-κB, which, in turn, down-regulates the expression of metastasis-related genes. The Chongqing group then confirmed the strong anti-metastatic effect of **1** (3.0 mg Kg^{-1}) *in vivo* in abolishing the metastasis into the liver of CT26 cells implanted in the spleen [15]. Some reduction in the volume of the primary tumour in the spleen was also observed. Selective killing of cell lines carrying mutated BRCA1 or BRCA2 by inhibitors of PARP-1 was first reported in 2005 [151,152] and olaparib **7** is licensed for treatment of BRCA-mutant tumours as a single agent [47]. One may speculate about the involvement of BRCA in the apparent anti-tumour activity of **1** but the BRCA-status of the CT26 line has not been reported. Finally in the context of cancer, it has been shown that inhibition of the mono(ADP-ribosyl)transferase ART1 acts synergistically with inhibition of PARP-1 with **1** to promote apoptosis in CT26 cells treated with cis-platin; down-regulation of NF-κB and of COX-2 was observed [153]. ART1 is inhibited weakly by the weak PARP-1 inhibitor 3-AB **6** [154]. Unfortunately, the direct inhibitory effect of 5-AIQ **1** on ART1 has not been reported but it is known not to affect the expression of the ART1 protein [130]. siRNA silencing of

ART1 has an anti-proliferative effect in CT26 cells [155] and one might question whether the observed anti-tumour effect of **1** may be due to simultaneous inhibition of ART1 and PARP-1.

2.5.7. 5-AIQ and fertility

The effects of 5-AIQ **1** on fertility and on development of the foetus are mixed. Treatment of female mice with **1** stimulates the production of mature oocytes, in a dose-dependent manner (1.2 mg Kg^{-1} and 3.0 mg Kg^{-1}) [156]. Qian *et al.* also report that the number of foetuses produced by female mice treated with **1** was higher than for control mice and that these foetuses showed no signs of abnormalities. These effects were absent in mice in which the gene encoding for PARP-1 had been knocked out, confirming that **1** was acting *via* inhibition of this enzyme. By contrast, Osada *et al.* reported that inhibition of PARP-1 with PJ34 or with **1** caused developmental arrest at the pronuclear envelope breakdown stage of fertilisation, citing effects at the spindle bundles and defective polymerisation of tubulins [157]. One may speculate whether these effects are due to inhibition of PARP-1 or to the weak collateral inhibition of the tankyrases (PARP-5a and PARP-5b) by these compounds [31,92,158], as the tankyrases are involved in the correct functioning of the mitotic spindle [159]. PJ34 and **1** were observed to cause changes in the methylation of histone H3 (increased at Lys^4 and decreased at Lys^9) and hypomethylation of DNA in the pro-embryo soon after fertilisation [160]. It was suggested that inhibition of PARP-1 may be a target in contraception [159] but the dichotomy of observed effects would have to be resolved before this approach is viable.

5-AIQ **1** appears to be devoid of genotoxicity *in vitro* and *in vivo*, according to a study by Vinod *et al.* [161].

