Cytotoxic, Thrombolytic and Antibacterial Evaluation of Synthesized Substituted and Un-Substituted Selenium-*N*-Heterocyclic Carbene Adducts

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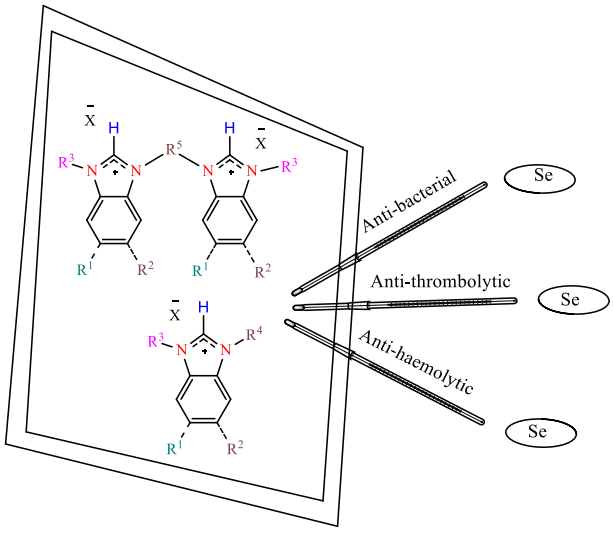
Summary: Alkyl-substituted azolium salts (**1-8**) and their Se-*N*-heterocyclic carbene (Se-*N*-Het-*C*) adducts (**9-12**) were obtained in very reasonable yields.
All synthesized azolium salts and their Se-*N*-Het-*C* adducts were characterized by different spectroscopic techniques such as FT-IR, ^{1}HNMR, ^{13}CNMR, and elemental analysis. It was found that all synthesized Se-*N*-Het-*C* adducts were stable at room temperature in both air and moisture. *In-vitro* these compounds **(5-12)** were assessed for their antimicrobial potential against *Bacillus subtilis (B. subtilis), Macrococcus brunensis (M. brunensis),* and *Bacillus cereus (B. cereus) in vitro*. Results of MIC and inhibition zone values revealed that the majority of the Selenium *N*-Heterocyclic carbene adducts were active against *Bacillus subtilis* (*B. subtilis*) than *Macrococcus brunensis* (*M. brunensis*) and *Bacillus cereus* (*B. cereus*) whereas opposite in the azolium salts **(5-8).** Compounds **5-8** have an inhibition zone of 16±0.1-26±0.3mm against all tested bacterial strains while selenium-NHC adducts **9-12** have a zone of inhibition (16±0.2 to 25 ± 0.4mm). Adduct **12** showed good activity against all tested strains with ZI values 25 ± 0.1, 22± 0.5, 17 ± 0.3 mm and MIC values 17 ± 0.2, 16 ± 0.4 and 18 ± 0.3 µg/mL against *Bacillus subtilis* (*B. subtilis*), *Macrococcus brunensis* (*M. brunensis*) and *Bacillus cereus* (*B. cereus*) respectively. Adduct **10** showed the highest thrombolysis i-e 86.9% and adduct **12** showed good hemolysis i-e 0.51%. Overall results of thrombolysis and cytotoxicity studies revealed that the compounds are safe for preclinical studies of mouse blood *in vitro*.

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



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 them with antimicrobial drugs [2]. These drugs have a substantial impact on human health by treating and preventing the spread of microbial infections [3]. Drugs develop resistance due to their prolonged use which is why researchers design new drugs with better inhibition power and lower adverse effects [3-9]. Metal-based antimicrobial

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treatments are effective against bacterial resistance and may be considered future antimicrobial drugs [6, 10]. Selenium is a metalloid, an essential trace element for animals [11], 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 an antimicrobial drug [12, 13]. However, the possibility of targeting Se delivery in biological systems remains a challenge. Recently, Selenium-*N*-Heterocyclic carbene adducts (Se-NHC) have been synthesized to see their donor abilities and biological potential [14]. In the past, azolium salts were used primarily as a versatile ligand in the synthesis of organometallic compounds, but these are now being used as an alternative to phosphine in organometallic chemistry.

