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FULL PAPER

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Synthesis and anticancer properties of novel hydrazone derivatives incorporating pyridine and isatin moieties

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Abstract

Nine novel hydrazone derivatives (**4a–i**) incorporating pyridine and isatin moieties were synthesized through one-pot, four-component heterocyclic condensation reactions. The structures of all new compounds (**2a–e**, **3a**, **3c–e**, and **4a–e**) were identified by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and Fourier-transform infrared spectroscopic techniques and elemental analysis. Cell viability assays for the tested hydrazone derivatives were performed and the log IC_{50} values of the compounds were calculated after a 24-h treatment. All hydrazide derivatives tested showed a promising antitumor activity against A-2780 cells as compared with the standard drug docetaxel with a log IC_{50} value of 0.2200 μM ($p < .05$). Seven of the examined compounds (**4b–e**, **4g–i**) showed high cytotoxic activity against A-2780 cells as compared with the standard drug docetaxel. Whereas the log IC_{50} of docetaxel was 0.2200 μM for A-2780 cells at 24 h, the IC_{50} values of these compounds were −0.4987, −0.4044, −0.8138, −0.3868, −0.6954, −0.4751, and 0.1809 μM, respectively. Three of the compounds, **4b**, **4d**, and **4i**, showed high cytotoxic activity against MCF-7 cells as compared with docetaxel ($p < .05$). Whereas the log IC_{50} of docetaxel was 0.2400 μM for MCF-7 cells at 24 h, the log IC_{50} values of compounds **4b**, **4d**, and **4i** were −0.1293, −0.1700, and 0.2459 μM, respectively.

KEYWORDS
anticancer activity, hydrazone derivatives, isatin derivatives, pyridine derivatives

1 | INTRODUCTION

Due to the increase in cancer incidence, scientific research on cancer morbidity and mortality and improvement in the quality of life of cancer patients is increasing rapidly. Cancer is a serious health concern in all societies, regardless of wealth or social status.

In 2018, 18.1 million people worldwide suffered from cancer and 9.6 million patients died from the disease. By 2040, these figures will almost double, and the largest increase will be noticed in low- and middle-income countries where more than two-thirds of world cancer cases occur.[1] Over the last two decades, hydrazide–hydrazone derivatives have been known as one of the most important groups in medicinal chemistry,[2–5] and they are present in a large number of compounds due to their diverse biological properties such as anti-inflammatory,[6] antimicrobial,[7,8] analgesic,[9] anti-HIV,[10] anticonvulsant,[11,12] antileishmanial,[13,14] antimalarial,[15] antitumor,[16–19] and antitubercular activities.[20] Furthermore, hydrazide–hydrazones are increasingly considered to be a valuable core in medicinal chemistry.[21–24] However, isatin is a natural product discovered in the early 19th century in the genus *Isatis* and in *Couroupita guianensis* Aubl. plants,[25,26] and it is known as oxidized indole that attracts much attention as a building block in the design of countless compounds. Isatin has a wide variety of biological and pharmacological activities such as antifungal and

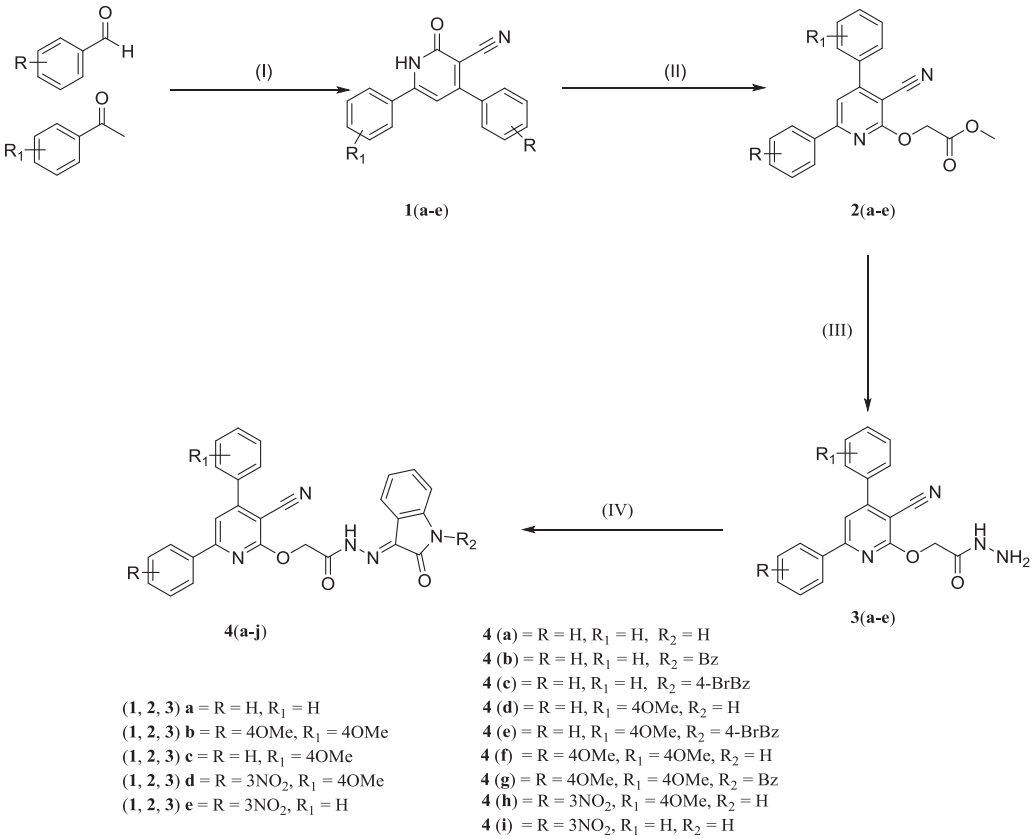
antibacterial properties[27] and potent caspase inhibitory[28,29] and anticancer activities.[30] Schiff bases of isatin have a wide range of pharmacological activities including antismallpox,[31] anti-inflammatory,[32] antibacterial,[33] antiviral,[34] and antitubercular activities[35]; they also act as a GAL3 receptor antagonist.[36] However, 3-cyano-2-pyridones show remarkable biological and pharmacological properties, particularly antidepressant,[37] anticancer,[38,39] antimicrobial activity,[40] and p38 MAP kinase inhibitory capabilities.[41] Although the developments in chemotherapy increase the fight against cancer, numerous side effects of the drugs used pose serious problems in cancer treatment. A great number of drugs have been used to treat cancer, such as *cis*-platinum and ruthenium compounds, but good treatable values have not been achieved with existing drugs. To eliminate these disadvantages, increasing numbers of studies are continuously being carried out in this area. We have also synthesized and tested anticancer activities of many heterocyclic compounds against several cell lines such as A-2780 (human ovarian),[42,43] PC-3 (human prostate),[43] DU-145 (human prostate),[42] A549 (human lung cancer),[44] and BEAS-2B (human lung cancer).[44] As a part of these research works, we planned to synthesize a number of new hydrazone derivatives incorporating pyridine and isatin moieties and examine their anticancer activities against A-2780 and MCF-7 cell lines.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthetic pathways utilized to prepare the new hydrazone derivatives are depicted in Scheme 1.

