Article

Synthesis, Characterization, and Anti-Cancer Activity of Some New *N*′-(2-Oxoindolin-3-ylidene)-2-propylpentane hydrazide-hydrazones Derivatives

Ayman El-Faham [1,2,*], Muhammad Farooq [3,*], Sherine N. Khattab [2], Nael Abutaha [3], Mohammad A. Wadaan [3], Hazem A. Ghabbour [4] and Hoong-Kun Fun [4,5]

[1] Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

[2] Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Alexandria 21321, Egypt; E-Mail: Sh.n.Khattab@gmail.com

[3] Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; E-Mails: nabutaha@ksu.edu.sa (N.A.); Wadaan@ksu.edu.sa (M.A.W.)

[4] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia; E-Mails: ghabbourh@yahoo.com (H.A.G.); hfun.c@ksu.edu.sa (H.-K.F.)

[5] X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, Penang 11800, Malaysia

*** Authors to whom correspondence should be addressed; E-Mails: aymanel_faham@hotmail.com (A.E.-F.); fmuhammad@ksu.edu.sa (M.F.); Tel.: +966-114-673-195 (A.E.-F.); Fax: +966-114-675-992 (A.E.-F.).

Academic Editor: Jean Jacques Vanden Eynde

Abstract: Eight novel *N*′-(2-oxoindolin-3-ylidene)-2-propylpentane hydrazide-hydrazone derivatives **4a**–**h** were synthesized and fully characterized by IR, NMR (^{1}H-NMR and ^{13}C-NMR), elemental analysis, and X-ray crystallography. The cyto-toxicity and *in vitro* anti-cancer evaluation of the prepared compounds have been assessed against two different human tumour cell lines including human liver (HepG2) and leukaemia (Jurkat), as well as in normal cell lines derived from human embryonic kidney (HEK293) using MTT assay. The compounds **3e**, **3f**, **4a**, **4c**, and **4e** revealed promising anti-cancer activities in tested human tumour cells lines (IC$_{50}$ values between 3 and 7 μM) as compared to the known anti-cancer drug 5-Fluorouracil (IC$_{50}$ 32–50 μM). Among the tested compounds, **4a** showed specificity

against leukaemia (Jurkat) cells, with an IC$_{50}$ value of 3.14 µM, but this compound was inactive in liver cancer and normal cell lines.

**Keywords:** valproic acid; isatin; hydrazide-hydrazone; anti-cancer activity

1. Introduction

Hydrazide-hydrazone derivatives are molecules containing a highly reactive group (CO-NH-N=CH) and considered to be a good candidate for development of a new drug [1]. Recently, hydrazide-hydrazones have been considered to be of great interest in medicinal chemistry due to their diverse biological properties, including anti-microbial [2–4], anti-mycobacterial [5,6], anti-convulsant [7], analgesic [8], anti-inflammatory [9], anti-platelet [10], anti-tubercular[10–13], and anti-tumoral activities [14–19]. In addition, hydrazide-hydrazones have been reported to elicit anti-cancer [18–21] and anti-HIV properties [22] and they are therefore increasingly considered to be of great value in medicinal chemistry [16,23–27].

Because of the remarkable biological value of isatin as an important constituent of bioactive compounds exhibiting caspase inhibitory [28,29], antibacterial, and antiproliferative activity [30], Schiff bases of isatin derivatives have antismallpox [31] and GAL3 receptor antagonist capabilities [32]. In addition, the analogous of isatin derivatives displayed inhibitory activity against eLF2 kinase activator [33], TNF-α, CDK2 [34] and SARS protease [35]. Isatin also displays antiviral [36], anti-inflammatory, analgesic [37], and anticonvulsant activities [38].

Valproic acid (VPA, **1**) has an important biological value as a potent antiepileptic molecule [39–41], as well as showing inhibition of angiogenesis both *in vitro* and *in vivo* [42–45]. VPA also acts as a powerful histone deacetylase inhibitor [46,47] and induces differentiation and apoptosis in a variety of malignant cells *in vitro* [48]. Clinical trials with VPA have focused on acute myeloid leukaemia and the myelodysplastic syndromes. When it was used as mono therapy or in combination with all-trans retinoic acid, which synergizes *in vitro*, VPA achieved hematologic improvement in a subset of patients [48].

As a continuation to our previously reported data [49–51], herein we designed eight isatin hydrazide-hydrazone derivatives, considering some of the factors responsible for such activity, including (i) the presence of isatin moiety; (ii) the presence of the hydrazide-hydrazone functionality; and (iii) valproic acid moiety (Figure 1).
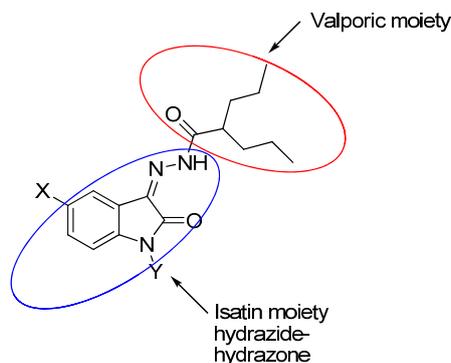

Figure 1. Structure of the target product.

All the prepared compounds were assessed against two different human tumour cell lines, including human liver (HepG2) and leukaemia (Jurkat), as well as in normal cell lines derived from human embryonic kidney (HEK293) using MTT assay.

2. Results and Discussion

2.1. Chemistry

Compound **2** was prepared following the reported method [49] and condensed with isatin derivatives **3a–h** in the presence of 2–3 drops of glacial HOAc and ethanol as the solvent to afford products **4a–h** (Scheme 1). The structures of all synthesized compounds were in a good agreement with their spectral data.
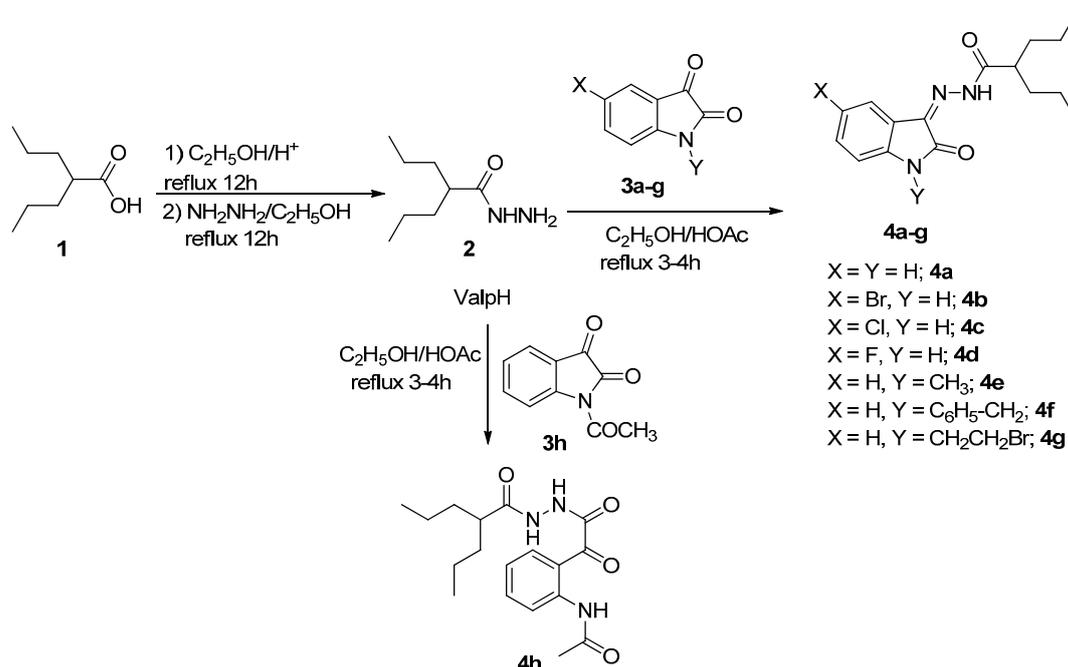

Scheme 1. Synthesis of *N'*-(2-oxoindolin-3-ylidene)-2-propylpentanehydrazide Derivatives.

