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Identification of aminoethyl pyrrolo dihydroisoquinolinones as novel poly(ADP-ribose) polymerase-1 inhibitors

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ABSTRACT

PARP inhibitors have been demonstrated to retard intracellular DNA repair and therefore sensitize tumor cells to cytotoxic agents or ionizing radiation. We report the identification of a novel class of PARP1 inhibitors, containing a pyrrolo moiety fused to a dihydroisoquinolinone, derived from virtual screening of the proprietary collection. SAR exploration around the nitrogen of the aminoethyl appendage chain of **1** led to compounds that displayed low nanomolar activity in a PARP1 enzymatic assay.

Poly(ADP-ribose)polymerases (PARPs) play significant roles in various cellular functions including DNA repair and control of RNA transcription. Poly(ADP-ribose)polymerase-1 (PARP1) is the most abundant nuclear enzyme of the PARP family. Its activation represents one of the earliest cellular responses to DNA damage, since it is able to recognize and rapidly bind to DNA strand breaks.[1] Binding activates the enzyme which, using nicotinamide adenine dinucleotide (NAD+) as a substrate, catalyzes the formation of long branched chains of poly(ADP-ribose) onto a variety of nuclear target proteins including PARP itself.[2] This process leads to the formation of poly-ADP-ribose chains. The formation of these negatively charged polymers is suggested to act as a molecular beacon and to facilitate recruitment of the base excision repair (BER) machinery to the site of the DNA damage,[3] upon which DNA repair occurs.

PARP1 plays a pivotal role in a number of cellular processes, thus it is regarded as a target for treating several important disease states, including cerebro-vascular disease, reperfusion injury[4] and cancer.[5]

In cancerous cells, inhibition of PARP1 would retard the repair of DNA strand breaks and therefore sensitize tumor cells to the effects of radiation and chemotherapeutic agents, by suppressing the repair of potentially lethal damage.[6] Recent studies have indicated that, in certain genetic backgrounds, tumor cells may be sensitive to the effects of PARP inhibition, also in the absence of cytotoxic chemotherapeutics.[7] These results suggest that PARP inhibitors could represent potential drug candidates for treatment of cancer, both as monotherapy in predefined patient populations and as combination therapies.

The vast majority of PARP1 inhibitors described to date bind to the nicotinamide binding site, since they are competitive with NAD+, and prevent PAR polymer formation.[1]

Early PARP inhibitors were analogs of 3-amino-benzamide (Fig. 1, **A**),[8] since it was observed that the benzamide functionality was crucial for the specific binding to the enzymatic site, forming 3 key H-bonds to the enzyme. In particular, several studies disclosed an increasing binding affinity when the carboxamide group, which is normally free to rotate, was restricted into a lactam (Fig. 1, **B**–**D**).[9]

In our company a research program was initiated aiming at developing a portfolio of different PARP1 inhibitors. In this article, we describe the synthesis, SAR study and preliminary biological evaluation of a novel series of PARP1 inhibitors that contains an aminoethyl-pyrrole fused to a dihydroisoquinolinone (Fig. 2, **1**).

Having shown previously that ligand based virtual screening can be used successfully to extract novel scaffolds,[10,11] this drug discovery program was started with the virtual screening of our

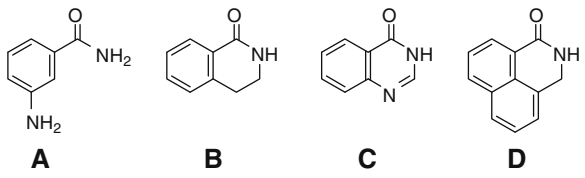

Figure 1. PARP1 Inhibitors.

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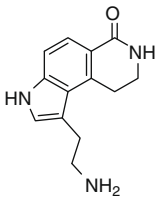


1

Figure 2. Lead identified by virtual screening of proprietary collection.

proprietary sample collection. To train the machine learning methods 200 known inhibitors were selected from the MDL Drug Data Report database,[12] patents and publications as actives and a set of 10,000 compounds representing the Merck collection were used as negatives. All molecules were described by topological atom pair descriptors.[13] All activities were treated as binary, either active or not active. This dataset was then used to train the support vector machine classifier (SVM).[14] The resulting SVM model was employed to predict potential actives from the Merck compound collection. The resulting potential PARP1 inhibitors were then tested firstly at fixed 1 μM concentration tested in the PARP1 enzyme assays, and subsequently active compounds were titrated with dose response curves.[15] Among the compounds identified aminoethyl-pyrrolodihydroisoquinolinone derivative **1** proved to be a potent PARP1 inhibitor (IC_{50} = 40 nM).

