Asian Journal of Chemistry; Vol. 38, No. 7 (2026), 1707-1712
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2026.35560

Synthesis, Characterisation of New Indolyl-1,2,4-triazole Derivatives and their Synergistic Anticancer Activity with Adriamycin against MCF-7 Cells

KARISHMA S. KAMBLE, LALIT G. RATHI*, and NILESH A. KARANDE

Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha-442001, India

*Corresponding author: E-mail: rathilg@rediffmail.com

Received: 3 January 2026 *Accepted*: 9 May 2026 *Published online*: 3 July 2026 AJC-22396

A series of novel indolyl-1,2,4-triazole derivatives **7a-l** were synthesised through a multistep pathway starting from 2-methyl-4-substituted anilines. Initial formylation furnished the corresponding indole precursors, which were further transformed into 5-substituted indoles and subsequently *N*-alkylated to yield 1,5-disubstituted-1*H*-indoles. These intermediates were converted into the corresponding indole-3-carbonitriles, which underwent cyclocondensation with appropriate acid hydrazides to provide the target indolyltriazole frameworks. Following this, the *in vitro* cytotoxicity of the synthesised derivatives **7a-l** was assessed in breast cancer at the cellular level on the MCF-7 cell line. Some of the synthesised derivatives showed excellent to good inhibitory activity with GI_{50} < 10 to 52.9 μg/mL when compared to the standard drug Adriamycin (GI_{50} < 10 μg/mL). Significant suppression of cell growth was observed by compounds **7h-k**, which exhibited 50% inhibition of cell growth at a concentration of less than 10 μg/mL, suggesting their potential as a therapeutic agent against breast cancer. The synergistic potential of the most active compound **7k** was evaluated in combination with adriamycin and the combination exhibited significantly higher cytotoxicity against the MCF-7 cell line than compound **7k** alone.

Keywords: Indolyl-1,2,4-triazole, Anti-breast cancer, Molecular docking, Triazole, Indole, HER 2.

INTRODUCTION

Among the various heterocyclic scaffolds explored in anticancer drug design, the indole nucleus has emerged as a privileged structure due to its unique electronic configuration and ability to interact with diverse biological targets [1]. Indole derivatives, widely distributed in natural products and synthetic compounds, exhibit a broad spectrum of pharmacological activities, including potent anticancer effects. Clinically relevant examples include the natural alkaloids vincristine and vinblastine, isolated from *Catharanthus roseus*, which have long been used in chemotherapy. Furthermore, several marine-derived indole compounds, such as 6-bromoisatin and eudistomin K, have demonstrated promising cytotoxic activity against cancer cell lines [2].

The therapeutic relevance of indole-based scaffolds is further exemplified by several approved and investigational drugs. Sunitinib [3] is widely used in the treatment of renal cell carcinoma and gastrointestinal stromal tumors, while obatoclax mesylate [4] has shown potential in inducing apoptosis in hematological malignancies. Nintedanib targets multiple signaling pathways, including VEGFR, PDGFR and FGFR, and is employed in various cancers such as non-small cell lung cancer (NSCLC) [5-7]. Other significant compounds include panobinostat [8] and osimertinib [9], which have demonstrated efficacy in multiple myeloma and NSCLC, respectively. Furthermore, apaziquone [10] has been explored for bladder cancer therapy, while *N*-hydroxy indole-2-carboxylates [11] represent a novel class of inhibitors targeting human lactate dehydrogenase (hLDH5), a key enzyme involved in cancer metabolism.

In parallel, the 1,2,4-triazole ring system has attracted considerable interest due to its diverse biological activities, including antimicrobial, anti-inflammatory and anticancer properties. The incorporation of triazole moieties into drug-like molecules often enhances metabolic stability, bioavailability and binding affinity toward biological targets. Hybridisation strategies combining indole with other five-membered heterocycles have yielded compounds with enhanced anticancer profiles [12]. For instance, indolyloxazole-containing natural products such as Labradorin analogues have demonstrated significant growth inhibition against human lung cancer cell lines,

while indolyl-thiazole derivatives like camalexin have shown promising activity against breast cancer models [13,14].

Motivated by these findings and the ongoing need for improved therapeutic agents, the present study focuses on the rational design and synthesis of a novel series of indole–1,2,4-triazole hybrid derivatives. Structural modifications were systematically introduced to optimize their pharmacological potential. The synthesised compounds were subjected to comprehensive biological evaluation for their anticancer activity against the human breast cancer MCF-7 cell line. The results demonstrate that these hybrids exhibit significant cytotoxic activity, highlighting their potential as promising candidates for further development in breast cancer therapy.

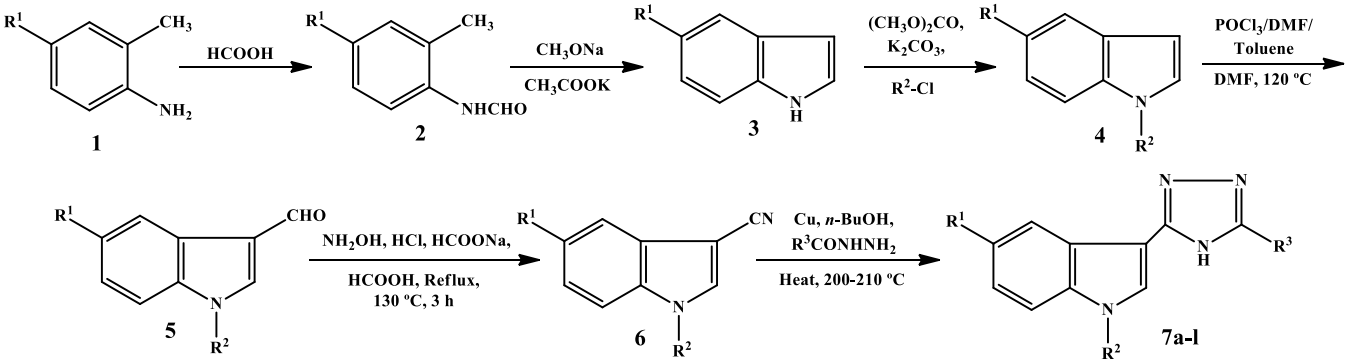
EXPERIMENTAL

All chemicals employed were procured from Sigma-Aldrich and Loba Chemie Pvt. Ltd. Differential scanning calorimetry (DSC4000, Perkin-Elmer) was used to determine the melting point. TLC plates (silica gel 60 F_{254}, Sigma Aldrich, Germany), were employed to monitor the progress of the reaction using chloroform and methanol (9.5:0.5, v/v ratio) as solvent system. The Shimadzu 8400S FT-IR spectrometer with KBr optics was used to record the infrared (IR) spectra. Bruker Avance NEO 500 MHz NMR spectrophotometer was utilised to acquire NMR spectra. The frequency used to record ^{1}H NMR and ^{13}C NMR spectra were 500 MHz and 125 MHz, respectively. Electrospray ionisation mass spectrometer (ESI-MS, Waters Corporation, U.K.) was used to obtain mass spectra.

General procedure for the synthesis of indolyl-1,2,4-triazoles (7a-l): A mixture of compound **6** (0.01 mol) and the appropriate substituted hydrazide (0.01 mol) was dissolved in *n*-butanol (10 mL) and refluxed in a round-bottom flask in the presence of a catalytic amount of copper catalyst. The reaction mixture was refluxed until completion, involving C–N bond cleavage, hydrazide insertion and subsequent triazole ring cyclisation. The reaction progress was monitored by silica gel TLC using $CHCl_3$: CH_3OH (9.5:0.5, v/v) as the mobile phase. On cooling, the reaction mixture was filtered, washed with water and purified by column chromatography, leading to the formation of **7a-l** (**Scheme-I**).

3-(4*H*-1,2,4-Triazol-3-yl)-1*H*-indole (7a): Yield: 60%; m.p.: 282-286 ºC; IR (KBr, ν_{max}, cm^{-1}): 3267 (NH), 3057 (CH-Ar), 1637 (C=C-Ar), 1550 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.13 (s, 1H, NH), 9.38 (s, 1H, NH), 8.78 (s, 1H, CH), 8.68 (s, 1H, CH), 8.17 (d, *J* = 4.55 Hz, 1H, CH), 7.55 (d, *J* = 7.05 Hz, 1H, CH), 7.28 (t, *J* = 6.4 Hz, 1H, CH), 7.14 (t, *J* = 11.85 Hz, 1H, CH); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 158.90, 157.04, 137.04, 126.41, 125.12, 121.82, 121.08, 119.18, 112.37, 106.12; MS (ESI, *m/z*): [$C_{10}H_8N_4$] calcd.: 185.20; found: 185.32.

3-(5-Phenyl-4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7b): Yield: 80%; m.p.: 203-205 ºC; IR (KBr, ν_{max}, cm^{-1}): 3259 (NH), 3005 (CH-Ar), 1638 (C=C-Ar), 1578 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.13 (s, 1H, NH), 9.98 (s, 1H, NH), 8.68 (s, 1H, CH), 8.38 (d, *J* = 5.75 Hz, 2H, 2CH), 8.26 (d, *J* = 3.5 Hz, 1H, CH), 7.57 (d, *J* = 10.81 Hz, 1H, CH), 7.53 (t, *J* = 3.1 Hz, 2H, 2CH), 7.30 (t, *J* = 11.5 Hz, 1H, CH), 7.25 (t, *J* = 1.55 Hz, 1H, CH), 7.23 (t, *J* = 7.6 Hz, 1H, CH); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 157.68, 157.63, 136.69, 132.99, 131.20, 129.99, 129.94, 128.31,128.23, 126.74, 125.28, 121.59, 121.39, 119.39, 111.99, 106.98; MS (ESI, *m/z*): [$C_{16}H_{12}N_4$] calcd.: 262.29, found: 262.40.



Compound	R^1	R^2	R^3
7a	H	H	H
7b	H	H	C_6H_5
7c	H	H	4-OHC_6H_4
7d	H	H	CH_3
7e	OCH_3	H	H
7f	OCH_3	H	C_6H_5
7g	OCH_3	H	4-OHC_6H_4
7h	OCH_3	H	CH_3
7i	H	CH_3	H
7j	H	CH_3	C_6H_5
7k	H	CH_3	4-OHC_6H_4
7l	H	CH_3	CH_3

Scheme-I: Synthesis of indolyl-1,2,4-triazole derivatives (**7a-l**)

4-(5-(1*H*-Indol-3-yl)-4*H*-1,2,4-triazol-3-yl)phenol (7c): Yield: 60%; m.p.: 239-241 ºC; IR (KBr, ν_{max}, cm^{-1}): 3300 (OH), 3203 (NH), 3018 (CH-Ar), 1643 (C=C-Ar), 1569 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.16 (s, 1H, NH), 9.82 (s, 1H, NH), 8.68 (s, 1H, CH), 8.17 (d, *J* = 1.5 Hz, 1H, CH), 7.96 (d, *J* = 4.45 Hz, 2H, 2CH), 7.86 (d, *J* = 1.75 Hz, 1H, CH), 7.55 (t, *J* = 6.15 Hz, 1H, CH), 7.25 (t, *J* = 2.55 Hz, 1H, CH), 6.85 (d, *J* = 3.2 Hz, 2H, 2CH), 5.38 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 158.11, 157.62, 156.96, 136.31, 135.20, 130.31, 130.47, 126.31, 125.68, 125.08, 121.59, 119.11, 116.26, 112.28, 106.58, 79.30; MS (ESI, *m/z*): [$C_{16}H_{12}N_4O$] calcd.: 277.10, found 277.34.

3-(5-Methyl-4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7d): Yield: 80%; m.p.: 294-296 ºC; IR (KBr, ν_{max}, cm^{-1}): 3244 (NH), 3122.64 (CH-Ar), 2849 (CH), 1634 (C=C-Ar), 1525 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.19 (s, 1H, NH), 9.98 (s, 1H, NH), 8.68 (s, 1H, CH), 8.17 (d, *J* = 6.3 Hz, 1H, CH), 7.55 (d, *J* = 5.5 Hz, 1H, CH), 7.28 (t, *J* = 6.15 Hz, 1H, CH), 7.24 (t, *J* = 8.45 Hz, 1H, CH), 2.29 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 159.81, 157.16, 136.25, 126.44, 125.18, 121.59, 121.19, 119.09, 112.53, 100.58, 13.30; MS (ESI, *m/z*): [$C_{11}H_{10}N_4$] calcd.: 199.09, found 199.73.

5-Methoxy-3-(4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7e): Yield: 58%; m.p.: 187-189 ºC; IR (KBr, ν_{max}, cm^{-1}): 3288 (NH), 3075 (CH-Ar), 2898 (CH), 1610 (C=C-Ar), 1569 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm: 10.16 (s, 1H, NH), 9.98 (s, 1H, NH), 8.88 (s, 1H, CH) 8.54 (s, 1H, CH), 7.56 (s, 1H, CH), 7.28 (d, *J* = 7.15 Hz, 1H, CH), 6.75 (d, *J* = 3.9 Hz, 1H, CH), 3.82 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 158.34, 157.31, 154.19, 129.84, 128.39, 126.74, 112.88, 112.39, 108.78, 106.93, 55.35; MS (ESI, *m/z*): [$C_{11}H_{10}N_4O$] calcd.: 215.09, found 215.81.

5-Methoxy-3-(5-phenyl-4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7f): Yield: 75%; m.p.: 196-198 ºC; IR (KBr, ν_{max}, cm^{-1}): 3272 (NH), 3071 (CH-Ar), 2846 (CH), 1610 (C=C-Ar), 1571 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.08 (s, 1H, NH), 8.98 (s, 1H, NH), 8.24 (s, 1H, CH), 8.16 (d, *J* = 5.45Hz, 2H, 2CH), 7.88 (t, *J* = 4.45 Hz, 2H, 2CH), 7.59 (d, *J* = 5.35 Hz, 1H, CH), 7.48 (s, 1H, CH), 7.45 (d, *J* = 1.25 Hz, 1H, CH), 6.78 (d, *J* = 2.35 Hz, 1H, CH), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 157.81, 157.32, 154.31, 132.58, 131.30, 130.21, 129.31, 129.22, 128.51, 126.74, 125.59, 125.28, 112.88, 112.25, 108.38, 106.28, 56.36; MS (ESI, *m/z*): [$C_{17}H_{14}N_4O$] calcd.: 292.11, found 292.47.

4-(5-(5-Methoxy-1*H*-indol-3-yl)-4*H*-1,2,4-triazol-3-yl)-phenol (7g): Yield: 70%; m.p.: 248-250 ºC; IR (KBr, ν_{max}, cm^{-1}): 3315 (OH), 3274 (NH), 3062 (CH-Ar), 2810 (CH), 1618 (C=C-Ar), 1535 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.18 (s, 1H, NH), 9.99 (s, 1H, NH), 8.86 (s, 1H, CH), 7.92 (d, *J* = 3.3 Hz, 2H, 2CH), 7.67 (s, 1H, CH), 7.45 (d, *J* = 2.15 Hz, 1H, CH), 6.69 (d, *J* = 2.7 Hz, 2H, 2CH), 6.45 (d, *J* = 10.25 Hz, 1H, CH), 5.55 (s, 1H, OH), 3.83 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 157.99, 157.39, 154.88, 135.20, 134.31, 129.99, 129.78, 129.39, 128.39, 127.84, 127.34. 125.28, 112.89, 112.48, 108.89, 106.19, 54.30; MS (ESI, *m/z*): [$C_{17}H_{14}N_4O_2$] calcd.: 308.12, found 308.85.

5-Methoxy-3-(5-methyl-4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7h): Yield: 80%; m.p.: 192-194 ºC; IR (KBr, ν_{max}, cm^{-1}): 3230 (NH), 3042 (CH-Ar), 2825 (CH), 1639 (C=C-Ar), 1552 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.19 (s, 1H, NH), 9.91 (s, 1H, NH), 8.74 (s, 1H, CH), 7.56 (s, 1H, CH), 7.23 (d, *J* = 10 Hz, 1H, CH), 6.88 (d, *J* = 5.9 Hz, 1H, CH), 3.83 (s, 3H, OCH_3), 2.83 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 159.98, 157.70, 154.88, 129.48, 128.39, 125.28, 112.69, 112.28, 108.89, 106.19, 54.36, 13.54; MS (ESI, *m/z*): [$C_{12}H_{12}N_4O$] calcd.: 229.10, found 229.25.

1-Methyl-3-(4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7i): Yield: 65%; mp:122-124 ºC; IR (KBr, ν_{max}, cm^{-1}): 3272 (NH), 3082 (CH-Ar), 2872 (CH), 1636 (C=C-Ar), 1523 (NH); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 9.95 (s, 1H, NH), 8.24 (s, 1H, CH), 8.18 (d, *J* = 11.9 Hz, 1H, CH), 7.60 (d, *J* = 11.85 Hz, 1H, CH), 7.43 (t, *J* = 2.7 Hz, 1H, CH), 7.25 (t, *J* = 2.1 Hz, 1H, CH), 7.18 (s, 1H, CH), 3.43 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) = 158.46, 157.71, 136.59, 129.31, 128.88, 121.76, 121.43, 119.82,109.8, 106.74, 34.12; MS (ESI, *m/z*): [$C_{11}H_{10}N_4$] calcd.: 199.09, found 199.65.

1-Methyl-3-(5-phenyl-4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7j): Yield: 70%; m.p.: 207-209 ºC; IR (KBr, ν_{max}, cm^{-1}): 3268 (NH), 3067 (CH-Ar), 2942 (CH), 1637 (C=C-Ar), 1527 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 9.28 (s, 1H, NH), 8.25 (d, *J* = 8.7 Hz, 1H, CH), 8.13 (d, *J* = 1.35 Hz, 2H, 2CH), 7.67 (d, *J* = 4.65 Hz, 1H, CH), 7.59 (t, *J* = 3.65 Hz, 1H, CH), 7.56 (t, *J* = 6.15 Hz, 1H, CH), 7.53 (t, *J* = 3.25 Hz, 2H, 2CH), 7.42 (d, *J* = 7.7 Hz, 1H, CH), 7.19 (s, 1H, CH), 3.64 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 157.69, 157.58, 136.52, 132.54, 131.20, 129.29, 129.17, 128.87, 127.57, 127.34, 127.19, 121.78, 121.52, 119.81, 109.63, 106.25, 34.33; MS (ESI, *m/z*): [$C_{17}H_{14}N_4$] calcd.: 275.13, found 275.63.

4-(5-(1-Methyl-1*H*-indol-3-yl)-4*H*-1,2,4-triazol-3-yl)-phenol (7k): Yield: 80%; m.p.: 227-229 ºC; IR (KBr, ν_{max}, cm^{-1}): 3382 (OH), 3288 (NH), 3025 (CH-Ar), 2854 (CH), 1612 (C=C-Ar), 1533 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 9.92 (s, 1H, NH), 8.17 (d, *J* = 6.2 Hz, 1H, CH), 7.97 (d, *J* = 2.9 Hz, 2H, 2CH), 7.49 (d, *J* = 6.55 Hz, 1H, CH), 7.45 (t, *J* = 4.25 Hz, 1H, CH), 7.43 (t, *J* = 3.7 Hz, 1H, CH), 7.19 (s, 1H, CH), 6.68 (d, *J* = 3.35 Hz, 2H, 2CH), 5.28 (s, 1H, OH), 3.76 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm: 159.49, 157.99, 157.49, 136.90, 130.99, 130.31, 129.44, 128.39, 125.28, 122.98, 122.69, 119.68, 116.89, 116.58, 109.19, 106.22, 34.40; MS (ESI, *m/z*): [$C_{17}H_{14}N_4O$] calcd.: 291.12, found 291.34.

1-Methyl-3-(5-methyl-4*H*-1,2,4-triazol-3-yl)-1*H*-indole (7l): Yield: 75%; m.p.: 287-289 ºC; IR (KBr, ν_{max}, cm^{-1}): 3274 (NH), 3009 (CH-Ar), 2889 (CH_3), 1618 (C=C-Ar), 1537 (NH); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 9.92 (s, 1H, NH), 8.21 (d, *J* = 4 Hz, 1H, CH), 7.71 (d, *J* = 5.5 Hz, 1H, CH), 7.48 (d, *J* = 2.05 Hz, 1H, CH), 7.45 (t, *J* = 3 Hz, 1H, CH), 7.19 (s, 1H, CH), 3.76 (s, 3H, NCH_3), 2.22 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 159.49, 157.59, 136.20, 129.89, 128.99, 121.84, 121.34, 119.89, 109.40, 106.89, 34.19, 13.49; MS (ESI, *m/z*): [$C_{12}H_{12}N_4$] calcd.: 213.25, found 213.56.

Biological activity

Cytotoxic and synergistic activity evaluation: The cytotoxic activity of the synthesised indolyl-1,2,4-triazole derivatives (**7a-l**) was evaluated against the human breast cancer

cell line (MCF-7) using the sulphorhodamine B (SRB) assay at concentrations ranging from 10 to 80 μg/mL [15-18]. Based on the cytotoxicity results, the most active compound was further assessed for its synergistic potential in combination with the clinically used anticancer drug adriamycin. The combination study was performed on MCF-7 cells using the SRB assay at concentrations of 10, 20, 40 and 80 μg/mL, with the test compound and adriamycin administered in a 1:1 ratio. The cytotoxic effects of the combination treatment were compared with those of the individual agents to evaluate potential synergistic interactions.

RESULTS AND DISCUSSION

The synthesis of a novel series of indolyl-1,2,4-triazole derivatives (**7a-l**) through a multistep heterocyclic synthetic route, as illustrated in **Scheme-I**, is successfully achieved. The synthetic sequence began with 2-methyl-4-substituted anilines (**1**), which were converted into the corresponding indole intermediates (**2**) according to the reported procedure [12]. Subsequent cyclisation afforded the 5-substituted-1H-indoles (**3**) [15]. These intermediates underwent *N*-alkylation to yield the corresponding 1,5-disubstituted indoles (**4**) [16], which were then transformed into 1,5-disubstituted indole-3-carboxaldehydes (**5**) [17]. Further conversion of the aldehydes produced the corresponding 1,5-disubstituted indole-3-carbonitriles (**6**), serving as key intermediates for the synthesis of the target compounds [12]. Cyclocondensation of the nitrile intermediates with appropriately substituted acid hydrazides furnished the target indolyl-1,2,4-triazole derivatives (**7a-l**).

Biological activity: The *in vitro* cytotoxicity of the synthesised compounds **7a-l** was assessed in terms of the concentration of drug causing 50% inhibition of cell growth (GI_{50}) in the breast cancer at the cellular level on the MCF-7 cell line (Table-1). Compounds **7h-k** exhibited extremely significant growth suppression ($p < 0.001$) even at the lowest tested dose (10 μg/mL) compared with the control value, producing < 30% growth. The cytotoxic activity increased dose-dependently and transitioning to negative growth values, which is indicative of strong cytotoxicity to cancerous cell at higher concentrations (Fig. 1). Their GI_{50} values (< 10 μg/mL) further support their high potency, mimicking the strong cytotoxic effects of the standard drug and reinforcing their potential as promising lead structures for further optimisation.

In contrast, compounds **7e-g** showed limited inhibitory activity, with percent control growth remaining above 70% across the concentration range and GI_{50} values exceeding 80 μg/mL, suggesting minimal anticancer potential within the tested window. Compounds **7a-d** and **7l** displayed intermediate efficacy, achieving partial inhibition at mid-to-high concentrations, but their GI_{50} values (17.5-52.9 μg/mL) indicate weaker potency compared with the leading candidates.

Across the series, compounds **7h-k** emerged as the most active compounds, exhibiting percent growth values comparable to and in some cases exceeding, the inhibitory performance of the reference drug adriamycin at equivalent concentrations. These findings emphasize the importance of specific substituent patterns in modulating biological activity. The cytotoxic impact on breast cancer cells was found to be significantly enhanced by substituents at the N-1 and C-12 regions in the indolyl-1,2,4-triazole skeleton. Methyl substitution at C-12 (compound **7h**) exhibited significant inhibition compared to unsubstituted analogs. Compound **7k**, with a methyl group at N-1 and a phenolic group on the triazole ring, showed the highest activity against MCF-7 cells. The observed variations in the anticancer activity among the synthesised indolyl-1,2,4-triazole derivatives **7a-l** indicate that structural modifications influence their biological performance. These findings provide a basis for further structural refinement and detailed mechanistic investigations to develop more potent analogues against breast cancer cells.

Synergistic effect: The synergistic potential of compound **7k** in combination with adriamycin (doxorubicin) was evaluated against breast cancer cells (Table-2 and Fig. 2). The combination exhibited enhanced cytotoxic activity compared with compound **7k** alone across the tested concentration range.

TABLE-1
In Vitro PERCENT GROWTH CONTROL AND GI_{50} VALUES OF COMPOUNDS **7a-l** AND ADRIAMYCIN IN THE MCF-7 CELL LINE

Compounds	Percent control growth on MCF-7 cell line[#]				Drug concentrations (μg/mL) causing 50% inhibition of cell growth (GI_{50})
	10 μg/mL	20 μg/mL	40 μg/mL	80 μg/mL	
Control	100	100	100	100	–
7a	94.2 ± 2.1	74.9 ± 2.3	21.9 ± 0.5*	-4.0 ± 0.2**	32.7
7b	91.5 ± 2.5	21.4 ± 4.2*	-1.4 ± 0.3**	-8.0 ± 0.5**	33.2
7c	88.7 ± 4.1	15.3 ± 0.4*	-3.5 ± 1.2**	-9.1 ± 0.4**	17.5
7d	80.1 ± 3.8	17.1 ± 4.0*	2.6 ± 0.7**	-8.8 ± 0.9**	28.5
7e	82.8 ± 2.6	80.2 ± 5.8	71.9 ± 3.6	65.8 ± 3.7	> 80
7f	70.8 ± 2.1	60.6 ± 3.6	47.9 ± 4.7	43.2 ± 2.5	52.9
7g	84.5 ± 3.8	81.6 ± 4.5	77.6 ± 3.8	71.1 ± 2.4	> 80
7h	20.3 ± 2.9*	9.7 ± 1.3**	3.8 ± 0.2**	-5.2 ± 0.3**	< 10
7i	26.0 ± 2.7*	15.1 ± 1.7*	6.9 ± 1.6**	-3.4 ± 2.8**	< 10
7j	29.6 ± 1.9	18.6 ± 1.2	9.7 ± 1.1*	-6.3 ± 0.9**	44.6
7k	18.2 ± 1.4**	7.2 ± 1.6**	-9.1 ± 0.4**	-25.4 ± 1.2**	< 10
7l	91.3 ± 1.0	54.2 ± 1.4	8.8 ± 0.1**	3.9 ± 0.6**	36.2
Standard	13.1 ± 0.7**	7.1 ± 1.2**	-3.2 ± .0.6**	-24.1 ± 1.9**	< 10

[#]Values are represented as mean ± S.D., (n = 3), Standard: Adriamycin, (–) negative value indicates higher tumor growth inhibition, $^{*}p < 0.01$, $^{**}p < 0.001$.

