Cytotoxic Constituents of the Marine Invertebrates Collected from Korean Waters

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1. Cytotoxic sterols and saponins from the starfish *Certonardoa semiregularis*

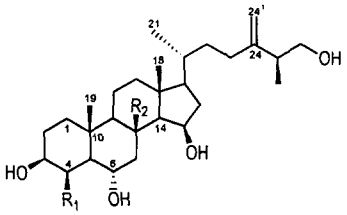
Starfish is known to yield a large number of unique bioactive metabolites such as steroids. These steroids have been reported to exhibit cytotoxic, hemolytic, antiviral, antifungal, and antimicrobial activities. In our search for bioactive metabolites from the starfish *Certonardoa semiregularis* (Family Linckiidae) collected from Korean waters, 65 steroids and two taurine derivatives have been isolated from the brine shrimp active fraction of the MeOH extract.

The structures of the compounds were elucidated as 60 new steroids, five known steroids (**6**, **13**, **22**, **31**, **58**), a new taurine derivative (**67**), and a known taurine derivative (**66**). Compounds **51**–**57** are the first examples of 15-keto sterols from starfish. The side chains of **10**, **11**, and **61** were first encountered in naturally occurring sterols. Compounds **17**–**21** contain previously undescribed 2-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-3-*O*-sulfonato-*β*-D-xylopyranosyl unit as a sugar moiety. The sugar moiety 2,4-di-*O*-methyl-*β*-D-xylopyranosyl-(1→2)-*β*-D-xylofuranosyl unit in compounds **23**–**28** was also unprecedented. The nonsubstituted xylofuranosyl unit in compound **32** has not been encountered in the starfish saponins.

The compounds were tested for cytotoxicity against five human solid cancer cell lines [human lung cancer (A549), human ovarian cancer (SK-OV-3), human skin cancer (SK-MEL-2), human CNS cancer (XF498), and human colon cancer (HCT15)].

Compounds **16**, **34**, **37**, **38**, **40**, **44**, **46**, and **56** showed significant cytotoxicity against all of the five human cancer cell lines (Table 1). Especially the cytotoxicity of compound **16** was comparable to that of doxorubicin. Sterols were generally more potent than their corresponding saponins. However, no clear structure-activity relationship was perceived.

Certain sterols of similar structure to compounds **1–11** were previously reported to show antimicrobial activity. Therefore, compounds **1–11**, along with compounds **26–28** and **32** were assayed for antibacterial activity against 20 clinically isolated strains. Most of these compounds displayed only weak antibacterial activity against *Streptococcus pyogenes* 308A, *Pseudomonas aeruginosa* 1771, and *Pseudomonas aeruginosa* 1771M. Some sulfated compounds and compounds **17a**, **22**, and **25** were evaluated for antiviral activity since certain sulfated sterols from marine invertebrate were reported to be active. It was reported that sterols sulfated exclusively on the A and B rings were much more potent than other sulfated sterols. However, the antiviral activity of these compounds was insignificant within the range of non-cytotoxic concentration. Only weak antiviral activity against HSV was observed in compounds **17a**, **29**, and **30**. Compounds **17** and **19** rather exhibited cytotoxicity against the MT-4 cell.



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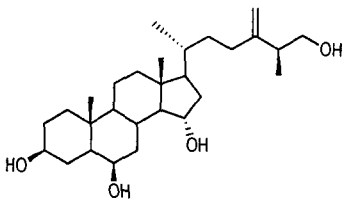
2 R_1=OH R_2=H
3 R_1=H R_2=OH
4 R_1=H R_2=H



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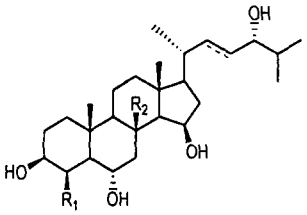

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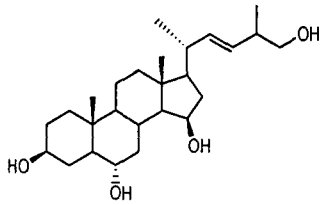
8 R_1=H R_2=H, 22,23-dehydro
13 R_1=OH R_2=OH, 22,23-dehydro



