SYNTHESIS AND ANTHELMINTIC EVALUATION OF [2,5']-BIS-HETEROCYCLES AS BENGAZOLE ANALOGS

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ABSTRACT

As part of our search for compounds as candidates for anticancer or antiparasitic drugs employing molecular simplification, we reported the preparation of uncommon [2,5'] bis-heterocycles employing efficient synthetic strategies. The synthesized compounds presents little modifications of the Bengazole heterocyclic fragment in order to preserve the biological properties to been employed in Bengazole derivatives analogs preparation. A limitation in the oxidation of 2-benzyl-oxazolines is described. We also presents the anthelmintic activities of these synthetized compounds.

Keywords: Bengazole, [2,5']bithiazole, [2,5']bis-heterocycle, Hantzsch, anthelmintic activity.

RESUMEN

Como parte de la búsqueda de compuestos candidatos a fármacos de uso contra el cáncer o parásitos empleando como estrategia la simplificación molecular, presentamos la preparación de los sistemas poco comunes [2,5'] bis-heterocíclicos, empleando estrategias sintéticas eficientes. Los compuestos sintetizados presentan en sus estructuras modificaciones simples del fragmento bis-heterocíclico de los Bengazoles con el fin de preservar las propiedades biológicas para ser empleados en la síntesis de análogos a derivados de Bengazoles. Se describe la limitante de oxidar oxazolinas que presenten sustituyentes de tipo bencílico en posición 2 del heterociclo. También presentamos los resultados obtenidos del ensayo de actividad antihelmíntica sobre los compuestos sintetizados.

Palabras claves: Bengazole, [2,5']bitiazol, [2,5']bis-heterociclo, Hantzsch, actividad antihelmintica.

INTRODUCTION

Natural products play an important role in drug development particularly in anticancer, antibiotics and antiparasitic drugs (Newman and Cragg, 2012). Its structural diversity is a source of inspiration for drug discovery and the preparation of analogs as simplified, synthetically more accessible and stable models are broadly described in the literature (Molinski et al., 2009; Njardarson et al., 2004).
Bis-1,3-azole scaffolds linked by different chain length and connectivity points between the rings, are present in numerous natural products with interesting biological activities (Davyt and Serra, 2010; Jin, 2006; Yeh, 2004). Representative examples include Cystothiazole A, with a [2,4'] bithiazole system (Ojika et al., 1998); Bengazoles containing an uncommon [2,5'] bioxazole (Adamaczeski et al., 1988; Rodriguez et al., 1993; Rudi et al., 1994); Leucamide A a cyclic heptapeptide with a [2,4'] oxazole-thiazole system (Kehraus et al., 2002); Largazole a depsipeptide containing a [2,4'] thiazoline-thiazole system (Taori et al., 2008); and cyclic peptides containing 1,3-azoles as Venturamide A (Linington et al., 2007).

The most common moiety is the [2,4'] bis-1,3-azole, consistent with the biogenesis of these heterocycles which are derived from Ser, Thr or Cys peptide by cyclodehydration and oxidation process (Riego et al., 2005). There are many synthetic methods reported for the preparation of (2,4-disubstituted) oxazoles (Taylor and Wipf, 2003). On the other hand, there are few methods to prepare (2,5-disubstituted) oxazoles. The most efficient methodology was developed by Schöllkopf (Schöllkopf and Gerhart, 1968; Schöllkopf and Schöder, 1971), starting from isocyanides, and then the use of TosMIC (tosylmethylisocyanide) reagent was pioneered by van Leusen (van Lausen et al., 1972).

Bengazoles are a representative family of natural products containing the uncommon [2,5'] bioxazole, so several academic groups have been involved in the synthesis of some members. Molinski (Mulder et al., 1999) and Shiioiri’s group (Chittari et al., 2003) reported the synthesis of bis-1,3-oxazole of Bengazole A and Deacylbengazole respectively with an optimized modification of Vedejs methods (Vedejs and Monahan, 1996) from 5-oxazolecarboxaldehyde using Schöllkopf methodology. Ley and coworkers (Bull et al., 2007) had explored different routes to completed the total synthesis of Bengazole A and B and the best result was obtained employing TosMIC and ethyl glyoxylate.

