Immunomodulatory Agents: Dioxothiadiazabicyclo[3.3.0]octanes and Their 2-Spiro Derivatives

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A series of 6,8-dioxo-3-thia-1,7-diazabicyclo[3.3.0]octanes and a series of 6,8-dioxo-3-thia-1,7-diazabicyclo[3.3.0]octane 2-spiro derivatives were synthesized from L-(−)-*R*-cysteine ethyl ester in two steps. The synthetic route involved condensation of the amino acid with an appropriate aldehyde or ketone, then a further condensation of the resultant ethyl thiazolidine-4-carboxylate with an isocyanate or an isothiocyanate. The proliferative response to human lymphocyte mitogen (phytohemagglutinin) was used as a primary screening assay for most of the thiadiazabicyclic compounds in comparison with levamisole. Furthermore, the most active compounds were tested for ability to release soluble receptors (sRIL-2) after mitogenic stimulation of T cells and for ability to activate macrophage oxidative metabolism measured by chemiluminescence. Most compounds were active in all three tests and some showed dose-dependent activity.

Keywords immunomodulatory agent; thiadiazabicyclic compound; dioxothiadiazabicyclo[3.3.0]octane; hydantoin; T lymphocyte; interleukin-2 receptor

Since the first report[1] that the anthelmintic levamisole was able to increase the immune response in experimental animals, other synthetic substances structurally related to levamisole has been synthesized and their immunological activity investigated. Various synthetic compounds structurally unrelated to levamisole also stimulated cell or humoral immunity,[2,3] including some amidic derivatives of *R*-(−)-thiazolidine-4-carboxylic acid.[4]

We focused our attention on bicyclic compounds with a bridgehead nitrogen atom. Some of them have been reported to exhibit significant activities on the central nervous system, such as psychotropic or anticonvulsant properties.[5-7] Furthermore a series of imidazo[1,5-*c*]thiazolidin-3-spiro-4′-piperidine derivatives with potential activity on the central nervous system[8] have been patented. In a previous paper[9] we reported immunomodulating properties of thiadiazabicyclic compounds containing a bridgehead nitrogen atom using a kinetic method based on CD2 receptor mobilization of human T lymphocyte. The introduction of a double bond in the ring system failed to improve the activity but increased the cytotoxicity.[10] Considering possible interactions between the brain and the immune system[11] we extended our investigations to 6,8-dioxo-3-thia-1,7-diazabicyclo[3.3.0]-octanes including in their ring system the hydantoin moiety. This bicyclic ring system is reported to contain the essential structural features necessary for immunomodulation.[12]

Chemistry

The dioxo-thiadiazabicyclic compounds **8**—**14** were obtained in good yields in two steps from the ethyl ester of L-(−)-*R*-cysteine as shown in Chart 1. When allowed to react in ethanol with an aldehyde or a ketone, the ester undergoes a condensation–cyclization reaction leading to the formation of a thiazolidinic heterocycle; the mechanism of this reaction has been discussed.[13] The use of formaldehyde as a carbonyl reagent led to the ethyl thiazolidine-4-carboxylate (**1**); the use of aliphatic ketones led to the 2-substituted ethyl thiazolidine-4-carboxylates

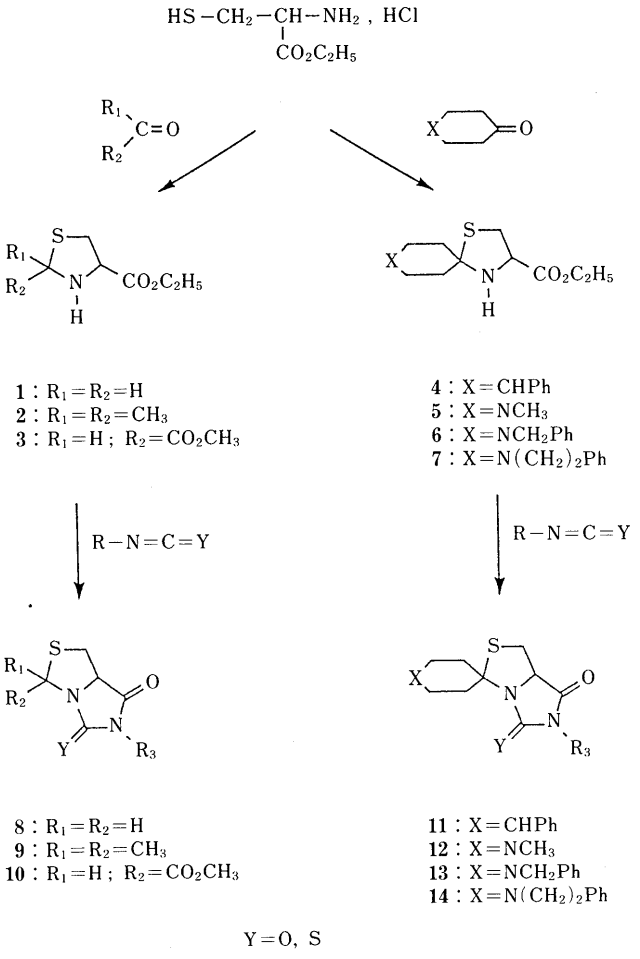
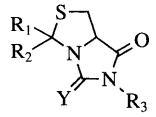


