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(6-Methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acid and related compounds exhibiting anti-inflammatory activity

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Base-promoted hydrolysis of methyl or ethyl esters **1a–c** gave the (6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)- and (5-ethyl-6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acids **2a, b**. Under the reaction of ester **1a** or acid **2a** with nucleophilic reagents a series of derivatives **3**–**7** of acid **2a** were synthesized and evaluated for their anti-inflammatory activity. Most of them were found to be more active than acetylsalicylic acid, and compounds **2a**, **6a**, **b**, **7a**, **f** were significantly more active than ibuprofen. The compounds exhibiting the best anti-inflammatory activity showed negative inotropic effect.

1. Introduction

The survey of recent reports showed that the heteroaromatic acids and their derivatives, also those with acetic acid moiety attached to nitrogen atom of heterocyclic system, possess anti-inflammatory activity [1–4]. Anti-inflammatory activity of 3-pyrimidinylacetic acids and their derivatives has not yet been investigated. Furthermore, it was interesting to examine the inotropic activity of the above mentioned compounds because 3-pyrimidinylpyrazoles exhibited strong positive inotropic effects [5]. Therefore, the evaluation of the anti-inflammatory activity of (6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acid (**2a**) and related compounds as well as the testing of most active ones for inotropic effects was the aim of the present work.

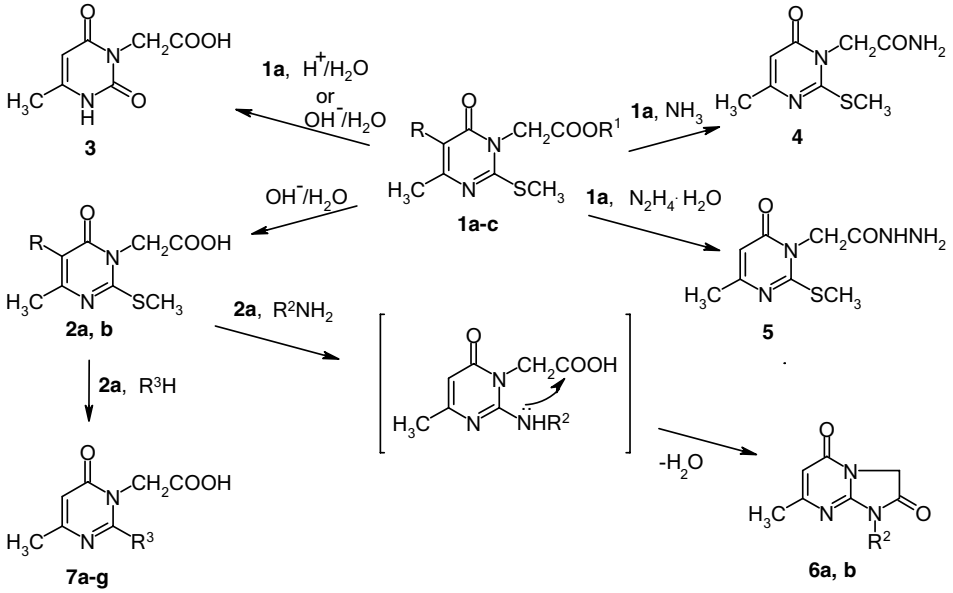
2. Investigations, results and discussion

2.1. Chemistry

Being interested in structure-pharmacological activity relationships we synthesized new compounds **1b**, **2b**, **6a**, **b**, **7a–g** and resynthesized compounds **1a**, **c** [6, 7], **2a**, **3–5** [8] earlier reported. Synthesis of the compounds is outlined in the Scheme.

