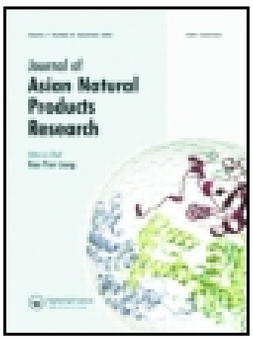

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"Click" reaction mediated synthesis of costunolide and dehydrocostuslactone derivatives and evaluation of their cytotoxic activity

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ABSTRACT

As part of pharmacological–phytochemical integrated studies on medicinal plants from Indian flora, costunolide (**1**) and dehydrocostus lactone (**2**), were isolated as major phytochemicals from *Saussurea lappa*, a plant traditionally used in different Asian systems of medicine. A series of 1,4-disubstituted-1,2,3-triazoles conjugates were synthesized through diastereo selective Michael addition followed by regioselective Huisgen 1,3-dipolar cycloaddition reactions. All these triazolyl derivatives (**5a**–**5j**) & (**7a**–**7j**) were well characterized using modern spectroscopic techniques and evaluated for their anticancer activity against a panel of five human cancerous celllines. The results indicated that all the analogs displayed moderate cytotoxic activity.

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KEYWORDS
Saussurea lappa; costunolide; dehydrococtuslactone; Miachael addition; cytotoxic activity

1. Introduction

Cancer is one of the most serious threats against human health in the world. The mortality and morbidity of cancer patients is the second highest among all diseases in the world. Over the past few decades, extensive research has led to the development of a plethora of chemotherapeutic agents; however, none of these agents are capable of completely eliminating cancer [1–3]. The limitations of current anticancer drugs, increased incidence, and rapid development of drug resistance [4] have highlighted the need for the discovery of new anticancer agents, preferably with novel mechanisms of action. To identify new chemical entities (NCEs) for a more effective treatment of cancer, drug designers can follow many strategies, but the crucial decision is always the selection of a suitable starting point from the vast chemical space [5,6]. In this respect, natural products can be viewed as evolved privileged structures with potent biological activities and biologically pre-validated leads. This is especially true in the cancer field, where about half the drugs are of natural origin including two of the most important anticancer agents taxol and taxotere [7]. The antitumor potentials of sesquiterpene lactones derivatives mainly found in the Astraceae family are widely known of which are costunolide, dehydrocostuslactone, and parthenolide (Figure 1).

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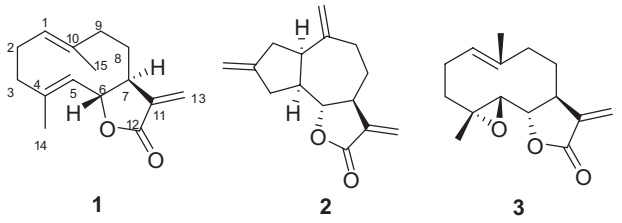

Figure 1. Structures of costunolide (**1**), dehydrocostus lactone (**2**), and parthenolide.

Until now, about 4000 sesquiterpene lactones with more than 30 carbon frameworks have been isolated from Astraceae family of plants [8–11]. Many of the species of this family plant have been used therapeutically for centuries in the Indian traditional systems of medicine for the treatment of various ailments and SLs are sesquiterpene lactones were considered as potential therapeutics of interest in combating multiple disorders.

As part of the ongoing efforts to discover potential leads from Indian medicinal plants [12–20], we have isolated large quantities of costunolide (**1**) and dehydrocostuslactone (**2**) (Figure 1) as a bioactive markers from *Saussurea lappa* [21] which prompted us to synthesize derivatives and screen for the anticancer activity. These two markers manifested an array of biological activities which includes cytotoxic, antioxidant, anti-inflammatory, antibacterial, and antifungal activities.

Recently, development of hybrid or conjugate molecules between two different types of moieties emerged as a new approach in the discovery of new cytotoxic agents, as they consists of high potency and also different alkylation sites which are essential to play vital role in tumor treatment [22,23]. Thus, this work was undertaken to synthesize triazole conjugates of costunolide, using metal-catalyzed "cycloaddition" reactions, and study their cytotoxic properties. The triazole products are more than just passive linkers; they readily associate with biological targets, through hydrogen bonding, improved solubility, and dipole interactions [24]. In addition, the chemical processes and reactions involved are concise and straightforward, which make it feasible for the scale-up production of required derivatives of interest. Herein, we report Cu-mediated Huisgen-click reaction of costunolide (**1**) and dehydrocostuslactone (**2**) for the synthesis of triazolyl derivatives and their *in vitro* anticancer activities against a panel of cancer cell lines.

2. Results and discussion

2.1. Chemistry

Costunolide (**1**) and dehydrocostus lactone (**2**), isolated from *Saussurea lappa,* were used as the starting material for all the products presented here and the numbering of compounds was given in accordance with IUPAC rules for sesquiterpene lactones (Figure 1). As shown in Scheme 1, the method adopted for the synthesis of 1,2,3-triazole conjugates were based on a Huisgen 1,3-dipolarcycloaddition reaction (click reaction) of alkynes and azides. Initially, costunolide and dehydrocostuslactone were subjected to Michael addition using various azides in acetonitrile under reflux at its highly reactive a-methylene-c-lactone motif to give corresponding azides [25]. Subsequently, all these azides used in a standard Cu(I)-catalyzed alkyne–azide [3 + 2] cyclo addition, with a series of 20 terminal alkynes to

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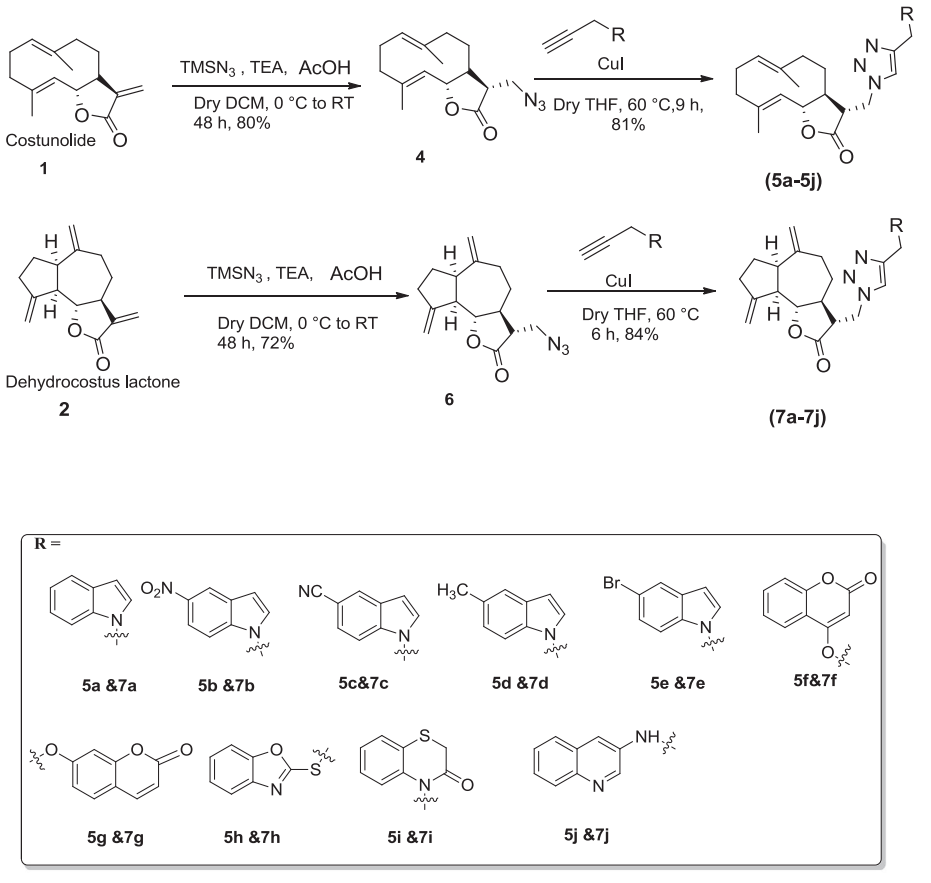

Scheme 1. "Click" reaction mediated synthesis of costunolide (**1**) and dehydrocostus lactone (**2**) conjugates.

afford the target compounds (**5a**–**5j** & **7a**–**7j**) in good yields (Table 1). The alkyne building blocks used in the click reaction were synthesized in a few steps from simple precursors. All of the reactions proceeded well in 6–9 h to give products withexcellent yields. All of the compounds were purified through silica gel column chromatography (HPLC purity > 95%) and were fully characterized [25] by IR, NMR, and high-resolution mass spectral analysis (see Supporting Information).

2.2. Biological activity

All of the triazole conjugates (**5a**–**5j** & **7a**–**7j**) were screened for *in vitro* anticancer activity against SIHA, PANC 1, MDA-MB-231, IMR-32, DU-145, and A549 cancer cell lines using sulforodamine B assay and anticancer effects are expressed in terms of the GI_{50} values (denoting the concentration that causes 50% inhibition in cell growth). In this screen, growth inhibitory effects of target compounds were measured as a function of the variation in optical density as a percentage of control. The clinically applied anticancer agent, doxorubicin, was used as positive control for cytotoxicity assays at concentrations of 100 and 100 μg/ml in each 96-well plate. The values represent averages of three independent

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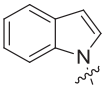
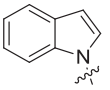
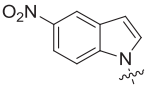
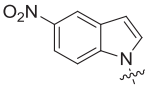
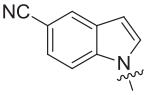
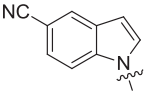
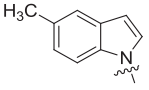
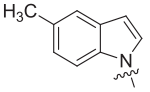
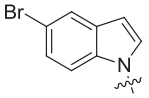
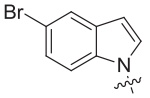
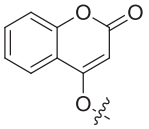
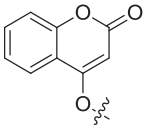
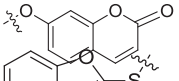
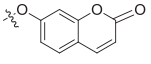
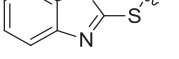
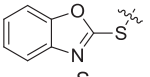
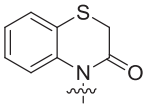
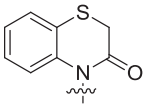
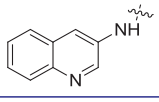
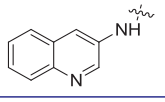
Table 1. Optimization of Click reactions on costunolide and dehydrocostuslactone.

