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SYNTHESIS, CHARECTERIZATION AND BIOLOGICAL EVALUATION OF POTENTIAL METALLO-B-LACTAMASE INHIBITORS

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ABSTRACT

β – lactam anti-infective agents are the most commonly used antibacterial drug and developing resistance to these medications is a worry. Metallo-β-lactamases are a various set of enzymes that catalyze the hydrolysis of a broad range of β-lactam drugs including carbapenems. This decent variety is reflected in the perception that the enzyme mechanism varies in light of whether may be a couple zincs are bound in the active site which, in turn, is reliant on the subclass of β-lactamase. The spread of the genes encoding these enzymes among Gram-negative bacteria has made them a vital reason for resistance. Thereare right now no clinically accessible inhibitors to inhibit metallo-β-lactamase activity. The reason for this investigation is to make various types of MBL inhibitors, and test for their exercises on various microorganism. In this examination our point is to combination some more up to date 2-substituted-3-mercaptopropanoic acids subordinates as metallo β – lactamase inhibitor.

KEYWORDS: β-lactamase; antibiotic resistance; carbapenem; zinc metallo-enzyme.

INTRODUCTION

The increase in antibioticresistance among gram-negative microorganisms is an eminent case of how microscopic organisms can acquire, keep up, and express new hereditary data that can give resistance from one or a few anti-infection agents. This hereditary plasticity can happen both inter and intra-generically. Gram-negative bacterial resistance potentially now levels

with or usurps that of gram-positive bacterial resistance and has provoked calls for similarinfection control measures to check their dissemination.[1]

β-Lactamases of class B are metallo-proteins, additionally called metallo-β-lactamases. These proteins utilize a zinc-bound hydroxyl group as the nucleophile to advance the hydrolysis of an exceptionally wide range of β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems.

Rather than serine β-lactamases, MBLs use not less than one yet more regularly two Zn^{2+}ions in their dynamic site to catalyze the hydrolysis of β-lactam rings. In 2009, another carbapenem-safe Klebsiella pneumoniae strain was found in New Delhi (India) by a group of researchersguide by Yong. It was later named as NDM-1.[2] This represented another challenge to develop new MBL inhibitors as none of the anti-infection agents were compelling against NDM-1.

MBLs are arranged into three subclasses (B1, B2 and B3).[3] This classification depends on substrate selectivity and amino acidsequence, especially the amino acid ligands that chelate the Zn^{2+} ions.[4,5]

MBLs can hydrolytically degrade the greater part of the β-lactam antibiotics including more current age β-lactam antibiotics like cephalosporins and carbapenems.[6,7] Clinical inhibitors of serine β-lactamase, for example, clavulanic acid, sulbactam and tazobactam are likewise substrates of MBLs.[6,8] Additionally, none of the serine β lactamase inhibitors are compelling against MBLs.

One potential system for beating the impacts of MBLs is to co-administer MBL inhibitors with β-lactam antibiotics. Inhibitors avoid binding of the MBL enzyme to β-lactam antibiotics and anticipate degradation and deactivation of antibiotics.

Despite the fact that there are clinically accessible inhibitors for serine β-lactamase, there are no clinically accessible inhibitors for MBLs. Furthermore, none of the inhibitors for serine β-lactamases are successful against MBL enzymes.[9] The aim of this investigation is to outline and orchestrate inhibitors against MBL enzymes.

MATERIALS AND METHOD

Experimental procedure

Meltingpoints were resolved on an electro thermal mechanical assembly by open capillaries and are uncorrected. Thin-layer chromatography was expert on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Perception was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a Shimadzu-fouriertransform infra-red (FTIR)-8400 Spectrophotometer utilizing KBr plate. ^{1}H NMR spectra were recorded on a Bruker DPX-400 MHz spectrometer. Chemical shiftingare communicated in δ ppm downfield from TMS as an internal standard.

