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Short communication

Imidazo[2,1-*b*]thiazole derivatives. XI. Modulation of the CD_2-receptor of human T trypsinized lymphocytes by several imidazo[2,1-*b*]thiazoles

S Harraga, L Nicod, JP Drouhin, A Xicluna, JJ Panouse, E Seilles, JF Robert*

Equipe de Chimie thérapeutique, Faculté de Médecine et de Pharmacie, place St-Jacques, 25030 Besançon Cedex, France

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Summary — About 40 substituted imidazo[2,1-*b*]thiazoles were obtained in order to study their *in vitro* immunological effect on the modulation of the expression of human T trypsinized lymphocytes by the CD_2 receptor. A synthetic program was developed to introduce either an oxygenated function, such as ester (**11**, **14**), acid (**12**) and arylketonic groups (**9**, **13**, **15**), or two groups, such as an aryl and an ester (**1**, **6**, **8**), an acid (**3**, **7**) or a hydrazide (**2**). These compounds were examined by an E-rosette-forming-cell test, and display a positive drug efficacity index, suggesting a regeneration effect on the expression of CD_2 receptors. The following structural parameters are favourable: an aryl moiety on the C-6 with a methoxy or nitro group; and an ethyl ester on the C-3, a double bond to the 2,3-position (the 5,6-position is ineffective). Acid and hydrazide functions or the loss of phenyl group on the C-6 decrease this activity. If the aryl group is on the C-3 or C-2 side chain, the activity is weaker and more so for the latter. However, the most interesting derivatives are less immunostimulating than levamisole hydrochloride.

immunomodulator / imidazo[2,1-*b*]thiazole / human T lymphocyte /CD_2 receptor

The discovery of antihelminthic properties of 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (tetramisole) by Janssen Pharmaceutica [1, 2, 3] provoked a great deal of research on imidazothiazole derivatives. Levamisole, the levogyre enantiomeric form of tetramisole, was later described as an immunomodulator by G and M Renoux [4]. The discovery of this new activity was the beginning of applications in anticancer therapy [5] or therapy against rheumatoid arthritis [6]. The toxicity of levamisole, however, reduced the maximum prescribed dose of these agents.

We have developed a program for the synthesis of new substituted imidazo[2,1-*b*]thiazoles with the presence of both an aryl group and oxygenated functional groups in various positions on the ring. Several compounds that are unsubstituted on the phenyl group were prepared and published by our team [7, 8, 9, 10]. However, many of these were not described in detail and are studied in this work. Thus we will repeat the principle of their synthesis.

T cells can be activated either through a CD_3-T-cell receptor (TCR) complex or through CD_2 (sheep erythrocyte receptor). The CD_2 molecules can also deliver a signal for the induction of T-cell proliferation, in addition to the CD_3-TCR pathway. This alternative pathway involves the CD_2 receptor and causes the cells to become responsive to interleukin 2 (IL–2) by inducing the expression of IL–2 receptors and probably plays a central role in T-lymphocyte functions [11, 12].

Trypsinized T cells lose their ability to form a normal number of E-rosette-forming cells (E-RFC) [13]. Immunomodulating drugs have been claimed to interfere with the expression and regeneration of receptors at the surface of T cells [14]. In previous

*Correspondence and reprints

studies, we suggested that the modulation of E-RFC with trypsinized lymphocytes by several drugs could be proposed as a simple *in vitro* test to screen the immunopharmacological effect of compounds [15,16].

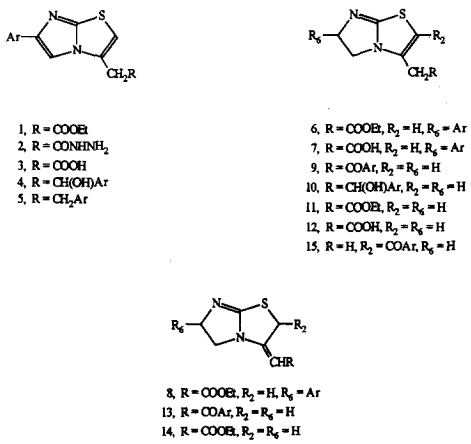
The aim of this study was to investigate the immunological effect of the imidazothiazoles **1–15** (scheme 1) on the modulation of the expression of human trypsinized T lymphocytes by the CD_2 receptor using an E-RFC test, and to propose an initial SAR approach.

