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Facile construction of fused benzimidazole-isoquinolinones that induce cell-cycle arrest and apoptosis in colorectal cancer cells

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ABSTRACT

Colorectal cancer (CRC) is one of the most frequent, malignant gastrointestinal tumors, and strategies and effectiveness of current therapy are limited. A series of benzimidazole-isoquinolinone derivatives (BIDs) was synthesized and screened to identify novel scaffolds for CRC. Of the compounds evaluated, **7g** exhibited the most promising anti-cancer properties. Employing two CRC cell lines, SW620 and HT29, **7g** was found to suppress growth and proliferation of the cell lines at a concentration of ~20 μM. Treatment followed an increase in G_2/M cell cycle arrest, which was attributed to cyclin B1 and cyclin-dependent kinase 1 (CDK1) signaling deficiencies with simultaneous enhancement in p21 and p53 activity. In addition, mitochondrial-mediated apoptosis was induced in CRC cells. Interestingly, **7g** decreased phosphorylated AKT, mTOR and 4E-BP1 levels, while promoting the expression/stability of PTEN. Since PTEN controls input into the PI3K/AKT/mTOR pathway, antiproliferative effects can be attributed to PTEN-mediated tumor suppression. Collectively, these results suggest that BIDs exert antitumor activity in CRC by impairing PI3K/AKT/mTOR signaling. Against a small kinase panel, **7g** exhibited low affinity at 5 μM suggesting anticancer properties likely stem through a non-kinase mechanism. Because of the novelty of BIDs, the structure can serve as a lead scaffold to design new CRC therapies.

1. Introduction

Human colorectal cancer (CRC) is the most predominant digestive malignant tumor in the world and accounts for approximately 10% of all tumors.[1] Metastatic CRC is the fourth leading cause of cancer-related mortality, which exceeds 600,000 deaths annually.[2] Although mortality has decreased from advances in screening, early detection, and innovative treatment strategies, relapses from treatment-resistant CRC remain frequent.[3,4] Therefore, it is imperative to further study the mechanisms governing CRC progression and resistance and identify tractable therapies that can further reduce CRC morbidity and mortality.

Nitrogenous heterocycles are prolific in medicinal and organic chemistry.[5–7] Exploring fast and expeditious routes to generate diverse, nitrogenous heterocycles is paramount in drug discovery. Due to distinct and compelling therapeutic properties, natural and synthetic benzimidazole and isoquinolinone derivatives have been widely investigated in medicinal and organic chemistry.[8–10] Benzimidazole-isoquinolinone derivatives have been synthesized as potential inhibitors of phosphodiesterase 4 (PDE4) employing an innovative amberlyst-15 catalyst.[11] Similar derivatives were developed with antimicrobial activity against *Trypanosoma cruzi* (*T. cruzi*) epimastigotes (GI_{50} value is 0.5 μM).[12] These compounds also exhibited anti-inflammatory properties and anticancer activity against ovary (IGROV-1), breast (MCF-7) and CNS (SF-295) human cancer cell lines.[13]

Multicomponent reactions (MCRs) have attracted considerable attention as a powerful tool to access highly functionalized heterocyclic skeletons of chemical and biomedical importance.[14–16] Particularly, the Ugi reaction is well known as a versatile and highly efficient synthetic tool with extremely green synthetic methods, such as mild reaction conditions, no metal catalysts, short reaction times, high yields, and atom economy.[17] We have an interest in employing the Ugi cascade reaction for the formation of new C–N/C–C bonds to construct heterocycles for drug discovery efforts.[18,19] In continuation of our work in search of potent anticancer scaffolds, a series of fused benzimidazole-isoquinolinone derivatives (BIDs) were synthesized and screened for

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CRC anticancer activity, which we report herein.

The PI3K/AKT/mTOR signaling pathway is a very important signal transduction cascade engaged in a wide range of biological processes, such as cell survival, proliferation, apoptosis, cellular metabolism and the stress response.[20,21] This signaling pathway is of particular importance to CRC because 20% of all CRCs harbor a mutation in the PIK3CA gene. The mutation constitutively activates the PI3K/AKT/mTOR pathway causing an increase in pro-survival gene expression.[22] Previous studies discovered that activation of the PI3K/AKT signaling-axis inhibits colorectal cancer cell apoptosis.[23] On the contrary, inhibiting the activation of PI3K/AKT and its downstream target mTOR results in the initiation of apoptosis and autophagy. Furthermore, previous studies suggest that small molecule inhibition of the PI3K/AKT/mTOR signaling pathway could be utilized as a therapeutic strategy to treat CRC.[24,25]

In this present study, we first determined the effect of BIDs on the growth of two colorectal cancer cell lines, SW620 and HT29. We found that compound **7g** could inhibit the growth of SW620 and HT29 cells in a time and concentration dependent manner. Furthermore, we found compound **7g** induced G_2/M cell cycle arrest and apoptosis in both SW620 and HT29 cells. To understand the mechanism behind growth inhibition in CRC, we examined PI3K/AKT/mTOR signaling and found that activity of AKT was inhibited in both SW620 and HT29 cells. In addition, the phosphorylation level of mTOR decreased as well as activity of downstream signaling partners, such as 4E-BP, in a concentration-dependent manner. Taken together, this data suggests compound **7g** inhibited the growth of CRC and induced apoptosis through a PI3K/AKT/mTOR dependent mechanism.

