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Azole-hydrazone derivatives: Design, synthesis, *in vitro* biological evaluation, dual EGFR/HER2 inhibitory activity, cell cycle analysis and molecular docking study as anticancer agents

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ABSTRACT

In this research, three series of azole-hydrazone derivatives namely, benzimidazole, benzoxazole and benzothiazole were designed and synthesized. Their structures were confirmed by elemental analysis and spectroscopic techniques. Stereochemical configuration of the synthesized compounds (*Z*/*E*) was determined. The new derivatives were tested *in vitro* against both human breast adenocarcinoma (MCF-7) and human hepatic adenocarcinoma (HepG2) cell lines. The most active compounds **3h** (IC_{50} = 0.067 μM against MCF-7) and **3l** (IC_{50} = 0.027 μM against HepG2) were further tested for Epidermal Growth Factor Receptor (EGFR) inhibitory activity. The most active **3h** on EGFR was then screened for HER2 and VEGFR enzymes. Caspase-3/9 protein level expression were measured for the two compounds **3h** and **3l**. Cell cycle analysis showed pre G1 apoptosis and cell cycle arrest at G2/M phase. Up-regulation of Bax and down-regulation of Bcl-2 protein expression level confirmed apoptosis. Molecular docking analysis was performed for all the synthesized compounds inside the active site of EGFR.

1. Introduction

Cancer is a lethal disease especially in developed countries. In 2030, it is expected that, the mortality rate will be increased to be 13.1 million deaths [1]. The risk of cancer disease may affect people at all ages and tends to be increased with age. Such a killer disease is caused by abnormalities in the genetic material of cells. Cancer cells are characterized by three main properties, uncontrolled proliferation, lake of differentiation and a capability to invade many tissues in other locations in body (metastasis) [2].

There is always a real challenge for chemists and oncologists with cancer chemotherapy and antitumor agents. This is due to the non-selectivity, acute toxicity and cellular drug resistance of many anticancer agents. So, there is a continuing need for designing and developing new chemotherapeutic agents for cancer treatment [3]. Receptor tyrosine kinases (RTKs) – cell surface receptors bind to polypeptide growth factors such as cytokines and hormones – play a critical role in the development and progression of many types of cancer [4].

The millstone in the control of cellular proliferation is protein tyrosine kinases. Many of transforming oncogenes (e.g.: Src and Abl) possess tyrosine kinase activity. Moreover, the response of many cells to growth factors is initiated by RTKs activation [5].

Overexpression of certain RTKs (e.g.: the epidermal growth factor (EGF) receptor tyrosine kinases) shows an inverse correlation with survival especially in breast, colon and bladder cancers. Thus, inactivation of specific TKs in certain cancers, represent a potential strategy for designing new antiproliferative drugs [6].

Many research articles reported that 4-anilinoquinazolines (erlotinib and gefitinib) were the first inhibitor of EGFR and compounds containing benzothiazole core could also act as EGFR-TK inhibitors *via* competing with ATP for binding at the catalytic domain of EGFR-TK [6–9].

Moreover, it was found that flavones, isoflavones, genistein and quarecetin are ATP-competitive inhibitors at the ATP-binding site of kinases [10–12]. From a comparison between polyhydroxyla ted-2-phenybenzothiazoles and the adenine part of ATP, it was suggested that substituted benzothiazoles might mimic the ATP-competitive binding of genisitein and quarecetin at TKs [2]. As a result, 2-(4-aminophenyl)benzothiazoles (CJM 126) represent a new important class of antitumor agents against human breast cancer cell lines [13–15]. On the other hand, benzimidazole derivatives are endowed with a wide biological activities especially antitumor activity against a variety of cancer cell lines. Thus, albendazole (methyl-5-propylthio-1*H*-benzimidazole-2-yl carbamate)- an

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anthelmintic drug- can also inhibit cell proliferation in hepatocellular carcinoma *in vitro* and *in vivo* [16].

Schiff bases of benzothiazole and benzimidazole or hydrazine derivatives of benzoxazole, for example, 2-[(4-substituted methyl benzylidine)hydrazine]-benzoxazoles (**I**) act as anticancer agents [17–19].

In the quest to find better anticancer agents, and as a continuation of our previous studies on anticancer screening [20–26], we kept in mind: (1) the biological importance of benzothiazoles and isosteric benzoxazoles and benzimidazoles as EGFR-TK inhibitors (competitive inhibitor to ATP for EGFR active site) and their widely known anticancer agents, (2) as well as benzohydrazide, with their anticancer activity against several cell lines. We designed and synthesized a new family of compounds containing 4-aminophenyl-2-benzothiazole/imidazole or oxazole skeleton with phenyl spacer (to mimic compound CJM 126) and attached to benzohydrazide derivatives aiming at finding new potent anticancer agents. All the synthesized compounds were *in vitro* evaluated for antitumor activity against both human adenocarcinoma (MCF-7) and hepatic carcinoma (HepG2) cell lines. To explore the possible anticancer mechanism of the synthesized compounds, EGFR, HER2 and VEGFR enzymatic assays were screened. Molecular docking study of the synthesized compounds inside the active site of EGFR enzyme was done. Cell cycle analysis and apoptosis through Caspase-3 and Caspase-9 expression levels were performed. Gene expression of Bax and Bcl-2 was also determined (see Fig. 1).

