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Short communication

Synthesis and hypolipidemic activity of *N*-pyridinyl borodipeptidylamides

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Summary — A series of borodipeptide derivatives **7–9**, which contain a 6-amino-2,4-dimethylpyridine moiety, was prepared in 3 steps. They were evaluated as hypolipidemic agents in rodents at 20 mg/kg per day. The methioninamide and phenylalaninamide derivatives were the most potent compounds demonstrating hypocholesterolemic and hypotriglyceridemic activities in rats. After 14 days, the activity of these compounds was superior to that of clofibrate, at a dose of 200 mg/kg per day.

6-amino-2,4-lutidine / borodipeptide / hypolipidemic activity

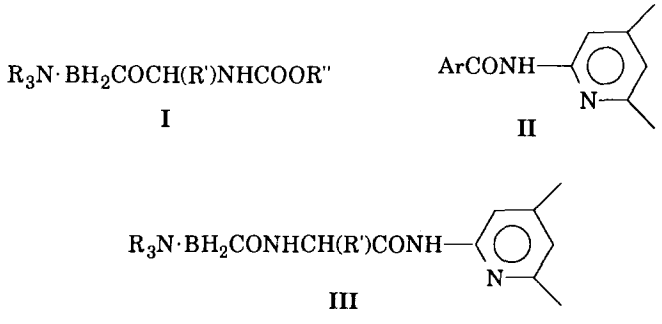
Introduction

The biological activity of substituted borane adducts of amines is now well established [1–4]. In particular, a number of amine carboxyborane derivatives exhibit potent pharmacological effects as antineoplastic [5–8], hypolipidemic [9], antiinflammatory and antiarthritic [10] agents.

Recently, a series of amine carboxyboranes including their amides and esters was reported to lower significantly serum total cholesterol in the mouse; they reduced VLDL and LDL fractions and increased the HDL fraction 14 days after initiation of the treatment [11]. Boron-containing hypolipidemic agents inhibit *de novo* regulatory enzymes for lipid synthesis and block the deposition of hepatic cholesterol esters [11], and may therefore be clinically beneficial in treating atherosclerosis. Moreover, these boron derivatives demonstrated activity in lowering serum triglyceride levels. Borodipeptides **I** were also shown to exhibit antiinflammatory activity but were more interesting as hypolipidemic agents [12].

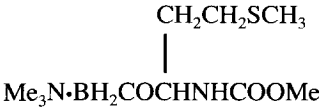
On the other hand, it was shown previously [13] that benzamides **II** issued from 6-amino-2,4-lutidine elicit antiinflammatory properties, partly mediated by inhibition of reactive oxygen species (ROS) production or their scavenging [14]. ROS and free radicals have been implicated in the development of the atherosclerotic lesions [15], and such compounds, which act as antioxidants, may prevent its progression by limiting production of LDL peroxidation and the subsequent transformation of macrophages into foam cells.

These results prompted us to carry out the coupling of the peptidoborane structure **I** with 6-amino-2,4-lutidine to generate new compounds **III** incorporating boron-containing amino acids and our pharmacophore. This paper reports the synthesis of various *N*-pyridinylborodipeptidylamides **III**, the structural requirements for hypolipidemic activity in rodents, and the evaluation of the antiinflammatory effect of the trimethylamine carboxyborane derivatives (**III**: $R_3N = Me_3N$).



