

Synthesis, Antibacterial and Anticancer Activities Evaluation of New 4-Thiazolidinone-Indolin-2-One Analogues

Mahshid Hamzehloueian¹, Yaghoob Sarrafi², Mahdieh Darroudi^{2,3},
Marjan Azimzadeh Arani⁴, Reza Nezamdoost Darestani⁵, Fatemeh Safari^{5,*},
Alireza Foroumadi^{4,6,*}

¹ Department of Chemistry, Jouybar Branch, Islamic Azad University, Jouybar, Iran

² Department of Organic Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran

³ Department of Physiology, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran

⁴ Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

⁵ Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran

⁶ Drug Design and Development Research Center, The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran, Iran

* Correspondences: fsafari@guilan.ac.ir (F.S.); aforoumadi@yahoo.com (A.F.)

Scopus Author ID 7003549932

Received: 14.10.2021; Revised: 15.11.2021; Accepted: 18.11.2021; Published: 5.12.2021

Abstract: A series of novel thiazolidinone-isatin hybrids have been synthesized through the Knoevenagel reaction of isatin derivatives with synthesized thiazolidinone scaffolds and then evaluated for their *in vitro* antibacterial effects on *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*). Cytotoxic effects of the compounds on non-small-cell lung cancer cells (A549 cells), breast epithelial cancer cell line (MCF-7), and prostate cancer cells (PC3 cells) were investigated. Among compounds tested for antibacterial activity, *S. aureus* was susceptible to compound **7d**. The most potent compounds against A549, MCF-7, and PC3 tumor cells were found to be **7g**. DAPI staining of all cancer cell lines treated with compound **7g**, associated with cell death. We finally confirmed that apoptosis occurred in A549 cells by up-regulated *Bax* expression and down-regulated *Bcl-2* expression from the mitochondrial pathway of apoptosis by using the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) method. Our findings suggested that compound **7g** may be a good target in designing cancer therapy strategies.

Keywords: isatin; thiazolidinone; isatin-thiazolidinone hybrids; apoptosis; cancer cells.

© 2021 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Cancer is an enormous worldwide health burden, touching every region and socioeconomic level. Globally, about one in every eight deaths is due to cancer. There were 9.6 million deaths from cancer worldwide in 2018 - more than those due to combined HIV/AIDS, malaria, and tuberculosis. Chemotherapy, the use of medicinal agents to destroy cancer cells, is the current approach to treating malignancies. Targeted cancer therapies progression is one of the most relevant challenges to chemists and oncologists. Isatin and its derivatives have been proven to be versatile substrates acting as a precursor for synthesizing a multitude of heterocyclic compounds as promising pharmacological agents [1-15]. Furthermore, thiazolidinone derivatives have been the subject of considerable studies due to

their biological activities [16-19], such as antimicrobial [20], antiviral [21-23], antifungal [24-26], and antituberculosis properties [27-30]. It has been known that many efforts devoted to adopting a hybridization approach for the development of diverse isatin-thiazolidine/thiazolidinone [31-38].

Taking the above into account, we have become interested in the combination of isatin and thiazolidinone cores to synthesize a novel series of 5-(2-oxoindolin-3-ylidene)-2-(arylimino)thiazolidin-4-ones 7a-h through the Knoevenagel condensation of derivatives of 4-thiazolidinone and oxindole in a facile procedure.

2. Materials and Methods

2.1. Chemistry part.

Melting points were determined on an Electrothermal 9100 apparatus and uncorrected. IR spectra were recorded on an FT-IR Bruker Vector 22 spectrophotometer. The ^1H and ^{13}C NMR spectra were measured using the Bruker DRX-400 AVANCE instrument (400.1 MHz for ^1H , 100.6 MHz for ^{13}C) with CDCl_3 as a solvent. Mass spectra were run on a Finnigan-Matt 8430 mass spectrometer operating at an ionization potential of 70 eV.

2.1.1. General procedure for the synthesis of arylthioureas 2.

To a solution of ammonium thiocyanate (11 mmol) in dry acetone (5mL) benzoyl chloride (10 mmol) was added over 5 min at 56 °C. The reaction mixture was stirred in reflux for 15 min. Then a solution of aniline (20 mmol) in dry acetone (2.5 mL) was added to the gently reflux mixture, and the mixture continuously stirred in reflux for 20 min. The mixture was poured into cold water (70 mL) with stirring, and the resulting yellow precipitate was filtered off and washed with H_2O and then cold $\text{H}_2\text{O}/\text{MeOH}$. The solid was added to the hot solution of 10% NaOH (aq) and stirred at 90 °C for 30 min. After removing insoluble material by filtration, the filtrate was acidified with concentrated HCl (aq) then made slightly basic with aqueous ammonia. Upon standing, the solution deposits the crystalline product 2a-b. Arylthioureas (2a) m.p.: 153-154 °C. IR (KBr, cm^{-1}) $\nu = 3480, 2921, 1632, 1182$. 4-Cl arylthioureas (2b) m.p.: 187-189, IR (KBr, cm^{-1}) $\nu = 3443, 3006, 1673, 1284, 1233$.

2.1.2. General procedure for the synthesis of arylaminothiazol-4-ones 3

To a stirred solution of arylthioureas 2 (6 mmol) in ethanol (20 mL) at 60 °C, bromoacetate (7.8 mmol) was added, and the reaction mixture was continuously stirred at 70 °C for 5 h. After completion of the reaction, the mixture was cooled down to the ambient temperature then neutralized with ammonia solution. Water was added dropwise to the mixture with stirring until the precipitate formed. The precipitated solid was filtered off and dried, then triturated with petroleum ether (2/3) to deliver yellow crystals. The products are recognized as a tautomeric mixture in DMSO. 2-(phenylamino) thiazol-4(5H)-one (3a). Yield: (71%), yellow crystals. Mp: 178.4-179.5 °C. 2-((4-chlorophenyl) amino) thiazol-4(5H)-one (3b). Yield: (65%), brown crystals. M.p: 221-230 °C. ^1H NMR (DMSO- d_6): δ (ppm) 12.34 (s, 0.5 \times 1H), 11.22 (s, 0.5 \times 1H), 7.82 (br, 2 \times 0.5H), 7.64 (d, $J = 8.4\text{Hz}$, 2H), 7.46 (br, 2 \times 0.5H), 7.17 (d, $J = 8.4\text{Hz}$, 2H), 4.18 (s, 0.5 \times 2H), 4.10 (s, 0.5 \times 2H). MS: $m/z = 226.0$ (M^+).

