Journal of Research in Pharmacy

Research Article

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# Synthesis and potential antitumor activities of mandelic acid linked 2-aryl-1,3-thiazolidin-4-ones

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Received: 11 February 2022 / Revised: 4 April 2022 / Accepted: 4 April 2022

**ABSTRACT**: In our pursuit to discover new anticancer agents, we have synthesized a new series of 2-aryl-1,3-thiazolidin-4-one derivatives carrying a mandelic acid part. Compounds **3a-h** and **4a-j** have been synthesized with the cyclization of mandelic acid-derived Schiff bases, their structures have been elucidated with spectral methods ($^{1}$H-NMR, $^{13}$C-NMR, and LC/MS-APCI) and elemental analyzes (C, H, N). Five compounds **3d, 3e, 4e, 4f,** and **4g** chosen as prototypes were evaluated against the full panel of 58 human tumor cell lines in the National Cancer Institute's *in vitro* primary cytotoxicity assay (Bethesda, Maryland). Among the tested compound, **3e** showed good cytotoxic effects against melanoma cell line UACC-62. Compounds **3d**, **4e**, **4f**, and **4g** showed moderate cytotoxic effects, especially against leukemia cell line, RPMI-8226, non-small cell lung cancer cell lines NCI-H23 and HOP-92, central nervous system (CNS) cancer cell lines SF-295 and SNB-75, melanoma cell line UACC-62, and ovarian cancer cell line IGROV1. It is considered that the *in vitro* test results of selected compounds are promising and an additional mandelic acid moiety to core structure 2-aryl-1,3-thiazolidin-4-one and different modifications may result in effective agents in anticancer treatment.

**KEYWORDS**: 1,3-Thiazolidin-4-one; mandelic acid; synthesis; anticancer agents; *in vitro* cytotoxicity assay.

## 1. INTRODUCTION

Cancer is the name of a group of diseases, which is characterized by an uncontrolled division of the body's cells that is able to break the healthy cell cycle and endogenous control mechanism [1,2]. According to the GLOBOCAN (The Global Cancer Observatory) platform of the World Health Organization (WHO), cancer is one of the foremost leading causes of death globally and nearly 19.3 million cases and 10 million deaths in 2020 were accounted [3]. In addition to oncologic surgery, current systematic treatments like chemotherapy, immunotherapy, and anti-angiogenic agents present options for the treatment of cancer [4]. But the existence of multiple drug resistance mechanisms against these treatments [5] and side effects [6] have revealed the need for new anticancer agents.

Like many heterocyclics, thiazolidin-4-ones have always fascinated medicinal chemists because of their therapeutic diversity. Up to date, this attractive core structure has been investigated many times for its antimycobacterial [7], antibacterial [8], antiparasitic [9], antifungal [10], antiviral [11,12], anticancer [13,14], and other possible activities. Similarly, Güzel-Akdemir's research groups reported the synthesis of some 1,3-thiazolidin-4-one derivatives fused with different structures outlined in **Figure 1**. These compounds showed a wide range of biological effects such as anticancer (i.e. compound **A**) [15], antibacterial (i.e. compound **B**) [16], antifungal and anticancer activities through fungal and human carbonic anhydrase (CgNce103 and hCA) inhibition (i.e. compound **C**) [17,18], and antimycobacterial (i.e. compounds **D**, **E**, **F,** and **G**) [19–22] activities.

Based on the studies explained above and summarized in **Figure 1**, in this study, we have been inspired by the mandelic acid part of compound **C** and the 2-aryl-1,3-thiazolidin-4-one core of compound **A**, and followed the idea to take advantage of both structures to design new anticancer agents. Herein we report the synthesis and structural elucidation of two small series of 2-aryl-1,3-thiazolidin-4-ones and share the *in vitro* primary cytotoxicity test results of **3d**, **3e**, **4e**, **4f**, and **4g**, against different human tumor cell lines of **3d**, **3e**, **4e**, **4f**, and **4g**, selected by the National Cancer Institute (NCI), Bethesda, Maryland among the 18 new compounds.

**How to cite this article:** Demir-Yazıcı K, Güzel-Akdemir Ö. Synthesis and potential antitumor activities of mandelic acid linked 2-aryl-1,3-thiazolidin-4-ones. J Res Pharm. 2022; 26(4): 931-940.


ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.191

The primary results showed that chosen prototypes performed diverse inhibitory activities against different tumor cell lines, which makes the new derivatives as potent anticancer agent candidates.

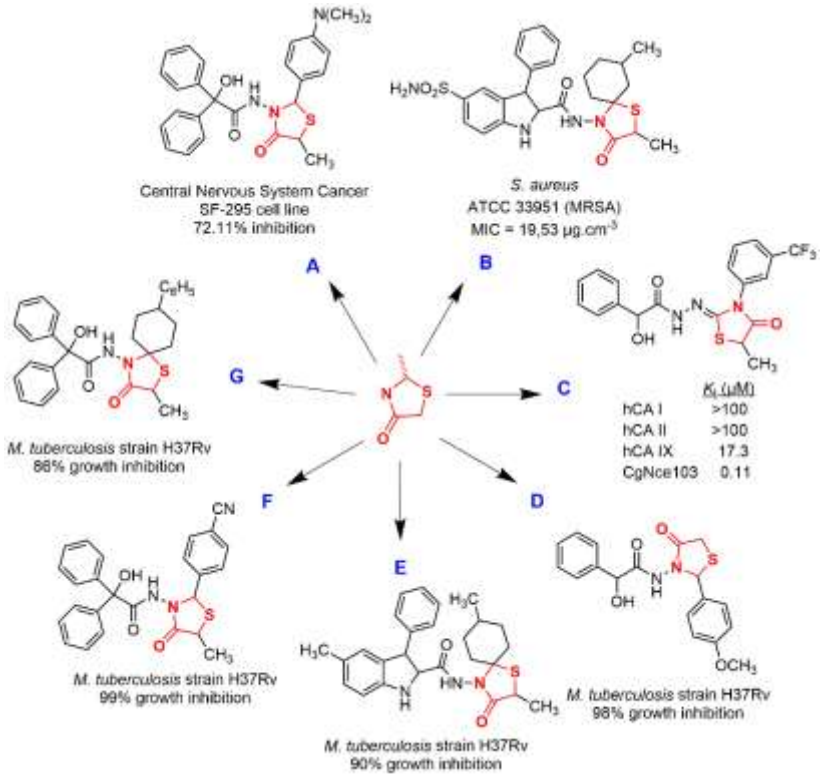
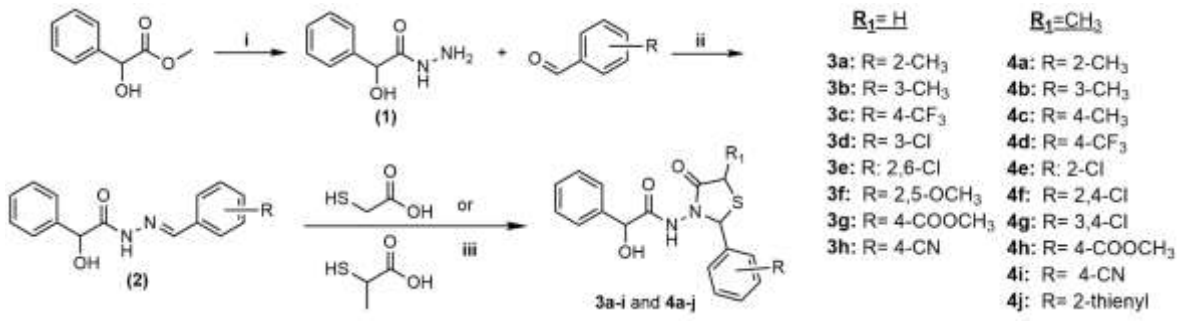