As part of our search for compounds as candidates for anticancer or antiparasitic drugs employing molecular simplification (Scarone et al., 2004; Sellanes et al., 2006; Mahler et al., 2006; Sellanes et al., 2007; Incerti et al., 2008; Peña et al., 2011), we reported our results on the synthesis of [2,4'] and [2,5'] bis-heterocycles with an ethylene bridge between the rings as scaffolds for Bengazole analogs using Robison-Gabriel, van Lausen and cyclodehydration-oxidation reactions (Scarone et al., 2009).

(Figure 1)
Bengazoles 1-10 were found to be very active against *Candida albicans*, with MIC values from 0.8 to 1.5 \( \mu g/mL \), and the hydrophilic derivative bengazole 11 (no esterification with a fatty acid) was inactive in these assays (Fernández et al., 1999). Bengazoles have been evaluated for their cytotoxicity in the NCI’s 60 cell lines screen and Bengazole A has shown in vitro potency against two human tumor cell lines. In contrast, Bengazole Z (11, figure 1) was inactive. Furthermore, Bengazole A shows complete anthelmintic activity at a 50 \( \mu g/mL \) against *Nippostrongylus brasiliensis* (Adamczeski et al., 1988; Rodriguez et al., 1993).

In the present work, we report our investigations to the synthesis of [2,5’]-bis-heterocycles of type 1 with a methylene between the rings. (Scheme 1) The bioisosterism between thiazole and oxazole rings and the use of a straightforward synthetic route employing commercially available reagents as starting materials were considered. Due to our interest in antiparasitic drugs, we present an *in vitro* preliminary screening of the effect on the L₄ larvae of *Nippostrongylus brasiliensis* of these bis-heterocycles and intermediates.

**MATERIAL AND METHODS**

IR spectra were recorded on a Shimadzu FTIR 8101A spectrophotometer. \(^1^H NMR\) and \(^{13}^C NMR\) spectra were recorded on Bruker Avance DPX-400. Chemical shifts are related to TMS as an internal standard. Mass spectra (EIMS) were obtained on a GCMS Shimadzu QP-2010 plus. Flash column chromatography was carried out with Silica gel 60 (J.T. Baker, 40 \( \mu \)m average particle diameter). All reactions and chromatographic separations were monitored by TLC, conducted on 0.25 mm Silica gel plastic sheets (Macherey/Nagel, Polygram SIL G/UV 254). TLC plates were analyzed under 254 nm UV light, iodine vapor, p-hydroxybenzaldehyde spray or ninhydrine spray. Yields are reported for chromatographically and spectroscopically (*\(^1^H\) and \(^{13}^C\) NMR) pure compounds.

**Ethyl 2-(2,4-dimethylthiazol-5-yl) acetate (8):** Ethyl levulinate (7.0 mmoles) in diethyl ether (2 ml) was cooled to 0ºC and bromine (0.18 ml, 3.5 mmoles) was added dropwise with stirring. The reaction mixture was stirred at room temperature overnight, washed with water (4x 5ml) and dried.

**Scheme 1:** Retrosynthetic analysis
with Na₂SO₄. Evaporation in vacuo of the diethyl ether gave α-bromoketones (5 and 6) in 1:1 mixture by ¹H NMR spectroscopy. The α-bromoketones were dissolved in dry EtOH (4 ml) and thioacetamide (7.0 mmoles) was added. The mixture was refluxed for 2 h, after cooling to room temperature the EtOH was evaporated under reduced pressure. The residue was dissolved in EtOAc (10 ml) and extracted with water (10 ml). The organic layer was dried with Na₂SO₄, filtered and concentrated in vacuo.

Flash chromatography (AcOEt: n-hexane, 1:4) afforded 8 in 50 % yield. Rf = 0.42. ¹H NMR (400 MHz, CDCl₃) δ 1.27 (t, J = 7.1 Hz, 3H), 2.32 (s, 3H), 2.63 (s, 3H), 3.69 (s, 2H), 4.18 (q, J = 7.8, 8.8 Hz, 1H), 4.54 (dd, J = 7.8, 8.8 Hz, 1H), 4.77 (dd, J = 7.8, 10.6 Hz, 1H), 6.51 (d, J = 6.1 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 19.1, 33.8, 52.9, 54.8, 63.1, 122.0, 150.0, 164.3, 169.6, 170.6. EIMS (70 eV), m/z (%) 272 (M⁺, 14), 153 (24), 127 (100), 86 (31), 61 (61). IR: v max/cm⁻¹ (liquid film): 1074, 1207, 1369, 1544, 1647, 1743, 2945, 2953, 3288.

