Asian Journal of Chemistry; Vol. 27, No. 11 (2015), 3925-3929

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.18871

Metal Complexes of Ceftriaxone as Potent Medication Against Ailment

MUHAMMAD DANISH[1,*], MUHAMMAD ASAM RAZA[1], SEHRISH[1] and NARGIS ANJUM[2]

[1]Department of Chemistry, University of Gujrat, Hafiz Hayat Campus, Gujrat, Pakistan
[2]Department of Chemistry, University of Sargodha, Sargodha, Pakistan

*Corresponding author: E-mail: drdanish62@gmail.com

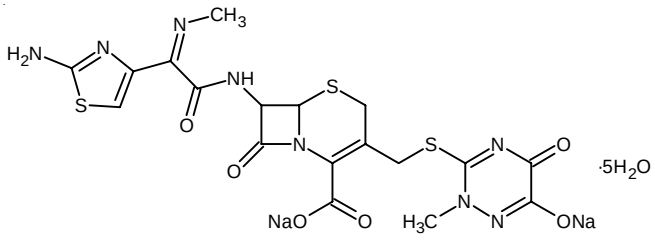
Received: 21 November 2014; *Accepted*: 8 June 2015; *Published online*: 16 July 2015; AJC-17377

Metal-ceftriaxone complexes were synthesized by reacting sodium salt of ceftriaxone with metallic salts or organometallic halides in a common organic solvent that requires a few hours of stirring at certain temperature. These were then purified from an appropriate solvent/mixture of solvents using thin layer chromatography technique. Melting point and FTIR studies indicated the synthesis of the complexes. Infrared studies of the complexes confirmed that ligand bonded to metal ion through the carboxylate ion and the oxygen of the β-lactam thiozolidine ring. These studies have shown that complexes of Co, Ni, Fe, Cu and Sn possess octahedral geometry having d^2sp^3 hybridization. In the spectrum mode of UV/visible spectrophotometer, λ_{max} was determined, close resemblance in the λ_{max} values of ligand and complexes showed that chromophoric groups (C=O, C=C, C=N) were similar in both cases. Antibacterial studies have been carried out against *Escherichia coli* and *Bacillus subtilis*. SH-2 exhibited maximum antimicrobial activity against *E. coli* (7-29 mm). Antioxidant activity was carried out using diphenyl picryl hydrazyl (DPPH) as free radical, SH-5 and SH-7 showed excellent activity (96.03 ± 1.1 % and 87.00 ± 0.9 % respectively). Enzyme inhibition potential activity was checked against three available enzymes and it was concluded from results that SH-5 metal complex of Cu is more potent inhibitor than others (67.92 ± 1.5 %, 84.16 ± 1.7 % and 69.77 ± 1.3 % against protease, AChE and BChE respectively).

Keywords: Metal complexes, Ceftriaxone, DPPH, Proteases, AChE, BChE.

INTRODUCTION

Ceftriaxone is the member of third-generation cephalosporin agents with broad spectrum activity, used for treating Gram-positive as well as Gram-negative bacteria[1]. It is safer, efficient and has good trading in various countries under different names. Ceftriaxone sodium is sold under the name Rocephin, administered both intravenously as well as intramuscularly to treat various diseases[2].



Most commonly used to treat pneumonia, bacterial meningitis, urinary tract, skin, bone, ear infection, typhoid fever, cancroids and gonorrhea[3-6]. The use of antimicrobial drugs for the control of infection was almost entirely a development of the 20th century[7]. Antibiotics exert an effect in the patient that is either bactericidal or bacteriostatic. Those antibiotics that are generally bacteriostatic at concentrations that are achieved clinically (*e.g.*, erythromycin, tetracyclines) inhibit bacterial cell replication but do not kill the organisms. Other antibiotics (cephalosporins, aminoglycosides) are usually bactericidal; they cause bacterial cell death and lysis[8]. Treatment with bacteriostatic drugs stops bacterial growth, thereby allowing the host defenses to catch up in their battle. The selective toxicity of antibiotics means that they must be highly effective against the microbe but have minimal or no toxicity to humans.

