Letters in Drug Design & Discovery, 2018, *15*, 1224-1229

LETTER ARTICLE

Synthetic Aspects and First-time Assessment of 2-amino-1,3-selenazoles Against *Mycobacterium tuberculosis*

Victor Facchinetti[a,b], Marcus V.N. de Souza[b,*] Ana C.S. Nery[b], Stephane L. Calixto[c], Juliana T. Granato[c], Elaine S. Coimbra[c], Maria C.S. Lourenço[d], Claudia R.B. Gomes[b] and Thatyana R.A. Vasconcelos[a]

[a]*Departamento de Química Orgânica, Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal Fluminense, Outeiro de São João Batista, s/no, Centro, Niterói, 24020-141, Rio de Janeiro, Brazil;* [b]*Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Farmanguinhos, Rua Sizenando Nabuco 100, Manguinhos, 21041-250, Rio de Janeiro, RJ, Brazil;* [c]*Departamento de Parasitologia Microbiologia e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Rua José Lourenço Kelmer, s/n- Campus Universitário, Bairro São Pedro, 36036-900-Juiz de Fora, MG, Brazil;* [d]*Fundação Oswaldo Cruz, Instituto Nacional de Infectologia-INI, Serviço de Bacteriologia. Av. Brazil, 4365, Manguinhos, 21045-900-Rio de Janeiro, RJ, Brazil*

Abstract: ***Background*:** 2-aminoselenazoles became an important core in medicinal chemistry after the discovery of Ebselen and Ethaselen. Therefore, many researchers have reported the synthesis of small selenazole intermediates *via* Hantzsch cyclization using a wide array of methodologies and catalysts.

***Methods*:** In this work, we investigated the formation of 2-aminoselenazoles on various organic solvents and in water, under catalyst-free conditions. Moreover, these molecules and their 2-aminothiazoles analogues were assessed *in vitro* for their antitubercular activity against *Mycobacterium tuberculosis* and the results compared.

***Results*:** Instant reactions were observed when using polar aprotic solvents and all selenazoles were synthesized in water using sonochemistry. Furthermore, two selenazoles and one thiazole displayed activity in the μM range and the selenium heterocycles seems to be more potent than their sulphur analogues.

***Conclusion*:** This is the first study of selenazoles against *M. tuberculosis*. It is noteworthy that 2-amino-1,3-selenazoles are interesting synthetic intermediates that could be incorporated into novel prototypes against tuberculosis.

ARTICLE HISTORY

Received: May 31, 2017
Revised: November 23, 2017
Accepted: January 23, 2018

DOI:
10.2174/1570180815666180209153925

Keywords: Tuberculosis, selenazole, synthesis, methodology, ultrasound, hantzsch cyclization.

1. INTRODUCTION

Selenium is an essential nutrient found in some selenoproteins [1], which can be obtained from food as a trace element. The body of an adult contains 5-15 mg of selenium, with four different selenium containing blood plasma components found in kinetic studies [2-7].

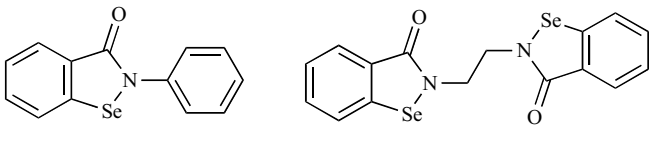
The effects of this element are controversial. Insufficient intake of selenium can lead to a deficient status; ingestion of this element in high quantities can lead to poisoning and toxic effects [8, 9]. Nevertheless, scientists believe that selenium can prevent some age-related pathologies, acting both as a heavy metal detoxifying agent and as an anti-inflammatory [10].

Interest in the synthesis of selenium-containing heterocycles has grown after the advent of Ebselen and Ethaselen (Fig. **1**), which can act as safe, potent antioxidants [11]. Moreover, many selenazoles are being reported for their interesting pharmacological activity, such as antitumor, anti-melanogenic and anti-inflammatory.

In 2015, our research group has published a review article highlighting the synthesis and biological properties of 1,3-selenazoles [12]. Although, many researchers have published articles on the formation of simple and complex 1,3-selenazoles [13-18] it seems that the aspects regarding the formation of this nucleus *via* Hantzsch's reaction and how they relate to the 1,3-thiazole synthesis, are not yet fully understood.

