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Synthesis and Anticancer Evaluation of Novel Indole Based Arylsulfonylhydrazides against Human Breast Cancer Cells

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Cite This: *ACS Omega* 2022, 7, 42036−42043

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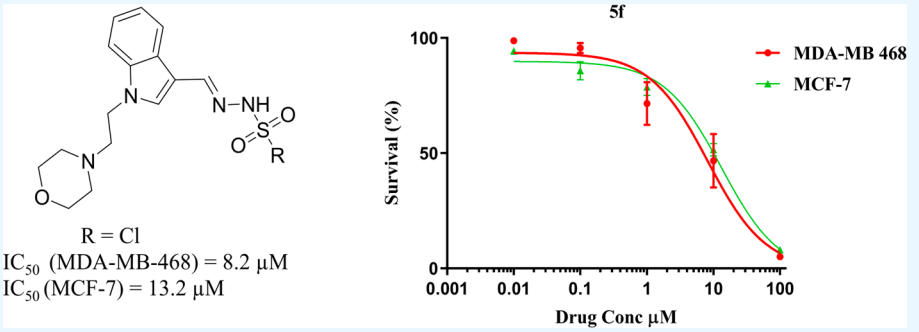

ABSTRACT: A series of novel indole based sulfonohydrazide derivatives (**5a−k**) containing morpholine heterocyclic ring were synthesized through multistep chemical reactions. The target compounds (**5a−k**) were prepared by the reaction of substituted phenyl sulfonylhydrazides (**2a−k**) with morpholine derivative of indole 3-carboxaldehyde. All the target compounds were screened for their anticancer activity *in vitro* against the estrogen receptor-positive breast cancer line MCF-7 and triple-negative breast cancer cell line, MDA-MB-468. It was found that among all the evaluated compounds, the chemotype 4-chloro-*N*′-((1-(2-morpholinoethyl)-1*H*-indol-3-yl)methylene)benzenesulfonohydrazide (**5f**) showed promising inhibition of both MCF-7 and MDA-MB-468 cancer cells with the respective IC_{50} values of 13.2 μM and 8.2 μM. Compound **5f** was found to be nontoxic against HEK 293 noncancerous cells in the studied concentration range, therefore indicating that such chemotypes inhibit the proliferation of cancerous cells selectively and significantly.

1. INTRODUCTION

Cancer is a class of complex diseases in which cells undergo rapid, uncontrollable, and pathological proliferation by disruption of the principles of normal cell division. Cancer has been considered one of the most dangerous diseases, which threatens human lives and is the second leading cause of death globally.[1] Therefore, researchers have developed several novel anticancer agents. and substantial numbers of compounds have been approved by the U.S. Food and Drug Administration (FDA) for cancer treatment. However, the emergence of drug resistance[2] and undesirable off target effects are significant factors associated with approved treatment chemotherapy regimens. Consequently, it is essential for medicinal chemists to develop new anticancer agents having high specificity and increased potency with the least off-target effects and limited resistance issues.[3,4] Breast cancer is one of the most commonly diagnosed types of cancer among women, with millions of new cases identified worldwide every year. It is classified into a few major molecular subtypes according to hormone and growth factor receptor expression. Hormone dependent breast cancer (estrogen receptor, progesterone receptor, HER2 receptor positive, and triple positive), which comprises about two-thirds of breast cancer cases, has several options for treatment. The activation of these receptors by female hormones induces the proliferation of cancer cells, so blocking it with receptor-specific therapy is an effective treatment strategy. However, not all breast cancer subtypes overexpress hormone receptors or become resistant, and thus they do not respond to receptor-specific therapy, necessitating the use of anticancer drugs.[5]

In addition, triple negative breast cancer (TNBC), that accounts for 10−15% of all cases, is characterized by the lack of expression of hormone receptors and considered more aggressive with limited options for specific and effective therapy due to less validated biomarkers.[6] Moreover, the high breast cancer mortality rate is primarily due to the inefficient current treatment (radiotherapy, systematic chemotherapy, and surgical procedures). Although preventive measures are taken, and several drugs have been developed, it is still

Received: June 22, 2022
Accepted: September 27, 2022
Published: November 10, 2022

