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# SYNTHESIS AND EFFECTS OF SOME NEW 2-ARYL-THIAZOLE AMMONIUM SALTS ON ISOLATED ILEUM MOTILITY

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Quaternary ammonium compounds are considered among the substances which can influence the contractility of smooth muscles from the digestive tract, acting via cholinergic mechanisms. Based on structural considerations, we synthesized twelve new compounds, derivatives of 2-aryl-thiazole, being substituted in the 2 and 4 positions. In order to elucidate the structure of the obtained compounds, MS determinations and $^1$H-NMR spectra were realized. The effect on the smooth muscles was tested using the guinea pig isolated ileum experimental model. The obtained results showed that the contractile effects or the antispasmodic activity of the compounds are influenced by the nature of the substituent in the 4 position, and the intensity of the effect is related to the type of substituent on the phenyl, from the 2 position of the thiazolic nucleus.

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*Keywords:* Quaternary ammonium salts, 2-aryl-thiazole, Contractile, Antispasmodic

## 1. Introduction

The quaternary ammonium group and the thiazole moiety constitute the core structure of a number of biologically interesting compounds [1-3].

The thiazole ring is present in a variety of therapeutic agents. These agents can exhibit significant anti-cancerous, antimicrobial, anti-diabetic, anti-inflammatory, antiviral or analgesic activity [4-6].

On the other hand, the quaternary ammonium group is present in antiseptic, antispasmodic or spasmodic compounds [7-12].

The present study represents a continuation of our previous research that showed the favorable effect of the association of the thiazole nucleus with a quaternary ammonium group in the same molecules. These compounds showed antiseptic and antifungal activity [13-16].

We have synthesized new 2-aryl-thiazolic compounds which contain in 4 position a quaternary ammonium group, which is included in a non-aromatic heterocyclic core (Piperazine, Piperidine, Morpholine, N-Methyl-Piperidine, N-Methyl-Morpholine, N-Methyl-Piperazine) or in an aromatic one (Pyridine).

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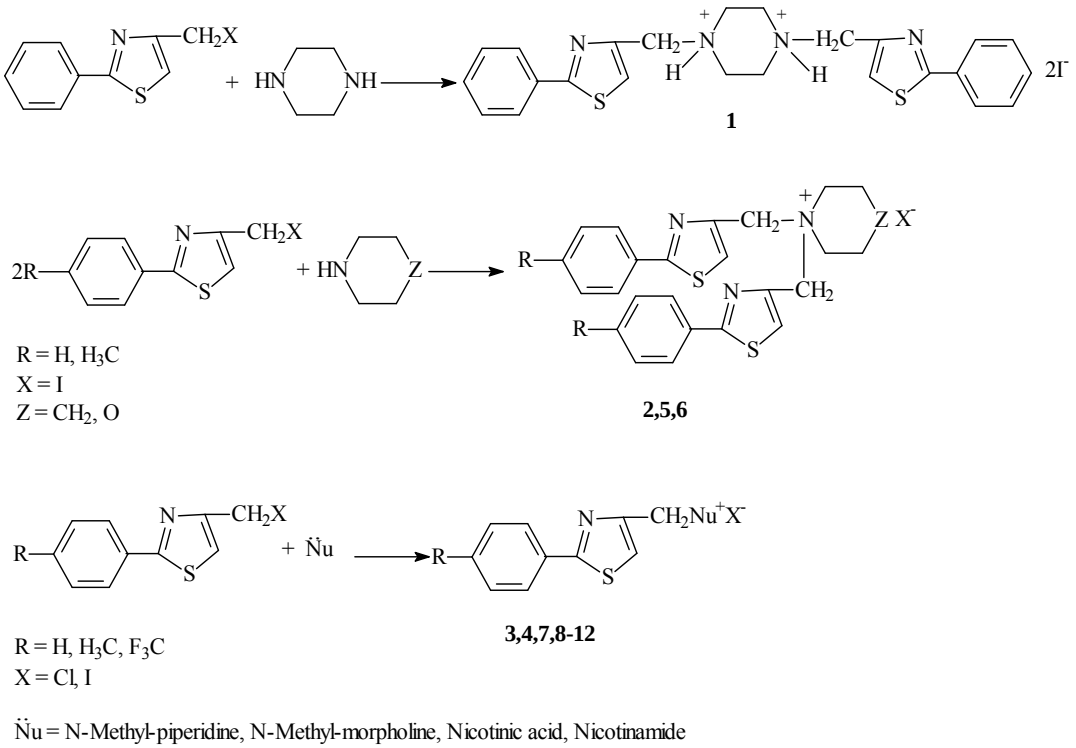
## 2. Experimental

### 2.1. Chemistry

We synthesized new quaternary ammonium compounds by alkylation of some nitrogen containing heterocyclic cores with 2-aryl-4-halogenomethyl-thiazole derivatives.

