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Synthesis and pharmacological study of new 3,4-dihydro-2*H*,6*H*-pyrimido-[2,1-*b*][1,3]thiazines

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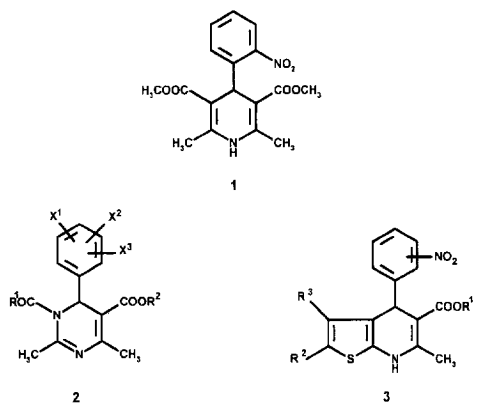
Summary — A series of racemic pyrimido-thiazine derivatives was synthesized and many of their in vivo activities found to be comparable to acetylsalicylic acid and aminophenazone in an antiinflammatory model and an antipyretic test. Analogues **7a** and **7e** are the most potent in rat carrageenin and yeast fever assays. These compounds did not inhibit prostaglandin biosynthesis in vitro.

pyrimidothiazine/antiinflammatory/antipyretic

Introduction

Dihydropyridines (eg, nifedipine, 1, scheme 1) are well-known calcium-channel blockers. Several related compounds [1,2], such as 2 and 3, have also been proven to be effective in this field of therapy. After considering the structural features of **1**, **2** and **3**, we decided to synthesize the pyrimidothiazines **7–9** and assay these compounds for the same profile. Contrary to expectations, the synthesized compounds showed only low activity as calcium-channel blockers. According the literature [3], analogous derivatives are antiinflammatories. So we evaluated our compounds in this area and they proved to be promising molecules. Generally it has been proposed that the non-steroidal antiinflammatory drugs act mainly through the inhibition of prostaglandin biosynthesis. Some of our compounds showed in vivo activities against fever and acute inflammation but lacked in vitro activities on prostaglandin synthetase in rat brain.

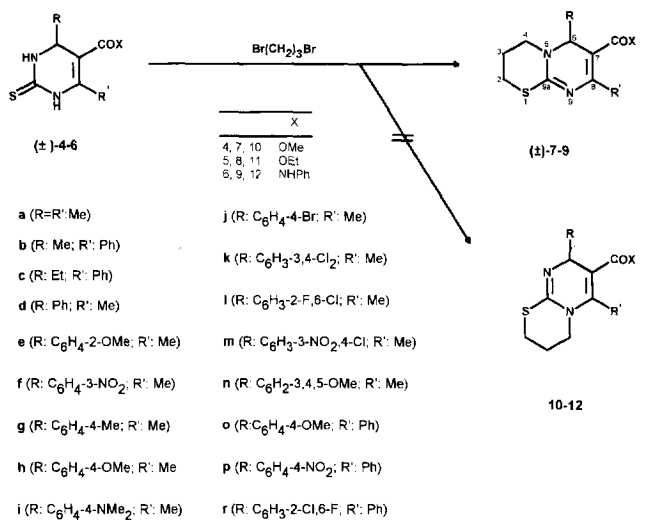


Scheme 1.

Chemistry

2-Thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxylic acid derivatives **4–6** were reacted with 1,3-dibromopropane to give 3,4-dihydro-2*H*,6*H*-pyrimido[2,1-*b*]-[1,3]thiazines **7–9** (scheme 2). The structure of the products was assigned on the basis of their IR, ^{1}H-NMR and UV spectroscopic data (characteristic spectra are given in table I, physical data in table II, and analytical data in table III).

