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Synthesis and cytotoxic activity of some 2-(2,3-dioxo-2,3-dihydro-1*H*-indol-1-yl)acetamide derivatives

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Abstract: Isatin, 1*H*-indoline-2,3-dione, an endogenous compound, is also a synthetically versatile molecule that possesses a diversity of biological activities including anticonvulsant, antibacterial, antifungal, antiviral, anticancer, and cytotoxic properties. Based on the promising cytotoxic activity studies on *N*-substituted isatin derivatives, a series of 18 derivatives of 2-(2,3-dioxo-2,3-dihydro-1*H*-indol-1-yl)-*N*-phenylacetamide were designed, synthesized, and characterized according to their analytical and spectral data. All of the compounds were evaluated for their cytotoxic activity against MCF7, A549, HeLa, and HEK293 cell lines by real time cell analyzer. Etoposide was used as a standard compound. Briefly, *ortho* substitutions gave better results compared to *meta* and *para* substitutions on the *N*−phenyl ring and compounds bearing *ortho* substitutions were more effective on MCF7 cell lines than A549 and HeLa cell lines. 2-(2,3-Dioxo-2,3-dihydro-1*H*-indol-1-yl)-*N*-(2-isopropylphenyl)acetamide was the most active compound against all the tested cell lines.

Key words: Isatin, acetamide, anilide, cytotoxic activity, anticancer

1. Introduction

Cancer is known as one of the most lethal diseases as it is responsible for more than 20% of all deaths in developed countries.[1] High mortality rates, serious side effects, deficiencies of the available chemotherapeutics, and high costs during treatment clearly underscore the need to develop new anticancer agents.

Isatin, one of the most studied nuclei for cytotoxic activity, is an endogenous compound found in blood, tissues, and various organs.[2−4] The synthetic versatility of isatin derived at C-2, C-3, and *N* positions has led to a wide variety of pharmacological responses including cytotoxic, anticancer, antibacterial, antiviral, anti-HIV, anticholinesterase, antiinflammatory, antihypertensive, antihypoxic, antiulcer, anticonvulsant, COX-2, and carboxylesterase inhibitor activities.[2−8] Among these activities, cytotoxic activity studies on isatin derivatives have been accelerated after the FDA approval of C-3 derivative of isatin, oxindole sunitinib malate. Although sunitinib is a C-3 derivative of isatin, none of the other studies related to C-3 derivatives led to compounds more active than C-2 and/or *N*-substituted analogues.[4,9] On the other hand, a literature survey on cytotoxic activity studies of *N*-alkyl isatin derivatives reveals the importance of *N*-substitution. In addition, SAR studies demonstrated that the introduction of an aromatic ring with 1 to 3 carbon atom linkers at the *N* atom enhances the cytotoxic activity.[9−11]

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Substituted anilides were also studied for their cytotoxic activity and the results suggested that the activity depends on the nature and the positions of the substituents on the N-phenyl ring.[12]

In this context, a group of N-phenylisatin-1-acetamide derivatives bearing diverse substitutions with different electronic and hydrophobic natures on the phenyl ring were designed and synthesized. Chemical structures of the title compounds were confirmed by IR, ^{1}H NMR, and ESI-MS spectra, and elemental analysis.

The cytotoxic activity of the final compounds was screened against MCF7, A549, HeLa, and 293T cell lines by real-time cell analyzer (RTCA).

2. Experimental

2.1. Chemistry

Melting points were determined on a Barnstead Electrothermal IA9100 melting point apparatus (USA) and are uncorrected. The IR spectra of the compounds were recorded as potassium bromide pellets on a Jasco FT/IR-400 spectrometer (Jasco, Tokyo, Japan). The NMR spectra were recorded on a Varian AS 400 Mercury Plus NMR (Varian Inc., Palo Alto, CA, USA). Chemical shifts were reported in parts per million (δ). J values were given in hertz (Hz). Mass spectra (electron spray ionization (ESI)) were measured on a Waters Micromass ZQ connected to a Waters Alliance HPLC (Waters Corporation, Milford, MA, USA). Elemental analyses (C, H, and N) were performed using a Leco CHNS-932 (Leco, St. Joseph, MI, USA).