**Figure 1.** Previously synthesized 1,3-thiazolidin-4-one derivatives combined with different structures and biological activities.

## 2. RESULTS

### 2.1. Chemistry

The synthetic procedure of new 2-aryl-1,3-thiazolidin-4-ones has been summarized in **Scheme 1**. As explained in our previous study [19], the reaction of 2-hydroxy-2-phenylacetohydrazide (**1**) with suitable aromatic aldehydes has resulted in Schiff bases (**2**) which give a cyclocondensation reaction with mercaptoethanoic/2-mercaptopropanoic acid to yield targeting compounds **3a-h**, and **4a-j**, respectively.




**Scheme 1.** Synthesis pathway and substitution patterns of new 2-aryl-1,3-thiazolidin-4-ones. i) hydrazine hydrate, ethanol, reflux, 6h; ii) ethanol, reflux, 4h; iii) dry benzene, reflux, 6h.

Chemical structures of new compounds have been determined with spectral analysis.

The IR spectra exhibited OH/N-H and C=O bands in the 3555-3205 and 1688-1640 $cm^{-1}$ regions, showing the common OH/NH and CONHN functions of **3a-h** and **4a-j**. Together with additional absorptions between the 1725-1687 $cm^{-1}$ region due to the characteristic lactam C=O functionality confirmed the success of the desired 4-thiazolidinone compounds [20,23,24].

The distinctive $^1$H-NMR signals of 4-thiazolidinones, i.e., the methylene (SC*H*$_2$) protons as two singlets or two doublets (δ 3.71-3.72, 3.82-3.89 ppm) for compounds **3a-h**, and methine (SC*H*CH$_3$) protons as two quartets (δ 3.99-4.03, 4.02-4.16 ppm) for compounds **4a-j** have been detected. Observation of these double signals is owing to the interactions of non-equivalent geminal methylene protons and methine protons with the chiral center at position 2 [25]. Signals that appeared at 5.69-6.11 ppm in the $^1$H-NMR spectra were attributed to 4-thiazolidinone C$_2$-H group [26].

$^{13}$C-NMR (APT) data of selected prototypes **4a**, **4h**, and **4j** also supported the structure of 4-thiazolidinone ring via SCH, *C*H, and C=O resonances appeared at 39.0-39.2, 56.52-60.5 and 171.7-172.3 ppm, respectively [25].

Abundant (M-H)$^-$ ions were observed in the APCI and ESI mass spectra of the selected compounds **4a**, **4d**, **4h,** and **4j** (4a: m/z 355, 4d: 409, 4h: m/z 399, and 4j: m/z 349) confirmed their molecular weights.

### 2.2. Antitumor Activity

Compounds **3d**, **3e**, **4e**, **4f**, and **4g** chosen by the National Cancer Institute were screened *in vitro* for antitumor activity against the National Cancer Institute's 58 human tumor cells, as outlined in **Table 1**. The primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch of the National Cancer Institute (Bethesda) [27,28]. For the compounds, the 50% growth inhibition (GI$_{50}$) and total growth inhibition (TGI) were obtained for each cell line. The log$_{10}$ GI$_{50}$ and log$_{10}$ TGI were then determined, defined as the mean of the log$_{10}$'s of the individual GI$_{50}$ and TGI values. Negative values indicated the most sensitive cell lines. Compounds having values –4 and < -4 were declared to be active.

