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Short Communication

# Synthesis and chemiluminescent high throughput screening for inhibition of acetylcholinesterase activity by imidazo[2,1-*b*]thiazole derivatives

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**Abstract**

The synthesis of a new series of imidazo[2,1-*b*]thiazole derivatives is described. They were tested as acetylcholinesterase inhibitors by means of a chemiluminescent method suitable for high throughput screening. The compounds without quaternization had no appreciable inhibitory potency probably because they are poorly soluble in water. The corresponding quaternized compounds were good inhibitors with activity related to the spacer employed.


*Keywords:* Imidazo[2,1-*b*]thiazole; Bisammonium salts; Chemiluminescence; Antiacetylcholinesterase activity; Neuromuscular transmission; Alzheimer's disease; Miastenia gravis; Glaucoma

## 1. Introduction

Acetylcholinesterase (AChE) inhibitors have been widely used as therapeutic agents in disease states such as Alzheimer's, miastenia gravis and glaucoma [1–4]. They are widely used in anesthesia to reverse the skeletal muscle relaxation induced by non-depolarizing neuromuscular blocking agents [5]. By inhibiting the hydrolysis of acetylcholine (ACh), AChE inhibitors increase the levels of ACh in the neuromuscular junction facilitating cholinergic neurotransmission and recovery of muscle function. Despite the widespread use of neostigmine, a reversible inhibitor of AChE ($IC_{50}$ = 11.3 nM) that is used as a reversal agent for neuromuscular block in surgical anesthesia [5], there is a need for an equally effective reversal agent with reduced cardiovascular side effects [6,7]. Also, the use of bis-quaternary acetylcholinesterase inhibitors may be a strategy for development of effective drugs for treatment of Alzeimer's disease [8,9].

In a prior report the 6-methylimidazo[2,1-*b*]thiazole derivatives (**3a**–**7a**) were known to act on neuromuscular transmission [10]. Two symmetrical bisammonium salts arising from 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid that contained a four-carbon spacer (the amide **5a** and the ester **6a**) showed a unique activity. At low doses (0.50–1 mg $kg^{-1}$) they released ACh at the presynaptic level, with a stimulating effect (37–50% increase in contractile force) that was not related to activity on the muscle. By contrast, at high doses (16–40 mg $kg^{-1}$) both compounds produced a blockade (50–100%) by acting at the postsynaptic level. Based on the data obtained, the effect at low doses could be due to ACh release or AChE inhibition. The biochemical evaluation for **3a**–**7a** was performed using a chemiluminescent (CL) method suitable for high throughput screening of AChE inhibitors [11,12]. To further optimize the activity observed, a new series of imidazo[2,1-*b*]thiazole derivatives with different spacers was prepared (**8**–**15**, **8a**–**15a**) (see Scheme 1) to investigate the effect of different distances between the two portions of the imidazothiazoles. These compounds were tested with the same CL method. Quaternary compounds are not considered useful drugs for the treatment of the Alzheimer's disease since they do not cross the blood–brain barrier but the authors of the already mentioned review [8] underline that as early as 1992 Rosenberry asserted that compounds such as ambenonium chloride are important tools for exploring AChE catalytic mechanism and supposed that simple analogs could penetrate the blood-brain barrier [13].

