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Synthesis, characterization, SAR, antioxidant, anti-acetylcholinesterase and anti-butyrylcholinesterase activities of cephradine Schiff bases

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Abstract: The objective of this study was to deal with the evaluation of 7-(2-(benzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid based schiff bases as a new class of enzyme inhibitors. In this connection, a series of Schiff bases of cephradine with substituted aromatic aldehydes was synthesized and characterized using FTIR, ^{1}HNMR and ^{13}CNMR. The *in-vitro* biological activities including free radical scavenging potential using DPPH assay, acetyl cholinesterase and butyryl cholinesterase inhibition potential were evaluated. Two compounds of the series 1g and 1h were found to be active against AChE whereas no derivative was active against BChE while the whole series showed excellent 1, 1-diphenyl-2-picrylhydrazyl scavenging activity. All the synthesized compounds were found to be non-toxic and present passive gastrointestinal absorption. Furthermore, the study suggests that the synthesized cephradine derivatives exhibit inhibitory potential against different biologically relevant enzyme targets.

Keywords: Acetylcholinesterase and butyrylcholinesterase, antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl, cephradine.

INTRODUCTION

Alzheimer's is elucidated via diverse indications in the brain, including amyloid plaques in huge numbers, neuronal cell defeat, and vascular damage due to extensive plaque deposition (Pandey and Ramakrishnan, 2020). The pathological process of Alzheimer's disease initiates as a result of β - amyloid peptide (Aβ) deposition which causes impairment in synapses (Selkoe, 2002, Zhang *et al.,* 2009, Klein, 2006). Following synapse, acetylcholine is hydrolyzed by acetyl cholinesterase (Silman and Sussman, 2008). Acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) are a group of enzymes containing serine hydrolases functioning to hydrolyze neurotransmitter and ultimately deferral of the cholinergic nerve impulse (Soreq and Seidman, 2001, Rosas-Ballina *et al.,* 2011). It has been hypothesized that AChE may possibly play a part in increasing the rate of Aβ formation and may participate in amyloid accretion in the brain during AD (García-Ayllón *et al*., 2008). BChE plays a vital part in the development of disease. Both of these appear to have extensive roles in the (CNS) central nervous system. BChE, the sister enzyme of AChE, is a serine hydrolase that act as catalyst to hydrolyze the choline esters, together with acetylcholine (Ballard *et al.,* 2005a, Darvesh and Hopkins, 2003, Ballard *et al.,* 2005b). Subsequently with cholinesterase inhibitors the treatment strategy is the selective inhibition of AChE and BChE (Darreh-Shori *et al.,* 2006, Lane *et al.,* 2006, Schliebs and Arendt, 2006). The potent dual inhibitors are inevitably required to treat AD, and researchers are continually synthesizing AChE and BChE inhibitors (dos Santos *et al*., 2018).

Free radicals, both endogenous and exogenous, have been associated with mutagenesis, cardiovascular diseases, carcinogenesis, and ageing (Singh and Singh, 2008). Free radical scavenging method (DPPH) presents the primary means to evaluate the antioxidant potential of a compound (Kedare and Singh, 2011). Extensive research has been going on the antioxidants as they have protective effects on cells reducing the burden of oxidative stress, (Lobo *et al*., 2010) and development of potent antioxidants (Kumar *et al.,* 2017).

Schiff bases serve as template to a number of biologically important compounds; including but not limited to antiviral (Appelt *et al.,* 2013), antitumoral (Mladenova *et al.,* 2002), antioxidant (Kumar *et al.,* 2017), and also inhibition activities acetylcholinesterase and butyrylcholinesterase (Rahim *et al.,* 2016). Imines or Schiff bases can easily be produced by condensation reactions of C=O groups and primary amines. The presence of imine (C=N) linkage in Schiff bases is mainly responsible for biological activity (Kumar *et al.,* 2017).

Previously we reported the synthesis of sulfonamides based Schiff bases as potent urease inhibitors (Hamad *et al.,* 2020). The present study aims to synthesize novel Schiff based cephradine derivatives to explore the

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antioxidants, AChE and BChE inhibition potential of these newly synthesized derivatives.

