Synthesis, Antibacterial, Thrombolytic and Cytotoxic Evaluation of Substituted and Un-substituted Selenium-n-heterocyclic Carbene Adducts

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Research Article

Keywords: Benzimidazolium salts, Se–NHC adducts, Antibacterial, Hemolytic, Thrombolysis

Posted Date: January 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1211884/v1

Abstract

N-heterocyclic carbene salts bearing alkyl substituents (**1-8**) and their selenium *N*-heterocyclic carbene adducts (**9-12**) were synthesized and characterized by elemental analysis, FT-IR, NMR (^{1}HNMR, ^{13}CNMR) spectroscopic techniques. All the adducts were found to be stable in air and moisture at room temperature. Compounds (**5-12**) were evaluated against *Bacillus subtilis Macrococcus brunensis and Bacillus cereus in vitro. The biological assay revealed* that antibacterial activity of Selenium-*N*-heterocyclic carbene adducts are comparatively better than the salts. MIC and inhibition zone values showed that *Bacillus subtilis* is more active to selenium adducts (**9-12)** than *Macrococcus brunensis and Bacillus cereus* whereas opposite in the salts (**5-8)**. *In vitro* studies of hemolysis and thrombolysis demonstrated that the synthesized compounds are innocuous for pre-clinical trials to mouse blood.

Introduction

Microbial infection is a common complication in contaminated wounds and extensive soft tissue damage [1]. It is very necessary to control such infections by treating with antimicrobial drugs [2]. These drugs have a substantial impact on human health by treating and preventing the spread of microbial infections [3]. Bacteria develop drug resistance due to their prolong use that's why researchers design new drugs with better inhibition power and lower adverse effects [3-6]. Metal based antimicrobial treatments have been found to be effective against bacterial resistance and may be consider as future antimicrobial drug [7, 6]. Selenium is a metalloid, essential trace element for animals [8] and has attracted a growing interest in medicinal chemistry due to their escalating reports representing their selectivity, biocompatibility and high efficacy e.g ebselen (under ten years' clinical trials) is considered as antimicrobial drug [9, 10]. However, the possibility of targeting Se delivery in biological systems remains a challenge. Recently, Selenium-*N*-Heterocyclic. To evaluate the donor capabilities of NHC ligands, carbene adducts have been designed and synthesized. [11]. In the past, NHC was used primarily as a versatile ligand in the synthesis of organometallic compounds, but is now used as an alternative to phosphine in organometallic chemistry.

Previously we synthesized benzimidazolium based Se- NHC carbenes adducts and evaluated their biological potential, some compounds showed themselves as potent antibacterial agents therefore now we synthesize eight novel unsubstituted and substituted benzimidazolium based Se-NHC adducts and tested *in vitro* against hemolytic, thrombolytic and various microbial strains to evaluate the effect of substitution on biological potential. By knowing the mechanism of Se-NHCs as antimicrobials, researchers may design strong anti-bacterial to ameliorate and prevent microbial infections.

Experimental

Materials and Methods

All analytical grade chemicals, reagents and solvents were taken from Merk (Germany) /and were utilized as such with no purification. Benzimidazole, 5-methyl benzimidazole, 5, 6-dimethyl benzimidazole, Selenium, 1,4-dibromobutane, propyl bromide, 2-ethyl hexyl bromide, butyl bromide, hexyl bromide, DPPH (1,1-diphenyl-2-picrylhydrazyl) were purchased from Sigma Aldrich. N-Alkylated substituted and unsubstituted benzimidazolium based salts were prepared according to our reported method with minor modification[3, 7, 10]. FTIR Spectrum's of compounds (**5-12**) were recorded in the range 4000–600 cm^{-1} using an Agilent spectrometer and NMR spectra were recorded in d_6-DMSO and d_6-Chloroform on Bruker 125.1 and 500 MHz spectrometers uses tetramethylsilane (TMS) as an internal reference.