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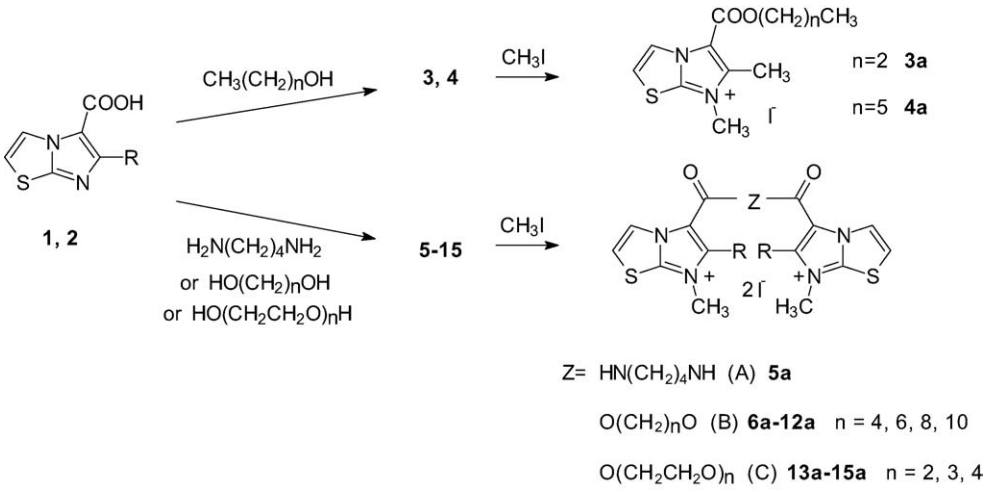


Scheme 1. (R see Table 1).

## 2. Chemistry

The new compounds were prepared from *6*-methyl and 6-phenyl-imidazo[2,1-*b*]thiazole-5-carboxylic acids [14] using the procedure previously reported [10]. The corresponding 1-acylimidazole, prepared by reacting the acid with 1,1′-carbonyldiimidazole (DCI), gave the alkyl carboxylate in fairly good yields (see Table 1) after reaction with 1,6-hexanediol (**10**), 1,8-octanediol (**8**, **11**), 1,10-decanediol (**9**, **12**), diethylene glycol (**13**), triethylene glycol (**14**), or tetraethylene glycol (**15**) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as activator of the alcohol. The quaternary salts **8a**–**15a** were prepared by quaternization of the tertiary bases **8**–**15** with a large excess of refluxing iodomethane [10] (see Scheme 1).

## 3. Biological results and conclusion

The biological activity of the compounds was evaluated by means of a high throughput CL assay based on a series of coupled enzymatic reactions involving AChE, choline oxidase and horseradish peroxidase, with luminol as the CL substrate [11,12]. Assay validation was performed using tacrine, a known AChE inhibitor [15]. The $IC_{50}$ values are reported in Table 3 and represent the mean ± S.D. of at least three independent measures. Most of the compounds without quaternization (**4**, **7**–**9**, **11**–**15**) were poorly soluble in water or water/DMSO and were not assayed for the AChE activity, however compounds **3**, **5**, **6**, **10** were soluble and showed weak activity ($IC_{50}$ = 20–70 μM). Most of the quaternized compounds were acetylcholinesterase inhibitors with significant

