Asian Journal of Chemistry; Vol. 24, No. 5 (2012), 2321-2323

ASIAN JOURNAL OF CHEMISTRY

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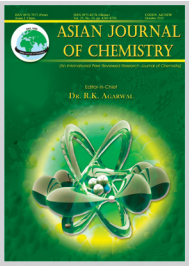

Synthesis of a Brucine-Dihydropyrimidine Derivative Using the Multi-Component System

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(*Received*: 10 June 2011; *Accepted*: 23 December 2011) AJC-10891

In this study, abrucine derivatives was synthesized. The route involved preparation of a brucine-dihydropyrimidine derivative using brucine, benzaldehyde and thiourea in the presence of hydrochloric acid. The antibacterial activity of brucine derivative was evaluated *in vitro* with both Gram positive and Gram negative bacteria using the dilution method and the minimum inhibitory concentration. The results indicate that ^{1}H NMR spectrum of brucine derivative showed signals at 385 and 391 ppm for methoxy groups at 7.31-7.40 ppm for phenyl group bound to dihydropyrimidine ring at 5.25 (-CH) and 7.60 ppm (-NH) involved in the dihydropyrimidine ring. Other results showed that bacterial growth of *Salmonella typhi* was inhibited by the compounds brucine derivative in a dose-dependent manner. Nevertheless, the bacterial growth of *V. cholerae*, *E. coli*, *K. pneumoniae* and *S. aureus* was not blocked by the brucine derivative. All these data indicate that the brucine derivative only induces antibacterial activity against *S. tiphy*; this phenomenon possibly can be due to expression of a dihydropyrimidine amidohydrolase, which could exert enzymatic activity on the dihydropyrimidine ring of brucine derivative in *V. cholerae*, *E. coli*, *K. pneumoniae* and *S. aureus*, which brings consequently that bacterial growth of this microorganism is not inhibited.

Key Words: Brucine, Derivative, Benzaldehyde, Thiourea, Antibacterial.

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 micro-organisms[7,8], unfortunately, prolonged antibiotic therapy can induce bacterial resistance, because some bacteria have developed ways to circumvent the effects of antibiotics[9,10]. In the research of new alternative therapeutic for the control of bacterial resistance, have been developed[11,12] for example, some dihydropyrimidine derivatives were synthesized as antibacterial drugs[13]. In this sense, there are reports[14], which show the synthesis of some dihydropyrimidine derivatives against *S. typhimurium*, *E. coli* and *S. aureus*. In addition, other studies show the synthesis of several 1,6-dihydropyrimidine derivatives as antibacterial agents against *Klebsiella pneumoniae* and *Proteus mirabilis*[15]. Other reports showed the preparation of a series of *N*-Mannich bases of 3,4-dihydropyrimidine-2(1*H*)-thiones and its antibacterial activity on *E. coli* and *B. subtilis*[16]. Additionally, there are studies, which shown the synthesis and antimicrobial activity of some derivatives of 5-substituted indole dihydropyrimidines[17]. All these studies show that dihydropyrimidine derivatives induce antibacterial activity against several microorganism. This study reports the synthesis of a new brucine-dihydropyrimidine derivative using a multi-componentsystem (brucine, benzaldehyde and thiourea). In addition, its antibacterial activity on both Gram positive and Gram negative was evaluated using the NCCLS (now CLSI) method with some modifications[18].

EXPERIMENTAL

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 (IR) 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.

Strychnidine [10,11-e]-3-phenyl-3,4-dihydro-1*H*-pyrazine-2-thione (4): A solution of brucine (200 mg, 0.50

