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Short communication

Synthesis and mitogenic activity of new imidazo[2,1-*b*]thiazoles

A Andreani[1], M Rambaldi[1], A Locatelli[1], AM Isetta[2]

[1]*Dipartimento di Scienze Farmaceutiche, University of Bologna, Via Belmeloro 6, Bologna;*
[2]*Immunology Line R&D, Farmitalia-Carlo Erba, Nerviano Milan, Italy*

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Summary — Eight new imidazo[2,1-*b*]thiazoles were synthesized in order to evaluate their ability to stimulate the proliferation of thymic lymphocytes. The 2-chloro (**5–8**) proved more active than the 2,3-dimethyl derivatives (**1–4**): in particular compounds **6** and **7** were more active than levamisole.

Résumé — **Synthèse et activité mitogène de nouveaux imidazo[2,1-*b*]thiazoles.** *Huit imidazo[2,1-b]thiazoles ont été synthétisés en vue d'évaluer leur capacité à stimuler la prolifération de lymphocytes du thymus. Les composés chlorés en position 2 (**5–8**) se sont avérés plus actifs que les composés diméthylés en position 2,3 (**1–4**). Les composés **6** et **7** se sont en particulier montrés plus actifs que le lévamisole.*

imidazo[2,1-*b*]thiazoles / levamisole / mitogenic activity / immunomodulation

Introduction

Modulation of the immune response is employed for the therapy of an increasing number of diseases. This may be achieved with natural or synthetic products as pointed out by numerous reviews on this topic [1–10] and levamisole, (–)2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole [11, 12], is one of the most known synthetic immunomodulators. We have prepared a number of imidazo[2,1-*b*]thiazoles and described their activity as antitumor [13–17], antiinflammatory [18], cardiotonic [19–23] and diuretic agents [24]. In this paper we report the synthesis of new imidazo[2,1-*b*]thiazoles and their effect on thymocyte proliferation compared to that of levamisole; among the numerous immunointerfering activities reported for levamisole, we chose this test [25] because we found it highly reproducible and easy to perform.

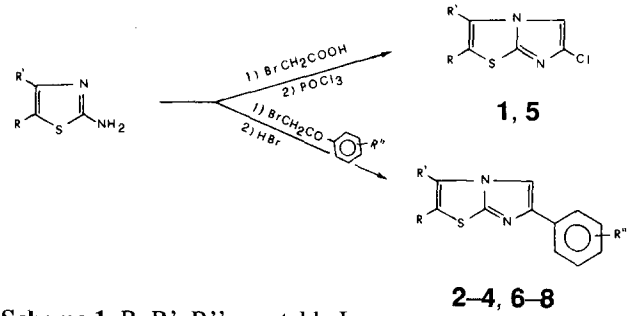
Chemistry

The starting material for the synthesis of these new derivatives was 2-amino-4,5-dimethylthiazole or 2-amino-5-chlorothiazole: it was treated with bromoacetic acid and then with phosphorus oxychloride in order to obtain the 6-chloro derivatives **1**, **5** or with the appropriate α-bromoacetophenone in order to prepare the other 6-substituted derivatives **2–4**, **6–8** (scheme 1).

The analytical data of compounds **1–8** are reported in tables I, II.

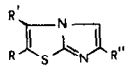
Results and Discussion

Table III reports the ^3H thymidine incorporation caused by the 8 imidazo[2,1-*b*]thiazoles tested in comparison to levamisole. The 6-chloro derivatives (**1**, **5**) and the 6-(3-nitro-4-chloro)-phenyl derivatives (**4**, **8**) were inactive. Compounds bearing a *p*-tolyl



Scheme 1. R, R', R'': see table I

Table I. Compounds **1–8**.



