Synthesis of 1-nicotinoylo-3-(meta-nitrophenyl)- thiourea derivatives with anti-inflammatory activity

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Abstract. Thiourea derivatives are used in many industries: medicine, agriculture, engineering, rubber industry, organic synthesis. On their basis, various preparations for the needs of the national economy were obtained. The latest studies of thiourea derivatives, carried out at the present time, are prompted not only by theoretical, but also by practical needs. From this point of view, thiourea derivatives are of undoubted interest in substances with different biological activities. They are widely used in agriculture and have been used as herbicides, fungicides, insecticides, bactericides, dyes, growth stimulants, etc. Of particular interest is the use of these compounds in medicine as antitumor, antiviral, anti-inflammatory, antiarrhythmic, vasodilator and other drugs. Today, most of the derivatives of thiourea, urea, the search for new highly effective low-toxic biologically active compounds based on them is constantly ongoing, as can be judged by the large number of publications in the world scientific and patent literature. This article proposes a simple and convenient method for the preparation of 1-nicotinoyl-3-(m-nitrophenyl)-thioureas based on heterocyclic thioisocyanate with nitroaniline in a dimethylformamide medium, in rather high yields. And also studied the anti-inflammatory activity of the synthesized drug.

1. Introduction

Thiourea derivatives have found widespread applications in various industries, including medicine, agriculture, engineering, rubber, and organic synthesis [1, 2]. Over time, numerous preparations for national economic needs have been developed based on these derivatives. In recent times, the interest in studying thiourea derivatives has grown significantly, driven not only by theoretical considerations but also practical requirements.

The versatility of thiourea derivatives is evident in their diverse biological activities, making them attractive for numerous applications. In agriculture, they have been harnessed as herbicides, fungicides, insecticides, bactericides, dyes, and growth stimulants, among other uses. Additionally, these compounds have garnered particular attention in the field of medicine due to their potential as antitumor, antiviral, anti-inflammatory, antiarrhythmic, and vasodilator agents, among others [1-5].

The search for new and highly effective, low-toxic, biologically active compounds continues to drive research on derivatives of thiourea and urea. This is evident from the extensive number of publications in the global scientific and patent literature [6-14]. The ongoing efforts in exploring these compounds hold promising prospects for developing novel applications across various industries and further advancing our understanding of their potential in addressing critical practical needs.

Therefore, the development of this branch of organic chemistry is the highest urgent task, requiring new developments in synthesis, technology and scientifically based approaches. The synthesis of new compounds based on aromatic amines and isothiocyanates, as well as their practical application, has broad prospects in solving top-priority tasks: the development, first of all, medicine and the pharmaceutical industry, as well as agriculture, the national economy and the growth of the welfare of the people of the Republic of new Uzbekistan.

Chemistry, technology and the properties of thiourea derivatives attract the attention of world researchers engaged in the search for biologically active substances. A number of works are devoted to this direction. Nevertheless, to date, there is no information in the literature on the synthesis of [1-nicotinoyl-3-(m-nitrophenyl)-thiourea] derivatives, as

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well as waste-free production technology, nicotinoylonitrophenylthiourea derivatives for the search for highly effective biologically active substances.

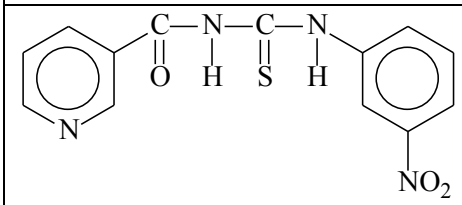
This article proposes a simple, convenient method for the preparation of 1-nicotinoyl-3-(m-nitrophenyl)-thioureas based on heterocyclic thioisocyanate with nitroaniline in a dimethylformamide medium, in fairly high yields.

2. Materials and methods

The reaction was carried out in a dimethylformamide medium at a temperature of 40-45 °C for 0.5 hours. The reaction mixture is kept under stirring for 4 hours at 80 °C. Physico-chemical parameters of the drug (1) are given in Table 1.

