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Original paper

Imidazo[2,1-*b*]benzothiazoles 3: syntheses and immunosuppressive activities of 2-(*m*-acyloxyphenyl)imidazo[2,1-*b*]benzothiazoles

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Summary — A series of acyl derivatives of 2-(*m*-hydroxyphenyl)imidazo[2,1-*b*]benzothiazole **2**, a selective immunosuppressive agent for delayed type hypersensitivity (DTH), was prepared and tested for its suppressive activity of DTH in mice after oral administration. Six compounds **3e, f, k, n, p** and **q** were found to be more potent immunosuppressive agents than **2** but they showed no effect on humoral immunity as in the case of **2.**

Résumé — **Imidazo[2,1-*b*]benzothiazoles 3: synthèse et activité immunosuppressive de (*m*-acyloxyphényl)-2 imidazo[2,1-*b*]-benzothiazoles.** *Une série de dérivés acylés de (*m*-hydroxyphényl)imidazo[2,1-*b*]benzothiazole 2, agents immuno-dépressifs sélectifs pour l'hypersensibilité retardée (DTH), a été préparée et testée sur des souris après administration orale pour leur activité dépressive sur la DTH. Six composés* **3e**, ***f***, ***k***, ***n***, ***p*** *et* ***q*** *se sont révélés être des agents immunodépressifs plus actifs que 2 et n'ont eu aucun effet sur l'immunité humorale comme dans le cas du dérivé* **2.**

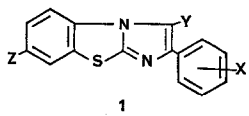
imidazo[2,1-*b*]benzothiazoles / immunosuppressive activity / delayed type hypersensitivity (DTH)

Introduction

We have previously reported [1] that some 2-phenylimidazo[2,1-*b*]benzothiazoles **1** show significant suppression of delayed type hypersensitivity (DTH) without inhibition of humoral immunity in mice, when administered orally, and that 2-(*m*-hydroxyphenyl)imidazo[2,1-*b*]benzothiazole **2** is the most active compound among them.

In a further study to find more active compounds, we focused on the modification of the hydroxy group of **2** and found some acyl derivatives of **2** to be more potent than **2** in suppressing DTH response.

In this paper, we describe the synthesis and immunological activity of 2-(*m*-acyloxyphenyl)imidazo[2,1-*b*]benzothiazoles **3**.



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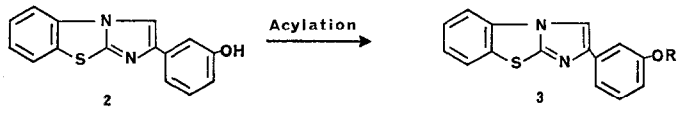


2 3

Fig. 1.

Chemistry

All the 2-(*m*-acyloxyphenyl)imidazo[2,1-*b*]benzothiazoles **3** were prepared by acylation of **2** as shown in Schemes 1—3.

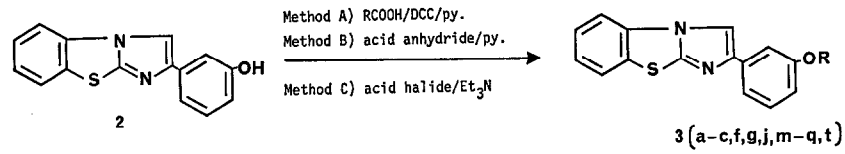
Treatment of **2** with the carboxylic acids in the presence of dicyclohexyl carbodiimide (DCC) in pyridine afforded **3n—q, t** (Method A). Treatment of **2** with the acid anhydrides in pyridine yielded **3a, b, f, g** and **j** (Method B) and with the acid halides in the presence of triethyl amine in tetrahydrofuran gave **3c** and **m** (Method C) (Scheme 1).

Several compounds, **3d, e, h, i, k** and **l**, which have the carboxy moiety in the acyl group were obtained as shown in Scheme 2.

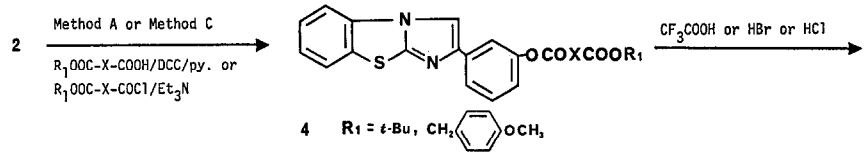
Treatment of **2** with the half esters (in Method A) or with the acid halides of the half esters (in Method C) afforded the asymmetrical diesters **4**, which were converted into **3d, e, h, i, k** and **l** by removing the protecting group R_1.