Moreover, with the resurgence of tuberculosis (TB) in the 21st century, mainly due to the advent of multidrug resistant *Mycobacterium tuberculosis* strains and the high mortality among patients co-infected with TB and HIV, it is urgent to

*Address correspondence to this author at the Instituto de Tecnologia em Fármacos-Farmanguinhos, Fiocruz-Fundação Oswaldo Cruz, P.O. Box: 21041-250, Rio de Janeiro, Brazil; Tel/Fax: (55) 2139772404, (55) 2125602518; E-mail: marcos_souza@far.fiocruz.br



Ebselen **Ethaselen**

Fig. (1). Ebselen and ethaselen.

develop new, potent, less toxic and cheaper drugs, with new mechanisms of action. In this context, to the best of our knowledge, there are no reports of selenazole activity studies against TB.

Therefore, in this work we investigated the synthetic aspects underlying the formation of 2-aminoselenazoles in various solvents without catalysts, and herein report, for the first time, the activity of these synthetic intermediates against *Mycobacterium tuberculosis* ATTC 27294 and compare it to the activity of their 2-aminothiazole analogs.

2. MATERIALS AND METHODS

2.1. Chemistry

All reagents were used as obtained from commercial suppliers without further purification. Ultrasound reactions were carried out in a Multiwave Eco-Sonics QR750 ultrasonic generator (20 kHz, 750 W) equipped with a converter/transducer, and titanium oscillator (horn, diameter = 4 mm). Melting points were determined on a Buchi Melting Point B-545 and are uncorrected. NMR spectra were recorded using a Varian Unity 300 and 500 (Varian Inc., Palo Alto, CA, USA) or on Bruker DRX 400 spectrometers (Bruker, Billerica, MA, USA) in DMSO-d6, MeOD or Acetone-*d6* with TMS as the internal standard. Mass spectrometry analyses were performed on MS micromass ZMD using electrospray ionization. Samples were introduced by the standard direct insertion probe method. All spectral data and melting points for the synthesized compounds have been previously published [19].

2.1.1. General Procedure for the Synthesis of 5g in Various Solvents

2-bromoacetophenone **1g** (199mg, 1mmol), selenourea **3** (147mg, 1.2mmol) and 5mL of the corresponding solvent (water, DMF, MeCN, MeOH, EtOH or Dichloromethane) were added to a round bottle flask under magnetic stirring at room temperature. After completion (TLC), the solvents were concentrated under reduced pressure and the solids filtered and washed with 1mL of water.

2.1.2. General Procedure for the Preparation of Compounds 4a-g and 5a-g in Water

Corresponding 2-bromoacetophenones **1a-g** (199-278mg; 1mmol), thiourea **2** (91.2mg; 1.2 mmol) or selenourea **3** (147mg; 1.2mmol) and 5mL of water were added to a round bottom flask and reactions proceeded under ultrasonic irradiation in a Multiwave Eco-sonics QR750 ultrasonic generator (20 kHz, 750 W) equipped with a converter/transducer, and titanium oscillator (horn, diameter = 4 mm) for 10 minutes. Afterwards, the mixture was cooled down and the products were filtered and washed with 1mL of water, furnishing the desired thiazoles or selenazoles.

2.1.2.1. 4-(4-methoxyphenyl)thiazol-2-amine hydrobromide (4a)

Beige solid; Yield US: 98%; mp 228-231ºC. ^{1}H NMR (400 MHz, acetone-*d6*): δ = 7.80-7.76 (m, 2H, Ph), 6.93-6.89 (m, 2H, Ph), 6.76 (s, 1H, H5), 6.35 (sl, 2H, N$\underline{H}_2$), 3.80 (s, 3H, OC$\underline{H}_3$). ^{13}C NMR (100 MHz, acetone-*d6*): δ = 169.7 (C2), 160.9 (C4′), 152.5 (C4), 130.1 (Ph), 128.8 (Ph), 115.4 (C3′), 101.2 (C5), 56.4 (OCH3) MS (ESI): *m/z* = 206.9 ([M]$^+$, 100%).

2.1.2.2. 4-(4-fluorophenyl)thiazol-2-amine hydrobromide (4b)

White solid; 53%; Yield US: 57%; mp 236-237ºC. ^{1}H NMR (400 MHz, acetone-*d6*): δ = 7.92-7.86 (m, 2H, Ph), 7.15-7.08 (m, 2H, Ph), 6.91 (s, 1H, H5), 6.43 (sl, 2H, N$\underline{H}_2$). ^{13}C NMR (100 MHz, acetone-*d6*): δ = 169.1 (C2), 163.0 (d, J = 242.8 Hz, C4′), 150.6 (C4), 132.8 (d, J = 3.1 Hz, C1′), 128.5 (d, J = 7.9 Hz, C2′), 115.9 (d, J = 21 Hz, C3′), 102.6 (d, J = 1.1 Hz, C5). MS (ESI): *m/z* = 194.9 ([M]$^+$, 100%).