Synthesis of Pro-ligands

Stirred a solution of substituted and un substituted benzimidazole (benzimidazole, 5-methyl benzimidazole and 5,6 dimethyl benzimidazole) with KOH (0.71 g, 2.6 mM) in 20 mL DMSO for 30 min. After complete mixing added 1-bromopropane, 1-bromobutane, 1-bromohexane and stir it for 3 hrs. After that, the reaction mixture was poured in 200mL distilled water. After 2-5 minutes turbidity appeared, oily product or white powder obtained **(1-4)**. Extracted the product with a chloroform using separating funnel if the product is oily layer. After evaporation of chloroform. If precipitates appeared filter it with three piles of filter paper (sand wash it with distilled water and dry it in an oven and keep these for further use

Synthesis of salts

1, 3-dipropyl 5-methyl benzimidazolium dibromide (5)

A solution of 5-methyl-1-propyl-benzimidazole (1 g, 5.25mmol) and 4 mL bromopropane was dissolved in 30 mL 1,4 dioxane and reflux it for 18 h at 100 ^oC. White precipitates appeared. Filtered the precipitates and washed with 1,4 dioxane and dried in an oven. Yield: 2.1g (81%); M.P = 119-121^oC. FTIR (ATR, υ, cm^{-1}): 3009, 2932, 2782, 1456, 1434, 1265, 823, 610. ^1H NMR (500 MHz, DMSO-d_6, δ ppm) 1.03 (6H, m, CH_3), 2.04 (4H, m, CH_2), 2.62 (3H, s, CH_3), 4.43 (4H, m, CH_2), 7.5 (2H, t, Ar-H), 7.42 (2H, d, Ar-H, J =7.86), 9.14 (1H, s, NCHN). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 10.9, 21.8 (R-CH_3), 22.6, 23.1 (R-CH_2), 48.7, 48.9 (NCH_2), 67.0, 112.6, 112.7, 128.8, 129.4, 131.5, 138.0 (Ar-C), 142.0 (NCN). Anal. Calcd. for $C_{14}H_{21}N_2Br_2$: C, 77.37; H, 9.74; N, 12.89 Found: C, 77.35; H, 9.76; N, 12.93

Synthesis of 1-butyl-3-(5-(3-butyl-5,6-dimethyl-1H-benzo[d]imidazol-3-ium-1-yl)pentyl)-5,6-dimethyl-1H-benzol imidazolium bromide (6)

Followed the same procedure as 5, but using 1-butyl-5,6-dimethyl-benzimidazole (1.5 g, 7.2 mM) and di-bromo pentane (0.5mL, 3.7mM) as second alkyl halide. White powder obtained. Yield: 2.8g (79%), M.P: 74-76 ^oC. FTIR (ATR, v, cm^{-1}): 3386, 2934, 2871, 1559, 1486, 1454, 1362, 1205, 853, 684. ^1H NMR (500 MHz, DMSO-d_6, δ ppm) 1.02 (6H, t, CH_3, J = 7.20 Hz), 1.47 (4H, m, CH_2), 1.76, (2H, m, CH_2), 2.05 (4H, m, CH_2), 2.24 (4H, q, CH_2), 2.48 (12H, d, Ar-H, J = 12.10 Hz 4.5 (4H, t, J = 7.5 Hz, CH_2), 4.7 (4H, t, CH_2, J = 7.5 Hz), 7.4-7.7 (2H, s, Ar-H), 11.1 (2H, s, NCHN). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 13.54 (CH_3), 19.8,

20.5, 20.7, 28.1, 31.3, (R-CH_2) 46.7, 47.5 (Ar-CH_3), 112.5, 113.3 (CH_2), 129.8, 129.9, 137.2, 137.4 (Ar-C), 141.1 (NCN). Anal. Calcd. for $C_{31}H_{46}Br_2N_4$: C, 58.68; H, 7.31; N, 8.83 Found: C, 58.72; H, 7.30; N, 8.80

Synthesis of 1-(2-ethylhexyl)-3-hexyl-2,3-dihydro-1H-benzo[d]imidazol-1-ium bromide (7)

Followed the same procedure as above but using 1-hexyl-benzimidazole (1g, 4.3mM). and 2-ethyl hexyl bromide (1mL, 5.7mM) as second alkyl halide. 3 were obtained as lemon yellow color liquid on evaporation in fume hood which was converted in shiny lemon yellow sticky gel. Yield: 1.11 g (69%). FTIR (ATR, v, cm^{-1}): 2960, 2874, 1495, 1460, 1375, 1361, 1280, 1260, 792, 780. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm) 0.91 (3H, t, CH_3, J=7.67 Hz), 1.23 (10H, m, CH_2), 1.81 (2H, m, CH_2), 4.22 (2H, m, CH_2), 5.10 (2H, t, CH_2), 7.41(1H), 7.53, 7.74, 7.81 (1H, Ar-H), 9.91 (1H, s, NCHN). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 14.21, 22.31, 25.84 (CH_3), 26.17, 28.98, 29.93, 31.09, 44.51, 47.12 (CH_2), 110.82, 114.27, 119.71, 121.71, 122.55, 126.93, 134.17 (Ar-C), 142.62 (N=C=N). Anal. Calcd. for $C_{21}H_{37}BrN_2$: C, 63.46; H, 9.38; N, 7.05 Found : C, 63.42; H, 9.40; N, 7.01.