Chart 1. Synthetic Routes to Azabicyclic Compounds

TABLE I. Physical Constants of 6,8-Dioxo-3-thia-1,7-diazabicyclo[3.3.0]octanes

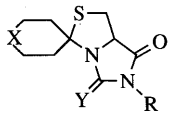


Compd. No.	R_1	R_2	R_3	Y	Yield (%)	mp (°C)	$[\alpha]_D^{20}$ (°)	Formula (M.W.)	Calcd (Found) C	H	N	S	X
8a	H	H	Ph	O	59	149	−70	$C_{11}H_{10}N_2O_2S$ (234)	56.41 (56.53	4.27 4.27	11.96 12.01	13.67 13.71)	
8b	H	H	4-ClPh	O	49	136	−51	$C_{11}H_9ClN_2O_2S$ (268.5)	49.16 (49.16	3.38 3.31	10.42 10.30	11.93 11.45	13.19 13.18)
8c	H	H	4-FPh	O	50	140	−68	$C_{11}H_9FN_2O_2S$ (252)	52.38 (52.25	3.57 3.61	11.11 11.10	12.68 12.54	7.53 7.25)
8d	H	H	4-CH_3Ph	O	52	163	−65	$C_{12}H_{12}N_2O_2S$ (248)	58.06 (57.98	4.87 4.84	11.29 11.34	12.90 12.76)	
8e	H	H	Ph	S	48	140	−69	$C_{11}H_{10}N_2OS_2$ (250)	52.78 (52.72	4.03 4.04	11.20 11.17	25.60 24.57)	
8f	H	H	4-CH_3OPh	O	61	130	−177	$C_{12}H_{12}N_2O_3S$ (264)	54.53 (54.32	4.58 4.62	10.61 10.31	12.12 12.07)	
8g	H	H	CH_3	O	67	175	−75	$C_6H_8N_2O_2S$ (172)	41.86 (41.80	4.68 4.59	16.28 16.34	18.60 18.49)	
8h	H	H	*n*-C_4H_9	O	53	160	−92	$C_9H_{14}N_2O_2S$ (214)	50.45 (50.64	6.59 5.20	13.07 12.99	14.96 14.93)	
8i	H	H	Cyclohexyl	O	75	110	−88	$C_{11}H_{16}N_2O_2S$ (240)	54.98 (54.65	6.71 7.12	11.66 11.75	13.34 13.28)	
8j	H	H	CH_2Ph	O	58	165	−91	$C_{12}H_{12}N_2O_2S$ (248)	58.06 (58.16	4.87 4.92	11.29 11.11	12.90 12.74)	
9a	CH_3	CH_3	Ph	O	60	151	−85	$C_{13}H_{14}N_2O_2S$ (262)	59.52 (59.32	5.38 5.55	10.69 10.48	12.21 12.31)	
9b	CH_3	CH_3	4-ClPh	O	60	144	−78	$C_{13}H_{13}ClN_2O_2S$ (296.5)	52.61 (52.71	4.42 4.32	9.44 9.28	10.79 10.56	11.97 11.87)
9c	CH_3	CH_3	4-FPh	O	55	138	−89	$C_{13}H_{13}FN_2O_2S$ (280)	55.71 (55.48	4.67 4.71	10.00 9.89	11.43 11.65	6.79 6.73)
9d	CH_3	CH_3	4-CH_3Ph	O	60	147	−77	$C_{14}H_{16}N_2O_2S$ (276)	60.85 (60.92	5.84 5.75	10.14 10.12	11.59 11.48)	
9e	CH_3	CH_3	Ph	S	50	141	−82	$C_{13}H_{14}N_2OS_2$ (278)	56.09 (55.68	5.07 5.15	10.06 10.21	23.03 23.60)	
10a	H	$COOCH_3$	Ph	O	75	110	−55	$C_{13}H_{12}N_2O_4S$ (292)	53.42 (53.58	4.14 4.19	9.58 9.46	10.96 11.01)	
10b	H	$COOCH_3$	4-ClPh	O	73	176	−44	$C_{13}H_{11}ClN_2O_4S$ (326.5)	47.78 (47.80	3.39 3.27	8.57 8.48	9.80 9.58	10.87 10.87)
10c	H	$COOCH_3$	4-CH_3Ph	O	70	114	−48	$C_{14}H_{14}N_2O_4S$ (306)	54.90 (55.04	4.61 4.65	9.15 9.11	10.46 10.37)	
10d	H	$COOCH_3$	Cyclohexyl	O	57	86	−51	$C_{13}H_{18}N_2O_4S$ (298)	52.33 (52.30	6.08 6.01	9.40 9.38	10.74 10.81)	
10e	H	$COOCH_3$	CH_3	O	77	128	−50	$C_8H_{10}N_2O_4S$ (230)	41.74 (41.70	4.38 4.31	12.17 11.97	13.92 14.99)	
10f	H	$COOCH_3$	*n*-C_4H_9	O	70	66	−52	$C_{11}H_{16}N_2O_4S$ (272)	48.53 (48.55	5.92 6.00	10.29 10.34	11.76 11.77)	

2 and **3**, whereas cyclanones such as cyclohexanone and piperidone gave rise to the 2-substituted spiro ethyl thiazolidine-4-carboxylates **4**—**7**. This first step is followed by condensation of the ethyl thiazolidine-4-carboxylates with an isocyanate or an isothiocyanate at room temperature in anhydrous pyridine to give the bicyclic derivatives **8**—**10** and the 2-spiro derivatives **11**—**14** (Tables I and II). All bicyclic compounds were crystallized from ethanol or purified by column chromatography.

Spectral data for the dioxothia-diazabicyclo-octanes **8**—**10** are summarized in Table III and those for the 2-substituted spiro derivatives in Table IV. They are consistent with the proposed structures. In the infrared spectra two large absorption bands attributable to the carbonyl groups of the sequencce >N–CO–NR_3–CO can be observed at 1700 and 1770 cm^{-1}. In the proton nuclear magnetic resonance spectra the signal of the H_a, $H_{a'}$ protons appears at 3.3 ppm as a multiplet. The signal of the H_b proton appears at 4.6—4.8 ppm as a quadruplet except for 2-unsubstituted compounds **8a**—**j** or geminal 2-dimethyl derivatives **9a**—**e**, where it is shifted to 5.0—5.2 ppm. Coupling constants for the ABX system of the H_a, $H_{a'}$, H_b protons in the thiazolidinic ring determined for **10a** were $J_{AB}=11.5$ Hz, $J_{AX}=10.8$ Hz, and $J_{BX}=6.8$ Hz, and for **11a**, **13a**, **14a** they were $J_{AB}=11$—11.5 Hz, $J_{AX}=9.7$—11.6 Hz, and $J_{BX}=6.4$—7.3 Hz. From the spectral data for compounds **10a**—**f**, the existence of two diastereoisomers for these compounds may be postulated. Furthermore, all compounds exhibited a specific optical rotation so that it may be inferred that the configuration

TABLE II. Physical Constants of 6,8-Dioxo-3-thia-1,7-diazabicyclo[3.3.0]octane-2-Spiro Derivatives



