Asian Journal of Chemistry; Vol. 24, No. 5 (2012), 2173-2176

ASIAN JOURNAL OF CHEMISTRY

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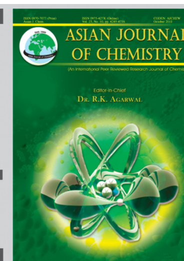

Design and Synthesis of Two Brucine Derivatives and Evaluation of Its Antibacterial Activity Against *Escherichia coli* and *Salmonella typhi*

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(*Received*: 17 May 2011; *Accepted*: 16 December 2011) AJC-10853

In this study two brucine derivatives were synthesized. The first step was achieved by the reaction of brucine with ethylenediamine using boric acid as catalyst to form an imine group (Schiff base) involved in the compound **3**. The second step involves the coupling of ethylenediamine to brucine by the method reported by Mannich, using formaldehyde to form the compound **4**. The antibacterial activity of both brucine derivatives was evaluated *in vitro* on *Escherichia coli* and *Salmonella typhi* using the dilution method and the minimum inhibitory concentration. The results showed that bacterial growth of *Escherichia coli* was inhibited by the compounds **3** and **4** in a dose-dependent manner. Nevertheless, the bacterial growth of *Salmonella typhi* only was blocked by the compound **3**. These data suggest that functional groups involved in the structure of **3** are specific for its antibacterial activity.

Key Words: Brucine derivatives, *Escherichia coli*, *Salmonella typhi*.

INTRODUCTION

Infectious diseases are one of the main causes of morbidity-mortality in the world[1-3]. Several causal agents, such as *S. typhi* and *E. coli*[4,5] among others[6], have been shown to accelerate the progression of infectious diseases. Although there are many therapeutic agents for treatment of these bacterial microorganisms[7,8], unfortunately, prolonged antibiotic therapy can induce bacterial resistance, because some bacteria have developed ways to circumvent the effects of antibiotics[9,10]. For example, several studies indicate that *S. typhiis* not sensitive to chloramphenicol and co-trimoxazole *in vivo*[11]. Additionally, other studies[12] made in Vietnam showed that *S. typhi* may be resistant to quinolones. It is important to mention that also the quinolones and chloramphenicol has development bacterial resistance to other type of bacteria such as *E. coli*.[13,14]. Therefore, antibiotic resistance can be considered a serious threat for the human health; this fact requires an international approach to its management. In this sense, new drugs have been developed for control of bacterial resistance[15,16] for example, several compounds with free amino groups have been developed as potential therapeutic agents for infectious diseases[17-20], which mimic the antibacterial behaviour of some endogenous peptide antibiotics[21]. This task includes selective interaction of the free amino group with some components involved in the bacterial membrane[22], which bring consequently death cell. It is important to mention that also been suggested than membrane selectivity is primarily derived from ionic recognition of negatively charged bacterial membranes[23]. Therefore, in this work the antibacterial activity of two brucine derivatives on *S. typhi* and *E. coli* was evaluated according to NCCLS (now CLSI)[24] with some modifications.

EXPERIMENTAL

Chemical synthesis: Brucine and the other compounds evaluated in this study were purchased from Sigma-Aldrich Co. Ltd. The melting points for the different compounds were determined on an Electrothermal (900 model). Infrared spectra were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in DMSO-d_6 using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GC Polaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

***N*^{1}-(2,3-dimethoxystrychnidin-10-yliden)-ethane-1,2-diamine:** A solution of brucine (70 mg, 0.18 mmol), ethyl-

enediamine (50 mg, 0.50 mmol) and boric acid (31 mg, 0.50 mmol) in 10 mL of methanol was stirring for 48 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 55 % of product, m.p. 144-146 ºC; IR (KBr, ν_{max}, cm^{-1}) : 3380, 1210; ^1H NMR (300 MHz, DMSO-d_6) δ_H : 1.42 (m, 1H), 1.81-1.89 (m, 2H), 2.23-2.37 (m, 2H), 2.67-2.74 (m, 2H), 2.83-3.01 (m, 2H), 3.09 (t, 2H, *J* = 6.5 Hz), 3.14-3.26 (m, 2H), 3.52-3.58 (m, 3H), 3.64 (t, 2H, *J* =6.5 Hz), 3.69 (m, 2H), 3.85 (s, 3H), 3.91 (s, 3H), 4.30 (broad, 2H), 4.70-580 (m, 2H), 5.88 (s, 1H), 7.50 (s, 1H) ppm. ^{13}C NMR (300 MHz, DMSO-d_6) δ_C: 26.68 (C-17), 28.12 (C-7), 29.22 (C-18), 39.96 (C-14), 40.70 (C-27), 45.72 (C-15), 45.80 (C-3), 50.67 (C-11),51.25 (C-26), 52.24 (C-13), 56.20 (C-32), 56.29 (C-30), 59.99 (C-16), 64.67 (C-8), 65.06 (C-4), 79.27 (C-2), 98.33 (C-20), 105.72 (C-23), 127.87 (C-9),129.27 (C-24), 139.02 (C-19), 140.31 (C-10), 143.15 (C-22),147.70 (C-21), 147.80 (C-6), ppm. MS (70 ev): *m/z* 436.18 [M+] 378.54, 188.20; Anal. calcd. for $C_{25}H_{32}N_4O_3$: C, 68.78; H, 7.39; N, 12.83; 0, 10.99. Found: C, 68.74; H, 7.38.