2.1.3. General procedure for the synthesis of title compounds 7

To a stirred solution of isatins 4a-c (2mmol) and thiazolidinone derivatives 3a-b (2.4 mmol) in ethanol (20 mL), malononitrile (0.25 mmol) and triethylamine (1 mmol) were added. The reaction mixture was refluxed for 3 h. After completion of the reaction (monitored by TLC), the mixture was allowed to cool to ambient temperature. Then the generated solid was filtered off, washed with hot ethanol and recrystallized from an appropriate solvent to obtain 7a-h.

5-(2-oxoindolin-3-ylidene)-2-(phenylamino) thiazol-4(5H)-one (7a): Yield: 0.61 g (91%), Red solid, m.p: 171-172 °C. ¹H NMR (400 MHz, DMSO-d₆): δ = 7.13 (d,d, J = 7.6, 8.4 Hz, 1H, ArH), 7.22 – 7.36 (m, 4H, ArH), 7.47 (d, 2J = 7.6 Hz, 2H, ArH), 7.57 (d, 2J = 7.6 Hz, 1H, ArH), 7.75 (d, 2J = 8.4 Hz, 1H, ArH), 8.79 (s, 1H, NH), 11.75 (bs, 1×0.5H, NH), 11.87 (bs, 1×0.5H, NH) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ= 115.93, 121.74, 123.35, 126.47, 128.29, 128.97, 129.19, 130.68, 131.96, 132.23, 137.39, 147.39, 159.47, 116279, 175.35 ppm. IR (KBr, cm⁻¹) ν = 3430, 3131, 1698, 1580, 1506, 1170. MS: m/z = 320.0 (M⁺).

2-((4-chlorophenyl) amino)-5-(2-oxoindolin-3-ylidene) thiazol-4(5H)-one (7b): Yield: 0.61 g (87%), Brown solid. m.p: 198.1-199.5 °C. ¹H NMR (400 MHz, DMSO-d₆): δ= 7.10 ~ 7.05 (m, 3H, ArH), 7.47 (d, 2J = 7.6 Hz, 2H, ArH), 7.75 (d, 2J = 8.4 Hz, 2H, ArH), 8.23 (s, 1H, ArH), 9.23 (s, 1H, NH), 11.54 (bs, 1×0.5H, NH), 11.75 (bs, 1×0.5H, NH) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ= 120.49, 121.71, 125.26, 125.84, 127.49, 128.27, 129.96, 131.87, 132.06, 138.93, 142.21, 153.09, 156.28, 163.49, 169.25 ppm. IR (KBr, cm⁻¹) ν = 3439, 3102, 1667, 1596, 1176. MS: m/z = 356.1 (M⁺).

5-(5-bromo-2-oxoindolin-3-ylidene)-2-(phenylamino) thiazol-4(5H)-one (7c): Yield: 0.71 g (87%), Brown solid, decomposed at: 221-222.5 °C. ¹H NMR (400 MHz, DMSO-d₆): δ= 6.83 (d, 2J = 8.8 Hz, 2H, ArH), 6.93 (m, 2H, ArH), 7.11 (d, 2J = 8.4 Hz, 1H, ArH), 7.47 (t, 2J = 8.8 Hz, 2H, ArH), 8.10 (s, 1H, CH), 8.44 (s, 1H, NH), 11.03 (bs, 1×0.5H, NH), 11.49 (bs, 1×0.5H, NH) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ= 113.32, 115.87, 121.90, 125.23, 125.93, 126.43, 128.60, 129.22, 131.13, 136.32, 137.46, 138.39, 143.85, 156.06, 162.32, 167.37, 178.04 ppm. IR (KBr, cm⁻¹) ν = 3443, 3115, 1685, 1605, 1591, 1176, 823. MS: m/z = 400.3 (M⁺).

5-(5-chloro-2-oxoindolin-3-ylidene)-2-(phenylamino) thiazol-4(5H)-one (7d): Yield: 0.61 g (88%), Red solid, m.p: 205-206.4 °C. ¹H NMR (400 MHz, DMSO-d₆): δ= 7.22 (d, 2J = 8.8 Hz, 2H, ArH), 7.36 – 7.41 (m, 3H, ArH), 7.52 (d, 2J = 8.4 Hz, 2H, ArH), 7.88 (t, 2J = 8.4 Hz, 1H, ArH), 8.07 (s, 1H, ArH), 8.37 (s, 1H, NH), 10.98 (bs, 1×0.5H, NH), 11.09 (bs, 1×0.5H, NH) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ= 120.68, 121.76, 125.26, 125.76, 126.47, 128.29, 129.13, 129.96, 137.23, 137.39, 142.03, 153.09, 158.23, 161.75, 173.15 ppm. IR (KBr, cm⁻¹) ν = 3430, 3101, 1641, 1594, 1176. MS: m/z = 355.8 (M⁺).

5-(5-chloro-2-oxoindolin-3-ylidene)-2-((4-chlorophenyl) amino) thiazol-4(5H)-one (7e): Yield: 0.66g (85%), Brown solid, m.p: 199-200.5 °C. ¹H NMR (400 MHz, DMSO-d₆): δ= 6.91 (d, 2J = 8.8 Hz, 2H, ArH), 7.04 (d, 2J = 8.8 Hz, 2H, ArH), 7.11 (d, 2J = 8.0 Hz, 1H, ArH), 7.38 (d, 2J = 8.0 Hz, 1H, ArH), 7.79 (s, 1H, CH), 8.00 (s, 1H, ArH), 8.44 (s, 1H, NH), 10.97 (bs, 1×0.5H, NH), 11.27 (bs, 1×0.5H, NH) ppm. ¹³C NMR (100 MHz, DMSO-d₆O): δ= 120.91, 121.02, 125.28, 125.81, 127.48, 129.40, 129.96, 130.82, 136.94, 138.92, 144.02, 153.09, 158.81, 158.23, 164.75, 170.15 ppm. IR (KBr, cm⁻¹) ν = 3428, 3107, 1668, 1592, 1535, 1253, 1138. MS: m/z = 388.9 (M⁺).