Preparation of metallo β – lactamase inhibitor

A. Reductive amination

General procedure

The aldehyde (1.5 eq) was mixed to a suspension of cysteine (1.0 eq) and $NaBH_3CN$ (1.0 eq) in methanol (5 ml for each 2 mmol) at room temperature and stirred for 22-26 hours. A white precipitateobtained and was filtered of and washed with methanol. During the reaction, the evolving gas was risen through a mixture of 6 ml chlorine dye (asolution of roughly 3-6 % sodium hypochlorite, NaClO) and 5 ml 10 % NaOH.

Preparation of compound 1a (2-(benzylamino)-3-mercaptopropionic acid

Benzaldehyde about 0.33 g was added to a suspension of 0.25 g cysteine and $NaBH_3CN$ (1.0 eq) in methanol (5 ml per 2 mmol) at room temperature and stirred for 22-26 hours. A white precipitate formed and was filtered of and washed with methanol, gave compound 1a

Yield: 0.084mmol, 1.02 g, 84.0 %; Colour :white solid. Melting point: 114ºC

^{1}H NMR (D_2O with NaOH, 400 MHz): δ 7.46 – 7.01 (m, 5H), 3.62 (d, *J* = 12.7 Hz, 1H), 3.47 (d, *J* = 12.7 Hz, 1H), 2.90 (dd, *J* = 8.3, 5.0 Hz, 1H), 2.64 (dd, *J* = 12.6, 5.0 Hz, 1H), 2.45 (dd, *J* = 12.7, 8.4 Hz, 1H).

13C NMR (D2O with NaOH, 400 MHz): δ 181.7, 128.6, 128.5, 127.1, 125.8, 67.8, 51.2, 28.4.

IR: 3027 cm^{-1}, 1606 cm^{-1}

Preparation of compound 1b [3-mercapto-2-(phenethylamino)propionic acid]

Phenylacetaldehyde of 0.37 g was added to a suspension of and 0.25 g of cysteine and $NaBH_3CN$ (1.0 eq) in methanol (5 ml per 2 mmol) at room temperature and stirred for 22-26 hours produced compound 1b.

Yield: 0.098 mmol, 1.02 g, 84 % (11.4 %; Colour: White ; Texture: solid.; Mlting point: 122 ^{o}C

^{1}H NMR (D2O with NaOH, 400 MHz): δ 7.19 (m, 5H), 2.86 (dd, *J* = 8.0, 5.4 Hz, 1H), 2.67 (t, *J* = 4.3 Hz, 4H), 2.58 (dd, *J* = 12.5, 5.3 Hz, 2H), 2.41 (dd, *J* = 12.5, 8.0 Hz, 1H).

13C NMR (D2O with NaOH, 400 MHz): δ 181.84, 140.31, 128.81, 128.63, 126.24, 68.60, 48.87, 35.11, 28.19.

IR: 2803 cm-1, 1581 cm^{-1}

B. Sulfonation of amino acids

General procedure

The sulfonyl chloride (1 eq) was mixed to a solution of the amino acid (1.2 eq) in aqueous K_2CO_3 (2-3 eq). Under vigorous stirring, the mixture was heated to 70° C for 30 min, cooled in an icebath, and acidified (HCl) to pH 2.5 by dropwise addition of concentrated HCl under stirring. The precipitate was collected by filtration and washed with a minimum of cold water.

Preparation of compound 2a [3-(acetamidomethylthio)-2-(4-methylphenyl sulfonamide) propanoic acid]

Para-toluene sulfonyl chloride of 0.15 g was added to a solution of and, 0.25 g S-acetamidomethyl-L-cysteine HClin aqueous K_2CO_3 (2-3 eq) under vigorous stirring, the mixture was heated to 70° C for 30 min, cooled in an ice bath, and acidified (HCl) to pH 2.5 by dropwise addition of concentrated HCl under stirring. gave compound 2a.

