Accepted Manuscript

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PII: S0968-0896(16)31395-5
DOI: http://dx.doi.org/10.1016/j.bmc.2016.12.010
Reference: BMC 13437

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 1 August 2016
Revised Date: 22 October 2016
Accepted Date: 8 December 2016

Please cite this article as: Subba Rao, A.V., Swapna, K., Pasha Shaik, S., Lakshma Nayak, V., Srinivasa Reddy, T., Sunkari, S., Basha shaik, T., Bagul, C., Kamal, A., Synthesis and Biological Evaluation of *cis*-Restricted Triazole/Tetrazole Mimics of Combretastatin-Benzothiazole Hybrids as Tubulin Polymerization Inhibitors and Apoptosis Inducers, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.12.010

Graphical Abstract

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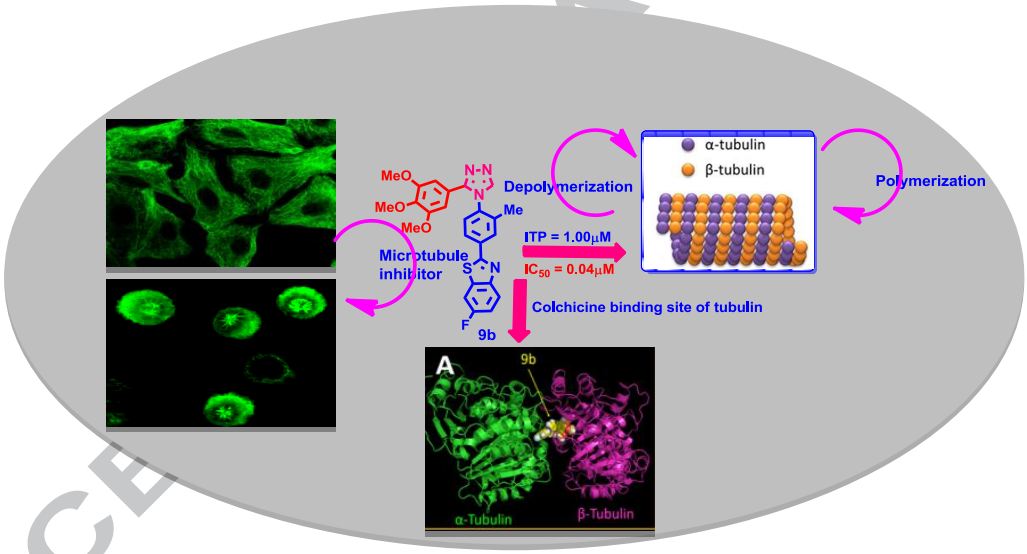

Bioorganic & Medicinal Chemistry
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Combretastatin A-4;
Benzothiazole;
Triazoles and Tetrazoles;
Antiproliferative activity;
Tubulin polymerization;
Colchicine competition assay;
Apoptosis;

ABSTRACT

A series of colchicine site binding tubulin inhibitors were synthesized by the modification of the combretastatin pharmacophore. The ring B was replaced by the pharmocogically relevant benzothiazole scaffolds, and the *cis* configuration of the olefinic bond was restricted by the incorporation of a triazole and tetrazole rings which is envisaged by the structural resemblance to a tubulin inhibitor like combretastatin (**CA-4**). These compounds were evaluated for their antiproliferative activity on selected cancer cell lines and an insight in the structure activity relationship was developed. The most potent compounds (**9a** and **9b**) demonstrated an antiproliferative effect comparable to that of **CA-4**. Mitotic cell cycle arrest in G2/M phase revealed the disruption of microtubule dynamics that was confirmed by tubulin polymerization assays and immunocytochemistry studies at the cellular level. Western blot analysis revealed that these compounds accumulate more tubulin in the soluble fraction. The colchicine competitive binding assay and the molecular docking studies suggested that the binding of these mimics at the colchicine site of the tubulin is similar to that of **CA-4**. Moreover, the triggering of apoptotic cell death after mitotic arrest was investigated by studying their effect by Hoechst staining, Annexin-V-FITC assay, mitochondrial membrane potential, ROS generation and caspase-3 activation.

1. Introduction

Small molecules that disrupt microtubule/tubulin dynamics are used widely in cancer treatment. Hence discovery and development of newer small molecules with antitubulin activity have attracted medicinal chemists for past few years.[1] Microtubules are one of the key structural components of the cytoskeleton in eukaryotic cells comprising of α and β-tubulin heterodimers. They play a crucial role in various cellular processes and have emerged as an attractive and viable target in the development of anticancer drugs mainly due to their indispensability in mitotic cell division.[2] Generally, drugs that target microtubules and bind to the tubulin binding sites thereby stabilizing or destabilizing microtubule assembly. There are four main binding sites of tubulin[3] which includes the *taxane* and *laulimalide/pelouside A* sites for the microtubule stabilizing agents,[4,5] the *vinca domain*,[6] *maytansine binding site*[7] and the *colchicine domain*[8,9] for the destabilizing agents. Interfering with the dynamic stability of microtubules, these agents act as spindle poisons arresting the dividing cells in G2/M phase of the cell cycle, causing mitotic catastrophy and finally leading to apoptotic cell death. Some of the well-known naturally occuring tubulin binding ligands that affect the microtubule dynamics by binding to distinct colchicine domain of tubulin are colchicine (**1**) and combretastatin A-4 (**2a**) (Figure **1**).[10,11] Combretastatin A-4 (**CA-4**), natural vicinally diaryl *cis*- stilbenoid phenol isolated from the bark of the South African tree *Combretum caffrum* is one of the simplest and highly effective molecule among the structurally diverse microtubule disturbing agents affecting microtubule dynamics by binding to the colchicine site. **CA-4** strongly inhibits tumor cell growth, including MDR cancer cell lines,[12] and exhibits remarkable strong inhibition of tubulin polymerization as well as potent cytotoxicity against murine lymphocytic leukemia, human ovarian and colon cancer cell lines resulting in vascular shutdown in solid tumor.[13-17] The limitation of poor aqueous solubility of **CA-4**[18,19] circumvented with the development of a prodrug, CA-4 phosphate (**2b**) and other synthetic analogues like OXI4531 (**2c**), both of which are currently undergoing clinical trials.[20] However, evidences of

tumor re-growth, isomerization of *cis* double bond and significant toxicity towards normal cells resulted in a surge of research endeavors in the development of more effective and safer **CA-4** based new molecules acting on microtubules with desired properties.[21-23] In recent years **CA-4** emerged as a preferred lead compound in the development of new inhibitors of tubulin polymerization because of its high potency and ease of synthesis.[22,23] Similar to other colchicine site binders, **CA-4** has three important pharmacophoric substructures, the two hydrophobic rings (A and B) and the linking olefinic bridge with appropriate dihedral angle resulting from the cis configuration. It was previously postulated that the presence of trimethoxy substitution on ring A is crucial for efficient binding at colchicine site.[10] On the other hand, the ring B was found to be tolerant to structural modification and a number of reports defined this ranging from substituted phenyl ring to heterocyclic and nonsubstituted aromatic rings.[13,24] Importantly, the olefinic bond with cis configuration plays a fundamental role in binding at the colchicine site by positioning the rings at appropriate distance to maximize interactions.[25] Several attempts have been reported to modify this cis-olefinic bond to prevent its isomerization under amenable conditions. This mostly included either modification of the olefinic bond by the introduction of saturation, substituents and its replacement with a three to six membered ring system, which resulted in cis restricted analogues of **CA-4**.[23,26-29]

Our recent research studies have been mainly focused on the synthesis, evaluation and mechanistic aspects of newer molecules based on different heterocyclic scaffolds as potential anticancer agents.[30,31] In particular, the targeting of tubulin polymerization by new diversified ligands based on **CA-4** has led to promising and interesting results.[32] Herein, we describe different modifications on **CA-4** scaffold, specifically the incorporation of benzothiazole moieties of pharmacological relevance by replacement of ring B. The rationale behind this is the significance of these moieties as privileged structures and the opportunity offered by them as anchors that could be harnessed for further diversification, besides they are present in a number of antitumor agents such as (5F-203) (**3**)[33] and its prodrug, phortress (**4**)[34] which is water soluble and chemically stable, is found to rapidly and quantitatively revert to its parent amine in mice, rats and dogs (*in vivo*). Thus, clinical evaluation of phortress has demonstrated potent and selective antitumor activity via a different mechanism of action which provides substantial scope for the development of benzothiazole-based derivatives as anticancer agents. Moreover, the structural resemblance to **CA-4**, a colchicine site binder was envisaged by the replacement of the olefinic bond by a triazole and tetrazole rings to maintain the cis configuration and provide optimal conformational geometry for interaction with the colchicine binding site. Such replacements of the olefinic bond with a ring system have been reported to provide potential advantage of promoting tubulin binding activity and tumor selectivity besides minimizing toxicity.[13] In addition, the constitutive role of the trimethoxybenzene moiety (ring A) is ambiguous in tubulin binding.[35] Considering the biological importance of these moieties, an attempt has been made in the present study to synthesize *cis* restricted triazole/tetrazole analogues of **CA-4** and benzothiazole (CAB) hybrids. The resulting *cis*-restricted benzothiazole mimics of CA-4 were evaluated for antiproliferative activity and examined for their structure activity relationship (SAR) followed by studies to elucidate the mechanism of action which included cell cycle progression, tubulin polymerization assay and molecular docking studies. Further to confirm the induction of apoptotic cell death by the potent mimics like **9a** and **9b**, studies such as Hoechst staining performed.

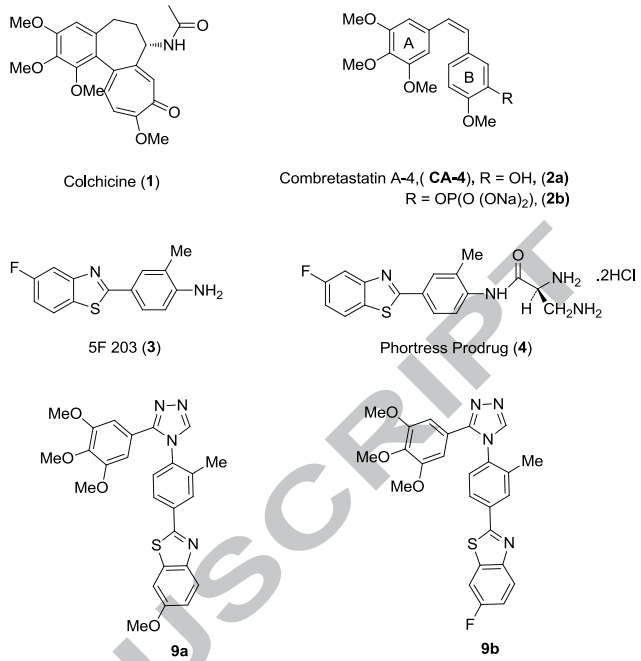


Figure 1: Colchicine binding microtubule inhibitors (**1**, **2a**, **2b**, **9a** and **9b**) and antitumor benzothiazoles (**3**) and its prodrug (**4**).

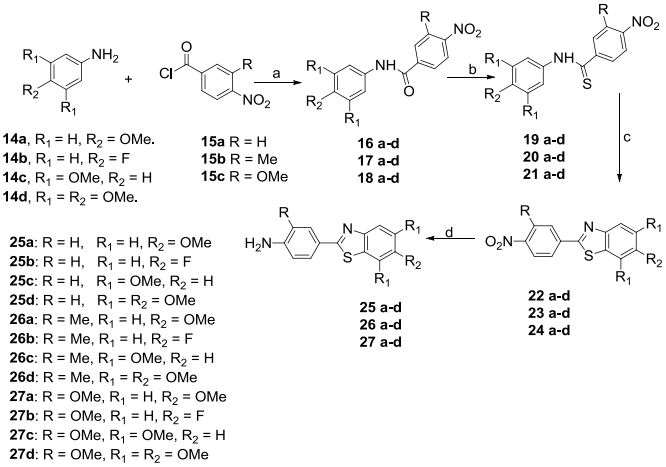
2. Results and Discussion

2.1 Chemistry

Scheme 1

Synthesis of substituted 2-(4-amino-phenyl) benzothiazoles (25-27a-d)

The synthesis of triazole and tetrazole mimics of CA-4 linked benzothiazole hybrids (**5-13a-d**) was accomplished by the synthetic route illustrated in Schemes **1**, **2** and **3**. The preparation of various substituted 2-(4-aminophenyl)benzothiazole precursors **25-27(a–d)**, was achieved by the Jacobson's thioanilide radical cyclization. Thus, reaction of substituted anilines (**14a–d**) with substituted p-nitrobenzoylchlorides (**15a-c**) in pyridine gave the benzanilides **16-18(a–d)** which were further converted to their corresponding thiobenzanilides **19-21(a–d)** using Lawesson's reagent. These were cyclized by using Jacobson's method to afford nitrobenzothiazole derivatives **22-24(a–d)** using potassium ferricyanide and 10% NaOH solution which upon reduction of these nitro compounds with stannous chloride yielded the corresponding substituted 2-(4-amino-phenyl) benzothiazoles **25-27(a–d)** as illustrated in Scheme **1**. These key intermediates were utilized in the synthesis of the triazole and tetrazole mimics of CA-4 benzothiazole hybrids.

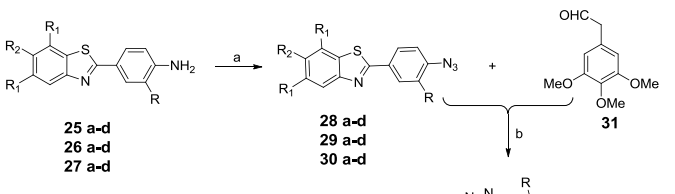


Scheme 1: *Reagents and conditions*: (a) Pyridine, reflux, 3 h; (b) Lawesson's reagent, toluene, reflux, 8 h; (c) $K_3Fe(CN)_6$, aq. NaOH, EtOH, 90 °C, 2-3 h; (d) $SnCl_2 \cdot 2H_2O$, EtOH, 3 h.

Scheme 2

Synthesis of 1, 4-disubstituted 1,2,3-triazoles 5-7(a-d)

To access the 1,4-disubstituted 1,2,3-triazole mimics of CA-4 benzothiazole hybrids the same intermediates **25-27(a-d)** were employed as depicted in Scheme **2**. These amines were further converted to azides by using sodium nitrite in HCl followed by sodium azide to afford corresponding azides **28-30(a–d)**. 1,4-Disubstituted 1,2,3-triazoles **5-7(a–d)** were synthesized by exposing trimethoxy phenyl acetaldehyde (**31**) to azides in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene DBU in DMSO[36] as illustrated in Scheme **2**.



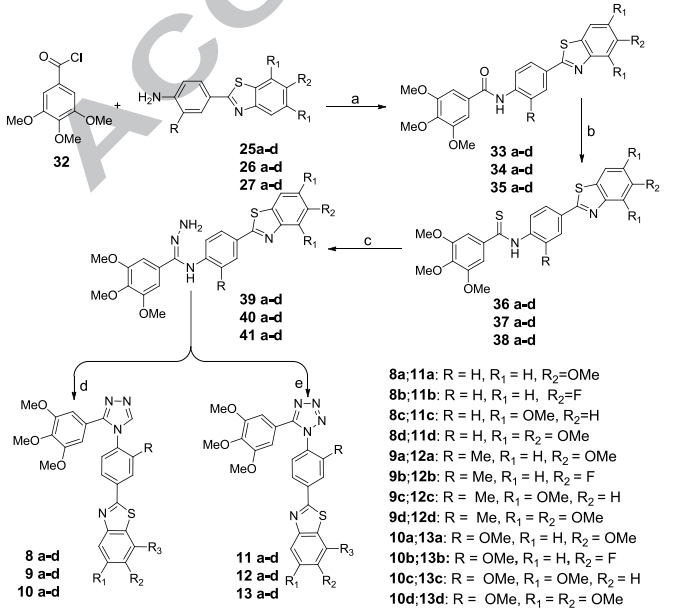
5a: R = H, R_1 = H, R_2= OMe
5b: R = H, R_1 = H, R_2 = F
5c: R = H, R_1 = OMe, R_2 = H
5d: R = H, R_1 = R_2 = OMe
6a: R = Me, R_1 = H, R_2 = OMe
6b: R = Me,R_1 = R_3 = H, R_2 = F
6c: R = Me, R_1 = OMe, R_2 = H
6d: R = Me, R_1 = R_2 = OMe
7a: R = OMe,R_1 = H, R_2 = OMe
7b: R = OMe,R_1 = H, R_2 = F
7c: R = OMe, R_1 = OMe, R_2 = H
7d: R = OMe, R_1 = R_2 = OMe

Scheme 2: *Reagents and conditions*: a) $NaNO_2$, aq. HCl, 0-5 °C, NaN_3, 1 h; b) DBU, DMSO, 1 h.

Scheme 3

Synthesis of 3,4-disubstituted 1,2,4-triazoles and 1,5-disubstituted 1,2,3,4-tetrazoles 8-10 (a-d) and 11-13(a-d)

The key intermediates of 2-(4-aminophenyl) benzothiazoles **25-27(a–d)** were coupled with trimethoxy benzoyl chloride (**32**) to give corresponding trimethoxy substituted benzothiazole amides **33-35(a–d)** which upon further conversion of their carbonyl functionality to its analogous thiocarbonyl group employing Lawesson's reagent under refluxing toluene provides thioamides **36-38(a–d)** in good yields.



8a;**11a**: R = H, R_1 = H, R_2=OMe
8b;**11b**: R = H, R_1 = H, R_2=F
8c;**11c**: R = H, R_1 = OMe, R_2=H
8d;**11d**: R = H, R_1 = R_2 = OMe
9a;**12a**: R = Me, R_1 = H, R_2 = OMe
9b;**12b**: R = Me, R_1 = H, R_2 = F
9c;**12c**: R = Me, R_1 = OMe, R_2 = H
9d;**12d**: R = Me, R_1 = R_2 = OMe
10a;**13a**: R = OMe, R_1 = H, R_2 = OMe
10b;**13b**: R = OMe, R_1 = H, R_2 = F
10c;**13c**: R = OMe, R_1 = OMe, R_2 = H
10d;**13d**: R = OMe, R_1 = R_2 = OMe

Scheme 3: *Reagents and conditions*: (a) Pyridine, reflux, 12 h; (b) Lawesson's reagent, toluene, reflux, 8 h; (c) $NH_2NH_2 \cdot 8H_2O$, EtOH, rt, 12 h; (d) trimethylorthoformate, EtOH, reflux, 3 h; (e) $NaNO_2$, AcOH, rt, 4 h.