The IR spectra of the compounds **4a–g** reveal absorption bands in the region 3195–3217 cm^{-1} corresponding to the (NH), a band at 1720 and 1688 cm^{-1} corresponding to the C=O group, and a peak around 1596 cm^{-1} related to the C=N bond. The NMR of all the products **4a–g** are in good agreement with their structures (Figures S1–S7).

As a prototype ^{1}H-NMR of **4g** showed multiple peaks at δ 0.86–0.89, 1.27–1.29, 1.40–1.44, 1.56–1.60 ppm, and a broad singlet at δ 2.51 ppm corresponding to the valporic acid moiety (2 CH$_3$, 4 CH$_2$, and CH, respectively). Also, two triplet peaks were observed at δ 3.77 and 4.20 ppm corresponding to the two methylene group (CH$_2$-CH$_2$Br), respectively. The observed peaks in the aromatic region at δ 7.18 (t, 1H, Ar-H), 7.32 (d, 1H, Ar-H), 7.53 (t, 1H, Ar-H), and 7.88 (brs, 1H, ArH) are related to the isatin moiety, while the broad singlet peak at δ 12.28 ppm is related to the NH. The ^{13}C-NMR of **4g** showed peaks at δ 14.5, 20.6, 29.9, 35.3, 39.7, 42.2, and 46.5 ppm, corresponding to the valporic acid moiety and the two methylene groups, in addition to seven peaks related to the aromatic carbons and imino function group. The two peaks at δ 169.8 and 176.0 ppm corresponded to the carbonyl groups of the isatin moiety and the hydrazide group, respectively.

It is expected that compound **4g** could adopt two different geometrical isomers (*Z* and *E*) as shown in Figure 2A. Therefore, it is considered worthwhile to model the compounds using molecular mechanics MM2 calculations. In addition, quantum chemical calculations were carried out with the GAUSSIAN 98 suite of programs. Geometry optimizations were carried out using the DFT level (B3LYP/6-31G **) of theory to assess the relative stability of the *Z* and *E* isomeric species. Calculated relative energies of **4g** *Z* and *E* isomers are −3585.4860873 au and 3585.4635100 au, respectively. Computed energies indicate the stability of the *Z* isomer over the *E* one by 0.0225773 au (14.1675 kcal/mol) (Figure 2B).
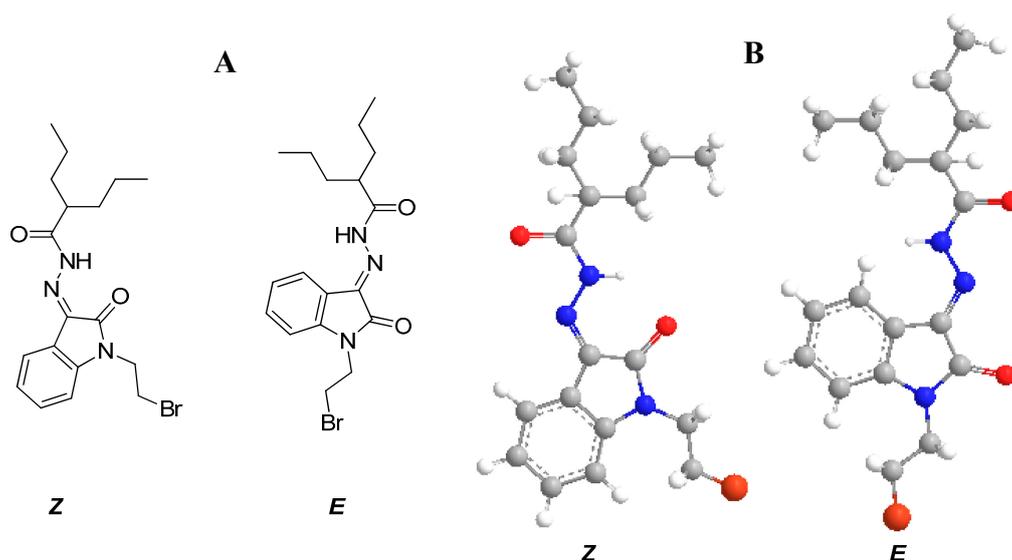
Figure 2. (**A**) The expected geometrical isomers, *Z* and *E* forms, of *N*′-(1-(2-bromoethyl)-2-oxoindolin-3-ylidene)-2-propylpentane hydrazide; (**B**) The expected geometrical isomers, the 3D structure of *Z* and *E* forms, of *N*′-(1-(2-bromoethyl)-2-oxoindolin-3-ylidene)-2-propyl pentane hydrazide.

The X-ray single crystal structure determination of compound **4g** (CCDC: 996592) confirmed that the structure exists in the *Z*-conformer rather than the *E*-conformer (Figure 3). The details of data collection and structure refinement are listed in Table 1, and the X-ray structures are shown in Figure 3. Compound **4g** crystallized from ethanol in the triclinic space group *P*-1. All bond lengths and angles are in normal ranges [52].

In the crystal packing (Figure 4), the molecules are linked by intermolecular C17A—H17B···Br1B, C17B—H17D···O2B and C18A—H18B···O2A hydrogen bonds (Table 2) into two-dimensional networks parallel to the bc plane (Figure 4 and Table 2). The asymmetric unit contains two molecules of the compound with disorder in one of the ethylene arms. The molecular structure of compound **4g** is composed of a 2-oxoindoline ring (C1/N1/C2-C8) which is linked with two side chains at N1 and C8. The two molecules in the asymmetric unit are with Z configuration about the C8=N2 double bond (Figure 3). The Z configuration of **4a** is stabilized with intramolecular hydrogen N3—H3N···O2 (Table 2). The single bond N2—N3 is clearly characterized by the distances of 1.355 (3) Å and 1.361 (4) Å, respectively, for molecules A and B. The double bond of C8=N2 is characterized by the distances of 1.298 (3) Å and 1.283 (4) Å, for molecules A and B, respectively.
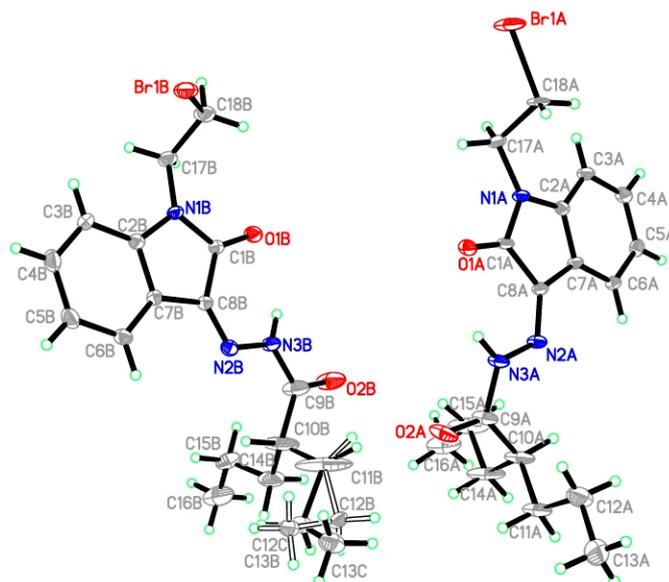
Figure 3. ORTEP diagram of the asymmetric unit consisting of two symmetry-independent molecules. The ellipsoids are drawn at the 50% probability level with hydrogen atoms being shown as spheres of arbitrary radii. One of the molecules has disorder in the side chain C11B—C13B.

Table 1. Details of data collection and structure refinement for compound **4g**.