The starting 4,6-disubstituted-2-thioxo-1,2,3,4-tetra hydropyrimidine-5-carboxylic acid derivatives **4–6** are readily accessible by condensing an aldehyde, thiourea and an acetoacetic acid derivative [4, 5]. Some 2-thioxopyrimidines obtained by this way are listed in tables I–IV. **4a** and **5b** are new compounds. Starting compounds **4–6** were transformed into pyrimido[2,1-*b*][1,3]thiazines **7–9** by alkylation with 1,3-dibromopropane. The same orientation of cyclization was established by Kappe et al [6] in the reaction of compound **5d** with 1,2-dibromoethane. The cyclization proved to be regioselective, giving in every case a single, isolable product. DNOE (differential nuclear Overhauser effect) measurements allowed us to assign **7–9** and to exclude **10–12** as the structures



Scheme 2.

of the isolated compounds. Irradiation of the (C-4)CH_2 group of **8b** resulted in an enhanced signal intensity of the (C-6)CH group, indicating the close steric arrangement of these groups. Similarly, when the (C-4)CH_2 signal of **8d** was saturated, the intensity of the (C-6)CH signal increased. With the inverse experiments the result was the same. According to the literature [7] characteristic differences were expected between the UV spectra of compounds containing cross-conjugated (eg, **10–12**) and 'ordinary' conjugated (eg, **7–9**) chromophores. Since the UV spectra of all the pyrimidothiazines obtained were practically identical with those of compounds **8b** and **8d**, it can be assumed that all of them have the same 'ordinary' conjugated chromophore system. The significant bathochromic shifts of the longest wavelength UV absorption bands of compounds **7–9** as compared to compounds **4–6** (table I) point to the fact that the chromophore system is increased. The UV spectra of compounds **8a**, **8b** and **8d** are very similar, indicating that the chromophore system is little influenced by the presence or the position of the phenyl group. This can be rationalized by supposing that the phenyl groups of **8b** and **8d** are forced out of the chromophore system by the large ethoxycarbonyl group.

Pharmacological Results and Discussion

In vivo studies.

Acute antiinflammatory activity was tested on several compounds by inhibition of the carrageenin-induced paw edema in rats, according to the method of Winter et al [8]. As shown in table IV, some molecules exhibited good antiinflammatory properties. The activities of compounds **7a, 7e, 7i, 8i** and **8l** were more potent than those of acetylsalicylic acid and aminophenazone, while eight analogues (**7d,h,k,n** and **8d,h,j,m**) were effective in a range similar to that of acetylsalicylic acid or aminophenazone. Four of the most potent six derivatives (**7a, 7e, 8i** and **8l**) showed greater efficacy than phenylbutazone, but they did not reach the efficacy of indomethacin.

The compounds listed in table IV were evaluated against fever produced in rats by brewer's yeast. Significant antipyretic effects were observed with a number of derivatives. Several molecules (**4a, 7a, d,e, f,h,i** and **8c,i,l,n**) were more active than acetylsalicylic acid and paracetamol, but less active than indomethacin. The antipyretic effect of seven of the potent derivatives (**4a, 7a,d,e,h,i** and **8i**) were comparable to that of aminophenazone. The activities of compounds **7k, m,n, 8d** and **8f** were equal to those of acetylsalicylic acid and paracetamol. These analogues did not influence the normal body temperature of rats.

In vitro studies

As the molecules produced strong antipyretic effects, their inhibition of cyclooxygenase enzyme in the microsomal fraction of rat-brain homogenate were examined. Prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) is one of the characteristic metabolites of the prostaglandin synthetic pathway released by the stimulated cells. $PGF_{2\alpha}$ is a known mediator of inflammation, and cyclooxygenase enzyme inhibitors are potential drugs for the treatment of inflammatory diseases. Rat-brain prostaglandine synthetase was stimulated by noradrenaline, and the formation of $PGF_{2\alpha}$ was measured by radioimmunoassay. Table IV shows that none of these compounds decreased the formation of $PGF_{2\alpha}$ significantly.

Conclusion

A series of novel pyrimidothiazine derivatives has been synthesized. Many of them inhibited carrageenin edema and decreased yeast-induced pyrexia in rats. In contrast to the well-known non-steroidal antiinflammatory drugs, these analogues did not hinder the cyclooxygenase enzyme activity. Since these compounds have no CO inhibitory properties it is possible that they do not damage the gastrointestinal mucosa. Further studies will be required to elucidate their mode of action.

