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Research paper

Synthesis and cytotoxic studies of novel 5-phenylisatin derivatives and their anti-migration and anti-angiogenic evaluation

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ABSTRACT

A number of 5-arylisatin derivatives were synthesized in 5–6 steps from readily available starting materials. Their structures were confirmed by ^{1}H NMR and ^{13}C NMR as well as LC/MS. The cytotoxicity of these novel isatins against human leukemia K562 cells were evaluated by MTT assay *in vitro*. SAR studies indicated that the N-substituted benzyl and C-5 substituted phenyl groups greatly enhance their cytotoxic activity, whereas an intact carbonyl functionality on C-3 present in the parent ring is required to maintain such a potency. Particularly, N-(p-methoxybenzyl)-5-(p-methoxyphenyl)isatin (compound **2m**) showed the highest antitumor activity against K562 cell lines ($IC_{50} = 0.03\ \mu M$). Moreover, treatment with compound **2m** significantly inhibited liver cancer HepG2 cells proliferation and migration, which could also reduce the human umbilical vein endothelial cells (HUVEC) tube formation. In conclusion, compound **2m** exhibited very good cancer cells proliferation inhibition by angiogenesis responses *in vitro*, and **2m** might be a promising angiogenesis inhibitor for cancer treatment.

1. Introduction

Isatins are a class of structurally versatile molecules and their core structure appears in many biologically active molecules and pharmaceutical agents. Isatin derivatives have been reported to possess a broad spectrum of activities such as anticancer [1,2], antidepressant [3], anticonvulsant [4], antifungal [5], anti-HIV [6] and anti-inflammatory [7], etc. In the last decade, N-substituted isatins have attracted increasing attention from both industry and academia. Vine et al. has reported that some N-benzyl isatin derivatives were more cytotoxic than their N-H counterparts toward some lymphoma cells as well as a series of human cancer cell lines, including human leukemic (K562, U937 and Jurkat), liver (HepG2), breast (MDA-MB-231 and MCF-7), prostate (PC-3) and colorectal (HCT-116) cell lines [8]. Chen's and Chiyanzu's groups reported that N-substituted isatin derivatives exhibited inhibition activities against SARS CoV 3CLpro and parasitic cysteine proteases [9,10]. Later on, Limpachayaporn and Liu found that 5-sulfonylisatin analogues could act as caspase-3,7 inhibitors and SARS-CoV 3C-like protease inhibitors [11,12]. In addition, Chinnasamy's group found that some N-1 and C-5 disubstituted molecules such as 1-(substituted benzylidene)-3-(1-(morpholino/piperidinomethyl)-2,3-dioxoindolin-5-yl)urea derivatives exhibited antiepileptic activity and neurotoxicity [13]. Previously, we reported some 5-(2-carboxyethenyl)isatin derivatives as anticancer agents, and found that the combination of a 5-[trans-2-(methoxycarbonyl)ethen-1-yl] group and a 1-(4-methoxybenzyl) group (compound **5**–**61**, Fig. 1) in isatin significantly enhanced it's cytotoxic activity [14]. However, further research on this molecule indicated that the presence of a metabolically unstable acrylic ester moiety on the C-5 position is the key to its in *vivo* efficacy. In order to further improve the antitumor property of this type of 1,5-disubstituted isatin molecules, we have synthesized a serious of isatin derivatives and studied their cytotoxicity. The results showed that although the substituent of 5-methacrylate could increase the antitumor activity of the target compound, it is not stable in metabolic processes (unpublished data). Therefore, we report a series of new compounds in this paper, which can maintain good anti-tumor efficacy even without the use of 5-methacrylate substituents. Among them, compound **2m** was shown as the most active compound against human leukemia K562 cells. Moreover, the molecular mechanism

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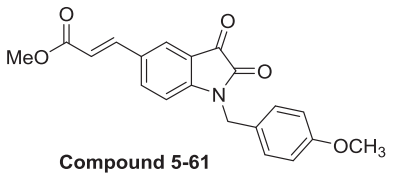


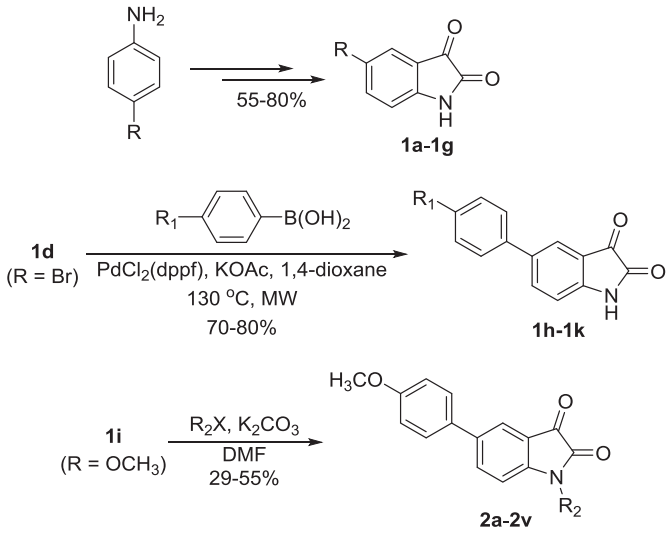
Fig. 1. The structure of 1,5-disubstituted isatin derivative (compound **5**–**61**).

of the cytotoxic activity of compound **2m** was explored on anti-migration and anti-angiogenesis.

