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Synthesis and biological evaluation of substituted 2-phenyl-2*H*-indazole-7-carboxamides as potent poly(ADP-ribose) polymerase (PARP) inhibitors

Rita Scarpelli *,†, Julia K. Boueres, Mauro Cerretani, Federica Ferrigno, Jesus M. Ontoria, Michael Rowley, Carsten Schultz-Fademrecht, Carlo Toniatti, Philip Jones

IRBM—Merck Research Laboratories Rome, Via Pontina km 30,600, Pomezia 00040, Rome, Italy

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ABSTRACT

A potent series of substituted 2-phenyl-2*H*-indazole-7-carboxamides were synthesized and evaluated as inhibitors of poly (ADP-ribose) polymerase (PARP). This extensive SAR exploration culminated with the identification of substituted 5-fluoro-2-phenyl-2*H*-indazole-7-carboxamide analog **48** which displayed excellent PARP enzyme inhibition with IC_{50} = 4 nM, inhibited proliferation of cancer cell lines deficient in BRCA-1 with CC_{50} = 42 nM and showed encouraging pharmacokinetic properties in rats compared to the lead **6**.

Poly(ADP-ribose) polymerases (PARPs) are cell signaling enzymes that catalyze the transfer of ADP-ribose units from nicotinamide adenine dinucleotide (NAD^+) to itself plus a number of acceptor proteins (e.g., histones H1 and H2B, DNA polymerases α and β).[1] PARP-1, the best characterized member of the PARP family which currently comprises 18 members,[2] is an abundant nuclear DNA-binding enzyme implicated in cellular responses to DNA damage. Upon formation of DNA single strand breaks, PARP catalyzes the polymerization of NAD^+ into long poly(ADP-ribose) (PAR) polymers on various proteins, including PARP-1 itself. The presence of the negatively charged polymers leads to a chromatin relaxation, facilitating the access of DNA break repair proteins to the site of damage.[3]

PARP inhibitors (PARPi) have been shown to enhance the cytotoxic effects of ionizing radiation and DNA-damaging chemotherapy agents, such as methylating agents and topoisomerase I inhibitors.[4–6] Recent in vitro and in vivo evidence suggests that PARPi could be used not only as chemo/radiotherapy sensitizers, but also as single agents to selectively kill cancers defective in DNA double strand break repair, specifically cancers with mutations in the breast cancer-associated genes (BRCA-1 and BRCA-2).[7]

Most of the PARPi in development mimic the nicotinamide moiety of NAD^+. There are currently at least six PARPi in clinical trial development,[8] including: ABT-888 (**1**),[9] AG-014699 (**2**),[10] AZD-2281 (**3**),[11] MK-4827 (**4**)[12] (Fig. 1), plus INO-1001[13] and BSI-201[14] both of whose structures have not been disclosed yet.

We have recently reported the identification of a novel class of 2-phenyl-2*H*-indazole-7-carboxamide PARPi, such as compounds **5** and **6** (Fig. 2), and the optimization to MK-4827 (**4**).[12] These compounds incorporate a nitrogen atom within the [6,5]-bicyclic heteroaromatic ring which locks the carboxamide group into the desired conformation by an intramolecular hydrogen bond.

The lead compound **5** was a moderate PARP enzyme inhibitor, with IC_{50} = 25 nM[12] and its corresponding methylaminomethyl analog **6** resulted in 5-fold improvement in enzyme activity (IC_{50} = 5 nM) and inhibited the proliferation of BRCA-1 deficient (BRCA1-) HeLa cells (CC_{50} = 500 nM), displaying good selectivity, >10-fold, over the corresponding BRCA-1 wild type (BRCA1wt) HeLa cells.

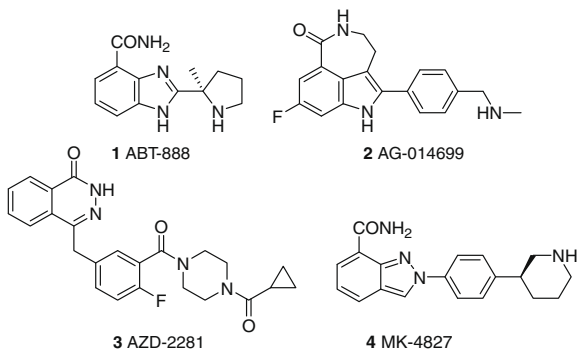

Figure 1. Some PARP inhibitors currently in clinical trials.