Table I. The characteristic UV maximum [nm, (ε x 10^{-3}), in ethanol], IR carbonyl frequencies [cm^{-1}, in KBr discs] and ^{1}H-NMR chemical shifts (δ_{TMS} = 0 ppm) of compounds **4–5** and **7–9** in $CDCl_3$ solution[a] at 250 MHz.

Compound	*λ max*	*ν C=O band*	*CH_3 t (3H)*[b]	*CH_3 s (3H)*[c]	*OCH_2 dq (2H)*[d]	*CH s (1H)*
4a	299 (16.0)	1660	3.64s	2.31	–	4.15q
5b	303 (13.8)	1690	0.91	1.42[d]	3.94	4.52q
5d	304 (15.8)	1670	1.10	2.30	4.02	5.18
7a	342 (9.4)	1685	3.70s	2.26	–	4.28q
7d	357 (9.3)	1691	3.60s	2.33	–	5.15
7e	350 (8.3)	1698	3.55s	2.37	–	5.74
7f	360 (8.9)	1661	3.65s	2.34	–	5.30
7g	334 (9.0)	1690	3.61s	2.33[e]	–	5.11
7h	350 (9.6)	1687	3.61s	2.33	–	5.09
7i	355 (9.4)	1692	3.60s	2.32	–	5.03
7k	354 (8.5)	1668	3.64s	2.33	–	5.12
7m	355 (8.4)	1665	3.66s	2.33	–	5.25
7n	350 (7.7)	1696	3.66s	2.33	–	5.09
8b	353[f]	1670	0.89	1.31[d]	3.90	4.35q
8c	358 (8.3)	1680	0.88	–	3.90	4.36t
8d	353 (10.2)	1660	1.20	2.35	4.07	5.16
8f	353 (8.8)	1685	1.23	2.35	4.10	5.32
8h	350 (9.7)	1685	1.20	2.35	4.07	5.10
8i	355 (9.4)	1693	1.20	2.33	4.05	5.03
8j	332 (9.2)	1690	1.21	2.33	4,08	5.12
8k	360 (9.3)	1660	1.22	2.34	4.10	5.12
8l	363 (9.9)	1682	1.12	2.31	4.03	6.11
8m	356 (7.7)	1660	1.24	2.33	4.10	5.24
8n	354 (8.6)	1685	1.25	2.34	4.11	5.11
8o	358 (6.4)	1690	0.76	–	3.72	5.24
8p	380 (6.2)	1655	0.82	–	3.84	5.41
8r	368 (9.0)	1655	0.80	–	3.78	6.26
9d	314 (7.7)	1695	–	1.90	–	6.19
9l	322 (8.8)	1625	1.88	–	–	6.22

Further signals: NH (1,3): ~7.9 and ~8.0 (2 x bs, 2 x 1H) (**4a**), ~9.65 and 10.35 (2 x bs, 2 x 1H) (**5b**), CH_3 (6): 1.18 (d, 3H, J = 6.2 Hz) (**7a**), CH_2 (6): 1.75 (dq, 2H) (**8c**), OCH_3 (Ar-6): 3.88 (s, 3H) (**7e**), 3.83 (4') and 3.84 (3', 5') (s, 3 x 3H) (**8n**), 3.79 (s, 3H) (**8h**), 3.74 (s, 3H) (**8o**), NCH_3 (Ar-6) 2.93 (s, 6H) (**8i**). All ^{1}H-NMR spectra (except for **4a**) contain the signals of the aryl substituents with the expected shifts, multiplicities and intensities. Similarly, the methylene signals of the thiazine ring appear in the intervals 2.0–2.35 (one or two multiplets of 2: or 1:1 H-intensity for CCH_2C group), 2.8–3.10 (1/2 m, 2/2 x 1H for SCH_2 groups) and 3.05–3.60 ppm (1/2m, 2/2 x 1H for NCH_2 groups) respectively. [a]Solvent DMSO-d_6 for **5b, 8o, 9l, 9d**. [b]Ester group, CH_3CH_2: J = 7.1 Hz (except **4a, 7a, 7d–n**). [c] Position 4 for **5b**, position 6 for **4a, 5d, 8b**, position 8 for **7a, 7d–n, 8d-n, 9l, 9d**. [d]Ester group, AB-part of an ABX_3 spin system due to diastereotopy of the *O*-methylene hydrogens arising from the asymmetric structures of these molecules. In the cases of **5d**, **5b** and **8b, c** this anisotropy is not observable: the signal is a q (A_2 part on A_2X_3 spin system). [e]CH_3 (8) and CH_3 (Ar-6), (s, 6H). [f]Owing to the low solubility of the compound, it is not possible to measure the value of the molar extinction.