11-[(2-Amino-ethylamino)-methyl]-2,3-dimethoxy-strychnidin-10-one: A solution of brucine (200 mg, 0.50 mmol), ethylenediamine (60 mg, 1.00 mmol), in 10 mL formaldehyde was gently refluxed for 48 h and then cooled to room temperature. After the solvent was removed under vacuum and the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 46 % of product, m.p. 150-152 ºC; IR (KBR, ν_{max}, cm^{-1}) : 3320, 1720; ^1H NMR (300 MHz, DMSO-d_6) δ_H : 1.42-1.44 (m, 2H), 1.52-1.77 (m, 4H), 1.79-1.98 (m, 2H), 2.31 (m, 1H), 2.65 (t, 2H, *J* = 6.5 Hz), 2.73 (m, 1H), 2.85 (t, 2H, *J* = 6.5 Hz), 2.90-2.95 (m, 2H),3.03 (broad, 3H), 3.04-3.23 (m, 3H), 3.43-3.67 (m, 3H), 3.80 (s, 3H), 3.89 (m, 2H), 3.91 (s, 3H), 3.97 (m, 1H), 4.02 (m, 1H), 5.80 (m, 1H), 6.61 (s, 1H), 7.35 (s, 1H) ppm. ^{13}C NMR (300 MHz, DMSO-d_6) δ_C : 26.60 (C-17), 34.46 (C-18), 41.38 (C-29), 42.28 (C-14), 45.51 (C-3), 48.46 (C-26), 48.72 (C-7), 50.67 (C-11), 50.70 (C-13), 51.91 (C-15), 52.01 (C-28), 56.20 (C-34), 56.50 (C-32), 59.99 (C-16), 62.07 (C-8), 63.76 (C-4), 83.06 (C-2), 100.02 (C-20), 106.20 (C-23), 122.94 (C-24), 127.87 (C-9), 135.27 (C-19), 139.98 (C-10), 146.08 (C-22), 148.90 (C-21), 168.10(C-6) ppm. MS (70 ev): m/z 466.16 [M+] 435.54, 322.40, 153.18; Anal. calcd. for $C_{26}H_{34}N_4O_4$: C, 66.93; H, 7.35; N, 12.01; 0, 13.72. Found : C, 66.91; H, 7.38.

Biological evaluation

Strains: The microorganisms in this study belonged to the strain bank at the Department of Pharmaco-Chemistry at the Facultad of Ciencias Químico-Biológicas of the Universidad Autonoma de Campeche. The strains are certified by the center for disease control in Atlanta and were as follows; *S. Typhi* (ATCC 23564) and *E. coli* (ATCC 25922). The strains were kept under refrigeration at 4 ºC for its conservation in a mixture of culture mediums (caseine peptone [2.5 g/L], extract of meat [1.5 g/L] and columbia agar base [4.2 g/L]).

Antimicrobial agents: Brucine derivatives and the other compounds studied were dissolved in methanol and diluted with distilled water. Cefotaxime, gentamycin and ciprofloxacin were used as control drugs.

Antimicrobial activity: The evaluation of antimicrobial activities from the compounds involved in this study was tested in triplicate (three times, n = 9) using the NCCLS[24] method with some modifications. The bacterial species were incubated on McConkey (*E. coli*) and Brain Heart Infusion (*S. Typhi*) agars for 24 h at 37 ºC. After 24 h, it was determined whether growth had taken place or not. In addition, a series of tubes were prepared, the first of which contained 2 mL of culture medium (tripticase soye) at double concentration and the remainder (11 tubes), contained the same quantity of medium at single concentrations. From the first tube (double concentration) an aliquot of 2 mL of the studied compound (1 mg/mL) was added and stirred, from this tube an aliquot of 2 mL was taken and added to the following tube (simple concentration) and the process was successively repeated until the last 2 mL of dissolution had been used up. After this process, each tube was inoculated with 0.1 mL of the bacterial suspension, whose concentration corresponded to McFarland scale (9 × 108 cells/mL) and all the tubes were incubated at 37 ºC for 24 h. Subsequently, a loop was taken from each of them and inoculated into the appropriate cultures for different bacterial organisms and were incubated for 24 h at 37 ºC. After such time, the minimum inhibitory concentration (MIC) was evaluated to consider the antimicrobial effect of all compounds.