Entry	Costunolide alkyne partners[a]	Time[b]	%yield[c]	Entry	Dehydrocostus lactone alkyne partners[a]	Time[b]	% yield[c]
5a		14	84	7a		8	93
5b		12	78	7b		6	97
5c		13.5	82	7c		9	92
5d		15	78	7d		18	82
5e		14	87	7e		20	78
5f		11.5	92	7f		12	91
5g		12	89	7j		14	88
5h		10	92	7k		16	84
5i		12	78	7i		1.17	79
5j		14	92	7j		18	78

[a]different alkyne partners.
[b]time in hr.
[c]% yield.

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experiments, each with duplicate samples. As shown in Table 2, it is clear that majority of the derivatives synthesized displayed higher anticancer activity than the parent compounds, costunolide (**1**) and dehydrocostuslactone (**2**) against the tested cell lines. Out of the entire library of compounds tested, compounds **5g** & **7b** showed potent activity against MDA-MB-231 cell lines with the GI_{50} of 0.12 and 0.16 μM, respectively. Compounds **5d** and **7h** displayed moderate activity against SIHA, IMR-32, and DU-145 cell lines ranging from 0.49–0.57 μM. Similarly, compounds **7b**, **7c**, and **7h** showed moderate activity against IMR-32, MDAMB-231, and A549 cell lines ranging from 0.2–0.88 μM. These preliminary studies laid a solid foundation for further lead optimization of this class of compounds by a systematic chemical modification including the synthesis of water-soluble compounds to improve their overall pharmaceutical properties. However, additional studies to improve

Table 2. Biological activities (GI50 in μM/ml) of costunolide **1** and dehydrocostuslactone (**2**).

	SIHA	PANC 1	MDA-MB-231	IMR-32	DU-145	A549
Sample	GI_{50}	GI_{50}	GI_{50}	GI_{50}	GI_{50}	GI_{50}
5a	10.0 ± 0.5	0.66 ± 0.08	0.7 ± 0.06	15.1 ± 0.9	15.1 ± 0.09	0.12 ± 0.02
5b	1.1 ± 0.09	3.1 ± 0.06	4.8 ± 0.03	12.9 ± 0.3	2.6 ± 0.07	2.3 ± 0.07
5c	> 100	11.3 ± 0.1	0.64 ± 0.02	4.7 ± 0.1	3.0 ± 0.02	31.6 ± 0.5
5d	0.49 ± 0.04	5.5 ± 0.03	2.5 ± 0.05	0.62 ± 0.04	2.4 ± 0.06	2.0 ± 0.09
5e	4.8 ± 0.08	1.3 ± 0.05	2.7 ± 0.09	1.7 ± 0.2	1.1 ± 0.03	0.68 ± 0.01
5f	5.3 ± 0.1	3.2 ± 0.07	2.0 ± 0.4	12.1 ± 0.05	4.6 ± 0.1	1.8 ± 0.07
5g	2.4 ± 0.09	1.3 ± 0.06	**0.12 ± 0.05**	**0.7 ± 0.09**	21.5 ± 0.4	0.71 ± 0.01
5h	27.2 ± 0.3	2.3 ± 0.09	> 100	0.57 ± 0.01	0.57 ± 0.1	1.3 ± 0.02
5i	53.7 ± 0.9	> 100	1.4 ± 0.05	5.2 ± 0.03	2.6 ± 0.05	> 100
5j	5.0 ± 0.2	4.5 ± 0.7	4.2 ± 0.08	3.1 ± 0.06	31.6 ± 0.4	2.7 ± 0.03
Costunolide	1.1 ± 0.05	0.26 ± 0.04	0.56 ± 0.02	4.2 ± 0.1	0.65 ± 0.02	7.4 ± 0.08
7a	1.1 ± 0.02	4.1 ± 0.08	2.8 ± 0.4	1.8 ± 0.2	28.5 ± 0.2	2.1 ± 0.03
7b	21.9 ± 0.15	2.0 ± 0.03	**0.16 ± 0.01**	0.20.06	8.3 ± 0.07	1.7 ± 0.05
7c	> 100	13.8 ± 0.5	0.4 ± 0.09	2.9 ± 0.1	58.5 ± 0.9	11.5 ± 0.07
7d	10.5 ± 0.1	28.0 ± 0.7	44.2 ± 0.9	1.3 ± 0.03	3.6 ± 0.1	18.8 ± 0.06
7e	2.8 ± 0.03	1.9 ± 0.05	> 100	0.68 ± 0.01	1.8 ± 0.04	2.2 ± .09
7f	6.5 ± 0.02	1.4 ± 0.01	19.2 ± 0.2	> 100	1.9 ± 0.07	1.2 ± 0.02
7g	1.0 ± 0.02	2.9 ± 0.1	2.1 ± 0.06	4.6 ± 0.04	17.7 ± 0.08	2.6 ± 0.09
7h	7.4 ± 0.06	0.95 ± 0.02	3.7 ± 0.2	19.5 ± 0.3	> 100	0.88 ± 0.05
7i	12.9 ± 0.5	9.1 ± 0.1	10.7 ± 0.5	> 100	> 100	7.2 ± 0.04
7j	> 100	1.7 ± 0.08	> 100	12.5 ± 0.5	4.1 ± 0.01	1.6 ± 0.07
Dehydrocostus lactone	2.4 ± 0.09	1.2 ± 0.2	0.88 ± 0.09	4.1 ± 0.08	3.4 ± 0.03	3.9 ± 0.03
Doxorubicin	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

the biological activity of lead compounds through the incorporation of multiple substituents on the aromatic ring and by replacing the phenyl ring with hetero aromatic rings are underway in our laboratory.

In conclusion, we have successfully synthesized 20 analogs of costunolide (**1**) and dehydrocostuslactone (**2**) and these analogs were evaluated for their anticancer activities. Few of the compounds exhibited more significant anticancer activity than the parent compound costunolide (**1**) and dehydrocostuslactone (**2**). The results are an indicative of the fact that compound **5g** proved to be the best analog with GI_{50} of < 0.12 μM against MDA MB-231 cell line. However, further studies need to be carried out to improve the biological activities of these revealing the exact mechanism of action and will be taken up in the future in our laboratory.

3. Experimental

3.1. General experimental procedures

Melting points were recorded on polmon melting point apparatus (Polmon Instruments Pvt. Ltd, India). Optical rotations were recorded on a JASCO DIP 300 digital polarimeter at 25 °C (JASCO Labor-und Datentechnik GmbH-Germany). IR spectra were recorded in a Nicolet 6700 FT-IR (Thermo Electron Corp, USA). UV spectra were measured with an Optizen 3220 UV spectrophotometer (Mekasys Co. Ltd, Daejon, Korea). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-300 MHz spectrometer (Bruker, Rheinstetten, Germany) at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively, using TMS as internal standard. The chemical shifts are expressed as (δ) values in parts per million (ppm) and the coupling constants (J) are given in hertz (Hz). Spin multiplicities are described as s

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(singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). ESIMS was performed using a Thermo Finnigan LCQ mass spectrometer (Thermo Electron, San Jose, CA, USA). HRESIMS data were collected with a LCT Premier time-of-flight mass spectrometer (Waters Corp., Milford, MA, USA). Column chromatography was carried out using silica gel (100–200 mesh, 200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) and precoated silica gel plates (Merck, 60 F254, Germany) were used for preparative TLC. Normal-phase silica M gel G TLC plates (UV 254) were used for fraction detection. The spots were visualized using UV light at 254 nm and 10% EtOH–sulfuric acid spray reagent. All the reagents and solvents were purchased from Sigma-Aldrich (Sigma-Aldrich Fine Chemicals Ltd, USA) and Finar (Finar chemicals limited, India).

3.2. Extraction and isolation of costunolide & dehydrocostus lactone from *Saussurea lappa*

The roots of *Saussurea lappa* were collected from Srinagar forest region, J&K, India. It was authenticated by Dr. K. Madhava Chetty, and a voucher specimen of *S. lappa* was deposited in the herbarium of the Botany Department, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The roots of *Saussurea lappa* were powdered in a pulvarizer (5 kg) and extracted with hexane in a Soxhlet apparatus for 72 h. The resulting extract was filtered to get 30 g of costunolide.

