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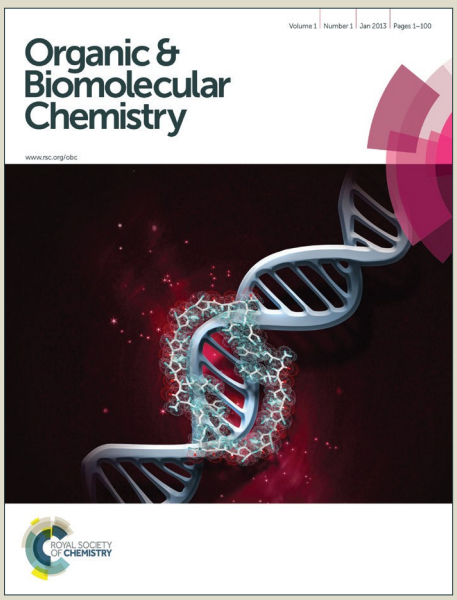

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ARTICLE

Identification of Gallic acid based Glycoconjugates as a novel anti-tumor agents targeting tubulin polymerization

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A novel class of gallic acid based glycoconjugates were designed and synthesized as potential anticancer agents by economical and eco-friendly method. Among all the compounds screened, compound **2a** showed potent anticancer activity against breast cancer cells. The latter resulted in tubulin polymerization inhibition and induced G2/M cell cycle arrest, generation of reactive oxygen species, mitochondrial depolarization and subsequent apoptosis in breast cancer cells. In addition, ultraviolet–visible spectroscopy and fluorescence quenching studies of compound with tubulin confirmed direct interaction of compounds with tubulin. Molecular modeling studies revealed that it binds at a colchicine binding site in tubulin. Further, **2a** also exhibited potent *in vivo* anticancer activity in LA-7 syngenic rat mammary tumor model. Current data projects its strong candidature to be developed as anticancer agent.

Introduction

Microtubules, the dynamic structures that undergo continuous assembly and disassembly are important in maintaining the architecture of the cell and, together with microfilaments and intermediate filaments, they form the cytoskeleton. These dynamic tubulin polymers also play important roles in cell motility, intracellular transport, cell cycle, and many other cellular processes including cell division.[1,2] Due to active participation of microtubules in the formation of dynamic spindle apparatus during cell division, it has been considered as an interesting target for anticancer therapeutics.[3] A large number of compounds of both natural and synthetic origin have been reported to inhibit cancer progression by microtubule depolymerization.[4] These are termed as microtubule binding agents which inhibit cellular proliferation by directly interacting with microtubules and thereby stabilizing/destabilizing microtubule dynamics during the course of cancer progression and called as anti-mitotic agents.[5] Vinca, taxane and colchicine are the three most studied molecule families with the available binding sites in tubulin.[6,7] However, clinical usages of these tubulin polymerization inhibitors are restricted due to high toxicity, complex and high cost synthesis, poor solubility and drug resistance.[8] Therefore, there is an urgent need of new molecules which use readily available and highly economical substrates with potent antitumor activity while low toxicity against normal cells.

Carbohydrates, the structurally diverse molecules due to their involvement in molecular recognition and intracellular function, provide a viable source for chemical libraries in drug discovery and development.[9] In recent years, targeting a notable effect called as Warburg effect which is defined as a presence of high rate of aerobic glycolysis and marked glucose avidity in cancerous cells proved to be an efficient strategy in anticancer therapy.[10] Carbohydrates are associated with the stigma of weak bonding and its inability to cross cell membrane have always been excluded in drug discovery in its native form.[11] Therefore to enhance bioavailability and to enable therapeutic concentration of the drug to reach blood stream, esters are used for the masking of polar hydroxyl groups of the monosaccharides by hydrophobic acyl groups.[12] After reaching in blood, these esters are prone towards enzymatic hydrolysis[13] and hence used as important prodrug moiety in pharmaceutical industry.[14] In view of these points, glycoconjugation has emerged out as one of the most effective strategy for targeting the cancerous cells by linking pharmacophoric moiety to glycosyl unit.[15] The glycosylation of bioactive molecules of both synthetic and natural origin most often improves pharmacological properties and ADMET

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Additional information includes spectral copies, HPLC chromatogram of compounds, 2D NMR spectral copies. This material is available free of charge via the Internet at http://pubs.rsc.org.
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parameters.[16] Several successful attempts have been made by glycosylating paclitaxel[17] and 4′-demethylepipodophyllotoxin[18] to improve their water solubility as well as reduced toxicity. Interestingly it is found that protected sugars such as diacylated etopophos[19] i.e. tafluposide has enhanced biological activity as compare to etopophos whereas Gu*iet al* [20] has found that diphyllin glycoside having acetylated D-quinovose sugar moiety is a potent topoisomerase II an inhibitor. Very recently 4′-azido-D-glucoside derivative of perrilyl alcohol has been reported as potent antiproliferative agent.[21] Naturally occurring polyphenols have been studied in great details due to their interesting physicochemical properties and wide range of biological activities.[22] Among naturally occuring polyphenols, the gallic acid (3,4,5-trihydroxybenzoic acid) has extensively been studied and many of its derivatives as phytomedicines are endowed with numerous biological functions such as neuroprotection,[23a] anti-bacterial,[23b] anti-viral,[23c] anti-inflammatory,[23d] antimelanogenic and antioxidant,[23e] as well as anticancer activities in various cell lines.[24] It has been demonstrated that gallates selectively kill cancer cells by apoptosis and are non-toxic to normal cells.[25] Gallic acid esters of sugars particularly D-glucose constitute another important class of natural products, tannins in different forms. 3,4,5-Trimethoxy phenyl ring, a key structural component in many well-known anticancer drugs and lead molecules such as Combretastatin A4, Podophyllotoxin, Colchicine, Phenstatin and found to be crucial for their anti-proliferative and tubulin polymerization inhibitory activity (Figure.1).[26] 1,2,3-triazole is an effective and attractive functional group for bioconjugate chemistry[27] and known to exhibit several key features of biological importance such as inertness toward hydrolytic cleavage or redox modification, as well as mimicking the peptide bond basicity and hydrogen bond acidity.[28].Moreover, several biologically active molecules having 1,2,3-triazole as a peptide bond surrogate have displayed a wide range of biological activities such as anti-viral[29], anti-cancer[30] and as antibiotic agents[31] as well as glycoconjugates having 1,2,3-triazole backbone are known to inhibit galectins[32], bind with RNA[33] and exhibit α-glucosidase inhibitory activity.[34] In recent times, glycoconjugates consisting of 3,4,5-trimethoxybenzene with amide-triazole linker have been shown to bind to the angiogenic growth factors FGF-1, FGF-2 and VEGF.[35] Keeping in mind the above view and in continuation of our endeavour to develop carbohydrate based new chemical entities as chemotherapeutic agents we embarked upon a new series of glycoconjugtes consisting of sugar, gallic acid trimethyl ether and triazole scaffolds and evaluated for their antiproliferative activity.

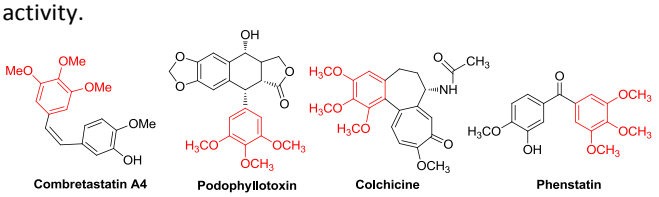


Figure 1. Structures of representative trimethoxy phenyl unit bearing tubulin inhibitors bound to the colchicine-binding site

Results and Discussion

Compounds design and synthesis

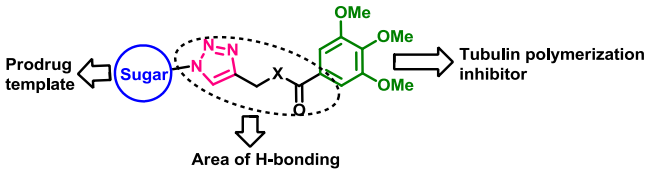
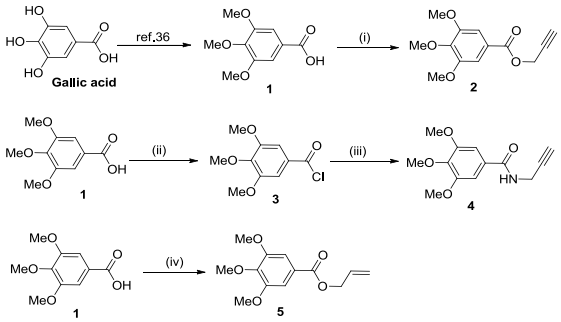


Figure 2. Design of gallic acid based glycoconjugates

Gallic acid derived glycoconjugates synthesized during this study (**2a-2i**), (**4a-4i**), (**2a′-2d′**) and (**4a′-4d′**) comprise 3 core structural units: (i) a sugar moiety, (ii) 1,2,3-triazole moiety, and (iii) 3,4,5-trimethoxyphenyl unit. The sugar moiety provides a common prodrug template, while the 3,4,5-trimethoxy phenyl unit acts as an important pharmacophore for tubulin polymerization inhibition and 1,2,3-triazole linker a bioisostere of amide bond serves as a biocompatible, nonlabile covalent spacer between the sugar and 3,4,5-trimethoxy phenyl unit (Figure.2). To further investigate the role of sugar moiety and triazole ring; few molecules were synthesized (**2j, 4j, 5, 6, 7a, 7f, 7g, 7a′, 7g**′). To check the importance of acetylated analogues, isopropylidene protected glycoconjugates derived from galactose (**2h** and **4h**) and xylose (**2i** and **4i**) were synthesized.The synthesis of the triazole linked 3,4,5-trimethoxy gallic acid derivative was successfully carried out by performing a highly regioselective procedure of 1,3-dipolar cycloaddition (click chemistry) by using Cu(I)-catalyzed azide-alkyne cycloaddition reaction.[27] Following this strategy, the synthesis of alkynyl derivatives was carried out as outlined in the **scheme.1**. Methylation followed by hydrolysis of gallic acid yielded the 3,4,5-trimethoxy benzoic acid (**1**).[36] Propargylation of **1** using propargyl bromide and K_2CO_3 yielded the *O*-propynyl ester (**2**) in excellent yield. Synthesis of *N*-propynyl amide derivative (**4**) of gallic acid was carried out by the coupling of 3,4,5-trimethoxy benzoyl chloride (**3**) and propargyl amine. Similarly *O*-propenyl ester (**5**) was prepared by treating **1** with allyl bromide as reported in scheme 1.



Reagents and conditions: (i) Propargyl bromide (1.1 equiv.), K_2CO_3 (1 equiv.), dry DMF, rt, 4h (ii) Thionyl chloride (5.48 equiv.), benzene, reflux, 12h (iii) Propargyl amine (1.0 equiv.), Et_3N (1 equiv.), DMAP (2 mol%), 0°C, 3h, 85% (iv) Allyl bromide (1.1 equiv.), K_2CO_3 (1 equiv.), dry DMF, rt, 4h, 97%

Scheme1. Synthesis of alkynyl derivatives of gallic acid

A pool of 9 azido sugars (**a-i**) and benzyl azide (**j**) was prepared from benzyl bromide and respective monosaccharides and disaccharide, D-glucose, D-ribose, D-galactose, D-mannose, D-xylose, and D-cellobiose by the procedure already reported in the literature (Figure.3).[37]

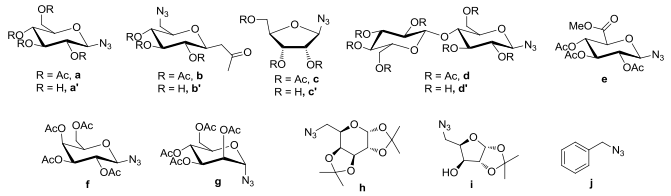
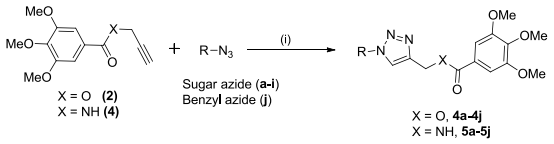


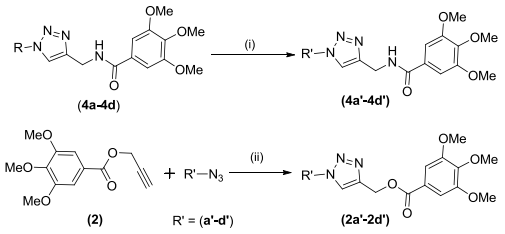
Figure 3. Azides used for click reaction: **a-d** (R= Ac) and **a'-d'** (R = H)

Ester and amide series of gallic acid derivatives (**2a-2j**) and (**4a-4j**) were synthesized by reacting scaffolds **2** and **4** with the azides from azide pool (**a-j**) respectively (scheme 2). 1,3-dipolar cycloaddition reaction was carried out using 10 mol% of $CuSO_4.5H_2O$ and 20 mol% of sodium ascorbate in 1:1 ratio of *t*-BuOH and water as solvent at ambient temperature (scheme 2). Reactions were generally complete following 4-5 hours of vigorous stirring (TLC). The deacetylation of the peracetylated amide analogues (**4a-4d**) were carried out using Zemplén[38] conditions, with methanolic sodium methoxide to liberate the fully deprotected sugar analogues **4a'-4d'** in good yields. The Zemplén deacetylation was found to be unsuitable for the synthesis of ester series of the glycoconjugate (**2a'-2d'**) owing to the cleavage of the ester bond. To overcome this problem the azido sugars panel (**a-d**) were first deacetylated to give the fully deprotected azido sugars (**a'-d'**) which were then reacted with scaffold **2** to give the non-acetylated glycoconjugate of the ester series (**2a'-2d'**) (**scheme 3**).



Reagents and conditions: (i) Azide (1.0 mmol), alkyne (1.0 mmol), $CuSO_4.5H_2O$ (10 mol%), Na-ascorbate (20 mol%), 1:1 t-BuOH/H_2O, rt, 4h, 80-98%

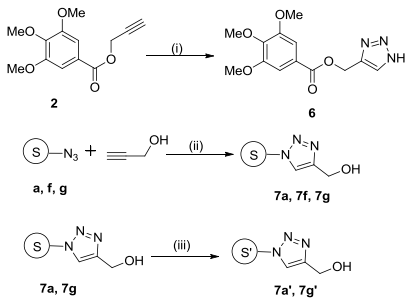
Scheme 2. Synthesis of triazole analogues of gallic acid



Reagents and conditions: (i) NaOMe, MeOH, rt, 15-30 mins, 90-98%, (ii) Azide (1.0 mmol), alkyne (1.0 mmol), $CuSO_4.5H_2O$ (10 mol%), Na-ascorbate (20 mol%), 1:1 t-BuOH/H_2O, rt, 4h, 80-98%

Scheme 3. Synthesis of fully deacetylated glycoconjugates

To gain insight into the structure activity relationship, four fragments i.e. a (triazole conjugated sugars derivative without trimethoxy benzoic acid), b (trimethoxy benzoic acid), c (triazole conjugated trimethoxy benzoic acid derivative without sugar part) and d (*O*-propenyl ester of trimethoxy benzoic acid) were prepared. Synthesis of fragments b and d were carried out as shown in scheme 1. Triazole conjugated derivative of trimethoxy ether of gallic acid without sugar part (fragment c, **6**) was synthesized by reacting *O*-propynyl ester (**2**) with $TMSN_3$, yielded product **6** in 52% yield (scheme 4). Synthesis of fragment **a** i.e. sugar conjugated triazole derivatives (**7a**, **7f**, **7g**) were carried out by reacting sugar azides (**a**, **f** and **g**) with propargyl alcohol using high yielding click chemistry. Subsequently deacetylation of compounds **7a** and **7g** afforded **7a'** and **7g'** respectively (scheme 4).



Reagents and conditions: (i) $TMSN_3$, CuI, DMF/MeOH (9:1), 100 °C, 10-12h, 52% (ii) Azide (0.53 mmol), alkyne (2.24 mmol), $CuSO_4.5H_2O$ (20 mol%), Na-ascorbate (40 mol%), 1:1 *t*-BuOH/H_2O, 40 °C, 2h, 83-91%, (iii) NaOMe, MeOH, rt, 15-30 mins, 95-97%

Scheme 4. Synthesis of fragments of final molecule.

The structures of these compounds (Figure 4) were established on the basis of their ^1H and ^{13}C NMR spectroscopy and high resolution mass spectrometry (HRMS), further to confirm the structure; several 2D NMR experiments were also carried out, which established the complete correlation of the synthesized molecules (**Supporting Information**).

