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Isolation, Characterization of Undescribed Alkaloids, and Semisynthetic Modifications of Cytotoxic Pyranoacridone Alkaloids from *Glycosmis pentaphylla*

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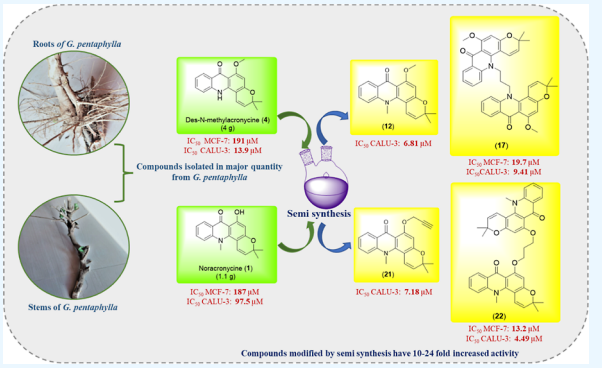
ABSTRACT: Two undescribed alkaloids (**10** and **11**), along with nine known alkaloids (**1**−**9**), have been isolated from the stem and root bark of *Glycosmis pentaphylla*. Among them are carbocristine (**11**), a carbazole alkaloid first time isolated from a natural source, and acridocristine (**10**), a pyranoacridone alkaloid first time isolated from the genus "*Glycosmis*". *In vitro* cytotoxicity of isolated compounds has been analyzed on breast cancer (MCF-7), lung cancer (CALU-3), and squamous cell carcinoma cell lines (SCC-25). The results demonstrated that compounds are moderately active. In order to study the structural activity relationship of majorly isolated compounds, semisynthetic modifications have been done on majorly isolated compounds such as des-*N*-methylacronycine (**4**) and noracronycine (**1**) to synthesize 11 semisynthetic derivatives (**12**−**22**) on functionalizable −NH and −OH groups of the pyranoacridone scaffold at 12th and 6th positions. Semisynthetic derivatives are explored on the same cell lines as isolated compounds, and the results exhibit that semisynthetic compounds showed potent cytotoxic activity compared with naturally isolated compounds. In the case of CALU-3, the dimer at −OH position of noracronycine (**1**), i.e., compound **22**, showed 24-fold better activity with an IC_{50} of 4.49 μM compared with noracronycine (**1**) with IC_{50} 97.5 μM. In MCF-7, the dimer at −OH position of noracronycine (**1**), i.e., compound **22**, showed 14-fold better activity with an IC_{50} of 13.2 μM compared with noracronycine (**1**) with IC_{50} 187 μM.



INTRODUCTION

Genus *Glycosmis* belongs to the family Rutaceae, composed of 51 accepted species and 22 varieties. *Glycosmis* is typically an evergreen glabrous shrub found throughout the tropical forests at low altitudes, in the sub-tropical Himalayas, and across warm or temperate regions worldwide.[1] Among these 51 accepted species, *Glycosmis pentaphylla* is one of the most explored species with a wide range of phytochemical constituents and biological applications. *G. pentaphylla* is a perennial shrub indigenous to the tropical and subtropical regions of China, India, Sri Lanka, Bangladesh, Indonesia, Myanmar, Malaysia, Thailand, the Philippines, Java, Vietnam, Sumatra, Borneo, and Australia.[2,3] These plants are traditionally used to treat a variety of ailments including rheumatism, urinary tract infections, chest pain, anemia, cough, liver disorders, inflammation, pain, fever, jaundice, bronchitis, bone fractures, toothache, gonorrhea, diabetes, cancer, and other chronic diseases.[4,5] An extensive literature search on phytochemistry of *G. pentaphylla* revealed that various parts of this plant were reported to contain at least 354 secondary metabolites such as acridone, carbazole, quinolone, and quinazoline types of alkaloids, flavonoids, phenolic glycosides, quinones, furoquinolines, terpenoids, steroids, sulfur-containing amides, gums, reducing sugars, tannins, saponins, and fatty derivatives.[5] Pharmacological evidence showed that *G. pentaphylla* had anti-oxidant, anti-inflammatory, antihyperlipidemic, anticancer, antifungal, anthelmintic, antimutagenic, antibacterial, mosquitocidal, antidiabetic, analgesic, antipyretic, anti-arsenicosis, and wound healing properties.[6]

The selection of this plant for the isolation and evaluation of potential secondary metabolites for anticancer activity is based on previous studies that revealed that alkaloids are the primary bioactive constituents of *G. pentaphylla* that exhibits significant cytotoxicity activity against various cancer cell lines. Few examples such as glycoborinine, a carbazole alkaloid, demonstrated potent dose- and time-dependent cytotoxicity against human liver cancer cells (Hep G2).[7] Dimeric carbazole alkaloids, namely, biscarbalexine A, glycosmisine A, and glycosmisine B, showed dose-dependent anticancer activity

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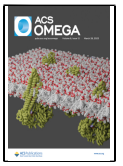

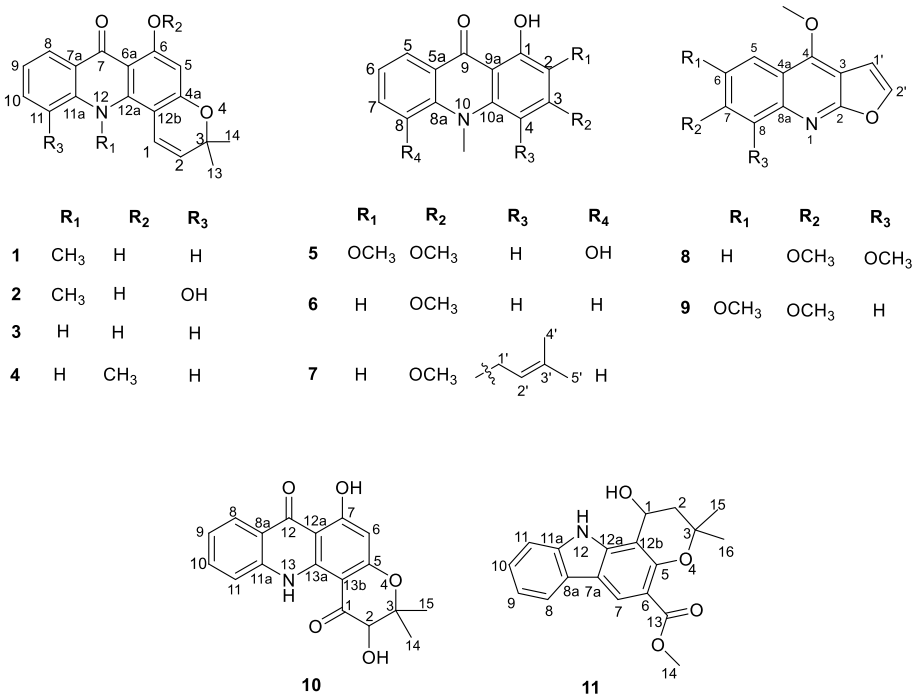

Figure 1. Structures of isolated compounds from *G. pentaphylla* (stem and root bark).

against Hep-G2, human liver cancer (Huh-7), and alveolar adenocarcinoma cells (A549).[8] Glycopentalone exhibited prominent antiproliferative activity against hepatocarcinoma cells (Hep-3B).[9] Apart from the alkaloids, bioactive amides such as methylgerambullin, glycopentamide J, and glycopentamide H showed potent activity against cancerous Hep-G2 hepatocytes with IC_{50} values of 7.47 ± 0.91, 8.01 ± 3.79, and 9.22 ± 0.06 μM, respectively. Sulfur-containing glycopentamide derivatives such as glycopentamide B, C, E, G, K, M, N, O, P, and R showed potent anticancer activity with IC_{50} values ranging from 11.46 ± 4.13 to 16.23 ± 0.80 μM.[10] On the other hand, this plant is also traditionally used to treat cancer.[5] Furthermore, according to the literature, this plant contains a few structurally interesting, unexplored scaffolds such as dimeric acridones, carbazoles, flavanols, and a rare group of flavanocoumarins that can be investigated further for biological activities such as anti-cancer activity. As a result, efforts were made to isolate previously unknown secondary metabolites with cytotoxic activity.[1,5]