Table 1
Compounds **5**–**15**

| Compound | Z-n | R | Formula | MW | M.p. (°C) or References |
|---|---|---|---|---|---|
| **5** | A | $CH_3$ | $C_{18}H_{20}N_6O_2S_2$ | 416.5 | [10] |
| **5a** | A | $CH_3$ | $C_{20}H_{26}I_2N_6O_2S_2$ | 700.4 | [10] |
| **6** | B-4 | $CH_3$ | $C_{18}H_{18}N_4O_4S_2$ | 418.5 | [10] |
| **6a** | B-4 | $CH_3$ | $C_{20}H_{24}I_2N_4O_4S_2$ | 702.4 | [10] |
| **7** | B-6 | $CH_3$ | $C_{20}H_{22}N_4O_4S_2$ | 446.5 | [10] |
| **7a** | B-6 | $CH_3$ | $C_{22}H_{28}I_2N_4O_4S_2$ | 730.4 | [10] |
| **8** | B-8 | $CH_3$ | $C_{22}H_{26}N_4O_4S_2$ | 474.6 | 124–126 |
| **8a** | B-8 | $CH_3$ | $C_{24}H_{32}I_2N_4O_4S_2$ | 758.5 | 217–218 Dec. |
| **9** | B-10 | $CH_3$ | $C_{24}H_{30}N_4O_4S_2$ | 502.7 | 93–95 |
| **9a** | B-10 | $CH_3$ | $C_{26}H_{36}I_2N_4O_4S_2$ | 786.5 | 216–217 Dec. |
| **10** | B-6 | $C_6H_5$ | $C_{30}H_{26}N_4O_4S_2$ | 570.7 | 148–150 |
| **10a** | B-6 | $C_6H_5$ | $C_{32}H_{32}I_2N_4O_4S_2$ | 854.6 | 200–201 Dec. |
| **11** | B-8 | $C_6H_5$ | $C_{32}H_{30}N_4O_4S_2$ | 598.7 | 112–114 |
| **11a** | B-8 | $C_6H_5$ | $C_{34}H_{36}I_2N_4O_4S_2$ | 882.6 | 165–166 Dec. |
| **12** | B-10 | $C_6H_5$ | $C_{34}H_{34}N_4O_4S_2$ | 626.79 | Oil |
| **12a** | B-10 | $C_6H_5$ | $C_{36}H_{40}I_2N_4O_4S_2$ | 910.7 | 174–175 Dec. |
| **13** | C-2 | $CH_3$ | $C_{18}H_{18}N_4O_5S_2$ | 434.5 | 159–161 |
| **13a** | C-2 | $CH_3$ | $C_{20}H_{24}I_2N_4O_5S_2$ | 718.4 | 216–217 Dec. |
| **14** | C-3 | $CH_3$ | $C_{20}H_{22}N_4O_6S_2$ | 478.6 | 145–147 |
| **14a** | C-3 | $CH_3$ | $C_{22}H_{28}I_2N_4O_6S_2$ | 762.4 | 200–202 Dec. |
| **15** | C-4 | $CH_3$ | $C_{22}H_{26}N_4O_7S_2$ | 522.6 | 91–92 |
| **15a** | C-4 | $CH_3$ | $C_{24}H_{32}I_2N_4O_7S_2$ | 806.5 | 152–153 Dec. |

Table 2
IR and $^1$H-NMR of compounds **8**–**15** (th = thiazole, ar = aromatic)