According to modern theory of free radical biology and medicine, reactive oxygen species are involved in several disorders[3]. The harmful action of the free radicals can, however, be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids *etc.*), nitrogen compounds (alkaloids, chlorophyll

derivatives), or carotenoids as well as ascorbic acid[9-11]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity[12,13].

Enzyme is a protein molecule acting as catalyst in enzymatic reactions. Enzyme inhibition is a science of enzyme-substrate reaction influenced by the presence of any organic chemical or inorganic metal or biosynthetic compound due to their covalent or non-covalent interactions with enzyme active site[14]. The activity of many enzymes can be inhibited by the binding of specific site by small molecules and ions. This means of inhibiting enzyme activity serves as a major control mechanism in biological systems. The regulation of allosteric enzymes typifies this type of control. In addition, many drugs and toxic agents act by inhibiting enzymes. Inhibition by particular chemicals can be a source of insight into the mechanism of enzyme action: specific inhibitors can often be used to identify residues critical for catalysis[15]. This study was designed in order to check the biological potential of metal complexes of ceftriaxone in terms of antibacterial, antioxidant and enzyme inhibition potential.

EXPERIMENTAL

Diphenyl picrylhydrazyl (DPPH), 5,5'-dithio*bis*[2-nitrobenzoic acid] (DTNB), protease and DMSO were purchased from Sigma-Aldrich (USA) while acetylcholine esterase (AChE) and butyrylcholine esterase (BChE) were obtained from Biochemistry Laboratory, Mayo Hospital, Lahore, Pakistan. All other chemicals and solvents used were of analytical grade from Merck. UV/visible spectrophotometer (UV-4000, Shimadzu) and FTIR (Shimadzu, IR-Prestige-21) were used during studies.

Synthetic scheme: The new compounds were prepared according to literature method[16,17]. Complexes of transition metal were prepared by drop-wise addition of aqueous solution of sodium salt of ceftriaxone to an aqueous solution of transition metal salt (copper chloride, nickel chloride, cobalt nitrate and iron sulphate) in a 250 mL round bottom flask equipped with a water condenser and magnetic stirring bar. The reflux was carried out for about 3 h till complete precipitation occurred. After the formation of precipitates, the reaction mixture was cooled, filtered and the residue was dried comp-letely. The residue was purified by slow precipitation from suitable solvent or solvent mixture. Complexes of organotin halides with sodium salt of ligand acid were prepared by the addition of appropriate triorganotin chloride in methanol to the reaction mixture dropwise with constant stirring. The reaction mixture was then refluxed for 4-6 h, cooled and filtered. The filtrate was concentrated on rotary evaporator and then kept for crystallization. Recrystalization was carried out from a suitable solvent.

Characterization: In the spectrum mode of UV/visible spectrophotometer, λ_{max} was determined. Blank solution was used to correct base line in the range of 190-800 nm. Then sample cell was filled with very dilute sample solution and scan was obtained for UV/visible spectrum (plot of wavelength *vs*. absorbance). λ_{max} was detected from the peak maximum of the spectrum[18].

Antibacterial studies: The representative samples were individually tested against two bacterial strains including *Escherichia coli* and *Bacillus subtilis* using agar well diffusion method. Bacterial strains were available and maintained in the Microbiology laboratory, Department of Zoology, University of Gujrat, Gujrat, Pakistan. Strains were cultured overnight at 37 °C in nutrient agar medium[19].

Antioxidant activity: The radical scavenging ability was measured by using the method of Shahwar *et al.*[20]. 100 µL of the synthesized compound (5 mg/mL in DMSO) was added into 2 mL methanolic solution of DPPH. The reaction mixture was incubated at room temperature for 0.5 h. The decrease in absorbance at 517 nm was noted after 0.5 h.

The percentage scavenging of radical was determined by the following formula:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{A-B}}{\text{A}} \times 100$$

where A is the optical density of blank and B is the optical density of sample.

