The Analgesic Activity of 8-(Trifluoromethyl)-1,2,3,4,5-benzopentathiepine-6-amine and Its Hydrochloride

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Abstract: 8-(Trifluoromethyl)-1,2,3,4,5-benzopentathiepine-6-amine **1a** and its hydrochloride revealed significant analgesic activity in the acetic acid-induced writhing test, and ED_{50} of the compound **1a** was 0.66 mg/kg. In the hot plate pain test, the reliable analgesic activity was found in hydrochloride **1a*HCl**, whereas the free base **1a** proved to be inactive. Based on the results of the naloxone test, the analgesic effect of the compound **1a*HCl** in both tests is mediated with the opioidergic system. Both the free base **1a** and its hydrochloride did not show the anti-inflammatory activity. Replacement of the CF_3 group for the fluorine atom resulted in complete loss of analgesic activity in the acetic acid-induced writhing test.

Keywords: Analgesic activity, Benzopentathiepine, Acetic acid-induced writhing test, Hot plate test, Opioidergic system.

INTRODUCTION

Pain is a common symptom, which non-steroidal anti-inflammatory drugs are usually used to remove [1]. However, chronic use of these medications produces significant adverse effects such as gastro- and nephrotoxicity, renal impairment, etc. In recent years medications have started to be used for pain control, which were originally developed for other purposes, including antidepressants and anti-seizure drugs [2]. According to data on total value and market share by sales in the seven major pharmaceutical markets of major pain drug classes in 2009, shares of sales of antidepressants and anticonvulsant medications for pain relief were 11% and 13%, respectively [3]. Usually antidepressants are applied to control chronic pain [4] and fibromyalgia [5], but research in the hot plate model demonstrated [6] that the antidepressant Paroxetin might be efficient for acute pain relief and its mechanism probably involves the opioid and serotoninergic systems.

It was recently found that 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepine-6-amine **1a** showed a high anticonvulsant and anxiolytic activity [7]. The available data indicate the involvement of the 5-HT brain system in the mechanism of action of this compound [8]. The study of acute toxicity of the compound **1a** revealed that at oral administration in rats, LD_{50} of this agent is more than 1000 mg/kg. In the present paper, we first studied the analgesic activity of the compound **1a** using the acute pain models.

MATERIALS AND METHODS

Chemistry

8-(Trifluoromethyl)-1,2,3,4,5-benzopentathiepine-6-amine **1a**, 8-(fluoro)-1,2,3,4,5-pentathiepin-6-amine **1b** and 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepine-6-amine hydrochloride **1a*HCl** were synthesized in accordance with previously published methodic [7-9].

Pharmacology

Animals

All studies were carried out on non-breeding albino mice (male) weighting 20-25 g, 8 animals in each group (SPF-vivarium of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences). Mice were maintained at 22-25 ^{o}C on a 12 h light-dark cycle with food and water available ad libitum. All work with animals was performed in strict accordance with the legislation of the Russian Federation, the regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the requirements and recommendations of the Guide for the Care and Use of Laboratory Animals.

Analgesic Tests

Agents were dissolved in saline containing 0.5% Tween 80 just before use and were administered p.o. 1 h before testing. Naloxone (Sigma) was administered (dose of 1 mg/kg s.c.) 50 min after administration of the test agents (10 min before testing). Analgesic activity of test agents was assessed using acetic acid-induced writhing test and hot plate test.

In the acetic acid-induced writhing test, the pain reaction was determined by the number of abdominal convulsions, recorded from the 5th to the 8th min following the acetic acid injection (0.75%, 0.1 mL/mouse) [10]. The percentage of pain reaction inhibition was calculated according to the following equation: % inhibition = 100 x (A – B)/A, where A is the mean number of writhes in the control group, and B is the mean number of writhes in the test group.

