ELSEVIER

Il Farmaco 54 (1999) 95–100

IL FARMACO

Antiinflammatory agents: new series of *N*-substituted amino acids with complex pyrimidine structures endowed with antiphlogistic activity

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Received 4 March 1998; accepted 30 November 1998

Abstract

A series of *N*-methyl-*N*-pyrimidin-2-yl glycines **2a–e**, having the pyrimidine ring fused with a cyclohexane [*N*-methyl-*N*-(5,6,7,8-tetrahydroquinazolin-2-yl)glycine], cyclohexene [*N*-methyl-*N*-(5,6-dihydroquinazolin-2-yl)glycine], 1,2,3,4-tetrahydronaphthalene [*N*-methyl-*N*-(5,6-dihydrobenzo[e]quinazolin-2-yl)glycine], benzopyrane [*N*-methyl-*N*-(5-phenyl-5*H*-[1]benzopyrano[4,3-*d*]pyrimidin-2-yl)glycine] and benzothiopyrane [*N*-methyl-*N*-(5*H*-[1]benzothiopyrano[4,3-*d*]pyrimidin-2-yl)glycine] ring, was prepared and tested for antiinflammatory activity. With the same purpose a number of *N*-5*H*-[1]benzopyrano[4,3-*d*]pyrimidin-2-yl substituted amino acids **3a–e**, having a different chain length and branching were also synthesized and tested. All the described products **2** and **3** showed an appreciable antiphlogistic activity, particularly **2b** and **2c**.

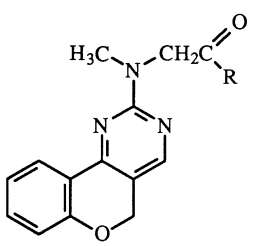
Keywords: Complex pyrimidinyl amino acids; Complex pyrimidines supporting amino acid chains; Antiinflammatory agents with a complex pyrimidinyl amino acid structure; Antiphlogistic agents

1. Introduction

We have previously described, during a study of 5*H*-[1]benzopyrano[4,3-*d*]pyrimidine derivatives, a series of *N*-methylglycinamides **1b–g** showing interesting pharmacological activities [1]. In particular, the free acid **1a** exhibited a reasonable degree of antiinflammatory activity.

Owing to our current interest in the search of new antiphlogistic agents, we decided to investigate more deeply the potential of *N*-pyrimidin-2-yl substituted amino acids **2a–e** and **3a–e** having the pyrimidine moiety fused with various hydroaromatic rings.

In order to verify the influence of the different configurations of the ring system on the biological activity, we first fixed an *N*-methylglycine moiety in the 2-position of the pyrimidine nucleus. However, with the aim of evaluating the contribution of the amino acid portion, we subsequently selected the studied 5*H*-[1]benzopyrano[4,3-*d*]pyrimidine framework as a basic support, and we introduced some amino acid substituents of different length and branching in the 2-position of the pyrimidine ring (1):



1

	R
a	OH
b	$NHCH(CH_3)_2$
c	$N(CH_3)_2$
d	$N(C_2H_5)_2$
e	1-Pyrrolidinyl
f	1-Piperidinyl
g	4-Morpholinyl

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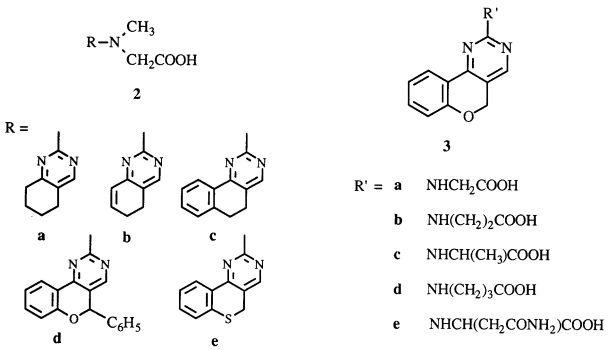
0014-827X/99/$ - see front matter
PII: S0014-827X(98)00109-8



2. Chemistry

To obtain compounds **2** we utilized our well-known synthetic route [1], starting with the corresponding 1,3-bielectrophiles dimethylaminomethylene ketones by reaction with the binucleophile creatine in basic medium (see Scheme 1).

