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Antiproliferative Effects of a Series of Pyrazolines on Lung Cancer †

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Abstract: Lung cancer is one of the most diagnosed cancers worldwide and the development of anticancer agents for the treatment of lung cancer is an important task for researchers. For this purpose, herein pyrazoline-based compounds **1–18** were investigated for their cytotoxic effects on A549 human lung adenocarcinoma and CCD-19Lu human lung fibroblast cell lines using MTT assay. According to the results, 1-(4-methoxyphenyl)-3-(2-furanyl)-5-(4-methylphenyl)-2-pyrazoline (**7**) was identified as the most promising anticancer agent due to its selective inhibitory effect on A549 cell line with an IC_{50} value of 138.63 μg/mL when compared with cyclophosphamide (IC_{50} = 309.97 μg/mL).

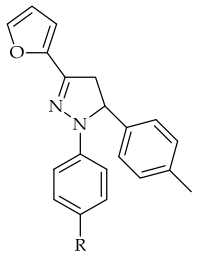
Keywords: lung cancer; antiproliferative activity; pyrazoline

1. Introduction

Lung cancer is among the most deadly cancers with an estimated 1.6 million deaths each year. Smoking, radon exposure, occupational exposures, long-term and accumulated exposure to air pollution, personal or family history serve as risk factors for a person to develop lung cancer. The treatment procedures change due to the molecularly heterogeneous and complex formation of lung cancer. In general, patients with stage I, II, and III undergo surgery to remove the tumor, whereas cytotoxic combination chemotherapy is the first-line treatment for patients with stage IV. However, during the recent years treatment has changed from classical chemotherapy to targeted and better tolerated therapy [1,2].

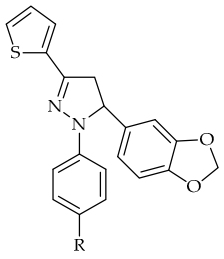
Pyrazoline is a five membered heterocyclic ring containing two adjacent nitrogen atoms. Diversely substituted pyrazoline derivatives have been reported to display various pharmacological effects including anticancer, antibacterial, antifungal, antiamoebic, antiviral, anti-inflammatory, analgesic, antidepressant, antiepileptic, antioxidant and antidiabetic activities. In particular, some pyrazoline-containing agents possess chemopreventive properties in addition to their cytotoxic effects on different types of cancer ranging from lung, breast, brain, bone, mouth, stomach, liver, bladder, pancreas, cervix, colon, rectum to prostate cancers [3].

In the current work, 1-(phenyl/4-substituted phenyl)-3-(2-furanyl/thienyl)-5-aryl-2-pyrazolines (**1–18**) (Figure 1), which had been synthesized previously by our research group [4], were evaluated for their cytotoxic effects against A549 human lung adenocarcinoma and CCD-19Lu human lung fibroblast cells.



1-9

Compound	R
1	H
2	CN
3	F
4	Cl
5	Br
6	CH_3
7	OCH_3
8	SO_2CH_3
9	SO_2NH_2



10-18

Compound	R
10	H
11	CN
12	F
13	Cl
14	Br
15	CH_3
16	OCH_3
17	SO_2CH_3
18	SO_2NH_2

Figure 1. The structures of pyrazoline-based compounds **1–18**.

2. Materials and Methods

2.1. Chemistry

1-(Phenyl/4-substituted phenyl)-3-(2-furanyl)-5-(4-methylphenyl)-2-pyrazolines (**1–9**) were obtained via the cyclization of 1-(2-furanyl)-3-(4-methylphenyl)-2-propen-1-one with phenylhydrazine hydrochloride derivatives in the presence of acetic acid. 1-(Phenyl/4-substituted phenyl)-3-(2-thienyl)-5-(1,3-benzodioxol-5-yl)-2-pyrazolines (**10–18**) were also synthesized via the ring closure reaction of 1-(2-thienyl)-3-(1,3-benzodioxol-5-yl)-2-propen-1-one with phenylhydrazine hydrochloride derivatives in the presence of acetic acid. The synthetic protocol and spectral data of 1-(phenyl/4-substituted phenyl)-3-(2-furanyl/thienyl)-5-aryl-2-pyrazolines (**1–18**) were reported previously by our research group [4].

2.2. Anticancer Activity

2.2.1. Cell Culture

A549 Human lung adenocarcinoma cells (ATCC® CCL-185™) and CCD-19Lu human lung fibroblast cells (ATCC® CCL-210™) were obtained from American Type Culture Collection (ATCC). A549 cells were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% fetal bovine serum and 1% penicillin/streptomycin and CCD-19Lu cells were grown in EMEM 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% CO_2 atmosphere. Compounds **1–18** and cyclophosphamide (positive control) were dissolved in dimethyl sulfoxide (DMSO) and diluted to working concentrations with fresh medium. Control group (solvent control) was prepared with medium containing 0.1% DMSO.

2.2.2. Cell Viability Assay

Cell viability was determined by MTT [(3-(4,5-Dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide] assay to obtain IC_{50} concentrations of compounds **1–18** and cyclophosphamide as described previously [5]. Cells were grown in 96-well plates at a density of 5×10^3 cells per well and subjected to different concentrations of the compounds and cyclophosphamide (400, 200, 100, 50 and 25 μg/mL). After 24 h incubation, MTT solution was added to wells to reach a final concentration of 0.5 mg/mL. The cells were incubated for another 4 h and then current medium was changed with 100 μL DMSO solution. The absorbance values were measured at 540 nm using a Cytation 3 Cell Imaging

Multi-Mode Reader (BioTek, Winooski, VT, USA). Cell survival rates were expressed as the percentage of the DMSO (0.1%) solvent control.