Chemistry

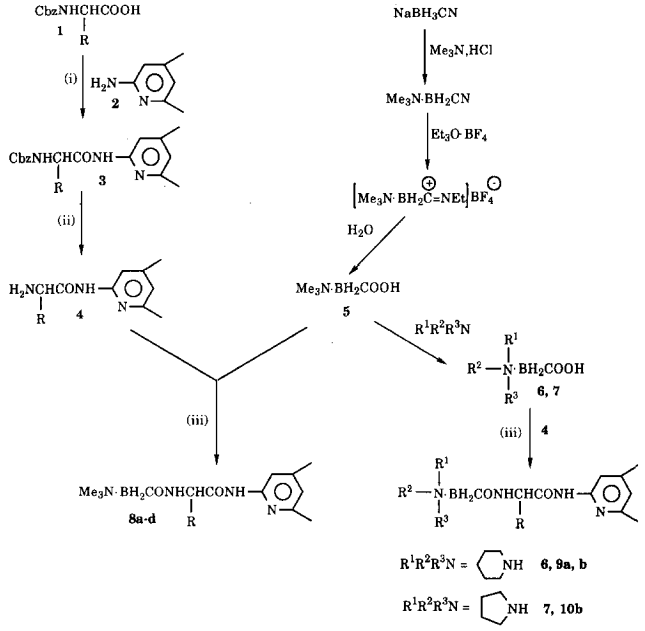
The synthetic pathway followed for the preparation of the title compounds is shown in scheme 1. The N^2-benzyloxycarbonyl-*N*-(4,6-dimethylpyridin-2-yl) α-amino amides **4** were synthesized by carbonyldiimidazole coupling of (*S*)-*N*-benzyloxycarbonyl α-amino acids **1** with 6-amino-2,4-lutidine **2** at room temperature. The *N*-blocking group (Cbz) was then removed by catalytic hydrogenation, over 10% palladium-on-charcoal, at atmospheric pressure. The *N*-pyridinylborodipeptidylamides **8–10** were obtained by reaction of amine carboxyboranes **5–7** with *N*-(4,6-dimethylpyridin-2-yl) α-amino amides **4** using the PPh_3/CCl_4 method [12]. The intermediate formed upon reaction of acid with PPh_3/CCl_4 is expected to be the acyloxyphosphonium salt $[Ph_3POC(O)BH_2NR_3)]Cl$. The reaction of the nitrogen of the amine at the carbonyl carbon then afforded the boron compounds **8a–d**, **9a–b** and **10b** in fairly good yields (table I). Attempts to synthesize these amides using dicyclohexylcarbodiimide as a condensing agent [16] resulted in much lower yields. The boron dipeptide **11** was also synthesized for pharmacological comparison purposes, using the sequence described by Sood *et al* [12].



11

The trimethylamine carboxyborane **5** [3] was obtained at room temperature by hydrolysis of the corresponding *N*-ethylnitrilium salt resulting from the action of triethyloxonium tetrafluoroborate on the trimethylaminocyanoborane (scheme 1). Condensation of sodium cyanoborohydride with trimethylamine hydrochloride in refluxing THF, afforded the intermediate nitrile, in quantitative yield [17]. Compound **5** undergoes a facile exchange reaction, by gentle reflux in excess amine, leading, for example, to the piperidine and pyrrolidine carboxyboranes **6** and **7** [8].

Structural assignments of compounds are based on IR, ^{1}H-NMR and ^{13}C-NMR spectra (see *Experimental protocols*). In particular, the ^{1}H-NMR spectra of compounds **3b**, **8b–d**, **9b** and **10b** are characterized by 2 distinct signals, which are due to the 2 non-equivalent protons of the 3-CH_2. The ^{11}B-NMR spectra show a single broad signal at about 3.5 ppm from the most intense singulet of decaborane, indicating the presence of a single compound.

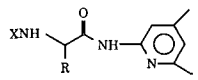


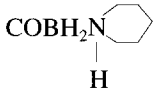
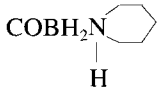
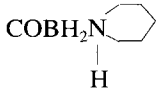
Scheme 1.

Pharmacological study and discussion

Hyperlipidemic Sprague–Dawley male rats were administered *N*-lutidinyl borodipeptidylamides **8–10**, their precursors **3** and the borodipeptide **11** [12], at 20 mg/kg per day ip for 14 d. Clofibrate, a reference hypolipidemic drug, was experimented in the same conditions at 150 mg/kg per day. Serum total cholesterol and triglyceride contents were determined after 10 and 14 d drug treatment (table II). The Cbz-protected α-aminocarboxamides **3a** and **3b** afforded no hypocholesterolemic activity. Conversely, after incorporation of a trimethylamineborylcarbonyl moiety into **3**, the corresponding amides **8a–d** exhibited a significant cholesterol lowering effect (25 to 65%). Replacement of trimethylamine by pyrrolidine did not seem to be beneficial (compared compound **10b** with **8b**), and insertion of a piperidine grouping induced a detrimental effect (compounds **9a** and **9b**). Derivatives **8** that incorporate a bulky R residue (**8b–d**) afforded potent activity in lowering serum cholesterol by 48 to 65%; compound **8b**, with a methionyl grouping, was the most active of all the amides (65% reduction). Comparison of this amide with the corresponding methyl ester **11** (Spielvogel's derivative [12]) enables us to state that the former caused a higher reduction 14 d after administration at 20 mg/kg per day: 65 *versus* 45% ($p < 0.05$). As illustrated by the absence of activity of *N*-lutidinyl

Table I. Physical data of synthesized compounds **3** and **8–10**.



