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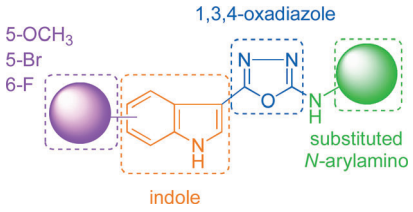
Rationally simple: Coupling of two key scaffolds in medicinal chemistry, the indole and 1,3,4-oxadiazole ring systems, gave rise to 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles with IC_{50} values in the nanomolar range against a panel of tumor cell lines. Preliminary structure–activity relationship studies indicate potential for improved selectivity through further manipulation of the oxadiazole C-2 and C-5 positions.



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Synthesis and Biological Evaluation of 2-Arylamino-5-(3′-Indolyl)-1,3,4-Oxadiazoles as Potent Cytotoxic Agents

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Synthesis and Biological Evaluation of 2-Arylamino-5-(3′-Indolyl)-1,3,4-Oxadiazoles as Potent Cytotoxic Agents

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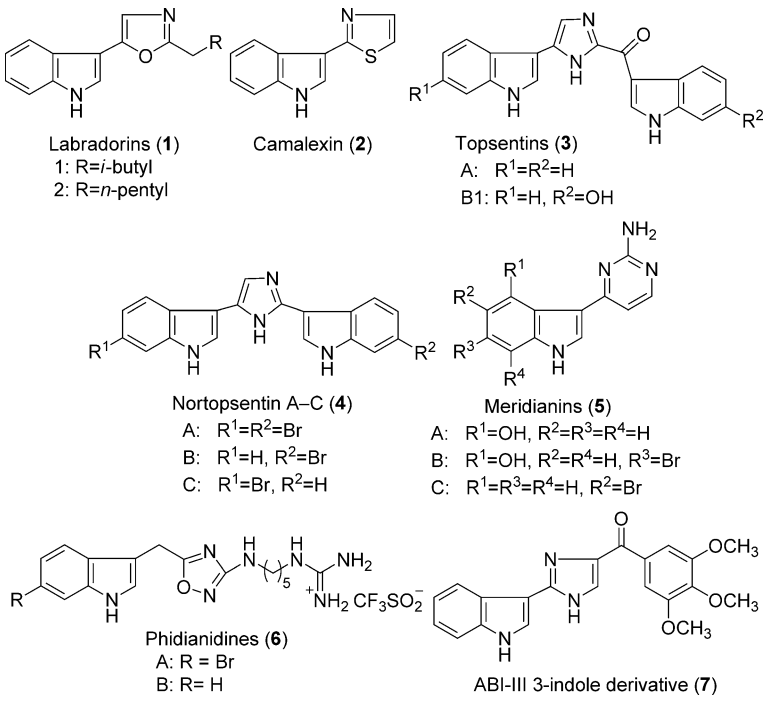
The indole nucleus is a privileged scaffold that is highly prevalent in natural and synthetic compounds of medicinal interest.[1] It is found in many clinical therapeutic agents (e.g., indomethacin, indorenate and indoramin) and endogenous biologically active substances (e.g., serotonin, melatonin, tryptophan and brassinin). Molecules containing an indole nucleus display a diverse range of biological activities, such as anticancer, antidiabetic, antirheumatoidal, antioxidant and antiviral properties,[2a] and are known to play a crucial role in the immune system.[2b,3] In recent years, several indole-containing heterocyclic compounds with interesting activities have been isolated or synthesized. For example, Labradorins (**1**), indolyloxazole-derived natural products, have been reported to exhibit potent in vitro growth inhibition of human cancer cells with GI_{50} values of 40.8 μM (non-small-cell lung carcinoma) and 25.8 μM (pancreatic adenocarcinoma).[4] Camalexin (**2**) is a characterstic phytoalexin of *Arabidopsis thaliana* that is induced by a variety of plant pathogens.[5] Topsentins (**3**), a class of bis-(indole)alkaloid natural products isolated from the marine sponge *Topsentina genitrix*, inhibit the growth of leukemia cells in vitro.[6] Nortopsentins A–C (**4**) exhibit in vitro cytotoxicity against murine leukemia (P388) cells with IC_{50} values of 16660, 20670 and 4500 μM, respectively.[7] Meridianins (**5**) and their analogues have shown good anticancer activities against human breast adenocarcinoma (MCF-7) cells (IC_{50} = 1.10 μM).[8] In 2011, Gavagnin et al. reported the isolation of Phidianidines A and B (**6**) from the marine opisthobranch mollusk *Phidiana militaris*; these natural products were found to display high cytotoxicity against both tumor and nontumor cell lines.[9] A recent report from Li et al. described a series of 2-aryl-4-benzoyl-imidazoles (ABI-III) where replacement of the aryl ring with an indole nucleus enhanced the antiproliferative activity; the 3-indole derivative (**7**) showed remarkable activity aganist melanoma and prostate cancers through inhibition of tubulin polymerization, with typical IC_{50} values around 3.8 nM.[10] Given the observed improvement in activity imparted by the introduction of an indole scaffold on to an imidazole, a similar design strategy might be used for other nitrogen-containing heterocycles that are reported to possess desirabale physiochemical properties and pharmacokinetic profile, such as oxadiazoles (e.g., IMC-094332 (**8**) in Scheme 1),[11] to yield potent cytotoxic agents.