Different cyanopyridinones **1a–e** were prepared as a starting material via one-pot, four-component heterocyclic condensation process, as reported in the literature.[39] The reaction of **1a–e** with methyl bromoacetate in the presence of anhydrous K_2CO_3 in dimethylformamide (DMF) yielded the corresponding methyl ester derivatives **2a–e**. In infrared data, the presence of $\upsilon_{(COOR)}$ at around 1750 cm^{-1} and the disappearance of the amide carbonyl band at position 2 of the pyridine core prove that the desired ester derivatives (**2a–e**) are synthesized. The singlet peaks around 5.00 ppm for compounds **2a–e** were assigned to the OCH_2 protons of the acetyl group, whereas the singlet signals around 3.70 ppm were assigned to the methyl protons of the ester group for compounds **2a–e**. The ^{13}C nuclear magnetic resonance (NMR) spectra of **2a–e** showed three signals around δ 168.9, 55.47, and 52.24 ppm, corresponding to (C=O, ester), (OCH_3), and CH_2O, respectively. The reaction of esters (**2a–e**) with hydrazine monohydrate in boiling THF yielded the corresponding acetohydrazides **3a–e**. The optimization studies were



SCHEME 1 Synthetic pathway for compounds **1–4**. Reagents and conditions: (I) $CNCH_2COOEt/CH_3COONH_4$/EtOH/reflux; (II) methyl bromoacetate/anhydrous K_2CO_3/dimethylformamide/RT; (III) N_2H_4 100%/tetrahydrofuran/reflux; (IV) appropriate isatin derivative/1,4-dioxane/10% *p*-toluenesulfonic acid/RT

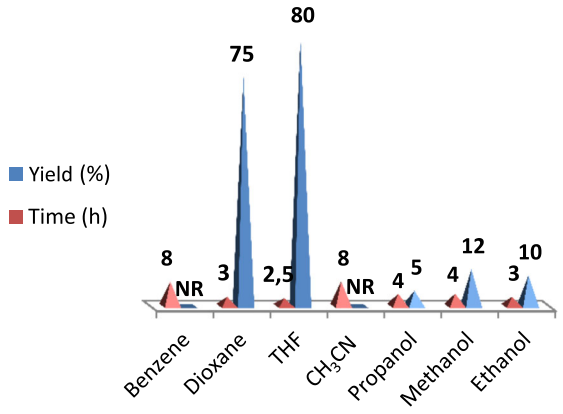


FIGURE 1 The effect of solvent with time in the reaction. THF, tetrahydrofuran

accomplished for the current reaction using **2a** (1.0 eq) and hydrazine monohydrate (4 eq). The use of THF has proven to be essential to achieve maximum conversion for this reaction. Attempts to use other solvents (such as ethanol, methanol, propanol, dioxane, CH_3CN, and benzene) resulted in lower yields or no reaction (Figure 1). The use of protic solvents such as ethanol, methanol, and propanol generated a side product, as indicated in Scheme 2. The infrared spectra of **3a**–**e** show an absorption peak at around 3333.42 and 1675.80 cm^{-1} for NH/NH_2 and the amide carbonyl groups, respectively, which confirms the presence of the hydrazide group. The ^1H NMR data confirm the presence of the NH_2 and NH peaks in the regions 4.33–4.37 and 9.43–9.45 ppm, respectively, and the disappearance of OCH_3 protons. The ^{13}C NMR spectra of compounds **3a**–**e** confirmed the absence of the methoxy carbon atom.

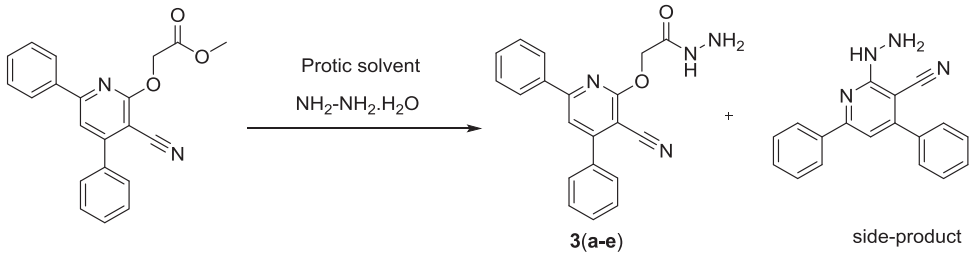
The condensation of hydrazides **3a**–**e** with different isatins in dioxane containing 10% PTSOH (*p*-toluenesulfonic acid) at room temperature afforded the corresponding isatin Schiff base derivatives **4a**–**i**. The infrared spectra of these compounds showed the appearance of NH stretching bands in the range of 3301–3089 cm^{-1}. The ^1H NMR spectra of **4a**–**i** also showed two D_2O exchangeable signals due to the NH group of isatin (**4a**, **4d**, **4f**, **4h**, and **4i**) in the range of 10.90–10.93 ppm and NH of the hydrazide linker in the range of 11.66–11.80 ppm. All other spectral data were in accordance with the assumed structures.

According to the literature,[45] the *N*-substituted hydrazones may exist as *Z*/*E* geometrical isomers about C=N double bonds and as *cis*/*trans* amide conformers (Scheme 3). In this respect, the ^1H NMR spectra of **4a**–**i** in dimethyl sulfoxide (DMSO)-d_6 showed only the presence of *E* conformers; however, the spectra for compounds **4i** and **4g** revealed two sets of protons at 13.49–12.61 and 5.80–5.61 ppm, which are attributed to CO–NH amide and OCH_2 due to *cis* and *trans* conformers. According to the literature, the *cis* conformers of amide were assigned to upfield signals of CO–NH and OCH_2 protons, whereas the downfield peaks were caused by the *trans* conformer.

The *N*-benzylisatin derivatives used in this study were prepared from isatin and an appropriate benzyl halide in the presence of anhydrous K_2CO_3 and KI in DMF, according to the literature.[46,47] The structures of all isatin derivatives were confirmed by ^1H NMR and ^{13}C NMR, and melting point data and all spectral and analytical data were in accordance with the literature values.[46,47]