2. Results and discussion

2.1. Chemistry

The starting materials **1a-c** [27,28], the key intermediates **2a-c** and the new synthesized compounds **3a-m** were depicted in Scheme 1. The substituted phenyl hydrazine hydrochlorides **2a-c** were prepared by diazotization of aromatic amines of benzimidazole, benzoxazole and benzothiazole derivatives **1a-c** followed by reduction with acidic stannous chloride. The structure of compounds **2a-c** was confirmed by IR spectra which displayed peaks at 3435–3204 cm^{-1} indicating NH and NH_2. ^1H NMR of hydrazine compounds **2a-c** showed the appearance of D_2O exchangeable peaks corresponding to NH and NH_2 protons in the aromatic region.

Condensation of substituted phenyl hydrazine hydrochlorides **2a-c** with equimolar amounts of different aromatic aldehydes in absolute ethanol afforded the target compounds **3a-m**. The structure of compounds **3a-m** was investigated by elemental and spectral analyses. The IR spectra revealed NH peaks in the range 3467–3200 cm^{-1}. ^1H NMR of compounds **3a-m** confirmed the proposed structure by the presence of single D_2O exchangeable peaks at δ 9.76–11.38 ppm corresponding to NH protons in addition to another singlet peak at δ 7.69–8.28 ppm indicating azomethine proton (N=CH). Additional D_2O exchangeable peaks at δ 9.76–9.88 ppm appeared in ^1H NMR spectrum of compounds **3a, 3f** and **3k** corresponding to phenolic OH protons. ^1H NMR of **3d** and

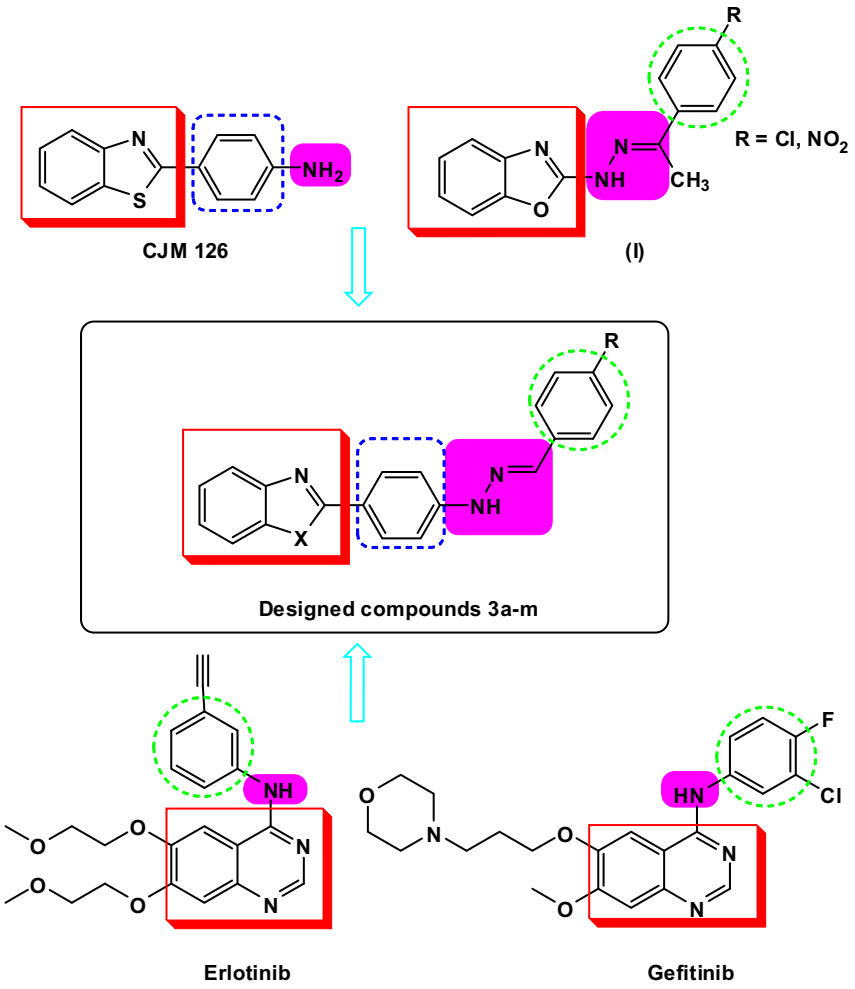

Fig. 1. Reported antitumor agents and the new designed compounds **3a-m.**