2. Chemistry

Firstly, the target compounds were synthesized according to the procedures outlined in Scheme 1. Monosubstituted isatins **1a**-**1g** were obtained in two steps with a improved yield of 55–80% [15]. Moreover, 5-Bromoisatin (**1d**) was coupled with six different boronic acids by microwave-assistant Suzuki coupling reaction to afford compounds **1h**-**1k** in 70–80% isolated yield [16]. Secondly, N-alkylation of **1i** led to the synthesis of compounds **2a-2v** in good to excellent yield (75–90%). Thirdly, compound **3a-3c** were prepared from isatin derivatives by suzuki-coupling reaction (Scheme 2). Finally, compound **4a-4s** were prepared from 5-bromo-1-(4-methoxybenzyl)indoline-2,3-dione by Suzuki-coupling (Scheme 3).

Compounds **5a-5k** (Scheme 4) were prepared from **2m** in good yield in one or two steps. Compound **2m** was reduced with $NaBH_4$ in methanol under room temperature to afford compound **5a**. Compound **5b** was prepared by treatment of the compound **2m** with methylmagnesium bromide in tetrahydrofuran at −78 °C. Compound **2m** was reduced with hydrazine monohydrate in ethanol under reflux to afford compound **5c**. Compound **5d** and **5e** were prepared by treatment of the compound **2m** with (triphenylphosphoranylidene)acetate in tetrahydrofuran at room temperature. Compound **5f** was synthesized by treatment of the compound **2m** with ethylene glycol in toluene at 110 °C. Compound **5g** was prepared by treatment of the compound **2m** with diethylaminosulfur trifluoride in DCM at reflux. Compound **5h** was synthesized by treatment of the compound **2m** with hydroxylamine hydrochloride in ethanol at reflux. The compounds **5i-5k** were prepared by the treatment of **5c** with benzaldehyde or substituted benzaldehyde and pyridine in ethanol.



Scheme 1. Synthesis of isatin derivatives (**1a**-**1k**, **2a**-**2v**).

The data obtained from ^{1}H NMR, ^{13}C NMR and Mass Spectrometry confirmed the proposed structures (Spectral data results are provided in supporting information).

3. Results and discussion

When contemplating a new class of 1,5-substituted isatin structure that could avoid the metabolically liabile acrylic acid ester group found in compound **5**–**61** while maintaining its biological potency, we envisaged a general structure compound **5**–**61** (Fig. 1) [14]. It can bear a various of aromatic groups at the C-5 position, that carry some structural similarity in terms of their size and conjugation property, whereas the *in vivo* Michael addition liability of the 5-acrylic acid ester group could be eliminated.

As illustrated in Fig. 2, with the isatin as the core structure of our drug design, this strategy would allow us to start with the modification of the substitution pattern of the benzene ring, especially at C-5, followed by N-derivatization and then C-3 variation.

The *in vitro* antitumor activities of the 5-substituted isatins **1a-1k** against two human tumor cells K562 and HepG2 were first evaluated. As shown in Table 1, the cytotoxic activities of the 5-phenyl substituted compounds are far better than the parent nucleus. Among them, compound **1i** exhibited the highest cytotoxic activity against HepG2 cell lines ($IC_{50} = 0.96\ \mu M$).

After compound **1i** was identified as the potential lead, our SAR study was then focused on the N-derivatization based on the hypothesis that the combination of C-5 substitution with N-alkylation may further enhance their cytotoxic potency. A series of 5-(4-methoxyphenyl) N-substituted derivatives (Scheme 1) were screened for their *in vitro* cytotoxic activity.

As can be seen from the data in Table 2, introduction of simple aliphatic groups at N-1 (**2a-2c**) did not significantly enhance their potency. Moreover, a *p*-methoxyphenyl group at N-1 significantly diminished the potency. However, the introduction of benzyl groups into the N-1 position (**2d-2v**) resulted in much better inhibitory activities, with 10–300 folds improvement compared to that of **1i**, pointing to the tendency that the N-1 position needs a relatively sterically larger group with certain lipophilic property to increase the potency. Further examination of the structure difference and the cytotoxic data of the compounds **2d** and **2f-2v** against K562 showed that the substituted benzyl group did increase the potency. The improvement in potency does not seem to be related to the electron density of the substituent groups (**2f**, **2r** and **2h**, **2l**); however, the substituents' size (**2k**, **2p**) and location (**2n** and **2m**, **2o**) plays a more significant role. Moreover, the excellent cytotoxic activity for the compounds containing N-1 para or ortho mono methoxy benzyl group (**2m**, **2o**), dimethoxy benzyl group (**2q**) as well as a piperonyl group (**2v**) indicated that hydrogen bond receptor in this direction of the molecule could significantly enhance the potency (8–25 folds, compared to that of **2d**).

With the identification of the p-methoxybenzyl group as an optimal substituent at N-1, our attention then turned back to the p-methoxyphenyl substitution on the benzene ring. Specifically, we were interested in finding out how the location would influence the cytotoxic potency. For this purpose, a series of isatins bearing p-methoxyphenyl group(s) at various position on the benzene ring were synthesized and their cytotoxic activities were examined. The compounds **3a-3c** were synthesized following the procedure similar to that of **2m** (Scheme 2) and their potency were listed in Table 3. The 4-, 6- and 7-monosubstituted compounds **3a-3c** exhibited much weaker potency than that of **2m** (>220 folds), which may suggest that the N-1, C-5 disubstituted pattern in **2m** possess a much favorable 2-dimensional orientation toward the biological targets.

The SAR analysis of the compounds in Table 3 revealed that the