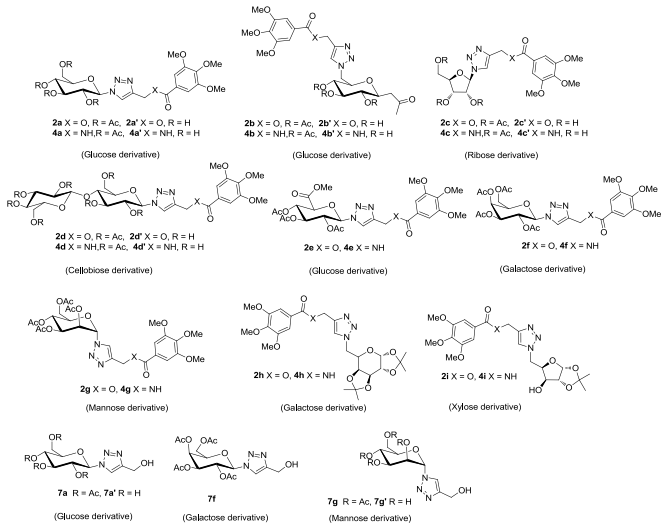


Figure 4. Structures of synthesized glycoconjugates

Biological Evaluation

In Vitro anti-proliferative activities.

All the synthesized compounds were evaluated for their anticancer activities against different cancer cell lines MCF-7 and MDA-MB-231 (human breast cancer), LA-7 (rat mammary cancer), 4T1 (mouse mammary cancer), PC-3 and DU-145 (human prostate cancer), HeLa (human cervical cancer) and HEK-293 (human

embryonic kidney) cells using MTT assay to assess cell proliferation

Table 1. *In Vitro* Anticancer activities of compounds (**2a-4d′**) against different cancer cells

	IC_{50} (μM)[a]							
Entry	**MCF-7**	**MDA-MB-231**	**LA-7**	**4T1**	**PC-3**	**DU-145**	**HeLa**	**HEK-293**
2a	20±2.43	4.2±1.3	9.4±2.65	24.2±3.87	18.3±3.67	10.5±1.76	26±4.34	40±1.27
2b	15±2.14	10±3.01	11±1.78	20.1±1.98	18.6±4.34	34.2±2.57	20.1±2.67	>50
2c	>50	43.2±1.34	>50	45±3.78	39.3±4.34	>50	>50	>50
2d	13.2±1.24	>50	24.1±3.76	32.4±3.56	45.2±2.76	23±3.45	39±3.45	>50
2e	>50	6.2±2.76	10±2.17	19.4±2.19	33.4±2.33	37.2±2.56	26.5±3.23	>50
2f	>50	>50	>50	>50	>50	>50	>50	>50
2g	>50	>50	>50	>50	>50	>50	>50	>50
2h	>50	>50	43±5.7	>50	>50	42.5±4.67	>50	>50
2i	41.1±2.3	>50	38.2±3.43	45.6±2.76	37.2±5.23	>50	>50	>50
2j	>50	>50	>50	>50	43.4±7.61	48.5±4.49	38.1±4.61	39.1±7.52
4a	>50	>50	>50	>50	>50	>50	>50	>50
4b	>50	36.9±2.52	>50	>50	26.2±7.32	>50	>50	35.5±8.81
4c	>50	>50	>50	>50	>50	>50	>50	>50
4d	>50	39.1±7.89	>50	>50	45.8±7.19	>50	>50	>50
4e	48.8±4.79	>50	>50	>50	36.6±4.84	>50	>50	>50
4f	>50	>50	>50	>50	>50	>50	>50	>50
4g	>50	>50	>50	>50	>50	>50	>50	>50
4h	>50	>50	>50	>50	>50	>50	>50	>50
4i	>50	>50	>50	>50	>50	>50	>50	>50
4j	>50	45.5±2.93	>50	>50	>50	47.5±4.65	34.7±3.27	36.8±5.46
2a′	>50	>50	>50	>50	>50	37.1±3.12	25.7±2.91	>50
2b′	>50	45.6±3.37	>50	>50	>50	>50	>50	>50
2c′	>50	39.0±5.95	45.9±9.32	>50	>50	>50	>50	>50
2d′	>50	>50	>50	>50	>50	30.0±5.21	>50	>50
4a′	46.4±5.38	>50	>50	>50	>50	>50	>50	>50
4b′	>50	>50	>50	>50	>50	>50	>50	>50
4c′	>50	>50	>50	>50	>50	>50	>50	>50
4d′	>50	32.2±4.00	>50	>50	>50	>50	>50	>50
5	>50	34±1.37	>50	39±2.12	40±3.32	42±2.34	45±1.16	>50
6	41±2.54	33±3.43	37±3.12	35±3.45	42±2.37	43±2.62	35±2.16	>50
7a	15±3.45	20.5±3.21	19.2±2.1	16.2±2.3	28.5±3.2	22±4.1	21±2.2	>50
7f	>50	45.2±2.13	>50	>50	53.1±3.32	>50	>50	>50
7g	>50	>50	>50	48.2±1.43	>50	>50	>50	>50
7a′	31±3.24	42±2.32	45±3.34	40±1.32	32±2.45	33±1.62	34±2.45	>50
7g′	>50	>50	>50	>50	>50	>50	>50	>50
1	IN	IN	IN	IN	IN	IN	IN	IN
2	IN	IN	IN	IN	IN	IN	IN	IN
4	IN	IN	IN	IN	IN	IN	IN	IN
Gallic acid	>40.3±5.38	>50	>50	>50	>50	>50	>50	>50
Nocodazol	0.45±0.34	1.2±1.11	2.1±0.62	1.3±0.52	2.3±1.21	2.1±0.54	0.7± 1.13	5.6±1.11
Paclitaxel	0.89±0.12	1.4±0.21	1.8±0.13	2.0±0.16	1.1±0.11	0.87±0.13	0.65±0.13	15.3±2.35

[a] *IC_{50} values are concentration required to inhibit tumor cell proliferation by 50%. Values are reported as the ± SD of at least 3 independent experiments. IN-Inactive*

whereas nocodazole and paclitaxel were used as positive control (inducing microtubule destabilization and stabilization respectively (Table.1). Among all the compounds screened, 3 compounds **2a**, **2b** and **2e** displayed notable anti-proliferative activity in breast cancer cells (**Table.1**), specifically against highly aggressive and metastatic MDA-MB-231 breast cancer cells. The IC_{50} values of **2a**, **2b** and **2e** in MDA-MB-231 cells at 24 h were 4.2±1.3 µM, 10±3.01 µM and 6.2±2.76 µM respectively, without any apparent cytotoxicity whereas nocodazole and paclitaxel were found to be cytotoxic at this dose level. A moderate activity against breast cancer cells MCF7 is shown by **2b** and **2d** having IC_{50} values 15±2.14 µM and 13.2±1.24 µM respectively. MDA-MB-231 is triple negative (ER, PR, and HER2 negative) whereas MCF7 is ER, PR positive and HER2 negative and hence MDA-MB-231 is more prone to chemotherapeutic agents.[39] Interestingly, compounds **2a**, **2b** and **2e** were also the most active compounds against LA-7 cells having IC_{50} values 9.4±2.65 µM, 11±1.78 µM and 10±2.17 µM respectively. In addition, compound **2a** showed a broad spectrum of moderate to good anti-proliferative activity against various cancer cell lines, especially against prostate cancer cells DU-145 with IC_{50} values 10.5±1.76 µM. It is observed that gallic acid, which is active at higher doses and trimethoxy ether of gallic acid are found to be inactive under similar experimental conditions. Compound **7a** shows moderate to good activity in different cell lines whereas compound **6** and **7a'** showed moderate activity. The IC_{50} values of **7a** in MDA-MB-231 and MCF-7 cells at 24 h were 20.5±3.21 µM and 15±3.45 µM respectively, without any apparent cytotoxicity.

It has been observed that ester linkage is important for the anti-proliferative activity of these glycoconjugates. Apart from this, there are several crucial factors responsible for the anti-proliferative activity of these molecules. It was found out that *O*-propynyl ester (**2**) and *N*-propynyl amide (**4**) were inactive whereas *O*-propenyl ester (**5**, fragment d) showed somehow better activity. Gallic acid and trimethoxy ether of gallic acid (**1**, fragment b) were inactive under similar experimental conditions. Whereas little improvement in the anti-proliferative activity was observed by replacing alkene of **5** with a triazole ring (**6**, fragment c) which emphasise the importance of triazole ring. It is also observed that benzyl conjugated derivatives (**2j** and **4j**) were found to be inactive. Furthermore, acetylated glycoconjugates derived from glucose and glucuronic methyl ester (which is derived from glucose) are more active and more selective against the MDA-MB-231 cell line. It was also observed that acetylated analogue of glucose conjugated triazole derivative (**7a**, fragment a) showed good antiproliferative activity as compared to other sugar conjugated derivatives. Whereas deacetylated analogue (**7a'**) found to be less activie. All these facts conclude the importance of acetyl group as a masking agent and glucose molecule in these anti-proliferative agents. These all facts indicated the association of all fragments a, b, c and d is crucial for the designing of highly active derivatives.

To gain further insight into the anti-proliferative activity against metastatic breast cancer, we investigated the effect of compound **2a** on cell cycle using Propidium iodide (PI) in conjunction with Annexin V FITC to discriminate whether cells are viable (AV^-/PI^-), early apoptotic (AV^+/PI^-), late apoptotic (AV^+/PI^+) or necrotic (AV^-/PI^+) through differences in plasma membrane integrity and permeability.[40] Results demonstrated that treatment of MDA-MB-231 cells with **2a** resulted in a dose dependent statistically significant increase in the number of early apoptotic (AV^+/PI^-) and late apoptotic (AV^+/PI^+)/necrotic cells (AV^-/PI^+) with subsequent decrease of live cells (AV^-/PI^-). In contrast to this no change was seen in necrotic cells (PI^-) as compared to untreated vehicle control (Figure 5A). Further the effects induced by compound **2a** on cell cycle progression in a 24 h treatment at different concentration were determined using flow cytometry based total DNA content analysis in MDA-MB-231 cells using PI staining (Figure 5B).

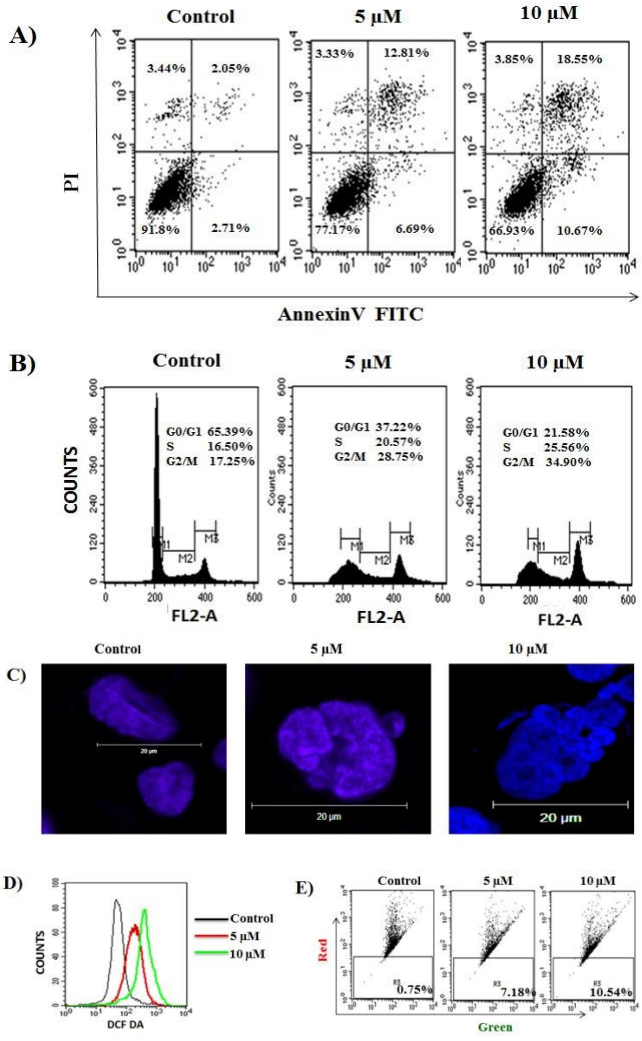


Figure 5. Effects of compound **2a** in MDA-MB-231 cells. Cells were treated with **2a** at the indicated concentration for 24 h (A) Flow cytometric analysis of apoptotic cells after treatment of MDA-MB-231 cells. Cells were harvested and stained with annexin V FITC and PI to differentiate apoptotic and necrotic cells by flow cytometry (B) Effect of compound **2a** on cell cycle progression in MDA-MB-231 cells were fixed and stained with PI before analyzing with flow cytometry. (C) Nuclear morphology of cells after treatment with **2a** Cells were fixed and stained with DAPI. Photographs were taken by confocal microscope (D) Cells were fixed and stained with DCFH-DA dye to check the ROS level by flow cytometry. (E) Mitochondrial membrane depolarization was checked using JC-1 dye by flow cytometry following treatment with **2a** for 24 h.

Result demonstrated a significant G2/M arrest in a concentration dependent manner, with an increasing population in peak corresponding to G2/M phase. The cell cycle arrest in G2/M phase was accompanied by a comparable reduction in the proportions of the cells in other phases of the cell cycle (Figure 5B). Moreover, increased nuclear fragmentation and nuclear bodies was seen in cells treated with compound **2a** as compared to untreated vehicle control as evident from DAPI staining clearly suggests induction of apoptosis (Figure 5C). Collectively, these data suggest that **2a** inhibits growth of MDA-MB-231 by arresting the proliferation of cells and subsequent induction apoptosis. Reactive Oxygen Species (ROS) is a key mediator of apoptotic signaling and many anticancer agents have demonstrated to exert their cytotoxic effects by the generation of reactive oxygen species (ROS).[41] Therefore, we investigated the role of compound **2a** in inducing the production of ROS that could potentially lead to the cytotoxic effect in the MDA-MB-231 cells. ROS generation was detected using ROS sensitive and cell permeable, non-fluorescent probe Dichlorodihydrofluorescein diacetate (DCFH-DA) dye by flow cytometer (**Figure 5D**). After treatment with **2a** at 5 µM and 10 µM concentrations, a dose dependent increase of fluorescent product DCF was observed by successive cleavage of DCFH-DA by intracellular esterases and oxidation of resulting product into DCF which can be measured by flow cytometry confirming ROS generation as compared to untreated vehicle control. Furthermore, an important event related to apoptosis, mitochondrial membrane potential ($\Delta\Psi_{mt}$) was analyzed using JC-1 dye by flow cytometry in MDA-MB-231 cells following **2a** treatment (Figure 5E). Compound **2a** induced concentration dependent depolarization of the mitochondrial membrane potential (low $\Delta\Psi_{mt}$), a characteristic feature of the intrinsic signaling pathway that initiates the apoptosis. In the lower half of the histogram, an increase in the percentage of cells emitting green fluorescence in a dose dependent manner as compared to untreated vehicle control cells can be attributed to mitochondrial membrane depolarization.

Immunofluorescence Staining.

The apparent mitotic phase arrest and subsequent induction of apoptosis in cancer cells treated with compound **2a** prompted us to examine the effects of this compound on the dynamics of microtubule polymerization in intact cells. We started by assessing the integrity of the microtubule network by immunofluorescence microscopy, using an α-tubulin antibody to stain cellular microtubules and with 4′,6-diamidino-2-phenylindole (DAPI) for DNA. Visualization upon immunofluorescence staining of MDA-MB-231 cells by confocal microscopy displayed a well-developed array of hairlike microtubule network of slim fibrous microtubules (red) wrapped around the cell nucleus (blue) in the DMSO treated control cells. On the contrary, cells treated with compound **2a**, at different concentration and after 24 h of incubation (5 µM and 10 µM) had a tubulin staining pattern that was diffuse and disorganized (Figure 6). Compound **2a** treatment at lower concentration of 5 µM lead to disorganization of tubulin as compared to untreated vehicle control. However, this effect is more pronounced at 10 µM concentration. Same pattern was observed when cells were treated with 1.2 µM concentration of Nocodazole which acts as a tubuline polymerization inhibitor. Data showed compound **2a** inhibited microtubule assembly and interfere with mitosis of MDA-MB-231 cells as observed by multipolarization of spindle and multinucleation phenomena. Furthermore, concentration dependent disruption of microtubule suggesting microtubule might be an effective target for **2a**.