Synthesis of 3-butyl-1-octyl-1H-benzimidazolium bromide (8)

Followed the same procedure as 1 but using 1-butyl-benzimidazole (0.87 mL, 8.4mM) and octyl bromide (0.99mL, 5.7mM). The reaction mixture was refluxed for 48 hr continuously. A thick brown fluid is obtained by rotary evaporator. Yield 1.27g (71%), M.P 75 ^{o}C. FTIR (ATR, v, cm^{-1}): 3386, 2934, 2871, 1559, 1486, 1454, 1362, 1205, 853, 684. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm)1.02 (6H, t, CH_3, J = 8.07 Hz), 1.48 (4H, m, CH_2), 1.76 (2H, m, CH_2), 2.05 (4H, m, CH_2), 2.24 (4H, q, CH_2), 2.48 (12H, d, CH_3, J = 12.10 Hz), 4.51 (4H, t, J = 7.5 Hz, CH_2), 4.70 (4H, t, J = 7.5 Hz, CH_2), 7.42, 7.71 (2H, s, Ar-H), 11.11 (2H, s, NCHN). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 9.0, 56.5 62.4 (R-CH_2), 117.1, 120.1, 122.3, 123.0, 126.6, 11.7 120.1 122.3 123.0 126.6 (R-CH_3), 127.0 , 127.1, 128.2-128.9, 129.1, 129.3, 129.5, 129.6, 134.3, 138.9, 139.1, 139.3, 142.6 143.3 (Ar-C), 144.0 (N=C=N). Anal. Calcd. for $C_{19}H_{31}BrN_2$: C, 62.12; H, 8.51; N, 7.63 Found: C, 62.18; H, 8.49; Br, N, 7.60

Synthesis of selenium adducts

Synthesis of 5-methyl-1, 3-dipropyl-benzimidazole-2(3H)-selenone (9)

5 (0.8g, 2.69 mM) was added in 50 mL distilled water and dissolved it on heating using 100 mL flask. After that, selenium powder (0.32 g, 4.06 mM) and Na_2CO_3 (0.71g, 6.7 mM) were added and refluxed for 7 h. White color precipitates were separated and dried. Washing was done by distilled water (3 × 5 mL). M.P= 89-91^{o}C. FTIR (ATR, v, cm^{-1}): 3010, 2923, 2782, 1464, 1424, 1265, 823, 610. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm) 0.93 (6H, t, CH_3, J=7.81 Hz), 1.85 (2H, m, CH_2), 2.01 (2H, m, CH_2), 4.31 (2H, t, CH_2), 4.56 (4H, t, CH_2), 7.03 (1H, s, Ar-H), 7.11, 7.42 (2H, d, Ar-H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 11.0, 11.3, 21.5, 21.8 (CH_3), 22.7, 22.8 (Ar-CH_3), 47.9, 48.1, 48.8, 48.9 (N-CH_2, CH_2), 109.3, 109.8, 112.6, 112.7 (Ar-

CH_3), 129.4, 131.1, 131.5, 133.1, 138.1, (Ar-C), 164.5 (C=Se). Anal. Calcd. for $C_{14}H_{20}N_2Se$: C, 56.95; H, 6.83; N, 9.49 Found C, 56.91; H, 6.78; N, 9.56

Synthesis of 1-butyl-3-octyl-1H-benzimidazole-selenone (10)

'Synthesis of 10 followed the same procedure as 9 but using 6 (1.6g, 2.71 mM) and selenium powder (0.321 g, 4.07mM) and Na_2CO_3 (0.572g, 5.39 mM) and refluxed it for 5 h. After 5 h some black oily layer present above the reaction mixture Now separate it by solvent extraction method using chloroform. A dark brown solution is obtained. Filter it by using three layers of filter paper then from celite. Thick brown colour liquid is obtained. Yield: 0.51g (64 %). FTIR (ATR, υ, cm^{-1}): 3010 2923, 2782, 1464, 1424, 1265, 823, 610. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm) 1.46 (6H, t, CH_3), 1.96 (12H, m, CH_2), 4.52 (4H, m, CH_2), 6.41 (4H, t, CH_2), 7.37 (2H, d, Ar-H, *J*= 11.28 Hz), 7.86 (2H, t, Ar-H, *J*= 8.57 Hz). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 34.7 (Ar-CH_3), 42.6 (N-CH_2, CH_2), 107.6, 121.1, 126.7, 127.1, 127.3, 129.1 129.8, 138.2, (Ar-C), 153.4 (C=Se). Anal. Calcd. for $C_{19}H_{30}N_2Se$: C, 62.45; H, 8.28; N, 7.67 Found: C, 62.46; H, 8.32; N, 7.71

