Anticancer and antituberculosis effects of 5-fluoro-1H-indole-2,3-dione 3-thiosemicarbazones

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ABSTRACT

Background and Aims: The aim of this study was to screen the in vitro anticancer/antituberculosis activities of 5-fluoro-1-methyl/ethyl-1H-indole-2,3-dione 3-thiosemicarbazones.

Methods: A549/U-87MG cell lines were used for the anticancer activity of the compounds, while CCD-19Lu cell line was used to determine their cytotoxic effects. In antituberculosis activity studies using MTB H37Rv cell line, BJ cell line was used to determine the cytotoxic effects. MTT assay was used to obtain IC50 values.

Results: 6a, 6b, 6h, 6l, 6n, 7c, 7k and 7l were found to be highly effective against A549 cell line compared to cisplatin whereas 6d, 6h, 6l, 6n, 7d and 7f were found to be effective against U-87MG cell line compared to cisplatin. It was also determined that 6a, 6b and 7l have near-standard activity and 6b, 7b and 7l were not cytotoxic on BJ cell line.

Conclusion: While determining effective compounds in anticancer studies, it was concluded that active compounds can be reached by modifications in compounds in antituberculosis studies.

Keywords: Anticancer activity, antituberculosis activity, 5-fluoro-1H-indole-2,3-diones

INTRODUCTION

1H-Indole-2,3-dione and its derivatives have a broad spectrum of biological properties including anticancer, antiviral and antimicrobial activities. There are several reports on the anticancer activities of 1H-indole-2,3-dione 3-thiosemicarbazone derivatives (Karali, 2002; Hall et al., 2009; Hall et al., 2011; Priyanka, Manasa & Sammaiah, 2014; Pape et al. 2016; Karalı et al., 2017). A pharmacophore analysis of the active compounds revealed that 1H-indole-2,3-dione 3-thiosemicarbazone moiety, aromatic/hydrophobic features at the N4 position of the thiosemicarbazone, introduction of electron-withdrawing groups at position 5 of 1H-indole-2,3-dione and alkylation of position 1 of 1H-indole-2,3-dione were essential for anticancer activity (Vine, Locke, Ranson, Pyne & Bremner, 2007; Matesic et al., 2008; Sabet, Mohammadpour, Sadeghi & Fassihi, 2010, Pervez, Saia, Iqbal, Yaqub, & Khan, 2011; Pervez, Saia, Iqbal, Yaqub, & Khan, 2013; Lin et al. 2013). Researchs also showed that N4-phenyl substituted thiosemicarbazone derivatives were significantly more active than N4-alkyl and N4-cycloalkyl thiosemicarbazone derivatives (Hall et al., 2009; Hall et al., 2011). 5-Fluoro-1H-indole-2,3-dione 3-[4-(4-methoxyphenyl)thiosemicarbazone] have been reported as selective MDR1 activity (Hall et
It has been found that derivatives of the 5-fluorosatin ring with methylene/ethylene bridges with fluoronucleon derivatives such as gatifloxacin, balofloxacin and 8-methoxyciprofloxacin have significant antituberculous activity (Feng et al., 2010; Banerjee et al., 2011). 5-Nitro-5-methyl-5-trifluoromethoxysatin 3-thiosemicarbazone derivatives showed antituberculous activity (Karali et al., 2007; Güzel, Karali & Salman, 2008).

In this study, in vitro the antitumor and antituberculosis activities of 5-fluoro-1-methyl/ethyl-1H-indole-2,3-dione-3-[4-(4-substituted phenyl)thiosemicarbazones] derivatives, which were previously synthesized by our research, were screened. The structures of all the synthesized compounds were determined by analytical and spectral methods (Sevinçli, Duran, Özbil & Karali, 2020). The molecular and isomeric structures of 6h and 6j were determined by X-ray single crystal diffraction analysis (Atioğlu, Sevinçli, Karali, Akkurt & Ersanli, 2017a; Atioğlu, Sevinçli, Karali, Akkurt & Ersanli, 2017b). In the study where A549 (human lung adenocarcinoma cells) and U-87 MG (human glioblastoma cells) cell lines were used to determine the antitumor activities of the compounds, CCD-19Lu (human normal lung fibroblast cells) cell line were used to determine cytotoxic effects. Cisplatin was used as a positive control. While Mycobacterium tuberculosis (MTB) H37Rv (ATCC 27294) cell line was used to determine antituberculosis activities, a BJ (human healthy fibroblast cell line) cell line was used to determine the cytotoxic effects of some compounds on healthy cells. Rifampicin was used as a standard to evaluate antituberculosis activities.