**Table 1.** *In vitro* tumor cell growth inhibition of **3d**, **3e**, **4e, 4f** and **4g**.

| Panel/cell line | **3d** | | **3e** | | **4e** | | **4f** | | **4g** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | log$_{10}$GI$_{50}$ | log$_{10}$TGI | log$_{10}$GI$_{50}$ | log$_{10}$TGI | log$_{10}$GI$_{50}$ | log$_{10}$TGI | log$_{10}$GI$_{50}$ | log$_{10}$TGI | log$_{10}$GI$_{50}$ | log$_{10}$TGI |
| Leukemia | | | | | | | | | | |
| CCRF-CEM | >-4.00 | >-4.00 | -4.36 | >-4.00 | >-4.00 | >-4.00 | -4.18 | >-4.00 | -4.26 | >-4.00 |
| K-562 | -4.56 | >-4.00 | -4.53 | -4.12 | -4.23 | >-4.00 | -4.40 | >-4.00 | -4.24 | >-4.00 |
| MOLT-4 | -4.11 | >-4.00 | -4.60 | -4.09 | -4.30 | >-4.00 | -4.46 | >-4.00 | -4.57 | -4.09 |
| RPMI-8226 | **-4.64** | >-4.00 | -4.36 | >-4.00 | -4.35 | >-4.00 | -4.41 | >-4.00 | **-4.60** | -4.18 |
| SR | | | | | -4.40 | >-4.00 | | | | |
| HL-60(TB) | | | | | -4.08 | >-4.00 | | | | |
| Non-small cell lung cancer | | | | | | | | | | |
| A549/ATCC | -4.45 | >-4.00 | **-4.67** | -4.09 | -4.28 | >-4.00 | -4.47 | >-4.00 | -4.53 | -4.07 |
| EKVX | -4.19 | >-4.00 | -4.22 | >-4.00 | >-4.00 | >-4.00 | -4.16 | >-4.00 | -4.36 | >-4.00 |
| HOP-62 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.07 | >-4.00 |
| HOP-92 | >-4.00 | >-4.00 | **-4.68** | -4.00 | >-4.00 | >-4.00 | -4.44 | >-4.00 | -4.39 | >-4.00 |
| NCI-H226 | -4.09 | >-4.00 | -4.42 | >-4.00 | >-4.00 | >-4.00 | -4.36 | >-4.00 | -4.47 | >-4.00 |
| NCI-H23 | -4.25 | >-4.00 | **-4.70** | >-4.00 | - | - | -4.19 | >-4.00 | -4.48 | >-4.00 |
| NCI-H322M | >-4.00 | >-4.00 | -4.18 | >-4.00 | - | - | >-4.00 | >-4.00 | -4.22 | >-4.00 |
| NCI-H460 | -4.37 | >-4.00 | -4.39 | >-4.00 | -4.17 | >-4.00 | -4.32 | >-4.00 | -4.48 | >-4.00 |
| NCI-H522 | -4.17 | >-4.00 | -4.59 | >-4.00 | -4.26 | >-4.00 | -4.38 | >-4.00 | -4.55 | -4.01 |
| Colon cancer | | | | | | | | | | |
| COLO 205 | >-4.00 | >-4.00 | -4.47 | >-4.00 | | | >-4.00 | >-4.00 | -4.35 | >-4.00 |
| HCC-2998 | | | -4.19 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.43 | >-4.00 |
| HCT-116 | -4.08 | >-4.00 | -4.44 | >-4.00 | -4.02 | >-4.00 | -4.21 | >-4.00 | -4.37 | >-4.00 |
| HCT-15 | -4.16 | >-4.00 | -4.51 | >-4.00 | -4.17 | >-4.00 | -4.21 | >-4.00 | -4.34 | >-4.00 |
| HT29 | >-4.00 | >-4.00 | -4.48 | >-4.00 | >-4.00 | >-4.00 | -4.38 | >-4.00 | -4.49 | >-4.00 |
| KM12 | -4.04 | >-4.00 | -4.47 | >-4.00 | >-4.00 | >-4.00 | -4.29 | >-4.00 | -4.37 | >-4.00 |
| SW-620 | >-4.00 | >-4.00 | -4.14 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.11 | >-4.00 |
| CNS cancer | | | | | | | | | | |
| SF-268 | >-4.00 | >-4.00 | -4.22 | >-4.00 | >-4.00 | >-4.00 | -4.09 | >-4.00 | -4.25 | >-4.00 |
| SF-295 | **-4.65** | >-4.00 | -4.43 | >-4.00 | -4.49 | >-4.00 | **-4.63** | >-4.00 | **-4.61** | -4.17 |
| SF-539 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.14 | >-4.00 |
| SNB-19 | >-4.00 | >-4.00 | -4.15 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.03 | >-4.00 |
| SNB-75 | -4.19 | >-4.00 | -4.49 | >-4.00 | >-4.00 | >-4.00 | -4.49 | >-4.00 | **-4.61** | -4.05 |
| U251 | -4.23 | >-4.00 | -4.43 | >-4.00 | -4.17 | >-4.00 | -4.21 | >-4.00 | -4.39 | >-4.00 |

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**Table 1.** *In vitro* tumor cell growth inhibition of **3d**, **3e**, **4e, 4f** and **4g** (continuation)

| Panel/cell line | **3d** | | **3e** | | **4e** | | **4f** | | **4g** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $log_{10}GI_{50}$ | $log_{10}TGI$ | $log_{10}GI_{50}$ | $log_{10}TGI$ | $log_{10}GI_{50}$ | $log_{10}TGI$ | $log_{10}GI_{50}$ | $log_{10}TGI$ | $log_{10}GI_{50}$ | $log_{10}TGI$ |
| Melanoma | | | | | | | | | | |
| LOX IMVI | >-4.00 | >-4.00 | -4.19 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.26 | >-4.00 |
| MALME-3M | >-4.00 | >-4.00 | -4.50 | >-4.00 | - | - | -4.15 | >-4.00 | -4.34 | >-4.00 |
| M14 | >-4.00 | >-4.00 | -4.45 | >-4.00 | -4.03 | >-4.00 | -4.37 | >-4.00 | -4.37 | >-4.00 |
| SK-MEL-2 | >-4.00 | >-4.00 | -4.30 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.34 | >-4.00 |
| SK-MEL-28 | >-4.00 | >-4.00 | -4.20 | >-4.00 | >-4.00 | >-4.00 | -4.12 | >-4.00 | -4.41 | >-4.00 |
| SK-MEL-5 | >-4.00 | >-4.00 | -4.48 | >-4.00 | -4.19 | >-4.00 | -4.23 | >-4.00 | -4.42 | >-4.00 |
| UACC-257 | >-4.00 | >-4.00 | -4.51 | -4.02 | -4.31 | >-4.00 | -4.21 | >-4.00 | -4.46 | >-4.00 |
| UACC-62 | -4.64 | >-4.00 | **-5.15** | -4.36 | -4.35 | >-4.00 | **-4.62** | >-4.00 | **-4.60** | -4.06 |
| Ovarian cancer | | | | | | | | | | |
| IGROV1 | >-4.00 | >-4.00 | -4.39 | >-4.00 | -4.14 | >-4.00 | **-4.64** | -4.11 | -4.55 | -4.13 |
| OVCAR-3 | -4.47 | >-4.00 | -4.67 | -4.12 | -4.45 | >-4.00 | -4.54 | >-4.00 | -4.59 | -4.11 |
| OVCAR-4 | -4.18 | >-4.00 | -4.54 | >-4.00 | >-4.00 | >-4.00 | -4.55 | -4.01 | -4.49 | -4.04 |
| OVCAR-5 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.15 | >-4.00 |
| OVCAR-8 | -4.08 | >-4.00 | -4.28 | >-4.00 | >-4.00 | >-4.00 | -4.20 | >-4.00 | -4.47 | >-4.00 |
| SK-OV-3 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | | | >-4.00 | >-4.00 | >-4.00 | >-4.00 |
| Renal cancer | | | | | | | | | | |
| 786-O | >-4.00 | >-4.00 | -4.16 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.10 | >-4.00 |
| A498 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.17 | >-4.00 | >-4.00 | >-4.00 | -4.11 | >-4.00 |
| ACHN | -4.17 | >-4.00 | -4.55 | >-4.00 | -4.25 | >-4.00 | -4.31 | >-4.00 | -4.48 | >-4.00 |
| CAKI-1 | -4.04 | >-4.00 | **-4.62** | >-4.00 | -4.35 | >-4.00 | -4.22 | >-4.00 | -4.51 | >-4.00 |
| RXF 393 | -4.23 | >-4.00 | -4.53 | >-4.00 | >-4.00 | >-4.00 | -4.37 | >-4.00 | -4.52 | -4.08 |
| SN12C | >-4.00 | >-4.00 | -4.46 | >-4.00 | >-4.00 | >-4.00 | -4.07 | >-4.00 | -4.36 | >-4.00 |
| TK-10 | >-4.00 | >-4.00 | -4.10 | >-4.00 | **-4.51** | >-4.00 | -4.21 | >-4.00 | -4.38 | >-4.00 |
| UO-31 | -4.12 | >-4.00 | -4.53 | >-4.00 | -4.13 | >-4.00 | -4.31 | >-4.00 | -4.44 | >-4.00 |
| Prostate cancer | | | | | | | | | | |
| PC-3 | -4.22 | >-4.00 | **-4.53** | >-4.00 | -4.34 | >-4.00 | -4.37 | >-4.00 | -4.46 | >-4.00 |
| DU-145 | -4.08 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.23 | >-4.00 |
| Breast cancer | | | | | | | | | | |
| MCF7 | >-4.00 | >-4.00 | -4.35 | >-4.00 | -4.26 | >-4.00 | -4.24 | >-4.00 | -4.33 | >-4.00 |
| MDA-MB 231/ATCC | -4.06 | >-4.00 | **-4.72** | >-4.00 | >-4.47 | >-4.00 | -4.34 | >-4.00 | -4.50 | >-4.00 |
| HS 578T | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.07 | >-4.00 | >-4.00 | >-4.00 | -4.29 | >-4.00 |
| MDA-MB 435 | >-4.00 | >-4.00 | -4.60 | >-4.00 | >-4.00 | >-4.00 | -4.13 | >-4.00 | -4.38 | >-4.00 |
| BT-549 | >-4.00 | >-4.00 | -4.10 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | >-4.00 | -4.35 | >-4.00 |
| T-47D | -4.17 | >-4.00 | -4.39 | >-4.00 | - | - | >-4.00 | >-4.00 | -4.17 | >-4.00 |
| MG MID | -4.12 | -4.0 | -4.38 | -4.01 | -4.13 | -4.0 | -4.22 | -4.0 | -4.37 | -4.02 |
| Delta | 0.53 | 0 | 0.77 | 0.35 | 0.38 | 0 | 0.42 | 0.11 | 0.24 | 0.16 |
| Range | 0.65 | 0.0 | 1.15 | 0.36 | 0.51 | 0.0 | 0.64 | 0.11 | 1.61 | 0.18 |

