

# SULPHUR ANALOGS OF PYRIMIDINE BASES: NEW 2-THIO- 6- ALKOXYCARBONYLURACILS AND 2-THIO- 6- HYDROXYAMINOCARBONYLURACILS

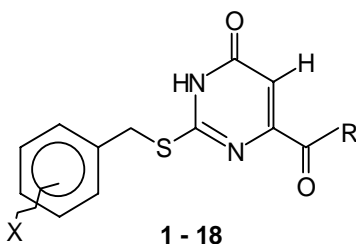
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## Introduction:

The title compounds (**1-18**) constitute a subclass of derivatives of orotic acid (6-carboxyuracil, vitamin B<sub>13</sub>), an important representative of pyrimidine compounds, which takes crucial part in metabolic processes in living organisms [1] and exhibits various physiological effects [2]. Its sulphur analog — 2-thioorotic acid — is also of interest because of its biological activity and chelating power with metal cations. The great interest with this compound results in continuous search of its new derivatives, modified both in the position 2 of uracil ring (sulphur atom) and in the position 6 (carboxylic group).

Among derivatives of carboxylic acids on the special attention deserve hydroxamic acids. These compounds possess a wide spectrum of biological activities including antibacterial, anti-inflammatory, antifungal and antitumor properties [3-5] and are also known as stimulators of growth of several plants [6]. Hydroxamates have been identified as selective inhibitors of matrix metalloproteinases [7] and other enzymes, for example 5-lipoxygenase, an enzyme involved in the biosynthesis of mediators of allergic disorders [8]. Wide range of physiological properties of sulphur analogs of pyrimidine bases (2-thiouracil, 2-thioorotic acid) as well as activity of hydroxamic acids encouraged me to obtain (*via* methyl esters) new hydroxamic acids, rooted from substituted 2-benzylthioorotic acids, containing in the phenyl ring chloro, bromo or nitro substituents in the positions *ortho*, *meta* or *para*. The substrates have been synthesized previously in our laboratory [9].



Compound	X	R	Compound	X	R
<b>1</b>	<i>o</i> -Cl	OCH <sub>3</sub>	<b>10</b>	<i>o</i> -Cl	NHOH
<b>2</b>	<i>m</i> -Cl	OCH <sub>3</sub>	<b>11</b>	<i>m</i> -Cl	NHOH
<b>3</b>	<i>p</i> -Cl	OCH <sub>3</sub>	<b>12</b>	<i>p</i> -Cl	NHOH
<b>4</b>	<i>o</i> -Br	OCH <sub>3</sub>	<b>13</b>	<i>o</i> -Br	NHOH
<b>5</b>	<i>m</i> -Br	OCH <sub>3</sub>	<b>14</b>	<i>m</i> -Br	NHOH
<b>6</b>	<i>p</i> -Br	OCH <sub>3</sub>	<b>15</b>	<i>p</i> -Br	NHOH
<b>7</b>	<i>o</i> -NO <sub>2</sub>	OCH <sub>3</sub>	<b>16</b>	<i>o</i> -NO <sub>2</sub>	NHOH
<b>8</b>	<i>m</i> -NO <sub>2</sub>	OCH <sub>3</sub>	<b>17</b>	<i>m</i> -NO <sub>2</sub>	NHOH
<b>9</b>	<i>p</i> -NO <sub>2</sub>	OCH <sub>3</sub>	<b>18</b>	<i>p</i> -NO <sub>2</sub>	NHOH

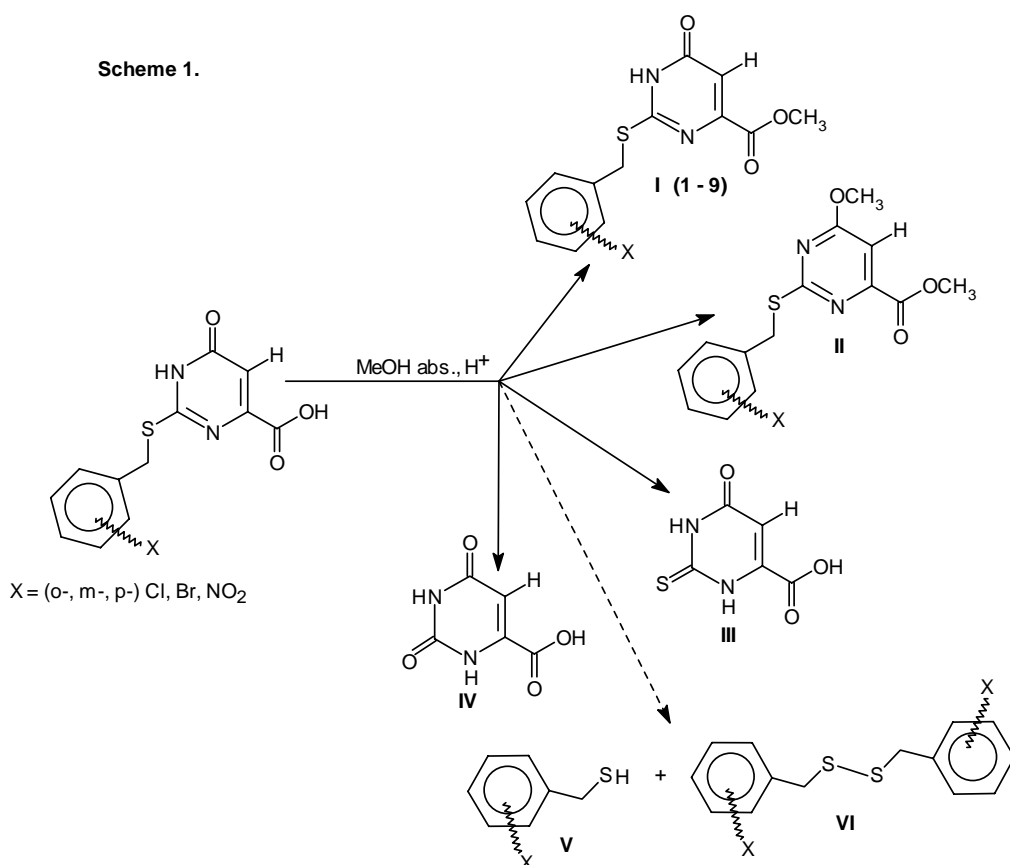
My aim was to examine the reactivity of the above 2-benzylthioorotic acids under the conditions of esterification reaction, to receive X-substituted 2-benzylthio-6-