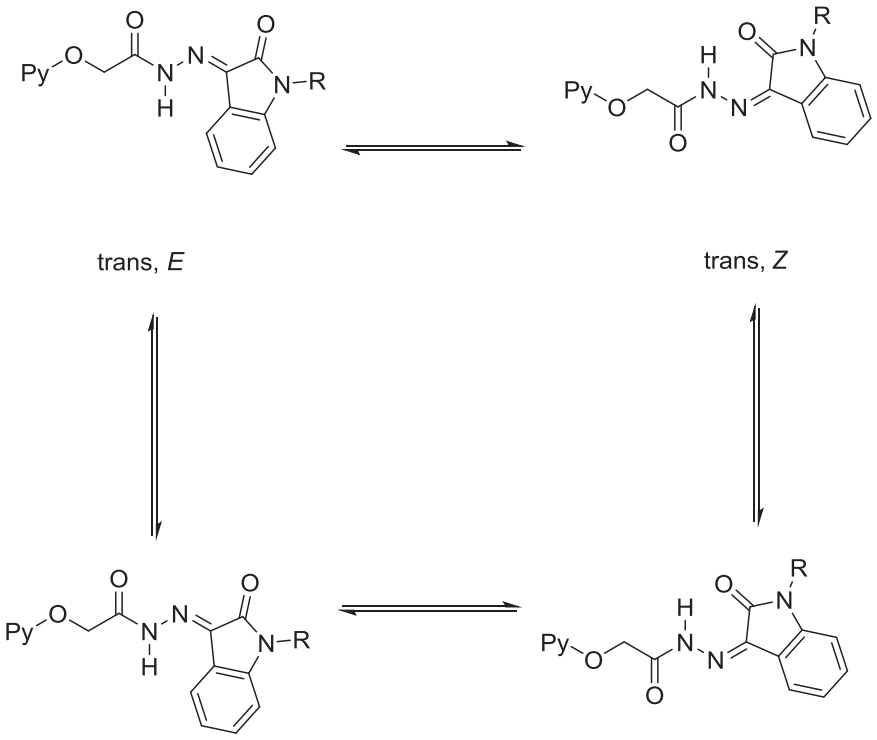
2.2 | Cytotoxicity study

Cytotoxic activities of nine new hydrazone derivatives including pyridine and isatin moieties were determined using human ovarian (A-2780) and human breast cancer (MCF-7) cell lines. To obtain the cytotoxic properties of the newly synthesized hydrazone derivatives on A-2780 and MCF-7 cells, the respective cell lines were incubated with increasing concentrations (0–100 μg/ml) of the compounds for 48 h and then subjected to an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Among the tested nine hydrazone derivatives, those bearing hydrogen atoms for R, R_1, and R_2 substituents and with 4-methoxy substituent for R, R_1, and hydrogen for R_2 at position 1 of the isatin moiety showed a lower cytotoxicity on A-2780 cells than the other compounds and the standard drug docetaxel at 0.1 μM. A nearly similar result was obtained at 1 μM concentration for the A-2780 cell line. The cell viability results of A-2780 and MCF-7 cells after a 48-h treatment with the nine hydrazone derivatives are shown in Tables 1 and 2, respectively. As shown in Table 1, all tested hydrazone derivatives demonstrated an anticancer activity against the A-2780 cell line at 0.1, 1, 10, and 100 μM concentrations with $p < .05$, except compounds **4a** and **4f** at 0.1 μM concentration. Similar to A-2780 cell line results, all tested compounds show a cytotoxic activity against MCF-7 cell line at 0.1, 1, 10, and 100 μM concentrations with $p < .05$, except compounds **4a**, **4f**, and **4h** at 0.1 μM concentration. Among the tested nine hydrazone derivatives,



SCHEME 2 The condensation of ester with hydrazine under protic solvents conditions

SCHEME 3 *E/Z* geometrical isomers about the C=N double bond and *cis/trans* amide conformers of the *N*-substituted hydrazones



compounds **4b** and **4d** showed a better cytotoxicity against MCF-7 cells as compared with the standard drug docetaxel with an inhibitory logarithmic 50 (log IC_{50}) value of 0.24 μM.

A time-dependent cell viability assay for the tested hydrazone derivatives was performed, and the log IC_{50} values of the compounds were calculated after a 24-h treatment. Log IC_{50} values calculated for the A-2780 and MCF-7 cells, based on the results of MTT assays for a 24-h interaction of hydrazone derivatives, are presented in Table 3. As can be seen in Table 3, all tested hydrazone derivatives showed promising antitumor activity against A-2780 cells as compared with the standard drug docetaxel with a log IC_{50} value of 0.22 μM. Among the tested nine hydrazone derivatives, compounds **4b** and **4d** showed better cytotoxicity against MCF-7 cells as compared with the standard drug docetaxel with a log IC_{50} value of 0.24 μM.

3 | CONCLUSIONS

Nine new hydrazone derivatives were synthesized successfully and their anticancer activities were evaluated against human cancer cell lines (A-2780 and MCF-7). Seven out of the nine compounds (**4b**, **4c**, **4d**, **4e**, **4g**, **4h**, and **4i**) showed better anticancer activity than the

TABLE 1 The cell viability results of A-2780 cells after a 48-h treatment with nine hydrazone derivatives[a]

A2780 cell viability (%)						
Compound no.	**Control**	**Solvent (DMSO)**	**0.1 μM**	**1 μM**	**10 μM**	**100 μM**
4a	100.00 ± 3.90	94.72 ± 2.39	83.60 ± 6.70	64.85 ± 5.21*	42.47 ± 3.98*	37.76 ± 5.44*
4b	100.00 ± 3.90	94.72 ± 2.39	55.11 ± 8.36*	23.48 ± 4.43*	18.53 ± 2.70*	17.59 ± 3.08*
4c	100.00 ± 3.90	94.72 ± 2.39	44.80 ± 1.48*	39.28 ± 5.30*	30.10 ± 2.49*	9.74 ± 1.08*
4d	100.00 ± 3.90	94.72 ± 2.39	21.45 ± 5.13*	21.23 ± 1.10*	18.08 ± 5.14*	13.45 ± 1.34*
4e	100.00 ± 3.90	94.72 ± 2.39	50.24 ± 7.65*	38.11 ± 3.64*	19.50 ± 3.29*	12.64 ± 3.05*
4f	100.00 ± 3.90	94.72 ± 2.39	103.44 ± 7.64*	67.46 ± 5.78*	27.82 ± 3.84*	11.78 ± 0.79*
4g	100.00 ± 3.90	94.72 ± 2.39	31.21 ± 7.96*	25.77 ± 4.95*	23.87 ± 7.15	20.97 ± 5.44
4h	100.00 ± 3.90	94.72 ± 2.39	50.34 ± 9.10*	27.44 ± 3.50*	38.35 ± 2.09*	13.03 ± 3.67*
4i	100.00 ± 3.90	94.72 ± 2.39	58.50 ± 5.06*	61.93 ± 3.23*	25.76 ± 3.30*	12.27 ± 2.00*
Docetaxel (ref. drug)	100.00 ± 3.90	94.72 ± 2.39	60.21 ± 5.97	40.13 ± 6.58	14.12 ± 2.73	0.48 ± 0.02

Abbreviation: DMSO, dimethyl sulfoxide.

[a]The changes in cell viability caused by hydrazone derivatives are compared with the control data. Each data point is an average of eight viability measurements.

*$p < .05$.

TABLE 2 The cell viability results of MCF-7 cells after a 48-h treatment with nine hydrazone derivatives[a]

MCF-7 cell viability (%)						
Compound no.	**Control**	**Solvent (DMSO)**	**0.1 μM**	**1 μM**	**10 μM**	**100 μM**
4a	100.00 ± 5.32	95.79 ± 2.47	76.51 ± 9.13	65.60 ± 6.61*	64.37 ± 3.77*	46.87 ± 2.59*
4b	100.00 ± 5.32	95.79 ± 2.47	53.98 ± 6.15*	44.46 ± 4.47*	39.14 ± 3.09*	34.32 ± 3.30*
4c	100.00 ± 5.32	95.79 ± 2.47	70.19 ± 6.91*	68.06 ± 2.10*	51.57 ± 3.50*	53.38 ± 3.55*
4d	100.00 ± 5.32	95.79 ± 2.47	50.95 ± 2.55*	43.11 ± 4.16*	42.22 ± 1.96*	44.05 ± 3.32*
4e	100.00 ± 5.32	95.79 ± 2.47	69.37 ± 3.95*	68.48 ± 5.68*	57.96 ± 4.97*	49.57 ± 5.74*
4f	100.00 ± 5.32	95.79 ± 2.47	84.42 ± 4.70	71.87 ± 6.84*	53.51 ± 7.44*	51.73 ± 7.16*
4g	100.00 ± 5.32	95.79 ± 2.47	63.21 ± 6.60*	54.36 ± 3.57*	50.44 ± 1.93*	53.13 ± 3.75*
4h	100.00 ± 5.32	95.79 ± 2.47	80.37 ± 2.73	57.02 ± 6.97*	56.52 ± 5.12*	45.03 ± 1.79*
4i	100.00 ± 5.32	95.79 ± 2.47	58.96 ± 4.60*	51.89 ± 4.92*	44.88 ± 2.13*	42.56 ± 2.11*
Docetaxel (ref. drug)	100.00 ± 5.32	95.79 ± 2.47	64.56 ± 6.14	36.85 ± 4.92	20.01 ± 3.09	0.88 ± 0.04

Abbreviation: DMSO, dimethyl sulfoxide.