In order to discard the effect of methanol (solvent) on the bacterial species studied, a series of the same number of tubes was prepared in parallel, to which 2 mL of methanol at 60 % was added to the first and corresponding successive dilutions were added in the same way as before. In addition a control series was also performed using distilled water at pH 7.0.

Statistical analysis: The obtained values are expressed as mean ± SE. The lineal regression analysis was used.

RESULTS AND DISCUSSION

In this study we report a straightforward route for the synthesis of two brucine derivatives. The first step was achieved by the synthesis of imine group (Schiff base) involved in the compound **3** (Fig. 1). It is important to mention that there are several procedures for the synthesis of imines which are described in the literature[25-27]; despite its wide scope, several method ssuffer from several drawbacks such as specific reagents which are very expensive. Nevertheless, there are reports to synthesis of imine using other type of reagents, for example the synthesis of imines by reaction of 5-*tert*-butyl-3-(5-*tert*-butyl-2-hydroxy-3-vinyl-benzyl)2-hydroxy-benzaldehyde with benzene-1,2-diamine using boric acid as catalyst[28]. Therefore, in this study the synthesis of the compound **3** was developed by the reaction of brucine with ethylenediamine to yield **3**. The results indicate that ^1H NMR spectrum of **3** showed signals at 1.42-2.37, 2.67-301, 3.14-3.58, 3.69 and 4.70-7.50 ppm corresponding to protons presents in the heterocyclic rings. In addition, other signals at 3.09 and 3.64 ppm for methylenes involved in the arm bound to brucine nucleus were found. Other signals at 3.85 and 3.91 ppm for methoxy groups

were shown. Finally, a signal at 4.30 ppm corresponding to amino group was found. The ^{13}C NMR spectra displays chemical shifts at 26.60-39.96, 45.72-50.67, 52.24, 59.99-148.96 ppm for the carbons of methylenes groups presents in the heterocyclic rings. The chemical shifts of the methylenes involved in the arm bound to brucine nucleus were found out at 40.70 and 51.25 ppm. Finally, several signals at 56.20 and 56.50 ppm for the carbons of methoxy groups; at 147.80 for imino group (N=C) were found. Additionally, the presence of brucine derivative (compound **3**) was further confirmed from mass spectrum which showed a molecular ion at m/z 436.18. The second step involves thecoupling of ethylenediamine to brucine by the reported method[29], using formaldehyde to form the compound **4** (Fig. 2), which has characteristic aspacer arm with free amino group involved in its chemical structure. The structural chemistry of these compounds involves an activated methyl group in heterocyclic rings[30]. The ^{1}H NMR spectra of compound **4** showed signals at 1.42-2.31, 2.73, 2.90, 3.04-3.67 and 3.97-7.35 ppm corresponding to protons presents in the heterocyclic rings. In addition, three signals at 2.65, 2.85 and 2.94 ppm were found for methylenes involved in arm bound to brucine nucleus. Finally, several chemical shifts at 3.80 and 3.91 for methoxy groups; at 3.03 ppm for amino group were found. The ^{13}C NMR spectra displays chemical shifts at 26.60-34.46, 42.28-45.51, 48.72-51.91 and 59.99-148.90 ppm for the carbons of methyls groups presents in the heterocyclic rings. In addition, another chemical shifts at 41.38, 48.46 and 52.01 ppm for methyls groups involved in arm bound to brucine nucleus were found. Finally several signals at 56.20 and 56.50 ppm for carbons corresponding to methoxy groups; at 168.10 for ketone group were found. In addition, the presence of the compound **4** was further confirmed from mass spectrum, which showed a molecular ion[16] at m/z 466.