The synthesis of the title compounds was realized in 2 steps. First, substituted anilines and benzylamine were reacted with 2-chloroacetyl chloride according to the reported procedures to obtain the intermediates, ω-chloroanilides and ω-chlorobenzylamide; then they were condensed with isatin to yield the title compounds (Figure).[13,14]

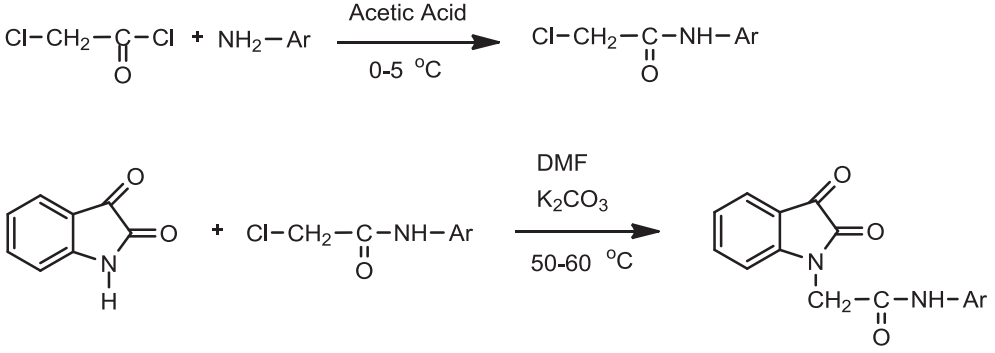


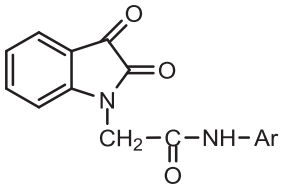
Figure. Synthesis of compounds **1**–**18**.

2.2. General procedure for the synthesis of the title compounds (1–18)

According to the reported procedure, isatin (10 mmol) and K_2CO_3 (14.5 mmol) were stirred at 50–60 °C for 1 h in 6–8 mL of DMF; then ω-chloroanilides or ω-chlorobenzylamide (11 mmol) and KI (2 mmol) were added and heated at 60 °C.[8,11] After confirming the end of the reaction by TLC, the mixture was poured into ice-water. The precipitated crude product was filtered and washed successively with cold water. Compounds **1**, **15** (DMF:H_2O, 1:1), **2**–**7**, **17**, and **18** (EtOH) were crystallized from the crude product. Compounds **8**–**10** (CH_2Cl_2:acetone, 100:1), **14** ($CHCl_3$:MeOH, 95:5), and **16** (EtOAc:Hxn, 5:1) were purified by column chromatography and crystallized from EtOH:H_2O (1:1). For compounds **11**–**13**, the crude product in EtOAc

was washed with 12.5% HCl; residue was crystallized from DMF:H_2O (1:1). Reaction times, yields, and melting points are presented in Table 1.

Table 1. Reflux times, yields, and melting points of the title compounds.



Comp.	Ar	Reaction time (h)	Mp (°C)	Yield (%)
1	phenyl	5	245[a]	78
2	2-methylphenyl	5	257[b]	18
3	3-methylphenyl	3	266	60
4	4-methylphenyl	3	252[c]	75
5	2-methoxyphenyl	3	195	76
6	3-methoxyphenyl	4	242	58
7	4-methoxyphenyl	4	235	56
8	2-chlorophenyl	6	344	25
9	3-chlorophenyl	9.5	275	8
10	4-chlorophenyl	9	273	36
11	2-nitrophenyl	4.5	218[d]	11
12	3-nitrophenyl	3.5	318	55
13	4-nitrophenyl	4	285[e]	34
14	2-ethylphenyl	4	252	10
15	2-isopropylphenyl	6	171	65
16	2,6-dimethylphenyl	4	323	11
17	2,6-dichlorophenyl	2	308	18
18	benzyl	3	214	65

[a] 215–219 °C from DMF[9], [b] 222–226 °C from DMF[9], [c] 225–228 °C from DMF[9], [d] 215–221 °C from DMF[9], [e] 222–225 °C from DMF[9].