Synthesis of 1-(2-ethylhexyl)-3-hexyl-1H-benzimidazole-2(3H)-selenone (11)

Synthesis followed the same procedure as above but using 7 (1g, 3.2mM), selenium metal powder (0.24 g, 3.04mM) and Na_2CO_3 (0.44g, 4.15 mM) and refluxed for 4 h. After 4 h dark brown oily layer is formed. To remove unreacted selenium metal filter, the reaction mixture by using celite. Extarction of oily layer was done by solvent extraction method using chloroform as solvent. Washing was done by acetonitrile (3 × 5 mL). A thich dark brown liquid is obtained. Filter it by using three layers of filter paper then from celite. Light brown colour liquid is obtained. Yield: 0.61 g (71 %). FTIR (ATR, υ, cm^{-1}): 3137, 2934, 1508, 1456, 1200, 1070, 1002, 880, 837, 636, 622. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm) 1.46 (6H, t, CH_3), 1.96 (8H, m, CH_2), 3.63 (8H, m, CH_2) 4.12 (1H, m, CH_2), 2.41 (4H, m, CH_2), 7.37 (2H, m, Ar-H), 7.86 (2H, d, *J* = 8.57 Hz, Ar-H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 22.5, 26.1 (Ar-CH_3), 36.5, 43.6, 45.9 (N-CH_2), 109.0, 110.0, 123.1, 132.2, 133.0 (Ar-C), 169.8 (C=Se). Anal. Calcd. for $C_{21}H_{34}N_2Se$: C, 64.10; H, 8.71; N, 7.12 Found: C, 64.12; H, 8.69; N, 7.08

Synthesis of 3,3'-(pentane-1,5-diyl) bis(1-butyl-5,6-dimethyl benzimidazole-2(3H)-selenone) (12)

Synthesis of 12 followed the same procedure as 9 but using **8** (0.6g, 0.94 mM) and selenium metal powder (0.3 g, 3.8 mM) and Na_2CO_3 (0.4g, 3.8 mM) for 5 hr. After 5 hr some black oily layer was present above the reaction mixture. Black bead was present with magnetic stirrer. Now separate it by solvent extraction method using chloroform. Bead dissolved in chloroform. A black solution is obtained. Filter it by using three layers of filter paper. Light yellow color solution is obtained. Pass it from celite. Lighter color is obtained. Cover it with paraffin film with two small holes for evaporation. After ten days' beige color powder is obtain. Yield: 0.71 g (68 %). M.P= 96-98 ^{o}C. FTIR (ATR, υ, cm^{-1}): 3011, 2924, 2781, 1463, 1425, 1261, 821, 609. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm) 0.96 (6H, t, CH_3), 1.28 (14H, m, CH_2), 1.98 (4H, t, CH_2) 4.52 (4H, m, CH_2), 6.39 (4H, s, CH_2), 7.62- 7.75 (4H, t, Ar-H, *J* = 8.57 Hz), 7.91 (2H, d, *J*=6.11 Hz),

8.24 (2H, d, *J*= 3.54 Hz, Ar-H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 22.6, 22.8, 25.5 (CH_3-R), 37.8, 39.4, 39.5, 49.5 (CH_2-R), 112.5, 113.2 (CH_3), 129.6, 129.7 137.1, 137.3, 140.4 (Ar-C), 162.4 (C=Se). Anal. Calcd. for $C_{31}H_{44}N_4Se_2$: C, 59.04; H, 7.03; N, 8.88. Found: C, 59.01; H, 7.09; N, 8.80

EXPERIMENTAL PHYSICOCHEMICAL PROPERTIES

Antibacterial study

Bacterial potential of compounds **5-12** were evaluated against *Bacillus subtilis, Macrococcus brunensis and Bacillus cereus*, as previously described (Wiegand et al., 2008), using the inhibition zone and minimum inhibitory concentration (MIC) test. Bacterial suspensions were prepared by inoculating fresh LB broth with a loop full of fresh colonies of Luria Bertani (LB) agar and growing overnight at 37 °C. Overnight, 100 ml of bacterial culture was transferred to10 ml fresh

LB broth and incubated at 37°C till

the turbidity met the mcfarland standards of 0.5. The final MIC concentration evaluated for each sample was then adjusted using bacterial cultures. The MIC concentrations examined were 1.0 mg per ml, 500 mg per ml, 250 mg per ml, 125 mg per ml, 62.50 mg per ml, 31.25 mg per ml, and 15.63 mg per ml. After an overnight incubation at 37 °C, bacterial growth and inhibition were observed.