The five-membered oxadiazole ring system is an important structural motif, found in a wide range of biologically potent compounds and known to have applications in medicinal chemistry as bioisosteres for ester and amide functionalities.[12–14] In particular, 2-arylamino-1,3,4-oxadiazoles are important chemical entities due to their interesting biological properties[15,16] and various applications in materials science as photosensitizers and organic light-emitting diodes.[17] Indolyl-1,3,4-

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oxadiazoles (i.e., **9** in Scheme 1), reported as analogues of naturally occurring Labradorins (**1**), show in vitro cytotoxicity in the micromolar range.[18] Furthermore, 2-arylamino-5-(2,4-dihydroxy-phenyl)-1,3,4-thiadiazoles (e.g., FABT (**10**) in Scheme 1) have also emerged as potent cytotoxic agents.[19]

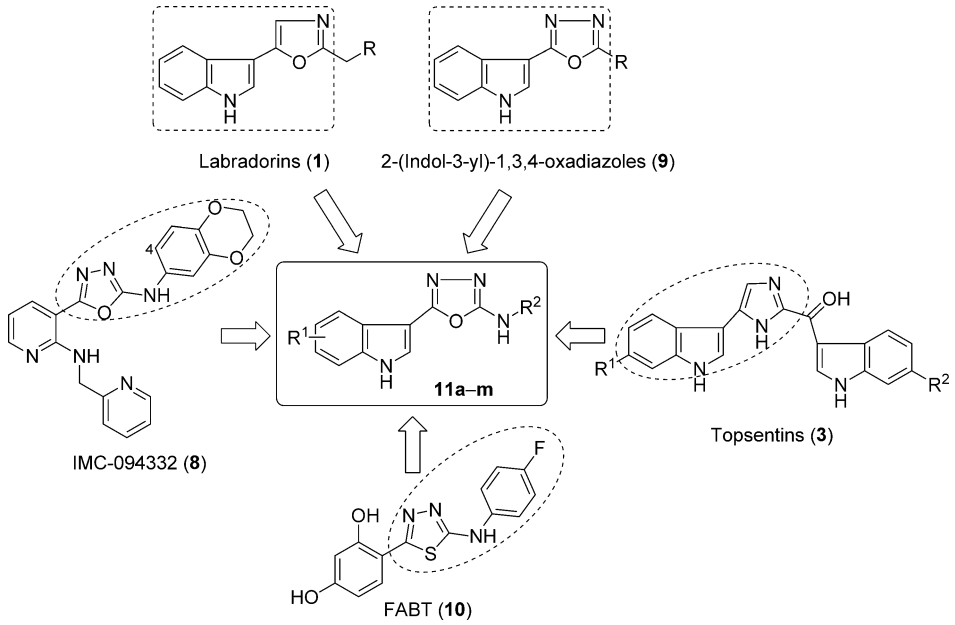
Cancers are among the most harmful diseases and are a leading cause of death worldwide.[20a] Over the past decade, several chemotherapeutic agents have been identified including paclitaxel, docetaxel, ixabepilone and doxorubicin. However, due to drug resistance, toxicity, low bioavailability, and solubility problems associated with the drugs currently in clinical use, there is a need to develop more effective drugs with decreased side effects.[20b] Several bis(indolyl)-thiophenes,[21a] pyrazoles,[21b] furans,[21c] isoxazoles,[21d] pyrroles,[21e] indolyl-phenyl and azaindolylphenyl thiazoles[21f] have been reported as antiproliferative agents. Moreover, several indolyloxadiazoles and indolylthiadiazoles have been identified as anticancer agents.[22]

On the basis of the above-mentioned observations regarding the potential of oxadiazoles and indoles and our own previous observations regarding the importance of the indole motif in conferring anticancer activity together with interesting cytotoxicity, a series of 2-arylamino-1,3,4-oxadiazoles (**11a**–**m**) were designed, incorporating both the indolyloxadiazole and arylamino-oxadiazole key scaffolds, with an aim to obtain potent and selective cytotoxic agents (Scheme 1).

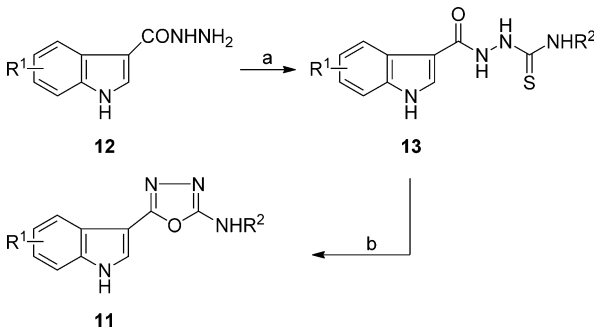
2-Arylamino-1,3,4-oxadiazoles can be prepared either by cyclodehydration of semicarbazides or cyclodesulfurization of thiosemicarbazides. Cyclodehydration to form 1,3,4-oxadiazoles has previously been achieved by using concentrated sulfuric acid, *para*-toluenesulfonyl chloride (tosyl chloride), phosphorous oxychloride, thionylchloride, bromine, and methyl *N*-(triethylammoniumsulfonyl)carbamate (Burgess reagent).[23,24] Cyclodesulfurization of acylthiosemicarbazides to form 1,3,4-oxadiazoles typically involves the use of mercury and lead salts, iodine in the presence of base, tosyl chloride in combination with pyridine, 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent), 2-iodoxybenzoic acid (IBX), hydantoin-potassium iodide, and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in the presence of *N*,*N*-diisopropylethylamine (DIEA).[14,25–27] In the literature, the required intermediate acylthiosemicarbazides, prepared from reaction of a carboxylic acid and a thiosemicarbazide, were cyclized to the corresponding 1,3,4-oxadiazoles by employing coupling reagents, such as carbodiimides 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl), *N*,*N*′-dicyclohexylcarbodiimide (DCC), and PS-carbodiimide.[27] Alternatively, 2-arylamino-1,3,4-oxadiazoles were synthesized by amination of oxadiazol-2-ones using phosphorous oxychloride and an appropriate amine.[28] Recently, Telvekar et al. reported the preparation of 2-arylamino-1,3,4-oxadiazoles by using activated iodine(III) species, generated in situ from iodobenzene and oxone in the presence of triethylamine.[26b] Given the versatility of this last method, synthesis of the designed 2-arylamino-1,3,4-oxadiazoles (**11a**–**m**) was carried out from readily available indolylhydrazides (**12**) via iodobenzene diacetate (IBD)-mediated desulfurization of the intermediate acylthiosemicarbazides (**13**) (Scheme 2).