3. Cytotoxic activity

3.1. Cell types and culture conditions

Human embryonic kidney cells (HEK293), human breast cancer cells (MCF7), and human epithelial cervical cancer cells (HeLa) were kindly provided by Dr Shengyun Fang (University of Maryland, Baltimore, MD, USA). The human lung carcinoma cell line (A549) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle Medium with high glucose. These media were supplemented with 10% fetal bovine serum, 50 U/mL penicillin, 50 μg/mL streptomycin, and L-glutamine (2 mmol/L). All the tissue culture reagents were purchased from Biological Industries (Israel). Each cell type was cultivated at 37 °C in a humidified incubator with 5% CO_2.

3.2. Determination of cell viability by RTCA

The xCELLigence system was used according to the instructions of the supplier (Roche Applied Science). Cells were grown and expanded in tissue culture flasks. After reaching 70%–80% confluence, cells were washed with PBS and detached from the flasks by trypsin/EDTA treatment. Subsequently, 100 μL of cell culture media was added into each well of E-plate 96 at room temperature. Then, E-plate 96 was connected to the RTCA-MP station and the background impedance was measured. To determine the effect of the test compounds, 5000 cells for each cell line were seeded. After 30 min of incubation at room temperature, E-plates were placed back into the RTCA-MP station. Cells were grown and the electrical impedance was measured every 30 min. Approximately 18 h after seeding, when the cells were in the log growth phase, the cells were exposed to test compounds at different concentrations (10, 20, 40, 60, 100 μM). Controls received only dimethyl sulfoxide (DMSO) with a final concentration of 0.20%. Measurements were performed every 2 min for 2 h and then every 30 min in order to visualize the fast drug response and late drug response, respectively. The electrical impedance measured by the RTCA software of the xCELLigence system was reflected as a dimensionless parameter called the cell index (CI) value. Growth curves were normalized to the CI at the last measured time point before compound addition for each well. IC50 values were determined using RTCA software performing a curve fitting of the sigmoidal dose-response equation. All the experiments were run for 150 h and done in triplicate.

4. Results and discussion

4.1. Chemistry

Eighteen *N*-phenylisatin-1-acetamide derivatives were synthesized in order to appraise their cytotoxic activity (Figure). The structures of the title compounds were confirmed by spectral (IR, ^{1}H NMR, and ESI-MS) and elemental analysis.

Among the synthesized compounds, **6**, **14**, and **17** are novel. Compounds **1**–**4**, **11**, **13**, and **18** were reported previously.[15] Compounds **5**, **7**–**10**, **12**, **15**, and **16** are listed in the literature with registry numbers CASRN 302968-17-8, 609794-52-7, 61764-48-2, 893653-42-4, 444792-13-6, 303045-63-8, 685845-29-8, and 617697-23-1, respectively, but corresponding scientific reference data are not available.

In the IR spectra, the stretching and bending bands are confirmative frequencies indicating the presence of the amide structure for the title compounds. Amide I vibrations arising mainly from a carbonyl stretching band (1731–1607 cm^{-1}) and amide II bands resulting from N-H bending (1615–1331 cm^{-1}) were detected within the expected frequencies. Similarly, N-H stretching bands of amide were seen between 3372 and 3219 cm^{-1}. The carbonyl group's stretching bands of the isatin ring were also observed between 1743 and 1727 cm^{-1} (Table 2).

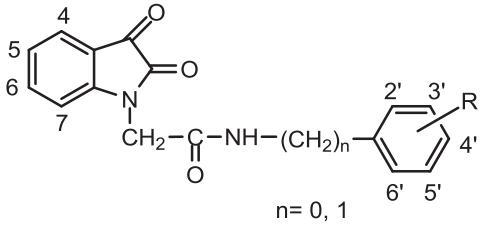
The ^{1}H NMR spectra of the title compounds were recorded in DMSO-d6 solution and are in complete agreement with the expected resonance signals in terms of chemical shifts and integrations. In the aliphatic region, besides the proton signals of substituents on the phenyl ring, the methylene protons in acetamide derivatives are observed as singlets. Depending on the nature of the substituents and substitution patterns on the *N*-phenyl ring, the aromatic protons of certain compounds are observed in distinct chemical shifts with expected splitting patterns as doublets, triplets, or multiplets integrating more than one proton due to the close chemical shifts.

