Studies on the Chemical Transformations of Rotenoids. **6** Synthesis and Antitumor-Promoting Activity of [1]Benzofuro[2,3-*d*]pyridazines fused with 1,2,4-Triazole, 1,2,4-Triazine and 1,2,4-Triazepine

Shin-ichi Nagai*, Satoshi Takemoto, Taisei Ueda and Kanako Mizutani

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

Yasuhiro Uozumi

Institute for Molecular Sciences, Myodaiji, Okazaki 444-8585, Japan

Harukuni Tokuda

Department of Biochemistry, Kyoto Prefectural University of Medicine, Kawaramachi-dori, Hirokoji, Kamigyo-ku, Kyoto 602-0841, Japan

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[1]Benzofuro[2,3-*d*]pyridazines fused with 1,2,4-triazole (**6** and **7**), 1,2,4-triazine (**8**-**10**) and 1,2,4-triazepine (**12**) were prepared by the ring closure of 4-hydrazino-[1]benzofuro[2,3-*d*]pyridazine (**5**), derived from naturally occurring rotenone. Compounds (**1a** and **1b**) exhibited significant inhibitory activity against 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBA-EA) activation in Raji cells. In contrast, the fused [1]benzofuro[2,3-*d*]pyridazines except **6c** and **8** were quite inactive.

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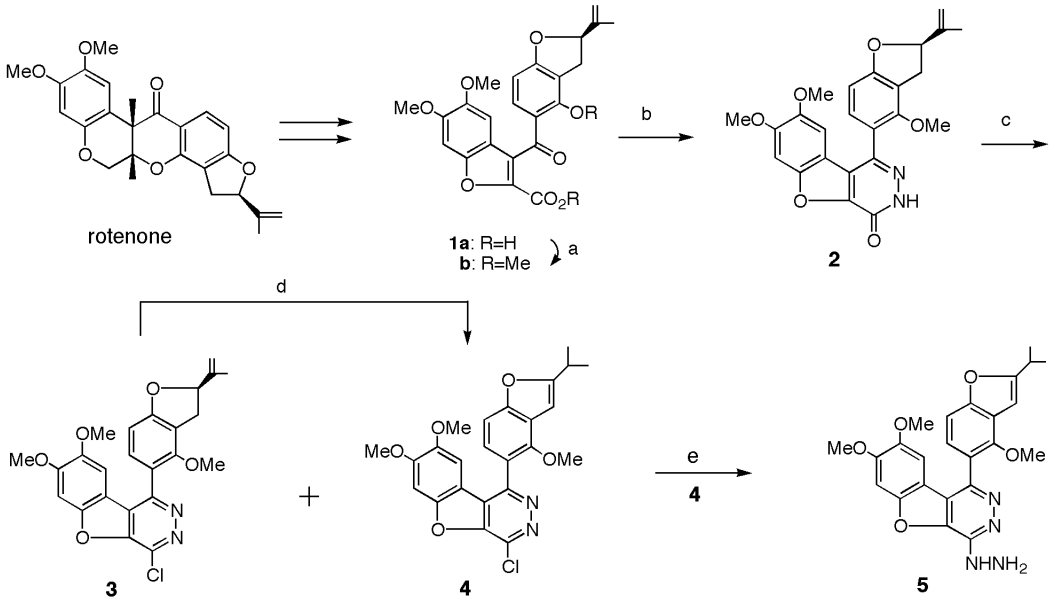
Rotenone is an abundant natural product, occurring in the roots of tropical plants belonging to *Legminosae* family and is known to be useful insecticides and nonpersistent in the enviroment. In a previous paper [1], we reported the synthesis and the cytotoxic activity against L 1210 and P 388 leukemia cells of pyridazinobenzofurans fused with imidazole and pyrimidine. Recently, Fang and Casida elucidated anticancer activity of rotenoids by examination of inhibitory activty of NADH ubiquinone oxidoreductase and phorbol ester-induced decarboxylase activities [2]. In continuation of our studies on the chemical transformations and evaluation of pharmacologically active rotenone derivatives, we now report the synthesis of [1]benzofuro[2,3-*d*]pyridazines fused with 1,2,4-triazole, 1,2,4-triazine and 1,2,4-triazepine and their inhibitory activity against 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) activation in Raji cells.

Heterocyclic hydrazines are excellent intermediate for construction of novel condensed heterocyclic ring systems.

