NEW THEOPHYLLINE DERIVATIVES WITH POTENTIAL PHARMACOLOGICAL ACTIVITY

LENUŢĂ PROFIRE1*, VALERIU ŞUNEL4, DAN LUPAŞCU1, MIHAELA CRISTINA BAICAN3, NELA BIBIRE3, CORNELIA VASILE5

1Pharmaceutical Chemistry Department, 2Physics Department, 3Analytical Chemistry Department, Faculty of Pharmacy, „Gr. T. Popa” University of Medicine and Pharmacy, Universitatii Street, 16, 700115, Iasi, Romania
4Department of Organic Chemistry and Biochemistry, Faculty of Chemistry, „Al. I. Cuza” University, B-dul Carol I 11, Iasi, 700506, Romania
5„P. Poni” Institute of Macromolecular Chemistry, Gr. Gica Voda Alley, 41 A, 700487 Iasi, Romania
*corresponding author: nprofire@yahoo.com

Abstract
Theophylline, a methylxanthine compound, is known as an efficient bronchodilator drug, having also anti-inflammatory and immunomodulatory effects. In order to improve its pharmacological profile and to reduce also the serious side effects that appear at high concentrations, new theophylline derivatives have been synthesized. The new compounds are obtained in two steps by the reaction of 8-substituted-theophyllines with epoxy-propyl-acetaminophen. The chemical structure of the synthesized compounds has been elucidated by their 1H-NMR spectra. The potential bronchodilator effects of the synthesized compounds have been also established.

Keywords: theophylline, spectral data, bronchodilator effect.

Introduction
Theophylline, also known as 1,3-dimethylxanthine, is a proven bronchodilator drug used in therapy for respiratory diseases such as asthma or chronic pulmonary obstructive disease (COPD) [4, 5, 9-12]. One of the action mechanisms of theophylline is that of adenosine receptor antagonism.
Theophylline is a non-specific adenosine antagonist, antagonizing A1, A2, and A3 receptors almost equally [1]. It is unclear if this mechanism is significant because, enprophylline, another methylxanthine drug, which does not antagonize the adenosine receptors, is a more potent bronchodilator than theophylline [3, 7]. Adenosine antagonism may, however, be responsible for some of the toxicity associated with theophylline, such as arrhythmia, central nervous stimulation, gastric acid hypersecretion and diuresis [1]. Another proposed mechanism of action includes a non-specific inhibition of phosphodiesterase enzymes, producing an increase in intracellular cyclic AMP (3'-5'-cyclic adenosine monophosphate) [2]. The pharmacological profile of theophylline includes also anti-inflammatory [8] and immunomodulatory effects [6]. At patients with asthma or COPD the administration of theophylline was associated with substantial reductions in sputum neutrophils, interleukin-8 concentrations and neutrophil chemotaxis [6, 13].

To continue our research [14-16] and in order to improve the pharmacological profile of theophylline and also to reduce the serious side effects that appear at high concentrations, new theophylline derivatives have been synthesized.

**Materials and methods**

**Chemistry**

All starting materials were purchased from the Aldrich Chemical Company and used without purification. Melting points were measured in open capillary tubs on a Melt-Temp R apparatus equipped with a digital thermometer and are uncorrected. The ¹H-NMR spectra were recorded using a BRUKER AVANCE DRX apparatus at 400 MHz using solutions in DMSO-d6 as solvent. Chemical shifts were recorded as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane as internal standard.

*The synthesis of 4-(2,3-epoxi-propyl)-acethaminophen*

To a solution of sodium hydroxide (1.5 g, 0.0375 mol) in water there were added 4.5 g of acetaminophen (0.0298 mol), and then 3.5 ml (0.0447 mol) of epichlorhydrine dropwise. The reaction mixture was stirred at room temperature for 16 hours. It was obtained a white precipitated that was separated by vacuum filtration and than it was washed with water. Recrystallization from ethanol gave pure white solid compound, mp 116-117°C.
The synthesis of 7-[2-hydroxy-3-(4-acetyl-amino)-phenoxy]-propyl-
theophylline derivatives. General procedure.

2.07 g (0.01 mol) of 4-(2,3-epoxy-propyl)-acetaminophen were
solved in ethanol under heating and then an ethanol solution of theophylline
and 8-R-theophylline respectively was added. The reaction mixture was
heated under reflux for 10 hours and then the solvent was removed by
distillation under reduced pressure to ¼ from the initial volume. The rough
products were separated by filtration under vacuum, dried and recrystallized
from ethanol.

Biological study

The potential bronchodilator effects were studied using
precontracted Wistar rat tracheal strips according to the methods described
in literature [2, 4, 17]. The substances were administered as
dimethylsulfoxide solutions in concentration between 10^-10M and 10^-3 M
using theophylline as reference. The control contraction of the tracheal
strips was obtained using a solution of carbachol 10^-5M.

Results and discussion

The synthesis of the theophylline derivatives was performed in two
steps. Firstly, the acetaminophen (1) was turned into its phenoxy form (2) in
an alkaline medium, which further reacted with epichlorhydrine to form 4-
(2,3-epoxy-propyl)-acetaminophen (3) (Figure 1).

Secondly, this intermediary (3) reacts in mild conditions at the
boiling temperature of the ethyl alcohol with 8-substituted theophylline (4-
10) (8-R-theophylline; R=hydrogen, bromo, nitro, pyrrolidinyl, piperidinyl,
morpholinyl, imidazolyl radicals) (Figure 2).