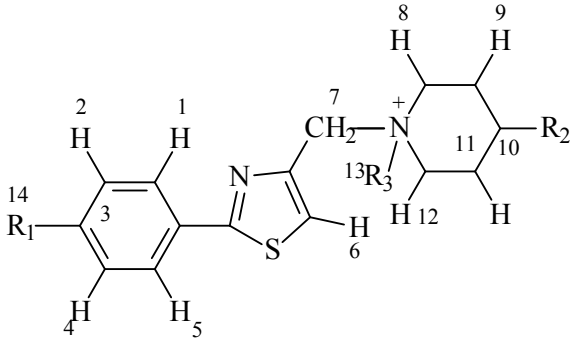
The synthetic procedure for obtaining quaternary ammonium compounds was already described in our previous works [13, 16].

Depending on the nucleophilic component, we obtained new tertiary and quaternary ammonium salts which are illustrated in *Scheme 1*:




*Scheme 1: The synthetic procedure*

Melting points were determined using a capillary melting point apparatus (Electrothermal IA 9000) and are uncorrected. Elemental analyses were performed by an Elemental Analysen Systeme GmbH VarioEL. MS spectra were recorded on a LC-MSD-Trop-VL mass spectrophotometer and $^1$H-NMR spectra were recorded on a $^1$H-NMR Bruker Avance apparatus.



*Scheme 2: General structure and numbering of the hydrogen atoms for the compounds analyzed by $^1$H-NMR*

*2.1.1. 1,4-Bis[(2-phenyl-1,3-thiazol-4-yl)methyl]piperazine-1,4-diium diiodide* **(1)**

White-yellowish powder. 17.19% yield. Melting point: 148-149°C. Anal. calcd. for $C_{24}H_{26}I_2N_4S_2$(MM): C, 41.87; H, 3.81; I, 36.87; N, 8.14; S, 9.32. Found: C, 41.78; H, 3.99; I, 36.81; N, 8.10; S, 9.26. MS: molecular peak m/z 433.4, fragmentation peaks m/z 174.2 (2-aryl-thiazole nucleus), 347.2 (2 components of 2-aryl-thiazole nucleus) 215.1, 229.1, 244.1, 258.2, 314.2. $^1$H-NMR ($D_2O$): δ (ppm) = 8.03 (H-1 and H-5), 7.51 (H-2 and H-4), 7.41 (H-3), 7.94 (H-6), 4.93 (H-7/2H$^+$), 3.9 (H-8), 3.62 (H-9), 3.55 (H-11), 3.47 (H-12), 2.0 (H-10 and H-13).

*2.1.2. 1,1-Bis[(2-phenyl-1,3-thiazol-4-yl)methyl]piperidin-1-ium iodide* **(2)**

Yellowish powder. 55.13% yield. Melting point: 173-175°C. Anal. calcd. for $C_{25}H_{26}IN_3S_2$ (MM): C, 53.66; H, 4.68; I, 22.68; N, 7.51; S, 11.46. Found: C, 53.64; H, 4.63; I, 22.65; N, 7.62; S, 11.41. MS: molecular peak m/z 432.3, fragmentation peaks m/z 175 (2-aryl-thiazole nucleus), 347.2 (2 components of 2-aryl-thiazole nucleus), 245.2, 257.4. $^1$H-NMR ($D_2O$): δ (ppm) = 8.01 (H-1 and H-5), 7.55 (H-2 and H-4), 7.4 (H-3), 7.53 (H-6), 4.86 (H-7/2H$^+$), 3.48 (H-8/2H$^+$ and H-12/2H$^+$), 1.69 (H-9/2H$^+$ and H-11/2H$^+$), 2.05 (H-10/2H$^+$).

*2.1.3. 1-Methyl-1-{[2-(4-methylphenyl)-1,3-thiazol-4-yl]methyl}piperidin-1-ium iodide* **(3)**

White-yellowish powder. 74.14% yield. Melting point: 190.3°C. Anal. calcd. for $C_{17}H_{23}IN_2S$ (MM): C, 49.28; H, 5.59; I, 30.63; N, 6.76; S, 7.74. Found: C, 49.25; H, 5.56; I, 30.6; N, 6.72; S, 7.71. MS: molecular peak m/z 287, fragmentation peaks m/z 188 (2-aryl-thiazolic nucleus substituted with $CH_3$), 118.2, 161.2, 249.4. $^1$H-NMR ($D_2O$): δ (ppm) = 7.94 (H-1 and H-5), 7.52 (H-2 and H-4), 8.07 (H-6), 4.67 (H-7/2H$^+$), 3.56 (H-8/2H$^+$ and H-12/2H$^+$), 1.93 (H-9/2H$^+$ and H-11/2H$^+$), 1.59 (H-10/2H$^+$), 3.10 (H-13/3H$^+$), 2.43 (H-14/3H$^+$).