Compound	*X*	*R*	*Molecular formula* M_r	*Yield (%)*	*Mp (°C)*	*Recrystallization solvent*[a]
3a	Cbz	H	$C_{17}H_{19}N_3O_3$, 313.35	80	103	A
3b	Cbz	$CH_2CH_2SCH_3$	$C_{20}H_{25}N_3O_3S$, 387.50	70	87	A
8a	$COBH_2NMe_3$	H	$C_{13}H_{23}BN_4O_2$, 278.16	55	106	B
8b	$COBH_2NMe_3$	$CH_2CH_2SCH_3$	$C_{16}H_{29}BN_4O_2S$, 352.31	60	120	B
8c	$COBH_2NMe_3$	CH_2Ph	$C_{20}H_{29}BN_4O_2$, 368.28	51	82	B
8d	$COBH_2NMe_3$	$CH_2CH(CH_3)_2$	$C_{17}H_{31}BN_4O_2$, 334.27	58	oil	
9a		H	$C_{15}H_{25}BN_4O_2$, 304.20	58	140	B
9b		$CH_2CH_2SCH_3$	$C_{18}H_{31}BN_4O_2S$, 378.34	30	125	B
10b		$CH_2CH_2SCH_3$	$C_{17}H_{29}BN_4O_2S$, 364.32	48	119	B

[a]After purification by silica-gel chromatography eluting with diethyl ether (**3a**, **3b** and **8d**) or Et_2O and afterwards a 9.5:0.5 CH_2Cl_2/EtOH mixture (**8a–c**, **9a**) or a 5.5:4.0:0.5 Et_2O/CH_2Cl_2/EtOH mixture (**9b** and **10b**). Recrystallization solvents A: $(Me_2CH)_2O$; B:Et_2O.

α-aminocarboxamides **3a** and **3b** and the moderate activity demonstrated by boropeptidylamides **8a** and **9a**, the simultaneous presence of a boron moiety and a bulky R residue seems necessary to induce a marked hypolipidemic activity.

Similar structural requirements were generally observed for hypotriglyceridemic activity. Nevertheless, in the trimethylamine series **8**, the glycyl derivative **8a** elicited a high activity (48% reduction) and the most potent amide, compound **8c**, resulted from incorporation of a phenylalanine residue. This compound was more effective than clofibrate at 150 mg/kg per day considering both hypotriglyceridemic and hypocholesterolemic activities: 59 and 60% *versus* 47 and 55%, respectively, after 14 d administration. It is noteworthy that, after oral administration (150 to 200 mg/kg per day for 16 d), clofibrate exhibits only a moderate hypocholesterolemic effect (10–20%) but it lowers more significantly (25–30%) the serum triglyceride level [9, 18–20].

The antiinflammatory activity of the trimethylamine series **8** was assessed by inhibition of the carrageenen-induced rat-paw edema [21] after oral administration at 35 mg/kg. Edema was significantly inhibited by the 4 test compounds **8a–d** (table III); the glycinamide **8a** exhibited the highest potency, affording 99% inhibition. It has been stated previously that the *N*-(4,6-dimethylpyridin-2-yl) benzamide derivatives exert their antioedematous effect partly by a 'redox type' mechanism. As *in vitro* and *in vivo* studies support the hypothesis that there is an oxidative modification of LDL that targets it for uptake by the macrophage through the scavenger receptor [22], it is obvious that such compounds could have some utility for reducing atherosclerotic lesions.

Conclusion

Although a larger set of compounds is necessary to gain a better insight into the stuctural requirements for

Table II. Hypolipidemic activity of test compounds 20 mg/kg per day (ip) in rats after 14 d administration ($N = 10$).

