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Synthesis of Bio-Active Guanidines by using Dioxane- Dibromide (Ddb) Under Ultrasound conditions

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Abstract

Over the decades guanidine and its derivatives have gained wide applications in organic synthesis, in the field of immunology andorgano electronics. In this paper, we highlight the facile conversion of 1,3-disubstituted thioureas to symmetric and non-symmetric guanidine derivatives by using dioxane-dibromide (DDB) as an oxidant with various amines under ultrasound conditions. The guanidines were obtained with quantitative yield, less reaction time, we also described further elaboration of this method for the synthesis of protected guanidine derivatives. All synthesized guanidines were tested for their invitro antimicrobial activity against Staphylococcus aureus, Staphylococcus albus, Klebsiella pneumonia Salmonella typhiand antifungal activity against Candida albicans, Aspergillusclavatusduring 48 h incubation period.

Keywords: 1,3-disubstituted thioureas, guanidine, DDB, ultrasound (Sonication), antimicrobial activity.

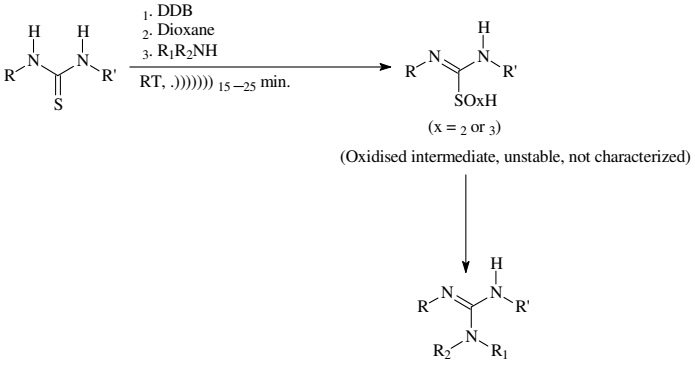
Introduction

Recently guanidine derivatives attracted much attention since it is found in a wide array of natural and synthetic bioactive compounds[1,2,3]. Because of resonance stabilization, guanidines were considered as super bases and successfully employed in organic synthesis[4]. They are useful in the production of agrochemicals, in pharmaceutical industry[5]and in rubber industry (DPG – Diphenyl guanidine)[6]. Geihe *et al* synthesized a new class of noncovalent guanidiniumrich amphipathic oligocarbonates, which delivers and release siRNA in cells, makes this an attractive strategy for biological tool development as imaging, diagnostics[7] and therapeutic applications[8-10]. Also the synthetic guanidine moieties are useful in the construction of molecular recognition devices, sensors, organic materials and phase-transfer catalysts in organic synthesis[11, 12]. Isobe *et al* reported that by introduction of chirality in one of the nitrogen atoms[13-15], the resulting chiral guanidine molecules were effective in catalytic[16-19] and stoichiometric asymmetric synthesis[20, 21].The guanidine containing molecules used in therapeutics as a drug which include cardiovascular, antihistamine, anti-inflammatory, antidiabetic, antibacterial, antiviral, and antineoplastic medicines[22, 23]. Berlinck *et al* showed that polysubstituted guanidine functionality play as a key component for the expression of biological activity in numerous natural compounds[24,25].

The wide range of biological activities displayed by guanidines has motivated the development of novel reagents and different synthetic scheme for their preparation. Nearly all possible synthetic routes were exploited to prepare guanidines and substituted guanidines[26,27]. Thioureas and isothioureas are used as common starting materials for the synthesis of guanidines and its derivatives. Reagents like DIC, EDCI, Hg^{2+}, Bismuth catalyst activate the thioureas and comfortably afford guanidine (75%-97%)[28].

Ultrasound promoted synthesis has attracted much attention during the past few decades, it is an efficient and innocuous technique for the activation of various chemical reactions which enhances selectivity and product yield but also shortens the reaction time and minimizes the undesired side products[29]. This can be considered as a processing aid in terms of energy conservation and minimizing the waste as compared to conventional heating[29,30]. In this context we decided to explore the efficient, simple and fast synthesis of guanidine compounds.