Scheme 1

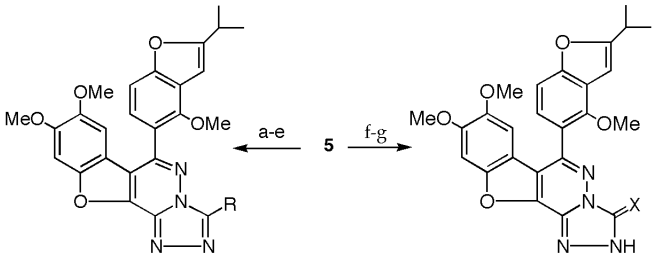


We then started with the preparation of 4-hydrazino-[1]benzofuro[2,3-*d*]pyridazine (**5**) as shown in Scheme 1.

[1]Benzofuro[2,3-*d*]pyridazin-4-one (**2**) prepared *via* methylation of rotenononic acid (**1a**) and subsequent condensation of **1b** with hydrazine hydrate, was boiled in phosphorous oxychloride, followed by dropwise addition of potassium hydroxide solution provided a mixture of 4-chloro[1]benzofuro[2,3-*d*]pyridazine (**3**) and the constitutional isomer (**4**) in 14 % and 74 % yields respectively. The formation of **4** was unambiguously rationalized by isomerization of the asymmetric carbon of **3** under strong acidic reaction conditions. Since isomerization of **3** was very difficult to prevent and separation of two isomers was rather troublesome, the minor chloro compound **3** was converted to **4** on treatment with concentrated sulfuric acid-glacial acetic acid. The hydrazino intermediate **5** was readily prepared by hydrazinolysis of **4** with hydrazine hydrate in 80 % yield. Synthesis of [1,2,4]-triazolo[1]benzofuro[2,3-*d*]pyridazines (**6** and **7**) was successfully carried out with employing appropriate condensing reagents as shown in Scheme 2.

Ring closure of **5** with triethyl orthoformate proceeded within 4 hours at 60° to give **6a**. Treatment of **5** with cyanogen bromide gave 3-amino compound **6b**. The presence of amino group was ascertained by ir and ^{1}H nmr spectra. Heating of **5** in boiling acetic acid or benzoyl chloride resulted in formation of 3-methyl or 3-phenyl-1,2,4-triazines (**6c** and **6d**) respectively. Ethoxycarbonyl derivative (**6e**) was obtained by reaction of **5** with ethyl

Scheme 2

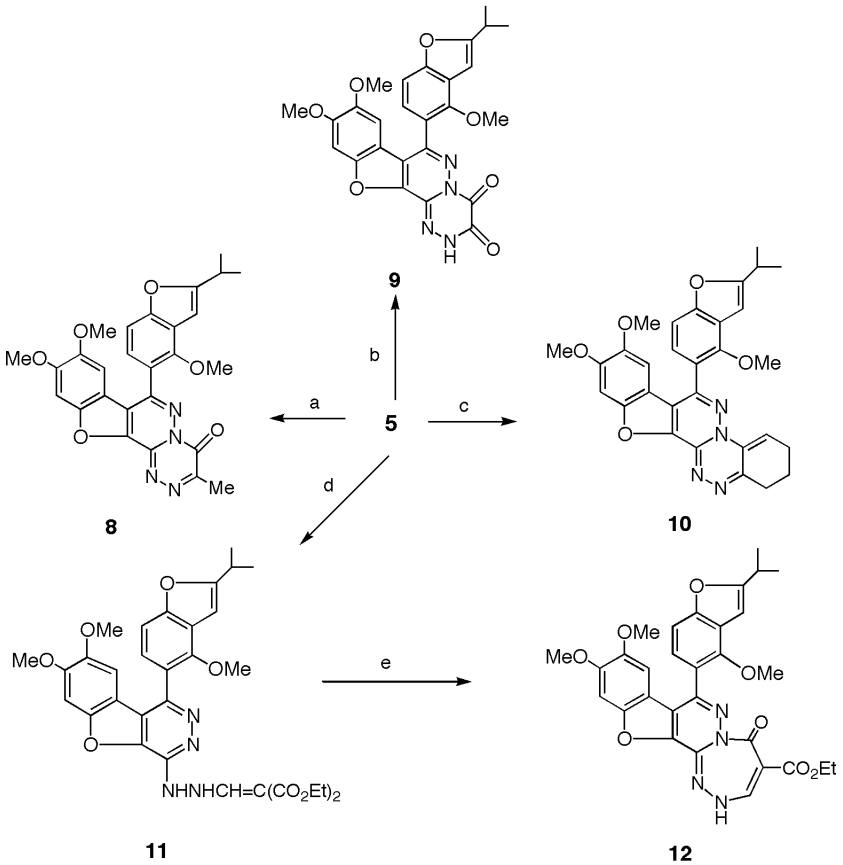


6a: R=H (a)
b: R=NH_2 (b)
c: R=CH_3 (c)
d: R=C_6H_5 (d)
e: R=CO_2Et (e)

