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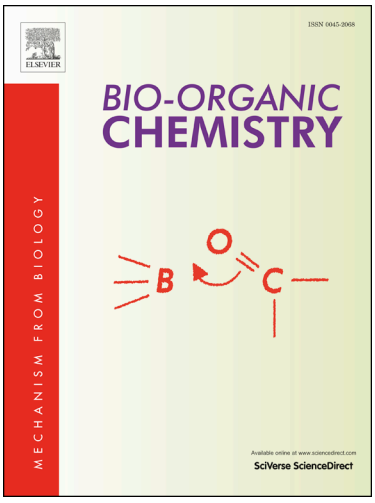

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Synthesis and Investigation of inhibitory activities of Imidazole Derivatives Against sthe Metallo-β-Lactamase IMP-1

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Abstract

Mutations in bacteria can result in antibiotic resistance due to the overuse or abuse of β-lactam antibiotics. One strategy which bacteria can become resistance toward antibiotics is secreting of metallo β-lactamase enzymes that can open the lactam ring of the β-lactam antibiotic and inactivate them. This issue is a threat for human health and one strategy to overcome this situation is co-administration of β-lactam antibiotics with an inhibitor. So far, no clinically available inhibitors of metallo β-lactamases (MBLs) reported and the clinically inhibitors of serine β-lactamase are useless for MBLs. Accordingly, finding a potent inhibitor of the MBLs being very important. In this study, imidazole derivatives primarily were synthesized and their inhibitory activity were measured. Later in silico binding model was used to predict the configuration and conformation of the ligands into the active site of enzyme. Two molecules demonstrated with IC_{50} of 39 μM and 46 μM against MBL (IMP-1).

Introduction

Since their discovery in 1930, β-lactam antibiotics have become the standard treatment for bacterial infections. The use of antibiotics is not monitored strictly in many parts of the world and more than 50% of antibiotics are used without prescription worldwide.[1-8] Due to the clinical overuse of antibiotics, many bacteria have become resistance against β-lactam antibiotics with emergence of bacterial pathogens by secreting β-lactamases (enzymes that inactivate these antibiotics by hydrolyzing their β-lactam ring) which cause a matter of great concern. According to the mechanism of action, β-Lactamases are classified into serine-β-lactamases (Classes A, C and D) and MBLs (Class B). MBLs are further divided into three subclasses, B1, B2, and B3, depending on their amino acid sequences and metal occupancies with one or two zinc (II) ions in the active site.[1-9] The inactivation of β-lactam antibiotics by MBLs, is a significant threat to public health. This issue can be controlled by either discovery of new antibiotics or the use of an inhibitor co-administered with β-lactam antibiotics.[10, 11] At the current time, none of them are clinically available. Moreover, MBLs are not inhibited by clavulanic acid, an inhibitor commonly co-administered with β-lactam antibiotics for serine β-lactamases. Therefore, there are currently no clinically available inhibitors of MBLs. In addition, MBLs are characterized by αββα quaternary structural fold. The zinc ion(s) containing active site is located at the edge of the ββ sheets in a pocket. The distance between two zinc ions is roughly 3.4-4.4 Å for most of the MBLs. There is a bridging water molecule between two zinc ions and the activated water molecule plays an important role in the hydrolysis of

antibiotics. In the active site of subclass B1, one of the zinc ions is coordinated by three histidines (His116, His118 and His196) and a water molecule with the arrangement of tetrahedral while the second zinc ion is coordinated by Asp120, Cys221, His263 and two water molecules with the geometry of distorted trigonal bipyramidal (Figure 1).[12-20] The aim of the research is to introduce a new series of MBLs inhibitors derived from Imidazole and investigate their inhibitory activity.

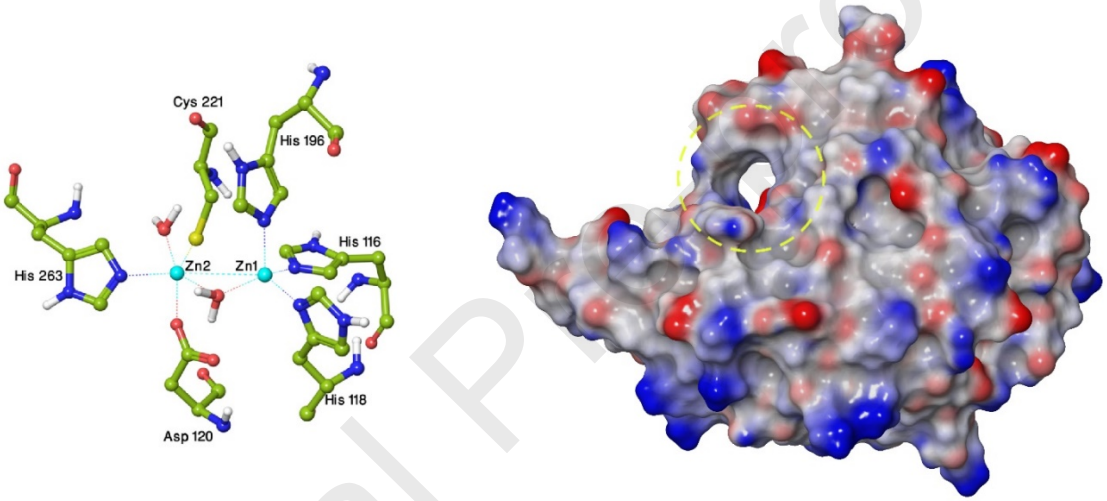


Figure 1: Representative active-site pocket of MBL (IMP-1, subclass B1 of metallo β-lactamase (MBLs), PDB Code: 1JJT). The active site is shown with yellow dash circle and the left picture shows zinc ions and the amino acids of the active site of IMP-1.

Inhibitors of MBLs have been reported in different categories such as thiols,[21-27] sulphates,[28] dicarboxylic acids,[29-34] trifluoromethylketones and alcohols,[35] hydroxamates,[36] tetrazoles,[37] sulfonamides and sulfonyl hydrazones.[38] Furthermore, compounds containing a zinc-binding sulfur atom comprise the first category of MBLs inhibitors.[39]

Chemistry

In this study, to find a new series of inhibitors, two imidazole molecules (Figure 2), reported by Vella *et al*.[40] with IC_{50} at the concentration of 1 mM against IMP-1, were used as the lead compounds for synthesis of further analogues.