MATERIALS AND METHODS

Chemistry

Cephradine was mixed with absolute ethanol and then different substituted aldehydes were added along with 1% solution of KOH as a catalyst. The mixture was refluxed for 3 h (Scheme 1). The solids formed under room temperature were subject to filtration and recrystallized from methanol to obtain compounds 1(a-h) in better yields. The final compounds were dried using a VacuumTherm (Thermo Scientific) vacuum oven overnight. All the solvents and reagents are of high purity and have been purchased from Sigma-Aldrich, Fluorochem, Alfa Aesar and Fisher Scientific. Melting points were recorded using a Gallen kamp melting point apparatus and are uncorrected. ^{1}H and ^{13}C nuclei nuclear magnetic (NMR) analyses were performed on a Spectrospin 400 MHz spectrometer (Bruker) equipped with a Sample Xpress (from Bruker) auto sampler system, using deuterated solvents for the preparation of the samples. The obtained spectra were analyzed using Topspin 7.1 software (Bruker). The chemical shifts were reported relative to trimethylsilane (TMS), used as a standard (0.00 ppm). Signals were identified and described as singlet (s), doublet (d), triplet (t) (Picconi *et al*., 2019). Fourier transform Infrared (FTIR) spectrum of samples was recorded using KBr pellet press method by Bruker TENSOR 27 FTIR spectrophotometer. Glassware used was dried in a UN55 oven (Memmert) at 200°C. The yields (%) given are on the basis of 1.0mM of each reactant used.

In-vitro Biological studies

AChE and BChE inhibitory Activity

Ellman's method (Ellman *et al.,* 1961) was employed for AChE and BChE inhibition assay. The reaction mixture consists of 60µL Na_2HPO_4 buffer (50mM, pH 7.7), 10µL test sample (0.5mM) and 10µL enzyme (0.005 unit AChE, 0.5 unit BChE, Sigma Inc.). The mixture was mixed and pre-incubated for 10 min at 37°C and read at 405 nm. Then 10µL substrate (0.5mM) (acetylthiocholine iodide / butyrylthiocholine chloride, Sigma Inc.), afterward 10µL 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (0.5mM) from Sigma Inc. was added, that is used for the initiation of reaction. After 15 min at 37°C the incubation time was completed, the absorbance was measured, using a 96-well plate reader (Synergy HT, Biotek, USA). The whole procedure was performed in triplicate using their respective reference controls. Eserine (0.5mM) was the positive control. The percent (%) inhibition of active compounds was calculated using the following equation.

Inhibition (%) = Control -Test / Control ×100

The inhibitory concentrations were calculated using the EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA) was used to calculate IC_{50}.

STATISTICAL ANALYSIS

All the experiments were performed in triplicate and the standard error of mean was calculated by Microsoft Excel 2010.

Antioxidant activity

(2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

Antioxidant activity of the derivatives was evalauted using DPPH assay (Chen *et al.,* 2006). 2.0 mL (0.2mg/mL in methanol) DPPH mixed with 1.0 mL (0.5 mg/mL in methanol) sample solution. UV-Visible spectrophotometer was used to monitor reduction in the free radicals concentration, at 517 nm. Absorbance of blank sample (1.0 mL methanol + 2.0 mL DPPH solution) was also measured. Percent inhibition (%) was calculated by the following formula:

Inhibition (%) = $[(A_B–A_A)/A_B] \times 100$

Where,

A_B =Absorbance of blank

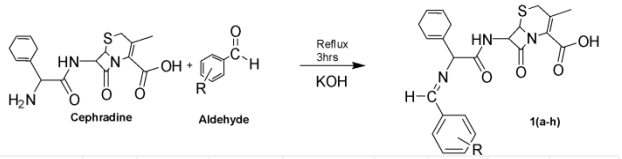
A_A =Absorbance of test sample

Standard antioxidant Ascorbic acid was tested and used as reference.

RESULTS

Synthesis and Characterization

The targeted motifs were synthesized by solubilizing cephradine in methanol (25-30 ml) using round bottom flask, into which substituted aromatic aldehyde was added. The pH of reaction mixture at the range of 7-8 using 1% potassium hydroxide solution in methanol. After the completion of reflux time (3hr), the developed product was filtered and recrystallized from methanol (Scheme-1). After optimizing the reaction conditions, a series of Schiff bases of cephradine and substituted aromatic aldehydes was prepared and characterized using FTIR, ^{1}H NMR and ^{13}C NMR. Synthesis of the compounds 1(a-h) is presented in Scheme 1 and the characterization is in section below.



