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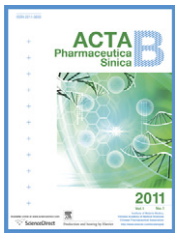

ORIGINAL ARTICLE

Synthesis and cytotoxic evaluation of substituted 3-(3′-indolyl-/3′-pyridyl)-isoxazolidines and bis-indoles

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3-Pyridylisoxazolidines;
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Modified nucleosides;
Cytotoxic activity

Abstract Regio- and stereoselective 1,3-dipolar cycloadditions of *C*-(3-indolyl)-*N*-phenylnitrone (**10**) were carried out with different mono-substituted, disubstituted and cyclic dipolarophiles under mono-mode microwave irradiation to obtain substituted 3-(indol-3′-yl)-*N*-phenyl-isoxazolidines (**16–22**). Reactions of nitrone (**10**) with allenic esters under similar conditions afforded, via a domino process, bis-indole derivatives (**23a–c**) along with compounds **24** and **25**. Similarly, reactions of *C*-(3-pyridyl)-*N*-phenylnitrone (**26**) with mono-substituted, disubstituted and cyclic dipolarophiles were carried out in refluxing dry toluene to obtain substituted 3-(3′-pyridyl)-*N*-phenylisoxazolidines (**27–34**). Some of the compounds (**16f**, **18b**, **23a**, **23c**, **27c** and **29f**) display significant cytotoxicity against a number of human cancer cell lines.

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1. Introduction

Modified nucleoside analogs (NAs) having modified bases were among the first chemotherapeutic agents to be introduced for the treatment of cancer[1–4]. Over the years, modified nucleosides have expanded the scope of antiviral and anticancer chemotherapeutics[5–11]. Presently, enormous effort is centered on structural modifications of hydroxyl moiety, heterocyclic bases and furanose ring of nucleosides leading to the discovery of a number of antiviral and anticancer agents[12–16]. Recently, isoxazolidine moiety has emerged as valuable analog of the furanose ring and substituted isoxazolidines have been found to display valuable antiviral and anticancer properties[5,14,15]. Indole alkaloids have also emerged as important candidates for a wide range of biological activities, including antimicrobial, antiviral and antitumor[17]. Various analogs of the natural pimprinine alkaloids (Fig. 1) are potent inhibitors of HIV-1 integrase. Indole based molecule is an aromatase inhibitor and has been used to treat breast cancer. Spiro-indoline-isoxazolidines are known to display anti-invasive activity against human mammary carcinoma cells[18]. Bis-indole derivatives such as bis(indolyl)thiazoles and bis(indolyl)pyrimidines also exhibit cytotoxic activity[19,20].

At the same time pyridinyl isoxazolidines (Fig. 2) such as pyrinodemin-A, chromano-piperidine fused isoxazolidine[21] and pyridinyl-isoxazolidine[22,23] exhibit good anticancer activities.

In continuation of our efforts in the search of newer cytotoxic agents, particularly isoxazolidines[15,16], it was decided to resynthesize the earlier reported 3-pyridyl-/3-indolyl-isoxazolidines and bis-indoles (**17a**, **18a-d**, **19-21**, **23b**, **23c**, **24**, **27a-e**, **30**, **32** and **33**)[24] along with some new derivatives (**16f**, **17d**, **23a** and **27h**) and evaluate their cytotoxic activities against various human cancer cell lines.

2. Results and Discussion

Initially, *C*-(3-indolyl)-*N*-phenylnitrone (**10**) was synthesized by reacting 3-formylindole and *N*-phenylhydroxylamine and characterized spectroscopically[24]. It was reacted with mono-substituted, disubstituted and cyclic dipolarophiles, and allenic esters under microwave irradiation leading to the synthesis of isoxazolidines (**16**–**22**) and bis-indole derivatives (**23a–c**) along with compounds (**24**, **25**). All compounds (**16**–**25**, Scheme 1) were characterized spectroscopically (^{1}H and ^{13}C NMR, IR and MS) and microanalytical data[24]. The structure of compound **21**, earlier assigned on the basis of NMR spectral data, was confirmed by X-ray crystallographic analysis (Fig. 3).

Further, the *C*-(3-pyridyl)-*N*-phenylnitrone (**26**) was obtained by reacting 3-formylpyridine with *N*-phenylhydroxylamine in dry benzene and characterized spectroscopically. It was reacted with mono-substituted, disubstituted and cyclic dipolarophiles by refluxing in dry toluene for 24–36 h to obtain, after column chromatographic separation, compounds **27**–**34**. All purified products (**27**–**34,** Scheme 2) were characterized by spectroscopic techniques (^{1}H and ^{13}C NMR, IR and MS) and microanalytical data[25].

All synthesized compounds (**16f**, **17a**, **17d**, **18a–d**, **19–21**, **23a-c**, **24**, **27a–e**, **27h**, **29f**, **29g**, **30**, **32** and **33**) were evaluated for their cytotoxic activity against various human cancer cell lines according to the protocol of Skehan et al[26]. Indole-based isoxazolidines (**16–24**) were evaluated against various human cancer cell lines such as cancer of the breast (MCF-7), ovary (IGROV-1), lung (A-549, HOP-62) and colon (HCT-15 and SW-620). The cytotoxic effects are reported as percent growth inhibition (Table 1) and IC_{50} values (μM, concentration required to inhibit cancer cell proliferation by 50% after exposure of cells to test compounds) have also been determined (Table 2); paclitaxel, adriamycin and mitomycin-C were used as positive controls.

