Bioorganic & Medicinal Chemistry Letters 19 (2009) 4050–4054

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and SAR of novel tricyclic quinoxalinone inhibitors of poly(ADP-ribose)polymerase-1 (PARP-1)

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ARTICLE INFO

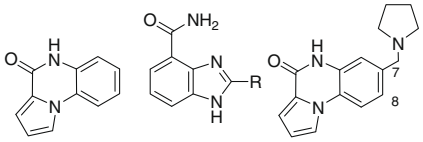
Article history:
Received 21 April 2009
Revised 3 June 2009
Accepted 5 June 2009
Available online 13 June 2009

Keywords:
PARP-1
Poly(ADP-ribose)polymerase
Quinoxalinone
Nuclear enzyme
DNA repair
Programmed cell death
Nicotinamide

ABSTRACT

Based on screening hit **1**, a series of tricyclic quinoxalinones have been designed and evaluated for inhibition of PARP-1. Substitutions at the 7- and 8-positions of the quinoxalinone ring led to a number of compounds with good enzymatic and cellular potency. The tricyclic quinoxalinone class is sensitive to modifications of both the amine substituent and the tricyclic core. The synthesis and structure–activity relationship studies are presented.

Poly(ADP-ribose)polymerases (PARPs) are a family of nuclear enzymes (17 members) involved in the regulation of a number of cellular processes involving DNA repair and programmed cell death. PARP-1, the main isoform, is one of the most abundant proteins in the nucleus and can be activated up to 100-fold by DNA strand breaks. This ubiquitous 116-kDa protein consists of 3 domains: an amino(N)-terminal DNA-binding domain, an automodification domain, and a carboxy(C)-terminal domain. The N-terminal zinc fingers of PARP-1 recognize single and double-stranded DNA breaks. The oxidative stress induced by DNA damage catalyzes the synthesis of poly ADP-ribose polymers on acceptor proteins with NAD^+ as the substrate. This cellular ADP-ribose transfer is an essential component of DNA repair and the maintenance of genomic integrity.[1] PARP-1 is primarily responsible for the catastrophic depletion of NAD^+ and ATP observed under high oxidative stress which culminates in cell dysfunction and death. Since tumor cells often have compromised DNA repair mechanisms, they are more dependent than normal cells on PARP-1 for DNA repair. Thus, using a PARP-1 inhibitor to shut down the DNA repair mechanism has the potential to enhance the therapeutic benefit of DNA-damaging anticancer drugs or ionizing radiation.[2–7]



1 2 9c

A considerable number of novel PARP inhibitors have been described in the literature over the past decade.[8] Many of the recent medicinal chemistry efforts have focused on tricyclic and tetracyclic carboxamide scaffolds. In general, these inhibitors mimic the binding mode of nicotinamide with the carboxamide group forming hydrogen bonds with Gly-863 and Ser-904 in the PARP enzyme.[4–9] Utilizing screening hit **1,** whose core has a potency comparable to the previously described benzimidazole carboxamide core **2** (R = H, PARP-1 K_i = 0.240 μM),[10] a series of tricyclic quinoxalinone analogues were explored leading to the identification of **9c**. This compound displayed excellent enzymatic and cellular potency. As seen in the PARP-1 X-ray co-crystal structure (Fig. 1), the quinoxalinone scaffold accesses a binding region previously not filled by the benzimidazole inhibitors. Access to this new 'northern' binding pocket may provide insights into the activity of **9c** and its analogues.

The synthesis of pyrroloquinoxalinone **9c** and its analogues is shown in Scheme 1. Attempts to arylate methyl pyrrole-2-carbox-

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doi:10.1016/j.bmcl.2009.06.016