In the current research work, five pyranoacridones (**1**−**4**) and **10**, three acridone alkaloids (**5**−**7**), two furoquinoline (**8**−**9**), and a carbazole alkaloid (**11**) have been isolated from the stem bark and root bark of *G. pentaphylla*. Among them, carbocristine [methyl 1,2,3,11-tetrahydro-1-hydroxy-3,3 dimethylpyrano[3,2-*a*] carbazole-5-carboxylate] (**11**) (10 mg), a carbazole alkaloid first time isolated from natural origin, and acridocristine [1-*oxo*-2-hydroxy-1,2-dihydro-12-desmethylnoracronycine] (**10**) (20 mg), a pyranoacridone isolated first time from the genus "*Glycosmis*" along with nine known alkaloids (**1**−**9**) were present. Noracronycine (**1**) is a pyranoacridone alkaloid isolated from both stem and root bark, and it is one of the majorly isolated compounds of about 1.1 g. 5-Hydroxynoracronycin (**2**) is a pyranoacridone first time isolated (20 mg) from the *G. pentaphylla* species. A known pyranoacridone alkaloid des-*N*-methylnoracronycine (**3**) has been isolated from the stem and root bark of *G. pentaphylla* with a significant quantity of 1.3 g. Des-*N*-methylacronycine (**4**) was another major compound isolated in 4 g from the root bark of *G. pentaphylla*. 5-Hydroxyarborinine (**5**) (10 mg) is a known acridone alkaloid isolated from the stem bark. 1-Hydroxy-3-methoxy-10-methyl-9-acridone (**6**) (30 mg) has been isolated and characterized for the first time from the root bark of *G. pentaphylla*. 3-*O*-Methoxyglycocitrine II (**7**) (100 mg) was isolated first from the *G. pentaphylla* species. Skimmianine (**8**) (200 mg) and kokusaginine (**9**) (150 mg) are furoquinoline alkaloids isolated from the stem bark and root bark of *G. pentaphylla*, respectively. All these naturally isolated compounds are explored on various cell lines such as breast cancer (MCF-7), lung cancer cell lines (CALU-3), and squamous cell carcinoma cell lines (SCC-25) for their cytotoxic activity. In order to study the structural activity relationship of majorly isolated pyranoacridones, we did simple semisynthetic modification at their free −NH and −OH positions for majorly isolated compounds such as des-*N*-methylacronycine (**4**) and noracronycine (**1**) to synthesize 11 semisynthetic derivatives. These semisynthetic compounds were further explored on the same cell lines as natural ones to evaluate cytotoxic activity. Semisynthetic compounds showed potent cytotoxicity compared with naturally isolated compounds.

RESULTS AND DISCUSSION

Dried coarse powder of the stem (2.8 kg) and root bark (1.3 kg) was subsequently extracted with hexane at room temperature to defat nonpolar constituents. After removing the hexane extract, the remaining plant material was subjected to methanolic extraction for 3 days at room temperature. The methanolic extract was filtered and concentrated under reduced pressure to get a concentrated methanolic extract, which was further taken for chromatographic purification to obtain two undescribed alkaloids and nine known alkaloids. Semisynthetic modifications of majorly isolated compounds have been performed. The chemical structures of the isolated

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alkaloids and semisynthetic compounds were elucidated based on the spectroscopic and spectrometric analysis, especially 2D NMR and high-resolution mass spectrometry.

Compound **1** was obtained as a yellow crystalline solid with the molecular formula of $C_{19}H_{17}NO_3$ as determined by the high-resolution electrospray ionization mass spectrometry (HRESIMS) ion at m/z 308.1283 $[M + H]^+$ (calculated for 308.1281). By comparing mass and NMR data with published data, compound **1** was identified as noracronycine (Figure 1).[11] Compound **2** was obtained as a brown crystalline powder with the molecular formula of $C_{19}H_{17}NO_4$ as determined by the HRESIMS ion at m/z 324.1231 $[M + H]^+$ (calculated for 324.1230). This compound was characterized as 5-hydroxynoracronycin by comparing the mass and NMR data with published data reported in the literature.[12] Compound **3** was isolated as an orange crystalline solid with the molecular formula of $C_{18}H_{15}NO_3$ as determined by the HRESIMS ion at m/z 294.1125 $[M + H]^+$ (calculated for 294.1125). This was characterized as des-*N*-methylnoracronycine by comparing the mass and NMR data with published data in the literature.[13] Compound **4** was obtained as a fluorescence yellow powder with the molecular formula of $C_{19}H_{17}NO_3$ as determined by the HRESIMS ion at m/z 308.1276 $[M + H]^+$ (calculated for 308.1281). Compound **4** was characterized as des-*N*-methylacronycine by comparing the mass and NMR data with published data in the literature.[14] As these four compounds have a similar pyranoacridone scaffold, the delta values of each proton and carbon, along with their positions, are given in Tables 1 and 2 for the comparison of compounds based on the scaffold similarity, for the better understanding.

Table 1. ^{1}H NMR (500 MHz, *J* in HZ) Data of Compound 1 ($CDCl_3$), Compounds 2 and 3 (DMSO-d_6), and Compound 4 (CD_3OD)

position	1	2	3	4
1	6.53 1H, d (9.6)	6.70 1H, d (9.2)	7.10 1H, d (10.2)	7.01 1H, d (9.5)
2	5.49 1H, d (9.6)	5.68 1H, d (9.2)	5.75 1H, d (10.3)	5.70 1H, d (9.5)
5	6.23 1H, s	6.14 1H, s	6.05 1H, s	6.29 1H, s
$-OR_2$-6	14.7 1H, s	14.4 1H, s	14.6 1H, s	3.92 3H, s
8	8.32 1H, dd (8.0, 1.5)	7.67 1H, d (7.4)	8.18 1H, d (9.2)	8.28 1H, d (8.3)
9	7.27 1H, t (6.9)	7.22 1H, m	7.31 1H, t (6.3)	7.23 1H, m (6.3)
10	7.68 1H, t (6.9)	7.28 1H, m	7.79 1H, m	7.63 1H, m
$-R_3$-11	7.40 1H, d (8.5)	10.5 1H, s	7.76 1H, m	7.62 1H, m
$-NR_1$-12	3.88 3H, s	3.75 3H, s	11.1 1H, s	
13, 14 ($-2CH_3$)	1.53 6H, s	1.48 6H, s	1.44 6H, s	1.48 6H, s

Compound **5** was isolated as a brown-color solid with the molecular formula of $C_{16}H_{15}NO_5$ as determined by the HRESIMS ion at m/z 302.1008 $[M + H]^+$ (calculated for 302.1023). It was characterized as 5-hydroxyarborinine by comparing the mass and NMR data with previous reports.[15] Compound **6** was isolated as fluorescence yellow powder with the molecular formula of $C_{15}H_{13}NO_3$ as determined by the HRESIMS ion at m/z 256.0957 $[M + H]^+$ (calculated for 256.0968). The basic acridone scaffold of compound **6** is similar to that of compound **5**, as shown in Figure 1, except for the absence of $-OCH_3$ at C-2 and $-OH$ at C-1 positions. As

Table 2. ^{13}C NMR (125 MHz) Data of Compound 1 ($CDCl_3$), Compounds 2 and 3 (DMSO-d_6), and Compound 4 (CD_3OD)

position	1	2	3	4
1	122.1	120.1	116.0	116.7
2	123.0	124.1	124.8	127.7
3	76.4	76.7	77.1	78.3
4a	161.6	160.7	159.2	159.9
5	97.9	97.0	96.2	94.9
6	165.3	163.7	163.8	164.0
6a	107.0	106.3	104.0	107.9
$-OR_2$-6				56.3
7	181.2	181.3	180.6	179.2
7a	121.6	123.7	117.6	123.4
8	126.2	115.3	125.7	127.2
9	121.6	123.6	121.9	122.7
10	134.0	120.5	134.0	134.1
11	116.2	148.5	118.8	117.8
11a	144.4	136.6	137.8	141.3
12a	144.9	147.2	140.9	141.6
12b	101.0	101.9	98.0	101.2
-NR_1-12	43.7	48.5		
13, 14 ($-2CH_3$)	27.0	26.7	27.5	28.0

^{13}C values of this compound were not reported, we have performed distortionless enhancement by polarization transfer (DEPT) and further 2D experiments such as heteronuclear single quantum coherence spectroscopy (HSQC) to know proton−carbon attachment. The adjacent protons are confirmed by correlation spectroscopy (COSY) correlations, and the position of $-OH$, $-OCH_3$, and $-NCH_3$ is confirmed by heteronuclear multiple bond correlation (HMBC) corrections. We did not observe any nuclear overhauser effect (NOE) correlations in this compound. The major 2D correlations observed to characterize this compound are represented in Figure 2. Thus, the structure of **6** was confirmed as 1-hydroxy-3-methoxy-10-methyl-9-acridone based on the above-mentioned observations and reported

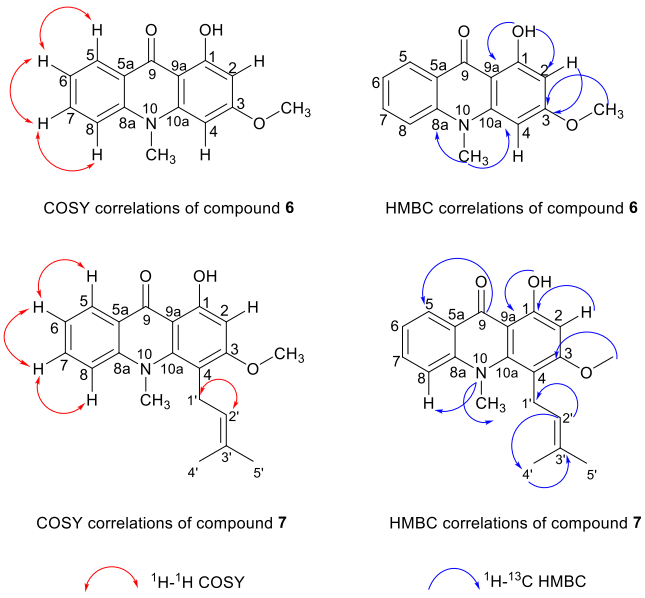

Figure 2. ^{1}H−^{1}H COSY and ^{1}H−^{13}C HMBC correlations of compounds **6** and **7**.