The synthetic way to **3** was different owing to the unavailability or unreactivity of the relevant guanidines. Therefore, we prepared the corresponding 2-methylthiopyrimidines **4** by using *S*-methylisothiourea with 3-(dimethylaminomethylene)-4-chromanone. Having ascertained that the thiomethyl group in the 2-position of the pyrimidine ring was not directly replaceable with amine reagents [2], we resorted to the methylsulfinyl derivative **5**, which is more prone to the nucleophile attack [3], according to the procedure in Scheme 2.

All the above mentioned compounds were obtained in good yields and characterized on the basis of their spectroscopic and elemental (C, H, N) data.

3. Pharmacology

Compounds **2a–e** and **3a–e** were submitted to a preliminary screening for antiinflammatory activity, evaluated by carrageenan-induced paw edema in rats.

4. Experimental

4.1. Chemistry

Melting points were determined with a Büchi 530 apparatus. IR spectra were measured in $CHCl_3$ solution or in KBr with a Perkin–Elmer 398 spectrophotometer. ^1H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solution on a Hitachi Perkin–Elmer R-600 (60 MHz) instrument; chemical shifts are reported as δ (ppm) relative to TMS as internal standard; J in Hz. Analyses for C, H, N were within $\pm 0.3\%$ of the theoretical values.

2-(Dimethylaminomethylene)cyclohexanone [4], 6-(dimethylaminomethylene)-2-cyclohexen-1-one [4], 2-(dimethylaminomethylene)-3,4-dihydro-1(2*H*)-naphthalenone [5], 3-(dimethylaminomethylene)-4-chromanone [1] and 3-(dimethylaminomethylene)-2-phenyl-4-chromanone [6] were prepared following the literature references.

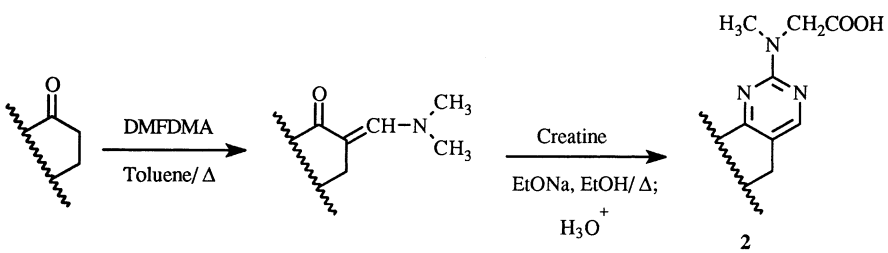
4.1.1. 3-(Dimethylaminomethylene)-4-thiochromanone

A solution of 4-thiochromanone (3.28 g, 20 mmol) and dimethylformamide dimethylacetal (DMF DMA) (3.0 g, 25 mmol) was refluxed for 3 h; the excess DMF DMA was then evaporated under reduced pressure to obtain a yellow amorphous solid which was suspended in diethyl ether, filtered and recrystallized from absolute ethanol. Yield: 3.80 g (88%), yellow crystals, m.p. 112–113°C (lit. [7] 112–113°C via the corresponding hydroxymethylene derivative).

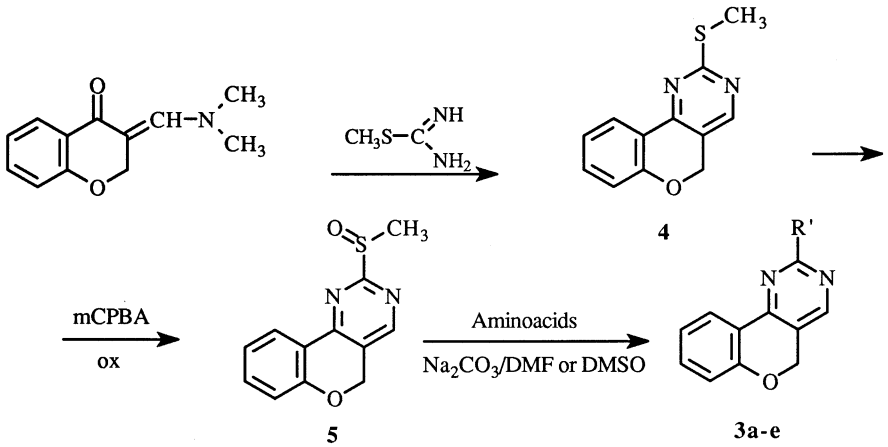
4.1.2. General procedure for the synthesis of N-substituted N-methylglycines **2a–e**