Indole-based isoxazolidines were found to be highly active against colon and lung cancer cells, moderately active against ovarian cancer and less active against breast cancer cell. Initially, compounds (**16–24**) were evaluated at 100 μM; however, compounds that showed high cytotoxic activity at 100 μM were further evaluated at concentrations of 50 and 10 μM. Compound **16f** showed inhibition of 90% at 100 μM and 79% at 50 μM against colon cell line (SW-620) with IC_{50}

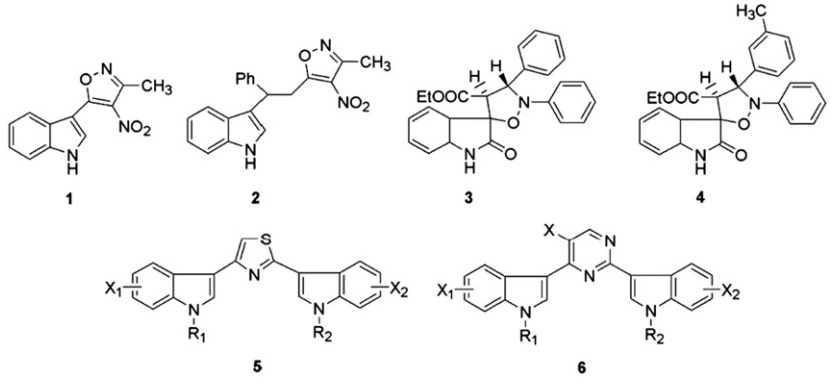


Figure 1 Some indole based anticancer agents.

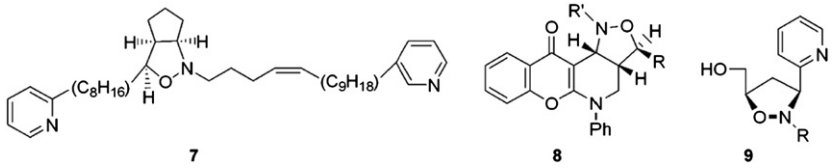
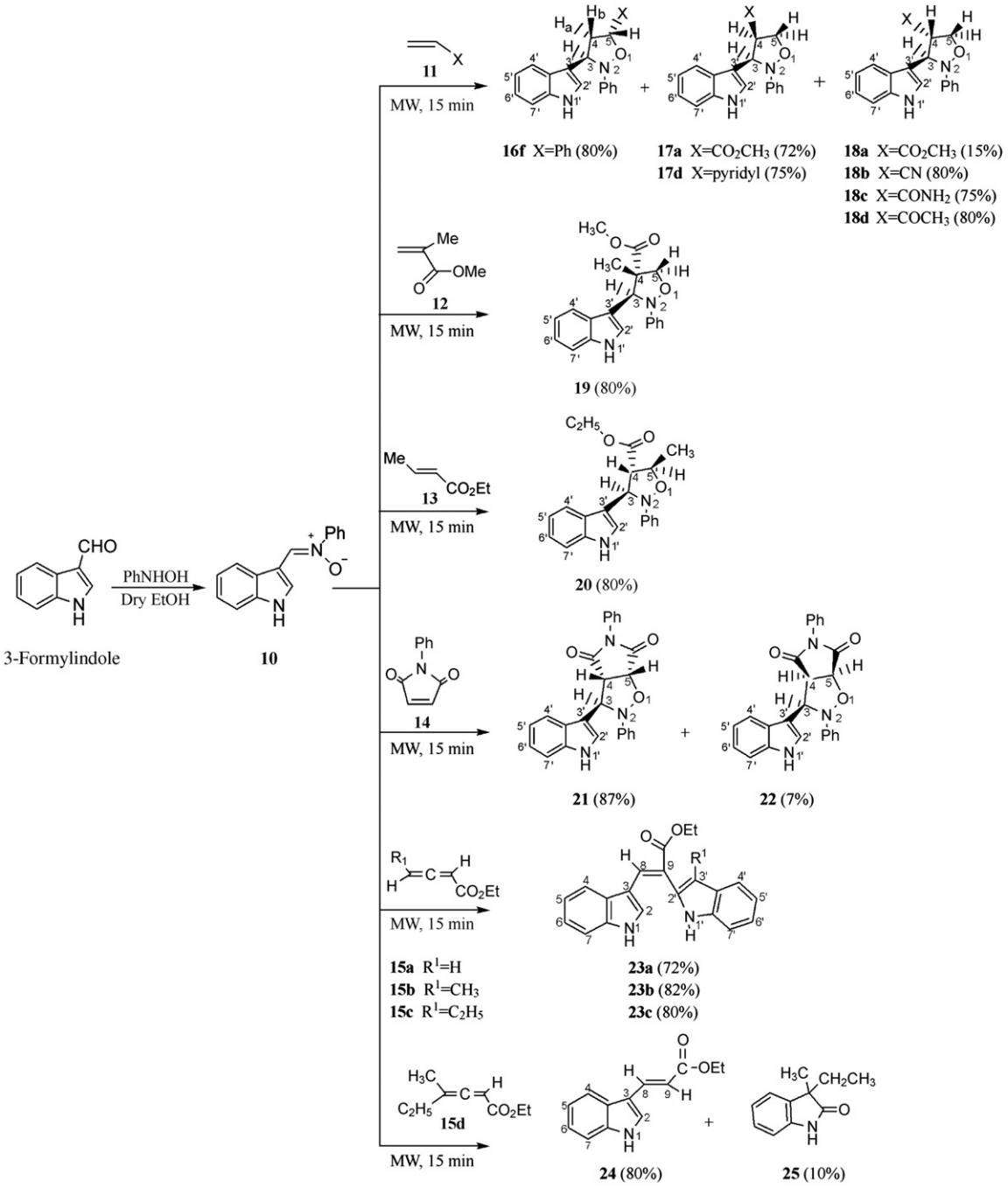