There are a variety of synthetic routes are available using activated Se sources for the preparation of Se-based compounds which are usually toxic and unstable while solvents used as reaction mediums are non-greener [15]. However, using *Se* and H_2O as a reaction medium is thought to be comparatively a greener approach [16-19] for the preparation of selenium compounds. Previously we synthesized benzimidazolium-based Se-NHC carbene adducts and evaluated their biological potential, where some of the test compounds proved to be potent antibacterial agents [16]. Hence in the current investigation, we synthesized eight novel unsubstituted and substituted benzimidazolium-based Se-NHC adducts in a greener way and tested *in vitro* against hemolytic, thrombolytic and various microbial strains to evaluate the effect of substitution on biological potential.

Experimental

Materials and Methods

All analytical grade chemicals, reagents, and solvents were taken from Merk (Germany) and were utilized as such with no purification. Azole (benzimidazole, 5/5, 6-dimethyl benzimidazole). Selenium, 1,4-dibromo butane, 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 [3, 10, 13]. FTIR Spectrum of compounds (**5-12**) was 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 use tetramethylsilane (TMS) as an internal reference.

Synthesis of Pro-ligands

Stirred a solution of substituted and unsubstituted 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, and 1-bromhexine and stir it for 3 hrs. After that, the reaction mixture was poured into 200 mL of distilled water. After 2-5 minutes turbidity appeared, and an oily product or white powder was obtained **(1-4)**. Extracted the product with chloroform using a separating funnel if the product is an 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)**

5-methyl-1-propyl-benzimidazole (1 g, 5.25mmol) and 4 mL bromopropane were added in 1,4 dioxane (20mL) and reflux for 18 h at 100 °C. White precipitates settled at the bottom of the flask. Filtered it and washed it with 1,4 dioxane and dried it in an oven. Yield: 2s.1g (81%); M.P = 119-121°C. FTIR (ATR, υ, cm^{-1}): 3009, 2932, 2782, 1456, 1434, 1265, 823, 610. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm) 1.03 (m, 6H), 2.04 (m, 4H), 2.62 (s, 3H), 4.43 (m, 4H), 7.5 (t, 2H), 7.42 (d, 1H, *J* =7.86), 9.14 (s, 1H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 10.9, 21.8, 21.8, 22.7, 22.8, 48.7, 48.9, 67.0, 112.6, 112.7, 128.8, 129.4, 131.5, 138.0, 142.0. Analytical Calculated 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 dibromo pentane (0.5mL, 3.7mM) as second alkyl halide. The white powder was obtained. Yield: 2.8g (79%), M.P: 74-76 °C. FTIR (ATR, υ, cm^{-1}): 3386, 2934, 2871, 1559, 1486, 1454, 1362, 1205, 853, 684. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm) 1.28 (t, 6H, *J* = 7.20 Hz), 1.67 (m, 4H), 2.24, (m, 8H), 2.67 (m, 12H), 4.54 (q, 4H), 4.58 (m, 4H), 4.57 (t, 4H, *J* = 7.5 Hz), 7.71 (s, 2H), 7.75 (s, 2H), 9.45 (s, 2H). ^{13}C NMR

(125.1 MHz, DMSO-d_6, δ ppm) 13.5, 19.2, 22.8, 28., 31.3, 47.0, 47.6, 113.0, 113.9, 127.0,127.2, 131.2, 131.3, 142.3. Analytical Calculated 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 a lemon yellow color liquid on evaporation in a fume hood which was converted into a lemon yellow sticky gel. Yield: 1.11 g (69%). M.P: 86-88 ^oC. FTIR (ATR, υ, cm^{-1}): 2960, 2874, 1495, 1460, 1375, 1361, 1280, 1260, 792, 780. ^1H NMR (500 MHz, DMSO-d_6, δ ppm) 1.48 (t, 6H, *J*=7.67 Hz), 1.78 (m, 12H), 3.23 (d, 9H), 5.02 (m, 4H), 8.20 (d, 2H), 8.65(d, 2H), 10.42 (s,1H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 14.0, 22.1, 25.8, 28.4, 28.5, 28.6, 31.2,46.8, 113.8, 126.6, 131.2,142.1 Analytical Calculated 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 a rotary evaporator. Yield 1.27g (71%), M.P 75 ^oC. FTIR (ATR, υ, cm^{-1}): 3386, 2934, 2871, 1559, 1486, 1454, 1362, 1205, 853, 684. ^1H NMR (500 MHz, DMSO-d_6, δ ppm)1.01 (t, 6H, *J* = 8.07 Hz), 1.48 (m, 4H), 1.76 (m, 2H), 2.05 (m, 4H), 2.24 (q, 4H), 2.48 (d,12H, *J* = 12.10 Hz), 4.52 (t, 4H *J* = 7.5 Hz), 4.56 (t, 4H, *J* = 7.5 Hz), 7.44 (d, 2H), 7.61 (t, 2H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 19.0, 39.4, 58.5 62.4, 111.7, 120.1, 122.3, 123.0, 126.6, 11.7 120.1 122.3 123.0 126.6, 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). Analytical Calculated for $C_{19}H_{31}BrN_2$: C, 62.12; H, 8.51; N, 7.63 Found: C, 62.18; H, 8.49; N, 7.60

