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Identification of substituted pyrazolo[1,5-*a*]quinazolin-5(4*H*)-one as potent poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors

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ABSTRACT

A novel series of pyrazolo[1,5-*a*]quinazolin-5(4*H*)-one derivatives proved to be a potent class of PARP-1 inhibitors. An extensive SAR around the 3-position of pyrazole in the scaffold led to the discovery of amides derivatives as low nanomolar PARP-1 inhibitors.

Poly(ADP-ribose) polymerase-1 (PARP-1) is an abundant nuclear enzyme, discovered four decades ago, with an important role in the cellular life cycle.[1] It is involved in many fundamental processes including DNA repair, transcriptional regulation as well as governing cell death.[2] The activation of PARP-1 occurs upon binding of the enzyme to single or double strand DNA breaks, causing the formation of long and branched poly(ADP-ribose) polymers using nicotinamide adenine dinucleotide (NAD^+) as substrate. PARP-1 catalyzes the addition of these long branched chains onto itself and on other target proteins acceptors.[3] These PAR chains serve to recruit other DNA repair machinery to the site of damage, and the DNA breaks are thereafter repaired by base excision repair (BER). The role of PARP-1 in the regulation of DNA integrity has made it an attractive target for cancer therapy. Indeed, PARP-1 inhibitors have been shown to sensitize cells to cytotoxic agents such as temozolomide, topoisomerase inhibitors and cisplatin, as well as ionizing radiation.[4] Moreover several recent articles have demonstrated that cells deficient in BRCA1 and BRCA2 proteins, involved in the repair of DNA double-strand breaks by homologous recombination, are highly sensitive to PARP-1 inhibitors.[5] Several PARP-1 inhibitors are currently being explored in the clinic as mono- and combination therapy in cancer patients.[6]

The first PARP-1 inhibitors identified (3-substituted benzamides **1**, Fig. 1) are structural analogues of the nicotinamide moiety of NAD^+.[7] However these compounds have a low potency and specificity. Further studies revealed that restriction of the carboxamide, which is normally free to rotate, to the biologically-active anti-conformation provided an increase in binding affinity.[8] Based on these studies, research focused on the design of PARP-1 inhibitors in which the carboxamide group could be locked into the required conformation. This was achieved with ring closure of the carboxamide into bicyclic systems. Bicyclic lactams such as quinazolinones (**2a**, Fig. 1) and isoquinolinones (**2b**) are two example of 'first generation' PARP-1 inhibitors.[9] Further development of these bicyclic systems is represented by the related tricyclic lactam **3**. Elaboration of these core structures in order to enhance potency, improve pharmacokinetic properties and increase aqueous solubility is reported in literature.[10]

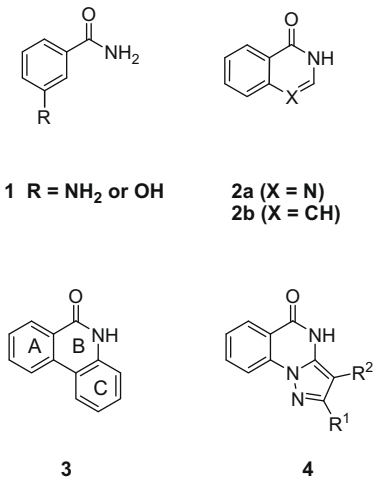

Figure 1. Structure of PARP-1 inhibitors.

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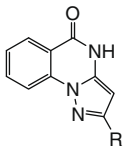
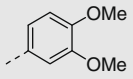
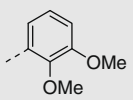
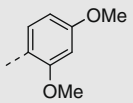
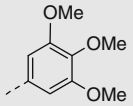
We envisaged the possibility to develop novel tricyclic lactam compounds as potential PARP-1 inhibitors by replacement of the C ring of **3** with a 5-member nitrogen containing heterocycle. Here we report the synthesis and biological evaluation of cyclic pyrazole derivatives **4**, in which one of the pyrazole nitrogens is embedded in the quinazolinone **2a**. The rational for this was based on their synthetic accessibility, as they could be readily prepared, and on the potentially improved physiochemical properties as a result of the inclusion of the weakly basic pyrazole motif.