Based on the critical analysis of literature, we selected Dioxan-dibromide (DDB) as an oxidant and offer the benefits of sonication method for the synthesis of guanidines. The reaction is envisaged to proceed through the formation of sulphonic acid and acted on by the amine to yield guanidines. No attempt was made to isolate the oxidized thiourea intermediates owing to their instability. The reaction is presumed to proceed as per the scheme below.



Scheme-1
Synthesis of Guanidine

Material and Methods

General: All chemicals were purchased from Fluka and Aldrich. The melting points were measured using a differential scanning calorimeter (Shimadzu DSC-50) and are uncorrected[1]. H NMR and ^{13}C spectra were measured on a Bruker AVANCE (400 MHz) spectrometer using TMS as the internal standard and in the specified deuterated solvents. Chemical shifts were expressed in ppm and Infrared (IR) spectra were recorded as KBr pellets on a Perkin-Elmer1 FTIR spectrometer. Elemental analyses were measured on a HERAEUS (CHNO, Rapid) analyzer.

Ultrasonic process equipment: Sonication reaction was performed in a Shanghai Branson-BUG40-06-ultrasound cleaner and energy is transmitted to the reaction vessel through the liquid medium. The internal dimension of the ultrasonic cleaner tank is 48 X 28 X 20 cm with liquid holding capacity of 5 L. The reactions were carried out in a round-bottomed flask of 50 mL capacity, RB flask was placed at the center of the cleaner, 2 cm above from the position of the transducer to get the maximum ultrasound energy. The addition or removal of water controlled the temperature of the water bath. The temperature of the water bath was controlled at 25–30^oC. All the experimental parameters were done at 40 kHz with output power of 250 W.

General procedure for the preparation of Dioxane dibromide: Dioxane dibromide was comfortably prepared by following the reported procedure[31], Bromine (3 ml, 9.3g, 58.1 mmol) was added drop wise to ice-cold dioxane (8 ml, 8.1 g, 92 mmol) under stirring after 15 min. an orange-colored solid appeared. Reaction mixture was allowed to stir at RT for a further 2 hours. The orange product was filtered, washed with dioxane, dried in a desiccator under reduced pressure (yield 9.3 g, 65%) and stored in a refrigerator at below 5^oC for several months. DDB is a stable crystalline solid, orange color was due to charge transfer transitions between dioxane and bromine. The structure was confirmed by elemental analysis. Mpt = 62^oC, Yield 10 g. Anal. Calcd. for dioxane-dibromide ($C_4H_8O_2Br_2$): C, 19.5: H, 3.25; O, 13.008; Br, 64.2, Found: C, 19.3; H, 3.30; O, 12.98; Br, 64.0.

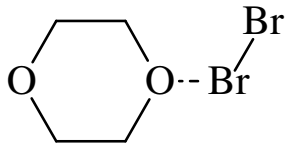


Figure-1
Dioxane-dibromide

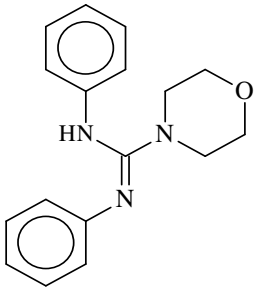
Synthesis of substituted guanidines by using Dioxan dibromide: To a stirred solution of 1,3-disubstituted thiourea in THF (100mmol, 2.28g, 25cm^3) Dioxan-dibromide (1.0 Molar eq.) was added in portions over 5 min. The reaction mixture was irradiated with an ultrasound probe for 20-30 min., (reaction was monitored by TLC by every 5 min). After the reaction, solvent was evaporated under reduced pressure by using rotary evaporator, yields gummy solid and guanidine was extracted with ethyl acetate and water. The crude product was further purified by flash column chromatography. The spectral data for the compounds 1,2,7,8,9,10 are presented as below, the compounds 3,4,5,6 already well characterized and reported[6,28].