https://doi.org/10.1021/acsomega.2c03908
ACS Omega 2022, 7, 42036−42043

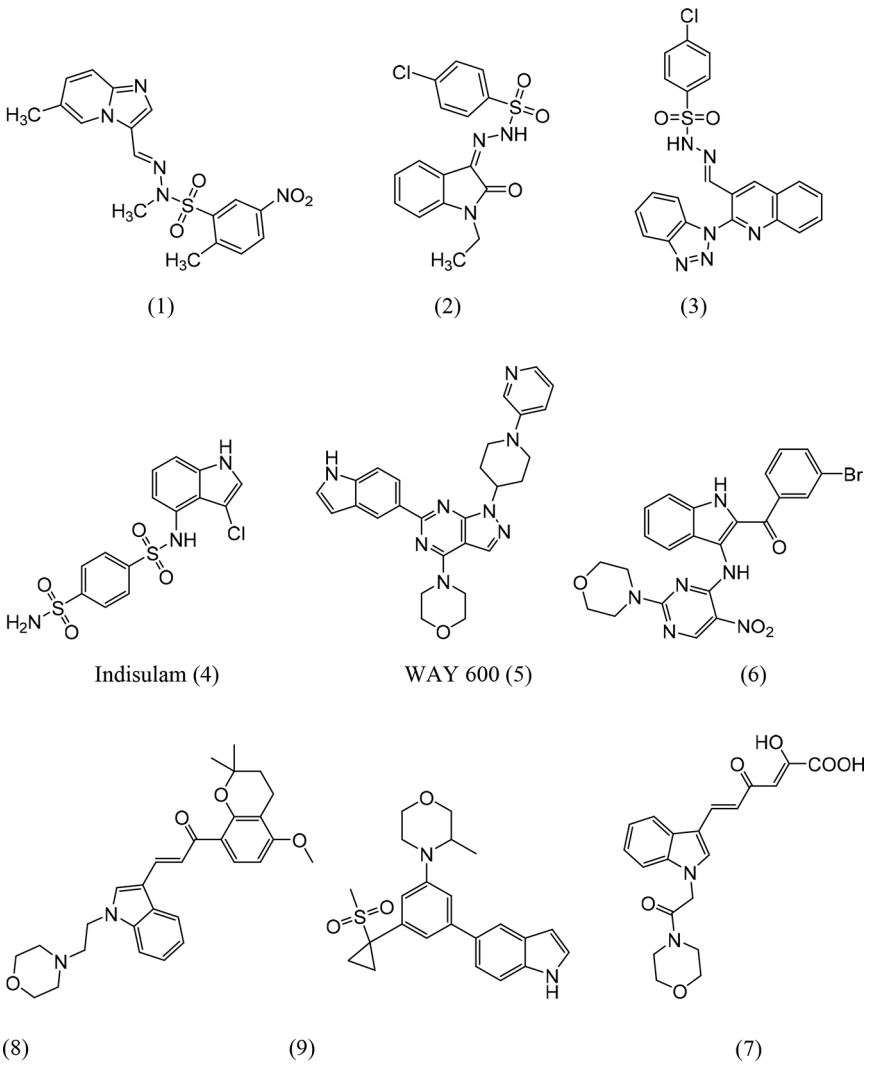

Figure 1. Some compounds containing indole, morpholine, and sulfonohydrazide scaffolds that are reported as anticancer agents.

necessary to develop new antineoplastic drugs that are more effective and selective toward cancer cells.

In anticancer drug discovery, heterocycles have played a very important role and have been considered for the development of novel lead compounds.[7] In particular, nitrogen-based heterocycles are a prominent source of biologically active compounds and important structural motifs in anticancer drug design,[8,9] featuring in almost three-quarters of the heterocyclic anticancer agents approved by the FDA.[10,11] Of all the nitrogen heterocycles, indole is one of the most important scaffolds, and the derivatives of it have demonstrated strong ability to induce cell death in a number of cancer cell lines. Over the last few decades, indole and its derivatives have been shown to modulate a number of biological pathways implicated in the progression of cancer.[12] These include the prevention of cell signaling, normal cell cycle progression, tumor vascularization, and DNA repair, as well as the ability to induce cellular oxidative stress and cell death. Due to the versatility of indole, it has been a highly "privileged scaffold" for the design and development of anticancer agents.[13,14]

The two most important naturally occurring indole alkaloids, vincristine and vinblastine, are used in the management of melanoma, acute lymphoblastic leukemia, small and nonsmall cell lung cancer, Hodgkin's lymphoma, etc. Various other indole derivatives like indole-3-carbinol and DIM (3,3′-diindolylmethane) are known to be efficacious in a number of cancers.[15] So far, many natural and synthetic indole based molecules have been discovered as promising anticancer agents used in clinic or clinical evaluations, suggesting its prominent place in anticancer drug development, and paved a faithful way to develop effective novel potent compounds.[16−19] Furthermore, among nitrogen-containing six-membered heterocycles, morpholine has also proved to be the most useful framework for biological activities. Indole and morpholine heterocycles were ranked in the ninth and 17th positions, of the top 25 most frequent nitrogen heterocycles among the U.S. FDA approved drugs.[20]

Appropriately substituted morpholines have long been known to possess a wide range of biological actions ranging from analgesic, anti-inflammatory, antioxidant, antiobesity, and anti-hyperlipidemic to antimicrobial, anti-neurodegenerative, and anticancer activity.[21] The main structural feature responsible for bioactivity of morpholine is the presence of oxygen atom, which is capable of participating in donor−acceptor-type interactions with the substrate and thereby forms a strong complex with its target. This oxygen atom also complements the pharmacophoric performance of morpholine by reducing the basicity of nitrogen. Morpholine is introduced

https://doi.org/10.1021/acsomega.2c03908
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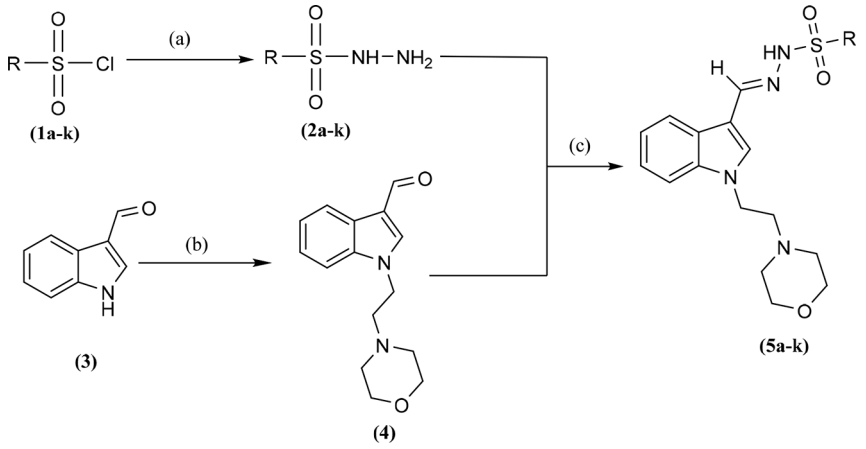
Scheme 1. [a]



[a]Reagents and conditions (a) $NH_2NH_2.H_2O$, THF, 0 °C; 3 h. (b) choloroethyl morpholine, K_2CO_3, CH_3CN, room temperature, 24−48 h. (c) ethanol, glacial acetic acid, reflux 80 °C, 4−7 h.