The first tricyclic compound tested in the PARP-1 enzyme assay to probe the hypothesis was the commercially available methyl derivative **5**[11] which, pleasingly, showed good activity (IC_{50} = 0.3 μM) comparable to the (5*H*) phenanthridin-6-one (**3**, literature, IC_{50} = 0.52 μM).[10a] This encouraging result prompted us to target a library of analogs with different substituents in position 2 of the tricyclic system. The results in PARP-1 inhibition assay are summarized in Table 1.

Replacement of the methyl group of **5** with higher alkyls, such as *i*-butyl (**6**) and cyclohexyl (**7**) gave compounds basically equipotent with **5**. When a phenyl substituent was introduced in position 2 of the tricyclic system (**8**) a 3-fold boost in activity was achieved, displaying an IC_{50} = 0.1 μM. The isomeric phenyl derivative (**4**, R_1 = H, R_2 = Ph) was inactive (IC_{50} > 1 μM) so no further isomeric derivatives were explored. The effects of further substitutions on the phenyl ring of **8** were investigated. 4-methyl (**9**) and 4-ethyl (**10**) substitutions provided 5-fold less active compounds with respect to **8**, while 4-propyl and 4-*t*-butyl derivatives displayed a marked drop in PARP-1 potency (**11** and **12**). Similarly, a drop in activity was observed also by the 3- and 4-fluoro derivatives (**13** and **14**), while the other halogen substitutions

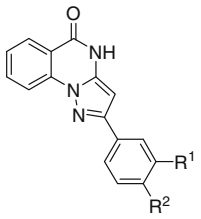
Table 1
SAR study on 2-position of the pyrazolo[1,5-*a*]quinazolin-5(4*H*)-one

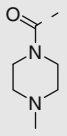
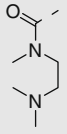
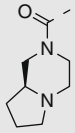
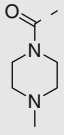
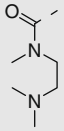
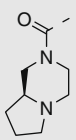


Compd	R	PARP-1 IC_{50}[a,b] (μM)
5	Me	0.3
6	^{i}Bu	0.4
7	Cyclohexyl	0.2
8	Ph	0.1
9	4-Me–Ph	0.5
10	4-Et–Ph	0.5
11	4-Pr–Ph	>1.0
12	4-^{t}Bu–Ph	>1.0
13	3-F–Ph	>1.0
14	4-F–Ph	>1.0
15	3-Cl–Ph	0.5
16	4-Cl–Ph	0.5
17	3-Br–Ph	0.6
18	4-Br–Ph	0.7
19	4-OCF_3–Ph	>1.0
20	4-CN–Ph	>1.0
21		0.2
22		0.1
23		0.2
24		0.03
25		0.15
26		0.2

[a] Values are means of two experiments. SD is ±30%.
[b] See Ref. 17 for PARP-1 inhibition assay.

Table 2
SAR study on *meta* and *para* position of **8**



Compd	R^1	R^2	PARP-1 IC_{50}[a] (μM)
8	H	H	0.1
27		H	1.0
28		H	0.8
29		H	0.5
30	H		1.4
31	H		1.3
32	H		2.5

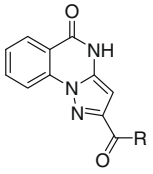
[a] See note a–b in Table 1.

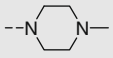
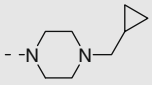
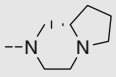
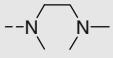
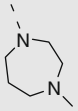
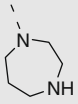
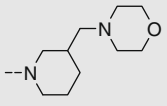
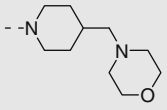
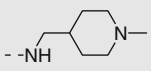
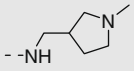
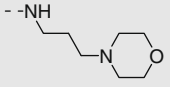
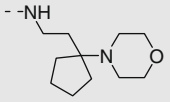
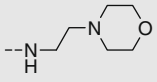
(**15–18**) provided compounds with an IC_{50} ranging between 0.5 and 0.7 μM. Likewise, different substitutions such as trifluoromethoxy (**19**) and cyano (**20**) in *para* position proved to be not tolerated.