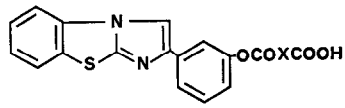
In this method, the selection of the protecting group R_1 was very important, because the alkaline hydrolysis of **4** would result in reversion to the starting compound **2**. The intermediates **4** have a sulfur atom in their structure, so the hydrogenating cleavage was difficult. With this in mind, we selected *tert*-butyl and *p*-methoxybenzyl groups as the protecting group R_1, which are easily removed by acidic hydrolysis (Method D).

The amino acyl derivatives **3r** and **s** were also obtained in a manner similar to that described in Method D by protecting the amino function with *tert*-butyloxycarbonyl group which is also easily removed by acidic conditions (Scheme 3).

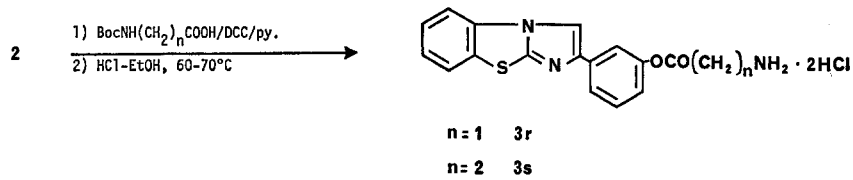


Scheme 1.





Scheme 2.



Scheme 3.

Biological Results and Discussion

The effects of new compounds on DTH (cell-mediated immunity) induced by picryl chloride in mice were determined by oral administration at doses of 25 and 200 mg/kg and the results, together with comparative data for **2**, are summarized in Table I.

Some hemiester derivatives, the hemimalonate **3e**, hemisuccinate **3f** and hemiglutarate **3g**, were more potent than **2** and the rank order of potency was **2** < **3g** < **3f** < **3e**. These results indicated that the decrease in spacer between two carbonyl groups would yield compounds more potent than **2**. However, the hemioxalate **3d**, which lacks a methylene group between two carbonyl groups, showed no activity. The introduction of the methyl **3h** or methoxy **3i** group at the carbon atom in the methylene group of **3e**, which showed the most potent activity, resulted in a decrease of activity. Compound **3j**, in which a middle methylene group of **3g** is replaced by an oxygen atom showed no activity. The hemifumarate **3k** was more potent than **2** but its *cis*-isomer (hemimaleate) **3l** was less active than **3k**.

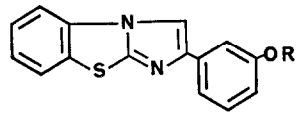
Some of the asymmetrical diesters **3m**, **n**, **p** and **q** showed activity and **3n**, **p** and **q** were more potent than **2**. In contrast to the results for hemiesters (**3k** and **3l**), the *cis*-isomer **3q** was as potent as the *trans*-isomer **3p**.

In contrast to the results obtained with the hemiester and asymmetrical diester, the amino and alkyl acyl derivatives **3r**—**t** and **3a**—**c** had lower levels of activity (**3a**, **c**, **r** and **s**) or showed no improvement in activity (**3t**) and the propionyl derivative **3b** enhanced the DTH response.

None of the compounds, at any of the doses tested, showed significant effects on IgE and hemagglutinating antibody formation in mice.

In summary, a series of 2-(*m*-acyloxyphenyl)imidazo-[2,1-*b*]benzothiazoles were investigated for their suppressive activity on the DTH response following oral administration and 6 compounds **3e**, **f**, **k**, **n**, **p** and **q** were more potent than **2** and showed no effect on humoral immunity.

Table I. Chemical characteristics and effects on the DTH response of **3**.



