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Research paper

Synthesis and pharmacological evaluation of some new fluorine containing hydroxypyrazolines as potential anticancer and antioxidant agents

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ABSTRACT

Breast cancer is probably the most prevalent cancer in women. The development of resistance to therapeutic agents and lack of targeted therapy for breast cancer cells provide motivation to identify new compounds for the treatment. With this objective in mind, a new series of 3-fluoro-4-methoxyphenyl group based 1,3,5-trisubstituted aryl-5-hydroxypyrazoline analogues **4a–l** was synthesized through multi-step reaction sequence. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, LC-MS and elemental analysis. They were screened for their *in vitro* anticancer and *in vitro* antioxidant activities. Among the tested compounds **4h, 4c** and particularly **4i** displayed promising cytotoxic effect on breast cancer cell lines. The compounds were also found to possess antioxidant activity when tested against DPPH free radical. Overall, this work has contributed to the development of promising leads for anticancer and antioxidant activities.

1. Introduction

Cancer, the uncontrolled, rapid and pathological proliferation of abnormal cells, is among the major worldwide health problems. Despite significant advances in diagnostic and therapeutic techniques, it is now the second major fatal ailment liable for 8.2 million deaths in 2012 [1]. Breast cancer is one of the most commonly diagnosed cancers, accounting for ~20% of all malignancies worldwide and over half a million women develop breast cancer every year. In India, almost 100,000 women are diagnosed with breast cancer every year and a rise to 131,000 cases is predicted by 2020 [2,3]. In general, breast cancer is broadly classified as an endocrine receptor (i.e., estrogen receptor or progesterone receptor), positive or negative. Numerous studies have revealed that estrogens are predominantly active in the initiation and proliferation of breast cancer. Many women ultimately develop metastatic breast carcinoma, which is essentially an incurable disease and the prognosis has changed little over the past decade [4]. Among all the current therapeutic methods, chemotherapy has become one of the most significant treatment modalities in cancer management. Many current breast cancer chemotherapy treatments are often associated with side effects and the development of drug resistance in cancer cells, whereby majority of the patients succumb to their disease within 2 years of diagnosis. Hence, there is a great need for novel small molecules with the potential to effectively manage the different breast cancer subtypes [5–7].

In the last few decades, research has been focussed on chemically synthesized or natural product derived compounds as anticancer entities. Heterocyclic ring systems have emerged as powerful scaffolds for many biological evaluations. Heterocyclic compounds provide scaffolds on which pharmacophores can arrange to yield potent and selective drugs [8]. Triaryl substituted heterocyclic class of compounds represented by structure **A** (Fig. 1) have attracted considerable interest in the development of potential anticancer agents. Pyrazole and pyrazoline are prominent structural motifs found in numerous antitumor agents [9]. Several 1,3,5-triaryl-4-alkyl-pyrazoles have been evaluated as breast cancer treatments with the goal of reducing both toxicity and increasing

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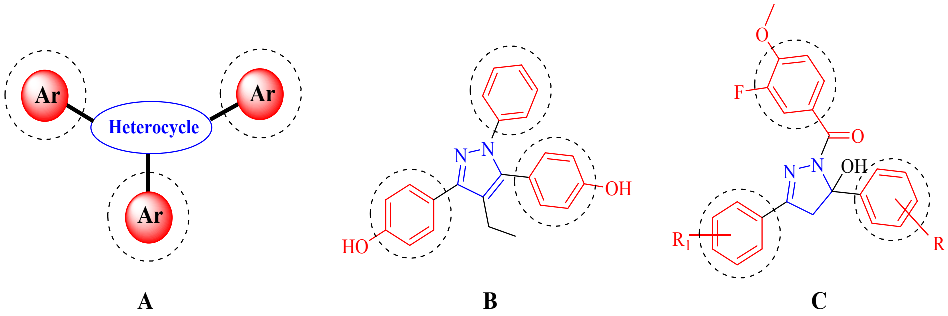

Fig. 1. Structural relatedness of hydroxypyrazolines (**C**) and triaryl substituted pyrazole (**B**) analogues with the triaryl substituted heterocyclic model (**A**).

the response rate as an antitumor agent [10]. Ying Huang and John Katzenellebogen found that triaryl-substituted pyrazole represented by structure **B** (Fig. 1), was good ligand for ER [11]. Since the development of triaryl substituted heterocyclic template has formed the backbone of a multitude of non-steroidal estrogen agonists as well as antagonists.

Numerous studies have demonstrated that in addition to cancer, oxidative stress on the cell has increased dramatically. The oxidative stress reflects an imbalance between the oxidants and the antioxidant favoring the oxidants implies damage of all essential bio-compounds like proteins, DNA and membrane lipids and can result in cell death [12]. There is increasing evidence showing the involvement of oxidative stress induced by free radicals and reactive oxygen species (ROS) in a variety of diseases and pathophysiological events including inflammation, cancer, myocardial infraction, arthritis and neurodegenerative disorders [13]. Antioxidants can minimize or inhibit the oxidative damage by interrupting the free radical formation or terminating the chain reaction. Antioxidants may slow or possibly prevent the development of the above mentioned diseases [14,15]. On the other hand, many chemotherapeutic agents act by producing free radicals, causing oxidative stress in normal cells [16]. A mono therapy of an anticancer drug with antioxidant properties will probably become more advantageous from the pharmaco-economic point of view.