Figure 2 Some biologically active pyridinyl isoxazolidines.



Scheme 1 Synthesis of substituted 3-indolyl-isoxazolidines and bis-indoles.

of 26.6 μM; 86% at 100 μM and 63% at 50 μM against colon cell line (HCT-15) with IC_{50} of 32.5 μM; 71% at 100 μM and 66% at 50 μM against lung cell line (A-549) with IC_{50} of 41.1 μM; 72% at 100 μM and 56% at 50 μM against ovarian cancer cell line (IGROV-1) with IC_{50} of 41.4 μM. Compound **17d** showed inhibition of 63% at 100 μM and 37% at 50 μM against colon cell line (SW-620) with IC_{50} of 74.8 μM. Compound **18b** showed inhibition of 86% at 100 μM and 58% at 50 μM against colon cell line (SW-620) with IC_{50} of 35.4 μM; 70% at 100 μM and 65% at 50 μM against colon cell line (HCT-15) with IC_{50} of 36.8 μM; 74% at 100 μM and 54% at 50 μM against lung cell line (A-549) with IC_{50} of 43.4 μM. Compound **18d** showed inhibition of 76% at 100 μM and 30% at 50 μM against colon cell line (SW-620) with IC_{50} of 68.2 μM; 81% at 100 μM and 34% at 50 μM against colon cell line (HCT-15) with IC_{50} of 66.1 μM.

Indole derivatives have been reported to suppress the proliferation of various cancer cell lines at the concentration range of 50 and 100 μM, including those of breast[27–29], colon[30–32], prostate[33–35] and endometrium[36], by targeting a wide spectrum of signaling pathways, cell cycle progression and cell proliferation and survival[37–40]. Further, it has been observed that indole-3-carbinol and its metabolite 3,3′-diindolylmethane (DIM) inhibit chemical-induced tumorigenesis in

mammary gland, liver, lung, cervix and gastrointestinal tract in different animal model studies[41–47]. Studies using *in vitro* models demonstrated that indole derivatives exert anticancer effects by inhibiting the formation of free radicals, shifting estrogen metabolism towards the less estrogenic metabolite 2-hydroxyestrone, inducing G1/S arrest of the cell cycle and apoptosis, suppressing tumor cell migration, invasion and angiogenesis[48]. In clinical trials, indole derivatives have shown promising efficacy for the prevention of breast cancer, vulvar intraepithelial neoplasia and human papilloma virus-induced cervical cancer[49–52]. These preclinical studies demonstrate the translational value of indole derivatives in cancer prevention and therapy[34]. From the present investigations it emerges that monosubstituted, 3-indolyl-isoxazolidines (**16f**, **17a**, **17d**, **18a–d**) showed better cytotoxic activities than disubstituted compounds (**19**, **20**) and bicyclic compound (**21**). Further, it was found that compounds having X=–CN, –Ph, and –$COCH_3$, which are electron withdrawing groups, were found to be more active against various human cancer cell lines.

