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Design, synthesis and anticancer activity of novel dihydrobenzofuro[4,5-*b*][1,8]naphthyridin-6-one derivatives

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ABSTRACT

On the basis of the chemical structures of psorospermin with a xanthone template and acronycine derivatives with an acridone template, *rac*-**1** and *rac*-**2** constructed on an 1,2-dihydrobenzofuro[4,5-*b*][1,8]naphthyridin-6(11*H*)-one scaffold were designed and synthesized as potential anticancer agents. Their anticancer activities were evaluated against five human cancer cell lines. *Rac*-**2** showed similar anticancer activity to doxorubicin and *rac*-**1** exhibited even higher anticancer activity against LNCaP (IC_{50} = 0.14 μM), DU145 (IC_{50} = 0.15 μM), PC3 (IC_{50} = 0.30 μM) and MCF-7 (IC_{50} = 0.26 μM) cancer lines than doxorubicin and *rac*-**2**. Also, *rac*-**1** revealed very potent anticancer activity (IC_{50} = 0.15 μM) against MCF-7/ADR cell (doxorubicin-resistant breast cancer cell) lines and induced G2/M phase arrest of the cell cycle in MCF-7/ADR cells.

Psorospermin (Fig. 1), a natural product isolated from the tropical plant *Psorospermum febrifugum*, consists of a xanthone scaffold[1] (Fig. 2) and was reported to exhibit potent anticancer activity against several human and murine cancer cell lines in vitro and in vivo.[2,3] Acronycine, an alkaloid first isolated from *Acronychia baueri* Schott (*Rutaceae*) consists of an acridone scaffold and showed a broad spectrum of activity against a variety of solid tumors including sarcoma, myeloma, carcinoma and melanoma.[4,5] However, the poor clinical potency of this alkaloid prompted medicinal chemists to synthesize numerous acronycine derivatives with higher clinical anticancer activity and less cytotoxicity to normal cells than acronycine. Among them, S23905-1 (Fig. 1) exhibited much higher anticancer activities against murine P388 leukemia and colon 38 adenocarcinoma in vivo as well as L1210 cell lines in vitro than acronycine itself.[6]

On the other hand, the discovery of acronycine epoxide (Fig. 1) isolated from several *Sarcomelicope* species has supported that acronycine might exert its anticancer in vivo activities through biotransformation to the corresponding epoxide, acronycine epoxide.[7] According to the previous studies, psorospermin is a selective DNA alkylating agent and its oxirane ring acts as an electrophile reacting with the N-7 of guanine base[8] and S23906-1 is also an alkylating agent reacting with the N-2 of guanine base of DNA.[9]

Recently, we synthesized novel oxiranyl and thiiranyl phenolic derivatives constructed on various templates such as naphthalene, 9*H*-carbazole, benzophenone and anthraquinone as potential alkylating agents and the compounds exhibited better to a little less potent anticancer activities against MDA-MB-231, LNCaP, Du145 and PC3 cell lines than doxorubicin.[10] This result implies that introduction of electrophiles such as epoxypropyl and thioepoxypropyl groups into a flat scaffold may provide an ability of DNA alkylation. The flat scaffold seems to contribute to insertion and stacking between the base pairs of the DNA double helix.

On the basis of the hypothesis that the structural combination of two compounds possessing similar biological profiles could afford synergistically increased pharmacological activity, replacement of the xanthone scaffold of psorospermin with an acridone scaffold (Fig. 2) was first considered for the target structure. However, the existence of protonated nitrogen atoms on the structure of DNA alkylators in biological pH might increase the binding affinity through the electrostatic attraction between the protonated nitrogen atom of the DNA alkylators and the phosphate anions of the DNA skeleton. Therefore, insertion of an additional nitrogen atom to the acridone template is expected to enhance binding affinity between the target compounds and the duplex DNA, which in turn may improve selectivity of the target compounds toward DNA as alkylators. On the basis of these findings and hypothesis, we have finally designed and synthesized novel dihydrobenzof