Readily available indolylhydrazides **12** were prepared from the correspoding indole-3-carboxylates and hydrazine hydrate. Reaction of indolylhydrazides **12** with aryl isothiocyanates in ethanol gave acylthiosemicarbazides **13** in almost quantitative yields. Subsequent cyclodesulfurization of acylthiosemicarbazides **13** with IBD in acetonitrile afforded final compounds **11a**–**m** with yields ranging from 72–85%. Attempts using other hypervalent iodine reagents such as Dess–Martin periodinane (DMP), *o*-iodoxybenzoic acid (IBX) and [bis(trifluoroacetoxy)-iodo]benzene required extended reaction times (8–10 h) and gave compounds **11** in comparatively poor yields (40–50%). It was also observed that the reaction in other solvents including dichloromethane and chloroform remained incomplete, probably due to poor solubility of acylthiosemicarbazides **13**. The optimized protocol was used to prepare 2-arylamino-1,3,4-oxadiazoles **11a**–**m**.

2-Arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles **11a**–**m** were assessed for their in vitro cytotoxicity against human embryonic kidney cells (HEK293), and a panel of cancer cell lines: cervical (HeLa), prostate (LNCaP and PC3) and breast (MCF-7 and MDA-MB-231. Doxorubicin was evaluated in parallel as a positive control, and the IC_{50} values were



Scheme 1. Design strategy of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles. The dashed boxes indicate the key core structure present in previously reported compounds.



Scheme 2. Optimized protocol for the preparation of 2-arylamino-1,3,4-oxadiazoles **11 a**–**m** via cyclodesulfurization of acylthiosemicarbazides **13**. *Reagents and conditions*: a) R^2NCS, EtOH, 60 °C 2–3 h, 85–95%; b) $PhI(OAc)_2$, CH_3CN, RT, 3–4 h, 72–85%. For R groups, see Table 1.

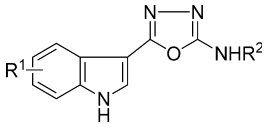
determined from the results of the MTT assay (Table 1). The results indicate that substituents at the C-2 and C-5 positions of the 1,3,4-oxadiazole ring have significant impact on the potency of derivatives **11 a**–**m**. Most of the compounds exhibited significant anticancer activities in the cell lines tested, with IC_{50} values in the nanomolar to micromolar range.

The structure–activity relationships (SAR) were probed through altering substituents in the indole (R^1) and *N*-arylamino (R^2) moieties. Unsubstituted derivative **11 a** ($R^1 = H$; $R^2 = Ph$) was found to be active against the cancer cell lines tested. In general, electron-donating groups on the phenyl ring of the *N*-arylamino moiety (R^2) were found to be beneficial for the activity. Analogue **11 b** with methyl substituent displayed enhanced activity against all cell lines tested, but was particularly active in HeLa cells with an IC_{50} value in the nanomolar range. Introduction of *para*-methoxy (**11 c**) or trimethoxy (**11 d**) group in the anilino ring led to compounds with significantly improvement cytotoxic activity, particularly against prostate cancer cell line (PC-3 and LNCaP). The trimethoxy derivative (**11 d**) was also found to exhibit potent cytotoxicity in HEK293 cells, with an IC_{50} value of 0.1 μM.

Compounds **11 e** and **11 g** with an electronegative substituent (F and Cl, respectively) on the phenyl group of the C-2 *N*-arylamino motif (R^2) exhibited good cytotoxicity across all cell lines tested, however, the cytotoxicity observed against MCF-7 (**11 e**, $IC_{50} = 0.4$ μM; **11 g**, $IC_{50} = 0.1$ μM) and HeLa (**11 e**, $IC_{50} = 1.0$ μM; **11 g**, $IC_{50} = 0.3$ μM) cancer cell lines was particularly potent. Furthermore, *para*-chloro analogue **11 g** showed improved activity against a pancreatic cancer cell line (PC-3, $IC_{50} = 0.2$ μM), whereas *para*-fluoro analogue **11 e** was found to be selectively cytotoxic against HeLa and MCF-7 cells. Replacement of the methyl moiety in compound **11 b** by a trifluoromethyl group (**11 f**) imparted improved activity against LNCaP cells, however, activity was decreased to varying degrees against the other cell lines tested.