Chemistry

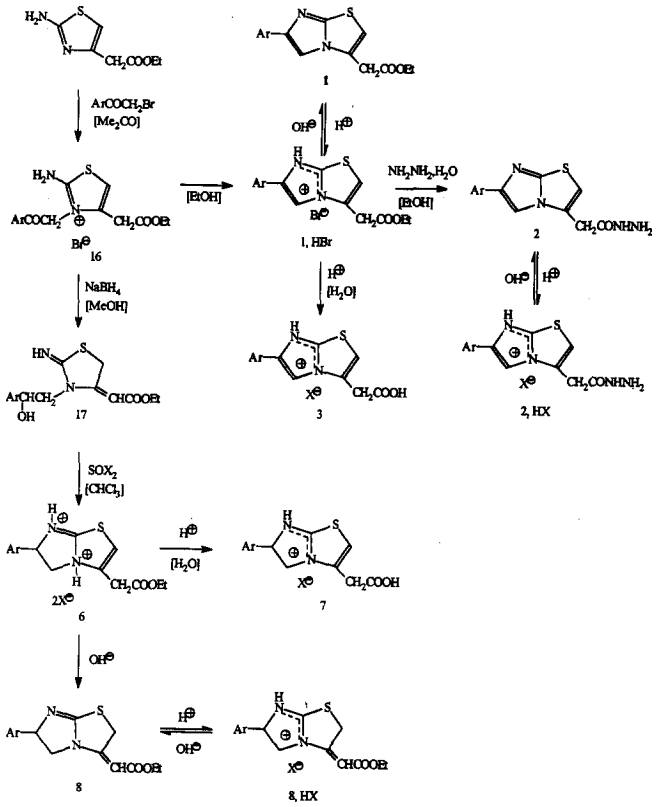
The starting compound for the preparation of **1**, **2**, **3**, **6**, **7**, and **8**, was ethyl(2-aminothiazol-4-yl)acetate, which was described in more detail by Steude [17], Lespiau [18] and Hamel [19]. We used the condensation between ethyl 4-chloro-3-oxobutanoate and *iso*-thiourea in acetone at room temperature [20] (scheme 2).

Ethyl(2-aminothiazol-4-yl)acetate was condensed for 24 h with a substituted phenacylbromide in acetone at room temperature, according to the procedure of Feffer and King [21], to obtain the corresponding thiazolium bromide **16** [7, 8]. Several of the compounds **16** were described by Sawhney *et al* [22]. Upon warming in boiling ethanol for 5–20 min, compounds **16** gave the corresponding imidazo[2,1-*b*]thiazole hydrobromides **1**HBr. The bases were obtained by addition of alkaline solutions up to pH 8–9 (NH_4OH, Na_2CO_3) on the **1**HBr salts. Action of 6 N hydrochloric acid in refluxing water for 30 min hydrolysed the esters **1**HBr to acid salts **3**.

The **1**HBr compounds were treated with hydrazine hydrate in refluxing ethanol, which led to the hydrazides **2** [23]. Action of gaseous HCl in acetone gave the hydrochloride salt of **2**, which is more soluble in water than the corresponding base.



Scheme 1.



Scheme 2.

Compounds **16** permit access to imidazo[2,1-*b*]thiazoles **6**. Using sodium borohydride in methanolic solution, according to the procedure described by Janssen [2] and later by Robert and Panouse [9] for the preparation of **17a**, it is possible to prepare compounds **17** in good yields. When **17** was refluxed in chloroform with a large excess of thionyl chloride, it was cyclized into ethyl(6-aryl-5,6-dihydroimidazo-[2,1-*b*]thiazol-3-yl)acetate dihydrochlorides **6**. In alkaline solution (NH_4OH), **6** gave the corresponding (6-aryl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazol-3-ylidene)acetic ethyl esters **8**, *via* double bond migration [9]. Acidification gave **8**HX by action of 6 N hydrochloric acid in refluxing water for 1 h; the esters **6** were hydrolysed in acid salts **7**.

To obtain the derivatives **9–15**, the starting compound was the (4,5-dihydro-1*H*-imidazol-2-yl)-thiol or 2-mercaptoimidazoline (scheme 3).