5-(5-bromo-2-oxoindolin-3-ylidene)-2-((4-chlorophenyl) amino) thiazol-4(5H)-one (7f): Yield: 0.75 g (87%), Brown solid, m.p: 221.5-223 °C. ¹H NMR (400 MHz, DMSO-d₆): δ= 7.09 (d, 2J = 8.4 Hz, 2H, ArH), 7.47 (d, 2J = 7.6 Hz, 2H, ArH), 7.75 (d, 2J = 8.4 Hz, 1H, ArH), 8.24 (d, 2J = 8.4 Hz, 1H, ArH), 8.80 (s, 1H, ArH), 9.16 (s, 1H, NH), 11.27 (bs, 1×0.5H, NH), 11.75 (bs, 1×0.5H, NH) ppm. ¹³C NMR

(100 MHz, DMSO-d₆): δ = 113.48, 118.35, 121.81, 125.66, 128.74, 129.48, 130.35, 131.72, 132.24, 137.66, 139.49, 143.65, 157.47, 162.48, 171.07, 176.51 ppm. IR (KBr, cm⁻¹) ν = 3428, 3138, 1639, 1516, 1263, 1153. MS: m/z = 433.9 (M⁺). 5-(5-nitro-2-oxoindolin-3-ylidene)-2-(phenylamino) thiazol-4(5H)-one (7g): Yield: 0.66 g (89%), Red solid, decomposed at 186-188 °C. ¹H NMR (400 MHz, DMSO-d₆): δ = 7.23 (t, 3J, J = 7.2 Hz, 1H, ArH), 7.33(d, 2J = 7.6 Hz, 2H, ArH), 7.35~7.44 (m, 3H, ArH), 7.64 (d, 2J = 8.0 Hz, 1H, ArH), 8.00 (s, 1H, CH), 8.45 (s, 1H, NH), 11.53 (bs, 1×0.5H, NH), 12.08 (bs, 1×0.5H, NH) ppm. ¹³C NMR (100 MHz, DMSO): δ = 120.11, 121.27, 125.72, 128.85, 129.33, 129.74, 133.06, 137.38, 143.27, 148.11, 158.00, 164.27, 172.87, 178.62 ppm. IR (KBr, cm⁻¹) ν = 3391, 3152, 1709, 1635, 1585, 1522, 1344, 1134. MS: m/z = 366.2 (M⁺). 2-((4-chlorophenyl) amino)-5-(5-nitro-2-oxoindolin-3-ylidene) thiazol-4(5H)-one (7h): Yield: 0.60 g (79%), Brown solid, m.p: 203-204 °C. ¹H NMR (400 MHz, DMSO-d₆): δ = 7.06 (d, 2J = 7.6 Hz, 2H, ArH), 7.21(d, 2J = 7.6 Hz, 2H, ArH), 7.61 (d, 2J = 6.0 Hz, 1H, ArH), 8.01 (d, 2J = 6.0 Hz, 1H, ArH), 8.37 (s, 1H, CH), 9.47 (s, 1H, NH), 11.54 (bs, 1×0.5H, NH), 12.29 (bs, 1×0.5H, NH) ppm. ¹³C NMR (100 MHz, DMSO): δ = 121.74, 123.35, 125.48, 128.35, 129.24, 129.91, 136.44, 143.48, 153.48, 159.74, 164.07, 172.14, 176.07, 178.14 ppm. IR (KBr, cm⁻¹) ν = 3432, 3202, 1641, 1597, 1508, 1381, 1247, 1176, 827. MS: m/z = 400.7 (M⁺).

2.2. Biological part.

2.2.1. Determination of the antibacterial activity of synthetic compounds using the disk diffusion method.

Antibacterial activity of the prepared synthetic compounds against the Gram-negative bacteria (*E. coli*; ATCC: 15922) and the Gram-positive bacteria (*S. aureus*; PTCC: 1112, International No. ATCC6538P) were examined by disk diffusion assay. Bacterial cultures were obtained from Persian Type Culture Collection, Tehran, Iran (PTCC). Isolated pure colonies from freshly grown bacteria were transferred from the plates into the sterile normal saline solution, and it was vortexed to form homogenous bacterial suspensions. Based on 0.5 Mcfarland standard unit, the turbidity of suspensions was adjusted and poured over Mueller–Hinton agar (MHA) plates. Then, sterile filter paper disks were placed over these plates and impregnated with the tested compounds. Tetracycline was a positive control, and sterile distilled water was a negative control. Incubate in an incubator at 37 °C in 24h for bacteria (Table 1). The MIC results were also determined.

2.2.2. Cell lines and culture

Human non-small-cell lung cancer A549 cells, human breast cancer MCF-7 cells, and prostate cancer PC3 cells were received from Pasture Institute, Tehran, Iran. MCF-7 cells were grown in RPMI 1640 medium, A549 cells, and PC3 cells were grown in DMEM medium. All media contain 10% fetal bovine serum (FBS), penicillin G, streptomycin 100 μ g/mL, and 1% L-Glutamine. The cells were cultured and incubated under humidified 5% CO₂ atmosphere at 37 °C [39].

2.2.3. MTT assay.

The viability of cancer cell lines after treatment with related compounds was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide or MTT assay (MTT assay kit, Bio IDEA, Cat No:BI1017, Iran) in accordance with our previous study [39].

2.2.4. DAPI staining assay.

DAPI staining assay was used to determine chromatin changes. A549 cells were seeded in six-well plates (5×10^4 cells/well) containing 12 mm cover-slips and subsequently treated for H1 compound (Sample or treated cells) and DMSO (Control or untreated cells) for 24 h. Cells then were fixed with 3.7% paraformaldehyde, permeabilized in 0.5% (w/v) Triton X-100, 1% BSA (w/v) for 5 min, washed in PBS, and stained with DAPI (Sigma-Aldrich, USA). All images were taken by an inverted fluorescent microscope (Nikon Eclipse Ti-E) [39].

2.2.5. RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR).

For quantitative real-time RT-PCR analysis, after 48 h of treatment with 40 μ M of compound (**7g**), A549 cells were lysed, and the total RNA was extracted using 500 μ L of Trizol® reagent according to the protocol provided by the manufacturer (Invitrogen Life Technologies, Carlsbad, CA, USA) followed by reverse transcription into cDNA according to manufactures protocol (ReveretAid M-Mulv reverse transcriptase kit, Thermo Fisher Scientific, MA, USA). Real-time RT-PCR was then performed to amplify cDNA using SYBR Green dye universal master mix (Bioron GmbH, Germany), on a Light Cycler 480 (Roche) using the primers for GAPDH-F: 5'-CAA GGT CAT CCA TGA CAA CTTTG-3', R:5'-GTCCACCACCCTGTTGCTGTAG-3'; BAX-F:5'-GTCGCCCTTTTCTACTTTGCC -3', R: 5'-CTCCCGCCACAAAGATGGTCA-3'and BCL2-F: 5'-CCCCTCGTCCAAGAATGCAA-3', R: 5'- TCTCCCGGTTATCGTACCCTG-3'for forty cycles. Thermal conditions of the PCR were described previously [39].