Compound	4g
Empirical formula	$C_{18}H_{24}BrN_3O_2$
Formula weight	349.21
Temperature (K)	100
Wavelength (A)	Mo Kα radiation, λ = 0.71073 Å
Crystal system, Space group	Triclinic, *P*-1
Crystal	Plate, yellow
a (Å)	8.8126 (11)
b (Å)	14.4104 (18)
c (Å)	14.479 (2)
α (°)	92.002 (4)
β (°)	97.498 (4)
γ (°)	92.598 (4)
Volume (Å³)	1819.6 (4)
Z, D_{calc} (Mg·m⁻³)	4, 1.275
F (000)	816
Crystal size (mm)	0.58 × 0.41 × 0.12
θ Range for data (°)	2.33–28.11
Collection Limiting indices	−12 ≤ h ≤ 12, −20 ≤ k ≤ 20, −20 ≤ k ≤ 20
Reflections collected/unique	81,300/6824 [R_{int} = 0.136]
restraints/parameters	112/469
Goodness-of-fit on F^2	1.009
Final R indices[I > 2s(I)]	R1 = 0.053, wR2 = 0.135
Absorption correction	Multi-scan, SADABS V2012/1 (Bruker AXS Inc., Madison, WI, USA)
Instrument	Bruker APEX-II D8 venture diffractometer
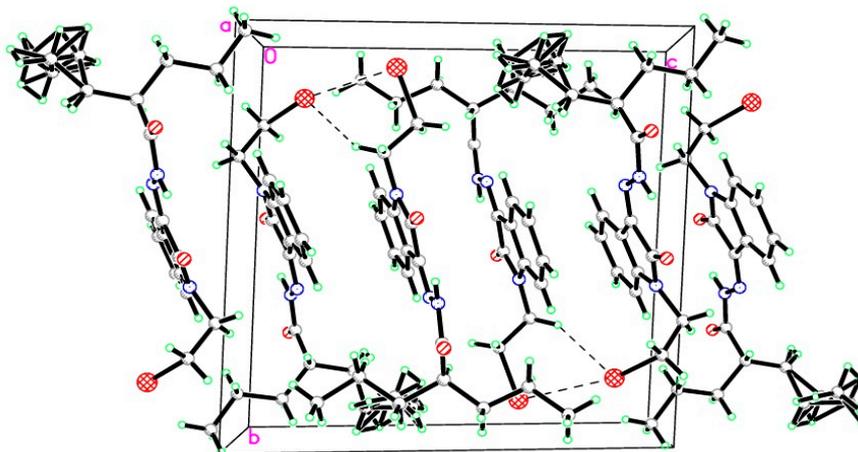
Figure 4. Crystal packing showing intermolecular hydrogen bonds as dashed lines.

Table 2. Hydrogen-bond geometry (Å, °).

D—H⋯*A*	*D*—H	H⋯*A*	*D*⋯*A*	*D*—H⋯*A*
N3B–H3NB⋯O1B	0.81 (3)	2.10 (3)	2.779 (3)	141 (3)
N3A–H3NA⋯O1A	0.86 (3)	2.11 (3)	2.799 (3)	138 (3)
C10A–H10A⋯N2A	0.9800	2.3700	2.815 (4)	107.00
C10B–H10B⋯N2B	0.9800	2.3600	2.833 (4)	109.00
C17A–H17A⋯O1A	0.9700	2.5700	2.930 (3)	102.00
C17A–H17B⋯Br1B [i]	0.9700	2.9100	3.779 (3)	149.00
C17B–H17D⋯O2B [ii]	0.9700	2.4100	3.139 (4)	131.00
C18A–H18B⋯O2A [iii]	0.9700	2.2900	3.096 (3)	140.00

Symmetry codes: ([i]) $x - 1, y, z$; ([ii]) $-x + 1, -y + 1, -z$; ([iii]) $-x + 1, -y + 1, -z + 1$.

Reaction of valporic acid hydrazide **2** with *N*-acetylisatin **3h** proceeds in a different way, where the ring opening occurred to afford the product **4h**. The IR and NMR data proved its structure in the open form, due to the attack of the hydrazide group on C2 instead of C3 [53]. The IR spectrum of **4h** reveals absorption bands in the region 3310 and 3223 cm^{-1} corresponding to the NH, a band at 1720, 1709, 1688, and 1608 cm^{-1} corresponding to the C=O and C=N group, respectively. ^{1}H-NMR of **4h** showed multiple peaks at δ 0.86–0.89, 1.27–1.29, 1.40–1.44, 1.56–1.60, and a broad singlet peak at δ 2.21 ppm corresponding to the valporic acid moiety (2 CH$_3$, 2 CH$_2$, and 2CH$_2$, CH, respectively). The observed peak at δ 2.16 ppm is corresponding to the *N*-acetyl group. The observed peaks in the aromatic region at δ 7.28 (t, 1H), 7.68 (t, 1H), 7.99 (d, 1H), and 8.17(d, 1H) ppm, are related to the phenyl ring, while the two singlet peaks at δ 10.03 and 10.72 ppm corresponded to the two NH. The ^{13}C-NMR of **4h** showed peaks at δ 14.6, 20.6, 29.9, 35.3, 43.8, and 46.5 ppm corresponding to the valporic acid moiety group, while the acetyl group was observed at δ 25.0 ppm, in addition to six peaks related to the aromatic carbons at δ 121.5, 121.8, 123.8, 133.6, 135.9, and 140.3 ppm. The three peaks observed at δ 164.1, 169.6, and 174.7 ppm related to the three CONH groups, while the peak observed at δ 193.0 ppm is related to the α-ketoamide group.

2.2. Anti-Cancer Activity

The cyto-toxicity activity was measured *in vitro* in HepG2, Jurkat, and HEK 293 cells using the MTT 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan colorimetric assay. For comparison, 5-Fluorouracil

(5-FU) was used as a standard anti-cancer drug. Treatment with dimethylsulfoxide (DMSO) was used as a control for the cancer cells.

The anti-cancer activity of the new prepared compounds against the HepG2 cell line revealed that compounds **3a**, **3h**, **4a**, **4b**, **4f**, **4g**, and **4h** showed no anti-cancer activity. Similarly, the starting compounds **1** and **2** did not show promising anti-cancer activity in HepG2 cells. Compound **3e** exhibited higher potency against HepG2 cell line with $IC_{50} = 2.82 \pm 40.7$ μM, which is much lower than the IC_{50} value of 5-FU (32.15 ± 48.1 μM). Moreover, the results showed that compounds **3f**, **4c**, and **4e** were also found to be potent and selective against HepG2 with IC_{50} values of 3.13 ± 42.0, 4.39 ± 43.2, and 7.61 ± 44.6 μM, respectively, which are also much lower than 5-FU (Figure 5 and Table 3).

The anti-cancer profile of newly synthesized compounds was also tested against leukaemia cells (Jurkat cells, originated from human T lymphocytes). The compound **4c** was most active, with an IC_{50} value of just 2.90 ± 36.3 μM, which was much lower than the positive control (5-FU $IC_{50} = 51.16 \pm 47.4$ μM). Similarly, the compounds **3f**, **4a**, and **4c** showed strong activity and IC_{50} values were much lower as compared to 5-FU (Table 3 and Figure 5). The order of activity was **3f**, **4a**, **4e**, and **3e** in ascending order (Table 3 and Figure 5). The compound **1** showed a weaker level of activity against Jurkat cells (IC_{50} 79.35 ± 40.7 μM) and, indeed, this was much weaker than 5-FU (IC_{50} 51.16 ± 47.4 μM).
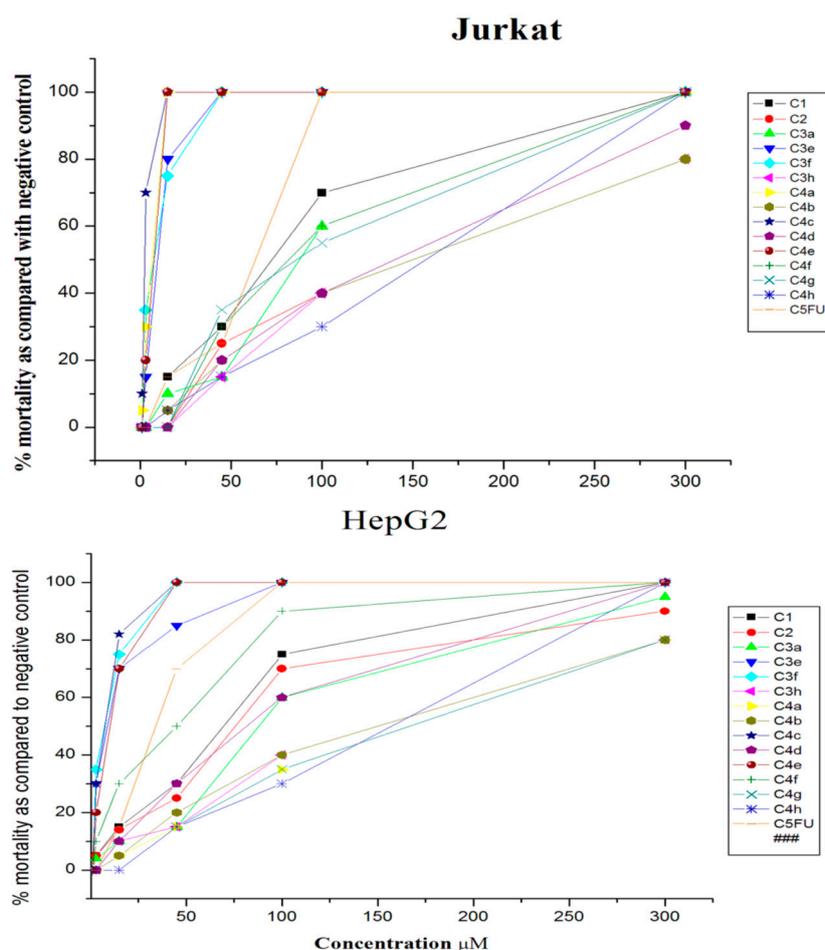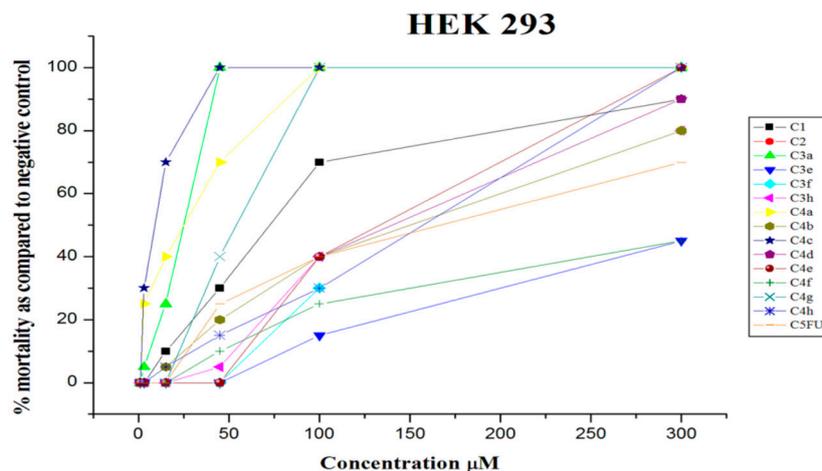

