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Preliminary communication

Synthesis and anti-tumor activity of 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives *in vitro*

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ABSTRACT

A series of novel 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives were synthesized and their cytotoxic activity against A549, H460, HT-29 and SMMC-7721 cell lines was evaluated *in vitro*. Among them, ten compounds (**10**, **11**, **14**, **16**, **17**, **26**, **27**, **29**, **30** and **31**) displayed excellent anti-tumor activity against different cell lines. The most promising compound **27** showed strong anti-tumor activity against A549, H460, HT-29 and SMMC-7721 cell lines with IC_{50} values of 22, 0.23, 0.65 and 0.77 nM, which were 2.6-, 83-, 1.1×10^3- and 2.0×10^3- fold more active than MX-58151 (IC_{50} values of 0.058, 0.019, 0.70 and 1.53 μM), respectively.

1. Introduction

The mortality and morbidity of cancer patients are the second highest among all diseases in the world, after heart disease [1,2]. The demand for new lead compounds in this therapeutic area is higher than ever before. It has encouraged scientists to search for anti-tumor agents with novel chemical eternity and new model of action. 2-Amino-3-cyanopyridine derivatives are known to have multiple biological activities, such as anti-microbial [3], cardiotonic [4], anti-inflammatory [5] and anti-parkinsonism properties [6]. We are much interested in the anti-tumor activity of these compounds owing to their different types of mechanisms of action. As reported, several 4,6-diaryl-substituted and tricyclic 2-amino-3-cyanopyridines exhibited potent anti-tumor activity against human breast cancer cell lines T-47D and ZR-75-1 and significant inhibitory activity of mitogen activated protein kinase-activated protein kinase 2 (MK-2) in μM range [7–10]. Therefore, optimizations of 2-amino-3-cyanopyridines may lead to more active anti-tumor chemotherapeutics. Considerable attention has been focused on modification of 4,6-diaryl-2-amino-3-cyanopyridines. Generally, in these literatures, the aryl group at 6-position of pyridine is always phenyl or other single heteroaromatic ring including furan-2-yl, thiophen-2-yl and pyrrol-2-yl groups, and there are hardly any studies referring to compounds bearing a bicyclic core structure so far. In view of the attractive pharmacological activity present of indole and its analougues in several drugs [11,12], the bicyclic indole core was introduced into the 4,6-diaryl-2-amino-3-cyanopyridines to identify more potent anti-tumor agents.

Herein, we reported the synthesis and cytotoxic activity of 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives (Fig. 1) against A549, H460, HT-29 and SMMC-7721 cell lines *in vitro*.

2. Chemistry

3-Bromo-4,5-dimethoxybenzaldehyde was synthesized *via* the reaction of 5-bromovanillin with dimethyl sulfate (Scheme 1).

Due to the different substituents on the indole core, two methods were used to synthesize the 1-(1*H*-indol-3-yl)ethanones with the starting materials of their corresponding anilines and benzaldehydes. The general synthetic routes of substituted 1-(1*H*-indol-3-yl)ethanones is illustrated in Scheme 2. The substituted phenylhydrazine hydrochlorides (**1a**–**1b**) were prepared *via* a three-step reaction starting from the corresponding aniline. Substituted anilines were diazotized with sodium nitrite under acidic conditions, reduced by sodium bisulfite and acidified with hydrochloric acid to afford intermediates **1a**–**1b**. By a nucleophilic addition reaction of **1a**–**1b** with ethyl pyruvate, Schiff's bases ethyl

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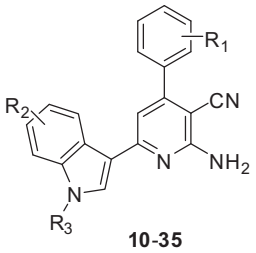


Fig. 1. Structures of target compounds **10**–**35**.

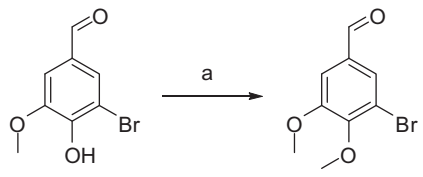
2-(2-phenylhydrazono)propanoates (**2a**–**2b**) were synthesized, which were then cyclized in PPA to afford the key intermediates ethyl 1*H*-indole-2-carboxylates (**3a**–**3b**). In an alternative approach, ethyl bromoacetate and sodium azide were heated to 63 °C in acetone to give ethyl 2-azidoacetate (**7**) as a colorless oil, which was then treated with substituted benzaldehyde using EtONa in ethanol to form the corresponding ethyl 2-azido-3-phenylacrylates (**8c**–**8d**) as yellow solids. Cyclization by heating in xylene then led to the required ethyl 1*H*-indole-2-carboxylates (**3a**–**3b**). Furthermore, hydrolysis of **3a**–**3d** yielded 1*H*-indole-2-carboxylic acids (**4a**–**4d**), which were decarboxylated to give 1*H*-indoles (**5a**–**5d**). Thereafter, substituted 1-(1*H*-indol-3-yl) ethanones (**6a**–**6d**) were successfully obtained *via* the reaction of **5a**–**5d** with glacial acetic acid and acetic anhydride, which were then reacted with iodomethane to afford the corresponding 1-(1-methyl-1*H*-indol-3-yl)ethanones (**9b**–**9e**).