Introduction of a methoxy group at the indole 5 position of compound **11 c** to give analogue **11 h** led to a tenfold improvement in activity against HEK293 and MCF-7 cells, and fourfold improvement against MDA-MB-231 cells. The same modification to trimethoxy analogue **11 d** to give compound **11 i** resulted in significantly improved cytotoxicity against HeLa cells ($IC_{50} < 0.001$ μM). The presence of a bromo substituent at the indole 5 position was found to be beneficial for activity. 5-Bromoindoles **11 j** and **11 k** displayed improved activity against

Table 1. In vitro cytotoxicity data for 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles **11 a**–**m** against a panel of cell lines.[a]



Compd	R^1	R^2	IC_{50} [μM][b]					
			HEK293	HeLa	LNCaP	MCF-7	PC3	MDA-MB-231
11 a	H	C_6H_5	1.5 ± 0.01	2.2 ± 0.04	3.0 ± 0.01	1.6 ± 0.01	4.7 ± 0.40	**0.8 ± 0.01**
11 b	H	4-$CH_3C_6H_4$	2.4 ± 0.04	< **0.001**[c]	2.8 ± 0.01	0.2 ± 0.01	**0.8 ± 0.07**	**0.2 ± 0.01**
11 c	H	4-$OCH_3C_6H_4$	1.0 ± 0.01	3.1 ± 0.1	**0.5** ± 0.01	1.0 ± 0.01	**0.1 ± 0.01**	7.6 ± 0.19
11 d	H	3,4,5-$(OCH_3)_3C_6H_2$	**0.1 ± 0.01**	**0.5 ± 0.01**	**0.9 ± 0.01**	**0.5 ± 0.03**	**0.2 ± 0.01**	**0.9 ± 0.06**
11 e	H	4-FC_6H_4	7.2 ± 0.14	1.0 ± 0.04	11.2 ± 0.13	**0.4 ± 0.03**	9.7 ± 0.38	4.3 ± 0.31
11 f	H	4-$CF_3C_6H_4$	10.6 ± 0.1	**0.2 ± 0.01**	**0.6 ± 0.01**	**0.2 ± 0.01**	2.7 ± 0.01	5.8 ± 0.1
11 g	H	4-ClC_6H_4	4.7 ± 0.14	**0.3 ± 0.03**	1.0 ± 0.06	**0.1 ± 0.01**	**0.2 ± 0.02**	1.5 ± 0.06
11 h	5-OCH_3	4-$OCH_3C_6H_4$	**0.1** ± 0.01	10.3 ± 0.30	4.6 ± 0.03	**0.1** ± 0.004	**0.7 ± 0.01**	1.9 ± 0.04
11 i	5-OCH_3	3,4,5-$(OCH_3)_3C_6H_2$	**0.3 ± 0.01**	< **0.001**[c]	**0.7** ± 0.03	6.4 ± 0.4	2.0 ± 0.1	55.0 ± 3.5
11 j	5-Br	4-$OCH_3C_6H_4$	**0.6 ± 0.006**	**0.1 ± 0.002**	**0.2** ± 0.01	1.1 ± 0.01	**0.2 ± 0.01**	**0.9 ± 0.03**
11 k	5-Br	3,4,5-$(OCH_3)_3C_6H_2$	1.1 ± 0.01	1.7 ± 0.03	**0.6** ± 0.01	1.4 ± 0.01	**0.2 ± 0.01**	**0.3 ± 0.03**
11 l	H	$CH_2C_6H_5$	1.1 ± 0.02	**0.8 ± 0.03**	5.7 ± 0.47	< **0.001**[c]	**0.1 ± 0.01**	64.4 ± 0.51
11 m	6-F	4-$OCH_3C_6H_4$	**0.2 ± 0.01**	1.5 ± 0.12	3.7 ± 0.07	**0.1 ± 0.003**	**0.1 ± 0.01**	**0.8 ± 0.01**
Doxorubicin			**0.4 ± 0.02**	**0.9 ± 0.04**	**0.3 ± 0.01**	1.4 ± 0.06	**0.7 ± 0.02**	1.5 ± 0.11
Labradorin 1			–	–	–	16.23[d]	–	–

[a] Human embryonic kidney cells (HEK293), and cervical (HeLa), prostate (LNCaP and PC3) and breast (MCF-7 and MDA-MB-231) cancer cells. [b] IC_{50} values were obtained from dose–response curves by nonlinear regression using a curve fitting program (GraphPad Prism 5.0). Data are the mean ± standard error of the mean (SEM) of at three independent experiments performed in triplicate. SEM was < 10% for all values. IC_{50} values < 0.1 μM are given in bold. [c] Due to the potent nature of the compound and the limitations of the assay used, the IC_{50} value could not be accurately determined. [d] Data converted from the half maximal growth inhibition (GI_{50}) value of 3.9 μg mL^{-1} given in Ref. [4].

the MDA-MB-231 cell line over the unsubstituted indole analogues **11 c** and **11 d**, however derivative **11 j** with a single methoxy group in the *para* position of the *N*-arylamino moiety (R^2) also exhibited a 30-fold improvement in cytotoxicity against the HeLa cell line. Compound **11 l** with an unsubstituted indole ($R^1 = H$) and an *N*-benzyl moiety in the R^2 position exhibited cytotoxicity in the nanomolar range against MCF-7 cells and good cytotoxicity against all other cell lines test with the except of MDA-MB-231. Incorporation of a fluoro substituent at the 6 position of the indole ring was found to be beneficial for activity. 6-Fluoroindole **11 m** showed improved cytotoxicity over analogue **11 c** against HEK293 cells ($IC_{50} = 0.2$ μM) and breast cancer cell lines (MCF-7, $IC_{50} = 0.1$ μM; MDA-MB-231, $IC_{50} = 0.8$ μM).