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literature.[16] Compound **7** was obtained as an orange crystalline solid with the molecular formula of $C_{20}H_{21}NO_3$ as determined by the HRESIMS ion at m/z 324.1596 $[M + H]^+$ (calculated for 324.1594). The acridone scaffold of compound **7** is similar to that of compounds **5** and **6**, as shown in Figure 1, except for the presence of a prenyl group at C-4. The two $-CH_3$ signals of the prenyl group (at C-4′ and C-5′) were observed at δ 1.75 (3H, s) and δ 1.76 (3H, s), and the corresponding carbon signals were observed at δ 18.2 and δ 25.8, respectively. In ^{1}H NMR at δ 3.41 2H, d (J = 5.3), the $-CH_2$ signal of prenyl (C-1′) was observed, and its corresponding carbon was observed at δ 27.2 in ^{13}C NMR. At δ 5.32 1H, t (J = 5.2), the $-CH$ signal of prenyl (C-2′) was observed, and its corresponding carbon was observed at δ 124.5. As both the protons at (C-1′) and (C-2′) have the same J values of 5.3, it was confirmed that these protons are coupled with each other and are nearby protons. As ^{13}C values of this compound were not reported, we have performed DEPT and 2D experiments such as HSQC to know proton−carbon attachment. The adjacent protons are confirmed by COSY correlations, and the position of $-OH$, $-OCH_3$, $-NCH_3$, and prenyl was confirmed by ^{13}C HMBC and ^{15}N HMBC corrections. The key correlations observed by 2D experiments to characterize this compound are represented in Figure 2. Thus, the structure of compound **7** was confirmed as 3-*O*-methoxyglycocitrine II based on the above-mentioned correlations and reported literature.[17] These three compounds have a similar acridone scaffold, and the delta values of each proton and carbon, along with their positions, are given in Tables 3 and 4 for the comparison of compounds based on the scaffold similarity.

Table 3. ^{1}H NMR (500 MHz, J in HZ) Data of Compound 5 (DMSO-d_6) and Compounds 6 and 7 ($CDCl_3$)

position	5	6	7
1 (−OH)	14.1 1H, s	14.8 1H, s	14.7 1H, s
2-R_1	3.71 3H, s	6.31 1H, s	6.39 1H, s
3-R_2	3.93 3H, s	3.91 3H, s	3.91 3H, s
4-R_3	6.48 1H, s	6.31 1H, s	(1′−5′) given below
5	7.65 1H, d (7.8)	8.46 1H, d (9.4)	8.33 1H, d (7.5)
6	7.18 1H, t (7.8)	7.29 1H, t (8.7)	7.27 1H, t (9.0)
7	7.25 1H, d (7.8)	7.72 1H, t (8.8)	7.69 1H, t (7.5)
8-R_4	10.4 1H, s	7.48 1H, d (8.5)	7.40 1H, d (7.5)
10($-NCH_3$)	3.73 3H, s	3.79 3H, s	3.81 3H, s
(4-R_3) 1′			3.41 2H, d (5.3)
(4-R_3) 2′			5.32 1H, t (5.1)
(4-R_3) 3′			
(4-R_3) 4′			1.75 3H, s
(4-R_3) 5′			1.76 3H, s

Compound **8** was obtained as a pale-yellow crystalline solid with the molecular formula of $C_{14}H_{13}NO_4$ as determined by the HRESIMS ion at m/z 260.0917 $[M + H]^+$ (calculated for 260.0917). Compound **9** was obtained as a white crystalline solid with the molecular formula of $C_{14}H_{13}NO_4$ as determined by the HRESIMS ion at m/z 260.0921 $[M + H]^+$ (calculated for 260.0917). Compounds **8** and **9** were characterized as skimmianine and kokusaginine, respectively, by comparing the mass and NMR data with those reported in the literature.[18] The delta values of each proton and carbon, along with their positions, are given in Tables 5 and 6 for the better understanding.

Table 4. ^{13}C NMR (125 MHz) Data of Compound 5 (DMSO-d_6) and Compounds 6 and 7 ($CDCl_3$)

position	5	6	7
1	159.4	166.2	163.9
2	129.6	94.1	93.4
3	159.6	166.1	165.5
4	93.9	90.2	106.7
5	115.3	126.9	126.2
5a	123.7	121.2	121.5
6	122.9	121.5	121.6
7	120.0	134.2	134.0
8	148.2	114.6	116.5
8a	136.9	142.5	147.0
9	181.6	180.9	182.0
9a	105.3	105.4	107.2
10 ($-NCH_3$)	46.2	34.2	44.1
10a	141.6	144.9	146.3
R_1	59.9		
R_2	56.3	55.7	56.1
1′			27.2
2′			124.5
3′			131.8
4′			18.2
5′			25.8

Table 5. ^{1}H NMR (500 MHz, J in HZ) Data of Compounds 8 and 9 ($CDCl_3$)

position	8	9
4-OCH_3	4.44 3H, s	4.44 3H, s
5	8.02 1H, d (9.3)	7.48 1H, s
6-R_1	7.24 1H, d (9.1)	4.02 3H, s
7-R_2	4.03 3H, s	4.03 3H, s
8-R_3	4.11 3H, s	7.34 1H, s
1′	7.05 1H, d (3.2)	7.04 1H, d (2.8)
2′	7.59 1H, d (3.2)	7.57 1H, d (2.8)

Table 6. ^{13}C NMR (125 MHz) Data of Compounds 8 and 9 ($CDCl_3$)

position	8	9
2	164.5	163.2
3	102.1	102.2
4	157.3	155.6
4 ($-OCH_3$)	59.1	58.9
4a	115.0	113.0
5	118.3	100.3
6	112.1	147.8
6-R_1		56.10
7	152.3	152.6
7-R_2	56.9	56.14
8	142.1	106.8
8-R_3	61.8	
8a	141.6	142.7
1′	104.7	104.7
2′	143.1	142.5

Compound **10** was obtained as fluorescence yellow powder with the molecular formula of $C_{18}H_{15}NO_5$ as determined by the HRESIMS ion at m/z 326.1036 $[M + H]^+$ (calculated for 326.1023). We did not get hits with this molecular weight in the entire *Glycosmis* genus during the initial dereplication process, so we ran a Sci-Finder search and discovered a few

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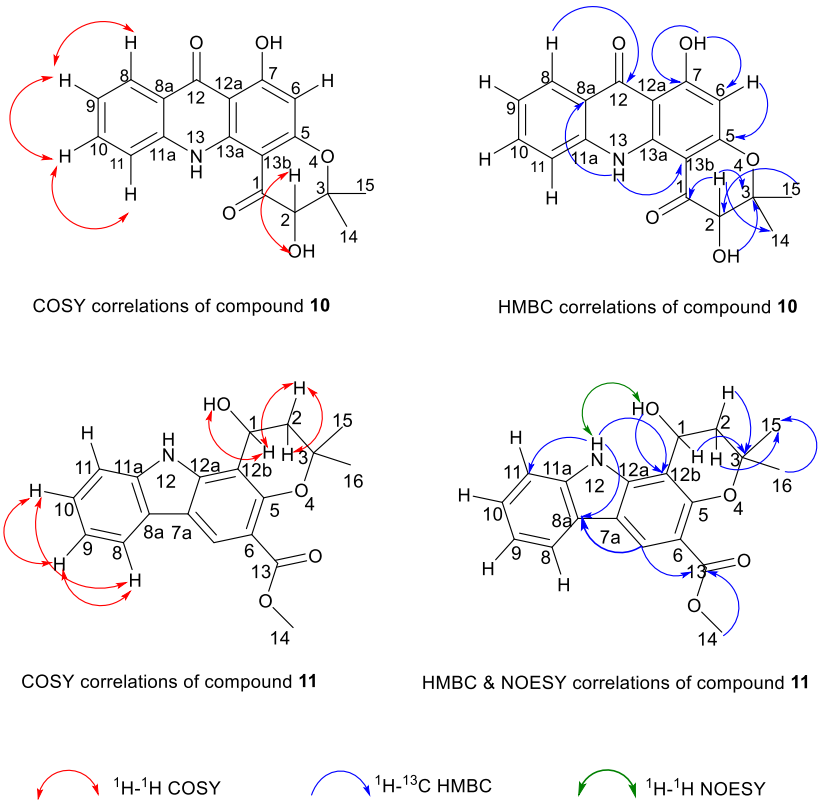

Figure 3. ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMBC, and ^{1}H−^{1}H NOESY correlations of compound **10** and **11**.