Compd. No.	R	X	Y	Yield (%)	mp (°C)	$[\alpha]_D^{20}$ (°)	Formula (M.W.)	Calcd (Found)				
								C	H	N	S	X
11a	Ph	CHPh	O	87	182	−65	$C_{22}H_{22}N_2O_2S$ (378)	69.82 (69.70	5.86 5.80	7.41 7.38	8.47 8.37)	
11b	4-ClPh	CHPh	O	78	222	−47	$C_{22}H_{21}ClN_2O_2S$ (412.6)	64.00 (64.07	5.13 5.13	6.79 6.79	7.76 7.66	8.61 8.70)
11c	4-FPh	CHPh	O	70	148	−55	$C_{22}H_{21}FN_2O_2S$ (396)	66.65 (66.48	5.34 5.15	7.07 7.01	8.08 7.99	4.80 4.75)
11d	4-CH_3Ph	CHPh	O	72	174	−54	$C_{23}H_{24}N_2O_2S$ (392)	70.38 (70.49	6.16 6.13	7.14 7.12	8.16 8.11)	
11e	Ph	CHPh	S	58	175	−61	$C_{22}H_{22}N_2OS_2$ (394)	66.96 (67.12	5.67 5.36	7.10 7.05	16.24 16.20)	
11f	4-ClPh	CHPh	S	55	212	−48	$C_{22}H_{21}ClN_2OS_2$ (428.9)	61.61 (61.54	4.93 4.87	6.53 6.38	14.94 14.87)	
12a	Ph	NCH_3	O	65	135	−66	$C_{16}H_{19}N_3O_2S$ (317)	60.55 (60.81	6.03 5.48	13.24 13.36	10.10 10.21)	
12b	4-ClPh	NCH_3	O	70	182	−56	$C_{16}H_{18}ClN_3O_2S$ (351.9)	54.62 (54.76	5.16 4.81	11.94 11.67	9.12 9.14	10.12 10.15)
12c	4-FPh	NCH_3	O	60	159	−72	$C_{16}H_{18}FN_3O_2S$ (335)	57.30 (57.36	5.41 5.11	12.53 12.51	9.56 9.62	5.66 5.75)
12d	4-CH_3Ph	NCH_3	O	60	153	−69	$C_{17}H_{21}N_3O_2S$ (331)	61.61 (61.75	6.39 6.01	12.68 12.38	9.67 9.56)	
12e	Ph	NCH_3	S	45	147	−67	$C_{16}H_{19}N_3OS_2$ (333)	57.63 (57.49	5.74 5.44	12.61 12.28	19.22 19.15)	
13a	Ph	NCH_2Ph	O	65	150	−68	$C_{22}H_{23}N_3O_2S$ (393)	67.15 (67.25	5.89 5.59	10.68 10.68	8.16 8.15)	
13b	4-ClPh	NCH_2Ph	O	66	170	−59	$C_{22}H_{22}ClN_3O_2S$ (427.9)	61.78 (61.72	5.15 5.17	9.82 9.82	7.49 7.48	8.28 8.30)
13c	4-FPh	NCH_2Ph	O	65	155	−59	$C_{22}H_{22}FN_3O_2S$ (411)	64.23 (64.18	5.39 5.32	10.22 10.15	7.79 7.81	4.62 4.36)
13d	4-CH_3Ph	NCH_2Ph	O	61	148	−63	$C_{23}H_{25}N_3O_2S$ (407)	67.79 (67.74	6.18 6.20	10.32 10.19	7.86 7.89)	
13e	Ph	NCH_2Ph	S	64	137	−66	$C_{22}H_{23}N_3OS_2$ (409)	64.52 (64.62	5.66 5.44	10.27 10.28	15.65 15.39)	
14a	Ph	$N(CH_2)_2Ph$	O	55	140	−68	$C_{23}H_{25}N_3O_2S$ (407)	67.79 (67.78	6.18 6.17	10.32 10.21	7.86 7.90)	
14b	4-ClPh	$N(CH_2)_2Ph$	O	53	152	−59	$C_{23}H_{24}ClN_3O_2S$ (441.9)	62.51 (62.51	5.47 5.39	9.51 9.49	7.25 7.31	8.02 8.01)
14c	4-FPh	$N(CH_2)Ph$	O	50	140	−70	$C_{23}H_{24}FN_3O_2S$ (425)	64.92 (64.88	5.69 5.38	9.88 9.64	7.53 7.36	4.47 4.32)
14d	4-CH_3Ph	$N(CH_2)_2Ph$	O	51	146	−71	$C_{24}H_{27}N_3O_2S$ (421)	68.38 (68.38	6.46 6.33	9.98 9.94	7.60 7.66)	
14e	Ph	$N(CH_2)_2Ph$	S	45	137	−69	$C_{23}H_{25}N_3OS_2$ (423)	65.22 (65.31	5.95 5.88	9.92 9.91	15.13 15.06)	

of the chiral carbon of the L-(−)-*R*-cysteine is preserved through the two steps of synthesis, giving the chiral C-5 in the cyclic system.

Biological Results and Discussion

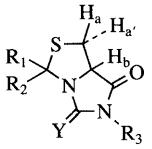
Phytohemagglutinin-induced lymphocyte proliferation[14,15] was used as a primary screening test to assess the immunomodulating activity of most of the synthesized thiadiazabicyclic compounds. The activity was compared with that of levamisole.

Activation of the immune system leads to increased metabolic activity of macrophages, which can be analyzed in terms of activation of chemiluminescence in response to stimulation by opsonized yeast stimulus (zymosan).[16,17] Thus, some of these compounds were tested for ability to enhance mouse peritoneal macrophage activation.

Soluble interleukin-2 receptors (sRIL-2) are known to be released after *in vitro* mitogenic and antigenic stimulation of T cells and can be evaluated by a sandwich enzyme-linked immunosorbent assay (ELISA) of culture supernatants.[18,19] Thus we examined the correlation between soluble IL-2 receptor levels and the immunomodulating effect of some compounds.

The results in the phytohemagglutinin-induced lymphocyte proliferation test, expressed as stimulation index (SI), are summarized in Table V. The activity of levamisole, used as reference, is maximum at concentrations between

TABLE III. Spectral Data for 6,8-Dioxo-3-thia-1,7-diazabicyclo[3.3.0]octanes



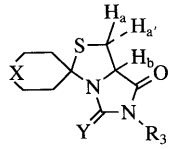
8: $R_1=H_c$, $R_2=H_{c'}$
9: $R_1=R_2=CH_3$
10: $R_1=H_c$, $R_2=CO_2CH_3$