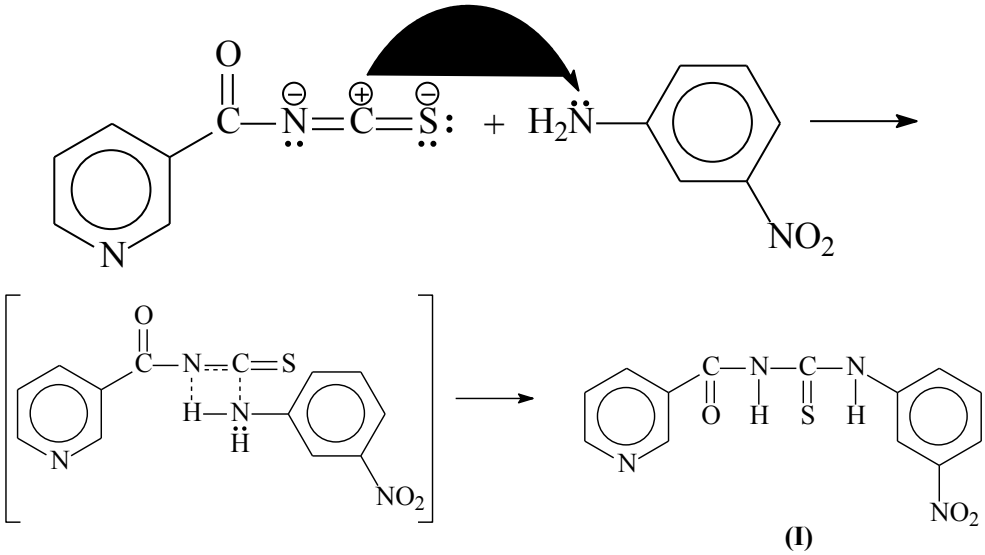
High density, selectivity and easy group electron cloud mobility of cause its high reactivity. The yield of product (I) was 94%. As expected, products were obtained in good yields by the AN reaction mechanism. The physicochemical characteristics of thiourea derivatives are apparently due to the high density and easy mobility of the conjugate electron cloud of group, resulting in an increase in the positive charge on the carbon atom of the thiocyanate group, facilitating attack on that carbon atom or by stabilizing the transition state.

Table 1. Physico-chemical parameters of the drug (I).

Compound	Outcome, %	T.melting, ^{0}C	R_f	Brutto Formula	Element analysis, %		M_M
					N	N	
	94	106-107	0.67	$C_{13}H_{10}N_4O_3S$	18.54	18.36	302.31

However, in our cases, the N–H group of the imine, having a free pair, attacks the electrophilic center in the thioisocyanate molecule with the formation of an intermediate product (B), which then rearranges into the final reaction product.

Based on our proposals and literature data, the probable mechanism of the interaction of m-nitroaniline with nicotinoylisothiocyanate can be represented by the scheme:



(I)

Purification of the starting reagent was carried out using preparative thin layer chromatography on Al_2O_3 in system of ($HCOOH:CH_3COCH_3:CHCl_3$)=0,5:4,5:1,0. To prove 1-nicotinoyl-3-(m-nitrophenyl)-thiourea, the method of IR and UV spectroscopy was used.

The UV spectrum of [1-nicotinoyl-3-(m-nitrophenyl)-thiourea] has characteristic absorption bands in the region of 207-358 nm, which corresponds in structure and name. A band appears in the spectrum in the region of 207 nm due to the mono-substituted nicotinic ring. Absorption bands in the long-wavelength part of the spectrum, due to the π-$\pi^{\#}$ transition, which indicates the absence of a double bond in its molecule. The absorption band in the region of 207 nm is due to the excitation of electrons of the monosubstituted nicotine ring

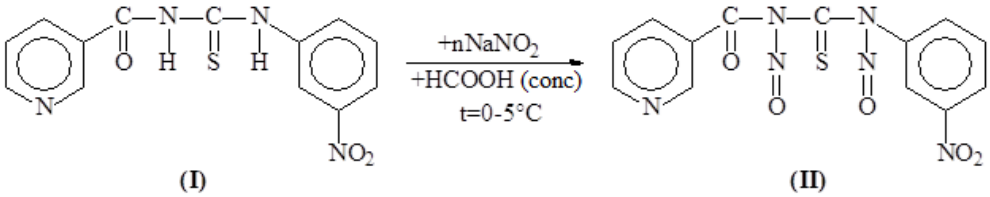
Chromatography is carried out on a thin layer of alumina on a glass substrate. R_f=0.67 in the solvent system - benzene: ethyl acetate=4:1. The development of chromatograms is carried out with iodine.