The reaction of thioamides with hydrazine hydrate affords amidrazones **39-41(a–d)**. These amidrazones were used for next step without purification. Finally, the intramolecular cyclization was performed by using trimethylorthoformate in refluxing conditions with catalytic amount of H_2SO_4 furnishing the desired cis-restricted 1,2,4-triazoles[28] **8-10(a-d)**, whereas 1,2,3,4-tetrazoles **11-13(a-d)** were achieved by using sodium nitrate in AcOH as illustrated in Scheme **3**.

3. Biological activity

3.1 Antiproliferative activity

MTT assay[37] was performed to evaluate the cytotoxic effects of all newly synthesized analogues of 1,4-disubstituted 1,2,3-triazoles and 3,4-disubstituted 1,2,4-triazoles and 1,5-disubstituted 1,2,3,4-tetrazoles against selected human cancer cell lines viz., prostate (DU-145, cervix (HeLa), lung adenocarcinoma (A549), liver (HepG2) and breast (MCF-7), using **CA-4** as reference compound and the IC50 (μM) values are presented in Table **1**.

Most of the compounds from these series displayed potent broad spectrum growth inhibitory effects against all the tested cancer cell lines. In the SAR studies it was considered to investigate whether the presence of 1,2,3-triazoles, 1,2,4-triazoles and 1,2,3,4-tetrazoles influences the activity along with the other substituents on the benzothiazole ring. It was observed that the compounds (**8-10a-d**) containing 1,2,4-triazole ring on olefinic bridge showed relatively strong inhibitory effect than the bioisosters of 1,2,3-triazoles and 1,2,3,4-tetrazoles with IC_{50} values in the range of 0.048-14.67 μM against some representative cancer cell lines. Among them, compound **9a** and **9b** with a methyl substitution on C'-3 position of the 2-phenylbenzothiazole moiety and methoxy and fluoro substitution on C'-6 position of benzothiazole moiety exhibited promising activity with a IC_{50} value of 0.054 and 0.048 μM against the lung cancer cell line. The replacement of 1,2,4-triazole bridge with 1,2,3-triazole and 1,2,3,4- tetrazole resulted in moderate activity and compound **7c** (1,2,3-triazole) and **12b** (1,2,3,4-tetrazole) were the most potent drugs among them exhibiting IC_{50} values of 0.246 and 0.243 μM respectively against the lung cancer cell line. The substitution effect on 2-phenylbenzothiazole ring by these studies reaffirmed the importance of 1,2,4 –triazole ring on the cytotoxic effect by these CA-4 linked benzothiazole hybrids. We then proceeded to explore the influence of substituents on the phenyl ring as well as benzothiazole ring with respect to antiproliferative activity.

Further, compounds **5-13(a-d)** allowed us to investigate the SAR effects of electron-withdrawing (F) and electron-releasing (OMe), as well as substituents like (H, Me and OMe) on the phenyl ring at the C'3-position of the benzothiazole ring. It was observed that among different substituents studied on phenyl ring attached to benzothiazole ring, methyl group (R = Me) at C'3-position exhibited significant increase in activity compared to other substituents (H, OMe) with irrespective of triazole and tetrazole rings. Based on this findings, we introduced some other substituents on the benzothiazole ring and studied their effect on the antiproliferative activity. Initially, we introduced a methoxy group at the C-6 position and the resulting compound showed pronounced activity on the A549 cell line compared to other cell lines and this compound (**9a**) exhibited significant cytotoxic activity with IC_{50} values of 0.054 μM which has 1,2,4-triazole

ring. The effect of different bridge rings revealed that the bioisosteric replacement of the 1,2,4-triazole by a 1,2,3-

Table **1**: [a]IC_{50} (μM) values for Combretastatin-Benzothiazole hybrids **5-13(a-d)**

Bridge	Compound	DU-145[b]	HeLa[c]	A549[d]	HepG2[e]	MCF-7[f]
1,2,3	**5a**	12.30 ±0.8	11.48 ±0.6	6.30 ±0.5	12.38 ±0.97	22.95 ±1.73
Triazole	**5b**	8.12 ±0.5	3.46 ±0.4	1.41 ±0.1	7.76 ±0.8	10.71 ±0.9
	5c	9.33 ±0.4	9.05 ±0.8	7.61 ±0.9	17.37 ±2	19.95 ±1.1
	5d	14.79 ±0.9	11.48 ±0.5	8.54 ±0.5	15.48 ±0.7	9.12 ±0.03
	6a	18.19 ±1.05	13.10 ±0.8	15.85 ±2.12	20.14 ±1.2	22.00 ±0.9
	6b	7.33 ±0.41	6.76 ±0.8	3.38 ±0.4	8.05 ±0.7	10.81 ±0.8
	6c	10.20 ±0.7	8.12 ±0.6	7.76 ±0.6	15.48 ±0.6	18.78 ±0.09
	6d	15.84 ±1.21	11.35 ±1.07	9.77 ±0.9	18.57 ±1.04	20.77 ±0.06
	7a	18.62 ±1.54	11.14 ±1.58	10.69 ±1.05	16.44 ±1.73	15.48 ±1.32
	7b	14.16 ±0.19	10.14 ±1.21	6.02 ±0.7	13.93 ±0.9	16.14 ±1.14
	7c	0.41 ±0.06	0.37 ±0.02	0.24 ±0.01	1.47 ±0.09	4.36 ±0.09
	7d	15.89 ±1.34	17.80 ±1.03	11.00 ±1.1	29.87 ±1.12	27.78 ±2.12
1,2,4	**8a**	11.22 ±0.9	10.02 ±0.98	7.24 ±0.9	11.64 ±0.75	9.50 ±0.9
Triazole	**8b**	8.21 ±0.5	7.00 ±0.4	6.58 ±0.7	10.41 ±0.62	12.52±0.64
	8c	11.84 ±0.7	4.13 ±0.5	5.04 ±0.2	9.65 ±0.34	8.21 ±0.7
	8d	0.46 ±0.08	0.21 ±0.01	0.091±0.04	1.27 ±0.03	3.15 ±0.1
	9a	0.48 ±0.03	0.16 ±0.01	0.054±0.01	0.67 ±0.06	1.13 ±0.05
	9b	0.28 ±0.02	0.15 ±0.06	0.048±0.009	0.30 ±0.02	1.41 ±0.06
	9c	1.04 ±0.09	0.93 ±0.04	0.26 ±0.06	2.54 ±0.4	5.35 ±0.3
	9d	0.70 ±0.07	0.21 ±0.01	0.23 ±0.04	1.25 ±0.06	2.99 ±0.3
	10a	11.60 ±0.8	8.76 ±0.3	9.06 ±0.4	9.05 ±0.63	14.67 ±0.87
	10b	7.58 ±0.7	2.81 ±0.5	1.22 ±0.06	2.75 ±0.6	2.49 ±0.3
	10c	10.21 ±0.6	6.45 ±0.4	7.24 ±0.4	9.20 ±0.8	8.57 ±0.5
	10d	11.53 ±0.8	5.51 ±0.2	3.46 ±0.3	7.41 ±0.9	10.54 ±0.89
1,2,3,4	**11a**	9.12 ±0.9	6.60 ±0.4	3.80 ±0.4	9.05 ±0.7	11.16 ±0.9
Tetrazole	**11b**	4.57 ±0.5	2.23 ±0.3	2.07 ±0.5	7.95 ±0.7	8.57 ±0.8
	11c	19.95 ±0.9	10.23 ±0.9	7.24 ±0.9	22.38 ±1.64	31.62 ±2.79
	11d	7.24 ±0.8	6.16 ±0.7	3.54 ±0.6	8.51 ±0.72	8.68 ±0.8
	12a	15.88 ±1.53	10.96 ±1.11	4.78 ±0.3	10.47 ±0.7	12.64 ±1.09
	12b	1.14 ±0.05	0.97 ±0.05	0.24±0.03	1.69 ±0.1	3.23 ±0.2
	12c	11.74 ±0.7	8.70 ±0.7	5.38 ±0.5	9.74 ±0.8	17.15 ±1.32
	12d	14.79 ±1.76	9.81 ±0.3	7.41 ±0.8	23.44 ±2	17.54 ±1.27
	13a	5.67 ±0.3	4.56 ±0.5	2.25 ±0.06	1.67 ±0.08	3.45 ±0.3
	13b	4.77 ±0.8	4.36 ±0.7	1.38 ±0.04	1.24 ±0.01	1.52 ±0.1
	13c	13.45 ±0.6	11.74 ±2.21	9.24 ±0.8	17.00 ±1.43	24.73 ±1.54
	13d	10.82 ±1.52	5.79 ±0.6	3.80 ±0.1	15.96 ±1.61	16.64 ±0.5
	CA-4	0.069±0.006	0.005±0.001	0.058±0.001	0.009±0.001	0.043±0.001

[a] 50% Inhibitory concentration and the values are average of three individual experiments after 48 h of drug treatment. [b]prostate cancer. [c]cervical cancer. [d] lung cancer. [e] liver cancer. [f] breast cancer.

triazole and 1,2,3,4-tetrazole ring on the olefinic bridge resulted in total loss of antiproliferative activity in most of the cell lines. It is important to point out that the introduction of an additional methoxy group i.e., disubstitution at C5 and C7-position, having 1,2,4-triazoles, produced 6-fold reduction in antiproliferative activity against all the cancer cell lines. Whereas in case of 1,2,3-triazoles and 1,2,3,4-tetrazoles a slight reduction (1-2-fold) in antiproliferative activity observed in all the cancer cell lines with some exceptions in certain cell lines. Moreover, introduction of additional methoxy groups i.e

trisubstitution at C5, C6 and C7-position having 1,2,4-triazoles, produced a 3-fold reduction in antiproliferative activity against all the cancer cell lines. Surprisingly when electron withdrawing substituents (F) are incorporated at C-6 position on the benzothiazole ring, a substantial increase in the cytotoxic potency was observed in all cases (1,2,3-triazole, 1,2,4-triazole and 1,2,3,4- tetrazoles). Compound **9b** with a fluoro substituent on C-6 position of the benzothiazole ring and a methyl substituent on the C'-3 position of the phenyl ring is the most active hybrid exhibiting an IC_{50} ranging from 0.048 to 1.41 μM against different cancer cell lines. On other hand, unsubstituted phenyl ring (R = H) of the benzothiazole counterparts (**5a-d**, **8a-d** and **11a-d**), an identical pattern of activity was observed wherein the 1,2,4-triazole ring plays a relatively stronger influence on the activity compared to compounds having moieties 1,2,3-triazole and 1,2,3,4-tetrazoles. This compound **8d** showed significant activity with IC_{50} values in the range of 0.0991-3.155 μM compared to other compounds like **5a-d** and **11a-d**. Moreover, we also introduced electron–releasing group (R = OMe) on phenyl ring of benzothiazole (**7a-d**, **10a-d** and **13a-d**), having 1,2,3-triazole ring where in compound **7c** showed significant activity with IC_{50} values in the range of 0.246-4.365 μM, amongst the other compounds like **10a-d** and **13a-d**. Interestingly, hybrids **9a** and **9b** exhibit promising antiproliferative activity against A549 cancer cell line.

Overall, SAR studies of these *cis*-restricted triazole and tetrazole mimics of CA-4 linked benzothiazole hybrids suggest that the superior activity of 1,2,4-triazole ring in place of the olefinic bridge compared to other heterocyclic moieties is in agreement with some similar studies on CA-4 analogues described in the earlier studies.[8] The considerations that play a crucial role in the antiproliferative activity are summarized in Fig. **2**.

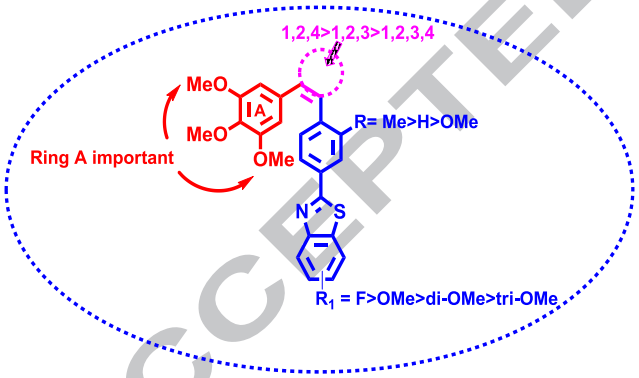


Figure 2: SAR of CA-4 benzothiazole hybrids.

3.2. Analysis of Cell cycle Arrest

Cell cycle analyses were performed using flow cytometry[38] to examine the effect of the cell cycle distribution profiles of A549 cells by these hybrids (**9a** and **9b**) as a manifestation of their antiproliferative action. Initially, cell cycle analysis was performed using the cell permeable DNA binding dye propidium iodide to determine whether the antiproliferative effect resulted from cell cycle arrest. For this purpose, A549 cells were treated with **9a** and **9b** at concentrations of 50 nM as well as 100 nM for 48 h to study the distribution of cells at different phases of cell cycle as shown in Fig. **3**. The results demonstrated that compared to untreated cells (panel A), the cells treated with **9a** (Panel D and E) and **9b** (Panel F and G) displayed significant arrest in G2/M phase with increasing concentrations of **9a** and **9b** which were not only comparable but superior to **CA-4** (Panel B and C). Compounds **9a** and **9b** arrested 59.5 % and 65.5 % cells, respectively, in G2/M phase compared to 58.7 % blocked by **CA-4** as shown in Table **2**. Therefore, treatment with compounds **9a** and **9b** causes a clear G2/M arrest pattern in a concentration-dependent manner, with a concomitant decrease in cells found in other phases of the cell cycle. These results demonstrate that the growth inhibitory effect of these hybrids are induced because of the arrest in G2/M phase during cell cycle progression in a concentration dependent manner.

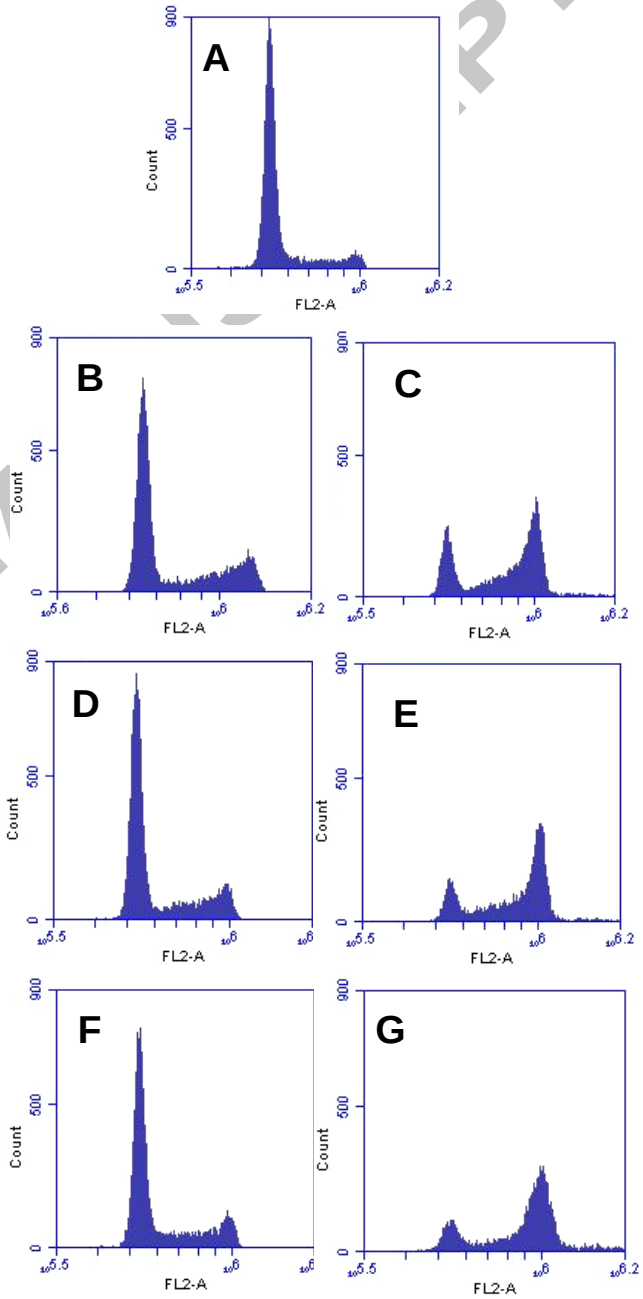


Figure 3: FACS analysis of cell cycle distribution of A549 cells after treatment with **CA-4**, **9a** and **9b** at 50 nM and 100 nM for 48 h. Cell cycle analysis was performed employing propidium iodide as indicated under Materials and Methods.

Table 2: Distribution of cell cycle phases following treatment with compounds (**CA-4**, **9a** and **9b**) in A549 cells was quantified by flow cytometry.

Panel: Compd	Con(nM)	% of cells in phase		
		G0/G1	S	G2/M
A: Control		78.5	9.4	12.1
B: **CA-4**	50	56.1	12.6	31.3
C: **CA-4**	100	26.1	15.9	58.7
D: **9a**	50	63.7	10.6	25.9
E: **9a**	100	21.5	17.6	59.5
F: **9b**	50	60.6	14.7	25.1
G: **9b**	100	19.8	15.4	65.5

3.3. Inhibition of Tubulin Polymerization

The G2/M phase arrest of cells takes place due to the perturbation in the mitosis cell division machinery and the tubulin is one of the important structural proteins in the mitosis process. Therefore, as **9a** and **9b** showed significant antiproliferative activity as well as arrest in the G2/M phase in the cell cycle analysis, it is likely that they would inhibit the polymerization of tubulin.[39] The progression in tubulin polymerization was investigated by monitoring the increase in fluorescence emission at 420 nM at 3 μM concentration employing CA-4 as the positive control (Fig. **4**). Compounds **9a** and **9b** inhibited tubulin polymerization by 72.89 %, 74.43 % respectively, which is comparable to CA-4. Furthermore, we decided to elucidate the action of **9a** and **9b** towards tubulin by determining their IC_{50} values at different concentrations. It is observed that **9a** and **9b** significantly inhibit the tubulin assembly with IC_{50} values of 1.67 and 1.00 μM as shown in Table **3**. These results suggest that antiproliferative activity as well as cell cycle analysis correlates well with the effect on the inhibition of tubulin polymerization and these results support that compounds **9a** and **9b** are potent inhibitors of tubulin assembly.