Compd. No.	IR (KBr) cm^{-1}	^{1}H-NMR (60 MHz, $CDCl_3$) δ ppm					
	(C=O)	H_a, $H_{a'}$	H_b	H_c, $H_{c'}$	H_3C C H_3C	$COOCH_3$	R_3
8a	1700 1700	3.3 (m)	5.1 (dd)	4.3 (m)			7.4 (s, 5H)
8b	1700 1775	3.3 (m)	5.0 (dd)	4.4 (m)			7.3 (m, 4H)
8c	1700 1775	3.3 (m)	5.0 (dd)	4.4 (m)			7.3 (m, 4H)
8d	1710 1775	3.4 (m)	5.15 (dd)	4.3 (m)			2.4 (s, 3H) 7.3 (s, 4H)
8e	1470 1770	3.3 (m)	5.0 (dd)	4.4 (m)			7.3 (s, 5H)
8f	1700 1770	3.3 (m)	5.1 (dd)	4.4 (m)			3.8 (s, 3H) 6.8 (m, 2H) 7.2 (m, 2H)
8g	1710 1775	3.3 (m)	5.2 (dd)	4.4 (m)			3.05 (s, 3H)
8h	1700 1750	3.3 (m)	5.0 (dd)	4.2 (m)			0.9 (m, 3H, CH) 1.45 (m, 4H) 3.3 (m, 2H)
8i	1700 1770	3.3 (m)	5.1 (dd)	4.3 (m)			1.1—2.1 (m, 10H) 3.4 (m, 1H)
8j	1710 1770	3.3 (m)	5.1 (dd)	4.2 (m)			4.7 (s, 2H) 7.3 (s, 5H)
9a	1710 1775	3.3 (m)	5.1 (dd)		1.5 (s) 1.6 (s)		7.4 (s, 5H)
9b	1700 1775	3.3 (m)	5.1 (dd)		1.5 (s) 1.6 (s)		7.4 (s, 4H)
9c	1700 1770	3.3 (m)	5.1 (dd)		1.5 (s) 1.6 (s)		7.4 (s, 4H)
9d	1710 1770	3.3 (m)	5.1 (dd)		1.5 (s) 1.6 (s)		2.4 (s, 3H) 7.3 (s, 4H)
9e	1470 1770	3.3 (m)	5.1 (dd)		1.5 (s) 1.6 (s)		7.4 (s, 5H)
10a	1710 1735 1775	3.3 (m)	4.7 (q)	5.1 (s, 1H)		3.8 (s)	7.5 (s, 5H)
10b	1710 1735 1770	3.3 (m)	4.4 (q)	5.5 (s, 1H)		3.85 (s)	7.4 (s, 4H)
10c	1710 1735 1770	3.3 (m)	4.4 (q)[a] 4.8 (q)[b]	5.4 (s)[a] 5.1 (s)[b]		3.85 (s)[a] 3.80 (s)[b]	2.4 (s, 3H), 7.3 (s, 4H)[a]
10d	1700 1740 1770	3.3 (m)	4.6 (q)[a] 4.3 (q)[b]	5.1 (s)[a] 5.25 (s)[b]		3.80 (s)[a] 3.85 (s)[b]	1.1—2.1 (m, 10H) 3.5 (m, 1H)
10e	1710 1740 1770	3.3 (m)	4.7 (q)[a] 4.3 (q)[b]	5.1 (s)[a] 5.3 (s)[b]		3.80 (s)[a] 3.85 (s)[b]	3.0 (s, 3H)[a]
10f	1700 1735 1770	3.3 (m)	4.6 (q)[a] 4.3 (q)[b]	5.1 (s)[a] 5.3 (s)[b]		3.80 (s)[a] 3.85 (s)[b]	0.9 (m, CH_3) 1.5 (m, $2CH_2$) 3.4 (m, CH_2)

a) For the major diastereoisomer, ratio 70/30—60/40. *b*) For the minor diastereoisomer.

1 and 20 μg/ml ($p<0.05$), then rapidly decreases ($p>0.05$). At any concentration used, the highest stimulation indexes were obtained in the presence of compounds **8b**, **8d**, **13b** and **14d** ($p<1\times10^{-3}$). Compound **8b** exhibits a maximum at a concentration of 40 μg/ml, but its activity is always above 1.00. The activity of **8d** is dose-dependent and significant, so this compound appeared to be a good candicate for further investigations. Compounds **11b** and

TABLE IV. Spectral Data for 6,8-Dioxo-3-thia-1,7-diazabicyclo[3.3.0]octane-2-Spiro Derivatives



11: X=CHPh
12: X=NCH_3
13: X=NCH_2Ph
14: X=$N(CH_2)_2Ph$

Compd. No.	IR (KBr) cm^{-1} (C=O)	^{1}H-NMR (60 MHz, $CDCl_3$) δ ppm H_a, $H_{a'}$	H_b	X	R_3
11a	1710, 1770	3.2 (m)	4.8 (dd)	1.7—2.3 (m, 8H), 3.0 (m, 1H), 7.3 (s, 5H)	7.4 (s, 5H)
11b	1710, 1775	3.2 (m)	4.8 (dd)	1.7—2.3 (m, 8H), 3.0 (m, 1H), 7.3 (s, 5H)	7.4 (s, 4H)
11c	1710, 1770	3.2 (m)	4.8 (dd)	1.7—2.3 (m, 8H), 3.0 (m, 1H), 7.3 (s, 5H)	7.4 (s, 4H)
11d	1710, 1770	3.2 (m)	4.8 (dd)	1.7—2.3 (m, 8H), 3.0 (m, 1H), 7.3 (s, 5H)	2.4 (s, 3H), 7.3 (s, 4H)
11e	1470, 1770	3.2 (m)	4.7 (dd)	1.7—2.3 (m, 8H), 3.0 (m, 1H), 7.3 (s, 5H)	7.4 (s, 5H)
11f	1470, 1770	3.2 (m)	4.7 (dd)	1.7—2.3 (m, 8H), 3.0 (m, 1H), 7.3 (s, 5H)	7.4 (s, 4H)
12a	1710, 1770	3.2 (m)	4.6 (dd)	1.9—2.8 (m, 8H), 2.3 (s, 3H)	7.4 (s, 5H)
12b	1710, 1770	3.2 (m)	4.6 (dd)	1.9—2.8 (m, 8H), 2.3 (s, 3H)	7.4 (s, 4H)
12c	1710, 1770	3.2 (m)	4.6 (dd)	1.9—2.8 (m, 8H), 2.3 (s, 3H)	7.4 (s, 4H)
12d	1710, 1770	3.2 (m)	4.6 (dd)	1.9—2.8 (m, 8H), 2.3 (s, 3H)	2.3 (s, 3H), 7.4 (s, 4H)
12e	1470, 1770	3.2 (m)	4.6 (dd)	1.9—2.8 (m, 8H), 2.3 (s, 3H)	7.4 (s, 5H)
13a	1710, 1770	3.2 (m)	4.7 (dd)	1.9—2.8 (m, 8H), 3.5 (s, 2H), 7.3 (s, 5H)	7.4 (s, 5H)
13b	1710, 1770	3.2 (m)	4.7 (dd)	1.9—2.8 (m, 8H), 3.5 (s, 2H), 7.3 (s, 5H)	7.4 (s, 4H)
13c	1710, 1770	3.2 (m)	4.7 (dd)	1.9—2.8 (m, 8H), 3.5 (s, 2H), 7.3 (s, 5H)	7.4 (s, 4H)
13d	1700, 1775	3.2 (m)	4.7 (dd)	1.9—2.8 (m, 8H), 3.5 (s, 2H), 7.3 (s, 5H)	2.3 (s, 3H), 7.4 (s, 4H)
13e	1470, 1770	3.2 (m)	4.6 (dd)	1.9—2.8 (m, 8H), 3.5 (s, 2H), 7.3 (s, 5H)	7.4 (s, 5H)
14a	1700, 1770	3.2 (m)	4.6 (dd)	1.9—2.6 (m, 12H), 7.2 (s, 5H)	7.4 (s, 5H)
14b	1710, 1775	3.2 (m)	4.6 (dd)	1.9—2.6 (m, 12H), 7.2 (s, 5H)	7.4 (s, 4H)
14c	1700, 1770	3.2 (m)	4.6 (dd)	1.9—2.6 (m, 12H), 7.3 (s, 5H)	7.3 (s, 4H)
14d	1710, 1775	3.2 (m)	4.6 (dd)	1.9—2.6 (m, 12H), 7.1 (s, 5H)	2.3 (s, 3H), 7.3 (s, 4H)
14e	1475, 1775	3.2 (m)	4.6 (dd)	1.9—2.6 (m, 12H), 7.1 (s, 5H)	7.4 (s, 5H)

11d exhibit a constant activity and are more potent at intermediate concentrations of 10 and 20 μg/ml. Compounds **13a** and particularly **13b** showed good stimulatory activity at low concentrations, whereas the activity of **13d** seems independent of the concentration. Compound **14b** is active from low concentrations, whereas **14d** has dose-dependent activity.