2.2.3. Statistical Analysis

Graphics were drawn with Graphpad Prism 6.0 software and statistically analyzed using one-way ANOVA and Tukey's post hoc test. Results are expressed as mean ± standard deviation and the means of three independent experiments (n = 8), n.s; $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ were considered significant compared to the control group.

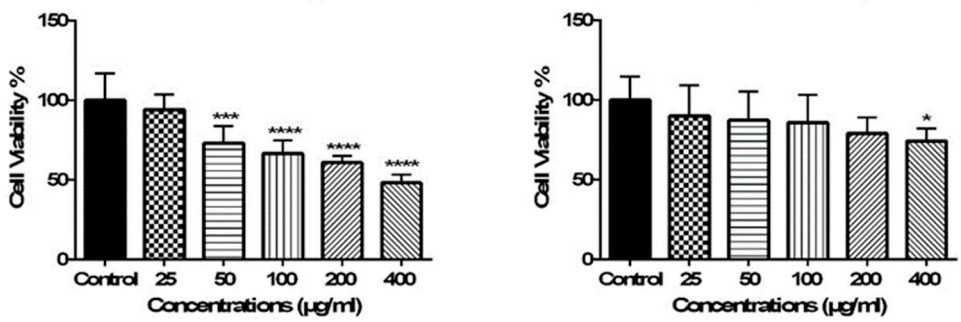
3. Results and Discussion

Treatment with compounds **6**, **7**, **10**, **12**, **13**, **14**, **15** and **18** for 24 h reduced cell viability significantly in A549 cell line with IC_{50} values of 361.87, 138.63, 361.49, 374.47, 217.27, 341.87, 229.69, 297.35 μg/mL, respectively when compared with the positive control cyclophosphamide (IC_{50} = 309.97 μg/mL) as shown in Table 1. On the other hand, compound **18** also reduced cell viability in CCD-19Lu cell line with an IC_{50} value of 357.51 μg/mL indicating low selectivity to cancer cells (Table 1).

Table 1. IC_{50} values of compounds **1–18** and cyclophosphamide.

Compound	IC_{50} (μg/mL)	
	A549 Cell Line	**CCD-19Lu Cell Line**
1	>400	>400
2	>400	>400
3	>400	>400
4	>400	>400
5	>400	>400
6	361.87	>400
7	138.63	>400
8	>400	>400
9	>400	>400
10	361.49	>400
11	>400	>400
12	374.47	>400
13	217.27	>400
14	341.87	>400
15	229.69	>400
16	>400	>400
17	>400	>400
18	297.35	357.51
Cyclophosphamide	309.97	>400

In general, compounds **10–18** exhibited more significant cytotoxic effects than compounds **1–9** against A549 cell line in relation to the presence of thiophene and 1,3-benzodioxole moieties. Among compounds **1–9**, only 4-methylphenyl substituted compound **6** and 4-methoxyphenyl substituted compound **7** showed notable anticancer activity. In particular, compound **7** was found as the most promising anticancer agent among all compounds with an IC_{50} value of 138.63 μg/mL. The cytotoxic effects of compound **7** on A549 and CCD-19Lu cell lines were depicted in Figure 2. Compounds **10**, **12**, **13**, **14**, **15** and **18** showed notable anticancer activity. Among these compounds, 4-chlorophenyl substituted compound **13** and 4-methylphenyl substituted compound **15** were determined as the most effective agents. Therefore, it can be concluded that the 4-methylphenyl moiety at the 1st position of the pyrazoline scaffold enhanced anticancer activity as observed in compounds **6** and **15**.



(**a**) (**b**)

Figure 2. Cytotoxic effects of compound **7** on cell viability of A549 (**a**) and CCD-19Lu (**b**) cell lines. The results are the means of three independent experiments. The error bars represent the standard deviations ((*n* = 8), $P > 0.05$ not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ compared to the control group).

In an attempt to predict the physicochemical properties of compounds **1–18**, in silico Absorption, Distribution, Metabolism and Excretion (ADME) studies were carried out previously by our research group [4] using QikProp module of Schrödinger's Molecular modelling package. The results indicated that all compounds were within the acceptable range intended for human use, making these derivatives as promising drug candidates.

Author Contributions: Conceptualization, B.S., M.D.A. and A.Ö.; methodology, B.S., E.K.T., M.D. and A.Ö.; software, B.S. and E.K.T.; validation, A.Ö. and M.D.; formal analysis, B.S., A.Ö., E.K.T. and M.D.; investigation, B.S.; resources, M.D.A., M.D. and A.Ö.; data curation, B.S., M.D.A., E.K.T., M.D. and A.Ö.; writing—original draft preparation, B.S.; writing—review and editing, B.S., M.D.A., E.K.T., M.D. and A.Ö.; visualization, B.S., E.K.T. and M.D.; supervision, M.D.A. and A.Ö.

Conflicts of Interest: The authors report no conflicts of interest.

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