methoxycarbonyluracils (methyl esters **1 – 9**) and to transform them into corresponding 2-benzylthio-6-hydroxyaminocarbonyluracils (hydroxamic acids **10 – 12**). The research of antimicrobial properties of the compounds studied is being in progress.

### Experimental part

At first, a conventional method was elaborated to synthesize methyl esters of 2-(chloro, bromo or nitro) benzylthioorotic acids **1-9**. Starting material was refluxed with boiling absolute methanol and catalytic amount of concentrated sulphuric acid to the entire dissolution of the substrate. The reaction mixture was evaporated on the vacuum rotary evaporator to 1/10 of previous volume. TLC monitoring showed a complicated mixture of products. The obtained mixture was purified and products separated using silica gel column chromatography. The set of products received (**I-VI**) is presented on the **Scheme 1**.

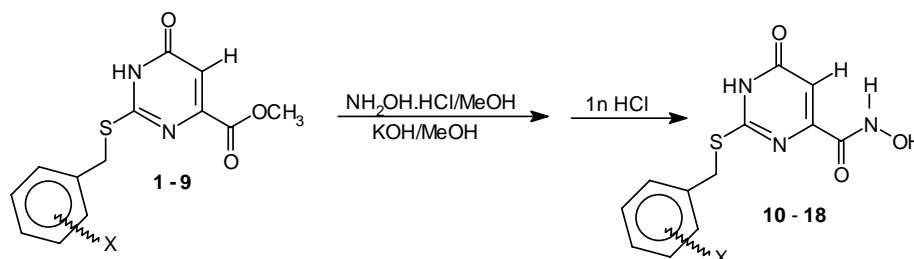
In the second method of esterification 2-*o*-chlorobenzylthioorotic acid was submitted to the action of thionyl chloride in absolute methanol at room temperature. After standing over night only products **I** and **II** were isolated from the reaction mixture. All products were identified using electron ionization mass spectrometry, electrospray MS and  $^1\text{H}$  NMR.



The purified esters **1-9** were dissolved (or suspended) in methanol and added to the previously prepared mixture of saturated solutions of hydroxylamine hydrochloride in methanol and potassium hydroxide in methanol (2:1) and then stirred magnetically over three days. After this time the reaction mixture was neutralized with 1N HCl and gave the positive result of the  $\text{FeCl}_3$  test (coloured complex) for hydroxamic acids. The neutral or slightly acidic solution containing one of the hydroxamic acids **10 – 18** was

gently evaporated under reduced pressure and cooled in the refrigerator. The precipitated solid was filtered, washed with small amount of cold absolute methanol and dried.

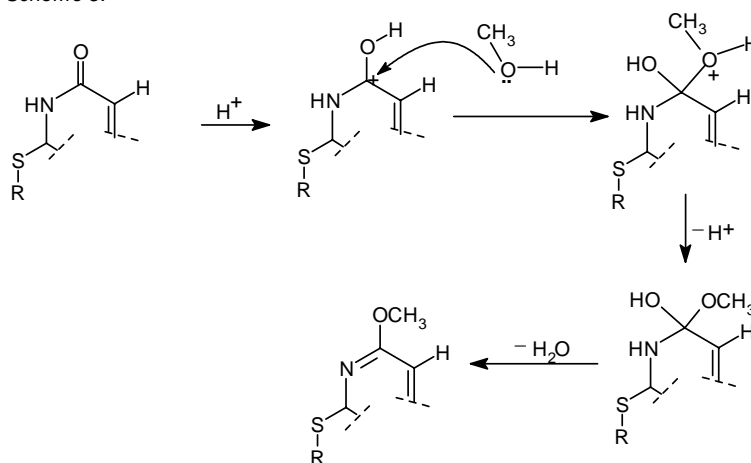
Scheme 2.