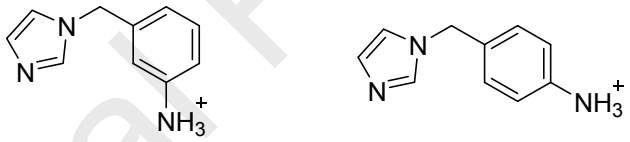
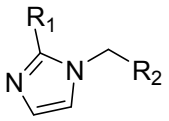


Figure 2: Lead compounds with IC_{50} at the concentration of 1 mM against IMP-1.[40]

Accordingly, a series of imidazole analogues with electron -donor and -acceptor (Figure 3) were synthesized and their inhibitory activity evaluated against IMP-1. In total, twenty-four molecules and lead compounds were synthesized and ^{1}H NMR and ^{13}C NMR spectra and HRMS were recorded. Some of these compounds are new and were not already reported in the literature. All chemicals required for the process is commercially available with inexpensive price which is an advantage to produce the final compound in a single

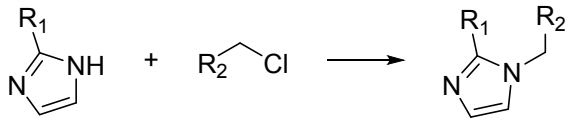
step. The other advantages of these chemicals are their stability as they are not easily oxidized when access to fresh air or dissolved in water. Furthermore, because of the hydrogen -donor and -acceptor groups, they are expected to be soluble in water at the time of assay. These molecules were synthesized in one step by three different procedures.



1	R_1=H, R_2=phenyl	**14**	R_1=CH_3, R_2=3-nitrophenyl
2	R_1=CH_3, R_2=phenyl	**15**	R_1=H, R_2=4-nitrophenyl
3	R_1=H, R_2=4-methylphenyl	**16**	R_1=CH_3, R_2=4-nitrophenyl
4	R_1=CH_3, R_2=4-methylphenyl	**17**	R_1=H, R_2=2-chloro-5-nitrophenyl
5	R_1=H, R_2=2-chlorophenyl	**18**	R_1=CH_3, R_2=2-chloro-5-nitrophenyl
6	R_1=CH_3, R_2=2-chlorophenyl	**19**	R_1=H, R_2=3-anilinium **(reference molecule)**
7	R_1=H, R_2=4-chlorophenyl	**20**	R_1=CH_3, R_2=3-anilinium
8	R_1=CH_3, R_2=4-chlorophenyl	**21**	R_1=H, R_2=4-anilinium **(reference molecule)**
9	R_1=H, R_2=4-methoxyphenyl	**22**	R_1=CH_3, R_2=4-anilinium
10	R_1=CH_3, R_2=4-methoxyphenyl	**23**	R_1=H, R_2=2-chloro-5-anilinium
11	R_1=H, R_2=1-naphthyl	**24**	R_1=CH_3, R_2=2-chloro-5-anilinium
12	R_1=CH_3, R_2=1-naphthyl	**25**	R_1=H, R_2=4-hydroxyphenyl
13	R_1=H, R_2=3-nitrophenyl	**26**	R_1=CH_3, R_2=4-hydroxyphenyl

Figure 3: two reference compounds and other imidazole derivatives

Compounds **1-18** were synthesized with the general procedure reported by Salvio *et al*.[41] with the yield of 55-93% and the synthetic pathway of the reaction is shown in Scheme 1. The ^{1}H NMR and ^{13}C NMR spectra were recorded and were in agreement with compounds that already reported. For all known and new compounds, LRMS and HRMS (positive mode) spectra were also recorded to make sure about the purity of these compounds as a key factor at the time of assay.



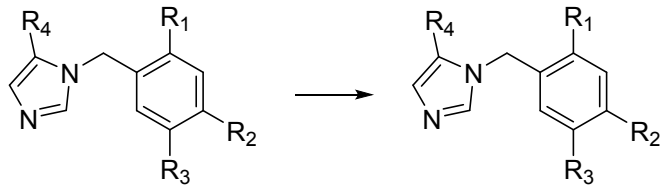
27 R_1=H
28 R_1=CH_3

29 R_2=phenyl
30 R_2=4-methylphenyl
31 R_2=2-chlorophenyl
32 R_2=4-chlorophenyl
33 R_2=4-methoxyphenyl
34 R_2=1-naphthyl
35 R_2=3-nitrophenyl
36 R_2=4-nitrophenyl
37 R_2=2-chloro-5-nitrophenyl

1 R_1=H, R_2=phenyl
2 R_1=CH_3, R_2=phenyl
3 R_1=H, R_2=4-methylphenyl
4 R_1=CH_3, R_2=4-methylphenyl
5 R_1=H, R_2=2-chlorophenyl
6 R_1=CH_3, R_2=2-chlorophenyl
7 R_1=H, R_2=4-chlorophenyl
8 R_1=CH_3, R_2=4-chlorophenyl
9 R_1=H, R_2=4-methoxyphenyl
10 R_1=CH_3, R_2=4-methoxyphenyl
11 R_1=H, R_2=1-naphthyl
12 R_1=CH_3, R_2=1-naphthyl
13 R_1=H, R_2=3-nitrophenyl
14 R_1=CH_3, R_2=3-nitrophenyl
15 R_1=H, R_2=4-nitrophenyl
16 R_1=CH_3, R_2=4-nitrophenyl
17 R_1=H, R_2=2-chloro-5-nitrophenyl
18 R_1=CH_3, R_2=2-chloro-5-nitrophenyl

Scheme 1: Reagents and conditions: Dry CH_3CN, K_2CO_3, reflux, and yield=55-93%.

Compounds **19-24** were synthesized in one step with the general procedure reported by Roumen *et al*.[42] using Pd/C, HCl, H_2 in ethanol shown in Scheme 2. These molecules were prepared in a single step by a range yield of 80-92% and their chemical structures were investigated by ^{1}H NMR and ^{13}C NMR spectra as well as recording LRMS and HRMS (positive mode) spectra to investigate the purity of these compounds.

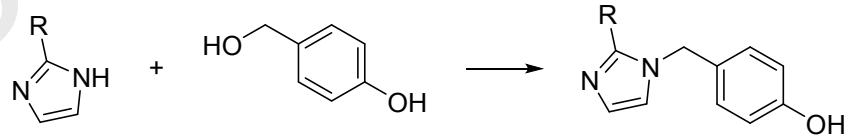


13 R_1=H, R_2=H, R_3=NO_2, R_4=H
14 R_1=H, R_2=H, R_3=NO_2, R_4=CH_3
15 R_1=H, R_2=NO_2, R_3=H, R_4=H
16 R_1=H, R_2=NO_2, R_3=H, R_4=CH_3
17 R_1=Chloro, R_2=H, R_3=NO_2, R_4=H
18 R_1=Chloro, R_2=H, R_3=NO_2, R_4=CH_3

19 R_1=H, R_2=H, R_3=NH_3^+, R_4=H
20 R_1=H, R_2=H, R_3=NH_3^+, R_4=CH_3
21 R_1=H, R_2=NH_3^+, R_3=H, R_4=H
22 R_1=H, R_2=NH_3^+, R_3=H, R_4=CH_3
23 R_1=Chloro, R_2=H, R_3=NH_3^+, R_4=H
24 R_1=Chloro, R_2=H, R_3=NH_3^+, R_4=CH_3

Scheme 2: Reagents and conditions: Pd/C (5%), H_2, ethanol, HCl, reflux, 24h, yield: 80-92%.