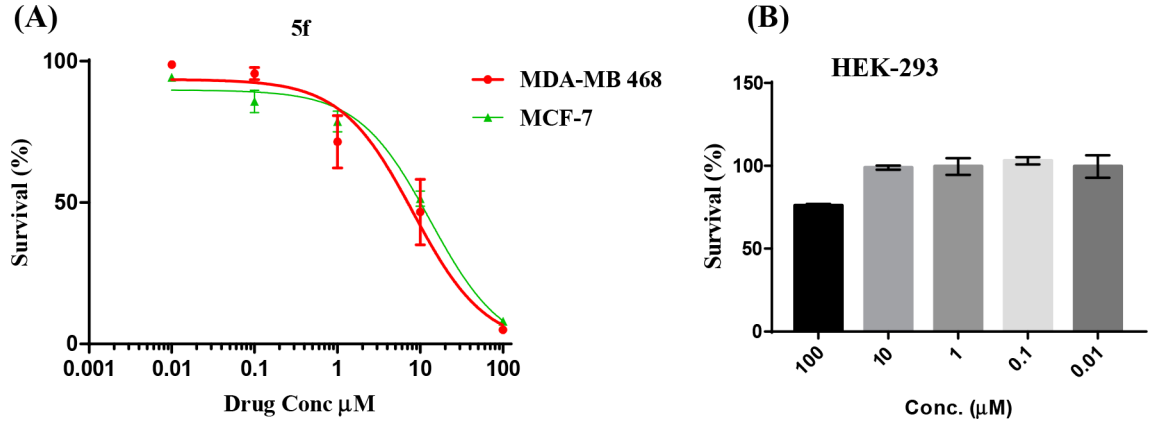


Figure 2. Cell viability studies: (A) Effect of compound **5f** on the viability of MCF-7 and MDA-MB-468 cancer cells lines vs concentration of compound **5f**. (B) Graphical representation for cell viabilities of HEK 293 cells. Each of the selected cells was treated with increasing concentrations of compound **5f** for 48 h.

in drug molecules for enhanced bioavailability and aqueous solubility.[22,23] To a medicinal chemist morpholine is a moiety of immense significance and has been used as a core scaffold and capping fragment to design new drug molecules.[22]

Likewise, as an important nitrogen-containing compound in organic synthesis, sulfonohydrazide derivatives have emerged as the significant class of compounds explored for drug discovery and widely employed to construct C−C, C−N, and C−S bonds.[24,25] Compounds containing sulfonohydrazide structural units have been recognized as potential anticancer,[26] antifungal,[27] antibacterial,[28] antituberculosis,[29] anti-anxiety,[30] and anti-arrhythmic agents.[31] As illustrated in Figure 1, numerous compounds containing indole, sulfonohydrazide, and morpholine moieties have been reported to exhibit potential anticancer activity in which they acted as inhibitors against many biological targets such as compound **1** acting as PI3Kinase p110α inhibitor,[32] compounds **2** and **3** displaying significant anticancer activity against HCT116 colon cancer and DAN-G pancreas cancer cell line,[33,34] compound **4** indisulam, an indole sulfonamide derivative, being used as an anticancer agent for the treatment of lung cancer and exhibiting promising carbonic anhydrase inhibitory activity,[35] compound **5** WAY-600, bearing a morpholine ring, a selective inhibitor of mTOR and clinically used for the treatment of cancer, and compounds **6**−**7** being indole derivatives containing morpholine moiety, which showed considerable antiproliferative activities against cancer cell lines and acting as potent tubulin polymerization inhibitor.[36,37]

Furthermore, other morpholine-linked indole derivatives **8**−**9** possessed significant inhibitory activity toward ATR protein kinase[38] and terminal deoxynucleotidyl transferase (tdt).[39,40] Keeping under consideration the broad-spectrum biological activity of indole, morpholine. and sulfonohydrazide based compounds, these motifs were integrated with each other by going through the multistep chemical reactions to develop the novel compounds and to evaluate their anticancer activity.

2. CHEMISTRY

The synthetic strategy employed for the synthesis of title compounds (**5a**−**k**) is dipicted in Scheme 1. The starting material, substituted phenyl sulfonylhydrazides (**2a**−**k**), were prepared by the reaction of differently substituted benzene-sulfonyl chlorides (**1a**−**k**) with hydrazine monohydrate. Further, the indole 3-carboxaldehyde (**3**) was reacted with chloroethyl morpholine in the presence of potassium carbonate at room temperature to get the intermediate compound (**4**). Substituted phenyl sulfonylhydrazides (**2a**−**k**) were then reacted in the presence of glacial acetic acid with intermediate

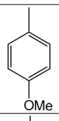
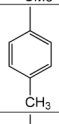
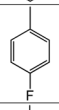
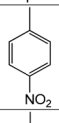
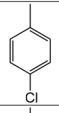
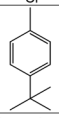
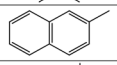
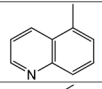
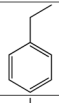
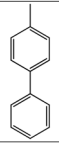
https://doi.org/10.1021/acsomega.2c03908
ACS Omega 2022, 7, 42036−42043

4 for the introduction of azomethein group to get the target compounds (**5a−k**). The formation of target compounds was confirmed on the basis of ^{1}H NMR, ^{13}C NMR, and ESI mass spectroscopy.