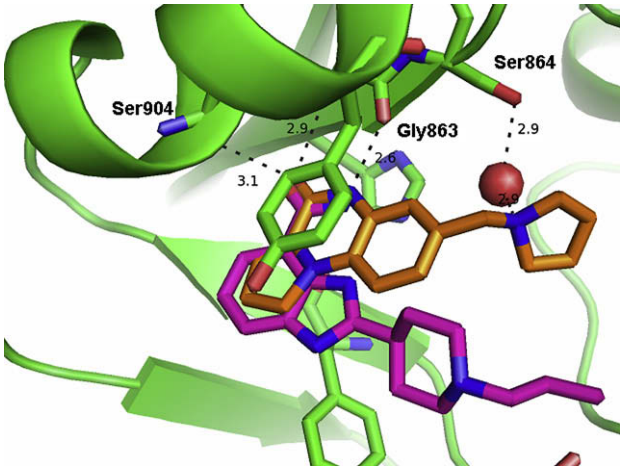
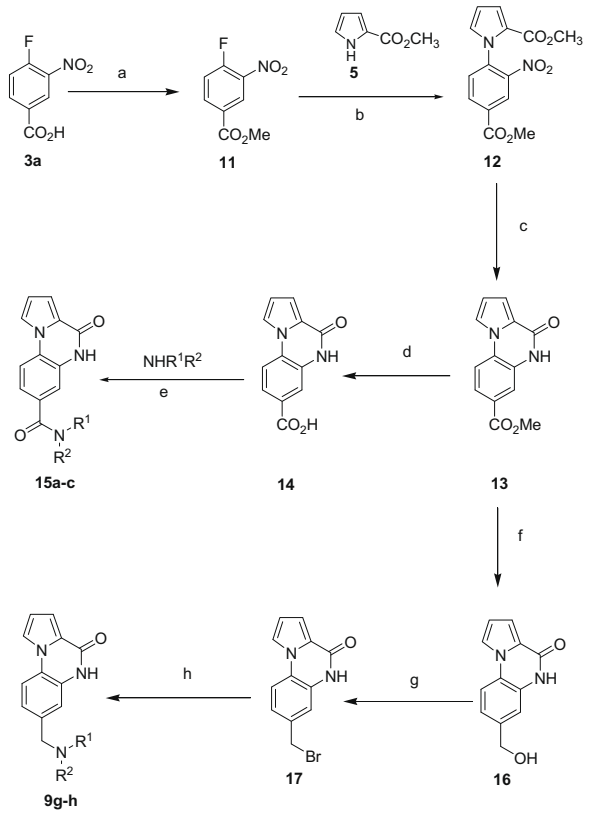


Figure 1. X-ray of **2** (R = 4-(*N*-propylpiperidine), purple) and **9c** (orange) in PARP-1 binding site.[11]

ylate (**5**) with fluoronitrobenzoic acid **3a** or **3b** were unsuccessful. The reaction was facilitated by conversion of the benzoic acid to either the Weinreb amide **4** (Scheme 1) or the methyl ester **11** (Scheme 2). Weinreb amide **4** and methyl pyrrole-2-carboxylate (**5**) were heated under basic conditions to give arylpyrrole **6**. Reduction of the nitro group and subsequent cyclization formed the quinoxalinone core. The Weinreb amide **7** was reduced to aldehyde **8** with lithium aluminum hydride. Introduction of the amine substituents at the 7- and 8-positions was made via reductive aminations of aldehyde **8** with various amines to give compounds 9a–**f** and **10a–c** (Table 1).

Alternatively, compounds **9g–h** were synthesized via bromide **17** shown in Scheme 2. Methyl ester **13** was reduced to alcohol **16** then converted to bromide **17** with PBr_3. Reaction with amines resulted in compounds **9g–h** (Table 1). Saponification of ester **13** to give carboxylic acid **14** followed by amide formation gave the desired amides **15a–c** (Table 1).

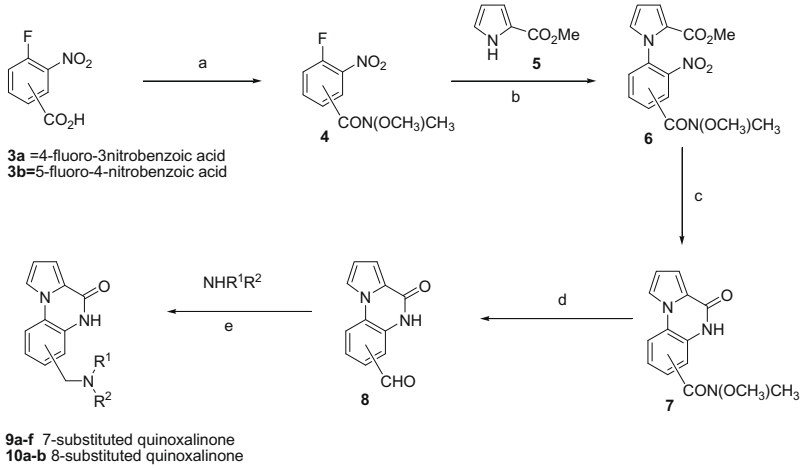
Imidazoquinoxalinones **23a–b** were prepared from N-arylation of ethyl imidazole-2-carboxylate (**18**) with methyl 4-fluoro-3-nitrobenzoate (**11**) as shown in Scheme 3. As in the pyrroloquinoxalinone series, the quinoxalinone core was constructed by reduction of the nitro group followed by cyclization. Reduction of methyl ester **20** followed by reaction with PBr_3 gave bromide **22**