All tested new 2-aryl-1,3-thiazolidin-4-one derivatives showed antitumor activity against different tumor cell lines ($log_{10}GI_{50}$ values < -4). Among the compounds tested, **3d** exhibited the best cytotoxicity on leukemia, on cell line RPMI-8226 ($log_{10}GI_{50}$ value –4.64) and CNS (central nervous system) cancer, on cell line SF-295 ($log_{10}GI_{50}$ value –4.65). Compound **3e** performed the largest number of effective toxicity and the most effective growth inhibition among the tested tumor cell lines: Against non-small cell lung cancer on cell lines NCI-H23 ($log_{10}GI_{50}$ value –4.70), HOP-92 ($log_{10}GI_{50}$ value –4.68) and A549/ATCC ($log_{10}GI_{50}$ value –4.67), on melanoma cell line UACC-62 ($log_{10}GI_{50}$ value –5.15), renal cancer on cell line CAKI-1 ($log_{10}GI_{50}$ value –4.63), prostate cancer on cell line PC-3 ($log_{10}GI_{50}$ value –4.53), and breast cancer on cell line MDA-MB 231/ATCC ($log_{10}GI_{50}$ value –4.72) Compound **4f** showed cytotoxicity on CNS cancer cell line (SF-295, $log_{10}GI_{50}$ value –4.63), melanoma (UACC-62, $log_{10}GI_{50}$ value –4.62) and the best inhibitory activity against ovarian cancer cell line (IGROV1, $log_{10}GI_{50}$ value –4.64). Compound **4g** exhibited cytotoxicity on leukemia cell line (RPMI-8226, $log_{10}GI_{50}$ value –4.60), two CNS cancer cell lines (SF-295, $log_{10}GI_{50}$ value –4.61; SNB-75, $log_{10}GI_{50}$ value –4.61), and melanoma cell line (UACC-62, $log_{10}GI_{50}$ value –4.60). And lastly, compared with other selected

http://dx.doi.org/10.29228/jrp.191
**J Res Pharm 2022; 26(4): 931-940**

compounds, **4e** performed less cytotoxicity on the aforementioned tumor cell lines but showed slight inhibitory activity against renal cancer on cell line TK-10 ($log_{10}$ $GI_{50}$ value –4.51).

## 3. CONCLUSION

Our small new 2-aryl-1,3-thiazolidin-4-one collection with 18 members has been successfully synthesized and their chemical structures have been proven by spectral analyses and supported with previous studies. As expected, due to the biologically active 4-thiazolidinone scaffold they carried, selected and tested compounds showed a wide range of antitumor activity from moderate to good against different 58 tumor cell lines. As a result, it is right to say that further investigations on different varieties of 4-thiazolinones with diverse structures like mandelic acid moiety seem strongly probable to lead to potent anticancer agents.

## 4. MATERIALS AND METHODS

### 4.1. Chemistry

DL methyl 2-hydroxy-2-phenylacetate, all used aromatic aldehyde derivatives, mercaptoacetic acid, and 2-mercaptopropionic acid were commercially available. The key intermediate 2-hydroxy-2 phenylacetohydrazide was obtained according to the referred literature [29]. Melting points of newly synthesized compounds were measured by the open capillary method with a Buchi 530 melting point apparatus and uncorrected. Infrared spectra (KBr disk) were recorded as $\upsilon_{max}$ $cm^{-1}$ using a Perkin-Elmer 1600 FTIR. $^{1}$H-NMR and $^{13}$C-NMR (APT) were performed on Bruker AC 200 / 250 MHz, Bruker DPX 400 MHz, and Varian$^{UNITY}$INOVA 500 MHz spectrometers. Elemental analyses were conducted with a Leco 932 elemental analyzer. Mass spectra (LC/MS-APCI and ESI) were recorded on a Finnigan ™ LCQ ™ Mass Spectrometer in the negative or positive ionization mode.