## Results and discussion

2-benzylthiorotic acids, containing X substituent (X = Cl, Br or NO<sub>2</sub>) in the position *ortho*, *meta* or *para* of phenyl ring turned out to be susceptible to decomposition in the conditions of esterification reactions (acidic methanol, temperature 63-64°C). One of the side reactions observed is debenzylation to the 2-thiorotic acid **III** (Scheme 1), the other is hydrolysis of C<sub>sp2</sub>-S bond of benzylthiorotic acid, which yielded the orotic acid **IV** and was accompanied with formation of appropriate X-benzyl-mercaptane (**V**) and/or di-(X-benzyl)-disulphide (**VI**), substances of unpleasant smell. Surprisingly, 2-(*o*-, *m*- or *p*-)(chloro, bromo or nitro)benzylthio-4-methoxy-6-methoxycarbonylpyrimidines (compounds **II**) are also formed in this reaction. The possible mechanism of creating these compounds (similar to the reaction of the hemiacetal formation) is shown on Scheme 3.

Scheme 3.



As a consequence of side reactions, the laborious separation – silica gel column chromatography – was needed to isolate desirable esters from the reaction mixture and the yield received was about 15 percent. To the quick identification of compounds obtained especially mass spectral methods (EIMS and ESIMS) appeared useful and convenient. Characteristic parameters of the obtained new 2-benzylthio-6-methoxycarbonyluracils **1-9** are collected in Table 1.

The isolated and purified esters **1 – 9** were used further to obtain hydroxamic acids as described earlier. Some characteristics of products **10 – 18** (X substituted 2-benzylthio-6-hydroxyaminocarbonyluracils) are shown in Table 2.

**Table 1.** Structure, thin layer chromatography (phase A\* = BuOH- AcOH-H<sub>2</sub>O 5:2:3), melting points and molecular ions (EIMS, m/z) of esters **1 - 9**

Compound	Substituent X	Molecular formula	R <sub>f</sub> (TLC) phase A*	m.p. °C	M <sup>+</sup> m/z
1	<i>o</i> -Cl	C <sub>13</sub> H <sub>11</sub> N <sub>2</sub> O <sub>3</sub> SCl	0,95	240-246	310/312
2	<i>m</i> -Cl		0,96	228-229	
3	<i>p</i> -Cl		0,95	233-237	
4	<i>o</i> -Br	C <sub>13</sub> H <sub>11</sub> N <sub>2</sub> O <sub>3</sub> SBr	0,95	243-245	354/356
5	<i>m</i> -Br		0,96	239-240	
6	<i>p</i> -Br		0,95	240-243	
7	<i>o</i> -NO <sub>2</sub>	C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> O <sub>5</sub> S	0,95	244-247	321
8	<i>m</i> -NO <sub>2</sub>		0,96	239-241	
9	<i>p</i> -NO <sub>2</sub>		0,96	247-249	

**Table 2.** Characteristic data for hydroxamic acids **10 - 18**

Compound	Substituent X	Molecular formula	Molecular weight	m.p. °C	Complex with Fe <sup>3+</sup> (colour)
10	<i>o</i> -Cl	C <sub>12</sub> H <sub>10</sub> N <sub>3</sub> O <sub>3</sub> SCl	311	126-129	violet
11	<i>m</i> -Cl			120-123	
12	<i>p</i> -Cl			116-118	
13	<i>o</i> -Br	C <sub>12</sub> H <sub>10</sub> N <sub>3</sub> O <sub>3</sub> SBr	355	128-131	violet
14	<i>m</i> -Br			124-126	
15	<i>p</i> -Br			123-126	
16	<i>o</i> -NO <sub>2</sub>	C <sub>12</sub> H <sub>10</sub> N <sub>4</sub> O <sub>5</sub> S	322	128-130	dark red to brown
17	<i>m</i> -NO <sub>2</sub>			125-127	
18	<i>p</i> -NO <sub>2</sub>			132-135	

#### Summary:

New organic compounds, derivatives of pyrimidine bases, rooted from 2-thioorotic acid and expected to possess significant physiological activity, were received.

The main difficulty in the synthesis of hydroxamic acids appeared the first step, i. e. the esterification of substituted 2-benzylthioorotic acids and proper separation of products. The side reactions took place due to the lability of sulphur-carbon bonds in the applied conditions (presence of strong concentrated acid, raised temperature). Other synthetic routes to the desired esters should be investigated to make this synthesis more selective and efficient. Synthesis of hydroxamic acids proceeds mostly unambiguously, although it requires observing strictly basicity/acidity conditions.

Electrospray ionization mass spectrometry turned out to be convenient to the identification of isolated main and by-products of above-mentioned reaction as well as to evaluate the degree of purity or kind of contaminations of compounds studied (variety of pseudo molecular ions, protonated and deprotonated, are easily detected).

#### References:

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