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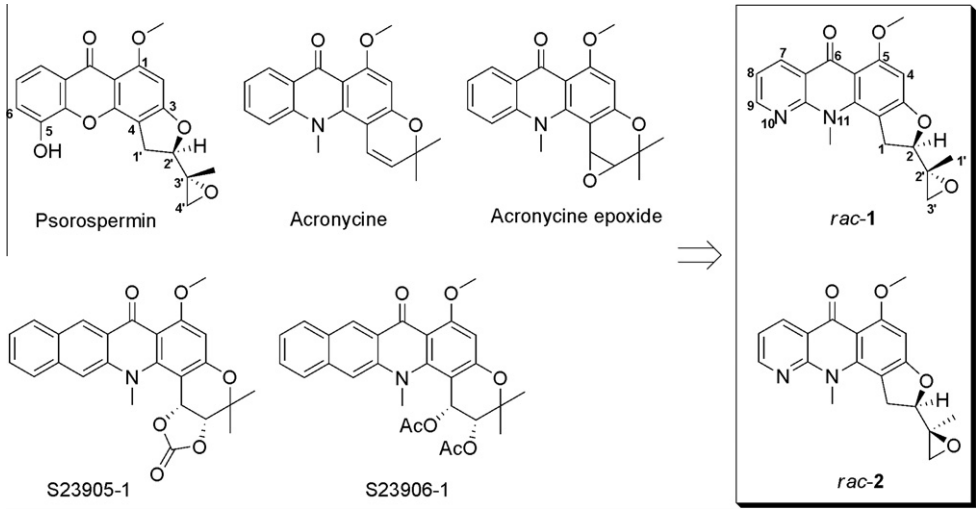


Figure 1. The rationale for the design of the target compounds, *rac*-**1** and *rac*-**2**.

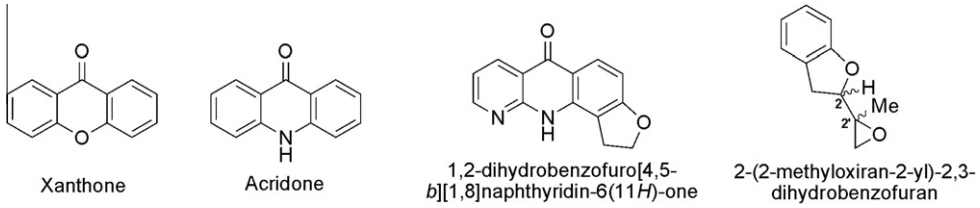
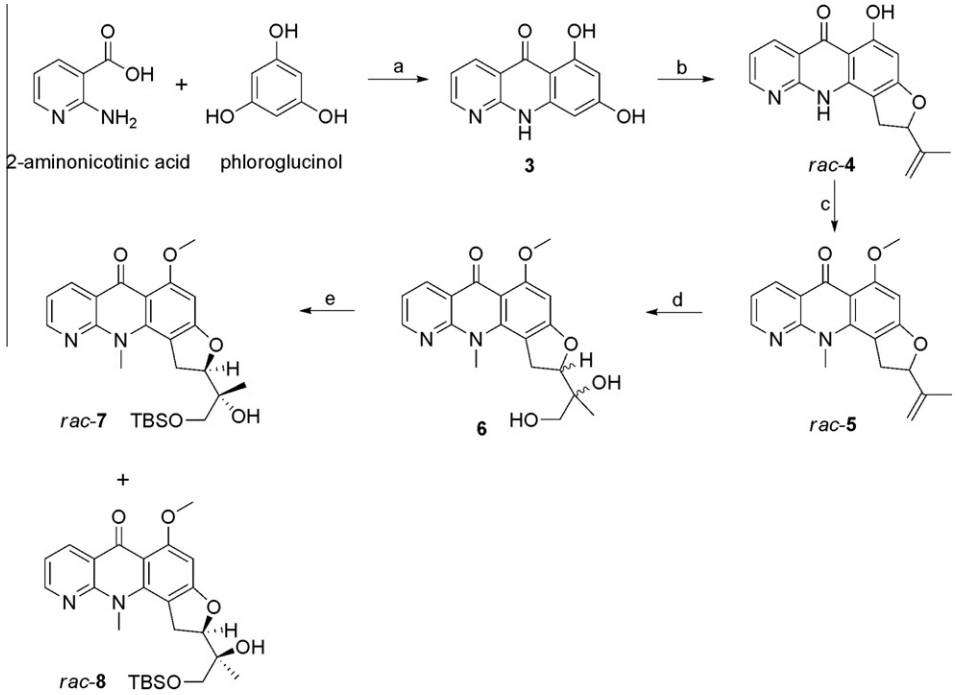


Figure 2. Structures of xanthone, acridone and benzofuran analogs.