Yield : 0.25 g, 0.72 mmol, 86 %; Melting point : 112^{o} C

^{1}H NMR (CD3OD, 400 MHz): δ 7.75 (d, J = 7.6 Hz, 2H), 7.34 (d, J = 7.8 Hz, 2H), 4.25 (s, 2H),

4.08 (t, J = 6.4 Hz, 1H), 2.96 (dd, J = 14.0, 5.5 Hz, 1H), 2.85 (dd, J = 13.9, 7.3 Hz, 1H), 2.41 (s, 3H), 1.95 (s, 3H).

^{13}C NMR (CDCl3, 400 MHz): δ 171.63, 171.62, 143.30, 137.74, 129.13, 126.85, 55.98, 40.74, 33.53, 21.20, 20.05.

IR: 3402 cm-1, 3280 cm-1, 2959 cm-1, 1732 cm^{-1}

Preparation of compound 2b (3-hydroxy-2-(4-methylphenylsulfonamido)propanoic acid)

Para-toluene sulfonyl chloride 0.48 g was added to a solution of serine (0.32 g) in aqueous K_2CO_3 (2-3 eq) under vigorous stirring, the mixture was heated to 70° C for 30 min, cooled in

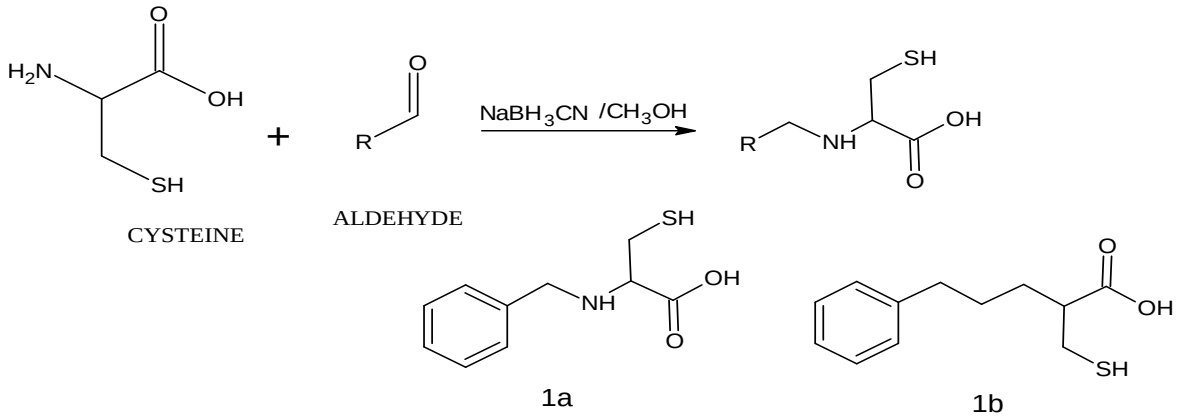
an ice bath, and acidified (HCl) to pH 2.5 by dropwise addition of concentrated HCl under stirring. gave compound 2b