Creatine monohydrate (2.09 g, 14 mmol) was added to a solution of sodium (0.3 g, 14 mmol) in anhydrous ethanol (30 ml) and the mixture was stirred at room temperature (r.t.) for 15 min. The dimethylaminomethylene derivative of the suitable ketone (10 mmol), dissolved in anhydrous ethanol (25 ml), was slowly added and the suspension refluxed for 10 h. Then, the solution was concentrated, poured into ice-water (50 ml) and acidified to pH of ca. 4 with 1 M HCl. After acidification, **2d** and **2e** were obtained directly as crude solids which were filtered and recrystallized. In the case of **2a–c**, the acid solution was saturated by NaCl and extracted with $CHCl_3$, the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure. The crude solids thus obtained were then recrystallized.

Yields, melting points, recrystallization solvents, IR and ^1H NMR spectral data of **2a–e** are reported in Table 1.



Scheme 1.



Scheme 2.

4.1.3. 2-Methylthio-5H-[1]benzopyrano[4,3-d]pyrimidine ***4***

S-Methylisothiourea sulfate (2.78 g, 10 mmol) and anhydrous sodium acetate (1.6 g, 20 mmol) were suspended in dry DMF (20 ml). 3-(Dimethylaminomethylene)-4-chromanone (2.03 g, 10 mmol) was added immediately and the mixture was stirred at 80–90°C for 12 h. After cooling, the mixture was poured in water (10 ml) and stirred at r.t. to obtain a white-ivory precipitate which was filtered, dissolved in CH_2Cl_2 and washed with water. The organic solution was dried ($MgSO_4$) and evaporated under reduced pressure: the yellow oil obtained was purified by flash chromatography on Florisil (100–200 Mesh) with CH_2Cl_2 as eluent. Finally the crude oil was recrystallized by diethyl ether/petroleum ether (1:1). Yield: 1.40 g (61%) white crystals, m.p. 81–82°C.

IR ($CHCl_3$): 2930–2850 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.63 (s, 3H, CH_3), 5.21 (s, 2H, CH_2O), 6.90–7.70 (m, 3H, H-7 + H-8 + H-9), 8.10–8.30 (m, 1H, H-10), 8.32 (s, 1H, H-4). *Anal.* $C_{12}H_{10}N_2OS$ (C, H, N).

4.1.4. 2-Methylsulfinyl-5H-[1]benzopyrano[4,3-d]-pyrimidine ***5***

Compound **4** (2.30 g, 10 mmol) was dissolved in $CHCl_3$ (20 ml) and cooled in a salt–ice-water bath (-10°C). *m*-Chloroperoxybenzoic acid (mCPBA) 85% (1.98 g, 10 mmol) was then added in small portions at regular time intervals. The solution was stirred at -10°C for 4 h and then allowed to stand at 0°C for 12 h. The chloroformic solution was washed twice with a saturated $NaHCO_3$ solution, dried ($MgSO_4$) and evaporated under reduced pressure. A yellow oil was obtained (2.64 g) which crystallized by adding diethyl ether. The ivory solid was then recrystallized from anhydrous ethanol. Yield: 1.49 g (61%), m.p. 121–125°C.

IR ($CHCl_3$): 2860, 1055 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.00 (s, 3H, CH_3SO), 5.36 (s, 2H, CH_2O), 6.90–7.75 (m, 3H, H-7 + H-8 + H-9), 8.15–8.45 (m, 1H, H-10), 8.67 (s, 1H, H-4). *Anal.* $C_{12}H_{10}N_2O_2S$ (C, H, N).

4.1.5. General procedure for the synthesis of N-(5H-[1]benzopyrano[4,3-d]pyrimidin-2-yl)amino acids ***3a–e***

To a suspension of **5** (1.23 g, 5 mmol) in dry DMF, for **3a** and **3c,** or dry DMSO, for **3b, 3d** and **3e**, Na_2CO_3 (1.05 g, 10 mmol) and the suitable amino acid (glycine, β-alanine, L-alanine, γ-aminobutyric acid, L-asparagine) (10 mmol) were added in succession. The mixture was refluxed for 4 h and then poured into ice-water. Any solid was then filtered off and the basic solution was acidified with 1 M HCl or glacial acetic acid to pH of ca. 4: amorphous, pale yellow solids precipitated, which were filtered and washed with water. In the case of **3c**, an oil was obtained which was extracted with $CHCl_3$; the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to obtain a solid residue. All the recovered solids were finally recrystallized from the proper solvent.