Figure 5. *Cont.*

Figure 5. Dose-dependent responses of hydrazide-hydrazone derivatives in three cell lines. Each line represents the mean of three different experiments representing the % of mortality as compared to mock treated control cells.

Table 3. *In vitro* cytotoxicity activity of the synthesized compounds in two cancer and one normal cell line(s) as measured with MTT assay *.

Compd. No.	IC_{50} (µM)		
	HepG2	Jurkat	HEK293
1	69.74 ± 40.8	79.35 ± 40.7	62.98 ± 38.2
2	68.50 ± 37.1	N.A.	N.A.
3a	N.A.	N.A.	16.73 ± 50.0
3e	2.82 ± 40.7	7.13 ± 46.0	N.A.
3f	3.13 ± 42.0	3.13 ± 42.0	N.A.
3h	N.A.	N.A.	N.A.
4a	N.A.	3.15 ± 43.3	N.A.
4b	N.A.	N.A.	26.08 ± 31.3
4c	4.39 ± 43.2	2.90 ± 36.3	4.77 ± 42.7
4d	N.A.	N.A.	N.A.
4e	7.61 ± 44.6	3.19 ± 46.9	N.A.
4f	N.A.	N.A.	N.A.
4g	N.A.	N.A.	45.43 ± 48.9
4h	N.A.	N.A.	N.A.
5-FU	32.15 ± 48.1	51.16 ± 47.4	N.A.
DMSO	N.A.	N.A.	N.A.

* Data were expressed as the mean ± standard deviation (SD) of six independent experiments; N.A.: IC_{50} values more than 100 µM was considered as No Activity.

Finally the cyto-toxicities of these compounds were screened in the normal human embryonic kidney (HEK293) cell line, in order to assess whether these compounds are active only in cancer cells or whether they possess toxicity against normal cells as well. The results revealed that the compound **4c** turned out to be most toxic by disrupting the cell survival of normal cells (HEK 293) with IC_{50} 4.77 ± 42.7 µM. The compounds **3a**, **4b**, and **4g** also possessed some level of cyto-toxicity against HEK 293 cells with IC_{50} values 16.73 ± 50.0, 26.08 ± 31.3, and 45.43 ± 48.9 µM, respectively. The IC_{50} values of these

compounds in HEK cells, however, are much higher compared to their activity in cancer cells (Table 3 and Figure 5). The compounds **3e**, **3f**, **4a**, and **4e** showed no activity in HEK 293 cells, but strong activity in liver (HepG2) and leukaemia (Jurkat) cancer cells, suggesting that these compounds are potent and potentially viable anti-cancer molecules.

3. Experimental Section

3.1. Chemistry

3.1.1. Materials

The solvents used were of HPLC reagent grade. Melting points were determined with Melting points were obtained in open capillary tubes using a MEL-Temp melting point apparatus (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) and are uncorrected and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier transform instrument (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) as KBr pellets. Nuclear Magnetic resonance spectra (^{1}H-NMR and ^{13}C-NMR spectra) were recorded on 400 MHz JEOL spectrometer (JEOL Ltd., Tokyo, Japan) at room temperature. Chemical shifts are reported in parts per million (ppm) and are referenced relative to residual solvent (e.g., CHCl$_3$ at δ 7.26 ppm for CDCl$_3$, DMSO at δ 2.50 ppm for DMSO-d_6). Spin multiplicities are represented by the following signals: singlet (s), broad singlet (br s), doublet (d), broad doublet (br d), doublet of doublets (dd), triplet (t), doublet of triplets (dt), and multiplet (m). Elemental analyses were performed on a Perkin-Elmer 2400 elemental analyzer (PerkinElmer Inc., Waltham, MA USA), and the values found were within ±0.3% of the theoretical values. Follow-up of the reactions and checks of the purity of the compounds was done by TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck Millipore, Billerica, MA, USA) and the spots were detected by exposure to UV-lamp (Company Seven, Montpelier, MD, USA) at λ 254 nm for a few seconds. The compounds were named using Chem. Draw Ultra version 11, Cambridge soft Corporation. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Center and allocated with the deposition numbers: CCDC 996592 for compound **4g**. CCDC 996592 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk).

3.1.2. Synthesis of Valproic Hydrazide **2**
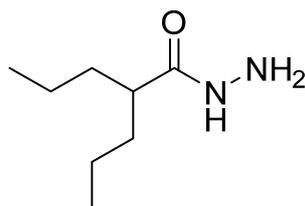
The product was prepared according to the reported procedure [50] and obtained as white needle crystals, in 85%, yield; mp 123–124 °C; IR (KBr): 3284 (NH), 1631 (CO, amide) cm^{-1}; ^{1}H-NMR (CDCl$_3$) δ (ppm): 0.86 (t, J = 4.4 Hz, 6H, 2CH$_3$), 1.14–1.39 (m, 6H, 3CH$_2$), 1.53–1.604 (m, 2H, CH$_2$), 1.95–2.01

(m, 1H, CH), 3.95 (brs, 2H, NH$_2$), 7.05 (s, 1H, NH). ^{13}C-NMR (CDCl$_3$) δ (ppm): 14.11, 20.86, 35.05, 45.61, 177.06.

3.1.3. Synthesis of Compounds 3e–h

Compounds **3e–h** were prepared following the reported procedure [54]. The entire prepared compounds were in a good agreement with the reported data.

3.1.4. General Method for Preparation of *N′*-(2-Oxoindolin-3-ylidene)-2-propylpentane Hydrazide Derivatives 4a–h

A solution of valproic hydrazide **2** (316 mg, 2 mmol) in ethanol (20 mL) was added to a solution of substituted isatin **3a–h** (1 mmol) in ethanol (20 mL), and glacial acetic acid (2 drops); the reaction mixture was refluxed for 3–4 h. The product was separated out on cooling, filtered, and recrystallized from ethanol or ethylacetate to afford *N′*-(2-oxoindolin-3-ylidene)-2-propylpentanehydrazide derivatives **4a–h**.