Protease inhibition assay: Protease inhibition assay was carried out according to the method of Jedinak *et al.*[22] with some modification[21,22]. Tris buffer (100 mM) of pH 7.5 was prepared by dissolving 12.1 g of tris(hydroxymethyl)aminomethane in distilled water and adjusted pH 7.5 with HCl (5 M). The stock solution of trypsin was prepared by dissolving 2 mg of trypsin in 10 mL of 1.0 mM HCl. Nα-benzoyl-DL-arginine-para-nitroanilide hydrochloride (BApNA) was dissolved in DMSO (20 mg/mL). Enzyme (0.3 mL) and inhibitor (100 µL) was incubated at 37 °C for 15 min then 0.6 mM substrate was added and final volume was made 2.5 mL with tris buffer. The reaction mixture was incubated at 37 °C for 0.5 h. The reaction was quenched by adding 30 % acetic acid (0.1 mL) and read the absorbance at 410 nm using UV/visible spectrophotometer. Phenylmethanesulfonylfluoride (PMSF) was used as a positive inhibitor. The percentage inhibition was calculated by using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance (blank) - Absorbance (test)}}{\text{Absorbance (blank)}} \times 100$$

Acetylcholine/butyrylcholine esterase assay: Acetylcholine/butyrylcholine esterase inhibitory activity was measured by spectrophotometric method of Shahwar *et al.*[21,23]. The reaction mixture contained 1500 µL of (100 mM) tris buffer (pH 7.8), 100 µL of DTNB, 100 µL (100 µL) of test compound solution and 100 µL of cholinesterase solution, which were mixed and incubated for 15 min (37 °C). The reaction was initiated by the addition of 100 µL acetylcholine/butyrylthiocholine iodide. The hydrolysis of acetylcholine/butyrylthiocholine iodide was monitored at 412 nm after 0.5 h. Galanthamine was used as positive control. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{E-S}}{\text{E}} \times 100$$

where E is the activity of enzyme without test compound and S is the activity of enzyme with test compound.

RESULTS AND DISCUSSION

Electronic spectra: The value of λ_{max} for ligand under study and synthesized complexes are presented in Table-1. The value of λ_{max} for ligand under study was 291-298 nm that indicated the presence of chromophoric groups like C=O, C=C, C=N. The unsaturation of these groups is responsible for $\pi \rightarrow \pi^*$ transitions. The close resemblance in the λ_{max} values of ligand and complexes showed that chromo-phoric groups are similar in both cases. According to Woodward-Fisher rule the contribution of unsaturated groups (C=O, C=C, C=N) towards the λ_{max} is also in the favour of λ_{max} value. A very little difference in λ_{max} values of complexes from that of the value of ligand may arise due to the coordination of metal ions with ligands to form metal complexes[24].

IR spectroscopy: The infrared spectra of the ligand and its complexes were recorded as KBr pellets in the range 4000-200 cm^{-1}. IR absorptions of these complexes were assigned in comparison with that of free acids and their sodium salts. The absorption bands were assigned by comparison with earlier reports[25] and important frequencies such as ν(CO) lactam, ν(C=O)-NH, $\nu(COO)_{asym}$, $\nu(COO)_{sym}$ and ν(Sn-O) are listed in Table-2. A marked shifting is observed in the –COO frequencies for the complexes as compared to free acid. This vibration is very important in the prediction of the bonding mode of the ligand. In comparison to free acids and their sodium salts, a remarkable change in absorption values of group $\nu(COO)_{asym}$ is observed indicating that this group is directly involved in complexation with metal in form of carboxylate ion thus indicating coordination through that group[24]. IR values of group $\nu(COO)_{asym}$ lowers due to delocalization of π-electrons in the adjacent C=C and C=O bonds, this delocalization increases single bond character in them hence lowers force constants resulting in lowering of the frequencies of C=O and C=C groups. The lactam ν(CO) bond appears in range of 1780-1170 cm^{-1} in free acids and their sodium salts while in spectra of complexes this bond is shifted to lower absorptions indicating that bonding occurs through the oxygen atom of lactam C=O group. The asymmetric stretching absorptions of carboxylate group and lactam group are decreased depending on the nature of metal ion. The shifting of absorptions due to amide carbonyl band ν(C=O)-NH in complexes is not remarkable in comparison to free acids and their salts thus indicating that ligand coordination with metal ions takes place through oxygen atom of lactam C=O group rather than amide carbonyl group[24]. Absorptions near 1300-1000 cm^{-1} indicate the presence of C-O group which is confirmation of ester formation. In the synthesized complexes a marked shifting of this group is observed which also favours completion of complex formation by forming esters[26]. The IR studies of ceftriaxone ligand and its respective complexes suggested that this antibiotic acts as dianionicpentadentate N_2O_3 chelating agent and the sixth position of the octahedral seems to occupy by solvent molecule[27,28]. It is bonded to metal ion through the carboxylate ion and oxygen atom of lactam C=O group rather than amide carbonyl group thus indicating coordination through these groups. The overlapped amide (C=O) and triazole (C=O) bands were recorded. The presence of M-N vibration in range of 475-450 cm^{-1} provide evidence that thiazole moiety is bonded to metal ion through $–NH_2$ group. Their characteristic vibrations are given in Table-2. In case of all R_2SnL_2 derivatives two ν(Sn-C) bands are observed that indicates the non-linear configuration of the alkyl groups around the tin as a consequence of distorted octahedral geometry of the molecule. The terminal NH_2 does not seem coordinating in case of all organotin. Exact geometry of these complexes cannot be ascertained in the absence of crystallography report.