Thus, with 1-aryl-4-bromobutane-1,3-diones or ethyl-4-halo-3-oxobutanoate, the 2-mercaptoimidazoline led to the *S*-substituted 4,5-dihydroimidazoles **18** and **19**, respectively. Reflux of **18** and **19** in ethanol for 4–6 h, gave the imidazo[2,1-*b*]thiazoles **9** [24] or **11** [7]. The corresponding base was not released in alkaline solution (NH_4OH, Na_2CO_3). We observed an allylic

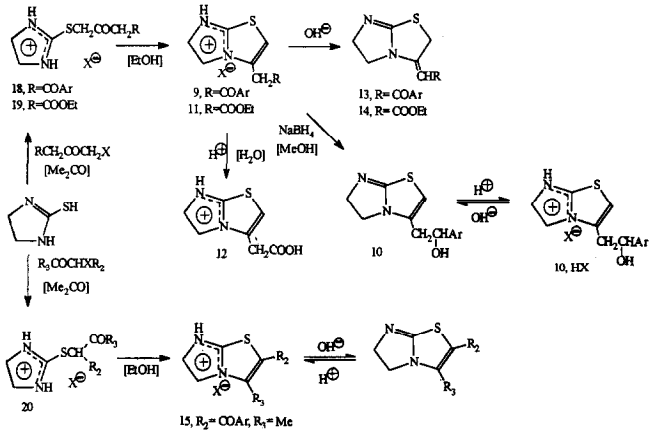
transposition with formation of 1-aryl-2-(2, 3, 5, 6-tetrahydroimidazo[2,1-*b*]thiazol-3-ylidene)ethan-1-ones **13** [24, 25] or the ethyl (2, 3, 5, 6-tetrahydroimidazo[2,1-*b*]thiazol-3-ylidene) acetate **14** [26, 27].

Treatment with HBr (45%) in refluxing water of the ethyl ester **11** led to the corresponding acid **12**. Blackshire and Sharpe [28] described a method of obtaining the same compound by action of methanesulfonic acid in hot water on **11**.

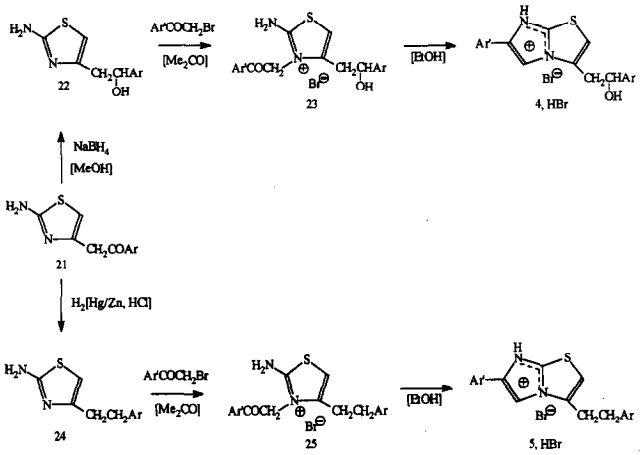
The action of sodium borohydride on **9** provoked the reduction of 1-aryl-2-(5,6-dihydroimidazo[2,1-*b*]thiazol-3-yl) ethanol **10** [24]. The corresponding hydrochlorides were obtained by using gaseous HCl in acetone.

2-Mercaptoimidazoline was also a starting material for other 5,6-dihydroimidazo[2,1-*b*]thiazoles substituted on both C-2 and C-3. 1-Aryl-2-bromobutane-1,3-diones condensed with the 2-mercaptoimidazoline to give the *S*-substituted imidazolines **20**, which are isomers of **18**, when the reaction was carried out in acetone at room temperature [10]. These compounds were cyclized in corresponding dihydroimidazo[2,1-*b*]thiazoles **15** in refluxing ethanol for 2–12 h. It was possible to access **15** directly by warming the 2-mercaptoimidazoline and the appropriate arylbromobutanedione, but the yields were lower.

The starting product for the preparation of imidazo[2,1-*b*]thiazoles **4** was 2-(2-aminothiazol-4-yl)-1-arylethanone **21**, which was synthesized from thiourea and 1-aryl-4-bromobutane-1,3-dione according to the procedure described for ethyl(2-aminothiazol-4-yl) acetate (scheme 4). After $NaBH_4$-reduction of **21** to **22**, we obtained **23** by action of the appropriately substituted phenacylbromide. Finally **23** led to **4**HBr in refluxing ethanol [8]. Alternatively, **21** was reduced by the Clemmensen reaction to **24** and warmed in refluxing ethanol to give **5**HBr [8].