Compound	*Inhibition (%)*			
	Serum cholesterol		*Serum triglycerides*	
	day 10	*day 14*	*day 10*	*day 14*
3a	22 ± 20	28 ± 11	NA[a]	NA[a]
3b	21 ± 20	27 ± 10	NA[a]	20 ± 14
8a	40 ± 18	25 ± 2	45 ± 15	48 ± 2
8b	48 ± 8	65 ± 3[b]	4 ± 8	38 ± 7
8c	24 ± 11	60 ± 3[b]	14 ± 6	59 ± 4[b]
8d	23 ± 10	48 ± 3	16 ± 4	43 ± 3
9a[c]	–	28 ± 11	–	11 ± 45
9b[c]	17 ± 2	29 ± 10	16 ± 8	19 ± 10
10b	36 ± 8	37 ± 4	37 ± 11	38 ± 3
11	59 ± 16	45 ± 4	51 ± 6	50 ± 1
Clofibrate (150 mg/kg per day)	67 ± 5	55 ± 9	69 ± 9	47 ± 1

[a]NA: non-active; [b]significantly different from the value of compound **11** [12] ($p < 0.05$); [c]at 20 mg/kg dose, toxic effects were present in animals.

Table III. Antiinflammatory activity of test compounds **8** at 35 mg/kg *per os* in rats.

Compound	*Carrageenen-induced rat-paw edema inhibition (%)*
8a	99 ± 0
8b	80 ± 5
8c	73 ± 6
8d	96 ± 4

cholesterol-lowering activity, the present results suggest that a trimethylamine boron moiety and steric crowding at the R are necessary for potent activity in these *N*-lutidinylboropeptidylamides. Potentially useful hypolipidemic agents should significantly lower LDL and VLDL cholesterol content and increase HDL cholesterol content. Work is now in progress to evaluate the effect of borodipeptidylamides **8a–d** on the different lypoprotein fractions. Finally, investigation of their ability to inhibit LDL modification and oxidation will give information about the interest of the 6-amino-2,4-lutidine pharmacophore in limiting atherosclerosis progression.

Experimental protocols

Chemistry

Melting points were determined in open capillary tubes with a Büchi apparatus. IR spectra were obtained on a Beckman IR 4230 spectrometer using KBr pellets or using the compound on a NaCl disk. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC 250 and chemical shifts are reported in δ units (ppm) relative to internal tetramethylsilane. ^{11}B-NMR spectra were recorded on a Bruker ARX 400 and chemical shifts are given in δ values downfield from the most intense singlet of internal decaborane. Elemental analyses for C, H, N were performed using a Perkin Elmer C, H, N 240.

General procedure for the synthesis of N^2-benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl) α-amino amides 3

A solution of (*S*)-*N*-benzyloxycarbonyl α-amino acid (10 mmol) and carbonyldiimidazole (10 mmol) in anhydrous THF was stirred for 1 h at room temperature and then 6-amino-2,4-lutidine (10 mmol) was added. Stirring at room temperature was continued for 10 h. The solvent was removed *in vacuo*. Purification of the residue, by chromatography on silica gel, using ether as the eluent, gave the product as a white solid.

N^2-Benzyloxycarbonyl-N-(4,6-dimethylpyridin-2-yl) glycinamide ***3a***. IR (KBr) ν 3220 (NH), 1710 (C=O) and 1670 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.33 (3H, s, 4-CH_3), 2.39 (3H, s, 6-CH_3), 4.05 (2H, d, $J = 5.0$ Hz, CH_2NH), 5.16 (2H, s, CH_2Ph), 5.58 (1H, m, NH), 6.75 (1H, s, H^5), 7.36 (5H, m, Ph), 7.82 (1H, s, H^3), 8.34 (1H, m, pyr NH). Anal $C_{17}H_{19}N_3O_3$ (C, H, N).

*N^2-Benzyloxycarbonyl N-(4,6-dimethylpyridin-2-yl) methioninamide **3b***. IR (KBr) ν 3320 (NH), 3200 (NH), 1730 (C=O) and 1660 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.84 (1H, m, 1H 3-CH_2), 2.14 (3H, s, SCH_3), 2.16 (1H, m, 1H 3-CH_2), 2.31 (3H, s, 4-CH_3), 2.40 (3H, s, 6-CH_3), 2.59 (2H, m, CH_2CH_2S), 4.54 (1H, m, CH), 5.12 (1H, d, J = 12.2 Hz, 1H CH_2Ph), 5.16 (1H, d, J = 12.2 Hz, 1H CH_2Ph), 5.67 (1H, d, J = 7.9 Hz, NH), 6.75 (1H, s, H^5), 7.35 (5H, m, Ph), 7.83 (1H, s, H^3), 8.55 (1H, m, pyr NH). Anal $C_{20}H_{25}N_3O_3S$ (C, H, N).