*4.1.1. Synthesis of 2-Hydroxy-2-phenyl-N'-[(substituted phenyl)methylene]acetohydrazides (**2**)*

6.6 mmol of a selected aromatic aldehyde was added to 30 ml ethanolic solution of 6 mmol **1**, and heated under reflux for 4 h. The obtained precipitate was either washed with ethanol or recrystallized from ethanol for purification [19].

*4.1.2. General procedure for synthesis of 2-Hydroxy-N-(5-methyl-/4-oxo-2-substitutedphenyl-1,3-thiazolidin-3-yl)-2-phenylacetamides (**3-4**)*

A mixture of 5 mmol **2** and 20 mmol mercaptoacetic acid or 2-mercaptopropionic acid was refluxed in 30 ml dry benzene for 6 h using a Dean-Stark water separator. The remaining solvent was evaporated, then the obtained crude product was triturated with saturated $NaHCO_3$ solution until $CO_2$ evolution stopped. Yielded crude product was refrigerated overnight, washed with $H_2O$, dried, and crystallized from EtOH/ $H_2O$ [19].

*2-Hydroxy-N-[2-(2-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide (**3a**)*

Yield %14; m. p. 213-215 °C. IR (ν, $cm^{-1}$): 3334 (OH, NH); 1711, 1681 (C=O). $^{1}$H -NMR (500 MHz, DMSO-$d_6$, δ, ppm): 2.12 (3H,s, -$CH_3$); 3.71, 3.82 (2H, 2d, *J*=15.73Hz, $SCH_2$); 4.96 (1H, d, *J*=5.37Hz, C<u>H</u>OH); 6.01 (1H, s, thiaz. $C_2$-H); 6.15 (1H, d, *J*=5.37Hz, CHO<u>H</u>); 7.10-7.23 (8H, m, Ar-H); 7.47 (1H, s, Ar-H); 10.35 (1H, s, CONH). Anal. Cald. for $C_{18}H_{18}N_2O_3S$ (342.41): C, 63.14; H, 5.30; N, 8.18. Found: C, 62.52; H, 5.34; N, 8.07.

*2-Hydroxy-N-[2-(3-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide (**3b**)*

Yield 18%; m.p. 220-222 °C. IR (ν, $cm^{-1}$): 3333 (NH); 1709, 1670 (C=O). $^{1}$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 2.23 (3H, s, $CH_3$); 3.77 (2H, dd, *J*=16.0 Hz, thiaz. $C_5$-H); 4.99 (1H, s, C*H*OH); 5.69 (1H, s, thiaz. $C_2$-H); 6.14 (1H, br s, CHO<u>H</u>); 7.09-7.23 (9H, m, Ar-H); 10.27 (1H, s, CONH). Anal. Calcd. for $C_{18}H_{18}N_2O_3S$ (342.41): C, 63.14; H, 5.30; N, 8.18. Found: C, 62.96; H, 5.34; N, 8.12.

*2-Hydroxy-N-{4-oxo-2-[4-(trifluoromethyl)phenyl]-1,3-thiazolidin-3-yl}-2-phenylacetamide (**3c**)*

Yield 33%; m.p. 207-209 °C. IR (ν, $cm^{-1}$): 3567, 3348 (OH, NH); 1710, 1673 (C=O). $^{1}$H -NMR (500 MHz, DMSO-$d_6$, δ, ppm): 3.71, 3.82 (2H, 2d, *J*=15.73Hz, $SCH_2$); 4.86 (1H, d, *J*=4.39 Hz, C<u>H</u>OH); 5.92 (1H, d, *J*=7.32 Hz, thiaz. $C_2$-H); 6.15 (1H, d, *J*=5.37 Hz, CHO<u>H</u>); 7.10-7.21 (5H, m, Ar-H); 7.52 (1H, d, *J*=8.29 Hz, Ar-H); 7.58 (2H,

t, *J*=7.56 Hz, Ar-H); 7.66 (1H, d, *J*=8.30 Hz, Ar-H); 10.27 (1H, s, CONH). Anal. Calcd. for $C_{18}H_{15}F_3N_2O_3S$ (396.38): C, 54.54; H, 3.81; N, 7.07. Found: C, 54.10; H, 3.32; N, 6.88.

*2-Hydroxy-N-[2-(3-chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide* ***(3d)***

Yield 17%; m.p. 195-196 °C. IR (ν, $cm^{-1}$ ): 3333 (NH); 1710, 1671 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 3.69, 3.88 (2H, 2d, *J*=5.37 Hz, thiaz. $C_5$-H); 4.95 (1H, s, C*H*OH); 5.75 (1H, s, thiaz. $C_2$-H); 6.19 (1H, s, CHO<u>H</u>); 7.16-7.22 (5H, m, Ar-H); 7.30-7.33 (2H, m, Ar-H); 7.36-7.38 (1H, m, Ar-H); 7.47 (1H, s, Ar-H); 10.35 (1H, s, CONH). Anal. Calcd. for $C_{17}H_{15}ClN_2O_3S.½H_2O$ (371.85): C, 54.51; H, 4.33; N, 7.53. Found: C, 55.17; H, 3.90; N, 7.64 [19].

*2-Hydroxy-N-[2-(2,6-dichlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide* ***(3e)***

Yield 32%; m.p. 184 °C. IR (ν, $cm^{-1}$ ): 3301 (NH); 1713, 1679 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 3.73, 3.88 (2H, 2d, *J*=15.8 Hz, thiaz. $C_5$-H); 5.00 (1H, s, C*H*OH); 6.11 (1H, s, thiaz. $C_2$-H), 6.26 (1H, br s, CHO<u>H</u>); 7.23 (5H, s, Ar-H); 7.40 (1H, d, *J*=8.8 Hz, Ar-H); 7.58 (1H, s, Ar-H); 7.66 (1H, d, *J*=8.3 Hz, Ar-H); 10.47 (1H, s, CONH). Anal. Calcd. for $C_{17}H_{14}Cl_2N_2O_3S$ (397.28): C, 51.40; H, 3.55; N, 7.05. Found: C, 51.19; H, 3.06; N, 6.89 [19].