Codes	1a	1b	1c	1d	1e	1f	1g	1h
R	H	2-OH, 4-F	4-NO_2	4-OCH_3	2-F, 4-Cl	2-Cl, 4-F	2-Br, 4-F	2,4-Cl

Scheme 1: Synthesis of the schiff bases of cephradine using aromatic aldehydes.

Table 1: Free Radical scavenging activity of the synthesized cephradine schiff bases.

S. No.	Code	%age scavenging activity	Sr. No.	Code	%age scavenging activity
1	*1a*	90.73	5	*1e*	92.88
2	*1b*	88.04	6	*1f*	70.22
3	*1c*	89.56	7	*1g*	41.33
4	*1d*	77.93	8	*1h*	79.62
Ascorbic Acid		80.00			

Table 2: AChE and BChE inhibition studies of the synthesized cephradine derivatives.

S. No.	Code	AChE		BChE	
		Inhibition (%) at 0.5 mM	IC_{50} (µM)	Inhibition (%) at 0.5 mM	IC_{50} (µM)
1.	*1a*	13.96±0.45	-	11.69±0.51	-
2.	*1b*	38.56±0.42	-	27.84±0.46	-
3.	*1c*	25.72±0.37	-	19.63±0.41	-
4.	*1d*	48.96±0.61	-	42.78±0.36	-
5.	*1e*	45.73±0.39	-	25.96±0.51	-
6.	*1f*	43.56±0.52	-	35.82±0.63	-
7.	*1g*	56.83±0.71	375.42±0.56	28.64±0.39	-
8.	*1h*	68.47±0.64	164.89±0.48	32.56±0.27	-
9.	*Eserine*	91.46±1.25	0.19±0.05	83.75±1.16	0.62±0.08

7-(2-(benzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1a)

Orange red crystalline solid; m.p; 129-131 ºC ; FT-IR (cm^{-1}), 3398, 3293 ν(NH), 3060 ν(CH), 1684 ν(C=O), 1657 ν(C=N), 1599,1490 ν(CH=CH); ^{1}H NMR (DMSO_d*6*,ppm), 1.91s(−CH_3), 2.56d: 3.18s, 3.25s (−CH_2), 4.12d, 5.69d, 5.71t, 5.93-5.94m (-CH), 7.26t ,7.32t; 7.63d (-C_6H_5), 8.12s (-NH-), 8.72s,(-CH), 10.17s(-OH); ^{13}C NMR (DMSO-d*6*, ppm), 21.5, 49.1, 53.8, 54.8, 54.9, 55.0, 85.3, 112.8, 112.9, 126.5, 128.9, 129.2, 131.8, 133.1, 133.8, 137.8, 160.2, 160.2, 174.0, 191.1.

7-(2-(4-fluoro-2-hydroxybenzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1b)

Orange crystalline powder; m.p; 78-80ºC; FT-IR (cm^{-1}), 3391, 3287 ν(NH), 3088 ν(CH), 1706 ν(C=O), 1647 ν(C=N), 1567,1465 ν(CH=CH); ^{1}H NMR (DMSO_d*6*,ppm), 2.28s(-CH_3), 2.53d: 3.08s, 3.16s (-CH_2), 3.48d, 5.43d, 5.44-5.45m, 5.47t; (-CH), 6.29d; 6.56d, 7.38d (-C_6H_5), 7.37s (-NH-), 7.39s,(-CH), 9.98s(-OH); ^{13}C NMR (DMSO−d*6*, ppm), 15.6, 30.5, 35.5, 50.0, 55.5, 67.5, 109.6, 111.3, 122.9, 123.9, 126.8, 129.7, 135.7, 136.7, 139.1, 155.0, 157.2, 158.0, 158.8, 162.2 .

7-(2-(4-nitrobenzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1c)

Orange crystalline powder; m.p; 109-111ºC; FT-IR (cm^{-1}), 3289 ν(NH), 2978 ν(CH), 1701 ν(C=O), 1650 ν(C=N), 1532,1466 ν(CH=CH); ^{1}H NMR (DMSO_d*6*,ppm), 2.28s(-CH_3), 2.49d: 3.02s, 3.15s (-CH_2), 5.44d, 5.46 t, 5.48d; (-CH), 7.26d, 7.79d, (-C_6H_5), 7.80s (-NH-), 7.81s,(-NCH), 9.77s(-OH) ;^{13}C NMR (DMSO−d*6*, ppm), 15.5, 39.4, 39.5, 39.9, 40.4, 40.6, 56.5, 115.6, 116.8, 116.9, 129.0, 132.5, 132.9, 133.54, 135.5, 136.7, 139.8, 142.5, 167.5, 192.2.