(Z) N′-(2-Oxoindolin-3-ylidene)-2-propylpentane Hydrazide **4a**
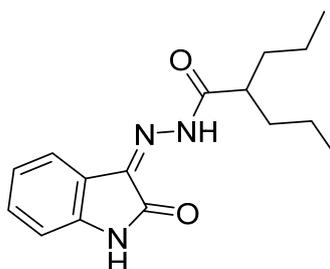
The product was obtained as yellow solid, in 89% yield; mp 175–176 °C. IR (KBr): 3194 (NH), 1720 (CO), 1663 (C=N), 1602 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.85-0.86 (m, 6H, 2 CH$_3$), 1.26–1.27 (m, 4H, 2 CH$_2$), 1.40–1.42 (m, 2H, CH$_2$), 1.56–1.58 (m, 2H, CH$_2$), 2.51 (brs, 1H, CH), 6.90 (d, *J* = 4.6 Hz, 1H, Ar-H), 7.06 (brs, 1H, Ar-H), 7.38 (brs, 1H, ArH), 8.02 (brs,1H, ArH), 10.80 (s, 1H, NH), 11.08 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.5, 20.6, 35.1, 39.8,111.2, 115.9, 122.2, 126.6, 133.1, 144.3, 165.3, 169.80, 172.5. *Anal.* Calcd for C$_{16}$H$_{21}$N$_3$O$_2$: C, 66.88; H, 7.37; N, 14.62; found: C, 66.70; H, 7.44; N, 14.78.

(Z) N′-(5-Bromo-2-oxoindolin-3-ylidene)-2-propylpentane Hydrazide **4b**
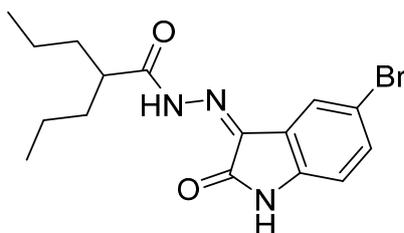
The product was obtained as yellow solid, in 86% yield; mp 172–173 °C; IR (KBr): 3222 (NH), 1729 (CO), 1663 (C=N), 1609 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ (ppm): 0.85–0.89 (m, 6H, 2 CH$_3$), 1.27–1.29 (m, 4H, 2 CH$_2$), 1.40–1.43 (m, 2H, CH$_2$), 1.57–1.59 (m, 2H, CH$_2$), 2.50 (brs, 1H, CH), 6.90 (brs, 1H, Ar-H), 7.54 (brs, 1H, ArH), 8.02 (brs, 1H, ArH), 10.91 (s, 1H, NH), 11.31 (s, 1H, NH); ^{13}C-NMR

(DMSO-d_6) δ (ppm): 14.6, 20.6, 35.1, 39.8, 112.9, 117.5, 122.2, 128.7, 135.1, 144.3, 166.3, 170.80, 172.5. *Anal.* Calcd for $C_{16}H_{20}BrN_3O_2$: C, 52.47; H, 5.50; N, 11.47; found: C, 52.66; H, 5.61; N, 11.23.

(*Z*) *N'*-(5-Chloro-2-oxoindolin-3-ylidene)-2-propylpentane Hydrazide **4c**
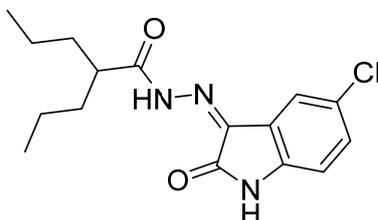
The product was obtained as yellow solid, in 88% yield; mp 154–156 °C; IR (KBr): 3233 (NH), 1720 (CO), 1689 (C=N), 1620 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.87–0.93 (m, 6H, 2 CH$_3$), 1.26–1.29 (m, 4H, 2 CH$_2$), 1.40–1.43 (m, 2H, CH$_2$), 1.57–1.59 (m, 2H, CH$_2$), 2.51 (brs, 1H, CH), 6.91 (m, 1H, Ar-H), 7.42 (m, 1H, ArH), 8.2 (brs, 1H, ArH), 10.91 (s, 1H, NH), 11.35 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.6, 20.6, 35.3, 39.7, 112.8, 118.5, 122.0, 126.2, 134.1, 145.3, 165.3, 170.80, 172.5. *Anal.* Calcd for $C_{16}H_{20}ClN_3O_2$: C, 59.72; H, 6.26; N, 13.06; found: C, 59.55; H, 6.18; N, 13.33.

(*Z*) *N'*-(5-Fluoro-2-oxoindolin-3-ylidene)-2-propylpentane Hydrazide **4d**
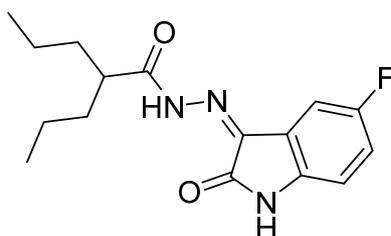
The product was obtained as yellow solid in 89% yield; mp 180–182 °C; IR (KBr): 3181 (NH), 1721 (CO), 1686 (C=N), 1630 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86–0.89 (m, 6H, 2 CH$_3$), 1.26–1.27 (m, 4H, 2 CH$_2$), 1.40–1.43 (m, 2H, CH$_2$), 1.57–1.59 (m, 2H, CH$_2$), 2.50 (brs, 1H, CH), 6.88 (m, 1H, Ar-H), 7.24 (m, 1H, ArH), 8.2 (brd, 1H, ArH), 10.81 (s, 1H, NH), 11.23 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.6, 20.6, 35.3, 39.7, 112.8, 118.5, 119.8, 124.2, 134.1, 140.3, 162.3, 169.80, 172.5. *Anal.* Calcd for $C_{16}H_{20}FN_3O_2$: C, 62.94; H, 6.60; N, 13.76; found: C, 63.13; H, 6.77; N, 13.52.

(*Z*) *N'*-(1-Methyl-2-oxoindolin-3-ylidene)-2-propylpentane Hydrazide **4e**
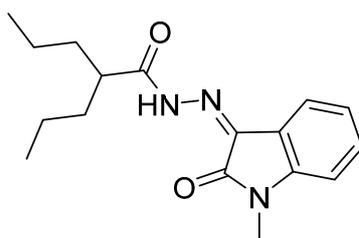
The product was obtained as yellow solid, in 89% yield; mp 154–156 °C; IR (KBr): 1720 (CO), 1689 (C=N), 1620 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.85–0.87 (m, 6H, 2 CH$_3$), 1.24–1.29 (m, 4H, 2 CH$_2$), 1.40–1.43 (m, 2H, CH$_2$), 1.56–1.59 (m, 2H, CH$_2$), 2.50 (brs, 1H, CH), 3.18 (s, 3H, CH$_3$), 7.08–7.14 (m, 2H, Ar-H), 7.46 (m, 1H, ArH), 8.20 (brs, 1H, ArH), 11.23 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6)

δ (ppm): 14.5, 20.6, 27.6, 35.3, 39.7, 115.3, 119.6, 122.7, 129.2, 131.1, 134.8, 164.0, 169.80, 171.5. *Anal.* Calcd for $C_{17}H_{23}N_3O_2$: C, 67.75; H, 7.69; N, 13.94; found: C, 67.63; H, 7.78; N, 14.21.
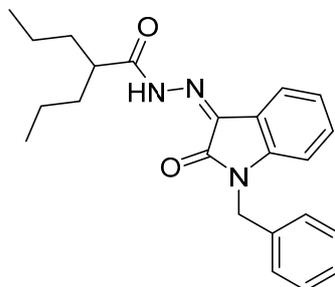
(*Z*) *N*′-(1-Benzyl-2-oxoindolin-3-ylidene)-2-propylpentane Hydrazide **4f**

The product was obtained as yellow solid, in 83% yield; mp 170–172 °C; IR (KBr): 1718 (CO), 1686 (C=N), 1620 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86-0.89 (m, 6H, 2 CH$_3$), 1.27–1.29 (m, 4H, 2 CH$_2$), 1.40–1.44 (m, 2H, CH$_2$), 1.56–1.60 (m, 2H, CH$_2$), 2.51 (brs, 1H, CH), 4.96 (s, 2H, CH$_2$), 7.00–7.20 (m, 2H, Ar-H), 7.33–7.39 (m, 6H, ArH), 8.20 (brs, 1H, ArH), 11.28 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.6, 20.6, 35.3, 39.7, 43.2, 115.4, 122.8, 122.9, 126.8, 127.8, 128.1, 129.2, 129.3, 136.8, 144.2, 164.2, 170.5. *Anal.* Calcd for $C_{23}H_{27}N_3O_2$: C, 73.18; H, 7.21; N, 11.13; found: C, 73.41; H, 7.40; N, 10.94.