Moreover, in the aromatic region, the protons of isatin are recorded with relevant splitting patterns and integration values and the N-H protons are observed between δ 10.96 and 9.59 ppm. The NMR data of the title compounds are summarized in Table 3.

Table 2. Formulae and IR and ESI-MS data of the title compounds.

Comp. no.	Formula	IR (cm^{-1})	MS m/e (% intensity)
1	$C_{16}H_{12}N_2O_3$	3320, 1743, 1721, 1683, 1615, 1606	281[M+H] (100%)
2	$C_{17}H_{14}N_2O_3$	3259, 1740, 1666, 1612, 1539, 1473	295[M+H] (100%)
3	$C_{17}H_{14}N_2O_3$	3266, 1739, 1668, 1616, 1562, 1486	295 [M+H] (100%)
4	$C_{17}H_{14}N_2O_3$	3349, 1727, 1689, 1612, 1552, 1469	295 [M+H] (100%)
5	$C_{17}H_{14}N_2O_4$	3367, 1741, 1679, 1612, 1535, 1459	311 [M+H] (100%)
6	$C_{17}H_{14}N_2O_4$	3326, 1741, 1722, 1677, 1612, 1558, 1469	311 [M+H] (100%)
7	$C_{17}H_{14}N_2O_4$	3326, 1741, 1722, 1677, 1612, 1558, 1469	311 [M+H] (100%)
8	$C_{16}H_{11}ClN_2O_3$	3250, 1728, 1665, 1607, 1589, 1542	316[M+H] (79.6%), 318[M+H+2] (32.7%)
9	$C_{16}H_{11}ClN_2O_3$	3319, 1741, 1687, 1610, 1547, 1470	316[M+H] (100%), 318[M+H+2] (29.3%)
10	$C_{16}H_{11}ClN_2O_3$	3331, 1740, 1720, 1693, 1612, 1552	316[M+H] (100%), 318[M+H+2] (36%)
11	$C_{16}H_{11}N_3O_5$	3329, 1740, 1697, 1612,1581, 1500,1464, 1331	326[M+H] (25%)
12	$C_{16}H_{11}N_3O_5$	3323, 1734, 1689, 1608, 1552,1520, 1468	326[M+H] (27.4%)
13	$C_{16}H_{11}N_3O_5$	3329, 1728, 1702, 1615, 1598, 1558, 1511, 1346	326[M+H] (17.1%)
14	$C_{18}H_{16}N_2O_3$	3280, 1735, 1666, 1614, 1538, 1471	309 [M+H] (100%)
15	$C_{19}H_{18}N_2O_3$	3239, 1740, 1659, 1611, 1537, 1473	323[M+H] (98.8%)
16	$C_{18}H_{16}N_2O_3$	3257, 1743, 1731, 1664, 1612, 1538	309[M+1] (100%)
17	$C_{16}H_{10}Cl_2N_2O_3$	3239, 1735, 1670, 1612, 1575, 1535, 1467	349 [M+H] (57%)
18	$C_{17}H_{14}N_2O_3$	3372, 1735, 1670, 1612, 1562, 1471	295 [M+H] (92%)

Table 3. ^{1}H NMR data of the title compounds.



Comp.	NMR
1	**^{1}H NMR (DMSO-d6):** δ 10.20 (1H, *s*, NH), 7.69–7.65 (1H, *m*, H-6), 7.62 (1H, *dd*, J = 0.78, 7.41 Hz, H-4), 7.55 (2H, *d*, J = 7.8 Hz, H-2', H-6'), 7.32 (2H, *t*, J = 7.8 Hz, H-3', H-5'), 7.19–7.14 (2H, *m*, H-4', H-5), 7.08 (1H, *t*, J = 7.41 Hz, H-7), 4.57 (2H, *s*, -CH_2-) ppm.
2	**^{1}H NMR (DMSO-d6):** δ 9.65 (1H, *s*, NH), 7.7 (1H, *td*, J = 1.17, 7.8 Hz, H-6), 7.61 (1H, *d*, J = 7.4 Hz, H-4), 7.28 (1H, *d*, J = 7.8 Hz, H-6'), 7.22–7.09 (5H, *m*, H-3', H-4', H-5', H-5, H-7), 4.58 (2H, *s*, -CH_2-), 2.15 (3H, *s*, CH_3) ppm.
3	**^{1}H NMR (DMSO-d6):** δ10.1 (1H, *s*, NH), 7.67 (1H, *td*, J = 1.17, 7.8 Hz, H-6), 7.61 (1H, *d*, J = 7.02 Hz, H-4), 7.38 (1H, *s*, H-2'), 7.34 (1H, *d*, J = 8.58 Hz, H-6'), 7.21–7.13 (3H, *m*, H-5, H-7, H-5'), 6.89 (1H, *d*, J = 7.41 Hz, H-4'), 4.55 (2H, *s*, -CH_2-), 2.26 (3H, *s*, CH_3) ppm.