*General procedure for the synthesis of boron compounds **8***
Trimethylamine-carboxyborane (10 mmol), *N*-(4,6-dimethylpyridin-2-yl) α-amino amides **4** (10 mmol) and triphenylphosphine (11 mmol) were dissolved in anhydrous acetonitrile. To this solution, triethylamine (10 mmol) and carbon tetrachloride (10 mmol) were added and the mixture was stirred at room temperature. After 10 h, the solvent was removed under reduced pressure. Purification of the residue by chromatography on silica gel gave the desired products.

*N-(4,6-Dimethylpyridin-2-yl) N-[(trimethylamine boryl) carbonyl]glycinamide **8a***. IR (KBr) ν 3405 (NH), 3240 (NH), 2320 (BH), 1680 (C=O) and 1610 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.29 (3H, s, 4-CH_3), 2.37 (3H, s, 6-CH_3), 2.78 (9H, s, $N(CH_3)_3$), 4.04 (2H, d, J = 5.9 Hz, CH_2), 6.15 (1H, m, NH), 6.70 (1H, s, H^5), 7.81 (1H, s, H^3), 8.62 (1H, m, pyr NH). ^{13}C-NMR ($CDCl_3$) δ 21.12 (4-CH_3), 23.77 (6-CH_3), 42.94 (CH_2), 52.36 ($N(CH_3)_3$), 111.43 (C^3), 120.15 (C^5), 149.58 (C^4), 150.24 (C^2), 156.24 (C^6) 169.35 (C=O). ^{11}B-NMR (DMSO) δ 3.56. Anal $C_{13}H_{23}BN_4O_2$ (C, H, N).

*N-(4,6-Dimethylpyridin-2-yl) N-[(trimethylamine boryl) carbonyl]methioninamide **8b***. IR (KBr) ν 3390 (NH), 3200 (NH), 2350 (BH), 1685 (C=O) and 1605 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.93 (1H, m, 1H, 3-CH_2), 2.10 (3H, s, SCH_3), 2.22 (1H, m, 1H, 3-CH_2), 2.24 (3H s, 4-CH_3), 2.36 (3H, s, 6-CH_3), 2.57 (2H, m, SCH_2), 2.76 (9H, s, $N(CH_3)_3$), 4.83 (1H, m, CH), 6.03 (1H, d, J = 7.9 Hz, NH), 6.69 (1H, s, H^5), 7.80 (1H, s, H^3), 8.89 (1H, s, pyr NH). ^{13}C-NMR ($CDCl_3$) δ 16.05 (SCH_3), 21.80 (4-CH_3), 24.47 (6-CH_3), 31.24 (CH_2), 31.59 (CH_2), 51.87 (CH), 53.03 ($N(CH_3)_3$), 112.04 (C^3), 120.82 (C^5), 150.25 (C^4), 151.30 (C^2), 157.03 (C^6), 171.71 (C=O). ^{11}B-NMR (DMSO) δ 3.56. Anal $C_{16}H_{29}BN_4O_2S$ (C, H, N).

*N-(4,6-Dimethylpyridin-2-yl) N-[(trimethylamine boryl) carbonyl]phenylalaninamide **8c***. IR (KBr) ν 3390 (NH), 3370 (NH), 2360 (BH), 1680 (C=O) and 1610 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.38 (3H, s, 4-CH_3), 2.44 (3H, s, 6-CH_3), 2.74 (9H, s, $N(CH_3)_3$), 3.15 (1H, dd, J = 7.8 and 14.0 Hz, 1H, 3-CH_2), 3.36 (1H, dd, J = 5.8 and 14.0 Hz, 1H, 3-CH_2), 5.14 (1H, m, CH), 6.34 (1H, d, J = 7.8 Hz, NH), 6.78 (1H, s, H^5), 7.34 (5H, m, Ph), 7.95 (1H, s, H^3), 9.23 (1H, s, pyr NH). ^{13}C-NMR ($CDCl_3$) δ 21.85 (4-CH_3), 24.46 (6-CH_3), 37.88 (CH_2), 52.98 ($N(CH_3)_3$), 53.61 (CH), 112.08 (C^3), 120.87 (C^5), 127.27, 129.15, 129.98 (CH), 137.93 (C quart), 150.30 (C^4), 151.23 (C^2), 156.97 (C^6) and 171.60 (C=O). ^{11}B-NMR (DMSO) δ 3.55. Anal $C_{20}H_{29}BN_4O_2$ (C, H, N).