compounds with this molecular weight in related genera across the entire Rutaceae family. Based on the functional groups observed in ^{1}H NMR and ^{13}C NMR, we found a compound that matched this molecular weight. This compound has 18 carbons and 15 hydrogens based on ^{1}H NMR and ^{13}C NMR spectral data, which match the compound's molecular formula. There is an aromatic −OH signal present at C-7 at δ 15.4 (1H, s), and the −OH-attached carbon (C-7) is observed at δ 170.5. At δ 6.21 (1H, d), an aliphatic −OH signal is present with a J value of 5.4 observed as a doublet at C-2. At δ 4.28 (1H, d), a −CH proton as a doublet with J value 5.4 was observed at C-2. Based on the COSY correlations (Figure 3), we found that δ 6.21 1H, d (5.4) and δ 4.28 1H, d (5.4) are the −OH and −CH signals that are adjacent to each other, and they both show coupling with each other. In ^{1}H NMR, there is a proton at δ 12.9 (1H, s), which was found to be the −NH signal of the compound. At δ 1.34 (3H, s) and δ 1.51 (3H, s) are two $-CH_3$ signals present in the compound at C-14 and C-15, and their corresponding carbons are present at 19.8 and 25.5, respectively. We have performed DEPT and 2D experiments, such as HSQC, to know proton−carbon attachment. The adjacent protons are confirmed by COSY correlations, and the position of −OH and −NH was confirmed by ^{13}C HMBC and ^{15}N HMBC corrections. The key correlations observed by 2D experiments to characterize this compound are represented in Figure 3. The delta values of each proton and carbon, along with their positions, are given in Table 7. Thus, the structure of compound **10** was confirmed and named acridocristine [1-*oxo*-2-hydroxy-1,2-dihydro-12-desmethylnoracronycine] by comparing the mass and NMR data with those reported in the literature.[19]

Compound **11** was obtained as a white crystalline powder with the molecular formula of $C_{19}H_{19}NO_4$ as determined by the HRESIMS ion at m/z 326.1395 $[M + H]^+$ (calculated for 326.1387). The $-OCH_3$ signal of the carboxylate present in the structure was obtained at 3.78 (3H, s), and its corresponding carbon signal was observed at 55.5. In ^{1}H NMR, δ 2.19 dd (6.0) and δ 2.04 dd (6.4) are the two proton signals of $-CH_2$, which are attached to the same carbon of δ 41.8 which is confirmed by DEPT and HSQC experiments. At δ 5.67 (1H, d), an aliphatic −OH signal is present with J value 6.3 as a doublet. At 5.08 (1H, d), a −CH proton is observed as

Table 7. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compound 10 (DMSO-d_6)

position	^{1}H NMR	^{13}C NMR
1		194.2
2	2a-6.21 1H, d (5.4); 2b-4.28 1H, d (5.4)	75.1
3		84.1
5		167.0
6	6.12 1H, s	96.5
7 (−OH)	15.4 1H, s	170.5
8	8.25, 1H, d (9.7)	125.3
8a		120.7
9	7.46 1H, m	124.2
10	7.85 1H, m	135.1
11	7.91 1H, d (9.7)	119.4
11a		139.8
12		180.5
12a		103.7
13	12.9 1H, s	
13a		143.8
13b		96.9
14	1.34 3H, s	19.8
15	1.51 3H, s	25.5

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a doublet with a J value of 6.2. Based on the COSY correlations (Figure 3), we found that δ 5.67 (1H, d) (6.3) and δ 5.08 (1H, d) (6.3) are the −OH and −CH signals that are adjacent to each other, and they both show coupling with each other. At 175.1, the carbonyl carbon at C-13 was observed. We have observed a key NOESY correlation between the −NH signal at 10.2 (1H, s) and −OH at δ 5.67 (1H, d), which helped us fix the position of the −OH signal only at the C-1 position. We have performed 2D experiments, such as HSQC, to know proton−carbon attachment. The adjacent protons are confirmed by COSY correlations, and the position of two −OH and −NH is confirmed by ^{13}C HMBC and ^{15}N HMBC corrections. The key correlations observed by 2D experiments to characterize this compound are represented in Figure 3. The delta values of each proton and carbon, along with their positions, are given in Table 8. Thus, the structure of compound **11** was confirmed and named carbocristine [methyl 1,2,3,11-tetrahydro-1-hydroxy-3,3dimethylpyrano[3,2-*a*]-carbazole-5-carboxylate] by comparing the mass and NMR data with those reported in the literature.[20] The ^{1}H, ^{13}C, 2D NMR, and HRESIMS spectra are given in the Supporting Information for all the compounds.

Table 8. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compound 11 (DMSO-d_6)

position	^{1}H NMR	^{13}C NMR
1	(1a = OH)-5.67 1H, d (6.3); 1b-5.08 1H, q (6.2, 1.0)	59.3
2	2a-2.19 1H, dd (6.0); 2b-2.04 1H, dd (6.4)	41.8
3		75.9
5		161.3
6		143.4
7	6.12 1H, s	93.4
7a		122.2
8	8.09 1H, d (8.0)	125.8
8a		106.4
9	7.19 1H, m	121.1
10	7.59 1H, m	132.4
11	7.60 1H, d (8.2)	117.1
11a		139.3
12	10.2 (1H, s)	
12a		157.3
12b		101.4
13		175.1
14	3.78 3H, s	55.5
15	1.43 3H, s	27.3
16	1.32 3H, s	26.5

Semi-Synthesis of Des-*N*-methylacronycine (4) and Noracronycine (1). According to the literature reports, acridone derivatives can intercalate DNA and inhibit topoisomerases I and II. Acronycine has a pyranoacridone scaffold with strong cytotoxic activity. It advanced to phase I and II clinical trials for the treatment of solid tumors.[21] The compounds **1** and **4** have a similar pyranoacridone scaffold to acronycine, so better targeted functionality substitution may lead to increased cytotoxic activity. To our delight, noracronycine (**1**) and des-*N*-methylacronycine (**4**) are the majorly isolated compounds from this plant. These two compounds have functionalizable free −NH and −OH groups at the 12th and 6th positions, respectively (Figure 1). Simple substitutions such as methyl, ethyl, allyl, prenyl, and propargyl at both −NH and −OH positions have been done to check the cytotoxic effect of substitution on two different positions (12th and 6th positions). In addition to this, two dimer (**17** and **22**) compounds were synthesized to check their *in vitro* cytotoxicity. The general procedure followed for the synthesis of these derivatives has been provided in the Experimental Section. The general schemes for the semi-synthesis of des-*N*-methylacronycine (**4**) and noracronycine (**1**) have been given below (Figures 4−7).

***In Vitro* Cytotoxicity Evaluation.** As most of these compounds are unexplored for cytotoxic activity, isolated and semisynthetic compounds were explored on various cancer cell lines, such as the human breast cancer cell line (MCF-7), human lung cancer cell line (CALU-3), and human squamous cell carcinoma cell line (SCC-25), for their cytotoxic activity. The results showed that the cytotoxic activity of semisynthetic compounds **12**−**22** was higher than that of isolated compounds **1**−**11** (Table 9).

In the case of MCF-7 cell lines, the three-membered acridone scaffold containing naturally isolated compounds **5**−**7** showed mild cytotoxic activity with an IC_{50} of 90.9, 140, and

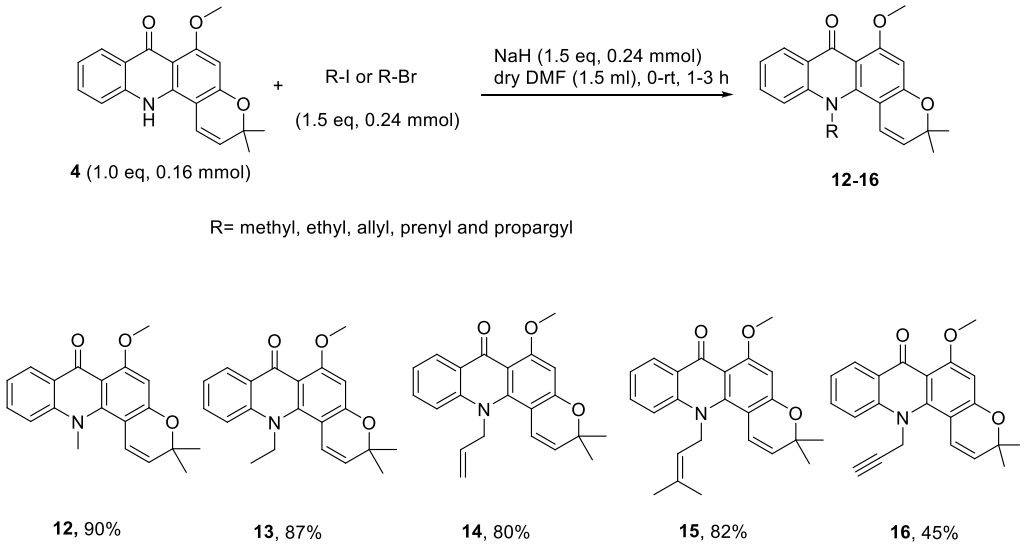

Figure 4. General scheme for the synthesis of compounds **12**−**16**.