The general procedure reported by Vallée *et al*.[43] was used for the synthesis of compounds **25** and **26** and the synthetic pathway is shown in Scheme 3. The mixture of starting materials was heated up to 160 °C for half an hour without addition of solvent to produce the products a range yield of 80-90%. These compounds are known and their ^{1}H NMR and ^{13}C NMR spectra were in agreement with the reference.[43] For both molecules LRMS and HRMS (positive mode) were recorded for the purpose of purity assessment.



27 R_1=H
28 R_1=CH_3

56

25 R=H
26 R=CH_3

Scheme 3: Reagents and conditions: 160 °C, 30 min, yield: 80-90%.

Biological Evaluation

Enzyme Expression and Purification

The IMP-1 enzyme, lacking the first 21 signal peptide amino acid residues, was expressed and purified using the protocol of Vella *et al*.[40] Details of the expression and purification of the enzyme are presented in the Supplementary data.

Kinetic Assays

CENTA (sodium (7R)-3-(((3-carboxylato-4-nitrophenyl)thio) methyl)-8-oxo-7-(2-(thiophen-2-yl)acetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate) was used as the substrate for the study of kinetic assay and it was synthesized by a procedure reported by Bebrone *et al*.[44] Inhibition assays for IMP-1 were performed in 96 well 400 µL multi-titre plates using a multi-plate spectrophotometer and the hydrolysis rate of CENTA was measured at λ=405 nm. The initial screening assay was performed in duplicate at concentrations of 1 mM, 500µM, 250µM, 100µM, 50µM and 10µM of inhibitors, with CENTA (a type of cephalosporin) as the substrate and HEPESX (50 mM HEPES, 0.1 M NaCl, 100 mM $ZnCl_2$, pH 7.0) as the buffer at 25 °C. At the time of assay, some compounds were insoluble at the concentration of 1 mM but were soluble at the other concentrations. The final concentrations of enzyme and CENTA were 5 nM and 70 µM respectively. Bovine serum albumin (final concentration of 20 µg/ml) was added to

the enzyme to stability. For each well, 196 µL substrate and 4 µL inhibitor were added followed by the addition of 200 µL of enzyme. The rate of hydrolysis of substrate was recorded for 5 minutes. As per discussed, MBLs catalyze the hydrolysis of β-lactam antibiotics (Figure 4) which eventually lead to their inactivation against bacteria. When CENTA is hydrolyzed by MBLs, a yellow chromophore compound (3-carboxy-4-nitrobenzenethiolate, $\varepsilon = 6400\ M^{-1}\ cm^{-1}$) is released which is detectable at the wavelength of 405 nm. (Figure 4)

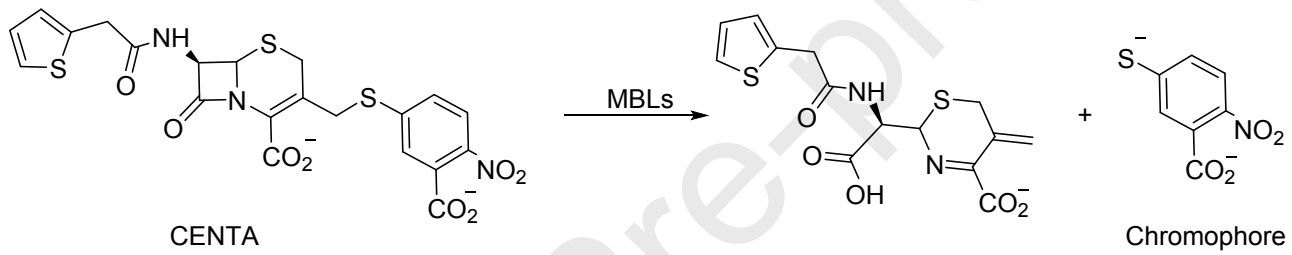


Figure 4: Hydrolysis of CENTA by MBLs and releasing a yellow chromophore.

The results indicate that nearly 90% of all compounds are at least 5 to 10-fold more active than reference compounds (Table 1). In addition, more than half of the synthesized molecules show IC_{50} nearly 100 µM while the reference molecules show IC_{50} at 1 mM. Furthermore, compounds with R = methyl group are more potent than analogues with hydrogen group. This issue can be explained by the better fitting or locking of these inhibitors into the active site of enzyme.

For calculation of IC_{50}, the reciprocal rate (1/V) plotted versus concentration of inhibitors in Excel and the trendline equation was found. IC_{50} was then estimated using the fitted line equation where the x-intercept=IC_{50}.

In this group, compound **16** with the nitro substitution, an electron donor group, in para position of aromatic phenyl ring shows the highest inhibition activity with IC_{50} of 39 μM. Compound **12** is the second potent enzyme-based inhibitor with IC_{50} of 46 μM.

Table 1: IC_{50} of imidazole derivatives **1-26** against IMP-1

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
1	>100	**14**	84.7
2	>100	**15**	>100
3	>100	**16**	39.0
4	89.3	**17**	>100
5	>100	**18**	>100
6	88.6	**19***	970
7	88.1	**20**	64.6
8	81.5	**21***	962
9	>100	**22**	64.7
10	92.5	**23**	>100
11	>100	**24**	>100
12	46.0	**25**	>100
13	>100	**26**	97.6

*Reference compounds

Computational Modeling

For the study of computational modeling, the crystal structure of IMP-1 enzyme (PDB code: 1JJT) was downloaded from Protein Data Bank (PDB) and Chain B was deleted as the topologies of the active sites of both chain A and chain B are identical. Further elimination for Chain A was carried out by removing water molecules (solvents), single Zinc ion and the coordinated ligand of the active site. The cavity of the active site with two zinc ions was set by MolDock for the investigation of docking study. To predict the conformation and configuration of ligands into the active site of enzyme, synthesized molecules as well as reference compounds were docked into the active site of IMP-1. The computational modeling showed that all compounds are in proper size to fit into the active site of IMP-1 as it is located in the pocket of enzyme and apparently larger molecules can't get easily access to this part. The in-silico modeling shows different configurations and conformations for this series of compounds into the active site. For instance, compounds that have nitro substitution are coordinated into zinc ions via the oxygen of nitro group while the other molecules without electron-donor groups are coordinated into zinc ions via the lone pair of nitrogen of imidazole. Moreover, the molecules with electron-donor groups can create further interactions with amino acids of the active sites. The investigation of computational modeling of ligand **16** (figure 5) predicted that one of the oxygen atoms of nitro group is coordinated to both zinc atoms with the predicted distances of 5.10 Å (Zn1) and 3.36 Å (Zn2) and the other oxygen of nitro group predicted to form hydrogen bonds with Gly232 and Lys224 with the distances of 1.70 Å and 2.04 Å

respectively. The aromatic ring of this ligand can also create a π-π interaction with aromatic ring of TRP64 which predicted to increase the potency of inhibition activity.