7-(2-(4-methoxybenzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1d)

Orange crystalline solid; m.p; 99-101ºC; FT-IR (cm^{-1}), 3289 ν(NH), 2970 ν(CH), 1657 ν(C=N), 1542,1487 ν(CH=CH); ^{1}H NMR (DMSO_d*6*,ppm), 1.84s, 3.91s (-CH_3), 2.451d, 2.48t: 3.05s, 3.14s (-CH_2), 3.95d, 5.46t, 6.69d; (-CH), 6.88d, 7.16d (-C_6H_5), 7.42s (-NH-), 7.43s,(-NCH), 10.03s(-OH); ^{13}C NMR (DMSO-d*6*, ppm), 14.4, 26.6, 39.4, 39.6, 40.4, 40.6, 56.6, 60.1, 102.1, 115.8, 119.1, 124.7, 129.8, 130.0, 138.6, 138.9, 155.2, 159.7, 164.4, 167.2, 189.8.

7-(2-(4-chloro-2-fluorobenzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1e)

Yellow crystalline powder; m.p; 104-106ºC; FT-IR (cm^{-1}), 3307 ν(NH), 2982 ν(CH), 1649 ν(C=N), 1567, 1464 ν(CH=CH) ; ^{1}H NMR (DMSO_d6,ppm), 2.32s (-CH_3), 2.50t, 2.52d, 3.20s (-CH_2), 3.45d, 5.44d, 5.49t, 5.52d (-CH), 7.13d, 7.16d, 7.53d, (-C_6H_5), 7.69s (-NH-), 7.71s,(-NCH), 9.99s (-OH); ^{13}C NMR (DMSO−d6, ppm), 15.5, 39.6, 39.7, 39.9, 40.6, 56.5, 62.2, 117.8, 118.1, 120.2, 121.1,123.1, 126.1, 126.2, 131.3, 132.3, 142.1, 155.5, 159.3, 166.8, 168.2, 187.4.

7-(2-(2-chloro-4-fluorobenzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1f)

Orange crystalline solid; m.p; 69-71ºC; FT-IR (cm^{-1}), 3419 ν(NH), 2975 ν(CH), 1655 ν(C=N), 1694, 1489 ν(CH=CH); ^{1}H NMR (DMSO_d6,ppm), 2.09s (-CH_3), 2.51t, 2.67d, 2.69s, 3.17s (-CH_2), 4.17d, 4.18d, 5.69t, 5.70d, (-CH), 7.13d, 7.14s, 7.53d, (-C_6H_5), 7.97s (-NH-), 7.99s (-NCH), 10.27s (-OH); ^{13}C NMR (DMSO−d6, ppm), 19.0, 27.4, 39.4, 39.8, 40.6, 56.5, 62.2, 116.0, 116.2, 117.2, 118.5, 118.8, 121.3, 132.5, 132.7, 133.4, 137.5, 152.3, 155.4, 167.1 , 167.4 , 189.0.

7-(2-(2-bromo-4-fluorobenzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1g)

Orange crystalline powder; m.p; 84-86ºC; FT-IR (cm^{-1}), 3411 ν(NH), 2973 ν(CH), 1700, 1465 ν(CH=CH); 1654 ν(C=N), ^{1}H NMR (DMSO_d6,ppm), 1.07s (-CH_3), 2.53d, 2.54t, 3.19s (-CH_2), 4.01d, 4.41d, 5.49t, 5.49-5.50m, 5.53d (-CH), 7.14s, 7.18d, 7.65d, (-C_6H_5), 7.80s (-NH-), 8.43s (-NCH), 10.00s (-OH); ^{13}C NMR (DMSO−d6, ppm), 15.6, 27.9, 39.4, 39.9, 40.6, 56.5, 64.2, 116.6, 119.9, 120.5, 121.8, 123.1, 124.7, 132.9, 133.2, 136.6, 137.1, 152.1, 155.2 , 161.6 , 165.7, 190.8.