3.3. Typical experimental procedure for the synthesis of costunolide and dehydrocostuslatone derivatives

3.3.1. Experimental procedure for the preparation of azide

Trimethylsilylazide (4.9 ml, 5.0 mmol), anhydrous CH_2Cl_2 (10 ml), and acetic acid (2.58 ml, 5.0 mmol) were added to a round bottom flask. The mixture was stirred at room temperature for 20 min, and the costunolide or dehydrocostuslactone (1.0 mmol) were added in anhydrous CH_2Cl_2, and then catalytic amount of triethylamine (0.17 ml, 0.2 mmol) was added dropwise slowly to the reaction mixture. Then, the reaction mixture was stirred at room temperature for 18 h. The TLC indicated consumption of reactants. The reaction mixture was poured into ice cold water (15 ml) and then it was extracted with CH_2Cl_2 (10 ml × 3). The organic layers were combined, washed with saturated sodium bicarbonate solution (10 ml × 3) and brine (10 ml × 3), then it was dried over anhydrous sodium sulfate, and the combined organic layers were concentrated under reduced pressure. The resulting crude residue was purified using silica gel column chromatography (60:120 silica gel mesh). The product was eluted (hexane: ethyl acetate = 92:8) to afford the product as yellow oil.

3.3.2. Experimental procedure for the preparation of alkyne partners (4a–4j)

To a solution of amine or alcohol in DMF, Cs_2CO_3 was added at 0 °C, and the reaction mixture was stirred for 30 min at the same temperature. Propargyl bromide was added slowly to the reaction mixture at 0 °C and it was stirred at RT for 2 h. The progress of the reaction was monitored by TLC; it indicated the consumption of the reactants on TLC. The reaction mixture was poured into ice cold water (15 ml) and then it was extracted with ethyl acetate (10 ml × 3). The organic layers were combined, washed with saturated sodium bicarbonate solution (10 ml × 3) and brine (10 ml × 3), then it was dried over anhydrous sodium sulfate,

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and the combined organic layers were concentrated under reduced pressure. The resulting crude residue was purified using silica gel column chromatography (60:120 silica gel mesh). The product was eluted (Hexane: Ethyl acetate = 92:8) to afford the product.

3.3.3. General procedure for the synthesis of compounds

To a solution of compound **4** (1.0 mmol) in dry THF, the appropriate terminal alkynes (1.5 mmol) followed by catalytic amount of CuI under inert atmosphere was added. The resulting mixture was stirred at RT until the reactants were consumed on the TLC. The reaction mixture was poured into ice water (5 ml) and extracted with ethyl acetate (10 ml × 3). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and filtered to remove the solid. The mother liquid was concentrated under reduced pressure, and the residue was purified with chromatography to afford the products.

3.3.4. General procedure for the synthesis of derivatives of compound 2

To a solution of compound **6** (1.0 mmol) in dry THF, the appropriate terminal alkynes (1.5 mmol) followed by catalytic amount of CuI under inert atmosphere was added. The resulting mixture was stirred at RT until the reactants was consumed on the TLC. The progress of the reaction was monitored by TLC. The reaction mixture was poured into ice water (5 ml) and was extracted with ethyl acetate (10 ml × 3). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and filtered to remove the solid. The mother liquid was concentrated under reduced pressure, and the residue was purified with chromatography to afford the products.

3.4. Spectral, chemical, and physical properties of costunolide and dehydrocostus lactone (5a–5j) & (7i–7j)

3.4.1. (3aR,6E,10E,11aS)-3-((4-((1H-Indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)-6,10-dimethyl-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5a)

White solid; mp 152–156 °C; $[\alpha]_D^{25}$ +101.7 (*c* 0.176, $CHCl_3$); IR (KBr) υ_{max} 3781, 3139, 2927, 2857, 2493, 1765, 1613, 1512, 1483, 1461, 1381, 1311, 1191, 1050, 751, 666, 509 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60 (1H, d, J = 1.8 Hz, H-13′), 7.44 (s, 1H, H-5′), 7.38 (d, 1H, J = 8.2 Hz, H-15′), 7.17(d, 1H, J = 8.5, 3.2 Hz, H-8′), 7.09 (d, 1H, J = 7.7 Hz, H-9′), 6.50 (d, 1H, J = 3.2 Hz, H-14′), 5.38 (d, 2H, J = 4.4 Hz, H-1,H-5), 4.69–4.66 (m, 1H, H-6), 4.62–4.61 (brs, 1H, H-6′), 4.55 (t, 1H, J = 18.2 Hz, H-13), 4.34 (d, 1H, J = 10.0 Hz, H-13), 2.64–2.60 (m, 1H, H-7), 2.28–2.12 (m, 5H, overlapped), 1.97–1.89 (m, 2H, H-2a, H-2b), 1.80–1.76 (m, 2H, H-3a, H-3b), 1.63(s, 3H, H-14), 1.58–1.49(m, 2H, H-8a, H-8b), 1.36 (s, 3H, H-15), 1.27–1.24 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 175.0 (C, C-11), 144.9 (CH, C-10), 141.6 (C, C-4), 132.6 (C, C-10′), 136.4 (C, C-11′), 128.7(C, C-4′), 127.6 (CH, C-8′), 127.1 (CH, C-9′), 126.1 (CH, C-1), 123.3 (CH, C-5), 121.8 (CH, C-13′), 121.0 (CH, C-15′), 119.6 (CH, C-14′), 109.4 (CH, C-12′), 81.8 (CH, C-6), 48.6 (CH, C-6′), 48.4 (CH, C-12), 47.3 (CH_2, C-13), 41.8 (CH, C-7), 40.4 (CH_2, C-3), 39.3 (CH_2, C-8), 29.6 (CH_2, C-9), 27.9 (CH_2, C-2), 26.0 (C-3), 17.2 (CH3, C-14), 16.0 (CH3, C-15);. HRESIMS: *m/z* 453.2261 $[M+Na]^+$ (calcd for $C_{26}H_{30}N_4O_2Na$, 453.2261).

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3.4.2. 3aR,6E,10E,11aS)-6,10-Dimethyl-3-((4-((5-nitro-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5b)

Yellow solid, mp 158–161 °C; $[\alpha]_D^{25}$ +22.38 (*c* 0.21, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.57 (d, 1H, *J* = 2.2 Hz, H-13′), 8.10 (dd, 1H, *J* = 2.1, 2.8 Hz, H-12′), 7.60 (s, 1H, H-5′), 7.47 (d, *J* = 9.8 Hz, H-8′), 7.35 (d, 1H, *J* = 3.3 Hz, H-15), 6.70 (d, 1H, *J* = 3.0, Hz, H-9′), 5.45 (d, 1H, *J* = 3.5 Hz, H-1, H-5), 4.70 (t, 2H, *J* = 6.4 Hz, H-13a, H-13b), 4.58 (t, 1H, *J* = 18.4 Hz, H-6a′), 4.29 (d, 1H, *J* = 19.5 Hz, H-6b′), 2.71–2.68 (m, 1H, H-12), 2.35–2.12 (m, 2H, Overlapped), 2.44–2.07 (m, 3H, Overlapped), 1.94–1.84 (m, 3H, Overlapped), 1.82–1.76 (m, 1H, H-2a), 1.67 (brs, 1H, H-2b), 1.64 (s, 3H, H-14), 1.55–1.48 (m, 1H), 1.36 (s,1H, H-15), 1.27–1.21(m, 2H, Overlapped); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.1 (C, C-11), 143.5 (CH, C-15′), 141.8 (C, C-11′), 138.5 (C, C-10), 136.3 (C, C-4), 130.9 (C, C-4′), 128.0 (C, C-14 ′), 127.6 (C, C-13′), 127.1 (C, C-10′), 125.9 (CH, C-1), 123.5 (CH, C-5), 118.2 (C, C-13), 117.5 (C, C-12′), 104.6 (C, C-9′), 81.9 (CH, C-6), 60.3 (CH, C-7), 48.5 (CH, C-6′), 48.4 (CH, C-12), 46.5 (CH2, C-13), 40.4 (CH_2, C-3), 39.3 (CH_2, C-8), 29.6 (CH_2, C-9), 26.1 (CH_2, C-12), 17.2 (CH_3, C-14), 16.0 (CH_3, C-15);. HRESIMS: *m/z* 498.6825 $[M+Na]^+$ (calcd for $C_{26}H_{29}N_5O_4Na$(M+Na), 498.6825).

3.4.3. 1-((1-(((3aR,6E,10E,11aS)-6,10-Dimethyl-2-oxo-2,3,3a,4,5,8,9,11a-octahydrocyclodeca[b]furan-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indole-5-carbonitrile (5c)

Pale yellow solid; mp 152–156 °C; $[\alpha]_D^{25}$ +23.79 (*c* 0.29, $CHCl_3$); IR (KBr) υ_{max} 3453, 3137, 3103, 2926, 2855, 2219, 1948, 1764, 1611, 1455, 1259, 1091, 801 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.94 (s, 1H, H-15′), 7.57 (s, 1H, H-5′), 7.49 (d, 1H, *J* = 8.5 Hz, H-12′), 7.41 (d, 1H, *J* = 8.5 Hz, H-13′), 7.32 (d, 1H, *J* = 3.3 Hz, H-8′), 6.59 (d, 1H, *J* = 3.3 Hz, H-9′), 5.43 (d, 2H, *J* = 3.2 Hz, H-1, H-5), 4.69–4.65 (m, 2H, H-6a′, H-6b′), 4.58 (t, 1H, *J* = 18.5 Hz, H-6), 4.29 (d, 2H, *J* = 10.0 Hz, H-13a, H-13b), 2.71–2.66 (m, 1H, H-12), 2.31–2.13 (m, 2H, Overlapped), 2.04 (s, 1H), 1.99–1.96 (m, 2H, H-3a, H-3b), 1.94–1.87 (m, 2H, H-10a, H-10b), 1.64 (s, 3H, H-14), 1.54–1.47 (m, 2H, H-2a, H-2b), 1.36 (s, 3H, H-15), 1.27–1.24 (m, 2H, H-8a, H-8b):^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.1 (C, C-11), 143.7 (C, C-11′), 141.7 (C, C-10), 137.2 (C, C-4), 136.3 (C, C-4′), 130.0 (C, C-10′), 128.4 (C, C-8′), 127.0 (C, C-15′), 126.4 (C, C-1), 125.9 (C, C-5), 124.6 (C, C-13′), 123.5 (C, C-5′), 120.5 (C, C-12′), 111.0 (C, C-CN), 110.4 (C, C-9′), 102.9 (C, C-14′), 81.8 (CH, C-6), 48.4 (CH2, C-6′), 46.5 (CH, C-7), 41.9 (CH, C-12), 40.4 (CH_2, C-13), 39.3 (CH_2, C-3), 29.6 (CH_2, C-3), 27.8 (CH_2, C-8), 25.9 (CH_2, C-9), 17.1(CH3, C-14), 15.9 (CH3, C-15); HRESIMS: *m/z* 479.2347 $[M+Na]^+$ (calcd for $C_{27}H_{30}N_5O_2Na$, 479.2347).