2.2.6. Statistical analysis

Data were analyzed using SPSS (ver. 22, Chicago, IL, USA), and graphs were generated using Graph Pad Prism 7 software. Data were expressed as means \pm standard deviation (SD). Experiments were performed in triplicate. Comparisons between groups were performed using an independent sample t-test. The value of $p < 0.05$ was considered statistically significant [39].

3. Results and Discussion

3.1. Results.

3.1.1. Compounds synthesis

The synthetic strategy to achieve target compounds 7a-h is outlined in Scheme. 1. According to the previously reported article, the key intermediates 3a-b were synthesized starting from commercially available anilines 1 [40]. The first step was the synthesis of arylthioureas 2 from aniline derivatives using ammonium thiocyanate and benzoyl chloride in dry acetone. 2-Aryliminothiazolidin-4-ones 3a-b were obtained by ring closure of 2 with ethyl 2-bromoacetate. Condensation of compounds 3a-b with isatin derivatives 4a-d in the presence

of malononitrile in ethanol under reflux afforded title compounds 7a-h in good to excellent yields. A mechanistic rationalization for the reaction between compounds 3 and 4 is provided in Scheme. 2. The first step is the Knoevenagel condensation reaction of isatins 4a-d and malononitrile delivering the 2-(2-oxoindolin-3-ylidene)malononitriles 5a-d. The electrophilic carbon atom of 5 may undergo nucleophilic addition of 2-Aryliminothiazolidin-4-ones 3a-b through a Michael type addition reaction to form the intermediates 6a-h. Subsequent elimination of malononitrile lead to the 4-thiazolidinone- indolin-2-one conjugates 7a-h [41].

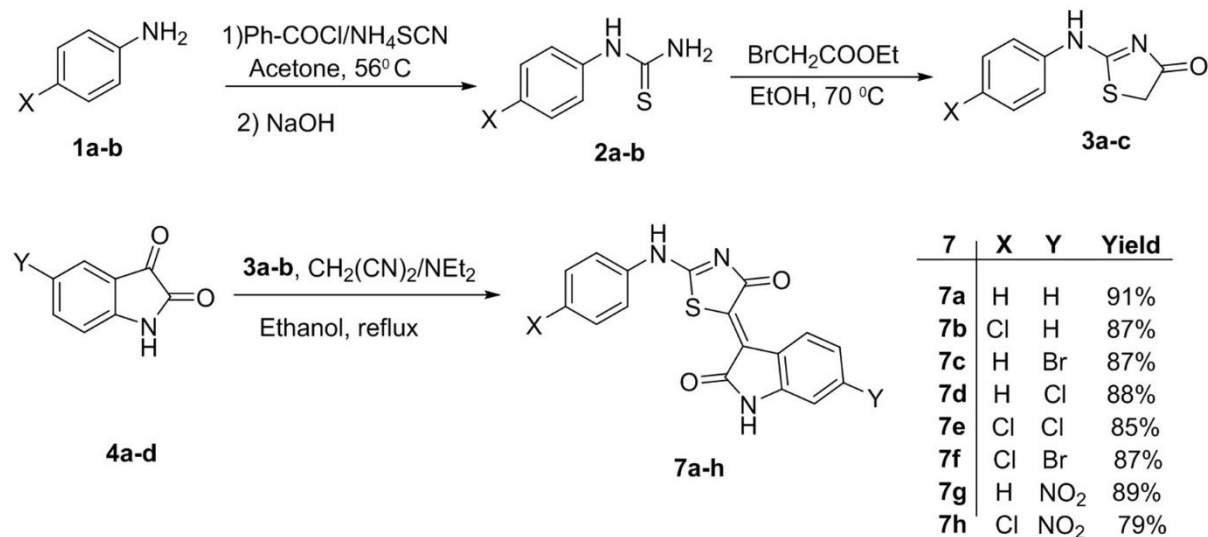


Figure 1. Synthesis of target hybrids 7a-h.

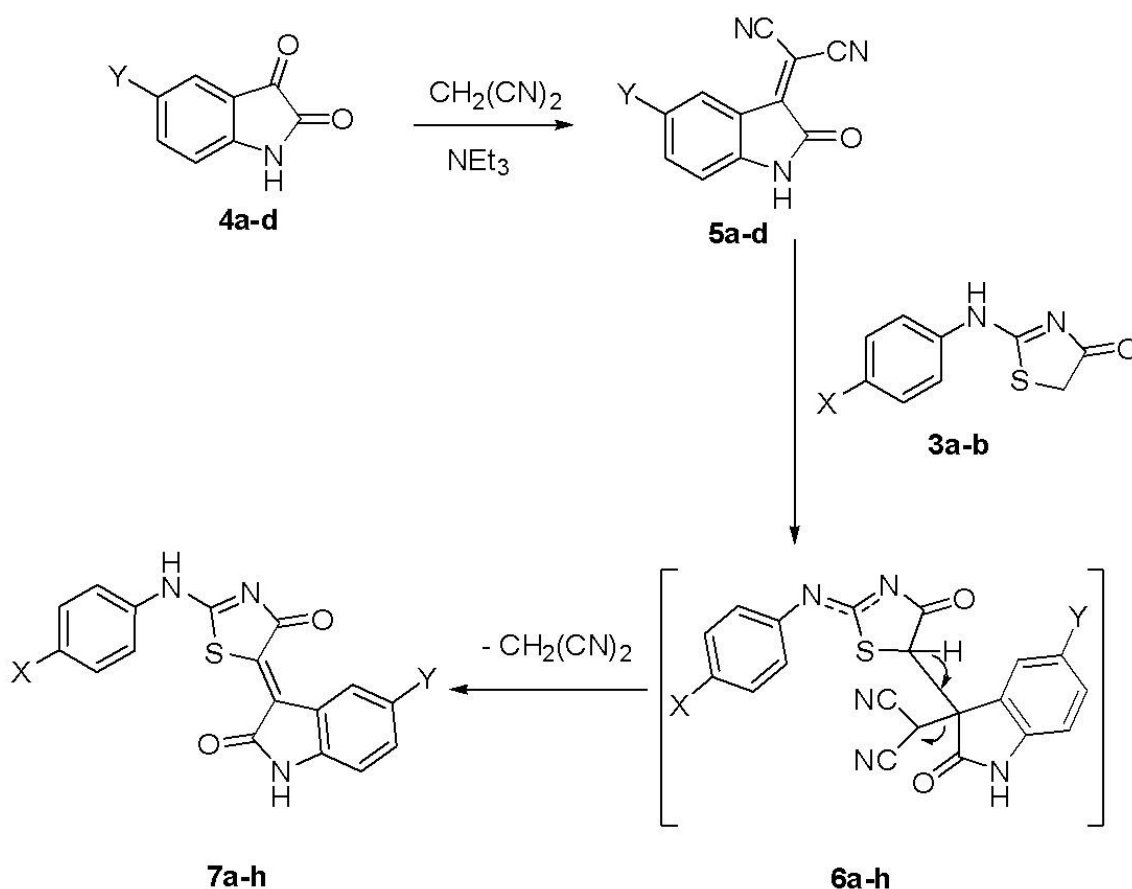


Figure 2. Proposed mechanism for the formation of the title compounds 7a-h from 2-Aryliminothiazolidin-4-ones 3a-b and isatins 4a-d.

