Ameliorative effect of 5H-benzo[h]thiazolo[2,3-b]quinazoline and indeno[1,2-d]thiazolo[3,2-a]pyrimidine analogues against urethane induced lung carcinoma in albino Wistar rats

SUMMARY OF THESIS

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SUMMARY

Lung cancer (LC) stands as the most prevalent and lethal solid cancer, primarily due to uncontrolled respiratory proliferation. The biomarkers for potential targeted treatment of LC include epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), Kirsten rat sarcoma viral oncogene (KRAS), mesenchymal-epithelial transition factor (MET), human epidermal growth factor receptor-2 gene (HER2, also known as ERBB2), proto-oncogene tyrosine-protein kinase ROS (ROS1), B-Raf protein (BRAF), RET proto-oncogene (RET), neurotrophic receptor tyrosine kinase-1 (NTRK1), Mediator of RNA polymerase II transcription subunit-1 (MED1 gene), vascular endothelial growth factor receptor-2 (VEGFR2), HER3 (HER3 knows as ERBB3), and insulin-like growth factor receptor-1 (IGF-1R). Biomarkers also have potential targeted therapeutic treatments for LC like-serine/threonine kinase-11 (STK11), receptor tyrosine-protein kinase (ERBB3 known as HER3), KRAS, BRAF, phosphatidylinositol 3-kinase (PI3K), phosphatase and tensin homolog (PTEN), and retinoblastoma (RB) gene. Traditional chemotherapy treatments face significant limitations such as low bioavailability, non-specific targeting, and drug resistance.

5-FU or 5-fluro-2, 4-pyrimidinedione is a chemotherapeutic drug frequently delivered either by a single medication or in combination with other therapeutic regimens. 5-FU is an anticancer antimetabolite drug primarily derived from thiazolo-pyrimidines that comprise a thiazole and a pyrimidine ring, which inhibit the proliferation of cancer cells. This has led to considerable hope for the development of new chemical moieties with pyrimidine ring. Therefore, identifying or synthesizing new chemical moieties that target molecular pathways is a key step in the development of new treatment approaches.

Urethane (Ethyl Carbamate; EC) is the widely used chemical to induce LC in mice and rats, and cause tumor formation in a variety of cell types, including the skin, hepatic, mammary gland, and lymphoid tissue.

In recent years, thiazolo[2,3-b]quinazoline analogues have garnered interest for their anticancer potential in various cancer types. This study evaluates the anti-inflammatory and anticancer properties of quinazoline analogues synthesized in our laboratory. Among the 15 compounds screened against the A549 cell line, 7A (*4-(6,7-dihydro-5H-benzo[h]thiazolo[2,3-b]quinazolin-7-yl)phenol*) and 9A (*7-(4-chlorophenyl)-3-methoxy-9-methyl-6,7-dihydro-5H-benzo[h]thiazolo[2,3-b]quinazoline*) emerged as the most effective. These compounds feature a fusion of three bio-dynamically privileged heterosystems with a bridgehead nitrogen atom, enhancing their cytotoxic effects.

Molecular docking studies confirmed the strong binding affinity and stability of 7A and 9A with target proteins. 7A demonstrated the highest binding affinity with caspase-9 (-7.2 kcal/mol), caspase-3 (-6.9 kcal/mol), and IL-6 (-6.6 kcal/mol). In contrast, 9A showed superior binding affinity with caspase-3 (-7.7 kcal/mol), caspase-9 (-7.3 kcal/mol), and caspase-8 (-7.0 kcal/mol). The structural efficacy of 7A and 9A is attributed to specific substitutions, such as 4-hydroxyphenyl in 7A and -CH3, -OCH3, and -Cl in 9A.

Acute toxicity studies on albino Wistar rats revealed that 7A and 9A are safe at administered doses. Pharmacokinetic studies indicated better oral bioavailability due to enhanced absorption of the substituted quinazoline moiety from the gastrointestinal tract. Subsequent in-vivo studies against urethane-induced LC in rats showed significant efficacy in weight restoration and tumor reduction, particularly with 7A at a dose of 10mg/kg. Morphological analysis and SEM revealed better restoration of tissue architectures in the 9A-treated group compared to 7A.

Histopathological analysis indicated that treatment with 7A and 9A restored healthy tissue architecture, reduced lung proliferation, and restored tissue integrity. The urethane metabolite vinyl carbamate leads to ROS formation and DNA damage, promoting cancer progression. 7A and 9A reduced levels of ProC and TBARs, highlighting their antioxidant potential. Additionally, these compounds restored the activity of SOD and GSH, confirming their free radical scavenging capabilities.

The study further scrutinized pro-inflammatory (IL-2, IL-6, IL-10, IL-1β) and apoptotic (caspase-3, caspase-9) markers. Treatment with 7A and 9A normalized the elevated IL-2 levels observed in the cancer group and demonstrated chemotherapeutic effects consistent with previous reports. The reduction in IL-10 levels in the cancer group after treatment with 7A and 9A aligns with the anti-inflammatory effects reported in clinical studies.

The findings suggest that urethane induces inflammatory signaling and anti-apoptotic mechanisms, which were mitigated by 7A and 9A through the up-regulation of caspase-3 and caspase-9 levels. Immunoblotting confirmed the down-regulation of IL-6, STAT3, and Bcl-2, initiating apoptotic mechanisms. The part A study concludes that 7A and 9A exhibit significant anticancer activity by regulating intrinsic apoptotic pathways, supported by protein and gene expression analyses.