7a: X=O (f)
b: X=S (g)

a, $CH(OEt)_3$; b, BrCN; c, CH_3CO_2H; d, C_6H_5COCl; e, $ClCOCO_2Et$; f, Im_2CO; g, CS_2

Scheme 3



a, CH_3COCO_2H; b, ClCOCOCl; c, 1,2-cyclohexanedione;
d, $EtOCH=C(CO_2Et)_2$; e, tert-butylbenzene, reflux

chlorooxoacetate. On heating of **5** with 1,1'-carbonyldiimidazole in toluene produced **7a** which was confirmed to exist as a keto form on the basis of ir and ^{1}H nmr spectra. Similar ring closure of **5** with carbon disulfide was achieved by heating in toluene to yield **7b**.

Compounds in which the six and seven-membered rings are annelated to the pyridazine ring, were prepared as shown in Scheme 3. Condensation of **5** with pyruvic acid in ethanol gave [1,2,4]triazino[1]benzofuro[2,3-*d*]pyridazin-4-one (**8**), ^{1}H nmr of which showed the methyl signal at δ 2.90. The 3,4-dione analog **9** was obtained when **5** was condensed with oxalyl chloride in pyridine. Cyclocondensation of **5** with 1,2-cyclohexanedione in boiling ethanol provided pentacyclic compound **10**. The structure of **10** was confirmed by ^{1}H nmr spectrum which showed a new signal at δ 5.97 attributable to cyclohexene proton. Condensation of **5** with diethyl ethoxymethylenemalonate, followed by heating of hydrazinomethylenemalonate (**11**) in *tert*-butylbenzene, gave 1,2,4-triazepino compound **12** although the isolated yield was low. The mass spectrum of **12** showed the molecular ion peak at m/z 572 and the presence of ethoxy carbonyl group was confirmed by ^{1}H nmr spectrum.

It is noteworthy that the fused [1]benzofuro[2,3-*d*]pyridazines (**6-10** and **11**) developed deep blue colors on thin layer chromatography (silica gel, 60, F_{254}, 0.25 mm, Merck) and were clearely distinguishable from **5**.

Table 1

Relative Ratio of Rotenoids (**1**) and Fused Pyridazinobenzofurans (**2-10** and **12**) Activation with Respect to Positive Control [a] Concentration (mol ratio/TPA)]

Compound	1000	500	100	10
	% to control (% viability)			
rotenone	19.2 (70)	37.8	78.3	100
1a	0.00 (80)	30.2	88.8	92.3
1b	6.90 (80)	33.7	72.5	97.3
2	21.9 (80)	39.9	76.1	100
3	28.4 (80)	48.7	82.8	100
4	29.1 (80)	45.5	84.5	100
5	33.2 (80)	48.8	88.4	100
6a	12.2 (80)	32.5	70.1	100
6b	26.8 (80)	40.8	79.4	100
6c	11.4 (80)	30.4	67.8	97.0
6d	20.5 (80)	37.5	75.2	100
6e	18.5 (80)	36.0	73.7	100
7a	15.7 (70)	86.9	72.3	100
7b	20.4 (80)	39.2	78.8	100
8	10.0 (80)	85.2	72.6	100
9	13.2 (80)	37.1	72.9	100
10	19.8 (80)	39.2	78.3	100
12	25.7 (70)	40.0	79.9	100

[a] Values are EBV-EA activation (%) ± s.d. s (± 5.0%) in the presence of test compound relative to the positive control (100). The activation was caused by TPA (32 pmol/mL). Values in parentheses represent the viability % of Raji cells measured through Trypan Blue staining, followed by counting of the surviving cells 48 hours after the concomitant treatment of the cells with TPA, *n*-butylate, and test compounds in a 0.25% phosphorous buffer solution (pH 7.2).

The inhibitory activity of EBV-EA activation (Table 1) was assayed using the previously reported method [3] which is known as an excellent *in vitro* screening. Rotenononic acid (**1a**) and methyl rotenononate (**1b**) exhibited strong inhibitory activities. Among the fused [1]benzofuro[2,3-*d*]pyridazines, compounds (**6c** and **8**) were slightly active whereas other compounds were ineffective on inhibitory activity. These results indicate that the presence of free hydroxy group may be essential for inhibitory activity. In contrast, the introduction of methyl groups on the triazole or triazine rings decreased the activity and the presence of amino groups led to disappearence of activity. Rotenone was not so effective as expected.