Interestingly, the presence of the electron-donating groups was tolerated for PARP-1 activity as demonstrated by compounds **21–23**. In particular, the trimethoxy compound **24** proved to be a low nanomolar inhibitor, displaying IC_{50} = 0.03 μM, and to be 3-fold more active then the parent phenyl derivative **8**. Introduction of phenyl bioisosters such as thiophene and furan (**25** and **26**) retained the PARP-1 activity of the phenyl analog **8**.

It is widely reported that most of the tricyclic unsaturated PARP-1 inhibitors show limited solubility in both organic and

Table 3
SAR of 5-oxo-4,5-dihydropyrazolo[1,5-*a*]quinazoline-2-carboxamides



Compd	R	PARP-1 IC_{50}[a] (μM)
33		0.3
34		0.3
35		0.6
36		0.1
37		0.01
38		0.03
39		0.7
40		1.1
41		0.07
42		0.08
43		0.14
44		0.09
45		0.14

[a] See note a–b in Table 1.

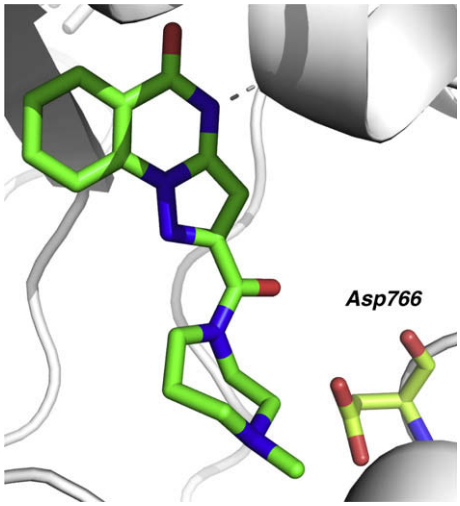
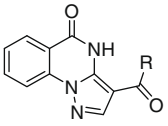
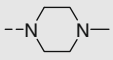
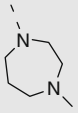
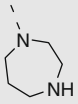
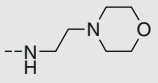


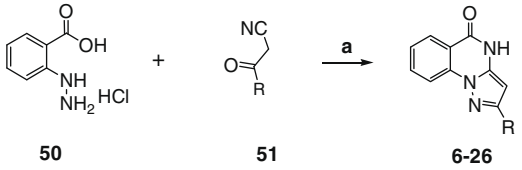
Figure 2. Model of **37** in the NAD binding site of PARP enzyme (pdb code: 2pax). Derivative **37** was manually placed in the NAD binding site and, to obtain the minimum conformation, a conformational analysis was performed using the MMFF forcefield[18] as implemented in Macromodel 7.0.[19]

Table 4
SAR of 5-oxo-4,5-dihydropyrazolo[1,5-*a*]quinazoline-3-carboxamides



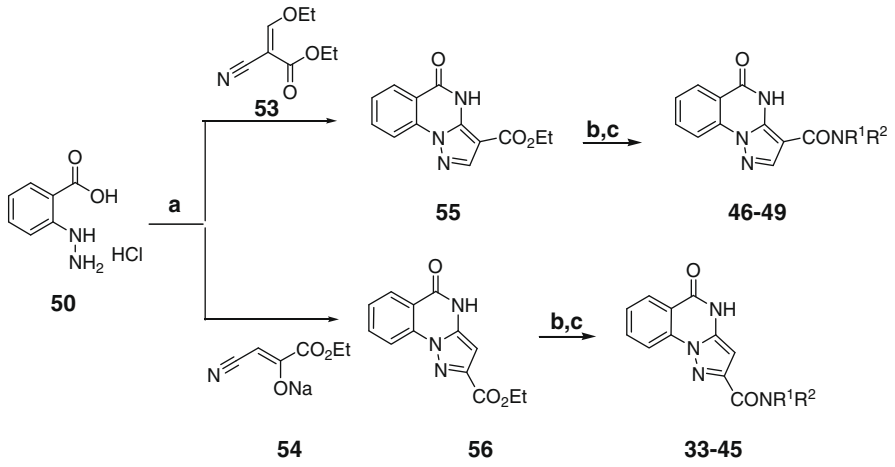
Compd	R	PARP-1 IC_{50}[a] (μM)
46		>1.0
47		>1.0
48		>1.0
49		>1.0