To study the reactivity at N-H reaction centers of [1-nicotinoyl-3-(m-nitrophenyl)-thiourea], we carried out rare reactions: N,N^1-dinitrosation, N,N^1-dichlorination, N,N^1-dialkylation.

3 Results and discussion

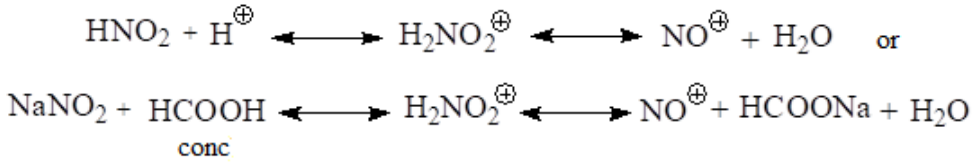
The N,N^1 dinitrosation reaction (I) is relatively little studied in the world literature [10-14].

According to the literature data and the data of our own research, during N-nitrosation, nitrogen atoms react, which are directly connected, on the one hand, with the residues of nicotinic acid and, on the other hand, with m-nitrophilic substituted thiourea groups. As a result of the reaction of N,N^1-dinitrosation of [1-nicotinoyl-3-(m-nitrophenyl)-thiourea] (II) with $NaNO_2$ (in excess) 98% HCOOH at a temperature of 0-5 ^{o}C, N,N^1-dinitroso-substituted (II) in 49.6% yields. N,N^1 - dinitrosation proceeds by the mechanism of electrophilic substitution (S_E).

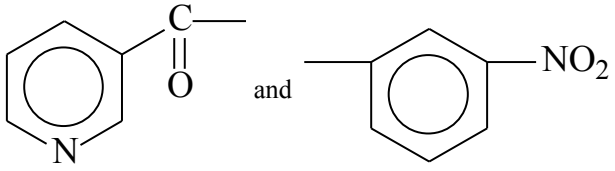


(I) (II)

The attacking agent is the nitrosonium ion, since nitrous acid, which is the most common nitrosating agent, does not exist in a free form, sodium nitrite and strong acid (HCOOH) are used to carry out the process. The resulting nitrous acid, by attaching a proton, generates an ion.



N,N^1 -dinitrosation is carried out by cooling the reaction mixture. An increase in temperature is undesirable, since this reduces the yield of the target product and sometimes affects the direction of the reaction. In this reaction, the low yield (59.6%) apparently explains the effect of EA group.

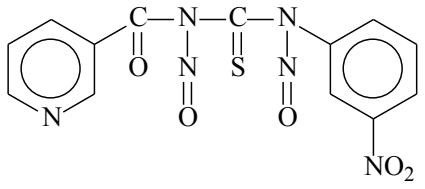


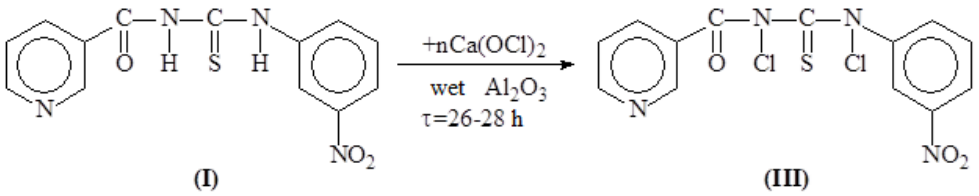
The identification of the target product of N,N^1-dinitroso compounds is carried out by the absorption bands>N–N=O groups. A characteristic band in the region of 1500-1420 cm^{-1} for >N-N=O groups (Table 2).

The structure is proven by elemental analysis data and IR spectroscopy data. In the IR spectrum of the obtained compound, there are absorption bands that are characteristic of vibrations of aromatic and heterocyclic rings. IR spectrum (KBr), ν, cm^{-1}: 1070 (C=S).

Derivatives [1-nicotinoyl-3-(m-nitrophenyl)-thiourea](I) are the most valuable raw materials for the further synthesis of various biologically active substances of compounds used in engineering, agriculture, and also have an N-H group reaction center for reactions nucleophilic - electrophilic substitutions (SN and SE).
We have developed a stable and environmentally friendly method for the implementation of N,N^1-dichlorination of a thiourea derivative - calcium hypochlorite on wet alumina.