3.1.2. The evaluation of antibacterial effects of synthetic compounds.

Antibacterial effects of compounds 7a-h were evaluated *in vitro* using the disk diffusion method against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. Compound 7d showed an inhibition zone of 9 mm against *S. aureus* using the disk diffusion method. MIC assay was used to determine the lowest concentration for inhibition. The MIC value of 7d was 20 mM. *E. coli* was resistant to all synthetic compounds (Table 1). Tetracycline and DMSO were used as positive and negative controls, respectively.

Table 1. Antimicrobial activity of synthesized compounds using disc diffusion test at concentration 50 mM (diameter of zone of inhibition in mm), R: resistant.

Compounds	Inhibition zone (mm)	
	<i>S. aureus</i>	<i>E. coli</i>
7a	R	R
7b	R	R
7c	R	R
7d	9 mm	R
7e	R	R
7f	R	R
7g	R	R
7h	R	R
Tetracycline	29 mm	25 mm
DMSO	0	0

3.1.3. *In vitro* cytotoxic activity evaluation of synthetic compounds.

At first, to compare anticancer properties, the synthesized compounds 7a-h were evaluated against A549 cells, MCF-7 cells, and PC3 cells lines using the MTT colorimetric assay. The activity is expressed as 50% growth inhibitory concentration (IC₅₀) values at 48 h, and the results are presented in Table 2. We used DMSO (1%) as negative control and Etoposide as a positive control. Among the different synthetic compounds in terms of chemical structure, compound 7g (IC₅₀ = 40 μM in MCF-7 and A549, 50 μM in PC3) showed the highest cytotoxicity against different cancer cell lines. Next, DAPI staining showed clear morphological changes and fragmentation in the chromatin within the nucleus of treated cells, but their morphology is not altered in untreated cells (or control). Our results indicated that compound 7g induced cell death (Figure 1). Finally, we confirmed that apoptosis mediated by mitochondria induces in A549 cells. Our results demonstrated that up-regulated expression of *Bax* and down-regulation expression of *Bcl-2* (Figure 2A, 2B).

Table 2. *In vitro* cytotoxic activities of compounds 7a-h against A549, PC3, and MCF-7 cancer cell lines. Data represent mean ± SD of three independent experiments.

Compounds	Cell lines (IC ₅₀ /μM)		
	A549 cells	MCF-7 cells	PC3 cells
7a	> 100	> 100	> 100
7b	90±0.009	> 100	> 100
7c	> 100	> 100	> 100
7d	90±0.021	> 100	> 100
7e	> 100	> 100	> 100
7f	> 100	> 100	> 100
7g	40±0.017	40±0.002	50±0.011
7h	100±0.026	> 100	> 100
Etoposide	60±0.01	30±0.01	40±0.06

3.2. Discussion.

In the current study, synthesis and screening of antibacterial and anticancer activities of thiazolidinone-isatin hybrids 7a-h are performed. Our results demonstrated that the synthesized compounds have inhibition effects on cell viability on three cancer cell lines, MCF-7, A549, and PC3. Interestingly, compound 7g has inhibitory effects on A549 cancer cells at a concentration lower than Etoposide as a drug reference (40 μ M in 7g). We found that compound 7g can induce apoptotic mediated mitochondria pathway. It was previously found that the balance between the anti-apoptotic Bcl-2 protein and pro-apoptotic is important for activating apoptosis. Furthermore, the down-regulation of Bcl-2 and/or up-regulation of Bax disturbs this balance and leads to apoptosis. Using qRT-PCR, we could detect the upregulation of Bax expression and the downregulation of Bcl2 expression. Moreover, it is unknown why compound 7g has inhibitory effects on the A549 cancer cell line, and thereby, more experiments will be needed to clarify the related molecular mechanisms.