(*Z*)-*N*′-(1-(2-Bromoethyl)-2-oxoindolin-3-ylidene)-2-propylpentane Hydrazide **4g**
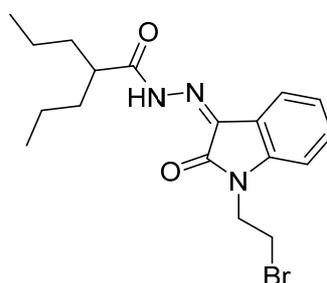
The product was obtained as yellow crystals, in 83% yield; mp 90–91 °C; IR (KBr): 1700 (CO), 1686 (C=N), 1608 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86–0.89 (m, 6H, 2 CH$_3$), 1.27–1.29 (m, 4H, 2 CH$_2$), 1.40–1.44 (m, 2H, CH$_2$), 1.56–1.60 (m, 2H, CH$_2$), 2.51 (brs, 1H, CH), 3.77 (t, *J* = 6.8 Hz, 2H, CH$_2$-CH$_2$Br), 4.20 (t, *J* = 6.4 Hz, 2H, CH$_2$-CH$_2$Br), 7.18 (t, *J* = 8.4 Hz, 1H, Ar-H), 7.32 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.53 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.88 (brs, 1H, ArH), 12.28 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.5, 20.6, 29.9, 35.3, 39.7, 42.2, 46.5, 110.9, 119.7, 122.7, 123.2, 134.1, 142.9, 164.0, 169.80, 176.0. *Anal.* Calcd for $C_{18}H_{24}BrN_3O_2$: C, 54.83; H, 6.13; N, 10.66; found: C, 55.12; H, 6.23; N, 10.90.

N-(2-(2-Oxo-2-(2-(2-propylpentanoyl)hydrazinyl)acetyl)phenyl) Acetamide **4h**
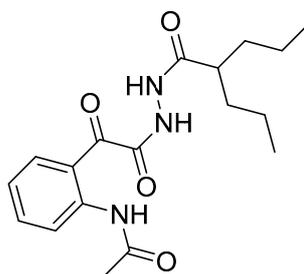
The product was obtained as yellowish white solid, in 83% yield; mp 180–181 °C; IR (KBr): 3310 (NH), 3223(NH), 1720 (CO), 1709 (CO), 1686 (C=N), 1608 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86–0.89 (m, 6H, 2 CH$_3$), 1.27–1.29 (m, 4H, 2 CH$_2$), 1.40–1.44 (m, 2H, CH$_2$), 1.56–1.60 (m, 2H, CH$_2$), 2.16 (s, 3H, CH$_3$), 2.21 (brs, 1H, CH), 7.28 (t, J = 8.0 Hz, 1H, Ar-H), 7.68 (t, J = 8.0 Hz, 1H, Ar-H), 7.99 (d, J = 7.3 Hz, 1H, Ar-H), 8.17(d, J = 8.1 Hz, 1H, ArH), 10.03 (s, 1H, NH), 10.64 (s, 1H, NH), 10.72 (s, 1H, NH);^{13}C-NMR (DMSO-d_6) δ (ppm): 14.6, 20.6, 25.0, 29.9, 35.3, 43.8, 46.5, 121.5, 121.8, 123.8, 133.6, 135.9, 140.3, 164.1, 169.6, 174.7, 193.0. *Anal.* Calcd for C$_{18}$H$_{25}$N$_3$O$_4$: C, 62.23; H, 7.25; N, 12.10; found: C, 62.55; H, 7.42; N, 12.38.

3.2. X-ray Crystallography

Single crystals were obtained by slow evaporation from ethanol. A good crystal with a suitable size was selected for X-ray diffraction analysis. Data were collected on a D8 Venture area diffractometer equipped with graphite monochromatic MoK/α radiation (λ = 0.71073 Å) at 100 K. Cell refinement and data reduction were done by Bruker SAINT (Bruker, Madison, WI USA); the program used to solve structure and refine structure is SHELXS-97 [55]. The final refinement was performed by full-matrix least-squares techniques with anisotropic thermal data for non-hydrogen atoms on F^2. All the hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Multi-scan absorption correction was applied by use of SADABS software [56].

3.3. Biology

3.3.1. Cell Culture and Cell Viability Assay

The stock concentration of the entire compound in DMSO was 10 mM and this concentration was used to prepare the working dilution. The final DMSO concentration used in the experiments was ≤0.5% as the working concentration. Human liver cancer cell lines (HepG2), human leukemia cell line (Jurkat), and human embryonic kidney cell line (HEK293) cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM: Life technologies cat #11995073) supplemented with 10% Fetal bovine serum (FBS: Life technologies cat #16000044) in a humidified incubator with 5% CO$_2$ at 37 °C. Around 2×10^3 cells were seeded in each well of a 24-well cell culture plate and were allowed to adhere and grow for 24 h. The Jurkat cells grow in suspension so they were cultured and allowed to grow overnight before the addition of compounds. The compounds in serial dilutions (1, 3, 15, 45, 100, and 300 μM) were added after 24 h of culture and the cells were cultured for another 24 h at 37 °C. The cell viability was determined in each experiment using MTT 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan colorimetric assay. Briefly, the treated or untreated cells were trypsenized and centrifuged, and the resulting pellet was re-suspended in 100 μL of DMEM serum-free medium and incubated at 37 °C for 2 h. After incubation, 20 μL of MTT solution (5 mg/mL in PBS: Sigma Aldrich cat #M2003) was added to each well and further incubated for 2 h. The plate was centrifuged at 40,000 rpm for 10 min then the medium was removed from each well and 2-propanol containing 0.04 M HCl was added to dissolve the formazan produced in the cells. The optical density of the formazan product in solution was measured with a microplate reader at 540 nm. The experiment was conducted in triplicate. Data were calculated as percent of cell viability by the following formula:

% cell viability = (Mean absorbance in test wells/Mean absorbance in control wells) × 100   (1)

The IC_{50} values were calculated from the means of six different concentrations by Bio Data Fit 1.02 using the software Bio Tool Kit (Version 300; Chang Bioscience Inc., Castro Valley, CA, USA) and online program http://ic50.tk/index.html.

3.3.2. Statistics

All data were analyzed using Origin (Version 6.1052; Origin Lab Corp Northampton, MA, USA). One-way ANOVA analysis of variance and student T TEST was used to compare different experimental groups, and data were considered statistically significant for *p* values less than 0.05.

4. Conclusions

In conclusion, the screening results from human cancer and normal cell lines suggested that isatin derivatives were remarkably influenced by various substituents on the isatin ring and at the *N*-terminal of the hydrazide-hydrazone moiety. The data revealed that replacement of *N*-hydrogen at position 1 of the isatin moiety **3a** by methyl and benzyl of the targeted compound (**3e** and **3f**) noticeably enhanced the activity against the selected two cell lines, HepG2 and Jurkat, for compound **3f**. The presence of the valproic acid moiety as hydrazide-hydrazone derivatives **4a** make the compound more targeted towards Jurakt cells *in vitro*. Introducing the chlorine atom to the molecule **4c**, meanwhile, increased the reactivity more significantly than with **4a** and **3a** towards the three cell lines HepG2, Jurkat, and HEK293. Furthermore, the methyl group in the same analogous compound **4e** increased the reactivity towards the two cell lines HepG2 and Jurkat more than the benzyl group **4f**. The rest of the compounds had no effect on the three cell lines. Further studies to assess the effect of more derivatives on various cancer cell biomarkers are currently underway in our lab.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/08/14638/s1.