Table-1
Guanidines with different substituents and % Yield

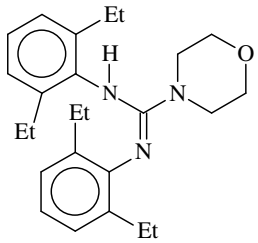
No	Substrate (R)	(R^1)	(R^2)	*Yield (%)
1	Phenyl	Morpholinyl	H	90
2	2,6-Diethyl phenyl	Morpholinyl	H	93
3	Phenyl	C_2H_5	C_2H_5	88
4	Phenyl	*i*-C_3H_7	*i*-C_3H_7	90
5	Phenyl	C_6H_{11}	H	92
6	Phenyl	C_6H_5 CH_2	H	95
7	Phenyl	C_6H_5 CH_2	Pbf	94
8	C_6H_5 CH_2	4-methoxy phenyl	Pbf	96
9	Phenyl	Morpholinyl	Pbf	95
10	4-methoxy phenyl	Morpholinyl	Pbf	95

*- Isolated yield under the above experimental conditions,

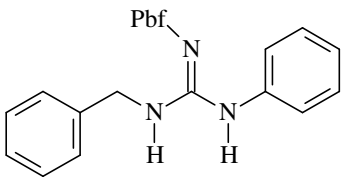
Spectral data for compounds: Compound1: N, N'-diphenyl-N"-morpholinyl guanidine: M.pt: 130°- 131°C, ν $_{max}$ (KBr) cm^{-1}: 3348, 3174, 2358, 8H 1597, 1521, 1321, 1212, 700, 656, 502. Mass values: m/z: 282 (M^+ +1); 281 (M^+); 189,145, 93, 77. ^{1}H NMR values: δ_H ($CDCl_3$/TMS) 7.3-6.8 (10H, Ar, m and 1H, -NH exchangeable with D_2O), 3.6 [(4H, -$O(CH_2)_2$, as triplet], 3.3 [4H, -$N(CH_2)_2$, as triplet]. Elemental analysis: (Found: C, 72.13; H, 7.01; N, 14.75; O, 5.71; $C_{17}H_{19}N_3O$ requires C,72.56%; H, 6.80%; N, 14.93%; O, 5.69).



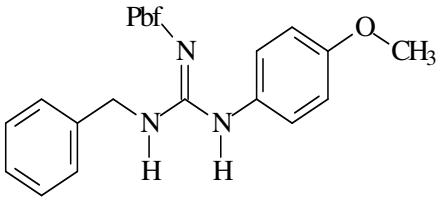
Compound 2: N, N'- (2,6,-diethyl) phenyl-N"-morpholinyl guanidine: M.pt: 142°-144°C, ν_{max}(KBr) cm^{-1}: 3325, 2835, 2930, 1563, 1485, 1231, 685, 525. Mass values: m/z: 394 (M^+ +1); 393 (M^+); 363,306, 291, 248, 158. ^{1}H NMR values: δ_H ($CDCl_3$/TMS) 7.3-6.9 (6H, Ar, m and 1H, -NH exchangeable with D_2O), 3.8-3.6 [(4H, -$O(CH_2)_2$, as triplet], 3.5-3.4 [4H, -$N(CH_2)_2$, as triplet], 3.1-2.9 (8H, 4 X –CH_2, as multiplet). Elemental analysis: (Found: C, 76.25; H, 9.03; N, 10.58; $C_{25}H_{35}N_3O$ requires C, 76.29%; H, 8.96%; N, 10.67%).



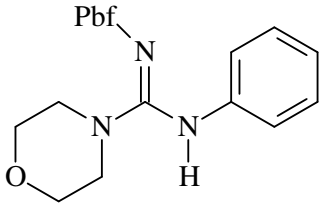
Compound 7: N-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-N'-benzyl-N"-phenyl guanidine: MS: 478.2 $(M+H)^+$, ^{1}H NMR (300 MHz, $CDCl_3$): δ_H1.48 (s, 6H), 2.10 (s, 3H), 2.55 (s, 3H), 2.60(s, 3H), 2.96(s, 2H), 4.44-4.46 (d, J=5.9Hz, 2H), 7.13-7.19 (m, 4H), 7.23-7.29 (m, 4H), 7.35-7.40(m, 2H), ^{13}C NMR (75MHz, CDCl3): δ 12.4, 17.9, 19.2, 28.5, 43.1, 45.2, 86.3, 117.3, 124.4, 125.8, 127.4, 127.5, 128.6, 130.0, 132.3, 133.0, 135.5, 137.6, 138.5, 153.2, 158.6. Anal. Calcd for $C_{27}H_{31}N_3O_3S$: C, 67.90; H, 6.54; N, 8.80. Found: C, 67.74; H, 6.62; N, 8.96.



