BRIEF REPORT

Three-component Castagnoli-Cushman reaction with ammonium acetate delivers 2-unsubstituted isoquinol-1-ones as potent inhibitors of poly(ADP-ribose) polymerase (PARP)

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ABSTRACT

An earlier described three-component variant of the Castagnoli-Cushman reaction employing homophthalic anhydrides, carbonyl compound and ammonium acetate was applied towards the preparation of 1-oxo-3,4-dihydroisoquinoline-4-carboxamides with variable substituent in position 3. These compounds displayed inhibitory activity towards poly(ADP-ribose) polymerase (PARP), a clinically validated cancer target. The most potent compound (PARP1/2 IC₅₀ = 22/4.0 nM) displayed the highest selectivity towards PARP2 in the series (selectivity index = 5.5), more advantageous ADME parameters compared to the clinically used PARP inhibitor Olaparib.

INTRODUCTION

Poly(ADP-ribose) polymerase (PARP) enzymes are regarded as important targets for the development of anticancer drugs owing to the clinical success of PARP inhibitors Olaparib, Talazoparib, Niraparib and Rucaparib approved for cancer treatment. PARP1 and PARP2 are two key enzymes that are critical for repairing single-strand breaks (“nicks”) in the DNA – a mechanism which is critical for the survival of both normal and cancer cells. It is generally accepted that, despite the non-selectivity of the approved PARP inhibitors, it is the inhibition of PARP1 that is responsible for the manifestation of their clinical efficacy. Normal cells do not divide as frequently as cancer cells, which allows them to survive PARP1 inhibition. However, tumour cells with certain mutations that are synthetically lethal with PARP1 inhibition are efficiently killed by the drugs of this class. This notion motivated the development of selective PARP1 inhibitors such as NMS-P118 reported by Nerviano Medical Sciences.

The majority of PARP1 inhibitors, including the above-mentioned “paribs”, were designed to mimic the nicotinamide moiety of NAD⁺ (from which the adenine ribose unit of poly(ADP-ribose) originates) with which the inhibitors compete for the NAD⁺-binding site of PARP1. This mimicry is achieved via the use of either a rotationally constrained primary benzamide (as in Niraparib and NMS-P118) or a benzamide motif embedded in a ring (as in Olaparib, Talazoparib and Rucaparib). Another characteristic feature noticeable in some of the advanced PARP1 inhibitors is the presence of a fluorine atom (the “magic fluorine” highlighted in blue) in the meta-position of the NAD⁺-mimicking benzamide moiety (Figure 1). This “magic fluorine” has been shown to enhance binding to the target.

Recently, we discovered 1-oxo-3,4-dihydroisoquinoline-4-carboxamides as a novel chemotype capable of delivering potent PARP inhibitors. After screening the amide residues around the basic core, we identified compound 1a as a lead structure for further development. It displayed a potent inhibition profile towards PARP1 (IC₅₀ = 156 nM) and PARP2 (IC₅₀ = 70.1 nM).
Figure 1. Clinically used PARP1 inhibitors, advanced clinical candidate NMS-P118 (NAD$^+$-mimicking motif is highlighted in red, NAD$^+$ structure shown).

Figure 2. Previously discovered series of PARP1/2 inhibitors 1 with the lead compound 1a, the newly designed series of inhibitors 2 bearing substitutions at position 3 and its envisioned synthesis via the 3C-CCR.
compound 1a displayed a better microsomal and plasma stability in vitro compared to clinically used PARP1 inhibitor Olaparib. Encouraged by this finding, we continued looking for ways to improve the potency profile of the 1-oxo-3,4-dihydroisoquinoline-4-carboxamide series. Carboxamide 1a bore no substituents in position 3 of the 1-oxo-3,4-dihydroisoquinoline core. Therefore, we considered exploring the structure-activity relationships within the series 2 where the 3-mono- and 3,3-disubstituted scaffolds could be screened while the 1,4'-bipiperidine carboxamide would remain unchanged. Access to derivatives 2 was envisioned via the amidation of carboxylic acids 3 which, in turn, could be prepared via the recently reported three-component Castagnoli-Cushman reaction (3 C-CCR) of homophthalic anhydride (HPA), carbonyl compounds and ammonium acetate. Herein, we describe the results obtained in the course of realising this strategy.

Results and discussion

Twelve carboxylic acids 3a-l were synthesised in diastereomerically pure (trans-configured, based on their vicinal coupling constants of methine protons) form as described previously and subjected to amidation with 1,4'-bipiperidine using 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) to activate the carboxylic group. Resulting amides 2a-l were obtained in moderate to excellent yields (Scheme 1).