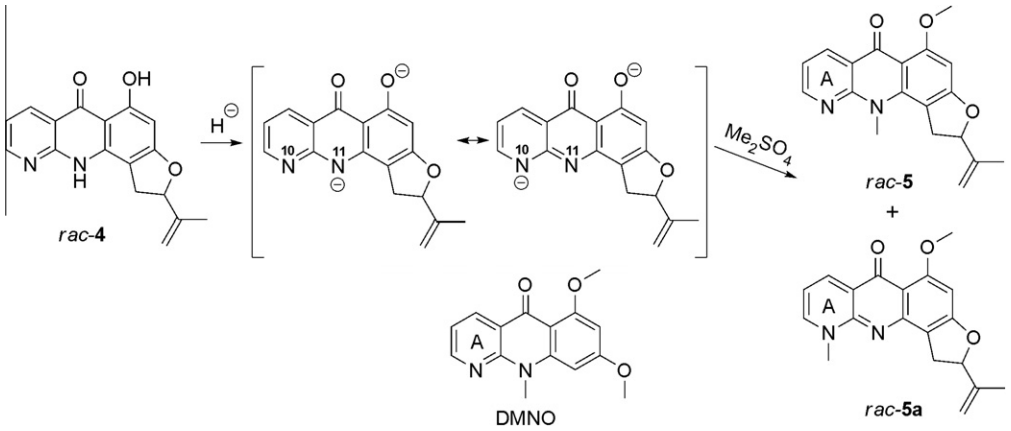
uro[4,5-*b*][1,8]naphthyridin-6-one derivatives (Figs. 1 and 2), *rac*-**1** and *rac*-**2** bearing an oxiranyl substituent as a potential electrophile, with anticipation that the plausible protonated nitrogen atoms at 10- and/or 11-positions of *rac*-**1** and -**2** in biological pH might increase the binding affinity with the DNA helix. Also, anticancer activities of *rac*-**1** and *rac*-**2** were evaluated against human prostate cancer cell lines (LNCaP, DU145 and PC3) and breast cancer cell lines (MCF-7 and doxorubicin-resistant MCF-7/ADR). Moreover, we examined the effect of *rac*-**1** and *rac*-**2** on regulation of cell cycles against MCF-7/ADR cell lines.