(6-Methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acid (**2a**) was obtained from methyl (**1a**) [6, 7] or ethyl (6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetate (**1b**) under basic hydrolysis reaction. Earlier ester **1a** was hydrolyzed to acid **2a** in aqueous potassium carbonate solution at 40 °C for 6 h [8]. Now we determined, that hydrolysis of ester **1a** without substitution of the sensitive 2-methylsulfanyl group to hydroxy can be accomplished even at reflux. Hydrolysis of methyl

Scheme



1: R = H (**a**, **b**), C_2H_5 (**c**); R^1 = CH_3 (**a**, **c**), C_2H_5 (**b**);
2: R = H (**a**), C_2H_5 (**b**);
6: R^2 = C_4H_9 (**a**), $CH_2C_6H_5$ (**b**);
7: R^3 = $N(C_2H_5)_2$ (**a**), $N(C_3H_7)_2$ (**b**), $N(C_4H_9)_2$ (**c**), N⟨ ⟩ (**d**), N⟨ ⟩ (**e**), N⟨ ⟩ (**f**), N⟨ ⟩O (**g**).

ester **1a** proceeded for 10 min, ethyl ester **1b** for 40 min. Under analogous conditions hydrolysis of ester **1c** gave acid **2b**. Stronger conditions of hydrolysis (either acidic or basic) of ester **1a** led to the formation of acid **3** [8]. The reaction of ester **1a** with ammonia or hydrazine hydrate yielded amide **4** and hydrazide **5** [8], respectively. Also we investigated reactions of acid **2a** with primary and secondary amines. The pathway of reaction and the structure of formed compounds depended on the nature of the amine used. Primary – butyl- or benzylamine – reacted with acid **2a** under heating for 1 h to lose methanethiol with the further cyclization of formed 2-amino substituted acid into imidazo[1,2-a]pyrimidine-2,5-diones **6a**, **b**. Secondary amines – aliphatic diethyl-, dipropyl-, dibutylamine or heterocyclic – pyrrolidine, hexamethyleneimine and morpholine – reacted with acid **2a** to give 2-amino substituted acids **7a**–**g**.

Structures of the new synthesized compounds are in agreement with their IR, ^{1}H NMR spectral and elemental analysis data.

2.2. Pharmacological activity

Anti-inflammatory activity was studied by carrageenin- and bentonite-induced paw oedema in rats [9, 10]. The results are given in Table 1. As reference substances in experiments acetylsalicylic acid and ibuprofen were used. All tested compounds, except **7c**, reduced carrageenin- or bentonite-induced hind paw oedema in rats. Compound **2b** decreased carrageenin-induced oedema and was inactive in the bentonite test. Most of the compounds showed anti-inflammatory activity higher than acetylsalicylic acid, and compounds **2a**, **6a**, **b**, **7a**, **f** higher than that of ibuprofen. Among the compounds examined in this study (6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acid (**2a**) possesses the best anti-inflammatory effect, e.g., it decreased carregeenin-induced and bentonite-induced rats paw oedema by 47.8 and 52.7%, respectively. Replacement of the carboxy group in acid **2a** with different substituents decreased anti-inflammatory activity, e.g., ester **1a** reduced carrageenin- and bentonite-induced oedema by 31.3 and 37.0% respectively, then the amide **4** showed still less anti-inflammatory activity (16.2% in carrageenin and 24% in bentonite test). Hydrazide **5** had the same activity as acid **2a** in the bentonite test (50.5%), but by hydrazide **5** the carrageenin-induced oedema was lowered only by 23.5%. Substitution of the methylsulfanyl group in the pyrimidine ring by a hydroxy (compound **3**) also significantly decreased anti-inflammatory activity. The "structure-anti-inflammatory activity" relationship among the compounds **7a**–**c** can be established depending on the length of the carbon atoms chain in the secondary aliphatic amines, i.e., the longer the chain, the lower activity. Thus, compound **7a** bearing a diethylamino group at the second position of the pyrimidine ring was the most active of all and is similar to acid **2a**. It decreased carrageenin- and bentonite-induced paw oedema in rats by 45.3 and 44.5% respectively, while dipropyl substituted **7b** decreased inflammation only by 12.1 and 10.9%, respectively. Furthermore, the compound **7c** bearing a dibutylamino substituent was inactive at all. Introduction of the heterocyclic amine into the second position of the pyrimidine ring also decreased activity (**7d**–**g**), though the most active of them, **7f**, bearing a hexamethyleneimine moiety, exceeded anti-inflammatory activity of ibuprofen and was a little weaker than the acid **2a**. Compound **7f** decreased carrageenin and bentonite-induced oedema by 44.8 and 39.3%, respectively. The presence of an ethyl group in the fifth position of the pyrimidine ring, likely, determined weaker activity of the acid **2b** in response to carrageenin-induced oedema (18.9%) and total inactivity in the bentonite test. High anti-inflammatory activity, significantly higher than that of ibuprofen, exhibited compounds **6a**, **b** containing cyclic amide structure. Compound **6a** with a butylamine substituent in its molecule decreased carrageenin-induced paw oedema by 52.9% and bentonite-induced by 44.4%. It must be noted, unfortunately, that these compounds **6a**, **b** were more toxic than other derivatives (LD_{50} for **6a** – 785 mg/kg, for **6b** – 890 mg/kg). Compounds **6a**, **b** were more toxic than acetylsalicylic acid, but did not exceed the toxicity of ibuprofen. All the other tested compounds were less toxic than ibuprofen and most of them (except **5**) were less toxic than acetylsalicylic acid (Table 1).