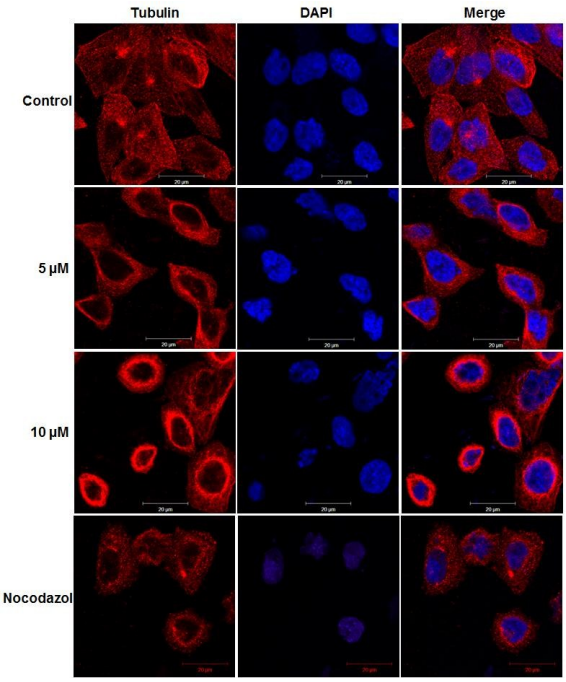


Figure 6. Effect of tested compound **2a** on microtubule dynamic in MDA-MB-231 cells. Cells were treated with 5 and 10 µM of **2a** for 24 h, fixed and probed against tubulin. 1.2 µM Nocodazol used as a positive control. The cells were counterstained with DAPI and visualized by confocal microscopy.

Microtubule Polymerization Inhibition.

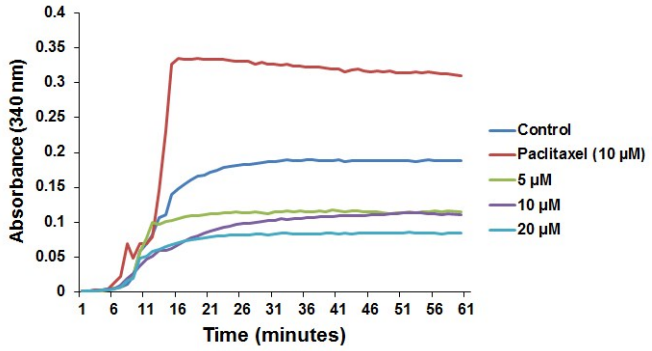


Figure 7. Effects of compound **2a** on microtubule polymerization in a cell free system. Tubulin was incubated with 5, 10 and 20 µM of **2a** at 37^{0}C in polymerization buffer.10 µM paclitaxel used as a positive control. Polymerization of microtubule was monitored kinetically by spectrophotometer.

To obtain a more direct indication of the effects of the compound **2a** on tubulin, tubulin polymerization inhibition assay was performed. Tubulin was incubated with the **2a** (5 µM, 10 µM and 20 µM) and its effect on dynamic of tubulin polymerization was measured by the change of absorbance at 340 nm during 1 hour reaction at 37°C using spectrophotometer (Figure 7). Paclitaxel was used as a positive

control. Results demonstrated that compound **2a** inhibited tubulin polymerization as destabilizer, unlike the positive control paclitaxel. Significant difference in term of polymerization inhibition was observed at all the concentration of compound **2a** as compared to negative vehicle control. However, no difference was seen between 5 and 10 µM. The tubulin polymerization inhibitor EC_{50} of compound **2a**, was 20 µM. Collectively, these results suggest that compound **2a** directly destabilizes and inhibits microtubule depolymerization that may play a part in their anti-proliferative activity.

UV-visible absorption spectroscopy.

Ultraviolet-visible (UV-vis) absorption spectroscopy is greatly used for steady-state studies of protein-drug interaction.[42,43] Therefore, to test interaction of **2a** with tubulin, **2a** was titrated by increasing concentration of tubulin and spectra were recorded by UV-vis spectroscopy (**Figure 8A**). Absorption peak of **2a** centered at 222 nm. Addition of Tubulin drastically decreased maximal absorption peak of **2a** due to complete masking, clearly indicates interaction of **2a** with tubulin.

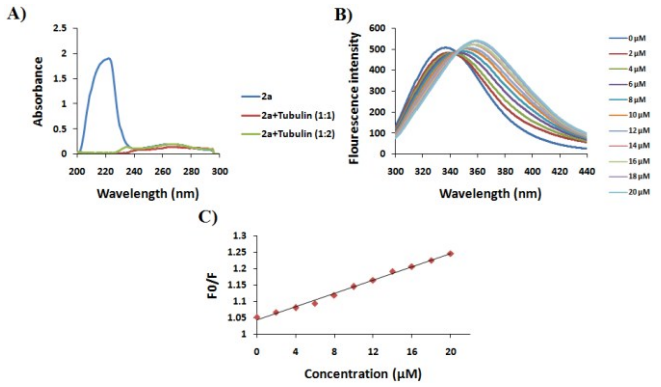


Figure 8. Effect of compound **2a** on tubulin interaction. (A) Compound **2a** was incubated with increasing ratio of Tubulin (1:0, 1:1 and 1:2) and UV-vis spectra were recorded by spectrophotometry (B) Tubulin (5 µM) was incubated with increasing concentration of compound (0-20 µM) with increment of 2 µM. Tubulin was excited at 295 nm and fluorescence emission spectra was recorded by fluorescence spectrophotometer. (C) Stern–Volmer plot of fluorescence of tubulin by **2a**.

Intrinsic Fluorescence Measurements.

The aromatic fluorophores, tryptophan, tyrosine, and phenylalanine contribute in intrinsic fluorescence and thus used for studying drug and protein binding.[44] Therefore, to investigate interaction between tubulin and **2a**, fluorescence quenching was performed by titrating tubulin with **2a** and fluorescence spectra was recorded by fluorimetry (Figure 8B). The mmaximum wavelength of tubulin was in the range of 330–340 nm clearly indicates that tubulin was properly folded and tryptophan is buried in a hydrophobic core.[45] Addition of compound **2a** in tubulin (1:1 ratio) drastically changed the fluorescence emission spectrum. Further addition of compound linearly shifted the fluorescence emission spectra clearly indicate the binding of **2a** to tubulin. In the presence of **2a**, fluorescence spectra of tubulin shifted towards red with the decrease of fluorescence intensity (Figure 8B). Overall, these data clearly suggest that **2a** directly bind to tubulin protein.

To further understand the interaction and quenching process, the ratio of fluorescence intensity (F0/F) was plotted in presence and in absence of **2a** using Stern-Volmer equation.[46]

$$\frac{F0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q]$$

Where F0 and F are the maximum fluorescence intensities in the absence and presence of the quencher i.e. **2a**, τ_0 is the excited state lifetime in the absence of quencher, k_q is quenching constant, [Q] is the concentration of **2a**, K_{SV} is the Stern–Volmer quenching constant. Change in the value of F0/F was plotted against concentration of **2a**. The fluorescent intensity was directly proportional to **2a**concentration. The linearity of the Stern–Volmer plot suggested that only one type of binding or quenching process occurs, either static or dynamic (Figure 8C).

Molecular Modeling Studies.

Molecular modeling studies were then performed to investigate the potential interactions of the most active compound of the series **2a**, as well as other active compounds **2b** and **2e** with the colchicine binding site of α, β-tubulin.

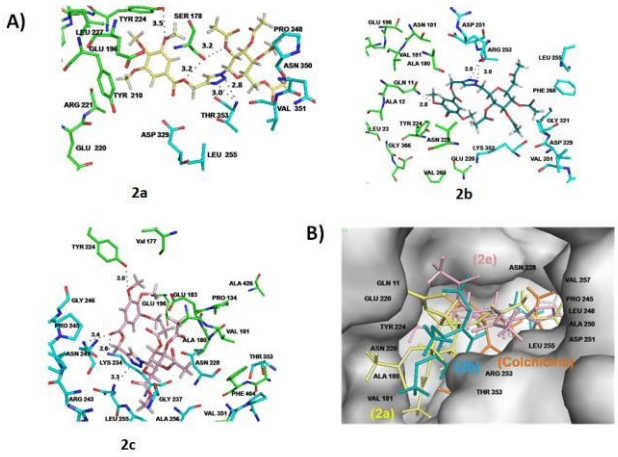


Figure 9. Docking analysis. (A) Predicted docking modes for **2a**, **2b** and **2e** binding in the CS. The *α* and *β* – subunits of tubulin are shown as green and cyan sticks respectively. Polar interactions are shown as black dots. The lead compounds used for the docking study are shown as yellow skeleton (**2a**), blue skeleton (**2b**) and pink skeleton (**2e**) respectively where O-atom, N-atom and H-atoms are depicted in red, blue and gray colors respectively. All compounds are surrounded by the amino acid residue like TYR 224, GLN 11, SER 178(α -subunit), ASN 245, THR 353, ALA 250 and ASP 251(β- subunit). (B) Surface representation of tubulin showing inhibitors **2a**, **2b**, **2e** and colchicine (orange) docked into the colchicine binding site of tubulin.

We performed docking simulations, using the SYBYL X-1.2 package (Tripos Inc., USA) and the Surflex-Dock (SFXC) module. **2a**, **2b** and **2e** were docked inside the colchicine binding site. Docking studies suggest that the most active compound **2a**, occupies the colchicine binding site with the X-ray structure complex of DAMA-Colchicine-α, β-tubulin (PDB code: 1SA0).[35] The α and β subunits of tubulin are shown as green and cyan sticks respectively. Detailed analysis of the inhibitor-tubulin complex revealed several key interactions that are crucial for the binding of tubulin polymerization inhibitors. N-atoms of the triazole formed two hydrogen bonds with the Thr353 amino acid, whereas the O-atom of glucosyl moiety and ester bond

formed hydrogen bonds with Ser178. The hydroxyl group of the Tyr224 formed a hydrogen bond with the O-atom of one of the methoxy group of **2a**. Similar interaction patterns are also shown by compounds **2b** and **2e** (Figure 9A).Thus, the docking studies suggest that the compound(s) preferably occupy the colchicine binding pocket of tubulin and provide a possible rationale for the high potency and anti-proliferative activity exhibited by **2a** in biological assays.

In Vivo Pharmacokinetic Profile.

In order to check the efficacy of the lead compound **2a**, the pharmacokinetic properties were evaluated in male Sprague Dawley rats weighing 250 ± 25 g at a single 5.0 mg/kg dose administered intravenously (IV).

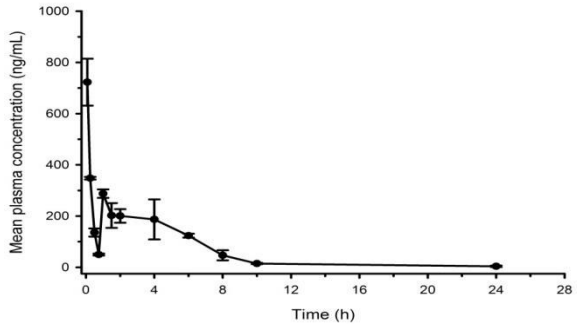


Figure 10. Time versus plasma concentration profile of compound **2a** in male *Sprague Dawley* rats (n=3) following single intravenous (IV, 5 mg/kg) administration. Bar represents SEM.

Table 2. *In vivo* pharmacokinetic parameters of compound **2a** in male *Sprague Dawley* rats[a].

Parameters	
AUC (ng h/mL)	1570.7 ± 224.1
V_{ss} (L/kg)	14.3 ± 3.6
Clearance (L/h/kg)	3.1 ± 0.3
$t_{1/2}$ (h)	4.3 ± 1.0

[a]Each value represents the average of three rats dosed intravenous (5 mg/kg); Values are mean ± SD. $AUC_{0-\infty}$ = area under the plasma concentration-time curve, V_{ss} = volume of distribution at steady-state and $t_{1/2}$ = terminal elimination half-life.

Following intravenous administration the compound was monitored up to 24h. Due to the multiple peak phenomenon, the plasma concentration-time profile was subjected to non-compartmental analysis using Phoenix WinNonlin (version 6.3; Certara Inc, Missouri, USA) and the calculated pharmacokinetic parameters are shown in Table 2. The time versus plasma concentration profile of compound **2a** is shown in figure 10. The volume of distribution (14.3 ± 3.6 L/kg) is higher than the total blood volume of rat (0.054 L/kg; Davies and Morris) and systemic clearance (3.1 ± 0.3 L/h/Kg) is also higher than the total hepatic blood flow in rats (2.9 L/h/kg; Davies and Morris)[48] indicating extravascular distribution along with the extrahepatic elimination of the drug.

In Vivo Anti-tumor Activity.

In vitro results indicated that compound **2a** considerably inhibit cell cycle arrest and apoptosis in highly malignant breast cancer cells. These results firmly prompted us to test it for *in vivo* efficacy. Therefore, to evaluate *in vivo* antitumor efficacy of compound **2a**, a syngenic rat mammary tumor model was generated using LA-7 cells (**Figure 11**).

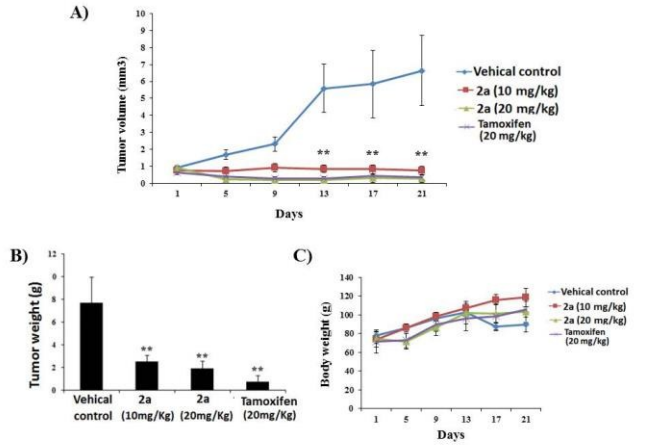


Figure 11. *In Vivo* antitumor effect of compound **2a** in syngenic rat mammary tumor model. LA-7 cells were injected in the mammary fat pad and allowed to develop tumor for one week. 10 mg/kg and 20 mg/kg body weight of the compound was injected daily intra peritoneally and tumor size was measured. Effect of indicated treatment on (A) Tumor volume, (B) Tumor weight and (C) Body weight of the animal.

In this approach LA-7 cells ($6x10^6$ cells) were injected in the mammary fat pad of SD rat. After one week when tumour reached a measurable size, 24 animals were randomly categorized into one of 4 groups (six animals per group). Two groups were injected daily via intraperitoneally with compound **2a** at doses of 10mg/kg and 20 mg/kg respectively. In a third group, tamoxifen was given at 20 mg/kg, while the fourth group received vehicle only. As shown in figure 11 compound **2a** exhibited approximately 80% tumor growth inhibition at a dose of 10 mg/kg as compared to untreated vehicle group within 21 days of treatment window. In terms of tumor growth curve, a dose dependent regression of tumor was observed. 20 mg/kg dose caused complete tumor regression in some of the rats suggesting potency of **2a**. During compound treatment, effect on animal's body weight was also noted. No weight loss was observed as well as no significant mortality and/or toxicity were seen in both the selected dose. Collectively, these data suggest a compound **2a** as a potential anticancer drug candidate selectively against breast cancer while safe towards normal cells.

Conclusions

In conclusion, we designed and synthesized a novel class of gallic acid based glycoconjugates using highly regioselective CuAAC chemistry and evaluated them for their anticancer activities. The mode of action of this series has been revealed via tubulin inhibition assays. The docking studies support that the identified compounds interact with several important residues in the colchicine binding site of tubulin. Compounds

2a, **2b**, and **2e** showed significant anticancer activity in MDA-MB-231 cell line. Further, the lead compound **2a** exhibits anti-proliferative activity (IC_{50} = 4.2 µM) against MDA-MB-231 cells and induce apoptotic cell death by inhibiting the tubulin polymerization leading to arrest at G2/M phase of the cell cycle. The induction of apoptosis was further confirmed by loss of mitochondrial membrane potential, ROS generation, and increased nuclear fragmentation. Immunocytochemistry staining assay demonstrated that **2a** disrupted the microtubule network. UV-visible absorption spectroscopy and intrinsic fluorescence measurements showed direct interaction of **2a** with tubulin. Finally, the representative compound **2a** significantly regress tumor growth of syngenic mammary tumor model, which demonstrated the *in vivo* efficacy of **2a**, without any apparent cytotoxicity, suggesting the selectivity of these molecules toward growth of cancerous cells.