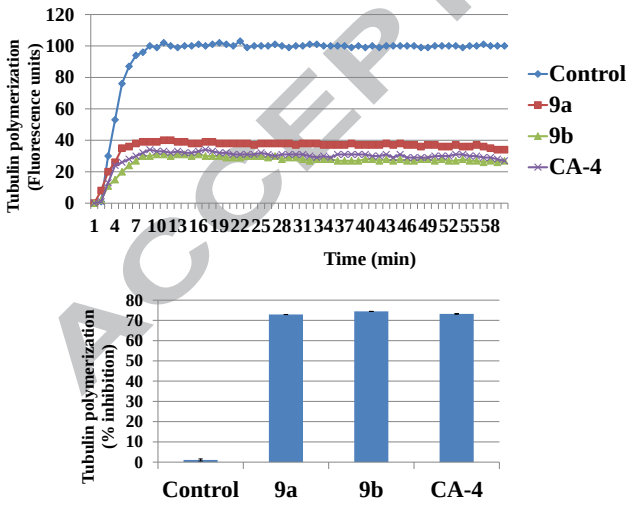


Figure 4: Effect of compounds on tubulin polymerization: Tubulin polymerization was monitored by the increase in fluorescence at 360 nm (excitation) and 420 nm (emission) for 1 h at 37 °C. Synthesized compounds **9a** and **9b** and **CA-4** were included at a final concentration of 3 μM **9a** and **9b** and **CA-4** used as positive control. Percentage of tubulin polymerization inhibition compared to control.

Table 3. Inhibition of tubulin polymerization (IC_{50}) of mimics **9a, 9b** and **CA-4**.

Compound	IC_{50} [a] ± SD (in μM)	% of Tubulin inhibition
9a	1.67±0.04	72.89
9b	1.00±0.04	74.43
CA-4	1.60±0.07	73.20

Note: [a] Concentration of drug to inhibit 50% of tubulin assembly. Values indicated are the mean ± SD of two different experiments performed in triplicates. Statistical analysis was performed using Graph Pad Prism software version 5.01.

3.4. Immunocytochemistry

Spindle poisons exert their action through the disruption of chromosome separation during mitosis.[40] To validate the effect of these compounds on the disruption of microtubule dynamics in living cells, studies were carried out to examine the in situ effects of **9a** and **9b** on cellular microtubules. A549 cells seeded on sterile cover slips were treated with these compounds and **CA-4** was employed as a standard at a concentration of 50 nM for 48 h. The confocal images depicted in Fig. **5** showed that the untreated lung cancer cell line displayed the normal distribution of microtubules. However, cells treated with compounds **9a** and **9b** showed disrupted microtubule organization (Fig. **5**), thus demonstrating the inhibition of tubulin polymerization. The density of microtubules was pronounced at the cell periphery with disorganized central networks and the standard **CA-4** also showed disrupted microtubule organization. This immunofluorescence study showed that the level of tubulin polymerization inhibition for both **9a** and **9b** was comparable to that of **CA-4**.

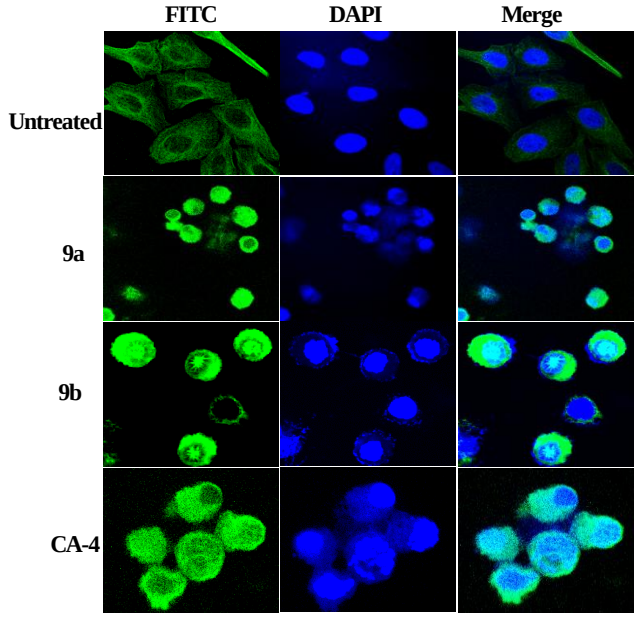


Figure 5: A549 cells were treated with compounds **9a** and **9b** at 50 nM concentration for 48 h followed by staining with α-tubulin antibody. Microtubule organization was clearly observed by green colour tubulin network like structures in untreated cells (UT) and was found to be disrupted in cells treated with compounds **9a** and **9b**. **CA-4** was used as positive control.

3.5. Distribution of soluble versus polymerized tubulin in cells

Microtubules exhibit dynamic equilibrium with free tubulin monomers in the cells. Pharmacological agents exploit this property of microtubules to exert their anticancer effects.[41] Since these mimics inhibit the tubulin polymerization and

disturbs the microtubule dynamics, we elucidated the levels of soluble versus polymerized forms of tubulin in A549 cells following treatment with 1μM of **9a** and **9b** for 48 h. In addition, cells were treated with combretastatin A-4 (**CA-4**, 1 μM) and paclitaxel (1 μM) as positive controls and DMSO as negative in parallel experiments. Subsequently, these soluble and polymerized fractions were collected and subjected to Western blot analysis. This analysis reveals that the amount of tubulin protein in both soluble and polymerized fractions was approximately the same in DMSO treated cells. The standard combretastatin A-4 treated cells exhibited a shift of tubulin from the polymerized fraction into the soluble fraction. In comparison, paclitaxel a microtubule polymerization agent showed more amount of tubulin in the polymerized fraction. As expected the cells treated with **9a** and **9b** significantly increased the tubulin content in the soluble fraction. Specifically, **9b** treated cells showed a more distinct shift in tubulin balance, with almost all tubulin present in the soluble fraction similar to that of the positive control. Therefore, increased tubulin in soluble fraction of cells treated by these hybrids corroborated with the inhibition of tubulin assembly and arrest of cells in G2/M phase as shown in Fig. **6**.

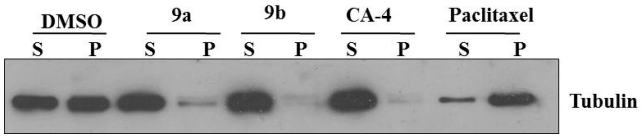


Figure 6: A549 cells were treated with 1 μM of **9a** and **9b** for 48 h. **CA-4** and paclitaxel were used as reference standards. Levels of tubulin was detected by Western blot analysis.

3.6. Competitive Colchicine binding assay

These hybrids showed similar inhibitory effects on tubulin polymerization to that of **CA-4** and which prompted us to investigate whether these compounds bind to the colchicine site of the tubulin by employing fluorescence based assay. Tubulin (3 μM) was incubated with various concentrations of **9a** and **9b** (0–20 μM) in the presence of colchicine (3 μM) with **CA-4** as positive control at 37 °C for 60 min and the fluorescence of tubulin–colchicine complex was monitored at 435 nm by exciting at 350 nm. We observed an increase in fluorescence of tubulin–colchicine complex in the presence of these hybrids. This is in the agreement with a previous investigation in which **CA-4** is reported to give fluorescence upon binding at the colchicine site.[42] Therefore, the experiment was carried out both in the presence and absence of colchicine to obtain fluorescence values of the desired tubulin–colchicine complex, the fluorescence values of tubulin–test compounds complex was subtracted from the tubulin–test compounds–colchicine complex. It was observed that at low concentration these hybrids (**9a** and **9b**) and **CA-4** showed significant affinity towards colchicine site, whereas at higher concentrations (10 μM and 15 μM) **9b** and **CA-4** showed more binding affinity while **9a** showed lower binding affinity as shown in Fig. **7**. Compared to the positive control **CA-4**, compound **9b** exhibited more affinity towards colchicine site whereas **9a** has less affinity. Vinblastine was used as a negative control, which is known to bind at a different site and has no effect on tubulin–colchicine complex. Therefore this study indicates that **9a** and **9b** bind at the colchicine site of the tubulin.

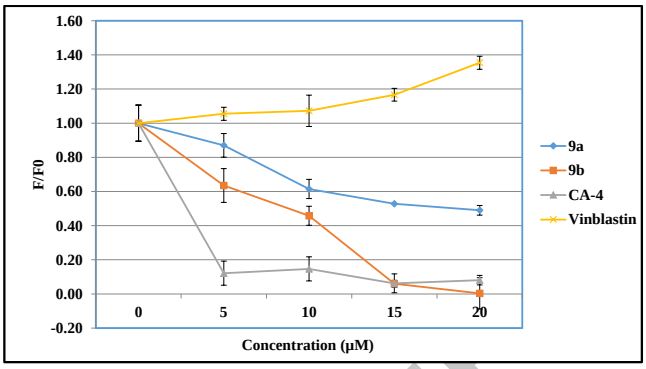


Figure 7: Fluorescence based colchicine competitive binding assay of conjugates **9a** and **9b** were carried out at various concentrations containing 3 μM of tubulin and colchicine for 60 min at 37 °C. Combretastatin A4 was used as a positive control whereas vinblastine was used as negative control which binds at vinca site. Fluorescence values are normalized to DMSO (control).

3.7. Hoechst staining

Apoptosis is one of the major pathways of programmed cell death. Chromatin condensation, nuclear shrinking, and fragmented nuclei are some of the characteristics of apoptotic cells. Disruption of microtubule formation leads to cell cycle arrest in the G2/M phase followed by apoptotic cell death.[43] To investigate the apoptotic inducing effect of these hybrids (**9a** & **9b**) and **CA-4**, Hoechst staining assay was carried out on A549 cells. Hoechst 33258 is a cell membrane permeable nuclear staining dye, which emits blue fluorescence and stains the live cell nuclei as light blue, whereas the apoptotic cell nuclei appear as bright blue due to chromatin condensation. A549 cells were treated with **9a**, **9b** and **CA-4** at their IC_{50} concentration (50 nM) for 48 h and stained with Hoechst. The results from Fig. **8** indicated that in the control group, the untreated cells did not show obvious morphological changes (all the cells exhibited uniform rounded cell morphology), however in compound treated cells exhibited typical apoptotic morphology such as highly condensed nuclei (brightly stained) as indicated by arrows. This observation demonstrates that these hybrids **9a**, **9b** and **CA-4** are able to induce apoptosis in A549 cells.

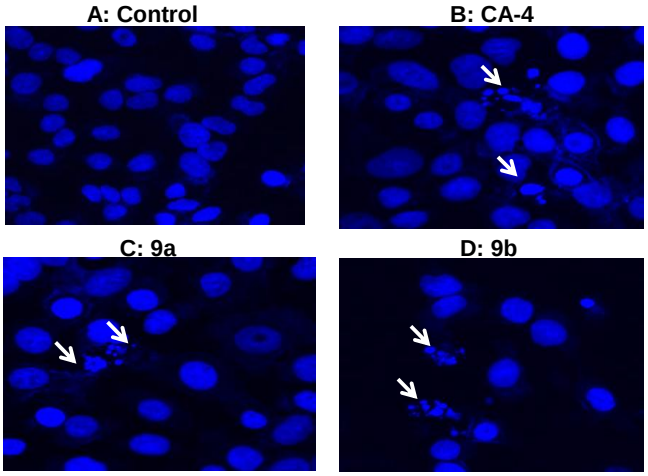


Figure 8: Hoechst staining in A549 cells. A: Untreated control cells (Control, A549), B: **CA-4** (50 nM), C: **9a** (50 nM) and D: **9b** (50 nM).

3.8. Annexin V-FITC for apoptosis

To characterize the mode of cellular death induced by **9a** and **9b**, we performed a biparametric cytofluorimetric analysis assay[44] by using propidium iodide (PI), which stains DNA and enters only dead cells, and fluorescent immunolabeling of the protein annexin-V, which binds to phosphatidyl serine (PS) in a highly selective manner. Dual staining for annexin-V and with PI permits discrimination between live cells (annexin-V−/PI−), early apoptotic cells (annexin-V+/PI−), late apoptotic cells (annexin-V+/PI+) and necrotic cells (annexin-V−/PI+). A549 cells were treated with these hybrids for 48 h at 50 and 100 nM concentrations and CA-4 was employed as a reference compound to examine the apoptotic effect. After treatment, it was observed that **9a** and **9b** significantly induced apoptosis in A549 cells as shown in Fig. **9**. As depicted in Fig. **9**, both **9a** and **9b** induced an accumulation of annexin-V positive cells in comparison with the control in a concentration dependent manner.

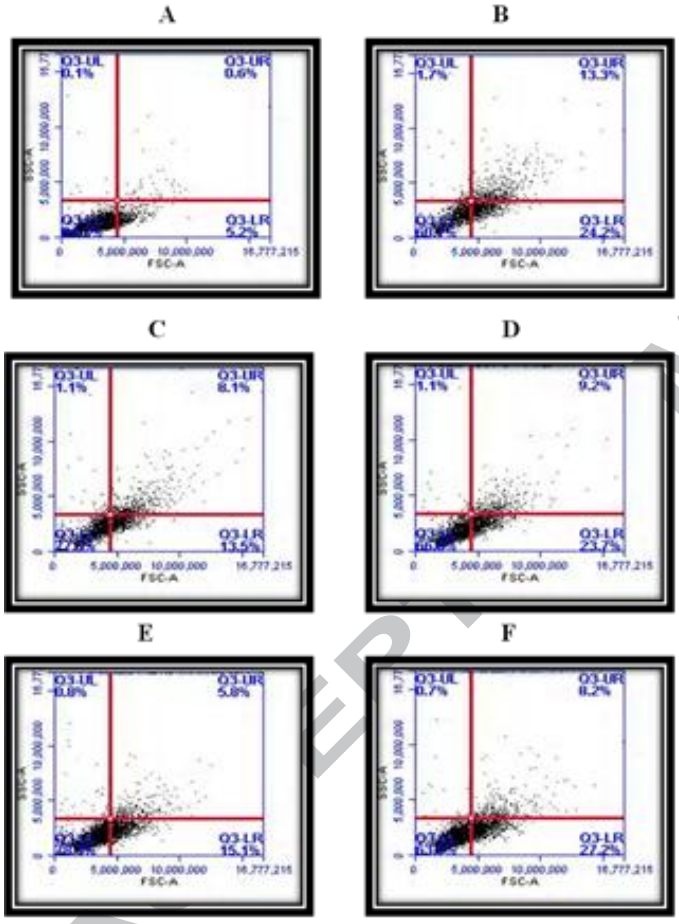


Figure 9: Annexin V-FITC staining; A: Untreated control cells (A549), B: **CA-4** (100 nM), C: **9a** (50 nM) and D: **9a** (100 nM), E: **9b** (50 nM) and F: **9b** (100 nM).

Table 4: Apoptotic effects of compounds **9a**, **9b** and CA-4 in A549 lung cancer cells.[a]

Sample	Concentration	UL(%)	UR(%)	LL(%)	LR(%)
Control		0.1	0.6	94.2	5.2
CA-4	100 nM	1.7	13.3	60.8	24.2
9a	50 nM	1.1	8.1	77.3	13.5
9a	100 nM	1.1	9.2	66.0	23.7
9b	50 nM	0.8	5.8	78.3	15.1
9b	100 nM	0.7	8.2	63.9	27.2

Note: [a]Determined by Annexin V–FITC assay; data are the quantification of the FACS results shown in **Figure 5**.

3.9. Measurement of mitochondrial membrane potential (ΔΨm)

Mitochondria play an essential role in the propagation of apoptosis.[45] It is well established that at an early stage, apoptotic stimuli alter the mitochondrial transmembrane potential (Δψmt). Δψmt was monitored by the fluorescence of the dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine (JC-1). Mitochondrial changes, including loss of mitochondrial membrane potential (ΔΨm), are key events that take place during drug-induced apoptosis. JC-1 has the unique property of forming orange fluorescent aggregates locally and spontaneously under high mitochondrial ΔΨM, whereas the monomeric form fluoresces in green.[46] When a collapse of ΔΨM occurs, JC-1 is only found in monomeric form. In this study we have investigated the involvement of mitochondria in the induction of apoptosis by **9a**, **9b** and **5F-203**. Treated A549 cells in the presence of compounds **9a** and **9b** after 48 h, it was observed that a dramatic shift in fluorescence in a dose-dependent manner (50 and 100 nM) compared to the control cells, indicating depolarization of mitochondrial membrane potential (Fig. **10**).

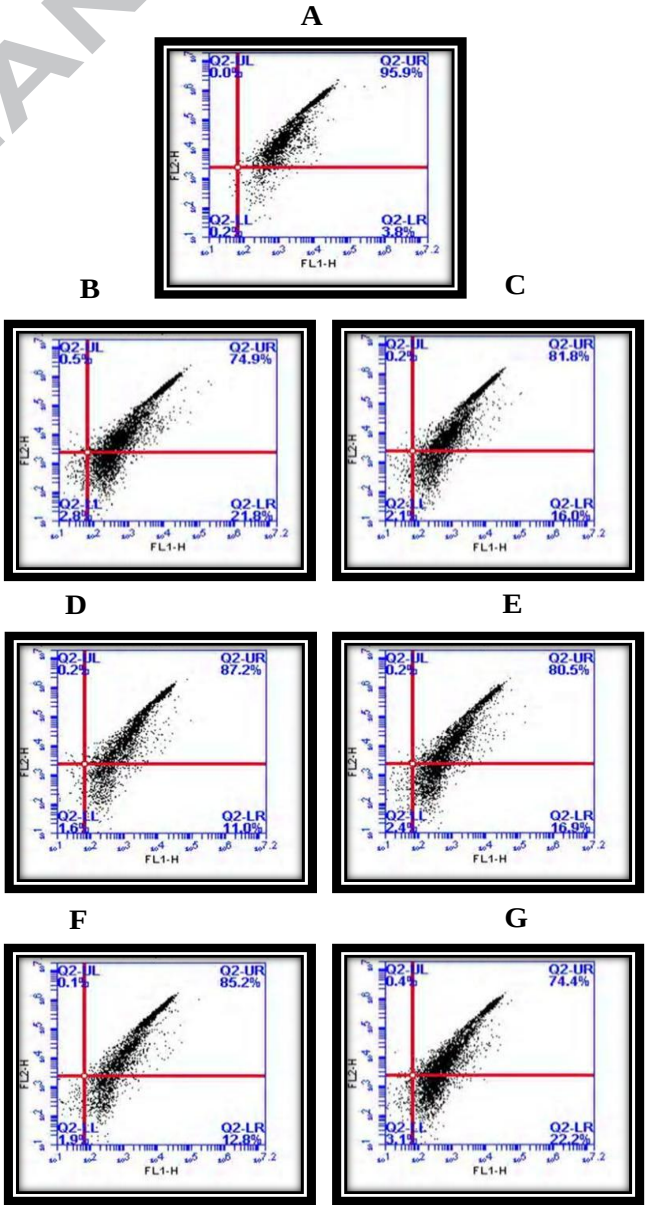


Figure 10: Drops in mitochondrial membrane potential (ΔΨm) was assessed by JC-1 staining of A549 cells treated with compounds **9a** and **9b** and samples were then subjected to flow cytometry analysis on a FACScan (Becton Dickinson). A: Untreated control cells (A549), B: **CA-4** (100 nM), C: **5F-203** (100 nM) D: **9a** (50 nM) and E: **9a** (100 nM), F: **9b** (50 nM) and G: **9b** (100 nM).