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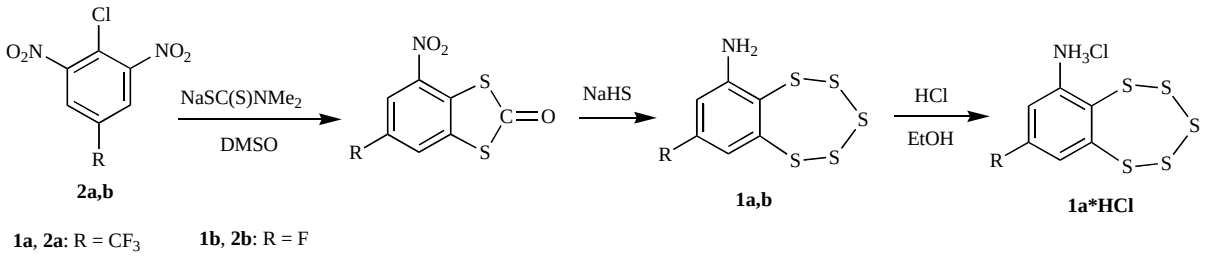

2a,b **1a,b** **1a*HCl**

1a, **2a**: R = CF_3 **1b**, **2b**: R = F

Scheme 1. Synthesis of compounds **1a,b**.

In the hot plate test, animals were placed individually on a metallic plate warmed to 54±0.5 ^{o}C and the time until either licking of the hind paw or jumping occurred was recorded by a stopwatch [11].

Anti-Inflammatory Activity

Anti-inflammatory activity of the compounds was studied in inflammation model induced by injection of 0.1% histamine (0.05 mL) into the aponeurosis of the hind limb [11]. Test compounds were administered 1 h before injection of the inflammatory agent. The total dose used for agent was 10.0 mg/kg, and the total dose for sodium diclofenac was 20 mg/kg. Mice were killed 5 h after the inflammatory episode; the hind limbs were severed and then weighed. The inflammation percentage was calculated by the equation: % inflammation = 100 x (M_{il}-M_{nil})/M_{nil}; where M_{il} is inflamed limb mass, and M_{nil} is non-inflamed limb mass.

RESULTS AND DISCUSSION

The compound **1a** was synthesized from commercially available 4-chloro-3,5-dinitrobenzotrifluoride **2a** in a two-stage process, in accordance with the procedure proposed earlier (Scheme **1**) [7, 9]. Hydrochloride **1a*HCl** was obtained by the interaction of **1a** with HCl in absolute ethanol [8]. To find the effect of the CF_3 replacement in the aromatic ring on biological activity, we also tested the compound **1b**, where the CF_3 group is replaced for the fluorine atom (Scheme **1**) [9].

Analgesic activity of the agents was studied using standard models of experimental pain, including the acetic acid-induced writhing test (intraperitoneal administration of 0.75% acetic acid, 0.1 ml per one animal) and the hot plate pain test (T= 54±0.5 ^{o}C) in doses of 0.5, 1.0, 5.0 and 10.0 mg/kg orally [10, 11].

In the acetic acid-induced writhing test, which represents the model of acute visceral pain, the compound **1a** in the 10 mg/kg showed reliable analgesic activity (Table **1**) as it significantly reduced the number of convulsions provoked by intraperitoneal administration of acetic acid. In the dose of 5 mg/kg, the compound **1a** only insignificantly, and with a low degree of reliability, decreased the number of the convulsions. However, further decreasing the dose **(**to 1 and 0.5 mg/kg) led to a pronounced increase of the analgesic effect. Interestingly, for hydrochloride **1a*HCl,** another dynamics of the dose-effect dependence was observed: raising the dose increased its analgesic activity. At the replacement of the CF_3 group for the fluorine atom, the compound **1b** in the dose of 10 mg/kg, no reliable analgesic effect was found.

Table 1. Percent Analgesic Activity (Acetic Acid-Induced Writhing Test)

Compound	Dose (mg/kg)	Pain inhibition (%)
1a	10	29$^{\#}$
1a	5	6
1a	1	54$^{\#}$
1a	0.5	50§
1a*HCl	10	45$^{\#}$
1a*HCl	5	41$^{@}$
1a*HCl	1	27$^{\#}$
1a*HCl	0.5	28§
1b	10	18
Diclofenac sodium	10	90$^{\#}$

$^{@}P < 0.05$; $^{\S}P < 0.01$; $^{\#}P < 0.001$ in comparison with control.