Acknowledgments

Author Contributions

Ayman El-Faham and Sherine Khattab carried out the synthesis and designed the proposed methods and analyzed the data statistically together. Hazem Ghabbour and Hoong-Kun Fun carried out X-ray method and the characterization; Muhammad Farooq, Nael Abutaha, and Mohammad Wadaan carried out all the biological activities studies. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Olayinka, O.A.; Craig, A.O.; Obinna, C.N.; David, A.A. Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives. *Bioorg. Med. Chem.* **2010**, *18*, 214–221.

2. Rollas, S.; Gulerman, N.; Edeniz, H. Synthesis and antimicrobial activity of some new hydrazones of 4-fluorobenzoic acid hydrazide and 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines. *Farmaco Prat.* **2002**, *57*, 171–174.

3. Cukurovali, A.; Yilmaz, B.; Gur, S.; Kazaz, C. Synthesis, antibacterial and antifungal activity of some new thiazolylhydrazone derivatives containing 3-substituted cyclobutane ring. *Eur. J. Med. Chem.* **2006**, *41*, 201–207.

4. Capilla, J.; Serena, C.; Javier, F.; Ortoneda, T.; Guarro, J. Efficacy of voriconazole in treatment of systemic scedosporiosis in neutropenic mice. *Antimicrob. Agents Chemother.* **2003**, *47*, 3976–3978.

5. Mamolo, M.G; Falagiani, V.; Zampieri, D.; Vio, U.; Banfi, E.; Scialino, G. Synthesis and antimycobacterial activity of (3,4-diaryl-3*H*-thiazol-2-ylidene)-hydrazide derivatives. *Farmaco Prat.* **2003**, *58*, 631–637.

6. Govindasami1, T.; Pandey, A.; Palanivelu, N.; Pandey1, A. Synthesis, characterization and antibacterial activity of biologically important vanillin related hydrazone derivatives. *Int. J. Org. Chem.* **2011**, *1*, 71–77.

7. Dimmock, J.R.; Vasishtha, S.C.; Stables, J.P. Anticonvulsant properties of various acetylhydrazones, oxamoylhydrazones and semicarbazones derived from aromatic and unsaturated carbonyl compounds. *Eur. J. Med. Chem.* **2000**, *35*, 241–248.

8. Lima, P.C.; Lima, L.M.; Silva, K.C.; Leda, P.H.; Miranda, A.L.P.; Fraga, C.A.M.; Barreiro, E.J. Synthesis and analgesic activity of novel *N*-acylarylhydrazones and isosters, derived from natural safrole. *Eur. J. Med. Chem.* **2000**, *35*, 187–203.

9. Salgin, G.U.; Gokham, K.N.; Gostal, O.; Koysal, Y.; Kilici, E.; Isik, S.; Aktay, G.; Ozalp, M. 1-Acylthiose-micarbazides, 1,2,4-triazole-5(4*H*)-thiones, 1,3,4-thia-diazoles and hydrazones containing 5-methyl-2-benzoxazolinones: Synthesis, analgesic-anti-inflammatory and antimicrobial activities. *Bioorg. Med. Chem.* **2007**, *15*, 5738–5751.

10. Silva, G.A.; Costa, L.M.M.; Brito, F.C.B.; Miranda, A.L.P.; Barreiro, E.J.; Fraga, C.A.M. New class of potent antinociceptive and antiplatelet 10*H*-phenothiazine-1-acylhydrazone derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 3149–3158.

11. Imramovsky, A.; Polanc, S.; Vinsova, J.; Kocevar, M.; Jampitek, J.; Reckova, Z.; Kaustova, J.A. A new modification of anti-tubercular active molecules. *Bioorg. Med. Chem.* **2007**, *15*, 2551–2559.

12. Janin, Y. Antituberculosis drugs: Ten years of research. *Bioorg. Med. Chem.* **2007**, *15*, 2479–2513.

13. Du Toit, L.C.; Pillay, V.; Danckwerts, M.P. Tuberculosis chemotherapy: Current drug delivery approaches. *Respir. Res.* **2006**, *7*, 118–136.

14. Savini, L.; Chiasserini, L.; Travagli V.; Pellerano, C.; Novellino, E.; Consentino, S.; Pisano, M.B. New α-(*N*)-heterocyclichydrazones: Evaluation of anti-cancer, anti-HIV and antimicrobial activity. *Eur. J. Med. Chem.* **2004**, *39*, 113–122.

15. El-Hawash, A.M.; Abdel, W.A.E.; El-Dewellawy, M.A. Cyanoacetic acid hydrazones of 3-(and 4-) acetyl-pyridine and some derived ring systems as potential antitumor and anti-HCV agents. *Arch. Pharm.* **2006**, *339*, 14–23.

16. Wahba, W.; Nahed, W.; El-Sayed, N.E.; Mohareb, R.M. Synthesis and anti-tumor evaluation of novel hydrazide and hydrazide-hydrazone derivatives. *Acta Pharm.* **2013**, *63*, 45–57.

17. Mohareb, R.M.; Fleita, D.H.; Sakka, O.K. Novel synthesis of hydrazide-hydrazone derivatives and their utilization in the synthesis of coumarin, pyridine, thiazole and thiophene derivatives with antitumor activity. *Molecules* **2011**, *16*, 16–27.

18. Terzioglu, N.; Gursoy, A. Synthesis and anti-cancer evaluation of some new hydrazone derivatives of 2,6-dimethylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbohydrazide. *Eur. J. Med. Chem.* **2003**, *38*, 781–786.

19. Boga, C.; Fiume, L. ; Baglioni, M. ; Bertucci, C.; Farina, C.; Kratz, F.; Manerba, M.; Naldiand, M.; Stefano, G. Characterization of the conjugate of the (6-maleimidocaproyl) hydrazone derivative of doxorubicin with lactosaminated human albumin by ^{13}C-NMR spectroscopy. *Eur. J. Pharm. Sci.* **2009**, *38*, 262–269.

20. El-Sabbagh, O.I.; Rady, H.M. Synthesis of new acridines and hydrazones derived from cyclic α-diketone for cytotoxic and antiviral evaluation. *Eur. J. Med. Chem.* **2009**, *44*, 3680–3686.

21. Zhang, H.Z.; Drewe, J.; Tseng, B.; Kasibhatla, S.; Cai, S.X. Discovery and SAR of indole-2-carboxylic acid benzylidene-hydrazides as a new series of potent apoptosis inducers using a cell-based HTS assay. *Bioorg. Med. Chem.* **2004**, *12*, 3649–3655.

22. Vicini, P.; Incerti, M.I.; Collaand, P.L.; Loddo. R. Anti HIV evaluation of benzo[*d*]isothiazole hydrazones. *Eur. J. Med. Chem.* **2009**, *44*, 1801–1807.

23. Rahman, V.M.; Mukhtar, S.; Ansari, W.H.; Lemiere, G. Synthesis, stereochemistry and biological activity of some novel long alkyl chain substituted thiazolidin-4-ones and thiazan-4-one from 10-undecenoic acid hydrazide. *Eur. J. Med. Chem.* **2005**, *40*, 173–184.

24. Kaushik, D.; Khan, S.A.; Chawla, G.; Kumar, S. *N′*-[(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene] 2/4-substituted hydrazides: Synthesis and anticonvulsant activity. *Eur. J. Med. Chem.* **2010**, *45*, 3943–3949.

25. Xia, Y.L.; Chuan-Dong, F.; Zhao, B.X.; Zhao, J.; Shin, D.S. ; Miaom, J.Y. Synthesis and structure–Activity relationships of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives as potential agents against A549 lung cancer cells. *Eur. J. Med. Chem.* **2008**, *43*, 2347–2353.

26. Menendez, C.; Chollet, A.; Rodriguez, F.; Inard, C.; Pasca, M.R.; Lherbet, C.; Baltas, M. Chemical synthesis and biological evaluation of triazole derivatives as inhibitors of InhA and antituberculosis agents. *Eur. J. Med. Chem.* **2012**, *52*, 275–283.

27. Nerkar, A.G.; Saxena, A.K.; Ghone, S.A.; Thaker, A.K. In Silico Screening, synthesis and *in vitro* evaluation of some quinazolinone and pyridine derivatives as dihydrofolate reductase inhibitors for anti-cancer activity. *J. Chem.* **2009**, *6*, 97–102.