Methyl 2-(2,4-dimethylthiazol-5-yl) methyl]oxazoline-4-carboxylate (11): A solution of β-hydroxyamide 10 (0.37 mmol) in dry CH₂Cl₂ (4 mL) at -78 °C under N₂, DAST (0.05 mL, 0.40 mmol) was added dropwise. After stirring for an hour, the reaction mixture was quenched with K₂CO₃ (0.17 g, 1.23 mmol) at -20 °C. After warming to room temperature, the mixture was further diluted with saturated aqueous solution of Na₂CO₃ and then extracted with CH₂Cl₂ (4x 20 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated in vacuo. Flash chromatography (EtOAc: MeOH, 4:0.5) afforded thiazole-oxazole (11) in 96% yield. Rf = 0.44. ¹H NMR (400 MHz, CDCl₃) δ 2.36 (s, 3H), 2.63 (s, 3H), 3.75 (d, J = 6.2 Hz, 2H), 3.81 (s, 3H), 4.45 (dd, J = 8.8, 10.6 Hz, 1H), 4.54 (dd, J = 7.8, 8.8 Hz, 1H), 4.77 (dd, J = 7.8, 10.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 19.1, 25.6, 52.7, 68.0, 70.0, 122.3, 149.6, 163.7, 171.4, 176.1, 196.0.

Methyl 2-(2,4-dimethylthiazol-5-yl) methyl]oxazoline-4-carboxylate (1a): Oxazoline 11 (0.37 mmol) was dissolved in dry CH₂Cl₂ (2 mL). The reaction mixture was cooled at -20 °C and BrCCl₃ (1.4 mmol, 3.5 eq.) was slowly added. Then it was allowed to reach 0 °C and DBU (1.4 mmol, 3.5 eq.) was slowly dripped. The reaction mixture stirred at room temperature overnight. Then it was quenched with a saturated solution of NaHCO₃ and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Flash chromatography (AcOEt) afforded thiazolet oxazole 1 in 32% yield. Rf = 0.56 (AcOEt). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 2.39 (s, 3H), 2.63 (s, 3H),
3.93 (s, 3H), 4.24 (s, 2H), 8.18 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ (ppm): 14.9, 19.1, 25.5, 52.3, 122.4, 133.3, 144.3, 149.6, 161.5, 162.4, 163.9. EIMS (70 eV), m/z (%) 252 (M+, 100), 220 (27), 192 (48), 126 (74), 112 (34), 85 (39). IR $\nu_{\text{max}}$/cm$^{-1}$ (liquid film): 1109, 1139, 1199, 1321, 1437, 1585, 1740, 2853, 2928.

2-(2,4-dimethylthiazol-5-yl)acetamide (12): To a solution of acid 9 (4.6 mmol) in dry THF (15 mL) was added DIPEA (5.6 mmol) under N$_2$ at 0 ºC. The reaction mixture was stirred at 0 ºC for 30 minutes before 2,2,2-trichloroethyl chloroformate (5.6 mmol) was added rapidly and continued stirring for 30 minutes. Then aqueous NH$_3$ solution (1.7 mL) in THF (1.7 mL) was added. The resulting reaction mixture was allowed to reach room temperature and stirred for additionally 16 h. The reaction mixture was concentrated in vacuo and the resulting residue was partitioned between EtOAc (40 mL) and H$_2$O (40 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL) and the combined organic layers were washed with saturated aqueous NaHCO$_3$ solution (30 mL), water (30 mL), brine (30 mL); dried with Na$_2$SO$_4$ and concentrated in vacuo. Flash chromatography (EtOAc: n-hexane, 3:1) afforded amide 12 in 53% yield. $^{1}$H NMR (400 MHz, CDCl$_3$) δ (ppm): 2.36 (s, 3H), 2.67 (s, 3H), 3.67 (s, 2H), 5.55 (bs, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.9, 19.1, 33.4, 122.6, 150.0, 164.3, 171.4.