Table 3. Continued.

Comp.	NMR
4	**^{1}H NMR (DMSO-d6):** δ 10.1 (1H, *s*, NH), 7.65 (1H, *td*, J = 1.17, 7.7 Hz, H-6), 7.59 (1H, *d*, J = 7.4 Hz, H-4), 7.40 (2H, *d*, J = 8.58 Hz, H-2', H-6'), 7.14–7.10 (4H, *m*, H-5, H-7, H-3', H-5'), 4.52 (2H, *s*, -CH_2-), 2.23 (3H, *s*, CH_3) ppm.
5	**^{1}H NMR (DMSO-d6):** δ 9.59 (1H, *s*, NH), 7.80 (1H, *d*, J = 7.02 Hz, H-6'), 7.66 (1H, *td*, J = 1.56, 7.8 Hz, H-6), 7.58 (1H, *d*, J = 7.02 Hz, H-4), 7.16–7.03 (4H, *m*, H-3', H-4', H-5, H-7), 6.87 (1H, *td*, J = 1.56, 7.6 Hz, H-5), 4.64 (2H, *s*, -CH_2-), 3.82 (3H, *s*, CH_3) ppm.
6	**^{1}H NMR (DMSO-d6):** δ 10.20 (1H, *s*, NH), 7.65 (1H, *td*, J = 1.17, 7.8 Hz, H-6), 7.59 (1H, *d*, J = 7.41 Hz, H-4), 7.24–7.11 (4H, *m*, H-5, H-7, H-2', H-6'), 7.06 (1H, *d*, J = 8.9 Hz, H-5'), 6.64 (1H, *dd*, J = 2.3, 8.19 Hz, H-4'), 4.55 (2H, *s*, -CH_2-), 3.693 (3H, *s*, CH_3) ppm.
7	**^{1}H NMR (DMSO-d6):** δ 10.03 (1H, *s*, NH), 7.64 (1H, *t*, J = 7.41 Hz, H-6), 7.59 (1H, *d*, J = 7.8 Hz, H-4), 7.42 (2H, *d*, J = 8.58 Hz, H-2', H-6'), 7.14 (1H, *t*, J = 7.41 Hz, H-5), 7.11 (1H, *d*, J = 7.8 Hz, H-7), 6.86 (2H, *d*, J = 8.97 Hz, H-3', H-5'), 4.51 (2H, *s*, -CH_2-), 3.70 (3H, *s*, CH_3) ppm.
8	**^{1}H NMR (DMSO-d6):** δ 9.92 (1H, *s*, NH), 7.68 (1H, *td*, J = 1.56, 7.80 Hz, H-6), 7.60–7.57 (2H, *m*, H-4, H-6'), 7.50–7.48 (1H, *m*, H-3'), 7.31 (1H, *td*, J = 1.56, 7.8 Hz, H-5'), 7.21 (1H, *td*, J = 1.56, 7.8 Hz, H-4'), 7.18–7.13 (2H, *m*, H-5, H-7), 4.62 (2H, *s*, -CH_2-) ppm.
9	**^{1}H NMR (DMSO-d6):** δ 10.39 (1H, *s*, NH), 7.74 (1H, *t*, J = 1.95 Hz, H-2'), 7.68 (1H, *td*, J = 1.17, 6.63 Hz, H-6), 7.62 (1H, *d*, J = 7.41 Hz, H-4), 7.45 (1H, *d*, J = 8.97 Hz, H-6'), 7.36 (1H, *t*, J = 8.19 Hz, H-5'), 7.19–7.14 (3H, *m*, H-7, H-5, H-4'), 4.59 (2H, *s*, -CH_2-) ppm.
10	**^{1}H NMR (DMSO-d6):** δ 10.32 (1H, *s*, NH), 7.67–7.54 (4H, *m*, H-2', H-6', H-6, H-4), 7.37–7.35 (2H, *m*, H-3', H-5'), 7.17–7.11 (2H, *m*, H-7, H-5), 4.56 (2H, *s*, -CH_2-) ppm.