* Corresponding author. Tel.: +39 01071781233; fax: +39 0107170187.
E-mail address: rita.scarpelli@iit.it (R. Scarpelli).
† Present address: Department of Drug Discovery and Development, Italian Institute of Technology, Via Morego, 30, 16163 Genoa, Italy.

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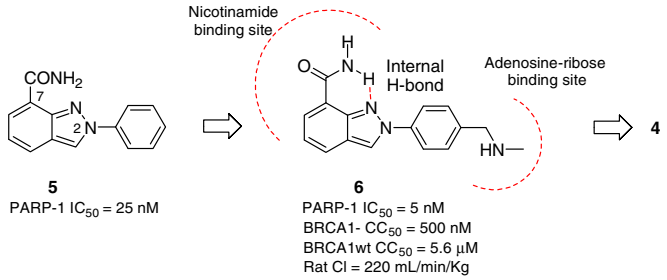
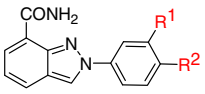


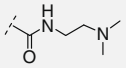
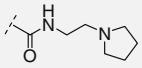
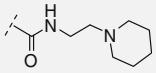
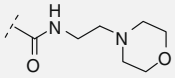
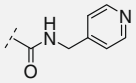
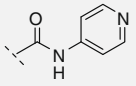
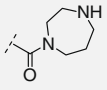
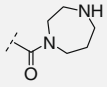
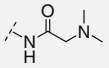
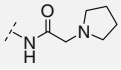
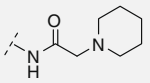
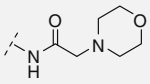
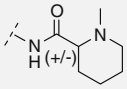
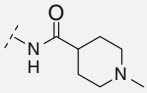
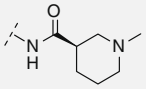
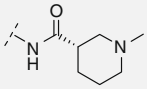
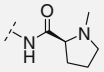
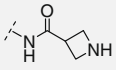
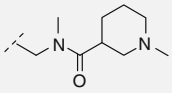
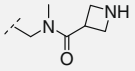
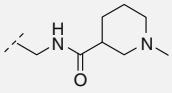
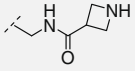
Figure 2. Development of novel indazole PARP inhibitors.[12]

Herein we describe a further investigation on this series of PARPi, conducting an extensive SAR exploration around the indazole scaffold, with the aim to find compounds with improved potency, selectivity, cell-based activity and optimal pharmacokinetic profile. Furthermore, we also report exploration of alternative groups on the pendant phenyl ring, knowing that the major route of metabolism of **6** in rats occurs through extra-hepatic oxidation by CYP450 1A1 and 1A2 of the methylaminomethyl group.[12]

The SAR exploration started with the preparation of analogs bearing alternative substituents at the *meta*- and *para*-positions of the pendant phenyl ring, such as carboxamides and anilides (Table 1), in the hope of picking up additional binding interactions in the adenosine-ribose binding pocket (Fig. 2). We intentionally excluded substitution at the *ortho*-position of the phenyl ring, based on previously reported results,[12] which demonstrated that functionalization adjacent to the indazole ring system was detrimental for activity. We also decided to focus our attention on studying the effect of polar groups with a view to maintaining good physicochemical properties and improve metabolic stability.

Table 1
SAR study around the pendant phenyl ring



Compd	R^1	R^2	PARP-1[12] IC_{50} (nM)[a]	BRCA1-[12] CC_{50} (nM)[a]	BRCA1wt[12] CC_{50} (nM)[a]
6	H		5	500	5600
7	H		10	5400	8900
8	H		20	3300	8000
9	H		38	3000	6400
10	H		54	7600	>20,000
11	H		10	3900	8700
12	H		30	7300	>50,000
13	H		22	>10,000	>20,000
14		H	40	>20,000	>20,000
15	H		15	1500	5000
16	H		10	930	2800
17	H		20	1400	3000
18	H		30	5000	>6000
19	H		46	2600	6700
20	H		10	3600	8400
21	H		30	820	4300
22	H		20	1300	6000
23	H		17	1200	5000
24	H		10	920	>20,000
25	H		25	8600	>20,000
26	H		18	>20,000	>20,000
27	H		22	2300	9400
28	H		65	>20,000	>20,000

[a] Values are means of >2 experiments with standard deviations <30%.

From this preliminary SAR exploration, we were pleased to demonstrate a general tolerability of different substituents on the pendant phenyl ring of the indazole scaffold, as reported in Table 1.