Synthesis of selenium adductsSynthesis 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 on heating using a 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 with distilled water (3 × 5 mL). Yield: 0.62g (71 %). M.P= 89-91^oC. FTIR (ATR, υ, cm^{-1}): 3010, 2923, 2782, 1464, 1424, 1265, 823, 610. ^1H NMR (500 MHz, $CDCl_3$- δ ppm) 1.03 (s, 3H), 1.63 (t, 6H), 4.56 (m, 4H), 5.68 (t, 4H), 7.34 (m, 2H), 7.45 (s, 1H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 11.0, 11.3, 21.5, 21.8, 22.7, 22.8, 47.9, 48.1, 48.8, 48.9, 109.3, 109.8, 112.6, 112.7, 129.4, 131.1, 131.5, 133.1, 138.1, 164.5 (C=Se). Analytical Calculated 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 is present above the reaction mixture Now separate it using chloroform via the solvent extraction method. A dense brown soln is achieved. Filter it by using three layers of filter paper from celite. A thick brown color liquid is obtained. Yield: 0.51g (64 %). %). M.P= 86 ^oC. FTIR (ATR, υ, cm^{-1}): 3010 2923, 2782, 1464, 1424, 1265, 823, 610. ^1H NMR (500 MHz, DMSO-d_6, δ ppm) 1.46 (t, 6H), 1.96 (m, 8H), 3.02 (t, 8H), 5.15 (t, 4H), 7.48 (d, 2H, *J*= 11.28 Hz), 7.86 (t, 2H, t, *J* = 8.57 Hz). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 34.7, 42.6, 107.6, 121.1, 126.7, 127.1, 127.3, 129.1 129.8, 138.2, 153.4 (C=Se). Analytical Calculated 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), *Se* (*M*) powder (0.24 g, 3.04mM), and Na_2CO_3 (0.44g, 4.15 mM) was refluxed for 4 h. Dense brown oily layers appeared after 4 hrs. Celite was used to filter unreacted *Se* (*M*). An oily layer was extracted in chloroform via the solvent extraction method. Acetonitrile (3 × 5 mL) was used to wash. A dark brown liquid is obtained. Filter it by using three layers of filter paper instead of celite. The light brown color liquid is obtained. Yield: 0.61 g (71 %). %). M.P= 82^o C. FTIR (ATR, υ, cm^{-1}): 3137, 2934, 1508, 1456, 1200, 1070, 1002, 880, 837, 636, 622. ^1H NMR (500 MHz, $CDCl_3$, δ ppm) 0.98 (t, 6H), 1.46 (m, 12H), 1.98 (m, 4H), 5.51 (m, 4H), 7.28 (t, 2H), 7.43 (t, 2H), 7.87 (d, 2H), 8.16 (d, 2H, *J* = 8.57 Hz, Ar-H). ^{13}C NMR (125.1 MHz, $CDCl_3$, δ ppm) 22.5, 26.1, 36.5, 43.6, 45.9, 109.0, 110.0, 123.1, 132.2, 133.0, 169.8 (C=Se). Analytical Calculated 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 *Se* (M) powder (0.299 g, 3.8 mM) and Na_2CO_3 (0.4g, 3.8 mM) was refluxed for 5 hrs. A little black oily layer appeared above the reaction mixture after 5hrs. A black bead was present with a 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. A light yellow color solution is obtained. Pass it from celite. The lighter color is obtained. Cover it with paraffin film with two small holes for evaporation. After ten days' beige color powder is obtained. Yield: 0.71 g (68 %). M.P= 96-98 ^oC. FTIR (ATR, υ, cm^{-1}): 3011, 2924, 2781, 1463, 1425, 1261, 821, 609. ^1H NMR (500 MHz, DMSO-d_6, δ ppm) 2.68 (t, 4H), 3.23 (s, 12H) 4.92 (m, 14H), 5.39 (t, 4H, *J*=6.11 Hz), 5.39 (t, 4H, *J* = 8.57 Hz), 7.62- 7.75 (t, 4H, t), 7.91 (d, 2H), 8.17 (s, 2H), 8.25 (s, 2H). ^{13}C NMR (125.1 MHz, DMSO-d_6, δ ppm) 22.5, 22., 22.9, 25.5, 37.8, 39.4, 39.5, 49.5, 120.0, 120.6, 128.3, 128.4134.8, 165.6 (C=Se). Analytical Calculated 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.