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ACS Omega 2023, 8, 11201−11212

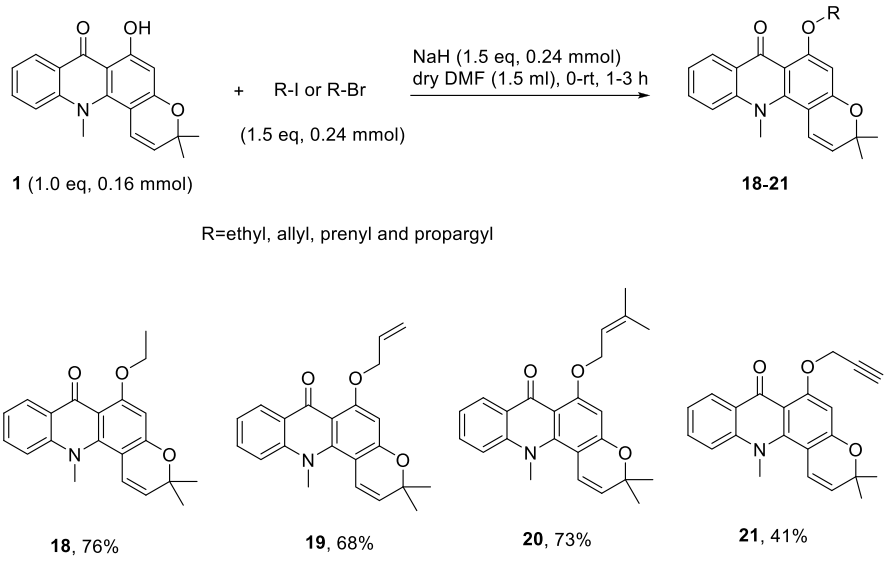

Figure 5. General scheme for the synthesis of compounds **18**−**21**.

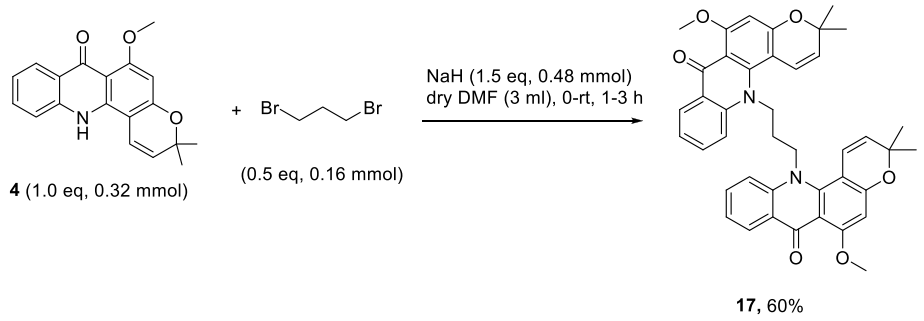

Figure 6. Scheme for the synthesis of compound **17**.

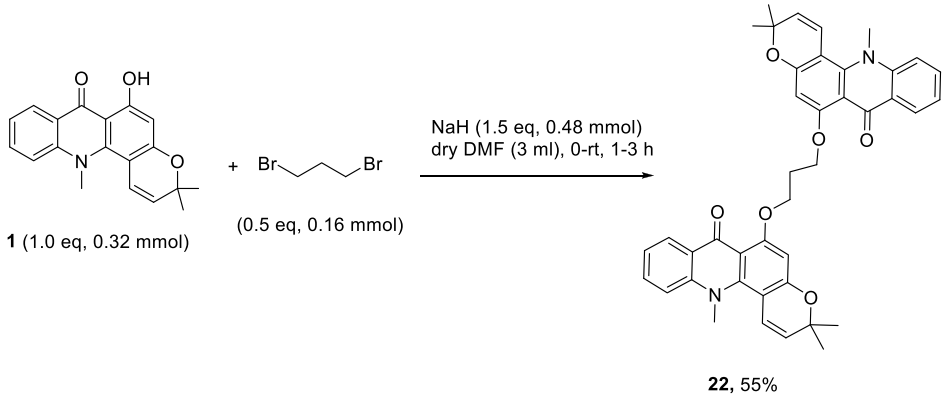

Figure 7. Scheme for the synthesis of compound **22**.

527 μM, respectively. The two furoquinoline alkaloids **8** and **9** showed an IC_{50} of 166 and 122 μM activity, respectively. The four-membered basic scaffold pyranoacridone with $-NCH_3$ at the 12th position and the −OH group at C-6, i.e., noracronycine (**1**), showed an IC_{50} of 187 μM in MCF-7 cell lines. The substitution of −OH at C-11 on noracronycine (**1**), i.e., 5-hydroxynoracronycin (**2**), has increased the activity of noracronycine (**1**) from 187 to 92.3 μM. Substitution of −NH instead of $-NCH_3$ at the 12th position of noracronycine (**1**), i.e., des-*N*-methylnoracronycine (**3**), has increased the activity from 187 to 106 μM. Substitution of −NH at the 12th position and $-OCH_3$ at C-6 of noracronycine (**1**), i.e., des-*N*-methylacronycine (**4**), has shown an IC_{50} of 191 μM. Breakdown of the double bond at the pyrane ring of des-*N*-methylnoracronycine (**3**) and substitution of the ketone at C-1 and −OH at C-2, i.e., acridocristine (**10**), have been found to be significantly inactive (IC_{50} of 390 μM), which demonstrated that the pyrane ring is essential for the activity of these compounds. The above-mentioned naturally isolated molecules are found to be mildly to moderately active. In order to enhance the cytotoxic activity of the pyranoacridones, semi-synthetic modifications have been performed at the free −NH position of des-*N*-methylacronycine (**4**) and −OH position of noracronycine (**1**). Substitution of methyl, ethyl, and propargyl

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ACS Omega 2023, 8, 11201−11212

Table 9. *In Vitro* Cytotoxicity Evaluation of Compounds 1−22 in MCF-7, CALU-3, and SCC-25 Cell Lines

compound	IC_{50} (μM) in MCF-7	IC_{50} (μM) in CALU-3	IC_{50} (μM) in SCC-25
1	187	97.5	14.1
2	92.3	75.1	29.7
3	106	10.3	18.8
4	191	13.9	21.2
5	90.9	69.7	16.1
6	140	36.7	24.6
7	527	98.1	86.8
8	166	84.6	45.5
9	122	34.1	20.8
10	390	61.7	7.6
11	413	78.7	13.9
12	38.2	6.81	28.6
13	35.6	13.4	40.6
14	>100	31.2	50.6
15	>100	106	57.8
16	63.5	13.5	36.3
17	19.7	9.41	17.0
18	61.8	8.56	20.8
19	37.5	7.66	15.7
20	63.5	12.3	18.7
21	33.0	7.18	16.4
22	13.2	4.49	12.9

groups at the −NH position of des-*N*-methylacronycine (**4**), i.e., compounds **12**, **13**, and **16**, has increased the activity of des-*N*-methylacronycine (**4**) from IC_{50} 191 μM to 38.2, 35.6, and 63.5 μM, respectively. The dimer at −NH position of des-*N*-methylacronycine (**4**), i.e., compound **17**, showed increased activity of compound **4** from IC_{50} 191 to 19.7 μM, which is around 10-fold better activity. Substitution of ethyl, allyl, prenyl, and propargyl groups at −OH position of noracronycine (**1**), i.e., compounds **18**, **19**, **20**, and **21**, has increased the activity of noracronycine (**1**) from an IC_{50} of 187 μM to 61.8, 37.5, 63.5, and 33.0 μM, respectively. The dimer at −OH position of noracronycine (**1**), i.e., compound **22**, showed 14-fold better activity with an IC_{50} of 13.2 μM compared with noracronycine (**1**) with IC_{50} 187 μM.

In the case of the human lung cancer cell line (CALU-3), naturally isolated compounds such as des-*N*-methylnoracronycine (**3**) and des-*N*-methylacronycine (**4**) have shown potent activity with an IC_{50} of 10.3 and 13.9 μM, respectively. Substitution of methyl and ethyl groups at −NH position of des-*N*-methylacronycine (**4**), i.e., compounds **12** and **13**, has slightly increased the activity of des-*N*-methylacronycine (**4**) from an IC_{50} of 13.9 to 6.81 and 13.4 μM, respectively. Allyl substitution at the −NH position of des-*N*-methylacronycine (**4**), i.e., compound **14**, has decreased activity from 13.9 to 31.2 μM. Substitution of the prenyl group, i.e., compound **15**, decreased the activity drastically to 106 μM (IC_{50}). Substitution of the propargyl group and dimer at −NH of des-*N*-methylacronycine (**4**), i.e., compounds **16** and **17**, has slightly increased the activity from an IC_{50} of 13.9 to 13.5 and 9.41 μM, respectively. Substitution of ethyl, allyl, prenyl, and propargyl groups at −OH position of noracronycine (**1**), i.e., compounds **18**, **19**, **20**, and **21**, has increased the activity of noracronycine (**1**) from an IC_{50} of 97.5 μM to 8.56 μM, 7.66 μM, 12.3 μM, and 7.18 μM, respectively. The dimer at −OH position of noracronycine (**1**), i.e., compound **22**, showed 24-fold better activity with an IC_{50} of 4.49 μM compared with noracronycine (**1**) with an IC_{50} of 97.5 μM.