Compounds **8d** and **13b** were tested for ability to release soluble interleukin-2 receptor from the supernatant of 24 h culture of human T lymphocytes stimulated *in vitro* by phytohemagglutinin. If the incubation is extended to 48 or 72 h, only the highest concentrations provoke higher release of soluble interleukin-2 receptor than that obtained in the reference assay. A low increase (4 to 6 pM) can not be considered as significant since the accuracy of the assay is 5 pM. After a 24 h incubation, maximum releases of sRIL-2 were observed with compounds **8d** and **13b** at 80 μg/ml and levamisole at 20 μg/ml. At these concentrations, the increase in sRIL-2 was ranged from 17% to 22% (Table VI). The increase in sRIL-2 release was dose-dependent, and compound **13b** seemed to induce the highest enhancement. These results were correlated with those otbained in the phytohemagglutinin (PHA)-induced lymphocyte transformation tests. This assay seems more useful as a complementary one rather than as a basic screening test.

Finally the effect of some compounds on macrophage oxidative metabolism was investigated through determination of the chemiluminescence index of mice peritoneal macrophages (Table VII). All tested compounds exhibited a significant stimulating activity, much higher than that of levamisole. Moreover, all compounds potent in the phytohemagglutinin-induced T lymphocyte proliferation assay were also potent in the chemiluminescence test, as if there were two kinds of cellular targets, T lymphocytes and macrophages.

A comparison of these results with those previously reported[9] for thia-7-diaza-1,3 bicyclo[3.3.0]octanes indicates that the 6,8-dioxo group on the hydantoin heterocycle significantly improves the immunomodulating activity. The influence of other structural features is unclear. It seems nevertheless that 4′ substitution on the N-7 phenyl group interferes with the immunological effect. The most potent compounds are those bearing a 4′-chloro or a 4′-methyl substituent on the N-7 phenyl nucleus. Those bearing a 4′-fluoro substituent are not more potent than the unsubstituted derivative.

Experimental

General Remarks Melting points were determined on a Kofler apparatus without correction. Infrared (IR) spectra were recorded on a Beckman 4240 spectrophotometer in KBr disks for solids and as liquid films for oils. Proton magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian EM 360A spectrometer with tetramethylsilane as an internal standard, The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quadruplet, m=multiplet. Optical rotations were measured on a Perkin–Elmer model 241 polarimeter in chloroform. Microanalyses were performed by Service Central d'Analyses du CNRS, 69390 Vernaison, France. When required, the separation of crude reaction products was performed by chromatography on a silica gel column (70—230 mesh).

L-(−)-*R*-cysteine ethyl ester hydrochloride was purchased from Aldrich Chemical Company, mp 123—125 °C, $[\alpha]_D^{20}$ −7.9° (c=1, 1 N HCl), M_r=185.5.

TABLE V. Stimulation Index (SI) of Human T Lymphocytes Stimulated *in Vitro* by Phytohemagglutinin (25 μg/ml) Obtained at Increasing Concentrations of Compounds