mmol), thiourea (70 mg, 0.91 mmol) and benzaldheyde (100 µL, 0.98 mmol), in 10 mL of ethanol was stirred for 10 min at room temperature. Then 1 mL hydrochloric acid was added and the mixture was stirred for 48 h at room temperature. The reaction mixture was evaporated to a smaller volume, 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 76 % of product **4**. m.p. 70-72 ºC: IR (KBR, ν_{max}, cm^{-1}) : 3430, 2810; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H : 1.42-1.50 (m, 2H), 1.69-1.74 (m, 3H), 1.81-1.89 (m, 2H), 2.02-2.10 (m, 2H), 2.23-2.47 (m, 2H), 2.67-2.74 (m, 2H), 3.30-3.82 (m, 3H), 3.85 (s, 3H), 3.91 (s, 3H), 3.97 (m, 1H), 4.84 (d, 1H), 5.25 (m, 1H), 5.86 (d, 1H), 7.27 (m, 1H), 7.31-7.40 (m, 5H), 7.60 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, DMSO-d_6) δ_C : 29.68 (C-4), 32.02 (C-21), 33.01 (C-3), 39.70 (C-20), 40.75 (C-19), 48.75 (C-8), 49.26 (C-9), 52.24 (C-7), 56.20 (C-32), 56.29 (C-30), 56.88 (C-5), 64.67 (C-22), 67.36 (C-10), 69.22 (C-26), 71.30 (C-2), 76.27 (C-18), 97.23 (C-13), 103.97(C-16), 120.95 (C-28), 126.43 (C-17), 127.87 (C-37), 128.10 (C-39, C-35), 129.27 (C-36, C-38), 133.70 (C-23), 135.50 (C-34), 140.31 (C-12), 143.32 (C-15), 147.20 (C-14), 180.34 (C-25) ppm. EI-MS *m/z*: 542.75 (M^+ 12), 203.27, 189.21. Anal. calcd. for $C_{31}H_{34}N_4O_3S$: C, 68.61; H, 6.31; N, 10.32; O, 8.84; S, 5.91. Found: C, 68.58; H, 6.34.

Biological activity: The microorganisms in this study belonged to the strain bank at the Departament of Pharmaco-Chemistry at the Faculty of Chemical Biological Sciences of the Universidad Autonóma de Campeche. These strains were certified by the Centers for Disease Control and Prevention in Atlanta (USA) and were *V. cholerae* O1 (ATCC 14547), *S. typhi* (ATCC 23564) and *S. aureus* (ATCC 25923), *K. pneumoniae* (ATCC 700603) 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 [42/L]).

Antimicrobial agents: Brucine derivative was dissolved in methanol and diluted with distilled water. Cefotaxime, gentamycin, methicillin and ciprofloxacin were used as standard drugs.

Antimicrobial activity: Evaluation of the antimicrobial effect of different compounds on the bacterial species was performed according to NCCLS with some modifications[18]. *E. coli*, *S. tiphi* and *K. pneumoniaeisolate* were cultured on McConkey agar and the strain of *V. cholerae* on brain heart infusion (BHI) agar for 24 h at 37 ºC. In addition, a series of tubes were prepared, the first of which contained 2 mL of culture medium (trypticase soy) 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 Mc-Farland 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 the different 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 to pH 7.

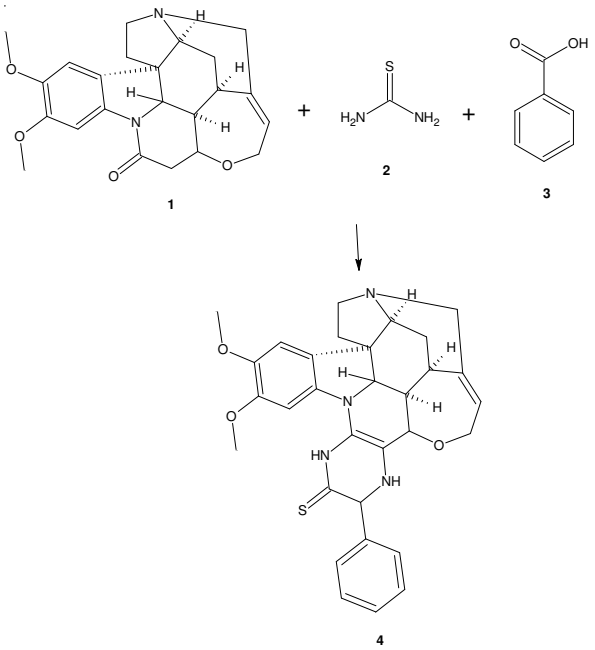
RESULTS AND DISCUSSION

Many procedures for synthesis of dihydropyrimidine derivatives are available in the literature. The most widely practiced methods employ boric acid[19], silica sulfuric acid[20], poly(4-vinylpyridine-co-divinylbenzene)-Cu(II) complex[21], H_2SO_4[22], silica triflate[23] and phosphorus pentoxide[24]. Nevertheless, despite their wide scope, these procedures suffer from several drawbacks; some reagents are of limited stability and preparation can be dangerous. Therefore, in this work we report a straightforward route for synthesis of a new brucine derivative using the three-component system such as brucine, benzaldehyde and thiourea in presence of hydrochloric acid as catalyst (**Scheme-I**). The ^{1}H NMR spectrum of the brucine derivative shows signals at 1.42-3.82, 3.97-4.84 and 5.86-7.27 and 0.78 ppm for brucine nucleus. In addition, other signals at 385 and 391 ppm for methoxy groups; at 7.31-7.40 ppm for phenyl group bound to dihydropyrimidine ring. Finally, the spectrum contains two signals at 5.25 (-CH) and 7.60 ppm (-NH) involved in the dihydropyrimidine ring. The ^{13}C NMR spectra displays chemical shifts at 29.68-52.24, 56.88-67.36, 71.30-126.43, 133.70-147.20 ppm for brucine nucleus. In addition, several signals at 69.22, 120.95, 133.70 and 180.34 ppm for carbons involved in the dihydropyrimidine ring; at 127.87-135.50 ppm for phenyl group bound to dihydropyrimidine ring were found. Finally, the spectrum contains two signals at 56.20 and 56.29 ppm for methoxy groups. In addition, the presence of brucine derivative was further confirmed from mass spectrum which showed a molecular ion at *m/z* 542.75.