3.10. Effect on intracellular ROS generation

Mitochondrial membrane depolarization is associated with mitochondrial production of ROS.[47] Therefore, we investigated whether ROS production increased after treatment with the test compounds. We analyzed the production of ROS by flow cytometry utilizing fluorescence indicator: DCFDA (2,7-dichlorodihydrofluorescein diacetate).[48] Treatement of A549 cells in the presence of compounds **9a** and **9b** at 50 and 100 nM and 5F-203 100 nM concentrations for 48 h, the level of ROS was significantly increased. The results presented in Fig. **11**, where it can be observed that both **9a** and **9b** induced the production of significant amounts of ROS in comparison with control cells, which agrees with the previously described dissipation of Δψm.

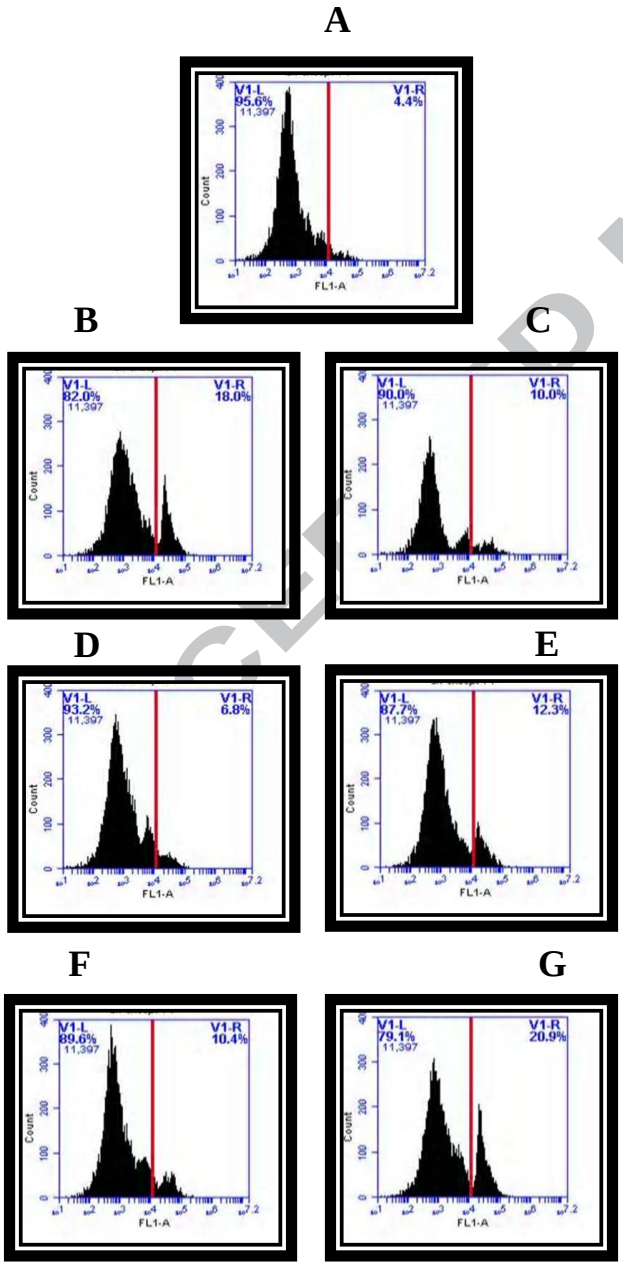


Figure 11: Production of ROS in human lung cancer cells (A549); A: Untreated control cells (A549), B: **CA-4** (100 nM), C: **5F-203** (100 nM) D: **9a** (50 nM) and E: **9a** (100 nM), F: **9b** (50 nM) and G: **9b** (100 nM), the cells were stained with DCFDA and analyzed by flow cytometry.

The ratio of DCF-positive cells for compounds **9a** and **9b** was 6.8 and 10.4 % and 12.3 and 20.9 % respectively at 50 and 100 nM concentrations, whereas **CA-4** and **5F-203** 18.0 and 10.0 respectively. The test results evidenced that these compounds had enhanced the generation of ROS in A549 cells. Altogether, these results indicate that these compounds induced apoptosis through the mitochondrial pathway. In this context, it is interesting to note that many other antimitotic compounds induce apoptosis through the mitochondrial (intrinsic) pathway.[48]

3.11. Effect on activation of caspase 3

Caspases, are a family of cysteine-aspartic proteases that are crucial mediators of apoptosis. Among them, caspase-3 is well understood among the mammalian caspases in terms of its specificity and its role in apoptosis. Furthermore, there are some reports [45,49,50] that indicate that the cell cycle arrest at G2/M phase takes place by the induction of cellular apoptosis. Hence, it was considered of interest to understand the correlation of cytotoxicity with that to apoptosis by these hybrids (**9a** and **9b**).

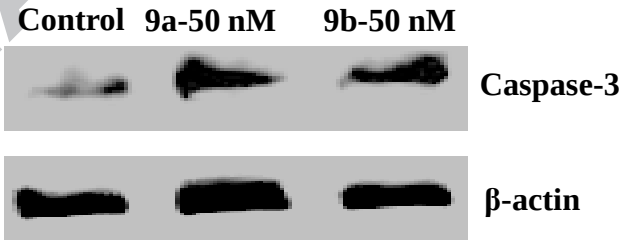


Figure 12: Effect of **9a** and **9b** on caspase-3 level. A549 cells were treated with these compounds at 50 nM concentration for 48 h and western blot analysis was performed. β-actin was used as internal loading control.

In this study, A549 cells were treated with them at 50 nM concentration for 48 h and Western blot analysis was performed. An increase in the levels of caspase-3 was observed upon treatment of cells with these hybrids compare to control. This results clearly indicates that **9a** and **9b** induced apoptosis in A549 cells by activation of caspase-3 as shown in Fig. **12**.

4. Molecular Modeling

To understand the binding mode and SAR for these hybrids, some molecular modeling studies were performed on all molecules. As these hybrids have a similarity to **CA-4** (an inhibitor of tubulin polymerization), we performed the docking studies of on the tubulin protein and the tubulin crystal structure was obtained from the Protein Data Bank (PDB ID 3E22).[51] Necessary corrections to the protein were carried out using Protein Preparation Wizard from Schrodinger package. Geometry of the molecule was optimized in Gaussian 09 using PM3 semi-empirical method.[52] Further docking studies were performed using AutoDock 4.2 docking software.[53] The molecular docking studies have been performed on all the molecules. The highest ranking pose of **9b** shows that molecule fits well in the colchicine binding domain (Fig. **13B**). The trimethoxyphenyl group was at the hydrophobic site deep in the β chain and amino acid close in contact with this group are Val238, Thr239, Cys241, Leu242, Ala250, Leu252,

Leu255, Ala316, Ala317, Val318, Thr353, Ala354, Ile378 of β chain. The benzothiazole is extended towards the α,β interface of the tubulin and forms close contact with Gln11, Ser178 and Tyr224 of α chain and Glu247 and Gln248 residues of β chain. However, 1-phenytriazole is in close contact with Asn101, Ala180 and Val181 residues of α chain and Asn258, Met259 and Lys352 residues of β chain. Superimposed pose of **9b** with cocrystal ligand shows the trimethoxyphenyl ring is at the same position whereas c ring of colchicine is at the same position to that of triazole ring (Fig. **13C**). Interestingly **9a** and **9b** have shown a similar binding pose (Fig. **13D**). However the 3D geometry of 1,2,3-triazole and 1,2,4-triazole were totally different, where 1,2,3-triazole is having slightly bent geometry while 1,2,4-triazole is having L shaped. Due to this geometrical difference, compounds from these series accommodate different binding poses Fig. **13E**. If we compare the binding pose of **9b** and **6b** reveal that both compounds having opposite binding pose. For **9b** trimethoxy phenyl was in hydrophobic site whereas for **6b** trimethoxyphenyl group in at interface of α- β chain. This may be the reason for the least activity of compounds from this series. Besides geometry of 1,2,4-triazole and 1,2,3,4-tetrazole is similar, therefore their binding poses are also similar (Fig. **13F**). Thus the docking studies suggest that these hybrids fit well in the colchicine binding domain of the tubulin. Compounds with 1,2,3-triazole moiety having different binding pose than the remaining compounds and having lesser activity.

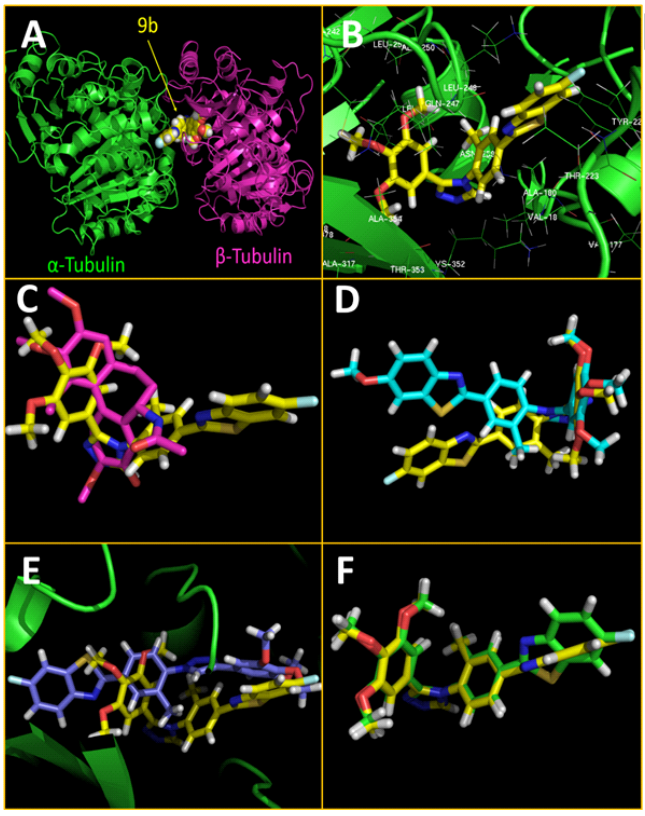


Figure 13: A) Docking pose of **9b** (yellow) in colchicine binding domain of tubulin where α-chain is in green color and β-chain in pink color B) Docking pose of **9b** (Yellow) and protein in green color. C) Superimposed pose of **9b** with cocrystal ligand colchicine (pink) D) Superimposed pose of **9b** with **9a** (cyan) E) Superimposed pose of **9b** with **6b** (blue) F) Superimposed pose of **9b** with **12b** (green).

5. Conclusion

In the present investigation, a series of *cis*-restricted triazole/tetrazole mimics of CA-4 linked benzothiazole hybrids were designed, synthesized and evaluated for their cytotoxic potential against selected human cancer cell lines. These hybrids exhibited promising activity and the lead compounds like **9a** and **9b** displayed potent IC_{50} values in the range of 0.054 and 0.048 μM respectively, against human lung cancer cell line (A549). The importance of 1,2,4-triazole ring on CA-4 bridge in comparision to 1,2,3-triazole and 1,2,3,4-tetrazole rings is evident from the SAR analysis. The methyl group on C'-3 position of the benzothiazole moiety having either electron withdrawing group (F) or electron releasing group (OMe) is essential for imparting potent antiproliferative activity. In the case of **9a** having methoxy group on C-6 position as well as in **9b** having a fluoro group on C-6 position of benzothiazole moiety exhibited significant activity. The SAR provided a useful insight that could be utilized in developing improved leads based on these hybrid scaffolds.FACS analysis demonstrated that these hybrids arrest of cell cycle progression in G2/M phase in a concentration dependent manner. Tubulin polymerization assay showed that they are potent inhibitors of tubulin polymerization. Westren blot analysis revealed that these hybrids remarkably inhibit tubulin polymerization. To understand the mode of binding, colchicine competitive binding assay and molecular docking studies were undertaken which demonstrated that these hybrids bind at the colchicine site of the tubulin. Interestingly,the triggering of apoptotic cell death was investigated by studying their effect by Hoechst staining, Annexin-V-FITC assay, mitochondrial membrane potential, ROS generation and caspase-3 activation. Moreover, **9b** has a potential to be taken up for preclinical studies for its development as a lead towards the treatment of lung cancer.

6. Experimental Section

Chemistry

All reagents, starting materials and solvents were obtained from commercial suppliers and used as such without further purification. Reactions were monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates (60 F254). Visualization was accomplished with either UV light, iodine adsorbed on silica gel or by immersion in ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde, 2, 4-DNP, KMnO4, ninhydrin solution followed by heating with a heat gun for ~15 seconds. Column chromatography was performed with Merck 60–120 mesh silica gel. Deuterated solvents for NMR spectroscopic analyses were used as received. All ^{1}H NMR and ^{13}C NMR spectra were obtained using a 300 MHz, 400 MHz, and 500 MHz spectrometer and coupling constants were measured in Hertz. All chemical shifts were quoted in ppm relative to TMS, using the residual solvent peak as a reference standard. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. EI-MS spectra were recorded on Micro mass, Quattro LC using ES I+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High -resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Chemical nomenclature was generated using Chembiodrawultra 12.0.

6.1.1 General Method A for the Synthesis of substituted Benzanilides 16-18(a-d):

Substituted 4–Nitrobenzoyl chlorides (**15a-c**) (0.029 mol) were added slowly to a solution of the appropriately substituted

anilines (**14a**–**d**) (0.04 mol) in pyridine (110 mL). The resulting solution was stirred under reflux for 3 h and then poured into ice water (400 mL).The precipitate formed was collected and washed with 2N HCl (100 mL), followed by water and methanol, to afford the respective benzanilides **16-18(a-d)** as a yellow solids.

6.1.2. N-(4-Methoxyphenyl)-4-nitrobenzamide (16a):

The compound **16a** was synthesized by following general procedure **A**, as a yellow solid; Yield 85%; mp: 199-202 °C; ^{1}H NMR (300 MHz, DMSO): δ 10.47 (s, 1H), 8.36 (d, *J* = 8.8 Hz, 2H), 8.17 (d, *J* = 8.8 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 3.75 (s, 3H); MS (ESI): *m/z* 273 $(M+H)^+$;

6.1.3. N-(4-Methoxyphenyl)-3-methyl-4-nitrobenzamide (17a):

The compound **17a** was synthesized by following general procedure **A**, as a yellow solid; Yield 71%; mp: 152-154 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.91 (s, 1H), 7.83 (s, 1H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 2H), 6.90 (d, *J* = 9.0 Hz, 2H), 3.82 (s, 3H), 2.64 (s, 3H); MS (ESI): *m/z* 287 $(M+H)^+$.

6.1.4. 2-Methoxy-N-(4-methoxyphenyl)-4-nitrobenzamide (18a):

The compound **18a** was synthesized by following general procedure **A**, as a yellow solid; Yield 85%, mp: 156-158 ° C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.86 (d, *J* = 8.3 Hz, 1H), 7.65 (s, 1H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.38 (d, *J* = 8.3 Hz, 1H), 6.92 (d, *J* = 9.0 Hz, 2H), 4.00 (s, 3H), 3.82 (s, 3H); MS (ESI): *m/z* 303 $(M+H)^+$;

6.1.5. N-(4-Fluorophenyl)-4-nitrobenzamide (16b):

The compound **16b** was synthesized by following general procedure **A**, as a yellow solid; Yield 88%; mp: 164 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (d, *J* = 8.7 Hz, 2H), 8.04 (d, *J* = 8.6 Hz, 2H), 7.89 (s, 1H), 7.61 (dd, *J* = 8.6, 4.7 Hz, 2H), 7.10 (t, *J* = 8.6 Hz, 2H); (ESI): *m/z* 261 $(M+H)^+$;

6.1.6. N-(4-Fluorophenyl)-3-methyl-4-nitrobenzamide (17b):

The compound **17b** was synthesized by following general procedure **A**, as a yellow solid; Yield 91%; mp: 160-162 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.96 (s, 1H), 7.84 (s, 1H), 7.78 (d, *J* = 8.3 Hz, 1H), 7.60 (dd, *J* = 8.8, 4.7 Hz, 2H), 7.11 – 7.05 (m, 2H), 2.65 (s, 3H); MS (ESI): *m/z* 297 $(M+Na)^+$.

6.1.7. N-(4-Fluorophenyl)-2-methoxy-4-nitrobenzamide (18b):

The compound **18b** was synthesized by following general procedure **A**, as a yellow solid; Yield 82%; mp: 175-178 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO): δ 10.18 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.81 – 7.71 (m, 3H), 7.68 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.06 (t, *J* = 8.7 Hz, 2H), 4.05 (s, 3H); MS (ESI): *m/z* 291$(M+H)^+$.

6.1.8. N-(3,5-Dimethoxyphenyl)-4-nitrobenzamide (16c):

The compound **16c** was synthesized by following general procedure **A**, as a yellow solid; Yield 88%; mp: 211-213 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO): δ 10.22 (s, 1H), 8.31 (d, *J* = 8.9 Hz, 2H), 8.20 (d, *J* = 8.9 Hz, 2H), 7.07 (d, *J* = 2.2 Hz, 2H), 6.25 (t, *J* = 2.2 Hz, 1H), 3.80 (s, 6H); MS (ESI): *m/z* 303 $(M+H)^+$.

6.1.9. N-(3,5-Dimethoxyphenyl)-3-methyl-4-nitrobenzamide (17c):

The compound **17c** was synthesized by following general procedure **A**, as a yellow solid; Yield 72%; mp: 189-192 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.81 (s, 1H), 7.75 (dd, *J* = 8.4, 1.5 Hz, 1H), 6.87 (d, *J* = 2.0 Hz, 2H), 6.29 (t, *J* = 2.2 Hz, 1H), 3.79 (s, 6H), 2.62 (s, 3H); MS (ESI): *m/z* 317 $(M+H)^+$.

6.1.10. N-(3,5-Dimethoxyphenyl)-2-methoxy-4-nitrobenzamide (18c):

The compound **18c** was synthesized by following general procedure **A**, as a yellow solid; Yield 73%; mp: 176-179 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (d, *J* = 8.3 Hz, 2H), 7.66 (d, *J* = 1.4 Hz, 1H), 7.37 (dd, *J* = 8.3, 1.7 Hz, 1H), 6.88 (d, *J* = 2.1 Hz, 2H), 6.31 (t, *J* = 2.2 Hz, 1H), 4.02 (s, 3H), 3.81 (s, 6H); MS (ESI): *m/z* 333 $(M+H)^+$.

