Istanbul J Pharm 50 (3): 176-180
DOI: 10.26650/IstanbulJPharm.2020.0086
Original Article

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

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Cite this article as: Sevincli, Z. S., Canturk, Z., Dikmen, M., & Karali, N. (2020). Anticancer and antituberculosis effects of 5-fluoro-1*H*-indole-2,3-dione 3-thiosemicarbazones. *Istanbul Journal of Pharmacy, 50* (3), 176-180.

ABSTRACT

Background and Aims: The aim of this study was to screen the *in vitro* anticancer/antituberculosis activities of 5-fluoro-1-methyl/ethyl-1*H*-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 IC_{50} values.
Results: 6a, **6b**, **6g**, **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** did not show cytotoxic effects on CCD-19Lu cell line. The antituberculosis effects of the compounds were investigated against MTB H37Rv cell groups using rifampicin as standard. It was determined that **6b**, **6c**, **6g-k**, **6n**, **7b**, **7j** 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-1*H*-indole-2,3-diones

INTRODUCTION

1*H*-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 1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives (Karalı, 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 1*H*-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 1*H*-indole-2,3-dione and alkylation of position 1 of 1*H*-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, Saira, Iqbal, Yaqub, & Khan, 2011; Pervez, Saira, Iqbal, Yaqub, & Khan, 2013; Lin et al. 2013). Researchs also showed that N^4-phenyl substituted thiosemicarbazone derivatives were significantly more active than N^4-alkyl and N^4-cycloalkyl thiosemicarbazone derivatives (Hall et al., 2009; Hall et al., 2011). 5-Fluoro-1*H*-indole-2,3-dione 3-[4-(4-methoxyphenyl)thiosemicarbazone] have been reported as selective MDR1 activity (Hall et

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Submitted: 08.09.2020
Revision Requested: 14.10.2020
Last Revision Received: 27.10.2020
Accepted: 18.11.2020

al., 2010; Brimacombe, Fales, Gottesman, Hall & Handley, 2012). It has been found that derivatives of the 5-fluoroisatin ring with methylene/ethylene bridges with fluoroquinolone 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-trifluoromethoxyisatin 3-thiosemicarbazone derivatives showed antituberculous activity (Karalı et al., 2007; Güzel, Karalı & Salman, 2008).

In this study, *in vitro* the anticancer and antituberculosis activities of 5-fluoro-1-methyl/ethyl-1*H*-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 & Karalı, 2020). The molecular and isomeric structures of **6h** and **6j** were determined by X-ray single crystal diffraction analysis (Atioğlu, Sevinçli, Karalı, Akkurt & Ersanlı, 2017a; Atioğlu, Sevinçli, Karalı, Akkurt & Ersanlı, 2017b). In the study where A549 (human lung adenocarsinoma cells) and U-87 MG (human glioblastoma cells) cell lines were used to determine the anticancer 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% CO_2 atmosphere. Cisplatin was used as positive control. Substances and positive control were dissolved in dimethyl sulfoxide (DMSO) and diluted to working concentrations with fresh medium. The control group (solvent control) was prepared with medium containing 0.1% DMSO like as working concentrations.

The viability of the cells were assessed by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Yellow MTT is reduced to purple formazan in the mitochondria of living cells. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore, the conversion can be directly related to the number of viable (living) cells (Mosmann, 1983; Dikmen, Öztürk & Öztürk, 2011).

In order to obtain IC_{50} concentrations of the samples, the cell viability was determined by a MTT assay. In short, cells were grown in 96-well plates at a density of 5×10^3 cells per well. A total of 70–80% confluent cells (after 24 h) were treated with concentrations (400, 200, 100 and 50 µM) of substances for 24 h in the growth medium. After 24 h incubation, a 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 the current medium was removed and 100 µL of DMSO solution was added. The absorbance values were measured at 540 nm using a Cytation 3 Cell Imaging Multi-Mode Reader (BioTek). The measured absorbance directly correlates with the number of viable cells. The cell viability rates were expressed as the percentage of the DMSO (0.1%) solvent control and then IC_{50} concentrations were calculated according to the analysis result. In the experiment, each group was performed in 8 wells of three independent experiments.