3.4.4. (3aR,6E,10E,11aS)-6,10-Dimethyl-3-((4-((5-methyl-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5d)

Brown gummy; $[\alpha]_D^{25}$ +19.17 (*c* 0.48, $CHCl_3$); IR (KBr) υ_{max} 3782, 3376, 3137, 2959, 2856, 1764, 1668, 1607, 1453, 1380, 1331, 1296, 1260, 1159, 1091, 1048, 864, 799, 759, 605, 509 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.43 (s, 2H, H-5′, H-15′), 7.39 (d, 1H, *J* = 1.5 Hz, H-8′), 7.14 (d, 1H, *J* = 3.2 Hz, H-12′), 7.01 (d, 1H, *J* = 7.0 Hz, H-13′), 6.42 (dd, 1H, J = 1.7, 2.4 Hz, H-5), 5.38 (d, 1H, *J* = 5.3 Hz, H-1), 4.70–4.67 (m, 1H, H-6), 4.65–4.63 (m, 1H, H-13a), 4.57 (q, 1H, *J* = 17.9 Hz, H), 4.34 (d, *J* = 7.9 Hz, 1H), 2.76–2.72 (m, 1H), 2.30–2.11 (s, 3H, Overlapped), 2.0 (s, 3H, H-16′), 2.03–1.98 (t, 2H, *J* = 19.2 Hz, H-3a, H-3b), 1.98–1.89 (m, 2H, H-9a, H-9b), 1.83–1.79 (m, 2H, H-2a, H-2b), 1.60 (s, 3H, H-14), 1.37 (s, 3H, H-15),

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1.27–1.24 (m, 2H, H-8a, H-8b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.0 (C, C-11), 141.5 (C, C-10), 136.4 (C, C-4), 134.1 (C, C-11′), 129.1 (C, C-4′), 128.8 (C, C-14′), 127.7 (C, C-1), 127.1 (C, C-5), 126.1 (C, C-5′), 123.4 (C, C-10′), 123.2 (C, C-13′), 120.6 (C, C-15′), 109.1 (C, C-12′), 106.2 (C, C-8), 101.5 (C, C-9′), 81.8 (CH, C-6), 48.7 (CH_2, C-6′), 48.4 (CH, C-7), 46.7 (CH, C-12), 41.9 (CH_2, C-13), 40.4 (CH_2, C-3), 39.3 (CH_2, C-8), 27.9 (CH_2, C-9), 26.0 (CH_2, C-2), 21.3 (CH3, C-16′), 17.2 (CH3, C-14), 16.0 (CH_3, C-15); HRESIMS: *m/z* 467.9581 $[M+Na]^+$ (calcd for $C_{27}H_{32}N_4O_2Na$, 467.9581).

3.4.5. (3aR,6E,10E,11aS)-3-((4-((5-Bromo-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl) methyl)-6,10-dimethyl-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5e)

Yellow solid, mp 171–173 °C; $[\alpha]_D^{25}$ +24.53 (*c* 0.15, $CHCl_3$); IR (KBr) υ_{max} 3782, 2924, 2854, 1765, 1677, 1455, 1373, 1260, 1219, 1162, 1019, 772 cm^{-1};^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.7 (s, 1H, H-5′), 7.49 (brs, 1H, H-12′), 7.26 (brs, 1H, H-15′), 7.18 (d, 1H, *J* = 3.2 Hz, H-8′), 7.06 (d, 1H, *J* = 3.2 Hz, H-13′), 6.44 (d, 1H, *J* = 3.0 Hz, H-9′), 5.37 (brs, 2H, H-5, H-1), 4.65 (q, 2H, *J* = 4.6 Hz, H-6a′, H-6b′), 4.55 (t, 1H, *J* = 18.8 Hz, H-6), 4.29 (d, 2H, *J* = 9.6 Hz, H-13a, H-3b), 2.68–2.61 (m, 1H, H-7), 2.16 (s, 2H, Overlapped), 1.97–1.78 (m, 3H, Overlapped), 1.63 (s, 3H, C-14), 1.36 (s, 3H, C-15), 1.27–1.24 (m, 2H, H-8a, H-8b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.6 (C, C-11), 144.4 (C, C-10′), 128.9 (C, C-4), 124.6 (C, C-10′), 123.4 (C, C-4′), 124.1(CH, C-12′), 123.3(C, C-5′), 123.2(C, C-10), 123.1(C, C-10′), 122.7 (CH, C-13′), 114.2(CH, C-14′), 111.0 (CH, C-15′), 108.7 (CH, C-5′), 101.6 (CH, C-12′), 100.2 (CH, C-9′), 80.2 (CH, C-6), 54.1 (CH_2, C-6′), 50.3 (CH_2, C-7), 49.4 (CH, C-12), 47.9 (, C-13), 47.1 (CH_2, C-3), 46.7 (CH_2, C-8), 42.1 (CH_2, C-9), 41.4 (CH_2, C-2), 22.6 (CH_3, C-14), 17.8 (CH_3, C-15); HRESIMS: *m/z* 531.1366 $[M+Na]^+$ (calcd for $C_{26}H_{29}BrN_4O_2Na$, 531.1366).

3.4.6. (3aR,6E,10E,11aS)-6,10-Dimethyl-3-((4-((2-oxo-2H-chromen-4-yloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5f)

White solid, mp 164–166 °C; $[\alpha]_D^{25}$ +24.55 (*c* 0.15, $CHCl_3$); IR (KBr): υ_{max} 2924, 1763, 1717, 1621, 1564, 1452, 1376, 1219, 1104, 931, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.94 (s, 1H, H-5′), 7.77 (dd, 1H, *J* = 1.3, 1.5 Hz, H-14′), 7.56 (td, 1H, *J* = 1.5, 8.4 Hz, H-16′), 7.32(dd, 1H, *J* = 1.3, 1.4 Hz, H-17), 7.23 (td, 1H, *J* = 1.5, 8.4 Hz, H-15′), 5.87 (s, 1H, H-9′), 5.37 (brs, 2H, H-5, H-1), 5.34 (s, 2H, H-6a′, H-6b′), 4.84–4.76 (m, 3H, overlapped), 2.81–2.74 (m, 1H, H-7), 2.43–2.36 (m, 1H, H-3a), 2.31–2.21 (m, 1H, H-3b), 2.10 (m, 2H, H-9a, H-9b), 2.17–1.93 (m, 4H, overlapped), 1.67 (s, 3H), 1.40 (s, 3H), 1.27–1.24 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.2 (C, C-11), 168.2(C, C-9′), 164.9 (C, C-10′), 141.8 (C, C-8′), 136.4 (C, C-12′), 132.5 (C, C-4′), 127.2 (C, C-10), 126.0 (C, C-4), 125.0 (CH, C-5′), 123.9 (CH, C-16′), 123.1 (CH, C-15′), 116.7 (CH, C-5), 114.5 (CH, C-1), 113.9 (C, C-8′), 112.8 (CH, C-14′), 109.6 (C, C-13′), 82.0 (CH, C-6), 48.6 (CH_2, C-6′), 48.5 (CH, C-12), 46.6 (CH, C-7), 40.4 (CH_2, C-13), 39.3 (CH_2, C-3), 29.6 (CH_2, C-8), 28.0 (CH_2, C-9), 26.0 (CH_2, C-2), 25.6 (CH3, C-14), 17.2 (C, C-15); HRESIMS: *m/z* 475.2107 $[M+Na]^+$ (calcd for $C_{27}H_{29}N_3O_5Na$, 475.2107).

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3.4.7. (3aR,6E,10E,11aS)-6,10-Dimethyl-3-((4-((2-oxo-2H-chromen-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5g)

White solid, mp 173–175 °C; $[\alpha]_D^{25}$ +3.33 (*c* 0.0.06, $CHCl_3$); IR (KBr): υ_{max} 3634, 2961, 1260, 1219, 1023, 772, 685 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.85 (s, 1H, H-5′), 7.64 (d, 1H, *J* = 9.6 Hz, H-17′), 7.39 (d, 1H, *J* = 9.3 Hz, H-12′), 6.95 (m, 2H, H-13′, H-9′), 6.27 (d, 1H, *J* = 9.4 Hz, H-16′), 5.37 (brs, 2H, H-5, H-1), 5.32 (s, 2H, H-6′), 4.79 (dd, 1H, *J* = 4.4, 9.1Hz, H-6), 4.63 (t, 2H, *J* = 18.6 Hz, H-13a′, H-13b′), 4.41 (brs, 1H, H-12), 2.76–2.72 (m, 1H, H-7), 2.38–1.90 (m, 5H, overlapped), 1.82–1.78 (m, 2H, H-3a, H-3b), 1.66 (s, 3H, H-14), 1.39 (s, 3H, H-15), 1.27–1.24 (m, 1H, H-8a, H-8b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.1 (C, C-11), 166.3 (C, C-15′), 151.4 (C, C-8′), 143.4 (C, C-10′), 143.2 (C, C-17′), 141.7 (C, C-4′), 136.4 (C, C-10), 128.8 (C, C-4), 127.2 (CH, C-5′), 126.0 (CH, C-12′), 124.6 (CH, C-1), 113.4 (CH, C-5), 112.9 (CH, C-16′), 112.4 (CH, C-11′), 102.0 (CH, C-13′), 101.6 (CH, C-9′) 81.9 (CH, C-6), 62.1 (CH_2, C-6′), 48.6 (CH, C-7), 48.5 (CH, C-12), 46.6 (CH_2, C-13), 46.4 (CH_2, C-3), 39.4 (CH_2, C-8), 29.6 (CH_2, C-9), 28.8 (CH_2, C-2), 26.0 (CH_3, C-14), 17.5 (CH_3, C-15); HRESIMS: *m/z* 498.6984 $[M+Na]^+$ (calcd for $C_{27}H_{29}N_3O_5Na$, 498.6984).