28. Chu, W.; Zhang, J.; Zeng, C.; Rothfuss, J.; Tu, Z.; Chu, Y.; Reichert, D.E.; Welch, M.J.; Mach, R.H. *N*-Benzylisation sulfonamide analogues as potent caspase-3 inhibitors: Synthesis, *in vitro* activity, and molecular modeling studies. *J. Med. Chem.* **2005**, *48*, 7637–7647.

29. Chu, W.; Rothfuss, J.; Chu, Y.; Zhou, D.; Mach, R.H. Synthesis and *in vitro* evaluation of sulfonamide isatin Micheal acceptors as small molecule inhibitors of caspase-6. *J. Med. Chem.* **2009**, *52*, 2188–2191.

30. Chohan, Z.H.; Pervez, H.; Rauf, A.; Khan, K.M.; Supuran, C.T. Isatin-derived antibacterial and antifungal compounds and their transition metal complexes. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 417–423.

31. Pirrung, M.C.; Pansare, S.V.; das Sarma, K.; Keith, K.A.; Kern, E.R. Combinatorial optimization of isatin-β-thiosemicarbazones as anti-poxvirus agents. *J. Med. Chem.* **2005**, *48*, 3045–3050.

32. Konkel, M.J.; Lagu, B.; Boteju, L.W.; Jimenez, H.; Noble, S.; Walker, M.W.; Chandrasena, G.; Blackburn, T.P.; Nikam, S.S.; Wright, J.L.; *et al.* 3-Arylimino-2-indolones are potent and selective galanin GAL3 receptor antagonists. *J. Med. Chem.* **2006**, *49*, 3757–3758.

33. Natarajan, A.; Fan, Y.H.; Chen, H.; Guo, Y.; Iysere, J.; Harbinski, F.; Christ, H. Aktas, W.J.; Halperin, Y.A. 3,3-Diaryl-1,3-dihydroindol-2-ones as antiproliferatives mediated by translation initiation inhibition. *J. Med. Chem.* **2004**, *47*, 1882–1885.

34. Matheus, M.E.; Violante, F.D.A.; Garden, S.J.; Pinto, A.C.; Fernandes, P.D. Isatins inhibit cyclooxygenase-2- and inducible nitric oxide synthesis in a mouse macrophage cell line. *Eur. J. Pharmacol.* **2007**, *556*, 300–306.

35. Zhou, L.; Liu, Y.; Zhang, W.; Wei, P.; Huang, C.; Pei, J.; Yuan, Y.; Lai, L. Isatin compounds as noncovalent SARS coronavirus 3C-like protease inhibitors. *J. Med. Chem.* **2006**, *49*, 3440–3443.

36. Jarrahpour, A.; Khalili, D.; de Clercq, E.; Salmi, C.; Brunel, J.M. Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new *bis*-Schiff bases of isatin and their derivatives. *Molecules* **2007**, *12*, 1720–1730.

37. Sharaf, O.A. Some pharmacological activities of new substituted pyrolloindoles, Indolothiazepine and azole derivatives. *Bull. Fac. Pharm.* **1997**, *35*, 79–82.

38. Verma, M.; Pandeya, S.N.; Singh, K.N.; Stables, J.P. Anticonvulsant activity of Schiff bases of Isatin derivatives. *Acta Pharm.* **2004**, *54*, 49–56.

39. Bersudsky, Y.; Applebaum, J.; Gaiduk, Y.; Sharony, L.; Mishory, A.; Podberezsky, A.; Agam, G.; Belmaker, R.H. Valnoctamide as a valproate substitute with low teratogenic potential in mania: A double-blind, controlled, add-on clinical trial. *Bipolar Disord.* **2010**, *12*, 376–382.

40. Bialer, M. New antiepileptic drugs that are second generation to existing antiepileptic drugs. *Expert Opin. Investig. Drugs* **2006**, *15*, 637–647.

41. Bialer, M.; Yagen, B. Valproic acid: Second generation. *Neurotherapeutics* **2007**, *4*, 130–137.

42. Michaelis, M.; Michaelis, U.R.; Fleming, I.; Suhan, T.; Cinatl, J.; Blaheta, R.A.; Hoffmann, K.; Kotchetkov, R.; Busse, R.; Nau, H.; *et al.* Valproic acid inhibits angiogenesis *in vitro* and *in vivo*. *Mol. Pharm.* **2004**, *65*, 520–527.

43. Osuka, S.; Takano, S.; Watanabe, S.; Yamamoto, T.; Matsumura, A. Valproic acid inhibits angiogenesis *in vitro* and glioma angiogenesis *in vivo* in the brain. *Neurol. Med. Chir. (Tokyo)* **2012**, *52*, 186–193.

44. Zgouras, D.; Becker, U.; Loitsch, S.; Stein, J. Modulation of angiogenesis-related protein synthesis by valproic acid. *Biochem. Biophys. Res. Commun.* **2004**, *316*, 693–697.

45. Kitazoe, K.; Abe, M.; Hiasa, M.; Oda, A.; Amou, H.; Harada, T.; Nakano, A.; Takeuchi, K.; Hashimoto, T.; Ozaki, S.; *et al.* Valproic acid exerts anti-tumor as well as anti-angiogenic effects on myeloma. *Int. J. Hematol.* **2009**, *89*, 45–57.

46. Farooq, M.; Sulochana, K.N. ; Pan, X.; To, J.; Sheng, D.; Gong, Z.; Ge, R. Histone deacetylase 3 (hdac3) is specifically required for liver development in zebrafish. *J. Dev. Biol.* **2008**, *317*, 336–353.

47. Gottlicher, M.; Minucci, S.; Zhu, P.; Kramer, O.H.; Schimpf, A.; Giavara, S.; Sleeman, J.P.; lo Coco, F.; Nervi, C.; Pelicci, P.G.; *et al.* Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *Eur. Mol. Biol. Org. J.* **2001**, *20*, 6969–6978.

48. Kuendgen, A.; Gattermann, N. Valproic acid for the treatment of myeloid malignancies. *Cancer* **2007**, *110*, 943–954.

49. Farooq, M.; El-Faham, A.; Ahmad, M.; Sami, A.S.; Wadaan, M.A. Teratogenic profile of valproic acid and newly synthesized derivatives in zebrafish embryos. *Int. J. Agric. Biol.* **2012**, *14*, 894–900.

50. El-Faham, A.; Farooq, M.; Khattab, S.N.; Elkayal, A.M.; Ibrahim, M.F.; Abutaha, N.; Wadaan, M.A.M.; Hamed, E.A. Synthesis and biological activity of Schiff base series of valproyl, *N*-valproyl glycinyl, and *N*-valproyl-4-aminobenzoyl hydrazide derivatives. *Chem. Pharm. Bull.* **2014**, *62*, 591–599.

51. Farooq, M.; El-Faham, A.; Khattab, S.N.; Elkayal, A.M.; Ibrahim, M.F.; Abutaha, N.; Baabbad, A.; Wadaan, M.A.M.; Hamed, E.A. Biological screening of novel derivatives of valproic acid for anti-cancer and antiangiogenic properties. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 7785–7792.

52. Allen, F.H.; Kennard, O.; Watson, D.G.; Brammer, L.; Orpen, A.G.; Taylor, R. Tables of bond lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds. *J. Chem. Soc. Perkin Trans.* **1987**, *2*, S1–S19.

53. El-Faham, A.; Khattab, S.N.; Ghabbour, H.A.; Fun, H.K.; Siddiqui, M.R. Microwave irradiation: Synthesis and characterization of α-ketoamide and *bis* (α-ketoamide) derivatives via the ring opening of *N*-acetylisatin. *Chem. Cent. J.* **2014**, *8*, 1–10.

54. El-Faham, A.; Elzatahry, A.A.; Al Othman, Z.A.; Elsayed, E.A. Facile method for the synthesis of silver nanoparticles using 3-hydrazino-isatin derivatives in aqueous methanol and their antibacterial activit*y. Int. J. Nanomedicine* **2014**, *9*, 1167–1174.

55. Sheldrick, G.M. A short history of *SHELX. Acta Crystallogr. A* **2008**, *64*, 112–122.

56. *Bruker*, *APEX2, SAINT*, *SADABS*; Bruker AXS Inc.: Madison, WI, USA, 2009.

Sample Availability: Samples of the compounds **4 a**–**h** are available from the authors.