6.1.11. 4-Nitro-N-(3,4,5-trimethoxyphenyl)benzamide (16d):

The compound **16d** was synthesized by following general procedure **A**, as a yellow solid; Yield 88%; mp: 220-224 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO): δ 10.12 (s, 1H), 8.32 (d, *J* = 8.8 Hz, 2H), 8.20 (d, *J* = 8.8 Hz, 2H), 7.19 (s, 2H), 3.88 (s, 6H), 3.81 (s, 3H); MS (ESI): *m/z* 333 $(M+H)^+$.

6.1.12. 3-Methyl-4-nitro-N-(3,4,5-trimethoxyphenyl)benzamide (17d):

The compound **17d** was synthesized by following general procedure **A**, as a yellow solid; Yield 76%; mp: 210-214 °C;^{1}H NMR (500 MHz, $CDCl_3$): δ 8.23 (s, 1H), 8.03 (s, 1H), 8.02 (s, 1H), 7.51 (s, 1H), 6.85 – 6.58 (m, 2H), 3.84 – 3.82 (m, 6H), 3.81 – 3.80 (m, 3H), 2.44 – 2.42 (m, 3H); MS (ESI): *m/z* 347 $(M+H)^+$.

6.1.13. 2-Methoxy-4-nitro-N-(3,4,5-trimethoxyphenyl)benzamide (18d):

The compound **18d** was synthesized by following general procedure **A**, as a yellow solid; Yield 77%; mp: 178-180 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO): δ 9.42 (s, 1H), 7.86 (d, *J* = 8.3 Hz, 1H), 7.73 (s, 1H), 7.58 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.09 (s, 2H), 4.02 (s, 4H), 3.85 (s, 7H), 3.81 (s, 3H); MS (ESI): *m/z* 363 $(M+H)^+$.

6.2. General Method B for the Synthesis of Substituted Thiobenzanilides 19-21(a-d):

To a solution of substituted benzanilides **16-18(a-d)** (6g, 0.018 mol) in toluene (40 mL), Lawesson's reagent (0.9 eq) was added and reflux it for 8 h. After completion of the reaction, cool to rt and solvent was removed in vaccuo and work up with ethylacetate and water. Separation of the organic layer and evaporation followed by column chromatography gave yellow solids **19-21(a-d)**

6.2.1. N-(4-methoxyphenyl)-4-nitrobenzothioamide (19a):

The compound **19a** was synthesized by following general procedure **B**, as a yellow solid; Yield 84%; mp: 173-175 °C; MS (ESI): *m/z* 289 $(M+H)^+$.

6.2.2. N-(4-methoxyphenyl)-3-methyl-4-nitrobenzothioamide (20a):

The compound **20a** was synthesized by following general procedure **B**, as a yellow solid; Yield 72%; mp: 160-162 °C; MS (ESI): *m/z* 303 $(M+H)^+$.

6.2.3. 2-Methoxy-N-(3-methoxyphenyl)-4-nitrobenzothioamide (21a):

The compound **21a** was synthesized by following general procedure **B**, as a yellow solid; Yield 69%; mp: 170-171 °C; MS (ESI): *m/z* 319 $(M+H)^+$.

6.2.4. N-(4-fluorophenyl)-4-nitrobenzothioamide (19b):

The compound **19b** was synthesized by following general procedure **B**, as a yellow solid; Yield 80%; mp: 143-145 °C; MS (ESI): *m/z* 277 $(M+H)^+$.

6.2.5. N-(4-fluorophenyl)-3-methyl-4-nitrobenzothioamide (20b):

The compound **20b** was synthesized by following general procedure **B**, as a yellow solid; Yield 77%; mp: 164-165 °C; MS (ESI): *m/z* 291 $(M+H)^+$.

6.2.6. N-(3-fluorophenyl)-2-methoxy-4-nirobenzothioamide (21b):

The compound **21b** was synthesized by following general procedure **B**, as a yellow solid; Yield 73%; mp: 166-168 °C; MS (ESI): *m/z* 307 $(M+H)^+$.

6.2.7. N-(3,5-dimethoxyphenyl)-4-nitrobenzothioamide (19c):

The compound **19c** was synthesized by following general procedure **B**, as a yellow solid; Yield 90%; mp: 138-140 °C; MS (ESI): *m/z* 319 $(M+H)^+$.

6.2.8. N-(3,5-dimethoxyphenyl)-3-methyl-4-nitrobenzothioamide (20c):

The compound **20c** was synthesized by following general procedure **B**, as a yellow solid; Yield 74%; mp: 178-180 °C; MS (ESI): *m/z* 333 $(M+H)^+$.

6.2.9. N-(3,5-dimethoxyphenyl)-2-methoxy-4-nitrobenzothioamide (21c):

The compound **21c** was synthesized by following general procedure **B**, as a yellow solid; Yield 84%; mp: 198-200 °C; MS (ESI): *m/z* 349 $(M+H)^+$.

6.2.10. 4-Nitro-N-(3,4,5-trimethoxyphenyl)benzothioamide (19d):

The compound **19d** was synthesized by following general procedure **B**, as a yellow solid; Yield 84%; mp: 198-200 °C; MS (ESI): *m/z* 349 $(M+H)^+$.

6.2.11. 3-Methyl-4-nitro-N-(3,4,5-trimethoxyphenyl)benzothioamide (20d):

The compound **20d** was synthesized by following general procedure **B**, as a yellow solid; Yield 72%; mp: 136-138 °C; MS (ESI): *m/z* 363 $(M+H)^+$.

6.2.12. 2-Methoxy-4-nitro-N-(3,4,5-trimethoxyphenyl)benzothioamide (21d):

The compound **21d** was synthesized by following general procedure **B**, as a yellow solid; Yield 76%; mp 136-138 °C; MS (ESI): *m/z* 379 $(M+H)^+$.

6.3. General Method C for the Jacobson Synthesis of Substituted 2-(4-Nitrophenyl) benzothiazoles 22-24(a-d):

A solution of the substituted thiobenzanilides **19-21(a-d)** (0.017 mol) in aqueous sodium hydroxide (8 eq in 50 mL of water) containing ethanol (3 mL) was added dropwise to a pre–heating solution of potassium ferricyanide (4 eq) in water (30 mL) taken in a 250 mL RB flask at 90 °C over a period of 1 h. The resulting solution was stirred at 90 °C for a further 2 h and then cooled to room temperature. The precipitate formed was filtered and washed with water. Products were purified by column chromatography (ethylacetate/hexane) and to furnish the 4-nitrophenyl-benzothiazoles **22-24(a-d)** as yellow solids.

6.3.1. 6-Methoxy-2-(4-nitrophenyl)benzo[*d*]thiazole (22a):

The compound **22a** was synthesized by following general procedure **C**, as a yellow solid; Yield 62%; mp: 216-217 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (d, *J* = 8.9 Hz, 2H), 8.21 (d, *J* = 8.9 Hz, 2H), 8.00 (d, *J* = 9.0 Hz, 1H), 7.39 (d, *J* = 2.5 Hz, 1H), 7.15 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.92 (s, 3H); MS (ESI): *m/z* 287 $(M+H)^+$.

6.3.2. 6-Methoxy-2-(3-methyl-4-nitrophenyl)benzo[*d*]thiazole (23a):

The compound **23a** was synthesized by following general procedure **C**, as a yellow solid; Yield 64%; mp: 195-197 °C; ^{1}H NMR (300 MHz, CDCl3): δ 8.10 (d, *J* = 8.5 Hz, 1H), 8.04 (s, 1H), 7.99 (t, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 2.4 Hz, 1H), 7.15 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.93 (s, 3H), 2.72 (s, 3H); MS (ESI): *m/z* 301 $(M+H)^+$.

6.3.3. 6-Methoxy-2-(3-methoxy-4-nitrophenyl)benzo[*d*]thiazole (24a):

The compound **24a** was synthesized by following general procedure **C**, as a yellow solid; Yield 66%; mp: 201-203 °C; ^{1}H NMR (300 MHz, $CDCl_3$):δ 7.99 (dd, *J* = 11.3, 8.7 Hz, 2H), 7.90 (d, *J* = 1.2 Hz, 1H), 7.60 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.40 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.12 (s, 3H), 3.93 (s, 3H); MS (ESI): *m/z* 317 $(M+H)^+$.

6.3.4. 6-Fluoro-2-(4-nitrophenyl)benzo[*d*]thiazole (22b):

The compound **24b** was synthesized by following general procedure **C**, as a yellow solid; Yield 65%; mp: 194-195 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.39 – 8.32 (m, 2H), 8.27 – 8.20 (m, 2H), 8.08 (dd, *J* = 8.9, 4.8 Hz, 1H), 7.64 (dd, *J* = 7.9, 2.5 Hz, 1H), 7.38 – 7.27 (m, 1H); MS (ESI): *m/z* 275 $(M+H)^+$.

6.3.5. 6-Fluoro-2-(3-methyl-4-nitrophenyl)benzo[*d*]thiazole (23b):

The compound **23b** was synthesized by following general procedure **C**, as a yellow solid; Yield 67%; mp: 220-224 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (d, *J* = 8.5 Hz, 1H), 8.06 (dd, *J* = 8.7, 5.0 Hz, 2H), 7.99 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.62 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.28 (dt, *J* = 8.8, 2.3 Hz, 1H), 2.71 (s, 3H); MS (ESI): *m/z* 289 $(M+H)^+$.

6.3.6. 6-Fluoro-2-(3-methoxy-4-nitrophenyl)benzo[*d*]thiazole (24b):

The compound **24b** was synthesized by following general procedure **C**, as a yellow solid; Yield 63%; mp: 189-201 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (dd, *J* = 9.0, 4.8 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 1.3 Hz, 1H), 7.61 (ddd, *J* = 11.9, 8.2, 2.0 Hz, 2H), 7.32 – 7.25 (m, 1H), 4.11 (s, 3H); MS (ESI): *m/z* 305 $(M+H)^+$.

6.3.7. 5,7-Dimethoxy-2-(4-nitrophenyl)benzo[*d*]thiazole (22c):

The compound **22c** was synthesized by following general procedure **C**, as a yellow solid; Yield 60%; mp: 238-239 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37 – 8.32 (m, 2H), 8.24 (d, J = 8.9 Hz, 2H), 7.22 (d, J = 1.9 Hz, 1H), 6.56 (d, *J* = 1.9 Hz, 1H), 3.99 (s, 3H), 3.92 (s, 3H); MS (ESI): *m/z* 317 $(M+H)^+$.

6.3.8. 5,7-Dimethoxy-2-(3-methyl-4-nitrophenyl)benzo[*d*]thiazole (23c):

The compound **23c** was synthesized by following general procedure **C**, as a yellow solid; Yield 65%; mp: 223-225 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 (dd, *J* = 1.9, 1.2 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.06 – 7.02 (m, 2H), 6.83 (d, *J* = 2.4 Hz, 1H), 3.93 (s, 3H), 3.80 (s, 3H), 2.65 (s, 3H); MS (ESI): *m/z* 330 $(M+H)^+$.

6.3.9. 5,7-Dimethoxy-2-(3-methoxy-4-nitrophenyl)benzo[*d*]thiazole (24c):

The compound **24c** was synthesized by following general procedure **C**, as a yellow solid; Yield 64%; mp: 219-221 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$):δ 7.95 (d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 1.4 Hz, 1H), 7.63 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.20 (d, *J* = 1.9 Hz, 1H), 6.55 (d, *J* = 1.9 Hz, 1H), 4.10 (s, 3H), 3.98 (s, 3H), 3.92 (s, 3H);MS (ESI): *m/z* 347 $(M+H)^+$.

6.3.10. 5,6,7-Trimethoxy-2-(4-nitrophenyl)benzo[*d*]thiazole (22d):

The compound **22d** was synthesized by following general procedure **C**, as a yellow solid; Yield 66%; mp 160-168 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.8 Hz, 2H), 7.40 (s, 1H), 4.14 (s, 3H), 3.99 (d, *J* = 6.4 Hz, 6H); MS (ESI): *m/z* 347 $(M+H)^+$.

6.3.11. 5,6,7-Trimethoxy-2-(3-methyl-4-nitrophenyl)benzo[*d*]thiazole (23d):

The compound **23d** was synthesized by following general procedure **C**, as a yellow solid; Yield 69%; mp 176-179 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, *J* = 8.4 Hz, 1H), 7.86 (s, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.63 – 7.38 (m, 1H), 7.37 (s, 1H), 4.11 (d, *J* = 5.7 Hz, 6H), 3.97 (d, *J* = 5.6 Hz, 6H); MS (ESI): *m/z* 361 $(M+H)^+$.

6.3.12. 5,6,7-Trimethoxy-2-(3-methoxy-4-nitrophenyl)benzo[*d*]thiazole (24d):

The compound **24d** was synthesized by following general procedure **C**, as a yellow solid; Yield 66%; mp 185-187 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, *J* = 8.4 Hz, 1H), 7.86 (s, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.63 – 7.38 (m, 1H), 7.37 (s, 1H), 4.11 (d, *J* = 5.7 Hz, 6H), 3.97 (d, *J* = 5.6 Hz, 6H); MS (ESI): *m/z* 377 $(M+H)^+$.

6.4. General Method D for the Reduction of Substituted 2–(4–Nitrophenyl)benzothiazoles 25-27(a-d):

To a solution of substituted 2–(4–nitrophenyl)benzothiazoles (3 g, 8.66 mmol) in ethanol, tin(II) chloride dihydrate (3 eq, 25.98 mmol) was added and refluxed it for 3 h. The solvent was removed under vacuum and the resulting oil taken up in choloform (75 mL) was quenched with aq. $NaHCO_3$ solution. The resulting organic layer was separated and evaporated to leave a residue of the amine which was purified by column chromatography (eluent: ethylacetate/hexane).

6.4.1. 4-(6-Methoxybenzo[*d*]thiazol-2-yl)aniline (25a):

The compound **25a** was synthesized by following general procedure **D**, as a yellow solid; Yield 92%; mp 191-193 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (t, *J* = 8.6 Hz, 3H), 7.34 (d, *J* = 2.4 Hz, 1H), 7.07 (dd, *J* 8.9, 2.5 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 2H), 3.99 (s, 1H), 3.90 (s, 3H); MS (ESI): *m/z* 257 $(M+H)^+$.

6.4.2. 4-(6-Methoxybenzo[*d*]thiazol-2-yl)-2-methylaniline (26a):

The compound **26a** was synthesized by following general procedure **D**, as a yellow solid; Yield 95%; mp 151-153 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (d, *J* = 8.9 Hz, 1H), 7.78 (d, *J* = 1.2 Hz, 1H), 7.69 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.31 (d, *J* = 2.5 Hz, 1H), 7.04 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.71 (d, *J* = 8.3 Hz, 1H), 3.91 (s, 1H), 3.87 (s, 3H), 2.23 (s, 3H); MS (ESI): *m/z* 271 $(M+H)^+$.

6.4.3. 2-Methoxy-4-(6-methoxybenzo[*d*]thiazol-2-yl)aniline (27a):

The compound **27a** was synthesized by following general procedure **D**, as a yellow solid; Yield 85%; mp 181-183 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO): δ 7.78 (d, *J* = 8.9 Hz, 1H), 7.47 (s, 2H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.03 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 1H), 5.32 (s, 1H), 3.92 (s, 3H), 3.85 (s, 3H); MS (ESI): *m/z* 287 $(M+H)^+$.

6.4.4. 4-(6-Fluorobenzo[*d*]thiazol-2-yl)aniline (28b):

The compound **28b** was synthesized by following general procedure **D**, as a yellow solid; Yield 88%; mp 152-153 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (dd, *J* = 8.9, 4.8 Hz, 1H), 7.85 (d, *J* = 8.6 Hz, 2H), 7.52 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.17 (td, *J* = 8.9, 2.6 Hz, 1H), 6.73 (d, *J* = 8.6 Hz, 2H), 4.02 (s, 2H); MS (ESI): *m/z* 245 $(M+H)^+$.

6.4.5. 4-(6-Fluorobenzo[*d*]thiazol-2-yl)-2-methylaniline (29b):

The compound **29b** was synthesized by following general procedure **D**, as a yellow solid; Yield 92%; mp 203-205 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (dd, *J* = 8.9, 4.8 Hz, 1H), 7.85 (d, *J* = 8.6 Hz, 2H), 7.52 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.17 (td, *J* = 8.9, 2.6 Hz, 1H), 6.73 (d, *J* = 8.6 Hz, 2H), 4.02 (s, 2H); MS (ESI): *m/z* 259 $(M+H)^+$.

6.4.6. 4-(6-Fluorobenzo[*d*]thiazol-2-yl)-2-methoxyaniline (30b):

The compound **30b** was synthesized by following general procedure **D**, as a yellow solid; Yield 83%; mp 191-193 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (dd, *J* = 8.9, 4.8 Hz, 1H), 7.56 (d, *J* = 1.8 Hz, 1H), 7.51 (dd, *J* = 8.1, 2.6 Hz, 1H), 7.41 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.16 (td, *J* = 8.9, 2.6 Hz, 1H), 6.72 (dd, *J* = 8.1, 4.1 Hz, 1H), 4.16 (s, 2H), 3.97 (s, 3H). MS (ESI): *m/z* 276 $(M+H)^+$.

6.4.7. 4-(5,7-Dimethoxybenzo[*d*]thiazol-2-yl)aniline (28c):

The compound **28c** was synthesized by following general procedure **D**, as a yellow solid; Yield 90%; mp 150-152 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.90 – 7.85 (m, 2H), 7.14 (d, *J* = 2.0 Hz, 1H), 6.75 – 6.69 (m, 2H), 6.45 (d, *J* = 2.0 Hz, 1H), 3.98 (s, 1H), 3.95 (s, 3H), 3.88 (s, 3H); MS (ESI): *m/z* 286 $(M)^+$.

6.4.8. 4-(5,7-Dimethoxybenzo[*d*]thiazol-2-yl)-2-methylaniline (29c):

The compound **29c** was synthesized by following general procedure **D**, as a yellow solid; Yield 91%; mp: 142-145 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.75 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.16 (d, *J* = 2.0 Hz, 1H), 6.73 (d, *J* = 8.3 Hz, 1H), 6.47 (d, *J* = 2.0 Hz, 1H), 3.97 (s, 3H), 3.96 – 3.93 (m, 1H), 3.91 (s, 3H), 2.26 (s, 3H); MS (ESI): *m/z* 301 $(M+H)^+$.