*N-(4,6-Dimethylpyridin-2-yl) N-[(trimethylamine boryl) carbonyl]leucinamide **8d***. IR (film) ν 3390 (NH), 3320 (NH), 2360 (BH), 1680 (C=O) and 1610 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 0.91 (3H, d, J = 6.5 Hz, CH_3), 0.94 (3H, d, J = 6.5 Hz, CH_3), 1.50 (1H, m, 1H, 3-CH_2), 1.71 (1H, sept J = 6.6 Hz, $CH(CH_3)_2$), 1.77 (1H, m, 1H, 3-CH_2), 2.26 (3H, s, 4-CH_3), 2.35 (3H, s, 6-CH_3), 2.74 (9H, s, $N(CH_3)_3$), 4.65 (1H, m, CH), 5.80 (1H, d, J = 7.8 Hz, NH), 6.66 (1H, s, H^5), 7.81 (1H, s, H^3), 8.94 (1H, m, pyr NH). ^{13}C-NMR ($CDCl_3$) δ 21.82 (4-CH_3), 22.67 (6-CH_3), 23.66 (CH_3), 24.44 (CH_3), 25.60 (CH), 40.86 (CH_2), 51.34 (CH), 53.06 ($N(CH_3)_3$), 112.10 (C^3), 120.72 (C^5), 150.33 (C^2), 151.49 (C^4), 156.94 (C^6) and 172.62 (C=O). ^{11}B-NMR (DMSO) δ 3.56. Anal $C_{17}H_{31}BN_4O_2$ (C, H, N).

*N-(4,6-Dimethylpyridin-2-yl) N-[(piperidine boryl) carbonyl]-glycinamide **9a***
Compound **9a** was prepared according to the general method from piperidine carboxyborane **6**. IR (KBr) ν 3300 (NH), 3250 (NH), 2360 (BH), 1670 (C=O) and 1620 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.50 (6H, m, pip H), 2.30 (3H, s, 4-CH_3), 2.37 (3H, s, 6-CH_3), 2.50 (2H, m, pip H), 3.20 (2H, m, pip H), 4.06 (2H, d, J = 5.9 Hz, CH_2), 4.60 (1H, m, pip NH), 6.22 (1H, m, NH), 6.70 (1H, s, H^5), 7.81 (1H, s, H^3), 8.73 (1H, m, pyr NH). ^{13}C-NMR ($CDCl_3$) δ 21.86 (4-CH_3), 23.37 (CH_2), 24.47 (6-CH_3), 25.66 (CH_2), 44.10 (CH_2NH), 52.94 (CH_2), 112.18 (C^3), 121.00 (C^5), 150.48 (C^4), 151.21 (C^2), 156.96 (C^6), 169.73 (C=O). ^{11}B-NMR (DMSO) δ 3.56. Anal $C_{15}H_{25}BN_4O_2$ (C, H, N).

*N-(4,6-Dimethylpyridin-2-yl) N-[(piperidine boryl) carbonyl]-methioninamide **9b***
Compound **9b** was prepared according to the general method from piperidine carboxyborane **6**. IR (KBr) ν 3270 (NH), 2350 (BH), 1680 (C=O) and 1610 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.60 (6H, m, pip H), 1.92 (1H, m, 1H, 3-CH_2), 2.09 (3H, s, SCH_3), 2.21 (1H, m, 1H, 3-CH_2), 2.28 (3H, s, CH_3), 2.37 (3H, s, CH_3), 2.57 (4H, m, SCH_2 + 2 pip H), 3.47 (2H, m, pip H), 4.62 (1H, m, pip NH), 4.89 (1H, m, CH), 6.13 (1H, d, J = 7.8 Hz, NH), 6.69 (1H, s, H^5), 7.81 (1H, s, H^3), 9.00 (1H, m, pyr NH). Anal $C_{18}H_{31}BN_4O_2S$ (C, H, N).