2.1.2.3. 4-(p-tolyl)thiazol-2-amine hydrobromide (4c)

Pale yellow solid; Yield US: 85% mp 285-287ºC. ^{1}H NMR (400 MHz, acetone-*d6*): δ = 7.77-7.71 (m, 2H, Ph), 7.16 (d, *J* = 7.9 Hz, 2H, Ph), 6.85 (s, 1H, H5), 6.38 (sl, 2H, N$\underline{H}_2$), 2.32 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 170.1 (C2), 139.1 (C4), 139.0 (Ph), 129.5 (Ph), 125.9 (Ph), 125.6 (Ph), 101.8 (C5), 20.7 (CH3). MS (ESI): *m/z* = 190.9 ([M]$^+$, 100%).

2.1.2.4. 4-(4-nitrophenyl)thiazol-2-amine hydrobromide (4d)

Yellow solid; Yield US: 65% mp 235-238ºC. ^{1}H NMR (400 MHz, MeOD): δ = 8.32 (d, *J* = 8.9 Hz, 2H, H3'), 7.95 (d, *J* = 8.9 Hz, 2H, H2'), 7.30 (s, 1H, H5). ^{13}C NMR (125 MHz, DMSO-*d6*): δ = 168.54 (C2), 147.46 (C4), 145.92 (C4′), 140.61 (C1′), 126.19 (Ph), 123.83 (Ph), 106.46 (C5). MS (ESI): *m/z* = 221.9 ([M]$^+$, 100%).

2.1.2.5. 4-(4-chlorophenyl)thiazol-2-amine hydrobromide (4e)

Pale yellow solid; Yield US: 98% mp 235-238ºC. ^{1}H NMR (400 MHz, MeOD): δ = 7.66 (d, *J* = 8.6 Hz, 2H, Ph), 7.53 (d, *J* = 8.6 Hz, 2H, Ph), 7.13 (s, 1H, H5). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 170.0 (C2), 133.7 (C4), 129.6 (Ph), 128.9 (Ph), 128.2 (Ph), 127.5 (Ph), 103.5 (C5). MS (ESI): *m/z* = 210.9; 211.8 ([M]$^+$,100%; [M + 1]$^+$, 33%).

2.1.2.6. 4-(4-bromophenyl)thiazol-2-amine hydrobromide (4f)

White solid; Yield US: 75% mp 226-227ºC. ^{1}H NMR (400 MHz, MeOD): δ = 7.71-7.65 (m, 2H, Ph), 7.61-7.56 (m, 2H, Ph), 7.13 (s, 1H, H5). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 168.27 (C2), 148.58 (C4), 134.05 (Ph), 131.27 (Ph), 127.48 (Ph), 120.01 (Ph), 102.32 (C5). MS (ESI): *m/z* = 256.8; 254.9 ([M]+, 100%; [M + 2]$^+$, 98%).

2.1.2.7. 4-phenylthiazol-2-amine hydrobromide (4g)

White solid; Yield US: 82% mp 220-222ºC. ^{1}H NMR (400 MHz, acetone-*d6*): δ = 7.86 (d, *J* = 7.2 Hz, 2H, Ph),

7.35 (dd, *J* = 7.6, 7.6 Hz, 2H, Ph), 7.25 (t, *J* = 7.3 Hz, 1H, H4'), 6.93 (s, 1H, H5), 6.41 (sl, 2H, N$\underline{H}_2$). ^{13}C NMR (100 MHz, acetone-*d6*): δ = 169.0 (C2), 151.7 (C4), 136.2 (Ph), 129.2 (Ph), 128.0 (Ph), 126.6 (Ph), 102.4 (C5). MS (ESI): *m/z* = 177.0 ([M + H]$^+$, 100%).