As shown in Scheme 1, compound **3** with a benzo[*b*][1,8]naphthyridinone template was obtained in 38% yield by heating 2-aminonicotinic acid and phloroglucinol without a solvent in a sealed tube at 230 °C. Treatment of **3** with dibromoisoprene and sodium hydride in methanol formed fused dihydrofuran, in 42% recovered yield, as a racemate form.[11] It is worthy to note that the process of *N*,*O*-dimethylation using dimethyl sulfate in the presence of NaH afforded two isomeric compounds, *rac*-**5**[12] and *rac*-**5a**[13] in an 1:1.3 ratio, separable on silica gel column chromatography (Scheme 2). Treatment of *rac*-**4** with sodium hydride might give a nitrogen anion capable of acting as an ambident nucleophile at N-10 and N-11 positions. Therefore, it is possible for two constitutional isomers, *rac*-**5** and *rac*-**5a** to be generated. Whereas the A-ring protons of *rac*-**5a** were observed at δ 8.68 (1H), 7.77 (1H) and 6.54 (1H) ppm, respectively, those of *rac*-**5** were observed at δ 8.64 (2H) and 7.07 (1H) ppm. Protons of A-ring of *rac*-**5a** resonated at more up-field region than those of *rac*-**5**, implying that the A-ring of *rac*-**5a** is not aromatic and *rac*-**5** is our desired compound. In addition, chemical shifts of the A-ring protons of *rac*-**4** were similar to those of *rac*-**5** (δ 8.75, 8.49 and 7.32 ppm), suggesting that *rac*-**5** preserves a desired 1,2-dihydrobenzofuro[4,5-*b*][1,8]naphthyridin-6(11*H*)-one template. Chemical shift pattern (δ 8.65, 8.53 and 7.08 ppm) of the A-ring protons of DMNO[14] (Scheme 2) also confirms that *rac*-**5** is the desired compound. 1,2-Dihydroxylation of *rac*-**5** with OsO_4 in the presence of NMO serving as a co-oxidant furnished four stereoisomers (two diastereomeric racemates) inseparable on silica gel column chromatography. A temporary protection of the primary hydroxyl group with a TBS group allowed the diasteromeric mixture to be separated on silica gel column chromatography, giving *rac*-**7** and *rac*-**8** in 26 and 20% yields, respectively, from *rac*-**5**. The relative stereochemistry of the obtained racemates was determined from that of the corresponding final compounds, *rac*-**1** and *rac*-**2**. Synthesis of the target compounds, *rac*-**1** and *rac*-**2** from *rac*-**7** and *rac*-**8** is depicted in Scheme 3. Fluoride anion from TBAF gently removed the TBS group of each racemate to afford the corresponding diol compounds, in which only the primary hydroxyl groups were regioselectively mesylated by treatment with mesyl chloride in pyridine to give *rac*-**9** and *rac*-**10**, respectively. Finally, epoxidation reactions were conducted using K_2CO_3 in the presence of 18-Crown-6 to generate the target compounds, *rac*-**1** and *rac*-**2** in 61 and 62% yields, respectively, from *rac*-**7** and *rac*-**8**, respectively.[15] Relative stereochemistry of *rac*-**1** and *rac*-**2** was assigned on the basis of information from the literature[16] reported by Cassady and his co-workers. According to the literature, (2*R*,2′*R*)-compound of 2-(2-methyloxiran-2-yl)-2,3-dihydrobenzofuran (Fig. 2) showed bigger difference in ^{1}H NMR chemical shifts between hydrogens of the 3′-methylene than that of (2*R*,2′*S*)-compound (0.24 vs 0.11 ppm). The differences in *rac*-**1** and *rac*-**2** were 0.26 and 0.10 ppm, respectively, indicating that *rac*-**1**[17] is (2*R**,2′*R**)-compound and *rac*-**2**[18] is (2*R**,2′*S**)-compound.

Anticancer activity[19] of *rac*-**1** and *rac*-**2** was evaluated against LNCaP, DU145, PC3, MCF-7/ADR and MCF-7 cancer cell lines after 24 and 48 h incubation, respectively (Table 1). Interestingly, the final diasteroisomeric compounds, *rac*-**1** and *rac*-**2** exhibited the differences in anticancer activity against several cancer cell lines.



Scheme 1. Synthesis of *rac*-**7** and *rac*-**8** with a dihydrobenzofuro[4,5-*b*][1,8]naphthyridinone scaffold. Reagents and conditions: (a) neat, 230 °C, 38% yield; (b) dibromoisoprene, NaH, MeOH, rt, 21% yield (recovered yield: 42%); (c) Me_2SO_4, NaH, DMF, rt, 25% yield for *rac*-**5**, 32% yield for *rac*-**5a** (See Scheme 2); (d) OsO_4, NMO, acetone/H_2O (4:1), rt; (e) TBSCl, imidazole, DMAP, DMF, 75 °C, 26% yield (two-steps yield) for *rac*-**7**, 20% yield (two-steps yield) for *rac*-**8** from *rac*-**5**.