Table 1: Anti-inflammatory activity (50 mg/kg p.o.) and acute toxicity (LD_{50}) data for compounds 1a, 2a, b, 3–5, 6a, b, 7a–g

Compd.	0.1 ml of 1% carrageenin solution		0.1 ml of 5% bentonite suspension		LD_{50} (mg/kg)
	Cross-section of rat paw (relative units)	Inhibition of rat paw oedema (%) over control	Cross-section of rat paw (relative units)	Inhibition of rat paw oedema (%) over control	
Control	94.3	0	95.0	0	
1a	64.7	31.3	59.8	37.0	1367
2a	49.2	47.8	44.9	52.7	1765
2b	75.4	18.9	96.1	0	
3	75.7	19.7	66.6	29.8	
4	79.0	16.2	72.2	24.0	
5	72.1	23,5	47.0	50.5	>1000
6a	44.4	52.9	52.8	44.4	785
6b	52.1	44.7	59.1	37.7	890
7a	51.5	45.3	52.7	44.5	>1500
7b	82.8	12.1	84.6	10.9	
7c	95.1	0	96.3	0	
7d	73.0	22.5	76.9	19.1	>1600
7e	70.1	25.6	72.4	23.8	>1800
7f	52.0	44.8	57.6	39.3	1231
7g	66.5	29.4	75.0	21.0	>1500
Acetylsalicylic acid	77.21	19.8	74.71	21.6	1216
Ibuprofen	59.69	38.0	75.57	20.7	500

Table 2: Effect of cumulative doses of compounds 2a, 6a, b, 7a, f on contractile force in papillary muscles of rat hearts

Compd.	Force of contraction ($P/P_0 \pm$ SEM,%)				EC_{50} (mol/l)
	1×10^{-6} mol/l	1×10^{-5} mol/l	1×10^{-4} mol/l	5×10^{-4} mol/l	
2a (5)	98.5 ± 4.9	85.8 ± 7.2	$62.7 \pm 6{,}5$	32.6 ± 5.8	6.5×10^{-3}
6a (4)	80.3 ± 2.0	63.4 ± 3.5	46.4 ± 1.9	44.7 ± 2.3	1×10^{-5}
6b (4)	81.0 ± 4.0	68.5 ± 2.6	55.0 ± 1.9	36.3 ± 0.9	1.7×10^{-4}
7a (4)	$86.3 \pm 6{,}0$	60.2 ± 4.4	43.5 ± 2.8	34.0 ± 2.8	1.25×10^{-5}
7f (5)	114.6 ± 1.9	80.0 ± 4.7	62.7 ± 2.7	45.5 ± 4.5	1.38×10^{-5}

Data are means ± SEM. Numbers in parentheses: number of experiments. P, contraction force at a given concentration; P_0, the control (100%).