2.6. Substituted analogues of 5-AIQ

2.6.1. 5-NH$_2$-Substituted analogues of 5-AIQ

Several sets of analogues of 5-AIQ substituted at the exocyclic 5-NH$_2$ have been reported to have been prepared and evaluated for different applications. Compound **37** (Schemes 2 and 4) was evaluated as a member of a series of 4-substituted coumarins by Rambabu *et al.*, who found it to be a very weak inhibitor of cyclooxygenases [84]. The 5-acylamido-isoquinolin-1-ones **38** (R = Me, cyclopropyl, Ar, (substituted)Bn, BnO) were used as intermediates by Chen *et al. en route* to the corresponding 5-acylaminoisoquinoline-1,3,4-triones **45**, which were potent inhibitors of caspase-3 (IC$_{50}$ = 40 to 356 nM) [85]. Good selectivity was seen *vs.* other proteases but the compounds were effectively non-selective between the caspase isoforms [85]. Sunderland *et al.* found that similar compounds **38** (R = Ar, adamantyl, Bu^t, cHex, thiophene) were inhibitors of PARP-1 and PARP-2, with the benzamide **38** (R = Ph) being 9.3-fold selective for inhibition of PARP-2 [78]. In a comparative study, this benzamide was more selective than the corresponding ester **46**, which had earlier been claimed to be highly selective [162] but which is probably hydrolytically unstable in aqueous solution. These researchers also hydrolysed the ester **41** to the carboxylic acid **47**, which showed moderate potency against both isoforms [78]. The group of Rehwinkel and Krolikiewicz [67,86,87] used an imine linkage to join a variety of pharma-
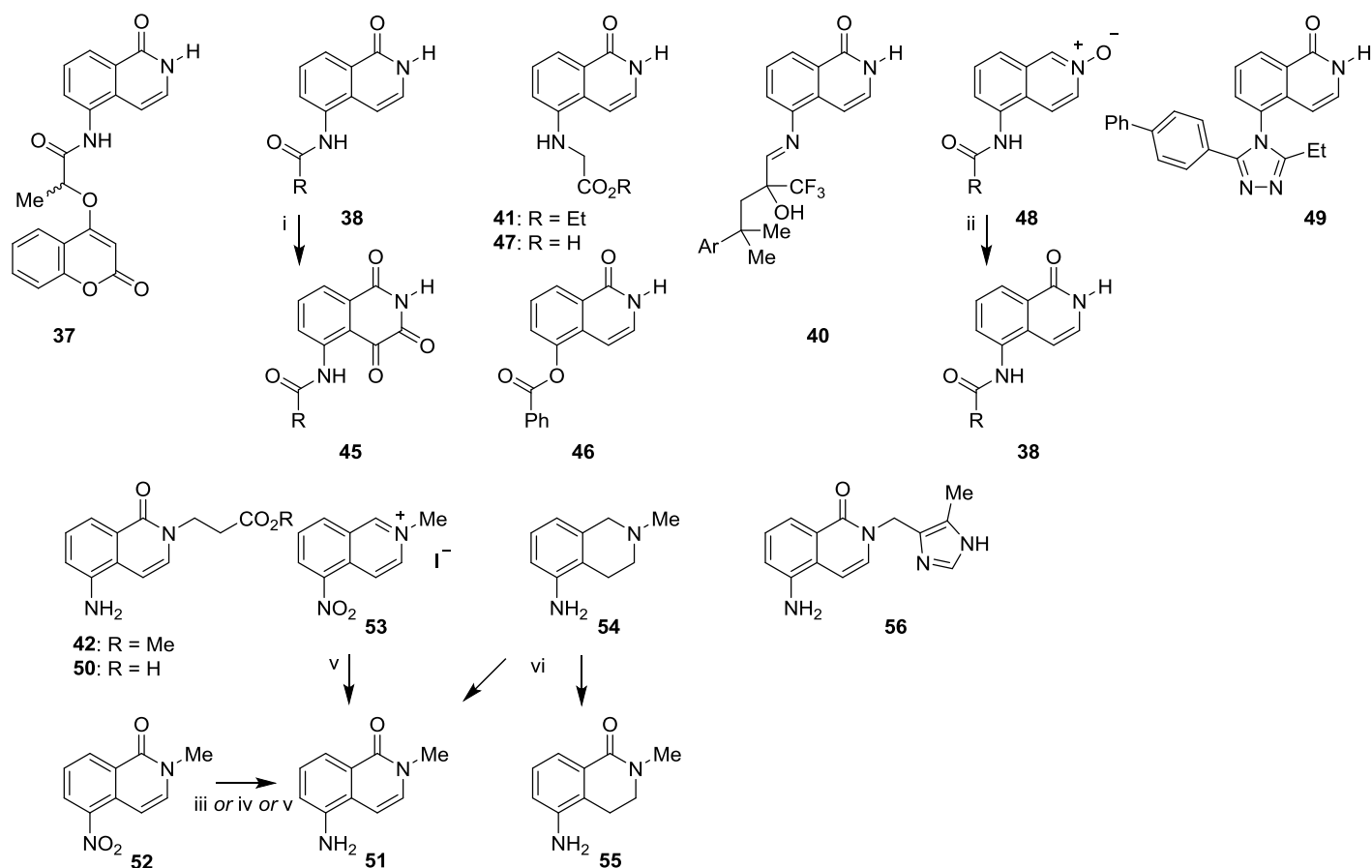
Scheme 4. Structures of reported N-substituted analogues of 5-AIQ and associated chemistry. *Reagents*: i, $K_2Cr_2O_7$ [85]; ii, hv [162]; iii, $N_2H_4 \cdot H_2O$, Raney Ni [165]; iv, H_2, Pd/C [166]; v, $TiCl_3$ [167,168]; vi, Hg^{2+} / EDTA [169].