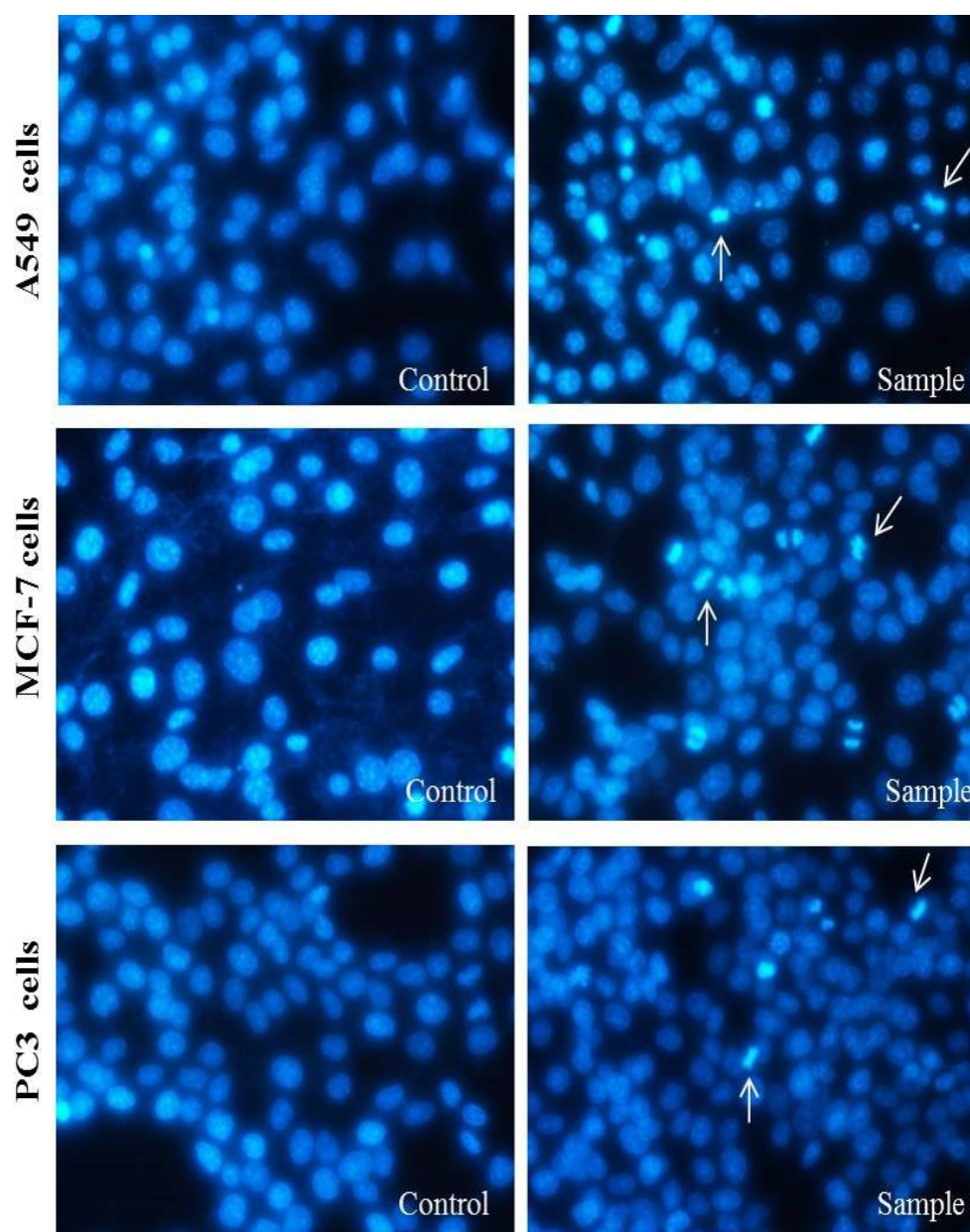


Figure 3. Inverted fluorescent microscopy images of chromatin damages occurrence in the nucleus of treated cells with compound 7g and DMSO (1%), which have been stained with DAPI in different cancer cell lines. The experiments were performed three times (original microscope magnification, 40X, Scale bar, 10 μ M).

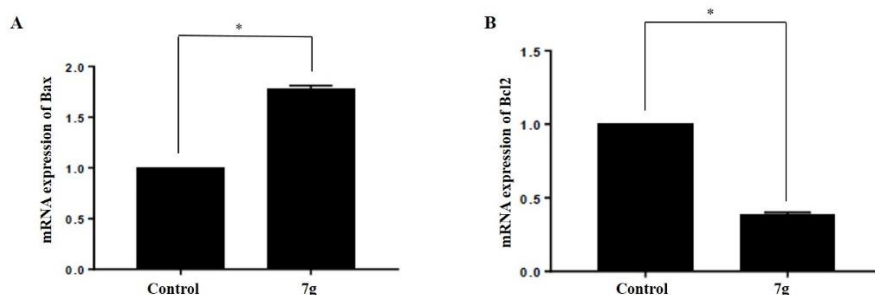


Figure 4. Relative expression of *Bax* mRNA and relative expression of *Bcl-2* mRNA in A549 cell line after treatment with compound 7g were shown. Data represent mean \pm SD of three independent experiments. $p < 0.05$ was considered to be statistically significant (A) and (B).

In the case of inhibitory effects, compound 7d also showed weak antibacterial activity against *S. aureus* with minimum inhibitory concentration (MIC) 20 mM. It seems due to the structural differences of the bacterial cell wall (i.e., the presence of the outer membrane with lipid, lipopolysaccharide, and lipoprotein content in Gram-negative bacteria), *E. coli* was resistant to our synthetic compounds, and only *S. aureus* was sensitive to compound 7d. Thus, it seems that 7d compound may be a possible candidate to design drugs against *S. aureus* infection.

4. Conclusions

In this study, we suggested that among eight compounds with thiazolidinone-isatin hybrids structure, 7d and 7g compounds may be considered potent candidates to design drugs for inhibiting *S. aureus* infection and cancer treatment strategies. However, more experiments will be required to find the molecular mechanisms of the related compounds to inhibit bacterial infection and tumor growth.

Funding

This research received no external funding.

Acknowledgments

This work was supported by the University of Guilan and Iran's National Elites Foundation (INEF).

Conflicts of Interest

The authors declare that there is no conflict of interest.

References

1. Sagnou, M.; Mavroidi, B.; Kaminari, A.; Boukos, N.; Pelecanou, M. Novel Isatin Thiosemicarbazone Derivatives as Potent Inhibitors of β -Amyloid Peptide Aggregation and Toxicity. *ACS Chem Neurosci* **2020**, *11*, 2266-2276. <https://doi.org/10.1021/acchemneuro.0c00208>.
2. Yousef, M.A.; Ali, A.M.; El-Sayed, W.M.; Qayed, W.S.; Farag, H.H.A.; Aboul-Fadl, T. Design and synthesis of novel isatin-based derivatives targeting cell cycle checkpoint pathways as potential anticancer agents. *Bioorg Chem* **2020**, *105*, 104366. <https://doi.org/10.1016/j.bioorg.2020.104366>.
3. Chauhan, G.; Pathak, D.P.; Ali, F.; Bhutani, R.; Kapoor, G.; Khasimbi, S. Advances on Synthesis, Derivatization and Bioactivity of Isatin: A Review. *Curr Org Synth* **2020**, *24*, <https://doi.org/10.2174/1570179417666200924150907>.