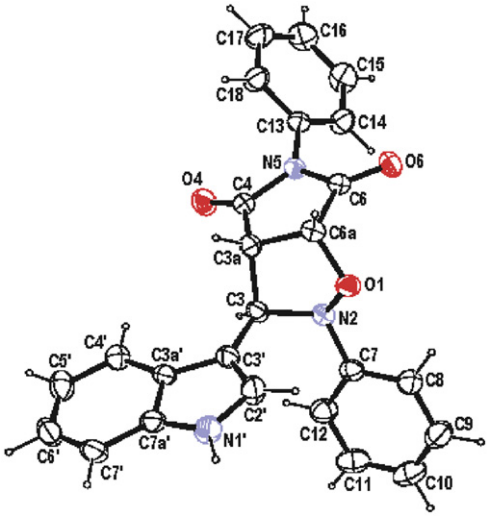
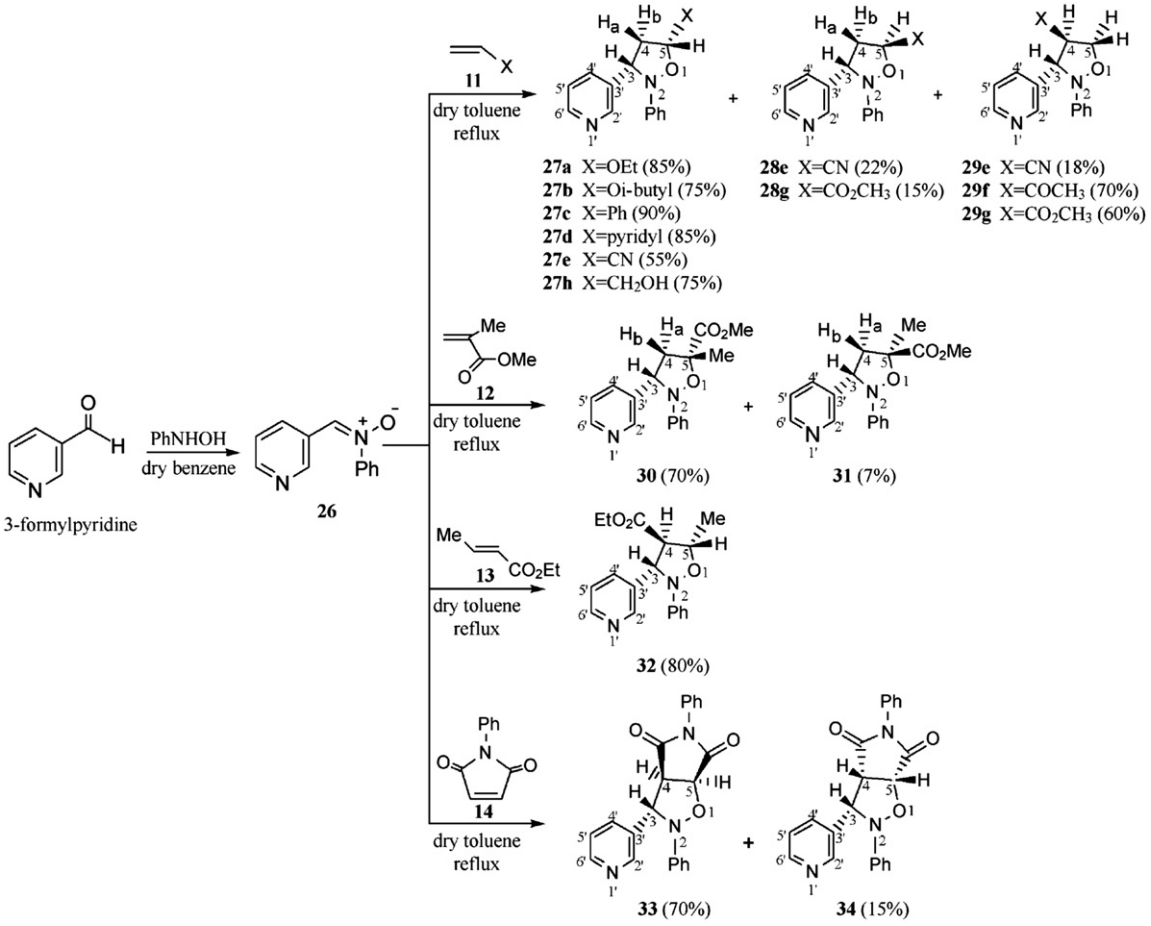


Figure 3 ORTEP view of **21**.

Bis-indole derivatives were similarly evaluated against the human cancer lines; these bis-indoles were mainly active against colon (HCT-15, SW-620) cancer cells. Compound **23a** showed inhibition of 61% at 100 μM and 58% at 50 μM against colon cell line (SW-620) with IC_{50} of 39.7 μM. Compound **23c** showed inhibition of 75% at 100 μM and 46% at 50 μM against colon cell line (SW-620) with IC_{50} of 39.7 μM; 85% at 100 μM and 56% at 50 μM against colon cell line (HCT-15) with IC_{50} of 46.6 μM. Although in most of the cases the exact mechanism of cytotoxic activity is not known for indole derivatives, isoxazolidines and bis-indoles; however, the varied modes of action have been reported, which include the inhibition of NAD^+-dependent histone deacetylases[53], inhibition of cyclin dependent kinases[54], DNA binding at



Scheme 2 Synthesis of substituted 3-pyridyl-isoxazolidines.

Table 1 *In vitro* cytotoxicity of compounds **16f**, **17a**, **17d**, **18a–d**, **19–21**, **23a–c** and **24** against human cancer cell lines.

Compound No.	Conc. (μM)	Percent growth inhibition against human cancer cell lines (%)					
		MCF-7	IGROV-1	A-549	HOP-62	HCT-15	SW-620
16f	10	–	5	9	–	15	10
	50	–	56	66	–	63	79
	100	–	72	71	–	86	90
17a	100	22	27	–	4	–	–
17d	10	–	13	7	–	0	7
	50	–	25	24	–	14	37
	100	–	58	49	–	54	63
18a	100	39	23	–	51	–	–
18b	10	–	5	9	16	15	10
	50	–	33	54	–	65	58
	100	–	48	74	–	70	86
18c	100	56	43	–	16	–	–
18d	10	–	12	7	–	0	10
	50	–	27	29	–	34	30
	100	–	43	52	–	81	76
19	100	20	21	–	22	–	–
20	10	–	0	0	–	3	6
	50	–	11	13	–	30	16
	100	–	55	54	–	56	39
21	100	11	3	–	6	–	–
23a	10	–	0	0	–	16	21
	50	–	11	27	–	26	58
	100	–	36	40	–	42	61
23b	100	21	23	–	18	–	–
23c	10	–	12	–	–	–	9
	50	–	27	30	–	56	46
	100	–	62	62	–	85	75
24	100	57	42	–	29	–	–
Paclitaxel	10	–	52	59	54	–	–
Adriamycin	1	75	–	–	–	80	70
Mitomycin-C	10	–	–	60	–	–	–

Table 2 IC_{50} value for the compounds 3-indolyl-isoxazolidines and bis-indoles against various human cancer cell lines.