*2.1.4. 1-Methyl-1-({2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-4-yl}methyl)piperidin-1-ium chloride* **(4)**

Brownish powder. 64.17% yield. Melting point: 219.4°C. Anal. calcd. for $C_{17}H_{20}ClF_3N_2S$ (MM): C, 54.18; H, 5.35; Cl, 9.41; F, 15.12; N, 7.43; S, 8.51. Found: C, 54.19; H, 5.32; Cl, 9.42; F, 15.13; N, 7.41; S, 8.5. MS: molecular peak m/z 341.4, fragmentation peaks m/z 242.0 (2-aryl-thiazolic nucleus substituted with $CF_3$), 145.0, 215.0, 260.0. $^1$H-NMR ($D_2O$): δ (ppm) = 8.19 (H-1 and H-5), 7.91 (H-2 and H-4), 8.24 (H-6), 4.86 (H-7/2H$^+$), 3.48 (H-8 and H-12), 1.96 (H-9 and H-11), 1.85 (H-10), 3.12 (H-13/3H$^+$).

*2.1.5. 4,4-Bis [(2-phenyl-1,3-thiazol-4-yl)methyl]morpholin-4ium iodide* **(5)**

White powder. 56.93% yield. Melting point: 173-175°C. Anal. calcd. for $C_{24}H_{24}IN_3OS_2$ (MM): C, 51.34; H, 4.31; I, 22.6; N, 7.48; O, 2.85; S, 11.42. Found: C, 51.35; H, 4.32; I, 22.57; N, 7.47; O, 2.83; S, 11.37. MS: molecular peak m/z 434.4, fragmentation peaks m/z 174.2 (2-aryl-thiazolic nucleus), 347.1 (2 components of 2-aryl-thiazolic nucleus) 156.2, 244.1, 259.1. $^1$H-NMR ($D_2O$): δ (ppm) = 7.94 (H-1 and H-5), 7.57 (H-2 and H-4), 7.41 (H-3), 7.35 (H-6), 4.96 (H-7/2H$^+$), 3.78 (H-8/2H$^+$ and H-12/2H$^+$), 4.18 (H-9/2H$^+$ and H-11/2H$^+$).

*2.1.6. 4,4-Bis ({[2-(4-methylphenyl)-1,3-thiazol-4-yl]methyl})morpholin-4-ium iodide* **(6)**

Yellowish powder. 75.71% yield. Melting point: 230.7°C. Anal. calcd. for $C_{26}H_{28}IN_3OS_2$ (MM): C, 52.97; H, 4.79; I, 21.53; N, 7.13; O, 2.71; S 10.88. Found: C, 52.96; H, 4.74; I, 21.51; N, 7.19; O, 2.76; S, 10.81. MS: molecular peak m/z 462.3, fragmentation peaks m/z 375.3 (2 components of 2-aryl-thiazolic nucleus substituted with $CH_3$) 226.1, 273.2, 345.3, 419.2. $^1$H-NMR ($D_2O$): δ (ppm) = 7.92 (H-1 and H-5), 7.86 (H-2 and H-4), 7.90 (H-6), 4.97 (H-7/2H$^+$), 3.78 (H-8 and H-12), 3.98 (H-9 and H-11), 2.38 (H-14/3 H$^+$).

*2.1.7. 4-Methyl-4-{[2-(4-methylphenyl)-1,3-thiazol-4-yl]methyl}morpholin-4-ium* **(7)**

White powder. 56.19% yield. Melting point: 219.9-220.1°C. Anal. calcd. for $C_{16}H_{21}N_2SOI$ (MM): C, 46.15; H, 5.04; N, 6.73; S, 7.69; O, 3.84; I, 30.52. Found: C, 47.25; H, 5.21; N, 6.62; S, 7.61; O, 3.73; I, 31.54. MS: molecular peak m/z 289.2, fragmentation peaks m/z 188.2 (2-aryl-thiazolic nucleus), 214.1, 245.1, 275.3. $^1$H-NMR ($D_2O$): δ (ppm) = 7.88 (H-1 and H-5), 7.37 (H-2

and H-4), 8.06 (H-6), 4.88 (H-7/2H$^+$), 3.54 (H-8/2H$^+$ and H-12/2H$^+$), 3.06 (H-9/2H$^+$ and H-11/2H$^+$), 3.39 (H-13/3H$^+$), 2.37 (H-3/3H$^+$).

*2.1.8. 4-Methyl-4-({2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-4-yl}methyl)morpholin-4-ium chloride* **(8)**

Yellowish powder. 24.84% yield. Melting point: 229.2°C. Anal. calcd. for $C_{16}H_{18}ClF_3N_2OS$ (MM): C, 50.73; H, 4.79; Cl, 9.36; F, 15.04; N, 7.39; O, 4.22; S, 8.46. Found: C, 50.75; H, 4.74; Cl, 9.41; F, 15.05; N, 7.36; O, 4.2; S, 8.48. MS: molecular peak m/z 343.2, fragmentation peaks m/z 242.0 (2-aryl-thiazolic nucleus substituted with $CF_3$) 100.1, 259.9. $^1$H-NMR ($D_2O$): δ (ppm) = 7.92 (H-1 and H-5), 7.97 (H-2 and H-4), 8.22 (H-6), 4.83 (H-7/2H$^+$), 4.06 (H-8 and H-12), 3.52 (H-9), 3.48 (H-11), 3.21 (H-13/3H$^+$).