MATERIAL AND METHODS

Anticancer activity studies

U-87 MG (ATCC number HTB-14™), A549 (ATCC number CCL-185™) and CCD-19Lu (ATCC number CCL-210™) cell lines were obtained from the American Type Culture Collection. All the cell lines were grown in EMEM (Eagle’s Minimum Essential Medium) supplemented with 2 mM L-glutamine, 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in a humidified incubator with a 5% CO2 atmosphere. Compounds were dissolved in DMSO so- lution and the concentration-dependent cytotoxic effect (Karali, 2020).

Antituberculosis activity studies

MTB H37Rv (ATCC 27294) was purchased from the American Type Culture Collection (ATCC) cell bank. BD BACTEC™ MGIT™ (mycobacterial growth indicator tubes) were reconstituted in tubes in seven days. Later on, a special medium, ATCC® Medium 1395: Middlebrook 7H9, was prepared for anti-tuberculosis activity test, ADC (albumin-dextrose-catalase) and oleic acid were added to enrich the medium. The developed microorganisms were adjusted to McFarland Standard No. 1 and prepared for experiments. The test substances were prepared at concentrations of 0.97-500 μg/mL. Rifampicin (Sigma, R3501) was used as a positive control. Materials in 96-well plates and MTB H37Rv were incubated for 7 days at 37°C. Then, 20 μL MT was added vigorously and left to incubate for 24 h under the same conditions. At the end of the 24th h, the vitality conditions were compared (Foongladda et al., 2002; Raut, Narang, Mendiratta, Narang & Deotale, 2008).

A BJ cell line (ATCC® CRL-2522™) was obtained from the American Type Culture Collection. The cells were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% foetal bovine serum, 1% penicillin/streptomycin at a temperature of 37°C in a humidified incubator with a 5% CO2 atmosphere. Compounds were dissolved in DMSO solution and the concentration-dependent cytotoxic effect was studied (0.97- 500 μg/mL). The BJ cells were inoculated into 96-well culture plates at a density of 5x10^4 cells per well. Then, after 24 h of incubation, 10 μL of MTT solution (5 mg/ mL) was added to each well and the plates were incubated for a further 4 h (Mosmann 1983). The formazan crystals produced, which are converted with dye, are solubilized with DMSO. The culture plates were inserted in Cytation 3 Cell Imaging Multi-Mode Reader (BioTek) and the absorbance was measured at 540 nm. The percent values of cell proliferations were calculated relative to controls, whose cell proliferations were accepted as 100%.

RESULTS AND DISCUSSION

Chemistry

The structures of 6a-n and 7a-n were confirmed by analytical and spectral (IR, 1H NMR, 13C-NMR, HSQC-2D, HMBC-2D, HRMS-ESI+ and LCMS-ESI+) data (Sevinçli, Duran, Özbil & Karali, 2020).
The molecular and isomeric structures of 6h and 6j were determined by X-ray single crystal diffraction analysis and the stable isomer was found to be in Z configuration (Figures 1 and 2) (Atıoglu, Sevincli, Karali, Akkurt & Ersanli, 2017a; Atıoglu, Sevincli, Karali, Akkurt & Ersanli, 2017b).