Compd. No.	Concentrations of tested compounds (μg/ml)						*p* value
	1	5	10	20	40	80	
Levamisole	1.04[a]	1.08	1.10	1.13	0.95	0.65	NS
($n=11$)	(1.02—1.07)[b]	(1.05—1.12)	(1.09—1.15)	(1.11—1.21)	(0.8—1)	(0.5—0.76)	
8a	1.02	1.03	1.01	1.01	1.01	1.01	NS
($n=4$)	(1.01—1.05)	(1.02—1.05)	(1.00—1.03)	(0.95—1.05)	(1.00—1.05)	(0.99—1.05)	
8b	1.05	1.03	1.09	1.08	1.13	1.05	$<1\times10^{-3}$
($n=4$)	(1.01—1.07)	(1.02—1.06)	(1.08—1.13)	(1.05—1.12)	(1.09—1.15)	(1.03—1.06)	
8c	1.04	1.02	1.02	1.04	1.03	1.06	$<2\times10^{-2}$
($n=4$)	(1.02—1.06)	(0.99—1.05)	(0.94—1.03)	(1.02—1.06)	(1.00—1.05)	(1.01—1.07)	
8d	1.07	1.09	1.11	1.15	1.22	1.25	$<1\times10^{-4}$
($n=8$)	(1.05—1.12)	(1.07—1.14)	(1.09—1.20)	(1.13—1.25)	(1.19—1.28)	(1.21—1.35)	
9a	0.98	0.93	1.02	1.04	0.93	0.98	NS
($n=4$)	(0.93—1.0)	(0.93—1.00)	(0.99—1.03)	(1.02—1.07)	(0.92—0.98)	(0.95—1.01)	
9b	1.00	1.03	1.00	1.08	1.05	1.01	<0.05
($n=4$)	(0.98—1.01)	(1.00—1.07)	(0.98—1.02)	(1.01—1.08)	(1.02—1.08)	(1.00—1.05)	
9c	1.01	1.03	1.05	0.97	0.98	1.00	NS
($n=4$)	(0.98—1.03)	(1.00—1.03)	(1.01—1.08)	(0.95—1.01)	(0.96—1.02)	(0.99—1.03)	
9d	1.00	1.02	1.02	1.02	1.03	1.00	NS
($n=4$)	(0.97—1.03)	(1.01—1.07)	(1.00—1.06)	(1.00—1.05)	(0.98—1.06)	(0.99—1.07)	
11a	1.00	1.02	1.03	0.98	0.98	0.99	NS
($n=4$)	(0.98—1.03)	(1.00—1.05)	(1.01—1.07)	(1.01—1.06)	(0.95—1.01)	(0.95—1.00)	
11b	1.02	1.09	1.13	1.06	1.08	1.00	<0.05
($n=4$)	(1.00—1.04)	(1.06—1.13)	(1.07—1.15)	(1.03—1.09)	(1.03—1.15)	(0.99—1.03)	
11c	1.00	0.98	1.01	1.03	1.05	1.00	NS
($n=4$)	(0.98—1.03)	(0.97—1.01)	(1.00—1.06)	(0.99—1.05)	(1.03—1.07)	(0.98—1.05)	
11d	0.99	1.00	1.09	1.11	1.09	1.05	<0.05
($n=4$)	(0.97—1.01)	(0.98—1.03)	(1.04—1.11)	(1.07—1.13)	(1.07—1.10)	(1.03—1.08)	
12a	1.02	1.03	1.01	0.99	0.96	0.90	NS
($n=4$)	(1.00—1.05)	(1.01—1.06)	(0.98—1.05)	(0.97—1.01)	(0.94—1.00)	(0.87—0.93)	
12b	0.95	0.99	1.01	0.98	0.97	0.95	NS
($n=4$)	(0.93—0.98)	(0.96—1.04)	(0.99—1.06)	(0.95—1.02)	(0.95—1.00)	(0.93—0.98)	
12c	0.98	1.02	1.00	1.01	0.95	0.99	NS
($n=4$)	(0.96—1.02)	(0.98—1.05)	(0.97—1.04)	(0.98—1.04)	(0.92—0.97)	(0.95—1.05)	
12d	1.02	1.03	1.01	1.03	0.97	0.98	NS
($n=4$)	(1.00—1.05)	(0.91—1.06)	(1.00—1.04)	(1.00—1.06)	(0.95—1.01)	(0.95—1.04)	
13a	1.07	1.15	1.17	1.07	0.98	0.95	NS
($n=6$)	(1.02—1.09)	(1.09—1.21)	(1.11—1.25)	(1.03—1.09)	(0.94—1.00)	(0.91—0.97)	
13b	1.15	1.16	1.23	1.16	1.12	1.07	$<1\times10^{-3}$
($n=8$)	(1.09—1.23)	(1.12—1.21)	(1.17—1.25)	(1.13—1.24)	(1.08—1.15)	(1.00—1.10)	
13c	0.95	0.95	0.98	1.01	1.00	0.97	NS
($n=6$)	(0.91—0.98)	(0.92—0.97)	(0.95—1.00)	(0.99—1.03)	(0.98—1.05)	(0.95—1.04)	
13d	1.05	1.06	1.05	1.05	1.07	1.02	$<1\times10^{-2}$
($n=7$)	(1.01—1.07)	(1.01—1.09)	(1.02—1.08)	(1.01—1.09)	(1.04—1.10)	(0.97—1.05)	
14a	1.01	1.08	1.09	1.05	1.05	1.02	$<1\times10^{-2}$
($n=5$)	(0.98—1.05)	(1.04—1.12)	(1.07—1.14)	(1.02—1.09)	(1.01—1.11)	(0.98—1.06)	
14b	1.07	1.05	1.09	1.09	1.06	1.02	$<1\times10^{-2}$
($n=6$)	(1.01—1.11)	(1.02—1.09)	(1.07—1.14)	(1.07—1.13)	(1.04—1.14)	(1.00—1.09)	
14c	1.02	1.05	1.05	1.09	1.11	1.04	<0.05
($n=6$)	(1.00—1.05)	(1.02—1.08)	(1.01—1.07)	(1.05—1.13)	(1.09—1.14)	(1.01—1.07)	
14d	1.02	1.07	1.05	1.10	1.15	1.17	$<1\times10^{-2}$
($n=8$)	(1.00—1.06)	(1.02—1.11)	(1.03—1.14)	(1.08—1.15)	(1.12—1.18)	(1.12—1.21)	

n, number of tests carried out on different donors. *a*) Median. *b*) ( ), extreme values. NS: Not significant.

Ethyl Thiazolidine-4-carboxylate (1) A suspension of L-(−)-*R*-cysteine ethyl ester hydrochloride (5.60 g, 0.03 mol) in ethanol (50 ml) was treated with 5 ml of 37% aqueous formaldehyde solution (0.03 mol). The mixture was stirred for 30 min at room temperature and then at 70 °C for 30 min; the reaction mixture was cooled and evaporated. The resulting crystals were collected by filtration, washed with ether and then dissolved in water (50 ml). The aqueous solution was neutralized with sodium carbonate, then the aqueous layer was extracted with ether (2 × 50 ml) and the combined organic layers were dried over sodium sulfate, filtered and evaporated. The residue was distilled *in vacuo* to give a colorless liquid, yield 80%, bp 125 °C (13 mmHg) lit.[20] 124—125 °C/13 mmHg. IR (NaCl): 1750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t), 2.6 (1H, s), 3.3 (2H, q), 4.1 (2H, q), 4.3 (3H, m). *Anal.* Calcd for $C_6H_{11}NO_2S$: C, 44.70; H, 6.88; N, 8.70; S, 19.88. Found: C, 44.68; H, 6.88; N, 8,75; S, 19.75.

Ethyl 2,2-Dimethylthiazolidine-4-carboxylate (2) This compound was prepared in the same manner as described for **1**. Colorless liquid, yield 80%, bp 120 °C/13 mmHg. IR (NaCl): 1750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t), 1.5 (3H, s), 1.6 (3H, s), 2.6 (1H, s), 3.3 (2H, m), 4.1 (2H, q), 4.3 (1H, m). *Anal.* Calcd for $C_8H_{15}NO_2S$: C, 50.77; H, 7.99; N, 7.40; S, 16.93. Found: C, 51.35; H, 7.83; N, 7.35; S, 16.99.

Ethyl 2-Methoxycarbonylthiazolidine-4-carboxylate (3) Methyl glyoxylate (2.65 g, 0.03 mol), was added to a suspension of L-(−)-*R*-cysteine ethyl ester hydrochloride (5.60 g, 0.03 mol) in ethanol (50 ml). The mixture was stirred for 30 min at room temperature then at 50 °C for 1 h, cooled and evaporated. The residue was dissolved in water (50 ml) and the aqueous layer was washed with ether (2 × 20 ml). The aqueous layer was separated, neutralized with sodium carbonate, then extracted

TABLE VI. Concentrations of Soluble Interleukin-2 Receptor (sRIL-2) (pM) Released from Supernatant of 24 h Culture of Human T Lymphocytes from 3 Healthy Blood Donors, Stimulated *in Vitro* with Phytohemagglutinin

Compd.	Concentrations of tested compounds (μg/ml)				
	0	5	20	40	80
8d	48	50	55	56	58
Blood donor 1	(0%)[a]	(4%)[a]	(12%)[a]	(14%)[a]	(17%)[a]
13b	175	180	200	212	225
Blood donor 2	(0%)[a]	(2.7%)[a]	(12.5%)[a]	(17.4%)[a]	(22.2%)[a]
Levamisole	89	102	110	104	ND
Blood donor 3	(0%)[a]	(12.7%)[a]	(19%)[a]	(14.4%)[a]	

pM = picomol per liter (1 pM = 42 pg/ml). ND = Not determined.

a) Value % of: [(sRIL-2 concentration obtained with compound) − (sRIL-2 concentration obtained without compound)]/(sRIL-2 concentration obtained without compound).