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-tuberculous 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 MTT 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% CO_2 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 $5x10^3$ 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, ^{1}H NMR, ^{13}C-NMR, HSQC-2D, HMBC-2D, HRMS-ESI+ and LCMS-ESI+) data (Sevinçli, Duran, Özbil & Karalı, 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) (Atioğlu, Sevinçli, Karalı, Akkurt & Ersanlı, 2017a; Atioğlu, Sevinçli, Karalı, Akkurt & Ersanlı, 2017b).

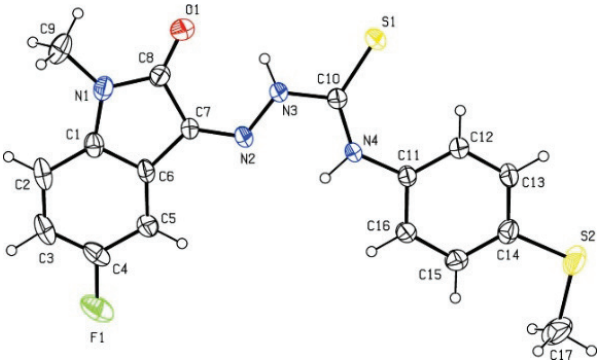

Figure 1. View of the molecular structure of **6h**, with the atom labelling

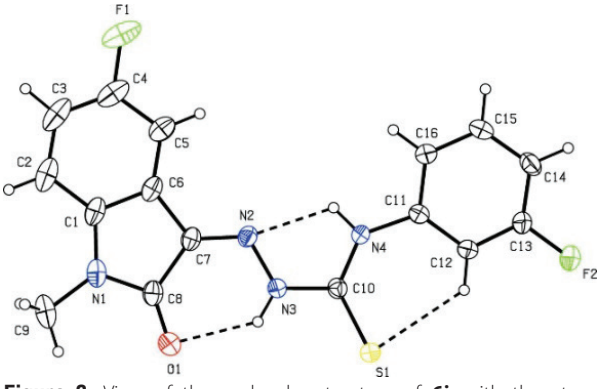

Figure 2. View of the molecular structure of **6j**, with the atom labelling.

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**, **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**, **7c** and **7k** 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_2 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 nonsubstituted **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 **6h** (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).

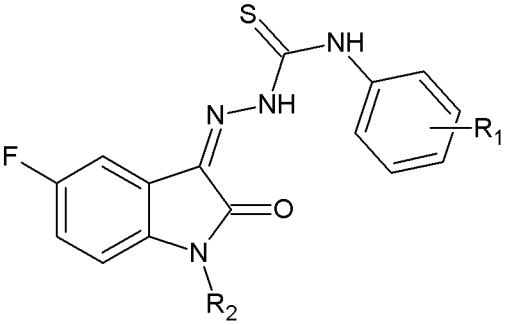

Figure 3. General structures of **6a-n** and **7a-n**.

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**, **6i**, **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-ethly substituted **6e** and **7e,** R_1 nonsubstituted **7a**, R_1 4-methly 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).

Table 1. Anticancer activities of 6a-n and 7a-n against A549/ U-87 MG cell lines and cytotoxic effect for CCD-19Lu cell line.