Yields, melting points, recrystallization solvents, IR and ^1H NMR spectral data of **3a–e** are reported in Table 2.

4.2. Pharmacology

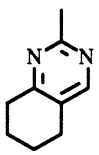
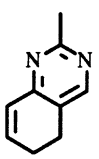
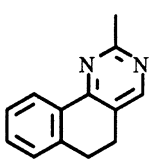
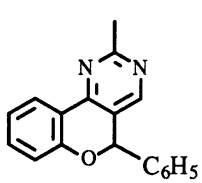
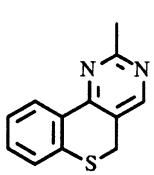
Antiinflammatory activity was evaluated by standard procedure [8] (Table 3); for the most active compounds the respective mentioned ED_{25} and ED_{50} were determined by administration of three dosages (12.5, 25 and 50 mg/kg).

5. Results and discussion

All the title compounds reported proved to be active antiinflammatories, the most effective being **2b** and **2c** with an ED_{25} at 2 h of 23.38 (19.35–28.24) and 11.23 (8.26–15.28) mg/kg, respectively, and with an ED_{50} at 4 h of 33.27 (27.38–40.44) and 25.05 (14.51–43.27) mg/kg, respectively.

Comparing these results for derivatives **2a–e** and **3a–e**, we can infer that the best degree of antiinflammatory activity was elicited by the *N*-methylglycine derivatives similar to **1a**, which turned out to be the most effective compound having an ED_{50} at 4 h of 17.00 (13.75–20.92) mg/kg [1]. In fact, all the compound

Table 1
Physical and spectral data of *N*-substituted-*N*-methylglycines **2**

Comp.R	Formula	Yield (%)	M.p. (°C)	IR (cm^{-1})	^1H NMR (δ)
2a 	$C_{11}H_{15}N_3O_2$	72	174[a]	2930, 1725[d]	1.64–2.12 (m, 4H, 2 CH_2), 2.42–2.94 (m, 4H, 2 CH_2), 3.26 (s, 3H, CH_3), 4.30 (s, 2H, CH_2N), 8.12 (s, 1H, H-4), 11.26 (bs, 1H, OH, disappears with D_2O)[e]
2b 	$C_{11}H_{13}N_3O_2$	62	165–166[b]	2930, 2600–2200, 1720, 1630[f]	2.10–2.90 (m, 4H, 2 CH_2), 3.18 (s, 3H, CH_3), 4.32 (s, 2H, CH_2N), 5.70–6.10 (m, 1H, H-7), 6.41 (d, $J = 10$, 1H, H-8), 8.06 (s, 1H, H-4), 12.35–12.90 (very bs, 1H, OH, disappears with D_2O)[g]
2c 	$C_{15}H_{15}N_3O_2$	74	198 (dec.)[c]	2930, 2550–2100, 1712[f]	2.82 (bs, 4H, 2 CH_2), 3.26 (s, 3H, CH_3), 4.37 (s, 2H, CH_2N), 7.20–7.60 (m, 3H, H-7 + H-8 + H-9), 8.10–8.50 (m, 2H, H-4 + H-10), 12.30–12.65 (bs, 1H, OH, disappears with D_2O)[g]
2d 	$C_{20}H_{17}N_3O_3$	62	170–173 (dec.)[a]	3490, 2600–2200, 1730[f]	3.33 (s, 3H, CH_3), 4.43 (s, 2H, CH_2N), 5.45 (bs, 1H, NH^+ inner salt, disappears with D_2O), 6.21 (s, 1H, H-5), 6.90–7.90 (m, 3H, H-7 + H-8 + H-9), 7.46 (s, 5H, C_6H_5), 7.80 (s, 1H, H-4), 8.22 (m, 1H, H-10)[e]
2e 	$C_{14}H_{13}N_3O_2S$	49	193[c]	2920, 2550–2200, 1715[f]	3.28 (s, 3H, CH_3), 3.99 (s, 2H, CH_2S), 4.39 (s, 2H, CH_2N), 7.20–7.60 (m, 3H, H-7 + H-8 + H-9), 8.10–8.60 (m, 2H, H-4 + H-10), 12.60 (bs, 1H, OH, disappears with D_2O)[g]

[a] From ethanol 96%.
[b] From anhydrous ethanol.
[c] From ethanol/ethyl acetate (1:1).
[d] In $CHCl_3$.
[e] In $CDCl_3$.
[f] In KBr.
[g] In DMSO-d_6.