Hemocompatibility Assay

Benzimidazolium based salts and their respective selenium adducts (5-12) were investigated according to 10993-4 ISO standards by evaluating hemolytic potentials after exposing to blood of normal mouse using previously developed method with minor modification [21]. Blood samples of different concentrations were centrifuge for 5 min at 5000 rpm and were added in erythrocyte suspension which is prepared by adding PBS and incubate it for 30 minutes and after that centrifuged it for 5 min at 13000 rpm, at 540nm free hemoglobin from supernatant was investigated using spectrophotometer. PBS and X-100 Triton were taken as control (negative and positive). Percentage hemolysis was measured according to the following equation [22]

$$\% \text{ Hemolysis} = \frac{\text{AOs} - \text{ACn}}{\text{ACp}} \times 100$$

AOs=Sample's absorbance

ACn=Negative control's absorbance

ACp=Positive control's absorbance

Thrombolytic assay

Thrombolysis was performed according to already reported method with little modification [20]. 1 mL of normal mouse blood was incubated at 37°C for 40 min and allowed it to clot. After formation of clot, serum was removed and the weight of clot is measured by equation

$$\%\, Thrombolysis = X/Y + Z$$

Now, 10 mL of DMSO and 100 µL solution of test sample was added in clot and incubated for 3

hr at 37°C. Afterward, by discarding the liquid, the tube was weighed (X) again. Percentage of thrombolysis was calculated by taking ratio of X and Y. Sreptokinase and Distilled water were used as control (positive and negative).

X = weight of colt before lysis

Y = weight of clot after lysis

Z = Weight of eppendorf

Results And Discussion

Synthesis

Attempts to synthesize substituted and un-substituted benzimidazolium salts **(5-8)** and their respective selenium adducts **(9-12)** according to our previously reported methods with little changes [12, 13, 10] (Scheme 1) and for their chemical structures see supplementary data (Figure S1). All the compounds **(5-12)** were found to be stable inair and moisture after keeping in an open menvironment for seven consecutive days

Characterization

The synthesized compounds (1-8) were preliminary characterized by FTIR, some distinct spectral changes were observed prior and after the addition of selenium to benzimidazolium salt which could be the indication of successful synthesis. Only representative data of preligand (1, 4), benzimidazolium salts (5, 6) And their corresponding selenium adducts (9,10) were shown in Figure S2

NMR spectra (^{1}H & ^{13}C) of compounds (1-12) were recorded in deuterated solvents (chloroform and DMSO) depending upon their organic and inorganic nature of solubilities. The ^{1}H NMR salts spectras (5-8) displayed that the acidic proton peak (NCHN) at 9-10 δ ppm vanished in selenium adducts (5-8) due to its replacement in adducts with selenium. Moreover, ^{13}C NMR spectra of the salts (5-8) and selenium NHC adducts (9-12) showed distinct changes in chemical shift values (142±2 to 159±5δ ppm) as N*C*HN carbon changes to NC*Se*N in Se-NHC adducts (Figure S3-S17). But it is generally seen that, the changing in size of substituted alkyl chain at benzimidazole, chemical shift values also altered (range 153.0-180.6 δ ppm) (Figure 1).

Biological studies

In vitro antibacterial potential

Disk diffusion and broth dilution methods were used to evaluate the zones of inhibition (at 100µL conc.) and minimum inhibition concentration (MIC, at different concentrations) respectively for the synthesized compounds (5-12) against *Bacillus subtilis, Macrococcus brunensis and Bacillus cereus* using a ciprofloxacin as reference drug (Table 1). Salts **5-8 have smaller inhibition zone** (8–15mm) against all tested bacterial strains than selenium-NHC adducts **9-12** having zone of inhibition (19–29 mm) which is comparable to ciprofloxacin (reference drug). The change in activities of salts and adducts may be due to the replacement of halide with selenium in selenium-NHC bonding that is responsible for the enhancement of lipophilicity of adducts. MIC values also support the results of inhibition zones (Table 1). Selenium-NHC carbene adducts **9-12** displayed comparable activity (MIC 17 ± 0.3 – 27 ± 0.1 µg/mL) to the reference drug [14] ciprofloxacin (MIC 11 ± 0.5–15 ± 0.2 µg/mL) against all the tested strains whereas benzimidazolium salts **1-4** displayed lesser activity (MIC 33 ± 0.6–48 ± 0.7 µg/mL) compared to selenium adducts. It was observed that salts showed relatively stronger antibacterial activity against *Bacillus subtilis, Macrococcus brunensis and Bacillus cereus* while vice versa in selenium adducts.