11	**^{1}H NMR (DMSO-d6):** δ 10.53 (1H, *s*, NH), 7.95 (1H, *dd*, J = 1.17, 8.19 Hz, H-3'), 7.72–7.66 (2H, *m*, H-5', H-6'), 7.614–7.559 (2H, *m*, H-4, H-6), 7.416–7.373 (1H, *m*, H-4'), 7.17 (1H, *t*, J = 7.410 Hz, H-5), 7.09 (1H, *d*, J = 7.8 Hz, H-7), 4.592 (2H, *s*, -CH_2-) ppm.
12	**^{1}H NMR (DMSO-d6):** δ 10.96 (1H, *s*, NH), 8.561–8.55 (1H, *m*, H-2'), 7.96–7.91 (2H, *m*, H-4', H-6'), 7.68 (1H, *td*, J = 1.17, 7.8 Hz, H-6), 7.70–7.62 (2H, *m*, H-4, H-5'), 7.20–7.17 (2H, *m*, H-5, H-7), 4.64 (2H, *s*, -CH_2-) ppm.
13	**^{1}H NMR (DMSO-d6):** δ 10.83 (1H, *s*, NH), 8.24 (2H, *d*, J = 9.36 Hz, H-3', H-5'), 7.82 (2H, *d*, J = 9.36 Hz, H-2', H-6'), 7.68 (1H, *td*, J = 1.17, 6.63 Hz, H-6), 7.63 (1H, *d*, J = 6.63 Hz, H-4), 7.19 (2H, *d*, J = 7.80 Hz, H-5, H-7), 4.66 (2H, *s*, -CH_2-) ppm.
14	**^{1}H NMR (DMSO-d6):** δ 9.60 (1H, *s*, NH), 7.68 (1H, *td*, J = 1.17, 7.8 Hz, H-6), 7.59 (1H, *d*, J = 7.41 Hz, H-4), 7.22–7.13 (6H, *m*, H-5, H-7, H-3', H-4', H-5', H-6'), 4.55 (2H, *s*, CH_2CO), 2.51–2.48 (2H, *m*, CH_2CH_3), 1.05 (3H, *t*, J = 7.6 Hz, CH_3) ppm.
15	**^{1}H NMR (DMSO-d6):** δ 9.66 (1H, *s*, NH), 7.71–7.67 (1H, *m*, H-6), 7.60 (1H, *d*, J = 7.41 Hz, H-4), 7.29 (1H, *d*, J = 7.8 Hz, H-5'), 7.18–7.12 (5H, *m*, H-2', H-3', H4', H-5, H-7), 4.55 (2H, *s*, -CH_2-), 3.051–3.017 (1H, *m*, isopro-CH-), 1.073 (6H, *d*, J = 6.63 Hz, 2 × CH_3) ppm.
16	**^{1}H NMR (DMSO-d6):** δ 9.57 (1H, *s*, NH), 7.70 (1H, *t*, J = 7.8 Hz, H-6), 7.62 (1H, *d*, J = 7.41 Hz, H-4), 7.21–7.16 (2H, *m*, H-2', H-4'), 7.09–7.03 (3H, *m*, H-5, H-7, H-3'), 4.54 (2H, *s*, -CH_2-), 2.09 (6H, *s*, 2 × CH_3) ppm.
17	**^{1}H NMR (DMSO-d6):** δ 10.19 (1H, *s*, NH), 7.71 (1H, *td*, J = 1.17, 7.8 Hz, H-6), 7.61 (1H, *dd*, J = 0.78, 7.4 Hz, H-4), 7.53 (2H, *d*, J = 8.19 Hz, H-3', H-5'), 7.35 (1H, *t*, J = 7.8 Hz, H-4'), 7.18 (1H, *t*, J = 7.6 Hz, H-5), 7.12 (1H, *d*, J = 7.8 Hz, H-7), 4.58 (2H, *s*, -CH_2-) ppm.
18	**^{1}H NMR (DMSO-d6):** δ 8.72 (1H, *t*, J = 5.85 Hz, NH), 7.65 (1H, *td*, J = 1.17, 7.8 Hz, H-6), 7.59 (1H, *d*, J = 7.02 Hz, H-4), 7.32–7.20 (4H, *m*, H-2', H-3', H-5', H-6'), 7.15 (2H, *t*, J = 7.41 Hz, H-5, H-7), 7.06 (1H, *d*, J = 8.19 Hz, H-4'), 4.40 (2H, *s*, -CH_2-), 4.29 (2H, *d*, J = 5.85 Hz, -CH_2-phenyl) ppm.

