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ORIGINAL ARTICLE

Synthesis of *N*-Mannich bases of berberine linking piperazine moieties revealing anticancer and antioxidant effects

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Abstract A new Mannich base series of piperazine linked berberine analogues was furnished in this study to screen the antioxidant and anticancer potential of the resultant analogues. Alkoxy group at a C-9 position of berberine was converted to hydroxyl functionality to enhance the ability of final scaffolds binding to the target of drug action mainly through hydrophobic effect, conjugation effect, whereas Mannich base functionality was introduced on the C-12 position of berberine. Scaffolds were investigated for their free radical scavenging antioxidant potential in FRAP and DPPH assay, whereas tested to check their Fe^{+3} reducing power in ABTS assay. The radical scavenging potential of the final derivatives **4a**–**j** was found excellent with IC_{50}s, < 13 μg/mL and < 8 μg/mL in DPPH and ABTS assay, respectively, whereas some analogues showed significant Fe^{+3} reducing power with absorption at around 2 nm in the FRAP assay. Anticancer effects of titled compounds were inspected against cervical cancer cell line Hela and Caski adapting SRB assay, in which analogues **4a**–**j** presented < 6 μg/mL of IC_{50}s, and > 30 of therapeutic indices, thus exerting low cytotoxic values against Malin–Darby canine kidney (MDCK) cell lines at CC_{50}s > 125 μg/mL. Hence, from the bioassay outcomes it can be stated that these analogues are dual active agents as the scavengers of reactive oxygen species and inhibitors of the cancerous cells as compounds with halogen functional group have overall good pharmacological potential in assays studied in this research. Correct structure of the final compounds was adequately confirmed on the basis of FT-IR and ^{1}H NMR as well as elemental analyses.

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1. Introduction

Reactive oxygen species (ROS) carrying an unpaired electron in an atomic orbital are endogenous stimuli capable to interact with DNA which is the primary reason for the occurrence of cancer (Valko et al., 2004). One of the reasons for the

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generation of ROS responsible for extreme uncertainty and high reactivity is some kind of pathological states and physiochemical circumstances. These species can accept or donate an electron with adjunct molecules and can act as oxidants or reductants, important in the pathogenesis of many different diseases (Droge, 2002; Valko et al., 2006, 2007; Cheeseman and Slater, 1993). Interaction of ROS with DNA results into the damage of cell and homeostatic disruption which may outcomes into deleterious events such as cardiovascular disorders, diabetes, cancer, cirrhosis etc. (El-Gazzar et al., 2009; Kashif et al., 2008). "Oxidative stress" which is an imbalance toward the pro-oxidative state is the responsible factor to damage very important biological components like essential proteins, lipids and nucleic acids (Lobo et al., 2010) thereby furnishing excess quantities of reactive oxygen species. The process of cancer and its treatments create an discrepancy between anti-oxidant protection and toxic production.

Antioxidants can reduce oxidative stress caused carcinogenesis by an immediate scavenging of ROS and have an important role in the enhancement of these infected conditions. They are stable components and able to donate an electron to unstable ROS, and thus ROS can be neutralized and can no longer affect essential biological species (Mc Cord, 2000). The chain reactions often constructed by ROS can be broken by antioxidant molecules by the donation of an electron which stops catalysts of ROS which initiated chain reactions and as a result ROS can be quickly destroyed. By such means, antioxidant molecules works as an enzyme inhibitor, a radical scavenger, singlet oxygen quencher, hydrogen and electron donor, peroxide decomposer, synergist, and metal-chelating agents (Rice-Evans and Diplock, 1993; Krinsky, 1992). The aforementioned facts imply that scaffolds which hold dual antioxidant and anticancer activities are of tremendous significance in the current medication finding improvement. In fact, discovery of anticancer molecules from natural product leads is a major theme of the current decade worldwide with a reason that cancer is responsible for numbers of deaths in most developed, developing and underdeveloped countries.

Cancer is responsible for numbers of death each year worldwide, more specifically with the highest death rate in Asia and then Europe. As per the World Health Organization, death rate were 6.2 million in 1997 and 7.6 million in 2008, which means 13% of all fatalities were due to melanoma and that the international melanoma rate may improve by 50% to 15 million new cases by 2030. Among women, cervical cancer is the most typical which is responsible for an approximated 527,624 new cases and 265,653 fatalities in 2012. Cervical melanoma is the 2nd most typical females' melanoma in females older 15 to 44 years in Globe (Bray et al., 2013).