No.	R	mp °C	Method[a]	Yield (%)	Formula	Anal.	Recryst.[b]	Dose (mg/kg)	Increase in ear thickness, 1/100mm±SE	Inhib. (%)
2	H							control	6.2±0.8	
								25	5.3±0.8	14.5
								200	2.8±0.9[e]	54.8[e]
3a	$COCH_3$	101—102	B	51	$C_{17}H_{12}N_2O_2S$	CHNS	A	control	5.3±0.5	
								50	4.2±1.1	20.0
								400	4.2±0.7	20.0
3b	$COCH_2CH_3$	119—121	B	74	$C_{18}H_{14}N_2O_2S$	CHNS	A	control	7.6±0.7	
								12.5	6.2±0.9	18.4
								100	4.4±0.7[c]	42.1[c]
3c	COC_5H_{11}	oil	C	63	$C_{21}H_{20}N_2O_2S$	CHNS	F	control	7.6±0.7	
								25	4.6±1.8	39.4
								200	5.3±0.8	30.2
3d	COCOOH	165—167	D	32	$C_{17}H_{10}N_2O_4S$	HNS:C[d]	B	control	4.7±0.6	
								25	3.2±0.7	31.9
								200	3.7±0.8	21.3
3e	$COCH_2COOH$	159—161	D	49	$C_{18}H_{12}N_2O_4S$	CHNS	C	control	6.6±0.9	
								12.5	3.1±0.9[e]	53.0[e]
								100	1.4±0.9[e]	78.8[e]
3f	$CO(CH_2)_2COOH$	138—139	B	68	$C_{19}H_{14}N_2O_4S$	CNHS	B	control	7.6±0.7	
								25	4.8±1.1	36.8
								200	3.0±0.9[e]	60.5[e]
3g	$CO(CH_2)_3COOH$	168—171	B	77	$C_{20}H_{16}N_2O_4S$	CHNS	D	control	6.6±0.9	
								25	5.3±0.7	19.7
								200	2.8±0.6[e]	57.6[e]
3h	$COCH(CH_3)COOH$	143—146	D	66	$C_{19}H_{14}N_2O_4S$ HCl	CHNSCl	B	control	6.0±0.4	
								25	4.7±0.4	21.7
								200	3.2±1.0[e]	46.7[e]
3i	$COCH(OCH_3)COOH$	110—113	D	59	$C_{19}H_{14}N_2O_5S$	CHNS	E	control	4.7±0.6	
								25	2.6±1.0	44.1
								200	3.8±1.1	19.1
3j	$COCH_2OCH_2COOH$	185—190	B	70	$C_{19}H_{14}N_2O_5S$	CHN	B	control	4.5±0.6	
								25	5.6±1.0	−24.4
								200	3.3±1.0	20.7
3k	COCH=CHCOOH *trans*	247—250	D	53	$C_{19}H_{12}N_2O_4S$	CHNS	C	control	8.8±1.1	
								25	2.8±1.3[c]	68.2[c]
								200	1.9±0.7[e]	88.6[e]
3l	COCH=CHCOOH *cis*	250—253	D	52	$C_{19}H_{12}N_2O_4S$ HCl	CHNSCl	B	control	6.0±0.4	
								25	3.6±1.3[c]	40.0[c]
								200	5.1±0.9	15.0
3m	$COCOOC_2H_5$	120—121	C	50	$C_{19}H_{14}N_2O_4S$	CHNS	F	control	8.5±1.2	
								25	5.6±1.1	34.1
								200	4.0±0.8[e]	52.9[e]
3n	$CO(CH_2)_2COOC_2H_5$	70—74	A	60	$C_{21}H_{18}N_2O_4S$	CHNS	F	control	8.5±1.2	
								25	4.4±1.1[c]	48.2[c]
								200	3.0±0.3[e]	64.7[e]
3o	$CO(CH_2)_4COOC_2H_5$	90—92	A	35	$C_{23}H_{22}N_2O_4S$	CHNS	F	control	8.5±1.2	
								25	6.0±1.0	29.4
								200	6.0±0.6	29.4
3p	$COCH=CHCOOC_2H_5$ *trans*	130—130.5	A	63	$C_{21}H_{16}N_2O_4S$	CHNS	G	control	8.8±1.1	
								25	3.2±0.3[e]	63.6[e]
								200	3.5±0.9[e]	60.2[e]
3q	$COCH=CHCOOC_2H_5$ *cis*	126—128	A	50	$C_{21}H_{16}N_2O_4S$	CHNS	F	control	8.8±1.1	
								25	4.8±0.9[c]	45.5[c]
								200	1.3±0.4[e]	85.2[e]
3r	$COCH_2NH_2$	185—190	D	64	$C_{17}H_{13}N_3O_2S$ 2HCl H_2O	CHNS	E	control	4.7±0.6	
								25	5.7±1.2	−21.3
								200	3.2±0.6	31.9
3s	$CO(CH_2)_2NH_2$	157—160	D	48	$C_{18}H_{15}N_3O_2S$ 2HCl	CHNS	E	control	4.5±0.6	
								25	4.2±0.4	6.7
								200	5.5±1.1	−22.0
3t	$COCH_2N(CH_3)_2$	210—212	A	42	$C_{19}H_{17}N_3O_2S$ HCl	CHNCl	B	control	7.6±0.7	
								25	4.4±0.5[e]	42.1[e]
								200	4.0±1.3[c]	47.4[c]