2.1.2.8. 4-(4-methoxyphenyl)-1,3-selenazol-2-amine hydrobromide (5a)

Salmon solid; Yield US: 58%; mp 229-231ºC. ^{1}H NMR (400 MHz, MeOD): δ = 7.56 (d, *J* = 8.9 Hz, 2H, Ph), 7.22 (s, 1H, H5), 7.04 (d, *J* = 8.9 Hz, 2H, Ph), 3.85 (s, 3H, OC$\underline{H}_3$). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 174.7 (C2), 160.4 (C4′), 138.5 (C4), 127.81 (Ph), 122.7 (Ph), 114.9 (C3′), 104.7 (C5), 55.8 (OCH3) MS (ESI): *m/z* = 254.9 ([M+H]$^+$, 100%).

2.1.2.9. 4-(4-fluorophenyl)-1,3-selenazol-2-amine hydrobromide (5b)

Pale yellow solid; Yield US: 75%; mp 236-238ºC. ^{1}H NMR (400 MHz, acetone-*d6*): δ = 7.92-7.86 (m, 2H, Ph), 7.42 (s, 1H, H5), 7.09 (t, *J* = 8.9 Hz, 2H, Ph), 6.71 (sl, 2H, N$\underline{H}_2$). ^{13}C NMR (100 MHz, acetone-*d6*): δ = 171.5 (C2), 162.2 (d, J = 244.0 Hz, C4′), 150.1 (C4), 132.7 (d, J = 3.0 Hz, C1′), 127.7 (d, J = 8.0 Hz, C2′), 114.6 (d, J = 22 Hz, C3′), 105.0 (d, J = 2.0 Hz, C5). MS (ESI): *m/z* = 243.0 ([M+H]$^+$, 100%).

2.1.2.10. 4-(p-tolyl)-1,3-selenazol-2-amine hydrobromide (5c)

Brown solid; Yield US: 70%; mp 285-288ºC. ^{1}H NMR (400 MHz, acetone-*d6*): δ = 7.76-7.72 (m, 2H, Ph), 7.37 (s, 1H, H5), 7.17-7.12 (m, 2H, Ph), 6.65 (sl, 2H, N$\underline{H}_2$), 2.31 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 169.1 (C2), 150.7 (C4), 136.0 (Ph), 133.1 (Ph), 128.9 (Ph), 125.7 (Ph), 104.8 (C5), 20.7 (CH3). MS (ESI): *m/z* = 238.9 ([M+H]$^+$, 100%).

2.1.2.11. 4-(4-nitrophenyl)-1,3-selenazol-2-amine hydrobromide (5d)

Yellow solid; Yield US: 78%; mp 236-239ºC. ^{1}H NMR (400 MHz, MeOD): δ = 8.36 (d, *J* = 8.9 Hz, 2H, H3'), 7.89 (d, *J* = 8.9 Hz, 2H, H2'), 7.70 (s, 1H, H5). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 170.2 (C2), 147.6 (C4), 145.8 (C4′), 141.0 (C1′), 126.6 (Ph), 123.9 (Ph), 111.4 (C5). MS (ESI): *m/z* = 269.9 ([M+H]$^+$, 100%).

2.1.2.12. 4-(4-chlorophenyl)-1,3-selenazol-2-amine hydrobromide (5e)

Salmon solid; Yield US: 98%; mp 237-239ºC. ^{1}H NMR (400 MHz, acetone-*d6*): δ = 7.88 (s, 2H, Ph), 7.51 (s, 1H, H5), 7.36 (d, *J* = 8.6 Hz, 2H, Ph), 6.73 (sl, 2H, N$\underline{H}_2$). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 170.0 (C2), 149.9 (C4), 135.0 (Ph), 131.7 (Ph), 128.8 (Ph), 128.0 (Ph), 107.1 (C5). MS (ESI): *m/z* = 258.8 ([M+H]$^+$, 100%).

2.1.2.13. 4-(4-bromophenyl)-1,3-selenazol-2-amine hydrobromide (5f)

Pale brown solid; Yield US: 97%; mp 223-226ºC. ^{1}H NMR (400 MHz, MeOD): δ = 7.67 (d, *J* = 8.6 Hz, 2H, Ph), 7.56 (d, *J* = 8.6 Hz, 2H, Ph), 7.44 (s, 1H, H5). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 174.1 (C2), 137.9 (C4), 131.9 (Ph), 129.2 (Ph), 127.9 (Ph), 122.2 (Ph), 107.5 (C5). MS (ESI): *m/z* = 232.8 ([M+H]$^+$, 100%).