Table II. Physical data for compounds **4–5** and **7–9**

Compound	*Mp(°C)*[a]	*Yield (%)*	*Reaction time(h)*
4a	204–206	45	40
5b	227–230	30	40
5d	207–209	60	25
7a	92–94	85	32
7d	186–188	87	17
7e	132–137	66	15
7f	177–179	84	13
7g	174–178	78	25
7h	185–186	85	36
7i	128–130	78	32
7k	151–153	95	23
7m	146–148	98	13
7n	137–139	55	32
8b	Oil[b]	45	25
8c	70–73	86	28
8d	110–112	80	25
8f	163–165	93	30
8h	148–150	52	13
8i	127–129	87	30
8j	150–153	75	13
8k	116–118	97	26
8l	129–131	95	20
8m	125–130	92	13
8n	101–102	84	28
8o	180–182	80	9
8p	190–192	45	6
8r	168–170	92	5
9d	220–223	45	6
9l	210–215	97	7

[a]Crystallized from water or *i*-propanol (**8h, 7h, 8o**) or ethanol (**8p**). Recrystallized from ethanol (**5d, 7f, 8h, 8i, 9l, 8n**), DMF/water (**5b, 8f, 7h, 7i, 8j, 8k, 8l, 9l, 8m, 8r**), methanol (**4a, 7k, 7n, 8p**), *i*-propanol (**8c, 8d, 7d**), acetonitrile (**7a**) or acetone (**7g**). [b]Purified by column chomatography, column packing silica gel, eluent: *n*-hexane/ethylacetate (1:1). Method: B: **9l** and **8r**; A: the others.

Table III. Analytical data for compounds **4–5** and **7–9**

Compound	*Mol formula*	*Mol weight*
4a	$C_8H_{12}N_2O_2S$	200.3
5b	$C_{14}H_{16}N_2O_2S$	276.4
5d	$C_{14}H_{16}N_2O_2S$	276.4
7a	$C_{11}H_{16}N_2O_2S$	240.3
7d	$C_{16}H_{18}N_2O_2S$	302.39
7e	$C_{17}H_{20}N_2O_3S$	332.42
7f	$C_{16}H_{17}N_3O_4S$	347.93
7g	$C_{17}H_{20}N_2O_2S$	316.42
7h	$C_{17}H_{20}N_2O_3S$	332.42
7i	$C_{18}H_{23}N_3O_2S$	345.46
7k	$C_{16}H_{16}Cl_2N_2O_2S$	371.29
7m	$C_{16}H_{16}ClN_3O_4S$	381.83
7n	$C_{19}H_{24}N_2O_5S$	392.47
8b	$C_{17}H_{20}N_2O_2S$	316.42
8c	$C_{18}H_{22}N_2O_2S$	330.45
8d	$C_{17}H_{20}N_2O_2S$	316.42
8f	$C_{17}H_{19}N_3O_4S$	361.42
8h	$C_{18}H_{22}N_2O_3S$	346.45
8i	$C_{19}H_{25}N_3O_2S$	359.49
8j	$C_{17}H_{19}BrN_2O_2S$	395.39
8k	$C_{17}H_{18}Cl_2N_2O_2S$	385.32
8l	$C_{17}H_{18}ClFN_2O_2S$	368.86
8m	$C_{17}H_{18}ClN_3O_4S$	395.86
8n	$C_{20}H_{26}N_2O_5S$	406.50
8o	$C_{23}H_{24}N_2O_3S$	408.52
8p	$C_{22}H_{21}N_3O_4S$	423.49
8r	$C_{22}H_{20}ClFN_2O_2S$	430.93
9d	$C_{21}H_{21}N_3O_4S$	423.49
9l	$C_{21}H_{19}ClFN_3OS$	415.91