cophores onto the isoquinolin-1-one core in **40**, aiming to prepare anti-inflammatory agents. An interesting synthesis of **38** (R = Et, Bn) by photolytic Polonovski rearrangement of the precursor N-oxides **48** was disclosed [163], as part of an attempt to access 4-methoxyindole. Incorporation of the 5-N in a 1,2,4-triazole (in **49**) led to inhibitors of Gly transporter-1 [164].
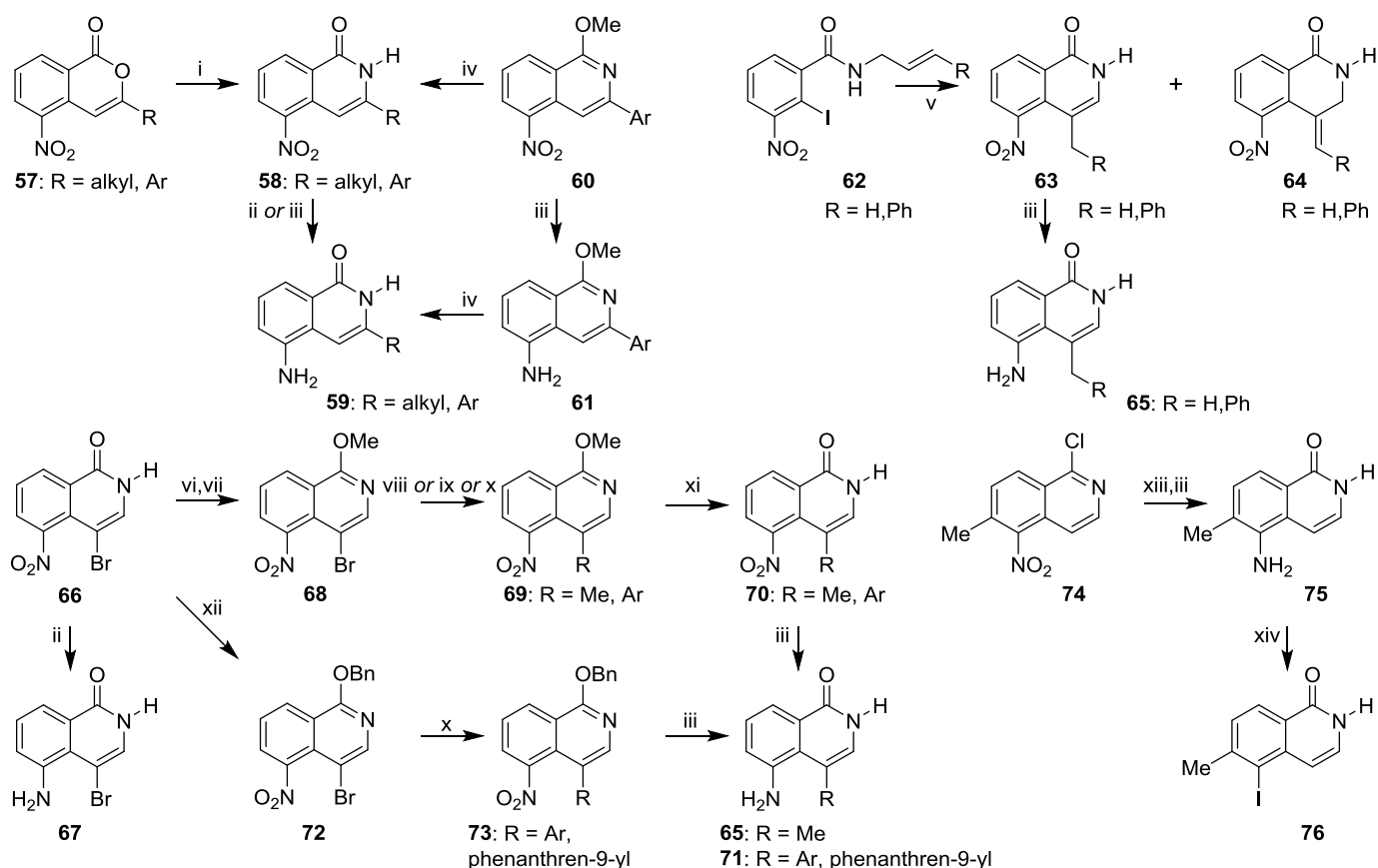
2.6.2. 2-N-Substituted analogues of 5-AIQ