Scheme 3.



Scheme 4.

Immunopharmacology

The determination of the expression of the CD_2 receptor was described in detail by Refouvelet *et al* in a previous study [15]. The method was based on the potential capacity of several compounds to induce regeneration and modulation of CD_2 receptors at the surface of trypsinized human T lymphocytes (LT) using the E-RFC test.

The effect of trypsination and various compounds on E-RFC was assessed after a 2-h incubation. The E-RFC percentages obtained with trypsinized LT after incubation with a compound (X), trypsinized LT (basal level: T) and untrypsinized LT (C) from the same blood donor were determined.

The results were expressed by a 'drug efficacity index' (I)

$$I = \frac{X - T}{C - T} \times 100$$

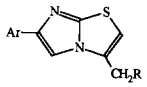
X: % of trypsinized LT + tested compound
C: % of untrypsinized LT
T: % of trypsinized LT

The mean values of I of the tested compounds and tetramisole (reference compound) obtained with 4 assays on 4 blood donors are listed in tables I, II, and III.

Discussion

Although levamisole hydrochloride was 10-fold more active [15], we used tetramisole as a reference, because the studied compounds were achiral or were obtained in a racemic form.

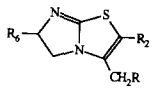
Table I. Physical properties and mean values of I for compounds **1**, **2**, **3**, **4**, and **6**.



Compound	Ar	R	% Yield[a]	mp°c	Formula	Mr	I	I(ref)
1a HBr[b]	Ph	COOEt					5	25
1b HBr	3-MePh	COOEt	75	201-203	$C_{16}H_{16}N_2O_2S$,HBr	381	12	25
1c HBr	4-MePh	COOEt	85	208-210	$C_{16}H_{16}N_2O_2S$,HBr	381	18	25
1d HBr	2-MeOPh	COOEt	60	230-232	$C_{16}H_{16}N_2O_3S$,HBr	397	33	28
1e HBr	4-MeOPh	COOEt	72	202-204	$C_{16}H_{16}N_2O_3S$,HBr	397	22	18
1f HBr	4-BrPh	COOEt	82	192-194	$C_{15}H_{13}BrN_2O_2S$,HBr	446	12	28
1g HBr	4-ClPh	COOEt	88	194-196	$C_{15}H_{13}ClN_2O_2S$,HBr	401.5	15	28
1h HBr	4-FPh	COOEt	86	182-184	$C_{15}H_{13}FN_2O_2S$,HBr	385	13	18
1i HBr	3-NO_2Ph	COOEt	80	182-184	$C_{15}H_{13}N_3O_4S$,HBr	412	42	35
1j HBr	4-NO_2Ph	COOEt	85	208-210	$C_{15}H_{13}N_3O_4S$,HBr	412	38	35
1k HBr	3-AcNHPh	COOEt	70	178-180	$C_{17}H_{17}N_3O_3S$,HBr	424	15	28
1l HBr	4-AcNHPh	COOEt	58	> 260	$C_{17}H_{17}N_3O_3S$,HBr	424	12	28
2a HCl	Ph	$CONHNH_2$	70	> 260	$C_{13}H_{12}N_4OS$,HCl	308.5	19	25
2b	3-MePh	$CONHNH_2$	52	180-182	$C_{14}H_{14}N_4OS$	286	< 1	18
2c	4-Meph	$CONHNH_2$	58	177-179	$C_{14}H_{14}N_4OS$	286	< 1	18
2d	2-MeOPh	$CONHNH_2$	45	205-207	$C_{14}H_{14}N_4O_2S$	302	33	28
2e	4-MeOPh	$CONHNH_2$	52	182-184	$C_{14}H_{14}N_4O_2S$	302	14	35
2f	4-BrPh	$CONHNH_2$	88	219-221	$C_{13}H_{11}BrN_4OS$	351	18	35
2g	4-ClPh	$CONHNH_2$	86	214-216	$C_{13}H_{11}ClN_4OS$	306.5	20	35
2h	4-FPh	$CONHNH_2$	70	138-140	$C_{13}H_{11}FN_4OS$	290	15	25
2i	3-NO_2Ph	$CONHNH_2$	80	229-231	$C_{13}H_{11}N_5O_3S$	317	16	32
2j	4-NO_2Ph	$CONHNH_2$	80	173-175	$C_{13}H_{11}N_5O_3S$	317	15	32
2k	3-AcNHPh	$CONHNH_2$	75	210-212	$C_{15}H_{15}N_5O_2S$	329	< 1	30
2l	4-AcNHPh	$CONHNH_2$	70	> 260	$C_{15}H_{15}N_5O_2S$	329	18	32
3a HCl	Ph	COOH	60	> 260	$C_{13}H_{10}N_2O_2S$,HCl	294.5	< 1	29
4 HBr[c]	Ph	CH(OH)Ph					10	30
6 HBr[d]	Ph	CH_2Ph					20	25

