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1*H*-Pyrazolo[3,4-*b*]quinolin-3-amine derivatives inhibit growth of colon cancer cells *via* apoptosis and sub G1 cell cycle arrest

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ABSTRACT

A series of 1*H*-pyrazolo[3,4-*b*]quinolin-3-amine derivatives were synthesized and evaluated for anticancer efficacy in a panel of ten cancer cell lines, including breast (MDAMB-231 and MCF-7), colon (HCT-116, HCT-15, HT-29 and LOVO), prostate (DU-145 and PC3), brain (LN-229), ovarian (A2780), and human embryonic kidney (HEK293) cells, a non-cancerous cell line. Among the eight derivatives screened, compound **QTZ05** had the most potent and selective antitumor efficacy in the four colon cancer cell lines, with IC_{50} values ranging from 2.3 to 10.2 μM. Furthermore, **QTZ05** inhibited colony formation in HCT-116 cells in a concentration-dependent manner. Cell cycle analysis data indicated that **QTZ05** caused an arrest in the sub G1 cell cycle in HCT-116 cells. **QTZ05** induced apoptosis in HCT-116 cells in a concentration-dependent manner that was characterized by chromatin condensation and increase in the fluorescence of fluorochrome-conjugated Annexin V. The findings from our study suggest that **QTZ05** may be a valuable prototype for the development of chemotherapeutics targeting apoptotic pathways in colorectal cancer cells.

Cancer still remains the leading cause of human deaths worldwide, with 8.2 million reported deaths (around 13% of all deaths) in 2012.[1] Despite an increasing understanding of the molecular biology of cancer and the consequent increase in the development of anticancer compounds, most of the chemotherapeutic drugs currently used for treating many types of cancer are often rendered ineffective due to the development of drug resistance during treatment.[2] Furthermore, treatment may be discontinued as a result of drug-related toxicities.[2] Therefore, there is a continuous need for the development of novel chemotherapeutic drugs for cancer therapy that can surmount these aforementioned limitations.

Functionalized quinolines and their hetero-fused analogues represent an important class of organic molecules and they are present in numerous natural products.[3,4] Pyrazoloquinolines, which have quinoline and pyrazole moieties in their molecular framework, are an excellent example of this class of compounds that have a broad spectrum pharmacological efficacies.[5–13] Notably, we are interested in the antitumor efficacy of pyrazoloquinolines, which is mediated by inhibition of topoisomerase II,[7] CHK1 kinase,[14] DNA-dependent protein kinase,[15] Oncogenic *Ras* protein[16] and by induction of apoptosis.[17]

During the course of our investigations on polycyclic quinolines with antitumor efficacy, we reported the discovery of IND-2, a pyrimido [1″,2″:1,5]-pyrazolo[3,4-*b*]quinoline (Fig. 1) with potent cytotoxic and apoptosis-inducing properties in colon cancer cells.[18] Thus, in this study, we chose to evaluate 1*H*-pyrazolo[3,4-*b*]quinolin-3-amines, the major substructural unit and synthetic precursor of IND-2, for their anticancer efficacy (Fig. 1). Eight pyrazolo[3,4-*b*]quinolin-3-amine derivatives (Fig. 1), bearing different substituents on the fused benzo ring, were synthesized and their anticancer efficacy determined in a panel of 10 cancer cell lines.

The synthesis of 1*H*-pyrazolo [3,4-*b*]quinolin-3-amines was accomplished, using a three-step protocol, as shown in Scheme 1. The first step involved the synthesis of the starting material, 2-chloroquinoline-3-carbaldehydes (**2a–2h**), from suitable acetanilides (**1a–1h**). This was

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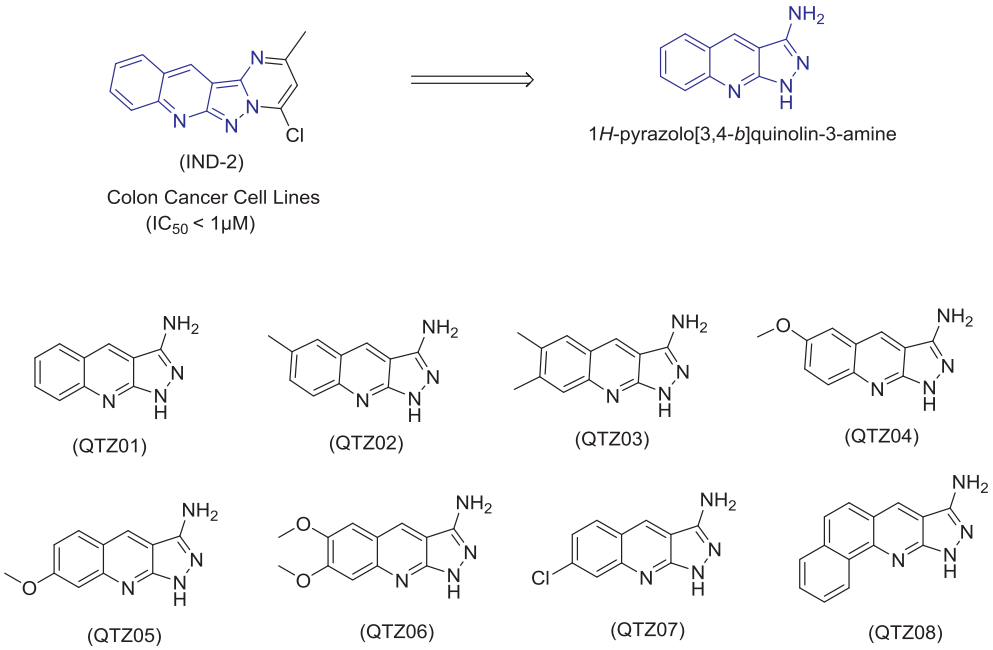
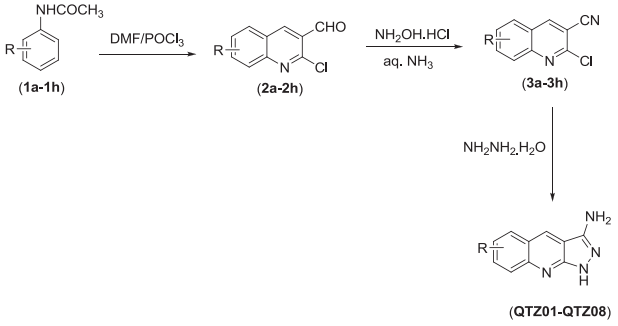