| Compound | IR: $\nu_{max}$ (cm$^{-1}$) | $^1$H-NMR:[a] $\delta$ (ppm) in DMSO-d$_6$ |
|---|---|---|
| **8** | 1670, 1320, 1230, 1140, 1100 | 1.36 (8H, m, 4$CH_2$), 1.71 (4H, qui, 2$CH_2$, $J$ = 6.5), 2.48 (6H, s, 2$CH_3$), 4.27 (4H, t, 2COO$CH_2$, $J$ = 6.5) 7.43 (2H, d, th, $J$ = 4.4), 8.06 (2H, d, th, $J$ = 4.4) |
| **8a** | 1705, 1595, 1370, 1230, 1120 | 1.37 (8H, m, 4$CH_2$), 1.77 (4H, qui, 2$CH_2$, $J$ = 6.3), 2.70 (6H, s, 2$CH_3$), 3.95 (6H, s, 2$CH_3$I), 4.40 (4H, t, 2COO$CH_2$, $J$ = 6.3) 7.89 (2H, d, th, $J$ = 4.1), 8.42 (2H, d, th, $J$ = 4.1) |
| **9** | 1670, 1320, 1230, 1135, 1095 | 1.32 (12H, m, 6$CH_2$), 1.69 (4H, qui, 2$CH_2$, $J$ = 6.6), 2.49 (6H, s, 2$CH_3$), 4.25 (4H, t, 2COO$CH_2$, $J$ = 6.6), 7.43 (2H, d, th, $J$ = 4.4), 8.05 (2H, d, th, $J$ = 4.4) |
| **9a** | 1700, 1580, 1230, 1150, 825 | 1.33 (12H, m, 6$CH_2$), 1.76 (4H, qui, 2$CH_2$, $J$ = 6.7), 2.71 (6H, s, 2$CH_3$), 3.95 (6H, s, 2$CH_3$I), 4.40 (4H, t, 2COO$CH_2$, $J$ = 6.7) 7.89 (2H, d, th, $J$ = 4.2), 8.43 (2H, d, th, $J$ = 4.2) |
| **10** | 1685, 1120, 750, 685, 655 | 1.17 (4H, m, 2$CH_2$), 1.55 (4H, qui, 2$CH_2$, $J$ = 6.2), 4.19 (4H, t, 2COO$CH_2$, $J$ = 6.2), 7.39 (6H, m, ar), 7.52 (2H, d, th, $J$ = 4.4), 7.77 (4H, m, ar), 8.20 (2H, d, th, $J$ = 4.4) |
| **10a** | 1690, 1120, 950, 820, 655 | 0.78 (4H, s, 2$CH_2$), 1.30 (4H, qui, 2$CH_2$, $J$ = 6), 3.75 (6H, s, 2$CH_3$I), 4.14 (4H, t, 2COO$CH_2$, $J$ = 6), 7.64 (10H, s, ar), 8.00 (2H, d, th, $J$ = 4.2), 8.59 (2H, d, th, $J$ = 4.2) |
| **11** | 1695, 1315 1225, 1110, 680 | 1.16 (8H, s, 4$CH_2$), 1.57 (4H, qui, 2$CH_2$, $J$ = 6.3), 4.20 (4H, t, 2COO$CH_2$, $J$ = 6.3), 7.39 (6H, m, ar), 7.52 (2H, d, th, $J$ = 4.4), 7.77 (4H, m, ar), 8.20 (2H, d, th, $J$ = 4.4) |
| **11a** | 1720, 1220, 1130, 835, 730 | 0.88 (4H, s, 2$CH_2$), 1.00 (4H, s, 2$CH_2$), 1.38 (4H, qui, 2$CH_2$, $J$ = 5.3), 3.74 (6H, s, 2$CH_3$I), 4.16 (4H, t, 2COO$CH_2$, $J$ = 5.3), 7.63 (10H, s, ar), 7.98 (2H, d, th, $J$ = 3.5), 8.58 (2H, d, th, $J$ = 3.5) |
| **12** | 1675, 1225, 1130, 750, 685 | 1.18 (12H, s, 6$CH_2$), 1.57 (4H, qui, 2$CH_2$, $J$ = 6.3), 4.21 (4H, t, 2COO$CH_2$, $J$ = 6.3), 7.40 (6H, m, ar), 7.53 (2H, d, th, $J$ = 4.4), 7.79 (4H, m, ar), 8.20 (2H, d, th, $J$ = 4.4) |
| **12a** | 1710, 1205, 1120, 825, 690 | 0.93 (4H, m, 2$CH_2$), 1.11 (8H, s, 4$CH_2$), 1.40 (4H, qui, 2$CH_2$, $J$ = 6.4), 3.74 (6H, s, 2$CH_3$I), 4.17 (4H, t, 2COO$CH_2$, $J$ = 6.4), 7.64 (10H, s, ar), 7.99 (2H, d, th, $J$ = 4.1), 8.58 (2H, d, th, $J$ = 4.1) |
| **13** | 1685, 1235, 1148, 717, 666 | 2.40 (6H, s, 2$CH_3$), 3.83 (4H, t, 2$CH_2$, $J$ = 4.6), 4.41 (4H, t, 2$CH_2$, $J$ = 4.6), 7.33 (2H, d, th, $J$ = 4.4), 7.97 (2H, d, th, $J$ = 4.4) |
| **13a** | 1725, 1230, 1122, 835, 738 | 2.69 (6H, s, 2$CH_3$), 3.94 (6H, s, 2$CH_3$I), 4.54 (8H, m, 4$CH_2$), 7.84 (2H, d, th, $J$ = 4.2), 8.35 (2H, d, th, $J$ = 4.2) |
| **14** | 1715, 1250, 1153, 764, 682 | 2.44 (6H, s, 2$CH_3$), 3.63 (4H, s, 2$CH_2$), 3.76 (4H, t, 2$CH_2$, $J$ = 4.6), 4.34 (4H, t, 2$CH_2$, $J$ = 4.6), 7.39 (2H, d, th, $J$ = 4.4), 8.00 (2H, d, th, $J$ = 4.4) |
| **14a** | 1696, 1127, 835, 748, 661 | 2.70 (6H, s, 2$CH_3$), 3.64 (4H, s, 2$CH_2$), 3.80 (4H, t, 2$CH_2$, $J$ = 4.7), 3.94 (6H, s, 2$CH_3$I), 4.50 (4H, t, 2$CH_2$, $J$ = 4.7), 7.88 (2H, d, th, $J$ = 4), 8.39 (2H, d, th, $J$ = 4) |
| **15** | 1700, 1291, 1148, 840, 687 | 2.48 (6H, s, 2$CH_3$), 3.55 (8H, s, 4$CH_2$), 3.73 (4H, m, 2$CH_2$), 4.35 (4H, m, 2$CH_2$), 7.41 (2H, d, th, $J$ = 4.4), 8.03 (2H, d, th, $J$ = 4.4) |
| **15a** | 1700, 1245, 825, 738, 666 | 2.70 (6H, s, 2$CH_3$), 3.57 (8H, m, 4$CH_2$), 3.79 (4H, m, 2$CH_2$), 3.94 (6H, s, 2$CH_3$I), 4.49 (4H, m, 2$CH_2$), 7.88 (2H, d, th, $J$ = 4.2), 8.40 (2H, d, th, $J$ = 4.2) |