Scheme 2. Reagents and conditions: (a) $SOCl_2$, MeOH; (b) Cs_2CO_3, DMF, 80 °C; (c) 10% Pd/C, EtOH; (d) 5 equiv LiOH, 5:1 THF/MeOH; (e) HATU, DIPEA, DMF; (f) LAH, 0 °C to rt; (g) PBr_3, dioxane, 0 °C to rt; (h) 3 equiv amine, CH_3CN.

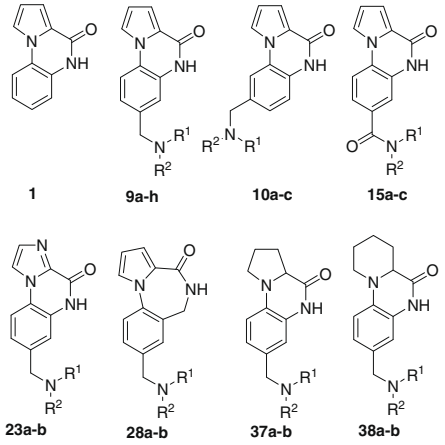
which was reacted with various amines to give compounds **23a–b** (Table 1).

Ring expansion of the tricylic core to give the pyrrolodiazepinone scaffold is shown in Scheme 4. The acetal protected fluorocyanobenzaldehyde **25** was heated under basic conditions with pyrrole **6** to give N-arylated pyrrole **26.** The key intermediate **27** was obtained after Raney nickel reduction of the nitrile, cyclization and deprotection of the aldehyde. Reductive amination of aldehyde **27** with the requisite amines gave compounds **28a–b**.



Scheme 1. Reagents and conditions: (a) (1) $(COCl)_2$, CH_2Cl_2; (2) NH(OMe)Me·HCl; (b) Cs_2CO_3, DMF, 80 °C; (c) 10% Pd/C, EtOH; (d) 1 M LAH in THF, 0 °C for 0.5 h; (e) $Na(OAc)_3BH$, HOAc, CH_2Cl_2.

Table 1
SAR of tricyclic quinoxalinones



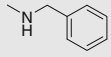
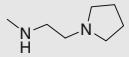
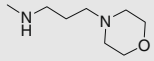
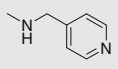
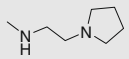
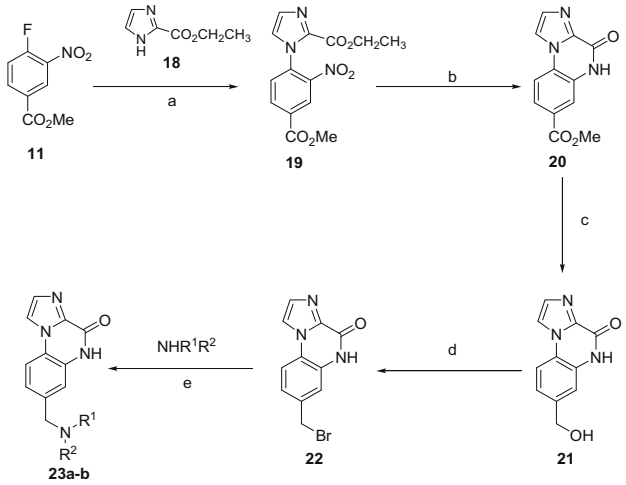
Compds	–NR¹R²	PARP-1[a] K_i (μM)	Cellular[a] EC_{50} (μM)
1	—	0.238	—
9a		0.037	0.039
9b		0.009	0.011
9c		0.005	0.006
9d		0.026	0.014
9e		0.161	0.164
9f		0.698	—
9g		0.133	0.171
9h		0.023	0.040
10a		0.031	0.025
10b		0.047	0.031
10c		0.048	0.029
15a		0.149	—
15b		0.023	0.280
15c		0.239	—
23a		0.280	—
23b		0.519	—

Table 1 (*continued*)

Compds	–NR¹R²	PARP-1[a] K_i (μM)	Cellular[a] EC_{50} (μM)
28a		0.589	—
28b		1.12	—
37a		0.096	>1
37b		0.190	0.646
38a		0.115	0.058
38b		0.063	0.039

[a] Mean of at least two determinations.