2.1.2.14. 4-phenyl-1,3-selenazol-2-amine hydrobromide (5g)

Salmon solid; Yield US: 73%; mp 220-221ºC. ^{1}H NMR (400 MHz, MeOD): δ = 7.63 (dd, *J* = 7.8, 1.8 Hz, 2H, Ph), 7.51-7.47 (m, 3H, Ph), 7.39 (s, 1H, H5). ^{13}C NMR (125 MHz, DMSO-*d6*): δ = 169.4 (C2), 149.4 (C4), 134.5 (C1′), 131.2 (Ph), 128.3 (Ph), 127.5 (Ph), 106.6 (C5). MS (ESI): *m/z* = 224.0 ([M + H]$^+$, 100%).

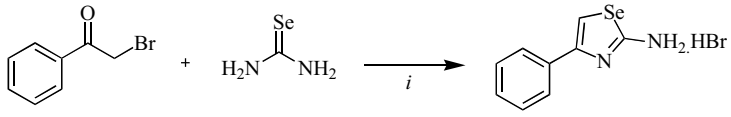
2.2. Biology

The antimycobacterial activity of both 2-aminoselenazoles and 2-aminothiazoles was assessed against *M. tuberculosis* ATTC 27294 using the Micro plate Alamar Blue Assay (MABA) [20]. This is a nontoxic methodology, which uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods [21, 22]. For this procedure, 200 mL of sterile deionized water was added to all outer-perimeter wells of 96 sterile well plates (falcon, 3072: Becton Dickinson, Lincoln Park, NJ) to minimize evaporation of the medium in the test wells during incubation. 96 plates received 100 mL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and successive dilutions of the compounds **4** and **5** were made directly on the plate. The final drug concentrations tested were 0.01 to 20.0 μg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for five days. 25 mL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% tween 80 were then added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The Minimal Inhibitory Concentration (MIC) was defined as the lowest drug concentration, which prevented a color change from blue to pink. MIC values represent means of three separate experiments and the variation coefficient of the method is 9.8 %.

Cytotoxicity assays were performed in murine peritoneal macrophages and the viability of cells was determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT - Sigma Chemical Co., St. Louis, MO, USA) method [23]. Peritoneal macrophages were obtained from BALB/c mice previously inoculated with 3% thioglycollate medium (Sigma Chemical Co., St. Louis, MO, USA). The macrophages were used for the cytotoxicity assay in a concentration of $2x10^6$ cells/mL ($2x10^5$cells/well) in 96-well culture plates in RPMI 1640 medium supplemented with 10% inactivated fetal bovine serum, at 33°C and 5% CO_2 atmosphere. After 24 hours, the adherent macrophages were incubated with several concentrations of the compounds for 72 hours, at 37°C in 5% CO_2. Then, the cells were incubated with 10 μL of a 5mg/ml solution of MTT during two hours at 37°C in 5% CO_2. The reaction was stopped by adding 100 μL of acid isopropanol and the absorbance was measured at 570 nm (Multiskan MS microplate reader, LabSystems Oy, Helsink, Finland). All procedures performed in this assay were approved by the Ethics Committee for Animal Handling (013/2015-CEUA) of the Federal University of Juiz de Fora.

Table 1. Ultrasound synthesis of 4-phenyl-1,3-selenazol-2-amine hydrobromide.

Methodology	Total Time (Min)	Number of Pulses	Potency (% MAX)	Isolated Yield (%)
I	20	-	-	41
II	15	1	20%	33
III	10	1	50%	72
IV	5	1	50%	60
V	10	1	90%	70
VI	6	1	90%	58
VII	6	3	90%	56



i: Various Solvents, r.t.

4-phenyl-1,3-selenazol-2-amine hydrobromide

Solvent	Yield (%)	Polarity Index	Reaction Time
H_2O	82	9.0	50 min
DMF	95	6.4	Instant
MeCN	96	5.8	Instant
MeOH	78	5.2	< 1 min
EtOH	74	5.1	< 1 min
CH_2Cl_2	80	3.1	5 min

Scheme 1. Synthesis of 4-phenyl-1,3-selenazol-2-amine hydrobromide in various solvents.