Methyl 2-((2,4-dimethylthiazol-5-yl)methyl)thiazole-4-carboxylate (1b): Lawesson’s reagent (2.24 mmol) was added to a solution of amide 12 (1.4 mmol) in dry THF (15 mL) and the reaction mixture was stirred under N$_2$ atmosphere at room temperature for 24 h. The reaction was quenched by addition of saturated aqueous NaHCO$_3$ and was stirred for 1 h before been extracted with EtOAc (3 x 15 mL). The organic layers were washed with brine (20 mL); dried with Na$_2$SO$_4$ and the solvent evaporated under reduced pressure. Flash chromatography (EtOAc) afforded thioamide 13 in 89% yield. A solution of ethyl bromopiruvate (2.1 mmol), thioacetamide 13 (1.1 mmol) and pyridine (3.2 mmoles) in dry EtOH (4 mL) under N$_2$, was refluxed during 6 hours. Then, the reaction mixture was concentrated under vacuo and HCl aq. (sol. 5%) was added until pH 4. The aqueous layer was extracted with Et$_2$O (4 x 20 mL), and the combined organic layers were dried over Na$_2$SO$_4$, filtered and concentrated under vacuo. Flash chromatography (AcOEt: n-hexane, 3:1) afforded bithiazole 1 in 41% yield. $R_f$ = 0.52. $^{1}$H NMR (400 MHz, CDCl$_3$) δ (ppm): 1.43 (t, J= 7.1 Hz, 3H), 2.39 (s, 3H), 2.67 (s, 3H), 4.45 (q, J= 7.1 Hz, 2H), 4.47 (s, 2H), 8.10 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ (ppm): 14.4, 15.0, 19.2, 30.4, 61.6, 125.4, 127.9, 147.1, 149.8, 161.6, 164.4, 170.6. EIMS (70 eV), m/z (%) 282 (M+, 81), 236 (17), 208 (100), 167 (6), 126 (25), 85 (21). IR $\nu_{\text{max}}$/cm$^{-1}$ (liquid film): 1093, 1205, 1240, 1319, 1477, 1719, 1719, 2924, 2980.

Anthemintic Assay: Animal protocol was in conformity with Uruguayan Law No. 18611 (http://www.presidencia.gub.uy/web/leyes/2009/EC1395.pdf) and harmonized with The Canadian Guidelines on Animal Care. The experimental protocol of the study was reviewed and approved by IACUC of Facultad de Química - Universidad de la República, Uruguay, under approval number 06-05-09 (http://www.chea.udelar.edu.uy).

Parasite specimens of *N. brasiliensis* L4 were obtained from rat small intestines as per the procedure described previously for the *in vitro* model of anthelmintic activity developed by our team (Gordon et al., 1997). Briefly, Wistar rats were infected subcutaneously with 5000 L3 larvae of *N. brasiliensis* and euthanized by cervical dislocation at 72 h post-infection. L4 parasites were recovered from intestines, washed, and kept in a 24-well tissue culture plate (disposable plates of 24 wells x 2 mL, pfs SIGMA) containing 1.8 mL of culture medium and 50 L4s suspended in 0.2 mL of medium per well. Samples in each well
were dissolved and diluted to the desired final concentration by the addition of 10 µL of DMSO. Controls with and without the addition of DMSO were also included. Plates were incubated at 37°C and the number of dead parasites read under an inverted microscope (Nikon TS 100) on day five. Readings were corrected against DMSO controls (corrections amounting to less than 15%). Results were analyzed by ANOVA and Tukey’s post-run test (p < 0.05). The mean effective concentration (EC_{50})—corresponding to 50% of dead parasites—was calculated for each product by the probit method at a confidence level of 95% by means of Prism GraphPad 5.00 software (2008, San Diego, USA), and later confirmed experimentally.

RESULTS AND DISCUSSION

Synthesis of ring A: Thiazole ring is a very important scaffold in medicinal chemistry, so several methods were developed for the synthesis of this ring by Hantzsch, Cook-Heilbron, Gabriel among others (Zagade and Senthilkumar, 2011). However, the most widely used and relied method for the preparation of 2,4-disubstituted thiazoles is Hantzsch’s synthesis.

Thiazoles have been used previously in our group as building blocks to the synthesis of natural products analogs (Peña et al., 2011; Peña et al., 2012). Now, we decided to explore the Hantzsch’s reaction for the synthesis of 2,5-disubstituted thiazoles. Overend protocol (Overend et al., 1950) was employed to prepare both α-bromoketones (5 and 6) which were obtained as a 1:1 mixture. These compounds were used, without further purification, in Hantzsch’s reactions with thioacetamide. Thiazoles 7 and 8 were obtained in excellent yield (1:1 relationship). (Scheme 2) Then, thiazoles were purified by flash chromatography and we continue our synthetic route employing thiazole 8.

