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Synthesis, molecular modeling, and biological evaluation of novel RAD51 inhibitors

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ABSTRACT

RAD51 recombinase plays a critical role for cancer cell proliferation and survival. Targeting RAD51 is therefore an attractive strategy for treating difficult-to-treat cancers, e.g. triple negative breast cancers which are often resistant to existing therapeutics. To this end, we have designed, synthesized and evaluated a panel of new RAD51 inhibitors, denoted IBR compounds. Among these compounds, we have identified a novel small molecule RAD51 inhibitor, **IBR120**, which exhibited a 4.8-fold improved growth inhibition activity in triple negative human breast cancer cell line MBA-MD-468. **IBR120** also inhibited the proliferation of a broad spectrum of other cancer cell types. Approximately 10-fold difference between the IC_{50} values in normal and cancer cells were observed. Moreover, **IBR120** was capable of disrupting RAD51 multimerization, impairing homologous recombination repair, and inducing apoptotic cell death. Therefore, these novel RAD51 inhibitors may serve as potential candidates for the development of pharmaceutical strategies against difficult-to-treat cancers.

1. Introduction

Targeting DNA damage repair pathway as a novel means of treating cancer, especially difficult-to-treat cancers, has attracted much attention in the past a few years. Homologous recombination (HR) is one of the most prominent repair pathways and is required for cancer cells to undergo continuous proliferation under stress. Therefore, various efforts have been taken to inhibit or modulate this pathway, in the hope of rendering cancer cells vulnerable [1–4]. As one of the key players in HR, RAD51 is essential for DNA repair, proliferation and survival. RAD51 protein level is elevated in many cancer cells, contributing to their resistance to chemotherapy and the continuous cell proliferation [5–14]. Therefore, RAD51 inhibition has been pursued by several groups in search of a novel potential treatment for difficult-to-treat-cancers [2,3,15].

Recently, our group identified and validated a small molecule synthetic alkaloid, named **IBR2** (Fig. 1), which showed interesting RAD51 inhibition activities both *in vitro* and *in vivo* [15]. RAD51 was rapidly degraded in **IBR2**-treated cancer cells, and the homologous recombination repair was impaired, subsequently leading to cell death. Therefore, **IBR2** represents a novel class of direct and specific RAD51 inhibitors [1–3,15]. The IC_{50} values of the original **IBR2** were in the range of 12–20 μM for most tested cancer cell lines. Its molecular scaffold contains a chiral center and the structure-activity relationship of this chirality has not been explored in our previous studies. It is well accepted that the interaction between chiral molecules and biological systems can provide more

Abbreviations: DMAP, 4,4-dimethylaminopyridine; XTT, sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro-) benzene sulfonate; IBR, RAD51 inhibitor.

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[1] These authors contributed equally to this work. HC, XLQ, and JZ designed, synthesized, and characterized all compounds under the guidance of ARC; XEG and CMH performed XTT assay and HR assay; JZ performed the gel filtration assay, QSAR analysis, and molecular modeling. JZ and WHL wrote the paper with inputs from all authors.

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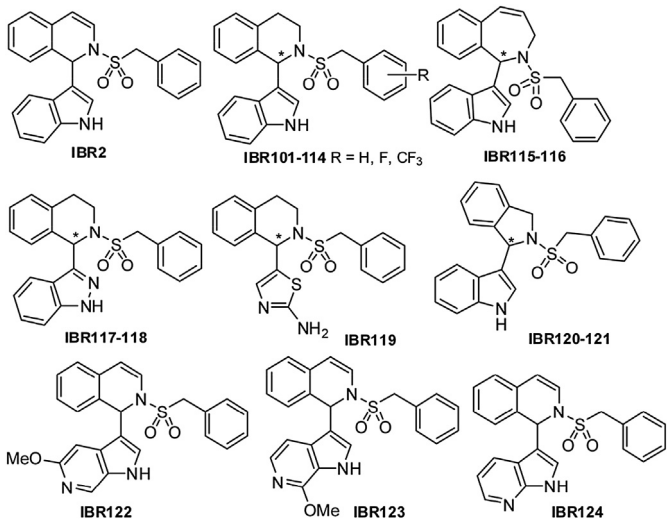

Fig. 1. Structures of **IBR2** and its analogues **IBR101–124**.

mechanistic insight and important clues for drug discovery and development [16]. Therefore, we sought to develop efficient and stereo-selective synthetic routes such that more potent analogues can be identified [17]. In this report, several types of **IBR2** analogues **IBR101–124** were designed, synthesized, modeled, and biologically evaluated. These new compounds constitute a focused compound library representing a diversity of modified scaffold or substituents (Fig. 1). As a result, a 4.8-fold improved RAD51 inhibitor was identified, which was able to inhibit the growth of triple-negative breast cancer cells and a panel of other malignancies. This finding may provide support for innovative methods and developments in cancer treatment.

2. Results and discussion

2.1. Synthesis of *IBR2* analogues

1,2,3,4-tetrahydroisoquinoline indole alkaloids **IBR101–114** were stereoselectively synthesized by the addition of *N*-Boc-3-bromo-indole **1** to the chiral benzylidene sulfinamide **2** as key steps [17]. 2,3-dihydro-1*H*-benzo[*c*]azepine analogues **IBR115–116** were stereoselectively synthesized using the bifunctional cinchona alkaloid-thiourea catalyzed addition of unprotected indole **3** to the sulfonyl amide **4** (Scheme 1) [17,18].