3.4.8. (3aR,6E,10E,11aS)-3-((4-((Benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)methyl)-6,10-dimethyl-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5h)

White solid, mp 168–171 °C; $[\alpha]_D^{25}$ +65.26 (*c* 0.1, $CHCl_3$); IR (KBr): υ_{max} 2922, 1445, 1215, 1019, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.97 (d, 1H, *J* = 8.0 Hz, H-16′), 7.85 (s, 1H, H-5′), 7.73 (d, 1H, *J* = 7.9 Hz, H-13′), 7.42 (td, 1H, *J* = 0.9, 7.1 Hz, H-14′), 7.30 (td, 1H, *J* = 0.9, 7.1 Hz, H-15′), 4.72 (brs, 2H, H-5, H-1), 4.60 (brs, 1H, H-6), 4.65 (s, 2H, H-6′), 4.58–4.54 (m, 1H, H-13a, H-13b), 4.35–4.33 (m, 1H, H-12), 2.71–2.67 (m, 1H, H-7), 2.36–2.10 (m, 5H, overlapped), 2.01–2.67 (m, 1H, H-8a), 1.90–1.86 (m, 1H, H-8b), 1.63 (s, 3H, H-14), 1.35 (s, 3H, H-15), 1.27–1.24 (m, 2H, H-3a, H-3b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.0 (C, C-11), 163.9 (C, C-8′), 151.9 (C, C-11′), 141.7 (C, C-10′), 141.5 (C, C-10), 136.4 (C, C-4), 127.1 (C, C-4′), 126.1 (C, C-1), 124.3 (C, C-5), 123.9 (C, C-5′), 118.6 (C, C-15′),116.3 (C, C-14′), 115.4 (C,C-16′), 109.9 (C,C-13′), 81.8 (CH, C-6), 48.7 (CH, C-7), 48.5 (CH_2, C-12), 46.8 (C, C-13), 40.4 (CH_2, C-3), 39.3 (CH_2, C-8), 29.6 (CH_2, C-9), 28.0 (CH_2, C-6′), 26.6 (CH_2, C-2), 17.1 (CH3, C-14), 16.0 (CH_3, C-15); HRESIMS: *m/z* 487.1778 $[M+Na]^+$ (calcd for $C_{25}H_{28}N_4O_3SNa$, 487.1778).

3.4.9. 4-((1-(((3aR,6E,10E,11aS)-6,10-Dimethyl-2-oxo-2,3,3a,4,5,8,9,11a-octahydrocyclodeca[b]furan-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-2H-benzo[b][1,4]thiazin-3(4H)-one (5i)

Yellow gummy; $[\alpha]_D^{25}$ +43.23 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.75 (s, 1H, H-5′), 7.67 (dd, 1H, *J* = 0.9, 7.3 Hz, H-13′), 7.34 (td, 1H, *J* = 1.3, 5.2 Hz, H-14′), 7.24 (dd, 1H, *J* = 1.5, 6.2 Hz, H-16′), 7.03 (td, 1H, *J* = 1.0, 6.4 Hz, H-15′), 5.30 (s, 2H, H-6a′, H-6b′), 4.73–4.71 (m, 3H, H-6, H-13a, H-13b), 4.59 (t, 1H, *J* = 9.7 Hz, H-12), 4.42 (brs, 2H, H-6a′, H-6b′), 3.39 (brs, 2H, brs, 2H, H-9a′, H-9b′), 2.75–2.71 (m, 1H, H-7), 2.29–2.19 (m, 2H, overlapped), 1.77–1.72 (m, 1H), 1.66 (s, 3H), 1.36 (s, 3H), 1.27–1.25 (m, 2H, H-3a′, H-3b′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.8 (C, C-11), 165.3 (C, C-8′), 144.4 (C, C-10), 141.4 (C, C-4), 139.6 (C, C-12′), 136.4 (C, C-4′), 128.1 (CH, C-16′), 127.4 (C, C-11′), 127.0 (CH, C-5′), 126.3 (CH, C-13′), 124.8 (CH, C-1), 123.7 (CH, C-5), 123.3 (C, C-14′), 118.5 (C, C-15′), 81.7 (CH, C-6), 48.9 (CH, C-6′), 48.3 (CH, C-7), 47.0 (CH, C-12), 41.2 (CH, C-13), 40.5 (CH_2, C-3),

39.3 (CH_2, C-9′), 31.5 (CH_2, C-8), 28.0 (CH_2, C-9), 25.9 (CH_2, C-2), 17.1 (CH_3, C-14), 16.0 (CH_3, C-15); HRESIMS: *m/z* 501.1932 $[M+Na]^+$ (calcd for $C_{26}H_{30}N_4O_3SNa$, 501.1932).

3.4.10. (3aR,6E,10E,11aS)-6,10-dimethyl-3-((4-((quinolin-3-ylamino)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3,3a,4,5,8,9-hexahydrocyclodeca[b]furan-2(11aH)-one (5j)

Yellow solid, mp 177–181 °C; $[\alpha]_D^{25}$ +90.75 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.50 (brs, 1H, H-NH), 7.95–7.92 (m, 1H, H-9′), 7.71 (s, 1H, H-5′), 7.64 (s, 1H, H-15′), 7.43–7.40 (m, 2H, H-11′, H-14′), 7.12 (dd, 2H, *J* = 1.5, 6.2 Hz, H-12′, H-13′), 4.76–4.74 (m, 3H, overlapped), 4.62–4.55 (m, 4H, overlapped), 4.29 (d, 2H, *J* = 9.8 Hz, H-13a, H-13b), 2.74–2.67 (m, 1H, H-7), 2.39–1.77 (m, 4H, overlapped), 1.35 (s, 3H, H-14), 1.25 (s, 3H, H-15), 0.92–0.82 (m, 2H, H-8a, H-8b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.1 (C, C-11), 143.1 (CH, C-9′), 142.0 (C, C-11′), 141.7 (C, C-10), 140.7 (C, C-8′), 136.3 (C, C-4), 128.7 (C, C-12′), 127.1 (CH, C-13′), 127.0 (CH, C-17′), 126.1 (C, C-15′), 125.9 (CH, C-4′), 125.2 (C, C-1), 124.5 (C, C-5), 123.2 (CH, C-16′), 121.2(C, C-17′), 118.6 (CH, C-14′), 110.9 (CH, C-5′), 81.9 (CH, C-6), 48.6 (CH, C-7), 48.4 (CH, C-12), 46.5 (CH_2, C-13), 40.4 (CH_2, C-6′), 40.3 (CH_2, C-3), 39.3 (CH_2, C-9), 29.6 (CH_2, C-2), 17.2 (CH_3, C-14), 16.4 (CH_3, C-15); HRESIMS: *m/z* 458.2545 $[M+Na]^+$ (calcd for $C_{27}H_{31}N_5O_2Na$, 458.2545).

3.4.11. (3R,3aS,6aR,9aS,9bR)-3-((4-((1H-Indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl) methyl)-6,8-dimethylenedecahydroazuleno[4,5-b]furan-2(9bH)-one (7a)

Brown gummy, $[\alpha]_D^{25}$ -15.00 (*c* 0.04, $CHCl_3$); IR (KBr): υ_{max} 2926, 2856, 1768, 1612, 1512, 1462, 1313, 1260, 1183, 1094, 1011, 895, 772, 685 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.61 (d, 1H, *J* = 7.9 Hz, H-8′), 7.40 (s, 1H, H-5′), 7.37 (d, 1H, *J* = 8.8 Hz, H-7′), 7.18 (d, 2H, *J* = 8.5 Hz, H-9′, H-12′), 7.09 (td, 1H, *J* = 3.6, 7.9 Hz, H-11′), 6.51–6.50 (td, 1H, *J* = 7.8 Hz, H-10′), 5.41 (brs, 2H, H-15a, H-15b), 5.10 (q, 1H, *J* = 3.4 Hz, H-14a), 5.01 (q, 1H, *J* = 3.4 Hz, H-14b), 4.89 (q, 2H, *J* = 3.3 Hz, H-6a′,H-6b′), 4.83 (brs, 1H, H-6), 4.73 (brs, 1H, H-1), 4.65–4.55 (m, 2H, H-11, H-7), 4.33 (t, 2H, *J* = 18.7 Hz, H-13a, H-13b), 3.91 (t, 1H, *J* = 9.8 Hz, H-5), 2.7–2.68 (m, 1H, H-3a), 2.63–2.59 (m, 1H, H-3b), 2.51–2.44 (m, 1H, overlapped), 1.94–1.84 (m, 3H, H-9a, H-9b, H-8a), 1.82–1.76 (m, 1H, H-8b), 1.63 (brs, 1H, H-2a), 1.27–1.24 (m, 1H, H-2b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.2 (C, C-12), 164.4 (C, C-4), 161.0 (C, C-10), 155.8 (C, C-11′), 147.1 (C, C-5′), 146.6 (CH, C-8′), 145.6 (CH, C-9′), 142.7 (CH, C-1′), 141.2 (CH, C-11′), 140.4 (CH, C-13′), 139.1 (CH, C-15′), 128.9 (CH, C-14′), 121.5 (CH_2, C-12′), 115.2(C, C-14), 112.3(CH_2, C-15), 81.0 (CH, C-6), 68.1 (CH, C-5), 67.9 (CH, C-6′), 66.2 (CH, C-11), 61.3 (CH, C-1), 55.9 (CH_2, C-7), 58.8 (CH_2, C-13), 47.4 (CH_2, C-9), 45.4 (CH_2, C-3), 36.6 (CH_2, C-2), 35.4 (CH_2, C-8); HRESIMS: *m/z* 451.7385 $[M+Na]^+$ (calcd for $C_{26}H_{28}N_4O_2Na$, 451.7385).

