Synthesis and Calcium Antagonistic Activity of Some New 2-Thioxo-1,2,3,4-tetrahydropyrimidine Derivatives

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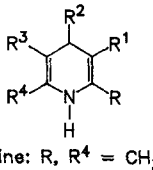
A series of 1,2,3,4-tetrahydro-6-methyl-4-(substituted phenyl)-2-thioxo-5-pyrimidinecarboxylic acid methyl esters were synthesized by condensing thiourea with methyl acetoacetate and nonsubstituted or differently substituted benzaldehydes in absol. ethanol using HCl as a catalyst according to the *Biginelli* reaction. The structures of the compounds were confirmed by elemental and spectroscopic analysis. - The compounds were evaluated for their calcium antagonistic activity on the basis of their potency in inhibiting [^{3}H] PN 200-110 binding on microsomes obtained from rat skeletal muscle.

Synthese und calciumantagonistische Aktivität von einigen neuen 1,2,3,4-Tetrahydropyrimidin-2-thion Derivaten

Eine Reihe von 1,2,3,4-Tetrahydro-6-methyl-4-(substituierten phenyl)-2-thioxo-pyrimidin-5-carbonsäure-Methylestern wurde synthetisiert. Die Synthesen wurden nach der Biginelli-Reaktion durch Kondensation von Thioharnstoff mit Methylacetoacetat und nichtsubstituierten bzw. unterschiedlich substituierten Benzaldehyden und HCl als Katalysator in absol. Ethanol durchgeführt. Die Strukturaufklärung der Substanzen erfolgte durch Elementar- und spektroskopische Analyse. - Die calciumantagonistische Aktivität der Verbindungen wurde aufgrund ihrer Fähigkeit bestimmt, die Bindung von [^{3}H] PN 200-110 an Microsomen zu hemmen; die Microsomen wurden aus Skelettmuskeln der Ratte isoliert.

Calcium channel blockers are important drugs in the treatment of angina[1] and hypertension[2] because of their vasodilator properties. Since the discovery of nifedipine which has 1,4-dihydropyridine structure **1**, as a vasodilator[3,4,5], antihypertensive[6], and calcium channel blocker[7], a number of studies were performed on **1**[8-12]. Also the similar pharmacological properties of various 1,4-dihydro-[13,14], 2-oxo-[10,15] or 2-thioxo-3,4-dihydro-[15], 1,2,3,4-tetrahydro-[16], 2-oxo-[10,17,18] or 2-thioxo-1,2,3,4-tetrahydropyrimidine[18,19] derivatives have been investigated.

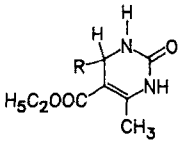


1 (Nifedipine: R, R^4 = CH_3, R^1, R^3 = $COOCH_3$, R^2 = 2-NO_2-C_6H_4

In this study, we synthesized some new 2-thioxo-1,2,3,4-tetrahydropyrimidine derivatives and tested their calcium antagonistic activity.

Results and Discussion

The first series of tetrahydropyrimidine compounds **2** were prepared by *Biginelli* in 1983 by condensing urea, an aldehyde and a β-keto ester (ethyl acetoacetate)[20].



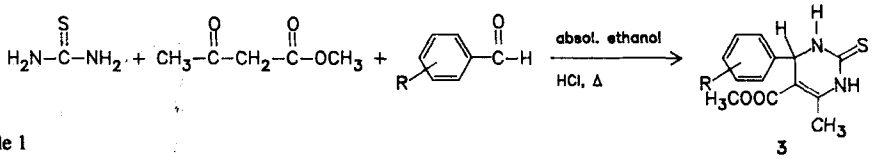
R: Alkyl, aryl and aralkyl groups

2

In 1929 *Hinkel* and *Hey* modified *Biginelli's* method by using thiourea instead of urea[21]. We used this method to synthesize our compounds (Tab. 1) by refluxing thiourea, methyl acetoacetate and nonsubstituted or differently substituted benzaldehydes in absol. ethanol and HCl (Scheme 1).

Folkers et al.[22] studying the preparation of tetrahydropyrimidine derivatives, report that 3-5 h were enough to synthesize 2-oxo-4-(substituted phenyl)-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine derivatives, but we observed that most of our derivatives required a longer time of reaction (Tab. 1). We found that the mechanism of these reactions was in accordance with *Biginelli's*[20] *and Folker* and *Johnson's*[23] reports; we reacted thiourea first with methyl acetoacetate and obtained methyl β-thioureidocrotonate (**4**), then with 4-methoxybenzaldehyde yielding 4-methoxybenzalbisthiourea (**5**) (Scheme 2).

