Biochemistry & Physiology: Open Access

Ali El-Remaily, et al., Biochem Physiol 2014, 3:3
DOI: 10.4172/2168-9652.1000139

Research Article Open Access

Synthesis of Dihydroimidazole Derivatives under Solvent Free Condition and Their Antibacterial Evaluation

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Rec date: Jul 03, 2014, **Acc date:** Jul 25, 2014, **Pub date:** Aug 01, 2014

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Abstract

Reaction of 2-guanidinobenzoxazole with several halogenated active methylene compounds has revealed formation of the corresponding dihydroimidazole derivatives under solvent free conditions in excellent yield. The anti-bacterial evaluation of the newly synthesized products against different types of Gram-positive and Gram-negative bacteria was performed. Most of products showed high inhibitory effect. The structure of all compounds has been characterized by on IR, 1H-NMR, 13C-NMR, Mass spectrum and some X-ray diffractions.

Keywords: Guanidylbenzoxazol; Dihydroimidazole, Halogenated active methylenes; Anti-bacterial

Introduction

Imidazole containing drugs exhibit different types of pharmacological and biological activities like metronidazole and nitrosoimidazole as bactericidal, 1-vinyl imidazole as fungicidal, megazol as trypanocidal, imidazole-2-one as antileishmanial [1,2], dantrolene sodium as muscle relaxant [3], denzimol and nafimidone as anticonvulsants [4]. In addition clonidine, guanfacine and newly synthesize lofexidine hydrochloride act as α2-agonists, digitoxin and digoxin have been used as positive ionotropic agents for the treatment of congestive heart failure [5]. The importance of dihydroimidazole moieties especially in biochemistry is recently increasing, since they are found in many biologically active compounds [6-10]. They are also used in organic synthesis as synthetic intermediates [11,12], chiral auxiliaries [13,14], and chiral ligands [15-17]. Therefore, several methodologies for synthesizing such significant compounds have already been developed, mainly by using nitriles [18-22] and esters [23-25] as starting substrates. However, these previous methods have several drawbacks, namely the need for a high reaction temperature, acidic conditions, and the use of metal cyanide for preparation of the nitrile compounds that limits their uses. Although several new methods have been recently developed [26-28], they need rather special starting materials such as azalactones [26], 2-aryl-1,1-dibromoethanes [27], and amino amides [28]. The development of mild and efficient methods is still strongly desirable. Dihydroimidazoles are reported to exhibit diverse biological and pharmacological properties. Examples of these include α-receptor stimulation, vasodepressor activity, α-adrenergic inhibition, and sympathomimetic, antihistaminic, histamine-like, and cholinomimetic activity [29,30]. Dihydroimidazoles, such as midaglizole, deriglidole, and efaroxan have been found to be potent antihyperglycemic agents [31]. In this context and as part of our on-going efforts to synthesize small bio-active heterocyclic molecules from modified guanidines and halogenated active methylene compounds [32-34] we report in this study the synthesis of C2-amino benzoxazole substituted dihydroimidazoles in addition to screening of their inhibitory effect on different types of Gram-positive and Gram-negative bacteria.

Materials and Methods

All melting points were recorded on Melt-Temp II melting point apparatus. IR spectra were measured as KBr pellets on a Shimadzu DR-8001 spectrometer. 1H-NMR spectra were recorded on a bruker at 300 MHz using TMS as an internal reference and DMSO-d6 as a solvent. Mass spectra were performed on a Shimadzu GCMS-QP 1000 mass spectrometer at 70 eV. The elemental analyses were carried out on a Perkin-Elmer 240C Microanalyzer. All compounds were checked for their purity on TLC plates. X-ray was measured on Bruker APEX2; cell refinement: Bruker SAINT; program(s) used to solve structure: SHELXS97; program(s) used to refine structure: SHELXL97; molecular graphics: XSEED.

Modified procedure for preparation of 2-benzoxazolylguanidine (1)

Method A: A mixture of o-aminophenol (1 mol), dicyandiamide (1 mol), concentrated hydrochloric acid (2 mols) and water (15 ml) was heated under reflux for 3 hours. The reaction mixture was allowed to cool down and treated with 10%NaOH to afford the solid product 1 which was recrystallized from $CHCl_3$ in give colorless crystals in 95% yield.