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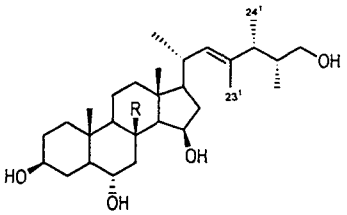

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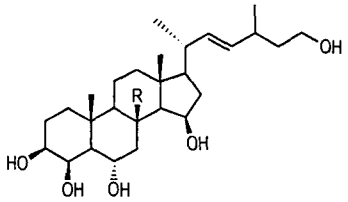

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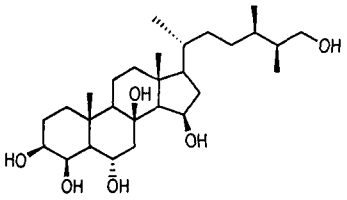

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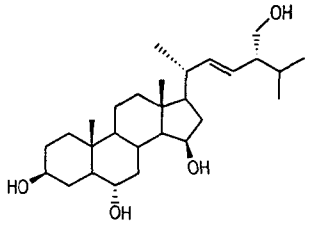

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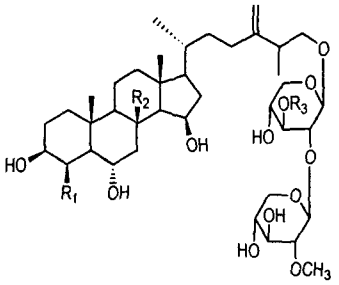

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17a R_1=OH R_2=OH R_3=H
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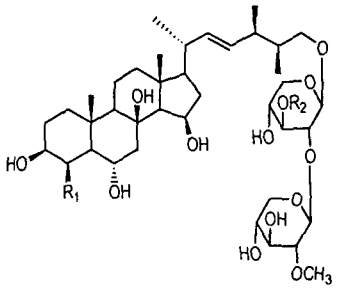
19 R_1=H R_2=OH R_3=SO_3Na



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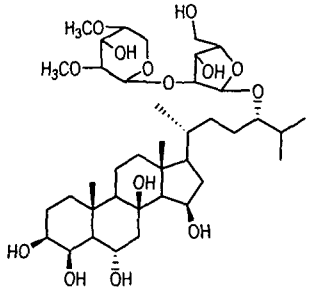

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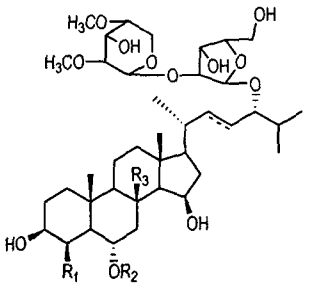

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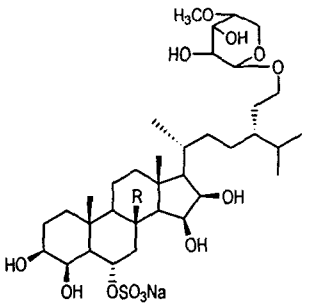
25 R_1=OH R_2=H R_3=H
26 R_1=OH R_2=H R_3=OH
27 R_1=H R_2=H R_3=H
28 R_1=OH R_2=H R_3=H, 22,23-dehydro



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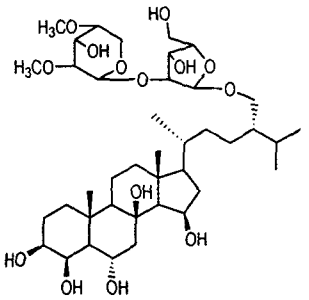

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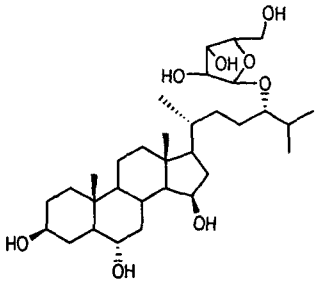

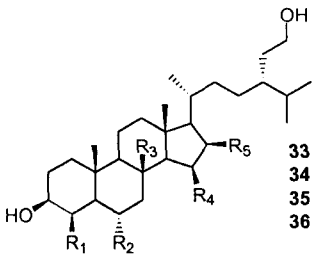

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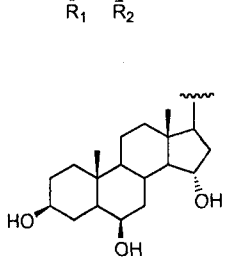