**Figure 1.** View of the molecular structure of 6h, with the atom labelling.

**Figure 2.** View of the molecular structure of 6j, with the atom labelling.

**Biological activity**

A549 and U-87 MG cell lines were used for the anticancer effects of the compounds, while CCD-19Lu cell line was used to determine their cytotoxic effects. Cisplatin was used as positive control. 6a, 6b, 6g, 6h, 6l, 6n, 7b, 7c, 7k and 7l (IC$_{50}$= 10.6-58.8 mM) were found to be highly effective against A549 cell line compared to cisplatin (IC$_{50}$= 70.3 mM). It was determined that 6a, 6b, 6g, 6h, 6l, 6n, 7b, 7c, 7k and 7l were highly effective against A549 cell line compared to cisplatin at 51.2, 26.8, 16.4, 35.8 and 10.6 µM, respectively. The R$_1$ methyl substituted derivatives 6a-n are generally more effective than the R$_2$ ethyl substituted derivatives 7a-n against the A549 cells. Whereas, among the R$_2$ ethyl substituted derivatives, R$_1$ 4-methyl substituted 7c and R$_1$ 4-fluorine substituted 7k have higher efficiency than the corresponding R$_2$ methyl substituted derivatives 6c and 6k, but these compounds have higher cytotoxicity. R$_1$ 3-chloro substituted 6l and 7l were found to be effective against A549 cell line compared to cisplatin at 20.6 and 58.8 µM, respectively. This result indicates that the chlorine atom in position 3 of the phenyl ring plays an important role in the activity. While 7l had a selective and nontoxic effect, the selectivity decreased and toxicity increased at 6l. It was also determined that R$_1$ non-substituted 6a, R$_1$ 3-methyl substituted 6b and 3-chloro substituted 7l showed selective effects on the A549 cell line while not showing cytotoxic effects (IC$_{50}$= >400 mM) on CCD-19Lu cell line. 6b was found to be the most effective, selective and nontoxic compound against A549 cell line. It was also determined that 6a, 6b and 7l did not show cytotoxic effects on CCD-19Lu cell lines. 6d, 6h, 6l, 6n, 7d and 7f (IC$_{50}$= 34.9-93.9 mM) were found to be effective against U-87 MG cell line compared to cisplatin (IC$_{50}$= 96.7 mM). The results showed that R$_1$ 4-trifluoromethyl substituted 6d (IC$_{50}$= 46.6 mM) and 7d (IC$_{50}$= 34.9 mM) are more effective than cisplatin against U-87 MG. These results showed that the trifluoromethyl group at the 4 position of the phenyl ring and the R$_2$ ethyl substitution contributed to the activity. The efficiency and selectivity of 7d was higher than 6d, and its toxicity was lower than 6d. R$_1$ 3-methoxy substituted 7f (IC$_{50}$= 91.1 mM) were found to be effective against U-87 MG whereas R$_1$ 4-methylthio substituted 6l (IC$_{50}$= 93.9 mM), R$_1$ 3-chloro substituted 6l (IC$_{50}$= 84.7 mM) and R$_1$ 4-bromo substituted 6n (IC$_{50}$= 61.4 mM) were found to be more effective than cisplatin against both A549 and U87-MG cell lines (Table 1).

**Figure 3.** General structures of 6a-n and 7a-n.

The antituberculosis effects of the compounds were investigated on the MTB H37Rv (ATCC 27294) cell group using rifampicin (IC$_{50}$= 25.00 µg/mL) as standard. 6b, 6c, 6g, 6h, 6l, 6j, 6k, 6n, 7b, 7j and 7l were found effective with IC$_{50}$ value of 31.25 µg/mL. R$_1$ 3-methyl substituted 6b and 7b and R$_1$ 3-fluoro substituted 6j and 7j were found to be effective at 31.25 µg/mL. In this way it was determined that these groups played an important role in the activity. Also R$_1$ 4-methyl substituted 6c, R$_1$ 4-methoxy substituted 6g, R$_1$ 3-thiomethyl substituted 6h, R$_1$ 4-trifluoromethoxy substituted 6i, R$_1$ 4-fluoro substituted 6k, R$_1$ 4-bromo substituted 6n and R$_1$ 3-chloro substituted 7l were found effective at 31.25 µg/mL. Whereas, R$_1$ 4-trifluoromethyl substituted 6d and 7d, R$_1$ 4-ethyl substituted 6e and 7e, R$_1$ nonsubstituted 7a, R$_1$ 4-methyl substituted 7c, R$_1$ 3-methoxy substituted 7f, 4-trifluoromethoxy substituted 7i, R$_1$ 4-fluoro substituted 7k and R$_1$ 4-chloro substituted 7m showed the activity at 62.5 µg/mL (Table 2). It was also determined that R$_1$ 3-methyl substituted 6b and 7b and R$_1$ 3-chloro substituted 7l selected as a prototype did not show cytotoxic effects with IC$_{50}$ value of >500 µg/mL on BJ cell line (Table 3).
CONCLUSIONS

In summary, the anticancer and antituberculosis activities of 5-fluoro-1H-indole-2,3-dione 3-thiosemicarbazones derivatives previously synthesized by our research group have been performed and promising results have been obtained. The R1 unsubstituted 6a, R1 3-methyl substituted 6b and R1 3-chloro substituted 7l compounds are selective and nontoxic effects on the A549 cell line. In addition, the prototype
selected antituberculosis effective R₁ 3-methyl substituted 6b and R₂ 3-chloro substituted 7b compounds were found to be nontoxic on BJ cell line. These results show the importance of R₁ 3-methyl and 3-chlorine substitution.

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