3. CELL VIABILITY ASSAY

Cell viability studies showed a significant decrease in the proliferation of selected cancer cells. We have evaluated the antiproliferative potential of synthesized compounds on MCF-7 and MDA-MB-468 human breast cancer cell lines with the help of MTT assay. Treatment of compounds inhibited the viability of cancer cells in a dose-dependent manner (Figure 2). IC_{50} values of each compound (**5a−k**) after 48 h treatment are given in Table 1. Interestingly, it was found that all compounds inhibited the viability of cells as the concentration of compounds increases, but compound **5f** inhibited the viability of MDA-MB-468 and MCF-7 cells more prominently as evident by their IC_{50} values. The IC_{50} value of **5f** is found to be 8.2 μM, and 13.2 μM for MDA-MB-468 and MCF-7 cells, respectively. On the other hand in the case of HEK-293 cells, it was found that the studied compounds did not inhibit the proliferation of these cells in the studied concentration range (100−0.01 μM). These results suggested that the synthesized compounds specifically show significant toxicity toward cancerous cells MCF-7 and MDA-MB-468 only and confirmed that **5f** is biocompatible for healthy cells.

Table 1. *In Vitro* Inhibition Profile of the Compounds (5a−k) against MCF-7 and MDA-MB-468 breast cancer cells

Compound	R	MCF-7 IC_{50} (μM)	MDA-MB-468 IC_{50} (μM)
5a		62.75	43.32
5b		61.14	40.81
5c		73.15	47.02
5d		32.59	15.00
5e		82.03	37.07
5f		13.2	8.2
5g		67.84	29.63
5h		29.93	53.38
5i		79.13	32.20
5j		62.53	50.63
5k		17.3	31.98
Doxorubicin	-	0.06	0.08

In chemotherapy, minimal cytotoxicity to noncancerous cells and high cytotoxicity toward cancerous cells is desirable. The selectivity index (SI) therefore reflects the differential cytotoxicity of a compound to noncancerous cells and cancerous cells (SI = IC_{50} of a pure compound in noncancer cell line/IC_{50} of the same pure compound in cancer cell line). The higher the SI value of a compound is, the greater its selectivity.[41,42] SI values calculated in relation to HEK-293, noncancerous cells for compound **5f**, provided SI values of 36.6 and 58.9 for MDA-MB 468 and MCF-7 cells, respectively, demonstrating that this compound is selectively toxic toward cancerous cells.

4. RESULTS AND DISCUSSION

Novel arylsulfonohydrazides (**5a−k**) were synthesized by going through the multistep chemical reactions. Various sulfonohydrazides were grafted with 1-[2-(morpholin-4-yl)-ethyl]-1*H*-indole-3-carbaldehyde to get the novel compounds (**5a−k**) (Scheme 1). Phenyl rings containing electron with drawing and electron donating groups were attached to the indole-morpholine hybrids through the SO_2N_2 group. All the synthesized compounds (**5a−k**) were evaluated against MCF-7, MDA-MB-468 cancer call lines, and HEK-293 noncancerous cell line. Cells were exposed to different concentrations of compounds (**5a−k**) to determine the effect. The molecules (**5a−k**) inhibited the activity of MCF-7 cells in the range of 13.2−82.03 μM. It was found that compound **5f** having a *p*-chlorophenyl substituent showed significant inhibition of MCF-7 cells followed by biphenyl substituent containing compound **5k**, and these compounds prevented the proliferation of MCF-7 cells with IC_{50} values of 13.2 μM and 17.3 μM, respectively.

Compounds **5e** and **5i** containing *p*-nitrophenyl and quinoline groups showed least antiproliferative effect for MCF-7 cells exhibiting the respective IC_{50} values of 82.03 and 79.13 μM. However, all the title compounds inhibited the growth of estrogen receptor (ER)-negative MDA-MB-468 cancerous cells in the range of 8.2 to 53.38 μM. The compound **5f** having *p*-chlorophenyl substituent inhibited the growth of MDA-MB-468 cancer cells significantly and the observed minimal inhibition concentration was 8.2 μM. The compound **5f** inhibited the growth of both MCF-7 and MDA-MB-468 cells prominently. Following compound **5f**, the compound **5d** having *p*-fluorophenyl substituent inhibited the growth of MDA-MB-468 cells with IC_{50} value of 15 μM. The compounds **5j** and **5h** of the series containing benzyl and naphthyl substituent showed least inhibition of investigated MDA-MB-468 cancer cells.

Compound **5j** inhibited the growth of MDA-MB-468 cell line with IC_{50} value of 50.63 μM, whereas the compound **5h** inhibited the growth of these cells with 53.38 μM. As the activity of the compound (**5a−k**) varied with the change in substituent; therefore in terms of structure activity relationship, the activity of the compounds of this series is substituent dependent. All the title compounds (**5a−k**) were nontoxic to

https://doi.org/10.1021/acsomega.2c03908
ACS Omega 2022, 7, 42036−42043

the HEK-293 cell line indicating that these compounds inhibited the growth of cancerous cells selectively. Therefore, the synthesized arylsulfonohydrazides (**5a−k**) could be treated as the significant anticancer candidates for the inhibition of breast cancer.

