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Design, synthesis and biological evaluation of novel 1,3,4-trisubstituted pyrazole derivatives as potential chemotherapeutic agents for hepatocellular carcinoma

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ABSTRACT

A series of novel 1,3,4-trisubstituted pyrazole derivatives were synthesized and evaluated for their cytotoxic activity against three different cancer cell lines namely HCT116, UO-31 and HepG2. Compounds **3b**, **3d**, **7b** and **9** showed excellent anticancer activity against all the tested cancer cell lines and had better cytotoxic activities than the reference drug, Sorafenib. Therefore, these compounds were chosen to be further evaluated in a panel of HCC cell lines. Among them, **3b** and **7b** were the most active compounds against HCC cells used here. Further studies on the mechanism demonstrated that **3b** and **7b** induced apoptosis in addition to induction of cell cycle arrest at G2/M phase in HepG2 and Huh7 cells. Consistent with these results, caspase-3 assay was done and the results revealed that the pro-apoptotic activity of the target compounds could be due to the stimulation of caspases-3. In addition, CDK1 inhibition assay was done and it was found that compounds **3b** and **7b** inhibited CDK1 activities with IC_{50} values of 2.38 and 1.52 μM, respectively. Finally, pyrazole derivatives **3b** and **7b** showed potent bioactivities, indicating that these compounds could be potent anticancer drugs in the future.

1. Introduction

Cancer is one of the main causes of death worldwide. It is the second leading cause of death after cardiovascular diseases [1]. Hepatocellular carcinoma (HCC), one of the most common cancers, is considered as the second major cause of cancer-related death. Though the treatment strategies have been largely improved, the prognosis of HCC patients is still unoptimistic [2]. Globally, the death rate from all other common cancers (such as lung, breast and prostate cancers) is declining, whereas the mortality rate from liver cancer is increased by 2.8 and 3.4 percentages per year in men and women, respectively [3]. At an advanced stage, Sorafenib, a multikinase inhibitor, is the only drug approved by FDA for the treatment of HCC which can only prolong patient survival for a short period of time (2–3 months) [4]. Multidrug resistance of cancer cells to conventional chemotherapy is the main cause of the HCC chemotherapy failure. Therefore, the development of novel anticancer agents is of great significance.

Cell cycle deregulation is frequently accompanied by altered CDKs activity in many cancers. CDKs are one of the serine-threonine kinases involved in the regulation of cell cycle in the liver. They bind to their partner proteins (cyclins) in order to phosphorylate the Rb protein which helps in the release of transcription factors to ensure cell cycle transition. The alterations in the activities of CDKs lead to the transformation of normal cells toward cancerous state [5,6]. Therefore, design and synthesis of therapeutic agents targeting CDKs have been the subject of recent drug discovery research studies and a number of CDK inhibitors have been considered in several clinical trials [7]. On the other hand, apoptosis is an important biological phenomenon in tissue homeostasis. The inactivation of apoptosis is important in cancer progression and development of drug resistance [8]. The role of apoptotic cell death in response to chemotherapy is also of great importance [9].

Pyrazoles constitute an important heterocyclic family that has been found to possess significant antitumor activity with excellent IC_{50} [10]. As shown in Fig. 1, pyrazole-containing drugs Ruxolitinib [11] and Crizotinib [12] were approved for the treatment of non-small-cell lung cancer and myelofibrosis, respectively. Studies concerning structure–activity relationships have shown that both the skeleton of pyrazole and the type of peripheral substituents play an important role in its biological effects [13]. Among the reported studies, a series of 1,3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives [14] was synthesized and evaluated for their cytotoxic activity. From these compounds, compound **I** showed potent cytotoxic efficacy against MCF-7 cell line (GI_{50} = 0.13 μM). It caused cell