Previous research demonstrated that bond between selenium and benzimidazolium salts plays very momentous role in biological activity in addition with other factors e.g solubility and degree of polymerization [7]. In another research outcomes scientists described that redox selenium compound covalently attached to peptides and bacterial phage to kill bacteria by generating superoxide radicals [15]. Broad spectrum antibacterial Se-NHC adducts might be due to exchange phenomenon with thiol group (Sulfur) of some protein such as thioredoxin reductase of bacteria. Pathogenic bacteria died due to the inhibition of TrxR as it inhibits GSH system [16]. In another mechanism researchers analyzed that there is an interaction with the free thiol group of a cysteine, after the scission of the Se-N bond of ebselen [17]. The NHC moiety in selenium-NHC adduct only participate as carrier in biological system for the transport of selenium ion to the site of action, therefore antibacterial potential of selenium adducts depends on the release of selenium ions and ease of salt exchange process [7]. In addition, the type of bacterial strain and nature of the selenium adduct is also involved in antibacterial activity. Thus making it impossible to make a general statement about structure-activity relationships for the development of antibacterial activity [18]. Table 1 shows the susceptibility of B. cereus to selenium adduct from smaller MICs and larger inhibition zones. In addition, the presence of benzimidazole's aromatic ring increases its activity by increasing lipophilicity, helping selenium ions penetrate into the cell membrane, enter cells of microorganisms and destroy the function of organelles respiratory and metabolic processes [19, 14]. The cellular function of microorganisms can be pretentious by the interaction of selenium ions with cellular DNA and proteins, whereas adducts interact with the thiol (S) group of enzymes to cause denaturation. Many investigations demonstrated that replication is effective when the DNA helix is relaxed, whereas replication is impaired when the DNA is in a condensed form. When selenium ions permeate microbial cells, DNA molecules condense, preventing their replication and leading to cell death.[3, 6].

Table 1. ZI and MIC values of Compounds (**5-12**) *and*

ZI (mm)				MIC (µg/mL)		
Comp.	*Bacillus subtilis*	*Macrococcus brunensis.*	*Bacillus cereus*	*Bacillus subtilis*	*Macrococcus brunensis.*	*Bacillus cereus*
5	8 ± 1.2	10 ± 0.4	8 ± 0.2	21 ± 0.5	35 ± 0.6	38±0.1
6	7 ± 0.2	9 ± 0.3	6 ± 0.6	35 ± 0.4	33 ± 0.6	n.a
7	6 ± 0.1	8 ± 0.5	9 ± 0.1	45 ± 0.4	46 ± 0.7	43 ± 0.5
8	9 ± 0.3	11 ± 0.4	10 ± 0.3	43 ± 0.3	38 ± 0.6	48 ± 0.7
9	23 ±01	24 ±03	16±0.1	16±0.2	18 ±0.3	20 ±0.1
10	21 ± 0.5	23 ± 0.2	18 ± 0.4	19 ± 0.6	15 ± 0.9	23 ± 0.7
11	25 ± 0.4	28 ± 0.1	23 ± 0.6	24 ± 0.5	20 ± 0.3	27 ± 0.1
12	22 ± 0.5	25 ± 0.1	17 ± 0.5	17 ± 0.3	16 ± 0.4	18 ± 0.3
C	27 ± 0.4	36 ± 0.1	25 ± 0.9	14 ± 0.2	12 ± 0.5	11 ±0.3

C= Control (Ciprofloxacin)

Hemolytic potential

Hemolysis experiments were performed to check the interaction between salts/selenium NHC adducts with normal erythrocytes of mouse. Hemolysis is actually **the** destruction of **many** red blood cells with **the release of** red blood **cells, which occurs** when **the** blood **comes into** contact with **the surface of a** foreign **body.** A compound shows excellent blood compatibility if it has less value of hemolysis rates. The essential post-implantation phenomenon is compound-blood interaction. In vitro blood compatibility assessments of salts / adducts were performed by direct contact with erythrocytes using phosphate buffered saline. See Figure 2. The values are shown in Table S2. The hemolysis of the synthesized compound (**5-12**) varied from 0.52 to 3.42% relative to normal mouse erythrocytes, indicating no adverse effects on the compound and thus safe for clinical trials [23] It was also observed that the salts have higher hemolytic potential than selenium adducts.