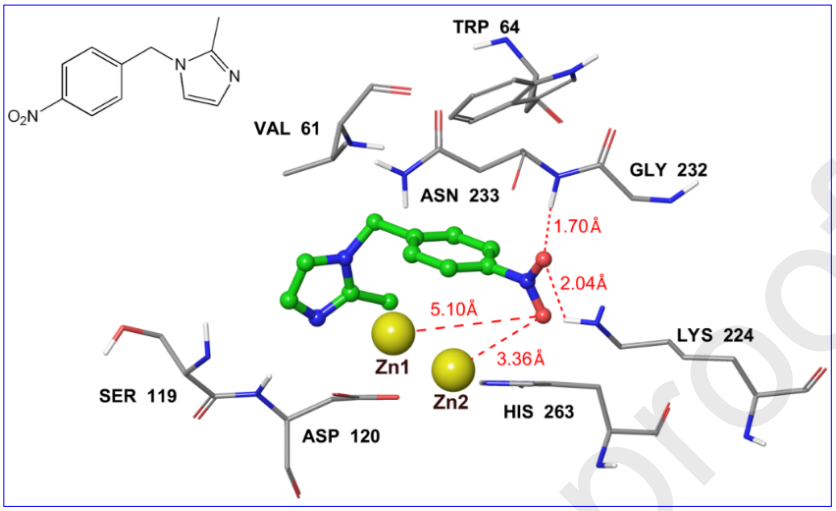


Figure 5: Interactions of ligand **16** at the active site of IMP-1 (PDB: 1JJT). Atom colors are as follows: blue–nitrogen, red–oxygen, grey–carbon and yellow-zinc atom (of IMP-1), and red–oxygen, blue–nitrogen, green–carbon, and yellow–sulfur (on inhibitor).

Conclusion

In order to introduce a new class of inhibitors of IMP-1, twenty-four molecules derived from imidazole were synthesized and their inhibitory activity and IC_{50} were measured. The results demonstrated that all molecules are more potent than reference molecules against IMP-1. Furthermore, two compounds show the best IC_{50} with the values of 39 µM and 46 µM in this series of molecules. The study demonstrated that the addition of the electron-donor group to imidazole derivatives can increase the potency of inhibitory activity against IMP-1 with better coordination to zinc ions of the active site.

Experimental

Synthesis

All chemicals were purchased from Sigma-Aldrich, Merck and Fluka chemical companies. All NMR experiments were recorded on Bruker AVANCE 500, 400 or 300 MHz spectrometers. Chemical shifts are reported in parts per million (ppm) on a δ scale and referenced to the residual solvent peak (^{1}H, 7.24 ppm, ^{13}C, 77.0 ppm for $CDCl_3$; ^{1}H, 2.49 ppm, ^{13}C, 39.5 ppm for DMSO-d_6; ^{1}H, 3.30 ppm, ^{13}C, 49.5 ppm for CD_3OD). Coupling constants (*J*) are reported in Hertz. Multiplicities of the peaks are abbreviated as follows: s for singlet, bs for broad singlet, d for doublet, t for triplet, and q for quartet. Low- and high- resolution EI-MS were measured on a Finnigan MAT 900 XL-Trap mass spectrometer in positive and negative ionization mode. LR-ESI were recorded on a Bruker HCT 3D Ion Trap and HR-ESI were performed on a BrukerMicrOTof-Q with the DIONEX Ultimate 3000 LC in positive and negative electrospray ionization mode, with CH_3OH as solvent. ^{1}H NMR and ^{13}C NMR spectra are shown in the Supplementary data.

General procedure 1:

Compounds **1-18** were synthesized using the following general procedure reported by Salvio *et al.*[41] A mixture of potassium carbonate (2.0 g, 14.4 mmol), imidazole derivatives **27-28** (32.9 mmol) and compounds **29-37** (4.7 mmol) in dry acetonitrile (50 ml) was heated under reflux and the progress of the reaction was monitored by TLC. Then

the solvent was evaporated under the vacuum and the residue was dissolved in DCM (100 ml) *. The organic layer was washed with saturated sodium bicarbonate aqueous solution (2×50 ml), passed through diatomaceous earth, dried over sodium sulfate, evaporated under the vacuum, and purified by flash column chromatography (ethyl acetate-hexane 0-100%)** to give the products **1-18**.

*Note 1: Ethyl acetate (200 ml) was used for compounds **17** and **18**.

Note 2: Compounds **17 and **18** (ethyl acetate-methanol 0-100%)

General procedure 2:

The general procedure reported by Roumen *et al*.[43] was modified and used (HCl 2M was added in the reaction) for the synthesis of hydrochloride salt of the molecules **19-24**. A mixture of compounds **13-18** (4.6 mmol), HCl 2M (4 ml), Pd/C 5% (100 mg) in ethanol (20 ml) was hydrogenated under reflux at atmospheric pressure for 24 hours. The organic solvent was evaporated under the vacuum and water (50 ml) was added and stirred for 10 minutes and the solid impurity was filtered off then water phase was washed with ethyl acetate (2×50 ml), passed through diatomaceous earth, and evaporated under the vacuum and dried in oven at 40 °C to give the products **19-24**.

General procedure 3:

Compounds **25-26** were synthesized using the following general procedure reported by Vallée *et al*.[43] A mixture of imidazole derivatives **27-28** (40 mmol) and 4-hydroxybenzyl alcohol **56** (1.0 g, 8.0 mmol) was heated at 160 °C for half an hour which resulted dark brown oil. The mixture of the reaction was then cooled down to 50-80° C and added to a stirring hot water (100 ml) resulted brown solid residue which was filtered off. Then the residue was transferred to a round bottom flask and ethyl acetate (2×50 ml) was added and stirred for 10 min and then it was filtered off and dried in the oven at 40 °C to give the products **25-26**.

