On the anti-inflammatory activity of some novel N,N-disubstituted triazene derivatives

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The acute toxicity and anti-inflammatory activity of 11 potassium salts of sulfobenzene and sulfonaphthalene 3,3-disubstituted triazenes (50 mg/kg *per os*)has been examined on the carrageenin- and bentonite-induced edema in rats, using. All compounds were found to exhibit the anti-inflammatory activity significantly exceeding that of acetylsalicylic acid and ibuprofen, and to be less toxic than these reference drugs. The derivative of N-methylpiperazine showed the most pronounced anti-inflammatory activity.

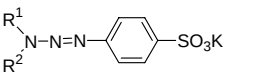
Key words: triazenes, sulfoaryl derivatives, anti-inflammatory activity, carrageenin-and bentonite induced edema in rats

INTRODUCTION

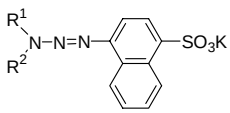
Though non-steroidal anti-inflammatory drugs (NSAIDs) proved to be sufficiently effective in therapy, the search for new active compounds is still in progress [1–3]. The renewed interest is related to the recent findings on the mechanism of NSAIDs action *via* selective inhibition of the cyclooxygenases COX-1 and COX-2, resulting in inhibition of prostaglandin synthesis in the inflammation site [4, 5]. The chemical structure of the active ingredients of NSAIDs shows a great variety, and the search for new active substances is proceeding in the classes of, for example, aromatic compounds [6], sulfonamides [1, 7], heterocycles [8], derivatives of succinic and amino acids [9, 10], etc.

Aryldimethyl triazenes, in addition to their known antitumour activity and low toxicity, also exhibit a well-pronounced anti-inflammatory action [11, 12]. These data induce a further interest to the anti-inflammatory activity of related triazenes.

Therefore the objective of this work was to study the anti-inflammatory activity of the potassium salts of sulfobenzene 3,3-disubstituted triazenes, containing various substituents at triazene function (**1–8**) and to compare their activity with that of the related derivatives of naphthalene (**9–11**):



	$R^1 = R^2$ or $R^1 + R^2$
1	$-CH_3$
2	$-(CH_2)_4-$
3	$-(CH_2)_5-$
4	$-(CH_2)_6-$
5	$-(CH_2)_2O(CH_2)_2-$
6	$-(CH_2)_2N(H)(CH_2)_2-$
7	$-(CH_2)_2N(CH_3)(CH_2)_2-$
8	$-(CH_2)_2N(N{=}N{-}C_6H_4{-}SO_3K)(CH_2)_2-$



	$R^1 = R^2$ or $R^1 + R^2$
9	$-CH_3$
10	$-(CH_2)_5-$
11	$-(CH_2)_2O(CH_2)_2-$

The anti-inflammatory action of these compounds has been examined on two experimental models: carrageenin- and bentonite-induced edema in rats. For comparison, the well-known anti-inflammatory drugs, acetylsalicylic acid and ibuprofen, were studied too. Acute toxicity was evaluated only for the most active compounds (**4, 7–9**).

MATERIALS AND METHODS

Substances

Melting points were determined in open glass capillaries and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL 90 instrument in D_2O with DSS as an internal standard. Multiplicity of signals is expressed as s (singlet), m (multiplet) or bs (broad singlet).

The synthesis and physicochemical properties of compounds **1–8** are given in [13]. Compounds **9–11** were prepared according to the following procedure. To a stirred suspension of 0.01 mol of 4-sulphonaphthalene diazonium chloride obtained analogously to 4-sulfobenzene diazonium chloride [14], to 20 ml of water at room temperature 0.05 mol of the appropriate amine was added, and stirring was continued for 10 min. Then the reaction temperature was gradually raised to 50°, and after 10 min a clear solution was obtained. The solution was then treated with 0.1 mol of KOH in 20 ml of methanol, the mixture was heated to reflux and left to crystallize. Crystals were collected by filtration, washed with acetone and recrystallized from methanol-water.

4-(3,3-Dimethyltriazeno)naphthalene sulfonic acid, potassium salt, **9**. Yield 55%; m.p. 255–257 °C; NMR (D_2O), δ, ppm: 3.50 (6H, bs, two CH_3), 7.20–9.10 (6H, m, Ar).