[a] See note a–b in Table 1.



50 **51** **6-26**

Scheme 1. Synthesis of derivatives in Table 1. Reagents and conditions: (a) acetic acid, MW, 150 °C, 5 min. Yield 30–65%.



Scheme 2. Synthesis of carboxamides in Tables 3 and 4. Reagents and conditions: (a) DMF, MW, 140 °C, 105 min; (b) 2 M NaOH, MeOH, MW, 90 °C, 5 min; (c) PS-DCC, HOBt, DMF, R^1R^2NH, rt, 60 min.

aqueous solvents, probably due to the planarity of the tricyclic core.[12] In an attempt to improve solubility while maintaining or possibly increasing potency, we thought to introduce polar amides in the *meta* and *para* positions of **8**, bearing a basic amino functionality as solubilizing group.

As shown in Table 2, piperazine compound **27** caused a 10-fold drop in activity compared to **8** (1.0 μM vs 0.1 μM). Also open analogue **28** and fused bicyclic piperazine **29** proved to be less potent with respect to the unsubstituted phenyl. The detrimental effect of the introduction of an amide substituent on the phenyl ring was even more evident in the 4-position, that produced compounds with an $IC_{50} > 1.0$ μM (see **30–32**, Table 2).

Based on the above disappointing results we envisaged the possibility of a steric restriction in that region of the molecule so we thought to introduce the polar amide groups directly linked to the tricyclic core (R^1 and R^2 in Fig. 1, structure **4**), removing the phenyl spacer.

Interestingly, substitutions at the C-2 position resulted in submicromolar inhibitors: substituted piperazines **33** and **34** (see Table 3) displayed $IC_{50} = 0.3$ μM against PARP-1, comparable to the lead compound **5** bearing a single methyl. The fused bicyclic piperazine **35** showed lower activity, while the open analogue **36** proved to be 3-fold more active than **33**. A further improvement in activity was achieved with the diazepane derivatives **37** and **38** which were low nanomolar PARP-1 inhibitors ($IC_{50} = 10$ and 30 nM, respectively), resulting to be the most potent of this class of compounds, presumably due to a beneficial interaction of the amine with acid residues of the protein. Modeling studies of **37** suggest that Asp766, which is close to the basic amine, can change its conformation in order to maximize its charge/charge interactions with the ligand, and thus contribute to binding (Fig. 2). Derivative **37** was tested for its ability to inhibit the formation of PAR polymers in HeLa cells upon stimulation of DNA damage with H_2O_2 where it showed an $EC_{50} = 3.9$ μM.

Alkylated piperidines **39** and **40** were only moderately active, while good potency was achieved with methyl piperidine (**41**) and methyl pyrrolidine (**42**) substitutions. Aliphatic derivatives **43, 44** and **45** showed comparable good potency indicating that both the branching and the length of the spacer between the amide nitrogen and the basic nitrogen did not dramatically affect the activity, although all three were less active than diazepanes **37** and **38**.

Taking into account the above results, substitutions in the 3-position of tricyclic system were also explored. Installation of the best amide fragments previously described dramatically diminished the inhibitory activity (Table 4), in line with the result on the 3-phenyl analogue.