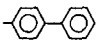
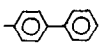
Compound	R	R'	R''	Formula (mw)	Mp (°C)
1	CH_3	CH_3	Cl	$C_7H_7ClN_2S$ (186.7)	128–130
2	CH_3	CH_3		$C_{14}H_{14}N_2S$ (242.3)	183–186
3	CH_3	CH_3		$C_{19}H_{16}N_2S$ (304.4)	200–203
4	CH_3	CH_3		$C_{13}H_{10}ClN_3O_2S$ (307.7)	219–224
5	Cl	H	Cl	$C_5H_2Cl_2N_2S$ (193.1)	162–165
6	Cl	H		$C_{12}H_9ClN_2S$ (248.7)	202–204
7	Cl	H		$C_{17}H_{11}ClN_2S$ (310.8)	249–251
8	Cl	H		$C_{11}H_5Cl_2N_3O_2S$ (314.1)	197–203

Table II. IR and ^{1}H-NMR data of compounds **1–8**; i.t. = imidazo[2,1-*b*]thiazole.

	ν_{max} (cm^{-1})	δ (ppm) in DMSO-d_6
1	1280,1225, 950,750	2.39(6H,s,CH_3) 8.18(1H,s,i.t.)
2	1545,1305, 820,725	2.34(3H,s,C_6H_4-CH_3) 2.35(6H,s,CH_3) 7.45(2H,d,arom.,J=9Hz) 8.01(2H,d,arom.,J=9Hz) 8.40(1H,s,i.t.)
3	1180,840 765,730	2.39(6H,s,CH_3) 7.72(3H,m,arom.) 8.0(4H,m,arom.) 8.25(2H, m,arom.) 8.55(1H,s,i.t.)
4	1515,1300 1190,730	2.40(6H,s,CH_3) 8.05(1H,d,arom.,J=9Hz) 8.42(1H,dd,arom., J=9Hz,J=2Hz) 8.77(1H,s,i.t.) 8.78(1H,d,arom.,J=2Hz)
5	1330,1245, 990,700	8.21(1H,s,i.t.) 8.59(1H,s,i.t.)
6	1545,990, 820,730	2.38(3H,s,CH_3) 7.45(2H,d,arom.,J=9Hz) 8.01(2H,d,arom., J=9Hz) 8.48(1H,s,i.t.) 8.58(1H,s,i.t.)
7	1545,1185, 990,730	7.72(3H,m,arom.) 8.0(4H,m,arom.) 8.25(2H,m,arom.) 8.59 (1H,s,i.t.) 8.61(1H,s,i.t.)
8	1515,1190 990,725	8.05(1H,d,arom.,J=9Hz) 8.42(1H,dd,arom.,J=9Hz,J=2Hz) 8.65(1H,s,i.t.) 8.77(1H,s,i.t.) 8.78(1H,d,arom.,J=2Hz)

group in position 6 (**2**, **6**) showed a very different behaviour: compound **2** proved toxic, whereas compound **6** increased the proliferation of thymic lymphocytes and this activity was higher than that of levamisole. Compound **3** proved more active than levamisole only at low doses (< 25 µg/ml), while the other biphenylyl derivative, bearing a chlorine in position 2 (**7**), was even more active than **6**. From these preliminary results it already seems clear that 2-chloro-6-phenylimidazo[2,1-*b*]thiazole may be a suitable moiety in the development of new immunostimulants: the synthesis of analogs bearing different substituents at the phenyl ring is now in progress.

Experimental protocols

Chemistry

The melting points were taken on an Electrothermal apparatus and are uncorrected. Elemental analyses of the new compounds (**1–8**) were within ± 0.4% of the theoretical values. The IR spectra were recorded on a Perkin–Elmer 298 instrument and the ^{1}H-NMR spectra on a Varian EM 390 (90 MHz) using TMS as an internal standard. α-Bromo-4-methylacetophenone and α-bromo-4-phenyl-acetophenone were commercially available products; α-bromo-3-nitro-4-chloroacetophenone was prepared from α-bromo-4-chloroacetophenone according to the literature [26, 27].