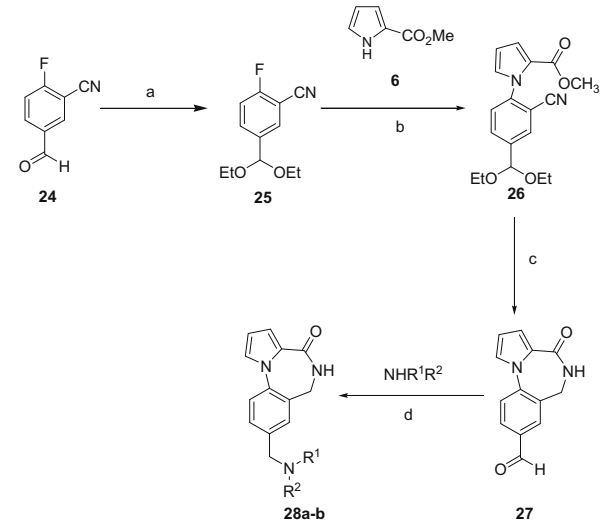


Scheme 3. Reagents and conditions: (a) Cs_2CO_3, DMF, 55 °C; (b) 10% Pd/C, EtOH; (c) 1 M LAH in THF; (d) PBr_3, dioxane, 0 °C to rt; (e) 3 equiv amine, CH_3CN.

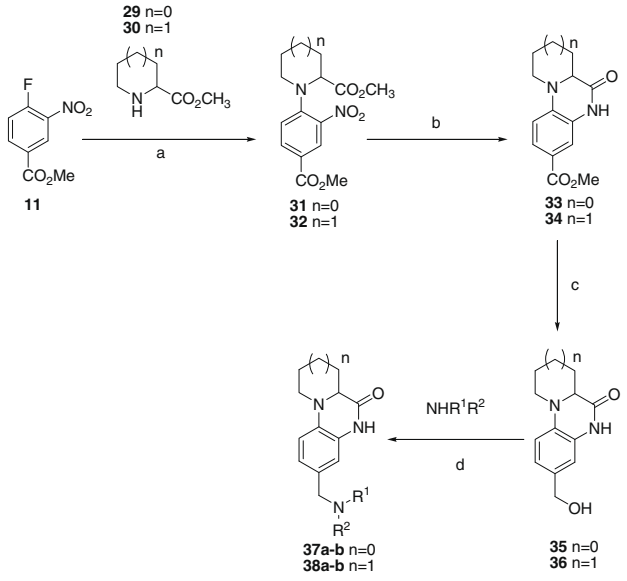
Analogs **37a–b** and **38a–b** were prepared as shown in Scheme 5. N-arylation of pyrrolidine **29** and piperidine **30** gave compounds **31** and **32** which were reduced and cyclized to give the tetrahydropyrroloquinoxalinone and tetrahydropyridoquinoxalinone cores, respectively. Methyl esters **33** and **34** were reduced with LAH to give the corresponding alcohols **35** and **36**. Conversion of the alcohol to the amines via the bromide was unsuccessful due to the apparent instability of the bromide. The amines were therefore prepared from the alcohol using standard Mitsunobu conditions to give **37a–b** and **38a–b** (Table 1).

Compound **1** was identified as a hit from a high-throughput screening assay (PARP-1 K_i = 0.238 μM). The tricyclic quinoxalinone core possesses potency equivalent to the benzimidazole carboxamide core **2** which we have investigated extensively in our labs, (R = H, PARP-1 K_i = 0.240 μM).[10] In an attempt to gain access to a new binding pocket and potentially obtain potency through interactions with additional residues in this pocket, we functionalized the 7- and 8-positions of the tricyclic quinoxalinone core with an amine containing side chain.

The compounds were initially evaluated for inhibition of PARP-1 enzyme activity.[12] Inhibitors with sufficient enzymatic activity



Scheme 4. Reagents and conditions: (a) $CH(OEt)_3$, NH_4NO_3, EtOH, Δ; (b) Cs_2CO_3, DMF, 55 °C; (c) (1) Raney Ni, 7 M NH_3 in MeOH; (2) 2 M HCl, THF; (d) $Na(OAc)_3BH$, HOAc, CH_2Cl_2.