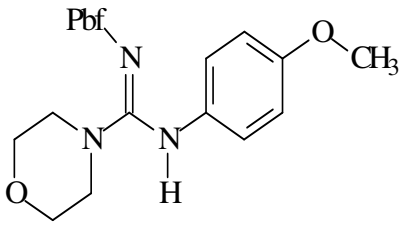
Compound 8: N-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-N'-benzyl-N"-(4-methoxyphenyl) guanidine: MS: 494.2 $(M+H)^+$, ^{1}H NMR (300 MHz, $CDCl_3$): δ_H1.47 (s, 6H), 2.09 (s, 3H), 2.54 (s, 3H), 2.59(s, 3H), 2.96(s, 2H), 3.81(s, 3H), 6.88-6.91 (m, 2H), 7.15-7.20 (m, 3H), 7.23-7.32 (m, 4H). ^{13}C NMR (75MHz, CDCl3): δ 12.3, 17.9, 19.2, 28.4, 43.1, 55.4, 86.3, 114.8, 117.3, 123.3, 124.4, 125.5, 127.0, 129.0, 132.3, 132.8, 136.2, 138.6, 151.3, 158.7. Anal. Calcd for $C_{27}H_{31}N_3O_4S$: C, 65.70; H, 6.33; N, 8.51. Found: C, 65.65; H, 6.30; N, 8.37.



Compound 9: N-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-N'-phenyl-N"-(morpholine-4) guanidine: MS: 458.2 $(M+H)^+$, ^{1}H NMR (300 MHz, $CDCl_3$): δ_H1.40(s, 6H), 2.04(s, 3H), 2.44 (s, 3H), 2.61(s, 3H), 2.79(s, 2H), 3.33-3.36(t, J=5.1 Hz), 3.57-3.60(t, J=4.4Hz, 4H), 6.70-6.73 (d, J=8.1 Hz, 2H), 7.03-7.08(t, J=8.1 Hz, 1H), 7.20-7.25 (m, 2H).^{13}C NMR (75MHz, CDCl3): δ 12.3, 18.2, 19.1, 28.5, 43.0, 46.9, 66.0, 86.4, 117.7, 120.0, 120.1, 124.4, 124.7, 129.4, 132.4, 138.4, 139.0, 155.4, 158.9. Anal. Calcd for $C_{24}H_{31}N_3O_4S$: C, 63.00; H, 6.83; N, 9.18. Found: C, 63.19; H, 6.76; N, 9.38.



Compound 10: N-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-N'-(4-methoxyphenyl)-N"-(morpholine-4) guanidine MS: 458.2 $(M+H)^+$, ^{1}H NMR (300 MHz, $CDCl_3$): δ_H1.40(s, 6H), 2.04(s, 3H), 2.44 (s, 3H), 2.61(s, 3H), 2.79(s, 2H), 3.33-3.36(t, J=5.1 Hz), 3.57-3.60(t, J=4.4Hz, 4H), 6.70-6.73 (d, J=8.1 Hz, 2H), 7.03-7.08(t, J=8.1 Hz, 1H), 7.20-7.25 (m, 2H).^{13}C NMR (75MHz, CDCl3): δ 12.3, 18.2, 19.1, 28.5, 43.0, 46.9, 66.0, 86.4, 117.7, 120.0, 120.1, 124.4, 124.7, 129.4, 132.4, 138.4, 139.0, 155.4, 158.9. Anal. Calcd for $C_{24}H_{31}N_3O_4S$: C, 63.00; H, 6.83; N, 9.18. Found: C, 63.19; H, 6.76; N, 9.38.