In particular, both carboxamide derivatives, **7**–**14** and anilide derivatives, **15**–**24** proved to be tolerated, although both classes of compounds generally displayed weaker activity than **6**. With regard to the carboxamides **7**–**14**, a small set of different polar substituents was explored, including secondary and tertiary amides bearing basic centers, such as Me_2N, pyrrolidine, morpholine, piperidine and 1,4-diazepane. These analogs displayed IC_{50}s around 10–55 nM, although in cells they showed only micromolar anti-proliferation activity in BRCA1- HeLa cells with a narrow selectivity over BRCA1wt HeLa cells. Substitution at the *para*-position was confirmed to be better tolerated than the isomeric *meta*-position, as shown for the pair **13** and **14**, whereby **14** displayed

2-fold weaker activity, IC_{50} = 40 nM, than the *para*-isomer **13**, IC_{50} = 22 nM.

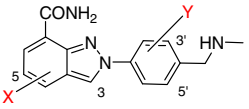
Although the anilides **15**–**24** showed similar enzymatic activity to the carboxamides **7**–**14**, only the anilides showed submicromolar inhibitory activity against the proliferation of BRCA1- HeLa cells, displaying modest selective cell growth inhibition. In particular, **21** and **24** while showing moderate enzymatic inhibition, with IC_{50} = 30 and 10 nM, respectively, both demonstrated inhibition of the proliferation of the BRCA1- HeLa cells in the submicromolar range. While the *N*-methylpiperidine **21** demonstrated only 5-fold selective cytotoxicity for BRCA1- HeLa cells, the corresponding azetidine **24** showed 20-fold selective cytotoxicity between BRCA-1 silenced and BRCA1wt cells. The (*R*)-*N*-methyl piperidine **21** proved to have the preferential stereochemistry, as enantiomer **22** displayed around 2-fold weaker activity. Unfortunately, despite **24** showed good rat microsome stability (Cl_{int} = 1.0 μL/min/mg), in vivo in rat PK this derivative suffered from a lack of oral bioavailability (*F* = 1%). On the other hand, **21** displayed a good rat PK profile with low clearance in vivo (Cl = 23 mL/min/kg) and good oral bioavailability (*F* = 47%). While, the corresponding (*S*)-enantiomer **22** showed higher clearance in rat (Cl = 42 mL /min/kg).

The two best fragments identified in the anilide series, piperidine and azetidine groups, were selected and the corresponding amide derivatives of compound **6** were prepared. Unfortunately, these amides derivatives **25** and **26**, resulted to be less active in the cellular assays compared to **21** and **24**. The same trend was observed for the corresponding *des*-methylated amide analogs, **27** and **28**.

We then moved our attention to study the effect of the introduction of small substituents in different positions of the methylaminomethyl indazole series, specifically exploration on the pendant phenyl ring and also the 3-, 4-, 5- and 6-positions of the indazole core, with the aim to identify compounds with improved potency and PK profile (Table 2).

First, we investigated different substituents in the *meta*-position of the pendant phenyl ring, focusing on derivatives with diverse physicochemical properties, such as CF_3, OMe, OH and F. We were pleased to identify the fluorinated analog **32** which showed similar potency in PARP enzyme assay compared to **6**. Moreover, the introduction of the fluoro group improved cellular activity and **32** displayed 2-fold improvement in anti-proliferation activity against BRCA1- HeLa cells (CC_{50} = 200 nM), maintaining 17-fold selectivity against their BRCA-1 proficient HeLa counterparts.

Table 2
SAR study around the indazole core and the pendant phenyl ring



Compd	X	Y	PARP-1 IC_{50} (nM)[a]	BRCA1- CC_{50} (nM)[a]	BRCA1wt CC_{50} (nM)[a]
6	H	H	5	500	5600
29	H	3′-CF_3	5	6200	>20,000
30	H	3′-OMe	4	1900	11,000
31	H	3′-OH	12	3400	>10,000
32	H	3′-F	4	200	3800
33	3-Cl	H	20	5900	11,000
34	4-Cl	H	180	5200	4300
35	5-Cl	H	5	260	1000
36	4-F	H	8	2500	6300
37	5-F	H	1.4	94	1800
38	6-F	H	20	1600	>10,000
39	4,5-F	H	25	2600	>5000
40	4-OMe	H	>1000	3400	5500
41	4-OH	H	540	>20,000	>20,000
42	5-F	2′-F	6	230	4200
43	5-F	3′-F	2	26	1500

[a] Values are means of >2 experiments with standard deviations <30%.

