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Anti-inflammatory action of sulfoaryl 3,3-disubstituted triazenes in rat experimental edema models

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Received March 3, 2003, accepted April 5, 2003

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Pharmazie 58: 730–732 (2003)

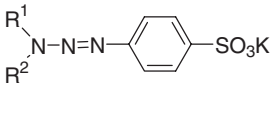
The acute toxicity and anti-inflammatory activity of eleven potassium salts of sulfobenzene and sulfonaphthalene 3,3-disubstituted triazenes have been examined in rats with carrageenin- and bentonite-induced edema using a 50 mg/kg p.o. dose. All compounds were found to exhibit anti-inflammatory activity significantly exceeding that of acetylsalicylic acid and ibuprofen, and were less toxic than these reference drugs. The most pronounced anti-inflammatory activity was shown by the potassium salt of 4-(piperazin-1-ylazo)benzenesulfonic acid.

1. Introduction

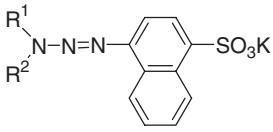
Aromatic triazenes are well known and have been widely studied due to their important antitumour effects and low toxicity [1–4]. In recent years this class of compounds has received attention in a search for potential HIV-1 inhibitors [5]. 4-(3,3-dimethyltriazeno)benzoic acid potassium salt (DM-COOK) [6], a highly active antimetastatic and antidisseminative compound, which has been proposed for use as a substitute for the clinically used compounds 5-(3,3-dimethyltriazeno)imidazole-4-carboxamide (dacarbazine, DTIC), has been shown to possess high antiinflammatory action on carrageenin induced edema in guinea pigs, comparable with that of indometacin and phenylbutazone [7]. The sodium salt of 4-(3,3-dimethyltriazeno)benzenesulfonic acid also exhibits a pronounced antiinflammatory effect [7]. N-acylated derivatives of (3,3-dimethyltriazeno)benzenesulphonamide have been studied on the adjuvant arthritis model in rats, and it was observed that the activity of these compounds depends on the substituent in the sulfonamide group [8]. Several of them were found to be more active than the reference compounds acetylsalicylic acid and phenylbutazone. Here it should be emphasized that all the compounds investigated had the 3,3-dimethyltriazeno group.

In the search for new potential antiinflammatory agents among triazene (aminoazo) derivatives it seems promising to study compounds having modifications both in the triazene function, and in the aromatic system. Therefore in this work we present experimental data on the anti-inflammatory activity of the potassium salts of sulfobenzene 3,3-disubstituted triazenes, containing various substituents at the triazene function (**1**–**8**) and compare their activity with related newly synthesized naphthalene derivatives (**9**–**11**).

The anti-inflammatory action of these compounds was examined in two experimental models: carrageenin- and bentonite-induced edema in rats [9, 10]. For comparison, the well-known anti-inflammatory drugs, acetylsalicylic acid and ibuprofen, were also studied. Acute toxicity was evaluated only for the most active compounds (**4, 7**–**9**).



	$R^1 = R^2$ 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	$-(CH_2)_2O(CH_2)_2-$

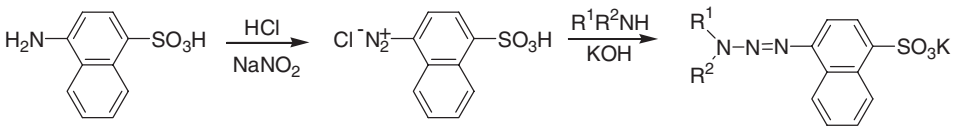
2. Investigations, results and discussion

2.1. Chemistry

The synthesis and physicochemical properties of compounds **1**–**8** are described elsewhere [11]. The naphthalene derivatives **9**–**11** were obtained in an analogous manner by diazoaminocoupling of 4-sulfonaphthalenediazonium chloride (DS-SO_3H) with dimethylamine, morpholine or piperidine according to the Scheme.

DS-SO_3H was obtained by a procedure similar to that described in [12]. Products **9** and **10** were isolated as potassium salts after addition of KOH in methanol to the reaction mixture. The structures of the newly synthesized

Scheme



DS-SO$_3$H **9 - 11**

compounds are in agreement with their elemental analysis data and ^{1}H NMR spectra.