In the case of SCC-25 cell lines, both naturally isolated and semisynthetic molecules showed similar activity profiles. The first-time isolated natural compounds such as carbocristine (**11**) and acridocristine (**10**) showed potent cytotoxic effects with an IC_{50} of 13.9 and 7.6 μM, respectively. Semisynthetic derivatives at the −NH position of des-*N*-methylacronycine (**4**) did not show significant increase in cytotoxic activity except the dimer at −NH position of des-*N*-methylacronycine (**4**), i.e., compound **17**, which showed slightly increased activity of (**4**) from IC_{50} 21.2 to 17.0 μM. Similarly, semisynthetic derivatives at the −OH position of noracronycine (**1**) did not show a significant increase in cytotoxic activity except the dimer at the −OH position of noracronycine (**1**), i.e., compound **22**, which showed slightly increased activity of (**1**) from IC_{50} 14.1 to 12.9 μM.

In conclusion, an undescribed carbazole alkaloid carbocristine (**11**) and an unexplored pyranoacridone alkaloid acridocristine (**10**) along with four known pyranoacridones (**1**−**4**), three acridone alkaloids (**5**−**7**), and two furoquinoline alkaloids (**8**−**9**) were isolated from the stem and root bark of *G. pentaphylla*. As most of these compounds are unexplored for cancer, these naturally isolated compounds were explored on various cell lines such as the human breast cancer cell line (MCF-7), human lung cancer cell line (CALU-3), and human squamous cell carcinoma cell line (SCC-25) for their cytotoxic activity. In the case of MCF-7 cell lines, naturally isolated pyranoacridone compounds such as noracronycine (**1**), 5-hydroxynoracronycin (**2**), des-*N*-methylnoracronycine (**3**), des-*N*-methylacronycine (**4**), and acridocristine (**10**) showed mild to moderate cytotoxicity with an IC_{50} of 187, 92.3, 106, 191, and 390 μM, respectively. To enhance the cytotoxicity of these pyranoacridones, semi-synthetic modifications have been performed at the free −NH position of des-*N*-methylacronycine (**4**) and −OH position of noracronycine (**1**). Substitution of methyl and ethyl at the −NH position of des-*N*-methylacronycine (**4**), i.e., compounds **12** and **13**, has increased the activity of des-*N*-methylacronycine (**4**) from IC_{50} 191 to 38.2 and 35.6 μM, respectively. The dimer at −NH position of des-*N*-methylacronycine (**4**), i.e., compound **17**, showed increased activity of compound **4** from IC_{50} 191 to 19.7 μM, which is around 10-fold better activity. The dimer at −OH position of noracronycine (**1**), i.e., compound **22**, showed 14-fold better activity with an IC_{50} of 13.2 μM compared with noracronycine (**1**) with an IC_{50} of 187 μM. In the case of the human lung cancer cell line (CALU-3), naturally isolated compounds such as des-*N*-methylnoracronycine (**3**) and des-*N*-methylacronycine (**4**) showed potent activity with an IC_{50} of 10.3 and 13.9 μM, respectively. Substitution of methyl and ethyl groups at −NH position of des-*N*-methylacronycine (**4**), i.e., compounds **12** and **13**, has slightly increased the activity of des-*N*-methylacronycine (**4**) from an IC_{50} of 13.9 to 6.81 and 13.4 μM, respectively. Substitution of the propargyl group and dimer at −NH of des-*N*-methylacronycine (**4**), i.e., compounds **16** and **17**, has slightly reduced the activity from an IC_{50} of 13.9 to 13.5 and 9.41 μM, respectively. Substitution of ethyl, allyl, prenyl, and propargyl groups at −OH position of noracronycine (**1**), i.e., compounds **18**, **19**, **20**, and **21**, increased the activity of noracronycine (**1**) from an IC_{50} of 97.5 to 8.56, 7.66, 12.3, and 7.18 μM, respectively. The dimer at −OH position of noracronycine (**1**), i.e., compound **22**, showed 24-fold better

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ACS Omega 2023, 8, 11201−11212

activity with an IC_{50} of 4.49 μM compared to noracronycine (**1**) with an IC_{50} of 97.5 μM. In the case of SCC-25 cell lines, naturally isolated compounds such as carbocristine (**11**) and acridocristine (**10**) showed potent cytotoxic effects with an IC_{50} of 13.9 and 7.6 μM, respectively. The dimer at −NH position of des-*N*-methylacronycine (**4**), i.e., compound **17**, showed slightly increased activity of (**4**) from IC_{50} 21.2 to 17.0 μM. The dimer at −OH position of noracronycine (**1**), i.e., compound **22**, showed slightly increased activity of (**1**) from IC_{50} 14.1 to 12.9 μM. Based on the *in vitro* cytotoxic assay data presented above, we can conclude that semisynthetic pyranoacridone derivatives outperformed naturally isolated compounds. Compounds **12**, **17**, **21**, and **22** could be lead molecules for the development of novel anticancer drugs. In the future, better-targeted functionality substitutions on various positions of pyranoacridone molecules may result in more potent anticancer molecules.

EXPERIMENTAL SECTION

General Experimental Procedures. HRESIMS spectra were recorded on an Agilent Q-TOF spectrometer in the positive (ESI^+) ion mode. In addition, ^1H NMR and ^{13}C NMR spectra were recorded on Bruker 500 and 125 MHz spectrometers, respectively, using tetramethylsilane (1% v/v solution in the respective solvent) as an internal standard. Column chromatography was carried out with 100−200 or 230−400 mesh size silica and eluted using EtOAc: hexane as the mobile phase. Fractions were monitored by thin layer chromatography (TLC) using Merck TLC plates pre-coated with 250 μm thickness silica gel 60 F254 plates and visualized under UV 254 or 365 nm. *p*-Anisaldehyde and Wagner's and Dragendorff's reagents were freshly prepared and used as the TLC spraying reagent. All the other reagents, like starting materials and bases, were used as received from Sigma-Aldrich. Ethyl acetate, hexane, methanol, and diethyl ether were purchased from Fisher Scientific, Qualigen, and used as received. Anhydrous solvent dimethylformamide (DMF) was obtained from Sigma-Aldrich and used as received. All deuterated solvents were purchased from Sigma-Aldrich.

Plant Materials. The stem bark and root bark of *G. pentaphylla* (Rutaceae) were collected from the forest region of Mannangidinne, Andhra Pradesh, South India (GPS coordinates: 14°53′29″N 80°02′25″E), in March 2020. The fruits were identified by Professor Hitesh Solanki, Gujarat University. Its certificate specimen number is (GU/BOT/RG1).

Extraction and Isolation. The stem bark (8.5 kg) and root bark (4 kg) of *G. pentaphylla* were dried under shade, and the dried plant material was subjected to a mechanical grinder to afford a dried coarse powder of the stem bark (2.8 kg) and root bark (1.3 kg). Both the plant materials were subsequently extracted with hexane at room temperature for 3 days for defatting of nonpolar constituents. The hexane extract was filtered and concentrated under reduced pressure to obtain the concentrated hexane extract. The remaining plant material after hexane extraction was subjected to methanolic extraction for 3 days at room temperature. The methanolic extract was filtered and concentrated under reduced pressure to get the concentrated methanolic extract, which further proceeded to column chromatography. The crude methanolic extract was directly loaded on open-column chromatography with 230−400 mesh silica. The column was started with hexane, and polarity was slowly increased with ethyl acetate. Pooled fractions 5−9 obtained in 2% v/v ethyl acetate: hexane afford an orange crystalline solid of compound **7** (100 mg). Fractions 15−19 in 5% v/v ethyl acetate: hexane afforded a yellow crystalline solid, which was further purified by hexane washing to get yellow crystals of compound **1** (1.1 g). Pooled fractions of 32−37 in 5% v/v ethyl acetate: hexane gave brown crystals of compound **2** (20 mg). Fractions 38−47 in 7% v/v ethyl acetate: hexane gave orange crystals of compound **3** (1.3 g). Pooled fractions of 52−58 in 7% v/v ethyl acetate: hexane afford brown crystals of compound **5** (10 mg). Fractions 62−69 in 10% v/v ethyl acetate: hexane gave pale-yellow crystals of compound **8** (200 mg). Pooled fractions of 71−75 in 12% v/v ethyl acetate: hexane afford white crystals of compound **9** (150 mg). Fractions 76−79 in 15% v/v ethyl acetate: hexane gave compound **6** (30 mg). Pooled fractions of 81−105 in 20% v/v ethyl acetate: hexane afford fluorescent yellow powder of compound **4** (4 g). All these above-mentioned compounds were obtained in the methanolic extract of the stem bark and root bark. The root bark methanolic extract afforded two additional compounds, which were not obtained from stem barks. Pooled fractions of 21−31 in 7% v/v ethyl acetate: hexane gave fluorescent yellow powder of compound **10** (20 mg). Fractions 106−112 in 45% v/v ethyl acetate: hexane afford compound **11** (10 mg). In order to study the structural activity relationship of pyranoacridones, semi-synthetic modifications have been done on −OH and −NH groups present at 6th and 12th positions of majorly isolated compounds such as noracronycine (**1**) and des-*N*-methylacronycine (**4**), respectively. The detailed procedure for the synthesis of semisynthetic derivatives given below and related schemes are given in Figures 4−7.