Compounds with higher expressed anti-inflammatory activity (**2a**, **6a, b**, **7a, f**) were evaluated for cardiotonic action. Preliminary studies of cardiotonic activity indicated, that they possess a negative inotropic effect (Table 2).

3. Experimental

3.1. Chemistry

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on a FT-IR Spectrum BX (Perkin-Elmer, Sweden) in nujol and ^{1}H NMR spectra – on a BS-587A (80 MHz, Czechoslovakia) in $CDCl_3$ with TMS as an internal standard. Chemical shifts (δ) are reported in ppm, coupling constants (J) are given in Hz. All new compounds were analyzed for C, H and N and the results were in an acceptable range.
The synthesis of compounds **1a** [6, 7], **1c** [7], **2a**, **3–5** [8] was reported earlier.

3.1.1. Ethyl (6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)-acetate (**1b**)

To a stirred suspension of 17.8 g (0.1 mol) sodium salt of 4-hydroxy-6-methyl-2-methylsulfanylpyrimidine [7] in 50 ml CCl_4, 16.7 g (11.1 ml, 0.1 mol) of ethyl bromoacetate were added portionwise. The reaction mixture was refluxed under stirring for 7 h. The precipitate was filtered off, the filtrate evaporated to dryness. The residue was poured into 200 ml H_2O. The precipitate was filtered off, washed with H_2O, dried at room temperature and recrystallized from hexane. Yield 18.9 g (72%), m.p. 72.5–74.5 °C. IR (ν, cm^{-1}): 1688, 1745 (C=O). ^{1}H NMR (δ, ppm): 1.25 (3 H, t–J = 7 Hz, CH_3), 2.23 (3 H, s, CH_3), 2.57 (3 H, s, SCH_3), 4.2 (2 H, q–J = 7 Hz, CH_2), 4.79 (2 H, s, NCH_2), 6.07 (1 H, s, CH).

3.1.2. (6-Methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acid (**2a**) *[8] and (5-ethyl-6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acid* (**2b**)

A mixture of 0.01 mol ester **1a–c**, 4.14 g (0.03 mol) K_2CO_3 and 16 ml H_2O was refluxed for 10 min (esters **1a**, **c**) or 40 min (ester **1b**). The solution was cooled in an ice-water bath and acidified with conc. HCl to pH 3. The formed precipitate was filtered off, washed with cold H_2O and recrystallized from H_2O. **2b**: yield 64%, m.p. 194–196 °C. IR (ν, cm^{-1}): 1619, 1751 (C=O), 3474 (OH). ^{1}H NMR (δ, ppm): 1.08 (3 H, t–J = 6 Hz, CH_3), 2.29 (3 H, s, CH_3), 2.46 (2 H, q–J = 6 Hz, CH_2), 2.58 (3 H, s, SCH_3), 4.84 (2 H, s, NCH_2), 8.26 (1 H, s, OH).

3.1.3. 1-Butyl (or benzyl)-7-methyl-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-2,5-diones (**6a, b**) *and (2-amino substituted 6-methyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acids* (**7a–g**)