TABLE VII. Chemiluminescence Index (CLI) of Mice Peritoneal Macrophages Treated or not Treated with Tested Compounds

Compd.	CLI treated 20 mg/kg	CLI not treated	*p* value
Levamisole	2.4[a]	2.0	0.05
$n=6$	(1.5—3.6)[b]	(0.5—4.5)	
8b	7.0	2.5	0.03
$n=6$	(0.4—11.3)	(0.0—6.7)	
8d	30.0	4.6	<0.02
$n=6$	(6.7—38.0)	(0.5—9.0)	
11b	14.9	2.5	<0.02
$n=6$	(2.4—60.0)	(0.7—6.7)	
13d	84.5	4.6	<0.02
$n=6$	(9.7—99.0)	(0.1—11.0)	
14b	10.9	3.5	0.04
$n=6$	(4.3—20.6)	(0.2—16.0)	
14d	13.2	3.5	<0.04
$n=6$	(5.9—32.8)	(0.2—16.0)	

a) Median. *b*) ( ), extreme values.

with chloroform (2 × 50 ml), and the combined organic layers were dried over sodium sulfate, filtered and evaporated.The residue was purified by trituration with ether to give pale yellow crystals, yield 70%, mp 60 °C. IR (KBr): 1740 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t), 2.7 (1H, t), 3.2 (2H, m), 3.8 (3H, s), 3.9 (1H, m), 4.15 (2H, q), 5.0 (1H, s). *Anal.* Calcd for $C_8H_{13}NO_4S$: C, 43.82; H, 5.94; N, 6.39; S, 14.61. Found: C, 43.85; H, 5.73; N, 6.30; S, 14.80.

The other 2-substituted ethyl thiazolidine-4-carboxylates (**4**—**7**) were prepared in the same manner as described for **3**.

Ethyl 4-Phenylcyclohexane-1-spiro-2′-thiazolidine-4′-carboxylate (4) Colorless powder, yield 90%, mp 104 °C. IR (KBr): 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.25 (3H, t), 1.4—2.2 (8H, m), 2.6 (1H, s), 2.8—3.4 (3H, m), 4.0—4.5 (3H, m), 7.3 (5H, s). *Anal.* Calcd for $C_{17}H_{23}NO_2S$: C, 66.85; H, 7.59; N, 4.59; S, 10.49. Found: C, 66.75; H, 7.64; N, 4.37; S, 10.72.

Ethyl *N*-Methylspiro[piperidine-4,2′-thiazolidine]-4′-carboxylate (5) Pale yellow oil, yield 70%, bp 104 °C/0.1 mmHg. IR (NaCl): 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.25 (3H, t), 1.7—2.2 (4H, m), 2.3 (3H, s), 2.4—2.8 (5H, m), 3.2 (2H, m), 3.9—4.4 (3H, m). *Anal.* Calcd for $C_{11}H_{20}N_2O_2S$: C, 54.07; H, 8.25; N, 11.46; S, 13.10. Found: C, 54.54; H, 8.12; N, 11.25; S, 13.48.

Ethyl *N*-Benzylspiro[piperidine-4,2′-thiazolidine]-4′-carboxylate (6) Pale yellow crystals, yield 75%, mp 72 °C. IR (KBr): 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.25 (3H, t), 1.7—2.2 (4H, m), 2.2—2.8 (5H, m), 3.2 (2H, m), 3.5 (2H, s), 3.9—4.4 (3H, m), 7.3 (5H, s). *Anal.* Calcd for $C_{17}H_{24}N_2O_2S$: C, 63.72; H, 7.55; N, 8.74; S, 10.0. Found: C, 63.56; H, 7.40; N, 8.54; S, 9.26.

Ethyl *N*-Phenethylspiro[piperidine-4,2′-thiazolidine]-4′-carboxylate (7) Pale yellow crystals, yield 65%, mp 72 °C. IR (KBr): 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.25 (3H, t), 1.8—2.2 (4H, m), 2.2—2.7 (5H, m), 2.7—3.0 (4H, m), 3.2 (2H, m), 3.9—4.4 (3H, m), 7.25 (5H, s). *Anal.* Calcd for $C_{18}H_{26}N_2O_2S$: C, 64.64; H, 7.84; N, 8.38; S, 9.59. Found: C, 64.67; H, 7.95; N, 8.50; S, 9.60.

6,8-Dioxo-7-phenyl-3-thia-1,7-diazabicyclo[3.3.0]octane (8a) A mixture of ethyl thiazolidine-4-carboxylate (**1**) (4.83 g, 0.03 mol) and phenyl isocyanate (4.17 g, 0.035 mol) in pyridine (50 ml) was stirred at room temperature for 24 h. The crude compound contained some 1,3-diphenylurea, which was extracted with 25 ml of dichloromethane at room temperature. After evaporation of the solvent, the residue was recrystallized from ethanol to give white needles. For physical properties see Table I. All other compounds (**8b**—**j**) were prepared similarly and recrystallized from ethanol.

2,2-Dimethyl-6,8-dioxo-7-phenyl-3-thia-1,7-diazabicyclo[3.3.0]octane (9a) This compound was prepared from ethyl 2,2-dimethylthiazolidine-4-carboxylate (**2**, 5.67 g, 0.03 mol), according to the above procedure. All other compounds were prepared similarly and recrystallized from ethanol (Tables I and III).

2-Methoxycarbonyl-6,8-dioxo-7-phenyl-3-thia-1,7-diazabicyclo[3.3.0]-octane (10a) A mixture of ethyl 2-methoxycarbonylthiazolidine-4-carboxylate (**3**, 6.51 g, 0.03 mol) and phenyl isocyanate (4.17 g, 0.035 mol) in pyridine (50 ml) was stirred at room temperature for 12 h. After evaporation of the solvent, the residue was dissolved in ether (100 ml) and 1 ml of concentrated hydrochloric acid was added. The reaction mixture was heated on a steam bath for 30 min. After evaporation of the solvent, the crude compound was purified by column chromatography (ethyl acetate–hexane, 5 : 5). All other compounds (**10b**—**f**) were prepared similarly. See Tables I and III for physical constants and spectral data.

6,8-Dioxo-7-phenyl-3-thia-1,7-diazabicyclo[3.3.0]octane-2-spiro-1′-(4′-phenylcyclohexane) (11a) A mixture of ethyl 4-phenylcyclohexane-1-spiro-2′-thiazolidine-4′-carboxylate (**4**, 9.15 g, 0.03 mol) and phenyl isocyanate (4.17 g, 0.035 mol) in pyridine (50 ml) was stirred at room temperature for 48 h. The solvent was then evaporated *in vacuo* and the residue was dissolved in dichloromethane (25 ml). The solution was filtered, washed with water and dried. After evaporation of the solvent, the crude compound was purified by column chromatography (ethyl acetate–hexane, 5 : 5). All other compounds (**11b**—**e**) were prepared similarly. See Tables II and IV for physical constants and spectral data.