[a]The changes in cell viability caused by hydrazone derivatives are compared with the control data. Each data point is an average of eight viability measurements.

*$p < .05$.

standard drug docetaxel at a concentration of 0.1 μM against the A-2780 cell line. The log IC_{50} of docetaxel was 0.2200 μM for A-2780 cells at 24 h, whereas the IC_{50} values of compounds **4b**, **4c**, **4d**, **4e**, **4g**, **4h**, and **4i** were −0.4987, −0.4044, −0.8138, −0.3868, −0.6954, −0.4751, and 0.1809 μM, respectively. Three out of the nine compounds (**4b**, **4d**, and **4i**) at a concentration of 0.1 μM showed better anticancer activity against the MCF-7 cancer cell line than the standard drug. The log IC_{50} of docetaxel was 0.2400 μM for MCF-7 cells at 24 h, whereas the log IC_{50} values of compounds **4b**, **4d**, and **4i** were −0.1293, −0.1700, and 0.2459 μM, respectively. The outcomes of this study indicate that the newly studied hydrazone derivatives may act as potential drug candidates for cancer treatment, and the results of the present study are encouraging us to continue our anticancer activity screening with further modification in their structure in the future.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

The starting materials and reagents used in the reactions were supplied commercially by Aldrich, Acros, ABCR, and Merck. The human breast (MCF-7) cancer cell line and female ovarian (A-2780) cancer cell line were retrieved from the American Type Culture Collection (ATCC). The nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra (see the Supporting Information) were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). The assignment of exchangeable protons (NH) was confirmed by the addition of D_2O. Elemental analyses were performed by a LECO CHNS 932 Elemental Analyzer. The infrared spectra were recorded with ATR equipment in the range of 4000–650 cm^{-1} on a Perkin Elmer Spectrum One Fourier-transform infrared spectrophotometer. Melting points (mp) were measured in open capillary tubes and were uncorrected, using a Gallenkamp MPD350.BM3.5 apparatus. 1-Benzylindoline-2,3-dione,[46,47] 1-(4-bromobenzyl)indoline-2,3-dione,[46,47] and compound **3b**[48] were prepared according to the published procedures.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

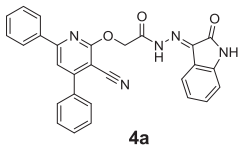
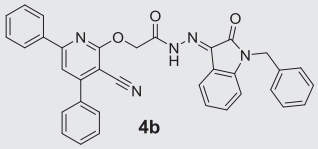
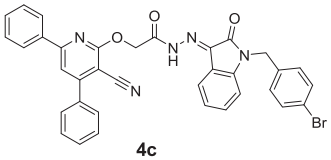
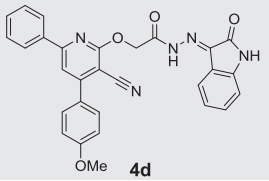
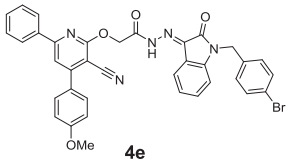
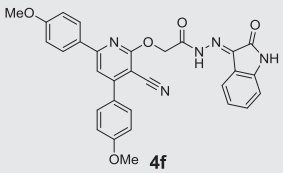
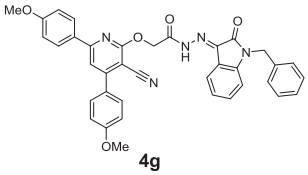
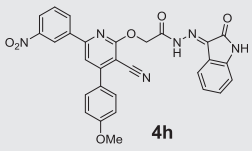
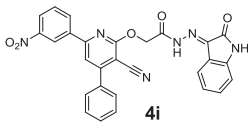
4.1.2 | General procedure for the synthesis of the products 2a–e

A mixture of an appropriate pyridin-2(1*H*)-one (**1a–e**; 0.01 mol) and anhydrous K_2CO_3 (0.015 mol) was stirred at room temperature in DMF (10 ml) for 1 h, and then 0.011 mol of methyl bromoacetate was added. The reaction mixture was stirred for an additional 3 h and poured into ice-cooled water. The obtained product was filtered off, dried, and crystallized from ethanol/acetone (2:1).

*Methyl 2-[(3-cyano-4,6-diphenylpyridin-2-yl)oxy]acetate (**2a**)*

White crystals, yield 89%, mp: 157–159°C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (dd, J = 6.8, 3.0 Hz, 2H, Ar–H), 7.70–7.64 (m, 2H, Ar–H), 7.58–7.45 (m, 7H, Ar–H, pyridine C5–H), 5.11 (s, 2H, OCH_2), 3.81 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 164.14 ($\underline{C}OOCH_3$), 158.67, 152.85, 152.37, 132.05, 131.37, 125.95, 125.42, 124.31, 124.22, 123.68, 122.50, 110.28, 109.70 (Ar–C), 88.54 (CN), 58.85 (OCH_2), 47.51 ($COO\underline{C}H_3$). ν(C–O–C) aliphatic ether: 1145.43 cm^{-1}, ν(C=O) ester: 1733.12 cm^{-1}, ν(CN): 2226.56 cm^{-1},

TABLE 3 Log IC_{50} (μM) concentrations calculated for A-2780 and MCF-7 cells in the GraphPad Prism 6 program of hydrazone derivatives

	log IC_{50} calculation		
	Compound	A-2780	MCF-7
 4a	4a	0.7816	1.660
 4b	4b	−0.4987	−0.1293
 4c	4c	−0.4044	1.465
 4d	4d	−0.8138	−0.1700
 4e	4e	−0.3868	1.598
 4f	4f	0.4636	1.551
 4g	4g	−0.6954	1.085
 4h	4h	−0.4751	1.267
 4i	4i	0.1809	0.2459
	Docetaxel	0.2200	0.2400

$_{\nu}$(C–H) aromatic: 2959.37 cm^{-1}. Anal. calcd. for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13. Found: C, 74.10; H, 4.95; N, 8.21.

*Methyl 2-[(3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl)oxy]-acetate (**2b**)*

White solid, yield 75%, mp: 130–132°C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, J = 9.0 Hz, 2H, Ar–H), 7.63 (d, J = 8.8 Hz, 2H, Ar–H), 7.43 (s, 1H, pyridine C5–H), 7.04 (d, J = 8.8 Hz, 2H, Ar–H), 6.98 (d, J = 9.0 Hz, 2H, Ar–H), 5.07 (s, 2H, OCH_2), 3.87 (s, 6H, $2OCH_3$), 3.79 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.04 (COOCH3), 163.47, 161.73, 161.16, 157.07, 156.45, 129.90, 129.42, 128.78, 128.51, 115.61, 114.45, 114.29, 113.15 (Ar–C), 91.75 (CN), 63.55 (OCH_2), 55.46 ($2OCH_3$), 52.21 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1140.53, 1170.69, 1182.51 cm^{-1}, $_{\nu}$(C=O) ester: 1761.85 cm^{-1}, $_{\nu}$(CN): 2220.54 cm^{-1}, $_{\nu}$(C–H) aromatic: 2838.91, 2949.49 cm^{-1}. Anal. calcd. for $C_{23}H_{20}N_2O_5$: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.98; H, 5.02; N, 6.94.