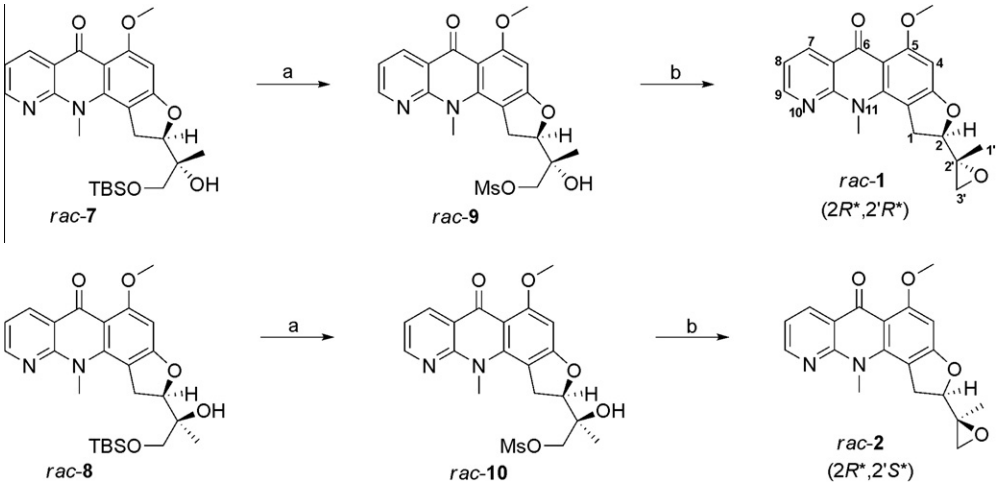


Scheme 2. Plausible mechanism of formation of *rac*-**5** and *rac*-**5a**.

Rac-**2** showed 1.5- to 15-fold more potent anticancer activity than doxorubicin used a positive control against most cancer cell lines after 48 h incubation except DU145 and PC3 cell lines and its IC_{50} values were in the range of 0.84–2.15 μM. It is notable that the anticancer activity ratio of *rac*-2 to doxorubicin after 24 h incubation is much better than that after 48 h incubation. Anticancer activity of *rac*-**2** against LNCaP, DU145, MCF-7/ADR and MCF-7 cell lines were 2- to 3-fold higher than that against PC3 cell lines. *Rac*-**1** displayed much more potent anticancer activity than *rac*-**2** and doxorubicin and its IC_{50} values revealed submicromolar concentrations in all cancer cell lines tested. Especially, it is notable that *rac*-**1** exhibited significant anticancer activity against doxorubicin-resistant breast cancer cell lines (MCF-7/ADR). Anticancer activities of *rac*-**1** were 4- to 7-fold more potent than those of *rac*-**2**, implying that (2*R**,2′*R**)-stereochemistry is better for anticancer activity than (2*R**,2′*S**)-stereochemistry. This relationship between stereochemistry and anticancer activity is consistent with the results obtained from psorospermin.[15]

Effects of *rac*-**1** and *rac*-**2** on the cell cycle progression were examined by Flow cytometry.[20] *Rac*-**1** (0.2 μM) induced an accumulation of cells in the G2/M phases of the cell cycle with a concomitant decrease in the percentage of MCF-7/ADR cells in the G1 phase. A total of 22% of the untreated MCF-7/ADR cells were in the G2/M phases, compared with 42% of cells cultured with 0.2 μM *rac*-**1**. However, *rac*-**2** (0.2 μM) did not induce G2/M phase arrest of the cell cycle in MCF-7/ADR cells, whereas slightly increased S phase of cell population in MCF-7/ADR cells (Table 2).

We have designed dihydrobenzofuro[4,5-*b*][1,8]naphthyridin-6-one as a novel potential anticancer scaffold and synthesized its derivatives with an oxiranyl functional group capable of serving an electrophile. *Rac*-**2** with (2*R**,2′*S**)-stereochemistry exhibited similar anticancer activity to doxorubicin against all cancer cell



Scheme 3. Synthesis of *rac*-**1** and *rac*-**2** with a dihydrobenzofuro[4,5-*b*][1,8]naphthyridin-6-one scaffold. Reagents and conditions: (a) (i) TBAF, THF, rt; (ii) MsCl, pyridine, 0 °C; (b) K_2CO_3, 18-Crown-6, acetone, 61% yield for *rac*-**1** from *rac*-**7**, 62% yield for *rac*-**2** from *rac*-**8**.

Table 1
Cytotoxicities of *rac*-**1** and *rac*-**2** against five human cancer cell lines after 24 and 48 h incubation[a]

Compd	IC_{50} (μM)									
	LNCaP[b]		DU145[c]		PC3[d]		MCF-7/ADR[e]		MCF-7[f]	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
rac-**1**	0.48 ±0.05	0.14 ±0.01	0.55 ±0.06	0.15 ±0.02	0.56 ±0.04	0.30 ±0.02	0.49 ±0.05	0.15 ±0.02	0.66 ±0.07	0.26 ±0.03
rac-**2**	2.48 ±0.15	0.86 ±0.05	2.36 ±0.02	0.84 ±0.05	4.67 ±0.38	2.15 ±0.31	2.21 ±0.17	0.92 ±0.08	1.84 ±0.11	1.02 ±0.12
Doxo[g]	7.42 ±0.48	1.66 ±0.03	6.24 ±0.32	0.58 ±0.06	4.10 ±0.35	1.22 ±0.06	>20	13.6 ±0.12	8.94 ±0.54	1.51 ±0.02

[a] Assay was done in triplicate.
[b] Prostate cancer cell lines (AR-positive).
[c] Prostate cancer cell lines.
[d] Prostate cancer cell lines (AR-negative).
[e] Breast cancer cell lines (adriamycin-resistant).
[f] Breast cancer cell lines (ER-positive).
[g] Doxorubicin.