Antibacterial study

The antibacterial potential of compounds **5-12** was evaluated against *B. subtilis, M. brunensis and B. 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, bacterial culture (100 mL) was transferred to 10 ml fresh LB broth and incubated at 37°C till the turbidity reached 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 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 to determine the hemolytic potentials by treating the blood of normal mice with synthesized adducts using a previously developed method with minor modification [32]. Blood samples of different concentrations were centrifuged for 5 min at 5000 rpm and were added in erythrocyte suspension which is prepared by adding PBS and incubating it for 30 minutes after that centrifuged for 5 min at 13000 rpm, at 540 nm free hemoglobin from the supernatant was investigated using a spectrophotometer. Negative and positive control (PBS and X-100 Triton) were taken. Percentage hemolysis was measured according to the following equation [33].

$$\% \text{ Hemolysis} = \frac{\text{ODS}-\text{ODN}}{\text{ODP}} \times 100$$

ODS = Optical density of the sample
ODN = Optical density of the -Ve control
ODP = Optical density of the +Ve control

Thrombolytic assay

Previously reported methods developed by Coordination Chemistry Lab (CCL) were adopted for the thrombolytic assay [34].

Results and Discussion

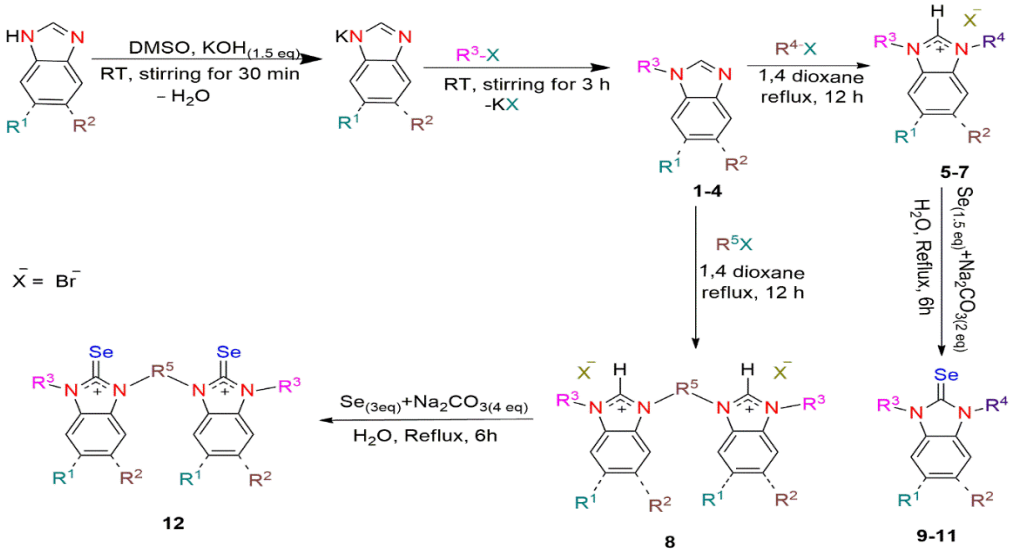
Synthesis

Attempts to synthesize substituted and un-substituted benzimidazolium salts **(5-8)** and their respective Se-*N*-Het-*C* adducts **(9-12)** according to our previously reported methods with little changes [13, 20, 21] (Scheme-1) and for their chemical structures see supplementary data (Fig S1). The initial indications for the successful synthesis of desired compounds (**5-12**) were the physical states, solubility, and a difference in the melting points (M.P.) of azolium salts and their respective substituted and un-Se-*N*-Het-*C* adducts. The solubility of azolium salts was high in polar solvents like water, methanol, and ethanol while the solubility of their respective Se-*N*-Het-*C* adducts **(9-12)** was high in non-polar solvents like *n*-hexane, diethyl ether, chloroform, and dichloromethane. The desired compounds were successfully formed in appropriate yields as white solid (compound **5-6**), yellow (compound **7),** and brown gel (compound **8**). Furthermore, selenium *N*-heterocyclic carbene adducts appeared as white solid (compound **9**), and brown gel (compound **10-11**) while **12** as beige colored solid. All the compounds **(5-12)** were found to be stable in air and moisture after keeping in an open environment for seven consecutive days.