Method B: To a solution of 50 mmol of o-aminophenol in 50 mL of 10% hot sulfuric acid, 75 mmol of dicyandiamide was added with stirring. The reaction mixture was heated for 20 min and then 10 mL of 50% sodium hydroxide solution was added. After heating for additional 15 min, the reaction mixture was cooled down to the ambient temperature. The resulting solid product was collected by filtration, washed with water and dried under vacuum. The prepared compound (97% yields) was sufficiently pure and used without further purification.

Method C: A mixture of o-aminophenol (1 mol), dicyandiamide (1 mol), 8 drops of concentrated hydrochloric acid and ethanol (15 ml)

was heated under reflux for 3 hours. The reaction mixture was allowed to cool down then treated with 10% NaOH to afford the pure product 1 in 98% yields.

General procedure for preparation of compounds 2 – 7

A mixture of compound 1 (50 mmol) and 10 ml of halogenated active methylene compounds such as phenacyl bromide, chloro acetyl chloride, chloro acetone, ethyl bromo acetate, chloro acetonitril and bromo malononitril, in addition to few drops of catalytic glacial acetic acid was refluxed for 4 hours. The resulting solid product was collected by filtration, dried under vacuum, washed with cold ethanol and recrystallized from ethanol in excellent yield.

Synthesis of N-[(2Z)-4-phenyl-1,3-dihydro-2H-imidazol-2-ylid-ene]-1,3-benzoxazol-2-amine 2

Yield 90%, mp 255°C ; IR: cm^{-1} 3288, 3178 (2NH); 1HNMR: δ 09.74 (s,1H, NH), 7.78-7.09 (br, 10H, 2 arom + NH), 6.33 (s, 1H, CH); MS m/z (%): M+ 276 (10.20), 190 (55.10), 165 (22.70), 150 (50.50), 96 (100) ; Anal. Calc. For $C_{16}H_{12}N_4O$ (276.29): C(69.55%) H(4.38%) N(20.28%). Found: C(69.61%) H(4.45%) N(20.01%).

Synthesis of N-[(2Z)-4-chloro-1,3-dihydro-2H-imidazol-2-ylid-ene]-1,3- benzoxazol -2-amine 3

Yield 75%, mp 235°C ; IR: cm^{-1} 3389, 3221 (2NH); 1HNMR: δ 09.23 (s,1H, NH), 7.70-7.10 (br, 5H, arom + NH), 6.62 (s, 1H, CH); Anal. Calc. For $C_{10}H_7ClN_4O$ (234.64): C(51.19%) H(3.01%) Cl(15.11) N(23.88%). Found: C(51.23%) H(3.10%) Cl(15.00) N(23.65%).

Synthesis of N-[(2Z)-4-methyl-1,3-dihydro-2H-imidazol-2-ylid-ene]-1,3- benzoxazol -2-amine 4

Yield 84%, mp 255°C ; IR: cm^{-1} 3388, 3210 (2NH); 1HNMR: δ 10.04 (s, 1H, NH), 7.70-7.07 (br, 5H, arom + NH), 6.64 (s,1H, CH) , 2.56 (s, 3H, CH_3); Anal. Calc. For $C_{11}H_{10}N_4O$ (214.22): C(61.67%) H(4.71%) N(26.15%). Found: C(61.78%) H(4.66%) N(26.01%).

Synthesis of (2Z)-2-(1,3- benzoxazol -2-ylimino)imidazolidin-4-ol 5

Yield 90 %, mp 250°C ; IR: cm^{-1} 3431 (OH), 3287, 3176 (2NH); 1HNMR: δ 11.15 (s, 1H, OH), 10.12 (s, 1H, NH), 7.87-7.06 (br, 5H, arom + NH), 6.66 (s, 1H, CH); Anal. Calc. For $C_{10}H_8N_4O_2$ (216.20): C(55.55%) H(3.73%) N(25.91%). Found: C(55.69%) H(3.84%) N(25.77%).