Compound No.	IC_{50} (μM)			
	IGROV-1	A-549	HCT-15	SW-620
16f	41.4	41.1	32.5	26.6
17d	>100	>100	96.3	74.8
18b	>100	43.4	36.8	35.4
18d	>100	94.7	66.1	68.2
20	95	96.4	89.3	>100
23a	>100	>100	43.8	39.7
23c	72.4	75.1	46.6	39.7
Paclitaxel	4.5	4.1	–	–
Adriamycin	–	–	0.1	0.5
Mitomycin-C	–	0.4	–	–

adinine–thymidine deoxynucleotide rich region in concentration/substituent manner[55], inhibition of topoisomerase I having potencies similar to comptothecin[56], and inhibition of tubulin action[57].

The cytotoxic activity of pyridine-based isoxazolidines **27(a–e, h)**, **30**, **32**, **33** against various human cancer cell lines are reported as percent growth inhibition (Table 3) and IC_{50} values (Table 4) using paclitaxel, adriamycin and mitomycin-C as the controls. Compound **27b** showed inhibition of 80% at 100 μM and 41% at 50 μM against lung cancer cell line (A-549) with IC_{50} of 72 μM. Compound **27c** showed inhibition of 87% at 100 μM and 80% at 50 μM against human glioblastoma cell line (SF-295) with IC_{50} of 38 μM; 76% at 100 μM and 16% at 50 μM against breast cell line (MCF-7); 72% at 100 μM and 45% at 50 μM against lung cell line (A-549) with IC_{50} of 64 μM. Compound **29f** showed inhibition of 98% at 100 μM and 92% at 50 μM against lung cell line (HOP-62) with IC_{50} of 27 μM; 94% at 100 μM, 87% at 50 μM and 78% at 10 μM against human glioblastoma cell line (SF-295) with IC_{50} of 4.35 μM; 82% at 100 μM and 77% at 50 μM against ovarian cancer cell line (IGROV-1) with IC_{50} of 35 μM; 84% at 100 μM and 68% at 50 μM against breast cancer cell line (MCF-7) with IC_{50} of 54 μM; 79% at 100 μM and 55% at 50 μM against lung cancer cell line (A-549) with IC_{50} of 55 μM.

In general pyridine-based compounds are reported to be active against various types of cancer such as leukemia, lung, colon, ovarian, prostate, breast and renal[58]. Several compounds have been found to exert anticancer action through the inhibition of protein kinases (CDK1, CDK5 and GSK-3) while others have shown inhibitory activity against

Table 3 *In vitro* cytotoxicity of compounds **27(a–e, h)**, **29f**, **29g**, **30**, **32**, **33** against human cancer cell lines.

Compound No.	Conc (μM)	Percent growth inhibition against human cancer cell lines				
		MCF-7	IGROV-1	A-549	HOP-62	SF-295
27a	100	46	35	39	10	37
27b	10	9	17	5	2	14
	50	26	36	41	28	40
	100	73	51	80	39	64
27c	10	0	10	2	18	8
	50	16	58	45	30	80
	100	76	80	72	54	87
27d	100	44	52	34	6	38
27e	100	43	31	16	5	39
27h	100	32	9	13	11	22
29f	10	11	15	10	23	78
	50	68	77	55	92	87
	100	84	82	79	98	94
29g	100	30	41	22	4	32
30	100	43	19	6	18	26
32	100	27	29	18	7	30
33	100	36	35	13	14	24
Paclitaxel	10	–	52	59	54	–
Adriamycin	1	75	–	–	–	73
Mitomycin-C	10	–	–	60	–	–

Table 4 IC_{50} value for the compounds 3-pyridyl-isoxazolidines against various human cancer cell lines.

Compound No.	IC_{50} (μM)				
	MCF-7	IGROV-1	A-549	HOP-62	SF-295
27b	90	>100	72	>100	69
27c	>100	51	64	>100	38
29f	54	35	55	27	4.35
Paclitaxel	–	4.5	4.1	2.8	–
Adriamycin	0.2	–	–	–	1
Mitomycin-C	–	–	0.4	–	–

topoisomerase I and II[59]. In the present case, pyridine-based isoxazolidines **27(a–e, h)**, **30**, **32**, **33** were highly active against ovarian (IGROV-1), breast (MCF-7) and human glioblastoma (SF-295) cancer cells. Based on the observed cytotoxic activity of 3-pyridyl-isoxazolidines against various human cancer cell lines, it was observed that monosubstituted, 3-pyridyl-isoxazolidines (**27a–e**, **27h**, **29f**, **29g**) showed better cytotoxic activity than disubstituted compounds (**30**, **32**) and cyclic compound (**33**). Further, it was found that compounds having X=–Ph, and $COCH_3$ were found to be more active against human cancer cell lines than others. Cytotoxic results also reveal that the compounds having *syn* isomeric form showed better activity than *trans* form, and that **29f**, as the only single dominant regioisomer formed with *trans* orientation, was active against human cancer cell lines such as breast, lung, CNS and ovary.