Then we reacted **4** with 4-methoxybenzaldehyde and **5** with methyl acetoacetate, respectively, and we got compound **3c** at the end of both reactions.



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Scheme 1: For R see Table 1

  0365-6233/91/0303-0135 $ 3.50 + .25/0

Tab. 1: Physical and analytical data of compounds 3a-3j

Compd.	R	M.p. (°C)	Yield% (Reac. time, hours)	Recryst.[a]	Formula	Elementary analysis Calc. %	Found %
3a	H	224-25	47(8)	A	$C_{13}H_{14}N_2O_2S$	C: 59.52	C: 59.21
						H: 5.38	H: 5.26
						N: 10.68	N: 10.51
						S: 12.22	S: 12.31
3b	2-OCH_3	246-47	87(1)	A	$C_{14}H_{16}N_2O_3S$	C: 57.52	C: 57.29
						H: 5.52	H: 5.51
						N: 9.58	N: 9.33
						S: 10.97	S: 10.90
3c	4-OCH_3	177-78	62(9)	A	$C_{14}H_{16}N_2O_3S$	C: 57.52	C: 57.19
						H: 5.52	H: 5.56
						N: 9.58	N: 9.29
						S: 10.97	S: 11.12
3d	4-CH_3	152-53	58(9)	B	$C_{14}H_{16}N_2O_2S$	C: 60.85	C: 61.23
						H: 5.84	H: 5.59
						N: 10.13	N: 9.74
						S: 11.60	S: 11.24
3e	4-$NHCOCH_3$	266-67	77(3)	C	$C_{15}H_{17}N_3O_3S$	C: 56.41	C: 56.32
						H: 5.37	H: 5.27
						N: 13.16	N: 12.77
						S: 10.04	S: 9.94
3f	2-Cl	176-77	50(8)	A	$C_{13}H_{13}ClN_2O_2S$	C: 52.61	C: 52.69
						H: 4.41	H: 4.43
						N: 9.44	N: 9.43
						S: 10.80	S: 10.95
						Cl:11.95	Cl:12.26
3g	3-Cl	240-41	43(8)	A	$C_{13}H_{13}ClN_2O_2S$	C: 52.61	C: 52.49
						H: 4.41	H: 4.34
						N: 9.44	N: 9.27
						S: 10.80	S: 10.93
						Cl:11.95	Cl:12.27
3h	4-Cl	138-39	55(8)	D	$C_{13}H_{13}ClN_2O_2S$	C: 52.61	C: 52.39
						H: 4.41	H: 4.49
						N: 9.44	N: 9.34
						S: 10.80	S: 10.79
						Cl:11.94	Cl:12.25
3i	4-Br	158-59	54(10)	A	$C_{13}H_{13}BrN_2O_2S$	C: 45.76	C: 45.91
						H: 3.84	H: 3.92
						N: 8.21	N: 7.84
						S: 9.39	S: 8.99
						Br:23.42	Br:23.20
3j	4-NO_2	225-26	68(9)	E	$C_{13}H_{13}N_3O_4S$	C: 50.81	C: 50.44
						H: 4.26	H: 4.35
						N: 13.67	N: 13.31
						S: 10.43	S: 10.65

[a] A: recrystallized from ethanol; B: recrystallized from benzene; C: washed with ethyl acetate; D: recrystallized from a mixture of acetonitril-water (2:1); E: recrystallized from a mixture of ethyl acetate-benzene (1:1).

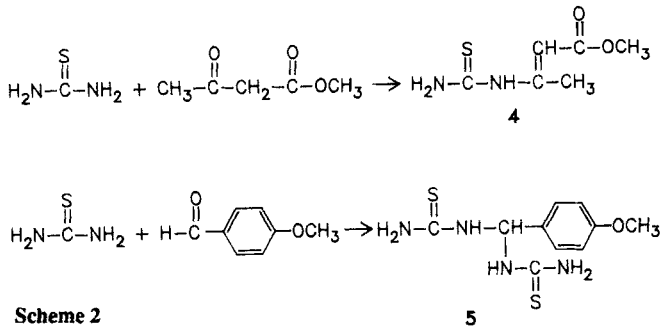
The structure of the compounds was confirmed by spectral data and elemental analysis (Table 1 and Table 2).