Taken together, these *in vitro* and *in vivo* data suggest that compound **2a** can be transformed into promising lead molecule for drug development against breast cancer and deserve further research and development.

Experimental

General methods

Commercially available reagent grade chemicals were used as received. All reactions were monitored by TLC on E. Merck Kieselgel 60 F254, with detection by UV light, spraying 20% aq $KMnO_4$ solution and/or spraying 4% ethanolic H_2SO_4. Column chromatography was performed on Silica Gel (60–120 mesh, E. Merck). IR spectra were recorded as thin films or in KBr solution with a Perkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker DRX 500 MHz, 400 MHz, 300 MHz, 125 MHz and 100 MHz instruments, respectively, in $CDCl_3$ and DMSO-d_6. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as the internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), dd (double doublet), m (multiplet); J in Hertz. ESI mass spectra were performed using a Quattro II (Micromass) instrument. HRMS spectra were performed using a mass spectrometer Q-TOF. The purity of all tested compounds was characterized by HPLC analysis (Discovery HS C-18 HPLC system The HPLC system consisted of a pump (LC-10AT VP with FCV-10AL VP), degasser (DGU-14A) and auto-injector (SIL-HTc, fixed with a 100 µl loop) (Shimadzu, Japan). Eluents were monitored at 260 nm with UV-Vis multiple wavelength detector and chromatograms were integrated using Class-VP (version 6.12 SP5) software (Shimadzu, Japan).). Individual compounds with a purity of >95% were used for subsequent experiments (see the Supporting Information).

Procedure for the synthesis of Prop-2-yn-1-yl-3,4,5-trimethoxybenzoate (2)

Propargyl bromide (0.79 ml, 10.42 mmol) was added dropwise to a cooled solution of 1 (2.0g, 9.43 mmol) and K_2CO_3 (1.30g, 9.43 mmol) in 10 ml dry DMF. The mixture was stirred for 30 mins at 0ºC then for 4 h at room temperature. After completion of reaction (TLC) the reaction mixture was diluted with ethyl acetate and the organic layer was washed with water and brine solution. The combined organic layer was dried and evaporated under reduced pressure to give the crude mass. Purification by column chromatography using 60-120 mesh silica gel and ethyl acetate: hexane (1:9) as eluant gave the titled compound **2** (2.30 g, 97%) as a white solid, mp 86-88 ºC; R_f (30% EtOAc/Hexane) 0.5; HPLC (purity 96,51%): t_R = 3.175 min.; IR (KBr) cm^{-1}: 670, 759, 1217, 1416, 1461, 1717, 3020, 3305; ^1H NMR (400 MHz, $CDCl_3$): δ 7.24 (s, 2H, Ph.H), 4.84 (d, *J* = 2.44 Hz, 2H, -CH_2O), 3.83 (s, 9H, 3×OCH_3), 2.45 (t, *J* = 2.44 Hz, 1H, -CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.1, 152.9 (2C), 142.6, 124.2, 107.1(2C), 77.7, 75.0, 60.7, 56.1 (2C), 52.4; HRMS (ESI): MH^+, found 251.0914. $C_{13}H_{14}O_5[M+H^+]$ requires 251.0914

Procedure for the synthesis of 3,4,5-trimethoxy-*N*-(prop-2-yn-1-yl)benzamide (4)

At 0 ºC, 3,4,5-trimethoxybenzoyl chloride 3 (2.00 g, 8.70 mmol) dissolved in approx. 10 ml CH_2Cl_2, was added to a solution of propargylamine (0.56 ml, 8.70 mmol), Et_3N (1.21 ml, 8.70 mmol) and DMAP (0.02 g, 0.17 mmol) in CH_2Cl_2. The reaction mixture is stirred at 0 ºC for 30 mins and further 3hrs at ambient temperature. After hydrolysis extraction of the aqueous phase with CH_2Cl_2 and drying of the organic phase with Na_2SO_4 gave crude residue. Scratching of the crude product with ethyl acetate and filteration gave the titled compound 4 (1.91 g, 88%) as a brown solid, mp 168-170ºC; R_f (60% EtOAc/Hexane) 0.4; HPLC (purity 100%): t_R = 2.683 min.; IR (KBr) cm^{-1}: 669, 758, 1215, 1411, 1654, 2399, 3019, 3445; ^1H NMR (400 MHz, DMSO-d_6): δ 8.56 (br.s, 1H, -NH), 7.14 (s, 2H, Ph.H), 4.07-4.05 (m, 2H, -$NHCH_2$), 3.81 (s, 6H, 2×OCH_3), 3.74 (s, 3H, -OCH_3), 2.38 (br.s, 1H, -CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.7, 153.0 (2C), 140.5, 129.3, 105.2 (2C), 81.7, 73.3, 60.5, 56.4 (2C), 28.9; HRMS (ESI): MH^+, found 250.1065. $C_{13}H_{15}NO_4[M+H^+]$ requires 250.1074

General procedure for the synthesis of compounds (2a-2j, 4a-4j)

A mixture of acid (1.0 equiv.) and alkyne (1.0 equiv.) was suspended in a *t*-BuOH and water mixture (1:1) and kept for stirring at room temperature. To the stirring reaction mixture freshly prepared solution of sodium ascorbate (20 mol %) in 500 µL and freshly prepared solution of $CuSO_4.5H_2O$ (10 mol %) in 200 µL water were sequentially added. The reaction mixture was stirred at ambient temperature for 3-4 h. After completion of the reaction (TLC), the reaction mixture was extracted with EtOAc and water. The organic layer was dried (anhyd Na_2SO_4) and evaporated under reduced pressure to give a crude mass. The latter was purified by silica gel (60-120 mesh) column chromatography using ethyl acetate/hexane (1:1) as eluent to give the cycloaddition products detailed below.

Preparation of deprotected glycosyl triazole (2a'-2d' and 4a'-4d').

Compounds 4a'-4d' were prepared by treating the acetylated precursors 4a-4d (final concentration of ~0.1-0.2 M) with dry methanolic sodium methoxide (final pH 9-12). Reactions were found to be complete within 15-30 mins by TLC. Neutralization of the solution by Amberlite IR-120 ion-exchange resin, followed by filtration and evaporation of the filtrate to

dryness, afforded pure material. Likewise, the analogous esters 2a'-2d' were prepared by deprotecting the peracetylated glycosyl azides a-d in the same way as described above, prior to the cycloaddition reaction with 2 (see Scheme 2).

1-(6'-Azido-1',6'-dideoxy-*β*-D-glucopyranos-1'-yl)-propan-2-one (b')

It was obtained by treating the acetylated precursor[34] b(0.10 g, 0.27 mmol) with dry methanolic sodium methoxide (final pH 9-12). Reactions were found to be complete within 15-30 mins by TLC. Neutralization of the solution by Amberlite IR-120 ion-exchange resin, followed by filtration and evaporation of the filtrate to dryness, afforded pure compound b' (0.064 g, 97%) as a white fluffy solid; mp 116-118ºC; R_f (10% MeOH/$CHCl_3$) 0.4; IR (KBr) cm^{-1}: 669, 1070, 1157, 1636, 1715, 2104, 3396; ^1H NMR (400 MHz,DMSO-d6): δ 5.12 (t, *J* = 4.98 Hz, 2H, 2×-OH), 5.01 (d, *J* = 4.59 Hz, 1H, -OH), 3.60-3.55 (dt, J_1= 9.44 Hz, J_2 = 2.69 Hz, 1H, $H_{1'}$), 3.40-3.26 (m, 4H, -CH_2CO, $H_{2'}$, $H_{3'}$), 3.05-2.99 (m, 1H, $H_{5'}$), 2.93-2.89 (m, 1H, $H_{4'}$), 2.79-2.74 (dd, J_1 = 15.73 Hz, J_2 = 9.57 Hz, 1H, $H_{6'}$), 2.44 (dd, J_1 = 15.73 Hz, J_2= 9.57 Hz, 1H, $H_{6''}$), 2.11 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ 207.1, 79.4, 78.0, 76.6, 73.9, 71.5, 51.9, 46.6, 30.8; molecular formula: $C_9H_{16}O_6$.

1-(2',3',4',6'-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2a)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar a (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 2a (0.24 g, 95%) as a white solid; mp 108-112ºC; R_f (60% EtOAc/Hexane) 0.4; HPLC (purity 95.85%): t_R = 2.942 min.; IR (KBr) cm^{-1}: 669, 759, 1216, 1416, 1755, 3021; ^1H NMR (400 MHz, $CDCl_3$): δ 7.84 (s, 1H, triazole.H), 7.22 (s, 2H, Ph.H), 5.81 (d, *J*= 9.01 Hz, 1H, $H_{1'}$), 5.43 (d, *J* = 12.52 Hz, 1H, CH_2O), 5.38-5.33 (m, 3H, CH_2O, $H_{2'}$, $H_{3'}$), 5.15-5.13 (m, 1H, $H_{4'}$), 4.24-4.20 (dd, J_1 = 12.65 Hz, J_2 = 5.02 Hz, 1H, $H_{6'}$), 4.09-4.05 (dd,J_1 = 12.65 Hz, J_2 = 1.09 Hz, 1H, $H_{6''}$), 3.94-3.90 (m, 1H, $H_{5'}$), 3.81 (s, 9H, 3×OCH_3), 2.00 (s, 3H, -OAc), 1.98 (s, 3H, -OAc), 1.94 (s, 3H, -OAc), 1.77 (s, 3H, -OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.5, 169.0, 168.5, 165.8, 152.9 (2C), 143.6, 142.5, 124.5, 122.3, 107.0 (2C), 85.8, 75.3, 72.5, 70.3, 67.6, 61.3, 60.7, 57.8, 56.1 (2C), 20.5, 20.4 (2C), 20.0; HRMS (ESI): MH^+, found 624.2035. $C_{27}H_{33}N_3O_{14}[M+H^+]$ requires 624.2035.

1-*β*-D-glucopyranosyl-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2a')

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar a' (0.08 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (5% MeOH/$CHCl_3$), gave the titled compound 2a' (0.16 g, 85%) as a yellow sticky solid; R_f (10% MeOH/$CHCl_3$) 0.4; HPLC (purity 99.45%): t_R = 2.892 min.; IR (KBr) cm^{-1}: 669, 759, 1216, 1414, 1459, 1710, 3020, 3399; ^1H NMR (400 MHz, DMSO-d6): δ 8.38 (s, 1H, triazole.H), 7.24 (s, 2H, Ph.H), 5.56 (d, *J* = 9.24 Hz, 1H, $H_{1'}$), 5.44 (d, *J* = 6.01 Hz, 1H, -OH), 5.41 (s, 2H, CH_2O), 5.31 (d, *J* = 4.45 Hz, 1H, -OH), 5.21 (d, *J* = 5.31 Hz, 1H, -OH), 4.70 (br.s, 1H, -OH), 3.81 (s, 6H, 2×OCH_3), 3.78-3.77 (m, 1H, $H_{2'}$), 3.72 (s, 3H, OCH_3), 3.46 (br.s, 2H, $H_{6'}$, $H_{6''}$), 3.44 (br.s, 1H, $H_{3'}$), 3.41-3.36 (m, 1H, $H_{5'}$), 3.27-3.21 (m, 1H, $H_{4'}$); ^{13}C NMR (100 MHz, DMSO-d6): δ 165.6, 153.2 (2C), 142.4, 124.8, 124.6, 107.1 (2C), 87.9, 80.3, 77.3, 72.4, 69.9, 61.1, 60.6, 58.3, 56.5 (2C), 46.2; HRMS (ESI): MH^+, found 456.1610. $C_{19}H_{25}N_3O_{10}[M+H^+]$ requires 456.1613.

1-({[1',6'-dideoxy-2',3',4'-tri-*O*-acetyl-*β*-D-glucopyranosyl-1'-yl]-propan-2''-one}-6'-yl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2b)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar b (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), Purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 2b (0.25 g, 91%) as a white solid; mp 152-154 ºC; R_f (60% EtOAc/Hexane) 0.35; HPLC (purity 95.38%): t_R = 2.833 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1416, 1464, 1718, 1755, 2399, 3019; ^1H NMR (400 MHz, $CDCl_3$): δ 7.71 (s, 1H, triazole.H), 7.30 (s, 2H, Ph.H), 5.45-5.44 (m, 2H, CH_2O), 5.21-5.17 (t, *J*= 9.45 Hz, 1H, $H_{1'}$), 4.88-4.81 (m, 2H, $H_{2'}$, $H_{3'}$), 4.59-4.55 (dd, J_1 = 14.75 Hz, J_2 = 2.23 Hz, 1H, $H_{6'}$), 4.31-4.25 (dd, J_1 = 14.48 Hz, J_2 = 8.08 Hz, 1H, $H_{6''}$), 3.95-3.92 (m, 1H, $H_{4'}$), 3.88 (s, 10H, 3×OCH_3, $H_{5'}$), 2.67-2.61 (dd, 1H, J_1 = 16.67 Hz, J_2 = 8.91Hz, -CH_2CO), 2.51-2.46 (dd, 1H, J_1 = 16.67 Hz, J_2 = 3.34 Hz, -CH_2CO), 2.08 (s, 3H, CH_3CO), 2.05 (s, 3H, -OAc), 2.00 (s, 3H, -OAc), 1.98 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.0, 170.0, 169.7 (2C), 166.0, 152.9 (2C), 143.0, 142.4, 125.3, 124.7, 107.0 (2C), 76.1, 73.7, 73.6, 71.2, 69.7, 60.8, 57.9, 56.2 (2C), 50.9, 45.2, 30.4, 20.6, 20.5, 20.5; HRMS (ESI): MH^+, found 622.2243. $C_{28}H_{35}N_3O_{13}[M+H^+]$ requires 622.2243.

1-({[1',6'-dideoxy-*β*-D-glucopyranosyl-1'-yl]-propan-2''-one}-6'-yl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-l-*H*-1,2,3-triazole (2b')

It was obtained by the reaction of 2 (0.10 g, 0.40mmol), azido sugar b' (0.10 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), Purification by column chromatography using 60-120 mesh silica gel (5% MeOH/$CHCl_3$), gave the titled compound 2b' (0.17 g, 84%) as a yellow sticky solid; R_f (10% MeOH/$CHCl_3$) 0.3; HPLC (purity 100%): t_R = 2.450 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1416, 1464, 1712, 2399, 3019, 3399; ^1H NMR (400 MHz, DMSO-d6): δ 7.99 (s, 1H, triazole.H), 7.25 (s, 2H, Ph.H), 5.37 (br.s, 2H, $H_{1''}$,-OH), 5.15 (d, *J* = 5.17 Hz, 1H, -OH), 5.11 (d, *J* = 4.17 Hz, 1H, -OH), 4.74 (d, *J* = 13.97 Hz, 1H, $H_{6'}$), 4.36-4.30 (m, 1H, $H_{6''}$), 3.83 (s, 6H, 2×OCH_3), 3.74 (s, 3H, OCH_3), 3.50 (d, *J* = 9.63 Hz, 1H, -CH_2O), 3.45 (d, *J* = 10.39 Hz, 1H, -CH_2O), 3.21-3.20 (m, 1H, $H_{2'}$), 3.10-2.98 (m, 2H, $H_{3'}$, $H_{4'}$), 2.92-2.86 (m, 1H, $H_{5'}$), 2.73 (d, *J* = 15.14 Hz, 1H, -CH_2CO), 2.35-2.29 (m, 1H, -CH_2CO), 1.86 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, DMSO-d6): δ 207.3, 165.5, 153.2 (2C), 142.3, 142.0, 126.1, 124.9, 107.0 (2C), 78.4, 77.9, 76.6, 74.0, 72.0, 60.6, 58.3, 56.4 (2C), 51.7, 46.6, 30.2; HRMS (ESI): MH^+, found 496.1928. $C_{22}H_{29}N_3O_{10}[M+H^+]$ requires 496.1926.