Characterization

The synthesized compounds **(1-8)** were preliminarily characterized by FTIR, and some distinct

spectral changes were observed before and after the addition of selenium to azolium salts which could be an indication of successful synthesis. For example, comparing spectra of benzimidazolium salts with respective organoselenium compounds, it can be observed that distinct changes were observed in the region 1350-1450 cm^{-1} due to variation in C-N stretch due to bonding of selenium with the carbene carbon (NC=Se) [17, 22, 23]. Such changes provide clear evidence of the formation of desired organoselenium compounds. Only representative data of pre-ligands **(1, 4),** azolium salts **(5, 6),** and their corresponding selenium adducts **(9, 10)** are shown in Fig 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 spectra **(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 (Fig S3-S17). But it is generally seen that the changing in size of the substituted alkyl chain at benzimidazole, chemical shift values also altered (range 153.0-180.6 δ ppm) (Fig 1).



Compounds	Alkyl group (R)				
	R^1	R^2	R^3	R^4	R^5
1	Methyl	H	Propyl	–	–
2	Methyl	Methyl	Butyl	–	–
3	H	H	Hexyl	–	–
4	H	H	Butyl	–	–
5, 9	Methyl	H	Propyl	Propyl	–
6, 10	H	H	Butyl	Octyl	–
7, 11	H	H	Hexyl	2-ethyl hexyl	–
8, 12	Methyl	Methyl	Butyl	Butyl	Pentyl

Scheme-1: Synthesis of benzimidazolium-based salts (5-8) and their respective selenium adducts (9-12).