Synthesis of N-[(2Z)-4-amino-1,3-dihydro-2H-imidazol-2-ylid-ene]-1,3- benzoxazol -2-amine 6

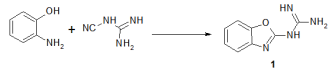
Yield 89 %, mp 290°C ; IR: cm^{-1} 3382, 3242, 3177 (2NH, NH_2); 1HNMR: δ 09.88 (s, 1H, NH), 7.71-7.07 (br, 5H, arom + NH), 5.65 (s, 1H, CH) , 4.72 (s, 2H, NH_2); Anal. Calc. For $C_{10}H_9N_5O$ (215.21): C(55.81%) H(4.22%) N(32.54%). Found: C(55.98%) H(4.34%) N(32.39%).

Synthesis of (2Z)-4-amino-2-(1,3- benzoxazol -2-ylimino)-2,3-dihydro-1H-imidazole-5-carbonitrile 7

Yield 88 %, mp 310°C ; IR: cm^{-1} 3388, 3277, 3176 (2NH, NH_2), 2222 (CN); 1HNMR: δ 10.10 (s, 1H, NH), 7.80-7.07 (br, 5H, arom + NH), 5.36 (s, 2H, NH_2); Anal. Calc. For $C_{11}H_8N_6O$ (240.22): C(55.00%) H(3.36%) N(34.98%). Found: C(55.12%) H(3.44%) N(34.81%).

Results

We have modified the reported synthesis of 2-benzoxazolylguanidine 1 in different methods A, B and C based on the reaction of cyanoguanidine with 2-aminophenol (Scheme 1) in excellent yield (see experimental section). The physical and spectral data of 1 was in complying with the reported data [35-37]. Moreover, we have reported its x-ray analyses of compound 1 [38] (Figure 1).



Scheme1: Synthesis illustration of 2-benzoxazolylguanidine 1

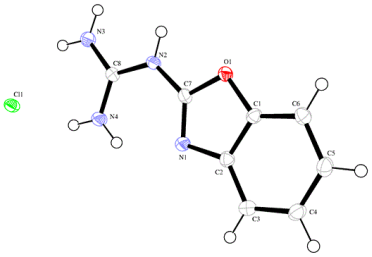
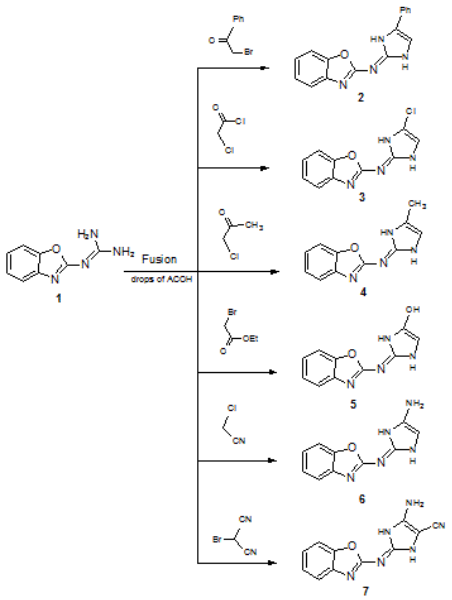


Figure 1: x-ray crystal images of compound 1



Scheme 2: Reaction illustration of halogenated active methylenes with 2 benzoxazolylguanidine

Reaction of compound 1 with halogenated active methylenes such as phenacyl bromide, chloro acetyl chloride, chloro acetone, ethyl bromo acetate, chloro acetonitril and bromo malononitril, in presence of a few drops of glacial acetic acid as a benign catalyst afforded the formation of dihydroimidzole drevatives 2 - 7 respectively (Scheme 2).

The *in vitro* inhibitory effect of compounds 2-7 on broad spectrum of bacteria representing different types of Gram-positive and Gram-negative bacteria, such as Bacillus cereus, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Micrococcus roseus* was evaluated (Table 1) using agar diffusion method (cup and plate method) [39-42]. DMSO was used as solvent control. All plates were incubated at 37±0.5°C for 24 h.

