Synthesis, Characterization and Anticancer Potential of the Selected Biginelli Hybrids and Their Nanocomposites

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Abstract: Cancer is one of the most important health problems of today, which ranks second in the list of known deaths. Leukemia remains one of the most important health problems of today, ranking second among known causes of cancer-related death. Although there are treatments that can be effective, new targets and drugs with high selectivity and efficacy are still needed, particularly for non-solid tumors such as leukemia. Many reported antileukemic agents suffer from poor selectivity and bioavailability. Pyrimidines, as fundamental components of DNA and RNA, have attracted significant attention in drug discovery and development. Among the pyrimidine family, Biginelli-derived tetrahydropyrimidines (THPMs) hold particular importance due to their broad pharmacological potential, including anticancer activity. In this study, THPM molecules, and their nanocomposites were synthesized and characterized by NMR, elemental analysis, DSC, TGA, FTIR, MS, and subsequently assessed for their *in vitro* anticancer activities. The cytotoxic effects were assessed in K-562, THP-1, and MOLT-4 leukemia cell lines using the MTT assay. Results demonstrated that selected THPM derivatives exhibited potent and selective cytotoxicity in distinct leukemia subtypes: compounds **4b**, **4e**, **4j**, and **4k** were most active in CML (K-562) cells: **4c**, **4e**, and **4g** in AML (THP-1) cells; and **4b**, **4g**, **4j**, and **4k** in ALL (MOLT-4) cells. The observed selectivity is likely driven by phenotypic and genotypic differences among leukemia cell lines, underscoring the therapeutic potential of THPM scaffolds as targeted antileukemic agents. These findings provide a strong basis for further mechanistic studies and preclinical evaluation.

Keywords: Tetrahydropyrimidines, Anticancer, Selectivity, Nanocomposites, Leukemia

1. Introduction

Cancer is one of the most important health problems of today, which ranks second in the list of known deaths. Although there are treatments that can be effective in cancer treatments, new targets and new drugs are needed. Pyrimidines acts as a potential framework for DNA and RNA, elucidating its importance in drug discovery and development [1]. From pyrimidines family is very important are Biginelli hybrids

tetrahydropyrimidines- THPMs (former name-dihydropyrimidines). They were originally synthesized in XIX century in multicomponent chemical reaction proposed by Pietro Biginelli [2,3]. These scaffolds have attracted imposing interest of medicinal chemists considering their diverse therapeutic and pharmacological properties. Straight forward synthesis of THPMs led to discovery of many significant products such as: antidiabetic, calcium channel blockers [4], adrenergic receptor antagonists, anti-inflammatory, antiviral, antioxidant, and anti-SARS THPMs agents [5]. Nowadays, an "ideal" drug against non-solid cancer (e.g. leukemia) that possesses significant antitumor activity and selectivity has not been identified yet. THPM derivatives have high cytotoxic effects at low concentrations not only in solid tumours but also in suspended leukaemia cell lines such as Molt-4 (acute lymphoblastic leukemia cell line) [6]. In addition to cytotoxic activity studies, they have effects on cancer signalling pathways. THPM derived compounds have been reported to reduce the phosphorylation of Akt and the expression of key downstream effectors of Akt kinase [7]. Characteristic features of nanocomposite compounds such as high bioavailability and low overall cytotoxic effects make them potential anti-cancer agents. Nanomaterial forms of compounds exhibiting a wide range of pharmacological activities such as THPMs can maximise these effects.

2. Methodlogy

2.1 Synthesis and Characterization of Tetrahydropyrimidines

THPM derivatives were synthesized by project partners in Serbia using the Biginelli reaction. Reagent-grade alkyl halides, *p*-tolyl chloride, ethyl 4-chloroacetoacetate, urea, *N*-methyl urea, vanillin, and various solvents (ethanol, acetone, glacial acetic acid and DCM) were used. Melting points were measured using a Mel-Temp device, and IR spectra were obtained using a Perkin–Elmer Spectrum 3 FT-IR/FIR spectrometer. ^{1}H and ^{13}C NMR analyses were performed at 288 K using a Bruker Ascend 400 MHz device in DMSO-d6. Mass spectrometric analyses were performed using a Shimadzu LC-MS/MS system according to methods reported in the literature. A total of eight THPM derivatives (**4a**, **4b**, **4c**, **4e**, **4g**, **4h**, **4j**, and **4k**) were successfully synthesized and characterized.