A mixture of 2.14 g (0.01 mol) acid **2a** and 0.01 mol corresponding amine was heated for 1 h in an oil bath at 120–200 °C. The reaction mixture was cooled, the glassy paste was mixed up with 20 ml ether. The precipitate was filtered off and recrystallized.
6a (reaction temp. 120–140 °C): yield 69%, m.p. 97–98 °C (H_2O). IR (ν, cm^{-1}): 1689, 1703, 1752 (C=O). ^{1}H NMR (δ, ppm): 0.98 (3H, t–J = 6 Hz, CH_3), 1.2–1.87 [4 H, m, $(CH_2)_2$], 2.29 (3 H, s, CH_3), 3.76 (2 H, t–J = 6 Hz, NCH_2), 4.44 (2 H, s, NCH_2), 6.03 (1 H, s, CH).
6b (reaction temp. 190–200 °C): yield 65%, m.p. 149–150 °C (i-PrOH). IR (ν, cm^{-1}): 1680, 1698, 1749 (C=O). ^{1}H NMR (δ, ppm): 2.29 (3 H, s, CH_3), 4.38 (2 H, s, NCH_2), 4.87 (2 H, s, NCH_2), 5.97 (1 H, s, CH), 7.2–7.57 (5 H, m, H_{arom}).
7a (reaction temp. 140–180 °C): yield 70%, m.p. 166–167 °C (toluene-hexane). IR (ν, cm^{-1}): 1637, 1672, 1718 (C=O), 3121, 3165, 3231 (OH). ^{1}H NMR (δ, ppm): 0.96–1.4 [6 H, m, $(CH_3)_2$], 2.07 (3 H, s, CH_3), 3.38 [4 H, q–J = 7 Hz, $N(CH_2)_2$], 4.7 (2 H, s, NCH_2), 5.49 (1 H, s, CH), 10.28 (1 H, broad s, OH).
7b (reaction temp. 130–170 °C): yield 56%, m.p. 180–181 °C (H_2O). IR (ν, cm^{-1}): 1643, 1652, 1714, 1739 (C=O), 3090, 3167, 3238, 3385 (OH). ^{1}H NMR (δ, ppm): 0.93 [6 H, q–J = 7 Hz, $(CH_3)_2$], 1.39–1,92 [4 H, m, $(CH_2)_2$], 2.07 (3 H, s, CH_3), 3.27 [4 H, t–J = 7 Hz, $N(CH_2)_2$], 4.71 (2 H, s, NCH_2), 5.52 (1 H, s, CH), 10.09 (1H, broad s, OH).
7c (reaction temp. 160–180 °C): yield 54%, m.p. 127–128 °C (hexane-CCl_4). IR (ν, cm^{-1}): 1633, 1655, 1712, 1724 (C=O), 3107, 3178, 3249, 3397 (OH). ^{1}H NMR (δ, ppm): 0.94 [6 H, t–J = 6 Hz, $(CH_3)_2$], 1.1–1.9 [8 H, m, $(CH_2)_4$], 2.05 (3 H, s, CH_3), 3.1–3.46 [4 H, m, $N(CH_2)_2$], 4.68 (2 H, s, NCH_2), 5.51 (1 H, s, CH), 10.38 (1 H, s, OH).
7d (reaction temp. 130–155 °C): yield 57%, m.p. 250–252 °C (CH_3OH-ether). IR (ν, cm^{-1}): 1627, 1655, 1727 (C=O), 3119, 3169, 3216, 3353 (OH). ^{1}H NMR (δ, ppm): 1.74–2.12 [4 H, m, $(CH_2)_2$], 2.08 (3 H, s, CH_3), 3.38–3.62 [4 H, m, $N(CH_2)_2$], 4.64 (2 H, s, NCH_2), 5.49 (1 H, s, CH), 10.07 (1 H, broad s, OH).
7e (reaction temp. 130–160 °C): yield 65%, m.p. 175–176 °C (CH_3OH-ether). IR (ν, cm^{-1}): 1629, 1659, 1722 (C=O), 3094, 3130, 3180, 3232 (OH). ^{1}H NMR (δ, ppm): 1.65 [6 H, s, $(CH_2)_3$], 2.1 (3 H, s, CH_3), 3.49 [4 H, s, $N(CH_2)_2$], 4.73 (2 H, s, NCH_2), 5.53 (1 H, s, CH), 9.9 (1 H, broad s, OH).
7f (reaction temp. 160–180 °C): yield 60%, m.p. 190–191 °C (CH_3OH-ether). IR (ν, cm^{-1}): 1613, 1646, 1667, 1728 (C=O), 3098, 3160, 3244(OH). ^{1}H NMR (δ, ppm): 1.64 [8 H, s, $(CH_2)_4$], 2.08 (3 H, s, CH_3), 3.51 [4 H, s, $N(CH_2)_2$], 4.73 (2 H, s, NCH_2), 5.52 (1 H, s, CH), 10.11 (1 H, broad s, OH).
7g (reaction temp. 190–200 °C): yield 63%, m.p. 194–196 °C (CH_3OH). IR (ν, cm^{-1}): 1631, 1660, 1727 (C=O), 3110, 3170, 3240(OH). ^{1}H NMR (δ, ppm): 2.14 (3 H, s, CH_3), 3.7 [8 H, s, $N(CH_2)_2$, $O(CH_2)_2$], 4.74 (2 H, s, NCH_2), 5.57 (1 H, s, CH), 9.61 (1 H, s, OH).