Among the nitrogen heterocycles, pyrazoline and their derivatives are acknowledged to possess a wide range of bioactivities [17]. Pyrazolines are used as anticancer [18,19], antioxidant [20], antitumor [21], anti-inflammatory [22], trypanocidal [23], MAO-B inhibitors [24], agonist of cannabinoid receptors [25] and antidepressant agents [26]. Therefore, the pyrazoline motif makes up the core structure of numerous biologically active compounds. Many fluorinated organic molecules often exhibit remarkable physical and biological properties which originate from the C–F bond with a wide range of applications [27]. Around 20% of all licensed pharmaceutical products over the last 50 years contain a fluorine atom. In the recent past, it was found that fluorinated drugs are metabolically non-degradable, thereby, enhancing the rate of absorption and transfer of the drug to the active site in the body [28]. Since there are very few naturally occurring fluorine-containing compounds and there being a great demand for fluorinated chemicals worldwide, it is necessary to synthesize fluorinated organic compounds. Recently Sharma et al., reported synthesis and *in vitro* antitumor activity of novel fluorine containing pyrazoles and pyrazolines. These compounds exhibited excellent cytotoxicity against MCF-7 breast cancer cell line [29]. Sarojini and co-workers revealed that fluorine containing hydroxypyrazolines showed potential antiproliferative activity [30]. Based on these aspects, it was decided to report the synthesis and study of *in vitro* anticancer and *in vitro* antioxidant activities of a new series of 1,3,5-trisubstituted aryl-5-hydroxypyrazoline analogues.

2. Chemistry

The hydroxypyrazolines **4a–l** were synthesized according to the literature method [30] as shown in Scheme 1. In the first step, synthesis of chalcones was carried out by the well-known Claisen–Schmidt reaction, and the products were purified by recrystallization from ethanol (60–70% yield). Chalcones **1a-l** were converted to chalcone dibromides **2a-l**. The intermediate 3-fluoro-4-methoxybenzohydrazide **3** was prepared according to the literature procedure [31]. Condensation of chalcone dibromides **2a-l** with 3-fluoro-4-methoxybenzohydrazide **3** in the presence of triethylamine using absolute ethanol as solvent of reaction gave the desired product. Further purification was done by recrystallization from ethanol.

The structures of the hydroxypyrazolines **4a–l** were determined by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and LC-MS spectral studies (Table 1). In the IR spectrum of hydroxypyrazoline **4c,** a broad absorption band around 3373 cm^{-1} indicated the presence of the hydrogen bonded hydroxyl group in the compound. The amide carbonyl stretching frequency was observed at 1614 cm^{-1}. The shift in the frequency of lower values could be explained on the based on the mesomeric shift and intramolecular hydrogen bonding. The other prominent absorption bands observed in the IR spectrum are 3097 (Ar C–H), 2935 (C–H), 1566 (C=C) and 1176 (C–F) cm^{-1}. The ^{1}H NMR spectrum of **4c** showed that the proton of the hydroxyl group resonated as a singlet at δ 5.44. The methylene protons of hydroxypyrazoline ring appeared as two doublets centred at δ 3.57 and δ 3.66 with a geminal coupling constant ($J = 18$ Hz). The appearance of the two doublets clearly reveals the magnetic non-equivalence of the two protons of the CH$_2$ group adjacent to a chiral centre. The two sharp singlets at δ 3.86 and δ 3.96 assigned for methoxy protons. The four protons of *p*-anisyl moiety resonated as two doublets at δ 6.97 and δ 7.69 ($J = 8.8$ Hz). Two doublets and one double-doublet at δ 7.02 ($J = 8.4$ Hz), δ 7.39 ($J = 2$ Hz), and δ 7.37 ($J = 2.4$ Hz) were due to the aromatic protons of the 2,4-dichlorophenyl moiety. The protons of 3-fluoro-4-methoxyphenyl ring resonated as complex multiplets at δ 7.85–7.94. Further, the ^{13}C NMR spectra of **4c** confirmed the presence of a pyrazoline ring by exhibiting signals at δ 48.9 and δ 91.1 as a singlet attached to *sp*^{3} carbons due to C-4 and C-5 carbons, respectively. The two signals due to O–CH$_3$ carbons appear at δ 55.8 and δ 56.6, and a signal due to the carbonyl carbon appears at δ 162.8. Other aromatic carbons were observed in the expected regions. The LC-mass spectral data of compound **4c** provided further evidence of its correct structure. The molecular ion peak was observed at 487 as expected for *m/z* value [M–H]$^-$. Elemental analysis of C, H, and N are within ±0.1% of the predicted values. In the same way, the structures of all the final compounds were confirmed by their characterization data.