Fig. 1. Design strategy and eight 1*H*-pyrazolo[3,4-*b*]quinolin-3-amines reported in this study.



Scheme 1. Synthesis of l*H*-pyrazolo[3,4-*b*]quinolin-3-amine derivatives.

followed by subsequent conversion to the corresponding 2-chloroquinoline-3-carbonitriles (**3a–3h**), using well-established procedures as described previously.[18–20] The obtained 2-chloroquinoline-3-carbonitriles on addition with hydrazine hydrate under reflux conditions produced 1*H*-pyrazolo[3,4-*b*]quinolin-3-amines in good yields (**QTZ01–QTZ08**).[8]

The IR spectra of the synthesized 1*H*-pyrazolo[3,4-*b*]quinolin-3-amines displayed two characteristic peaks for the NH and NH_2 groups in the region 3424–3325 cm^{-1} and 3300–3256 cm^{-1}, respectively. In the ^1H NMR spectra, the signal for NH and NH_2 appeared as singlets at δ 11.24–12.5 ppm (D_2O exchangeable) and δ 5.52–5.96 ppm (D_2O exchangeable), respectively. The ^1H NMR spectra of compounds also showed a singlet in the region δ 8.28–8.72 ppm due to the presence of the C13 proton of the 1*H*-pyrazolo[3,4-*b*]quinoline ring. Further, the presence of singlet, doublet, triplet and multiplets at around δ 6.77–8.03 ppm in the compounds indicated aromatic protons in the benzo ring of the pyrazolo[3,4-*b*]quinolinyl moiety. The structures assigned for the compounds were also supported by the elemental analysis and mass spectral data.

The cytotoxicity of synthesized compounds was determined in a panel of ten cancer cell lines, including breast (MDAMB-231 and MCF-7), colon (HCT-116, HCT-15, HT-29 and LOVO), prostate (DU-145 and PC3), brain (LN-229), ovarian (A2780), and a non-cancerous cell line, human embryonic kidney (HEK293) (Table 1). The cytotoxicity was determined by MTT assay using a series of different concentrations for each compound was used (0, 0.1, 0.3, 1, 3, 10, 30, and 100 μM). The results are expressed as IC_{50} and are summarized in Table 1.

According to the cytotoxicity data in Table 1, the compounds **QTZ01**, **QTZ05**, **QTZ06** and **QTZ08** displayed variable efficacy on the inhibition of the growth of the human tumor cell lines HCT-116, HCT-15, HT-29, LOVO, LN-229 and A2780. Compound **QTZ05**, with a 7-OCH_3 substituent in the benzo ring of the 1*H*-pyrazolo[3,4-*i*]quinolin-3-amine moiety, was the most potent in the series. **QTZ05** had broad spectrum cytotoxic efficacy in colon cancer cells, with IC_{50} values ranging from 2.3 to 10.2 μM. **QTZ05** also displayed excellent selectivity for colon cancer cells compared to non-cancerous HEK293 cells, with 20, 5, 11 and 4-fold greater selectivity for HCT-116, HCT-15, HT-29 and LOVO cells, respectively (Table 1).

Fig. 2 illustrates the cytotoxicity of **QTZ05** in colon cancer cells compared to normal cells. Furthermore, **QTZ05**, at 7.6 μM, also significantly inhibited the growth of the brain cancer cell line. **QTZ01**, the unsubstituted derivative, also inhibited LN-229 cells at concentration similar to that of **QTZ05**. However, neither **QTZ05** nor **QTZ01** had significant cytotoxic efficacy in other cancer cell lines (Table 1). Compound **QTZ06**, with a 6,7-dimethoxy substitution, had moderate cytotoxic efficacy against the colon cancer cell lines (HCT-15 and HT-29 (IC_{50} < 20 μM) and A2780 ovarian cancer cells (IC_{50} = 19.6 μM) (Table 1). In contrast, compound **QTZ08**, a benzo fused derivative, had selective and moderate growth inhibitory efficacy in A2780 cancer cells (IC_{50} = 13.6 μM) (Table 1).

Overall, the results of the MTT assay indicates that compound **QTZ05** was the most promising candidate for further mechanistic investigations in terms of its potent cytotoxicity on colon cancer cell lines.

First, we determined the cytotoxicity of **QTZ05** on HCT116 cells over time. The cytotoxicity of different concentrations of **QTZ05** (0, 10, 20, and 30 μM) at different times (every 15 min up to 72 h) was