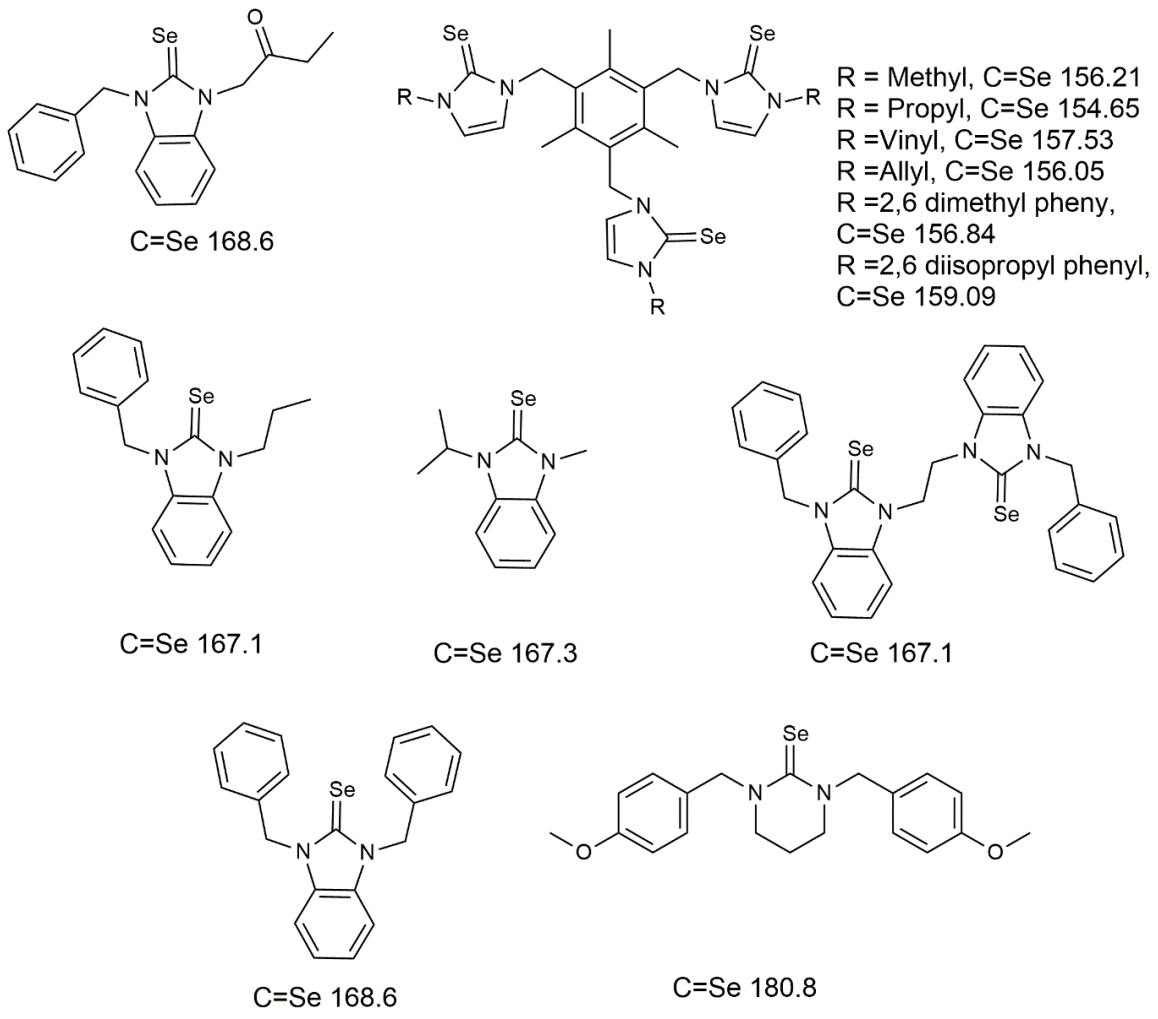


Fig 1: General presentation of chemical shift values ranges from 153.0-180.6 δ ppm.

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

Comp.	Zones of inhibition (mm)			MIC (µg/mL)		
	B. subtilis.	*M. brunensis.*	*B. cereus.*	*B. subtilis.*	*M. brunensis.*	*B. cereus*
5	**18 ± 1.2**	**20 ± 0.4**	**18 ± 0.2**	**21 ± 0.5**	**35 ± 0.6**	**38±0.1**
6	**17 ± 0.2**	**19 ± 0.3**	**16 ± 0.6**	**35 ± 0.4**	**33 ± 0.6**	**n.a**
7	**16 ± 0.1**	**18 ± 0.5**	**19 ± 0.1**	**45 ± 0.4**	**46 ± 0.7**	**43 ± 0.5**
8	**26 ± 0.3**	**19 ± 0.4**	**20 ± 0.3**	**23 ± 0.3**	**18 ± 0.6**	**28 ± 0.7**
9	**23 ±01**	**20 ±03**	**16±0.2**	**16±0.2**	**17 ±0.3**	**20 ±0.1**
10	**21 ± 0.5**	**18 ± 0.2**	**18 ± 0.4**	**19 ± 0.6**	**15 ± 0.9**	**23 ± 0.7**
11	**25 ± 0.4**	**22 ± 0.1**	**21 ± 0.6**	**24 ± 0.5**	**20 ± 0.3**	**27 ± 0.1**
12	**22 ± 0.5**	**21 ± 0.1**	**27 ± 0. 3**	**17 ± 0.2**	**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), na= not active