[a]Calculated from ethyl(2-aminothiazol-4-yl)acetate for **1** and **3** from **1**HBr for **2**; [b]ref [7]; [c]ref [8]; [d]ref [8].

Table II. Physical properties and mean values of I for compounds **6**, **7**, **9**, **10**, **11**, **12**, and **15**.



Compound	R	R_2	R_6	%Yield[a]	mp°c	Formula	Mr	I	I(ref)
6a 2HCl[b]	COOEt	H	Ph					< 1	20
6b 2HCl	COOEt	H	3-MePh	53	230-232	$C_{16}H_{18}N_2O_2S,2HCl$	375	< 1	33
6c 2HCl	COOEt	H	4-MePh	38	150-152	$C_{16}H_{18}N_2O_2S,2HCl$	375	10	19
6d 2HCl	COOEt	H	2-MeOPh	30	193-195	$C_{16}H_{18}N_2O_3S,2HCl$	391	< 1	19
6e 2HCl	COOEt	H	4-MeOPh	42	190-192	$C_{16}H_{18}N_2O_3S,2HCl$	391	20	19
6f 2HCl	COOEt	H	4-BrPh	60	256-258	$C_{15}H_{15}BrN_2O_2S,2HCl$	440	9	28
6g 2HCl	COOEt	H	4-ClPh	53	170-180	$C_{15}H_{15}ClN_2O_2S,2HCl$	395.5	32	28
6h 2HCl	COOEt	H	4-FPh	15	190-194	$C_{15}H_{15}FN_2O_2S,2HCl$	379	< 1	19
6i 2HCl	COOEt	H	3-NO_2PH	62	> 260	$C_{15}H_{15}N_3O_4S,2HCl$	406	35	28
6j 2HCl	COOEt	H	4-NO_2Ph	45	> 260	$C_{15}H_{15}N_3O_4S,2HCl$	406	32	28
7 HCl	COOH	H	Ph	82	> 260	$C_{13}H_{12}N_2O_2S,HCl$	296.5	15	25
9 HBr[c]	COPh	H	H					15	30
10[d]	CH(OH)Ph	H	H					10	25
11 HBr[e]	COOEt	H	H					< 1	25
12 HBr	COOH	H	H	90	> 260	$C_7H_8N_2O_2S,HBr$	265	< 1	36
15 HBr[f]	H	COPh	H					< 2	36

[a]Calculated from **17** for **6**, from **6a** for **7** and from **11**HBr for **12**HBr; [b]ref [9]; [c]ref [24]; [d]ref [24]; [e]ref [7]; [f]ref [10].

After 2 h incubation with trypsinized LT, most of the compounds had a positive drug efficacity index. This observation confirms their immunostimulant effect on the expression of the CD_2 receptor and on the regeneration of impaired CD_2 by trypsin in particular. Nevertheless, most show a weaker effect than tetramisole hydrochloride.

However, the presence of an aryl group appears to be advantageous and compounds **11**, **12**, and **13** are weakly active. If the aryl group was on C-6, as in tetramisole, the nature of the substituents changed the immunological response. Methoxy groups (**1d**, **1e**, **2d**, **2e**) and nitro groups (**1i**, **1j**, **6i**) increase notably the activity and therefore index compound > index tetramisole. The effect of halogens was inconsistent: either they increased the regeneration of the CD_2 receptor, like chlorine, **6g**, or they had no significant effect, such as bromine and fluorine.