To evaluate the calcium antagonistic activity, the effects of the compounds on the binding of [^{3}H] PN 200-110 (Isradipine; 4-(Benz-2-oxa-1,3-diazole)-4-dihydro-2,6-dimethyl-pyridine-3,5-dicarboxylic acid 3-isopropyl, 5-methyl ester) to microsomal fractions of rat skeletal muscle were determined.

Tab. 2: IR, ^{1}H-NMR and Mass spectral data of compounds **3a-3j**

Compd.	IR (KBr) (cm^{-1})	^{1}H-NMR (DMSO-d_6) (ppm)	Mass (% relative abundance)
3a	3317(N-H) 1665(C=O) 1580(C=S)	2.30(3H;s;CH_3),3.50(3H;s;COO-CH_3),5.20(1H;s;py* H-4),7.20-7.40(5H;m;ph**),9.70(1H;s;N_1-H),10.30(1H;s;N_3-H)	262(75),247(21),231(4), 203(26),185(100),153 (14),126(7),77(12)
3b	3152(N-H) 1713(C=O) 1581(C=S)	2.30(3H;s;CH_3),3.50(3H;s;OCH_3), 3.80(3H;s;$COOCH_3$),5.30(1H;s;py H-4),6.80-7.10(3H;m;ph H-3,H-5, H-6),7.20-7.30(1H;m;ph H-4), 9.30(1H;s;N_1-H),10.30(1H;s;N_3-H)	292(100),277(28),261 (24),233(55),185(61), 153(14),126(13),84(36)
3c	3327(N-H) 1664(C=O) 1557(C=S)	2.30(3H;s;CH_3),3.50(3H;s;OCH_3), 3.70(3H;s;$COOCH_3$),5.10(1H;s;py H-4),6.90(2H;d;ph H-3,H-5),7.10 (2H;d;ph H-2,H-6),9.60(1H;s;N_1-H),10.30(1H;s;N_3-H)	292(100),277(51),261 (12),233(76),185(69), 153(14),126(13),84(48)
3d	3329(N-H) 1700(C=O) 1576(C=S)	2.25(3H;s;CH_3),3.50(3H;s;COO-CH_3),5.10(1H;s;py H-4),6.90-7.30(4H;m;ph),9.60(1H;s;N_1-H), 10.30(1H;s;N_3-H)	276(88),261(35),245(7), 217(45),185(100),153 (17),126(10),91(13),84 (12)
3e	3202(N-H) 1685(C=O) 1580(C=S)	2.00(3H;s;CH_3),2.30(3H;s;NHCO-CH_3),3.50(3H;s;$COOCH_3$),5.10(1H; s;py H-4),7.10(2H;d;ph H-2,H-6), 7.50(2H;d;ph H-3,H-5),9.60(1H; s;N_1-H),9.90(1H;s;N_3-H),10.30 (1H;s;$NHCOCH_3$)	319(100),304(24),287(8) 260(60),185(84),153(12), 126(11)
3f	3380(N-H) 1681(C=O) 1566(C=S)	2.30(3H;s;CH_3),3.50(3H;s;COO-CH_3),5.60(1H;s;py H-4),7.00-7.60(4H;m;ph),9.60(1H;s;N_1-H), H),10.40(1H;s;N_3-H)	296(51),281(17),237(22), 185(100),153(13),126(9) 84(12)
3g	3316(N-H) 1663(C=O) 1576(C=S)	2.30(3H;s;CH_3),3.50(3H;s;COO-CH_3),5.20(1H;s;py H-4),7.00-7.25(2H;m;ph H-2,H-4),7.30-7.50(2H;m;ph H-5,H-6),9.70 (1H;s;N_1-H),10.45(1H;s;N_3-H)	296(46),281(13),265(4), 237(17),185(100),153(15) (15),126(8),84(16)
3h	3320(N-H) 1696(C=O) 1572(C=S)	2.30(3H;s;CH_3),3.50(3H;s;COO-CH_3),5.20(1H;s;py H-4);7.20 (2H;d;ph H-2,H-6),7.55(2H;d; ph H-3,H-5),9.70(1H;s;N_1-H), 10.40(1H;s;N_3-H)	296(60),281(26),265(6), 237(30),185(100),153 (14),126(10),101(5),84 (23)
3i	3310(N-H) 1668(C=O) 1571(C=S)	2.30(3H;s;CH_3),3.50(3H;s;COO-CH_3),5.20(1H;s;py H-4)),7.15 (2H;d;ph H-2,H-6),7.55(2H;d; ph H-3,H-5),9.70(1H;s;N_1-H), 10.40(1H;s;N_3-H)	341(39),326(18),310(3), 282(18),185(100),153(13) 126(8),84(29)
3j	3169(N-H) 1716(C=O) 1580(C=S)	2.50(3H;s;CH_3),3.65(3H;s;COO-CH_3),5.85(1H;s;py H-4),7.50 (2H;d;ph H-2,H-6),8.25(2H;d; ph H-3,H-5),9.60(1H;s;N_1-H), 10.50(1H;s;N_3-H)	307(46),292(7),276(22), 248(100),202(22),185(4), 84(55)