Finally, we investigated the mechanism of cell death in MDA-MB-231 cells using two compounds: **11 a** and **11 e**, selected for their potency against this cell line ($IC_{50} = 0.8$ and 4.3 μM, respectively). After incubation for 48 h, MDA-MB-231 cells were fixed and stained with propidium iodide, and their nuclear morphology was analyzed using fluorescence microscopy. While DMSO-treated control cells revealed normal healthy nuclei, inhibitor-treated cells showed apoptotic nuclei (Figure 1), suggesting that apoptosis is the major mechanism

Figure 1. Propidium iodide staining of MDA-MB-231 cells treated with compounds **11 a** and **11 e** for 48 h. DMSO was used as a control. Scale: 10 μm.

by which these 2-arylamino-1,3,4-oxadiazoles promote cell death in MDA-MB-231 cancer cells. Some recent studies on arylamino-1,3,4-oxadiazoles and analogues have shown that the antiproliferative activities of these oxadiazoles could be due to the inhibition of tubulin polymerization or inhibition of kinases, such as GSK3β, VEGFR-2 and CK2.[11b, 29a] Another study by Ziedan et al. has shown that indole-based 3,5-disubstituted oxadiazole promote apoptosis, which is consistent with our findings.[29b] However, the exact molecular target of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles remains unknown at this stage, and further mechanism of action studies are currently underway.

In summary, a convenient and general protocol for the synthesis of a range of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles in high yields by employing IBD-mediated oxidative desulfurization of readily available acylthiosemicarbazides **13** has been developed. Results from anticancer screening showed that compounds **11 a**–**m** possess significant antiproliferative activity against a panel of cell lines. The most potent compounds (**11 b**, **11 i** and **11 l**), with *N*-(tolyl), *N*-(trimethoxyphenyl) and *N*-(benzyl) motifs at the C-2 position of the 1,3,4-oxadiazole core, exhibited IC_{50} values in the nanomolar range against most cell lines tested. Interestingly, compounds **11 i** and **11 l** although exceedingly potent against most cell lines tested, were not as cytotoxic in MDA-MB-231 cells. Compounds with electron-withdrawing substituents in the C-2 *N*-aryl ring (**11 e**–**g**) exhibited a greater range of IC_{50} values across the tested cell lines, indicating potential selective cytotoxicity. Also, the introduction of electron-donating (MeO) and electron-withdrawing (Br and F) substituents in the indolyl ring resulted in derivatives with enhanced cytotoxicity, and again, the spread of IC_{50} values suggests some potential selectivity. Finally, preliminary mechanism of action studies indicated that these compounds induce apoptosis in MDA-MB-231 cancer cells, though further studies are required to confirm the molecular mechanism of these agents.

Experimental Section

Chemistry

All laboratory grade reagents were obtained commercially from Aldrich or Spectrochem. The reactions were monitored by thin layer chromatography (TLC) performed on commercially available Merck precoated plates (silica gel 60 F_{254}, 0.25 mm). Melting points (mp) were determined on an Ez Melt melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker advance II (400 MHz) spectrophotometer using [D_6]DMSO as a solvent. Mass spectra (MS) were obtained on a Bruker ProFLEX III MALDI-TOF (matrix: 2,5-dihydroxybenzoic acid (2,5-DHB)) or a ABI Sciex 5800 ESI-TOF mass spectrometer. Analyses were performed on a Waters 515 HPLC system equipped with a reverse-phase Sunfire C18 column (5 μm, 4.6 mm × 250 mm) and photodiode array detectors, at a flow rate of 1 mL min^{-1} and a gradient of solvent A (MeOH) and solvent B (CH_3CN). HPLC traces for all final products (**11 a**–**m**) are provided in the Supporting Information.

Synthesis of indole-3-carbohydrazides (12): A solution of indole-3-carboxylic acid (1 mmol) in EtOH (20 mL) was treated with a catalytic amount of concentrated sulfuric acid (0.2 mL), and the reaction was heated at reflux for 20 h. After completion, the solvent was removed in vacuo, and the residue was extracted with EtOAc (2 × 15 mL). The combined organic extracts were washed with saturated aq $NaHCO_3$ (25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the corresponding ester in good yield (85–95%). The ester was used in the subsequent step without further purification A solution of the ester (1 mmol) and $NH_2NH_2{\cdot}H_2O$ (2 mmol) in EtOH (15 mL) was heated at reflux for 4 h. The reaction mixture was cooled to RT, and the resultant solid was isolated by filtration and recrystallized from EtOH to obtain pure hydrazides **12**.

Indole-3-carbohydrazide (12 a): White solid (850 mg, 85%): R_f = 0.30 (hexane/EtOAc, 1:9); mp: 232–234 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 11.31$ (s, 1H), 9.14 (s, 1H), 8.17 (d, $J = 7.1$ Hz, 1H), 7.95 (d, $J = 2.8$ Hz, 1H), 7.41 (d, $J = 8.1$ Hz, 1H), 7.20–6.99 (m, 2H), 3.95 ppm (s, 2H); IR (KBr): $\tilde{\nu} = 3256$, 3109, 1660, 1607, 1583, 1523, 1433, 1240, 736 cm^{-1}; MS (ESI): *m/z* (%): 175.2 $[M]^+$ (100).

5-Methoxyindole-3-carbohydrazide (12 b): White solid (900 mg, 90%): $R_f = 0.28$ (hexane/EtOAc, 1:9); mp: 178–179 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 11.68$ (s, 1H), 9.64 (s, 1H), 7.61 (s, 1H), 7.49 (s, 1H), 7.40 (d, $J = 8.0$ Hz, 2H), 4.18 (s, 2H), 3.86 ppm (s, 3H); IR (KBr): $\tilde{\nu} = 3340$, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724 cm^{-1}; MS (ESI): *m/z* (%): 206.2 $[M+H]^+$ (100).