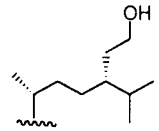

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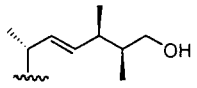

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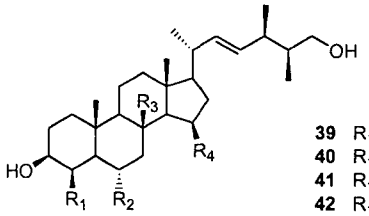

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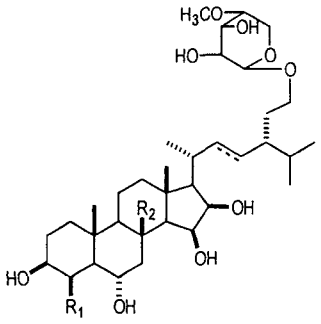
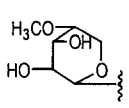
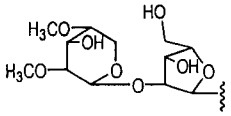
41 R_1=OH R_2=OH R_3=H R_4=OH
42 R_1=OH R_2=OH R_3=OH R_4=OH



43 R_1=H R_2=H
44 R_1=H R_2=OH
45 R_1=OH R_2=H
46 R_1=OH R_2=H 22,23-dehydro
47 R_1=OH R_2=OH



A



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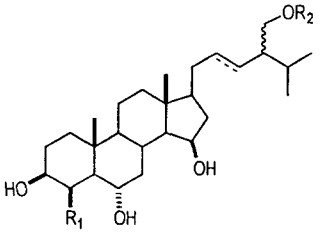

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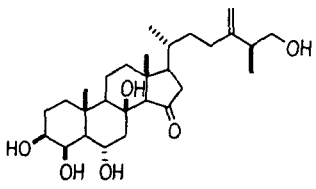
50 R_1=H R_2=**B** 22,23-dehydro 24 *R*



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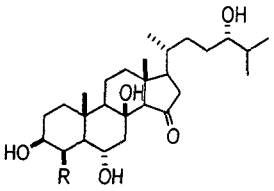

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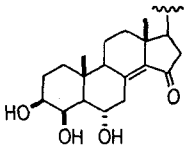
53 R=H



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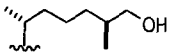

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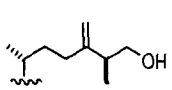

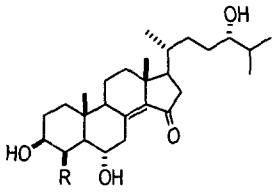

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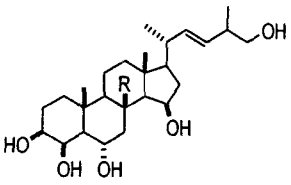

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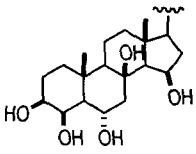

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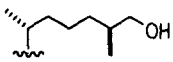

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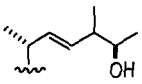

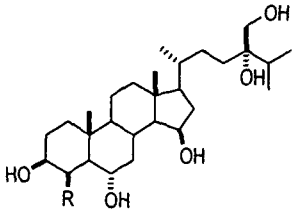

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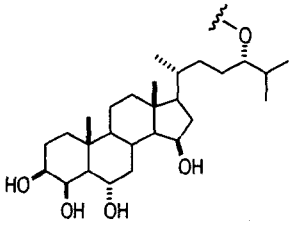

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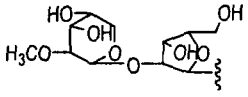

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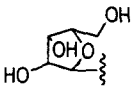
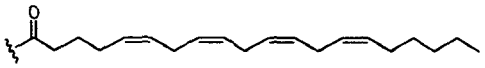


66 R=



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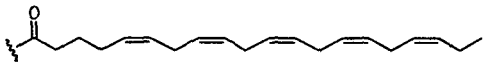