Microbiological screening: The size of inhibition diameter was measured to determine the susceptibility of strains of bacterium towards ceftriaxone and its metal complexes. Bactericidal diameter measured for ligands and metal-complexes shows that they have good activity as bactericides. The average results

TABLE-1
PHYSICAL AND λ_{max} VALUES OF LIGAND/COMPLEXES

Compound	Code	Colour	State	Solubility	Yield (%)	m.p. (°C)	λ_{max} (nm)
Ligand	SH-1	Yellowish	Powder	C_2H_5OH	–	152	291
Bu_3SnL	SH-2	Orange	Powder	C_2H_5OH	71	146	297
Me_2SnL	SH-4	Pink	Powder	C_2H_5OH	75	266	295
CuL	SH-5	Green	Powder	C_2H_5OH	74	284	297
NiL	SH-6	Green	Powder	C_2H_5OH	62	257	297
CoL	SH-7	Pink	Powder	C_2H_5OH	61	268	297
FeL	SH-8	Brown	Powder	C_2H_5OH	68	252	296
Me_3SnL	SH-10	Yellow	Powder	C_2H_5OH	64	129	297

TABLE-2
KEY IR VIBRATIONS (cm^{-1}) OF CEFTRIAXONE COMPLEXES

Compound	ν(C=O) β-lactam	ν(C=O) triazole	$\nu(COO)_{asym}$	$\nu(COO)_{sym}$	ν(C-O)	ν(Sn-O)
SH-1	1747	1571	1606	1392	1035	466
SH-2	1770	1512	1668	1344	1037	468
SH-4	1749	1546	1658	1373	1047	–
SH-5	1759	1533	1670	1350	1043	–
SH-6	1764	1541	1666	1354	1039	–
SH-7	1764	1548	1664	1382	1039	–
SH-8	1691	1442	1512	1220	1118	–
SH-10	1681	1431	1535	1338	1037	445

are shown in Table-3. The results have shown that the metal complexes exhibited two types of behaviour compared with ligands against the same bacteria and under the identical experimental conditions. For example, some of metal-complexes showed higher bactericidal activity than that of corresponding ligand and some metal-complexes have less activity than the ligand. These differences may arise due to steric, electronic, pharmacokinetic factors along with mechanistic pathways. SH-2 which is complex of Bu_3SnCl exhibited maximum activity. The antibacterial screening data showed that the complexes exhibit antimicrobial properties and we noted that the metal chelates exhibited more inhibitory effects than the parent ligand. The increased activity of the metal chelates can be explained on the basis of chelation theory. It is known that chelation tends to make the ligand act as powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand alone. It is observed that, in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligand and there may be *p*-electron delocalization over the whole chelating space. This increases the lipophilic character of the metal chelate and favours its permeation through the lipoid layer of the bacterial membranes. The increased lipophilic character of these complexes seems to be responsible for their enhanced potent antibacterial activity. It may be suggested that these complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms. It has also been proposed that the ultimate action of the toxicant is the denaturation of one or more proteins of the cell, which as a result, impairs normal cellular processes. There are other factors which also increase the activity. These include solubility, conductivity and bond length between the metal and ligand.