The mass spectra of the title compounds were recorded by using ESI positive mode and the $[M+H]^+$ ions of the compounds are in complete agreement with the calculated molecular weights (Table 2).

Purity levels of the compounds were determined by elemental analysis (C, H, N) and the results are within $\pm 0.4\%$ of the calculated values (Table 4).

Table 4. Elemental analysis of the title compounds.

Elemental analysis (% calculated)				
Comp.	Formula	%C	%H	%N
1	$C_{16}H_{12}N_2O_3$	68.56 (68.49)	4.32 (4.379)	9.99 (9.967)
2	$C_{17}H_{14}N_2O_3$	69.16 (69.38)	4.60 (4.79)	9.42 (9.52)
3	$C_{17}H_{14}N_2O_3 \times 0.075C_2H_5OH$	69.17 (69.19)	5.25 (4.86)	9.44 (9.41)
4	$C_{17}H_{14}N_2O_3 \times 0.05C_2H_5OH$	68.92 (69.38)	5.01 (4.79)	9.48 (9.52)
5	$C_{17}H_{14}N_2O_4$	65.13 (65.80)	4.46 (4.55)	8.85 (9.03)
6	$C_{17}H_{14}N_2O_4$	65.54 (65.80)	4.22 (4.55)	8.98 (9.03)
7	$C_{17}H_{14}N_2O_4$	65.46 (65.80)	4.31 (4.55)	8.98 (9.03)
8	$C_{16}H_{11}ClN_2O_3$	60.69 (61.06)	3.704 (3.52)	8.776 (8.90)
9	$C_{16}H_{11}ClN_2O_3$	60.81 (61.06)	3.361 (3.52)	8.821 (8.90)
10	$C_{16}H_{11}ClN_2O_3$	60.92 (61.06)	3.18 (3.52)	8.995 (8.90)
11	$C_{16}H_{11}N_3O_5 \times 0.2\ H_2O$	58.38 (58.43)	3.432 (3.49)	12.89 (12.78)
12	$C_{16}H_{11}N_3O_5 \times 0.1\ H_2O$	58.36 (58.75)	3.338 (3.45)	12.83 (12.85)
13	$C_{16}H_{11}N_3O_5 \times 0.1\ H_2O$	58.65 (58.75)	3.404 (3.45)	12.75 (12.85)
14	$C_{18}H_{16}N_2O_3$	69.75 (70.12)	5.01 (5.23)	9.00 (9.09)
15	$C_{19}H_{18}N_2O_3$	71.16 (70.79)	5.896 (5.63)	8.784 (8.69)
16	$C_{18}H_{16}N_2O_3$	69.68 (69.31)	5.587 (5.30)	8.924 (8.98)
17	$C_{16}H_{10}Cl_2N_2O_3$	54.75 (55.04)	3.24 (2.89)	8.01 (8.02)
18	$C_{17}H_{14}N_2O_3$	69.42 (69.38)	5.08 (4.79)	9.51 (9.52)

4.2. Cytotoxic activity

The synthesized derivatives were screened for their cytotoxic activity against some tumor cell lines (MCF7, A549, HeLa) and one nontumor cell line (HEK293) by real-time cell assay. Etoposide was used as a standard compound (Table 5).