Experimental protocols

Chemical synthesis

Uncorrected melting points were determined on a Kofler-Boetius microapparatus. UV spectra were taken on a Pye-Unicam SP-8-150 model spectrophotometer. IR spectra were measured in KBr discs on a Bruker IFS-113 V model vacuum optic FT-spectrophotometer coupled with an Aspect 2000 computer. ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solution, on a Bruker WM-250-FT model NMR spectrometer at 250 MHz, using the deuterium signal of the solvent as the lock and $SiMe_4$ as internal standard. Elemental analyses were carried out in the analytical laboratory of EGIS Pharmaceuticals Ltd. All chiral compounds were obtained as racemic mixtures.

*Preparation of tetrahydropyrimidin-5-carboxylic acid derivatives **4–6***

The new 2-thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxylic acid derivatives **4a** and **5b** were obtained similarly to the procedure described in the literature [4]. A mixture of acetaldehyde (44.0 g, 1.00 mol), thiourea (76.0 g 1.00 mol) and an acetoacetic acid derivative (1.00 mol) was stirred in a solution of hydrochloric acid in 2-propanol (1000 mL, 0.2g/mL) at room temperature. The resulting crystalline product was filtered and washed with 2-propanol and recrystallized (see table II).

*General method for the synthesis of compounds **7–9***

*Method A: Compounds **7–9**, excluding **8r** and **9l**.* A mixture of the appropriate 2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid derivative **4–6** (0.10 mol), 1,3-dibromopropane (30.3 g, 15.3 mL 0.15 mol), potassium carbonate (27.6 g,

Table IV. Pharmacological data for compounds **4–5** and **7–9.**

Compound	*Carrageenin edema*	*Yeast fever*		*CO assay effect (%) 10^{-5} M*
	ID_{30} (mg/kg po)	*Dose (mg/kg po)*	*Effect (°C)*	
4a	>50	50	–1.6	nt
5b	>200	200	–0.2	nt
7a	8.5	50	–1.9	–16.2
7d	40.6	100	–1.9	–12.0
7e	16.3	50	–1.6	–17.7
7f	>200	200	–1.9	nt
7g	≈200	200	–0.6	nt
7h	66.3	100	–1.8	+27.5
7i	27.2	100	–1.8	+6.5
7k	≈110	200	–1.2	+22.0
7m	>140	200	–1.2	nt
7n	≈110	200	–1.0	–20.2
8c	≈200	200	–1.8	0
8d	≈100	100	–1.2	0
8f	≈160	200	–1.3	0
8h	57.7	200	–0.8	–28.7
8i	10.4	100	–1.8	–17.9
8j	76.5	nt	–	+1.8
8k	>200	nt	–	nt
8l	14.4	30	–1.2	+7.5
8m	≈100	200	–0.6	+10.6
8n	≈50	200	–1.7	–16.7
8o	>200	200	–0.1	nt
8p	≈160	200	–0.7	–24.1
8r	>200	200	+0.2	nt
9d	≈200	200	–0.7	nt
9l	>200	200	–0.7	nt
Indomethacin	2.9	10	–1.2	–84.5
Phenylbutazone	24.3	200	–1.8	–34.5
Aminophenazone	43.6	200	–2.7	nt
		100	–2.0	
		50	–1.9	
Acetylsalicylic acid	62.4	200	–1.2	nt
		100	–1.0	
Paracetamol	195.3	200	–1.5	nt
		100	–1.3	

nt = not tested

0.20 mol), potassium iodide (2.0 g, 0.012 mol), dimethylformamide (50 mL) and methyl ethyl ketone (500mL) was refluxed with stirring for the time indicated in table II. After cooling to room temperature the precipitated mineral salts were filtered off and the filtrate was evaporated in vacuo. The residue was triturated with a solvent, and recrystallized (for the solvents and other data see table II).