Table 2
Physical and spectral data of *N*-(5*H*-[1]benzopyrano[4,3-d]pyrimidin-2-yl)amino acids **3**

Comp.	R	Formula	Yield (%)	M.p. (°C)	IR (cm^{-1})	^1H NMR (δ)
3a	$NHCH_2COOH$	$C_{13}H_{11}N_3O_3$	78	225 (sub.)[a]	3250, 2550–2200, 1715[f]	4.05 (d, $J=6$, 2H, CH_2N), 5.19 (s, 2H, CH_2O), 6.86–7.75 (m, 4H, H-7+H-8+H-9+NH, 1H disappears with D_2O), 8.00–8.25 (m, 1H, H-10), 8.29 (s, 1H, H-4), 12.00–12.60 (bs, 1H, OH, disappears with D_2O)[g]
3b	$NH(CH_2)_2COOH$	$C_{14}H_{13}N_3O_3$	70	190–191[b]	3415–3285, 2600–2200, 1715[f]	2.64 (t, $J=6$, 2H, CH_2), 2.74 (near q, $J=6$, 2H, CH_2NH, t after exchange with D_2O), 5.14 (s, 2H, CH_2O), 6.48 (t, $J=6$, 1H, NH, disappears with D_2O), 6.85–7.60 (m, 3H, H-7+H-8+H-9), 8.05–8.35 (m, 2H, H-4+H-10), 9.60–10.55 (very bs, 1H, OH, disappears with D_2O)[e]
3c	$NHC(CH_3)HCOOH$	$C_{14}H_{13}N_3O_3$	63	222–223 (dec.)[c]	3270, 1710[d]	1.56 (d, $J=7$, 3H, CH_3), 4.70 (mc, 1H, CH), 5.12 (s, 2H, CH_2O), 6.21 (d, $J=8$, 1H, NH, disappears with D_2O), 6.80–7.70 (m, 3H, H-7+H-8+H-9), 8.00–8.35 (m, 2H, H-4+H-10), 9.10–9.70 (bs, 1H, OH, disappears with D_2O)[e]
3d	$NH(CH_2)_3COOH$	$C_{15}H_{15}N_3O_3$	67	178–181[a]	3340, 2600–2200, 1680[f]	1.50–2.10 (m, 2H, CH_2), 2.10–2.60 (m, 2H, CH_2), 3.15–3.70 (m, 2H, CH_2), 5.18 (s, 2H, CH_2O), 6.90–7.70 (m, 4H, H-7+H-8+H-9+NH, 1H disappears with D_2O), 8.05–8.35 (m, 2H, H-4+H-10), 9.10–9.60 (bs, 1H, OH, disappears with D_2O)[g]
3e	$NHC(CH_2CONH_2)\times HCOOH$	$C_{15}H_{14}N_4O_4$	54	196–197[c]	3240, 2600–2300, 1720[f]	2.68 (d, $J=6$, 2H, CH_2), 4.45–4.95 (m, 1H, CH), 5.20 (s, 2H, CH_2O), 6.80–7.80 (m, 7H, H-7+H-8+H-9+NH_2+NH_2^+ inner salt, 4H disappear with D_2O), 8.00–8.40 (m, 2H, H-4+H-10)[g]

[a] From ethanol 96%.
[b] From ethanol/ethyl acetate (1:1).
[c] From diethyl ether.
[d] In $CHCl_3$.
[e] In $CDCl_3$.
[f] In KBr.
[g] In DMSO-d_6.

3b–c (with the exception of **3a** that has a close similarity and a comparable level of activity with **1a**) were only moderately effective. The length and/or the complexity of the amino acid chain is evidently not the decisive factor. For a modulation of the antiinflammatory activity, the nature of the cyclic moiety supporting the *N*-methylglycine chain seems to be the most important factor.