5. CONCLUSION

A series of novel sulfonohydrazides incorporating indole and morpholine heterocyclic scaffolds was synthesized by going through multistep chemical reactions. These compounds were evaluated against MDA-MB-468 and MCF-7 cancer cells. It was found that among all the compounds; compound **5f** containing the *p*-chlorophenyl substituent was more active compared to the other compounds of the series. Compound **5f** inhibited the proliferation of MCF-7 breast cancer cells with an IC_{50} value of 13.2 μM, whereas it inhibited the growth of MDA-MB-468 cancerous cells with an IC_{50} value of 8.2 μM. It was found that all the evaluated compounds were nontoxic toward HEK 293 cells in concentrations up to 100 μM. The compounds inhibited the growth of breast cancer cells selectively and significantly. The integration of active pharmacophores used in this study lead to the development of novel antiproliferative agents.

6. EXPERIMENTAL PROTOCOLS

All the required chemicals were purchased from Merck and Aldrich Chemical Co. (USA). The reagents were of analytical grade and were used without further purification. Precoated aluminum sheets (Silica gel 60 F254, Merck Germany) were employed for thin-layer chromatography (TLC). The synthesized compounds were visualized on TLC using ultraviolet (UV) light (λ = 254 nm). The melting points of all the compounds were determined on a Veego instrument with model specifications REC-22038 A2 and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol-500 MHz and Jeol-100 MHz spectrometer, respectively, using DMSO-d_6 as a solvent, and tetramethylsilane (TMS) was employed as the internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; t: triplet: m, multiplet; ar: aromatic. Mass spectra of all the compounds were recorded by ESI-MS (AB-Sciex 2000, Applied Biosystem).

6.1. Procedure Used for Synthesis of Arylsulfonylhydrazides (2a−k). A mixture of substituted sulfonyl chlorides (**1a−k**) (26 mmol) and hydrazine monohydrate (66 mmol, 99%) was stirred at 0−5 °C in THF (20 mL) for 1−2 h. The reaction progress was monitored using TLC. After the completion of the reaction the solvent was evaporated *in vacuo*. The residue thus obtained was washed with water, extracted with dichloromethane, and dried over anhydrous sodium sulfate. The product was recrystallized from methanol.

6.2. Procedure Used for Synthesis of Morpholine Derivative of Indole 3-Carboxaldehyde (4). To a stirred solution of indole-3-carbaldehyde (3) (1.72 mmol) in dry acetonitrile at room temperature, potassium carbonate (8.60 mmol) was added. After 15−20 min, *N*-(2-chloroethyl) morpholine hydrochloride (3.44 mmol) was added dropwise to the resulting mixture and the reaction mixture was heated to reflux for 28 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the mixture was cooled to room temperature followed by extraction with ethyl acetate and water. The organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was recrystallized from ethanol and ethyl acetate to afford compound **4**.

6.3. Procedure Used for the Synthesis of Morpholine Based Indolyl 3-Sulfonohydrazide Hybrids (5a−k). The *N*-substituted indole 3-carboxaldehyde (**4**) (3 mmol) prepared above and different arylsulfonylhydrazides (**2a−k**) (1 mmol) were taken in ethanol in the presence of catalytic amount of glacial acetic acid and refluxed at 80 °C for 4−7 h. The progress of the reaction was monitored by using TLC visualized under ultraviolet wavelength of 254 nm. On completion of reaction, the reaction mixture was cooled to room temperature and then extracted with ethyl acetate and water. The ethyl acetate layer was dried by anhydrous Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude product was crystallized from chloroform and ethanol to get the title sulfonohydrazides (**5a−k**).

4-Methoxy-N′-((1-(2-morpholinoethyl)-1H-indol-3-yl)-methylene)benzenesulfonohydrazide ***5a***. Yield: 82%; white solid; m.p.: 220−221 °C; M.F.: $C_{22}H_{26}N_4O_4S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.75 (s, 1H, −NH), 8.55 (s, 1H, Ar−H), 7.97 (d, 1H, J = 8.25 Hz, Ar−H), 7.93 (d, 1H, J = 2.75 Hz, Ar−H), 7.77−7.75 (m, 2H, Ar−H), 7.46 (d, 1H, J = 8.25 Hz, Ar−H), 7.22−7.19 (m, 1H, Ar−H), 7.15−7.12 (m, 3H, Ar−H), 3.83 (s, 3H, -OCH_3), 3.54−3.53 (m, 4H, morph-CH_2), 3.41−3.40 (m, 2H, −CH_2), 2.49−2.46 (m, 2H, −CH_2), 2.45−2.39 (m, 4H, morph-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 163.04, 155.84, 137.11, 132.94, 130.61, 127.43, 124.28, 122.93, 121.82, 121.06, 114.22, 112.11, 111.07, 66.18, 56.17, 55.79, 53.36, 47.69; ESI-MS (m/z): [M^+ + 1] 443.20; Anal. Calcd For $C_{22}H_{26}N_4O_4S$: C, 59.71; H, 5.92; N, 12.66; S, 7.25. Found: C, 60.01; H, 5.72; N, 12.91; S, 6.95.