Compounds	R_1	R_2	IC_{50} (μM)		
			A549	U-87 MG	CCD-19Lu
6a	H	CH_3	**51.2**	>400	**>400**
6b	3-CH_3	CH_3	**26.8**	>400	**>400**
6c	4-CH_3	CH_3	91.2	165.4	302.0
6d	4-CF_3	CH_3	76.0	**46.6**	155.0
6e	4-C_2H_5	CH_3	172.8	>400	>400
6f	3-OCH_3	CH_3	94.9	354.8	73.2
6g	4-OCH_3	CH_3	**16.4**	182.2	267.8
6h	4-SCH_3	CH_3	**50.1**	**93.9**	160.9
6i	4-OCF_3	CH_3	187.0	150.9	176.5
6j	3-F	CH_3	97.4	384.1	205.1
6k	4-F	CH_3	>400	>400	303.0
6l	3-Cl	CH_3	**20.6**	**84.7**	98.3
6m	4-Cl	CH_3	136.9	244.6	359,6
6n	4-Br	CH_3	**35.5**	**61.4**	91.8
7a	H	C_2H_5	144.2	>400	>400
7b	3-CH_3	C_2H_5	211.2	285.8	>400
7c	4-CH_3	C_2H_5	**35.8**	>400	170.0
7d	4-CF_3	C_2H_5	316.0	**34.9**	198.1
7e	4-C_2H_5	C_2H_5	>400	>400	>400
7f	3-OCH_3	C_2H_5	247.2	**91.1**	250.6
7g	4-OCH_3	C_2H_5	>400	>400	>400
7h	4-SCH_3	C_2H_5	365.0	>400	>400
7i	4-OCF_3	C_2H_5	118.1	>400	268,8
7j	3-F	C_2H_5	90.7	>400	260.2
7k	4-F	C_2H_5	**10.6**	196.9	93.3
7l	3-Cl	C_2H_5	**58.8**	>400	**>400**
7m	4-Cl	C_2H_5	>400	173,8	>400
7n	4-Br	C_2H_5	>400	>400	>400
Cisplatin			**70.3**	**96.7**	**>400**

Table 2. Antituberculosis activities of 6a-n ve 7a-n against MTB H37Rv.

Compounds	R_1	R_2	IC_{50} (μg/mL)
			MTB H37Rv (ATCC 27294)
6a	H	CH_3	500,00
6b	3-CH_3	CH_3	**31,25**
6c	4-CH_3	CH_3	**31,25**
6d	4-CF_3	CH_3	**62,50**
6e	4-C_2H_5	CH_3	**62,50**
6f	3-OCH_3	CH_3	500
6g	4-OCH_3	CH_3	**31,25**
6h	4-SCH_3	CH_3	**31,25**
6i	4-OCF_3	CH_3	**31,25**
6j	3-F	CH_3	**31,25**
6k	4-F	CH_3	**31,25**
6l	3-Cl	CH_3	500
6m	4-Cl	CH_3	500
6n	4-Br	CH_3	**31,25**
7a	H	C_2H_5	**62,50**
7b	3-CH_3	C_2H_5	**31,25**
7c	4-CH_3	C_2H_5	**62,50**
7d	4-CF_3	C_2H_5	**62,50**
7e	4-C_2H_5	C_2H_5	**62,50**
7f	3-OCH_3	C_2H_5	**62,50**
7g	4-OCH_3	C_2H_5	500
7h	4-SCH_3	C_2H_5	500
7i	4-OCF_3	C_2H_5	**62,5**
7j	3-F	C_2H_5	**31,25**
7k	4-F	C_2H_5	**62,50**
7l	3-Cl	C_2H_5	**31,25**
7m	4-Cl	C_2H_5	**62,50**
7n	4-Br	C_2H_5	500
Rifampicin			**25**

Table 3. Cytotoxic effect of 6b, 7b and 7l on BJ cell line.

Compounds	R_1	R_2	IC_{50} (μg/mL)
			BJ (ATCC® CRL-2522™)
6b	3-CH_3	CH_3	>500
7b	3-CH_3	C_2H_5	>500
7l	3-Cl	C_2H_5	>500

CONCLUSIONS

In summary, the anticancer and antituberculosis activities of 5-fluoro-1-methyl/ethyl-1*H*-indole-2,3-dione 3-[4-(4-substituted phenyl)thiosemicarbazones] derivatives previously synthesized by our research group have been performed and promising results have been obtained. The R_2 methyl substituted derivatives **6a-n** are generally more effective than the R_2 ethyl substituted derivatives **7a-n** against the A549 and U-87 MG cell lines. However, antituberculosis activity is generally increased with R_2 ethyl substitution. It is one of the most important findings that the R_1 unsubstituted **6a**, R_1 3-methyl substituted **6b** and R_1 3-chloro substituted **7l** compounds are selective and nontoxic effects on the A549 cell line. In addition, the prototype