*2.1.9. 3-Carbamoyl-1-[(2-phenyl-1,3-thiazol-4-yl)methyl]pyridin-1-ium iodide* **(9)**

Yellowish powder. 92.27% yield. Melting point: 206.1°C. Anal. calcd. for $C_{16}H_{14}IN_3OS$ (MM): C, 45.4; H, 3.33; I, 29.98; N, 9.93; O, 3.78; S, 7.58. Found: C, 45.32; H, 3.36; I, 30.06; N, 9.9; O, 3.71; S, 7.55. MS: molecular peak m/z 296.2, fragmentation peaks m/z 174.1 (2-aryl-thiazolic nucleus) 104.3, 206.1. $^1$H-NMR ($D_2O$): δ (ppm) = 7.81 (H-1 and H-5), 7.38 (H-2 and H-4), 7.42 (H-3), 8.12 (H-6), 6.1 (H-7/2H$^+$), 9.38 (H-8), 8.33 (H-9), 9.01 (H-10), 9.59 (H-12), 8.21 (H$^+$ amydic group).

*2.1.10.3-Carbamoyl-1-{[2-(4-methylphenyl)-1,3-thiazol-4-yl]methyl}pyridin-1-ium iodide* **(10)**

Yellowish powder. 36.29% yield. Melting point: 259.6°C. Anal. calcd. for $C_{17}H_{16}IN_3OS$ (MM): C, 46.69; H, 3.69; I, 29.02; N, 9.61; O, 3.66; S, 7.33. Found: C, 46.67; H, 3.6; I, 29.08; N, 9.65; O, 3.58; S, 7.29. MS: molecular peak m/z 310.3, fragmentation peaks m/z 188.0 (2-aryl-thiazolic nucleus substituted with $CH_3$). $^1$H-NMR ($D_2O$): δ (ppm) = 7.78 (H-1 and H-5), 7.31 (H-2 and H-4), 8.02 (H-6), 6.09 (H-7/2H$^+$), 9.37 (H-8), 8.35 (H-9), 9.02 (H-10), 9.67 (H-12), 2.35 (H-14/3H$^+$), 8.21 (H$^+$ amydic group).

*2.1.11. 3-Carboxy-1-[(2-phenyl-1,3-thiazol-4-yl)methyl]pyridin-1-ium iodide* **(11)**

Orange powder. 96.45% yield. Melting point: 188.7°C. Anal. calcd. for $C_{16}H_{13}IN_2O_2S$ (MM): C, 45.3; H, 3.09; I, 29.91; N, 6.6; O, 7.54; S, 7.56. Found: C, 45.28; H, 3.06; I, 29.89; N, 6.63; O, 7.52; S, 7.55. MS: molecular peak m/z 297.2, fragmentation peaks m/z 174.1 (2-aryl-thiazolic nucleus), 206.2. $^1$H-NMR ($D_2O$): δ (ppm) = 7.82 (H-1 and H-5), 7.39 (H-2 and H-4), 7.47 (H-3), 6.96 (H-6), 5.26 (H-7/2H$^+$), 9.15 (H-8), 8.33 (H-9), 8.99 (H-10), 9.55 (H-12), 12.60 (H$^+$ carboxylic group).

*2.1.12. 3-Carboxy-1-{[2-(4-methylphenyl)-1,3-thiazol-4-yl]methyl}pyridin-1-ium iodide* **(12)**

Brown powder. 34.23% yield. Melting point: 209.4°C. Anal. calcd. for $C_{17}H_{15}IN_2O_2S$ (MM): C, 46.59; H, 3.45; I, 28.95; N, 6.39; O, 7.30; S, 7.32. Found: C, 46.56; H, 3.40; I, 28.99; N, 6.42; O, 7.28; S, 7.3. MS: molecular peak m/z 311.1, fragmentation peaks m/z 188.2 (2-aryl-thiazolic nucleus substituted with $CH_3$), 118.3S. $^1$H-NMR ($D_2O$): δ (ppm) = 7.81 (H-1 and H-5), 7.31 (H-2 and H-4), 7.96 (H-6), 6.16 (H-7/2H$^+$), 9.41 (H-8), 8.39 (H-9), 9.01 (H-10), 9.74 (H-12), 2.34 (H-14), 12.60 (H$^+$ carboxylic group).

### 2.2. Pharmacological evaluation

#### 2.2.1. Materials and methods

Quaternary ammonium compounds are considered among the substances which can influence the contractility of smooth muscles from the digestive tract, acting via cholinergic mechanisms.