*Methyl 2-{[3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}-acetate (**2c**)*

White crystals, yield 92%, mp: 148–150°C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (d, J = 9.0 Hz, 2H, Ar–H), 7.67–7.62 (m, 2H, Ar–H), 7.55–7.49 (m, 3H, Ar–H), 7.45 (s, 1H, pyridine C5–H), 6.98 (d, J = 9.0 Hz, 2H, Ar–H), 5.08 (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 3.79 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 168.99 ($COOCH_3$), 163.36, 161.83, 157.28, 156.87, 136.28, 130.06, 129.27, 129.00, 128.83, 128.40, 115.26, 114.33, 113.45 (Ar–C), 92.15 (CN), 63.58 (OCH_2), 55.47 (OCH_3), 52.24 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1140.53, 1172.51 cm^{-1}, $_{\nu}$(C=O) ester: 1760.38 cm^{-1}, $_{\nu}$(CN): 2223.11 cm^{-1}, $_{\nu}$(C–H) aromatic: 2842.39, 2950.17 cm^{-1}. Anal. calcd. for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48. Found: C, 71.25; H, 4.90; N, 7.61.

*Methyl 2-{[3-cyano-6-(4-methoxyphenyl)-4-(3-nitrophenyl)pyridin-2-yl]oxy}acetate (**2d**)*

Yellow solid, yield 77%, mp: 190–192°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.60 (t, J = 1.9 Hz, 1H, Ar–H), 8.47–8.40 (m, 1H, Ar–H), 8.26–8.20 (m, 1H, Ar–H), 8.16 (d, J = 8.9 Hz, 2H, Ar–H), 7.94 (s, 1H, pyridine C5–H), 7.90 (t, J = 8.0 Hz, 1H, Ar–H), 7.09 (d, J = 9.0 Hz, 2H, Ar–H), 5.19 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.75 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.28 ($COOCH_3$), 163.20, 162.23, 157.61, 154.45, 148.39, 137.58, 135.81, 130.97, 129.65, 128.85, 125.21, 124.09, 115.31, 114.86, 114.17 (Ar–C), 91.63 (CN), 64.10 (OCH_2), 55.91 (OCH_3), 52.42 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1149.23, 1176.64 cm^{-1}, $_{\nu}$(N–O) nitro: 1531.41 cm^{-1}, $_{\nu}$(C=O) ester: 1787.33 cm^{-1}, $_{\nu}$(CN): 2217.02 cm^{-1}, $_{\nu}$(C–H) aromatic: 2837.24, 2950.66 cm^{-1}. Anal. calcd. for $C_{22}H_{17}N_3O_6$: C, 63.01; H, 4.09; N, 10.02. Found: C, 63.69; H, 4.09; N, 10.02.

*Methyl 2-{[3-cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl]oxy}-acetate (**2e**)*

Yellow solid, yield 72%, mp: 170–172°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.64 (t, J = 1.9 Hz, 1H, Ar–H), 8.47–8.41 (m, 1H, Ar–H), 8.26 (d, J = 8.2 Hz, 1H, Ar–H), 8.20 (dd, J = 6.7, 3.0 Hz, 2H, Ar–H), 8.05 (s, 1H, pyridine C5–H), 7.92 (t, J = 8.0 Hz, 1H, Ar–H), 7.59–7.52 (m, 3H, Ar–H), 5.23 (s, 2H, OCH_2), 3.75 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.23 ($COOCH_3$), 163.25, 157.75, 154.76, 148.42, 137.44, 136.50, 135.86, 131.56, 131.00, 129.47, 127.90, 125.31, 124.18, 115.23, 115.12 (Ar–C), 92.96 (CN), 64.18 (OCH_2), 52.44 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1155.06 cm^{-1}, $_{\nu}$(N–O) nitro: 1528.48 cm^{-1}, $_{\nu}$(C=O) ester: 1754.76 cm^{-1}, $_{\nu}$(CN): 2225.46 cm^{-1}, $_{\nu}$(C–H) aromatic: 3092.87 cm^{-1}. Anal. calcd. for $C_{21}H_{15}N_3O_5$: C, 64.78; H, 3.88; N, 10.79. Found: C, 65.04; H, 4.02; N, 10.69.

4.1.3 | General procedure for the synthesis of the products 3a–e

A mixture of **2a–e** (0.01 mol) and hydrazine monohydrate (0.04 mol, 1 ml) was boiled in tetrahydrofuran (10 ml) for 2.5–3 h. After cooling, the formed precipitate was filtered off, dried, and crystallized from ethanol/DMF (2:1) to give the target compounds **3a–e**.

*2-[(3-Cyano-4,6-diphenylpyridin-2-yl)oxy]acetohydrazide (**3a**)*

Yellowish white crystals, yield 80%, mp: 227–228°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (s, 1H, NH), 8.21 (dd, J = 6.7 Hz, 3.3 Hz, 2H, Ar–H), 7.85 (s, 1H, pyridine C5–H), 7.77–7.75 (m, 2H, Ar–H), 7.61–7.52 (m, 6H, Ar–H), 5.04 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.06 ($CONH–NH_2$), 163.77, 157.49, 156.88, 136.90, 136.25, 131.22, 130.61, 129.40, 129.38, 129.10, 128.03, 115.75, 114.67 (Ar–C), 93.10 (CN), 64.81 (OCH_2). $_{\nu}$(C–O–C) aliphatic ether: 1142.28 cm^{-1}, $_{\nu}$(C=O) amide: 1675.80 cm^{-1}, $_{\nu}$(CN): 2224.20 cm^{-1}, $_{\nu}$(C–H) aromatic: 2934.87, 3051.01 cm^{-1}, $_{\nu}$(N–H): 3282.25, 3333.42 cm^{-1}. Anal. calcd. for $C_{20}H_{16}N_4O_2$: C, 69.76; H, 4.68; N, 16.27. Found: C, 70.29; H, 4.91; N, 16.31.

*2-{[3-Cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}-acetohydrazide (**3c**)*

White solid, yield 73%, mp: 209–211°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44 (s, 1H, NH), 8.19 (d, J = 8.9 Hz, 2H, Ar–H), 7.80–7.69 (m, 3H, Ar–H, pyridine C5–H), 7.64–7.55 (m, 3H, Ar–H), 7.06 (d, J = 8.9 Hz, 2H, Ar–H), 5.01 (s, 2H, OCH_2), 4.33 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.14 ($CONH–NH_2$), 163.71, 161.94, 157.31, 156.64, 136.41, 130.51, 129.76, 129.35, 129.30, 129.06, 115.94, 114.76, 113.66 (Ar–C), 91.89 (CN), 64.72 (OCH_2), 55.87 (OCH_3). $_{\nu}$(C–O–C) aliphatic ether: 1141.80, 1171.45 cm^{-1}, $_{\nu}$(C=O) amide: 1665.12 cm^{-1}, $_{\nu}$(CN): 2217.43 cm^{-1}, $_{\nu}$(N–H): 3281.79 cm^{-1}. Anal. calcd. for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96. Found: C, 68.15; H, 4.91; N, 14.90.