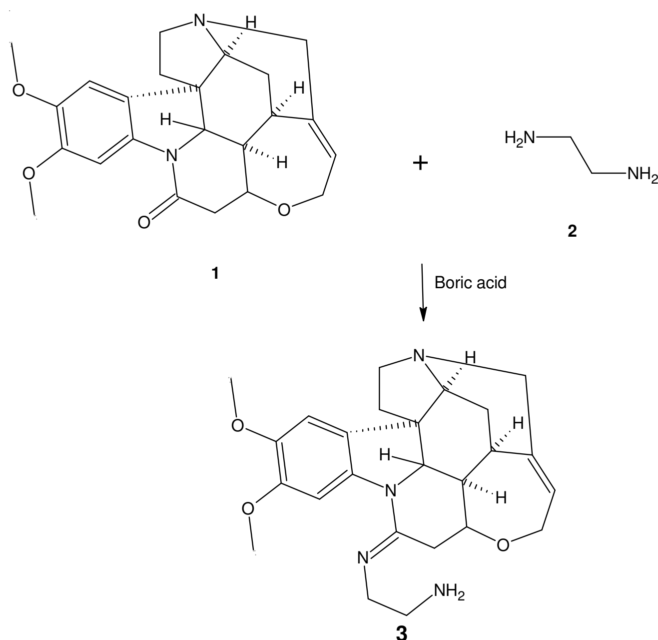

Fig. 1. Synthesis of brucine-etylendiamine derivative (**3**). Reaction of brucine (**1**) with ethylenediamine to form compound **3** using boric acid as catalyst

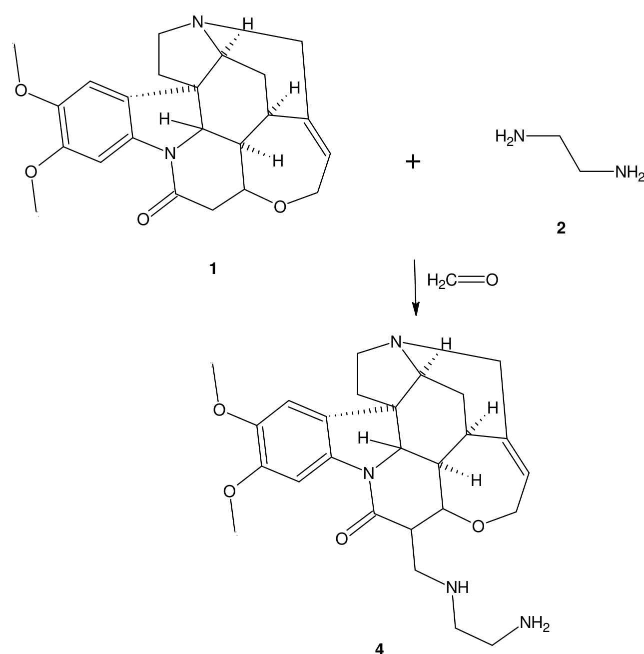

Fig. 2. Synthesis of brucine-ethylendiamine derivative (**4**). Reaction of brucine (**1**) with ethylenediamine to form **4** using Mannich reaction

Biological evaluation: The antibacterial activity of two brucine derivatives on *S. typhi* and *E. coli* was evaluated by means of dilution method and the minimum inhibitory concentration (MIC)[27], using gentamycin, cefotaxime and ciprofloxacines control in this study. The results obtained (Fig. 3) indicate that bacterial growth of *E. coli* was inhibited with cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamycin (MIC= 1.34×10^{-5} mmol/mL) and ciprofloxacin (MIC = 3.01×10^{-3} mmol/mL). In addition, the bacterial growth of *E. coli* in presence of the compound **3** (MIC = 2.26×10^{-3} mmol/mL) was blocked. These data indicate that antibacterial activity induced by the brucine derivative was lower in comparison with cefotaxime (β-lactam antibiotic) and gentamycin (inhibitor of synthesis of protein); nevertheless, the antibacterial activity of compound **3** was high in comparison with ciprofloxacin. This phenomenon can be due mainly to the different molecular mechanism involved and the characteristic chemical structure of the compounds studied in this work.

In order to evaluate if the changes in the position of arm bound to brucine (C_{13}) may induce antibacterial activity different, the compound **4** was used as pharmacological tool. The results showed that in presence of compound **4** the bacterial growth of *E. coli* (MIC = 2.13×10^{-3} mmol/mL) was blocked. The experimental data suggest that two brucine derivatives has similar antibacterial effect and this phenomenon was not affected by change in the position of arm bound to brucine nucleus.