Based on structural considerations, nine compounds were biologically evaluated in the experimental model of guinea-pig isolated ileum, using acetylcholine as a reference contractile agent with a cholinergic mechanism. To compare the importance of the substituent in the 4 position, we tested 16 compounds, 12 newly synthesized molecules and four other compounds (**13**, **14, 15** and **16**) for which the synthesis was presented in our previous papers [13, 16].

For the isolated ileum model, nine groups of male guinea-pigs (n=5) with a mean weight of 350 g were used. The animals were kept in standard laboratory conditions, on a 12-h light/dark cycle, the access to food and water being prohibited 24h before the experiment. In the day of the experiment, the animals were sacrificed by cervical dislocation under general anesthesia, and fragments of ileum were obtained by surgical techniques. Two centimetres long fragments of ileum were placed in organ baths containing Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$ and 11 mM glucose per L), at $37^0$C, gassed with a mixture of oxygen 95% and carbon dioxide 5% v/v, and were subsequently connected to a transducer-amplifier system coupled with a multiple channel recorder (Kipp and Zonnen). The resting tension was set at 1g, the experiment being conducted in isotonic conditions [17].

After a 45 minutes resting period, concentration-response curves were constructed with acetylcholine (Sigma Chemicals) at concentrations of $10^{-8}$M, $10^{-7}$M, $10^{-6}$M, $10^{-5}$M and $10^{-4}$M respectively. Then, concentration-response curves were constructed for each of the nine tested compounds (**1, 2, 5, 6, 7, 13, 14, 15,** and **16,** dissolved in DMSO), using the same molar concentrations. The contractile response for each compound was calculated as percentage of the contractile response obtained with acetylcholine. The $pD_2$ parameter (negative logarithm of EC50) was calculated from the graphical representation of the concentration-response curves for the compounds with significant effects. Also, for the most active compounds (**1, 5, 13, 14, 15, and 16**) the concentration-response curves were reconstructed in the presence of a$10^{-5}$M atropine solution in the organ bath [18, 19].

For compounds **9, 10, 11** and **12**, which have nicotinic acid or nicotinamide in their structures, a possible antispasmodic effect was investigated. Thus, concentration-response curves were constructed for acetylcholine ($10^{-8}$ M, $10^{-7}$M, $10^{-6}$ M, $10^{-5}$M and $10^{-4}$M) using ileum fragments pre-incubated with a $10^{-5}$M solution of each of the four mentioned compounds [20].

All the biological experiments were approved by the Ethics Commission of the ”Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca and were conducted according to the EC directive 86/609/EEC, which regulates the use of laboratory animals.

The results were expressed as mean values ± s.e.m. and the statistical interpretation of the results was performed by „t” Student test, p values≤0.05 being considered statistically significant.

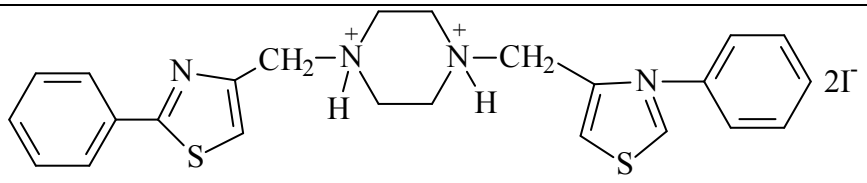
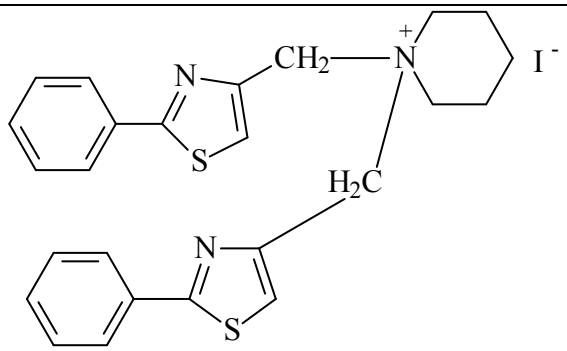
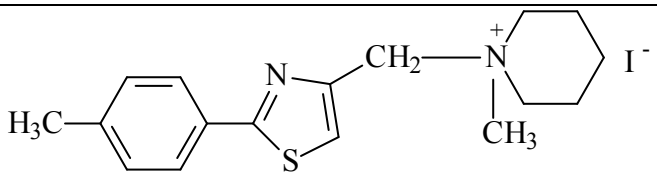
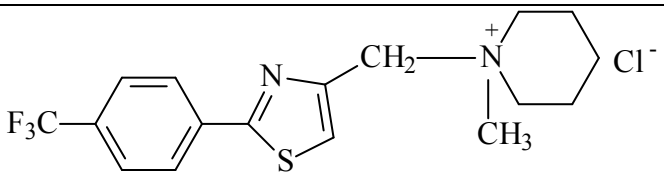
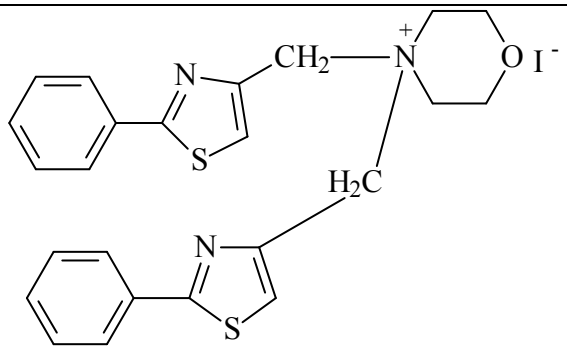
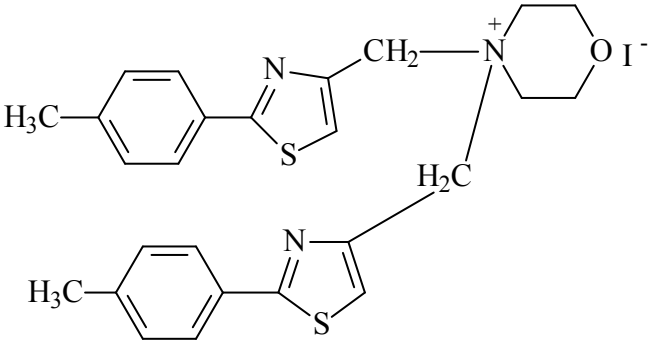
## 3. Results and discussions