4-(3,3-Pentamethylenetriazeno)naphthalene sulfonic acid, potassium salt, **10**. Yield 53%; m.p. 225–228 °C; NMR (D_2O), δ, ppm: 1.34 (6H, bs, $(CH_2)_3$), 3.50 (4H, br.m, CH_2NCH_2).

4-(3,3-Oxydiethylenetriazeno)naphthalene sulfonic acid, potassium salt, **11**. Yield 60%; m.p. 250 °C (dec.); NMR (D_2O), δ, ppm: 3.56 (8H, s, $(CH_2)_2O(CH_2)_2$), 7.09–8.73 (6H, m, Ar).

Animals

Young male BALB/c strain mice (body mass 22–24 g) and adult male Wistar strain rats (body mass 180–200 g) were clinically healthy. They were obtained from the breeding unit of the Institute of Immunology and kept under standard housing conditions in the Vivarium of the Faculty of Medicine of Vilnius University. The animals were acclimatized to laboratory conditions for at least 5 days prior to the test. Then they were randomised into treatment groups and housed in standard smal polycarbonate cages with chipped hardwood bedding. Throughout the study the animals were cared for in accordance with European Convention and Guide for the Care and Use of Laboratory Animals and the Lithuanian Laws [15–17]. The animals were supplied with food (standard ration) and tap water *ad libitum*. The response of each animal to killing procedure was recorded by the same person using the cerebral dislocation method [18].

Toxicity tests

In the acute toxicity test, the survival of mice (5 in each group) administered the graduated single dose levels of each compound was observed for 8 days [19]. The LD_{50} value was determined by the accepted Litchfield and Wilcoxon method [20].

Anti-inflammatory activity tests

For these tests adult male Wistar strain rats were used. Each experiment was performed with five groups of rats, 10 rats in each. All tested compounds and reference drugs were suspended in 0.5% carboxymethylcellulose (CMC) solution and administered orally at a dose of 50 mg/kg.

Carrageenin-induced hind paw edema in rats was produced by the method of Winter et al. [21]. Carrageenin 1% solution in steril 0.9% NaCl solution in the volume of 0.1 ml was injected subplantary into the right hind paw 1 h before administration of the test compounds. Animals of the control group received only 0.5% CMC solution. Hind paw volume was measured with an electronic oncograph immediately before and 1, 2, 3 and 5 h after injection of a flagogenic agent. The results were compared with those of the control rats.

Analogously bentonite-induced hind paw edema [22] was studied. Bentonite suspension (5% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was used.

The obtained data were evaluated statistically using Student's t test. The level of $p \leq 0.05$ was adopted as the test of significance.

RESULTS AND DISCUSSION

Evaluation of the acute toxicity showed that all compounds selected for the study (**4, 7–9**) were low toxic ($LD_{50} > 1500$ mg/kg), *i.e.* less toxic than acetylsalicylic acid ($LD_{50} \approx 1200$ mg/kg) and much less toxic than ibuprofen ($LD_{50} \approx 500$ mg/kg).

The effects caused by p.o. administration of the test compounds as well as reference drugs at a dose of 50 mg/kg on the carrageenin- and bentonite-induced edema development are given in Table. Each reported value is expressed as the percentage inhibition (±S.E.) of the mean increase of paw volume in the treated animals as compared with untreated controls. The results were statistically significant ($p \leq \leq 0.05$) during the whole period of observation (5 h).

In general, all the compounds studied 5 h after the injection of flagogenic agent exhibited a high anti-inflammatory activity, which significantly excee-

Table. **Time-dependent anti-inflammatory action (50 mg/kg, p.o.) of compounds 1–11 compared to control in rats expressed as inhibition of rat paw edema (%)**