3.2. Pharmacology

3.2.1. Anti-inflammatory activity

Adult male Wistar strain rats weighing 180–220 g and male BALB/C strain mice weighing 18–22 g were used. The animals were allowed food and water *ad libitum*. They were housed in rooms at 18–20 °C with a 12 h light/dark cycle and relative humidity of 55–60%. The animals were randomly allocated into groups at the begining of all the experiments. All test compounds and the reference drugs were administered orally suspended in 0.5% carboxymethylcellulose solution. Carrageenin-induced hind paw oedema in rats was produced by the method of Winter et al. [9]. Carrageenin solution (1.0% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was injected subcutaneously into the subplanar region of the right hind paw 1 h after administration of the test compound. Control animals received only 0.5% carboxymethylcellulose solution. Right hind paw volume was measured with an electronic onkograph immediately before and 1, 2, 3 and 5 h after the carrageenin injection. The increase observed was compared with that of control rats. Each experiment was made with five group of rats, 10 animals each (the first one served as control). Bentonite-induced hind paw oedema was analogously studied [10]. Bentonite suspension (5% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was used. The data were evaluated statistically using Student's t-test. A level of $p < 0.05$ was adopted as the test of significance.
The tests of acute toxicity of the compounds were done on mice fasted for 24 h water *ad libitum*. Groups of 6 mice were treated perorally with the test compound at various dose levels. The animals were watched for mortality and symptoms until 8th day [11].

3.2.2. Inotropic activity

Adult male rats were stunned with a blow on the head, their chest was opened rapidly, the heart was quickly removed and immersed in Tyrode's solution, pH 7.4, containing (in mmol/l): NaCl (160), KCl (4.6), $CaCl_2$ (1.5), $MgCl_2$ (1), Tris·Cl (5), glucose (10). The left ventricular papillary muscle was severed and placed in an organ bath with the temperature maintained at 37 °C. All solutions for dissection and perfusion were equilibrated with pure oxygen. The maximal volume of perfusion of the heart

preparations was about 4.5 ml/min. Electrostimulator ESU-2 (Kursk, Russia) electrically stimulated the muscles by square wave pulses of a voltage of 20% above threshold, 10 ms duration at a frequency of 1 Hz. The force of the isometric contraction was measured by force displacement transducer 6MX2B (Electrical engineering works, Moscow, Russia) and recorded by recorder H3030–4 (Work of Measuring Instruments, Krasnodar, Russia).

To obtain a concentration-dependent response of investigated compounds, each papillary muscle after a 1 h equilibration period was tested in cumulative doses of studied agents. Quantitatively, the contraction force (P) was expressed as respective ratios to the initial value of the contraction force (P_0) under normal conditions. The index of potency employed to describe the inotropism in these experiments was the EC_{50}, the negative logarithm of the concentration of the test agent required for a 50% decrease in the minimal force of contraction in experimental conditions.

For contractility studies all tested compounds were dissolved in a minimum amount of DMSO and diluted with Tyrode's solution. The maximum final concentration of the vehicle in the medium did not exceed 0.33%.

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