selected antituberculosis effective R_1 3-methyl substituted **6b** and **7b** and R_1 3-chloro substituted **7l** compounds were found to be nontoxic on BJ cell line. These results show the importance of R_1 3-methyl and 3-chlorine substitution.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Z.Ş.S., N.K., Z.C., M.D.; Data Acquisition- Z.C., M.D.; Data Analysis/Interpretation- Z.Ş.S., N.K., Z.C., M.D.; Drafting Manuscript- Z.Ş.S., N.K.; Critical Revision of Manuscript- Z.Ş.S., N.K., Z.C., M.D.; Final Approval and Accountability- Z.Ş.S., N.K., Z.C., M.D.; Technical or Material Support- Z.Ş.S., N.K., Z.C., M.D.; Supervision- Z.Ş.S., N.K.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was financially supported by Scientific and Technological Research Council of Turkey (TÜBİTAK). (Grant number: 1003-215S011)

REFERENCES

- Atioğlu, Z., Sevinçli, Z. Ş., Karalı, N., Akkurt, M., & Ersanlı C. C. (2017a). 2-(5-Fluoro-1-methyl-2-oxoindolin-3-ylidene)-N-[4-(methylsulfanyl)phenyl]hydrazine-1-carbothioamide. IUCrData, *2*(5), x170671.
- Atioğlu, Z., Sevinçli, Z. Ş., Karalı, N., Akkurt, M., & Ersanlı C. C. (2017b). (2Z)-2-(5-Fluoro-1-methyl-2-oxoindolin-3-ylidene)-N-(3-fluorophenyl)hydrazine-1-carbothioamide. *IUCrData*, *2*(6), x170900.
- Banerjee, D., Yogeeswari, P., Bhat, P., Thomas, A., Srividya, M., & Sriram, D. (2011). Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. *European Journal of Medicinal Chemistry*, *46*(1), 106–121.
- Brimacombe K., Fales H. M., Gottesman M. M., Hall, M. D., & Handley, M. (2012). *W.O. Patent No. 2012033601 A1*.
- Dikmen, M., Özturk, N., & Özturk, Y. The antioxidant potency of Punica granatum L. Fruit peel reduces cell proliferation and induces apoptosis on breast cancer. *Journal of Medicinal Food*, *14*(12), 1638–1646.
- Feng, L. S., Liu, M. L., Wang, B., Chai, Y., Hao, X. Q., Meng, S., & Guo, H. Y. (2010). Synthesis and *in vitro* antimycobacterial activity of balofloxacin ethylene isatin derivatives. *European Journal of Medicinal Chemistry, 45*, 3407–3412.
- Foongladda, S., Roengsanthia, D., Arjrattanakool, W., Chuchottaworn, C., Chaiprasert, A., & Franzblau, S. G. (2002). Rapid and simple MTT method for rifampicin and isoniazid susceptibility testing of *Mycobacterium tuberculosis. The International Journal of Tuberculosis and Lung Disease*, *6*(12), 1118–1122.
- Güzel, Ö., Karalı, N., & Salman, A. (2008). Synthesis and antituberculosis activity of 5-methyl/ trifluoromethoxy-1H-indole-2,3-dione 3-thiosemicarbazone derivatives. *Bioorganic & Medicinal Chemistry*, *16*(19), 8976–8987.
- Hall, M. D., Salam, N. K., Hellawell, J. L., Fales, H. M., Kensler, C. B., Ludwig, J. A. … Gottesman, M. M. (2009). Synthesis, activity, and pharmacophore development for isatin-β-thiosemicarbazones with selective activity toward multidrug-resistant cells. *Journal of Medicinal Chemistry*, *52*(10), 3191–3204.
- Hall, M. D., Gottesman, M. M., Hellawell, J. L., Ludwig, J. A., Fales, H. M., Salam, N. K., & Szakács, G. (2010). *U.S. Patent Application No. 12/867,206*.
- Hall, M. D., Brimacombe, K. R., Varonka, M. S., Pluchino, K. M., Monda, J. K., Li, J. … Gottesman, M. M. (2011). Synthesis and structure-activity evaluation of isatin-β-thiosemicarbazones with improved selective activity toward multidrug-resistant cells expressing P-glycoprotein. *Journal of Medicinal Chemistry, 54*, 5878–5889.