*Method B: compounds **8r** and **9l**.* A mixture of the appropriate 2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid derivative **4–6** (0.10 mol), 1,3-dibromopropane (23.2 g, 11.7 mL, 0.115 mol), and potassium carbonate (13.8 g, 0.10 mol) in dimethyl formamide (200 mL) was stirred at 70 °C for the time indicated in table II. After cooling to room temperature the precipitated mineral salts were filtered off and the filtrate was evaporated in vacuo. The residue was triturated with water and recrystallized from a mixture of dimethylformamide and water (table II).

Pharmacological evaluation

Carrageenin paw edema.
The method of Winter et al [8] was followed. Three male and three female Wistar rats weighing 150–180 g were used. The animals were fasted and hydrated orally with 30 mL/kg of tap water 60 min prior to po administration of the test compound in 10 mL/kg 0.4% hydroxypropyl methyl cellulose solution (Methocell F 4 M Dow Chemical Company, USA).

One hour later 0.1 mL 1% (w/v) carrageenin (Viscarin 402, Marine Colloids Inc, Springfield, USA) was injected into the subplantar surface of the right hind paw of each animal. The paw volumes were determined plethysmographically prior to and 3 h following the phlogistic agent. Edema was calculated as the difference between the two measurements. Inhibition of swelling was determined by comparing the change in hind paw volume in compound- and vehicle-treated rats. ID_{30} values were determined by plotting the log dose versus percentage response as compared to vehicle-treated animals.

Brewer's yeast-induced fever in rats

A modification of the method of Smith and Hambourger [9] was used. Female Wistar rats weighing 150–200 g were used. To acclimatize the animals to the experimental conditions, they were taken out of the cage on the day preceding the test and a probe inserted 4 cm into the rectum. Rectal temperatures were recorded by an electric thermometer. Two millilitres of 20% (w/v) aqueous brewer's yeast (Buszesz, Budapest, Hungary) suspension was injected subcutaneously. Food was removed from the cage, and water was given ad libitum. Rectal temperatures were determined 17 h later. This reading was taken as the zero time rectal temperature. The animals were then divided into groups of six (only rats with a temperature rise greater than 1.0 °C were involved in the study). The compounds were administered orally (in 10 mL/kg 0.4% hydroxypropyl methyl cellulose solution; Methocell F 4 M Dow Chemical Company, USA). Thereafter rectal temperatures were measured after 1, 3 and 5 h. The experiments were performed in separate groups of animals, each consisting of six animals treated with the same dose of test compound or the vehicle (control). In each animal the differences between the original fever temperature (0 h) and temperatures determined at sutsequent times (1, 3, 5 h) were calculated.

Cyclooxygenase (CO) assay

The microsomal fraction (4 mg/mL protein) of homogenates of Wistar-rat brain was used as a source of cyclooxygenase enzyme. The prostaglandin synthesis was stimulated by noradrenaline. The incubation mixtures (0.5 mL containing 0.05 M Tris-HCl buffer pH ≠ 7.4, and 0.15 M NaCl) were incubated with the test compounds and 10^{-3} M noradrenaline at 37 °C for 15 min with shaking. The reaction was terminated by the addition of indomethacin (final concentration 10^{-3} M) and placing the samples in an ice bath. After centrifugation at 10 000 g at 0 °C for 10 min, the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) content of the supernatant was determined by radioimmuno assay (^{3}H-$PGF_{2\alpha}$ RIA kit was obtained from Izinta Ltd, Budapest).

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