Semi-Synthesis of Majorly Isolated Alkaloids. *General Procedure for the Synthesis of Compounds **12**−**16** and **18**−**21**.* Pyranoacridone, such as noracronycine (**1**) or des-*N*-methylacronycine (**4**) (0.16 mmol, 1.0 equiv), was dissolved in anhydrous DMF (1.5 mL). Sodium hydride dispersed in oil (0.24 mmol, 1.5 equiv) was added, and the mixture was stirred at 0 °C for 30 min. Next, bromo/iodide compounds (0.24 mmol, 1.5 equiv) were added, and the reaction mixture was stirred from 0 °C to room temperature for 1−3 h. After completion of the reaction, distilled deionized cold water (20 mL) was added, and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to get a crude solid of the reaction mixture. It was then subjected to column chromatography (silica gel 100−200 mesh size, ethyl acetate: pet ether) for further purification to get the desired compounds **12**−**16** and **18**−**21** in 90−45%.

*Spectral Data. 6-Methoxy-3,3,12-trimethyl-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**12**).* Column chromatography (SiO_2 100−200, eluted with 15% ethyl acetate) afforded the desired product as a yellow crystalline solid (46 mg, 90% yield). mp 176−178 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 8.29 (1H, d, J = 7.7 Hz), 7.73 (1H, t, J = 7.5 Hz), 7.61 (1H, d, J = 8.8 Hz, 1H), 7.29 (1H, t, J = 8.8 Hz), 6.71 (1H, d, J = 9.7 Hz), 6.41 (1H, s), 5.63 (1H, d, J = 9.6 Hz), 3.93 (3H, s), 3.91 (3H, s), 1.54 (6H, s) ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.1, 164.1, 161.4, 148.1, 146.0, 134.4, 127.4, 125.8, 124.3, 123.1, 122.8, 117.8, 110.9, 104.5, 95.4, 77.8, 56.4, 44.8, 26.4. HRESIMS: m/z calcd for $C_{20}H_{20}NO_3$ $[M + H]^+$ 322.1438; found, 322.1435.

*12-Ethyl-6-methoxy-3,3-dimethyl-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**13**).* Column chromatography (SiO_2 100−200, eluted with 12% ethyl acetate) afforded the desired product as a pale-yellow crystalline solid (47 mg, 87%

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ACS Omega 2023, 8, 11201−11212

yield). mp 194−199 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (1H, d, J = 9.9 Hz), 7.58 (1H, t, J = 8.5 Hz), 7.45 (1H, d, J = 8.4 Hz), 7.22 (1H, t, J = 8.8 Hz), 6.50 (1H, d, J = 9.6 Hz), 6.32 (1H, s), 5.55 (1H, d, J = 9.6 Hz), 4.35 (2H, q, J = 7.0 Hz), 3.97 (3H, s), 1.53 (6H, s), 0.91 (3H, t, J = 7.0) ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.2, 162.6, 159.1, 147.0, 143.5, 132.2, 128.1, 127.4, 124.1, 122.1, 121.5, 118.0, 112.4, 104.4, 94.7, 76.5, 56.4, 50.6, 27.1, 13.1. HRESIMS: m/z calcd for $C_{21}H_{22}NO_3$ $[M + H]^+$ 336.1594; found, 336.1580.

*12-Allyl-6-methoxy-3,3-dimethyl-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**14**).* The general procedure (A) was followed. Column chromatography (SiO_2 100−200, eluted with 10% ethyl acetate) afforded the desired product as a pale-yellow crystalline solid (45 mg, 80% yield). mp 198−204 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (1H, d, J = 8.6 Hz), 7.55 (1H, t, J = 7.5 Hz), 7.44 (1H, d, J = 8.6 Hz), 7.22 (1H, t, J = 7.5 Hz), 6.68 (1H, d, J = 9.6 Hz), 6.34 (1H, s), 5.76 (1H, m), 5.53 (1H, d, J = 9.6 Hz), 5.19 (2H, t, J = 12.5, 16.1 Hz), 4.83 (2H, d, J = 3.5 Hz), 3.97 (3H, s), 1.52 (6H, s) ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.7, 163.0, 159.2, 146.8, 143.8, 134.2, 132.3, 127.3, 126.7, 123.8, 122.2, 121.5, 118.4, 117.8, 111.3, 103.7, 94.8, 76.3, 57.9, 56.4, 27.0. HRESIMS: m/z calcd for $C_{22}H_{22}NO_3$ $[M + H]^+$ 348.1594; found, 348.1591.

*6-Methoxy-3,3-dimethyl-12-(3-methylbut-2-en-1-yl)-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**15**).* Column chromatography (SiO_2 100−200, eluted with 7% ethyl acetate) afforded the desired product as a white crystalline solid (50 mg, 82% yield). mp 210−214 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.34 (1H, d, J = 10 Hz), 7.55 (1H, t, J = 10 Hz), 7.41 (1H, d, J = 10 Hz), 7.21 (1H, t, J = 10 Hz), 6.60 (1H, d, J = 9.6 Hz), 6.31 (1H, s), 5.52 (1H, d, J = 9.6 Hz), 5.12 (1H, t, J = 7.5 Hz), 4.81 (2H, d, J = 6.2 Hz), 3.97 (3H, s), 1.60 (3H, s), 1.58 (3H, s), 1.53 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.2, 163.1, 159.3, 147.1, 144.2, 136.3, 132.4, 127.5, 127.2, 123.9, 122.3, 121.9, 121.2, 117.8, 111.8, 104.1, 94.8, 76.6, 56.6, 54.1, 27.3, 25.9, 18.6. HRESIMS: m/z calcd for $C_{24}H_{26}NO_3$ $[M + H]^+$ 376.1907; found, 376.1889.

*6-Methoxy-3,3-dimethyl-12-(prop-2-yn-1-yl)-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**16**).* Column chromatography (SiO_2 100−200, eluted with 10% ethyl acetate) afforded the desired product as a pale-white crystalline solid (25 mg, 45% yield). mp 152−154 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.32 (1H, d, J = 8.5 Hz), 7.63 (1H, t, J = 8.5 Hz), 7.57 (1H, d, J = 6.8 Hz), 7.27 (1H, t, J = 6.8 Hz), 7.04 (1H, t, J = 5.9 Hz), 6.74 (1H, d, J = 9.4 Hz), 6.34 (2H, s), 5.53 (1H, d, J = 9.7 Hz), 5.25 (2H, d, J = 6.2 Hz), 3.97 (3H, s), 1.53 (6H, s) ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.9, 162.5, 159.1, 144.4, 143.4, 132.3, 127.3, 126.4, 123.7, 122.8, 121.6, 117.4, 111.3, 104.8, 104.3, 95.5, 91.7, 85.7, 76.6, 56.4, 27.4. HRESIMS: m/z calcd for $C_{22}H_{20}NO_3$ $[M + H]^+$ 346.1432; found, 346.1426.

*6-Ethoxy-3,3,12-trimethyl-3,12-dihydro-7H-pyrano[2,3-c]-acridin-7-one (**18**).* Column chromatography (SiO_2 100−200, eluted with 12% ethyl acetate) afforded the desired product as a yellow crystalline solid (41 mg, 76% yield). mp 166−169 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (1H, d, J = 8.3 Hz), 7.62 (1H, t, J = 8.3 Hz), 7.36 (1H, d, J = 8.3 Hz), 7.22 (1H, t, J = 8.3 Hz), 6.55 (1H, d, J = 8.3 Hz), 6.29 (1H, s), 5.51 (1H, d, J = 8.3 Hz), 4.17 (2H, q, J = 6.8 Hz), 3.83 (3H, s), 1.59 (3H, t, J = 7.0 Hz), 1.52 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.3, 162.5, 159.3, 147.0, 144.7, 133.1, 127.4, 125.6, 123.0, 122.1, 121.9, 116.0, 110.8, 103.0, 95.2, 76.4, 65.0, 44.5, 26.2, 14.8. HRESIMS: m/z calcd for $C_{21}H_{22}NO_3$ $[M + H]^+$ 336.1589; found, 336.1581.