- Karalı, N. (2002). Synthesis and primary cytotoxicity evaluation of new 5-nitroindole-2,3-dione derivatives. *European Journal of Medicinal Chemistry*, *37*(11), 909–918.
- Karalı, N., Gürsoy, A., Kandemirli, F., Shvets, N., Kaynak, F. B.,Özbey, S. … Dimoglo, A. (2007). Synthesis and structure–antituberculosis activity relationship of 1*H*-indole-2,3-dione derivatives. *Bioorganic & Medicinal Chemistry, 15*(17), 5888–5904.
- Karalı, N., Akdemir, A., Göktaş, F., Eraslan-Elma, P., Angeli, A., Kızılırmak, M. & Supuran, C. T. (2017). Novel sulfonamide-containing 2-indolinones that selectively inhibit tumor-associated alpha carbonic anhydrases. *Bioorganic & Medicinal Chemistry*, *25*(14), 3714–3718.
- Lin, H. H., Wu, W. Y., Cao, S. L., Liao, J., Ma, L., Gao, M. ... Xu, X. (2013). Synthesis and antiproliferative evaluation of piperazine-1-carbothiohydrazide derivatives of indolin-2-one. *Bioorganic & Medicinal Chemistry Letters*, *23*(11), 3304–3307.
- Matesic, L., Locke, J. M., Bremner, J. B., Pyne, S. G., Skropeta, D., & Ranson, M. (2008). N-phenethyl and N-naphthylmethyl isatins and analogues as *in vitro* cytotoxic agents. *Bioorganic & Medicinal Chemistry, 16*, 3118–3124.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55–63.
- Pape, V. F. S., Tóth, S., Füredi, A., Szebényi, K., Lovrics, A., Szabó, P. … Szakács, G. (2016). Design, synthesis and biological evaluation of thiosemicarbazones, hydrazinobenzothiazoles and arylhydrazones as anticancer agents with a potential to overcome multidrug resistance. *European Journal of Medicinal Chemistry*, *117*, 335–354.
- Pervez, H., Saira, N., Iqbal, M. S., Yaqub, M., & Khan, K. M. (2011). Synthesis and toxicity evaluation of some N4-aryl substituted 5-trifluoromethoxyisatin-3-thiosemicarbazones. *Molecules*, *16*(8), 6408–6421.
- Pervez, H., Saira, N., Iqbal, M. S., Yaqub, M., & Khan, K. M. (2013). Synthesis and biological evaluation of some N 4-aryl-substituted 5-fluoroisatin-3-thiosemicarbazones. *Medicinal Chemistry Research*, *22*(12), 5878–5889.
- Priyanka, K. B., Manasa, C., & Sammaiah, G. (2014). Synthesis and evaluation of new isatin derivatives for cytotoxic activity. *World Journal of Pharmacy and Pharmaceutical Sciences, 3*, 2393–2402.
- Raut, U., Narang, P., Mendiratta, D. K., Narang, R., & Deotale, V. (2008). Evaluation of rapid MTT tube method for detection of drug susceptibility of *Mycobacterium tuberculosis* to rifampicin and isoniazid. *Indian Journal of Medical Microbiology, 26*, 222–227.
- Sabet, R., Mohammadpour, M., Sadeghi, A. & Fassihi, A. (2010). QSAR study of isatin analogues as *in vitro* anti-cancer agents, *European Journal of Medicinal Chemistry, 45*, 1113–1118.
- Sevinçli, Z. Ş., Duran, G., Özbil, M., & Karalı, N. (2020). Synthesis, molecular modeling and antiviral activity of novel 5-fluoro-1*H*-indole-2,3-dione 3-thiosemicarbazones. *Bioorganic Chemistry*, In press, https://doi.org/10.1016/j.bioorg.2020.104202
- Vine, K. L., Locke, J. M., Ranson, M., Pyne, S. G., & Bremner, J. B. (2007). An investigation into the cytotoxicity and mode of action of some novel N-alkyl-substituted isatins. *Journal of Medicinal Chemistry, 50*, 5109–5117.