Synthesis of ring B: The ethyl ester hy-
Thiolytic cleavage of thiazole 8 afforded the carboxylic acid 9 as reagent to amide bond formation with the L-serine methyl ester hydrochloride employing N,N-dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt) as coupling reagents. β-hydroxyamide 10 was obtained in 54% yield. (Scheme 3) It is important to highlight that if the triethylamine (Et3N) : L-serine methyl ester hydrochloride relationship (1:1) is increased the reaction results in a mixture of inseparable products.

Next step involved the cyclodehydration of 10 using DAST to afford oxazoline 11 in excellent yield. Then, we oxidized oxazoline 11 using Williams’ protocol (Williams et al., 1997) to obtain oxazole 1a in low 32% yield.

A bibliographic revision was carried out in order to explain the low yield for oxazoline oxidation. Ley’s group reported 47% as the best yield for oxazoline oxidation in the total synthesis of Bengazoles A and B. Xi’s group (Xi et al., 2005) reported yield between 31-46% for the oxidation reactions of 2-benzyl-oxazolines. On the other hand, Cossu and co-workers (Cossu et al., 1994) reported an unusual reactivity of 4-carboxyamido-2-benzyl-oxazolines in the aim to obtain the 4-nitrile derivatives and assumed the presence of an equilibrium between two species with the presence of a hydrogen bond, probably due to the presence of exocyclic protons in position 2 of the heterocycle. (Scheme 4)

In the case of oxazoline 11 (I, scheme...
5), we propose an equilibrium with the oxazolidine II, stabilized by an hydrogen bond and consequently the desired oxazole synthesis proceed in poor yield.

For the synthesis of the bisthiazole of type 1, we decided to employ the widely used Hantzsch’s methodology. Amide 12 was prepared from acid 9 (Scheme 6), employing 2,2,2-trichloroethyl chloroformiate/aqueous ammonia in moderate yield. Further thionation of amide 12 with Lawesson’s reagent, allowed us to obtain the thioamide 13 in good yield. Then, Hantzsch’s reaction using ethyl bromo pyruvate afforded bis-thiazole 1b.

The anthelmintic effect on the parasitic stage (L₄) of *Nippostrongylus brasiliensis* was evaluated using Gordon protocol (Gordon et al., 1997; Jenkins et al., 1980). The results are summarized in Table 1 which includes the activities of some previously prepared [2, 5’]bis-heterocycles.

![Scheme 5: Species in equilibria](image)

![Scheme 6: Synthesis of ring B to obtain 1b](image)
Table 1: Anthelmintic activities of synthetic derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>MW</th>
<th>LogP</th>
<th>LC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Albendazole</td>
<td>265</td>
<td>2.55</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>Bengazole A</td>
<td>524</td>
<td>2.76</td>
<td>90*</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>272</td>
<td>0.46</td>
<td>3.27 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>252</td>
<td>2.42</td>
<td>69.2 ± 0.6</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>268</td>
<td>3.79</td>
<td>56.6 ± 0.3</td>
</tr>
</tbody>
</table>

*EC100 reported by Jenkins

Even though the overall level of activity was moderate, some remarks can be made. The open intermediate 10 (entry 3) show a 10-fold increase in activity compared with bis-heterocycles type 1. The presence of a thiazole instead an oxazole slight increases the anthelmintic activity if we compared data of entries 4 and 5.

CONCLUSIONS

We have applied a straightforward synthetic method to obtain [2,5'] bi-1,3-azoles linked by a methylene bridge. The synthesis of [2,5'] bis-heterocycle 1a was performed in just 5 steps with 16% overall yield and [2,5'] bis-heterocycle 1b was obtained in 5 step with 21% overall yield.

We proposed an explanation to the low yield in the oxidation of 2-benzyl-oxazolines and thus could be a limitation of this reaction.

Our preliminary evaluation of the anthelmintic activity demonstrated a broad distribution of anthelmintic effects. Insights gained from these studies will serve for further preparations of new analogs of these natural products. These compounds will be usefull for a fragment-based lead discovery (Rees et al., 2004) in order to improve the biological effects in a next-generation series.

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