Interestingly, the most active derivatives, **2b** and **2c**, both have a certain degree of unsaturation in their cyclic structure, **2b** being more active than the corresponding dihydro analogue **2a**. On the other hand, the carbocyclic **2c** proved to be more effective than the hetero substituted analogues **2d** and **2e**. It is also interesting to note that the thio-isostere **2e**, when compared with the strictly correlated **1a**, shows a less important level of activity.

Finally, an increase of lipophilicity in the cyclic structure causes a decrease of antiphlogistic activity, as it is evidenced by the phenyl substituted **2d** showing the least activity.

To conclude, concerning antiinflammatory activity in these complex pyrimidine derivatives, the best amino acid side chain was *N*-methylglycine and the best supporting cyclic structure was a dihydroaromatic carbocyclic moiety.

Acknowledgements

The authors would like to thank A. Panaro for the microanalyses, and F. Tuberoni and C. Rossi for the IR and NMR spectra. Financial support from MURST (Cofinanziamento Programma Nazionale) and CNR (Rome) is gratefully acknowledged.

Table 3
Antiinflammatory activity by carrageenan-induced rat paw edema test [a] of compounds **2a–e** and **3a–e**

Comp.	Dose (mg/kg p.o.)	Edema volume [ml ± S.E.[b]] at the following times (h) after treatment (% inhibition activity in parenthesis)				
		0	1	2	3	4
Control	–	1.6 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.6 ± 0.1
Indomethacin	5	1.5 ± 0.1	1.6 ± 0.1 (72)	1.6 ± 0.1 (81)	1.6 ± 0.1 (86)	1.6 ± 0.1 (89)
2a	50	1.5 ± 0.1	1.8 ± 0.1 (20)	2.0 ± 0.1 (11)	2.0 ± 0.1 (34)	2.1 ± 0.1 (35)
2b	12.5	1.4 ± 0.1	1.7 ± 0.1 (0)	1.9 ± 0.1 (5)	2.0 ± 0.1 (16)	2.1 ± 0.1 (19)
	25	1.5 ± 0.1	1.8 ± 0.1 (20)	1.9 ± 0.1 (27)	2.0 ± 0.1 (34)	2.0 ± 0.1 (47)
	50	1.6 ± 0.1	1.8 ± 0.1 (48)	1.9 ± 0.1 (49)	1.9 ± 0.1 (62)	2.0 ± 0.1 (60)
2c	12.5	1.6 ± 0.1	1.9 ± 0.1 (28)	2.0 ± 0.1 (32)	2.1 ± 0.1 (38)	2.2 ± 0.1 (40)
	25	1.4 ± 0.1	1.6 ± 0.1 (44)	1.7 ± 0.1 (43)	1.8 ± 0.1 (44)	1.8 ± 0.1 (54)
	50	1.5 ± 0.1	1.7 ± 0.1 (48)	1.8 ± 0.1 (46)	1.9 ± 0.1 (46)	1.9 ± 0.1 (56)
2d	50	1.4 ± 0.1	1.7 ± 0.1 (16)	1.9 ± 0.1 (3)	2.0 ± 0.1 (14)	2.0 ± 0.1 (31)
2e	50	1.4 ± 0.1	1.7 ± 0.1 (16)	1.8 ± 0.1 (24)	1.9 ± 0.1 (28)	1.9 ± 0.1 (42)
3a	50	1.3 ± 0.1	1.5 ± 0.1 (40)	1.6 ± 0.1 (38)	1.7 ± 0.1 (38)	1.7 ± 0.1 (50)
3b	50	1.4 ± 0.1	1.6 ± 0.1 (44)	1.7 ± 0.1 (43)	1.8 ± 0.1 (44)	1.9 ± 0.1 (42)
3c	50	1.3 ± 0.1	1.6 ± 0.1 (8)	1.7 ± 0.1 (16)	1.7 ± 0.1 (38)	1.8 ± 0.1 (39)
3d	50	1.5 ± 0.1	1.8 ± 0.1 (20)	1.9 ± 0.1 (27)	1.9 ± 0.1 (46)	2.0 ± 0.1 (47)
3e	50	1.4 ± 0.1	1.7 ± 0.1 (16)	1.8 ± 0.1 (24)	1.9 ± 0.1 (28)	1.9 ± 0.1 (42)

[a] Each compound was tested on a group of five albino rats (180–250 g). Compounds were given by gastric probe 30 min before carrageenan (0.1 ml of 1% solution).
[b] S.E. was always less than ± 0.1 ml and so were rounded up to this value.

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