Synthesis of optically pure indazole derivative **IBR117** embarked on the reaction of the chiral benzylidene sulfinamide **2** [17] and 3-Bromoindazole **5**. The desired indazolylated adduct **6** was obtained in medium yield (43%) and diastereoselectivity (65% *dr*) (Scheme 2) [19]. Protection of **6** with Boc_2O gave compounds **7** and **8** in 66% and 14% yields, respectively. To our delight, the diastereoisomers **7** and **8** could be readily separated by silica gel column chromatography. Then, starting with chiral sulfinamide **8**, HCl-mediated removal of the *tert*-butanesulfinyl group provided the amine product **9** in 90% yield. Subjecting the amine **9** to Pd/C-catalyzed hydrogenation followed by exposure of the resultant alcohol to CbzCl/DIPEA gave the compound **10** in 61% yield over 2 steps. Alcohol **10** was further mesylated to give compound **11** in 91% yield. Once hydrogenation of the mesylate **11** was carried out with Pd/C as catalyst in MeOH, the desired cyclic amine **12** was isolated in 94% yield, which led directly to **IBR117** in 61% yield via deprotection with NaOMe/MeOH and subsequent benzylsulfonylation. Utilizing similar reaction procedures, the *S* configuration indazole derivative **IBR118** was also prepared starting from chiral sulfinamide **7** in 37% yield over 5 steps (Scheme 3).

Chiral thiazolylamine derivative **IBR119** was synthesized starting from *N*-Boc-2-amino-5-bromothiazole **17** and chiral benzylidene sulfinamide **2** (Scheme 4). Addition proceeded well by treatment of compound **2** with *N*-Boc-2-amino-5-lithiothiazole (prepared *in situ* via reaction of 17 with *n*-BuLi) at −78 °C, and the desired sulfinamides **18** and **19** were provided in 39% yield with medium diastereoselectivity (59% *dr*). Diastereoisomers **18** and **19** (1:4 isolated molar ratio) were then successfully separated through silica gel chromatography (isolated yield: 8% and 31%, respectively). The major diastereomer compound **19** was chosen for further modification. *tert*-Butanesulfinyl group of **19** was removed by treatment with 4 M HCl in dioxane and the amine **20** was obtained in almost quantitative yield. Benzylsulfonylation of **20** gave compound **21** in 97% yield, which was subjected to Pd/C-catalyzed hydrogenation to afford the alcohol **22** in 80% yield. Mesylation of **22** by treatment with MsCl/DMAP/DIPEA followed by exposure of resultant mesylate to KHDMS in THF furnished the desired cyclic compound **23**. Synthesis of **IBR119** was finally accomplished via TFA-mediated removal of Boc protecting group in **23**.

Synthesis of isoindolinyl derivative **IBR120** started from the indole-derivated compound **24**, which was prepared according to our previous report [17,18]. A direct OsO_4–$NaIO_4$ mediated oxidative cleavage reaction on unprotected compound **24** in the presence of 2, 6-lutidine [20] was carried out, followed by reduction with $NaBH_4$ and resulted in alcohol **25** (82%). Alcohol **25** was then treated with mesyl chloride and an appropriate base in DCM to give the corresponding mesylate, which was used as an intermediate for the following cyclisation reaction to form **IBR120**. Our first attempt on cyclization of alcohol **25** using KHMDS as a base in THF gave a racemic product. Reaction conditions with milder bases were then investigated (Table S1 in Supporting information). Reactions with Et_3N in DCM at r.t. or in dioxane under reflux did not facilitate the conversion. The use of K_2CO_3 in MeOH gave racemic cyclization product in 42% yield. Finally, with Hünig's base in acetonitrile, the desired product **IBR120** was obtained in 53% yield with >95% ee (Scheme 5). Utilizing the similar reaction procedures, the *S* configuration isomer **IBR121** was also prepared starting from the indole-derivated compound **26** (Scheme 6). Chiral HPLC analysis confirmed high enantiomeric purities of **IBR120** and **IBR121** (See Figs. S1–S3 in Supporting Information for representative HPLC profiles).

To explore possible effects of other indole bioisosteres on the bioactivity, racemic azaindole derivatives **IBR122–124** were synthesized using a facile one-pot synthetic method as described in our previous report [15], starting from azaindoles **27**, **28**, and **29**, respectively (Scheme 7).

2.2. *IBR2* analogues inhibit growth of triple-negative human breast cancer

Triple-negative breast cancer is often easy to metastasize and difficult to treat using existing therapeutics [21,22]. As a broad spectrum anti-cancer agent, **IBR2** was able to inhibit a number of difficult-to-treat cancers [15]. To test the possibility of inhibiting triple-negative breast cancer, we screened the newly synthesized **IBR2** analogues **IBR101–124** of their growth inhibition abilities using an XTT assay. As shown in Table 1, most of these synthetic **IBR2** analogues can inhibit the growth of triple-negative human breast cancer cell line MBA-MD-468.

We found that the half inhibitory concentrations (IC_{50}) of 1,2,3,4-tetrahydroisoquinoline analogues **IBR101, 102, 103, 105,**