3. RESULTS AND DISCUSSION

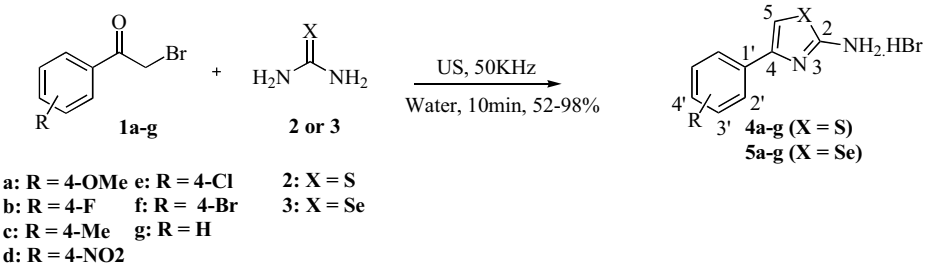
In our continuous attempts on the synthesis of pharmacologically important heterocycles, we have been studying the aspects regarding the synthesis of 2-amino-1,3-selenazoles from selenourea and α-bromoketones [19]. In the present work, we report the formation of this core in some common solvents under catalyst-free condition at room temperature. It should be mentioned that we were inspired by a similar work published in 2011 by Srinivasan and co-workers regarding the synthesis of 2-aminothiazoles [24]. In their paper, authors established that thiazole formation is fast at room temperature when polar solvents are used.

It is noteworthy that 1,3-selenazole synthesis seems to follow the same pattern described previously for their thiazole counterparts, albeit a bit faster in some cases. Instant reactions and high yields were observed in the formation of 4-phenyl-1,3-selenazol-2-amine hydrobromide under polar aprotic solvents with high polarity index such as DMF and acetonitrile. Moreover, the reaction was very fast in all other organic solvents (Scheme **1**). On the other hand, reaction did not proceed as smoothly in water due to solubility issues; even so, good yields were obtained after 50 minutes (Scheme **1**).

In 2007, Narender and co-workers reported the synthesis of 1,3-selenazoles in water using supramolecular catalysts such as *β*-cyclodextrin, in an elegant method to avoid the reagents' solubility issue [13]. Therefore, we decided to continue our studies with the goal of carrying out this reaction in water, in shorter times without using any catalyst. In this context, ultrasound reactors are interesting tools to increase reaction rates, mainly due to the cavitation effect. Reaction between selenourea and 2-bromo-1-phenylethanone (1:1.2), as in Scheme **1**, was carried out in various sonication conditions in order to establish the best methodology for this procedure and results are summarized in Table **1**. This reaction was also carried out under conventional heating (80ºC) (methodology **I**) to evaluate the importance of sonication for this synthesis. It is noteworthy that yields increased up to 1.8-fold, lower reaction times were needed when compared to conventional heating, and 50% of the maximum potency (375W) seemed to be optimal for this reaction. Moreover, splitting the total reaction time into shorter pulses did not affect reaction yields when comparing **VI** and **VII**.

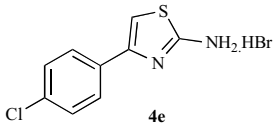
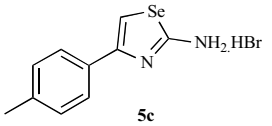
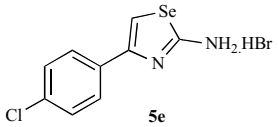
All compounds described in Scheme **2**, including the 2-amino-thiazoles **4a-g**, were synthesized *via* ultrasound in water, using methodology **III**, in good to excellent yields and their structures were analyzed by ^{1}H, ^{13}C NMR and ESI-MS analysis.

All synthesized compounds were assessed *in vitro* against *M. tuberculosis* ATCC 27294 strain. Compounds **4e**, **5c** and **5e** displayed moderate activity in the micromolar range (Table **2**), while the other compounds did not inhibit growth in the tested concentrations. It is interesting to notice that



Scheme 2. Synthesis of 2-aminothiazoles and 2-aminoselenazoles.

Table 2. MIC value for compounds 4e, 5c and 5e.

Compound	mLog P	MIC (ATCC 27294)
 **4e**	2.58	476 μM
 **5c**	2.35	210 μM
 **5e**	2.83	389 μM
Rifampicin	-	1.2 μM

these small intermediates exhibited lower MIC values, being 1.2 (**5e**) and 2.2 (**5c**) fold more potent than the sulfur derivative **4e**. Furthermore, active compounds did not seem to be cytotoxic.

Methyl and chloro substituents in the phenyl moiety of the structures seem to be important for the activity of this series. Moreover, a mLog P ranging from around 2.35 to 2.83 might be also playing a role in the biological activity, as mLog P of all inactive compounds, except for compound **5f**, fall outside of these boundaries.