7-(2-(4-chloro-2-hydroxybenzylideneamino)-2-(cyclohexa-1,4-dienyl)acetamido)-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1h)

Orange crystalline solid; m.p; 114-116ºC; FT-IR (cm^{-1}), 3414 ν(NH), 2973 ν(CH), 1698, 1463 ν(CH=CH); 1654 ν(C=N), ^{1}H NMR (DMSO_d6,ppm), 1.03s (-CH_3), 2.40d, 2.58t, 3.15s, (-CH_2), 4.23s, 4.90d, 5.11t, 5.51t, 5.61d; (-CH), 7.50d,7.51s, 7.53d, (-C_6H_5), 7.74s (-NH-), 7.76s (-NCH), 10.15s (-OH); ^{13}C NMR (DMSO-d6, ppm), 19.0, 29.3, 39.4, 39.6, 40.6, 56.5, 67.4, 120.2, 121.1, 125.5, 128.8, 129.1, 130.8, 131.5, 131.6, 134.1, 137.6, 140.2, 161.3, 162.5 , 166.2, 189.3.

DPPH Radical Scavenging Assay

The results of the radical scavenging activity of the synthesized derivatives are tabulated in table 1.

Enzyme Inhibition Studies

AChE and BChE Inhibition Studies

The results of the AChE and BChE Inhibition studies are presented in (table 2). Compounds 1g and 1h showed a significant potential for inhibition, while all other compounds were found to be inactive.

DISCUSSION

Schiff bases synthesized by the modification of marketed therapeutic agents have exhibited multiple pharmacological effects and more specifically schiff bases have a lot of potential to inhibit enzymes and displayed remarkable results as antioxidants (Kausar *et al*., 2019). Considering these remarkable pharmacological effects of Schiff bases we synthesized the novel derivatives of cephradine by using variety of aromatic aldehydes. Furthermore, antioxidant and enzyme inhibition activities were performed.

The antioxidant activity was explored by using DPPH free radical scavenging percentage of all the derivatives and structure activity relationship has shown that variation in inhibition potential of compounds is due to the varying substituents and their positions on the aromatic ring of attached benzaldehyde. All the compounds displayed significant antioxidant activity except 1g. Derivative 1e showed maximum radical scavenging activity of 92.88% having F at C-2 and Cl at C-4 as in another study the Schiff bases exhibited remarkable antioxidant effects (Khan *et al*., 2015). By inter-changing the position of F and Cl to (2Cl, 4F) in 1f the activity reduced to 70.22%. 1a was the second most active compound of the series with 90.73% scavenging activity. It has no attached substituent at the phenyl ring. By substitution of the methoxy group at C-4 in 1d and two chlorine atoms at C-2 and C-4 in 1h the activity was enhanced from 77.93% to 79.62%. When this methoxy group is replaced by NO_2 in 1c and substituting OH at C-2 and F at C-4 in 1b the activity increases up to 89.56% and 88.04% respectively. Interestingly, most of the compounds showed activity near to or better than the standard ascorbic acid having value of 80%.

Moreover, anticholinesterase potential of synthesized motifs was explored and the structure activity relationship studies of the prepared schiff bases have indicated that by varying the position and nature of the substituents on the phenyl ring their activities have changed. Among the tested series, two compounds viz 1g and 1h have been found active while the rest of the series was found inactive against AChE. Amongst these two 1g is found moderately active against AChE with IC_{50} of 375.42±0.56 as compared to standard eserine having IC_{50} value of 0.19±0.05. While the compound 1h significantly inhibited AChE (IC_{50} = 164.89±0.48). 1h is more active as compared to 1g as it has two Cl moieties at C-2 and C-4 position while the other one has a Br at C-2 and a F at C-4 of phenyl ring. 1f was found inactive in comparison to 1g although it has F at C-4 but at C-2 it has Cl not Br which rendered it inactive. While, most of the compounds showed weak inhibitory potential against BChe having % inhibition in the range of 11.69 to 32.56 due to the varying position and nature of the attached groups on the aryl ring of the aldehydes and similar type of results were observed by (Lolak and Akocak, 2020) against the cholinesterase enzyme.

CONCLUSION

Present work was done to prepare and characterize the Schiff base derivatives and to evaluate their antioxidant

and enzyme inhibition potential. The spectroscopic characterization data has confirmed the formation of Schiff bases of cephradine with benzaldehyde and it's C-2 and C-4 substituted derivatives. According to the results of the biological evaluation studies the compounds 1g and 1h showed significant AChE inhibition activities and the whole series except 1g was found to have excellent antioxidant activities even more than the standard ascorbic acid. The newly synthesized derivatives can be taken up as lead models for future drug developments and derivatization.

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