1.0. ABSTRACT

**Background and Introduction:** Colorectal cancer (CRC) is the third most common cancer in men (746,000 cases) and the second most common in women (614,000 cases). According to recent statistics, about 60% of CRC cases are observed in developed countries. The number of CRC-related deaths has increased to approximately 694,000 worldwide, making CRC as the fourth most common cause of deaths due to cancer. The existing therapies are either toxic and fail to retard the growth of advanced tumors, or specific to certain tumors expressing oncogenic proteins. Therefore, therapeutic agents that can inhibit majority of CRC are required immediately. Nrf2, a key transcription factor which regulate oxidative stress in cells, is a proteins with dual functions. While activation of Nrf2 is essential to mitigate the ROS in cells thereby prevent the transformation of normal cells in to cancerous ones, inhibition of Nrf2 is required for treating advanced tumors. Therefore, in this study we have synthesized tetrahydrocarbazoles (THCs) and oxazines and studied their ability to modulate Nrf2 *in silico* and *in vitro*. Our hypothesis is that THCs and Oxazines bind to Nrf2-Keap1 complex thereby facilitate the release of Nrf2. The released Nrf2 promotes the transcription of enzymes that degrade ROS, which otherwise promote transformation of normal cells to cancerous ones. However, at higher doses, these compounds inhibit cell viability through apoptosis induction. The rationale for this hypothesis is that THCs and Oxazines interact with Nrf2-Keap1 complex to prevent the degradation of Nrf2. Since Nrf2 has a role in tumorigenesis, growth and metastasis targeting this protein helps in the prevention and treatment of cancers

**Objectives:**
1. Assess the effect of targeting Nrf2 on colorectal carcinoma cell proliferation
2. Synthesis and characterization of novel Tetrahydro carbazoles (THC).
3. Determine the cytotoxic potential of novel THC and already reported 1,2 Oxazine compounds on colorectal carcinoma cell line HCT-116
4. Assess the binding potential of THC compounds and 1,2 Oxazines to keap1 binding site in Nrf2 using *in-silico* molecular docking; and measure the efficacy of selected molecules to modulate NRF2 and its target genes NQO1 and GST.
5. Assess the efficacy of selected THC compounds and 1,2 Oxazines for inhibiting the Ehrlich Ascites Carcinoma (EAC) cell growth in Swiss albino mice using liquid tumor model.
Methodology:
THCs were synthesized as detailed. To a stirred mixture of cyclohexene-1-one (1) (2mmol) and methyldimethylmalonate (2) (2mmol) in 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF₄]) (2mL) iodine (10mol%) was added and stirred for 2hr. Completion of the reaction was monitored by TLC. Substituted phenyl hydrazine (4a-k) (2mmol) was added to the reaction mass and heated at 50°C for 2hrs. The solid obtained was filtered under vacuum and washed with diethyl ether. The obtained solid products (5a-j) were re-crystallized in ethanol and structures determined with NMR and Mass spectrometry. Oxazines were synthesized as detailed by Srinivas et al., 2015. The in silico studies were carried out to determine the binding ability of THCs and Oxazines to Nrf2-Keap proteins. Furthermore, the ability of THCs and Oxazines for modulating the Nrf2 expression was carried out by measuring the NQO1 activity and real-time PCR. Targeted knockdown of Nrf2 was achieved using Lipofectamine RNAi Max reagent loaded with siRNAs. The effect on cell growth was assessed using MTT assay. In vivo, the most potent THC 5b and Oxazines COM and API were assessed for efficacy using EAC model.

Results: Colorectal carcinoma cell line HCT116 showed the NQO1 (a direct transcriptional target of Nrf2 and an indicator of Nrf2 function) activity which is much higher than breast cancer cell lines, but, lesser than lung carcinoma cell line A549. Targeted inhibition of Nrf2 using siRNA reduced the proliferation of HCT116 cells indicating that Nrf2 is a potential therapeutic target in cancer cell lines expressing elevated functionally active protein. Hence, a scheme was developed to synthesize and test the efficacy of oxazines and THCs for modulating the Nrf2 in colorectal carcinomas. Among these synthetic molecules the compound 5b showed better cell killing ability when tested against HCT116 and A549 cancer cells. In silico, 5b showed better binding to Nrf2-Keap1 interaction site indicating its ability to modulate Nrf2 and its target genes. Likewise, among various oxazines synthesized API and COM showed better cell killing in vitro and binding to Nrf2 in silico. Analysis of 5b, API and COM in animals showed better tumor growth inhibition as evidenced by a significant decrease in the animal body weight, tumor cell number and ascites fluid.

Conclusion: In conclusion, results of this study showed the potential of tetrahydrocarbazole 5b and oxazine derivatives API and COM for inhibiting tumor growth in vitro as well as in vivo, hence, could be considered for further development.

Key words: Colorectal cancer, Tetrahydrocarbazoles, Oxazines, Characterization, Cytotoxicity, Molecular docking.