In conclusion, we have prepared the novel [1]benzofuro[2,3-*d*]pyridazines fused with 1,2,4-triazole, 1,2,4-triazine and 1,2,4-triazepine. Rotenoids (**1a-b**) showed antitumor-promoting activity.

EXPERIMENTAL

Melting points were determined using a Yanagimoto micro melting point apparatus and are uncorrected. The infrared spectra were measured with a JASCO IRA-2 spectrometer. The ^{1}H nmr spectra were recorded with a JEOL EX-270 and JEOL JMN-LA 400 spectrometers using tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL JMS-DX 300 spectrometer.

1-(2*R*-2-Methylethenyl-4-methoxy-2,3-dihydrobenzofuran-5-yl)-4-chloro-7,8-dimethoxy [1]benzofuro[2,3-*d*]pyridazine (**3**) and 1-(2-Isopropyl-4-methoxybenzofuran-5-yl)-4-chloro-7,8-dimethoxy[1]benzofuro[2,3-*d*]pyridazine (**4**).

A suspension of **2** [1] (0.62 g, 1.9 mmole) in phosphorous oxychloride (10 ml) was refluxed for 3 hours and evaporated to dryness. To a solution of the residual dark oil in dioxane (30 ml) was added dropwise 20 % potassium hydroxide solution (12 ml) during 1.5 hours. The mixture was extracted with chloroform and the solvent was distilled from the extract. The residue was chromatographed on silica gel (chloroform-ethly acetate). The first compound to be eluted was recrystallized from ethanol to give **4** as white crystalline powders, mp 166-168°, yield 0.48 g (74 %); ^{1}H nmr (deuteriochloroform): δ 1.40 (d, 6H, J=7, $(CH_3)_2$CH), 3.14 (sept, 1H, J=7, $(CH_3)_2CH$), 3.69 (s, 3H, 4'-OCH_3), 3.85 and 4.01 (each s, 6H, 7 and 8-OCH_3), 6.62 (s, 1H, 3'-H), 6.83 (s, 1H, 5-H), 7.25 (s, 1H, 8-H), 7.36 (d, 1H, J=8, 6'-H), 7.48 (d, 1H, J=8, 7'-H); ms: m/z 452 (M^+), 454 (M^++2).

Anal. Calcd. for $C_{24}H_{21}N_2ClO_5$: C, 63.65; H, 4.67; N, 6.19. Found: C, 63.49; H, 4.80; N, 6.28.

Further elution gave white powders. Recrystallization from methanol gave **3** as colorless needles, mp 177-179° (177-179.5° [1]), yield 0.09 g (14 %). ^{1}H nmr spectrum of **3** was identical with that of an authentic **3**.

Isomerization of **3** to **4**.

A mixture of **3** (0.06 g, 0.13 mmole) in concentrated sulfuric acid (0.1 ml) and glacial acetic acid (1.8 ml) was stirred at 90° for 5 minutes and poured onto ice-water. The precipitates were extracted with ethyl acetate. The solvent was distilled from the extract and the residue was recrystallized from ethanol to give

0.057 g (95 %) of white crystalline powders, whose spectral data were identical with those of authentic **4**.

1-(2-Isopropyl-4-methoxybenzofuran-5-yl)-4-hydrazino-7,8-dimethoxy[1]benzofuro[2,3-*d*]pyridazine (**5**).

A mixture of **4** (0.2 g, 0.44 mmole) and hydrazine hydrate (0.22 g, 4.4 mmoles) in ethanol (15 ml) was refluxed for 4 hours and evaporated to dryness. The residual viscous oil was chromatographed on silica gel (chloroform) to give light brown solids. Recrystallization from chloroform-hexane (1:1) gave light brown crystalline powders, mp 145-147°, yield 0.16 g (80 %); ^{1}H nmr (deuteriochloroform): δ 1.40 (d, 6H, J=7, $(CH_3)_2$CH), 3.15 (sept, 1H, J=7, $(CH_3)_2CH$), 3.69 (s, 3H, 4'-OCH_3), 3.82 and 4.01 (each s, 6H, 7 and 8-OCH_3), 6.26 (br s, 3H, $NHNH_2$), 6.61 (s, 1H, 3'-H), 6.83 (s, 1H, 5-H), 7.27 (s, 1H, 8-H), 7.36 (d, 1H, J=8, 6'-H), 7.46 (d, 1H, J=8, 7'-H); ms: m/z 448 (M^+).