Table 2. Physico-chemical parameters of compounds (II)

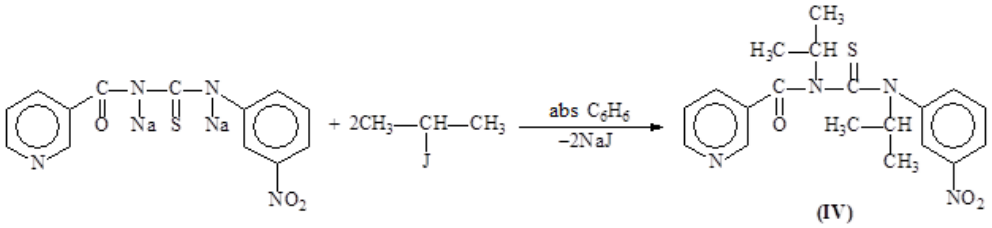
Structural formula	Output, %	T.melting^0	Form of	M_M	Element analysis, %	
					N	N
	59.6	204(decomp.)	$C_{13}H_8N_7SO_5$	374.06	26.20 S 8.57	26.11 S 8.61



(I) **(III)**

Output connections (III) - 68,7 %; T_{mp}= 94-95 ºC. R_f= 0,69.

Isopropylation at N-H groups in thioureas (I) with isopropyl iodides is of undoubted interest for establishing the reactivity of containing compounds (I). Isopropylation reactions were carried out by the interaction of N,N^1-disodium derivative of compounds (I) with isopropyl iodide in anhydrous benzene at a temperature of 30-320 C, and isopropyl iodide was added dropwise with stirring for 2.5-3.0 hours according to the following reaction scheme:

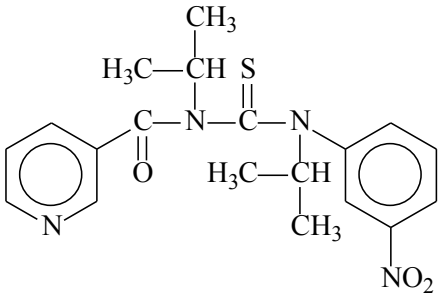


(IV)

The yield of compounds (IV) was 53.4 %. Tmp=66-67 ºC. The occurrence of the isopropylation reaction exclusively at the N,N^1 nitrogen atom is apparently explained by the relatively easy dissociation of sodium at this atom due to the presence of neighboring carbonyl and thiocarbonyl groups. Physico-chemical parameters of compounds (IV) are given in Table 3.
In a three-necked flask with a capacity of 250 ml, equipped with a reflux condenser with a calcium chloride tube, an automixer and an addition funnel, 3.28 g (0.02 mol) of nicotinoylthioisocyanate in 50 ml of dimethylformamide are placed. The contents of the flask are heated on a water bath to 40-45^{0}C and, without stopping stirring, 3.04 g (0.022 mol) of metanitroaniline in 50 ml of dimethylformamide are added dropwise over 30 minutes. The reaction mixture is kept under stirring for 4 hours at 80^{0}C. After the completion of the reaction, the mixture is cooled to room temperature, transferred to a beaker with a capacity of 500 ml, and 150 ml of water are added. The precipitate is filtered off, washed with water and 10% hydrochloric acid solution. The resulting product is dried over concentrated acid in a desiccator. [(1-Nicotinyl-3-(methanitrophenyl)-thiourea) recrystallized from benzene has a melting point of 105-107^{0}C. The yield of product (I) is 5.68 g (94.01% from theoretical**).** R_f =0,67.

Chromatography is carried out on a thin layer of alumina on a glass substrate. .R_f=0,67 in the solvent system - C_6H_6:$CH_3COOC_2H_5$ = (4:1). The development of chromatograms is carried out with iodine.

Table 3. Physico-chemical parameters of compounds (IV)

Structural formula	Output, %	T.melting, °C	R_f	Form	Element analysis, %				$M_м$
					N	S	N	S	
	53.4	66-67	0.59	$C_{19}H_{22}SN_4O_3$	14.50	8.30	14.42	8.33	386.06

In total, 3.02 g (0.01 mol) -[(1-nicotinoyl-3-(metha-nitrophenyl)-thiourea] (I) dissolved in 100 ml of formic acid is placed with a stirrer into a three-necked flask equipped with a reflux condenser, with a thermometer. at 0-5^{0}C, 0.5 g $NaNO_2$ is added in portions in excess within 3.5-4.0 hours after the end of the reaction, the contents are poured into a liter jar, 250 ml of cold water are added, and a precipitate begins to precipitate. The precipitate is filtered off, washed with benzene and dried The identity of N,N^1-dinitroso-[(1-nicotinoyl-3-(meta-nitrophenyl)-thiourea] was determined by TLC on Silufol plates. Yield (II) -59.6% (of theory). =204-206 (diff.).