*2-Hydroxy-N-[2-(2,5-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide* ***(3f)***

Yield 14%; m.p. 135 °C. IR (ν, $cm^{-1}$): 3492, 3281 (OH, NH); 1687, 1652 (C=O). $^1$H-NMR (200 MHz, DMSO-$d_6$, δ, ppm): 3.66 (2H, s, $SCH_2$); 3.64, 3.70 (6H, 2s, $OCH_3$); 5.00 (1H, s, <u>CH</u>OH); 6.04 (1H, s, thiaz. $C_2$-H); 6.24 (1H, s, CHO<u>H</u>); 7.01-7.02 (3H, m, Ar-H); 7.22 (5H, s, Ar-H); 10.45 (1H, s, CONH). Anal. Calcd. for $C_{19}H_{20}N_2O_5S.H_2O$ (406.46): C, 56.12; H, 5.46; N, 6.89. Found: C, 51.29; H, 5.04; N, 6.90.

*Methyl 4-[3-(2-hydroxy-2-phenylacetamido)-4-oxo-1,3-thiazolidin-2-yl]benzoate* ***(3g)***

Yield 36%; m.p. 132 °C. IR (ν, $cm^{-1}$): 3555, 3228 (OH, NH); 1713; 1686 (C=O). $^1$H-NMR (200 MHz, DMSO-$d_6$, δ, ppm): 3.71, 3.74 (2H,2 d, *J*=2.93 Hz. $SCH_2$); 3.87 (3H, s, $COOCH_3$); 4.94 (1H, s, C<u>H</u>OH); 5.82 (1H, s, thiaz. $C_2$-H); 7.17 (5H, d, *J*=6.9 Hz, Ar-H); 7.43-7.48 (2H, m, Ar-H); 7.82-7.93 (2H, m, Ar-H); 10.27 (1H, s, CONH). Anal. Calcd. for $C_{19}H_{18}N_2O_5S.½H_2O$ (395.43): C, 57.71; H, 4.84; N, 7.08. Found: C, 57.46; H, 4.61; N, 6.85.

*2-Hydroxy-N-[2-(4-cyanophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide* ***(3h)***

Yield 40%; m.p. 166 °C. IR (ν, $cm^{-1}$): 3508, 3257 (OH, NH); 1722, 1673 (C=O), 2226 (C≡N). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 3.72, 3.89 (2H, 2dd, $J_1$=16.1, $J_2$=3.9 Hz. $SCH_2$); 4.93 (1H, d, J=3.91 Hz, C<u>H</u>OH); 5.83 (1H, s, thiaz. $C_2$-H); 6.25 (1H, d, J=4.39 Hz, CHO<u>H</u>); 7.18-7.24 (5H, m, Ar-H); 7.58 (2H, d, J=6.58 Hz, Ar-H); 7.79 (2H, d, J=6.83 Hz, Ar-H); 10.39 (1H, s, CONH). Anal. Calcd. for $C_{18}H_{15}N_3O_3S$ (353.39): C, 61.20; H, 4.28; N, 11.89. Found: C, 61.00; H, 4.18; N, 11.46.

*2-Hydroxy-N-[5-methyl-2-(2-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide* ***(4a)***

Yield 24%; m.p. 133 °C. IR (ν, $cm^{-1}$): 3528, 3229 (OH, NH); 1715, 1640 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 1.47 (3H, d, *J*=6.8 Hz, thiaz. $C_5$-$CH_3$); 2.07 (3H, s, 2-$CH_3$); 4.01 (1H, q, *J*=6.8 Hz, thiaz. $C_5$-H); 4.92 (1H, s, C*H*OH); 6.01 (1H, s, thiaz. $C_2$-H); 7.13-7.29 (9H, m, Ar-H and CHO*H*); 7.54 (1H, br s, Ar-H); 10,35 (1H, s, CONH). $^{13}$C-NMR [APT (decoupled), 125.6 MHz, DMSO-$d_6$, δ, ppm]: 18.9 ($CH_3$); 19.9 (thiaz. $CH_3$); 39.2 (thiaz. $C_5$); 73.5 (CH-OH); 127.2, 127.4, 128.2, 128.6, 129.1 (aromatic <u>C</u>H); 141.1 (aromatic =C); 171.7 (amide C=O); 172.3 (thiaz. C=O). MS-APCI (150 eV, m/z, %): 355 ([M-H]$^-$, 13), 267 (58), 221 (16), 133 (100). Anal. Calcd. for $C_{19}H_{20}N_2O_3S.½H_2O$ (365.45): C, 62.44; H, 5.79; N, 7.67. Found: C, 63.02; H, 5.63; N, 7.72.

*2-Hydroxy-N-[5-methyl-2-(3-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide* ***(4b)***

Yield 32%; m.p. 167 °C. IR (ν, $cm^{-1}$): 3243 (OH, NH); 1723, 1684 (C=O); $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 1.49 (3H, d, *J*=6.8 Hz, thiaz. $C_5$-$CH_3$); 2.29 (3H, s, 3-$CH_3$); 3.98, 4.06 (1H, 2q, *J*=6.8 Hz, thiaz. $C_5$-H); 4.93 (1H, s, C<u>H</u>OH); 5.71 (1H, s, thiaz. $C_2$-H); ); 6.21 (1H, s, CHO<u>H</u>); 7.10-7.30 (9H, m, Ar-H); 10.31 (1H, s, CONH). Anal. Calcd. for $C_{19}H_{20}N_2O_3S$ (356.44): C, 64.02; H, 5.66, 7.87. Found: C, 64.09; H, 5.77; N, 7.79.

*2-Hydroxy-N-[5-methyl-2-(4-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide* ***(4c)***

Yield 34%; m.p. 186 °C. IR (ν, $cm^{-1}$): 3232 (OH, NH); 1725, 1688 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 1.51 (3H, d, *J*=6.8 Hz, thiaz. $C_5$-$CH_3$); 2.34 (3H, s, 4-$CH_3$); 3.98, 4.05 (1H, 2q, *J*=6.8 Hz, thiaz. $C_5$-H); 4.94 (1H, d, *J*=5.1 Hz, C<u>H</u>OH); 5.73 (1H, s, thiaz. $C_2$-H); 6.25 (1H, d, *J*=5.1 Hz, CHO<u>H</u>); 7.16-7.31 (9H, m, Ar-H); 10.33

(1H, s, CONH). Anal. Calcd. for $C_{19}H_{20}N_2O_3S.½H_2O$ (365.45): C, 62.44; H, 5.79; N, 7.67. Found: C, 63.58; H, 5.46; N, 7.37.