3.4.12. (3R,3aS,6aR,9aS,9bR)-6,8-Dimethylene-3-((4-((5-nitro-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)decahydroazuleno[4,5-b]furan-2(9bH)-one (7b)

Pale yellow solid, mp 169–171 °C; $[\alpha]_D^{25}$ +26.70 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.57 (d, 1H, *J* = 2.0 Hz, H-12′), 8.10 (d, 1H, *J* = 6.9 Hz, H-13′), 7.57 (s, 1H, H-5′), 7.45 (s, 1H, H-10′), 6.70 (d, 1H, *J* = 3.2 Hz, H-8′), 6.63 (d, 1H, *J* = 3.0 Hz, H-9′), 5.46 (brs, 2H, H-15a, H-15b), 5.12 (q, 2H, *J* = 3.3 Hz, H-6a′, H-6b′), 5.04–5.00 (brs, 2H, H-14a, H-14b), 4.84 (brs, 1H, H-6), 4.80 (brs, 1H, H-1), 4.66 (d, 1H, *J* = 4.3 Hz, H-11), 4.33 (t, 2H, *J* = 18.7 Hz, H-13a, H-13b), 3.94 (t, 2H, *J* = 19.0 Hz, H-7, H-5), 2.06–1.78 (m, 5H,overlapped), 1.35–1.27 (m, 2H, H-8b, H-2b); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.4 (C, C-12), 155.4

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(C, C-4), 148.7 (C, C-10), 142.8 (C, C-14′), 137.4 (CH, C-10′), 134.6 (C, C-11′), 130.7 (C, C-5′), 128.9 (C, C-15′), 125.4(C, C-1′), 122.9 (CH, C-8′), 119.7 (CH, C-12′), 115.1 (CH, C-13′), 107.8 (CH, C-9′), 108.1 (CH_2, C-14), 108.0 (CH2, C-15), 87.5 (CH, C-6), 86.9 (CH, C-5), 56.9 (CH, C-6′), 56.8 (CH, C-11), 55.6 (CH, C-1), 47.6 (CH, C-7), 43.8 (CH_2, C-13), 40.2 (CH_2, C-9), 38.4 (CH_2, C-3), 36.6 (CH_2, C-2), 27.0 (CH_2, C-8). HRESIMS: *m/z* 496.2386 $[M+Na]^+$ (calcd for $C_{26}H_{27}N_5O_4Na$, 496.2386).

3.4.13. 1-((1-(((3R,3aS,6aR,9aS,9bR)-6,8-Dimethylene-2-oxododecahydroazuleno[4,5-b]furan-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indole-5-carbonitrile (7c)

Yellow solid, mp 165–167 °C; $[\alpha]_D^{25}$ +29.32 (*c* 0.59, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 1H, H-10′), 7.54 (s, 1H, H-5′), 7.49 (d, 1H, *J* = 8.2 Hz, H-13′), 7.41(dd, 1H, *J* = 1.5, 8.5Hz, H-12′), 7.32 (d, 1H, *J* = 3.2Hz, H-8′), 6.60–6.59 (m, 1H, H-9′), 5.40 (brs, 2H, H-15a, H-15b), 5.12 (q, 2H, *J* = 3.3 Hz, H-6a′, H-6b′), 5.05 (brs, 2H, H-14a, H-14b), 4.85 (brs, 1H, H-6), 4.78–4.74 (brs, 1H, H-1), 4.65–4.64 (brs, 2H, H-7, H-5), 4.33 (t, 2H, *J* = 18.7 Hz, H-13a, H-13b), 3.93 (t, 1H, *J* = 18.7 Hz, H-11), 2.72–2.64 (m, 1H, H-13), 2.51–2.38 (m, 3H, overlapped), 2.18–2.17 (m, 2H, H-9b, H-3a), 2.08–2.0 (m, 1H, H-3b), 1.97–1.77 (m, 2H, H-8a, H-2a), 1.33–1.23 (m, 2H, H-8b, H-2b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.2 (C, C-12), 150.6 (C, C-4), 148.4 (C, C-10), 142.8 (C, C-14′), 136.5 (C, C-4′), 129.6 (C, C-15′), 127.7 (CH, C-8′), 125.5 (CH, C-5′), 123.5 (CH, C-10′), 122.9 (CH, C-12′), 115.2 (CH_2, C-14), 112.6 (CH_2, C-15), 111.9 (C, C-CN), 110.0 (CH, C-13′), 108.2 (CH, C-11′), 100.4 (CH, C-9′), 84.5 (CH, C-6), 50.7 (CH, C-5), 46.9 (CH2, C-6′), 46.7 (CH, C-11), 45.9 (CH, C-1), 43.7 (CH, C-7), 41.0 (CH_2, C-13), 39.2 (CH_2, C-9), 39.0 (CH_2, C-3), 36.2 (CH_2, C-2), 31.6 (CH_2, C-8). HRESIMS: *m/z* 476.5536 $[M+Na]^+$ (calcd for $C_{26}H_{30}N_4O_2Na$, 476.5536).

3.4.14. (3R,3aS,6aR,9aS,9bR)-3-((4-((5-methyl-1H-Indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)-6,8-dimethylenedecahydroazuleno[4,5-b]furan-2(9bH)-one (7d)

Pale brown solid, mp 168–170 °C; $[\alpha]_D^{25}$ +13.37 (*c* 0.95, $CHCl_3$); IR (KBr): υ_{max} 2960, 2923, 2853, 1737, 1461, 1260, 1091, 1021, 865, 801 cm^{-1}: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.38 (s, 1H, H-10′), 7.22 (s, 1H, H-5′), 7.14 (d, 1H, *J* = 3.2 Hz, H-10′), 7.10 (d, 1H, *J* = 3.2 Hz, H-13′), 7.0 (dd, 2H, *J* = 1.3, 7.0 Hz, H-11′, H-12′), 6.42 (dd, 1H, *J* = 0.7, 2.4 Hz, H-9′), 5.39 (brs, 2H, H-15a, H-15b), 5.12 (q, 2H, *J* = 3.3 Hz, H-6a′, H-6b′), 5.04–5.06 (brs, 2H, H-14a, H-14b), 4.83 (brs, 1H, H-6), 4.73 (brs, 1H, H-1), 4.63–4.58 (m, 2H, H-7, H-5), 4.35–4.31 (t, 2H, *J* = 18.7 Hz, H-13a, H-13b), 2.12 (s, 3H, H-ph-CH_3) 1.97–1.78 (m, 4H, overlapped), 1.65–1.50 (m, 2H, H-8a, H-2a), 1.27–1.40 (m, 2H, H-8b, H-2b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.6 (C, C-12), 151.3 (C, C-4), 149.3 (C, C-10), 145.2 (C, C-14′), 128.9 (C, C-4′), 127.7 (C, C-11′), 123.3 (CH, C-8′), 122.9 (C, C-15′), 120.7 (CH, C-12′),118.2(C, C-1′), 112.1 (CH, C-5′), 111.2(C, C-13′), 109.2 (CH, C-10′), 109.0 (CH_2, C-14), 101.5 (CH_2, C-15), 85.6 (CH, C-6), 51.4 (CH, C-5), 48.1 (CH_2, C-6′), 47.1 (CH, C-11), 46.7 (CH, C-1), 44.4 (CH, C-7), 41.9 (CH_2, C-13), 48.1 (CH_2, C-9), 37.1 (CH_2, C-3), 32.4 (CH_2, C-2), 31.9 (CH_2, C-8), 23.8 (CH_2, C-9) HRESIMS: *m/z* 465.2375 $[M+Na]^+$ (calcd for $C_{27}H_{30}N_4O_2Na$, 465.2375).