In total, 3.02 g (0.01 mol)[(1-nicotinoyl-3-(metha-nitrophenyl)thiourea] (I), 60 ml of CCl_4, 25 0 g of wet alumina and added dropwise 6.0 g of calcium hypochlorite at a temperature of 40 °C for 1.0 hour. Then the reaction mass was left for 24 hours, the residue was filtered, washed with ether and alcohol.-3-(meta-nitrophenyl)- thiourea]. Yield of compounds - 58.7% (from theoretical);T. melting. =94-95^{0}C. R_f=0,69.

To prove the structure of N,N^1-dichloro-substituted(III), elemental analysis was carried out with silver salts (solution $AgNO_3$). 3.02 g (0.01 mol) of compounds (I) are added to CH_3ONa (from 0.031 g/mol Na and 30 ml of absolute CH_3OH). The mixture is stirred for 2 hours at a temperature of 20^{0}C for 2 hours at a temperature of 40^{0}C, the precipitate is filtered off, washed with absolute CH_3OH and the product (IIIa) is obtained. Yield (IIIa) -48.3% (from theory). 1.67 g of (IIIa) are taken up in 20 ml of dry DMF, 3.5 ml (0.02 mol) of isopropyl iodide are added dropwise with stirring.

The mixture is stirred for 10.0 hours while heating on a boiling water bath, cooled and poured with 25 ml of water, the precipitate is separated and recrystallized from 30% alcohol, dried and (IV) is obtained with a yield of 53.4 % (from theory). T. pl. =66-67^{0}C; R_f=0,59.

The study of the antiphlogistic activity of the new compound is carried out on the model of carrageenan edema. Experiments are carried out on outbred rats of both sexes weighing 140-200 g. Inflammation is caused by the introduction of 0.2 ml of a 1% solution of carrageenan aponeurosis of the ankle joint. The volume of the paws is measured oncomometrically before causing inflammation and 1;2;3;4;5;24;48 and 72 hours after it. The test compound is administered orally using a metal probe at the most effective dose, i. at a dose of 100 mg/kg.

It has been established that the proposed compound inhibits the development of carrageenan edema by 57.9 %.

Structural analogue-4(3)[1-(nicotinoyloxypropynyl-1)-3-(nicotinoyloxyme-thylene)-pyrazole] at a dose of 50 mg/kg reduces the intensity of carrageenan edema by 33.2%. The anti-inflammatory activity of the base object - voltaren is 49.9%. The results of the experiments are shown in Table 4.

Acute toxicity is determined on white mice weighing 17-22 g with the calculation of LD50 according to the method of Litchfield and Wilcoxon. [1-(Nicotinoyl)-3-(methanitrophenyl)-thiourea] is a low-toxic compound, since it does not cause lethal outcomes when administered orally at a dose of 1500 mg/kg.

Table 4. Effect of 1-(nicotinoyl)-3-(methanitrophenyl)-thiourea, 4(3)-nicotinoyl-oxypropynyl-1)-3-(nicotinoyloxymethylene)-pyrazole and voltarene on carrageenan inflammation and their acute toxicity*

Compounds	Dose, mg/kg	Anti-inflammatory activity, %	LD50 mg/kg
1- (nicotinoylo-) -3- (meta-nitrophenyl) -thiourea	100	57.9	1500
4 (3) -Nicotinoyl-oxypropynyl-1) -3-	50	33.2	375

(nicotinoylooxy-methylene) -pyrazole			(246.7-570.0)
Voltaren	10	49.9	180 (162.2-199.8)

***** - acute toxicity of drugs is studied with the intragastric route of administration.

As can be seen from Table 5, compound (I) is 1.74 times superior to 4(3)-[1-(nicotinoyloxypropynyl-1)-3-(nicotinoyloxymethylene)-pyrazole] and 1.16 times Voltaren in anti-inflammatory action. Acute toxicity is 8.3 times less than that of Voltaren

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