Overall, the thiazolo[2,3-b]quinazoline analogues 7A and 9A demonstrated substantial antiproliferative activity against LC, suggesting their potential as novel therapeutic agents for LC treatment. Further modifications to the thiazolo[2,3-b]quinazoline moiety could provide new approaches for treating LC and other malignancies.

In the second study, we used previously synthesized series of thiazolo[3,2-a] pyrimidine ring containing molecules using rational drug design strategies. The thiazolo[3,2-a]pyrimidine

compounds are known to have various significant biological properties such as anticancerous, antimicrobial, antipsychotic, anti-inflammatory, anti-parkinson's, antidepressant, and anti-HIV.

The synthesized compounds were screened against A549 lung cancer cells to evaluate their anti-cancer potential. Among the 15 compounds, 9B (*8-methoxy-5-(3,4,5-trimethoxyphenyl)-5,6-dihydroindeno[1,2-d]thiazolo[3,2-a]pyrimidine*) and 12B (*5-(4-chlorophenyl)-5,6-dihydroindeno[1,2-d]thiazolo[3,2-a]pyrimidine*) exhibited the highest cytotoxic effects in the SRB assay.

Molecular docking studies revealed that 9B and 12B had good binding affinity and stability with proteins such as IL-6, Cyt-C, caspase-9, and caspase-3. These compounds showed strong binding activity with caspase-9 (-7.5 kcal/mol and -7.3 kcal/mol) and caspase-3 (-7.4 kcal/mol and -7.2 kcal/mol) through hydrogen bonds, indicating their potential to initiate and execute apoptosis.

Acute toxicity studies of compounds 9B and 12B showed no toxic effects at doses of 5, 10, and 15 mg/kg. Therefore, a dose of 10 mg/kg was selected for further assessments, comparable to 5-FU. Pharmacokinetic studies showed that 12B had better oral bioavailability than 9B, suggesting their potential as orally absorbed compounds.

The significant cytotoxic potential, binding affinity, safety profile, and oral bioavailability of 9B and 12B warranted further in-vivo evaluations against ethyl carbamate (EC)-induced lung cancer in albino Wistar rats. 12B demonstrated better efficacy in weight restoration at a dose of 10 mg/kg compared to 9B. Various studies have indicated that alterations in lipid metabolism are characteristic of carcinogenesis, with higher triglyceride (TG) and lower high-density lipoprotein (HDL) levels predisposing to higher LC incidence. Increased total

cholesterol (TC) and low-density lipoprotein (LDL) levels are associated with cancer growth and cellular proliferation.

EC is metabolized to vinyl carbamate, leading to reactive oxygen species (ROS) formation and DNA damage, laying the foundation for cancer development. Administration of 9B and 12B reduced ROS and oxidative stress markers while restoring antioxidant enzyme activities, indicating their potential to reduce EC-induced oxidative stress.

Histopathological analysis revealed that 9B and 12B treatments preserved lung tissue architecture and reduced alveolar damage compared to the EC-treated control group. The efficacy of these compounds was further supported by the up-regulation of caspase-3 and caspase-9 levels, indicating restored apoptotic machinery. Immunoblotting showed decreased expression of pro-inflammatory markers (IL-6, STAT3, Bcl-2) and increased expression of apoptotic markers (Cyt-C, caspase-9, caspase-3) in the treatment groups, confirming their anticancer activity through intrinsic apoptotic mechanisms.

In conclusion of part B, compounds 9B and 12B demonstrated significant anticancer potential against EC-induced lung cancer in albino Wistar rats by modulating lipid metabolism, oxidative stress, and inflammatory markers, and restoring apoptotic pathways. These findings suggest that 9B and 12B could serve as potential lead molecules for the development of lung cancer therapies.

NMR-based metabolomic studies were done to understand the metabolic changes during the LC state. In MetaboAnalyst software, the HMDB and BMRB database followed by using OPLSDA to effect metabolic changes between groups. Biochemicals directly involved with different pathways i.e. glycolysis, neo-glycogenesis, phosphatidylinositol, and other metabolic pathways in the TCA cycle during CRC condition. The box-cum-whisker plots of the OPLSDA analysis by NMR experiment differentiated between disruptions of serum

metabolites in non-treated and treated groups' recovery after treatment. In metabolic study, urethane-induce LC samples demonstrated the Warburg effect with a low level of glucose and a higher level of lactate. The decreased level of citrate and 3-hydroxybutyrate explored the LC progression. Conversely, the significantly elevated level of lactate, betain, and glutamine demonstrated their significance in the advancement of cancer. After treatment, the altered level of all cancer metabolic markers successfully began to recover to non-cancerous conditions.

The study concludes that out of 30 compounds (15 compounds of thiazolo[2,3-b]quinazoline analogue and remaining 15 of thiazolo[3,2-a]pyrimidine ring containing analouges) 7A, 9A, 9B and 12B exhibit significant anticancer activity against in lung carcinoma model of albino Wistar rat. Thus we postulate that 7A, 9A, 9B and 12B might be used as novel treatment option for LC treatment.