Docking of **1** into the PARP enzyme crystal structure (Fig. 3) reveals a tight overlap with the known PARP inhibitor **D**[9] and the possibility of a hydrogen bond between the indole NH and Glu 988 (2.8 Å).

Furthermore, the presence of the additional pyrrole ring on the isoquinolinone scaffold enhances the lipophilic surface area, resulting in a better lipophilic interaction within the hydrophobic cavity of the enzyme binding site.

Thus, **1** appeared to be a promising starting point and prompted us to explore the SAR around the amino group in the appending chain, with the hypothesis that this side chain could extend out into the adenosine binding pocket and pick up additional interactions. A number of functionalizations were investigated (amino, amide and urea derivatives) and the most relevant results are reported in Tables 1–3.

Alkylation of the amino group of **1** with isopropyl (**2**) and cyclohexyl (**3**) were tolerated (IC_{50} = 82 and 85 nM, respectively).

Introduction of a benzyl group, as in **4**, caused a threefold drop in activity compared to **1**, and small loss of potency occurred when the phenyl ring was substituted for oxazole (**5**). A further activity loss was observed upon introduction of a pyrazole (**6**), a methyl pyrazole (**7**) or a quinoline (**8**). A tertiary amine such as diethyl amine **9** was tolerated but suffered from a threefold drop of potency.

To further explore the SAR, a series of amides (Table 2) was synthesized. Previously reported studies revealed that incorporation of additional ring systems into the design of PARP inhibitors, thus expanding the planar structure, often led to poor aqueous solubility. To overcome this problem a possible approach was to introduce basic substituents.[9c] For this reason, the amides were chosen to include a protonatable nitrogen into the structure. Cyclic

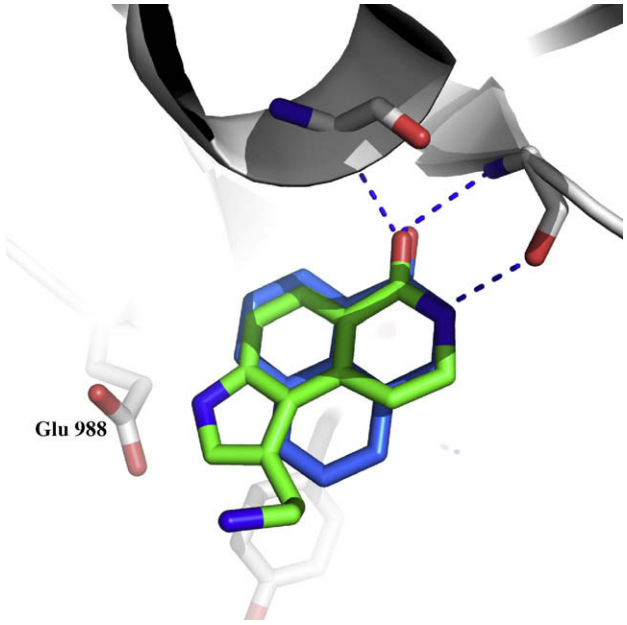
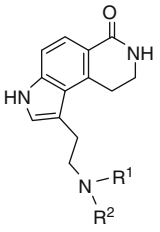
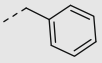
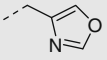
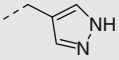
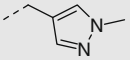
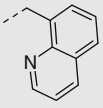


Figure 3. Docking of **1** (in green) and **D** (in blue) in the NAD binding site of PARP enzyme (pdb code: 2pax). **1** and **D** were manually placed in the NAD binding site and, to obtain the minimum conformation, a conformational analysis was performed using the MMFF forcefield[20] as implemented in Macromodel 7.0.[21]

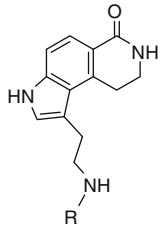
Table 1
Structure–activity relationships for amine functionalized derivatives[a]

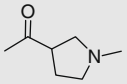
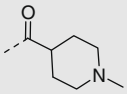
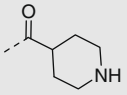
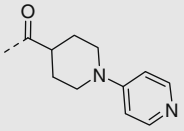
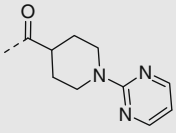
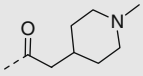
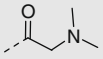
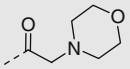
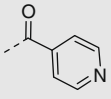
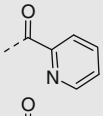
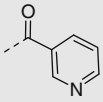


Compound	R^1	R^2	PARP1 IC_{50}[b] (nM)
1	H	H	40
2		H	82
3		H	85
4		H	110
5		H	180
6		H	340
7		H	>500
8		H	>500
9			130

[a] Results are the mean of at least two independent experiments, and std. dev. are <30% mean.
[b] Enzymatic assay performed as described in Ref. 11.