Clearly, the assessed compounds are not as potent *in vitro* as the first line drug rifampicin, however, it should be noted that the vital first line drug pyrazinamide displays a pH dependent *in vitro* MIC ranging from 41 μM up to 325.2 μM when pH varies from 5.5 to 5.95 [25]. Moreover, these 2-aminoselenazoles did not show any cytotoxicity. Selenazoles could be easily incorporated into drug-like structures, and might have a new mechanism of action, which would be desirable in order to avoid resistance. Our research group is carrying out further studies in order to establish a possible mechanism of action for these structures.

CONCLUSION

In summary, we have established that 2-aminoselenazole Hantzsch's synthesis is highly dependent on the solubility of the reagents and on the polar index of the solvents. We have attempted to carry out this reaction in water without catalysts, but the reaction was slow under conventional heating. On the other hand, fast reactions and good yields were obtained under sonication.

The biological activity of selenazoles against *M. tuberculosis* was assessed for the first time. These small molecules were capable of inhibiting growth in a micromolar range and remarkably, selenazoles were up to 2.2 fold more potent than the thiazoles analogues in the tested conditions. It is noteworthy that selenazoles may become important structures in the search for new prototypes against TB.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in this assay were approved by the Ethics Committee for Animal Handling (013/2015-CEUA) of the Federal University of Juiz de Fora.

HUMAN AND ANIMAL RIGHTS

No humans were used in this research. All animal research procedures followed were in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors thank Farmanguinhos, INI, Universidade Federal de Juiz de Fora and FAPERJ for the financial support of the research. Fellowships granted to A.C.S.N., E.S.C., M.V.N.S. and T.R.A.V. by CNPq. Fellowships granted to S.L.C. and J.T.C. by CAPES.

REFERENCES

[1] Wessjohann, L.A.; Schneider, A.; Abbas, M.; Brandt, W. Selenium in chemistry and biochemistry in comparison to sulfur. *Biol. Chem*., **2007**, *388*, 997-1006.

[2] Flohé, L. The selenoprotein glutathione peroxidase. In: *Glutathione: Chemical, Biochemical and Medical Aspects - Part A*. Dolphin, D.; Poulson, R.; Avramovic, O.J.; Eds. Wiley Online Library, **1989**, pp. 643-731.

[3] Knekt, P.; Aromaa, A, Maatela, J.; Alfthan, G., Aaran, R.K.; Hakama, M.; Hakulinen, T.; Peto, R.; Teppo, L. Serum selenium and subsequent risk of cancer among Finnish men and women. *J. Natl. Cancer Inst.*, **1990**, *82*, 864-868.

[4] Virtamo, J.; Huttunen, J.K. Micronutrients and cardiovascular disease. In: *Trace Elements in Health and Disease*. Aitio, A.; Aro, A.; Järvisalo, J.; Vainio, H., Eds. Cambridge UK: The Royal Society of Chemistry, **1991**, pp. 127-140.

[5] Willett, W.C.; Stampfer, M.J.; Hunter, D.; Colditz, G.A. The epidemiology of selenium and human cancer. In: *Trace Elements in Health and Disease*. Aitio, A; Aro A; Järvisalo, J; Vainio, H., Eds. Cambridge, UK: The Royal Society of Chemistry **1991**, pp. 141-155.

[6] Kok, F.J.; van Poppel, G.; Melse, J.; Verheul, E.; Schouten, E.G.; Kruyssen, D.H.C.M.; Hofman, A. Do antioxidants and polyunsaturated fatty acids have a combined association with coronary atherosclerosis? *Atherosclerosis*, **1991**, *31*, 85-90.

[7] Rayman, M.P. The importance of selenium in human health. *Lancet*, **2000**, *356*, 233-241.

[8] Muller, D.; Desel, H. Acute selenium poisoning by paradise nuts (Lecythis ollaria). *Hum. Exp. Toxicol.*, **2010**, *29*, 431-434.

[9] Klapec, T.; Mandić, M.L.; Grgić, J.; Primorac, L.; Ikić, M.; Lovrić, T.; Grgić, Z.; Herceg, Z. Daily dietary intake of selenium in Eastern Croatia. *Sci. Total Environ.*, **1998**, *217*, 127-136.

[10] Akbaraly, N.T.; Arnaud, J.; Hininger-Favier, I.; Gourlet, V.; Roussel, A.-M.; Berr, C. Selenium and mortality in the elderly: Results from the EVA study. *Clin. Chem.*, **2005**, *51*, 2117-2123.