Turning attention to the indazole core, compounds bearing a chlorine atom in the 3- and 4-position of the indazole ring, such as **33** and **34,** showed a loss of activity compared to the unsubstituted analog **6**, with IC_{50} = 20, 180 and 5 nM, respectively. Moreover, it should be noted that, for compounds bearing Br or F substituents at the 3- and 4-positions, we observed a tendency for these compounds to undergo nucleophilic aromatic substitution. This fact could be exploited synthetically, and **40** and **41** were prepared through methoxy displacement on the 4-fluoro substituted scaffold, followed by deprotection. Unfortunately, these modifications were not tolerated. On the other hand, the isomeric 5-chloro substituted analog **35** although it maintained activity, lost selectivity between BRCA1- and BRCA1wt HeLa cells, displaying only a 4-fold window.

Similar observations were made with fluorine substitution whereby the 4-substituted compound **36** (IC_{50} = 8 nM) was less active than unsubstituted **6**, as was the 6-fluoro substituted derivative **38** (IC_{50} = 20 nM). In contrast, **37**, the 5-fluoro substituted analog proved to be a very potent PARP enzyme inhibitor. In fact, this structural change has led to 5-fold increase in enzymatic activity, IC_{50} = 1.4 nM, and double-digit anti-proliferation activity in the BRCA1- HeLa cells (CC_{50} = 94 nM). This 5-fluoro analog displayed nearly 20-fold selectivity against BRCA proficient cells (CC_{50} = 1800 nM).

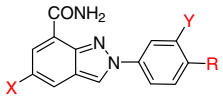
Having observed the beneficial effect of the 5-fluoro substitution, we decided to combine this structural feature with fluoro-substituents in the *ortho*- and *meta*-positions of the pendant phenyl ring (Table 2). The combination with the *ortho*-fluoro as in **42** proved to be ineffective for improving enzyme activity, IC_{50} = 6 nM, although in comparison to **6** it showed modestly improved cellular activity, CC_{50} = 230 nM. Instead, the *meta*-fluoro derivative **43** proved to be a very potent PARPi (IC_{50} = 2 nM), with excellent anti-proliferation activity in BRCA1- cells and high selectivity over BRCA-1 proficient HeLa cells, >50-fold. Unfortunately, these compounds, showed poor rat PK profile due to the presence of the highly unstable methylaminomethyl group, displaying clearance value in large excess of rat liver blood flow.

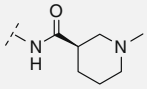
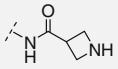
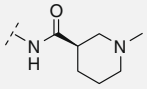
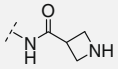
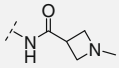
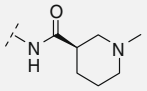
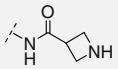
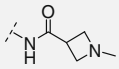
At this point, keeping in mind the encouraging potency and improved PK properties of anilides **21** and **24**, we were interested in preparing the corresponding *mono*- and *di*-fluorinated analogs (Table 3). Interestingly, both **44** and **45** were confirmed to show improved activity compared to **21** and **24** both in enzyme and cellular assays. Specifically, the 5-fluoro substitution gave a 2-fold boost in activity with both compounds displaying anti-proliferation activity in BRCA1- HeLa cells with CC_{50} = 520 and 310 nM, respectively. Although the *N*-methylated azetidine **46** showed similar enzymatic activity compared to **45**, this structural modification resulted in lost of activity in the cellular assays and selectivity between BRCA1- and BRCA1wt HeLa cells.

Moreover, **44** showed also a good rat PK profile with low-moderate clearance (Cl = 26 mL/min/kg) and good oral bioavailability (*F* = 66%). Similar to azetidine **24**, **45** when dosed in rats showed low clearance (Cl = 13 mL/min/kg), but minimal oral bioavailability (*F* = 3%).