Antimicrobial screening: The antibacterial activity of the synthesized compounds 1-10 was tested against gram-positive bacteria i.e. *Staphylococcus aureus*, *Staphylococcus albus*, gram-negative bacteria i.e., *Klebsiellapneumoniae*, *Salmonella typhi* using a nutrient agar medium[32]. The antifungal activity of the compounds was screened against *Candia albicans* and *Aspergillusclavatus*using dextrose agar medium[33]. The sterilized medium (autoclaved at 121°C for 15 min.) was inoculated with the suspension of the microorganisms and poured into a petri dish to give a depth of 3-4 mm. The paper impregnated with the synthesized guanidine compounds (300µg/ml in DMF) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37° for 24 h and 48 h for antibacterial and antifungal activity, respectively. Amicacin (300 µg/ml) was used in anti-bacterial activity studies, whereas fluconazole (300 µg/ml) was used in antifungal activity studies, as reference compounds. After incubation, the relative susceptibility of the micro-organisms to the potential antimicrobial agent is demonstrated by a clear zone of growth inhibition around the disc. The inhibition zone caused by the various compounds on the micro-organisms was measured and the activity rated on the basis of statistical analysis with help of ANOVA method. The observed zone of inhibition is presented in table 2.

Results and Discussion

Ultrasound sonicator is an ideal non-conventional energy source and its mediated chemical reactions have great potentials to be a green chemistry tool which reduce environmental waste and use fewer chemical ingredients. The high yield, mild reaction condition should make it a valuable extension to current synthetic methodologies and further applied to synthesis of Pbf − protected guanidines. These products were easily synthesized in a shorter reaction time, less percentage of side products was formed in comparison to the conventional methods. The synthesized guanidine derivatives were screened "*in vitro*" for antimicrobial activity. From the data presented in Table 2, it is clear that compounds 1, 2, 9 and 10 were found highly active against *Staphylococcus aureus, Staphylococcus albus, Streptococcus* as compared to the standard drug (Amicacin), but shows only moderate activity against *Klebsiella pneumonia* and *Salmonella typhi*. Other compounds exhibit moderate to good antibacterial activity against all organisms. Similarly, compounds 9 and 10 exhibits good antifungal activity against *Candida albicans* and *Aspergillusclavatus* as compared to the standard drug used (Fluconazole). The remaining compounds are moderately active against these two micro-organisms (*C. albicans* and *A. clavatus*).

Table-2
Antimicrobial activity of compounds 1-10, Zone of inhibition (mm) (activity index)*

Compd.	Antibacterial activity Zone of inhibition (mm)				Antifungal activity Zone of inhibition (mm)	
	S.aureus	*S.albus*	*K.pneumoniae*	*S. typhi*	*C.albicans*	*A.clavatus*
1	23	21	22	19	21	23
2	21	24	23	18	22	21
3	17	15	16	14	16	15
4	16	14	13	16	15	17
5	16	12	14	11	13	10
6	17	16	15	14	12	14
7	15	19	16	13	10	11
8	17	19	15	12	11	10
9	24	20	19	21	23	22
10	22	23	24	20	21	22
C_1	26	25	30	24	------	-------
C_2	-----	-----	------	-------	28	29

*Activity index = Inhibition area of the sample / Inhibition area of the standard. C_1 = Amicacin; C_2 = Fluconazole, Diameter of disc is 5 mm

Conclusion

In summary we have demonstrated the novel synthesis of N,N'-substituted guanidine compounds by employing DDB under sonication condition which meets our afore- mentioned goals. The reaction proceed through oxidation mechanism, hence we report an efficient synthesis of guanidines using dioxane dibromide. Furthermore, with the ease of recovery and recycling of dioxane-dibromide after the reaction makes these reagents environmentally benign reagent. An efficient synthesis of N, N'-substituted guanidine derivatives were developed by using DDB under ultrasound condition.

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