6.4.9. 4-(5,7-Dimethoxybenzo[*d*]thiazol-2-yl)-2-methoxyaniline (30c):

The compound **30c** was synthesized by following general procedure **D**, as a yellow solid; Yield 82%; mp: 173-175 °C;

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, *J* = 1.6 Hz, 1H), 7.47 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.13 (d, *J* = 1.9 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.45 (d, *J* = 1.9 Hz, 1H), 4.13 (s, 2H), 3.96 (d, *J* = 6.8 Hz, 6H), 3.89 (s, 3H); MS (ESI): *m/z* 317 $(M+H)^+$.

6.4.10. 4-(5,6,7-Trimethoxybenzo[*d*]thiazol-2-yl)aniline (28d):

The compound **28d** was synthesized by following general procedure **D**, as a yellow solid; Yield 89%; mp: 145-147 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, *J* = 8.6 Hz, 2H), 7.30 (s, 1H), 6.73 (d, *J* = 8.6 Hz, 2H), 4.09 (s, 3H), 3.99 (s, 2H), 3.94 (d, *J* = 4.4 Hz, 6H); MS (ESI): *m/z* 317 $(M+H)^+$.

6.4.11. 2-Methyl-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)aniline (29d):

The compound **29d** was synthesized by following general procedure **D**, as a yellow solid; Yield 90%; mp: 150-152 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79 (s, 1H), 7.71 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.30 (s, 1H), 6.71 (d, *J* = 8.2 Hz, 1H), 4.09 (s, 3H), 3.94 (d, *J* = 7.0 Hz, 8H). MS (ESI): *m/z* 331 $(M+H)^+$.

6.4.12. 2-Methoxy-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)aniline (30d):

The compound **30d** was synthesized by following general procedure **D**, as a yellow solid; Yield 87%; mp 141-143 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (s, 1H), 7.47 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.31 (s, 1H), 6.75 (d, *J* = 8.1 Hz, 1H), 4.14 (s, 2H), 4.11 (s, 3H), 4.02–3.90 (m, 9H); MS (ESI): *m/z* 347 $(M+H)^+$.

6.5. General Method E for the Conversion amines to azides 25-27(a-d):

Substituted anilines **25-27(a-d)** (150 mg, 1 mmol) was dissolved in 2N HCl (7 mL) in a 25 mL round-bottomed flask and cooled to 0°C in an ice bath for 5min. To this stirred solution aq.$NaNO_2$ (3 mmol) was added portion wise and mixture was stirred for 30 min followed by NaN_3 (3 mmol) was added to the reaction mixture. The resulting solution was stirred at room temperature for 1 h. After completion of the reaction, neutralized with aq.$NaHCO_3$ solution (25 ml) followed by extract with etyhyl acetate. The crude product was purified by column chromatography with hexane/ethyl acetate (1:9) to afford the pure products as yellow solids **28-30(a-d)**.

6.6. General Method F for the Synthesis of Substituted N-(4-(benzo[*d*]thiazol-2-yl)phenyl)-3,4,5-trimethoxybenzamide 33-35(a-d):

Trimethoxy benzoyl chloride (**32**) (0.027 mol) were added slowly to a solution of the appropriately substituted anilines **25-27(a-d)** (0.027 mol) in pyridine (15 mL). The resulting solution was stirred under reflux for 8 h and then poured into ice water (100mL).The precipitate formed was collected and washed with 2N HCl (100 mL), followed by water and methanol, to afford the respective benzanilides **33-35(a-d)** as a white solids.

6.6.1. 3,4,5-Trimethoxy-N-(4-(6-methoxybenzo[*d*]thiazol-2yl)phenyl)benzamide (33a):

The compound **33a** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 72%; mp180-183 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO): δ) 7.99 (q, *J* = 8.9 Hz, 4H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.74 (s, 1H), 7.43 (d, *J* = 2.4 Hz, 1H), 7.33 (s, 2H), 7.08 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.96 (s, 6H), 3.90 (s, 3H), 3.87 (s, 3H); MS (ESI): *m/z* 451 $(M+H)^+$.

6.6.2. 3,4,5-Trimethoxy-N-(4-(6-methoxybenzo[*d*]thiazol-2-yl)-2-methylphenyl)benzamide (34a):

The compound **34a** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 72%; mp 203-204 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4 Hz, 1H), 7.98 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 7.73 (s, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.12 (s, 2H), 7.11 – 7.07 (m, 1H), 3.95 (s, 6H), 3.93 (s, 3H), 3.90 (s, 3H), 2.43 (s, 3H); MS (ESI): *m/z* 465 $(M+H)^+$.

6.6.3. 3,4,5-Trimethoxy-N-(2-methoxy-4-(6-methoxybenzo[*d*]thiazol-2-yl)phenyl)benzamide (35a):

The compound **35a** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 76%; mp 198-201 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (d, *J* = 8.4 Hz, 1H), 8.57 (s, 1H), 7.93 (d, *J* = 8.9 Hz, 1H), 7.75 (d, *J* = 1.6 Hz, 1H), 7.60 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.13 (s, 2H), 7.08 (dt, *J* = 15.9, 7.9 Hz, 1H), 4.08 (s, 3H), 3.96 (s, 6H), 3.93 (s, 3H), 3.90 (s, 3H). MS (ESI): *m/z* 481 $(M+H)^+$.

6.6.4. N-(4-(6-Fluorobenzo[*d*]thiazol-2-yl)phenyl)-3,4,5-trimethoxybenzamide (33b):

The compound **33b** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 75%; mp 156-178 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (d, *J* = 8.6 Hz, 2H), 7.99 (dd, *J* = 8.6, 5.1 Hz, 2H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.58 (dd, *J* = 8.1, 2.5 Hz, 1H), 7.26 – 7.18 (m, 1H), 7.10 (s, 2H), 3.93 (d, *J* = 4.2 Hz, 9H); MS (ESI): *m/z* 439 $(M+H)^+$.

6.6.5. N-(4-(6-Fluorobenzo[*d*]thiazol-2-yl)-2-methylphenyl)-3,4,5-trimethoxybenzamide (34b):

The compound **34b** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 72%; mp 201-203 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (d, *J* = 8.5 Hz, 1H), 8.01 (dd, *J* = 8.9, 4.8 Hz, 2H), 7.92 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.78 (s, 1H), 7.60 (dd, *J* = 8.1, 2.5 Hz, 1H), 7.22 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.14 (s, 2H), 3.96 (s, 6H), 3.94 (s, 3H), 2.45 (s, 3H); MS (ESI): *m/z* 453 $(M+H)^+$.

6.6.6. N-(4-(6-Fluorobenzo[*d*]thiazol-2-yl)-2-methoxyphenyl)-3,4,5-trimethoxybenzamide (35b):

The compound **35b** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 69%; mp 205-208 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.68–8.54 (m, 2H), 7.98 (s, 1H), 7.76 (s, 1H), 7.62 (d, *J* = 9.8 Hz, 2H), 7.25 (d, *J* = 10.6 Hz, 2H), 7.13 (s, 2H), 4.08 (s, 3H), 3.95 (d, *J* = 9.6 Hz, 9H). MS (ESI): *m/z* 469 $(M+H)^+$.

6.6.7. N-(4-(5,7-Dimethoxybenzo[*d*]thiazol-2-yl)phenyl)-3,4,5-trimethoxybenzamide (33c):

The compound **33a** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 68%; mp 180-185 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09 (d, *J* = 8.6 Hz, 2H), 7.98 (s, 1H), 7.78 (d, *J* = 8.6 Hz, 1H), 7.17 (d, *J* = 2.0 Hz, 1H), 7.10 (s, 2H), 7.04 (s, 1H), 6.50 (d, *J* = 1.9 Hz, 1H), 3.97 (s, 3H), 3.94 – 3.89 (m, 9H), 3.76 (s, 3H); MS (ESI): *m/z* 481 $(M+H)^+$.

6.6.8. N-(4-(5,7-Dimethoxybenzo[*d*]thiazol-2-yl)-2-methylphenyl)-3,4,5-trimethoxybenzamide (34c):

The compound **34c** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 70%; mp 210-213 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.74 (s, 1H), 7.18 (d, *J* = 1.8 Hz, 1H), 7.12 (s, 2H), 6.50 (d, *J* = 1.8 Hz, 1H), 3.97 (s, 3H), 3.95 (s, 6H), 3.93 (s, 3H), 3.90 (s, 3H), 2.43 (s, 3H); MS (ESI): *m/z* 495 $(M+H)^+$.

6.6.9. N-(4-(5,7-dimethoxybenzo[*d*]thiazol-2-yl)-2-methoxyphenyl)-3,4,5-trimethoxybenzamide (35c):

The compound **35c** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 72%; mp 201-203 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (d, *J* = 8.4 Hz, 1H), 8.57 (s, 1H), 7.74 (d, *J* = 1.7 Hz, 1H), 7.67 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.17 (d, *J* = 2.0 Hz, 1H), 7.13 (s, 2H), 6.50 (d, *J* = 2.0 Hz, 1H), 4.06 (s, 3H), 3.96 (d, *J* = 3.6 Hz, 9H), 3.93 (s, 3H), 3.90 (s, 3H). MS (ESI): *m/z* 511 $(M+H)^+$.

6.6.10. 3,4,5-Trimethoxy-N-(4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzamide (33d):

The compound **33d** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 71%; mp 145-150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 8.6 Hz, 1H), 7.48 (s, 1H), 7.17 (d, *J* = 2.0 Hz, 1H), 7.10 (s, 2H), 7.04 (d, *J* = 1.9 Hz, 1H), 4.09 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 3.78 (s, 6H); MS (ESI): *m/z* 511 $(M+H)^+$.

6.6.11. 3,4,5-Trimethoxy-N-(2-methyl-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzamide (34d):

The compound **34d** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 71%; mp 160-165 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (d, *J*= 8.4 Hz, 1H), 8.06 – 7.95 (m, 1H), 7.94 – 7.80 (m, 1H), 7.77 (s, 1H), 7.34 (s, 1H), 7.03 (s, 2H), 4.11 (s, 3H), 3.96 (s, 3H), 3.93 (s, 3H), 3.87 (s, 3H), 3.78 (s, 6H), 2.43 (s, 3H); MS (ESI): *m/z* 525 $(M+H)^+$.

6.6.12. 3,4,5-Trimethoxy-N-(2-methoxy-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzamide (35d):

The compound **35d** was synthesized by following general procedure **F**, as a pale yellow solid; Yield 73%; mp 176-178 °C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO): δ 10.16 (s, 1H), 7.95 – 7.65 (m, 4H), 7.19 (s, 2H), 4.07 (s, 3H), 3.87 (s, 6H), 3.78 (s, 3H), 3.23 (m, 9H); MS (ESI): *m/z* 541 $(M+H)^+$.

6.7. General Method G for the Synthesis of Substituted N-(4-(benzo[*d*]thiazol-2-yl)phenyl)-3,4,5-trimethoxybenzothioamide 36-38(a-d):

To a solution of substituted of N-(4-(benzo[*d*]thiazol-2-yl) phenyl)-3,4,5-trimethoxybenzamide **33-35(a-d)** (1g, 0.018 mol) in toluene, Lawesson's reagent (0.9eq) was added and reflux it for 8h. After cooling solvent was removed in rotavapor and workedup with ethylacetate and water. Separation of the organic layer and evaporation followed by column chromatography gave yellow solids **36-38(a-d).**

6.7.1. 3,4,5-Trimethoxy-N-(4-(6-methoxybenzo[*d*]thiazol-2-yl)phenyl)benzothioamide (36a):

The compound **36a** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 68%; mp 206-210 °C; MS (ESI): *m/z* 467 $(M+H)^+$.

6.7.2. 3,4,5-Trimethoxy-N-(4-(6-methoxybenzo[*d*]thiazol-2-yl)-2-methylphenyl)benzothioamide (37a):

The compound **37a** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 66%; mp 212-214 °C; MS (ESI): *m/z* 481 $(M+H)^+$.

6.7.3. 4-Methoxy-N-(2-methoxy-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzothioamide (38a):

The compound **38a** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 69%; mp 203-204 °C; MS (ESI): *m/z* 497 $(M+H)^+$.

6.7.4. N-(4-(6-Fluorobenzo[*d*]thiazol-2-yl)phenyl)-3,4,5-trimethoxybenzothioamide (36b):

The compound **36b** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 68%; mp 188-201 °C; MS (ESI): *m/z* 439 $(M+H)^+$.

6.7.5. N-(4-(6-Fluorobenzo[*d*]thiazol-2-yl)-2-methylphenyl)-3,4,5-trimethoxybenzothioamide (37b):

The compound **37b** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 64%; mp 210-213°C; MS (ESI): *m/z* 469 $(M+H)^+$.

6.7.6. 4-Fluoro-N-(2-methoxy-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzothioamide (38b):

The compound **38b** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 67%; mp 223-225°C; MS (ESI): *m/z* 485 $(M+H)^+$.

6.7.7. N-(4-(5,7-Dimethoxybenzo[*d*]thiazol-2-yl)phenyl)-3,4,5-trimethoxybenzothioamide (36c):

The compound **36c** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 69%; mp 199-200 °C; MS (ESI): *m/z* 497 $(M+H)^+$.

6.7.8. N-(4-(5,7-Dimethoxybenzo[*d*]thiazol-2-yl)-2-methylphenyl)-3,4,5-trimethoxybenzothioamide (37c):

The compound **37c** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 67%; mp 175-176 °C; MS (ESI): *m/z* 511 $(M+H)^+$.

6.7.9. 3,5-Dimethoxy-N-(2-methoxy-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzothioamide (38c):

The compound **38c** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 69%; mp 187-189 °C; MS (ESI): *m/z* 527 $(M+H)^+$.

6.7.10. 3,4,5-Trimethoxy-N-(4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzothioamide (36d):

The compound **36d** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 67%; mp 175-176°C;MS (ESI): *m/z* 527 $(M+H)^+$.

6.7.11. 3,4,5-Trimethoxy-N-(2-methyl-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzothioamide (37d):

The compound **37d** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 72%; mp 199-200 °C; MS (ESI): *m/z* 541 $(M+H)^+$.

6.7.12. 3,4,5-Trimethoxy-N-(2-methoxy-4-(5,6,7-trimethoxybenzo[*d*]thiazol-2-yl)phenyl)benzothioamide (38d):

The compound **38d** was synthesized by following general procedure **G**, as a pale yellow solid; Yield 69%; mp 211-213 °C; MS (ESI): *m/z* 557 $(M+H)^+$.

6.8. General Method H for the Synthesis of Substituted amidrazones 39-41(a-d):

To a stirred solution of substituted thioamides **36-38(a-d)** (1 eq) diisolved in 20 mL of EtOH and $NH_2NH_2 \cdot H_2O$ (5 eq) was added slowly with vigorously stirring at room temperature for 16 h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, the solvent and excess hydrazine was evaporated under vacuum, and the syrup was dissolved in $CHCl_3$ (30 mL). The organic layer was washed with water (1 x 10 mL) and brine (1 x 10 mL), and was dried over Na_2SO_4. The organic layer was concentrated under reduced pressure to afford **39-41(a-d)** as yellow solids which were directly used in the next step without purification.

6.9. General procedure I for Synthesis of 1, 4-di substituted 1,2,3-triazoles 5-7(a-d):

To stirred solution of 0.05 mmol of catalyst DBU in DMSO (1.0 mL), was added 0.75 mmol of 2-phenyl benzothiazole azides **28-30(a-d)** and 0.5 mmol of 3,4,5-tri methoxyphenyl acetaldehyde (**31**) and the reaction mixture was stirred at 25 °C for 1 h. The crude reaction mixture was worked up with aqueous NH_4Cl solution and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. Pure organo-click products **5-7(a-d)** were obtained by column chromatography (silica gel, mixture of hexane/ethyl acetate).

6.9.1. 5-Methoxy-2-(4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (5a):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a pale yellow solid. Mp: 152-155 °C; (134 mg), Yield: 80%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (d, *J* = 2.9 Hz, 2H), 8.19 (s, 1H), 7.95 (dd, *J* = 13.7, 8.8 Hz, 3H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.16 (s, 2H), 7.12 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.97 (s, 6H), 3.91 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.49, 158.11, 153.75, 148.63, 148.57, 138.47, 138.07, 136.59, 134.01, 128.53, 125.59, 123.93, 120.50, 117.08, 116.08, 104.13, 103.11, 61.01, 56.31, 55.83; MS (ESI): *m/z* 475 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{23}N_4O_4S$ calcd 475.14345, found 475.14310 $(M+H)^+$.

6.9.2. 6-Methoxy-2-(3-methyl-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (6a):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a pale yellow solid. Mp: 145-147 °C; (135 mg), Yield: 82%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 – 8.19 (m, 1H), 8.11 (s, 1H), 8.03 – 7.90 (m, 3H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.16 (d, *J* = 6.3 Hz, 2H), 7.15 – 7.10 (m, 1H), 3.96 (s, 6H), 3.91 (s, 6H), 2.41 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.64, 158.15, 153.79, 148.66, 147.77, 136.69, 135.09, 134.53, 130.10, 128.61, 126.47, 125.70, 124.03, 120.80, 120.61, 117.08, 116.15, 104.16, 103.07, 61.04, 56.33, 55.88, 18.29; MS (ESI): *m/z* 489 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{25}N_4O_4S$ calcd 489.15910, found 489.15912 $(M+H)^+$.

6.9.3. 6-Methoxy-2-(3-methoxy-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (7a):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a pale yellow solid. Mp: 191-194 °C; (125 mg), Yield: 78%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (s, 1H), 7.78 (d, *J* = 9.0 Hz, 1H), 7.53 (s, 1H), 7.41 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.29 (d, *J* = 2.3 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.02 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.75 (s, 2H), 3.93 (s, 3H), 3.86 (d, *J* = 2.6 Hz, 6H), 3.68 (s, 6H); MS (ESI): *m/z* 505 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{25}N_4O_5S$ calcd 505.15402, found 505.15324 $(M+H)^+$.

6.9.4. 5-Fluoro-2-(4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (5b):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a pale yellow solid. Mp: 182-184 °C; (138 mg), Yield: 81%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 2H), 8.23 (d, *J* = 1.7 Hz, 1H), 8.04 (dd, *J* = 8.9, 4.8 Hz, 1H), 7.96 (d, *J* = 8.6 Hz, 2H), 7.62 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.29 – 7.24 (m, 1H), 7.16 (s, 2H), 3.97 (s, 6H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.82, 161.72, 159.76, 153.79, 150.75, 148.68, 138.54, 136.21, 136.12, 133.64, 128.88, 125.53, 124.47, 124.40, 120.66, 117.06, 115.52, 115.33, 108.12, 107.90, 103.14, 61.04, 56.34; MS (ESI): *m/z* 463 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{24}H_{20}N_4O_3FS$ calcd 463.12347, found 463.12304 $(M+H)^+$.