Table 3
Inhibition of acetylcholinestearase activity [a]

| Compound | $IC_{50}$ (μM) |
|---|---|
| **3** [10] | 70 ± 15 |
| **3a** [10] | 10 ± 3 |
| **4a** [10] | 3.5 ± 0.7 |
| **5** [10] | 40 ± 6 |
| **5a** [10] | 13 ± 4 |
| **6** [10] | 45 ± 15 |
| **6a** [10] | 30 ± 6 |
| **8a** | 0.8 ± 0.1 |
| **10** | 20 ± 4 |
| **10a** | 1.4 ± 0.5 |
| **13a** | 1.8 ± 0.4 |
| **14a** | 1.8 ± 0.4 |
| **15a** | 2.8 ± 0.4 |
| **Tacrine** | 0.20 ± 0.03 [b] |

[a] Mean ± S.D. of at least three independent measures.
[b] Used as the reference compound [15].

activity. The improvement in activity shown by the quaternized compounds were most likely dependant on an interaction of the positive charged imidazo[2,1-*b*]thiazoles with an active site of the enzyme. The prior activity seen for **5a** and **6a** ($IC_{50}$ = 13, 30 μM, respectively) suggests that the increase in contractile force at low doses [10] was due more to ACh release than AChE inhibition.

The effect of the spacers was comparable for all the quaternized compounds with the C type (**13a**–**15a**) giving good inhibition ($IC_{50}$ = 1.8–2.8 μM) and the most active compounds being the B-type (**8a**, **10a**, $IC_{50}$ = 0.8, 1.4 μM, respectively). Among this limited number of compounds, the nature of the spacer influenced biological activity more than the length of the spacer itself or the nature of the substituent at the 6 position.

## 4. Experimental section

### *4.1. Chemistry*

The melting points are uncorrected. Elemental analyses (C, H, N) were within ± 0.4% of the theoretical values. Bakerflex plates (silica gel IB2-F) were used for TLC: a mixture of chloroform/methanol in various proportions was used as the eluent and exposure to a UV lamp or iodine vapor was used as the visualization method. The IR spectra were recorded in Nujol on a Nicolet Avatar 320E.S.P.; $\nu_{max}$ is expressed in cm$^{-1}$ (see Table 2). The $^1$H-NMR spectra were recorded in $(CD_3)_2SO$ on a Varian Gemini (300 MHz); the chemical shift (referenced to solvent signal) is expressed in $\delta$ (ppm) and $J$ in Hz. (see Table 2).