ED_{50} in the test of acetic acid-induced writhing test constituted 0.66 mg/kg for the agent **1a**. To compare, ED_{50} of acetylsalicylic acid is 155 mg/kg, of metamizole sodium, 55 mg/kg, and diclofenac sodium, 5 mg/kg [12].

In the hot plate test, the compounds **1a** and **1b** appeared to be inefficient. At the same time, hydrochloride **1a*HCl** in doses of 1 and 0.5 mg/kg significantly (by 50 and 47%, respectively) increased the latent time of the pain response (Table **2**). It is interesting that in the dose of 10 mg/kg, this agent did not show reliable analgesic activity.

The studies conducted demonstrated that the compound **1a*HCl** has the broad spectrum of antinociceptive effect, expressing high analgesic activity both in the visceral pain test and thermal pain test. To define the recruitment of the opioid system in the mechanism of analgesic activity of that compound, its activity was studies using models of pain stimuli against the background of administering naloxone, the opioid receptor antagonist.

Treatment of animals with naloxone 10 min prior to testing completely blocked analgesic activity of the compound **1a*HCl** in the acetic acid-induced writhing and hot plate tests (Table **3**). Thus, the obtained results suggest that analgesic effect of the compound **1a*HCl** is mediated by the opioidergic system.

It appeared that the compound **1a*HCl** did not possess anti-inflammatory activity in histamine inflammation model (Table **4**) enabling the supposition that its ability to inhibit

Table 2. Percent Analgesic Activity (Hot Plate Test)

Compound	Dose (mg/kg)	Mean ± SD	Control	Protection, (%)
1a	10	19.0 ± 1.0	19.5 ± 2.2	0
1a	1	13.6 ± 1.1	10.8 ± 1.1	21
1a	0.5	13.2 ± 1.5	13.2 ± 0.9	0
1a*HCl	10	23.9 ± 2.0	22.8 ± 3.1	5
1a*HCl	1	17.9 ± 2.8	11.9 ± 1.6	50[@]
1a*HCl	0.5	17.5 ± 2.5	11.9 ± 1.6	47[@]
1b	10	20.5 ± 1.4	19.9 ± 1.8	3
Diclofenac sodium	10	33.4 ± 2.3	20.4 ± 2.2	64[§]

[@]$P < 0.05$; [§]$P < 0.01$.

Table 3. Interaction of 1a*HCl (1 mg/kg) with Naloxone (1 mg/kg)

Compound	Acetic acid-induced writing test		Hot plate test	
	Mean ± SD	Control	Mean ± SD	Control
Naloxone	13.9 ± 0.8	13.4 ± 1	11.3 ± 0.7	10.6 ± 1
Naloxone +1a*HCl	13.3 ± 1	13.4 ± 1	11.6 ± 0.8	10.6 ± 1
1a*HCl	10.6 ± 0.5[§]	13.4 ± 1	14.5 ± 1.2[§]	10.6 ± 1

[§]$P < 0.01$.

Table 4. Inflammation Percentage

Compound	Dose (mg/kg)	Inflammation percentage ± SD
Control		21.1 ± 2.1
Diclofenac sodium	20	12.5 ± 1.8[@]
1a*HCl	10	21.0 ± 2.4

[@]$P < 0.05$.

the pain reaction caused by chemical disturbance of the peritoneum is preconditioned only by its analgesic activity.

CONCLUSION

The compound **1a** both in the form of free base and hydrochloride showed significant analgesic activity in the acetic acid-induced writhing test. In the hot plate test, sole hydrochloride demonstrated the reliable analgesic activity and only at low dose (0.5 and 1 mg/kg). It seems that the analgesic effect of the compound **1a*HCl** is mediated by the opioid system. Both the free base **1a** and its hydrochloride did not have anti-inflammatory activity. The replacement of the CF_3 group for the fluorine atom resulted in complete loss of analgesic activity in the acetic acid-induced writhing test.

CONFLICT OF INTEREST

Declared none.

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