6.9.5. 6-Fluoro-2-(3-methyl-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (6b):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a yellow solid. Mp: 153-155 °C; (140 mg), Yield: 84%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (s, 1H), 8.05 (dd, *J* = 8.8, 4.7 Hz, 1H), 8.02 (d, *J* = 1.8 Hz, 1H), 8.00 (s, 1H), 7.63 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.30 – 7.25 (m, 1H), 7.17 (s, 2H), 3.96 (s, 6H), 3.91 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.94, 161.72, 159.76, 153.78, 150.71, 147.80, 138.40, 138.24, 136.27, 136.18, 134.62, 134.58, 130.35, 126.51, 125.90, 124.50, 124.43, 120.80, 115.51, 115.32, 108.11, 107.89, 103.06, 61.02, 56.31, 18.33; MS (ESI): *m/z* 477 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{22}N_4O_3FS$ calcd 477.13912, found 477.13924 $(M+H)^+$.

6.9.6. 6-Fluoro-2-(3-methoxy-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (7b):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a white solid. Mp: 139-1141 °C; (124 mg), Yield: 76%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.09 – 7.99 (m, 2H), 7.94 (s, 1H), 7.72 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.63 (dd, *J* = 8.0, 2.4 Hz, 1H), 7.33 – 7.23 (m, 2H), 7.16 (s, 2H), 4.11 (s, 3H), 3.97 (s, 6H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 168.77, 162.95, 159.35, 158.81, 158.27, 153.72, 151.86, 145.86, 145.60, 138.92, 133.23, 127.55, 126.13, 123.82, 121.70, 121.29, 120.44, 116.65, 112.86, 111.53, 109.07, 103.78, 60.79, 56.57, 55.96; MS (ESI): *m/z* 493 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{22}N_4O_4FS$ calcd 493.13403, found 463.13327 $(M+H)^+$.

6.9.7. 5,7-Dimethoxy-2-(4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (5c):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a white solid. Mp: 138-141 °C; (127 mg), Yield: 79%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 1H), 8.23 (s, 2H), 7.93 (d, *J* = 8.6 Hz, 2H), 7.20 (d, *J* = 1.9 Hz, 1H), 7.15 (s, 2H), 6.53 (d, *J* = 1.9 Hz, 1H), 3.97 (d, *J* = 4.3 Hz, 9H), 3.91 (d, *J* = 3.3 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.23, 160.63, 155.76, 154.36, 153.74, 148.56, 138.46, 138.28, 133.94, 128.65, 125.57, 120.48, 117.05, 116.43, 103.09, 97.59, 97.31, 61.01, 56.30, 56.01, 55.82; MS (ESI): *m/z* 505 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{25}N_4O_5S$ calcd 505.15402, found 505.15364 $(M+H)^+$.

6.9.8. 5,7-Dimethoxy-2-(3-methyl-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (6c):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a white off solid. Mp: 163-167 °C; (120 mg), Yield: 76%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.21 (s, 1H), 8.12 (dd, *J* = 8.2, 1.8 Hz, 1H), 8.01 (d, *J* = 7.0 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.27 (s, 1H), 7.17 (s, 2H), 6.62 (s, 1H), 4.10 (s, 3H), 3.97 (s, 6H), 3.91 (s, 2H), 2.44 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.08, 172.78, 163.03, 160.53, 155.66, 154.26, 153.77, 152.62, 147.75, 146.46, 139.60, 133.24, 130.12, 128.05, 126.32, 125.74, 121.11, 120.90, 109.82, 104.47, 103.08, 97.60, 97.37, 61.01, 57.02, 56.30, 56.03, 55.84, 18.30; MS (ESI): *m/z* 519 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{27}N_4O_5S$ calcd 519.16967, found 519.16994 $(M+H)^+$.

6.9.9. 5,7-Dimethoxy-2-(3-methoxy-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (7c):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a white solid. Mp: 120-122 °C; (112 mg), Yield: 72%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 8.04 (d, *J* = 8.1 Hz, 1H), 8.00 (s, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.22 (d, *J* = 2.0 Hz, 1H), 7.17 (s, 2H), 6.55 (d, *J* = 2.0 Hz, 1H), 3.98 (d, *J* = 7.0 Hz, 10H), 3.92 (d, *J* = 3.9 Hz, 7H), 2.41 (s, 3H); MS (ESI): *m/z* 535 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{27}N_4O_6S$ calcd 535.16458, found 535.16487 $(M+H)^+$.

6.9.10. 5,6,7-Trimethoxy-2-(4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (5d):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a white off solid. Mp: 151-154 °C; (120 mg), Yield: 77%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 1H), 8.22 (d, *J* = 8.4 Hz, 2H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.37 (s, 1H), 7.16 (s, 2H), 4.12 (s, 3H), 3.97 (t, *J* = 4.4 Hz, 12H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.88, 154.26, 153.70, 150.41, 148.54, 146.70, 140.03, 138.35, 138.17, 133.80, 128.49, 125.56, 120.49, 117.12, 102.99, 100.69, 61.50, 60.98, 60.62, 56.28, 56.24; MS (ESI): *m/z* 535 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{27}N_4O_6S$ calcd 535.16458, found 535.16416 $(M+H)^+$.

6.9.11. 5,6,7-Trimethoxy-2-(3-methyl-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (6d):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a white off solid. Mp: 174-177 °C; (124 mg), Yield: 81%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 8.00 (s, 1H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.39 (s, 1H), 7.17 (s, 2H), 4.13 (s, 3H), 3.98 (s, 3H), 3.96 (s, 9H), 3.91 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.01, 154.28, 153.72, 150.38, 147.71, 146.72, 140.09, 138.29, 137.91, 134.83, 134.49, 130.04, 126.43, 125.72, 125.60, 120.82, 120.60, 102.97, 100.73, 61.51, 60.98, 60.63, 56.29, 56.25, 18.26; MS (ESI): *m/z* 535 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{27}N_4O_6S$ calcd 535.16458, found 535.16416 $(M+H)^+$.

6.9.12. 5,6,7-Trimethoxy-2-(3-methoxy-4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)phenyl)benzo[*d*]thiazole (7d):

Prepared following the procedure **I** and purified by column chromatography using EtOAc/hexane and was isolated as a white off solid. Mp: 166-169 °C; (119 mg), Yield: 79%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.39 (s, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 7.90 (d, *J* = 1.5 Hz, 1H), 7.73 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.38 (s, 1H), 7.16 (s, 2H), 4.13 (s, 3H), 4.09 (s, 3H), 3.97 (t, *J* = 2.9 Hz, 12H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.88, 154.26, 153.70, 150.41, 148.54, 146.70, 140.03, 138.35, 138.17, 133.80, 128.49, 125.56, 120.49, 117.12, 102.98, 100.69, 61.50, 60.98, 60.62, 56.28, 56.24; MS (ESI): *m/z* 565 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{28}H_{29}N_4O_7S$ calcd 565.17515, found 565.17533 $(M+H)^+$.

6.10. General Method J for the Synthesis of Substituted 1,2,4-Triazole 8-10(a-d):

The amidrazones **39-41(a-d)** (1 mmol) was dissolved in Ethanol (10 mL) and trimethylorthoformate (5 mmol) was added at room temperature. Several drops of sulfuric acid were added as catalyst, and the solution was stirred vigorously at refluxing conditions for 3 h. After neutralization, the alcohol was evaporated and the triazole was purified by column chromatography ($CHCl_3$/MeOH) solvent system to obtain respective triazoles **8-10(a-d)** as a yellow solids.

6.10.1. 6-Methoxy-2-(4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (8a):

This compound was prepared according to the method **J**. (154mg), Yield: 75%; White yellow solid, mp: 88-92 °C**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.19 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 9.0 Hz, 1H), 7.41 (d, *J* = 8.6 Hz, 3H), 7.15 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.73 (s, 2H), 3.93 (s, 3H), 3.87 (s, 3H), 3.67 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.78, 158.28, 153.22, 148.55, 144.38, 139.50, 136.65, 136.04, 134.87, 128.48, 126.45, 124.08, 121.05, 116.28, 105.93, 104.10, 60.92, 56.00, 55.84; MS (ESI): *m/z* 475 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{23}N_4O_4S$ calcd 475.14345, found 475.14122 $(M+H)^+$.

6.10.2. 6-Methoxy-2-(3-methyl-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (9a):

This compound was prepared according to the method **J.** (152mg), Yield: 74%; White yellow solid, mp: 96-100 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (s, 1H), 8.08 (s, 1H), 8.04 (d, *J* = 8.1 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 2.3 Hz, 1H), 7.15 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.75 (s, 1H), 3.92 (s, 3H), 3.83 (s, 3H), 3.62 (s, 6H), 2.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.96, 158.32, 153.24, 148.58, 144.52, 139.49, 136.69, 136.36, 135.66, 135.55, 129.98, 128.15, 126.13, 124.09, 121.49, 116.32, 104.77, 104.15, 60.91, 55.93, 55.89, 17.67; MS (ESI): *m/z* 489 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{25}N_4O_4S$ calcd 489.15910, found 489.15642 $(M+H)^+$.

6.10.3. 5-Methoxy-2-(3-methoxy-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (10a):

This compound was prepared according to the method **J.** (152mg), Yield: 74%; White yellow solid, mp: 96-100 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (s, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.85 (s, 1H), 7.63 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.38 (d, *J* = 2.3 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.14 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.75 (s, 2H), 3.92 (s, 3H), 3.84 (d, *J* = 2.6 Hz, 6H), 3.66 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.07, 158.33, 154.59, 153.10, 148.47, 139.31, 136.72, 136.35, 135.64, 135.55, 129.78, 128.45, 126.38, 125.25, 121.89, 116.32, 110.34, 104.77, 104.13, 60.91, 56.20, 55.97, 55.88; MS (ESI):

m/z 505 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{25}N_4O_5S$ calcd 505.15402, found 505.15154 $(M+H)^+$.

6.10.4. 6-Fluoro-2-(4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (8b):

This compound was prepared according to the method **J**. (145 mg), Yield: 70%; White Yellow solid, mp: 120-122 °C**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (s, 1H), 8.22 (d, *J* = 8.5 Hz, 2H), 8.06 (dd, *J* = 9.0, 4.8 Hz, 1H), 7.64 (dd, *J* = 8.0, 2.4 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.34 – 7.24 (m, 1H), 6.73 (s, 2H), 3.87 (s, 3H), 3.67 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.16, 165.12, 162.46, 159.18, 153.26, 152.93, 150.69, 150.67, 144.34, 139.58, 136.57, 136.28, 136.13, 134.42, 128.76, 126.53, 124.66, 124.54, 121.04, 115.73, 115.40, 108.20, 107.84, 105.99, 60.93, 56.01; MS (ESI): *m/z* 463 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{24}H_{20}N_4O_3FS$ calcd 463.12347, found 463.12012 $(M+H)^+$.

6.10.5. 6-Fluoro-2-(3-methyl-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (9b):

This compound was prepared according to the method **J**. (148 mg), Yield: 72%; White yellow solid, mp: 199-200 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (s, 1H), 8.10 (s, 1H), 8.07 – 8.03 (m, 2H), 7.63 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.31 – 7.26 (m, 1H), 6.75 (s, 2H), 3.83 (s, 3H), 3.63 (s, 6H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.31, 161.83, 159.85, 153.27, 152.96, 150.68, 144.41, 139.54, 136.54, 136.15, 135.12, 130.27, 128.28, 126.38, 124.63, 124.56, 121.41, 115.70, 115.51, 108.17, 107.95, 104.81, 60.93, 55.94, 17.72; MS (ESI): *m/z* 477 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{22}N_4O_3FS$ calcd 477.13912, found 477.13675 $(M+H)^+$.

6.10.6. 5-Fluoro-2-(3-methoxy-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (10b):

This compound was prepared according to the method **J**. (148 mg), Yield: 72%; White yellow solid, mp: 199-200 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (s, 1H), 8.05 (dd, *J* = 9.0, 4.8 Hz, 1H), 7.86 (d, *J* = 1.7 Hz, 1H), 7.65 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.62 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.36 – 7.33 (m, 1H), 7.31 – 7.28 (m, 1H), 6.75 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.66 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.40, 162.48, 159.20, 154.64, 153.13, 150.56, 136.33, 136.19, 128.72, 125.70, 124.63, 124.51, 121.73, 120.26, 115.75, 115.42, 110.59, 108.20, 107.85, 105.22, 60.90, 56.23, 55.98; MS (ESI): *m/z* 493 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{22}N_4O_4FS$ calcd 493.13171, found 493.13403 $(M+H)^+$.

6.10.7. 5,7-Dimethoxy-2-(4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (8c):

This compound was prepared according to the method **J**. (137 mg), Yield: 67%; White yellow solid, mp: 197-198 °C**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.21 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 1.6 Hz, 1H), 6.72 (s, 2H), 6.55 (d, *J* = 1.7 Hz, 1H), 3.98 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.65 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.51, 160.74, 155.73, 154.37, 153.23, 152.88, 144.37, 139.62, 136.26, 134.87, 128.59, 126.50, 120.91, 116.55, 106.01, 97.65, 97.55, 60.89, 56.00, 55.81; MS (ESI): *m/z* 505 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{25}N_4O_5S$ calcd 505.15402, found 505.15196 $(M+H)^+$.

6.10.8. 5,7-Dimethoxy-2-(3-methyl-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (9c):

This compound was prepared according to the method **J**. (132 mg), Yield: 64%; White yellow solid, mp: 188-190 °C **;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (s, 1H), 8.12 (s, 1H), 8.06 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.20 (d, *J* = 1.9 Hz, 1H), 6.75 (s, 2H), 6.55 (d, *J* = 1.9 Hz, 1H), 3.98 (s, 3H), 3.92 (s, 3H), 3.83 (s, 3H), 3.62 (s, 6H), 2.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.74, 160.78, 155.73, 154.40, 153.24, 152.93, 144.48, 139.46, 136.40, 135.92, 135.51, 130.09, 128.20, 126.23, 121.45, 116.57, 104.73, 97.57, 60.92, 56.07, 55.91, 17.69; MS (ESI): *m/z* 519 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{27}N_4O_4S$ calcd 519.16967, found 519.16720$(M+H)^+$.

6.10.9. 5,7-Dimethoxy-2-(3-methoxy-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (10c):

This compound was prepared according to the method **J**. (132 mg), Yield: 64%; White yellow solid, mp: 188-190 °C **;** ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.86 (d, *J* = 1.5 Hz, 1H), 7.68 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.20 (d, *J* = 1.9 Hz, 1H), 6.75 (s, 2H), 6.55 (d, *J* = 1.9 Hz, 1H), 3.98 (s, 3H), 3.92 (s, 3H), 3.83 (s, 6H), 3.65 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.82, 160.73, 155.59, 154.53, 154.35, 153.07, 139.21, 136.56, 128.64, 125.48, 121.86, 120.03, 116.55, 110.43, 105.01, 97.52, 60.91, 56.16, 56.04, 55.92, 55.83; MS (ESI): *m/z* 535 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{27}N_4O_6S$ calcd 535.16458, found 535.16216$(M+H)^+$.

6.10.10. 5,6,7-Trimethoxy-2-(4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (8d):

This compound was prepared according to the method **J**. (142mg), Yield: 69%; White yellow solid, mp: 162-164 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (s, 1H), 8.19 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.37 (s, 1H), 6.72 (s, 2H), 4.12 (s, 3H), 3.97 (s, 3H), 3.86 (s, 6H), 3.66 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.21, 154.41, 153.20, 152.90, 150.33, 146.71, 144.41, 144.37, 139.45, 136.21, 134.73, 128.47, 126.51, 121.04, 120.68, 105.86, 100.75, 61.52, 60.93, 60.68, 56.32, 55.98; MS (ESI): *m/z* 535 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{27}N_4O_6S$ calcd 535.16458, found 535.16226 $(M+H)^+$.

6.10.11. 5,6,7-Trimethoxy-2-(3-methyl-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (9d):

This compound was prepared according to the method **J**. (139 mg), Yield: 68%; White yellow solid, mp: 104-106 °C**;** ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1H), 8.09 (s, 1H), 8.04 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.37 (s, 1H), 6.75 (s, 2H), 4.12 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.83 (s, 3H), 3.62 (s, 6H), 2.07 (s, 3H); ^{13}C NMR (75 MHz, DMSO): δ 163.47, 153.37, 152.76, 152.45, 148.65, 137.49, 135.38, 135.19, 133.57, 123.92, 117.28, 115.44, 98.08, 96.99, 60.10, 55.67, 29.00**;** MS (ESI): *m/z* 549 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{28}H_{29}N_4O_6S$ calcd 549.18023, found 549.17997 $(M+H)^+$.

6.10.12. 5,6,7-Trimethoxy-2-(3-methoxy-4-(3-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)phenyl)benzo[*d*]thiazole (10d):

This compound was prepared according to the method **J**. (139 mg), Yield: 68%; White yellow solid, mp: 104-106 °C**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.83 (d, *J* = 1.4 Hz, 1H), 7.66 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.41 – 7.30 (m, 2H), 6.76 (s, 2H), 4.13 (s, 3H), 3.98 (d, *J* = 6.5 Hz, 6H), 3.83 (d, *J* = 9.2 Hz, 6H), 3.66 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.48, 154.56, 154.43, 153.05, 150.23, 146.69, 144.92, 140.25, 139.21, 136.46, 128.65, 125.37, 121.84, 120.71, 119.93,

110.29, 105.00, 100.72, 61.51, 60.89, 60.66, 56.32, 56.14, 55.91; MS (ESI): *m/z* 565 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{28}H_{29}O_7N_4S$ calcd 565.17515, found 565.16529 $(M+H)^+$.

6.11. General Method K for the Synthesis of Substituted 1,2,3,4-Triazole 11-13(a-d):

The amidrazones **39-41(a-d)** was dissolved in 5 ml of AcOH and $NaNO_2$ (2 eq) was added portion wise slowly at room temperature for 10 min., and the reaction mixture was stirred for 4 h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, neutralize with saturated $NaHCO_3$ solution, and extracted with ethyl acetate (3 x 15 mL). The combined extract was washed with water (1 x 10 mL) as well as brine (1x10 mL) and was dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60-120 mesh) using ethyl acetate-hexane (4:6) as eluent to afford compound **11-13(a-d)** as a pale yellow solids.