Table 2
Structure–activity relationships of the amide series[a]

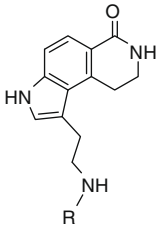


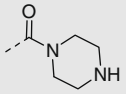
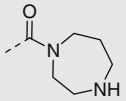
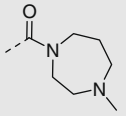
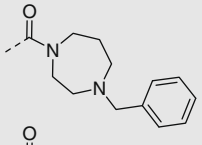
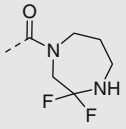
Compound	R	PARP1 IC_{50} (nM)
10		53
11		60
12		160
13		41
14		120
15		100
16		130
17		230
18		97
19		44
20		45

[a] See notes in Table 1.

amino groups, such as *N*-methyl pyrrolidine (**10**) and *N*-methyl piperidine (**11**) retained similar potency to the lead **1**. Moving from the tertiary to secondary amine (**12**), an adverse impact on the inhibition of PARP1 was observed. The study of the effect of substitution on the piperidine ring showed that a pyridyl-moiety, as in **13**, produced an inhibitor displaying similar activity to the lead **1**, whilst the introduction of a pyrimidine (**14**) resulted in a threefold loss in potency.

Table 3
Structure–activity relationships of the bioisosteric amide replacement: urea analogs[a]



Compound	R	PARP1 IC_{50} (nM)
21		25
22		27
23		58
24		230
25		390

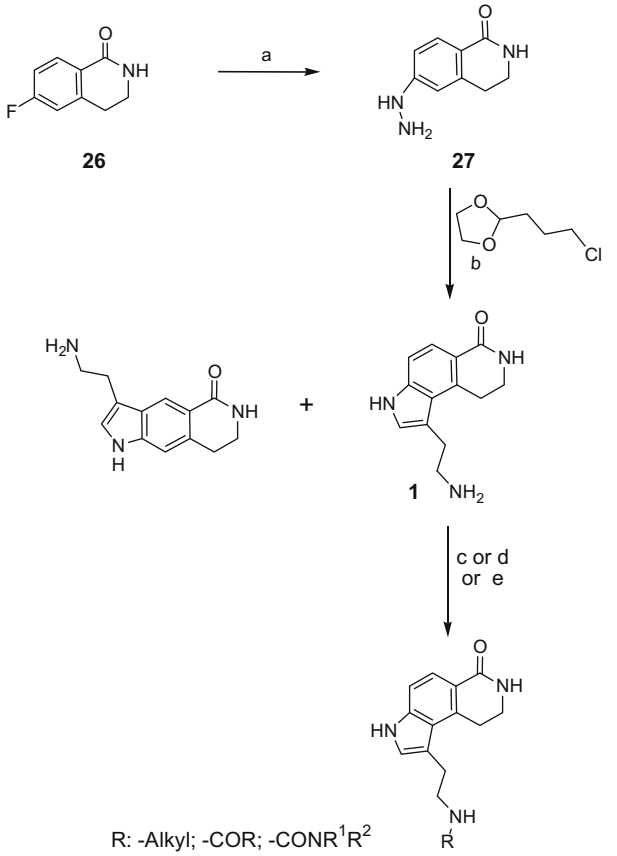
[a] See notes in Table 1.

The position of the basic amine had little influence on activity as a methylene spacer between the carbonyl and the piperidine ring proved to be not beneficial (compare **11** with **15**).

The *N*,*N*-dimethylglycinamide **16** displayed comparable activity, while its close analog morpholine **17**, bearing a less basic amine, proved to be a weaker PARP1 inhibitor. Turning attention to aromatic amines, of the three pyridine amides, the 4-pyridyl-derivative (**18**) proved to be the least active (IC_{50} = 97 nM), while the other regioisomers **19** and **20** were equipotent with lead **1** (IC_{50} = 44 and 45 nM, respectively).