Table 1. Cytotoxicity of the Compounds against Human Solid Tumor Cell Lines[a]

compound	A549	SK-OV-3	SK-MEL-	XF498	HCT15
1	3.1	7.3	4.1	4.4	4.5
2	3.6	6.9	4.9	4.7	6.4
3	14.1	20.2	15.1	13.3	23.9
4	5.3	8.5	5.5	4.8	9.6
5	3.9	4.9	4.1	4.1	4.2
6	11.3	15.6	12.4	11.4	19.4
7	3.8	4.4	3.8	4.3	5.5
8	3.6	3.6	3.4	3.7	4.0
9	7.1	9.4	6.4	8.6	10.6
10	5.1	8.8	5.3	7.3	6.3
11	8.0	10.3	7.1	11.4	12.5
14	4.3	6.0	4.3	4.5	4.2
15	>30	>30	>30	>30	>30
16	0.15	0.08	0.09	0.07	0.01
doxorubicin	0.02	0.17	0.02	0.06	0.06
17	>30	>30	6.7	>30	>30
19	25.0	25.9	3.8	19.4	15.8
20	>30	>30	>30	>30	>30
doxorubicin	0.02	0.16	0.02	0.08	0.06
21	>30	>30	>30	>30	>30
22	>30	>30	>30	>30	>30
23	>30	>30	>30	>30	>30
24	>30	>30	>30	>30	>30
25	>30	>30	16.1	>30	>30
26	>30	>30	10.6	>30	24.5
27	7.5	6.8	5.8	6.4	3.9
28	>30	>30	9.7	25.4	43.4
29	>30	>30	>30	>30	>30
30	>30	>30	>30	>30	>30
31	>30	>30	16.3	>30	>30
32	8.0	8.4	7.7	7.2	8.2
doxorubicin	0.02	0.17	0.02	0.08	0.06

[a]Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer. Compounds were assayed in several separate batches.

Table 1. Continued

compound	A549	SK-OV-3	SK-MEL-	XF498	HCT15
33	1.36	1.33	0.68	0.84	2.48
34	0.82	0.90	0.40	0.43	1.25
35	8.32	6.59	5.32	6.92	17.7
36	3.30	3.50	2.50	3.48	4.39
37	0.48	0.83	0.28	0.40	1.26
38	0.54	0.69	0.40	0.33	0.73
39	1.75	1.51	0.48	1.22	1.25
40	0.15	0.16	<0.10	0.08	0.25
41	2.33	2.64	2.23	2.21	2.71
42	1.75	1.13	0.67	0.89	2.54
43	1.25	2.00	0.68	0.74	2.28
44	0.87	0.89	0.26	0.35	2.17
45	3.41	3.29	2.36	3.53	8.20
46	0.83	0.74	0.30	0.45	2.76
47	4.26	3.06	2.39	3.69	8.32
48	6.61	3.71	2.10	3.00	5.04
49	4.44	4.32	2.93	3.82	8.13
50	4.13	4.92	3.85	4.58	6.14
doxorubicin	0.01	0.08	0.08	0.10	0.17
51	12.0	10.9	5.57	4.95	11.6
52	6.94	6.85	4.20	4.10	5.82
53	5.43	12.3	7.30	12.6	13.6
54	3.80	4.10	3.40	2.90	4.20
55	>30	>30	>30	>30	>30
56	0.43	0.22	0.17	0.12	0.48
57	4.58	6.65	4.66	3.80	7.10
58	>30	>30	>30	>30	>30
59	12.5	12.1	7.13	14.7	10.4
60	11.7	16.2	5.24	18.6	20.3
61	17.0	16.4	5.70	28.4	19.3
62	>30	>30	8.3	0.52	7.20
64	3.65	2.80	0.82	0.52	7.20
65	>30	>30	8.3	>30	>30
doxorubicin	0.04	0.12	0.05	0.12	0.18

[a]Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer. Compounds were assayed in several separate batches.