2.2 MTT Cell Viability Assay

The cytotoxic effects of THPM derivatives were evaluated by MTT assay. Stock solutions (50 mM) were prepared in DMSO (Sigma, D2650), aliquoted, and stored at −20 °C. Leukemia cell lines (K562, THP-1, MOLT-4) were seeded at a density of 5×10^3 cells per well in 96-well plates and incubated for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. Cells were then treated with THPM derivatives at final concentrations of 40 nM and 20 nM for 48 h and 72 h. After treatment, 20 μL of MTT reagent was added to each well and incubated for 4 h, followed by the addition of 10% SDS to dissolve formazan crystals overnight. Absorbance was measured at 570 nm using a microplate

spectrophotometer, and GI_{50}, TGI, and LC_{50} values were calculated according to the NCI drug screening protocol.

3. Results and Discussion

The cytotoxic effects of THPM derivatives were evaluated in K-562 (CML), THP-1 (AML), and MOLT-4 (ALL) cell lines. Eight compounds, prepared in DMSO (50 mM, -20°C), were applied at different concentrations for 48 and 72 h. Cytotoxic activities were assessed by the MTT assay according to the NCI protocol and expressed as IC_{50} values. A time-dependent increase in cytotoxicity was observed, with most compounds exhibiting lower IC_{50} values at 72 h compared to 48 h. Among the tested derivatives, compounds **4a**, **4b**, and **4g** demonstrated the strongest cytotoxic activity. Particularly, compound **4b** exhibited remarkable potency against MOLT-4 cells, with IC_{50} values of 1.21 μM at 48 h and 1.07 μM at 72 h, indicating high effectiveness and selectivity. Compound 4a also maintained consistently low IC_{50} values, reaching approximately 2 μM across all three cell lines at 72 h. Compound 4g was highly active as well, with notable efficacy against MOLT-4 cells (1.85 μM at 72 h). Compounds 4c, 4e, and 4h exhibited moderate cytotoxic effects; 4c maintained IC_{50} values of 2–5 μM at 72 h, 4e increased from ~8–9 μM at 48 h to 3–4 μM at 72 h, and 4h improved in K-562 and MOLT-4 cells (IC_{50} 2.20 μM and 4.66 μM, respectively). In contrast, compounds 4j and 4k were the least effective, with IC_{50} values above 8 μM after 72 h, indicating limited cytotoxic potential. Overall, compounds **4a**, **4b**, and **4g** represent the most promising derivatives with potent and consistent activity across leukemia subtypes, while 4c, 4e, and 4h show moderate effects, and **4j** and **4k** remain comparatively weak.

4. Conclusions

This study demonstrates that THPM derivatives exert potent and time-dependent cytotoxic effects across different leukemia subtypes. In CML (K-562) cells, compounds **4a**, **4b**, and **4h** showed the strongest activity, with IC_{50} values falling below 3 μM at 72 h. In AML (THP-1) cells, compounds **4a**, **4b**, **4c**, and **4g** were the most effective, exhibiting IC_{50} values in the range of 1.8 to 2.6 μM. In ALL (MOLT-4) cells, compounds **4a**, **4b**, and **4g** were particularly active, with IC_{50} values close to 1–2 μM. In contrast, compounds **4j**, **4k**, and partially 4e displayed comparatively weaker cytotoxicity across all cell lines. These findings highlight the selective and subtype-specific cytotoxic potential of THPM derivatives, underscoring their promise as candidate scaffolds for targeted antileukemic therapies. Further mechanistic and preclinical investigations are warranted to explore their therapeutic applicability.

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