Yield: 1.08 g, 4.4 mmol, 85 %; Melting point: 115º C

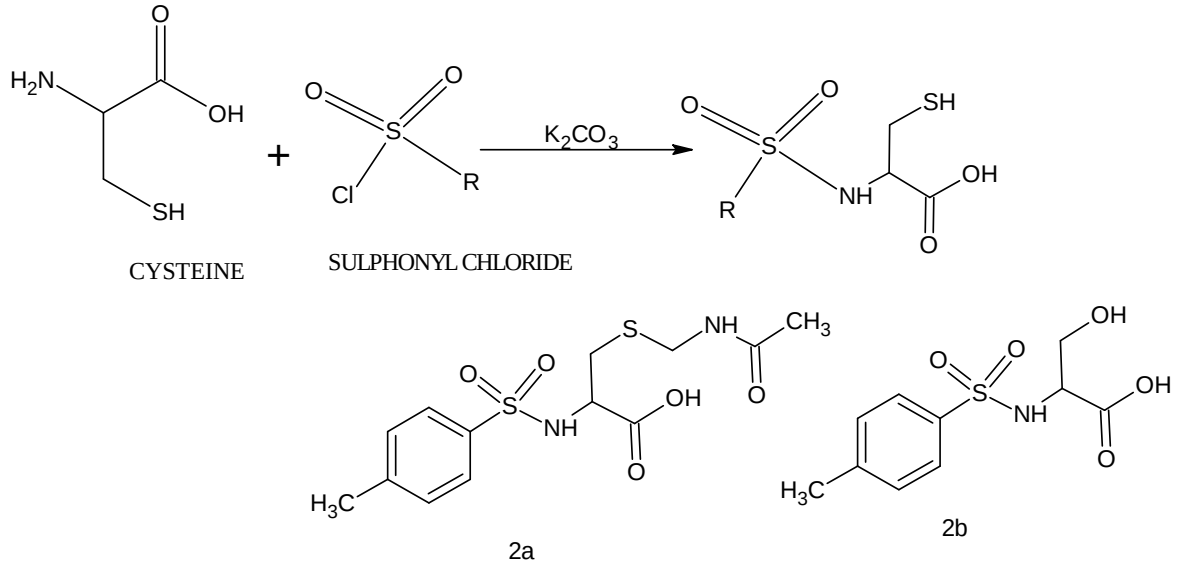
^{1}H NMR (DMSO, 400 MHz): δ 7.90 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 8 Hz 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 3.46 (m, 3H), 2.34 (s, 3H).

^{13}C NMR (DMSO, 400 MHz): δ 171.76, 143.00, 138.66, 129.83, 126.98, 62.48, 58.41, 39.96, 21.41, 3.78.

IR: 2916 cm-1, 1579 cm^{-1}



Scheme – 1 Reductive amination.



Scheme – 2 Sulfonation of amino acids.

Antimicrobial activity

The bacterial growth inhibition was evaluated by disk diffusion method[10,11] 5 µl of concentrations of 100µg/mL each MBL inhibitor (IMBL) 1a-b and 2a-b against Pseudomonas

aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 27853. Solvent DMSO was used as solvent control, Mueller-Hinton agar (MHA) broth was used as media for antibacterial test. Standard drugs like gentamicin was used for comparison purpose. After incubation at 37 ºC in 24h for bacteria, the zones of inhibition were measured. The bioactivity data of the screening test of synthesized compounds is given in Table 1. The minimum inhibitory concentration (MIC) values of the active compounds screened from the Table1. The MIC was the lowest concentration of test compound that was able to inhibit visible growth of the bacteria and was determined in triplicates. The MIC results were displayed in Table-2.

Tablet 1: Antimicrobial activity of Synthesized Compounds (diameter of zone of inhibition in mm).

Compound	Zone of inhibition in mm		
	S.aureus	P.aeruginosa	E.coli
1a	11	8	12
1b	16	13	14
2a	7	8	10
2b	10	11	10
Gentamicin	27	23	22

Tablet 2: Antibacterial activity of synthetic compounds (minimum inhibitory concentration in µg/mL).

Compound	Minimum Inhibitory Concentration in µg/ml		
	S.aureus	P.aeruginosa	E.coli
1a	32	32	32
1b	32	64	64
2a	32	32	64
2b	32	64	32
Gentamicin	2	4	1

RESULTS AND DISCUSSION

Four new metallo β – lactamase inhibitors (1a,1b,2a and 2b) were synthesized by reductive amination and sulphonation of aminoacid. The percentage yield was 84%, 86% and 85% respectively for compound 1a and 1b, 2a and 2b. All four compounds were screened for their antimicrobial activity against three selective bacteria stain Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 27853 using gentamicin as standard control. The minimum inhibitory concentration (Table – 2) and zone of inhibition

(Table – 1) was measured. Compound 1b and 2b shows maximum antibacterial activity among the four on compare with standard gentamicin.

CONCLUSION

In conclusion, four (1a, 1b, 2a, 2b) new biologically active metallo β – lactamase were synthesized for the first time in this study. The structures of novel compounds were determined by FT-IR, 1H NMR and 13C NMR spectroscopic techniques and analytical methods.

Further study will be required to know the exact mechanism of action and their structure activity relationship.

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