*2-Hydroxy-N-{5-methyl-4-oxo-2-[2-(trifluoromethyl)phenyl]-1,3-thiazolidin-3-yl}-2-phenylacetamide (**4d**)*

Yield 33%; m.p. 200-202 °C. IR (ν, $cm^{-1}$): 3464, 3234 (OH, NH); 1722, 1688 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 1.49, 1.51 (3H, 2d, *J*= 7.0 Hz, thiaz. $C_5$-$CH_3$); 4.03, 4.12 (1H, 2q, *J*=6.8 Hz, thiaz. $C_5$-H ); 4.88 (1H, s, CHOH); 5.91 (1H, d, *J*=7.3 Hz, thiaz. $C_2$-H); 6.24 (1H, s, CHOH); 7.12-7.21 (5H, m, Ar-H), 7.60 (2H, d, *J*=7.8 Hz, Ar-H); 7.69 (2H, t, *J*=7.8 Hz, Ar-H); 10.32 (1H, s, CONH). MS-APCI (150 eV, m/z, %): 409 ([M-H]$^-$, 60), 321 (100), 275 (43), 187 (51). Anal. Calcd. for $C_{19}H_{17}F_3N_2O_3S$ (410.41): C, 55.60; H, 4.18, N, 6.83. Found: C, 55.28; H, 3.55; N, 6.60.

*2-Hydroxy-N-[5-methyl-2-(2-chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide (**4e**)*

Yield 10%; m.p. 182 °C. IR (ν, $cm^{-1}$): 3346 (OH, NH); 1719, 1674 (C=O). $^1$H-NMR (400 MHz, DMSO-$d_6$, δ, ppm): 1.45 (3H, d, *J*=7.0 Hz, thiaz. $C_5$-$CH_3$); 4.02, 4.08 (1H, 2q, *J*=6.9 Hz, thiaz. $C_5$-H); 4.96 (1H, s, CHOH); 6.08 (1H, s, thiaz. $C_2$-H); 6.13 (1H, br s, CHOH); 7.21-7.27 (5H, m, Ar-H); 7.33-7.36 (2H, m, Ar-H); 7.41-7.44 (1H, m, Ar-H); 7.63-7.66 (1H, m, Ar-H); 10.45 (1H, br s, CONH). Anal. Calcd. for $C_{18}H_{17}ClN_2O_3S$ (376.85): C, 57.37; H, 4.55, N, 7.43. Found: C, 57.32; H, 4.40; N, 7.62.

*2-Hydroxy-N-[2-(2,4-dichlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide (**4f**)*

Yield 19%; m.p. 175 °C. IR (ν, $cm^{-1}$): 3378 (OH, NH); 1722, 1681 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 1.48, 1.51 (3H, 2d, J= 6.8 Hz, thiaz. $C_5$-$CH_3$); 4.04, 4.15 (1H, 2q, *J*=7.1 Hz, thiaz. $C_5$-H); 4.94 (1H, d, *J*=6.3 Hz, CHOH); 5.74 (1H, d, *J*=6.3 Hz, thiaz. $C_2$-H); 6.27 (1H, br.s, CHOH); 7.18-7.29 (5H, m, Ar-H); 7.37 (1H, dd, *J*=8.0,1.7 Hz, Ar-H); 7.60 (1H, dd, *J*=8.3, 2.9 Hz, Ar-H); 7.66 (1H, m, Ar-H); 10.42 (1H, s, CONH). Anal. Calcd. for $C_{18}H_{16}Cl_2N_2O_3S$ (411.30): C, 52.56; H, 3.92, N, 6.81. Found: C, 52.40; H, 3.53; N, 6.66.

*2-Hydroxy-N-[2-(3,4-dichlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide (**4g**)*

Yield 35%; m.p. 194 °C. IR (ν, $cm^{-1}$): 3378, 3258 (OH, NH); 1722, 1681 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 1.47, 1.50 (3H, 2d, *J*= 6.83 Hz, thiaz. $C_5$-$CH_3$); 4.02, 4.13 (1H, 2q, *J*=6.83 Hz, thiaz. $C_5$-H), 4.93 (1H, d, *J*=6.35 Hz, CHOH); 5.75 (1H, d, *J*=5.61 Hz, thiaz. $C_2$-H); 6.27 (1H, s, CHOH); 7.17-7.24 (5H, m, Ar-H); 7.35 (1H, dd, *J*=8.30,1.95 Hz, Ar-H); 7.57 (1H, dd, *J*=8.29, 2.93 Hz, Ar-H); 7.63, 7.66 (1H, 2d, *J*=2.44 Hz, Ar-H),;10.42 (1H, s, CONH). Anal. Calcd. for $C_{18}H_{16}Cl_2N_2O_3S$ (411.30): C, 52.56; H, 3.92, N, 6.81. Found: C, 52.51; H, 3.63; N, 6.84.

*Methyl 4-[3-(2-hydroxy-2-phenylacetamido)-5-methyl-4-oxo-1,3-thiazolidin-2-yl]benzoate (**4h**)*

Yield 42%; m.p. 178 °C. IR (ν, $cm^{-1}$): 3222 (OH, NH); 1717, 1686 (C=O). $^1$H-NMR (400 MHz, DMSO-$d_6$, δ, ppm): 1.48 (3H, t, *J*=7.0 Hz, thiaz. $C_5$-$CH_3$); 3.86 (3H, s, $OCH_3$); 4.04, 4.12 (1H, 2q, *J*=6.9 Hz, thiaz. $C_5$-H); 4.90 (1H, s, CHOH); 5.81 (1H, s, thiaz. $C_2$-H); 6.22 (1H, s, CHOH); 7.13-7.29 (5H, m, Ar-H); 7.53 (2H, d, *J*=8.1 Hz, Ar-H); 7.91 (2H, d, *J*=8.2 Hz, Ar-H); 10.36 (1H, s, CONH). $^{13}$C-NMR [APT (decoupled), 125 MHz, DMSO-$d_6$, δ, ppm]: 19.9 (thiaz. $CH_3$); 39.2 (thiaz. $C_5$); 52.9 ($OCH_3$); 60.5 (thiaz. $C_2$); 73.5 (CH-OH); 127.4, 128.2, 128.6, 129.1, 129.9 (aromatic CH); 141.1, 143.3, 144.2 (aromatic =C); 166.6 (ester C=O), 171.6 (amide C=O), 172.3 (thiaz. C=O). MS-APCI (150 eV, m/z, %): 399 ([M-H]$^-$, 32), 311 (100), 265 (32), 177 (71). Anal. Calcd. for $C_{20}H_{20}N_2O_3S$ (400.44): C, 59.99; H, 5.03; N; 7.00. Found: C, 60.48; H, 5.43; N, 6.83.