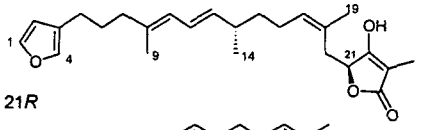
2. Cytotoxic pyrrolo- and furanoterpenoids from two sponges of *Sarcotragus* genus

Marine sponges of the order Dictyoceratida have frequently afforded a wide variety of linear sesterterpenes, many of which contain furanyl and tetronic acid termini. In our study on the cytotoxic compounds of two sponges of *Sarcotragus* genus (family Ircinidae, order Dictyoceratida), 29 new linear terpenoids, two known terpenoids (**4**, **6**), and three cyclitol derivatives (**32–34**) were isolated. The pyrrolosesterterpenes (**9–15**, **28–31**) were chemically unique incorporating a pyrrole ring in place of the furan ring. They might be biosynthesized by condensation of furanosesterterpene and amino acid derived unit. Unlike other common furanosesterterpenes, compounds **16–18**, **24**, **25**, **and 27** were carrying an oxidized furan ring similar to that found in manoalide. The gross structures of the compounds were elucidated by the aid of COSY, HMQC, and HMBC experiments while the absolute configuration of the tetronic acid moiety was proposed by comparison of the NMR and CD data of each diastereomeric pair.

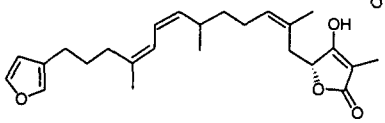
The isolated compounds were evaluated for cytotoxicity and showed a marginal to significant activity against a small panel of five human tumor cell lines (Table 2). Of the compounds tested, the derivatives with the tetronic acid function (**4–13**, **17**, **18**, **23**) exhibited higher potencies than the others (**19–22**, **24–26**, **30**, **31**), though the presence or absence of this moiety may not be the only determining factor as other tetronic acid containing compounds (**1–3**, **14–16**) also had lower potencies. Of the compounds, bisfuranosesterterpenes with conjugated tetronic acid function (**4–7**) showed the highest potency. Compound **5** (ircinin-1) was subjected to further evaluation of activity on cell cycle modulation in the SK-MEL-2 cell. Ircinin-1 was shown to arrest G1 phase and to

induce apoptosis by modulation of p21 expression and subsequent cellular process.

1



8 21*R*



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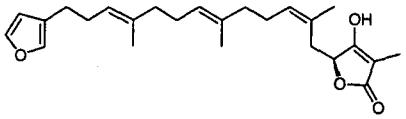

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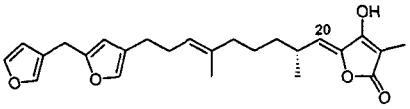

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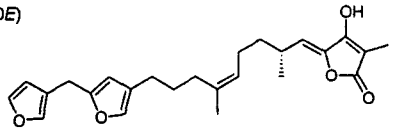
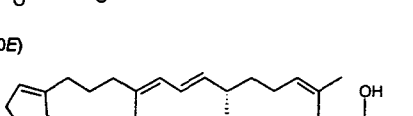
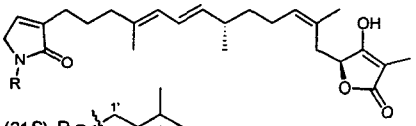


5 (20*E*)

6

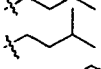


7 (20*E*)



9 (21*S*), R =

10 (21*R*), R =



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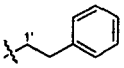

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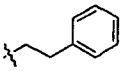

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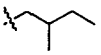

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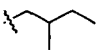
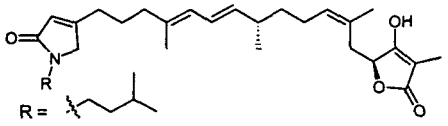


15 R =



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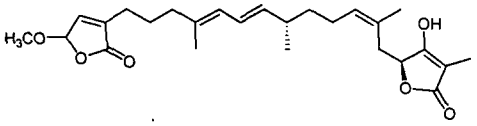

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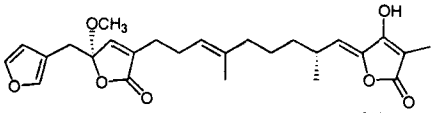

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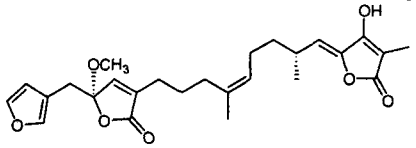

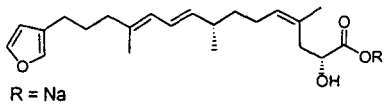

19 R = Na

20 R = CH_3

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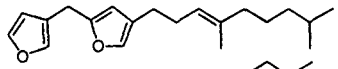

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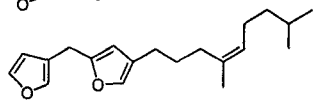