1-Benzyl-1*H*-imidazole **(1)**

Pale yellow solid, 0.61 g (82 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (1H, s, NCHN), 7.37-7.25 (3H, m, Ph-H), 7.16-7.08 (2H, m, Ph-H), 7.06 (1H, s, NCH<u>CH</u>NCH_2), 6.87 (1H, s, N<u>CH</u>CHNCH_2), 5.08 (2H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.4 (Ar-C), 136.1 (Ar-C), 129.7 (Ar-C), 128.9 (Ar-C), 128.2 (Ar-C), 127.2 (Ar-C), 119.2 (Ar-C), 50.7 (CH_2). HRMS calculated for $C_{10}H_{11}N_2$ $[M+H]^+$ 159.0922, found 159.0924. m.p. 71-73 °C (lit. m.p. 71-72 °C).[41] The NMR spectra are in agreement with the literature.[41]

1-Benzyl-2-methyl-1*H*-imidazole **(2)**

Pale yellow oil, 0.65 g (80 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30-7.17 (3H, m, Ph-H), 6.98 (2H, d, *J*=1.5 Hz, Ph-H), 6.88 (1H, d, *J*=1.3 Hz, NCH<u>CH</u>NCH_2), 6.76 (1H, d, *J*=1.4 Hz, N<u>CH</u>CHNCH_2), 4.95 (2H, s, CH_2), 2.45 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$):

δ 144.5 (Ar-C), 136.1 (Ar-C), 128.6 (Ar-C), 127.6 (Ar-C), 126.9 (Ar-C), 126.3 (Ar-C), 119.6 (Ar-C), 49.3 (CH_2), 12.8 (CH_3). HRMS calculated for $C_{11}H_{13}N_2$ $[M+H]^+$ 173.1073, found 173.1077. The NMR spectra are in agreement with the literature.[45]

1-(4-Methylbenzyl)-1*H*-imidazole **(3)**

Brown solid, 0.66 g (81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (1H, s, NCHN), 7.08 (2H, d, *J*=7.9 Hz, Ph-H), 7.04-6.94 (3H, m, Ph-H & NCHCHNCH$_2$), 6.81 (1H, t, *J*=1.2 Hz, NCHCHNCH$_2$), 4.97 (2H, s, CH_2), 2.27 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.7 (Ar-C), 137.1 (Ar-C), 132.9 (Ar-C), 129.4 (Ar-C), 129.3 (Ar-C), 127.0 (Ar-C), 118.9 (Ar-C), 50.2 (CH_2), 20.8 (CH_3). HRMS calculated for $C_{11}H_{13}N_2$ $[M+H]^+$ 173.1073, found 173.1076. m.p. 49-50 °C (lit. m.p. 49-50 °C).[46] The NMR spectra are in agreement with the literature.[46]

2-Methyl-1-(4-methylbenzyl)-1*H*-imidazole **(4)**

Brown oil, 0.69 g (79%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.09 (2H, d, *J*=7.9 Hz, Ph-H), 6.94-6.86 (3H, m, Ph-H & NCHCHNCH$_2$), 6.77 (1H, d, *J*=1.3 Hz, NCHCHNCH$_2$), 4.94 (2H, s, CH_2), 2.28 (6H, s, both CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.7 (Ar-C), 137.5 (Ar-C), 133.2 (Ar-C), 129.4 (Ar-C), 127.1 (Ar-C), 126.5 (Ar-C), 119.7 (Ar-C), 49.3 (CH_2), 20.9 (Ar-Me), 13.0 (Imidazole-Me). HRMS calculated for $C_{12}H_{15}N_2$ $[M+H]^+$ 187.1230, found 187.1227.

1-(2-Chlorobenzyl)-1*H*-imidazole **(5)**

Brown oil, 0.78 g (86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48 (1H, s, NCHN), 7.35-7.29 (1H, m, Ph-H), 7.25-7.11 (2H, m, Ph-H), 7.01 (1H, t, *J*=1.1 Hz, NCHCHNCH$_2$), 6.90-6.84 (2H, m, Ph-H & NCHCHNCH$_2$), 5.14 (2H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.4 (Ar-C), 133.8 (Ar-C), 132.8 (Ar-C), 129.6 (Ar-C), 129.5 (Ar-C), 129.4 (Ar-C), 128.8 (Ar-C), 127.2 (Ar-C), 119.1 (Ar-C), 48.1 (CH_2). HRMS calculated for $C_{10}H_{10}ClN_2$ $[M+H]^+$ 193.0527, found 193.0534. R_f= 0.13 (20/80 ethyl acetate/petroleum ether). The NMR spectra are in agreement with the literature.[47]

1-(2-Chlorobenzyl)-2-methyl-1*H*-imidazole **(6)**

Brown solid, 0.83 g (85 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (1H, dd, *J*=7.3, 1.3 Hz, Ph-H), 7.24-7.10 (2H, m, Ph-H), 6.92 (1H, d, *J*=1.3 Hz, NCHCHNCH$_2$), 6.77 (1H, d, *J*=1.3 Hz, NCHCHNCH$_2$), 6.60 (1H, d, *J*=6.5 Hz, Ph-H), 5.07 (2H, s, CH_2), 2.28 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.0 (Ar-C), 134.0 (Ar-C), 132.3 (Ar-C), 129.5 (Ar-C), 129.1 (Ar-C), 127.6 (Ar-C), 127.5 (Ar-C), 127.3 (Ar-C), 119.8 (Ar-C), 47.2 (CH_2), 12.8 (CH_3). HRMS calculated for $C_{11}H_{12}ClN_2$ $[M+H]^+$ 207.0684, found 207.0687. m.p=62.5-63.5 °C.

1-(4-Chlorobenzyl)-1*H*-imidazole **(7)**

Pale yellow oil, 0.84 g (93 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (1H, s, NCHN), 7.06 (2H, d, *J*=8.5 Hz, Ph-H), 6.88 (3H, m, Ph-H & NCHCHNCH$_2$), 6.67 (1H, t, *J*=1.3 Hz,

NCHCHNCH$_2$), 4.84 (2H, s, CH$_2$). ^{13}C NMR (75 MHz, CDCl$_3$): δ 136.7 (Ar-C), 134.3 (Ar-C), 133.3 (Ar-C), 129.2 (Ar-C), 128.4 (Ar-C), 128.1 (Ar-C), 118.6 (Ar-C), 49.3 (CH$_2$). HRMS calculated for $C_{10}H_{10}ClN_2$ $[M+H]^+$ 193.0527, found 193.0533. The NMR spectra are in agreement with the literature.[46]

1-(4-Chlorobenzyl)-2-methyl-1*H*-imidazole **(8)**

Brown viscous oil, 0.85 g (88 %). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.29 (2H, t, *J*=13.6 Hz, Ph-H), 6.98-6.92 (3H, m, Ph-H & NCHCHNCH$_2$), 6.80 (1H, d, *J*=1.4 Hz, NCHCHNCH$_2$), 5.00 (2H, s, CH$_2$), 2.31 (3H, s, CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 144.9 (Ar-C), 134.8 (Ar-C), 133.9 (Ar-C), 129.2 (Ar-C), 128.0 (Ar-C), 127.4 (Ar-C), 119.8 (Ar-C), 49.1 (CH$_2$), 13.0 (CH$_3$). HRMS calculated for $C_{11}H_{12}ClN_2$ $[M+H]^+$ 207.0684, found 207.0685.