Anal. Calcd. for $C_{24}H_{24}N_4O_5$: C, 64.28; H, 5.39; N, 12.49. Found: C, 64.39; H, 5.51; N, 12.58.

6-(2-Isopropyl-4-methoxybenzofuran-5-yl)-8,9-dimethoxy-[1,2,4]triazolo[3,4-*b*][1]benzofuro[2,3-*d*]pyridazine (**6a**).

A solution of **5** (0.03 g, 0.07 mmole) in triethyl orthoformate (2 g, 13.5 mmoles) was stirred at 60° for 4 hours and evaporated to dryness. The residue was recrystallized from ethanol to give light brown prisms, mp 208-210°, yield 0.022 g (73 %); ^{1}H nmr (deuteriochloroform): δ 1.41 (d, 6H, J=7, $(CH_3)_2$CH), 3.15 (sept, 1H, J=7, $(CH_3)_2CH$), 3.59 (s, 3H, 4'-OCH_3), 3.90 and 4.00 (each s, 6H, 8 and 9-OCH_3), 6.52 (s, 1H, 3'-H), 6.65 (s, 1H, 5-H), 7.30 (s, 1H, 8-H), 7.36 (d, 1H, J=8, 6'-H), 7.40 (d, 1H, J=8, 7'-H), 9.23 (s, 1H, 3-H); ms: m/z 458 (M^+).

Anal. Calcd. for $C_{25}H_{22}N_4O_5$: C, 65.49; H, 4.84; N, 12.22. Found: C, 65.58; H, 4.99; N, 12.29.

6-(2-Isopropyl-4-methoxybenzofuran-5-yl)-3-amino-8,9-dimethoxy[1,2,4]triazolo[3,4-*b*][1]benzofuro[2,3-*d*]pyridazine (**6b**).

A mixture of **5** (0.03 g, 0.07 mmole) and cyanogen bromide (0.08 g, 0.8 mmole) in 75 % methanol (3 ml) was stirred at room temperature for 7 hours and evaporated to dryness. The residue was dissolved in chloroform and washed with 5 % sodium bicarbonate solution. Chloroform was separated and distilled from the extract. The residue was recrystallized from ethanol-hexane (1:1) to give light orange crystalline powders, mp 152-154°, yield 0.025 g (78 %); ir (potassium bromide): 3345 and 3282 (NH_2) cm^{-1}; ^{1}H nmr (deuteriochlorform): δ 1.40 (d, 6H, J=7, $(CH_3)_2$CH), 3.14 (sept, 1H, J=7, $(CH_3)_2CH$), 3.69 (s, 3H, 4'-OCH_3), 3.85 and 4.01 (each s, 6H, 8 and 9-OCH_3), 4.97 (br s, 2H, NH_2), 6.62 (s, 1H, 3'-H), 6.83 (s, 1H, 5-H), 7.25 (s, 1H, 8-H), 7.36 (d, 1H, J=8, 6'-H), 7.48 (d, 1H, J=8, 7'-H); ms: m/z 473 (M^+).

Anal. Calcd. for $C_{25}H_{23}N_5O_5$; C, 63.42; H, 4.90; N, 14.79. Found: C, 63.29; H, 4.99; N, 14.70.

6-(2-Isopropyl-4-methoxybenzofuran-5-yl)-3-methyl-8,9-dimethoxy[1,2,4]triazolo[3,4-*b*][1]benzofuro[2,3-*d*]pyridazine (**6c**).

A solution of **5** (0.02 g, 0.04 mmole) in acetic acid (3 ml) was refluxed for 5 hours and evaporated to dryness. The residue was recrystallized from ethanol to give colorless plates, mp 216-218°, yield 0.014 g (67 %); ^{1}H nmr (deuteriochloroform): δ 1.41 (d, 6H, J=7, $(CH_3)_2$CH), 2.90 (s, 3H, 3-CH_3), 3.16 (sept, 1H, J=7, $(CH_3)_2CH$), 3.59 (s, 3H, 4'-OCH_3), 3.83 and 3.94 (each s, 6H, 8 and 9-OCH_3), 6.49 (s, 1H, 3'-H), 6.64 (s, 1H, 5-H), 7.28 (s, 1H, 8-H), 7.38 (d, 1H, J=8, 6'-H), 7.50 (d, 1H, J=8, 7'-H); ms: m/z 472 (M^+).

Anal. Calcd. for $C_{26}H_{24}N_4O_5$: C, 66.09, H, 5.12; N, 11.86. Found: C, 66.28; H, 5.29; N, 11.81.