1-(2',3',5'-tri-*O*-acetyl-*β*-D-ribofuranosyl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2c)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar c (0.12 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01g, 0.04 mmol) and sodium ascorbate (0.02g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml). Purification by column chromatography using 60-120 mesh silica gel (30% EtOAc/Hexane), gave the titled compound 2c (0.21 g, 95%) as a white solid; mp 68-70 ºC; R_f (70% EtOAc/Hexane) 0.3; HPLC (purity 95.65%): t_R = 2.950 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1416, 1463, 1590, 1709, 1752, 3019; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (s, 1H, triazole.H), 7.27 (s, 2H, Ph.H), 6.02 (d, J = 9.01 Hz, 1H, $H_{1'}$), 5.77 (br.s, 1H, $H_{4'}$), 5.49 (d, J = 12.70 Hz, 1H, CH_2O), 5.42 (d, J = 12.94 Hz, 1H, CH_2O), 5.37-5.34 (dd, J_1= 9.01 Hz, J_2 = 2.66 Hz, 1H, $H_{3'}$), 5.19-5.15 (m, 1H, $H_{2'}$), 4.07-4.03 (dd, J_1 = 11.20 Hz, J_2 = 5.31 Hz, 1H, $H_{5'}$), 3.99 (d, J = 10.63 Hz, 1H, $H_{5''}$), 3.88 (s, 6H, 2×OCH_3), 3.87 (s, 3H, OCH_3), 2.21 (s, 3H, -OAc), 2.04 (s, 3H, -OAc), 1.86 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 169.1, 168.5, 165.8, 152.9 (2C), 143.5, 142.5, 124.5, 122.6, 107.0 (2C), 83.6, 68.3, 67.9, 65.7, 63.7, 60.7, 57.8, 56.2 (2C), 20.6, 20.4, 20.1; HRMS (ESI): MH^+, found 552.1824. $C_{24}H_{29}N_3O_{12}[M+H^+]$ requires 552.1824.

1-(*β*-D-ribofuranosyl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2c')

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar c' (0.07 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml). Purification by column chromatography using 60-120 mesh silica gel (5% MeOH/$CHCl_3$), gave the titled compound 2c'(0.15 g, 87%) as a white sticky solid; R_f (10% MeOH/$CHCl_3$) 0.4; HPLC (purity 100%): t_R = 2.475 min.; IR (KBr) cm^{-1}: 669, 759, 1410, 1702, 2401, 3020, 3399; ^{1}H NMR (400 MHz, DMSO-d6): δ 8.40 (s, 1H, triazole.H), 7.24 (s, 2H, Ph.H), 5.67 (d, J_1 = 8.97 Hz, 1H, $H_{1'}$), 5.42 (s, 2H, CH_2O), 5.15-5.12 (m, 2H, 2×-OH), 4.91 (d, J = 4.91, 1H, -OH), 4.05-4.02 (m, 2H, $H_{2'}$,$H_{3'}$), 3.83 (s, 6H, 2×OCH_3), 3.74 (s, 3H, OCH_3), 3.71-3.64 (m, 2H, $H_{5'}$, $H_{5''}$), 3.62-3.59 (m, 1H, $H_{4'}$); ^{13}C NMR (100 MHz, DMSO-d6): δ 165.5, 153.2 (2C), 142.4, 142.3, 124.8, 124.7, 107.0 (2C), 85.3, 71.5, 69.5, 66.8, 65.6, 60.6, 58.3, 56.5 (2C); HRMS (ESI): MH^+, found 426.1508. $C_{18}H_{23}N_3O_9[M+H^+]$ requires 426.1507.

1-{4'-*O*-(2'',3'',4'',6''-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-2',3',6'-tetra-*O*-acetyl-*β*-D-glucopyranosyl}-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2d)

It was obtained by the reaction of 2 (0.10g, 0.40mmol), azido sugar d (0.26 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml). Purification by column chromatography using 60-120 mesh silica gel (50% EtOAc/Hexane), gave the titled compound 2d (0.32 g, 87%) as a white solid; mp 188-192 ºC; R_f (70% EtOAc/Hexane) 0.2; HPLC (purity 95.13%): t_R = 2.950 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1413, 1586, 1653, 3019; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (s, 1H, triazole.H), 7.28 (s, 2H, Ph.H), 5.82-5.79 (m, 1H, $H_{1'}$), 5.45 (s, 1H, $H_{2'}$), 5.43 (s, 1H, $H_{3'}$), 5.40-5.37 (m, 2H, CH_2O), 5.15 (t, J = 9.42 Hz, 1H, $H_{3''}$), 5.07 (t, J = 9.70 Hz, 1H, $H_{4''}$), 4.93 (t, J = 9.08 Hz, 1H, $H_{2''}$), 4.57 (d, J = 7.87 Hz, 1H, $H_{1''}$), 4.51 (d, J = 12.20 Hz, 1H, $H_{6b'}$), 4.40-4.35 (dd, J_1 = 12.50 Hz, J_2 = 4.33 Hz, 1H, $H_{6b''}$), 4.07-4.04 (m, 1H, $H_{4'}$), 3.91 (s, 1H, $H_{5'}$), 3.89 (s, 2H, $H_{6a'}$, $H_{6a''}$), 3.89 (s, 9H, 3×OCH_3), 3.69-3.66 (m, 1H, $H_{5''}$), 2.10 (m, 6H, 2×-OAc), 2.03 (m, 6H, 2×-OAc), 2.01 (s, 3H, -OAc), 1.98 (s, 3H, -OAc), 1.84 (s, 3H, -OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.0 (2C), 169.4, 169.1, 168.9 (2C), 165.9, 152.9(2C), 143.4, 142.5, 124.5, 122.6, 107.0(2C), 100.7, 85.5, 76.0, 75.7, 72.8, 72.2, 72.1, 71.5, 70.4, 67.7, 61.6, 61.5, 60.8, 57.8, 56.2 (2C), 20.7, 20.6, 20.4 (3C), 20.3, 20.1; HRMS (ESI): MH^+, found 912.2876. $C_{39}H_{49}N_3O_{22}[M+H^+]$ requires 912.2880.

1-(4'-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2d')

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar d' (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (10% MeOH/$CHCl_3$), gave the titled compound 2d' (0.20 g, 81%) as a yellow white solid; mp 106-108 ºC; R_f (15% MeOH/$CHCl_3$) 0.4; HPLC (purity 100%): t_R = 2.317 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1638, 2399, 3019, 3400; ^{1}H NMR (400 MHz, DMSO-d6): δ 8.42 (s, 1H, triazole.H), 7.25 (s, 2H, Ph.H), 5.67 (d, J = 9.15 Hz, 1H, $H_{1'}$), 5.59 (d, J = 6.10 Hz, 1H, -OH), 5.43 (s, 2H, CH_2O), 5.27 (d, J = 4.73 Hz, 1H, -OH), 5.05 (d, J = 3.76 Hz, 1H, -OH), 5.02 (d, J = 4.94 Hz, 1H, -OH), 4.90 (d, J = 1.77 Hz, 1H, -OH), 4.68 (t, J = 5.81 Hz, 1H, -OH), 4.62 (t, J = 4.70 Hz, 1H, -OH), 4.33 (d, J = 7.91 Hz, 1H, $H_{1''}$), 3.83 (s, 6H, 2×OCH_3), 3.79-3.72 (m, 5H, OCH_3,$H_{2'}$, $H_{6b'}$), 3.69-3.62 (m, 2H, $H_{6b''}$, $H_{5'}$), 3.61-3.41 (m, 4H, $H_{6a'}$,$H_{3'}$,$H_{4'}$, $H_{6a''}$), 3.25-3.16 (m, 2H, $H_{5''}$, $H_{3''}$), 3.11-3.01 (m, 2H, $H_{4''}$, $H_{2''}$). ^{13}C NMR (100 MHz, DMSO-d6): δ 165.5, 153.2 (2C), 142.4, 129.1, 124.8, 124.6, 107.0 (2C), 103.5, 87.4, 80.0, 78.2, 77.3, 76.9, 75.6, 73.7, 72.1, 70.5, 61.5, 60.6, 60.3, 58.3, 56.5 (2C); HRMS (ESI): MH^+, found 618.2142. $C_{25}H_{35}N_3O_{15}[M+H^+]$ requires 618.2141.

1-(2',3',4'-tri-*O*-acetyl-*β*-D-glucopyranuronic acid methyl ester)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2e)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar e (0.14 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml). Purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 2e (0.23 g, 93%) as a white solid; mp 164-166 ºC; R_f (70% EtOAc/Hexane) 0.4; HPLC (purity 95.24%): t_R = 2.992 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1637, 1759, 2399, 3019, 3400; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H, triazole.H), 7.23 (s, 2H, Ph.H), 5.87-5.83 (m, 1H, $H_{1'}$), 5.43-5.37 (m, 4H, CH_2O, $H_{2'}$, $H_{3'}$), 5.31-5.26 (m, 1H, $H_{4'}$), 4.26 (d, J = 9.70 Hz, 1H, $H_{5'}$), 3.82 (s, 9H, 3×OCH_3), 3.67 (s, 3H, CH_3), 1.99 (s, 3H, -OAc), 1.97 (s, 3H, -OAc), 1.78 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 169.2, 168.7, 166.0, 165.9, 152.9(2C), 143.7, 142.4, 124.5, 122.6, 107.0 (2C), 85.5, 74.9, 71.8, 70.0, 68.9, 57.8, 56.2 (2C), 53.1, 20.4, 20.4, 20.0; HRMS (ESI): MH^+, found 610.1879. $C_{26}H_{31}N_3O_{14}[M+H^+]$ requires 610.1879.

1-(2',3',4',6'-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2f)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar f (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml). Purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 2f (0.23 g, 94%) as a white solid; mp 70-74 ºC; R_f

(60% EtOAc/Hexane) 0.3; HPLC (purity 96.85%): t_R = 2.942 min.; IR (KBr) cm^{-1}: 669, 759, 1127, 1219, 1416, 1460, 1753, 3021; ^1H NMR (400 MHz, $CDCl_3$): δ 7.98 (br.s, 1H, triazole.H), 7.29 (s, 2H, Ph.H), 5.86 (d,*J*= 9.29 Hz, 1H, $H_{1'}$), 5.52-5.47 (m, 3H, CH_2O, $H_{2'}$), 5.42 (d, *J*= 12.75 Hz, 1H, $H_{6'}$), 5.26-5.23 (dd, J_1 = 10.26 Hz, J_2 = 3.33 Hz, 1H, $H_{6''}$), 4.27-4.18 (m, 2H, $H_{3'}$,$H_{4'}$), 4.14-4.09 (m, 1H, $H_{5'}$), 3.89 (s, 6H, 2×OCH_3), 3.87 (s, 3H, OCH_3), 2.23 (s, 3H, -OAc), 2.03 (s, 3H, -OAc), 2.00 (s, 3H, -OAc), 1.85 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.0, 169.8, 169.0, 166.0, 152.9 (2C), 143.5, 142.3, 124.5, 122.7, 106.9 (2C), 86.3, 74.1, 70.6, 67.8, 66.8, 61.2, 60.9, 57.8, 56.2 (2C), 20.7, 20.6, 20.5, 20.2; HRMS (ESI): MH^+, found 624.2035. $C_{27}H_{33}N_3O_{14}[M+H^+]$ requires 624.2035.

1-(2',3',4',6'-tetra-*O*-acetyl-*α*-D-mannopyranosyl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2g)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar g (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 2g (0.22 g, 89%) as a white solid; mp 66-70 ºC; R_f (60% EtOAc/Hexane) 0.3; HPLC (purity 95.27%): t_R = 2.933 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1417, 1464, 1654, 1752, 3019; ^1H NMR (400 MHz, $CDCl_3$): δ 7.89 (s, 1H, triazole.H), 7.29 (s, 2H, Ph.H), 5.99 (br.s, 1H, $H_{1'}$), 5.93-5.89 (m, 2H, $H_{2'}$, $H_{3'}$), 5.53 (d, *J* = 12.65 Hz, 1H, -CH_2O), 5.48 (d, *J* = 12.65 Hz, 1H, -CH_2O), 5.35 (t, *J* = 8.73 Hz, 1H, $H_{4'}$), 4.37-4.33 (dd, J_1 = 12.36 Hz, J_2 = 5.41 Hz, 1H, $H_{6'}$), 4.07 (d, *J* = 12.36 Hz, 1H, $H_{6''}$), 3.90 (s, 10H, $H_{5'}$, 3×OCH_3), 2.17 (s, 3H, -OAc), 2.06 (s, 9H, 3×-OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 169.5 (2C), 169.1, 166.0, 152.9 (2C), 143.6, 142.6, 124.5, 124.4, 107.0(2C), 83.5, 72.3, 68.6, 68.2, 66.0, 61.4, 60.8, 57.6, 56.2 (2C), 20.6 (2C), 20.54, 20.52; HRMS (ESI): MH^+, found 624.2034. $C_{27}H_{33}N_3O_{14}[M+H^+]$ requires 624.2035.

1-(6'-deoxy-1',2',3',4'-di-*O*-isopropylidene-*α*-D-galactopyranos-6'-yl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2h)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar h (0.11 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (30% EtOAc/Hexane), gave the titled compound 2h (0.20 g, 93%) as a white solid; mp 84-88 ºC; R_f (60% EtOAc/Hexane) 0.5; HPLC (purity 100%): t_R = 2.650 min.; IR (KBr) cm^{-1}: 670, 760, 1216, 1416, 1711, 3020; ^1H NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H, triazole.H), 7.29 (s, 2H, Ph.H), 5.52-5.40 (m, 3H, $H_{1'}$,CH_2O), 4.66-4.60 (m, 2H, $H_{2'}$,$H_{3'}$), 4.50-4.42 (dd, J_1 = 14.31 Hz, J_2 = 8.49 Hz, 1H, $H_{6'}$), 4.34-4.32 (dd, J_1 = 4.64 Hz, J_2 = 2.31 Hz, 1H, $H_{6''}$), 4.20 (d, *J* = 7.66 Hz, 2H, CH_2O), 3.89 (s, 9H, 3×OCH_3), 1.49 (s, 3H, CH_3), 1.36 (s, 6H, 2×CH_3), 1.27 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 152.8 (2C), 142.5 (2C), 125.2, 124.7, 109.8, 108.9, 107.0 (3C), 71.1, 70.7, 70.3, 67.1, 60.7, 58.1, 56.1 (2C), 50.5, 25.9 (2C), 24.8, 24.3; HRMS (ESI): MH^+, found 536.2239. $C_{25}H_{33}N_3O_{10}[M+H^+]$ requires 536.2239.

1-(5'-deoxy-1',2'-*O*-isopropylidene-*α*-D-xylofuranos-5'-yl)-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2i)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), azido sugar i (0.09 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 2i (0.17 g, 89%) as a white solid; mp 116-120 ºC; R_f (60% EtOAc/Hexane) 0.5; HPLC (purity 100%): t_R = 2.733 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1417, 1464, 1711, 3019; ^1H NMR (400 MHz, $CDCl_3$): δ 7.83 (s, 1H, triazole.H), 7.27 (s, 2H, Ph.H), 5.95 (d, *J* = 2.77 Hz, 1H, $H_{1'}$), 5.44-5.43 (m, 2H, CH_2O), 4.81-4.76 (dd, J_1 = 13.03 Hz, J_2 = 5.54 Hz, 1H, $H_{5'}$), 4.56-4.55 (m, 2H, $H_{5''}$, $H_{3'}$), 4.45 (br.s, 1H, $H_{4'}$), 4.23 (br.s, 1H, $H_{2'}$), 3.88-3.87 (m, 9H, 3×OCH_3), 3.12 (br.s, 1H, -OH), 1.42 (s, 3H, CH_3), 1.28 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 152.9 (2C), 142.9, 142.3, 125.6, 124.5, 112.0, 106.9 (2C), 105.1, 85.2, 79.1, 74.5, 60.9, 57.8, 56.2 (2C), 48.6, 26.7, 26.1; HRMS (ESI): MH^+, found 466.1821. $C_{21}H_{27}N_3O_9[M+H^+]$ requires 466.1820.

1-benzyl-4-(3,4,5-trimethoxybenzoyloxymethyl)-1*H*-1,2,3-triazole (2j)

It was obtained by the reaction of 2 (0.10 g, 0.40 mmol), benzyl azide j (0.05 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (20% EtOAc/Hexane), gave the titled compound 2j (0.15 g, 98%) as a white solid; mp 94-96 ºC; R_f (50% EtOAc/Hexane) 0.5; HPLC (purity 96.62%): t_R = 3.117 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1416, 1464, 1709, 1796, 2400, 3019; ^1H NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H, triazole H), 7.37-7.34 (m, 3H, Ph H), 7.28-7.27 (m, 4H, Ph H), 5.51 (s, 2H, CH_2O), 5.42 (s, 2H, -CH_2Ar), 3.88 (s, 3H, OCH_3), 3.86 (s, 6H, 2×OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 152.9 (2C), 143.2, 142.4, 134.3, 129.1 (2C), 128.8, 128.1 (2C), 124.6, 123.9, 107.0 (2C), 60.8, 58.0, 56.2 (2C), 54.2; HRMS (ESI): MH^+, found 384.1554. $C_{20}H_{21}N_3O_5[M+H^+]$ requires 384.1554.