*6-(Allyloxy)-3,3,12-trimethyl-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**19**).* Column chromatography (SiO_2 100−200, eluted with 10% ethyl acetate) afforded the desired product as a pale-white crystalline solid (38 mg, 68% yield). mp 124−126 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (1H, d, J = 8.5 Hz), 7.62 (1H, t, J = 8.5 Hz), 7.37 (1H, d, J = 8.5 Hz), 7.24 (1H, t, J = 7.4 Hz), 6.55 (1H, d, J = 9.5 Hz), 6.30 (1H, s), 6.15 (1H, m), 5.73 (1H, d, J = 13.3 Hz), 5.52 (1H, d, J = 9.6 Hz), 5.37 (1H, d, J = 12.2 Hz), 4.69 (2H, d, J = 4.8 Hz), 3.83 (3H, s), 1.54 (6H, s) ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.3, 162.0, 161.4, 159.4, 147.0 144.5, 132.7, 132.6, 127.4, 125.6, 123.1, 122.1 122.0, 118.6, 116.0, 111.0, 103.3, 95.6, 76.5, 69.9, 45.0, 27.0. HRESIMS: m/z calculated for $C_{22}H_{22}NO_3$ $[M + H]^+$ 348.1589; found, 348.1577.

*3,3,12-Trimethyl-6-[(3-methylbut-2-en-1-yl)oxy]-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**20**).* Column chromatography (SiO_2 100−200, eluted with 10% ethyl acetate) afforded the desired product as a yellow crystalline solid (44 mg, 73% yield). mp 142−144 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (1H, d, J = 8.0 Hz), 7.61 (1H, t, J = 8.3 Hz), 7.36 (1H, d, J = 8.2 Hz), 7.23 (1H, t, J = 8.3 Hz), 6.55 (1H, d, J = 9.7 Hz), 6.31 (1H, s), 5.67 (1H, d, J = 8.0 Hz), 5.51 (1H, t, J = 9.6 Hz), 4.68 (2H, d, J = 8.0 Hz), 3.82 (3H, s), 1.80 (3H, s), 1.75 (3H, s), 1.54 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.2, 162.5, 159.2, 147.0, 144.7, 137.3, 132.6, 127.4, 125.6, 122.9, 122.1, 121.9, 119.8, 115.9, 110.9, 103.0, 95.4, 76.4, 66.0, 44.4, 27.0, 25.5, 18.5. HRESIMS: m/z calcd for $C_{24}H_{26}NO_3$ $[M + H]^+$ 376.1902; found, 376.1886.

*3,3,12-Trimethyl-6-(prop-2-yn-1-yloxy)-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one (**21**).* Column chromatography (SiO_2 100−200, eluted with 7% ethyl acetate) afforded the desired product as a pale-yellow crystalline solid (23 mg, 41% yield). mp 153−155 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (1H, d, J = 8.4 Hz), 7.63 (1H, t, J = 8.5 Hz), 7.36 (1H, d, J = 7.7 Hz), 7.24 (1H, t, J = 7.2 Hz), 6.88 (1H, t, J = 5.8 Hz), 6.57(1H, d, J = 9.3 Hz) 6.55 (1H, s), 5.53 (1H, d, J = 9.3 Hz), 5.46 (2H, d, J = 5.8 Hz), 3.84 (1H, s), 1.55 (6H, s) ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.9, 160.1, 158.8, 146.6, 144.7, 132.9, 127.9, 125.4, 123.8, 122.1, 121.8, 117.3, 115.5, 109.5, 104.1, 98.4, 88.4, 76.5, 57.1, 43.8, 26.9. HRESIMS ion at m/$[M + H]^+$ (calcd for). HRESIMS: m/z calculated for $C_{22}H_{20}NO_3$ $[M + H]^+$ 346.1432; found, 346.1435.

*General Procedure for the Synthesis of Compounds **17** and **22**.* Pyranoacridone such as noracronycine (**1**) or des-*N*-methylacronycine (**4**) (0.32 mmol, 1.0 equiv) was dissolved in anhydrous DMF (3 mL) under a nitrogen atmosphere. Sodium hydride dispersed in oil (0.48 mmol, 1.5 equiv) was added, and the mixture was stirred at 0 °C for 30 min. 1,3 dibromopropane (0.16 mmol, 0.5 equiv) was added, and the reaction mixture was stirred from 0 °C to room temperature for 1−3 h. After completion of the reaction, distilled deionized cold water (50 mL) was added, and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to get a crude solid of the reaction mixture. It was then subjected to column chromatography (silica gel 100−200 mesh size, ethyl acetate: pet ether) for further purification to get the desired compounds **17** and **22**.

*Spectral Data. 12,12′-(Propane-1,3-diyl)bis(6-methoxy-3,3-dimethyl-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one) (**17**).* Column chromatography (SiO_2 100−200, eluted with 7% ethyl acetate) afforded the desired product as a pale-white crystalline solid (60 mg, 60% yield). mp 187−190 °C; ^{1}H

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ACS Omega 2023, 8, 11201−11212

NMR (500 MHz, $CDCl_3$): δ 8.37 (2H, d, J = 7.8 Hz), 7.55 (2H, t, J = 7.8 Hz), 7.42 (2H, d, J = 7.8 Hz), 7.22 (2H, t, J = 7.2 Hz), 6.66 (2H, d, J = 9.7 Hz), 6.34 (1H, s), 5.75 (2H, m), 5.50 (2H, d, J = 9.6 Hz), 4.84 (4H, dt, J = 5.1, 1.8 Hz), 3.98 (6H, s), 1.52 (12H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.6, 162.9, 159.1, 146.7, 143.7, 134.1, 132.2, 127.2, 126.6, 123.8, 122.1, 121.4, 118.3, 117.7, 111.3, 103.6, 94.7, 76.2, 57.8, 56.3, 26.9. HRESIMS: m/z calcd for $[C_{41}H_{38}N_2O_6-C_{19}H_{16}N0_3]^+$ 348.1600; found, 348.1587.

*6,6′-[Propane-1,3-diylbis(oxy)]bis(3,3,12-trimethyl-3,12-dihydro-7H-pyrano[2,3-c]acridin-7-one) (**22**).* Column chromatography (SiO_2 100−200, eluted with 10% ethyl acetate) afforded the desired product as a pale-yellow crystalline solid (22 mg, 55% yield). mp 124−126 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (2H, d, J = 9.6 Hz), 7.62 (2H, t, J = 9.6 Hz), 7.37 (2H, d, J = 9.6 Hz), 7.24 (2H, t, J = 8.0 Hz), 6.55 (2H, d, J = 9.6 Hz), 6.29 (2H, s), 6.16 (2H, m), 5.50 (2H, d, J = 9.6 Hz), 4.69 (4H, dt, J = 4.8, 1.8 Hz), 3.83 (6H, s), 1.54 (12H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.3, 162.0, 159.2, 147.0, 144.7, 132.7, 132.6, 127.4, 125.6, 123.1, 122.1, 122.0, 118.0, 116.0, 110.9, 103.2, 95.6, 76.5, 69.9, 44.5, 27.0. HRESIMS: m/z calcd for $[C_{41}H_{38}N_2O_6-C_{19}H_{16}NO_3]^+$ 348.1600; found, 348.1584.

In Vitro Cytotoxicity Assay. Breast cancer cells (MCF-7), lung cancer cells (CALU-3), and squamous cell carcinoma cells (SCC-25) were obtained from ATCC. MCF-7 cells and CALU-3 cells were grown in Eagle's minimum essential medium supplied with 10% fetal bovine serum (FBS), and SCC-25 cells were grown in Dulbecco's modified Eagle's medium/F12 supplied with 400 ng/μL hydrocortisone and 10% FBS. All the cells were cultured in a humidified incubator with 5% CO2 and kept at 37 °C. All the cytotoxicity experiments were conducted in 96-well plates. All the compounds were dissolved in media with a maximum of 0.5% dimethyl sulfoxide (DMSO). All the compounds were tested at 0.01−100 μM concentrations. CALU-3 cells were seeded at a density of 4×10^4 cells/well, MCF-7 cells were seeded at a density of 4×10^4 cells/well, and SCC-25 cells were seeded at a density of 1×10^4 cells/well. After overnight attachment, 100 μL of media containing the predetermined concentrations of compounds was added to the wells in triplicate. Following 96 h incubation, 20 μL of the 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent was added to all the wells and allowed to incubate for 4 h. Following the incubation, 10% W/V sodium dodecyl sulfate in 0.01 M HCl was added to the wells, and the plate was incubated overnight. The next day, absorbance in each well was determined at 590 nm using a plate reader. The absorbance in control wells (i.e., treated with media with no compounds) was used to calculate the percentage viability of cells in each well treated with the compound. The "% viability versus concentration" data was fitted to the cell killing equation provided in PRISM software to calculate the IC50 value of each compound in each of the cancer cell lines.[22,23]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c08100.

^{1}H, ^{13}C NMR, and HRESIMS spectra for compounds **1**−**22** and 2D NMR spectra for compounds **6**, **7**, **10**, and **11** (PDF)

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Notes

The authors declare no competing financial interest.

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