Table 2
Cell cycle analysis of MCF-7/ADR cells by flow cytometry

Compounds Cell cycle	Percentage (%)							
	Control	*Rac*-**1** (μM)			*Rac*-**2** (μM)			Doxo 5 μM
		0.05	0.1	0.2	0.05	0.1	0.2	
G1	62.15 ±3.14	63.48 ±3.29	57.41 ±1.82	51.23 ±2.14	68.37 ±3.65	64.64 ±3.97	55.16 ±2.23	53.26 ±4.21
S	15.34 ±0.98	12.37 ±1.42	8.97 ±0.15	7.68 ±0.37*	11.16 ±0.62	13.60 ±0.97	16.55 ±0.85	21.92 ±1.81
G2/M	22.51 ±1.50	24.15 ±1.87	33.68 ±1.09	41.09 ±1.95*	20.47 ±1.43	21.76 ±0.63	28.30 ±0.97	24.62 ±0.96

The MCF-7/ADR cells were treated with the indicated concentrations of test compounds for 48 h. The cells stained with propidium iodine (PI) were subjected to flow cytometric analysis to determine the cell distributions at each phase of the cell cycle. The data is reported as the mean ± SEM of three independent experiments. A one-way ANOVA with Newman–Keuls post hoc test was used to determine statistical significance.
* *p*-value <0.05 was considered significant *p* value.

lines tested, whereas *rac*-**1** with (2*R**,2′*R**)-stereochemistry revealed higher anticancer activity than both *rac*-**2** and doxorubicin. *Rac*-**1** was found to induce the accumulation of cells in the G2/M phases of the cell cycle of MCF-7/ADR cells. Further studies on the inhibition mechanism of *rac*-**1** and *rac*-**2** in molecular levels and their possibility capable of acting as topoisomerase inhibitors are in progress in our laboratory and the results will be reported in due course. Further studies on the separation of the two enantiomers *rac*-**1** and *rac*-**2**, their cytotoxicity assays, their inhibition mechanism in molecular levels and their possibility capable of acting as topoisomerase inhibitors are in progress and the results will be reported in due course.