The 2-substituted pyrazoloquinazolone derivatives in Table 1 were prepared according to the literature as described in Scheme 1.[13] The tricyclic pyrazoloquinazolone amides in Tables 3 and 4 were obtained by derivatization of the corresponding carboxylic acids. The tricyclic esters **55**[14] and **56**[15] were synthesized as described in Scheme 2. Condensation of benzoic acid hydrazide (**50**) with ethyl 2-cyano-3-ethoxyacrylate (**53**) or with sodium 1-cyano-3-ethoxy-3-oxoprop-1-en-2-olate (**54**), respectively, under microwave irradiation provided esters **55** and **56**. Hydrolysis with NaOH under microwave irradiation followed by amide coupling of the resulting carboxylic acid with the appropriate amines, using polymer supported carbodiimide as coupling reagent, led to the final carboxamides (**33–45** and **46–49**).[16]

In conclusion, we have reported the synthesis and biological evaluation of a new class of tricyclic lactam PARP-1 inhibitors containing a pyrazole ring fused to the isoquinolinone (**4**, Fig. 1). SAR at the 2-position of the tricyclic system with alkyl and phenyl substituents provided submicromolar inhibitors (**24,** $IC_{50} = 30$ nM). Introduction of polar amides in position 2 of the tricyclic lactam led to the identification of derivatives such as **37** as low nanomolar PARP-1 inhibitors ($IC_{50} = 10$ nM).

References and notes

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11. Compound commercially available from Bionet.
12. PARP-1 inhibition 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.
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18. *Synthesis of* **56**: A 0.5 M solution of 2-hydrazinobenzoic acid hydrochloride in DMF was treated with sodium l-cyano-3-ethoxy-3-oxoprop-l-en-2-olate (1 equiv). The reaction mixture was heated at 140 °C under microwave irradiation for 105 min and then poured into water. The resulting solid was isolated and dried under vacuum (yield = 66%). ^{1}H NMR (300 MHz, DMSO-d_6, 300 K) δ 1.32 (t, J = 7.1 Hz, 3H), 4.34 (q, J = 7.1 Hz, 2H), 6.26 (s, 1H), 7.58 (t, J = 7.5 Hz, 1H), 7.92 (t, J = 7.5 Hz, 1H), 8.10–8.20 (m, 2H), 12.34 (br s, 1H). MS m/z (EI+): 258 $(M+H)^+$.
19. *As an example, synthesis of* **38**. A solution of **56** in MeOH 0.6 M was treated with 2 N NaOH (3.5 equiv). The reaction mixture was heated at 90 °C under microwave irradiation for 5 min, then poured into water and acidified with 6 N HCl to pH 2. The solid formed was isolated to afford quantitatively 5-oxo-4,5-dihydropyrazolo[1,5-á]quinazoline-2-carboxylic acid. ^{1}H NMR (400 MHz, DMSO-d_6, 300 K) δ 6.23 (s, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.87–7.97 (m, 1H), 8.10–8.20 (m, 2H), 12.31(br s, 1H), 13.19 (br s,1H). MS m/z (EI+): 230 $(M+H)^+$. To the above carboxylic acid in DMF (0.1 M), HOBt (1.7 equiv) and PS-DCC (1.7 equiv) were sequentially added. The resulting suspension was stirred at rt for 60 min, then a solution of *tert*-butyl 1,4-diazepane-1-carboxylate (0.7 equiv) in DMF was added and the reaction mixture was stirred at rt for 24 h. Filtration and evaporation of volatiles provided Boc protected **38** that was purified by RP-HPLC. Addition of TFA and evaporation afforded **38** (yield = 58%). ^{1}H-NMR (300 MHz, DMSOd_6 + 2%TFA, 300 K) δ 2.00–2.18 (m, 2H), 3.18–3.55 (m, 4H), 3.70 (t, J = 5.7 Hz, 1H), 3.80–3.87 (m, 1H), 4.0 (t, J = 5.7 Hz, 1H), 4.08–4.18 (m, 1H), 6.18 (d, J = 8.4 Hz, 1H), 7.48–7.58 (m, 1H), 7.85–7.92 (m, 1H), 8.03–8.10 (m, 1H), 8.14–8.20 (m, 1H), 8.76 (br s, 2H). MS m/z (EI+): 312 $(M+H)^+$.