3. Conclusions

A variety of substituted 3-pyridyl-/3-indolyl-isoxazolidine and bis-indole derivatives were synthesized by reaction of nitrones with various olefinic/allenic dipolarophiles. The major isomers, some minor isomers and bis-indoles obtained were evaluated for their cytotoxic activities against various human cancer cell lines. Some of the compounds display significant cytotoxic activities against various human cancer cell lines. For instance, 3-indolyl-isoxazolidine **16f** and **18b** are active against the ovarian, lung and colon cancer cell lines, whereas, 3-pyridyl-isoxazolidines **27c** is active against glioblastoma cells and compound **29f** is active against breast, lung, glioblastoma and ovarian cancer cell lines. Bis-indole derivatives **23a** and **23c** are active against colon. These 'lead' compounds can be used for further anticancer drug development and their mode of action studies.

4. Experimental

4.1. General methods

Starting materials and reagents were purchased from commercial suppliers and used after further purification (crystallization/distillation). Bruker AC-200 FT (200 MHz) and JEOL (300 MHz) NMR spectrophotometer were used for recorded the ^1H and 1^3C NMR (75 MHz) Chemical shifts are reported in ppm, tetramethylsilane used as the internal standard and

J values in Hertz. IR spectra were recorded on Shimadzu 8400S FT-IR spectrophotometer (KBr. cm^{-1}). Mass spectra, EI and ESI methods, were recorded on Shimadzu GCMS-QP-2000A and Bruker Daltonics Esquire 300 mass spectrometers, respectively. Elemental analyses were carried out on a Thermoelectron EA-112 elemental analyzer and are reported in percent atomic abundance. All melting points are uncorrected and measured in open glass capillaries using Veego Precision Digital Melting Point Apparatus. X-ray analysis was recorded at Bruker SMART APEX diffractometer equipped with low-temperature device and the structure was solved by direct methods using SHELXS 97 software.

4.2. *Chemistry*

4.2.1. *Synthesis of C-(3-Indolyl)-N-phenylnitrone* **(10)**

3-Formylindole (2.8 mmol) was dissolved in dry ethanol (30 mL) to the clear solution. *N*-phenyl-hyroxylamine hydrochloride (2.8 mmol) was added and the contents were allowed to stand at room temperature overnight. Solvent was evaporated under vacuum to obtain the viscous yellow oil, which was crystallized in chloroform–ether (1:2) to obtain nitrone as a light yellow powder (yield 90%). The nitrone (**10**) was dried under vacuum and stored under refrigeration[24].

4.2.2. *General procedure for microwave irradiation of C-(3-indolyl)-N-phenylnitrone* **(10)** *with various dipolarophiles*

Mixture of *C*-(3-indolyl)-*N*-phenylnitrone (**10**, 1.28 mmol) and various dipolarophiles (**11**, **12**, **13**, **14**, 1.0 equ.) were dissolved in a 50-mL conical flask and the contents was placed in the cavity of the microwave reactor and irradiated. Progress of completion of reaction was monitored by TLC. After the completion of reaction, the residues were loaded onto silica (60–120, mesh column packed in hexane); elution of column using hexane-chloroform (gradient) afforded the pure products. The reported yields are based on isolated pure products and relative proportions determined in the mixtures by ^{1}H NMR spectroscopy[24].

4.2.3. *General procedure for microwave irradiation of C-(3-Indolyl)-N-phenylnitrone* **(10)** *with various allenic esters*

In a 150-mL round bottom flask, the *C*-(3-indolyl)-*N*-phenylnitrone (**10**, 1.28 mmol) and allenic esters (**15a–d**, 1.0 equ.) were added, and flask was fitted with a condenser in the cavity of the microwave reactor. After closing the cavity of the reactor with the cavity lid, the contents were irradiated (150W, 100 °C) for 3 min (1 min hold time and 2 min running time) till all the nitrone was consumed as monitored by TLC. After completion of the reaction, the residues were loaded onto silica gel column (60–120 mesh, column packed in hexane); elution of column using hexane–chloroform (gradient) afforded the pure products. The reported yields are based on isolated pure products and relative proportions determined in the mixtures by ^{1}H NMR spectroscopy[24].

4.2.4. *Synthesis of C-(3-Pyridyl)-N-phenylnitrone* **(26)**

3-Formylpyridine (3.0 g, 2.8 mmol) was dissolved in dry benzene (30 mL) and to the clear solution. *N*-Phenyl-hydroxylamine hydrochloride (4.08 g, 2.8 mmol) was added and the contents were allowed to stand at room temperature, after 30 min nitrone (**26**) precipitated out as a light yellow solid, which was filtered (5.2 g, 95%). mp 86–88 °C.