Finally, the desired compounds were prepared in good yields *via* a one-pot reaction between a substituted benzaldehyde, 1-(1*H*-indol-3-yl)ethanone or 1-(1-methyl-1*H*-indol-3-yl)ethanone, malononitrile and ammonium acetate, according to methods previously described in the literature (Scheme 3) [13]. All target compounds were purified by column chromatography on silica gel. The chemical structures of target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR and MS spectra (Table 1).

3. Biological evaluation

All compounds, along with the reference compound MX-58151 [14], were tested for their *in vitro* cytotoxic activity against A549 (human non-small-cell lung cancer cell line), H460 (human lung cancer cell line), HT-29 (human colorectal cancer cell line) and SMMC-7721 (human liver cancer cell line) using MTT assay. The results expressed as IC_{50} were summarized in Table 2. The IC_{50} values are the average of three independent experiments.

As shown in Table 2, most of the prepared compounds exhibited moderate to strong cytotoxic activity against all four cancer cell lines. Among them, compounds **17** and **27** were superior to MX-58151 against all four cell lines, while compounds **10**, **11**, **26** and **31** were more active than MX-58151 against H460, HT-29 and SMMC-7721 cells. 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-6-(1-methyl-1*H*-indol-3-yl)nicotinonitrile (**27**), the most promising compound, showed strong anti-tumor activity against A549, H460, HT-29 and SMMC-7721 cell lines with IC_{50} values of 22, 0.23, 0.65 and 0.77 nM, which were 2.6-, 83-, 1.1×10^3- and 2.0×10^3- fold more active than MX-58151 (IC_{50} values of 0.058, 0.019, 0.70 and 1.53 μM), respectively.



Scheme 1. Reagents and conditions: (a) Dimethyl sulfate, K_2CO_3, acetone, reflux, 6 h.

Interestingly, the cytotoxic activity of compounds **10**–**35** against H460 and HT-29 cells was much higher than those against A549 and SMMC-7721. On the other hand, compounds **16**, **17**, **29** and **30** exhibited excellent selectivity for H460 cells, while compounds **10**, **11**, **14**, **26**, **27** and **31** followed by compounds **17**, **13** and **30** were the most potent of the tested compounds against HT-29 cells. Furthermore, compound **27** was further far stronger against SMMC-7721 cell line than others.

The present study investigated the effect of several substituents on indole ring and benzene ring. In most cases, compounds with methyl group at the 1-postion of indole (**26**–**35**) displayed slightly higher cytotoxic activity than those of the corresponding compounds without substituent at the same position (**10**–**12**, **15**–**18** and **22**–**24**). Nevertheless, introduction of 5,6,7-trimethoxy group to indole (**21**–**25**, **33**–**35**) led to a dramatic decrease in anti-tumor activity. It can be concluded that halogen or no substituent on indole ring was benefit for cycotoxicity. The results suggested that the anti-tumor activity might be impacted by the size or electronic effects of substituents on indole ring. The position of substituents on indole ring also played an important role on the pharmacological activity. Generally, substituents at the 5-position (**15**–**17**, **29**) were preferred in comparison to those at the 6-position (**18**–**20**, **32**), particularly in terms of their activity against H460 cells. On the other hand, the trisubstituted phenyl groups were chosen to study the effect of substituents on benzene ring, including 2,3,4-trimethoxyphenyl, 3,4,5-trimethoxyphenyl and 3-bromo-4,5-dimethoxyphenyl. It was obvious that the cytotoxic activity was improved by replacement of the methoxy group at the 3-positon with a bromo group (**10** *vs* **11**, **15** *vs* **16**, **18** *vs* **19**, **26** *vs* **27**, **29** *vs* **30**).

4. Conclusion

In summary, a series of 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives were synthesized and screened for their cytotoxic activity against four human cell lines (A549, H460, HT-29 and SMMC-7721). Among them, ten compounds (**10**, **11**, **14**, **16**, **17**, **26**, **27**, **29**, **30** and **31**) displayed excellent anti-tumor activity against different cell lines. The most promising compound **27** showed potent anti-tumor activity against A549, H460, HT-29 and SMMC-7721 cell lines with IC_{50} values of 22, 0.23, 0.65 and 0.77 nM, which were 2.6-, 83-, 1.1×10^3- and 2.0×10^3- fold more active than MX-58151 (IC_{50} values of 0.058, 0.019, 0.70 and 1.53 μM), respectively. The pharmacological data indicated that introduction of indole core improved the anti-tumor activity of the 4,6-diaryl-2-amino-3-cyanopyridines. From the preliminary structure activity relationships, we may conclude that introduction of methyl group to the 1-postion of indole slightly enhance the cytotoxic activity, while halogen or no substituent on indole ring was favorable in this region. Furthermore, introduction of halogen groups into benzene ring was essential for their cytotoxic activity and the 3-bromo-4,5-dimethoxy group was the best of 3,4,5-trisubstituted groups. Studies on the mechanism of action of these compounds are in progress and will be reported upon in the future.

5. Experimental protocols

5.1. Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent