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RESEARCH ARTICLE

A novel series of substituted 1,2,3-triazoles as cancer stem cell inhibitors: Synthesis and biological evaluation

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Abstract

An alarming increase in global death toll resulting from cancer incidents, particularly due to multidrug resistance and reduced efficacy as a consequence of target mutations, has compelled us to look for novel anticancer agents. Cancer stem cells (CSCs), contributing majorly to the chemoresistance and tumor relapse, seem to the main culprits. In the present investigation, new chemical entities (NCEs) belonging to four novel chemical series (**A**: 4′-allyl-2′-methoxyphenoxymethyl-1,-2,3-triazoles; **B**: 4′-acetamidophenoxymethyl-1,2,3-triazoles; **C**: naphthalene-1′-yloxymethyl-1,2,3-triazoles, and **D**: naphthalene-2′-yloxymethyl-1,2,3-triazoles) were synthesized via Copper (I)-catalyzed alkyne-azide cycloaddition reaction and evaluated for *in vitro* anticancer activity. A total of 30 NCEs (**39–68**) were screened at 10 μM concentration in cell viability assay against cancer cell lines such as breast (MDA-MB-231), prostate (PC-3), glioma (U87 MG), along with cervical (SiHa) and lung (A549). The NCEs from Series C (**56–60**) and D (**61–68**) were more potent than those in Series A (**39-45**) and Series B (**46–55**) at the tested concentration. Furthermore, NCEs with >80% inhibition at 10 μM were evaluated for dose response. A total of five NCEs, **48**, **56**, **61**, **65** and **66**, were further assessed in soft-agar assay and found to be relatively potent (IC_{50} < 10 μM). Finally, the hits were screened in sphere assay to identify potential CSC inhibitors against mammospheres (MDA-MB-231) and prostatospheres (PC-3). More so, the hits were also evaluated to understand in vitro cytotoxicity against normal cells using mouse embryonic fibroblast cell line (NIH/3T3) and human peripheral blood mononuclear cells (*h*PBMCs). Overall, hits **56** and **61** exhibited potent anticancer as well as CSC inhibitory activities with notably less toxicity toward NIH/3T3 and *h*PBMCs. On the whole, our arduous study led to the identification of potential hits with anticancer and CSC inhibitory activities, with minimal or no toxicity to normal cells.

KEYWORDS

1,2,3-triazoles, breast cancer, cancer stem cells, click chemistry, CSCs, prostate cancer, soft-agar assay, sphere assay

1 | INTRODUCTION

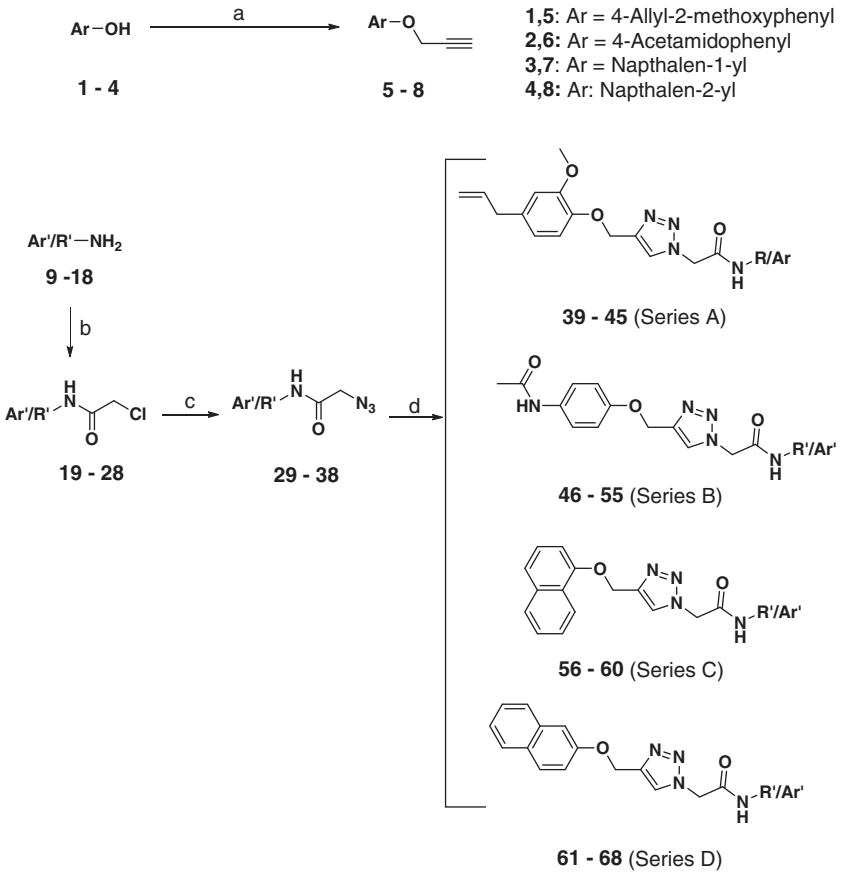
Cancer, a group of heterogeneous diseases, is characterized by uncontrolled growth of supposedly abnormal cells having the potential to invade and migrate to nearby tissues or other body parts culminating into metastasis. The battle to conquer cancer is one of the prime challenges faced by modern medicine. Some forms of cancer are better treated due to the availability of enhanced diagnostics tools. Recent chemotherapeutic regimens, including hormonal therapy along with adjuvant therapies like radiation and surgical proficiency, have shown remarkable success against primary tumors. Despite these, the battle against the swiftly growing disease is yet to be won. In spite of using the costliest drug or treatment available in the market, the patients' suffering is nowhere near the end. The horrifying statistics of 2018 proves the urgency of the matter. In 2018 alone, 17 million new cases were registered worldwide while the death toll due to various cancer was up to 9.6 million (Cancer Research UK, 2020). More so, 62% increase in incidence rate projected for the next decade is a significant cause of concern for the developed, developing and underdeveloped nations.

The two primary concerns confound our ability to combat this dreadful disease. Despite the availability of advanced treatment, resistance to recent line of chemotherapeutics agents has resulted in an increased incidence of therapy failure. However, the primary nemesis remains the phenomenon of metastasis and tumor recurrence leading to reduced survival rate. Drug resistance has majorly been attributed to the genetic and biochemical mechanisms including mutations in the target proteins. Thus, the target identification technique has become one of the recent trends in drug discovery for cancer chemotherapy (Hati et al., 2016; Kumar et al., 2017). However, recent awareness of intratumoral heterogeneity has piqued the interest to understand resistance, recurrence and relapse better. It is now known that a tumor consists of a complex ecosystem of heterogeneous cell mass that controls the function of the tumor as a whole. Majority of this cell mass has limited self-renewal capacity; however, a small subpopulation of these heterogeneous cell mass, known as cancer stem cells (CSCs) or cancer-initiating cells (CICs), has been the focus of cancer research in last few decades (Kharkar, 2017). These CICs are capable of self-renewal and have multilineage differentiation potential to initiate and sustain tumor growth. Having exceptional properties such as slow cell cycle, high expression of drug-resistant transporters, increased ability to efflux cytotoxic agents, high DNA repair capacity, and so on; CSCs are inherently resistant to recent line of chemo/radio therapies. Being less susceptible compared to the bulk tumor, CSCs are the prime reason for conventional treatment failure and increased incidence of relapse. The extensive ongoing research provides a robust platform on hallmarks of CSCs that differentiates it from normal stem cells and other normal cells of the body. These unique features of CSCs can be used as a leverage to selectively inhibiting CSCs while preserving the functionality of normal stem cells, thus, making it an essential target in anticancer therapy. Specifically targeting CSCs would attenuate their ability to survive conventional chemotherapy resulting in relapse. Due to the unique nature of these CSCs, exterminating the evil at its root will possibly help us in winning the battle against cancer.

In light of the role of CSCs in metastasis and relapse, many groups throughout the research fraternity worldwide (including the authors') are working on developing anti-CSC therapeutics (Yadav et al, 2019; Gavade et al., 2020). Table S1 lists few promising clinical candidates (alone or in combination) at various stages of development. The search for an effective anti-CSC agent continues. In the author's lab, while working on a lead from 2-azetidinones as potential anti-CSC agents, we tested a focussed library series of structurally diverse molecules having chloroacetamide warhead with potential anti-CSC activities (Padhariya, Athavale, Srivastava, & Kharkar, 2020). The positive result in breast and prostate CSCs motivated us further to modify the molecules to gain the upper hand over the activity given the nature of the warheads.

Continuing with our efforts to design more potent anti-CSC agents, we adopted few strategies to speed up the process of designing and constructing a diverse library of new chemical entities (NCEs). Designing of molecules containing two or more pharmacophoric features or privileged substructures is an important strategy in drug design and development. More so, the inclusion of heterocyclic pharmacophore linked with a promising framework could overall improve the efficacy of the series. The best way forward was to use click chemistry. Sharpless et al. defined click chemistry as "the regiospecific reactions that can be performed easily to yield pure compounds with less efforts" (Kolb, Finn, & Sharpless, 2001). The most widely used click chemistry reaction is the Cooper (I)-catalyzed azide alkyne cycloaddition reaction to obtain 1,4-disubstituted 1,2,3-triazole ring from a terminal alkyne and an azide (He, Xie, Tang, Li, & R Chen, 2012). The 1,2,3-triazole ring is an attractive pharmacophore for medicinal chemists, due to its stability to extreme oxidative or reducing conditions and to metabolic degradations (Dheer, Singh, & Shankar, 2017). Naturally, a variety of molecules bearing this *N*-containing heterocyclic ring, including anticancer agents, were synthesized by click chemistry as key step (Sahu, Purushothaman, Thiruvenkatam, & Kharkar, 2019). The triazole ring is reported to enhance anticancer activity due to its noncovalent interactions, namely, hydrophobic interactions, dipole–dipole interactions, van der Waals interaction, H-bonding, and so on with the putative biological target (Xu, Zhao, & Liu, 2019).

Intrigued by the results of 1,2,3-triazole ring containing moieties, a new series was conceptualized to target the CSCs. Based on our knowledge and previous experience with the chloroacetamide derivatives, we designed molecules belonging to four different Series **A**, **B**, **C**, and **D** (Scheme 1). In Series **A**, eugenol was attempted to explore potential anti-CSC activity. Eugenol is a primary constituent of the essential oil clove and is traditionally used to relieve mild pain, to reduce sepsis as well as an antibacterial agent (Ghosh et al., 2005). Derivatives of eugenol and combination of eugenol with a chemotherapeutic agent have been reported to suppress malignant cell lines (Bezerra, Militão, De Morais, & De Sousa, 2017;



SCHEME 1 General scheme for synthesis substituted 1,2,3-triazole derivatives (**39–68**). Reagents and conditions: a, propargyl chloride, K_2CO_3, DMF, RT; b, chloroacetyl chloride, TEA, THF 0°C to RT; c, NaN_3, DMF, RT; d, **5**–**8**, $Cu(OAc)_2.H_2O$, t-BuOH:H_2O (3:1). TEA, triethylamine; THF, tetrahydrofuran

Carrasco et al., 2008; Hussain et al., 2011; Pisano et al., 2007). Islam et al. reported the increase in anti-CSCs activity of cisplatin in presence of eugenol in breast CSCs (Islam et al., 2018). Series **B** involved 4-acetylaminophenoxymethyl derivatives linked to the 1,2,3-triazole moiety. Acetaminophen or paracetamol is reported to enhance antiproliferative and apoptosis-inducing potential in combination with anticancer agents in primary cancer cells (Bundscherer, Malsy, Gruber, Graf, & Sinner, 2018; Posadas, Santos, & Ceña, 2012). More so, it is also reported to suppress CSCs in human breast carcinoma cell line MDA-MB-231 (Takehara, Hoshino, Namba, Yamakawa, & Mizushima, 2011). In both Series **C** and **D**, we incorporated α- and β-naphthol core structures, respectively, based on our earlier findings (Sahu et al., 2019) and the reported literature of naphthol derivatives as anticancer agents (Abdelrahim, Mohan, Albarrati, Makeen, & Safhi, 2016; Das, Reddy, Kashanna, Mamidyala, & Kumar, 2012; Osowole, Kempe, Schobert, & Effenberger, 2011).

Thus, based on reported cytotoxicity of acetaminophen, naphthols, eugenol, and our in-house results of chloroacetamide derivatives as well as molecules with 1,2,3-triazoles moiety; it encouraged and prompted us to explore and screen the designed 1,2,3-triazole hybrid molecules with aforementioned substructures for their potential anti-cancer and anti-CSC activities. Herein, we report NCEs containing 1,2,3-triazole derivatives exhibiting comparative anti-CSC activity against breast and prostate cancer.

2 | MATERIAL AND METHODS

2.1 | Chemistry

A straight forward route to synthesize 1,2,3-triazole derivatives was adapted using click chemistry, a copper-catalyzed addition reaction between, prop-2-ynyl derivatives and the 2-azido-*N*-substituted acetamide derivatives (Scheme 1).

2.1.1 | General

All reagents or materials procured were of the highest commercially available purity from commercial sources such as Sigma-Aldrich (Steinheim, Germany), Spectrochem (Mumbai, India), Alfa Aesar (Karlsruhe, Germany), or Merck (Darmstadt, Germany) and were used without any further purification unless explicitly mentioned. Solvents procured commercially were dried as per reported procedures prior to use. The reactions were performed under inert (N_2) atmosphere and progress was monitored by thin-layer chromatography (aluminium plate coated with silica gel 60F254; Merck Millipore, Billerica, MA) and visualized under short ultraviolet light (λ 254). All intermediates were purified by recrystalization using appropriate solvents and final compounds were purified by performing column chromatography using silica

gel 60 (#230–400) using a mixture of 5% methanol in dichloromethane as eluent. Melting points of the final compounds were determined on Veego VMP DS (General Trading Co., Nagpur, India) and are uncorrected. The ATR spectra were recorded using Fourier transform infrared with UATR on Perkin Elmer spectrum 2 instrument (Waltham, MA). The purity of the compounds (≥95%) was determined using Agilent 1220 Infinity HPLC system (Santa Clara, CA), using a C_{18} Kromasil® column (25 cm × 4.6 mm, 5 μm, 100 Å pore size) using isocratic mobile phase (A) acetonitrile and (B) water (80:20, vol/vol) with flow rate of 1 ml/min. For NMR spectra, DMSO-d_6 was used as solvent and tetramethylsilane as an internal standard. ^{1}H-NMR spectra were recorded on Bruker Advance 400 (400 MHz) spectrometer; ^{13}C-NMR spectra were obtained on Bruker Advance 400 (100 MHz) instrument with chemical shifts expressed in δ (ppm). Mass spectra (MS) were recorded on a Shimadzu 8040 LC–MS/MS system (Japan), using electrospray ionization (ESI) mode or other ionization mode, as stated explicitly and high-resolution mass spectra (HRMS) were recorded on Bruker microTOF QII high-resolution mass spectrophotometer using ESI mode.

2.1.2 | General procedure for the syntheses of prop-2-ynyl derivatives (5–8)

To the stirred solution of substituted phenols **1–4** (10 mmol) in dry DMF (20 ml), anhydrous K_2CO_3 (30 mmol) was added along with catalytic amount of KI, and the mixture was stirred further for 30 min at RT. To the stirred solution, propargyl chloride (15 mmol) was added and reaction was stirred for additional 12 hr. The reaction was monitored by TLC using 7:3 petroleum ether/EtOAc as the mobile phase. After completion, the reaction was quenched with crushed ice (10 g). The solid was filtered followed by recrystallization from absolute ethanol. Yield: 70–80%.

2.1.3 | General procedure for the syntheses of chloroacetamide derivatives (19–28)

To the stirred solution of substituted amines **9–18** (1 mmol) and triethylamine (TEA) (1.5–3 mmol) in anhydrous tetrahydrofuran (THF) (10 ml) on ice-bath, cold solution of chloroacetyl chloride (1.5 mmol) in anhydrous THF (5 ml) was supplemented dropwise. The temperature was maintained below 5°C throughout addition. The resulting solution was stirred further for 12–15 hr at room temperature. After completion of reaction, as monitored by TLC, triethylammonium chloride was filtered off and the filtrate was concentrated under reduced pressure and quenched with ice-cold water. The solid precipitated was filtered at suction and washed to neutral filtrate using cold water. The crude was then quickly purified by column chromatography using 20–30% EtOAc/petroleum ether as eluent. Yield: 80–90%.

2.1.4 | General procedure for the syntheses of 2-azido-*N*-substituted acetamide derivatives (29–38)

To the stirred solution of choroacetamide derivatives **19–28** (10 mmol) in anhydrous DMF, sodium azide (25 mmol) dissolved in minimum DMF, was carefully transferred and the reaction was continued to stir overnight at RT. Upon reaction completion as indicated by TLC, cold brine solution was added to the reaction and the crude obtained was filtered at suction pump and washed with cold water. Yield 80–90%.

2.1.5 | General procedure for the syntheses of 1,2,3-triazole derivatives (39–68)

To the mixture of *t*-butanol: water (3:1, 5–10 mL), 2-azido-*N*-substituted acetamide derivative **29–38** (1 equiv.) was dissolved and stirred for 30 mins (Sahu et al., 2019). To the above stirred solution, prop-2-ynyl derivative **5–8** (1 equiv.) and $Cu(OAc)_2$ (20 mol%) were added and stirred further for about 8 hr at RT with continuous TLC monitoring using 5% methanol in dichloromethane as mobile phase. The reaction was then quenched using crushed ice and diluted with cold brine solution. Using separating funnel, the diluted mixture was extracted twice with ethylacetate (30 ml). The organic layers were collected and then dried by passing through the bed of anhydrous sodium sulphate and further concentrated in vacuo to yield the corresponding crude compounds **39–68**. The compounds were then purified by passing them through silica column using mixture of 5% MeOH in DCM as eluent.

*Synthesis of 2-(4-((4-allyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-chloro-phenyl)acetamide (**39**)*

The title compound was synthesized from 2-azido-*N*-(4-chlorophenyl) acetamide (0.3 g, 1.485 mmol), 4-allyl-2-methoxy-1-prop-2-ynyloxybenzene (0.31 g, 1.485 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **39** as white solid. Yield: 76.89%; TLC R_f = 0.43 (DCM:MeOH, 95:5); mp: 251–253°C; purity (HPLC): >99%; ATR cm^{-1} 3,267.35 (amide N—H, str), 3,192.89 (Ar═C—H, str), 1,694.25 (amide —C═O, str), 1,549.43 (Ar—C═C, str), 1,400.58 (N═N, str), 1,254.64 (Ar—O—CH_2, str), 854.14 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.62 (s, 1H), 8.22 (s, 1H), 7.64–7.56 (m, 2H), 7.42–7.34 (m, 2H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.68 (dd, *J* = 8.2 and 2.0 Hz, 1H), 5.94 (ddt, *J* = 16.8, 10.0, and 6.7 Hz, 1H), 5.35 (s, 2H), 5.11–4.97 (m, 4H), 3.71 (s, 3H), 3.29 (d, *J* = 6.9 Hz, 3H); MS (ESI +ve): *m/z* 413.0 $[M + H]^+$, 435.0 $[M + Na]^+$.

*Synthesis of 2-(4-((4-allyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-fluoro-phenyl)acetamide (**40**)*

The title compound was synthesized from 2-azido-*N*-(4-fluorophenyl) acetamide (0.3 g, 1.546 mmol), 4-allyl-2-methoxy-1-prop-2-ynyloxybenzene (0.31 g, 1.546 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **40** as off-white solid.

Yield: 71.67%; TLC R_f = 0.34 (DCM:MeOH, 95:5); mp: 246–248°C; purity (HPLC): >99%; ATR cm^{-1}3,285.39 (amide N—H, str), 3,136.05 (Ar═C—H, str), 1,682.42 (amide —C═O, str), 1,508.31 (Ar—C═C, str), 1,400.58 (N═N, str), 1,215.88 (Ar—O—CH_2, str), 833.20 (C—F, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.54 (s, 1H), 8.22 (s, 1H), 7.60 (dd, *J* = 8.8 and 5.0 Hz, 2H), 7.17 (t, *J* = 8.7 Hz, 2H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.69 (dd, *J* = 8.2 and 1.9 Hz, 1H), 5.94 (ddt, *J* = 17.0, 10.1, and 6.8 Hz, 1H), 5.34 (s, 2H), 5.11–4.98 (m, 4H), 3.72 (s, 3H), 3.29 (d, *J* = 6.7 Hz, 2H); MS (ESI +ve): *m/z* 397 $[M + H]^+$, 419 $[M + Na]^+$.

*Synthesis of 2-(4-((4-allyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(3,4-dichloro-phenyl)acetamide (**41**)*

The title compound was synthesized from 2-azido-*N*-(3,-4-dichlorophenyl)acetamide (0.4 g, 1.63 mmol), 4-allyl-2-methoxy-1-prop-2-ynyloxybenzene (0.33 g, 1.63 mmol), and $Cu(OAc)_2$ (20 mol %) according to the general procedure 2.1.5 to yield **41** as off-white solid. Yield: 21.23%; TLC R_f = 0.39 (DCM:MeOH, 95:5); mp: 223–225°C; purity (HPLC): >98%; ATR cm^{-1} 3,337.67 (amide N—H, str), 3,144.27 (Ar C═C, str), 1,688.60 (NH—C═O, str), 1,511.89 (C═C, str), 1,481.38 (N═N, str), 1,260.12 (Ar—O—CH_2, str), 542.86 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm δ 10.78 (s, 1H), 8.22 (s, 1H), 7.95 (d, *J* = 2.4 Hz, 1H), 7.60 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.47 (dt, *J* = 8.9, 2.4 Hz, 1H), 7.05 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.79 (d, *J* = 2.1 Hz, 1H), 6.69 (dd, *J* = 8.2, 2.2 Hz, 1H), 5.93 (dtd, *J* = 17.2, 6.8, 3.8 Hz, 1H), 5.37 (d, *J* = 2.4 Hz, 2H), 5.12–4.98 (m, 4H), 3.72 (d, *J* = 2.4 Hz, 3H), 3.29 (d, *J* = 6.8 Hz, 2H); MS (ESI +ve): *m/z* 447 $[M + H]^+$, 469 $[M + Na]^+$.

*Synthesis of 2-(4-((4-allyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-hydroxy-5-methylphenyl)acetamide (**42**)*

The title compound was synthesized from 2-azido-*N*-(2-hydroxy-5-methylphenyl)acetamide (0.3 g, 1.456 mmol), 4-allyl-2-methoxy-1-prop-2-ynyloxybenzene (0.29 g, 1.456 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **42** as off-white solid. Yield: 86.13%; TLC R_f = 0.41 (DCM:MeOH, 95:5); mp: 178–180°C; purity (HPLC): >98%; ATR cm^{-1} 3,274.38 (amide N—H, str), 3,274.38 (O—H, str), 2,974.73 (Ar C═C, str), 1,677.01 (NH—C═O, str), 1,511.84 (C═C, str), 1,419.40 (N═N, str), 1,260.65 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 9.68 (s, 2H), 8.19 (d, *J* = 1.7 Hz, 1H), 7.65 (s, 1H), 7.05 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.77 (dd, *J* = 13.6, 2.1 Hz, 3H), 6.68 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.93 (dddd, *J* = 13.2, 10.0, 8.1, 5.8 Hz, 1H), 5.41 (s, 2H), 5.11–4.98 (m, 4H), 3.72 (m, 3H), 3.36–3.26 (m, 2H), 2.15 (s, 3H); MS (ESI +ve): *m/z* 409 $[M + H]^+$, 431$[M + H]^+$.

*Synthesis of 2-[4-(4-allyl-2-methoxy-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-indan-5-yl-acetamide (**43**)*

The title compound was synthesized from 2-azido-*N*-(2,3-dihydro-1H-indan-5-yl)acetamide (0.3 g, 1.387 mmol), 4-allyl-2-methoxy-1-prop-2-ynyloxybenzene (0.25 g, 1.387 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **43** as off-white solid. Yield: 76.56%; TLC R_f = 0.31 (DCM:MeOH, 95:5); mp: 202–204°C; purity (HPLC): >99%; ATR cm^{-1} 3,282.05 (amide N—H, str), 2,944.66 (Ar═C—H, str), 1,672.08 (NH—C═O, str), 1,514.85 (Ar—C═C, str), 1,418.04 (N═N, str), 1,233.88 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.35 (s, 1H), 8.21 (s, 1H), 7.48 (s, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.79 (s, 1H), 6.68 (d, *J* = 8.2 Hz, 1H), 5.94 (ddt, *J* = 16.9, 10.3, and 6.9 Hz, 1H), 5.31 (s, 2H), 5.05 (d, *J* = 32.0 Hz, 4H), 3.72 (s, 3H), 3.29 (d, *J* = 6.8 Hz, 2H), 2.80 (q, *J* = 8.1 Hz, 4H), 1.99 (p, *J* = 7.4 Hz, 2H); MS (ESI +ve): *m/z* 419 $[M + H]^+$, 441$[M + Na]^+$.

*Synthesis of 2-(4-((4-allyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-(4-bromo-phenyl)propan-2-yl)acetamide (**44**)*

The title compound was synthesized from 2-azido-*N*-[1-(4-bromophenyl)-1-methylethyl]acetamide (0.4 g, 1.352 mmol), 4-allyl-2-methoxy-1-prop-2-ynyloxybenzene (0.27 g, 1.352 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **44** as off-white solid. Yield: 78.26%; TLC R_f = 0.35 (DCM: MeOH, 95:5); mp: 197–199°C; purity (HPLC): >98%; ATR cm^{-1} 3,310.17 (amide N—H, str), 2,974.73 (Ar═C—H, str), 1,693.51 (NH—C═O, str), 1,508.68 (C═C, str), 1,400.58 (N═N, str), 1,260.30 (Ar—O—CH_2, str), 546.93 (C—Br, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 8.68 (s, 1H), 8.08 (s, 1H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.77 (s, 1H), 6.66 (d, *J* = 8.2 Hz, 1H), 6.00–5.85 (m, 1H), 5.13 (s, 2H), 5.10–4.97 (m, 4H), 3.69 (s, 3H), 3.28 (d, *J* = 6.8 Hz, 2H), 1.55 (s, 6H); MS (ESI +ve): *m/z* 499 $[M + H]^+$, 521 $[M + Na]^+$.

*Synthesis of 2-(4-((4-allyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-bromo-6-nitrophenyl)acetamide (**45**)*

The title compound was synthesized from 2-azido-*N*(2-bromo-6-nitrophenyl)acetamide (0.3 g, 1.007 mmol), 4-allyl-2-methoxy-1-prop-2-ynyloxybenzene (0.2 g, 1.007 mmol), and $Cu(OAc)_2$ (20 mol %) according to the general procedure 2.1.5 to yield **45** as white solid. Yield: 79.13%; TLC R_f = 0.37 (DCM:MeOH, 95:5); mp: 184–186°C; purity (HPLC): >98%; ATR cm^{-1} 3,214.64 (amide N—H, str), 2,944.66 (Ar C═C, str), 1,682.83 (NH—C═O, str), 1,512.33 (C═C, str), 1,459.23 (N═N, str), 1,258.84 (Ar—O—CH_2, str), 794.83 (C—Br, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.78 (s, 1H), 8.17 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.99 (d, *J* = 8.2 Hz, 1H), 7.48 (t, *J* = 8.1 Hz, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 6.78 (d, *J* = 1.9 Hz, 1H), 6.67 (d, *J* = 8.3 Hz, 1H), 5.93 (ddt, *J* = 17.0, 10.3, 6.8 Hz, 1H), 5.42 (s, 2H), 5.04 (d, *J* = 29.9 Hz, 4H), 3.70 (d, *J* = 1.6 Hz, 3H), 3.28 (d, *J* = 6.9 Hz, 2H); MS (ESI +ve): *m/z* 502 $[M + H]^+$, 524 $[M + Na]^+$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-chlorophenyl)- acetamide (**46**)*

The title compound was synthesized from 2-azido-*N*-(4-chlorophenyl)- acetamide (0.1 g, 0.476 mmol), *N*-(4-prop-2-ynyloxyphenyl)acetamide (0.09 g, 0.476 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **46** as white solid. Yield: 71.28%; TLC R_f = 0.43 (DCM:MeOH, 95:5); mp: 240–242°C; purity (HPLC): >99%; ATR cm^{-1} 3,322.83 (amide N—H, str), 2,953.92 (C—H str), 1,677.28, 1,655.28 (NH—C═O, str), 1,510.58 (C═C, str), 1,402.45 (N═N, str), 1,224.21 (Ar—O—CH_2, str), 830.32

(C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.75 (s, 1H), 9.75 (s, 1H), 8.25 (s, 1H), 7.6 (d, 2H), 7.4 (dtd, 4H), 6.9 (d, 2H), 5.3 (s, 2H), 5.1 (s, 2H), 2.0 (s, 3H); MS (ESI –ve): *m/z* 398.0 $[M-H]^-$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-fluorophenyl)-acetamide (**47**)*

The title compound was synthesized from 2-azido-*N*-(4-fluorophenyl)-acetamide (0.1 g, 0.515 mmol), *N*-(4-prop-2-ynyloxyphenyl)acetamide (97 mg, 0.515 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **47** as white solid. Yield: 82.91%; TLC R_f = 0.26 (DCM:MeOH, 95:5); mp: 233–235°C; purity (HPLC): >99%; ATR cm^{-1} 3,279.04 (amide N—H, str), 3,091.79 (Ar═C—H str), 1,679.23, 1,656.99 (amide —C═O, str), 1,513.76 (Ar C═C, str), 1,412.19 (N═N, str), 1,230.18 (Ar—O—CH_2, str), 511.00 (C—F, str); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 9.79 (s, 1H), 8.23 (s, 1H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.51–7.44 (m, 2H), 7.17 (t, *J* = 7.9 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 2H), 5.33 (s, 2H), 5.11 (s, 2H), 1.99 (s, 3H); MS (ESI + ve): *m/z* 384.0 $[M + H]^+$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(3,4-dichloro-phenyl)acetamide (**48**)*

The title compound was synthesized from 2-azido-*N*-(3,-4-dichlorophenyl)acetamide (0.3 g, 1.224 mmol), *N*-(4-prop-2-ynyloxyphenyl)acetamide (0.23 g, 1.224 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **48** as white solid. Yield: 84.83%; TLC R_f = 0.42 (DCM:MeOH, 95:5); mp: 210–212°C; purity (HPLC): >99%; ATR cm^{-1} 3,298.85 (amide N—H, str), 2,924.75 (Ar═C—H str), 1,681.87, 1,657.37 (NH—C═O, str), 1,514.82 (Ar—C═C, str), 1,474.18 (N═N, str), 1,245.33 (Ar—O—CH_2, str), 817.99 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.79 (s, 1H), 9.79 (s, 1H), 8.23 (s, 1H), 7.94 (s, 1H), 7.59 (d, *J* = 8.7 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 3H), 6.97 (d, *J* = 8.5 Hz, 2H), 5.37 (s, 2H), 5.11 (s, 2H), 2.00 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 167.719, 164.809, 153.749, 142.648, 138.438, 132.841, 131.121, 130.873, 126.177, 125.272, 120.439, 119.296, 114.640, 61.113, 52.155, 23.789; MS (ESI +ve): *m/z* 434.0 $[M + H]^+$, 456.0 $[M + Na]^+$. HRMS (ESI) calculated for $C_{19}H_{17}Cl_2N_5O_3$ $[M + Na]^+$ 456.0708, found: 456.0604.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-hydroxy-5-methylphenyl)acetamide (**49**)*

The title compound was synthesized from 2-azido-*N*-(2-hydroxy-5-methylphenyl)acetamide (0.1 g, 0.485 mmol), *N*-(4-prop-2-ynyloxyphenyl)-acetamide (91.7 mg, 0.485 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **49** as white solid. Yield: 81.21%; TLC R_f = 0.36 (DCM:MeOH, 95:5); mp: 168–170°C; purity (HPLC): >99%; ATR cm^{-1} 3,256.61 (amide N—H, str), (C—H str), 1,673.8, 1,654.12 (NH—C═O, str), 1,563.63 (C═C, str), 1,402.96 (N═N, str), 1,238.36 (Ar—O—CH_2, str), 820.02 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 9.79 (s, 1H), 9.64 (d, *J* = 10.0 Hz, 2H), 8.21 (s, 1H), 7.65 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.75 (s, 2H), 5.41 (s, 2H), 5.10 (s, 2H), 2.15 (s, 3H), 1.99 (s, 3H); MS (ESI +ve): *m/z* 394.0 $[M–H]^-$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(2,3-dihydro-1H-inden-5-yl)acetamide (**50**)*

The title compound was synthesized from 2-azido-*N*-(2-hydroxy-5-methylphenyl)acetamide (0.1 g, 0.463 mmol), *N*-(4-prop-2-ynyloxyphenyl)acetamide (87 mg, 0.463 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **50** as white solid. Yield: 85.43%; TLC R_f = 0.32 (DCM:MeOH, 95:5); mp: 192–194°C; purity (HPLC): >99%; ATR cm^{-1} 3,300.23 (amide N—H, str), 2,944.20 (Ar═C—H str), 1,674.93, 1,660.07 (amide —C═O, str), 1,515.53 (Ar —C═C, str), 1,416.99 (N═N, str), 1,249.74 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.34 (s, 1H), 9.78 (s, 1H), 8.22 (s, 1H), 7.48 (s, 3H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 2H), 5.31 (s, 2H), 5.11 (s, 2H), 2.80 (d, 4H), 1.99 (s, 5H); MS (ESI +ve): *m/z* 406.0 $[M + H]^+$, 428.0 $[M + Na]^+$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-(4-bromo-phenyl)propan-2-yl)acetamide (**51**)*

The title compound was synthesized from 2-azido-*N*-(2-(4-bromophenyl)propan-2-yl)acetamide (0.1 g, 0.336 mmol), *N*-(4-prop-2-ynyloxyphenyl)-acetamide (87 mg, 0.336 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **51** as white solid. Yield: 79.42%; TLC R_f = 0.31 (DCM:MeOH, 95:5); mp: 185–186°C; purity (HPLC): >99%; ATR cm^{-1} 3,292.58 (amide N—H, str), 3,065.53 (Ar═C—H str), 1,677.67, 1,661.94 (amide —C═O, str), 1,507.78 (Ar —C═C, str), 1,315.78 (N═N, str), 1,222.26 (Ar—O—CH_2, str), 522.71 (C—Br, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 9.77 (s, 1H), 8.68 (s, 1H), 8.08 (s, 1H), 7.45 (ddd, *J* = 7.7, 5.1, 2.6 Hz, 4H), 7.32–7.24 (d, 2H), 6.98–6.86 (d, 2H), 5.13 (dd, 4H), 1.99 (s, 3H), 1.55 (s, 6H); MS (ESI +ve): *m/z* 486 $[M + H]^+$, 510 $[M + Na]^+$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-bromo-6-nitro-phenyl)acetamide (**52**)*

The title compound was synthesized from 2-azido-*N*-(2-bromo-6-nitrophenyl)acetamide (0.1 g, 0.336 mmol), *N*-(4-prop-2-ynyloxyphenyl)acetamide (87 mg, 0.336 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **52** as white solid. Yield: 87.26%; TLC R_f = 0.33 (DCM:MeOH, 95:5); mp: 176–178°C; purity (HPLC): >99%; ATR cm^{-1} 3,254.21 (amide N-H, str), 3,078.53 (Ar═C—H str), 1,682.90, 1,661.82 (NH—C═O, str), 1,509.94 (Ar—C═C, str), 1,414.63 (N═N, str), 1,232.04 (Ar—O—CH_2, str), 512.33 (C—Br, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.78 (bs, 1H), 9.78 (s, 1H), 8.19 (s, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 8.2 Hz, 1H), 7.48 (t, *J* = 8.7 Hz, 3H), 6.96 (d, *J* = 8.5 Hz, 2H), 5.42 (s, 2H), 5.09 (s, 2H), 1.99 (s, 3H); MS (ESI –ve): *m/z* 487.0 $[M–H]^-$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(6-chloropyrazin-2-yl)acetamide (**53**)*

The title compound was synthesized from 2-azido-*N*-(6-chloropyrazin-2-yl)acetamide (0.1 g, 0.472 mmol), *N*-(4-prop-2-ynyloxyphenyl)acetamide (89 mg, 0.472 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **53** as white solid. Yield: 73.21%; TLC R_f = 0.42 (DCM:MeOH, 95:5); mp: 228–230°C; purity (HPLC): >99%; ATR cm^{-1} 3,338.74 (amide N-H,

str), 2,945.75 (Ar ═ C—H str), 1,706.91, 1,652.33 (amide -C═O, str), 1,544.12 (Ar C═C, str), 1,415.17 (N═N, str), 1,225.16 (Ar—O—CH_2, str), 834.95 (C—Cl, str); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 9.78 (s, 1H), 9.21 (s, 1H), 8.54 (s, 1H), 8.24 (s, 1H), 7.48 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 5.46 (s, 2H), 5.12 (s, 2H), 1.99 (s, 3H); MS (ESI +ve): m/z 402.0 $[M + H]^+$, 424 $[M + Na]^+$.

*Synthesis of 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(5-(trifluoro-methyl)-1,3,4-thiadiazol-2-yl)acetamide (**54**)*

The title compound was synthesized from 2-azido-*N*-(5-trifluoromethyl-1,3,4-thiadiazol-2-yl)acetamide (0.1 g, 0.386 mmol), *N*-(4-prop-2-ynyloxyphenyl)acetamide (75 mg, 0.386 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **54** as white solid. Yield: 75.36%; TLC R_f = 0.31 (DCM:MeOH, 95:5); mp: 237–240°C; purity (HPLC): >99%; ATR cm^{-1} 3,274.77 (amide N—H, str), 3,154.99 (Ar ═ C—H str), 1,679.01, 1,660.37 (amide —C═O, str), 1,511.55 (Ar C═C, str), 1,413.79 (N═N, str), 1,232.93 (Ar—O—CH_2, str), 1,045.93 (C—F, str); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.8 (bs, 1H), δ 9.78 (s, 1H), 8.25 (s, 1H), 7.48 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 5.60 (s, 2H), 5.21 (s, 2H), 1.98 (s, 3H); MS (ESI +ve): m/z 442 $[M + H]^+$, 464 $[M + Na]^+$.

*Synthesis of methyl 2-(2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)acetamido)-thiophene-3-carboxylate (**55**)*

The title compound was synthesized from methyl 2-(2-azidoacetamido)thiophene-3-carboxylate (0.1 g, 0.416 mmol), *N*-(4-prop-2-ynyloxyphenyl)-acetamide (78 mg, 0.416 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **55** as white solid. Yield: 81.23%; TLC R_f = 0.48 (DCM:MeOH, 95:5); mp: 190–192°C; purity (HPLC): >99%; ATR cm^{-1} 3,301.69 (amide N—H, str), 3,186.75 (C—H str), 1,734.66 (ester—C═O, str), 1,676.18 (NH—C═O, str), 1,512.28 (C═C, str), 1,305.28 (N═N, str), 1,237.54 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.12 (s, 1H), 9.78 (s, 1H), 8.28 (s, 1H), 7.52–7.44 (d, 2H), 7.20 (d, J = 5.7 Hz, 1H), 7.08 (d, J = 5.4 Hz, 1H), 7.01–6.94 (d, 2H), 5.68 (s, 2H), 5.13 (s, 2H), 3.83 (s, 3H) 2.00 (s, 3H); MS (ESI –ve): m/z 428.0 $[M–H]^-$.

*Synthesis of N-(4-chlorophenyl)-2-(4-((naphthalen-1-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**56**)*

The title compound was synthesized from 2-azido-*N*-(4-chlorophenyl) acetamide (0.3 g, 1.428 mmol), 1-(prop-2-yn-1-yloxy)naphthalene (0.26 g, 1.428 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **56** as white solid. Yield: 89.12%; TLC R_f = 0.31 (DCM:MeOH, 95:5); mp: 191–192°C; purity (HPLC): >98%; ATR cm^{-1} 3,262.53 (amide N—H, str), 3,131.44 (Ar ═ C—H, str), 1,665.56 (NH—C═O, str), 1,549.40 (Ar—C═C, str), 1,396.97 (N═N, str), 1,242.64 (Ar—O—CH_2, str), 827.44 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.64 (s, 1H), 8.38 (s, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.56–7.40 (m, 4H), 7.39 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 7.5 Hz, 1H), 5.39 (s, 4H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 164.398, 153.483, 142.629, 137.353, 134.031, 128.829,127.453, 127.351, 126.449, 126.210, 126.135, 125.309, 124.882, 121.491, 120.775, 120.281, 105.745, 61.607, 52.214; MS (ESI +ve): m/z 393.0 $[M + H]^+$, 415.0 $[M + Na]^+$. HRMS (ESI) calculated for $C_{21}H_{17}ClN_4O_2$ $[M + Na]^+$ 415.1040, found: 415.0946.

*Synthesis of N-(4-fluorophenyl)-2-(4-((naphthalen-1-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**57**)*

The title compound was synthesized from 2-azido-*N*-(4-fluorophenyl) acetamide (0.3 g, 1.546 mmol), 1-(prop-2-yn-1-yloxy)naphthalene (0.28 g, 1.546 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **57** as white solid. Yield: 78.94%; TLC R_f = 0.33 (DCM:MeOH, 95:5); mp: 189–191°C; purity (HPLC): >99%; ATR cm^{-1} 3,271.03 (amide N—H, str), 3,093.39 (Ar ═ C—H, str), 1,662.08 (amide -C═O, str), 1,507.36 (Ar—C═C, str), 1,338.16 (N═N, str), 1,242.76 (Ar—O—CH_2, str), 519.35 (C—F, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.55 (s, 1H), 8.37 (s, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.60 (dt, J = 7.9 and 3.8 Hz, 2H), 7.48 (tdd, J = 16.6, 13.5, 11.4, and 7.4 Hz, 4H), 7.24–7.13 (m, 3H), 5.37 (s, 4H); MS (ESI + ve): m/z 377.0 $[M + H]^+$, 399.0 $[M + Na]^+$.

*Synthesis of N-(3,4-dichlorophenyl)-2-(4-((naphthalen-1-yloxy) methyl)-1H-1,2,3-triazol-1-yl)acetamide (**58**)*

The title compound was synthesized from 2-azido-*N*-(3,-4-dichlorophenyl)acetamide (0.3 g, 1.224 mmol), 1-(prop-2-yn-1-yloxy)naphthalene (0.22 g, 1.224 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **58** as white solid. Yield: 87.13%; TLC R_f = 0.33 (DCM:MeOH, 95:5); mp: 167–169°C; purity (HPLC): >99%; ATR cm^{-1} 3,345.60 (amide N-H, str), 2,922.08 (Ar═C—H, str), 1,691.00 (amide —C═O, str), 1,594.46 (C═C, str), 1,393.07 (N═N, str), 1,239.48 (Ar—O—CH_2, str), 769.58 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.81 (s, 1H), 8.37 (s, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.96 (s, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 8.7 Hz, 1H), 7.56–7.40 (m, 5H), 7.21 (d, J = 7.4 Hz, 1H), 5.39 (d, J = 8.9 Hz, 4H); MS (ESI + ve): m/z 427.0 $[M + H]^+$, 449.0 $[M + Na]^+$.

*Synthesis of N-(2-hydroxy-5-methylphenyl)-2-(4-((naphthalen-1-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**59**)*

The title compound was synthesized from 2-azido-*N*-(2-hydroxy-5-methylphenyl)acetamide (0.3 g, 1.456 mmol), 1-(prop-2-yn-1-yloxy) naphthalene (0.26 g, 1.456 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **59** as white solid. Yield: 71.91%; TLC R_f = 0.35 (DCM:MeOH, 95:5); mp: 173–175°C; purity (HPLC): >98%; ATR cm^{-1} 3,276.34 (amide N—H, str), 3,051.64 (—OH, str), 1,677.40 (amide —C═O, str), 1,554.54 (Ar—C═C, str), 1,357.91 (N═N, str), 1,241.65 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 9.64 (s, 2H), 8.35 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.66 (s, 1H), 7.56–7.41 (m, 4H), 7.21 (d, J = 7.1 Hz, 1H), 6.76 (s, 2H), 5.45 (s, 2H), 5.37 (s, 2H), 2.16 (s, 3H); MS (ESI +ve): m/z 389.0 $[M + H]^+$, 411.0 $[M + Na]^+$.

*Synthesis of N-(2,3-dihydro-1H-inden-2-yl)-2-(4-((naphthalen-1-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**60**)*

The title compound was synthesized from 2-azido-*N*-(indan-2-yl)acetamide (0.25 g, 1.157 mmol), 1-(prop-2-yn-1-yloxy)naphthalene

(0.21 g, 1.157 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **60** as white solid. Yield: 75.46%; TLC R_f = 0.32 (DCM:MeOH, 95:5); mp: 204–206°C; purity (HPLC): >99%; ATR cm^{-1} 3,263.79 (amide N—H, str), 3,054.08 (Ar C═C, str), 2,954.68 (C—H str), 1,666.24 (NH—C═O, str), 1,539.01 (C═C, str), 1,397.77 (N═N, str), 1,271.29 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.36 (s, 1H), 8.36 (s, 1H), 8.12 (d, *J* = 8.1 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.49 (s, 4H), 7.44 (s, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.18 (dd, *J* = 21.0 and 7.7 Hz, 2H), 5.36 (d, *J* = 11.5 Hz, 4H), 2.81 (q, *J* = 8.4 Hz, 4H), 2.03–1.95 (m, 2H); MS (ESI +ve): *m/z* 399.0 $[M + H]^+$, 421.0 $[M + Na]^+$.

Synthesis of N-*(4-chlorophenyl)-2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**61**)*

The title compound was synthesized from 2-azido-*N*-(4-chlorophenyl) acetamide (0.3 g, 1.428 mmol), 2-(prop-2-yn-1-yloxy)naphthalene (0.26 g, 1.428 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **61** as white solid. Yield: 76.21%; TLC R_f = 0.35 (DCM:MeOH, 95:5); mp: 224–226°C; purity (HPLC): >99%; ATR cm^{-1} 3,261.85 (amide N—H, str), 3,081.49 (Ar ═ C—H str), 1,672.63 (amide—C═O, str), 1,550.50 (Ar—C═C, str), 1,306.28 (N═N, str), 1,258.44 (Ar—O—CH_2, str), 740.21 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.63 (s, 1H), 8.32 (s, 1H), 7.83 (d, *J* = 8.3 Hz, 3H), 7.65 (d, 2H), 7.56 (s, 1H), 7.45 (t, 1H), 7.35 (m, 3H), 7.24 (d, 1H), 5.37 (s, 2H), 5.30 (s, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 164.377, 155.926, 142.398, 137.338, 134.182, 129.348, 128.826, 128.576, 127.500, 127.360, 126.737, 126.407, 123.679, 120.775, 118.663, 107.142, 61.057, 52.197; MS (ESI +ve): *m/z* 393.0 $[M + H]^+$, 415.0 $[M + Na]^+$; HRMS (ESI) calculated for $C_{21}H_{17}ClN_4O_2$ $[M + H]^+$ 393.1040, found: 393.1104.

Synthesis of N-*(4-fluorophenyl)-2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**62**)*

The title compound was synthesized from 2-azido-*N*-(4-fluorophenyl) acetamide (0.3 g, 1.546 mmol), 2-(prop-2-yn-1-yloxy)naphthalene (0.28 g, 1.546 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **62** as white solid. Yield: 74.78%; TLC R_f = 0.36 (DCM:MeOH, 95:5); mp: 220–222°C; purity (HPLC): >98%; ATR cm^{-1} 3,272.84 (amide N—H, str), 3,222.18 (Ar ═ C—H, str), 1,668.30 (amide —C═O, str), 1,507.43 (Ar—C═C, str), 1,308.84 (N═N, str), 1,217.13 (Ar—O—CH_2, str), 475.65 (C—F, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.54 (s, 1H), 8.31 (s, 1H), 7.83 (t, *J* = 6.3 Hz, 3H), 7.60 (dd, *J* = 8.6, 4.6 Hz, 2H), 7.55–7.42 (m, 2H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.23–7.12 (m, 3H), 5.35 (d, *J* = 2.9 Hz, 2H), 5.29 (d, *J* = 3.0 Hz, 2H); MS (ESI +ve): *m/z* 377.0 $[M + H]^+$, 399.0 $[M + Na]^+$.

Synthesis of N-*(3,4-dichlorophenyl)-2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**63**)*

The title compound was synthesized from 2-azido-*N*-(3,4-dichlorophenyl)acetamide (0.3 g, 1.22 mmol), 2-(prop-2-yn-1-yloxy) naphthalene (0.22 g, 1.22 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **63** as white solid. Yield: 82.36%; TLC R_f = 0.31 (DCM:MeOH, 95:5); mp: 173–175°C; purity (HPLC): >99%; ATR cm^{-1} ATR cm^{-1} 3,307.53 (amide N—H, str), 3,273.55 (Ar═C—H, str), 1,693.82 (amide —C═O, str), 1,538.66 (Ar —C═C, str), 1,473.53 (N═N, str), 1,212.48 (Ar—O—CH_2, str), 829.91 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.79 (s, 1H), 8.31 (s, 1H), 7.95 (s, 1H), 7.83 (d, *J* = 7.9 Hz, 3H), 7.60 (dd, *J* = 8.8 and 3.0 Hz, 1H), 7.56–7.42 (m, 3H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.20 (dd, *J* = 8.9 and 3.1 Hz, 1H), 5.38 (d, *J* = 3.1 Hz, 2H), 5.29 (d, *J* = 3.2 Hz, 2H); MS (ESI +ve): *m/z* 427 $[M + H]^+$.

Synthesis of N-*(2-hydroxy-5-methylphenyl)-2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**64**)*

The title compound was synthesized from 2-azido-*N*-(2-hydroxy-5-methylphenyl)acetamide (0.3 g, 1.46 mmol), 2-(prop-2-yn-1-yloxy) naphthalene (0.26 g, 1.46 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **64** as white solid. Yield: 78.21%; TLC R_f = 0.32 (DCM:MeOH, 95:5); mp: 171–173°C; purity (HPLC): >97%; ATR cm^{-1} 3,052.49 (O—H, str), 2,922.66 (amide N—H, str), 2,852.96 (Ar ═ C—H str), 1,654.10 (amide —C═O, str), 1,560.27 (Ar—C═C, str), 1,300.67 (N═N, str), 1,255.62 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 9.64 (d, *J* = 3.8 Hz, 2H), 8.28 (s, 1H), 7.83 (dd, *J* = 9.0, 4.0 Hz, 3H),7.65 (s, 1H), 7.53 (s, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.35 (t, *J* = 7.7 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 1H), 6.75 (s, 2H), 5.43 (s, 2H), 5.28 (s, 2H), 2.15 (s, 3H); MS (ESI +ve): *m/z* 389 $[M + H]^+$, 411 $[M + Na]^+$.

Synthesis of N-*(2,3-dihydro-1H-inden-2-yl)-2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**65**)*

The title compound was synthesized from 2-azido-*N*-(2,3-dihydro-1*H*-inden-2-yl)acetamide (0.25 g, 1.157 mmol), 2-(prop-2-yn-1-yloxy) naphthalene (0.21 g, 1.157 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **65** as white solid. Yield: 84.12%; TLC R_f = 0.29 (DCM:MeOH, 95:5); mp: 208–210°C; purity (HPLC): >99%; ATR cm^{-1} 3,195.24 (amide N—H, str), 3,037.44 (Ar ═ C—H, str), 2,920.09 (C—H str), 1,662.07 (amide—C═O, str), 1,552.66 (Ar—C═C, str), 1,297.69 (N═N, str), 1,261.60 (Ar—O—CH_2, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.40 (s, 1H), 8.31 (s, 1H), 7.83 (d, *J* = 8.5 Hz, 3H), 7.56–7.42 (m, 3H), 7.33 (dd, *J* = 25.7, 8.0 Hz, 2H), 7.18 (dd, *J* = 19.8, 8.6 Hz, 2H), 5.29–5.34 (dd, 4H), 2.80 (q, *J* = 8.3 Hz, 4H), 1.98 (p, *J* = 7.5 Hz, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 163.824, 155.936, 144.294, 142.335, 139.012, 136.583, 134.187, 129.342, 128.572, 127.498, 126.734, 126.402, 126.344, 124.296, 123.671, 118.661, 117.385, 115.452, 107.127, 61.061, 52.224, 32.445, 31.758, 25.102; MS (ESI +ve): *m/z* 399 $[M + H]^+$, 421 $[M + Na]^+$. HRMS (ESI) calculated for $C_{24}H_{22}N_4O_2$ $[M + H]^+$ 399.1743, found: 399.1816.

Synthesis of N-*(2-(4-bromophenyl)propan-2-yl)-2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**66**)*

The title compound was synthesized from 2-azido-*N*-(2-(4-bromophenyl)propan-2-yl)acetamide (0.3 g, 1.013 mmol), 2-(prop-2-yn-1-yloxy)naphthalene (0.185 g, 1.013 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **66** as white solid. Yield: 76.45%; TLC R_f = 0.34 (DCM:MeOH, 95:5);

mp: 168–170°C; purity (HPLC): >99%; ATR cm^{-1} 3,303.41 (amide N—H, str), 3,085.60 (Ar C═C, str), 2,920.22 (C—H str), 1,676.57 (NH—C═O, str), 1,557.69 (C═C, str), 1,466.84 (N═N, str), 1,255.59 (Ar—O—CH_2, str), 625.83 (C—Br, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 8.69 (s, 1H), 8.17 (s, 1H), 7.85–7.77 (m, 3H), 7.53–7.41 (m, 4H), 7.39–7.25 (m, 3H), 7.18 (dd, J = 8.6, 2.8 Hz, 1H), 5.24 (s, J = 2.9 Hz, 2H), 5.16 (s, J = 3.0 Hz, 2H), 1.55 (s, J = 3.0 Hz, 6H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 164.302, 155.918, 146.622, 142.163, 134.162, 130.764, 129.308, 128.543, 127.479, 127.146, 126.707, 126.378, 126.179, 123.644, 119.128, 118.640, 107.068, 61.002, 54.924, 51.820, 28.980; MS (ESI +ve): m/z 479 $[M + H]^+$, 481 $[M + H + 2]^+$. HRMS (ESI) calculated for $C_{24}H_{23}BrN_4O_2$ $[M + Na]^+$ 501.1004, found: 501.0920.

Synthesis of N-*(2-bromo-6-nitrophenyl)-2-(4-((naphthalen-2-yloxy) methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (**67**)*

The title compound was synthesized from 2-azido-*N*-[1-(4-bromophenyl)-1-methylethyl]acetamide (0.3 g, 1.002 mmol), 2-(prop-2-yn-1-yloxy)-naphthalene (0.183 g, 1.002 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **67** as white solid. Yield: 89.56%; TLC R_f = 0.31 (DCM:MeOH, 95:5); mp: 223–225°C; purity (HPLC): >99%; ATR cm^{-1} 3,262.17 (amide N—H, str), 2,941.54 (Ar═C—H, str), 1,688.61 (NH—C═O, str), 1,513.21 (C═C, str), 1,457.21 (—NO_2, str), 1,352.95 (N═N, str), 1,252.95 (Ar—O—CH_2, str), 470.34 (C—Br, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 10.80 (s, 1H), 8.27 (d, J = 1.7 Hz, 1H), 8.13–8.06 (m, 1H), 8.02–7.95 (m, 1H), 7.82 (dd, J = 8.1, 4.8 Hz, 3H), 7.55–7.42 (m, 3H), 7.35 (t, J = 7.5 Hz, 1H), 7.19 (dt, J = 9.1, 2.1 Hz, 1H), 5.44 (s, 2H), 5.27 (s, 2H); MS (ESI +ve): m/z 482 $[M + H]^+$.

Synthesis of N-*(6-chloropyrazin-2-yl)-2-(4-((naphthalen-2-yloxy) methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (**68**)*

The title compound was synthesized from 2-azido-*N*-(6-chloropyrazin-2-yl)acetamide (0.3 g, 1.415 mmol), 2-(prop-2-yn-1-yloxy)naphthalene (0.25 g, 1.415 mmol), and $Cu(OAc)_2$ (20 mol%) according to the general procedure 2.1.5 to yield **67** as white solid. Yield: 84.39%; TLC R_f = 0.38 (DCM:MeOH, 95:5); mp: 203–205°C; purity (HPLC): >98%; ATR cm^{-1} 3,050.66 (amide N—H, str), 3,019.67 (Ar ═ C—H, str), 1,697.29 (NH—C═O, str), 1,545.58 (C═C, str), 1,459.82 (C═N in ring, str), 1,408.98 (N═N, str), 1,216.95 (Ar—O—CH_2, str), 832.974 (C—Cl, str); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.67 (s, 1H), 9.21 (s, 1H), 8.54 (d, J = 2.0 Hz, 1H), 8.32 (d, J = 2.1 Hz, 1H), 7.83 (t, J = 7.4 Hz, 3H), 7.55 (s, 1H), 7.42 (t, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.20 (dd, J = 8.9, 2.6 Hz, 1H), 5.48 (s, 2H), 5.30 (s, 2H); MS (ESI +ve): m/z 395.0 $[M + H]^+$, 417.0 $[M + Na]^+$.

2.2 | Biology

2.2.1 | Cell lines and reagents

All the cell lines: MDA-MB-231 (breast adenocarcinoma), PC-3 (prostate carcinoma), U87 MG (glioblastoma astrocytoma), SiHa (cervical carcinoma), A549 (lung adenocarcinoma) and NIH/3T3 (mouse embryonic fibroblast) were purchased from National Centre for Cell Science (Pune, India). On receipt, the cell lines were passaged in our lab and the earliest passaged cells were cryopreserved in liquid nitrogen container for future use. The cell lines used in culture were passaged for fewer than 8 weeks and were carefully maintained as described. The cells were maintained in Dulbecco's modified Eagle medium/F12 (HiMedia, Catalogue No.: AL223A) complete media with 10% fetal bovine serum (Gibco Life Technologies, Grand Island, NY) and penicillin–streptomycin (50 U/ml, 50 mg/ml; HiMedia, Catalogue No.: A002) at 37°C, CO_2 (5%) and air (95%). Around 40–50% confluency of cultured cells were used for seeding during the assays.

Other reagents used during cell line studies included fibroblast growth factor (Sigma-Aldrich, Catalogue No.: F029125), epidermal growth factor (Sigma-Aldrich, Catalogue No.: E9644), insulin (Sigma, Catalogue No.: 19278), Dulbecco's phosphate-buffered saline (HiMedia, Catalogue No.: TL1006), trypan blue (TCI 93).

2.2.2 | Cell viability assay

Cytotoxic activity of the compounds was evaluated by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on the above-mentioned cell lines using doxorubicin HCl, cisplatin and sunitinib as controls. Briefly, logarithmically growing cells were seeded in 96-well plate (seeding density: MDA-MB-231:10,000 cells/well, PC-3: 8,000 cells/well, U87 MG: 5,000 cells/well, SiHa: 10,000 cells/well, A549: 5,000 cells/well, NIH-3T3: 10,000 cells/well) and incubated for 24 hr in humidified condition (5% CO_2) at 37°C and then observed under microscope. Test compounds with appropriate dilution were then added to the wells in triplicate along with DMSO as vehicle control. After 48 hr of test compound exposure in 5% CO_2 humidified condition at 37°C, the assay plate was centrifuged twice at 3,000 rpm for 3 min and the supernatant was discarded. Later, each well was treated with 100 μl of MTT reagent (5 mg/ml) and it was further incubated for 4 hr (in 5% CO_2 atmosphere at 37°C). Following incubation, the plate was centrifuged and the supernatant was aspirated off and 200 μl of DMSO was added in each well to solubilize the formazan crystals. The absorbance was then measured at 540 and 630 nm (background scan) using EPOCH 2 Biotek microplate reader. A concentration of 10 μM of test compounds was used for initial screening in all cell lines. The ones with >80% inhibition were taken forward for IC_{50} determination (test compound concentration inhibiting 50% of the cell population). To determine the IC_{50} value of test compounds in respective cell lines, a total of six concentrations ranging from 0.01 to 100 μM in triplicate were used. The IC_{50} was then calculated by regression analysis and expressed in μM using a mean of triplicate.

2.2.3 | Soft-agar assay

Briefly, a 100 μL mixture of 1:1 dilution of 2× complete media and 1.2% Bacto™ agar were transferred onto each well of the 96-well

microtiter assay plate. The cells (10 μl with seeding efficiency: MDA-MB-231: 7,500 cells/well, and PC-3: 5,000 cells/well) were mixed with 2× medium (20 μl), 0.8% of Bacto™ agar (30 μl) and appropriate concentration of test compound (1.6 μl) in a vial and transferred onto the solidified prelayers in the plates to incubate it further for (5% CO_2, 37°C) 1 week to grow and form colonies. Midway through the week, a feeding layer consisting of 50 μl of suitable 2× medium was added. Once the incubation period was over, 16 μl of alamarBlue® (1.5 mg/ml) was added to all the wells to quantify the developed colonies and the plates were placed in the incubator for 24 hr at 37°C before measuring absorbance at 630 nm. The IC_{50} (test compound concentration inhibiting 50% of cell population) was determined by regression analysis.

2.2.4 | Sphere assay

One of the unique properties of CSCs is that it can survive and can be cultured in serum-free media. Declustered suspension of CSCs under nonadherent conditions in the serum-free medium can grow and form spheres. For both the cell lines used in the assay, two different media mammosphere media (MDA-MB-231) and prostosphere media (PC-3) were used. The media were prepared as per our previously reported procedure. (Padhariya et al., 2020).

Briefly, the trypsinized cells were declustered using both 100- and 40-μm cell strainer to make a uniform single-cell suspension. Dilution of 2,000 cells/100 μl in respective mammosphere (for MDA-MB-231) and prostatosphere (for PC-3) medium was prepared and plated onto each well of 96-well suspension plates and incubated under humidified condition (37°C, 5% CO_2) for 24 hr. Once the media was set, 2 μl of test compounds per 100 μl of the respective medium was added to each well and again incubated under the same condition for 72 hr. After incubation, the spheres formed were again exposed to the test compounds in two rounds of 2.5 μl/50 μl and 3 μl/50 μl of the respective culture media and incubated for 72 hr each time at 37°C, 5% CO_2. At the end of 7 days of the experimental period, live primary spheres were counted manually under phase-contrast microscope (Figure 1) to determine % viability for each concentration by using Formula (1) and a comparative graph of % viable spheres formed at the given concentration to the positive control was then plotted.

$$\%\text{Viability} = \frac{\text{Number of spheres formed per well} \times 100}{\text{Number of spheres formed in control well}}. \quad (1)$$

2.2.5 | Human peripheral blood mononuclear cells assay

The assay was performed on lymphocytes obtained from human blood, stabilized by ethylenediaminetetraacetic acid, of healthy donors upon approval from the Institutional Ethics Committee (Reg. No: ECR/138/Inst/MH/2013). Briefly, the fresh blood was defribinated and laid gradually on HiSep™ LSM1077 and centrifuged for half an hour at low speed. The buffy-colored lymphocyte layer was carefully transferred to a new collection tube and washed with a diluent buffer solution to reduce platelet contamination. After discarding the supernatant, the diluent buffer was used to resuspend the pellet and viability was determined using hemocytometer. % Viability compared to the control group was determined by MTT assay as described above (seeding density 0.7 million/ml).

2.3 | Statistical analysis

All results were expressed as mean ± SD from experiments carried out in triplicates. Statistical analysis was performed using a two-way analysis of variance followed by Bonferroni post-test with the help of GraphPad Prism software (Version 6.0).

3 | RESULTS AND DISCUSSION

3.1 | Chemistry

In extension to our previous work on the synthesis of small molecules as anti-CSC agents, we adopted a simple and efficient synthetic route

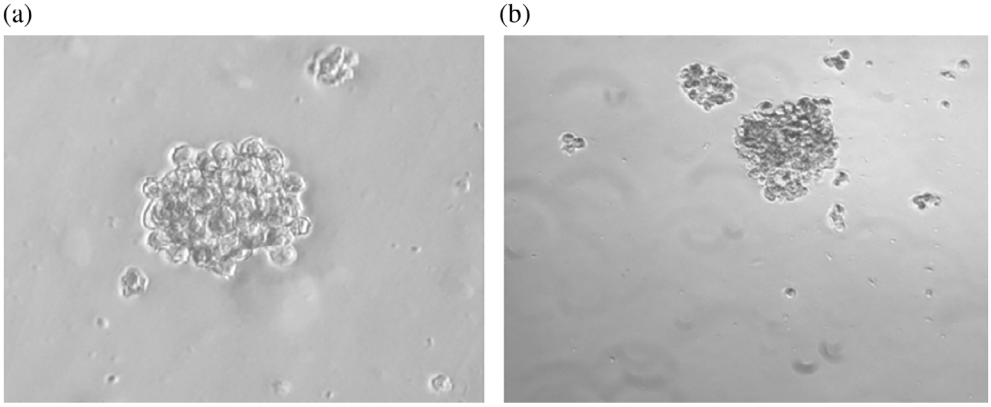
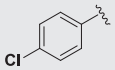
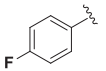
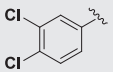
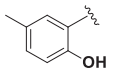
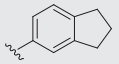
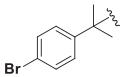
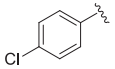
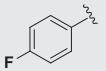
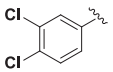
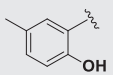
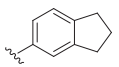


FIGURE 1 Phase contrast microscopy images (×20 magnification) of (a) mammospheres (MDA-MB-231) (b) prostatospheres (PC-3)

TABLE 1 Structural features, % inhibition (10 μM)[a] and median effect[b] of 1,2,3-triazole derivatives (**39–68**) on viability of various human cancer cell lines[c]

			MDA-MB-231		PC-3		U87 MG		SiHA		A-549	
Compounds	Series	R′/Ar′	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)
Cisplatin	–	–	Nt	44.55 ± 1.8		21.53 ± 4.24		22.40 ± 9.14		55.33 ± 7.62		21.36 ± 2.78
Doxorubicin HCl	–	–		0.59 ± 0.09		0.23 ± 0.12		0.17 ± 0.01		1.57 ± 0.18		1.08 ± 0.52
Sunitinib	–	–		2.00 ± 0.04		2.87 ± 0.05		3.86 ± 0.04		Nt		Nt
39	A		72.30 ± 1.25		66.32 ± 1.86		62.38 ± 1.57		60.17 ± 2.45		52.88 ± 1.51	
40	A		70.63 ± 1.09		71.79 ± 0.95		65.45 ± 2.57		62.12 ± 2.38		48.37 ± 2.45	
41	A		64.68 ± 0.78		58.33 ± 1.17		76.44 ± 1.31		45.12 ± 1.39		33.17 ± 1.31	
42	A		69.37 ± 1.57		73.66 ± 1.28		59.54 ± 1.22		52.55 ± 1.67		41.69 ± 2.29	
43	A		74.28 ± 1.41		63.10 ± 2.04		71.31 ± 1.84		49.11 ± 0.57		68.62 ± 1.45	
44	A		59.15 ± 0.31		69.22 ± 1.35		63.12 ± 1.96		23.86 ± 1.40		46.25 ± 1.57	
45	A		63.64 ± 0.18		62.33 ± 2.03		39.89 ± 1.15		63.69 ± 2.12		51.14 ± 1.02	
46	B		68.34 ± 0.35		60.59 ± 0.29		52.33 ± 0.41		65.36 ± 0.98		57.27 ± 1.79	
47	B		52.16 ± 0.93		58.84 ± 0.62		45.70 ± 0.28		68.25 ± 0.27		62.98 ± 0.41	
48	B		82.98 ± 0.82	3.59 ± 0.23	80.03 ± 1.09	2.53 ± 0.11	66.75 ± 0.34		68.33 ± 0.97		56.24 ± 0.65	
49	B		47.89 ± 0.62		37.28 ± 0.38		52.54 ± 0.94		61.37 ± 0.70		42.56 ± 0.95	
50	B		78.45 ± 0.78		52.35 ± 0.88		63.74 ± 0.77		53.29 ± 0.97		45.65 ± 0.49	

(Continues)

TABLE 1 (Continued)

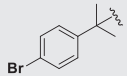
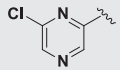
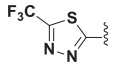
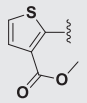
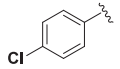
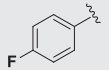
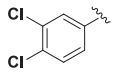
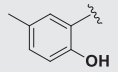
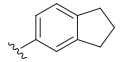
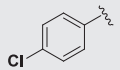
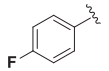
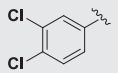
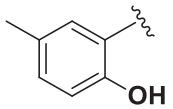
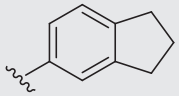
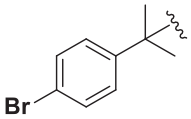
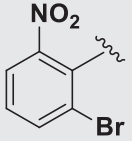
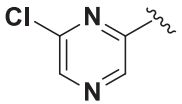
Compounds	Series	R′/Ar′	MDA-MB-231		PC-3		U87 MG		SiHA		A-549	
			% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)
51	B		74.28 ± 0.36		59.31 ± 0.69		64.37 ± 0.23		58.77 ± 0.55		37.40 ± 0.49	
52	B		37.76 ± 1.09		45.52 ± 0.29		52.87 ± 0.48		47.54 ± 0.42		34.36 ± 0.32	
53	B		64.78 ± 1.12		45.52 ± 0.29		69.41 ± 0.91		54.50 ± 0.42		34.47 ± 0.41	
54	B		48.93 ± 0.47		61.23 ± 0.19		52.91 ± 0.79		46.70 ± 0.77		51.30 ± 0.65	
55	B		72.09 ± 0.95		63.61 ± 0.91		71.94 ± 0.40		39.83 ± 0.55		48.54 ± 0.43	
56	C		80.12 ± 0.31	2.16 ± 0.16	72.31 ± 0.48		82.75 ± 1.20	7.24 ± 0.27	46.23 ± 1.04		49.78 ± 0.82	
57	C		73.98 ± 1.65		39.25 ± 0.91		54.42 ± 0.59		59.65 ± 0.53		21.35 ± 0.26	
58	C		71.34 ± 0.87		56.35 ± 0.21		63.85 ± 0.78		48.38 ± 0.32		51.32 ± 0.63	
59	C		69.23 ± 0.77		58.78 ± 1.32		62.36 ± 0.72		51.57 ± 0.88		47.71 ± 0.53	
60	C		59.41 ± 0.55		52.23 ± 0.82		40.24 ± 0.98		45.34 ± 1.59		39.56 ± 1.62	
61	D		84.08 ± 0.49	1.47 ± 0.07	81.89 ± 0.53	3.12 ± 0.8	81.50 ± 0.52	4.17 ± 0.09	73.33 ± 0.56		60.34 ± 0.53	
62	D		74.57 ± 0.33		47.22 ± 0.40		39.28 ± 0.20		47.96 ± 0.64		31.36 ± 1.48	
63	D		70.23 ± 0.79		45.67 ± 0.51		58.54 ± 0.53		41.38 ± 1.73		34.45 ± 1.67	

TABLE 1 (Continued)

Compounds	Series	R′/Ar′	MDA-MB-231		PC-3		U87 MG		SiHA		A-549	
			% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)	% Inhibition (10 μM)	IC_{50} (μM)
64	D		65.34 ± 0.34		53.31 ± 0.53		40.33 ± 0.87		68.29 ± 0.75		56.44 ± 0.79	
65	D		68.42 ± 0.98		83.58 ± 0.31	4.14 ± 0.14	85.93 ± 0.46	6.6 ± 0.11	56.39 ± 0.85		48.35 ± 0.35	
66	D		83.93 ± 0.67	1.63 ± 0.27	75.67 ± 0.65	Nt	80.05 ± 0.73	5.87 ± 0.18	62.48 ± 0.76	Nt	47.59 ± 0.36	Nt
67	D		41.92 ± 0.79	Nt	57.63 ± 0.11		67.32 ± 0.33	Nt	47.25 ± 1.25		52.48 ± 0.79	
68	D		38.41 ± 0.43		41.55 ± 0.54		52.57 ± 0.46		54.19 ± 0.63		38.69 ± 1.46	

Abbreviation: Nt, not tested.

[a]Determined by MTT assay, all data expressed as ±*SD* (results are average of triplicate analysis).

[b]Determined by MTT assay, all data expressed as ±*SD* (results are average of triplicate analysis). IC_{50} value was determined when the inhibitory rate of compound was higher than 80% at 10 μM concentration.

[c]Cancer cell line origin: Breast adenocarcinoma: MDA-MB-231; prostate carcinoma: PC-3; glioblastoma astrocytoma: U87 MG, cervical carcinoma: SiHA and lung carcinoma: A-549.

TABLE 2 Median effect of hits on viability in human breast and prostate cancer cell lines in soft-agar assay[a]

Compounds	IC_{50} (μM)[b]	
	MDA-MB-231[c]	PC-3[d]
Cispaltin	33.19 ± 0.11	26.18 ± 0.08
Doxorubicin	1.08 ± 0.09	1.48 ± 0.21
Sunitinib	2.8 ± 0.07	2.64 ± 0.25
48	4.68 ± 0.08	1.57 ± 0.18
56	6.34 ± 0.05	2.9 ± 0.22
61	>100	12.13 ± 0.18
65	7.26 ± 0.07	14.49 ± 0.28
66	11.67 ± 0.05	24.21 ± 0.05

[a]Determined by soft-agar assay.
[b]Compounds tested in triplicate.
[c]Breast adenocarcinoma: MDA-MB-231.
[d]Prostate carcinoma: PC-3.

to synthesize title compounds **39–68**. The substituted phenols **1–4** were *O*-alkylated using propargyl chloride in the presence of anhydrous K_2CO_3 in dry DMF to yield corresponding prop-2-ynyl derivatives **5–8** according to general procedure mentioned in Section 2.1.2. In a separate set of reactions as per general procedures in Sections 2.1.3 and 2.1.4, various substituted amines **9–18** bearing electron-donating and -accepting groups were converted to their respective 2-azido-*N*-substituted acetamide derivatives **19–28** by acetylation with chloroacetyl chloride followed by reaction with NaN_3 in anhydrous DMF. Finally, the 2-azido-*N*-substituted acetamide and prop-2-ynyl derivatives were used to carry out 1,3-dipolar cycloaddition reaction using a catalytic amount of $Cu(OAC)_2.H_2O$ in *t*-BuOH: H_2O (3:1) mixture to yield title compounds **39–68** (80–90% yield) as per previously described procedure in Section 2.1.5. Although an effort was made to synthesize 1,2,3-triazoles from all possible combinations of 2-azido-*N*-substituted acetamide **19–28** and prop-2-ynyl derivatives **5–8**, few reactions did not undergo as expected and purification of the same did not yield the desired product. The final sets of purified and structurally characterized molecules (Data S1) were screened in a battery of assays.

3.2 | Biology

3.2.1 | Cell viability in MTT assay

Initially, all the 30 molecules were screened at 10 μM concentration in cell viability (MTT) assay against various cancer cell lines: breast (MDA-MB-231), prostate (PC-3), glioma (U87 MG), cervical (SiHa), and lung (A549). The optimal plating densities of each cell line are mentioned in Section 2.2.2. Doxorubicin HCl, cisplatin and sunitinib were used as positive controls and the vehicle DMSO as a negative control. While doxorubicin HCl and cisplatin represented conventional chemotherapy modality, sunitinib was used as a representative anti-CSC drug (Yuan et al., 2016). The results are shown in Table 1. Molecules displaying >80% inhibition at 10 μM were further taken up for IC_{50} determination. Overall, at 10 μM most of the molecules exhibited better activity against breast (MDA-MB-231), prostate (PC-3), glioma (U87 MG) compared to cervical (SiHa) and lung (A549) cell line (Table 1). More so, molecules in Series C (**56–60**) and Series D (**61–68**) were found to more active than Series B (**46–55**) while Series A (**39–45**) were comparatively less active at tested concentration.

In Series A, molecules displayed comparative activity in breast and prostate cell line. The presence of electron-withdrawing substituent on the phenyl ring such as 4-Cl (**39**) and 4-F (**40**) showed improved activity in all cell lines compared to 3,4-Cl_2 (**41**) with an exception in U78 MG (76%). The activity improved when the phenyl ring was substituted with an indanyl ring (**43**). In Series B as well, molecules showed preference to inhibit breast and prostate cancer cell lines. Similar to Series A, molecules with phenyl ring substitution as 4-Cl (**46**), 4-F (**47**) and 3,4-Cl_2 (**48**) were found to be more active overall. More so, compound **48** exhibited around 80% inhibition at 10 μM in both breast (82%) and prostate (80%) cell line. While the IC_{50} value was comparable to sunitinib in MDA-MB-231 (3.5 μM), it was fourfold less in case of PC-3 (8.6 μM). Replacing substituted phenyl ring with heterocylic ring (**54**) clearly diminished the activity. A similar trend was observed in Series C, where 4-Cl phenyl substituted molecule (**56**) displayed >80% inhibition at 10 μM in both MDA-MB-231 and U87 MG cell line. In the case of MDA-MB-231, the IC_{50} value was found to be less than sunitinib (2.1 μM). However, in the case of Series D, three molecules **61**, **65**, and **66** exhibited >80% inhibition at 10 μM concentration in three cell lines: breast, prostate, and glioma. In case of MDA-MB-231, two molecules exhibited improved activity compared to sunitinib (**61**:1.4 μM, **66**:1.6 μM).

3.2.2 | Soft-agar assay

Title compounds exhibiting >80% inhibition at 10 μM in the respective cell line were carried forward for evaluation in soft-agar assay (SAA), also known as anchorage-independent assay (Fukazawa, Mizuno, & Uehara, 1995). A normal cell line in culture differs from a transformed cell line in various aspects with respect to growth parameters. Normal cells require adhesion to extracellular matrix and basement membrane for survival and growth and undergo apoptosis in suspension media. On the other hand, tumor cells can flourish without cell-substratum support and evade anoikis resulting in uncontrolled cell growth. A normal cell can survive, grow, and differentiate only in proper tissue surrounding; however, tumor cell is competent for survival irrespective of their original environment. To understand the hallmark characteristic of cell transformation, that is, uncontrolled cell growth and tumorigenecity, an important characteristic of CSCs, in a semisolid media without attachment to substratum, in vitro anchorage-independent growth is the best way to correlate with their tumorigenic nature in vivo (Shin, Freedman, Risser, & Pollack, 1975).

The superiority of testing anticancer molecules in three-dimensional (3D) format like SAA over monolayer culture to validate

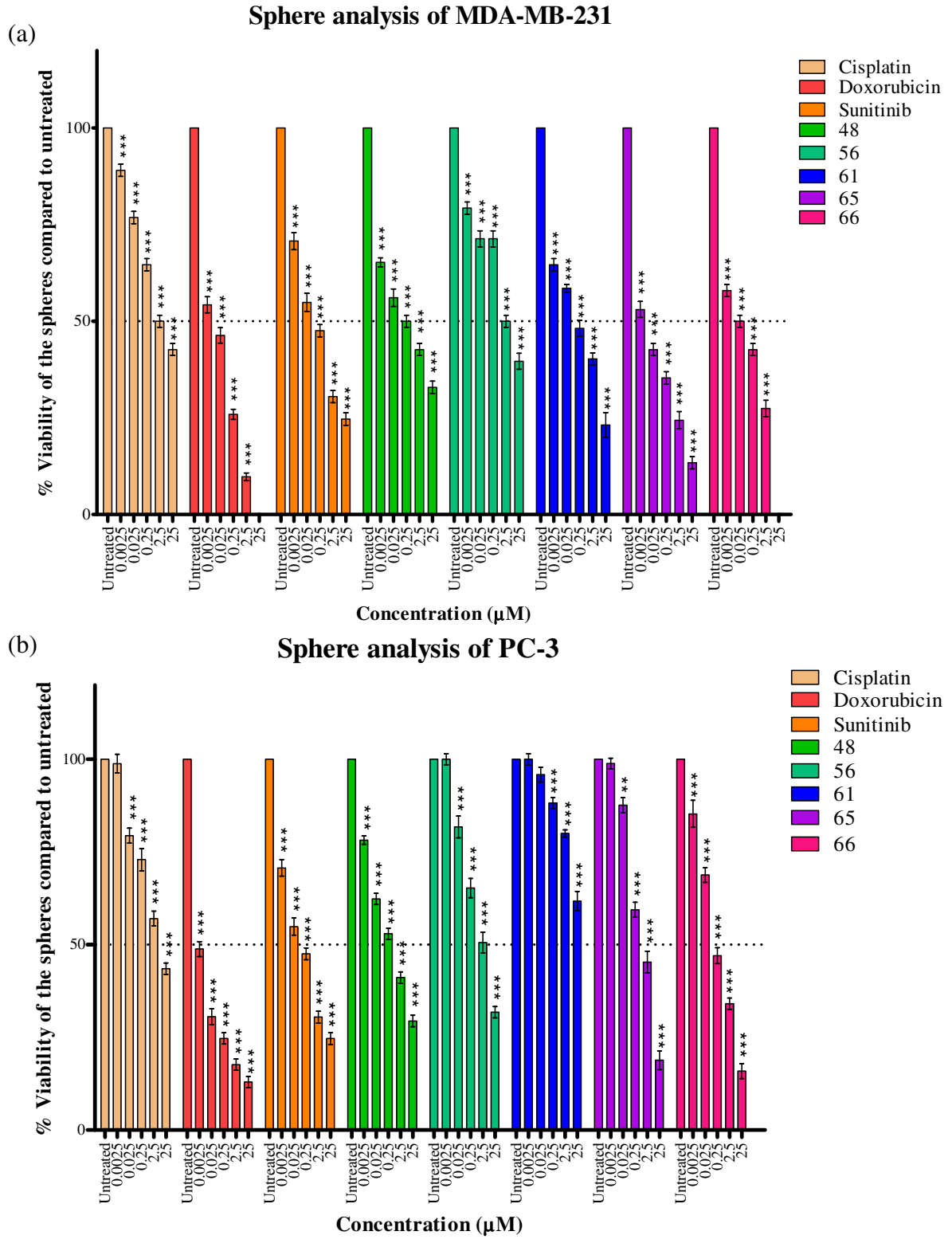


FIGURE 2 Results from sphere assay of the hits. (a) % Viability of mammospheres (MDA-MB-231); (b) % viability of prostatospheres (PC-3) compared to the untreated. (The data are expressed as mean ± *SD* [*n* = 4]. Asterisk above columns indicate statistically significant difference compared to control. ***$p < .001$)

cell transformation is used since 1977 in clonogenic assay developed by Hamburger and Salmon (1977) and Hamburger (1987). Since then, SAA is widely being used to identify potential antitumor agents and to evaluate the stemness properties of CSCs (Fukazawa, Noguchi, Murakami, & Uehara, 2002; Horibata, Vo, Subramanian, Thompson, & Coonrod, 2015; Seyfrid et al., 2019). More so, the traditional method

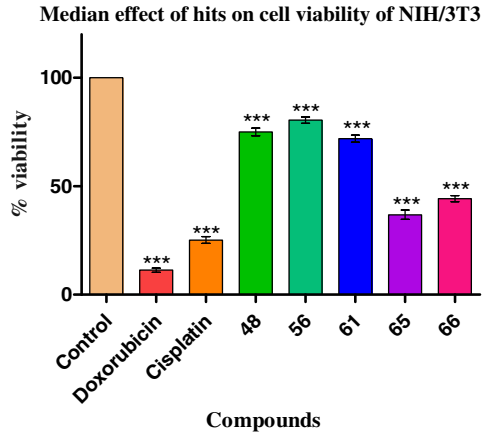


FIGURE 3 % Cell viability of the hits against NIH/3T3 cell line (IC_{50} ± *SD*, *n* = 3) (Asterisk above columns indicate statistically significant difference compared to control. ***$p < .05$)

TABLE 3 Median effect of the hits on lymphocyte viability (hPBMC assay)

Compound code	IC_{50} (μM)
Doxorubicin	11.07 ± 0.12
Cisplatin	59.43 ± 0.07
Sunitinib	2.8 ± 0.07
48	4.84 ± 0.07
56	>100
61	>100
65	10.21 ± 0.06
66	7.31 ± 0.07

is also modified with time to overcome time-consuming, labour intensive process with HTS to meet current oncology drug-target screening demand (Fukazawa et al., 1995; Ke et al., 2004).

In the current study, representative cell lines from the breast (MDA-MB-231) and prostate (PC-3) cancer types were used with their optimized cell plating density. The IC_{50} (μM) values were calculated based on the capacity of the molecules to inhibit 50% colony-formation compared to the control (Table 2).

Compound **48** (MDA-MB-231:4.6 μM and PC-3:1.5 μM) was the most potent against the indicated cell line. Compound **56** also showed activity comparable to sunitinib in the case of PC-3 (2.9 μM). Overall, most of the tested compounds showed IC_{50} < 10 μM. The positive results encouraged us to evaluate the hits in sphere-formation or sphere assay to understand their potential effect on CSCs.

3.2.3 | Sphere assay

3D bioassays mimic and simulate *in vivo* conditions better than the known 2D monolayer cell culture assays (Chen et al., 2012). To study the effect of test molecule specifically on CSCs, more harsh conditions are used to eliminate any chance of growth of cancer cells. A serum-free gel matrix supports the growth of only CSCs influencing the formation of spheres under such stringent conditions (Pastrana, Silva-Vargas, & Doetsch, 2011). The Sphere assay is widely used 3D-assay to evaluate the anti-CSC effect of NCEs (Bahmad et al., 2018; Morales et al., 2013).

In the present study, the hits from MTT and SAA were screened at five different concentrations (0.0025, 0.025, 0.25, 2.5 and 25 μM) against breast (MDA-MB-231) and prostate (PC-3) CSCs. (Figure 2a, b). Interestingly, most of the hits (**48**, **56**, **61**, **65**, and **66**) demonstrated moderate activity at 2.5 μM by inhibiting >50% spheres in both the cell lines. In particular, compounds **61**, **65**, and **66** inhibited >50% spheres at 25 nM concentration against the MDA-MB-231 cell line. Notably, **65** exhibited better inhibition profile than sunitinib (Figure 2a). In the case of PC-3 cell line, except **61**, all four NCEs exhibited moderate activity with >50% sphere inhibition at 2.5 μM, while only **66** displayed high activity at 0.25 μM. Overall, the hits were found to be more active against mammosphere than prostatosphere.

3.2.4 | Human peripheral blood mononuclear cells assay

The primary side effect of anticancer therapy is the destruction of normal cells. While a potential drug candidate should be toxic to the target cell, it should be nontoxic to the normal body cells. To understand the toxic effect on normal cells, potential hits (**48**, **56**, **61**, **65**, and **66**) were also screened against human peripheral blood mononuclear cells (hPBMCs) in lymphocyte assay and NIH/3T3 (mouse embryonic fibroblast) cell line by MTT assay in two different experiments.

In NIH/3T3 cell line, the hits (**48**, **56**, **61**, **65**, and **66**) were screened at five different concentrations (0.01, 0.1, 1, 10, and 100 μM) using cisplatin and doxorubicin as positive control. Hits **48**, **56**, and **61** found to have IC_{50} > 75 μM, while **65** and **66** had IC_{50} < 45 μM. Overall, **48**, **56**, and **61** were nearly threefold less toxic than doxorubicin under the same condition against NIH/3T3 cell line (Figure 3).

In lymphocyte assay, the hits were screened against hPBMCs using both doxorubicin and sunitinib as positive controls and IC_{50} values were calculated (highest concentration tested 100 μM) (Table 3). While **56** and **61** showed no toxicity toward hPBMCs (IC_{50} > 100 μM); hit **48** (IC_{50} = 4.84 μM), **65** (IC_{50} = 10.21 μM), and **66** (IC_{50} = 7.31 μM) were relatively toxic compared to the positive controls sunitinib (IC_{50} = 2.8 μM) and doxorubicin (IC_{50} = 11.07 μM).

4 | CONCLUSIONS

Our original hypothesis to use a hybrid structure with more than one pharmacophoric group to direct activity against CSCs led to interesting results. In the present investigation, a total of 30 (**39–68**) 1,2,3-triazole ring containing derivatives belonging to four related chemical series, were designed and synthesized using click chemistry

approach. While most of the synthesized molecules were active against breast, prostate and glioma cell lines, five hits displayed single-digit micromolar activity. Further screening the hits in sphere assay, the hits were also found to be active against mammospheres and prostatospheres with IC_{50} ranging from as low as 0.025 to 2.5 μM. Among all, **56** exhibited potent anticancer as well as anti-CSCs activity without affecting the tested normal cells NIH/3T3 and human lymphocytes. Further studies to improve pharmacokinetic and drug-like properties needs to be addressed. Overall, combining small pharmacophoric groups with the triazole functionality helped us to identify potential hits as anti-CSC agents.

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CONFLICT OF INTEREST

Authors declare no conflict of interest from a financial or commercial standpoint.

AUTHOR CONTRIBUTIONS

Prashant S. Kharkar envisaged the project. Prashant S. Kharkar and Sangeeta Srivastava monitored the progress of the project. Komal N. Padhariya synthesized, characterized, purified, and partly carried out the biological evaluation. Maithili Athavale and Sangeeta Srivastava carried out the majority of the biological evaluation. Prashant S. Kharkar and Sangeeta Srivastava contributed to the manuscript compilation and critical reading.

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SUPPORTING INFORMATION

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