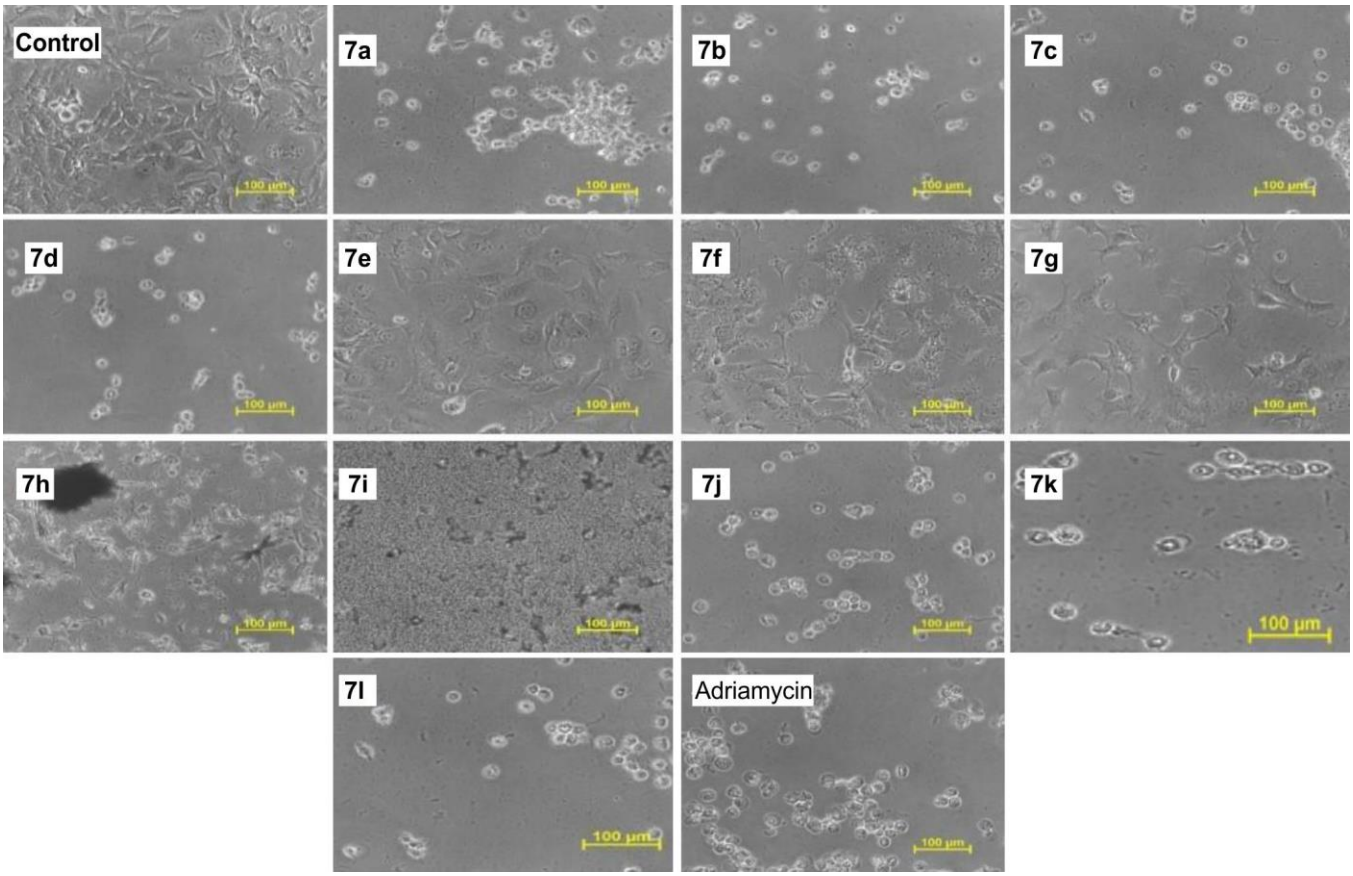


Fig. 1. Microscopic images of MCF-7 cells following treatment with compounds **7a-l** and Adriamycin

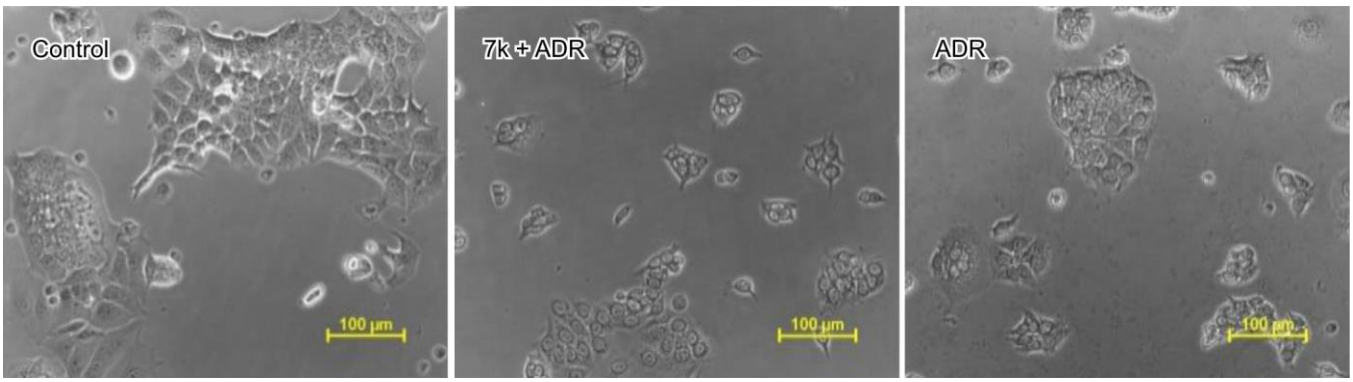


Fig. 2. Schematic illustration of the growth kinetics and tumour spheroid formation of MCF-7 cells *in vitro*

TABLE-2
PERCENT CONTROL GROWTH AND GI_{50} VALUES OF COMBINATION OF THE COMPOUND **7k** AND ADRIAMYCIN ON THE MCF-7 CELL LINE

Compounds	Human breast cancer cell line MCF-7				GI_{50}
	Control growth (%)				
	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	
7k + ADR	13.7	3.7	-27.5	-31.5	<10

At 10 and 20 µg/mL, growth inhibition values of 13.7% and 3.7%, respectively, were observed, indicating moderate anti-proliferative effects. A pronounced increase in cytotoxicity was evident at 40 µg/mL, where a growth value of -27.5% was recorded, reflecting substantial inhibition of cancer cell proliferation. Further enhancement was observed at 80 µg/mL, with a growth value of -31.5%, demonstrating a significant reduction in cell viability. These findings suggest that co-administration of compound **7k** with adriamycin may potentiate anticancer efficacy, indicating a favourable synergistic interaction and highlighting its potential for the development of improved therapeutic strategies against breast cancer.

Conclusion

A series of indolyl-1,2,4-triazole derivatives (**7a-l**) was successfully synthesised and evaluated for *in vitro* anti-breast cancer activity against the MCF-7 cell line. Among the tested compounds, derivatives **7h-k** exhibited more than 50% growth

inhibition at concentrations below 10 μg/mL, indicating promising anticancer potential. Significantly, compound **7k** demonstrated the highest activity across the broader concentration range of 20-80 μg/mL, suggesting a more sustained and potent antiproliferative effect than the other synthesised analogues. Furthermore, the combination of compound **7k** with the standard anticancer drug adriamycin produced significantly enhanced cytotoxicity compared to compound **7k** alone at all tested concentrations. These findings indicate that compound **7k** represents a promising lead molecule and that its combination with adriamycin may offer an effective strategy for improving therapeutic outcomes in breast cancer treatment.