*2-{[3-Cyano-6-(4-methoxyphenyl)-4-(3-nitrophenyl)pyridin-2-yl]oxy} acetohydrazide (**3d**)*

Yellow solid, yield 89%, mp: 236–238°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44 (s, 1H, NH), 8.59 (t, J = 2 Hz, 1H, Ar–H), 8.46–8.39 (m, 1H, Ar–H), 8.23–8.18 (m, 3H, Ar–H, pyridine C5–H), 7.92

(t, J = 7.9 Hz, 2H, Ar–H), 7.08 (d, J = 9.0 Hz, 2H, Ar–H), 5.03 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.03 (CONH–NH_2), 163.60, 162.09, 157.70, 154.15, 148.38, 137.74, 135.76, 131.03, 129.87, 129.10, 125.17, 124.00, 115.61, 114.78, 113.84 (Ar–C), 92.05 (CN), 64.79 (OCH_2), 55.90 (OCH_3). ν(C–O–C) aliphatic ether: 1150.79, 1171.99 cm^{-1}, ν(N–O) nitro: 1516.22 cm^{-1}, ν(C=O) amide: 1665.56 cm^{-1}, ν(CN): 2218.28 cm^{-1}, ν(C–H) aromatic: 2836.60, 3016.19 cm^{-1}, ν(N–H): 3290.90 cm^{-1}. Anal. calcd. for $C_{21}H_{17}N_5O_5$: C, 60.14; H, 4.09; N, 16.70. Found: C, 59.85; H, 4.16; N, 16.18.

*2-{[3-Cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl]oxy}-acetohydrazide (**3e**)*

Yellow solid, yield 69%, mp: 219–221°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (s, 1H, NH), 8.61 (t, J = 1.8 Hz, 1H, Ar–H), 8.45 (dd, J = 8.3, 1.5 Hz, 1H, Ar–H), 8.26–8.21 (m, 3H, Ar–H), 8.00 (s, 1H, pyridine C5–H), 7.96–7.88 (m, 1H, Ar–H), 7.56–7.53 (m, 3H, Ar–H), 5.06 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.98 (CONH–NH_2), 163.65, 157.86, 154.41, 148.41, 137.59, 136.70, 135.78, 131.41, 131.06, 129.42, 128.11, 125.25, 124.06, 115.41, 114.85 (Ar–C), 93.34 (CN), 64.89 (OCH_2). ν(C–O–C) aliphatic ether: 1158.46 cm^{-1}, ν(N–O) nitro: 1542.40 cm^{-1}, ν(C=O) amide: 1665.97 cm^{-1}, ν(CN): 2219.83 cm^{-1}, ν(C–H) aromatic: 2954.09 cm^{-1}, ν(N–H): 3299.03 cm^{-1}. Anal. calcd. for $C_{20}H_{15}N_5O_4$: C, 61.69; H, 3.88; N, 17.99. Found: C, 59.83; H, 4.04; N, 17.35.

4.1.4 | General procedure for the synthesis of the products 4a–j

A mixture of isatin derivative (0.01 mol) and hydrazides 3**a-e** (0.01 mol) in dioxane (10 ml) containing 10% PTSOH as catalyst was stirred at room temperature for 3 h. Then the resulting solid was filtered, washed with ethanol, filtered and recrystallized from ethanol/DMF.

*(E)-2-[(3-Cyano-4,6-diphenylpyridin-2-yl)oxy]-N′-[(2-oxoindolin-3-ylidene)acetohydrazide] (**4a**)*

Yellow solid, yield 70%, mp: 243–245°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.67 (s, 1H, NH), 10.90 (s, 1H, NH_{isatin}), 8.22–8.10 (m, 3H, Ar–H), 7.88 (s, 1H, pyridine C5–H), 7.83–7.77 (m, 2H, Ar–H), 7.67–7.58 (m, 3H, Ar–H), 7.48–7.38 (m, 2H, Ar–H), 7.32 (t, J = 7.6 Hz, 2H, Ar–H), 7.08 (t, J = 7.5 Hz, 1H, Ar–H), 6.96 (d, J = 7.8 Hz, 1H, Ar–H), 5.70 (s, 2H, OCH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.93 (CONHN), 163.65 ($CONH_{isatin}$), 157.44, 157.14, 144.45, 136.78, 136.18 (Ar–C), 133.38 (C=N_{imine}), 131.23, 130.67, 129.39, 129.22, 129.17, 127.91, 126.78, 122.25, 115.67, 115.63, 114.83, 111.20 (Ar–C), 92.72 (CN), 66.83 (OCH_2). ν(C–O–C) aliphatic ether: 1146.83 cm^{-1}, ν(C=N) imine: 1608.97 cm^{-1}, ν(C=O) amide: 1717.75 cm^{-1}, ν(CN): 2228.78 cm^{-1}, ν(C–H) aromatic: 2837.66 cm^{-1}, ν(N–H): 3147.71 cm^{-1}. Anal. calcd. for $C_{28}H_{19}N_5O_3$: C, 71.03; H, 4.04; N, 14.79. Found: C, 70.87; H, 4.04; N, 14.82.

*(E)-N′-(1-Benzyl-2-oxoindolin-3-ylidene)-2-[(3-cyano-4,6-diphenylpyridin-2-yl)oxy]acetohydrazide (**4b**)*

Yellow solid, yield 68%, mp: 217–219°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.79 (s, 1H, NH), 8.27 (d, J = 7.6 Hz, 1H, Ar–H), 8.16 (d, J = 7.2 Hz, 2H, Ar–H), 7.89 (s, 1H, pyridine C5–H), 7.84–7.77 (m, 2H, Ar–H), 7.67–7.60 (m, 3H, Ar–H), 7.48–7.26 (m, 9H, Ar–H), 7.14 (t, J = 7.6 Hz, 1H, Ar–H), 7.09 (d, J = 8.0 Hz, 1H, Ar–H), 5.70 (s, 2H, OCH_2), 5.01 (s, 2H, NCH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.80 (CONHN), 163.64 ($CONCH_{2\ isatin}$), 157.46, 157.15, 144.40, 136.79, 136.61, 136.18 (Ar–C), 133.18 (C=N_{imine}), 131.21, 130.68, 129.40, 129.22, 129.20, 129.18, 128.02, 127.92, 127.73, 126.65, 122.95, 115.67, 115.21, 114.87, 110.44 (Ar–C), 92.73 (CN), 43.20 (NCH_2). ν(C–O–C) aliphatic ether: 1143.54 cm^{-1}, ν(C=N) imine: 1675.58 cm^{-1}, ν(C=O) amide: 1694.48, 1724.92 cm^{-1}, ν(CN): 2225.44 cm^{-1}, ν(N–H): 3198.56 cm^{-1}. Anal. calcd. for $C_{35}H_{25}N_5O_3$: C, 74.59; H, 4.47; N, 12.43. Found: C, 74.47; H, 4.46; N, 12.48.

*(E)-N′-[1-(4-Bromobenzyl)-2-oxoindolin-3-ylidene]-2-[(3-cyano-4,6-diphenylpyridin-2-yl)oxy]acetohydrazide (**4c**)*

Yellow solid, yield 71%, mp: 228–230°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.81 (s, 1H, NH), 8.27 (d, J = 7.6 Hz, 1H, Ar–H), 8.19–8.11 (m, 2H, Ar–H), 7.89 (s, 1H, pyridine C5–H), 7.83–7.77 (m, 2H, Ar–H), 7.65–7.60 (m, 3H, Ar–H), 7.55 (d, J = 8.4 Hz, 2H, Ar–H), 7.49–7.27 (m, 6H, Ar–H), 7.15 (t, J = 7.6 Hz, 1H, Ar–H), 7.08 (d, J = 8.0 Hz, 1H, Ar–H), 5.73 (s, 2H, OCH_2), 4.98 (s, 2H, NCH_2). ν(C–O–C) aliphatic ether: 1143.67 cm^{-1}, ν(C=N) imine: 1672.08 cm^{-1}, ν(C=O) amide: 1690.59, 1725.73 cm^{-1}, ν(CN): 2226.51 cm^{-1}, ν(C–H) aromatic: 2982.40 cm^{-1}, ν(N–H): 3220.55 cm^{-1}. Anal. calcd. for $C_{35}H_{24}BrN_5O_3$: C, 65.43; H, 3.77; N, 12.44. Found: C, 65.42; H, 4.46; N, 12.48.