Types of Bacteria	Compound 2			Compound 3			Compound 4		
	Concentrations			Concentrations			Concentrations		
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm
Bacillus cereus	0.8 cm	0.9 cm	1.1 cm	0.6 cm	0.8 cm	1.4 cm	0.7 cm	0.9 cm	1.4 cm
Bacillus subtilis	1 cm	1.5 cm	2.1cm	0.9cm	1.3 cm	1.8 cm	1.1 cm	1.5 cm	1.8 cm
Escherichia coli	0.8 cm	1.5 cm	2.0 cm	0.7 cm	0.9 cm	1.1 cm	0.7 cm	1.5 cm	1.9 cm
Micrococcus luteus	0.4 cm	0.6 cm	0.9 cm	0.5 cm	0.8 cm	1.2 cm	0.6 cm	0.7 cm	1.1 cm
Staphylococcus aureus	0.6 cm	0.7 cm	0.8 cm	0.6 cm	0.7 cm	0.9 cm	0.6 cm	0.9 cm	1.2 cm
Pseudomonas aeruginosa	0.4 cm	0.7 cm	0.9 cm	0.5 cm	0.7 cm	0.9 cm	1.0 cm	1.5 cm	1.8 cm
Micrococcus roseus	0.6 cm	0.9 cm	1.1 cm	0.9 cm	1.4 cm	1.9 cm	0.5 cm	0.7 cm	1.0 cm
Types of Bacteria	Compound 5			Compound 6			Compound 7		
	Concentrations		Concentrations			Concentrations			
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm
Bacillus cereus	0.7 cm	0.8 cm	1.1 cm	0.6 cm	0.8 cm	0.9 cm	0.8 cm	0.9 cm	1.2 cm
Bacillus subtilis	1 cm	1.5 cm	1.9 cm	1 cm	1.5 cm	1.8 cm	1 cm	1.3 cm	1.7 cm
Escherichia coli	1.1 cm	1.7 cm	2.2 cm	0.7 cm	1 cm	1.3 cm	0.9 cm	1.2 cm	1.9 cm
Micrococcus luteus	0.4 cm	0.6 cm	0.7 cm	0.5 cm	0.7 cm	0.9 cm	0.6 cm	0.7 cm	0.8 cm
Staphylococcus aureus	0.6 cm	0.7 cm	0.8 cm	0.6 cm	0.7 cm	0.8 cm	0.5 cm	0.7 cm	0.9 cm
Pseudomonas aeruginosa	0.7 cm	1 cm	1.3 cm	0.5 cm	0.7 cm	0.9 cm	0.6 cm	0.7 cm	0.8 cm
Micrococcus roseus	0.3 cm	0.7 cm	1.0 cm	0.7 cm	0.8 cm	1.1 cm	0.4 cm	0.7 cm	0.9 cm

Table 1: Anti-bacterial evaluation of dihydroimidazole compounds 2-7.

Discussion

The reaction mechanism of formation the C2-amino benzoxazol substituted imidazoles 2-7 was proceeding via elimination of the corresponding hydrogen halide and elimination of either water molecules as in 2-4 or molecule of ethanol as in 5 while addition on cyano group resulted in ultimately the amino dihydroimidazoles 6 and 7.

IR spectra of compounds 2-7 illustrated absorption peaks at range between 3287 and 3178 cm^{-1} corresponding to NH groups in dihydroimidazole ring and confirmed in 1H-NMR spectra as appeared as singlet peak between 9.23-10.10 ppm while CH of dihydroimidazole ring was observed between 5.65-6.66 ppm as singlet peak for compounds 2-7.

All compounds were dissolved in DMSO. In order to ensure that the solvent had no effect on bacterial growth or enzymatic activity, negative control tests were performed using DMSO at the same concentrations. The zone of inhibition of compounds was measured using cm scale. The results in Table 1 revealed that compound 4 showed the highest inhibitory effect against all types of bacteria and compound 7 showed the lowest inhibitory effects against all types of bacteria (Table 1).

Conclusion

In this study, we report an efficient and environmentally friendly synthetic method for bio-active derivatives of dihydroimidazoles. N-[(2Z)-4-methyl-1,3-dihydro-2H-imidazol-2-ylid-ene]-1,3- benzoxazol -2-amine showed the highest inhibitory effect against all types of Gram-positive and Gram-negative bacteria.

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