ACKNOWLEDGEMENTS

The corresponding author extends sincere appreciation to the All India Council for Technical Education (AICTE), New Delhi, India, for their invaluable financial support (Grant letter No. 8-177/FDC/RPS/(Rural)/POLICY-1/2021-22). The first author expresses gratitude to Rashtra Sant Tukdoji Maharaj Nagpur University, for providing RTMNU Memorial Research Fellowship 2023 (Vikas/Javak/Rathod/2023-24/737). The authors also acknowledge Tata Memorial Centre, Mumbai, India and SAIF, Chandigarh, Panjab, India, for their contribution to the research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

REFERENCES

1. D. Kumar, N.M. Kumar, M.P. Tantak, M. Ogura, E. Kusaka and T. Ito, *Bioorg. Med. Chem. Lett.*, **24**, 5170 (2015); https://doi.org/10.1016/j.bmcl.2014.09.085
2. W.M. Eldehna, G.S. Hassan, S.T. Al-Rashood, H.M. Alkahtani, A. Almehizia and G.H. Al-Ansary, *Mar. Drugs*, **18**, 190 (2020); https://doi.org/10.3390/md18040190
3. R. Kurzrock and D.J. Stewart, *Clin. Cancer Res.*, **23**, 1137 (2017); https://doi.org/10.1158/1078-0432.CCR-16-1968
4. A.M. Almehdi, S.S.M. Soliman, A.-N.A. El-Shorbagi, A.D. Westwell and R. Hamdy, *Int. J. Mol. Sci.*, **24**, 14656 (2023); https://doi.org/10.3390/ijms241914656
5. A. Jamadar, S.M. Suma, S. Mathew, T.A. Fields, D.P. Wallace, J P. Calvet and R. Rao, *Cell Death Dis.*, **12**, 947 (2021); https://doi.org/10.1038/s41419-021-04248-9
6. F. Hilberg, U. Tontsch-Grunt, A. Baum, L.A. T. Le, R.C. Doebele, S. Lieb, D. Gianni, T. Voss, P. Garin-Chesa, C. Haslinger and N. Kraut, *J. Pharmacol. Exp. Ther.*, **364**, 494 (2018); https://doi.org/10.1124/jpet.117.244129
7. M.C. Riesco-Martinez, A. Sanchez-Torre and R. Garcia-Carbonero, *Expert Opin. Investig. Drugs*, **26**, 1295 (2017); https://doi.org/10.1080/13543784.2017.1385762
8. E. Eleutherakis-Papaiakovou, N. Kanellias, E. Kastritis, E. Terpos, M. Gavriatopoulou and M.A. Dimopoulos, *J. Oncol.*, **2020**, 7131802 (2020); https://doi.org/10.1155/2020/7131802
9. L. Yi, J. Fan, R. Qian, P. Luo and J. Zhang, *Int. J. Cancer*, **145**, 284 (2019); https://doi.org/10.1002/ijc.32097
10. F. Carames Masana and T.M. de Reijke, *Expert Opin. Pharmacother.*, **18**, 1781 (2017); https://doi.org/10.1080/14656566.2017.1392510
11. A. Kryshchyshyn-Dylevych, M. Garazd, A. Karkhut, S. Polovkovych and R. Lesyk, *Synth. Commun.*, **50**, 2830 (2020); https://doi.org/10.1080/00397911.2020.1786124
12. D. Kumar, M.K. Narayanam, K.H. Chang and K. Shah, *Chem. Biol. Drug Des.*, **77**, 182 (2011); https://doi.org/10.1111/j.1747-0285.2010.01051.x
13. A. Nagarsenkar, S.K. Prajapti, S.D. Guggilapu, S. Birineni, S.S. Kotapalli, R. Ummanni and B.N. Babu, *Med. Chem. Commun.*, **7**, 646 (2016); https://doi.org/10.1039/C5MD00513B
14. M.R. Mohamed, M.E. Shoman, T.F. Ali and G.F. Abuo-Rahma, *Lett. Drug Des Discov.*, **21**, 3332 (2024); https://doi.org/10.2174/0115701808288928240226104445
15. V. Vichai and K. Kirtikara, *Nat. Protoc.*, **1**, 1112 (2006); https://doi.org/10.1038/nprot.2006.179
16. J. Kode, J. Kovvuri, B. Nagaraju, S. Jadhav, M. Barkume, S. Sen, N.K. Kasinathan, P. Chaudhari, B.S. Mohanty, J. Gour and D.K. Sigalapalli, *Bioorg. Chem.*, **105**, 104447 (2020); https://doi.org/10.1016/j.bioorg.2020.104447
17. F. Kholiya, S. Chatterjee, G. Bhojani, M. Barkume, N.K. Kasinathan, S. Sen, J. Kode and R. Meena, *Carbohydr. Polym.*, **240**, 116282 (2020); https://doi.org/10.1016/j.carbpol.2020.116282
18. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney and M.R. Boyd, *J. Natl. Cancer Inst.*, **82**, 1107 (1990); https://doi.org/10.1093/jnci/82.13.1107