The ester derivatives **1** and **6** had a higher activity than the hydrazide compounds **2**, and acids **3** and **7**. The reduction of the 5,6-double bond was not significant. In the same way, the 2,3,5,6-tetrahydroimidazothiazoles **8** were weakly active even if there was an aryl group on the C-6 like in tetramisole.

When the aryl group was on a side chain (**9** and **15**), the substituent effects were not significant.

From these observations, it is difficult to propose an SAR approach. We can state that the presence of an

aryl group in the 6 position, like in tetramisole, is a good parameter, especially with a methoxy or nitro group. These substituents could permit a better penetration into cells. Nevertheless, this study confirms the interest of imidazo[2,1-*b*]thiazol cycle for an *in vitro* immunoactivity on human LT, although levamisole is the best compound in this series.

We believe that product of the condensation of a thiazole ring with an imidazole ring remains an important structural element for immunological properties. We are presently developing a program on thiadiazabicycloalkanes, which have the same heterocyclic coalescence as imidazo[2,1-*b*]thiazole [29, 30].

Experimental protocols

Chemistry

Melting points were determined on a Kofler bloch and are uncorrected. IR spectra were recorded on a Philips Unicam SP 1100 infrared spectrophotometer. ^{1}H-NMR spectra were determined on a Perkin–Elmer R24A (60 MHz) or Brucker AC 200 (200 MHz); spectrometric chemical shifts are reported in ppm relative to internal Me_4Si. Elemental analyses (C, H, N, and halogen) were in agreement with calculated values (within ± 0.4%).

Ethyl(6-arylimidazo [2,1-b]thiazol-3-yl) acetate hydrobromides 1HBr
These compounds were obtained according to the procedure described by Robert *et al* [7].

(6-Arylimidazo[2,1-b]thiazol-3-yl)acetohydrazides 2
These compounds were prepared according to the procedure described by Kühmstedt *et al* [23].

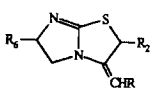
To obtain the corresponding hydrochloride salt, a solution of **2** (0.005 mol) in acetone was stirred with 2 ml 6 N HCl for 30 min. The precipitated salt was filtered off, washed with acetone and recrystallized from ethanol. Compound **2a**HCl: yield 70%; mp > 260°C; IR (cm^{-1}): 3400, 3300, 3280 (NH), 1650 (C=O); ^{1}H-NMR (DMSO-d_6 + TFA): 3.80 (s, CH_2), 6.90 (s, H^2), 8.30 (s, H^5), 7.20–7.90 (m, 5H, Ph), 9.90 (s, NH_2, NH, NH$^+$).

(6-Arylimidazo[2,1-b]thiazol-3-yl)acetic acids 3
To a suspension of **1**HBr (0.005 mol) in 20 ml water, was added 5 ml concentrated HCl. The mixture was refluxed for 30 min. The solution was concentrated to 5 ml. After cooling the hydrochloride salt of **3** crystallized (table III). Compound **3a**HCl: yield 60%; mp > 260°C; IR (cm^{-1}): 3400 (NH$^+$), 1720 (C=O). ^{1}H-NMR (DMSO-d_6): 4.20 (s, CH_2-COOH), 7.50 (s, H^2), 8.50 (s, H^5), 7.30–7.90 (m, 5H, Ph), 11 (s, broad, OH and NH$^+$).

Ethyl(6-aryl-5,6-dihydroimidazo[2,1-b]thiazol-3-yl)acetate dihydrochloride 6
This compound was obtained according to the procedure described by Robert and Panouse [9].

(6-Phenyl-5,6-dihydroimidazo[2,1-b]thiazol-3-yl)acetic acid hydrochloride 7HCl
A solution of **6a**·2HCl (1.80 g, 0.005 mol) in 50 ml water was treated with 5 ml 6 N HCl and refluxed for 1 h. After cooling, the product was collected and washed with acetone, yielding 1.2 g (82%) of 7HCl: mp > 260°C; IR (cm^{-1}): 3000 (NH$^+$), 1750 (C=O); ^{1}H-NMR (DMSO-d_6 + TFA): 7.50 (s, H^2), 8.60 (s, H^5), 7.40–7.90 (m, 5H, Ph), 10.50 (s, NH$^+$, OH).