Scheme 5. Reagents and conditions: (a) Cs_2CO_3, DMF, 60 °C; (b) 10% Pd/C, EtOH; (c) 1 M LAH in THF, 0 °C to rt; (d) PPh_3, DBAD, THF.

($K_i \leqslant 0.200$ μM) were further investigated in a PARP-1 cellular assay.[13] The PARP-1 enzymatic and cellular data are summarized in Table 1.

Both the isopropyl analogue **9d** and the cyclopropyl analogue **9a** are relatively potent inhibitors of PARP-1, showing more than sixfold improvement in enzyme activity (K_i = 0.026 μM and K_i = 0.037 μM, respectively) as compared to the hit **1**. Although **9d** and **9a** display comparable activity, the isopropyl analogue is three times more potent in the cellular assay. Replacement of the alkyl side chain with a benzyl group as in **9e** resulted in the loss of both enzymatic and cellular activity (K_i = 0.161 μM and EC_{50} = 0.164 μM). Cyclic amines **9b** and **9c** showed a significant increase in potency (K_i = 0.009 μM and K_i = 0.005 μM, respectively), whereas the addition of a carbonyl as in lactam **9f** dramatically decreased the potency (K_i = 0.698 μM). Increasing the length of the side chain as illustrated by **9g–h**, as well as, installation of a carbonyl group as in amides **15a–c** had deleterious effects on the PARP-1 activity (K_i = 0.023–0.239 μM).

The 8-position of the quinoxalinone core was also explored as demonstrated in **10a–c**. In general, the analogues substituted in the 7-position were more potent with **9b** and **9c** displaying the best activity.

Modification of the quinoxalinone core by the installation of a second nitrogen as in the imidazoquinoxalinones **23a–b** dramatically decreased the enzymatic potency (K_i = 0.280 μM and K_i = 0.519 μM, respectively). These analogues were even less active than the initial screening hit **1** (K_i = 0.238 μM). Increasing the ring size of the lactam by one carbon also led to a marked decrease in activity as seen in **28a–b** (K_i = 0.589 μM and K_i = 1.12 μM, respectively). The pyrrolidine analogues **37a–b** and the piperidine analogues **38a–b** displayed diminished activity and were only slightly more potent than the screening hit (K_i = 0.063–0.190 μM).

An overlay of the X-ray co-crystal structures of **9c** and a benzimidazole carboxamide **2** (R = 4-(*N*-propylpiperidine) is shown in Figure 1. The pyrrolidine nitrogen of **9c** is positioned to access a new binding pocket through a water-mediated hydrogen bond interaction with Ser-864. Analogues substituted in the 8-position are not in the proper orientation to access this binding region and in general, they show a sixfold decrease in activity compared to **9b** and **9c**. The previously described benzimidazole carboxamide series does not occupy this new binding region. Within the quinoxalinone series, it is clear that the Ser-864 interaction is important. However, it should be noted that **9c** (K_i = 5 nM) and **2** (K_i = 8 nM)[10a] have comparable enzyme inhibitory activities (see also **3a** in Ref. 10b). Thus, this interaction is series dependent and not required for potent inhibition.

In summary, we have modified a novel screening hit **1**, to lead to compounds with improved enzymatic and cellular potency against PARP-1. The quinoxalinone scaffold appears to be very sensitive to modifications to both the amine side chain and the tricyclic core. Acceptable enzymatic and cellular potency in this class has been limited. Enlarging the ring system or the addition of nitrogens was not tolerated. Further exploitation of the new 'northern' binding pocket will be addressed in future publications.

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11. The coordinates of compound **9c** have been deposited in the RCSB Protein Data Bank (PDB ID code 3GJW).
12. The enzyme assay was conducted in buffer containing 50 mM tris pH 8.0, 1 mM DTT and 4 mM $MgCl_2$. PARP reactions contained 1.5 mM [^{3}H]-NAD^+, 200 nM biotinylated histone H1, 200 nM slDNA and 1 nM PARP-1 enzyme. The details are described in Ref. 10b.
13. C41 cells were treated with test compound for 30 min. PARP was activated by damaging DNA with 1 mM H_2O_2 for 10 min. The details are described in Ref. 10b.