In order to probe for the minimal substitution at position 3, we aimed to synthesise 3-methyl-substituted 1-oxo-3,4-dihydroisoquinoline-4-carboxylic acid 3m. Unfortunately, the 3 C-CCR protocol did not furnish the desired product, likely due to the high volatility of acetaldehyde. Thus, for the preparation of 3m, we resorted to the alternative approach recently reported by Shaw and co-workers. It involves in situ generation of N-(4-...
Table 1. Inhibitory activity of compounds 2a-m vs. PARP1 and PARP2.

| Compound | R1 | R2 | PARP1 (IC50, nM) | PARP2 (IC50, nM) | SI
|----------|----|----|------------------|------------------|---
| 2a       | Ph | H  | 87.9 ± 10.2      | 69.5 ± 12.8      | 1.26
| 2b       | 4-FC6H4 | H | 55.4 ± 8.8      | 39.9 ± 7.3      | 1.39
| 2c       | Et | H  | 44.6 ± 5.6      | 11.0 ± 2.2      | 4.05
| 2d       | i-Pr | H | 159.9 ± 18.6    | ND               | –
| 2e       | Cyclopentyl | H | 26.4 ± 4.1      | 18.3 ± 4.5      | 1.44
| 2f       | t-Bu | H | 24.1 ± 6.0      | –                | –
| 2g       | i-Bu | H | 87.0 ± 8.3      | 104.9 ± 20.8    | 0.83
| 2h       | Me | Me | 4.0 ± 7.4      | –                | –
| 2i       | -(CH2)3- | H | 12.1 ± 7.2    | –                | –
| 2j       | -(CH2)O(CH2)2- | Me | 5.1 ± 6.6    | –                | –
| 2k       | -(CH2)5- | H | 10.6 ± 9.7    | –                | –
| 2l       | Cyclohexyl | H | >500            | >500             | –
| 2m       | Me | H  | 95.7 ± 17.7      | 29.2 ± 3.4      | 3.28
| 1a       | H  | H  | 156.5 ± 5.8      | 70.1 ± 7.6      | 2.23
| Olaparib |      |     | 2.8 ± 0.3       | 0.7 ± 0.2        | 3.98

The data are presented as the mean value ± SD obtained in triplicate (n = 3)

SI = IC50 (PARP2)/IC50 (PARP1)

*The value = % inhibition at 1 μM concentration of the inhibitor.

**Data from [6].

Table 1. Inhibitory activity of compounds 2a-m vs. PARP1 and PARP2 enzymes.

It is apparent from the data presented in Table 1 that some small alkyl groups introduced in position 3 of the 1-oxo-3,4-dihydroisoquinoline-4-1,4'-bipiperidine carbamoyl scaffold increase compounds’ potency towards PARP1 and PARP2 (cf. 2c, 2e (the most potent inhibitor in the series) and 2m vs. 1a) while bulkier (cyclo)alkyl groups do not change (2d) or ablate (2f and 2l) the inhibitor’s potency. Similarly, disubstituted (2h) and spirocyclic (2i-k) analogs do not inhibit PARP1 in the concentration range relevant for drug development. In light of the bulky substituents at position 3 being detrimental to the inhibitor’s activity, it came as a surprise that 3-aryl-substituted analogs 2a-b displayed better potency compared to their unsubstituted counterpart (1a).

We attempted to rationalise some of the observed structure-activity relationships by performing docking simulation of the inhibitors’ binding to the PARP1 active site. Figure 3 displays the binding poses of the most potent compound 2e as well as its active (2b) and inactive (2h and 2l) analogs. Compounds 2e and 2b displayed a favourable network of lipophilic contacts in the PARP-1 active cavity (Figure 2(A,B)). The 3,3-dimethyl substitution in 2h causes a transition to another metastable conformation with the loss of hydrophobic interactions with Val762 and destabilisation of electrostatic contacts with Glu763/Asp766 (Figure 2(C)). In the case of 2l, the cyclopentyl moiety appears to induce a conformational rearrangement of molecule. The resulting reorientation of the 1,4'-bipiperidine moiety leads to the loss of the hydrophobic contacts with Val762 and π-stacking interaction with Tyr907 (Figure 2(D)).

Thus, we have identified a new lead compound based on unsubstituted 1-oxo-3,4-dihydroisoquinoline core, 2e, which inhibited PARP1 with an IC50 of 26.4 ± 4.1 nM and PARP2 with an IC50 of 18.3 ± 4.5 nM, which corresponded to the selectivity index (SI) of 1.44. Mindful of the advantageous influence of the fluorine atom positioned in the meta-position relative to the NAD1'-mimicking lactam (or carboxamide) moiety, we set off to synthesise a 7-fluoro analog of compound 2e, 4-[[1,4'-bipiperidine]-1'-carbonyl]-3-cyclopentyl-7-fluoro-3,4-dihydroisoquinolin-1(2H)-one (6).