5-Bromoindole-3-carbohydrazide (12c): White solid (900 mg, 90%): $R_f = 0.32$ (hexane/EtOAc, 1:9); mp: 255–257 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.68$ (s, 1H), 9.78 (s, 1H), 7.62 (s, 1H), 7.52 (s, 1H), 7.46 (d, $J = 8.00$ Hz, 2H), 4.23 ppm (s, 2H); IR (KBr): $\tilde{\nu} =$ 3340, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724 cm^{-1}; MS (ESI): *m/z* (%): 253.0 $[M]^+$ (100).

6-Fluoroindole-3-carbohydrazide (12d):[22a] Off-white solid (850 mg, 85%): $R_f = 0.33$ (hexane/EtOAc, 1:9); mp: 204–205 °C; IR (KBr): $\tilde{\nu} = 3310$, 3163, 3070, 2931, 1620, 1542, 1442, 1218, 1141, 778, 724 cm^{-1}.

Synthesis of 1-(indole-3-carbonyl)-4-arylthiosemicarbazides (13): Indole-3-carbohydrazide **12** (2.8 mmol) and aryl isothiocyanate (2.8 mmol) were added to a round-bottom flask containing EtOH (10 mL), and the reaction was stirred at 60 °C for 2–3 h. The resultant solid was isolated by filtration and dried in vacuo to obtain thiosemicarbazides **13** in good yield (85–95%). The products were obtained in sufficient purity to be used without further purification in the next step. Characterization data for thiosemicarbazides **13a–m** are given in the Supporting Information.

Synthesis of 2-arylamino-5-(indolyl)-1,3,4-oxadiazoles (11a–m): A solution of thiosemicarbazide **12** (0.85 mmol) in dry CH_3CN (5 mL) was cooled to 10 °C and treated portionwise with iodobenzene diacetate (1 mmol). The reaction was stirred for 3–4 h at RT. Upon completion, the solvent was removed in vacuo, and the residue was triturated with hexane to afford crude product. Recrystalization from EtOH gave pure compound **11** in good yield (72–85%).

5-(1*H*-Indol-3-yl)-*N*-phenyl-1,3,4-oxadiazol-2-amine (11a): Light brown powder (133 mg, 75%): $R_f = 0.45$ (hexane/EtOAc, 4:6); mp: 212–214 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.73$ (s, 1H), 9.87 (s, 1H), 8.07 (d, $J = 7.3$ Hz, 1H), 7.86 (s, 1H), 7.98 (d, $J = 2.8$ Hz, 1H), 7.52 (t, $J = 8.2$ Hz, 3H), 7.22 (td, $J = 13.8$, 6.5 Hz, 2H), 7.13 ppm (d, $J = 8.3$ Hz, 2H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): $\delta = 158.6$, 156.0, 139.5, 136.9, 129.5, 126.9, 124.4, 123.0, 121.8, 121.2, 120.7, 117.3, 112.8, 100.3 ppm; IR (KBr): $\tilde{\nu} = 3193$, 1620, 1550, 1450, 1178, 750, 694 cm^{-1}; MS (ESI): *m/z* (%): 277.1 $[M+H]^+$ (100); HPLC: $t_R =$ 2.67 min (97.7% purity).

5-(1*H*-Indol-3-yl)-*N*-(4-methylphenyl)-1,3,4-oxadiazol-2-amine (11b): Redish brown powder (191 mg, 78%): $R_f = 0.50$ (hexane/EtOAc, 4:6); mp: 240–242 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta =$ 11.71 (s, 1H), 10.22 (s, 1H), 8.12 (d, $J = 7.3$ Hz, 1H), 7.87 (d, $J =$ 2.8 Hz, 1H), 7.52–7.54 (m, 3H), 7.18–7.25 (m, 2H), 7.14 (d, $J =$ 8.3 Hz, 2H), 2.29 ppm (s, 3H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): $\delta =$ 158.2, 155.5, 136.3, 136.2, 130.2, 129.1, 125.5, 123.9, 122.4, 120.5, 120.3, 116.8, 111.9, 100.2, 20.3 ppm; IR (KBr): $\tilde{\nu} = 3178$, 1612, 1581, 1504, 1420, 1160, 954, 748 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}N_4O$: 291.1246, found: 291.1237; HPLC: $t_R = 3.15$ min (98.0% purity).

5-(1*H*-Indol-3-yl)-*N*-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (11c): Light brown powder (67 mg, 75%): $R_f = 0.45$ (hexane/EtOAc, 4:6); mp: 215–217 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.83$ (s, 1H), 10.27 (s, 1H), 8.09 (d, $J = 7.0$ Hz, 1H), 7.93 (s, 1H), 7.56–7.51 (m, 3H), 7.27–7.21 (m, 2H), 6.96 (d, $J = 8.8$ Hz, 2H), 3.73 ppm (s, 3H); IR (KBr): $\tilde{\nu} = 3340$, 3139, 1635, 1570, 1512, 1458, 1226, 1141, 1030, 833, 794 cm^{-1}; MS (ESI): *m/z* (%): 307.1 $[M+H]^+$ (100); HPLC: $t_R = 3.48$ min (98.3% purity).