6-(2-Isopropyl-4-methoxybenzofuran-5-yl)-3-phenyl-8,9-dimethoxy[1,2,4]triazolo[3,4-*b*][1]benzofuro[2,3-*d*]pyridazine (**6d**).

A solution of **5** (0.02 g, 0.04 mmole) in benzoyl chloride (1 g, 7.1 mmoles) was refluxed for 6 hours and evaporated to dryness. The residue was chromatographed on silica gel (chloroform) to give orange powders. Recrystallization from ethanol gave orange crystals, mp 226-227°, yield 0.01 g (42 %); ^{1}H nmr (deuteriochloroform): δ 1.42 (d, 6H, J=7, $(CH_3)_2$CH), 3.16 (sept, 1H, J=7, $(CH_3)_2CH$), 3.62 (s, 3H, 4'-OCH_3), 3.93 and 4.00 (each s, 6H, 8 and 9-OCH_3), 6.57(s, 1H, 3'-H), 6.66 (s, 1H, 5-H), 7.30 (s, 1H, 8-H), 7.50 (m, 5H, phenyl protons), 8.10 (d, 1H, J=8, 6'-H), 8.61 (d, 1H, J=8, 7'-H); ms: m/z 534 (M^+).

Anal. Calcd. for $C_{31}H_{26}N_4O_5$; C, 69.65; H, 4.90; N, 10.48. Found: C, 69.77; H, 5.08; N, 10.39.

6-(2-Isopropyl-4-methoxybenzofuran-5-yl)-3-ethoxycarbonyl-8,9-dimethoxy[1,2,4]triazolo[3,4-*b*][1]benzofuro[2,3-*d*]-pyridazine (**6e**).

A mixture of **5** (0.02 g, 0.04 mmole) and ethyl chlorooxoacetate (0.03 g, 0.22 mmole) in pyridine (5 ml) was stirred at room temperature for 4 hours, then at reflux for 3 hours and evaporated to dryness. The residue was recrystallized from ethanol to give light brown prisms, mp 195-197°, yield 0.01 g (42 %); ^{1}H nmr (deuteriochloroform): δ 1.40 (d, 6H, J=7, $(CH_3)_2$CH), 1.51 (t, 3H, J=8, CH_2CH_3), 3.16 (sept, 1H, J=7, $(CH_3)_2CH$), 3.69 (s, 3H, 4'-OCH_3), 3.84 and 4.00 (each s, 6H, 8 and 9-OCH_3), 4.60 (q, 2H, J=8, CH_2CH_3), 6.60 (s, 1H, 3'-H), 6.73 (s, 1H, 5-H), 7.27 (s, 1H, 8-H), 7.37 (d, 1H, J=8, 6'-H), 7.47 (d, 1H, J=8, 7'-H) ; ms: m/z 530 (M^+).

Anal. Calcd. for $C_{28}H_{26}N_4O_7$: C, 63.39; H, 4.94; N, 10.56. Found: C, 63.30; H, 5.00; N, 10.68.

6-(2-Isopropyl-4-methoxybenzofuran-5-yl)-8,9-dimethoxy-2,3-dihydro[1,2,4]triazolo[3,4-*b*][1]benzofuro[2,3-*d*]pyridazin-3-one (**7a**).

A suspension of **5** (0.03 g, 0.07 mmole) and 1,1'-carbonyldiimidazole (0.015 g, 0.09 mmole) in toluene (5 ml) was refluxed for 2.5 hours and evaporated to dryness. The residue was recrystallized from ethanol to give yellow prisms, mp>270°, yield 0.025 g (79 %); ir (potassium bromide): 3135 (NH), 1720 (C=O) cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.40 (d, 6H, J=7, $(CH_3)_2$CH), 3.14 (sept, 1H, J=7, $(CH_3)_2CH$), 3.60 (s, 3H, 4'-OCH_3), 3.92 and 3.98 (each s, 6H, 8 and 9-OCH_3), 6.44 (s, 1H, 3'-H), 6.60 (s, 1H, 5-H), 7.11 (s, 1H, 8-H), 7.30 (d, 1H, J=8, 6'-H), 7.39 (d, 1H, J=8, 7'-H), 7.71 (br s, 1H, NH); ms: m/z 474 (M^+).

Anal. Calcd. for $C_{25}H_{22}N_4O_6$: C, 63.29; H, 4.67; N, 11.81. Found. C, 63.20; H, 4.80; N, 11.82.

6-(2-Isopropyl-4-methoxybenzofuran-5-yl)-8,9-dimethoxy-2,3-dihydro[1,2,4]triazolo[3,4-*b*][1]benzofuro[2,3-*d*]pyridazine-3-thione (**7b**).