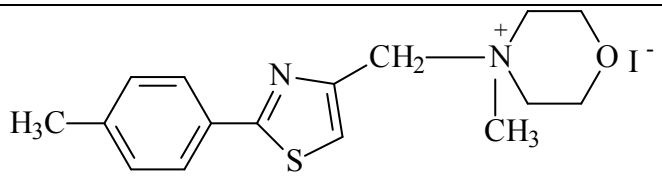
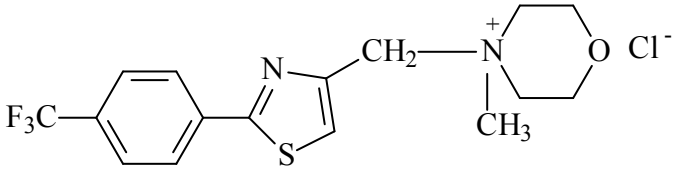
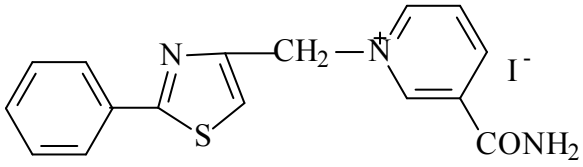
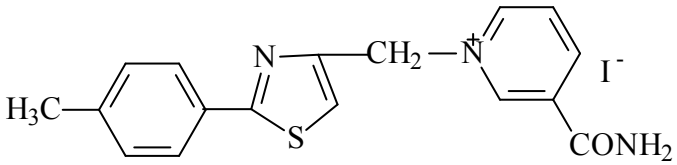
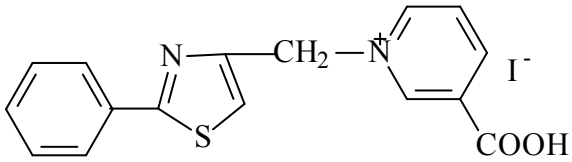
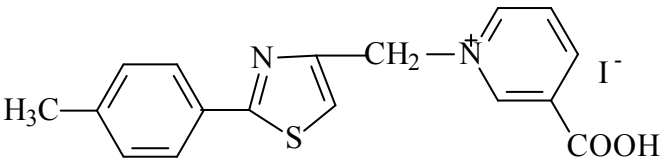
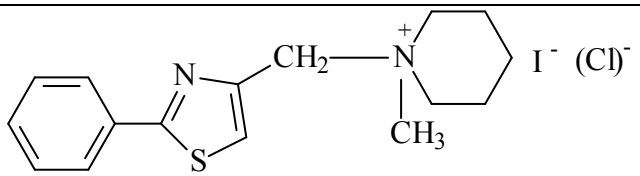
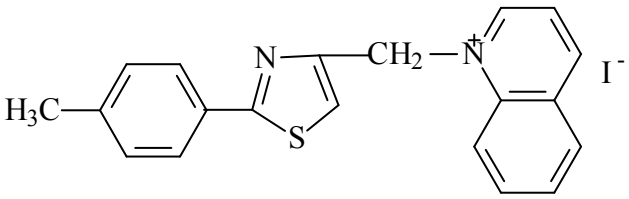
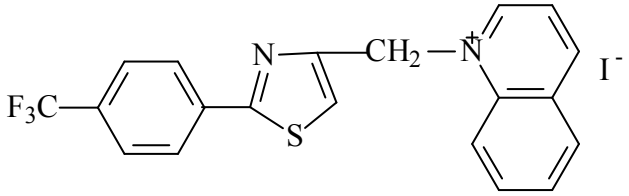
### 3.1. Chemistry