[a]See the Experimental protocols.
[b]A = recrystallized from a mixture of toluene—*n*-hexane: B = washed with ether; C = washed with H_2O; D = washed with ethyl acetate; E = washed with 2-propanol; F = purified by column chromatography on silica gel with a mixture of toluene—ethyl acetate (9:1); G = recrystallized from toluene.
[c]Statistical significance of the data was estimated using Student's *t* test ($p < 0.05$).
[d]Analyzed for pyridinium salt, calcd: C: 60.35. Found: C: 59.42.
[e]Statistical significance of the data was estimated using Student's *t* test ($p < 0.01$).

Table II. IR and ^1H NMR data for **3**.

Compd.	$\nu_{max.}$ (cm^{-1})	δ (ppm): im = imidazole ph = phenyl
3a	1730(C=O)	$CDCl_3$ 2.29(3H,s,CH_3CO);6.8-7.8(8H,m,ph);7.92(1H,s,im)
3b	1750(C=O)	$CDCl_3$ 1.28(3H,t,CH_2CH_3);2.62(2H,q,CH_2CH_3);6.8-7.8(8H,m,ph);7.94(1H,s,im)
3c	1764(C=O)	$CDCl_3$ 0.94(3H,t,CH_3);1.1-1.6(4H,m);1.6-2.0(2H,m);2.58(2H,t,$COCH_2$);6.8-7.8(8H,m,ph);7.96(1H,s,im)
3d	1760(C=O)	DMSO-d_6 6.7-8.1(8H,m,ph);8.88(1H,s,im)
3e	1768,1714(C=O)	DMSO-d_6 3.72(2H,s,$COCH_2$CO);6.9-8.1(8H,m,ph);8.84(1H,s,im);13.0(1H,br.s,COOH)
3f	1740,1730(C=O)	DMSO-d_6 2.5-3.2(4H,m,CH_2CH_2);6.9-8.1(8H,m,ph);8.76(1H,s,im)
3g	1754,1712(C=O)	DMSO-d_6 1.88(2H,q,$CH_2CH_2CH_2$);2.42(2H,t,$COCH_2CH_2$);2.70(2H,t,$COCH_2CH_2$);7.0-8.1(8H,m,ph);8.84(1H,s,im)
3h	1722(C=O)	DMSO-d_6 1.46(3H,d,$CHCH_3$);3.86(1H,q,$CHCH_3$);7.0-8.2(8H,m,ph);9.08(1H,s,im)
3i	1740(C=O)	DMSO-d_6 3.50(3H,s,OCH_3);4.88(1H,s,$CHOCH_3$);6.8-8.2(8H,m,ph);8.82(1H,s,im)
3j	1776,1732(C=O)	DMSO-d_6 4.28(2H,s,CH_2);4.58(2H,s,CH_2);7.0-8.2(8H,m,ph);8.88(1H,s,im)
3k	1740,1729(C=O)	DMSO-d_6 6.98(2H,s,vinyl H);7.0-8.2(8H,m,ph);8.83(1H,s,im)
3l	1754,1722(C=O)	DMSO-d_6 7.0(2H,s,vinyl H);7.0-8.2(8H,m,ph);9.01(1H,s,im)
3m	1780,1752(C=O)	$CDCl_3$ 1.47(3H,t,CH_2CH_3);4.50(2H,q,CH_2CH_3);7.0-8.0(8H,m,ph);7.97(1H,s,im)
3n	1756,1736(C=O)	$CDCl_3$ 1.30(3H,t,CH_2CH_3);2.6-3.1(4H,m,CH_2CH_2);4.22(2H,q,CH_2CH_3);6.9-7.9(8H,m,ph);7.97(1H,s,im)
3o	1754,1726(C=O)	$CDCl_3$ 1.28(3H,t,CH_2CH_3);1.6-2.0(4H,m,CH_2CH_2);2.41,(2H,t,$COCH_2$);2.63(2H,t,$COCH_2$);4.18(2H,q,CH_2CH_3); 6.9-7.9(8H,m,ph);7.96(1H,s,im)
3p	1730(C=O)	$CDCl_3$ 1.34(3H,$COCH_2CH_3$);4.30(2H,q,$COCH_2CH_3$);7.02(2H,s,vinyl H);7.0-7.8(8H,m,ph);7.92(1H,s,im)
3q	1734(C=O)	$CDCl_3$ 1.36(3H,t,CH_2CH_3);4.34(2H,q,CH_2CH_3);7.08(2H,s,vinyl H);7.0-7.9(8H,m,ph);7.96(1H,s,im)
3r	3452(NH_2)1777(C=O)	DMSO-d_6 4.15(2H,br.d,CH_2NH_2);7.08-8.2(8H,m,ph);9.0(1H,s,im)
3s	1754(C=O)	DMSO-d_6 2.5-2.8(2H,m,$COCH_2$);2.8-3.2(2H,m,CH_2NH_2);6.7-8.2(8H,m,ph);8.94(1H,s,im)
3t	1760(C=O)	DMSO-d_6 3.0(6H,s,$N(CH_3)_2$);4.62(2H,s,CH_2N);7.0-8.2(8H,m,ph);8.95(1H,s,im)