A mixture of **5** (0.03 g, 0.07 mmole) and carbon disulfide (0.5 g, 6.6 mmoles) in pyridine (2 ml) was refluxed for 3 hours and evaporated to dryness. The residue was recrystallized from

ethanol-hexane (1:1) to give light brown crystalline powders, mp>270°, yield 0.024 g (73 %); ir (potassium bromide): 3118 (NH) cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.40 (d, 6H, J=7, $(CH_3)_2CH$), 3.14 (sept, 1H, J=7, $(CH_3)_2CH$), 3.59 (s, 3H, 4'-OCH_3), 3.97 and 4.00 (each s, 6H, 8 and 9-OCH_3), 6.51 (s, 1H, 3'-H), 6.61 (s, 1H, 5-H), 7.25 (s, 1H, 8-H), 7.44 (d, 1H, J=8, 6'-H), 7.46 (d, 1H, J=8, 7'-H), 9.57 (br s, 1H, NH); ms: m/z 490 (M^+).

Anal. Calcd. for $C_{25}H_{22}N_4O_5S$: C, 61.21; H, 4.52; N, 11.42. Found: C, 61.06; H, 4.39; N, 11.51.

7-(2-Isopropyl-4-methoxybenzofuran-5-yl)-3-methyl-9,10-dimethoxy-4*H*-[1,2,4]triazino[3,4-*b*][1]benzofuro[2,3-*d*]pyridazin-4-one (**8**).

A mixture of **5** (0.03 g, 0.07 mmole) and pyruvic acid (0.02 g, 0.2 mmole) in ethanol (2 ml) was refluxed for 6 hours and evaporated to dryness. The residue was recrystallized from ethanol to give light yellow crystalline powders, mp 178-180°, yield 0.03 g (91 %); ir (potassium bromide): 1630 (CO) cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.41 (d, 6H, J=7, $(CH_3)_2CH$), 2.90 (s, 3H, 3-CH_3), 3.15 (sept, 1H, J=7, $(CH_3)_2CH$), 3.60 (s, 3H, 4'-OCH_3), 3.94 and 4.00 (each s, 6H, 9 and 10-OCH_3), 6.59 (s, 1H, 3'-H), 6.80 (s, 1H, 5-H), 7.24 (s, 1H, 8-H), 7.32 (d, 1H, J=8, 6'-H), 7.37 (d, 1H, J=8, 7'-H); ms: m/z 500 (M^+).

Anal. Calcd. for $C_{27}H_{24}N_4O_6$: C, 64.79; H, 4.83; N, 11.19. Found: C, 64.87; H, 4.95; N, 11.08.

7-(2-Isopropyl-4-methoxybenzofuran-5-yl)-9,10-dimethoxy-2,3-dihydro-4*H*-[1,2,4]triazino[3,4-*b*][1]benzofuro[2,3-*d*]pyridazine-3,4-dione (**9**).

To a solution of **5** (0.03 g, 0.07 mmole) in dry pyridine (1 ml) was added dropwise at 0° a solution of oxalyl chloride (0.08 g, 0.6 mmole) in pyridine (0.5 ml) by a syringe. After being stirred for 2 hours at 60° , the mixture was evaporated to dryness. The residue was mixed with water (5 ml) and extracted with chloroform. The solvent was distilled from the extract and the residue was chromatographed on silica gel (chloroform). Recrystallization from chloroform-hexane (1:1) gave yellow crystalline powders, mp 130-132°, yield 0.013 g, (39 %); ir (potassium bromide): 1625 (CO) cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.40 (d, 6H, J=7, $(CH_3)_2CH$), 3.15 (sept, 1H, J=7, $(CH_3)_2CH$), 3.69 (s, 3H, 4'-OCH_3), 3.94 and 4.00 (each s, 6H, 9 and 10-OCH_3), 6.42 (s, 1H, 3'-H), 6.83 (s, 1H, 5-H), 7.23 (s, 1H, 8-H), 7.36 (d, 1H, J=8, 6'-H), 7.48 (d, 1H, J=8, 7'-H); ms: m/z 502 (M^+).

Anal. Calcd. for $C_{26}H_{22}N_4O_7$: C, 62.15; H, 4.41; N, 11.15. Found: C, 62.30; H, 4.52; N, 11.18.

12-(2-Isopropyl-4-methoxybenzofuran-5-yl)-9,10-dimethoxy-3,4-dihydro-2*H*-benzotriazino[3,4-*b*][1]benzofuro[2,3-*d*]-pyridazine (**10**).