1-(2',3',4',6'-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4a)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar a (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (50% EtOAc/Hexane), gave the titled compound 4a (0.23 g, 90%) as a white solid; mp 134-138 ºC; R_f (70% EtOAc/Hexane) 0.3; HPLC (purity 100%): t_R = 2.675 min.; IR (KBr) cm^{-1}: 668, 756, 1215, 1433, 1640, 1756, 3020; ^1H NMR (400 MHz, $CDCl_3$): δ 7.94 (s, 1H, triazole.H), 7.24 (s, 1H, -NH), 7.03 (s, 2H, Ph.H), 5.87 (d, *J* = 8.49 Hz, 1H, $H_{1'}$), 5.45-5.37 (m, 2H, -CH_2NH), 5.25 (t, *J* = 9.43 Hz, 1H, $H_{2'}$), 4.70 (m, 2H, $H_{3'}$, $H_{4'}$), 4.32-4.28 (dd, J_1 = 12.50 Hz, J_2 = 4.72 Hz, 1H, $H_{6'}$), 4.14-4.11 (dd, J_1 = 12.56 Hz,J_2 = 1.53 Hz, 1H,$H_{6''}$), 4.02-3.98 (m, 1H, $H_{5'}$), 3.85 (s, 9H, 3×OCH_3), 2.06 (s, 6H, 2×-OAc), 2.02 (s, 3H, -OAc), 1.84 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 169.9, 169.3, 168.8, 167.1, 153.1 (2C), 145.5, 141.0, 129.2, 121.3, 104.5 (2C), 85.7, 75.1, 72.6, 70.4, 67.6, 61.4, 60.8, 56.2 (2C), 35.2, 20.6, 20.4 (2C), 20.1; HRMS (ESI): MH^+, found 623.2195. $C_{27}H_{34}N_4O_{13}[M+H^+]$ requires 623.2195.

1-(*β*-D-glucopyranosyl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4a')

Title compound was prepared according to general procedure 2 by treating the acetylated compound 4a (0.10 g, 0.16 mmol) with NaOMe in methanol to give the fully deprotected compound 4a' (0.07 g, 81%) as a red solid; mp 104-106 ºC; R_f (15% MeOH/$CHCl_3$) 0.3; HPLC (purity 100%): t_R = 2.358 min.; IR (KBr) cm^{-1}: 669, 759, 1215, 1384, 1638, 3019, 3400; ^1H NMR (400 MHz, DMSO-d_6): δ 9.07-9.04 (t, *J* = 4.85 Hz, 1H,-NH), 8.10 (s, 1H, triazole.H), 7.22 (s, 2H, Ph.H), 5.51 (d, *J*= 9.22 Hz, 1H, $H_{1'}$), 5.40 (d, *J* = 5.82 Hz, 1H, -OH), 5.28 (d, *J* = 3.74 Hz, 1H, -OH), 5.20 (d, *J* = 5.13 Hz, 1H, -OH), 4.70 (brs, 1H, -OH), 4.53 (d, *J* = 5.06 Hz, 2H, -CH_2NH), 3.81 (s, 6H, 2×OCH_3), 3.76 (d, *J* = 6.24 Hz, 1H, $H_{2'}$), 3.69 (s, 3H, OCH_3), 3.44-3.42 (m, 3H, $H_{5'}$, $H_{6'}$, $H_{6''}$), 3.38-3.37 (m, 1H, $H_{3'}$), 3.24-3.22 (m, 1H, $H_{4'}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.2, 153.0 (2C), 145.5, 140.5, 129.5, 122.6, 105.3 (2C), 87.8, 80.2, 77.3, 72.4, 69.9, 61.1, 60.5, 56.4 (2C), 35.2; HRMS (ESI): MH^+, found 455.1782. $C_{19}H_{26}N_4O_9$[M+H^+] requires 455.1773.

1-({[1',6'-dideoxy-2',3',4'-tri-*O*-acetyl-*β*-D-glucopyranosyl-1'-yl]-propan-2''-one}-6'-yl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4b)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar b (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (50% EtOAc/Hexane), gave the titled compound 4b (0.22 g, 86%) as a white solid; mp 166-164 ºC; R_f (70% EtOAc/Hexane) 0.3; HPLC (purity 100%): t_R = 2.633 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1496, 1654, 1755, 2399, 3019; ^1H NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H, triazole.H), 7.35 (brs, 1H, -NH), 7.12 (s, 2H, Ph.H), 5.20 (t, *J*= 9.29 Hz, 1H, $H_{1'}$), 4.88 (d, *J* = 9.33 Hz, 1H, $H_{2'}$), 4.84 (d, *J* = 9.63 Hz, 1H, $H_{3'}$), 4.71 (t, *J* = 4.57 Hz, 2H, -CH_2NH), 4.58 (d, *J* = 13.44 Hz, 1H, $H_{6'}$), 4.30-4.24 (dd, J_1 = 14.45 Hz, J_2 = 8.43 Hz, 1H, $H_{6''}$), 3.94 (d, *J* = 4.40 Hz, 1H, $H_{5'}$), 3.87 (s, 6H, 2×OCH_3), 3.86 (s, 3H, OCH_3), 3.82 (d, *J* = 8.28 Hz, 1H, $H_{4'}$), 2.67-2.61 (dd, J_1 = 16.86 Hz, J_2 = 9.33 Hz, 1H, CH_2CO), 2.51-2.46 (dd, J_1= 16.55 Hz, J_2 = 2.01 Hz, 1H, CH_2CO), 2.09 (s, 3H, CH_3), 2.02 (s, 3H, -OAc), 1.99 (s, 6H, 2×-OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.8, 170.0, 169.7 (2C), 167.1, 153.0 (2C), 144.7, 140.9, 129.3, 123.9, 104.6 (2C), 76.3, 73.6, 73.6, 71.2, 69.8, 60.8, 56.2 (2C), 50.9, 45.1, 35.5, 30.3, 20.6, 20.5, 20.5; HRMS (ESI): MH^+, found 621.2402. $C_{28}H_{36}N_4O_{12}$[M+H^+] requires 621.2402.

1-({[1',6'-dideoxy-*β*-D-glucopyranosyl-1'-yl]-propan-2''-one}-6'-yl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4b')

Title compound was prepared according to general procedure 2 by treating the acetylated compound 4b (0.10 g, 0.16 mmol) with NaOMe in methanol to give the fully deprotected compound 4b' (0.07 g, 92%) as a red solid; mp 134-138 ºC; R_f (15% MeOH/$CHCl_3$) 0.3; HPLC (purity 98.37%): t_R = 2.350 min.; IR (KBr) cm^{-1}: 669, 769, 1215, 1403, 1499, 1645, 3019,3400, 3745; ^1H NMR (400 MHz, DMSO-d_6): δ 8.94 (s, 1H, -NH), 7.74 (s, 1H, triazole.H), 7.23 (s, 2H, Ph.H), 5.35 (br.s, 1H, $H_{1'}$), 5.13 (d, *J* = 15.12 Hz, 1H, $H_{6'}$), 5.09 (br.s, 1H, $H_{2'}$) 4.70 (d, *J* = 13.49 Hz, 1H, $H_{6''}$), 4.48 (br.s, 2H, 2×-OH), 4.32-4.27 (m, 1H, -OH), 3.82 (s, 6H, 2×OCH_3), 3.70 (s, 3H, OCH_3), 3.44 (br.s, 1H, $H_{3'}$), 3.18 (br.s, 2H, -CH_2NH), 2.99 (br.s, 1H, $H_{5'}$), 2.89 (br.s, 1H, $H_{4'}$), 2.72 (d, *J* = 15.72 Hz, 1H, -CH_2CO), 2.35-2.29 (m, 1H, -CH_2CO), 1.87 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 207.4, 165.9, 152.9 (2C), 145.2, 140.3, 129.7, 124.1, 105.3 (2C), 78.5, 77.8, 76.6, 74.0, 71.9, 60.5, 56.4 (2C), 51.5, 46.6, 35.2, 30.3; HRMS (ESI): MH^+, found 495.2079. $C_{22}H_{30}N_4O_9$[M+H^+] requires 495.2086.

1-(2',3',5'-tri-*O*-acetyl-*β*-D-ribofuranosyl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4c)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar c (0.12 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 4c (0.20 g, 92%) as a white solid; mp 94-98 ºC; R_f (60% EtOAc/Hexane) 0.3; HPLC (purity 100%): t_R = 2.667 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1493, 1645, 1752, 2399, 3019; ^1H NMR (400 MHz, $CDCl_3$): δ 7.86 (s, 1H, -NH), 7.03 (s, 3H, triazole.H, Ph.H), 6.02 (d, *J* = 8.19 Hz, 1H, $H_{1'}$), 5.81 (s, 1H, $H_{4'}$), 5.41 (d, *J* = 8.19 Hz, 1H, $H_{2'}$), 5.20-5.18 (m, 1H, $H_{3'}$), 4.72 (br.s, 2H, -CH_2NH), 4.07-4.04 (m, 1H, $H_{5'}$), 4.01 (d, *J* = 10.39 Hz, 1H, $H_{5''}$), 3.88 (s, 6H, 2×OCH_3), 3.87 (s, 3H, OCH_3), 2.22 (s, 3H, -OAc), 2.06 (s, 3H, -OAc), 1.88 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 169.3, 168.7, 167.1, 153.1 (2C), 145.3, 141.0, 129.2, 121.4, 104.4 (2C), 83.6, 68.3, 67.8, 65.7, 63.7, 60.8, 56.2 (2C), 35.3, 20.6, 20.5, 20.2; HRMS (ESI): MH^+, found 551.1987. $C_{24}H_{30}N_4O_{11}$[M+H^+] requires 551.1984.

1-*β*-D-ribofuranosyl-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4c')

Title compound was prepared according to general procedure 2 by treating the acetylated compound 4c (0.10 g, 0.18 mmol) with NaOMe in methanol to give the fully deprotected compound 4c' (0.07 g, 93%) as a red sticky solid; R_f (15% MeOH/$CHCl_3$) 0.4; HPLC (purity 100%): t_R = 2.408 min.; IR (KBr) cm^{-1}: 668, 756, 1038, 1082, 1215, 1435, 1642, 2436, 3020, 3383; ^1H NMR (400 MHz, DMSO-d_6): δ 9.04-9.03 (t, *J* = 6.02 Hz, 1H, -NH), 8.07 (s, 1H, triazole.H), 7.21 (s, 2H, Ph.H), 5.61 (d, *J* = 8.55 Hz, 1H, $H_{1'}$), 4.52 (d, *J* = 2.31 Hz, 1H, -OH), 4.51 (d, *J* = 2.31 Hz, 1H, -OH), 4.00-3.97 (m, 3H, -OH, -CH_2NH), 3.81 (s, 9H, 3×OCH_3), 3.69 (s, 5H, $H_{2'}$, $H_{3'}$, $H_{4'}$,$H_{5'}$,$H_{5''}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 157.7 (2C), 150.2, 145.2, 134.3, 127.3, 110.0 (2C), 90.0, 76.2, 74.2, 71.5, 70.2, 65.3, 61.2 (2C), 40.0; HRMS (ESI): MH^+, found 425.1667. $C_{18}H_{24}N_4O_8$[M+H^+] requires 425.1667.

1-{4'-*O*-(2'',3'',4'',6''-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-2',3',6'-tetra-*O*-acetyl-*β*-D-glucopyranosyl}-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4d)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar d (0.27 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (60% EtOAc/Hexane), gave the titled compound 4d (0.30 g, 82%) as a white solid; mp 110-116 ºC; R_f (80% EtOAc/Hexane) 0.3; HPLC (purity 100%): t_R = 2.742 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1493, 1654, 1757, 2399, 3019; ^1H NMR (500 MHz, $CDCl_3$) δ 7.81 (s, 1H, triazole.H), 7.03 (s, 2H, Ph.H), 6.98 (t, *J* = 5.52 Hz, 1H, -NH), 5.79 (dd, J_1= 6.15 Hz, J_2= 2.60 Hz, 1H, $H_{1'}$), 5.39 (m, 2H, $H_{2'}$, $H_{3'}$), 5.14 (t, *J* = 9.80 Hz, 1H, $H_{3''}$), 5.08 (t, *J* = 9.58 Hz, 1H, $H_{4''}$), 4.96 (t, *J* = 8.20 Hz, 1H, $H_{2''}$),

4.70 (m, 2H, -CH_2NH), 4.55 (d, *J* = 8.42 Hz, 1H, $H_{1''}$), 4.50 (d, *J* = 12.33 Hz, 1H, $H_{6b'}$), 4.36 (dd, J_1= 12.60 Hz, J_2= 4.28 Hz, 1H, $H_{6b''}$), 4.12 (dd, J_1= 12.33 Hz, J_2= 4.78 Hz, 1H, $H_{6a'}$), 4.07 (dd, J_1= 12.60 Hz, J_2= 2.00 Hz, 1H, $H_{6a''}$), 3.93 (m, 2H, $H_{4'}$,$H_{5'}$), 3.88 (s, 6H, 2×OCH_3), 3.87 (s, 3H, OCH_3), 3.69 (m, 1H, $H_{5''}$), 2.10 (s, 3H, -OAc), 2.09 (s, 3H, -OAc), 2.04 (s, 6H, 2×-OAc), 2.01 (s, 3H, -OAc), 1.99 (s, 3H, -OAc), 1.85 (s, 3H, -OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.4, 170.3, 169.7, 169.5, 169.2, 167.3, 153.4 (2C), 145.4, 141.3, 129.4, 121.4, 104.6 (2C), 101.0, 85.8, 76.2, 75.9, 73.0, 72.4, 72.3, 71.7, 70.8, 67.9, 61.7 (2C), 61.1, 56.5 (2C), 35.5, 20.9, 20.8, 20.7, 20.6, 20.4. HRMS (ESI): MH^+, found 911.3041. $C_{39}H_{50}N_4O_{21}$[M+H^+] requires 911.3040.

1-(4′-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4d′)

Title compound was prepared according to general procedure 2 by treating the acetylated compound 4d (0.10 g, 0.11 mmol) with NaOMe in methanol to give the fully deprotected compound 4d′ (0.06 g, 91%) as a red solid; mp 74-76 ºC; R_f (15% MeOH/$CHCl_3$) 0.3; HPLC (purity 99.33%): t_R = 2.275 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1636, 2399, 3019, 3400; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.04 (t, *J* = 5.75 Hz, 1H, -NH), 8.12 (s, 1H, triazole.H), 7.24 (s, 2H, Ph.H), 5.60 (d, *J* = 9.55 Hz, 1H, $H_{1'}$), 5.52 (d, *J* = 6.15 Hz, 1H, -$OH_{2'}$), 5.25 (d, *J* = 5.01 Hz, 1H, $OH_{2''}$), 5.03 (d, *J* = 5.10 Hz, 1H. $OH_{3''}$), 5.00 (d, *J* = 5.47 Hz, 1H, $OH_{4''}$), 4.88 (d, *J* = 2.30 Hz, 1H, $OH_{3'}$), 4.68 (t, *J* = 4.90 Hz, 1H, $OH_{6'}$), 4.62 (t, *J* = 5.36 Hz, 1H, $OH_{6''}$), 4.53 (dd, J_1= 5.75 Hz, J_2= 2.60 Hz, 2H, -CH_2NH), 4.30 (d, *J* = 7.80 Hz, 1H, $H_{1''}$), 3.85 (m, 1H, $H_{2'}$), 3.82 (s, 6H, 2×OCH_3), 3.74 (m, 1H, $H_{6b'}$), 3.71 (m, 1H, $H_{6b''}$), 3.70 (s, 3H, OCH_3), 3.63 (m, 1H, $H_{5'}$), 3.60 (m, 1H, $H_{6a'}$), 3.55 (m, 1H, $H_{3'}$), 3.50 (t, *J* = 7.80 Hz, 1H, $H_{4'}$), 3.40 (m, 1H, $H_{6a''}$), 3.23 (m, 1H, $H_{5''}$), 3.16 (m, 1H, $H_{3''}$), 3.07 (m, 1H, $H_{4''}$), 3.01 (m, 1H, $H_{2''}$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.9, 152.9 (2C), 145.5, 140.4, 129.5, 122.5, 105.3 (2C), 103.5, 87.3, 79.9, 78.1, 77.2, 76.8, 75.6, 73.7, 72.1, 70.4, 61.4, 60.4, 60.3, 56.4 (2C), 35.2; HRMS (ESI): MH^+, found 617.2304. $C_{25}H_{36}N_4O_{14}$[M+H^+] requires 617.2301.