Compounds **12a**—**e**, **13a**—**e**, and **14a**—**e** were prepared in the same manner as described for **11a**. See Tables II and IV for physical constants and spectral data.

Proliferative Responses to Mitogen (Phytohemagglutinin): Lymphocyte Transformation Test Peripheral blood lymphocytes taken from healthy donors were separated by centrifugation on a Ficoll-Hypaque gradient. Cells were cultured in 199 medium including 10% human AB serum and 2 mM L-glutamine at a concentration of 10^6 lymphocytes/ml in culture tubes. Phytohemagglutinin (PHA) was used at a final concentration of 25 μg/ml to effect submaximal T-cell stimulation and 54 μg/ml for maximal T-cell stimulation.

These incubations were carried out with or without potential immunomodulating substances at various concentrations (1, 5, 10, 20, 40, 80 μg/ml) for 3 d. Cultures were harvested on glass filters after 72 h with 0.1 μCi of [*methyl*-^{3}H]thymidine per tube for the final 18 h of the assay. The incorporated radioactivity was determined with a β scintillation counter and expressed as net CPM. Results were expressed as a SI determined by using the following formula:

$$SI = \frac{\text{CPM (T-cells stimulated by PHA and compound)}}{\text{CPM (T-cells stimulated by PHA)}}$$

Soluble Interleukin-2 Receptor In parallel, lymphocyte cultures obtained from 3 healthy blood donors were used in order to determine the concentrations of soluble interleukin-2 receptor released. At various times of culture (24, 48 and 72 h), cells were harvested and the supernatants were frozen at −70 °C. Concentration of soluble interleukin-2 receptor was measured by ELISA using an immunoenzymatic assay kit (Immunotech, Marseille, France). Soluble interleukin-2 receptor concentrations of the test samples were determined by comparison with a standard curve and expressed as pM/l.

Chemiluminescence Test Swiss mice received the compounds intraperitoneally at 20 mg/kg, and phosphate-buffered saline solution (PBS) was administered to a control group. The mice were killed 3 d after injection. Macrophages were obtained by peritoneal lavage with 5 ml of PBS.

Measurement of the chemiluminescence reaction was performed according to the literature.[16] Briefly, a suspension of one hundred isolated cells was transferred into a counting tube previously filled with 10 μl of Hanks balanced salt sodium (HBSS) with or without the appropriate diluted simultating agent (zymosan, opsonized). The tube was placed into a dry bath at 37 °C under constant agitation. A 10 min incubation period was chosen for zymosan. After the incubation, 50 μl of luminol were added and the tube was shaken gently then immediately introduced into the photometer counting chamber. The third value obtained on the photometer read-out was found to be optimal and was therefore considered as the maximal light intensity (MLI), which is expressed in relative light units (RLU) by the photometer (Biolumat, Berthold Instruments, Wildbad, Germany). The chemiluminescence index (CLI) was defined as follows:

$$\frac{\text{MLI (stimulated cells)} - \text{MLI (unstimulated cells)}}{\text{MLI (unstimulated cells)}}$$

Because of the small sample size, results were expressed as median and extreme values. Statistical comparisons were made by using the non parametric Mann–Whitney U test.[21]

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References

1) G. Renoux, M. Renoux, *C. R. Acad. Sci.*, **272**, D, 349 (1971).
2) J. P. Devlin, K. D. Hargrave, *Tetrahedron*, **45**, 4327 (1989).
3) L. A. Radov, D. Kamp, D. Sloane, R. P. Julien, C. M. Clemens, R. J. Murray, *J. Immunopharmacol.*, **10**, 609 (1988).
4) Poli Industria Chimica, European Patent Appl., E.P. 0276752 A_1-03.08.1988 and E.P. 0383 180 A_2-16.08 (1990) [*Chem. Abstr.*, **114**, 81818*v* (1991)].
5) N. Margoum, P. Tronche, P. Bastide, J. Bastide, C. Rubat. *Eur. J. Med. Chem.*, **19**, 415 (1984).
6) L. Diafi, J. Couquelet, P. Tronche, D. Gardette, J. C. Gramain, *J. Heterocycl. Chem.*, **27**, 2181 (1990).
7) L. Diafi, C. Rubat, P. Coudert, P. Bastide, N. Margoum, P. Tronche, *Eur. J. Med. Chem.*, **26**, 231 (1991).
8) Yoshitomi Pharm. Ind., Japan. Patent, 9 159 573, 11.08.1973; Ger. Patent 2 425 306, 2.04 (1975) [*Chem. Abstr.*, **82**, 170884*b* (1975)].
9) B. Refouvelet, P. Tronche, J. Couquelet, J. F. Robert, G. Bonnefoy-Claudet, J. Panouse-Perrin, *Eur. J. Med. Chem.*, **22**, 11 (1987).
10) E. H. Bahaji, P. Tronche, J. Couquelet, S. Harraga, J. Panouse-Perrin, C. Rubat, *Chem. Pharm. Bull.*, **39**, 2126 (1991).
11) R. Ader, D. Felten, N. Cohen, *Ann. Rev. Pharmacol. Toxicol.*, **30**, 561 (1990).
12) M. E. Kammuller, W. Seinen, *Int. J. Immunopharmacol.*, **10**, 997 (1988).
13) R. G. Kallen, *J. Am. Chem. Soc.*, **93**, 6236 (1971).
14) J. W. Hadden, E. M. Hadden, R. G. Coffey, *Infect. Immun.*, **13**, 382 (1976).
15) M. Birouk, S. Harraga, J. Panouse-Perrin, J. F. Robert, M. Darmelincourt, F. Theobald, R. Mercier, J. J. Panouse, *Eur. J. Med. Chem.*, **26**, 91 (1991).
16) B. Descamps-Latscha, A. T. Nguyen, R. M. Golub, M. N. Feuillet-Fieux, *Ann. Immunol.* (Inst. Pasteur), **133C**, 319 (1982).
17) J. C. Schleupner, L. A. Glascow, *Infect. Immun.*, **1978**, 886.
18) E. P. Tam, R. D. Hinsdill, *Fundam. Appl. Toxicol.*, **14**, 542 (1990).
19) L. A. Rubin, C. C. Kurman, M. E. Fritz, W. E. Biddison, B. Boutin, R. Yarchoan, D. L. Nelson, *J. Immunol.*, **135**, 3172 (1985).
20) T. A. Crabb, M. J. Hall, R. O. Williams, *Tetrahedron*, **29**, 3389 (1973).
21) G. W. Snedecor, W. G. Cochran, "Statistical Methods in Medical Research," ed. by Blackwell Scientific Publications, Oxford, 1971, p. 593.