Compound	Carrageenin-induced edema				Bentonite-induced edema			
	after 1 h	after 2 h	after 3 h	after 5 h	after 1 h	after 2 h	after 3 h	after 5 h
1	17 ± 2	17 ± 2	38 ± 4	43 ± 5	14 ± 1	20 ± 2	40 ± 4	47 ± 5
2	17 ± 1	53 ± 5	16 ± 2	27 ± 3	2 ± 1	49 ± 5	23 ± 1	26 ± 3
3	21 ± 2	22 ± 3	33 ± 4	40 ± 4	20 ± 2	31 ± 3	39 ± 4	47 ± 5
4	63 ± 6	28 ± 3	16 ± 2	54 ± 5	52 ± 5	27 ± 3	15 ± 1	50 ± 4
5	20 ± 2	20 ± 2	32 ± 3	66 ± 7	9 ± 1	18 ± 2	28 ± 3	43 ± 4
6	28 ± 3	27 ± 2	28 ± 3	42 ± 4	18 ± 2	25 ± 3	23 ± 3	38 ± 4
7	71 ± 7	60 ± 6	51 ± 6	54 ± 5	48 ± 5	64 ± 7	52 ± 5	56 ± 6
8	40 ±3	45 ± 4	45 ± 5	50 ± 5	48 ± 5	43 ±4	42 ± 4	47 ± 5
9	32 ± 4	25 ± 2	31 ± 3	41 ± 4	29 ± 3	27 ±3	30 ± 3	40 ± 4
10	0 ± 1	24 ± 2	31 ±3	29 ± 3	21 ± 2	38 ± 4	37 ± 4	14 ± 1
11	18 ± 2	3 ± 1	18 ± 2	31 ± 3	9 ± 1	11 ± 1	14 ± 2	31 ± 3
Acetylsalicylic acid	11 ± 1	15 ± 2	21 ± 2	26 ± 2	11 ± 1	21 ± 2	21 ± 2	28 ± 3
Ibuprofen	28 ± 3	31 ± 2	31 ± 3	33 ± 3	21 ± 2	23 ± 2	19 ± 2	20 ± 2

ded that of reference drugs. According to the time-dependence of the effects they fell into three groups: compounds with gradual increase of activity (**1, 3, 5**), resembling acetylsalicylic acid; compounds of almost stable activity (**6, 7, 8, 9**), resembling ibuprofen; and compounds of variable activity (**2, 4, 10, 11**). The influence of the substituents in the triazene group was not pronounced, however, some regularities could be noticed. Thus, in the compounds of the first group the highest activity at the end of experiment in carrageenin-induced edema model was exhibited by the derivative containing a morpholine group (**5**), but it was less active in the bentonite-induced edema model. The piperidine derivative (**3**) in both models showed nearly the same activity as dimethyltriazene (**1**). The hexamethylene derivative (**4**) showed a high activity at the beginning of the experiment, but it varied in time. The compounds of the second group, especially derivatives of piperazine (**6, 7, 8**), deserve special attention. In addition to their high activity, these compounds retained an almost constant action throughout the whole experiment. Here it should be noted that methylation of the imino group in **6** as well as introduction of the second triazene function (**8**) caused a positive effect. The influence of the naphthalene ring as compared to a benzene analogues was indefinite. For instance, the activity of the dimethyl derivative of naphthalene (**9**) was about the same as that of benzene (**1**), but both other derivatives (**10, 11**) were less active.

In conclusion, the results of this study demonstrated that potassium salts of sulfoaryl 3,3-disubstituted triazenes, especially the derivatives of piperazine (**6–8**), exhibit a higher anti-inflammatory activity than acetylsalicylic acid and ibuprofen. Compounds with a gradually increasing activity (**1, 3, 5**) and compounds with almost constant activity (**6–9**) could be expected to be active also in chronic inflammation and are the subject of further more detailed examination.

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APIE KAI KURIŲ NAUJŲ N,N-DIPAKEISTŲ TRIAZENŲ DARINIŲ PRIEŠUŽDEGIMINĮ AKTYVUMĄ

S a n t r a u k a

Ištirtas 11 sulfobenzen- ir sulfonaftalen-3,3-dipakeistų triazenų kalio druskų priešuždegiminis aktyvumas bandymuose su karagenino ir bentonito sukeltomis žiurkių edemomis, naudojant peroraliai 50 mg/kg dozę. Nustatyta, kad dauguma tirtųjų junginių yra daug aktyvesni už acetilsalicilo rūgštį ir ibuprofeną, o jų toksiškumas yra mažesnis nei minėtų vaistų. Aktyviausias junginys yra N-metilpiperazino darinys.