4-Methyl-N′-((1-(2-morpholinoethyl)-1H-indol-3-yl) methylene) benzenesulfonohydrazide ***5b***. Yield: 88%; white solid; m.p.: 235−236 °C; M.F.: $C_{22}H_{26}N_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.75 (s, 1H, −NH), 8.55 (s, 1H, Ar−H), 7.97 (d, 1H, J = 7.55 Hz, Ar−H), 7.93 (d, 1H, J = 2.75 Hz, Ar−H), 7.73 (d, 2H, J = 8.25 Hz, Ar−H), 7.47 (d, 1H, J = 8.25 Hz, Ar−H), 7.42 (d, 2H, J = 8.25 Hz, Ar−H), 7.22−7.19 (m, 1H, Ar−H), 7.15−7.12 (m, 3H, Ar−H), 3.55−3.53 (m, 4H, morph-CH_2), 3.44−3.41 (m, 2H, −CH_2), 2.49−2.47 (m, 2H, −CH_2), 2.45−2.39 (m, 4H+3H, morph-CH_2,-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 155.61, 143.90, 137.06, 132.99, 132.92, 129.43, 128.35, 124.21, 122.84, 121.74, 120.94, 112.04, 110.99, 66.15, 56.14, 53.31, 47.60, 21.06; ESI-MS (m/z): [M^+ + 1] 427.50; Anal. Calcd For $C_{22}H_{26}N_4O_3S$: C, 61.95; H, 6.14; N, 13.14; S, 7.52. Found: C, 62.12; H, 5.89; N, 12.91; S, 7.77.

*N′-((1-(2-Morpholinoethyl)-1H-indol-3-yl)methylene)-benzenesulfonohydrazide (****5c****)*. Yield: 91%; white solid; m.p.: 245−246 °C; M.F.: $C_{21}H_{24}N_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.94 (s, 1H, −NH), 8.76 (s, 1H, Ar−H), 7.98 (d, 1H, J = 2.75 Hz, Ar−H), 7.91 (d, 1H, J = 7.55 Hz, Ar−H), 7.82 (d, 2H, J = 7.55 Hz, Ar−H), 7.77−7.49 (m, 1H, Ar−H), 7.66−7.63 (m, 2H, Ar−H), 7.49 (d, 1H, J = 8.25 Hz, Ar−H), 7.23−7.19 (m, 1H, Ar−H), 7.13−7.10 (m, 1H, Ar−H), 3.83 (s, 4H, morph-CH_2), 3.70 (s, 2H, −CH_2), 3.17 (s, 2H, −CH_2), 2.49 (s, 4H, morph-CH_2 missing); ^{13}C NMR (75 MHz, DMSO-d_6): 159.12, 137.15, 135.01, 133.95, 133.80, 129.23, 128.55, 124.28, 123.05, 121.83, 121.16, 112.25, 110.77, 63.74, 53.34, 51.55, 45.80; ESI-MS (m/z): [M^+ + 1] 413.10; Anal. Calcd For $C_{21}H_{24}N_4O_3S$: C, 61.14; H, 5.86; N, 13.58; S, 7.77. Found: C, 60.88; H, 6.12; N, 13.73; S, 8.05.

*4-Fuoro-N′-((1-(2-morpholinoethyl)-1H-indol-3-yl) methylene)benzenesulfonohydrazide (****5d****)*. Yield: 83%; white

https://doi.org/10.1021/acsomega.2c03908
ACS Omega 2022, 7, 42036−42043

solid; m.p.: 210−211 °C; M.F: $C_{21}H_{23}FN_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.97 (s, 1H, −NH), 8.79 (s, 1H, Ar−H), 7.99 (d, 1H, J = 2.75 Hz, Ar−H), 7.91−7.87 (m, 3H, Ar−H), 7.54−7.47 (m, 3H, Ar−H), 7.23−7.20 (m, 1H, Ar−H), 7.15−7.12 (m, 1H, Ar−H), 3.83 (m, 4H, morph-CH_2), 3.74 (m, 2H, −CH_2), 3.25 (m, 2H+4H, CH_2, morph-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 166.63, 164.63, 137.61, 134.38, 132.15, 132.07, 131.82, 124.75, 123.48, 122.25, 121.69, 117.09, 116.90, 112.71, 111.15, 63.84, 51.62; ESI-MS (m/z): [M^+ + 1] 470.50; Anal. Calcd For $C_{21}H_{23}FN_4O_3S$: C, 58.59; H, 5.39; N, 13.01; S, 7.45. Found: C, 58.62; H, 5.33; N, 13.03; S, 7.30.

*N′-((1-(2-Morpholinoethyl)-1H-indol-3-yl)methylene)-4-nitrobenzenesulfonohydrazide (**5e**).* Yield: 89%; yellow solid; m.p.: 232−233 °C; M.F: $C_{21}H_{23}N_5O_5S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.80 (s, 1H, −NH), 8.59 (s, 1H, Ar−H), 8.45−8.53 (m, 2H, Ar−H), 8.13−8.11 (m, 2H, Ar−H), 7.97 (d, 1H, J = 2.75 Hz, Ar−H), 7.91 (d, 1H, J = 8.25 Hz, Ar−H), 7.47 (d, 1H, J = 7.6 Hz, Ar−H), 7.23−7.19 (m, 1H, Ar−H), 7.16−7.13 (m, 1H, Ar−H), 3.54−3.52 (m, 4H+2H, morph-CH_2, −CH_2), 2.49 (m, 2H, −CH_2), 2.40 (m, 4H, morph-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 156.70, 150.74, 141.94, 137.60, 133.90, 130.37, 124.79, 124.65, 123.45, 122.17, 121.69, 112.63, 111.24, 66.64, 56.51, 53.77, 47.84; ESI-MS (m/z): [M^+ + 1] 458.50; Anal. Calcd For $C_{21}H_{23}N_5O_5S$: C, 55.71; H, 5.07; N, 15.31; S, 7.01. Found: C, 55.95; H, 5.23; N, 15.50; S, 6.82.