1-(4-Methoxybenzyl)-1*H*-imidazole **(9)**

Pale yellow solid, 0.80 g (90%). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.42 (1H, s, NCHN), 7.03 (3H, t, *J*=8.5 Hz, Ph-H & NCHCHNCH$_2$), 6.85-6.78 (3H, m, Ph-H & NCHCHNCH$_2$), 4.97 (2H, s, CH$_2$), 3.73 (3H, s, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ 159.3 (Ar-C), 137.1 (Ar-C), 129.5 (Ar-C), 128.7 (Ar-C), 128.0 (Ar-C), 119.0 (Ar-C), 114.2 (Ar-C), 55.1 (CH$_3$), 50.1 (CH$_2$). HRMS calculated for $C_{11}H_{12}N_2NaO$ $[M+Na]^+$ 211.0842, found 211.0846. m.p. 57-59 °C (lit. m.p. 57-58 °C).[48] The NMR spectra are in agreement with the literature.[48]

1-(4-Methoxybenzyl)-2-methyl-1*H*-imidazole **(10)**

Brown viscous oil, 0.85 g (89%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.93 (2H, d, *J*=11.0 Hz, Ph-H), 6.84 (1H, d, *J*=1.3 Hz, NCHCHNCH$_2$), 6.78 (2H, d, *J*=11.0 Hz, Ph-H), 6.72 (1H, d, *J*=1.3 Hz, NCHCHNCH$_2$), 4.87 (2H, s, CH_2), 3.69 (3H, s, CH_3O), 3.25 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.0 (Ar-C), 144.5 (Ar-C), 128.1 (Ar-C), 127.9 (Ar-C), 126.9 (Ar-C), 119.5 (Ar-C), 114.0 (Ar-C), 55.0 (CH_3O), 49.0 (CH_2), 12.9 (CH_3). HRMS calculated for $C_{12}H_{15}N_2O$ $[M+H]^+$ 203.1179, found 203.1179.

1-(Naphthalen-1-ylmethyl)-1*H*-imidazole **(11)**

Dark brown viscous oil, 0.88 g (90 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93-7.81 (3H, m, naphthyl), 7.66 (1H, s, NCHN), 7.56-7.47 (2H, m, naphthyl), 7.43 (1H, dd, *J*=8.2, 7.0 Hz, naphthyl), 7.18 (1H, d, *J*=7.0 Hz, naphthyl), 7.09 (1H, s, NCHCHNCH$_2$), 6.92 (1H, s, NCHCHNCH$_2$), 5.59 (2H, s, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.3 (Ar-C), 133.8 (Ar-C), 131.0 (Ar-C), 130.8 (Ar-C), 129.4 (Ar-C), 129.0 (Ar-C), 128.9 (Ar-C), 127.1 (Ar-C), 126.4 (Ar-C), 126.3 (Ar-C), 125.4 (Ar-C), 122.3 (Ar-C), 119.5 (Ar-C), 48.9 (CH_2). HRMS calculated for $C_{14}H_{13}N_2$ $[M+H]^+$ 209.1073, found 209.1077. The NMR spectra are in agreement with the literature.[49]

2-Methyl-1-(naphthalen-1-ylmethyl)-1*H*-imidazole **(12)**

Dark brown solid, 0.84 g (80 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90-7.71 (3H, m, naphthyl), 7.56-7.46 (2H, m, naphthyl), 7.33 (1H, dd, *J*=8.2, 7.2 Hz, naphthyl), 6.94 (1H,

d, *J*=1.3 Hz, NCHCHNCH$_2$), 6.75 (1H, dd, *J*=7.1, 1.0 Hz, naphthyl), 6.72 (1H, d, *J*=1.4 Hz, NCHCHNCH$_2$), 5.39 (2H, s, CH$_2$), 2.32 (3H, s, CH$_3$). ^{13}C NMR (100 MHz, CDCl$_3$): δ 144.9 (Ar-C), 133.4 (Ar-C), 131.5 (Ar-C), 130.2 (Ar-C), 128.8 (Ar-C), 128.4 (Ar-C), 127.2 (Ar-C), 126.6 (Ar-C), 126.0 (Ar-C), 125.3 (Ar-C), 124.1 (Ar-C), 122.0 (Ar-C), 119.8 (Ar-C), 47.2 (CH$_2$), 12.8 (CH$_3$). HRMS calculated for $C_{15}H_{15}N_2$ $[M+H]^+$ 223.1230, found 223.1234. m.p. 103.5-104.5 °C.

1-(3-Nitrobenzyl)-1*H*-imidazole **(13)**

Brown solid, 0.67 g (70 %). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.18-8.08 (2H, m, Ph-H), 7.82 (1H, s, NCHN), 7.73-7.60 (2H, m, Ph-H), 7.25 (1H, t, *J*=1.4 Hz, NCHCHNCH$_2$), 6.93 (1H, t, *J*=1.0 Hz, NCHCHNCH$_2$), 5.36 (2H, s, CH$_2$). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 147.9 (Ar-C), 140.1 (Ar-C), 137.6 (Ar-C), 134.1 (Ar-C), 130.3 (Ar-C), 129.0 (Ar-C), 122.7 (Ar-C), 122.1 (Ar-C), 119.6 (Ar-C), 48.5 (CH$_2$). HRMS calculated for $C_{10}H_{10}N_3O_2$ $[M+H]^+$ 204.0768, found 204.0767. m.p. 95-97 °C (lit. m.p. 95-97 °C).[50] The NMR spectra are in agreement with the literature.[51]

2-Methyl-1-(3-nitrobenzyl)-1*H*-imidazole **(14)**

Brown solid, 0.91 g (89 %). ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.14 (1H, m, Ph-H), 7.95 (1H, s, Ph-H), 7.51 (1H, t, *J*=8.0 Hz, Ph-H), 7.29 (1H, d, *J*=8.0 Hz, Ph-H), 6.97 (1H, s, NCHCHNCH$_2$), 6.84 (1H, s, NCHCHNCH$_2$), 5.14 (2H, s, CH$_2$), 2.31 (3H, s, CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 148.6 (Ar-C), 144.8 (Ar-C), 138.6 (Ar-C), 132.3 (Ar-C),

130.1 (Ar-C), 128.1 (Ar-C), 123.0 (Ar-C), 121.5 (Ar-C), 119.7 (Ar-C), 48.9 (CH_2), 13.1 (CH_3). HRMS calculated for $C_{11}H_{12}N_3O_2$ $[M+H]^+$ 218.0924, found 218.0929. m.p. 82-83 °C.