Bioisosteric amide replacement with a urea moiety was also investigated and the results are reported in Table 3. Knowing that small cyclic amines gave potent PARP1 inhibitors, piperazine **21** and diazepane **22** were prepared and shown to display twofold improvement compared to amide **12** (IC_{50} = 25 and 27 nM, respectively). In this case methylation of the nitrogen of the diazepane **22** led to **23** which resulted in a twofold loss in potency. The inclusion of a basic amine was important as when the nitrogen in **22** was benzylated (**24**), a significant drop of activity was observed. A marked reduction of the pK_a of the amine, by difluoro-substitution on the homopiperazine (**25**), proved to be detrimental for activity (compare **25** with **22**) and a 13-fold loss of activity was observed.

The most active compounds **21** and **22** were tested on other two PARP isoforms (PARP2 and PARP3) and on two other proteins which share homology with PARP catalytic domain (Tankyrase and vPARP). Both **21** and **22** resulted single digit nanomolar inhibitors of PARP2 (IC_{50} = 32 and 17 nM, respectively), whilst they proved to be not active up to 500 nM on PARP3, vPARP and Tankyrase.[16] Several of the most potent compounds were tested in cells for their ability to inhibit PAR formation upon DNA damage caused



Scheme 1. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, μW (115 °C, 15 min); (b) EtOH/H_2O, rt, 24 h; (c) when R = alkyl: aldehyde or ketone, MP-triacetoxyborohydride, DMF, μW (80 °C, 10 min); (d) when R = COR: RCOOH, PL-Mukaiyama, PS-dimethylaminopyridine (PS-DMAP), DCM/DMF, rt, 24 h; (e) when R = $CONR^1R^2$: (i) CDI, DIPEA, DMF, μW (80 °C, 5 min); (ii) HNR^1R^2, DIPEA, μW (80 °C, 5 min).

by addition of hydrogen peroxide, unfortunately the compounds were inactive at 5 μM.

The synthetic route for the synthesis of the pyrrolo-dihydroisoquinolone scaffold is outlined in Scheme 1.

The treatment of **26**[17] with hydrazine, upon microwave irradiation at 115 °C for 15 min, provided compound **27** (yield: 85%) that, after reaction with 2-(3-chloropropyl)-1,3-dioxolane, gave rise to a mixture of two regioisomers (1:1 ratio, by HPLC). Their separation by HPLC provided the desired pyrrolo-tetrahydroisoquinolinone **1** (yield: 32%).[18] All the derivatives in Tables 1–3 were prepared by functionalization of **1**.

Alkyl derivatives (Table 1) were obtained by reductive amination of **1** with the appropriate aldehyde or ketone.

Amide compounds in Table 2 were prepared by amide coupling reaction of **1** with the appropriate carboxylic acid. Activation of **1** with 1,1′-carbonyldiimidazole (μW, 80 °C, 5 min), followed by addition of the appropriate amine upon microwave irradiation, afforded urea analogs (Table 3).[19]

In conclusion, the synthesis and preliminary biological evaluation of a novel class of pyrrolo-isoquinolinones as PARP1 inhibitors have been described. After a virtual screening of the proprietary collection, compound **1** was identified as potent PARP1 inhibitor (IC_{50} = 40 nM). A variety of functionalizations on the terminal nitrogen of the pendant 2-aminoethyl chain were evaluated. The introduction of alkyl and acyl groups resulted in compounds with low nanomolar activity in the PARP1 enzymatic assay (Tables 1 and 2). Urea derivatives, such as compounds **21** and **22**, proved to be the most promising PARP1 inhibitors in the series displaying an IC_{50} = 25 and 27 nM, respectively (Table 3).