Experimental protocols

Chemistry

Melting points were determined on a Yanaco MP-3 melting apparatus and are uncorrected. IR spectra were obtained on a Hitachi 215 infrared spectrometer. Proton NMR spectra were recorded on a JEOL FX-900 (90 MHz) spectrometer, using tetramethylsilane as an internal standard. Elemental analyses for C, H, N were made on a Yanagimoto CHN Coder MY-3 and those for S and Cl were performed on a Metrohm 660 Conductometer and Metrohm 672 Titroprocessor, respectively, by the Measurement Division in this research facility and values are within ±0.4% of the theoretical ones. In general, organic extract was dried over anhydrous $MgSO_4$ and solvent was evaporated under reduced pressure.

*Mono-*p*-methoxybenzyl malonate*
To a mixture of malonic acid (10.4 g, 100 mmol) and *p*-methoxybenzyl alcohol (6.9 g, 50 mmol) in ethyl acetate (100 ml) was added a mixture of DCC (10.3 g, 50 mmol) in ethyl acetate (20 ml) below 10°C and the reaction mixture was stirred overnight at room temperature. The insoluble materials were removed by filtration. The filtrate was washed with water and extracted with aqueous $NaHCO_3$ (50 ml, containing 4.2 g of $NaHCO_3$). The extract was washed with ethyl acetate and acidified with 4 N HCl (16.8 ml) and extracted with ethyl acetate. The extract was washed with water, dried and concentrated. The residue was recrystallized from toluene to yield 7.01 g (62.6%), mp: 78°C. ^1H NMR ($CDCl_3$): 3.38 (2H, s, CH_2); 3.70 (3H, s, OCH_3); 5.02 (2H, s, CH_2); 6.72 and 7.16 (4H, ABq, protons of benzene ring); 11.12 (1H, s, COOH).

Method A: 2-[m-*(3-ethoxycarbonylpropionyloxy)phenyl*]*imidazo*[*2,1*-b]*benzothiazole* ***3n***
To a mixture of **2** (10 g, 37.6 mmol) and mono-ethyl succinate (obtained from succinic anhydride and absolute ethanol [2]) (9 g, 61.6 mmol) in pyridine (100 ml) was added DCC (11 g, 53.4 mmol) at 15—25°C and the reaction mixture was stirred for 3 h at 20—25°C. The precipitate was removed by filtration and the filtrate was concentrated. Toluene (100 ml) was added to the residue and the toluene solution was washed with water (100 ml × 2), dried and concentrated. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate, 9/1) to yield 8.9 g (60%), mp: 70—74°C. Anal. $C_{21}H_{18}N_2O_4S$ (C, H, N, S). The spectroscopic data are reported in Table II.