*(E)-2-{[3-Cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}-N′-[(2-oxoindolin-3-ylidene)acetohydrazide] (**4d**)*

Yellow solid, yield 80%, mp: 230–232°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.67 (s, 1H, NH), 10.92 (s, 1H, NH_{isatin}), 8.21 (d, J = 7.7 Hz, 1H, Ar–H), 8.15–8.05 (m, 2H, Ar–H), 7.82–7.72 (m, 3H, Ar–H, pyridine C5–H), 7.65–7.57 (m, 3H, Ar–H), 7.44 (t, J = 7.7 Hz, 1H, Ar–H), 7.10 (t, J = 7.2 Hz, 1H, Ar–H), 6.97 (d, J = 7.8 Hz, 1H, Ar–H), 6.79 (d, J = 8.8 Hz, 2H, Ar–H), 5.67 (s, 2H, OCH_2), 3.71 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.95 (CONHN), 163.56 ($CONH_{isatin}$), 161.85, 157.24, 156.87, 144.47, 136.33 (Ar–C), 133.38 (C=N_{imine}), 130.56, 129.59, 129.35, 129.11, 126.80, 122.27, 115.85, 115.66, 114.54, 113.80, 111.21 (Ar–C), 91.51 (CN), 66.82 (OCH_2), 55.73 (OCH_3). ν(C–O–C) aliphatic ether: 1146.56, 1170.17 cm^{-1}, ν(C=N) imine: 1584.37 cm^{-1}, ν(C=O) amide: 1715.81 cm^{-1}, ν(CN): 2222.83 cm^{-1}, ν(C–H) aromatic: 2839.13 cm^{-1}, ν(N–H): 3170.35 cm^{-1}. Anal. calcd. for $C_{29}H_{21}N_5O_4$: C, 69.18; H, 4.20; N, 13.91. Found: C, 68.95; H, 4.21; N, 14.03.

*(E)-N′-[1-(4-Bromobenzyl)-2-oxoindolin-3-ylidene]-2-{[3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}acetohydrazide (**4e**)*

Yellow solid, yield 71%, mp: 212–214°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.80 (s, 1H, NH), 8.28 (d, J = 7.7 Hz, 1H, Ar–H),

8.14–8.06 (m, 2H, Ar–H), 7.82–7.74 (m, 3H, Ar–H, pyridine C5–H), 7.64–7.59 (m, 3H, Ar–H), 7.53 (d, J = 8.3 Hz, 2H, Ar–H), 7.46 (t, J = 7.8 Hz, 1H, Ar–H), 7.33 (d, J = 8.3 Hz, 2H, Ar–H), 7.16 (t, J = 7.6 Hz, 1H, Ar–H), 7.09 (d, J = 8.0 Hz, 1H, Ar–H), 6.77 (d, J = 8.8 Hz, 2H, Ar–H), 5.67 (s, 2H, OCH_2), 4.98 (s, 2H, NCH_2), 3.66 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.82 (CONHN), 163.57 ($CONCH_{2\ isatin}$), 161.84, 157.27, 156.91, 144.20, 136.33, 136.09 (Ar–C), 133.20 ($C{=}N_{imine}$), 132.09, 130.57, 130.02, 129.60, 129.36, 129.14, 129.11, 126.70, 123.06, 121.15, 115.84, 115.27, 114.55, 113.86, 110.39 (Ar–C), 91.55 (CN), 55.69 (OCH_3), 42.61 (NCH_2). ν(C–O–C) aliphatic ether: 1142.76, 1175.30 cm^{-1}, ν(C=N) imine: 1675.11 cm^{-1}, ν(C=O) amide: 1693.27, 1724.24 cm^{-1}, ν(CN): 2224.31 cm^{-1}, ν(C–H) aromatic: 2974.03 cm^{-1}, ν(N–H): 3143.95 cm^{-1}. Anal. calcd. for $C_{36}H_{26}BrN_5O_4$: C, 64.29; H, 3.90; N, 10.41. Found: C, 64.21; H, 3.89; N, 10.53.

*(E)-2-{[3-Cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl]oxy}-N′-[(2-oxoindolin-3-ylidene)acetohydrazide] (**4f**)*

Yellow solid, yield 70%, mp: 240–242°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H, NH), 10.93 (s, 1H, NH_{isatin}), 8.21 (d, J = 7.7 Hz, 1H, Ar–H), 8.14–8.03 (m, 2H, Ar–H), 7.80–7.70 (m, 3H, Ar–H, pyridine C5–H), 7.44 (t, J = 7.8 Hz, 1H, Ar–H), 7.16 (d, J = 8.8 Hz, 2H, Ar–H), 7.10 (d, J = 7.3 Hz, 1H, Ar–H), 6.96 (d, J = 7.8 Hz, 1H, Ar–H), 6.78 (d, J = 8.9 Hz, 2H, Ar–H), 5.66 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.96 (CONHN), 163.67 ($CONH_{isatin}$), 161.76, 161.30, 157.00, 156.44, 144.46 (Ar–C), 133.36 ($C{=}N_{imine}$), 130.72, 129.52, 129.23, 128.53, 128.38, 126.79, 125.98, 122.72, 116.15, 115.65, 114.79, 114.50, 113.49, 111.21 (Ar–C), 91.04 (CN), 67.49 (OCH_2), 55.89 (OCH_3), 55.71 (OCH_3). ν(C–O–C) aliphatic ether: 1141.86, 1171.08, 1189.61 cm^{-1}, ν(C=N) imine: 1603.46 cm^{-1}, ν(C=O) amide: 1721.22, 1750.23 cm^{-1}, ν(CN): 2226.39 cm^{-1}, ν(C–H) aromatic: 2946.79 cm^{-1}, ν(N–H): 3301.03, 3393.82 cm^{-1}. Anal. calcd. for $C_{30}H_{23}N_5O_5$: C, 67.54; H, 4.35; N, 13.13. Found: C, 67.47; H, 4.35; N, 13.19.

*N′-(1-Benzyl-2-oxoindolin-3-ylidene)-2-{[3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl]oxy}acetohydrazide (**4g**)*

Yellow solid, yield, mp: 168–170°C, ^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.38 (s, 1H, $NH_{cis\ conformer}$), 12.61 (s, 1H, $NH_{trans\ conformer}$), 8.20–8.04 (m, 2H, Ar–H), 7.79–7.57 (m, 4H, Ar–H, pyridine C5–H), 7.46–7.24 (m, 6H, Ar–H), 7.21–7.02 (m, 4H, Ar–H), 7.02–6.77 (m, 2H, Ar–H), 5.78 (s, 2H, $OCH_{2\ trans\ conformer}$), 5.35 (s, 2H, $OCH_{2\ cis\ conformer}$), 5.00 (s, 2H, NCH_2), 3.86 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 161.86 (CONHN), 161.33 ($CONCH_{2\ isatin}$), 161.14, 157.06, 156.50, 143.26, 136.06 (Ar–C), 132.21 ($C{=}N_{imine}$), 130.71, 129.57, 129.20, 128.33, 128.15, 127.88, 123.85, 121.26, 119.57, 115.96, 114.79, 114.61, 113.75, 111.02 (Ar–C), 91.21 (CN), 66.83 (OCH_2), 55.90 (OCH_3), 55.72 (OCH_3), 43.06 (NCH_2). ν(C–O–C) aliphatic ether: 1142.92, 1170.32 cm^{-1}, ν(C=N) imine: 1610.41 cm^{-1}, ν(C=O) amide: 1698.07, 1729.42 cm^{-1}, ν(CN): 2215.51 cm^{-1}, ν(C–H) aromatic: 2963.10 cm^{-1}, ν(N–H): 3225.70 cm^{-1}. Anal. calcd. for $C_{37}H_{29}N_5O_5$: C, 71.26; H, 4.69; N, 11.23. Found: C, 71.34; H, 4.70; N, 11.39.