As observed in the methylaminomethyl series, an interesting additive effect of substitution at the *meta*-position of the pendant phenyl ring was achieved and both *di*-fluorinated analogs **47** and **48** displayed improved properties compared to their mono-substituted counterparts, **44** and **45**. The piperidine analog **47**, although only a modest PARPi (IC_{50} = 12 nM), displayed good cellular activity BRCA1- CC_{50} = 440 nM, while azetidine **48** was a potent PARP enzyme inhibitor (IC_{50} = 4 nM) and showed double digit nanomo-

Table 3
SAR study around the indazole core and the pendant phenyl ring

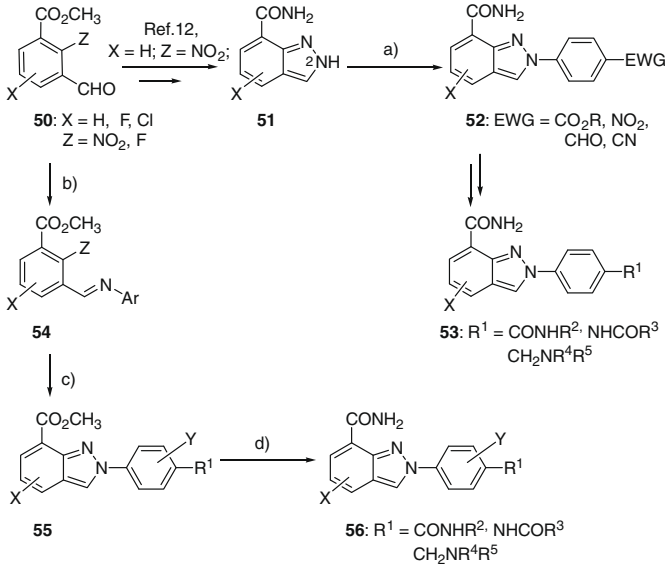


Compd	X	Y	R	PARP-1 IC_{50} (nM)[a]	BRCA1- EC_{50} (nM)[a]	BRCA1wt EC_{50} (nM)[a]
21	H	H		30	820	4300
24	H	H		10	920	>20,000
44	F	H		16	520	2700
45	F	H		5	310	5900
46	F	H		6	720	2600
47	F	F		12	440	1800
48	F	F		4	42	650
49	F	F		6	480	1600

[a] Values are means of >2 experiments with standard deviations <30%.

lar activity against BRCA1- HeLa cells (CC_{50} = 42 nM) and 15-fold selectivity. As expected, the *N*-methylated azetidine **49** showed the same trend observed for compound **46**.

Moreover, compounds **47** and **48** when dosed in vivo showed similar rat PK profile to **44** and **45**. In particular, **48**, which showed to be more potent and selective than **47**, showed a low clearance (Cl = 14 mL/min/kg) although being poorly oral bioavailable (F = 2%).



Scheme 1. Reagents and conditions: (a) *p*-F-C_6H_4-EWG, K_2CO_3, DMF, 200 °C, 10 min, microwave (20–90%); (b) $ArNH_2$, EtOH, reflux; (c) NaN_3, DMF, 90 °C, (40%, over two steps); (d) NH_3, MeOH, 60 °C, sealed tube, quantitative.

An efficient procedure was developed which allowed Rapid Analog Synthesis to be performed via a regioselective N-(2)-arylation of **51** under microwave assisted S_NAr conditions (Scheme 1),[15] using the appropriate activated fluorophenyl derivative in DMF in presence of K_2CO_3. This procedure allowed us to rapidly introduce versatile substituents (e.g., CO_2R, NO_2, CHO and CN) on the *para*-position of the phenyl ring. Further chemical manipulations (e.g., basic hydrolysis/amide coupling, reduction/amide coupling and reductive amination for CO_2R, NO_2, and CHO, respectively) allowed us to synthesize the required derivatives **53**. Alternatively, aldehyde **50** was treated with the appropriate substituted aniline in EtOH at reflux to give the corresponding imine **54** which was converted to the bicyclic indazole system by treatment with NaN_3 in DMF at elevated temperature.[12] The corresponding carboxamides **56** were obtained by treatment of **55** with a 7 M methanolic NH_3 solution at reflux in a sealed tube. This alternative procedure allowed us to overcome the limitation of the microwave assisted S_NAr when using doubly substituted fluorophenyl derivatives. Together these two synthetic routes allowed us to explore the effect of different substituents in all the possible positions of the indazole scaffold.

In conclusion, we have extensively explored the SAR on the indazole scaffold and the pendant phenyl ring and we have identified compound **48** which was shown to be a potent PARP enzyme inhibitor with IC_{50} = 4 nM, inhibiting the proliferation of the a BRCA1- HeLa cells in the double-digit nanomolar range, CC_{50} = 42 nM, displaying good selectivity over the corresponding BRCA1wt HeLa cells. We also demonstrated the feasibility to overcome the oxidative metabolic issue encountered with **6** as demonstrated with **48** that displayed low clearance in rats.

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