4.2.5. *General procedure for the reaction of nitrone* **(26)** *with various dipolarophiles*

Reactions of nitrone with various dipolarophiles were carried out by mixing nitrone (**26**, 1.5 mmol) with dipolarophiles (**11**, **12**, **13**, **14**, 1 equ.) in a dry toluene (50 mL) and reaction mixtures were refluxed with constant stirring, until all the nitrone was consumed. After the completion of reactions as monitored by TLC, the solvent was completely removed under reduced pressure. The products were purified by column chromatography (silica gel 60–120 mesh, 20 g, column packed in hexane). The reported yields are based on isolated pure products and relative proportions determined in the mixture by ^{1}H NMR spectroscopy.

4.3. *Characterization of new products*

4.3.1. *Anti-3′-(2,5-diphenyl-isoxazolidin-3-yl)-1-H-indole* ***(16f)***

Colorless solid; Yield (400 mg, 80%), mp. 168–170 °C; IR ($CHCl_3$): 3369, 3190, 2879, 1645, 1590, 1456, 1456, 1433, 1268, 767 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03 (br, s, 1H, NH), 7.65 (d, 1H, J=8.1 Hz, C4′H), 7.41–6.86 (m, 14H, Ar–Hs), 5.23–5.14 (m, 2H, C3H & C5H), 3.22 (ddd, J_{gem}=12.9 & J=7.2, 1.5 Hz, C4Ha), 2.61 (ddd, J_{gem}=12.9 & J=4.8, 2.1 Hz C4Hb); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 150.2 (q), 148.7 (q), 135.9 (C7′a), 131.6 (C3′a), 128.9 (CH), 126.9 (CH), 126.7 (CH), 125.4 (CH), 122.4 (CH), 122.3 (C2′), 121.5 (C5′), 121.3 (C4′), 119.7 (C6′), 116.1 (CH), 114.2 (C3′), 111.3 (C7′), 80.4 (C5), 65.7 (C3), 46.7 (C4). MS (ESI) *m*/*z*: 340 $[M]^+$; Anal. Calcd. for $C_{23}H_{22}N_2O$: C 81.45; H 5.90; N 8.20. Found: C 81.49; H 5.95; N 8.90.

4.3.2. *Syn-3′-(2-phenyl-5-pyridin-4-yl-isoxazolidin-3-yl)-1-H-indole* ***(17d)***

Light brown viscous oil (375 mg, 75%); IR ($CHCl_3$): 3257, 3062, 2923, 2852, 1650, 1596, 1494, 1438, 1377, 1244, 752 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.29 (br, s, 1H, NH), 7.50–7.25 (m, 15H, Ar–Hs), 5.30 (d, 1H, J=8.1 Hz, C3H), 4.55 (dd, 1H, J_{gem}=10.1 & J=6.2 Hz, C5Ha), 4.39 (dd, 1H, J_{gem}=10.1 & J=8.4 Hz, C5Hb), 3.38 (unresolved dd, J~8.1 & 5.7 C4H); ^{13}C NMR ($CDCl_3$, 75 MHz): 157.4 (q), 152.8 (CH), 150.4 (q), 139.1 (C7′a), 129.2 (C3′a), 128.7 (CH), 125.6 (CH), 124.0 (C2′), 123.4 (CH), 122.7 (C5′), 122.4 (C6′), 121.8 (C4′), 115.1 (CH), 114.2 (C3′), 111.7 (C7′), 89.4 (C5), 68.4 (C3), 49.4 (C4). MS (ESI) *m*/*z*: 341 $[M]^+$; Anal. Calcd. for $C_{22}H_{19}N_3O$: C 77.40; H 5.61; N 12.31. Found: C 77.43; H 5.64; N 12.35.

4.3.3. *Anti-1-[3-(1H-indol-3′-yl)-4-methyl-2-phenyl-isoxazolidin-4-yl]-ethanone* ***(18e)***

Brownish viscous oil (400 mg, 80%); IR ($CHCl_3$): 3343, 3292, 2927, 2856, 2362, 1713, 1653, 1532, 1489, 1429, 1362, 1244, 746 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.16 (br, s, 1H, NH), 7.79 (d, 1H, J=3.0 Hz, Ar-H), 7.37–7.00 (m, 9H, Ar–Hs), 5.06 (d, 1H, J=5.4 Hz, C3H), 4.46 (dd, 1H, J_{gem}=10.6 & J=3.4 Hz, C5Ha), 4.29 (dd, 1H, J_{gem}=10.6 & J=8.6 Hz, C5Hb), 3.85 (unresolved dd, J~6.9 & 3.4 C4H), 2.16 (s, 1H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): 204.4 (C=O),

150.2 (q), 136.9 (C7′a), 129.0 (C3′a), 128.6 (CH), 125.3 (CH), 122.7 (C2′), 122.6 (C5′), 120.9 (C4′), 119.6 (C6′), 116.1 (C3′), 115.5 (CH), 111.4 (C7′), 68.2 (C5), 66.1 (C3), 64.7 (C4), 29.6 (CH_3). MS (ESI) *m/z*: 306 $[M]^+$; Anal. Calcd. for $C_{19}H_{18}N_2O_2$: C 74.49; H 5.92; N 9.14. Found: C 74.52; H 5.94; N 9.16.