Method B: 2-[m-*(4-carboxybutyryloxy)phenyl*]*imidazo*[*2,1*-b]*benzothiazole* ***3g***
A mixture of **2** (8 g, 30.1 mmol) and glutaric anhydride (10 g, 87.7 mmol) in pyridine (25 ml) was heated at 100—110°C for 30 min. The reaction mixture was concentrated and chilled 5% acetic acid—water (200 ml) was added and the mixture was extracted with ethyl acetate (200 ml). The extract was washed with water, dried and concentrated to 70 ml. The separated crystals were collected by filtration and dried to yield 8.8 g (77%), mp: 168—171°C. Anal. $C_{20}H_{16}N_2O_4S$ (C, H, N, S). The spectroscopic data are reported in Table II.

*Method C: 2-(*m-*ethoxalyloxyphenyl)imidazo*[*2,1*-b]*benzothiazole* ***3m***
To a mixture of **2** (4 g, 15 mmol) and triethylamine (1.52 g, 15 mmol) in tetrahydrofuran (THF) (50 ml) was added a mixture of ethoxalyl chloride (2.1 g, 15.4 mmol) in THF (10 ml) below −10°C and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated and toluene (200 ml) was added to the residue. The toluene solution was washed with water, dried and concentr-

ated. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate, 9/1) to yield 2.75 g (50%), mp: 120—121°C. Anal. $C_{19}H_{14}N_2O_4S$ (C, H, N, S). The spectroscopic data are reported in Table II.

*Method D: 2-(*m*-carboxyacetoxyphenyl)imidazo[2,1-*b*]benzothiazole* **3e**
To a mixture of 2-[*m*-(*p*-methoxyphenylmethoxycarbonylacetoxy)-phenyl]imidazo[2,1-*b*]benzothiazole (11.5 g, 43.2 mmol), which was obtained from **2** and mono-*p*-methoxybenzyl malonate by Method A, in dichloromethane (5 ml) were added anisole (4 ml) and trifluoroacetic acid (50 ml) at 3—15°C and the reaction mixture was stirred for 1 h at the same temperature and concentrated. THF (100 ml) was added to the residue. 2 N HCl—EtOH (20 ml) at 3—15°C was added to the THF solution. The precipitate was collected by filtration and dried to give **3e**·HCl (9.1 g), which was suspended in water and CH_3COONa (2.1 g) was added. After stirring for 30 min at 48—51°C, the crystals were collected by filtration and washed with ethyl acetate and water and dried to give **3e** (7.4 g, 48.7%), mp: 159—161°C. Anal. $C_{18}H_{12}N_2O_4S$ (C, H, N, S). The spectroscopic data are reported in Table II.

Pharmacology

Delayed-type hypersensitivity reaction induced by picryl chloride in mice
Male ICR mice (7 weeks old) were sensitized [3] by applying 0.1 ml of 7% picryl chloride solution in acetone to the shaved abdomen. After a 7 day sensitization period, the mice were challenged by painting the inside of each ear with 0.02 ml of 1% picryl chloride solution in olive oil. The ear thickness was measured with a dial thickness gauge before and 24 h after the challenge, and differences were calculated. In order to see the effects on the sensitization phase of DTH, test compounds were administered orally from day 0 to day 2 post-immunization.

Antibody formation in mice
Immunization was performed according to the method of Levine and Vaz [4]. Briefly, female BDF_1 mice weighing 17—19 g were injected i.p. with 10 μg of dinitrophenyl—ovalbumin (DNP—OA) and 1 mg of alum in 0.2 ml of saline and bled from the tail vein 7 and 14 days later. Equal volumes of individual serum samples from mice in the same group were pooled and used for the antibody assay. In order to see the effects on the primary antibody response, the test compounds were administered orally from day 0 to day 4 post-immunization. Five mice were used for each dose level.

The anti-DNP (IgE) and anti-DNP antibody (IgM and IgG) titers were determined by rat passive cutaneous anaphylaxis (PCA) reaction and phytohemagglutinin (PHA) tests, respectively. Detailed procedures of the tests were described previously [5].

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