2. Results and discussion

2.1. Results

2.1.1. Chemistry

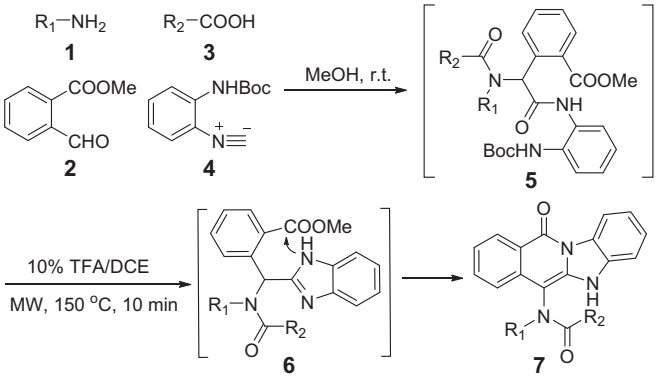
We conducted an Ugi four component reaction (U-4CR) using an amine **1**, methyl 2-formylbenzoate **2**, carboxylic acid **3** and isonitrile **4** in methanol. After stirring overnight at room temperature, the solvent was removed under a stream of nitrogen, and the crude Ugi product **5** was deprotected and cyclized. Intermediate **6** was used without purification and reacted under microwave irradiation using 10% TFA/DCE at 150 °C for 15 min.[19] A series of fused benzimidazole-isoquinolinones (BIDs) were obtained using these conditions. The results indicate that the Ugi product can be reacted crude without significantly impacting deprotection and cyclization yields. This two-step, one-pot procedure is mild and convenient and affords the desired BIDs in yields of 52–82% (Table 1).

2.1.2. Compound *7g* inhibits proliferation and viability of colorectal cancer cells in time and concentration dependent manners

In order to evaluate the potential of BIDs to inhibit CRC growth, growth inhibition (GI_{50}) values were determined in two CRC cell lines. As shown in Table 2, compound **7g** obtained the best growth inhibition (SW620 and HT29 are 23.78 and 24.13 μM, respectively) and was selected for further studies. SW620 and HT29 cells were treated with compound **7g** at different concentrations (0, 12.5, 25, 50, 100, 200 μM) and evaluated for viability at different time points (12, 24, 48 and 72 h). The results indicate that **7g** inhibits the growth of SW620 and HT29 cells in a time and concentration dependent manner (Fig. 1A).

To investigate the effect of proliferative inhibition of compound **7g** on SW620 and HT29, immunofluorescent analysis was conducted. As shown in Fig. 1B–D, immunofluorescence confirmed that **7g** inhibited the proliferation of SW620 and HT29 in a concentration-dependent manner. Subsequently, **7g** was evaluated in a colony formation assay to determine effects on clonogenicity of SW620 and HT29 cells. Colony size and numbers were dramatically reduced after exposure to compound **7g** for two weeks (Fig. 1E).

Table 1
Structures, yields and chemical properties of fused benzoimidazole-isoquinolines **7a–n**.



Entry	R_1	R_2	Comp.	Yield (%)[a]	CLogP	tPSA
1	Cyclopropyl	2-thienyl	**7a**	82	3.799	52.65
2	*i*Bu	2-thienyl	**7b**	52	4.8955	52.65
3	*i*Bu	2-furanyl	**7c**	72	4.0715	61.88
4	2-BrC_6H_4	2-furanyl	**7d**	63	7.5791	52.65
5	2-BrC_6H_4	Acetyl	**7e**	75	4.672	52.65
6	2-BrC_6H_4	3-pyridyl	**7f**	71	5.5395	65.01
7	*i*Bu	3-pyridyl	**7g**	72	3.7945	65.01
8	*i*Bu	2-$COOHC_6H_4$	**7h**	76	4.9034	89.95
9	Bzl	Ph	**7i**	62	6.0965	52.65
10	Ph	Ph	**7j**	71	6.2375	52.65
11	Phenethyl	4-pyridyl	**7k**	67	4.4345	65.01
12	H	Ph	**7l**	66	3.8747	61.44
13	2-BrC_6H_4	2-furanyl	**7m**	76	5.8165	61.88
14	2-BrC_6H_4	methyl	**7n**	78	3.5115	52.65

[a] Isolated yield (%) from the one-pot procedure.

Table 2
GI_{50} of BIDs against colorectal cancer cell lines.

Entry	Comp.	GI_{50} (μM)[a]	
		SW620	HT29
1	**7a**	47.60 ± 3.76	51.30 ± 5.29
2	**7b**	44.05 ± 7.37	49.52 ± 6.86
3	**7c**	53.46 ± 4.63	52.80 ± 5.90
4	**7d**	46.03 ± 4.61	43.73 ± 7.59
5	**7e**	46.89 ± 7.16	57.20 ± 5.25
6	**7f**	57.87 ± 5.25	61.27 ± 7.42
7	**7g**	**23.78 ± 4.06**	**24.13** ± 3.88
8	**7h**	52.70 ± 5.94	59.80 ± 6.65
9	**7i**	69.70 ± 7.14	74.30 ± 4.32
10	**7j**	> 100	> 100
11	**7k**	48.90 ± 6.06	52.30 ± 5.82
12	**7l**	> 100	> 100
13	**7m**	> 100	> 100
14	**7n**	> 100	> 100

[a] Each GI_{50} value was calculated from 3 independent experiments conducted in sextuplicate.

2.1.3. Compound *7g* induces G_2/M cell cycle arrest of human colorectal cancer cells by decreasing cyclin B1 and CDK1

In order to explore the effects on cell cycle progression, SW620 and HT29 cells were treated with compound **7g** at four concentrations (0, 25, 50, 75 μM) for 48 h and then analyzed by flow cytometry. The average G_2/M proportions of SW620 and HT29 increased in a concentration-dependent manner. However, there was no significant difference in either cell line at lower concentrations. These results indicate that compound **7g** can induce cell cycle arrest of CRC cells at the G_2/M checkpoint.