3.4.15. (3R,3aS,6aR,9aS,9bR)-3-((4-((5-Bromo-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)-6,8-dimethylenedecahydroazuleno[4,5-b]furan-2(9bH)-one (7e)

Pale brown gummy; $[\alpha]_D^{25}$ +15.56 (*c* 0.82, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.72 (m, 1H, H-13′), 7.43 (s, 1H, H-5′), 7.25 (s, 1H, H-10′), 7.19 (d, 1H, *J* = 3.2 Hz, H-8′), 6.45 (d, 2H,

J = 3.0 Hz, H-12′, H-9′), 5.38 (brs, 2H, H-15a, H-15b), 5.06–5.05 (brs, 2H, H-14a, H-14b), 5.02 (q, 2H, J = 3.3 Hz, H-6a′, H-6b′), 4.84 (m, 1H, H-6), 4.74 (m, 1H, H-1), 4.71 (brs, 1H, H-1), 4.66–4.58 (m, 2H, H-7, H-5), 3.90 (t, 2H, J = 18.8 Hz, H-13a, H-13b), 2.71–2.60 (m, 2H, H-9a, H-3a), 2.49–2.35 (m, 2H, H-9b, H-3b),1.97–1.76 (m, 2H, m, 2H, H-8a, H-2a), 1.27–1.21 (m, 2H, H-8b, H-2b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.9 (C, C-12), 151.2 (C, C-4), 149.1 (C, C-10), 144.4 (C, C-14′), 134.3 (C, C-4′), 130.4 (C, C-15′), 128.9 (CH, C-8′), 127.6 (CH, C-5′), 124.5 (CH, C-12′), 123.4 (CH, C-10′), 113.0 (C, C-11′), 112.1 (CH_2, C-14), 110.9 (CH_2, C-15), 109.2 (CH, C-13′), 101.5 (CH, C-9′), 85.5 (CH, C-6), 77.2 (CH, C-5), 76.9 (CH2, C-6′), 48.1 (CH, C-11), 47.0 (CH, C-7), 45.9 (CH, C-1), 41.2 (CH_2, C-13), 39.6 (CH_2, C-9), 39.2 (CH_2, C-3), 36.7 (CH_2, C-2), 31.9 (CH_2, C-8) HRESIMS: m/z 529.3619 $[M+Na]^+$ (calcd for $C_{26}H_{27}BrN_4O_2Na$, 529.3619).

3.4.16. (3R,3aS,6aR,9aS,9bR)-6,8-Dimethylene-3-((4-((2-Oxo-2H-chromen-4-yloxy) methyl)-1H-1,2,3-triazol-1-yl)methyl)decahydroazuleno[4,5-b]furan-2(9bH)-one (7f)

White solid, mp 152–156 °C; $[\alpha]_D^{25}$ +101.7 (c 0.176, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.92 (s, 1H, H-5′), 7.77 (dd, 1H, J = 1.1, 6.7 Hz, H-15′), 7.54 (td, 1H, J = 1.5, 6.9 Hz, H-13′), 7.32–7.30 (m, 2H, H-12′, H-14′), 5.87 (s, 1H, H-9′), 5.34 (brs, 2H, H-15a, H-15b), 5.08 (q, 2H, J = 3.3 Hz, H-6a′, H-6b′), 5.03–5.01 (brs, 2H, H-14a, H-14b), 4.8 (brs, 1H, H-7), 4.79–4.77 (m, 3H,overlapped), 4.00 (t, 2H, J = 3.2 Hz, H-13a, H-13b), 2.83–2.79 (m, 1H, 9a), 2.77–2.72 (m, 1H, H-3a), 2.63–2.60 (m, 1H, H-9b), 2.17–2.13 (m, 1H, H-3b), 2.09 (m, 1H, H-8a), 2.08 (m, 1H, H-2a), 1.41–1.31 (m, 1H, H-8b), 1.25 (m, H-2b, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.0 (C, C-12), 164.8 (C, C-10′), 162.5 (C, C-17′), 153.2 (C, C-8′), 151.2 (C, C-4), 149.2 (C, C-10), 142.0 (C, C-4′), 132.5 (CH, C-5′), 124.9 (CH, C-13′), 123.9 (CH,C-14′), 123.0 (CH, C-15′), 116.7 (CH, C-16′), 115.3 (CH_2, C-14), 112.3 (CH_2, C-15), 108.4(C, C-14′), 91.2 (CH, C-9′), 85.7 (CH, C-6), 62.6 (CH, C-5), 51.5 CH_2, C-6′), 48.4 (CH, C-11), 47.0 (CH, C-7), 46.8 (CH_2, C-13), 44.4 (CH_2, C-1), 37.2 (CH_2, C-9), 32.4 (CH_2, C-3), 32.0 (CH_2, C-2), 30.0 (CH_2, C-8); HRESIMS: m/z 496.5639 $[M+Na]^+$ (calcd for $C_{27}H_{27}N_3O_5Na$, 496.5639).

3.4.17. (3R,3aS,6aR,9aS,9bR)-6,8-Dimethylene-3-((4-((2-oxo-2H-chromen-7-yloxy) methyl)-1H-1,2,3-triazol-1-yl)methyl)decahydroazuleno[4,5-b]furan-2(9bH)-one (7g)

Pale yellow gummy; $[\alpha]_D^{25}$ +128.89 (c 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.85 (s, 1H, H-5′), 7.63 (d, 1H, J = 7.7 Hz, H-12′), 7.42 (d, 1H, J = 7.3 Hz, H-13′), 7.31(brs, 1H, H-9′), 7.24 (d, 2H, J = 7.4 Hz, H-14′, H-15′), 5.90 (brs, 2H, H-13a, H-13b), 5.31 (brs, 2H, H-15a, H-15b), 4.98 (brs, 2H, H-14a, H-14b), 4.81 (brs, 2H, H-6a′, H-6b′), 4.71–4.68 (m, 3H, overlapped), 4.60 (m, 1H, H-5), 3.91 (t, 1H, J = 18.7 Hz, H-11), 2.74–2.64 (m, 2H, H-1, H-7), 2.53–2.32 (m, 1H, overlapped), 1.95–1.76 (m, 3H, overlapped), 1.28–1.15 (m, 2H, H-8b, H-2b). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.8 (C, C-12), 174.4 (C, C-14′), 151.3 (C, C-8′), 149.3 (C, C-4), 144.6 (C, C-10), 135.3 (C, C-4′), 134.9 (CH, C-12′), 126.1 (C, C-16′), 124.4 (CH, C-5′), 124.1 (CH, C-10′), 121.6 (CH, C-11′), 121.0 (C, C-17′), 118.2 (CH, C-9′), 116.5 (CH, C-13′), 112.0 (CH_2, C-14), 109.2 (CH_2, C-15), 85.7 (CH, C-6), 51.4 (CH, C-5), 48.2 (CH_2, C-6′), 47.2 (CH, C-11), 46.7 (CH, C-7), 43.4 (CH_2, C-13), 42.3 (CH_2, C-1), 37.3 (CH_2, C-9), 32.4 (CH_2, C-3), 32.0 (CH_2, C-2), 30.0 (CH_2, C-8); HRESIMS: m/z 496.6426 $[M+Na]^+$ (calcd for $C_{27}H_{27}N_3O_5Na$, 496.6426).

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3.4.18. (3R,3aS,6aR,9aS,9bR)-6,8-Dimethylene-3-((4-((2-oxo-2H-chromen-7-yloxy) methyl)-1H-1,2,3-triazol-1-yl)methyl)decahydroazuleno[4,5-b]furan-2(9bH)-one (7h)

White solid, mp 168–170 °C; $[\alpha]_D^{25}$ +128.89 (*c* 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.85 (s, 1H, H-5′), 7.62 (td, 2H, *J* = 3.3, 7.7 Hz, H-11′, H-12′), 7.43 (dd, 2H, *J* = 3.7, 7.3 Hz, H-13′,H-10′), 5.31 (brs, 2H, H-15a, H-15b), 5.04 (q, 2H, *J* = 3.5 Hz, H-6a′, H-6b′), 4.98 (brs, 2H, H-14a, H-14b), 4.81(brs, 2H, H-13a, H-13b), 4.71–4.68 (m, 3H, overlapped), 4.60 (m, 1H, H-5), 3.92 (t, 1H, *J* = 18.6 Hz, H-11), 2.74–2.64 (m, 2H, H-1, H-7), 2.53–2.32 (m, 2H, overlapped), 1.95–1.76 (m, 3H, overlapped), 1.28–1.15 (m, 2H, H-8b, H-2b). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.8 (C, C-12), 174.4 (C, C-8′), 151.3 (C, C-10′), 149.3 (C, C-4), 144.6 (C, C-10), 135.3 (C, C-11′), 134.9 (C, C-4′), 126.1 (CH, C-15′), 124.4 (CH, C-14′), 124.1 (CH, C-5′), 121.6 (CH, C-16′), 121.0 (CH, C-13′), 118.7(C, C-17′), 112.0 (CH_2, C-14), 109.4 (CH2, C-15), 109.2 (CH_2, C-15′), 85.7 (CH, C-6), 51.4 (CH, C-5), 48.2 (CH, C-11), 47.2 (CH, C-1), 46.7 (CH, C-7), 44.4 (CH_2, C-13), 37.3 (CH_2, C-6′), 32.4 (CH_2, C-9), 32.0 (CH_2, C-3), 30.0 (CH_2, C-2), 29.6 (CH_2, C-8); HRESIMS: *m/z* 496.6426 $[M+Na]^+$ (calcd for $C_{27}H_{27}N_3O_5Na$, 496.6426).