1-(4-Nitrobenzyl)-1*H*-imidazole **(15)**

Brown solid, 0.86 g (90 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.19 (2H, d, *J*=8.5 Hz, Ph-H), 7.55 (1H, s, NCHN), 7.25 (2H, d, *J*=8.5 Hz, Ph-H), 7.11 (1H, t, *J*=1.2 Hz, NCH<u>CH</u>NCH$_2$), 6.88 (1H, t, *J*=1.2 Hz, N<u>CH</u>CHNCH$_2$), 5.23 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 147.8 (Ar-C), 143.4 (Ar-C), 137.5 (Ar-C), 130.4 (Ar-C), 127.7 (Ar-C), 124.2 (Ar-C), 119.2 (Ar-C), 49.9 (CH_2). HRMS calculated for $C_{10}H_{10}N_3O_2$ $[M+H]^+$ 204.0768, found 204.0770. m.p. 55-56 °C (lit. m.p. 55-56 °C).[52] The NMR spectra are in agreement with the literature.[53]

2-Methyl-1-(4-nitrobenzyl)-1*H*-imidazole **(16)**

Black solid, 0.90 g (88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (2H, d, *J*=8.8 Hz, Ph-H), 7.16 (2H, d, *J*=8.6 Hz, Ph-H), 7.00 (1H, s, NCH<u>CH</u>NCH$_2$), 6.85 (1H, s, N<u>CH</u>CHNCH$_2$), 5.16 (2H, s, CH_2), 2.32 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.7 (Ar-C), 143.4 (Ar-C), 127.7 (Ar-C), 127.2 (Ar-C), 124.5 (Ar-C), 124.3 (Ar-C), 119.9 (Ar-C), 49.1 (CH_2), 12.9 (CH_3). HRMS calculated for $C_{11}H_{12}N_3O_2$ $[M+H]^+$ 218.0924, found 218.0927. m.p. 96-97 °C (lit. m.p. 95-96 °C). The NMR spectra are in agreement with the literature.[54]

1-(2-Chloro-5-nitrobenzyl)-1*H*-imidazole **(17)**

Brown solid, 0.61 g (55 %). ^{1}H NMR (500 MHz, CD_3OD): δ 8.17 (1H, dd, *J*=8.8, 2.6 Hz, Ph-H), 7.89 (1H, d, *J*=2.7 Hz, Ph-H), 7.83 (1H, s, NCHN), 7.70 (1H, d, *J*=8.8 Hz, Ph-H), 7.19 (1H, t, *J*=1.3 Hz, NCHCHNCH$_2$), 7.05 (1H, t, *J*=1.2 Hz, NCHCHNCH$_2$), 5.46 (2H, s, CH_2). ^{13}C NMR (125 MHz, CD_3OD): δ 148.4 (Ar-C), 140.9 (Ar-C), 139.2 (Ar-C), 138.0 (Ar-C), 132.1 (Ar-C), 129.8 (Ar-C), 125.5 (Ar-C), 125.0 (Ar-C), 121.1 (Ar-C), 48.8 (CH_2). HRMS calculated for $C_{10}H_9ClN_3O_2$ $[M+H]^+$ 238.0378, found 238.0386. m.p. 107-109 °C. The NMR spectra are in agreement with the literature.[51]

1-(2-Chloro-5-nitrobenzyl)-2-methyl-1H-imidazole (**18**)

Dark brown solid, 0.65 g (55 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.16 (1H, dd, *J*=8.8, 2.7 Hz, Ph-H), 7.72 (1H, d, *J*=8.7 Hz, Ph-H), 7.56 (1H, d, *J*=2.6 Hz, Ph-H), 7.06 (1H, d, *J*=1.3 Hz, NCHCHNCH$_2$), 6.94 (1H, d, *J*=1.3 Hz, NCHCHNCH$_2$), 5.37 (2H, s, CH_2), 2.34 (3H, s, CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 148.5 (Ar-C), 146.7 (Ar-C), 140.5 (Ar-C), 137.9 (Ar-C), 132.2 (Ar-C), 127.8 (Ar-C), 125.3 (Ar-C), 123.9 (Ar-C), 121.5 (Ar-C), 48.0 (CH_2), 12.5 (CH_3). HRMS calculated for $C_{11}H_{11}ClN_3O_2$ $[M+H]^+$ 252.0534, found 252.0537. m.p. 88-90 °C.

1-(3-Aminobenzyl)-*1H*-imidazol-3-ium **(19)**

Pale brown solid, 0.79 g (82 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.39 (1H, s, NHCHN), 7.79 (1H, t, *J*=1.7 Hz, NHCHCHN), 7.72 (1H, t, *J*=1.7 Hz, NHCHCHN), 7.45 (1H, t,

J=7.8 Hz, Ph-H), 7.34-7.23 (3H, m, Ph-H), 5.50 (2H, s, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 136.8 (Ar-C), 135.5 (Ar-C), 134.8 (Ar-C), 130.2 (Ar-C), 126.0 (Ar-C), 122.4 (Ar-C), 122.1 (Ar-C), 121.4 (Ar-C), 120.3 (Ar-C), 51.0 (CH_2). HRMS calculated for $C_{10}H_{12}N_3$ $[M]^+$ 174.1031, found 174.1034. m.p. 217-218 °C.

1-(3-Aminobenzyl)-2-methyl-*1H*-imidazol-3-ium **(20)**

Pale yellow solid, 0.82 g (80 %). ^1H NMR (300 MHz, DMSO-d_6): δ 8.28-8.18 (2H, m, Ph-H), 7.81-7.66 (3H, m, Ph-H & NHCHCHN), 7.62 (1H, d *J*=2.1 Hz, NHCHCHN), 5.56 (2H, s, CH_2), 2.63 (3H, s, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 148.0 (Ar-C), 144.6 (Ar-C), 136.7 (Ar-C), 134.6 (Ar-C), 130.6 (Ar-C), 123.4 (Ar-C), 122.9 (Ar-C), 122.2 (Ar-C), 118.3 (Ar-C), 48.9 (CH_2), 10.5 (CH_3). HRMS calculated for $C_{11}H_{14}N_3$ $[M]^+$ 188.1188, found 188.1191. m.p. 219-220 °C.

1-(4-Aminobenzyl)-*1H*-imidazol-3-ium **(21)**

Brown solid, 0.81 g (82 %). ^1H NMR (300 MHz, DMSO-d_6): δ 9.34 (1H, t, *J*=1.5 Hz, NHCHN), 7.78 (1H, t, *J*=1.6 Hz, NHCHCHN), 7.68 (1H, t, *J*=1.6 Hz, NHCHCHN), 7.45 (2H, d, *J*=8.5 Hz, Ph-H), 7.27 (2H, d, *J*=8.5 Hz, Ph-H), 5.43 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 135.3 (Ar-C), 134.0 (Ar-C), 133.6 (Ar-C), 129.5 (Ar-C), 122.9 (Ar-C), 121.9 (Ar-C), 120.2 (Ar-C), 50.9 (CH_2). HRMS calculated for $C_{10}H_{12}N_3$ $[M]^+$ 174.1031, found 174.1032. m.p. 209-210 °C. The ^1H NMR spectrum of neutral compound has been reported in the literature.[42]

1-(4-Aminobenzyl)-2-methyl-*1H*-imidazol-3-ium **(22)**

Brown solid, 0.84 g (80 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (2H, d, *J*=8.5 Hz, Ph-H), 7.75 (1H, d, *J*=2.0 Hz, NHCHCHN), 7.61 (1H, d, *J*=2.0 Hz, NHCHCHN), 7.56 (2H, d, *J*=8.7 Hz, Ph-H), 5.60 (2H, s, CH_2), 2.59 (3H, s, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 147.3 (Ar-C), 144.6 (Ar-C), 142.2 (Ar-C), 128.9 (Ar-C), 124.0 (Ar-C), 122.3 (Ar-C), 118.5 (Ar-C), 49.0 (CH_2), 10.4 (CH_3). HRMS calculated for $C_{11}H_{14}N_3$ $[M]^+$ 188.1188, found 188.1193. m.p. 183-185 °C.