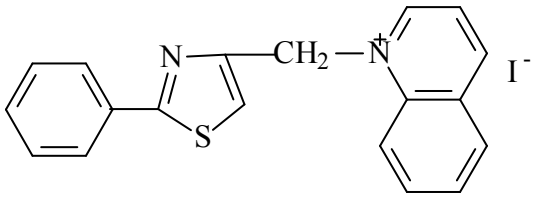
The new compounds are soluble in ethanol, dimethylsulphoxide (DMSO), slightly soluble in water, heavily soluble in chloroform, insoluble in ether.

The proposed structures of the newly synthesized molecules were in accordance with the data obtained from $^1$H-NMR, mass spectrometry and elemental analysis, given below.

*Table I. The chemical structures of the synthesized compounds*

| Compound | Structure |
|---|---|
| **1** |  |
| **2** |  |
| **3** |  |
| **4** |  |
| **5** |  |
| **6** |  |

| Compound | Structure |
|---|---|
| **7** |  |
| **8** |  |
| **9** |  |
| **10** |  |
| **11** |  |
| **12** |  |
| **13[16]** |  |
| **14[13]** |  |
| **15[13]** |  |

| Compound | Structure |
|---|---|
| **16[13]** |  |

Good reaction yields were obtained in the case of derivatives which have in the 4 position of the benzene nucleus, from the 2-aryl thiazole nucleus, electron donating groups (e.g. $CH_3$) and smaller yields were obtained for the derivatives in which this position is substituted by electron withdrawing groups (e.g. $CF_3$).

### 3.2. Pharmacological activity on the guinea-pig isolated ileum

The results of the experiments are shown in *Tables II, III and IV*.

*Table II. Contractile response (as % of Ach response) and pD2 values for the tested compounds.*

| **Comp.** | **Contractile response of the isolated ileum (% of Ach response) (Mean±s.e.m.)** | | | | | **$pD_2$** |
|---|---|---|---|---|---|---|
| | *$10^{-8}$ M* | *$10^{-7}$ M* | *$10^{-6}$ M* | *$10^{-5}$ M* | *$10^{-4}$ M* | |
| 1 | 20.90±5.94* | 24.64±2.74* | 32.77±9.55* | 46.12±8.42* | 50.34±6.22* | 4.0 |
| 2 | 0.4±0.32 | 0.50±0.28 | 1.06±0.40 | 1.66±0.67 | 1.70±0.62 | - |
| 5 | 13.35±1.71 | 18.54±3.89* | 24.62±3.49* | 38.10±3.28* | 42.52±1.27* | - |
| 6 | 0.43±0.21 | 0.45±0.18 | 1.10±0.62 | 1.10±0.37 | 2.00±0.91 | - |
| 7 | 0.98±0.41 | 1.36±0.32 | 2.3±1.43 | 1.8±1.03 | 1.9±0.94 | - |
| 13 | 25.91±4.54* | 31.34±4.86* | 34.15±10* | 41.61±7.28* | 58.09±6.81* | 4.3 |
| 14 | 21.81±3.21* | 24.28±3.12* | 29.17±7.85* | 45.51±10.97* | 50.73±8.03* | 4.0 |
| 15 | 19.01±1.81* | 25.22±2.47* | 35.33±1.85* | 41.75±2.71* | 52.62±2.20* | 4.2 |
| 16 | 27.71±3.05* | 33.64±2.60* | 38.60±4.88* | 54.01±5.9* | 61.25±8.50* | 5.2 |

(*p ≤0.05: statistically significant)

A significant contractile effect was produced by compounds **1, 5, 13, 14, 15** and **16.** The other compounds (**2, 6** and **7**) demonstrated reduced and non-significant effects. According to pD2 parameter values, the most potent contractile effect was produced by compound **16** (pD2=5.20), followed by compound **13** (pD2=4.3), **15** (pD2=4.2) and the compounds **1** and **4** (pD2=4.0). For compound **5**, the pD2 value could not be calculated.