6.11.1. 6-Methoxy-2-(4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (11a):

This compound was prepared according to the method **K**. (145 mg), Yield: 71%; Brown color solid, mp: 170-175 °C**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.5 Hz, 2H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 2.3 Hz, 1H), 7.14 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.81 (s, 2H), 3.92 (s, 3H), 3.89 (s, 3H), 3.70 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.65, 158.34, 153.54, 153.50, 148.59, 140.72, 136.72, 135.84, 135.69, 128.33, 125.97, 124.14, 120.19, 118.13, 116.31, 106.39, 104.11, 60.96, 56.15, 55.83; MS (ESI): *m/z* 476 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{24}H_{22}N_5O_4S$ calcd 476.13870, found 476.13701 $(M+H)^+$.

6.11.2. 6-Methoxy-2-(3-methyl-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (12a):

This compound was prepared according to the method **K**. (136 mg), Yield: 66%; Brown color solid, mp: 160-164 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (s, 1H), 8.07 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.99 (d, *J* = 9.0 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.39 (d, *J* = 2.5 Hz, 1H), 7.15 (dt, *J* = 4.9, 2.5 Hz, 1H), 6.84 (s, 2H), 3.92 (s, 3H), 3.86 (s, 3H), 3.65 (s, 6H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.80, 154.34, 149.83, 149.48, 144.54, 136.62, 132.73, 132.33, 131.54, 125.95, 124.08, 122.10, 120.13, 114.10, 112.38, 101.35, 100.08, 56.97, 52.00, 51.87, 13.57; MS (ESI): *m/z* 490 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{24}N_5O_4S$ calcd 490.15435, found 490.15241 $(M+H)^+$.

6.11.3. 6-Methoxy-2-(3-methoxy-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (13a):

Prepared following the procedure K and purified by column chromatography using EtOAc/hexane and was isolated as a Brown colour solid. (104 mg), Yield: 68%; Mp: 172-175 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.20 (d, *J* = 1.9 Hz, 1H), 6.81 (s, 2H), 6.55 (d, *J* = 1.9 Hz, 1H), 3.99 (s, 3H), 3.92 (s, 3H), 3.89 (s, 3H), 3.70 (s, 6H). MS (ESI): *m/z* 506 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{24}N_5O_5S$ calcd 506.14856, found 506.14678 $(M+H)^+$.

6.11.4. 6-Fluoro-2-(4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (11b):

This compound was prepared according to the method **K**. (134 mg), Yield: 65%; Brown color solid, mp: 195-197 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27 – 8.24 (m, 2H), 8.05 (dd, *J* = 9.0, 4.8 Hz, 1H), 7.63 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.62 – 7.59 (m, 2H), 7.31 – 7.28 (m, 1H), 6.81 (s, 2H), 3.90 (s, 3H), 3.70 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.07, 161.87, 159.90, 153.60, 150.70, 140.72, 136.33, 136.23, 135.24, 128.65, 126.07, 124.73, 124.66, 118.12, 115.75, 115.55, 108.17, 107.96, 106.37, 61.05, 56.20; MS (ESI): *m/z* 464 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{23}H_{19}N_5O_3FS$ calcd 464.11871, found 464.11628 $(M+H)^+$.

6.11.5. 6-Fluoro-2-(3-methyl-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (12b):

This compound was prepared according to the method **K**. (136 mg), Yield: 66%; Brown color solid, mp: 162-164 °C**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 8.12 – 8.03 (m, 2H), 7.64 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.30 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.84 (s, 2H), 3.86 (s, 3H), 3.65 (s, 6H), 2.11 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.82, 153.48, 150.63, 136.49, 136.02, 135.85, 130.21, 128.19, 126.31, 124.67, 124.55, 118.02, 115.75, 115.42, 108.19, 107.83, 105.39, 60.93, 55.98, 17.56; MS (ESI): *m/z* 478 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{24}H_{21}N_5O_3FS$ calcd 478.13436, found 478.13234 $(M+H)^+$.

6.11.6. 6-Fluoro-2-(3-methoxy-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (13b):

This compound was prepared according to the method **K**. (136 mg), Yield: 66%; Brown color solid, mp: 162-164 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (dd, *J* = 9.0, 4.8 Hz, 1H), 7.88 (d, *J* = 1.7 Hz, 1H), 7.77 – 7.75 (m, 1H), 7.63 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.60 (d, *J* = 8.1 Hz, 1H), 7.30 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.86 (s, 2H), 3.86 (s, 3H), 3.78 (s, 3H), 3.68 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.26, 161.89, 159.92, 154.56, 153.37, 150.59, 140.46, 137.31, 136.39, 136.30, 128.88, 125.78, 124.72, 124.64, 120.38, 118.93, 115.76, 115.57, 110.71, 108.17, 107.96, 105.26, 60.98, 56.31, 56.08; MS (ESI): *m/z* 494 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{24}H_{21}N_5O_4FS$ calcd 494.12928, found 494.12721 $(M+H)^+$.

6.11.7. 5,7-Dimethoxy-2-(4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (11c):

This compound was prepared according to the method **K**. (135 mg), Yield: 66%; Brown color solid, mp: 191- 195 °C**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.20 (d, *J* = 1.9 Hz, 1H), 6.81 (s, 2H), 6.55 (d, *J* = 1.9 Hz, 1H), 3.99 (s, 3H), 3.92 (s, 3H), 3.89 (s, 3H), 3.70 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.41, 160.74, 155.74, 154.37, 153.54, 153.49, 140.70, 136.09, 135.64, 128.44, 126.00, 118.10, 116.63, 106.36, 97.66, 97.59, 60.97, 56.14, 56.01, 55.80; MS (ESI): *m/z* 506 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{25}H_{24}N_5O_5S$ calcd 506.14927, found 506.14715 $(M+H)^+$.

6.11.8. 5,7-Dimethoxy-2-(3-methyl-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (12c):

This compound was prepared according to the method **K**. (137 mg), Yield: 67%; Brown color solid, mp: 210-213 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.74 (s, 1H),7.12 (s, 1H), 6.50 (s, 2H), 3.97 (d, *J* = 0.6 Hz, 3H), 3.95 (d, *J* = 1.1 Hz, 6H), 3.93 (d, *J* = 0.7 Hz, 3H), 3.90 (d, *J* = 0.7 Hz, 3H), 2.43 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.75, 165.42, 160.38, 155.75, 154.22, 153.40, 141.40, 138.33, 130.58, 130.15, 129.16, 129.01, 126.21, 122.59, 106.37, 104.52, 97.35, 96.79, 60.99, 56.36, 55.94, 55.76, 17.83. MS (ESI): *m/z* 520 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{26}N_5O_5S$ calcd 520.15867, found 520.15778 $(M+H)^+$.

6.11.9. 5,7-Dimethoxy-2-(3-methoxy-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (13c):

This compound was prepared according to the method **K**. (137 mg), Yield: 67%; Brown color solid, mp: 210-213 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (d, *J* = 8.4 Hz, 1H), 8.57 (s, 1H), 7.74 (d, *J* = 1.7 Hz, 1H), 7.67 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.17 (d, *J* = 2.0 Hz, 1H), 7.13 (s, 2H), 6.50 (d, *J* = 2.0 Hz, 1H), 4.06 (s, 3H), 3.96 (d, *J* = 3.6 Hz, 9H), 3.93 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.88, 165.05, 160.35, 155.68, 154.26, 153.33, 148.19, 141.36, 130.40, 130.18, 129.23, 121.12, 119.49, 108.05, 104.57, 97.33, 96.74, 60.97, 56.40, 56.23, 55.92, 55.76. MS (ESI): *m/z* 536 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{26}N_5O_5S$ calcd 536.15455, found 536.15326 $(M+H)^+$.

6.11.10. 5,6,7-Trimethoxy-2-(4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (11d):

This compound was prepared according to the method **K**. (138 mg), Yield: 67%; Brown color solid, mp: 150-152 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.5 Hz, 2H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.37 (s, 1H), 6.81 (s, 2H), 4.12 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.89 (s, 3H), 3.70 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.10, 154.45, 153.52, 150.35, 146.71, 140.65, 140.31, 135.98, 135.54, 128.33, 126.05, 120.77, 120.21, 118.09, 106.32, 100.83, 61.50, 60.98, 60.66, 56.32, 56.13; MS (ESI): *m/z* 536 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{26}H_{26}N_5O_6S$ calcd 536.15983, found 536.15782 $(M+H)^+$.

6.11.11. 5,6,7-Trimethoxy-2-(3-methyl-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (12d):

This compound was prepared according to the method **K**. (141 mg), Yield: 69%; Brown color solid, mp: 200-202 °C**;** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (d, *J* = 8.6 Hz, 1H), 7.98 (s, 1H), 7.92 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.35 (s, 1H), 7.12 (s, 2H), 3.96 (s, 3H), 3.95 (s, 9H), 3.95 (s, 3H), 3.93 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.39, 150.44, 149.22, 148.89, 146.32, 142.73, 140.43, 136.25, 135.44, 132.40, 131.76, 131.39, 125.96, 124.19, 122.09, 117.36, 116.69, 100.69, 96.75, 57.53, 56.90, 56.68, 52.33, 51.88, 13.68; MS (ESI): *m/z* 550 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{28}N_5O_6S$ calcd 550.16923, found 550.16784 $(M+H)^+$.

6.11.12. 5,6,7-Trimethoxy-2-(3-methoxy-4-(5-(3,4,5-trimethoxyphenyl)-1H-tetrazol-1-yl)phenyl)benzo[*d*]thiazole (13d):

This compound was prepared according to the method K. (141 mg), Yield: 69%; Brown color solid, mp: 200-202 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, *J* = 1.4 Hz, 1H), 7.77 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.38 (s, 1H), 6.87 (s, 2H), 4.13 (d, *J* = 4.8 Hz, 3H), 3.98 (d, *J* = 5.9 Hz, 6H), 3.87 (s, 3H), 3.78 (s, 3H), 3.68 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.29, 154.47, 153.30, 150.24, 146.70, 140.34, 137.60, 128.80, 125.38, 120.84, 120.06, 118.91, 110.43, 105.13, 100.77, 61.52, 60.95, 60.68, 56.33, 56.23, 56.00; MS (ESI): *m/z* 566 $(M+H)^+$; HRMS (ESI *m/z*) for $C_{27}H_{28}N_5O_6S$ calcd 566.16423, found 550.16554 $(M+H)^+$.

7. Biology

7.1. Antiproliferative activity

The cytotoxic activity of the compounds was determined by using MTT assay.[54,55] Cells were seeded in 100 µl DMEM, supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 °C in a CO_2 incubator. After incubation cells were treated with test compounds for 48 h. After 48 h of incubation, 10 µl MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide) (5 mg/mL) was added to each well and the plates were further incubated for 4 h. Then the supernatant from each well was carefully removed, formazon crystals were dissolved in 100 µl of DMSO and absorbance at 570 nm wavelength was recorded.

7.2. Cell cycle analysis

Flow cytometric analysis (FACS) was performed to evaluate the distribution of the cells through the cell-cycle phases. A549 cells were incubated for 48 h with compounds **9a** and **9b** at concentrations of 50 and 100 nM. Untreated and treated cells were harvested, washed with phosphate-buffered saline (PBS), fixed in ice-cold 70% ethanol, and stained with propidium iodide (Sigma–Aldrich). Cell-cycle analysis was performed by flow cytometry (Becton Dickinson FACS Caliber instrument) [56].

7.3. Tubulin polymerization assay

A fluorescence based in vitro tubulin polymerization assay was performed according to the manufacturer's protocol (BK011, Cytoskeleton, Inc.). Briefly, the reaction mixture in a total volume of 10 µl contained PEM buffer, GTP (1 µM) in the presence or absence of test compounds. Tubulin polymerization was followed by a time dependent increase in fluorescence due to the incorporation of a fluorescence reporter into microtubules as polymerization proceeds. Fluorescence emission at 420 nm (excitation wavelength is 360 nm) was measured by using a Varioscan multimode plate reader (Thermo scientific Inc.). CA-4 was used as reference compound and to determine the IC_{50} values of the compounds against tubulin polymerization, the compounds were pre-incubated with tubulin at varying concentrations (0.5, 1, 2, 3, 5 µM). Assays were performed under similar conditions as employed for polymerization assays as described above. [57]

7.4. Immunocytochemistry

A549 cells were seeded on a glass cover slip, incubated for 48 h in the presence or absence of test compounds **9a**, **9b** and CA-4 at a concentration of 50 nM. Cells grown on coverslips were fixed in 3.5% formaldehyde in phosphate-buffered saline (PBS) pH 7.4 for 10 minutes at room temperature. Cells were permeabilized for 6 minutes in PBS containing 0.5% Triton X-100 (Sigma) and 0.05% Tween-20 (Sigma). The permeabilized cells were blocked with 2% BSA (Sigma) in PBS for 1 h. Later, the cells were incubated with the primary antibody for tubulin from Sigma at 1:200 diluted in blocking solution for 4 h at room temperature. Subsequently the antibodies were removed and the cells were washed thrice with PBS. Cells were then incubated with the FITC labeled anti-mouse secondary antibody (1: 500) for 1 h at room temperature. Cells were washed thrice with PBS and mounted in medium containing DAPI. Images were captured using an Olympus confocal microscope and analyzed with Provision software.

7.5. Western blot analysis of soluble versus polymerized tubulin.

Cells were seeded in 12-well plates at 1×10^5 cells per well in complete growth medium. Following treatment of cells with respective compounds (**9a**, **9b** and CA-4) for a duration of 48 h, cells were washed with PBS and subsequently soluble and insoluble tubulin fractions were collected. To collect the soluble tubulin fractions, cells were permeabilized with 200 µL of pre-warmed lysis buffer [80 mM Pipes-KOH (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA, 0.2% Triton X-100, 10% glycerol,

0.1% protease inhibitor cocktail (Sigma-Aldrich)] and incubated for 3 min at 30 °C. Lysis buffer was gently removed, and mixed with 100 µL of 3 × Laemmli's sample buffer (180 mM Tris-Cl pH 6.8, 6% SDS, 15% glycerol, 7.5% β-mercaptoethanol and 0.01% bromophenol blue). Samples were immediately heated to 95 °C for 3 min. To collect the insoluble tubulin fraction, 300 µL of 1 × Laemmli's sample buffer was added to the remaining cells in each well, and the samples were heated to 95 °C for 3 min. Equal volumes of samples were run on an SDS-10% polyacrylamide gel and were transferred to a nitrocellulose membrane employing semidry transfer at 50 mA for 1 h. Blots were probed with mouse anti-human α-tubulin diluted 1:2000 ml (Sigma) and stained with a rabbit anti-mouse secondary antibody coupled with horseradish peroxidase, diluted 1:5000 ml (Sigma). Bands were visualized using an enhanced chemiluminescence protocol (Pierce) and radiographic film (Kodak).

7.6. Colchicine competition assay

The test compounds (**9a** and **9b**) of various concentrations 5 µM, 10 µM, 15 µM and 20 µM were incubated with 3 µM tubulin in the presence and absence of 3 µM colchicine in 30 mM Tris buffer for 60 min at 37 °C. CA-4 was used as a positive control whereas vinblastine was used as negative control which binds at the taxane site. After incubation the fluorescence of tubulin–colchicine complex was determined by using Tecan multimode reader with excitation wavelength of 350 nm and emission wavelength of 435 nm. 30 mM Tris buffer was used as blank which was subtracted from all the samples and the fluorescence values are normalized to DMSO (control).[58]

7.7. Hoechst staining

Human lung cancer A549 cells (5 $X10^4$ cells/well) at their growth phase were seeded in 12 well plates and allowed to adhere for 48 h. The culture medium containing the test compounds **9a** and **9b** at their IC_{50} concentration were added to the cells and incubated for 48 h at 37 ºC. After 48 h, culture medium was removed; cells were washed with PBS and fixed with 4 % para formaldehyde solution at 4 ºC for 10 min. The cells were washed twice with PBS and stained with Hoechst 33258 (5 µg/mL) for 30 min at room temp. The excess dye was removed by washing twice with PBS and cells were examined for morphological changes under fluorescence microscope using 350 nm excitation and 460 nm emission (Nikon, magnification 10X).

7.8. Annexin staining assay for apoptosis

A549 cells were seeded in six-well plates and allowed to grow overnight. The medium was then replaced with complete medium containing compounds **9a** and **9b** at 50 and 100 nM concentrations. After 48 h of drug treatment, cells from the supernatant and adherent monolayer cells were harvested by trypsinization, washed with PBS at 5000 rpm. Then the cells were stained with Annexin V-FITC and propidium iodide using the Annexin-V-FITC apoptosis detection kit (Sigma Aldrich). Flow cytometry was performed for this study as described earlier [59].

7.9. Mitochondrial membrane potential

A549 cells were cultured in six-well plates after treatment with compounds **9a** and **9b** at 50 and 100 nM concentrations and 5F-203 100 nM concentration for 48 h. After 48 h of treatment, cells were collected by trypsinization and washed with PBS followed by resuspending in JC-1 (5 µg/ml) and incubated at 37 °C for 15 min. Cells were rinsed three times with medium and suspended in pre warmed medium. The cells were then subjected to flow cytometric analysis on a flow cytometer (Becton Dickinson) in the FL1, FL2 channel to detect mitochondrial potential [60].

7.10. ROS generation

The production of ROS (reactive oxygen species) was measured by flow cytometry using DCFDA (2',7'dichlorofluorescindiacetate) as previously described [61]. In this study A549 cells were treated with compounds **9a** and **9b** at 50 and 100 nM concentrations and 5F-203 100 nM concentration for 48 h. After treatment, cells were incubated with DCFDA (2 µM) at 37 °C for 30 min and then measured with the flow cytometer (FACS).

7.11. Western blot analysis of caspase 3

After treatment with test compounds **9a** and **9b** at 50 and 100 nM concentrations for 48 h. Protein was isolated with RIPA (radioimmunoprecipitation assay) buffer. Protein (50 µg per lane) was applied in 10 % SDS-PAGE (Sodium dodecyl sulfate Polyacrylamide gel electrophoresis). After electrophoresis, the protein was transferred to a polyvinylidine difluoride (PVDF) membrane (Thermo Scientific Inc.) and blocked with BSA (bovine serum albumin). The membrane was washed with TBST for 5 min, then primary antibody was added. After 24 h, the membrane was incubated with the corresponding horseradish peroxidase labeled secondary antibody and incubated for another 1 h. Membranes were washed with TBST three times for 15 min and the protein blots were visualized with chemiluminescence reagent (Thermo Scientific Inc.). Images were captured by using the chemiluminescence (vilber lourmat) [62].

Acknowledgents

The authors A.V.S.R and S. P. S. would like to thank to CSIR, New Delhi, India for the award of research fellowships. The authors are also thankful to CSIR for financial support under the 12th Five Year plan project "Affordable Cancer Therapeutics (ACT)" (CSC0301).

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