Modi et al. reported research including some isatin-*N*-phenylacetamide derivatives during our ongoing study. According to their article, compounds **1**, **2**, **4**, **11**, and **13** were evaluated for cytotoxic activity against MCF7 and VERO cell lines and these compounds displayed greater than 50% survival after an exposure time of 72 h and had not been further evaluated for finding IC50 values.[15]

In our study, the activity results demonstrated that the synthesized compounds are more active against MCF7 than A549 and HeLa cell lines. Among these compounds, under the set of studied substituents, the *ortho* substitution seems more critical than *meta* and *para* positions to support the activity since *ortho* substitution of chloro, nitro, methoxy, and isopropyl led to more active compounds than *meta* and *para* substituted derivatives. The contribution of *ortho* substitution to IC50 values in decreasing order is as follows: 2-OCH_3, 2-NO_2 >2-$CH(CH_3)_2$ >2-Cl >2-C_2H_5 >2,6-dichloro >2,6-dimethyl >2-methyl. This order supports the idea that *ortho*

Table 5. Cytotoxic activities of the title compounds.

Comp.	IC50 MCF -7 (μM)	IC50 A549 (μM)	IC50 HeLa (μM)	IC50 HEK293 (μM)
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	88	-	-	>100
5	11	>100	94	47
6	-	-	-	-
7	85	>100	>100	>100
8	32	>100	64	85
9	61	-	96	>100
10	-	-	-	>100
11	11	>100	28	46
12	>100	-	-	-
13	68	-	96	>100
14	49	>100	77	>100
15	18	54	30	27
16	64	-	93	>100
17	60	-	64	>100
18	47	>100	98	65
Etoposide	11	7	11.4	2.4

MCF7: Human mammary gland adenocarcinoma (nonmetastatic) cell line, **A549**: carcinomatous human alveolar basal epithelial cell line, **HeLa**: Human epithelial carcinoma cell line, **293T**: human renal epithelial cell line.
"-" Nondetectable activity

substituents capable of hydrogen bonding, intramolecularly (with amide proton by leading to a conformation state critical for desired biological interactions) and/or intermolecularly (with corresponding target molecular site), can favor the enhancement of cytotoxic activity. Similarly, the contribution of the bulky substituents at the *ortho* position to cytotoxic activity could well be related to the conformational preferences to support the desired biological interaction, since increasing the size of the alkyl substituent enhances the activity (see compounds **2**, **14**, and **15** in Table 5).

Compared to the reference compound etoposide, compounds **5** and **11** possess equal IC50 values in MCF7 cell lines and display less cytotoxicity against nontumoral HEK293 cell lines. This result suggests that, among the cell lines studied, compounds **5** and **11** have more selective cytotoxic activity compared to etoposide. Compound **15**, which has an IC50 value close to that of etoposide in MCF7 cell lines, is the third most active compound in the series but the close IC50 value against MCF7 and HEK293 cells indicates nonselective cytotoxic activity.

In terms of the A549 cell line, the only active compound with an IC50 value less than 100 μM is compound **15**. The rest of the synthesized compounds did not show any beneficial cytotoxic activity against A549 cell lines.

The most active compounds against the HeLa cell line are compounds **11** and **15**, with IC50 values of 28

μM and 30 μM, respectively. None of the synthesized compounds yield better activity than etoposide in this cell line.

The cytotoxic behaviors of the synthesized compounds were also assessed against a human embryonic kidney cell line (HEK293). The screening results indicate that, in general, the cytotoxic tendency of the compounds was decreased in normal human cells, indicating more selective behavior in tumor cell lines.

5. Conclusion

Substituents and their positions on the *N*-phenyl ring seem to have a direct impact on the cytotoxic activity of 2-(2,3-dioxo-2,3-dihydro-1*H*-indol-1-yl)-*N*-phenylacetamide derivatives. In general, more bulky or hydrogen bonding substituents at the *ortho* position seem to yield more active compounds against the studied cell lines of MCF7 and HeLa (see compounds **11** and **15**).

Those results will be utilized for further derivatization of the title compound in order to optimize the cytotoxic activity and to yield compounds to serve as leader templates for *N*-phenylisatin-1-acetamide.

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