The new synthesized compounds have been characterized based on
the molecular formula, molecular weight, yield and the control of purity was
achieved by melting point determination using the Melt-Temp R apparatus
and differential scanning calorimetry (DSC) analysis (Table I).
The synthesis of new theophylline derivatives

The new compounds are solid, crystallized, white or light yellow, soluble at room temperature in dimethylformamide, dimethylsulfoxide and by heating in inferior alcohols, acetone, dioxane; insoluble in water, chloroform, benzene, diethyl ether.

The chemical structure has been proved by $^1$H-NMR spectroscopy; the characteristic bands are given in Table II.

### Table I

<table>
<thead>
<tr>
<th>Comp No.</th>
<th>R</th>
<th>Molecular Formula</th>
<th>Molecular weight (g/mol)</th>
<th>Yield (%)</th>
<th>Melting point ($T_m$) (°C)</th>
<th>$T_{DSC}$ peak (°C)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>-H</td>
<td>$C_{16}H_{21}N_{3}O_{5}$</td>
<td>387.41</td>
<td>90.08</td>
<td>202-205</td>
<td>204</td>
<td>65.9</td>
</tr>
<tr>
<td>12</td>
<td>-Br</td>
<td>$C_{16}H_{30}N_{3}O_{5}$</td>
<td>466.31</td>
<td>92.21</td>
<td>220-222</td>
<td>219</td>
<td>37.8</td>
</tr>
<tr>
<td>13</td>
<td>-NO$_2$</td>
<td>$C_{16}H_{20}N_{6}O_{7}$</td>
<td>432.41</td>
<td>81.01</td>
<td>211-213</td>
<td>208</td>
<td>56.8</td>
</tr>
<tr>
<td>14</td>
<td>-N</td>
<td>$C_{20}H_{26}N_{6}$</td>
<td>456.46</td>
<td>72.36</td>
<td>268-270</td>
<td>267</td>
<td>54.6</td>
</tr>
<tr>
<td>15</td>
<td>-N</td>
<td>$C_{20}H_{26}N_{6}$</td>
<td>470.53</td>
<td>72.34</td>
<td>227-229</td>
<td>231</td>
<td>57.4</td>
</tr>
<tr>
<td>16</td>
<td>-N</td>
<td>$C_{20}H_{26}N_{6}O_{6}$</td>
<td>472.51</td>
<td>68.56</td>
<td>228-230</td>
<td>220</td>
<td>24.9</td>
</tr>
<tr>
<td>17</td>
<td>-N</td>
<td>$C_{20}H_{23}N_{5}$</td>
<td>453.57</td>
<td>61.73</td>
<td>266-267</td>
<td>265</td>
<td>48.2</td>
</tr>
</tbody>
</table>
Table II

$^1$H-NMR spectra (DMSO-d$_6$, δ ppm)

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>δ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>8.12</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>R*</td>
</tr>
<tr>
<td>15</td>
<td>R**</td>
</tr>
<tr>
<td>16</td>
<td>R***</td>
</tr>
<tr>
<td>17</td>
<td>R****</td>
</tr>
</tbody>
</table>

R* - pirrolidinyl (4H, m, 1.59; 4H, t, 2.80); R** - piperidinyl (6H, m, 1.50; 4H, t, 2.76); R*** - morpholinyl (4H, t, 2.90; 4H, t, 3.67); R**** - imidazolyl (1H, s, 8.15; 2H, d, 6.90).

The study of the effects of the new theophylline compounds on carbachol-precontracted tracheal strips established that all tested compounds are more active than theophylline at $10^{-3}$M concentration. The Structure–Activity Relationships study showed that the most favorable influence on the bronchodilator effect of theophylline have bromo and imidazolyl radicals from the 8$^{th}$ position, the corresponding compounds being 6.5 times and respectively 2.5 times more active than theophylline (Table III).
Table III
The effect of theophylline derivatives on tracheal strips precontracted with carbachol (10^{-5} M)

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>R</th>
<th>Solvent mixture</th>
<th>Solution conc.</th>
<th>Retained contraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>-H</td>
<td>DMSO: water (1:9)</td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 12.10±3.70</td>
</tr>
<tr>
<td>12</td>
<td>-Br</td>
<td>DMSO: water (3:7)</td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 2.75±1.00</td>
</tr>
<tr>
<td>13</td>
<td>-NO₂</td>
<td>DMSO: water (1:9)</td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 8.74±2.80</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>DMSO: water (3:2)</td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 8.56±2.24</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>DMSO: water (3:7)</td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 8.20±2.13</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>DMSO: water (1:9)</td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 8.40±2.38</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>DMSO: water (3:7)</td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 7.30±2.00</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Water</td>
<td></td>
<td>10^{-10}, 10^{-4} M 10^{-3} M</td>
<td>100 41.00±7.40 18.00±2.70</td>
</tr>
<tr>
<td>Carbachol</td>
<td></td>
<td></td>
<td>10^{-5}</td>
<td>100</td>
</tr>
</tbody>
</table>

Conclusions
Following the improvement of the pharmacological profile of theophylline we obtained some new theophylline derivatives starting from 8-R-theophyllines and epoxy-propyl-acetaminophen. The synthesized compounds have been characterized from some physical properties and the chemical structure was proved by $^1$H-NMR spectral data.

The study of the potential bronchodilator effects established that all tested compounds are more active than theophylline at 10^{-3} M concentration; the most favorable effect having bromo and imidazolyl radicals from the 8th position of xanthine structure, the corresponding compounds being 6.5 times and 2.5 times respectively more active than theophylline.

Acknowledgement
The authors are grateful to the Romanian Ministry of Education, the National Centre for Programme Management (CNMP), Programme “Partnerships
References


Manuscript received: August 30th 2009