*4-Chloro-N′-((1-(2-morpholinoethyl)-1H-indol-3-yl)-methylene)benzenesulfonohydrazide (**5f**).* Yield: 90%; white solid; m.p.: 223−224 °C; M.F: $C_{21}H_{23}N_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.78 (s, 1H, −NH), 8.56 (s, 1H, Ar−H), 7.95 (d, 1H, J = 2.75 Hz, Ar−H), 7.92 (d, 1H, J = 7.55 Hz, Ar−H), 7.86−7.84 (m, 2H, Ar−H), 7.72−7.70 (m, 2H, Ar−H), 7.47 (d, 1H, J = 8.25 Hz, Ar−H), 7.22−7.19 (m, 1H, Ar−H), 7.15−7.12 (m, 1H, Ar−H), 3.54−3.53 (m, 4H, morph-CH_2), 3.48−3.45 (m, 2H, −CH_2), 2.47−2.45 (m, 2H, −CH_2), 2.39 (m, 4H, morph-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 156.59, 139.02, 137.59, 135.24, 133.69, 130.72, 129.72, 124.68, 123.41, 122.17, 121.60, 112.61, 111.36, 66.65, 56.56, 53.79, 47.99; ESI-MS (m/z): [M^+ + 1] 447.10; Anal. Calcd For $C_{21}H_{23}ClN_4O_3S$: C, 56.43; H, 5.19; N, 12.54; S, 7.17. Found: C, 56.15; H, 4.95; N, 12.49; S, 7.26.

*4-(tert-Butyl)-N′-((1-(2-morpholinoethyl)-1H-indol-3-yl)-methylene)benzenesulfonohydrazide (**5g**).* Yield: 85%; brown solid; m.p.: 237−238 °C; M.F: $C_{25}H_{32}N_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.74 (s, 1H, −NH), 8.52 (s, 1H, Ar−H), 7.94−7.91 (m, 2H, Ar−H), 7.78−7.76 (m, 2H, Ar−H), 7.63 (d, 1H, J = 8.25 Hz, Ar−H), 7.46 (d, 1H, J = 8.25 Hz, Ar−H), 7.21−7.18 (m, 1H, Ar−H), 7.12−7.09 (m, 1H, Ar−H), 3.55−3.53 (m, 4H, morph-CH_2), 3.49−3.46 (m, 2H, −CH_2), 2.49−2.47 (m, 2H, −CH_2), 2.46−2.39 (m, 4H, morph-CH_2), 1.29 (s, 9H, −CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 156.43, 154.62, 137.06, 133.29, 132.73, 128.24, 125.86, 124.21, 122.85, 121.78, 120.87, 112.04, 111.04, 66.16, 56.21, 53.35, 47.42, 34.97, 30.79; ESI-MS (m/z): [M^+ + 1] 469.50; Anal. Calcd For $C_{25}H_{32}N_4O_3S$: C, 64.08; H, 6.88; N, 11.96; S, 6.84. Found: C, 63.80; H, 6.97; N, 11.68; S, 6.65.

*N′-((1-(2-Morpholinoethyl)-1H-indol-3-yl)methylene)-naphthalene-2-sulfonohydrazide (**5h**).* Yield: 89%; white solid; m.p.: 242−243 °C; M.F: $C_{25}H_{26}N_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.74 (s, 1H, −NH), 8.57 (d, 2H, J = 8.25 Hz, Ar−H), 8.16−8.12 (m, 2H, Ar−H), 8.06 (d, 1H, J = 8.25 Hz, Ar−H), 7.95−7.92 (m, 2H, Ar−H), 7.88−7.86 (m, 1H, Ar−H), 7.73−7.66 (m, 2H, Ar−H), 7.45 (d, 1H, J = 8.25 Hz, Ar−H), 7.20−7.17 (m, 1H, Ar−H), 7.07−7.04 (m, 1H, Ar−H), 3.54−3.53 (m, 4H, morph-CH_2), 2.49−2.47 (m, 2H, −CH_2), 2.46−2.39 (m, 4H, morph-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6); 154.80, 137.09, 134.66, 133.19, 132.93, 131.63, 129.93, 129.40, 129.19, 128.92, 127.94, 127.71, 124.20, 123.58, 122.93, 121.80, 120.95, 112.10, 111.04, 66.18, 56.23, 53.36, 47.46; ESI-MS (m/z): [M^+ + 1] 463.50; Anal. Calcd For $C_{25}H_{26}N_4O_3S$: C, 64.91; H, 5.67; N, 12.11; S, 6.93. Found: C, 65.05; H, 5.43; N, 11.93; S, 7.18.

*N′-((1-(2-Morpholinoethyl)-1H-indol-3-yl)methylene)-quinoline-5-sulfonohydrazide (**5i**).* Yield: 91%; white solid; m.p.: 257−258 °C; M.F: $C_{24}H_{25}N_5O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.38 (s, 1H, −NH), 9.04−9.03 (m, 1H, Ar−H), 8.62−8.61 (m, 1H, Ar−H), 8.46−8.44 (m, 1H, Ar−H), 8.28−8.26 (m, 1H, Ar−H), 8.10 (s, 1H, Ar−H), 7.86−7.83 (m, 1H, Ar−H), 7.63−7.60 (m, 2H, Ar−H), 7.41 (d, 1H, J = 8.25 Hz, Ar−H), 7.31 (d, 1H, J = 8.25 Hz, Ar−H), 7.07−7.04 (m, 1H, Ar−H), 6.90−6.87 (m, 1H, Ar−H), 4.34 (t, 2H, J = 6.85 Hz, −CH_2), 3.64−3.62 (m, 4H, morph-CH_2), 2.69 (t, 2H, J = 6.85 Hz, −CH_2), 2.55−2.50 (m, 4H, morph-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 151.51, 143.19, 140.25, 136.91, 136.77, 136.08, 134.21, 134.00, 130.16, 128.54, 125.57, 123.78, 122.47, 122.43, 121.55, 120.15, 111.67, 111.52, 66.33, 57.37, 53.64, 46.08; ESI-MS (m/z): [M^+ + 1] 464.50; Anal. Calcd For $C_{24}H_{25}N_5O_3S$: C, 62.18; H, 5.44; N, 15.11; S, 6.92. Found: 61.99; H, 5.18; N, 14.92; S, 7.10.