Biological evaluation: The bacterial activity of brucine derivative was compared with the antibacterial effect of cefotoxime, gentamycin and ciprofloxacin (controls) in such bacterial microorganism studied. The results obtained (Fig. 1) indicate that bacterial growth of *S. tiphy* was inhibited by cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamycin (MIC = 1.34×10^{-5} mmol/mL) and ciprofloxacin (MIC = 3.01×10^{-4} mmol/mL. In addition, in presence of brucine derivative (MIC = 9.21×10^{-4} mmol/mL) the bacterial growth of *S. tiphy* was blocked in a dose-dependent way.

On the other hand, it is important to mention that the antibacterial activity of brucine derivative against *V. cholerae*, *E. coli*, *K. pneumoniae* and *S. aureus* was evaluated. Nevertheless, the bacterial growth of these microorganisms was not inhibited in presence of the brucine derivative (data not showed). All these data indicate that the brucine derivative

only induces antibacterial activity against *S. tiphy*. This phenomenon possibly can be due toexpression of a dihydropyrimidine amidohydrolase which could exert enzymatic activity on the dihydropyrimidine ring of brucine derivative in *V. cholerae*, *E. coli*, *K. pneumoniae* and *S. aureus* what brings consequently that bacterial growth of this microorganism is not inhibited. This hypotheses is availed by other type of studies, which suggest that dihydropyrimidine can be hydrolyzed *via* dihydro-pyrimidine amidohydrolase[25], in addition it is important to mention that dihydropyrimidine amidohydrolase has been found in several microorganism[26-28].



Scheme-I Synthesis of brucine derivative (4) using the multi-componentsystem (brucine, benzaldehyde and thiourea) using hydrochloric acid as catalyst

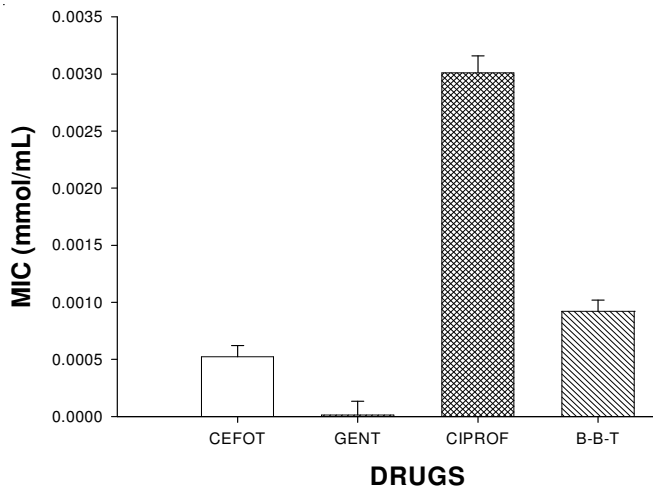


Fig. 2. Antibacterial effects induced by the brucine derivative (B-B-T) and controls (cefotaxime, CEFOT; gentamycin, GENT; ciprofloxacin, CIPROF) on *S. tiphi*. The results obtained indicate that bacterial growth of *S. tiphi* was inhibited by cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamicin (MIC = 1.34×10^{-5} mmol/mL) and ciprofloxacin (MIC = 3.01×10^{-4} mmol/mL. In addition, in presence of brucine derivative (MIC = 9.21×10^{-4} mmol/mL) the bacterial growth of *S. tiphi* was blocked in a dose-dependent way. Each bar represents the mean ± S.E. of 9 experiments

Conclusion

In conclusion, we reported an easy procedure for the synthesis of a brucine derivative. In addition, experimental data suggest that dihydropyrimidine ring involved in the brucine derivative is the responsibly of antibacterial activity against *S. tiphi*.

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