Hydrolysis of the ester function of the 2-N-substituted 5-aminoisoquinolin-1-one **42** provided the acid **50** (Scheme 4) Remarkably for a compound which does not fit the general pharmacophore for inhibition of PARPs in that it lacks the lactam N-H (Scheme 1), **50** is almost as potent as is the parent **1** against PARP-1 (IC_{50} = 550 nM) and has good activity for inhibition of PARP-2 [78]. 2-N-methyl-5-AIQ **51** has been accessed synthetically in several ways. Horning *et al*. reduced the 5-nitroisoquinolin-1-one **52** to **51** with hydrazine and Raney nickel [165], whereas Henry *et al*. effected the same conversion by catalytic hydrogenation [166]. Somei *et al*. used the reduction of **52** to **51** as an example in their development of the use of $TiCl_3$ as a reagent for the selective high-yielding reduction of aromatic nitro compounds [167]. Attempting to adapt this procedure to selective reduction of the nitro group of the quaternary isoquinolinium salt **53**, Knefeli *et al*. unexpectedly achieved the complex oxidation / reduction sequence leading to the isoquinolinone **51** [168].

By contrast, Möhrle and Biegholdt selectively oxidised the tetrahydroisoquinoline **54** to a mixture of the 3,4-dihydroisoquinolin-1one **55** and the isoquinolin-1-one **51**; surprisingly, the arylamine was untouched by the oxidising Hg(II) complex [169]. The presence of the 5-NH_2 in **56** abolishes the 5-HT_3-antagonist activity of the 2-N-imidazolylmethylisoquinolin-1-one unit, whereas primary amines are tolerated well at the 6-, 7- or 8-positions [170].

2.6.3. 3-Substituted analogues of 5-AIQ

Scheme 5 shows the C-substituted analogues of 5-AIQ and the associated chemistry. By far the largest group of C-substituted derivatives known are the 3-alkyl and 3-aryl-5-aminoisoquinolin-1-ones **59**, owing to their potent biological activity. The 3-substituted-5-nitroisocoumarins **57** have been prepared by Hg^{2+}-catalysed ring-closure of methyl 2-arylethynyl-3-nitrobenzoate [82], tandem Castro-Stevens – ring-closure of 2-halo-3-nitrobenzoic acid and arylethynes [82] or tandem Hurtley coupling – retro-aldol – ring-closure [171] processes. The oxygen is displaced with ammonia under forcing conditions, giving **58**, in which the nitro group can be reduced to afford the 3-substituted-5-aminoisoquinolinones **59** [92,172]. Paine *et al*. devised a more efficient route to these important targets, assembling the 3-aryl-1-methoxy-5-nitroisoquinolines **60** by Suzuki couplings of arylboronic acids with 3-bromo-1-methoxy-5-nitroisoquinoline [92]. Demethylation unmasked the lactam (giving **58** (R = Ar). Alter-

Scheme 5. Structures of reported C-substituted analogues of 5-AIQ and associated chemistry. *Reagents*: i, NH₃, MeOCH₂CH₂OH, Δ [82,170]; ii, SnCl₂ [170,171]; iii, H₂, Pd/C [90,92,172]; iv, HBr, AcOH [92]; v, Pd(PPh₃)₄, Et₃N, Bu₄NCl, DMF, Δ [90]; vi, (COCl)₂, DMF, (CH₂Cl)₂ [171]; vii, NaOMe [171]; viii, BuLi, THF, MeI, -78°C [171]; ix, SnMe₄, Pd₂(dba)₃, SPhos, PhMe, Δ [171]; x, ArB(OH)₂, Pd₂(dba)₃, SPhos, PhMe, Δ [171]; xi, aq. HBr [171]; xiii, NaOBn [171]; xiii, aq. HCl, THF, Δ [172]; xiv, NaNO₂, aq. HCl, KI [172].

natively, **60** were reduced to the 5-aminoisoquinolines **61**, which were demethylated to give **59** (R = Ar) [92].