1-(5-Amino-2-chlorobenzyl)-*1H*-imidazol-3-ium **(23)**

Pale yellow solid, 1.06 g (92 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.35 (1H, t, *J*=1.3 Hz, NHCHN), 8.38 (1H, d, *J*=2.7 Hz, Ph-H), 8.28 (1H, dd, *J*=8.8, 2.8 Hz, Ph-H), 7.85 (1H, d, *J*=8.8 Hz, Ph-H), 7.80 (1H, t, *J*=1.7 Hz, NHCHCHN), 7.74 (1H, t, *J*=1.7 Hz, NHCHCHN), 5.71 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 146.6 (Ar-C), 140.1 (Ar-C), 136.1 (Ar-C), 134.0 (Ar-C), 131.3 (Ar-C), 126.2 (Ar-C), 125.6 (Ar-C), 122.3 (Ar-C), 120.2 (Ar-C), 49.2 (CH_2). HRMS calculated for $C_{10}H_{11}ClN_3$ $[M]^+$ 208.0642, found 208.0645. m.p. 233-234 °C.

1-(5-Amino-2-chlorobenzyl)-2-methyl-*1H*-imidazol-3-ium **(24)**

Black solid, 1.12 g (92 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26 (1H, dd, *J*=8.8, 2.8 Hz, Ph-H), 8.11 (1H, d, *J*=2.7 Hz, Ph-H), 7.87 (1H, t, *J*=8.8 Hz, Ph-H), 7.64 (1H, d, *J*=2.0 Hz, NHCHCHN), 7.58 (1H, d, *J*=2.0 Hz, NHCHCHN), 5.60 (2H, s, CH_2), 2.66 (3H, s,

CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 146.7 (Ar-C), 145.2 (Ar-C), 139.7 (Ar-C), 133.7 (Ar-C), 131.4 (Ar-C), 125.3 (Ar-C), 125.3 (Ar-C), 122.2 (Ar-C), 118.4 (Ar-C), 47.7 (CH_2), 10.6 (CH_3). HRMS calculated for $C_{11}H_{13}ClN_3$ $[M]^+$ 222.0798, found 222.0799. m.p. 199-201 °C.

4-((1*H*-imidazol-1-yl)methyl)phenol **(25)**

Pale brown solid, 1.12 g (80%). ^1H NMR (400 MHz, DMSO-d_6): δ 9.47 (1H, bs, OH), 7.68 (1H, s, NCHN), 7.10-7.01 (3H, m, Ph-H & NCH<u>CH</u>NCH_2), 6.86 (1H, t, *J*=1.0 Hz, N<u>CH</u>CHNCH_2), 6.71 (2H, d, *J*=8.5 Hz, Ph-H), 5.02 (2H, s, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.0 (Ar-C), 137.1 (Ar-C), 129.1 (Ar-C), 128.5 (Ar-C), 127.9 (Ar-C), 119.3 (Ar-C), 115.3 (Ar-C), 49.1 (CH_2). HRMS calculated for $C_{10}H_{10}N_2NaO$ $[M+Na]^+$ 197.0685, found 197.0689. m.p. 210-212 °C (lit. m.p. 210-211 °C). The NMR spectra are in agreement with the literature.[52]

4-((2-Methyl-1*H*-imidazol-1-yl)methyl)phenol **(26)**

Brown solid, 1.25 g (90%). ^1H NMR (300 MHz, DMSO-d_6): δ 9.46 (1H, bs, OH), 7.05 (1H, d, *J*=1.3 Hz, NCH<u>CH</u>NCH_2), 6.99 (2H, d, *J*=8.6 Hz, Ph-H), 6.75-6.68 (3H, m, Ph-H & N<u>CH</u>CHNCH_2), 4.97 (2H, s, CH_2), 2.22 (3H, s, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.8 (Ar-C), 143.6 (Ar-C), 128.6 (Ar-C), 127.6 (Ar-C), 126.2 (Ar-C), 120.0 (Ar-C), 115.4 (Ar-C), 48.3 (CH_2), 12.8 (CH_3). HRMS calculated for $C_{11}H_{13}N_2O$ $[M+H]^+$

189.1022, found 189.1022. m.p. 209-210 °C. The NMR spectra are in agreement with the literature.[52]

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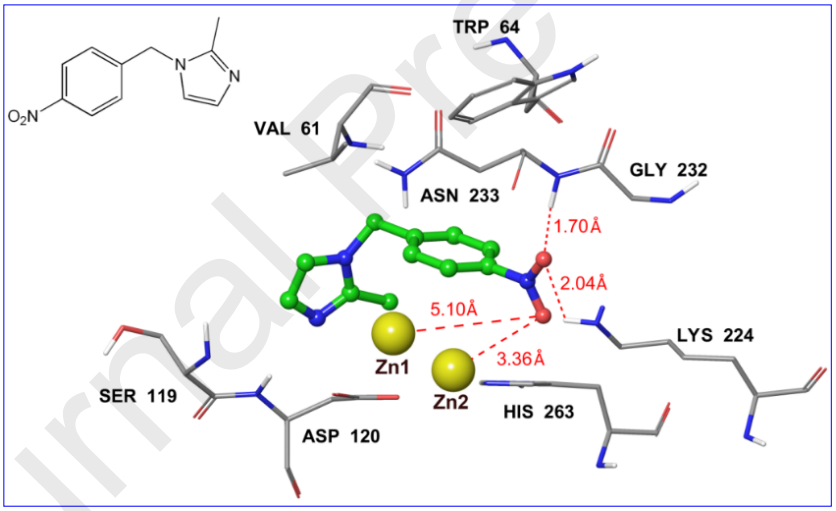
Research Highlight:

In this study, we described synthesis of a new series of Metallo β-lactamases inhibitors derived from imidazole compound. Some molecules are new organic compounds and two molecules show potent inhibitory activity with IC50 of 27 μM and 35 μM against IMP-1, (a subclass B1 of MBLs). The analytical structures of the synthesized compounds were approved by ^{1}H-NMR, ^{13}C-NMR and high-resolution mass spectroscopy.

Yours Sincerely,
Associate Professor Hadi Adibi

Graphical Abstract



IC_{50} = 39 μM against IMP-1