This required that 7-homophthalic anhydride (7) be synthesised. Adaptation of its synthesis from the literature13 involved the preparation of 3-(4-fluorophenyl)propionic acid (8) via the Knoevenagel condensation and hydrogenation, 2) indanone ring closure followed by methoxylaylation to give compound 9, 3) oxidative fragmentation of the latter on treatment with hydrogen peroxide and cyclodehydration of 2-(carboxymethyl)-5-fluoro benzoic acid (10). The 3-C-CCR involving 7-F-HPA (7) followed by amida tion (as described above for the preparation of compounds 2a-l) furnished 7-fluoro analog 6 in modest yield over 2 steps (Scheme 3).

To our delight, when tested for inhibition of PARP1 and PARP2, compound 6 indeed displayed an improved potency profile towards both enzymes (PARP1 IC50 = 22.0 ± 3.2 nM, PARP2 IC50 = 4.3 ± 0.5 nM, SI = 5.1). Moreover, this compound can be regarded as a relatively rare PARP2-selective inhibitor which can enrich the toolbox of compounds needed for the investigation of the physiological role of PARP2 inhibition.

We were curious to compare the physicochemical and ADMET properties of the newly discovered lead compound 6 to those of more potent, clinically used PARP1 inhibitor Olaparib. Indeed, as was noted previously, the sheer potency of pharmacological agents is not a sole determinant of the pharmacodynamic efficacy and should be considered in combination with the overall candidate’s profile.

While, of course, the approved PARP1 inhibitor Olaparib is well within the limits of druglikeness as defined by Lipinski18 (and so is compound 6), the two compounds are quite similar in terms of molecular weight and lipophilicity. Quite reassuringly, compound 6 displayed similar stability in plasma to that of Olaparib. However, the metabolic stability of the former is significantly higher (Table 2).
Figure 3. Binding poses of compounds 2e (A), 2b (B), 2h (C) and 2l (D) in the active site of PARP1; crystal structure of PARP1 with inhibitor NMS-P118 (PBD ID 4ZZZ) was used for docking.

Scheme 3. Synthesis of 3-cyclopropyl-substituted 1-oxo-7-fluoro-3,4-dihydroisoquinoline-4-carboxamide 6. Reagents and conditions: a. malonic acid, morpholine (10 mol%), pyridine, 110 °C, 4 h; b. H₂ (1 atm), 10% Pd/C, MeOH/THF, r. t., 12 h; c. (COCl)₂, DMF (cat.), DCM, r. t., 2 h; d. AlCl₃, DCM, 0 °C then reflux, 2 h; e. (COCl)₂, NaOMe/MeOH, TolH, r. t., 16 h; f. 30% aq. H₂O₂, KOH/H₂O, r. t. then 50 °C, 3 h; g. TFAA, EtOAc, r. t., 3 h; h. cyclopropane carboxaldehyde, NH₄OAc, MeCN, 80 °C; i. 1,4'-bipiperidine, HATU, Et₃N, DMF, r. t., 16 h.
Table 2. Experimentally determined ADME parameters and calculated molecular characteristics for the lead compound 6 as well as clinically used drug Olaparib.

| Compound | Solubility a (μM) | HLM Stability b | Plasma Stability c | PPB d | MW | cLogP | HBD e | HBA f |
|----------|-------------------|-----------------|-------------------|-------|----|-------|-------|-------|
| Olaparib | >100              | 1.1             | 15                | 100   | 90.2| 434.5 | 2.52  | 1     |
| 6        | >100              | 0.1             | 3.8               | 100   | 71.0| 399.4 | 2.59  | 1     |

aSolubility (μM) in pH 7.4 0.01 M phosphate buffer solution; bStability in the presence of hepatic S9 fraction (μL/min/mg); cStability in the presence of human liver microsomes (μL/min/mg); d% compound remaining after incubation (4 h) with human plasma; eplasma protein binding (% bound).

In summary, we have further optimised the inhibitory potency of an earlier discovered lead 1a based on a 1-oxo-3,4-dihydroisoquinoline-4-carboxamide scaffold by exploring various substitutions at position 3. This exploration was reliant on the earlier described three-component variant of the Castagnoli-Cushman reaction of ammonium acetate. The profiling of various 3-mono- and 3,3-disubstituted analogs of compound 1a for PARP1 and PARP2 inhibition allowed establishing structure-activity relationships and led to the identification of 3-cyclopropyl substituent as the preferred periphery pattern. Introduction of a fluorine atom in the position 7 of the 1-oxo-3,4-dihydroisoquinoline core (inspired by the presence of this “magic fluorine” in several clinical candidates and approved drugs) further improved the potency profile and led to 1-oxo-7-fluoro-3,4-dihydroisoquinoline-4-carboxamide 6 as the new lead compound. It displayed 5.5-fold selectivity towards PARP2, good metabolic and plasma stability as well as better plasma protein binding, compared to Olaparib.

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Disclosure statement

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