The 3-alkyl- and 3-aryl analogues **59** of 5-AIQ were developed and optimised as part of a major study towards potent and selective inhibitors of the tankyrases (PARP-5a and PARP-5b) [92]. Particularly potent were compounds carrying *para*-substituted phenyl at the 3-position, with **59** (R = 4-MePh) having IC₅₀ = 39 nM *vs*. tankyrase-1 and IC₅₀ = 34 nM *vs*. tankyrase-2. Isoform-selectivity was moderate-to-good for the 3-aryl compounds, with **59** (R = 4-MePh) showing IC₅₀ = 880 nM *vs*. PARP-1. Interestingly, several of these 3-aryl-5-AIQs were markedly cytotoxic towards tumour cell lines, with IC₅₀ values in the low μM range.

2.6.4. 4-Substituted analogues of 5-AIQ

4-Methyl and 4-aryl derivatives of 5-AIQ have been approached by two distinct synthetic routes (Scheme 5). Dhami *et al*. carried out Pd-catalysed C=C bond migration and intra-molecular Heck reaction of the N-allyl- and N-cinnamyl-2-iodobenzamides **62** [90] Isomeric mixtures of **63** and **64** were formed, with the ratios depending on the rate of heating to 150°C, contributing to the conclusion that π-allyl-Pd species were involved in the cyclisation. Reduction gave 5-amino-4-methylisoquinolin-1-one and 5-amino-4-benzyliso-quinolin-1-one **65**.

Sunderland *et al*. used intermolecular Pd-catalysed coup-lings to approach the targets [172]. The 4-Br in **66** was intro-duced by electrophilic bromination of 4-nitroisoquinolinone **20**. Reduction furnished 4-bromo-5-AIQ **67**. Neither **66** nor **67** was an effective coupling partner, so the polar lactam was masked as the 1-methoxyisoquinoline **68**, a strategy also used [92] for the couplings at the 3-position. Halogen-Li exchange and quench with iodomethane gave **69** (R = Me) in poor yield but Pd-catalysed Stille and Suzuki couplings eff-iciently provided **69** (R = Me, Ar). Buchwald-Hartwig coup-ling was also achieved. Demethylation and reduction led to the targets **65** (R = Me) and **71** (R = Ar). A shorter route *via* the 1-benzyloxy-4-bromo-intermediate **72** was also reported [172]. This route facilitated coupling to sterically crowded boronic acids, including preparation of analogue **73** (R = phenanthren-9-yl). Reduction of the nitro group and unmask-ing the lactam were achieved simultaneously. The 4-substit-uted-5-AIQs were evaluated biochemically, with most show-ing inhibition of both PARP-1 and PARP-2 in the low μM range.

2.6.5. 6-Substituted analogue of 5-AIQ

6-Methyl-5-AIQ **75** has been used as an intermediate in the assembly of a series of selective inhibitors of the mutant B-Raf, which contain aminoisoquinoline units [173]. Hydro-lysis of the imino-chloride unit of **74**, followed by reduction,

provided **75**. A Sandmeyer reaction then introduced the 5-iodine in **76** for later coupling reactions.

3. CONCLUSIONS

5-AIQ **1** is a development of the first "gold-standard" inhibitor of PARP-1, 3-AB **6**, in which the critical amide is constrained to be coplanar with the benzene ring by incorporation into a lactam ring. This coplanarity brings increased potency against PARP-1 but the retention of the primary arylamine (5-NH$_2$ in **1**) maintains the excellent physico-chemical properties, especially the water-solubility as the hydrochloride salt. Efficient synthetic routes to **1** have been devised, making the agent readily accessible and available through commercial suppliers.

5-AIQ is moderately potent against its original target enzyme, PARP-1, with IC$_{50}$ = *ca.* 300 nM, depending on the nature of the assay. There is no selectivity for this isoform, however, with similar potency demonstrated for inhibition of PARP-2. The agent binds less well to the tankyrases (PARP-5a and PARP-5b), with IC$_{50}$ = *ca.* 10 μM for inhibition of these isoforms. Off-target inhibition of non-PARP enzymes (MMP-2, diphtheria toxin) is weak.