5-(1*H*-Indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (11d): Light brown powder (73 mg, 80%): $R_f = 0.45$ (hexane/EtOAc, 3:7); mp: 263–265 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta =$ 11.67 (s, 1H), 10.23 (s, 1H), 8.14 (d, $J = 7.5$ Hz, 1H), 7.85 (s, 1H), 7.51 (d, $J = 7.6$ Hz, 1H), 7.26–7.18 (m, 2H), 7.03 (s, 2H), 3.85 (s, 6H), 3.70 ppm (s, 3H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): $\delta = 158.2$, 155.5, 152.9, 136.3, 135.1, 131.9, 125.7, 123.9, 122.4, 120.6, 120.3, 112.0, 99.9, 94.6, 60.1, 55.5 ppm; IR (KBr): $\tilde{\nu} = 3494$, 3209, 1650, 1560, 1504, 1450, 1234, 1126, 955, 817, 748 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{19}N_4O_4$: 367.1406, found: 367.0526; HPLC: $t_R = 2.85$ min (98.2% purity).

***N*-(4-Fluorophenyl)-5-(1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine (11e)**: Light brown powder (72 mg, 81%): $R_f = 0.45$ (hexane/EtOAc, 5:5); mp: 215–217 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.58$ (s, 1H), 10.24 (s, 1H), 8.15 (d, $J = 7.0$ Hz, 1H), 7.81 (d, $J = 3.4$ Hz, 1H), 7.68–7.64 (m, 2H), 7.51–7.49 (m, 1H), 7.26–7.18 (m, 2H), 7.09–7.02 ppm (m, 2H); IR (KBr): $\tilde{\nu} = 3409$, 3294, 1643, 1581, 1535, 1488, 1350, 1126, 940, 740 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{12}FN_4O$: 295.0995, found: 295.1038; HPLC: $t_R = 2.97$ min (99.0% purity).

5-(1*H*-Indol-3-yl)-*N*-[4-(trifluoromethyl)phenyl]-1,3,4-oxadiazol-2-amine (11f): Off-white powder (77 mg, 85%): $R_f = 0.55$ (hexane/EtOAc, 4:6); mp: 206–208 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta =$ 11.57 (s, 1H), 10.65 (s, 1H), 8.17 (d, $J = 7.6$ Hz, 1H), 7.89 (d, $J =$ 11.2 Hz, 2H), 7.83 (d, $J = 7.7$ Hz, 2H), 7.59 (d, $J = 7.7$ Hz, 1H), 7.51 (d, $J = 7.4$ Hz, 1H), 7.28–7.19 ppm (m, 2H); IR (KBr): $\tilde{\nu} = 3425$, 3271, 1643, 1572, 1427, 1326, 1249, 1118, 804, 725 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{12}F_3N_4O$: 345.0963, found: 345.0812; HPLC: $t_R = 3.13$ min (98.9% purity).

***N*-(4-Chlorophenyl)-5-(1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine (11g)**: Off-white powder (69 mg, 77%): $R_f = 0.50$ (hexane/EtOAc, 4:6); mp: 237–239 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.85$ (s, 1H), 10.67 (s, 1H), 8.09 (d, $J = 7.6$ Hz, 1H), 7.96 (s, 1H), 7.67 (d, $J =$ 7.6 Hz, 2H), 7.54 (d, $J = 7.7$ Hz, 1H), 7.43 (d, $J = 7.4$ Hz, 2H), 7.27–7.24 ppm (m, 2H); IR (KBr): $\tilde{\nu} = 3409$, 3294, 1643, 1581, 1535, 1488, 1215, 1126, 740, 680 cm^{-1}; MS (ESI): *m/z* (%): 311.2 $[M+H]^+$ (100); HPLC: $t_R = 3.20$ min (99.2% purity).

5-(5-Methoxy-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (11h): Off-white powder (69 mg, 77%): $R_f = 0.35$ (hexane/EtOAc, 3:7); mp: 210–212 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.51$ (s, 1H), 10.05 (s, 1H), 8.10 (s, 1H), 7.77 (d, $J =$ 2.6 Hz, 1H), 7.61 (s, 1H), 7.56 (d, $J = 8.8$ Hz, 1H), 7.39 (d, $J = 8.8$ Hz, 1H), 6.90–6.78 (m, 3H), 3.86 (s, 3H), 3.77 ppm (s, 3H); IR (KBr): $\tilde{\nu} =$ 3317, 3178, 1650, 1581, 1512, 1419, 1234, 1180, 970, 720 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{17}N_4O_3$: 337.1301, found: 337.1664; HPLC: $t_R = 3.10$ min (98.7% purity).

5-(5-Methoxy-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (11i): Light brown powder (66 mg, 72%): $R_f = 0.30$ (hexane/EtOAc, 3:7); mp: 250–252 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.31$ (s, 1H), 10.06 (s, 1H), 8.03 (d, $J = 8.8$ Hz, 1H), 7.59 (s, 1H), 6.99 (s, 2H), 6.90 (d, $J = 8.6$ Hz, 1H), 6.77 (d, $J = 8.6$ Hz, 1H), 3.81 (s, 6H), 3.78 (s, 3H), 3.67 ppm (s, 3H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): $\delta = 161.3$, 156.3, 153.3, 152.9, 137.2, 137.1, 132.0, 125.1, 121.3, 118.4, 110.5, 106.9, 95.0, 94.6, 60.1, 55.6, 55.0 ppm; IR (KBr): $\tilde{\nu} = 3217$, 3186, 1658, 1604, 1350, 995, 833, 702 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{21}N_4O_5$: 397.1512, found: 397.141; HPLC: $t_R = 2.88$ min (97.2% purity).