4.3.4. *2-(1 H-Indol-2′-yl)-3-(1 H-indol-3-yl)-acrylic acid ethyl ester* ***(23a)***

Brown viscous oil, yield (360 mg, 72%); IR ($CHCl_3$): 3375, 3056, 2927, 1654, 1596, 1458, 1242, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.20 (s, 1H, C8H), 8.14 (br, s, 1H, NH), 7.80 (unresolved dd, 2H, $J=7.8$ Hz, Ar–Hs), 7.52 (br, s, 1H, NH), 7.44−7.02 (m, 7H, Ar-Hs), 6.00 (d, 1H, $J=2.4$ Hz, C_2H), 4.04(q, 2H, $J=7.2$ Hz, OCH_2), 1.14 (t, 3H, $J=7.2$ Hz, OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.7 (C=O), 136.9 (C8), 136.8 (C9), 136.2 (C7′a), 135.9 (C7a), 128.1 (C3′a), 127.0(C3a), 124.6 (C2′), 124.4 (C2), 122.8 (C4′), 121.7 (C4), 119.6 (C5), 118.7 (C5′), 117.8 (C6′), 117.4 (C6), 111.7 (C7′), 111.3 (C7), 98.0 (C3), 63.29 (–OCH_2), 13.9 (CH_3). MS (ESI) *m/z* : 330.3 $[M]^+$; Anal. Calcd. for $C_{21}H_{18}N_2O_2$: C 76.34; H 5.49; N 8.48. Found: C 76.35; H 5.54; N 8.50.

4.3.5. *Syn-5-hydroxymethyl-2-phenyl-3-(3′-pyridyl)-isoxazolidine* **(27*h*)**

Light brown viscous oil (350 mg, 70%). IR ν_{max} ($CHCl_3$): 3410, 3396, 3043, 2922, 2246, 1596, 1489, 1453.9, 1427, 1322, 1261, cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): $\delta=8.67$(br s, 1H, Ar–H), 8.51(d, 1H, $J=8.1$ Hz, Ar–H), 7.87(d, 1H, $J=8.1$ Hz, Ar–H), 7.30-7.16(m, 3H, Ar-H), 7.01−6.93(m, 3H, Ar–H), 4.81(dd, $J=8.1$ & 6.3 Hz, C3H), 4.48(dd, $J=5.4$ & 3.0 Hz, C5H), 3.75-3.66(m, 2H, CH_2OH), 3.37(br, s, 1H, OH), 2.48 (ddd, 1H, $J_{gem}=12.6$ Hz and $J=8.1$ & 5.6 Hz, C4Ha), 2.04 (ddd, 1H, $J_{gem}=12.6$ Hz and $J=5.8$ & 3.0 Hz, C4Hb); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 150.06(C2′), 148.4(C6′), 148.1(q), 138.1(C3′), 134.4(C4′), 128.8(CH), 123.7(C5′), 122.3(CH), 115.7(CH), 68.2(C3), 62.5(C5), 58.1(CH_2OH) 49.3(C4). MS (ESI) *m/z* : 256 $[M]^+$; Anal. Calcd. For $C_{15}H_{16}N_2O_2$: C 75.56; H 7.13; N 11.01. Found: C 67.24; H 5.41; N 10.99.

4.4. *Cytotoxic activity*

For the evaluation of cytotoxicity, the compounds were dissolved in DMSO and stock solutions of 2×10^4 μM were prepared. Stock solutions were further diluted with complete growth medium supplemented with 50 μg/mL gentamicin to obtain test concentrations of 10, 50 and 100 μM. Adriamycin and paclitaxel were dissolved in DMSO and stock solutions of 2×10^3 μM were prepared. Mitomycin-C was dissolved in double distilled water and a stock solution of 2×10^3 μM was prepared. All cells were maintained in RPMI-1640 medium, supplemented with fetal bovine serum (10%), 100 units/mL penicillin and 100 μg/mL streptomycin (complete medium). The cells were seeded into 96 well cell culture plates (1×10^4 cells/100 μL/well) and incubated in CO_2 incubator (37 °C, 5% CO_2, 95% relative humidity) for 24 h. After 24 h, compounds **16f**, **17a**, **17d**, **18a–d**, **19–21**, **23a–c**, **24**, **27a–e**, **29f**, **29g**, **30**, **32, 33**, and positive controls (100 μL/well) were added in quadruplets and the plates were further incubated in CO_2 incubator for 48 h. Suitable controls were also included in each experiment. After 48 h chilled trichloro acetic acid (50% *w/v*, 50 μL) was laid gently on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells. All the contents of the wells were gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloro acetic acid, growth medium, low molecular weight metabolites and serum proteins etc. The plates were air-dried. Sulphorhodamine-B (0.4% SRB in 1% acetic acid, 100 μL/well) was added to each well of the 96 well plates for 30 min. Excess of the dye was washed off using 1% acetic acid and the plates were air-dried. Tris buffer (10 mM, pH 10.5, 100 μL/well) was added to each well and plates were shaken on a mechanical stirrer for 10 min and optical density was recorded on an ELISA reader at 540 nm. Viability of cells was evaluated by trypan blue exclusion method immediately before setting up the experiment for cytotoxicity determination. Cells with >98% viability were used in the assay.

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