*2-Hydroxy-N-[2-(4-cyanophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-phenylacetamide (**4i**)*

Yield 47%; m.p. 191 °C. IR (ν, $cm^{-1}$): 3264 (OH, NH); 1713, 1681 (C=O), 2228 (C≡N). $^1$H-NMR (400 MHz, DMSO-$d_6$, δ, ppm): 1.50 (3H, t, *J*=7.7 Hz, thiaz. $C_5$-$CH_3$); 4.05, 4.16 (1H, 2q, *J*=5.8 Hz, thiaz. $C_5$-H); 4.95 (1H, s, CHOH); 5.84 (1H, s, thiaz. $C_2$-H); 6.29 (1H, s, CHOH); 7.19-7.26 (5H, m, Ar-H); 7.57-7.62 (2H, m, Ar-H); 7.75 (1H, d, *J*=8.2 Hz, Ar-H); 7.83 (1H, d, *J*=8.2 Hz, Ar-H); 10.44 (1H, br.s, CONH). Anal. Calcd. for $C_{19}H_{17}N_3O_3S$ (367.41): C, 62.11; H, 4.66; N; 11.44. Found: C, 61.71; H, 4.23; N, 11.18.

*2-Hydroxy-N-{5-methyl-4-oxo-2-[4-(3-thienyl)phenyl]-1,3-thiazolidin-3-yl}-2-phenylacetamide (**4j**)*

Yield 27%; m.p. 176 °C. IR (ν, $cm^{-1}$): 3205 (OH, NH); 1724, 1688 (C=O). $^1$H-NMR (500 MHz, DMSO-$d_6$, δ, ppm): 1.47, 1.50 (3H, 2d, *J*=6.8 Hz, thiaz. $C_5$-$CH_3$); 3.99, 4.03 (1H, 2q, *J*=6.8 Hz, thiaz. $C_5$-H); 4.99 (1H, d, *J*=4.2 Hz, CHOH); 6.00 (1H, d, *J*=6.1 Hz, thiaz. $C_2$-H); 6.28 (1H, d, *J*=4.9 Hz, CHOH); 6.90-7.02 (2H, m, Ar-H); 7.28-7.32 (5H, m, Ar-H); 7.59-7.66 (1H, m, Ar-H); 10.41 (1H, s, CONH). $^{13}$C-NMR [decoupled, 125 MHz, DMSO-$d_6$, δ, ppm]: 20.1 (thiaz. $CH_3$); 39.0 (thiaz. $C_5$); 56.52 (thiaz. $C_2$); 73.36 (CH-OH); 127.4, 127.5, 128.3, 128.61, 128.9, 129.3, 129.7 (aromatic CH); 141.1, 142.0 (aromatic C); 171.6 (amide C=O), 171.7 (thiaz. C=O). MS-ESI (150 eV,

m/z, %): 349 ([M+H]$^+$, 100). Anal. Calcd. for $C_{16}H_{16}N_2O_3S_2$ (348.43): C, 55.15; H, 4.63; N; 8.04. Found: C, 55.38; H, 4.37; N, 7.87.

### 4.2. *In vitro* Cancer Screening Method

An RPMI 1640 medium including 2 mM L-glutamine and fetal bovine serum (5%) were prepared to grow the human tumor cell lines for the cancer screening panel. The human tumor cell lines were inoculated into 96 well microtiter plates for a standard screening procedure, in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. Before the addition of experimental drugs, the microtiter plates were incubated at 37° C, 5 % $CO_2$, 95 % air and 100 % relative humidity for 24 h after cell inoculation. At the end of the 24 h, to measure the cell population for each cell line of two plates at the time of drug addition (Tz) whole cell line was fixed *in situ* with TCA (trichloroacetic acid). To reach the desired final maxima test concentration, experimental drugs were solubilized in DMSO (dimethyl sulfoxide) at 400-fold and kept frozen before use. At the moment of the drug addition, an aliquot of concentrate was defrosted and with a medium included 50 µg/ml gentamicin was diluted two times to reach the final maximum test concentration. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. 100 µl aliquots aforementioned drug dilutions were added to suitable microtiter which have already 100 µl of the medium, resulting in desired final drug concentrations. After that the plates were incubated for an additional 48 h at 37°C, 5 % $CO_2$, 95 % air, and 100 % relative humidity. The assay was ended for adherent cells by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was eliminated, and the plates were washed five times with tap water and air dried. 0.4% (w/v) 100 µl of Sulforhodamine B (SRB) solution in 1 % acetic acid was added to each well, and for 10 minutes at room temperature plates were incubated. After staining, to remove the unbound dye, plates were washed five times with 1 % acetic acid and air-dried. The bound stain was then solubilized with 10 mM trizma base, and at a wavelength of 515 nm, the absorbance was recorded in an automated plate reader. The methodology for suspension cells was similar except that the test was terminated by fixing settled cells at the bottom of the wells by adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti) absorbance were measured and the growth (%, percentage) for each drug concentration level were calculated using absorbances. The growth inhibition (percentage) was calculated as:

$$[(Ti-Tz)/(C-Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz$$

$$[(Ti-Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz.$$

For all experimental agents, three-dose reponse parameters were determined. The $GI_{50}$ (growth inhibition of 50 %) was determined from the formula [(Ti-Tz)/(C-Tz)] x 100 = 50, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The $LC_{50}$ value, the drug concentration resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning was calculated from [(Ti-Tz)/Tz] x 100 = -50. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

**Acknowledgements:** We thank the Drug Research and Development, Division of Cancer Research, National Cancer Institute, Bethesda, Maryland, for the anticancer activity screening of the compounds.

**Author contributions:** Concept – Ö.G.-A.; Design – Ö.G.-A.; Supervision – Ö.G.-A; Resources – Ö.G.-A; Materials – Ö.G.-A; Data Collection and/or Processing – Ö.G.-A., K.D.-Y.; Analysis and/or Interpretation – Ö.G.-A., K.D.-Y.; Literature Search – Ö.G.-A., K.D.-Y..; Writing – Ö.G.-A., K.D.-Y.; Critical Reviews – Ö.G.-A., K.D.-Y.

**Conflict of interest statement:** The authors declared no conflict of interest.

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