*(E)-2-{[(3-Cyano-6-(4-methoxyphenyl)]-4-[(3-nitrophenyl)pyridin-2-yl]oxy}-N′-(2-oxoindolin-3-ylidene)acetohydrazide (**4h**)*

Yellow solid, yield 66%, mp: 224–226°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.66 (s, 1H, NH), 10.90 (s, 1H, NH_{isatin}), 8.62 (m, 1H, Ar–H), 8.47–8.40 (m, 1H, Ar–H), 8.29–8.17 (m, 2H, Ar–H), 8.16–8.07 (m, 2H, Ar–H), 7.96–7.86 (m, 2H, Ar–H, pyridine C5–H), 7.44 (t, J = 7.7 Hz, 1H, Ar–H), 7.10 (t, J = 7.5 Hz, 1H, Ar–H), 6.96 (d, J = 7.9 Hz, 1H, Ar–H), 6.81 (d, J = 8.8 Hz, 2H, Ar–H), 5.66 (s, 2H, OCH_2), 3.72 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.95 (CONHN), 163.48 ($CONH_{isatin}$), 162.01, 157.63, 154.42, 148.41, 144.48, 137.69, 135.82 (Ar–C), 133.39 ($C{=}N_{imine}$), 130.99, 129.71, 128.95, 126.79, 125.19, 124.09, 122.27, 115.65, 115.51, 114.58, 114.01, 111.21 (Ar–C), 91.70 (CN), 64.33 (OCH_2), 55.76 (OCH_3). ν(C–O–C) aliphatic ether: 1148.39, 1173.92 cm^{-1}, ν(N–O) nitro: 1536.99 cm^{-1}, ν(C=N) imine: 1606.06 cm^{-1}, ν(C=O) amide: 1669.94, 1722.80 cm^{-1}, ν(CN): 2225.58 cm^{-1}, ν(C–H) aromatic: 2967.74 cm^{-1}, ν(N–H): 3089.46 cm^{-1}. Anal. calcd. for $C_{29}H_{20}N_6O_6$: C, 63.50; H, 3.68; N, 15.32. Found: C, 63.43; H, 3.67; N, 15.35.

*2-{[3-Cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl]oxy}-N′-(2-oxoindolin-3-ylidene)acetohydrazide (**4i**)*

Yellow solid, yield 69%, mp: 280–282°C, ^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.49 (s, 1H, $NH_{cis\ conformer}$), 12.70 (s, 1H, $NH_{trans\ conformer}$), 11.35 (s, 1H, NH_{isatin}), 8.65–8.61 (m, 1H, Ar–H), 8.44 (d, J = 8.0 Hz, 1H, Ar–H), 8.26 (d, J = 7.8 Hz, 1H, Ar–H), 8.22–7.95 (m, 3H, Ar–H, pyridine C5–H), 7.92 (t, J = 7.9 Hz, 1H, Ar–H), 7.64–7.32 (m, 5H, Ar–H), 6.99 (m, 2H, Ar–H), 5.80 (s, 2H, $OCH_{2\ trans\ conformer}$), 5.39 (s, 2H, $OCH_{2\ cis\ conformer}$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.41 (CONHN), 163.02 ($CONH_{isatin}$), 157.83, 154.72, 148.43, 143.08, 137.46, 136.54, 135.85 (Ar–C), 132.42 ($C{=}N_{imine}$), 131.48, 131.02, 129.30, 128.00, 125.31, 124.15, 123.16, 121.41, 120.00, 115.26, 115.21, 115.18, 111.72 (Ar–C), 93.06 (CN), 63.67 (OCH_2). ν(C–O–C) aliphatic ether: 1141.09 cm^{-1}, ν(N–O) nitro: 1526.53 cm^{-1}, ν(C=N) imine: 1617.90 cm^{-1}, ν(C=O) amide: 1691.35, 1729.31 cm^{-1}, ν(CN): 2226.86 cm^{-1}, ν(N–H): 3204.30 cm^{-1}. Anal. calcd. for $C_{28}H_{18}N_6O_5$: C, 64.86; H, 3.50; N, 16.21. Found: C, 64.77; H, 3.48; N, 16.26.

4.2 | Cytotoxicity study

4.2.1 | Cell lines and culture conditions

The human cancer lines, ovarian cancer (A-2780) and prostate cancer (MCF-7), were used for in vitro screening experiments. Both cell lines were both purchased from the ATCC. All cells were fed in 25- and 75-cm^2 flasks with the RPMI-1640 medium (containing 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin) 2 days apart. In cells with a carbon dioxide (5% CO_2) incubator (Panasonic), the cells maintained at 37°C and in a humid environment were separated from the flasks using a solution of trypsin–EDTA (Sigma-Aldrich) when confluent. The viability of the cells was determined using 0.4% trypan blue and experiments were started when the viability was above 90%.

4.2.2 | MTT assay

Antitumor activities of these substances were evaluated by MTT assay.[30] Cells were removed using a trypsin–EDTA solution from flasks and counted by a hemocytometer to determine cytotoxic effects. Furthermore, 15×10^3 cells per well were plated in 96-well plates including 200 µl of the RPMI-1640 medium. Cells were incubated at 37°C in a CO_2 incubator for 24 h to adhere to a 96-well plate base. When the incubation ended, concentrations of 0.1, 1, 10, and 100 µM of the hydrazone derivatives were added to the wells in which the cells were contained. Incubation with cancer cells for 24 h at 37°C in a CO_2 incubator was performed to determine the effects of different concentrations of hydrazone derivatives on cell viability for 24 h. When the incubation was over, 0.5 mg/ml of MTT solution in sterile phosphate-buffered solution was prepared and added to 96-well plates. After the addition of MTT, the plates were incubated again for 3 h. After this time, incubation was stopped by adding DMSO to the wells, and the optical densities of the cells in the plates were read on a spectrophotometer (Synergy HTX) at a wavelength of 550 nm.[31] The cell viability percentage was calculated by proportioning the absorbance values obtained from hydrazone derivative-applied wells to that of control group. MTT trials were performed 10 times in triplicate on different days, and the log IC_{50} values of the applied compounds were calculated on the basis of MTT results using the GraphPad Prism 6 program on a computer.

4.3 | Statistical analysis

The IBM SPSS Statistics 24.0 (Windows) package program was used in the analysis. Conformity to normal distribution was evaluated by the Shapiro–Wilk test. Intergroup comparisons of quantitative variables were measured by Kruskal–Wallis *H* test. When significant statistical differences were determined between groups, multiple comparisons were made with Bonferroni correction Mann–Whitney *U* test. All *p* values <.05 were considered statistically significant. Log IC_{50} values of melatonin and agomelatine were calculated using GraphPad Prism 6 program on a computer based on the MTT results obtained from the experiments.

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CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

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