1-(2',3',4'-tri-*O*-acetyl-*β*-D-glucopyranuronic acid methyl ester)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4e)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar e (0.14 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (50% EtOAc/Hexane), gave the titled compound 4e (0.21 g, 85%) as a white solid; mp 116-118 ºC; R_f (70% EtOAc/Hexane) 0.3; HPLC (purity 100%): t_R = 2.275 min.; IR (KBr) cm^{-1}: 670, 759, 1216, 1651, 1758, 3021; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H, triazole.H), 7.28 (s, 1H, -NH), 7.09 (s, 2H, Ph.H), 5.94 (d, *J*= 8.58 Hz, 1H, $H_{1'}$), 5.53-5.48 (m, 2H, $H_{2'}$, $H_{4'}$), 5.38-5.33 (m, 1H, $H_{3'}$), 4.68 (d, *J* = 5.12 Hz, 2H, -CH_2NH), 4.36 (d, *J* = 9.98 Hz, 1H, $H_{5'}$), 3.86 (s, 9H, 3×OCH_3), 3.73 (s, 3H, CH_3), 2.06 (s, 3H, -OAc), 2.04 (s, 3H, -OAc), 1.85 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 169.3, 168.7, 167.0, 166.2, 153.1 (2C), 145.5, 141.0, 129.2, 121.4, 104.5 (2C), 85.3, 74.7, 71.8, 70.1, 68.9, 60.8, 56.2 (2C), 53.1, 35.3, 20.4, 20.4, 20.1; HRMS (ESI): MH^+, found 609.2038. $C_{26}H_{32}N_4O_{13}$[M+H^+] requires 609.2039.

1-(2',3',4',6'-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4f)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar f (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (50% EtOAc/Hexane), gave the titled compound 4f (0.22 g, 87%) as a white solid; mp 104-108 ºC; R_f (70% EtOAc/Hexane) 0.3; HPLC (purity 100%): t_R = 2.658 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1653, 1753, 3019; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H, triazole.H), 7.27 (s, 1H, -NH), 7.05 (s, 2H, Ph.H), 5.83 (d, *J*= 8.87 Hz, 1H, $H_{1'}$), 5.52-5.47 (m, 2H, $H_{2'}$, $H_{4'}$), 5.26-5.23 (dd,J_1= 9.97 Hz, J_2 = 2.49 Hz, 1H, $H_{3'}$), 4.77 (d, *J* = 13.30 Hz, 1H, -CH_2NH), 4.66 (d, *J* = 11.64 Hz, 1H, -CH_2NH), 4.24-4.17 (m, 2H, $H_{6'}$, $H_{6''}$), 4.14-4.09 (m, 1H, $H_{5'}$), 3.86-3.84 (m, 9H, 3×OCH_3), 2.23 (s, 3H, -OAc), 2.03 (s, 3H, -OAc), 2.00 (s, 3H, -OAc), 1.86 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.9, 169.6, 168.8, 167.0, 153.1(2C), 145.5, 141.0, 129.2, 121.4, 104.5 (2C), 86.3, 74.0, 70.4, 68.0, 66.8, 61.1, 60.7, 56.2 (2C), 35.2, 20.6, 20.5, 20.4, 20.1; HRMS (ESI): MH^+, found 623.2193. $C_{27}H_{34}N_4O_{13}$[M+H^+] requires 623.2195.

1-(2',3',4',6'-tetra-*O*-acetyl-*α*-D-mannopyranosyl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4g)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar g (0.15 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (50% EtOAc/Hexane), gave the titled compound 4g (0.22 g, 88%) as a white solid; mp 74-78 ºC; R_f (70% EtOAc/Hexane) 0.3; HPLC (purity 95.90%): t_R = 2.667 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1654, 1752, 3019; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (s, 1H, triazole.H), 7.03 (s, 2H, Ph.H), 7.00-6.97 (t, *J* = 5.57 Hz, 1H, -NH), 5.99 (d, *J* = 2.07 Hz, 1H, $H_{1'}$), 5.94-5.88 (m, 2H, -$NHCH_2$), 5.40-5.35 (t, *J* = 5.88 Hz, 1H, $H_{2'}$), 4.82-4.66 (m, 2H, $H_{3'}$,$H_{5'}$), 4.39-4.33 (dd, J_1 = 12.53 Hz, J_2 = 5.47 Hz, 1H, $H_{6'}$), 4.07-4.02 (dd, J_1 = 12.53 Hz, J_2 = 2.55 Hz, 1H, $H_{6''}$), 3.92-3.90 (m, 7H, 2×OCH_3, $H_{4'}$), 3.87 (s, 3H, OCH_3), 2.18 (s, 3H, -OAc), 2.08 (s, 3H, -OAc); 2.06 (s, 3H, -OAc), 2.05 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 169.6, 169.5 (2C), 167.1, 153.1 (2C), 145.3, 141.0, 129.0, 123.2, 104.5 (2C), 83.6, 72.1, 68.8, 68.2, 65.8, 61.5, 60.8, 56.2 (2C), 35.1, 20.6 (3C), 20.5; HRMS (ESI): MH^+, found 623.2193. $C_{27}H_{34}N_4O_{13}$[M+H^+] requires 623.2195.

1-(6'-deoxy-1',2',3',4'-di-*O*-isopropylidene-*α*-D-galactopyranos-6'-yl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4h)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar h (0.11 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (40% EtOAc/Hexane), gave the titled compound 4h (0.19 g, 87%) as a white solid; mp 96-102 ºC; R_f (70% EtOAc/Hexane) 0.4; HPLC (purity 100%): t_R = 2.800 min.; IR (KBr) cm^{-1}: 669, 760, 1126, 1652, 1750, 3019; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (s, 1H, triazole.H), 7.61 (br.s, 1H, -NH), 7.08 (s, 2H, Ph.H), 5.49 (d, *J* = 4.85 Hz, 1H, $H_{1'}$), 4.72-4.54 (m, 4H, $H_{3'}$, -CH_2NH, $H_{6'}$), 4.47-4.42 (m, 1H, $H_{6''}$), 4.31-4.30 (dd, J_1 = 4.89 Hz, *J* = 2.49 Hz, 1H, $H_{2'}$), 4.17-4.16 (m, 2H, $H_{4'}$, $H_{5'}$), 3.85-3.84

(m, 9H, 3×OCH_3), 1.48 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.27 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0, 153.0 (2C), 144.4, 140.8, 129.4, 123.8, 109.9, 109.0, 104.5 (2C), 71.0, 70.7, 70.2, 67.0, 60.8, 56.2 (2C), 50.5, 35.3, 25.9, 25.8, 24.8, 24.4; HRMS (ESI): MH^+, found 535.2396. $C_{25}H_{34}N_4O_9$[M+H^+] requires 535.2399.

1-(5'-deoxy-1',2'-*O*-isopropylidene-*α*-D-xylofuranos-5'-yl)-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4i)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), azido sugar i (0.09 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (50% EtOAc/Hexane), gave the titled compound 4i (0.16 g, 85%) as a sticky yellow solid; R_f (70% EtOAc/Hexane) 0.4; HPLC (purity 100%): t_R = 2.517 min.; IR (KBr) cm^{-1}: 670, 759, 1217, 1416, 1752, 3021; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H, triazole.H,), 7.17-7.13 (t, *J* = 5.16 Hz, 1H, -NH), 7.03 (s, 2H, Ph.H), 5.97 (d, *J* = 3.63 Hz, 1H, $H_{1'}$), 4.77-4.66 (m, 3H, $H_{2'}$, -NHCH_2), 4.59-4.53 (m, 2H, $H_{6'}$,$H_{6''}$), 4.49-4.44 (dt, J_1 = 6.92 Hz, J_2 = 2.66 Hz, 1H, $H_{4'}$), 4.17 (brs, 1H, -OH), 3.88 (s, 6H, 2×OCH_3), 3.87 (s, 3H, OCH_3), 3.63 (d, *J* = 3.63 Hz, 1H, $H_{3'}$), 1.44 (s, 3H, CH_3). 1.29 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.4, 153.1 (2C), 144.6, 141.0, 129.0, 124.2, 111.9, 105.0, 104.7 (2C), 85.3, 79.1, 74.3, 60.8, 56.2 (2C), 48.7, 35.2, 26.7, 26.1; HRMS (ESI): MH^+, found 465.1980. $C_{21}H_{28}N_4O_8$[M+H^+] requires 465.1980.

1-Benzyl-4-(3,4,5-trimethoxybenzoylaminomethyl)-1*H*-1,2,3-triazole (4j)

It was obtained by the reaction of 4 (0.10 g, 0.40 mmol), benzyl azide j (0.05 g, 0.40 mmol), $CuSO_4{\cdot}5H_2O$ (0.01 g, 0.04 mmol) and sodium ascorbate (0.02 g, 0.08 mmol) in 1:1 *tert*-Butanol-water (5 ml), purification by column chromatography using 60-120 mesh silica gel (30% EtOAc/Hexane), gave the titled compound 4j (0.15 g, 96%) as a white solid; mp 152-156 ºC; R_f (50% EtOAc/Hexane) 0.4; HPLC (purity 97.05%): t_R = 2.733 min.; IR (KBr) cm^{-1}: 669, 757, 1215, 1637, 3019; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (brs, 1H, -NH), , 7.66 (s, 1H, triazole.H), 7.35 (brs, 3H, Ph.H), 7.27-7.25 (m, 2H, Ph.H), 7.11 (s, 2H, Ph.H), 5.48 (s, 2H, CH_2O), 4.64 (s, 1H, CH_2Ph), 4.62 (s, 1H, CH_2Ph), 3.85 (s, 3H, OCH_3), 3.79 (s, 6H, 2×OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1, 153.0 (2C), 145.4, 140.9, 134.3, 129.2, 129.1 (2C), 128.8, 128.1 (2C), 122.9, 104.5 (2C), 60.8, 56.1 (2C), 54.2, 35.1; HRMS (ESI): MH^+, found 383.1714. $C_{20}H_{22}N_4O_4$[M+H^+] requires 383.1714.

Prop-2-en-1-yl-3,4,5-trimethoxybenzoate (5)

Allyl bromide (0.08 ml, 1.04 mmol) was added dropwise to a cooled solution of **1** (0.20 g, 0.94 mmol) and K_2CO_3 (0.13 g, 0.94 mmol) in 10 ml dry DMF. The mixture was stirred for 30 mins at 0 ºC then for 4 h at room temperature. After completion of reaction (TLC) the reaction mixture was diluted with ethyl acetate and the organic layer was washed with water and brine solution. The combined organic layer was dried and evaporated under reduced pressure to give the crude mass. Purification by column chromatography using 60-120 mesh silica gel and ethyl acetate: hexane (1:9) as eluant gave the titled compound **5** (0.23 g, 97%) as a white solid, mp 46-48 ºC; R_f (30% EtOAc/Hexane) 0.6; IR (KBr) cm^{-1}: 669, 931, 1128, 1332, 1414, 1460, 1503, 1592, 1710, 3020; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (s, 2H, Ph.H), 6.09-5.99 (m, 1H, H_2), 5.43-5.37 (m, 1H, $H_{3'}$), 5.31-5.27 (m, 1H, $H_{3''}$), 4.83-4.82 (m, 1H, $H_{1'}$), 4.81 (m, 1H, $H_{1''}$), 3.90 (s, 9H, 3×OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 152.9 (2C), 142.3, 132.2, 125.1, 118.2, 106.9 (3C), 65.7 (2C), 60.9, 56.2 (2C). HRMS (ESI): MH^+, found 253.1068. $C_{13}H_{16}O_5$[M+H^+] requires 253.1071.

(1*H*-1,2,3-triazol-4-yl)methyl-3,4,5-trimethoxybenzoate (6)[49]

It was prepared according to procedure reported in a literatureTo a stirred solution of ethynyl substrate **2** (0.37 g, 1.47 mmol) and CuI (0.01 g, 0.07 mmol) in DMF/MeOH solution (4 mL, 9:1) under nitrogen atmosphere, trimethylsilyl azide was added (0.30 ml, 2.20 mmol). The resulting solution was stirred at 100 °C for 10−12 h. After consumption of the ethynyl substrate, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude residue was purified with silica gel column chromatography to obtain the desired product as white solid (0.23 g, 52%). mp. 136–138 ºC; R_f (40% EtOAc/Hexane) 0.4; IR (KBr) cm^{-1}: 495, 671, 928, 1127, 1334, 1462, 1506, 1591, 1712, 2401, 3021, 3436, 3683; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.05-12.96 (br.s, 1H, -NH), 7.87 (s, 1H, triazole.H), 7.32 (s, 2H, Ph.H), 5.52 (s, 2H, -CH_2), 3.92 (s, 3H, OCH_3), 3.90 (s, 6H, 2×OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 152.9 (2C), 142.5, 124.5, 107.0 (3C), 60.9, 57.6, 56.2 (3C). HRMS (ESI): MH^+, found 294.1084. $C_{13}H_{15}N_3O_5$[M+H^+] requires 294.1084.

General procedure for the synthesis of compounds 7a, 7f, 7g[50]

Compounds **7a**, **7f** and **7g** were prepared as reported in literature. To a vigorously stirring suspension of glycosyl azide (0.20 g, 0.53 mmol) in tert-butyl alcohol (1.1 mL) was added propargyl alcohol (0.13 mL, 2.24 mmol, 4.2 equiv). The reaction was initiated by the addition of a solution of $CuSO_4.5H_2O$ (0.027 g, 0.11 mmol, 0.2 equiv) and sodium ascorbate (0.043 mg, 0.21 mmol, 0.4 equiv) in distilled H_2O (1.1 mL). The deep yellow suspension was stirred vigorously at 40 °C for 2 h. At this time, TLC indicated reaction completion (1:1 ethyl acetate/hexanes). Distilled H_2O (20 mL) was added and the aqueous layer extracted twice with CH_2Cl_2 (2-50 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to afford a crude yellow solid residue, which was The crude product was purified by 60-120 silica gel chromatography using hexane:ethyl acetate, 1:1 as the eluent to give the pure product.

4-Hydroxymethyl-1-(2',3',4',6'-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1,2,3-triazole (7a)[50a]

It was prepared by general procedure as mentioned above yielded **7a** as white solid (0.21 g, 91%). mp. 151–153 ºC (lit. mp: 148–150 ºC); R_f (70% EtOAc/Hexane) 0.4; IR (KBr) cm^{-1}: 627, 1036, 1377, 1521, 1602, 1754, 2401, 3414, 3684; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H, triazole.H), 5.89-5.87 (m, 1H, $H_{1'}$), 5.46-5.40 (m, 2H, $H_{3'}$, $H_{2'}$), 5.27-5.22 (m, 1H, $H_{4'}$), 4.80 (s, 2H, -CH_2OH), 4.32-4.27 (dd, J_1 = 12.66 Hz, J_2 = 5.03 Hz, 1H, $H_{6''}$), 4.17-4.13 (dd, J_1 = 12.57 Hz, J_2 = 1.77 Hz, 1H, $H_{6'}$), 4.03-3.98 (m, 1H, $H_{5'}$), 2.08 (s, 3H, -OAc), 2.07 (s, 3H, -OAc), 2.03 (s, 3H, -OAc), 1.88 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 169.9, 169., 169.0, 148.4, 120.0, 8.7, 75.1, 72.6, 70.3, 67.7, 61.5, 56.5, 20.6, 20.5, 20.4, 20.1. HRMS (ESI): MH^+, found 430.1448. $C_{17}H_{23}N_3O_{10}$[M+H^+] requires 430.1456.