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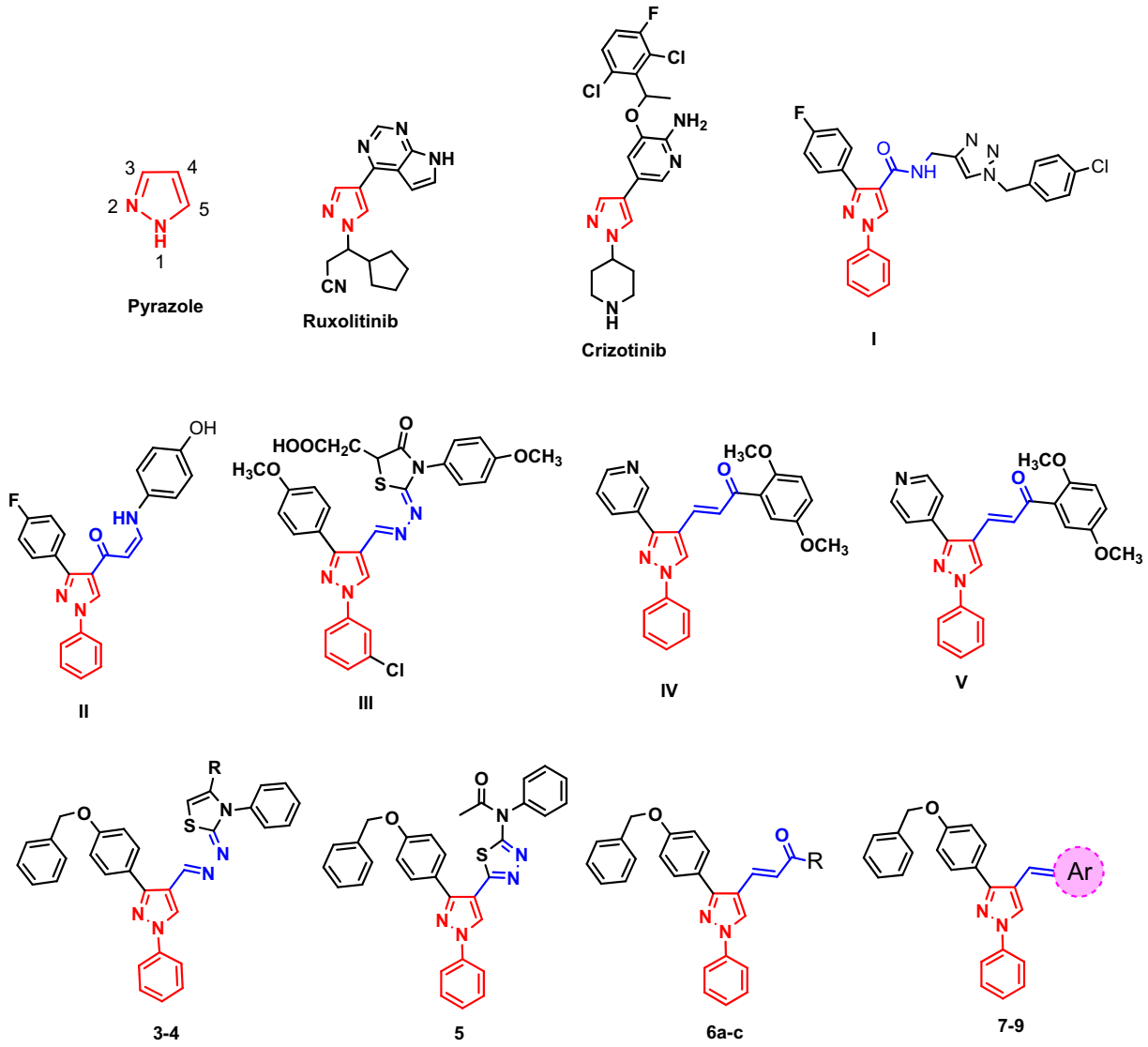

Fig. 1. Structure of the lead anticancer pyrazole derivatives and the designed target oyrazoles 3–9.

cycle arrest in the G2/M phase through reduction of CDK1 expression levels in addition to induction of apoptosis. In another study, Reddy *et al* revealed that compound **II** had potent growth inhibition with IC_{50} (A549) 1.50 μM. Flow-cytometry analysis showed that compound **II** led to G2/M cell cycle arrest and induction of apoptosis [15]. Moreover, Nossier et al. [16] reported the *in vitro* cytotoxic activities of some substituted thiazolidinonyl and thiazolyl pyrazole derivatives, compound **III** has the most potent activity with IC_{50} 0.2 μM against HepG2 and 3.1 μM against PC-3. Recently, pyrazolic chalcone derivatives **IV** and **V** were reported to have antiproliferative activity with IC_{50} values smaller than 5 μM against some HCC cells. They caused cell cycle arrest at G2/M phase followed by the apoptotic cell death [7].

Based on these findings and with the attempt to find potent anticancer agents, the synthesis of a series of pyrazole derivatives was adopted and evaluated as anticancer agents. In the present work, we report the synthesis and biological activities of novel series of 1,3,4-trisubstituted pyrazoles **2**–**9** in which; (*i*) phenyl is attached to the pyrazole ring at position 1; (*ii*) benzyloxyphenyl moiety is attached to the central pyrazole ring at position 3 and (*iii*) different substituents are attached to the pyrazole ring at position 4.

2. Results and discussion

2.1. Chemistry

The target compounds were synthesized according to the steps outlined in Schemes 1 and 2. Condensation of 3-(4-(benzyloxy) phenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**1**) with phenyl thiosemicarbazide in absolute ethanol [17] gave the corresponding thiosemicarbazone derivative **2**. The structure of **2** was well established from its micro-analytical and spectral data. The IR spectrum revealed the appearance of two NH bands at 3147 and 3296 cm^{-1} with a concomitant disappearance of the C=O band of the parent **1**. Its ^1H NMR spectrum (DMSO d_6) showed singlet at δ 8.30 ppm for CH=N proton and two singlet signals at δ 9.78 and 11.71 ppm corresponding to two NH protons (exchangeable with D_2O). Cyclization of **2** either by phenacyl bromides or methyl bromoacetate according to a reported method [18,19] furnished the novel thiazole derivatives **3a-d** and **4**, respectively. Elemental and spectral analyses of **3a-d** and **4** were in agreement with their structures. ^1H NMR spectrum of **3b** showed singlet at δ 2.47 ppm corresponding to methyl protons. In addition, its mass spectrum represented the molecular ion peak M^+ at *m*/*z* 617 (26.65%). Compound **4**