3.4.19. 4-((1-(((3R,3aS,9bS)-6,9-Dimethylene-2-oxododecahydroazuleno[4,5-b]furan-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-2H-benzo[b][1, 4]thiazin-3(4H)-one (7i)

Brown semi solid; $[\alpha]_D^{25}$ +20.00 (*c* 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93–7.90 (dd, 1H, *J* = 3.6, 8.0 Hz, H-12′), 7.79 (s, 1H, H-5′), 7.73 (td, 1H, *J* = 3.8, 7.9 Hz, H-13′), 7.46–7.40 (m, 1H, H-11′), 7.32–7.29 (m, 1H, H-14′), 5.32 (brs, 2H, H-15a, H-15b), 5.04 (brs, 2H, *J* = 1.3, 3.5 Hz, H-6a′, H-6b′), 4.99 (brs, 2H, H-14a, H-14b), 4.81 (brs, 2H, H-13a, H-13b), 4.61–4.55 (brs, 1H, H-6), 4.51–4.55 (m, 1H, H-5), 3.91 (t, 1H, *J* = 18.6 Hz, H-11), 3.65 (s, 2H, H-9a′, H-9b′), 2.72–2.61(m, 1H, H-1), 2.49–2.31 (m, 2H, overlapped), 1.94–1.76 (m, 3H, overlapped), 1.25 (m, 2H, H-8b, H-2b); ^{13}CNMR ($CDCl_3$, 75 MHz) δ 174.8 (C, C-12), 168.4 (C, C-8′), 163.8 (C, C-4), 151.9 (C, C-10), 151.3 (C, C-12′), 149.3 (CH, C-16′), 144.1 (C, C-4′), 141.7 (CH, C-14′), 124.3 (CH, C-13′), 124.1 (C, C-11′), 124.0 (CH, C-5′), 118.5 (CH, C-10′), 112.0 (CH, C-15′), 109.9 (CH_2, C-14), 109.1 (CH_2, C-15), 85.7 (CH,C-6), 51.4 (CH, C-5), 48.2 (CH_2, C-6′), 47.2 (CH, 11), 46.7 (CH, C-1), 44.9 (CH, C-7), 37.3 (CH_2, C-13), 32.4 (CH_2, C-9′), 32.0 (CH_2, C-9), 29.9 (CH_2, C-3), 29.1 (CH_2, C-2), 26.6 (CH_2, C-8); HRESIMS: *m/z* 485.3451 $[M+Na]^+$ (calcd for $C_{27}H_{26}N_3O_5NaS$, 485.3451).

3.4.20. (3R,3aS,6aR,9aS,9bR)-6,8-Dimethylene-3-((4-((quinolin-3-ylamino)methyl)-1H-1,2,3-triazol-1-yl)methyl)decahydroazuleno[4,5-b]furan-2(9bH)-one (7j)

Yellow solid, mp 182–184 °C; $[\alpha]_D^{25}$ +90.75 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.42 (brs, 1H, H-9′), 7.80 (dd, 1H, *J* = 1.6, 6.4 Hz, H-17′), 7.71 (s, 1H, H-5′), 7.64–7.61 (s, 1H, H-13′), 7.41 (d, *J* = 1.6 Hz, 2H, H-11′, H-14′), 7.12 (dd, *J* = 1.5, 6.2 Hz, 2H, H-11′, H-12′), 6.43 (s, 1H, H-15′), 5.26 (brs, 2H, H-15a, H-15b), 4.91 (brs, 2H, H-14a, H-14b), 4.78 (q, 2H, *J* = 3.5 Hz, H-6a′, H-6b′), 4.66 (brs, 2H, H-13a, H-13b), 4.54 (brs, 1H, H-6), 4.47–4.42 (m, 1H, H-5), 3.84 (t, 2H, *J* = 18.7 Hz, H-7, H-1), 2.49–2.31 (m, 2H, overlapped), 1.94–1.76 (m, 3H, overlapped), 1.25 (m, 2H, H-8b, H-2b); ^{13}CNMR ($CDCl_3$, 75 MHz) δ 174.2 (C, C-12), 142.8 (C, C-4), 141.6 (C, C-10), 141.3 (CH, C-9′), 140.4 (C, C-11), 136.0 (C, C-8), 127.9 (C, C-4′), 126.8 (CH, C-13′), 126.4 (CH, C-17′), 125.8 (CH, C-5′), 124.6 (CH, C-7′), 123.6 (CH, C-15′),119.6 (CH, C-16′),116.4 (CH, C-14′), 111.2 (CH_2, C-14),108.3 (CH_2, C-15), 81.1 (CH, C-6), 48.3 (CH, C-5), 48.0 (CH, C-11), 46.5 (CH, C-1), 46.0 (CH, C-7), 40.2 (CH_2,

C-13), 39.4 (CH_2, C-6′), 29.9 (CH_2, C-9), 28.4 (CH_2, C-3), 17.0 (CH_2, C-2), 16.3 (CH_2, C-8); HRESIMS: *m/z* 456.5694 $[M+Na]^+$ (calcd for $C_{27}H_{29}N_5O_2Na$, 456.5694).

3.5. Cell lines and cell cultures

3.5.1. Evaluation of the anti-proliferative activity against SIHA, PANC1, MDA MB-231, IMR 32, DU-145, and A549 cell lines

All cell lines (SIHA, PANC1, MDA MB-231, IMR-32, DU-145, and A549) used in this study were purchased from the American Type Culture Collection, United States. The synthesized compounds were evaluated for their *in vitro* anti-proliferative activity against six different human cancer cell lines. A protocol of 48h continuous drug exposure was used, and a SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100 µl aliquots at plating densities depending on the doubling time of individual cell lines. The micro liter plates were incubated at 37 °C, 5% $CO_{2,9}$5% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 h with different doses (0.01, 0.1, 1, 10, and 100 µM) of prepared derivatives. After 48-h incubation at 37 °C, cell mono layers were fixed by the addition of 10% (wt/vol) cold trichloroacetic acid and incubated at 4 °C for 1 h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for OD determination at 510 nm using a micro plate reader (Enspire, Perkin Elmer, USA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$[(Ti\text{-}Tz)/(C\text{-}Tz)] \times 100$ for concentrations for which $Ti \geq Tz$

$[(Ti\text{-}Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50% (GI50) was calculated from $[(Ti\text{-}Tz)/(C\text{-}Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti\text{-}Tz)/Tz] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] A.S. Sreedhar and P. Csermely, *Pharmacol. Ther.* **101**, 227 (2004).
[2] J.A. McCubrey, L.S. Steelman, S.L. Abrams, J.T. Lee, F.Chang, F.E. Bertrand, P.M. Navolanic, D.M. Terrian, R.A. Franklin, A.B. D'Assoro, J.L. Salisbury, M.C. Mazzarino, F. Stivala, and M. Libra, *Adv. Enzy. Regul.* **46**, 249 (2006).
[3] P.M. Pechan, *FEBS Lett*. **280**, 1 (1991).
[4] D.G. Lloyd, G. Golfis, A.J. Knox, D. Fayne, M.J. Meegan, and T.I. Oprea, *Drug Disc. Today*. **11**, 149 (2006).
[5] D.J. Newman, G.M. Cragg, and K.M Snader, *J. Nat. Prod.* **66**, 1022 (2002).
[6] F.E. Koehn and G.T. Carter, *Nat. Rev. Drug Disc.* **4**, 206 (2005).
[7] D.J. Newman, *J. Med. Chem.* **51**, 2589 (2008).
[8] A.K. Picman, *Biochem. Syst. Ecol.* **14**, 255 (1986).
[9] G.W. Ivie, D.A. Witzel, R.F. Keeler, and A.T. Tu, *Marcel Dekker Curr. Org. Chem*. **17**, 543 (1983).
[10] T.J. Schmidt, *Curr. Org. Chem*. **3**, 577 (1999).
[11] D. Chaturvedi, Challenge and Scope of Natural Products in Medicinal Chemistry. 313 (2011).
[12] C. Han, F.J. Barrios, M.V. Riofski, and D.A. Colby, *J. Org. Chem*. **74**, 7176 (2009).
[13] B. Siva, G. Suresh, B. Poornima, A. Venkanna, K.S. Babu, K.R. Prasad, L.P. Reddy, A.S. Sreedhar, and C.V. Rao, *Tetrahedron Lett*. **54**, 2934 (2013).
[14] M.S.A. Rao, G. Suresh, P.A. Yadav, K.R. Prasad, V.L. Naik, S. Ramakrishna, C.V. Rao, and K.S. Babu, *Tetrahedron Lett*. **53**, 6241 (2012).
[15] P.A. Yadav, G. Suresh, K.R. Prasad, M.S.A. Rao, and K.S. Babu, *Tetrahedron Lett*. **53**, 773 (2012).
[16] P.P. Reddy, R.R. Rao, K. Rekha, K.S. Babu, J. Shashidhar, G. Shashikiran, V.V. Lakshmi, and J.M. Rao, *Bioorg. Med. Chem. Lett.* **19**, 192 (2009).
[17] A. Hymavathi, K.S. Babu, V.G.M. Naidu, S. Ramakrishna, P.V. Diwan, and J.M. Rao, *Bioorg. Med. Chem. Lett.* **9**, 5727 (2009).
[18] G. Suresh, K.S. Babu, M.S. Apparao, V.R.S. Rao, P.A. Yadav, V.L. Naik, and S. Ramakrishna. *Tetrahedron Lett*. **52**, 5016 (2011).
[19] G. Suresh, K.S. Babu, V.R.S Rao, M.S. Apparao, V.L. Naik, and S. Ramakrishna, *Tetrahedron Lett.* **52**, 1273 (2011).
[20] V.R.S. Rao, G. Suresh, K.S. Babu, S.S.N. Raju, M.V.P.S. VishnuVardhan, S. Ramakrishna, and J.M. Rao, *Tetrahedron*. **67**, 1885 (2011).
[21] A. Robinson, T.V. Kumar, E. Sreedhar, V.G.M. Naidu, S. Ramakrishna, P.V. Srinivas, and J.M. Rao, *Bioorg. Med. Chem. Lett.* **18**, 4015 (2008).
[22] A. Kamal, V. Devaiah, K.L. Reddy, and M.S. Kumar, *Bioorg. Med. Chem.* **13**, 2021 (2005).
[23] J.M. Noh, S.Y. Kwak, H.S. Seo, J.H. Seo, B.G. Kim, and Y.S.Lee, *Bioorg. Med. Chem. Lett.* **19**, 5586 (2009).
[24] Q. Zhang, Y. Lu, Y. Ding, J. Zhai, Q. Ji, Q. Ma, M. Yang, H. Fan, J. Long, Z. Tong, Y. Shi, Y. Jia, and B. Han, *J.Med. Chem.* **55**, 8757 (2012).
[25] A. Li, A. Sun, and R. Liu, *J. Chromatogr. A*. **1076**, 193 (2005).

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