Table III. Mean values of I for compounds **8**, **13**, and **14**.



Compound	*R*	R_2	R_6	*I*	*I (ref)*
8 HCl[a]	COOEt	H	Ph	12	25
13[b]	COPh	H	H	17	25
14[c]	COOEt	H	H	10	25

[a]Ref [9]; [b]ref [24]; [c]ref [7].

Ethyl(6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b]thiazol-3-ylidene)acetate 8
This compound was prepared as described by Robert and Panouse [9].

Ethyl(6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b]thiazol-3-ylidene)acetate hydrochloride 8HCl
This compound was prepared as described by Robert and Panouse [9].

1-Phenyl-2-(6-phenylimidazo[2,1-b]thiazol-3-yl)ethanol hydrobromide 4HBr
This compound was prepared as described by Robert *et al* [8].

6-Phenyl-3-(2-phenylethyl)imidazo[2,1-b]thiazole hydrobromide 5HBr
This compound was prepared as described by Robert *et al* [8].

1-Phenyl-2-(5,6-dihydroimidazo[2,1-b]thiazol-3-yl)ethan-1-one hydrobromide 9HBr
This compound was prepared as described by Hablouj *et al* [24].

1-Phenyl-2-(5,6-dihydroimidazo[2,1-b]thiazol-3-yl)ethanol 10
This compound was prepared as described by Hablouj *et al* [24].

1-Phenyl-2-(2,3,5,6-tetrahydroimidazo[2,1-b]thiazol-3 ylidene)ethan-1-one 13
This compound was prepared as described by Hablouj *et al* [24].

Ethyl(5,6-dihydroimidazo[2,1-b]thiazol-3-yl)acetate hydrobromide 11
This compound was prepared as described by Robert *et al* [7].

(5,6-Dihydroimidazo[2,1-b]thiazol-3-yl)acetic acid hydrobromide 12HBr
Treatment of **11** (2.93 g, 0.01 mol) with 10 ml HBr (45% in aqueous solution) in boiling water for 30 min, gave 2.2 g (90%) of the hydrobromide salt **12**HBr: mp > 260°C; IR (cm^{-1}): 3300 (OH), 3050 (NH$^+$), 1740 (C=O); ^{1}H-NMR (DMSO-d_6 + TFA): 4.00 (s, CH_2-COOH), 4.50 (s, 4H imidazoline), 6.95 (s, H^2), 10.10 (s, NH$^+$, OH).

Ethyl(2, 3, 5, 6-tetrahydroimidazo[2,1-b]thiazol-3-ylidene) acetate ***14***
This compound was prepared as described by Robert *et al* [7, 26].

Phenyl(3-methyl-5,6-dihydroimidazo[2,1-b]thiazol-2-yl)-acetone hydrobromide ***15HBr***
This compound was prepared as described by Robert and Panouse [10].

Immunopharmacology

Cell preparation
Normal human mononuclear cells (LT) were collected from blood donors and were isolated on a Ficoll-hypaque gradient ($d = 1.077$) and adjusted to 10^3 cells/mm^3 in RPMI–1640. The cells were incubated in the presence of an equal volume of a 0.5% trypsin solution (Merieux, Lyon, France) in phosphate buffer saline solution (PBS). The mixture was shaken manually every 5–7 min. After 30 min incubation at 37°C, the cells were washed in RPMI–1640 and in RPMI containing a 5% complement-free human AB serum, which had been previously absorbed with sheep erythrocytes. The cells were suspended in the latter medium at the concentration of 10^3 cells/mm^3, regulated according to the volume of drug solution added to the medium in the different assays. Control cells underwent a similar handling in absence of trypsin.

Drug addition
The drugs were dissolved in RPMI–1640 to a concentration of 4.6×10^{-6} mmol/ml. They were added to trypsinized LT just before incubation for culture. Tetramisole was used as a reference compound.

E-rosette forming cells
Determination of the effects of trypsination and various drugs on E-RFC were asssessed after 2 h incubation. A sample of the cells was washed with RPMI–1640. The cells were resuspended in RPMI–1640 with 0.1 ml of 0.5% sheep erythrocytes and incubated at 4°C overnight. The pellets were gently resuspended and the percentage of E-RFC obtained with trypsinized and untrypsinized cells were determined in a haemocytometer.

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