[11] Wang, L.; Yang, Z.; Fu, J.; Yin, H.; Xiong, K.; Tan, Q.; Jin, H.; Li, J.; Wang, T.; Tang, W.; Yin, J.; Cai, G.; Liu, M.; Kehr, S.; Becker, K.; Zeng, H. Ethaselen: A potent mammalian thioredoxin reductase 1 inhibitor and novel organoselenium anticancer agents. *Free Rad. Biol. Med.*, **2012**, *52*, 898-908.

[12] Facchinetti, V.; Nery, A.; Avellar, M.; Gomes, C.R.B.; De Souza, M.V.N.; Vasconcelos, T.R.A. Highlights on the synthesis and biological activity of 1,3-selenazoles. *Curr. Org. Synth.*, **2015**, *12*(2), 140-149.

[13] Narender, N.; Reddy M.S.; Kumar V.P.; Reddy V.P.; Nageswar, Y.V.D.; Rao K.R. Supramolecular synthesis of selenazoles using selenourea in water in the presence of β-cyclodextrin under atmospheric pressure. *J. Org. Chem.*, **2007**, *72*, 1849-1851.

[14] Madhav, J.V.; Kuarm, B. S.; Rajitha, B. Solid-state synthesis of 1,3-selenazoles employing $CuPy_2Cl_2$ as a lewis acid catalyst. *Synth. Commun.*, **2008**, *38*(20), 3514-3522.

[15] Potewar, T.M.; Ingale, S.A.; Srinivasan K.V. An efficient and ecofriendly synthesis of 2-amino-1,3-selenazoles in an ionic liquid/water system under ambient conditions. *ARKIVOC*, **2008**, *xii*, 117-125.

[16] Zavozin, A.G.; Ignat'ev, N.V.; Schulte, M.; Zlotin S.G. Synthesis of thiazole derivatives bearing an incorporated Z-5-aminopent-3-enoic acid fragment. *Tetrahedron Lett.*, **2013**, *69*(34), 6975-6980.

[17] Das, B.; Reddy, V.S.; Ramu, R. A rapid and high-yielding synthesis of thiazoles and aminothiazoles using ammonium-12-molybdophosphate. *J. Mol. Catal. A: Chem*., **2006**, *252*(1), 235-237.

[18] Kabalka, G.W.; Mereddy, A.R. Microwave promoted synthesis of functionalized 2-aminothiazoles. *Tetrahedron Lett.*, **2006**, *47*, 5171-5172.

[19] Facchinetti, V.; Avellar, M.; Nery, A.; Gomes, C.R.B.; Vasconcelos, T.R.A; De Souza, M.V.N. An eco-friendly, hantzsch-based, solvent-free approach to 2-aminothiazoles and 2-aminoselenazoles. *Synthesis*, **2016**, *48*(3), 437-440.

[20] Franzblau, S.G.; Witzig, R.S.; McLaughlin, J.C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M.T.; Cook, M.B.; Quenzer, V.K.; Ferguson, R.M.; Gilman, R.H. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar blue assay. *J. Clin. Microbiol.*, **1998**, *36*, 362-366.

[21] Vanitha, J.D.; Paramasivan, C.N. Evaluation of microplate Alamar blue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. *Diagn. Microbiol. Infect. Dis.*, **2004**, *49*, 179-182.

[22] Reis, R.S.; Neves, Jr. I.; Lourenço, S.L.S.; Fonseca, L.S.; Lourenço, M.C.S. Comparison of flow cytometric and Alamar blue tests with the proportional method for testing susceptibility of *Mycobacterium tuberculosis* to rifampin and isoniazid. *J. Clin. Microbiol.*, **2004**, *42*, 2247-2248.

[23] Mossman, N. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity. *J. Immunol. Meth.*, **1988**, *65*, 55-63.

[24] Dighe, S.N.; Chaskar, P.K.; Jain, K.S.; Phoujdar, M.S.; Srinivasan, K.V. A remarkably high-speed solution-phase combinatorial synthesis of 2-substituted-amino-4-aryl thiazoles in polar solvents in the absence of a catalyst under ambient conditions and study of their antimicrobial activities. *ISRN Org. Chem.*, **2011**, 2011, 434613.

[25] Salfinger M.; Heifets L.B. Determination of pyrazinamide MICs for *Mycobacterium tuberculosis* at different pHs by the radiometric method. *Antimicrob. Agents Chemother.*, **1988**, *32*(7), 1002-1004.