*N-(4,6-Dimethylpyridin-2-yl) N-[(pyrrolidine boryl) carbonyl]-methioninamide **10b***
Compound **10b** was prepared according to the general method from pyrrolidine carboxyborane **7**. IR (KBr) ν 3280 (NH), 2330 (BH), 1665 (C=O) and 1610 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.86 (4H, m, pyrr H), 1.92 (1H, m, 1H, 3-CH_2), 2.11 (3H, s, SCH_3), 2.17 (1H, m, 1H, 3-CH_2), 2.29 (3H, s, 4-CH_3), 2.37 (3H, s, 6-CH_3), 2.58 (2H, m, SCH_2), 2.62 (2H, m, pyrr H), 3.34 (2H, m, pyrr H), 4.87 (1H, m, CH), 5.47 (1H, m, pyrr NH), 6.11 (1H, d, J = 8.0 Hz, NH), 6.70 (1H, s, H^5), 7.82 (1H, s, H^3), 9.04 (1H, m, pyrr NH). Anal $C_{17}H_{29}BN_4O_2S$ (C, H, N).

Biological methods

1-Hypolipidemic screenings in hyperlipidemic rat
Evaluation of this activity was carried out using rats maintained on a high CH diet, according to a protocol comparable to those frequently used for antilipidemic studies as regards dose, drug administration period and measurement days [2, 9, 11, 22, 23]. Male Sprague–Dawley rats weighing 200 ± 20 g were evenly distributed into groups of 10. Food and water were provided *ad libitum*. All experiments started 2 d after grouping the animals and continued for 24 d. At day 0, the animals were placed on a commercial diet (UAR 214 B) containing cholesterol (4.5% cholesterol, 37% butter, 20% casein, 18.9% dextrose, 9.1% cellulose fiber, 7.3% USP XIV, 1.8% Na cholate, 1% vitamin mix and 0.4% choline chloride; w/w) which produced a 'hyperlipidemic' state [23]. Rats were maintained on this diet for a period of 10 d prior to drug or vehicle treatment (period necessary to observe high serum cholesterol and triglyceride levels in rat) and for the remainder of the experiment (14 d). The test compounds were suspended in an aqueous 1% carboxymethylcellulose solution. Groups of

rats received 20 mg/kg of test compounds, 150 mg/kg of clofibrate or 1% carboxymethylcellulose solution intraperitoneally once daily for 14 consecutive days.

All rats were fasted 16–18 h before blood collection. Blood was drawn (orbital plexus) from rats under light ether anaesthesia 1 d before and 10 d after drug treatment. After 14 d of treatment, blood was collected by exsanguination from the abdominal aorta of rats. All blood samples were placed on ice, and after clotting, serum was separated by centrifugation at 2500 *g* for 10 min. The serum samples were kept at 4°C and analyzed in 48 h. Serum total cholesterol and triglyceride contents were determined by commercial kits Biotrol (cholesterol enzymatic color, A01379 [24]; triglyceride enzymatic Trinder, A02555 [25, 26]).

The results were expressed in inhibition percentage as compared to control group only for days 10 and 14.

Statistical evaluation

The results are given with mean and SEM. Data obtained were evaluated by an analysis of variance. Differences between groups were determined using Student's *t*-test or Mann–Whitney test.

2-Carrageenen-induced rat-paw edema

Wistar CF male rats (150 ± 10 g) were used; food and water were given *ad libitum* unless otherwise stated. The inhibitory activity of the studied molecules on carrageenen-induced rat-paw edema was determined according to the method of Winter *et al* [21], with slight modifications. The drugs were orally administrated (35 mg/kg), 1 h before injection of 0.95 ml of a 1% suspension of carrageenen in saline into the subcutaneous tissues of one hind paw. The other hind paw was injected identically with 0.95 ml of a saline solution. Since the hydration state of animals can modify the intensity of swelling, rats were fasted 24 h before experiment, and water (1.5 ml/100 g body weight) was orally administrated twice (20 h and 4 h) before injections. Volumes of both hind paws of control and treated animals were measured with a plethysmograph, 3 h after injections. Rats were kept in the same experimental conditions.

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