*6-Chloro derivatives **1**, **5***

The substituted 2-amino-thiazole (15 mmol) was dissolved in 20 ml EtOH and refluxed for 6 h with the equivalent of bromoacetic acid (2.1 g). The resulting precipitate was collected and refluxed for 3 h with 30 ml $POCl_3$. The reaction mixture was evaporated under reduced pressure and the residue poured onto ice; the solution was basified with 20% NH_4OH and the crude 6-chloroimidazo[2,1-*b*]thiazole thus obtained was crystallized from ethanol with a yield of ≈ 40%.

*6-Phenyl derivatives **2–4**, **6–8***

The substituted 2-amino-thiazole (15 mmol) was dissolved in 30 ml acetone and treated with 15 mmol of the appropriate α-bromoacetophenone. The mixture was refluxed for 3 h and the resulting precipitate was collected and treated with 50 ml of 2 N HBr. After 1 h reflux, the solution was basified with 20% NH_4OH: the crude imidazo[2,1-*b*]thiazole bearing a substituted phenyl ring in position 6 was collected and crystallized from ethanol with a yield of ≈ 30%.

Mitogenic activity

Thymic lymphocytes were collected from 8-wk-old, C57B1/6 female mice (CRI), and cultivated *in vitro* in the presence of a suboptimal dose of the mitogen concanavalin A (5 µg/ml). The compounds, which were insoluble in water, were dissolved in DMSO at the concentration of 5 mg/ml, then diluted in complete medium (RPMI 1640 supplemented with penicillin, streptomycin, L-glutamine and 10% FCS). 0.1 ml of the cell suspension (7.5 x 10^5) was set up in 96-well microtiter plates with 0.1 ml of 2-fold dilutions of the products and the mixture incubated at 37°C in a humidified, 5% CO_2 incubator for 72 h. Methyl-3H-thymidine (3H TdR, 0.2 µCi/well) was added during the last 18 h of incubation, then the cultures were harvested on glass fiber filters by using an automated cell harvester (Skatron). The cell proliferation was expressed as the mean ± standard deviation of radioactivity (cpm) taken from triplicate cultures.

Table III. Mitogenic activity of compounds **1–8** on thymic lymphocytes. Cell harvested from 3 mice, were incubated *in vitro* for 3 d with different concentrations of the test compound, the last 18 h in the presence of 0.2 μCi of ^{3}H-thymidine. Data are reported as mean count per minute (cpm) from triplicate cultures ± standard deviation of the mean. In parenthesis, the T/C x 100 (treated/control x 100) value is reported.

Product	*^{3}H-thymidine uptake (cpm ± SD) μg/ml of the products*					
	50	*25*	*12.5*	*6.25*	*3.125*	*0*
1	1589**± 58(45)	3033 ±245(85)	3612 ±252(101)	3635 ±205(102)	3628 ±395(102)	
2	202**±135 (6)	370**± 77(10)	361**± 28 (10)	993**±167 (28)	3836 ±854(108)	
3	3393 ±213(95)	4398* ±574(124)	5187**±241(146)	5556**±490(156)	5900**±392(166)	
4	1119**±144(31)	1823**±144(51)	2234**±194 (63)	3219 ±186 (90)	4078 ±109(115)	
5	1277**±171(36)	3482 ±252(98)	4038 ±163(113)	4044 ±407(114)	4199 ±363(118)	
6	6644**±161(187)	6557**±731(184)	7031**±220(198)	7179**±543(202)	6700**±566(188)	
7	6910**±276(194)	8057**±114(226)	9077**±323(255)	8648**±512(243)	7664**±299(215)	
8	2771**± 45(78)	2966 ±179(83)	3248 ±319(91)	3502 ±170(98)	4291* ±205(205)	
levamisole	5238**±600(147)	5395**±238(152)	4262* ±483(120)	3915 ±195(110)	4091 ±126(115)	
diluent						3558±344

$^xP < 0.05$; $^{xx}P < 0.01$ (Dunnet's *t* test)

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