- Zhang, Y.Z.; Du, H.Z.; Liu, H.L.; He, Q.S.; Xu Z. Isatin dimers and their biological activities. *Arch Pharm (Weinheim)* **2020**, *353*, e1900299, <https://doi.org/10.1002/ardp.201900299>.
- Annageldiyev, C.; Gowda, K.; Patel, T.; Bhattacharya, P.; Tan, S.F.; Iyer, S.; Desai, D.; Dovat, S.; Feith, D.J.; Loughran, T.P.; Jr, Amin, S.; Claxton, D.; Sharma, A. The novel Isatin analog KS99 targets stemness markers in acute myeloid leukemia. *Haematologica* **2020**, *105*, 687-696, <https://doi.org/10.3324/haematol.2018.212886>.
- Hou, Y.; Shang, C.; Wang, H.; Yun, J. Isatin-azole hybrids and their anticancer activities. *Arch Pharm (Weinheim)* **2020**, *353*, e1900272, <https://doi.org/10.1002/ardp.201900272>.
- Yang, M.; Liu, H.; Zhang, Y.; Wang, X.; Xu, Z. Moxifloxacin-isatin Hybrids Tethered by 1,2,3-triazole and their Anticancer Activities. *Curr Top Med Chem* **2020**, *20*, 1461-1467, <https://doi.org/10.2174/1568026620666200128144825>.
- Da Silva, F.M.; Jones, J. Organic Reaction in Water. Part 31: Diastereoselectivity in Michael Additions of Thiophenol to Nitro Olefins in Aqueous Media. *J. Braz. Chem. Soc* **2001**, *12*, 135-137, <https://doi.org/10.1590/S0103-50532001000200002>.
- Dounay, A.B.; Hatanaka, K.; Kodanko, J.J.; Oestreich, M.; Overman, L.E.; Pfeifer, L.A.; Weiss, M.M. Catalytic asymmetric synthesis of quaternary carbons bearing two aryl substituents. Enantioselective synthesis of 3-alkyl-3-aryl oxindoles by catalytic asymmetric intramolecular Heck reactions. *J. Am. Chem. Soc.* **2003**, *125*, 6261-6271, <https://doi.org/10.1021/ja034525d>.
- Toyota, M.; Ihara, M.; Wong, K.T.; Tan, B.K.H.; Sim, K.Y.; Goh, S.H.; Garden, S.J. Recent progress in the chemistry of non-monoterpenoid indole alkaloids. *Nat. Prod. Rep* **1998**, *15*, 327, <https://doi.org/10.1039/a815327y>.
- Marti, C.; Carreira, E.M. Construction of Spiro[pyrrolidine-3,3'-oxindoles] – Recent Applications to the Synthesis of Oxindole Alkaloids. *European J. Org. Chem* **2003**, 2209-2219, <https://doi.org/10.1002/ejoc.200300050>.
- Nair, V.; Mathai, S.; Augustine, A.; Viji, S.; Radhakrishnan, K.V. The Huisgen Reaction of Azomethine Ylide to Isatins: A Facile Synthesis of Spiro-oxindoles, *Synthesis (Stuttg)* **2004**, *16*, 2617-2619, <https://doi.org/10.1055/s-2004-831219>.
- Hilton, S.T.; Ho, T.C.T.; Pljevaljcic, G.; Jones, K. A new route to spirooxindoles, *Org. Lett* **2000**, *2*, 2639-2641. <https://doi.org/10.1021/ol0061642>.
- Patel, A.; Bari, S.; Talele, G.; Patel, J.; Sarangapani, M. Synthesis and antimicrobial activity of some new isatin derivatives, *Iran. J. Pharm. Res* **2010**, 249-254, <http://doi.org/10.22037/IJPR.2010.685>.
- Grewal, A.S. Isatin derivatives with several biological activities. *Int. J. Pharm. Res* **2014**, *6*, 1-7, https://www.researchgate.net/profile/Ajmer-Grewal/publication/261873183_Isatin_Derivatives_with_Several_Biological_Activities/links/542500770cf26120b7ac4d40/Isatin-Derivatives-with-Several-Biological-Activities.pdf.
- Tahmasvand, R.; Bayat, P.; Vahdaniparast, S.M.; Dehghani, S.; Kooshafar, Z.; Khaleghi, S.; Almasirad, A.; Salimi, M. Design and synthesis of novel 4-thiazolidinone derivatives with promising anti-breast cancer activity: Synthesis, characterization, *in vitro* and *in vivo* results. *Bioorg Chem* **2020**, *104*, 104276, <https://doi.org/10.1016/j.bioorg.2020.104276>.
- Türe, A.; Ergül, M.; Ergül, M.; Altun, A.; Küçükgül, İ. Design, synthesis, and anticancer activity of novel 4-thiazolidinone-phenylaminopyrimidine hybrids. *Mol Divers* **2021**, *25*, 1025-1050, <https://doi.org/10.1007/s11030-020-10087-1>.
- Genc; Bilgili, H.; Taslimi, P.; Akyuz, B.; Tuzun, B.; Gulcin, İ. Synthesis, characterization, biological evaluation, and molecular docking studies of some piperonyl-based 4-thiazolidinone derivatives. *Arch Pharm (Weinheim)* **2020**, *353*, e1900304, <https://doi.org/10.1002/ardp.201900304>.
- Nayak, P.; Kachroo, M. Design, Synthesis and *In vitro* Biological Activity of Some New 1,3- thiazolidine-4-one Derivatives as Chemotherapeutic Agents using Virtual Screening Strategies. *Curr Comput Aided Drug Des* **2020**, *16*, 757-771, <https://doi.org/10.2174/1573409916666200116102359>.
- Eroglu, B.; Ozadali-Sari, K.; Unsal-Tan, O.; Dharmarajan, S.; Yogeewari, P.; Balkan, A. Novel thiazolidinone-azole hybrids: design, synthesis and antimycobacterial activity studies. *Iran. J. Pharm. Res.* **2016**, *15*, 783-790, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5316256/>.
- Buemi, M.R.; Gitto, R.; Ielo, L.; Pannecouque, C.; De Luca, L. Inhibition of HIV-1 RT activity by a new series of 3-(1,3,4-thiadiazol-2-yl)thiazolidin-4-one derivatives. *Bioorg Med Chem* **2020**, *28*, 115431, <https://doi.org/10.1016/j.bmc.2020.115431>.
- Barreca, M.L.; Chimirri, A.; De Luca, L.; Monforte, A.M.; Monforte, P.; Rao, A.; Zappala, M.; Balzarini, j.; De Clercq, E.; Pannecouque, C.; Witvrouw, M. Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV-1 agents, *Bioorganic Med. Chem. Lett* **2001**, *11*, 1793-1796, [https://doi.org/10.1016/S0960-894X\(01\)00304-3](https://doi.org/10.1016/S0960-894X(01)00304-3).
- Rao, A.; Balzarini, J.; Carbone, A.; Chimirri, A.; De Clercq, E.; Monforte, A.M.; Monforte, P.; Pannecouque, C.; Zappalà, M. Synthesis of new 2,3-diaryl-1,3-thiazolidin-4-ones as anti-HIV agents. *Farmaco* **2004**, *59*, 33-39, <https://doi.org/10.1016/j.farmac.2003.09.001>.
- Güzel-Akdemir, Ö.; Carradori, S.; Grande, R.; Demir-Yazıcı, K.; Angeli, A.; Supuran, C.T.; Akdemir, A. Development of Thiazolidinones as Fungal Carbonic Anhydrase Inhibitors. *Int J Mol Sci* **2020**, *21*, 2960,