Despite the improvement in the area of melanoma research, both developing and developed countries are in the hold of this dangerous illness and still there is a need to discover and create therapeutic agents potentially effective against several types of cancer. It has identified that natural products signify the wealthiest source of high substance variety, offering the foundation for recognition of novel scaffolding components that provides starting points for rational drug design (Koehn and Carter, 2005). Natural products are small-molecule secondary metabolites that contribute to organism survival. This can be one of the factors that initiatives have been instructed to find appealing cancer therapeutic agents from natural resources. According to the latest evaluation, ~49% of cancer medication was either through natural products or their derivatives that are used as chemotherapeutic drugs (Newman and Cragg, 2012). The latest review states that there are 12 approved natural product anticancer agents (Basmadjian et al., 2014). Berberine, an isoquinoline plant alkaloid is acquired from different plant species including *Hydrastis canadensis* L., (Ranuncufaceae), *Berberineeris* species (Berberidaceae) and *Arcungelisia flaw* (Menispermaceae). Reports show that berberine has been observed to be efficient towards osteosarcoma, lung, liver, prostate and breast cancer (Wang et al., 2011; Patil et al., 2010) by using transcriptional control of some oncogene and carcinogenesis-related gene appearance and contacts with both DNA and RNA. It regulates ROS production and activates nuclear factor-B that make it responsible for the inhibitor of cancer cell growth because it affects N-acetyltransferase, cyclooxygenase-2, and topoisomerase activities and gene/protein expression. Berberin has efficacies as the most potent anticancer natural product alkaloid as it inhibits tumourigenic microorganisms, regulates oncogene and carcinogenesis-related gene expression, inhibits telomerase, inhibits cyclooxygenase-2, suppresses tumour cell proliferation, impacts cytochrome c discharge and caspase initial as well as enhances the possibilities of fighting against multidrug resistance problems (Sun et al., 2009). Naturally occurring berberine as well as their synthetic analogues have been shown to demonstrate interesting and different activities including antimicrobial, antileukemic, anti ulcerous, and enzyme-inhibiting, anti-inflammatory, anti-diarrhoea, glucose-lowering, cholesterol-lowering, neuroprotective, antidepressant, Alzheimers disease-ameliorating (Verpoorte, 1998; Bodiwala et al., 2011; Kuo et al., 2004; Yin et al., 2002; Leng et al., 2004; Peng et al., 2007; Cui et al., 2009; Kulkarni and Dhir, 2007; Asai et al., 2007) etc. It exhibits activity on LDLR (Yang et al., 2008) and is found to reveal cytotoxicity against HeLa, SVKO3, Hep-2 cancer cell lines (Orfila et al., 2000) and anti-leishmaniasis activity (Vennerstrom et al., 1990). Thus, in a view of the above-mentioned therapeutic role of berberine, their scaffolds have attracted significant interest in recent years.

Recent reports suggest that this piperazine derivative effectively prevents melanoma cell growth and causes caspase-dependent apoptosis via suppressing multiple signalling pathways implicated in cancer (Edward and Zhonglin, 2013). Moreover, alkaloids bearing piperazine residue are previously studied to deliver significant anticancer effects (Kohmoto et al., 1988). Some recent reports suggested that substituting different electron withdrawing and electron donating functional group bearing piperazine moieties to the heterocyclic or aromatic core results in analogues demonstrating appreciable anticancer efficacies (Patel and Park, 2013; Patel et al., 2011). According to the extensive variety of scientific actions associated with berberine and the piperazine, the mixture of these two moieties in the same compound is an exciting task for the growth of new pharmacologically effective agents. Derivatization has been conducted via developing *N*-Mannich bases as the scientific results of this scaffold is well known, particularly as antioxidant (Malhotra et al., 2012; Ma et al., 2013; Dong et al., 2012) and anticancer agents (Bala et al., 2014; Venkateshwarlu et al., 2014). *N*-Mannich bases equipped with piperazine linkage was recently attempted and adapted