TABLE-3
ANTIBACTERIAL ACTIVITIES OF LIGAND AND COMPLEXES

Compound	Zone of inhibition (mm)	
	Escherichia coli	*Bacillus subtilis*
SH-1	14-18 (H.A)	13-14 (M.A)
SH-2	07-29 (H.A)	07-20 (M.A)
SH-4	04-05 (L.A)	05-18 (M.A)
SH-5	03-07 (L.A)	03-06 (L.A)
SH-6	07-09 (L.A)	15-19 (M.A)
SH-7	14-27 (H.A)	11-16 (M.A)
SH-8	06-10 (L.A)	15-28 (H.A)
SH-10	04-07 (L.A)	05-1 (M.A)

H.A: Highly active, M.A: Moderately active, L.A: Less active

Antioxidant activity: Antiradical activity of the synthesized compounds was evaluated using DPPH as free radical. DPPH is stable free radical and being used to check the radical scavenging potential of synthesized as well natural compounds. It was observed that about 80 % of the targeted compounds are active as antioxidant. SH-5 compound showed antioxidant activity equal to that of gallic acid which was used as standard. SH-4 and SH-8 showed low response (15.56 ± 0.7 and 33.96 ± 1.0 %) in this study. The order of reactivity of the synthesized molecules is as SH-5 > SH-10 > SH-7 > SH-6 > SH-1 > SH- 2 > SH- 8 > SH- 4 (Table-4, Figs. 1 and 2). DPPH was used as stable free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule[29]. DPPH is a stable-free radical containing an odd electron in its structure and usually used for detection of the radical scavenging activity in chemical analysis[30]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants[31].

TABLE-4
ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS

Compounds	Inhibition (%)*	IC_{50} (µg/mL)
SH-1	65.01 ± 2.5	49 ± 2
SH-2	58.30 ± 1.9	57 ± 3
SH-4	15.56 ± 0.7	–
SH-5	96.03 ± 1.1	13 ± 1
SH-6	76.14 ± 1.5	27 ± 2
SH-7	87.00 ± 0.9	24 ± 2
SH-8	33.96 ± 1.0	22 ± 2
SH-10	88.19 ± 1.4	20 ± 1
Gallic acid	93.11 ± 1.0	13 ± 1