*py: pyrimidine; **ph: phenyl



Scheme 2

All compounds, inhibited [^{3}H] PN 200-110 binding and their K_i values are given in Tab. 3. Nifedipine, used as reference antagonist, was found most potent in displacing [^{3}H] PN 200-110. Among our compounds **3f**, although weak, showed greater and significant affinity to dihydropyridine binding site in comparison with the others.

Although the compounds have an asymetric center on the fourth position, we did not separate the enantiomers; so in the present study, their influence on pharmacological activity was not determined. Our next study will be concerned with the separation and the comparison of the activities of the enantiomers. The most potent compound, **3f**, was chosen for this purpose.

Experimental Part

Chemistry

Melting points: Thomas-Hoover capillary melting point apparatus, uncorrected. - IR spectra: IRFT Bruker IFS 88 spectrometer (KBr pellets). - ^{1}H-NMR spectra: Bruker AC 200 (200 MHz) spectrometer, DMSO-d_6, TMS as internal standard. Chemical shifts: δ units. Mass spectra: VG Analytical 70-250 S spectrometer, 70 eV. - Elemental analysis: Fondax-Actam & Cie, Puteaux, France.

Tab. 3: Pharmacological results

Compd.	Ki*(Molar)
3a	$5 \cdot 10^{-5}$
3b	$> 10^{-4}$
3c	$5 \cdot 10^{-5}$
3d	$4 \cdot 10^{-5}$
3e	10^{-4}
3f	$4.7 \cdot 10^{-6}$
3g	$2 \cdot 10^{-5}$
3h	$2 \cdot 10^{-3}$
3i	$2.5 \cdot 10^{-5}$
3j	$2.5 \cdot 10^{-5}$
Nifedipine	10^{-8}

*K_i value represents the concentration of the compounds required to cause 50% inhibition of [^{3}H] PN 200-110 binding in rat skeletal muscle

1,2,3,4-Tetrahydro-6-methyl-4-(substituted phenyl)-2-thioxo-5-pyrimidinecarboxylic acid methyl esters

A solution of thiourea (3.8 g, 0.05 mol), benzaldehyde or substituted benzaldehyde (0.05 mol), methyl acetoacetate (8.7 g, 0.05 mol), absol. ethanol (20 ml) and 37% HCl (4 drops) was refluxed for an appropriate period and allowed to stand 24 h for crystallization. The product was filtered and washed with 50% ethanol (25 ml). Then it was recrystallized from proper solvent (Tab. 1).

Pharmacology

Preparation of membranes

The membranes were microsomes obtained from rat skeletal muscle according to *Fosset* et al[24]. Briefly, the muscles were weighed and homogenized in 4 volumes of 20 mM MOPS (3-[*N*-Morpholino]propane sulfonic acid)/KOH buffer, at pH 7.4, containing 0.3 M sucrose, 1 mM EDTA and PMSF (Phenylmethylsulfonyl fluoride) 1/1000 at 4°C. The homogenate was centrifuged at 3200xg for 10 min. The pellet was eliminated and the supernatant was recentrifuged at 15000xg for 20 min. The new supernatant was brought to 0.6 M KCl by adding the solid salt, stirred for a few min, and centrifuged at 10000xg for 45 min. The resulting pellet was suspended in 2 ml of the buffer, homogenized and centrifuged again at 45000xg for 45 min. The new pellet obtained corresponds to the microsomal fraction used in binding studies.

Binding assay

Binding assay was performed at 20°C on microsomal fractions by using [^{3}H] PN 200-110 as radioligand. Increasing concentrations of the compounds tested were added into the incubation medium containing [^{3}H] PN 200-110. Activities were determined by displacement of [^{3}H] PN 200-110 from its specific binding sites. K_i values given in the text represent the concentrations of the compounds required to cause 50% inhibition of [^{3}H] PN 200-110 binding. The incubations were performed in dublicate in at least two independent experiments. Nifedipine was used as the reference antagonist.

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