- <https://doi.org/10.3390/ijms21082960> .
25. Cesur, N.; Cesur, Z.; Ergenc, N.; Uzun, M. Synthesis and Antifungal Activity of Some 2-Aryl-3-substituted 4-Thiazolidinones. *Arch. Pharm.* **1994**, *327*, 271–272, <https://doi.org/10.1002/ardp.19943270414>.
 26. Fahmy, H.T. Synthesis of some new triazoles as potential antifungal agents. *Boll. Chim. Farm.* **2000**, *140*, 422–7, <https://pubmed.ncbi.nlm.nih.gov/11822232/>.
 27. Thompson, A.M.; Blaser, A.; Anderson, R.F.; Shinde, S.S.; Franzblau, S.G.; Ma, Z.; Denny, W.A.; Palmer, B.D. Synthesis , Reduction Potentials , and Antitubercular Activity of Ring A / B Analogues of the Bioreductive Synthesis , Reduction Potentials , and Antitubercular Activity of Ring A / B. *J. Med. Chem.* **2009**, *52*, 637–645, <https://doi.org/10.1021/jm801087e>.
 28. Babaoglu, K.; Page, M.A.; Jones, V.C.; McNeil, M.R.; Dong, C.; Naismith, J.H.; Lee, R.E. Novel inhibitors of an emerging target in Mycobacterium tuberculosis; substituted thiazolidinones as inhibitors of dTDP-rhamnose synthesis. *Bioorg Med Chem Lett* **2003**, *13*, 3237-30, [https://doi.org/10.1016/S0960-894X\(03\)00673-5](https://doi.org/10.1016/S0960-894X(03)00673-5).
 29. Ulusoy, N. Synthesis and antituberculosis activity of cycloalkylidenehydrazide and 4-aza-1-thiaspiro[4.5]decan-3-one derivatives of imidazo[2,1-b]thiazole. *Arzneimittelforschung* **2002**, *52*, 565–571, <https://doi.org/10.1055/s-0031-1299931>.
 30. Kucukguzel, S.G.; Oruc, E.E.; Rollas, S.; Sahin, F.; Ozbek, A. Synthesis, characterisation and biological activity of novel 4-thiazolidinones, 1, 3, 4-oxadiazoles and some related compounds. *Eur. J. Med. Chem.* **2002**, *37*, 197–206, [https://doi.org/10.1016/s0223-5234\(01\)01326-5](https://doi.org/10.1016/s0223-5234(01)01326-5).
 31. Kaur, R.; Kumar, R.; Dogra, N.; Kumar, A.; Yadav, A.K.; Kumar, M. Synthesis and studies of thiazolidinedione-isatin hybrids as α -glucosidase inhibitors for management of diabetes. *Future Med Chem* **2021**, *13*, 457-485, <https://doi.org/10.4155/fmc-2020-0022>.
 32. Ramshid, P.K.; S. Jagadeeshan, S.; Krishnan, A.; Mathew, M.; Asha Nair, S.; Radhakrishna Pillai, M. Synthesis and *in vitro* evaluation of some isatin-thiazolidinone hybrid analogues as anti-proliferative agents. *Med. Chem. (Los. Angeles)* **2010**, *6*, 306–312, <https://doi.org/10.2174/157340610793358909>.
 33. Kaminsky, D.; Khylyuk, D.; Vasylenko, O.; Zaprutko, L.; Lesyk, R. A facile synthesis and anticancer activity evaluation of spiro [thiazolidinone-isatin] conjugates. *Sci. Pharm* **2011**, *79*, 763–778, <https://doi.org/10.3797/scipharm.1109-14>.
 34. Havrylyuk, D.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. Synthesis of new 4-thiazolidinone-, pyrazoline-, and isatin-based conjugates with promising antitumor activity. *J. Med. Chem* **2012**, *55*, 8630–8641, <https://doi.org/10.1021/jm300789g>.
 35. Wang, S.; Zhao, Y.; Zhang, G.; Lv, Y.; Zhang, N.; Gong, P. Design , synthesis and biological evaluation of novel 4-thiazolidinones containing indolin-2-one moiety as potential antitumor agent. *Eur. J. Med. Chem.* **2011**, *46*, 3509–3518, <https://doi.org/10.1016/j.ejmech.2011.05.017>.
 36. El-Naggar, M.; Eldehna, W.M.; Almahli, H.; Elgez, A.; Fares, M.; Elaasser, M.M.; Abdel-Aziz, H.A. Novel thiazolidinone/thiazolo[3,2-a] benzimidazolone-isatin conjugates as apoptotic anti-proliferative agents towards breast cancer: One-pot synthesis and *in vitro* biological evaluation. *Molecules* **2018**, *23*, 1420, <https://doi.org/10.3390/molecules23061420>.
 37. Wang, F.; Liu, Z.; Wang, J.; Tao, J.; Gong, P.; Bao, X.; Zhao, Y.; Wang, Y. The interaction of 4-thiazolidinone derivatives containing indolin-2-one moiety with P-glycoprotein studied using K562 cell lines. *European journal of medicinal chemistry* **2015**, *101*, 126-32, <https://doi.org/10.1016/j.ejmech.2015.06.002>.
 38. Liu, Z.; Hou, Y.; Zhang, G.; Xu, N.; Mi, B.; Gong, P.; Zhao, Y. Design, synthesis and antitumor activity of novel indolin-2-one derivatives containing 4-thiazolidinone moiety. *Chemical Research in Chinese Universities* **2015**, *31*, 235-43, <https://doi.org/10.1007/s40242-015-4335-8>.
 39. Rahmani, Z.; Safari, F. Evaluating the *in vitro* therapeutic effects of human amniotic mesenchymal stromal cells on MiaPaca2 pancreatic cancer cells using 2D and 3D cell culture model. *Tissue Cell* **2020**, *68*, 101479, <https://doi.org/10.1016/j.tice.2020.101479>.
 40. Mahboobi, S.; Sellmer, A.; Ho, H.; Schmidt, M.; Maier, T.; Höcher, H.; Eichhorn, E.; Bär, T.; Schmidt, M.; Maier, T.; Stadlwieser, J.F.; Beckers, T.L. [4- (Imidazol-1-yl) thiazol-2-yl] phenylamines . A Novel Class of Highly Potent Colchicine Site Binding Tubulin Inhibitors : Synthesis and Cytotoxic Activity on Selected Human Cancer Cell. *J. Med. Chem* **2006**, *49*, 5769–5776, <https://doi.org/10.1021/jm060545p>.
 41. Behbehani, H.; Ibrahim, H.M. 4-Thiazolidinones in heterocyclic synthesis: synthesis of novel enaminones, azolopyrimidines and 2-arylimino-5-arylidene-4-thiazolidinones. *Molecules* **2012**, *17*, 6362–6385, <https://doi.org/10.3390/molecules17066362>.