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Synthesis and Characterization of New Hexahydroquinoline Derivatives, *In Silico* Determination of Their Inhibitory Effects on Transforming Growth Factor Beta (TGF-β) and Their Effects on Oxidative Stress *In Vitro*

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1. Introduction

Hypertension is the biggest risk factor for atherosclerosis, which is a chronic vascular inflammatory disease. Normal endothelial cellular functions are disturbed in atherosclerosis.

1,4-dihydropiridines (1,4-DHPs) are an important class of bioactive molecules. Studies on 1,4-DHP ring system have come by a new dimension after nifedipine and later amlodipine were introduced. Since then, several modifications were experimented on 1,4-DHP ring and investigation of other pharmacological activities along with their cardiovascular effects has gained speed. Hexahydrokinolines, the analogues of 1,4-DHP, are now intensively investigated for their calcium channel blocking activities. In the recent years, their inhibitory effects on transforming growth factor beta (TGF-β), their anti-atherogenic and anti-inflammatory effects were also discovered.

The aim of this study was to evaluate the effects of 1,4-DHP derivatives on TGF-β *in silico*. In addition, the cytotoxic and oxidative stress-producing effects of 1,4-DHP derivatives (with a general formula of alkyl 4-(2-fluoro-4-(trifluoromethyl) phenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate) were determined in mouse 3T3 fibroblast cells.

2. Materials and methods

Six compounds (RG-104, RG-105, RG-106, RG-107, RG-109, and RG-111) were synthesized by modified Hantzsch reaction. Exact structures of the compounds were elucidated by IR and ^{1}H-NMR, ^{13}C-NMR and HRMS analysis. The absorption bands at around 3300 cm^{-1} confirmed N-H in the ring system, around 1700 cm^{-1} confirmed ester groups and around 1600 cm^{-1} confirmed ketone groups. As expected ^{1}H-NMR spectra of the compounds displayed singlet signals belonging to $-CH_3$ groups at 0.9 ppm, multiplet signals belonging to aromatic protons at 7.0 ppm and singlet signals belonging to –NH groups at 9 ppm. The peaks belonging to other protons were seen in expected values of the compounds' spectra. The ^{13}C-NMR spectra of the compounds displayed the appropriate number of resonances that exactly fitted the number of non-equivalent carbon atoms. *In silico* studies were performed by using SwissADME in order to obtain the primary targets of these compounds.

Cytotoxicity was determined by using MTT assay. Reactive oxygen species (ROS) formation was determined spectrofluorometrically. Studies on their oxidative potentials were performed at both inhibitory concentration 20 (IC_{20}) and at the predicted peak plasma concentrations (10 ng/ml), which were evaluated according to the plasma concentrations of azelnidipine, which is a structurally similar calcium channel blocker. Total glutathione levels, lipid peroxidation and protein oxidation were evaluated by using spectrophotometric commercial kits.

Experimental data were analyzed with ANOVA followed by the Mann-Whitney U test by using SPSS. The $p < 0.05$ were considered as significant.

3. Results

In silico studies showed that primary effect of the compounds could be TGF-β inhibition. Cytotoxicity experiments showed that all of the compounds had inhibitory concentration 50s (IC_{50}s) >100 μM. The IC_{50} doses and IC_{20} doses are shown in Table 1.

None of the compounds caused increases in intracellular ROS production at both IC_{20} and predicted peak plasma concentrations.

Table 1 *Inhibitory concentrations 50 and 20 of the 1,4-DHP derivatives.*

Compound	IC_{50} (μM)	IC_{20} (μM)
RGD-104	104.7	178.3
RGD-105	152.7	239.5
RGD-106	111.9	196.8
RGD-107	100.7	168.1
RGD-109	114.4	259.7
RGD-111	129.4	280.2

None of the compounds caused increases in lipid peroxidation and protein oxidation and decreases in total GSH levels in both IC_{20} and predicted peak plasma concentrations.

4. Conclusions

Transforming growth factor beta is a major orchestrator of the fibro-proliferative response to tissue damage. At later stages of response, it negatively regulates fibrosis. In advanced lesions, TGF-β might be important in arterial calcification.

In this study, newly synthesized 1,4-DHP derivatives are evaluated for their biological activities both *in silico* and *in vitro*. Their primary target was found to be TGF-β. Inhibition of TGF-β may have several consequences, including the prevention of atherosclerotic plaques and inflammation.

These compounds did not cause cytotoxic effects even at concentrations above 50 μM. Their IC_{50} values were >100 μM (RGD-105>RGD-111> RGD-109>RGD-106> RGD-104> RGD-107).

The drug candidates also did not lead to oxidative stress at IC_{20} concentrations and their predicted peak plasma concentrations. However, more studies are needed to confirm their both *in vitro* and *in vivo* effects. Their TGF-β inhibitory effects should be confirmed with mechanistic studies. Moreover, their effects on other proteins in the TGF-β pathway should be investigated.

References

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