In vitro thrombolytic activity

Thrombolytic potential was evaluated by published method [20]. The maximum clot lysis was achieved when both positive control and clots were incubated for 180 minutes at 37°C (83 %). Clot lysis was reduced in clots treated with sterile distilled water (100 µL) (negative control) (31%). The thrombolytic activity of salts and selenium adducts was found to be comparable to that of positive control streptokinase. These findings (Figure 3) show that benzimidazolium salts have lesser thrombolytic

activity than selenium-NHC adducts and can thus be used in preclinical studies as active thrombolytic drugs. Tabulated values of % clot lysis can be seen in Table S3.

Conclusion

New unsubstituted and substituted benzimidazolium salts and Se NHC-carbene adducts have been designed and synthesized in good yields. They were characterized by elemental analysis, FTIR, ^{1}H-NMR, ^{13}C NMR. The salt showed stronger antibacterial activity against *Bacillus cereus* than *Bacillus subtilis* and *Macrococcus brunensis*, a selenium adducts, showed a stronger antibacterial effect against *Bacillus* subtilis and *Macrococcus brunensis*. The results of Hemolysis and thrombolysis assays have shown that the compound is safe for preclinical studies of mouse blood *in vitro*.

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Scheme

Scheme 1 is available in supplementary section.

Figures

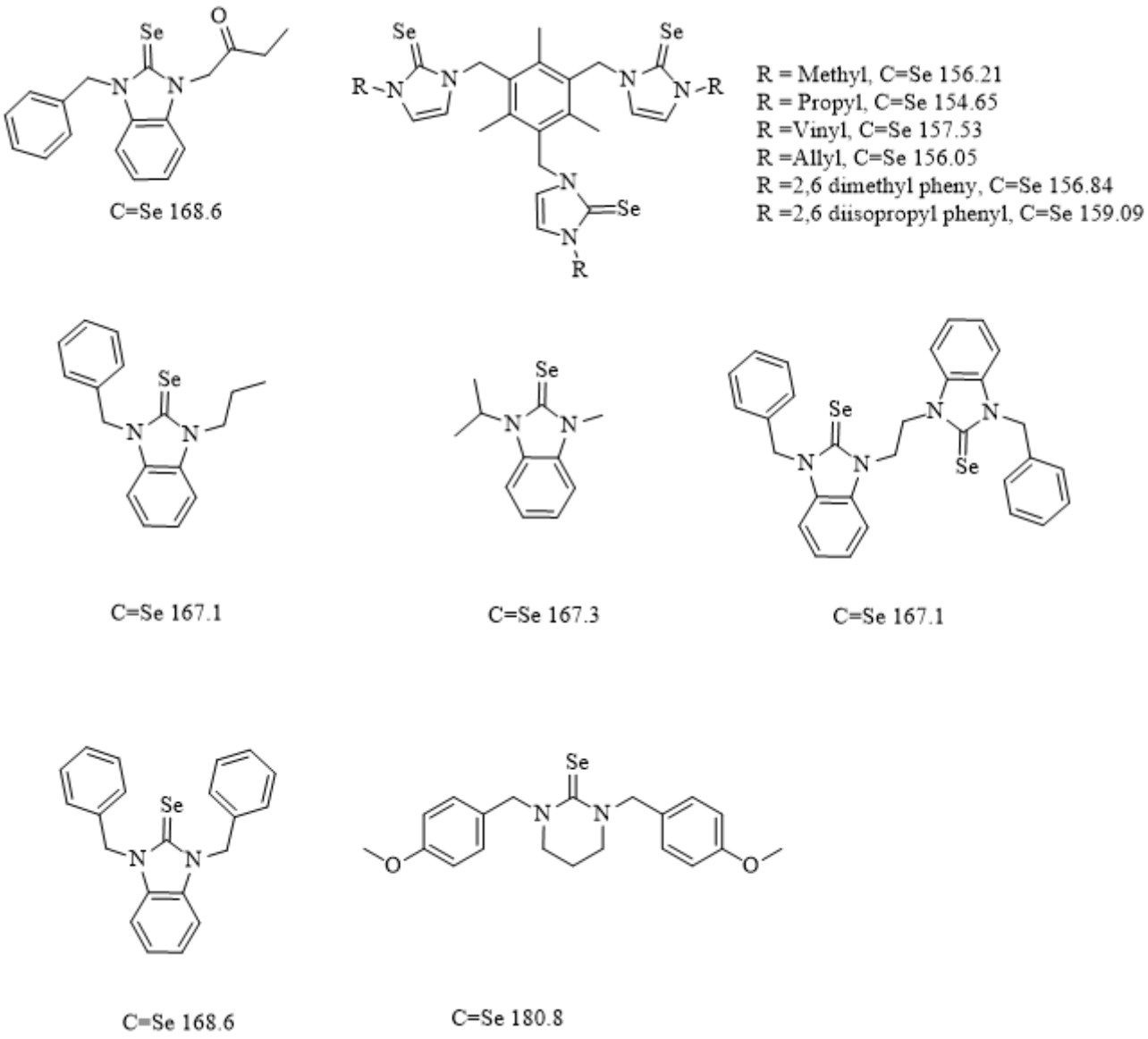

Figure 1

General presentation of chemical shift values ranges from 153.0-180.6 δ ppm.

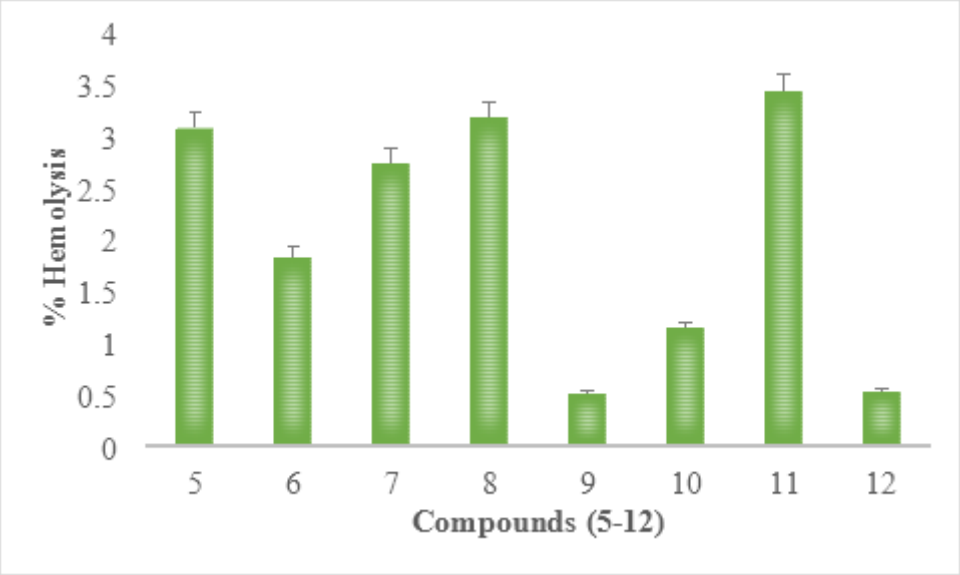

Figure 2

Percentages of hemolytic activity of NHC salts **5-8** and Se-NHC adducts **9-12**.

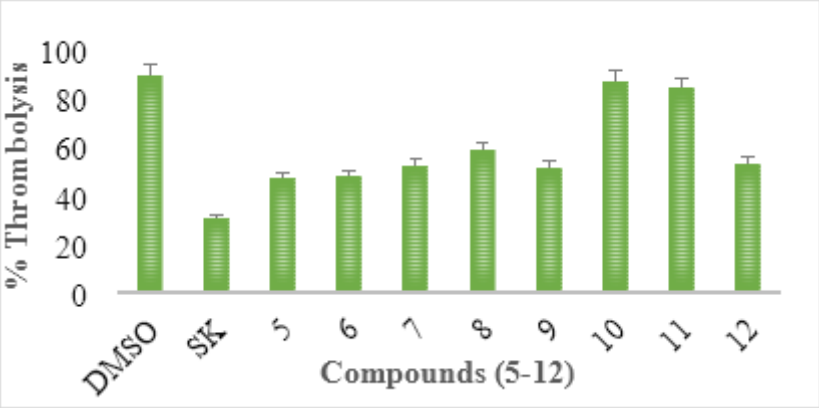

Figure 3

Percentage thrombolysis of NHC salts **5-8**. and Se-NHC adducts **9-12**

Supplementary Files

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