A mixture of **5** (0.025 g, 0.06 mmole) and 1,2-cyclohexanedione (0.015 g, 0.13 mmole) in ethanol (2 ml) was refluxed for 3 hours and evaporated to dryness. The residue was chromatographed on silica gel (chloroform) and recrystallized from hexane to give light brown crystalline powders, mp 108-110°, yield 0.021 g (77 %); ^1H nmr (deuteriochloroform); δ 1.40 (d, 6H, J=7, $(CH_3)_2CH$), 2.37, 2.72 and 3.17 (each m, 6H, $CH_2CH_2CH_2$), 3.13 (sept, 1H, J=7, $(CH_3)_2CH$), 3.66 (s, 3H, 4'-OCH_3), 3.92 and 3.96 (each s, 6H, 9 and 10-OCH_3), 5.97 (t, 1H, J=6, cyclohexene proton), 6.54 (s, 1H, 3'-H), 6.61 (s, 1H, 5-H), 7.20 (s, 1H, 8-H), 7.34 (d, 1H, J=8, 6'-H), 7.52 (d, 1H, J=8, 7'-H); ms: m/z 524 (M^+).

Anal. Calcd. for $C_{30}H_{28}N_4O_5$: C, 68.69; H, 5.38; N, 10.68. Found: C, 68.73; H, 5.39; N, 10.77.

Diethyl [1-(2-Isopropyl-4-methoxybenzofuran-5-yl)-7,8-dimethoxy[1]benzofuro[2,3-*d*]pyridazine-4-yl]hydrazinemethylenemalonate (**11**).

A mixture of **5** (0.03 g, 0.07 mmole) and diethyl ethoxymethylenemalonate (0.02 g, 0.09 mmole) in ethanol (2 ml) was refluxed for 1 hour and evaporated to dryness. The residue was chromatographed on silica gel (chloroform) and recrystallized from chloroform-hexane (1:1) to give light yellow crystalline powders, mp 111-114°, yield 0.018 g (59 %); ^1H nmr (deuteriochloroform): δ 1.39-1.41 (m, 12H, $(CH_3)_2CH$ and 2 x OCH_2CH_3)), 3.14 (sept, 1H, J=7, $(CH_3)_2CH$), 3.69 (s, 3H, 4'-OCH_3), 3.85 and 4.01 (each s, 6H, 7 and 8-OCH_3), 4.24 and 4.33 (each q, 4H, J=8, 2 x OCH_2CH_3), 6.62 (s, 1H, 3'-H), 6.83 (s, 1H, 5-H), 7.25 (s, 1H, 8-H), 7.36 (d, 1H, J=8, 6'-H), 7.48 (d, 1H, J=8, 7'-H); ms: m/z 618 (M^+).

Anal. Calcd. for $C_{32}H_{34}N_4O_9$: C, 62.13; H, 5.54; N, 9.06. Found: C, 62.31; H, 5.59; N, 9.14.

4-Ethoxycarbonyl-8-(2-isopropyl-4-methoxybenzofuran-5-yl)-10,11-dimethoxy-2*H*,5*H*-[1,2,4]triazepino[3,4-*b*][1]benzofuro[2,3-*d*]pyridazin-5-one (**12**).

A solution of **11** (0.03 g, 0.05 mmole) in *tert*-butylbenzene (2 ml) was refluxed for 1 hour and evaporated to dryness. The residue was recrystallized from ethanol to give orange crystalline powders, mp 121-123°, yield 0.01 g (36 %); ^1H nmr (deuteriochloroform): δ 1.40 (m, 9H, $(CH_3)_2CH$ and OCH_2CH_3)), 3.16 (sept, 1H, J=7, $(CH_3)_2CH$), 3.59 (s, 3H, 4'-OCH_3), 3.86 and 3.93 (each s, 6H, 10 and 11-OCH_3), 4.39 (q, 2H, J=8, OCH_2CH_3), 6.64 (s, 1H, 3'-H), 6.76 (s, 1H, 5-H), 7.23 (s, 1H, 8-H), 7.36 (d, 1H, J=8, 6'-H), 7.39 (br s, 1H, 3-H), 7.46 (d, 1H, J=8, 7'-H); ms: m/z 572 (M^+).

Anal. Calcd. for $C_{30}H_{28}N_4O_8$: C, 62.93; H, 4.93; N, 9.79. Found: C, 63.02; H, 4.90; N, 9.63.

In Vitro EBV-EA Activation Experiments. The inhibition of EBV-EA activation was assayed using the same method described previously [3].

REFERENCES AND NOTES

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