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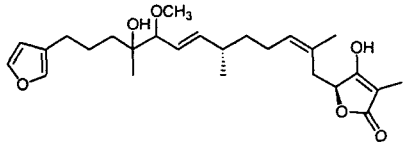

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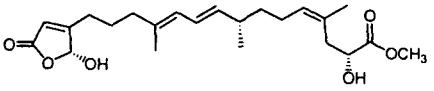

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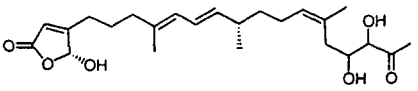

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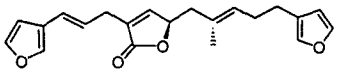

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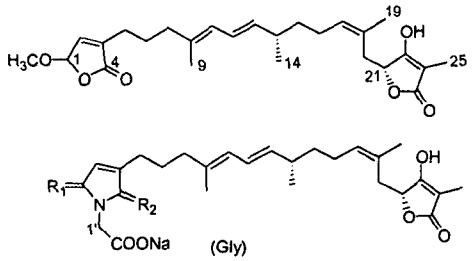
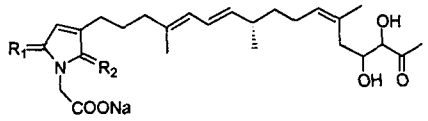


28 $R_1 = H_2$, $R_2 = O$

29 $R_1 = O$, $R_2 = H_2$



30 $R_1 = H_2$, $R_2 = O$

31 $R_1 = O$, $R_2 = H_2$

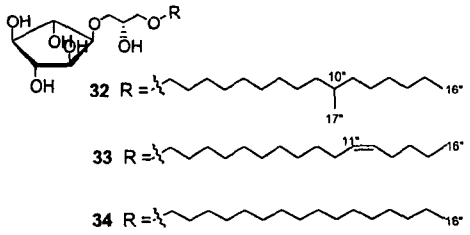


Table 2. Cytotoxicity of the Compounds against Human Solid Tumor Cell Lines[a]

compound	A549	SK-OV-3	SK-MEL-	XF498	HCT15
1	29.7	22.1	>30	24.8	27.2
2	10.1	11.3	7.8	8.9	9.0
3	16.9	26.8	16.3	20.4	27.5
4	3.7	6.6	9.0	5.4	6.9
5	5.0	9.4	10.2	6.5	9.8
6	3.8	5.9	5.8	3.7	4.7
7	3.8	6.2	8.3	5.0	7.3
8	12.3	9.6	5.6	9.8	6.5
9	15.1	5.3	4.1	5.5	5.0
10	4.3	4.0	3.4	3.9	3.8
11	6.3	6.7	4.3	5.2	4.9
12	16.8	13.1	4.8	10.5	5.4
13	19.0	6.9	3.8	5.4	5.3
14	27.1	26.8	15.9	25.2	22.3
15	>30	25.9	13.2	>30	21.6
16	24.1	15.2	7.6	20.1	10.5
17	9.1	10.0	5.1	7.6	7.3
18	6.7	6.8	5.9	6.3	6.1
19	24.8	23.3	25.7	25.9	23.7
20	18.1	10.0	7.8	24.3	8.7
21,22[b]	>30	26.8	6.2	29.6	23.9
23	9.0	8.4	9.9	11.3	10.1
cisplatin	0.72	1.23	2.26	1.03	1.10
doxorubicin	0.02	0.16	0.02	0.13	0.06
24	19.4	>30.0	10.9	>30.0	21.7
25	6.8	14.9	3.0	11.5	4.6
26	7.9	32.3	4.5	11.8	4.2
27	>30.0	>30.0	10.9	>30.0	33.0
28	>30.0	>30.0	>30.0	>30.0	>30.0
29	>30.0	>30.0	>30.0	>30.0	>30.0
30,31	>30.0	>30.0	>30.0	>30.0	>30.0
32	>10.0	9.5	>10.0	9.8	9.4
33	11.5	5.1	7.9	7.5	10.5
34	4.8	5.3	4.6	4.3	5.3
doxorubicin	0.04	0.15	0.06	0.19	0.24

[a]Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer. [b]Obtained as an inseparable mixture.