5-(5-Bromo-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (11j): Brown powder (69 mg, 75%): $R_f = 0.32$ (hexane/EtOAc, 3:7); mp: 263–265 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 12.65$ (s, 1H), 10.32 (s, 1H), 8.36–8.32 (m, 2H), 8.18 (s, 1H), 7.61 (s, 1H), 7.55 (d, $J = 8.6$ Hz, 1H), 7.45 (dd, $J = 8.6$, 1.9 Hz, 1H), 4.08 (s, 3H), 3.99 (s, 3H), 3.94 ppm (s, 3H); IR (KBr): $\tilde{\nu} = 3260$, 3240, 1643, 1550, 1512, 1427, 1242, 1141, 686 cm^{-1}; HRMS (MALDI):

m/z $[M+H]^+$ calcd for $C_{19}H_{18}BrN_4O_4$: 447.0491, found: 446.9796; HPLC: $t_R = 3.21$ min (98.7% purity).

5-(5-Bromo-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (11k): Light brown powder (73 mg, 78%): $R_f = 0.35$ (hexane/EtOAc, 3:7); mp: 199–201 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.67$ (s, 1H), 9.98 (s, 1H), 9.47 (d, $J = 7.7$ Hz, 1H), 8.36–8.28 (m, 1H), 8.17 (d, $J = 9.0$ Hz, 1H), 7.45–7.25 (m, 3H), 6.86 (dd, $J = 8.7$, 8.6 Hz, 2H), 3.77 ppm (s, 3H); IR (KBr): $\tilde{\nu} = 3240$, 3109, 1674, 1473, 1419, 1311, 1226, 1134, 933, 765 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{14}BrN_4O_2$: 387.0280, found: 387.0356; HPLC: $t_R = 2.96$ min (97.3% purity).

***N*-Benzyl-5-(1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine (11l)**: Off-white powder (68 mg, 76%): $R_f = 0.45$ (hexane/EtOAc, 3:7); mp: 268–270 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.73$ (s, 1H), 9.87 (s, 1H), 8.07 (d, $J = 7.3$ Hz, 1H), 8.00 (d, $J = 7.6$ Hz, 1H), 7.86 (s, 1H), 7.48–7.47 (m, 1H), 7.41–7.33 (m, 3H), 7.30–7.23 (m, 3H), 4.44 ppm (s, 2H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): $\delta = 162.5$, 155.8, 139.2, 136.6, 128.8, 127.8, 127.5, 126.4, 124.5, 122.9, 121.0, 120.7, 112.8, 100.8, 46.7 ppm; IR (KBr): $\tilde{\nu} = 3217$, 3186, 1658, 1604, 1350, 995, 833, 702 cm^{-1}; MS (ESI): *m/z* (%): 291.3 $[M+H]^+$ (100); HPLC: $t_R = 2.99$ min (98.3% purity).

5-(6-Fluoro-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (11m): Off-white powder (72 mg, 80%): $R_f = 0.50$ (hexane/EtOAc, 3:7); mp: 259–261 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.74$ (s, 1H), 10.13 (s, 1H), 8.06 (dd, $J = 8.7$, 5.5 Hz, 1H), 7.84 (s, 1H), 7.54 (d, $J = 9.0$ Hz, 2H), 7.23 (dd, $J = 9.6$, 2.0 Hz, 1H), 7.02–6.97 (m, 1H), 6.88 (d, $J = 9.0$ Hz, 2H), 3.74 ppm (s, 3H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): $\delta = 158.5$, 158.2, 155.1, 154.1, 136.4, 136.3, 132.2, 126.3, 121.4, 121.3, 120.7, 118.3, 114.0, 109.2, 109.0, 100.3, 99.5, 98.3, 98.0, 55.0 ppm; IR (KBr): $\tilde{\nu} = 3201$, 3155, 1620, 1551, 1419, 1218, 756, 700 cm^{-1}; HRMS (MALDI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{14}FN_4O_2$: 325.1101, found: 325.1384; HPLC: $t_R = 2.91$ min (97.7% purity).

Biology

MTT assay: Human prostate (LNCaP and PC3), cervical (HeLa), and breast (MCF-7 and MDA-MB-231) cancer cell lines, and human embryonic kidney cells (HEK293) were cultured in RPMI-1640 media supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. The cells were seeded in 96-well plates at a density of 4×10^3 cells per well, and incubated in a humidified atmosphere of 5% CO_2 in a tissue culture incubator. After 12 h, cells were incubated with various concentrations of test compound (10 nM–1 mM) for 48 h. The final DMSO concentration was 0.05% in each sample including the control. After 48 h, 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well to a final concentration of 0.2 mg mL^{-1}, and the plates were incubated for an additional 30 min. The cells were washed twice with phosphate-buffered saline (PBS) and then lysed in DMSO (100 μL). The absorbance was measured at 570 nm using Tecan Spectrafluor Plus. The experiment was performed three times in triplicate, and IC_{50} values were determined from dose–response curves by nonlinear regression using a curve fitting program (GraphPad Prism 5.0).

Nuclear staining using propidium iodide: MDA-MB-231 cells plated on coverslips were treated either with 0.01% DMSO (control), or 10 μM test compound (**11a** or **11e**) and incubated for 48 h. After treatment, cells were fixed with cold MeOH for 5 min, followed by rehydration in PBS and and permeabilization using 0.1% Triton X-100 in PBS plus 2% BSA. Cells were treated with 0.1 μg mL^{-1} RNase A in PBS for 1 h, rinsed, and stained with 2.5 μg mL^{-1} propidium iodide in PBS for 1 h. Before mounting with Mowiol, coverslips were washed twice with PBS and once with H_2O. Each experiment was repeated three independent times. Images were taken using a Nikon Eclipse E600 microscope (Nikon Instruments, Melville, NY, USA).

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