*100 µL samples (5 mg/mL DMSO), – not calculated

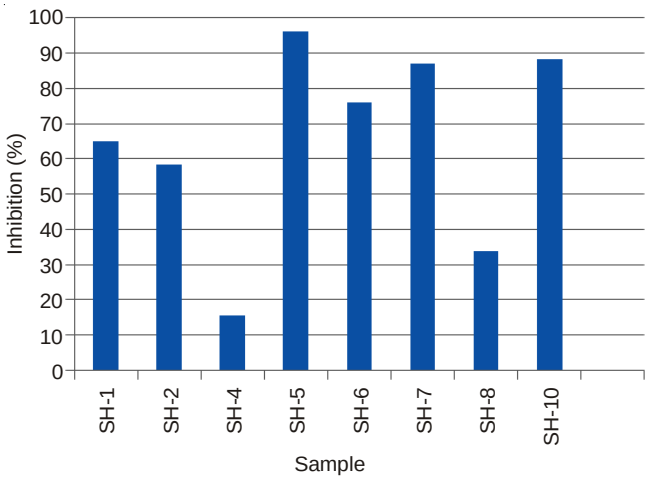


Fig. 1. Antioxidant activity of synthesized compounds

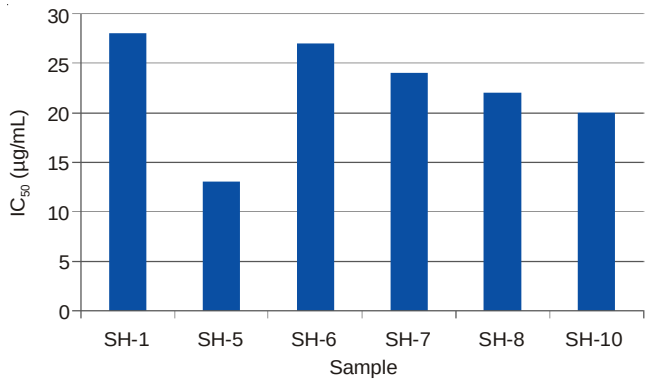


Fig. 2. IC_{50} values of synthesized compounds

Enzyme inhibition study: Enzyme inhibition potential of the above mentioned compounds were carried out against three enzymes; proteases, acetylcholine esterase and butyrylcholine esterase according to reported spectrophotometric protocols. Some compounds exhibited better results in one enzyme but remained inactive against others. In protease activity six compounds showed activity more than 40 %. SH-5 and SH-7 exhibited protease inhibition potential greater than 50 % (67.92 ± 1.5 and 59.43 ± 1.3 respectively). In AChE and BChE only SH-5 showed maximum activity; 84.16 ± 1.7 % and 69.77 ±

TABLE-5
ENZYME INHIBITION POTENTIAL OF SYNTHESIZED COMPOUNDS

Code	Protease enzyme		AChE enzyme		BChE enzyme	
	Inhibition (%)*	IC_{50} (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)
SH-1	37.02 ± 1.1	–	19.40 ± 1.0	–	11.17 ± 0.6	–
SH-2	35.59 ± 0.9	–	16.19 ± 0.6	–	9.80 ± 0.5	–
SH-4	41.69 ± 1.0	–	8.42 ± 1.0	–	19.92 ± 0.9	–
SH-5	67.92 ± 1.5	160 ± 5	84.16 ± 1.7	110 ± 4	69.77 ± 1.3	205 ± 2
SH-6	14.22 ± 0.6	–	27.59 ± 0.8	–	17.90 ± 0.6	–
SH-7	59.43 ± 1.3	190 ± 4	28.03 ± 0.9	–	20.53 ± 1.0	–
SH-8	23.06 ± 0.9	–	23.89 ± 0.8	–	24.83 ± 0.3	–
SH-10	39.87 ± 1.0	–	2.97 ± 0.5	–	8.21 ± 0.7	–
PMSF	81.21 ± 1.1	16 ± 1	–	–	–	
Galanthamine	–	–	87.12 ± 1.2	13 ± 1	82.07 ± 0.9	25 ± 1

*100 µL samples (5 mg/mL DMSO), – not calculated

1.3 % against AChE and BChE respectively (Table-5, Fig. 3). All other compounds exhibited weak response against these two tested enzymes which may be due to lack of binding capacity to the active site of the enzyme or to the substrate. Our findings are with agreements with previous studies that metal complex are good enzyme inhibitors and can be used for medicinal purposes.

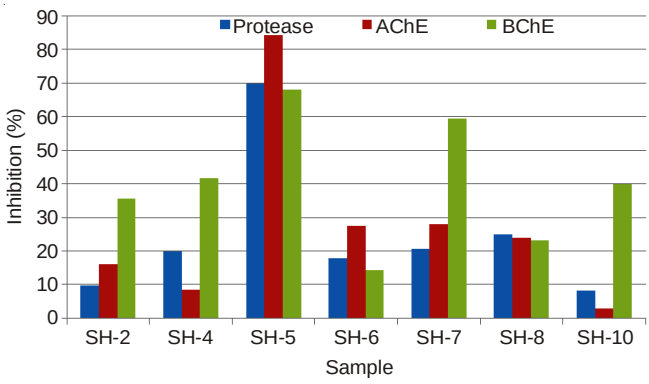


Fig. 3. Enzyme inhibition potential of the synthesized compounds

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