Acknowledgments

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15. The ability of compounds to inhibit PARP1 activity was tested using a modified literature procedures (Cheung A., Zhang J. *Analytical Biochemistry* **2000**, *282*, 24), using PARP1 enzyme isolated from HeLa cells. Enzyme assay was conducted in buffer containing 25 mM Tris pH 8.0, 1 mM DTT, 1 mM Spermine, 50 mM KCl, 0.01% Nonidet P-40 and 1 mM $MgCl_2$. PARP reactions contained 0.1 μCi [^{3}H]-NAD (200,000 DPM), 1.5 μM NAD^+, 150 nM biotinylated NAD^+, 1 μg/mL activated calf thymus, and 1–5 nM PARP-1. Auto reactions utilizing SPA bead-based detection were carried out in 50 μL volumes in white 96-well plates. Compounds were prepared in 11-point serial dilution in 96 well plate, 5 μL/well in 5% DMSO/H_2O (10× concentrated). Reactions were initiated by adding first 35 μL of PARP-1 enzyme in buffer and incubating for 5 min at rt, then 10 μL of NAD^+ and DNA substrate mixture. After 3 h at rt these reactions were terminated by the addition of 50 μL Streptavidin-SPA beads (2.5 mg/ml in 200 mM EDTA pH 8). After 5 min, they were counted using a TopCount microplate scintillation counter. IC_{50} data was determined from inhibition curves at various substrate concentrations.
16. The PARP isoforms were assayed using a TCA protocol using [^{3}H]-NAD and activated calf thymus (PARPs 2 and 3 only). hPARP-2 was expressed using a baculovirus, hPARP-3 was available from Alexis Biochemical ALX-201-170, while the catalytic domains of the human vPARP (aa. 209-661) and human Tankyrase 1 (aa. 1013-132) were expressed in *Escherichia Coli*.
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18. *Synthesis of compound* **1**. Step 1: A suspension of 6-fluoro-3,4-dihydroisoquinolin-1(2*H*)-one (1 equiv) (obtained as described in Ref. 12) and hydrazine monohydrate (25 equiv) was irradiated in a microwave oven at 110 °C for 15 min. The reaction mixture was cooled to rt, water was added and the resulting precipitate was filtered and washed with diethyl ether. MS (EI+) $C_9H_{11}N_3O$ calcd: 177, found: 178 $(M+H)^+$. Step 2: A solution of 6-hydrazino-3,4-dihydroisoquinolin-1(2*H*)-one (1 equiv) and 2-(3-chloropropyl)-1,3-dioxolane (1 equiv) in EtOH/H_2O (5:1) was stirred at rt for 24 h. After aqueous work up, a crude mixture of the two isomers 1-(2-aminoethyl)-3,7,8,9-tetrahydro-6*H*-pyrrolo[3,2-*f*]isoquinolin-6-one and 3-(2-aminoethyl)-1,6,7,8-tetrahydro-5*H*-pyrrolo[2,3-g]isoquinolin-5-one was obtained. This mixture was purified by RP-HPLC (column Symmetry C18 7 μm, gradient A: H_2O + 0.1% TFA; B: MeCN + 0.1% TFA) to afford the desired product (first eluted isomer), as its trifluoroacetate salt. ^{1}H NMR (400 MHz, DMSO-d_6, 300 K): δ 11.27 (br s, 1H), 7.81 (br s, 3H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.62 (br s, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 7.25 (s, 1H), 3.48-3.38 (m, 2H), 3.30–3.20 (m, 2H), 3.17–3.00 (m, 4H). MS *m/z* (EI+) $C_{13}H_{15}N_3O$ calcd: 229, found: 230 $(M+H)^+$.
19. *As an example, synthesis of* **21**: A 0.3 M solution of **1** (1 equiv), DIPEA (1.1 equiv) and CDI (1 equiv) in DMF was heated in a microwave oven at 80 °C for 5 min. This mixture was added to a 0.4 M solution of *tert*-butyl 1,4-diazepane-1-carboxylate and DIPEA (1.1 equiv) in DMF and heated in a microwave at 80 °C for 5 min. The crude mixture was purified by RP-HPLC (column Waters X-TERRA MS C18, gradient A: H_2O + 0.1% TFA; B: MeCN + 0.1% TFA). During evaporation of the HPLC fractions Boc-deprotection occurred, providing **21** as its trifluoroacetate salt (yield: 65%). ^{1}H NMR (300 MHz, DMSO-d_6+ 2% TFA, 300 K): δ 11.11 (s, lH), 8.68 (br s, 2H), 7.63 (d, *J* = 8.6 Hz, lH), 7.59 (br s, lH), 7.24 (d, *J* = 8.6 Hz, lH), 7.20–7.16 (m, lH), 6.93–6.83 (m, lH), 3.53–3.46 (m, 4H), 3.44–3.38 (m, 2H), 3.34–3.27 (m, 4H), 3.10–3.01 (m, 4H), 2.99–2.92 (m, 2H). $C_{18}H_{23}N_5O_2$ calcd: 341, found: 342 $(M+H)^+$.
20. Halgren, T. A. *Curr. Opin. Struct. Biol.* **1995**, *5*, 205.
21. MacroModel Version 7.0: Schroedinger Inc., Portland OR 97201. http://www.schrodinger.com/Products/macromodel.html.