Biological studies

In vitro antibacterial potential

Broth dilution and disk diffusion methods were used to evaluate the zones of inhibition (100µL) and minimum inhibition concentration (MIC) respectively for the synthesized compounds (**5-12**) against *B. subtilis*, *M. brunensis* and *B. cereus* using ciprofloxacin as the reference drug (Table-1). Compounds **5-8** have an inhibition zone of 16±0.1 to 26±0.3mm against all tested bacterial strains while selenium-NHC adducts **9-12** have a zone of inhibition (16±0.2 to 25 ± 0.4mm). The change in activities of azolium salts and adducts may be due to the replacement of acidic proton and counter ion (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). Adduct **12** showed good activity against all tested strains with ZI value 25 ± 0.1, 22 ± 0.5, 17 ± 0.3 mm and MIC values 17 ± 0.2, 16 ± 0.4, and 18 ± 0.3 µg/mL against *B. Subtilis*, *M. Brunensis* and *B. cereus* respectively. Selenium-NHC carbene adduct **12** displayed pronounced activity (ZI=27 ± 0.6 mm) to the reference drug [24] ciprofloxacin (MIC 25 ± 0.9 mm) against B. cereus. Previous research demonstrated that the bond between selenium and benzimidazole plays a very momentous role in biological activity in addition to other factors e.g solubility and degree of polymerization [10]. In another research outcome, scientists described that redox selenium compound covalently attached to peptides and bacterial phage to kill bacteria by generating superoxide radicals [25]. Broad spectrum antibacterial Se-NHC adducts might be due to the exchange phenomenon with the thiol group (Sulfur) of some proteins such as thioredoxin reductase of bacteria. Pathogenic bacteria died due to the inhibition of TrxR as it inhibits the GSH system [26]. In another mechanism, researchers analyzed that there is an interaction with the free thiol group of cysteine, after the scission of the Se-N bond of ebselen [27]. The NHC moiety in selenium-NHC adduct only participate as a carrier in a biological system for the transport of selenium ion to the site of action, therefore the antibacterial potential of selenium adducts depends on the release of selenium ions and ease of azolium, salt exchange process [10]. 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 [28]. Table-1 shows the susceptibility of B. cereus to selenium adduct from smaller MICs and larger inhibition zones. In addition, the presence of aromatic substitution on the benzimidazole ring increases its activity by increasing lipophilicity, helping selenium ions pierce into the cell membrane of microorganisms and destroy the function of organelles' respiratory and metabolic processes [24, 29]. 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].

Hemolytic potential

Hemolysis experiments were carried out to plaid the interaction between azolium salts and Se-*N*-Het-*C* adducts with normal erythrocytes of the mouse. Hemolysis destroys RBCs 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 azolium salts/adducts were performed by direct contact with erythrocytes using PBS (Fig 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 [6, 30]. In the comparison of hemolytic potential between NHC salt and selenium adduct, it was seen that the NHC salts have more hemolytic potential.

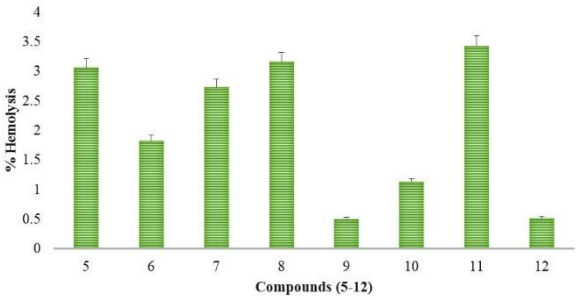


Fig.. 2: Percentages of hemolytic activity of NHC salts **5-8** and Se-NHC adducts 9-12.

In vitro thrombolytic activity

The thrombolytic potential was gaged by a published method [31]. The maximum clot lysis (83 %) was achieved when both positive control and clots were incubated at 37 °C for 3 hours. Clot lysis was reduced to 31% when clots were cured with distilled water taken as a negative control. Results revealed that the thrombolytic activity of salts and selenium adducts was analogous to streptokinase (positive control). Results (Fig 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.

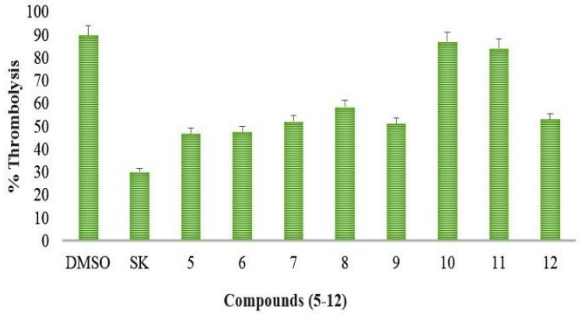


Fig**.** 3: Percentage thrombolysis of NHC salts **5-8**. and Se-NHC adducts **9-12**.

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. Compounds **5-8** have an inhibition zone of 16±0.1-26±0.3mm against all tested bacterial strains while selenium-NHC adducts **9-12** have a zone of inhibition (16±0.2 ± 25 ± 0.4mm). Adduct **12** showed good activity against all tested strains with ZI values 25 ± 0.1, 22± 0.5, 17 ± 0.3 mm and MIC values 17 ± 0.2, 16 ± 0.4, and 18 ± 0.3 µg/mL against *B. subtilis, M. brunensis and B. cereus* respectively. The results of Hemolysis and thrombolysis assays have shown that the compounds are safe for preclinical studies on mouse blood *in vitro*.

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