The excellent water-solubility and other properties of the hydrochloride salt of **1** lead to good cellular uptake, as demonstrated in several functional studies and by measurement of apparent IC$_{50}$ against poly(ADP-ribosyl)ation in cells. This PARP-1-inhibitory potency in cells gives **1** strong activity in models of oxidative damage to DNA. In these models, the dramatic depletion of substrate NAD$^+$ by over-activated PARP-1 in cells is diminished or abolished by **1**. These cellular effects are translated into the remarkable potency of **1** in protecting against ischaemia-reperfusion injury in *in-vivo* models of a wide range of disease states. Of particular note is the significant protective activity of **1** at 30 μg Kg^{-1} in a rat model of haemorrhagic shock [73]. Organs become ischaemic during transplantation and **1** protects effectively against damage and loss of function consequent to reperfusion in the recipient animal.

Anti-inflammatory activity is also seen for 5-AIQ **1** in rodent models of arthritis and several other inflammatory disease states, including models of asthma and drug-induced lung inflammation and of damage to the spinal cord. This activity is largely due to down-regulation of the activity of NF-κB, which is regulated by PARP-1. Inhibition of PARP-1 activity with **1** therefore leads to down-regulation of expression of several effectors of inflammation, including ICAM-1, P-selectin and the matrix metalloproteinases.

The expression of proteins involved in angiogenesis is also down-regulated by treatment with 5-AIQ **1**, leading to inhibition of the angiogenesis process itself. Similarly, although **1** has only very modest direct anti-cancer activity *in vivo*, the down-regulation of the expression of MMP-2, MMP-9, P-selectin and ICAM-1, *inter alia*, leads to remarkable anti-metastatic activity in a mouse model [15]. Some synergy has been noted between inhibition of PARP-1 with **1** and inhibition of ART1 with *meta*-iodobenzylguanidine (MIBG), suggesting that these enzymes may be linked in some regulatory processes.

3-Substituted analogues of 5-AIQ **1** have been investigated thoroughly for their ability to inhibit the tankyrases (PARP-5a,b) [92]. 3-(*para*-Substituted-phenyl)-5-AIQs are particularly potent against these isoforms and show selectivity for the tankyrases over PARP-1 and PARP-2.

Several inhibitors of PARP-1 (*e.g.* olaparib **7**, rucaparib **8**, veliparib **9** and niraparib **10**) have entered clinical use or are in late stage clinical trial, largely with restricted indications (BRCA-mutant cancer). The very broad range of therapeutic activities demonstrated *in vivo* by the leading model agent 5-AIQ **1**, mediated by inhibition of PARP-1, points to much wider potential for therapeutic applications of PARP-1-inhibitory drugs in a diverse human diseases.

LIST OF ABBREVIATIONS

3-AB, 3-aminobenzamide; 5-AIQ, 5-aminoisoquinolin-1-one; Ac, acetyl; ADP, adenosine diphosphate; Akt, protein kinase B; ART1, ADP-ribosyl transferase-1; Ar, aromatic; BNCT, boron neutron capture therapy; BRCA, breast cancer type-1 susceptibility protein; BUN, blood urea nitrogen; DEAD, diethyl azodicarboxylate; DiBAL-H, diisobutylaluminium hydride; DMF, dimethylformamide; DMFDMA, dimethylformamide dimethylacetal / N-(dimethoxymethyl)dimethylamine; DNA, deoxyribonucleic acid; EAE, experimental autoimmune encephalomyelitis; Et, ethyl; FDA, Food and Drugs Administration; FGF, fibroblast growth factor; HATU, (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate); HUVEC, human umbilical vein endothelial cell; IC$_{50}$, concentration to inhibit activity by 50%; ICAM-1, intercellular adhesion molecule-1; logP, logarithm (base 10) of the octanol / water partition coefficient; IκB, inhibitory protein-κB; Me, methyl; MMP, matrix metalloproteinase; mRNA, messenger ribonucleic acid; MVP, major vault protein; NAD$^+$, nicotinamide adenine dinucleotide; NF-κB, nuclear factor-κB; NuMA, nuclear mitotic apparatus protein; PARP, poly(ADP-ribose) polymerase; Pr^i, prop-2-yl; TEP-1, telomerase-associated protein-1; TGF-β, transforming growth factor-β; THF, tetrahydrofuran; TLR2, toll-like receptor 2; TNF-α, tumour necrosis factor α; VEGF-C, vascular endothelial growth factor-C; vPARP, vault PARP;

CONFLICT OF INTEREST

The author declares no conflict of interest in preparing this review.

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