The presence of a $10^{-5}$M atropine solution in the organ bath, significantly decreased the contractile effect produced by the compounds **1, 5, 13, 14, 15** and **16**, as presented in the table below:

*Table III. Contractile response (as % of Ach response) for the tested compounds in the presence of a $10^{-5}$M atropine solution.*

| Comp. | Contractile response of the isolated ileum (% of Ach response) in the presence of a $10^{-5}$M atropine solution (Mean±s.e.m.) | | | | |
|---|---|---|---|---|---|
| | $10^{-8}$ M | $10^{-7}$ M | $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M |
| 1 | 2.97±0.32* | 8.40±1.03* | 14.75±3.33* | 18.63±4.11* | 20.13±4.29* |
| 5 | 2.34±0.45 | 9.61±1.75* | 11.89±2.44* | 15.67±8.23 | 17.11±4.89* |
| 13 | 8.50±2.11* | 10.71±3.04* | 20.93±5.45* | 22.05±7.19* | 24.03±5.72* |
| 14 | 8.00±2.62* | 11.23±3.50* | 15.40±4.83* | 17.05±6.05* | 19.11±7.95* |
| 15 | 8.55±2.15* | 12.43±2.95* | 17.97±4.34* | 20.11±5.14* | 22.33±6.48* |
| 16 | 7.03±2.35* | 13.20±3.55* | 18.39±4.15* | 21.15±6.05* | 27.05±7.75* |

(*p ≤0.05: statistically significant)

Among the compounds with nicotinic acid or nicotinamide moieties (**9, 10, 11** and **12**), with presumed antispasmodic effect, only the compounds **9** and **12** produced statistically significant effects, inhibiting the contractions induced by acetylcholine, as presented in the table below:

*Table IV. Contractile response produced by Ach alone (control) and in the presence of $10^{-5}$M solutions of compounds 9, 10, 11 and 12*

| Comp. | Contractile response of the isolated ileum produced by Ach in the presence of $10^{-5}$M solutions of compounds 9, 10, 11 and 12 (Mean±s.e.m.) | | | | |
|---|---|---|---|---|---|
| | $10^{-8}$ M | $10^{-7}$ M | $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M |
| control | 6.91±1.14* | 30.07±7.75* | 45.48±13.55* | 80.29±12.42* | 100.1±14.65* |
| 9 | 2.34±0.45 | 19.27±1.75* | 32.57±2.44* | 60.54±8.23 | 77.19±6.89* |
| 10 | 6.50±3.12 | 29.71±9.53* | 40.73±8.93 | 72.89±9.55 | 94.45±11.13 |
| 11 | 7.05±1.44 | 31.77±8.90 | 45.25±8.13 | 77.62±9.85 | 99.05±14.35 |
| 12 | 2.83±0.75 | 20.88±4.33* | 33.45±6.91* | 47.91±8.60* | 71.50±8.42* |

(*p ≤0.05: statistically significant)

The concentration-response curves constructed for the compounds with contractile activity, in the presence of a $10^{-5}$M atropine solution, showed a significant decrease of the contractile effect, which indicates a pharmacodynamic antagonism, further research being necessary to clarify the contractile mechanism.

The correlation of the obtained results with the chemical structures of the compounds, revealed the importance of the substituent in the 4 position of the thiazole ring for the biological activity. Thus, the quinolinium salts (**14, 15** and **16**) are active, the intensity of the effect being influenced by the substitution of the phenyl moiety in the 2 position of the thiazole ring. The contractile effect was more intense when the phenyl moiety in the 2 position of the thiazole ring did not include any substituent, which suggests its involvement in a hydrophobic bond. A substitution of this moiety with electron donating groups ($CH_3$) or electron withdrawing groups ($CF_3$) decreased the biologic effect, probably due to steric impediments.

The N-Methyl-Piperidine ring, associated with the substitution of the thiazole nucleus in the 2 position with a phenyl ring, favourably influenced the contractile effect. For the piperazine compounds, only the iodhydrate (**1**) presented a contractile effect.

The bis-thiazolyl quaternary ammonium compounds **(2**, **5** and **6**) for which the quaternary nitrogen atom is included in the morpholine ring, presented a reduced effect. This can be explained by the existence of steric impediments, caused by the voluminous substituent at the quaternary nitrogen atom, and by electron withdrawing effect of the oxygen atom from the morpholine ring.

The compounds **9, 10, 11** and **12** which are pyridinium salts substituted in position meta with carboxyl or formamide groups, did not produce any contractile effect on the isolated guinea-pig ileum. Moreover, compounds **9** and **12** decreased the basal tonus of the isolated ileum. Further testing demonstrating a significant inhibition of acetylcholine-induced contractions is necessary.

## 4. Conclusion

Twelve new tertiary and quaternary ammonium derivatives of the 2-aryl-thiazole core were synthesized. Their structure was elucidated by elemental analysis, melting points and spectral determinations (MS, $^{1}$H-NMR). The spectral determinations validated the structures attributed to the synthesized compounds.

The contractile effects of nine compounds (**1, 2, 5, 6, 7, 13, 14, 15** and **16**) were investigated on isolated guinea-pig ileum. Also, the antispasmodic potential of other four compounds with nicotinic acid or nicotinamide moieties (**9, 10, 11** and **12**) was investigated, using the same experimental model.

Five of the tested compounds (**1, 13, 14, 15** and **16**) presented statistically significant contractile effects on the isolated guinea-pig ileum, the pD2 parameter values being situated between 4.0 and 5.2. The compounds **9** and **12** presented significant antispasmodic effects on the isolated guinea-pig ileum.

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