On the other hand, in alternative experiments the antibacterial activity of two brucine derivatives was evaluated on *S. typhi* using the same controls. The results (Fig. 4) showed that the bacterial growth of *S. typhi* was blocked in presence of cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamycin (MIC = 1.34×10^{-5} mmol/mL) and ciprofloxacin (MIC = 3.01×10^{-3} mmol/mL). Additionally, the bacterial growth of *S. typhi* in presence ofcompound **3** (MIC = 2.26×10^{-3} mmol/mL) was

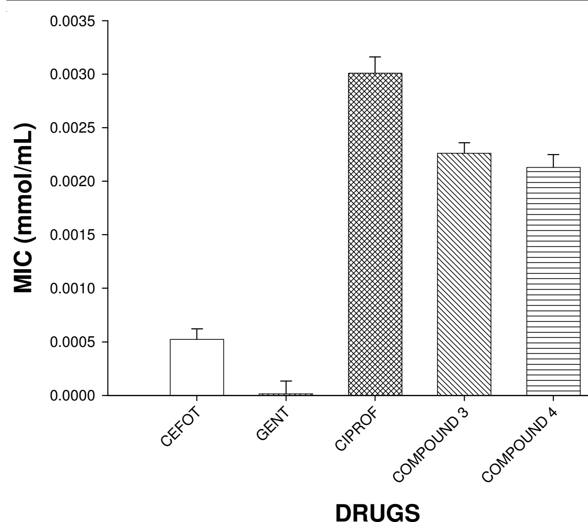

Fig. 3. Antibacterial effects induced by two brucine derivatives (compounds **3** and **4**) and controls (cefotaxime, CEFOT; gentamycin,GENT; ciprofloxacin, CIPROF) on *E. coli*. Experimental datashowed that *E. coli* was susceptible to cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamycin (MIC = 1.34×10^{-4} mmol/mL) and ciprofloxacin (MIC = 3.01×10^{-3} mmol/mL). Inaddition, the bacterial growth of this microorganism in the presence of thecompounds **3** (MIC = 2.26×10^{-3} mmol/mL; $p = 0.005$) and **4** (MIC = 2.13×10^{-3} mmol/mL; $p = 0.006$) was inhibited significantly. Each bar represents the mean ± S.E. of 9 experiments

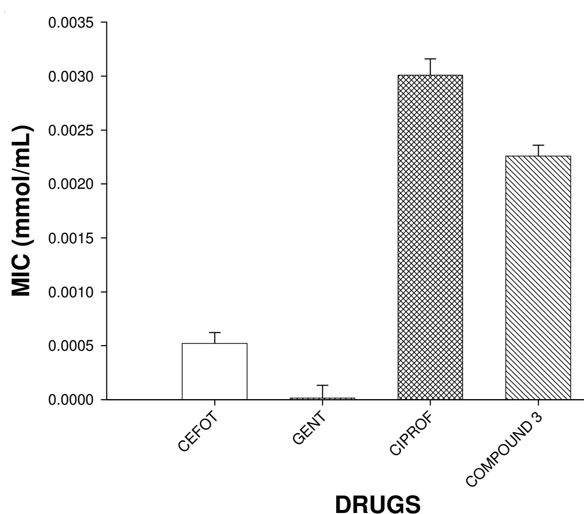

Fig. 4. Antibacterial activity exerted by brucine derivative (compound **3**)and controls (cefotaxime, CEFOT; gentamycin, GENT; ciprofloxacin, CIPROF) on *S. typhi*. The resultsshowed that *S. typhi* was susceptible to cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamycin (MIC = 1.34×10^{-4} mmol/mL) and ciprofloxacin (MIC = 3.01×10^{-3} mmol/mL). Additionally, the bacterial growth of this microorganism in the presence of the compound **3** (MIC = 2.26×10^{-3} mmol/mL)was inhibited significantly($p = 0.006$). Each bar represents the mean ± S.E. of 9 experiments

blocked; nevertheless, in presence of compound **4** the bacterial growth of *S. typhi* was not inhibited. These data indicate that ketone group could not be necessary to antibacterial effect on *S. typhi*. Analyzing this hypothesis, the antibacterial activity of brucine (compound **1**) was evaluated. The results showed that bacterial growth of *S. typhi* was not inhibited in presence of brucine (data not shown). All these data suggest that antibacterial activity of two brucine derivatives on *E. coli* can depend on the nature of the free amino group or imine group involved in the compound **3**. This functional group could be a membrane-perturbing agent whose antibacterial activity is induced, possibly, by the interaction with some substance that plays a vital role in the growth and development of *E. coli*, such as happened with other compounds, which induce antibacterial effect on this microorganism[19]. In addition, the antibacterial activity of compound **3** on *S. typhy* apart of free amino group, may depend on imine group and the hydrophobic region of brucine, which bring consequently changes in the antibacterial activity of brucine derivatives.

Conclusion

Experimental data suggest that antibacterial activity of two brucine derivatives depend of functional groups involved in their chemical structure, in addition of the hydrophobic region of brucine nucleus, in order to interact with the cell surface and perturb bacterial growth of *E. coli* and *S. typhi*.

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