Acknowledgments

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12. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66–8.62 (m, 2H), 7.17 (dd, 1H, J = 4.5, 7.5 Hz), 6.35 (s, 1H), 5.24 (t, 1H, J = 9.0 Hz), 5.13 (s, 1H), 4.98 (s, 1H), 4.12 (s, 3H), 3.97 (s, 3H), 3.93 (dd, 1H, J = 9.5, 14.5 Hz), 3.55 (dd, 1H, J = 8.0, 14.5 Hz), 1.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.46, 166.11, 164.14, 152.10, 152.07, 143.38, 141.12, 136.57, 117.81, 113.78, 113.16, 109.64, 102.91, 89.49, 86.40, 56.53, 38.11, 35.62, 17.34.
13. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (dd, 1H, J = 2.0, 7.2 Hz), 7.77 (dd, 1H, J = 1.6, 6.4 Hz), 6.54 (t, 1H, J = 6.8 Hz), 6.24 (s, 1H), 5.28 (t, 1H, J = 8.8 Hz), 5.08 (s, 1H), 4.89 (s, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.43 (dd, 1H, J = 10.0, 15.2 Hz), 3.41 (dd, 1H, J = 8.0, 15.2 Hz), 1.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.57, 164.70, 163.77, 150.99, 149.69, 144.38, 143.24, 143.19, 141.49, 123.62, 112.04, 111.37, 108.45, 88.43, 87.74, 56.33, 40.52, 33.24, 17.43.
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17. *(2R*)-5-Methoxy-11-methyl-2-((2R*)-2-methyloxiran-2-yl)-1,2-dihydrobenzofuro[4,5-b][1,8]naphthyridin-6(11H)-one*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (m, 2H, 7-H, 9-H), 7.15 (dd, 1H, J = 7.6, 4.8 Hz, 8-H), 6.30 (s, 1H, 4-H), 4.73 (dd, 1H, J = 10.0, 7.6 Hz, 2-H), 4.12 (s, 3H, NCH_3), 3.94 (s, 3H, OCH_3), 3.80 (dd, 1H, J = 14.4, 10.0 Hz, 1-Ha), 3.64 (dd, 1H, J = 14.4, 7.6 Hz, 1-Hb), 2.97 (d, 1H, J = 4.8 Hz, 3′-Ha), 2.71 (d, 1H, J = 4.8 Hz, 3′-Hb), 1.42 (s, 3H, 1′-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.46, 165.88, 164.22, 152.15, 151.84, 144.66, 136.60, 119.27, 117.80, 109.82, 102.22, 89.48, 84.90, 57.78, 56.56, 51.24, 35.58, 35.23, 17.17; HRMS (FAB+) calcd for $C_{19}H_{19}N_2O_4$ $(M+H)^+$: 339.1345, found: 339.1351.
18. *(2R*)-5-Methoxy-11-methyl-2-((2S*)-2-methyloxiran-2-yl)-1,2-dihydrobenzofuro[4,5-b][1,8]naphthyridin-6(11H)-one*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (m, 2H, 7-H, 9-H), 7.15 (dd, 1H, J = 7.2, 4.8 Hz, 8-H), 6.30 (s, 1H, 4-H), 4.70 (dd, 1H, J = 9.6, 7.6 Hz, 2-H), 4.11 (s, 3H, NCH_3), 3.93 (s, 3H, OCH_3), 3.80 (dd, 1H, J = 14.8, 9.6 Hz, 1-Ha), 3.62 (dd, 1H, J = 14.8, 7.6 Hz, 1-Hb), 2.84 (d, 1H, J = 4.4 Hz, 3′-Ha), 2.74 (d, 1H, J = 4.4 Hz, 3′-Hb), 1.42 (s, 3H, 1′-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.47, 165.58, 164.17, 152.17, 151.81, 144.68, 136.58, 119.24, 117.91, 109.82, 102.56, 89.47, 85.00, 56.90, 56.56, 52.71, 35.59, 34.65, 16.81; HRMS (FAB+) calcd for $C_{19}H_{19}N_2O_4$ $(M+H)^+$: 339.1345, found: 339.1336.
19. *Cell culture and cytotoxicity test.* The human cancer cell lines were purchased from American Type Culture Collection (Rockville, MD) and maintained in a humidified atmosphere at 37 °C in 5% CO_2. The cells was grown in RPMI-1640 (Gibco, Rockville, MD) media containing 10% heat-inactivated fetal bovine serum (FBS), 1.25 mM HEPES and 1% penicillin/streptomycin. Cytotoxicity was determined by the MTT (Promega, Madison, WI) assay according to the manufacturer's protocol. Briefly, cells were seeded in 96-well microtiter plates at a density of 2×10^3 cells per well. After 48 h incubation, cells were treated with various concentrations of *rac*-1 and *rac*-2, cultured for for 24 and 48 h, respectively. At the end of the treatment period, 15 μL of the MTT (5 mg/mL) reagent was added to each well. After 4 h incubation at 37 °C, the supernatant was aspirated, and formazan crystals were dissolved in 100 μL DMSO at 37 °C for 10 min with gentle agitation. The absorbance per well was measured at 540 nm with a VERS Amax Microplate Reader (Molecular Devices Corp.). Assay was done in triplicate. The IC_{50} values were then determined for each drugs from a plot of log (drug concentration) versus percentage of loss of viability.
20. *Flow cytometry*. Cancer cells were exposed to *rac*-**1** and *rac*-**2** for 48 h. Total number of cells was collected, washed, and suspended in cold PBS. The cells were fixed in chilled 75% methanol and stained with propidium iodine (PI) solution (100 μg/mL RNase and 10 μg/mL PI in PBS). Data acquisition and analysis was performed using a flow cytometry system (Becton Dickinson, San Jose, CA).