*N′-((1-(2-Morpholinoethyl)-1H-indol-3-yl)methylene)-1-phenylmethanesulfonohydrazide (**5j**).* Yield: 90%; white solid; m.p.: 236−237 °C; M.F: $C_{22}H_{26}N_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.69 (s, 1H, −NH), 8.38 (s, 1H, Ar−H), 8.21 (d, 1H, J = 7.55 Hz, Ar−H), 7.90 (d, 1H, J = 2.75 Hz, Ar−H), 7.49 (d, 1H, J = 8.25 Hz, Ar−H), 7.36 (s, 4H, Ar−H), 7.24−7.19 (m, 2H, Ar−H), 4.67 (s, 2H, −CH_2), 3.63 (t, 2H, J = 6.85 Hz, −CH_2), 3.55−3.53 (m, 4H, morph-CH_2), 2.39−2.36 (m, 2H+4H, −CH_2, morph-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 149.08, 137.09, 131.85, 131.03, 129.00, 128.51, 128.33, 124.25, 122.81, 121.80, 120.92, 112.01, 111.27, 66.16, 56.05, 53.66, 53.34, 45.85. ESI-MS (m/z): [M^+ + 1] 427.50; Anal. Calcd For $C_{22}H_{26}N_4O_3S$: C, 61.95; H, 6.14; N, 13.14; S, 7.52. Found: C, 62.14; H, 6.06; N, 13.05; S, 7.45.

*N′-((1-(2-Morpholinoethyl)-1H-indol-3-yl)methylene)-[1,1′-biphenyl]-4-sulfonohydrazide (**5k**).* Yield: 92%; white solid; m.p.: 248−249 °C; M.F: $C_{27}H_{28}N_4O_3S$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.79 (s, 1H, −NH), 8.60 (s, 1H, Ar−H), 8.02−7.93 ((m, 6H, Ar−H), 7.75 (d, 2H, J = 7.60 Hz, Ar−H), 7.52−7.42 (m, 4H, Ar−H), 7.23−7.14 (m, 2H, Ar−H), 3.55−3.51 (m, 4H+2H, morph-CH_2, −CH_2), 2.51−2.48 (m, 4H, morph-CH_2), 2.41 (s, 2H, −CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 155.55, 144.84, 138.35, 137.10, 134.73, 133.00, 129.20, 129.04, 128.69, 127.19, 127.15, 124.25, 122.90, 121.79, 121.06, 112.10, 111.01, 66.17, 56.18, 53.34, 47.55; ESI-MS (m/z): [M^+ + 1] 489.26; Anal. Calcd For $C_{27}H_{28}N_4O_3S$: C, 66.37; H, 5.78; N, 11.47; S, 6.56. Found: C, 66.58; H, 5.59; N, 11.53; S, 6.37.

6.4. Cell Proliferation Study. MDA-MB-468 (8000 cells/well), MCF-7 (8000 cells/well), and HEK 293 (5000 cells/well) cells were seeded in DMEM media supplemented with 10% FBS in a 96 well plate. Cells were left for 24 h in an incubator maintained at 37 °C, 5% CO_2, and humidified atmosphere. Later, cells were treated with compounds (**5a**−**k**) at different concentrations and incubated for additional 48 h. For biocompatibility on HEK 293 cells, (**5a**−**k**) compounds were exposed to the cells. Following incubation, viability of each group was quantified using MTT assay. For MTT assay,

https://doi.org/10.1021/acsomega.2c03908
ACS Omega 2022, 7, 42036−42043

10 μL MTT reagent (5 mg/mL) was added per well and incubated for 2−4 h. Later, media was discarded from each well and crystals developed from MTT reagent were dissolved in 100 μL DMSO. Absorbance was measured at 570 nm/690 nm using ELISA plate reader and % survival was calculated as follows:

$$\text{Survival}\,(\%) = \text{OD}_{570-690\ \text{of treated group}} / \text{OD}_{570-690\ \text{of control group}} \times 100$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03908.

^{1}HNMR and ^{13}CNMR spectra of the compounds **5a−k** (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Aysha Gaur is thankful to Department of Science & Technology, New Delhi, India for the award of INSPIRE fellowship (F. No. IF180426).

■ ABBREVIATIONS

FDA, food and drug administration; PI3Kinase p110α, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; mTOR, mammalian target of rapamycin; ATR, ataxia telangiectasia and Rad3-related protein; ESI-MS, electrospray ionization mass spectrometry; DMSO, dimethyl sulfoxide; s, singlet; d, doublet; t, triplet; m, multiplet; morph, morpholine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; FBS, fetal bovine serum; MCF-7, Michigan cancer foundation-7; HEK 293, human embryonic kidney 293

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https://doi.org/10.1021/acsomega.2c03908
ACS Omega 2022, 7, 42036−42043