2.2. Pharmacological activity

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

In general, all the compounds studied exhibited high anti-inflammatory activity 5 h after the injection of the flagogenic agent, significantly exceeding that of the reference drugs. Based on the time-dependence of the effects they fall into three groups: compounds with a gradual increase of activity (**1**, **3**, **5**), resembling acetylsalicylic acid; compounds of almost stable activity (**6**–**9**), resembling ibuprofen; and compounds of variable activity (**2, 4, 10, 11**). Influence of the substituents in triazene group is not clearly expressed, although some regularities may be noted. Thus, in the compounds of the first group the highest activity at the end of the experiment on the carrageenin-induced edema model is exhibited by the derivative containing a morfoline group (**5**), but it is less active in the bentonite-induced edema model. The piperidine derivative (**3**) has about the same activity in both models as dimethyltriazene (**1**). The hexamethylene derivative (**4**) possesses high activity at the beginning of the experiment, but it varies over time. The compounds of the second group, especially derivatives of piperazine (**6**–**8**), deserve special attention. In addition to their high activity, the compounds retain an almost constant action during the whole experiment. Here it should be noted that methylation of the imino group in **6** as well as the introduction of the second triazeno function (**8**) causes a positive effect. The influence of the naphthalene ring as compared with benzene analogues is indefinite. For instance, the activity of the dimethyl derivative of naphthalene (**9**) is about the same as that of benzene (**1**), but both other derivatives (**10**, **11**) are less active.

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

In conclusion, the results of this study demonstrate that potassium salts of sulfoaryl 3,3-disubstituted triazenes, especially derivatives of piperazine (**6**–**8**), have an advantage in anti-inflammatory activity over acetylsalicylic acid and ibuprofen. Compounds with a gradual increase of activity (**1**, **3**, **5**), and compounds with almost constant activity (**6**–**9**) might be expected to be active in chronic inflammation also and are the subject of further more detailed examination.

3. Experimental

3.1. Chemistry

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** have been described previously [11]. Compounds **9**–**11** were prepared according to the following procedure: To a stirred suspension of 0.01 mol of DS-SO$_3$H, obtained analogously to 4-sulfobenzene diazonium chloride [12], in 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 °C, 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, and this mixture was heated to reflux, and left to crystallize. Crystals were collected by filtration, washed with acetone and recrystallized from methanol-water.

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

Compd.	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

4-(3,3-Dimethyltriazeno)naphthalene sulfonic acid, potassium salt, **9.** Yield 55%; m.p. 255–257 °C; NMR (D_2O), δ, ppm: 3.50 (6 H, bs, two CH_3), 7.20–9.10 (6 H, m, Ar).
4-(Piperidin-1-ylazo)naphthalene sulfonic acid, potassium salt, **10.** Yield 53%; m.p. 225–228 °C; NMR (D_2O), δ, ppm: 1.34 (6 H, bs, $(CH_2)_3$), 3.50 (4 H, br.m, CH_2NCH_2).
4-(Morpholin-4-ylazo)naphthalene sulfonic acid, potassium salt, **11.** Yield 60%; m.p. 250 °C (dec.); NMR (D_2O), δ, ppm: 3.56 (8 H, s, $(CH_2)_2O(CH_2)_2$), 7.09–8.73 (6 H, m, Ar).

3.2. Pharmacology

3.2.1. 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 breedingunit 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. The animals were then randomised into treatment groups and housed in standard small polycarbonate cages with chipped hardwood bedding. They were supplied with food (standard ration) and tap water *ad libitum*.

3.2.2. Toxicity tests

In the acute toxicity test the survival of mice (5 in each group) administered graduated single doses of each compound (p.o.) was observed for 8 days. The LD_{50} value was determined by the an accepted method of Litchfield and Wilcoxon [13].

3.3.3. Anti-inflammatory activity tests

Adult male Wistar strain rats were used for these tests. Each experiment was performed with five groups of rats, 10 rats each. All test compounds and reference drugs were suspended in 0.5% carboxymethylcellulose (CMC) solution and administered orally in a dose of 50 mg/kg. Carrageenin-induced hind paw edema in rats was produced by the method of Winter et al. [9]. 0.1 ml of carrageenin solution (1% in sterile 0.9% NaCl solution) was injected subplantary into the right hind paw 1 h before the administration of the test compounds. Animals of the control group received only 0.5% CMC solution. Hind paw volume was measured with an electronic onkograph immediately before and 1, 2, 3 and 5 h after injection of the flagogenic agent. The results were compared with those of the control rats.
Bentonite-induced hind paw edema [10] was studied analogously. 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. A level of $p \leq 0.05$ was adopted as the test of significance.

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