1-(*β*-D-Glucopyranosyl)-4-hydroxymethyl-1,2,3-triazole (7a')[50a]

It was obtained by treating the acetylated precursor **7a** (0.10 g, 0.27 mmol) with dry methanolic sodium methoxide (final pH 9-12). Reactions were found to be complete within 15-30 mins by TLC. Neutralization of the solution by Amberlite IR-120 ion-exchange resin, followed by filtration and evaporation of the filtrate to dryness, afforded pure compound **7a'** (0.058 g, 97%) as a white fluffy solid; mp 160-162ºC (lit. mp: 162–163 ºC); R_f (10% MeOH/$CHCl_3$) 0.4; IR (KBr) cm^{-1}: 627, 849, 928, 1022, 1334, 1475, 1522, 1602, 2399, 3019, 3428, 3684; ^{1}H NMR (400 MHz, DMSO-d6): δ 8.11 (s, 1H, triazole.H), 5.51 (d, *J* = 9.12 Hz, 1H, $H_{1'}$), 5.34 (d, *J* = 5.77 Hz, 1H, -OH), 5.24 (d, *J* = 4.14 Hz, 1H, -OH), 5.20-5.17 (t, *J* = 5.05 Hz, 1H, -OH), 5.12 (d, *J* = 5.05 Hz, 1H, -OH), 4.60 (br.s, 1H, $H_{2'}$), 4.53 (d, *J* = 4.91 Hz, 2H, CH_2OH), 3.78-3.67 (m, 2H, $H_{6''}$, $H_{2'}$), 3.44-3.38 (m, 3H, $H_{5'}$, $H_{3'}$, $H_{6'}$), 3.24-3.22 (m, 1H, $H_{4'}$); ^{13}C NMR (100 MHz, DMSO-d6): δ 148.2, 122.3, 87.8, 80.3, 77.4, 72.5, 70.0, 61.2, 55.3. HRMS (ESI): MH^+, found 262.1007. $C_9H_{15}N_3O_6$[M+H^+] requires 262.1034.

4-Hydroxymethyl-1-(2',3',4',6'-tetra-*O*-acetyl-*β*-D galactopyranosyl)-1,2,3-triazole (7f)[50b]

It was prepared by general procedure as mentioned above yielded **7f** as white solid (0.20 g, 87%). mp. 148–150 ºC (lit. mp: 148–150 ºC); R_f (70% EtOAc/Hexane) 0.4; IR (KBr) cm^{-1}: 627, 1052, 1215, 1420, 1522, 1645, 1752, 2399, 3019, 3409, 3681; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (s, 1H, triazole.H), 5.86 (d, *J* = 9.34 Hz, 1H, $H_{1'}$), 5.57-5.52 (m, 2H, $H_{3'}$, $H_{2'}$), 5.27-5.24 (dd, J_1 = 10.27 Hz, J_2 = 3.37 Hz, 1H, $H_{6''}$), 4.81 (s, 2H, -CH_2OH), 4.25-4.11 (m, 3H, $H_{4'}$, $H_{6'}$, $H_{5'}$), 2.21 (s, 3H, -OAc), 2.04 (s, 3H, -OAc), 2.01 (s, 3H, -OAc), 1.90 (s, 3H, -OAc), ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.35, 169.96, 169.81, 169.21, 148.38, 120.20, 86.26, 74.02, 70.77, 67.95, 66.88, 61.21, 56.53, 20.61, 20.47, 20.26. HRMS (ESI): MH^+, found 430.1477. $C_{17}H_{23}N_3O_{10}$[M+H^+] requires 430.1456.

4-Hydroxymethyl-1-(2',3',4',6'-tetra-*O*-acetyl-*α*-D-mannopyranosyl)-1,2,3-triazole (7g)[50c]

It was prepared by general procedure as mentioned earlier yielded **7g** as white solid (0.19 g, 83%). mp. 120–124 ºC (lit. mp: 122–124 ºC); R_f (70% EtOAc/Hexane) 0.4; IR (KBr) cm^{-1}: 627, 927, 1062, 1216, 1423, 1521, 1603, 1753, 2401, 3022, 3411, 3684; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H, triazole.H), 6.03 (d, *J*= 2.69 Hz, 1H, $H_{1'}$), 5.96-5.89 (m, 2H, $H_{3'}$, $H_{2'}$), 5.40-5.36 (m, 1H, $H_{4'}$), 4.85 (s, 2H, -CH_2OH), 4.40-4.36 (dd, J_1= 12.49 Hz, J_2 = 5.44 Hz, 1H, $H_{6''}$), 4.09-4.05 (dd, J_1= 12.49 Hz, J_2 = 2.53 Hz, 1H, $H_{6'}$), 3.94-3.90 (m, 1H, $H_{5'}$), 2.19 (s, 3H, -OAc), 2.10 (s, 3H, -OAc), 2.08 (s, 3H, -OAc), 2.06 (s, 3H, -OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.53, 169.72, 169.65, 169.45, 148.39, 122.13, 83.58, 72.17, 68.81, 68.27, 66.03, 61.58, 56.42, 20.71, 20.67, 20.57. HRMS (ESI): MH^+, found 430.1483. $C_{17}H_{23}N_3O_{10}$[M+H^+] requires 430.1456.

1-(*α* -D-Manopyranosyl)-4-hydroxymethyl-1,2,3-triazole (7g')[50d]

It was obtained by treating the acetylated precursor **7g** (0.10 g, 0.27 mmol) with dry methanolic sodium methoxide (final pH 9-12). Reactions were found to be complete within 15-30 mins by TLC. Neutralization of the solution by Amberlite IR-120 ion-exchange resin, followed by filtration and evaporation of the filtrate to dryness, afforded pure compound **7g'** (0.057 g, 95%) as a sticky off white solid; R_f (10% MeOH/$CHCl_3$) 0.4; IR (KBr) cm^{-1}: 669, 928, 1068, 1215, 1403, 1644, 2399, 3019, 3400, 3674; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H, triaole.H), 5.89 (br.s, 1H, $H_{1'}$), 5.25 (d, *J* = 14.45 Hz, 2H, $H_{6'}$, $H_{6''}$), 5.04 (br.s, 1H, -OH), 4.98 (br.s, 1H, -OH), 4.61 (br.s, 1H, -OH), 4.54 (s, 2H, CH_2OH), 4.40 (br.s, 1H, -OH), 3.83 (br.s, 1H, $H_{3'}$), 3.61 (br.s, 1H, $H_{2'}$), 3.55 (br.s, 2H, $H_{4'}$, $H_{5'}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.4, 122.9, 86.2, 78.5, 71.6, 68.7, 67.9, 61.2, 55.4. HRMS (ESI): MH^+, found 262.1035. $C_9H_{15}N_3O_6$[M+H^+] requires 262.1034.

Materials & Methods

Cell culture and reagents

MDA-MB-231 and MCF-7 (human breast cancer cell lines) LA-7 (rat mammary cancer cell line), 4T1 (mouse mammary cancer cell line), PC-3 and DU-145 (human prostate cancer cell lines), HeLa (human cervical cancer cell line) originally obtained from American type of cell culture collection (ATCC), USA and stock was maintained in laboratory. Normal human epithelial kidney cell line (HEK-293) was obtained from culture facility (CSIR-CDRI). Cells were maintained in culture medium supplemented with 10% fetal bovine serum (GIBCO BRL Laboratories, New York, USA) and 1% Penicillin-Streptomycin solution (Sigma Chemical Co., St. Louis, MO, USA) at 37^{0}C with 5% CO_2.

Cell proliferation assay

Evaluation of anticancer properties of compounds was determined by MTT assay. Briefly Cells were seeded in 96-well plate with a density of 1 x10^4 cells per well and were allowed to grow for 24 h. After 24 h, cells were treated with different concentration of compounds for further 24 h. At the end of treatment 20 µL of MTT (5 mg/ml) was added to each well and incubated for 3h. At the end of incubation media along with MTT was removed and 200 µL dimethyl sulfoxide was added to dissolve the formazan crystal and absorbance was recorded at 540 nm by plate reader.[51]

Cell cycle analysis

Effect of **2a** on distribution of the cells in different phases of the cell cycle was studied using propidium iodide staining method (Sigma-Aldrich, USA). MDA-MB-231 cells (1×10^6 cells) were seeded in six well plates and allowed to grow for 24 h. After 24 h, cells were treated with compound **2a** for 24 h. All the cells, including floating cells were harvested and fixed in cold 70% ethanol at 4 ^{0}C for 1h. Cells were then centrifuged, resuspended in PBS and incubated with 30 µg RNAse A and 30 µg PI (Sigma-Aldrich) for 30 minutes at room temperature and analyzed by flow cytometry BD FACSCalibur flow cytometer.[52]

Apoptosis analysis

Effect of compound **2a** on apoptosis was evaluated using AnnexinV FITC/PI kit (Sigma-Aldrich). MDA-MB-231 cells (1 X10^6) were seeded in six-well plates and allowed to grow for 24 h. Cells were treated with compound **2a** for 24 h. After 24 h cells were harvested by trypsinization, washed with PBS and resuspended in binding buffer. Then the cells were stained with AnnexinV FITC and propidium iodide using the AnnexinV/PI apoptosis detection kit for 10 minutes at room temperature. Samples were aquired using FACScaliber (BD biosciences).[53]

Mitochondrial Membrane Potential Analysis

Effect of compound **2a** on MMP was evaluated using JC-1 staining. Briefly MDA-MB-231 cells (1 X10^6) were seeded in six well plates and allowed to grow for 24 h. After 24 h cells were treated with compound **2a** for 24 h. At the end of treatment cells were harvested, washed with PBS and stained with 5 µg of JC-1 for 30 minutes in dark at room temperature. Cells were acquired by FACScaliber(BD biosciences).[54]

Reactive Oxygen Species (ROS) Analysis

Effect of compound **2a** on ROS generation was checked by DCFH-DA staining by flow cytometry. MDA-MB-231 cells (1 X10^6) were seeded in six well plates incubated for 24 h. Cells were treated with compound **2a** for 24 h. After 24 h, cells were harvested, washed with PBS and fixed with chilled methanol. Then cells were stained with 10 µg of DCFH-DA dye for 30 minutes in dark at room temperature. At the end of incubation, cells were centrifuged and re-suspended in PBS and samples were acquired by FACScaliber (BD biosciences).[55]

Tubulin Polymerization Assay

Effect of compound **2a** on tubulin polymerization was evaluated using tubulin polymerization assay kit (cytoskeleton, USA) as per manufactures instructions. Briefly, tubulin protein along with different concentration (5 µM, 10 µM and 20 µM) of compound **2a** was incubated with pre-warmed 96-well micro-plates at 37^{o}C. Samples were mixed properly by shaking and polymerization was monitored kinetically by recording absorbance at 340 nm every min for 1 h using Spectramax plate reader.The tubulin polymerization EC_{50} is the concentration at which rate polymerization inhibition is 50% calculated using Microsoft Excel.[56]

UV-Vis spectrophotometry

Interaction of compound **2a** with tubulin protein was evaluated by UV-Vis spectrophotometry.[57] Compound **2a** (5 µM) was titrated with different concentration (5 µM and 10 µM) of tubulin protein and absorption spectra were recorded in a range of 200-300 nm. Respective blanks were subtracted.

Fluorescence quenching assay

Binding of compound **2a** with tubulin protein was evaluated using a fluorescence quenching assay by fluorescence spectrometer.[58] Tubulin protein (5 µM) was titrated with compound **2a** (0-20 µM) and fluorescence emission spectra were recorded in a range of 300-400 nm at a excitation wave length of 295 nm. Respective blanks were subtracted.

Immunofluorescence staining

Effect of compound **2a** on tubulin dynamics was evaluated using immunofluorescence staining by confocal microscopy. MDA-MB-231 cells were grown on cover slips for 24 h and treated with compound **2a** for 24h at indicated concentration. At the end of treatment, cells were fixed with 4% paraformaldehyde, permeabilized in 0.5% Triton X-100 in PBS and blocked with 2% BSA. Subsequently, cells were probed overnight against tubulin (Life technologies) at 4^{0}C. Thereafter, cells were washed with PBS and incubated with secondary Alexa Fluor 594 anti-mouse antibody (Life Technologies) along with DAPI for 1 h at room temperature. Images were acquired using a Carl Zeiss LSM 510 META confocal microscope equipped with a Plan Apochromat 63x oil/1.4 NA DIC objective.[59]

Molecular modelling studies

All the molecular studies were performed by simulation of synthetic compounds into the colchicine binding site in tubulin. The studies were performed with the SYBYL X-1.2 package (Tripos Inc., USA) and the Surflex-Dock (SFXC) module was used to perform molecular docking. Different crystal structures of colchicine domain inhibitors have been reported; in our study, the X-ray structure of the DAMA-colchicine-α,β-tubulin complex was used (PDB code 1SA0). All structural images were prepared using PyMOL.[60]

In Vivo Pharmacokinetic Assay

The pharmacokinetic studies of **2a** were carried out in young and healthy male *Sprague Dawley* rats weighing 250 ± 25 g. The rats were obtained from the Laboratory Animal Division of the Institute and were housed in plastic cages under standard laboratory conditions with a regular 12 h day-night cycle. Standard pelleted laboratory chow (Goldmohar Laboratory Animal Feed, Lipton India Ltd, Chandigarh, India) and water were allowed *ad libitum.* The rats were acclimatized to this environment for at least two days before conducting the experiments. Solution formulation containing 12.5 mg compound was prepared by dissolving the compound in 250 µL of dimethyl sulfoxide (50% v/v) and propylene glycol (50% v/v). The formulation was administered intravenously to conscious rats (via the caudal vein) at a dose of 5 mg/kg. Blood (approx. 150 µl) was collected by excising the tail at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10 and 24 h post dose from each rat in heparinized microtubes (Axygen, CA, USA) containing 10 µL of 50% w/v potassium fluoride solution. The tubes were centrifuged immediately at 5000 rpm for 10 min at 4°C and the plasma was separated into clean and neatly labeled tubes. All samples were stored at -80°C until analysis. The plasma (50 µL; blank, spiked or test) was extracted twice with 1 mL of hexane: ethyl acetate (60:40, %v/v), followed by vortex-mixing and centrifugation. The supernatant was evaporated to dryness in Turbo vap LX (Caliper, Massachusetts, USA). The residue was reconstituted in 100 µl mobile phase, and centrifuged. Clear supernatant (80 µL) was transferred into HPLC vials and 10 µL was injected on to LC-MS/MS system. A Shimadzu UFLC pump (LC-20AD) with online degasser (DGU-20A3), an auto-sampler (SIL-HTc) with a temperature-controlled peltier-tray and a triple quadrupole API 4000 Q trap mass spectrometer (Applied Biosystems, Toronto, Canada) was used for analysis on a Discovery HS C-18 column (5 µm, 100 x 4.6 mm id) preceded with a guard column (5 µm, 50 x 4.0 mm, id) packed with the same material under isocratic condition at a flow rate of 0.6 mL/min. The mobile phase [85%acetonitrile in aqueous ammonium acetate buffer (0.01M)] was degassed by ultrasonication for 15 min before use. LC-MS/MS system was equilibrated for approximately 20 min before commencement of analysis. The column oven temperature was 40°C. Total analysis time was 3 min per

sample. The mass spectral analysis was performed using multiple reaction monitoring mode (MRM), *m/z* 624.4 precursor ion to the *m/z* 331.1 was used for quantification for 2a and *m/z* 180.1 precursor ion to the *m/z* 138.2 was used for quantification of internal standard (phenacetin, 50 ng/mL) in the positive ionization mode at 4500 V spray voltage. Data acquisition and quantitation were performed using analyst software (version 1.4.2; AB Sciex, Toronto, Canada).

In vivo syngenic tumor model

Animal studies were approved by the Institutional Animal Ethics Committee (IAEC). Syngenic rat mammary tumor model was developed as per earlier method.[61] LA-7 cells (6×10^6 cells) in 200µL PBS were injected in the mammary fat pad of female Sprague Dawley rats. After one week when the tumor becomes measurable, rats were randomly grouped in three groups. Animals were treated with 10 mg/kg and 20 mg/kg of body weight for 21 day intra peritoneal (i.p.). Tumor volumes were measured using vernier calipers every third day. Tumor volumes were calculated using the formula $V=[(\text{Length}) \times (\text{Width})^2]/2$.

Statistical Analysis

Data are expressed as mean±SEM with at least three independent experiments. The data obtained in experiments with multiple treatments were subjected to one-way ANOVA followed by post hoc Newman-Keuls multiple comparison test of significance using Graph Pad Prism 3.02 software.

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Supporting Information

Additional information includes spectral copies, HPLC chromatogram of compounds, 2D NMR spectral copies. This material is available free of charge via the Internet at http://pubs.rsc.org.

Author Contributions

[1]Authors contributed equally. This manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Abbreviations used

PI, propidium iodide; AV, Annexin V; MMP, mitochondrial membrane potential; ROS, reactive oxygen species; DCFH-DA, Dichlorodihydrofluorescein diacetate; DAPI, 4′,6-diamidino-2-phenylindole; SD, Sprague Dawley; DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; IC_{50}, half-maximal inhibitory concentration; NMR, nuclear magnetic resonance; IR, infrared spectroscopy; TLC, thin-layer chromatography.

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Abstract Figure

Identification of Gallic acid based Glycoconjugates as a novel anti-tumor agents targeting tubulin polymerization

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