*4.1.1. Alkyl carboxylates **8–15***

DCI (22 mmol) was added to a suspension of 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid (**1**, 20 mmol) or 6-phenylimidazo[2,1-*b*]thiazole-5-carboxylic acid (**2**, 20 mmol) in anhydrous $CHCl_3$ (50 ml) and held at reflux under stirring until effervescence ceased. After cooling 1-acylimidazole was treated with the appropriate alcohol (20 mmol) or diol (10 mmol) and DBU (22 mmol). The reaction mixture was stirred under reflux for 4 h, cooled, and partitioned between $CHCl_3$ and water. The organic layer was washed with water to neutrality, dried and concentrated under reduced pressure. The residue was crystallized from ethanol (**8**, **10**, **11**), methanol (**13**, **15**) and ethyl acetate (**14**) or purified by column chromatography (**9** $SiO_2$/ethyl acetate; **12** $Al_2O_3$/$CHCl_3$) with a yield of 35% (**8**, **9**, **11**) and 60% (**10**, **12–15**).

*4.1.2. Quaternary salts **8a–15a***

Tertiary bases **8–15** (7–10 mmol) and $CH_3I$ (60 ml) were stirred under reflux using a coil condenser cooled at –2 °C. The reactions were allowed to proceed until TLC analysis of the crude mixture (methanol solution) indicated both the lack of the tertiary base and the presence of a single adduct. The required time ranged from 120 (**8a**, **9a**) to 240 h (**10a–15a**). The reaction mixtures were then cooled and the suspended solid filtered and washed with $Et_2O$ in order to remove the excess $CH_3I$. Additional purification from traces of unreacted tertiary base or monoquaternary salt was achieved by treating a saturated ethanol solution with $Et_2O$ (**8a**, **9a**) or by recrystallization with $H_2O$ (**10a–12a**) or by washing with acetone (**13a–15a**) with a yield of 80–90%.

*4.2. Biological assay*

AChE (EC 3.1.1.7, type V-S from Electric Eel), choline oxidase (ChOX, EC 1.1.3.17, from Alcaligenes species), horseradish peroxidase (HRP, EC 1.11.1.7, type VI-A, from Horseradish), luminol (5-amino-2,3-dihydro-1,4-phtalazinedione sodium salt) and ACh chloride were purchased from Sigma. All other reagents and solvents used were of analytical grade. The high throughput CL assay procedure, performed in black polystyrene 384-well microtiter plates (Black Cliniplate 384, Labsystems Oy, Helsinki, Finland) was described in detail elsewhere [11,12]. Briefly, 5 μl of scalar dilutions of the AChE inhibitors in DMSO/water 1:10 (v/v) were dispensed in duplicate to each well. Then, 40 μl of a CL cocktail containing 0.0625 $U.ml^{-1}$ AChE, 0.75 $U.ml^{-1}$ ChOX, 0.25 $U.ml^{-1}$ HRP, and 75 mM luminol were added to the wells. The CL reaction was started by adding 10 μl of a 10 mM water solution of ACh to each well, then the CL signal from the whole 384-well microtiter plate was measured at 1-min time intervals by using a low-light imaging luminograph (NightOWL, PerkinElmer Berthold, Bad Wildbad, Germany). Upon evaluation of the CL intensity values for each well at different times, the slopes of the luminescence kinetic profiles within 5 min after the start of the CL reaction were calculated. The AChE inhibition in each well was then determined by using a calibration curve obtained in the same analytical session in wells with different amounts of AChE. Finally, the $IC_{50}$ values were evaluated by fitting the experimental inhibition data with a four-point dose–response curve.

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